

FLAVONOIDS

**Chemistry, Biochemistry
and Applications**

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Chemistry, Biochemistry and Applications

Edited by
Øyvind M. Andersen
Kenneth R. Markham



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Editors

Oyvind M. Andersen, a full professor of chemistry since 1993, has specialized in the chemistry of natural compounds. Since 2002, he has been head of the Department of Chemistry, University of Bergen, Norway. He received his Ph.D. degree in 1988 from the University of Bergen. He is author or coauthor of 100 journal articles, seven book chapters, and five patents, and has supervised over 40 M.Sc. and Ph.D. students in the fields of anthocyanins and other flavonoids. The activities of Dr. Andersen's research group have led to the establishment of several flavonoid-based companies. Most of his research projects concentrate on structural elucidation of new compounds, while others are methodology based and relate to NMR spectroscopy and various chromatographic techniques. Some of the projects focus on anthocyanin properties with the objective of exploring their pharmaceutical potential and their use as colorants in food.



Kenneth R. Markham (Ken) has recently retired from his position as group leader and distinguished scientist at Industrial Research Ltd., a New Zealand Crown Research Institute. Following completion of his B.Sc. and M.Sc. (Hons) at Victoria University, Wellington, New Zealand, and his Ph.D. in chemistry (University of Melbourne, Australia, 1963), he returned to Chemistry Division, D.S.I.R., in Lower Hutt, New Zealand, to work on xanthone chemistry. His interest in flavonoids began during a 2-year postdoctoral work with Professor Tom Mabry at the University of Texas at Austin (1965–1967). The coauthored book *The Systematic Identification of Flavonoids* resulted from this interaction. On his return to New Zealand, Dr. Markham established and led the “Natural Products” section, predominantly devoted to the study of flavonoids with particular emphasis on the chemotaxonomy of New Zealand bryophytes, ferns, and gymnosperms. This work was carried out in conjunction with both local botanists and colleagues at the University of Saarbrücken (Professors Zinsmeister, Mues, and Geiger). In 1979, he worked as a visiting scientist with Professor Jeffrey Harborne at the University of Reading. This again resulted in the publication of a popular book, *Techniques of Flavonoid Identification*. Dr. Markham's flavonoid research continued at Industrial Research Ltd. and has led him into studies as diverse as plant chemotaxonomy, plant evolution, plant UV protection, historical Antarctic ozone levels, propolis and bee pollen bioactives, G.E. modification of flower color, and subcellular chemistry, to name but a few. His work has been reported in some 280 publications, including 18 invited chapters and two books, and has been recognized over the years through awards such as the Chemical Society's Easterfield Medal, Fellowship of the Royal Society of New Zealand, a Ministerial Award for Excellence in Scientific Research, and the 1999 Pergamon Phytochemical Prize.



Preface

It is with great pleasure that we accepted the offer by CRC Press to assemble and edit this compilation of reviews on flavonoids and their properties and functions for the present volume. We considered the volume timely in that the last book of this general type, *The Flavonoids — Advances in Research Since 1986* (edited by Jeffrey B. Harborne), appeared over a decade ago. Since then, advances in the flavonoid field have been nothing short of spectacular. These advances are particularly evident in the contributed chapters that cover: the discovery of a variety of new flavonoids; the application of advanced analytical techniques; genetic manipulation of the flavonoid pathway; improved understanding of flavonoid structures and physiological functions in plants and animals; and, perhaps most importantly, the significance of flavonoids to human health.

Whilst the updating aspect of the chapters is seen as the prime contribution of this book, an effort also has been made to include a summary of previous knowledge in the field to enable the reader to place new advances in this context. Chapters 1 and 2 review the application of contemporary isolation, quantification, and spectroscopic techniques in flavonoid analysis, while Chapter 3 is devoted to molecular biology and biotechnology of flavonoid biosynthesis. Individual chapters address the flavonoids in food (Chapter 4) and wine (Chapter 5), and the impact of flavonoids and other phenolics on human health (Chapter 6 and, in part, Chapter 16). Chapter 8 reviews newly discovered flavonoid functions in plants, while Chapter 9 is the first review of flavonoid–protein interactions. Chapters 10 to 17 discuss the chemistry and distribution of the various flavonoid classes including new structures reported during 1993 to 2004. A complete listing of all known flavonoids within the various flavonoid classes are found in these later chapters and the Appendix, and to date a total of above 8150 different flavonoids has been reported.

It is difficult to overstate the importance of recent advances in research on flavonoids, and we are sure that the information contained within this book will prove to be invaluable to a wide range of researchers, professionals, and advanced students in both the academic and industrial sectors.

We are greatly indebted to our authors, and are delighted that so many of the world's leading researchers in a variety of flavonoid-related fields have been willing, so generously, to share their knowledge and experience with others through their contribution to this volume. We are also very grateful to Lindsey Hofmeister, Erika Dery, Jill Jurgensen, and Tanya Gordon at Taylor and Francis, and Balaji Krishnasamy at SPI Publisher Services for their support and interest throughout the preparation of this book.

Øyvind M. Andersen and Kenneth R. Markham

Historical Advances in the Flavonoid Field — A Personal Perspective

Having been associated with flavonoid research for the past 40 years, and having witnessed the spellbinding changes that have taken place in the field during this time, it is too tempting by far not to take this opportunity to document for future researchers a brief personal perspective on developments in the flavonoid field over this period. I emphasize that this is

but a personal perspective on progress, and as such will surely exhibit some bias and deficiencies. To the aggrieved I offer my apologies.

In the early 1960s, flavonoids were widely viewed as metabolic waste products that were stored in the plant vacuole. Whilst there was interest at that time in their function as flower colorants, and in their distribution between plant taxa, the earliest investigations of their biosynthesis had just begun. In this respect it is informative to note that Tom Geissman's 1962 compilation of reviews in *The Chemistry of Flavonoid Compounds* includes nothing at all on biological function, and details only paper chromatography and absorption spectroscopy as analytical tools. At this time too, information on flavonoid distribution within the plant kingdom was still incomplete. For example, even as late as 1969 Bate-Smith wrote (in *Chemical Plant Taxonomy* edited by T. Swain) that flavonoids are rarely found in any but vascular plants. But within a few years of this statement, Markham, Porter, and others reported the widespread presence of flavonoids in mosses and liverworts and even their occurrence in an alga, *Nitella hookeri*. This, incidentally, remains the sole example of the occurrence of flavonoids in algae. To date, flavonoids have been found in all major categories of green plants except for the Anthocerotae.

By 1967 little had changed with regard to the application of physical techniques to flavonoid structure determination, with NMR and GLC yet to make an impact on the field. About this time, however, there was an upsurge in the application of flavonoid distribution to the emerging field of chemotaxonomy. Leading these researches were groups at the University of Texas at Austin (led by Tom Mabry, Ralph Alston, and Billy Turner) and at the University of Reading (led by Jeffrey Harborne). Alston and Turner's pioneering work on the tracking of plant hybridization through flavonoid analyses is still quoted today, as also is much of the anthocyanin structure and flavonoid distributional work detailed by Harborne in his 1967 book, *Comparative Biochemistry of the Flavonoids*.

Rapid advances in the application of physical techniques to flavonoid structure analysis began appearing in the literature in the mid- to late-1960s, and these were well documented in a series of books beginning in 1970 with Mabry, Markham, and Thomas' *The Systematic Identification of Flavonoids*, which reviewed, for the first time, the considerable advances made in the application of shift reagents in UV-visible absorption spectroscopy, the use of GLC for sugar analyses, and the application of ^1H NMR spectroscopy to flavonoid structure analysis. At this time, CCl_4 -soluble TMS ether derivatives were widely used for NMR studies in the absence of readily obtainable deuterated solvents. These same derivatives, together with permethylated derivatives, were commonly also used to make flavonoid glycosides sufficiently volatile for early applications of electron impact mass spectrometry to flavonoid structure analysis (first summarized in the 1974 "Advances" book, *The Flavonoids*, Chapter 3). Contemporaneously, and detailed in the above volume, rapid advances were being reported in the structure analysis of C-glycosides (Chopin and Brouillant, Chapter 12) and in the biosynthesis of flavonoids (Hahlbrock and Grisebach, Chapter 16). A growing awareness of the physiological, metabolic, and evolutionary value of flavonoids was also beginning to emerge. Tony Swain, for example, was in the initial stages of formulating his innovative interpretations of the evolution of flavonoids, and the part that their "chemoecology" played in the evolution of plants (e.g., see Chapter 20 in *The Flavonoids*, 1974).

The next major advance in flavonoid structural techniques was the application of ^{13}C NMR spectroscopy. This has arguably had the greatest impact on flavonoid structure analysis since the invention of paper chromatography around 1900. For the first time, complete flavonoid structures, including flavonoid aglycones together with sugar types and linkages, could be determined using a single technique. Admittedly, the development of advanced two-dimensional techniques has further revolutionized structure analysis since the earliest applications of this technique to flavonoids in the mid-1970s, and the appearance of

the first review article by Markham and Chari in 1982 (*The Flavonoids — Advances in Research*, Chapter 2). Advances in technology have diminished the sample size required for spectral analysis by more than 100 times for both ^{13}C and ^1H NMR techniques. In the early 1960s, 100-mg samples were required for proton work and in the late 1970s the same sized samples were required for carbon-13 studies.

Modern flavonoid researchers will also be aware of the impact that the more recently developed mass spectrometry techniques such as FAB, MALDI-TOF, and electrospray have had on the ability of researchers to elucidate complex flavonoid glycosidic structures through the ready determination of accurate molecular weights and limited fragmentation patterns. Similarly, the development of advanced methods of separation such as capillary electrophoresis, HPLC, and, latterly, HPLC-MS, has recently revolutionized the qualitative and quantitative analysis of flavonoid mixtures. Chemotaxonomic studies involving comparative distributional data have accordingly been vastly facilitated. Techniques such as those referred to above have enabled previously intractable flavonoid structural problems to be solved. Particularly good examples of this are to be found amongst the many complex structures currently being reported for “blue” flower pigments based on anthocyanins elaborated in an often intricate manner with large numbers of sugars, acyl groups, other flavonoids, and occasionally including metal ions.

Returning once again to the questions of function and uses, the old concept of flavonoids being merely the by-products of cellular metabolism, which are simply compartmentalized in solution in the cell vacuole, is well and truly past its use-by date. For a start, studies have revealed that flavonoids are also commonly found on the outer surfaces of leaves and flowers, albeit only the aglycone form. Additionally, flavonoids have been shown over the past few years to be found in the cell wall, the cytoplasm, in oil bodies, and associated with the nucleus and cell proteins, as well as in the vacuole. Even in the vacuole, flavonoids are not necessarily found free in solution. For example, protein-bound flavonoids have been isolated from lisianthus and other flowers in which a structurally specific binding has been identified (in anthocyanic vacuolar inclusions). It is probable that flavonoid location and specific protein binding properties will ultimately prove to relate directly to their function in plants.

Amongst the many functions now known to be performed by plant flavonoids are those of UV protection, oxidant or free radical protection, modulation of enzymic activity, allelopathy, insect attraction or repulsion, nectar guides, probing stimulants, viral, fungal, and bacterial protection, nodulation in leguminous plants, pollen germination, etc., and it is likely that this is only the tip of the iceberg. Flavonoids, it would seem, have been vital components of plants, ever since their (purported) development at the time plant life emerged from the aquatic environment, and needed protection from UV light in an atmosphere lacking today's protective ozone layer. The continued widespread accumulation of flavonoids by virtually all land-based green plants lends support to this view.

Intriguingly, it is now possible to exert some precise control over plant flavonoid composition. Manipulation of the flavonoid biosynthetic pathway in plants via genetic engineering has progressed rapidly in recent years. This has been expedited by the extensive information made available through the earlier studies of flavonoid biosynthesis pioneered in the 1960s and 1970s (see above). Genetic manipulation of the flavonoid pathway in plants has enormous potential to, for example, produce new flower colors, enhance the nutritional value of crops, and improve crop protection from UV light, microorganisms, insects, and browsing animals. Indeed, much of this work has been underway for some time and shows great promise.

Plant flavonoids have been shown in recent years to be of vital significance to mankind as well as to plants. They have been strongly implicated as active contributors to the health benefits of beverages such as tea and wine, foods such as fruit and vegetables, and even,

recently, chocolate. The widely lauded “Mediterranean diet,” for example, is thought to owe much of its benefits to the presence of flavonoids in the food and beverages. In the early 1990s, Hertog published aspects of the “Zutphen Elderly Study,” and, in so doing, provided for the first time a sound epidemiological correlation between high food flavonoid intake and a lowering in the risk of coronary heart disease. This study also produced the first reliable estimates of average daily flavonoid intake at around 23 mg, a figure much lower than the 1000 mg that had been proposed in the 1970s. The major sources of flavonoids (in the Dutch population) were found to be tea, onions, and apples.

Other potential health benefits of dietary flavonoids are too numerous to mention here. Suffice it to say that our understanding of the importance of flavonoids in the human diet is continuing to advance rapidly. One suspects that much of the physiological activity associated with flavonoids can be attributed to (i) their proven effectiveness as antioxidants and free radical scavengers, (ii) to their metal complexing capabilities (a capability that drove early advances in absorption spectroscopy and NMR studies), and (iii) to their ability to bind with a high degree of specificity to proteins.

Because of the incredible advances that have taken place, my involvement in flavonoid studies over the past 40 years has been exciting and stimulating. I feel privileged to have been part of the discovery process. During this period flavonoids as a natural product group have risen from relative obscurity (at least in the popular media) to such prominence that educated people in the West are now not only aware of the name, but also aware of the publicized health benefits associated with their consumption.

At an academic level too, although flavonoid structure elucidation is rapidly becoming a mature science thanks to technological advances, studies of their bioavailability and physiological activity in both animals and plants is likely to become the new frontier. Exciting advances in the understanding of this physiological activity will undoubtedly lead to the more widespread application of flavonoids in the improvement of human health and in crop quality. A major influence, especially in the latter, is likely to be brought about through skilful genetic manipulation of the flavonoid biosynthetic pathway. We await this progress with eager anticipation.

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1 Separation and Quantification of Flavonoids

Andrew Marston and Kurt Hostettmann

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1.1 INTRODUCTION

Essential to the study of flavonoids is having the means available for their separation (analytical and preparative) and isolation. The importance of this aspect of flavonoid research can be seen in the number of review articles that refer to their chromatography.^{1–6} However, the information is usually spread out over chapters in books or occurs in isolated sections devoted to individual classes of these polyphenols.

This chapter, therefore, aims to present a brief unified summary of general techniques, with reference to the different categories of structure: flavones and flavonols (and their glycosides), isoflavones, flavanones, chalcones, anthocyanins, and proanthocyanidins.

In earlier times, thin-layer chromatography (TLC), polyamide chromatography, and paper electrophoresis were the major separation techniques for phenolics. Of these methods, TLC is still the workhorse of flavonoid analysis. It is used as a rapid, simple, and versatile method for following polyphenolics in plant extracts and in fractionation work. However, the majority of published work now refers to qualitative and quantitative applications of high-performance liquid chromatography (HPLC) for analysis. Flavonoids can be separated,

quantified, and identified in one operation by coupling HPLC to ultraviolet (UV), mass, or nuclear magnetic resonance (NMR) detectors. Recently, the technique of capillary electrophoresis (CE) has been gaining attention.

One feature that is of immense benefit for flavonoid analysis is the presence of the phenyl ring. This excellent chromophore is, of course, UV active and provides the reason why flavonoids are so easy to detect. Their UV spectra are particularly informative, providing considerable structural information that can distinguish the type of phenol and the oxidation pattern.

A number of techniques have been used for the preparative separation of flavonoids. These include HPLC, Diaion, Amberlite XAD-2 and XAD-7, and Fractogel TSK/Toyopearl HW-40 resins, gel filtration on Sephadex, and centrifugal partition chromatography (CPC).⁷ The choice of methods and strategies varies from research group to research group and depends often on the class of flavonoid studied.

1.2 EXTRACTION

Flavonoids (particularly glycosides) can be degraded by enzyme action when collected plant material is fresh or nondried. It is thus advisable to use dry, lyophilized, or frozen samples. When dry plant material is used, it is generally ground into a powder. For extraction, the solvent is chosen as a function of the type of flavonoid required. Polarity is an important consideration here. Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are extracted with chloroform, dichloromethane, diethyl ether, or ethyl acetate, while flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol–water mixtures. Glycosides have increased water solubility and aqueous alcoholic solutions are suitable. The bulk of extractions of flavonoid-containing material are still performed by simple direct solvent extraction.

Powdered plant material can also be extracted in a Soxhlet apparatus, first with hexane, for example, to remove lipids and then with ethyl acetate or ethanol to obtain phenolics. This approach is not suitable for heat-sensitive compounds.

A convenient and frequently used procedure is sequential solvent extraction. A first step, with dichloromethane, for example, will extract flavonoid aglycones and less polar material. A subsequent step with an alcohol will extract flavonoid glycosides and polar constituents.

Certain flavanone and chalcone glycosides are difficult to dissolve in methanol, ethanol, or alcohol–water mixtures. Flavanone solubility depends on the pH of water-containing solutions.

Flavan-3-ols (catechins, proanthocyanidins, and condensed tannins) can often be extracted directly with water. However, the composition of the extract does vary with the solvent — whether water, methanol, ethanol, acetone, or ethyl acetate. For example, it is claimed that methanol is the best solvent for catechins and 70% acetone for procyanidins.⁸

Anthocyanins are extracted with cold acidified methanol. The acid employed is usually acetic acid (about 7%) or trifluoroacetic acid (TFA) (about 3%). The use of mineral acid can lead to the loss of attached acyl groups.

Extraction is typically performed with magnetic stirring or shaking but other methods have recently been introduced to increase the efficiency and speed of the extraction procedure. The first of these is called pressurized liquid extraction (PLE). By this method, extraction is accelerated by using high temperature and high pressure. There is enhanced diffusivity of the solvent and, at the same time, there is the possibility of working under an inert atmosphere and with protection from light. Commercially available instruments have extraction vessels with volumes up to about 100 ml. In a study involving medicinal plants, solvent use was

reduced by a factor of two.⁹ The optimization of rutin and isoquercitrin recovery from older (*Sambucus nigra*, Caprifoliaceae) flowers has been described. Application of PLE gave better results than maceration — and shorter extraction times and smaller amounts of solvent were required.¹⁰ PLE of grape seeds and skins from winemaking wastes proved to be an efficient procedure for obtaining catechin and epicatechin with little decomposition, provided the temperature was kept below 130°C.¹¹

As its name suggests, supercritical fluid extraction (SFE) relies on the solubilizing properties of supercritical fluids. The lower viscosities and higher diffusion rates of supercritical fluids, when compared with those of liquids, make them ideal for the extraction of diffusion-controlled matrices, such as plant tissues. Advantages of the method are lower solvent consumption, controllable selectivity, and less thermal or chemical degradation than methods such as Soxhlet extraction. Numerous applications in the extraction of natural products have been reported, with supercritical carbon dioxide being the most widely used extraction solvent.^{12,13} However, to allow for the extraction of polar compounds such as flavonoids, polar solvents (like methanol) have to be added as modifiers. There is consequently a substantial reduction in selectivity. This explains why there are relatively few applications to polyphenols in the literature. Even with pressures of up to 689 bar and 20% modifier (usually methanol) in the extraction fluid, yields of polyphenolic compounds remain low, as shown for marigold (*Calendula officinalis*, Asteraceae) and chamomile (*Matricaria recutita*, Asteraceae).¹⁴

Ultrasound-assisted extraction is a rapid technique that can also be used with mixtures of immiscible solvents: hexane with methanol–water (9:1), for example, is a system used for the Brazilian plant *Lychnophora ericoides* (Asteraceae). The hexane phase concentrated less polar sesquiterpene lactones and hydrocarbons, while the aqueous alcohol phase concentrated flavonoids and more polar sesquiterpene lactones.¹⁵

Microwave-assisted extraction (MAE) has been described for the extraction of various compounds from different matrices.¹⁶ It is a simple technique that can be completed in a few minutes. Microwave energy is applied to the sample suspended in solvent, either in a closed vessel or in an open cell. The latter allows larger amounts of sample to be extracted. A certain degree of heating is involved.¹⁷

1.3 PREPARATIVE SEPARATION

1.3.1 PRELIMINARY PURIFICATION

Once a suitably polar plant extract is obtained, a preliminary cleanup is advantageous. The classical method of separating phenolics from plant extracts is to precipitate with lead acetate or extract into alkali or carbonate, followed by acidification. The lead acetate procedure is often unsatisfactory since some phenolics do not precipitate; other compounds may co-precipitate and it is not always easy to remove the lead salts.

Alternatively, solvent partition or countercurrent techniques may be applied. In order to obtain an isoflavonoid-rich fraction from *Erythrina* species (Leguminosae) for further purification work, an organic solvent extract was dissolved in 90% methanol and first partitioned with hexane. The residual methanol part was adjusted with water to 30% and partitioned with *t*-butyl methyl ether–hexane (9:1). This latter mixture was then chromatographed to obtain pure compounds.¹⁸

A short polyamide column, a Sephadex LH-20 column, or an ion exchange resin can be used. Absorption of crude extracts onto Diaion HP-20 or Amberlite XAD-2 (or XAD-7) columns, followed by elution with a methanol–water gradient, is an excellent way of preparing flavonoid-rich fractions.

1.3.2 PREPARATIVE METHODS

One of the major problems with the preparative separation of flavonoids is their sparing solubility in solvents employed in chromatography. Moreover, the flavonoids become less soluble as their purification proceeds. Poor solubility in the mobile phase used for a chromatographic separation can induce precipitation at the head of the column, leading to poor resolution, decrease in solvent flow, or even blockage of the column.

Other complications can also arise. For example, in the separation of anthocyanins and anthocyanin-rich fractions, it is advisable to avoid acetonitrile and formic acid — acetonitrile is difficult to evaporate and there is a risk of ester formation with formic acid.

There is no single isolation strategy for the separation of flavonoids and one or many steps may be necessary for their isolation. The choice of method depends on the polarity of the compounds and the quantity of sample available. Most of the preparative methods available are described in a volume by Hostettmann et al.⁷

Conventional open-column chromatography is still widely used because of its simplicity and its value as an initial separation step. Preparative work on large quantities of flavonoids from crude plant extracts is also possible. Support materials include polyamide, cellulose, silica gel, Sephadex LH-20, and Sephadex G-10, G-25, and G-50. Sephadex LH-20 is recommended for the separation of proanthocyanidins. For Sephadex gels, as well as size exclusion, adsorption and partition mechanisms operate in the presence of organic solvents. Although methanol and ethanol can be used as eluents for proanthocyanidins, acetone is better for displacing the high molecular weight polyphenols. Slow flow rates are also recommended. Open-column chromatography with certain supports (silica gel, polyamide) suffers from a certain degree of irreversible adsorption of the solute on the column.

Modifications of the method (dry-column chromatography, vacuum liquid chromatography, VLC, for example) are also of practical use for the rapid fractionation of plant extracts. VLC with a polyamide support has been reported for the separation of flavonol glycosides.¹⁹

Preparative TLC is a separation method that requires the least financial outlay and the most basic equipment. It is normally employed for milligram quantities of sample, although gram quantities are also handled if the mixture is not too complex. Preparative TLC in conjunction with open-column chromatography remains a straightforward means of purifying natural products, although variants of planar chromatography, such as centrifugal TLC,⁷ have found application in the separation of flavonoids.

Other combinations are, of course, possible, depending on the particular separation problem. Combining gel filtration or liquid–liquid partition with liquid chromatography (LC) is one solution. Inclusion of chromatography on polymeric supports⁷ can also provide additional means of solving a difficult separation.

Several preparative pressure liquid chromatographic methods are available. These can be classified according to the pressure employed for the separation:

- High-pressure (or high-performance) LC (>20 bar/300 psi)
- Medium-pressure LC (5 to 20 bar/75 to 300 psi)
- Low-pressure LC (<5 bar/75 psi)
- Flash chromatography (ca. 2 bar/30 psi)

1.3.2.1 High-Performance Liquid Chromatography

HPLC is becoming by far the most popular technique for the separation of flavonoids, both on preparative and analytical scales. Improvements in instrumentation, packing materials, and column technology are being introduced all the time, making the technique more and more attractive.

The difference between the analytical and preparative methodologies is that analytical HPLC does not rely on the recovery of a sample, while preparative HPLC is a purification process and aims at the isolation of a pure substance from a mixture.

Semipreparative HPLC separations (for 1 to 100 mg sample sizes) use columns of internal diameter 8 to 20 mm, often packed with 10 μm (or smaller) particles. Large samples can be separated by preparative (or even process-scale) installations but costs become correspondingly higher.

Optimization can be performed on analytical HPLC columns before transposition to a semipreparative scale.

The aim of this chapter is not a detailed description of the technique and instrumentation but to show applications of HPLC in the preparative separation of flavonoids. Some representative examples are given in Table 1.1. In a 1982 review of isolation techniques for flavonoids,³ preparative HPLC had at that time not been fully exploited. However, the situation is now very different and 80% of all flavonoid isolations contain a HPLC step. Approximately 95% of reported HPLC applications are on octadecylsilyl phases. Both isocratic and gradient conditions are employed.

1.3.2.2 Medium-Pressure Liquid Chromatography

The term “medium-pressure liquid chromatography” (MPLC) covers a wide range of column diameters, different granulometry packing materials, different pressures, and a number of

TABLE 1.1
Preparative Separations of Flavonoids by HPLC

Sample	Column	Eluent	Reference
Phenolics from <i>Picea abies</i>	Nucleosil 100–7C ₁₈ 250 × 21 mm	MeOH–H ₂ O, gradient	21
Chalcones from <i>Myrica serrata</i>	LiChrosorb Diol 7 μm , 250 × 16 mm	MeOH–H ₂ O, 55:45	20
	Nucleosil 100–7C ₁₈ 250 × 21 mm	MeOH–H ₂ O, 76:24	
Flavones from <i>Tanacetum parthenium</i>	LiChrospher RP-18 250 × 25 mm	CH ₃ CN–H ₂ O, 3:7	22
Flavone glycosides from <i>Lysionotus pauciflorus</i>	LiChrosorb RP-18 250 × 10 mm	CH ₃ CN–H ₂ O, 1:4	23
Flavonoid glucuronides from <i>Malva sylvestris</i>	Spherisorb ODS-2 5 μm , 250 × 16 mm	CH ₃ CN–H ₂ O–THF–HOAc, 205:718:62:15	24
Flavonol galloyl-glycosides from <i>Acacia confusa</i>	Hyperprep ODS 250 × 10 mm	CH ₃ CN–H ₂ O, gradient	25
Flavanones from <i>Greigia sphacelata</i>	LiChrospher Diol 5 μm , 250 × 4.6 mm	Hexane–EtOAc, 7:3	26
Prenylated flavonoids from <i>Anaxagorea luzonensis</i>	Asahipack ODP-90 10 μm , 300 × 28 mm	CH ₃ CN–H ₂ O, 45:55	27
Prenylated isoflavonoids from <i>Erythrina vogelii</i>	μ Bondapak C ₁₈ 10 μm , 100 × 25 mm	MeOH–H ₂ O, isocratic	28
Biflavones from <i>Cupressocyparis leylandii</i>	LiChrospher RP-18 7 μm , 250 × 10 mm	MeOH–H ₂ O, 72:28	29
Anthocyanin glycosides	Spherisorb ODS-2 10 μm , 250 × 10 mm	MeOH–5% HCOOH	30
Proanthocyanidins and flavans from <i>Prunus prostrata</i>	Eurosphere 80 RP-18 7 μm , 250 × 16 mm	CH ₃ CN–H ₂ O (+0.1% TFA), 1:4, 3:17	31

commercially available systems. In its simplest form, MPLC is a closed column (generally glass) connected to a compressed air source or a reciprocating pump. It fulfills the requirement for a simple alternative method to open-column chromatography or flash chromatography, with both higher resolution and shorter separation times. MPLC columns have a high loading capacity — up to a 1:25 sample-to-packing-material ratio³² — and are ideal for the separation of flavonoids.

In MPLC, the columns are generally filled by the user. Particle sizes of 25 to 200 μm are usually advocated (15 to 25, 25 to 40, or 43 to 60 μm are the most common ranges) and either slurry packing or dry packing is possible. Resolution is increased for a long column of small internal diameter when compared with a shorter column of larger internal diameter (with the same amount of stationary phase).³³ Choice of solvent systems can be efficiently performed by TLC³⁴ or by analytical HPLC. Transposition to MPLC is straightforward and direct.³⁵

Some applications of MPLC to the separation of flavonoids are shown in Table 1.2.

1.3.2.3 Centrifugal Partition Chromatography

Various countercurrent chromatographic techniques have been successfully employed for the separation of flavonoids.⁷ Countercurrent chromatography is a separation technique that relies on the partition of a sample between two immiscible solvents, the relative proportions of solute passing into each of the two phases determined by the partition coefficients of the components of the solute. It is an all-liquid method that is characterized by the absence of a solid support, and thus has the following advantages over other chromatographic techniques:

- No irreversible adsorption of the sample
- Quantitative recovery of the introduced sample
- Greatly reduced risk of sample denaturation

TABLE 1.2
Separation of Flavonoids by Medium-Pressure Liquid Chromatography

Sample	Column	Eluent	Reference
Chalcones from <i>Piper aduncum</i>	Silica gel 800 × 36 mm	Hexane–TBME–CH ₂ Cl ₂ –EtOH, 99:0.4:0.3:0.3	36
Flavonoids from <i>Sophora moorcroftiana</i>	RP-18 20 μm	MeOH–H ₂ O, 3:1	37
Flavonol glycosides from <i>Epilobium</i> species	RP-18 15–25 μm 460 × 26 mm	MeOH–H ₂ O, 35:65	38
Dihydroflavonoid glycosides from <i>Calluna vulgaris</i>	Polyamide SC-6 460 × 26 mm RP-18 20–40 μm 460 × 15 mm	Toluene–MeOH MeOH–H ₂ O	39
Prenylflavonoid glycosides from <i>Epimedium koreanum</i>	RP-8 460 × 26 mm	MeOH–H ₂ O, 2:3	40
Prenylated isoflavonoids from <i>Erythrina vogelii</i>	RP-18 15–25 μm 500 × 40 mm	MeOH–H ₂ O, 58:42, 60:40	41
Biflavonoids from <i>Wikstroemia indica</i>	RP-18 300 × 35 mm	MeOH–H ₂ O, 55:45 → 95:5	42

- Low solvent consumption
- Favorable economics

It is obvious, therefore, that such a technique is ideal for flavonoids, which often suffer from problems of retention on solid supports such as silica gel and polyamide.

Countercurrent distribution, droplet countercurrent chromatography, and rotation locular countercurrent chromatography are now seldom used but CPC, also known as centrifugal countercurrent chromatography, finds extensive application for the preparative separation of flavonoids. In CPC, the liquid stationary phase is retained by centrifugal force instead of a solid support (in column chromatography). Basically, two alternative designs of apparatus are on the market⁴³: (a) rotating coil instruments; (b) disk or cartridge instruments.

Although most CPC separations are on a preparative scale, analytical instruments do exist.⁴⁴ However, these are mostly used to find suitable separation conditions for scale-up.

There are numerous examples of preparative separations of flavonoids^{7,45} and some are listed in Table 1.3.

An example of the separation of flavonoid glycosides by CPC is shown in Figure 1.1. The leaves of the African plant *Tephrosia vogelii* (Leguminosae) were first extracted with dichloromethane and then with methanol. Methanol extract (500 mg) was injected in a mixture of the two phases of the solvent system and elution of the three major glycosides was achieved within 3 h.⁵⁸

The technique of CPC was also employed as a key step in the purification of 26 phenolic compounds from the needles of Norway spruce (*Picea abies*, Pinaceae). An aqueous extract of needles (5.45 g) was separated with the solvent system CHCl₃-MeOH-*i*-PrOH-H₂O (5:6:1:4), initially with the lower phase as mobile phase and then subsequently switching to the upper phase as mobile phase. Final purification of the constituent flavonol glycosides, stilbenes, and catechins was by gel filtration and semipreparative HPLC.²⁰

TABLE 1.3
Separation of Flavonoids by Centrifugal Partition Chromatography

Sample	Solvent System	Reference
Flavonoids from <i>Hippophae rhamnoides</i>	CHCl ₃ -MeOH-H ₂ O, 4:3:2	46
Flavonol glycosides from <i>Vernonia galamensis</i>	CHCl ₃ -MeOH- <i>n</i> -BuOH-H ₂ O, 7:6:3:4	47
Flavonol glycosides from <i>Picea abies</i>	CHCl ₃ -MeOH- <i>i</i> -PrOH-H ₂ O, 5:6:1:4	20
Flavonol glycosides from <i>Polypodium decumanum</i>	<i>n</i> -BuOH-EtOH-H ₂ O, 4:1.5:5	48
Flavone C-glycosides from <i>Cecropia lyratiloba</i>	CHCl ₃ -MeOH- <i>n</i> -BuOH-H ₂ O, 10:10:1:6	49
Biflavonoids from <i>Garcinia kola</i>	EtOAc- <i>n</i> -BuOH-MeOH-H ₂ O, 35:10:11:44	50
Isoflavones from <i>Astragalus membranaceus</i>	<i>n</i> -Hexane-EtOAc-MeOH-H ₂ O, 2:8:5:5	51
	EtOAc-EtOH- <i>n</i> -BuOH-H ₂ O, 15:5:3:25	
	EtOAc-EtOH-H ₂ O, 5:1:5	
Isoflavones from <i>Glycine max</i>	CHCl ₃ -MeOH-H ₂ O, 4:3:2	52
	CHCl ₃ -MeOH- <i>n</i> -BuOH-H ₂ O, 8:6:1:4	
Anthocyanidins from <i>Ricciocarpos natans</i>	<i>n</i> -Hexane-EtOAc- <i>n</i> -BuOH-HOAc-HCl 1%, 2:1:3:1:5	53
Proanthocyanidins from <i>Stryphnodendron adstringens</i>	EtOAc- <i>n</i> -PrOH-H ₂ O, 35:2:2	54
Proanthocyanidins from <i>Cassipourea gummiflua</i>	<i>n</i> -Hexane-EtOAc-MeOH-H ₂ O, 8:16:7:10	55
Anthocyanins from plants	<i>n</i> -BuOH-TBME-CH ₃ CN-H ₂ O, 2:2:1:5	56
Polyphenols from tea	<i>n</i> -Hexane-EtOAc-MeOH-H ₂ O, 3:10:3:10	57

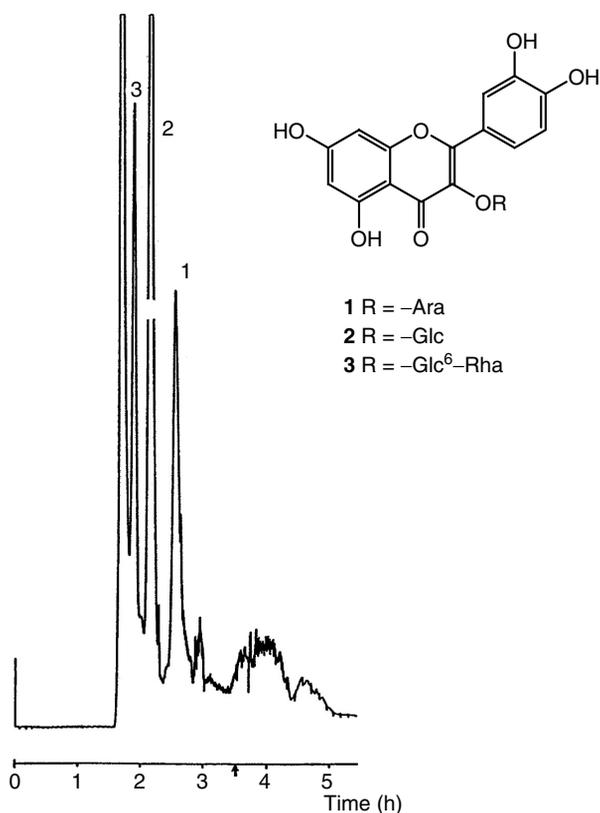
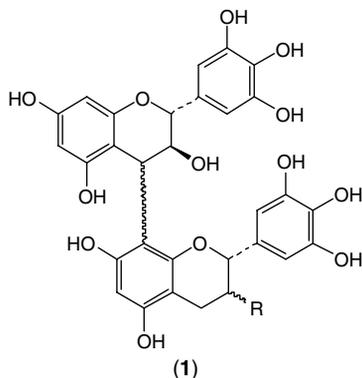


FIGURE 1.1 Separation of flavonol glycosides from *Tephrosia vogelii* (Leguminosae) with a Quattro CPC instrument. Solvent system, CHCl_3 -MeOH-EtOH- H_2O (5:3:3:4); mobile phase, upper phase; flow-rate, 3 ml/min; detection, 254 nm; sample, 500 mg MeOH extract. (From Sutherland, I.A., Brown, L., Forbes, S., Games, G., Hawes, D., Hostettmann, K., McKerrell, E.H., Marston, A., Wheatley, D., and Wood, P., *J. Liq. Chrom. Relat. Technol.*, 21, 279, 1998. With permission.)

Four pure isoflavones were obtained from a crude soybean extract after CPC with the solvent system CHCl_3 -MeOH- H_2O (4:3:2) (Figure 1.2). The isoflavones were isolated in amounts of 5 to 10 mg after the introduction of 150 mg of sample.⁵²

A combination of gel filtration, CPC, and semipreparative HPLC was reported for the isolation of eight dimeric proanthocyanidins of general structure **1** from the stem bark of *Stryphnodendron adstringens* (Leguminosae). The CPC step involved separation with the upper layer of EtOAc-*n*-PrOH- H_2O (35:2:2) as mobile phase.⁵⁴



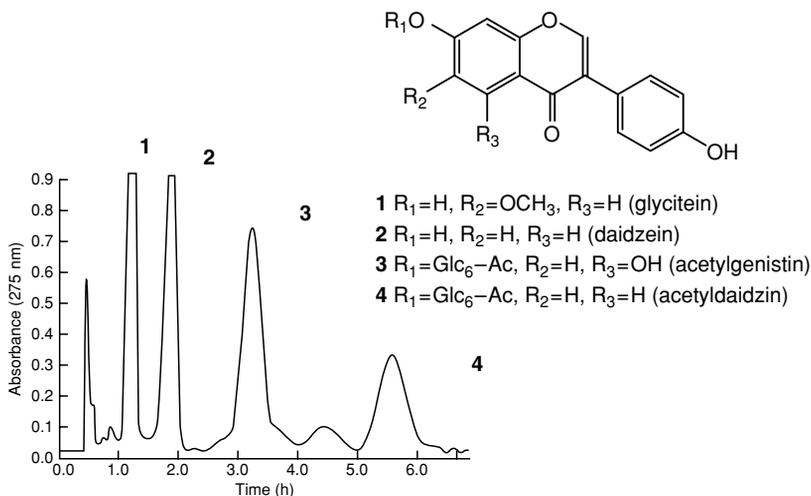


FIGURE 1.2 Separation of a crude soybean extract with a multilayer CPC instrument. Solvent system, $CHCl_3$ -MeOH- H_2O (4:3:2); mobile phase, lower phase; flow rate, 2 ml/min; detection, 275 nm; sample, 150 mg. (From Yang, F., Ma, Y., and Ito, Y., *J. Chromatogr.*, 928, 163, 2001. With permission.)

1.4 ANALYTICAL METHODS

A herbal product contains multiple constituents that might be responsible for its therapeutic effects. It is thus necessary to define as many of the constituents as possible in order to understand and explain the bioactivity. The concept of “phytoequivalence” has been introduced in Germany to ensure consistency of phytotherapeutics.⁵⁹ According to this concept, a chemical profile for a herbal product is constructed and compared with the profile of a clinically proven reference product. Since many of these preparations contain flavonoids, it is essential to have adequate analytical techniques at hand for this class of natural product.

Knowledge of the flavonoid content of plant-based foods is paramount to understanding their role in plant physiology and human health. Analytical methods are also important to identify adulteration of beverages, for example. And flavonoids are indispensable markers for chemotaxonomic purposes.

Various analytical methods exist for flavonoids. These range from TLC to CE. With the introduction of hyphenated HPLC techniques, the analytical potential has been dramatically extended. Gas chromatography (GC) is generally impractical, due to the low volatility of many flavonoid compounds and the necessity of preparing derivatives. However, Schmidt et al.⁶⁰ have reported the separation of flavones, flavonols, flavanones, and chalcones (with frequent substitution by methyl groups) by GC.

Quantification aspects are discussed under individual techniques.

1.4.1 SAMPLE PREPARATION

Sample preparation is included in sample handling⁶¹ and is rapidly becoming a science in itself. The initial treatment of the sample is a critical step in chemical and biochemical analyses; it is usually the slowest step in the analysis. In the case of food and plant samples, the number and diversity of analytes is very high and efficient pretreatment is required to obtain enriched phenolic fractions.

Sample preparation methods should:⁶²

- Remove possible interferents (for either the separation or detection stages) from the sample, thereby increasing the selectivity of the analytical method.
- Increase the concentration of the analyte and hence the sensitivity of the assay.
- Convert the analyte into a more suitable form for detection or separation (if needed).
- Provide robust and reproducible methods that are independent of variations in the sample matrix.

The aim of sample preparation is that the components of interest should be extracted from complex matrices with the least time and energy consumption but with highest efficiency and reproducibility. Conditions should be mild enough to avoid oxidation, thermal degradation, and other chemical and biochemical changes. Some procedures — CE, for example — necessitate more rigorous sample pretreatment than others. On the other hand, TLC requires an absolute minimum of sample preparation.

As well as typical sample preparation methods such as filtration and liquid–liquid extraction,⁶¹ newer developments are now extensively used. The first of these is solid-phase extraction (SPE). This is a rapid, economical, and sensitive technique that uses several different types of cartridges and disks, with a variety of sorbents. Sample preparation and concentration can be achieved in a single step. Interfering sugars can be eluted with aqueous methanol on reversed-phase columns prior to elution of flavonoids with methanol.

Among the numerous applications of SPE are separations of phenolic acids and flavonoids from wines and fruit juices. Sep-Pak C₁₈ cartridges have been used for the fractionation of flavonol glycosides and phenolic compounds from cranberry juice into neutral and acidic parts before HPLC analysis.⁶³ Antimutagenic flavonoids were identified in aqueous extracts of dry spinach after removal of lipophilic compounds by SPE.⁶⁴

Anthocyanins were recovered from wine (after removal of ethanol) by elution from a C₁₈ cartridge with an aqueous eluent of low pH.⁶⁵

Different extracts of *Citrus* were subjected to SPE on C₁₈ cartridges to remove polar components. The retained flavonoids (mainly flavanones) were eluted with methanol–dimethyl sulfoxide, which enhanced solubility of hesperidin, diosmin, and diosmetin. Recoveries of eriocitrin, naringin, hesperidin, and tangeretin from spiked samples of mesocarp tissue exceeded 96%. Flavones were relatively abundant in the leaves.⁶⁶

SFE has long been of industrial importance but has only recently been introduced on a laboratory scale. Few applications have been reported for polyphenols but simpler phenolics have been extracted by this method, albeit with addition of methanol to the supercritical fluid.¹³ Some potential may be found for online SFE, since very clean extracts (but at low extraction efficiency for phenolic compounds) can be obtained.⁶⁷

Other innovations include PLE, MAE⁶⁸ (see Section 1.3.1), and solid-phase microextraction (SPME). SPME is a sampling method applied to GC, HPLC, and CE. It is based on adsorbent- or adsorbent-type fibers and lends itself well to miniaturization.⁶¹

1.4.2 THIN-LAYER CHROMATOGRAPHY

Paper chromatography and paper electrophoresis were once extensively used for the analysis of flavonoids,⁵ but now the method of choice for simple and inexpensive analytical runs is TLC. The advantages of this technique are well known: short separation times, amenability to detection reagents, and the possibility of running several samples at the same time. TLC is also ideally suited for the preliminary screening of plant extracts before HPLC analysis. An excellent general text on TLC methodology has been written by Jork et al.⁶⁹ A good

discussion presented by Markham in one of the earlier volumes on flavonoids describes TLC on silica gel and also two other supports, cellulose and polyamide (which now find less application).⁷⁰ In his chapter, solvent systems and spray reagents are described.

Many different solvent systems have been employed for the separation of flavonoids using TLC. Table 1.4 shows a selection for different classes of these polyphenols. Some solvent systems cited by Markham⁷⁰ are reproduced here because they still find application in the separation of flavonoids. Highly methylated or acetylated flavones and flavonols require nonpolar solvents such as chloroform–methanol (15:1). Widely distributed flavonoid aglycones, such as apigenin, luteolin, and quercetin, can be separated in chloroform–methanol (96:4) and similar polarity solvents. One system that is of widespread application for flavonoid glycosides is ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26). By the addition of ethyl methyl ketone (ethyl acetate–ethyl methyl ketone–formic acid–glacial acetic acid–water, 50:30:7:3:10), rutin and vitexin-2''-*O*-rhamnoside can be separated.⁷¹ Careful choice of solvent system also allows separation of flavonoid glycosides from their galactosidic analogs.⁷² This is especially important for the distinction of *C*-glucosides from *C*-galactosides. As an illustration, 8-*C*-glucosylapigenin (vitexin) can be separated from 8-*C*-galactosylapigenin with the solvent ethyl acetate–formic acid–water (50:4:10).⁷²

With regard to detection, brief exposure of the TLC plate to iodine vapor produces yellow-brown spots against a white background. And, as stated by Markham,⁷⁰ flavonoids appear as dark spots against a fluorescent green background when observed in UV light (254 nm) on plates containing a UV-fluorescent indicator (such as silica gel F₂₅₄). In 365 nm UV light, depending on the structural type, flavonoids show dark yellow, green, or blue fluorescence, which is intensified and changed by the use of spray reagents. One of the most important of these is the “natural products reagent,” which produces an intense fluorescence under 365 nm UV light after spraying with a 1% solution of diphenylboric acid-β-ethylamino ester (diphenylboryloxyethylamine) in methanol. Subsequent spraying with a 5% solution of polyethylene glycol-4000 (PEG) in ethanol lowers the detection limit from 10 μg (the average TLC detection limit for flavonoids) to about 2.5 μg, intensifying the fluorescence behavior. The colors observed in 365 nm UV light are as follows:

- Quercetin, myricetin, and their 3- and 7-*O*-glycosides: orange-yellow
- Kaempferol, isorhamnetin, and their 3- and 7-*O*-glycosides: yellow-green
- Luteolin and its 7-*O*-glycoside: orange
- Apigenin and its 7-*O*-glycoside: yellow-green

Further details about the use of the “natural products reagent” can be found in an article by Brasseur and Angenot.⁷³

Aqueous or methanolic ferric chloride is a general spray reagent for phenolic compounds and gives a blue-black coloration with flavonoids. Similarly, Fast Blue Salt B forms blue or blue-violet azo dyes.

For quantitative analyses, scanning of the TLC plate with a densitometer provides good results. The flavonoids, both aglycones and glycosides, in *Vaccinium myrtillus* and *V. vitis-idaea* (Ericaceae) were determined after TLC and densitometry at 254 nm.⁷⁴ With suitable spray reagents, detection limits of 20 ng can be achieved by densitometry.⁷⁵

Better resolution is obtained by chromatographing flavonoids on high-performance TLC (HPTLC) plates. Silica gel 60F₂₅₄, RP-18, or, less frequently, Diol HPTLC plates are used for separation purposes. Methanol–water eluents are indicated for HPTLC on RP-18 chemically bonded silica gel but some acid is generally added to avoid tailing. Polar glycosides require eluents containing a high percentage of water. Special HPTLC plates have been designed for

TABLE 1.4
Solvent Systems for Thin-Layer Chromatography of Flavonoids on Silica Gel

Sample	Eluent
Flavonoid aglycones	EtOAc- <i>i</i> -PrOH-H ₂ O, 100:17:13
	EtOAc-CHCl ₃ , 60:40
	CHCl ₃ -MeOH, 96:4
	Toluene-CHCl ₃ -MeCOMe, 8:5:7
	Toluene-HCOOEt-HCOOH, 5:4:1
	Toluene-EtOAc-HCOOH, 10:4:1
	Toluene-EtOAc-HCOOH, 58:33:9
	Toluene-EtCOMe-HCOOH, 18:5:1
	Toluene-dioxane-HOAc, 90:25:4
	Flavonoid glycosides
<i>n</i> -BuOH-HOAc-H ₂ O, 3:1:1	
EtOAc-MeOH-H ₂ O, 50:3:10	
EtOAc-MeOH-HCOOH-H ₂ O, 50:2:3:6	
EtOAc-EtOH-HCOOH-H ₂ O, 100:11:11:26	
EtOAc-HCOOH-H ₂ O, 9:1:1	
EtOAc-HCOOH-H ₂ O, 6:1:1	
EtOAc-HCOOH-H ₂ O, 50:4:10	
EtOAc-HCOOH-HOAc-H ₂ O, 100:11:11:26	
EtOAc-HCOOH-HOAc-H ₂ O, 25:2:2:4	
THF-toluene-HCOOH-H ₂ O, 16:8:2:1	
CHCl ₃ -MeCOMe-HCOOH, 50:33:17	
CHCl ₃ -EtOAc-MeCOMe, 5:1:4	
CHCl ₃ -MeOH-H ₂ O, 65:45:12	
CHCl ₃ -MeOH-H ₂ O, 40:10:1	
MeCOMe-butanone-HCOOH, 10:7:1	
MeOH-butanone-H ₂ O, 8:1:1	
Flavonoid glucuronides	EtOAc-Et ₂ O-dioxane-HCOOH-H ₂ O, 30:50:15:3:2
	EtOAc-EtCOMe-HCOOH-H ₂ O, 60:35:3:2
Flavanone aglycones	CH ₂ Cl ₂ -HOAc-H ₂ O, 2:1:1
Flavanone glycosides	CHCl ₃ -HOAc, 100:4
	CHCl ₃ -MeOH-HOAc, 90:5:5
Chalcones	<i>n</i> -BuOH-HOAc-H ₂ O, 4:1:5 (upper layer)
	EtOAc-hexane, 1:1
Isoflavones	CHCl ₃ -MeOH, 92:8
	CHCl ₃ -MeOH, 3:1
Isoflavone glycosides	<i>n</i> -BuOH-HOAc-H ₂ O, 4:1:5 (upper layer)
Dihydroflavonols	CHCl ₃ -MeOH-HOAc, 7:1:1
Biflavonoids	CHCl ₃ -MeCOMe-HCOOH, 75:16.5:8.5
	Toluene-HCOOEt-HCOOH, 5:4:1
Anthocyanidins and anthocyanins	EtOAc-HCOOH-2 <i>M</i> HCl, 85:6:9
	<i>n</i> -BuOH-HOAc-H ₂ O, 4:1:2
	EtCOMe-HCOOEt-HCOOH-H ₂ O, 4:3:1:2
	EtOAc-butanone-HCOOH-H ₂ O, 6:3:1:1
Proanthocyanidins	EtOAc-MeOH-H ₂ O, 79:11:10
	EtOAc-HCOOH-HOAc-H ₂ O, 30:1.2:0.8:8

this purpose, since normal plates can only accommodate aqueous methanol mixtures with up to about 40% water.

The European Pharmacopoeia stipulates TLC fingerprint analysis for the identification of plant drugs. This can be used, for example, in the case of hawthorn extracts (*Crataegus monogyna* and *C. laevigata*, Rosaceae), which contain flavone *O*-glycosides and flavone *C*-glycosides or passion flower extracts (*Passiflora incarnata*, Passifloraceae), which contain only flavone *C*-glycosides.

1.4.3 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

The method of choice for the qualitative and quantitative analysis of flavonoids is HPLC. Since its introduction in the 1970s, HPLC has been used for all classes of flavonoids and hundreds of applications have been published. Numerous reviews have also appeared, such as those by Hostettmann and Hostettmann,³ Merken and Beecher,⁷⁶ He,⁷⁷ and Cimpan and Gocan.⁷⁸

It is not the purpose of this chapter to go into the theory of HPLC, which is adequately covered in other texts, but to describe the applications of the method. This section will concentrate on analytical applications because semipreparative HPLC has been described in Section 1.3.2. Analytical HPLC finds use in the quantitative determination of plant constituents, in the purity control of natural products, and in chemotaxonomic investigations.

For the analytical HPLC of a given subclass of flavonoids (flavones, flavonols, isoflavones, anthocyanins, etc.), the stationary phase, solvent, and gradient have to be optimized.

A very high proportion of separations are run on octadecylsilyl bonded (ODS, RP-18, or C₁₈) phases. Some reported analyses use octasilyl bonded (RP-8 or C₈) phases but these are becoming increasingly rare. Flavonoid glycosides are eluted before aglycones with these phases, and flavonoids possessing more hydroxyl groups are eluted before the less substituted analogs. As solvents for application, acetonitrile–water or methanol–water mixtures, with or without small amounts of acid, are very common. These are compatible with gradients and UV detection. Occasionally, other solvents such as tetrahydrofuran, isopropanol, or *n*-propanol are used. Acid modifiers are necessary to suppress the ionization of phenolic hydroxyl groups, giving sharper peaks with less tailing. A study has shown that there are large differences in the effectiveness of C₁₈ columns for the separation of flavonoid aglycones and glycosides. While some columns give good results, others produce substantial band broadening and peak asymmetry.⁷⁹

Octadecylsilyl stationary phases with hydrophilic endcapping have been developed for the separation of very polar analytes, which are not sufficiently retained on conventional reversed-phase columns. Among numerous other applications, they have been demonstrated to be suitable for the separation of flavonol and xanthone glycosides from mango (*Mangifera indica*, Anacardiaceae) peels.⁸⁰

Normal phases (unmodified silica gel) are rarely employed, except for the occasional separation of weakly polar flavonoid aglycones, polymethoxylated flavones, flavanones, or isoflavones. The polymethoxylated flavones present in citrus fruits can, for example, be separated on silica gel columns. The big drawback is that solvent gradients cannot normally be run with normal phases.

Flavone *C*-glycosides generally elute with shorter retention times than the corresponding *O*-glycosides. Thus, vitexin (8-*C*-glucosylapigenin) elutes with a shorter retention time than apigenin 7-*O*-glucoside. Furthermore, 8-*C*-glycosylflavones elute with shorter retention times than the corresponding 6-*C*-glycosylflavones. Thus, apigenin 8-*C*-glucoside elutes earlier than apigenin 6-*C*-glucoside.

Flavanones elute before their corresponding flavones due to the effect of unsaturation between positions 2 and 3.

Isoflavones, chiefly found in the Leguminosae (such as soy, *Medicago sativa*, and red clover, *Trifolium pratense*) in the plant kingdom, are also successfully analyzed by HPLC on C₁₈ columns.⁷⁶

The anthocyanins exist in solution as various structural forms in equilibrium, depending on the pH and temperature. In order to obtain reproducible results in HPLC, it is essential to control the pH of the mobile phase and to work with thermostatically controlled columns. For the best resolution, anthocyanin equilibria have to be displaced toward their flavylium forms — peak tailing is thus minimized and peak sharpness improved. Flavylium cations are colored and can be selectively detected in the visible region at about 520 nm, avoiding the interference of other phenolics and flavonoids that may be present in the same extracts. Typically, the pH of elution should be lower than 2. A comparison of reversed-phase columns (C₁₈, C₁₂, and phenyl-bonded) for the separation of 20 wine anthocyanins, including monoglucosides, diglucosides, and acylated derivatives was made by Berente et al.⁸¹ It was found that the best results were obtained with a C₁₂ 4 μm column, with acetonitrile–phosphate buffer as mobile phase, at pH 1.6 and 50°C.

Applications of HPLC to the analysis of flavonoids in medicinal and other plants are summarized by Cimpan and Gocan.⁷⁸ From the methods listed, it is noteworthy that 90% of the separations use C₁₈ columns. The importance of flavonoids in foods (fruits, vegetables, and grains) means that it is indispensable to have suitable means of determining their content. The review by Merken and Beecher⁷⁶ gives an excellent summary (including full details of separation conditions) of applications of HPLC to the determination of flavones, flavonols, flavanones, isoflavones, anthocyanidins, catechins, and their respective glycosides in foods. Here again, virtually all separations are performed on RP-18 columns, with column lengths between 100 and 300 mm and with internal diameters between 2 and 5 mm. Granulometries vary from 3 to 10 μm, with most being 5 μm. Separation runs are generally up to 1 h in duration. For aglycones and glycosides of isoflavones, certain reported separations of soybean products (e.g., the work of Barnes et al.⁸²) have employed C₈ packings, but these are rare. Some applications are given in which two or more subclasses are analyzed simultaneously, such as flavanones, flavones, and flavonols in honey, and anthocyanins, catechins, and flavonols in fruit and wines.

In general, though, there is not a single HPLC method that can solve all flavonoid separation problems. However, Sakakibara et al.⁸³ claim to have found a method capable of quantifying every polyphenol in vegetables, fruits, and teas. For this purpose, they used a Capcell pak C18 UG120 (250 × 4.6 mm, S-5, 5 μm) column at 35°C. Gradient elution at a flow rate of 1 ml/min was performed over 95 min with solution A (50 mM sodium phosphate [pH 3.3] and 10% methanol) and solution B (70% methanol) as follows: initially 100% of solution A; for the next 15 min, 70% A; for 30 min, 65% A; for 20 min, 60% A; for 5 min, 50% A; and finally 100% B for 25 min. Vegetable material was extracted with 90% methanol containing 0.5% acetic acid. A typical HPLC profile for 28 reference polyphenols is shown in Figure 1.3. The method allowed the determination of aglycones separately from glycosides. Information could also be obtained about simple polyphenols in the presence of more complex polycyclic polyphenols. Quantitative determination was achieved for a total of 63 different food samples.

Within the domain of medicinal plants, preparations of *Ginkgo biloba* (Ginkgoaceae) are the most widely sold phytomedicines, with sales of over US\$ 1 billion in 1998.⁸⁴ These principally involve special extracts of the leaves. Flavonoids are, at least in part, responsible for the beneficial effects of Ginkgo extracts. Generally, enriched ginkgo extracts for the preparation of ginkgo products are standardized to contain 24% flavonoids and 6% terpene

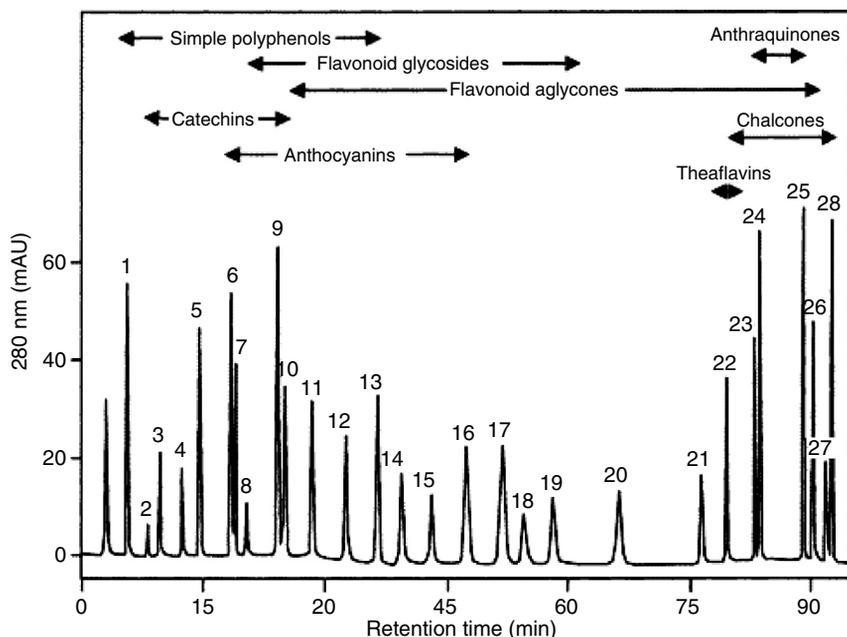


FIGURE 1.3 HPLC profile for 28 different polyphenols on a C_{18} column. Classes of compound are shown in the upper part of the chromatogram. (From Sakakibara, H., Honda, Y., Nakagawa, S., Ashida, H., and Kanazawa, K., *J. Agric. Food Chem.*, 51, 571, 2003. With permission.)

lactones and, therefore, the content of flavonoids and terpene lactones is one of the important parameters to assess the quality of ginkgo products. Fingerprint analysis for the quality control of Ginkgo preparations has shown that it is possible to separate six flavonoid aglycones, 22 flavonoid glycosides, and five biflavonoids in one run (Figure 1.4). This was achieved on a 100×4 mm Nucleosil 100- C_{18} 3 μ m column. In order to complete the run in 30 min, a ternary mobile phase was used, consisting of isopropanol-THF (25:65) (solvent A), acetonitrile (B), and 0.5% orthophosphoric acid (solvent C). A three-pump system was required to produce a complex elution gradient (1 ml/min, detection at 350 nm) starting with 15.0% A, 1.5% B, 83.5% C and ending with 0% A, 78.0% B, 22% C.⁸⁵

As few flavonoid glycosides are commercially available for reference purposes, their direct quantitative analysis is often impractical. It is thus common practice when investigating plant extracts to hydrolyze the glycosides and identify and quantify the released aglycones. This was the procedure adopted for the analysis of white onions (*Allium cepa*, Liliaceae) and white celery stalks (*Apium graveolens*, Apiaceae). Lyophilized plant material was extracted with 60% aqueous methanol and hydrolyzed with 1.2 M HCl before HPLC analysis on a Waters C_{18} Symmetry 150 \times 3.9 mm (5 μ m) column. As the mobile phase, a gradient of 15 to 35% acetonitrile in water adjusted to pH 2.5 with TFA was used. In nonhydrolyzed white onion extract, two major, nonidentified, flavonoid glycoside peaks were present. When the extract was hydrolyzed, these two peaks were replaced by a major peak corresponding to quercetin. Kaempferol was used as internal standard. In the analysis of nonhydrolyzed celery extract (isorhamnetin as internal standard), several peaks were observed. After hydrolysis the major peaks were due to apigenin, luteolin, and an unknown component. Quantification of the aglycones in fresh plant material was achieved by extrapolation. The limit of detection for endogenous quercetin and other flavonoid aglycones was ca. 3 μ g/g fresh mass.⁷⁹

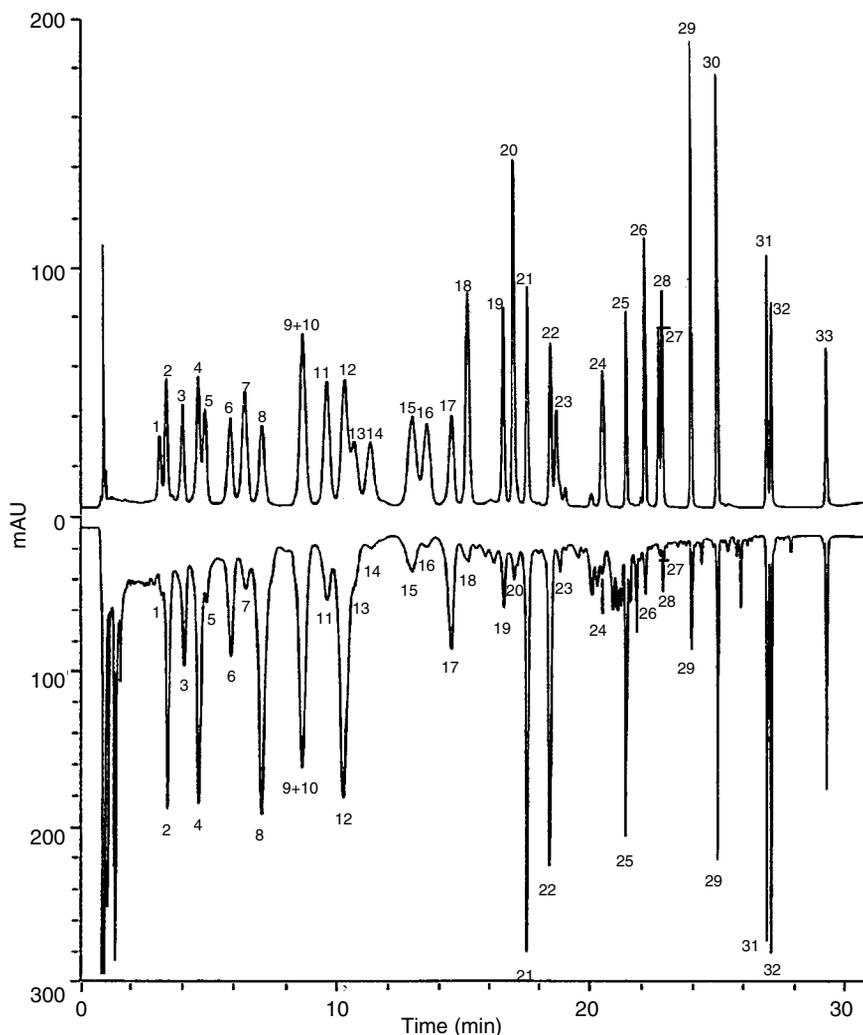


FIGURE 1.4 HPLC fingerprint of a therapeutically used dry extract of *Ginkgo biloba* leaves. Top chromatogram, pure reference flavonoids; bottom chromatogram, leaf extract. Identified flavonoids are numbered 1 to 33. (From Hasler, A., Sticher, O., and Meier, B., *J. Chromatogr.*, 605, 41, 1992. With permission.)

1.4.4 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–ULTRAVIOLET SPECTROPHOTOMETRY

The most frequently used detection method for HPLC is UV spectrophotometry. Routine detection in HPLC is typically based on measurement of UV absorption, or visible absorption in the case of anthocyanins. No single wavelength is ideal for all classes of flavonoids since they display absorbance maxima at distinctly different wavelengths. The most commonly used wavelength for routine detection has been 280 nm, which represents a suitable compromise.

With the introduction of diode-array technology in the 1980s, a further dimension is now possible because coupled LC–UV with diode array detection (DAD) allows the chromatographic eluent to be scanned for UV–visible spectral data, which are stored and can later be compared with a library for peak identification.⁸⁶ This increases the power of HPLC analysis

because with the information from the UV spectrum, it may be possible to identify the compound subclass or perhaps even the compound itself. UV spectral data of 175 flavonoids in several solvents can be found, for example, in a book by Mabry et al.¹ LC–UV with DAD enables simultaneous recording of chromatograms at different wavelengths. This improves the possibilities of quantification because detection can be performed at the wavelength maximum of the compound in question. These are typically to be found⁷⁶ at 270 and 330 to 365 nm for flavones and flavonols, at 290 nm for flavanones, at 236 or 260 nm for isoflavones, at 340 to 360 nm for chalcones, at 280 nm for dihydrochalcones, at 502 or 520 nm for anthocyanins, and at 210 or 280 nm for catechins.

Peak purity can also be determined. The spectra of eluting peaks obtained at the apex and both inflexion points of the peak can be compared in order to obtain a measure of the purity of the particular component of the sample.

LC–UV is valuable for the identification of isoflavones since their spectra differ in absorption properties from most of the other flavonoids. They have a C2–C3 double bond, with the B-ring at C3, which prevents conjugation of the phenyl group with the pyrone carbonyl group. This reduces the contribution of the B-ring to the UV spectrum and results in a peak of very low intensity in the 300 to 330 nm range (band I).

The analysis of catechins and proanthocyanidins by LC–UV presents certain problems. In general, only monomers and oligomers up to tetramers can be separated and detected as defined peaks. Polymeric forms, which may constitute the bulk of proanthocyanidins in many plant materials, are not well resolved. They give place to a drift in the baseline and the formation of characteristic humps in HPLC chromatograms. Furthermore, the spectral characteristics of these compounds do not allow easy detection and identification. Flavan-3-ols give absorption maxima at nonspecific wavelengths (270 to 290 nm) and they have lower extinction coefficients than other accompanying phenolics. Their quantification is thus not easy. The lack of reference proanthocyanidins implies that results have to be expressed with respect to other reference substances, normally catechin or epicatechin. This causes concomitant errors of quantification caused by the different extinctions shown by the individual flavan-3-ols. For reverse-phase HPLC of proanthocyanidin oligomers, the percentage of methanol or acetonitrile usually does not exceed 20%.

The coupling of HPLC with DAD allows online quantification of flavonoids in samples analyzed. Justesen et al.⁸⁷ have quantified flavonols, flavones, and flavanones in fruits, vegetables, and beverages in this fashion. The food material was extracted and then hydrolyzed to produce the corresponding flavonoid aglycones. These were analyzed on a Phenomenex C₁₈ column (250 × 4.6 mm, 5 μm) using a mobile phase of methanol–water (30:70) with 1% formic acid (solvent A) and 100% methanol (solvent B). The gradient was 25 to 86% B in 50 min at a flow rate of 1 ml/min. UV spectra were recorded from 220 to 450 nm. For each compound, peak areas were determined at the wavelength providing maximal UV absorbance. Quantification was performed based on external standards. A mixture of standards of known concentrations was analyzed in duplicate before and after the batch of samples. Peak areas were used to calculate the hydrolyzed food sample flavonoid aglycone content. Method validation indicated good day-to-day variability (reproducibility) and recoveries in the range of 68 to 103%. There was low recovery of myricetin standard, presumably because of degradation during hydrolysis. Detection by online mass spectrometry (MS) was also included to check possible interferences between flavonoids eluting at similar retention times.

While identification of the peaks in a LC–UV chromatogram is possible by comparing retention times and UV spectra with authentic samples or a databank, this might not be possible for compounds with closely related structures, and wrong conclusions might be drawn. It has been established that in order to complete the characterization of phenolic compounds, reagents inducing a shift of the UV absorption maxima can be used.¹

A postcolumn derivatization procedure, based on this technique, is possible by adding suitably modified shift reagents to the eluate leaving a HPLC column.⁸⁸ Direct information is provided about the flavonoid oxidation pattern and position of free phenolic hydroxyl groups. In the analysis of *Gentiana* (Gentianaceae) extracts, best results were obtained on a reversed-phase column with a methanol–water eluent at a pH of around 3.5, to avoid peak tailing. Classical shift reagents were adapted in order to be compatible with these conditions: sodium monohydrogenphosphate and potassium hydroxide were used as the weak and strong bases, respectively, instead of sodium acetate and sodium methanolate. In order to form a complex with the keto function, an aqueous solution of aluminum chloride was passed with the eluate through a reaction coil at 60°C. The presence of *ortho*-hydroxyl groups was shown with boric acid–sodium acetate. These shift reagents gave identical results to those obtained with classical shift reagents. The small amount of material required (50 to 100 µg of crude plant extract) in LC–UV postcolumn derivatization allows the analysis of very rare and small species, as well as single plant parts of herbarium samples.⁸⁸

To illustrate this approach, the online identification of flavonol glycosides in *Epilobium* (Onagraceae) species will be described. Certain of these willow-herbs have important implications in the treatment of benign prostatic hyperplasia and knowledge of their flavonoid content is an aid to their identification. The aerial parts of 13 different species were extracted first with dichloromethane and then with methanol. The flavonoid-containing methanol extracts were partitioned between *n*-butanol and water; the *n*-butanol fractions were then analyzed on a Novapak C₁₈ column (150 × 3.9 mm, 4 µm) with an acetonitrile–water gradient. TFA was added to give a pH of 3. Photodiode-array detection allowed the online recording of UV spectra (200 to 500 nm), all typical of flavonoids (Figure 1.5), except for ellagic acid (X in Figure 1.6). The HPLC chromatogram for *Epilobium angustifolium* is shown

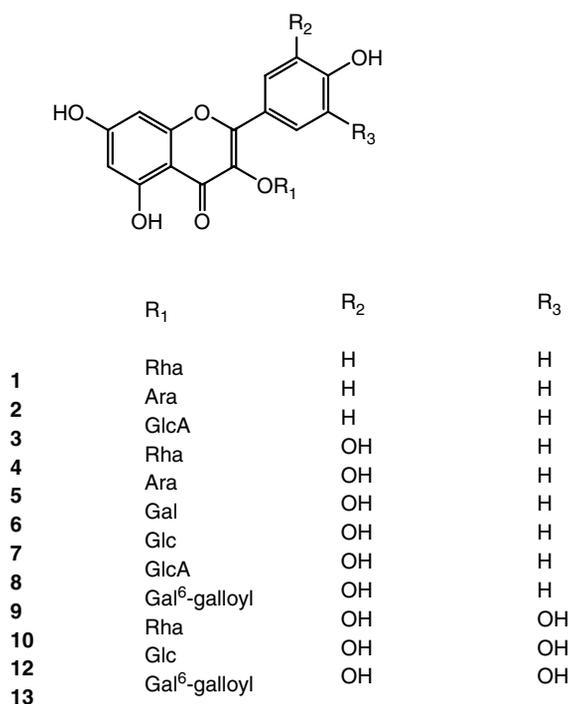


FIGURE 1.5 Flavonoid glycosides isolated from the aerial parts of *Epilobium angustifolium*.

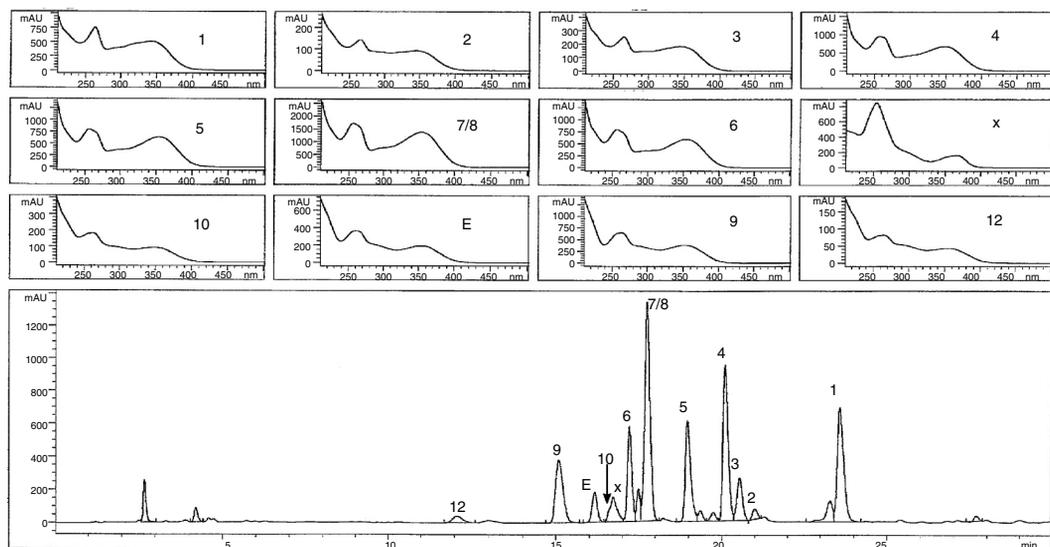


FIGURE 1.6 LC-UV chromatogram of a methanolic extract of the aerial parts of *Epilobium angustifolium* enriched by *n*-BuOH-H₂O partition. Column Novapak C₁₈ (150 × 3.9 mm, 4 μm); step gradient of CH₃CN-H₂O (containing 0.05% TFA): 0 min 10% CH₃CN, 4 min 12%, 12 min 12%, 16 min 18%, 30 min 25%; flow-rate 1 ml/min; chromatogram recorded at 350 nm. (From Ducrey, B., Wolfender, J.L., Marston, A., and Hostettmann, K., *Phytochemistry*, 38, 129, 1995. With permission.)

in Figure 1.6. Structure elucidation of flavonoid **E** was incomplete. In combination with shift reagents added postcolumn, LC-UV allowed determination of the hydroxylation pattern of flavonols and the position of the sugars on the aglycones. Figure 1.7 shows the UV spectra obtained online for **4** (quercitrin) after the addition of five different shift reagents. The shift of 11 nm of band II with weak base, 0.1 M Na₂HPO₄, was characteristic for a nonsubstituted 7-hydroxyl group. A 15 nm shift with boric acid reagent was typical for *ortho*-dihydroxyl groups on the B-ring. The shift of 42 nm of band I obtained for aluminum chloride without neutralization of the eluate was specific for a 5-hydroxyl substituent. Addition of aluminum chloride after neutralization gave a 56 nm shift of band I. This was due to a combination of an

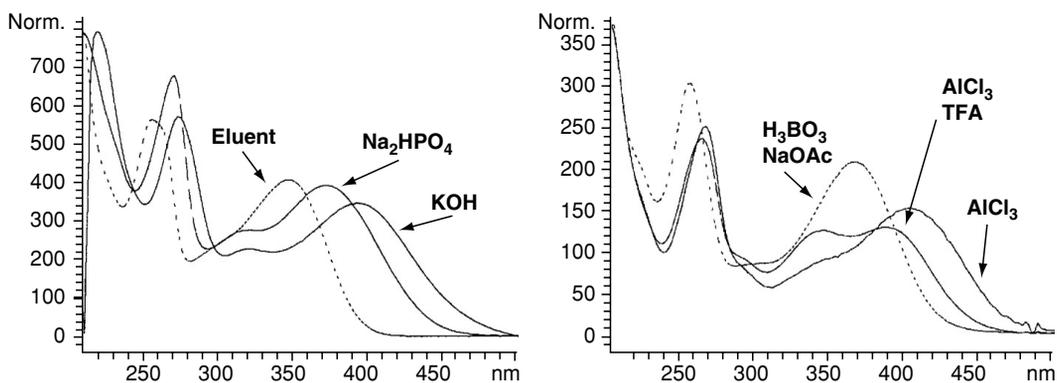


FIGURE 1.7. Online UV spectra of **4** obtained after postcolumn addition of different shift reagents. (From Ducrey, B., Wolfender, J.L., Marston, A., and Hostettmann, K., *Phytochemistry*, 38, 129, 1995. With permission.)

ortho-dihydroxyl group (C-3' and C-4') and to complex formation with the C-4 keto function and the 5-hydroxyl group. These data confirmed the aglycone to be quercetin. A similar procedure was adopted for the identification of the other flavonol glycosides — showing the presence of three different aglycones: kaempferol, quercetin, and myricetin. Thermospray LC–MS provided additional information on the molecular weight of the flavonol glycosides and their aglycones.³⁸

1.4.5 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY

Coupled HPLC–MS is one of the most important techniques of the last decade of the 20th century. The combination offers the possibility of taking advantage of chromatography as a separation method and MS as an identification tool. The amazing number of applications and the rapid drop in price (and size) of MS instruments has meant that the use of LC–MS is now extremely widespread. MS is one of the most sensitive methods of molecular analysis. Due to its high power of mass separation, very good selectivities can be obtained. However, the coupling between HPLC and MS has not been straightforward since the normal operating conditions of a mass spectrometer (high vacuum, high temperature, gas-phase operation, and low flow rates) are diametrically opposed to those used in HPLC, namely liquid-phase operation, high pressures, high flow rates, and relatively low temperatures.

In LC–MS, there are three general problems: the amount of column effluent that has to be introduced in the MS vacuum system, the composition of the eluent, and the type of compounds to be analyzed. Many interfaces have been developed in order to cope with these factors.⁸⁹ The interfaces must accomplish nebulization and vaporization of the liquid, ionization of the sample, removal of excess solvent vapor, and extraction of the ions into the mass analyzer. To date, no real universal interface has been constructed; each interface has characteristics that are strongly dependent on the nature of the compounds for which they are used. In LC–MS, the same rules that govern the ionization of pure compounds in the direct insertion mode are roughly preserved. Most interfaces work with reversed-phase HPLC systems, with a number of them suitable for the analysis of plant secondary metabolites. These include thermospray (TSP), continuous-flow fast-atom bombardment (CF-FAB), and electrospray (ES).⁹⁰ They cover the ionization of relatively small nonpolar products (aglycones, MW 200) to highly polar molecules (glycosides, MW 2000). Contrary to TSP or CF-FAB, where the source is in the vacuum region of the mass spectrometer, in ES the ion source is at atmospheric pressure. Atmospheric pressure ionization (API) has rendered LC–MS more sensitive and easy to handle. An API interface or source consists of five parts: (a) the liquid introduction device or spray probe; (b) the actual atmospheric pressure ion source region, where the ions are generated by means of electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), or by other means; (c) an ion sampling aperture; (d) an atmospheric pressure-to-vacuum interface; (e) an ion optical system, where the ions are subsequently transported into the mass analyser.⁸⁹ ESI and APCI are soft ionization techniques that generate mainly molecular ions for relatively small plant metabolites such as flavonoids.

Mass spectral data provide structural information on flavonoids and are used to determine molecular masses and to establish the distribution of substituents between the A- and B-rings. A careful study of fragmentation patterns can also be of particular value in the determination of the nature and site of attachment of the sugars in *O*- and *C*-glycosides. For flavonoid aglycones and glycosides with a limited number of sugar units (not more than three), TSP LC–MS analysis leads to a soft ionization, providing only intense $[M + H]^+$ ions for the aglycones and weak $[M + H]^+$ ions of glycosides (mono- or disaccharide), together with intense fragment ions due to the loss of the saccharide units, leading to the aglycone moiety $[A + H]^+$.

For example, the LC–UV–MS analysis of a methanol root extract of *Gentianella cabreræ* (Gentianaceae) gave a peak in the HPLC chromatogram (Figure 1.8), which had a UV spectrum characteristic of a xanthone (compound 2) and one which had a UV spectrum typical for a flavonoid (compound 1). In the TSP mass spectrum, the latter exhibited a protonated molecular ion $[M + H]^+$ at m/z 463 and fragments $[M + H - 90]^+$ and $[M + H - 120]^+$ at m/z 373 and 343, respectively, which were characteristic for the cleavage of flavone *C*-glycosides. According to these data, 1 was most probably a flavone *C*-glycoside substituted by three hydroxyl groups and one methoxyl group. Two isomeric flavones in the Gentianaceae, isoscaparin and swertiajaponin, fit with such data.⁹¹

In general, HPLC coupled with diode array and mass spectrometric detection provides an efficient method of rapid identification of flavonoids in a mixture. This technique now finds widespread application. By this means, 14 xanthone and flavonol glycosides were characterized online in a prepurified extract of mango (*Mangifera indica*, Anacardiaceae) peels, using ESI MS.⁸⁰ LC–UV–MS profiles of several medicinal plant extracts, including red clover (isoflavonoids), sour orange (flavanones), and astragalus (isoflavonoids and isoflavans), have been described by He.⁷⁷

Both ES and TSP are soft ionization methods and do not typically produce many fragments. This is useful in quantitative analysis or molecular mass determination but is of little use in structure elucidation. In this case, collision-induced dissociation (CID) or collision-activated dissociation methods can be employed.⁹² Fragmentation is induced in one of the high-pressure regions of the ion passageway from source to mass analyzer. Fragment ions produced by CID are very efficiently transported into the mass analyzer, providing a simple MS–MS method. With LC–MS, one analysis without CID and one with CID can be performed to obtain fragments of all components. CID is carried out to enhance fragmentation of the analytes either at the ES source (in-source CID) or in conjunction with tandem MS. In tandem MS, the first operation is to isolate a parent ion and the second is to determine the mass-to-charge ratio of the product ions formed after CID of the parent ion. The sequence of ion isolation and CID can be repeated many times in MSⁿ. Tandem MS and in-source CID give very similar product-ion mass spectra.

Since molecular weight information alone is insufficient for online structural determination of natural products, the fragmentation pathways of flavones and flavonols by fast-atom bombardment CID MS–MS have been documented.^{93–96}

The CID MS–MS and MSⁿ spectra of flavonoids have been systematically studied using hybrid quadrupole time of flight (Q-TOF) and ion trap (IT) mass analyzers, under various energy conditions, to generate fragment ions.⁹⁷ These two instruments were chosen because the CID process in beam and trap systems is not generated in the same way. The results demonstrated that, if for hydroxylated flavonoids the CID MS–MS spectra generated on both instruments were similar, for partially methoxylated derivatives, there were important differences. This is a hindrance to the creation of MS–MS databases exchangeable between instruments. Generally, fragments issued from C-ring cleavage were easier to observe on a Q-TOF instrument, while losses of small molecules were favored in IT-MS. MS–MS recorded in the positive ion mode were more informative than those obtained from negative ions. Online accurate mass measurements of all MS–MS fragments were obtained on the Q-TOF instrument, while the multiple-stage MSⁿ capability of the IT was used to prove fragmentation pathways. Molecular formulae with an accuracy of 1.8 ppm could be produced for isovitexin on the Q-TOF instrument.⁹⁷ It is worthy of note that high-resolution MS allows molecular formulae of compounds to be assessed directly; these can then be cross-checked with spectral libraries to provide identification of unknown components.

The application of tandem MS (LC–TSP MS–MS) can be illustrated for the online characterization of flavonoids from *Gentianella cabreræ*. The LC–UV–MS of the methanol

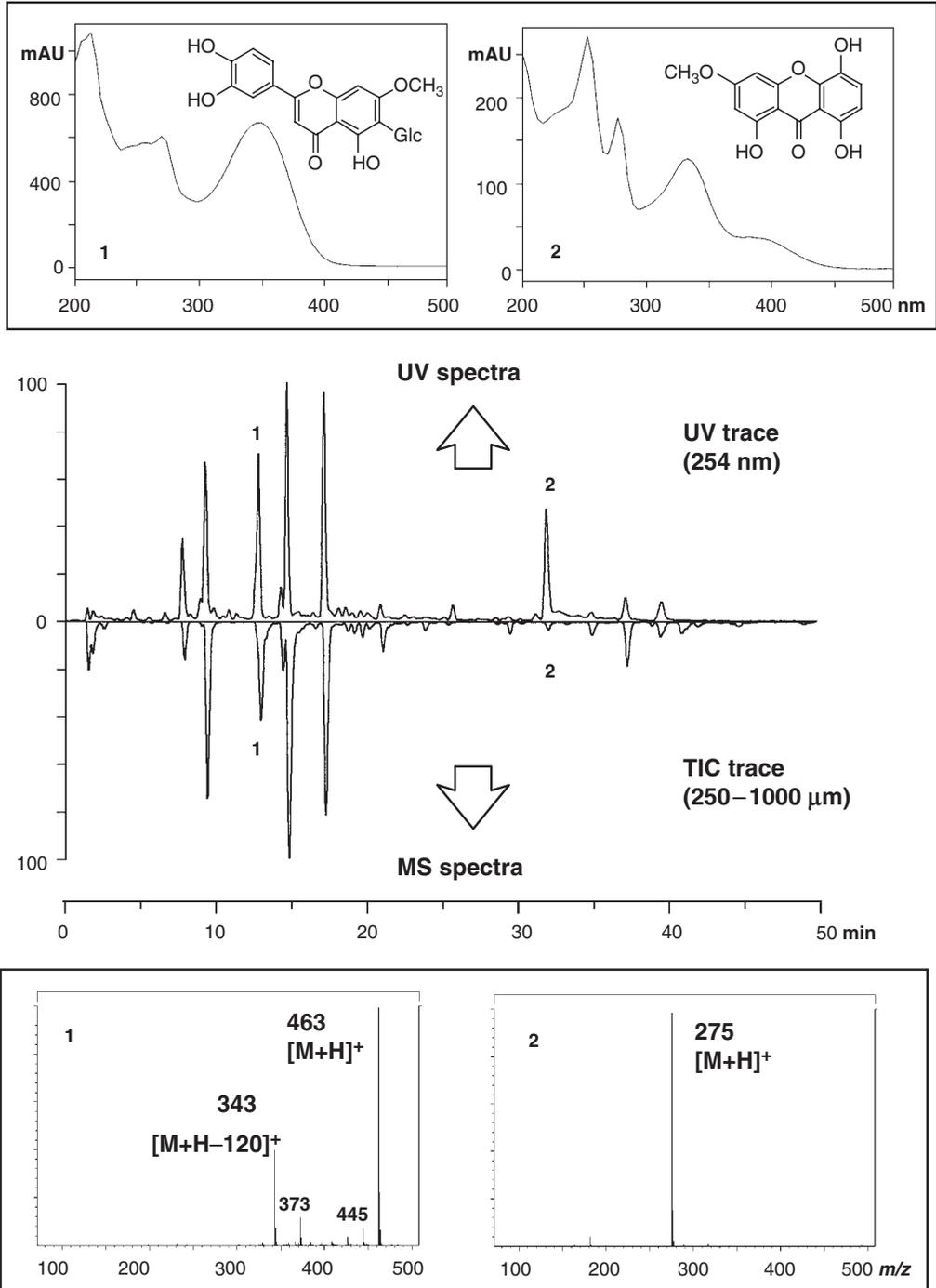


FIGURE 1.8 LC–UV–MS analysis of a methanolic extract of *Gentianella cabrerana*. The UV trace was recorded at 254 nm and the UV spectra from 200 to 500 nm. The LC–TSP–MS trace was recorded from 250 to 1000 μm. HPLC: Column Novapak C₁₈ (150 × 3.9 mm, 4 μm); gradient of CH₃CN–H₂O (containing 0.1% TFA) 5:95 → 65:35 in 50 min; flow-rate 1 ml/min; 0.5 M NH₄OAc (0.2 ml/min) postcolumn. TSP: positive ion mode; filament off; vaporiser 100°C; source 280°C. (From Hostettmann, K., Wolfender, J.L., and Rodriguez, S., *Planta Med.*, 63, 2, 1997. With permission.)

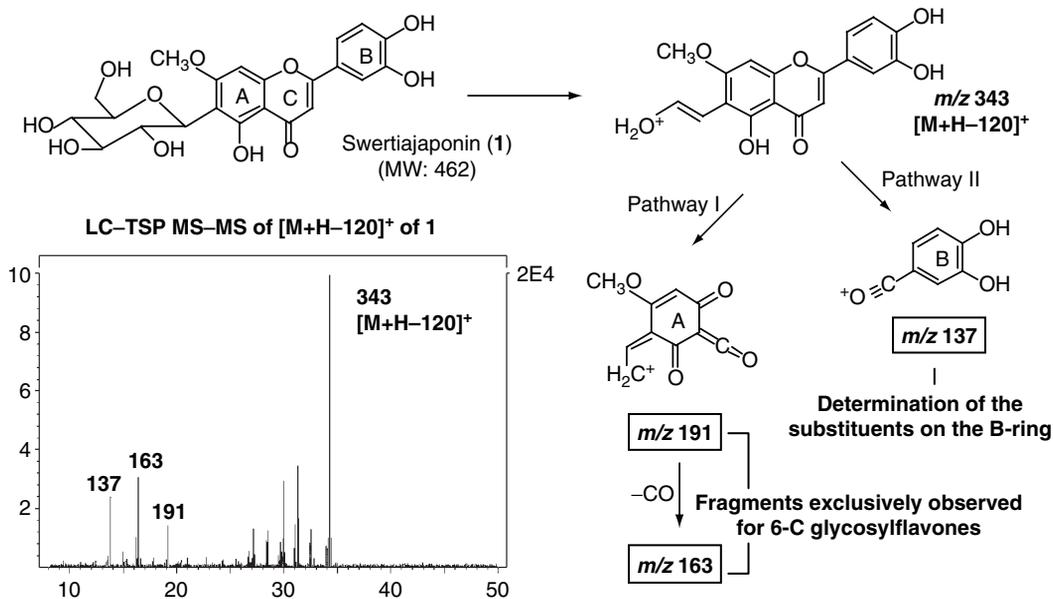


FIGURE 1.9 Online LC-TSP MS-MS of swertiajaponin (1) from the methanol extract of *Gentiana cabreræ*. The CID of the $[M + H - 120]^+$ ion at m/z 343 exhibited characteristic fragments for the substitution of the B-ring and for the glycosylation at C-6. (From Hostettmann, K., Wolfender, J.L., and Rodriguez, S., *Planta Med.*, 63, 2, 1997. With permission.)

extract indicated the presence of different flavonoid C-glycosides. LC-TSP MS data were insufficient to determine if compound **1** (Figure 1.9) was isoscoparin or swertiajaponin, two isomeric glycosides having a methoxyl group either at position C-3' or at C-7, respectively. In order to complete structure determination, LC-TSP MS-MS of **1** was performed on the intense $[M + H - 120]^+$ ion (Figure 1.9). The experiment was run on a triple quadrupole instrument, in which the first quadrupole was set in order to filter the ion $[M + H - 120]^+$. This ion was then selectively fragmented by CID with argon in the collision chamber (the second quadrupole). Finally, the spectrum was recorded by scanning the third quadrupole. A classic fragmentation of the aglycone moiety of the flavonoid gave an ion at m/z 137. This fragment was indicative of a B-ring substituted with two hydroxyl groups, confirming the localization of the methoxyl group of **1** on the A-ring. Ions at m/z 163 and 191 were specific for flavone C-glycosides having their saccharide moiety at C-6. Thus, the flavonoid could be identified as swertiajaponin.⁹¹

In a study of the Guinean medicinal plant *Dissotis rotundifolia* (Melastomataceae) by hyphenated HPLC techniques, online data showed the presence of isomeric pairs of C-glycosylflavones in the alcoholic or hydroalcoholic extracts but these could not be distinguished either by TSP LC-MS or their UV spectra. Figure 1.10 shows the TSP LC-MS analysis of a methanol extract of *D. rotundifolia*, with the corresponding UV spectra of the four separated glycosides (**1-4**). However, tandem MS (TSP LC-MS-MS) provided a means of differentiating the isomers. There were well-defined $[M + H]^+$ pseudomolecular ions and $[M + H - 120]^+$ ion fragments for all four C-glycosylflavones but only isorientin and isovitexin, the 6C-isomers, gave daughter ions at m/z 177 and m/z 149 from the $[M + H - 120]^+$ parent ion in the CID spectra (Figure 1.11) and hence could be distinguished from the 8C-isomers. The ions at m/z 177 and m/z 149 are produced after a retro-Diels-Alder fragmentation, which only occurs for isorientin and isovitexin (Figure 1.12). By performing two parent

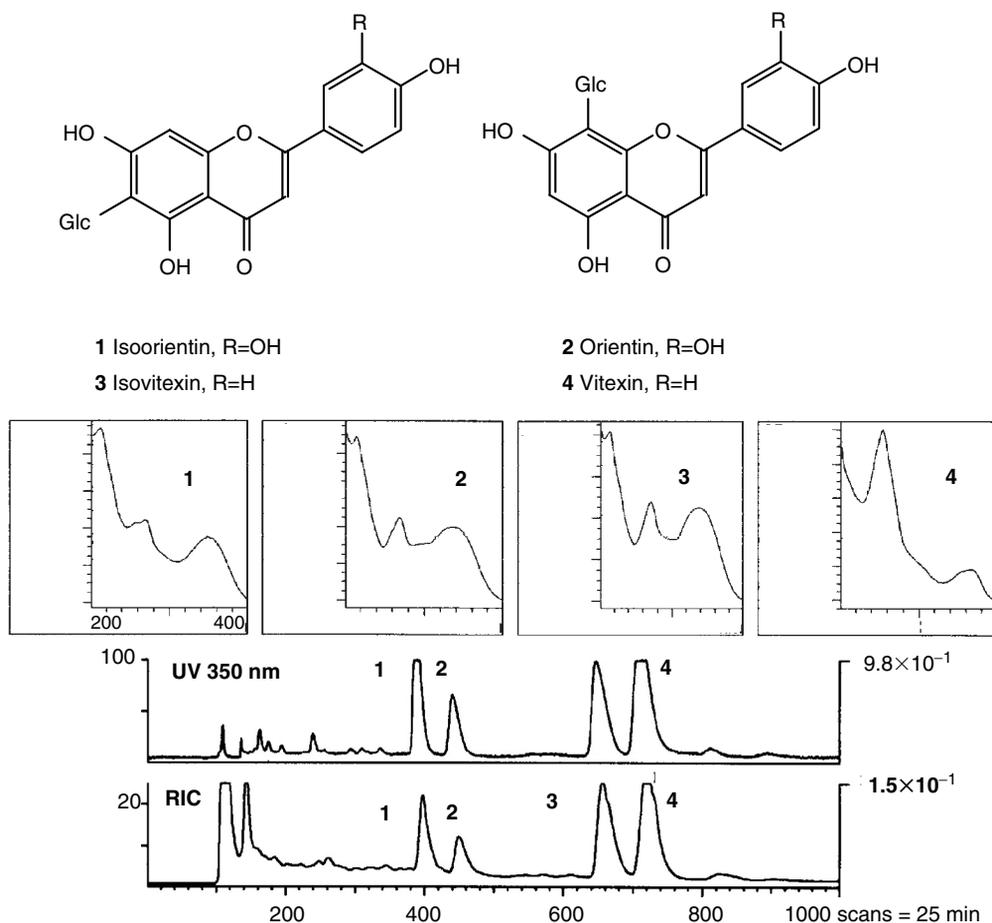


FIGURE 1.10 TSP LC–MS analysis of a methanol extract of *Dissotis rotundifolia*. Column Novapak C₁₈ (300 × 3.9 mm, 4 μm); CH₃CN–H₂O (14:86) (containing 0.05% TFA); flow rate 0.8 ml/min; detection 350 nm; RIC reconstructed ion current.

ion scan experiments with an isoorientin reference solution, it was possible to confirm that m/z 177 and m/z 149 were daughter ions of m/z 329. This is a rapid and simple means of distinguishing C-glycosylflavonoid isomers in plant extracts.⁹⁸

While typical flow rates for the HPLC analyses of flavonoids lie in the 1.0 to 1.5 ml/min range, the introduction of short columns containing stationary phases with smaller pore sizes (allowing narrower peaks to be obtained in shorter separation times) means that considerably lower flow rates are the trend. Not only is there a decrease in solvent consumption but coupling to mass spectrometers or NMR instruments is facilitated.

1.4.6 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–NUCLEAR MAGNETIC RESONANCE

Careful and critical use of the hyphenated techniques LC–UV–MS and LC–MS–MS can provide sufficient online information for the identification of small molecules such as flavonoids. However, in many cases, more data are required for an in-depth structural investigation and this can be supplied by the addition of an LC–NMR analytical capability (Figure 1.13). For practical purposes, LC–UV–MS and LC–UV–NMR are generally run as separate

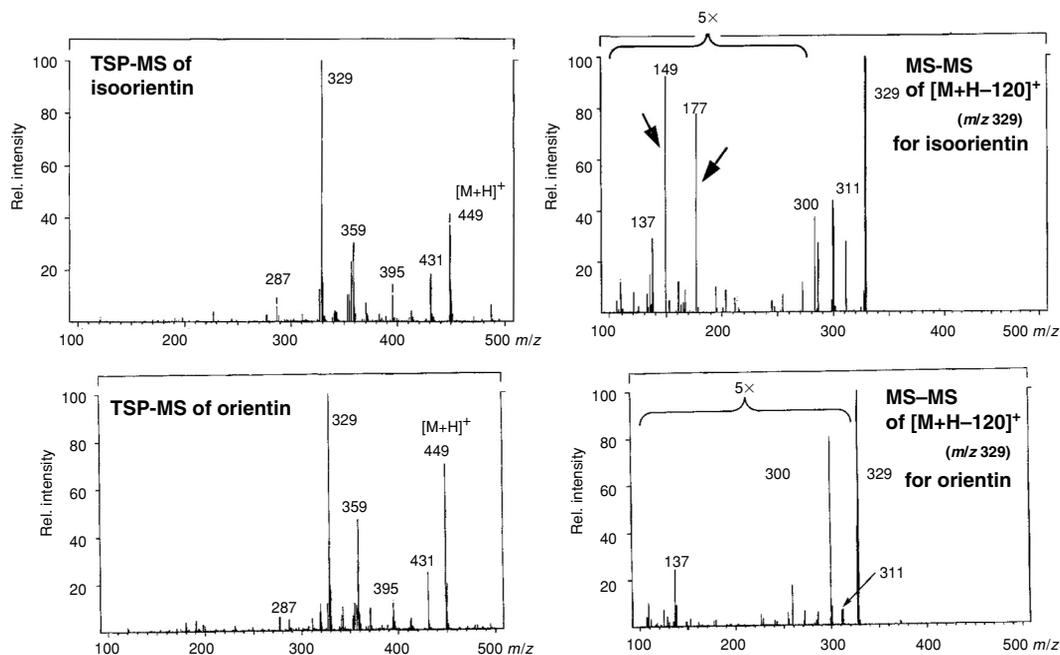


FIGURE 1.11 Online mass spectra of isoorientin (**1**) and orientin (**2**) in the TSP mode. The respective TSP MS–MS (CID) analyses of the parent $[M + H - 120]^+$ (m/z 329) ions are also shown.

operations. The coupling of HPLC with NMR spectroscopy, introduced around 1978, is one of the most powerful methods for the combined separation and structural elucidation of unknown compounds in mixtures.^{99,100}

At first, LC–NMR was little used because of its lack of sensitivity. However, recent progress in pulse field gradients and solvent suppression, improvement in probe technology, and the construction of high-field magnets have given a new impulse to the technique. While HPLC–NMR coupling is relatively straightforward (the samples flow in a nonrotating 60 to 180 μl glass tube connected at both ends with HPLC tubing) compared to LC–MS, the main problem of LC–NMR is the difficulty of observing analyte resonances in the presence of the much larger resonances of the mobile phase. This problem is magnified under typical reversed-phase HPLC operating conditions, where more than one protonated solvent is used and where the resonances change frequencies during analysis in the gradient mode. Furthermore, the continuous flow of sample in the detector coil complicates solvent suppression. These problems have now been overcome by the development of fast, reliable, and powerful solvent suppression techniques, such as WET (water suppression enhanced through T_1 effects),¹⁰¹ which produce high-quality spectra in both on-flow and stopped-flow modes. These techniques consist of a combination of pulsed-field gradients, shaped radiofrequency pulses, shifted laminar pulses, and selective ^{13}C decoupling, and are much faster than classical presaturation techniques previously used in the field. Thus, for typical reversed-phase HPLC analyses, nondeuterated solvents, such as methanol and acetonitrile, can be used, while water is replaced by D_2O .

The information provided by LC–NMR consists mainly of ^1H NMR spectra or ^1H – ^1H correlation experiments. Access to ^{13}C NMR is possible but is restricted only to a very limited number of cases where the concentration of the LC peak of interest is high and ^{13}C NMR data can be deduced indirectly from inverse detection experiments. Due to the low natural

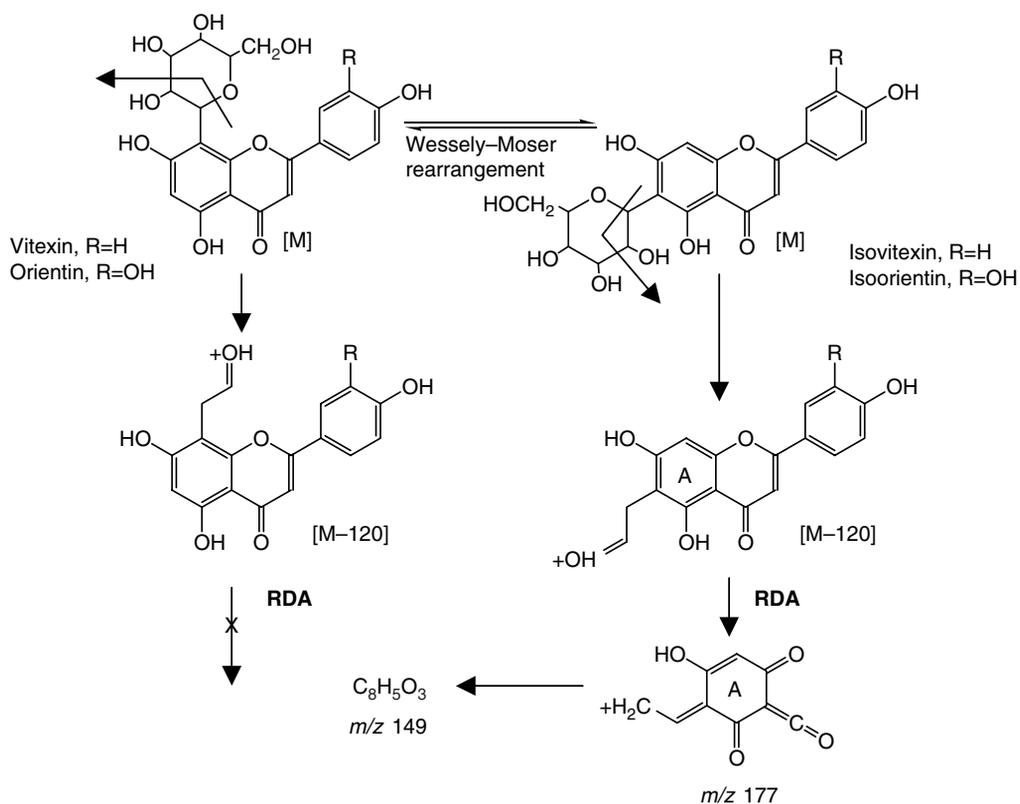


FIGURE 1.12 Specific fragmentations of the C-glycosylflavones isoorientin and isovitexin.

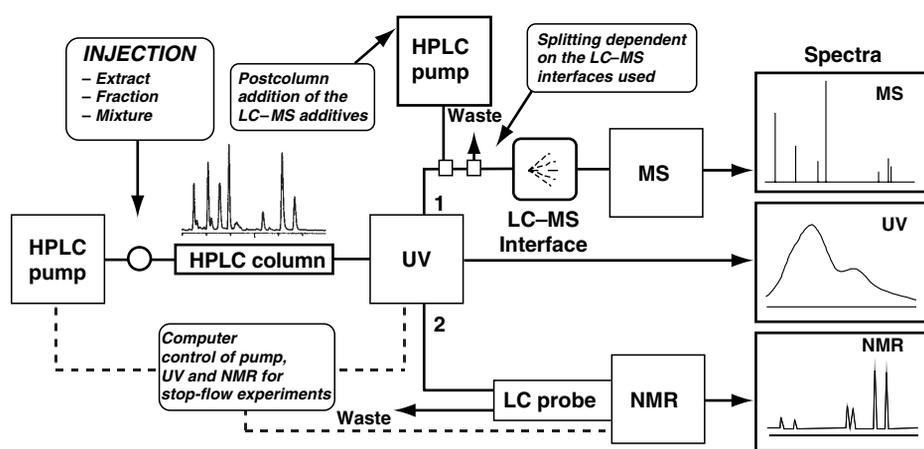


FIGURE 1.13 Schematic representation of the instrumentation used for LC-UV-MS (1) and LC-UV-NMR (2) analyses.

abundance of the ^{13}C isotope (1.1%), the sensitivity for direct measurement in the LC–NMR mode is insufficient.

LC–NMR can be operated in two different modes: on-flow and stopped-flow. In the on-flow mode, LC–NMR spectra are acquired continuously during the separation. The data are processed as a two-dimensional (2D) NMR experiment. The main drawback is the inherent low sensitivity. The detection limit with a 60 μl cell in a 500 MHz instrument for a compound with a molecular weight around 400 amu is 20 μg . Thus, on-flow LC–NMR runs are mainly restricted to the direct measurement of the main constituents of a crude extract and this is often under overloaded HPLC conditions. Typically, 1 to 5 mg of crude plant extract will have to be injected on-column.¹⁰² In the stopped-flow mode, the flow of solvent after HPLC separation is stopped for a certain length of time when the required peak reaches the NMR flow cell. This makes it possible to acquire a large number of transients for a given LC peak and improves the detection limit. In this mode, various 2D correlation experiments (COSY, NOESY, HSQC, HMBC) are possible.

The combination of HPLC with online UV, MS, and NMR detection has proved to be a very valuable tool for the analysis of natural products in extracts or mixtures.^{102,103} The field of flavonoids is no exception. The LC–NMR information obtained comes from the ^1H NMR spectra of selected peaks in the HPLC chromatogram. From LC–MS, A- or B-ring substitution can be deduced from the fragmentation pattern but the exact location of the substituent cannot be determined. However, for a flavonoid like apigenin, where only one hydroxyl group is located on the B-ring, ^1H NMR will give the substitution position because each of the three possibilities of localization of the hydroxyl group will give a unique splitting pattern. Much information can be derived about the nature and linkage positions of sugars. However, since D_2O is present in the eluent, exchangeable signals are not observed in the NMR spectrum.

An example of the LC–NMR stop-flow procedure is provided in the analysis of polyphenolics from the Chilean plant *Gentiana ottonis* (Gentianaceae).¹⁰⁴ Preliminary LC–UV analysis of a methanol extract of the roots showed the presence of several xanthenes (**2**, **4**, **6–8**; Figure 1.14), an iridoid (**1**), and two compounds (**3**, **5**) with UV spectra typical of flavonoids. TSP LC–MS provided the molecular weights of the latter two compounds and gave fragments characteristic for C-glycosides (losses of 90 and 120 amu). According to their UV spectra, **3** and **5** (MW 448 and 446) were, respectively, tri- and tetra-oxygenated flavone C-glycosides. In order to obtain further information for characterization of the polyphenols in the extract, LC–NMR was performed under the same conditions used for LC–UV–MS. However, water was replaced by D_2O and the amount injected was increased to 0.4 mg, which did not cause a noticeable loss in resolution. LC– ^1H NMR spectra were recorded for all the main peaks in the stop-flow mode and the number of transients for a good signal-to-noise ratio varied between 128 and 2048. For flavone C-glycoside **5** (MW 446), the LC– ^1H NMR spectrum (Figure 1.15) gave signals for six aromatic protons (δ 6.8 to 8.1), one methoxyl group (δ 4.0), and the C-glycoside moiety (δ 3.5 to 5.0). A pair of symmetric *ortho*-coupled protons (2H, δ 7.06, $J=8.3$, H-3',5' and 2H, δ 8.00, $J=8.3$, H-2',6') was characteristic for a B-ring substituted at C-4'. The singlet at δ 6.8 was attributed to H-3. A singlet at δ 6.9 was due to a proton either at position C-6 or C-8 on the A-ring. Thus, LC–UV–MS and stop-flow LC–NMR were not sufficient to fully ascertain the structure of **5**. In order to ascertain the position of C-glycosylation, an LC–MS–MS experiment was performed by choosing $[\text{M} + \text{H} - 120]^+$ as parent ion. This gave fragments at m/z 191 and 163, characteristic for 6-C-glycosylated flavones (described earlier). The fragment at m/z 121 indicated a monohydroxylated B-ring, confirming the methoxyl group to be on the A-ring. This combination of data allowed identification of **5** as 5,4'-dihydroxy-7-methoxy-6-C-glucosylflavone (swertisin).¹⁰⁴

If full metabolite profiling of a plant extract has to be performed, LC–NMR can be run in the on-flow mode. In order to obtain adequate NMR spectra of all constituents, the amount

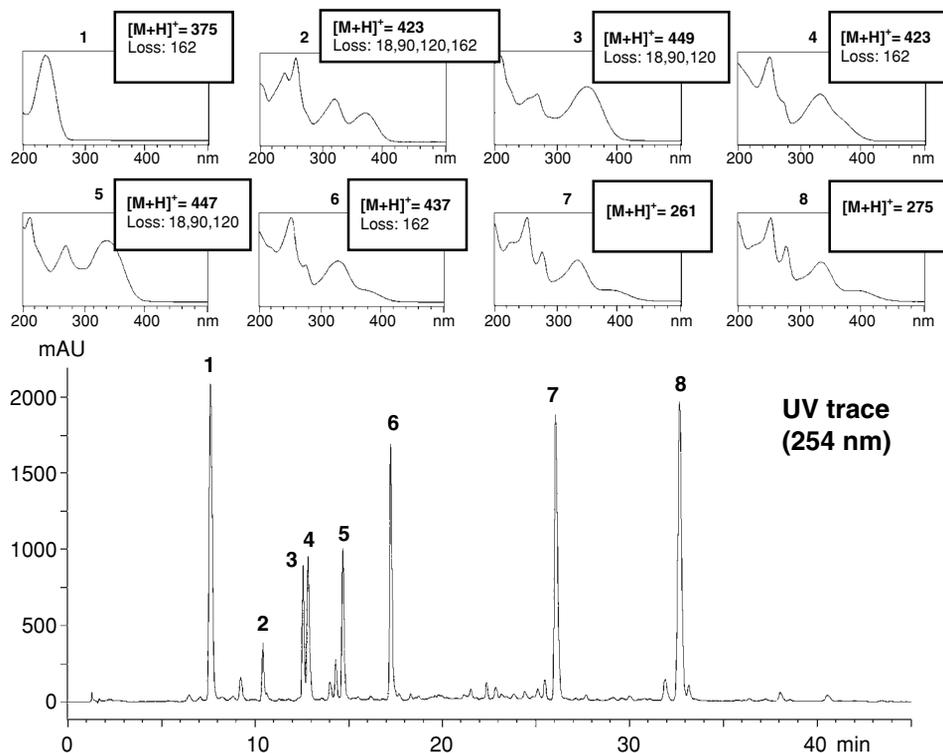


FIGURE 1.14 Online LC–UV of a methanol extract of *Gentiana ottonis*, with protonated molecular ions obtained for the main constituents by TSP LC–MS. Column Novapak C₁₈ (150 × 3.9 mm, 4 μm); gradient CH₃CN–H₂O (containing 0.05% TFA) 5:95 → 65:35 in 50 min; flow rate 0.8 ml/min. (From Wolfender, J.-L., Ndjoko, K., and Hostettmann, K., in *Methods in Polyphenol Analysis*, Santos-Buelga, C. and Williamson, G., Eds., Royal Society of Chemistry, Cambridge, 2003. With permission.)

of sample injected has to be increased — this produces overloading when compared with normal analytical HPLC conditions but gives the possibility of testing for biological activity (in conjunction with a microfractionation procedure). This was the approach adopted for the investigation of new antifungal constituents from *Erythrina vogelii* (Leguminosae), a medicinal plant of the Ivory Coast.¹⁰⁵ In order to rapidly identify the active principles from the antifungal dichloromethane extract of the roots, preliminary analysis by LC–UV and Q-TOF LC–MS was performed. Approximately 12 major peaks were observed in the HPLC chromatogram and from UV, MS, and MS–MS online data, these were shown to be prenylated isoflavones and isoflavanones. In order to obtain more information, on-flow LC–¹H NMR was performed by injecting 10 mg of crude extract onto an 8 mm C₁₈ radial compression column connected to the NMR instrument. At a low flow rate (0.1 ml/min), acquisition of ten LC–NMR spectra was possible. Of these ten peaks, five were found by simultaneous HPLC microfractionation to be associated with the antifungal activity of the extract. Interpretation of all online data, with emphasis on LC–NMR, allowed the identification of eight flavonoids, including a known isoflavone with antifungal activity and two putative new isoflavanones, also with antifungal activity. This dereplication procedure allowed the targeted isolation of the new antifungal compounds.¹⁰⁵

Applications of LC–NMR for the online identification of flavonoids are still few and far between, one reason probably being the high cost of the apparatus. However, several other

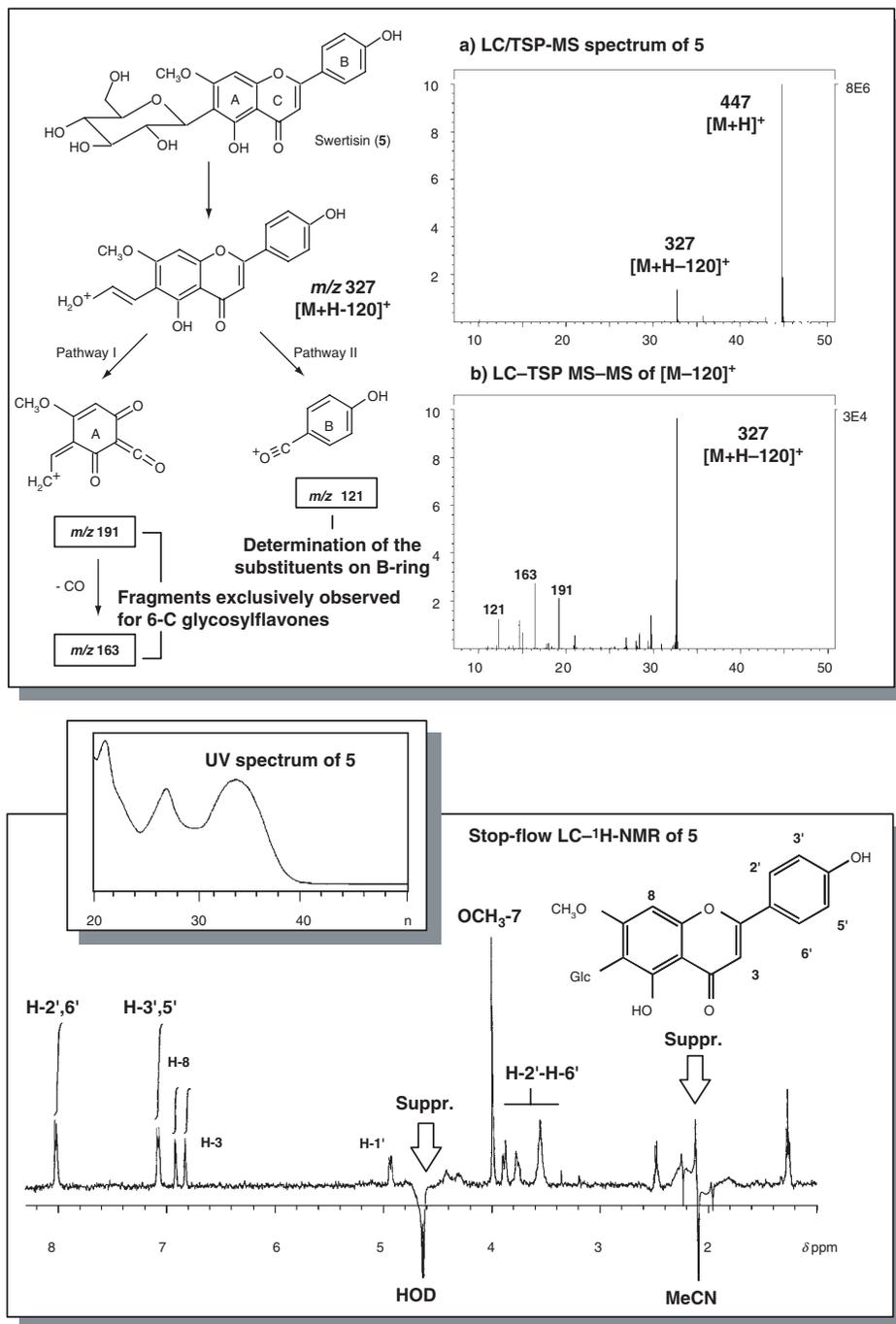


FIGURE 1.15 Stop-flow LC- 1H NMR spectrum of swertisin (5) from the methanol extract of the roots of *Gentiana ottonis*, together with the TSP LC-MS (a) and TSP LC-MS-MS (b) spectra. The LC-MS-MS analysis was performed using the fragment $[M + H - 120]^+$ (spectrum a) as parent ion. Characteristic daughter ions at m/z 121, 163, and 191 were observed, indicating the substitution on the A- and B-ring of the C-glycosylflavone. (From Wolfender, J.-L., Ndjoko, K., and Hostettmann, K., in *Methods in Polyphenol Analysis*, Santos-Buelga, C. and Williamson, G., Eds., Royal Society of Chemistry, Cambridge, 2003. With permission.)

examples do exist, in addition to those mentioned above. The technique has been successfully applied to the analysis of *Hypericum perforatum* (Guttiferae). Online identification of quercetin, several of its glycosides, and the biflavonoid I5,I18-biapigenin in an extract was possible.¹⁰⁶

1.4.7 CAPILLARY ELECTROPHORESIS

CE is an analytical technique that provides high separation efficiency and short run times. When compared to HPLC, however, CE generally exhibits much lower sensitivity, a tendency to overload with samples, and less reproducible quantitative data. In contrast to HPLC, method development is more time consuming in CE — involving investigation of types, pH and concentrations of electrolytes, types and concentrations of surfactants and organic modifiers, temperatures, and applied voltages. Several modes of CE are available: (a) capillary zone electrophoresis (CZE), (b) micellar electrokinetic chromatography (MEKC), (c) capillary gel electrophoresis (CGE), (d) capillary isoelectric focusing, (e) capillary isotachopheresis, (f) capillary electrochromatography (CEC), and (g) nonaqueous CE. The simplest and most versatile CE mode is CZE, in which the separation is based on differences in the charge-to-mass ratio and analytes migrate into discrete zones at different velocities.¹⁰⁷ Anions and cations are separated in CZE by electrophoretic migration and electro-osmotic flow (EOF), while neutral species coelute with the EOF. In MEKC, surfactants are added to the electrolyte to form micelles. During MEKC separations, nonpolar portions of neutral solutes are incorporated into the micelles and migrate at the same velocity as the micelles, while the polar portions are free and migrate at the EOF velocity.

Applications of CE for the analysis of phytochemicals have been well documented.^{108,109} CE is especially suitable for the separation of flavonoids as they are negatively charged at higher pH values.^{108,110} Suntornsuk¹¹⁰ has reviewed quantitative aspects and method validation of CE for flavonoids. Compared with HPLC, CE can provide an alternative analytical method when higher efficiency or higher resolution is required. For example, while TLC and HPLC analyses of passion flower do not provide adequate separation of all identified flavonoids, CE can fulfill the necessary requirements.¹¹¹ Separations of *Passiflora incarnata* (Passifloraceae) flavonoid glycosides were performed on a 50 μm internal diameter uncoated fused-silica capillary with 25 mM sodium borate buffer with 20% methanol (pH 9.5). The voltage was 30 kV and the temperature of the capillary maintained at 35°C. The CE instrument was equipped with a diode array detector. Twelve glycosides were satisfactorily separated within 13 min (Figure 1.16). For quantification, quercetin 3-*O*-arabinoside was used as internal standard. Calibration curves for internal standardization were established. The method was applied to the analysis of ten commercial samples of Passiflorae herba. They showed similar flavonoid patterns but differed quantitatively in individual flavonoid glycosides. Reproducibility was good, with a coefficient of variation (CV) of 2.83% for interday precision and a mean CV of 1.26% for migration time.¹¹¹

Other applications include the online coupling of capillary isotachopheresis and CZE for the quantitative determination of flavonoids in *Hypericum perforatum* (Guttiferae) leaves and flowers. This method involved the concentration and prepreparation of the flavonoid fraction before introduction into the CZE capillary. The limit of detection for quercetin 3-*O*-glycosides was 100 ng/ml.¹¹²

1.5 OUTLOOK

Preparative separations still present a challenge. There is no general, simple, straightforward strategy for the isolation of natural products, even if certain compounds are readily accessible by modern chromatographic techniques. Each particular separation problem has to be

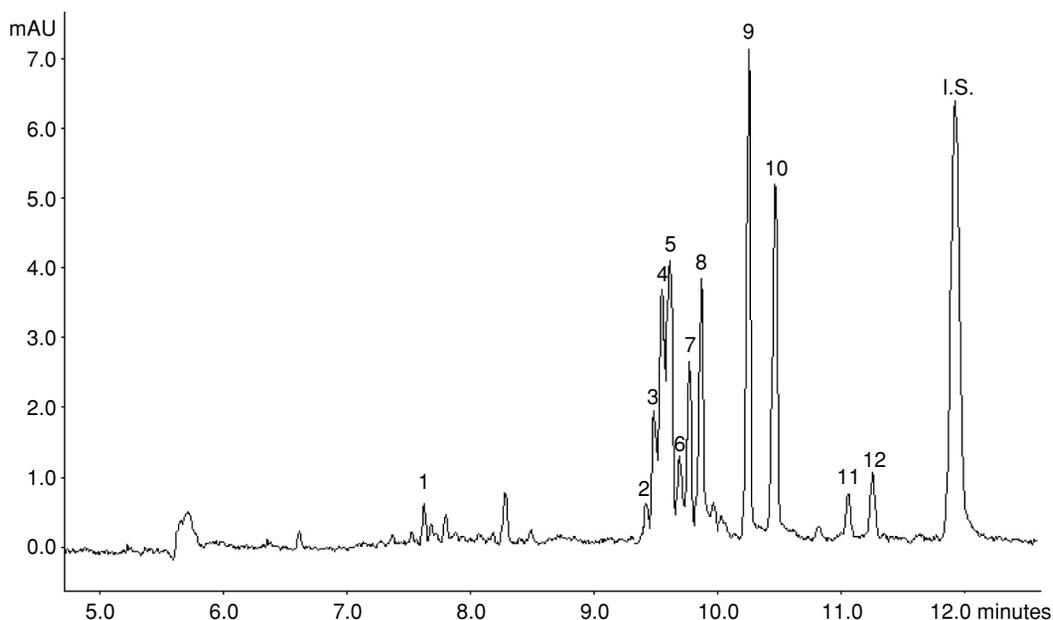


FIGURE 1.16 Electropherogram of a *Passiflorae herba* methanol extract. Capillary temperature 35°C; voltage 30 kV; electrolyte buffer 25 mM sodium tetraborate containing 20% MeOH (pH 9.5); UV detection at 275 nm; IS internal standard quercetin 3-*O*-arabinoside. (From Marchart, E., Krenn, L., and Kopp, B., *Planta Med.*, 69, 452, 2003. With permission.)

considered on its own and a suitable procedure has to be developed. In this respect, the flavonoids are no exception.

However, analytical separations of flavonoids are now routine. In quantitative measurements, the amounts of the individual components within a particular class of constituent need to be determined. Nowadays, this can easily be achieved through the use of GC, HPLC, and hyphenated techniques.

In HPLC, microbore operation is becoming popular, especially for LC–MS applications, because it allows smaller samples, faster separation times, and lower solvent consumption.

The trend is toward multiple hyphenation techniques like HPLC–UV–MS and HPLC–UV–NMR. These have an enormous potential for the rapid investigation of plant extracts.¹⁰⁰ Multiple hyphenation in a single system provides a better means of identification of compounds in a complex matrix.

Applications of LC–NMR are still scarce but the technique will become more widely used. The main effort for efficient exploitation of LC–NMR needs to be made on the chromatographic side, where strategies involving efficient preconcentration, high loading, stop-flow, time slicing, or low flow procedures have to be developed. Microbore columns or capillary separation methods, such as capillary LC–NMR, CE–NMR, and CEC–NMR, will find increased application, one reason being that the low solvent consumption will allow the use of fully deuterated solvents.

Other online HPLC techniques (such as LC–CD or LC–IR) are likely to be exploited. For example, a mixture of diastereoisomeric biflavonoids from the African plant *Gnidia involucreta* (Thymelaeaceae) could not be separated on a preparative scale by HPLC or crystallization. However, their analytical separation on a C₁₈ column was sufficient to run an online LC–CD investigation and provide stereochemical information about the individual isomers.¹¹³

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2 Spectroscopic Techniques Applied to Flavonoids

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2.1 INTRODUCTION

The purpose of this chapter is mainly to review the different spectroscopic techniques used for flavonoid analysis during the last decade. A typical analysis involving spectroscopic techniques embraces structural elucidation including determination of stereochemical attributes. However, it may also be aimed at tracing specific compounds and presenting quantitative aspects (see Chapter 1), or revealing color depiction. More than 7000 structures in various flavonoid classes have been reported in this book. Nearly half of them have been reported after 1993, which reflect that continual improvements in methods and instrumentation used for separation and structural elucidation have made it easier to use smaller flavonoid quantities to achieve results at increasing levels of precision. Recent attention regarding the variety of flavonoid structures (Chapters 10–17) and their potential properties (Chapters 4–9) has highlighted the need for understanding the physiological functions of individual flavonoids in plants and animals, and their importance to human health. Deciphering biological functions, including pharmaceutical functions, from structural flavonoid information is of increasing importance in our society.

From an analytical point of view, flavonoids may be grouped into various types of monomeric aglycones, bi-, tri-, and oligo-flavonoids including proanthocyanidins, *C*-alkylated flavonoids, flavonoids with different levels of *O*-methylation, and flavonoids with one or more saccharide units, which may include various types of acyl substituents (Chapters 10–17). The flavonoids under investigation may be part of complexes, may occur in complex matrices, and some flavonoids like the anthocyanins may exist on a variety of equilibrium forms. A successful characterization will thus follow a specific analytical route designed for the type of flavonoids under investigation, and the sort of information that is looked for. Without reference compounds the characterization of a novel compound will normally require more spectroscopic data than in the determination of a flavonoid that has been reported earlier. The amounts of flavonoids present in most plant tissues are relatively small even though the visual impression is quite striking. Methods for the characterization of individual flavonoids have traditionally reflected the lack of available material, and

sensitive chromatographic and spectroscopic techniques have achieved prominence in the characterization of flavonoids.^{1–3} Thus, the coupling of instruments performing chromatographic separations to those providing structural data (hyphenated methods), in particular high-performance liquid chromatography (HPLC) coupled to a diode-array detector, and a mass spectrometer or, more recently, a nuclear magnetic resonance (NMR) instrument, has had an enormous impact on structural studies involving flavonoids (see also Chapter 1).

Before a species is analyzed with respect to its flavonoid content, knowledge about earlier reports on the chemistry and flavonoid distribution within the genus and related species may be of value. The most exhaustive source for such information is *Chemical Abstracts*, and excellent reviews on structures and distribution of flavanoids have been compiled regularly.^{4–12} Several reviews have recently addressed the general field of flavonoid analysis.^{13–19} Among the earlier reviews in the field, we will particularly recommend consulting *Techniques of Flavonoid Identification* by Markham² and *Plant Phenolics* by Harborne.³ References to review articles on specific spectroscopic techniques applied on flavonoids will be cited under the various spectroscopic methods covered in this chapter. Spectroscopic information of importance is also presented in several other chapters in this book.

In this chapter, examples of the usefulness and recent applications of the different spectroscopic techniques applied on various flavonoids will be presented. Developments in NMR instrumentation including higher fields, high-temperature superconducting probes, low-temperature coils, better radiofrequency technology, as well as improvements of techniques and computing power have made NMR spectroscopy (Section 2.2) the most important tool for structural elucidation of flavonoids when these compounds are isolated in the milligram scale. Special effort has been made in this chapter to present assigned ¹H and ¹³C chemical shifts characteristic for the various flavonoid classes (Table 2.1–Table 2.6), and we present the first report of a 3D heteronuclear single-quantum coherence–total correlation spectroscopy (HSQC–TOCSY) spectrum applied to a flavonoid (Figure 2.6). Advances in mass spectrometry (MS) methodology have been shown to be extremely valuable for flavonoid analysis during the last two decades, especially the use of mild ionization techniques, which have improved the possibility of recording molecular ions and suppressed the detection limits by several orders of magnitude (Section 2.3). When flavonoid standards are not available, detailed structural information can be obtained by resorting to cone voltage fragmentation (by collision-induced dissociation (CID), tandem MS, etc.) and use of various types of mass analyzers.

Although vibrational spectroscopy (Section 2.4), infrared (IR) spectroscopy and Raman spectroscopy, is not routinely used in most flavonoids studies, the range of potential uses for these methods have been extended considerably by the development of microspectrometers with laser excitation, linked techniques, e.g., liquid chromatography (LC)–Fourier transform IR (FTIR), and two-dimensional (2D) correlation IR. Near-IR (NIR) spectroscopy has been shown to be an effective alternative method to conventional quantitative analysis of flavonoids in food, plant extracts, and pharmaceutical remedies. Absorption spectroscopy (ultraviolet, UV, or UV–Vis) (Section 2.5) will normally form part of any particular flavonoid analysis during the initial analytical stages; however, during the period of this review only minor advances in methodology were reported. In the flavonoid field, absorption spectroscopy provides most structural information about anthocyanins. Color measurements using CIE (Commission Internationale de l’Eclairage) specifications applied to pure anthocyanins and anthocyanins in plants and products derived thereof, determination of absolute configuration of flavonoid stereocenters by circular dichroism (CD) spectroscopy, and x-ray diffraction studies on solid flavonoid structures have been treated separately in Sections 2.6–2.8, respectively. Abbreviations are listed in Chapter 1.

2.2 NMR SPECTROSCOPY

2.2.1 INTRODUCTION

NMR spectroscopy is an extremely powerful analytical technique for the determination of flavonoid structures,^{20–23} but it is limited by poor sensitivity, slow throughput, and difficulties in analysis of mixtures. Recent developments have, however, made NMR arguably the most important tool for complete structure elucidation of flavonoids. Today, it is possible to make complete assignments of all proton and carbon signals in NMR spectra of most flavonoids isolated in the low milligram range. These assignments are based on chemical shifts (δ) and coupling constants (J) observed in 1D ^1H and ^{13}C NMR spectra combined with correlations observed as crosspeaks in homo- and heteronuclear 2D NMR experiments. Other nuclei like ^{17}O NMR spectroscopy has been used to study flavonoids only in a few cases. Natural abundance ^{17}O NMR spectra have been recorded for 11 methoxyflavones,²⁴ and ^{17}O NMR data for some 3-arylidenechromanones and flavanones have recently been discussed in terms of mesomeric and steric substituent interactions.²⁵ ^{17}O NMR spectroscopy has also been used to study the effect of sugar on anthocyanin degradation and water mobility in a roselle anthocyanin model system.²⁶

Advances in computing power have been an important factor for the success of more advanced NMR techniques. Running many scans and accumulating data may enhance weak signals since baseline noise, which is random, tends to cancel out. One of the main advantages to be gained from signal averaging combined with the use of Fourier transform methods and high-field superconducting magnets (up to 21 T) is the ability to routinely obtain ^{13}C NMR spectra. This carbon isotope exists in low abundance (1.108%) compared to the essentially 100% abundance of ^1H . NMR sensitivity also depends on magnetogyric constants, which for ^{13}C is only one-fourth of the value of ^1H . Thus, the sample amount required for ^{13}C NMR spectra is about ten times that for ^1H NMR spectra, and the number of scans is normally much higher. Progress in NMR instrumentation has been considerable during the last decade, and the recent development of cryogenic probe technology may further increase the signal-to-noise ratio by a factor of 3 to 4, as compared to conventional probes, leading to a possible reduction in experiment time of up to 16 or a reduction in required sample concentration by a factor of up to 4.²⁷ NMR instruments equipped with cryogenic probes have hitherto very rarely been used in structural elucidation of flavonoids.²⁸

Excellent compilation of NMR data on individual flavonoids has previously been presented,^{20–22} and some useful reviews in this field have also been published recently.^{23,29,30} Based on a database containing 700 ^{13}C spectra of flavonoids obtained from the literature, pattern recognition has been used to assemble compatible substructures according to related spectra.³¹ Some recent publications reporting flavonoid coupling constants include: NMR studies on flavones after the incorporation of ^{13}C at the carbonyl group, which allowed the measurement of two- and three-bond carbon–carbon coupling constants, ranging from 1.4 to 3.5 Hz, and the measurement of two-, three-, and four-bond carbon–hydrogen coupling constants, which ranged from 0.3 to 3.8 Hz;³² complete assignment of the ^1H and ^{13}C NMR spectra of several flavones and their proton–proton and carbon–proton coupling constants, including the extreme seven-bond long-range coupling between H-7 and H-3 in 6-hydroxyflavone (0.52 Hz) and flavone (0.27 Hz).³³ Typical one-bond ^1H – ^{13}C coupling constants of monosaccharides in anthocyanins have been observed within magnitudes of 125 and 175 Hz.³⁴

Mainly during the last decade structural information regarding flavonoids associated with other molecules has been reported. High-resolution ^1H magic angle spinning (MAS) NMR spectroscopy has been used to investigate the structural basis for the antioxidative effects of

five flavones and flavonols on the lipid molecules of cellular membranes.³⁵ A structural model of the solution complex between the flavonol kaempferol 7-neohesperidoside and a DNA dodecamer containing the *E. coli* wild-type lac promoter sequence (TATGTT) was obtained by simulated annealing for refinement based on distance constraints derived from nuclear Overhauser enhancement spectroscopy (NOESY) spectra.³⁶ The saturation transfer difference NMR technique has been used to investigate the binding of luteolin and its 7-*O*- β -D-glucopyranoside to a multi-drug-resistance transporter protein,³⁷ and NMR (TOCSY and NOESY spectra) has demonstrated molecular interaction of human salivary histatins with a flavan-3-ol ester, epigallocatechin gallate.³⁸ A review of NMR studies on the conformation of polyflavanoids and their association with proteins has been reported by Hemingway et al.³⁹

The use of NMR spectroscopy in the chemical analysis of food and pharmaceutical products is very advantageous because it is nondestructive, selective, and capable of simultaneous detection of a great number of low-molecular-mass components in complex mixtures. Conventional 1D and 2D NMR and high-resolution diffusion-ordered spectroscopy have recently been used for the characterization of selected Port wine samples of different ages.⁴⁰ NMR analysis of anthocyanins and amino acids has been used to differentiate wines according to the vine variety, geographic origin, and year of production.⁴¹ Multivariate statistical analysis of 2D NMR data of polyphenol extracts has been applied to differentiate grapevine cultivars and clones,⁴² and a ¹H NMR method has been utilized for quality control analyses of *Ginkgo* constituents, including flavonols.⁴³ A very interesting metabolomic approach based on chemometric analysis of ¹H NMR spectra of blood plasma has been used to investigate metabolic changes following dietary intervention with soy isoflavones in healthy premenopausal women under controlled environmental conditions.⁴⁴ With respect to future *in vivo* studies, the production of ¹³C-labeled anthocyanins in cell cultures is promising.⁴⁵

In this chapter, NMR solvents useful for flavonoid analysis are presented (Section 2.2.2), followed by an overview of various NMR techniques, including improved 2D and 3D NMR techniques with potential in structural elucidation of flavonoids (Section 2.2.3). The application of solid-state NMR and the coupled technique LC–NMR as tools for flavonoid analysis have been highlighted in separate sections (Sections 2.2.4 and 2.2.5). Tabulated compilations of recently published NMR data (both chemical shifts and coupling constants) on flavonoids belonging to various subclasses, including individually assigned data for rotameric flavone C-glycoside conformers (Table 2.1 and Table 2.2), complete assignments of ¹H and ¹³C chemical shifts of various flavonoid glycosyl (Table 2.3 and Table 2.4) and acyl (Table 2.5 and Table 2.6) moieties, reveal the importance of NMR for flavonoid structure elucidations. The names of the flavonoids listed in Table 2.1–Table 2.6 are collated in Table 2.7 and their structures are shown in Figure 2.8–Figure 2.16.

2.2.2 NMR SOLVENTS

The most frequently used NMR solvents for flavonoid analyses are hexadeuterodimethylsulfoxide (DMSO-*d*6) and tetradeuteromethanol (CD₃OD). Anthocyanins require the addition of an acid to ensure conversion to the flavylium form. For the analysis of relatively nonpolar flavonoids, solvents such as hexadeuteroacetone (acetone-*d*6), deuteriochloroform (CDCl₃), carbontetrachloride (CCl₄), and pentadeuteropyridine have found some application. The choice of NMR solvent may depend on the solubility of the analyte, the temperature of the NMR experiments, solvent viscosity, and how easily the flavonoid can be recovered from the solvent after analysis. In recent years, the problem of overlap of solvent signals with key portions of the NMR spectrum has been reduced by solvent suppression and the application of improved 2D and 3D NMR techniques.

Most flavonoids in the milligram scale are dissolved relatively easily in DMSO-*d*₆. The solvent peak (39.6 ppm) and the residual solvent signal (2.49 ppm) are used as secondary references for ¹³C and ¹H, respectively. DMSO is viscous at room temperature (m.p. 18°C), and may be the solvent of choice for recording of optimum nuclear Overhauser effects (NOEs) in 2D NOESY experiments at room temperatures.⁴⁶ However, the relatively high melting point (m.p. 18°C) makes low-temperature studies in this solvent impossible. Alternatively, the low freezing point of CD₃OD (m.p. -98°C) implies that low-temperature experiments can be performed with this solvent. It is also easy to recover the flavonoid after NMR analysis by evaporating the solvent (b.p. 65°C); however, in contrast to DMSO the relatively low boiling point of CD₃OD limits the possibilities of high-temperature experiments like those performed to study the equilibrium of rotameric conformers of flavone C-glycosides.⁴⁷ The solubility of several types of flavonoids is more restricted in CD₃OD than in DMSO; however, CD₃OD combined with various proportions (2 to 20%) of deuterio-trifluoroacetic acid, CF₃COOD, is at present the most common NMR solvent used for anthocyanins. However, the application of acidified NMR solvents may cause the exchange of exchangeable protons such as the methylene protons of malonyl residues and the anthocyanidin H-6 and H-8 with deuterium, thus also preventing the detection of ¹³C correlations of these signals in heteronuclear correlation experiments.²³ Other disadvantages with acidified solvents include (1) flavonoid glycoside hydrolysis and (2) anthocyanins acylated with dicarboxylic acids may be esterified by the NMR solvent during analysis.²³ Recent NMR investigations and theoretical calculations have compared the effect of acetone-*d*₆, DMSO-*d*₆, and CDCl₃ on the conformations of 4',7-di-hydroxy-8-prenylflavan.⁴⁸

2.2.3 NMR EXPERIMENTS

The purpose of a standard ¹H NMR experiment is to record chemical shifts, spin-spin couplings, and integration data, thus providing information about the relative number of hydrogen atoms. Applied to a flavonoid, this information may help in identifying the aglycone and acyl groups, the number of monosaccharides, and the anomeric configuration of the monosaccharides. However, for most flavonoids the information provided by a standard ¹H NMR experiment is insufficient for complete structural elucidation. Thus, ¹³C NMR experiments (spin-echo Fourier transform, SEFT, compensated attached proton test, CAPT, etc.) combined with various 2D NMR experiments, especially those using gradient techniques that imply increased sensitivity, have to be used for assignments of all ¹H and ¹³C NMR signals. For the NMR experiments described below, we recommend Braun et al.⁴⁹ for descriptions of the pulse programs and important acquisition and processing parameters. A protocol treating experimental details of modern NMR techniques for anthocyanin analysis has recently been published.²³

Two-dimensional NMR spectra are mainly produced as contour maps. These maps may be best imagined as looking down on a forest where all the trees (representing peaks in the spectrum) have been chopped off at the same fixed height. Two-dimensional NMR spectra are produced by homonuclear and heteronuclear experiments. Homonuclear ¹H-¹H correlated NMR experiments like the double-quantum filtered COSY (¹H-¹H DQF-COSY) and ¹H-¹H TOCSY experiments generate NMR spectra in which ¹H chemical shifts along two axes are correlated with each other. Values on the diagonal in these spectra correspond to chemical shifts that would have been shown in a 1D ¹H NMR experiment. It is the off-diagonal "spots," called crosspeaks, which present information that is new. These crosspeaks arise from coupling interactions between different ¹H nuclei. A crosspeak observed above the diagonal will normally also be found below the diagonal, thus producing a nearly symmetrical spectrum. The 1D ¹H NMR spectra may be placed as projections along the top and left parts

of the 2D NMR spectra. ^1H - ^1H NOESY (Figure 2.3) and rotating frame Overhauser effect spectroscopy (^1H - ^1H ROESY) (Figure 2.4) experiments are other examples of homonuclear NMR experiments. Heteronuclear NMR experiments are represented by ^1H - ^{13}C HSQC (Figure 2.1) and heteronuclear multiple bond correlation (^1H - ^{13}C HMBC) (Figure 2.2) experiments. The ^{13}C NMR spectrum (or ^{13}C projection) is displayed along one axis and the ^1H NMR spectrum (or ^1H projection) along the other. The ^1H - ^{13}C correlations are shown as crosspeaks in the spectrum. Contrary to homonuclear NMR experiments, there exists no diagonal and only one crosspeak is present for each correlation.

2.2.3.1 COSY and TOCSY

Two-dimensional ^1H - ^1H COSY (correlation spectroscopy) experiments allow determination of the protons that are spin-spin coupled, and the spectrum shows couplings between neighboring protons ($^2J_{\text{HH}}$, $^3J_{\text{HH}}$, and $^4J_{\text{HH}}$) revealed as crosspeaks in the spectrum. The ^1H - ^1H DQF-COSY experiment is a modification of the standard ^1H - ^1H COSY experiment. The main advantage of the DQF technique is that noncoupled proton signals are eliminated. The DQF technique eliminates the strong solvent signal and the often very strong H_2O signal associated therewith, which may overlap with flavonoid sugar signals. The DQF-COSY experiment is routinely used in flavonoid analysis to assign all the sugar protons. The use of a “sequential walk” approach may provide information on the relative positions of individual proton signals along a spin system.

The 2D homonuclear ^1H - ^1H TOCSY (Total Correlation Spectroscopy) experiment identifies protons belonging to the same spin system. As long as successive protons are coupled with coupling constants larger than 5 Hz, magnetization is transferred successively over up to five or six bonds. The presence of heteroatoms, such as oxygen, usually disrupts TOCSY transfer. Since each sugar ring contains a discrete spin system separated by oxygen, this experiment is especially useful for assignments of overlapped flavonoid sugar protons in the 1D ^1H NMR spectrum. It must be understood that the crosspeak intensity is not an indicator of the distance between the protons involved, and that all expected correlations may not appear in a TOCSY spectrum. To avoid this latter problem it may be helpful to record a second spectrum with another mixing time.

Most of the sugar proton signals found in flavonoids occurs in the narrow spectral region of 4.5 to 3.0 ppm. Thus, for complex flavonoids containing several sugar units, extensive overlap occurs in this part of the spectrum. ^1H - ^1H sugar coupling constants for such compounds can, however, be accessible by using the selective 1D TOCSY experiment, also known as the HOHAHA (homonuclear Hartman-Hahn) experiment. In the 1D TOCSY experiment, the resonances of one proton are selected and the signal formed is transferred in a stepwise process to all protons that are J -coupled to this proton. Instead of crosspeaks, magnetization transfer is seen as increased multiplet intensity. Thus, this 1D TOCSY spectrum looks like a normal ^1H NMR spectrum including only the protons that belong to the same spin system as the chosen proton.

TOCSY experiments have together with other NMR experiments been used for the structural elucidation of flavonols from, for instance, the Indian spice *Mammea longifolia*,⁵⁰ from *Erythrina abyssinica*,⁵¹ red onion,⁵² *Polygonum viscosum*,⁵³ *Morina nepalensis* var. *alba*,⁵⁴ *Centaurium spicatum*,⁵⁵ *Pisum sativum* (cv Solara) shoots,⁵⁶ leaves of *Canthium dicoccum*,⁵⁷ pericarps of *Sophora japonica*,⁵⁸ flavonol and chalcone glycosides from *Bidens andicola*,⁵⁹ a new flavonol glycoside and a new pterocarpan glucoside from *Ononis vaginalis*,⁶⁰ and a flavone and three iridoids from *Stachys spinosa*.⁶¹ TOCSY experiments have also been used for structural elucidation of anthocyanins produced in petals of genetically transformed lisianthus,⁶² from blue berries of *Vaccinium padifolium*,⁶³ and flowers of *Ipomoea asarifolia*,^{64,65} etc.

2.2.3.2 ^1H - ^{13}C Heteronuclear NMR Experiments

The HSQC experiment is a 2D one that correlates ^{13}C nuclei with ^1H nuclei within a molecule by means of the one-bond coupling between them (Figure 2.1). In HSQC, the ^1H magnetization is directly detected and the ^{13}C magnetization is indirectly detected, unlike the HETCOR experiment, in which ^1H magnetization is indirectly detected (F_1) and converted to ^{13}C magnetization, which is directly detected (F_2). There are several advantages in the manner in which magnetization is detected in the HSQC experiment, including increased sensitivity (<1.0 mg flavonoid sample is sufficient) and the ability to see long-range interactions between ^{13}C and ^1H nuclei using a variant called HMBC (heteronuclear multiple bond correlation). After the assignment of proton signals by a combination of ^1H - ^1H COSY and ^1H - ^1H TOCSY experiments, the one-bond ^1H - ^{13}C correlations observed in the HSQC spectrum allow the assignment of the corresponding ^{13}C signals. HSQC spectra have been recorded routinely for a variety of flavonoids during the last decade. A gradient-assisted heteronuclear multiple quantum correlation (GRASP-HMQC) optimized for the detection of $^1J_{\text{CH}}$ (147 Hz) and a long-range version thereof (GRASP-HMQC-LR) optimized for the detection of scalar coupling $^{2,3}J_{\text{CH}}$ (8, 5, or 3 Hz) used for the structural elucidation of an acylated flavonol glycoside⁶⁶ are alternatives to the HSQC and HMBC experiments.

The HMBC correlates proton nuclei with carbon nuclei that are separated by more than one bond (Figure 2.2). The experiment is normally optimized for $^3J_{\text{CH}}$ and $^2J_{\text{CH}}$ couplings; however, the intensity of the crosspeaks generated by this experiment

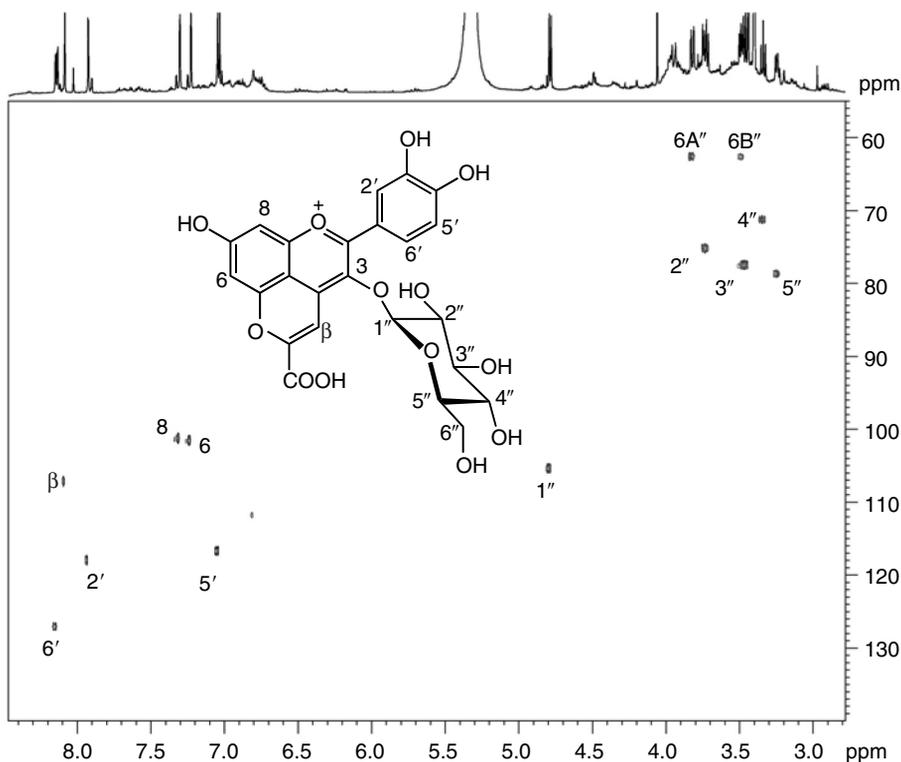


FIGURE 2.1 ^1H - ^{13}C heteronuclear single-quantum coherence spectrum of 5-carboxypyranocyanidin 3-glucoside, showing one-bond ^1H - ^{13}C correlation crosspeaks. The 1D ^1H NMR spectrum is included as a projection in the proton dimension.¹⁷⁶

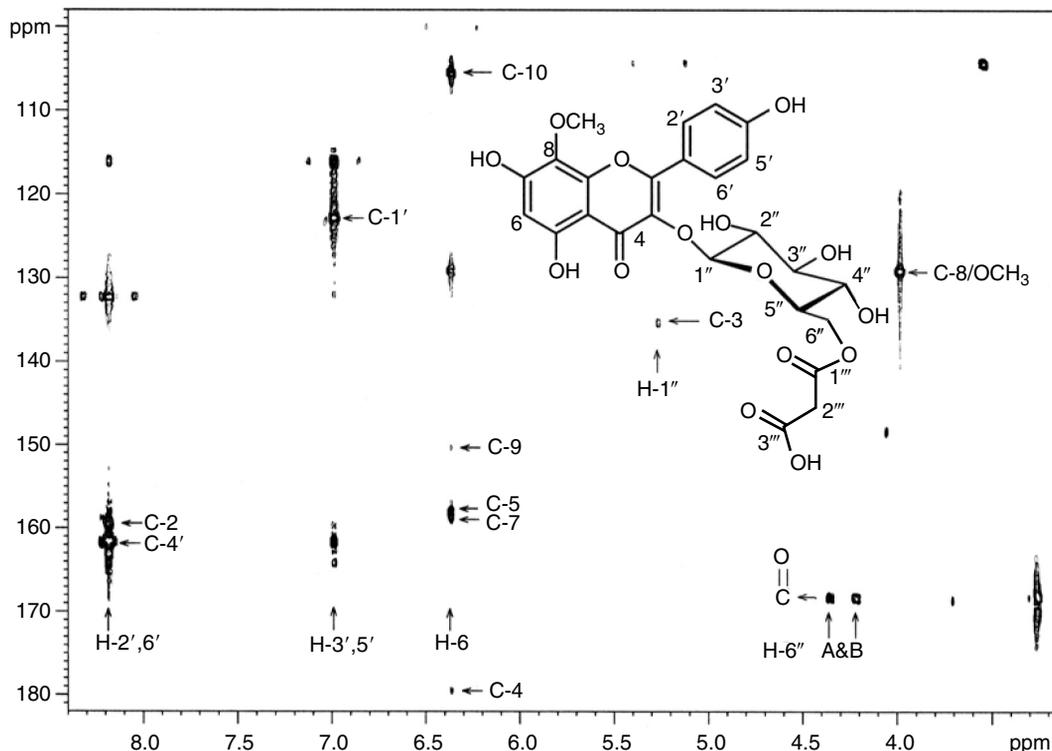


FIGURE 2.2 ^1H - ^{13}C heteronuclear multiple bond correlation spectrum of 8-methoxykaempferol 3-*O*-(6''-malonyl- β -glucopyranoside) (in CD_3OD) showing multiple bond correlations important for determination of linkages between subunits and assignments of aglycone ^{13}C resonances, respectively. Horizontal arrows indicate carbon assignments, while vertical arrows show proton assignments.¹⁷⁶

may sometimes be unexpected; some $^1J_{\text{CH}}$ couplings may occur as symmetrical doublets, while in rare cases crosspeaks caused by $^{n>3}J_{\text{CH}}$ couplings may be observed. In the aromatic region of the spectra some $^2J_{\text{CH}}$ couplings may be too small to be detected as crosspeaks.

Major applications of the HMBC experiment related to flavonoids include the assignment of resonances of nonprotonated carbon nuclei of the aglycone and potential acyl group(s) (Figure 2.2). Since long-range correlation of protonated carbon resonances also occurs with carbon nuclei that are separated by nonprotonated carbons or other heteronuclei like oxygen, the experiment is often used to determine the linkage points of the flavonoid building blocks (aglycone, sugar unit[s] and acyl moieties). The HMBC experiment has been successfully used for ^1H and ^{13}C NMR assignments and structural elucidations of a range of flavonoids including green tea flavonoids,⁶⁷ four new types of chalcone dimers isolated from *Myrcodruon urundeuva*,⁶⁸ diprenylated chalcones from the twigs of *Dorstenia barteri* var. *subtriangularis*,⁶⁹ flavonol and chalcone glycosides from *Bidens andicola*,⁵⁹ two unusual macrocyclic flavonols,⁷⁰ rare diastereoisomeric flavonolignans,⁷¹ three epicatechin glucuronides isolated from plasma and urine after oral ingestion of epicatechin,⁷² proanthocyanidin dimers,⁷³⁻⁷⁵ a symmetrical glycosylated methylene bisflavonoid *Blutaparon portulacoides*,⁷⁶ several peracetylated proanthocyanidin trimers,^{77,78} some flavones^{79,80} and isoflavones,⁸¹ 3-deoxyanthocyanins,⁸² many anthocyanins,^{65,83-87} some anthocyanin-flavonol complexes from flowers of chive,⁸⁸ etc.

The ^1H and ^{13}C NMR spectra of flavones and aurones are rather similar.^{20,21} However, these flavonoid subgroups may be distinguished by the long-range correlations found in their HMBC spectra.⁸⁹ The ^1H and ^{13}C NMR data of the (*E*) and (*Z*) isomeric pair of 4,6,3',4'-tetramethoxyaurone are included in Table 2.1 and Table 2.2. Gradient-enhanced (ge) HSQC and ge-HMBC studies of the flavonols quercetin and kaempferol and the flavone luteolin have been used to demonstrate that the strong intramolecular hydrogen bond of the $-\text{CO}(4)$ and $-\text{OH}(5)$ moieties persists over a wide range of aqueous solvent mixtures.⁹⁰ The use of reference deconvolution to suppress t_1 noise due to imperfections in spectrometer reproducibility has been described with particular emphasis on the use of HMBC applied on the proanthocyanidin, ent-epiafzelechin($4\alpha \rightarrow 8;2\alpha \rightarrow \text{O} \rightarrow 7$)-epicatechin.⁹¹

2.2.3.3 Improved Versions of the HMBC Experiment

One-bond correlations observed in the HMBC spectrum may, in some cases, provide useful structural information. On the other hand, incomplete suppression of the $^1J_{\text{CH}}$ correlations due to poor low-pass filter quality, which is often the case in the gradient-selected (gs) HMBC experiment, may complicate the spectrum considerably. Another problem associated with the gs-HMBC experiment is that the experiment may not be adjusted in a uniform manner to the wide range of multiple bond coupling constants (ranging from 1 to 25 Hz) of the compound. Thus, crosspeaks vital for the structural elucidation may be weak or totally absent from the spectrum.⁹² To overcome these problems, improved HMBC experiments including 3D HMBC, in which the third dimension is used to scan the whole range of $^nJ_{\text{CH}}$ coupling constants,⁹³ and the 2D experiments ACCORD-HMBC,⁹⁴ IMPEACH-MBC,⁹⁵ and CIGAR-HMBC,⁹⁶ have been developed. Only the last of these has been applied to flavonoids.⁹⁷ Recently, a $^2J, ^3J$ -HMBC experiment has been developed that allowed differentiation between $^2J_{\text{CH}}$ and $^3J_{\text{CH}}$ correlations.^{92,98}

2.2.3.4 Nuclear Overhauser Enhancement Spectroscopy

Protons that are close to each other in space may be observed as crosspeaks in a NOESY spectrum (Figure 2.3). Thus, the NOESY experiment proves to be a more sensitive alternative technique to HMBC for the determination of some linkages within a flavonoid. For instance, when sugars are attached to the 5 and 3' hydroxyls of the aglycone, the anomeric protons show crosspeaks to H-6 and H-2', respectively, while the anomeric proton of a sugar attached to the 7 position will exhibit crosspeaks to both H-6 and H-8. NOESY spectra have been used for unequivocal assignments of methoxyl group resonances and observation of restricted rotation of the B-ring of 2'-methoxyflavones,⁹⁹ and for the structural elucidation of two unusual macrocyclic flavonols.⁷⁰ Crosspeaks observed in a NOESY spectrum may reveal both intra- and intermolecular distances between flavonoid protons.¹⁰⁰ This type of information has been used for depiction of association mechanisms involving anthocyanins,^{101–104} and to show that some synthesized 8-*C*-glucosylflavones in DMSO-*d*₆ adopted conformations in which the H-2'' and H-4'' protons in the glucose moiety were oriented toward the B-ring in the flavone structure.¹⁰⁵ Based on NMR data and molecular dynamic simulations, a folded conformation was elucidated for the flavonol kaempferol 3-*O*-(2''(6'''-*p*-coumaroyl)glucosyl)-rhamnoside) in solution, implying a hydrophobic interaction between the aromatic nuclei of the aglycone and the acyl group.¹⁰⁶

The NOESY experiment has also been very useful for revealing the presence of rotational conformers of dimeric flavonoids and flavone *C*-glycosides (Figure 2.3).^{107–109} Strong exchange crosspeaks between equivalent protons of each conformer revealed the rotational equilibriums. This NOE phenomenon was first noted by Hatano et al.¹¹⁰ in two conformers of procyanidin dimers.¹¹⁰ The volume of the NOESY crosspeaks is related to the distance

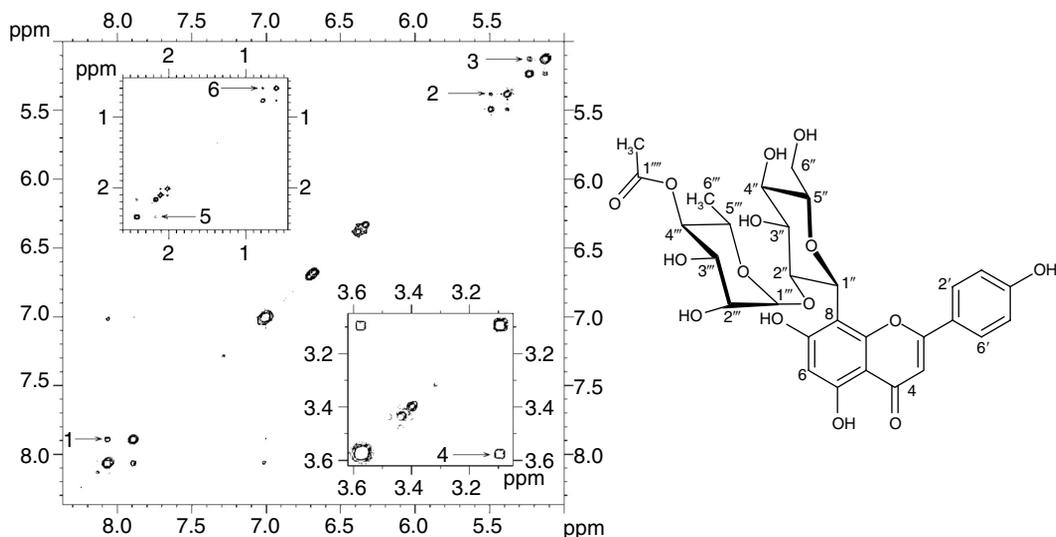


FIGURE 2.3 Expanded region of a NOESY spectrum of kaempferol 8-C-(2''-(4'''-acetyl)rhannosyl)-glucoside recorded in CD₃OD showing the exchange crosspeaks between equivalent protons of two rotameric conformers (denoted as A and B) due to rotational equilibrium. Arrows labeled by 1 to 6 show the exchange crosspeaks between each pair of the equivalent protons of H-2',6'A/H-2',6'B; H-1'''A/H-1'''B; H-1''A/H-1''B; H-3'''A/H-3'''B; H-5'''A/H-5'''B; and H-6'''A/H-6'''B, respectively. The relative proportions of A and B were 1.00:0.58 when dissolved in CD₃OD.¹⁷⁶

between the nuclei, and 3D distance information can be estimated based on the integration of the volume of the crosspeaks. Here, crosspeaks corresponding to two protons with a known distance are used as references for the calculation of distance. Thus, intermolecular association of two anthocyanin molecules¹⁰¹ and a structural model of the solution complex between a flavonol and a DNA dodecamer have been evidenced by NOESY NMR experiments and distance geometry calculations.³⁶

2.2.3.5 Rotating Frame Overhauser Effect Spectroscopy

Similar to the ¹H-¹H NOESY experiment, the ¹H-¹H ROESY experiment is useful for the determination of signals arising from protons that are close in space but not necessarily connected by chemical bonds. A ROESY spectrum yields through-space correlations via the rotating frame (also called rotational nuclear) Overhauser Effect (ROE). When one multiplet is irradiated, the intensities of multiplets arising from nearby nuclei are affected. The ROESY experiment (Figure 2.4) is especially useful for cases where the NOESY signals are weak because they are near the transition between negative and positive, which may be the case for flavonoids with masses of 800 to 2000 amu. The ROESY crosspeaks are negative; however, the ROESY (and NOESY) experiment may also yield crosspeaks arising from chemical exchange. These exchange peaks are always positive.

The ROESY experiment has together with other NMR experiments been used for structural elucidation of several new flavonoids including a pentaflavonoid, ochnachalcone, isolated from the stem bark of *Ochna calodendron*,¹¹¹ some flavones,^{79,80} some isoflavones from soybeans,¹¹² some flavonols from red onion,⁵² a flavonol glycoside and a pterocarpan glycoside from *Ononis vaginalis*,⁶⁰ a dimeric proanthocyanidin,¹¹³ some triacylated anthocyanins from *Ajuga reptans* flowers and cell cultures,⁸⁴ a rare anthocyanin from blue flowers of

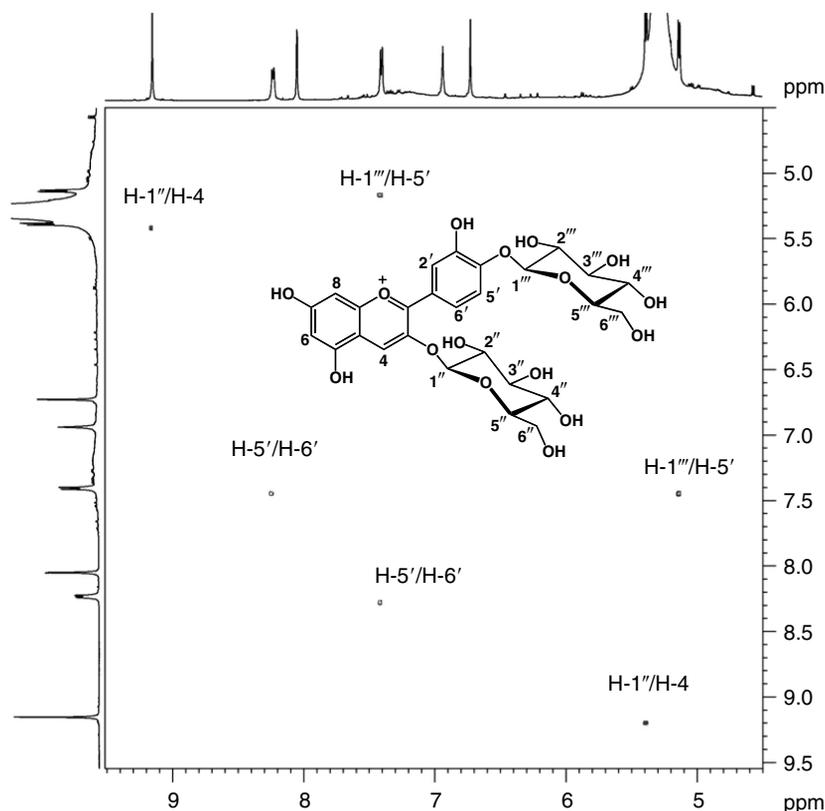


FIGURE 2.4 Expanded region of the ^1H - ^1H ROESY spectrum of cyanidin 3,4'-diglucoside, recorded in $\text{CD}_3\text{OD}-\text{CF}_3\text{COOD}$ (95:5, v/v) showing through-space correlations, which are important for determination of the linkages between the aglycone and the sugar units. Projections of the 1D ^1H NMR spectrum are included in both dimensions.⁵⁸²

Nymphaea caerulea,¹¹⁴ for seven natural anthocyanins stabilizing a DNA triplex,¹¹⁵ etc. Sequential analysis of the oligosaccharide structures of the flavonol tamarixetin-7-*O*-rutinoside has been performed by 1D multistep-relayed COSY-ROESY experiments.¹¹⁶ Selective excitation was performed by Gaussian-shaped soft pulses.

The ROESY experiment has often been used for revealing stereochemical aspects. The relative stereochemistry of two new biflavonoids isolated from the leaves of *Calycopteris floribunda* was deduced through NOE and ROESY experiments, comparative CD experiments, and optical rotation evaluations.¹¹⁷ A ROESY experiment was needed to confirm the H-2, H-3-*trans* relationship of the C- and F-rings of a procyanidin trimer, since these protons showed some rather unexpected coupling constants.⁷⁷ The experiment has recently been used in the room-temperature conformational analysis of a biflavanoid and a polyhydroxylated flavanone-chromone isolated from *Cratoxylum neriifolium*.¹¹⁸ Both compounds showed rotameric behavior due to the presence of a single bond between the highly substituted flavanone and flavanonol parts and the flavanone and chromone parts, respectively. Transverse-ROESY experiments in conjunction with theoretical (MM2) calculations have been used to support the proposal that the two rotamers of spinosin, a flavone C-glycoside, interchanged via rotation about the C6-C1'' bond.¹¹⁹ The structure of β -cyclodextrin (β -CD) inclusion complexes of naringin, naringin dihydrochalcone, and the aglycone of naringin dihydrochalcones has been determined from 2D ROESY and 1D ROE experiments.¹²⁰ A quenched

molecular dynamics (QMD)-ROSY study of a series of semibiosynthetically monoacylated anthocyanins produced in tissue cultures of *Daucus carota* has recently led to the identification of families of conformers of these flexible molecules that are of interest in work toward determining the mechanism for stabilization of color among these compounds in solution.¹²¹

2.2.3.6 Two- and Three-Dimensional HSQC-TOCSY, HSQC-NOESY, HSQC-ROESY, HMQC-NOESY, HMQC-ROESY

In recent years, several new 2D and 3D NMR techniques with significant potential in structural elucidation of flavonoids have been evolved.¹²² Taking advantage of the excellent resolution in the ¹³C dimension, interpretable NOESY, ROESY, or TOCSY data can be achieved from spectral regions with considerable overlap in the ordinary 2D NOESY, ROESY, or TOCSY spectra, respectively. The ¹³C chemical shift values of the individual monosaccharides in polyglycosylated flavonoids can, for instance, be identified and assigned by the 2D and 3D HSQC-TOCSY experiments due to the fact that each anomeric proton exhibits crosspeaks to all ¹³C resonances in the same spin system. The 2D HSQC-TOCSY, HSQC-NOESY, HSQC-ROESY, HMQC-NOESY, and HMQC-ROESY experiments are relatively sensitive, being only slightly less sensitive than the corresponding 2D HSQC and HMQC experiments, respectively. Despite their potential, as far as we know only 2D HSQC-TOCSY^{118,123,124} has, among these techniques, been applied in the structural elucidation of flavonoids. The sugar region of the 2D ¹H-¹³C gs-HSQC-TOCSY spectrum of malvidin 3-*O*-(6''-*O*- α -rhamnopyranosyl- β -glucopyranoside)-5-*O*- β -glucopyranoside, showing crosspeaks of most of the sugar signals, is presented in Figure 2.5. Here, we present the first report of a 3D HSQC-TOCSY spectrum applied to a flavonoid (Figure 2.6). The spectrum of an anthocyanin, pelargonidin 3-(6''-(4'''-*E*-*p*-coumaroyl- α -rhamnosyl)- β -glucoside)-5- β -glucoside, is recorded in CD₃OD-CF₃COOD (95:5; v/v). To visualize the potential of this experiment, a 2D plane derived from the 3D HSQC-TOCSY spectrum shows the ¹H-¹³C HSQC spectrum of all signals exhibiting TOCSY correlations to the 3-glucosyl anomeric proton, i.e., a ¹H-¹³C HSQC spectrum representing no more than the correlations of the 3-glucosyl unit.

2.2.4 SOLID-STATE NMR

Solid-state NMR is a fast and nondestructive method for identification of flavonoids in the solid state, provided that a sufficient amount of the sample is available (ca. 10 mg or more).¹²⁵ The development of high-resolution ¹H MAS NMR spectroscopy has had a substantial impact on the ability of researchers to analyze intact tissues. Rapid spinning of the sample relative to the applied magnetic field serves to reduce line broadening. Thus, it is possible to obtain very high quality NMR spectra of whole tissue samples with no sample pretreatment. Geometric data on solids, including determination of hydrogen positions, are usually obtained by x-ray analysis of crystals. However, according to the Cambridge Structural Database, x-ray data are available only for a limited number of flavonoids, most probably due to difficulties with growing single flavonoid crystals. Solid-state ¹³C NMR data of powder samples have been used to characterize the solid-state conformation of the flavones chrysin, apigenin, luteolin, and acacetin, the flavanones naringenin and hesperetin, and the flavonols galangin, kaempferol, quercetin, and myricetin (Figure 2.7).^{125,126} Based on cross-polarization magic angle spinning (CP-MAS) solid-state ¹³C NMR spectra, it was found that the locked conformations of the OH and OCH₃ groups in the solids resulted in increased shielding of carbon atoms adjacent to C-OH or C-OCH₃ hydrogens, thus enabling the determination of the orientations of these groups. The C5-OH (and C3-OH) hydroxyl

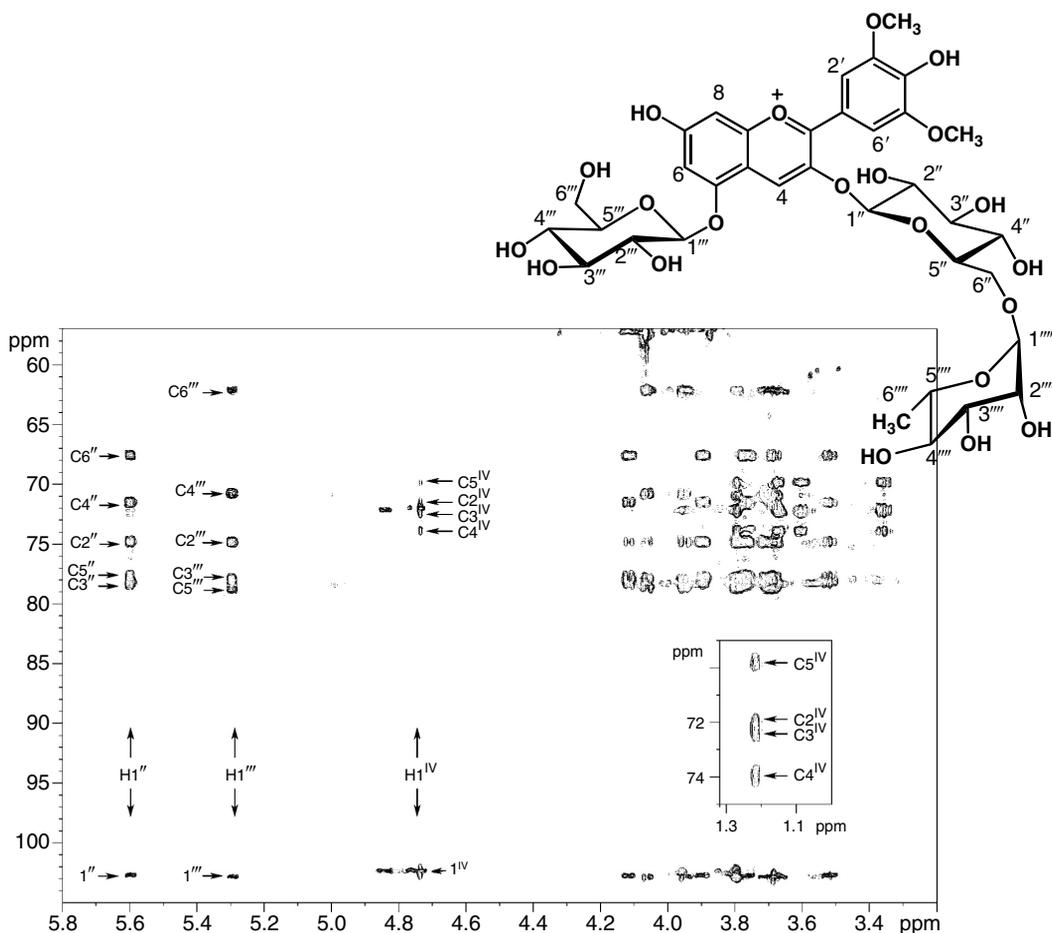


FIGURE 2.5 Region of the 2D ^1H - ^{13}C gs-HSQC-TOCSY spectrum of malvidin 3-*O*-(6''-*O*- α -rhamnopyranosyl- β -glucopyranoside)-5-*O*- β -glucopyranoside showing crosspeaks of most of the sugar signals. In this spectrum, each proton/carbon correlation shows crosspeaks to all ^1H and ^{13}C resonances belonging to the same spin system. The expanded region (boxed) reveals the correlation between H-6^{IV} and the carbons of the terminal rhamnose unit.¹⁷⁶

pointed toward oxygen in the carbonyl group, forming intramolecular hydrogen bonds. Considerations of the C2' and C6' carbon shielding suggested that the flavonol B-ring was not coplanar with the benzopyran fragment in kaempferol, acetin, and myricetin. More recently, assignments of ^{13}C CP-MAS NMR spectra of green and black tea, respectively, based on model compound spectra and literature data on various compounds including flavonoids, have made it possible to differentiate between commercial samples of these tea types.¹²⁷ Solid-state ^{13}C NMR and x-ray studies have also been applied on the complexes between the isoflavone genistein and various amines.¹²⁸

2.2.5 LIQUID CHROMATOGRAPHY-NMR

The coupling of chromatographic techniques such as HPLC with NMR, LC-NMR can, in principle, provide the molecular structures of compounds in mixtures (extracts) in just one online experiment. The use of LC-NMR in the flavonoid field has been reviewed by

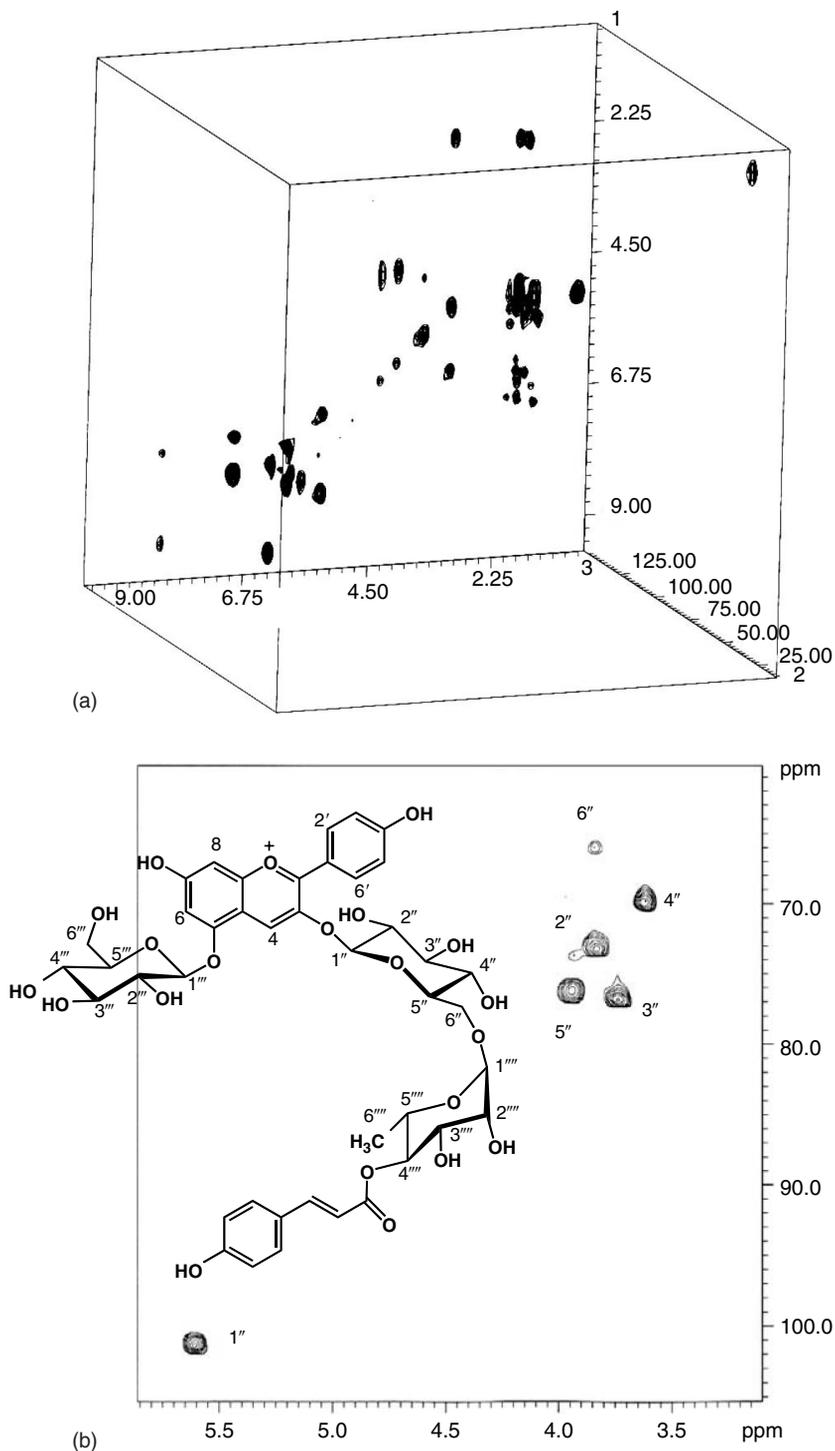


FIGURE 2.6 (a) 3D HSQC-TOCSY spectrum of pelargonidin 3-(6''-(4'''-*E-p*-coumaroyl- α -rhamnosyl)- β -glucoside)-5- β -glucoside recorded in $\text{CD}_3\text{OD}-\text{CF}_3\text{COOD}$ (95:5; v/v) at 298 K. (b) 2D plane derived from the 3D HSQC-TOCSY spectrum showing the $^1\text{H}-^{13}\text{C}$ HSQC spectrum of all signals exhibiting TOCSY correlations to the 3-glucosyl anomeric proton, i.e., the signals belonging to the 3-glucosyl unit.⁵⁸³

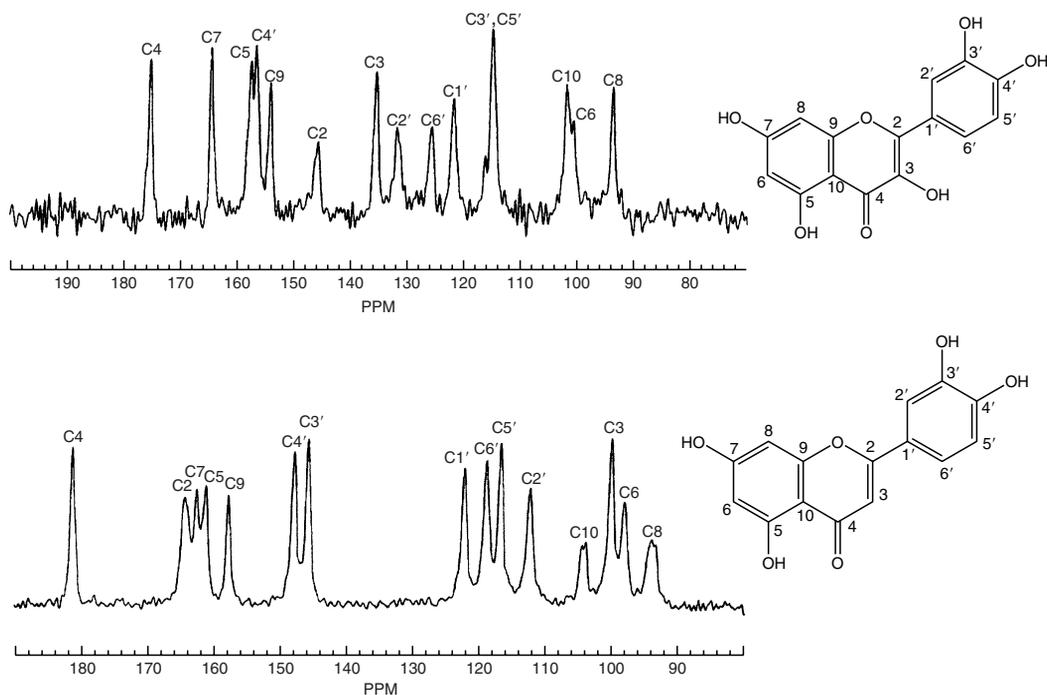


FIGURE 2.7 ^{13}C CP/MAS NMR spectra of the flavonol kaempferol (top) and the flavone luteolin (bottom). (Reprinted from Wawer, I. and Zielinska, A., *Magn. Reson. Chem.*, 39, 374, 2001. Copyright 2001 John Wiley & Sons, Ltd. With permission.)

Wolfender et al.,^{129–132} and this coupled technique is treated more comprehensively in Chapter 1. The LC–NMR technique is by nature rather insensitive; however, high-field magnets and recent improvements in solvent suppression, pulse field gradients, and probe technology have made it possible to achieve useful results on various flavonoid structures.^{28,133–141} The detection limit with a 60 μl cell in a 500 MHz instrument for a compound with a molecular weight of around 400 amu may typically be around 20 μg , and the information provided is hitherto mainly based on ^1H NMR spectra or ^1H – ^1H correlation experiments. However, a more recent system seems to be promising, affording increased sensitivity and thus shorter NMR acquisition time compared to conventional LC–NMR systems:²⁸ After LC separation of flavonoids in Greek oregano using ordinary nondeuterated solvents, solid-phase extraction was used for peak storage prior to the NMR analysis, and fully deuterated solvents were then used for flushing the trapped compounds into the NMR probe. Thus, the application of expensive deuterated solvents during HPLC separation and solvent suppression during NMR analysis are no longer necessary. Increased sensitivity was achieved using a newly developed cryogenic flow probe. Combining the data from the UV, MS, and NMR spectra, the flavonoids taxifolin, aromadendrin, eriodictyol, naringenin, and apigenin were identified.

2.2.6 NMR DATA ON FLAVONOID CLASSES

Assigned ^1H and ^{13}C chemical shifts characteristic for the various flavonoid classes are shown in Table 2.1–Table 2.6. The names of the flavonoids listed in Table 2.1–Table 2.6 are given in Table 2.7. The structures of the flavonoids listed in Table 2.1–Table 2.7 are shown in Figure 2.8–Figure 2.16.

TABLE 2.1
¹H NMR Spectral Data of Flavonoids Recorded in Various Solvents*

No.	Flavones	3	5	6	7	8	2'	3'	4'	5'	6'	Ref.
1	6-OH-Luteolin 4'-OMe-7-(2''- α -rha-6''-ac- β -glc) (D)	6.76 s	12.69 s OH			6.94 s	7.44 d 2.3		3.88 s	7.11 d 8.7	7.54 dd 8.6, 2.3	142
2	6-OH-Luteolin 7-(6''-(D)-ca)- β -glc (D)	6.61 s	12.74 br s OH			6.91 s	7.38 d 2.3		OMe	6.83 d 8.8	7.38 dd 8.8, 2.3	142
3	Isoscutellarein 7-(2''-(6''-ac)- β -all)- β -glc (D)	6.80 s	12.33 s OH	6.70 s			7.97 'd' 8.9	6.95 'd' 8.9		6.95 'd' 8.9	7.97 'd' 8.9	142
4	Isoscutellarein 4'-OMe-7-(2''-(6''-ao)- β -all)- β -glc (D)	6.89 s	12.28 s OH	6.71 s			8.09 'd' 8.9	7.14 'd' 8.9	3.88 s	7.14 'd' 8.9	8.09 'd' 8.9	142
5	Apigenin 4-(2''-(2''-fer-glu)-glu) (M)	6.87 s		6.19 d 1.9		6.51 d 1.9	7.98 'd' 8.7	7.10 'd' 8.7	OMe	7.10 'd' 8.7	7.98 'd' 8.7	143
6	Apigenin 7-glu-4-(2''-(2''-fer-glu)-glu) (M)	6.61 s		6.48 d 2.0		6.79 d 2.0	7.84 'd' 9.0	7.15 'd' 9.0		7.15 'd' 9.0	7.84 'd' 9.0	143
7	Apigenin 7-glu-4-(2''-(2''-E-cou-glu)-glu) (M)	6.61 s		6.46 d 2.0		6.78 d 2.0	7.83 'd' 9.0	7.14 'd' 9.0		7.14 'd' 9.0	7.83 'd' 9.0	143
8	Luteolin 3'- β -glc-4-(2''- α -rha- β -glc) (M)	6.71 s		6.23 d 1.3		6.52 d 1.3	7.98 d 1.7			7.34 d 8.0	7.70 dd 8.0, 1.7	144
9	Luteolin 3',4'-di- β -glc (M)	6.80 s		6.13 d 1.5		6.46 d 1.5	7.93 d 1.7			7.30 d 8.0	7.67 dd 8.0, 1.7	144
10	5,7,4'-tri-OH-3'-OMe-Flavone 8-C-(2''-O- β -glc- β -xylyl) (M)	6.53 s		6.22 s		C-(2''-O- β -glc- β -xylyl)	7.50 d 1.2	3.96 s OMe		6.88 d 7.2	7.42 dd 1.2, 7.2	145
11	5,7-di-OH-3'-OMe-4'-Acetoxyflavone 8-C-(2''-O- β -glc- β -xylyl) (M)	6.54 s		6.18 s		C-(2''-O- β -glc- β -xylyl)	7.62 d 1.4	4.00 s OMe	1.94 s acetoxy	6.92 d 8.4	7.47 dd 1.4, 8.4	145
12	Iso-orientin 3'-OMe (D)	6.55 s		C-glc		6.91 s	7.56 s	3.90 s OMe		6.96 d 8.6	7.57 d 8.6	146
13	8-C-p-OH-Benz-isovitexin 4'-glc (D)	6.92 s		C-glc		C-p-OH-benz 2,6 st	7.95 'd' 6.8	7.19 'd' 6.8		7.19 'd' 6.8	7.95 'd' 6.8	146
14	Apigenin 8-C-(2''-(4''-ac-rha)-glc) ^y (M)	6.68 s, 6.72 s		6.39 s, 6.34 s			8.07 'd' 9.0, 7.89 'd' 9.0	7.02 'd' 9.0, 7.00 'd' 9.0		7.02 'd' 9.0, 7.00 'd' 9.0	8.07 'd' 9.0, 7.89 'd' 9.0	109
15	Spinosin (D)	6.83, 6.84 s			3.89 s OMe	6.67, 6.80 s	7.97 'd' 8.7	6.95 'd' 8.7		6.95 'd' 8.7	7.97 'd' 8.7	147
16	6''-Fer-spinosin (D)	6.85, 6.83 s			3.84 s OMe	6.77, 6.67 s	7.81 'd' 7.9	6.89 'd' 7.9		6.89 'd' 7.9	7.81 'd' 7.9	147
17	Isoscoparin 7-glc (D)	6.58 s				6.47 s	7.56 br s	3.90 s OMe		7.38 br s	6.94 m	47
18	Carinoside (D)	6.63 s					7.45 s			6.90 d 8.0	7.56 br s	47
Flavonols												
19	Kaempferol 3-(6''- α -ara-glc) (D)			6.14 d 1.9		6.34 d 1.9	8.01 'd' 8.8	6.89 'd' 8.8		6.89 'd' 8.8	8.01 'd' 8.8	148
20	Kaempferol 3-(6''- α -ara-glc)-7-glc (D)			6.44 br s		6.76 br s	8.04 'd' 8.8	6.88 'd' 8.8		6.88 'd' 8.8	8.04 'd' 8.8	148

continued

TABLE 2.1
¹H NMR Spectral Data of Flavonoids Recorded in Various Solvents* — continued

No.	Flavones	3	5	6	7	8	2'	3'	4'	5'	6'	Ref.
21	Kaempferol 3-(2''-rha-6''-mal-glc) (M)		6.18 d 2.1			6.38 d 2.1	7.99 'd' 8.9	6.88 'd' 8.9		6.88 'd' 8.9	7.99 'd' 8.9	149
22	Kaempferol 3-glc-7-(2''-(6''-p-cou-glc-glc)) (M)		6.31 d 1.8			6.33 d 1.8	8.07 'd' 8.0	6.91 'd' 8.0		6.91 'd' 8.0	8.07 'd' 8.0	150
23	8-OMe-Kaempferol 3-(6''-mal-glc) (M)		6.37 s			3.99 s OMe	8.18 'd' 8.9	6.99 'd' 8.9		6.99 'd' 8.9	8.18 'd' 8.9	109
24	Quercetin (M)		6.27			6.47	7.82			6.97	7.72	52
25	Quercetin 4-glc (M)		6.25			6.44	7.81			7.35	7.75	52
26	Quercetin 3'-xyl (M)		6.27			6.47	8.17			7.06	7.91	151
28	Quercetin 3,4'-diglc (D)		6.14			6.35	7.64			7.20	7.57	52
29	Quercetin 3,7,4'-triglc (D)		6.43			6.80	7.67			7.21	7.62	52
30	Isorhamnetin 3-rut (M)		6.28			6.48	8.04			7.00	7.72	153
31	Isorhamnetin 3,7'-diglc (M)		6.48 d 2			6.77 d 2	7.93 d 2			6.88 d 8	7.62 dd 8, 2	154
32	Myricetin 3-(2''-rha-glc) (M)		6.17 d 2.0			6.35 d 2.0	7.23 s				7.23 s	149
33	Myricetin 3-(6''-p-cou-glc) (P)		6.70 d 2			6.81 d 2	8.44 d 2				8.52 d 2	70
34	Myricetin 7-(6''-gall-glc) (D)		6.42 d 2.5			6.72 d 2.5	7.28 s				7.28 s	155
27	Myricetin 3-(2''-ac-rha) (M)		6.32			6.48	7.08			OH	7.08	152
35	Laricitrin 3- α -ara-furanoside (D)		6.21 d 2.0			6.43 d 2.0	7.31 d 1.9				7.17 d 1.9	156
36	Laricitrin 3-glc (M)		6.27 d			6.45 d	7.62 d				7.38	153
37	Syringetin 3-(5''-glc- α -ara-furanoside) (D)		6.22 d 2.0			6.50 d 2.0	7.34 s			3.86 s OMe	7.34 s	156
38	Syringetin 3-(6''-ac-glc) (M)		6.24 d			6.44 d	7.61 s			4.03 s OMe	7.61 s	153
39	Syringetin 3-(6''-rha-gal) (M)		6.23 d 2.0			6.46 d 2.0	7.60 s			3.95 s OMe	7.60 s	157
40	Syringetin 6-C-glc (D)	9.51 s OH	13.02 s OH			6.53 s	7.48 s	3.83 s OMe	9.18 s OH	3.83 s OMe	7.48 s	158
	Aurones	α	4	5	6	7	2'	3'	4'	5'	6'	
41	6,3'-di-OH-4,4'-di-OMe-5-Me-Aurone (D)	6.59 s	4.05 s OMe	1.98 s Me		6.61 s	7.48 d 1.5		3.83 s OMe	7.02 d 8.4	7.31 dd	159
42	4,6,3',4'-tetra-OMe-Aurone (Z-form) (D)	6.69 s	3.92 ^b s OMe	6.34 d 1.7	3.89 ^b s OMe	6.72 d 1.7	7.55 d 1.7	3.83 ^b s OMe	3.82 ^b s OMe	7.07 d 7.9	7.54 dd	159
43	4,6,3',4'-tetra-OMe-Aurone (E-form) (D)	7.00 s	3.89 ^b s OMe	6.29 d 1.7	3.88 ^b s OMe	6.51 d 1.7	8.48 d 1.8	3.84 ^b s OMe	3.82 ^b s OMe	7.01 d 7.9	1.7, 8.0	159
44	6,3',4'-tri-OH-4-OMe-5-Me-Aurone (D)	6.54 s	4.03 s OMe	1.90 s Me		6.54 s	7.42 d 2.0			6.82 d 6.9	7.19 dd	160
45	Maecopsin (P)	3.06 s		5.76 d 1.5	5.73 d 1.5		7.00 'd' 8.4	6.57 'd' 8.4		6.57 'd' 8.4	7.00 'd' 8.4	161

TABLE 2.1
¹H NMR Spectral Data of Flavonoids Recorded in Various Solvents* — continued

No.	Flavones	3	4	5	6	7	8	2'	3'	4'	5'	6'	Ref.
Isoflavonoids													
73 [#]	Judacina 7-(6''-ac-glc) (D)	4.88 s	6.60 s	7.05 d 8.2	6.59 dd 8.2, 2.1	6.50 d 2.1	3.74 s OMe	6.82 s	6.90 s	6.90 s	6.90 s	179	
74	Tectorigenin 4-(6''-glc-glc) (P)	8.0 s	8.0 s	4.0 OMe	7.7 'd' 8.8	7.5 'd' 8.8	7.5 'd' 8.8	7.5 'd' 8.8	7.7 'd' 8.8	7.7 'd' 8.8	7.7 'd' 8.8	180	
75 ^b	7-OH-6-OMe-3',4'-methylenedioxy-Isoflavone 7-glc (D)	8.02 s	8.02 s	7.98 d 8.9	7.17 dd 8.5, 2.6	7.10 d 2.3	6.87 s	6.82 s	3.70 s OMe	3.70 s OMe	3.70 s OMe	181	
76	Irisjaponin A (C)	7.90 s	7.90 s	4.04 s OMe	6.54 s	6.54 s	3.84 s OMe	3.96 s OMe	6.63 s	6.63 s	6.63 s	182	
77	Irisjaponin B (C)	7.85 s	7.85 s	4.04 s OMe	6.53 s	6.53 s	3.84 s OMe	3.91 s OMe	7.02 d 8.6	7.02 d 8.6	7.02 d 8.6	182	
78	Junipegenin B (C)	7.89 s	7.89 s	4.04 s OMe	6.53 s	6.53 s	7.10 d 2.0	3.93 s OMe	7.03 dd	7.03 dd	8.3, 2.0	182	
Flavanones													
79 ^j	Matteucinol 7-(6''-apio-furanosyl)-β-glc) (P)	5.42 dd	2.89 dd	3ax	3.25 dd 17.0, 13.0	12.6 s OH	2.65 s Me	2.57 s Me	7.58 'd' 7.0	7.02 'd' 7.0	3.66 s OMe	183	
80 ^j	Hesperitin 7-(2''-gal-6''-rha-glc) (D)	5.45 m	2.78 dd	3.15 m	12.0 s OH	6.15 d 2.0	6.17 d 2.0	6.80 m	9.15 br s OH	3.75 s OMe	3.75 s OMe	184	
81 ^k	5,3'-di-OH-7,4'-di-OMe-Flavanone (D)	5.33 dd	2.81 d	3.04 dd	OH 12.0 s	6.08 d	3.95 ^b s OMe	6.05 d	7.25 s	5.77 s OH	3.85 ^b s OMe	185	
82 ^l	Naringenin 7-glc (M)	5.46 dd	2.83 dd	3.25 dd 13.0, 17.3	6.30 d 2.2	6.30 d 2.2	7.40 'd' 8.6	6.27 d 2.2	6.90 'd' 8.6	6.90 'd' 8.6	6.90 'd' 8.6	109	
83 ^m	Naringenin 7-(6''-gal-glc) (M)	2.9, 13.0	2.9, 17.3	3.30 dd 17.0, 12.5	6.18 d 2.5	6.18 d 2.5	7.30 'd' 7.5	6.20 d 2.5	6.80 'd' 7.5	6.80 'd' 7.5	6.80 'd' 7.5	155	
Dihydroflavonols and dihydroflavones													
84	Taxifolin 4'-glc (M)	5.08	4.59	3b	6	6	5.99 ^b	7.14	7.33	7.06	7.06	52	
85	Aromadendrin 7-glc (M)	5.11 d 11.7	4.68 d 11.7	6.32 d 2.2	6.30 d 2.2	6.30 d 2.2	7.44 'd' 8.6	6.91 'd' 8.5	6.91 'd' 8.5	7.44 'd' 8.6	7.44 'd' 8.6	186	
86	Ampelopsin 7-glc (M)	4.98 d 11.3	4.61 d 11.3	6.32 d 2.2	6.30 d 2.4	6.32 d 2.2	6.62 s	6.62 s	6.62 s	6.62 s	6.62 s	186	
87	2''-Ac-callunin (M)	4.63 d 11.4	5.03 d 11.4	5.93 s	5.93 s	5.93 s	7.39 'd' 8.6	6.81 'd' 8.6	6.81 'd' 8.6	7.39 'd' 8.6	7.39 'd' 8.6	187	
88	2 <i>R</i> ,3 <i>R</i> - <i>trans</i> -Aromadendrin 7-(6''-(4''-OH-2''-methylhebutanoyl)-glc) (D)	5.10 d 11.6	4.70 dd 11.6, 6.25	5.83 d 6.25, 3-OH, 11.80 s 5-OH, 9.60 s 4'-OH	6.15 s	6.15 s	7.34 'd' 8.5	6.81 'd' 8.5	6.81 'd' 8.5	7.34 'd' 8.5	7.34 'd' 8.5	188	
89	(2 <i>R</i> ,3 <i>S</i>)-(+)-3',5-di-OH-4',7-di-OMe-Dihydroflavonol (D)	6.03 d 2.2	4.80 d 2.2	11.47 s 5-OH, 3.80 s 7-OMe, 3.91 s 4'-OMe	6.16 d 2.3	6.29 d 2.3	6.77 d 2.0	9.28 s OH	6.95 d 8.3	6.67 dd 8.3, 2.0	6.67 dd 8.3, 2.0	189	
90	3-Desoxycallunin (M)	5.42 dd	3.04 dd 17.2, 11.8, 3.0	2.75 dd 17.2, 3.0	5.91 s	5.91 s	7.37 'd' 8.5	6.78 'd' 8.5	6.78 'd' 8.5	7.37 'd' 8.5	7.37 'd' 8.5	187	

Flavan glycosides																						
91	Catechin 3-(6'- <i>cin</i> -glc) (A)	2	4.90 d 6.5	3	4.19 m	4 α	2.91 <i>dd</i> 16.5, 5.5	4 β	2.76 <i>dd</i> 16.5, 7.0	5	6.04 d 2.5	6	5.91 d 2.5	8	5.91 d 2.5	2'	6.90 d 2.0	5'	6.81 d 8.5	6'	6.78 <i>dd</i> 8.5, 2.0	190
92	Catechin 3-(2'- <i>cin</i> -glc) (A)	2	4.65 d 8.0	3	4.21 m	4 α	2.90 <i>dd</i> 16.5, 5.5	4 β	2.63 <i>dd</i> 16.5, 8.0	5	6.03 d 2.5	6	5.85 d 2.5	8	5.85 d 2.5	2'	6.90 d 2.0	5'	6.81 d 8.0	6'	6.71 <i>dd</i> 8.0, 2.0	190
93	Catechin 3-(2',6'- <i>dicin</i> -glc) (A)	2	4.68 d 7.8	3	4.17 m	4 α	3.04 <i>dd</i> 16.5, 5.5	4 β	2.67 <i>dd</i> 16.5, 8.0	5	6.02 d 2.5	6	5.83 d 2.5	8	5.83 d 2.5	2'	6.90 d 2.0	5'	6.78 d 8.0	6'	6.73 <i>dd</i> 8.0, 2.0	190
94	Ananthoside (M)	2	4.97 d 5.9	3	4.15 m	4 α	2.87 <i>dd</i> 15.6, 4.8	4 β	2.82 <i>dd</i> 15.6, 6.2	5	6.36 <i>dd</i> 2.0, 8.3	6	6.33 d 2.0	8	6.33 d 2.0	2'	6.82 d 2.0	5'	6.76 d 8.3	6'	6.72 <i>dd</i> 8.3, 2.0	191
Prenylated flavonoids																						
95 ^a	Cajamin (D)	2	8.22 s	3a	2.87 m	3b	3.11 m	4a	2.89 m	4b	3.16 m	5	12.98 s OH	6	6.28 d 2.2	7	3.85 s OMe	8	6.24 d 2.2	2'	9.39 s OH	192
96 ^c	Indicanine C (D)	2	7.76 s	3a	2.87 m	3b	3.11 m	4a	2.89 m	4b	3.16 m	5	3.89 s OMe	6	6.60 s	7	5.85 s OH	8	6.60 s	2'	7.26 d 8.7	192
97 ^a	6-(1,1-di-Me-allyl)-7,4'-di-OH-Flavan (C)	2	4.97 m	3a	2.87 m	3b	3.11 m	4a	2.89 m	4b	3.16 m	5	7.03 brs	6	6.34 br s	7	5.85 s OH	8	6.34 br s	2'	7.15 br d 8.5	193
98 ^a	5 ^l (C)	2	3.16 d	3a	2.87 m	3b	3.11 m	4a	2.89 m	4b	3.16 m	5	OMe 3.73 s	6	6.62 m	7	5.85 s OH	8	6.62 m	2'	7.10 d 8.9	194
Pterocarpan and rotenoids																						
99 ^c	Maackiainin 3-(6'- <i>mal</i> -glc) (D)	1	7.38	2	6.71	4	6.54	6 α	3.58-3.61	6 β	4.27	6 α	3.58-3.61	7	6.97	10	6.52	11	6.52	11a	5.55	179
100 ^a	3,4,8,9-dimethylendioxy-Pterocarpan (C)	1	6.96	2	6.55	4	6.54	6 α	3.64	6 β	4.23	6 α	3.45	7	6.66	10	6.37	11	6.37	11a	5.43	195
101 ^c	Usararotenoid C (C)	1	7.70	2	6.41	4	6.41	6 α	4.45	6 β	4.37	6 α	4.60	7	6.71	10	6.71	11	6.71	11a	7.89	196
102 ^a	12a-Epimillettosin (C)	1	7.67	2	6.37	4	6.37	6 α	4.44	6 β	4.36	6 α	4.59	7	6.54	10	6.54	11	6.54	11a	7.74	197
103 ^a	(+)-Usararotenoid-B (C)	1	8.29	2	6.70	4	6.70	6 α	4.94	6 β	4.63	6 α	4.94	7	6.79	10	6.79	11	6.79	11a	8.05	196
Biflavonoids																						
104	[Catechin 3-glc-(4 α → 8)-Catechin 3-(2'- <i>cin</i> -glc)] (biflavanol) (A)	2	4.27 d 9.5	3	4.47 m	4	4.45 m	4' α /4' β	4.45 m	7	6.71	6 α	5.87 d 2.0	7	6.64 d 1.5	10	6.64 d 1.5	11	6.64 d 1.5	11a	6.33 <i>dd</i> 8.5, 1.5	190
		2	4.50 d 8.5	3	3.98 m	4	2.96 <i>dd</i> 16.5, 6.02.63	4' α /4' β	2.96 <i>dd</i> 16.5, 6.06 s	5	5.76 d 2.0	6	6.06 s	7	6.62 m	10	6.62 m	11	6.62 m	11a	6.39 br d 8.5	190
105	[catechin 3-glc-(4 α → 8)-Epicatechin 3-(6'- <i>cin</i> -glc)] (biflavanol) (A)	2	4.38 d 10.0	3	4.69 <i>dd</i> 10.0, 6.5	4	4.56 d 6.5	4' α /4' β	4.56 d 6.5	5	5.75 d 2.0	6	6.05 s	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190
		2	5.23 d 4.5	3	4.25 m	4	2.50 <i>dd</i> 17.0, 4.6	4' α /4' β	2.50 <i>dd</i> 17.0, 4.6	5	6.05 s	6	6.05 s	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.04 m	190
106	Amentoflavone (biflavone) (D)	2	6.81 s	3	6.81 s	4	2.71 <i>dd</i> 17.0, 4.6	4' α /4' β	2.71 <i>dd</i> 17.0, 4.6	5	6.18 d 2.5	6	6.18 d 2.5	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190
		2	6.76 s	3	6.76 s	4	2.71 <i>dd</i> 17.0, 4.6	4' α /4' β	2.71 <i>dd</i> 17.0, 4.6	5	6.18 d 2.5	6	6.18 d 2.5	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.04 m	190
107	Aulaciumin-biaureusidin (biaurone) (D)	2	6.86 s	3	6.86 s	4	2.71 <i>dd</i> 17.0, 4.6	4' α /4' β	2.71 <i>dd</i> 17.0, 4.6	5	6.18 d 2.5	6	6.18 d 2.5	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190
108	Cupressuflavone 7,7'- <i>di</i> -OMe (biflavone) (D)	2	6.86 s	3	6.86 s	4	2.71 <i>dd</i> 17.0, 4.6	4' α /4' β	2.71 <i>dd</i> 17.0, 4.6	5	6.18 d 2.5	6	6.18 d 2.5	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190
109	4,4',6-tri- <i>O</i> -methyl-2-Deoxymaepsosin-(2 → 7)-2,4,4',6-tetra- <i>O</i> -methylmaepsosin (bibenzofuranoid) (C)	2	3.24 s OMe	3	3.70 s	4	3.70 s	4-OMe/6-OMe	3.70 s	5	5.93 d 2.0	6	5.88 s	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190
		2	3.16 d 14.0,	3	3.16 d 14.0,	4	3.16 d 14.0,	4-OMe/6-OMe	3.16 d 14.0,	5	5.93 d 2.0	6	5.88 s	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190
		2	3.04 d 14.0	3	3.04 d 14.0	4	3.04 d 14.0	4-OMe/6-OMe	3.04 d 14.0	5	5.93 d 2.0	6	5.88 s	7	6.18 d 2.5	10	6.18 d 2.5	11	6.18 d 2.5	11a	6.70 <i>dd</i> 8.0, 1.5	190

continued

TABLE 2.1
¹H NMR Spectral Data of Flavonoids Recorded in Various Solvents* — continued

No.	Flavones	3	5	6	7	8	2'	3'	4'	5'	6'	Ref.
110	Catechin-(4 α \rightarrow 8)-pelargonidin 3-glc (anthocyanin-flavanol) [†] (M/T5)	4.81 d 9.2 4.62 d 9.3	4.35 t 9.2 4.61 m	9.14 s 9.21 s	6.90 s 6.79 s	ND	8.30 'd' 9.1 8.77 'd' 9.2 6.92 d 2.0 7.07 d 2.0	8.30 'd' 9.1 8.77 'd' 9.2	7.06 'd' 9.1 7.17 'd' 9.2 6.76 d 8.3	7.06 'd' 9.1 7.17 'd' 9.2 6.82 dd 8.3, 2.0 6.95 dd 8.3, 2.0	108	
111	2',4'',2''-tri-OH-4',4''-di-OMe-4-O-5''- Bichalcone (C) (Rhuschalcone 1) (bichalcone)	2/6' 2'/6''	3/5 3'/5''	3' 3'''	5' 5''	α α'	β β'	4-OMe 4''-OMe	3.85 s 3.82 s		202	
		7.70 'd' 8.8 7.61 'd' 8.6	6.90 'd' 8.8 6.80 'd' 8.7	6.45 d 2.4 6.69 s	6.53 dd 9.0, 2.5	7.71 d 15.4 7.93 s	7.83 d 15.4 7.85 d 15.5					

Notes: pg, pelargonidin; cy, cyanidin; dp, delphinidin; pn, peonidin; pt, petunidin; mv, malvidin; all, allopnyranose; ara, α -arabinopyranose; gal, galactopyranose; glc, glucopyranose; glu, glucuronic acid; rha, rhamnopyranose; xyl, xylopyranose; ac, acetyl; benz, benzoyl; caf, caffeoyl; cin, cinnamoyl; cou, *p*-coumaroyl; fer, feruloyl; gall, galloyl; mal, malonyl; sinap, sinapoyl; ND, not detected.

*C-*p*-OH-benz 2/6 7.07 'd' 7.7 3/5 7.90 'd' 7.7, CH₂ bridge 4.06 s. ^bAssignments may be reversed.
 *A, acetone-*d*6; C, CDCl₃; M, CD₃OD; M/T1, CD₃OD-CF₃COOD 99:1; M/T2, CD₃OD-CF₃COOD 98:2; M/T3, CD₃OD-CF₃COOD 95:5; M/T10, CD₃OD-CF₃COOD 90:10; M/T17, CD₃OD-CF₃COOD 5:1; D, DMSO-*d*6; D/T10, DMSO-*d*6-CF₃COOD 90:10; D/T20, DMSO-*d*6-CF₃COOD 80:20; P, pyridine-*d*5; T, CF₃COOD; ¹³C chemical shift values of OMe and Me groups are given in brackets.

[†]Rotamers: Top — chemical shift values of major rotamer.

[‡]5 = 3-(4-Hydroxyphenyl)-5-methoxy-6-(3,3-dimethylallyl)-2'',2''-dimethylchromene-(5',6'':8,7)-3-(propyl-2-one)-4*H*-1-benzo-2,3-dihydropyran-2,4-dione.
^b47: 1'' (3.56 d 7.0); α (3.33 t 7.4), ⁸⁷3: OCH₂O (5.99 s), ⁸⁷5: OCH₂O (6.00 s), ⁷⁹: 5' (7.02 'd' 7.0); 6' (7.58 'd' 7.0), ⁸⁰: 5' (6.80 m); 6' (6.80 m), ⁸¹: 5' (6.92 m); ⁸²: 5' (6.90 'd' 8.6); (2.96 t 7.4); α (3.33 t 7.4), ⁸⁷3: OCH₂O (5.99 s), ⁸⁷5: OCH₂O (6.00 s), ⁷⁹: 5' (6.63 d 2.3); 4' (9.31 s OH); 5' (6.36 dd 2.3, 8.3); 6' (6.96 d 8.3), ⁹⁶: 3' (6.82 'd' 8.7); 4' (7.19 s OH); 5' (6.82 'd' 8.7); 6' (7.40 'd' 8.6), ⁸³: 5' (6.80 'd' 7.5); 6' (7.30 'd' 7.5), ⁹⁵: 3' (6.63 d 2.3); 4' (9.31 s OH); 5' (6.36 dd 2.3, 8.3); 6' (6.96 d 8.3), ⁹⁶: 3' (6.82 'd' 8.7); 4' (7.19 s OH); 5' (6.82 'd' 8.7); 6' (7.26 'd' 8.7); 3'' (5.72 d 10); 4'' (6.72 d 10); 1''' (1.47 s), ⁹⁷: 3' (6.80 br d 8.5); 5' (6.80 br d 8.5); 2'' (6.15 br d 10.5, 17.7); 3''a (5.36 dd 1.0, 17.7); 3''b (5.30 dd 1.0, 10.5); 4'' (1.42 s), ⁹⁸: 3' (6.70 'd' 8.9); 5' (6.70 'd' 8.9); 7' (3.27 dd 13.9, 7.2); 8' (5.07 td 7.2, 1.2); 10' (1.75 s); 11' (1.64 s); 2'' (1.46 s, 1.45 s Me); 3'' (5.65 d 10.1); 4'' (6.74 d 10.1); CH₂COCH₃ (3.87 d 18.1); 3:5 d 18.1; CH₂COCCH₃ (2.20 s), ⁹⁹: 8.9 OCH₂O (5.92) ¹⁰⁰: 3.4 OCH₂O (5.84); 8.9 OCH₂O (5.93), ¹⁰¹: 2.3 OCH₂O (5.94); 1' (3.37); 2' (5.17); 4' (1.68); 5' (1.77); OMe (3.91), ¹⁰²: 2.3 OCH₂O (5.91); 4' (5.62); 5' (6.62); 2'-Me (2.1.48), ¹⁰³: 2.3 OCH₂O (5.93, 5.95); OMe (3.92, 3.79).

TABLE 2.2
¹³C NMR Spectral Data of Flavonoids Recorded in Various Solvents*

No.	Flavones	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'	OMe	Ref.
1	6-OH-Luteolin 4'-OMe-7-(2''-α-rha-6''-ac-β-glc) (D)	163.8	103.1	182.1	146.8	130.7	151.2	93.8	149.0	105.5	123.1	113.0	146.7	151.1	112.1	118.5	55.7	142
2	6-OH-Luteolin 7-(6''-E)-cauf-β-glc (D)	164.3	101.8	182.0	ND	130.3	151.1	93.2	148.9	105.7	121.0	112.7	145.9	150.3	115.5	118.5		142
3	Isoscutellarein 7-(2''-(6''-ac)β-sall)-β-glc (D)	164.0	102.6	182.2	152.1	99.5	150.4	127.5	143.7	105.6	121.1	128.5	115.9	161.3	115.9	128.5	55.5	142
4	Isoscutellarein 4'-OMe-7-(2''-(6''-ac)β-sall)-β-glc (D)	163.6	103.3	182.3	152.1	99.5	150.5	127.5	143.7	105.6	122.8	128.3	114.5	162.4	114.5	128.3		142
5	Apigenin 4-(2''-(2''-fer-glu)-glu) (M)	166.1	104.8	183.9	163.2	100.2	165.5	95.1	159.4	105.4	126.1	129.1	117.9	161.3	117.9	129.1		143
6	Apigenin 7-glu-4-(2''-(2''-fer-glu)-glu) (M)	165.9	104.9	184.0	162.9	101.3	164.5	96.0	158.9	107.3	125.8	129.2	117.9	161.4	117.9	129.2		143
7	Apigenin 7-glu-4-(2''-(2''-α-rha-β-glc) (M)	166.0	105.0	184.0	162.8	101.2	164.5	95.9	158.9	107.3	125.8	129.3	117.9	161.4	117.9	129.3		143
8	Luteolin 3'-β-glc-4-(2''-α-rha-β-glc) (M)	165.1	105.2	183.8	163.2	100.1	166.6	95.1	159.4	106.9	127.1	118.1	149.2	151.4	119.2	123.3		144
9	Luteolin 3',4'-di-β-glc (M)	165.4	105.5	183.5	163.0	100.0	166.0	95.0	159.6	106.7	126.8	117.8	149.1	151.0	118.9	123.0		144
10	5,7,4'-tri-OH-3'-OMe-Flavone-8-C-β-xyf-2''-O-glc (M)	166.00	104.11	184.12	162.69	100.75	164.66	104.04	156.84	105.43	123.97	110.81	149.30	151.73	116.72	121.78		145
11	5,7,4'-tri-OH-3'-OMe-4'-Acetoxyflavone-8-C-(2''-O-glc-β-xyf) (M)	166.22	104.23	184.15	162.61	100.88	164.66	105.41	156.80	105.60	124.26	111.19	149.41	151.79	116.73	121.81		145
12	Isoorientin 3'-OMe (D)	163.32	103.03	181.85	160.48	108.72	163.24	93.64	156.21	103.29	121.32	109.99	147.90	150.59	115.68	120.23	55.84	146
13	8-C- <i>p</i> -OH-Benz-isovitexin 4'-O-glc (D)	162.80	103.45	182.12	157.25	107.73	160.76	106.76	153.69	103.56	123.86	127.90	116.47	160.08	116.47	127.90		146
14	Apigenin 8-C-(2''-(4''-ac-rha)-glc) [†] (M)	166.66,	103.58,	184.11	162.82,	99.77,	164.12,	105.75,	157.80,	105.98,	123.36,	130.13,	117.01,	162.79	117.01,	130.13,		109
		165.95	104.21		163.20	101.32	164.34	105.61	156.34	104.74	123.32	129.52	117.15		117.15	129.52		
15	Spinosin [†] (D)	163.66	102.90,	181.82,	159.57,	108.58	163.72,	90.20,	156.85,	104.09,	120.92	128.34	115.59,	160.78,	115.59,	128.34	55.99,	147
		102.99	182.15	160.42	164.94	90.67	156.96		156.96	104.36		128.34	115.59	161.22	115.89	56.42		
16	6''-Fer-spinosin [†] (D)	163.6,	102.9,	181.7,	159.4,	108.7	165.1,	89.9,	156.8,	103.9,	121.1,	128.4,	115.70,	161.1	115.70,	128.4,	56.3,	147
		163.8	103.1	182.2	160.7	104.4	164.1	90.6	157.0	104.4	121.2	128.5	115.75		115.75	128.5	56.0	
17	Isoscaparin 7-glc (D)	164.3	104.7	181.7	159.2	110.2	162.2	93.4	156.2	102.9	121.2	121.2	149.3	160.1	115.3	118.8	60.2	147
	Flavonols	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'		
19	Kaempferol 3-(6''-α-ara-glc) (D)	156.0	133.1	177.0	161.0	98.9	165.4	93.7	156.4	103.5	120.8	130.7	115.0	159.8	115.0	130.7		148
20	Kaempferol 3-(6''-α-ara-glc)-7-glc (D)	157.0	133.3	177.4	160.8	99.2	162.8	94.5	155.9	105.6	120.0	130.8	115.3	160.8	115.3	130.8		148
21	Kaempferol 3-(2''-rha-6''-mal-glc) (M)	161.24	134.27	179.24	163.16	99.79	165.67	94.71	158.44	105.91	123.18	132.10	116.08	159.13	116.08	132.10		149
22	Kaempferol 3-glc-7-(2''-(6''- <i>p</i> -cou-glc)-glc) (M)	157.8	135.9	177.3	162.7	100.0	163.1	95.7	157.8	107.5	122.9	132.5	116.1	161.7	116.1	132.5		150
23	Quercetin (M)	148.00	137.21	179.33	162.50	99.25	165.34	94.40	158.22	104.52	124.15	115.99	148.75	146.21	116.22	121.67		52
24	8-OMe-Kaempferol 3-(6''-mal-glc) (M)	159.33	135.33	179.57	157.97	100.14	158.57	129.14	150.39	105.59	122.80	132.30	116.15	161.68	116.15	132.30		109
								(62.01)										
25	Quercetin 4'-glc (M)	148.04	137.89	177.36	162.47	99.31	165.66	94.47	158.19	104.54	127.60	116.49	147.83	146.79	117.62	121.26		52
26	Quercetin 3'-xyl (M)	147.17	137.47	177.36	162.53	99.30	165.65	94.42	158.17	104.55	124.31	118.54	146.32	150.49	117.19	124.92		151
28	Quercetin 3,4'-diglc (D)	155.96	134.26	174.82	161.64	99.16	164.65	94.08	156.83	104.53	124.92	115.86	147.67	146.52	116.97	121.42		52
29	Quercetin 3,7,4'-triglc (D)	156.27	134.20	177.62	160.90	99.48	162.99	94.49	156.12	105.61	124.42	116.73	147.72	146.22	115.52	120.40		52

continued

TABLE 2.2
¹³C NMR Spectral Data of Flavonoids Recorded in Various Solvents* — continued

No.	Flavones	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'	OMe	Ref.	
30	Isorhamnetin 3-rut	158.91	135.70	179.50	ND	100.61	163.28	95.43	159.03	105.67	123.28	114.78	151.19 (57.02)	148.62	116.40	124.25		153	
31	Isorhamnetin 3,7-diglc (M)	160.2	136.2	180.3	163.6	101.6	165.5	96.5	158.8	108.4	123.0	115.1	149.5 (57.5)	153.0	117.1	124.9		154	
32	Myricetin 3-(2''-rha-glc) (M)	158.48	134.71	179.31	163.20	99.65	165.62	94.45	158.34	105.97	122.43	109.87	146.46	137.74	146.46	109.87		149	
33	Myricetin 3-(6''-p-couglc) (P)	147.9	138.8	177.7	104.9	162.9	99.8	166.1	94.9	157.4	123.5	110.2	148.5	140.5	148.2	113.0		70	
34	Myricetin 7-(6''-gall-glc) (D)	148.2	136.2	176.3	160.3	98.8	162.5	94.6	156.0	105.0	121.1	107.9	146.1	136.2	146.1	107.9		155	
27	Myricetin 3-(2''-ac-rha) (M)	158.51	135.60	179.43	163.25	99.77	165.89	94.74	159.44	105.85	121.77	109.55	146.95	137.99	146.95	109.55		152	
35	Laricitrin 3- α -ara-furanoside (D)	156.4	133.4	177.7	161.3	98.6	164.2	93.6	156.9	103.9	119.8	109.5	147.8 (55.8)	137.4	145.7	105.6		156	
37	Syringetin 3-(5''-glc- α -ara-furanoside) (D)	156.4	133.3	177.6	161.2	98.7	164.2	94.0	157.1	104.0	119.7	106.6	147.5 (56.0)	138.6	147.5	106.6		156	
39	Syringetin 3-(6''-rha-gal) (M)	158.6	135.6	179.4	163.1	100.1	166.0	95.1	158.5	105.9	121.9	108.4	149.0 (57.5)	140.1	149.0	108.4		157	
40	Syringetin 6-C-glc (D)	146.2	135.8	176.0	159.6	108.1	163.0	93.3	155.0	102.7	120.7	106.0	147.7 (56.2)	138.2	147.7	106.0		158	
Aurones																			
41	6,3'-di-OH-4,4'-di-OMe-5-Me-Aurone (D)	145.96 ^a	178.75	156.87 (61.39)	117.04 (8.20)	165.68 ^b	93.30	165.30 ^b	105.31	110.29	124.94	111.41	146.61 ^a	149.34	112.16	123.95		159	
42	4,6,3',4'-tetraOMe-Aurone (Z-form) (D)	146.15	178.90	168.04 (55.59) ^a	89.89	168.74	94.38	158.82	104.21	110.29	124.94 ^b	111.89	148.75 (56.42) ^a	150.27	113.99	124.79 ^b		159	
44	6,3',4'-tri-OH-4-OMe-5-Me-Aurone (D)	145.52 ^a	178.76	156.86	116.05	165.12 ^b	93.26	165.63 ^b	105.50	110.94	123.59	111.40	145.55 ^a	147.80	117.66	124.3		159	
45	Maepsosin (P)	107.4	196.8	173.8	91.1	171.6	96.8	159.8	103.1	42.1	125.9	132.6	115.7	157.2	115.7	132.6		160	
46a	Maepsosin 6-O-glc diastereoisomer 1 (P)	107.7	197.0	174.6	93.3	171.4	97.6	158.4	103.6	42.1	125.6	132.5	115.8	157.2	115.8	132.5		161	
46b	Maepsosin 6-O-glc diastereoisomer 2 (P)	107.6	196.9	174.5	93.2	171.4	97.3	158.3	103.4	41.9	125.6	132.5	115.7	157.2	115.7	132.5		161	
47	Licoisgaurone (A)	147.4	182.9	123.4	112.9	167.7	113.1	163.6	114.9	112.0	125.722.6	118.8/146.2/122.1	132.8	148.1/18.1	116.5/25.8	125.5		89	
Chalcones and dihydrochalcones																			
48	3'-formyl-4',6'-di-OH-2'-OMe-5-Me-Chalcone (C)	147.0	125.0	193.0	108.2	167.1	108.5	165.7	109.3	169.0	136.0	128.7	129.2	132.1	129.2	132.1		162	
49	Chalcononaringenin 2',4'-diglc (P)	144.4	125.2	193.9	108.4	164.2	98.9	166.7	95.7	161.6	127.3	131.7	116.9	161.0	116.9	131.7		163	
50	2',4'-di-OH-4'-OMe-6'-glc Dihydrochalcone (D)	30.0	46.3	205.4	107.3	166.1	96.3	166.3	94.6	161.3	132.5	130.3	116.0	156.3	116.0	130.3		164	
51	2'-OH-3',4',6'-tri-OMe-Dihydrochalcone (A)	31.3	46.7	206.1	106.6	159.6	131.6	159.9	88.3	159.9	142.7	129.3 ^a	129.2 ^a	126.7	129.2 ^a	129.3 ^a		165	

	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'	
Anthocyanins																
53	165.0	145.8	135.8	156.6	106.2	169.8	97.5	157.0	113.3	120.5	136.1	118.1	167.4	118.1	136.1	167
54	164.58	145.59	136.90	159.2	103.64	170.41	95.25	157.76	113.28	121.24	118.43	147.50	155.89	117.39	128.48	168
55	164.17	145.62	136.21	159.06	103.48	170.45	95.23	157.63	113.24	121.20	118.40	147.44	155.88	117.43	128.42	168
56	164.42	145.10	136.76	159.37	103.62	170.65	94.93	157.78	113.15	120.83	117.71	147.58	155.88	117.31	128.50	169
58	164.38	145.98 ^a	135.68	159.38 ^b	103.30	170.24	95.13	157.64 ^b	113.87	120.01	112.71	147.57	144.91 ^a	147.57	121.71	171
62	164.7	146.2	134.9	156.6	106.2	169.6	97.4	157.1	113.3	119.8	109.6	149.9	146.3	147.7	114.2	173
											(57.2)					
65	163.98	146.05	134.56	156.65	105.55	169.88	97.68	157.14	113.14	119.50	110.87	149.82 ^c	147.27	149.82	110.87	174
											(57.31)			(57.31)		
63	164.89	146.00	136.38	157.34	106.11	170.01	97.70	156.87	113.47	119.58	110.95	149.84	147.21	149.84	110.95	174
											(57.26)			(57.26)		
66	164.92	146.40	135.46	156.58	106.03	169.8	97.65	157.47	113.47	119.65	111.07	149.93	147.37	149.93	111.07	174
											(57.31)			(57.31)		
67	173.8	112.0	149.9	158.6	105.5	172.1	98.5	160.2	114.5	121.6	134.1	118.9	168.3	118.9	134.1	82
68	173.1	113.0	149.7	158.2	105.3	171.3	98.5	159.6	113.7	121.9	117.0	148.7	157.2	118.8	126.6	82
69 ^e	166.46	135.95	150.44	154.48	101.77 ^m	169.33	101.53 ⁿ	154.48	111.00	120.95	135.04	117.40	165.75	117.40	135.04	175
70 ^d	166.09	136.02	149.84	154.31	101.78	169.44	101.45	154.32	110.71	121.31	118.21	147.09	154.59	117.02	127.27	176
71 ^e	165.01	135.25	109.79 ^a	153.07	101.30	168.40	101.05	153.20	109.67 ⁿ	120.33	118.5	146.14	153.89	116.89	126.52	177
72 ^f	163.4	134.8	109.3	152.7	100.8	168.2	101.1	152.7	109.5	118.4	109.4	148.2	143.9	148.2	109.4	178
											(56.5)			(56.5)		
Isoflavonoids																
73	67.6	129.1	120.8	117.6	127.2	109.4	157.6	103.4	153.8	119.1	152.5	95.5	147.7	141.0	107.5	179
											(56.5)					
74	153.6	122.5	180.9	105.0	153.9	132.9	154.3	95.1	153.9	125.5	130.8	117.1	158.4	117.1	130.8	180
						(60.3)										
112	152.6	122.5	174.9	114.9	126.2	114.9	161.2	112.6	157.1	123.0	130.0	114.9	157.1	114.9	130.0	203
113	153.0	123.3	174.5	116.2	127.3	115.1	162.5	102.2	157.3	124.7	116.4	146.0	147.5	111.9	119.7	204
75	154.7	121.7	174.2	118.3	126.8	115.3	161.3	103.4	157.0	122.6	110.9	140.3	147.9	95.5	152.8	181
											(55.6)					
76	152.5	120.0	181.2	106.5	154.8	130.4	155.2	93.3	153.5	118.6	149.4	147.3	143.5	145.9	108.9	182
						(56.2)					(60.9)	(61.2)	(61.3)	(61.2)		
77	152.5	120.3	181.3	106.5	154.3	130.3	155.0	93.2	153.5	117.0	151.5	142.3	154.3	107.2	125.8	182
						(56.1)					(60.8)	(60.9)	(61.2)			
78	152.6	121.3	181.3	106.5	154.0	130.4	155.2	93.2	153.4	121.3	112.3	148.9	149.3	111.2	123.3	182
						(56.0)					(60.9)	(60.9)	(60.9)			

continued

TABLE 2.2
¹³C NMR Spectral Data of Flavonoids Recorded in Various Solvents* — continued

No.	Flavones	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'	OMe	Ref.
Flavanones																		
79	Matteucinol 7-(6'-apio-furanosyl-β-glc)(P)	78.8	43.6	198.4	159.5	112.3 (9.4)	162.6	111.3 (10.0)	158.3	105.8	131.7	128.3	144.5	160.3 (55.2)	114.5	128.3		183
80	Hesperitin 7-(2''-gal-6''-rha-glc)(D)	78.49	42.13	197.17	163.10	96.48	165.17	95.65	163.15	103.42	130.96	114.24	148.61	146.52 (55.76)	112.06	118.11		184
81	5,3'-di-OH-7,4'-di-OMe-Flavanone (D)	79.0	41.9	196.7	167.4	95.3	146.2 (55.6)	93.7	162.7	102.5	120.5	113.7	163.1	149.1 (55.8)	112.1	130.6		185
82	Naringenin 7-glc (M)	80.62	44.13	198.52	159.07	96.92	167.00	97.99	164.93	104.91	130.85	129.09	116.33	164.59	116.33	129.09		109
83	Naringenin 7-(6''-gall-glc)(M)	78.8	42.1	197.3	163.0	96.4	165.1	95.3	163.0	103.4	128.6	128.7	115.3	157.8	115.3	128.7		155
Dihydroflavones and dihydroflavones																		
84	Taxifolin 4-glc (M)	85.55	73.70	198.22	168.69	97.39 ^a	165.72	96.32 ^a	164.35	102.19	133.96	116.57	147.22	148.37	118.51	120.70		52
85	Aromadendrin 7-glc (M)	85.2	73.8	199.5	164.3	98.3	167.3	97.0	164.9	103.2	129.0	130.4	116.1	159.3	116.1	130.4		186
87	2''-Ac-callunin (M)	85.2	73.0	198.0	159.5	98.1	162.2	127.8	155.0	101.7	129.3	130.9	116.3	161.3	116.3	130.9		187
88	2 <i>R</i> ,3 <i>R</i> - <i>trans</i> -Aromadendrin 7-(6''- (4''-OH,2''-methylbutanoyl)-glc)(D)	84.0	72.5	199.7	163.5	97.6	166.0	96.1	163.4	103.0	128.3	130.5	115.8	158.7	115.8	130.5		188
89	(2 <i>R</i> ,3 <i>S</i>)(+)-3',5'-di-OH-4',7'-di- OMe-Dihydroflavonol (D)	82.2	77.8	191.6	163.3	95.8	170.3 (57.0 ^a)	95.0	165.4	103.6	129.3	119.4	147.8	149.3 (56.9 ^a)	112.7	114.8		189
90	3-Desoxycallunin (M)	80.6	44.0	197.7	158.9	97.4	162.0	127.5	155.4	103.2	129.2	128.8	116.4	161.5	116.4	128.8		187
Flavan glycosides																		
91	Catechin 3-(6''-cin-glc)(A)	79.6	76.3	26.7	152.7	96.3	157.7	95.5	156.5	100.3	132.1	114.2	145.8	145.7	115.9	119.4		190
92	Catechin 3-(2''-cin-glc)(A)	80.4	74.8	27.7	157.1	96.3	157.8	95.5	156.7	100.5	131.9	115.4	145.8	145.8	115.8	120.1		190
93	Catechin 3-(2,6''-dicin-glc)(A)	80.2	75.6	24.7	157.0	96.3	157.7	95.4	156.6	100.4	131.7	115.3	145.7 ^a	145.6 ^a	115.8	120.1		190
94	Anaethoside (M)	80.7	76.9	31.0	131.5	109.6	158.0	103.5	155.9	112.4	132.2	114.8	146.3	146.4	116.3	119.6		191
Prenylated flavonoids																		
95 ^a	Cajaniin (D)	155.6	120.6	180.6	161.6	97.9	165.1 (56.1)	92.3	158.6	105.4	108.4	156.4	102.6	157.5	106.2			192
96 ^b	Indicanine C (D)	150.7	125.9	175.8	158.2 (62.9)	113.4	158.7	100.7	155.7	113.1	123.4	130.4	115.8	156.4	115.8			192
97 ^c	6-(1,1-di-Me-allyl)-7,4'-di-OH-Flavan (C)	85.0	41.5	34.9	122.4	124.3	155.3	99.8	159.8	118.7	130.0	115.8	154.7	115.8	115.8			193
98 ^d	5 ^c (C)	169.1	61.4	187.6	159.3 (62.1)	106.4	158.3	120.8	148.9	105.7	126.0	127.8	116.2	155.9	116.2			194
Pterocarpan and rotenoids																		
100 ^b	3,4,8,9-dimethylene-dioxo-Pterocarpan (C)	123.5		102.1	143.0	143.3	166.8		66.0	39.6	117.0	104.1	148.3	148.3	93.3	160.0		195
114 ^e	Isonorautenol (C)			66.5	39.4	76.5		132.2	109.8			160.2 ^a	103.7	157.2 ^a	112.4 ^b			205
115 ^f	Erybraedin A (C)			66.8	39.8	78.8		129.3	109.7			158.4 ^a	110.3	155.7 ^a	112.6 ^b			205
101 ^g	Usarotenoid C (C)	109.9	110.5	142.3	149.4	98.5	150.7		61.7	76.5	158.1	117.3	163.3	105.9				196
102 ^h	12a-Epimillettosin (C)	109.6		142.5	149.5	98.6	150.8		61.9	76.8	155.8	109.1	159.8	112.3				197
103 ^h	(+)-Usarotenoid-B (C)	111.2		142.9	149.8	99.2	151.5		62.9	78.2	155.2	137.6	159.5	107.4				197
Biflavonoids																		
2	3	4	5	6	7	8	9	10/α	1'	2'	3'	4'	5'	6'				
2	3	4	5	6	7	8	9	10'/α'	1''	2''	3''	4''	5''	6''				
82.5	81.2	37.5	158.7	97.0	157.2	97.5	157.2	107.2	132.2	116.9	145.8	146.5	116.6	120.9				190
81.8	76.3	29.6	155.2	96.6	155.6	109.3	155.3	103.0	131.4	116.8	145.9	146.4	116.2	121.0				

105	[Catechin 3-glc-(4 α → 8)-epicatechin 3-(2-cin-glc)] (A)	82.7	82.2	37.5	159.0	97.0	157.5	97.7	157.2	107.3	132.5	116.6	146.7	146.1	116.4	121.2	190
106	Amentoflavone (biflavone) (D)	77.7	74.2	25.3	155.8	96.4	154.9	109.5	153.9	101.7	131.8	114.1	145.7	145.9	116.4	119.0	198
		163.7	102.9	181.7	161.4	98.7	164.1	94.0	ND	103.6	120.9	131.4	120.0	ND	116.3	127.8	
107	Aulacommiumbiaureusidin (biaurone) (D)	163.8	102.6	182.2	161.5	98.9	160.5	104.0	159.7	103.7	121.4	128.2	115.8	161.0	115.8	128.2	199
		145.5	179.0	166.8	97.6	167.4	90.2	158.1	102.9	109.9	122.4	115.8	145.7	147.5	120.8	127.3	
108	Cupressuflavone 7, 7''-di-OMe (D)	145.9	180.1	165.8	108.7	165.2	90.5	158.1	102.9	109.5	123.7	117.5	145.5	147.5	115.8	124.0	200
		163.0	102.5	182.2	161.7	95.5	163.8 (56.5)	98.8	153.8	104.2	120.9	127.9	116.0	161.8	116.0	127.9	
		161.2	102.5	182.2	161.7	95.5	163.8 (56.5)	99.0	153.8	104.2	120.9	127.9	116.0	161.8	116.0	127.9	
109	4,4',6-tri- <i>O</i> -methyl-2-Deoxymaepsosin-(2 → 7)-2,4,4',6-tetra- <i>O</i> -methylmaepsosin* (bibenzofuranoid) (C)	91.3	197.5	158.5 ^a	92.2	169.2	88.3	174.5	106.2	41.6	127.2	132.2 ^b	113.4	158.4 ^b	113.4	132.2 ^b	201
		109.7	194.4	160.1 ^b	89.4	169.4 ^b	103.4	171.6	105.3	40.8	125.6	132.1 ^b	113.9	158.9	113.9	132.1 ^b	
110	Catechin-(4 α → 8)-pelargonidin 3-glc (anthocyanin-flavanol) (M/T5)	163.9	144.5	138.3	157.9	102.7	170.1	111.4	154.5	113.9	121.0	135.7	117.5	165.8	117.5	135.7	108
		163.6	144.6	39.0	158.2	103.4	169.9	ND	155.5	114.1	121.1	135.3	117.9	165.8	117.9	135.3	
		83.5	72.8	39.2	157.7	ND	ND	ND	154.0	104.3	131.6	115.5	146.0	146.3	115.5	120.3	
		84.0	72.6						154.1		116.2	146.1	146.1		115.5	121.1	
111	2',4-(2''-tri-OH-4',4''-di-OMe-4-O-5''-Bichalcone (Rhuschalcone I) (bichalcone) (C)	β	α	C = O	1'	2'	3'	4'	5'	6'	1	2	3	4	5	6	
		β'	α'	C = O'	1''	2''	3''	4''	5''	6''	1''	2''	3''	4''	5''	6''	
		143.8	118.6	191.5	113.9	166.6	101.1	166.1 (56.2)	107.7	130.9	129.4	130.7	115.8	158.6	115.8	130.7	202
		144.9	117.4	191.5	113.0	164.6	101.5	158.8 (55.6)	135.1	122.8	128.9	130.3	116.0	160.9	116.0	130.3	

Notes: pg, pelargonidin; cy, cyanidin; dp, delphinidin; pn, peonidin; mv, malvidin; all, allopyranose; ara, α -arabinopyranose; gal, galactopyranose; glu, glucopyranose; glu, glucuronic acid; rha, rhamnopyranose; xyl, xylopyranose; ac, acetyl; benz, benzoyl; caf, caffeoyl; cin, cinnamoyl; cou, *p*-coumaroyl; fer, feruloyl; gall, galloyl; mal, malonyl; sinap, sinapoyl; ND, not detected.

^{a,b,c}Assignments may be reversed.

*A, acetone-*d*6; C, CDCl₃; M, CD₃OD; M/T1, CD₃OD-CF₃COOD 99:1; M/T2, CD₃OD-CF₃COOD 98:2; M/T5, CD₃OD-CF₃COOD 95:5; M/T10, CD₃OD-CF₃COOD 90:10; M/T17, CD₃OD-CF₃COOD 5:1; D, DMSO-*d*6; D/T10, DMSO-*d*6-CF₃COOD 90:10; D/T20, DMSO-*d*6-CF₃COOD 80:20; P, pyridine-*d*5; T, CF₃COOD; ¹³C chemical shift values of OMe and Me groups are given in brackets.

¹Rotamers: Top — chemical shift values of major rotamer.

¹⁵5 = 3-(4'-Hydroxyphenyl)-5-methoxy-6-(3,3-dimethylallyl)-2'',2''-dimethylchromene-(5'',6'',8,7)-3-(propyl-2-one)-4*H*-1-benzo-2,3-dihydro-2,4-dione.
⁶⁹69: COOH (161.17); α (154.55); β (107.48), ⁷⁰70: COOH (161.44); α (155.7); β (107.4), ⁷¹71: COOH (160.52); α (154.46); β (106.8), ⁷²72: COOH (160.2); α (154.5); β (106.2), ⁹⁵95: 6' (132.2), ⁹⁶96: 6' (130.4); 2' (77.8); 3' (130.8); 4' (116.0); 1'' (116.0); 1'' (28.3), ⁹⁷97: 6' (130.9); 1'' (40.3); 2' (148.7); 3' (113.7); 4'/5' (27.6), ⁹⁸98: 6' (127.8); 7' (22.0); 8' (122.2); 9' (131.6); 10' (17.9); 2'' (78.3); 3'' (115.3); 4''/5'' (129.1); CH₂COCH₃ (205.9); CH₂COCH₃ (51.7); CH₂COCH₃ (28.9); 2''-Me (28.5, 28.4), ¹⁰⁰100: 11a (77.6); 11b (114.8); 3,4 OCH₂O (100.7); 8,9 OCH₂O (101.2), ¹⁰¹101: 11 (128.3); 11a (114.0); 12 (187.6); 12a (66.5); 2,3 OCH₂O (101.5); 1' (22.1); 2' (121.5); 3' (132.0); 4' (25.8); 5' (17.8); OMe (56.0), ¹⁰²102: 11 (129.9); 11a (110.8); 12 (189.9); 12a (66.6); 12b (113.7); 2,3 OCH₂O (101.8); 2' (78.0); 3' (129.4); 4' (115.6); 2''-Me (28.6), (28.4), ¹⁰³103: 11 (125.1); 11a (116.9); 12 (189.9); 12a (67.3); 12b (113.7); 2,3 OCH₂O (102.4); OMe (56.7), (61.2), ¹⁰⁴104: 1' (119.4^b); 2' (122.0); 3' (114.9^b); 4' (156.6^a); 5' (99.4); 6' (154.4^b); 2'' (78.4); 3'' (127.6); 4'' (122.1), 5'' (26.9), ¹⁰⁵105: 1' (118.8^b); 2' (122.3); 3' (108.0); 4' (153.9^a); 5' (114.9); 6' (155.5^a); 1'' (22.0); 2'' (121.7); 3'' (134.3); 4'' (25.0), 5'' (17.8); 1''' (23.1); 2''' (121.4); 3''' (134.9); 4''' (25.3); 5''' (17.8).

Glycosyl Moiety	Chemical Shift (ppm)/Coupling Constants (Hz)						Ref.
	H-1	H-2	H-3	H-4	H-5	H-6	
Glucosides							
3- <i>O</i> -β-glc (D)	5.51	3.19	3.22	3.03	3.07	3.54	52
3-(6''-mal-glc) (M)	5.26 d 7.3	3.56 m	3.54 m	3.42 m	3.49 m	3.30	109
7- <i>O</i> -β-glc (D)	4.87	3.23	3.26	3.12	3.37	4.21 dd 5.7, 11.8	52
7- <i>O</i> -β-(3- <i>p</i> -cou-glc) (D)	5.25 d 7.8	3.51 dd 7.8, 9.4	5.1 t 9.4	3.47 t ca. 9	3.62 m	3.46	206
4'- <i>O</i> -β-glc (D)	5.09	3.31	3.26	3.16	3.41	3.53 dd 5.4, 11.3	52
4'- <i>O</i> -β-glc (M)	5.00	3.61	3.60	3.53	3.57	3.46	52
6- <i>C</i> -β-glc (D)	4.57 d 9.7	4.03 t 9.4	3.19 t 8.6	3.11 t 9.2	3.15 m	3.68 dd 1.8, 12.0	109
6- <i>C</i> -β-glc (M)	4.99 d 9.9	4.26 t 9.9	3.57 m	3.57 m	3.50 m	3.40 dd 6.3, 12.0	109
8- <i>C</i> -β-glc [†] (D)	4.67 d 9.8 4.82 d 9.8	3.82 t 9.6 3.86 m	3.24 m 3.31 m	3.37 t 9.4 3.24 m	3.21 m 3.34 m	3.98 m 3.84 m	109
8- <i>C</i> -β-glc [‡] (M)	5.07 d 9.7 5.16 d 9.7	4.20 t 9.5	3.62 m 3.67 m	3.78 t 9.2 3.63 m	3.56 m 3.63 m	3.68 m 3.48 m	109
8- <i>C</i> -β-(2(6''-di-ac-glc) (D)	4.92 d 10	5.37 t 10	3.2-3.9 m			3.99 m 3.87 m 4.47 d 12 4.12 dd 12, 5	207
8- <i>C</i> -(2''-(4''-ac-α-rha)-β-glc) [†] (M)	5.13 d 9.9 5.24 d 9.9	4.31 dd 9.9, 8.6 4.18 dd 9.8, 8.9	3.77 dd 8.6, 9.2 3.84 m	3.72 t 9.2 3.65 m	3.57 dd (b) 5.9, 9.2 3.67 m	4.09 dd 1.4, 12.3 3.91 m	109
3- <i>O</i> -β-glc (M/T5)	5.40 d 7.8	3.75 dd 7.8, 9.2	3.61 t 9.2	3.51 m	3.64 m	4.04 dd 1.4, 12.3 3.91 m	170
3- <i>O</i> -(6''-mal-glc) (M/T5)	5.36 d 7.9	3.76 dd 7.9, 9.1	3.63 t 9.1	3.50 dd 9.1, 9.5	3.90 ddd 9.5, 7.3, 1.9	4.00 dd 2.0, 2.0 3.79 dd 12.0, 6.1	168
3- <i>O</i> -(6''-mal-glc) (D/T10)	5.41 d 7.0	3.53 t 8.5	3.43 t 9.1	3.25 t 9.0	3.48 dt 9.3	4.47 d 10.5 4.13 dd 12.2, 7.8	225
3- <i>O</i> -(6-rha-glc) (M/T5)	5.37 d 7.7	3.75 dd 7.7, 9.1	3.64 t 9.1	3.51 dd 9.1, 9.4	3.81 m	4.16 dd 11.3, 1.6 3.69 dd 11.3, 5.0	168
3-(2''-xyl-6''-mal-glc) (D/T10)	5.67 d 7.7	4.02 t 8.1	3.67 t 9.0	3.38 t 7.7	3.95 t 9.6	4.41 d 11.6 4.17 dd 11.6, 7.7	224
3-glc in Ternatin A3 (D/T10)	4.97 d 8	3.60 t 7	3.43 t 7	3.20-3.70 m	3.70-3.90 m	4.10-4.30 m 4.50-4.70 m	221
3-glc (M/T5)	4.82 d 7.7	3.77 dd 7.7, 9.3	3.49 dd 8.9, 9.3	3.37 dd 8.9, 9.8	3.27 ddd 2.3, 6.8, 9.8	3.85 dd 11.7, 2.3	176

3-(6''-rha-glc)-5-glc (M/T5)	5.60 d 7.7	3.77 m	3.69 m	3.52 t 9.4	3.90 ddd 2.1, 6.5, 9.0	4.12 dd 12.0, 2.1	174
3-(6''-rha-glc)-5-glc (M/T5)	5.30 d 7.7	3.79 m	3.68 m	3.68 m	3.71 m	3.77 m	174
5-O-β-glc (M/T5)	5.25 d 7.9	3.76 dd 7.9, 9.3	3.64 m	3.54 t 9.3	3.65 m	3.95 dd 12.3, 5.0 4.04 dd 12.0, 2.2	170
5-O-β-glc (D/T10)	5.10	3.45-3.55	3.37	3.24	3.37	3.83 dd 12.0, 5.8 3.74 3.50	220
5-O-(6''-ac-β-glc) (D/T10)	5.19 d 7.6	3.50 t 7.7	3.40 t 8.9	3.23 t 9.3	3.90 ddd 1.5, 9.0, 10.0	4.01 dd 12.0, 7.8 4.37 d 10.5	219
7-O-β-(3''-glc-6''-mal-glc) (M/T5)	5.53 d 7.7	3.55 m	3.69 m	3.52 m	3.67 m	4.49 dd 12.1, 1.8 4.35 dd 12.1, 5.9	170
3-[2''-(2'''-(E-caf)-glc)-6''-mal-gal]-7-[6'''-(E-caf)-glc]-3'-glu (D/T10)	5.35 d 7.0	3.42 m	3.36-3.42	3.36-3.42	3.90 m	4.30 m	222
3'-glc in Ternatin A3 (D/T10)	5.33 d 7	3.52 t 7	3.39 t 7	3.25 t 8	3.70-3.90 m	4.50 m	221
3,4'-di-O-β-glc (M/T5)	5.17 d 7.8	3.69 dd 7.8, 9.2	3.62 t 9.2	3.53 t 9.2	3.64 m	4.10-4.30 m 4.50-4.70 m	170
5'-glc in Ternatin A3 (D/T10)	5.33 d 7	3.52 t 7	3.39 t 7	3.25 t 8	3.70-3.90 m	4.04 dd 12.0, 2.1 3.84 dd 12.0, 5.7 4.50-4.70 m 4.50-4.70 m	221
Galactosides							
3-O-β-gal (D)	5.38 d 7.7	3.58 dd 9.6, 7.7	3.38 dd 9.6, 3.3	3.66 br d 3.3	3.34 dd 6.0, 6.3	3.46 dd 6.0, 10.6 3.38 dd 6.3, 10.6	109
3-O-β-(2''-O-glc-gal) (D)	5.68 d 7.7	3.82 dd 9.4, 7.7	3.64 dd 9.4, 3.4	3.70 dd 3.4, <1	3.29-3.44 m	3.29-3.44 m	215
3-O-β-(6''-O-rha-gal) (M)	5.16 d 7.8	3.92 dd 9.8, 7.8	3.66 dd 9.8, 3.5	3.90 dd 3.5, 1.0	3.74 t 6.3	3.84 dd 5.9, 10.2 3.50 dd 6.8, 10.2	109
3-O-β-gal (M/T5)	5.36 d 7.8	4.11 dd 7.8, 9.7	3.78 dd 9.7, 3.7	4.05 d 3.7	3.85 m	3.86 m	171
3-O-(6''-ac-gal) (M/T5)	5.34 d 7.7	4.11 dd 7.7, 9.6	3.78 dd 9.6, 3.4	4.04 dd 3.4, 1.0	4.15 ddd 1.0, 3.7, 8.3	3.86 m 4.43 dd 8.3, 11.8 4.37 dd 3.7, 11.8	171
3-[2''-(2'''-(E-caf)-glc)-6''-mal-gal]-7-[6'''-(E-caf)-glc]-3'-glu (D/T10)	5.49 d 7.6	4.24 m	3.69 m	3.74 br s	4.14 m	4.23 m	222
3'-(2''-gal-gal) (M/T5)	5.55 d 8.0	5.69 dd 8.0, 10.0	4.11 dd 10.0, 3.5	4.15 d 3.5	4.04 m	4.26 m 4.00 dd 11.4, 7.4 3.93 dd 11.4, 4.6	114
Glucuronides							
7-O-β-glu (D)	5.18 d 7.5	3.28 dd 8.5, 7.5	3.34 dd 9.4, 8.5	3.41 t 9.4	4.02 d 9.4	0.77 d 6.3	214
3-[2''-(2'''-(E-caf)-glc)-6''-mal-gal]-7-[6'''-(E-caf)-glc]-3'-glu (D/T10) (D/T10)	5.21 d 7.5	3.40 m	3.45 m	3.53 m	4.09 d 9.8	0.60 d 6.3	222
Rhamnosides							
8-C-(2''-(4'''-ac-α-rha)-β-glc) [†] (M)	5.39 d 1.8	3.91 m 3.80 dd	3.57 dd 3.3, 9.8	4.70 t 9.8	2.41 m 2.17 m	0.77 d 6.3	109
3-O-α-rha (M)	5.50 d 1.7	1.7, 3.3	3.10 dd 3.3, 9.8	4.63 t 9.8	3.64 dd 9.5, 6.2	1.08 d 6.2	152
3-O-α-(2''-ac-rha) (M)	5.44 d 1.5	4.35 dd 1.5, 3.3	3.92 dd 3.3, 9.5	3.47 t 9.5	3.48 dd 9.6, 6.2	1.05 d 6.3	152
5.49 d 1.5	5.53 dd 1.5, 3.4	3.97 dd 3.4, 9.5	3.37 m				

continued

TABLE 2.3
¹H NMR Spectral Data of Glycosyl Moieties of Flavonoid Glycosides Recorded in Various Solvents* — continued

Glycosyl Moiety	Chemical Shift (ppm)/Coupling Constants (Hz)						Ref.
	H-1	H-2	H-3	H-4	H-5	H-6	
3- <i>O</i> - α -(4''- <i>ae</i> - <i>rha</i>) (D)	5.20 d 1	4.04 dd 2, 5	3.75 dd 2, 10	4.73 t 10	3-3.7 m	0.72 d 7	208
7- <i>O</i> - α - <i>rha</i> (D)	5.64 br s	3.92 br s	3.64 m	3.27-3.30 m	3.43 m	1.21 d 6	209
3- <i>rha</i> (M/T5)	6.00	4.37	4.02	3.65	3.65	1.34	223
3-(6''- <i>rha</i> - <i>glc</i>)-5- <i>glc</i> (M/T5)	4.74 d 1.5	3.79 m	3.67 dd 3.5, 9.5	3.36 t 9.5	3.60 dd 9.5, 6.3	1.22 d 6.3	174
3- <i>O</i> -(6''- <i>rha</i> - <i>glc</i>) (M/T5)	4.75 d 1.6	3.89 dd 1.6, 3.1	3.71 m	3.41 m	3.65 m	1.25 d 6.3	168
Xylosides							
3- <i>O</i> - β - <i>xy</i> l (D)	5.31 d 7.4	3.34	3.14 t 8.7	3.31	2.91		210
					3.60		
3- <i>O</i> - α - <i>xy</i> l (D)	5.20	3.17	3.14	3.21	3.54		210
					2.89		
3'- <i>O</i> - β - <i>xy</i> l (M)	4.90 d 7.6	3.63 dd 7.6, 9.0	3.56 t 8.9	3.71 m	4.10 dd 11.4, 5.3		151
					3.48 dd 11.1, 10.5		
8- <i>C</i> -(2''- <i>glc</i> - β - <i>xy</i> l) (M)	5.02 d (10)	4.32	3.90	4.08	4.28		145
					3.88		
(2''- <i>xy</i> l)- <i>glc</i> (C + 1 dr D)	4.52 d 7.7	3.26 m	3.30 m	3.48 m	3.65 m		211
3-(2''- <i>xy</i> l)-6''- <i>mal</i> - <i>glc</i> (D/T10)	4.59 d 7.7	2.96 t 7.7	3.08 t 8.6	2.73 t 10.7	3.14 m		224
					3.22 dd 4.7, 10.7		
Arabinosides							
3'- <i>O</i> - α - <i>ara</i> -furanoside (A + D ₂ O)	4.12 s	3.78 br s	3.78 br s	4.20 m	3.62 br d 2.6		212
3- <i>O</i> - α - <i>ara</i> (P)	6.09 d 5.2	3.8-5.0 m	3.8-5.0 m	3.8-5.0 m	3.8-5.0 m		236
1- α - <i>ara</i> (D)	3.96 d 7.0	3.17 dd 8.6, 7.0	2.98 dd 8.6, 3.5	3.45 br s	3.52 dd 10.0, 4.0		213
					2.94 d 10.0		
3- <i>O</i> - α - <i>ara</i> (D)	5.22	3.73	3.48 dd 6.7, 3.1	3.62	3.18		210
					3.57		
3- <i>O</i> - α - <i>ara</i> -furanoside (D)	5.53	4.11	3.67	3.51	3.25		210
8- <i>C</i> - β - <i>ara</i> (D) (spectra recorded at 70°C)	4.81 d 9.5	4.04 br t	3.54 dd 9.0, 2.5	3.87 br s	3.93 dd 12.0, 1.5		47
					3.67 d 12.0		
Other sugars							
1- <i>O</i> - β -(6''- <i>ac</i> - <i>all</i>) (D)	4.93 d 8.0	3.27 dd 8.0, 2.6	3.93 t [†] 2.6	3.43 dd 10.0, 2.8	3.88 ddd 10.0, 4.8, 2.3	4.10 dd 12.0, 2.3	142
						4.04 dd 12.0, 4.8	
6- <i>C</i> - β -L- <i>bo</i> ivinoside (D)	5.33 dd 12.3, 3.1	1.50 ddd 4.3	3.86 ddd 3.4	3.25 ddd 4.3	4.04 q 6.0	1.17 d 6.0	216
6- <i>C</i> - β -D- <i>ol</i> ioside (D)	5.01 dd 11.7, 3.2	2.05 q 11.7	3.77 ddd 12.7,	3.46 d 2.4	3.65 q 6.4	1.18 d 6.4	217
		1.60 ddd 11.9,	5.2, 2.4				
		5.2, 3.2					
6- <i>C</i> - <i>ch</i> inovoside (D)	4.91, 9.4	4.28, 9.2	3.46, 9.1	3.22, 9.1	3.48, 6.2	1.36	218
6- <i>C</i> - <i>fu</i> coside (D)	4.92, 9.6	4.18, 9.4	3.64, 3.0	3.80 (small)	3.84, 6.4	1.34	218

Notes: all, allopyranose; ara, arabinopyranose; gal, galactopyranose; glc, glucopyranose; glu, gluconic acid; rha, rhamnopyranose; xyl, xylopyranose; ac, acetyl; caf, caffeeoyl; cou, *p*-coumaroyl; gall, galloyl; mal, malonyl; t, terminal.

*A, acetone-*d*6; C, CDCl₃; M, CD₃OD; M/T5, CD₃OD-CF₃COOD 95:5; D, DMSO-*d*6; D/T10, DMSO-*d*6-CF₃COOD 90:10; T, CF₃COOD; P, pyridine-*d*5.

[†]Rotamers: Top — chemical shift values of major rotamer. The parts in bold indicate the monosaccharide involved.

TABLE 2.4
¹³C NMR Spectral Data of Glycosyl Moieties of Flavonoid Glycosides Recorded in Various Solvents*

Glycosyl Moiety	Chemical Shift (ppm)						Ref.
	C-1	C-2	C-3	C-4	C-5	C-6	
Glucosides							
3- <i>O</i> -β-glc (D)	100.69	74.19	76.49	70.19	77.76	61.46	52
3-(6''-mal-glc) (M)	104.36	75.61	77.83	71.15	75.53	64.89	109
7- <i>O</i> -β-glc (D)	99.73	73.15	76.50	69.83	77.83	60.79	52
4'- <i>O</i> -β-glc (D)	101.56	73.55	76.49	69.88	77.19	61.04	52
4'- <i>O</i> -β-glc (M)	103.42	74.82	77.54	71.32	78.36	62.44	52
6- <i>C</i> -β-glc (D)	73.12	70.27	79.02	70.70	81.65	61.57	109
6- <i>C</i> -β-glc (M)	75.28	72.60	80.12	71.79	82.62	62.86	109
8- <i>C</i> -β-glc [†] (D)	73.45	70.89	78.70	70.25	81.74	61.27	109
	74.35	71.09	78.72	70.60	81.93	61.35	
8- <i>C</i> -β-glc [†] (M)	75.36	72.85	80.33	72.30	82.93	63.23	109
	76.28	72.68	79.84	71.29	82.62	63.51	
8- <i>C</i> -(2''-(4'''-ac-rha)-glc) [†] (M)	73.77	76.09	81.80	72.51	82.99	63.13	109
	75.30	76.29	81.45	71.50	83.00	62.45	
3- <i>O</i> -β-glc (M/T5)	103.72	74.79	78.14	71.08	78.77	62.36	170
3- <i>O</i> -(6''-mal-glc) (M/T5)	103.62	74.65	77.92	71.33	75.94	65.45	168
3-glc (M/T5)	105.59	75.39	77.57	71.44	78.91	62.80	176
3-(6''-rha-glc) (M/T5)	103.53	74.69	78.02	71.22	77.44	67.79	168
3-(6''-rha-glc)-5-glc (M/T5)	102.59	74.74	78.34	71.47	77.74	67.51	174
3-(6''-rha-glc)-5-glc (M/T5)	102.75	74.74	77.89	70.75	78.67	62.08	174
5- <i>O</i> -β-glc (M/T5)	102.7	74.6	78.1	71.2	78.6	62.6	170
5- <i>O</i> -(6''-ac-β-glc) (D/T10)	101.3	73.1	75.8	70.1	74.4	63.6	219
7- <i>O</i> -β-(3''-glc-6''-mal-glc) (M/T5)	95.3	74.9	87.3	71.2	75.0	65.4	170
3,4'-di- <i>O</i> -β-glc (M/T5)	102.3	74.5	77.8	71.1	78.3	62.4	170
Galactosides							
3- <i>O</i> -β-gal (D)	102.00	71.38	73.30	68.11	75.99	60.33	109
3- <i>O</i> -β-(6''-O-rha-gal) (M)	105.98	73.14	75.08	70.18	75.28	67.32	109
3- <i>O</i> -β-gal (M/T5)	104.63	72.16	74.87	70.14	77.80	62.35	171
3- <i>O</i> -(6''-ac-gal) (M/T5)	104.06	71.89	74.60	70.31	75.15	65.20	171
3'-(2''-gall-6''-ac-gal) (M/T5)	101.69	72.67	71.96	70.24	75.34	65.13	114
Glucuronide							
7- <i>O</i> -β-glu (M)	102.3	74.3	77.1	72.9	76.4	173.0	72
Rhamnosides							
3- <i>O</i> -α-rha (M)	103.64	71.90	72.15	73.37	72.07	17.67	152
3- <i>O</i> -α-(2''-ac-rha) (M)	100.36	73.39	70.49	73.50	72.11	17.70	152
8- <i>C</i> -(2''-(4'''-ac-rha)-glc) [†] (M)	101.15	72.09	70.02	75.16	67.27	17.84	109
	101.19	71.80			67.03	17.95	
3-rha (M/T5)	101.99	71.40	72.30	73.28	72.21	18.04	223
3-(6''-rha-glc) (M/T5)	102.19	71.87	72.44	73.92	69.77	17.87	168
3-(6''-rha-glc)-5-glc (M/T5)	102.21	71.93	72.28	73.86	69.77	17.94	174
Xylosides							
3- <i>O</i> -β-xyl (D)	102.5	74.3	77.0	70.2	66.9		210
3'- <i>O</i> -α-xyl (D)	97.5	69.5	71.3	65.1	61.7		210

continued

TABLE 2.4
¹³C NMR Spectral Data of Glycosyl Moieties of Flavonoid Glycosides Recorded in Various Solvents* — *continued*

Glycosyl Moiety	Chemical Shift (ppm)						Ref.
	C-1	C-2	C-3	C-4	C-5	C-6	
3'- <i>O</i> -β-xyl (M)	104.95	74.65	77.41	71.04	67.10		151
(2''-xyl-glc) (C + 1 drop D)	102.6	71.8	71.2	67.5	62.8		211
8- <i>C</i> -(2''-glc-β-xyl) (M)	76.04	81.34	77.04	70.17	72.10		145
Arabinosides							
3'- <i>O</i> -α-ara-furanoside (A + D ₂ O)	108.0	80.8	79.4	90.1	63.2		212
3- <i>O</i> -α-ara (P)	104.5	72.8	74.1	68.2	66.4		163
<i>t</i> -α-ara (D)	103.1	70.6	72.6	67.5	65.1		213
3- <i>O</i> -α-ara (D)	102.3	71.7	72.6	66.9	65.2		210
3- <i>O</i> -α-ara-furanoside (D)	108.6	83.0	77.6	86.8	61.6		210
Other sugars							
<i>t</i> - <i>O</i> -β-(6''-ac-all) (D)	102.5	71.4	70.7	66.8	71.5	63.5	142
6- <i>C</i> -β-boivinoside (M)	67.3	31.5	69.6	71.3	72.5	17.7	237
6- <i>C</i> -β-D-olioside (D)							217
6- <i>C</i> -chinovoside (D)							218
6- <i>C</i> -fucoside (D)	73.4	68.6	75.0	71.6	73.9	17.0	238

Notes: all, allopyranose; ara, arabinopyranose; gal, galactopyranose; glc, glucopyranose; glu, glucuronic acid; rha, rhamnopyranose; xyl, xylopyranose; ac, acetyl; mal, malonyl; t, terminal.

*A, acetone-*d*6; C, CDCl₃; M, CD₃OD; M/T5, CD₃OD-CF₃COOD 95:5; D, DMSO-*d*6; D/T10, DMSO-*d*6-CF₃COOD 90:10; T, CF₃COOD; P, pyridine-*d*5.

†Rotamers: Top — chemical shift values of major rotamer. The parts in bold indicate the monosaccharide involved.

2.3 MASS SPECTROMETRY

Modern mass spectrometric techniques are very well suited for the analysis of flavonoids isolated from plants and foodstuffs and in their *in vivo* metabolite forms (Table 2.8). Progress during the last two decades has made MS the most sensitive method for molecular analysis of flavonoids. MS has the potential to yield information on the exact molecular mass, as well as on the structure and quantity of compounds with the nature and within the mass range of flavonoids. Furthermore, due to the high power of mass separation, very good selectivities can also be obtained.

The purpose of the MS techniques is to detect charged molecular ions and fragments separated according to their molecular masses. Most flavonoid glycosides are polar, non-volatile, and often thermally labile. Conventional MS ionization methods like electron impact (EI) and chemical ionization (CI) have not been suitable for MS analyses of these compounds because they require the flavonoid to be in the gas phase for ionization. To increase volatility, derivatization of the flavonoids may be performed. However, derivatization often leads to difficulties with respect to interpretation of the fragmentation patterns. Analysis of flavonoid glycosides without derivatization became possible with the introduction of desorption ionization techniques. Field desorption, which was the first technique employed for the direct analysis of polar flavonoid glycosides, has provided molecular mass data and little structural information.²³⁹ The technique has, however, been described as “notorious for the transient

TABLE 2.5
Typical ^1H NMR Chemical Shift Values of Acyl Moieties of Acylated Flavonoid Glycosides Recorded in DMSO- d_6 (D) or CD $_3$ OD (M) with Various Proportions of CF $_3$ COOD (T) or Acetone (A)

	2	3	4	5	6	α	β	4-OH	Ref.
Acetyl (M/T10)	1.99 s								167
Malonyl (M/T10)	3.41 s								167
Succinyl (M/T2)	2.60 m	2.46 m							226
Malyl (M/T1)	2.80 dd 17, 10 4.40 dd 10, 2 2.50 dd 17, 2								227
4-OH-2-Methylethanoyl		2.34 t 6.5 4.54 d 2.4	3.45 q 6.5	6.06 s 5.44 s				4.4 t 5.4	188
Tartaryl (D/T10)	5.22 d 2.4								228
<i>p</i> -OH-Benzoyl (D/T10)	7.93 'd' 8.6				7.15 'd' 8.6				229
Galloyl (M/T5)	7.07 s				7.07 s				171
<i>E</i> -Cinnamoyl (A)	7.75 ddd 8.0, 1.5, 0.5	7.45 m	7.45 m	7.45 m	7.75 ddd 8.0, 1.5, 0.5	6.56 d 16.5	7.66 d 16.5		190
<i>E-p</i> -Coumaroyl (M/T17)	7.10 'd' 8.6	6.65 'd' 8.6		6.65 'd' 8.6	7.10 'd' 8.6	6.23 d 15.7	7.48 d 15.7		65
<i>Z-p</i> -Coumaroyl (D/T10)	7.09 'd' 8.6	6.31 'd' 8.6		6.31 'd' 8.6	7.09 'd' 8.6	6.37 d 13	5.72 d 13		173
<i>E</i> -Caffeoyl (M/T17)	6.95 d 2.0			7.12 d 8.6	6.71 dd 2.0, 8.6	5.94 d 15.7	7.18 d 15.7		65
<i>Z</i> -Caffeoyl (M/T5)	7.96 d 2.0			6.70 d 8.5	7.03 dd 2.0, 8.5	5.81 d 13	6.77 d 13		230
<i>E</i> -3,5-di-OH-Cinnamoyl (M/T17)	6.51 s		6.81 s		6.51 s	6.16 d 16.0	7.39 d 16.0		65
<i>E</i> -Feruloyl (D/T10)	7.08 d 2.0	OCH $_3$ 3.75 s		6.76 d 8.5	6.97 dd 2.0, 8.5	6.29 d 16.0	7.37 d 16.0		231
<i>E</i> -Sinapoyl (M/T5)	6.60 s	OCH $_3$ 3.80 s		OCH $_3$ 3.80 s	6.60 s	6.22 d 15.9	7.34 d 15.9		232

Notes: A, acetone- d_6 ; M, CD $_3$ OD; M/T1, CD $_3$ OD-CF $_3$ COOD 99:1; M/T5, CD $_3$ OD-CF $_3$ COOD 95:5; M/T10, CD $_3$ OD-CF $_3$ COOD 90:10; M/T17, CD $_3$ OD-CF $_3$ COOD 5:1; D, DMSO- d_6 ; D/T10, DMSO- d_6 -CF $_3$ COOD 90:10; T, CF $_3$ COOD.

TABLE 2.6
Typical ^{13}C NMR Chemical Shift Values of Acyl Moieties of Acylated Flavonoid Glycosides Recorded in DMSO- d_6 (D) or CD_3OD (M) with Various Proportions of CF_3COOD (T) or Acetone (A)

	1	2	3	4	5	6	α	β	C=O	Ref.
Acetyl (M/T10)	172.9	20.7								167
Malonyl (M/T10)	168.5	41.9	170.5							167
Oxalyl	169.95 ^a	168.53 ^a								233
Dioxalyl (M/T5)	158.1	172.4	172.4	174.8						234
Malyl (M/T1)	174.4	40.0	69.8	171.5						227
4-OH-2-Methylenebutanoyl	167.1	137.8	35.8	60.3	128					188
Tartaryl (D/T10)	171.7	74.2	69.7	167.9						228
<i>p</i> -OH-Benzoyl (D/T10)	120.4	131.3	115.3	162.0	115.3	131.3			165.3	235
Galloyl (M/T5)	120.83	110.48	146.33	140.14	146.33	110.48			168.11	171
<i>E</i> -Cinnamoyl (A)	135.7	129.2	129.9	131.0	129.9	129.2	119.6	145.1	166.3	190
<i>E-p</i> -Coumaroyl (M/T17)	125.0	130.1	115.8	159.8	115.8	130.1	115.5	144.8	166.2	65
<i>Z-p</i> -Coumaroyl (D/T10)	127.0	133.3	115.5	159.5	115.5	133.3	115.8	143.9	168.9	173
<i>E</i> -Caffeoyl (M/T17)	127.5	115.6	144.6	147.2	115.9	120.6	114.2	146.2	166.3	65
<i>E</i> -3,5-di-OH-Cinnamoyl (M/T17)	127.6	122.8	146.6	139.0	146.6	122.8	115.1	146.8	168.9	65
<i>E</i> -Feruloyl (D/T10)	125.6	111.7	148.0 (55.8)	149.5	115.7	123.1	114.1	145.6	166.7	231
<i>E</i> -Sinapoyl (M/T5)	125.80	106.51	148.92 (56.56)	139.44	148.92 (56.56)	106.51	115.00	146.94	168.42	232

Notes: A, acetone- d_6 ; M, CD_3OD ; M/T1, CD_3OD - CF_3COOD 99:1; M/T5, CD_3OD - CF_3COOD 95:5; M/T10, CD_3OD - CF_3COOD 90:10; M/T17, CD_3OD - CF_3COOD 5:1; D, DMSO- d_6 ; D/T10, DMSO- d_6 - CF_3COOD 90:10; T, CF_3COOD ; ^{13}C chemical shift values of OMe and Me groups are given in brackets.

nature of the spectra,²⁴⁰ and drawbacks related to the preparation of the MS samples have restricted application of this technique. Another method, desorption chemical ionization (DCI), provides rapid heating of the analyte and overcomes the problem of thermal decomposition inherent in conventional CI. The combined use of positive- and negative-ion DCI-MS has been shown to be an alternative approach for the structural characterization of flavonoid glycosides;²⁴¹ however, this method has been applied infrequently to flavonoid analysis in recent years.^{242,243} Plasma desorption mass spectrometry (PD-MS) is another MS method used for flavonoid analysis; however, its application has in recent years been limited to some papers on anthocyanins including deoxyanthocyanidins.²⁴⁴⁻²⁴⁶ Fast atom bombardment (FAB) MS is still popular for flavonoid analysis (Section 2.3.1.2). In this technique, the flavonoid is solubilized in a nonvolatile polar matrix and deposited on a copper target, which is bombarded with fast neutral energized particles such as xenon or argon and thereby inducing the desorption and ionization.

In parallel with these developments, other techniques have been introduced that were especially applicable to the combination of liquid chromatography with MS. The most interesting, from the point of view of structural studies of flavonoid glycosides, are thermospray (TSP) and atmospheric pressure ionization (API) methods, which include electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). TSP was the first

TABLE 2.7
Compounds Included in the NMR Tables. Individual Pigment Structures Are Shown in Figure 2.8–Figure 2.16

1. 6-OH-Luteolin 4'-methyl ether-7-(2''- α -rhamnoside-6'''-acetyl- β -glucoside)
2. 6-OH-Luteolin 7-(6''-(*E*)-caffeoyl)- β -glucoside
3. Isoscutellarein 7-(2''-(6'''-acetyl)- β -allosyl)- β -glucoside
4. Isoscutellarein 4'-methyl ether-7-(2''-(6'''-acetyl)- β -allosyl)- β -glucoside
5. Apigenin 4'-(2''-(2'''-feruloyl-glucuronyl)-glucuronide)
6. Apigenin 7-glucuronide-4'-(2''-(2'''-feruloyl-glucuronyl)-glucuronide)
7. Apigenin 7-glucuronyl-4'-(2''-(2'''-*E-p*-coumaroyl-glucuronyl)-glucuronide)
8. Luteolin 3'- β -glucoside-4'-(2''- α -rhamnosyl- β -glucoside)
9. Luteolin 3',4'-di- β -glucoside
10. 5,7,4'-tri-OH-3'-OMe-Flavone 8-C-(2''-*O*- β -glucosyl- β -xyloside)
11. 5,7-di-OH-3'-OMe-4'-Acetoxyflavone 8-C-(2''-*O*- β -glucosyl- β -xyloside)
12. Iso-orientin 3'-methyl ether
13. 8-*C-p*-OH-Benzoyl-isovitexin 4'-glucoside
14. Apigenin 8-C-(2''-(4'''-acetyl-rhamnosyl)-glucoside)
15. Spinosin
16. 6'''-Feruloyl-spinosin
17. Isoscoparin 7-glucoside
18. Carlinoside
19. Kaempferol 3-(6''- α -arabinosyl-glucoside)
20. Kaempferol 3-(6''- α -arabinosyl-glucoside)-7-glucoside
21. Kaempferol 3-(2''-rhamnosyl-6''-malonyl-glucoside)
22. Kaempferol 3-glucoside-7-(2''-(6'''-*p*-coumaroyl-glucosyl)-glucoside)
23. 8-OMe-Kaempferol 3-(6''-malonyl-glucoside)
24. Quercetin
25. Quercetin 4'-glucoside
26. Quercetin 3'-xyloside
27. Myricetin 3-(2''-acetyl-rhamnoside)
28. Quercetin 3,4'-diglucoside
29. Isorhamnetin 3-rutinoside
30. Quercetin 3,7,4'-triglucoside
31. Isorhamnetin 3,7-diglucoside
32. Myricetin 3-(2''-rhamnosyl-glucoside)
33. Myricetin 3'-(6''-*p*-coumaroyl-glucoside)
34. Myricetin 7-(6''-galloyl-glucoside)
35. Laricitrin 3- α -arabinofuranoside
36. Laricitrin 3-glucoside
37. Syringetin 3-(5''-glucosyl- α -arabinofuranoside)
38. Syringetin 3-(6''-acetyl-glucoside)
39. Syringetin 3-robinobioside
40. Syringetin 6-*C*-glucoside
41. 6,3'-di-OH-4,4'-di-OMe-5-Me-Aurone
42. 4,6,3',4'-tetra-OMe-Aurone (*Z*-form)
43. 4,6,3',4'-tetra-OMe-Aurone (*E*-form)
44. 6,3',4'-tri-OH-4-OMe-5-Me-Aurone
45. Maesopsin
46. Maesopsin 6-*O*-glucoside (two diastereoisomers)
47. Licoagroaurone

continued

TABLE 2.7
Compounds Included in the NMR Tables. Individual Pigment Structures Are Shown in Figure 2.8–Figure 2.16 — continued

48. 3'-formyl-4',6'-di-OH-2'-OMe-5-Me-Chalcone
49. Chalcononaringenin 2',4'-diglucoside
50. 2',4'-diOH-4'-OMe-6'-glucoside Dihydrochalcone
51. 2'-OH-3',4',6'-tri-OMe-Dihydrochalcone
52. Pelargonidin 3-glucoside-5-(6'''-acetyl-glucoside)
53. Pelargonidin 3-(6''-feruloyl-glucoside)-5-(6'''-malonyl-glucoside)
54. Cyanidin 3-(6''-malonyl-glucoside)
55. Cyanidin 3-rutinoside
56. Cyanidin 3-(2'',3''-digalloyl-glucoside)
57. Cyanidin 3,4'-diglucoside
58. Delphinidin 3-(6''-acetyl-galactoside)
59. Delphinidin 3'-(2''-galloyl-6''-acetyl-galactoside)
60. Peonidin 3-rutinoside
61. Petunidin 3,7-diglucoside
62. Petunidin 3-(6''-E-p-coumaroyl-glucoside)-5-(6'''-malonyl-glucoside)
63. Malvidin 3-(6''-E-p-coumaroyl-glucoside)-5-glucoside
64. Malvidin 3-(6''-Z-p-coumaroyl-glucoside)-5-glucoside
65. Malvidin 3-rutinoside-5-glucoside
66. Malvidin 3-(6''-(4'''-malonyl-rhamnosyl)-glucoside)-5-(6'''-malonyl-glucoside)
67. Apigeninidin 5-glucoside
68. Luteolinidin 5-glucoside
69. Carboxypyranopelargonidin 3-glucoside
70. Carboxypyranocyanidin 3-glucoside
71. Carboxypyranocyanidin 3-(6''-malonyl-glucoside)
72. Carboxypyranomalvidin 3-glucoside
73. Judaicin 7-(6''-acetyl-glucoside)
74. Tectorigenin 4'-(6''-glucosyl-glucoside)
75. 7-OH-6'-OMe-3',4'-methylenedioxyisoflavone 7-glucoside
76. Irisjaponin A
77. Irisjaponin B
78. Junipegenin B
79. Mattheucinol 7-(6''-apiofuranosyl-β-glucoside)
80. Hesperitin 7-(2''-galactosyl-6''-rhamnosyl-glucoside)
81. Persicogenin 5,3'-di-OH-7,4'-di-OMe-flavanone
82. Naringenin 7-glucoside
83. Naringenin 7-(6''-galloyl-glucoside)
84. Taxifolin 4'-glucoside
85. Aromadendrin 7-glucoside
86. Ampelopsin 7-glucoside
87. 2''-Accallunin
88. 2R,3R-trans-aromadendrin 7-(6''-(4'''-OH-2'''-methylenebutanoyl)-glucoside)
89. (2R,3S)-(+)-3',5-di-OH-4',7-di-OMe-Dihydroflavonol
90. 3-Desoxycallunin
91. Catechin 3-(6''-cinnamoyl-glucoside)
92. Catechin 3-(2''-cinnamoyl-glucoside)
93. Catechin 3-(2'',6''-dicinnamoyl-glucoside)
94. Anadanthoside

TABLE 2.7
Compounds Included in the NMR Tables. Individual Pigment Structures Are Shown in Figure 2.8–Figure 2.16 — continued

95. Cajanin
96. Indicanine C
97. 6-(1,1-di-Me-allyl)-7,4'-di-OH-Flavan
98. 3-(4'-hydroxyphenyl)-5-methoxy-6-(3,3-dimethylallyl)-2'',2''-dimethylchromene-(5'',6'':8,7)-3-(propyl-2-one)-4*H*-1-benzo-2,3-Dihydropyran-2,4-dione
99. Maackianin 3-(6''-malonyl-glucoside)
100. 3,4:8,9-Dimethylenedioxy-pterocarpan
101. Usararotenoid C
102. 12a-Epimillettosin
103. (+)-Usararotenoid-B
104. [Catechin 3-glucoside-(4 α \rightarrow 8)-catechin 3-(2''-cinnamoyl-glucoside)]
105. [Catechin 3-glucoside-(4 α \rightarrow 8)-epicatechin 3-(6''-cinnamoyl-glucoside)]
106. Amentoflavone
107. Aulacomnium-biaureusidin
108. Cupressuflavone 7,7''-dimethyl ether
109. 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2 \rightarrow 7)-2,4,4',6-tetra-*O*-Methylmaesopsin
110. Catechin-(4 α \rightarrow 8)-pelargonidin 3-glucoside
111. 2',2'',2'''-tri-OH-4',4'''-di-OMe-4-*O*-5'''-bichalcone (Rhuschalcone 1)
112. Puerarin (Daidzein 8-*C*-glucoside)
113. Calycosin
114. Isoneorautenol
115. Erybraedin A

method to combine true LC–MS compatibility with the ability to determine nonvolatile thermally labile compounds. The method has, for instance, enabled the analysis of mixtures of polar flavonoid glycosides,^{247,248} and allowed the detection of monomeric flavan-3-ols and dimeric proanthocyanidins.²⁴⁹ However, the application of this technique has some limitations related to the thermal stability of the compounds studied. This is connected to the high temperature in the TSP ion source, which is necessary for the efficient ionization of the molecules to be analyzed. In addition, the efficiency of ion production varies widely with compound type, and the flow rate and temperature of the inlet tube must be optimized for each type of compound. LC–TSP–MS has provided for the characterization of catechins and flavonoids from their CID spectra of the quasimolecular ion.²⁵⁰ In this study, flavonoids exhibited three types of ring cleavage in the pyran ring, and the differentiation among flavanone, flavone, and flavonol was possible. LC–TSP–MS has also been applied for the detection and identification of a wide range of other flavonoids (see Section 1.4.5). The technique has, however, been gradually phased out by ESI and APCI, which in recent years seem to be the most useful ionization techniques for the characterization of flavonoids (see Sections 1.4.5, 2.3.1.4, 2.3.2.2, 2.3.3, and 2.3.4). These also include matrix-assisted laser desorption ionization (MALDI) MS coupled with a time-of flight (TOF) mass analyzer, which is another soft ionization technique that allows the analysis of small quantities of flavonoids (see Sections 2.3.1.3, 2.3.3, and 2.3.4). The major advantages of most of the soft ionization techniques are that they include those of minute sample sizes, and the possibility of coupling MS with different chromatographic techniques, e.g., gas chromatography (GC–MS), capillary electrophoresis (CE–MS), and, in particular, liquid chromatography (LC–MS) (see Sections 1.4.5 and 2.3.2.2).

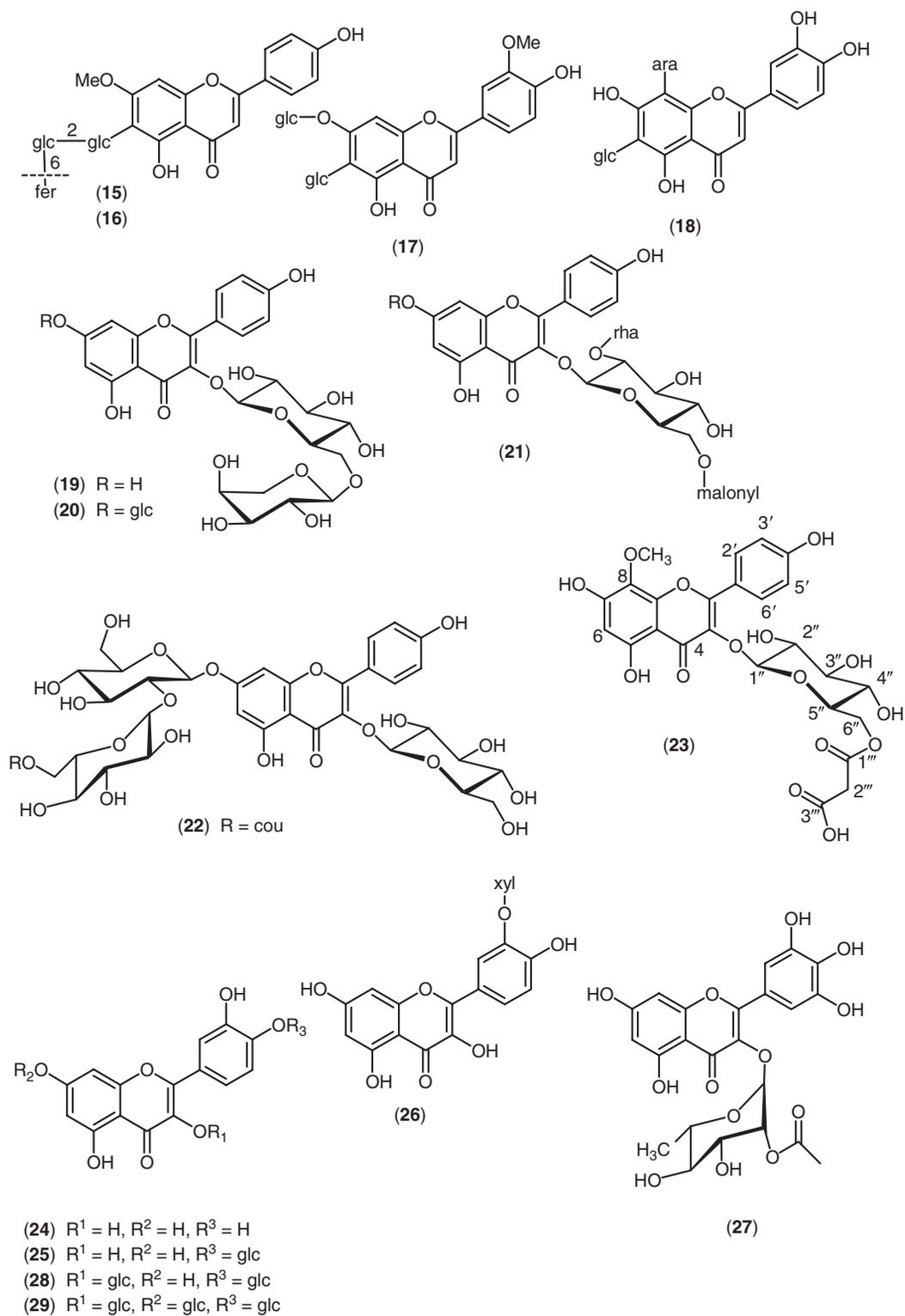


FIGURE 2.9 Structures of compounds 15–29. See Table 2.7 for names.

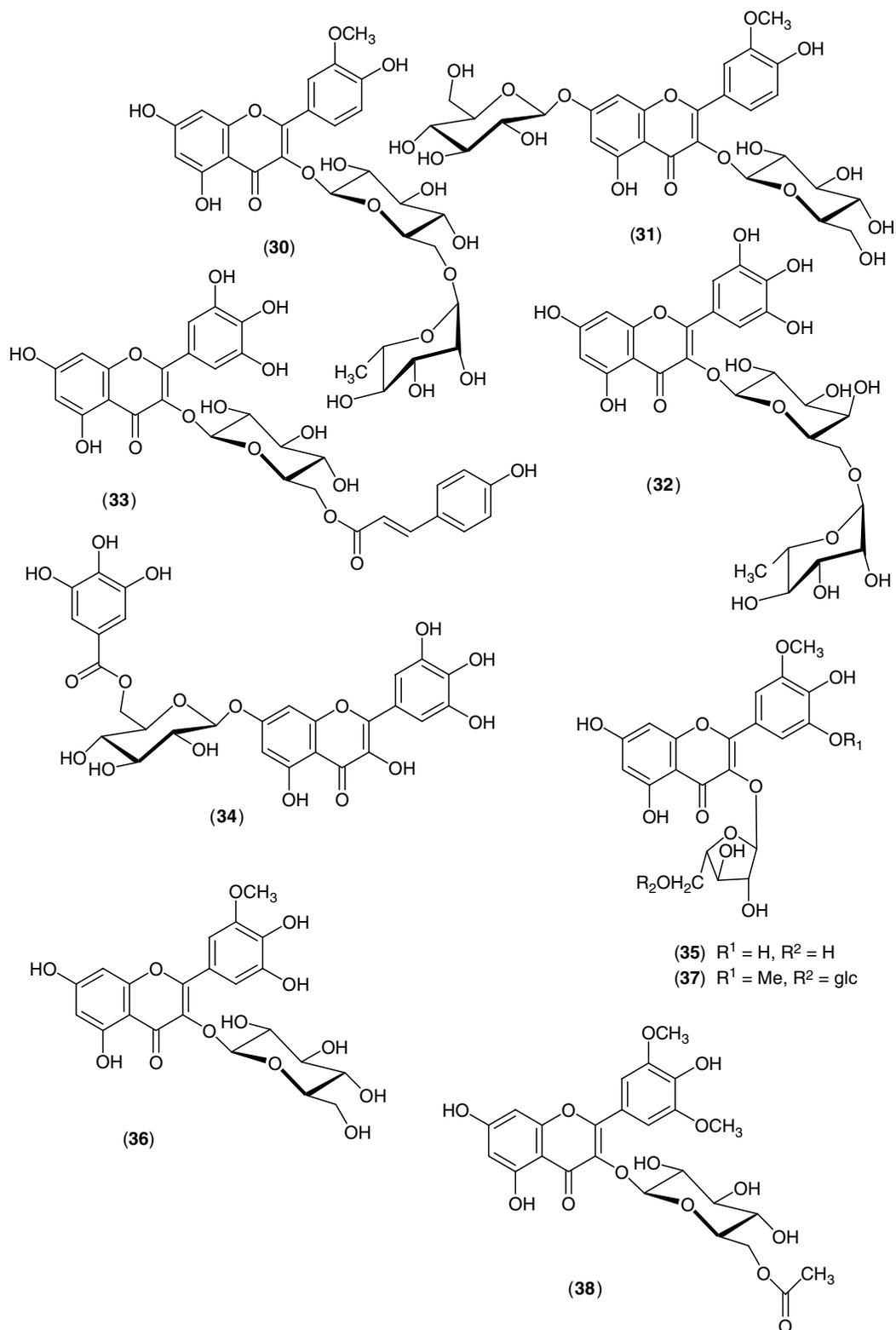


FIGURE 2.10 Structures of compounds 30–38. See Table 2.7 for names.

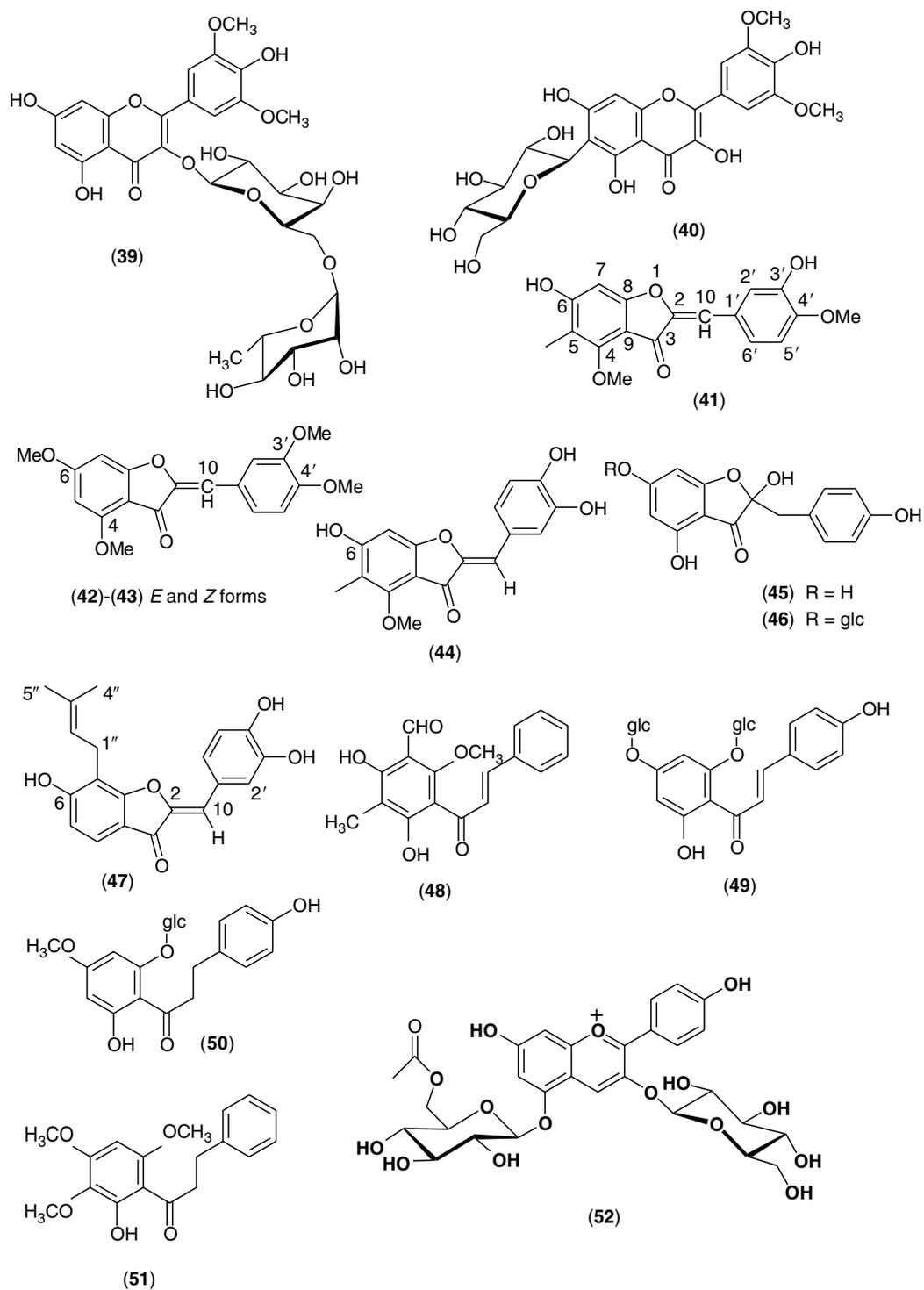


FIGURE 2.11 Structures of compounds 39–52. See Table 2.7 for names.

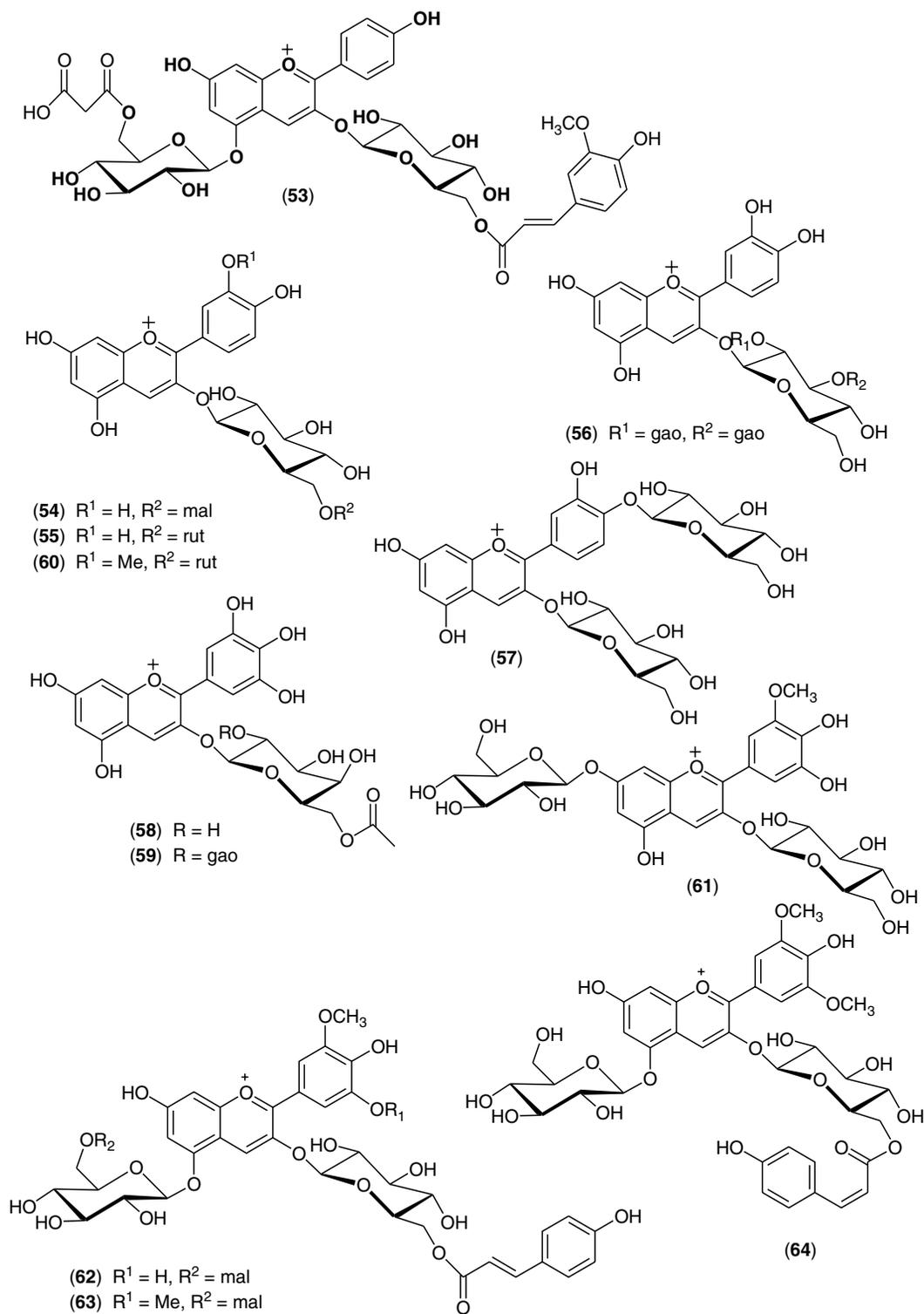


FIGURE 2.12 Structures of compounds 53–64. See Table 2.7 for names.

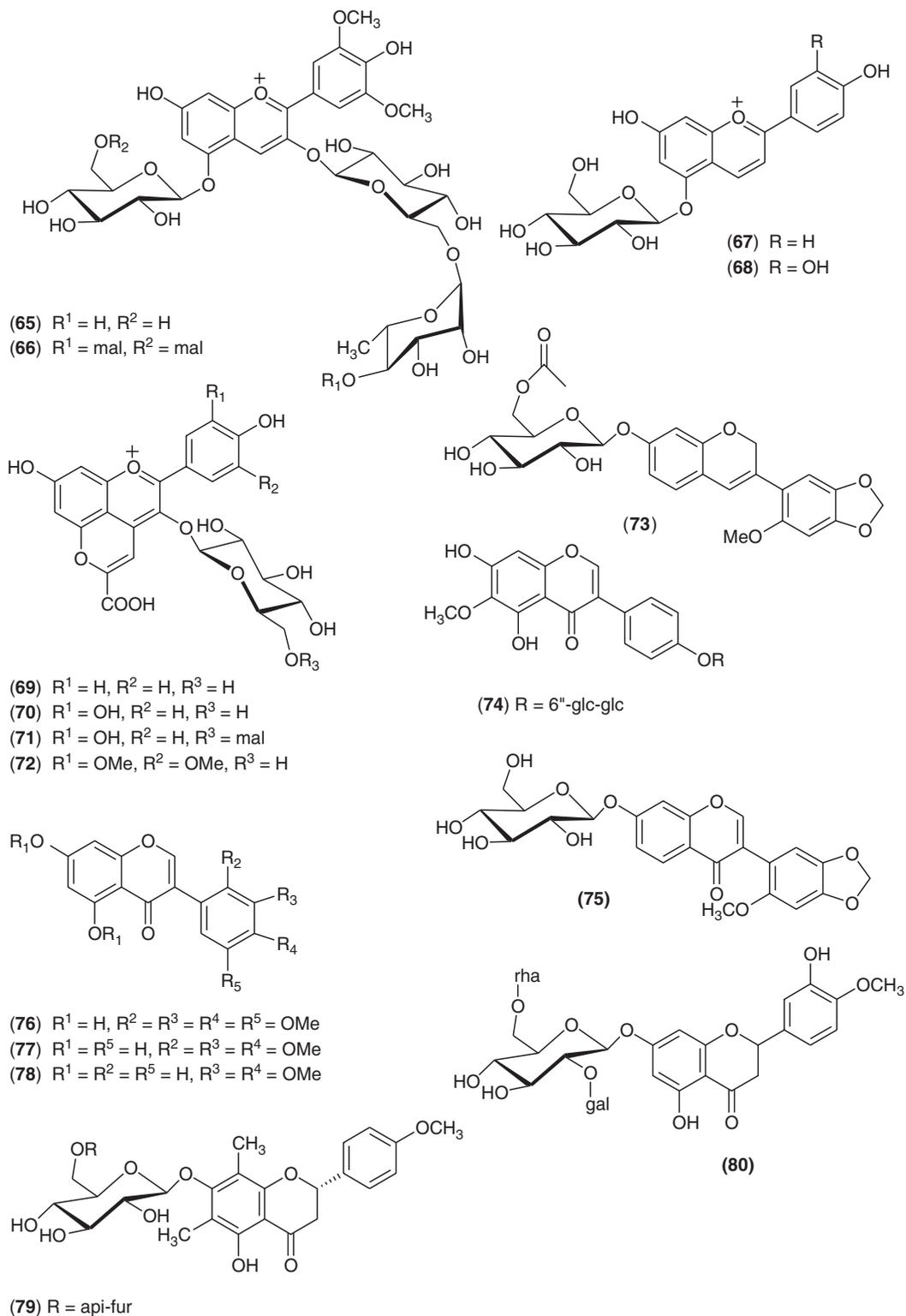


FIGURE 2.13 Structures of compounds 65–80. See Table 2.7 for names.

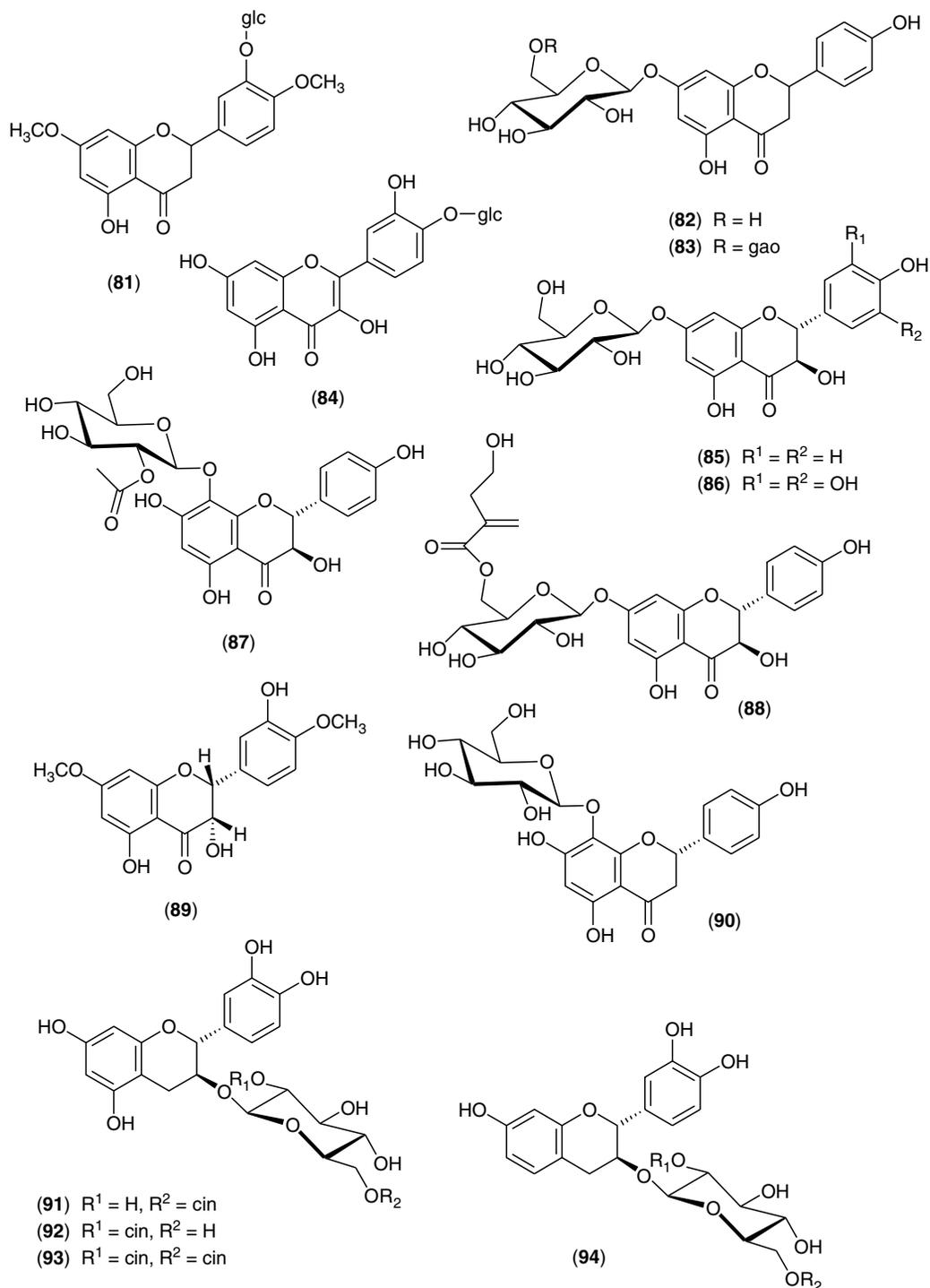


FIGURE 2.14 Structures of compounds 81–94. See Table 2.7 for names.

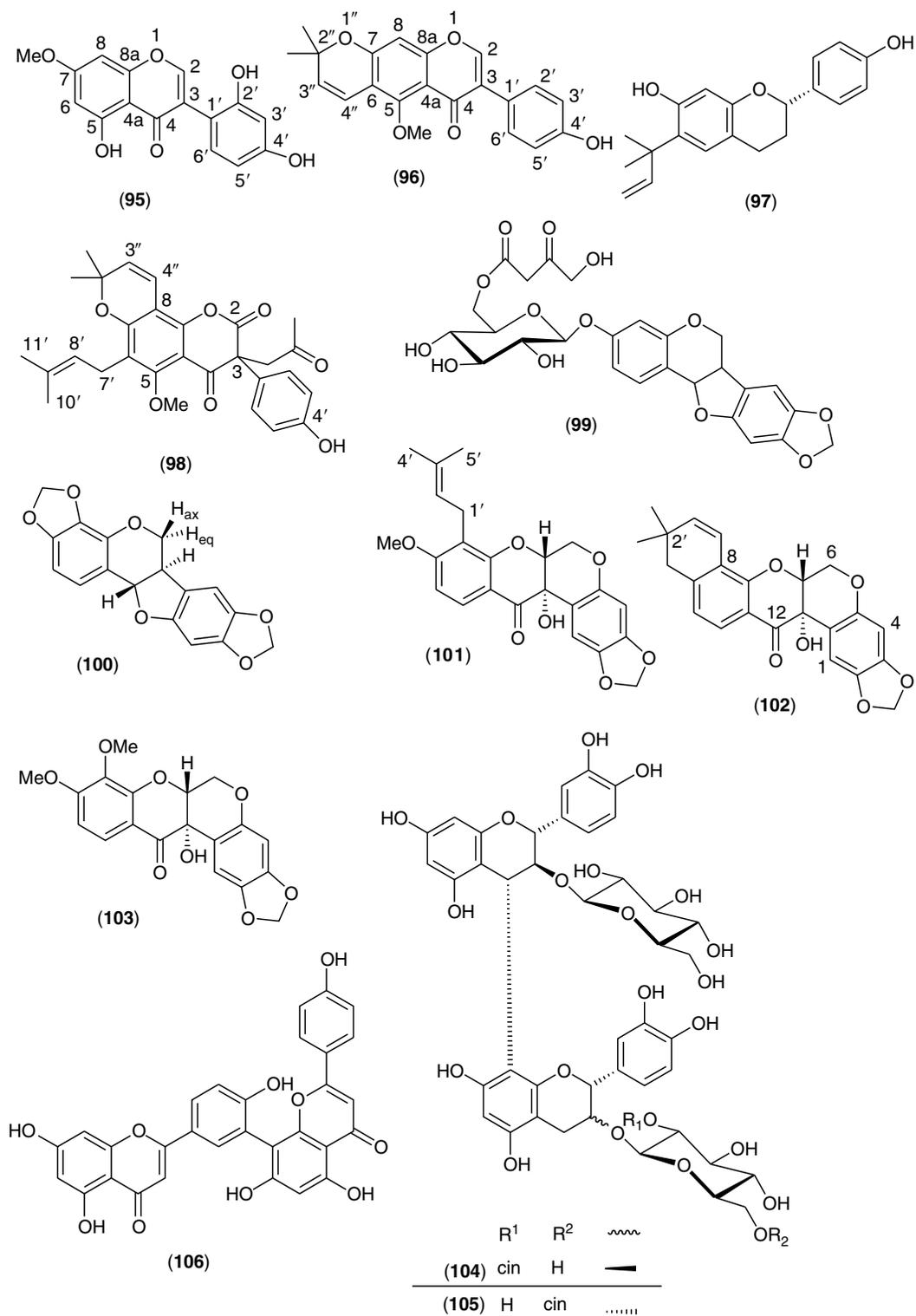


FIGURE 2.15 Structures of compounds 95–106. See Table 2.7 for names.

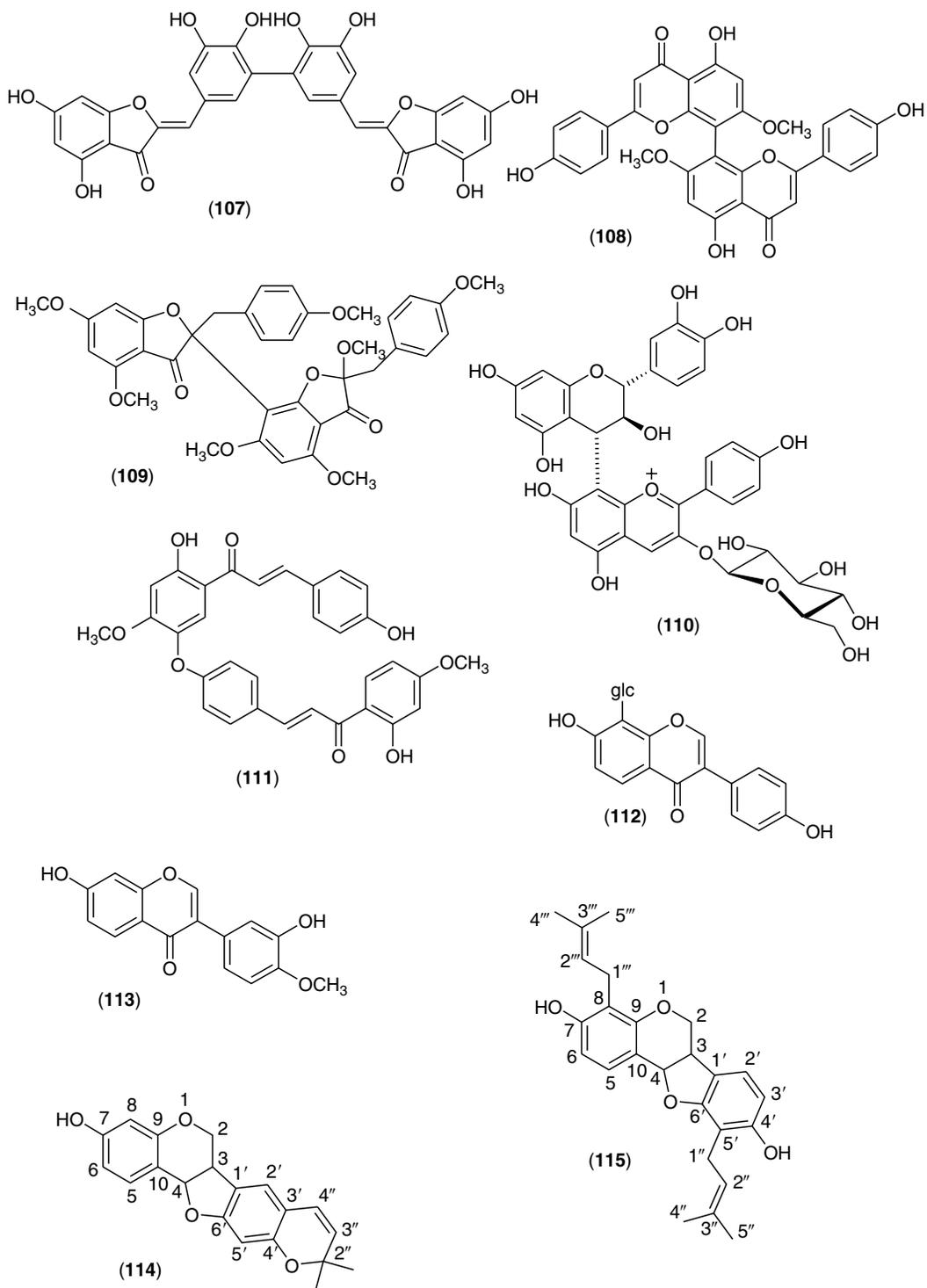


FIGURE 2.16 Structures of compounds 107–115. See Table 2.7 for names.

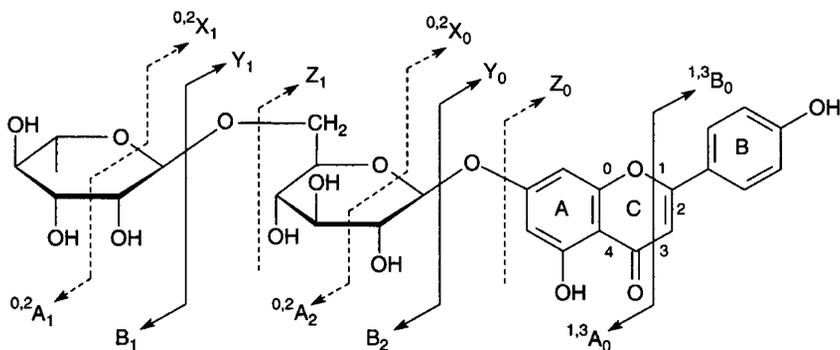


FIGURE 2.17 Ion nomenclature used for flavonoid glycosides (illustrated for apigenin 7-*O*-rutinoside). (Reprinted from Cuyckens, F. and Claeys, M., *J. Mass Spectrom.*, 39, 1, 2004. Copyright 2004 John Wiley & Sons, Ltd. With permission.)

In addition to giving accurate molecular masses of molecular ions, fragmentation patterns revealed by some MS methods may provide (a) structural information about the nature of the aglycone and substituents (sugars, acyl groups, etc.), (b) interglycosidic linkages and aglycone substitution positions, and (c) even some stereochemical information (Figure 2.17). The amount of structural information obtained for flavonoids from a mass spectrum depends on the ionization method used. The highest energy transfer occurs during EI of flavonoid aglycones, and in these cases fragmentation of molecular ions is normally seen. When soft ionization methods like ESI and APCI are applied on flavonoids in LC-MS and CE-MS systems, fragmentation of the flavonoids is not commonly seen in the spectra. However, the use of collision-induced dissociation tandem mass spectrometry (CID-MS-MS) allows detection of fragment ions.²⁵¹ During CID-MS-MS analysis precursor ions extracted in the first analyzer collide with atoms of an inert gas in the collision cell. The ionized fragments created are separated in the second analyzer. In another recent achievement, the fragment ions created can be further studied using an additional MS^{*n*} stage in a multistage tandem MS instrument with an ion trap (IT) analyzer (see Section 2.3.1.5).

At present, MS spectra alone rarely provide sufficient information for complete structural elucidation of novel complex flavonoids. Normally, MS techniques give little information about the configuration of the glycosidic linkage, and are reckoned not to be capable of distinguishing between diastereomeric sugar units. However, both FAB and ESI in combination with CID have recently been used for direct stereochemical assignment of hexose and pentose residues in acetylated flavonoid *O*-glycosides. The differentiation between a glucose residue and a galactose residue was, for instance, made by employing the $[m/z\ 127]/[m/z\ 109]$ peak intensity ratios and the relative abundance of a fragment ion at $m/z\ 271$.²⁵² Combined with data obtained by other spectroscopic techniques (especially NMR and UV), MS has proved to be an invaluable tool for the identification of novel flavonoids.

Exact mass measurement at high resolution is an important tool along with other spectroscopic methods to help confirm the structure of novel flavonoids. It is used as structural proof when elemental analysis is not possible, e.g., when studying minor components. When EI-MS can be used, 1 to 10 pmol samples are required for one measurement; however, when FAB-MS is used, 0.1 to 1 nmol is normally required. The use of ESI on a double focusing mass spectrometers and MALDI-TOF-MS requires smaller amounts of sample, and subpicomole amounts of flavonoids may be adequate.

MS techniques applied to flavonoids have been covered by several recent reviews. An excellent paper by Cuyckens and Claeys²⁵³ covers sample preparation, LC-MS analysis, and

tandem mass spectrometric procedures for the characterization of flavonoid aglycones, *O*-glycosides, *C*-glycosides, and acylated glycosides in a tutorial manner. Various aglycone fragmentation patterns obtained under EI conditions can be designated according to the nomenclature proposed by Ma et al.²⁵⁴ Similarly, ions formed from flavonoid glycosides can be denoted according to the nomenclature introduced by Domon and Costello (Figure 2.17).²⁵⁵ The different mass spectrometric techniques applied for the analysis of flavonoid glycosides have been reviewed by Stobiecki.²⁵⁶ The MS analysis of anthocyanidins and derived pigments, catechins and proanthocyanidins, and flavonols and flavonol glucuronide conjugates has recently been illustrated with case studies.²⁵⁷ Mass spectrometric methods for the determination of flavonoids in biological samples have been reviewed by Prasain et al.,²⁵⁸ and excellent reviews on tandem mass spectral approaches to the structural characterization of flavonoids have recently been reported.^{259,260} Several general reviews covering studies on flavonoids have included MS analysis applied to flavonoids,^{19,139,261,262} and important applications of the various MS techniques are covered in other chapters of this book.

The consecutive introduction and success of FAB, MALDI, APCI, ESI, new mass analyzers, as well as exciting coupled MS techniques, which progressively have drawn the main attention of mass spectroscopists for analysis of flavonoids in recent years, are described in more detail in the following sections. Some representative applications of these techniques for flavonoid analysis are presented in Table 2.9–Table 2.11.

2.3.1 MS INSTRUMENTATION AND TECHNIQUES

The three major parts of a mass spectrometer are the device for the introduction of the sample into the ionization chamber where the ionization takes place, the mass analyzer where the ions are separated according to their mass-to-charge ratios, and the detector, which can measure the quantity of negative or positive ions. The ionization methods used for flavonoid analysis can be classified as gas-phase methods including EI and CI, desorption methods including FAB and MALDI, and spray methods including ESI and APCI (Table 2.8). The different types of mass analyzers are double focusing magnetic sectors instruments, quadrupole mass filters (Q), quadrupole ion traps (Q-IT), TOF analyzers, and Fourier-transform ion-cyclotron resonance (FT-ICR).

2.3.1.1 Electron Impact and Chemical Ionization

The first conventional mode of MS involves EI ionization, in which the neutral flavonoid is impacted in the gas phase with an electron beam of 70 to 100 eV. Resulting mass spectra of the flavonoid aglycones are characterized by molecular ion peaks (M^+), and fragment ions from both the A and B rings. The use of a reactant gas in the ionization chamber, CI, normally results in the production of a more abundant molecular ion and simpler fragmentation patterns. General information about mass spectra of flavonoids recorded by these methods has been published by several authors.^{2,15,256,263} More specific mass spectra analyses of 39 polymethoxylated flavones have been obtained by GC–MS.²⁶⁴ In addition to the common fragmentation behavior of flavones under EI, such as retro-Diels–Alder reactions, which give characteristic fragments from the phenyl group of the flavone skeleton, new fragmentation pathways have been identified and proposed.²⁶⁴

EI and CI are normally unsuitable for most flavonoid glycosides because of their polarity and thermolability. Preparation of permethylated or trimethylsilylated derivatives may overcome this problem. However, derivatization often produces mixtures of partially derivatized compounds and may involve rearrangements,^{256,265} and usually only weak molecular ion signals is observed when permethylated compounds are studied under EI conditions.

TABLE 2.8
Common Mass Spectrometry (MS) Methods Used in Recent Years for Flavonoid Analyses

Ionization Source/ Sample Introduction	Means of Ionization	Particularities	Commonly Associated Mass Analyzers
Fast atom bombardment (FAB) Direct insertion probe or LC-MS	The flavonoid is dissolved in a liquid matrix. The sample is bombarded with a fast atom beam that desorbs molecular ions and fragments from the analyte. The spectrum often contains peaks from the matrix	Mass range up to 7000 Da. Exact mass measurements are usually done by peak matching. The accuracy of the mass is the same as obtained in EI, CI. Relatively low sensitivity. Molecular ions often absent	Quadrupole
Matrix assisted laser desorption ionization (MALDI) Direct insertion probe or continuous-flow introduction. Not easily compatible with LC-MS	The flavonoid is dissolved in a solution containing an excess of a matrix that has a chromophore that absorbs at the laser wavelength. The matrix absorbs the energy from the laser pulse and produces plasma that results in vaporization and ionization of the analyte. Some structural information can be obtained in a "postsource decay" mode, or by collisional activation	High mass range. Sample amount very low (picomoles or less). Mass accuracy (0.1 to 0.01%) is normally not as high as for other mass spectrometry methods. Recent developments in delayed extraction	Requires a mass analyzer that is compatible with pulsed ionization techniques Time of flight Fourier-transformed ion-cyclotron resonance
Electrospray ionization (ESI) Flow injection or LC-MS or CE-MS	The sample must be soluble, polar, and relatively clean. The sample solution is sprayed from a needle held at high voltage to form charged liquid droplets from which ions are desolvated. Multiply charged ions are usually produced	TOF allow higher resolving power and mass accuracy. The analysis is relatively insensitive to contaminants. MS-MS difficult	Quadrupole Ion trap Time of flight Fourier-transformed ion-cyclotron resonance
Atmospheric pressure chemical ionization (APCI) Flow injection or LC-MS or CE-MS	Similar interface to that used for ESI. In APCI, a corona discharge is used to ionize the analyte in the atmospheric pressure region. Ions are formed by charge transfer from the solvent as the solution passes through a heated nebulizer into the APCI source	High mass range. Best method for analyzing multiply charged compounds. Very low chemical background leads to excellent detection limits (femtomole to picomole). Can control presence or absence of fragmentation by controlling the interface lens potentials. Compatible with MS-MS methods	Quadrupole Ion trap Time of flight

Notes: MS techniques are usually designated by the ionization source producing the ions (e.g., FAB, MALDI, ESI, APCI) and by the mass analyzer (e.g., TOF, IT) used to sort them according to their m/z values (e.g., MALDI-TOF, ESI-TOF, ESI-IT). Two analyzers are used in series in tandem MS (MS-MS) techniques (e.g., ESI-Q-TOF).

2.3.1.2 Fast Atom Bombardment

FAB-MS (Table 2.8) has been widely used for the characterization of flavonoids solubilized in a variety of matrices, and normally involve the use of xenon or argon atoms for bombardment (Table 2.9). The matrix signals may complicate interpretation of the spectra. Nevertheless, when combined with CID of positive ions and tandem mass spectrometric techniques, FAB-MS can provide information on the aglycone moiety, the carbohydrate sequence, and

TABLE 2.9
Selected Papers in Mass Spectrometry Applied to Flavonoid Analysis with Fast Atom Bombardment Ionization

Analytes	Sample	Ionization Mode	Matrix	Ref.
Anthocyanins	Pure compounds	FAB (+)	a	273
Anthocyanins	Pure compounds	FAB (+)	<i>m</i> -Nitrobenzyl alcohol	84
Anthocyanins	<i>Malva silvestris</i> , purified extract	FAB (+)	Glycerol	274
Anthocyanin–flavanol dimers	Pure compounds	FAB (+)		275
Pyrananthocyanins	Pure compounds	FAB (+)	Glycerol	276
Anthocyanins, flavanols	Red wine	FAB (+)	Glycerol	277
Flavonols, proanthocyanindins				
Chalcones, flavanones,				
Flavonols	Pure compounds	FAB (+)	Glycerol	163
Flavanones	Pure compounds	FAB (+)	1 <i>N</i> HCl–glycerol	278
Flavones	Pure compounds	FAB (–)	Triethanolamine	279
Flavones	Pure compounds	FAB (+)	Nitrobenzyl alcohol	280
Flavone <i>C</i> -glycosides	Pure compounds	FAB (+)	1 <i>N</i> HCl–glycerol	281
Flavone <i>C</i> -glycosides	Pure compounds	FAB (+)	Glycerol and <i>m</i> -nitrobenzyl alcohol	145
Flavone <i>C</i> -glycosides	Pure compounds	FAB (+)	Glycerol	282
Flavone <i>O</i> -glycoside,				
Flavonols	Pure compounds	FAB (+)	Glycerol	252
Flavones, flavonols	Pure compounds	FAB (+)	Glycerol	254
Flavonols	Pure compounds	FAB (–)	2-Hydroxyethyl disulfide	283
Flavonols	Pure compound	FAB (+)	Nitrobenzene	284
Flavonols	Alkaline-earth metal complexes	FAB (±)	<i>m</i> -Nitrobenzyl alcohol	285
Flavonols	Pure compounds	FAB (+)	<i>m</i> -Nitrobenzyl alcohol	286
Flavonols	Pure compounds	FAB (+)	Lactic acid	287
Flavonols	Pure compounds	FAB (+)	Thioglycerol + NaI	288
Flavonols	Pure compounds	FAB (+)	Lactic acid	289
Flavonol sulfates	Pure compounds	FAB (+)		290
Flavonoids	Pure compounds	FAB (–)	Glycerol–thioglycerol	291
Flavonoids	Pure compounds	FAB (+)	<i>m</i> -Nitrobenzyl alcohol	292
Isoflavones	Pure compounds	FAB (+)	<i>m</i> -Nitrobenzyl alcohol	293
Isoflavone phosphates	Pure compounds	FAB (–)	<i>p</i> -Nitrobenzyl alcohol	294
Isoflavonoids, triflavonoids	Pure compounds	FAB (+)		295
Proanthocyanidins	Rat metabolites	FAB (–)		296

^aDissolved in methanol and formic acid with subsequent addition of a 1:1 mixture of dithioerythritol and dithiothreitol.

the glycosylation position of glycosides,^{266–269} and even stereochemical assignment of hexose and pentose residues in flavonoid *O*-glycosides.²⁵² An MS method based on the combined use of FAB and CID tandem MS has been used for the analysis of the fragmentation behavior of protonated 3-methoxyflavones.²⁶⁸ It was shown that several diagnostic ions allowed unambiguous localization of the functional groups in the A and B rings, and isomeric 3-methoxyflavones could be differentiated using this methodology. The principles of the technique together with the processes involved in ion formation, the effect of the liquid matrix, and the optimization of FAB conditions for flavonoids have been described in several studies.^{270–272} The value of FAB-MS in flavonoid analysis is demonstrated by some recent applications using both positive- and negative-ion modes (Table 2.9).

2.3.1.3 Matrix-Assisted Laser Desorption Ionization

MALDI-MS is considered a sensitive and powerful tool for the analysis of nonvolatile molecules (Table 2.8). It has greatly expanded the use of MS toward large molecules, and has revealed itself to be a powerful method for the characterization of both monomeric flavonoids as well as proanthocyanidins (Table 2.10). With this technique, fragmentation of the analyte molecules upon laser irradiation can be substantially reduced by embedding them in a light-absorbing matrix. As a result, intact analyte molecules are desorbed and ionized along with the matrix, and can be analyzed in a mass spectrometer. This soft ionization technique is mostly combined with TOF mass analyzers. A crucial factor that influences the quality of MALDI-TOF mass spectra is the crystallization of the analyte during sample preparation and the behavior of the matrix during laser irradiation. MALDI-MS can measure the mass of almost any molecule (masses up to 10^6 Da). The analysis can be performed in the linear or reflectron mode. Mass accuracy (0.1 to 0.01%) is not as high as for other MS methods; however, the analysis is relatively insensitive to contaminants. The amount of sample needed is very low (picomoles or less), and may involve only 1 to 2 μl of sample solution.²⁹⁷ The technique is fast to handle, often taking less than a minute for the actual analysis after sample preparation. The use of the MALDI technique has helped in obtaining vital information in many recent flavonoid analyses (Table 2.10).

Recent developments in delayed extraction TOF allow higher resolving power and mass accuracy, and this method in the reflector mode has been used for accurate measurement of the mass of several compounds including two prenylated flavonoids.²⁹⁸ However, the performance of the MALDI-TOF instrument was not better than those of the FAB and FT-ICR MS instruments, and insufficient to give acceptable accuracy for literature reporting.

The MALDI-MS technique has been extended with the so-called postsource decay method (PSD) by Spengler and coworkers.²⁹⁹ This technique, which allows the determination of the fragment ions formed from the decomposition of the precursor ions of high internal energy, has been used to study the fragmentation and the fragmentation mechanisms of the flavonol glycoside rutin cationized with different alkali metal ions.³⁰⁰ The technique permits the selection of a precursor molecule in a distinct mass window and the subsequent analysis of its fragments. The precursor ions passing the mass window can spontaneously fragment on their way to the detector due to the application of higher laser energies. All ions with lower and higher masses are deflected by the electrostatic device. The PSD-MALDI mass spectra of the cationized rutin molecules showed, depending on the cation, different fragmentation patterns with respect to both quality and quantity of the fragment ions formed.³⁰⁰ For a more specific sequential elucidation of individual proanthocyanidin chains, MALDI-TOF-MS has been extended to PSD fragmentation.³⁰¹ Recently, Keki et al.³⁰² have used PSD-MALDI MS-MS to deal with the fragmentation and the fragmentation mechanisms of paracetylated isoflavone glycosides cationized with proton and various metal ions. In a very

TABLE 2.10
Papers on Matrix-Assisted Laser Desorption Ionization Mass Spectrometry with Time of Flight Mass Analyzer Applied to Flavonoid Analysis

Analyte	Sample	Ionization Mode	Matrix	Ref.
Proanthocyanidins	American cranberry (<i>Vaccinium macrocarpon</i>) fruit	(+)	DHB	304
Proanthocyanidins	Mimosa (<i>Acacia mearnsii</i>) bark tannin, Quebracho (<i>Schinopsis balansae</i>) wood tannin	Linear (+)	DHB	305
Proanthocyanidins	American cranberry (<i>Vaccinium macrocarpon</i>) concentrate juice powder	Reflectron (+)	IAA	306
Proanthocyanidins	Ruby Red sorghum (<i>Sorghum bicolor</i>) grain	Reflectron (+)	IAA	307
Proanthocyanidins	Apple (<i>Malus pumila</i>)	Reflectron (\pm)	IAA/Ag ⁺	308
Proanthocyanidins	Coffee pulp (Arabica variety)	Linear	DHB	309
Proanthocyanidins	Grape seed	Reflectron (+)	IAA	310
Proanthocyanidins	Leaves or needles of willow (<i>Salix alba</i>), spruce (<i>Picea abies</i>), beech (<i>Fagus sylvatica</i>), lime (<i>Tilia cordata</i>)	linear (+)	DHB, dithranol, IAA	301
Proanthocyanidins	Grape seed	Reflectron (+)	DHB, IAA	311
Proanthocyanidins	Grape berries (variety Gamay)	(+)	DHB	312
Proanthocyanidins	Grapes (seeds, skins and stems), Quebracho (<i>Schinopsis balansae</i>) heartwood	Reflectron (+)	DHB	313
Proanthocyanidins	Grape seed	Reflectron (+)	DHB	314
Theaflavins, thearubigins	Black tea	Linear (+)	DHB, CHCA	303
Flavonol	Rutin	Reflectron (+)	DHB	300
Anthocyanins, 3-deoxyanthocyanidins	Sorghum (<i>Sorghum bicolor</i>) plant tissue	Linear (+)	CHCA	315
Anthocyanins	Highbush blueberries (<i>Vaccinium corymbosum</i>)	Linear (+)	THAP	316
Anthocyanins	Red wines, fruit juices	Linear (+)	THAP	317
Isoflavonoids	Soybeans, tofu, isoflavone supplements	Linear (+)	THAP, DHB	318
Flavonols	Yellow onion, green tea	Linear (\pm)	THAP, IAA	319
Flavonols	Almond (<i>Prunus dulcis</i>) seedcoat	Linear (+)	THAP	320
Flavonols	Almond (<i>Prunus amygdalus</i>) seedcoat	Linear (+)	THAP	321

Notes: CHCA, α -cyano-4-hydroxycinnamic acid; DHB, 2,5-dihydroxybenzoic acid; IAA, *trans*-3-indoleacrylic acid; THAP, 2,4,6-trihydroxyacetophenone monohydrate.

interesting paper, the structures of theaflavins (TFs) and thearubigins (TRs) from black tea have been revealed by the use of delayed pulsed ion extraction of ions generated via MALDI-TOF-MS.³⁰³ Spectra of standard TFs showed not only pseudomolecular ions but also ions resulting from fragmentation.

2.3.1.4 Electrospray Ionization and Atmospheric Pressure Chemical Ionization

ESI-MS (Table 2.8) was introduced by Yamashita and Fenn in 1984,³²² and this invention was recognized by The Royal Swedish Academy of Sciences with the award of The Nobel

Prize in Chemistry for 2002 partly to John B. Fenn for his pioneering work in ESI-MS. The mechanism of the transformation of ions in solution to ions in the gas phase prior to their mass analysis in a mass spectrometer together with instrumentation and applications of ESI have been reviewed by Cole.³²³

ESI is at present the most common technique used to analyze polar and nonvolatile flavonoids (from anthocyanins to condensed tannins), mainly because of the ease with which it can ionize polar and nonvolatile compounds (Table 2.11). The technique permits the detection of the molecular ion, either as a protonated molecule, $[M + H]^+$, adduct, $[M + Na]^+$, or as a deprotonated molecule, $[M - H]^-$, and causes only moderate fragmentation of the molecule as occurs with other higher-energy types of ionization techniques. MS with ESI ionization has been used for analysis of flavan-3-ols in plant extracts and in plasma samples with results achieving levels of detection of 20 ng.³²⁴ Furthermore, this technique provides structural information for highly polymerized compounds from the interpretation of the fragmentation profiles and from the level of charge the formed ions, which can either be monocharged or appear with multiple charges.³²⁵ There exists a very useful review on the principles, signal acquisition, and interpretation of proanthocyanidin spectra obtained by ESI-MS.³²⁶ In a recent study, Oliveira et al.³²⁷ described the use of ESI-MS, in combination with CID and tandem MS, for the structural characterization of anthocyanidins and anthocyanins.³²⁷ This technique has also been used in a fragmentation study of an flavone triglycoside, kaempferol-3-*O*-robinoside-7-*O*-rhamnoside,³²⁸ and for high mass resolution studies of a isoflavone glycoside, genistein-7-*O*-glucoside.³²⁹ The ability of ES to work with liquid sample introduction techniques has made it one of the most important detectors for HPLC and capillary zone electrophoresis (Section 2.3.2).

The APCI source (Table 2.8) has been used for the analysis of various flavonoids, especially flavonols, flavones, flavanones, and chalcones (Table 2.11). APCI is based on gaseous-phase ionization, and is most suitable for compounds that are partially volatile and have a medium polarity. Thus, the application of APCI with respect to analysis of condensed tannins and anthocyanins is more limited.²⁵⁷ Compared with ESI, APCI produces more fragment ions in the spectrum due to the harsher vaporization and ionization processes. More information about ESI and APCI can be found in Section 1.4.5.

2.3.1.5 Tandem (MS–MS) and Multiple (MS^{*n*}) Mass Spectrometry

In order to obtain fragmentation of ions produced by, for instance, ESI or APCI, these ions are accelerated inside the mass spectrometer so as to collide with molecules of the bath gas, usually helium.¹³⁹ Such CID of ions can be performed on all the ions emerging from the source, but this produces mixed CID spectra when more than one compound enters the source at the same time, as frequently occurs in LC–MS. To obtain pure CID spectra, the ion of interest (the precursor ion) needs to be isolated. Initially, the quadrupole mass filter was the ideal instrument to do this since its radiofrequency voltage can be set at a given value to only allow the selected precursor ion to pass through. This ion can then be accelerated into the bath gas in a collision cell (also of quadrupole design) and the products can be recorded using a third quadrupole operated in normal scanning mode. Therefore, MS–MS in space requires three quadrupoles.¹³⁹

Different MS–MS experiments of product ion scan, precursor ion scan, and neutral loss scan modes of selected flavonoids can be carried out in order to confirm the structure of flavonoids previously detected by the full-scan mode. In the product ion scan experiments, MS–MS product ions can be produced by CID of selected precursor ions in the collision cell of the triple-quadrupole mass spectrometer (Q2) and mass analyzed using the second analyzer of the instrument (Q3). However, in the precursor ion scan experiments, Q1 scans over all possible precursors of the selected ion in Q3 of the triple quadrupole. Finally, in neutral loss

scan experiments, both quadrupoles scan for a pair of ions that differ by a characteristic mass difference (neutral mass). The ESI-MS-MS experiments of product ion scan, neutral loss scan, and precursor ion scan modes have, for instance, enabled structural determination of the acylated flavonoid-*O*-glycosides and methoxylated flavonoids occurring in *Tagetes maxima*.³³⁰ In another example, MS-MS with positive CI has been applied successfully to problems involving trace analysis of citrus flavanones and metabolite identification.³³¹ Positive CI-MS-MS was compared to EI-MS-MS and found to be more advantageous in searching for the common daughter ion for flavanones (m/z 153) in complex matrices.

The two main methods for investigation of flavonoids using MS methods are direct infusion using a syringe, and flow injection either with or without chromatographic separation.²⁶⁰ The first method allows a long and thorough sample investigation, including the acquisition of data for several consecutive fragmentation steps (MS^{*n*} experiments). This method, which is used for structure characterization, requires a relatively large amount of purified flavonoid as the sample is normally infused with a flow rate of about 3 to 10 $\mu\text{l}/\text{min}$. Flow injections allow only a short investigation time for each signal, which may be too short for MS-MS experiments. However, the Q-IT has the potential to perform MS-MS in time within one analyzer.¹³⁹ Ions of a given mass-to-charge ratio can be isolated within a Q-IT and then excited such that they collide with bath gas and the resulting product ions are trapped and scanned out to the detector. Indeed, rather than being scanned out, the cycle of ion isolation and fragmentation can be repeated a number of times to achieve multistage MS (MS^{*n*}). The development of new, more powerful mass spectrometers has thus allowed one to obtain MS-MS spectra corresponding to the fragmentation of the molecular ion previously isolated in the ionization chamber in a selective way, and, moreover, to obtain MS^{*n*} spectra up to $n = 10$,²⁵⁷ which facilitates possibilities with respect to structural elucidation of unknown compounds. The application of ESI-MS^{*n*} in the analysis of the noncovalent complexes of cyclodextrins with quercetin 3-rutinoside (rutin) and quercetin has provided information about binding stoichiometry, as well as the relative stabilities and binding sites of the cyclodextrin-rutin complexes studied.³³² The diagnostic fragmentation pattern suggested that the specific inclusion complexes between rutin and the cyclodextrins could be confirmed by ESI-MS-MS alone without the need for solution-phase studies. The number of papers including MS-MS for flavonoid analysis has increased considerably in recent years, and some excellent reviews on tandem mass spectral approaches to the structural characterization of flavonoids have been reported.^{259,260} Some references to other applications of MS-MS and MS^{*n*} techniques are found elsewhere in this section.

2.3.1.6 Mass Analyzers

Quadrupole mass filters, Qs, which are still widely used for flavonoid analysis, isolate ions of a selected m/z ratio.^{330,333-336} They are mainly able to perform low-resolution mass analyses, and have a limited mass-to-charge range, typically up to m/z 4000, which, however, is appropriate for most flavonoid analyses. The ESI interfaces are most often used in combination with quadrupoles; on the other hand, in LC-ESI-MS, the quadrupole detector can be replaced by an IT or a TOF detector. In a Q-IT, ions are trapped in a cavity formed by three electrodes and are ejected through them by application of potentials, as a function of m/z values. The IT has the advantage that it can carry out sequential fragmentation first of the parent molecular ion and then of the daughter ions. Thus, it provides MS^{*n*} spectra by successive fragmentation of selected ions. The uses of IT analyzers has been described in several excellent papers.^{333,334,336-340}

The TOF analyzer separates ions by virtue of their different flight times over a known distance, depending on their m/z value.^{298,301,305,307,311,315,316,318,321,333,341,342} It supplies

accurate mass determination, and has theoretically an unlimited mass range. Hybrid instruments take advantage of easy creation and isolation of molecular ions of flavonoids. The quadrupole orthogonal time-of-flight (Q-TOF) mass spectrometer is related to triple-quadrupole instruments. Ions are generated with ESI or MALDI, selected in the first quadrupole, and fragmented by collision with argon gas, and the fragments accelerated orthogonally and injected into a TOF analyzer. The advantage of the TOF detector is its higher sensitivity and better mass accuracy (at least 20 ppm) than the quadrupole detector in a triple-quadrupole instrument.

FT-ICR mass spectrometers take advantage of ion-cyclotron resonance to select and detect ions. This analyzer can be used with both ESI and MALDI interfaces. Their particular advantages are their sensitivity, extreme mass resolution, and mass accuracy. The latter allows for the determination of the empirical formulae of compounds under 1000 Da. As far as we know, this analyzer has not been applied to flavonoids.

2.3.2 COUPLED TECHNIQUES INVOLVING MASS SPECTROMETRY

Complex plant extracts and biological fluids often require very effective and sensitive separation techniques to allow the identification of the various flavonoids in the samples. The coupling of instruments performing chromatographic separations, particularly HPLC, to those providing mass structural data has in recent years had an enormous impact in flavonoid chemistry. These coupled techniques are, above all, adept at targeted analyses; i.e., determining whether a specific component is present in a plant extract or a biological fluid. They are, for instance, ideally suited to studies in systematic phytochemistry in which the occurrences of, e.g., specific flavonoids are surveyed in taxonomic groups.¹³⁹ In this section, recent papers on high-performance LC-MS, GC-MS, and CE-MS applied in the flavonoid field are considered. The usefulness of LC-MS has been thoroughly covered in other chapters of this book (e.g., Sections 1.4.5 and 5.2).

2.3.2.1 Gas Chromatography Coupled to Mass Spectrometry

GC-MS is established as a routine technique for the analysis of flavonoid aglycones and is carried out with either EI or CI sources (see Section 2.3.1.1). Because of limited volatility, analysis of flavonoid glycosides by GC-MS has not generally found favor; however, improvements in GC column technology have increased the range of flavonoids amenable to GC-MS as underivatized compounds. Schmidt et al.³⁴³ analyzed 49 flavones, flavonols, flavanones, and chalcones without derivatization by GC and GC-MS (EI mode) using an OV-1 capillary column.³⁴³ Recently, lipophilic and thermolabile flavonoids in various plant extracts have been characterized directly by high-temperature high-resolution GC with cold on-column injection coupled with MS.^{344,345}

Employing chemical derivatization to increase volatility may extend the range of flavonoids that can be analyzed by GC-MS. However, derivatization may lead to the formation of more than one derivative from a single flavonoid. Frequently used derivatization methods are to silylate or methylate the hydroxyl groups of flavonoids. An in-vial simple and fast method for the combined methylation and extraction of phenolic acids and flavonoids in various plant extracts, followed by direct determination with GC-MS, includes the use of phase-transfer catalysis.³⁴⁶ Another GC-MS method has been developed for the determination of some flavonoid aglycones and phenolic acids in human plasma.³⁴⁷ The procedure involved extraction with ethyl acetate, followed by the derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane reagent. The trimethylsilyl derivatives formed were separated and quantified using GC-MS (EI). The average recovery was 79.3%, and the

method may be used in different matrices such as serum, urine, and tissues. An isotope dilution GC–MS method has been used for the identification and quantitative determination of unconjugated lignans and isoflavonoids in human feces.³⁴⁸ Following the formation of trimethylsilyl ethers, the samples were analyzed by combined capillary column GC–MS in the single-ion monitoring (SIM) mode, including corrections for all losses during the procedure using the deuterated internal standards.

2.3.2.2 High-Performance Liquid Chromatography Coupled to Mass Spectrometry

During the last decade, research efforts in the field of LC–MS have changed considerably. Technological problems in interfacing appear to be solved, and a number of interfaces have been found suitable for the analysis of flavonoids. These include TSP, continuous-flow fast-atom bombardment (CF-FAB), ESI, and APCI. LC–MS is frequently used to determine the occurrence of previously identified compounds or to target the isolation of new compounds (Table 2.11). LC–MS is rarely used for complete structural characterization, but it provides the molecular mass of the different constituents in a sample. Then, further structural characterization can be performed by LC–MS–MS and MS–MS analysis. In recent years, the combination of HPLC coupled simultaneously to a diode-array (UV–Vis) detector and to a mass spectrometer equipped with an ESI or APCI source has been the method of choice for the determination of flavonoid masses. Applications of LC–MS (and LC–MS–MS) in flavonoid analysis has recently been described in several excellent reviews,^{130,139,256,257,326,336,349–353} and the usefulness of these techniques have also been thoroughly covered in other chapters of this book (e.g., Chapters 1 and 5).

Selected papers on flavonoid analyses by LC coupled to positive- or negative-mode APCI or ESI are listed in Table 2.11. These two techniques are based on API, and their operational principle is that the column effluent from the LC is nebulized into an atmospheric-pressure ion-source region. Nebulization is performed pneumatically in a heated nebulizer (APCI), by means of the action of a strong electrical field (ESI), or by a combination of both. The ions produced from the evaporating droplets are, together with solvent vapor and nitrogen, sampled in an ion-sampling aperture by supersonically expanding into this low-pressure region before transportation to the mass analyzer. The mobile phase used contains easily ionized components (e.g., trifluoroacetic acid), from which a charge may be transferred to the flavonoid, $[M + H]^+$. Both sodium $[M + 23]^+$ and potassium $[M + 39]^+$ adducts may be seen in these spectra. Depending on the energy of the ion source, sugar moieties may fragment off the flavonoid. Mobile-phase flow in API interfaces may differ from nanoliters (nanoelectrospray) to milliliter per minute. The temperature control of the APCI desolvation process is far less critical than in TSP-MS. In this way, a wide range of flavonoids may be analyzed under the same conditions maintained at the APCI interface. In many cases, splitting of the eluate from the LC column is necessary in order to decrease the volume of solution entering the API source.

2.3.2.3 Capillary Electrophoresis Coupled to Mass Spectrometry

The first detection of ionic species in aqueous solutions by capillary zone electrophoresis combined with ESI-MS was applied with a quadrupole mass spectrometer.³⁸⁴ For the analysis of charged molecules, the high voltage applied to the electrospray needle is ideal in creating both the electrospray effect and closing the CE circuit. For uncharged molecules, though, the modified CE technique of capillary electrokinetic chromatography is necessary to achieve separation, which may create incompatibilities with ES.³⁸⁵

The CE–MS combination may provide valuable, structure-selective information about flavonoids in plant extracts; however, this coupled technique has hitherto found only very

TABLE 2.11
Selected Papers on LC–MS with Atmospheric Pressure Ionization Applied to Flavonoid Analysis

Analytes	Sample	LC Eluent	Ionization Mode	Mass Analyzer	Ref.
Anthocyanins	<i>Solanum stenotomum</i> tubers	ACN–H ₂ O, 0.1% TFA	ESI (+)	Q	354
Anthocyanins	Raspberry fruits	ACN–H ₂ O, 1% FA	APCI (+)	Q	335
Anthocyanins	Boysenberries	MeOH–H ₂ O, 5% FA	ESI (+)	Q	355
Anthocyanins	Grape juices	ACN–H ₂ O, 10% AA	ESI (+)	IT	356
Anthocyanins	Port wines	ACN–H ₂ O, 0.1% TFA	ESI (+)	Q	357
Anthocyanins	Purple corn	ACN–H ₂ O, 0.1% TFA	ESI (+)	Q	358
Flavones	Chamomile	ACN–H ₂ O, 0.1% TFA	ESI (±)	QqQ	359
Flavonols	Tomatoes	ACN–H ₂ O, 1% FA	APCI (–)	Q	360
Isoflavones and flavones	<i>Genista tinctoria</i>	ACN–H ₂ O, 0.1% AA	ESI (–)	Q	361
Isoflavones	Soy foods	ACN–H ₂ O, 0.1% TFA or 0.1% AA	APCI (±) and IS (±)	QqQ	362
Isoflavones	<i>Trifolium pretense</i>	ACN–H ₂ O, 0.2% AA	ESI (+)	Q	363
Isoflavones	<i>Trifolium pretense</i>	MeOH–10 mM ammonium formate buffer, pH 4.0	APCI (±)	IT and Q	364
Oligomeric anthocyanins	Grape skins	FA–H ₂ O–ACN	ESI (+)	QqQ	365
Flavonoid aglycones	Pure compounds	MeOH–H ₂ O, 0.1% FA	ESI (–)	IT	339
C-Glycosidic flavonoids	Pure compounds	ACN–H ₂ O, 0.5% AA	APCI (±) and ESI (±)	IT and Q-TOF	334
O- and C-Glycosidic flavonoids	<i>Sechium edule</i>	ACN–H ₂ O, 0.05% AA	ESI (–)	IT and Q	366
Flavonoids	Onion, blossom, and St. John's wort	ACN–H ₂ O, 20 mM TFA	ESI (+)	IT	367
Flavonoids	Pure compounds	MeOH–H ₂ O or ACN–H ₂ O, 0.1–0.4% FA or 10 mM AAc or 0.1% AH, 0.05% TFA	IS (±), APPI (±), and APCI (±)	QqQ	368
Flavonoids	Apples	MeOH–H ₂ O, 5% FA	ESI (+)	Q	369
Flavonoids	<i>Azima tetracantha</i>	ACN–H ₂ O–THF, 0.1% TFA	ESI (±)	QqQ	370
Flavonoids	<i>Oroxylum</i> seeds	ACN–H ₂ O, 0.2% FA	ESI (+)	IT and Q	371
Flavonoids	Tomatoes	ACN–H ₂ O–THF, 0.1% TFA	ESI (+)	QqQ	372
Flavonoids	Cocoa	ACN–H ₂ O, 0.1% FA	ESI (–)	QqQ	373
Flavonoids	Rooibos tea	ACN–H ₂ O, 0.1% AA	ESI (±)	Q	374
Flavonoids	Blood plasma and urine	ACN–H ₂ O, 2% AA	ESI (±)	QqQ	375
Flavonoids	Citrus	ACN–H ₂ O–ammonium acetate	ESI (+)	Q	376
Flavonoids	Barley	ACN–H ₂ O–MeOH, 1% AA	APCI (+)	Q	377
Flavonoids	Fresh herbs	MeOH–H ₂ O, 1% FA	APCI (–)	Q	378
Flavonoids	Red clover	ACN–H ₂ O, 0.25% AA	ESI (±)	Q	379
Flavonoids	Wood pulp, waste water	MeOH–H ₂ O, 0.5% AA	ESI (±)	QqQ	380
Flavonoids	Urine	MeOH–ACN–H ₂ O, 0.5% FA	APCI (–)	Q	381
Phenolic compounds	Soy, onions	ACN–H ₂ O, 10% FA	ESI (–)	Q	382
Phenolic compounds	Olives	MeOH–H ₂ O, 1% AA	ESI (±)	QqQ	383

Notes: AA, acetic acid; AAc, ammonium acetate; ACN, acetonitrile; AH, ammonium hydroxide; FA, formic acid; MeOH, methanol; TFA, trifluoroacetic acid; THF, Tetrahydrofuran; APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; ESI, electrospray ionization; IS, ion spray; IT, ion trap; Q, single quadrupole; QqQ, triple quadrupole; TOF, time of flight.

limited use in flavonoid analysis. Various isoflavones have been separated on an uncoated fused-SiO₂ CE column (110 cm × 75 mm i.d.) using 25 mM NH₄OAc buffer and UV and ESI-MS detection.³⁸⁶ The ESI-MS allowed recognition of the molecular masses of the isoflavones, as well as the presence of various functional groups according to observed losses from the [M – H][–] ion during CID by adjusting some MS parameters. Recently, a similar CE method has been established for the analysis of a flavonoid mixture obtained from plant extracts.³⁸⁷ This method used a fused-silica capillary and a buffer system consisting of 40 mM NH₄OAc, 15% MeCN (pH 9.5). After validation of the CE method in combination with a quadrupole mass spectrometer (with an electrospray interface and 0.1% triethylamine in 2-propanol–water [80:20 v/v] as sheath liquid in the negative-ion mode), MS detection showed sensitivity for hesperetin and naringenin similar to that of UV detection (0.4 to 0.6 mg/l). Employing external calibration allowed the reliable quantification of naringenin in a phytomedicine containing five different herbal drugs.

2.3.3 STRUCTURAL INFORMATION

The application of MS in structural elucidations of flavonoids has increased dramatically with recent developments related to soft ionization techniques, mass analyzers, and coupled MS techniques (see Sections 2.3.1 and 2.3.2). Based on relatively small flavonoid quantities, reports have provided the molecular mass in addition to structural information about the flavonoid skeleton,^{252,254,333,339,340,388–392} aglycone attachment points of glycosidic residues,³⁹² the types of carbohydrates present,^{252,388} and the types of interglycosidic linkages.^{262,340,390,391} The exact location of acyl groups in the glycosidic residue is, however, difficult to define on the basis of MS data. The numerous papers on structural information about flavonoids, predominantly focus on the use of CID in triple quadrupole or ion trap mass spectrometers, which allows generation and analysis of accurate daughter fragments, and on the usefulness of MALDI-MS. The reader will also find references to many other important papers in the field other than in Sections 2.3.3 and 2.3.4.

A considerable amount of information has been accumulated during the review period with respect to fragmentation studies of flavonoid aglycones and their glycosides using ionization techniques such as EI and CID (Figure 2.17).²⁵³ Tandem mass spectrometry with soft ionization methods such as FAB, ESI, and APCI have been used for the structural characterization of a variety of flavonoids, and both deprotonation^{339,340,378,380,393–396} and protonation^{252,254,340,380,390,397} modes combined with CID have been used (Table 2.9–Table 2.13). Due to their acidic nature, flavonoids usually give higher ion abundances upon deprotonation in the negative ESI mode than via protonation in the positive mode.

Fragmentation pathways of protonated and deprotonated molecules of *C*-glycosidic flavonoids obtained with CID tandem MS techniques have enabled important structural information to be obtained about different substitution patterns of sugars in this class of compounds.^{266,334,392} Fragment ions formed by the loss of water were more pronounced for 6-*C*-glycosyl flavonoids than for the corresponding 8-*C*-glycosyl flavonoids, due to the hydrogen bonds existing between the 2''-hydroxyl of the 6-*C*-sugar unit and either the 5- or 7-hydroxyl of the aglycone (Figure 2.18). Differentiation between *O*-glycosides, *C*-glycosides, and *O,C*-diglycosides have been achieved by examining the fragmentation patterns in their first-order positive-ion spectra or low-energy CID spectra (Figure 2.17).²⁵³ Diagnostic fragmentation patterns of flavonoids have also been reported based on a metal complexation mode in conjunction with CID.^{395,399–402} EI-MS of solutions containing a flavonoid, a transition metal salt, and an auxiliary ligand has resulted in differentiation between flavonoid isomers, as well as determination of the position of glycosylation. The CID patterns can be “tuned” by changing the auxiliary ligand,⁴⁰¹ however, little is known about the specific

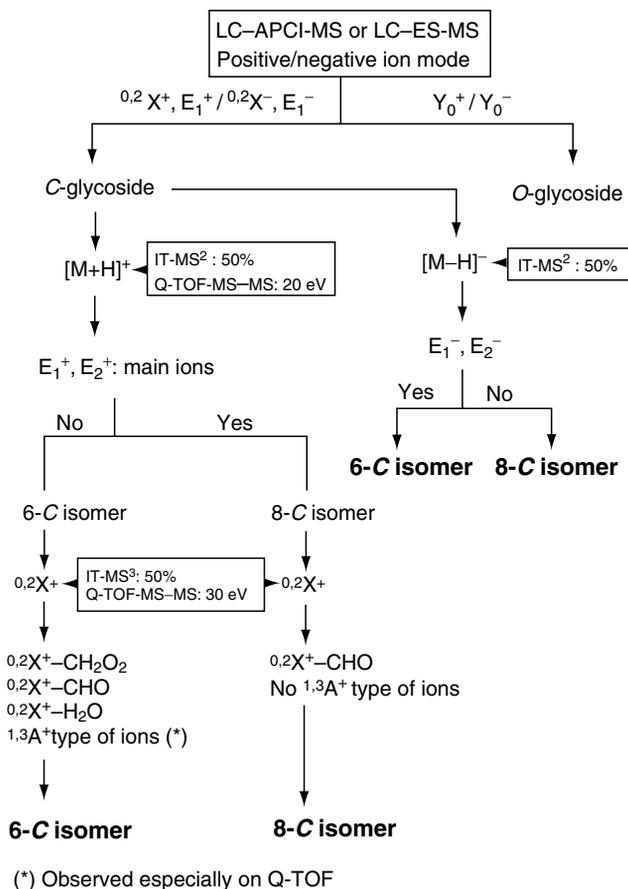


FIGURE 2.18 Identification of the 6-C and 8-C isomers of mono-C-glycosylflavones, respectively, based on diagnostic fragment ions observed in low-energy collision-induced dissociation tandem MS spectra. (Reprinted from Waridel, P. et al., *J. Chromatogr. A*, 926, 29, 2001. Copyright 2001 Elsevier Science B.V. With permission.)

structures of the metal complexes. Common auxiliary ligands used include 2,2'-bipyridine and 4,7-diphenyl-1,10-phenanthroline.

Many studies have dealt with the analysis of acylated flavonoid glycosides. MS analyses have mainly been used to obtain molecular mass information, but structure-specific information about the acyl group can be provided by neutral losses that are characteristic of the acyl group or the acylated glycosyl residue.²⁵³ Characteristic acyl-related product ions can be observed in the $[M+H]^+$ and $[M+Na]^+$ low-energy CID spectra and radical acid-related product ions at high-energy CID conditions, which provide information on the presence and identity of the acyl group and its position on the flavonoid backbone structure.⁴⁰³

MALDI-TOF-MS has been used to identify and quantify other anthocyanins in foods.^{317,404} When the anthocyanin content of highbush blueberries at different stages of anthocyanin formation were analyzed by both HPLC and MALDI-TOF-MS, it was found that both techniques provided comparable quantitative anthocyanin profiles.³¹⁷ While HPLC could distinguish anthocyanin isomers, MALDI-TOF-MS proved to be more rapid. MALDI-TOF-MS has also been used to identify the isoflavones in soy samples.³¹⁸ In a comparison of several matrices, 2',4',6'-trihydroxyacetophenone (THAP) and 2,5-dihydroxybenzoic acid

were found most suitable. Isoflavones were predominantly ionized in a protonated form with a very small amount of sodium or potassium adduct ions. Fragmentation occurred only through loss of glycosidic residues. The same authors have used the technique to identify flavonol glycosides in yellow onion bulbs and green tea.³¹⁹ THAP was chosen as the best matrix because it worked for crude sample extracts and ionized flavonol glycosides in both positive and negative modes. In the positive mode, multiple ion forms were observed for flavonol glycosides, including $[M + H]^+$, $[M + Na]^+$, $[M + K]^+$, and $[M - H + Na + K]^+$, with further fragmentation through loss of glycosidic residues. The negative mode for all flavonol glycosides resulted in $[M - H]^-$ ion formation without detectable fragmentation. MALDI-TOF-MS has been used for structural elucidation of some flavones,⁴⁰⁵ and a symmetrical glycosylated methylene bisflavonoid.⁴⁰⁶

Although proanthocyanidins are present as the second most abundant class of natural phenolic compounds after lignin,⁴⁰⁷ relatively few MS studies appear in the literature before 1993, due to the structural complexity of these compounds. During the review period considerable progress has been achieved through studies of proanthocyanidins in various foods and beverages.^{304,307–310} Using tandem MS coupled to reversed-phase HPLC (RP-HPLC), the proanthocyanidins of cocoa (*Theobroma cacao*) (Table 2.12),³⁷³ green tea (*Camellia sinensis*),⁴⁰⁸ and wine⁴⁰⁹ were identified. The negative-ion mode was found to be more sensitive and selective in the studies of proanthocyanidins than the positive-ion mode.⁴⁰⁹ Using LC-ESI-MS-MS analysis in the negative-ion mode, several new heterogeneous B-type proanthocyanidins containing (epi)afzelechin as subunits, including tetramers and pentamers, were identified in extracts of pinto beans, plums, and cinnamon (Table 2.13).⁴¹⁰ In MALDI analyses of Ruby Red sorghum,³⁰⁷ deionization of the proanthocyanidin fractions with the Dowex 50 \times 8–400 cation-exchange resin and subsequent addition of cesium trifluoroacetate (¹³³Cs) allowed the detection of exclusively $[M + Cs]^+$ ions in the spectra.

MALDI-TOF-MS has been used to characterize the molecular masses of condensed tannins with varying degrees of polymerization in unripe apples.³⁰⁸ The technique has provided evidence for a catechin pentadecamer using *trans*-3-indoleacrylic acid as matrix in the presence of silver ion. Even in the absence of silver ion, the dodecamer and undecamer were observed in the positive- and negative-ion modes, respectively. The technique has also been employed to determine molecular sizes of oligomeric proanthocyanidins in coffee pulp,³⁰⁹ and to characterize the polygalloyl polyflavan-3-ols (PGPF) in grape seed extracts.^{310,311} Masses corresponding to a series of PGPF units inclusive of nonamers were observed in the positive-ion reflectron mode, while masses of PGPF inclusive of undecamers were observed in the positive-ion linear mode, providing the first known evidence of PGPF of this size.³¹⁰ In another study, the MALDI-TOF mass spectra of the condensed tannins of leaves and needles from willow (*Salix alba*), spruce (*Picea abies*), beech (*Fagus sylvatica*), and lime (*Tilia cordata*) in dihydroxybenzoic acid as matrix have shown signals of polymers of up to undecamers.³⁰¹ Supporting observations from NMR spectroscopy, the mass spectra of the willow, and lime leaf condensed tannins were identified as polymers with mainly procyanidin–prodelphinidin ratios.

Because of their complex nature, a complete characterization of grape proanthocyanidins has so far eluded analytical chemists despite the effort devoted to it. A reliable method for total proanthocyanidin quantification and for supplying information regarding the molecular weight distribution of the most complex proanthocyanidins is still lacking. However, the potential role of the MALDI-TOF-MS technique for proanthocyanidin differentiation and as a quantification tool is promising.^{305,306,311–313,411} In a recent paper, an offline coupling of size-exclusion chromatography and MALDI has been carried out to measure differences between polystyrenes and procyanidins.³¹⁴ Polystyrenes are used as standards because no

TABLE 2.12
Liquid Chromatography–Electrospray Ionization Tandem Mass Spectrometric Study of the Flavonoids of Cocoa (*Theobroma cacao*) with Negative Ion Detection

Compound	MW	t_r (min)	MS–MS ions m/z (Relative Abundance, %)	DP (V)	CE (V)
Catechin	290	8.5	289 [M – H] [–] (40), 245 (100)	–50	–20
Epicatechin	290	11.8	289 [M – H] [–] (40), 245 (100)	–50	–20
Luteolin 6-C-glucoside (isorientin)	448	15.4	447 [M – H] [–] (65), 429 (65), 357 (100), 327 (100), 285 [A – H] [–] (50)	–60	–30
Luteolin 8-C-glucoside (orientin)	448	15.7	447 [M – H] [–] (30), 357 (70), 327 (100), 285 [A – H] [–] (20)	–60	–30
Apigenin 8-C-glucoside (vitexin)	432	17.8	431 [M – H] [–] (35), 341 (30), 311 (100), 269 [A – H] [–] (<5)	–55	–30
Apigenin 6-C-glucoside (isovitexin)	432	18.1	431 [M – H] [–] (15), 353 (<5), 341 (40), 311 (100), 269 [A – H] [–] (<5)	–55	–30
Quercetin 3-rutinoside (rutin)	610	18.2	609 [M – H] [–] (100), 301 [A – H] [–] (40)	–60	–30
Quercetin 3-galactoside (hyperoside)	464	18.4	463 [M – H] [–] (5), 301 [A – H] [–] (100)	–60	–38
Quercetin 3-glucoside (isoquercitrin)	464	19.0	463 [M – H] [–] (35), 301 [A – H] [–] (100)	–60	–32
Luteolin 7-glucoside	448	19.2	447 [M – H] [–] (100), 285 [A – H] [–] (100)	–60	–30
Kaempferol 3-rutinoside	594	21.3	593 [M – H] [–] (70), 285 [A – H] [–] (100)	–65	–45
Apigenin 7-rutinoside (isorhoifolin)	578	22.0	577 [M – H] [–] (20), 269 [A – H] [–] (100)	–60	–40
Naringenin 7-glucoside (prunin)	434	22.4	433 [M – H] [–] (70), 271 [A – H] [–] (100)	–60	–20
Kaempferol 3-glucoside	448	22.4	447 [M – H] [–] (90), 285 [A – H] [–] (100)	–60	–30
Quercetin 3-rhamnoside (quercitrin)	448	22.6	447 [M – H] [–] (25), 301 [A – H] [–] (100)	–60	–30
Naringenin 7-neohesperidoside (naringin)	580	22.6	579 [M – H] [–] (100), 459 (20), 271 [A – H] [–] (40)	–80	–35
Kaempferol 7-neohesperidoside	594	23.1	593 [M – H] [–] (20), 327 (5), 285 [A – H] [–] (100)	–70	–40
Apigenin 7-glucoside	432	23.3	431 [M – H] [–] (100), 269 [A – H] [–] (75)	–60	–35
Quercetin	302	33.0	301 [M – H] [–] (60), 151 (100)	–60	–35
Luteolin	286	33.1	285 [M – H] [–] (100), 217 (10), 199 (20), 175 (20), 151 (85), 133 (50), 107 (10)	–60	–35
Naringenin	272	34.5	271 [M – H] [–] (25), 177 (20), 151 (100), 119 (75), 107 (35), 93 (15), 83 (10)	–60	–30
Apigenin	270	34.8	269 [M – H] [–] (60), 151 (100)	–60	–35
Kaempferol	286	35.1	285 [M – H] [–] (100), 217 (40), 151 (85), 133 (75)	–60	–35
Isorhamnetin	316	35.4	315 [M – H] [–] (60), 300 (100), 151 (10)	–60	–30
Amentoflavone	538	36.3	537 [M – H] [–] (100), 375 (65)	–60	–40

Notes: DP, declustering potential; CE, collision energy. The CE values were optimized in such a way that the sensitivity of the multiple reaction monitoring signal was at the maximum.

Source: From Sanchez-Rabameda, F. et al., *J. Mass Spectrom.*, 38, 35, 2003. Copyright 2003 John Wiley & Sons, Ltd. With permission.

TABLE 2.13
Liquid Chromatography–Electrospray Ionization Mass Spectrometric Characteristics ([M – H][–] and Product Ions) of Selected Proanthocyanidin Tetramers and Pentamers from Pinto Beans, Plums, and Cinnamon, Respectively

Procyanidin Connection Sequence	[M – H] [–]	Product Ions
(Epi)Afz–(Epi)Afz–(Epi)Cat–(Epi)Cat	1121.3	849.0, 831.1, 577.2, 543.1
(Epi)Afz–(Epi)Cat–(Epi)Afz–(Epi)Cat	1121.2	849.0, 831.1, 561.1
(Epi)Afz–(Epi)Cat–(Epi)Cat–(Epi)Cat	1137.3	865.3, 577.2, 559.0
(Epi)Cat–(Epi)Afz–(Epi)Cat–(Epi)Cat	1137.3	849.3, 847.3, 577.2, 559.1
(Epi)Afz–(Epi)Afz–(Epi)Cat–(Epi)Cat–(Epi)Cat	1409.3	1119.3, 865.0, 831.1, 577.1
(Epi)Afz–(Epi)Cat–(Epi)Cat–(Epi)Cat–(Epi)Cat	1425.3	1153.3, 1135.7, 865.1, 577.1
(Epi)Cat–(Epi)Cat–A–(Epi)Cat–A–(Epi)Cat	1149.3	861.4
(Epi)Cat–(Epi)Cat–(Epi)Cat–A–(Epi)Cat	1151.2	863.5, 575.1
(Epi)Cat–(Epi)Cat–(Epi)Cat–(Epi)Cat–A–(Epi)Cat	1439.3	1151.3, 863.4, 575.2
(Epi)Afz–(Epi)Cat–A–(Epi)Cat–(Epi)Cat	1135.2	863.2, 847.2, 573.0
(Epi)Cat–A–(Epi)Cat–A–(Epi)Cat–(Epi)Cat	1149.3	859.2
(Epi)Cat–(Epi)Cat–A–(Epi)Cat–(Epi)Cat	1151.2	863.4, 861.2, 573.2
(Epi)Cat–A–(Epi)Cat–(Epi)Cat–(Epi)Cat	1151.2	861.3, 573.1
(Epi)Cat–A–(Epi)Cat–A–(Epi)Cat–(Epi)Cat–(Epi)Cat	1437.4	1147.4, 859.5
(Epi)Cat–(Epi)Cat–(Epi)Cat–A–(Epi)Cat–(Epi)Cat	1439.4	1151.2, 1149.4, 863.2, 575.1, 573.1
(Epi)Cat–(Epi)Cat–A–(Epi)Cat–(Epi)Cat–(Epi)Cat	1439.4	1151.3, 1149.4, 861.5, 577.2, 575.2, 573.1

Notes: The chirality of C-3 on the flavan-3-ols cannot be differentiated by MS. (Epi)afzelechin represents either afzelechin or epiafzelechin. Afz, afzelechin; Cat, catechin; A, A-type binding between the flavanol units, i.e., flavanols doubly linked by an additional ether bond between C-2 and O-7 in addition to the C4–C8 (or more rarely C4–C6) bond.

Source: From Gu, L. et al., *J. Mass Spectrom.*, 38, 1272, 2003. Copyright 2003 John Wiley & Sons, Ltd. With permission.

commercial procyanidin standards are available. Between 1000 and 8000 Da, there was good correlation between the MALDI and PS calibration curves. In this range, the PS calibration was correct and enabled true mass determination.

2.3.4 QUANTITATIVE CONSIDERATIONS

Recently, a method has been developed for faster evaluation of the total flavonoid content in plants and foodstuffs.³⁴¹ A TOF instrument has been used to acquire an *m/z* range of 220 to 700 with a generic gradient HPLC run, detecting both positive negative ions in alternating spectral acquisitions, and producing exact mass data during the whole run by using a Lock-Spray ESI source. Traditionally, most of the quantitative LC–MS methods utilize linear quadrupole or ion trap mass spectrometers, due to their good linear dynamic range. However, if a large number of compounds with different molecular weights is to be detected simultaneously in an LC–MS experiment using quadrupole instruments, the demand for scanning over the wide mass range decreases the sensitivity. On the other hand, if SIM is used to increase the sensitivity, the chromatographic resolution may become a problem. In view of the above considerations together with the moderately poor ionization efficiency of the flavonoids, higher detection limits with LC–MS than with LC–UV methods are usually observed. When using a TOF instrument, the necessity to compromise between sensitivity and chromatographic resolution was considerably reduced when data were acquired over a wide range

of m/z ratios. The compounds were quantified using quercetin, quercitrin, rutin, and kurmanine as external standards and dextromethorphan as an internal standard. The detection limits ranged from 0.01 to 0.04 mg/ml, while the quantification ranges obtained were 0.2 to 10 mg/ml for anthocyanins and 0.2 to 4 mg/ml for the other flavonoids.³⁴¹

The analytical performance of four modes of LC–MS, multiple MS (MS^n), and tandem MS operation (APCI and ESI with positive and negative ionizations) has been compared for two mass spectrometers, a triple-quadrupole and an ion-trap instrument.³³⁶ With 15 flavonoids as test compounds, the use of APCI in the negative-ion mode gave the best response, with the signal intensities and the mass-spectral characteristics not differing significantly between the two instruments. Under optimum conditions, full-scan limits of detection of 0.1 to 30 mg/l were achieved in the negative APCI mode. The main fragmentations observed in the MS^n spectra on the ion trap, or the tandem MS spectra on the triple quadrupole, were generally the same. The advantage of the former approach was the added possibility to ascertain precursor–product ion relations. The best results were obtained when methanol–ammonium formate (pH 4.0) was used as LC eluent.³³⁶ In another comparison based on five flavones or flavonols, the effect of nine different eluent compositions on the ionization efficiency has been studied using ion spray (IS), APCI, and atmospheric pressure photoionization (APPI) in positive- and negative-ion modes.³⁶⁸ It was shown that the eluent composition had a major effect on the ionization efficiency, and the optimal ionization conditions were achieved in positive-ion IS and APCI using 0.4% formic acid (pH 2.3) as a buffer, and in negative-ion IS and APCI using ammonium acetate buffer adjusted to pH 4.0. For APPI work, the eluent of choice appeared to be a mixture of organic solvent and 5 mM aqueous ammonium acetate. The limits of detection (LODs) were determined in scan mode, and it was shown that negative-ion IS with an eluent system consisting of acidic ammonium acetate buffer provided the best conditions for detection of flavonoids in MS mode, their LODs ranging between 0.8 and 13 μM for an injection volume of 20 μl .³⁶⁸

A rather sensitive RP–HPLC method combined with UV (270 nm) and ESI–MS detection has been established for the determination of flavonoids and other phenolic compounds in various biological matrices.³⁸² LODs based on UV data of flavonoids in onion and soybean were 6 to 42 pmol injected, which corresponded with analyte concentrations of 0.08 to 0.63 mg/l. It has also been reported that 12 dietary flavonoid glycosides and aglycones in human urine have been identified and quantified by LC–MS using MeOH–ACN–formic acid as eluent, and APCI in negative mode.³⁸¹ Calibration graphs were prepared for urine, and good linearity was achieved over a dynamic range of 2.5 to 1000 ng/ml. Selected ion monitoring offered a considerable gain in selectivity as well as sensitivity, and LODs were determined to be 0.25 to 2.5 ng/ml.³⁸¹ Wogonin metabolites (flavones) have been identified in rat plasma with an LC–ESI/IT method in multistage full-scan mode.⁴¹² On basis of this a sensitive LC–triple-quadrupole MS method using APCI in the selected reaction monitoring mode has been used to determine the concentration of wogonin and its major metabolite in rat plasma. The method had a lower limit of quantification of 0.25 ng/ml for wogonin, and has been successfully applied to a preclinical pharmacokinetic study after an oral administration of 5 mg/kg wogonin to rats. The quantitative method was validated with respect to linearity, precision, and accuracy.

Based on an in-vial derivatization method, the mass spectra of methylated flavonoids and other phenolics have been obtained via EI–MS at 70 eV.³⁴⁶ Detection was performed in the selective ion monitoring mode and peaks were identified and quantified using target ions. The detection limits ranged between 2 and 40 ng/ml, whereas the limits of quantitation fall in the range of 5 to 118 ng/ml, with flavonoids accounting for the lowest sensitivity due to their multiple reaction behavior.

Probably most important for plant extract analyses, MALDI–MS is remarkably tolerant of impurities making the direct analysis of crude extracts possible. Through its capability for

analyzing very small quantities of these compounds in unpurified samples, MALDI provides a sensitive means for the detection of flavonoid pigments in plant tissues. By analyzing a mixture of 3-deoxyanthocyanidins using MALDI-MS, sensitivities to the level of 15 pmol/ μ l have been attained for 3-deoxyanthocyanidins present in crude extracts from sorghum plant tissue, and as low as 5 pmol/ μ l for pure samples containing the anthocyanidin, pelargonidin, and the anthocyanin, malvin.³¹⁵

2.4 VIBRATIONAL SPECTROSCOPY (IR AND RAMAN)

Two different types of spectroscopic techniques are most frequently used to view the fundamental modes of molecular vibrations, namely mid-IR spectroscopy and Raman spectroscopy.⁴¹³ The first method measures the absorption, transmission, or reflection of IR radiation with wavelengths in the range of 2.5 to 25 μ m. The Raman method irradiates the sample with radiation of much shorter wavelengths and measures the fraction of scattered radiation for which the energy of the photon has changed. The vibrational spectra may serve as fingerprints of structure, composition, interactions, and dynamics. The reciprocal of wavelength, wavenumber (cm^{-1}), is commonly used to characterize the energy in the field of vibrational spectroscopy.

Systematic vibrational spectroscopy studies on flavonoids have occurred since the early 1950s, and most of them have been limited to a discussion on the hydroxyl and carbonyl absorption frequencies.⁴¹⁴ However, with the technical advances of the last two decades, the application of vibrational spectroscopy has become much more relevant in the field of flavonoid analysis.^{415,416} The implementation of FTIR spectroscopy has significantly enhanced the sensitivity, and Raman spectroscopy has benefited from the availability of holographic notch filters, which efficiently suppress the strong signal from elastically scattered (Rayleigh) radiation while maintaining the Raman-shifted intensity with minimal attenuation. Furthermore, high-powered NIR semiconductor lasers and sensitive charge-coupled devices have replaced inconvenient gas lasers and light-detection technologies. Ordinary Raman spectroscopy has drawbacks in that it requires high compound concentrations, and the recorded spectrum will correspond to all molecules present in the sample. In resonance Raman spectroscopy, this is overcome through the use of laser light with a frequency corresponding to the absorption maximum of the compound to be characterized. Finally, the increase in the computing power of standard computers has facilitated more sophisticated data evaluation of both IR and Raman spectra.

The following sections describe the applications of IR, Raman, and NIR spectroscopic techniques applied to the field of flavonoids in recent years.

2.4.1 IR AND RAMAN SPECTROSCOPIC TECHNIQUES IN STUDIES OF FLAVONOID STRUCTURES

IR and Raman spectroscopic techniques have been extensively used by Merlin, Cornard, and their coworkers to achieve structural information about flavonoid geometry.⁴¹⁷⁻⁴²⁰ These investigations have usually been accompanied by UV-Vis spectroscopic and x-ray crystallographic analysis, as well as quantum chemical calculations. The main focus has been on the effects of position and nature of substituent (hydroxyl or methoxyl groups) on the molecular structure, including investigations of the dihedral angle between the phenyl ring and the chromone part of the molecule. The vibrational spectra of various simple flavonoids in solid state have been compared with those obtained in solutions, and differences between the solid-state spectra and solution-state spectra were explained by the possibility of the formation of intramolecular hydrogen bonds present in the solid state and under specific solution conditions, or formation of intermolecular hydrogen bonds with the solvent (CH_3OH). The Raman

spectra were preferred in most of these studies because they were considerably less complex than the corresponding IR spectra. The structures of a variety of flavonoid–aluminum ion complexes, including the complexes of aluminum(III) with 3-hydroxyflavone,⁴²¹ 5-hydroxyflavone,⁴²² 3',4'-dihydroxyflavone,⁴²³ quercetin,⁴²⁴ and quercetin 3-glucoside,⁴²⁵ have also been examined by this research group. The influence of pH and Al³⁺ concentration on the complex formation was considered, and molecular conformations of both the free and complexed flavonoids were proposed. Recently, these flavonoids have been used as model compounds for the study of the behavior of humic substances toward Al(III) complexation.⁴²⁶ Other complexes between flavonoids and metal ions investigated by IR spectroscopy include an alumina–(+)-catechin solution system,⁴²⁷ and some prepared organotin(IV) complexes with the flavonoid glycosides, rutin, hesperidin, and 2',4',3-trihydroxy-5',4-dimethoxychalcone 4-rutinoside, and with the aglycones, quercetin, morin, hesperitin, and some flavones.⁴²⁸ The FTIR spectra of these latter complexes were consistent with the presence of Sn–O (phenol or carbohydrate) vibrations in the compounds, and the structures of the complexes were measured by Mössbauer spectroscopy.

FTIR spectroscopy has been used for the reexamination of the carbonyl stretching frequency of some simple hydroxyflavones in argon and methanol–argon matrices,⁴²⁹ and IR spectra have been recorded for some simple flavonoids including sulfonic acid derivatives.⁴³⁰ The Raman spectra of six common anthocyanidins and some of their glycosylated derivatives in acidic aqueous solutions have been compared.⁴³¹ Despite great similarity between these spectra, the anthocyanin substitution pattern could easily be recognized and the effect of glycosylation was clearly visible; 5-glycosylation seemed to cause a greater perturbation in the vibrational properties than that of 3-glycosylation. A more recent Raman spectroscopy study of the structure of anthocyanins in aqueous solutions as a function of temperature has also been presented.⁴³²

Among the new vibrational spectroscopic techniques applied to flavonoids, the hydrophilic extracts of Scots pinewood and two model compounds, the flavone chrysin and the stilbene pinosylvin, have been characterized using UV resonance Raman spectroscopy.⁴³³ Pinosylvin and chrysin were resonance enhanced by UV excitation. Both compounds showed very intense UV Raman bands due to alkene and aromatic structures at 1649 to 1635 cm⁻¹ and 1605 to 1600 cm⁻¹, respectively. In addition, aromatic and unsaturated structures of pinosylvin and chrysin showed bands at 1582 and 1549 cm⁻¹, respectively. A very useful multichannel spectrometer with microprobe and laser excitation either in the near-UV or visible range has been used for flavonoid analysis.⁴¹⁵ It combines high-detection sensitivity with rapid recording of spectra. The instrument is designed to record fluorescence emission or Raman scattering spectra of samples examined in the microscope. The study of anthocyanins in *Zebrina pendula* leaves has illustrated the possibility of recording absorption, fluorescence, and Raman spectra from the same living cell.⁴¹⁵

The frequencies of many vibrational normal modes do not depend only on the molecular structure. IR studies of 5-methyl-7-methoxy-isoflavone in 20 different organic solvents have been used to examine the solvent–solute interactions and to correlate solvent properties with the IR band shift.⁴³⁴ It was found that no linear relationship existed between the wavenumber of the C=O stretching band and the Kirkwood–Bauer–Magat solvent parameters. However, good correlations were observed between the wavenumbers of the C=O stretching band and the solvent acceptor number, and even better correlations between the wavenumbers of the C=O stretching band and the linear solvation energy relationships. A new chemistry model within density functional theory (called CHIH-DFT) has recently been used to predict the IR and UV–Vis spectra of quercetin.⁴³⁵ The predicted spectra were in good agreement with the previously reported experimental UV and IR spectra, and assignments of the principal peaks have been achieved.

2.4.2 IR AND RAMAN SPECTROSCOPIC TECHNIQUES IN STUDIES OF COMPLEXES INVOLVING FLAVONOIDS

In recent years, IR and Raman spectroscopic techniques have been applied for the characterization of flavonoid-containing systems with rather complex composition. A rapid analytical method involving attenuated total reflection (ATR) mid-IR spectroscopy and UV-Vis spectroscopy, combined with multivariate data analysis, has been applied for the discrimination of Austrian red wines.⁴³⁶ By analyzing phenolic extracts (obtained by C18 solid-phase extractions followed by elution with acidified methanol) of the various wines by mid-IR spectroscopy, almost complete discrimination of all samples was achieved. Furthermore, it was possible to establish class models for five different wine cultivars and to classify the test samples correctly. In another study, the Raman spectrum of *Artocarpus heterophyllus* heartwood has shown to exhibit two characteristic bands at 1247 and 745 cm^{-1} .⁴³⁷ Based on Raman measurements of pure flavones and related compounds, it was predicted that the Raman band at 1247 cm^{-1} was attributed to flavonoid-type compounds. In this case, no vibrational band corresponding to the characteristic Raman bands was observed by diffuse reflectance IR spectroscopy.⁴³⁷ By using solid-state FTIR and Raman spectroscopies an inclusion complex between 2',6'-dimethoxyflavone and formic acid has been identified.⁴³⁸ The broad and intense IR absorption observed in the range 3400 to 1900 cm^{-1} , assigned to the hydrogen-bonded OH-group stretching vibration, exhibited the characteristic ABC structure of strong hydrogen-bonded complexes in good agreement with previous x-ray data showing that *cis*-formic acid was strongly hydrogen bonded to 2',6'-dimethoxyflavone. The inclusion complex was quite unstable, and the IR spectrum clearly showed that formic acid disappeared after a period of a few months. The formation of some β -cyclodextrin inclusion complexes involving various flavanones has been investigated by FTIR and other methods.⁴³⁹ Changes in the characteristic IR bands of pure substances confirmed the existence of β -cyclodextrin-flavanon complexes as new compounds with different spectroscopic bands.

In an interesting application, the interaction of polyphenols with proline-rich proteins was studied using an automated flow injection system with FTIR detection to gain insight into chemical aspects related to astringency.⁴¹⁶ Agarose beads carrying the proline-rich protein were placed in the IR flow cell in such a way that the beads were probed by the IR beam. By using an automated flow system, the samples were flushed over the proteins in a highly reproducible manner. Simultaneously, any retardation due to polyphenol-protein interactions taking place inside the flow cell was monitored by IR spectroscopy.⁴¹⁶ Recent data obtained by FTIR experiments, fluorescence spectroscopy, CD experiments, and molecular modeling have suggested that the flavone scutellarein can strongly bind to the human serum albumin.⁴⁴⁰

2.4.3 TWO-DIMENSIONAL IR ANALYSIS

The introduction of generalized 2D correlation IR spectroscopy, 2D-IR, by Noda⁴⁴¹ has extended markedly the potential of using vibrational spectroscopy for flavonoid analysis. Analyses have been performed using ordinary FTIR spectrometers, and the coordinates of the two dimensions in these spectra both use frequency or wavenumber as units (Figure 2.19). Peaks in 2D-IR spectra might show the sensitivity for each IR band or each functional group and the correlation between the functional groups, even the order of the influence when the system is subjected to a given perturbation.⁴⁴¹ 2D-IR spectroscopy can simplify complex spectra consisting of many overlapping bands and enhance spectral resolution by spreading peaks along the second dimension, thus enabling extraction of information that cannot be obtained straightforwardly from 1D spectra. The method has hitherto not been applied to

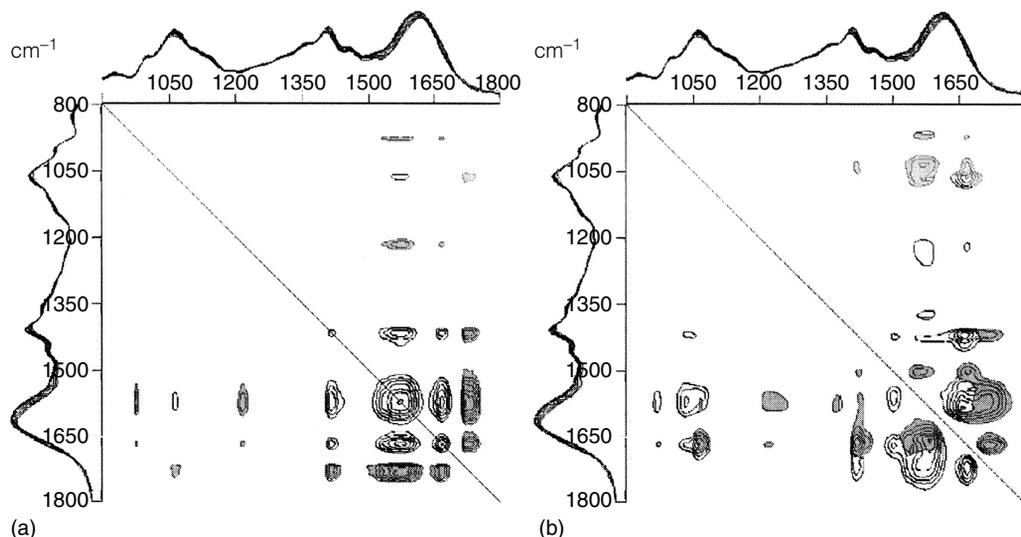


FIGURE 2.19 2D IR correlation spectra of the flavonoid-containing Chinese medicine “Qing Kai Ling.” (a) Synchronous IR spectrum; (b) asynchronous IR spectrum. In analogy to the corresponding 1D IR spectra, the units of the axis in both these 2D spectra are given in cm^{-1} . (Reprinted from Zuo, L. et al., *J. Pharm. Biomed. Appl.*, 30, 1491, 2003. Copyright 2003 Elsevier Science B.V. With permission.)

pure flavonoids; however, the traditional Chinese medicine “Qing Kai Ling” injection after deterioration has been distinguished from the original formulation using FTIR and 2D-IR (Figure 2.19).⁴⁴² It has been very difficult to distinguish IR spectra of injections before and after deterioration by using the conventional 1D approach. However, higher spectral resolution and more structural information provided by 2D-IR analyses have made this differentiation possible. According to the 2D correlation analysis, the band at 1611 cm^{-1} in the conventional IR spectra, in fact, consisted of the overlap of three bands at 1572 , 1667 , and 1729 cm^{-1} , which were assigned, respectively, to the alkaloids, flavone derivatives, and carbonyl compounds in the injection.⁴⁴²

2.4.4 COUPLED TECHNIQUES INVOLVING VIBRATIONAL SPECTROSCOPY

Trimethylsilyl derivatives of ten hydroxy- and methoxyhydroxyflavonoids have been studied by the GC-FTIR technique.⁴⁴³ The correlation found between retention and gas-phase IR data was used in structural identification of compounds having very similar chromatographic behavior. The shift of the carbonyl frequency gave information on the presence of substitution. Some hydroxy- and methoxy-substituted flavones have been studied following carbon dioxide supercritical fluid chromatography on polymethylsiloxane capillary columns using flame ionization and FTIR detection.⁴⁴⁴

The HPLC-FTIR technique has recently been used to identify six catechins and two methyl-xanthines present in green tea extracts.⁴⁴⁵ A reversed-phase separation of the compounds was performed on a C-18 column equilibrated at 30°C using an isocratic mobile phase of acetonitrile-0.1% formic acid (15:85), prior to introduction to the deposition interface linked to the FTIR detector. The solvent was evaporated at 130°C and spectra were collected every 6 sec during the run. Two distinct designs for HPLC-FTIR interfaces have been developed: flow cells and solvent elimination systems. Flow cell systems acquired spectra of the eluent in the solvent matrix through IR transparent, nonhydroscopic windows. The

spectrum of the solvent was then subtracted from the sample spectrum, with the result being the spectrum of the analyte of interest. Solvent elimination systems nebulized the HPLC eluent and removed the solvent before depositing the dried solutes onto an IR transparent surface. Due to the strong IR absorption of water and other solvent, solvent elimination systems are generally more sensitive than flow cell arrangements. Acid washing the drift tube was recognized as being essential to obtain reproducible catechin depositions.⁴⁴⁵

2.4.5 NEAR-INFRARED SPECTROSCOPY

NIR spectroscopy involves the measurement of wavelengths (800 nm to 2.5 μm) and intensity of the absorption of NIR light by a sample. This light span is energetic enough to excite overtones and combinations of molecular vibrations to higher energy levels. Several recent studies emphasize the potential of NIRS as a nondestructive and effective alternative method to conventional quantitative analysis of food, plant extracts, pharmaceutical remedies, etc. In one application, Huck et al.⁴⁴⁶ have reported the use of NIR for quantitative analysis of the water content, ethanol, and the flavone 3',4',5'-trimethoxyflavone in *Primulae veris* Flos extracts. First, a calibration set using a reference method was established, e.g., HPLC for quantification of the flavone. The values obtained were correlated by the use of special software (NIRCal) to analyze the NIR spectral data. This calibration set could then be used to quantify other new samples without the need for HPLC. An NIR reflectance spectroscopy technique has been developed for the prediction of procyanidins in cocoa beans (*Theobroma cacao*),⁴⁴⁷ for the estimation of the nasunin (anthocyanin) content in the skin of eggplant,⁴⁴⁸ to predict the content of the dihydrochalcone aspalathin in unfermented rooibos,⁴⁴⁹ condensed tannins concentrations in *Lotus uliginosus*,⁴⁵⁰ tannins and total phenolics content in forage legumes,⁴⁵¹ and for the simultaneous determination of alkaloids and phenolic substances including flavonoids in green tea.⁴⁵² NIRS has been used for the prediction of resistance in sugarcane to stalk borer *Eldana saccharina*.⁴⁵³ NIR spectra of 60 sugarcane clones varying in resistance to *E. saccharina* indicated that chlorogenates and flavonoids might be involved in the interaction between the insect and sugarcane. NIRS has also been used to estimate plant pigment content (anthocyanins, carotenoids, chlorophylls) in higher plant leaves.⁴⁵⁴ A rapid quantitative NIRS method was established for the determination of the constituents, including the biflavone I3,II8-biapigenin, in St. John's wort extracts,⁴⁵⁵ and for simultaneous determination of total flavonoids and total lactones in *Ginkgo* extracts.⁴⁵⁶ Recent developments of multichannel dispersive Raman microprobes using NIR excitation beyond 1000 nm and linear array detectors offering good sensitivity in the NIR region of the spectrum between 1000 and 1600 nm have been applied to various samples including flavone.⁴⁵⁷

2.5 ULTRAVIOLET-VISIBLE ABSORPTION SPECTROSCOPY

The application of standardized UV (or UV-Vis) spectroscopy has for years been used in analyses of flavonoids. These polyphenolic compounds reveal two characteristic UV absorption bands with maxima in the 240 to 285 and 300 to 550 nm range. The various flavonoid classes can be recognized by their UV spectra,² and UV-spectral characteristics of individual flavonoids including the effects of the number of aglycone hydroxyl groups, glycosidic substitution pattern, and nature of aromatic acyl groups have been reviewed in several excellent books.^{1,2,458}

Today, the major use of UV-Vis spectroscopy applied to flavonoids is in quantitative analyses, and the value of this method for some structural analyses is diminishing compared to the level of information gained by other modern spectroscopic techniques like NMR and

MS. This section will rather briefly concentrate on some of the more recent applications of UV absorption spectroscopy in the flavonoid field. It will mainly cover online UV absorption spectroscopy in chromatography (Section 2.5.1). Because of the current importance of UV–Vis in the study of anthocyanins, some more UV–Vis spectral details have been included related to this pigment group (Section 2.5.2). Section 2.5.3 indicates the recent use of this technique in studies of flavonoids interacting with other compounds.

2.5.1 ONLINE UV ABSORPTION SPECTROSCOPY IN CHROMATOGRAPHY

The combination of HPLC equipped with a UV–Vis DAD (see Section 1.4.4) has for the two last decades been the standard method for the detection of flavonoids in mixtures. This type of detector allows the simultaneous recording of chromatograms at different wavelengths. The HPLC–DAD (alternatively called LC–UV) method has, during the period of this review, been used for isolation, identification, screening, measurement of peak purity, or quantitative determinations of flavonoids in numerous studies, and there exist several excellent recent reviews in the field.^{459–461} In the absence of standards, the method offers spectral information about individual flavonoids by recording UV–Vis spectra wherein each peak is revealed in the chromatogram. However, during elution the mobile-phase composition may vary considerably, and the various LC methods may involve different solvents and solvent compositions. There may, for instance, be a 15 nm shift toward shorter wavelengths when water is substituted for methanol. Thus, the resulting spectra of the same flavonoid may be obtained in different solvents, aggravating precise identification based on agreement with literature data obtained on pure flavonoids in a standardized solvent. When it comes to alternative quantitative methods, various catechins have been separated with gradient RP–HPLC and quantified by UV (270 nm) and fluorescence (280/310 nm, excitation/emission) detection in series.⁴⁶² The combination of HPLC with online UV, MS, and NMR detection in LC–UV–MS⁽ⁿ⁾ (Sections 1.4.5 and 2.3.2.2) and LC–UV–NMR (Sections 1.4.6 and 2.2.5) has proved to be a very valuable tool for the analysis of natural products especially in extracts or mixtures.

The use of UV shift reagents such as AlCl_3 (5% in methanol)–HCl (20% aqueous), NaOMe (2.5% in methanol), and NaOAc (3 mg)– H_3BO_3 has proven to be very useful as guidelines for substitution patterns of many flavonoids; however, the use of these reagents has mainly been applicable for purified flavonoids.^{1,2} By adding suitably modified shift reagents to the eluate leaving a HPLC column, similar shifts of the UV absorption maxima of flavonoids in the eluate have been induced.⁴⁶³ Recently, Wolfender and his collaborators have introduced UV shift reagents by postcolumn addition in hyphenated LC–UV–MS analysis of flavonoid-containing crude extracts (Figure 2.20).^{131,141,464} The shifts observed were interpreted according to the rules previously established for the analysis of pure polyphenols,² and permitted the localization of the position of the hydroxyl groups on most of the compounds detected in the crude extracts that were analyzed. A screening method that allowed the determination of the flavonoid composition of plant and food extracts has been based on double online detection, first at 280 nm and then at 640 nm, after derivatization with *p*-dimethylaminocinnamaldehyde (1% in 1.5 M sulfuric acid in methanol).⁴⁶⁵ The colored adducts showed maximum absorption between 632 and 640 nm, thus preventing the interference of other colored compounds in the same extracts.

2.5.2 UV–VIS ABSORPTION SPECTROSCOPY ON ANTHOCYANINS

The UV–Vis spectral data on anthocyanins (typically measured in methanol containing 0.01% HCl) give important information about the nature of the aglycone and aromatic acyl

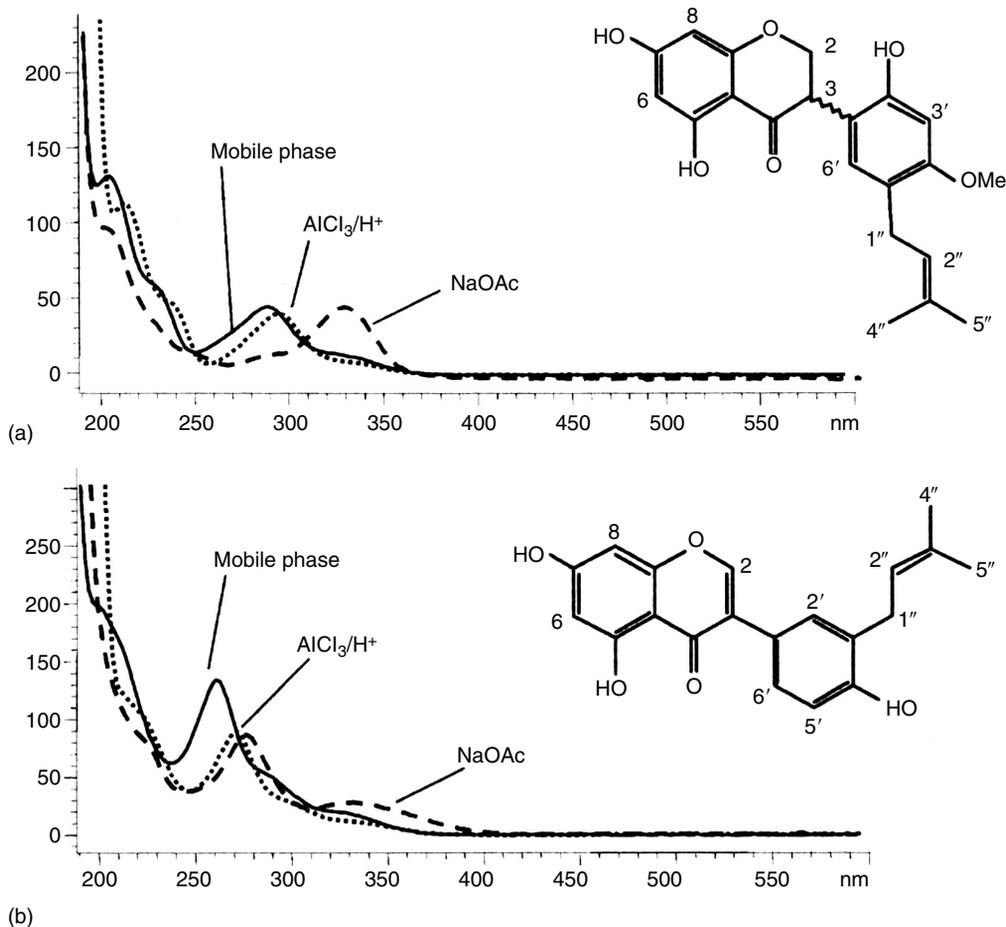


FIGURE 2.20 Complementary UV-DAD and shifted UV-DAD spectra with postcolumn addition of shift reagents of an isoflavanone (a) and an isoflavone (b) recorded online. The weak base NaOAc and acidic AlCl_3 , respectively, were used as shift reagents. The shifted UV spectra are superimposed on the original spectra for each compound. The observed shifts provide information about the flavonoid substitution in accordance to the rules established for pure compounds. (Modified from Wolfender, J.-L., Ndjoko, K., and Hostettmann, K., *J. Chromatogr. A*, 1000, 437, 2003. Copyright 2003 Elsevier Science B.V. With permission.)

groups.^{460,466} Anthocyanins with 4'-*O*-glycosylation have recently been identified,¹⁷⁰ and now UV-Vis spectral data for anthocyanins having glycosyl moieties connected to all the hydroxyl positions have been reported.

Some diagnostic information about the glycosidic substitution pattern may thus be revealed: anthocyanins with sugar unit(s) on the B-ring connected to the 3'-, 4'-, or 5'-hydroxyls seem to have their visible λ_{max} at shorter wavelengths (4 to 14 nm) than the corresponding anthocyanin 3-glycosides. A hypsochromic shift (12 nm) was observed for λ_{max} in the UV-Vis spectrum of cyanidin 3,4'-*O*-diglucoside, compared to the corresponding value of cyanidin 3-*O*-glucoside.¹⁷⁰ Also, λ_{max} in the spectra of the 3,7-*O*-diglucoside, 3,7,3'-*O*-triglucoside, and 3,7,3',5'-*O*-tetraglucoside of delphinidin have been observed at 537, 525, and 521 nm, respectively.^{467,468} Anthocyanin 5-*O*- and 7-*O*-glucosides seem to exhibit their

λ_{\max} at slightly longer wavelengths (5 to 9 nm) than the corresponding anthocyanin 3-*O*-glycosides; however, no similar effect on λ_{\max} has been observed for anthocyanin 3,5-*O*-diglycosides and anthocyanin 3,7-*O*-diglycosides. It is well known that absorption spectra of pelargonidin 3-*O*-glycosides show higher absorbances at wavelengths around 440 nm than found in the corresponding spectra of the other common anthocyanidin 3-*O*-glycosides. Similarly with the spectra of pelargonidin 3-*O*-glycosides, a shoulder has been observed around 440 nm in the UV-Vis spectra of cyanidin and delphinidin derivatives with *O*-glycosyl moieties on their B-rings.^{114,170} The relationship between color and substitution patterns in anthocyanidins has been investigated with the aim of developing quantitative structure-color models; and experimental data for the lowest UV transition in 20 substituted anthocyanidins have been reviewed.⁴⁶⁹ While hypsochromic effects from hydroxyl and methoxyl moieties at positions 6 and 8 were reported, it is interesting to note that a *C*-glycosyl moiety in the 8-position has the opposite effect producing a more bluish color.⁴⁷⁰

The anthocyanins change their forms and colors depending on pH, concentration, copigmentation, and metal ions. Various inter- and intramolecular association mechanisms may be involved. Many of the studies in the field of anthocyanin and flower color, which were covered excellently by Brouillard and Dangles,⁴⁷¹ involve UV-Vis absorption spectroscopy. The presence of acylation with cinnamic acids can be deduced by the appearance of a peak or shoulder in the 310 to 330 nm region, while this peak is not observed in the case of benzoic acids, which have their maximum absorption between 270 to 290 nm. The $A_{\lambda_{\max}(\text{acyl})}$ – $A_{\lambda_{\max}(\text{visible})}$ ratio may be a measure of the number of aromatic acids present in the anthocyanin.⁴⁶⁶ In the literature, there exist many recent examples on how aromatic acyl substituents of anthocyanins have caused bathochromic shifts in the UV-Vis spectra due to intra- or intermolecular ‘ π – π ’ stacking with the anthocyanidins.^{472–475} In these cases, either cinnamoyl moieties or polyacylation with benzoic acids are involved. However, no significant bathochromic effects are observed on λ_{\max} for cyanidin 3-(2''-galloylglucoside) and cyanidin 3-(2'',3''-digalloylglucoside), indicating the absence of intramolecular copigmentation for these pigments.¹⁶⁹ Dangles et al.⁴⁷⁶ have indeed reported that two sugars are necessary as spacers to allow folding of the acyl moiety leading to higher chromophore integrity. During the review period several covalent complexes between anthocyanins and other flavonoids have been identified.^{88,226,477–479} Significant bathochromic shifts of λ_{\max} (12 to 20 nm) in spectra of these complexes may reveal intramolecular ‘ π – π ’ stacking of the anthocyanidin with the flavone or flavonol moiety.

Absorption spectra have also been used in the reexamination of pH-dependent color and structural transformations in aqueous solutions of some nonacylated anthocyanins and synthetic flavylium salts.⁴⁸⁰ In a recent study, the UV-Vis spectra of flower extracts of *Hibiscus rosasinensis* have been measured between 240 and 748 nm at pH values ranging from 1.1 to 13.0.⁴⁸¹ Deconvolution of these spectra using the parallel factor analysis (PARAFAC) model permitted the study of anthocyanin systems without isolation and purification of the individual species (Figure 2.21). The model allowed identification of seven anthocyanin equilibrium forms, namely the flavylium cation, carbinol, quinoidal base, and *E*- and *Z*-chalcone and their ionized forms, as well as their relative concentrations as a function of pH. The spectral profiles recovered were in agreement with previous models of equilibrium forms reported in literature, based on studies of pure pigments.

Pigment stability measurements of anthocyanin-containing extracts and pure pigments is another application area of UV-Vis spectroscopy.^{482–485} Color and stability studies of the 3-glucosides of the six common anthocyanidins and petunidin 3-[6-(4-(*p*-coumaryl)rhamnopyranosyl)glucoside]-5-glucoside in aqueous solutions, during several months of storage, have revealed large variation between the pigments in particular at slightly acidic to slightly alkaline pH values.^{485,486}

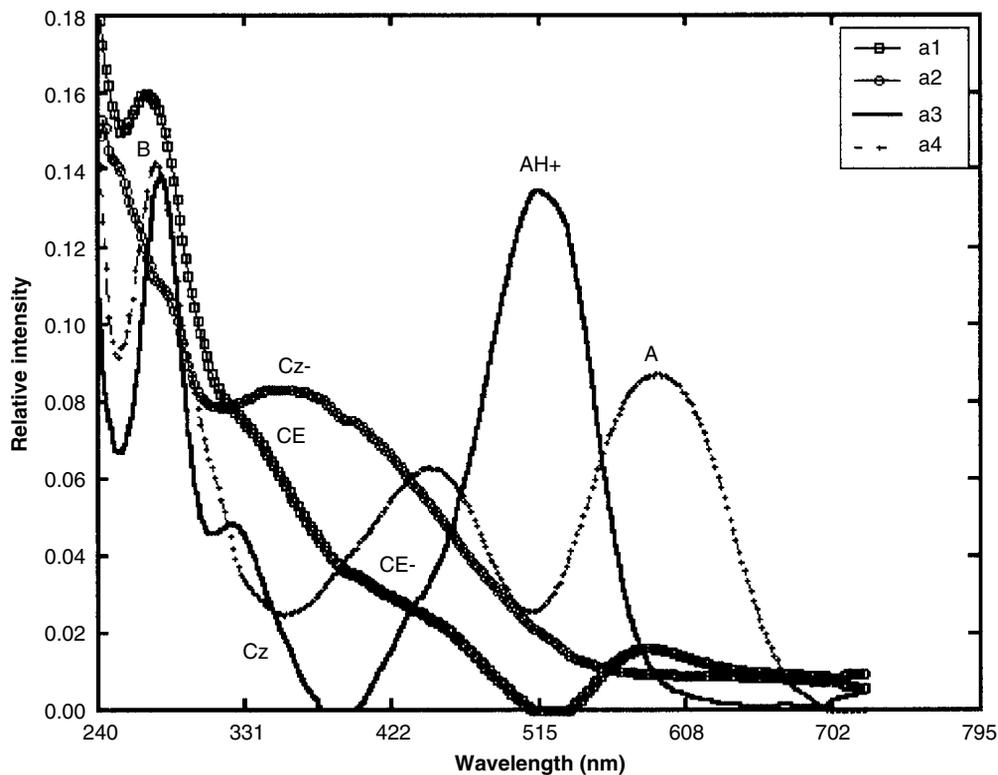


FIGURE 2.21 Spectral profiles recovered by the PARAFAC model at different pH values based on the deconvolution of UV–Vis absorption spectra, featuring the various anthocyanin secondary monomeric forms. (Reprinted from Levi, M.A.B. et al., *Talanta*, 62, 299, 2004. Copyright 2004 Elsevier Science B.V. With permission.)

Useful for quantification of anthocyanins, the molar absorption coefficients of several anthocyanins have been reviewed.^{460,488} However, these compilations reveal lack of uniformity between the reported values, most probably due to the unavailability of pure anthocyanins in sufficient quantities to allow reliable weighing under optimal conditions, and the lack of standardization of anthocyanin solvent used for measurements.

2.5.3 UV–VIS ABSORPTION SPECTROSCOPY INVOLVING FLAVONOIDS IN COMPLEXES

In a recent paper, the interaction of various simple flavonoids with an anionic surfactant, sodium dodecyl sulfate (SDS) in aqueous solution, has been studied through absorption spectroscopy as a function of the concentration of the surfactant above and below the critical micelle concentration.⁴⁸⁹ The approximate number of additive molecules (flavonoids) incorporated per micelle was estimated at a particular concentration of SDS. Incorporation of flavonoids in micelles shifted the UV absorption bands toward higher wavelengths, and the bathochromic shifts also depended upon the nature of the surfactant head group.

UV–Vis linear dichroism and (mid-)IR ATR IR analysis have been used to explain the possible association between DNA and the flavonols quercetin, rutin (quercetin-3-rutinoside), and morin (3,5,7,2',4'-pentahydroxyflavone) in solution.⁴⁹⁰ These nucleophilic flavonoids were shown to bind DNA by intercalation with an interaction having similar nature and geometry; however, under comparable conditions, quercetin exhibited a greater number

of intercalated chromophores. The sugar part of rutin, which was arranged out of the intercalation site, did not seem to represent any steric hindrance for the interaction with DNA. This is in accordance with the findings of Nerdal et al.,³⁶ who determined the detailed structure of the complex between the flavonol kaempferol 7-*O*-neohesperidoside and a DNA dodecamer containing the *E. coli* wild-type *lac* promoter sequence (TATGTT) using 2D NOESY NMR. DNA, which is a target for free radicals and reactive electrophilic groups, may thus be protected by the potential close relationship with flavonols and similar compounds.

The petals of a number of flowers contain similar intensely colored intravacuolar bodies referred to as anthocyanic vacuolar inclusions (AVIs), and the presence of AVIs has been shown to have a major influence on the color of flowers by enhancing both intensity and blueness.⁴⁹¹ In these studies, the anthocyanin–flavonol ratios in clarified extracts of blue-gray carnation have been determined by absorption spectroscopy. The levels of anthocyanin and flavonols were calculated from the absorption at 508 and 350 nm, respectively, using molar extinction coefficients of 36,000 and 15,000, respectively. Absorbance or reflectance spectra of inner and outer petal zones of purple lisianthus, measured with an integrating sphere connected to a spectroradiometer, indicated a distinct bluing of color in the AVI-rich inner petal. This bluing was evidenced by enhanced absorbance in the longer wavelength bands at 625 nm. Thus, in the outer petal, absorbance at 625 nm was 79% of the intensity of the 545 and 575 nm peaks whilst in the inner petal it was 95%. Reflectance and transmittance were determined at 2 nm intervals over the 400 to 1100 nm waveband.⁴⁹¹ Flowers of the rose cultivar Rhapsody in Blue display unusual colors, changing as they age, from a vivid red-purple to a lighter and duller purple.⁴⁹² Unexpectedly, the chemical basis of these colors is among the simplest, featuring cyanidin 3,5-diglucoside as the sole pigment and quercetin and kaempferol glycosides as copigments at a relatively low copigment–pigment ratio (about 3:1), which usually produces magenta or red shades in roses. It has been revealed that the color shift to bluer shades was coupled with the progressive accumulation of cyanidin 3,5-diglucoside into AVIs, the occurrence of which increased as the petals grew older. In these studies, spectral reflectance curves between 380 and 780 nm were recorded on circular petal areas (6 mm diameter) by a spectrophotometer. Each color was then numerically specified in the CIELAB scale (see Section 2.6). Spectroscopic measurements of live petals were based on transmission curves between 380 and 780 nm recorded using a spectrophotometer on petals fixed on a glass microscope slide (1 mm thickness), while spectroscopic curves of portions of individual epidermal cells were recorded between 400 and 700 nm at a 2.5 nm interval (bandwidth 10 nm) using a single-beam microphotometer with a continuous interferential filter.⁴⁹²

2.6 COLOR MEASUREMENTS USING COMMISSION INTERNATIONALE DE L'ÉCLAIRAGE SPECIFICATIONS

Color is a complex phenomenon, and in the evaluation of color the sample must be illuminated. In the light–sample interaction different physical phenomena are observed: transmission, absorption, scattering, refraction, etc. One way of describing sample color is to use numerical terms, which can be converted to CIE (Commission Internationale de l'Éclairage) color specifications. Using the CIELAB system, the principal attributes of sample colors are lightness (L^*), saturation (C^*), and hue (h_{ab}) (Figure 2.22). L^* considers color as a source of reflected light ranging from black ($L^*=0$) to white ($L^*=100$). C^* describes the chroma, which correlates to the degree of gray tone of the color. The higher the C^* value, the more saturated a color is. A very high L^* value (approximately 95 or more) combined with very low C^* (approximately 4 or less) corresponds to a virtually achromatic stimulus (i.e., white). The third parameter, namely h_{ab} , defines the tonality that we normally identify with the name of a

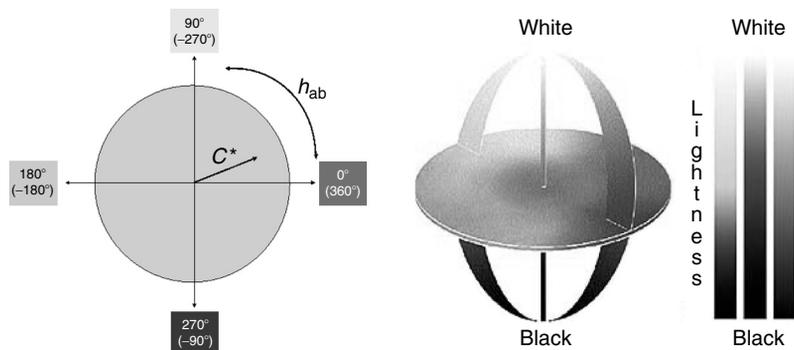


FIGURE 2.22 The three-dimensional CIELAB colour system. Left: h_{ab} (hue) describing the tonality of the color, where 0° corresponds to red, 90° corresponds to yellow, $\pm 180^\circ$ corresponds to green, and 270° (or -90°) corresponds to blue, C^* represents the chroma (or saturation), where 0 is a gray tone, while 100 is a pure hue. Right: the lightness (L^*) goes from black (0) to white (100).

color (where 0° corresponds to red color, 90° corresponds to yellow, 180° [or -180°] corresponds to green, and 270° [or -90°] corresponds to blue). From this model, it is easy to predict that, for instance, 45° corresponds to orange, whereas 315° (or -45°) corresponds to purple. Furthermore, determinations of colors also depend on the illuminant (e.g., D65) and observer conditions (e.g., 10°) under which the colors have been measured.^{493–496} During the review period, color measurements using the CIE system have been performed on anthocyanin-containing samples such as intact plant parts and products derived thereof, including red wines and fruit juices, as well as on pure anthocyanins. These analyses have included stability studies, factors influencing colors, and quantitative analyses. The effects of copigmentation on the color of anthocyanins have been extensively studied by Gonnet using the CIELAB parameters.^{495–497}

2.6.1 COLORIMETRIC STUDIES ON PURE ANTHOCYANINS

While most colorimetric analyses involving flavonoids have been performed on samples containing anthocyanins in mixtures with other compounds, some studies have been aimed at color analyses on pure pigments. First of all, it is obvious that the color characteristics of individual anthocyanins strongly depend on the anthocyanin concentration (Table 2.14). At pH values below 3.5, when the examined anthocyanins occur mainly in their flavylium forms, the hues of the same anthocyanin increase and the L^* values decrease considerably with increasing concentration. However, a remarkable effect was observed at high concentrations of cyanidin 3,5-diglucoside in buffer solutions at pH 2.5: the red color of the solution at 10^{-5} M (h_{ab} 358.4°) shifted to an orange hue (h_{ab} 48.3°) at 10^{-3} M and then back toward more reddish colors (h_{ab} 29.4°) at even higher concentrations (5×10^{-3} M), although the $\lambda_{vis-max}$ values of all these solutions remained at similar values (510 to 511 nm).⁴⁹⁵ Decreasing L^* values caused by increasing pigment concentration were also observed at pH values 3.5, 4.5, and 5.5; however, the pattern for the hue variation varied at these pH values,⁴⁹⁵ most probably due to different impact of the various equilibrium forms.

The 3-glucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, isolated from red grape skins, have been subjected to colorimetric analysis in model solutions imitating wine in the pH range 1.5 to 7.0.⁴⁹⁸ It was revealed that both increasing number of *O*-substituents and degree of methoxylation on the B-ring of anthocyanidins led to a color shift toward more purple hues at pH 1.5 (Table 2.14).⁴⁹⁸ The chroma of these pigments decreased

TABLE 2.14
Influence of Concentration, pH, and Solvent on Colors of Pure Anthocyanins Using CIELAB Parameters^a

Anthocyanin ^b	pH ^c	Conc. (mM)	L*	C*	h _{ab}	Ref.
Pg	0.1% HCIM	0.0154	87.1	22.1	357.3	488
Pg 3-glc	0.1% HCIM	0.0176	88.1	20.2	17.6	488
Pg 3-(2-glcglc)-5-glc	0.1% HCIM	0.0185	91.8	13.5	15.5	488
Pg 3-(2-glcglc)-5-glc	0.1% HCIM	0.0298	78.4	39.2	53.4	488
Pg 3-(2-glcglc)-5-glc + cou	0.1% HCIM	0.0173	85.3	30.3	16.4	488
Pg 3-(2-glcglc)-5-glc + fer	0.1% HCIM	0.0276	82.8	36.5	19.5	488
Pg 3-(2-glcglc)-5-glc + cou + mal	0.1% HCIM	0.0246	83.4	35.4	18.3	488
Pg 3-(2-glcglc)-5-glc + fer + mal	0.1% HCIM	0.0270	82.6	37.0	20.5	488
Pg 3-(6-rhaglc)-5-glc + cou	0.1% HCIM	0.0194	87.2	25.7	11.0	488
Pg	BpH 1.0	0.0154	90.0	16.7	22.7	488
Pg 3-glc	BpH 1.0	0.0176	90.3	17.6	44.0	488
Pg 3-(2-glcglc)-5-glc	BpH 1.0	0.0185	90.0	20.3	41.0	488
Pg 3-(2-glcglc)-5-glc	BpH 1.0	0.0298	81.8	56.0	53.5	488
Pg 3-(2-glcglc)-5-glc + cou	BpH 1.0	0.0173	86.4	26.8	23.3	488
Pg 3-(2-glcglc)-5-glc + fer	BpH 1.0	0.0276	83.4	33.7	24.1	488
Pg 3-(2-glcglc)-5-glc + cou + mal	BpH 1.0	0.0246	86.8	25.9	21.7	488
Pg 3-(2-glcglc)-5-glc + fer + mal	BpH 1.0	0.0270	83.1	33.9	22.1	488
Pg 3-(6-rhaglc)-5-glc + cou	BpH 1.0	0.0194	87.3	24.8	23.1	488
Pg 3-glc	BpH 1.1	0.10	76.6	86.9	58.7	175
5-CarboxypyranogPg 3-glc	BpH 1.1	0.10	87.5	48.7	61.1	175
Cy 3-glc	BpH 1.1	0.05	81.1	45.9	20.8	232
Cy 3-glc	BpH 1.1	0.15	63.7	81.9	42.4	232
Cy 3-(2-glcglc)-5-glc	BpH 1.1	0.05	81.5	46.7	12.8	232
Cy 3-(2-glcglc)-5-glc	BpH 1.1	0.15	69.5	78.6	38.0	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 1.1	0.05	66.0	69.5	-14.8	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 1.1	0.15	56.7	79.6	-9.7	232
Dp 3-glc	BpH 1.5	0.10	63.85	73.66	21.48	498
Cy 3-glc	BpH 1.5	0.10	70.4	69.0	29.5	498
Pt 3-glc	BpH 1.5	0.10	63.2	68.4	16.1	498
Pn 3-glc	BpH 1.5	0.10	73.2	61.1	24.5	498
Mv 3-glc	BpH 1.5	0.10	67.9	66.3	9.9	498
Cy 3,5-diglc	BpH 2.5	0.01	96.3	8.67	358.4	495
Cy 3,5-diglc	BpH 2.5	0.025	92.7	17.89	1.4	495
Cy 3,5-diglc	BpH 2.5	0.05	87.5	30.99	4.8	495
Cy 3,5-diglc	BpH 2.5	0.10	80.0	47.87	11.0	495
Cy 3,5-diglc	BpH 2.5	0.25	69.2	69.69	26.9	495
Cy 3,5-diglc	BpH 2.5	0.50	60.1	90.0	41.2	495
Cy 3,5-diglc	BpH 2.5	1.0	48.3	106.86	48.3	495
Cy 3,5-diglc	BpH 2.5	2.5	31.7	85.26	39.7	495
Cy 3,5-diglc	BpH 2.5	5.0	16.6	57.89	29.4	495
Cy 3-glc	BpH 3.0	0.05	82.3	40.5	5.7	232
Cy 3-glc	BpH 3.0	0.15	63.0	70.2	25.6	232
Cy 3-(2-glcglc)-5-glc	BpH 3.0	0.05	90.5	23.1	-2.8	232
Cy 3-(2-glcglc)-5-glc	BpH 3.0	0.15	76.9	52.9	4.4	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 3.0	0.05	66.5	67.9	-17.4	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 3.0	0.15	48.1	80.6	-5.2	232

continued

TABLE 2.14
Influence of Concentration, pH, and Solvent on Colors of Pure Anthocyanins Using CIELAB Parameters^a — *continued*

Anthocyanin ^b	pH ^c	Conc. (mM)	L*	C*	h _{ab}	Ref.
Dp 3-glc	BpH 3.5	0.10	88.2	20.6	-5.3	498
Cy 3-glc	BpH 3.5	0.10	88.2	22.9	8.1	498
Pt 3-glc	BpH 3.5	0.10	90.2	16.6	-4.0	498
Pn 3-glc	BpH 3.5	0.10	92.1	13.9	7.5	498
Mv 3-glc	BpH 3.5	0.10	93.3	11.8	-6.9	498
Cy 3,5-diglc	BpH 3.5	0.01	98.8	1.23	1.4	495
Cy 3,5-diglc	BpH 3.5	0.025	97.9	3.25	1.3	495
Cy 3,5-diglc	BpH 3.5	0.05	96.5	6.49	1.0	495
Cy 3,5-diglc	BpH 3.5	0.10	93.9	12.54	1.5	495
Cy 3,5-diglc	BpH 3.5	0.25	86.8	27.99	2.9	495
Cy 3,5-diglc	BpH 3.5	0.50	76.8	46.95	5.9	495
Cy 3,5-diglc	BpH 3.5	1.0	62.1	66.25	17.7	495
Cy 3,5-diglc	BpH 3.5	2.5	42.2	87.89	38.2	495
Cy 3,5-diglc	BpH 3.5	5.0	24.5	71.91	35.8	495
Cy 3-glc	BpH 4.1	0.05	89.5	22.1	-2.5	232
Cy 3-glc	BpH 4.1	0.15	69.6	49.8	5.0	232
Cy 3-(2-glcglc)-5-glc	BpH 4.1	0.05	96.5	6.3	-6.3	232
Cy 3-(2-glcglc)-5-glc	BpH 4.1	0.15	89.4	20.9	-8.0	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 4.1	0.05	72.5	50.5	-25.8	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 4.1	0.15	50.3	79.0	-22.2	232
Cy 3,5-diglc	BpH 4.5	0.01	99.0	0.33	16.8	495
Cy 3,5-diglc	BpH 4.5	0.025	98.9	0.65	14.7	495
Cy 3,5-diglc	BpH 4.5	0.05	98.4	1.33	9.4	495
Cy 3,5-diglc	BpH 4.5	0.10	97.4	2.70	5.9	495
Cy 3,5-diglc	BpH 4.5	0.25	94.7	7.03	3.1	495
Cy 3,5-diglc	BpH 4.5	0.50	89.7	14.28	2.3	495
Cy 3,5-diglc	BpH 4.5	1.0	79.9	28.73	0.5	495
Cy 3,5-diglc	BpH 4.5	2.5	58.8	54.10	3.4	495
Cy 3,5-diglc	BpH 4.5	5.0	35.9	67.79	17.2	495
Cy 3-glc	BpH 5.1	0.05	94.9	5.0	-16.8	232
Cy 3-glc	BpH 5.1	0.15	65.5	22.6	-50.0	232
Cy 3-(2-glcglc)-5-glc	BpH 5.1	0.05	98.2	1.5	-1.1	232
Cy 3-(2-glcglc)-5-glc	BpH 5.1	0.15	94.5	6.1	-13.6	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 5.1	0.05	82.7	24.7	-44.3	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 5.1	0.15	52.5	65.4	-41.2	232
Cy 3,5-diglc	BpH 5.5	0.01	99.4	0.19	26.8	495
Cy 3,5-diglc	BpH 5.5	0.025	99.1	0.45	22.9	495
Cy 3,5-diglc	BpH 5.5	0.05	98.7	0.98	23.1	495
Cy 3,5-diglc	BpH 5.5	0.10	97.6	2.11	24.1	495
Cy 3,5-diglc	BpH 5.5	0.25	94.6	5.39	22.5	495
Cy 3,5-diglc	BpH 5.5	0.50	90.3	10.41	12.1	495
Cy 3,5-diglc	BpH 5.5	1.0	80.6	20.97	7.9	495
Cy 3,5-diglc	BpH 5.5	2.5	57.2	45.79	9.1	495
Cy 3,5-diglc	BpH 5.5	5.0	31.6	62.39	16.9	495
Cy 3-glc	BpH 6.0	0.05	82.6	15.4	-21.2	232
Cy 3-glc	BpH 6.0	0.15	39.9	32.5	-47.1	232

TABLE 2.14
Influence of Concentration, pH, and Solvent on Colors of Pure Anthocyanins Using CIELAB Parameters^a — *continued*

Anthocyanin ^b	pH ^c	Conc. (mM)	L*	C*	<i>h</i> _{ab}	Ref.
Cy 3-(2-glcglc)-5-glc	BpH 6.0	0.05	96.6	4.0	-26.0	232
Cy 3-(2-glcglc)-5-glc	BpH 6.0	0.15	89.2	13.1	-28.9	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.0	0.05	72.1	38.1	-49.9	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.0	0.15	35.4	75.8	-43.0	232
Cy 3-glc	BpH 6.6	0.05	75.8	15.2	-17.1	232
Cy 3-glc	BpH 6.6	0.15	33.5	27.7	-50.7	232
Cy 3-(2-glcglc)-5-glc	BpH 6.6	0.05	80.7	25.9	-46.8	232
Cy 3-(2-glcglc)-5-glc	BpH 6.6	0.15	49.9	64.7	-42.3	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.6	0.05	76.0	29.4	-63.3	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.6	0.15	42.3	64.4	-58.2	232
Cy 3-glc	BpH 6.8	0.05	75.2	13.6	-14.8	232
Cy 3-glc	BpH 6.8	0.15	35.3	22.9	-46.8	232
Cy 3-(2-glcglc)-5-glc	BpH 6.8	0.05	73.2	34.7	-53.7	232
Cy 3-(2-glcglc)-5-glc	BpH 6.8	0.15	36.9	75.7	-46.3	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.8	0.05	75.3	28.4	-75.4	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.8	0.15	33.8	57.8	-67.4	232
Pg 3-glc	BpH 6.9	0.10	58.0	43.09	37.3	175
5-Carboxypyranopg 3-glc	BpH 6.9	0.10	81.2	37.5	34.8	175
Cy 3-glc	BpH 6.9	0.05	74.3	12.2	-13.8	232
Cy 3-glc	BpH 6.9	0.15	33.3	22.3	-50.0	232
Cy 3-(2-glcglc)-5-glc	BpH 6.9	0.05	69.3	38.3	-62.6	232
Cy 3-(2-glcglc)-5-glc	BpH 6.9	0.15	31.3	74.6	-51.5	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.9	0.05	72.2	31.2	-88.5	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 6.9	0.15	41.8	59.7	-72.1	232
Cy 3-glc	BpH 7.2	0.05	73.7	11.6	-15.8	232
Cy 3-glc	BpH 7.2	0.15	32.2	21.9	-47.0	232
Cy 3-(2-glcglc)-5-glc	BpH 7.2	0.05	65.4	42.2	-70.2	232
Cy 3-(2-glcglc)-5-glc	BpH 7.2	0.15	29.1	73.4	-56.1	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 7.2	0.05	69.3	34.7	-97.7	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 7.2	0.15	53.6	48.9	-87.7	232
Cy 3-glc	BpH 7.3	0.05	72.5	11.8	-24.2	232
Cy 3-glc	BpH 7.3	0.15	31.1	24.0	-39.7	232
Cy 3-(2-glcglc)-5-glc	BpH 7.3	0.05	64.4	43.2	-77.8	232
Cy 3-(2-glcglc)-5-glc	BpH 7.3	0.15	27.6	71.5	-59.8	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 7.3	0.05	63.2	41.4	-100.4	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 7.3	0.15	57.3	46.6	-97.4	232
Cy 3-glc	BpH 8.0	0.05	74.8	21.5	23.9	232
Cy 3-glc	BpH 8.0	0.15	38.7	47.2	24.1	232
Cy 3-(2-glcglc)-5-glc	BpH 8.0	0.05	67.1	52.8	-40.9	232
Cy 3-(2-glcglc)-5-glc	BpH 8.0	0.15	35.6	81.2	-34.2	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 8.0	0.05	57.8	43.4	-87.5	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 8.0	0.15	28.2	56.1	-77.4	232
Cy 3-glc	BpH 8.9	0.05	75.0	21.2	27.9	232
Cy 3-glc	BpH 8.9	0.15	38.7	46.9	26.9	232
Cy 3-(2-glcglc)-5-glc	BpH 8.9	0.05	67.3	49.1	-43.8	232
Cy 3-(2-glcglc)-5-glc	BpH 8.9	0.15	35.1	77.7	-34.8	232

TABLE 2.14
Influence of Concentration, pH, and Solvent on Colors of Pure Anthocyanins Using CIELAB Parameters^a — continued

Anthocyanin ^b	pH ^c	Conc. (mM)	L*	C*	h _{ab}	Ref.
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 8.9	0.05	56.3	35.0	-66.5	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 8.9	0.15	22.7	38.8	-79.4	232
Cy 3-glc	BpH 9.9	0.05	73.2	16.1	17.6	232
Cy 3-glc	BpH 9.9	0.15	39.3	40.6	23.4	232
Cy 3-(2-glcglc)-5-glc	BpH 9.9	0.05	80.6	17.2	-61.2	232
Cy 3-(2-glcglc)-5-glc	BpH 9.9	0.15	52.3	332.5	-46.9	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 9.9	0.05	55.3	20.2	-70.6	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 9.9	0.15	19.6	24.3	-73.9	232
Cy 3-glc	BpH 10.5	0.05	62.0	28.5	-62.8	232
Cy 3-glc	BpH 10.5	0.15	24.3	49.7	-53.6	232
Cy 3-(2-glcglc)-5-glc	BpH 10.5	0.05	83.3	14.4	-155.5	232
Cy 3-(2-glcglc)-5-glc	BpH 10.5	0.15	55.5	35.6	-171.4	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 10.5	0.05	57.2	47.2	-155.4	232
Cy 3-(2-(2-singlc)-6-singlc)-5-glc	BpH 10.5	0.15	27.6	42.3	-153.0	232

^aSome of the values need to be regarded as transient because some pigments degrade fast at some pH values. This is especially profound for nonacylated pigments in weak acid and neutral solutions.

^bPg, pelargonidin; Cy, cyanidin; Dp, delphinidin; Pn, peonidin; Pt, petunidin; Mv, malvidin; glc, glucose; gal, galactose; xyl, xylose; rha, rhamnose; cou, *p*-coumaric acid; fer, ferulic acid; sin, sinapic acid; mal, malonic.

with methoxylation of the hydroxyl groups on the B-ring. Changes in lightness (L^*) corresponding to color loss and a color shift toward purple/blue were observed when increasing the pH toward 7.

In a comparative analysis of pelargonidin and several pelargonidin glycosides with different glycosylation and acylation patterns dissolved in MeOH-HCl (99.9:0.1, v/v) and aqueous buffer solution (pH 1.0), hyperchromic effects leading to higher chroma, as well as lower hue values (more reddish colors), were observed for all pelargonidin derivatives when dissolved in acidified methanolic solutions (Table 2.14).⁴⁸⁸ The glycosylation pattern, the nature of the glycosyl substituents, as well as aromatic acylation showed significant impact on the colors of the solution. 3-Glycosylated pigments showed lower hues than the corresponding 3,5-diglycosylated pigments. The presence of cinnamoyl moieties lowered the hue value and increased the chroma, compared to similar nonacylated pigments. Contrary to this, aliphatic acylation with malonic acid had little effects on chroma and lightness, and these pigments exhibited lesser difference between the hue values obtained in aqueous and methanolic solutions, respectively.⁴⁸⁸ Similarly, the color properties of several cyanidin-based anthocyanins, all containing a 3-glycosyl residue with different overall substitution patterns, have been analyzed in various solutions with pH ranging from 0.45 to 6.00.⁴⁹⁹ Compared with cyanidin 3-glycosides without acylation, both acylation with cinnamic acids and glycosidic substitution at the 5-position shifted color tonalities (hue) toward purple. A small increase in color strength through acylation was also confirmed; however, it was proved that slightly different acyl moieties also affected color appearance.

An important conclusion from the colorimetric analysis of newly discovered pyrananthocyanins is that each of them seems to retain the original color over the whole pH region

1 to 7, which implies that they, in contrast to analogous common anthocyanins, remain in the flavyllium cationic equilibrium form to a significant extent even up to pH 7.^{175,500,501} The pyruvic acid pyrano-anthocyanin adducts adopt a more yellowish color than the corresponding pigments lacking the extra ring.^{175,500} In contrast to this, pyrano-anthocyanins formed from the reaction between cinnamic acids and anthocyanins achieved a more bluish color.⁵⁰¹

Studies of color and color stability of some anthocyanins, representing the structural variation of the common pigments isolated from many fruits and vegetables, have been performed at various pH values covering the pH region 1.0 to 10.5 and at two different concentrations (Table 2.14).²³² The hue angle shift toward bluish tones in freshly made samples of anthocyanins with 5-glucosidic substitution was amplified with aromatic acylation throughout the entire pH range except at pH 10.5. Of potential interest for weakly acidic food products, one of the pigments involved, namely cyanidin 3-(2''-(2'''-sinapoylglucosyl)-6''-sinapoylglucoside)-5-glucoside, maintained nearly the same h_{ab} , C^* , and L^* values during the whole period (98 days), in contrast to similar anthocyanins lacking the aromatic acyl moieties.

2.6.2 ANTHOCYANIN-BASED COLORS OF PLANTS AND PRODUCTS DERIVED THEREFROM

Colorimetric measurements have been applied to various analyses of red wines and model wine solutions.^{500,502–507} Accurate definitions of the wines have been achieved by the L^* (lightness) and a^* (redness) values, while the representation of ΔL^* against ΔC^* revealed the color differences between various wines.⁵⁰⁵ The color stability of wine and model wine solutions toward storage time and bleaching by sulfur dioxide has been extensively studied by Bakker et al.,^{502,504,507} while the color stability of a range of anthocyanin-containing extracts, including fruit juices, has been the subject of other colorimetric studies.^{508–515} It has, for instance, been shown that black carrot anthocyanins are applicable as food colorants in the acidic to weakly acidic pH region.⁵¹⁶

Colorimetric analyses performed in the reflection mode have been used to determine colors of various flowers^{492,517–521} as well as the colors and color development of fruits and berries.^{511,522–533} In an interesting study, the “cyclamen” red (or pink) colors of some carnation cultivars have been found to be based on the macrocyclic anthocyanin, pelargonidin 3,5-diglucoside (6'',6'''-malyl diester).⁵³⁴ The CIELAB coordinates revealed that these flowers showed similar colors as some rose cultivars, which, however, were mainly based on a very different pigment, cyanidin 3,5-diglucoside.

2.7 CIRCULAR DICHROISM SPECTROSCOPY

When polarized light is passed through a substance containing chiral molecules, the direction of polarization can be changed. This phenomenon is called optical activity. Optical activity of flavonoids is basically measured by two spectroscopic methods, optical rotation and dichroism. The first method observes a sample's effect on the *velocities* of right and left polarized light beams. Dichroism is defined as the *differential absorption* of radiation polarized in the two directions as a function of frequency. When applied to plane polarized light, it is called linear dichroism (LD) and for circularly polarized light, circular dichroism, CD. CD spectroscopy has during the last few decades been the most commonly used spectroscopic technique for extracting stereochemical information about flavonoids. CD measurements are simpler to perform than optical rotation measurements because they require only the detection of intensity variations, whereas rotation experiments require measurements of very small changes in electron polarization. Another benefit with CD spectroscopy is that this method, unlike optical rotation, is confined to the narrow absorption band of each chromo-

phore involved. Thus, it is easier to determine the contribution of individual chromophores, information vital to structural analysis. Derivatization of phenolic functionalities of flavonoids may also influence the sign of the optical rotation.

2.7.1 DETERMINATION OF ABSOLUTE FLAVONOID CONFIGURATION

The utilization of CD spectroscopy in the field of flavonoids has been reviewed by Lévai⁵³⁵ and Slade et al.⁵³⁶ The value of the method has primarily been related to establishing the absolute configuration of flavonoids and proanthocyanidins with stereogenic centers (chiral molecules), including the configuration of flavanones, dihydroflavonols, flavanols, flavans, isoflavans, isoflavanones, pterocarpanes, neoflavonoids, 4-arylflavan-3-ols, proanthocyanidins, various classes of biflavonoids, and auronols.^{107,117,535-545} These determinations have mainly been based on the sign of the observed CD bands (Figure 2.23) compared to reference compounds with defined stereochemistry. In general, Cotton effects observed in the 240 to 360 nm spectral region are similar for analogous flavonoid structures with the same configuration and independent of the substituents. Although optically active biflavonoids have been known since 1968, the first determination of the absolute configuration of optically pure biflavonoids was performed in 1995 (Figure 2.23),⁵³⁷ on the basis of CD spectroscopy and single-crystal x-ray crystallography.

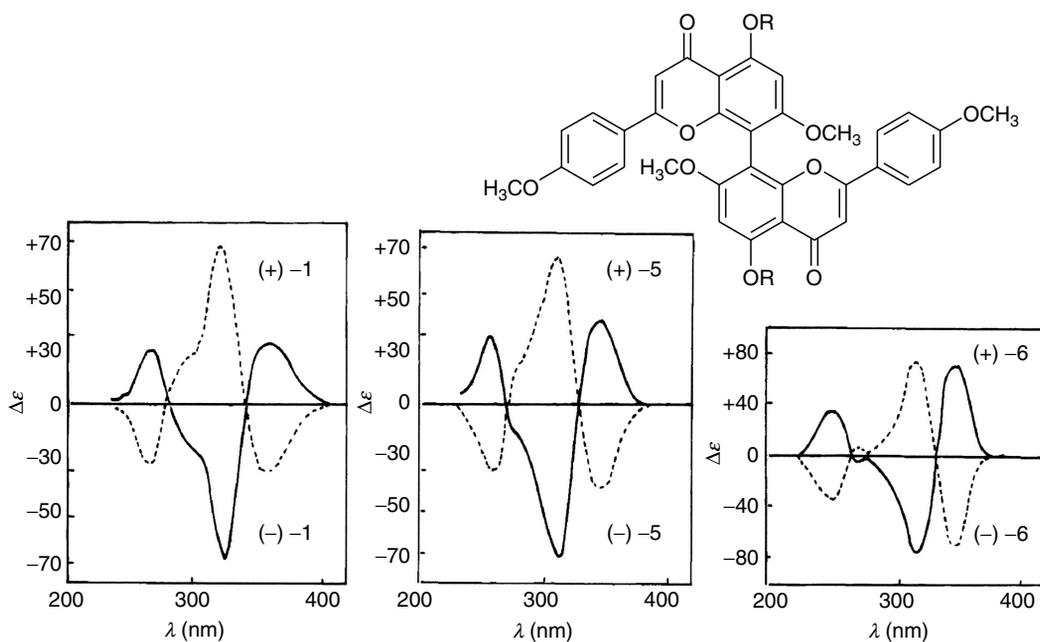


FIGURE 2.23 CD spectra of optically pure 8,8''-biflavones 5,5''-dihydroxy-4,4''',7,7''-tetramethoxy-8,8''-biflavone (1; R = H), its camphorsulphonate derivative (6; R = camphorsulphonate), and its dimethyl derivative, namely 5,5''-dihydroxy-4,4''',7,7''',5,5''-hexamethoxy-8,8''-biflavone (5; R = Me) (*c* 8 $\mu\text{g/ml}$, ethanol). These biflavones are optically active due to the atropisomerism of the biflavone moiety. The dashed lines refer to (+)-1, (+)-5, and (+)-6. The solid lines refer to (-)-1, (-)-5, and (-)-6. Cotton effects observed in the 240 to 360 nm spectral region are similar for the 8,8''-biflavones with the same configuration and independent of the substituents. (Reprinted from Zhang, F.J., Lin, G.Q., and Huang, Q.C., *J. Org. Chem.*, 60, 6427, 1995. Copyright 1995 American Chemical Society. With permission.)

During the review period, the benefits of coupled HPLC-CD as an analytical tool have been demonstrated in the flavonoid field. Based on HPLC-CD analysis, Antus et al.⁵⁴⁶ determined the absolute configuration of several *cis*-pterocarpanes with different substitution patterns. Using HPLC with a chiral stationary-phase column, coupled to a CD instrument, the separation of the C-2 diastereomers of naringin from *Citrus* juice and determination of their stereochemistry have also been achieved.⁵⁴⁷ In another interesting report, the absolute configuration of two new 3,8''-biflavonoid diastereomers from roots of *Gnidia involucrata* have been determined by online LC-CD measurements, which also implied revision of the absolute configurations of some known biflavanones.⁵⁴⁸

2.7.2 CIRCULAR DICHROISM IN STUDIES OF MOLECULAR FLAVONOID INTERACTION

The term chirality has a broader sense than involving just enantiomers and diastereoisomers. Chirality may arise from spatial isomerism resulting from the lack of free rotation around single or double bonds (chiral axis). Dichroism spectra have thus been valuable tools by providing conformational-related information about molecular shape, size, and electronic properties, binding parameters in molecular complexes, and the orientation of the chromophores.⁵⁴⁹ CD spectroscopy has, for instance, been used to gain insight into molecular association properties of anthocyanins involving aromatic stacking.^{550,551} More recent applications of dichroism spectroscopy have been related to evaluation of the interaction between the flavonol rutin with cyclodextrins,⁵⁵² interactions of the flavonoids quercetin, morin, rutin, and naringin with DNA,^{490,553-556} and studies of complexes between quercetin and human serum albumin, the most abundant carrier protein in blood.⁵⁵⁷ In this last case, it was revealed that when quercetin was bound to the asymmetric albumin environment, at least three induced CD bands appeared in the spectra. The induced CD bands were then utilized for calculation of the association constant and to probe the ligand binding site.

2.8 X-RAY CRYSTALLOGRAPHY

X-ray crystallography is the most accurate method for structural elucidation of flavonoids in the solid state. The method can only be applied to crystallized compounds, which has limited the number of flavonoid crystal structures reported.

2.8.1 X-RAY STUDIES ON FLAVONOID STRUCTURES

During the period of this review, the structure of several flavonoid aglycones including flavanonols,^{558,559} a flavanone,⁵³⁸ a prenylated chalcone,⁵⁶⁰ flavones,^{418,420,561,562} the desoxyanthocyanidin carajurin,⁵⁶³ and the conformation of the biflavonoid sciadopitysin in the solid state⁵⁶⁴ have been determined by x-ray analysis. The constitution and configuration of procyanidin B1 was proved by the x-ray analysis of its decaacetyl derivative.⁵⁶⁵ The structures of two flavones, two biflavonoids, and two flavanones have recently been investigated by single-crystal x-ray analysis.⁵⁶⁶ Crystallization of the two biflavonoids as their methanol and pyridine solvates, respectively, highlighted the role of the solvent molecules in stabilizing a crystal lattice. Intermolecular ' π - π ' interactions were observed in the crystal structures of the flavones and biflavonoids with centroid-centroid distances ranging from 3.70 to 3.81 Å and displacement angles ranging from 2.7 to 9.9°, but there was no ' π - π ' interaction in the flavanones.⁵⁶⁶ The flavonol mikanin-3-*O*-sulfate, in the form of its potassium salt, has been isolated from *Mikania micrantha*.⁵⁶⁷ The crystal structures of the 1:1 complex of potassium mikanin-3-*O*-sulfate (m-3-s) with methanol showed that the potassium ions in K(m-3-s)CH₃OH were bridged by O5, O7, and O8 to form a chain of face-sharing KO8

coordination polyhedra, from which the aglycone units were outstretched to form a polymeric molecular column. Adjacent molecular columns were linked by 'π-π' stacking between parallel, intercalating aglycon units to form layers, which were further interconnected into a 3D supramolecular assembly.⁵⁶⁷

Recent literature reports on the crystal structures of flavonoid glycosides are scarce; however, the x-ray structures of the flavonol quercetin 3-methyl ether 3'-glucoside⁵⁶⁸ and the flavone 6-hydroxyluteolin 7-rhamnoside⁵⁶⁹ have been reported. For the latter compound, crystals of the hepta-*O*-acetyl derivative were used. Cartormin, a rare semi-quinone chalcone sharing a pyrrole ring *C*-glycoside, has been isolated from *Carthamus tinctorius*, and its structure was established from various spectral data and a single-crystal x-ray analysis.⁵⁷⁰ Quercetin is known as a potent, competitive inhibitor of lipoxygenase LOX. Structural analysis has revealed that quercetin entrapped within this enzyme underwent degradation, and the resulting compound was identified by x-ray analysis as 3,4-dihydroxybenzoic acid positioned near the iron site.⁵⁷¹

The molecular structures of various synthesized flavonoid derivatives have been examined by x-ray crystallography in recent years,⁵⁷²⁻⁵⁷⁵ including a 3-methyl-substituted flavylum salt with photochromic properties⁵⁷⁶ and some phosphorylated flavones.⁵⁷⁷

2.8.2 X-RAY STUDIES ON COMPLEXES INVOLVING FLAVONOIDS

Flavocommelin is a flavonoid component of the blue pigment commelin, which has been isolated from the petals of *Commelina communis*. Commelin is composed of six molecules each of the anthocyanin malonylawobanin and flavocommelin, and two atoms of magnesium.⁵⁷⁸ The crystal structure of the octaacetate derivative of flavocommelin has been determined by x-ray diffraction.⁵⁷⁹ In the crystal, the molecules were arranged parallel to each other according to the periodicity of the crystal lattice. However, intermolecular stacking of the flavanone skeletons was not observed, which suggested that the hydrophilicity of the glucose moieties was one of the important factors governing the self-association. X-ray diffraction data collected at low temperature (130 K), using synchrotron radiation, have recently been used for determination of crystal architecture and conformational properties of the inclusion complex, neohesperidin dihydrochalcone-β-cyclodextrin.⁵⁸⁰ The complex was characterized by one aromatic part of neohesperidin dihydrochalcone deeply inserted into the hydrophobic cavity of β-cyclodextrin through the primary OH rim. The formation of other β-cyclodextrin inclusion complexes involving various flavanones,⁴³⁹ as well as the inclusion behavior of both 2-hydroxypropyl β-cyclodextrin and β-cyclodextrin in solution and solid-state toward quercetin,⁵⁸¹ have also been subjected to x-ray diffractometric analysis. Genistein and its amine complexes with morpholine and piperazine have been studied in the solid and liquid states by x-ray crystallography and ¹³C and ¹⁵N NMR spectroscopy.¹²⁸

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3.1 THE SCOPE OF THE REVIEW

Flavonoid biosynthesis is probably the best characterized of all the secondary metabolic pathways. Therefore, this chapter cannot hope to cover all the available information in detail as well as address recent results. Since 1994, tremendous progress has been made in the identification and analysis of genes or cDNAs for flavonoid biosynthetic enzymes and regulatory factors, in the analysis of enzyme structure and function, and the targeted manipulation of flavonoid production in transgenic plants. Thus, while a general overview of the biosynthetic pathway is presented in this chapter, the focus is on these recent advances. For further information on the biochemistry and classical genetics of flavonoids, the reader is referred to previous reviews as appropriate. In particular, the biochemistry and genetics of flavonoids were covered in detail in two editions of "The Flavonoids,"¹⁻³ and these excellent chapters are used as general references for much of the earlier literature. Also, Bohm⁴ provides an extensive review of flavonoid biosynthesis, including the biochemistry of enzyme

steps for which no cDNA clones are yet available. There are also several recent reviews on individual families of enzymes, such as the acyltransferases, to which reference is given. Furthermore, much biochemical information is available on the Internet, in particular the IUBMB (<http://www.chem.qmw.ac.uk/iubmb/enzyme>) and BRENDA (<http://www.brenda.uni-koeln.de>) databases. The EC numbers listed in Table 3.1 can be used to locate appropriate entries. Previous reviews in “The Flavonoids” have featured listings of genetic mutants affecting flavonoid biosynthesis for the classical model species. Much recent progress has been made with *Arabidopsis thaliana* (thale cress), and a listing of defined loci affecting flavonoid biosynthesis in this species is included in this chapter.

The advent of functional genomics has made it possible to analyze how genes or enzymes fit as part of larger families. Within the flavonoid pathway, important biosynthetic gene families include various transferases (for acyl, glycosyl, or methyl groups), reductases, and those for oxidative reactions — membrane-bound cytochrome P450-dependent mono-oxygenases (P450s) and soluble nonheme dioxygenases. Furthermore, the regulatory factors are also typically members of large gene families. Rather than present molecular phylogenies or a discussion of flavonoid gene evolution here, the reader is referred to other publications. In particular, Tanner⁵ presents recent molecular phylogenies for most of the flavonoid biosynthetic enzymes, and additional references are given for those gene families not included in that review.

3.2 OVERVIEW OF FLAVONOID BIOSYNTHESIS

The flavonoid pathway is part of the larger phenylpropanoid pathway, which produces a range of other secondary metabolites, such as phenolic acids, lignins, lignans, and stilbenes. The key flavonoid precursors are phenylalanine, obtained via the shikimate and arogenate pathways, and malonyl-CoA, derived from citrate produced by the TCA cycle. Most flavonoid biosynthetic enzymes characterized to date are thought to operate in enzyme complexes located in the cytosol. Flavonoid end products are transported to various subcellular or extracellular locations, with those flavonoids involved in pigmentation generally being transported into the vacuole.

There are many branches to the flavonoid biosynthetic pathways, with the best characterized being those leading to the colored anthocyanins and proanthocyanidins (PAs) and the generally colorless flavones, flavonols, and isoflavonoids. Genes or cDNAs have now been identified for all the core steps leading to anthocyanin, flavone, and flavonol formation, as well as many steps of the isoflavonoid branch, allowing extensive analysis of the encoded enzymes (Table 3.1). In addition, several DNA sequences are available for the modification enzymes that produce the variety of structures known within each class of compound.

Significant recent advances in our understanding of flavonoid biosynthesis include characterization of the formation of anthocyanidin 3-*O*-glucoside from leucoanthocyanidin, clarification of PA monomer formation, progress toward elucidating aurone and 3-deoxyflavonoid formation, the molecular characterization of several genes encoding enzymes that modify the flavonoid core structures, analysis of enzyme function, the determination of enzyme structures by x-ray crystallography and homology modeling, and the identification of several different groups of transcription factors regulating anthocyanin and PA biosynthesis. Data are also starting to emerge on the subcellular organization of the biosynthetic enzymes within the cytosol (and the role this may play in metabolic channeling), and transport mechanisms for the flavonoids within the cell. However, there are still major areas where data are lacking. Tertiary structures are available for only a few of the biosynthetic enzymes, little is known about the turnover or degradation of flavonoids, and details of post-transcriptional regulatory mechanisms are limited. Furthermore, the range of genes

TABLE 3.1
Flavonoid Biosynthetic Enzymes for which DNA Sequences Have Been Obtained

Enzyme	Abbreviation	EC number	Protein family	Ref. ^{1a}
<i>Flavonoid precursors</i>				
Acetyl-CoA carboxylase (cytosolic)	ACC	6.4.1.2	Biotin-containing carboxylases	8, 9
Phenylalanine ammonia-lyase	PAL	4.3.1.5	Ammonia-lyases	10
Cinnamate 4-hydroxylase	C4H	1.14.13.11	CytP450 (CYP73A)	15–17
4-Coumarate:CoA ligase	4CL	6.2.1.12	Adenylate-forming enzymes	10
Phenolic ester 3 β -hydroxylase	CYP93A3	1.14.13.–	CytP450 (CYP93A)	41
<i>The pathway to anthocyanins</i>				
Chalcone synthase	CHS	2.3.1.74	Polyketide synthase	44, 45
Chalcone isomerase	CHI	5.5.1.6	No named family	361
Flavanone 3 β -hydroxylase	F3H (FHT)	1.14.11.9	2-Oxoglutarate-dependent dioxygenase (2OGD)	64
Flavanone 4-reductase	FNR	1.1.1.234	NADPH reductase (RED)	69 ^b
Dihydroflavonol 4-reductase	DFR	1.1.1.219	RED	68, 69
Anthocyanidin synthase (leucoanthocyanidin dioxygenase)	ANS (LDOX)	1.14.11.19	2OGD	64, 77
UDP-Glc:anthocyanidin 3- <i>O</i> -glucosyltransferase/UDP-Glc:flavonol 3- <i>O</i> -glucosyltransferase ^c	F3GT	2.4.1.115/ 2.4.1.91	UDP- <i>O</i> -Glycosyltransferase (UGT)	362
UDP-Glc:anthocyanin 5- <i>O</i> -glucosyltransferase ^d	A5GT	2.4.1.–	UGT	88, 102
UDP-Glc:anthocyanin 3'- <i>O</i> -glucosyltransferase	A3GT	2.4.1.–	UGT	113
UDP-Rha:anthocyanidin 3- <i>O</i> -glucoside 6''- <i>O</i> -rhamnosyltransferase	A3RT	2.4.1.–	UGT	111, 112
SAM:anthocyanin 3'- <i>O</i> -methyltransferase	A3'OMT	2.1.1.–	SAM <i>O</i> -Methyltransferase (OMT)	Patent application WO03/062428
SAM:anthocyanin 3', 5'- <i>O</i> -methyltransferase	A3'5'OMT	2.1.1.–	OMT	Patent application WO03/062428
Hydroxycinnamoyl-CoA:anthocyanin 5- <i>O</i> -glucoside-6''- <i>O</i> -hydroxycinnamoyltransferase ^e	A5AT (Gt5AT)	2.3.1.153	Versatile acyltransferase (VAT)	121
Hydroxycinnamoyl-CoA:anthocyanidin 3- <i>O</i> -glucoside-6''- <i>O</i> -hydroxycinnamoyltransferase	A3AT (Pf3AT)	2.3.1.–	VAT	122
Malonyl-CoA:anthocyanin 5- <i>O</i> -glucoside-6''- <i>O</i> -malonyltransferase	A5MT (Ss5MaT1)	2.3.1.–	VAT	126
Malonyl-CoA:anthocyanidin 3- <i>O</i> -glucoside-6''- <i>O</i> -malonyltransferase	A3MT (Sc3MaT, Dm3MaT1, Dv3MaT)	2.3.1.–	VAT	123–125

Malonyl-CoA:anthocyanin 5- <i>O</i> -glucoside-4''- <i>O</i> -malonyltransferase	A5MT (Ss5MaT2)	2.3.1.-	VAT	406
Malonyl-CoA:anthocyanidin 3- <i>O</i> -glucoside-3''-6''- <i>O</i> -dimalonyltransferase	A3diMT (Dm3MaT2)	2.3.1.-	VAT	125
Flavonoid 3'-hydroxylase	F3'H	1.14.13.21	CytP450 (CYP75B)	105
Flavonoid 3',5'-hydroxylase	F3',5'H	1.14.13.-	CytP450 (CYP75A)	103
<i>Flavones, flavonols, and flavanones</i>				
Flavonol synthase	FLS	1.14.11.-	2OGD	146
Flavone synthase I	FNSI	1.14.11.-	2OGD	145
Flavone synthase II	FNSII	1.14.13.-	CytP450 (CYP93B)	143, 144
(2S)-Flavanone 2-hydroxylase	F2H	1.14.13.-	CytP450 (CYP93B)	223
UDP-Gal:flavonoid 3- <i>O</i> -galactosyltransferase	F3GalIT	2.4.1.-	UGT	156
UDP-Glc:flavonoid 7- <i>O</i> -glucosyltransferase ^f	F7GT	2.4.1.81/ 2.4.1.185	UGT	152, 153, 155
UDP-Glc:flavonoid 3,7,4'- <i>O</i> -glucosyltransferase ^g	UGT73G1	2.4.1.-	UGT	154
UDP-Rha:flavonol 3- <i>O</i> -rhamnosyltransferase	F3RT	2.4.1.159	UGT	153
UDP-Gal:flavonoid-3- <i>O</i> -galactosyltransferase/UDP-Gal:flavonol-3- <i>O</i> -galactosyltransferase	F3GalIT//PhF3GalIT	2.4.1.-	UGT	156, 158
UDP-Rha:flavanone 7- <i>O</i> -glucoside-2''- <i>O</i> -rhamnosyltransferase ^h	F7RT	2.4.1.-	UGT	407
SAM:flavonoid 7- <i>O</i> -methyltransferase	F7OMT	2.1.1.-	OMT	160
SAM:flavonol/dihydroflavonol 3',5'- <i>O</i> -methyltransferase	F3'5'OMT(CrOMT2)	2.1.1.149	OMT	167
SAM:flavonoid/HCA 3'- <i>O</i> -methyltransferase	F3'OMT (AfOMT1, CaOMT1)	2.1.1.-	OMT	164
SAM: 3'- <i>O</i> -methylflavonoid 4'- <i>O</i> -methyltransferase	F4'OMT (CrOMT6)	2.1.1.75	OMT	87
SAM:trimethylflavonol 3',5'- <i>O</i> -methyltransferase	F3'5'OMT (CaFOMT3)	2.1.1.-	OMT	161
SAM:flavonoid <i>O</i> -methyltransferase ⁱ	FOMT (PFOMT)	2.1.1.-	OMT	116
Flavonol 3- <i>O</i> -sulfotransferase	F3ST	2.8.2.25	Sulfotransferase	169
Flavonol 4'- <i>O</i> -sulfotransferase	F4ST	2.8.2.27	Sulfotransferase	169
Flavonoid 7- <i>O</i> -sulfotransferase	F7ST	2.8.2.28	Sulfotransferase	168
<i>Chalcones</i>				
Polyketide reductase	PKR (CHR, CHKR)	1.1.1.-	Aldo/keto-reductase	177
UDP-Glc:chalcone 2'- <i>O</i> -glucosyltransferase	C2'GT	2.4.1.-	UGT	Patent application WO03/18682
SAM:isoliquiritigenin/licodione 2'- <i>O</i> -methyltransferase	C2'OMT	2.1.1.65	OMT	221, 222

continued

TABLE 3.1
Flavonoid Biosynthetic Enzymes for which DNA Sequences Have Been Obtained — continued

Enzyme	Abbreviation	EC number	Protein family	Ref. ^a
<i>Aurones</i>				
Aureusidin synthase	AUS	1.21.3.6	Polyphenol oxidase	212
UDP-Glc:aureusidin 7- <i>O</i> -glucosyltransferase	AU7GT	2.4.1.–	UGT	Patent application WO00/49155
<i>Isoflavonoids</i>				
2-Hydroxyisoflavanone synthase (isoflavone synthase)	2HIS (IFS)	5.4.99.–	CytP450 (CYP93C)	180–182
Isoflavone reductase	IFR	1.3.1.45	RED	195, 196
Isoflavone 2'-hydroxylase	I2'H	1.14.13.53	CytP450 (CYP81E)	193
Isoflavone 3'-hydroxylase	I3'H	1.14.13.52	CytP450 (CYP81E)	194
SAM:isoflavone 7- <i>O</i> -methyltransferase	I7OMT	2.1.1.150	OMT	190
SAM:2,7,4'-trihydroxyisoflavone 4'- <i>O</i> -methyltransferase	H14'OMT	2.1.1.46	OMT	187
Vestitone reductase	VR	1.1.1.246	RED	200
Pterocarpan 6a-hydroxylase (3,9-dihydroxypterocarpan 6a-hydroxylase)	P6aH (DH6aH)	1.14.13.28	CytP450 (CYP93A)	202
SAM:pterocarpan 3- <i>O</i> -methyltransferase	P3OMT (HM3OMT)	2.1.1.–	OMT	203
Flavonoid 6-hydroxylase	F6H	1.14.13.–	CytP450 (CYP71D)	210
UDP-Glc:formononetin 7- <i>O</i> -glucosyltransferase	I7GT	2.4.1.–	UGT (UGT73F1)	209
<i>Proanthocyanidins</i>				
Leucoanthocyanidin reductase	LAR (LCR)	1.17.1.3	RED	133
Anthocyanidin reductase	ANR	1.1.1.–	RED	136, 137

^aReference is given only to the first publications on the isolation and characterization of the corresponding cDNA/gene.

^bSome DFRs show FNR activity also, and reference is given to the first example of isolation of a cDNA for such a DFR.

^cSome 3GTs analyzed will accept other flavonoids in addition to anthocyanidins as substrates, and so the term F3GT is used.

^dRecombinant A5GT proteins show varying degrees of anthocyanin substrate specificity.

^eThe nomenclature for the AATs follows Nakayama et al.¹²⁰ The positional numbering of the sugar hydroxyl that is modified is given followed by prime symbols to indicate which sugar is affected. The double and triple primes indicate the 3-*O*-glycosyl and 5-*O*-glycosyl, respectively. Recombinant AATs show varying degrees of substrate specificity.

^fA number of flavonoid-7-*O*-GT cDNAs have been isolated, many encoding recombinant proteins with wide substrate acceptance.

^gUGT73G1 can produce mono- or diglucosides with a wide range of flavonoid substrates, adding glucose to one or two of the indicated hydroxyls.

^hThe *Citrus* enzyme catalyzes 2''-*O*-rhamnosylation of the 7-*O*-glucoside of flavanones and flavones but not flavonols.

ⁱThe recombinant product is able to catalyze methylation at a variety of flavonoid hydroxyl positions.

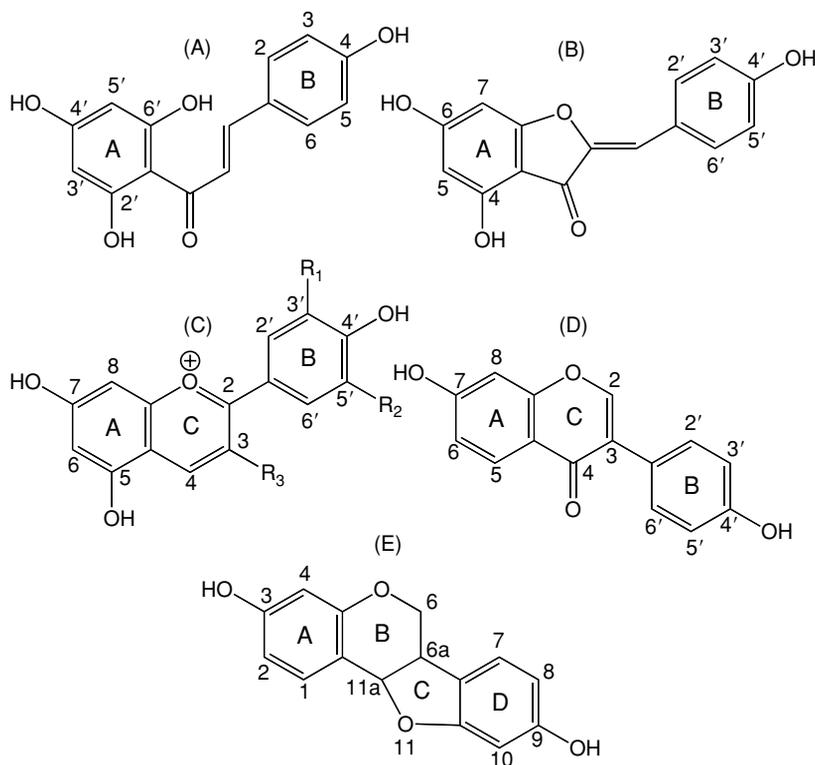


FIGURE 3.1 Base structures of a chalcone (A), an aurone (B), the main anthocyanidins (C), an isoflavonoid (D), and a pterocarpin (E). The lettering of the carbon rings is shown, as well as the numbering of the key carbons. Note that the numbering is different for each type of compound. For the majority of flavonoid types the numbering is as for the anthocyanidins, and for the cinnamic acids it is as for the chalcones. R_1 , R_2 , and R_3 substitutions determine the various common anthocyanidins. The common 3-hydroxyanthocyanidins ($R_3 = \text{OH}$) are pelargonidin (R_1 and $R_2 = \text{H}$), cyanidin ($R_1 = \text{OH}$ and $R_2 = \text{H}$), delphinidin (R_1 and $R_2 = \text{OH}$), peonidin ($R_1 = \text{OCH}_3$ and $R_2 = \text{H}$), petunidin ($R_1 = \text{OCH}_3$ and $R_2 = \text{OH}$), and malvidin (R_1 and $R_2 = \text{OCH}_3$). The rare 3-deoxyanthocyanidins ($R_3 = \text{H}$) are apigeninidin (R_1 and $R_2 = \text{H}$), luteolinidin ($R_1 = \text{OH}$ and $R_2 = \text{H}$), and tricetinidin (R_1 and $R_2 = \text{OH}$).

encoding secondary modification enzymes that have been characterized is still limited compared to the great array of known flavonoid structures. Nor have cDNAs or genes been published for some of the enzymes carrying out hydroxylation of the core flavonoid structure, such as at the C-8 and C-2' positions.

The key enzymes involved in the formation of the hydroxycinnamic acids (HCAs) from phenylalanine and malonyl-CoA are now discussed in detail, while later sections address the branches of the flavonoid pathway leading to anthocyanins, aurones, flavones, flavonols, PAs, and isoflavonoids. This is followed by brief reviews of the regulation of flavonoid biosynthesis and the use of flavonoid genes in plant biotechnology. To assist the reader, Figure 3.1 presents the carbon numbering for the various flavonoid types discussed.

3.3 BIOSYNTHESIS OF FLAVONOID PRECURSORS

The first flavonoids, the chalcones, are formed from HCA-CoA esters, usually 4-coumaroyl-CoA (Figure 3.2), in three sequential reactions involving the “extender” molecule

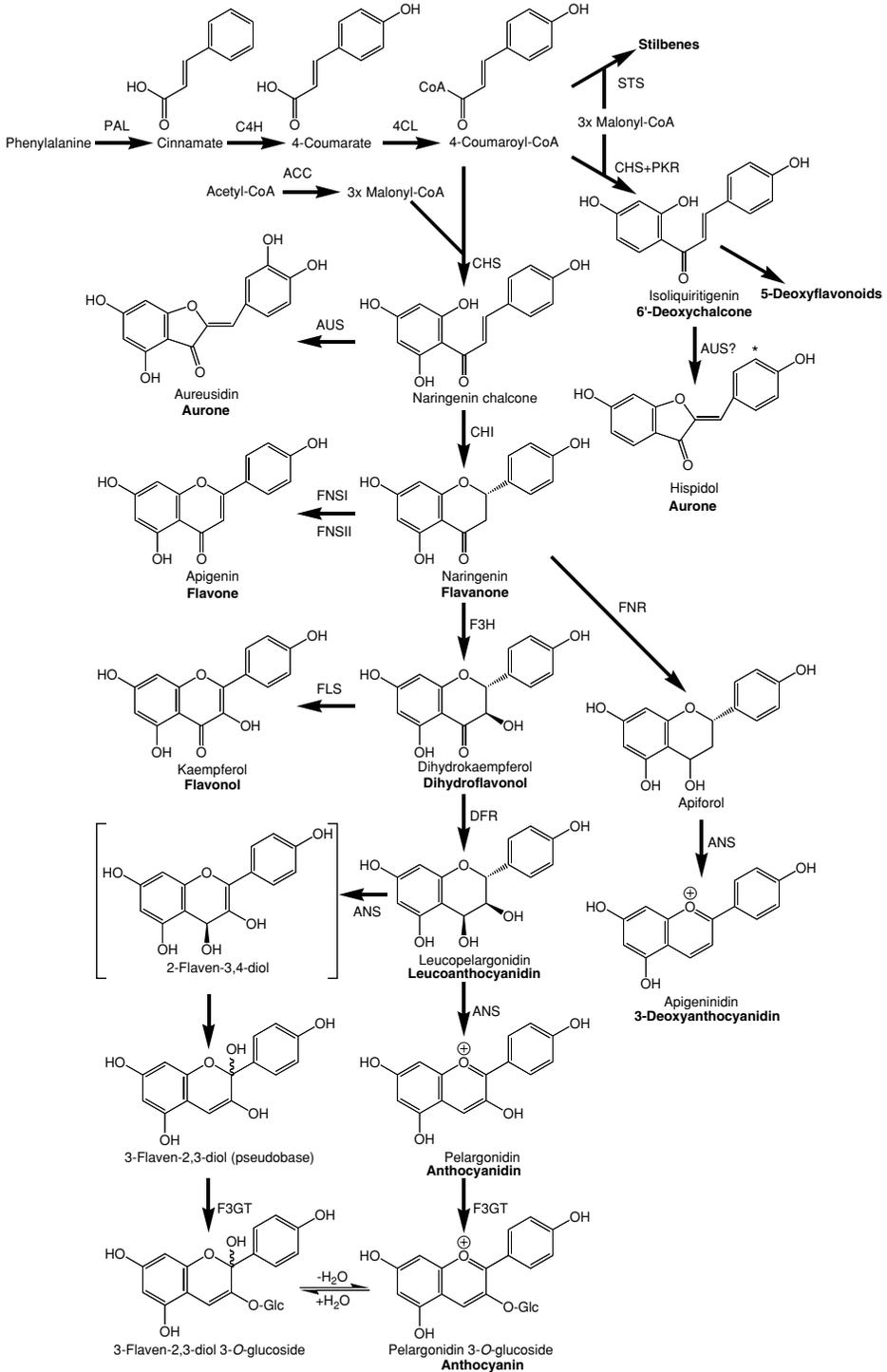


FIGURE 3.2 General phenylpropanoid and flavonoid biosynthetic pathways. The B-ring hydroxylation steps are not shown. For formation of anthocyanins from leucoanthocyanidins two routes are represented: a simplified scheme via the anthocyanidin (pelargonidin) and the likely *in vivo* route via the pseudobase. Enzyme abbreviations are defined in the text and in Table 3.1.

malonyl-CoA. In a few species, caffeoyl-CoA and feruloyl-CoA may also be used as substrates for chalcone formation.

4-Coumaroyl-CoA is produced from the amino acid phenylalanine by what has been termed the general phenylpropanoid pathway, through three enzymatic conversions catalyzed by phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), and 4-coumarate:CoA ligase (4CL). Malonyl-CoA is formed from acetyl-CoA by acetyl-CoA carboxylase (ACC) (Figure 3.2). Acetyl-CoA may be produced in mitochondria, plastids, peroxisomes, and the cytosol by a variety of routes. It is the cytosolic acetyl-CoA that is used for flavonoid biosynthesis, and it is produced by the multiple subunit enzyme ATP-citrate lyase that converts citrate, ATP, and Co-A to acetyl-CoA, oxaloacetate, ADP, and inorganic phosphate.⁶

Many other compounds are involved in flavonoid biosynthesis in some species, for example, as donors for methylation or aromatic or aliphatic acylation. For intact plants, these are generally accepted to be available in the cell for the reaction to proceed if the appropriate modification activity is present.

3.3.1 ACETYL-COA CARBOXYLASE

ACC (also termed the acetyl-CoA:carbon-dioxide ligase [ADP-forming]) catalyzes the ATP-dependent carboxylation of acetyl-CoA, with Mg^{2+} as a cofactor, to form malonyl-CoA. ACC activity is found in both the plastid, where a heteromeric enzyme supports fatty acid biosynthesis, and the cytoplasm, in which a homodimeric enzyme of around 250 kDa supplies malonyl-CoA for the synthesis of a range of compounds that includes the flavonoids.⁷ Full-length cDNAs for the plant cytoplasmic isoenzyme were first identified from *Medicago sativa* (alfalfa) using a probe from the human protein disulfide isomerase,⁸ and from *A. thaliana* using polymerase chain reaction (PCR) with degenerate oligonucleotide sequences based on nonplant ACC sequences.⁹ Sufficient amino acid similarity was found to the rate ACC to enable a full protein line-up and identification of putative binding sites for ATP, acetyl-CoA, and carboxybiotin. Sequences from several species are now available, and studies on the regulation of the gene are well advanced. ACC activity is induced in response to stimuli that increase flavonoid biosynthesis, such as ultraviolet (UV) light and fungal elicitors.

3.3.2 PHENYLALANINE AMMONIA LYASE

PAL is one of the best-characterized enzymes of plant secondary metabolism. It converts L-phenylalanine into *trans*-cinnamate (*E*-cinnamate) by the *trans*-elimination of ammonia and the pro-3S proton (see Ref. 4 for a full reaction discussion). The enzyme, which requires no cofactor, is a tetramer of 310–340 kDa.³ A cDNA for PAL was first isolated from *Petroselinum crispum* (parsley),¹⁰ and others have subsequently been isolated from numerous species. Often PAL is produced from a multigene family and is present in a variety of isoenzyme forms.

Enzymatic preparations for PAL from monocotyledonous species (monocots) can show a similar activity against tyrosine (tyrosine ammonia lyase, TAL), and TAL enzymatic preparations also show PAL activity. That a single enzyme may account for the observed co-occurring TAL and PAL activities was confirmed by Rösler et al.,¹¹ who showed the recombinant *Zea mays* (maize) PAL converted tyrosine to 4-coumarate directly, thus removing the requirement for the usual 4-hydroxylation step in phenylpropanoid biosynthesis.

Considerable progress has been made with elucidating the functional aspects of the PAL protein, assisted by the availability of the crystal structure for histidine ammonia lyase (HAL), which catalyzes a reaction similar to that of PAL, the conversion of L-histidine to

E-urocanic acid. Röther et al.¹² used the HAL structure to generate a homology-based model of PAL and to identify specific amino acids to target in PAL that may have an equivalent role in the active site and reaction mechanism. The same group has proposed a reaction mechanism involving electrophilic attack at the phenyl ring by a prosthetic group.¹³ K_m values in the order of 0.1 mM have been reported for recombinant PAL proteins.¹² PAL activity *in vivo* appears to be modulated by signals generated by the levels of cinnamic acid, altering both gene transcription and enzyme activity (reviewed in Ref. 14).

3.3.3 CINNAMATE 4-HYDROXYLASE

C4H catalyzes the hydroxylation of *trans*-cinnamate to *trans*-4-coumarate in the initial oxygenation step of phenylpropanoid biosynthesis, which introduces the 4'-hydroxyl that is common to most flavonoids. The isolation of a cDNA for C4H was first reported from *Helianthus tuberosus* (Jerusalem artichoke), *M. sativa*, and *Vigna radiata* (mung bean), based on purification and amino acid sequencing of the protein^{15,16} or similarity to other members of the same enzyme superfamily.¹⁷ Genes or cDNAs for C4H have subsequently been cloned from many species, with Hübner et al.¹⁸ listing over ten species and Gravot et al.¹⁹ noting 41 different C4H cDNAs in public databases.

Based on the amino acid sequences, two classes of C4H have been described for some species, with around 60% sequence similarity between the groups. The first sequence for the class II type was reported from *Phaseolus vulgaris* (French bean),²⁰ but they have also been found in other species (see, e.g., Ref. 21). The two C4H types differ at both terminal domains and in three internal domains, and it has been suggested that one type may be involved in stress responses and the other in vascular differentiation.^{20,21}

Enzyme characteristics have been examined for recombinant C4H proteins from several species, including those from *P. crispum*, *P. vulgaris*, *Ammi majus*, *H. tuberosus*, and *Ruta graveolens*.^{18–22} Similar K_m values toward cinnamate (2 to 10 μ M) are reported, and consistently high substrate specificity (although the V_{max} values vary between studies). Only 4-coumarate is found as the *in vitro* product, with no detectable 2- or 3-coumarate production.²²

C4H was one of the first plant enzymes of the P450 enzyme superfamily (EC 1.14.13.X) to be characterized at the encoding DNA sequence level. Several members of this family are involved in flavonoid biosynthesis, and it is worth mentioning some of the general features of this remarkable group of enzymes. P450s are heme-dependent mixed function mono-oxygenases that require O₂ and NADPH for activity. All those involved in flavonoid biosynthesis that have been characterized to date are A-type P450s localized in the microsomal fraction. P450s of plants require a second enzyme system as an additional redox partner for the transfer of electrons from NADPH to oxygen via the heme iron. This is typically the flavoprotein NADPH-P450 reductase, but sometimes NADH-dependent Cyt *b*₅ reductase is used in conjunction with Cyt *b*₅. Plant P450s are typically 50 to 60 kDa in size, and C4H is usually around 60 kDa. The general characteristics of P450s of plants are reviewed in more detail in several articles (such as in Refs. 23, 24).

P450s are grouped according to molecular phylogeny (rather than function) into families ($\geq 40\%$ amino acid positional identity), which for plants are given a number from CYP71 to CYP99 then CYP701 onwards, and subfamilies ($\geq 55\%$ amino acid identity) designated by a letter. Thus, C4H is in subfamily A of family 73 and termed CYP73A. Individual genes, either from different loci in a species or from different species, may then be numbered; e.g., the flavonoid 3',5'-hydroxylase (F3',5'H) cDNAs for the *Hf1* and *Hf2* loci of *Petunia hybrida* were named CYP75A1 and CYP75A3, and that from *Eustoma grandiflorum* (lisianthus) CYP75A5. Sequences from the same species with $>97\%$ identities are assumed to be allelic variants unless otherwise demonstrated.

P450 amino acid sequences contain several well-conserved regions, such as the heme binding domain (generally FxxGxxxCxG) and the proline rich region (PPxP) that forms a hinge between the membrane anchored N terminal and the rest of the protein. Rupasinghe et al.²⁵ have modeled the structure of C4H and three other *A. thaliana* P450s involved in phenylpropanoid biosynthesis, based on the amino acid sequences and crystal structures of other P450s. In addition to C4H these were the flavonoid 3'-hydroxylase (F3'H) and two enzymes involved in the monolignol pathway. The analysis showed that, despite low amino acid sequence identities in some cases, the enzymes displayed significant conservation in terms of structure and substrate recognition.

Linear furanocoumarins (psoralens) inhibit P450s as mechanism-based inactivators (suicide inhibitors). Thus, species that produce psoralens may have evolved C4H enzymes with enhanced tolerance to these compounds.^{18,19} Recombinant C4H from the psoralen-producing species *R. graveolens* showed markedly slower inhibition kinetics with psoralens, and possibly biologically significant tolerance, compared to C4H from a species that does not produce the compounds (*H. tuberosus*).¹⁹

3.3.4 4-COUMARATE:COA LIGASE

4CL activates the HCAs for entry into the later branches of phenylpropanoid biosynthesis through formation of the corresponding CoA thiol esters. 4-Coumarate, the product of C4H, is key for flavonoid biosynthesis, but 4CL will commonly accept other HCAs as substrates. Generally, 4-coumarate and caffeate are the preferred substrates, followed by ferulate and 5-hydroxyferulate, with low activity against cinnamate and none with sinapate. However, different isoenzymes have been identified that exhibit distinct substrate specificities, including within the same species. These include isoforms with a variant substrate-binding pocket that will accept sinapate,^{26,27} those that will not accept ferulate,²⁸ and isoforms that differ in their ability to accept 5-hydroxyferulate.²⁹ It is thought that the different isoenzymes may have distinct roles in metabolic channeling in flavonoid or lignin biosynthesis (see, e.g., Refs. 26, 30). Supporting an important role for the variant isoforms is the observation that 4CL is encoded by gene families in all species examined to date.³¹ The evolution of the 4CL gene family of plants is discussed extensively in Refs. 27, 31.

The formation of the HCA-CoA esters proceeds through a two-step reaction, with Mg²⁺ as a cofactor. In the first step, 4-coumarate and ATP form a coumaroyl-adenylate intermediate, with the simultaneous release of pyrophosphate. In the second step, the coumaroyl group is transferred to the sulfhydryl group of CoA, with the release of AMP. The reaction mechanism of 4CL is discussed in detail in Pietrowska-Borek et al.,³² including the newly discovered ability of recombinant *A. thaliana* 4CL2 (At4CL2) to synthesize mono- and diadenosine polyphosphates. Based on the presence of a highly conserved peptide motif, 4CL has been placed in the adenylate-forming superfamily that also includes acetyl-CoA synthetases, long-chain fatty acyl-CoA synthetases, luciferases, and peptide synthetases.^{28,31,33} 4CL contains amino acid motifs conserved either among the superfamily or just among 4CL sequences. Mutagenesis, domain swapping, and homology modeling analyses have shown the functional importance of some of these regions and identified amino acids important in the specificity shown toward the different substrates.^{28,34,35} This information has been used to modify the At4CL2 isoform to accept ferulate and sinapate,^{34,35} and to change the substrate specificities of the *Glycine max* (soybean) Gm4CL2 and Gm4CL3 isoforms.³³

The route to formation of flavonoids lacking 4'-hydroxylation of the B-ring has not been elucidated. However, one possible route is the direct use of cinnamate as a substrate by 4CL. Activity on cinnamate has been shown at low levels for some of the recombinant 4CL

proteins, and a separate cinnamoyl:CoA ligase with specific activity on cinnamate and not 4-coumarate has been characterized in some species.³⁶ Data with regard to subsequent enzymes accepting the alternative substrates are limited, but cinnamoyl-CoA is used by recombinant chalcone synthase (CHS) from several species, as well as CHS-like enzymes such as pinosylvin synthase (EC 2.3.1.146) (see, e.g., Refs. 37, 38). Indeed, the recombinant CHS from *Cassia alata* (ringworm bush) can use a wide range of substrates to make various products, including 4-deoxychalcones.³⁹ However, definitive evidence showing this route to 4'-deoxyflavonoids *in vivo*, from plant studies for example, has not been published.

3.3.5 MODIFICATION OF HYDROXYCINNAMIC ACID-CoA ESTERS

4-Coumaroyl-CoA is the major substrate for entry into flavonoid biosynthesis through the action of CHS. However, some CHS enzymes may also use caffeoyl-CoA or feruloyl-CoA;² and various HCA-CoA esters are used by the aromatic acyltransferases that modify the flavonoid glycosides. Although it might be expected that caffeoyl-CoA would be formed by direct 3-hydroxylation of 4-coumaroyl-CoA, and feruloyl-CoA by subsequent 3-*O*-methylation, varying biosynthetic routes may exist.^{3,40} In particular, recombinant CYP98A3 of *A. thaliana*, which encodes an enzyme that can add a hydroxyl at the C-3 position of 4-cinnamate derivatives, shows no activity toward 4-coumaroyl-CoA, and only low-level activity toward 4-coumarate, the preferred substrates being the 5-*O*-shikimate and 5-*O*-quininate esters.^{41–43} However, analysis of the corresponding mutant *ref8* supports an *in vivo* role for CYP98A3 in phenylpropanoid 3-hydroxylation. Similarly, the ferulate 5-hydroxylase (F5H, CYP84A1 of *A. thaliana*) shows greater preference toward coniferaldehyde and coniferyl alcohol than ferulate or feruloyl-CoA. In addition, *O*-methylation can occur both on caffeoyl-CoA and 5-hydroxyferuloyl-CoA and their respective aldehyde forms. Thus, a metabolic grid seems to prevail generally for the reactions on the HCAs, particularly with respect to formation of the monolignols. Some of the downstream flavonoid biosynthesis enzymes have been studied for acceptance of *O*-methylated substrates, in particular CHS (see Section 3.4.1), and some of the oxidoreductases (see Section 3.4.5).

3.4 FORMATION OF ANTHOCYANINS

The flavonoid pathway starts with the formation of the C₁₅ backbone by CHS. Chalcones are then generally directly or indirectly converted to a range of other flavonoids in a pathway of intersecting branches, with intermediate compounds being involved in the formation of more than one type of end product (Figure 3.2). This section discusses the genes and enzymes involved in the formation of the simplest common anthocyanins, 3-hydroxyanthocyanidin 3-*O*-glycosides, which require a minimum of five enzymatic steps subsequent to the formation of chalcone (Figure 3.2).

3.4.1 CHALCONE SYNTHASE

CHS carries out a series of sequential decarboxylation and condensation reactions, using 4-coumaroyl-CoA (in most species) and three molecules of malonyl-CoA, to produce a polyketide intermediate that then undergoes cyclization and aromatization reactions that form the A-ring and the resultant chalcone structure. The chalcone formed from 4-coumaroyl-CoA is naringenin chalcone. However, enzyme preparations and recombinant CHS proteins from some species have been shown to accept other HCA-CoA esters as substrates, such as cinnamoyl-CoA (see, e.g., Ref. 37). In particular, the *Hordeum vulgare* (barley) CHS2 cDNA encodes a CHS protein that converts feruloyl-CoA and caffeoyl-CoA at the highest rate, and cinnamoyl-CoA and 4-coumaroyl-CoA at lower rates.³⁸

The key role of CHS in flavonoid biosynthesis has made it a focus of research for many years, and it is now very well characterized. The isolation of a cDNA for CHS represented the first gene cloned for a flavonoid enzyme.^{44,45} *CHS* sequences, and a series of *CHS*-like sequences, have now been characterized from many species, and Austin and Noel⁴⁶ have identified close to 650 *CHS*-like sequences in public databases.

Native CHS is a homodimer with subunits of 40 to 44 kDa. The structure of the protein produced from the *CHS2* cDNA of *M. sativa* has been determined and the residues of the active site defined.⁴⁷ It belongs to the polyketide synthase (PKS) group of enzymes that occur in bacteria, fungi, and plants, and is a type III PKS. All the reactions are carried out at a single active site without the need for cofactors.⁴⁷⁻⁴⁹

PKSs are characterized by their ability to catalyze the formation of polyketide chains from the sequential condensation of acetate units from malonate thioesters. In plants they produce a range of natural products with varied *in vivo* and pharmacological properties. PKSs of particular note include acridone synthase, bibenzyl synthase, 2-pyrone synthase, and stilbene synthase (STS).⁴⁶ STS forms resveratrol, a plant defense compound of much interest with regard to human health. STS shares high sequence identity with CHS, and is considered to have evolved from CHS more than once.⁵⁰ Knowledge of the molecular structure of the *CHS*-like enzymes has allowed direct engineering of CHS and STS to alter their catalytic activities, including the number of condensations carried out (reviewed in Refs. 46, 51, 52). These reviews also present extensive, and superbly illustrated, discussions of CHS enzyme structure and reaction mechanism.

3.4.2 CHALCONE ISOMERASE

In a reaction that establishes the flavonoid heterocyclic C-ring, chalcone isomerase (CHI) catalyzes the stereospecific isomerization of chalcones to their corresponding (2*S*)-flavanones, via an acid base catalysis mechanism.^{53,54} Almost 40 years ago, the first flavonoid enzyme to be described was CHI (in the adopted hometown of the authors of this chapter).⁵⁵ Since then CHI has been analyzed in great detail, and surprisingly, it shows little similarity to other known protein sequences,⁵⁴ although CHI-like sequences have recently been reported from plants and other organisms.⁵⁶

CHI has a deduced molecular weight (MW) of 27 to 29 kDa, although possible *in vivo* modifications have been reported.⁵⁷ Two types of CHI have been identified, the more common CHI-I type, which can use only 6'-hydroxychalcone substrates, and the CHI-II type, which can catalyze isomerization of both 6'-hydroxy- and 6'-deoxychalcones. CHI-II is prevalent in legumes, although sequence analysis and recent transgenic results⁵⁸ suggest the activity also occurs in nonlegumes. Sequences from different species for the same type of CHI show amino acid identities of >70%, while between type I and type II identities of about 50% are found. Genes for both types of CHI occur as a cluster in *Lotus japonicus*,⁵⁹ and the presence of tandem gene copies suggests an origin for *CHI-II* by gene duplication and divergence from *CHI-I*.

The structure of the recombinant *M. sativa* CHI-II protein has been determined to 1.85 Å resolution. The progress of the reaction in the reactive site cleft has been elucidated, and a full reaction sequence proposed.⁵²⁻⁵⁴ However, the basis for the specificities toward 6'-hydroxy- and 6'-deoxychalcones was not resolved, although potentially key amino acid residues were identified.

With 6'-hydroxychalcones, such as naringenin chalcone, the isomerization reaction can readily occur nonenzymically to form racemic (2*R*,2*S*) flavanone. This occurs easily *in vitro* and has been reported to occur *in vivo* to the extent that moderate levels of anthocyanin can be formed.³ However, 6'-deoxychalcones are stable under physiological conditions, due to an

intramolecular hydrogen bond between the 2'-hydroxyl and the carbonyl group, and CHI-II is required to convert them to flavanones. CHI accelerates ring closure to a 10^7 -fold acceleration over the spontaneous reaction rate, but with significantly slower kinetics for the 6'-deoxychalcones than 6'-hydroxychalcones; and ensures formation of the biosynthetically required (2*S*)-flavanones.^{53,54,60} The requirement for CHI for significant flavonoid biosynthesis in some plants is well illustrated by the acyanic phenotype of the *transparent testa5* (*tt5*) *CHI* mutation of *A. thaliana*, and the increased levels of flavonols in *CaMV35S:CHI* transgenic plants of *Lycopersicon esculentum* (tomato).⁶¹

3.4.3 FLAVANONE 3 β -HYDROXYLASE

(2*S*)-Flavanones are converted stereospecifically to the respective (2*R*,3*R*)-dihydroflavonols (DHF) by flavanone 3 β -hydroxylase. Stereospecificity for (2*S*)-flavanones has been confirmed by analysis of the recombinant protein.⁶² Flavanone 3 β -hydroxylase is commonly abbreviated to F3H, which is what has been used in this chapter, but FHT is also used in the literature, which agrees with the nomenclature for phenylpropanoid biosynthesis proposed in Heller and Forkmann.³

F3H is a soluble nonheme dioxygenase dependent on Fe^{2+} , O_2 , and 2-oxoglutarate (2OG), and thus belongs to the family of 2OG-dependent dioxygenases (2OGDs). 2OGDs have been characterized from mammalian, microbial, and plant sources, and they all use four electrons generated from oxoglutarate decarboxylation to split di-oxygen and create reactive enzyme-iron species. The protein family is well represented in flavonoid biosynthesis, as can be seen from Table 3.1, and this will be discussed later. Further details on the 2OGD family are given in Refs. 62, 63.

A cDNA for F3H was first isolated from *Antirrhinum majus* (snapdragon),⁶⁴ and since then genes and cDNAs have been isolated from over a dozen other species.^{5,65} The native protein is a monomer of 41 to 42 kDa, although proteolysis during purification gave values of 34 to 39 kDa in early studies of the enzyme. Using sequence comparison and analysis of recombinant proteins good progress has been made in elucidating the tertiary structure of the enzyme, the nature of the active site, and the binding of 2OG and Fe^{2+} . Britsch et al.⁶⁵ identified 14 amino acids strictly conserved among F3H sequences from several species, including histidines with a putative role in Fe^{2+} binding. Mutation analysis of recombinant *P. hybrida* F3H showed that His220, Asp222, and His278 are indeed involved in Fe^{2+} binding in the active site, and that Arg288 and Ser290 are required for 2OG binding.^{66,67} Full characterization awaits determination of the crystal structure for the enzyme.

3.4.4 DIHYDROFLAVONOL 4-REDUCTASE

Dihydroflavonol 4-reductase (DFR) catalyzes the stereospecific conversion of (2*R*,3*R*)-*trans*-DHF to the respective (2*R*,3*S*,4*S*)-flavan-2,3-*trans*-3,4-*cis*-diols (leucoanthocyanidins) through a NADPH-dependent reduction at the 4-carbonyl. DNA sequences for DFR were first identified from *A. majus* and *Z. mays*,^{68,69} and the identity of the *Z. mays* sequence confirmed by *in vitro* transcription and translation of the cDNA and assay of the resultant protein.⁷⁰ DNA sequences have now been cloned from many species, with the size of the predicted protein averaging about 38 kDa. Stereospecificity to (2*R*,3*R*)-dihydroquercetin (DHQ) has been shown for some recombinant DFR proteins.⁷¹

DFR belongs to the single-domain-reductase/epimerase/dehydrogenase (RED) protein family, which has also been termed the short chain dehydrogenase/reductase (SDR) superfamily. This contains other flavonoid biosynthetic enzymes, in particular the anthocyanidin reductase (ANR), leucoanthocyanidin reductase (LAR), isoflavone reductase (IFR), and vestitone reductase (VR), as well as mammalian, bacterial, and other plant enzymes.^{72,73}

The preference shown by DFR toward the three common DHFs varies markedly between species, with some enzymes showing little or no activity against dihydrokaempferol (DHK) and others showing preference toward dihydromyricetin (DHM). In particular, DFR in *Cymbidium hybrida* (cymbidium orchids), *L. esculentum*, *Petunia*, and *Vaccinium macrocarpon* (cranberry) cannot efficiently reduce DHK,^{3,74,75} so that pelargonidin-based anthocyanins rarely accumulate in these species. However, DFR enzymes of many species accept all three DHFs, and DHM can be used by *Dendranthema* (chrysanthemum), *Dahlia variabilis*, *Dianthus caryophyllus* (carnation), *Matthiola*, and *Nicotiana* (tobacco) flowers even though delphinidin derivatives do not naturally occur in these ornamentals.^{3,76}

Some species contain a closely related enzyme activity to DFR that can act on flavanones, termed the flavanone 4-reductase (FNR), which may represent a variant DFR form. This is discussed in more detail in Section 3.4.7. 5-Deoxyleucoanthocyanidin compounds are known to occur in legumes, and analysis of two recombinant DFR proteins (MtDFR1 and MtDFR2) from *Medicago truncatula* (barrel medic) has found activity on the 5-deoxyDHF substrates fustin and dihydrorobinetin.⁷¹ Indeed, fustin was the preferred substrate of both recombinant enzymes. MtDFR1 and MtDFR2 showed distinct enzyme characteristics, and overexpression of MtDFR1 but not MtDFR2 promoted anthocyanin biosynthesis in flowers of *N. tabacum*.

Substrate specificity between DHK, DHQ, and DHM appears, based on chimeric DFR proteins formed using the *P. hybrida* and *Gerbera hybrida* sequences, to locate to a 26 amino acid region that may be the binding pocket for the B-ring, and as little as one amino acid change in this region can alter the specificity of the enzyme.⁷³

3.4.5 ANTHOCYANIDIN SYNTHASE

The role of anthocyanidin synthase (ANS) in the biosynthetic pathway is to catalyze reduction of the leucoanthocyanidins to the corresponding anthocyanidins. However, *in vivo* it is anthocyanidins in pseudobase form that are formed, as is described below. In this chapter, use of anthocyanidin should be taken to include the pseudobase form. Furthermore, although the name ANS is commonly used, the enzyme is also referred to in the literature as leucoanthocyanidin dioxygenase (LDOX), reflecting the reaction type.

Much of the information on ANS has come not from studies on enzyme extracts but from analysis of DNA sequences and recombinant proteins. Sequences for the ANS were first isolated using transposon generated mutant lines of *A. majus* and *Z. mays*.^{64,77} They encoded proteins of 40 to 41 kDa that were found to have similarity to 2OGDs, during a study on a nonflavonoid enzyme.⁷⁸ This sequence-based identification was confirmed by the *in vitro* assay of the recombinant *Perilla frutescens* protein,⁷⁹ and subsequent assays on recombinant ANS from a range of species that confirmed the requirement for Fe²⁺, 2OG, and ascorbate.^{80,81} Sequence comparisons show that ANS is more closely related to flavonol synthase (FLS), another 2OGD, than to F3H.

From extensive analysis of recombinant proteins, and the crystal structure of *A. thaliana* protein, detailed reaction mechanisms have been proposed.^{80–85} The ANS reaction likely proceeds via stereospecific hydroxylation of the leucoanthocyanidin (flavan-3,4-*cis*-diol) at the C-3 to give a flavan-3,3,4-triol, which spontaneously 2,3-dehydrates and isomerizes to 2-flaven-3,4-diol, which then spontaneously isomerizes to a thermodynamically more stable anthocyanidin pseudobase, 3-flaven-2,3-diol (Figure 3.2). The formation of 3-flaven-2,3-diol via the 2-flaven-3,4-diol was previously hypothesized by Heller and Forkmann.³ The reaction sequence, and the subsequent formation of the anthocyanidin 3-*O*-glycoside, does not require activity of a separate dehydratase, which was once postulated. Recombinant ANS and uridine diphosphate (UDP)-glucose:flavonoid 3-*O*-glucosyltransferase (F3GT, sometimes

abbreviated in the literature as UF3GT, UFGT, or 3GT) are sufficient for the formation of cyanidin 3-*O*-glucoside from leucocyanidin, at least under mildly acid conditions, that are to be expected in a vacuole.⁸⁰

Turnbull et al.^{81,83,86} used a range of substrates to study recombinant *A. thaliana* ANS activity. *Trans*-DHQ, a potential substrate that would occur *in vivo*, was converted to quercetin in a reaction equivalent to that of the FLS. Incubation with the physiological substrate (2*R*,3*S*,4*S*)-3,4-*cis*-leucocyanidin resulted in the formation of *cis*-DHQ, *trans*-DHQ, quercetin, and cyanidin, with cyanidin being a minor product. The acceptance of multiple substrates and generation of a variety of products fits with the proposed 3-hydroxylation mechanism of ANS and the suggested relatively large active site cavity.⁸⁵ The overlapping *in vitro* activities of 2OGDs of flavonoid biosynthesis are discussed further in Sections 3.6 and 3.14.

Some of the 2OGDs of flavonoid biosynthesis (F3H, FNSI, FLS, and ANS) have been studied for acceptance of *O*-methylated substrates.⁸⁷ Substrates methylated at the 3'-hydroxyl were accepted, while methylation at the 4'- or 7-hydroxyls reduced activities to varying degrees. Multiple methylation or methylation at other positions prevented acceptance of the substrate.

For 3-deoxyanthocyanin biosynthesis the 3-hydroxyl is, of course, lacking from the ANS substrates (e.g., apiforol). Whether a specific ANS is thus involved in 3-deoxyanthocyanin biosynthesis is not clear. However, it has been postulated that the reaction may still proceed through 3-hydroxylation, and initial results suggest recombinant ANS from species that do not produce 3-deoxyanthocyanins may still use apiforol as a substrate to produce apigeninidin (results of J-I. Nakajima and K. Saito of Chiba University, Japan, with the authors' coworkers in New Zealand).

3.4.6 FORMATION OF ANTHOCYANIDIN 3-*O*-GLYCOSIDE

The anthocyanidin pseudobases are thought to be too unstable to accumulate in the cell, and are converted to the stable anthocyanins in what might be regarded as the final essential biosynthetic step, *O*-glycosylation. In the majority of plants, the initial reaction is the transfer of a glucose residue from the energy-rich nucleotide sugar (UDP-glucose) to the 3-hydroxyl of the proposed pseudobase by F3GT. As mentioned in Section 3.4.5, the action of ANS and F3GT has been demonstrated to be sufficient to convert the leucoanthocyanidins to colored anthocyanins (in an acidic environment).⁸⁰ Although the addition of glucose at the 3-hydroxyl is the most common initial activity, 3-*O*-galactosylation is the first reaction in some species. No cDNAs for anthocyanidin 3-*O*-galactosyltransferases have been published, although such sequences have been lodged with the GenBank database (accession BAD06514). 3-*O*-Glycosylation is often only the first of multiple sugar additions, either at other positions of the anthocyanin core structure (both A- and B-rings) or on to previously added sugars, and other glycosylations are discussed in Section 3.4.9.1.

Commonly, the F3GT is designated a flavonoid GT, as enzyme preparations from several species,² the recombinant *Forsythia × intermedia* and *P. hybrida* enzymes,^{88,89} and the *A. majus* cDNA expressed *in vivo*,⁹⁰ can conjugate both anthocyanidin and flavonol substrates with high efficiency. However, F3GTs of some species, such as *Gentiana trifolia* and *Vitis vinifera* (grape),⁹¹⁻⁹³ may specifically or primarily act on anthocyanidin substrate, and should be termed UDP-glucose:anthocyanidin 3-*O*-glucosyltransferases (A3GTs) in reflection of this. Indeed, within the EC system separate classifications are given for A3GT (EC 2.4.1.115) and UDP-glucose:flavonol 3-*O*-glucosyltransferase (EC 2.4.1.91).

The F3GT is part of the UDPG-glycosyltransferase (UGT) family, which is family 1 of the glycosyltransferase superfamily (EC 2.4.1.X).^{94,95} UGTs have a central role in detoxifying or

regulating the bioactivities of a wide range of endogenous and exogenous low molecular weight compounds in both plants and mammals. In plants, they are generally monomeric, soluble enzymes of 50 to 60 kDa catalyzing *O*-glycoside formation, although cases of *C*-glycoside formation are known. Affinity for the sugar acceptor is usually high and that for the sugar donor typically lower. Conserved amino acids occur across the UGT family, in particular several histidine residues, and significantly conserved regions are illustrated in the alignment of the Plant Secondary Product Glycosyltransferase motif (PSPG-box) of 44 sequences in Ref. 95. The PSPG-box is thought to be common to UGTs involved in plant secondary metabolism, and may define a monophyletic group of genes. A nomenclature system for the UGTs has been suggested, similar to that for P450s, with groups of plant origin sharing >45% amino acid sequence identity numbered 71 to 100, subgroups with >60% identity given a letter, and the individual gene a number; e.g., the F3GT of *G. trifolia* is named UGT78B1 under this system (for details, see Refs. 95, 96). In general, the catalytic mechanism of UGTs is not well characterized, and no crystal structure of a plant enzyme has been published.

There are few reports of specific F3GT mutations, and none giving a phenotype in petal tissue, perhaps reflecting common redundancy in UGT activity. However, the first reported cloning of a plant UGT DNA sequence was based on the *F3GT bronze1 (bz1)* mutation of *Z. mays*, in which glycosylation does not occur and a brown pigment is formed in the kernels by the condensation of the anthocyanidins in the cytosol.⁴

3.4.7 FORMATION OF 3-DEOXYANTHOCYANIN

The biosynthesis of 3-deoxyanthocyanins has only been studied in any detail for two grass species, *Z. mays* and *Sorghum bicolor*, and one member of the Gesneriaceae, *S. cardinalis*. It is thought that 3-deoxyanthocyanin biosynthesis occurs through the activity of FNR, so that flavan-4-ols are formed. The flavan-4-ols are then converted to anthocyanins through the action of ANS and a A5GT. The evidence to date is that, in addition to FNR activity, 3-deoxyanthocyanin biosynthesis also requires a marked reduction in the potentially competitive F3H activity.

FNR is most likely a variant form of DFR that has dual DFR/FNR activity. The recombinant DFR enzymes of *Malus domestica* (apple), *Pyrus communis* (pear), and *Z. mays*, all species that can produce 3-deoxyflavonoids under some circumstances, show both DFR and FNR activity.^{97,98} In all three cases, DHFs were preferred as substrates over flavanones, supporting the need for a mechanism promoting flavanone production, i.e., a reduction in F3H activity. Enzymology studies for 3-deoxyanthocyanin-producing flower silks of *Z. mays* also show FNR activity occurs with only low levels of F3H activity.⁹⁸ The FNR was initially characterized in detail from the flowers of the Gesneriads *S. cardinalis* and *Columnnea hybrida*, in which 3-deoxyanthocyanins are found in great excess to 3-hydroxyanthocyanins.³ Recent studies on the recombinant *S. cardinalis* FNR show that this also has both DFR and FNR activity.⁹⁹

Gene expression studies in relation to 3-deoxyflavonoid biosynthesis are limited to *S. cardinalis*, *S. bicolor*, and *Z. mays*, but all are in general agreement with the proposed biosynthetic mechanism. In petals of *S. cardinalis* transcript abundance is very high for the *FNR* and very low for *F3H*, with that for *ANS* intermediate between the two.⁹⁹ A similar pattern is found for phlobaphene production in *Z. mays* kernels, but without expression of *ANS* (see Section 3.15.3). In *S. bicolor* a similar pattern of *DFR/FNR* and *ANS* expression coincident with low *F3H* transcript levels is seen during production of 3-deoxyflavonoids in response to fungal inoculation,¹⁰⁰ although it has been suggested that some biosynthetic variations occur.¹⁰¹

The 3-deoxyanthocyanidins are initially glucosylated at the 5-hydroxyl. Clones for UDP-glucose:anthocyanin 5-*O*-glucosyltransferases (A5GT) have been isolated from a range of species.^{88,102} However, all of these were found to require, at a minimum, prior 3-*O*-glucosylation of the substrate (see Section 3.4.9.1). This suggests that for 3-deoxyanthocyanin formation a specific A5GT may have evolved to accept the aglycone.

3.4.8 FLAVONOID 3'-HYDROXYLASE AND FLAVONOID 3',5'-HYDROXYLASE

In a few species, the B-ring hydroxylation pattern is thought to be fully or partially determined through the HCA-CoA ester used by CHS (see Section 3.4.1). However, most commonly hydroxylation at the C-3' and C-5' positions is determined at the C₁₅ level by the activity of two P450s, the F3'H and F3',5'H. Genes and cDNAs for both enzymes, sometimes informally referred to as the red and blue genes because of their impact on flower color, were first cloned and characterized from *Petunia*^{103–105} and subsequently from several other species (listed in Ref. 5). Based on sequence analysis, the F3'H and F3',5'H proteins are 56 to 58 kDa in size.

The F3'H recombinant proteins of *A. thaliana*, *P. hybrida*, and *P. frutescens* accept flavanones, flavones, and DHFs as substrates,^{105–107} as do enzyme preparations from plant tissues.³ Indeed, recombinant *P. frutescens* F3'H showed a similar K_m (about 20 μM) for naringenin, apigenin, and DHK.¹⁰⁷ The *A. thaliana* F3'H amino acid sequence has been used to generate a model of the enzyme and examine the active site architecture and substrate recognition, as discussed in Section 3.3.3.²⁵ Recombinant F3',5'H also accepts a range of substrates to carry out stepwise 3'- and 5'-hydroxylation, in particular converting naringenin or eriodictyol to pentahydroxyflavanone, DHK or DHQ to DHM, apigenin or luteolin to tricetin, and kaempferol or quercetin to myricetin.^{91,103,108,109} Recombinant F3',5'H from *P. hybrida* and *Catharanthus roseus* (Madagascar periwinkle) showed greatest activity with naringenin (K_m 7 μM) and apigenin, and decreasing activity against kaempferol and DHQ, with a preference for the 4'-hydroxylated substrates over the 3'4'-hydroxylated ones.^{108,109} F3',5'H has been shown to be able to use the alternative electron donor Cyt *b*₅, based on analysis of a knockout line of *P. hybrida* for the *Cytb*₅ gene (*difF*), which showed it is required for full activity of F3',5'H but not F3'H in flowers.¹¹⁰

3.4.9 ANTHOCYANIN MODIFICATION ENZYMES

Knowledge of the genetics and molecular biology of the genes encoding the enzymes that carry out modifications of the core anthocyanin structure has advanced greatly in recent years, based on research involving a wide variety of plant species. Of the classic model species, anthocyanin modification enzymes have been studied in detail only for *P. hybrida*, regarding the production of methylated and acylated anthocyanidin 3,5-*O*-glycosides. However, 99 UGT-like sequences have been identified in the genome of *A. thaliana*, and the research currently underway to characterize them by transgenic approaches and expression *in vitro* may identify other flavonoid GTs.⁹⁶

3.4.9.1 Anthocyanin Glycosyltransferases

Glycosylation at the 3-hydroxyl, or for 3-deoxyanthocyanidins at the 5-hydroxyl, has been discussed earlier as part of the core anthocyanin biosynthetic pathway. In this section, molecular data on the genes encoding enzymes that carry out subsequent glycosylations is reviewed. A great variety of anthocyanin glycosides occur, but at the time of the review by Heller and Forkmann³ only a few anthocyanin GTs had been characterized biochemically, and DNA sequences were only available for the F3GT. Since then genes or cDNAs have been

isolated for the UDP-rhamnose:anthocyanidin 3-*O*-glucoside *O*-rhamnosyltransferase (A3RT) from *P. hybrida*,^{111,112} A5GTs,^{88,102} and a UDP-glucose:anthocyanin 3'-*O*-glucosyltransferase (A3'GT).¹¹³

The recombinant A5GT from *P. hybrida* accepts only delphinidin 3-*O*-(4-coumaroyl)-rutinoside, consistent with the prior biochemical data.⁸⁸ In contrast, the recombinant A5GTs from *P. frutescens* and *Verbena hybrida* accept a range of 3-*O*-glycosides and acyl-glycosides, although prior 3-*O*-glycosylation is required.¹⁰² Phylogenetic alignment of UGT sequences with proven functionality shows that the A3GT and A5GT sequences form two distinct groups, with the A3RT falling well separated from either group.^{88,114} The separation of the A3RT is due to a distinctive PSPG-box, for the binding of UDP-rhamnose rather than UDP-glucose.

3.4.9.2 Anthocyanin Methyltransferases

There is a wide range of methylated flavonoids that is formed through the action of members of the *S*-adenosyl-L-methionine (SAM)-dependent methyltransferase (MT) family (EC 2.1.1.X). This large family includes enzymes targeting *O*, *C*, *N*, and *S* atoms in the methyl acceptor molecule. Those characterized to date for flavonoid biosynthesis are generally class II *O*-MTs (OMTs), which do not require Mg²⁺ and have MWs typically of 38 to 43 kDa, although one of the smaller MW class I Mg²⁺-dependent OMT types has also been reported.¹¹⁵ Although *C*-methylated flavonoids are known, there are no reports on the characterization of the enzymes. Excellent reviews of the plant OMT family are available in Refs. 116 and 117, which include presentations of molecular phylogenies, as does the review of Schröder et al.⁸⁷ Structural information on plant OMTs has been gathered from amino acid sequence analysis and the crystal structures for various animal OMTs and one plant OMT, whose crystal structure was solved to a 1.8 Å resolution.¹¹⁸ Significant conserved regions include motifs for SAM (LExGxGxG) and Mg²⁺ binding (KGTVL).¹¹⁷

The well-characterized anthocyanin-related OMTs are those acting at the 3'- and 5'-hydroxyls, encoded by the genes *Mt1*, *Mt2*, *Mf1*, and *Mf2* in *P. hybrida*.¹ Anthocyanins with methylation at the 5- or 7-hydroxyls are also known. The isolation of cDNAs for anthocyanin OMTs has been reported only in the patent literature to date (International Patent Application WO03/062428), although some cDNAs were mentioned in brief in Ref. 119. The patent describes *P. hybrida*, *Fuchsia*, *Plumbago*, and *Torenia* cDNAs encoding OMTs that act on the 3'- or 3',5'-hydroxyls. The recombinant proteins act on delphinidin 3-*O*-glucoside or delphinidin 3-*O*-rutinoside to produce the 3'- or 3',5'-*O*-methylated derivatives. Interestingly, the sequences are closer to those of class I OMTs than class II.

3.4.9.3 Anthocyanin Acyltransferases

Anthocyanin acyltransferases (AATs) catalyze transfer of either aromatic or aliphatic acyl groups from a CoA-donor molecule to hydroxyl residues of anthocyanin sugar moieties; and are part of the general group of acyltransferase enzymes (EC 2.3.1.X). A wide range of activities have been characterized, including enzymes using acetyl-CoA, caffeoyl-CoA, 4-coumaroyl-CoA, malonyl-CoA, and succinyl-CoA. Nakayama et al.¹²⁰ list six aromatic and 14 aliphatic AATs, and others, such as those of *Dendranthema × morifolium*, have been reported since. Following the publication of the first AAT cDNA in 1998,¹²¹ cDNA clones for two aromatic and six aliphatic AATs have been characterized at the molecular level (Table 3.1). Examples of reactions carried out by these enzymes are shown in Figure 3.3.

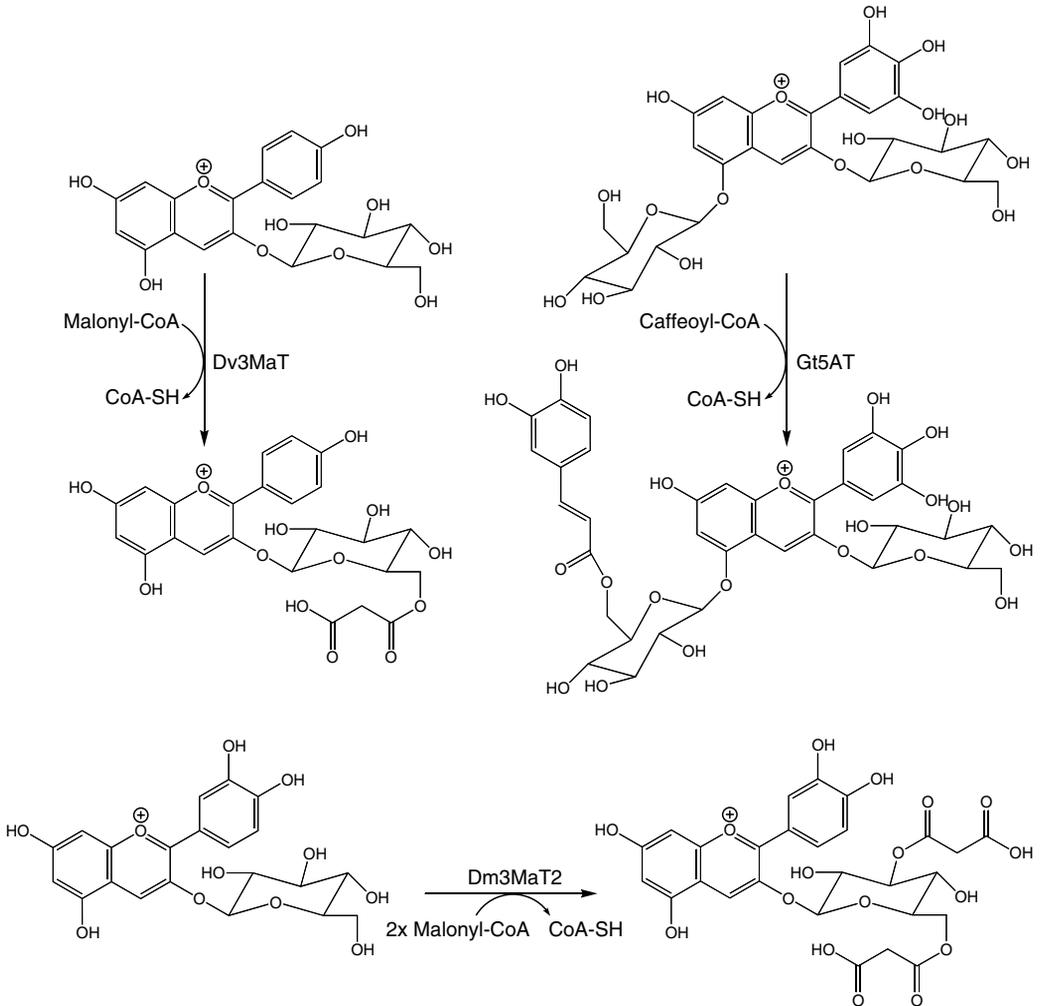


FIGURE 3.3 Examples of aliphatic and aromatic acylation of anthocyanins, as carried out by the malonyl-CoA:anthocyanidin 3-*O*-glucoside-6''-*O*-malonyltransferase (Dv3MaT from *D. variabilis*), malonyl-CoA:anthocyanidin 3-*O*-glucoside-3'',6''-*O*-dimalonyltransferase (Dm3MaT2 from *D. Xmorifolium*), and hydroxycinnamoyl-CoA:anthocyanin 5-*O*-glucoside-6'''-*O*-hydroxycinnamoyltransferase (Gt5AT from *G. triflora*).

Aromatic AAT cDNAs have been isolated from *G. triflora*¹²¹ and *P. frutescens*.¹²² The recombinant *G. triflora* protein (Gt5AT) can use either caffeoyl-CoA or 4-coumaroyl-CoA to introduce the HCA group to the glucose at the C-5 position of anthocyanidin 3,5-di-*O*-glucoside. Although specificity is shown with regard to the anthocyanin glycosylation and acylation pattern, the enzyme can accept pelargonidin, cyanidin, or delphinidin derivatives. That the number of hydroxyl groups on the B-ring does not affect reactivity is typical of AATs studied to date. The *P. frutescens* recombinant product (Pf3AT) was identified as a hydroxycinnamoyl-CoA:anthocyanidin 3-*O*-glucoside-6'''-*O*-hydroxycinnamoyltransferase, utilizing cyanidin 3-*O*-glucoside and cyanidin 3,5-di-*O*-glucoside (the putative substrates *in vivo*) as well as other anthocyanins. K_m values of 6 to 227 and 113 to 777 μM have been

reported for various anthocyanin substrates for the Pf3AT and Gt5AT, respectively, and 24 to 190 μM toward the HCA-CoA esters for a range of aromatic AATs.¹²⁰ The isolation and analysis of aromatic AAT cDNAs from additional species, including *P. hybrida* and *Lavandula angustifolia*, has been reported in the patent literature (International Patent Application WO96/25500).

Of the aliphatic AAT cDNAs characterized, three encode malonyl-CoA:anthocyanidin 3-*O*-glucoside-6''-*O*-malonyltransferases (A3MT) from *D. variabilis*, *Senecio cruentus* (cineraria), and *D. \times morifolium*.^{123–125} The *D. variabilis* and *S. cruentus* recombinant proteins (Dv3MaT and Sc3MaT) accept pelargonidin-, cyanidin-, or delphinidin 3-*O*-glucosides, but do not use anthocyanidin diglycosides. The *D. \times morifolium* recombinant protein (Dm3MaT1) also does not accept diglycosides as substrates, but will use a wide range of flavonoid monoglucosides, including anthocyanidin 3-*O*-glucosides and quercetin 3-*O*-glucoside. With regard to acyl donors, Dm3MaT1 has highest activity with malonyl-CoA but also shows significant activity with succinyl-CoA. Other acyl-CoA compounds are either accepted weakly or not at all. K_m values of 19 to 57 μM toward malonyl-CoA have been reported for the aliphatic AATs.¹²⁰

Other aliphatic AAT cDNAs characterized include ones encoding a malonyl-CoA:anthocyanin 5-*O*-glucoside-6'''-*O*-malonyltransferase (Ss5MaT) from *Salvia splendens* (scarlet sage) and a malonyl-CoA:anthocyanidin 3-*O*-glucoside-3'',6''-*O*-dimalonyltransferase (Dm3MaT2) from *D. \times morifolium*.^{125,126} Ss5MaT shows high specificity, accepting only “bisdemalonylsalvianin” (a pelargonidin 3,5-di-*O*-glucoside derivative), the endogenous substrate. Recombinant Dm3MaT2 produced two products from cyanidin 3-*O*-glucoside, cyanidin 3-*O*-(6''-*O*-malonylglucoside) and cyanidin 3-*O*-(3'',6''-*O*-dimalonylglucoside), suggesting it carries out sequential acylations at the glucose 6''- and 3''-hydroxyl groups. Both Dm3MaT2 and Ss5MaT show strong preference for malonyl-CoA as the acyl donor, but Ss5MaT also shows significant usage of succinyl-CoA.

All AATs characterized to date are monomers of approximately 50 to 52 kDa in size, and are members of the large versatile acyltransferase (VAT or VPAT) family of enzymes (also known as the BAHD superfamily) involved in many primary and secondary metabolite pathways of plants and fungi.^{120,127} Nakayama et al.¹²⁰ present detailed sequence comparisons among AATs and a molecular phylogeny for the VAT family. The three A3MT amino acid sequences share >70% amino acid identity. However, although the other AATs, both aliphatic and aromatic, show significant amino acid sequence identity, it is at a relatively low level. For example, Dm3MaT1 has 40% identity to Ss5MaT1, 38% to Gt5AT, and 37% to Pf3AT. Of three significant amino acid motifs identified for VATs, Motifs 1 (HxxxD) and 3 (DFGWG) are thought to be key for catalytic activity, while Motif 2 (YxGNC) might relate to recognition of the anthocyanin, as it is present only in AATs to date. The putative reaction mechanism and the possible role of conserved amino acids, based principally on mutagenesis of recombinant Ss5MaT1, are discussed in detail in Refs. 120, 126, 128. No three-dimensional structure has been published for a member of the VAT family.

The evidence suggests that AATs characterized to date are likely to be localized to the cytosol, with their deduced amino acid sequences lacking known vacuolar targeting sequences. However, it cannot be ruled out that some flavonoid ATs occur in the vacuole. Some serine carboxypeptidase-like (SCPL) enzymes, which use 1-*O*-acylglucosides as acyl group donors and catalyze acylation reactions in plant secondary metabolism, are located in the vacuole, e.g., sinapoylglucose:malate sinapoyltransferase.^{129,130} It has previously been proposed that anthocyanin malonylation may occur in the vacuole, and that an SCPL-like activity is involved in the acylation of cyanidin *O*-glycosides in *Daucus carota* (wild carrot) (discussed in Ref. 131).

3.5 FORMATION OF PROANTHOCYANIDINS AND PHLOBAPHENES

The PAs, or condensed tannins, are polymers synthesized from flavan-3-ol monomer units. The phlobaphenes are 3-deoxy-PAs formed from flavan-4-ol monomers. The biosynthesis of both types of PAs follows the biosynthetic route of anthocyanins from chalcones through to the branch points to flavan-3-ol and flavan-4-ol formation. In this section, the specific enzymes forming the monomers are discussed, along with a discussion on the polymerization process. Although the chemistry of tannins is described in detail elsewhere in this book, it is useful to briefly mention the nature of the monomer subunit types and the polymer forms.

Although many variant polymer forms have been reported, the most common ones consist of linear C-4 to C-8 linkages, with the linking bond at the C-4 being *trans* with respect to the 3-hydroxyl. The subunits are usually flavan-3-ol epimers for the 3-hydroxyl, being either 2,3-*trans* or 2,3-*cis*, with the latter being prefixed with “*epi*.” Most commonly these are (2*R*,3*S*)-*trans* or (2*R*,3*R*)-*cis*, although 2*S*-enantiomers do occur, being indicated by the “*ent*” prefix. Intermediates in the flavonoid pathway up to and including leucoanthocyanidins are 2,3-*trans* in stereochemistry. Flavan-3-ols may be formed by two biosynthetic routes, from either leucoanthocyanidins or anthocyanidins (Figure 3.4). The 2,3-*trans*-flavan-3-ols are produced from the leucoanthocyanidins by LAR, while the 2,3-*cis*-flavan-3-ols are produced from the anthocyanidins by ANR.

The common subunits are those with 3',4'-dihydroxylation of the B-ring (catechin and, most commonly, epicatechin) or 3',4',5'-trihydroxylation (gallocatechin and epigallocatechin). Monohydroxylation of the B-ring (at C-4'), producing the subunits of propelargonidin, is rare, as is the occurrence of subunits with hydroxyl patterns on the A- and C-rings varying from the common one of 3,5,7-hydroxylation. As the subunits for PA biosynthesis are formed

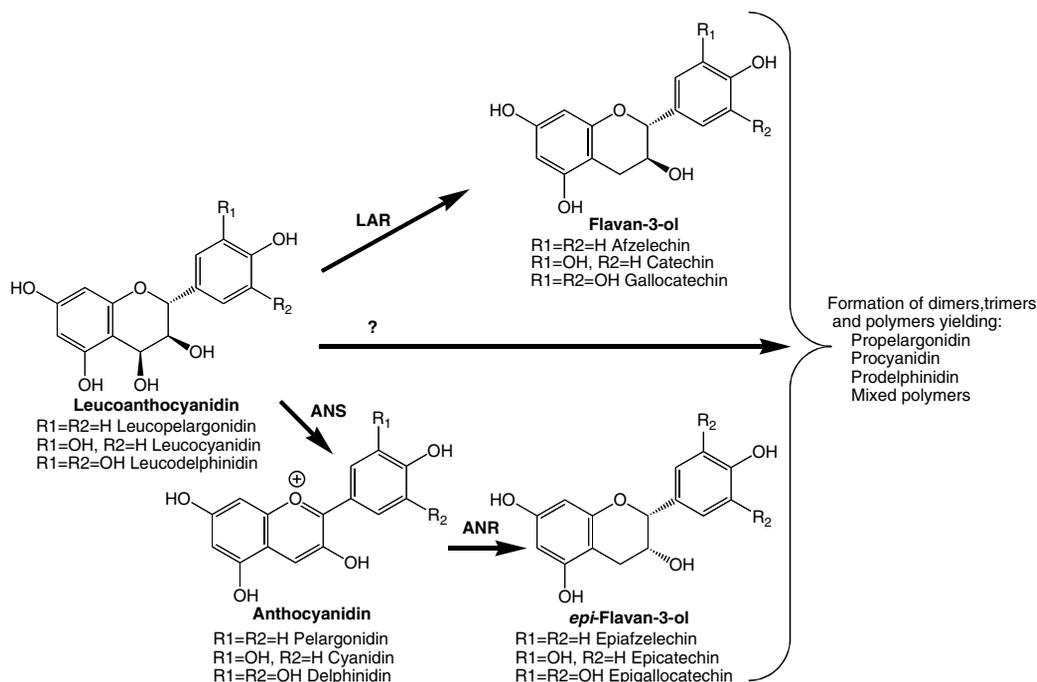


FIGURE 3.4 Biosynthetic route to proanthocyanidins from leucoanthocyanidins. The product of the ANS is given in the flavylum cation form. Enzyme abbreviations are defined in the text and in Table 3.1.

later in the pathway than flavanones, the hydroxylation status of the B-ring of the flavan-3-ols is likely to be determined by the action of the F3'H and F3',5'H on the precursors.

3.5.1 LEUCOANTHOCYANIDIN REDUCTASE

LAR removes the 4-hydroxyl from leucoanthocyanidins to produce the corresponding 2,3-*trans*-flavan-3-ols, e.g., catechin from leucocyanidin.^{5,132} Despite early biochemical characterization, it is only recently that a *LAR* cDNA was isolated and the encoded activity characterized in detail. Tanner et al.¹³³ purified LAR to homogeneity from *Desmodium uncinatum* (silverleaf desmodium), and used a partial amino acid sequence to isolate a *LAR* cDNA. The cDNA was expressed in *E. coli*, *N. tabacum*, and *Trifolium repens* (white clover), with the transgenic plants showing significantly higher levels of LAR activity than nontransformed plants.

LAR of *D. uncinatum* is a NADPH-dependent reductase with closest similarity to IFR (a reductase involved in isoflavonoid biosynthesis), and like IFR, DFR, and VR (another isoflavonoid reductase), LAR belongs to the RED protein family. It shares several conserved amino acid sequence motifs with other RED proteins, but has a C terminal extension of approximately 65 amino acids of unknown function. The protein is a monomer with a predicted MW of 42.7 kDa. The preferred substrate is 3,4-*cis*-leucocyanidin, although 3,4-*cis*-leucodelphinidin and 3,4-*cis*-leucopelargonidin are also accepted. Although 2,3-*cis*-3,4-*trans*-leucoanthocyanidins have not been shown to exist as substrates *in vivo*, if LAR accepted them it would raise the possibility of a route to the 2,3-*cis*-flavan-3-ols in addition to the route through ANR. The difficulty in synthesizing 2,3-*cis*-3,4-*trans*-leucoanthocyanidins *in vitro* has prevented a definitive test of LAR activity on these substrates. However, product inhibition is about 100 times greater with catechin than epicatechin, supporting ANR as the main route. There appears to be no LAR sequence or enzyme activity in *A. thaliana*, consistent with the lack of catechin derivatives in that species.¹³⁴

3.5.2 ANTHOCYANIDIN REDUCTASE

ANR converts anthocyanidins (presumably the pseudobase forms) to the corresponding 2,3-*cis*-flavan-3-ols, e.g., cyanidin to epicatechin. For many years little information was available on the formation of 2,3-*cis*-flavan-3-ols. However, the solution emerged from studies on the *banyuls* mutant of *A. thaliana*, which displays precocious accumulation of anthocyanins in the seed coat endothelium.¹³⁵ Initially thought to represent an anthocyanin regulatory gene, it was found that the locus encoded a DFR-like protein of 38 kDa, and as *banyuls* mutant plants also lack accumulation of PAs in the seed coat endothelium, it was suggested that the gene might encode LAR.¹³⁶ However, recombinant BANYULS from *A. thaliana* and *M. truncatula* did not show LAR activity, but rather reduced pelargonidin, cyanidin, and delphinidin to the corresponding 2,3-*cis*-flavan-3-ols.¹³⁷ Low-level production of *ent*-epiafzelechin, *ent*-epicatechin, and *ent*-epigallocatechin was also seen, although these were suspected to be artifacts of the analysis generated by chemical epimerization.¹³⁸

Like LAR, ANR is a member of the RED protein family. Full details of the protein structure and reaction mechanism are yet to be published. However, Xie et al.¹³⁸ compared the *A. thaliana* (AtANR) and *M. truncatula* (MtANR) ANR amino acid sequences and recombinant protein activities, and made suggestions on the possible reaction series. The two recombinant proteins showed significantly different kinetic properties, substrate specificities, and cofactor requirements. Although AtANR and MtANR share only 60% sequence identity, some well-conserved domains are evident, in particular the Rossmann dinucleotide-binding domain (GxxGxxG) near the N-termini. However, two amino acid variations did

occur in this domain, and this may account for the ability of MtANR to use either NADH or NADPH, while AtANR will use only NADPH. Both reduce pelargonidin, cyanidin, and delphinidin, with MtANR preferring cyanidin > pelargonidin > delphinidin and AtANR showing the reverse preference. With regard to *in vivo* activities, the low level of PAs (6% of wild-type levels) in the *F3'H* mutant of *A. thaliana* (*tt7*) suggests that AtANR, or other PA biosynthetic enzymes of *A. thaliana*, have a limited activity against pelargonidin.¹³⁴

The ANR reaction involves a double reduction at the C-2 and C-3 of the anthocyanidin, allowing the inversion of C-3 stereochemistry. Xie et al.¹³⁸ postulate four possible reaction mechanisms, proceeding via either flav-3-en-ol or flav-2-en-ol intermediates. The proposed reaction mechanisms are based on anthocyanidins (the flavylum cation forms) as the starting molecules; however, as the authors acknowledge, other forms of the anthocyanidin may exist *in vivo*. In particular, the 3-flaven-2,3-diol pseudobase is thought to be the more likely *in vivo* product of the ANS.

3.5.3 PROANTHOCYANIDIN POLYMERIZATION

Nonenzymatic studies of polymerization *in vitro* suggest that it starts by the attack of the C-4 of an electrophilic leucoanthocyanidin on the C-8 of the nucleophilic flavan-3-ol initiating subunit to form the initial dimer (the chemistry of the polymerization is reviewed in Refs. 4, 5, 132). Polymerization then continues by addition of leucoanthocyanidin extension subunits in the same C4–C8 route. This suggests that the great majority of the substrate for polymerization *in planta* may not need to pass through either the LAR or ANR biosynthetic steps. However, in most cases, the extension units are 2,3-*cis*, and leucoanthocyanins are 2,3-*trans*. Thus, it may not be leucoanthocyanidins that are the extension units *in planta*, but leucoanthocyanidin derivatives. Thus, intermediates proposed for polymerization include reactive (2*R*,3*S*) compounds derived from leucoanthocyanidins, such as quinone methide and carbocation products. Another, perhaps more likely, source for the common 2,3-*cis* extension units is intermediates of the ANS and ANR biosynthetic route, possibly also as quinone methide and carbocation products. Indeed, the *tannin-deficient seed4* (*tds4*) mutant of *A. thaliana*, which is for the *ANS* gene, accumulates unidentified possible PA intermediate compounds in the developing seed.¹³⁹ These seem to fail to be transported into the vacuole as usual and accumulate in the cytoplasm, triggering the formation of multiple small vacuoles. These studies and those of Grotewold et al.¹⁴⁰ on *Z. mays* cell lines suggest that the final steps of PA biosynthesis may be linked to developmental changes in the cell structure. Any enzymatic polymerization process would need to evolve a mechanism for avoidance of inhibitory protein–PA interactions. A membrane-bound biosynthetic complex associated directly with the accumulation of the polymers in the vacuole might achieve this.

The recent molecular characterization of the LAR and ANR has been a major advance in the understanding of PA biosynthesis. However, it is still not known whether the polymerization of PAs occurs spontaneously in all tissues or is enzyme catalyzed in some or all cases. Flavan-3-ols and leucoanthocyanidins will polymerize spontaneously *in vitro* (reviewed in Ref. 5), and no activities responsible for the formation of PA polymers have been described. Differences in PA composition and polymer length do occur between different tissues and during tissue development, and variations from the C4–C8 linkage pattern also occur. However, these variations are not necessarily evidence of an enzymatic mechanism of polymerization, as changes in the availability or reactivity of initiating and extension units might also generate such differences. Nevertheless, a nonenzymatic polymerization process seems unlikely, given that PAs occur in some species with specific arrangements of catechin and epicatechin units. Perhaps the best evidence for biosynthetic steps after LAR and ANR are the mutant lines that have been identified that prevent PA accumulation and appear to occur

after monomer formation. At least four PA mutations of *H. vulgare* (*ant25*, *ant26*, *ant27*, and *ant28*) appear to act after LAR, with evidence for *ant26* encoding a polymerization enzyme.¹⁴¹ In *A. thaliana*, *TDS1*, *TDS2*, *TDS3*, *TDS5*, and *TDS6* act after *Banyuls*,¹³⁹ and *TT9*, *TT10*, *TT11*, *TT13*, and *TT14* act after *TT12*, which encodes a vacuolar transporter for PAs.¹⁴² Although some of the *TDS* mutants may be allelic to the *TT* mutants, this still indicates a number of genetic steps postmonomer formation, which may include polymerization enzymes, regulatory factors, transport activities, and dirigent proteins that control stereospecificity of polymerization.

One other line of evidence supporting enzymatic polymerization comes from recent studies overexpressing ANR in transgenic plants. *N. tabacum* ANR transgenics are reported to have increased levels of at least four compounds that react with the PA stain dimethylaminocinnamaldehyde (DMACA) but no PA polymers, supporting the requirement for further enzymatic steps.^{5,133} However, lack of PA polymers may also be due to conditions in the transgenic tissues studied being unsuitable for spontaneous polymerization. Also, Xie et al.¹³⁷ reported results differing to these, as they found PA polymer-like DMACA staining compounds in leaves or petals of *A. thaliana* or *N. tabacum* *CaMV35S:ANR* transgenics, respectively, although it has been commented that more extensive analysis than what was presented is often required to confirm the PA polymer status.¹³²

3.6 FORMATION OF FLAVONES AND FLAVONOLS

A desaturation reaction forming a double bond between C-2 and C-3 of the C-ring is involved in the formation of both flavones and flavonols, and the respective substrates involved, (2*S*)-flavanones and (2*R*,3*R*)-DHF_s, differ only in the presence or absence of the 3-hydroxyl (Figure 3.2).

Two distinct FNS activities have been characterized that convert flavanones to flavones. In most plants FNS is a P450 enzyme (FNSII), but species in the Apiaceae have been found to contain the 2OGD FNSI. FNSII cDNAs were first isolated from *G. hybrida*, based on a differential display technique focusing on the conserved P450 heme-binding site,¹⁴³ and from *A. majus* and *T. fournieri* using another P450 cDNA as a probe.¹⁴⁴ Isolation of a cDNA for FNSI was first reported from *P. crispum*.¹⁴⁵ Although FNSI and FLS catalyze the equivalent 2,3-desaturation reaction, it is thought that FNSI is most likely to have evolved from the F3H in the Apiaceae.

Flavonols are formed from DHF_s by the FLS. A cDNA for FLS was first isolated from *P. hybrida* using degenerate PCR primers for conserved 2OGD sequences,¹⁴⁶ and indeed all FLS cDNAs identified to date encode 2OGD enzymes. However, the *Torenia* FNSII shows FLS activity, raising the suggestion that, analogous to flavone formation, there are two types of FLS.¹⁴⁴ The recombinant *Citrus unshiu* (Satsuma mandarin) FLS had K_m values of 45 and 272 μM for DHK and DHQ, respectively.

A similar reaction mechanism has been proposed for F3H, ANS, FLS, and FNSI, involving *cis*-hydroxylation at C-3 followed by dehydration, with (2*R*,3*S*)-*cis*-DHF_s as possible intermediates.^{81,84} Akashi et al.¹⁴⁴ have suggested that the FNSII reaction likewise involves a C-2 hydroxylation step followed by dehydration. However, the studies of Martens et al.^{143,145,147} suggest direct 2,3-desaturation of flavanones by FNSI and FNSII, as previously proposed from biochemical studies of FNSI.¹⁴⁸ A comparison of 59 2OGD amino acid sequences, including those for ANS, F3H, and FLS, identified three regions of high similarity and eight absolutely conserved amino acids.⁶² These include residues with proposed functions in Fe²⁺ and 2OG binding, and two others of unknown function that are required for enzyme activity.^{62,67}

Recent studies of members of the flavonoid 2OGD family show overlapping substrate and product selectivities *in vitro*. For example, the *C. unshiu* FLS has been termed a bifunctional

enzyme, as the recombinant protein has both FLS and F3H activity.⁶² Two groupings of flavonoid 2OGDs have been proposed, FLS/ANS and F3H/FNSI, with the former having wider substrate selectivity than the latter.^{62,81,147} The overlapping activities are discussed further in Section 3.14.

3.7 FLAVONE AND FLAVONOL MODIFICATION ENZYMES

Flavones and flavonols are the substrates for a range of modification reactions, including glycosylation, methylation, acylation, and sulfation. To date, genes and cDNAs have been cloned that represent activities specific to flavones or flavonols for all of these modifications except acylation. There are also several cDNAs isolated, the encoded proteins of which accept a range of flavonoid, and even nonflavonoid, substrates. However, *in vitro* activities of recombinant proteins may not reflect their *in vivo* role. Factors such as the abundance of the protein (temporally or spatially) in relation to the potential substrate, and the involvement of metabolic channeling (see also Section 3.14), affect *in vivo* activity. However, in various transgenic experiments endogenous flavonoid GTs have been shown to accept substrates that are not normally present in the recipient species, such as 6'-deoxychalcones and isoflavonoids,^{149–151} supporting broad substrate acceptance of some modification enzymes.

3.7.1 FLAVONE AND FLAVONOL GLYCOSYLTRANSFERASES

Clones have now been isolated for several types of UGT with *O*-glycosylation activity on flavones and flavonols. The recombinant proteins show wide substrate acceptance, including some that will accept both flavonoids and some of the biosynthetically unrelated betalain pigments. In general, the UGTs characterized in flavonoid biosynthesis show high regiospecificity but broad substrate acceptance, although there are exceptions.

A number of cDNAs encoding UGT activities against the 7-hydroxyl have been identified. NtGT2 from *N. tabacum* showed activity against several types of phenolic compounds.¹⁵² Despite having closest sequence identity with A5GT sequences, no significant activity with anthocyanins was found but it catalyzed the transfer of glucose to the 7-hydroxyl of flavonols, with a K_m of 6.5 μM for the aglycone kaempferol. The recombinant protein from the *UGT73C6* gene of *A. thaliana* also transfers glucose to the 7-hydroxyl of a range of flavonols and flavones, as well as the 6-hydroxyl of the unnatural 6-hydroxyflavone substrate. However, its *in vivo* activity is likely as a UDP-Glc:flavonol-3-*O*-glycoside-7-*O*-glucosyltransferase.¹⁵³ The recombinant *Allium cepa* (onion) UGT73G1 protein also showed wide regiospecificity, adding glucose to the 3-, 7-, and 4'-hydroxyls of a wide range of flavonoids, including chalcones, flavanones, flavones, flavonols, and isoflavones, producing both mono- and diglucosides.¹⁵⁴ The lack of triglucoside products suggests UGT73G1 accepts only aglycone or monoglucoside substrates. Recombinant protein from a UGT cDNA from the Chinese medicinal herb *Scutellaria baicalensis* also showed activity toward the 7-hydroxyl of flavonoids, among other substrates.¹⁵⁵ In contrast to these activities, recombinant protein from *A. cepa* UGT73J1 showed both high regiospecificity and tight substrate specificity, adding glucose at the 7-hydroxyl of only quercetin 3-*O*-glucoside (a flavonol) and genistein (an isoflavonoid) out of many flavonoid substrates tested.¹⁵⁴

A cDNA from *Vigna mungo* (black gram) seedlings encodes a protein with UDP-galactose:flavonoid 3-*O*-galactosyltransferase (F3GalT) activity.¹⁵⁶ A 20-fold preference for UDP-galactose over UDP-glucose was found with kaempferol as a substrate. It would accept DHFs and flavones at a lower efficiency (anthocyanins were not tested). Average amino acid sequence identity is around 35 to 45% with F3GTs and 23% to the A3RT of *P. hybrida*.

The *UGT78D1* gene of *A. thaliana* encodes a specific UDP-rhamnose:flavonol-3-*O*-rhamnosyltransferase with activity only on kaempferol and quercetin, out of various flavonoids tested.¹⁵³

Like many flavonoid UGTs, the UGTs reported to be involved in betalain biosynthesis in *Dorotheanthus bellidiformis* (Livingstone daisy) show precise regiospecificity but surprisingly wide substrate acceptance. Recombinant betanidin 5-*O*-glucosyltransferase will add a glucose to the 4'- and 7-hydroxyls of luteolin (a flavone) or quercetin, and the 4'-hydroxyl of cyanidin with lower efficiency;¹⁵⁷ betanidin 6-*O*-glucosyltransferase (B6GT) will add a glucose to the 3-hydroxyl of quercetin and cyanidin.¹¹⁴ B6GT shows marked divergence at the amino acid level from the previously characterized F3GTs (for a molecular phylogeny, see Ref. 114).

P. hybrida pollen accumulates kaempferol and quercetin 3-*O*-(2''-*O*-glucopyranosyl)-galactopyranosides, which are not prevalent elsewhere in the plant, by the action of flavonol 3-*O*-galactosyltransferase (PhF3GalT) and flavonol 3-*O*-galactoside-2''-*O*-glucosyltransferase (F2''GT). Miller et al.¹⁵⁸ isolated a cDNA for a pollen-specific gene from *P. hybrida* whose recombinant protein showed the same activity profile as the previously characterized PhF3GalT.¹⁵⁹ Unlike most of the GTs discussed previously, the PhF3GalT showed strong preference and high catalytic efficiency to kaempferol and quercetin, with other lower activities being limited to a range of flavonol aglycones. Notably, the PhF3GalT also catalyzed the reverse reaction, a deglycosylation. The enzyme, therefore, could be involved in modulating the abundance of a biologically active aglycone.

3.7.2 FLAVONE AND FLAVONOL METHYLTRANSFERASES

Methylation has been reported at all available hydroxyls of flavones and flavonols (the C-5, -6, -7, -8, -2', -3', -4', and -5' positions), and it can occur on both aglycone and glycoside substrates. Many of the corresponding enzyme activities have been described in detail, and typically show strong preferences with regard to substrate type and the position methylated.³ Recently, cDNA sequences have been identified for several flavonoid OMTs, allowing sequence-based analysis and examination of recombinant protein activities. All are members of the SAM-MT family described in Section 3.4.9.2.

Induced in leaves of *H. vulgare* in response to pathogen attack is an mRNA encoding a flavonoid 7-OMT with activity against flavanone, flavone, and flavonol aglycones, with the flavone apigenin the preferred substrate.¹⁶⁰ Caffeic acid or glycosylated flavonoids were not accepted as substrates. Gauthier et al.^{161,162} have characterized cDNA clones for two distinct enzyme activities of the semiaquatic freshwater plant *Chrysozplenium americanum* that methylate the 3' and 5' hydroxyls of flavonoids. Recombinant F3'OMT specifically methylated the 3' or 5'-hydroxyls of 3,7,4'-trimethoxyquercetin, accepting neither quercetin nor mono- or dimethylquercetin as substrates.¹⁶¹ However, in contrast, recombinant proteins from the two highly similar clones *CaOMT1* and *CaOMT2* showed 3'-OMT activity against luteolin and quercetin, and lower 3- or 5-OMT activities on caffeic and 5-hydroxyferulic acids, respectively.¹⁶² An *A. thaliana* cDNA, *AtOMT1*, was originally identified as encoding a HCA OMT,¹⁶³ and the deduced amino acid sequences from *CaOMT1*, *CaOMT2*, and *AtOMT1* show high sequence identity (around 85%), and even higher identity across putative sequence motifs relating to substrate specificity and binding.¹¹⁷ Recombinant protein from *AtOMT1* showed flavonol 3'-OMT activity, using quercetin and myricetin (flavonol aglycones) efficiently; however, it had much lower activity with luteolin and did not accept HCAs.¹⁶⁴ The initial identification of *AtOMT1* as a HCA OMT based on sequence similarity illustrates the potential problems in using sequence alone to predict function (discussed in detail for OMTs in Ref. 165).

A cDNA for an OMT (PFOMT) with wide substrate acceptance that includes flavonols and HCA derivatives has been identified from *Mesembryanthemum crystallinum* (ice plant).¹¹⁵

In contrast to the other flavonol OMTs reported, the deduced amino acid sequence of PFOMT has most similarity to caffeoyl-CoA OMTs (CCoAOMTs), and it is a class I OMT with a MW of 27 kDa and a requirement for a bivalent cation such as Mg^{2+} . A wide range of flavonols, including 6-hydroxykaempferol, quercetin, 6-hydroxyquercetin (quercetagenin), 8-hydroxyquercetin (gossypetin), myricetin, and quercetin 3-*O*-glucoside, were accepted as substrates by the recombinant protein, as were some flavones, flavanones, and HCA-CoA esters and glucosides. Generally, potential substrates with two free hydroxyls were accepted while those with a single free hydroxyl were not. The reaction product for quercetin was shown to be isorhamnetin (3'-methoxyquercetin), while with quercetagenin five different products with 5-*O*-, 6-*O*-, 3'-*O*-, 5,3'-*O*-, or 6,3'-*O*-methylation were generated. This range of substrate choice and products with the recombinant protein is wider than for the purified native enzyme, the major products of which are only the 6-*O*- and 6,3'-*O*-methyl ethers. This difference has been shown to be due to the N terminal region of the protein, as a recombinant protein with the first 11 N terminal amino acids removed shows the same enzyme characteristics as the native enzyme.¹⁶⁶ The dual methylation reaction suggests a large and flexible active site, which is rare for the OMTs characterized to date.

Ibdah et al.¹¹⁵ also examined the wider substrate acceptance of four recombinant CCoAOMTs; one each from *Stellaria longipes* (chickweed) and *A. thaliana* with high sequence similarity to PFOMT, and one each from *N. tabacum* and *V. vinifera* with lower sequence similarity. The CCoAOMT from *S. longipes* showed the same range of substrate acceptance as PFOMT, and the same range of products from quercetagenin. The *A. thaliana* protein efficiently accepted a similar wide range of substrates, but produced only the 6-*O*- and 6,3'-*O*-methyl ethers of quercetagenin. In contrast, the *N. tabacum* and *V. vinifera* enzymes showed strong preference for caffeoyl-CoA, although they would accept other flavonoids with much lower efficiencies. The *in vitro* activity pattern is reflected by phylogenetic analysis, which groups the *M. crystallinum* and *S. longipes* sequences separately to a large group of class I CCoAOMTs (which include the *N. tabacum* and *V. vinifera* sequences).

A cDNA, *CrOMT2*, encoding a flavonoid 3',5'-OMT has been identified from *C. roseus* (during a study of alkaloid biosynthesis). The encoded protein could sequentially methylate the 3'- and 5'-hydroxyls of both myricetin and DHM, and showed weaker activity against the 3'-hydroxyl of DHQ.¹⁶⁷ Recombinant F3H, FNS, FLS, and ANS were all able to use the 3'-*O*-methylated substrates.⁸⁷ 3',5' *O*-methylation is characteristic of both flavonol and anthocyanin glycosides of *C. roseus* (hirsutidin and malvidin glycosides occur), so it is possible that this represents the *in vivo* activity. Whether a separate anthocyanin 3',5'-OMT exists in *C. roseus*, or whether the *CrOMT2*-derived enzyme also methylates anthocyanins is not known. Schröder et al.⁸⁷ attempted to isolate a cDNA for anthocyanin 7-OMT from *C. roseus*, and in the process identified a clone (*CrOMT6*) encoding an OMT that specifically accepted 3'-*O*-methylated flavonoids as substrates, in particular flavanones, flavones, and flavonols, to produce the 3',4'-*O*-methylated derivatives. In a molecular phylogeny the flavonoid-related *CrOMT* sequences form a separate cluster from the *CaOMT* and *AtOMT1* sequences.

3.7.3 FLAVONE AND FLAVONOL SULFOTRANSFERASES

Flavonoids esterified with sulfate groups have been reported to occur in many plant species, in particular mono- to tetrasulfate esters of flavonols and flavones, and their methylated or glycosylated derivatives. These are likely generated by soluble sulfotransferases (STs), which transfer a sulfonate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS).¹⁶⁸ Two subgroups of STs have been reported. The first contains enzymes with generally wide substrate acceptance that are typically involved in detoxification of small metabolites. Enzymes of the second subgroup, which includes the flavonoid STs, show high specificity,

and in animals are involved in processes such as steroid transport and inactivation. The first plant STs characterized at the molecular level were the flavonol 3-*O*- and 4'-*O*-STs (F3ST and F4'ST) from *Flaveria chloreaefolia*,¹⁶⁹ followed by a second F3ST, BFST3, from *Flaveria bidentis*.¹⁷⁰ These enzymes form part of a group of flavonol STs that act sequentially to generate the range of flavonol polysulfates found in this genus. Strict specificities are shown to the 3-hydroxyl of flavonol aglycones (F3ST), or the 3', 4'- (F4'ST), or 7-hydroxyls of flavonol 3,3' or 3,4'-disulfates.¹⁷¹ Analysis of the recombinant proteins found the *F. chloreaefolia* F3ST used only flavonol aglycones as substrates (both kaempferol and quercetin, and the methylated rhamnetin and isorhamnetin), and the F4'ST used only the flavonol 3-*O*-sulfates. BFST3 recombinant protein showed similar activity to the *F. chloreaefolia* F3ST, except that kaempferol was not accepted as a substrate.

A range of ST cDNAs has been identified from functional genomics studies of *A. thaliana*,¹⁶⁹ including one (*AtST3*) that has been shown to encode a flavonoid 7-ST. Unlike BFST3, *AtST3* recombinant protein accepts a number of flavonol and flavone aglycones, as well as their 3-*O*-monosulfated derivatives.¹⁶⁹ However, strict specificity to the 7-hydroxyl was found.

The plant soluble STs have around 25 to 30% amino acid identity with mammalian soluble STs, and are of a similar size.¹⁶⁹ Comparisons between *F. chloreaefolia* F3ST and F4'ST, combined with mutational analysis and data from the crystal structure of mouse estrogen ST, have defined amino acid residues important for PAPS binding, substrate binding and catalysis, and the mechanism of sulfonate transfer.¹⁷²⁻¹⁷⁵ Sequence relatedness has been used to divide the STs into families and subfamilies in a similar manner as for P450s.¹⁶⁹

3.8 BIOSYNTHESIS OF 5-DEOXYFLAVONOIDS

A characteristic of legumes is the biosynthesis of 6'-deoxychalcones (chalcones lacking a hydroxyl at the C-6' position), which are the substrates for the production of 5-deoxyflavonoids. The formation of 6'-deoxychalcones requires the activity of polyketide reductase (PKR) (also known as chalcone reductase or chalcone ketide reductase) in conjunction with CHS. It is thought that CoA-linked polyketide intermediates diffuse in and out of the CHS active site, and while unbound are reduced to alcohols by PKR.⁴⁶ The resultant hydroxyl groups are then removed from the PKR products in the final cyclization and aromatization steps catalyzed by CHS.

PKR is a NADPH-dependent monomeric enzyme of 34 to 35 kDa belonging to the aldo- and keto-reductase superfamily.¹⁷⁶ The first isolation and characterization of a PKR cDNA was from *G. max*.¹⁷⁷ The *G. max* cDNA, and cDNAs from other species, have been used to confirm the PKR activity of the recombinant protein, and to produce larger protein amounts for structural analysis.¹⁷⁷⁻¹⁷⁹ Studies of the recombinant protein, and analysis of *35SCaMV:PKR* transgenic plants,^{58,149} have also shown that PKR is able to function with CHS proteins from nonlegume species that synthesize only the common 5-hydroxyflavonoids.

3.9 BIOSYNTHESIS OF ISOFLAVONOIDS AND THEIR DERIVATIVES

Principally found in legumes, isoflavonoids are a group of compounds that originate from flavanones. The factor differentiating isoflavonoids from other flavonoids is the linking of the B-ring to the C-3 rather than the C-2 position of the C-ring. Subsequent modifications can result in a wide range of structural variation, including the formation of additional heterocyclic rings. The additional rings are principally methylenedioxy or dimethylchromene types, formed from cyclization between vicinal hydroxyl and methoxyl or monoprenyl groups, respectively. The initial steps of isoflavonoid biosynthesis are now well characterized at the

molecular level, but there is limited progress on the later enzymatic steps that produce the wide range of complex derivatives found in different legume species. A general scheme for their biosynthesis is presented in Figure 3.5 and Figure 3.6.

3.9.1 2-HYDROXYISOFLAVANONE SYNTHASE

The entry into the isoflavonoid branch of the pathway occurs through the action of 2-hydroxyisoflavanone synthase (2HIS, also known as isoflavone synthase, IFS). 2HIS catalyzes both C-2 to C-3 aryl migration and hydroxylation of the C-2 of (2*S*)-flavanones to yield (2*R*,3*S*)-2-hydroxyisoflavanones. Dehydration of the 2-hydroxyisoflavanones, either spontaneously or through the action of the isoflavone dehydratase (IFD), then forms the isoflavones. The isoflavones formed will be either 5-hydroxy or 5-deoxy compounds, depending on whether they originate from the 6'-hydroxy- or the 6'-deoxychalcone pathways, respectively (e.g., genistein from naringenin and daidzein from liquiritigenin).

2HIS cDNAs were first isolated from *G. echinata* and *G. max* by a variety of functional genomics approaches.^{180–182} These cDNAs were used to identify additional sequences from many legumes and some nonlegume species (see, e.g., Ref. 181), and at the time of this review there were 2HIS sequences from 14 species in public databases. The cDNAs encode P450s that have been classified as part of the CYP93 family (CYP93C) that includes FNSII (CYP93B), flavanone 2-hydroxylase (F2H, CYP93B1), and pterocarpan 6a-hydroxylase (P6aH, CYP93A). Recombinant *G. max* 2HIS expressed in insect cells was able to form isoflavones without measurable accumulation of 2-hydroxyisoflavanone intermediates.¹⁸² This suggests that the dehydration either occurs spontaneously or as part of the 2HIS reaction, without the need for the previously proposed, and partially characterized,¹⁸³ IFD activity. However, when *G. echinata*, *G. max*, or *L. japonicus* cDNAs were expressed in yeast, 2-hydroxyisoflavanones could be identified.^{180,181} Liquiritigenin was converted at a greater efficiency than naringenin, although the extent of the variation in efficiency was different for recombinant 2HIS prepared from insect or yeast systems.

2HIS sequences from different species generally show high amino acid identity scores. Some of the amino acid sequences key to the reaction have been identified by computer analysis and mutational analysis of recombinant proteins.¹⁸⁴ Changing Ser310 of CYP93C2 to Thr gave increased formation of 3-hydroxyflavanone (i.e., DHF), usually a minor product of the 2HIS reaction. Furthermore, replacing Lys375 with Thr gave F3H-like activity producing only DHFs. Other residues are also important for the reaction, as introduction of the defined Ser and Lys residues into the F2H sequence did not confer the ability to carry out aryl migration or 3-hydroxylation. The results support the suggestion of Hashim et al.¹⁸⁵ that the reaction proceeds by a radical generation at C-3 followed by migration of the aryl group from C-2 to C-3, leaving the hydroxyl at C-2.

3.9.2 4'-O-METHYLATION

Subsequent to the 2HIS step, a series of reactions lead to a range of plant defense compounds whose exact structures vary between species, in particular pterocarpan — such as glyceollins in *G. max* and phaseollins in *P. vulgaris* (Figure 3.5–Figure 3.7). The glyceollins and phaseollins have a free 4'-hydroxyl, but in some legume species, such as *Cicer arietinum* (chickpea), the 4'-O-methylated versions of daidzein and genistein occur, named formononetin and biochanin A, respectively.

Akashi et al.¹⁸⁶ suggested that the 2-hydroxyisoflavanone product of 2HIS (e.g., 2,7,4'-trihydroxyisoflavanone that dehydrates to daidzein) might be the substrate for the 4'-O-methylation reaction *in vivo*, rather than daidzein itself. This biosynthetic route is

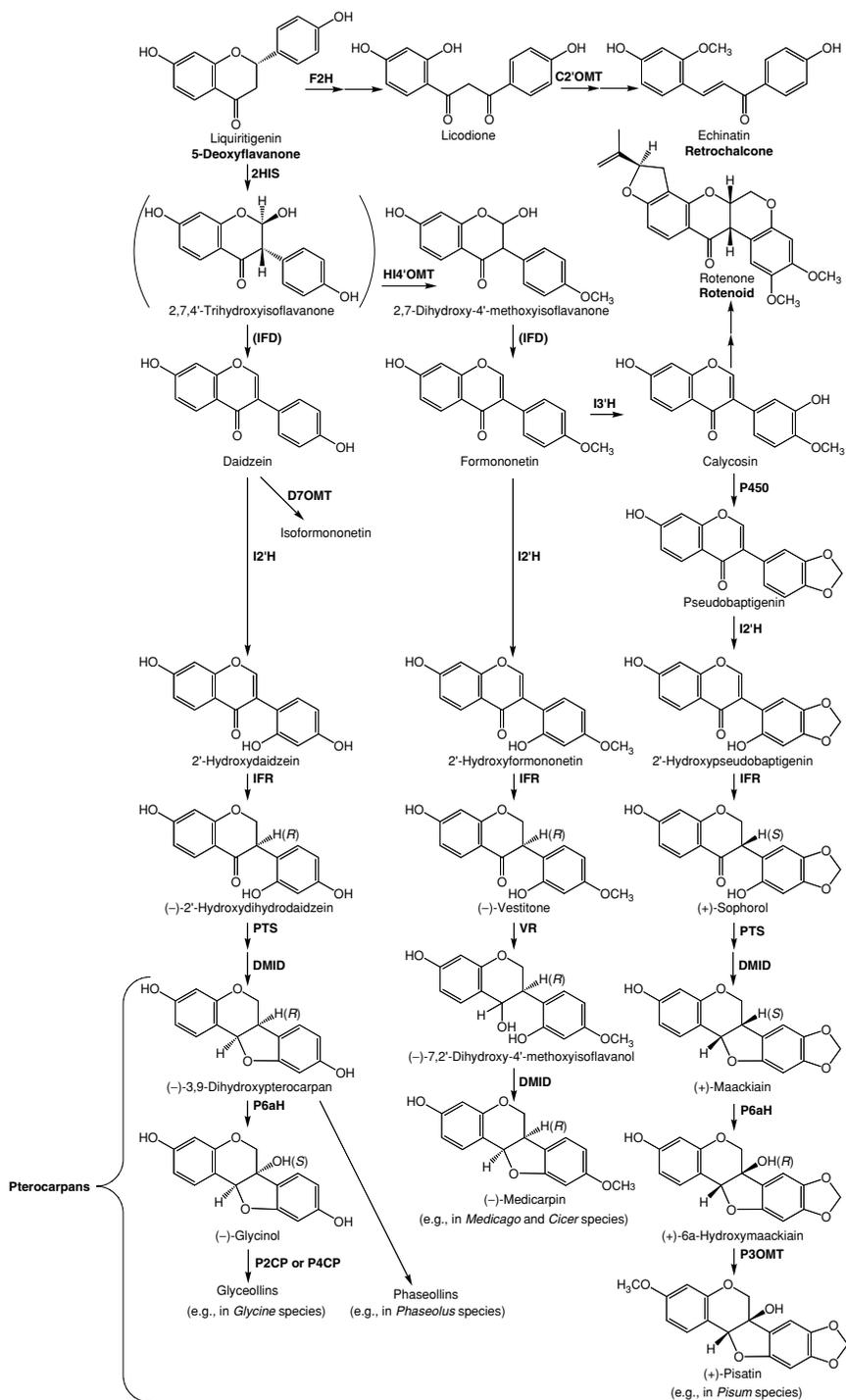


FIGURE 3.5 Biosynthetic route to isoflavonoids (and some derivatives) from the 5-deoxyflavanone liquiritigenin. A possible route to the retrochalcone echinatin is also shown. Unlabelled arrows indicate biosynthetic steps for which the enzyme(s) have not been characterized. Enzyme abbreviations are defined in the text and in Table 3.1, except for P2CP, pterocarpan 2-C-prenyltransferase; P4CP, pterocarpan 4-C-prenyltransferase.

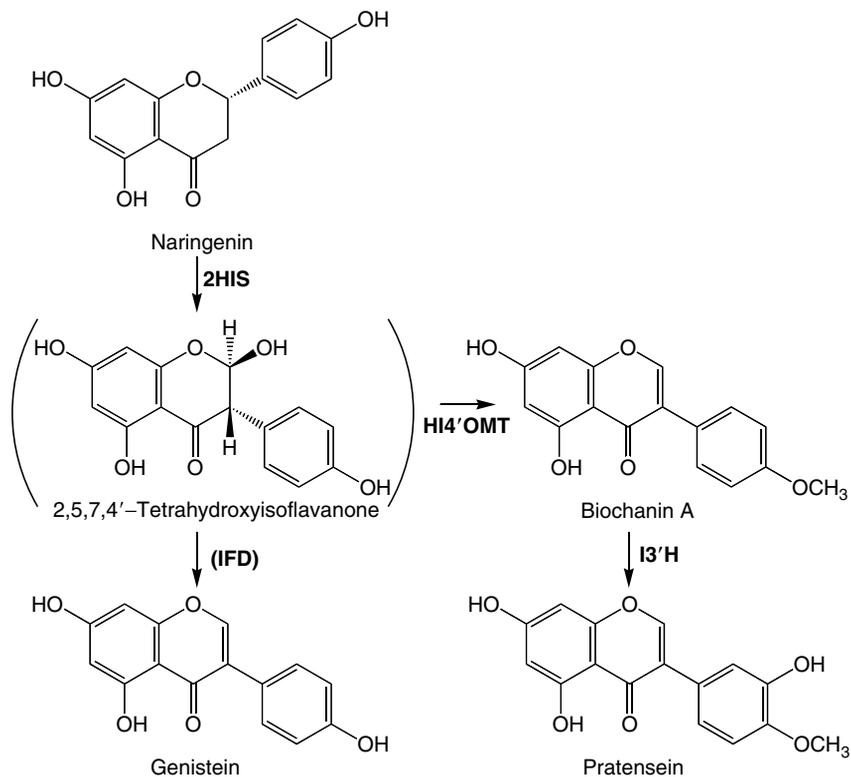


FIGURE 3.6 Biosynthetic route to isoflavones from the 5-hydroxyflavanone naringenin. Enzyme abbreviations are defined in the text.

supported by the isolation of a cDNA from *G. echinata* whose recombinant protein has 4'-OMT activity against 2,7,4'-trihydroxyisoflavanone but not daidzein, thus indicating it is a hydroxyisoflavanone 4'-OMT (HI4'OMT).¹⁸⁷

An alternative biosynthetic route for the introduction of the 4'-methoxyl has been suggested from studies of *M. sativa*. Initial studies of one of the four isoflavone OMTs (IOMTs) of *M. sativa* showed it had 7-*O*-methylation activity to the A-ring of daidzein, thus forming isoformononetin from daidzein. However, overexpression of this IOMT in transgenic *M. sativa* plants enhanced the biosynthesis of 4'-*O*-methylated isoflavonoids.^{188–190} The crystal structure of the IOMT¹¹⁸ indicates the enzyme could accept 2,7,4'-trihydroxyisoflavanone in addition to daidzein, with the SAM methyl donor arranged close to the 7- or 4'-hydroxyl of the respective possible substrates, supporting the observed *in vitro* and *in vivo* reactions. Furthermore, IOMT8 (one of the other *M. sativa* IOMTs) was shown to localize to the endomembranes, with which 2HIS is associated, after induction of isoflavonoid biosynthesis in transgenic *M. sativa* plants.¹⁹¹ Liu and Dixon¹⁹¹ have proposed that close physical association of 2HIS and the IOMT causes metabolic channeling of 2,7,4'-trihydroxyisoflavanone, ensuring its 4'-*O*-methylation and the subsequent formation of formononetin, rather than its dehydration to daidzein and subsequent 7-*O*-methylation.

HI4'OMT from *G. echinata* is thought to be distinct from the *M. sativa* IOMT, because a separate daidzein 7-OMT is present in *G. echinata*, prompting the suggestion that the IOMT be renamed D7OMT.¹⁸⁷ The HI4'OMT amino acid sequence is closely related to that of the SAM:(+)-6a-hydroxymaackiain 3-*O*-methyltransferase (HM3OMT), which carries out a similar reaction in (+)-pisatin biosynthesis in *Pisum sativum* (pea) (see Section 3.9.7). The

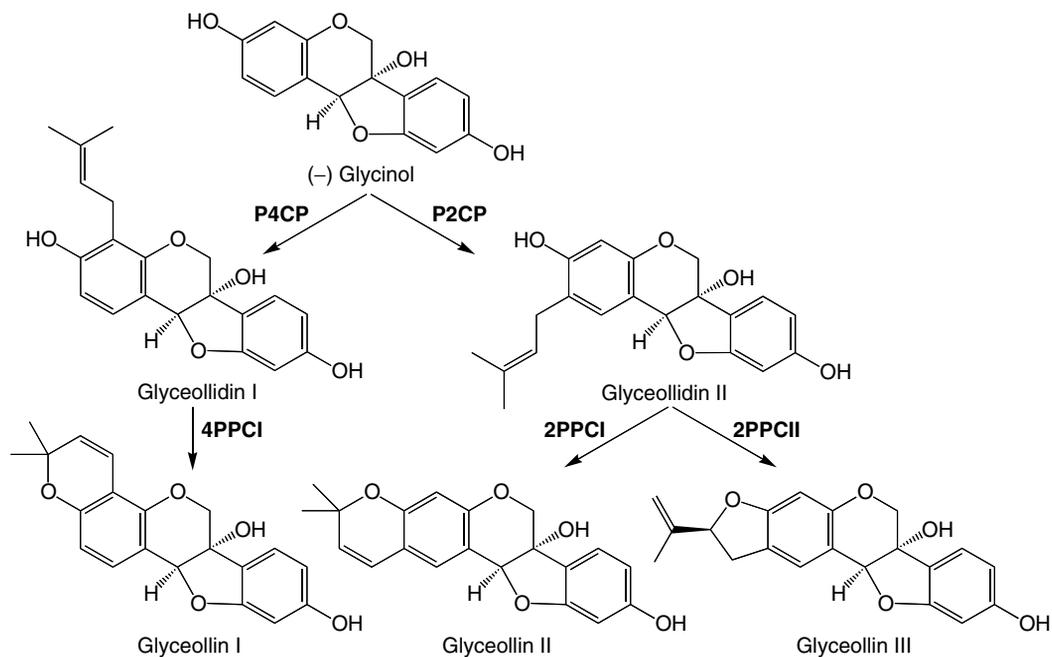


FIGURE 3.7 Biosynthetic route to complex glyceollin phytoalexins from glycinol. Enzyme abbreviations are P2CP, pterocarpan 2-C-prenyltransferase; P4CP, pterocarpan 4-C-prenyltransferase; 2PPCI/2PPCII, prenylpterocarpan cyclases acting at the C-2 prenyl group; 4PPCI, prenylpterocarpan cyclase acting at the C-4 prenyl group.

HI4'OMT protein showed activity against the 3-hydroxyl of a compound related to (+)-6a-hydroxymaackiain, (\pm)-medicarpin, suggesting HI4'OMT may be functionally identical to HM3OMT. However, the HM3OMT substrate is only found in species making (+)-pisatin. The *G. echinata* HI4'OMT cDNA was used to isolate HI4'OMT cDNAs from *L. japonicus*, *M. truncatula*, and other legumes. Both HI4'OMT and IOMT may be involved in formononetin biosynthesis, perhaps in the same tissues, and the formation of heterodimers of similar OMTs has been reported.¹⁹²

3.9.3 ISOFLAVONE 2'- AND 3'-HYDROXYLASE

Two P450s of the CYP81E subfamily, isoflavone 2'-hydroxylase (I2'H) and isoflavone 3'-hydroxylase (I3'H), catalyze key steps in the formation of the more complex isoflavonoids. Hydroxylation of formononetin at the C-3' position produces calycosin, a precursor for subsequent methylenedioxy bridge formation (yielding pseudobaptigenin) as part of the branches leading to maackiain- and pisatin-type phytoalexin end products, as well as to the rotenoids and other complex derivatives. Hydroxylation at the C-2' position of daidzein, formononetin, or pseudobaptigenin provides the hydroxyl required for C–O–C bridge formation that defines the pterocarpan (e.g., glycinol).

A cDNA encoding the I2'H was first identified among a group of cDNAs from *G. echinata* cell lines representing elicitor-induced mRNAs for P450s.¹⁹³ The recombinant protein catalyzed the 2'-hydroxylation of formononetin and daidzein, and had no activity with HCAs or flavanones. Subsequently, cDNA clones have been isolated from *M. truncatula* (*CYP81E7*) and other species.¹⁹⁴ *CYP81E7* expressed in transgenic *A. thaliana* conferred the

ability to form 2'-hydroxyformononetin when formononetin was supplied. A closely related cDNA to *CYP81E7*, *CYP81E9*, was found to encode the I3'H from *M. truncatula*.¹⁹⁴ Recombinant MtI2'H and MtI3'H showed preference to the 5-hydroxylated substrate biochanin A over the 5-deoxy equivalent, formononetin. MtI2'H also showed the expected activity with pseudobaptigenin, daidzein, and genistein, but with reducing preference in each case. For daidzein and genistein, a lesser 3'-hydroxylase activity was also found. The preferred substrates for recombinant MtI3'H were, in descending order, biochanin A, formononetin, and 2'-hydroxyformononetin. K_m values of 67 and 50 μM toward formononetin were found for MtI2'H and MtI3'H, respectively. The I2'H and I3'H genes are differentially expressed in *M. truncatula*, in terms of spatial patterns and response to biotic and abiotic stresses.¹⁹⁴ The data suggest a role for I3'H in the formation of compounds such as rotenoids in response to insect attack, and I2'H in biosynthesis of antimicrobial isoflavonoids.

3.9.4 ISOFLAVONE REDUCTASE

The 2'-hydroxyisoflavones are reduced to the corresponding isoflavanones by a NADPH-dependent isoflavone reductase (IFR). The isoflavanones are the final isoflavonoid intermediates of pterocarpan biosynthesis. Variant IFR activities between species are thought to contribute to the stereochemistry of the pterocarpan produced, in particular, (+)-maackiain in *P. sativum*, (-)-maackiain in *C. arietinum*, (-)-3,9-dihydroxypterocarpan in *G. max*, and (-)-medicarpin in *M. sativa*. The (-) indicates 6aR11aR stereochemistry.

Clones corresponding to IFR were first isolated from *M. sativa*, using an antibody raised against the *P. sativum* protein to screen an expression cDNA library,¹⁹⁵ from *C. arietinum* based on protein purification and sequencing,¹⁹⁶ and subsequently from *P. sativum*.¹⁹⁷ IFR has a calculated MW of 34 to 35 kDa, and is a member of the RED protein family. The recombinant *M. sativa* enzyme converted 2'-hydroxyformononetin to the expected (3R) isomer of vestitone, and would accept 2'-hydroxypseudobaptigenin, but not formononetin, pseudobaptigenin, or several other flavonoids tested. These are similar substrate preferences to those of the purified *C. arietinum* enzyme. Surprisingly, the recombinant *P. sativum* enzyme produced a (3R)-isoflavanone product, (-)-sophorol, from 2'-hydroxypseudobaptigenin, rather than the (3S)-stereoisomer, (+)-sophorol, that would be expected from the accumulation of (+)-maackiain in this species (Figure 3.5). Thus, it is possible that an epimerase is also involved in the biosynthetic pathway to the (+)-isoflavonoids, at least in *P. sativum*.

Using the crystal structures of two related RED enzymes of lignan biosynthesis, a provisional molecular model has been produced for the *M. sativa* IFR.¹⁹⁸ A smaller binding pocket in the protein, in comparison to the other enzymes, is suggested to account for the specific enantiomer binding and processing of IFR.

3.9.5 VESTITONE REDUCTASE

Based on analysis of enzyme preparations, the conversion of isoflavanones to pterocarpan was thought initially to be catalyzed by a single NADPH-dependent enzyme, termed the pterocarpan synthase (PTS). However, it was subsequently shown that in *M. sativa* the conversion of vestitone to medicarpin involves two enzymes, VR and 7,2'-dihydroxy-4'-methoxyisoflavanol (DMI) dehydratase (DMID).¹⁹⁹ The reaction series from vestitone to the pterocarpan is thought to proceed by the VR-catalyzed reduction of vestitone to DMI, followed by the loss of water and formation of the C-O-C bridge between the heterocycle and the B-ring, catalyzed by DMID.

Guo and Paiva²⁰⁰ used the amino acid sequence of purified VR to isolate a cDNA clone from *M. sativa*, which was then analyzed by expression in *E. coli*. VR, a monomeric 38 kDa

protein, is another member of the RED family. It converts (3*R*)-vestitone, but not (3*S*)-vestitone, to DMI *in vitro*, although the stereospecificity may vary with VR enzymes from other species.²⁰⁰ Little has been published on other VR cDNAs, although the *G. max* VR sequence is available in US patent #6,617,493. The isolation of DNA sequences for DMID has not been reported.

In some species, such as *C. arietinum* and *M. sativa*, the products of VR and DMID (maackiain and medicarpin) are the main pterocarpans phytoalexins. They are typically glucosylated and malonylated and stored in the vacuole.²⁰¹ In species such as *G. max*, *P. sativum*, and *P. vulgaris*, the pterocarpan are further converted by a series of reactions to species-specific compounds. For *G. max* and *P. sativum*, the initial reaction is a hydroxylation catalyzed by P6aH.

3.9.6 PTEROCARPAN 6A-HYDROXYLASE

The main phytoalexins in *G. max* (glyceollins) and *P. sativum* (pisatin) are pterocarpan hydroxylated at position C-6a, in a reaction carried out by the P450 P6aH (at least in *G. max*). Recombinant CYP93A1, from a cDNA isolated from elicitor-induced *G. max* cells, carries out the stereospecific and regioselective hydroxylation at the 6a position of (6*R*,11*aR*)-3,9-dihydroxypterocarpans, with a K_m of 0.1 μM , to yield 3,6a,9-trihydroxypterocarpans.²⁰² Given the specificity of the reaction, the encoded protein was termed the dihydroxypterocarpans 6a-hydroxylase (D6aH). P6aH activities are present in other species, but analysis of cDNA clones has not been published.

3.9.7 SAM:6A-HYDROXYMAACKIAIN 3-O-METHYLTRANSFERASE

Methylation of the 3-hydroxyl of (+)-6a-hydroxymaackiain by HM3OMT produces the major phytoalexin of *P. sativum*, (+)-pisatin. Two closely related cDNAs for HM3OMT were isolated from a cDNA library prepared from pathogen-induced *P. sativum* mRNA using antibodies prepared to the purified enzyme.²⁰³ The recombinant proteins had highest activity with (+)-6a-hydroxymaackiain, a lower activity with (+)-medicarpin, and low or no activity with (-)-6a-hydroxymaackiain, (-)-medicarpin, (-)-maackiain, isoliquiritigenin, daidzein, or formononetin. One of the HM3OMT proteins also had significant activity with (+)-maackiain although this is unlikely to be an *in vivo* substrate, as 3-*O*-methylmaackiain is not observed in *P. sativum* tissues.

3.9.8 PTEROCARPAN PRENYLTRANSFERASES

The formation of phytoalexins such as glyceollins and phaseollins requires *C*-prenylation by a range of pterocarpans prenyltransferase (PTP) activities, with dimethylallyl pyrophosphate (DMAPP) as the prenyl donor. For glyceollins and phaseollins, prenylation occurs at position C-2 or C-4 of glycinol or C-10 of 3,9-dihydroxypterocarpans.^{204,205} However, there are differing activities in other species. For example, in *Lupinus albus* (white lupin) a prenyltransferase acting at the C-6, -8, and -3' positions of isoflavones has been identified.²⁰⁶ PTPs have also been characterized in detail for the formation of prenylated flavanones in *Sophora flavescens* (see, e.g., Ref. 207). However, no cDNA clones for flavonoid-related prenyltransferases have been published to date.

3.9.9 PRENYLPTEROCARPAN CYCLASES

The final step of glyceollin and phaseollin formation is the cyclization of the prenyl residues of glyceollidins and phaseollidins, carried out by P450 prenylcyclases (Figure 3.7). These

activities have been studied in detail for the formation of three glyceollins (I, II, and III) from glyceollidin I and II, and it is thought that specific activities are involved in each reaction.²⁰⁸ However, no corresponding cDNA sequences have been published to date.

3.9.10 ISOFLAVONOID GLUCOSYLTRANSFERASES AND MALONYLTRANSFERASES

As mentioned previously, in some species the major phytoalexins are glycosylated and acylated. The final product of the isoflavonoid phytoalexin pathway in *M. sativa* is medicarpin 3-*O*-glucoside-6''-*O*-malonate, and a range of isoflavone 7-*O*-glucosides and their malonylated versions accumulate in *G. max*. There are few reports on molecular characterization of DNA sequences for the enzymes carrying out glycosylation or acylation of isoflavonoids, although some cloned GTs with wide substrate acceptance have been shown to act on isoflavones (see Section 3.7.1). However, a cDNA for one isoflavonoid-specific GT has been isolated from *G. echinata* using the *S. baicalensis* F7GT cDNA as a probe.²⁰⁹ The *UGT73F1* cDNA encodes a putative UDP-glucose:formononetin 7-*O*-glucosyltransferase. The recombinant protein accepted both formononetin and daidzein efficiently, and had little activity on other flavonoids tested. Glycosylation occurred at only one, as yet unassigned, position of daidzein, which is hydroxylated at C-7 and C-4'. Since the likely *in vivo* substrate is formononetin, which has only the 7-hydroxyl free, the enzyme was termed a formononetin 7GT. In a molecular phylogeny the *UGT73F1* sequence is located in a cluster with other stress-induced GTs.

3.9.11 FLAVONOID 6-HYDROXYLASE

The hydroxyl groups at C-5 and C-7 of the A-ring are introduced during the formation of chalcones by CHS (or at the C-7 alone if PKR is coactive). However, flavonoids also occur with C-6 and C-8 hydroxylation, including 6- and 8-hydroxyanthocyanins, flavonols, and isoflavonoids. Latunde-Dada et al.²¹⁰ have identified a cDNA representing an elicitor-induced P450 (CYP71D9) with flavonoid 6-hydroxylase (F6H) activity, which may be involved in the biosynthesis of isoflavonoids with 6,7-dihydroxylation of the A-ring. The recombinant protein did not act on the isoflavonoids or pterocarpan directly, but rather accepted flavanone and DHF substrates, including liquiritigenin, suggesting hydroxylation occurs prior to aryl migration of the B-ring. In support of this route, 2HIS was found to be able to use 6,7,4'-trihydroxyflavanone.

The F6H showed low activity against flavones and little action on the flavonol kaempferol. Nevertheless, it is possible that a similar biosynthetic route through hydroxylation of precursors, perhaps by the same F6H, is involved in the production of other 6-hydroxyflavonoids. However, an alternative activity of the 2OGD type has been characterized at the biochemical level from *C. americanum* that catalyzes the 6-hydroxylation of partially methylated flavonoids.²¹¹

3.10 FORMATION OF AURONES

Although it has long been thought, based on genetic mutant and biochemical evidence, that aurones are derived from chalcones, the biosynthetic mechanism has only recently been clarified, and some aspects of the enzymatic process still await *in vivo* proof. An mRNA from *A. majus*, specifically expressed in the petal epidermal cells, has been shown to encode a recombinant protein with aureusidin synthase (AUS) activity.²¹²⁻²¹⁴ AUS is a variant polyphenol oxidase (PPO) that can catalyze conversion of either 2',4',6',4'-etrahydroxychalcone (naringenin chalcone) or 2',4',6',3,4-pentahydroxychalcone to

aureusidin (3',4'-hydroxylated) or bracteatin (3',4',5'-hydroxylated), respectively²¹² (note that carbon numbering differs between chalcones and aurones — Figure 3.1). Thus, it carries out both B-ring hydroxylation and formation of the C-ring, and, indeed, the enzyme studies suggest 3,4,2'-hydroxylation of the chalcone substrate may be a requirement for the formation of the aurone.²¹⁴ How this observation relates to the biosynthesis of those aurones with no B-ring hydroxylation is not clear. The only studies to date published on the enzymology of B-ring deoxyaurone formation, in cell cultures of *Cephalocereus senilis* (old man cactus), did not address this step.²¹⁵ For 4-deoxyaurones, it is likely the 6'-deoxychalcones are acted on by AUS, as the compounds commonly co-occur and AUS preparations show significant activity on the 6'-deoxychalcones.²¹⁴

PPOs are typically plastid located copper-containing glycoproteins that have activity on a wide range of phenylpropanoids. It has been known for many years that PPOs can form aurones from chalcones, and this has been demonstrated recently with a recombinant tyrosinase from *Neurospora crassa*.²¹² However, AUS represents a specific variant to previously defined PPOs. In particular, it lacks a typical N terminal plastid localization signal, and it has been suggested that it may be located to the vacuole.²¹² How AUS would then compete with CHI for chalcone substrate, as may happen in species such as *A. majus* that can produce aurones and anthocyanins in the same petal cells, is not clear. However, recombinant AUS can use chalcone 4'-*O*-glucosides as substrates,²¹⁴ raising the possibility that aurone formation *in vivo* occurs in the vacuole from glucosylated substrates. Confirmation of the role of AUS awaits characterization of specific mutants, or the production of knockout or gain of function transgenic plants, and determination of the protein localization. Bifunctional PPOs may also be involved in the biosynthesis of betalains.²¹⁶

Aurones are commonly glucosylated at the 4- or 6-hydroxyl. International Patent Application WO00/49155 reports that the recombinant *S. baicalensis* F7GT¹⁵⁵ can glucosylate aureusidin at the equivalent 6-hydroxyl, and also details the cloning of similar 7-*O*-glucosyltransferase cDNAs from *A. majus* and *P. hybrida*. However, it is not clear how these activities relate to the aurone or chalcone 6-*O*-glucosyltransferase activity characterized from *Coreopsis grandiflora* that accepts both 4-deoxyaurones and 6'-deoxychalcones.²¹⁷

To date, in addition to *CHS* mutants, no mutants have been fully characterized that abolish aurone biosynthesis, although a commercial white-flowered line of *A. majus* was shown to lack *AUS* transcript in Nakayama et al.²¹² and there is a preliminary report of an EMS-generated aurone-specific mutant of *A. majus*.²¹⁸

3.11 OTHER ACTIVITIES OF FLAVONOID BIOSYNTHESIS

There are a few well-characterized modification enzyme activities that have not been detailed in the preceding sections on flavonols, flavones, anthocyanins, and isoflavonoids, and these are discussed here. Chalcones may be modified by hydroxylation, glycosylation, or methylation. The common 6'-deoxychalcone butein is hydroxylated at the C-3' position by a P450 enzyme thought to be distinct to the F3'H.²¹⁹ Methylation of the 2'-hydroxyl of isoliquiritigenin in legumes such as *M. sativa* and *P. sativum* forms one of the most active flavonoids for signaling to *Rhizobium* symbiots.²²⁰ As methylation prevents formation of the flavonoid C-ring, the OMT activity will help determine the balance between nodulation-related and defense-related flavonoids. Maxwell et al.²²¹ isolated a cDNA from *M. sativa* for a SAM:isoliquiritigenin 2'-OMT and demonstrated the encoded activity by assaying recombinant protein from *E. coli*. As would be expected from the symbiosis function, the gene is expressed primarily in developing roots. Haga et al.²²² have presented a short report on a cDNA for a similar OMT from *G. echinata*, which in addition to acting on isoliquiritigenin, may be involved in the methylation of licodione to form the retrochalcone echinatin (Figure 3.5).

F2H was postulated to be involved in the formation of the common flavones upon its identification from *G. echinata*, as the recombinant F2H catalyzed the formation of 2-hydroxynaringenin from (2*S*)-naringenin, which yielded apigenin upon acid treatment.^{180,223} Based on the subsequent identification of FNS sequences from *G. echinata* and other species, it now seems unlikely that F2H is involved in the formation of the common flavones. Rather, its *in vivo* role in *G. echinata* may be the 2-hydroxylation of liquiritigenin, which can then yield licodione upon hemiacetal opening, as part of the biosynthetic route to echinatin (see Figure 3.5). Thus, F2H could be the licodione synthase. The pathway to echinatin may continue by the methylation of licodione by the OMT described by Haga et al.²²² Alternatively, the F2H may be part of the biosynthetic pathway to flavone *C*-glycosides. 2-Hydroxyflavanones have been proposed to be direct precursors for flavone *C*-glycosides and the substrates for *C*-UGTs, thus giving a biosynthetic scheme in which glycosylation occurs prior to the formation of the flavone structure.^{143,224}

The flavonoid modification enzymes for which DNA sequences are available, and which we have already discussed in this chapter, represent only a few of the expected activities. Heller and Forkmann³ tabulated 20 to 30 characterized enzymes, and given the variety of flavonoid structures identified, this must still be only a small portion of the existing activities.

3.12 VACUOLAR IMPORTATION OF FLAVONOIDS

Although flavonoids are found in many cellular compartments, it is only the mechanisms for vacuolar import that have been characterized in any detail. Alternative import mechanisms have been found that involve direct uptake, carrier proteins, or secondary modifications triggering importation. While commonalities are found for the import of anthocyanins, flavones, flavonols, and PAs, differences have also been observed for the different types of flavonoid.

The best-characterized mechanism shares elements with general xenobiotic detoxification processes, which typically involve the addition of glycosyl, malonyl, or glutathione residues to form stable water-soluble conjugates, and the sequestration of these conjugates by ATP-binding cassette (ABC) transmembrane transporters.^{225,226} In particular, glutathione-*S*-transferase (GST, EC 2.5.1.18) activities have been shown to be required for the transport of anthocyanins and PAs in some species, mostly based on studies of mutants affected in *GST* genes. In the *bronze2* (*bz2*) mutant of *Z. mays* anthocyanins undergo oxidation and condensation in the cytosol, causing bronze kernel pigmentation.²²⁷ The *an9* mutant of *P. hybrida* has acyanic petals,²²⁸ while the *tt19* mutant of *A. thaliana* has reduced anthocyanin and PA accumulation in seedlings and seed, respectively.²²⁹ Although AN9 and BZ2 only share 12% amino acid identity and belong to different classes of GST (type-I and type-III, respectively), they show functional homology, being able to reciprocally complement the mutations and, furthermore, complement the mutant floral phenotype of the *flavonoid3* mutant of *D. carophyllus*.²³⁰ AN9 also complemented the anthocyanin phenotype of *tt19*, but it did not overcome the loss of PA production. Another indication that anthocyanin sequestration is linked to general detoxification processes is that alternatively spliced *Bz2* mRNAs accumulate in response to various stresses.²³¹

Interestingly, recombinant AN9 does not glutathionate cyanidin 3-*O*-glucoside or other flavonoids *in vitro*, and no anthocyanin–glutathione conjugates have been observed *in vivo*.²³² It has been suggested, therefore, that GST directly binds anthocyanins and escorts them to the vacuole, without glutathione addition being required.²³² However, the recombinant proteins of the *A. thaliana* ABC transporter proteins AtMRP1 and AtMRP2 can transport glutathionated anthocyanin *in vitro*, as well as other glutathione *S*-conjugates and chlorophyll

catabolites, supporting a role for glutathionation and subsequent import by ABC transporters.²³³

Vacuolar transport of PAs differs from that of anthocyanins in some species. In *A. thaliana*, the *TT12* locus encodes a transporter of the multidrug and toxic compound extrusion (MATE) type with 12 membrane-spanning domains, and mutations preventing *TT12* function prevent accumulation of PAs.¹⁴² Interestingly, a transcript with high sequence identity to *TT12*, *MTP77*, is upregulated in *L. esculentum* transgenics over-producing anthocyanin due to activation of an anthocyanin regulatory gene, suggesting a link between MATE transporters and anthocyanins in this species.²³⁴

There is also a preliminary report of a *P. frutescens* cDNA encoding a membrane protein of unknown function (8R6) that promotes anthocyanin uptake into protoplasts and anthocyanin accumulation when overexpressed in *A. thaliana* transgenics.²³⁵ Within the vacuole of some plants (but not *A. thaliana*) the anthocyanins may occur in protein containing bodies, termed anthocyanic vacuolar inclusions (AVIs), whose function is as yet unknown but may relate to transport activities.^{236,237}

An additional aspect of flavonoid transport to the vacuole is the coordination of the localization process with vacuole biogenesis. The major vacuole in a cell may grow by small pro-vacuolar vesicles that have budded off the plasma membrane, endoplasmic reticulum, or Golgi fusing with the tonoplast. Interestingly, the *tds4* mutation of *A. thaliana* (for ANS, preventing epicatechin production) prevents normal vacuole development and causes accumulation of small vesicles.¹³⁹ Other *tt* mutations that prevent PA accumulation but not epicatechin formation do not interfere with vacuole development, implying a link between epicatechin biosynthesis and vacuole development.¹³⁹

There are few studies on vacuolar importation of flavonoids other than anthocyanins and PAs. Klein et al. reported uptake of flavone glycosides by isolated *H. vulgare* primary leaf vacuoles via a vacuolar H⁺-ATPase linked mechanism,²³⁸ and by vacuoles from *Secale cereale* (rye) mesophyll via a possible ABC transporter mechanism.²³⁹ Li et al.²⁴⁰ found medicarpin conjugated to glutathione was also sequestered by an ABC transporter mechanism.

3.13 ENZYME COMPLEXES AND METABOLIC CHANNELING

It has long been thought that biosynthetic enzymes of plants are organized into macromolecular complexes in specific subcellular locations. It was suggested in the 1970s and 1980s that for flavonoid biosynthesis a multienzyme complex might exist, loosely associated with the endoplasmic reticulum and perhaps anchored through P450 enzymes such as C4H or F3'H (for coverage of the earlier literature, see Refs. 14, 57). This proposal has received support from more recent research, in particular affinity chromatography with flavonoid enzymes, yeast two-hybrid analysis, subcellular localization studies, and transgenic plants lacking individual phenylpropanoid enzymes.^{14,57,241,242} Activity studies also support direct enzyme association. For example, PKR is thought to act on an intermediate of the CHS reaction, suggesting close association of the two enzymes in the cell.^{46,57} Additionally, data from transgenic experiments support the occurrence of metabolic channeling and feedback loops in the early steps of phenylpropanoid biosynthesis, in particular for PAL and C4H.^{14,243,244}

A role for enzyme complexes in the metabolic channeling of substrate has been proposed, so that competition between alternate enzyme pathways can be managed and the production of a specific product from a range of possibilities is favored. Furthermore, isoforms of the different enzymes may assemble in particular complexes dedicated to specific classes of flavonoid.

3.14 ASSIGNING ENZYME FUNCTION FROM DNA SEQUENCE OR RECOMBINANT PROTEINS

As molecular studies of flavonoid biosynthesis have progressed, particular issues in assigning enzyme function from DNA sequence or recombinant protein activity have arisen that are worth commenting on briefly. Recent studies with recombinant flavonoid 2OGDs have shown overlapping substrate usage and product formation *in vitro*. For example, recombinant ANS shows overlapping activities with F3H and FLS, and recombinant FLS shows F3H and FNS activity.^{62,63,84–86} However, genetic mutants suggest that these *in vitro* activities may be less prevalent *in vivo*. For example, the still active *ANS* or *FLS* genes do not complement the acyanic phenotypes of *F3H* mutants of *A. majus* or *A. thaliana*, although it is possible (but unlikely) that the genes do not have the appropriate expression patterns either temporally or spatially. The UGT enzymes also may display broader *in vitro* activities than those *in vivo*.^{95,153} The reasons for the differences between the *in vitro* and *in vivo* observations are not clear. The *in vitro* assay conditions may not accurately replicate the *in vivo* environment, so that differential efficiencies with regard to the alternative substrates and isomers might not be correctly represented. Also, recombinant proteins may have small but important structural differences to the native enzyme, as well illustrated by studies of PFOMT¹⁶⁶ (see Section 3.7.2). Alternatively, the formation of the enzymes into complexes and the effects of metabolic channeling may control the different activities. For example, the action of F3GT on an intermediate of the ANS reaction may direct anthocyanin formation over flavonol formation, even though flavonols are the favored ANS product *in vitro*.

The second area worthy of comment is with regard to assignment of enzyme function based on amino acid sequence alone. In some cases sequence comparisons can be used to determine if a new cDNA encodes one of the well-known flavonoid biosynthetic enzymes. However, in some cases, sequence similarity is insufficient for reliable prediction of the encoded function. For example, the OMTs may have amino acid identities of above 85% but different activities.¹⁶⁵ For transcription factors such sequence-based assignments of function are significantly more difficult, as specific roles for a given factor may have arisen in particular species. Thus, analysis of genetic mutants and transgenic overexpression or “knockout” lines is still preferable for confirmation of the encoded activities.

3.15 REGULATION OF FLAVONOID GENE TRANSCRIPTION

The phenylpropanoid pathway involves many biosynthetic genes and several alternative branches from common precursors leading to different flavonoid types and other compounds. Such complexity requires fine-tuned control, allowing the alteration of flux as conditions vary. This control is often achieved by the coordinate regulation of multiple genes, with the groupings varying with not only the end product that is to be made, but also with respect to the species and the type and developmental stage of a tissue. Furthermore, individual biosynthetic genes may be regulated in response to a number of developmental and environmental signals. For example, in flowers the biosynthetic genes change their activities as a consequence of light and spatio-temporal developmental factors for the production of anthocyanins in the petal epidermal cells, coincident with flower fertility.

Studies to date have shown that increases in gene transcription rates generally precede flavonoid production. This observation, in conjunction with studies on flavonoid-related transcription factors (TFs), suggests that gene transcription is the key point for biosynthetic gene regulation, rather than translation or post-translational steps. Post-transcriptional regulation has been reported for flavonoid biosynthesis (reviewed in Ref. 14 and recently

featured in Ref. 245), and the extent of its role in controlling the pathway may come to light as methods for studying these aspects improve. In combination with metabolic channeling, post-transcriptional regulation would provide a means of fine control on the metabolic flux through the pathway to the accumulation of end products. Nevertheless, the overall data support the idea that it is changes in biosynthetic gene transcription rate that underpin major changes in flavonoid biosynthetic activity in most situations.

The transcription rate of a given gene is principally determined by interactions of TFs specific for that gene with the RNA polymerase II-containing holoenzyme and other components of the basal transcription machinery. Gene-specific TFs bind in a sequence-specific manner to motifs (*cis*-elements) within genes, usually in gene promoters, and increase (as activators) or decrease (as repressors) the rate of transcriptional initiation.^{246–248} TF activity may be modulated by a range of mechanisms, in particular competition with other TFs, direct interaction with coactivator or corepressor proteins (which themselves do not bind DNA), and reversible phosphorylation.

Research on the regulation of the flavonoid biosynthetic genes is at the forefront of general plant transcriptional regulation studies. There are data from a number of different plant species concerning the specific *cis*-elements and TFs involved, and some of the functional interactions between the different types of TF have been elucidated. In this section, the literature on regulation of flavonoid biosynthesis is reviewed in brief only, and the reader is referred to more detailed review articles where possible. For additional information on the aspects covered, as well as detailed discussion of the TF families, the reader is referred to other reviews, including a comprehensive review of the genomics of transcriptional regulation in *A. thaliana*.^{52,248–255}

3.15.1 ENVIRONMENTAL REGULATION OF EARLY BIOSYNTHETIC STEPS

Studies have predominantly focused on the regulation of *CHS*, presumably because *CHS* activity is a key flux point for flavonoid biosynthesis. *CHS* is commonly regulated coordinately with general phenylpropanoid genes in response to abiotic and biotic stresses. Much data are available for the *cis*-regulatory elements and associated TFs for regulation of *PAL*, *C4H*, *4CL*, and *CHS* in response to UV light and pathogen-related elicitors, and cross-talk between the different stress signaling pathways is being uncovered. Furthermore, the control of flavonoid gene expression during general photomorphogenesis is being characterized as part of studies in *A. thaliana* on the constitutive photomorphogenic (COP) regulatory system.

The COP system is composed of the COP9 signalosome, a multiprotein complex encoded by several *COP*, *De-etiolated* (*DET*), and *Fusca* genes, and at least four related proteins that act outside the COP9 complex — COP1, COP10, COP1 Interacting Protein (CIP), and DET1 (reviewed in Refs. 256–258). It is thought to act via ubiquitin-mediated, light-dependent degradation of downstream signaling components of many photoreceptors, as COP1 is a putative E3 ubiquitin ligase that interacts directly with several TFs. Affected proteins encoding phenylpropanoid regulators include AtMYB21 and HY5.^{259,260} *COP10* and *CIP* probably encode other components of the ubiquitin degradation pathway.

From promoter analysis and transgenic studies the light-responsive unit (LRU) of the *CHS* genes of *P. crispum* and *A. thaliana* has been shown to be necessary and sufficient for induction of gene expression in response to UV-A and blue light or UV-B (see, e.g., Refs. 261–264). The LRU contains an ACGT-containing element (ACE) and an MYB-recognition element (MRE). A variety of ACEs have been identified that are named from the last nucleotide in the recognition motif, including the A-box (TACGTA)-, C-box (GACGTC)-, G-box (CACGTG)-, and T-box (AACGTT)-type elements. Many basic region/leucine

zipper (bZIP) proteins have been shown to recognize and bind ACEs present in a range of promoters (reviewed in Refs. 265, 266). In *P. crispum*, at least seven bZIP proteins (referred to as common plant regulatory factors — CPRFs) bind the LRU ACE with varying affinities. In particular, PcCPRF1 is likely to be vital to the UV-induction process, as *PcCPRF1* transcript levels increase in abundance in response to UV light and precede the increase in *CHS* transcript abundance. In *A. thaliana*, the bZIP HY5 regulates a number of pathways, including that of the phenylpropanoids, and interacts directly with the ACE of light-responsive promoters.²⁶⁷ Homo- and heterodimers of CPRFs occur, and dimerization, along with phosphorylation, may be important to the subcellular localization and function of bZIP proteins.^{268,269} A number of two-repeat R2R3 MYB proteins (including AmMYB305 or AmMYB340 described in Section 3.15.4) and an unusual single-repeat MYB from *P. crispum*, PcMYB1,²⁶³ have been shown to activate phenylpropanoid gene promoters through interaction with the LRU MRE or related MREs (e.g., the P-box). The MREs are often not only involved in the response to environmental stimuli but also in the developmental and spatial control of gene expression.

MYB proteins with a repressive function are involved in the regulation of phenylpropanoid biosynthetic genes. The first indication of such a role came from overexpression of *A. majus AmMYB308* or *AmMYB330* in *N. tabacum*, which caused dramatic reductions in the levels of lignin.²⁷⁰ Analysis of an *A. thaliana* mutant for the orthologous gene *AtMYB4* showed increased *C4H* transcript levels, elevated levels of sinapate esters (HCA derivatives), and enhanced tolerance to UV-B exposure.²⁷¹ In *CaMV35S:AtMyb4* plants, the *C4H*, *4CL*, and *CHS* genes were all downregulated. Although white light is required for *AtMyb4* gene expression, transcript levels fall markedly within 24 h of exposure to UV-B. Deletion analysis and creation of fusion proteins indicate a role for the C terminal domain of the AtMYB4 protein in the repression function, perhaps through an “active” repressive effect on the basal transcription machinery.²⁷¹

The response to pathogen infection involves not only some of the *cis*-elements characterized for the UV-light response but also additional regulatory elements related to pathogen-associated stimuli. These include the H-box and a TGAC (W-box) sequence that interacts specifically with a WRKY TF.^{249,272,273} H-boxes have been associated with stress induction and tissue specificity of *PAL*, *4CL*, and *CHS* genes of a number of species. For example, the multiple H-boxes of the *P. vulgaris CHS15* gene promoter, along with the neighboring G-box and ACE, contribute to both tissue specificity of gene expression and induction in response to pathogen elicitation.^{272,273} Although the H-box (CCTACC) closely resembles MREs such as the P-box (e.g., CCACCTACCCC), the interacting TFs characterized to date are not MYB proteins. The KAP-1 and KAP-2 proteins of *P. vulgaris*, which interact specifically with this sequence in the *CHS15* promoter, have sequence similarity to the mammalian Ku autoantigen protein involved in control of DNA recombination and transcription.²⁷⁴ KAP-2 cDNAs have been isolated from *P. vulgaris* and *M. truncatula*, and the recombinant protein has been shown to activate H-box-containing promoters *in vitro*. KAP-2 transcript is constitutively present in *P. vulgaris* tissues, suggesting post-translational control of its activity, perhaps related to the previously observed elicitor-induced phosphorylation of the protein.²⁷⁵

Pathogen elicitation can also downregulate gene transcription, as evidenced by studies on the UV-light-inducible *CHS* and *ACC* genes of *P. crispum*.²⁷⁶ When plants are placed under both pathogen and UV-light stresses, the pathogen repression signal to these genes is dominant to the UV-light induction signal. For *ACC*, both signals converge on two very similar ACEs, suggesting the switch from activation to repression might be achieved through replacement of activating TFs with a repressor protein. UV-induced increases in PcCPRF1 transcript levels are prevented by elicitor treatment, while PcCPRF2 transcript levels are decreased by UV light and increased by elicitation, indicating such a regulatory system.

3.15.2 REGULATION OF ANTHOCYANIN BIOSYNTHESIS

Regulation of anthocyanin production involves transcriptional activators of the R2R3 MYB and the basic helix–loop–helix (bHLH) (or MYC) types (Table 3.2). This was first revealed by studies of the monocot *Z. mays*. It was found that the anthocyanin pathway is turned on in this species through the combined action of one member of the COLORED ALEURONE1 (C1)/PURPLE PLANT (PL) MYB family and one member of the RED1 (R)/BOOSTER1 (B) bHLH family.^{14,52} The members of the MYB and bHLH families are functionally redundant, and their specific expression patterns enable spatial and temporal control of anthocyanin biosynthesis.

Activation of the biosynthetic genes is dependent on direct interaction between the MYB and bHLH TFs within the transcriptional activation complex.^{14,52} The complex binds DNA through discrete *cis*-elements in the target gene promoters, one that is recognized specifically by the MYB member and one (the ARE) that is recognized by an as yet unidentified protein.^{277,278} The bHLH member functions in part through the ARE, and may be the protein that binds directly to it, or alternatively, interacts with a different protein that binds to it.²⁷⁸

Other species for which the elucidation of regulatory mechanisms controlling anthocyanin pigmentation is well underway are the eudicotyledon (dicot) species *A. majus* and *P. hybrida* (primarily for floral pigmentation) and *A. thaliana* (for vegetative pigmentation) (see Table 3.2). While TF genes equivalent to those in *Z. mays* are involved, the mechanism of regulation varies. Based on mutant analyses, the encoded TFs in these species control a subset of the anthocyanin biosynthetic genes, the late biosynthetic genes (LBGs). The early biosynthetic genes (EBGs) are under independent control, by as yet unidentified TFs. Partitioning of the pathway into separately regulated units allows for independent control of the production of other flavonoid types. In insect pollinated flowers, flavonoids have roles beyond pigmentation, e.g., flavones and flavonols are frequently the basis of nectar guides. For the floral models studied, the step in the pathway at which control by the defined TFs starts is at *F3H* or *DFR*, depending on whether there is predominate coproduction with anthocyanins of flavones or flavonols, respectively.^{64,89,119,279} In one of the few studies on fruit, the *A* regulatory gene of *Capsicum annuum* (bell pepper) was found to affect only transcript levels of LBGs.²⁸⁰ In *A. thaliana*, the LBGs are coregulated during induction of anthocyanins in seedlings exposed to white light and for PA production.^{281–283}

Modular control of the pathway is not a universal trait in dicot species. For anthocyanin production in vegetative tissues of *P. frutescens*, the biosynthetic genes are regulated as a single group, from *CHS* to *GSTs*, as occurs in *Z. mays*.^{235,284,285} Furthermore, single genes may be important points of control. In *Viola cornuta* *ANS* may be the main regulatory target,²⁸⁶ and in *V. vinifera* berries *A3GT* is regulated separately and may be the key step for triggering anthocyanin production in berries during ripening.^{287,288} Modular control has also been found in the monocot *Anthurium andraeanum*; in both spathe and spadix *DFR* is regulated separately from the other genes.²⁸⁹

As in *Z. mays*, families of MYB and bHLH TFs regulating anthocyanin production have been commonly found in the other species investigated to date. Through studies of these families it has become apparent that highly similar family members may vary subtly in their regulatory activity or act at different points in a regulatory hierarchy. In *A. majus*, differential activities of the family members give variations in floral pigmentation patterns, including a striking venation pattern determined by the *Myb* gene *Venosa*.²⁵⁰ Within the MYB and bHLH families in *P. hybrida*, there are indications that some exert transcriptional control over others,²⁹⁰ a complexity that has not been found in *Z. mays*²⁹¹ or reported for related TFs in other species. It remains to be determined whether these *P. hybrida* TFs also function as direct regulators of the anthocyanin biosynthetic genes.

TABLE 3.2
Transcription Factors that Regulate Flavonoid Biosynthetic Genes

Species	Metabolite	Protein Type	Name	Ref.
<i>Antirrhinum majus</i>	Phenylpropanoids (including flavonoids)	MYB	AmMYB305	319
		MYB	AmMYB308	270
		MYB	AmMYB330	270
		MYB	AmMYB340	319
	Anthocyanins	MYB	ROSEA1	250
		MYB	ROSEA2	250
		MYB	VENOSA	250
		bHLH	DELILA	363
		bHLH	MUTABILIS	250
		bHLH	MUTABILIS	250
<i>Arabidopsis thaliana</i>	Phenylpropanoids	MYB	AtMYB4 (repressor)	271
	Anthocyanins and proanthocyanidins	MYB	PAP1 (AtMYB75)	364
		MYB	PAP2 (AtMYB90)	364
		MYB	TT2 (AtMYB123)	283
		bHLH	TT8	282
		bHLH	GL3/EGL3 (AtMYC-2, AtMYC-146)	365, 366, 295, 367
		WD40 ^a	TTG1	293
		WRKY	TTG2	308
		MADS box	TT16	310
		WIP	TT1	311
		HD-GLABRA2	ANL2	300
	VPI-like	ABI3	303	
	<i>Capsicum annuum</i>	Anthocyanins	MYB	A
<i>Fragaria × ananasa</i>	Anthocyanins and flavonols	MYB	FaMYB1 (repressor)	297
<i>Gerbera hybrida</i>	Anthocyanins	MYB	GMYB10	368
		bHLH	GMYC1	369
<i>Ipomoea tricolor</i>	Anthocyanins	bHLH	IVORY SEED	408
<i>Lycopersicon esculentum</i>	Anthocyanins	MYB	ANT1	234
<i>Perilla frutescens</i>	Anthocyanins	MYB	MYB-P1	370
		bHLH	MYC-RP/GP	371
		WD40	PFWD	294
		MYB	AN2	372, 373
<i>Petunia</i>	Anthocyanins	MYB	AN4	372, 373
		bHLH	AN1	290
		bHLH	JAF13	290
		WD40	AN11	292
		MYB	Y	314
<i>Sorghum bicolor</i>	Phlobaphenes	MYB	Y	314
<i>Vitis vinifera</i>	Anthocyanins	MYB	MYBA	288
<i>Zea mays</i>	Anthocyanins	MYB	C1	374, 375
		MYB	PL	376
		bHLH	B	377
		bHLH	LC	378
		bHLH	R	379
		bHLH	SN	380
		bHLH	IN (repressor)	299
		VPI	VPI	301, 302
		WD40	PAC1	291

TABLE 3.2
Transcription Factors that Regulate Flavonoid Biosynthetic Genes — *continued*

Species	Metabolite	Protein Type	Name	Ref.
	Phlobaphenes/flavone	MYB	P1 (P)	312, 317, 381
	C-glycosides	MYB	P2	317

Note: The functions of the transcription factors have been confirmed (or indicated) by genetic mutant or plant transgenic studies. They are activators of transcription unless stated otherwise. Some are direct activators of the flavonoid biosynthetic genes, while others may encode factors upstream in a regulatory cascade.

^aThese WD40 proteins interact with the TFs for the regulation of the biosynthetic genes.

Another type of protein shown to be involved in the regulation of anthocyanin (and PA) synthesis is the WD repeat (WD40) protein.^{291–293} The WD40 proteins confirmed to date as being involved in flavonoid biosynthesis (Table 3.2) have relatively high sequence identity of around 60%, and form a distinct group within the WD40 family (a molecular phylogeny of WD40 sequences is presented in Ref. 291). Based on gene expression or genetic interaction studies, the WD40 proteins do not function through direct transcriptional control of the *MYB* and *bHLH* genes, even though overproduction of MYB or bHLH factors can partially overcome some of the phenotypes of lines mutated in the WD40 gene.^{291,292} Rather, they likely function as part of the MYB-bHLH transcriptional complex, perhaps providing a stabilizing influence.^{285,294–296}

It remains to be determined whether other types of TFs or regulatory proteins are also directly involved in the control of anthocyanin pigmentation. As mentioned previously, the TF binding the ARE *cis*-element has not been identified, although it potentially is the bHLH factor. Furthermore, although some R2R3 MYB and bHLH TFs with a repressive effect on transcription have been identified for anthocyanin biosynthesis, their role in the overall regulatory system, as well as that of repressor TFs in general, needs further characterization. FaMYB1 likely plays a role in the regulation of anthocyanin and flavonol production in *Fragaria Xananasa* (strawberry) fruit, and has structural features in common with the repressor TF AtMYB4, suggesting it operates through a direct repression mechanism.²⁹⁷ *CI-1* is a dominant negative allele of *CI* that lacks the C-terminal activation domain normally present, and probably represses anthocyanin biosynthetic genes “passively” through competition with activators for target promoter binding sites.²⁹⁸ Also in *Z. mays* is *Intensifier1 (In1)*, which encodes a bHLH protein similar to R.²⁹⁹ IN1 is suggested to have a repressive activity, as recessive mutations in the gene increase anthocyanin levels. It is worth noting that one of the suggested roles of the bHLH TFs is to relieve the MYB coactivators from the effect of an inhibitory factor, which is perhaps of the protein types described above.²⁷⁸

With regard to the regulation of the anthocyanin regulatory genes, progress has been made in dissecting signaling to phenylpropanoid-related TF genes during photomorphogenesis and flavonoid production in vegetative tissues and seeds. *ANTHOCYANINLESS2 (Anl2)* of *A. thaliana* encodes a homeobox protein of the HD-GLABRA2 group that is required for anthocyanin production in the subepidermal cells of vegetative tissues.³⁰⁰ Unlike the *tt* mutants, the *anl2* mutant is not altered in pigmentation of the seed coat, but it does have aberrant cellular organization in the roots. Given that homeodomain proteins are often involved in cell specification and pattern formation, *anl2* may encode an upstream regulator rather than a direct activator of the flavonoid-related *MYB* and *bHLH* genes. *Viviparous 1 (Vp1)* of *Z. mays* encodes a distinct type of TF that has been identified subsequently in a

number of species, including *A. thaliana* (*ABI3*).^{301–303} In *Z. mays* *Vp1* has multiple regulatory roles in seed development, including both up- and downregulation of gene expression. It is required for production of anthocyanins in the aleurone and upregulates the *C1* gene directly.³⁰⁴ Regulation of *C1* occurs through the *Sph cis*-element in the promoter, which is recognized by the B3 domain of VP1. VP1 likely acts as part of a complex of TFs, which may include 14-3-3 proteins.³⁰⁵

On a final note, it is being increasingly found that the anthocyanin-related factors regulate other processes. In this regard, the best characterized is the WD40 protein TRANSPARENT TESTA GLABRA1 (TTG1) of *A. thaliana*. In addition to anthocyanin pigmentation, a number of other processes occurring in epidermal cells are also dependent on TTG1, including trichome, seed mucilage, and PA production.^{293,306} In a similar vein, the WD40 ANTHOCYANIN11 (AN11) of *P. hybrida* also influences seed coat development, in conjunction with the activity of the bHLH anthocyanin regulator ANTHOCYANIN1 (AN1).³⁰⁷ These *P. hybrida* regulators, along with the MYB factor ANTHOCYANIN2 (AN2), also regulate vacuolar pH in the petal cells,³⁰⁷ a role that appears to be played by some of the anthocyanin MYB TFs of *A. majus* as well (K. Schwinn, unpublished data).

3.15.3 REGULATION OF PROANTHOCYANIDIN BIOSYNTHESIS

Although initial progress in understanding regulation of PA biosynthesis was made with *Z. mays*, it is now best characterized in *A. thaliana*, in which PAs accumulate in the testa of the seeds. There are at least six classes of regulatory proteins that have been shown to control transcription of the genes for the PA pathway: bHLH, MADS box, R2R3 MYB, WD40, WIP, and WRKY (Table 3.3).

The bHLH and MYB factors are thought to be direct activators of PA production. Knockout and gain of function experiments have demonstrated that TT2 (MYB) and TT8 (bHLH) interact to upregulate LBGs, but not EBGs such as *CHS*.^{137,282,283} TT2 may be a key determinant of the pattern of PA biosynthesis, as its transcript abundance shows much greater spatial and temporal variation than that of TT8. TT2 is also involved in regulating anthocyanin production during seedling development.

The WD40 protein is *TTG1* (described in the previous section). The WRKY TF is TTG2, which may function downstream of *TTG1*. Mutant lines for *ttg2* have reduced production of PAs and mucilage, and have fewer, less branched trichomes, although they are wild type for anthocyanin production in vegetative tissues.³⁰⁸ However, *ttg2* does not affect *ANR* gene expression, suggesting a late role in PA biosynthesis or a post-transcriptional regulatory role.³⁰⁹ *TT1*, encoding a member of the WIP subfamily of zinc finger proteins, is required for endothelium development, as *tt1* mutants have reduced PA production and altered seed coat morphology.³¹⁰ The MADS box protein TT16 (identical to BSISTER) also may be involved in endothelium development. Lines mutant for *tt16* have altered cell shape and reduced PA production and expression of *ANR*, but only in a specific region of the seed coat, and ectopic expression of *TT16* leads to ectopic PA accumulation.³¹¹ Overexpression of *TT2* can partially complement the *tt16* phenotype, restoring PA biosynthesis, suggesting *TT2* acts parallel or downstream of *TT16*.

The production of 3-deoxyflavonoids, in particular the 3-deoxy-PAs, in *Z. mays*, also involves R2R3 MYB transcription factors.^{312–315} In *Z. mays*, for the production of phlobaphenes, the MYB protein P1 activates *CHS* and *DFR*, and, presumably, the gene for the flavan-3-ol biosynthetic enzyme, but does not upregulate *F3H*.^{312,313} The regulatory activity of P1 does not require a bHLH coactivator, even though P1 recognizes the same promoter elements of the *DFR* gene as C1, the MYB TF regulating anthocyanin synthesis. Grotewold et al.³¹⁶ compared the amino acid sequence of P1 and C1 and were

TABLE 3.3
Genetic Loci of *A. thaliana* Involved in Flavonoid Biosynthesis for which Genetic Mutants and the Encoded Product Have Been Characterized

Locus	Gene Product	Ref. ^a
<i>Biosynthetic enzymes</i>		
<i>TT3</i>	DFR	382
<i>TT4</i>	CHS	383
<i>TT5</i>	CHI	382
<i>TT6</i>	F3H	384
<i>TT7</i>	F3'H	106
<i>TDS4/TT18</i>	ANS	134
<i>Banyuls</i>	ANR	136, 137
<i>Transporter activities</i>		
<i>TT12</i>	MATE transporter	142
<i>TT19</i>	GST	229
<i>Regulatory factors</i>		
<i>TT1</i>	WIP	311
<i>TT2</i>	MYB	259
<i>TT8</i>	bHLH	258
<i>TT16</i>	MADS box	310
<i>TTG1</i>	WD40	293
<i>TTG2</i>	WRKY	308
<i>ANL2</i>	HD-GLABRA2	300

^aReference to the first publication reporting detailed characterization of the corresponding cDNA or gene.

able to identify which amino acid residues in the C1 protein defined the interaction with the *Z. mays* bHLH proteins.

3.15.4 REGULATION OF THE PRODUCTION OF OTHER FLAVONOIDS

There are much less data on the regulation of flavonoids such as the isoflavonoids, flavones, and flavonols. Some of the genes regulating the EBGs in response to environmental stimuli, as discussed in Section 3.15.1, may be involved in controlling flavonol or flavone production. However, their specific role in relation to these compounds, and the regulation of genes encoding FLS and FNS in general, has not been studied widely.

The *P1* gene of *Z. mays*, which controls phlobaphene production, along with a closely related second gene *P2*, controls production of flavone *C*-glycosides in *Z. mays* flower silks by upregulating genes required for flavanone, and possibly flavone, biosynthesis but not the subsequent genes that are required for anthocyanin production.³¹⁷ Furthermore, *P1* or *P2* driven by the *CaMV35S* promoter induces production of flavone *C*-glycosides in transgenic cell lines of *Z. mays*.^{140,317} Flavonol production in *Z. mays* may be under separate regulatory control to anthocyanins or flavones.^{317,318} Two *A. majus* MYB proteins with relatively high sequence identity, AmMYB305 and AmMYB340, may be involved in regulation of the EBGs required for flavonol biosynthesis.³¹⁹ AmMYB305 has been shown to activate the promoters of *CHI* and *F3H*, and AmMYB340 to regulate *CHI* and bind the “P-box” MYB recognition element that is linked to petal-enhanced expression of phenylpropanoid genes.^{319,320} Like *P1* of *Z. mays*, the proteins did not require a bHLH partner for their binding or activating activities.

In *P. hybrida*, genetic and biochemical evidence suggests that *An1* and *An2*, which regulate the LBGs for anthocyanin production, also positively regulate *F3',5'H* and *Cytb5*, but do not affect *F3'H* expression.^{105,110} However, in leaves of *CaMV35S:Lc* transgenic *P. hybrida* both *F3'H* and *F3',5'H* are upregulated, but not *FLS*.³²¹ The *an4* mutation, which lacks activity of an anthocyanin regulator related to *An2* and results in acyanic pollen, does not effect the expression of *F3GalT*.³²² In *A. thaliana*, some of the genes for PA biosynthesis, such as *TTG1*, that are required for LBG expression do not affect expression of *FLS* or *F3'H*.²⁸³

The induction of isoflavonoid production in response to biotic signals is extensively characterized,³²³ and analysis of gene promoter *cis*-elements is well advanced for both the EBGs (see Section 3.15.1 and Ref. 323) and isoflavonoid-specific genes (see, e.g., Ref. 324). However, there is no information on the TFs factors involved specifically in isoflavonoid gene regulation. Introduction of a transgene (*CRC*) for a chimeric protein with activity of both the C1 and R Z. *mays* anthocyanin-related TFs into *G. max* did not alter the transcript levels for the isoflavonoid-specific genes *2HIS*, *IFR*, and *IOMT*, although *PKR*, *FLS*, and other phenylpropanoid genes were upregulated.³²⁵

3.16 GENETIC MODIFICATION OF FLAVONOID BIOSYNTHESIS

In addition to being carried out as part of fundamental studies, genetic modification (GM) of flavonoid production has been used to extend existing flower color ranges or induce vegetative anthocyanin pigmentation in ornamental crops, to modify production of plant-defense flavonoids, and to increase levels of flavonoids related to human and animal health. Indeed, the flavonoid pathway has been the target of more biotechnology research than probably any other plant secondary metabolite pathway. The major approaches have been to prevent or inhibit flavonoid production, redirect substrate within the pathway, introduce new flavonoid biosynthetic activities, and modulate pathway regulation. There are numerous examples of the GM of flavonoid biosynthesis using these approaches, and due to space limitations we discuss only some of them here. An extensive listing of published examples is provided in Table 3.4–Table 3.6. An emerging area in flavonoid biotechnology is the introduction of flavonoid biosynthesis into microorganisms (see, e.g., Ref. 326), but the focus of the following sections will be on GM approaches in plants.

3.16.1 PREVENTING FLAVONOID PRODUCTION

A reduction in, or the prevention of, flavonoid biosynthesis has been demonstrated many times, and in several species, by inhibiting production of a single flavonoid biosynthetic enzyme. This approach is reviewed in Refs. 76, 327, and examples are listed in Table 3.4. It is worth noting that the first published accounts of antisense or sense RNA inhibition of plant gene expression involved *CHS* in *P. hybrida*.^{328–330}

Commonly the target phenotype has been flower color. In addition to the expected white-flower phenotypes, in some species both ordered and erratic pigmentation patterns have been obtained (Table 3.4). Patterning only seems to occur in species that naturally have patterned varieties. Furthermore, some of the patterns show instability, not only within a particular plant but also in their inheritance (see, e.g., Ref. 331), which may limit the commercial usefulness of some of the more dramatic phenotypes.³³²

Approaches to inhibit anthocyanin production that target *CHS* can cause plant sterility, as flavonols play a role in fertility in some species.^{333–335} It is possible to inhibit anthocyanin production by targeting an enzyme such as *DFR*, which still allows the formation of flavonols and flavones. Sense or antisense *DFR* transgenes have been used to reduce or prevent anthocyanin production in several species (Table 3.4), with results similar to those for *CHS*

TABLE 3.4
Genetic Modification of Flavonoid Production Using Inhibition of Flavonoid Biosynthetic Gene Activity by Sense or Antisense RNA

Transgene	Species Modified	Phenotype Change	Ref.
<i>CHS</i> ^a			
Sense and antisense	<i>Dendranthema</i>	Flower color changed from pink to white	385
Sense	<i>Dianthus caryophyllus</i>	Flower color changed from pink to white	386
Antisense	<i>Eustoma grandiflorum</i>	Flower color changed from purple to white or patterns	387
Antisense	<i>Gerbera hybrida</i>	Flower color changed from red to pink or cream	388
Antisense	<i>Juglans nigra</i> × <i>J. regia</i>	Enhanced adventitious root formation	389
Antisense	<i>Lotus corniculatus</i>	Decreased flavonoids, enhanced PA production in root cultures	336
Antisense	<i>Petunia hybrida</i>	Flower color changed from red to white or patterns	328
Antisense	<i>Petunia hybrida</i>	Flower color changed from purple to pale purple or white	327
Sense	<i>Petunia hybrida</i>	Flower color changed from purple to white or patterns	329, 330
Sense	<i>Rosa hybrida</i>	Flower color changed from red to pale red	390
Sense and antisense	<i>Torenia fournieri</i>	Flower color changed from blue to pale blue or patterns	391, 392
Sense	<i>Torenia hybrida</i>	Flower color changed from blue to white or patterns	393
<i>F3H</i>			
Antisense	<i>Dianthus caryophyllus</i>	Flower color changed from orange to white	394
<i>DFR</i>			
Sense	<i>Petunia hybrida</i>	Flower color changed from purple to white or patterns	335
Antisense	<i>Solanum tuberosum</i>	Decreased anthocyanin levels	343
Sense or antisense	<i>Lotus corniculatus</i>	Decreased PA levels	337–339
Sense and antisense	<i>Torenia fournieri</i>	Flower color changed from blue to pale blue or patterns	391, 392
Sense	<i>Torenia hybrida</i>	Flower color changed from blue to white or patterns	393
<i>F3',5'H</i>			
Sense	<i>Petunia hybrida</i>	Flower color changed from dark blue to pale blue or pink	335, 395
Sense	<i>Torenia hybrida</i>	Flower color changed from blue to pink	393
<i>FLS</i>			
Antisense	<i>Eustoma grandiflorum</i>	Reddening effect on flower color	341
Antisense	<i>Petunia</i>	Flower color changed from purple to red	146
Antisense	<i>Petunia</i>	Flower color changed from white to pale pink	342
<i>FNSII</i>			
Antisense	<i>Torenia</i>	Paler flower color	350
<i>3RT</i>			
Antisense	<i>Petunia hybrida</i>	Flower color changed from purple to pink or patterns	111
<i>IFR</i>			
Sense or antisense	<i>Pisum sativum</i>	Reduced pisatin production	396
<i>HM3OMT</i>			
Sense or antisense	<i>Pisum sativum</i>	Reduced pisatin production and reduced pathogen resistance	396

^aMany examples of antisense or sense suppression of *CHS* gene activity in *P. hybrida* have been published, many using it as a phenotypic marker for studying the silencing process rather than through an interest in the effect on flavonoid biosynthesis or function. Only the first reports are referenced here.

TABLE 3.5
Genetic Modification of Flavonoid Production in Plants by the Introduction of Novel Flavonoid Biosynthetic Activities or by Increasing Endogenous Activities (All "Sense" Transgenes). Examples of Complementation of Genetic Mutants Are Not Featured

Transgene	Species Modified	Phenotype ^a	Ref.
<i>CHR</i>	<i>Nicotiana tabacum</i> <i>Petunia</i>	Flower color changed from pink to pale pink Flower color changed from white to pale yellow	58 149
<i>STS</i>	<i>Nicotiana tabacum</i>	Flower color changed from pink to pale pink	334
<i>AUS</i>	<i>Arabidopsis thaliana</i>	Seed color changed ^b	213
<i>CHI</i>	<i>Arabidopsis thaliana</i>	Increased flavonol levels	151
	<i>Lycopersicon esculentum</i>	Increased flavonol levels	61
<i>DFR</i>	<i>Forsythia Xintermedia</i>	Vegetative anthocyanin pigmentation increased	344
	<i>Lotus corniculatus</i>	PA types altered in cell cultures	338
	<i>Nicotiana tabacum</i>	Flower color changed from pink to dark pink	71, 75
	<i>Petunia</i>	Flower color changed from white to pink	342
	<i>Petunia</i>	Flower color changed from pale pink to orange or red	73, 345, 397, 398
	<i>Solanum tuberosum</i>	Increased anthocyanin levels	343
<i>DFR</i> and <i>ANS</i>	<i>Forsythia Xintermedia</i>	Vegetative and flower anthocyanin pigmentation increased or induced	89
<i>DFR</i> and <i>F3',5'H</i>	<i>Dianthus caryophyllus</i>	Flower color changed from pink or white to blue-purple	348
<i>F3'H</i>	<i>Petunia</i>	Flower color changed from lilac to pink	105
	<i>Torenia</i>	Reddening effect on flower color	350
<i>F3',5'H</i>	<i>Dianthus caryophyllus</i>	Flower color changed from pink to blue-purple	348
	<i>Nicotiana tabacum</i>	Change in pink shade of flowers	109, 349
	<i>Petunia</i>	Flower color changed from pale pink-red to magenta-deep red or patterns	146, 395, 399
<i>F3GT</i>	<i>Eustoma grandiflorum</i>	No change in visible phenotype ^c	90

<i>A3'GT</i> and <i>F5GT</i>	<i>Petunia</i>	No change in visible phenotype ^d	113
<i>Dn3MaT</i>	<i>Petunia</i>	No change in visible phenotype ^e	123
<i>ANR</i>	<i>Nicotiana tabacum</i>	Flower color changed from pink to white; PA-like compounds produced	137
<i>2HIS</i>	<i>Arabidopsis thaliana</i>	Genistein produced	151, 182, 346
	<i>Glycine max</i>	Isoflavone levels changed	347
	<i>Nicotiana tabacum</i>	Genistein produced	346
<i>2HIS</i> and <i>R/C1</i>	<i>Zea mays</i>	No change in phenotype	346
	<i>Zea mays</i>	Genistein produced in cell lines	346
<i>2HIS CHR</i> , and <i>R/C1</i>	<i>Zea mays</i>	Daidzein and genistein produced in cell lines	346
	<i>Arabidopsis thaliana</i>	Genistein produced	151
<i>2HIS</i> , <i>CHI</i> , and <i>Pap1</i>	<i>Arabidopsis thaliana (tt6/tt3)</i>	Genistein produced	151
	<i>Arabidopsis thaliana</i>	Genistein produced	151
	<i>Nicotiana tabacum</i>	No change in visible phenotype ^f	150
<i>IFR</i>	<i>Arabidopsis thaliana</i>	No change in visible phenotype ^g	194
<i>I2'H</i>	<i>Medicago sativa</i>	Enhanced production 4'- <i>O</i> -methylated isoflavonoid	189

^aOnly a general indication of the phenotype is given.

^bSeed color was restored in the *tt5* mutant.

^cA change in flavonoid glycosylation and acylation occurred.

^dOne new anthocyanin type, delphinidin 3,5,3'-tri-*O*-glucoside, was found.

^eAnthocyanins with novel malonylation were formed.

^fCell lines were able to biotransform exogenously supplied isoflavonoids.

^gTransgenic plants were able to convert exogenously supplied formononetin to 2'-hydroxyformononetin.

TABLE 3.6
Genetic Modification of Flavonoid Production in Stably Transformed Plants or Cell Lines by Introduction of Genes Encoding Transcription Factors that Regulate Flavonoid Biosynthetic Genes (All "Sense" Transgenes)

TF Type and Transgene ^a	Species Modified	Effect on Flavonoid Production ^b	Ref.
MYB, <i>Ant1</i>	<i>Lycopersicon esculentum</i>	Anthocyanins increased	234
	<i>Nicotiana tabacum</i>	Anthocyanins increased	234
MYB, <i>Cl</i>	<i>Arabidopsis thaliana</i>	No change in visible phenotype	355
	<i>Medicago sativa</i>	No change in visible phenotype	360
	<i>Nicotiana tabacum</i>	No change in visible phenotype	355
	<i>Trifolium repens</i>	Anthocyanins increased	400
MYB, <i>Gmyb10</i>	<i>Nicotiana tabacum</i>	Anthocyanins increased under high light conditions	369
MYB, <i>Myb.P12</i>	<i>Trifolium repens</i>	Anthocyanins increased	400
MYB, <i>Pap1</i> or <i>Pap2</i>	<i>Arabidopsis thaliana</i>	Anthocyanins and other phenylpropanoids increased	364
	<i>Nicotiana tabacum</i>	Anthocyanins increased	364
MYB, <i>Pap1</i> with EAR-motif repression domain	<i>Arabidopsis thaliana</i>	Anthocyanin and PA production inhibited	357, 358
MYB, <i>P1</i> or <i>P2</i>	<i>Zea mays</i> cell lines	3-Deoxyflavonoids, C-glycosylflavones, other phenylpropanoids and fluorescent compounds increased	140, 317
MYB, <i>Roseal</i>	<i>Eustoma grandiflorum</i>	Anthocyanins increased	401
	<i>Petunia</i>	Anthocyanins increased	401
bHLH, <i>B-Peru</i>	<i>Medicago sativa</i>	No change in visible phenotype	360
	<i>Trifolium repens</i>	Anthocyanins increased	400
bHLH, <i>Delila</i>	<i>Lycopersicon esculentum</i>	Anthocyanins increased	402
	<i>Nicotiana tabacum</i>	Anthocyanins increased	402

bHLH, <i>Lc</i>	<i>Arabidopsis thaliana</i>	Anthocyanins increased	355
	<i>Eustoma grandiflorum</i>	No change in visible phenotype	359
	<i>Medicago sativa</i>	Anthocyanins and PAs increased and flavones decreased under stress conditions	360
	<i>Lycopersicon esculentum</i>	Increased under high light levels	403
	<i>Nicotiana tabacum</i>	Anthocyanins increased	355
	<i>Pelargonium</i>	No change in visible phenotype	359
	<i>Petunia</i>	Anthocyanins increased	321
bHLH, <i>Myc-rp</i> and <i>Myc-gp</i>	<i>Lycopersicon esculentum</i>	Anthocyanins increased	371
	<i>Nicotiana tabacum</i>	Anthocyanins increased	371
bHLH, <i>Sn</i>	<i>Lotus corniculatus</i>	Anthocyanins increased	356
bHLH and transposon, <i>R</i> and <i>Tag1</i>	<i>Nicotiana tabacum</i>	Anthocyanins and proanthocyanidins increased. PAs increased in roots (and decreased in leaves of some lines)	354
bHLH and MYB, <i>C1</i> and <i>Delita</i>	<i>Arabidopsis thaliana</i>	Anthocyanins increased (variegated patterns)	404
bHLH and MYB, <i>C1</i> and <i>R</i>	<i>Glycine max</i>	No change in visible phenotype	402
bHLH and MYB, <i>C1</i> and <i>R</i> and suppressed <i>F3H</i>	<i>Zea mays</i> cell lines	Ratio of genistein to daidzein reduced	325
bHLH and MYB, <i>Lc</i> and <i>C1</i>	<i>Glycine max</i>	Anthocyanins, phlobaphenes, and C-glycosylflavones increased	140, 405
	<i>Arabidopsis thaliana</i>	Isoflavonoids increased	325
	<i>Arabidopsis thaliana</i>	Anthocyanins increased	355

^aExcept for MYB-Ph2, P1, and P2, the other TFs normally function as regulators of anthocyanin biosynthesis.

^bOnly a general indication of phenotype is given, and the full changes identified may include production of anthocyanin earlier in flower development than normal, increased anthocyanin production only under stress conditions, small increases in flavonoid levels in tissues already producing flavonoids, ectopic flavonoid production, changes in levels of nonflavonoid phenylpropanoids.

inhibition. Sense or antisense transgenes for CHS or DFR have also been used to reduce PA levels in transgenic root cultures of *Lotus corniculatus* (bird's foot trefoil).³³⁶⁻³³⁹

An alternative approach to RNA suppression for controlled reduction of flavonoid enzyme activity is the expression of single-chain antibody fragments targeted to a key enzyme or TF. Early attempts with enzymes such as DFR have been inconclusive as to the effectiveness of the technology.³⁴⁰

3.16.2 REDIRECTING SUBSTRATE IN THE FLAVONOID PATHWAY

The flavonoid pathway contains many branch points at which enzymes may compete for substrate, depending on the spatial and temporal occurrences of the enzymes and any metabolite channeling effects. Altering the balance of the competing activities may alter the levels of the different enzyme products and their derivatives. Alternatively, when a potential substrate is accumulating in tissues, a rate-limiting step may be overcome by increasing levels of the required enzyme.

Changes in the production rates of different branches of the pathway have been achieved by altering the competing activities of FLS and DFR, CHS and STS or PKR, and ANR and F3GT. Introduction of an antisense *FLS* transgene into *E. grandiflorum*,³⁴¹ *N. tabacum*,¹⁴⁶ or specific *Petunia* lines³⁴² reduced flavonol production and increased anthocyanin levels. *CaMV35S:DFR* transgenes increased anthocyanin content in flowers of *N. tabacum* and *Petunia* and tubers of *S. tuberosum*, and altered the type of PAs in *L. corniculatus* cell cultures.^{75,338,342,343} In *Forsythia*, anthocyanins accumulate in some vegetative tissues, but only flavonol glycosides are found in petals. Introduction of a *CaMV35S:DFR* transgene into *F. × intermedia* increased levels of anthocyanins in tissues which normally produce them, demonstrating that DFR is rate limiting for anthocyanin biosynthesis in these cells.³⁴⁴ The addition of a second sense transgene, for *M. incana ANS*, extended anthocyanin pigmentation to the flowers of the double transgenic lines.⁸⁹ 2HIS and F3H also potentially compete for substrate, and reduction of *F3H* transcript levels in transgenic *G. max* lines enhanced isoflavonoid accumulation (see Section 3.16.3.3).

STS from *V. vinifera* uses the same substrates as CHS, so that a *CaMV35S:STS* transgene introduced into *N. tabacum* reduced anthocyanin levels in the flowers of the transgenics, so that flowers were near white rather than the usual dark pink.³³⁴ Similarly, the introduction of *CaMV35S:PKR* transgenes diverted substrate into 6'-deoxychalcone production in transgenic *N. tabacum* and *Petunia*, significantly reducing floral anthocyanin biosynthesis and resulting in pale flower colors.^{58,149} A *CaMV35S:ANR* transgene introduced into *N. tabacum* changed the flower color of some transgenic lines from pink to white, presumably through competition with F3GT (see also Section 3.5.3).¹³⁷ Thus, the introduction of *STS*, *PKR*, or *ANR* transgenes offers a route for reduction of flavonoid biosynthesis, and may produce transgenics with more stable phenotypes than antisense RNA or sense-inhibition approaches.

The overproduction of an enzyme at a rate-limiting step to increase levels of specific flavonoids is well illustrated by the *Forsythia ANS* example described earlier, and also by the use of *CaMV35S:CHI* to increase flavonol levels in *A. thaliana* and *L. esculentum*.^{61,151} In particular, fruit of *L. esculentum* plants expressing *P. hybrida CHI* had up to a 78-fold increase in flavonol content, principally rutin, in fruit peel.

3.16.3 INTRODUCING NOVEL FLAVONOID COMPOUNDS

3.16.3.1 Chalcones, Aurones, and Flavonols

The biosynthesis of the yellow flavonoids has been targeted for biotechnology applications in commercial ornamental crops, such as *Cyclamen*, *E. grandiflorum*, *Impatiens*, and *Pelargonium*, which currently lack yellow-flowered varieties. The compounds targeted to date are the

aurones, which provide strong yellow colors, and the chalcones, which can provide yellow colors in certain circumstances. Aurones should offer excellent biotechnology prospects for generating yellow colors, as their direct precursors, chalcones, are ubiquitous intermediates in flavonoid biosynthesis, and cDNAs are available for the key biosynthetic activity, AUS. However, no experiments showing the use of the *AUS* cDNA to introduce aurone production in transgenic plants have been published.

A successful biotechnology route for directing chalcone accumulation has been the use of PKR to generate 6'-deoxychalcones, which are not accepted as substrates by CHI of many species. Introducing *CaMV35S:PKR* into a white-flowered line of *Petunia* generated transgenic lines that accumulated up to 50% of their petal (and pollen) flavonoids as 6'-deoxychalcones, changing the flower color from white to pale yellow.¹⁴⁹ Hydroxylation and glucosylation of the novel chalcones occurred at the C-3 and C-3' positions. In *N. tabacum*, the same approach resulted in the accumulation of the flavanone liquiritigenin in the petals at the expense of anthocyanin accumulation, indicating the endogenous CHI could accept the 6'-deoxychalcone substrate.⁵⁸

3.16.3.2 Altering Dihydroflavonol 4-Reductase Activity

As discussed in Section 3.4.4, in some species DFR can show strong preference to DHF substrate with di- or trihydroxylated B-rings, limiting the production of pelargonidin-based anthocyanins. Meyer et al.,³⁴⁵ in the first published case of GM of flower color, introduced a *Z. mays CaMV35S:DFR* transgene into a *P. hybrida* mutant that accumulated DHK, enabling reduction of DHK, and production of pelargonidin-based anthocyanins in the petals. Subsequent crosses of the transgenics to commercial *P. hybrida* cultivars led to F₂ lines with flower colors, including orange, novel to this species. Similar results have been obtained with other *DFR* transgenes in *P. hybrida* (Table 3.5). *DFR* transgenes have also been used to alter the type of PA accumulated³³⁸ and to encourage accumulation of delphinidin-derived anthocyanins (see Section 3.16.3.4).

3.16.3.3 Isoflavonoids

Modification of isoflavonoid biosynthesis may have a wide range of applications for improving not only plant defense characters but also the health benefits of food for humans. With the exception of IFD (which may not be required *in vivo*), cDNA clones are available for all of the enzymes needed for the production of the isoflavonoid vestitone. Furthermore, as VR cDNAs have been cloned, only clones for the DMID are lacking for the biosynthetic branch of the antifungal pterocarpan. To date, experiments have focused on 2HIS, but there has also been success using IFR, I2'H, and I7OMT.

Overexpression of 2HIS genes has not only successfully introduced isoflavonoid production into species that lack this branch of the flavonoid pathway, but also altered levels of these compounds in isoflavonoid-producing species (Table 3.5). The first reported GM experiment used *CaMV35S:G.max2HIS* to produce genistein in *A. thaliana*, although only at levels of around 2 to 4 $\mu\text{g g}^{-1}$ fresh weight.¹⁸² The level of genistein could be raised approximately threefold in the transgenics by UV-B induction of the general phenylpropanoid pathway,³⁴⁶ or up to 30-fold (50 $\mu\text{g g}^{-1}$) by using a mutant line (*tt3* or *tt6*) lacking *DFR* and *F3H* gene activity as the recipient of *CaMV35S:2HIS* and *CaMV35S:CHI-II* transgenes.¹⁵¹ However, these levels are still low compared to, for example, isoflavonoid levels of >4 mg g^{-1} fresh weight of *G. max* seeds.³²⁵ The experiments of Liu et al.¹⁵¹ suggest competing activities within flavonoid biosynthesis and the effects of metabolic channeling may significantly affect the ability to engineer isoflavonoid biosynthesis in nonlegumes. Several enzymes may use

flavanones as substrates in addition to the 2HIS, including F2H, F3H, F6H, FNR, FNS, F3'H, F3',5'H, and various GTs and OMTs. Thus, understanding the relationship between these activities will be important for future biotechnology approaches.

The *CaMV35S:G.max2HIS* transgene was also introduced into *N. tabacum* transgenic plants, resulting in genistein being produced in the petals (at $2 \mu\text{g g}^{-1}$ fresh weight) but not leaves.³⁴⁶ Cell lines of *Z. mays* only produced genistein when the 2HIS transgene was cointroduced with the chimeric CRC transgene, which induced general flavonoid biosynthesis.³⁴⁶ CRC encodes a fusion protein comprised of two *Z. mays* TFs that regulate anthocyanin biosynthesis. By introducing a third transgene, *CaMV35S:G.maxPKR*, low levels of daidzein could be produced along with genistein. Analysis in most experiments has included a hydrolysis to yield the aglycone (genistein or daidzein). However, when extraction without hydrolysis was performed, it was found that most isoflavonoids in *A. thaliana*, *N. tabacum*, and *Z. mays* transgenics were conjugated forms. Genistin and malonyl-genistin were found in *N. tabacum* petals³⁴⁶ and genistin, genistin-*O*-glucoside, genistin-*O*-rhamnoside, and a genistin glucose, rhamnose di-*O*-glycoside, in *A. thaliana*.¹⁵¹

A *CaMV35S:M.sativaIFR* transgene was used to generate transgenic *N. tabacum* cell lines, which were then analyzed for their ability to metabolize exogenously supplied isoflavonoids.¹⁵⁰ The transgenic cells could take up 2'-hydroxyformononetin and convert it to vestitone. Both 2'-hydroxyformononetin and vestitone accumulated as 2'- or 7-*O*-glucosides, demonstrating the activity of endogenous GTs on these compounds.

There are fewer publications on changing levels of isoflavonoids in isoflavonoid-producing species. Yu et al.³²⁵ introduced a CRC transgene into *G. max*, under the control of a seed-specific promoter. Overall levels of seed isoflavonoids were slightly raised, and there was a reduction in the ratio of genistein to daidzein. Accompanying the changes in isoflavonoid levels was an accumulation of isoliquiritigenin and liquiritigenin malonyl-glucose conjugates, as well as flavonols and PA-like compounds. There was also an association of the strong phenotypes with a visual color change in some seed. Suppression of *F3H* gene activity further raised isoflavonoid levels in the CRC transgenics. There is also a preliminary report that the introduction of a *CaMV35S:G.max2HIS* transgene into *G. max* resulted in plants with either reduced or enhanced levels of isoflavones.³⁴⁷ In addition, the role of the D7OMT in isoflavonoid biosynthesis has been studied by expression of a *CaMV35S:D7OMT* transgene in *M. sativa*.¹⁸⁹ Some transgenics showed higher transcript abundance for flavonoid biosynthesis enzymes after infection with *Phoma medicaginis*, as well as increased isoflavonoid levels and improved pathogen resistance.

3.16.3.4 Altering B-Ring Hydroxylation

An obvious approach for flavonoid biotechnology is to direct the accumulation of pelargonidin- or delphinidin-derived anthocyanins in species that may lack them by inhibiting the activity of the F3'H and/or F3',5'H or introducing the F3',5'H. Thus, it is no surprise that *F3',5'H* transgenes have been introduced into several species that lack the activity, including the leading ornamental crops *Dendranthema*, *Dianthus*, and *Rosa hybrida* (Table 3.5). Indeed, transgenic *Dianthus* with novel colors based on delphinidin-derived anthocyanins are now commercially available in Australia, Japan, and the United States.³⁴⁸ The amount of delphinidin produced in some of the first *F3',5'H* transgenics was relatively low, due to competition from the F3'H and the substrate preference of the endogenous DFR for DHK or DHQ. Much higher levels of delphinidin-derived anthocyanins were produced by introducing both a *F3',5'H* transgene and a transgene for a DFR that prefers DHM to DHK as substrate (such as that from *P. hybrida*) into a plant background that accumulates DHK (a *DFR-F3'H* double mutant) (International Patent Application WO96/36716). An alternative approach has been

demonstrated by Okinaka et al.,³⁴⁹ who generated transgenic *Nicotiana* in which over 99% of the anthocyanins accumulated were delphinidin based by using a *Campanula medium* cDNA for a *F3',5'H* that seems particularly efficient at generating delphinidin precursors. However, formation of blue flower colors involves many factors in addition to production of delphinidin-derived anthocyanins, and many of these are still out of reach of the current gene technology. In a similar approach to that for increasing delphinidin-derived anthocyanin levels, a *F3'H* transgene has been used to increase the amount of cyanidin-based anthocyanins in *T. hybrida*.³⁵⁰

Inhibition of the activities of *F3'H* or *F3',5'H* should enable production of pelargonidin-based anthocyanins in species that lack them. *Dendranthema* is one candidate for such an approach.³⁵¹ Of course, the subsequent enzymes would need to be able to use the substrates with only monohydroxylation of the B-ring, and in particular, DFR specificity would be a consideration for some species (e.g., *Cymbidium*). Reports of transgenic plants demonstrating this approach have yet to be published.

3.16.3.5 Secondary Modifications

Although there is a wide range of cDNAs available for secondary modifications of flavonoids, biotechnology applications to date have primarily focused on modifying anthocyanin production.

Glycosylation can affect the anthocyanin-based color of flowers through both direct effects on anthocyanin chemistry and indirect effects in which the change in glycosylation has ramifications on other modifications. This later effect is nicely illustrated by experiments with the *A3RT* and *F3GT*. Inhibition of *A3RT* gene activity in *P. hybrida* produced transgenics with altered flower color. The flowers had reduced levels of malvidin derivatives and increased levels of pigments based on delphinidin and petunidin, presumably due to the anthocyanin OMT being unable to use the nonrhamnosylated substrate.¹¹¹ Introduction of a *CaMV35S:A.majusF3GT* transgene into *E. grandiflorum* resulted in a change from flavonoid 3-*O*-galactosylation to 3-*O*-glucosylation, and an associated reduction in acylation of the flavonols, but no change in flower color.⁹⁰

Much of the focus on glycosylation activities for biotechnology has been with regard to generating blue flower colors in leading ornamental species, although published transgenic experiments to date have concentrated on model species. The *A3'GT* is involved in the formation of the blue anthocyanin gentiodelphin in the source species *G. triflora*,¹¹³ and thus is a good prospect for generating anthocyanins more suited to blue flower colors. Expression of *CaMV35S:A3'GT* and *CaMV35S:A5GT* transgenes together in *P. hybrida* resulted in the formation of a new anthocyanin type, delphinidin 3,5,3'-tri-*O*-glucoside, but only at low levels (2 to 6% of total anthocyanins). No change in flower color occurred.¹¹³

To date, published results are available for only one of the AAT cDNAs, the *Dv3MaT*, which was overexpressed in *P. hybrida*. Although the transgenics produced malonylated anthocyanins in the petals that were novel to *P. hybrida*, no change in flower color occurred.¹²³ Modification of the activity of anthocyanin OMTs has been reported only in the patent literature (International Patent Application WO03/062428).

3.16.4 MODULATING PATHWAY REGULATION

TFs have been used to modify not only the amount of flavonoids in transgenic plants and cell lines of several species but also their temporal and spatial distributions, including effective use in heterologous species. A key advantage of targeting TF activity is that several biosynthetic genes may be coordinately regulated, overcoming the need to introduce multiple transgenes for biosynthetic enzymes or to identify a rate-limiting step.

Anthocyanin production has been increased in transgenic plants of several species by introduction of anthocyanin-related *Myb* or *bHLH* cDNAs under the control of the *CaMV35S* promoter. Most experiments to date have used *C1* and *R/B* family transgenes from *Z. mays*; however, increasingly TF genes from other species are proving effective (Table 3.6). The *Z. mays* TF transgenes also increased the levels of flavonols in *L. esculentum*, PAs in *L. corniculatus* and various flavonoids, and other metabolites in *Z. mays* cell lines.^{140,352–354} Cointroducing transgenes for MYB and bHLH TFs that normally interact can enable higher levels of anthocyanin production, or novel spatial distribution, compared to the introduction of either transgene alone. The first example of this was for *A. thaliana*, in which plants with both *CaMV35S:C1* and *CaMV35S:Lc* transgenes had increased anthocyanin levels and novel distribution patterns not seen in plants with either transgene on its own.³⁵⁵ As would be expected from the role of some TFs in repressing gene expression, appropriate transgenes can also be used to downregulate sections of the phenylpropanoid pathway.^{270,271,297,356} Furthermore, TFs that normally increase transcription can be altered so that they repress it, through the addition of repression domains from other proteins, such as the ERF-associated amphiphilic repression motif.^{357,358}

For reasons that are not clear, the overexpression of anthocyanin-related TFs can enhance production of other flavonoids in addition to anthocyanins. It may be that the TFs have an uncharacterized role in regulating the other pathways as part of their usual function. Alternatively, they may increase flux into, or alter flux within, the flavonoid pathway so that substrate is fed into production of the nonanthocyanin end products. A further possibility is that the effects seen are due to the nonphysiological levels of TF protein produced through the use of strong promoters, such as *CaMV35S*. Excess levels of TF protein may promote activity on gene promoter sites additional to the usual targets, as demonstrated in *CaMV35S:AtMYB4* transgenic *A. thaliana*,²⁷¹ or interfere with the endogenous regulatory environment.

Although there has been much success with flavonoid-related TF transgenes, the technology has also proven unpredictable. This is well illustrated by the fact that different bHLH TFs, despite all being regulators of anthocyanin production, generate distinct phenotypes in recipient species (Table 3.6). Thus, one transgene may produce a phenotype in a given species while one with a similar sequence may not (see, e.g., Ref. 354), or one recipient species may develop a strong phenotype while the same transgene gives no increased pigmentation in other species (see, e.g., Refs. 321, 359). Such diverse responses likely reflect differences in the binding or activation characteristics of the introduced TFs, combined with variation in the type and activity of interacting endogenous TFs and the presence or absence of target promoter *cis*-sequences in the recipient species. The role of endogenous factors is demonstrated by the markedly different phenotypes of *M. sativa* and *P. hybrida* *CaMV35S:Lc* transgenics under varying environmental conditions.^{254,360}

3.17 CONCLUDING COMMENTS

Since 1994, remarkable progress has been made in our understanding of flavonoid biosynthesis. DNA sequences are now available for most of the biosynthetic enzymes required to form the common flavonoid types, such as anthocyanins, flavonols, flavones, PAs, isoflavonoids, and pterocarpanes. These have allowed three-dimensional structures to be established for some of the enzymes using homology modeling or x-ray crystallography. Furthermore, significant progress has been made in understanding some of the activities involved in transport of anthocyanins and PAs to the vacuole. The magnitude of the advances in molecular knowledge of the pathway is illustrated by the listing in Table 3.1 of over 50 flavonoid biosynthetic enzymes for which cDNAs or genes are available, compared to the

eight that were listed in the review by Forkmann,¹ which covered the literature up to 1991. Likewise, the same review listed cloned regulatory genes from only two species, *A. majus* and *Z. mays*, while Table 3.2 lists cloned genes from 12 species.

Biotechnology of the flavonoid pathway was in its infancy at the start of the 1990s, but is currently being widely applied to develop improved cultivars of ornamental, pasture, and food crops. In particular, the recent identification of the PA monomer biosynthetic genes should be of significant benefit to agriculture in the coming decade. As knowledge of flavonoids and human health progresses, one may anticipate that gene technology will be increasingly applied to improve the health characteristics of crop plants. Important factors in the future success of flavonoid biotechnology in achieving precision engineering of the pathway will be not only continuing advances in the identification of regulatory and biosynthetic genes, but also an improved understanding of metabolic channeling. This may apply particularly to the introduction of new branches of the pathway, such as that for the pterocarpan, to target crops.

3.18 ACKNOWLEDGMENTS

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4 Flavonoids in Foods

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4.1 INTRODUCTION

A “poor” diet is a major contributing factor to the etiology of chronic diseases such as heart disease and cancer.^{1,2} However, defining what constitutes a “healthy” diet remains contentious, as it is difficult to definitively ascribe beneficial and detrimental properties to the

diverse components of the many foods we consume. Nevertheless, considerable evidence indicates that adequate fruit and vegetable consumption has a role in maintaining health and preventing disease.^{3–9} Some of these protective effects may be due to flavonoids, which are widely distributed in plant-based foods at varying levels.^{10–13} For example, numerous *in vitro* investigations have demonstrated potent effects of flavonoids in mammalian systems that are potentially anticarcinogenic and antiatherogenic.^{14–16} These include antioxidant protection of DNA and low-density lipoprotein, modulation of inflammation, inhibition of platelet aggregation, estrogenic effects, and modulation of adhesion receptor expression.^{14–18} The role of flavonoids in health is extensively covered in Chapter 6.

From a nutritional perspective, the actual importance of flavonoids to health and disease remains unclear. Unlike the recognized micronutrients that can be obtained from plant-based diets, such as vitamin E and vitamin C, a lack of dietary flavonoids does not result in obvious deficiency syndromes. Consequently, the initial classification of some citrus flavonoids as “vitamin P”¹⁹ was later revoked.¹⁰ In addition, epidemiological studies relating intake of flavonoids to disease incidence or risk do not give consistent results. For example, the average combined intake of flavonols and flavones in a cross-cultural correlation study composed of 16 cohorts followed up for 25 years after initial baseline measurements collected around 1960²⁰ was found to be inversely associated with coronary heart disease mortality, statistically explaining 25% of the variability in rates across the cohorts. In contrast, increased risk with increasing flavonoid intake has also been observed (Table 4.1). Similarly, although some studies have found positive inverse relationships between flavonoid intake and several cancers, others have failed to demonstrate significant statistical associations (Table 4.2).

Early analysis and identification of flavonoids in plant materials and products led to estimated intakes of up to 1 g/day in the United States.¹⁰ This approximation included flavanones, flavonols, and flavones (160 to 175 mg/day), anthocyanins (180 to 215 mg/day), catechins (220 mg/day), and biflavans (460 mg/day). More recent estimates focusing on flavonol and flavone intake indicate that these early intake levels may be too high. One explanation for this is that flavonoid analysis initially employed semiquantitative spectrophotometric measurement.^{21–24} Analytical methodology has since progressed with the development of more sensitive and specific techniques. Optimized and better-validated sample preparation and hydrolysis techniques are now commonly used.^{25–30} For example, Hertog et al.²⁵ optimized and tested the completeness of acid hydrolysis and solvent extraction of flavonol glycosides to their free (aglycone) form before quantifying flavonol and flavone concentrations of freeze-dried fruit and vegetable samples. Reversed-phase high-pressure liquid chromatography (RP-HPLC) with ultraviolet (UV) detection³¹ has improved isolation and separation of compounds superseding thin layer chromatography isolation and quantification by measurement of UV spectral shifts in response to addition of colorimetric reagents.

Implementing such improved methodology on a selection of nine fruits, 28 vegetables, and several different beverages commonly consumed in the Netherlands³² generally provided lower values compared with earlier determinations.^{3,33} When flavonol and flavone intake was recalculated, levels of intake in the United States fell to 13 mg/day for this subgroup of flavonoids, which is about one tenth that of earlier estimates.²⁰ These Dutch compositional data, with the addition of local food preferences such as berries, have since been used in several studies to assess dietary intake and potential associations with disease incidence.^{34–38} Application of this limited dataset to epidemiological studies relating flavonoid intake to disease incidence has produced conflicting results (Table 4.1 and Table 4.2), probably reflecting, in part, the paucity of composition data for many foods.^{17,39} In addition,

TABLE 4.1
Epidemiological Studies Investigating Dietary Flavonoid Intake and Coronary Heart Disease Incidence and Mortality

Study	Flavonoid Class	Follow-up (Years)	No. of Cases	RR (95% CI) ^a	Ref.
<i>Cohort</i>					
Dutch, 693 men 805 men	Flavonols ^b /flavones ^c	5	Incidence (<i>n</i> = 38) Mortality (<i>n</i> = 43)	0.52 (0.22–1.23) 0.32 (0.15–0.71)	134
Finnish, 2,748 men and 2,385 women	Flavonols/flavones	26	Mortality, men (<i>n</i> = 324) Mortality, women (<i>n</i> = 149)	0.73 (0.41–1.32) 0.67 (0.44–1.00)	34
US, 34,789 men	Flavonols/flavones	6	Incidence (<i>n</i> = 486) Mortality (<i>n</i> = 105)	1.08 (0.81–1.43) 0.63 (0.33–1.20)	35
Welsh, 1,900 men	Flavonols	14	Incidence (<i>n</i> = 186) Mortality (<i>n</i> = 131)	1.0 (0.9–2.9) 1.6 (0.9–2.9)	135
Dutch, 4,807 men and women	Flavonols	5.6	Incidence (<i>n</i> = 146) Mortality (<i>n</i> = 30)	0.76 (0.49–1.18) 0.35 (0.13–0.98)	136
Finnish, 9,131 men and women	Flavonols/flavanones ^d [Quercetin]	15	Incidence (<i>n</i> = 806) Mortality (<i>n</i> = 681)	0.79 (0.64–0.98) [0.86 (0.70–1.05)] 0.93 (0.74–1.17) 0.79 (0.63–0.99)]	129
US, 34,492 women	Flavonols/flavones	10	Mortality (<i>n</i> = 438)	0.62 (0.44–0.87)	36
Dutch, 693 men	Catechins ^e	10	Incidence (<i>n</i> = 90)	0.70 (0.39–1.26)	137
Dutch, 805 men	Catechins	13	Mortality (<i>n</i> = 90)	0.49 (0.27–0.88)	138
USA, 34,492 women	Catechin/epicatechin Gallated catechins ^f		Mortality (<i>n</i> = 767)	0.85 (0.67–1.07) 0.76 (0.58–1.03) 1.00 (0.77–1.29)	

Note: Values in bold indicate statistical significance.

^aMultivariate adjusted relative risk (95% confidence intervals) highest vs. lowest quintile of intake.

^bQuercetin, kaempferol, and myricetin.

^cApigenin and luteolin.

^dHesperetin and naringenin.

^eCatechin, epicatechin, gallo catechin, epigallocatechin, epicatechingallate, and epigallocatechingallate.

^fGallo catechin, epigallocatechin, epicatechingallate, epigallocatechingallate.

TABLE 4.2
Epidemiological Studies Investigating Dietary Flavonoid Intake and Cancer Incidence

Study	Flavonoid Class	Follow-up (Years)	Site of Cancer	RR (95% CI)	Ref.
<i>Cohort</i>					
US, 34,651 women	Catechins	13	All causes	0.97 (0.88–1.06)	139
			Bronchus and lung (<i>n</i> = 549)	0.94 (0.72–1.23)	
			Colon (<i>n</i> = 635)	1.10 (0.85–1.44)	
Dutch, 728 men	Catechins	10	Lung (<i>n</i> = 12)	0.92 (0.41–2.07)	140
Dutch, 246 men	Flavonol/flavone	5	Epithelial (<i>n</i> = 30)	0.94 (0.56–1.59)	140
			All causes (<i>n</i> = 27)	1.21 (0.66–2.21)	
Finnish, 27,110 male Smokers	Flavonols/flavones	6	Alimentary and respiratory (<i>n</i> = 19)	1.02 (0.51–2.04)	141
			Lung (<i>n</i> = 791)	0.6 (0.4–0.7)	
			Urothelial (<i>n</i> = 156)	1.2 (0.7–1.8)	
			Renal cell (<i>n</i> = 92)	0.6 (0.4–1.1)	
			Prostate (<i>n</i> = 226)	1.3 (0.9–1.8)	
			Stomach (<i>n</i> = 111)	1.2 (0.7–1.9)	
Finnish, 9,959 men and women	Flavonols/flavones	20	Colorectal (<i>n</i> = 133)	1.7 (1.0–2.7)	142
			All sites (<i>n</i> = 997)	0.87 (0.70–1.09)	
			Lung (<i>n</i> = 151)	0.53 (0.29–0.97)	
<i>Case-control</i>					
Hawaii, men and women	Flavonols/flavanones [Quercetin]		Lung (582 cases and 582 controls)	OR (95% CI) 0.8 (0.5–1.4) [0.7 (0.4–1.1)]	143
Spanish, women	Flavonols		Lung (103 cases and 206 controls)	0.98 (0.44–2.19)	144

concentrations of flavonoids in foods can vary by many orders of magnitude due to the influence of numerous factors such as species, variety, climate, degree of ripeness, and postharvest storage.^{10,12,40}

Consequently, the aims in this chapter are to critically examine the available literature on the flavonoid composition of foods and to establish a food flavonoid database, which can be continually expanded as more information becomes available. By using predetermined selection criteria to ensure critical assessment of data quality, the intention is to provide researchers with an improved resource for use in studies exploring the relationships between flavonoid intake and health as well as highlighting important food groups where flavonoid content data are currently lacking.

4.2 DATABASE DEVELOPMENT

4.2.1 DATABASE DEVELOPMENT

To ensure comprehensive coverage of foods and relevant flavonoids, compilation of the flavonoid composition database followed a preset development profile (Figure 4.1). This was a multistage process that evolved from a review of two major food composition databases^{41–43} and from other early stage nutrient bases such as those for vitamin K⁴⁴ and

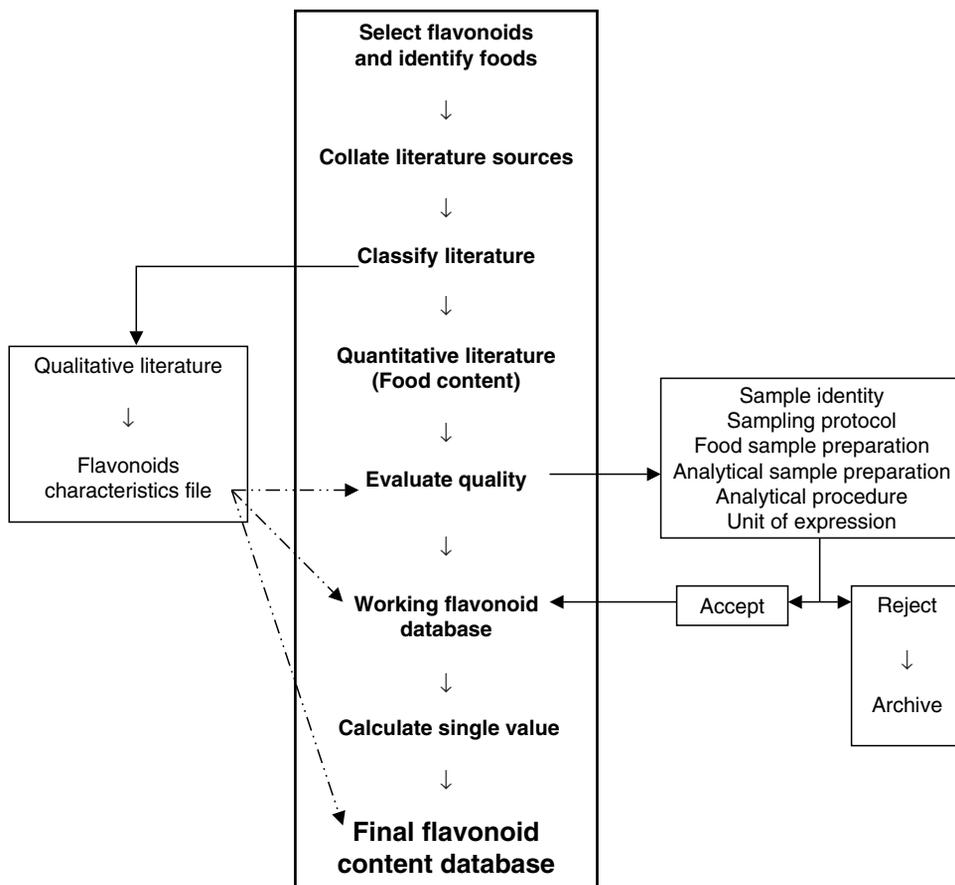


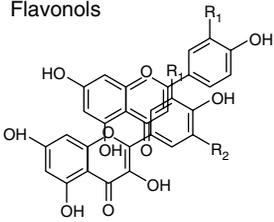
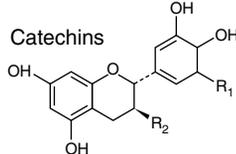
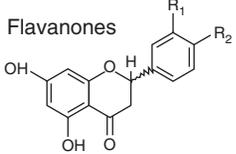
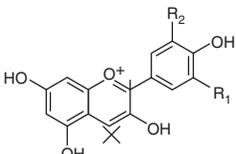
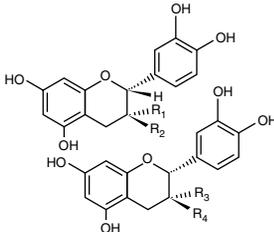
FIGURE 4.1 Schematic of selection and evaluation processes used to compile the flavonoid database.

carotenoids.^{42,45} Essentially, the database development profile consists of the selection of commonly consumed flavonoid-rich foods, collation and evaluation of available literature, and, finally, acceptance or rejection of flavonoid content values (Figure 4.1).

4.2.2 SELECTION OF FOODS AND FLAVONOID CLASSES

As all foods of plant origin potentially contain flavonoids^{3,10} and over 4000 individual compounds have previously been identified,¹¹ the development of a comprehensive flavonoid database is a huge task to undertake. Therefore, in the first instance, it was decided to focus on foods commonly available and eaten in Britain and to initially consider those flavonoid classes that have attracted most attention in relation to potential health benefits namely flavonols, flavones, catechins, flavanones, and anthocyan(id)ins.¹⁷ Table 4.3 outlines the compounds selected to represent the five main subclasses of flavonoids. Consumer dietary surveys of the UK population were initially consulted.^{46,47} Although a general indication of commonly consumed foods was found in these surveys, fruits and vegetables were presented as generalized groups such as “other fresh fruit.” The fruits assigned to this group were not clearly identified. Visiting local British supermarkets proved to be the most informative method of identifying products readily available to the consumer and proved a good starting point for the literature search for flavonoid composition data (Table 4.4).

TABLE 4.3
Compounds Included in Flavonoid Database

Flavonoid Subclass	Compound
Flavonols 	Quercetin ($R_1 = \text{OH}, R_2 = \text{H}$) Kaempferol ($R_1 = R_2 = \text{H}$) Myricetin ($R_1 = R_2 = \text{OH}$)
Flavones 	Apigenin ($R_1 = \text{H}$) Luteolin ($R_1 = \text{OH}$) (+)-Catechin (C) ($R_1 = \text{H}, R_2 = \text{OH}$) (-)-Epicatechin (EC) ($R_1 = \text{H}, R_2 = \text{OH}$) (-)-Epigallocatechin (EGC) ($R_1 = \text{OH}, R_2 = \text{OH}$) (-)-Epicatechingallate (ECG) ($R_1 = \text{H}, R_2 = -\text{OC}-\text{Ph}(\text{OH})_3$) (-)-Epigallocatechingallate (EGCG) ($R_1 = \text{OH}, R_2 = -\text{OC}-\text{Ph}(\text{OH})_3$) (-)-Gallocatechin (GC) ($R_1 = -\text{OC}-\text{Ph}(\text{OH})_3, R_2 = \text{H}$)
Flavanones 	Hesperetin ($R_1 = \text{OH}, R_2 = \text{OMe}$) Naringenin ($R_1 = \text{H}, R_2 = \text{OH}$)
Anthocyanidins 	Delphinidin ($R_1 = R_2 = \text{OH}$) Cyanidin ($R_1 = \text{OH}, R_2 = \text{H}$) Pelargonidin ($R_1 = R_2 = \text{H}$) Petunidin ($R_1 = \text{OMe}, R_2 = \text{OH}$) Peonidin ($R_1 = \text{OMe}, R_2 = \text{H}$) Malvidin ($R_1 = R_2 = \text{OMe}$)
Procyanidin B-type dimers 	B1: $R_1 = \text{OH}; R_2 = \text{H}; R_3 = \text{H}; R_4 = \text{OH}$ B2: $R_1 = \text{OH}; R_2 = \text{H}; R_3 = \text{OH}; R_4 = \text{H}$ B3: $R_1 = \text{H}; R_2 = \text{OH}; R_3 = \text{H}; R_4 = \text{OH}$ B4: $R_1 = \text{H}; R_2 = \text{OH}; R_3 = \text{OH}; R_4 = \text{H}$

4.2.3 COLLATION AND EVALUATION OF LITERATURE SOURCES

To ensure coverage of a broad spectrum of journals containing both original and review articles, bibliography databases, such as CAB abstracts, BIDS ISI and Embase, Medline and Current Contents Agriculture and Food Citation index, were searched. Terms used for all database searches were kept simple to limit the risk of missing any original publications (Table 4.5). Initially, each flavonoid subclass was entered and then cross-searched with the

TABLE 4.4
Foods of Plant Origin Commonly Consumed in the United Kingdom and Available in Local Supermarkets^{a,b}

Food Type	Food Item
Beverages	Tea, coffee, fruit and herbal drinks Cocoa or drinking chocolate, malted drinks Fruit juice concentrates, ready to drink fruit juices (still and carbonated) Beer, lager, wine, spirits, liqueurs, alco pops
Fresh fruits	
Normally available	Apples, bananas, oranges, grapefruit, pears, melons, grapes, lemon, strawberries
Seasonal	Satsumas, tangerines, clementines, peaches, pineapples, nectarines, plums, mangoes, raspberries, blueberries, blackcurrants, blackberries, rhubarbs
Exotic fruit section	Custard apple, passion fruit, pomegranate, sharon fruit, lychee, figs, cranberry, gooseberry
Fresh vegetables	
Normally available	Broccoli, carrots, onions, potatoes, courgette, mushrooms Lettuce (different varieties), tomatoes (different types), celery, cucumber, sweet pepper (red and green)
Seasonal	Spinach, winter greens, leeks, sude, turnip, Brussels sprouts, red cabbage, parsnips, corn-on-cob, red radish, mange tout, French beans, sweet potatoes, shallots, asparagus, chives
Specialist	Artichoke, squash, yam, celeric, fennel bulb, okra, oyster mushrooms
Fresh herbs	
Normally available	Oregano, basil, thyme, mint, coriander, rosemary, sage, dill

^aSupermarkets were visited at least once per month over 4 years. Also from Gregory et al.⁴⁶ and the National Household Consumption Survey.⁴⁷

^bProcessed food stuffs not included.

terms “composition” or “content.” Common and botanical names of foods (Appendix 1) were then individually added to the search profile. Food Science and Nutrition Journal Archives were also hand searched to ensure complete coverage of journals dating back to 1976. Pre-1976 literature was referred to for flavonoid characterization purposes only.

To ensure compatibility with the Royal Society of Chemistry’s food composition tables, predetermined screening procedures were used (Table 4.6), which were derived from those outlined for the nutrient tables.⁴¹ All publications and reports on flavonoid content of foods were subsequently evaluated employing the screening procedures (Table 4.6). In brief, inclusion criteria were (a) randomly selected food items purchased from various commercial outlets during different seasons of the year, (b) food samples prepared using normal domestic

TABLE 4.5
Search Strategy for Locating Flavonoid Publications

Search Term	Publications		
	Medline	Web of Science	CAB
(Flavonols or flavones or catechins or flavanols or anthocyanins or flavanones)	13,315	4,373	6,617
and (composition or content)	833	711	4,061
and (Malus or apple or apples)	17	44	190
and (Prunus or apricot or apricots)	2	17	158
and (Allium or onion or onions)	14	19	43
and (repeated for all foods outlined in Table 4.4)			

TABLE 4.6
Selection Criteria for Addition to Database

Criteria	Outlined in Manuscript
Sample identity	Foods common or varietal name Country of origin and locality with details of growing conditions if possible; e.g., British tomatoes grown in greenhouses
Sampling protocol	Place and time of sample collection; e.g., purchased from local market or commercial grower in September 1999 Number of samples collected and how these were obtained; e.g., 1 kg of fruit purchased from three shops
Food sample preparation	State of sample; e.g., raw, cooked, processed (canned or frozen), pickled Preparative treatment outlined; e.g., chopping or shredding
Analytical sample preparation	Storage conditions before analysis outlined; e.g., freeze dried and stored at -20°C Nature of sample analyzed; e.g., edible portion only Sample extraction and hydrolysis details; e.g., solvent extraction after freeze drying, with optimized acid or enzymatic hydrolysis
Analytical procedure	Preparation of flavonoid standards and use of internal standards Chromatographic separation and detection method used, ideally RP-HPLC with UV or fluorescent detection Outline of quality assurance procedures employed
Unit of expression	Method of calculating content value; e.g., based on retention time of standards, mean of duplicate samples, adjustment to recovery of internal standard Presentation of final value; e.g., mean \pm SD as fresh weight or dry weight with original moisture content

practices such as discarding nonedible portions of onions, (c) optimized sample extraction and hydrolysis conditions clearly outlined or cited, and (d) flavonoid separation and determination conducted using standard modern techniques with validation and quality assurance measures summarized.^{25,31} A worked example of the selection criteria as applied to four different onion composition studies is presented in Table 4.7. Acceptable papers were included into the working database (see Appendix 2), while manuscripts not meeting the criteria were archived and reasons for the exclusion noted.

The evaluation criteria applied during database development highlighted a lack of acceptable anthocyanin food content literature. Values were often presented as percentage of the total anthocyanin content.^{48,49} In addition, test samples were frequently gathered from noncommercial sources, such as horticultural research stations.^{50,51} Moreover, analytical procedures often employed spectral pH differential methodology rather than HPLC to estimate anthocyanin content.^{52,53} Consequently, although there is a substantial amount of characterization information with crude estimates of total anthocyanin content (Table 4.8), anthocyanins had to be excluded from the final database. Similarly, the flavanone eriodictyol was also excluded from the final database due to a lack of rigorously analyzed quantitative information.

The evaluation process also illustrated that food content data are increasingly presented as total glycosides of flavonoids such as quercetin-3-glucoside. If a study met all the criteria except presenting aglycone data, the values were converted to aglycone format using molecular weights, an approach validated by Price et al.⁵⁴ Additionally, values expressed in parts per million or milligrams per kilogram were converted to mg/100 g. Content levels presented as dry weight were only adjusted to fresh weight concentrations if the original moisture content was available. During the process of finalizing the database, to ensure all available flavonoid composition data had been gathered, literature determining proanthocyanidin content of foods was identified. Subsequently, content values for B-type procyranidin dimers

TABLE 4.7
Assessment of Four Onion Flavonol Composition Studies Showing Acceptance or Rejection for Database Inclusion

Criteria	Hertog et al. ³²	Leighton et al. ⁷⁹	Bilyk and Sapers ⁸⁹	Crozier et al. ⁹⁰
<i>Sample identity</i>				
Common/variety name	Identified	Identified	Identified	Identified
Number of varieties	Not reported	20	7	2
Country of origin	Netherlands	USA and Mexico	Not reported	UK
Growing conditions	Not reported	Identified	Not reported	Identified
<i>Sampling protocol</i>				
Place of purchase	Supermarket, grocer and local supermarket	Commercial growers and local suppliers	Not reported	Local supermarket
Year/season of purchase	Four times during 1991–1992	Not reported	Not reported	August 1994 and July 1995
Number of samples collected	One kilogram or three units from three locations	Not reported	Not reported	~250 g randomly selected
<i>Food sample preparation</i>				
State of sample	Raw	Raw	Raw	Raw and cooked
Preparative treatment	Fresh, chopped	Fresh, chopped	Fresh onion divided into rings, then shredded	Fresh, sliced
		Cooking methods — boiled and fried		Cooking methods — boiled, fried, and microwaved
<i>Analytical sample preparation</i>				
Storage conditions prior to analysis	Freeze dried, stored at -20°C for <4 months	Analyzed fresh?	Analyzed fresh?	Freeze dried, stored at -20°C
Nature of sample analyzed	Edible portion only	Edible portion only	Both edible and inedible sections	Edible portion only
Sample extraction and hydrolysis	1.2 M HCl in 50% methanol, hydrolyzed for 2 h at 90°C	Butanol phase partitioning	Methanol, followed by acid hydrolysis	1.2 M HCl in 50% methanol, hydrolyzed for 2 h at 90°C
Use of external and internal standards	External standards	External standards	External standards	External standards
<i>Analytical procedure</i>				
Isolation method	C18 column	XAD-2 column	Thin layer chromatography	C18 column
Method of analysis	RP-HPLC	HPLC	HPLC	RP-HPLC
Method of detection	UV-visible	Diode array	UV-visible	UV-visible
Quality assurance	Spiked standards recovery reported, identification on aglycone	Mass spectrometry confirming peak identity, quantitation based on retention time	Comparison of external standard retention times	Spiked standards recovery reported, identification on aglycone
Unit of expression	RP-HPLC retention times	RP-HPLC retention times		RP-HPLC retention times
Status	mg/kg fresh weight Accept	mg/kg fresh weight Accept	g/kg fresh weight Reject	mg/kg fresh weight Accept

TABLE 4.8
Anthocyanin Content of Some Plant-Based Food Items

Food	Content ^a (mg/100 g)	Anthocyanin ^b	Glycosides ^c
<i>Fruits</i>			
Red apple	0.1–0.2	Cyanidin	Glu; gala; arab
Apricot		Cyanidin	Glu
Bilberry	3.7	Malvidin; Dp; Cy; Pt; Pn	Glu; gala; arab
Blackberry	0.3–1.1	Cyanidin; Pg	Glu; gala; rut; arab; xyl
Blueberry	0.8–2.8	Malvidin; Cy; Dp; Pn; Pt	Arab; gala; glu; 6-ace-3-gly, samb; soph
Cherry	2.4	Cyanidin; Pn	Glu; rut; glu-rut; soph
Cranberry	0.1–3.6	Peonidin; Cy	Gala; glu; arab
Blackcurrants	1.3–4.0	Cyanidin; Dp	Glu; rut
Elderberry	2.0–10.0	Cyanidin	Samb; glu; samb-5-glu, 3,5-diglu, <i>p</i> -cou-glu-5-glu
Grapes (red/black)	0.7–1.1	Malvidin; Dp; Pt; Pn; Cy	Glu; glu-ace; glu- <i>p</i> -cou; rut
Litchi	0.5	Cyanidin; Mv	Glu; rut; ace-glu
Olive	—	Cyanidin	Glu; rut; caf-rut; glu-rut
Orange, blood	2	Cyanidin; Dp	Glu; mal-glu
Peach	0–0.1	Cyanidin	Glu; rut
Pear	—	Peonidin; Cy	Glu; rut
Plum	0.02–0.3	Cyanidin	Glu; rut; 3-ace-glu; gala
Pomegranate	—	Delphinidin; Cy; Pg	3,5-Diglu; glu
Raspberry	0.3–1.2	Cyanidin; Dp; Mv; Pg	3-Glu; soph, rut; 3-glu-rut
Strawberry	0.1–3.8	Pelargonidin; Cy	Rut; 3-ace-gly; glu
<i>Vegetables</i>			
Asparagus	—	Cyanidin; Pn	Glu; rut; glu-rut; 3,5-diglu; 3,5-caf-rham-glu
Red cabbage	0.7–0.9	Cyanidin	3,5-Diglu; soph-5-glu acylated with <i>p</i> -cou, fer, sin
Red lettuce	—	Cyanidin	Mal-glu; glu
Red onion	0.2	Cyanidin; Pn	Mal-glu; di-mal-lam; mal-lam; glu; mal-3''glu; arab
Red radish	1.5 (skin)	Pelargonidin; Cy	3,5-Diglu; soph-5-glu acylated with <i>p</i> -cou, fer, caf
Black beans	2.1	Delphinidin; Pt; Mv	Glu
Cocoa beans	0–1.0	Cyanidin	Arab; gala; arab-glu
Purple basil	0.2	Cyanidin, Pn	Glu; 3,5-glu; <i>p</i> -cou; mal; <i>p</i> -cou-glu-5-glu
<i>Miscellaneous</i>			
Red wine	0.01–0.5	Malvidin; Dp; Pt; Pn; Cy	Glu; ace-glu; <i>p</i> -cou-glu; 3,5-glu
Blackcurrant juice	0.02–0.1	Cyanidin	Glu; rut

^aCrude total anthocyanin content of edible portion of plant.

^bCy, cyanidin; Dp, delphinidin; Mv, malvidin; Pn, peonidin; Pg, pelargonidin.

^cGlu, glucoside; gala, galactoside; arab, arabinoside; rut, rutinoside; xyl, xyloside; rham, rhamnoside; soph, sophoroside; mal, malonyl; lam, laminaribioside; samb, sambubioside; *p*-cou, *p*-coumaroyl; ace, acetyl; caf, caffeoyl; fer, feruloyl; sin, sinapic.

(Table 4.3) — polymers of catechin and catechin esters — were found to meet the selection criteria and included in the database.

4.3 DATABASE OF FLAVONOIDS IN FOODS

The database (Table 4.9–Table 4.12) currently contains entries for 35 types of fruits, 31 vegetables, 26 beverages, eight different jams, three types of chocolate, and 12 herbs. Data are presented as average, minimum, and maximum values (mg/100 g) for each of the flavonoid

TABLE 4.9
Flavonoid Content of Fruits

	Flavonols			Flavones		Procyanidins	Catechins			Flavanones		
	Qu	K	My	Lut	Apig	B1-4	C	EC	EGCG	EC	Nar	Hesp
Apple	3.5 ^a	0.2	Tr	—	—	9.2	0.8	6.3	—	0.03	—	—
Eating	(2.0-7.2) ^b	(Tr-0.5)	Tr	—	—	(3.8-15.4)	Tr (Tr-0.1)	—	—	—	—	—
Peeled	Tr	Tr	Tr	—	—	7.7	0.9	6.2	—	—	—	—
Sauce	2.0	Tr	—	—	—	—	C	6.2	—	—	—	—
Apricots	2.6	C	—	—	—	0.1	2.6	3.0	—	—	—	—
	(2.5-2.6)	—	—	—	—	—	(0.3-5.0)	—	—	—	—	—
Avocado	—	—	—	—	—	0.02	—	0.3	—	—	—	—
	—	—	—	—	—	—	—	(0.1-0.6)	—	—	—	—
Banana	C	C	—	—	—	C	C	0.3	—	—	—	—
	—	—	—	—	—	—	—	(Tr-0.03)	—	—	—	—
Bilberry	2.9	1.6	1.0	—	—	C	C	C	—	—	—	—
	(1.7-4.1)	(Tr-4.7)	(Tr-1.8)	—	—	—	—	—	—	—	—	—
Blackberry	1.0	0.1	Tr	—	—	1.4	0.4	10.4	—	C	—	—
	(0.2-1.9)	(Tr-0.2)	Tr	—	—	(0.8-2.0)	(0.0-0.7)	(2.4-18.1)	—	—	—	—
Blackcurrants	2.9	1.6	1.0	—	—	C	C	0.5	—	—	—	—
	(3.7-7.9)	(Tr-1.7)	(Tr-13.1)	—	—	—	—	—	—	—	—	—
Blueberries	4.0	Tr	1.3	—	—	1.4	0.4	0.7	—	—	—	—
	(2.1-7.3)	Tr	(Tr-2.5)	—	—	(0.6-2.2)	(0.2-0.6)	(0.1-1.1)	—	—	—	—
Cherries	1.3	Tr	—	—	—	2.6	0.1	7.5	—	0.2	—	—
	(1.0-1.5)	Tr	—	—	—	—	—	(2.5-9.5)	—	—	—	—
Cranberries	13.0	Tr	10.1	—	—	C	C	4.2	—	—	—	—
	(7.3-17.2)	(Tr-0.2)	(0.4-14.2)	—	—	—	—	—	—	—	—	—
Custard apple	—	—	—	—	—	11.1	—	5.6	—	0.04	—	—
Elderberries	17.1	—	—	—	—	C	C	—	—	—	—	—

continued

TABLE 4.9
Flavonoid Content of Fruits — continued

	Flavonols				Flavones			Procyanidins		Catechins				Flavanones	
	Qu	K	My	Lut	Apig	B1-4	EGC	C	EC	EGCG	ECG	GC	Nar	Hesp	
Early fig	—	—	—	—	—	0.03	—	0.1	0.1	—	—	—	—	—	
Gooseberry	2.0	1.8	—	—	—	—	—	1.7	—	—	—	0.4	—	—	
Grapefruit	—	—	—	—	C	—	—	—	—	—	—	—	39.2	1.4	
	(Tr-1.4)												(25.4-53.0)	(1.3-1.5)	
Grapes															
Black	2.6	—	0.2	—	—	1.2	0.1	4.9	4.7	—	1.5	—	—	—	
	(1.5-3.7)		(Tr-0.5)			—	—	(0.8-8.9)	(0.7-8.6)		(0.2-2.8)				
Green	0.7	—	—	—	—	0.9	0.04	1.4	0.6	—	—	0.03	—	—	
	(0.2-1.2)					—	—	(0.4-2.5)	(0.1-1.0)						
Kiwi fruit	N	N	—	—	—	0.1	—	—	0.4	—	—	—	C	C	
						—			(0.3-0.5)						
Lemon	1.3	C	C	0.5	C	—	—	—	—	—	—	—	0.8	18.3	
	(Tr-2.8)			(Tr-1.5)									(0.5-1.3)	(17.0-20.6)	
Lime	0.4	—	—	—	—	—	—	—	—	—	—	—	3.4	43.0	
Lingonberry	12.6	—	—	—	—	C	C	C	C	C	C	C	—	—	
	(10.0-16.9)														
Mango	C	C	C	—	—	C	C	1.7	—	C	C	C	C	C	
Nectarine	N	C	C	—	—	C	C	2.8	N	C	C	C	—	—	
Olives	12.4	—	—	12.4	4.6	—	—	—	—	—	—	—	—	—	
	(6.0-12.2)			(6.0-12.2)	(2.0-7.1)										
Orange	1.4	—	—	C	—	—	—	—	—	—	—	—	12.5	38.5	
	(Tr-4.3)												(11.0-14.5)	(31.0-43.2)	

Peaches	0.5 (Tr-0.9)	C	—	—	3.2	—	1.4 (0.5-2.3)	0.7	—	0.01	—	—
Pears	0.5 (0.5-0.6)	C	—	—	0.7	—	0.1 (Tr-0.1)	1.5 (0.3-3.4)	—	—	—	—
Peeled	C	C	—	—	C	—	0.03	1.1	—	—	—	—
Persimmon	—	—	—	—	0.1	—	0.6	—	—	—	0.2	—
Plums, blue	1.2 (0.9-1.5)	—	—	—	16.1	—	4.3 (3.4-5.2)	3.6 (2.8-4.4)	—	—	—	—
Pomegranate	—	—	—	—	0.3	0.2	0.4	0.1	—	—	0.2	—
Raisins	3.6	3.2	—	—	—	C	3.0	0.7	C	C	—	—
Raspberries	0.7 (0.5-5.1)	C	—	—	2.8 (2.1-3.5)	1.1 (0.9-1.2)	0.5 (0.0-1.0)	5.0 (1.4-8.3)	—	—	—	—
Red currants	0.9 (0.6-1.3)	0.04 (Tr-0.1)	—	—	0.2	0.4	1.3	0.1	—	—	1.3 (1.0-1.7)	—
Rhubarb	C	C	—	—	C	C	2.2 (1.1-1.6)	0.5	C	0.6	C	—
Strawberry	0.6 (0.4-0.9)	0.8 (0.5-1.2)	—	C	1.9	0.2	3.0	—	—	—	0.1	—

Notes: Tr, below limit of detection; C, not quantified; N, known to be present; —, not known to be present; see Appendix 2 for references included in database. Qu, quercetin; K, kaempferol; My, myricetin; Lut, luteolin; Apig, apigenin; B1-4, B-procyanidins; EGC, epigallocatechin gallate; EC, catechin; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; GC, gallic acid; Nar, naringenin; Hesp, hesperetin.

^aAglycone mg/100 g fresh weight.

^bMinimum to maximum values included in database (values given in parentheses).

TABLE 4.11
Flavonoid Content of Beverages

	Flavonols			Flavones		Procyanidins		Catechins				Flavanones		
	Qu	K	My	Lut	Apig	B1-4	EGC	C	EC	EGCG	ECG	GC	Nar	Hesp
Apple juice	1.1 ^a (0.1-2.9) ^b	—	—	—	—	0.05	C	1.7 (Tr-5.1)	6.5 (Tr-19.3)	—	—	C	0.8	9
Black current juice fresh	1.6 (0.7-2.5)	C	2.1 (2.1-3.2)	—	—	C	C	C	—	—	—	C	—	—
Cacao drink	C	—	—	—	—	0.4	Tr	0.7	0.6	Tr	Tr	Tr	—	—
Chocolate milk	0.1	Tr	Tr	—	—	C	Tr	0.9	0.3	Tr	Tr	Tr	—	—
Coffee	Tr	—	Tr	—	—	—	Tr	—	Tr	—	—	—	—	—
Cranberry juice, canned	1.2	—	2.9	—	—	C	—	0.2	C	—	—	—	—	—
Cranberry juice, fresh	17.5	—	4.7	—	—	C	—	1.0	C	—	—	—	—	—
Grape juice, black	0.5 (0.4-0.5)	—	0.6	—	—	—	—	0.4	Tr	—	—	—	—	—
Grape juice, white	0.5 (0.4-0.5)	—	0.6	—	—	0.4	—	0.4	0.01 (Tr-0.01)	—	—	—	—	—
Grapefruit juice	0.3 (0.1-0.5)	C	—	—	C	—	—	—	—	—	—	—	23.0 (16.8-31.2)	0.5 (Tr-1.3)
Lemon juice	1.1	—	Tr	—	C	—	—	—	—	—	—	—	0.3	6.7
Lime juice	—	—	—	—	—	—	—	—	—	—	—	—	0.2	6.9
Orange juice (diluting)	0.6	—	—	—	C	—	—	—	—	—	—	—	3.3 (3.1-3.4)	21.2 (1.1-25.4)
Orange juice (fresh)	0.5 (0.1-1.0)	—	—	—	C	—	—	—	—	—	—	—	2.3 (0.8-2.9)	21.2 (9.0-38.2)

TABLE 4.12
Miscellaneous Foods

	Flavonols			Flavones		Procyanidins		Catechins				Flavanones		
	Qu	K	My	Lut	Apig	B1-4	EGC	C	EC	EGCG	EGC	GC	Nar	Hesp
<i>Jam</i>														
Apricot	0.8 ^a	0.1	—	—	—	C	—	0.5	0.5	—	—	—	—	—
Cherry	C	C	—	—	—	C	C	0.2	0.9	—	C	—	—	—
Forest fruit	—	—	—	—	—	—	—	0.1	1.6	—	—	—	—	—
Honey	0.2	0.1	0.1	0.3	0.03	—	—	—	—	—	—	—	C	—
	(Tr-0.4) ^b	(Tr-0.1)	(0.1-0.2)	(Tr-0.7)										
Peach	0.4	0.3	C	—	—	—	—	C	—	—	—	—	—	—
Plum	0.7	C	—	—	—	C	—	C	C	—	—	—	—	—
Raspberry	3.3	0.5	—	—	—	C	—	C	C	—	—	—	—	—
	(3.0-3.7)	(0.4-0.5)												
Strawberry	0.5	0.3	—	—	—	C	—	0.9	0	—	—	—	—	—
	(0.4-0.6)	(0.1-0.4)												
<i>Confectionary</i>														
Chocolate, dark	C	—	—	—	—	2.1	C	6.6	21.8	—	—	C	—	—
								(1.3-12.0)	(2.2-41.5)					
Chocolate, fancy and filled	C	—	—	—	—	C	C	2.2	6.3	—	—	C	—	—
Chocolate, milk	C	—	—	—	—	2.1	C	2.3	7.4	—	—	C	—	—
								(1.3-3.3)	(2.2-12.5)					

subclasses; i.e., flavonols, flavones, proanthocyanidins, catechins, and flavanones. All the flavonol, flavone, and flavanone content data have been determined after optimized hydrolysis of their respective glycosides to the aglycone. Catechin monomers such as epicatechin gallate occur naturally in their free form and are presented as mg catechin/100 g. Procyanidins are presented as their monomeric unit (–)epicatechin.

4.3.1 FRUITS

Catechins, flavonols, and proanthocyanidins are abundant in fruits. In contrast, flavanones and flavones are restricted to citrus varieties such as oranges and lemons (Table 4.9). In some fruits (e.g., apples), flavonols are principally present in the skin and hence peeling significantly reduces levels unlike catechins which are found in the flesh of fruits. Overall, catechins were the most abundant flavonoid, (+)-catechin (C) and (–)epicatechin (EC) being particularly prevalent. Black grapes (4.9 and 4.7 mg/100 g, C and EC, respectively) are one of the richest fruit sources of catechins followed by apples (0.8 and 6.3 mg/100 g, C and EC, respectively). Catechins are also relatively abundant in stone fruits, such as blue plums (4.3 and 3.6 mg/100 g, C and EC, respectively) and apricots (2.6 and 3.0 mg/100 g, C and EC, respectively). The gallic acid esters of catechin, (–)epigallocatechin, (–)epigallocatechin gallate, (–)epicatechin gallate, and (+)-gallocatechin, are relatively uncommon in fruits, with only berries, currants, and grapes containing small amounts. Strawberries were found to contain the most complex mixture of catechins, comprising catechin (75% of total catechins), ECG (18% of total catechins), EGC (5% of total catechins), and GC (3% of total catechins). Catechin esters have been characterized, but not measured, in some fruits such as nectarines and mangos. Type B procyanidins are also present in fruits, with apples (3.8 to 15.4 mg/100 g), plums (16.1 mg/100 g), and peaches (3.2 mg/100 g) containing the highest concentrations. However, citrus fruits do not appear to contain detectable levels of catechins or type B procyanidins.

Quercetin is the most common flavonol in fruits, elderberries (17.0 mg/100 g), lingonberries (12.6 mg/100 g), and cranberries (13.0 mg/100 g) being particularly rich sources. Berries and currants are also the fruits containing most kaempferol and myricetin. For example, these two flavonols account for 29 and 18%, respectively, of the total flavonol content of the bilberry. Although kaempferol and myricetin have also been identified in fruits such as peaches and pears, concentrations are generally too low to be readily quantified in the whole fruit. The skin of these fruits contains these flavonols in significant amounts; however, their flesh, which constitutes >70% of the fresh weight, does not. Consequently, when analyzed as normally eaten only trace levels are present.

Often termed the citrus flavonoids, flavanones are only found in citrus fruits such as oranges, grapefruit, and lemons. Although naringenin is present at greater concentrations than hesperetin in grapefruit (39.2 and 1.4 mg/100 g, respectively), the latter is the dominant form in oranges, lemons, and limes. Although citrus fruit also contains low levels of flavones, the olive is by far the richest source of luteolin and apigenin (12.4 and 4.6 mg/100 g, respectively).

4.3.2 VEGETABLES

Allium (e.g., onions), *Brassica* (e.g., broccoli and kale), and *Lactuca* (e.g., lettuce) varieties of vegetables and tomatoes (*Lycopersicon* species) are abundant sources of flavonols, primarily quercetin and kaempferol (Table 4.10). Flavones are also found in some vegetables such as celery, sweet peppers, and lettuce. Catechins and type B procyanidins, however, have not been found in leafy green or root vegetables but have been detected in legumes such as broad and green beans. The tomato is the only vegetable (although taxonomically a fruit) to possibly contain the flavanones naringenin and hesperetin.

Of the *Allium* species, shallots and red onions represent the richest potential source of quercetin containing 95 and 64 mg/100 g, respectively. *Brassica* vegetables including broccoli, kale, cabbage, and brussels sprouts tend to contain complex mixtures of flavonols, with significant quantities of kaempferol and myricetin glycosides present in addition to quercetin conjugates. Kale is a good example of this with mean levels of 11.5 mg quercetin/100 g and 34.1 mg kaempferol/100 g. Legumes such as green and broad beans also contain complex mixtures, mainly of flavonols and catechins. For example, broad beans contain (–)-epicatechin (30.0 mg/100 g), (+)-catechin (14.5 mg/100 g), (–)-galliccatechin (4.8 mg/100 g), and quercetin, myricetin, and kaempferol at concentrations below 3.0 mg/100 g.

Green chilli pepper is one of the few vegetables to contain both flavonols (quercetin, 11.39 mg/100 g) and flavones (luteolin, 2.7 mg/100 g) at detectable levels. Celery and sweet ball peppers are the main food sources of flavones independent of flavonols.

4.3.3 BEVERAGES

Catechins are often the most common flavonoids in beverages such as fruit juice, tea, and wine (Table 4.11). These tend to contain complex mixtures of simple catechins and their gallated esters. Type B procyanidins have frequently been characterized in beverages such as fruit juices; however, reliable quantitative data are limited. Flavonols are also present in most beverages while flavanones are again restricted to citrus juices such as grapefruit and orange. The presence of flavones in beverages is not well described with only some characterization information available in the literature.

Fruit juice contains both catechins and flavonols. Apple juice is one of the richest juice sources of catechins (containing 6.3 mg (–)-epicatechin/100 ml and 0.8 mg (+)-catechin/100 ml) whereas cranberry juice contains the most flavonols, mainly in the form of quercetin and myricetin (17.5 mg/100 ml and 4.7 mg/100 ml, respectively).

Tea is the only analyzed beverage to contain (–)-epigallocatechingallate (EGCG) in quantifiable amounts. EGCG and (–)-epicatechingallate (ECG) are the most abundant forms, each contributing 27% to the total catechin content (22.2 mg/100 ml) of black tea. Three flavonols (quercetin, kaempferol, and myricetin) are also found in tea. For example, 100 g of decaffeinated tea contains 5.2 mg quercetin, 2.4 mg kaempferol, and 0.1 mg myricetin.

Wine also contains a complex mix of catechins, flavonols, procyanidins, and flavanones. Red wine contains higher flavonoid levels than white or rosé wines. Procyanidins usually represent 50% of the flavonoids found in red wine, followed by catechins (37%). A similar profile is observed with beer where again procyanidins dominate accounting for 42% of total flavonoid content.

4.3.4 MISCELLANEOUS FOODS

Jam, confectionery, and herb compositional data are presented in Table 4.12. Honey contains low levels of both flavonols and flavones, and the presence of the flavanone naringenin has also been documented. Fruit jams also contain low levels of flavonols and catechins, which generally reflect the flavonoid profile of the whole fruit.

Quantitative data for chocolate are limited, but the available literature demonstrates that it is a good potential source of (+)-catechin, (–)-epicatechin, and type B procyanidins. Dark chocolate, for example, contains 6.6 mg, 21.8 mg, and 2.1 mg/100 g of catechin epicatechin, and procyanidins, respectively.

Compositional analysis data for herbs are also limited; however, these plants may be rich sources of flavones. For example, parsley is the major source of apigenin (217.9 mg/100 g) in the whole database, while sage and thyme are rich in luteolin (33.4 and 39.5 mg/100 g, respectively).

4.4 QUALITY AND COMPLETENESS OF FLAVONOID CONTENT DATA

The data in Table 4.9–Table 4.12 are comprehensive estimates of five classes of flavonoids in commonly available foods in the United Kingdom. Moreover, these estimates are derived from critically assessed published sources and the evaluation procedures adopted ensured the inclusion of content values for edible parts of plant materials available to the UK consumer. A USDA compiled database (<http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.pdf>) aimed primarily at the North American diet is also available. These databases are in contrast to another literature-derived database that is available for flavonoids⁵⁵ where data quality was not formally assessed and flavonoid values determined using semiquantitative methods were also included.

Nevertheless, the current database also has unavoidable limitations as the selection of appropriate food items and flavonoid values still required an element of operator judgment. For example, potentially useful information was excluded because (a) only experimental plants rather than commercially available varieties were analyzed (e.g., Ref. 56), (b) the country of origin did not supply the UK market (e.g., Ref. 57), (c) values were expressed only on a dry weight basis (e.g., Ref. 58), (d) final figures were presented as percentage of the total content (e.g., Ref. 59), and (e) the flavonoid contribution from the edible portions were difficult to separate from the whole fruit (e.g., Ref. 60). In addition, flavonoid data for several commonly consumed items are likely to be missing. For example, no flavanone data are available for satsumas, tangerines, and clementines, although these are seasonally abundant in UK supermarkets. There is also an overall lack of information on commonly consumed items such as herbs, fruit tea, and beer.⁶¹ Additionally, bias may be introduced due to the relative number of compositional studies relating to each of the different flavonoid subclasses. For example, there are five large studies comprehensively identifying flavonols and flavones in several foods and beverages,^{25,29,32,62–66} but fewer for catechins,^{67–69} procyanidins,⁶⁹ and flavanones.²⁹

4.4.1 FACTORS AFFECTING FLAVONOID CONTENT OF FOOD

Another potential source of error in the database relates to the possibility that the flavonoid content of fruits and vegetables analyzed in a particular study do not reflect “normal” levels in the products. Such regional differences are frequently cited in order to explain the apparent lack of association between dietary components and disease.^{2,70} The present database attempts to minimize this effect by including flavonoid values of products from countries known to export to the United Kingdom as over 50% of fresh produce consumed in this country is imported from the global market to ensure a year round supply.⁷¹ Apples, for example, are imported from 24 different countries including France, Argentina, New Zealand, and Canada with British varieties being available for 9 months of the year (freshinfo.com).

However, other factors affecting flavonoid levels such as analytical variations, environmental factors, species characteristics, and the effects of processing and storage are more difficult to take into account when compiling the database.

4.4.1.1 Analytical Variations

The methods of extraction and analysis can markedly affect the determination of flavonoids in foods.^{12,13,31,72–76} Rigorous application of the selection criteria (Table 4.6) may minimize this confounding effect. Overall, sample preparation and extraction techniques along the lines described by Merken and Beecher³¹ were considered acceptable. These included freeze-drying, extraction either with aqueous methanol containing an antioxidant such as BHT or

by solid-phase columns, filtration and the reduction of flavonol conjugates to the “free” aglycone by acid hydrolysis, enzyme digestion, or alkaline hydrolysis. Acceptable separation methodology normally involved RP-HPLC with UV, diode array, or electrochemical detection. Fluorescence detection, capillary zone electrophoresis, and micellar electrokinetic capillary chromatography were also included if identification and confirmation of eluted peaks was based on comparison with external standards, or if mass spectroscopy or nuclear magnetic resonance was used to confirm structural identity.

4.4.1.2 Environmental Factors

The flavonoid content of plant foods may be affected by growing conditions.^{3,10} For example, red wine produced in the warm, dry, and sunny conditions prevalent in the New World tend to contain more quercetin and myricetin (but less catechin) than the wines produced in the cooler and damper regions of Northern Europe.^{77,78} Similar regional and climatic effects on flavonoid content have been observed for many different fruits and vegetables.^{65,79–81} Concentrations of flavonol and flavanone monoglycosides, for example, are greatest on the surface of plants grown in or originating from arid and semiarid habitats.^{11,79,82} However, flavonoid profiles are also influenced by irrigation, which, for example, modifies concentrations and types of anthocyanins and catechins in berries.^{82,83}

Marked differences in flavonoid content can even occur within a single variety depending on numerous factors such as maturity at harvesting, storage, use of glass and polythene, and organic cultivation methods.^{32,65,68,84–86} This latter factor may be one reason why the flavonoid content of foods from Hungary are much higher than those from Western Europe, the enhanced levels reflecting the role of flavonoids in plants as insecticides and antimicrobial and antifungal agents.^{11,85} Interestingly, Hungary is one of the main suppliers of organic vegetables to UK supermarkets.

Such environmental influences may account for why, for example, quercetin levels in Spanish cherry tomatoes range from 3.8 mg/100 g to 20.0 mg/100 g during a single year⁸⁰ and why produce grown in polythene tunnels with reduced UV exposure contain 98% less flavonoid glycosides than when grown in the open air.^{10,11,40,82} The degree to which such environmental factors decrease the accuracy of the database is impossible to quantify but is likely to be minimized by the computation of average content values from a wide range of sources. This, in turn, is likely to reflect the average intake of a population exposed to a diverse range of products over the longer term.

4.4.1.3 Species Characteristics

Computation of a single value from a wide range of sources will also minimize analogous confounding effects of varietal differences as flavonoid subclasses can vary widely between different cultivars of fruits and vegetables.^{11,32,87,88} Examples of such differences include flavonols in berries,⁸⁹ flavones in honey⁹⁰ and olives,⁹¹ catechins in pears⁹² and apples,⁸⁶ procyanidins in apples⁹³ and blueberries,⁹⁴ and flavanones in citrus fruit⁹⁵ and grapefruit juice.⁹⁶ Typically, for example, the flavonol content of 12 chilli-pepper varieties ranges from 0 to 85 mg/100 g.⁹⁷ Such differences can be ascribed to: (a) genetic mutations influencing the synthesis and accumulation of flavonoids in tissue^{11,79}; (b) the degree of pigmentation,^{56,79,89,98–100} particularly in berries¹⁰¹ and onions³ (although this has been recently disputed^{54,56,79,80} as original determinations may have included the nonedible and anthocyanin-rich skin of the onion); (c) the stage of maturity,^{13,102} quercetin levels, for example, tending to decline as fresh peppers ripen¹⁰³ whereas fresh young tea leaves contain more catechin derivatives than older ones.¹⁰⁴

In addition, there are varietal effects on the degree and type of glycosylation of flavonoids.¹⁰⁵ For example, quercetin rhamnoside is the most abundant glycoside in Jonagold and Golden Delicious apples whereas in Cox's orange and Elstar varieties, quercetin galactoside and arabinoside dominate.⁸⁶ As the nature of the sugar attachment may influence the bioavailability of a particular flavonoid,^{106,107} it may ultimately be preferable for the database to show flavonoid content data as glycosides. At present, such information is lacking as the majority of studies employ hydrolysis to liberate the aglycone from the food matrix. Alternatively, once the biological significance of different glycosides have been determined, it may be possible to calculate conversion factors for flavonoids that are analogous to the current means of expressing tocopherol homologs as vitamin E equivalents.⁴¹

4.4.1.4 Processing and Storage

In general, industrially produced products such as tea, red wine, and fruit juice have significantly different flavonoid levels and profiles than the original fresh product.^{108–112} Processing and preservation can expose fresh products to increased risk of oxidative damage and the activation of oxidative enzymes such as polyphenol oxidase.^{92,113} In addition, procedures such as solvent extraction, sulfur dioxide treatment, pasteurization, enzymic clarification, heating, canning, irradiation, drying, and fermentation have been reported to affect procyanidin and catechin concentrations in fruit juice,^{108–110,114–117} quercetin glucosides, catechins, and procyanidins in grapes,¹¹⁸ procyanidin and flavonol levels in tomatoes and related sauces,¹¹⁹ and quercetin concentrations in berries.⁸¹

Domestic preparation procedures such as chopping, shredding, peeling, and cooking may also affect flavonoid content accounting, for example, for 21 to 54% losses of flavonols in onions^{54,120,121} as well as inducing glucosidase-mediated formation of monoglucosides and free quercetin from diglucosides.⁵⁶ Boiling is reported to lead to reduced flavonol contents of onions,^{54,80,120} broccoli,¹²² tomatoes,⁸⁰ asparagus, and green beans¹²⁰ although the effects of microwave cooking and frying may be less marked^{80,120} due to decreased leaching of flavonoids from the foods during these cooking procedures.⁵⁴ Therefore, to minimize the confounding effects of such procedures, where possible values obtained from cooked and processed products were also included in the database.

4.5 ESTIMATED DIETARY FLAVONOID INTAKE

4.5.1 ESTIMATION OF DIETARY FLAVONOID INTAKE

Investigation of the relationship between diet and the development of chronic diseases requires an assessment tool that provides a valid estimate of "usual intake."¹²³ There is a wide variety of techniques available to assess dietary intake either prospectively or retrospectively. These range from those that provide relatively precise measurements of individual diet such as weighed intake records or duplicate diets to those that broadly rank intake in large cohort studies into high, medium, and low categories such as diet history or food frequency questionnaires. The advantages and disadvantages of these measurements have been extensively discussed and reviewed by several key workers in the field of dietary assessment.^{2,123–125} Dietary assessment workers consistently agree that after selection of the most appropriate measurement tool, accurate and representative nutrient data on food composition are required.

4.5.2 PREVIOUS ESTIMATIONS OF DIETARY FLAVONOID INTAKE

An initial estimate of flavonoid intake of 1000 mg/day was calculated in the United States during the 1970s using semiquantitative food composition data (Table 4.13).¹⁰ This estimate was not questioned until the 1990s with the calculation of dietary flavonol and flavone

TABLE 4.13
Average Daily Flavonoid Intake in the United States
During 1971

Flavonoid	mg/day
Flavanones, flavones, flavonols	160–175 (110–121) ^a
Anthocyanins	180–215 (124–148)
Catechins	220 (152)
Biflavans	460 (317)
Total flavonoids	1020–1070 (703–738)

^aExpressed as quercetin-3-rhamnoside (converted to quercetin aglycone).

Source: Reproduced from Kuhnau, J., *World Rev. Nutr. Diet.*, 24, 117, 1976. With permission.

aglycone (including glycosides hydrolyzed to their free form) intake from Dutch composition data for 28 vegetables, nine fruits, and several beverages.^{32,126} Intake estimates for cohorts from seven countries ranged from 3 mg/day in Finland to 65 mg/day in Japan.²⁰ When the data of Kuhnau,¹⁰ originally expressed as quercetin-3-rhamnoside equivalents, are converted to quercetin aglycones (703 to 738 mg/day), flavanones, flavonols, and flavones contribute 110 to 121 mg/day (Table 4.13). This suggests that the earlier study somewhat overestimated dietary flavonoid intake.

Several dietary flavonoid intake studies have now been completed using the Dutch composition data often with additional estimates of flavonoid content of local food preferences such as berries (Table 4.14). Comparison of these intake studies indicates that quercetin is consistently the main contributor to flavonol and flavone intake. In the Netherlands, for example, quercetin accounts for 70% of the 23 mg/day total flavonol and flavone intake followed by kaempferol (17%), myricetin (6%), luteolin (4%), and apigenin (3%).¹²⁷

TABLE 4.14
Estimated Daily Dietary Flavonoid Intakes by Different Countries

Flavonoid	Country	mg/day ^a	Main Dietary Sources	Ref.
<i>Flavonols/flavones</i>				
	Netherlands	23	Tea (48%), onions (29%), apples (7%)	27
	United States	20–24	Tea (26%), onions (24%), apples (8%)	145
	United Kingdom (Wales)	26	Tea (82%), onions (10%)	135
	Finland	4	Apples and onions	34
	Spain	5	Tea (26%), onions (23%), apples (8%)	144
	Japan	16	Onions (46%), molokheya (10%), apples (7%), green tea (5%)	146
<i>Catechins</i>				
	Netherlands	50	Tea (83%), chocolate (6%), apples and pears (6%)	130
	United States	25	Tea (59%), apples and pears (26%)	139
<i>Flavanones</i>				
	Finland	20	Orange and grapefruit	129

Different countries obtain flavonoids from differing sources with, for example, green tea being the main contributor to intake in Japan, red wine in Italy, and apples in Finland.²⁰ However, the original dietary data for these investigations were collected during the early 1960s and may be outdated, as dietary patterns have changed during the last 40 years. For example, green tea consumption in Japan accounted for 80% of flavonol intake in 1960 but only 5% in 1997.⁵⁷ Table 4.14 outlines recent dietary flavonoid estimates and main dietary sources using data gathered after 1985. Interestingly, Japanese flavonol and flavone intake is reported to have fallen from 65 to 17 mg/day with onions, not green tea, now being the main dietary source. This may reflect increasing westernization of the Japanese diet.¹²⁸

4.5.3 ESTIMATION OF SCOTTISH DIETARY FLAVONOID INTAKE USING THE NEW COMPREHENSIVE FLAVONOID DATABASE

The flavonoid database described in this chapter was applied to 4-day weighed food records obtained from healthy Scottish men ($n = 41$) and women ($n = 52$) to provide a provisional estimate of flavonoid intake in Scotland. All subjects consumed foods containing flavonols, procyanidins, and catechins, dietary intakes of which are given in Table 4.15. The main flavonol consumed was quercetin, accounting for 66 and 63% of the total flavonol intake of 18.8 mg/day. Primary sources of flavonols were from black tea (42.7%), onions (14.3%), apples (10.2%), and lager (7.2%) (Table 4.16).

Procyanidins and catechins were primarily obtained from black tea (procyanidins >47.6% and catechins >57.6%) and apples (procyanidins >15.8% and catechins >7.5%) (Table 4.16). The main catechins consumed were EGCG (23%), ECG (22%), and EC (25%).

Flavones and flavanones were less frequently consumed during the 4-day collection period. Flavones were not consumed at all by 38 participants, while 29 people did not consume any citrus flavonoids — flavanones. The interquartile range of intake of flavones was relatively limited, ranging from 0.0 to 2.0 mg/day. Flavone consumption was not normally distributed and was negatively skewed toward a lack of consumption of foods rich in flavones such as olives and lettuce. Likewise, flavanone intake was also not normally distributed with a mean flavanone intake of 1 mg/day compared to the median intake of 1.2 mg/day. This is accounted for by the fact that the range of flavanone intakes was very wide (0 to 239 mg/day), 36% of participants not consuming any flavanone-rich foods. The main dietary

TABLE 4.15
Estimated Dietary Flavonoid Intake by UK (Scottish) Population ($n = 81$)

Flavonoid Subclass	Daily intake (mg) (Median [Range])
<i>Anthocyanins</i>	
Flavonols (quercetin + kaempferol + myricetin)	18.8 (1.9–51.3)
Flavones (apigenin + luteolin)	0.1 (0–6.7)
Proanthocyanidins (procyanidin B1 + B2 + B3 + B4)	22.5 (0–144.5)
Catechins (C + EC + EGC + ECG + EGCG + GC) ^a	59.0 (1.8–263.3)
Flavanones (hesperidin + naringenin)	1.2 (0–238.6)

^aC, catechin; EC, epicatechin; EGC, epigallocatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; GC, galocatechin.

TABLE 4.16
Main Dietary Sources of Flavonoids from UK (Scottish) Diet^a

Flavonols		Flavones		Procyanidins		Catechins		Flavanones	
Food Item	% ^b	Food Item	%	Food Item	%	Food Item	%	Food Item	%
Black tea	42.7	Sweet peppers ^c	24.4	Black tea	49.5	Black tea	63.6	Orange juice	37.8
Onion	14.3	Lettuce	18.1	Apples	20.6	Apples	11.2	White wine	19.2
Apples	10.2	Cheese and tom. pizza	11.9	Red wine	8.9	Red wine	4.9	Red wine	19.0
Lager	7.2	Vegetable soup	7.6	Lager	8.0	White wine	4.9	Orange	11.6
Tomato	2.4	Honey	5.7	Chocolate milk	4.4	Chocolate milk	4.1	Vegetable samosas	4.8
Baked beans	2.1	Scotch broth	3.7	Strawberries	0.9	Lager	3.9	Grapefruit juice	3.4
Red wine	2.8	Chilli cone carne	3.6	Peaches	0.7	Apple juice	2.7	Lemon juice	2.4
Orange Juice	2.0	Celery	2.8	Grapes	0.4	Grapes	1.8	Lemon sorbet	0.9

^aData collected from 40 males and 41 females using 4-day weighed intake records.

^bPercentage contribution of the top eight dietary flavonoid sources.

^cSweet peppers — total contribution of red, green, and yellow peppers.

sources of flavanones were orange juice (37.8%) and wine (red wine 19.0% and white wine 19.2%) (Table 4.16).

4.5.4 COMPARISON WITH OTHER ESTIMATES OF DIETARY FLAVONOID INTAKE

Several dietary flavonoid intake studies have now been completed using the Dutch composition data often with additional estimates of flavonoid content of local food preferences such as berries (Table 4.14). The intake data reported above are the first estimation of dietary intake of different types of flavonoids by a UK population. Flavonol, flavone, and catechin intakes were comparable with Dutch literature^{127,130} and the only other UK dietary flavonol investigation¹³¹ (Table 4.14). The proportional contribution by individual flavonols and catechins to total flavonol and total catechin intakes, respectively, were analogous with Dutch intake and tea was their main dietary source. The flavanone intake of 1.2 mg/day is less than Finnish estimates of 20.2 ± 27.6 mg/day (mean \pm SD) for men and women combined.¹²⁹ This may reflect differences in dietary preferences although it should also be noted that the Finnish data were calculated from a food frequency questionnaire, a dietary assessment method known to overestimate intake compared with weighed records. Procyanidin intake has not previously been estimated, as there has been a lack of reliable content values. However, Santo-Buelga and Scalbert⁷⁵ noted that a rough estimated intake of proanthocyanidins in Spain might range from 10 to >100 mg. If this estimate is correct, then the procyanidins type B1-4 measured here represents a small fraction of the total.

Identification of black tea as the primary source of flavonols (42.7%), procyanidins (49.5%), and catechins (63.6%) is again consistent with published literature for tea-drinking nations.^{130,131} For example, a study of 1900 Welsh men also observed that tea was the main dietary source of flavonols.¹³¹ Interestingly, 5.4 ± 3.0 cups of tea were consumed by these subjects per day between 1979 and 1983 whereas the Scottish participants reported consuming only 2.8 ± 2.4 cups of tea per day. This may reflect the current downward trend in tea consumption in the United Kingdom especially by adults under 50 years⁶¹ and also suggests that flavonols are obtained from other food sources in the Scottish diet. Hertog,¹³¹ for

example, did not report lager as a major source of flavonols in the Welsh population despite its widespread consumption.⁴⁶ This is possibly because lager was not included in the Dutch database.^{25,32,126}

4.5.5 FUTURE REQUIREMENTS TO IMPROVE DATABASE

The compilation of the database has further emphasized the diversity of potential dietary sources of flavonoids. It has also been used to give the first estimation of dietary intake of different types of flavonoids by a UK population. However, continual update is required to accommodate the increasingly varied purchasing patterns of foods in the United Kingdom⁶¹ as well as for use in countries with markedly different dietary habits. There is a current lack of directly analyzed flavonoid measurements for composite meals. Therefore, recipe calculations would have to include assumptions for cooking losses or gains, which could subsequently introduce bias when calculating dietary intake.¹³² Moreover, it is also important to confirm the flavonoid profile of composite dishes as they may contain unusual complex mixtures. For example, bolognese sauce contains all five flavonoid subclasses (Table 4.17) whereas no individual food in the database exhibits such a profile. Despite these reservations, calculating the flavonoid content of recipes may be reasonably robust. Fjellkner-Modig et al.,¹³³ found close agreement between the directly analyzed and calculated quercetin levels in moussaka. However, it is possible that flavonoid values for retail products such as canned soups and preprepared meals may be underestimated as recipes and proportions of ingredients for such products are usually unavailable. Similarly, there is only limited compositional analysis for fruit juices and herbal tea despite their growing popularity.⁶¹ Also, currently lacking are data on flavonoid contents of satsumas, tangerines, and clementines (which were consumed by 25% of subjects during the 4-day record collection period) and herbs commonly added to recipe dishes. Both are potentially good sources of flavonoids.

Despite these caveats, the database constructed in the present study is a comprehensive assessment of the currently available data on the flavonoid contents of foods.

4.6 CONCLUSIONS

A comprehensive and critical review of food flavonoid literature has led to the development of a food composition database for flavonols, flavones, procyanidins, catechins, and flavanones. This database can now be used and continuously updated to estimate flavonoid intake of populations, to identify dietary sources of flavonoids, and to assess associations between flavonoid intake and disease. However, there is a need for better food composition data for flavones, procyanidins, and flavanones as current literature is sparse particularly for citrus fruits, fruit juices, and herbs. In addition, anthocyanin food composition data are lacking although validated methods of determination are becoming available.

4.7 ACKNOWLEDGMENTS

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TABLE 4.17
Estimated Flavonoid Content of Bolognese Sauce Using Standard Recipe

Ingredients	Wt (g)	Flavonols			Flavones		Procyanidin			Catechins			Flavanones		
		Qu	K	Myr	Lut	Apig	BI-4	EGC	C	EC	EGCG	ECG	GC	Nar	Hesp
Clove garlic, crushed	0.8	Tr	—	—	—	—	—	—	—	—	—	—	—	—	—
Onions chopped	60.0	24.4	0.1	C ^a	—	C	—	—	—	—	—	—	—	—	—
Minced beef	500.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vegetable oil	10.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Carrots, chopped	40.0	C	C	—	—	—	—	—	—	—	—	—	—	—	—
Celery chopped	30.0	Tr ^b	Tr	Tr	0.5	1.8	—	—	—	—	—	—	—	—	—
Tomato puree	10.0	0.4	Tr	—	—	—	—	—	—	—	—	—	—	—	—
Canned tomatoes	397.0	1.2	Tr	—	—	—	—	—	—	—	—	—	—	—	—
Stock	125.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Red wine	125.0	1.2	0.1	0.7	—	—	18.8	0.4	8.2	4.8	Tr	0.5	2.2	0.7	
Salt	2.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pepper	1.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dried mixed herbs	1.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total raw	1303.8	27.2	0.2	0.7	0.5	1.8	18.8	0.4	8.2	4.8	0.0	0.5	2.2	0.7	
Cooked dish	886.6 ^c														
Flavonoid content (mg/cooked dish) ^d		13.6	0.1	0.4	0.2	0.9	9.4	0.2	4.1	2.4	0.0	0.3	1.1	0.3	
Flavonoid content (mg/100 g)		1.5	Tr	Tr	Tr	0.1	1.1	Tr	0.5	0.3	—	Tr	0.1	Tr	

Source: Ingredients — recipe taken from Holland, B. et al. The composition of foods, In: *McCance and Widdowson's The Composition of Foods*, Cambridge: Royal Society of Chemistry & MAFF, 1991. With permission.

^aC — Characterized but not quantified.

^bTr — below limit of detection (<0.1 mg/100).

^cCooked dish weight (g) = total raw weight — 32% weight loss during cooking.⁴¹

^dFlavonoid content (mg/cooked dish) = total raw flavonoid content × % nutrient cooking losses.⁴¹

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APPENDIX 1

BOTANICAL NAMES OF FRUITS

Common Name	Botanical Name
Apple	<i>Malus silvestris</i>
Apricot	<i>Prunus armeniaca</i>
Avocado	<i>Persea armeniaca</i>
Banana	<i>Musa acuminata</i>
Bilberry	<i>Vaccinium myrtillus</i>
Blackberry, European	<i>Rubus fruticosus</i>
Blackberry, western trailing	<i>Rubus ursinus</i>
Blueberry, high-bush	<i>Vaccinium corymbosum</i>
Blueberry, low-bush	<i>Vaccinium angustifolium</i>
Brambleberry	<i>Rubus arcticus</i>
Cherry, sour	<i>Prunus cerasus</i>
Cherry, sweet	<i>Prunus avium</i>
Cranberry	<i>Vaccinium macrocarpum</i>
Currants, black	<i>Ribes nigrum</i>
Currants, red	<i>Ribes rubrum, sativum</i>
Custard apple	<i>Annona acuminata</i>
Eggplant/aubergine	<i>Solanum melongena</i>
Elderberry	<i>Sambucus nigra</i>
Fig	<i>Ficus carica</i>
Gooseberry	<i>Ribes uva crispa, grossularia</i>
Grapefruit	<i>Citrus paradisi</i>
Grapes, European	<i>Vitis vinifera</i>
Lemon	<i>Citrus lemon</i>
Lime	<i>Citrus aurantifolia</i>
Mandarin	<i>Citrus reticulata</i>
Mango	<i>Mangifera indica</i>
Olive	<i>Olea europaea</i>
Orange, Seville	<i>Citrus aurantium</i>
Orange, Valencia	<i>Citrus sinensis</i>
Passion fruit	<i>Passiflora edulis</i>
Peach	<i>Prunus persica</i>
Pear	<i>Pirus communis</i>
Pepper, red	<i>Capsicum annuum</i>
Persimmon	<i>Diospyros kaki, virginiana</i>
Plum, blue	<i>Prunus domestica</i>
Plum, yellow	<i>Prunus salicina</i>
Pomegranate	<i>Punica granatum</i>
Raspberry, black	<i>Rubus occidentalis</i>
Raspberry, red	<i>Rubus idaeus, strigosus</i>
Rhubarb	<i>Rheum rhaponticum, tataricum</i>
Strawberry	<i>Fragaria vesca, ananassa</i>
Tangerine	<i>Citrus deliciosa</i>
Tomato	<i>Lycopersicum esculentum</i>

BOTANICAL NAMES OF VEGETABLES

Common Name	Botanical Name
Asparagus	<i>Asparagus officinalis</i>
Artichoke	<i>Cynara scolymus</i>
Broad bean	<i>Vicia faba</i>
Broccoli	<i>Brassica oleracea</i>
Kale	<i>Brassica oleracea</i> var. <i>botrytis</i>
Brussels sprouts	<i>Brassica oleracea</i> var. <i>gemmifera</i>
Endive	<i>Cichorium intybus</i>
Spinach	<i>Spinacea oleracea</i>
Fennel	<i>Foeniculum vulgare</i>
Leek	<i>Allium porrum</i>
Lettuce	<i>Lactuca sativa</i>
Parsley	<i>Petroselinum crispum, sativum, hortense</i>
Red cabbage	<i>Brassica oleracea capitata rubra</i>
Salad greens	<i>Valerianella olitoria</i>
Turnip	<i>Brassica rapa</i> var. <i>perviridis</i>
Carrot	<i>Daucus carota</i>
Celery	<i>Apium graveolens</i>
Onion	<i>Allium cepa</i>
Parsnip	<i>Pastinaca sativa</i>
Potato	<i>Solanum tuberosum</i>
Radish, red	<i>Raphanus sativus</i>
Sweet potato	<i>Ipomoea batatas</i>

BOTANICAL NAMES OF HERBS AND SPICES

Common Name	Botanical Name
Basil, sweet	<i>Ocimum basilicum</i>
Caper	<i>Capparis ovata, spinosa</i>
Caraway	<i>Carum carvi</i>
Chamomile	<i>Anthemis nobilis</i>
Chervil	<i>Torilis tenella, nodosa</i>
Coriander	<i>Coriandrum sativum</i>
Dill	<i>Anethum graveolens</i>
Hops	<i>Humulus lupulus</i>
Lemon balm	<i>Melissa officinalis</i>
Marjoram	<i>Majorana hortensis</i>
Mint	<i>Mentha var.</i>
Mustard	<i>Sinapis alba, nigra</i>
Oregano	<i>Origanum vulgare</i>
Parsley	<i>Petroselinum crispum</i>
Peppermint	<i>Metha spicata, piperita</i>
Rosemary	<i>Rosmarinus officinalis</i>
Sage	<i>Salvia officinalis</i>
Tarragon	<i>Artemisia dranunculus</i>
Thyme	<i>Thymus vulgaris, collinus, nummularis</i>
Watercress	<i>Nasturtium officinale</i>

BOTANICAL NAMES OF LEGUMES

Common Name	Botanical Name
Broad bean	<i>Vicia faba</i>
Chick pea	<i>Cicer arietinum</i>
French bean	<i>Vicia faba</i>
Kidney bean	<i>Phaseolus vulgaris, lunatus, radiatus</i>
Lima bean	<i>Phaseolus vulgaris, lunatus, radiatus</i>
Mung bean	<i>Phaseolus vulgaris, lunatus, radiatus</i>
Pea	<i>Pisum sativum</i>
Sesame	<i>Sesamum indicum</i>
Soyabean	<i>Glycine max</i>

APPENDIX 2

REFERENCES USED FOR DATABASE COMPILATION

TABLE 4.9
Flavonoid Content of Fruits

	Flavonols	Flavones	Procyanidins	Catechins	Flavanones
Apple					
Eating	32, 29		69	67, 69, 86	29
Peeled	32			67	
Sauce	32			67	
Apricots	32, 29		69	67, 69	
Avocado			69	67, 69	
Banana			69	67, 69	
Bilberry	65, 81, 150		69	67, 69	
Blackberries	101		69	67, 69	
Blackcurrants	29, 81, 150, 151		67	67	
Blueberries	29, 81, 101, 150		67, 69	67, 69	
Cherries	29, 32		67, 69	67, 69	
Cranberries	29, 32, 148, 150		67, 69	67, 69	
Custard apple			69	69	
Elderberries	150		69	69	
Early fig			67	67	
Gooseberry	150		67	67	
Grapefruit	29				29
Grapes					
Black	29, 32		67, 69	67, 69	
Green	29, 32		67, 69	67, 69	
Kiwi fruit			67, 69	67, 69	
Lemon	29, 148	29, 148, 152			29, 148
Lime	29				29
Lingonberry	81, 148, 150				
Mango				67	
Nectarine				67	
Olives	91, 153, 154	91, 153, 154			
Orange	29, 148				29, 148
Peaches	32, 60		67, 69	67, 69	
Pears	29, 32		67, 69	67, 69, 155	
Peeled	32		32	32	
Persimmon			67, 69	67, 69	
Plums, blue	29, 32		67, 69	67, 69	
Pomegranate			69	69	
Raisins	118			67	
Raspberries	29, 81, 150		67, 69	67, 69	
Red currants	29, 32		67, 69	67, 69	
Rhubarb			67	67	
Strawberry	32, 65, 81, 150		67, 69	67, 69	

TABLE 4.10
Flavonoid Content of Vegetables

	Flavonols	Flavones	Procyanidins	Catechins	Flavanones
Asparagus	156				
Asparagus, boiled	156				
Broad beans	32		67, 69	67, 69	
Broad beans, boiled	32		67	67	
Broad beans, canned	32				
Broccoli	29, 32, 63, 148, 157				
Broccoli, boiled	157				
Brussels sprouts	29, 32				
Celery stalks	32, 80				
Chilli pepper, green	97, 103				
Chilli pepper, red	97, 103				
Chives	89				
Cress	158				
Endive	32, 105			67, 69	
Green beans	27, 29, 88, 120			67, 69	
Green beans, boiled	120				
Green beans, processed	27, 32			67	
Kale	29, 32, 89				
Kale, processed	32				
Kidney beans, canned				67	
Leeks	29, 32, 89				
Leeks, boiled					
Lettuce	32, 80, 89, 105				
Lettuce, red	32, 105				
Onions, red	29, 54, 79, 148, 159, 160				
Onions, white	79, 160				
Onions, yellow	25, 29, 32, 54, 79, 80, 120, 148, 159, 160				
Fried	80				
Microwaved	80				
Boiled	54, 80				
Red cabbage	32, 89				
Red radish	89				
Shallots	79				
Spring onions	29				
Sweet pepper, green	29, 32	29, 32			
Sweet pepper, red		29, 32			
Sweet pepper, yellow	29, 32, 103	29, 32, 103			
Tomatoes	29, 32, 80, 161, 162				29, 161
Tomatoes, beef	80, 162				
Tomatoes, cherry	80, 162				
Tomatoes, cherry, canned	162				
Tomatoes, plum, canned	162				
Tomatoes, yellow	162				
White cabbage	32				
Watercress	158				
Yellow beans	27				

TABLE 4.11
Flavonoid Content of Beverages

	Flavonols	Flavones	Procyanidins	Catechins	Flavanones
Apple juice	109, 149, 163		109	67, 109, 149	29
Black currant juice fresh	81				
Cranberry juice, canned	117				
Cranberry juice, fresh	117				
Grape juice, black	163			67	
Grape juice, white				67	
Grapefruit juice	163				96, 164, 165
Lemon juice	163, 166				165, 166
Lime juice					165
Orange juice (diluting)	163				164, 169
Orange juice (fresh)	29, 163				29, 168, 169, 170
Pear juice	110		110	110	
Tomato juice	162, 163, 167				167
Tea, black	29, 148, 163, 171, 172		67	67, 69, 148	
Tea, decaffeinated	172				
Tea, Earl Grey	163			67	
Tea, green	148, 163		67	67, 148, 173	
Tea, forest fruit				67	
Tea, freeze dried	172				
Tea, Oolong	163			148	
Coffee	148		67, 69, 174	67, 69, 148	
Cacao drink			69	69	
Chocolate milk	163		67	67	
Beer	163, 166		69	67, 69, 166	
Cider			69	69	
Sherry			175	175, 176	
Wine, red	29, 77, 78, 148, 166, 177, 179		69, 180	67, 69, 148, 166, 178, 179, 181	
Wine, rose			69	69	
Wine, white	163, 166, 177, 178		69	67, 69, 166	166

TABLE 4.12
Miscellaneous Foods

	Flavonols	Flavones	Procyanidins	Catechins	Flavanones
<i>Jam</i>					
Apricot	182		67, 69	67, 69	
Cherry			67, 69	67, 69	
Forest fruit				67	
Honey	59, 60, 90, 183	60, 183			60
Peach	182			67, 69	
Plum	182		69	69	
Raspberry	184		69	69	
Strawberry	65, 182		67, 69	67, 69	
<i>Confectionary</i>					
Chocolate, dark			67, 69, 185	67, 69, 185	
Chocolate, fancy and filled			67	67	
Chocolate, milk			67, 69, 185	67, 69, 185	
<i>Herbs</i>					
Basil, sweet		186			
Coriander	187				
Dill	187				
Lemon balm		187			
Lovage	187				
Marjorum		186			
Mint	187	187			
Oregano		186			
Parsley	29, 148, 187	29, 148, 187			
Rosemary		186, 187			186
Sage		186			
Tarrogon	187	187			
Thyme	187	187			

5 Flavonoids in Wine

Véronique Cheynier

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5.1 INTRODUCTION

Flavonoids (*sensu largo*, i.e., including flavanoids) are important components of grapes and essential to wine quality. They are responsible for the color and astringency of red wines as well as for the yellow hue of oxidized white wines, and are also involved in the development of

haze and precipitates, and other technological problems (e.g., clogging of filtration membranes, adsorption on tank surface). They have nutritional and pharmacological properties, as discussed in detail in Chapter 6, and may play a part in the health benefits attributed to moderate wine consumption.

Grape flavonoid composition has been extensively studied since the pioneering work of Ribéreau-Gayon.^{1,2} It consists primarily of anthocyanins (in red varieties) and flavanols, along with smaller amounts of flavonols and dihydroflavonols. The main representatives of each of these classes are well known but a few additional minor compounds have been formally identified in recent papers. However, major advances in the last decade concern structural determination of grape proanthocyanidins. Such progress is largely due to the development of new analytical methods, including in particular coupling of high-performance liquid chromatography with mass spectrometric detection (HPLC–MS).

Wine composition depends not only on the type of grape used as raw material, which is influenced by varietal and agricultural factors, but also on the wine-making process, which determines extraction of flavonoids into the liquid phase and their subsequent reactions. The reactions of anthocyanins and proanthocyanidins play a major role in organoleptic changes taking place during wine aging. Conversion of grape anthocyanins to other pigments and its role in color changes, from the purple nuance of young wines toward the red-brown tint of matured wine, was described by Somers in 1971.³ Also, the decrease of astringency has long been ascribed to reactions of proanthocyanidins, based on their characteristic C–C bond-breaking and bond-making processes and on oxidation mechanisms.⁴ However, some of the usually acknowledged reaction products, namely direct anthocyanin–flavanol adducts³ and adducts in which the anthocyanin and flavanol moieties are linked through a methylmethine bridge, often called ethyl bridge,⁵ have only recently been demonstrated to occur in wine,^{6,7} whereas others such as the xanthylium ions arising from anthocyanin and flavanol reactions³ have not yet been confirmed. In addition, several so far unsuspected mechanisms have been unraveled in the last few years and the resulting products have been identified.^{6,8–15}

The determination of organoleptic properties of grape and wine flavonoids is another extremely active research area. The color properties of new wine pigments have been studied and compared to those of their anthocyanin precursors.^{16–19} The influence of proanthocyanidin structures on their taste has also been investigated. It was thus demonstrated that higher molecular weight proanthocyanidins are both water soluble and highly astringent,²⁰ ruling out earlier assumptions.²¹

This chapter will provide a detailed account of the newly acquired data on flavonoid composition and distribution in grapes, and on flavonoid reactions in wine and structures of the resulting products. It will first present recent advances in analytical procedures that have rendered such progress possible and, for many of them, have been developed on grape extracts before being more widely applied to other plant material. Finally, it will review briefly current knowledge on the properties associated with flavonoids and their derivatives in wine.

5.2 ANALYTICAL PROCEDURES

5.2.1 EXTRACTION AND FRACTIONATION OF FLAVONOIDS FROM GRAPE AND WINE

Extraction of flavonoids from grapes is classically carried out with organic solvents, starting from fresh, frozen, or freeze-dried material. The most commonly used solvents are methanol, ethanol, and acetone, which can be used pure or mixed with water. Extraction of anthocyanins is commonly achieved at low temperatures with acidified methanol. The use of acid maintains the anthocyanins in the most stable flavylium forms but may cause degradation of

acylated anthocyanins. The use of 1% HCl in methanol may induce partial hydrolysis of acylated grape anthocyanins²² whereas that of 0.1% HCl in methanol causes no degradation.²³ In our experience, 60% acetone in water was as efficient as acidified methanol for the extraction of anthocyanins from grape skins, as reported earlier for strawberry anthocyanins.²⁴ Proanthocyanidins and especially larger molecular weight polymers are also extracted from grape seeds,²⁵ skins,²⁶ and stems²⁷ with aqueous acetone (60 to 70%). Selective extraction of oligomers can be performed by using ethyl acetate–water (90:10, v/v)²⁸ but higher yields are obtained with methanol–water (60:40, v/v).²⁹

Fractionation of flavonoids from grape extracts or wines can be achieved by liquid–liquid extraction or solid-phase extraction (SPE) procedures. Liquid–liquid extraction using water and diethyl ether enables the recovery of flavanol monomers and flavanol aglycones in the organic phase, whereas anthocyanins, flavanol glycosides, and proanthocyanidins remain in the aqueous phase. Ethyl acetate extracts flavanol monomers and oligomers along with flavonols. SPE on tC18 Sep-Pak cartridges permits similar separations with significant reduction of solvent volumes and of fractionation time.³⁰ Successive elutions with diethyl ether, ethyl acetate, and methanol allow the separation of flavanol monomers and oligomers that are recovered, respectively, in diethyl ether and ethyl acetate, whereas proanthocyanidin polymers and anthocyanins are eluted together with methanol. However, the application of this procedure to wine flavonoids may lead to contamination of the ethyl acetate phase with *p*-coumaroylated anthocyanins and derived pigments present in red wines.³¹ The latter have been separated from genuine anthocyanins by extraction with isoamyl alcohol.³⁷

Liquid–liquid extraction has been developed further in counter current chromatography (CCC) procedures. CCC has been first used to separate flavanol monomers, dimers, oligomers (i.e., trimers and tetramers), and polymers from white wines using the partition between water and ethyl acetate.³² Recent refinements of the technique are based on the introduction of a centrifugal force in different types of equipment, namely multilayer coil counter current chromatography (MLCCC), high-speed counter current chromatography, and centrifugal partition chromatography. CCC facilitates the separation of various types of pigments from red wine.³³ The technique has been successfully upgraded for the isolation of anthocyanins at the preparative scale.³⁴ Its association with gradient elution using increasing percentage of acetonitrile in the organic phase much improved its resolution.³⁵

Fractionation of grape seed flavanols on a molecular weight basis has been achieved by low-pressure chromatography on Sephadex LH-20³⁶ and Fractogel (*syn* Toyopearl) TSK HW-40 (F)^{37,38} or TSK HW-50 (F),²⁶ using methanol to elute monomers and oligomers, up to the tetramers, and acetone–water (70:30, v/v) to recover larger molecular weight procyanidins with no further separation. Elution of procyanidins in increasing molecular weight order indicates that separation of these molecules on Sephadex and Toyopearl phases actually relies primarily upon adsorption rather than exclusion processes. Gel permeation chromatography protocols using dimethylformamide–3 *M* aqueous ammonium formate (95.5:0.5, v/v) solvent on a polystyrene–divinylbenzene column³⁹ and acetone–8 *M* urea (60:40, v/v) adjusted to pH 2 on a Toyopearl column⁴⁰ have been proposed but the resolution was very poor when tested on procyanidins from grape seeds. The application of the latter method to red wine allowed the recovery of early eluting pigments that were claimed to be tannin-derived pigments.⁴¹

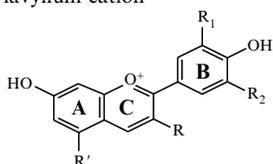
Flavanol monomers and oligomers from grape seeds^{25,42} and skins²⁶ have also been separated on a molecular weight basis using normal-phase HPLC methods adapted from a thin layer chromatography (TLC) procedure proposed for apple procyanidins.⁴³ However, no relationship between the chain length and the retention time could be established when comparing both extracts, due to differences in proanthocyanidin structures.⁴⁴ In addition, the presence of other ultraviolet (UV) absorbing material (e.g., anthocyanins, which elute

much later than procyanidin dimers under these chromatographic conditions) may lead to misinterpretation of the molecular weight distribution. Finally, proanthocyanidin fractions with different degrees of polymerization were obtained from grape seed and skin extracts by precipitation with chloroform–methanol (75:25, v/v) and gradual dissolution with increasing amounts of methanol in the solvent, but they showed important overlapping.^{45,46}

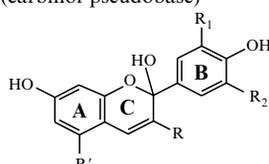
5.2.2 ANALYSIS OF LOWER MOLECULAR WEIGHT FLAVONOIDS

Flavonoids from grape extracts and wines are usually analyzed by reverse-phase HPLC coupled with diode array detection (DAD), enabling the distinction of the various classes of flavonoid compounds on the basis of their characteristic UV–visible spectrum.⁴⁷ For instance, grape anthocyanins (structures given in Figure 5.1) present absorption maxima in the range 510 to 528 nm as well as in the UV region (around 280 nm). Anthocyanins having two (cyanidin and peonidin; Figure 5.1, R₂ = H) or three (delphinidin, petunidin, malvidin; Figure 5.1, R₂ = OH, OCH₃) substituents on the B-ring can be distinguished as the former have absorption maxima about 10 nm lower than the latter in the aglycone (R = R' = OH),

Flavylium cation



Hemiketal (carbinol pseudobase)



	R ₁	R ₂	R'	R
Cyanidin 3-glucoside	OH	H	OH	<i>O</i> -Glucose
Petunidin 3-glucoside	OCH ₃	OH	OH	<i>O</i> -Glucose
Delphinidin 3-glucoside	OH	OH	OH	<i>O</i> -Glucose
Peonidin 3-glucoside	OCH ₃	H	OH	<i>O</i> -Glucose
Malvidin 3-glucoside	OCH ₃	OCH ₃	OH	<i>O</i> -Glucose
Cyanidin 3-acetylglucoside	OH	H	OH	<i>O</i> -Acetylglucose
Petunidin 3-acetylglucoside	OCH ₃	OH	OH	<i>O</i> -Acetylglucose
Delphinidin 3-acetylglucoside	OH	OH	OH	<i>O</i> -Acetylglucose
Peonidin 3-acetylglucoside	OCH ₃	H	OH	<i>O</i> -Acetylglucose
Malvidin 3-acetylglucoside	OCH ₃	OCH ₃	OH	<i>O</i> -Acetylglucose
Cyanidin 3- <i>p</i> -coumaroylglucoside	OH	H	OH	<i>O-p</i> -Coumaroylglucose
Petunidin 3- <i>p</i> -coumaroylglucoside	OCH ₃	OH	OH	<i>O-p</i> -Coumaroylglucose
Delphinidin 3- <i>p</i> -coumaroylglucoside	OH	OH	OH	<i>O-p</i> -Coumaroylglucose
Peonidin 3- <i>p</i> -coumaroylglucoside	OCH ₃	H	OH	<i>O-p</i> -Coumaroylglucose
Malvidin 3- <i>p</i> -coumaroylglucoside	OCH ₃	OCH ₃	OH	<i>O-p</i> -Coumaroylglucose
Peonidin 3- <i>p</i> -caffeoylglucoside	OCH ₃	H	OH	<i>O-p</i> -Caffeoylglucose
Malvidin 3- <i>p</i> -caffeoylglucoside	OCH ₃	OCH ₃	OH	<i>O-p</i> -Caffeoylglucose
Cyanidin 3,5-diglucoside	OH	H	<i>O</i> -Glucose	<i>O</i> -Glucose
Petunidin 3,5-diglucoside	OCH ₃	OH	<i>O</i> -Glucose	<i>O</i> -Glucose
Delphinidin 3,5-diglucoside	OH	OH	<i>O</i> -Glucose	<i>O</i> -Glucose
Peonidin 3,5-diglucoside	OCH ₃	H	<i>O</i> -Glucose	<i>O</i> -Glucose
Malvidin 3,5-diglucoside	OCH ₃	OCH ₃	<i>O</i> -Glucose	<i>O</i> -Glucose
Petunidin 3- <i>p</i> -coumaroylglucoside, 5-glucoside	OCH ₃	OH	<i>O</i> -Glucose	<i>O-p</i> -Coumaroylglucose
Cyanidin 3- <i>p</i> -coumaroylglucoside, 5-glucoside	OH	H	<i>O</i> -Glucose	<i>O-p</i> -Coumaroylglucose
Delphinidin 3- <i>p</i> -coumaroylglucoside, 5-glucoside	OH	OH	<i>O</i> -Glucose	<i>O-p</i> -Coumaroylglucose
Peonidin 3- <i>p</i> -coumaroylglucoside, 5-glucoside	OCH ₃	H	<i>O</i> -Glucose	<i>O-p</i> -Coumaroylglucose
Malvidin 3- <i>p</i> -coumaroylglucoside, 5-glucoside	OCH ₃	OCH ₃	<i>O</i> -Glucose	<i>O-p</i> -Coumaroylglucose

FIGURE 5.1 Structures of grape anthocyanins.

3-monoglucoside ($R = O\text{-glucose}$, $R' = OH$), and 3,5-diglucoside ($R = R' = O\text{-glucose}$) series. Glycosylation induces a hypsochromic shift of the absorption maximum while acylation with hydroxycinnamic acids is associated with a characteristic shoulder around 310 nm for *p*-coumaric acid ($R = O\text{-}p\text{-coumaroylglucose}$) and 325 nm for caffeic acid ($R = O\text{-caffeoylglucose}$). Coupling of HPLC with MS equipped with mild ionization techniques such as electrospray ionization (ESI-MS) or atmospheric pressure chemical ionization (APCI-MS) has become increasingly popular, as discussed below. Coupling of liquid chromatography with nuclear magnetic resonance (LC-NMR) certainly appears promising,⁴⁸ but has not yet to our knowledge been applied to grape or wine flavonoids.

5.2.2.1 HPLC Coupled to Mass Spectrometry

The first application of HPLC-ESI-MS to grape polyphenols was published in 1995.⁴⁹ Operation in the positive ion mode made it possible to detect 17 anthocyanins (namely the 3-glucoside, 3-acetylglucoside, and 3-*p*-coumaroylglucoside of cyanidin, delphinidin, petunidin, peonidin, and malvidin and the 3-caffeoylglucosides of the last two anthocyanidins) in a grape extract and characterize them through their typical fragmentation patterns obtained after increasing the orifice voltage, corresponding to the loss of sugar or acylated sugar residues. The reported mass signals were improperly interpreted as $[M + H]^+$ ions that could then arise from the anthocyanin quinonoidal bases but in fact corresponded to the flavylum cations (M^+) that predominate under the acidic conditions used in HPLC elution (7% HCOOH).

A year later, the first application of HPLC-ESI-MS analysis to a red wine allowed the detection of two new pigments at $m/z = 609$ and $m/z = 755$.⁵⁰ Their fragmentation patterns yielded the same aglycone (at $m/z = 447$) after the loss of glucose (-162) and *p*-coumaroylglucose (-308) moieties, respectively, showing that they were, respectively, the glucoside and *p*-coumaroylglucoside of the same anthocyanidin cation. Further studies (see Section 5.5.3.3) showed that they were phenylpyranomalvidin 3-glucoside and its *p*-coumaroylated derivative,⁸ confirming this hypothesis. Since then, a number of other anthocyanin-derived pigments have then been similarly detected and characterized in wine (see Section 5.5)^{6,7,14,15,51-53} or wine-like model systems.^{8,10,13,54-58} Performances of APCI and ESI sources operated in the negative and positive ion modes were compared on a series of standard compounds.⁵¹ The best detection of anthocyanins, as their cationic forms, was achieved with ESI in the positive ion mode, in acidified medium (10% formic acid).

The negative ion mode is usually preferred for uncharged flavonoids, including flavanols,^{27,59-61} flavonols,⁶² dihydroflavonols,²⁷ and flavanol reaction products,^{9,11,63,64} which are then detected as the deprotonated $[M - H]^-$ species. It also proved more sensitive for the detection of some new anthocyanin-derived pigments bearing a carboxylic group¹⁰ and for anthocyanin-flavanol adducts in which the anthocyanin moiety is not in the cationic form.⁷

Tandem mass spectrometry (MS-MS) and ion trap mass spectrometry (IT-MS, MSⁿ), which permit fragmentation patterns to be obtained on selected individual ions, are progressively replacing the classical ESI-quadrupole mass spectrometers in HPLC-MS coupling. MS-MS and MSⁿ have been successfully applied to identify anthocyanins in the juice of concord grape (*Vitis labrusca*),⁶⁵ and anthocyanins and flavanol glycosides in table grapes.⁶² Fragmentation patterns also provided insight on the sequences of flavanol units in proanthocyanidin oligomers⁶⁶ and of anthocyanin and flavanol units in flavanol-anthocyanin adducts.⁵⁸

Classical fragmentation patterns of flavonoid glycosides, based on the consecutive losses of sugar and acylglycoside residues, enable the determination of the nature of the aglycone and the mass and sequence of its substituents. Fragmentation patterns obtained from

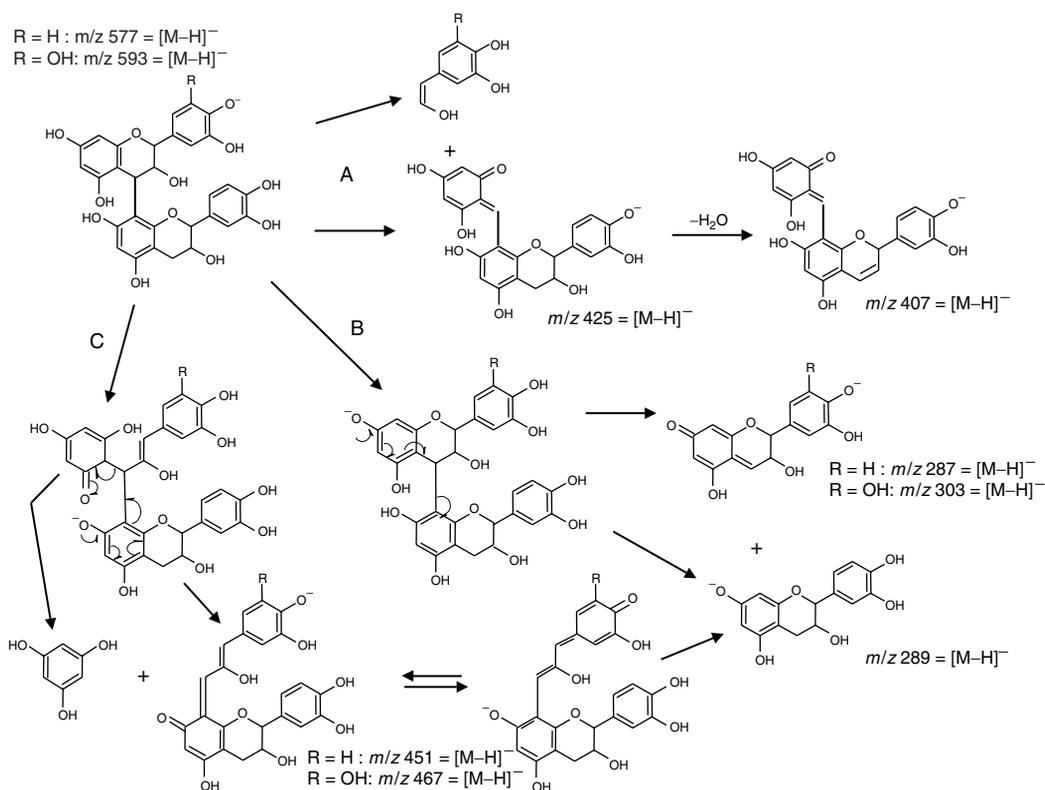


FIGURE 5.2 Mass fragmentation patterns of flavanol oligomers.

proanthocyanidins in the ESI-quadrupole apparatus are the same as in fast atom bombardment mass spectrometry (FAB-MS) operated in the negative ion mode, with one ion peak corresponding to the retro-Diels–Alder (RDA) fission (Figure 5.2, A) and two fragments generated by breakage of the interflavanic linkage, following the quinone methide process (Figure 5.2, B).⁶⁷ Fragmentation experiments performed with an ion trap mass analyzer generate additional fragments corresponding to the loss of a phloroglucinol moiety ($C_6H_6O_3$) from the upper unit (-126 a.m.u.) (Figure 5.2, C).⁶⁸

Analysis of dimeric proanthocyanidins with constitutive units showing different molecular weights demonstrates that RDA fission takes place specifically on the upper unit (substituted only in C4) and thus can be used to determine the sequence.^{66–68} Cleavage of the interflavanic linkage also yields different ion species, namely the quinone methide, detected as $[M - 3H]^-$ from the upper unit, and the pseudo-molecular ion $[M - H]^-$ from the lower unit, allowing distinction between both units as illustrated in Figure 5.3. Other useful fragmentations for proanthocyanidin characterization include the loss of galloyl moieties (-152 a.m.u.) in galloylated proanthocyanidins and that of the benzylthioether substituent (-124 a.m.u.) in derivatives obtained after thiolysis (see Section 5.2.2.2).

5.2.2.2 Analysis of Flavonoid Constitutive Moieties

However, MS does not allow to distinguish between isomers such as glucose and galactose in flavonoid glycosides, or (–)-epicatechin and (+)-catechin units in procyanidins. Sugars in glycosides can be identified by using specific glycosidases (e.g., β -glucosidase, α -rhamnosi-

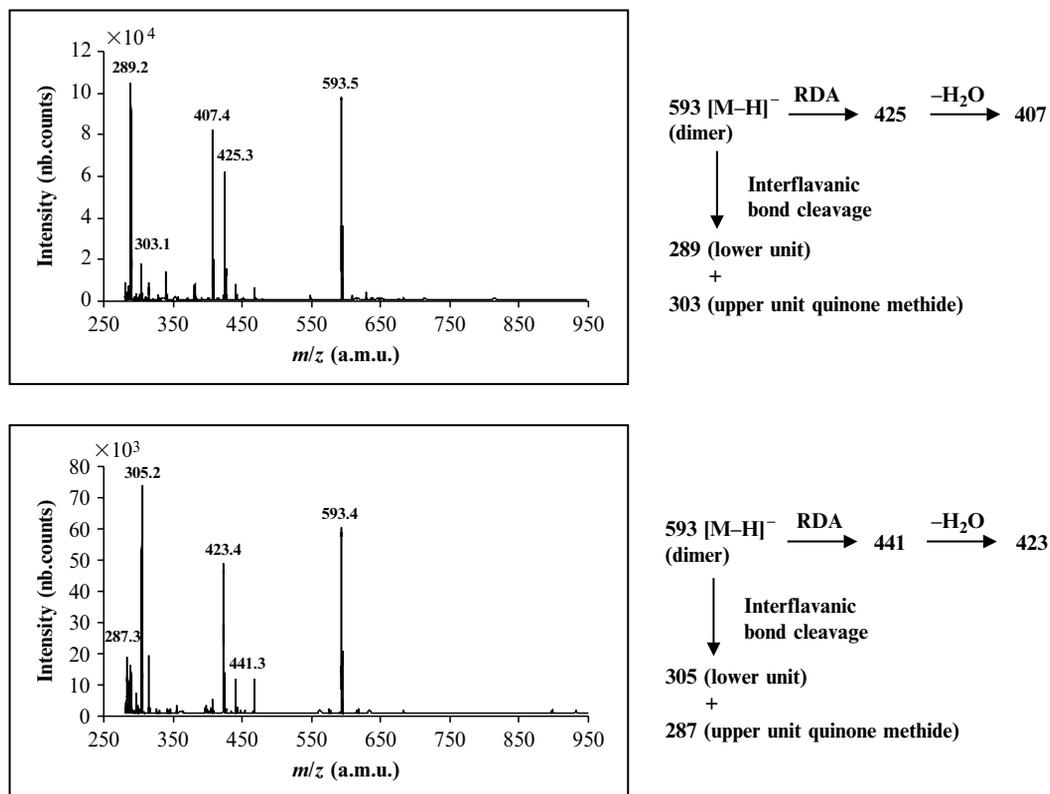


FIGURE 5.3 ESI-Q mass spectra obtained from two proanthocyanidin dimers.

dase) or by analyzing the sugar moiety released after acid-catalyzed cleavage of the glycosidic linkage. This has been achieved by comparison with reference compounds in TLC and more recently by gas chromatography (GC) analysis of the alditol acetate derivatives.⁵⁰ Further information on the linkage position on the sugar moiety was obtained by methylation of the sugar-free hydroxyl groups followed by acid hydrolysis, conversion of the partly methylated sugars to alditol acetates, and GC-MS analysis. Identification of *p*-coumaric acid in the *p*-coumaroylated anthocyanin derivative was carried out by alkaline hydrolysis followed by HPLC analysis of the released compounds.⁵⁰

Proanthocyanidin constitutive units can be determined by acid-catalyzed cleavage in the presence of a nucleophilic agent. Commonly used nucleophiles include benzylhydrosulfide (*syn* phenylmethanethiol, toluene- α -thiol), the method then referred to as thiolysis,⁶⁹ and 1,3,5-trihydroxybenzene (*syn*. phloroglucinol).^{70,71} Rupture of the interflavanoid bond in acidified methanol yields a carbocation from the upper and extension units of the molecule (initially substituted in C4) whereas the lower part (nonsubstituted in C4) is released as such. The carbocation then reacts with the nucleophiles to give a stable adduct. HPLC analysis of the reaction mixture allows the separation of lower units from derivatized units and, within each group, the distinction between isomers on the basis of their retention times.^{38,72,73} However, some epimerization, especially from (–)-epicatechin (2,3 *cis*) to its *trans* isomer, may take place when the reaction is carried out at high temperature. In addition, no information is available on the linkage position in dimeric species. Nevertheless, starting from the trimers, cleavage under mild conditions followed by HPLC analysis gives access to

constitutive dimers and thus to complete identification, provided that the linkage position in the released dimeric fragments can be unambiguously established.⁷³ Acylation with gallic acid is maintained under the mild acidic conditions used in thiolysis and phloroglucinolysis so that galloylated units present either in upper or in terminal positions can be determined. Tannase, i.e., galloyl acyl hydrolase, which cleaves the ester bond in galloylated flavanols, can also be used for identification purposes.^{38,74}

5.2.2.3 Nuclear Magnetic Resonance

Formal identification of proanthocyanidin dimers, including determination of the linkage position, can best be achieved by using two-dimensional NMR techniques.⁷⁵ Proton–proton and proton–carbon correlations enable to distinguish between the different flavanol moieties and to establish their sequence. In particular, attribution of the residual proton of the lower unit A-ring (H6 or H8) is based on long distance carbon–proton correlations established by heteronuclear multiple bond correlation (HMBC) experiments. The principle is as follows: the residual proton correlates with two carbons around 150 ppm, which are C5 and C7 in the case of H6 and C7 and C8a in the case of H8. C8a can be attributed and distinguished from C5 through its correlation with the H2 proton through the oxygen atom of the heterocyclic ring, as illustrated in Figure 5.4. It should, however, be pointed out that H2–H3 coupling constants cannot be relied upon to distinguish between 2–3 *cis* and 2–3 *trans* units within an oligomer.⁷⁶ In this case, less sophisticated methods such as thiolysis may prove complementary to NMR to unequivocally establish structures.

The same two-dimensional NMR approach has enabled the identification of tannin-like derivatives.^{8–10,77–80} Attribution of C8a, as described above, is not possible in the case of anthocyanins, due to the lack of H2 proton. Nuclear Overhauser effect spectroscopy experiments have been used as an alternative strategy to overcome this problem. For instance, observation of through space couplings between B-ring protons (H2', H6', OCH₃) and the protons of the methylenethine bond in a methylenethine-linked anthocyanin dimer is only possible in the case of a C8 linkage and thus allows one to rule out the C6-linkage position.¹³

5.2.3 ANALYSIS OF HIGHER MOLECULAR WEIGHT FLAVONOIDS

HPLC separation, as described above, is restricted to rather simple compounds that represent only a small proportion of flavonoids. Indeed, proanthocyanidin analysis becomes increasingly difficult as their molecular weight increases, due to the larger number of possible structures, smaller amounts of each individual compound, and poorer resolution of the chromatographic profiles. This is especially true of grape proanthocyanidins, which, unlike those of apple or cacao consisting only of epicatechin units, are based on four major

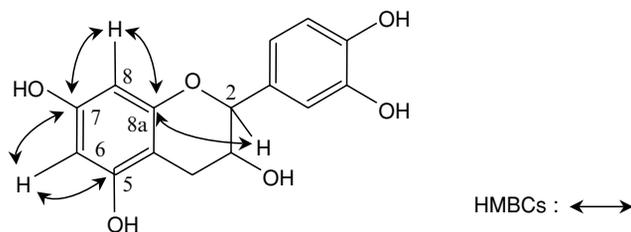


FIGURE 5.4 NMR HMBCs used to attribute flavanol H6 and H8 protons.

constitutive units and are thus extremely heterogeneous. Also, reactions of grape flavonoids in wine yield a large diversity of compounds that appear as an unresolved hump in the chromatographic profiles. To overcome this problem, analyses have been performed directly on grape or wine extracts, without prior HPLC separation.

5.2.3.1 Acid-Catalyzed Cleavage in the Presence of Nucleophilic Agents

The average composition of proanthocyanidin extracts can be determined by HPLC after acid-catalyzed cleavage in the presence of a nucleophilic agent, as described above. The most commonly used nucleophiles are benzylhydrosulfide^{7,25,26,81} and phloroglucinol,^{82,83} but cysteamine has recently been proposed.⁸⁴ Provided each unit can be individually quantified, after isolation and calibration, such methods allow one to determine proanthocyanidin content in a given tannin fraction, by summing the concentrations of all released units, to calculate their mean DP ($mDP = ([\text{upper and extension units}]_M + [\text{end units}]_M) / [\text{end units}]_M$) and give insight to the nature and average proportions of their constitutive units, including galloylated units.

Thiolysis also proved useful for the analysis of derived tannins. Methylmethine-linked tannin-like compounds resulting from acetaldehyde-mediated condensation of flavanols (see Section 5.5.3.2) yielded several adducts when submitted to acid-catalyzed cleavage in the presence of ethanethiol, providing information on the position of linkages in the original ethyl-linked compounds.^{64,85} Thiolysis of red wine extracts released benzylthioether derivatives of several anthocyanin–flavanol adducts,^{7,52} indicating that such structures were initially linked to proanthocyanidins. However, some of the flavonoid derivatives present in wine (e.g., flavanol–anthocyanins⁸⁶) are resistant to thiolysis, while others (e.g., flavanol–ethyl anthocyanins) were only partly cleaved.⁸⁷ Thiolysis, thus, appears as a rather simple, sensitive, and powerful tool for quantification and characterization of proanthocyanidins, but provides mostly qualitative data for their reaction products.

5.2.3.2 Mass Spectrometry

Mass spectrometry methods based on soft ionization techniques, ESI^{6,59,61,88,89} and matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF),^{90,91} have been successfully applied for the direct analysis of grape and wine extracts and for monitoring flavonoid reactions in model solution studies.^{13,59,63} They give access to the molecular weights of the different species present in a fraction or extract and, through fragmentation patterns, provide important information on their constitutive units. Description of the various MS techniques can be found in Chapters 1 and 2.

Analysis of a grape seed procyanidin extract by direct ESI-MS in the negative ion mode⁶ enabled to detect signals corresponding to not only $[M - H]^-$ ions of procyanidin oligomers (up to the pentamer) but also to doubly charged ($[M - 2H]^{2-}$) ions for higher molecular weight species (from DP5 to DP9). Arguments to distinguish between monocharged and doubly charged signals rely on the distance between the isotopic peaks due to the natural occurrence of carbon 13 (1%), as the spacing between the isotopic peaks is equal to one mass unit for monocharged ions and 0.5 mass units for doubly charged ions. The interpretation with higher charge states or several charge states in a single mass peak is limited by the resolution of the mass spectrometer. Monocharged signals of DP n species overlap with doubly charged signals of DP2 n species but those corresponding to doubly charged ions of species with uneven DP can be unambiguously attributed. ESI-MS analysis of a grape seed extract thus allowed detecting a complete series of oligomers, from DP2 to DP9, as well as the corresponding monogalloylated and digalloylated species. Analysis of grape seed tannins by

ESI-MS in the positive ion mode enabled to detect $[M + H]^+$ ions up to the galloylated pentamer.⁶¹ Analysis of wine proanthocyanidins showed the presence of additional series of species with 16 mass unit differences to signals given by grape seed procyanidins. These were attributed to the presence of (epi)galocatechin units in mixed procyanidin–prodelphinidin oligomers.⁵⁹

The analysis of proanthocyanidins has also been achieved by MALDI-TOF MS. This technique has a higher mass range than other MS techniques and produces only single ionization, unlike ESI-MS,⁹¹ but it requires incorporation of the sample in a matrix. Only *trans*-3-indolacetic acid (IAA) and 2,5 dihydroxybenzoic acid (DHB) have proven to be suitable matrices for proanthocyanidin analysis. It has been postulated that the intensity of each peak signal in the mass spectrum can be used as a measure of relative abundance but no calibration has been performed, because of the lack of available standards, to confirm this hypothesis. In addition, the charge density decreases as the molecular weight increases so that larger molecular weight tannins are more difficult to detect.⁹¹

ESI-MS was also applied to investigate acetaldehyde-induced condensation reactions in wine-like model solutions, enabling one to detect ethyl-linked catechin oligomers up to the hexamer as the monocharged $[M - H]^-$ ions^{60,92} and signals corresponding to dimeric, trimeric, and tetrameric species containing one anthocyanin and one, two, or three flavanol units as the M^+ flavylum ions.^{6,60,93} A tetrameric species containing two malvidin 3-glucoside units and two flavanol units was also found as the doubly charged M^{2+} ion at $m/z = 822$ ions.^{6,60} Similarly, ESI-MS analysis in the positive ion mode of a polymeric fraction arising from reaction of malvidin 3-glucoside with acetaldehyde allowed the detection of singly charged and doubly charged ions corresponding to methylmethine-linked malvidin 3-glucoside trimers and tetramers in which the constitutive anthocyanin moieties were present as different forms (i.e., flavylum ions, quinonoidal bases, hemiketal).¹³

Two ESI-MS studies aiming at profiling anthocyanin pigments in grape and wine extracts have been published.^{88,94} The first one used ESI-MSⁿ spectra in the positive ion mode to identify anthocyanins in grape extracts. MSⁿ was effective for differentiating most isobaric compounds but it did not allow one to distinguish between malvidin 3,5-diglucoside and malvidin 3-caffeoylglucoside, as they are based on the same aglycone. This was achieved by deuterium exchange experiments, leading to different mass shifts in agreement with the respective numbers of exchangeable protons in the molecules.⁹⁴ The second study used nano-electrospray tandem mass spectrometry (nano-ESI-MS-MS) to screen for potential anthocyanin-derived pigments in red wine polyphenol extracts purified by SPE. Replacement of conventional electrospray by nano-electrospray gives access to greater sensitivity while allowing one to work with smaller sample size. Neutral loss scanning for specific elimination masses was used for detection of particular derivatives (e.g., 162 for glucosyl derivatives).⁸⁸ Characteristic elimination masses provided by MS-MS analysis of anthocyanin aglycones served as signatures for the precursor anthocyanidin derivatives.

A recent study aiming at establishing wine fingerprints without any prior purification step compared positive ion and negative ion ESI combined with Fourier transformed (FT) ion cyclotron resonance mass spectrometry.⁹⁵ FT-MS provides mass resolving power and accurate mass determination, making it theoretically possible to assign molecular formula unambiguously. The positive ion mass spectra of red wines were dominated by anthocyanins but also showed proanthocyanidin dimers, flavonols, and dihydroflavonols (as their protonated forms and potassium adducts). Most of these compounds were previously known wine constituents but identification of some methoxylated flavanol and flavonol derivatives that have been reported for the first time requires confirmation. The negative ion spectra provided no further information on the flavonoid composition but exhibited greater variations among the wines and thus appear more promising for fingerprinting purposes.

5.3 GRAPE FLAVONOIDS

5.3.1 STRUCTURE AND DISTRIBUTION IN GRAPE

5.3.1.1 Anthocyanins

Grape anthocyanins (Figure 5.1) are based on five anthocyanidins, namely cyanidin, peonidin, petunidin, delphinidin, and malvidin. Those of *Vitis vinifera* are mostly 3-monoglucosides, whereas non-*vinifera* species also contain substantial amounts of 3,5-diglucosides.¹ The 3-acetylglucoside, 3-*p*-coumaroylglucoside, and 3-caffeoylglucoside of these anthocyanidins are also present in most grape varieties. The occurrence of malvidin 3-caffeoylglucoside has been known for many years, but that of other caffeoylglucosides has only recently been confirmed. Peonidin caffeoylglucoside has been detected in *V. vinifera* grapes by HPLC–MS.⁴⁹ The caffeoylglucosides of cyanidin, delphinidin, and petunidin are present only in trace amounts, but were identified in fractions obtained by MLCCC separation of a grape skin extract.³⁵ The 3,5-diglucosides and the 3-(*p*-coumaroylglucoside),5-glucosides of the five anthocyanidins were identified by ESI-MS–MS in Concord (*Vitis labrusca*) grape juice⁶⁵ and in hybrid grape varieties.⁹⁴ Anthocyanidin 3,5-diglucosides were also detected by MS in a red wine made from *V. vinifera* var. Dornfelder⁹⁶ and in grape skin extracts.³⁵ Additional signals found in the Dornfelder wine after fractionation were attributed to their acetyl and *p*-coumaroyl derivatives, the former reported for the first time in grape.⁹⁶

Anthocyanins are localized in skins, except in a few varieties, referred to as teinturier (e.g., *V. vinifera* var. Alicante Bouschet, var. Gamay Fréaux), that also contain anthocyanins in their pulp. In the skin, they are present in the first external layers of the hypodermal tissue,⁹⁷ and exclusively in the vacuoles.^{98,99} Light microscope observations have shown the presence of organites containing higher concentrations of anthocyanins in the vacuoles of the inner side of skins from ripe Pinot noir berries.¹⁰⁰ Resonance Raman microscopy spectra indicated that anthocyanins are mainly in the neutral quinonoidal form within these structures whereas they are present as flavylum cations in the outer cell vacuoles.¹⁰⁰ These intravacuolar bodies are similar to those described in red cabbage,¹⁰¹ and more recently in flower petals.¹⁰² They have been referred to as anthocyanoplasts,^{100,101} but this nomenclature is improper as it implies a membrane boundary that could not be detected by electron microscopy.^{102,103}

5.3.1.2 Flavanols

Flavan-3-ols are encountered in grape as monomers, oligomers, and polymers. Within the grape berry, they are localized mostly in seeds and skins although trace amounts of monomers and dimers have been detected in pulp, especially in the teinturier variety Alicante Bouschet.¹⁰⁴ Major monomers are (+)-catechin, (–)-epicatechin, and (–)-epicatechin 3-gallate.¹⁰⁵ Gallo-catechin¹⁰⁶ in *V. vinifera* and catechin 3-gallate¹⁰⁷ and gallo-catechin 3-gallate¹⁰⁸ in non-*vinifera* grapes have also been reported.

Grape seed proanthocyanidins are based on catechin, epicatechin, and epicatechin 3-gallate units and thus partly galloylated procyanidins.^{25,38,109} A number of B-type procyanidin dimers and trimers, including some galloylated derivatives, have been identified in grape^{38,109} in addition to the C4–C8-linked dimers (B1–B4).¹¹⁰ The presence of procyanidin A2 has also been reported,^{111,112} but its identification was based only on HPLC coelution with procyanidin A2 isolated from horse chestnut and has not been confirmed.¹¹² Finally, catechin–catechin 3-gallate was identified in non-*vinifera* varieties.^{108,113} Additional dimers based on both prodelfinidin and procyanidin units, presumably arising from grape skins, were also found in wine. Gallo-catechin–gallo-catechin, gallo-catechin–catechin, and catechin–gallo-catechin were tentatively identified in wine on the basis of their mass spectra and relative

retention times in HPLC⁶⁶ whereas epigallocatechin–catechin, epicatechin–gallocatechin, and epicatechin–epigallocatechin were characterized by mass fragmentation and thiolysis.⁸⁷ Flavanol monomers and oligomers identified in grape and wine extracts are listed in Table 5.1.

These lower molecular weight compounds make up only a relatively small proportion of grape proanthocyanidins, which consist mostly of higher oligomers and polymers, as in most other plant species.^{25,26,114,115} Heterogeneity of proanthocyanidins increases with their chain length, due to the diversity of constitutive units, linkage positions, and possible sequences.

TABLE 5.1
Flavanols Identified in Grapes and Wines

	Source	Ref.
<i>Monomers</i>		
(+)-Catechin	Berries	105
(-)-Epicatechin	Berries	105
(-)-Epicatechin gallate	Berries	105
(+)-Catechin gallate	Berries	107
(+)-Gallocatechin	Berries	106
(+)-Gallocatechin gallate	Berries	108
<i>Additional monomeric units in oligomers and polymers</i>		
(-)-Epigallocatechin	Skins, Stems	26
		27
(-)-Epigallocatechin gallate	Skins, Stems	26
		27
<i>Dimers</i>		
(-)-Epicatechin-(4 β -8)-(+)-catechin (B1)	Berries	110
(-)-Epicatechin-(4 β -8)-(-)-epicatechin (B2)	Berries	110
(+)-Catechin-(4 α -8)-(+)-catechin (B3)	Berries	110
(+)-Catechin-(4 α -8)-(-)-epicatechin (B4)	Berries	110
(-)-Epicatechin-(4 β -6)-(-)-epicatechin (B5)	Seeds	38
(+)-Catechin-(4 α -6)-(+)-catechin (B6)	Seeds	38
(-)-Epicatechin-(4 β -6)-(+)-catechin (B7)	Seeds	38
(+)-Catechin-(4 α -6)-(-)-epicatechin (B8)	Seeds	38
(-)-Epigallocatechin-(+)-catechin	Wine	87
(-)-Epicatechin-(+)-gallocatechin	Wine	87
(-)-Epicatechin-(+)-epigallocatechin	Wine	87
(-)-Epicatechin 3-gallate-(4 β -8)-(+)-catechin (B1 3-gallate)	Seeds	38
(-)-Epicatechin 3-gallate-(4 β -8)-(-)-epicatechin (B2 3-gallate)	Seeds	38
(-)-Epicatechin-(4 β -8)-(-)-epicatechin 3-gallate (B2 3'-gallate)	Seeds	109
(+)-Catechin-(4 α -8)-(-)-epicatechin 3-gallate (B4 3-gallate)	Seeds	38
(-)-Epicatechin 3-gallate-(4 β -8)-(-)-epicatechin 3-gallate (B2 3,3'-digallate)	Seeds	38
<i>Trimers</i>		
(-)-Epicatechin-(4 β -8)-(-)-epicatechin-(4 β -8)-(-)-epicatechin (C1)	Wine	32
(-)-Epicatechin-(4 β -8)-(-)-epicatechin-(4 β -8)-(+)-catechin	Wine	32
(-)-Epicatechin-(4 β -8)-(-)-epicatechin-(4 β -6)-(+)-catechin	Seeds	38
(-)-Epicatechin-(4 β -6)-(-)-epicatechin-(4 β -8)-(-)-epicatechin	Seeds	38
(-)-Epicatechin-(4 β -8)-(-)-epicatechin-(4 β -6)-(-)-epicatechin	Seeds	38
(-)-Epicatechin-(4 β -6)-(-)-epicatechin-(4 β -8)-(+)-catechin	Seeds	38
(-)-Epicatechin-(4 β -8)-(-)-epicatechin 3-gallate-(4 β -8)-(+)-catechin	Seeds	38

This results in poorer resolution in all separation methods and renders isolation and formal identification of individual compounds almost impossible beyond the tetramer.

Application of thiolysis to grape proanthocyanidin polymers showed that those extracted from seeds are partly galloylated procyanidins²⁵ whereas those of skins²⁶ and stems²⁷ consist of both procyanidins and prodelphinidins, confirming earlier results obtained by ¹³C NMR.¹¹⁶ The major constitutive units of grape skin proanthocyanidins are epicatechin and epigallocatechin. Their 3-gallates are also encountered as extension units whereas catechin and galocatechin are relatively more abundant in the terminal positions.²⁶ Much higher average degrees of polymerization were calculated in skins (about 30)²⁶ than in seeds²⁵ and stems²⁷ (around 10). The proportions of galloylated units are also quite different in skins (5%), stems (15%), and seeds (30%).

As discussed above, the development of mild MS techniques has led to further progress in the determination of proanthocyanidin size distribution. In particular, ESI-MS studies have demonstrated that prodelphinidin and procyanidin units coexist within the polymers, where they seem distributed at random.⁶⁰ A list of mass signals attributed to proanthocyanidins detected in grape or wine extracts is given in Table 5.2.

Flavanol content is higher in seeds than in skins but the contribution of the latter to the entire grape composition may exceed that of the former in some varieties showing small berry size. Microscopic observations have shown the presence of tannin aggregates in the vacuoles of skin cells. Most epidermal cells but only some hypodermal cells, which are more abundant in the external layers, contain tannins.¹¹⁷ In addition, tannins associated with vacuole membranes and to the cell walls were described.¹¹⁸ Whether these associations result from artifacts in preparation or extraction procedures or exist *in planta* is unknown and the structure of tannins present in the vacuole has not been compared to that of tannins linked to membrane material. Nevertheless, monitoring of tannin composition during grape maturation has shown that thiolysis yields gradually decrease as the berry ripens.¹¹⁹ Analysis of flavonoid extracts from unripe and ripe red berries by ¹³C NMR and ESI-MS suggested that proanthocyanidins in the ripe berry skins are associated with some polysaccharides and also with small amounts of anthocyanins.

5.3.1.3 Flavonols, Flavones, and Dihydroflavonols

Flavonols are found in grape skins as 3-glycosides of quercetin, kampferol, isorhamnetin, and myricetin.^{2,120,121} Small amounts of the corresponding aglycones have also been reported. The major flavonols in grape are quercetin 3-glucoside and quercetin 3-glucuronide.^{2,121–123} Other grape flavonols include myricetin 3-glucoside, kampferol 3-glucoside,² isorhamnetin glucoside, myricetin glucuronide, kampferol glucuronide, kampferol galactoside, kampferol glucosylarabinoside, quercetin glucosylgalactoside, quercetin glucosylxyloside,¹²¹ and quercetin 3-rhamnosylglucoside (i.e., rutin).^{120,124} Rutin identification was initially based only on comparison of HPLC retention time and UV-visible spectrum with those of a commercial standard and has therefore been considered questionable but it has recently been confirmed by MS-MS analysis in the skins of table grape⁶² and by ¹H NMR on the compound isolated from vine leaves.¹²⁵ The latter authors have also identified quercetin 3-galactosylrhamnoside and two flavone glycosides, namely luteolin 7-glucoside and apigenin 7-glucoside in vine leaves. Finally, dihydroquercetin 3-rhamnoside (astilbin) and dihydrokampferol 3-rhamnoside (engeletin) have been identified in grape skins¹²⁶ and stems.²⁷

5.3.2 IMPACT OF VARIETAL AND ENVIRONMENTAL FACTORS ON GRAPE FLAVONOIDS

Anthocyanin composition varies greatly within species and cultivars. The total content (from 500 mg/kg up to 3 g/kg) and the proportions of each anthocyanin are varietal characteristics

TABLE 5.2
Proanthocyanidin Signals Detected in Grape and Wine Extracts by ESI-MS in the Negative Ion Mode

DP	(epi)Catechin	Galloyl	(epi)Gallocatechin	Mass Signals	Ref.
2	2			$[M - H]^- = 577$	6
2	2	1		$[M - H]^- = 729$	6
2	1		1	$[M - H]^- = 593$	60
2			2	$[M - H]^- = 609$	60
3	3			$[M - H]^- = 865$	6
3	3	1		$[M - H]^- = 1017$	6
3	2		1	$[M - H]^- = 881$	60
3	1		2	$[M - H]^- = 897$	60
4	4			$[M - H]^- = 1153$	6
4	4	1		$[M - H]^- = 1305$	6
4	3		1	$[M - H]^- = 1169$	60
4	3	1	1	$[M - H]^- = 1321$	60
4	2		2	$[M - H]^- = 1185$	60
4	1		3	$[M - H]^- = 1201$	60
4			4	$[M - H]^- = 1217$	60
5	5			$[M - H]^- = 1441, [M - 2H]^{2-} = 720$	6
5	4		1	$[M - H]^- = 1457, [M - 2H]^{2-} = 728$	60
5	3		2	$[M - H]^- = 1473, [M - 2H]^{2-} = 736$	60
5	2		3	$[M - H]^- = 1489$	60
5	5	1		$[M - H]^- = 1595$	87
				$[M - 2H]^{2-} = 796$	6
5	5	2		$[M - 2H]^{2-} = 873$	6
6	6			$[M - H]^- = 1731$	87
6	5		1	$[M - H]^- = 1747$	87
6	4		2	$[M - H]^- = 1763$	87
6	6	1		$[M - 2H]^{2-} = 940$	6
6	6	3		$[M - 2H]^{2-} = 1093$	6
7	7			$[M - 2H]^{2-} = 1008$	6
7	7	1		$[M - 2H]^{2-} = 1084$	6
7	7	2		$[M - 2H]^{2-} = 1161$	6
7	7	3		$[M - 2H]^{2-} = 1237$	6
8	8	1		$[M - 2H]^{2-} = 1229$	6
8	8	3		$[M - 2H]^{2-} = 1381$	6
9	9			$[M - 2H]^{2-} = 1297$	6
9	9	1		$[M - 2H]^{2-} = 1373$	6
9	9	2		$[M - 2H]^{2-} = 1449$	6

and may be used to some extent as chemotaxonomy criteria.^{127,128} For instance, Pinot noir grapes contain no acylated anthocyanin. Detection of anthocyanin 3,5-diglucosides in wine is considered to attest the use of non-*vinifera* varieties in wine but improvement of the analytical methods has enabled to demonstrate that anthocyanin 3,5-diglucosides are also present in small amounts in *V. vinifera* varieties. Red and white varieties also present large differences in flavonol composition. Among some studied wine cultivars,¹²⁹ quercetin derivatives always predominate but myricetin derivatives and isorhamnetin 3-glucoside appeared restricted to red cultivars. No myricetin derivative was detected in a series of table grape varieties while isorhamnetin glucoside was found only in the white Superior seedless and Moscatel

Italia cultivars.⁶² Grape proanthocyanidin composition varies only slightly between varieties and no particular pattern was observed for red and white cultivars. The data are still too scarce to conclude whether it can serve for authentication purposes.

Grape flavonoid composition also varies with environmental conditions^{122,130,131} and changes throughout ripening. Anthocyanin biosynthesis starts rather late in grape maturation and shows maximum activity in the first weeks immediately after veraison (i.e., the beginning of berry softening and color change). Anthocyanin accumulation is regulated by both light exposure and temperature.¹³² It usually continues until harvest but a drop may be observed in the latest phase of maturation, especially in hot climates.^{132,133}

Treatment of grape berries with 2-phloroethylphosphonic acid, a compound known to enhance ethylene production, increased expression of genes encoding for major enzymes in the anthocyanin biosynthetic pathway, namely chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), leucoanthocyanin dioxygenase (LDOX), and UDPglucose-flavonoid 3-*O*-glucosyltransferase (UGT), and anthocyanin accumulation.¹³⁴ The transcript accumulation was sustained for 20 days after veraison while anthocyanin levels determined by HPLC analysis decreased, suggesting that modulation of anthocyanins may involve mechanisms other than transcriptional. In addition, red pigments determined by measuring absorbance values at 520 nm of acidified methanolic extracts increased over the same period of time. This may mean that anthocyanins were, in fact, converted to other pigment species that were not taken into account in the HPLC analysis but contributed to absorbance in the red region. Incorporation of anthocyanins in polymeric structures has been postulated to take place during grape maturation.¹³⁵ Anthocyanin oligomers were recently detected in grape skin extract by ESI-MS.¹³⁶

The concentration of proanthocyanidins is highest a few weeks after veraison both in skins and in seeds.⁸³ Accumulation of flavanol monomers is concomitant with that of proanthocyanidins in seeds. In skins, their maximum concentration is reached earlier and begins to decrease as proanthocyanidins accumulate. The decrease in proanthocyanidin concentration observed in the latest stages of grape maturation has been attributed to reduced extractability resulting from interactions with other cell constituents such as polysaccharides or proteins.^{83,119} Results regarding changes in proanthocyanidin composition during ripening appear contradictory: the average chain length has been reported to decrease,¹⁰⁹ increase,¹¹⁹ or remain constant^{83,137} from veraison to harvest, depending on the variety.¹³⁸ A temporary increase in epigallocatechin extension units was also observed in the skins of the red variety Shiraz immediately before veraison. Since these units are trihydroxylated on the B-ring like the majority of grape anthocyanins, a coordinated process between tannin and anthocyanin accumulation was suggested.⁸³

Flavanol synthesis occurs in two main periods; the first one around flowering and the second after the main period of anthocyanin biosynthesis.¹²³ In the latter phase, flavanol accumulation is highly dependent on environmental factors and, in particular, much increased by sun exposure of the berries.^{122,132}

Based on the currently available data, grape anthocyanin and flavanol profiles are determined by genetic characteristics whereas their content varies with the vine growing conditions. Available information still appears too limited and contradictory to draw any conclusion on the impact of genetic and environmental factors on grape proanthocyanidin composition.

5.4 EXTRACTION OF GRAPE FLAVONOIDS INTO THE WINE

Since flavonoids are localized almost exclusively in the solid parts of the cluster (skins, seeds, stems), their transfer into the must and wine is primarily determined by the extent of maceration allowed in the wine-making process. Thus, white wines are usually obtained by

direct pressing of red or white grapes whereas fermentation on skins, enabling extraction of anthocyanins from these tissues, is required to make red wine. Diffusion starts after crushing of the grape and continues until the wine is separated from the solid residue (marc) by racking or pressing. Diffusion kinetics depends both on the solubility of the compounds and on their localization in the berry. It is further modulated by other factors, including the concentration of alcohol and sulfur dioxide in the liquid phase, the temperature, and the extent of must homogenization.

White musts and wines made without maceration contain very low amounts of flavonoids. However, when making white wine from white grapes, skin contact at low temperature is sometimes performed before pressing and fermentation to increase extraction of volatile compounds and aroma precursors. After 4 h of skin contact, the concentration of flavanol monomers and dimers in must was increased threefold.¹³⁹ Delays between harvest and pressing, especially if sulfur dioxide is added to prevent oxidation, as well as thorough pressing, similarly result in increased concentrations of flavonoids in white musts and wines.^{140,141}

In red wine making, anthocyanins diffuse rapidly. Their concentration reaches a maximum after a few days and then steadily decreases as they are involved in various reactions.¹¹⁵ Monitoring of proanthocyanidin composition in the fermenting musts by HPLC after thiolysis demonstrated that proanthocyanidins from skins diffuse faster than those from seeds, due either to their localization or to the higher water solubility of prodelfphinidins compared to galloylated procyanidins.¹¹⁵ Extraction of proanthocyanidins from seeds starts after a lag phase, when the level of alcohol increases.¹⁴² Polymers with highest molecular weight also diffuse slower than those of lower molecular weight so that the average chain length gradually increases.¹¹⁵

Maceration at low temperature before fermentation starts enhanced extraction of anthocyanins and proanthocyanidins from skins whereas postfermentation maceration increased that of procyanidins from seeds. The levels of anthocyanins and proanthocyanidins recovered in wine at the end of fermentation represent about 40 and 20% of their amounts in grape, respectively.¹⁴³ Anthocyanin recoveries were lower after 3-week additional maceration, representing only 20% of the grape initial content. Extraction of the pomace hardly increased the recovery yield, indicating that a major proportion of flavonoids had been converted to other species or had been irreversibly adsorbed on the solid material during fermentation. This provides good evidence that anthocyanin and tannin reactions that have been reported to take place during aging actually start very early in the wine-making process. Degradation of anthocyanins and tannins is even faster when the wine is made with carbonic maceration, a process consisting in maintaining the grape under a carbon dioxide atmosphere for a few days as performed, for instance, in Beaujolais.¹⁴²

5.5 REACTIONS OF FLAVONOIDS IN MUSTS AND WINES

Changes in flavonoid composition involve both enzymatic and chemical processes. The former is restricted to the early stages of wine-making whereas the latter rapidly becomes prevalent as the enzymes become inactivated, and continues throughout aging. Whether they are biochemical or chemical, these processes rely primarily upon the reactivity of phenolic compounds, which is based on the reactivity of the phenol hydroxyl group itself but can be modulated by the presence of substituents. Additional reactions involve substituents or substitution bonds (e.g., enzymatic or acid-catalyzed hydrolysis of the glycosidic or ester linkages). A list of flavonoid derivatives formed by these reactions processes identified in wine or in wine-like model solutions is given in Table 5.3.

TABLE 5.3
Flavonoid Reaction Products Formed in Wine and Wine-Like Model Systems

Compound	Source	<i>m/z</i> (Parent and Fragment Ions)	λ_{\max}	Identification Methods	Ref.
<i>Anthocyanin reaction products</i>					
Caffeoyltartaric acid-malvidin 3- <i>O</i> -glc adducts (e.g., 5 1 , Figure 5.4)	Model solution	801 ($[M - 2H]^-$)/803 ($[M]^+$)	285, 327 (sh), 540	HPLC-DAD-ESI-MS	55
Flavylium form (five isomers)	Model solution	819 ($[M - H]^-$)	285, 325	HPLC-DAD-ESI-MS	55
Hemiketal form (six isomers)	Oxidized grape	803 ($[M]^+$)		HPLC-DAD-ESI-MS	142
Flavylium form (four isomers)	(gamay beaujolais)				
Caffeoyltartaric acid-peonidin 3- <i>O</i> -glc adducts (e.g., 5 2 , Figure 5.4)	Oxidized grape	773 ($[M]^+$)	285, 327 (sh), 540	HPLC-DAD-ESI-MS	142
Flavylium form (four isomers)	(gamay beaujolais)				
<i>F-A⁺ or A⁺-F adducts</i>					
(epi)Catechin-malvidin 3- <i>O</i> -glc	Red wines, model solutions	781 ($[M]^+$; 331)	280, 531	HPLC-DAD-ESI-MS	189
(epi)Catechin-peonidin 3- <i>O</i> -glc	Red wine extract ^a	767 ($[M]^+$)		HPLC-ESI-Q-MS	87
(epi)Catechin-petunidin 3- <i>O</i> -glc	Red wine extract ^a	751 ($[M]^+$)		HPLC-ESI-Q-MS	87
(epi)Catechin-malvidin 3- <i>O</i> -glc	Red wine extract ^a	781 ($[M]^+$; 619)		HPLC-ESI-Q-MS	87
(epi)Catechin-malvidin 3- <i>O</i> -(6- <i>O</i> -acetyl)glc	Red wine extract ^a	823 ($[M]^+$)		HPLC-ESI-Q-MS	87
(epi)Catechin-malvidin 3- <i>O</i> -(6- <i>O</i> - <i>p</i> -coumaroyl)glc	Red wine extract ^a	927 ($[M]^+$)		HPLC-ESI-Q-MS	87
(epi)Catechin-delphinidin 3- <i>O</i> -glc	Red wine extract ^a	781 ($[M]^+$)		HPLC-ESI-Q-MS	96
(epi)Catechin-cyanidin 3- <i>O</i> -glc	Red wine extract ^a	765 ($[M]^+$)			96
(epi)Catechin-petunidin 3- <i>O</i> -glc	Red wine extract ^a	7795 ($[M]^+$)			96
(epi)Catechin-peonidin 3- <i>O</i> -glc	Red wine extract ^a	779 ($[M]^+$)			96
<i>F-A⁺ adducts</i>					
(epi)Catechin-malvidin 3- <i>O</i> -glc (two isomers)	Red wine extract ^b	781 ($[M]^+$; 763, 619, 601, 493, 467)	282, 535	HPLC-DAD-ESI-MS	86
(epi)Catechin-malvidin 3- <i>O</i> -glc (two isomers)	Model solution	781 ($[M]^+$; 763, 619, 601, 493, 467)	282, 553, 535	HPLC-DAD-ESI-MS	58, 86
Epicatechin-malvidin 3- <i>O</i> -glc (5 3)	Synthesis	781 ($[M]^+$; 763, 619, 601, 493, 467)		HPLC-ESI-IT-MS	86

continued

TABLE 5.3
Flavonoid Reaction Products Formed in Wine and Wine-Like Model Systems — continued

Compound	Source	<i>m/z</i> (Parent and Fragment Ions)	λ_{\max}	Identification Methods	Ref.
Catechin-malvidin 3- <i>O</i> -glc (two isomers) (5 4)	Synthesis	781 ([M] ⁺ ; 763, 619, 601, 493, 467)		HPLC-ESI-IT-MS	86
Epicatechin-epicatechin-malvidin 3- <i>O</i> -glc (5 5)	Model solution	1069 ([M] ⁺)		HPLC-ESI-IT-MS	58
<i>A⁺-F adducts</i>					
Malvidin 3- <i>O</i> -glc-epicatechin (4-8)-epicatechin 3'- <i>O</i> -gallate (5 6)	Model solution	1221 ([M + H] ⁺ ; 1203, 1059, 1041, 907, 755, 617)		HPLC-ESI-IT-MS	58
Epicatechin (4-8)-(malvidin 3- <i>O</i> -glc (4-6)-epicatechin 3'- <i>O</i> -gallate) (5 7)	Model solution	1221 ([M + H] ⁺ ; 1203, 1059, 1041, 771)		HPLC-ESI-IT-MS	58
<i>A-Type anthocyanin-flavanol adducts</i>					
A-Type malvidin 3-glc-(epi)catechin (two isomers)	Red wine extract ^a	783 ([M + H] ⁺ ; 621, 631, 469)/781 ([M - H] ⁻)		HPLC-ESI-Q-MS	7
A-Type malvidin 3-glc-catechin (two isomers)	Model solution	783 ([M + H] ⁺ ; 621, 631, 469)		HPLC-ESI-Q-MS	7
Malvidin 3,5-di-glc-(2- <i>O</i> -7, 4-8)-catechin (5 8)	Model solution			¹ H, ¹³ C NMR	179
Malvidin 3-glc-(2- <i>O</i> -7, 4-8)-epicatechin (5 9)	Model solution	783 ([M + H] ⁺ ; 765, 747, 657, 631, 621, 495, 469, 451)/781 ([M - H] ⁻ ; 655, 629, 601, 583, 493)		HPLC-ESI-IT-MS, NMR	193
A-Type petunidin 3-glc-(epi)catechin (5 10)	Red wine extract ^a	769 ([M + H] ⁺)		HPLC-ESI-Q-MS	87
A-Type malvidin 3-acetylglc-(epi)catechin (5 11)	Red wine extract ^a	825 ([M + H] ⁺)		HPLC-ESI-Q-MS	87
A-Type malvidin 3-glc-procyanidin dimer (5 12)	Red wine extract ^a	1071 ([M + H] ⁺)		HPLC-ESI-Q-MS	87
Benzylthioether of A-type malvidin 3-glc-(epi)catechin (5 13)	Red wine extract ^a after thiolysis	903 ([M - H] ⁻ ; 779, 491, 287)		HPLC-ESI-Q-MS	87
<i>Anthocyanin dimers</i>					
Malvidin 3-glc dimer (A ⁺ -AOH)	Model solution	1071 ([M + H] ⁺)		HPLC-ESI-IT-MS	58
Malvidin-malvidin 3-glc (A ⁺ -AOH)	Model solution	839 ([M + H] ⁺)		HPLC-ESI-IT-MS	58
<i>Flavanol oxidation products</i>					
B-Type dehydrodicatichins					
Catechin-(6'-8)-catechin	Model solutions	577 ([M - H] ⁻)		FAB-MS, NMR	171
Catechin-(3'- <i>O</i> -8 or 4'- <i>O</i> -8)-catechin (two isomers)	Model solutions	577 ([M - H] ⁻)		FAB-MS, NMR	171
B-Type dehydrodicatichins	Model solutions	577 ([M - H] ⁻ ; 559, 533, 439, 425, 393)		ESI-MS-MS	172
A-Type dehydrodicatichins (two isomers)	Model solutions	575 ([M - H] ⁻)		FAB-MS, NMR	171

Methylmethine-bridged adducts

Epicatechin-CH-CH ₃ -malvidin 3-glc (two isomers)	Model solutions	809 (M ⁺)	280, 536, 280, 542	HPLC-DAD, FAB-MS	196
Catechin-CH-CH ₃ -malvidin 3-glc (two isomers)	Model solutions	809 (M ⁺)	280, 540	HPLC-DAD, FAB-MS	187
(epi)Catechin-CH-CH ₃ -malvidin 3-glc	Red wine	809 (M ⁺)	280, 540	HPLC-DAD-ESI-MS	6
Epicatechin-(8-(CH-CH ₃)-8)-malvidin 3-glc (5 14)	Model solutions	809 (M ⁺)		HPLC-DAD-ESI-MS, NMR	199
Catechin-(8-(CH-CH ₃)-8)-malvidin 3-glc (5 15)	Red wine ^b	781 (M ⁺)		HPLC-ESI-MS	52
(epi)Catechin-(CH-CH ₃)-delphinidin 3-glc (5 16)	Red wine ^b	795 (M ⁺)		HPLC-ESI-MS	52
(epi)Catechin-(CH-CH ₃)-petunidin 3-glc (5 17)	Red wine ^b	779 (M ⁺)		HPLC-ESI-MS	52
(epi)Catechin-(CH-CH ₃)-peonidin 3-glc (5 18)	Red wine ^b	1097 (M ⁺)		HPLC-ESI-MS	52
(epi)Catechin ₂ -(CH-CH ₃)-malvidin 3-glc (5 19)	Model solutions	1125 (M ⁺)		HPLC-ESI-MS	56
Malvidin 3-glc-(CH-CH ₃ -epicatechin) ₂	Model solutions	664 (M ²⁺)		HPLC-ESI-MS	56
(CH-CH ₃ -Malvidin 3-glc) ₂ -epicatechin	Model solutions	1441 (M ⁺)		HPLC-ESI-MS	56
Malvidin 3-glc-(CH-CH ₃ -epicatechin) ₃	Model solutions	822 (M ²⁺)		HPLC-ESI-MS	56
Malvidin 3-glc-(CH-CH ₃ -epicatechin) ₂ -CH-CH ₃ -malvidin 3-glc	Model solutions	605 (M - H ⁻) 607 (M + H ⁺) 605 (M - H ⁻)		HPLC-ESI-MS HPLC-ESI-MS	92 56
(epi)Catechin-CH-CH ₃ -(epi)catechin	Model solutions			NMR	77
Epicatechin-CH-CH ₃ -epicatechin	Model solutions				
(four isomers : 6-6, 8-8, 6-8 R, S)					
Catechin-CH-CH ₃ -catechin	Model solutions				
(four isomers : 6-6, 8-8, 6-8 R, S)					
(epi)Catechin-(CH-CH ₃ -(epi)catechin)	Red wine	605 (M - H ⁻)		HPLC-ESI-MS	6, 11
(epi)Catechin-(CH-CH ₃ -(epi)catechin) ₂	Model solutions	921 (M - H ⁻)		HPLC-ESI-MS	92
(epi)Catechin-(CH-CH ₃ -(epi)catechin) ₂	Red wine	921 (M - H ⁻)		HPLC-ESI-MS	6
(epi)Catechin-(CH-CH ₃ -(epi)catechin) ₃	Model solutions	1237 (M - H ⁻)		HPLC-ESI-MS	92
(epi)Catechin-(CH-CH ₃ -(epi)catechin) ₄	Model solutions	1553 (M - H ⁻)		HPLC-ESI-MS	92
(epi)Catechin-(CH-CH ₃ -(epi)catechin) ₅	Model solutions	1869 (M - H ⁻)		HPLC-ESI-MS	92
Malvidin 3-glc-(8-(CH-CH ₃)-8)-malvidin 3-glc (5 20)	Model solution, wine	1029 (M ⁺) : A ⁺ -AOH	280, 460 (sh), 536	HPLC-DAD-ESI-MS	13
		1011 (M ⁺) : A ⁺ -A			
		506 (M ²⁺) : A ⁺ -A ⁺			

continued

TABLE 5.3
Flavonoid Reaction Products Formed in Wine and Wine-Like Model Systems — continued

Compound	Source	<i>m/z</i> (Parent and Fragment Ions)	λ_{max}	Identification Methods	Ref.
Malvidin 3-glc-(CH-CH ₃ -malvidin 3-glc) ₂	Model solution	1529 ([M] ⁺ : A ⁺ -A ₂ 765 ([M] ²⁺) : (A ⁺) ₂ -A		HPLC-ESI-MS	13
Malvidin 3-glc-(CH-CH ₃ -malvidin 3-glc) ₃	Model solution	683 ([M] ³⁺) : (A ⁺) ₃ -A 1024 ([M] ²⁺) : (A ⁺) ₂ -A ₂ 1042 ([M] ²⁺) : (A ⁺) ₂ -AOH ₂ 865 ([M] ³⁺) : (A ⁺) ₃ -A ₂ 1283 ([M] ²⁺) : (A ⁺) ₂ -A ₃		HPLC-ESI-MS	13, 19
Malvidin 3-glc-(CH-CH ₃ -malvidin 3-glc) ₄	Model solution			HPLC-ESI-MS	19
<i>Pyranocanthocyanins</i>					
Pyranomalvidin 3-glc (5 21)	Wine	517 ([M] ⁺ ; 355)	270, 355, 498 280-490	FAB-MS, NMR	17
	Model solution			HPLC-DAD-ESI-MS	57, 149
Pyranopetunidin 3-glc	Model solution	503 ([M] ⁺)		HPLC-DAD-ESI-MS	57
Pyranopeonidin 3-glc	Model solution	487 ([M] ⁺)		HPLC-DAD-ESI-MS	57
Pyranocyanidin 3-glc	Model solution	473 ([M] ⁺)		HPLC-DAD-ESI-MS	57
Pyranodelphinidin 3-glc	Model solution	489 ([M] ⁺)		HPLC-DAD-ESI-MS	57
Pyranomalvidin 3-acetylglc (5 22)	Wine	559 ([M] ⁺ ; 355)	270, 355, 503	FAB-MS, NMR	17
(epi)Catechin pyranomalvidin 3-glc	Wine, marc	805 ([M] ⁺)		HPLC-ESI-MS	202
8-Catechin pyranomalvidin 3-glc (5 23)	Port wine	805 ([M] ⁺ ; 643, 491)	503	HPLC-DAD-ESI-MS, NMR	203
8-Epicatechin pyranomalvidin 3-glc (5 24)	Port wine	805 ([M] ⁺ ; 643, 491)	503	HPLC-DAD-ESI-MS, NMR	203
(epi)Catechin pyranomalvidin 3-acetylglc	Marc	847 ([M] ⁺)		HPLC-ESI-MS	202
(epi)Catechin pyranomalvidin 3- <i>p</i> -coumaroylglucoside	Wine, marc	951 ([M] ⁺)		HPLC-ESI-MS	202
(epi)Catechin pyranomalvidin 3- <i>p</i> -coumaroylglc (5 26)	Port wine	951 ([M] ⁺ ; 643)	280, 313, 503	HPLC-DAD-ESI-MS, NMR	53
8-Catechin pyranomalvidin 3- <i>p</i> -coumaroylglc (5 27)	Port wine	951 ([M] ⁺ ; 643)	280, 313, 503	HPLC-DAD-ESI-MS, NMR	53
8-Epicatechin pyranomalvidin 3- <i>p</i> -coumaroylglc (5 27)	Port wine	951 ([M] ⁺ ; 643)		HPLC-DAD-ESI-MS, NMR	53
Di(epi)catechin pyranomalvidin 3-glucoside	Wine, marc	1093 ([M] ⁺)		HPLC-ESI-MS	202
Epicatechin-(4-8)-epicatechin pyranomalvidin 3-glc	Model solution	1093 ([M] ⁺)		HPLC-ESI-MS	54
8-Catechin-(4-8)-catechin pyranomalvidin 3-glc (5 25)	Port wine	1093 ([M] ⁺)	520	HPLC-DAD-ESI-MS, NMR	203
(Epi)catechin ₂ pyranomalvidin 3-acetylglc	Marc	1135 ([M] ⁺)		HPLC-ESI-MS	202
(Epi)catechin ₂ pyranomalvidin 3- <i>p</i> -coumaroylglc	Marc	1239 ([M] ⁺)		HPLC-ESI-MS	202

8-Epicatechin-(4-8)-catechin-pyranomalvidin 3- <i>p</i> -coumaroylglc (5 28)	Port wine	1239 ([M] ⁺ ; 931)	280, 313, 512	HPLC-DAD-ESI-MS, NMR	53
((Epi)catechin) ₃ pyranomalvidin 3-glc	Marc	1381 ([M] ⁺)		HPLC-ESI-MS	202
((Epi)catechin) ₃ pyranomalvidin 3-acetylglc	Marc	1423 ([M] ⁺)		HPLC-ESI-MS	202
((Epi)catechin) ₃ pyranomalvidin 3- <i>p</i> -coumaroylglc	Marc	1527 ([M] ⁺)		HPLC-ESI-MS	202
((Epi)catechin) ₄ pyranomalvidin 3-glc	Marc	1669 ([M] ⁺)		HPLC-ESI-MS	202
4-Hydroxyphenyl pyranomalvidin 3-glc (5 29)	Wine, model solution	609 ([M] ⁺ ; 447)	280, 507	HPLC-DAD-MS ¹ H NMR	50 8
4-Hydroxyphenyl pyranomalvidin 3- <i>p</i> -coumaroylglc (5 30)	Wine, model solution	755 ([M] ⁺ ; 447)	280, 313 (sh), 507	HPLC-DAD-MS ¹ H NMR	50 8
4-Hydroxyphenyl pyranopetunidin 3-glc (5 31)	Wine	595 ([M] ⁺ ; 433)		ESI-MS-MS	88
4-Hydroxyphenyl pyranopetunidin 3-glc (5 32)	Wine	579 ([M] ⁺ ; 417)		ESI-MS-MS	88
4-Hydroxyphenyl pyranopetunidin 3-acetylglc (5 33)	Wine	637 ([M] ⁺ ; 433)		ESI-MS-MS	88
4-Hydroxyphenyl pyranopetunidin 3-acetylglc (5 34)	Wine	621 ([M] ⁺ ; 417)		ESI-MS-MS	88
4-Hydroxyphenyl pyranomalvidin 3-acetylglc (5 35)	Wine	651 ([M] ⁺ ; 447)		ESI-MS-MS	88
4-Hydroxyphenyl pyranopetunidin 3- <i>p</i> -coumaroylglc (5 36)	Wine	741 ([M] ⁺ ; 433)		ESI-MS-MS	88
4-Hydroxyphenyl pyranopetunidin 3- <i>p</i> -coumaroylglc (5 37)	Wine	725 ([M] ⁺ ; 417)		ESI-MS-MS	88
Catechyl pyranomalvidin 3-glc (5 38)	Wine	625 ([M] ⁺ ; 463)		ESI-MS-MS	88
Catechyl pyranomalvidin 3-acetylglc (5 39)	Wine	667 ([M] ⁺ ; 463)		ESI-MS-MS	88
Catechyl pyranomalvidin 3- <i>p</i> -coumaroylglc (5 40)	Wine	771 ([M] ⁺ ; 463)		ESI-MS-MS	88
Guaiacyl pyranomalvidin 3-glc (5 41)	Wine	639 ([M] ⁺ ; 477)		ESI-MS-MS	88
Guaiacyl pyranomalvidin 3-acetylglc (5 42)	Wine	681 ([M] ⁺ ; 477)		ESI-MS-MS	88
Guaiacyl pyranomalvidin 3- <i>p</i> -coumaroylglc (5 43)	Wine	785 ([M] ⁺ ; 477)		ESI-MS-MS	88
Syringyl pyranomalvidin 3-glc (5 44)	Wine	669 ([M] ⁺ ; 507)		ESI-MS-MS	88
Syringyl pyranomalvidin 3-acetylglc (5 45)	Wine	711 ([M] ⁺ ; 507)		ESI-MS-MS	88
Carboxy pyranomalvidin 3-glc (5 46)	Grape marc	559 ([M ⁺ - 2H ⁺] ⁺ ; 515; 353)	507	HPLC-ESI-MS	10
Carboxy pyranopetunidin 3-glc	Grape marc	529 ([M ⁺ - 2H ⁺] ⁺ ; 485; 323)	507	HPLC-ESI-MS	10
Carboxy pyranopetunidin 3-glc	Grape marc	547 ([M ⁺ - 2H ⁺] ⁺ ; 501; 339)	507	HPLC-ESI-MS	10
Carboxy pyranodelphinidin 3-glc	Grape marc	531 ([M ⁺ - 2H ⁺] ⁺ ; 487)	507	HPLC-ESI-MS	10
Carboxy pyranomalvidin 3-acetylglc (5 47)	Grape marc	601 ([M ⁺ - 2H ⁺] ⁺ ; 556; 353)	507	HPLC-ESI-MS	10
Carboxy pyranomalvidin 3- <i>p</i> -acetylglc (5 47)	Wine	603 ([M] ⁺ ; 561)	503, 370	HPLC-DAD-ESI-MS	293
Carboxy pyranomalvidin 3-caffeoylglc	Model solution	723 ([M ⁺ - 2H ⁺] ⁺ ; 556; 353)	514	HPLC-DAD-ESI-MS	57

continued

TABLE 5.3
Flavonoid Reaction Products Formed in Wine and Wine-Like Model Systems — continued

Compound	Source	m/z (Parent and Fragment Ions)	λ_{\max}	Identification Methods	Ref.
Carboxy pyranomalvidin 3- <i>p</i> -coumaroylglc	Model solution	705 ($[M^+ - 2H]^+$; 556, 353)	514	HPLC-DAD-ESI-MS	57
Carboxy pyranomalvidin 3- <i>p</i> -coumaroylglc	Model solution	707 ($[M]^+$; 561)	264, 306, 520	HPLC-DAD, FAB-MS	219
Carboxy pyranomalvidin 3- <i>p</i> -coumaroylglc (5 48)	Wine	707 ($[M]^+$; 561)	503, 370	HPLC-DAD-ESI-MS, NMR	293
Vinyl-epicatechin-(epi)catechin pyranomalvidin 3-glc (5 49)	Port wine	1119 ($[M]^+$; 957, 829, 667)	575	HPLC-DAD-ESI-MS, NMR	15
Vinyl-epicatechin-(epi)catechin pyranomalvidin 3- <i>p</i> -coumaroylglc (5 50)	Port wine	1265 ($[M]^+$; 957)	575	HPLC-DAD-ESI-MS, NMR	15
Vinylcatechin pyranomalvidin 3- <i>p</i> -coumaroylglc	Model solution	977 ($[M]^+$; 669)	575	HPLC-DAD-ESI-MS, NMR	15
<i>Products of condensation with other aldehydes</i>					
Catechin-(8-(CH-COOH)-8)-catechin (5 51)	Model solution	635 ($[M - H]^-$)	280	HPLC-DAD-ESI-MS, NMR	9
Catechin-(8-(CH-COO-CH ₂ -CH ₃)-8)-catechin	Model solution	663 ($[M - H]^-$)	280	HPLC-DAD-ESI-MS	222
Catechin-(6-(CH-COOH)-6)-catechin	Model solution	635 ($[M - H]^-$)	280	HPLC-DAD-ESI-MS, NMR	79
Catechin-(6-(CH-COOH)-8)-catechin (two isomers, <i>R</i> and <i>S</i>)	Model solution	635 ($[M - H]^-$)	280	HPLC-DAD-ESI-MS, NMR	79
8-Formylcatechin	Model solution	635 ($[M - H]^-$)	295, 340	HPLC-DAD-ESI-MS, NMR	213
6-Formylcatechin	Model solution	635 ($[M - H]^-$)	295, 340	HPLC-DAD-ESI-MS, NMR	213
6,8-Diformylcatechin	Model solution	635 ($[M - H]^-$)	295, 340	HPLC-DAD-ESI-MS, NMR	213
Catechin-(carboxymethine-catechin) ₂	Model solution	981 ($[M - H]^-$)	280		9
Catechin(-furfurylmeine)-catechin (four isomers)	Model solutions	657 ($[M - H]^-$; 505, 367, 289)	280	HPLC-DAD-ESI-MS	214
Catechin(5-hydroxymethylfurfuryl)-catechin (four isomers)	Model solutions	687 ($[M - H]^-$; 289)	280	HPLC-DAD-ESI-MS	214
Catechin(-furfurylmeine)-malvidin 3-glucoside (two isomers)	Model solution	859 ($[M^+ - 2H]^+$)	280, 450, 545	HPLC-DAD-ESI-MS	214
Catechin(5-hydroxymethylfurfurylmeine)-malvidin 3-glucoside (two isomers)	Model solution	889 ($[M^+ - 2H]^+$)	280, 450, 545	HPLC-DAD-ESI-MS	214

Catechin-(CH ₂)-malvidin 3-glucoside	Model solution	593 (M ⁺ ; 303)	280, 531	HPLC-DAD-ESI-MS	215
Catechin-(CH ₂ -CH ₃)-malvidin 3-glucoside	Model solution	621 (M ⁺ ; 331)	280, 541	HPLC-DAD-ESI-MS	215
Catechin-(CH-CH ₃) ₂ -malvidin 3-glucoside	Model solution	634 (M ⁺ ; 344)	280, 541	HPLC-DAD-ESI-MS	215
Catechin-(CH ₂ -CH-(CH ₃) ₂)-malvidin 3-glucoside	Model solution	649 (M ⁺ ; 359)	280, 541	HPLC-DAD-ESI-MS	215
Catechin-(CHCH ₃ -CH ₂ CH ₃)-malvidin 3-glucoside	Model solution	649 (M ⁺ ; 359)	280, 541	HPLC-DAD-ESI-MS	215
Catechin-(benzyl)-malvidin 3-glucoside	Model solution	669 (M ⁺ ; 379)	280, 541	HPLC-DAD-ESI-MS	215
<i>Xanthylum salts</i>					
Carboxy dicatechin xanthylum (5 52)	Model solution	617 (M ⁺)	273, 308 (sh), 444	HPLC-DAD-ESI-MS, NMR	217
Carboxy-dicatechin xanthylum (four isomers: 6-6, 6-8 R, S, 8-8)	Model solution	615 ([M ⁺ - 2H ⁺] ⁻ ; 571, 463, 419)			
	Model solution	615 ([M ⁺ - 2H ⁺] ⁻ ; 571, 463, 419)	273, 308 (sh), 444	HPLC-DAD-ESI-MS	12
Carboxy dicatechin xanthene (5 53)	Model solution	617 ([M - H] ⁻)		HPLC-DAD-ESI-MS	217
	Model solution	643 ([M ⁺ - 2H ⁺] ⁻)	277, 308 (sh), 459	HPLC-DAD-ESI-MS, NMR	222
Ethylcarboxy dicatechin xanthene (5 54) (four isomers)	Model solution	645 ([M - H] ⁻)		HPLC-DAD-ESI-MS	222
Ethylcarboxy dicatechin xanthene (5 55)	Model solution	587 ([M ⁺ - 2H ⁺] ⁻ ; 435)	280, 450	HPLC-DAD-ESI-MS, NMR	294
Hydroxy dicatechin xanthylum (three isomers)	Model solution	637 ([M ⁺ - 2H ⁺] ⁻)	280, 440	HPLC-DAD-ESI-MS	214
Furfuryl-dicatechin xanthylum	Model solution	639 ([M - H] ⁻)	280	HPLC-DAD-ESI-MS	214
Furfuryl dicatechin xanthene	Model solution	667 ([M ⁺ - 2H ⁺] ⁻)	280, 440	HPLC-DAD-ESI-MS	214
Hydroxymethylfurfuryl-dicatechin xanthylum	Model solution	669 ([M - H] ⁻)	280	HPLC-DAD-ESI-MS	214
Hydroxymethylfurfuryl dicatechin xanthene	Model solution				

5.5.1 REACTIVITY OF FLAVONOID COMPOUNDS

The reactivity of polyphenolic compounds is due, on the one hand, to the acidity of their phenolic hydroxyl groups and, on the other hand, to the resonance between the free electron pair on the phenolic oxygen and the benzene ring, which increases electron delocalization and confers the position of substitution adjacent to the hydroxyl group a partial negative charge and thus a nucleophilic character. The A-ring shared by all grape flavonoids possesses two nucleophilic sites, in C8 and C6 positions, due to activation by the hydroxyl groups of its phloroglucinol (1,3,5-trihydroxy)-type structure.

Anthocyanins are usually represented as the red flavylium cations (Figure 5.1, left). However, this form is predominant only in very acidic solvents ($\text{pH} < 2$) such as those used for HPLC analysis. In mildly acidic media, the flavylium cations undergo proton transfer and hydration reactions, respectively, generating the quinonoidal base and the hemiketal (*syn* carbinol) form (Figure 5.1, right) that can tautomerize to the chalcone.^{144,145} Thus, at wine pH, malvidin 3-glucoside occurs mostly as the colorless hemiketal (75%), the red flavylium cation, yellow chalcone, and blue quinonoidal base being only minor species.

The phloroglucinol A-ring of the anthocyanin hemiketal is nucleophilic, as described more generally for grape flavonoids, whereas the C-ring of the flavylium form, bearing a cationic charge in C2 or C4, reacts as an electrophile. Classical examples of nucleophilic addition reactions onto the flavylium cation are those of water and bisulfite that have long been known to result in anthocyanin bleaching. NMR studies demonstrated that addition of water occurs mostly in C2 position, the 4-carbinol being only a minor product,¹⁴⁶ whereas addition of sulfur dioxide yields the two C4-sulfonate adducts.¹⁴⁷ Addition with other nucleophiles normally occurs in C4 position but C2 adducts resulting from reaction of anthocyanins with acetone were isolated and characterized.¹⁴⁸

Another reaction of the flavylium cation has recently been demonstrated.^{8,10,57,149–151} It involves concerted addition of compounds possessing a polarizable double bond on the electron-deficient site C-4 and the oxygen of the 5-hydroxyl group of the anthocyanin. The new pigments thus formed, showing a second pyran ring, have been referred to as vitisins,¹⁵² but the term pyranoanthocyanins proposed by Lu and Foo¹⁵¹ is preferred.

Flavanols also react both as nucleophiles, through their A-ring, and as electrophiles, through the carbocations formed after acid-catalyzed cleavage of the interflavanoid linkages. The latter reaction, restricted to oligomers and polymers, was shown to occur spontaneously at wine pH values.^{4,153}

The basic forms of phenols (phenolate anions) are easily oxidized to semiquinone radicals through electron transfer. These radicals can then react with another radical to form an adduct through radical coupling or, in the case of *o*-diphenols, undergo a second oxidation step yielding *o*-quinones that are electrophiles as well as oxidants.^{154,155} Oxidation reactions are very slow in wine, due to the low proportion of phenolate ions at wine pH values, but take place extremely rapidly when oxidative enzymes are involved (see Section 5.5.2.2).

5.5.2 ENZYMATIC REACTIONS

Major enzymes catalyzing flavonoid reactions are oxidative enzymes (i.e., polyphenoloxidases and peroxidases) arising from grape but also from molds contaminating them. Various hydrolytic enzymes (glycosidases, esterases), excreted by the fermentation yeasts or fungi or present in preparations added for technological purposes (e.g., pectinases), are also encountered in wine.

5.5.2.1 Hydrolytic Enzymes

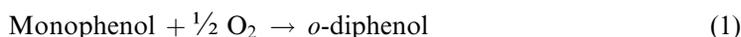
Pectinases and β -glucanases are the only enzymes allowed in wine-making by European legislation. They are used as clarification and filtration agents and also to release aroma compounds that are mostly present in grape as nonvolatile glycosidic precursors.¹⁵⁶ Pectolytic enzymes are also reported to increase extraction of phenolic compounds and wine color intensity.¹⁵⁷

Such rather crude commercial preparations often contain other activities, such as cinnamoyl esterases, tannin-acyl hydrolase (tannase), and glycosidases. Hydrolysis of flavonol glucosides catalyzed by β -glucosidase has been reported to induce haze formation, due to precipitation of insoluble flavonol aglycones.¹⁵⁸ Deglucosylation of anthocyanins results in discoloration, owing to instability of the resultant anthocyanidins. This can be further enhanced in the presence of cinnamoyl esterase hydrolyzing the linkage between *p*-coumaric acid and glucose in *p*-coumaroylated anthocyanins.¹⁵⁹ However, β -glucosidases from *Aspergillus niger* show different substrate affinity patterns so that activities needed to hydrolyze aroma precursors and plant cell wall polysaccharides can be separated from undesirable anthocyanase activities.¹⁶⁰

Tannase activity (tannin acyl hydrolase, EC 3.1.1.20) has been reported in numerous fungi, including *A. niger* and *Botrytis cinerea*.¹⁶¹ It catalyzes cleavage of the ester bond in galloylated flavanols. Its action in wine is rather weak, probably due to inhibition by high levels of phenolic compounds. In addition, some galloyl groups in procyanidin polymers are resistant to tannase hydrolysis, because of steric hindrance or other molecular interactions.¹⁶²

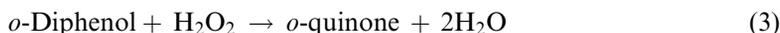
5.5.2.2 Enzymatic Oxidation and Subsequent Reactions

The major enzyme responsible for polyphenol oxidation in wine-making is grape polyphenoloxidase (PPO, *syn* catecholoxidase, EC 1.10.3.1), showing both cresolase (hydroxylating monophenols to *o*-diphenols (1)) and catecholase (oxidizing *o*-diphenols to *o*-quinones (2)) activities, using molecular oxygen as co-substrate



Laccase (EC 1.10.3.2), arising from fungal contamination by *B. cinerea*, which is the agent of gray and noble rot, also catalyzes reaction 2 and accepts a wider range of substrates, including, in particular, glycosylated flavonoids such as anthocyanins and also *p*-diphenols (oxidized to *p*-quinones).¹⁶³

Peroxidase (POD) catalyzes oxidation of a wide range of *o*-diphenolic substrates to *o*-quinones, using hydrogen peroxide as a co-substrate (3):



This activity is present in grape but has never been reported in musts where PPO is extremely active and the availability of hydrogen peroxide is limited. Studies performed on pear¹⁶⁴ suggest that POD may participate in enzymatic oxidation together with PPO that generate hydrogen peroxide through coupled oxidation processes.¹⁶⁵ It may also be involved in anthocyanin degradation occurring in postharvest storage. Decay of catalase activity, which catalyzes conversion of hydrogen peroxide to water, concomitant with anthocyanin degradation and induction of POD activity, has been observed in grapes submitted to carbonic maceration.¹⁴²

Flavonoids are not usually directly involved in enzymatic oxidation since they are very poor substrates for grape polyphenoloxidase compared to caffeoyltartaric acid,¹⁶⁶ which is also very abundant in grape musts. *ortho*-Diphenolic flavonoid aglycones and, in particular, flavanol monomers can be oxidized by grape PPO. Glycosylated flavonoids (anthocyanins, flavanol glycosides) and proanthocyanidins are poor substrates for grape PPO but may be oxidized by laccase and by peroxidase. Moreover, they can participate in oxidation reactions through coupled oxidation and nucleophilic addition processes with enzymatically generated quinones. In particular, kinetic studies showed that the quinone arising from enzymatic oxidation of caffeoyltartaric acid is a strong oxidant, capable of oxidizing most flavonoid *o*-diphenols, including catechins,¹⁶⁷ epicatechin gallate,¹⁶⁸ procyanidins,^{168,169} and cyanidin 3-glucoside¹⁷⁰ to the corresponding secondary *o*-quinones. Coupled oxidation of cyanidin 3-glucoside is illustrated in Figure 5.5(A). Such coupled oxidation reactions result in the recycling of caffeoyltartaric acid that is regenerated by reduction of its quinone and can then be reoxidized by PPO as long as enzyme activity and oxygen are available.

The electrophilic primary and secondary quinones undergo addition of nucleophiles, including flavonoids. For instance, nucleophilic addition of catechin to its enzymatically generated quinone yielded a catechin dimer in which the catechin moieties are linked through a C6'-C8 biphenyl linkage.¹⁷¹ This B-type dehydrodicatechin further oxidized to yellow pigments. Additional dehydrodicatechins arise from radical coupling of the catechin semi-quinones formed by retro-disproportionation, in which the catechin moieties are linked

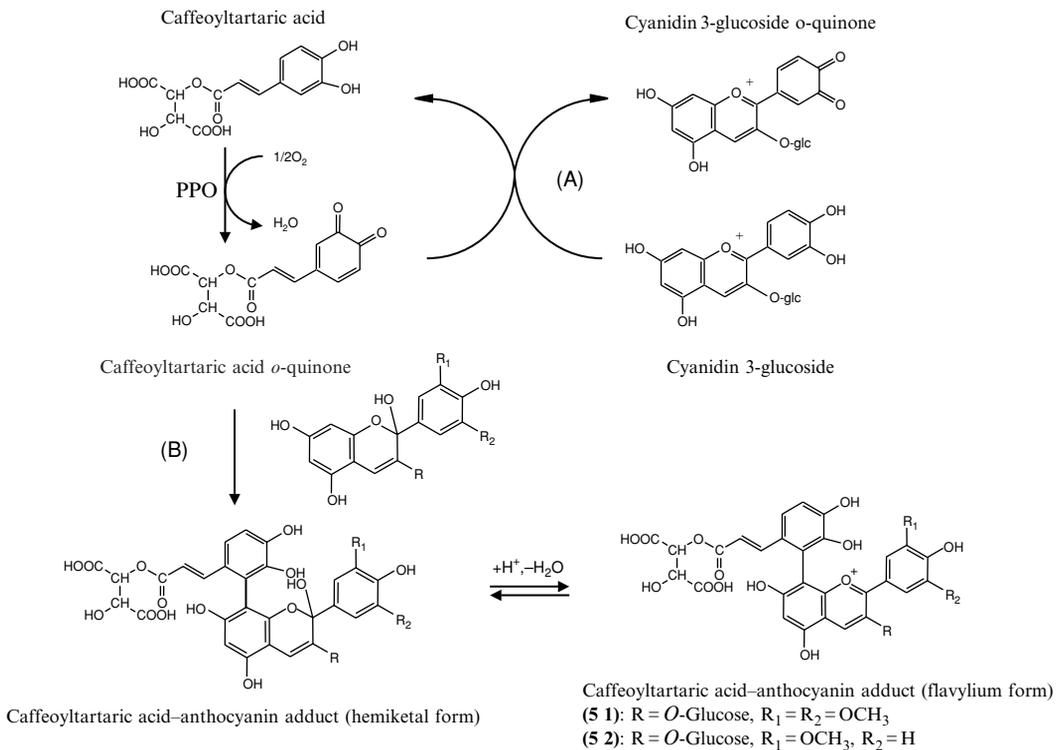


FIGURE 5.5 Anthocyanin reactions with caffeoyltartaric acid *o*-quinone coupled oxidation of cyanidin 3-glucoside (A) and nucleophilic addition of anthocyanins (B). Anthocyanin-caffeoyltartaric acid adducts are represented as one of the possible isomers, namely 2-(8-anthocyanin) caffeoyltartaric acid (**5 1**).

through phenyl-ether bonds (C3'-O-C8) or (C4'-O-C8), especially in solutions incubated at lower pH values. Catechin dimers resulting from oxidation can be distinguished from their procyanidin isomers on the basis of specific mass fragments obtained by ESI-MS, at m/z 451 for procyanidin dimers (cf. Figure 5.2) and at m/z 393 for dehydrodicatechins.¹⁷²

Addition of anthocyanins onto the caffeoyltartaric acid quinone formed by PPO oxidation was similarly demonstrated.^{55,170} Although malvidin 3-glucoside does not possess an *o*-diphenolic structure and is thus not susceptible to coupled oxidation, it was rapidly degraded when incubated with PPO and caffeoyltartaric acid. The apparent rate of caffeoyltartaric acid oxidation was not modified by the presence of malvidin 3-glucoside but the concentration of quinones was much lower, suggesting that they were trapped by nucleophilic addition of the anthocyanin (Figure 5.5, B).¹⁷⁰ LC-MS analysis of the oxidized solution allowed the detection of six malvidin 3-glucoside-caffeoyltartaric acid adducts both under the flavylium form ($[M]^+$ at $m/z = 803$ in the positive ion mode, $[M - 2H]^-$ at $m/z = 801$ in the negative ion mode) and the hydrated form ($[M - H]^-$ at $m/z = 819$ in the negative ion mode).⁵⁵ Comparison of the reaction rates at pH 1.7 and pH 3.4 confirmed that the addition involved the nucleophilic anthocyanin hemiketal, in agreement with the postulated mechanism. Unfortunately, no adduct was formed in sufficient amount to be isolated and identified. However, on the basis of quinone and anthocyanin reactivities, the linkages can be reasonably assumed to be in C8 and possibly C6 positions on the anthocyanin. Nucleophilic addition in positions 2 and 5 of caffeoyltartaric acid quinones has been observed earlier with glutathione.^{173,174} Nucleophilic addition in position 6,¹⁷⁵ as well as radical coupling leading to phenyl ether bonds in position 3 or 4,¹⁷¹ as demonstrated with catechin, are also possible. Finally, the side chain double bond of caffeoyltartaric acid can be the site of nucleophilic attack, as shown with caffeic acid.¹⁷⁶ However, the UV-visible spectra of all caffeoyltartaric acid-malvidin 3-glucoside adducts showed absorbance at 328 nm characteristic of the side chain conjugation of caffeic acid derivatives, ruling out this hypothesis. One of the possible isomers (2-(8-anthocyanin)caffeoyltartaric acid) (**5 1**) is shown in Figure 5.5. Four caffeoyltartaric acid-malvidin 3-glucoside adducts and four additional compounds detected at $m/z = 773$ in the positive ion mode, presumably corresponding to caffeoyltartaric acid-peonidin 3-glucoside adducts (**5 2**), were also detected after oxidation of Gamay beaujolais grapes.¹⁴²

5.5.3 CHEMICAL REACTIONS

Color and taste changes taking place during wine aging have long been ascribed to conversion of grape anthocyanins to polymeric pigments through addition reactions with flavanols.³ Three mechanisms have been postulated.

The first one involves nucleophilic addition of flavanols (in C8 or C6) onto the C4 position of the anthocyanin flavylium ion yielding 4-flavanyl-anthocyanins, which are also referred to as anthocyanin-flavanol (A-F) or anthocyanin-tannin (A-T) adducts (Figure 5.6). The formation of 4-phloroglucinyflavene and 4-flavanylflavene by addition of phloroglucinol and catechin, respectively, onto a flavylium was first demonstrated by Jurd.^{177,178} The latter was oxidized to the corresponding 4-flavanylflavylium by a second molecule of the flavylium salt whereas rearrangement of the former yielded a structure similar to A-type proanthocyanidins, in which the anthocyanin and phloroglucinol units are linked through both C-C and ether linkages. This rearrangement was also observed for the flavanylflavene formed from malvidin 3,5-diglucoside and catechin.¹⁷⁹ Finally, xanthylum salt structures were proposed for yellow-orange products formed by reaction of malvidin 3,5-diglucoside with catechin,⁵ phloroglucinol, epicatechin, and catechin-3-*O*-gallate¹⁸⁰ but no structural characterization was provided.

The second mechanism is based on nucleophilic addition onto the carbonium ion formed in acid solutions from flavan 3,4-diols¹⁸¹ or by cleavage of procyanidins.^{69,182} A condensation

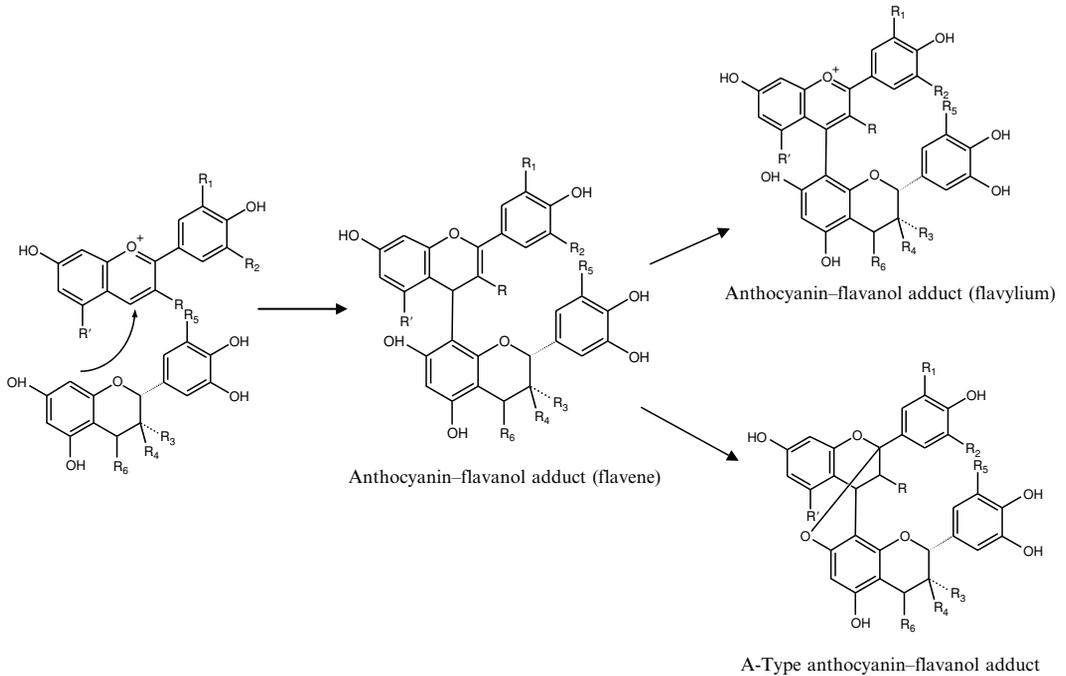


FIGURE 5.6 Postulated mechanism for formation of anthocyanin–flavanol adducts (flavanyl–anthocyanins). R, R', R₁, R₂ as in Figure 5.1; R₃ = H, OH, galloyl; R₄, R₅ = H, OH; R₆ = H, flavanyl.

product of 5,7,3',4'-tetramethoxyflavan 3,4-diol and phloroglucinol was prepared as a model proanthocyanidin and identified by ¹H NMR after methylation of the phloroglucinol hydroxyls, confirming the postulated addition mechanism.¹⁸³ Yellow products formed after acid treatment of this adduct were postulated to be xanthylium salts, resulting from oxidation of an intermediate xanthe.¹⁸⁴ According to these authors, a similar mechanism may explain the formation of so-called phlobaphene pigments from proanthocyanidins. Acid-catalyzed degradation of proanthocyanidins was also shown to take place at wine pH values.^{4,153} In the presence of large amounts of flavanol monomers, proanthocyanidin losses were much reduced and oligomeric species gradually replaced higher molecular weight polymers as the monomers added to the intermediate carbocation released by acid-catalyzed cleavage. Finally, addition of anthocyanins, either in the flavene form¹⁸¹ or in the hemiketal form,¹⁸⁵ onto the carbonium ion, leading to F–A adducts was described. The resulting flavene (F–A) or hemiketal (F–AOH) adducts both generate the corresponding flavylum (F–A⁺), through oxidation or dehydration reactions, respectively.

Reactions of anthocyanins and flavanols take place much faster in the presence of acetaldehyde^{5,186,187} that is present in wine as a result of yeast metabolism and can also be produced through ethanol oxidation, especially in the presence of phenolic compounds,¹⁸⁸ or introduced by addition of spirit in Port wine technology. The third mechanism proposed involves nucleophilic addition of the flavanol onto protonated acetaldehyde, followed by protonation and dehydration of the resulting adduct and nucleophilic addition of a second flavonoid onto the carbocation thus formed.⁵ The resulting products are anthocyanin–flavanol adducts in which the flavonoid units are linked in C6 or C8 position through a methyl-methine bond, often incorrectly called ethyl-link in the literature.

Earlier investigations relied upon model solution studies starting with grape components or related molecules and comparison of color characteristics of the reaction products with those of wine pigments, but none of these structures or reactions had been formally demonstrated in wine until recently. The development of more sensitive and selective analytical techniques, such as HPLC coupled to diode array detector and MS, has enabled the characterization of various wine components and to postulate the reaction mechanisms generating them. These mechanisms can then be investigated in model solution studies and characteristics of the resulting products compared with those of wine constituents. Conversely, products obtained in wine-like solutions serve to develop specific analytical tools as well as chromatographic and spectral data that are used for determination of new products in wine. Recent studies based on these complementary approaches have confirmed the occurrence of various expected structures, and discovered numerous others, as developed below.

5.5.3.1 Products of Direct Anthocyanin–Flavanol Addition

Pigments resulting from direct reaction of anthocyanins with flavanols were first detected in wine in 1999.^{87,189} HPLC–ESI–MS analysis of the aqueous phase recovered after isoamyl alcohol extraction of a 2-year-old cabernet sauvignon wine showed a series of signals at mass values corresponding to those of covalent adducts of flavanol monomers with the major grape anthocyanins as their flavylium form,⁸⁷ but did not allow to distinguish between F–A⁺ (Figure 5.7A) and A⁺–F (Figure 5.7B) derivatives. MSⁿ experiments performed on the major compound, detected at *m/z* 781, which was postulated to correspond to the flavylium form of an (epi)catechin–malvidin 3-glucoside adduct,^{19,86} indicated that the flavanol unit is in the upper position. To confirm this hypothesis and further characterize the structures, flavanol–anthocyanin adducts (F–A⁺) were prepared by hemisynthesis using two different approaches. Epicatechin–malvidin 3-glucoside (**5 3**) was obtained by incubating procyanidin B2 3'-*O*-gallate (epicatechin 3-gallate (4–8)-epicatechin) and malvidin 3-glucoside at pH 2.⁵⁸ Catechin–malvidin 3-glucoside (**5 4**) was synthesized by using a protocol adapted from the synthesis of procyanidin dimers,¹⁹⁰ involving nucleophilic addition of malvidin 3-glucoside onto the flavan 3,4-diol resulting from reduction of taxifolin.⁸⁶

These experiments demonstrated the occurrence in wine of F–A⁺ adducts arising from cleavage of procyanidins followed by nucleophilic addition of anthocyanins. Detection of epicatechin–epicatechin–malvidin 3-glucoside (**5 5**) (*m/z* 1069), resulting from addition of epicatechin–malvidin 3-glucoside onto the intermediate epicatechin carbonium ion, in the model solution containing B2 3'-*O*-gallate and malvidin 3-glucoside, is another argument in favor of this mechanism. F–A⁺ adducts were not detected at pH 3.8, meaning that their formation was limited by the rate of proanthocyanidin acid-catalyzed cleavage.⁵⁸ At this pH value, reaction of malvidin 3-glucoside with procyanidin B2 3'-*O*-gallate yielded two A⁺–F adducts (*m/z* 1221 in the positive ion mode) resulting from nucleophilic addition of the procyanidin dimer onto malvidin 3-glucoside. MSⁿ fragmentation experiments (Figure 5.8) established that malvidin 3-glucoside was linked to the epicatechin unit in one of them and to the epicatechin 3-*O*-gallate unit in the other.⁵⁸ The anthocyanin–procyanidin adducts were no longer detected after thiolysis. Two additional ions at *m/z* 903 and 933 were attributed to the benzylthioether of malvidin 3-glucoside–epicatechin and to malvidin 3-glucoside–epicatechin 3-*O*-gallate, confirming the postulated structures (**5 6**, **5 7**) as shown in Figure 5.9.

Additional compounds corresponding to anthocyanin dimers in which one of the anthocyanins is in the flavylium form and the other in the hydrated form were detected in the solutions incubated at pH 3.8. Such products arise from nucleophilic addition of the hemiketal onto the flavylium, confirming that, at this pH value, anthocyanins exist and react under both forms, as expected from their hydration equilibrium.

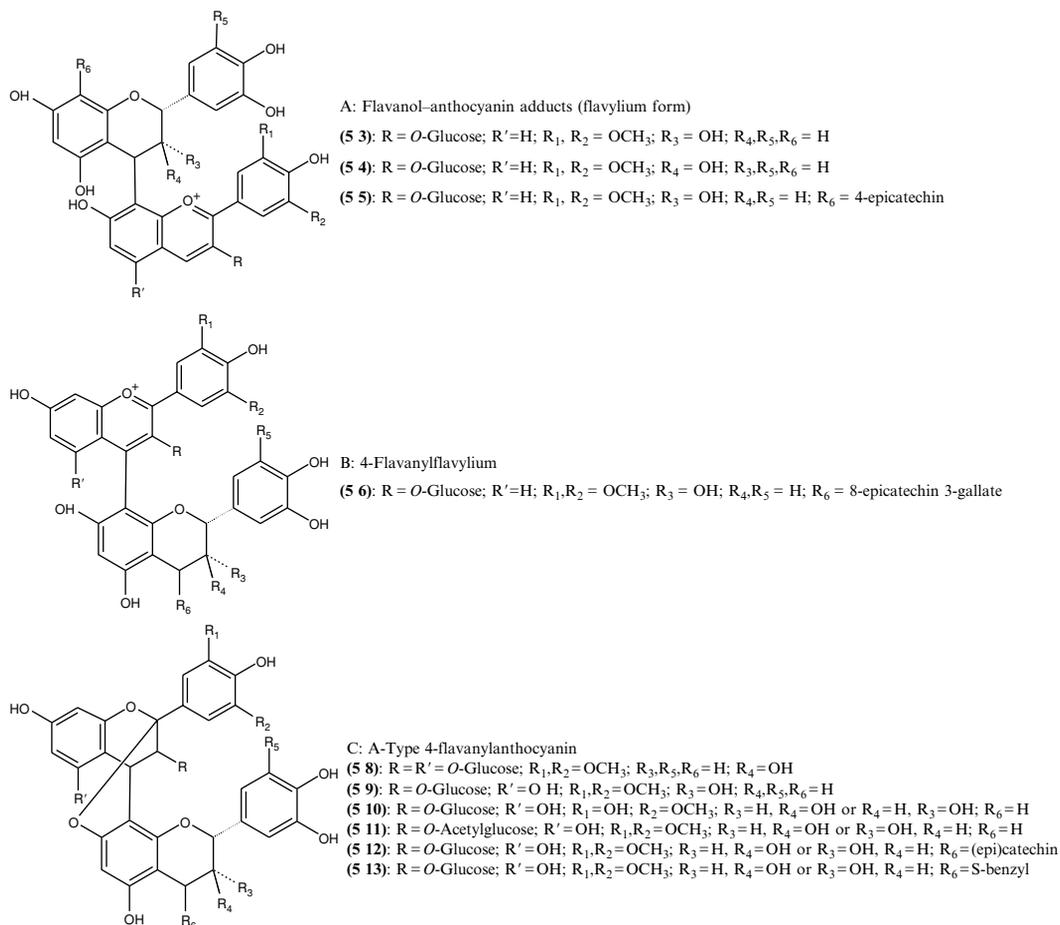


FIGURE 5.7 Structures of anthocyanin–flavanol and flavanol–anthocyanin adducts.

HPLC–MS analysis of the above-mentioned cabernet sauvignon wine extract⁷ also showed the presence of two compounds with the mass signals expected from the flavene forms of malvidin 3-glucoside–(epi)catechin adducts (MW = 782). This flavene structure (cf. Figure 5.6) has been postulated for a colorless product formed in wine-like model solutions containing catechin and malvidin 3-glucoside.^{191,192} Incubation of malvidin 3-glucoside and catechin in ethanol yielded two colorless compounds showing the same mass spectra and eluting at the same retention times as those found in the wine extract.⁷ Their resistance to thiolysis ruled out the postulated flavene structure and led to the proposal of a structure similar to A-type proanthocyanidins (Figure 5.7, C), as described earlier for a malvidin 3,5-diglucoside–catechin adduct.¹⁷⁹ Two-dimensional NMR spectrometry of the major adduct isolated from the model solution confirmed the postulated malvidin 3-glucoside (C2–O–C7, C4–C8) epicatechin structure (5 9).¹⁹³

Other A-type anthocyanin–flavanol adducts, namely petunidin 3-glucoside–(epi)catechin (5 10), malvidin 3-acetylglucoside–(epi)catechin (5 11), and malvidin 3-glucoside–procyanidin dimer (5 12), were similarly detected in the red wine extract.⁸⁷ Finally, a signal was detected at *m/z* 903 in the negative ion mode after thiolysis of the wine extract. Its characteristic fragments allowed to attribute it to the benzylthioether of A-type malvidin 3-glucoside–(epi)catechin (5 13) arising from thiolysis of A-type malvidin 3-glucoside–procyanidins.

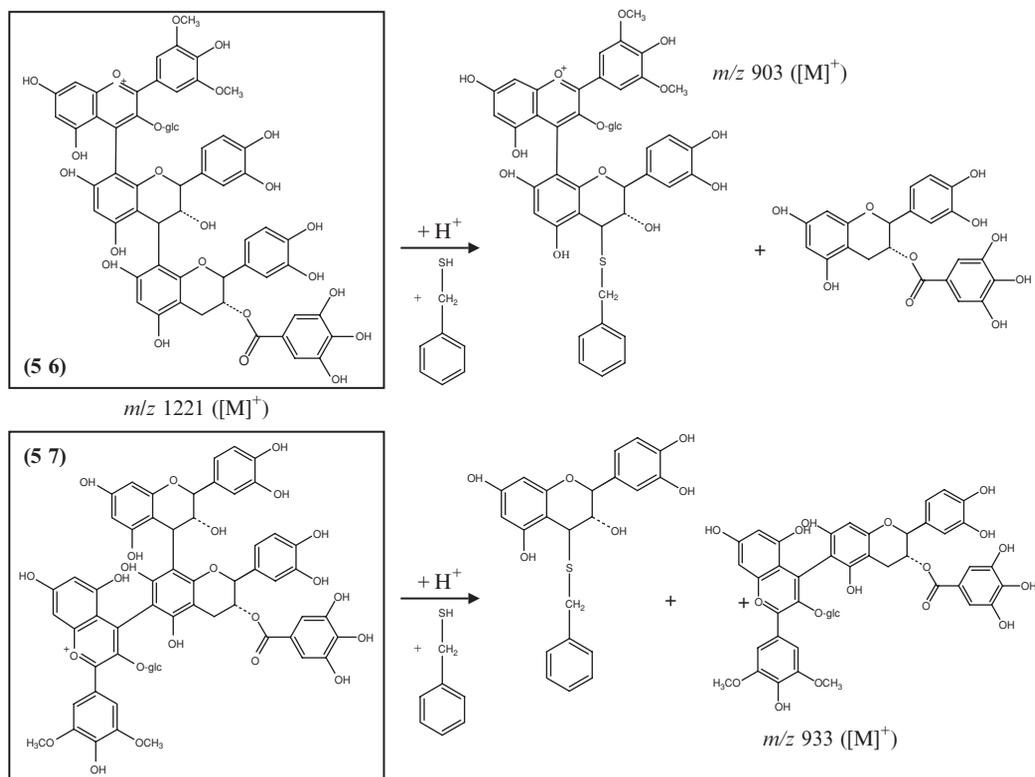


FIGURE 5.9 Hypothetical structures and thiolysis reaction of malvidin 3-glucoside–procyanidin B2 3'-gallate adducts.

unresolved late eluting compounds accumulated.⁵⁶ Their UV–visible spectra showed further a bathochromic shift in the visible region (λ_{\max} 555 nm) compared to those of the dimers (λ_{\max} 540 nm). Detection of mass signals corresponding to methylmethine-linked trimers containing two epicatechin and one malvidin 3-glucoside, one epicatechin and two malvidin 3-glucoside, and tetramers with three epicatechin and one malvidin 3-glucoside, or two epicatechin and two malvidin 3-glucoside, confirmed that the polymerization reaction continued.⁵⁶ Similar dimeric and trimeric adducts were formed by incubation of cyanidin 3-glucoside with (epi)catechin.⁹³ The presence of these various compounds and that of ethyl-linked flavanol dimers (Figure 5.10B) indicate that anthocyanin and flavanol units compete in the nucleophilic addition reaction. In addition, formation of trimers implies that both positions 6 and 8 are reactive, at least in flavanol units. Acetaldehyde-mediated reactions of procyanidins and anthocyanins were also demonstrated in model solutions, their rate increasing with the flavanol chain length.²⁰⁰

Monitoring of acetaldehyde-induced polymerization of catechin and epicatechin by HPLC–MS⁶³ demonstrated the formation of several methylmethine-linked flavanol dimers, trimers, and tetramers. Detection of the intermediate ethanol adducts confirmed the mechanism postulated by Timberlake and Bridle,⁵ which involves protonation of acetaldehyde in the acidic medium, followed by nucleophilic attack of the resulting carbocation by the flavan unit. The ethanol adduct then loses a water molecule and gives a new carbocation that undergoes nucleophilic attack by another flavanol molecule. Four dimers (C6–C6, C8–C8, and C6–C8, *R* and *S*) were formed from each monomeric flavanol.^{64,77} When both epicatechin and catechin units were present, additional isomers containing both types of units were

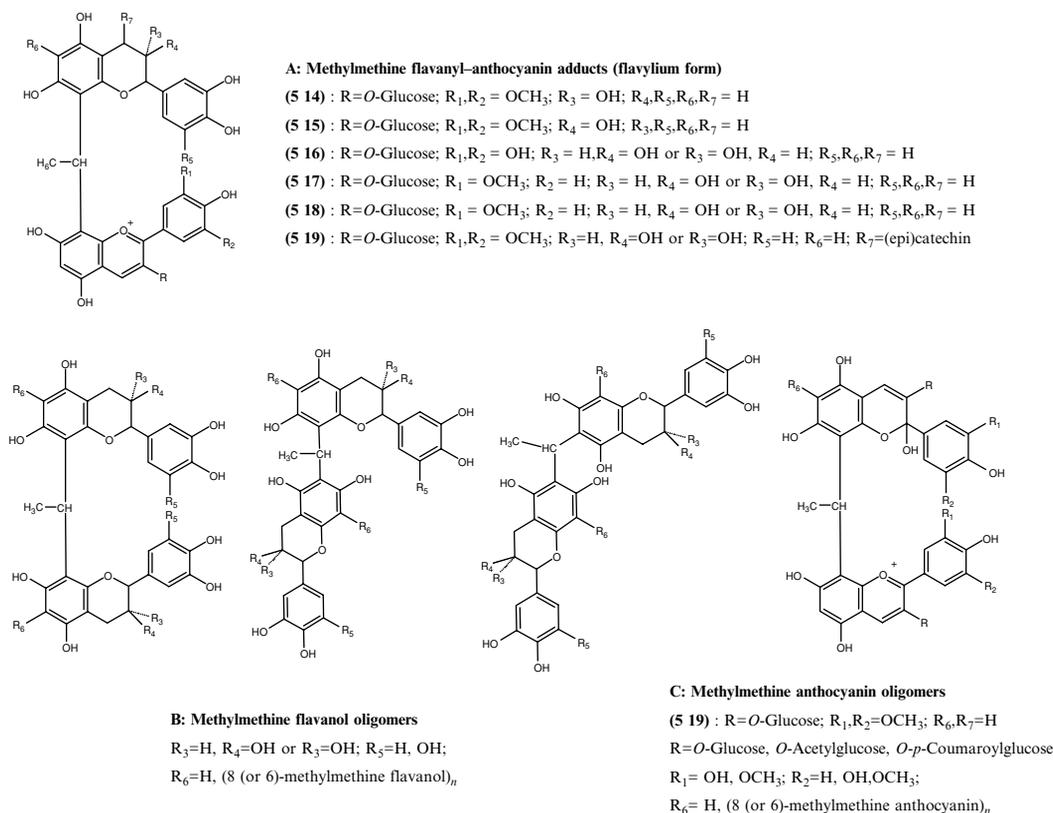


FIGURE 5.10 Structures of methylmethine-linked flavonoid derivatives.

formed. As the reaction was allowed to proceed, successive condensations led to numerous oligomers and polymers.⁶³

The methylmethine-bridged oligomers are rather unstable in wine-like media, due to the susceptibility of the methylmethine-flavanol bonds to acid-catalyzed cleavage.^{64,201} Methylmethine-linked anthocyanin-flavanol adducts are much more resistant than flavanol oligomers under the very acidic conditions used for thiolysis. However, when incubated at higher pH values (2.5 and 5), they underwent spontaneous cleavage and released malvidin 3-glucoside.¹⁸ The effect of pH is probably related to the higher proportions of adducts under the hemiketal form that is more favorable to acid-catalyzed cleavage than the flavylium form, as mentioned above.

Finally, the occurrence of methylmethine-linked malvidin 3-glucoside oligomers (Figure 5.10C) was also reported.¹³ HPLC-MS analysis of the major pigment formed after incubation of malvidin 3-glucoside and acetaldehyde in wine-like solutions at pH 3.2 gave signals at *m/z* 1029, 1011, and 506, which could be attributed to different forms of a methylmethine-linked malvidin 3-glucoside dimer, in which the anthocyanin units exist as one hydrated form and one flavylium form (as shown in Figure 5.10), one quinonoidal base and one flavylium, and two flavylium cations, respectively. NMR spectroscopic analysis of the dimer performed in 10% TFA in deuterated DMSO to ensure that it was in the bis-flavylium form demonstrated that it was a 8,8-methylmethine-linked malvidin 3-glucoside dimer (5 20). Other mass signals detected in the solution were attributed to methylmethine-linked malvidin 3-glucoside trimers, tetramers, and pentamers in which constitutive anthocyanin units were present as

different forms, namely flavylium (A^+), quinonoidal base (A), or hemiketal (AOH).^{13,19} NMR analysis of the oligomeric fraction confirmed that even in strongly acidic medium, the anthocyanin units in the oligomers were present as these different forms. As stated above for flavanol derivatives, the occurrence of polymeric species indicates that the C6 position can also participate in nucleophilic addition reactions although it is less reactive than the C8 position. This reactivity is attributable to the hemiketal units that are present in methylmethine anthocyanin polymers. In contrast, anthocyanin units in methylmethine-linked anthocyanin–flavanol adducts are mostly under the flavylium form and thus act as end points in the condensation process.

HPLC–ESI–MS analysis of red wines demonstrated the presence of (epi)catechin–(CH–CH₃)–malvidin 3-glucoside under the flavylium form (**5 14**, **5 15**),⁶ malvidin 3-glucoside–(CH–CH₃)–malvidin 3-glucoside under the hemiketal–flavylium form (**5 20**),¹³ and methylmethine-linked (epi)catechin dimers^{6,11} and trimers.⁶ Other signals detected were attributed to the flavylium forms of (epi)catechin–(CH–CH₃)–delphinidin 3-glucoside (**5 16**), (epi)catechin–(CH–CH₃)–petunidin 3-glucoside (**5 17**), (epi)catechin–(CH–CH₃)–peonidin 3-glucoside (**5 18**), and procyanidin dimer–(CH–CH₃)–malvidin 3-glucoside (**5 19**).⁵²

In addition to the purple methylmethine-linked adducts, pigments with maximum absorbance in the range 500 to 510 nm were observed in solutions containing acetaldehyde and anthocyanins.¹⁹⁷ A pyranomalvidin 3-glucoside structure (Figure 5.11, **5 21**) formed through an addition mechanism involving, on the one hand, the electronic deficient site C-4 and the 5-hydroxyl group of the malvidin 3-glucoside flavylium cation, and, on the other hand, the polarizable double of the acetaldehyde enolic form, followed by dehydration and rearomatization, was proposed for the major one on the basis of its mass and UV–visible spectra.¹⁵⁰ Simultaneously, the same product and its acetyl derivative (**5 22**) were isolated from wine and called vitisin B and acetylvitisin B.¹⁷ Their ¹H NMR data confirmed the postulated

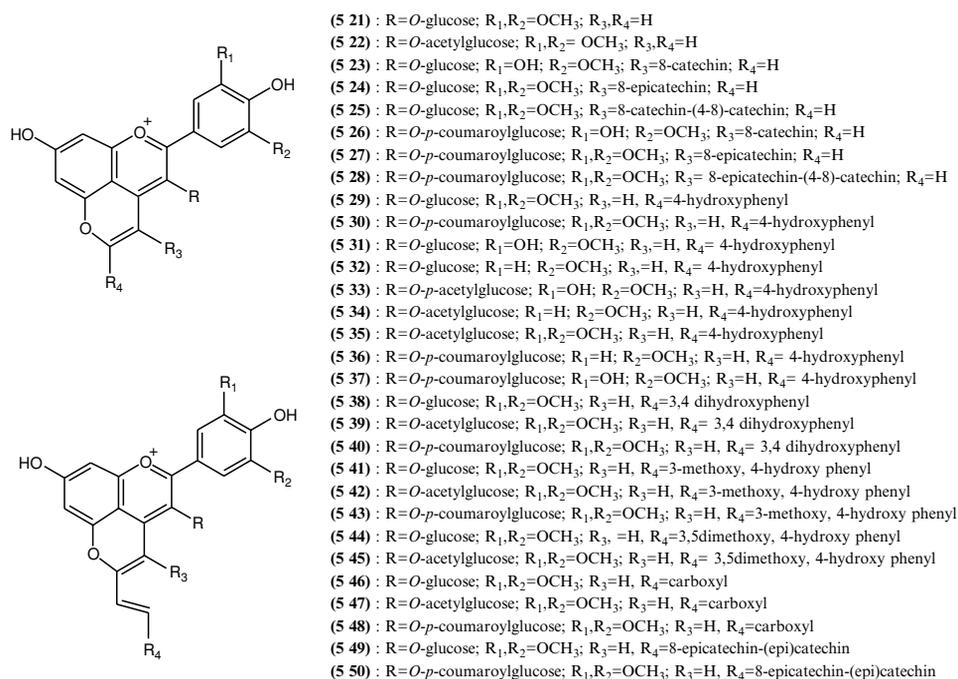


FIGURE 5.11 Structures of pyranoanthocyanins.

structure.¹⁷ Reaction of grape anthocyanin 3-glucosides with acetaldehyde yielded the corresponding series of pyranoanthocyanins that were also formed after yeast fermentation of a synthetic must containing anthocyanins.⁵⁷

Another product obtained by incubation of procyanidin dimer B2, malvidin 3-glucoside, and acetaldehyde in a model wine solution was tentatively identified to a pyranoanthocyanin–procyanidin adduct from its mass and UV–visible spectrum.⁵⁴ Other flavanyl-pyranoanthocyanins based on malvidin 3-glucoside or its acetyl and *p*-coumaroyl esters and (epi)catechin monomer through tetramer were detected in wine or marc extracts.²⁰² Complete structural identification was provided by mass spectrometry and two-dimensional NMR spectroscopy for the (+)-catechin, (–)-epicatechin, and procyanidin B3 (catechin–(4 α -8)–catechin) derivatives of pyranomalvidin 3-glucoside (**5 23**, **5 24**, **5 25**),²⁰³ and for the (+)-catechin, (–)-epicatechin, and procyanidin B1 (epicatechin–(4 β -8)–catechin) derivatives of pyranomalvidin 3-*p*-coumaroylglucoside (**5 26**, **5 27**, **5 28**)⁵³ isolated from Port wine. Flavanyl-pyranoanthocyanins may arise from nucleophilic addition of the flavanol onto the pyranoanthocyanin or from addition of the double bond of a vinylflavanol onto the anthocyanin.^{80,201}

5.5.3.3 Other Pyranoanthocyanins

The first two pyranoanthocyanin structures were detected in wine HPLC profiles in 1996.⁵⁰ Their UV–visible spectra showed a visible absorbance band with a more pointed shape and hypsochromically shifted compared to that of genuine anthocyanins, but differed from each other by the presence of a shoulder around 313 nm (of the least polar one), characteristic of *p*-coumaroylated derivatives. Their mass spectra (see Section 5.2.2.1) indicated that they were, respectively, the glucoside and *p*-coumaroylglucoside of the same anthocyanin-derived aglycone, as confirmed by hydrolysis experiments. Identical products were obtained by reaction of malvidin 3-glucoside and malvidin 3-*p*-coumaroylglucoside, respectively, with vinylphenol. ¹H NMR analysis demonstrated that they were phenylpyranomalvidin 3-glucoside (**5 29**) and phenylpyranomalvidin 3-*p*-coumaroylglucoside (**5 30**),⁸ formed by the addition of the vinylphenol double bond onto the flavylum followed by an oxidation step. The vinylphenol precursor is known to be present in wine as a result of decarboxylation of *p*-coumaric acid, catalyzed by a side yeast enzymatic activity.^{204,205} Similar products were obtained by reaction of vinylphenol with other grape anthocyanins¹⁶ and by reaction of other vinylphenol derivatives (e.g., vinylsyringol) with malvidin 3-glucoside.²⁰⁶

Analysis of anthocyanin derivatives by neutral loss scanning for precursor ions with elimination masses corresponding to glucosyl, acetylglucoside, and *p*-coumaroylglucoside residues detected guaiacyl (3-methoxy, 4-hydroxyphenyl), catechyl (3,4-dihydroxyphenyl), and syringyl (3,5-dimethoxy, 4-hydroxyphenyl) pyranoanthocyanins (**5 38–5 45**) in addition to the already reported vinylphenol adducts (**5 32–5 37**) in extracts from red grape skin and red wine.⁸⁸ These derivatives are produced by reaction of anthocyanins with vinylguaiacol, vinylcatechol, and vinylsyringol, which may be formed by decarboxylation of the corresponding hydroxycinnamic acids, i.e., ferulic acid, caffeic acid, and sinapic acid, known to be present in wine.²⁰⁷ Catechyl- and guaiacyl-pyranomalvidin 3-glucoside were actually obtained by this reaction and characterized by NMR.²⁰⁸ Nevertheless, the slow accumulation of catechyl pyranomalvidin 3-glucoside (called Pinotin A) observed during aging of Pinotage wine²⁰⁹ did not seem compatible with the fast rate reported for addition of vinylphenols onto anthocyanins.¹⁶ An alternative pathway involving reaction of anthocyanins with *p*-hydroxycinnamic acids was demonstrated.^{209,210}

Shortly after the discovery of phenyl pyranoanthocyanins, another series of anthocyanin derivatives showing similar UV–visible spectra, suggesting that they were also derived from a pyranoanthocyanin chromophore, and masses differing from those of grape anthocyanins by

an excess of 68 units was detected in marc.^{10,149,150,206} MS analysis of all these pigments gave characteristic fragments at -44 , corresponding to the loss of a carboxylic group, leading to the suggestion of a general carboxypyrananthocyanin structure. Signals from NMR analysis of the major product were in agreement with this hypothesis. The same malvidin 3-glucoside and malvidin 3-acetylglucoside derivatives were simultaneously detected in wine,¹⁷ but an alternative structure was proposed.¹⁵² Finally, the same product was obtained by reaction of pyruvic acid and malvidin 3-glucoside, definitively confirming the carboxypyrananthocyanin structure (**5 46**).¹⁰ Its formation mechanism involves addition of the double bond of the pyruvic acid enol form onto the flavylium cation followed by dehydration and rearomatization, as described above for acetaldehyde addition.

This mechanism can be extrapolated to other enolizable precursors potentially present in wine, including yeast metabolites such as α -ketoglutaric acid and 2-hydroxybutan-2-one,⁵⁷ but also to acetone, which can react with anthocyanins during solvent extraction procedures.^{57,151} The resulting products are pyrananthocyanins as presented in Figure 5.11, with $R_3 = \text{CH}_2\text{-COOH}$, $R_4 = \text{COOH}$; $R_3 = \text{H}$, $R_4 = \text{CHOH-CH}_3$; $R_3 = \text{H}$, $R_4 = \text{CH}_3$.

Finally, other pyrananthocyanin derivatives showing maximum absorption in the visible range at 575 nm were recently isolated from Port wine.¹⁵ The characterization of these blue pigments by ESI-MS and NMR showed that they consist of pyranomalvidin 3-glucoside (**5 49**) and its *p*-coumaroylated derivative (**5 50**) linked to a flavanol dimer through a vinyl bridge. Similar pigments were obtained by incubating catechin and carboxypyranomalvidin 3-*p*-coumaroylglucoside in ethanol-water (20:80, v/v) acidified at pH 2 under oxidative conditions. A mechanism involving nucleophilic addition of a vinylflavanol double bond onto the C10 carbon of carboxypyrananthocyanin followed by decarboxylation and oxidation was proposed.

5.5.3.4 Condensation Reactions with Other Aldehydes

The implication of aldehydes other than acetaldehyde in flavanol polymerization was first demonstrated in wine-like hydroalcoholic solutions containing catechin and tartaric acid, the major organic acid in wine, oxidized in the presence of catalytic amounts of iron.⁹ Replacement of tartaric acid and ethanol by other acids and solvents showed that tartaric acid was essential for this reaction. Reaction products showing identical retention times, UV and mass spectra were obtained in higher rates by incubating glyoxylic acid (COOH-CHO) with catechin. Analysis of the major one by two-dimensional NMR showed that it is a catechin dimer in which both catechin units are linked through a carboxymethine bond, in their C8 positions (**5 51**), as shown in Figure 5.12 (left). A mechanism involving oxidation of tartaric acid to glyoxylic acid, catalyzed by ferric ions, and condensation of glyoxylic acid with catechin, as described above for acetaldehyde-mediated condensation reactions, was thus postulated. Detection of the intermediate glyoxylic acid adducts and carboxymethine-linked trimers confirmed this hypothesis. Three other flavanol dimers in which the flavanol moieties are linked through C6-C6 or C6-C8 (*R* or *S*) carboxymethine bonds were also identified by NMR spectroscopy.⁷⁹ Copper ions can replace iron in metal catalysis of tartaric acid oxidation to glyoxylic acid.^{211,212} Other colorless products exhibiting characteristic UV spectra with absorption maxima at 295 nm and a shoulder at 340 nm were formed in the solution containing glyoxylic acid and catechin. Mass spectrometry and NMR analysis showed that they are 6- or 8-formylcatechin derivatives, probably arising from decarboxylation of the intermediate glyoxylic acid catechin adducts.²¹³

Several aldehydes, namely furfural, 5-hydroxymethylfurfural,²¹⁴ isovaleraldehyde, benzaldehyde, propionaldehyde, isobutyraldehyde, formaldehyde, and 2-methylbutyraldehyde,²¹⁵ were shown to react with catechin and malvidin 3-glucoside in the same way as acetaldehyde or

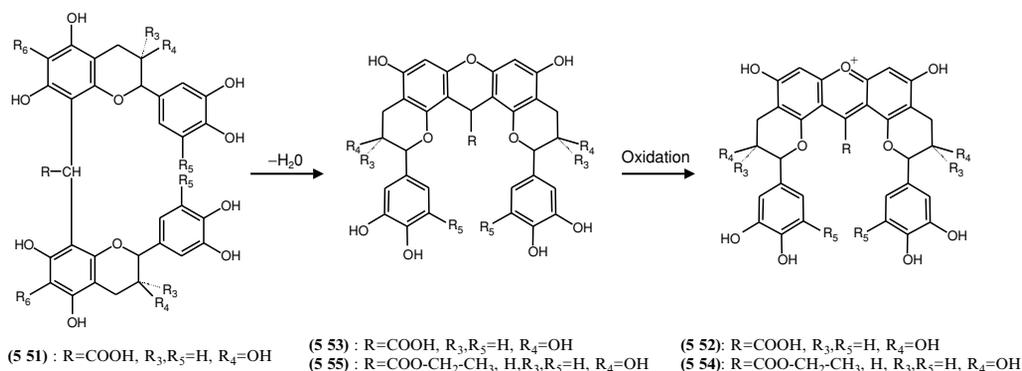


FIGURE 5.12 Structure of catechin-(8-(CH-COOH)-8)-catechin (5 51) and formation of its xanthenes (5 53) and xanthylum (5 52) derivatives.

glyoxylic acid. Furfural and 5-hydroxymethylfurfural are sugar degradation products that may be formed during toasting of oak barrels and present in barrel-aged wines, whereas all the others are constituents of spirits used in the production of fortified wines such as Port wine.

The carboxymethine-linked catechin dimers are unstable and proceed to yellow products, showing absorbance maxima around 440 and 460 nm.^{10,216} The major compound formed by incubating the (8–8) linked dimer in hydroalcoholic solution was isolated.²¹⁷ Its UV-visible spectrum in acidic medium showed two maxima at 273 and 444 nm that were bathochromically shifted after addition of sodium hydroxide, suggesting a xanthylum structure. Mass spectrometry data and complete assignment of the protons and carbons from two-dimensional NMR experiments led to the proposal of the xanthylum structure (5 52). This molecule may result from cyclization of the carboxymethine dimer followed by oxidation of the resulting xanthenes (5 53), which was also detected in the solution. The xanthenes were then obtained by reduction of the xanthylum and its structure confirmed by MS and NMR analysis.²¹⁷ The other pigment formed in hydroalcoholic solution was shown to be the ethyl ester of the carboxy xanthylum (5 54). Incubation of the other carboxymethine bis-catechin isomers yielded other isomers of the carboxy and ethylcarboxy xanthylum salts, through 5–7 or 7–7 dehydration.¹² Mass signals corresponding to the xanthenes and xanthylum arising from dehydration of the carboxymethine (epi)catechin dimers were found in wine stored with no special care to avoid oxidation,¹² demonstrating the occurrence of such reactions in wine. Furfuryl- and hydroxymethylfurfuryl-bridged catechin dimers also proceeded to the corresponding xanthenes and xanthylum salts²¹⁴ (structures as shown in Figure 5.12, with $R =$ furfuryl, hydroxymethylfurfuryl).

Other xanthylum salt structures in which one of the catechin A-rings was substituted with an hydroxyl (in 6 or 8 position) or an ethylcarboxy group (in C8) were proposed on the basis of their NMR and MS data and postulated to result from condensation of the formylcatechin derivatives.²¹³ Finally, carboxymethine-linked trimeric structures containing xanthylum and quinonoid moieties were postulated for pigments showing absorbance maxima at 560 nm, on the basis of their mass signals at m/z 959 and 961 in the positive ion mode.²¹⁸

5.5.4 FACTORS CONTROLLING FLAVONOID REACTIONS IN WINE

As discussed above, studies performed in wine-like model systems and identification of flavonoid-derived species in grape, musts, wines, or marcs have confirmed a number of reaction mechanisms and shown that they actually occur under conditions encountered in wine. Major processes thus demonstrated are based on

- Nucleophilic addition of anthocyanins and flavanols on electrophiles such as quinones, flavylium cations, protonated aldehydes, and carbocations resulting from acid-catalyzed cleavage of proanthocyanidins.
- Acid-catalyzed cleavage of proanthocyanidins and methylmethine-linked species, generating flavanol carbocations and vinylflavanols, respectively.
- Formation of pyranoanthocyanins through reaction of flavylium cations with compounds possessing a polarizable double bond, namely vinylphenol derivatives (including vinylflavanols and hydroxycinnamic acids) and enolizable aldehydes and ketones (e.g., acetaldehyde and pyruvic acid).

It should be emphasized that all known flavonoid derivatives are present in wines only in small amounts so that they account together for a minor proportion of wine polyphenol composition. However, detection of series of derivatives based on all grape anthocyanins or flavanols with different chain lengths suggests that each of the rather simple molecules identified can be considered as a marker of a whole group of similar structures. In addition, most of the primary structures formed are also highly reactive and can undergo acid-catalyzed cleavage or nucleophilic addition reactions similar to those of their precursors, leading to increased structural diversity.

As these reactions occur simultaneously in wine, flavonoid composition depends on the availability of the different precursors involved, the relative reaction rates, and product stability. The respective levels of anthocyanins and flavanols in wine are primarily determined by grape composition, itself influenced by the variety and degree of ripening (see Section 5.3.2), but can be modulated by extraction processes (see Section 5.4). Since both species compete in nucleophilic addition processes, high levels of anthocyanins should result in increased amounts of anthocyanin polymers and anthocyanin–flavanol adducts while high levels of flavanols favor the formation of flavanol polymers and their xanthylum derivatives. Other important precursors include yeast metabolites (e.g., aldehydes, pyruvic acid, vinylphenols) and oxidation products of ethanol (i.e., acetaldehyde) and tartaric acid (i.e., glyoxylic acid). Specific wine-making processes such as addition of wine spirits as performed in Port wine technology also increase the level of acetaldehyde, and possibly of other aldehydes, which explains why many of the flavanyl-pyranoanthocyanins^{53,203} as well as the vinylflavanyl-pyranoanthocyanins¹⁵ were first identified in these wines.

Finally, reactions of flavonoid and nonflavonoid precursors are affected by other parameters like pH, temperature, presence of metal catalysts, etc. In particular, pH values determine the relative nucleophilic and electrophilic characters of both anthocyanins and flavanols. Studies performed in model solutions showed that acetaldehyde-mediated condensation is faster at pH 2.2 than at pH 4 and limited by the rate of aldehyde protonation.⁶⁴ The formation of flavanol–anthocyanin adducts was also limited by the rate of proanthocyanidin cleavage, which was shown to take place at pH 3.2,¹⁵³ but not at pH 3.8.⁵⁸ Nucleophilic addition of anthocyanins was faster at pH 3.4 than at pH 1.7,⁵⁵ but still took place at pH values much lower than those encountered in wine, as evidenced by the formation of anthocyanin–caffeoyltartaric acid adducts,⁵⁵ methylmethine anthocyanin–flavanol adducts,^{18,56} and flavanol–anthocyanin adducts.⁵⁸ The formation of pyranoanthocyanins requiring the flavylium cation was faster under more acidic conditions, as expected, but took place in the whole wine pH range. Thus, the availability of either the flavylium or the hemiketal form does not seem to limit any of the anthocyanin reactions.

Methylmethine flavanol oligomers are more susceptible to acid-catalyzed cleavage than proanthocyanidins. C–C bonds between the methylmethine bridge and the anthocyanin unit are extremely resistant in strongly acidic medium (e.g., thiolysis conditions) where the anthocyanin is in the flavylium form⁵⁶ whereas they are rather labile at wine pH,¹⁸ probably

due to the higher proportion of anthocyanins in the hemiketal form. Cleavage of the methylmethine bonds results in rearrangement to other unstable ethyl-linked species⁶⁴ or to flavanyl-pyranoanthocyanins^{19,201} and vinylflavanyl-pyranoanthocyanins.¹⁵

Pyranoanthocyanins are extremely stable compared to anthocyanins and methylmethine-linked adducts but a decrease in their concentration was observed in model solutions,^{219,220} possibly due to their involvement in more complex structures. Higher temperatures speed up not only the formation of carboxypyrananthocyanins but also their degradation, so that higher concentrations were reached at temperatures 10 to 15°C, which are usual for wine storage.²²⁰ The rate of anthocyanin hemiketal isomerization to the unstable chalcone form is also highly influenced by temperature.²²¹

Oxidation is another important factor for the wine-aging process. Major oxidation reactions taking place in wine following oxygen exposure actually involve other wine constituents that are primarily ethanol and, in the presence of metal ions, tartaric acid⁹ rather than flavonoids, although phenolic compounds have been shown to participate in oxidation of ethanol to acetaldehyde.¹⁸⁸

Wine is exposed to oxygen in transfer operations such as racking or bottling. Some oxygen may also dissolve at the wine surface during storage, especially in barrels. A process referred to as micro-oxygenation, in which oxygen is supplied continuously in quantities small enough to avoid any accumulation, has been proposed to mimic oxidation conditions encountered in barrel aging. Monitoring of flavonoid composition in control and micro-oxygenated wines showed that concentration of anthocyanins and flavanols decreased during aging.⁵² Oxygenated wines contained significantly lower levels of anthocyanins and flavanols than the control wines. The concentration of methylmethine-linked species decreased in control wines, confirming the lability of these pigments,¹⁸ and increased in oxygenated wines as a result of acetaldehyde accumulation. Other derived pigments including pyranoanthocyanins and flavanol–anthocyanin adducts accumulated during storage and were significantly more abundant in the oxygenated wines, possibly because their formation mechanisms require an oxidation step to recover the flavylum moiety. Oxidation reactions are also responsible for the formation of yellow pigments from flavanols. Catechin auto-oxidation in wine-like medium yields the same products as enzymatic oxidation although much slower.²¹⁶ In the presence of iron or copper ions, oxidation of tartaric acid takes over, leading to carboxymethine-linked flavanol oligomers⁹ and xanthylium salts derived from them after oxidation of the intermediate xanthene,^{78,222} which are also formed by ascorbic acid-induced oxidation.^{223,224}

5.6 PHYSICOCHEMICAL AND ORGANOLEPTIC PROPERTIES OF GRAPE AND WINE FLAVONOIDS

5.6.1 IMPACT OF FLAVONOID REACTIONS ON WINE COLOR

The color of an anthocyanin solution is determined by the proportions of the different anthocyanin forms, namely red flavylum cation, violet quinonoidal bases, colorless water or sulfite adducts, and, finally, yellow chalcones. At wine pH, the C2-water adduct (hemiketal or carbinol and its open-chain *cis*-retrochalcone isomer) is actually the predominant form of malvidin 3-glucoside and other grape anthocyanin monoglucosides.¹⁴⁴ These species do not contribute red color. In addition, the chalcone is unstable. Cleavage of this open-chain form generates 2,4,6-trihydroxybenzaldehyde from the A-ring²²⁵ and hydroxycinnamic acids from the B-ring (e.g., syringic acid in the case of malvidin 3-glucoside).¹⁴⁴

Thus, the intense red wine color and its preservation over years require some pigment stabilizing mechanisms to take place. Such stabilization is achieved, on the one hand, through

complexation of the anthocyanin chromophores with other species and, on the other hand, through conversion of labile anthocyanins to more stable derived pigments. The former mechanism may be the first step leading to the latter.²²⁶

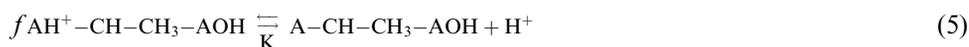
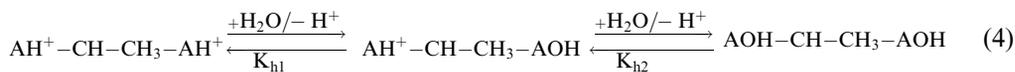
Molecules involved in association with anthocyanins can be an identical anthocyanin molecule, an aromatic acyl substituent in the anthocyanin itself, or another molecule, the processes referred to as self-association, intramolecular copigmentation, and intermolecular copigmentation, respectively. Their mechanisms have been thoroughly investigated and are described in detail in excellent reviews.^{227,228} The major driving force is hydrophobic vertical stacking to form π - π complexes from which water is excluded. Both the flavylium cations and quinonoidal bases but not the hemiketal form are planar hydrophobic structures that can stack to protect themselves from the water environment. The enhanced color intensity resulting from self-association or copigmentation is due to a shift of the hydration balance toward the pigment forms involved in these stable complexes. It can thus be expected to be particularly important in the wine pH range where hydrated forms normally predominate. The bathochromic effect often associated with copigmentation is attributed to the larger amount of quinonoidal base formed by deprotonation of the flavylium. The role of copigmentation in wine color can be estimated by comparing wine absorbance values in the visible range before and after disruption of copigmentation complexes by dilution in a wine-like buffer.²²⁹ Copigmentation has been reported to account for 30 to 50% of the color of young red wines, on the basis of such measurements.

The conversion of anthocyanins to the various pigments mentioned in the earlier sections increases the range of available colors. Moreover, substitutions of the C-ring as encountered in some of the derivatives impede nucleophilic addition of sulfite²³⁰ or water,^{231,232} thus increasing color stability.

Pyranoanthocyanins are orange pigments^{10,16,50,54,57,202} but further substitution with vinylbenzyl derivatives yield blue colors.^{15,233} These pigments are remarkably resistant to sulfite bleaching and hydration compared to anthocyanins.^{16,17} Color intensity was only reduced by half over the pH range 2.2 to 7.2, whereas a malvidin 3-glucoside solution is almost colorless at pH 4 to 5.5. Above pH 6, a bathochromic shift of the absorbance maximum in the visible region and the appearance of another maximum around 600 nm indicated the presence of neutral and anionic quinonoidal bases.¹⁷ Pyranoanthocyanins are also more stable over time than anthocyanins themselves,¹⁶ so that their contribution to wine color is expected to increase during aging. Carboxypyrananthocyanins were actually the major pigments detected in the HPLC profile of a grape marc extract¹⁰ and could serve as markers of changes taking place during wine aging.⁵²

The UV-visible spectra of anthocyanin oligomers and anthocyanin-flavanol adducts resulting from condensation with aldehydes are bathochromically shifted compared to those of their precursors⁵ (10 nm for linear substituents, 20 nm for branched substituents²¹⁵). The molar extinction coefficient of methylmethine-linked catechin-malvidin 3-glucoside adduct in 10% ethanol solution adjusted to pH 0.5 with hydrochloric acid (17,100)¹⁸ is slightly lower than that of malvidin 3-glucoside (20,200).²³⁴ The methylmethine-catechin derivative is much more resistant to discoloration through hydration and sulfite bleaching than genuine grape anthocyanins.¹⁸ Since the C-ring of the anthocyanin moiety in the dimer is not substituted, its greater protection against nucleophilic attack of water (and sulfites) may be due to stabilization through sandwich-type stacking as demonstrated for similar products obtained from a synthetic anthocyanin.²³⁵

The hydration and protonation reactions of the methylmethine-linked malvidin 3-glucoside dimer can be summarized as follows, with AH^+ , AOH , and A representing the flavylium, hemiketal, and quinonoidal base forms, respectively:



Spectrophotometric studies were conducted from pH 0.1 to 5.7. The thermodynamic constants calculated, assuming that the absorbance of AH^+-AH^+ was twice that of AH^+-AOH and that the second hydration constant (pK_{h2}) was equal to the proton transfer constant (pK), were 1.8 (pK_{h1}) for the first hydration reaction and 4.6 ($\text{pK}_{\text{h2}} \approx \text{pK}$) for the second,^{19,236} whereas the hydration and proton transfer constants calculated for malvidin 3-glucoside are 2.6 and 4.25, respectively.¹⁴⁴ Based on these hydration constants, the only significant form of the methyldimine dimer at wine pH is AH^+-AOH , in which one of the anthocyanin moieties is under the red flavylium form and the other one is hydrated, as predicted from mass spectrometry data. Thus, conversion of grape anthocyanins (75 to 80% colorless AOH, 20 to 25% red AH^+ in wine pH range) to the methyldimine bis-anthocyanin (50% AOH, 50% AH^+) may be responsible not only for a shift toward a more purple tint but also for a twofold increase in color intensity.

The flavylium ions of direct flavanol-anthocyanin adducts (i.e., A^+-F and $\text{F}-\text{A}^+$) and anthocyanin dimers (A^+-AOH) have the same UV-visible spectra as anthocyanins. Species in which the anthocyanin is substituted in the 4-position (A^+-F , A^+-AOH) are expected to be resistant to sulfite bleaching and hydration whereas $\text{F}-\text{A}^+$ is as susceptible to water addition as their anthocyanin precursor.²³⁷

Color changes were monitored in solutions containing malvidin 3-glucoside alone or with procyanidin B2 3'-gallate incubated at pH 2 and pH 3.8.⁵⁸ Absorbance values at 520 nm of the pH 2 solutions decreased over time and were highly correlated with the amount of malvidin 3-glucoside. However, the 520 nm absorbance values of the same solutions measured after adjusting the pH at 3.8 remained constant throughout the incubation period, meaning that formation of new pigments that are less susceptible to hydration and mostly present in colored forms at pH 3.8 compensated for the loss of malvidin 3-glucoside. Increased resistance to sulfite bleaching was also observed, especially in the solution containing both the pigment and the flavanol. Similar trends, with slightly higher proportions of sulfite bleaching resistant pigments, were observed in the solutions incubated at pH 3.8, containing A^+-F and A^+-AOH adducts. Browning (estimated by the 420 nm absorbance values) occurred in the solution containing B2 3'-gallate incubated at pH 2 and in both solutions incubated at pH 3.8. Absorbance values at 620 nm also increased over time, especially in solutions incubated at pH 3.8, suggesting that some of the derived pigments were under the quinonoidal form. Although the dimeric and trimeric reaction products detected in these solutions are present in too low amounts to explain their color properties, they may serve as markers of reaction processes leading to a whole range of pigments based on similar structures.

The influence of controlled oxygenation on color characteristics of red wine was studied and correlated with changes in flavonoid composition over a 7-month period.⁵² Pigments formed during aging were less red and more yellow and showed higher resistance to sulfite bleaching than their anthocyanin precursors, as described earlier,³ whereas those resulting from oxygenation were more purple. Higher levels of pyranoanthocyanins and methyldimine-linked pigments were associated with aging and oxidation, respectively, suggesting that both types of derivatives play a part in the observed color changes.

Browning of white wines was shown to be correlated to their flavanol content.^{139,238} Flavanol auto-oxidation and glyoxylic acid-mediated condensation resulting from oxidation of tartaric

acid may contribute to the browning process. The latter mechanism yields much more intense xanthylum yellow pigments^{12,218} and may also be involved in pinking of white wine,^{239,240} since some of the products resulting from glyoxylic acid-mediated reactions are purple pigments.²¹³

5.6.2 IMPACT OF FLAVONOID REACTIONS ON WINE TASTE PROPERTIES

The major organoleptic character associated with flavonoids is astringency although the lower molecular weight flavanols have also been reported to contribute bitterness.^{21,241,242} The physiological grounds of astringency that is described as drying, roughing, or puckering of the mouth mucosa are still obscure. However, it is generally accepted that it is not a taste perceived through recognition by taste receptors, but a tactile sensation.^{243–247}

Astringency of tannins results from their interactions with salivary proteins and glycoproteins, in particular proline rich proteins, causing a loss in the lubricating power of the saliva, or with the glycoproteins of the mouth epithelium. Flavonoid protein interactions are reviewed in Chapter 8. Briefly, the affinity of polyphenols for proteins depends primarily on the number of phenolic moieties, which are the major interactions sites in the molecule,^{248,249} the presence of several phenolic rings in a tannin molecule enabling it to build bridges between the proteins²⁵⁰ or with other polyphenols.²⁵¹ All flavonoids can precipitate proteins if present in sufficient amounts²⁵⁰ but precipitation increases with the degree of polymerization and the number of galloyl units in the polyphenol structure.^{162,252–256} Nevertheless, precipitation does not necessarily reflect astringency that might also be related to conformational changes in the protein structure induced by formation of soluble complexes with tannins.²⁵⁷

Spectroscopic methods such as NMR,^{251,258–262} MS,²⁶³ and light scattering^{262,264–266} have been used to study auto-association of flavonoids and their complexation with peptides in solution. Mechanisms involving hydrophobic interactions and hydrogen bonding were thus proposed. In addition, colloidal particles derived from flavanol aggregation might play an important role in tannin associations with macromolecules.^{264,267}

Within a series of flavanol monomers and dimers, self-association and formation of soluble complexes with peptides, detected by MS, increased with the chain length and with the presence of galloyl substituents.²⁶³ Aggregation of lower molecular weight flavanols increased with their molecular weight but particle size decreased with larger polymers.²⁶⁴ Methylmethine-linked catechin dimers also formed colloidal particles.²⁶⁸ Aggregation is strongly influenced by ethanol concentration and ionic strength. Moreover, the presence of polysaccharides was shown to modify flavanol aggregation^{264,265} as well as precipitation of pentagalloylglucose²⁶⁰ and wine proanthocyanidins²⁶⁹ by gelatins.

Proanthocyanidin astringency has been reported to increase with chain length, up to the decamer level, and to decrease beyond this value, as the polymers become insoluble.²¹ However, higher molecular weight proanthocyanidins (mDP > 20) were shown to be present in a red wine and selectively precipitated by proteins used as fining agents,^{270,271} meaning that they were soluble and presumably astringent.

Assessment of taste is achieved by sensory analysis, from very simple experiments such as triangular tests aiming at determining detection thresholds to complex descriptive analysis approaches. A method referred to as time–intensity that consists in recording continuously the intensity of a given sensation over time under standardized conditions has been applied to study flavonoid bitterness and astringency properties.^{247,272–279}

Recent studies performed using this method have shown that flavanol bitterness decreases from monomer to trimer.²⁴² Epicatechin was perceived more bitter than catechin and the C4–C6-linked catechin dimer more bitter than other procyanidin dimers with C4–C6 linkages. This may be due to the higher lipophilic character of these molecules facilitating their diffusion to the gustatory receptor.²¹ Bitterness of procyanidin fractions in 5% ethanol decreased with their

mean degree of polymerization (3, 10, 70).²⁸⁰ No bitterness was detected when tasting the same fractions in a wine-like solution containing 13% ethanol and acidified with tartaric acid.²⁰

Astringency was classically considered to increase with flavanol chain length and decrease beyond the octamer level,^{21,281} but, to our knowledge, this had never been confirmed experimentally as higher molecular weight fractions were not available. In fact, recent sensory studies performed on a series of proanthocyanidin fractions isolated from grape or apple and differing in chain length, galloylation rate, and content of epigallocatechin units showed that polymeric fractions (mDP 30, 70) were by far the most astringent.²⁰ Larger molecular weight proanthocyanidins extracted from apple (mDP 70), grape seeds (mDP 10, 20% galloylated units), and grape skins (mDP 20, 5% galloylated units, 20% prodelfinidin units) exhibited similar astringency when tasted at the same concentration (0.5 g/l) in a model wine medium, in spite of their large composition differences determined by thiolysis.²⁰ This confirmed earlier results obtained in citric acid solutions and in white wine.²⁸² The higher percentage of galloylation in the seed proanthocyanidins actually compensated for their lower molecular weight since the same fraction after degalloylation with tannase was scored similar to the mDP12 fraction from grape skins.

The decrease of astringency occurring during wine aging is usually ascribed to the conversion of proanthocyanidins to less astringent and eventually insoluble derivatives through polymerization reactions. However, the recent findings developed above suggest that this assumption has to be at least partly revised. On the one hand, astringency of flavonoid derivatives increases with their molecular weight so that reactions leading to higher molecular weight species may result in enhanced rather than decreased astringency. On the other hand, flavonoid reactions in wine yield not only larger molecules, through acetaldehyde-induced polymerization and formation of anthocyanin–flavanol adducts, but also lower molecular weight compounds through acid-catalyzed cleavage, especially if large amounts of monomeric flavonoids such as anthocyanins, are present. An average size reduction of wine molecules might be regarded as a possible alternative explanation for the loss of astringency associated with wine aging.

Very little is known of the sensory properties of the various tannin and anthocyanin-derived species identified. Conversion of proanthocyanidins to methylmethine-linked oligomers has been shown to occur during persimon ripening and postulated to participate in astringency reduction.^{85,283} However, a mDP5 methylmethine-linked catechin fraction obtained by acetaldehyde-mediated polymerization of catechin was equally astringent as equivalent chain length procyanidins.²⁸⁴

The astringency of wine tannin fractions appears to be correlated to the content of flavanol units released after thiolysis regardless of their environment in the original molecules.²⁸² Anthocyanins contributed neither bitterness nor astringency.²⁸⁵ Whether incorporation of anthocyanin moieties in tannin-derived structures affects their interactions with proteins and taste properties remains to be investigated.

Taste perception of flavanols is also greatly affected by other constituents of the medium. In particular, lowering of pH leads to a significant increase in astringency whereas increasing the level of ethanol enhances bitterness.^{286,287} The gustatory perception of tannins may also be altered by the presence of polysaccharides and proteins. A mechanism involving interaction of tannins with soluble pectins released during ripening, impeding their binding to salivary protein, has been proposed to explain changes occurring during fruit maturation.^{249,288,289} The formation of soluble and colloidal polysaccharide–tannin complexes in wine-like model systems was demonstrated by light scattering.²⁶⁴ Polysaccharides isolated from wine inhibited aggregation of flavanols, except type II rhamnogalacturoanane dimer, which enhanced it.²⁶⁴ Carbohydrates of different origins also solubilized flavanol–protein complexes, ionic polysaccharides being more effective.²⁹⁰ Similarly, analyses of the wines

before and after protein fining suggested that the reduction of astringency induced by fining was due to the presence of soluble tannin–protein complexes, along with removal of highly polymerized and highly galloylated tannins.^{271,291}

A sensory study based on an incomplete factorial design allowed to demonstrate that astringency of procyanidins was reduced in the presence of rhamnogalaturonan II added at levels encountered in wine but was modified neither by anthocyanins nor by the other wine polysaccharides (mannoproteins and arabinogalactan proteins).²⁹² Increase in ethanol level resulted in higher bitterness perception but had no effect on astringency.

The role of colloidal phenomena in astringency perception cannot be easily inferred from the available data. Aggregation kinetics and particle size as well as astringency intensity increased with the concentration of flavanols and with their chain length. Factors decreasing flavanol particle size such as presence of ethanol or of manoproteins and arabinogalactan proteins had no effect on astringency perception. In contrast, the presence of RGII and proteins, both of which increase particle size, reduced astringency perception, possibly because the flavanol involved in these aggregates could no longer interact with salivary or mouth proteins.

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6 Dietary Flavonoids and Health — Broadening the Perspective

Mike Clifford and J.E. Brown

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6.1 INTRODUCTION

Toward the end of the 20th century, epidemiological studies and associated meta-analyses suggested strongly that long-term consumption of diets rich in plant foods offered some protection against chronic diseases, especially cancer.^{1–7} Because uncontrolled production of free radicals was thought to be significantly implicated in the etiology of cancer,^{8–11} these observations focused attention on the possible role of radical scavenging and radical suppressing nutrients and nonnutrients in explaining the apparent benefit of such diets.^{12–15} The realization that free radicals were similarly implicated in the etiology of many other chronic diseases,^{16,17} along with the recognition of the “French Paradox”¹⁸ and the seminal papers from Hertog *et al.*,^{19,20} immediately focused attention on flavonoids and the foods and beverages rich therein. An unfortunate, but unintended side effect of these papers was the tendency of many investigators to think of dietary phenols, polyphenols, and tannins (PPT) as encompassing only the flavonoids, and the flavonoids *per se* to encompass only the three flavonols and two flavones that featured in those studies,^{19,20} but this is misleading and was never intended. This particular combination of events almost certainly resulted in many subsequent investigations adopting a too narrow focus.²¹

Subsequent epidemiological studies have supported the association between better health and long-term consumption of diets rich in foods of plant origin.^{22–29} However, whether this is because such diets minimize exposure to deleterious substances (e.g., oxidized cholesterol, pyrolysis mutagens, salt, saturated fat, etc.), or maximize intake of certain beneficial nutrients (e.g., isothiocyanates and other sulfur-containing plant constituents, mono-unsaturated fatty acids, and poly-unsaturated fatty acids, PPT, polyacetylenes, selenium, terpenes, etc.) or some combination as advocated in the “Polymeal” concept, remains unknown.^{30,31} An *in vitro* study indicates that there may be mechanistic basis for true synergy between PPT and isothiocyanates.³²

In contrast, more recent studies seeking to assess the suggested link between the consumption of flavonols and flavones, or other flavonoids, have given much less consistent results. Some studies have suggested a possible protective effect of flavonoids against vascular diseases^{33–37} or certain (but not all) cancers,^{38–43} whereas other studies have suggested no protective effect or even an increased risk in certain populations.^{44–50} Interestingly, an investigation of the relationship between the consumption of broccoli and other cruciferous vegetables and the risk of breast cancer in premenopausal women attributed the beneficial effects to isothiocyanates and not to the phenolic components,⁵¹ although these crops are good sources of dietary phenols^{52,53} including flavonoids,^{54–56} and a potential for synergy *in vivo* has been demonstrated.^{32,57}

In the same time period, various studies have suggested beneficial effects associated with raised consumption of other classes of dietary phenols. For example, increased coffee consumption has been linked with reduced incidence of type II diabetes.^{58–63} Similarly, increased consumption of lignans (or at least greater plasma concentrations of their metabolites) has been linked with reduced incidence of estrogen-related cancers in some^{64–66} but not all studies,^{67,68} and a prospective study was equivocal.⁶⁹ It has been suggested that this inconsistency might have a genetic basis.⁷⁰ Increased consumption of isoflavones has also been associated with decreased risk of estrogen-related cancers and vascular diseases.^{40,71}

This brief introduction demonstrates that the relationships between diet and health are far from simple and most certainly far from fully understood, but for a critical and detailed review of epidemiological data the reader is referred to an excellent paper by Arts and Hollman.⁷² The objective of the review that follows is to record recent changes in the perceived role of flavonoids as health-promoting dietary antioxidants and place these

observations in a broader context embracing other dietary phenols, and mechanisms other than simple radical scavenging and radical suppression.

6.2 THE DIVERSITY OF DIETARY PPT

PPT may be classified in several ways; for example, by biosynthetic origin, occurrence, function or effect, or structure.^{73–75} A classification based on structure and function will be used in this chapter.⁷⁶ Simple phenols are substances containing only one aromatic ring and bearing at least one phenolic hydroxyl group and possibly other substituents, whereas polyphenols contain more than one such aromatic ring. Phenols and polyphenols may occur as unconjugated aglycones or, as conjugates, frequently with sugars or organic acids, but also with amino acids, lipids, etc.⁷⁷ The commonest simple phenols are cinnamates that have a C₆–C₃ structure^{78,79} accompanied by C₆–C₂ and C₆–C₁ compounds, and a few unsubstituted phenols.^{80–82} In general, these occur as conjugates. Flavonoids are the most extensively studied polyphenols, all characterized by a C₆–C₃–C₆ structure, subdivided by the nature of the C₃ element into anthocyanins, chalcones, dihydrochalcones, flavanols, flavanones, flavones, flavonols, isoflavones, and proanthocyanidins. The flavanols and proanthocyanidins generally occur unconjugated but the others normally occur as glycosides. Since the seminal paper of Hertog et al.,⁸³ there has been a tendency to think of dietary PPT as encompassing only the flavonoids, and the flavonoids *per se* to consist only of the three flavonols and two flavones that featured in that study, but this is misleading and was never intended. It is not possible to say with precision just how many individual PPT occur regularly in human diets, but on present evidence a figure in excess of 200 seems reasonable.⁷⁷

The term “tannin” refers historically to crude plant preparations that are capable of converting hides to leather⁸⁴ and such preparations are not consumed as human food. However, the functional polyphenols contained therein at high concentration may also occur in certain foods and beverages but at comparatively low concentrations that would render them totally ineffective for producing leather. These polyphenols may be subdivided into the flavonoid-derived proanthocyanidins (condensed tannins)^{85,86} and the gallic acid-derived and ellagic acid-derived hydrolyzable tannins, this latter subgroup of more restricted occurrence in human food (but commoner in some animal feeds).⁸⁷ The phloroglucinol-derived phlorotannins, while never used for preparing leather, also have a limited occurrence in human food.⁸⁰ The more recent term “phytoestrogen” refers to substances with estrogenic or antiandrogenic activity at least *in vitro*, and encompasses some isoflavones, some stilbenes, some lignans, and some coumarins.⁸⁸ The lignans are not estrogen-active until transformed by the gut microflora.^{88,89} “Antioxidants” is a third function-based term much used to describe PPT, but individual compounds differ markedly in their ability to scavenge reactive oxygen species and reactive nitrogen species, and inhibit oxidative enzymes. Mammalian metabolites of PPT do not necessarily retain fully the antioxidant ability of the PPT found in plants and especially not that of their aglycones as commonly tested *in vitro*.^{90,91}

The PPT discussed above are substances found in healthy and intact plant tissues, and mainly have known structures. However, many traditional foods and beverages as consumed have been produced by more or less extensive processing of such plant tissues, resulting in biochemical or chemical transformations of the naturally occurring PPT. In some cases, for example, black tea, matured red wines, and coffee beverage, these transformations may be substantial, generating large quantities of substances not found in the original plant material. There have been significant advances in the last decade in the chemistry of both red wine^{92–97} and black tea.^{98–106} This includes the characterization of the first large-mass thearubigin derived from four flavanol monomers and containing three benzotropolone moieties,¹⁰⁷ and evidence that peroxidase-like reactions are involved in their production.¹⁰¹ The derived

polyphenols of matured wines are discussed eloquently by Cheynier in Chapter 5. However, the structures of the majority of these novel compounds have yet to be elucidated. Although often described as tannins, the beverages containing these substances are not functional tanning agents, and these substances should be referred to collectively as derived polyphenols (rather than tannins) until such time as their full structural characterization permits a more precise nomenclature.⁷⁶

6.3 THE INTAKE OF PPT

There have been several attempts to estimate the quantities of PPT consumed, either by using diet diaries or food frequency questionnaires and data on the typical composition of individual commodities^{33,36,41,47,50,86,108–113} or by diet analysis.^{42,46,83} In comparison with the comprehensive databases providing the content in the diet of the established micro- and macronutrients, data for the contents of PPT are much more limited. Those data that are available for PPT content have been obtained by many different methods of analysis, rarely take account of the effects of agricultural practice, season, cooking, or commercial processing, are not necessarily just for the edible portion, and may be for varieties of fruits and vegetables different from those consumed in a particular diet under investigation.^{71,110,111}

These are potentially serious limitations since quantitatively cultivars may differ substantially in composition, and the nonedible parts of fruits and vegetables may differ greatly both quantitatively and qualitatively, compared with the flesh or juice.¹¹⁴ Thermal processing of tea beverages results in significant flavanol epimerization and some food products present the consumer with epimers that do not occur naturally.^{115,116} In addition, domestic cooking and commercial processing may, in some cases, cause extensive leaching and destruction,^{56,117–122} although such data as are available sometimes are not completely in agreement^{56,122} and much remains to be investigated.¹²³

Data based on analysis of particular diets avoid these limitations but are usually restricted to a few PPT because of the difficulties and cost associated with quantifying so many individual compounds of known structure, to say nothing of the serious difficulties associated with quantifying the uncharacterized derived polyphenols.¹²⁴ When such data are available, they are usually for PPT as aglycones released by hydrolysis (to simplify the analysis still further) and generally for the flavonols and flavones first studied by Hertog et al.⁸³ since these are amongst the easiest to determine.^{33,34,36,38,39,41,46,47,71,125–127} There are more limited data for flavanones^{47,126} and isoflavones after hydrolysis,^{47,71} and flavanols, and proanthocyanidins^{86,128–130} (which occur as aglycones).

The lack of comprehensive and reliable food composition tables that encompass the PPT (and other nonnutrients) in commodities *as consumed* seriously inhibits the demonstration of statistical relationships between intake and health or disease and may be a factor contributing to the apparent inconsistency in the outcome of epidemiological studies (discussed above). This lack also impedes proper risk assessment of botanicals,¹³¹ as discussed below. A significant development is the creation of three online free-access databases. One provides data for contents in 205 commodities for most classes of flavonoids including the flavanol-derived theaflavins and thearubigins of black tea, but excluding isoflavones and dihydrochalcones.¹³² The second provides data for isoflavones in 128 commodities,¹³³ and the third for flavanols and proanthocyanidins in 225 commodities.¹³⁴ A similar development at the University of Surrey in the United Kingdom covers an even wider range of dietary phenols (including chlorogenic acids, benzoic acids, phenyl alcohols, lignans, and derived polyphenols) in some 80 commodities, but is not yet available online. Developed as part of an EU research program, the Vegetal Estrogens in Nutrition and the Skeleton (VENUS) database constructed in Microsoft Access 2000 contains the daidzein and genistein contents of 791

foods, more limited data for coumestrol, formononetin, and biochanin A, plus levels for the lignans matairesinol and secoisolariciresinol in 158 foods.¹³⁵

A commercial database covers cinnamates, flavonoids, isoflavones, and lignans plus many nonphenolic plant constituents.¹³⁶ Chapter 4 in this volume describes the development of a UK-focused database covering primarily anthocyanidins, flavanols, flavanones, flavones, and flavonols.

While recognizing the limitations (discussed above) of such an approach to estimating diet composition and the intake of PPT, using the University of Surrey database in conjunction with diet diaries available from their other studies^{137–139} has produced interesting data (Table 6.1) and insights. From Table 6.1 it is clear that PPT intakes may vary substantially, and that the flavones and flavonols, upon which most emphasis has so far been placed,^{33,34,36,38,39,41,46,47,71,83,125–127} form a comparatively small part of the total intake for the populations studied. The relatively low consumption of chalcones and dihydrochalcones, isoflavones, anthocyanins, and stilbenes reflects the comparatively low consumption of apples and ciders, soya products, dark berries, and red wines by these populations. The significant contributions made by the hydroxycinnamates (in these populations primarily reflecting coffee consumption^{78,79}) and derived polyphenols (in these populations primarily reflecting black tea consumption^{140–142}) are striking. In this context, “black tea” refers to the beverage prepared from the fermented leaf (as distinct from green tea) and not to the addition

TABLE 6.1
Mean Dietary Intakes of 14 Classes of PPT as Determined from Diet-Diaries and a Food Composition Database

PPT	103 UK Females Aged 20–30 Years ^a		50 UK Males Aged 27–57 Years ^b	
	Estimated as Conjugates	Estimated as Aglycones ^c	Estimated as Conjugates	Estimated as Aglycones ^c
Total, range	100–2300		30–2200	
Total, mean	780	451	1058	598
Hydroxybenzoates	15		23	
Hydroxycinnamates	353	176	670	335
Total flavonoids	210	105	205	103
Anthocyanins	5		9	
Chalcones and dihydrochalcones	0.7			
Flavanols	64		58	
Flavanones	22		89	
Flavones	72		17	
Flavonols	35		26	
Isoflavones	9		0.13	
Proanthocyanidins	7		6	
Ellagitannins	23			
Derived polyphenols	170	170	160	160
Stilbenes	9			
Lignans	0.04			

^aFrom Ref. 108.

^bFrom Ref. 113.

^cAglycones are estimated approximately by taking rutin as a representative flavonoid and 5-caffeoylquinic acid as a representative hydroxycinnamate and adjusting for the relevant molecular masses.

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or otherwise of milk to the beverage prior to consumption. This domination by PPT from black tea and coffee indicates the importance also of considering the hydroxycinnamates and derived polyphenols whenever assessing the dietary significance of PPT, and clearly shows the limitations of looking only at flavonols and flavones after hydrolysis no matter how precise *per se* the data for these aglycones might be.

It is important to stress that data for the composition of black tea and coffee beverage reflect exactly what is consumed (with the exception of the dregs left in the cup) since all transformations associated with processing and domestic preparation have already taken place. Moreover, NEODIET publications⁷⁷ are replete with analytical data from numerous sources for the composition of these beverages (thus better avoiding extreme values associated with any peculiarity of the material analyzed or method of analysis) compared with data for many fruits and vegetables. Accordingly, the estimated consumption figures obtained using this University of Surrey database are likely to be more accurate than would have been the case if solid foods were the major sources of PPT, and data were for raw foods rather than after cooking or processing. This argument applies also to the data for PPT delivered by wines and juices. Using this approach has led us to estimate typical mean intakes of PPT for the two populations so far studied to be in the range 450 to 600 mg calculated as aglycones.

6.4 ABSORPTION OF PPT AND THE NATURE OF THE PLASMA METABOLITES

6.4.1 INTRODUCTION

There have been comparatively few human studies using synthetic labeled forms of dietary PPT. In the absence of the comprehensive data that such studies provide, it is difficult to estimate the precise levels of absorption. Generally, estimates have been based on the urinary excretion of recognizable aglycones released after deconjugation with commercial sulfatase/ β -glucuronidase. A review of 97 bioavailability studies where original data have been recalculated to 50 mg aglycone equivalents found that excretion in urine ranged from 0.3 to 43% of the dose.¹⁴³ In some but not all studies this would include measurement also of the methylated forms, but data on amino acid conjugates are generally conspicuous by their absence. Generally, there would be no account of material that had been absorbed and excreted in bile, or absorbed after transformation to phenolic acids by the gut microflora, and even when phenolic acids have been fed there has rarely been any attempt to quantify the excretion of glycine or glutamine conjugates. Accordingly such data as are available, and hence used by Manach et al.,¹⁴³ will be underestimates of the true absorption, but it is impossible to judge by what amount. On present data,¹⁴³ gallic acid and isoflavones are the most well absorbed dietary PPT, though neither are major components of the European diet. Flavanols, flavanones, and flavonol glycosides are intermediate, whereas proanthocyanidins, flavanol galates, and anthocyanins are least well absorbed. Data for other classes are either nonexistent or inadequate — lack of data for chlorogenic acids and derived polyphenols, major contributors to total PPT intake, is a serious gap in our knowledge. The number of volunteers studied has generally been small (rarely more than ten), thus giving little insight into the between-person variation (whether phenotypic or genotypic), and very few studies have investigated the effects of repeat dosing at typical dietary levels which is how many PPT-rich commodities are consumed. All of these shortcomings must be addressed in future studies.

Although little studied, it is clear that the absorption of dietary PPT may be influenced by the matrix in which they are consumed, with enhanced excretion in urine of easily recognized mammalian conjugates observed when presented in foods with a higher fat content.^{144–148} In contrast, addition of milk to tea does not significantly affect absorption of either flavanols¹⁴⁹ or flavonols¹⁵⁰ despite suggestions that it might.⁴⁶ Alcohol seems not to affect the

absorption of (+)-catechin,^{151–153} resveratrol or quercetin aglycones,^{152,153} or malvidin-3-glucoside from red wine,¹⁵² but hastens (+)-catechin clearance from the plasma compartment either by more rapid excretion or more extensive methylation.¹⁵¹

Extensive studies in humans and animals have indicated that some PPT can be absorbed in the small intestine, for example, certain cinnamate conjugates,^{154,155} flavanols¹⁵⁶ (that occur naturally as aglycones), and quercetin-3-glucoside and quercetin-4'-glucoside.^{157–159} In contrast, quercetin, quercetin-3-galactoside, quercetin-3-rutinoside (rutin), naringenin-7-glucoside, genistein-7-glucoside, and cyanidin-3,5-diglucoside seem not to be.^{159,160} Mechanisms of absorption have not been completely elucidated but involve *inter alia* interaction of certain glucosides with the active sugar transporter (SGLT1) and luminal lactase–phloridzin hydrolase, passive diffusion of the more hydrophobic aglycones, or absorption of the glucoside and interaction with cytosolic β -glucosidase. Although varying with PPT subclass and matrix, when expressed relative to the total intake of PPT, only some 5 to 10% of the amount consumed is absorbed at this site. The major part of that absorbed in the duodenum (not less than 90 to 95% for every substance so far studied) enters the circulation as mammalian conjugates produced by a combination of methylation, sulfate conjugation, glucuronide conjugation plus glycine conjugation in the case of phenolic acids.⁹¹ Only a very small amount of the total PPT consumed, maximally 5 to 10%, enters the plasma as unchanged plant phenols.

The 90 to 95% of the total PPT ingested, plus any mammalian glucuronides excreted through the bile, pass to the colon where they are metabolized by the gut microflora. Transformations may be extensive, and include the removal of sugars, removal of phenolic hydroxyls, fission of aromatic rings, hydrogenation, and metabolism to carbon dioxide, possibly via oxaloacetate.¹⁶¹ A substantial range of microbial metabolites has been identified, including phenols and aromatic acids, phenolic acids, or lactones possessing 0, 1, or 2 phenolic hydroxyls and up to five carbons in the side chain.^{162–189} Many of these metabolites arise from flavonoids and nonflavonoids, requiring a broader approach. Certain *Eubacterium* spp. and *Peptostreptococcus* spp. are able to convert plant lignans to mammalian lignans.¹⁹⁰ *Eubacterium* is of particular interest since this species not only metabolizes dietary (poly)-phenols,^{183–185,187,190–194} but also produces butyrate,¹⁹⁵ a preferred energy source for colonic epithelial cells thought to play an important role in maintaining colon health in humans. Butyrate encourages differentiation of cultured colon cells and through PPAR γ activation decreases absorption by the paracellular route.¹⁹⁶ *Clostridium orbiscindens*, a somewhat atypical member of the genus, is also of interest for its ability to metabolize flavonoids.^{186,197} The yield of phenolic or aromatic acids is variable (up to ten times) between individuals, and for some individuals can vary with substrate,¹⁹⁸ but can be substantial (up to 50%) relative to the intake of PPT substrates.^{167,169,177–180,188,199,200}

There is evidence from cell culture studies that some of the aromatic or phenolic acids, e.g., benzoic, salicylic, *m*-coumaric, *p*-coumaric, ferulic, 3-hydroxyphenylpropionic, and 3,4-dihydroxyphenylpropionic, are transported actively by the monocarboxylate transporter MCT1,^{201–206} but gallic acid and intact 5-caffeoylquinic acid are not. These latter acids may enter by the paracellular route,^{207,208} but absorption by this route is thought to be inhibited by butyrate enhancing PPAR γ activation.¹⁹⁶ *In vitro*, green tea flavanols inhibit the active transport of phenolic acids by MCT1, but the significance *in vivo* of this observation is uncertain.²⁰⁹ Although these acids occur in the plasma primarily as mammalian conjugates some reports suggest that a variable portion may be present in the free form.^{155,210} Table 6.2 summarizes in a semiquantitative manner, so far as current knowledge allows, the fate of a “typical” daily consumption of some 450 to 600 mg of PPT (calculated as aglycones) previously defined in Table 6.1.

There have been significant advances in our knowledge of the PPT-derived metabolites that occur in plasma, i.e., identity of conjugating species, position(s) of conjugation, and

TABLE 6.2
Fate of Ingested PPT

	Aglycones (mg)
Estimated mean daily consumption (mg/day)(from Table 6.1)	450–600
● ~5 to 10% of intake absorbed in duodenum and excreted in urine. Of this	22–60
● 5 to 10% unchanged plant (poly)phenols, and	<6
● 90 to 95% mammalian conjugates	20–55
● ~90 to 95% fermented in colon (unabsorbed PPT + enteric and entero-hepatic cycling of glucuronides, etc.)	400–570
● ≤ Poorly defined and very variable portion (5 to 50%?) absorbed depending on individual's flora and substrates. Mainly mammalian conjugates of microbial metabolites	20–285

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concentrations achieved. It is now clear that there are some significant differences between humans and laboratory animals in this regard.^{144,211,212}

6.4.2 FLAVANOLS, FLAVANOL GALLATES, AND PROANTHOCYANIDINS

Flavanols are unique amongst the flavonoids because they occur in the diet as aglycones.^{55,85} Epimerization at C2 occurs between consumption and appearance in the plasma²¹³ but the chirality of the metabolites has not been measured routinely and it must be assumed that the data that follow have, unwittingly, been obtained on mixtures of isomers. The biological significance of the epimerization is unknown, although in rats, the novel epimers ((-)-catechin, (-)-gallocatechin, and their gallates) are more effective in inhibiting cholesterol absorption.²¹⁴ Nonconjugated (-)-epigallocatechin (up to 0.08 μM),¹⁵⁶ (-)-epigallocatechin gallate (EGCG) (up to 0.34 μM),^{215,216} and traces of (+)-catechin (<2 nM)²¹⁷ have been found in human plasma but free (-)-epicatechin has not.¹⁵⁶ Three (-)-epicatechin metabolites, (-)-epicatechin-3'-*O*-glucuronide, 4'-*O*-methyl(-)-epicatechin-3'-*O*-glucuronide, and 4'-*O*-methyl(-)-epicatechin-5 or 7-*O*-glucuronide have been purified from human urine,²¹¹ whereas the exact fate of (+)-catechin is not known although there is evidence for the formation of (+)-catechin sulfates, sulfo-glucuronides, and 4'-methylated conjugates in plasma and urine.^{147,218} In contrast, (-)-epicatechin gallate (ECG) and EGCG appear to be excreted in bile.^{149,156,219,220} ECG is extensively methylated by human liver catechol *O*-methyl transferase (COMT) at the 4' position and to a lesser extent at the 3' position.^{221,222} EGCG is metabolized first to the 4'-methyl ether and then to the 4',4''-dimethyl ether.²²² Glucuronidation on the B-ring or the D-ring (gallate ester) of EGCG greatly inhibited the methylation on the same ring, but glucuronidation on the A-ring of EGCG or EGC did not affect their methylation. Only a small proportion of the methylflavanols occurs unconjugated to glucuronide or sulfate, and total MeEGC in plasma exceeds total EGC by a factor of $\times 4$ to $\times 6$.²²¹

Traces of procyanidins B1 and B2 have been detected in plasma after hydrolysis of glucuronide and sulfate conjugates.^{223,224}

6.4.3 FLAVANOL GLYCOSIDES

In general, flavanols occur in the diet as glycosides.⁵⁵ Small amounts of unconjugated aglycones may be found in some red wines.²²⁵ Flavanol glycosides and quercetin aglycone have not been convincingly demonstrated in plasma,^{212,226,227} although kaempferol aglycone

has been observed.²²⁸ At least five different quercetin glucuronides²²⁹ as well as some sulfates and mixed conjugates, and four isorhamnetin (3'-methylquercetin) conjugates have been found in human plasma after consumption of foods containing quercetin glycosides. In these studies, isorhamnetin glucuronides accounted for ~30% of the total glucuronides, but only quercetin occurred in a sulfated form. Mixed conjugates accounted for some 11% of the total quercetin. Overall, one fifth of the absorbed quercetin was isorhamnetin and one third was sulfated, leaving some 45% as quercetin glucuronides or mixed conjugates. Some studies have failed to detect flavonol sulfates, but this may be due in part to their destruction by the use of acetone in sample preparation rather than nonformation.

The main kaempferol metabolite in human plasma is the 3-glucuronide.²²⁸ The three major metabolites of quercetin are quercetin-3-glucuronide, quercetin-3'-sulfate, and isorhamnetin-3-glucuronide (found at 0.1 to 1 μM). These are accompanied by lesser amounts of the 4'-glucuronides of quercetin and isorhamnetin, and several uncharacterized metabolites.²²⁶ Quercetin-7-glucuronide was not detected²²⁶ in human plasma, although it is a major metabolite in rats. At low quercetin doses (up to 10 mg), ~100% conversion to isorhamnetin has been reported,^{229,230} whereas at higher doses a quercetin-isorhamnetin ratio of 5:1 has been observed.²²⁶

Using human liver slices, it has now been shown that quercetin-3-glucuronide (a major human conjugate) and quercetin-7-glucuronide (a minor human but major rat conjugate) can both be converted to quercetin-3'-sulfate suggesting that there will be local and transient release of quercetin aglycone wherever β -glucuronidase is active,²³¹ and this might explain the detection of trace amounts of [2-¹⁴C]quercetin in liver.²³² The extent and rate of this transformation were increased when COMT was inhibited.²³¹

6.4.4 FLAVONES AND POLYMETHYLFLAVONES

Flavones occur widely in the diet as *O*-glycosides and *C*-glycosides, and in citrus fruits as unconjugated polymethyl-flavones.⁵⁵ Apigenin glucuronides have been detected in urine after volunteers consumed parsley.²³³ Luteolin aglycone administered to volunteers has been detected in plasma as a monoglucuronide accompanied by a trace of unconjugated luteolin.^{234,235} Chrysin is transformed primarily to the 7-glucuronide with much smaller yields of the 7-sulfate.²³⁶ Apigenin has been shown *in vitro* and *in vivo* to behave synergistically with sulforaphane, an isothiocyanate from cruciferae.^{32,57}

6.4.5 ISOFLAVONES

Isoflavone glycosides (of which a portion is acylated) are characteristic of legumes such as soya. Fermented soya products predominantly contain the aglycones whereas nonfermented products retain the β -glycosides.²³⁷ Soya derivatives are an important component of many processed foods, including human milk-replacers, and such usage increases the intake in western societies.²³⁸ In many dietary supplements and extracts, the contents of isoflavone aglycones as a percentage of total isoflavones may be significant (~2 to 15%) and a few may contain much larger proportions (>85%).²³⁹ The aglycones are more rapidly absorbed than the glycosides.²⁴⁰

Genistein-7-glucoside and daidzein-7-glucoside have not been found in human plasma²⁴¹ but the aglycones have been observed.²³⁹ Unconjugated plasma genistein may reach ~0.4 μM in males given an aglycone dose of 16 mg/kg body weight, but this is unlikely to be achieved from dietary sources even in Asian populations,²⁴² and concentrations of 50 to 100 nM are unlikely to be exceeded.²⁴³ Human metabolism of isoflavone glycosides produces genistein and daidzein 7-glucuronides/7-sulfates and 4',7-diconjugates (including diglucuronides and

mixed conjugates), with monoglucuronides predominant.^{244,245} Daidzein-4',7-diconjugates would not have an unconjugated phenolic hydroxyl so could not function as antioxidants. It has been suggested on the basis of studies *in vitro* that genistein may be converted to fatty acyl esters in human plasma,²⁴⁶ thus facilitating its incorporation into low-density lipoproteins (LDL) in a form not detected by routine methods of analysis.

Mean plasma isoflavone concentrations (after deconjugation) some 5 to 6 h after consumption of soya-based foods have been reported as follows: daidzein 0.5 to 3.1 μM ; genistein 0.3 to 4.1 μM ; glycitein 0.20 to 0.85 μM .^{247–250} Some data for the individual conjugates of daidzein and genistein have been reported. The average pattern was ~54% 7-glucuronide, 25% 4'-glucuronide, 13% monosulfates, 7% free daidzein, 0.9% sulfoglucuronides, 0.4% diglucuronide, and <0.1% disulfate.²⁵¹ A study by Shelnutt et al.²⁵² confirmed in plasma the greater prevalence of genistein monoglucuronides compared with genistein monosulfates (mean ratio ~5:1) whereas for daidzein the equivalent mean ratio was ~1.3:1. Also, for both aglycones, mixed conjugates appeared to account for some 45% of total conjugates. The gut flora metabolism is discussed below and more extensively in Chapter 4.

6.4.6 FLAVANONES

Flavanones occur as glycosides in the diet.¹¹⁴ Studies where volunteers consumed orange juice, grapefruit juice, or a meal containing cooked tomato paste have led to the detection in plasma of hesperetin and naringenin as mammalian conjugates. C_{max} values, after deconjugation, of up to 6 μM naringenin and up to 2.2 μM hesperetin have been recorded. Hesperetin occurred as glucuronides (87%) and sulfo-glucuronides (13%), and naringenin as glucuronides. Neither aglycone was found. The uptake of naringenin from tomato paste, relative to intake, was greater than from the citrus juices.^{146,253–257} Grapefruit juice, rich in naringenin, can inhibit clearance of CYP 3A4-metabolized drugs.²⁵⁸

6.4.7 CHALCONES, DIHYDROCHALCONES, AND RETROCHALCONES

The various chalcones have a comparatively restricted dietary occurrence, with apple and apple products as the major source.¹¹⁴ After consumption of alcoholic cider (1.1 l) a trace of phloridzin has been detected in the plasma of one out of six volunteers,²³⁰ but all six urines yielded phloretin after enzymic hydrolysis of mammalian conjugates. Studies *in vitro* with human microsomes indicate that the chalcone xanthohumol from hops is monoglucuronidated at C4 and C4',²⁵⁹ and some additional transformations of the aglycone have been observed using rat liver microsomes.²⁶⁰ Three glucuronides of licochalcone A, including the 4-glucuronide (*E* isomer) and 4'-glucuronide (*E* and *Z* isomers), have been detected in plasma and urine.²⁶¹ The glucuronide of a hydroxylated metabolite and mercapturic acid conjugates have been produced using rabbit and pig liver microsomes.²⁶²

6.4.8 ANTHOCYANINS

Anthocyanins occur in plants as (acylated) glycosides with the C3 hydroxyl always occupied, and sometimes the C5 hydroxyl also.²⁶³ It is now clear that anthocyanins are found in human plasma as the intact glycosides, rutinosides, sambubiosides, sophorosides, and caffeic acid conjugates of sophorosides.^{152,264–274} However, only some 0.01 to 0.2% of the dose is excreted in urine and maximal plasma concentrations for the six glycosides so far reported range from 1 to 129 nM with a mean total in one study of ≈ 150 nM for several glycosides consumed simultaneously. Alcohol appears to have little effect on the absorption of malvidin-3-glucoside, but plasma concentration appears to be a function of the dose. There

is a pronounced interindividual variation.¹⁵² More recently, evidence for methylation has also been presented, and a range of mammalian conjugates, anthocyanidin glucuronides, anthocyanidin sulfo-glucuronides, and anthocyanin glucuronides, have been characterized, demonstrating that some 2% of the dose may be absorbed.^{264,268} The relatively unstable free aglycones have not been detected. At plasma pH values, anthocyanins will be present as the pseudo-base or quinoidal base rather than the cation characteristic of acidic plant tissues.²⁶³ Since anthocyanins can exist in a retrochalcone form it is possible that some metabolism involves this chemical species (see above).

6.4.9 STILBENES

Resveratrol (3,5,4'-trihydroxystilbene) occurs in a limited number of foods and beverages (e.g., grapes, wines, and peanuts) either as the aglycone or the 3-glucoside (piceid). *Cis* and *trans* isomers are encountered. Some plant materials contain "oligomers" but these materials are rarely significant dietary components.⁸⁸ Resveratrol is sulfated and glucuronidated and very little free resveratrol is found even after large oral doses.^{153,275} Resveratrol-3-sulfate inhibits CYP3A4 *in vitro* (IC₅₀ 1 μ M).²⁷⁶

6.4.10 HYDROXYBENZOIC ACIDS, HYDROXYCINNAMIC ACIDS, AND ASSOCIATED CONJUGATES

Hydroxybenzoic acids are comparatively minor, but widespread components of the diet.⁸² The hydroxycinnamic acids, especially the chlorogenic acids of coffee, are a major contributor to the total dietary intake of PPT.⁷⁹

Chlorogenic acid (5-caffeoylquinic acid) is absorbed, apparently by the paracellular route,²⁰⁸ and can be detected unchanged in plasma.²⁷⁷ The concentrations are low (19 to 45 nM) and some studies have failed to detect this compound,¹⁵⁵ possibly because in the individuals studied absorption by the paracellular route was more limited,¹⁹⁶ or because it was hydrolyzed by commercial β -glucuronidases during sample work-up.¹⁴³ Mammalian conjugates of ferulic (max 200 nM) and sinapic acid (max 40 nM) have been detected in volunteers' plasma after consumption of cereals containing cinnamate-esterified arabinoxylans.²⁷⁸ The bioavailability of chlorogenic acid and other cinnamic acid conjugates is thus largely dependent on gut microflora metabolism.^{154,155,169,278–280}

Gut flora metabolism involves dehydroxylation, hydrogenation, and shortening of the cinnamate side chain, followed by mammalian methylation, and sulfate, glucuronide, and glycine conjugation. Recognized metabolites include monoglucuronides of caffeic, ferulic, isoferulic, and vanillic acids after consumption of coffee, artichoke, red wine, or cider.^{179,230,277,281–284} In one study,¹⁵⁵ free caffeic acid accounted for some 15 to 30% of the total (~506 nM). In contrast, after consumption of beer (500 ml), plasma was reported to contain *unconjugated* caffeic acid (0.03 to 0.30 μ M), vanillic acid (0.07 to 0.09 μ M), and syringic acid (up to 0.05 μ M).

Curcumin (diferuloylmethane) has very low oral bioavailability, but is rapidly absorbed and low nanomolar levels of the parent compound and its glucuronide and sulfate conjugates can be detected in human plasma and portal circulation after very high (nondietary) intakes (3.6 g/day for 1 week). Metabolic reduction occurs in the liver,^{285,286} and glutathione adducts have been observed *in vitro*.²⁸⁷

Gallic acid mono- and dimethyl ethers have been found as conjugates in human plasma after the consumption of either gallic acid, or beverages containing gallic acid or flavanol gallates.^{284,288,289} After consumption of 1.1 l of red wine, plasma treated with deconjugation enzymes contained gallic acid 4-*O*-methyl ether at concentrations up to 0.2 μ M.²⁸⁴

6.4.11 OLEUROPEIN, TYROSOL, AND HYDROXYTYROSOL

Oleuropein, a conjugate of hydroxytyrosol (3,4-dihydroxybenzyl alcohol), is a characteristic but very variable component of olives and olive oil.⁸¹ After consumption of 25 ml virgin olive oil, hydroxytyrosol, 3-*O*-methylhydroxytyrosol (homovanillyl alcohol), and homovanillic acid increase in plasma, as conjugates, predominantly glucuronide.^{290,291} Oleuropein may be deconjugated by the gut microflora.¹⁴⁴

6.4.12 HYDROLYZABLE TANNINS

Hydrolyzable tannins are comparatively restricted in the human diet⁸⁷ and there are no human metabolic data. Studies in rats have indicated that some 63% of a dose of 1 g/kg commercial tannic acid is excreted unchanged in the feces accompanied by small amounts of gallic acid, pyrogallol, and resorcinol. Plasma after enzymic hydrolysis was found to contain 4-*O*-methylgallic acid, pyrogallol, and resorcinol. Urine also contained a small amount of gallic acid after enzymic hydrolysis. The most notable observation from this study is the failure of the gut microflora to metabolize the galloylglucoses efficiently, at least at this substantial dose. The viability or composition of the gut microflora was not reported.²⁹²

Punicalagin is absorbed by rats, and ~3 to 6% of the dose has been characterized as metabolites in urine and feces.²⁹³ Plasma metabolites included punicalagin at ~30 $\mu\text{g/ml}$ (~15 nM) and glucuronides of methylether derivatives of ellagic acid. The major urine metabolites were 6*H*-dibenzo[*b,d*]pyran-6-one derivatives as aglycones or glucuronides. Punicalin, ellagic acid, and gallagic acid were reported in feces, along with 3,8-dihydroxy-6*H*-dibenzo[*b,d*]pyran-6-one. This metabolite has previously been reported in the feces of species (e.g., beaver) consuming large amounts of ellagitannins and is considered to be a hyaluronidase inhibitor. Metabolite production was biphasic. In the first 20 days the main metabolites in biological fluids were derived from punicalagin by hydrolysis and further conjugation (methyl ethers or glucuronic acid derivatives). Beyond 20 days the microflora metabolites start to appear in feces and their mammalian conjugates become the main metabolites in plasma and urine. This dramatic change could be explained by changes in the composition or biochemical competence of the gut microflora.

6.4.13 LIGNINS AND LIGNANS

Lignans are chiral cinnamate-derived glycosides of interest primarily because some are converted by the gut microflora (deglycosylations, *meta*-demethylations, and *para*-dehydroxylations without enantiomeric inversion) to the so-called mammalian lignans^{190,294–297} that after absorption are glucuronidated or sulfated^{244,298} and subject to phase II hydroxylations.²⁹⁷ The aglycones of enterodiol and enterolactone exhibit estrogenic activity²⁹⁹ and increased excretion of mammalian lignans has been associated with a decreased incidence of breast cancer.^{64,300}

The plant precursors of mammalian lignans include secoisolaricinol, laricresinol, matairesinol, 7-hydroxymatairesinol, pinoresinol, and lignin.^{301,302} Flaxseed³⁰³ and whole cereal grains^{297,304,305} are considered the most important dietary sources, but many others are known, for example, strawberry achenes, berries, coffee beans, tea leaves, etc.^{298,305–308}

6.4.14 DERIVED POLYPHENOLS

There have been very few studies on the absorption and metabolism of derived polyphenols despite them forming a very significant proportion of the PPT intake. Theaflavin absorption is unexpectedly rapid, but extremely limited, with a maximal plasma concentration, after

deconjugation, of 1 ng/ml 2 h after a dose of 700 mg.³⁰⁹ Extensive gut flora metabolism of derived polyphenols in black tea has been demonstrated by the detection in urine of substantial amounts of hippuric acid.^{154,167,198} Theasinensins A and D, B-ring linked dimers of EGCG, can be absorbed by mice, and have been found in mouse plasma.^{310,311}

6.4.15 PERSON-TO-PERSON VARIATION

The human data available are still comparatively limited. Most human studies show a considerable interindividual variation. Occasionally, a much greater variation is suggested, as, for example, the detection of phloridzin in the plasma of only one out of six volunteers,²³⁰ and the detection of curcumin sulfate in the feces of only one volunteer out of 15.³¹² The limited nature of the data available make it difficult to generalize with regard to either efficacy or safety.

6.5 PPT METABOLITES IN TISSUES

6.5.1 PSEUDO-PHARMACOKINETIC AND REDOX PROPERTIES

Since for the majority of dietary PPT neither the conjugates consumed nor their free aglycones are detectable in plasma, it is rarely possible to perform true pharmacokinetic analyses. Most so-called pharmacokinetic data that have been published relate to the concentrations of aglycones released after hydrolysis of mammalian conjugates in plasma or urine with commercial β -glucuronidase or sulfatase, and the data so obtained are better referred to as pseudo-pharmacokinetics. It must be noted that some glucuronides are insensitive to some commercial β -glucuronidases (although some can hydrolyze chlorogenic acids¹⁴³), and thus misleading data may be obtained. In addition, there are no convenient sources of enzymes to hydrolyze glycine or glutamine conjugates. These can be cleaved by 6 M HCl, but in some cases this process destroys the phenolic moiety.³¹³

Published human data are summarized in Table 6.3. Although the maximum concentration achieved transiently varies to some extent with PPT subclass and matrix in which it is consumed, it is unlikely that plasma metabolite concentrations will routinely exceed 10 μ M in total, and \sim 1 μ M for total aglycones. The reported T_{\max} values range from 1 to 2.5 h for substances absorbed in the duodenum,^{147,151,157,158,160,216,223,314-317} up to 5 to 12 h when microbial metabolism is a prerequisite.^{160,177,255} Published elimination half-lives are very variable, ranging from as low as 1 h^{221,318} to values in excess of 20 h.^{157,158,160} The very low values may be artifacts of observation periods less than the true half-life, whereas the very high values may be exaggerated because of a biphasic elimination reflecting significant enterohepatic circulation of glucuronides. Indeed, mammalian conjugates produced in the gastrointestinal epithelium do not necessarily enter the circulation — a significant portion is returned to the gut lumen^{231,319,320} where they may be deconjugated and further degraded by the gut microflora.

The effect of repeat dosing has rarely been studied. Repeated consumption of green or black tea produced only slight day-on-day accumulation of flavanols in plasma,^{149,321} and modest increase in the intrinsic resistance of isolated LDL to oxidation *ex vivo*,³²² suggesting that, in general, significant elimination occurs in a time period shorter than the interval between repeat doses.

Table 6.4 summarizes the concentrations of a range of endogenous (i.e., nondietary) simple phenols, including α -tocopherol, and ascorbate in plasma from healthy individuals. The total simple phenol and ascorbate concentration is between 159 and 380 μ M. The maximum additional concentration that is likely to be achieved from dietary sources, 3 to 22 μ M, is marginal by comparison adding only between 0.3 and 5% if it is assumed, quite reasonably, that the “typical” mean intake is taken over three equal meals. Many people consume a much smaller quantity of dietary PPT and even those consuming double the

TABLE 6.3
Plasma Pseudo-Pharmacokinetics After Consumption of Normal Portions of Rich Sources

PPT Subclass	C_{\max} (nM)	C_{\max} (nM)	Urine
	Unchanged ^a	Mammalian Conjugates	Excretion %
Anthocyanins	10–150	Traces	ND–0.1
Flavanols, low fat	40–140	1000–2000	0.5–4.0
Flavanols, high fat	150–220	Up to 6200	25–30
Flavonol glycosides	Minute traces	ND	0.5–2.5
Flavonol aglycones	Minute traces	350–1100	
Flavanone glycosides	Minute traces	120–1500	4–10
Isoflavone glycosides	Minute traces	900–4000	20–55
Isoflavone aglycones	10–150	500–6500	
Cinnamates and chlorogenic acids	Up to 120	Up to 500	1–2
Resveratrol aglycone	Minute traces	No quantitative data	64–70
Oleuropein	ND	Up to 60	55–60
Phenolic gut flora metabolites		20–60	Up to 50
Hypothetical total if all consumed in one meal	250–780	2890–21,720	

^a C_{\max} = maximum concentration achieved transiently in plasma.

Note: ND, not detected.

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TABLE 6.4
Plasma Concentrations (μM) of Endogenous (Nondietary) Phenols and Other Plasma Antioxidants

	Plasma Concentration in Healthy Individuals
Homogentisic acid	0.014–0.070 ^a
<i>p</i> -Hydroxyphenyl lactate	40–90 ^b
<i>p</i> -Hydroxyphenyl pyruvate	14–60 ^b
Tyrosine	60–130 ^{b,c}
Ascorbate	40–70 ^{d,e}
α -Tocopherol	5–30 ^f
Total endogenous phenols and antioxidants	159–380
Hypothetical total diet-derived phenols	3.1–22.5 ^g
Averaged over three meals gives a transient increase of between 0.3 and 5%.	~1–7.5 ^g
Many people consume much less	

^aFrom Ref. 523.

^bFrom Ref. 524.

^cFrom Ref. 525.

^dFrom Ref. 526.

^eFrom Ref. 527.

^fFrom Ref. 528.

^gFrom Table 6.3.

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average amount (450 to 600 mg calculated as aglycones) adopted in this review will only achieve a transient 5 to 10% increase in total plasma antioxidant content.

Many investigators have attempted to demonstrate increases in plasma antioxidant capability following the consumption of foods, beverages, or supplements rich in PPT. Table 6.5 summarizes the outcomes of 37 such studies.^{210,216,266,284,317,323–348} The test substances included a range of fruit and vegetable products, including juices, alcoholic beverages, tea, and chocolate. In view of the calculations presented in Table 6.3 and Table 6.4, it is perhaps not surprising that increases in plasma antioxidant capacity were often undetectable, and at best, small and transient. Moreover, in four studies that produced increases in plasma antioxidant capability it could be attributed, at least in part, to increased plasma ascorbate.^{336,343,346}

In view of these observations, it is instructive also to consider the redox potentials of PPT-derived mammalian metabolites that are known to reach plasma, and to compare these with the corresponding values for the endogenous plasma antioxidants. The polyphenols with the lowest redox potentials are flavonoids with vicinal hydroxyls in the B-ring, and conjugation extending to the A-ring, e.g., quercetin aglycone (330 mV at pH 7).³⁴⁹ If the conjugation does not extend beyond the B-ring, then the redox potential is significantly higher even for (–) epigallocatechin gallate (480 mV at pH 7)³⁵⁰ with three vicinal hydroxyls. The value rises again when there are only two vicinal hydroxyls (e.g., (+)-catechin 570 mV³⁵¹ or caffeic acid 540 mV³⁵¹), a single *para*-hydroxyl (e.g., hesperidin 720 mV³⁵¹), or isolated (*meta*) hydroxyls (e.g., resorcinol 810 mV³⁵²). These comparisons are extended to the endogenous (nondietary) plasma antioxidants in Table 6.6. Figure 6.1 illustrates the marked effects of mammalian and microbial metabolism on the redox potential of PPT aglycones that are frequently examined in *in vitro* systems designed to demonstrate their potent antioxidant properties.

Table 6.6 indicates that the diet-derived PPT metabolites are able thermodynamically to scavenge some or all of the damaging radicals should they come into contact. However, these metabolites are so hydrophilic, e.g., quercetin-3-glucuronide ($K = 0.008$)^{353,354} compared with quercetin ($K = 66$)^{353,354} and α -tocopherol ($K = 550$),³⁵⁵ that it is unlikely they will encounter lipid-derived radicals. However, any phenoxyl radicals generated will have to be removed either by transfer of the unpaired electron to an endogenous scavenger such as α -tocopherol, ascorbate, glutathione, or serum albumin, or by dismutation or disproportionation although these latter mechanisms seem somewhat unlikely *in vivo* because of the relatively low phenoxyl radical concentrations. The implied demand for α -tocopherol and ascorbate is particularly interesting, since two of the supplementation studies (Table 6.5) and a study in which rats were fed secoisolariciresinol produced reductions in plasma ascorbate or α -tocopherol,^{337,356} and the major sources of dietary PPT (coffee and black tea) supply neither. Moreover, it is known that for ~14% of the over-65 population subgroup in the United Kingdom the mean plasma ascorbate value is below 11 μM ,³⁵⁷ indicating biochemical depletion,³⁵⁸ suggesting that for heavy consumers of black tea or coffee within this population subgroup the transient concentration of PPT metabolites may approach or even exceed plasma ascorbate.

From the data assembled, it is difficult to envisage how diet-derived PPT metabolites can make a major contribution to radical scavenging in plasma compared with the contribution to be expected from the endogenous antioxidants in healthy individuals replete in ascorbate.^{21,359} An independent, but contemporaneous review of 93 intervention studies³⁶⁰ reached a similar conclusion with regard to foods and beverages and *in vivo* antioxidant effects. Herbal remedies and dietary supplements were sometimes more effective, reflecting PPT doses substantially above those achieved by diet alone, and frequently manifest through endpoints not directly associated with antioxidant effects. Contrary to the view expressed over the last decade there can now be little doubt, that if diets rich in fruits and vegetables are advantageous, at least in part by virtue of their content of PPT, then mechanisms other than antioxidant ability are implicated.^{21,361–363}

TABLE 6.5
The Outcome of 37 Studies^a in Which Volunteers Were Given Foods, Beverages, or Supplements Rich in PPT and Plasma was Analyzed for Total Antioxidant Activity

Thirty-seven studies, (poly)phenol-rich diet compared with control

- Thirteen studies (three high and three very high doses) showed no change in plasma antioxidant status *ex vivo*
- Twenty-four studies (ten low and seven moderate doses) showed small and transient increases in plasma antioxidant status *ex vivo*
- One showed reduction in plasma vitamin E
- One showed reduction in plasma ascorbate and glutathione

^aFrom Refs. 210, 216, 221, 255, 266, 284, 317, 318, 323–348.

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6.5.2 BINDING TO PLASMA PROTEINS

Data for the metabolites in plasma are generally for the unbound forms, but there is ample evidence that PPT bind noncovalently to proteins.³⁶⁴ Most studies on PPT–protein interaction have focused on protein utilization or astringency^{365–368} but a few studies have addressed binding to plasma proteins and lipoproteins.^{149,342,369–371} Strongest binding has been associated with 1,2-dihydroxyphenols and proline-rich proteins such as those characteristic of human saliva and structure–activity relationships have been reported.^{372,373}

Evidence has been presented from studies *in vitro* that unconjugated flavanols and proanthocyanidins bind preferentially to histidine-rich glycoprotein³⁷⁴ or to Apo-A1, the major protein in human LDL, but preferentially to transferrin in rat plasma.³⁷¹ However, although unconjugated flavanols may occur in human plasma, the majority of the phenolic metabolites, as discussed above, do not have a 1,2-dihydroxyphenol moiety and are relatively hydrophilic, and weak associations are therefore to be expected, possibly explaining why dialysis removes such metabolites from isolated LDL.³²³ Only traces of flavonoid-like substances have been recovered from plasma LDL of unsupplemented individuals. Tentatively, rutin (not confirmed by LC–MS and probably a misidentification) and quercetin-3-glucuronide were detected at 93 and 73 pmol/mg protein, respectively.³⁶⁹ Following green tea consumption (eight cups per day for 3 days), flavanols are associated primarily with the plasma proteins ($\sim 0.47 \mu M$) and high-density lipoproteins ($\sim 0.17 \mu M$), with lesser amounts in the LDL ($0.077 \pm 0.021 \mu M$) and least in the very low-density lipoproteins ($\sim 0.08 \mu M$). Feeding isoflavones in burgers or soya bars to provide doses ranging from ~ 0.03 to 1 mg/kg body weight resulted in less than 1% of total plasma isoflavones recovered from LDL proteins,³⁴² with genistein, daidzein, and equal concentrations of ~ 10 , ~ 3 , and up to 0.2 pmol/mg protein, respectively.³⁷⁵ Generally, the levels of PPT metabolites incorporated in LDL have been insufficient to increase its intrinsic resistance to oxidation *ex vivo*,^{149,323,337,376} although there have been exceptions.^{322,375}

6.5.3 EFFECTS ON THE VASCULAR SYSTEM

In the context of vascular disease, numerous studies have focused on the ability of phenolic compounds, as pure aglycones and as glycosides, to delay the oxidation of LDL *in vitro*.^{377–379} This work has been paralleled by studies investigating the propensity with which the consumption of PPT-rich foods and beverages reduce the oxidation of LDL *ex vivo*.^{149,322,337,341,348} The results of these studies, employing realistic PPT intakes, have

TABLE 6.6
A Summary of Published Data for Transient Maximal Plasma Concentrations of Diet-Derived (Poly)Phenols, Typical Plasma Concentrations of Endogenous Phenols and Antioxidants, and Associated Redox Potentials (pH 7)

Mammalian Metabolite Hydroxylation Pattern	Maximal Transient Concentration (μM)	Redox Potential (mV) at pH 7
1,2,3- <i>vic</i>	0.14 ^a	400–600 ^{d,e,f}
1,2- <i>vic</i>	0.8 ^a	500–650 ^{d,g,h}
Single <i>para</i> or isolated <i>meta</i> hydroxyls	10 ^a	700–1050 ^{d,e,g,i,j,k}
Blocked/inactive	?	Inactive
Damaging radicals		Redox Potential (mV) at pH 7
Hydroxyl radical		2310 ^h
Superoxide radical anion		1800 ^h
Alkoxy radical		1600 ^l
Alkyl-peroxy radical		1000 \pm 60 ^{h,l,m}
PUFA (bis-allylic) radical		600 ^h
Endogenous phenols and antioxidants in plasma	Typical plasma concentration (μM)	Redox Potential (mV) at pH 7
Endogenous <i>p</i> -phenols	114–280 ^a	\approx 700 ^{d,e,g,i,j,k}
α -Tocopherol	5–30 ^b	\approx 500 ^{d,h}
Ascorbate (depleted)	50–70 ^b (\leq 11) ^c	\approx 280 ^{e,h}
Glutathione		–276 ⁿ

^aFrom Table 6.3.

^bFrom Table 6.4.

^cFrom Ref. 357.

^dFrom Ref. 350.

^eFrom Ref. 352.

^fFrom Ref. 529.

^gFrom Ref. 351.

^hFrom Ref. 530.

ⁱFrom Ref. 531.

^jFrom Ref. 532.

^kFrom Ref. 533.

^lFrom Ref. 534.

^mFrom Ref. 535.

ⁿFrom Ref. 536.

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shown either a small decrease in LDL oxidizability or no change at all. However, it does not necessarily follow that the reductions in cardiovascular disease (CVD) associated with PPT intake, shown in epidemiological studies, will relate solely to the ability of phenols to modify LDL oxidizability. CVD engages a variety of cell types, including endothelial cells, vascular smooth muscle cells (VSMC), leukocytes, and platelets.³⁸⁰ Indeed, a key early event in atherogenesis is the adhesion of leukocytes to the arterial wall and their subsequent movement into the subendothelial space.³⁸⁰ This process is mediated via the expression of adhesion molecules on the surface of endothelial cells that are expressed constitutively but can be significantly induced by proinflammatory mediators, such as tumor necrosis factor- α (TNF-

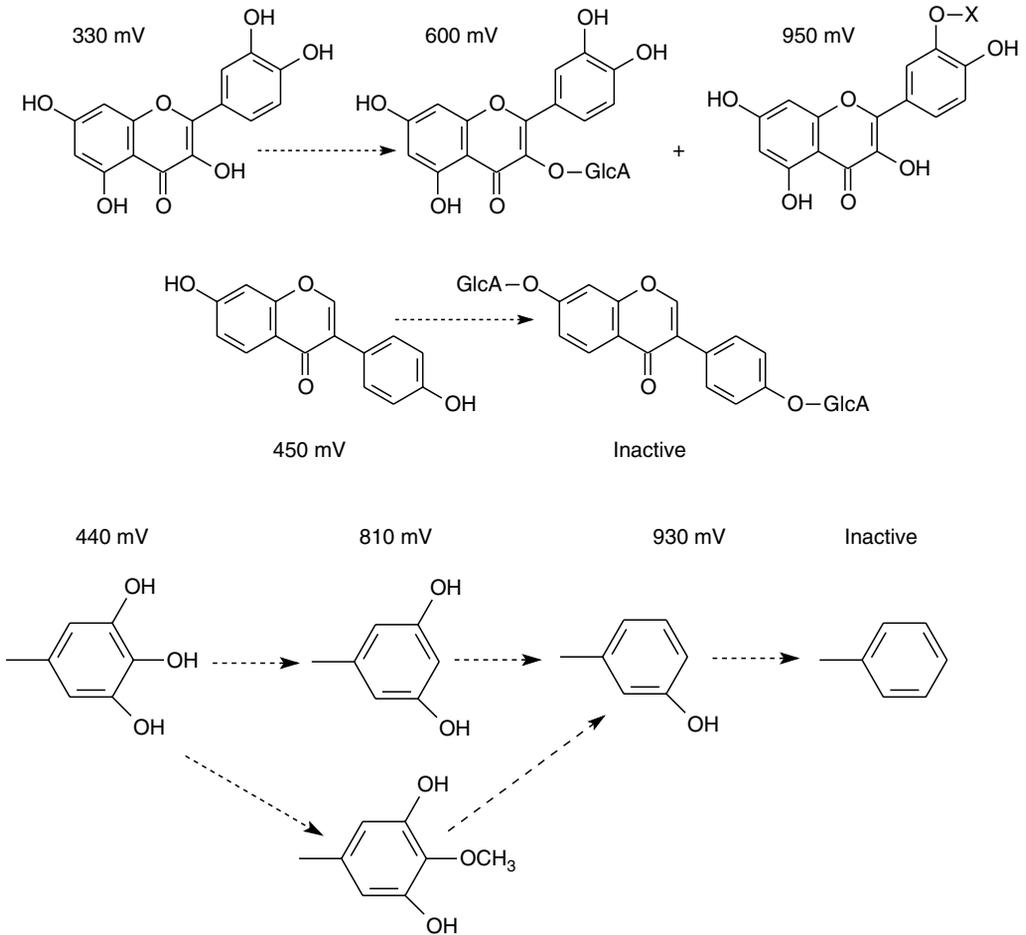


FIGURE 6.1 Illustration of the effects of mammalian metabolism and microbial metabolism on the redox potential of (poly)phenols found in plasma compared with their precursors in the diet and the aglycones commonly used in studies *in vitro*. (Reprinted from Clifford, M.N., *Planta Med.*, 12, 1103, 2004. With permission.)

α), interleukin-1 β (IL-1 β), and other stimuli. The redox-sensitive transcription factor, nuclear factor- κ B (NF- κ B), under the control of I κ B, is considered essential in mediating the response to TNF- α as many relevant genes contain NF- κ B binding sites including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein (MCP).³⁸¹ Mitogen-activated protein (MAP) kinases, activated via receptor tyrosine kinases, are also important in vascular gene regulation. Three main groups of MAP kinases exist and include extracellular signal regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38. These cell-signaling pathways are considered targets for the action of PPT and their effect on atherosclerotic and thrombotic pathways may be important. The modulation of these and other pathways by PPT will be discussed in the context of the vasculature and their potential impact on CVD.

Red wine consumption in humans reduces TNF- α -induced adhesion of monocytes to endothelial cells *ex vivo*³⁸² and is associated with the downregulation of monocyte adhesion molecules, in particular very late activation antigen-4 (VLA-4). Estruch et al.³⁸³ reported similar findings for VLA-4 and also showed that levels of MCP-1, VCAM-1, and ICAM-1 were decreased after red wine consumption. Interestingly, control studies with gin revealed no effect

on these variables indicating that red wine phenolic compounds were likely to be responsible. Other studies have shown that red wine prevents the activation of NF- κ B in human peripheral blood mononuclear cells induced by a fat-rich meal.³⁸⁴ Control studies with vodka highlighted the potential role of the phenolic component of red wine.³⁸⁴ Studies with tea have revealed little effect on markers of vascular inflammation³⁸⁵ although clear beneficial effects have been demonstrated in terms of other indices of vascular function.³⁸⁶ Grape seed proanthocyanidins reduce adhesion molecule expression in systemic sclerosis *in vivo*³⁸⁷ although the effect *in vitro* was only evident on VCAM-1 expression.³⁸⁸ Indeed, many PPT attenuate the endothelial production of VCAM-1 induced by either TNF- α or IL-1 β *in vitro*, including EGCG and ECG,³⁸⁹ oleuropein and hydroxytyrosol,³⁹⁰ apigenin and luteolin,³⁹¹ resveratrol,³⁹⁰ genistein,^{392,393} and a variety of gallates.³⁹⁴ Furthermore, TNF- α -induced MCP-1 secretion is inhibited by genistein,³⁹³ daidzein,³⁹² petunidin-3-glucoside, and delphinidin-3-glucoside³⁹⁵ as well as anthocyanin-rich berry extracts.^{396,397} These effects overall appear to reduce the ability of monocytes to adhere to TNF- α -activated endothelial cells.^{389,398} However, it is evident that while some PPT operate by preventing NF- κ B activation,^{384,394,396,398} others perhaps operate via a different system.³⁸⁹ Indeed, quercetin reduces ICAM-1 expression by preventing TNF- α activation of activator protein-1 (AP-1) via the JNK pathway.³⁹⁹ A common theme with many of the experiments discussed above is a lack of consideration for either the form of the PPT in the incubation or the concentration used. Many studies utilize concentrations far in excess of those attainable *in vivo*, for example, quercetin at 100 μ M³⁸⁴ and potentially dangerous if it were achieved since 20 μ M quercetin arising from intravenous infusion caused liver and kidney damage in cancer patients.⁴⁰⁰ Such *in vitro*–*in vivo* disparity highlights the need to evaluate the properties of mammalian metabolites at realistic concentrations. Some workers have tried to overcome this by assessing the effects of PPT metabolites produced by intragastric administration to rats.⁴⁰¹ Interestingly, the metabolites of (+)-catechin were more effective at reducing monocyte adhesion than the parent compound. However, the concentrations and identities of the metabolites used are not clearly stated (\sim 6 μ M for catechin) and appear to be higher than those normally present in plasma after a catechin-rich meal. Metabolites of the other flavanols, for which such concentrations might more easily be approached (see above), were not evaluated in this system, but quercetin metabolites were inactive.

The accumulation and proliferation of VSMC within the arterial wall is another key aspect of atherosclerosis in which PPT may also have a role. VSMC proliferation involves the activation of MAP kinases that regulate downstream targets related to cell cycle, proliferation, and migration.⁴⁰² Quercetin inhibits serum-induced VSMC proliferation, migration of VSMC from arterial explants, and platelet-derived growth factor (PDGF)-induced phosphorylation of p38 MAP kinase.⁴⁰³ Quercetin also reduces TNF- α -induced activation of matrix metalloproteinase-9 expression via ERK1/2, AP-1, and NF- κ B inhibition.⁴⁰⁴ EGCG reduces PDGF-BB-induced activation of MAP kinases in VSMC although no effect was observed with angiotensin II induction.⁴⁰⁵ Red wine polyphenols reduce vascular endothelial growth factor (VEGF) release in response to PDGF, an effect that also involves inhibition of the p38 MAP kinase pathway.⁴⁰⁶ A red wine extract and resveratrol have also been shown to reduce VSMC proliferation.⁴⁰⁷ Physiologically attainable concentrations of EGCG and EGC are effective at reducing VSMC proliferation⁴⁰⁸; however, in general the concentrations employed in these studies are greater than those that can be attained normally. Furthermore, these studies focus on aglycones rather than the metabolites present *in vivo*. It is interesting to note, however, that quercetin-3-glucuronide (10 μ M) inhibits angiotensin II JNK activation reducing AP-1 binding and a decrease in VSMC hypertrophy.^{409,410} On certain diets quercetin-3-glucuronide might reach a transient 1 μ M but it is not known whether long-term exposure to such a concentration might be modestly effective, or whether other quercetin metabolites might also contribute. Studies of this kind are required to assess the effects of PPT *in vivo*.

The effect with which PPT modulate the response of platelets is also pertinent to vascular disease, in particular, thrombosis. Resting platelets inhibit the respiratory burst of neutrophils whereas thrombin-activated platelets increase the respiratory burst. Quercetin and resveratrol at picomolar concentrations attenuate this response by preserving endothelial CD39/ATP-dase,⁴¹¹ and on present evidence (see above) such concentrations might be achieved locally following deglucuronidation at a site of inflammation.

The modulation of nitric oxide (NO) production by endothelial cells is a further route through which PPT may be important owing to the potent vasoprotective properties of NO. Polyphenol-rich beverages and extracts such as red wine,^{412–414} black tea,⁴¹⁵ cocoa,⁴¹⁶ and artichoke⁴¹⁷ can increase the expression and activity of endothelial NO synthase (eNOS) and consequently NO formation. Furthermore, EGCG,⁴¹⁸ resveratrol,⁴¹⁹ cyanidin-3-glucoside,^{420,421} genistein,⁴²² and luteolin and its 7-glucoside⁴¹⁷ have been shown to modulate NO production. Some phenols appear to operate via activation of the phosphatidylinositol-3 OH kinase/Akt pathway and an increase in intracellular calcium⁴²³ while others do not.⁴²² An upstream regulator may include p38 MAP kinase.⁴¹⁵ These effects have generally been demonstrated only at concentrations greater than 10 μM , although cyanidin-3-glucoside was effective at 0.1 μM ,⁴²⁴ a concentration that might reasonably be achieved *in vivo*. Genistein was effective *in vitro* at 1 μM ,⁴²² a concentration that might conceivably be approached following supplementation and local deglucuronidation, but the situation is far from straightforward. Deglucuronidation would be consequent upon inflammation and likely accompanied by superoxide radical anion leading to peroxynitrite formation from NO. Peroxynitrite is capable of initiating lipid peroxidation, and of nitrosating tyrosine residues in proteins, thus potentially interfering with cell signaling.⁴²⁵ Quercetin-3-glucuronide, one of the three major human conjugates of dietary quercetin glycosides, and quercetin that contemporaneously could be produced from it by deglucuronidation, have been shown *in vitro* to protect the vascular endothelium^{353,409,426,427} from the damaging effects of peroxynitrite and suppress peroxynitrite-induced consumption of lipophilic antioxidants in human LDL.⁴²⁶ A closer focus on plasma metabolites employed at realistic *in vivo* concentrations is required to disentangle these complexities and assess properly the importance of dietary PPT on NO production, cell signaling, and vascular tone.

Another human metabolite, quercetin-4'-glucuronide, inhibits xanthine oxidase *in vitro* at a concentration in plasma that on normal diets can realistically be approached ($K_i = 0.25 \mu\text{M}$).⁴²⁸ Various mammalian conjugates of quercetin suppress the formation of conjugated dienes,⁴²⁹ and some daidzein and genistein glucuronides bind to estrogen receptors and may occur *in vivo* at a sufficient concentration to exert a modest estrogenic effect.⁴³⁰

Although classically mammalian conjugates of drugs are viewed as significantly less active than the parent drug and therefore irrelevant physiologically, as illustrated by the examples above, this is not inevitably the case when PPT are considered. However, it should be noted that even when studies *in vitro* show an effect of a plasma metabolite at a concentration that might reasonably be expected *in vivo*, protein binding may impede or prevent this effect.

6.5.4 TRANSFORMATION OF PPT METABOLITES AFTER ABSORPTION

Although with few exceptions (some flavanols, isoflavones) PPT aglycones do not normally occur in tissues, it is now recognized that transient and local deconjugation could occur in plasma, liver, and probably other tissues. Studies using human liver slices have demonstrated the potential for hydrolysis of quercetin glucuronides and the transient release of quercetin.⁴³¹ β -Glucuronidase is released to the plasma from the liver⁴³² and from activated neutrophils and eosinophils under oxidative challenge.^{433,434} β -Glucuronidase is able *in vitro* to release luteolin from luteolin glucuronide.⁴³¹ The activity of β -glucuronidase is significantly raised in the

plasma of hemodialysis patients compared with healthy controls,⁴³³ and the lower plasma pH value associated with a site of inflammation is optimal for this enzyme.²³⁵ Collectively these data suggest that aglycones might be released from glucuronides *in vivo* raising the possibility of biologically significant aglycone-mediated interactions at sites of inflammation, but possibly not in healthy tissues or diseased tissues where inflammation has not occurred.⁴³⁵ It has been reported that estrogen-3-sulfates can be deconjugated *in vivo*,⁴³⁶ but whether this enzyme is able similarly to deconjugate sulfated PPT metabolites is unknown. *In vitro*, human CYP 1A2 and CYP 2C9 demethylate certain flavonoids,⁴³⁷ and hepatic demethylation of methoxyestradiol has been observed in hamsters,⁴³⁸ but it is not known whether the human isoforms can demethylate PPT *in vivo*. If so, then these enzymes may also generate the aglycones locally.

Gut microflora metabolites may also be important. As discussed above, the mammalian lignan aglycones, enterodiol and enterolactone, are estrogenic,²⁹⁹ and equol is more estrogenic than its dietary precursor, the isoflavone daidzein.⁴³⁹

Some C₆-C₃, C₆-C₂, and C₆-C₁ metabolites produced by the gut microflora from a wide range of dietary PPT inhibit platelet aggregation *in vitro*.^{440,441} Animal and studies *in vitro* also suggest that some C₆-C₂⁴⁴² and especially C₆-C₃⁴⁴³ metabolites interfere with various enzymes in the mevalonate pathway including HMG-CoA reductase, the rate-limiting enzyme in hepatic cholesterol biosynthesis, albeit at concentrations unlikely to occur in plasma. However, these observations are of interest since commodities rich in PPT that would yield such metabolites, and the metabolites when given *per os*, have been shown to inhibit platelet aggregation⁴⁴¹ or to have cholesterol-lowering activity in animal⁴⁴³⁻⁴⁴⁸ and human studies,⁴⁴⁹ and such gut flora metabolites may have contributed to the *in vivo* effect. Interference in the mevalonate pathway, particularly HMG-CoA reductase inhibition, may have broader human significance.⁴⁵⁰ Individual flora differ extensively in their yields of such metabolites^{167,180,183,184} or hippuric acid^{154,167,198} and this may be an important factor in interindividual response to diet that goes largely unrecognized in epidemiological studies. Modulation of the flora by dietary PPT, i.e., prebiotic effects, is discussed below.

Nonenzymic transformations should also be considered. Studies *in vitro* indicate that epigallocatechin gallate is transformed to B-ring linked dimers (theasinensins A and D and an oolongtheanin) in alkaline media such as plasma and intestinal fluid.³¹¹ The interaction of several dietary phenols and peroxy radicals⁴⁵¹⁻⁴⁵³ has been studied *in vitro* and several EGCG⁴⁵³ and genistein⁴⁵¹ transformation products have been identified. Although EGCG and genistein have been detected in plasma as aglycones it is not known whether such interactions occur *in vivo* where EGCG and genistein concentrations are comparatively low and other plasma constituents (e.g., α -tocopherol, ascorbate, and serum proteins) may interfere.

6.6 PPT PRIOR TO ABSORPTION

6.6.1 INTERACTION WITH TISSUES AND NUTRIENTS PRIOR TO ABSORPTION

Over the past decade, much emphasis has been placed on the role of dietary PPT on vascular health and disease. However, on the basis of the foregoing arguments it is clear that the major part of the plant PPT consumed never reach the plasma and systemic circulation. It is equally clear that the tissues most exposed to unchanged plant PPT are those of the oro-gastrointestinal tract, and hence the potential for biologically significant effects may be considerably greater here.

For example, the mouth is exposed to a substantial, albeit transient and spasmodic, flux of PPT. It has been reported that quercetin-4'-glucoside, quercetin-3-glucoside, phloretin-2'-glucoside, and genistein-7-glucoside can be rapidly hydrolyzed, and quercetin-3-rutinoside

slowly hydrolyzed, by mammalian or microbial enzymes in saliva. The rate of hydrolysis varied 20-fold across 17 volunteers.^{454,455} Quercetin thus released is a substrate for salivary peroxidase, transformed to a 2,3,5,7,3',4'-hexahydroxyflavanone-like compound and two "dimeric" species,⁴⁵⁶ but the significance of these transformations, for health or taste, is unknown.

Several black tea derived polyphenols, including theaflavin-3,3'-digallate, can inhibit *in vitro* the growth of human esophageal squamous carcinoma cells at concentrations near 20 μM ,¹⁰⁷ and theaflavin monogallates have *in vitro* inhibited growth of human colon cancer cell lines at 3 μM .⁴⁵⁷ The concentration of the four major theaflavins in a typical brew of black tea will individually exceed 3 μM and approach or exceed 20 μM and these *in vitro* observations are of interest especially with reference to the mouth and esophagus where tea beverage has not yet been diluted. Whether beneficial effects might accrue *in vivo* is uncertain, but evidence from animal studies suggests that diets rich in PPT that reach the colon may protect rodents from carcinogens,⁴⁵⁸⁻⁴⁶⁰ although the human epidemiological evidence for protection against cancer, as discussed above, is mixed.^{39,41,43} However, it must be noted that consumption of PPT-rich beverages at temperatures able to scald the esophagus and adjacent tissues can cause damage that predisposes to the development of cancer.⁴⁶¹ Fortunately, these damaging effects are not seen when the beverage is consumed at more modest temperatures,⁴⁶²⁻⁴⁶⁴ but these observations do raise the question of what might happen at preexisting sites of ulceration, especially as many dietary PPT would still retain their free *vic*-dihydroxyphenyl moieties and thus the ability to redox cycle.

PPT having 1,2-dihydroxyphenyl or α -hydroxy-keto (e.g., benzotropolone) or 1,3-diketo (e.g., curcumin)⁴⁶⁵⁻⁴⁶⁷ moieties are strong binders of divalent and trivalent metal ions, and have been blamed for impaired absorption of iron.^{468,469} While this may be significant for those who are already iron-deficient and dependent on poorly utilized inorganic iron supplements, for those on balanced western diets this may be of less significance than once thought,⁴⁷⁰ although still a cause for concern¹³¹ as the major sources of PPT are poor sources of ascorbate. Such metal binding might be one mechanism contributing to protection against carcinogenesis.⁴⁶⁰

Tannin-protein binding has long been known to have an economic impact on feed utilization by domestic animals, but adverse effects in humans have only rarely been demonstrated,¹³¹ although they may have been overlooked during famines. These interactions in the gastrointestinal tract were once thought to be comparatively nonspecific but evidence is accumulating that there may also be more specific interactions.⁴⁷¹

6.6.2 MODULATION OF THE GUT MICROFLORA — PREBIOTIC EFFECTS

As discussed above, the transformation of dietary PPT, including the generation of phytoestrogens, by the gut microflora has been known for many years. In contrast, the potential for prebiotic effects has only recently been recognized. Changes over 20 days in the metabolites produced from punicalagin have been interpreted as due to changes in the composition or biochemical competence of the gut microflora.²⁹³ Several studies indicate that regular consumption of green tea (poly)phenols influences the composition of the gut microflora in humans, pigs, and sheep; for example, lowering the colonic pH value, suppressing *Clostridium perfringens*, and increasing the proportion of bifidobacteria without inhibiting lactic acid bacteria,⁴⁷²⁻⁴⁷⁴ but the mechanisms are uncertain.⁴⁷⁵ However, assuming the colon contents are some 200 g and the daily intake of total PPT is 1 g, then if 90% passes to the colon concentrations in excess of 4 mg/g are plausible and such concentrations might influence which species grow efficiently. For example, black tea theaflavins are antibacterial against a wide range of organisms.⁴⁷⁶

Although the precise yield of aromatic and phenolic acids produced during the gut flora transformations of PPT are unknown, these metabolites may have a prebiotic or antimicrobial capability. Volunteer studies^{167,178} have demonstrated the production endogenously of up to 1100 mg benzoic acid per day, i.e., implying colon concentrations in excess of 5 mg/g (or 7 mg/g if expressed as 3-phenylpropionic acid, the putative immediate precursor). Six of 28 phenolic acids tested *in vitro* produced 50% inhibition of *Listeria monocytogenes* at concentrations in the range 1 to 2 mg/ml, and a further six at concentrations in the range 3 to 5 mg/ml, when applied in a medium at pH 6.2 and thus representative of the human large bowel where pH ranges from 5.7 in the cecum to 6.7 in the rectum.⁴⁷⁷ The properties of gut flora metabolites after absorption have been discussed above.

6.6.3 MODULATION OF THE POSTPRANDIAL SURGE IN PLASMA GLUCOSE — GLYCEMIC INDEX

There is a growing body of evidence suggesting that diets rich in PPT may influence the absorption and metabolism of glucose, resulting in a lower glycemic index⁴⁷⁸ than would otherwise be expected. Red wine,⁴⁷⁹ coffee,⁴⁸⁰ and apple juice⁴⁸¹ have all been shown in controlled volunteer studies to slow glucose absorption and reduce the postprandial surge in plasma glucose, an event known to be an independent risk factor for coronary heart disease.⁴⁸² Studies in which volunteers consumed normal portions of PPT-rich foods⁴⁸⁰ have also produced reductions in the postprandial concentrations of plasma insulin and glucose-dependent insulinotropic polypeptide (GIP) and elevation in the concentration of glucagon-like polypeptide-1 (GLP-1), and a polyphenol-enriched diet has been reported to reduce the incidence and severity of nephropathy in type II diabetics.⁴⁸³

A prospective study of 17,000 people suggested that the mean relative risk of developing type II diabetes was only 0.5 (0.35–0.72) in those individuals habitually consuming six or more cups of coffee per day compared with those consuming two or less ($p = 0.0002$).⁵⁸ The results of subsequent epidemiological studies on coffee consumption^{58–63} have been in good agreement.

The reduced glycemic index has been attributed to PPT-mediated inhibition of α -amylase,^{484,485} maltase,⁴⁸⁶ or α -glucosidase (sucrase),^{485,487} but the inhibition of these enzymes is not relevant when volunteers have been given glucose *per se*.^{480,481} These observations are more conveniently explained by an effect on the active glucose transporter (SGLT1) in the duodenum. Phloridzin, a dihydrochalcone glucoside characteristic of apples and apple products,¹¹⁴ but now known to be more widely distributed,⁴⁸⁸ competes for the active site both *in vitro* and *in vivo* when given intraperitoneally.^{489–492} Other dietary PPT (EGCG, EGC, and 5-caffeoylquinic acid) have been shown *in vitro* to dissipate the sodium gradient essential to the operation of SGLT1,^{493,494} and several quercetin glucosides have been shown to interact with it and thus to have the potential to interfere in glucose transport.^{495–501} While these effects on glucose absorption and the associated hormones (insulin, GIP, and GLP-1) are modest, they have been achieved in volunteers consuming sensible quantities of common dietary components (as distinct from effects seen only *in vitro* with high levels of PPT aglycones never seen in the diet). Such effects repeated daily, or even several times daily for say 30 years, might in part explain the reduced incidence of chronic disease, especially type II diabetes and the metabolic syndrome, in later life associated with diets rich in fruits and vegetables.

6.7 SAFETY ASSESSMENT OF DIETARY PPT

Normal dietary exposure over many years has highlighted two areas of concern arising through PPT binding of nutrients in the gastrointestinal tract. Impaired absorption of trace metals and impaired protein utilization have been referred to above.

However, in recent years PPT consumption has begun to change. The public has come to believe that diets rich in PPT are health promoting, and industry has increasingly marketed products and supplements rich in PPT. Such products range from traditional foods or beverages with a long history of safe consumption to botanical extracts in tablets or capsules marketed sometimes in an uncontrolled manner on the web.¹³¹ There is probably little cause for concern when some individuals increase their consumption of PPT-rich commodities such as wine, tea, cranberries, soya, etc., provided that they do not exceed levels that other populations have long been exposed to without evidence of harm. Even so, it is important that the possibility of genetic polymorphisms rendering some individuals more susceptible to adverse effects is not ignored,⁵⁰² and pharmaceutical-like preparations (tablets, capsules, drops) might present particular difficulties. Neonates lack many important detoxification systems, e.g., CYP 1A2, CYP 3A4, glucuronidation, glycine conjugation, and renal excretion, and are much more vulnerable.⁵⁰² CYP 3A4 is moderately variable in healthy adults with more significant ethnic variation and a reduced activity in the elderly, increasing the risk of an adverse reaction between PPT that inhibit this enzyme and drugs that require it for clearance, as discussed below.

While some PPT-rich botanicals are derived from conventional foods, beverages, or herbs, e.g., soya, rosemary, or green tea, others may be derived from materials that have no significant history of use as food or food ingredients despite a history of usage as herbal medicines in some area of the world, e.g., *Ginkgo biloba*.⁵⁰³ In some cases the supposed active principle(s) of such botanicals have been more or less purified and marketed in a form more concentrated than could be obtained from foods, with correspondingly increased bioavailability and bioefficacy.³⁶⁰ Mennen et al.¹³¹ have drawn attention to the availability on the web of “tablets or capsules containing 300 mg quercetin, 1 g citrus flavonoids or 20 mg resveratrol with suggested use of 1 to 6 tablets or capsules per day,” and point out that if such an exhortation is followed, then intakes of particular PPT could be some 100-fold higher than normally achieved on typical western diets.¹³¹ Nonculinary extraction processes may alter the composition compared with normal domestic practices, and an aqueous alcoholic extract of tea buds sold as a slimming aid had to be withdrawn from the market because of cases of severe liver toxicity that have not been observed following the consumption of conventional green tea brews.^{131,504} The toxic principle has not been identified,⁵⁰⁵ but it has been shown that EGCG, 4'-*O*-methyl-EGCG, and 4',4''-di-*O*-methyl-EGCG are potent inhibitors (IC₅₀ ~0.2 μM) of COMT, and thus might interfere with drug clearance requiring this enzyme.²²² It is well known that some flavonoid-rich commodities, e.g., grapefruit juice, can impair clearance of clinical drugs that are metabolized by CYP 3A4. This has been attributed variously to inhibition by naringenin, 6',7'-dihydroxybergamotol, and other undefined substances.^{258,506–510} Resveratrol-3-sulfate also inhibits CYP3A4 *in vitro* (IC₅₀ 1 μM).²⁷⁶

High doses of PPT with a *vic*-dihydroxy structure (e.g., protocatechuic acid, caffeic acid, quercetin) given orally to animals have produced forestomach and kidney tumors, and chronic nephropathy. Such PPT have relatively low redox potentials and redox cycle forming quinones or quinone-methides. In tissues low in glutathione, e.g., plasma, these quinones may be scavenged by ascorbate, but glutathione is able *in vitro* to out-compete ascorbate and in glutathione-rich tissues glutathionyl adducts form preferentially.⁵¹¹ If cellular glutathione is depleted, protein sulfhydryls are arylated. Candidate proteins identified include glutathione-S-transferase P1-1 (25% inhibition *in vitro* at 1 μM quercetin)⁵¹² and calcium ATPase,⁵¹¹ and their inhibition further depletes the endogenous cellular defenses against electrophiles, including ultimate carcinogens.^{511,513–519} Normally, as discussed above, only small amounts of unchanged PPT or PPT metabolites with unconjugated *vic*-dihydroxy structures reach the plasma but when the protection normally afforded by the alimentary tract is bypassed, as in

cancer patients given quercetin intravenously at a dose of 1700 mg/m², plasma quercetin reached 20 μM and some nephrotoxicity was observed.⁴⁰⁰

Diets rich in millet have been associated with endemic goiter in parts of West Africa where millet is a staple. The damage has been attributed to vitexin, a C-glycosyl flavone, that in rats has antithyroid activity and that *in vitro* inhibits thyroid peroxidase and the free radical iodination step in thyroid hormone biosynthesis.⁵²⁰ Isoflavones have produced similar antithyroid effects in rats, but clinical studies in adults have not.²⁴³ However, this remains a possible concern in infants fed soya-based milk-replacers, especially if iodine supply is compromised.

From the phytoestrogen standpoint (see also Chapter 4), isoflavone intakes of 0.2 to 5 mg/day, typical of western diets,^{238,243,521} and 20 to 120 mg/day, typical of Asian diets, appear to be safe, but there is concern that higher intakes can have adverse (antiandrogenic) effects on male and female fertility and sexual development *in utero* and *postpartum*.¹³¹ However, since genistein also inhibits tyrosine kinases its estrogenic effect is weaker than might otherwise have been expected.⁵²² The greatest cause for concern, however, is the potential for an antiluteinizing hormone effect in baby boys aged up to 6 months who on a body weight basis receive very high levels of isoflavones in soya-based infant formula.¹³¹ Although clinical evidence has not been produced to substantiate that this occurred, manufacturers have reformulated their soya infant formulas with low-isoflavone soya protein preparations.²⁴³ Nevertheless, the risk remains if ill-informed parents supplement their infants inappropriately.

Clearly, care must be exercised to ensure that dietary manipulation and supplementation do not produce oral loads able to swamp the body's defenses. For the reasons outlined above, a decision tree has been developed to assist in the risk assessment of botanical products.⁵⁰³

6.8 CONCLUSIONS AND FUTURE RESEARCH REQUIREMENTS

Although it is widely accepted that diets rich in fruits and vegetables are beneficial to health, the explanation(s) remain obscure. That dietary flavonoids are key drivers has achieved the status of dogma, but proof is lacking. It is clear that very little of the plant PPT ever reaches the tissues unchanged, and it is essential that the properties of the metabolites are properly addressed. In this regard, it is very interesting that the importance of the gut microflora in transforming dietary PPT has been rediscovered. It is now clear that cinnamates and derived polyphenols are the major dietary PPT for many populations, and these PPT share many gut flora metabolites with the flavonoids. For these two reasons these PPT deserve more attention in the future, and it would be illogical to consider flavonoids in isolation. Gut flora and mammalian metabolism combine to eradicate the powerful antioxidant capability shown *in vitro* by many aglycones, and coupled with the weak or zero antioxidant activity of the metabolites suggests that radical scavenging mechanisms are, at most, a minor part of the *in vivo* story, possibly restricted to sites of inflammation where localized deconjugation might occur. Nevertheless, there is good evidence that extensive initial conjugation is desirable since grossly elevated levels of powerful PPT-derived antioxidants could cause more harm than good. Supplementation must be approached with caution.

If PPT are beneficial to health, then explanations other than antioxidant effects or radical scavenging must be sought, and effects prior to absorption certainly deserve greater consideration since it is the tissues of the oro-gastrointestinal tract that see the greatest dose. Although as yet far from proven to be biologically significant, prebiotic effects, effects on the glycemic index, incretin hormones, and postprandial surge in glucose, and the effects of gut flora or mammalian metabolites on cell signaling systems, are beginning to receive serious consideration and systematic investigation. The preliminary results suggest that beneficial effects might be achieved at realistic dietary intakes. Even if unequivocally established, these

effects are likely to be modest, but with the potential possibly to yield health benefit over years or decades — fully in keeping with the epidemiology — they are unlikely to be curative. Other as yet unrecognized mechanisms may also operate at normal dietary levels.

The task remaining is massive. If significant progress is to be made cost-effectively greater coordination of efforts and resources will be necessary. For improved epidemiological data and more reliable risk assessment it is essential that more precise estimates are obtained of what actually reaches the mouth. The quantitative effects of commercial operations and culinary practice on the content of PPT must be better defined and the associated structural transformations associated with traditional processing or cooking need to be better understood. Attention must be focused on effects in humans, but animal studies with ^{14}C -labeled test compounds are essential in determining the fate of ingested material. Since polyphenols are fragmented by the gut, microflora studies may have to be performed with more than one position of labeling to determine the identity and disposition of these metabolites. The results will have to be confirmed in volunteers, perhaps using ^{13}C -labeled materials.

Volunteer studies must employ larger groups that are genetically defined and representative, and studies must be longer term, better to reflect long-term dietary practice. When ethically acceptable, volunteer studies comparing clinically defined at-risk groups with matched healthy controls could be informative. Eventually, studies must address the possibility of synergy, not only between classes of PPT, but also with other classes of nonnutrients, and multiple endpoints must be assessed so as to encompass as many disease states or mechanisms as possible.

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7 Isoflavonoids and Human Health

Helen Wiseman

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7.1 INTRODUCTION

Isoflavonoid phytoestrogens such as the soy isoflavones genistein and daidzein are plant-derived nonsteroidal estrogen mimics, often referred to as phytoestrogens (other phytoestrogens include lignans such as secoisolariciresinol, coumestans such as coumestrol, and prenylflavonoids such as 8-prenylnaringenin; see Figure 7.1), that are extensively investigated to determine their potential, particularly in the protection of human health.¹⁻⁹

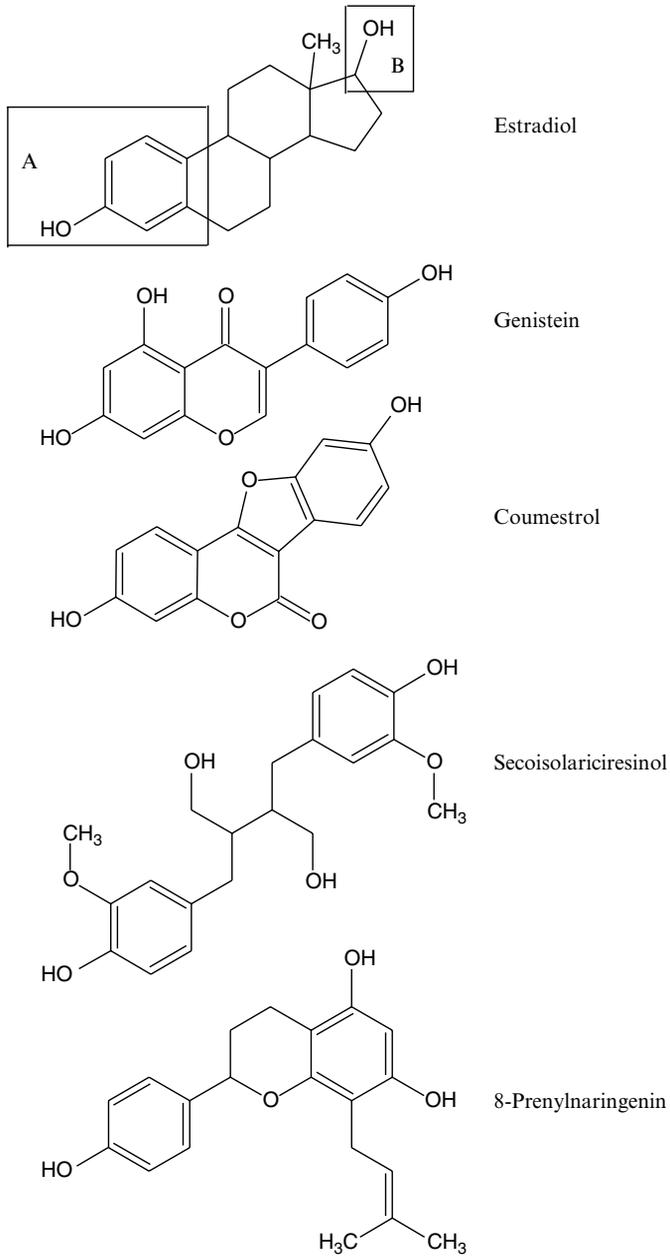
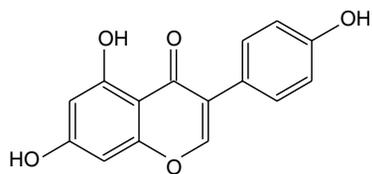


FIGURE 7.1 The structural relationship between phytoestrogens and 17β -estradiol.

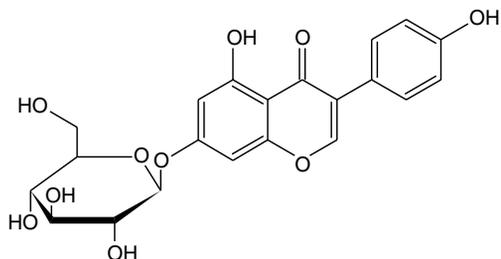
7.2 ISOFLAVONOIDS: DIETARY SOURCES AND INTAKES, METABOLISM, AND BIOAVAILABILITY

7.2.1 DIETARY SOURCES AND INTAKES

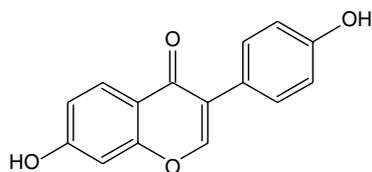
Isoflavonoids include the isoflavones genistein and daidzein, which occur mainly as the glycosides genistin and daidzin (see Figure 7.2), respectively, in soybeans and consequently in a wide range of soy-derived foods and to a lesser extent in other legumes.^{10–12} Traditional



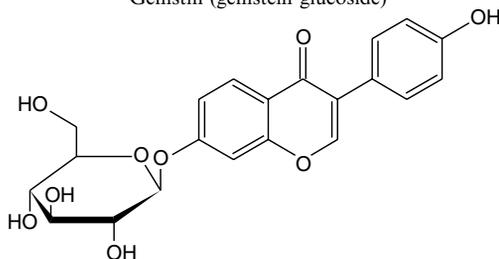
Genistein



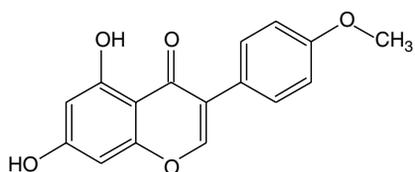
Genistin (genistein glucoside)



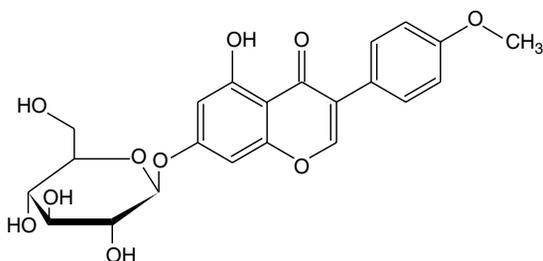
Daidzein



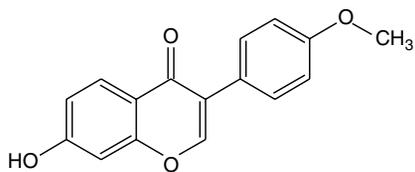
Daidzin (daidzein glucoside)



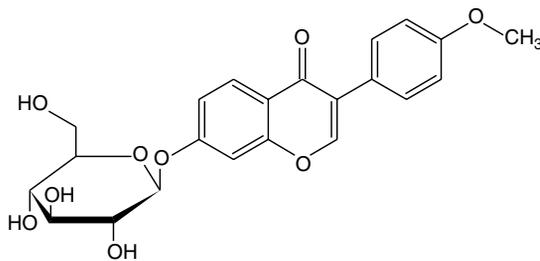
Biochanin A



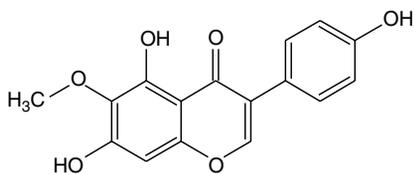
Ononin (biochanin A glucoside)



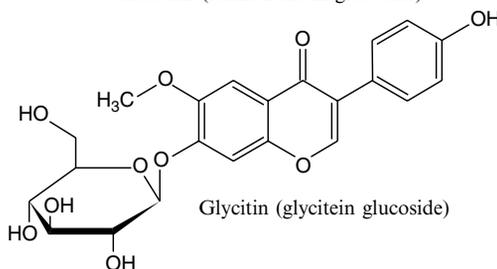
Formononetin



Sissotrin (formononetin glucoside)



Glycitein



Glycitin (glycitein glucoside)

FIGURE 7.2 Chemical structures of the main soy (genistein and daidzein) and red clover (biochanin A and formononetin) isoflavonoids (aglycones and glucosides).

soy foods are made from soy beans and include both fermented and nonfermented foods. Nonfermented soy foods contain isoflavones mostly present as β -glucosides, some of which are esterified with malonic acid or acetic acid. Fermented soy foods such as miso or tempeh contain mostly unconjugated isoflavones.¹³ Some alcoholic beverages such as beer contain significant amounts of isoflavones.¹⁴ The isoflavone aglycone and glucoconjugate content of high- and low-soy UK foods used in nutritional studies has been reported.¹¹ Soybeans (774 mg/kg isoflavones) and soybean-containing foods had the highest isoflavone content of the foods examined. The low-soy foods all contained very low concentrations (<8 mg/kg) of the isoflavone aglycones and glucoconjugates.¹¹

Dietary exposure estimates of isoflavones have been made from the 1998 UK Total Diet Study.¹⁵ Each Total Diet Study group consisted of composite samples, one for each of the 20 designated food groups. The composite samples were taken to represent the average consumption of all the individual food elements in each group, processed in the form that they were consumed in. In the Total Diet Survey, individual composites of the bread, processed meat, and fish food groups contained >5 mg/kg of the individual isoflavones, daidzein, genistein, and glycitein. In addition, individual composites from the groups, miscellaneous cereals, other vegetables, fruit products, and nuts contained >1 mg/kg of isoflavones. The Total Diet Survey sample collection model for the average adult consumer, following weighting for average consumption of food from each Total Diet Survey group, gave an estimated daily intake of 3 mg/day of combined isoflavone aglycones.¹⁵ This indicates that the UK dietary intake of isoflavone phytoestrogens is higher than previously estimated and this is likely to be due in part to the extensive use of soy products in processed foods.¹⁵ An investigation into the intake of dietary phytoestrogens by Dutch women indicates that pea, nuts, grain products, and soy products were the main sources of isoflavones; and also, despite their low concentrations of isoflavones compared to soy foods, coffee and tea, because of their high levels of consumption.¹⁶ The main sources of lignans were grain products, fruit and alcoholic beverages, and in this population the phytoestrogen intake consisted largely of lignans.¹⁶ Generally, in the diets of Europeans, food sources of lignans are more widely consumed than those of isoflavones and lignans may be the more important dietary source of phytoestrogens.¹⁷

Estimating dietary intakes of isoflavone phytoestrogens can be difficult because of inadequate information regarding the phytoestrogen contents of foods. The validation of a newly constructed phytoestrogen database containing more than 600 values given to foods, by using duplicate diet analysis together with measurements of isoflavone concentrations in urine and plasma, has shown that the 24-h urinary excretion and timed plasma concentrations of genistein and daidzein can be used as biomarkers of intake.¹⁸ However, it should be noted that the considerable variation found in the isoflavone content of isolated soy proteins used in food manufacture and in commercial milks exposes the limitations of using food databases for estimating daily isoflavone intakes.¹⁹

7.2.2 METABOLISM AND BIOAVAILABILITY

The metabolism and bioavailability of isoflavonoids is likely to be of crucial importance to their ability to help protect human health against disease.^{3,20,21} Many studies have been published on the metabolism and bioavailability of isoflavones in adults.²²⁻²⁵ The metabolism of isoflavones is of particular interest because the potency of isoflavone metabolites differs from that of the parent compounds.²⁶ The daidzein metabolite equol is three times as potent as is daidzein in an endometrial tumor line. Equol is also a more potent antioxidant *in vitro* (see Sections 7.3.5 and 7.4.2)²⁷⁻³⁰ and the clinical significance of the ability to form equol has been considered in depth.²⁰

Daidzin and genistin (and to a lesser extent glycitin) and their acetylglucosides and malonylglucosides are the predominant isoflavone forms in soy foods.¹¹ After ingestion isoflavones are hydrolyzed by mammalian lactase phlorizin hydrolase,²¹ which releases the aglycones daidzein, genistein, and glycitein. These may be absorbed or further metabolized by the gut microflora to metabolites, including the conversion of daidzein to the isoflavan equol or *O*-desmethylangolensin (*O*-DMA), and the conversion of genistein to *p*-ethyl phenol. More recently, 4-hydroxyphenyl-2-propionic acid was identified from the metabolism of genistein in rats.³¹ Studies have shown that particular bacterial groups are involved in the metabolism of the isoflavone glycosides.³²

Unconjugated isoflavones are absorbed quickly from the upper small intestine in rats,³³ whereas the glycoside conjugates are absorbed more slowly, which is consistent with their hydrolysis at more distal sites in the intestine to the unconjugated isoflavones.³⁴ Isoflavonoids, once absorbed, are rapidly converted to their β -glucuronides³³ and sulfate ester conjugates.³⁵ These biological conjugates circulate in the plasma and are excreted in the urine and feces. Indeed, intact sulfate and glucuronide isoflavone conjugates have been measured in human urine, following soy consumption.³⁶ Concentrations of the free aglycones of up to 22% of genistein and 18% of daidzein were observed, and the average pattern of daidzein conjugates was 54% 7-glucuronide, 25% 4'-glucuronide, 13% monosulfates, 7% free daidzein, 0.9% sulfoglucuronides, 0.4% diglucuronide, and 0.1% disulfate.³⁶

Plasma isoflavonoid levels in Japanese and Finnish men have been measured and the means of the total daidzein, genistein, *O*-DMA, and equol levels were approximately 17-, 44-, 33-, and 55-fold higher for the Japanese subjects compared to the Finnish ones.³⁷ In postmenopausal Australian women following consumption of soy flour, mean plasma levels of daidzein and equol of 68 and 31 ng/ml, respectively, were observed.³⁸ Interestingly, only 33% of subjects were able to metabolize daidzein to equol.³⁸ Increased concentrations of both genistein and daidzein were observed in male subjects following ingestion of a cake containing soy flour and cracked linseed, within 30 min of consumption, with maximum concentrations reached by 5.5 to 8.5 h.³⁹

The importance of the gut microflora in the metabolism of isoflavones has been demonstrated. Antibiotic administration blocks isoflavone metabolism and germfree animals do not excrete metabolites.⁴⁰ Moreover, only germfree rats colonized with microflora from a good equol producer excrete equol when fed soy.⁴¹

Interindividual variation in ability to metabolize daidzein to equol (more estrogenic and a more potent antioxidant than daidzein) could thus influence the potential health protective effects of soy isoflavones.²⁰ The extent of gut microflora metabolism in humans is variable, approximately 35% of a Western population can produce equol.^{23,38,42}

A variable metabolic response to isoflavones has been shown for subjects following consumption of soy flour; urinary excretion concentrations of genistein, daidzein, equol, and *O*-DMA were increased 8-, 4-, 45-, and 66-fold, respectively, compared to baseline.⁴³ Considerable interindividual variation in metabolic response was reported with the peak levels of equol showing the most variation.⁴³

In healthy young adults, when diets high or low in isoflavones (textured soy protein product containing 56 or 2 mg/day) were each consumed for 14 days separated by a 25-day washout period, considerable interindividual variation in metabolic response was found.²³ In addition, the good equol excretors (36% of subjects) consumed significantly less fat and more carbohydrate (also greater amounts of nonstarch polysaccharide, NSP) compared to the poor equol excretors.²³ Female equol excretors have been reported to consume a higher percentage of energy as carbohydrate and also greater amounts of plant protein and NSP than non-equol excretors.⁴²

Dietary modification, such as feeding wheat bran or soy protein, has been unsuccessful at changing equol-producing capability,⁴⁴ which indicates that the intestinal microflora of an individual is relatively stable and resistant to change.

The bioavailability of soy isoflavones has been shown to depend on the gut microflora.⁴⁵ Metabolism by the gut microflora is an important factor influencing the disposition of chemicals in the gut and can result in activation of substances to more biologically active products. The presence of different populations of microflora in the human gut may influence the bioavailability of isoflavones. The identification of the bacterial species involved in the conversion of daidzein to equol is of considerable importance and very challenging given the large number of bacteria present in the colon and small intestine. In a study that identified equol producers by culturing fecal flora from healthy Japanese adults after they consumed 70 g of tofu, three strains of bacteria were reported to convert pure daidzein to equol *in vitro*: the gram-negative *Bacteroides ovatus* spp., the gram-positive *Streptococcus intermedius* spp., and *Ruminococcus productus* spp.⁴⁶

Chronic consumption of a high-soy diet (104 mg isoflavones/day) has recently been compared with a low-soy diet (0.54 mg/day) in 76 healthy young adults; after the 10-week diet period, concentrations of the isoflavonoids in plasma, urine, and feces were significantly higher in the high-soy group than in the low-soy group.⁴⁷ Although interindividual variation in isoflavone metabolism was high (34% of subjects were good equol producers), intraindividual variation (assessed by comparing midpoint with endpoint results) in metabolism was low. Only concentrations of *O*-DMA in plasma and urine appeared to be influenced by sex, with men having significantly higher concentrations than women.⁴⁷ Other studies suggested that men and women respond differently to chronic exposure to isoflavones,^{48–50} although there are considerable inconsistencies in the data. Furthermore, chronic soy isoflavone consumption did not appear to induce many significant changes to the gut metabolism of isoflavones, other than effects on β -glucosidase activity (this was significantly higher in subjects who consumed the high-soy diet than in those who consumed the low-soy diet), suggesting that the gut bacteria and enzymes responsible for equol or *O*-DMA production are not inducible.⁴⁷

Further to the possible contribution that soy isoflavonoids may make to adult human health, the possible health consequences of early life soy exposure are also attracting attention.^{51,52} Although there have been many studies on the metabolism and bioavailability of isoflavonoids in adults, there is little information available in infants and children. The gut microflora in early childhood is very different to that in adulthood; therefore, it is important to characterize developmental changes in isoflavone metabolism in early life. The development of the microflora occurs gradually and it can take several years before an adult-type flora is established. This has important implications for isoflavone metabolism.

The urinary excretion of isoflavonoids has recently been investigated in 60 infants and children (aged 4 months to 7 years, divided into four age groups). The study compared infants and children who had been fed soy-based formulas in early infancy, to control (cows'-milk formula-fed) infants and children.⁵³ Infants aged 4 to 6 months, fed soy-based infant formulas (but not those fed cows'-milk formulas) were found to excrete considerable amounts of genistein, daidzein, and glycitein in urine, indicating that these compounds are well absorbed and that the required glucosidase activity has developed.⁵³ The majority of the soy-based infant formula-fed infants and about one half of the cows'-milk formula-fed group (after a soy challenge) were capable of converting daidzein to *O*-DMA. However, conversion of daidzein to equol was observed in very few children, even in the oldest age group (3 to 7 years). These findings indicate that there appears to be no lasting effect of early-life isoflavone exposure on isoflavone metabolism and have important developmental implications for isoflavonoid bioavailability.⁵³

7.3 ISOFLAVONOIDS AND CANCER PREVENTION

7.3.1 HORMONE-DEPENDENT CANCER PREVENTION BY ISOFLAVONOIDS

The possible role of isoflavonoids in the prevention of cancer and in particular hormone-dependent cancers such as breast and prostate cancer is currently extensively investigated.^{6,8,54–57} In addition, consumption of soy foods rich in isoflavones has been weakly associated with reduced colon cancer.^{54,58} Colon cancer risk is influenced by estrogen exposure; although the mechanism of action has not been fully elucidated, studies with estrogen receptor α (see Section 7.3.3) knockout mice indicate that it may be independent of estrogen receptor α .⁵⁸

Breast and prostate cancer is much less prevalent in Far Eastern countries, where there is an abundance of soy phytoestrogens in the diet, compared to Western ones. Emigration of people from Pacific Rim countries to the United States has been shown to increase their risk of breast and prostate cancer. The increase in prostate cancer risk in men occurs in the same generation, whereas for women the increase in breast cancer risk is observed in the next generation.⁵⁹ These changes in breast and prostate cancer risk have been mostly attributed to changes in diet, in particular the switch to a low-soy Western diet: in countries such as Japan, Korea, China, and Taiwan, the mean daily intake of soy products has been estimated to be in the range 10 to 50 g compared to only 1 to 3 g in the United States.⁶⁰ Increased soy intake has been associated with a lowered risk of breast cancer in two out of four epidemiological studies that examined a wide range of dietary components in relation to breast cancer risk: no significant effect was observed in the other two studies.^{1,60}

High urinary excretion of both equol and enterolactone (mammalian metabolite of plant lignans) has been found to be associated with a significant decrease in breast cancer risk in an epidemiological case-control study in breast cancer patients.⁶¹ Although this could suggest the possible importance of isoflavonoid and lignan metabolism in decreased breast cancer risk, the phytoestrogen excretion observed may just be a marker of dietary differences.¹

The possible protective effect of isoflavonoids against prostate cancer has recently been reviewed⁵⁶ and it is of particular interest that equol may be a novel antiandrogen that inhibits prostate growth and hormone feedback in rat studies.⁶² The role of isoflavonoids in the prevention of breast cancer is, however, the main focus of the next section of this chapter.

7.3.2 ESTROGENS AND RISK OF BREAST CANCER

Breast cancer is still a major cause of death for women in Western countries.⁶³ Breast cancer is thought to have many causes, ranging from gene profile to diet and lifestyle and mutations in particular tumor suppressor genes such as BRCA1, BRCA2, and p53 are likely to be of particular importance. A ribonucleotide reductase gene (*p53R2*) has been shown to be directly involved in the p53-dependent cell cycle checkpoint for DNA damage, thus clarifying the relationship between a ribonucleotide reductase activity involved in repair of damaged DNA and tumor suppression by p53.⁶⁴ Furthermore, the structural basis has been established for the recognition and repair by 8-hydroxyguanine DNA glycosylase of the oxidative DNA base damage product (and endogenous mutagen) 8-hydroxyguanine.⁶⁵

The role of endogenous estrogens in breast cancer risk is widely recognized. Different forms of estrogen metabolism result in the formation of mitogenic endogenous estrogens or the metabolic activation of estrogens that can result in carcinogenic free-radical-mediated DNA damage. Pregnancy appears to be important in breast cancer risk: women who have never been through pregnancy have the greatest risk of breast cancer. For those women who have been through pregnancy, multipregnancies are of no greater benefit than a single pregnancy and a pregnancy earlier in life is more protective than one later in life. This is

because of the differentiation of breast epithelial cells into milk-producing cells that occurs in the breast during pregnancy and the apoptosis of breast cells that occurs in the breast following pregnancy, thus providing a chance for elimination of mutated epithelial cells.¹

7.3.3 ESTROGEN RECEPTOR MEDIATED EVENTS

Estrogens play a vital role in the growth, development, and homeostasis of estrogen responsive tissues. The estrogen receptor mediates the biological activity of estrogens and is a ligand-inducible nuclear transcription factor: estrogen binds to the ligand-binding domain of the estrogen receptor resulting in either the activation or repression of target genes.^{66,67} The selective estrogen receptor antagonist raloxifene, structurally related to the anticancer drug tamoxifen,⁶³ can inhibit the mitogenic effects of estrogen in reproductive tissues, while maintaining the beneficial effects of estrogen in other tissues. The crystal structures of the ligand-binding domain of the estrogen receptor complexed to either 17 β -estradiol or to raloxifene have been reported,⁶⁸ thus providing structural evidence for the mechanisms of estrogen receptor agonism and antagonism. A combination of specific polar and nonpolar interactions enables the estrogen receptor to selectively recognize and bind 17 β -estradiol with great affinity. The estrogen receptor is the only steroid receptor able to additionally interact with a large number of nonsteroidal compounds, which frequently show a structural similarity to the steroid nucleus of estrogen, including phytoestrogens, and drug and environmental xenestrogens such as dioxins.⁶⁷ In particular, a phenolic ring analogous to ring A in estradiol is required (see Figure 7.1) and these structural features enable them to bind to estrogen receptors to elicit responses ranging from agonism to antagonism of the endogenous hormone ligand.⁶⁹

Originally, it was accepted that only one estrogen receptor existed (the classical estrogen receptor, ER α). This is in contrast to other members of the nuclear receptor superfamily where multiple forms have been found. A separate subtype, termed estrogen receptor β (ER β), has subsequently been identified in cDNA libraries from rat prostate and ovary tissues.⁷⁰ ER β shows a different tissue distribution from ER α . ER β was first reported to be strongly expressed in ovary, uterus, brain, bladder, testis, prostate, and lung.⁷¹ Expression of ER β appears to occur at different sites in the brain from ER α .⁷¹ Evidence has since been found, using reverse-transcription polymerase chain reaction, for the presence of ER β in normal human breast tissue⁷² and ER β has been shown to be highly expressed in rat breast tissue using specific antibodies.⁷³ ER β has also been found to be expressed in both bone^{74–76} and the cardiovascular system.⁷⁷

7.3.4 ANIMAL MODELS

Studies using animal models provided the initial experimental evidence that soy can prevent breast cancer.^{1,60} Results from 26 animal studies of experimental carcinogenesis have shown that in 17 of these studies (65%) protective effects were reported: the risk of cancer (incidence, latency, or tumor number) was greatly reduced, and no studies reported that soy intake increased tumor development.⁶⁰ In a rat model of breast cancer (7,12-dimethylbenz[*a*]anthracene (DMBA) induced), genistein administered in high doses by injection to young animals suppressed the number of mammary tumors observed over a 6-month period by 50% and delayed the appearance of the tumors,⁷⁸ indicating the likely importance of the timing of exposure to the protective components of soy. In later studies, similar levels of protection were achieved by adding genistein to the feed (0.25 g/kg) given to the mother such that the offspring were exposed to dietary genistein from conception to day 21 postpartum.⁷⁹ By contrast, when pregnant female rats were treated daily with subcutaneous injections of

genistein (doses given were 20, 100, or 300 $\mu\text{g}/\text{day}$) between days 15 and 20 of gestation, this *in utero* exposure was found to dose-dependently increase the incidence of DMBA-induced mammary tumors in female offspring.⁸⁰ However, when prepubertal rats were treated with 20 μg of genistein ($\sim 1 \text{ mg}/\text{kg}$ body weight) between postnatal days 7 and 20, this greatly reduced the multiplicity but not the incidence of DMBA-induced mammary tumors and 60% of the tumors that did occur were not malignant offspring.⁸¹ Furthermore, injection of prepubertal rats with genistein (500 $\mu\text{g}/\text{g}$ body weight) or estradiol benzoate (500 ng/g body weight) on days 16, 18, and 20 showed that both treatments resulted in significantly increased mammary gland terminal end buds and increased ductal branching compared to controls, indicating an ER-dependent action of genistein in mammary gland proliferation and differentiation, which could be protective against mammary cancer.⁸² Overall, these results indicate that genistein has very complex effects on carcinogen-induced mammary cancer in the rat model and great care is required in interpreting these results and drawing parallels with human breast cancer.

Biochanin A, found in certain subterranean clovers, including red clover, and converted to genistein by demethylation in the liver in addition to in the breast, was a good anticancer agent when administered following the carcinogen,⁸³ suggesting the benefits of isoflavones other than genistein may not be solely restricted to early life exposure. The fermented soy food miso (contains mostly unconjugated isoflavones) and tamoxifen acted together to cause an additive reduction in the number of mammary tumors in the rat model⁸⁴ and this may be of considerable importance for women on standard tamoxifen therapy.⁶³

In the mouse model of breast cancer (tamoxifen is estrogenic in this model), maternal genistein exposure (pregnant mice injected with 20 $\mu\text{g}/\text{day}$ between days 15 and 20 of gestation) resulted in similar effects to that of estrogen on mammary gland development,⁸⁵ and further studies are needed to determine whether these estrogenic changes could lead to an increased risk of breast tumors. In addition, when human breast cancer cells (MCF-7) are grown orthotopically in ovariectomized rats, addition of genistein to their diets resulted in an increase in the growth of the cancer cells.⁸⁶ This could have important implications (in relation to tumor reoccurrence) for the consumption of phytoestrogens including isoflavonoids by women who have had their ovaries removed following a diagnosis of breast cancer. But as the removal of the ovaries will greatly reduce the growth of breast cancer cells, then any increase in risk caused by dietary phytoestrogens would probably be less than if the ovaries remained intact.¹

7.3.5 MECHANISMS OF ANTICANCER ACTION OF ISOFLAVONOIDS

Genistein is a potent and specific *in vitro* inhibitor of tyrosine kinase action in the autophosphorylation of the epidermal growth factor (EGF) receptor⁸⁷ and is thus frequently used as a pharmacological tool. The EGF receptor is overexpressed in many cancers, in particular those with the greatest ability for metastasis⁸⁸ and it has therefore often been assumed that some of the anticancer effects of genistein are mediated via inhibition of tyrosine kinase activity; however, this is likely to be an oversimplification of the true *in vivo* situation.¹

Isoflavonoids have biphasic effects on the proliferation of breast cancer cells in culture; at concentrations greater than 5 μM , genistein exhibits a concentration-dependent ability to inhibit both growth factor-stimulated and estrogen-stimulated (reversed by 17 β -estradiol) cell proliferation.⁸⁹ Genistein at low concentrations can, in the absence of any estrogens, stimulate the growth of estrogen receptor-positive MCF-7 cells.^{90,91} Genistein does not, however, stimulate the growth of estrogen receptor-negative breast cancer cells,^{92,93} it only inhibits cell proliferation in these cell lines.⁹² Equol is a much more potent stimulator than daidzein of the expression of estrogen-specific genes.⁹⁴ It is of great interest that phytoestrogen-responsive

genes (PE-13.1 and pRDA-D) have been identified and characterized from MCF-7 cells and it may be possible to use these as molecular markers in elucidating the role phytoestrogens, including isoflavonoids, play in cancer prevention.⁹⁵

Although genistein is a much better ligand for ER β than for the ER α (20-fold higher binding affinity),⁷¹ it can also act as an estrogen agonist via both ER α and ER β in some test systems.^{96,97} However, genistein also behaves as a partial estrogen agonist in human kidney cells transiently expressing ER β , suggesting that it may be a partial estrogen antagonist in some cells expressing ER β .⁹⁸ Furthermore, although genistein binds to the ligand binding domain of ER β in a manner similar to that observed for 17 β -estradiol, in the ER β -genistein complex the AF-2 helix (H12) does not adopt the normal agonist type position, but instead takes up a similar orientation to that induced by ER antagonists such as raloxifene.⁹⁹ This suboptimal alignment of the transactivation helix is in keeping with the reported partial agonist activity of genistein via ER β .⁹⁸

Mechanisms other than those involving estrogen receptors are also likely to be involved in the inhibition of cell proliferation by genistein. This is because genistein inhibits both the EGF-stimulated as well as the 17 β -estradiol-stimulated growth of MCF-7 cells.⁸⁷ It has been suggested that the inhibitory action of genistein on cell proliferation involved effects on the autophosphorylation of the EGF receptor in membranes and demonstrated in membranes isolated from cells.⁸⁷ Although studies have shown that exposure to genistein can reduce the tyrosine phosphorylation of cell proteins in whole cell lysates, studies using cultured human breast and prostate cancer cells, have not, however, confirmed that genistein has a direct effect on the autophosphorylation of the EGF receptor.¹⁰⁰ Furthermore, *in vivo* studies in male rats have shown that genistein decreases the amount of EGF receptor present in the prostate, indicating that the observed decrease in tyrosine phosphorylation may be only a secondary effect of the influence of genistein on the expression or turnover of EGF receptor.^{1,101}

Many other mechanisms of action for isoflavonoids and genistein in particular have been suggested. These include inhibition of DNA topoisomerases,¹⁰² inhibition of cell cycle progression,¹⁰³ inhibition of angiogenesis,^{104,105} tumor invasiveness,¹⁰⁶ inhibition of enzymes involved in estrogen biosynthesis,¹⁰⁷ effects on the expression of DNA transcription factors c-fos and c-jun¹⁰⁸ and on transforming growth factor- β (TGF- β).^{103,109} Effects have also been reported on reactive oxygen species,^{28,110} oxidative membrane damage,³⁰ membrane rigidity,¹¹¹ similar to those found previously to contribute to the antioxidant action of tamoxifen,^{63,112,113} and oxidative damage *in vivo*.¹¹⁴

Antioxidant properties have been reported for isoflavones both *in vitro* and *in vivo*.^{28,30,110,114} Equol, in model membrane systems, was a more effective antioxidant than genistein or the parent compound daidzein³⁰ and shows structural similarity to the tocopherols.¹ Daidzein and genistein showed antioxidant action in primary and cancer lymphocytes (Jurkat cells), both isoflavones increased DNA protection against oxidative damage and decreased lipid peroxidation.¹¹⁵ Moreover, a protective effect was achieved at concentrations that can be achieved in plasma following soy consumption. An important aspect of cancer risk is the involvement of the inflammatory response, which involves the production of cytokines and proinflammatory oxidants such as the hypochlorous acid produced by neutrophils and peroxynitrite by macrophages, which react with phenolic tyrosine residues on proteins to form chloro- and nitrotyrosine.¹¹⁶ It has been reported that neutrophil myeloperoxidase chlorinates and nitrates isoflavones and enhances their antioxidant properties, thus soy isoflavones may have potentially protective benefits at sites of inflammation.^{116,117} Antioxidant action could also contribute to anticancer ability because reactive oxygen species could initiate signal transduction through the mitogen activated protein (MAP) kinases.^{1,118}

There have been a number of reports relating to the possible antioxidant effects of isoflavone consumption. Soy isoflavone consumption as a soy protein burger (56 mg

isoflavones/day for 17 days) decreased plasma F₂-isoprostane concentrations in healthy young adults.¹¹⁴ Consumption of a soy isoflavone supplement (50 mg isoflavones, twice a day for 3 weeks) decreased a biomarker of DNA oxidative damage (white cell 5-hydroxymethyl-2'-deoxyuridine concentrations) but did not alter plasma F₂-isoprostane concentrations.¹¹⁹ Furthermore, consumption of soy protein (110 mg isoflavones/day for 4 weeks) decreased plasma peroxide concentrations and increased total antioxidant status but did not effect a biomarker of oxidative DNA damage (urinary 8-hydroxy-2-deoxyguanosine concentrations).¹²⁰ It is of considerable interest that widely differing effects in relation to the potential benefits to human health are frequently reported for isoflavones consumed within the food matrix in soy foods, compared to those consumed in capsule or tablet form as dietary supplements (see Section 7.4.2).

Angiogenesis, the formation of new blood vessels, is normally an important process involved in productive function, development, and wound repair. Disease states, however, often involve persistent and unregulated angiogenesis. The growth and metastasis of tumors is dependent on angiogenesis. Genistein is a potent inhibitor of angiogenesis *in vitro*¹⁰⁴ and thus could have therapeutic applications in the treatment of chronic neovascular diseases including solid tumor growth¹²¹ and inhibition of neovascularization of the eye by genistein has been reported.¹⁰⁵ Recently, novel molecular targets for the inhibition of angiogenesis by genistein have been discovered including tissue factor, endostatin, and angiostatin.¹²²

Genistein may enhance the action of transforming growth factor- β (TGF β).^{103,109} This action may be a link between the effects of genistein in a variety of chronic diseases,¹ including atherosclerosis and hereditary hemorrhagic telangiectasia (the Osler–Weber–Rendu syndrome) in which defects in TGF β have been characterized.¹²³

7.3.6 CLINICAL STUDIES

Few studies have yet been reported on use of phytoestrogens as preventative agents for breast cancer. In one study, an isolated soy protein beverage (42 mg genistein and 27 mg daidzein) was administered daily for 6 months to healthy pre- and postmenopausal women and breast cancer risk factors were measured in nipple aspirate fluid (NAF).¹²⁴ Although no change in NAF was observed in postmenopausal women, premenopausal women showed an increase in NAF volume, which persisted even after treatment ended and indicates the isoflavones were having an undesirable estrogenic effect in the premenopausal women.¹²⁴ This provides some cause for concern that risk of premenopausal breast cancer may actually be enhanced by phytoestrogens,¹ although further studies are needed. Furthermore, in a study of 84 normal premenopausal women, consumption of a soy supplement (60 g soy, 45 mg total isoflavones) for 14 days resulted in a weak estrogenic response in the breast: nipple aspirate levels of apolipoprotein D were significantly lowered and pS2 levels were significantly raised.¹²⁵

Mammographic breast density has been consistently associated with risk for breast cancer. A review of case–control studies showed odds ratios for breast cancer in women with the highest versus the lowest mammographic breast density ranged from 2.1 to 6.0.¹²⁶ Although the reasons for this are not fully understood it is possible that breast density acts as a biomarker for the past and current reproductive and hormonal events that influence breast cancer risk.¹²⁷ Mammographic breast density can thus also be used as biomarker of estrogenic or antiestrogenic effects of a particular treatment on breast tissue.¹²⁸ Consumption of a dietary supplement that provided red clover-derived isoflavones (26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein) for 12 months did not increase mammographic breast density in postmenopausal women, suggesting neither estrogenic nor antiestrogenic effects, of this supplement at the dose given, on the breast.¹²⁸

7.4 PROTECTION BY ISOFLAVONOIDS AGAINST CARDIOVASCULAR DISEASE

7.4.1 CHOLESTEROL-LOWERING AND ISOFLAVONOIDS

Estrogen administration in postmenopausal women has been observed to produce cardio-protective benefits. The exact biomolecular mechanisms for this cardioprotection are unclear but it is likely that actions mediated both through the estrogen receptors, such as the beneficial alteration in lipid profiles and upregulation of the low-density lipoprotein (LDL) receptor, and independently of the estrogen receptors, such as antioxidant action, contribute to the observed cardioprotective effects of estrogens.

Lower incidence of heart disease has also been reported in populations consuming large amounts of soy products. Lowering of cholesterol is probably the best-documented cardio-protective effect of soy.^{129,130} Soy protein incorporated into a low-fat diet can reduce cholesterol and LDL-cholesterol concentrations and the soy isoflavones are likely to contribute to these effects.¹³¹ Soy isoflavones have been reported to improve cardiovascular risk factors in peripubertal rhesus monkeys,^{132,133} and inflammatory markers in atherosclerotic, ovariectomized monkeys.¹³⁴ The potential role of phytoestrogens, including isoflavonoids, as cardio-protective agents has been extensively reviewed.^{4,5,135}

A recent meta-analysis of eight randomized controlled trials of soy protein consumption in humans has found that with identical soy protein intake, high isoflavone intake led to significantly greater decreases in serum LDL cholesterol than low isoflavone intake, indicating that isoflavones have LDL-cholesterol-lowering effects that are independent of the soy protein.¹³⁶

Consumption of soy protein (40 g/day providing either 56 mg isoflavones/day or 90 mg isoflavones/day) or cesin and nonfat dry milk (40 g/day) by postmenopausal women for 6 months showed a significant decrease in non-high-density lipoprotein (HDL) cholesterol and a significant increase in mononuclear cell LDL receptor mRNA and HDL cholesterol in both of the soy isoflavone groups compared to the control group.¹³⁷ Indeed, consumption of soy protein (20 g/day containing 80 mg isoflavones/day for 5 weeks) in high-risk middle-aged men (45 to 59 years of age) in Scotland significantly decreased non-HDL cholesterol and blood pressure, compared to the control treatment.¹³⁸

However, studies in hypercholesterolemic subjects, using soy protein depleted of isoflavones have shown that soy protein independently of isoflavones can favorably affect LDL size, LDL particle distribution was shifted to a less atherogenic pattern,¹³⁹ and can decrease triglyceride concentrations, triglyceride fatty acid fractional synthesis rate, and cholesterol concentrations.¹⁴⁰

By contrast, a meta-analysis of randomized controlled trials indicates that consumption of isolated isoflavones did not appear to have any significant effect on serum cholesterol, suggesting further studies investigating possible interactions of isoflavones with other components of soy protein are needed.¹⁴¹ Indeed, a 12-month intervention with red clover-derived isoflavones (26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein) administered in the form of a dietary supplement found only modest protective benefits (decreases in triglycerides and plasminogen activator inhibitor type 1) in perimenopausal women.¹⁴² This could, however, relate to the use of isolated isoflavones consumed as a dietary supplement rather than in soy protein — see Section 7.4.2. This study also found interactions between the apolipoprotein E (apoE) genotype and treatment tended to be significant for changes in total and LDL cholesterol in 49- to 65-year-old women, with isoflavone treatment potentially beneficial.¹⁴² ApoE is an important factor influencing blood lipid profiles and the women were genotyped for polymorphisms in the gene encoding apoE to determine potential gene-treatment interactions.¹⁴²

It is of related interest that a cross-sectional study in 301 postmenopausal women (60 to 75 years of age) living in the Netherlands, where isoflavone and lignan intakes were assessed with a food frequency questionnaire covering habitual diet during the year prior to the study, reported that high intakes of isoflavones were associated with lower levels of the atherogenic lipoprotein Lp(a) but had little effect on plasma lipids (total cholesterol, LDL, and HDL cholesterol and triglycerides), suggesting that at low levels of intake dietary isoflavones have a limited effect on plasma lipids.¹⁴⁵

7.4.2 ANTIOXIDANT ACTION

Antioxidant action is one of the mechanisms that may contribute to the cardiovascular protective effects of soy and soy isoflavones. Antioxidant properties have been reported for isoflavones both *in vitro* and *in vivo* (see Section 7.3.5).

The oxidation hypothesis of atherosclerosis states that the oxidative modification of LDL (or other lipoproteins) is important and possibly obligatory in the pathogenesis of the atherosclerotic lesion; thus, it has been suggested that inhibiting the oxidation of LDL will decrease or prevent atherosclerosis and clinical sequelae.¹⁴⁴ LDL oxidation also has important implications for vascular health function. High concentrations of LDL may inhibit arterial function in terms of the release of nitric oxide from the endothelium and many of these effects are mediated by lipid oxidation products.¹⁴⁵ Furthermore, oxidized LDL inhibits endothelium-dependent nitric oxide-mediated relaxations in isolated rabbit coronary arteries.¹⁴⁶ Oxidized LDL induces apoptosis in vascular cells including macrophages and this is prevented by nitric oxide.¹⁴⁷

Isoprostanes are a relatively new class of lipids and are produced *in vivo* principally by a free radical-catalyzed peroxidation of polyunsaturated fatty acids.^{148–150} Isoprostanes are isomers of the conventional enzymatically derived prostaglandins. F₂-isoprostanes are the most studied species and are isomers of the enzyme-derived prostaglandin F_{2 α} . F₂-isoprostanes are considered to provide a reliable biomarker for oxidative stress and resultant oxidative lipid damage *in vivo* because of their mechanism of formation, chemical stability, and specific structural features that enable them to be distinguished from other free radical-generated products. Increased concentrations of F₂-isoprostanes have been consistently reported in association with cardiovascular risk factors such as chronic cigarette smoking, diabetes mellitus, and hypercholesterolemia. Furthermore, some F₂-isoprostanes possess potent biological activities indicating that they may also act as mediators of the cellular effects of oxidative stress. Oxidative stress may also lead to raised blood pressure, another cardiovascular risk factor, possibly via effects on arterial function.^{148–150}

The effect of dietary soy isoflavones on the F₂-isoprostane 8-*epi*-prostaglandin F_{2 α} (8-*epi*-PGF_{2 α} biomarker for *in vivo* lipid peroxidation) and on resistance of LDL to oxidation has been reported.¹¹⁴ In a randomized crossover study in 24 young healthy male and female subjects, consuming diets that were rich in soy that was high (56 mg isoflavones/day: 35 mg genistein and 21 mg daidzein) or low in isoflavones (2 mg isoflavones/day), each for 2 weeks, plasma concentrations of the F₂-isoprostane 8-*epi*-PGF_{2 α} were significantly lower after the high-isoflavone dietary treatment than after the low-isoflavone dietary treatment (326 \pm 32 and 405 \pm 50 ng/l, respectively, $P = 0.028$).¹¹⁴ The lag time for copper-ion-induced LDL oxidation was longer (48 \pm 2.4 and 44 \pm 1.9 min, respectively, $P = 0.017$).¹¹⁴

This increased resistance of LDL to oxidation is in agreement with the findings of a number of studies with dietary soy, including the increase in lag time in a study in six young healthy male and female subjects who consumed three soy bars per day (providing 57 mg total isoflavones/day: 36 mg genistein and 21 mg daidzein) for 2 weeks.¹⁵¹ Although HDL-derived 17 β -estradiol fatty acid esters have been shown to accumulate in LDL *ex vivo*¹⁵² and esterified

isoflavones can also be incorporated into LDL *ex vivo*,¹⁵³ it has not yet been shown that isoflavones can be esterified to LDL *in vivo*. Increased resistance to LDL oxidation has also been reported in a 12-week single open-group dietary intervention with soy foods (60 mg isoflavones/day) in 42 normal postmenopausal women.¹⁵⁴ A randomized crossover study in 25 hyperlipidemic male and female subjects, consuming soy-based breakfast cereals (168 mg total isoflavones/day) and control breakfast cereals, each for 3 weeks, reported decreased oxidized LDL (total conjugated diene content) following consumption of the soy-based breakfast cereal compared to the control.¹⁵⁵

In contrast to these clear antioxidant effects of dietary soy, which is likely to be mediated by soy isoflavones, when isoflavones are extracted from soy and used to make supplements, this appears to reduce their antioxidant efficacy, as indicated by a wide range of studies. A 4-month randomized crossover placebo-controlled study in 14 healthy premenopausal women using an isoflavone supplement to deliver 86 mg/day for two menstrual cycles reported no change in LDL lag time.¹⁵⁶ Furthermore, a number of studies investigating effects of soy isoflavone supplements on oxidative stress found no effect on F₂-isoprostane concentrations. Consumption of 55 mg isoflavones/day for 8 weeks by 59 male and female subjects (35 to 69 years of age) with high-normal blood pressure,¹⁵⁷ consumption of 50 mg isoflavones, twice a day for 3 weeks by 12 male and female subjects (22 to 56 years of age),¹¹⁹ and consumption of 30 mg isoflavones twice a day for 12 weeks by 36 postmenopausal subjects (H. Wiseman et al., unpublished results) all had no effect on F₂-isoprostane concentrations. To try and understand more fully how the form in which isoflavones are consumed influences their ability to protect human health, we have recently carried out a crossover study in healthy young women, comparing the effects of isoflavones within the food matrix with those in supplements, on biomarkers of oxidative stress, including F₂-isoprostanes (H. Wiseman et al., unpublished results).

7.4.3 ARTERIAL FUNCTION

Arterial function is vital to the prevention of ischemic changes in the organs that the arteries deliver blood to, and is particularly relevant to ischemic heart disease. A recent population-based study (the Rotterdam study) has shown arterial stiffness (or compliance) to be strongly associated with atherosclerosis at various sites in the vasculature (aorta and carotid artery).¹⁵⁸ Mechanisms of soy-mediated vascular protection may include effects on arterial function, including flow-mediated endothelium-dependent vasodilation (reflecting endothelial function) and systemic arterial compliance (reflecting arterial elasticity) and these have been measured in a number of studies. A randomized double-blind study administering either soy protein isolate (118 mg isoflavones/day) or caesin placebo for 3 months to 213 healthy male and postmenopausal subjects (50 to 75 years of age) showed a significant improvement in peripheral pulse wave velocity (reflecting peripheral vascular resistance and one component, together with systemic arterial compliance, of vascular function) but worsened flow-mediated vasodilation in the men and had no significant effect on the flow-mediated vasodilation in the postmenopausal women.¹⁵⁹ Furthermore, consumption of soy protein with isoflavones (107 mg isoflavones/day for 6 weeks) in a randomized, crossover study had favorable effects on the endothelium (postocclusion peak flow velocity of the brachial artery was significantly lower, consistent with a vasodilatory response) in healthy menopausal women.¹⁶⁰

In a placebo-controlled, randomized, crossover study with 21 peri- and postmenopausal women treated for 5 weeks with a supplement delivering 80 mg total soy isoflavones/day, a significant improvement was reported in systemic arterial compliance, but had no effect on flow-mediated vasodilation.¹⁶¹ This lack of an effect on flow-mediated vasodilation is in

agreement with a study of similar design, in 20 postmenopausal women and again using a supplement to provide 80 mg total soy isoflavones/day.¹⁶²

It is noteworthy that a cross-sectional study in 301 postmenopausal women (60 to 75 years of age) in the Netherlands, where isoflavone and lignan intakes were assessed with a food frequency questionnaire covering habitual diet during the year prior to the study, reported no associations between isoflavone intake and vascular function, including endothelial function, blood pressure, and hypertension, and this is in contrast to the observed protective effect of dietary lignan intake on blood pressure and hypertension.¹⁶³

7.4.4 CELLULAR EFFECTS

Vascular protection could also be conferred by the ability of genistein to inhibit proliferation of vascular endothelial cells and smooth muscle cells and to increase levels of growth factors,¹⁶⁴ including the cytokine transforming growth factor β (TGF- β). Phytoestrogens including the soy isoflavones genistein and daidzein and the daidzein metabolite equol have all been reported to inhibit growth and MAP kinase activity in human aortic smooth muscle cells¹⁶⁵ and thus may confer protective effects on the cardiovascular system by inhibiting vascular remodeling and neointima formation. TGF- β helps maintain normal vessel wall structure and promotes smooth muscle cell differentiation, while preventing their migration and proliferation. Genistein has been shown to increase TGF- β secretion by cells in culture¹⁶⁶ and, as previously suggested for tamoxifen,¹⁶⁷ increased TGF- β production may be a mediator of some of the cardioprotective effects of soy.¹⁶⁶ However, we have recently found no effect on plasma TGF- β concentrations following consumption of soy either high (56 mg/day) or low (2 mg/day) in isoflavones for 2 weeks in a randomized crossover study in young healthy subjects.¹⁶⁸

7.5 PROTECTION BY ISOFLAVONOIDS AGAINST OSTEOPOROSIS, COGNITIVE DECLINE, AND MENOPAUSAL SYMPTOMS?

7.5.1 OSTEOPOROSIS

Osteoporosis is a chronic disease in which the bones become brittle and break more easily. Postmenopausal women may suffer hip fractures caused by osteoporosis, which develops primarily as a consequence of the low estrogen levels that occur after the menopause. Premenopausal women are, therefore, protected by their estrogen levels against osteoporosis. Although calcium supplementation is important before the menopause, on its own it cannot stop bone loss in perimenopausal and postmenopausal women. Hormone replacement therapy (HRT) can be very effective, 0.625 mg/day of conjugated estrogens has been reported to prevent bone loss¹⁶⁹ and HRT is osteoprotective if taken postmenopausally for more than 24 months.¹⁷⁰ The drug ipriflavone (a synthetic isoflavone derivative) at a dose of 600 mg/day can prevent the increase in bone turnover and the decrease bone density in postmenopausal women.¹⁷¹ The protective effects of HRT, together with the finding that ER β is highly expressed in bone and appears to mediate a distinct mechanism of estrogen action,^{74,75} suggests that phytoestrogens may thus protect women against postmenopausal bone loss.¹⁷² The potential skeletal benefits of soy isoflavones has recently been reviewed.¹⁷³

Consumption by postmenopausal women (6-month parallel-group design) of soy protein (40 g/day providing either 56 mg isoflavones/day or 90 mg isoflavones/day) compared to cesin and nonfat dry milk (40 g/day) produced significant increases in bone mineral content and density in the lumbar spine (but not in any other parts of the body), but only in the higher isoflavone (90 mg/day) group compared to the control group.¹³⁷ Daily intake for 2 years of

two glasses of soymilk containing 76 mg of isoflavones has been reported to prevent lumbar spine bone loss in postmenopausal women.¹⁷⁴ Moreover, consumption of a red clover-derived isoflavone supplement (43.5 mg/day isoflavones) for 1 year significantly decreased loss of lumbar spine bone mineral content and bone mineral density and increased concentrations of the bone formation markers.¹⁷⁵ Similarly, consumption of a soy isoflavone supplement (80 mg/day isoflavones) for 1 year were found to have a beneficial effect on hip bone mineral content in postmenopausal Chinese women with a low initial bone mass.¹⁷⁶

Consumption of soy foods (providing 60 mg/day isoflavones) for 12 weeks by postmenopausal women has been found to significantly decrease clinical risk factors for osteoporosis (short-term markers of bone turnover) including decreased urinary *N*-telopeptide excretion (bone resorption marker) and increased serum osteocalcin (bone formation marker).¹⁵⁴ Furthermore, consumption of a soy isoflavone supplement containing 61.8 mg of isoflavones for 4 weeks by postmenopausal Japanese women significantly decreased excretion of bone resorption markers.¹⁷⁷

A study in 500 Australian women (aged 40 to 80 years) has shown that higher isoflavone intakes are associated with higher concentrations of bone alkaline phosphatase, a short-term marker of bone formation and turnover.¹⁷⁸

Treatment of postmenopausal women with osteoporosis with raloxifene (60 mg/day or 120 mg/day for 36 months) was found to significantly increase bone mineral density in the spine and femoral neck and decrease the risk of vertebral fracture compared to the placebo treatment.¹⁷⁹ Treatment with raloxifene increased the risk of venous thromboembolism compared to the placebo group and was also associated with a lower risk of breast cancer and did not cause breast pain or vaginal bleeding.¹⁷⁹

7.5.2 MENOPAUSAL SYMPTOMS AND COGNITIVE DECLINE

The estrogenic properties of phytoestrogens may also help with menopausal symptoms such as hot flushes and vaginitis.¹⁸⁰ An improvement in hot flushes with dietary supplementation with 45 g of raw soy flour per day has been reported; however, an improvement was also seen with white wheat flour (contains very little in the way of phytoestrogen content).¹⁸¹ Furthermore, consumption of a red clover-derived isoflavone supplement (80 mg/day isoflavones) has been reported to significantly decrease menopausal hot flush symptoms compared with placebo.¹⁸² However, two recent systematic reviews have reached differing conclusions; while the first review concludes that there is some evidence to support the efficacy of soy and soy isoflavone preparation for perimenopausal symptoms,¹⁸³ the second one concludes that isoflavone phytoestrogens available as soy foods, soy extracts, and red clover extracts do not improve hot flushes or other menopausal symptoms,¹⁸⁴ suggesting that further studies are needed.

Consumption of soy foods for 10 weeks (100 mg/day isoflavones) has been reported to improve human memory in young healthy adults^{185,186} and consumption of a soy isoflavone supplement for 12 weeks (60 mg/day isoflavones) to improve cognitive function in postmenopausal women.¹⁸⁷ By contrast, consumption of soy protein (99 mg isoflavones/day) for 12 months failed to improve cognitive function in postmenopausal women,^{188,189} suggesting further clinical trials are required to fully determine the possible health beneficial effects of isoflavones against cognitive decline.

7.6 ISOFLAVONOIDS: POTENTIAL RISKS

Phytoestrogens can cause infertility in some animals and thus concerns have been raised over their consumption by human infants. The isoflavones found in a subterranean clover species (in Western Australia) have been identified as the agents responsible for an infertility

syndrome in sheep.¹⁹⁰ Soy isoflavones in the diets of cheetahs in captivity has been shown to lead to their infertility.¹⁹¹ Most animals that are bred commercially and domestic animals, however, are fed diets containing soy (up to 20% by weight) without any apparent reproductive problems.¹ No reproductive abnormalities have been found in peripubertal rhesus monkeys¹³² nor in people living in countries where soy consumption is high. Indeed, the finding that dietary isoflavones are excreted into breast milk by soy-consuming mothers suggests that in cultures where the consumption of soy products is the normal dietary practice, breast-fed infants are exposed to high levels again without any adverse effects.¹⁹² Isoflavone exposure shortly after birth at a critical developmental period through breastfeeding may protect against cancer and may be more important to the observation of lower cancer rates in populations in the Far East than adult dietary exposure to isoflavones.¹⁹²

There have been some concerns expressed regarding the possible health consequences in adulthood (endocrinological and reproductive outcomes) of early-life isoflavone exposure from soy-based infant formula.¹⁹³ The daily exposure of infants to isoflavones in soy-based infant formulas is 6- to 11-fold higher on a body weight basis than the dose that has hormonal effects in adults consuming soy foods.¹⁹⁴ However, evidence from adult and infant populations indicates that dietary isoflavones in soy-based infant formulas do not adversely affect human growth, development, or reproduction.^{51,52,193,195-197}

Although toxicity from isoflavones may arise from their action as alternative substrates for the enzyme thyroid peroxidase¹⁹⁸ and people in southeast Asia would be protected by the dietary inclusion of iodine-rich seaweed products, a recent study has shown that isoflavone supplements do not affect thyroid function in iodine-replete postmenopausal women.¹⁹⁹ Considerations of the safety of soy isoflavones is an area of great interest in relation to their potential benefits to human health and has recently been comprehensively reviewed.²⁰⁰

7.7 ISOFLAVONOIDS AND HUMAN HEALTH: CONCLUSIONS

The important question of whether isoflavonoids should be used to protect human health clearly requires much more information to be provided by appropriate studies. Factors such as age and biological responsiveness to the different potential protective or even harmful effects of isoflavonoids (these will change with age) appear to play an important role.

Postmenopausal women may well benefit in terms of protection against heart disease and osteoporosis, from estrogen replacement therapeutics strategies that utilize isoflavonoids. Older men may also benefit from protection against problems such as prostate cancer and cardiovascular disease.

In 1999, the US FDA allowed health claims (on food labels) on the association between soy protein and reduced risk of coronary heart disease for foods containing ≥ 6.25 g of soy protein, assuming either four servings, or that a total of 25 g of soy protein are consumed daily. Furthermore, in 2002, the UK Joint Health Claims Initiative approved a health claim on the association between soy protein and cholesterol reduction, "the inclusion of at least 25 g of soy protein per day, as part of a diet low in saturated fat, can help reduce blood cholesterol levels" and it is important to note that this claim relates to soy protein that has retained its naturally occurring isoflavones.²⁰¹

An important *caveat* is that the epidemiological evidence suggesting protection from hormone-dependent cancer by isoflavone phytoestrogens is based on foods rather than isoflavone extracts, which in the future could include isoflavone-containing therapeutics. Indeed, the preparation of isoflavone extracts from soy protein could well result in the loss

of important components that act synergistically with the isoflavones. This approach could also result in daily isoflavonoid intake increased too far above normal dietary levels such that toxicity occurs.

The results of further extensive studies are thus clearly needed before further decisions can be made regarding the future of isoflavonoids in human health.

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8 Flavonoid Functions in Plants

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8.1 INTRODUCTION

The flavonoids are a remarkable group of plant metabolites. No other class of secondary product has been credited with so many — or such diverse — key functions in plant growth and development. Many of these tasks are critical for survival, such as attraction of animal vectors for pollination and seed dispersal, stimulation of *Rhizobium* bacteria for nitrogen fixation,

promotion of pollen tube growth, and the resorption of mineral nutrients from senescing leaves. Others provide a competitive edge to plants that grow under suboptimal environments. Flavonoids, for example, are known to enhance tolerance to a variety of abiotic stressors, they are employed as agents of defense against herbivores and pathogens, and they form the basis for allelopathic interactions with other plant species. The flavonoids are evidently extremely useful to plants, and it is not surprising, therefore, that species from all orders of the plant kingdom, from the basal liverworts to the most advanced angiosperms, invest significant amounts of metabolic energy into the production of these compounds.

The past decade has witnessed resurgence in research activity on the functions of flavonoids in plants. There are several reasons for this. First, advances in molecular biology, coupled with an improved knowledge of the pathway for flavonoid biosynthesis, have led to the production of plant mutants that are deficient or superabundant in one or more flavonoid pigments. Comparisons of mutant and wild-type phenotypes have permitted hypotheses for flavonoid function to be tested directly. Second, improvements in analytical techniques (e.g., high-performance liquid chromatography, liquid chromatography–mass spectrometry, and nuclear magnetic resonance spectroscopy) for flavonoid compounds have stimulated the search for novel compounds useful for the manipulation of flower color. These, in turn, prompted the discovery of hitherto unknown functions of flavonoids in plant reproduction. Third, concerns about the enlarging ozone hole and the increased exposure of biota to ultraviolet (UV) radiation led to the quest for sunscreens — and to the knowledge that some flavonoids play an important role in protecting plants from harmful UV-B levels. Fourth, there has been an explosive interest in flavonoids, particularly the anthocyanins, as potential nutritional supplements for humans. This contributed to the discovery of their antioxidant roles *in planta*. Finally, advances in field-portable instrumentation have enabled hypotheses for flavonoid function to be tested directly in the field.

In this chapter, we review experimental and theoretical evidence for the main hypotheses for flavonoid functions in plants. Our discussion distinguishes between the functions of the red and blue flavonoids (anthocyanins and 3-deoxyanthocyanins) and those of the colorless (or yellow) remainder. Recent evidence indicates that these two subsets differ markedly both in range and type of functions and, for many species, in their cellular location within the plant tissue. The property of anthocyanin molecules to absorb green light, for example, affords unique capabilities, such as the protection of chloroplasts from the damaging effects of strong irradiance, and as a visible cue to some animals. Flavonols and flavones, on the other hand, do not directly affect photosynthesis, but they can act as chemical signals or UV guides to attract or deter insects, and are highly effective UV filters. Thus, the comparison of the “colorful” versus “colorless” flavonoids provides an instructive insight into the divergent evolution of the roles of flavonoids from a common biosynthetic pathway.

8.2 ANTHOCYANINS AND 3-DEOXYANTHOCYANINS

8.2.1 DEFENSE

One of the longest-standing hypotheses for the presence of anthocyanins in leaves — that they serve to protect plants against herbivory or pathogenic attacks — has received strong theoretical support in recent years, yet still lacks compelling direct evidence. Anthocyanins have been implicated as aposematic colorants, as insect feeding-deterrents, and as antifungal agents, but there is as yet no good reason to believe that defense is the unifying, or indeed even a primary reason for the production of these pigments in vegetative tissues.^{1,2}

The anthocyanins, in contrast to certain other phenolic compounds (see Section 8.3.4), are not toxic to higher animal species.^{3,4} For the invertebrates, too, anthocyanin toxicity seems

to be the exception rather than the rule. The most abundant foliar anthocyanin, cyanidin-3-*O*-glucoside, has been reported to inhibit the growth of larvae of the tobacco budworm, *Heliothis virescens*, an important pest of cotton and other crops.⁵ However, anthocyanin-rich extracts did not influence the feeding behavior or survival rate of aphids, nor inhibit larval growth of the fruitworm.⁶⁻⁸ Fecundity was reduced in aphids that had been fed on preinfested, red portions of *Sorghum* leaves; the response could not be causally attributed to anthocyanins, however, because leaves that had turned red from water stress, rather than preinfestation, had no effect on fecundity.⁹ It seems unlikely, therefore, that anthocyanin toxicity plays any major role in the defense against insect grazers.

There is, nonetheless, evidence that some insects preferentially avoid eating red-leaved plants. California maple aphids *Periphyllus californiensis*, for example, have been observed to colonize the yellow-orange leaves of Japanese maples, yet almost entirely avoid red-leaved individuals.^{10,11} Hamilton and Brown¹² attributed such discriminatory behavior to the effects of aposematic coloration. The red colors in autumn leaves, they reasoned, serve as an honest signal of defensive commitment against insect herbivores. In their survey of the literature on 262 north-temperate tree species, autumn foliage coloration was noted to be strongest in those species that face a high diversity of damaging specialist aphids. The authors suggested that the red light reflected from anthocyanic leaves supplies a “pick on someone else” warning signal to the aphids, which are known to use color cues in host selection. One problem with this hypothesis, however, is that red light is thought to lie beyond the perception of most aphid species¹³; the red warning is being issued to a blind audience! An alternative explanation could be that the red coloration renders leaves unattractive to the insects. Aphids are most attracted to green or yellow light, the very waveband that is deficient in the spectrum that is reflected from anthocyanic leaves.¹⁴ Thus, red foliage may simply appear unpalatable to potential herbivores. The costs to the plant associated with the biosynthesis of anthocyanin pigment may be more than offset by the gains to be had from herbivore deterrence.

Similar hypotheses have been proposed to explain the presence of anthocyanins in the young leaves of growth flushes in many tropical, and some temperate plant species. When such leaves lack chlorophyll they are particularly conspicuous, at least to the human eye, varying from scarlet to red, crimson, mauve, or blue.¹⁵⁻¹⁸ The absence of chlorophyll means that flushing red leaves hold less nitrogen and other minerals than do young green leaves, and this may make them a less attractive meal for potential herbivores. Moreover, when chlorophyll is eventually synthesized, the leaves are already fully expanded and possess tough cellular features that would impair their digestibility.¹⁹ The red colors might, in addition, serve to undermine the visual camouflage of invertebrates; herbivory would be reduced by exposing the browsing insects to their predators.²⁰

For many species, however, leaf flushes contain appreciable amounts of chlorophyll in addition to the anthocyanins,²¹ and the pigment combination generates brown or almost black colors if in sufficient concentration. Dark colors can camouflage leaves against the exposed soil and litter of forest floors.^{22,23} They may also serve to mimic dead leaves.²⁴ Indeed, even the brilliant red and crimson flushing leaves might appear dark or dead to a potential herbivore, since most nonmammalian folivores lack red light receptors.²⁵ Juniper¹⁸ noted that flushing leaves often appear flaccid and hang down vertically from the branches “like wet facial tissue.” He argued that the color, texture, and orientation of such leaves would deter predation at this vulnerable stage in development by obscuring the characteristic “leaf cues” as perceived by an insect. Anthocyanin biosynthesis was considered a significant part of this complex strategy to ensure leaf longevity.

An alternative postulate for red coloration in tropical young leaves is that the anthocyanins protect against fungal infection. Such protection may be critical during the vulnerable

period of leaf expansion before the formation of a protective cuticle and lignified cell walls. In support of this hypothesis, Coley and Aide²⁶ showed that the leaf-cutting ant *Atta columbica* preferentially avoids red-leaved species. The ant cultivates fungus on acquired leaf pieces, and it is the fungus, rather than the leaves, that serves as the sole food source for larvae and much of the diet for workers and queen. The ant avoids cutting leaves that contain antifungal compounds, perhaps because of its dependence on the fungus. In feeding trials using leaf discs from 20 plant species, ant preference decreased significantly as the anthocyanin content increased. Moreover, when oat flakes impregnated with cyanidin chloride were supplied as the only food source, the ants again showed dosage-dependent avoidance. The authors suggested that even low concentrations of anthocyanins might be detrimental to fungal colonies.

There is not, however, very much *direct* evidence for fungicidal properties of the anthocyanin pigments. This is in sharp contrast to certain 3-deoxyanthocyanidins, which have been shown to be highly effective agents against both fungi and bacteria. Apigeninidin significantly inhibited the growth of *Fusarium oxysporum*, *Gibberella zeae*, *Gliocladium roseum*, *Alternaria solani*, and *Phytophthora infestans* on agar plates.²⁷ It also inhibited the growth of certain gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus faecalis*) and, to a lesser extent, gram-negative bacteria (*Escherichia coli*, *Serratia marcescens*, and *Shigella flexneri*) on agar.²⁸ Antifungal properties of apigeninidin have been postulated to play a role in mold resistance in *Sorghum* seeds, where the 3-deoxyanthocyanidin is abundant.

Although anthocyanins do not seem to have mycotoxic properties *per se*, they may still function to protect some plants from invading pathogens. Page and Towers²⁹ recently demonstrated a novel mechanism whereby a mix of cyanidin-3-*O*-glucoside and cyanidin-3-*O*-(6-*O*-malonylglucoside) could confer protection to silver beachwood (*Ambrosia chamissonis*). This plant holds large amounts of thiarubrine pigments in its stems, petioles, and roots. The thiarubrines, which are toxic to a variety of organisms including insects, bacteria, and fungi, contain a 1,2-dithiin chromophore that is highly unstable in light.^{30–33} Even short exposures to visible light or UV radiation convert the red thiarubrine A to 2,6-dithiabicyclo[3.1.0]hexane intermediates, and then to the colorless, inactive, thiophene A (Figure 8.1). *A. chamissonis* naturally grows at sunny locations along the Pacific coast of North America, and requires, therefore, a mechanism to prevent the decomposition of these photolabile defense compounds. In the stems and petioles, thiarubrine A is held in subepidermal laticifers surrounded by a sheath of anthocyanin-containing cells. The absorbance spectra for cyanidin-3-*O*-glucoside and thiarubrine A are strikingly similar in the visible region of the light spectrum. Thus, anthocyanins could contribute to defensive armory by intercepting quanta that would otherwise degrade the potent, toxic thiarubrines. Consistent with this hypothesis, surgical removal of the anthocyanic sheath led to rapid destruction of thiarubrine A under the light of a microscope.²⁹ Furthermore, thiarubrines in the roots, which would not normally experience high irradiances, are not enshrouded by anthocyanins. The metabolic investment into anthocyanin biosynthesis is evidently part of a coordinated strategy to ameliorate defense responses, rather than an extravagant byproduct of a saturated flavonoid pathway. It seems highly likely that other photolabile protectants could also benefit from an anthocyanin shield. The light-screening hypothesis for anthocyanin function as a defense agent presents an exciting new avenue for future research.

8.2.2 PROTECTION FROM SOLAR ULTRAVIOLET

The anthocyanins have often been included alongside other flavonoids and the hydroxycinnamic acids as potential UV protectants. Most anthocyanins absorb UV radiation between

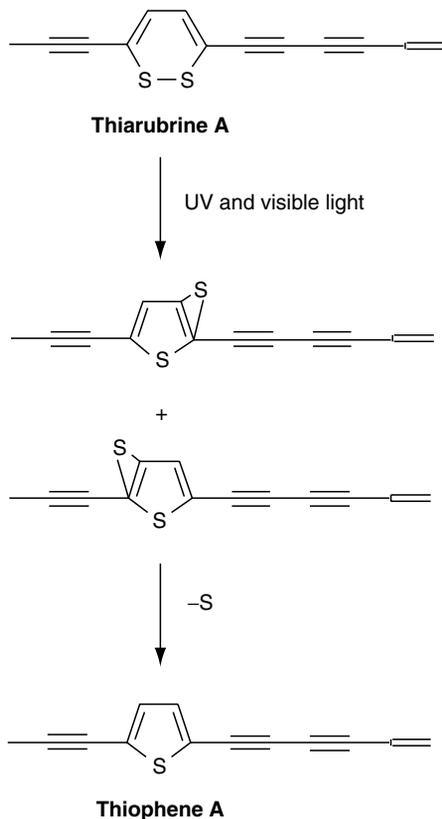


FIGURE 8.1 Conversion of the antimicrobial agent, thiarubrine A, to its photoproduct, thiophene A, by exposure to ultraviolet and visible radiation. (Redrawn from Page, J.E. and Towers, G.H.N., *Planta*, 215, 478, 2002. With permission.)

270 and 290 nm, and the acylated anthocyanins, in particular, are strong UV-B absorbers.^{34,35} Red leaves often reflect significantly less solar UV than do green leaves as a consequence of their higher UV absorbance.³⁶ If the energy of the absorbed UV is not transmitted to cellular organelles, then anthocyanins could serve to moderate the damage to DNA, proteins, and membranes in plants that grow naturally in high UV-B environments.

Consistent with a protective role against solar UV, anthocyanin biosynthesis has been observed many times to be induced or upregulated in plants following exposure to supplementary UV radiation,^{37–52} although exceptions have been noted.^{53–57} There are also reports describing more direct evidence for anthocyanin involvement in UV protection. For example, in cultured cells of the cornflower *Centaurea cyanus*, the presence of cyanidin 3-*O*-(6-*O*-malonyl) glucoside appreciably reduced the extent of damage to DNA following UV-B or UV-C irradiation.⁵⁸ Similarly, an anthocyanin-rich extract from red apple skins protected against UV-B-induced damage to plasmid DNA,⁵⁹ and flavonoids from a red-leafed maize mutant significantly impeded the dimerization of adjacent pyrimidines on the same strand of DNA, one of the key indicators of DNA damage (Figure 8.2), in tissues irradiated with UV-B or UV-C.⁶⁰ Mutants of *Arabidopsis thaliana* that are deficient in flavonoids, including the anthocyanins, but which have normal levels of sinapate esters, are more sensitive to UV-B radiation than is the wild type when grown under high irradiance.⁶¹ Finally, red-leafed *Coleus*

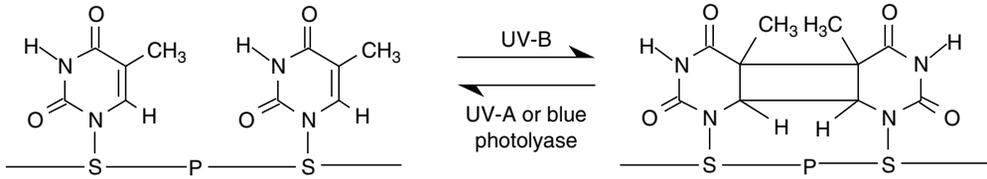


FIGURE 8.2 Formation of a thymine–thymine dimer by UV-B radiation, and repair by UV-A or blue light-activated photolyase.

varieties that had been pretreated with UV-B or UV-C retained greater photosynthetic efficiencies under strong light than did green-leaved varieties, indicating that their acylated anthocyanins effectively shielded the proteins associated with photosynthetic electron transport.⁶²

Notwithstanding these data, a substantial body of recent empirical evidence argues strongly against any major involvement of anthocyanins in the protection from UV. First, in most red-leaved species that have been examined, the anthocyanins do not reside at the most suitable cellular location for UV screening. An effective UV filter needs to remove the short-wavelength UV component before it reaches the chloroplasts, at the same time providing minimal attenuation of the photosynthetically active radiation.⁶³ Because chloroplasts are generally located in the subepidermal tissues of vegetative shoots, the outermost layer, known as the dermal system, is considered to be the most appropriate site for UV interception.⁶⁴ UV-screening pigments must be present in epidermal cell walls as well as in the vacuole if they are to prevent significant transmission of UV light to subjacent chlorenchyma.^{65–67} Colorless phenolic compounds, including certain flavonoids and hydroxycinnamic acids, have indeed been found in epidermal cell vacuoles, walls, cuticles, and trichomes in the leaves of many species (Table 8.1).^{68–80} The anthocyanins, too, have been found in epidermal cell vacuoles of roots,^{81,82} various floral organs,^{67,83,84} and some leaves, including *Arabidopsis*.⁸⁵ Cell-wall-bound anthocyanins and “anthocyanin-like pigments” have been observed in the leaves of certain liverwort and moss gametophytes.^{86–88} In most red-leaved species, however, the anthocyanins are present as solutions inside the vacuoles of palisade and spongy mesophyll cells, the very cells that require protection from the effects of excess UV radiation. This seems to be equally true of senescing leaves^{21,89} as of expanding and mature leaves.^{90–92} Thus, although the red cell vacuoles might well capture stray rays of UV light, their location within the internal tissues of the leaf precludes any major role as a UV filter.

Several studies involving plant mutants have also concluded that factors other than anthocyanin pigmentation contribute to UV protection. For example, Lois and Buchanan¹⁰³ identified in *Arabidopsis* a mutation that led to dramatic increases in sensitivity to UV radiation. The mutant was found to be deficient in certain flavonoids, particularly a rhamnosylated derivative of kaempferol, yet it held normal amounts of anthocyanin. Similarly, Klaper et al.¹⁰⁴ found that neither a deficiency nor a surfeit of anthocyanin influenced the growth responses of *Brassica rapa* mutants to supplementary UV-B treatment. The authors suggested that flavonoids other than the anthocyanins might respond more rapidly and to a greater extent to UV-B exposure. Red-leaved plants have been noted to contain high levels of colorless phenolic compounds which contribute significantly more than anthocyanins to the total absorbance of UV-A and UV-B radiation.¹⁰⁵

There is, moreover, evidence that for some species the presence of anthocyanins can impair, rather than enhance plant performance under UV radiation. Ryan et al.¹⁰⁶ noted that the transgenic *leaf color* line of *Petunia*, which had elevated levels of anthocyanins resulting from

TABLE 8.1
Examples of Plant Species from which UV-B Protective Flavonoids Have Been Identified

Plant Species	Flavonoid Location	Protective Flavonoids	Ref.
<i>Arabidopsis thaliana</i>	Epidermal cells	Kaempferol-3-gentiobioside-7-rhamnoside; kaempferol-3,7-dirhamnoside	94
<i>Hordeum vulgare</i>	Epidermal cells	Saponarin; luteonarin	95
<i>Brassica oleracea</i>	Epidermal cells	Cyanidin glycosides	96
<i>Zea mays</i>	Epidermal cells	Anthocyanins	60
<i>Gnaphalium luteo-album</i>	Leaf wax	Calycopterin; 3'-methoxycalycopterin	97
<i>Gnaphalium vira-vira</i>	Leaf wax	7-O-Methylaraneol	98
<i>Marchantia polymorpha</i>	Thallus cells	Luteolin-7-glucuronide; luteolin-3,4'-diglucuronide	99
<i>Sinapis alba</i>	Epidermal cells	Anthocyanin glycosides; quercetin glycosides	54
<i>Oryza sativa</i>	Epidermal cells	Iso-orientin acylated glucosides	100
<i>Pinus sylvestris</i>	Epidermal cells	3'',6''-di- <i>p</i> -Coumarylkaempferol-3-glucoside; 3'',6''-di- <i>p</i> -coumarylquercetin-3-glucoside	78
<i>Brassica napus</i>	Epidermal cells	Quercetin-3-sophoroside-7-glucoside; quercetin-3-sinapylsophoroside-7-glucoside	101
<i>Quercus ilex</i>	Leaf hairs	Acylated kaempferol glycosides	102

Source: Adapted from Harborne, J.B. and Williams, C.A., *Phytochemistry*, 55, 481, 2000. Copyright (2000), With permission from Elsevier.

the action of a maize flavonoid regulatory gene, grew slower than wild-type plants in a UV-B enriched environment. Likewise, the anthocyanic seedlings of *Impatiens capensis* accumulated less biomass and produced fewer flowers and fruits than did green seedlings after UV stress,¹⁰⁷ and purple-leaf mutants of rice grew less, and had more evidence of DNA damage after UV treatment than did green-leaved lines with a similar genetic background.^{46,108}

To understand the apparent detrimental effect of anthocyanins on the growth of UV-irradiated plants, Hada et al.¹⁰⁹ compared the rates of synthesis of cyclobutane pyrimidine dimers (CPDs) and of DNA repair in the near-isogenic lines of purple- and green-leaved rice. Short-term exposures to UV-B caused greater CPD production in the green than in the purple line. Under continuous UV exposure, however, the trend reversed; the purple line accumulated significantly more CPDs than did the green line. The difference was attributable to rates of CPD repair, which ran substantially faster in the green line. To repair UV-damaged DNA, plants employ photolyase, an enzyme that uses blue or UV-A light to re-monomerize the pyrimidine dimers (Figure 8.2). The anthocyanins in purple rice absorbed the energy of the incident UV-A, effectively preventing the photoactivation of photolyase. Thus, any short-term benefit to be gained from the absorption of UV-B by anthocyanins is offset by their property to limit the rate of DNA repair. Protection from the effects of solar UV cannot be the primary function of anthocyanins in shoots, although other flavonoids assume this role (see Section 8.3.1.1).

8.2.3 PHOTOPROTECTION

When plants receive more light energy than can be used for photochemistry, they show a characteristic decline in the quantum efficiency of photosynthesis, termed photoinhibition (reviewed in Ref. 110). Most plants experience photoinhibition at some point over the course of their lives, often on a regular or even a daily basis. The excess quanta from photoinhibitory

light fluxes can usually be channeled into quenching mechanisms such as xanthophyll pigments associated with the chloroplasts, which ultimately release the energy as heat. When the excess energy can be contained in this way, photoinhibition is said to be *dynamic*; it is reversible, usually short lived, and causes no long-term damage to the photosynthetic apparatus. However, prolonged exposures to strong light, particularly when combined with other environmental stressors such as cold or heat, can saturate the quenching mechanisms and lead to *chronic* photoinhibition. Reactive oxygen species are then generated, with the potential to destroy thylakoid membranes and to denature proteins associated with photosynthetic electron transport, particularly those of photosystem II. Characterized by chlorophyll bleaching or necrotic lesions, chronic photoinhibition can lead to long-term damage or even the death of the plant.

A substantial body of empirical evidence indicates that anthocyanins in leaves can reduce the severity of photoinhibitory damage to plants under stress. A photoprotective role for anthocyanins is hardly a new idea; as early as the 19th century, the German physiologist Pringsheim suggested that anthocyanins might protect the photosynthetic machinery by screening out the most damaging wavelengths (cited in Ref. 111). Only recently, however, have technological advances in field-portable instrumentation permitted the hypothesis to be tested directly. Foremost among these are the pulse-modulated chlorophyll fluorometers that are used to dissect photosynthetic function in the intact plant under field conditions. The kinetics of the generation and decay of chlorophyll fluorescence provide information on quantum efficiency, rates of electron transport, and energy quenching processes.^{112,113} Recent data suggest that chlorophyll fluorescence parameters can differ markedly between red and green leaves under light stress.

Central to the photoprotection hypothesis is the property of anthocyanins to absorb visible radiation. Solutions of anthocyanins in the vacuoles of living cells typically show strong absorbance in the 500 to 600 nm waveband (Figure 8.3). Consequently, red leaves absorb substantially more yellow-green light than do acyanic (green) leaves of comparable age and structure.^{14,62,92,114–120} The amount of extra light that is absorbed (often more than 20% greater than green leaves) is a direct function of the amount of anthocyanin present, irrespective of the location of the red cells within the leaf tissues. Interestingly, the amount of red light that is reflected from red leaves often only poorly correlates to anthocyanin content;

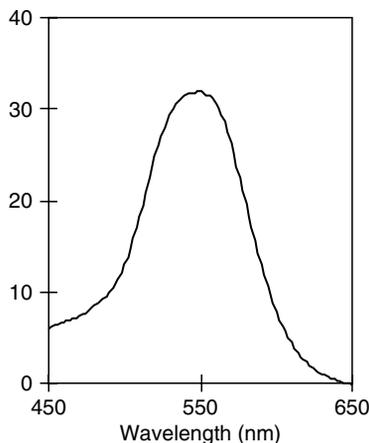


FIGURE 8.3 *In vivo* absorbance spectrum for a single anthocyanic cell vacuole in a leaf of *Aristotelia serrata*. (K.S. Gould, unpublished data.)

chlorophyll content and morphological and anatomical features of the leaf surface evidently influence red reflectance to the greater extent.

The fate of these extra absorbed green quanta is not known. It is very clear, however, that the energy is not transferable to the chloroplasts for use in photosynthesis. Profiles of the absorption of red, blue, and green light within the various leaf tissues have been compared recently for red and green leaves of the New Zealand tree *Quintinia serrata*.¹²¹ The presence of anthocyanin in a cell vacuole dramatically reduced the amount of green light that could penetrate down to the lowermost tissues in the leaf. When anthocyanins were present in the upper epidermal layer, green light utilization was restricted to an extremely narrow band of palisade mesophyll at the top of the leaf. Similarly, when anthocyanins were located in the palisade mesophyll layers, the subjacent spongy mesophyll cells were deprived of green light. Thus, although red leaves absorb the more green light in total, their photosynthetic tissues actually receive less green light than do those of green leaves. Consistent with previous observations of grape leaves,¹²² anthocyanins did not affect the distribution of blue light within the leaf, and they modified the gradient of red light only slightly.

Green light is an important contributor to photosynthesis, especially in the lower spongy mesophyll tissues.^{123,124} A deficit of green light should, therefore, reduce the overall photosynthetic productivity. Accordingly, red leaves have been found to have a lower quantum efficiency of photosynthesis, and a higher threshold irradiance at which saturation of photosynthesis is achieved, relative to green leaves. Red leaves can also display some physiological characteristics of shade plants, possibly as a result of having chloroplasts develop under the anthocyanin optical filter.^{121,125} The differences between photosynthesis of red and green leaves are usually small, however, and are often statistically insignificant,^{120,126–129} although more substantial differences have been noted for certain species.⁶²

Although anthocyanins seem to have only a modest effect on photosynthesis under nonsaturating light, they can nonetheless impact dramatically on the propensity for photoinhibition under conditions of strong light. By absorbing the green, high-energy photons that would otherwise excite chlorophyll *b*, the anthocyanins have the potential to reduce the frequency of dynamic photoinhibition, moderate the severity of chronic photoinhibition, and expedite photosynthetic recovery. Photoprotective advantages of red versus green leaves have been demonstrated many times in disparate plant species, as evidenced from the kinetics of chlorophyll fluorescence during and following light stress.^{115,129–134} For *Cornus stolonifera*, for example, 30-min exposure to white light at $1500 \mu\text{mol m}^{-2} \text{sec}^{-1}$ caused a 60% reduction in the quantum yield of red leaves, but an almost 100% reduction in acyanic leaves (Figure 8.4). When the light was turned off, quantum yields of the red leaves recovered to their maximum value after only 80 min, yet the acyanic leaves did not reach their pretreatment state even after 6 h.

Foliar anthocyanins are evidently an effective device for photoprotection under high light environments. They do not, however, substitute for the protection conferred by xanthophyll-cycle pigments. Recent experiments using flavonoid- or xanthophyll-deficient mutants of *A. thaliana* indicate that the xanthophylls have a greater role in the protection of plants from short-term light stress, but the flavonoids, including the anthocyanins, are the more effective photoprotectants in the long term.¹³⁵

The photoprotection hypothesis for anthocyanins is attractive because it can explain why red-leaved plants occur across such diverse environments. In the tropics, for example, anthocyanins in flushing red leaves may provide a critical photoprotective role to the nascent chloroplasts until adequate levels of xanthophylls have been synthesized.¹³⁶ In the understory, anthocyanins might protect shade-acclimated plants from the effects of sunflecks, which can be 2000-fold brighter than the usual light level, or canopy gaps caused by tree blowdown.^{131,137} A photoprotective function of anthocyanins would also benefit leaves in

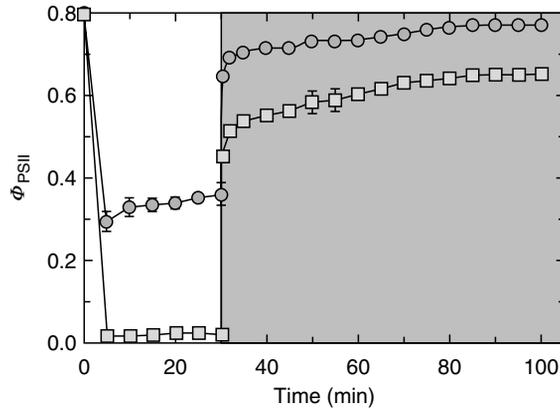


FIGURE 8.4 Changes in the quantum yield of photosystem II (ϕ_{PSII}) following irradiation of red (circles) and acyanic (squares) leaves of *Cornus stolonifera*. The strong, white light ($1500 \mu\text{mol m}^{-2} \text{sec}^{-1}$) was turned off after 30 min, as indicated by the shaded box. (Redrawn from Feild, T.S., Lee, D.W., and Holbrook, N.M., *Plant Physiol.*, 127, 566, 2001. With permission.)

sunny, cold climates where, because of reduced Calvin cycle activity, the absorbed light energy is in excess of the capacity to utilize ATP and NADPH in photosynthesis.¹³² The photoprotection hypothesis has even been used to explain the *de novo* synthesis of anthocyanins in the autumn foliage of deciduous trees. Autumn senescence involves the rapid liberation of nitrogen from the degradation of stroma proteins and thylakoid membranes, and its resorption into the branches. If degrading chlorophyll is not protected from light, however, reactive oxygen is formed that could jeopardize the resorptive process. As an optical screen, anthocyanins may provide photoprotection during the dismantling of the photosynthetic apparatus.^{115,138–142} In support of this hypothesis, the efficiency of nitrogen resorption from senescing leaves has been found to be significantly greater in wild type than in anthocyanin-deficient mutants of three deciduous woody species subjected to high light and low temperatures.¹⁴³

The photoprotection hypothesis is not without its critics,⁶² and it certainly does not account for every occurrence of anthocyanins, such as in the leaf veins, in stems and petioles, and in underground organs such as roots and tubers. Nonetheless, there is now a compelling body of empirical data consistent with a photoprotective function in the photosynthetic cells of leaves. Photoprotection might well be the primary role of anthocyanin in the vegetative shoots of many species.

8.2.4 ANTIOXIDANT ACTIVITY

Over the past decade, several hundred research articles have been published highlighting the antioxidant potency of anthocyanin-rich extracts from fruits and vegetables. It is evident from *in vitro* studies that the anthocyanins are exceptionally strong antioxidants; they scavenge almost all species of reactive oxygen and nitrogen with an efficiency up to four times greater than those of ascorbic acid and α -tocopherol.^{144–148} In experiments involving rats or animal cell cultures subjected to oxidative stress, anthocyanins have been found to reduce significantly the extent of DNA damage, lipid peroxidation, and low-density lipoprotein oxidation.^{149–153} It is reasonable, therefore, to ask whether anthocyanins might serve a similar function in plants — to ameliorate defense responses against oxidative stress caused by growing in unfavorable environments.

A growing body of experimental evidence does indeed indicate that anthocyanins contribute to the control of levels of reactive oxygen in plant cells. In apple fruit, for example, chlorophyll bleaching (a symptom of photooxidative damage) was considerably lower in red than in green regions after exposure to strong light.¹⁵⁴ Similarly, the combination of strong light and low temperatures caused significantly more lipid peroxidation in anthocyanin-deficient mutants of *Arabidopsis* than in wild type.¹³⁵ Gamma irradiation triggered the biosynthesis of anthocyanin in wild-type *Arabidopsis* plants, which were subsequently better scavengers of superoxide radicals than were nonirradiated or anthocyanin-deficient mutants.¹⁵⁵ Moreover, only those plants that contained both anthocyanin and ascorbic acid were able to grow normally and flower after gamma irradiation. Anthocyanins, therefore, can hold the key to survival under severe conditions of oxidative stress.

Anthocyanins have the potential to moderate the total oxidative load via three mechanisms. First, they can chelate to copper and iron, thereby decreasing the possibility of hydroxyl radical production from Haber–Weiss reactions.^{156–159} These chelates might also protect other low molecular weight antioxidants (LMWAs), such as ascorbate and α -tocopherol, from autoxidation by transition metals.¹⁶⁰ Anthocyanin–transition metal chelation has been demonstrated *in vitro* many times,^{161,162} but is unlikely to feature significantly *in planta*.

The property of anthocyanins to attenuate light presents a second possible mechanism for reducing the oxidative load. Chloroplasts, mitochondria, and peroxisomes are the chief sources of reactive oxygen in plant cells. Under conditions of excess light, (i) chlorophyll excitation leads to the production of singlet oxygen, (ii) electrons leak from reduced ferredoxin in photosystem I to molecular oxygen, forming superoxide radicals, and (iii) hydrogen peroxide may be produced in the peroxisomes by the C2 oxidative photosynthesis cycle.^{163–165} In theory, therefore, any process that serves to reduce the amount of light incident on chloroplasts, such as the optical masking of chlorophyll by anthocyanins, should also diminish the numbers of reactive oxygen species produced by photooxidation.^{115,166} The effectiveness of photoabatement by anthocyanins was demonstrated recently using a model system comprising suspensions of chloroplasts isolated from lettuce leaves.¹¹⁹ When used to shield the illuminated chloroplasts, a red cellulose filter whose optical properties approximated that of anthocyanin at pH 1 effected a 33% decline in numbers of superoxide radicals, and a corresponding 37% reduction in chlorophyll oxidation, as compared to unshielded chloroplasts. By absorbing green light and yet permitting the transmission of red and blue wavebands to the chloroplasts, anthocyanins are an efficient device to reduce the oxidative burden without seriously compromising photosynthesis.

The third mechanism for antioxidant activity of anthocyanins is through the direct scavenging or quenching of reactive oxygen and nitrogen. Cyanidin-3-*O*-glucoside, which is believed to be the most common anthocyanin in vegetative shoots, heads the antioxidant league for these pigments. Its high scavenging activity has been ascribed to the reducing power of the two ortho-related hydroxyl groups on the B ring, the oxonium ion in the C ring and the numbers and positions of free hydroxyl groups on the anthocyanin molecule.^{156,167} Anthocyanins that donate protons to free radicals become aryloxy radicals themselves. These may be stabilized through electron delocalization or via self-association,^{161,168} or else be regenerated to anthocyanins using other antioxidants such as ascorbic acid.¹⁶⁹

It could be argued, however, that the vacuolar location of the pigmented anthocyanins precludes any major role in free-radical scavenging *in planta*. Almost all free radicals originate from organelles, the plasma membrane, and the apoplast of plant cells. Cytoplasmic antioxidants, including ascorbate, α -tocopherol, and the extremely efficient enzyme superoxide dismutase, are therefore more optimally located to scavenge organelle-derived reactive oxygen. Nonetheless, a significant antioxidant advantage of anthocyanic cells over acyanic cells has been demonstrated *in vivo*. In microscopic examinations of mechanically injured leaf

tissues, red pigmented cells were found to eliminate H_2O_2 at rates substantially faster than those of green cells.¹⁷⁰ Similarly, slices of sweet potato tubers perfused with H_2O_2 showed rapid inactivation of the oxidant exclusively in those cells that held anthocyanin.¹⁷¹ In both of those studies, the responses were attributable to anthocyanins *per se*, rather than to other, possibly colorless phenolics associated with the red cells. It is significant that the fluorescent probes used to visualize H_2O_2 did not penetrate the tonoplast of the plant cells; therefore, changes in fluorescence associated with H_2O_2 scavenging were the result of cytoplasmic rather than vacuolar influences. Although anthocyanins are prominent as red solutions inside the cell vacuole, they are thought to be synthesized in the cytoplasm as an equilibrium of colorless tautomers.^{172,173} Hence, there is a real possibility that the scavenging of H_2O_2 is undertaken by colorless, cytosolic anthocyanins prior to their transport into the vacuole. Both the colorless and red forms of anthocyanin have strong antioxidant potentials, as measured by lipid peroxidation assays and by cyclic voltammetry.^{174–176} Colorless tautomers of cyanidin 3-*O*-(6-*O*-malonyl) glucoside at pH 7, the approximate pH of the cytosol, were shown to remove up to 17% of the superoxide radicals generated by irradiated suspensions of chloroplasts isolated from lettuce.¹¹⁹ Thus, the cytosolic anthocyanins have the potential to ameliorate a plant's defense system under conditions of oxidative stress.

Plant tissues normally hold a suite of enzymatic and LMWAs, any combination of which serves to protect membranes and DNA from the effects of reactive oxygen.¹⁷⁷ How important, therefore, is the contribution of anthocyanin to this antioxidant pool? From the limited data available, it appears that reliance on anthocyanins for antioxidant protection varies considerably among species and even across ecotypes. For example, in the shade plant *Elatostema rugosum*, anthocyanins contribute to the LMWA pool more than any other constituent phenolic, and total scavenging activity correlates linearly with anthocyanin content.¹⁷⁵ In contrast, green- and red-leafed morphs of the sun plant *Q. serrata* show similar ranges of scavenging capacities, and use hydroxycinnamic acids as the most active LMWA component.¹⁷⁶ For wild-type *Arabidopsis*, the contribution of anthocyanin to the total superoxide radical-scavenging activity was almost twice as great in the Landsberg *erecta* ecotype as in the Columbia ecotype.¹⁵⁵ Thus, it would seem that anthocyanin biosynthesis can enhance, but is not usually a prerequisite for protection from oxidative stress. It is likely to be of greatest use under harsh environments where the capacities for other methods of energy dissipation (e.g., via xanthophylls) and free radical scavenging (e.g., by enzymes) have been exhausted.

8.2.5 ANTHOCYANINS AS A GENERALIZED STRESS RESPONSE

There is a strong association between anthocyanin biosynthesis and plant stress. Almost all biotic and abiotic stressors — including herbivory, fungal and viral pathogens, wounding, temperature extremes, high light, UV radiation, mineral nutrient imbalances, drought, salinity, anoxia, ozone exposure, and herbicides — can cause anthocyanin levels to increase in vegetative shoots and roots.^{178–180} There is evidence that anthocyanins offer protection against many of these stressors. Anthocyanins, for example, have been associated with enhanced tolerance to chilling and freezing temperatures,^{181–186} to heavy metals,^{187–191} and to water stress.^{192–194} Along with certain other flavonoids and compounds such as abscisic acid, the jasmonates, and ethylene, it seems that the anthocyanins may function to mitigate the effects of general stress, and are therefore a useful component of the *general adaptation syndrome*.¹⁹⁵

There have been several attempts to provide a unifying hypothesis for the mechanism by which anthocyanins provide protection against such a diverse assortment of environmental stressors. Steyn et al.¹⁶⁶ argued that because light can become toxic to green tissues under

various conditions of abiotic and biotic stress, the photoprotective properties of anthocyanins are paramount. Chalker-Scott^{178,179} provided a compelling case for an osmoregulatory role of anthocyanins in plant cells, since most kinds of suboptimal environments induce water stress, either directly or indirectly. Antioxidant protection is a third potential candidate for the all-encompassing explanation for anthocyanin function,¹¹⁸ given that supernumerary reactive oxygen and nitrogen species are generated especially by plant tissues under stress. All of these hypotheses warrant further examination in plants from disparate taxonomic groups across diverse ecosystems. It seems equally possible, however, that there is no preeminent function of anthocyanins in vegetative tissues. Anthocyanins and 3-deoxyanthocyanins have been identified among various orders of bryophytes, the most primitive group of land plants with an estimated 470 million years of evolution.¹⁹⁶ It is likely, therefore, that these compounds have been “hijacked” over the course of evolution to perform an array of protective tasks that contribute in different ways and to different degrees to the physiology of plants.¹⁹⁷ Anthocyanins offer the potential for multifaceted, versatile, and effective protection to plants under stress.

8.3 COLORLESS FLAVONOIDS

8.3.1 STRESS PROTECTION

In common with the anthocyanins, the colorless and yellow flavonoids are also inducible by numerous, disparate stressors. The best known of these is probably exposure to UV radiation, although flavonoids also accumulate in response to wounding, pathogen infection, high light, chilling, ozone, or nutrient deficiency. Antioxidant protection provides a possible common thread linking all of these different responses.

8.3.1.1 Ultraviolet Radiation

Unlike the anthocyanins, the colorless flavonoids are thought to be primarily involved in the protection against UV radiation. UV light is a potential hazard to plants because it can damage DNA and impair various physiological processes.¹⁹⁸ Plants often respond to UV light by the activation of flavonoid biosynthetic genes.^{199–201} The flavones and flavonols are strongly UV absorbing and accumulate mainly in epidermal cells after UV induction, indicating that they may function as a shield for protecting photosynthetic tissues.^{200,202,203} Analyses of mutants defective in flavonoid biosynthesis have indicated the importance of flavonoids for UV tolerance.^{103,203} When *Arabidopsis* mutants lacking in flavonols were placed under UV light, growth was retarded.⁶¹ In rye (*Secale cereale*) seedlings, flavonoids have been shown to protect against the damaging effects of shortwave UV on the photosynthetic apparatus.⁸⁰

Fluorescence microscopy has shown that UV-B attenuating compounds are localized mainly in the upper epidermis (e.g., in *Brassica napus*).¹⁰¹ Interestingly, the smaller amounts of flavonoids present in mesophyll tissue and in the lower epidermis are not inducible by UV-B, but rather represent the constitutive fraction. Changes in the levels of flavonols have been observed not only inside epidermal cells, but also in hairs and epicuticular wax (Table 8.1). Flavonoids are generally present as water-soluble glycosides in cell vacuoles, but when present in the epicuticular wax on the leaf surface, flavonoids are nonglycosylated, are very often *O*-methylated, and are lipophilic. *O*-Methylation tends to shift the UV absorption properties to shorter wavelengths so that they typically absorb significantly in the 250 to 320 nm region. Thus, *O*-methylated flavonoids are better able to protect plant leaves from UV-B damage, as demonstrated in two *Gnaphalium* species.⁹⁸ One further site of flavonoid synthesis

is in the leaf hairs or trichomes on leaves. Pubescence enriched with acylated flavonol glycosides has been shown to provide UV protection in leaves of *Quercus ilex*.¹⁰²

Most studies on UV protection have focused on leaves; relatively few have studied other plant organs, such as fruit. Because the anatomy, histology, and physiology differ markedly between fruit and leaves, the two may also differ in UV shielding mechanisms. Studies on apple (*Malus pumila*) skins showed that flavonoids (mainly quercetin glycosides) accumulate during acclimation to strong sunlight and can serve as efficient UV-B screens.⁵⁷ Similarly, for berries of a white grape cultivar (*Vitis vinifera* L. cv. Bacchus), flavonols and hydroxycinnamic acids were identified as the main groups of UV-absorbing phenolics.²⁰⁴ The flavonols alone provided efficient shielding from UV-A, but the combination of flavonols and hydroxycinnamic acids was required for tolerance to UV-B. These results confirm that parts of the plant as dissimilar as fruits and leaves can share similar mechanisms of UV protection.

Many studies have shown that the flavonoids, along with certain other phenolics, increase in UV-B-treated plants, but until recently it has been difficult to assign a specific role for these compounds in UV-B resistance. Moreover, it has remained uncertain whether any one group of flavonoids was more important than others. Jaakola et al.²⁰⁵ examined the activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus*) leaves. The flavonol quercetin, various anthocyanins glycosides, and the hydroxycinnamic acids were shown collectively to play a predominant role in the defense against high solar radiation; all of these compounds increased markedly with greater exposure to sunlight. In contrast, levels of polymeric procyanidin decreased, indicating they do not participate in protecting leaf tissues from excess light. In soybean (*Glycine max*), the isoflavonoids are highly responsive to those wavelengths that are most affected by variations in ozone levels, suggesting that they function as UV protectants in the field.²⁰⁶

Recent work with several species (e.g., *Petunia*,²⁰⁷ *Arabidopsis*,²⁰⁸ *Vicia faba*,²⁰⁹ *B. napus*,¹⁰¹ *Oryza sativa*¹⁰⁰) has provided evidence that UV light can induce the preferential synthesis of those flavonols that have the higher levels of hydroxylation (e.g., quercetin instead of kaempferol, and isoorientin instead of isovitexin), rather than a general increase in all flavonols. For *B. napus*, quercetin glycosides are UV-B inducible and kaempferol glycosides form a constitutive shield.¹⁰¹ An increase in dihydroxylated compounds as a UV-B response has also been shown to apply to different populations within a species; in clover (*Trifolium repens*), for example, large differences between populations in both productivity and tolerance to UV-B stress were correlated to differences in amounts of quercetin.²¹⁰ The effect of UV-B on the levels of flavonol glycosides was synergistically enhanced by water stress. These changes were more pronounced for the *ortho*-dihydroxylated quercetin, rather than for the monohydroxylated kaempferol glycosides.²¹¹ Similarly, a *Petunia* mutant for which kaempferol was the predominant flavonol grew at a significantly slower rate under UV-B treatment than did the wild-type plants, which accumulated mainly quercetin.²⁰⁷ These observations suggest that quercetin confers a protective advantage that is not matched in the mutant, even though it has the higher overall flavonol levels. Hydroxylation does not affect the UV-absorbing properties *per se*, but it does affect the antioxidant capacities of these compounds. Thus, the selective advantage of quercetin under UV-B stress is likely to be related to its higher capacity to scavenge UV-generated free radicals.^{208,212} Interestingly, in Scots pine (*Pinus sylvestris*) both kaempferol and quercetin-3-glucoside increase after UV-B treatment, with kaempferol derivatives increasing in primary needles and quercetin derivatives increasing in cotyledonary needles.⁷⁸ The reason for these differences is not immediately apparent.

Some studies have shown no changes in flavonoid levels in response to UV. Originally, only the flavonoids were thought to serve as UV-screening pigments.⁶³ It is now clear, however, that other phenolics, such as hydroxycinnamic acids and their esters, are also

involved. The importance of each group of compounds seems to vary across plant species as well as between leaves of different developmental stages. One study has shown that for *Arabidopsis* the hydroxycinnamates are more effective UV-B protectants than flavonoids.²¹³ In contrast, Li et al.⁶¹ demonstrated for *Arabidopsis* that flavonoids are required in addition to other phenolic compounds *in vivo*. In rye (*S. cereale*), hydroxycinnamates in epidermal cells are the dominant UV-B protective compounds at the early stages of primary leaf development.⁶⁹ However, during subsequent leaf development and acclimation this function is increasingly replaced by epidermal flavonoids (e.g., isovitexin 2''-O-glycosides).

8.3.1.2 Temperature Stress

In addition to the induction of anthocyanin biosynthesis, chilling stress has also been shown to promote the formation of colorless flavonoids. Cold treatments (and drought stress) caused increases in levels of (–)-epicatechin and hyperoside (quercetin 3-galactoside) in two species of hawthorn, *Crataegus laevigata* and *C. monogyna*. Such treatments also enhanced the antioxidant capacity of the shoot extracts, and this may be the primary function of these cold-inducible flavonoids.²¹⁴

The flavonoids are also believed to play a role in the responses to heat stress. Recently, Coberly and Rausher²¹⁵ reported that flavonoids can mitigate the adverse effects of heat stress on fertilization and early seed maturation. Two possible mechanisms were suggested: (i) flavonoids promote the overall well-being of plants under heat stress (e.g., by scavenging free radicals); or (ii) heat stress inhibits one or more process directly involved in fertilization or ovule maturation, and flavonoids ameliorate these effects. As discussed in Section 8.3.2, flavonoids have been found to play key roles in plant fertility.

High temperatures have been shown to influence flavonoid gene expression.²¹⁶ There are also synergistic responses to the effects of high temperatures and UV-B. In cucumber (*Cucumis sativus* L.) seedlings, synthesis of a quercetin-like compound was preferentially enhanced after UV-B exposure.²¹⁷ When plants irradiated with low and medium doses of UV-B were subsequently heat stressed (46°C for 1 h), survival improved by 112 and 82% and shoot elongation increased by 35 and 40%, respectively, relative to the controls that received no UV-B. A synergism between UV-B tolerance and heat tolerance could be used by growers to precondition seedlings in UV-B-deficient greenhouses, and may benefit plants under the predicted global warming scenario.

8.3.1.3 Heavy Metal Tolerance

There is accumulating evidence that flavonoids participate in the resistance to high levels of metals in soils. Roots of maize (*Zea mays*) plants that had been exposed to aluminum have been found to exude high levels of phenolic compounds.^{218,219} The observation is consistent with the metal-binding activity of many flavonoids. It has been argued, however, that phenolics, including flavonoids, are unlikely to be effective in binding metals in an acid environment because H⁺ ions compete for complex formation. Kidd et al.²¹⁸ provided empirical evidence that certain flavonoids can form complexes with aluminum in the root. Indeed, morin, a pentahydroxyflavone, complexes with aluminum and is routinely used to stain for aluminum in the root apoplast.²²⁰ Catechin also forms stable aluminum complexes,²¹⁸ and green tea, which contains high levels of catechin, accumulates and tolerates high tissue levels of aluminum.

The distribution of aluminum in the root tips of the aluminum-tolerant forage legume, *Lotus pedunculatus* Cav., a species that also accumulates condensed tannin (proanthocyanidin), has also been investigated.²²¹ In osmium-fixed samples from high and low aluminum

concentrations, aluminum was generally found in association with osmium-binding vacuoles. Because of the high affinity of osmium for condensed tannin, it was hypothesized that condensed tannins possibly bind and detoxify aluminum in the root apices. Alternatively, in common with other abiotic stressors, high concentrations of metals such as copper and aluminum could result in the production of reactive oxygen species.²²² Plants with high flavonoid production may be able to combat this oxidative stress. It has also been suggested, however, that Al^{3+} has the potential to induce oxidative stress in plants by stimulating the prooxidant nature of endogenous phenolic compounds.²²³

8.3.1.4 Oxidative Stress

Antioxidant capacities are a feature of plant phenolics in general^{145,224} and like the anthocyanins, many of the colorless flavonoids are also thought to be key players in the abrogation of oxidative stress. Although they are localized largely in vacuoles,²²⁵ smaller pools of flavonoids are thought to occur in the cytoplasm.¹⁰¹ This has recently been demonstrated using mature petals of lisianthus (*Eustoma grandiflorum*), for which ~14% of the flavonoids reside within the cytoplasm, 30% in the cell wall, and 56% in the vacuole.²²⁶ Cytoplasmic flavonoids are better sited than vacuolar flavonoids to interact with reactive oxygen species generated by organelles,²²⁷ and they have a demonstrable antioxidant potential at the neutral pH of the cytosol.²²⁸ A vacuolar location would not, however, impede a second putative function of flavonoids, that of dissipating excess absorbed UV energy; indeed, it can be argued that an epidermal vacuolar location is ideal for that purpose.²⁰⁷

Under high-stress conditions, more H_2O_2 is produced by the chloroplasts, mitochondria, and peroxisomes than can be accommodated by peroxidase or catalase, and the oxidant can diffuse across the tonoplast into the cell vacuole. It has been proposed that vacuolar flavonoids in conjunction with peroxidase serve to scavenge excess H_2O_2 (Figure 8.5). Studies with the leaves of the tropical tree *Schefflera arboricola* have shown that quercetin and kaempferol glycosides, in particular, can act in this manner,¹⁶⁹ although all classes of flavonoids can act as radical scavengers to a greater or lesser extent.^{145,229} Since the oxidation of flavonols by H_2O_2 requires peroxidases, it might therefore be an advantage for the plant to have a colocalization of flavonoids and peroxidase in the vacuole.

8.3.2 REPRODUCTION AND EARLY PLANT DEVELOPMENT

Flavonoids often participate in plant reproduction, in the protection of reproductive tissues and seeds, and in seedling development. This may, in part, be due to their role in UV light shielding (thereby protecting DNA) and antioxidant properties, but other functions are also important.

8.3.2.1 Chemical Signals as Attractants for Pollination and Seed Dispersal

Plants that are insect pollinated generally have flowers with large, brightly colored petals, in contrast to most wind-pollinated plants for which flowers are small, dull, and often apetalous (e.g., *Petunia* versus maize).²⁰² Pigmentation presumably acts as a signal to attract pollinating insects or birds. The anthocyanins make the most obvious contribution to flower color,²³⁰ though other flavonoids can also assist in this. There are a few flavonoids that are yellow, e.g., chalcones and aurones, but these pigments are restricted to relatively few plant species. Yellow color can also be produced by flavonols following methylation, certain types of glycosylation, or certain A-ring hydroxylation patterns.²³¹ Yellow petal colors have also been shown to result from an aggregation of (colorless) flavonol glycosides on a protein matrix in the cytoplasm of epidermal cells.²²⁶ Various flavonols and flavones act as

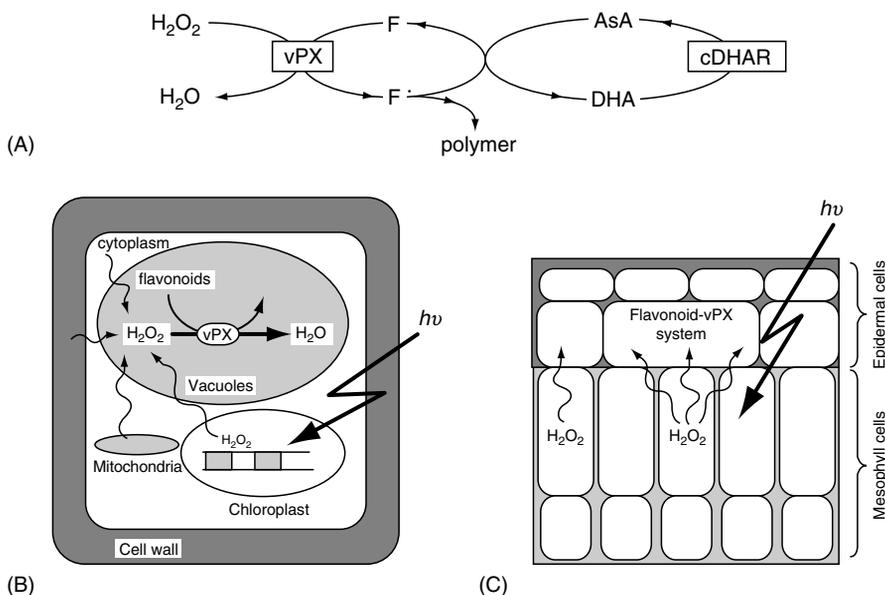


FIGURE 8.5 A proposed diagram for the protective function of flavonoids during stress (A). Scheme of the H_2O_2 -scavenging mechanism by flavonoids. vPX, vacuolar peroxidase; F, flavonoid; $F \cdot$, flavonoid radical; AsA, ascorbic acid; DHA, dehydroascorbic acid; $h\nu$, light energy; and cDHAR, cytosolic dehydroascorbic acid reductase. The diffusive nature of H_2O_2 enables vPX to scavenge it in vacuoles, even if the generating site is other than a vacuole (B). This concept can be expanded to cell-cell interaction. The photoproduced H_2O_2 may leak out from mesophyll cells and be scavenged in epidermal cells that have high flavonoid content (C). (Redrawn from Yamasaki, H., Sakihama, Y., and Ikehara, N., *Plant Physiol.*, 115, 1405, 1997. Copyright the American Society of Plant Biologists and reprinted with permission.)

copigments with anthocyanins leading to an intensification of flower color.²³¹ Colorless flavonoids may also provide “body” to flowers, to give a cream or ivory appearance in petals that would otherwise be translucent.²³¹

Petals and other floral organs often show intricate patterns under UV light. They are especially common among yellow flowered species, for which the principal yellow pigment is usually a carotenoid. However, the UV-absorbing regions that give rise to these patterns are colorless or yellow flavonol glycosides and chalcones, which quench the energy of light reflected from carotenoids in the chromoplasts.²³² UV patterning can also be provided by colorless flavonol glycosides and chalcones, e.g., in *Coronilla* and *Potentilla*.²³³ Flavonoids can determine which animal vector is attracted to effect pollination. In *Petunia integrifolia*, for example, flowers are pink and are pollinated by bees, while in the related species, *Petunia axillaris*, flowers are white and are pollinated by moths.²³⁴ It is assumed that these moths are attracted by the high amounts of fluorescent flavonols. Similarly, the corolla of *B. rapa* has a UV-absorbing zone in its center, known as a nectar guide, for attracting pollinating insects.²³⁵ The pigment responsible, identified as isorhamnetin 3,7-O-di- β -D-glucopyranoside, is present at 13-fold greater amounts in the basal parts of the petals than in the apical regions. This difference in flavonoid content is presumed to contribute to the visual attractiveness of *B. rapa* flowers to insect pollinators.

Flavonoids in fruit probably serve to attract frugivores that assist in seed dispersal. This is especially important for larger plants such as trees, for which seeds need to be transported some critical distance away from the parent to ensure germination.

8.3.2.2 Flavonoids in Pollen, and Their Role in Plant Sexual Reproduction

Anthocyanins, flavonols, or chalcones often accumulate in both the male and female sex organs in a flower, including the pollen grains.¹⁹² Insects can detect light from the UV to orange waveband,²³⁶ and are possibly attracted to pollen whose color contrasts against petals due to UV reflective or absorptive flavonoids. However, if pollen pigments were present primarily as insect attractants then these pigments should be redundant, and therefore unlikely to be present in wind-pollinated (anemophilous) species. Nevertheless, many anemophilous species do contain flavonoids.²³⁷ Indeed, there are relatively few plant species that only produce nonpigmented (white) pollen.

Alternative hypotheses for pollen pigments hold that flavonoids are involved in pollen-style incompatibility relationships via pollen germination, or else serve as growth regulators for pollen tube development. Certain flavonoids have also been postulated to ameliorate the effects of heat stress during fertilization.²¹⁵

In order to understand the role of flavonoids in pollen, flavonoid-deficient mutants have been produced through artificial methods, such as cosuppression, antisense expression, chemical mutagenesis, and ionizing radiation. Examples include *Petunia*^{238–241} and *A. thaliana*.²⁴² Most mutants produce white pollen that is sterile in self-pollinations (e.g., maize²⁴³). In *Petunia*, white pollen failed to produce functional pollen tubes both in an *in vitro* pollen germination assay and on stigmas from the same mutant.²⁴⁰ However, on wild-type stigmas the white pollen was functional and extracts from wild-type stigmas could “rescue” *in vitro* pollen tube growth, indicating that the stigma contains other factors that can complement the mutation.²⁴⁴ The active compound(s) in the stigma were shown to be flavonols. Similarly, flavonols (quercetin, kaempferol, myricetin), though no other class of flavonoid, have been shown to strongly promote pollen germination frequency and pollen tube growth of tobacco (*Nicotiana tabacum*) *in vitro*.²⁴⁵ In contrast to *Petunia*, the flavonoid-deficient mutant in *Arabidopsis* had fertile pollen. Ylstra et al.²⁴⁶ suggested that other phenylpropanoids or else compounds such as the (brassinoid-)phytosteroids might compensate for the absence of flavonoids. Song et al.²⁴⁷ showed that sinapate esters, rather than flavonoids, may have a physiological role in pollen tube growth in *Arabidopsis*.

Notwithstanding these studies, the precise function of flavonoids in fertilization and pollen tube growth remains unclear. It has been speculated that the flavonols form a gradient along which the growing pollen tubes are guided to their target, the ovule.¹⁹² However, there has been no direct experimental evidence to support this idea. It is interesting to note that certain mycorrhizal fungi demonstrate enhanced hyphal growth on a medium enriched in the same flavonols that promote pollen tube growth, and it is possible that there is a common mechanism of action, e.g., a regulatory effect on cell elongation.²⁴⁸ It has been postulated that flavonols play a structural role in the membranes of rapidly growing pollen tubes and that these may not be essential for pollen germinating with a short transmitting tract.²⁴⁹ However, in *Petunia* the absence of flavonoids interferes with the ability of pollen to penetrate the style. Derksen et al.²⁵⁰ noted that in flavonol-deficient pollen tubes of *Petunia hybrida*, the structure of the primary wall at the tip dramatically changed before it disrupted. It was concluded that flavonols act on precursors of the pollen tube wall and interfere with a cross-linking system in the wall, possibly via extensions.

The observations that flavonols are not involved in the fertilization process in certain species, and that this function can be completed using other compounds, suggest that flavonols only affect fertility indirectly.²³⁹ There are various examples of cross-talk between branch pathways of phenylpropanoid metabolism,^{61,251} or the shikimate pathway.²⁵² The absence of flavonols in maize and *Petunia* could affect the accumulation of other compounds that are more specifically required for male fertility. Thus, differences between species in terms of flavonoid

requirement for male fertility could relate to differences in the integration of metabolic pathways.²⁴² Nonetheless, virtually all plants accumulate flavonoids in pollen,²⁵³ including *Arabidopsis*, which suggests that they are of fundamental importance to the male gametophyte.

Another hypothesis for the role of pollen flavonoids is that they shield the vulnerable haploid genome from the mutagenic effects of UV light or, indeed, any auto- or photo-oxidative stress. There is only limited experimental evidence in support of this hypothesis for pollen. Indirect evidence has come from studies comparing flavonoid levels in pollen exposed to different UV light regimes. Pollen collected from UV-irradiated maize and Californian poppy (*Eschscholzia californica*) contained more flavonoids than did control pollen.^{254,255} However, in experiments comparing white pollen to pigmented pollen of *Ipomoea purpurea*, light was shown to have little discernible overall effect on fertilization success, with no effect on either pollen donor or pollen recipient.²¹⁵

8.3.2.3 Seeds and Seedling Development

The roles of flavonoids in seeds and seedling development have been reviewed previously.^{256,257} Ndakidemi and Dakora²⁵⁶ specifically discussed the role of flavonoids in legume seed development, viz: (i) nodulation; (ii) arbuscular mycorrhizal fungal development; (iii) resistance to microbial pathogens; (iv) defense against insect pests; (v) controlling the parasitic plant *Striga*; and (vi) as allelochemicals in controlling weeds in cropping systems. Many of these functions also apply to other plant parts, but there are also examples of flavonoid-enriched seed coats for which specific roles have been ascribed.

Other putative functions of flavonoids in seeds have received less attention. It was thought that flavonoids are necessary for embryo development, yet cosuppression of chalcone synthase (*Chs*) gene has been found not to cause female sterility.²⁵⁸ Experiments by Jorgensen et al.²⁵⁸ with *P. hybrida* led to the hypothesis that accumulation of dihydroflavonols in the seed coat inhibits embryo growth, either directly or indirectly. However, there appears to be no evidence of exactly how this may happen *in vivo*. There is some evidence that proanthocyanidins assist in the reinforcement of plant tissues; seeds of snap bean (*Phaseolus vulgaris*), which lack proanthocyanidins, are more sensitive to mechanical stress and water stress than are wild-type seeds.²⁵⁹ The flavonoids, particularly proanthocyanidins, in the seed coat contribute to the maintenance of seed dormancy as well as increasing seed longevity in storage.^{257,260,261} Testa pigmentation also appeared to confer resistance to solute leakage, imbibition damage, and attack by soil-borne fungi, thereby improving seed vigor and germination in legumes.^{262,263} High concentrations of phenolics in emerging seedlings have been associated with protection against UV-B damage at this critical stage of development.²⁶⁴

Kaempferol can increase seed production in *Petunia*, whether produced as a result of pollination or by wounding.²⁶⁵ It has been suggested that the reproductive function of flavonols may have evolved from a defensive role. Allelopathic flavonoids in the stigma may prevent introduction of pathogens into the pistil.

Seed and root exudates can affect growth of neighboring plants, including weeds in cropping systems. Various groups of compounds have been suggested as possible allelochemicals, including the flavonoids, although evidence for this is very limited. Nonetheless, the isoflavonoids medicarpin, 4-methoxymedicarpin, sativan, and 5-methoxysativan from alfalfa (*Medicago sativa*) seeds can inhibit seedling development in other species when released into the soil.^{266,267}

8.3.3 SIGNALING — A ROLE AS CHEMICAL MESSENGERS

It has been suggested that the synthesis of flavones, flavanones, and flavonols could have evolved primarily as chemical messengers, such as defense molecules.²⁶⁸ Indeed, flavonoids have recently been described as a novel class of hormones.²⁶⁹ Interestingly, in this regard, the

flavonoids share some structural and chemical features with the steroids, retinoids, thyroid hormone, prostaglandins, and fatty acids. A signaling capacity would explain many of the functions of flavonoids, both in plants and in humans.

Flavonoids are thought to be particularly useful as signals in plant–rhizosphere interactions. Roots are exposed to a variety of soil microorganisms that use plants as a food source or niche habitat. These relationships can ultimately be detrimental or beneficial to the plant, yet both types involve flavonoid synthesis. The stages of communication between interacting plants and microorganisms typically involve signal exchange and perception, followed by the invasion of the microbe and structural changes in the plant. The involvement of flavonoids as regulatory signals in such interactions has been reviewed previously.^{270–276} The main beneficial roles are in attracting microorganisms for nodulation and mycorrhizal association. The growth of other beneficial soil microflora has also been demonstrated; for example, luteolin and quercetin from alfalfa seed exudates promote growth of.²⁷⁷

8.3.3.1 Defense Against Pathogenic Microbes

The isoflavonoids, in particular, act as effective phytoalexins, which can be defined as small molecular weight antimicrobial compounds or biological stress metabolites. They can be constitutive, or else are inducible by wounding or biological attack. The constitutive versus inducible response varies between different species, and can also vary within the plant depending on age or environment. Isoflavonoids, especially the pterocarpan, isoflavans, isoflavones, and isoflavanones, are extremely toxic to fungal pathogens. These flavonoids inhibit fungal spore germination, germ tube elongation, and hyphal growth through causing damage to membrane systems.^{278,279} Other compounds such as kievitone and phaseollin isoflavonoids lead to mycelial leakage of metabolites and shrinkage of hyphal tip protoplasts.²⁸⁰ Antimicrobial activity has also been noted in the flavans, flavanones, 3-hydroxyflavanones, and flavonols.

Antimicrobial flavonoids are particularly rich in seed coats and in the sapwood of trees.²⁸¹ Various studies have shown that the seeds of grain legumes (e.g., *G. max*, *P. vulgaris*, *Pisum sativum*, *Canavalia ensiformis*, *Arachis hypogea* and *Cicer arietinum*) store antifungal isoflavonoids for defense against pathogen infection prior to germination.²⁵⁶ The isoflavonoid pterocarpan maackianin and pisatin (Figure 8.6) act as classical phytoalexins in the interaction between garden pea (*P. sativum*) and the fungal pathogen *Nectria haematococca*. Enzymes that detoxify maackianin and pisatin have been identified as fungal virulence factors.^{282,283} Pisatin induces a protein that appears to control the transcription of a fungal detoxification enzyme.²⁸⁴ In addition to serving as a cue for phytoalexin detoxification, pisatin, along with other flavonoid compounds, apparently stimulates the germination of spores of *N. haematococca*.²⁸⁵ *Sorghum* seeds that contain the higher concentrations of proanthocyanidins show enhanced tolerance to aflatoxin-producing *Aspergillus* fungus.²⁸⁶ The soybean isoflavones daidzein and genistein (Figure 8.6) stimulate chemotropic behavior in hyphal germination of the oomycete pathogen *Phytophthora sojae*, suggesting that they might help the hyphal tips of zoospores encysted adjacent to roots to locate their host.²⁸⁷

8.3.3.2 Roles in *Agrobacterium* Infection

Flavonoids from pollen and stigmas of *P. hybrida* (kaempferol 3-glucosylgalactoside, quercetin 3-glucosylgalactoside, rutin, myricetin 3-galactoside, narcissin, and apigenin 7-glucosides) have all been found to induce the *vir* region of the *Agrobacterium tumefaciens* Ti plasmid, which is responsible for virulence.²⁸⁸ Along with other *vir* inducing factors like cinnamic acids, these flavonoids might play an even more important role in the natural

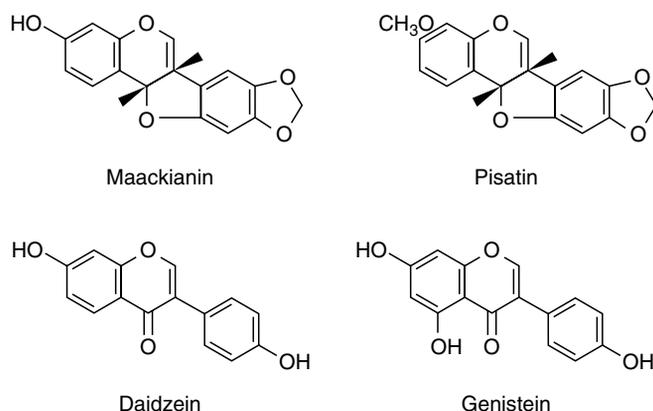


FIGURE 8.6 Structure of the phytoalexin isoflavonoid pterocarpan, maackianin, and pisatin from garden pea, and the isoflavones daidzein and genistein from soybean.

infection of plants by *Agrobacterium* than does acetosyringone, which until now has been found only in tobacco.

Agrobacterium tumefaciens-induced plant tumors accumulate considerable amounts of free auxin. The overproduction of auxin is required for tumor growth, and it appears that both the accumulation and maintenance of high auxin levels are dependent on flavonoids. To determine possible mechanisms by which high auxin concentrations are maintained, Schwalm et al.²⁸⁹ examined for associations between the distribution patterns of auxins and flavonoids, both in tumors from *T. repens* and in *A. thaliana* shoots. Tumor-specific flavones, isoflavones, and pterocarpan were detected, namely 7,4'-dihydroxyflavone (DHF), formononetin, and medicarpin. DHF was also the dominant flavone in high free-auxin regions of *Arabidopsis* leaf primordia. Moreover, these flavonoids were localized at the sites of strongest auxin-inducible chalcone synthase (CHS) genes. It was concluded that CHS-dependent flavonoid aglycones are possibly endogenous regulators of the basipetal auxin flux, thereby leading to free-auxin accumulation in *A. tumefaciens*-induced tumors. This, in turn, triggers vigorous proliferation and vascularization of the tumor tissues and suppresses their subsequent differentiation. The role of flavonoids in auxin regulation is discussed in more detail below.

8.3.3.3 Legume Nodulation

Flavonoid levels in plants can be affected by their nutritional status. Low nitrate concentrations, for example, induce flavonoid accumulation, which then serve as chemoattractants to nitrogen-fixing bacteria. Nodulation then restores the nitrogen economy of the plant.²⁹⁰ In *L. pedunculatus*, proanthocyanidins are not formed under high-nitrogen conditions,^{291,292} presumably because the requirement for nodulation is not so critical.

Flavonoids act as signal molecules in the early stage of legume–*Rhizobium* symbiosis (as reviewed in Refs. 273, 276). In legumes, flavonoid biosynthetic genes are active in young root cap cells and in the zones where root hairs emerge.²⁹³ Flavonoids are released into the soil, which then induce various *nod* genes in soil-borne rhizobia.^{294–296} *Nod* genes are responsible for the synthesis of lipochitin-oligosaccharides (LCOs), also called Nod factors, which are species-specific signaling molecules that initiate root nodule development. Genetic evidence indicates that a protein, termed NodD, functions as the receptor for the flavonoid signal.²⁹⁷ NodD protein from different bacteria differ in their response to distinct flavonoids excreted by their corresponding host plant, thus helping determine host specificity.²⁹⁸ The *nod* gene inducers so far identified from seeds and seed coats of legumes include flavones,^{294,299}

TABLE 8.2
Some *nod* Gene Inducers Released by Different Legumes

Source	Inducers	Ref.
<i>Medicago sativa</i>		
Root exudates	7,4'-Dihydroxyflavone; 4,4'-dihydroxy-3'-methoxychalcone; liquiritigenin; formononetin; formononetin-7- <i>O</i> -(6'- <i>O</i> -malonylglucoside); formononetin-7- <i>O</i> -glycoside	303, 306–308
Seed exudates	Luteolin; chrysoeriol; 5,3'-Dimethoxyluteolin; 5-Methoxyluteolin; Trigonelline; Stachydrine	294, 307, 309
<i>Phaseolus vulgaris</i>		
Root exudates	Genistein; genistein-7- <i>O</i> -glycoside; eriodictyol; naringenin; daidzein; coumestrol; liquiritigenin; isoliquiritigenin	304, 306, 310
Seed exudates	Delphinidin-3- <i>O</i> -glycosides; petunidin-3- <i>O</i> -glycosides; malvidin-3- <i>O</i> -glycosides; quercetin-3- <i>O</i> -glycosides; kaempferol-3- <i>O</i> -glycosides	304
<i>Pisum sativum</i>		
Seed rinse	Eriodictyol; apigenin-7- <i>O</i> -glucoside	311
<i>Glycine max</i>		
Root exudates	Isoliquiritigenin	312
Seed exudates	Genistein; genistein-7- <i>O</i> -(6'- <i>O</i> -malonylglucoside); daidzein-7- <i>O</i> -(6'- <i>O</i> -malonylglucoside); daidzein-2- <i>O</i> -glucoside with unidentified acylation	301, 313
<i>Trifolium repens</i>		
Seedling extract	7,4'-Dihydroxyflavone; geraldone (7,4'-dihydroxy-3'-methoxyflavone); 4'-hydroxy-7-methoxyflavone	295
<i>Vicia sativa</i>		
Root exudate	3,5,7,3'-Tetrahydroxy-4'-methoxyflavanone; 7,3'-dihydroxy-4'-methoxyflavanone; 4,2',4'-trihydroxychalcone; 4,4'-dihydroxy-2'-methoxychalcone; naringenin; liquiritigenin; 7,4'-dihydroxy-3'-methoxyflavanone; 5,7,4'-trihydroxy-3'-methoxyflavanone; 5,7,3'-trihydroxy-4'-methoxyflavanone	296, 300

Source: From Jain, V. and Nainawatee, H.S., *J. Plant Biochem. Biotechnol.*, 11, 1, 2002. With permission.

flavonones,³⁰⁰ isoflavones,^{301,302} flavonols,^{300,303} and anthocyanins.³⁰⁴ Details of specific flavonoids involved in *nod* gene induction are given in Table 8.2. Siqueira et al.³⁰⁵ also list a large number of examples of flavonoids involved in nodulation.

Certain flavonoids also act as inhibitors of *nod* gene expression.^{311,314,315} Examples of such compounds, which are also referred to as anti-inducers, are given in Table 8.3. The mechanism of action is often by competitive inhibition; chemical structures of these inhibitors are similar to those of *nod* inducers, and inhibition can be overcome by increasing the concentration of inducers.³¹⁴ Inhibitors are sometimes strain specific, acting on a few strains belonging to the same cross-inoculation group.³¹⁶ The isoflavone daidzein induces *nod* gene expression in *Bradyrhizobium japonicum* (nodulates soybeans)³⁰¹ but is an inhibitor in *Rhizobium trifolii* (nodulates clovers)³¹⁵ and *Rhizobium leguminosarum* (nodulates peas).³¹¹ These inhibitors may contribute to host specificity. The balance between stimulatory and inhibitory flavonoids in roots and root exudates may contribute to the regulation of nodulation. The environment may also influence whether compounds act as inducers or inhibitors of *nod* induction.³¹⁵ Spatiotemporal distribution of flavonoids in the rhizosphere and at the root surface is likely to determine the levels of induction of rhizobial *nod* genes.

TABLE 8.3
Some Major Antagonists of Inducers of Rhizobial and Bradyrhizobial *nod* Genes

Symbiotic Association	Antagonist Compounds	Ref.
<i>Medicago sativa</i> and <i>Rhizobium meliloti</i>	Umbelliferone, morin, quercetin	314
<i>Trifolium</i> spp. and <i>Rhizobium. leguminosarum</i> bv. <i>Trifolii</i>	Umbelliferone, formononetin	315
<i>Phaseolus vulgaris</i> and <i>Rhizobium leguminosarum</i> bv. <i>Phaseoli</i>	Umbelliferone, acetosyringone	316
<i>Pisum sativum</i> and <i>Rhizobium leguminosarum</i> bv. <i>Viciae</i>	Daidzein, genistein, kaempferol, acetovanollone, syringaldehyde	311
<i>Glycine max</i> and <i>Bradyrhizobium japonicum</i>	7-Hydroxy-5-methyl flavone, flavone, kaempferol, chrysin	316

Source: From Jain, V. and Nainawatee, H.S., *J. Plant Biochem. Biotechnol.*, 11, 1, 2002. With permission.

Some flavonoids may also play a broader role in *Rhizobium* infection, affecting other microbial processes such as by promoting growth,^{277,317} affecting metabolism^{317,318} and positive chemotaxis.³¹⁹

Nodule organogenesis is thought to involve auxin and possibly also cytokinin in the stimulation of cell divisions and regulation of root differentiation.³²⁰ Mathesius³²¹ tested whether those flavonoids that preferentially accumulate in cells undergoing early nodule organogenesis could affect peroxidase-driven auxin turnover, thereby explaining local changes in auxin distribution during nodule formation in white clover (*T. repens* cv. Haifa). A derivative of 7,4'-dihydroxyflavone (DHF), as well as free DHF, strongly inhibited auxin breakdown by peroxidase at concentrations estimated in the root tissue. Formononetin, an isoflavonoid accumulating in nodule primordia, accelerated auxin breakdown by peroxidase at concentrations estimated to be present in the roots. The data indicated that local changes in flavonoid accumulation could indeed regulate local auxin levels during nodule organogenesis. A model for the interaction of flavonoids with peroxidases has been proposed to explain changes in auxin during nodule development (Figure 8.7). A similar mechanism could be involved in lateral root and root gall development, and the wider role of flavonoids in auxin regulation is discussed below.

8.3.3.4 Mycorrhizal Fungi

The capacity for nitrogen fixation via nodulating bacteria is limited to relatively few plant species. In contrast, arbuscular mycorrhizal fungi (AMF) associations with roots occur in about 80% of plant species. The mutualistic association is important for improving the nutritional status of plants in soil where nutrients such as phosphate are limited. As with nodulation, exudates from seeds and seedlings affect plant infection by AMF, and flavonoids are one group of compounds present in such exudates. Nodulation and mycorrhizal formation on the same plant mutually increase each other's establishment, probably because of the involvement of flavonoids in both processes.³²² This may be due to flavonoids being stimulators of both. There are numerous reports of the effects of specific flavonoids on mycorrhizae in a wide range of plant species (Table 8.4). Effects of flavonoids have been demonstrated on spore germination, hyphal growth, and root colonization (reviewed in Refs. 271, 323). It has been commented that AMF might have genus- or even species-specific signal requirements during the AMF symbiosis, yet some flavonoids exhibit a general stimulatory effect on different AMF genera. For example, quercetin greatly stimulates hyphal development of

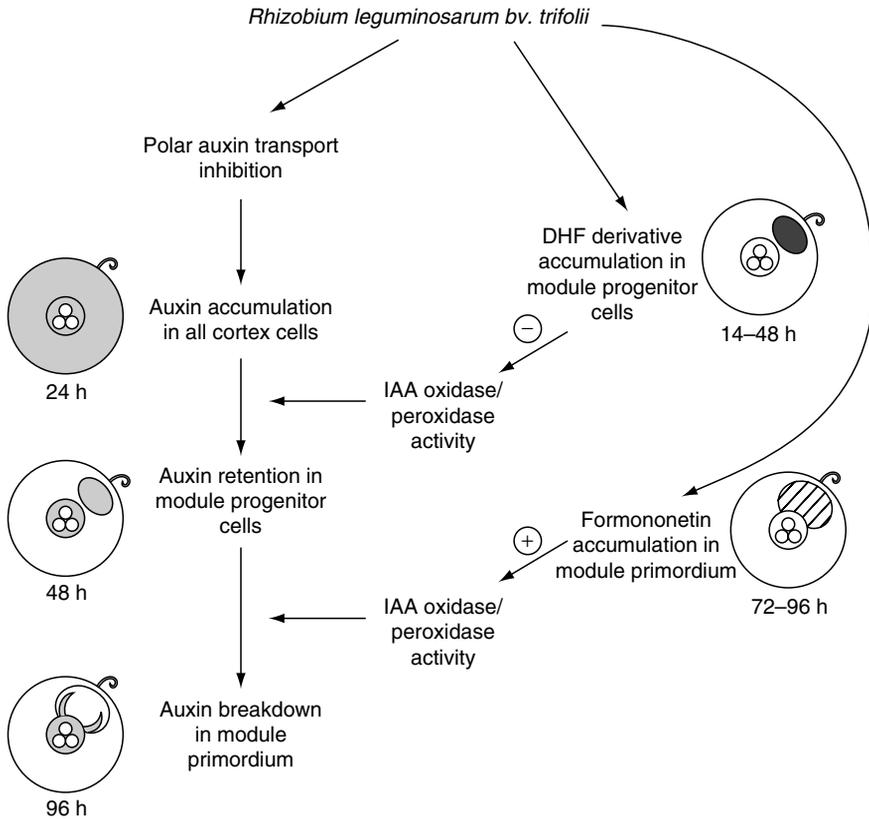


FIGURE 8.7 Model for the role of *Rhizobium*-induced flavonoids in the regulation of auxin balance during nodule formation in white clover. (Redrawn from Mathesius, U., *J. Exp. Bot.*, 52, 419, 2001. With permission of Oxford University Press.)

various species of fungi, e.g., *Gigaspora* and *Glomus*.³²⁴⁻³²⁶ However, studies with biochanin A showed stimulation of *Glomus*^{271,327} but not *Gigaspora* species.^{324,325}

Flavonoid levels or compositions change in the root from the early stages of appressoria formation through to the later stages of symbiosis when the AMF is well established. Thus, flavonoids have been suggested as signaling compounds during root colonization²⁷¹ although this has been debated considerably²⁴⁸. Larose et al.³³¹ presented various data that point

TABLE 8.4
Some Flavonoids Involved in Mycorrhizal Associations

Symbiotic Association	Flavonoids	Effect	Ref.
<i>Medicago sativa</i> and <i>Glomus</i> spp.	Quercetin and quercetin-3- <i>O</i> -galactoside	Promotion of spore germination, hyphal growth, and branching	277, 328
<i>Trifolium repens</i> and <i>Glomus</i> spp.	Formononetin and biochanin A	Promotion of root colonization, stimulation fungal growth	305, 327
<i>Eucalyptus globulus</i> and <i>Pisolithus</i> spp.	Rutin	Stimulation of hyphal growth	329
<i>Pinus sylvestris</i> and <i>Suillus</i> spp.	Root exudates	Enhanced spore germination	330

strongly toward the action of AMF-derived signal(s) on plant roots before root colonization. Two recent reports show a role of flavonoids as regulatory signals for the susceptibility of roots to AMF at the beginning of the formation of the symbiosis.^{332,333} Guenoune et al.³³² demonstrated that the flavonoid medicarpin accumulates in roots with high phosphate levels. Medicarpin exhibits a strong inhibitory effect on hyphal growth of *Glomus intraradices*, thereby preventing roots with a high phosphate status from being colonized by AMF. These data support the hypothesis that during early stages of colonization by *G. intraradices*, suppression of defense-related properties is associated with the successful establishment of AMF symbiosis. Akiyama et al.³³³ investigated melon (*Cucumis melo*) roots inoculated with or without the fungus *Glomus caledonium* under low phosphate conditions. Accumulation of a C-glycosylflavone, isovitexin 2''-O-beta-glucoside, occurred in phosphate-deficient, nonmycorrhizal roots but not in mycorrhizal roots, nor in phosphate-supplemented roots. This experiment indicated that the accumulation of the compound was caused by a phosphate deficiency. Treatment of roots with isovitexin 2''-O-beta-glucoside increased root colonization under both low and high phosphate conditions. These findings suggest that the phosphate deficiency-induced C-glycosylflavonoid is involved in the regulation of AMF colonization in melon roots.

Flavonoid accumulation during mycorrhizal associations has been shown both to vary over time and to be AMF specific. Larose et al.³³¹ studied the accumulation of flavonoids in roots of *M. sativa* after the exposure to *Glomus mosseae*, *G. intraradices*, or *Gigaspora rosea*. It was shown that flavonoid accumulation in *M. sativa* roots (i) is induced before root colonization, pointing toward the presence of a fungal-derived signal, (ii) varies according to the developmental stage of the symbiosis, and (iii) depends on the species of root-colonizing fungus.

Flavonoids are not always essential signal molecules in mycorrhizal symbioses, however; various other phenolic acids can elicit similar effects.²⁴⁸ Compounds that actively promote the growth of AMF (i.e., kaempferol, quercetin, and myricetin)^{324,325} are also required for pollen germination and growth of the germ tube.^{244,245} Thus, these compounds may have some general effect on cell elongation.²⁴⁸ It has been concluded that flavonoids stimulate, but are not essential for mycorrhiza formation.³³⁴

8.3.3.5 Parasitic Plants

Parasitic plants often use chemicals released by their host plant to stimulate seed germination, to locate the host, or for haustorial development. Many different compounds are involved, including strigolactones, quinones, coumarins, flavonoids, and other phenolics. Flavonoids contribute to signaling in some species but not others. Haustorial development in *Triphysaria versicolor* can be induced *in vitro* by the anthocyanidins petunidin, cyanidin, pelargonidin, delphinidin, as well as their glycosides obtained from the host plant.^{335,336} Anthocyanins are not usually found in root exudates, however, and thus the mechanism by which they affect natural signals for parasitic plants in the soil is not clear.

Flavonoids in the bark of the host *Malosma laurina* induce stem coiling in the parasitic plant *Cuscuta sublinclusa*.³³⁷ Similarly, xenogonins, the 2'-formononetin derivatives from *Astragalus* spp., attract a parasitic angiosperm *Agalinis purpurea* to the roots and subsequently initiate growth of the haustorium that allows the parasite to attach itself to its host.³³⁸ However, flavonoids are not always essential for parasitism, as demonstrated using *Orabanche aegyptiaca* on *Arabidopsis*.³³⁹ It has been argued that flavonoids have to be oxidized to quinines before they can actively induce haustorial development.^{335,340,341} Thus, the compelling data from the *in vitro* experiments do not necessarily recapitulate what happens *in vivo*.

Certain flavonoids are used by plants to protect them from invasion by parasites. For example, poplar (*Populus* spp.) cultivars produce a chemical barrier to parasitization by mistletoe (*Viscum album*).³⁴² Resistant poplar cultivars were significantly higher in flavonols and flavones compared to susceptible cultivars. Likewise, in *Streblus asper* the bark and wood of trees that are resistant to the parasite *Cuscuta reflexa* hold higher levels of flavonoids, as well as steroids and alkaloids.³⁴³

8.3.3.6 Regulation of Auxin

Auxins are implicated in many developmental and physiological responses, including regulation of the rate of organ elongation, phototropism, and gravitropism. The hormone might also assist plant stress responses through its involvement in stomatal aperture³⁴⁴ and by reallocating resources under poor growth conditions.³⁴⁵ Auxin moves from cell to cell in a polar fashion, exhibiting a basipetal polarity in stems and a more complex polarity in roots.³⁴⁶ Polar auxin transport is controlled by several types of proteins, including auxin influx and efflux carriers, which pump auxin into and out of plant cells, respectively. In the 1960s, it was shown that monohydroxy B-ring flavonoids were involved in degradation of indole acetic acid (IAA), whereas dihydroxy B-ring flavonoids inhibited IAA-degrading activity.^{347,348} There is now accumulating evidence for a role of flavonoids as endogenous regulators of auxin transport. However, they are not the sole regulators of auxin movement.

The development of nodules, lateral roots, and galls may all be mediated by plant flavonoids through a perturbation of the root auxin balance.³⁴⁹ Flavonoids are induced in root cortical cells before and during their division during the formation of nodules³⁵⁰ and galls.^{351,352} Flavonoids have been found to affect the activity of a peroxidase that regulates auxin turnover,³²¹ and they are inhibitors of auxin transport.^{349,353} Quercetin, apigenin, and kaempferol can outcompete auxins for binding sites on plasma membranes.³⁴⁹ Flavonoids may affect auxin distribution and local concentrations and thus modulate auxin-mediated processes that range from gene expression and ion transport to cell and organ differentiation. However, glycosylation interferes with binding and thus only aglycones are active inhibitors of auxin transport. Since the glycosidic form of flavonoids is most abundant in plant cells, their role remains unclear.

Evidence that flavonoids regulate auxin accumulation *in vivo* was obtained using the flavonoid-deficient mutant *tt4* of *Arabidopsis*.³⁵⁴ In whole seedling [¹⁴C]indole-3-acetic acid transport studies, the pattern of auxin distribution in this mutant was shown to be altered relative to that of wild-type plants. The defect appeared to be in auxin accumulation, as a considerable amount of auxin escaped from the roots through leakage from the root tip. Treatment of the *tt4* mutant with the missing intermediate naringenin restored normal auxin distribution and accumulation by the root. The ability of flavonoids to prevent auxin leakage from the tip provides compelling evidence that endogenous flavonoids are required for normal auxin accumulation by plant cells. Brown et al.³⁵³ also demonstrated that flavonoids act as endogenous regulators of auxin transport. Auxin transport measurements in the inflorescence and the hypocotyls of two different *tt4* mutants, which block flavonoid synthesis, indicate that auxin transport is elevated in the absence of endogenous flavonoids. Growth of plants on naringenin leads to growth and gravity inhibition consistent with inhibition of auxin transport.

Further studies are required to fully elucidate the role of flavonoids in auxin regulation *in vivo* to determine, for example, whether changes in the synthesis or deposition of specific flavonoids within the cell act to change the rate or direction of auxin transport.³⁵⁵ There is the question of how such different organs or developmental outcomes as nodules, lateral roots,

arbuscules, and root galls can involve such similar responses in the plant. How these differences in organogenesis are established remains largely unknown. One possibility is that gene duplication and formation of multigene families has allowed temporal and spatial differences in expression of different members of gene families from previous developmental pathways to symbiotic or pathogenic ones. These cell- and tissue-specific differences may confer specificity to different organs while maintaining gene function.

8.3.4 PROTECTION FROM INSECT AND MAMMALIAN HERBIVORY

Flavonoids, along with other phenolics, help protect plants from herbivory by both insects and mammals. Although one of the most studied groups of flavonoids in regard to this function is the isoflavonoids, other classes of flavonoids are apparently involved, including anthocyanins, flavones, flavonols, and proanthocyanidins (Table 8.5). Although most research has used leaves, protective flavonoids have also been found in other plant parts, such as the roots and seed coats. Insecticidal activity of flavonoids is achieved through various mechanisms including their effects as feeding deterrents,³⁵⁶ digestion inhibitors,³⁵⁷ and direct toxicity.^{358–360} Lipid-soluble flavonoids in leaves form phenolic resins that deter feeding by insects and can bind irreversibly with plant proteins to form flavonoid-based tannins that are unpalatable to herbivores.³ One of the best-known and commercially valuable flavonoid insecticide is the family of rotenoid isoflavonoids, in particular rotenone. These compounds are present in roots and aerial parts of many tropical species of Fabaceae including the genera *Derris*, *Lonchocarpus*, *Mundulea*, and *Tephrosia*.³⁶¹ Rotenone is potent against a wide range of pests including leaf-chewing beetles, caterpillars, flea beetles, and aphids. The metabolite acts by specifically inhibiting the NADH-dependent dehydrogenase step of the mitochondrial respiratory chain, thus impairing O₂ uptake by insects.³⁶¹

In addition to their being feeding deterrents, flavonol glycosides can also function as phagostimulants to insects. In some cases, the taste of the flavonoids may be associated with attraction or repellence of herbivores. Quercetin-3-glucoside, which occurs in the pollen of sunflower (*Helianthus annuus*), is phagoactive for the western corn rootworm (*Diabrotica virgifera*), which feeds on this pollen.³⁷⁹ However, it must be noted that flavonoids are not the only phagostimulant present in the pollen and there are some that are more active than the flavonol. There are cases where a feeding attractant becomes a deterrent as concentration increases. For example, in studies with clover (*Trifolium subterraneum*) and the red-legged earth mite (*Halotydeus destructor*), genistein showed 93% deterrence when supplied at a concentration of 0.08%, and 68% deterrence at 0.045%, yet was found to be an attractant to the mite at 0.01%.³⁸⁰

Some flavonoids, such as dicoumerol, can also serve as herbivore deterrents for mammals. Higher oligomeric forms of proanthocyanidins are feeding deterrents, or else they impair digestion due to their ability to precipitate proteins. This has been reviewed previously.^{381,382} Some mammals (various herbivores, though not carnivores) have adapted to a diet containing condensed tannins by the production of proline-rich proteins in the saliva.⁹³ These proteins have a strong affinity for tannins and bind them in the mouth so that the hydrogen-bonded complex passes through the stomach without causing any damage. There are examples where the tannin-binding capacity is restricted to condensed tannins and the reaction does not occur with hydrolyzable tannins.³⁸³ Scandinavian and North American moose can feed on twigs and bark from a range of trees and shrubs but they cannot eat tissue of *Rubus* and *Alnus*, which contain both classes of tannin. North American deer, on the other hand, can eat more widely as their salivary proteins can bind both types of tannins.³⁸⁴ Estrogenic isoflavonoids affect lambing rates in sheep³⁸⁵ and plants may have evolved these compounds to control the reproductive capacity of their foragers.

TABLE 8.5
Examples of Flavonoids Acting as Feeding Deterrents for Insects

Plant	Compound(s)	Insect Pest	Ref.
<i>Trifolium repens</i> Roots	Medicarpin (isoflavone)	Feeding deterrent to the beetle <i>Costelytra zealandica</i>	362
<i>Gossypium</i> sp. Buds	Cyanidin-3-glucoside (anthocyanin); gossypetin 8- <i>O</i> -rhamnoside and gossypetin 8- <i>O</i> -glucoside (flavonols)	Tobacco budworm, <i>Heliothis virescens</i>	5, 363
<i>Vigna unguiculate</i> Seed coats	Proanthocyanins	Resistance to cowpea weevil <i>Callosobruchus maculatus</i>	364
<i>Ulex europaeus</i> Root bark	Ulexones A (isoflavone)	Feeding deterrent for larvae of <i>Costelytra zealandica</i>	365
<i>Arachis hypogaea</i> Leaf bud petioles	Procyanidin	Affects fecundity of groundnut aphid (<i>Aphis craccivora</i>)	366
<i>Lonchocarpus castilloi</i> Heartwood	Castillen D (aurone) and castillen E (dihydrochalcone)	Termite <i>Cryptotermes brevis</i> feeding deterrence, but were not toxic	367
<i>Lotus pedunculatus</i> Roots, leaves	Vestitol (isoflavonoid)	<i>Heteronychus aratoo</i>	368
<i>Zea mays</i> Silks	Maysin (<i>C</i> -glycosyl flavone)	Corn earworm, <i>Helicoverpa zea</i>	369
<i>Melicope subunifoliolata</i> Leaves	Meliteratin (3,5-dimethoxy-3',4',6,7- bismethylendioxyflavone) and six other minor polyoxygenated flavones	Strong feeding deterrent activity against <i>Sitophilus zeamais</i> ; larvicidal activity against <i>Aedes aegypti</i>	370
<i>Nothofagus</i> spp. (Chile and New Zealand) Leaves	Galangin (flavonol) and the stilbene pinosylvin (appear to act in concert)	Deter feeding by leafrollers (<i>Ctenopsteustis obliquana</i> , <i>Epiphyas postvittana</i>)	371
<i>Pinus banksiana</i> Needles	Rutin and quercetin-3-glucoside (flavonol glycosides)	Reduced growth and increased mortality of gypsy moth (<i>Lymantria dispar</i>)	372
<i>Oryza sativa</i> Leaves	Schaftoside, isoschaftoside, neoschaftoside (glycoflavones)	Sucking deterrent to the brown plant hopper (<i>Nilaparvata</i> <i>lugens</i>)	373
<i>Glycine max</i> Leaves	Sakuranetin (flavanone) in conjunction with chlorogenic acid	Resistance to stem nematode (<i>Ditylenchus angustus</i>) attack	374
	Coumestrol, phaseollin, afrormosin (isoflavonoids)	Soybean looper (<i>Pseudophasia</i> <i>inclusens</i>); larvae of <i>Pectinophora gossypiella</i>	375, 376
	Daidzin and genistin (isoflavones) Rutin and quercetin-3-glucosylgalactoside (flavonols) and genistein (isoflavone)	Stink bug (<i>Nezara viridula</i>) Cabbage looper (<i>Trichoplusia ni</i>)	377 Cited in 93
<i>Mucuna</i> spp. Seeds	Tannins	Bruchids	359
<i>Triticum</i> spp. Stems and leaves	Tricin (flavone) and unidentified <i>C</i> -glycosyl-flavones	Aphids — <i>Schizaphis graminum</i> and <i>Myzus persicae</i>	378

8.4 CONCLUSIONS

Despite the resurgence in research activity on flavonoid function, many questions remain unanswered. Some functions are only partially understood, and there are probably many others not yet uncovered. For example, there have been several intriguing reports that describe correlations between flavonoid content and morphology. In *Antirrhinum*, the intensity of anthocyanin pigmentation in the flowers depends upon the shape of cells in the

petals.³⁸⁶ In maize, mutant endosperm cells show both an abnormality in shape and a blockage in anthocyanin biosynthesis, indicating a possible connection between flavonoid precursors and cell morphology.³⁸⁷ Although these may perhaps be explained by some of the activities described above (e.g., regulation of auxin), Tamagnone et al.³⁸⁸ have postulated a more direct function for flavonoid intermediates in tissue development.

Investigations into the medicinal properties of flavonoids have also revealed novel mechanisms of action; for example, in mediating nucleic acid strand scission, and the inhibition or induction of certain enzymes. It is unclear, however, if these functions have any physiological significance in the plant. Woo et al.³⁸⁹ have suggested that flavonoids in plants may affect gene expression by acting on a putative hormone receptor in the nuclear membrane, or else they could change the activity of regulatory proteins, such as tyrosine kinase, that are involved in cell division. This is being investigated further.

It is fascinating that this one class of secondary compounds has such a diversity of functions. Multigene families in the flavonoid pathway have presumably lead to specialization of flavonoid gene members in processes as disparate as signaling, defense, development, flower pigmentation, and cell wall modification.^{274,390} Different plant species may use different mechanisms to distribute flavonoids among subcellular compartments, and multiple mechanisms are used in individual species.³⁹¹ In addition to the flavonoids as a group displaying a diversity of functions, individual compounds also show multifarious functions. The different functions often share common mechanisms. For example, the ability of flavonoids to act as antioxidants is behind their role in combating many different types of stresses. Similarly, the role of flavonoids in regulation of auxin distribution influences plant responses to nodulating bacteria, mycorrhizal fungi, and *Agrobacterium*.

Future applications of analytical methodology and molecular biology techniques are likely to reveal much more about flavonoid function in plants in the coming decades. A more complete understanding of flavonoid function would provide the foundation for further manipulating plants to cope with environmental stress.

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9 Flavonoid–Protein Interactions

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9.1 GENERAL CONSIDERATIONS

Naturally occurring flavonoids are ubiquitous in all parts of plants where they display a huge structural diversity based on different C rings (Figure 9.1), aglycone substitutions by OH and OMe groups, and glycosidation and acylation patterns. In addition to their important biological roles in plants and plant–insect interactions, flavonoids have been thoroughly investigated during the last two decades because of their possible health effects in man via a diet rich in plant products.^{1,2} Indeed, flavonoids, especially flavanols, flavonols, and anthocyanins, are relatively abundant in human diet, partially bioavailable, and possibly involved in still incompletely understood mechanisms related to the prevention of cancers, cardiovascular diseases, and neurodegenerescence. However, whatever these mechanisms may be, most of them must be related to at least one of the two fundamental properties of flavonoids: their reducing ability (antioxidant properties by electron or H-atom donation) and their ability to interact with proteins.³ Flavonoid–protein interaction in plants is an important issue, for instance, in relation to flavonoid biosynthesis and flavonoid-mediated chemical defense mechanisms. However, this chapter will essentially deal with flavonoid–protein interactions in man and their possible implications for human health. Given the wealth of

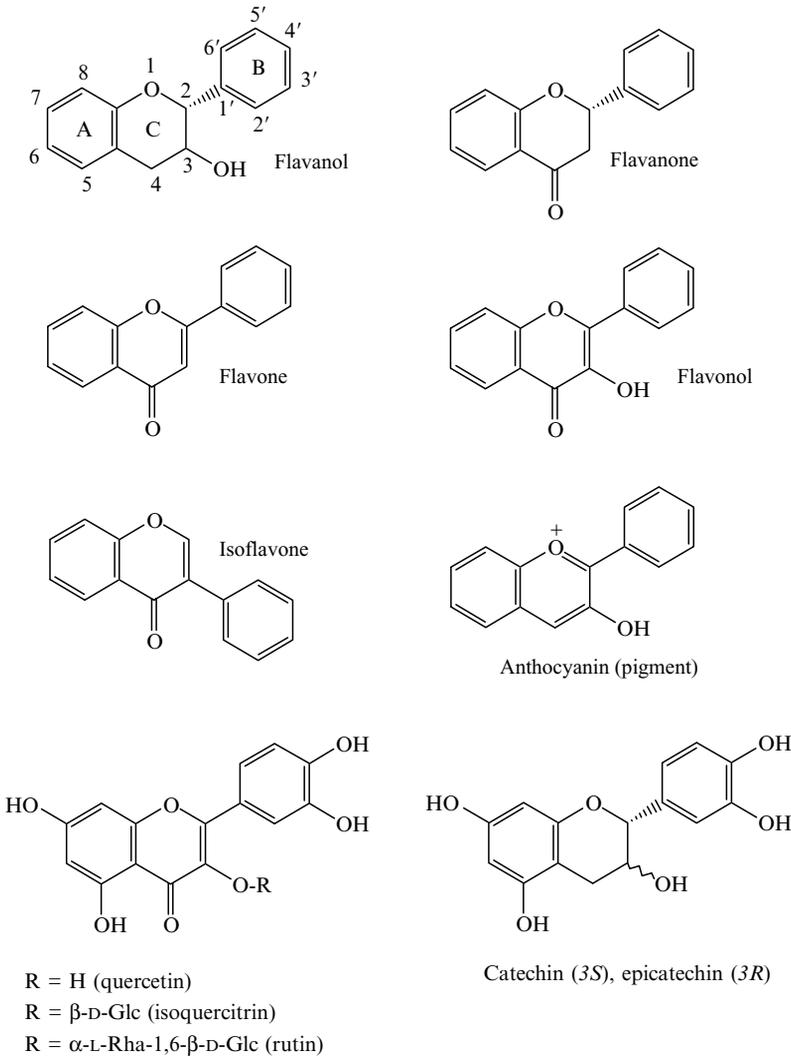


FIGURE 9.1 The main flavonoid classes and examples of common dietary flavonoids.

literature data in this important research field, this review is only aimed at providing a wide overview but by no means an exhaustive report.

9.1.1 THE BIOLOGICAL SIGNIFICANCE OF FLAVONOID-PROTEIN INTERACTIONS IN MAN

Biochemical studies devoted to the possible health effects of flavonoids try to assess either their nutritional value in the prevention of degenerative diseases or their therapeutic value as potential drugs. In the latter case, structure-activity relationships are typically established that include not only naturally occurring flavonoids but also synthetic analogs. In the former case, only dietary flavonoids are considered and bioavailability data must be kept in mind when it comes to building biologically relevant models. Fortunately, flavonoid bioavailability is a fast developing field and a lot of valuable information on the mechanisms by which flavonoids are absorbed from the intestine and metabolized (microbial catabolism, conjuga-

tion in liver and enterocytes) has emerged during the last 10 years.^{4,5} In fact, flavonoid-protein interactions involved in flavonoid bioavailability, in addition to interactions taking place before intestinal absorption (e.g., interactions with salivary proteins), are currently the sole binding processes with clear *in vivo* biological significance. However, little is known so far about the possible delivery of flavonoid conjugates to specific tissues. On the other hand, flavonoids have been very often pointed out as *in vitro* enzyme inhibitors and ligands of receptors involved in signal transduction.^{6,7} However, the biological relevance of these flavonoid-protein binding processes to the field of human nutrition still awaits *in vivo* validation. In particular, the crucial point would be the demonstration that the flavonoid in its bioavailable (typically conjugated) form can accumulate near the target protein in concentrations high enough for the interaction to take place. During the last decade, cell effects distinct from the antioxidant activity by radical scavenging and metal chelation have been frequently evoked to interpret flavonoid-mediated health effects. Indeed, the rather low circulating concentrations of dietary flavonoids (typically in the range 0.1 to 1 μM), and the demonstration that phenolic OH groups critical to the radical scavenging activity can be conjugated in the circulating forms, do not argue in favor of *in vivo* effects dominated by the antioxidant activity except, possibly, in the gastrointestinal tract where high concentrations of native flavonoids can accumulate after a meal. Hence, it can be speculated that most of the flavonoid-mediated health effects involve interactions of flavonoids with specific biological targets, mainly proteins. It is important to underline that the term flavonoid-protein interactions in the literature does not always refer to a direct molecular contact (complexation) but may point to regulation by flavonoids of gene expression for specific proteins. Cytochrome P450 enzymes are a good example of proteins whose function can be regulated by flavonoids via such diverse mechanisms.⁸

9.1.2 MOLECULAR INTERACTIONS RESPONSIBLE FOR FLAVONOID-PROTEIN COMPLEXATION

Intrinsically, the phenolic nucleus is a structural unit that is favorable to molecular (non-covalent) interactions with proteins. These interactions can be divided into two classes³:

- *Van der Waals interactions*: the nonpolar polarizable aromatic ring can develop strong dispersion interactions with amino acid residues displaying similar properties. These interactions are strengthened by the partial desolvation experienced by the two surfaces coming into contact and the simultaneous release of water molecules from the solvation shells where they are highly ordered to the bulk solvent where they develop more hydrogen bonds with other water molecules (hydrophobic effect). These two distinct processes (ligand-protein dispersion interactions and the hydrophobic effect) are often (erroneously) referred to collectively as “hydrophobic interactions.” The importance of dispersion interactions in the stability of the flavonoid-protein complexes could be reflected by the rather general trend emerging from structure-affinity relationships that the flat more polarizable (iso)flavones and flavonols (electron delocalization spread over the three rings) are generally better ligands than flavanones and flavanols (see below).
- *Electrostatic interactions*: in the case of phenols, hydrogen bonding is probably the most important interaction falling in this category. Indeed, the OH group can act as a hydrogen bond donor (via its acidic proton) and a hydrogen bond acceptor (via its nonconjugated lone pair lying in the plane of the phenolic nucleus) toward polar amino acid residues and peptide bonds. In addition, flavonoids having an electron-withdrawing 4-keto group possess a fairly acidic 7-OH group because of a strong electron delocalization in the corresponding phenolate anions. In the case of flavones and flavonols, a similar electronic effect also raises the acidity of the 4'-OH group.

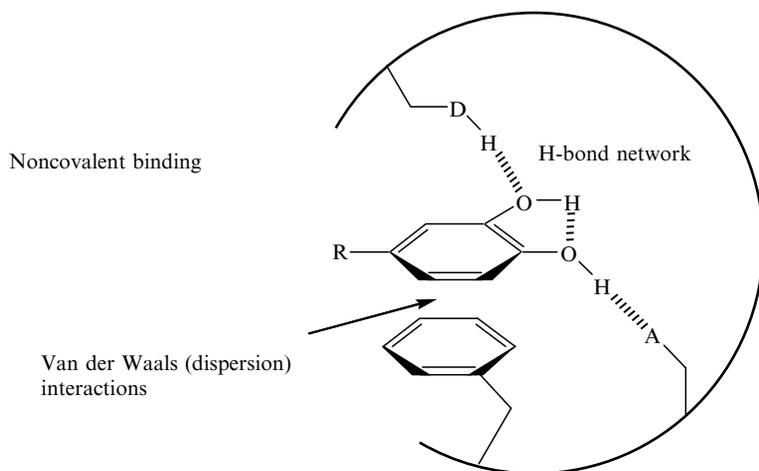


FIGURE 9.2 A schematic representation of molecular interactions at work in flavonoid–protein binding.

Hence, 4-keto flavonoids can be partially dissociated at neutral pH and eventually involved in attractive coulombic interactions with positively charged amino acid residues.

The main molecular interactions involved in phenol–protein interactions are arbitrarily represented in Figure 9.2. A real case of flavonoid–protein hydrogen bond network is depicted in Figure 9.3. It refers to one of the rare examples of a flavonoid–protein complex

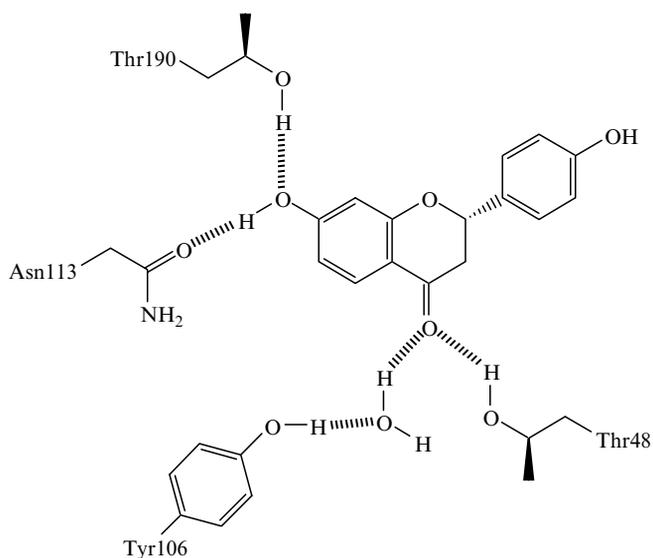


FIGURE 9.3 The hydrogen bond network in the (*S*)-4',7-dihydroxyflavanone–chalcone isomerase complex. (Adapted from Jez, J.M., Bowman, M.E., and Noel, J.P., *Biochemistry*, 41, 5168, 2002. With permission.)

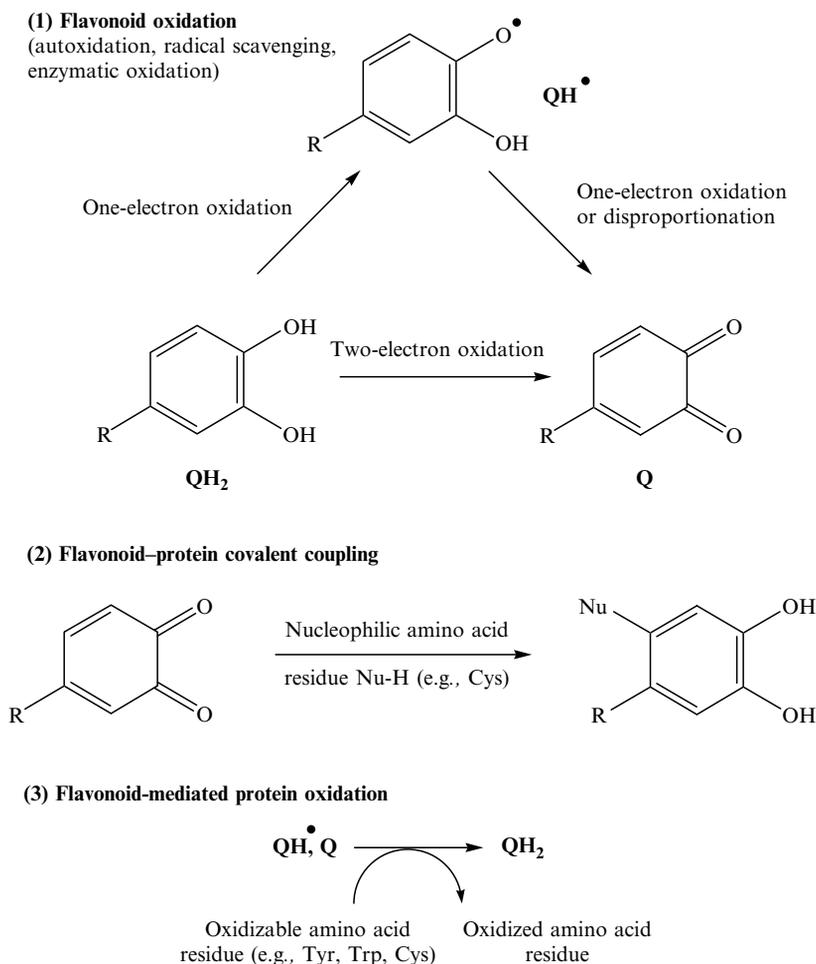


FIGURE 9.4 Flavonoid oxidation and protein modifications.

whose structure has been elucidated by x-ray crystallography, the complex of the plant enzyme chalcone isomerase with its product (*S*)-4',7-dihydroxyflavanone.⁹

Finally, in addition to these noncovalent and reversible interactions, flavonoid-protein redox reactions and oxidative covalent coupling may result from one- or two-electron oxidation of the flavonoid brought about by such mechanisms as: autooxidation (oxidation by dioxygen catalyzed by metal ion traces), scavenging of reactive oxygen species (ROS) (antioxidant activity) eventually produced by the protein itself, and enzymatic oxidation. Being both electrophilic and oxidizing, flavonoid oxidation products (aryloxy radicals, quinones, and quinonoid compounds) may react with nucleophilic or oxidizable amino acid residues, thereby irreversibly modifying the protein by covalent coupling or oxidation (Figure 9.4).

9.1.3 SPECIFICITY OF FLAVONOID-PROTEIN INTERACTIONS

Given the intrinsic propensity of the phenolic nucleus for developing molecular interactions, it is no surprise that examples of flavonoid-protein complexation are numerous and concern a wide variety of proteins. However, the question of their specificity deserves examination.

In the case of conformationally open proteins (random coils) with multiple binding sites for polyphenols such as proline-rich salivary proteins, binding constants are quite low for small polyphenols (gallates, catechin) but increase sharply when the number of polyphenolic nuclei increases (flavanol-3-*O*-gallates, oligomeric procyanidins, polygalloylglucose), thus allowing multiple molecular contacts along the protein chain with a preference for the hydrophobic proline residues.^{10,11} Similarly, oxidative polymerization of catechin or polycondensation with aldehydes produces a strong increase in the affinity for xanthine oxidase.^{12,13} Such trends reflect the approximately additive character of hydrogen bonding and Van der Waals interactions and suggest rather unspecific binding along an extended protein chain or at the surface of globular proteins.^{3,14} By contrast, structure–affinity relationships with various globular proteins having well-defined binding cavities (enzymes, receptors) clearly point to specific interactions with properly substituted flavonoids (generally, flavone, isoflavone, or flavanol aglycones) reaching dissociation constants in the range 1 nM to 1 μ M. Interestingly, a screening of 20 tannins including 10 flavanols for 16 brain receptors has shown a rather high specificity in the displacement of specific radioligands.¹⁵ For instance, only two receptors, 5HT1 and the β -adrenoceptor, are significantly inhibited (>60%) by more than one flavanol at the concentration of 10 μ M. In addition, whereas the β -adrenoceptor is inhibited by epicatechin and its 3'-deoxy analog, 5HT1 appears more sensitive to 4,8-procyanidin dimers. The origin of specific flavonoid–protein interactions may be traced to the structural resemblance between some properly substituted flavonoids and a variety of bioactive compounds including coenzymes (e.g., nucleotides), steroid hormones, and neurotransmitters (e.g., catecholamines) (Figure 9.5). An unexpected case of specific flavonoid–protein binding occurring *in vivo* can be found in the vacuoles of some flower

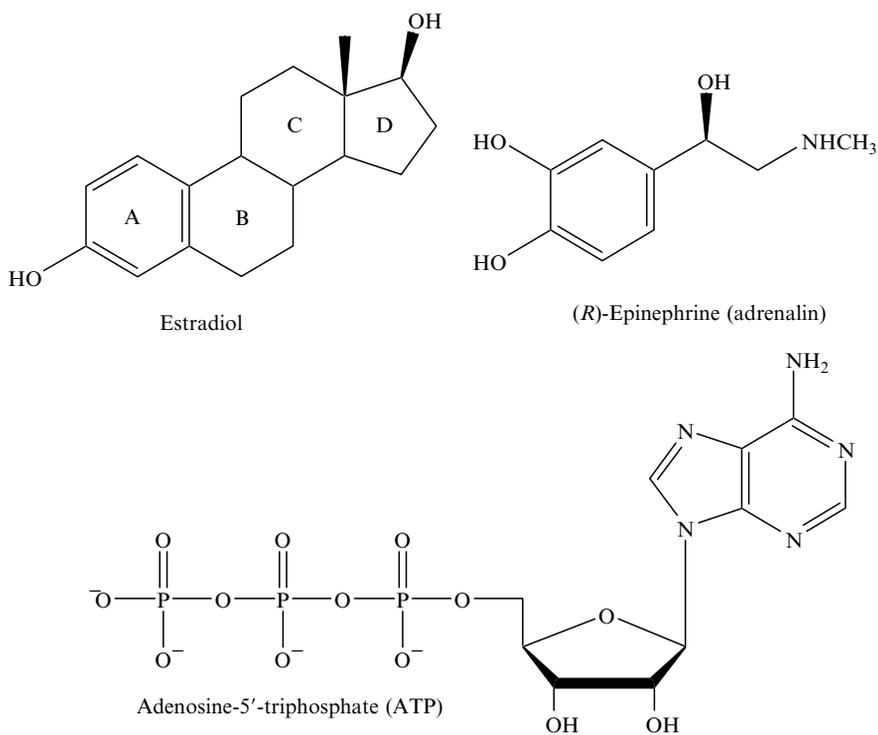


FIGURE 9.5 Structures of biologically important molecules with a formal structural resemblance to flavonoids.

petals (e.g., lisianthus and carnation) where specific anthocyanins are sequestered within protein matrices.¹⁶ In purple lisianthus, for instance, four minor acylated anthocyanin-3,5-diglycosides are tightly bound (noncovalent association) to a protein aggregate including three major components (MW 34 to 50 kDa) whereas their more abundant triglycoside analogs remain in solution.

9.2 EXAMPLES OF FLAVONOID–PROTEIN INTERACTIONS IN MAN

9.2.1 INTERACTIONS PRIOR TO INTESTINAL ABSORPTION

9.2.1.1 Interactions with Salivary Proteins and Astringency

The interaction of tannins (oligomeric procyanidins) with salivary proteins is a key step in the development of the oral sensation of dryness and roughness called astringency, which is typical of tannin-rich food. Basic salivary proteins are proline-rich proteins (25 to 42%) that can bind and precipitate polyphenols. They comprise a typical 19-residue sequence that is essentially constituted of proline, glutamine, and glycine residues. This sequence is repeated 5 to 15 times to form proteins of ~150 residues that display extended conformations with multiple binding sites for polyphenols.¹⁰ Polyphenol–salivary protein interactions are especially significant with procyanidins (condensed tannins) and galloylated derivatives of D-glucose (hydrolyzable tannins) that possess several 1,2-dihydroxy- or 1,2,3-trihydroxybenzene nuclei allowing multiple interactions with the protein. When the polyphenol concentration is increased to provide sufficient coating of the protein, interactions of the polyphenol molecules (probably under their self-associated form) with a second protein molecule lead to extensive aggregation to form colloidal particles of different sizes. Ultimately, precipitation occurs.

Detailed investigations by nuclear magnetic resonance^{4,5,10,11,17} using the typical proline-rich sequences (~20 residues including seven to eight Pro residues) have provided an outline of the main characteristics of the binding:

- The proline residues themselves are privileged sites for interactions with the phenolic nuclei. For instance, β -1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG) is able to stack one of its galloyl units on the most accessible face of the pyrrolidine heterocycle (that presenting the C α -H group). The interaction is essentially driven by dispersion forces and the hydrophobic effect with the possible participation of H bonds between some phenolic OH groups (donors) and the O atom of the tertiary amide group linking the Pro residue to the preceding residue.
- The size of the binding site is restricted to a proline residue for small polyphenols (propylgallate, flavanol monomers) only. As the polyphenol size increases, the number of binding sites is reduced. For instance, up to seven to eight epigallocatechin-3-*O*-gallate (EGCG) molecules (one per Pro residue) can be simultaneously bound to the 19-mer peptide (pH 3.8, 27°C, 10% DMSO). By contrast, the stoichiometry of the peptide–PGG complex is 1:2.
- Multidentate binding of the polyphenol is important for the formation of stable complexes. Indeed, taking into account polyphenol self-association and assuming identical peptide binding sites (probably, a crude approximation), dissociation constants can be estimated from binding isotherms expressing the chemical shift changes of sensitive peptide proton signals as a function of the polyphenol concentration. K_d values are 0.05, 2.4, 5.0, and 0.05 mM for PGG, EGCG, epicatechin, and its 4,8- β dimer (procyanidin B2), respectively (pH 3.8, 3°C, 10% dimethyl sulfoxide [DMSO], except for epicatechin [no DMSO]).

- Cooperativity between peptide binding sites may be important. Thus, the affinity of EGCG for the 19-mer peptide (eight Pro residues) is ~50 times as strong as for a 7-mer peptide with only one to two binding site(s) for EGCG. Similarly, complexation with a full-length basic salivary protein is also much stronger than with the peptide models.¹⁸ Hence, it may be proposed that salivary proteins wrap around the polyphenol molecules to allow multiple interactions. However, since proline residues provide rigidity to the protein backbone, this process is expected to take place with little loss in rotational freedom and little change in the extended conformation of the peptide.
- From the concentration dependence of the polyphenol chemical shifts as a function of the polyphenol concentration in the absence and presence of peptide, it is clear that polyphenol self-association is only weakly affected by the peptide. Thus, peptide–polyphenol binding probably involves noncovalent polyphenol oligomers that are more effective than the corresponding monomers at developing interactions with a second peptide molecule to trigger precipitation.
- Polyphenol–salivary protein interaction is essentially pH independent in the range 3.8 to 6.0 whereas the extent of precipitation is higher and the particle sizes larger at higher pH. Indeed, at higher pH, the net charge of the peptide moieties must be lower, thus favoring aggregation. On the other hand, at the highest pH values, partial autoxidation of EGCG may take place during the 24-h incubation of the polyphenol with the protein, thus providing electrophilic *o*-quinone intermediates that may couple to the nucleophilic residues of the protein. Hence, it cannot be excluded that the formation of covalent polyphenol–protein linkages is partially responsible for the increased aggregation at higher pH.

The influence of procyanidin structure on the extent of salivary protein precipitation has also been studied by light scattering techniques.¹⁹ Factors favoring precipitation are: catechin moieties rather than epicatechin moieties; the presence of 3-*O*-galloyl residues; a higher degree of polymerization; and 4,8-interflavan linkages rather than 4,6-interflavan linkages. However, it must be kept in mind that the extent of precipitation may not be a faithful reflection of the polyphenol–protein affinity in solution (see above).

Despite their very short sequence (7 to 38 amino acid residues for the 12 histatins identified so far), the histidine-rich salivary protein histatins have also been reported to precipitate tannins, eventually more efficiently than proline-rich proteins, especially at neutral pH and high tannin concentration.^{20,21} A detailed NMR analysis of the binding between EGCG and histatin 5, a 24-mer that is very rich in basic His, Lys, and Arg residues (~60%) and devoid of secondary structure, has revealed noncooperative binding of six to seven flavanol molecules with a dissociation constant of 1 mM (pH 3.0, 25°C).²²

9.2.1.2 Interactions in the Gastrointestinal Tract

The formation of insoluble tannin–dietary protein complexes and inhibition of digestive enzymes by tannins have been investigated for a long time for their implications in the low digestibility of nutrients in animals.²³ However, data on the structure and stability of the corresponding complexes seem scarce. In addition, the interactions of flavonoids other than tannins with dietary proteins and their possible consequences on flavonoid bioavailability and antioxidant activity in the gastrointestinal tract are essentially not documented. Regarding digestive enzymes, some properly hydroxylated flavone and flavonol aglycones (e.g., quercetin) have been reported to inhibit the protease trypsin with IC₅₀ values in the range 10 to 60 μM.²⁴

9.2.2 INTERACTIONS INVOLVED IN FLAVONOID BIOAVAILABILITY

9.2.2.1 Intestinal Absorption

After tannins, flavonoid glycosides are by far the most common dietary sources of flavonoids. However, intestinal absorption requires prior deglycosylation. This step, which does not take place in the gastric compartment, occurs in the large intestine as a result of microbial metabolism.²⁵ In addition, some flavonoid glycosides can be absorbed directly from the small intestine upon deglycosylation by the enzyme lactase phlorizin hydrolase (LPH) and subsequent passive diffusion of the aglycones through the enterocyte layer (route 1). However, a minor route (route 2) could also consist in active transport of the glycosides through the sodium-dependent intestinal D-glucose carrier SGLT1 and subsequent deglycosylation by cytosolic β -glucosidase. Using inhibitors of LPH and SGLT1, it was demonstrated that route 1 is the sole absorption mechanism for quercetin-3-glucoside (Q3Glc) whereas route 2 could contribute to the absorption of quercetin-4'-glucoside (Q4'Glc).²⁶ Hydrolysis of flavonoid glycosides by LPH has been studied in detail. This enzyme, which is present on the luminal side of the brush border of the small intestine, primarily catalyzes the hydrolysis of lactose. However, LPH also displays a second site for the hydrolysis of more lipophilic substrates such as the dihydrochalcone glycoside phlorizin. Selective inhibition of the phlorizin site only marginally affects the efficiency of LPH at hydrolyzing Q3Glc and Q4'Glc, thus demonstrating that deglycosylation mainly occurs at the lactase site.²⁷ Q3Glc and Q4'Glc have similar K_m (40 to 50 μM) and k_{cat} values and are more rapidly hydrolyzed than isoflavone glycosides. The ability of extracts of human small intestine and liver to hydrolyze flavonoid glycosides (likely due to the cytosolic broad-specificity β -glucosidase) was also investigated.²⁸ The flavonol Q4'Glc and the isoflavone genistein 7-glucoside are hydrolyzed with similar efficiencies by both tissues with apparent K_m values of 32 and 14 μM , respectively. By contrast, Q3Glc, quercetin-3,4'-diglucoside, kaempferol-3-glucoside, and rutin (Q-3- β -D-Glc-1,6- α -L-Rha) are not hydrolyzed (with the exception of a small proportion of Q3Glc with the small intestine extract). Hence, in the flavonol family, a Glc moiety at the 4' position and a free OH group at the 3 position seem required for activity.

The fraction of flavonoids that reaches the colon can be extensively metabolized by microflora enzymes. This may be an important step in flavonoid bioavailability, especially for flavonoids that are essentially not absorbed from the small intestine such as the oligomeric procyanidins.²⁹ Flavonoid glycosides (native forms) and glucuronides (excreted in the bile) can be hydrolyzed to the corresponding aglycones and further processed into phenolic acids.^{25,30,31} Both the flavonoid aglycones and their phenolic acid derivatives can then be absorbed from the colon. In fact, phenolic acids and their glucuronides are detected in much larger concentration in blood and urine than the conjugates of the flavonoid aglycones themselves, thus raising the possibility that the cleavage products are relevant active forms *in vivo*.^{31,32} However, no detailed biochemical information (enzyme identification and isolation for the determination of structure-activity relationships, K_m values, etc.) is currently available about the degradation of flavonoids by microflora enzymes.

9.2.2.2 Conjugation Enzymes

The detection of sulfates and glucuronides of quercetin and 3'-O-methylquercetin in the plasma of volunteers having absorbed a flavonol-rich meal³³ has prompted detailed investigations of quercetin metabolism in the enterocyte and liver. By recording the formation of the different quercetin glucuronides (QGlcU) from quercetin and UDP-glucuronic acid in the presence of human liver cell-free extract, it was shown that conjugation on the B ring (positions 3' and 4') takes place with low K_m values (0.5 to 1 μM), which suggest a high

affinity of quercetin for the corresponding isoforms of liver UDP-glucuronyl transferase.³⁴ Although quercetin has a lower affinity for isoforms catalyzing conjugation at the 7 position (together with marginal conjugation at the 3 position), the V_{\max} value is higher. Hence, at high quercetin concentration, glucuronidation at the 7 position may be predominant. Alternatively, quercetin and other flavonols have been shown to be substrates of purified isoforms of UDP-glucuronyl transferase.^{35,36} Using human liver cell-free extracts, it was also possible to demonstrate that quercetin glucuronides undergo further metabolism in the liver.³⁷ For instance, deconjugation to free quercetin takes place rapidly and is as efficient for each of the four glucuronides tested (positions 3, 3', 4', and 7). These results are in agreement with a preliminary investigation with human cell extracts from liver, small intestine, and neutrophils.³⁸ In addition, a detailed kinetic analysis with pure β -glucuronidase showed that Q4'GlcU ($K_m = 48 \mu M$) displays more affinity for the enzyme than its 3 and 7 regioisomers ($K_m = 167$ and $237 \mu M$, respectively) although deconjugation at the 3 position is faster based on the k_{cat}/K_m values. Hence, net glucuronidation of dietary flavonols in the liver could be the result of a balance between the activity of UDP-glucuronyl transferase and β -glucuronidase that would be normally shifted toward the former. Since conjugation dramatically alters the antioxidant activity of flavonoids and their interactions with proteins,^{33,34} their eventual deconjugation could be of great importance in the development of flavonoid-mediated health effects. Using intact hepatocytes, methylation of Q3GlcU and Q7GlcU at the 3' and 4' positions as a result of catalysis by catechol *O*-methyltransferase and formation of quercetin 3'-sulfate (following initial deglucuronidation) were also observed.³⁷ By contrast, Q4'GlcU is not metabolized under the same conditions.

9.2.2.3 Plasma Proteins

The absorption bands of flavonol conjugates detected in the plasma of rats fed quercetin or rutin are bathochromically shifted in comparison to typical flavonol absorption bands.³⁹ A similar phenomenon can be reproduced by adding serum albumin (SA) to flavonol solutions, thus suggesting that the circulating flavonols are bound to SA. Since then, several studies have investigated flavonol–albumin complexation. For instance, the human SA (HSA)–quercetin binding constant (high-affinity site) has been evaluated at physiological pH by circular dichroism ($14.6 \pm 2.1 \times 10^3 M^{-1}$, 37°C), ultracentrifugation ($267 \pm 33 \times 10^3 M^{-1}$, 20°C), and fluorescence ($50 \pm 8 \times 10^3 M^{-1}$, 25°C).^{40–43} These values reflect the moderate affinity of quercetin for albumin in line with similar observations for a variety of drugs and xenobiotics.⁴⁴ On the other hand, the sole flavonols that can be detected in plasma after a meal rich in quercetin or rutin are glucuronides and sulfoglucuronides of quercetin and 3'-*O*-methylquercetin.³³ Hence, investigating the binding of quercetin conjugates (or models of quercetin conjugates), rather than that of quercetin itself, is more biologically relevant. To that purpose, the influence of quercetin methylation, glycosidation, and sulfation on albumin binding has been assessed.^{42,43} (Table 9.1). Quercetin-7-*O*-sulfate retains a high affinity for BSA and HSA whereas additional sulfation of 4'-OH markedly weakens the binding to both albumins. Glycosidation and sulfation of 3-OH, methylation of 3'-OH (isorhamnetin), and deletion of 3'-OH (kaempferol) all significantly lower the affinity to BSA, the binding to HSA being much less affected. These observations outline the importance of a free OH at position 3' for a strong binding to BSA. Luteolin (no 3-OH) is as efficient as quercetin in binding BSA but not HSA. The influence of glycosyl and acyl substituents is also reflected in the large differences in affinity to BSA displayed by chrysin (strong binding competitively to quercetin), chrysin-7-*O*- β -D-glucoside and *p*-methoxycinnamic acid methyl ester (weak binding), and chrysin-7-*O*-(6-*O*-*p*-methoxycinnamoyl)- β -D-glucoside (strong binding noncompetitively to quercetin).⁴⁵

TABLE 9.1
Binding Constants (K) for Flavonoid–Serum Albumin Complexes
(1:1 Stoichiometry), pH 7.4 Phosphate–NaCl Buffer, 25°C

Flavonoid ^a	$K \pm SD (\times 10^3 M^{-1})$	
	BSA	HSA
Quercetin	134 ± 6 ^b	50 ± 8 ^c
Quercetin-7- <i>O</i> -sulfate	143 ± 29	87 ± 23
Quercetin-3- <i>O</i> -sulfate	21 ± 2	58 ± 5
Quercetin-4',7- <i>O</i> -disulfate	25 ± 2	6 ± 1 ^d
3'- <i>O</i> -Methylquercetin	24 ± 1 ^d	N/A
Quercetin-3- <i>O</i> -β-D-Glc	13 ± 4 ^d	16 ± 1
Rutin	11 ± 7 ^d	—
Kaempferol	12 ± 1 ^d	129 ± 13 ^d
Luteolin	143 ± 35	14 ± 3
Luteolin-7- <i>O</i> -β-D-Glc	N/A	N/A
Flavonoid^e		
4'- <i>O</i> -Methylquercetin	51 ± 2 ^d	75 ± 3 ^d
Diosmetin	66 ± 2	53 ± 1
Baicalein	148 ± 2 ^d	74 ± 4
Baicalein-7- <i>O</i> -β-D-GlcU	78 ± 1 ^d	117 ± 4
Genistein	30 ± 1	11 ± 1
Naringenin	49 ± 1	3 ± 1
Catechin	N/A	N/A

^aFlavonoids forming fluorescent complexes.

^bSD: standard deviation from four experiments.

^cFrom triplicates.

^dFrom duplicates. Otherwise SD from curve fitting.

^eAssuming competitive binding with quercetin (fluorescent indicator).

N/A, not applicable.

While nonfluorescent in its free state, SA-bound quercetin is strongly fluorescent in the visible range. Hence, quercetin can be used as a fluorescent probe for investigating the binding of flavonoids that are nonfluorescent in both their free and SA-bound states such as flavones and flavonols lacking a free 4'-OH, flavanones, and isoflavones^{42,43} (Table 9.1). Assuming competitive binding, it can be noticed that methylation of 4'-OH (quercetin vs. tamarixetin, luteolin vs. diosmetin) decreases the BSA binding constants by a factor 2 to 3. By contrast, the affinity for HSA of tamarixetin (4'-*O*-methylquercetin), identified as a quercetin metabolite in bile and urine, remains high. Baicalein (5,6,7-trihydroxyflavone) and its 7-*O*-β-D-glucuronide (baicalin) are tightly bound to both albumins. Thus, the relatively bulky glucuronide moiety that is typical of most flavonoid conjugates does not hamper the binding in this case. The isoflavone genistein appears as a poor albumin ligand. The lack of conjugation or a non-planar C ring in the flavanone naringenin may be responsible for its relatively low affinity for both albumins, especially HSA. These structural characteristics are even more pronounced in the flavanol catechin, which actually does not affect the fluorescence of albumin-bound quercetin, thus suggesting either no affinity for albumin or binding to a totally independent site.

Despite its moderate intensity (binding constants K in the range 10^4 to $10^5 M^{-1}$), the interaction between albumin and flavones and flavonols is strong enough to ensure a

quasi-total complexation of the circulating forms, owing to the respective concentrations of the partners (0.1 to 1 μM for the circulating forms, 0.6 mM for serum albumin). Hence, it may be reasonably proposed that flavonols and flavones circulate as albumin complexes rather than in their free form.

Additional investigations aimed at demonstrating specific binding to a particular SA site have been carried out. Displacement studies with known site markers (dansylasparagine and warfarin for subdomain IIA, ibuprofen and diazepam for subdomain IIIA)⁴³ are in agreement with preliminary results⁴¹: the quercetin high-affinity binding site appears to be located in subdomain IIA for both HSA and BSA. In the case of HSA, the quercetin binding cavity in subdomain IIA is large enough to accommodate additional ligands such as salicylate⁴⁰ and warfarin.^{41,43} Furthermore, this site, which is known to bind a large variety of xenobiotics, is lined by positively charged basic residues (Lys, Arg) and nonpolar residues (Tyr, Trp, Leu), in agreement with a complexation driven by dispersion interactions, the hydrophobic effect, and, possibly, attractive coulombic interactions involving partially dissociated flavonol molecules. Finally, flavonoid–albumin binding is noncompetitive to that of fatty acids.⁴⁰

Interactions with other plasma proteins are still poorly documented. Weak binding of quercetin with α 1-glycoprotein has been reported.⁴¹ Moreover, when a sample of human plasma is eluted on an EGCG-linked sepharose column, the sole proteins that are retained are fibronectin, fibrinogen, and histidine-rich glycoprotein.⁴⁶ Binding of EGCG to each of these plasma proteins was confirmed using dot binding assays on nitrocellulose sheets. In addition, the binding was shown to be restricted to flavanols having a 3-*O*-galloyl moiety. Interaction of catechin with the protein component (Apo A-1) of high-density lipoproteins has also been proposed from gel electrophoresis experiments and comparison with molecular mass markers.⁴⁷

9.2.3 INTERACTIONS POTENTIALLY INVOLVED IN CELLULAR HEALTH EFFECTS

9.2.3.1 ATP-Binding Proteins

Flavonoids are known to inhibit the function of many ATP-binding proteins,^{6,7} such as mitochondrial ATPase, myosin, Na/K and Ca plasma membrane ATPases, protein kinases, topoisomerase II, and multidrug resistance (MDR) proteins. In general, inhibition takes place through binding of the flavonoids to the ATP-binding site. Only two cases relevant to the inhibition of carcinogenesis by flavonoids^{48,49} will be discussed in detail.

9.2.3.1.1 Kinases

Phosphorylation of proteins at OH groups of serine, threonine, and tyrosine residues is an important mechanism of intracellular signal transduction involved in various cellular responses including the regulation of cell growth and proliferation.^{49,50} The reaction makes use of ATP as a phosphate donor and is catalyzed by protein kinases. For instance, growth factor hormones bind to extracellular domains of large transmembrane receptors that display a tyrosine kinase moiety in their intracellular portion. As a consequence of hormone–receptor binding, the receptor dimerizes and becomes active in the phosphorylation of proteins close to the membrane, thereby triggering a large number of signaling pathways themselves involving other PKs, such as PKC, a Ser/Thr PK, and mitogen-activated PKs (MAPKs). On the other hand, each phase of the cell cycle, during which the DNA is replicated and the chromosomes built and then separated, is characterized by intense bursts of phosphorylation controlled by highly regulated kinases called cyclin-dependent kinases (CDKs).

A possible mechanism for the potential anticarcinogenic effects of flavonoids could be their ability to inhibit various PKs (demonstrated with purified enzymes or cell extracts). For

instance, the isoflavone genistein has been shown to inhibit the epidermal growth factor (EGF) receptor in the submicromolar range by competing with ATP for its binding site.⁵¹ Similarly, butein (2',3,4,4'-tetrahydroxychalcone) appears as a specific inhibitor of tyrosine kinases (IC_{50} for EGF receptor = 65 μM) acting competitively to ATP and noncompetitively to the phosphate acceptor and having no affinity for Ser/Thr PKs such as PKC and the cAMP-dependent PKA.⁵² However, structural changes may affect both the selectivity toward different kinases and the binding site. For instance, silymarin, a flavanonol analog, was suggested to bind to the hormone-binding site in competition with EGF.⁵³ Recently, PKC was shown to be efficiently inhibited by flavones and flavonols having a 3',4'-dihydroxy substitution on the B ring (efficient concentrations 50 in the range 1 to 10 μM).^{54,55} Hydrogen bonding between these two OH groups and the enzyme seems a key determinant of the complexation that takes place in the ATP-binding site, in competition with the cofactor. Similar structure-activity relationships were also established in the inhibition by flavonoids of phosphoinositide 3-kinase (PI3-K), a lipid kinase catalyzing phosphorylation of inositol lipids at the D3 position of the inositol ring to form new intracellular lipid second messengers.⁵⁵ Flavonoids were also demonstrated to inhibit CDKs.⁵⁶ Consistently, studies with intact cells have shown that various flavonoids can cause cell cycle arrest in correlation to their ability to inhibit CDKs.^{53,57}

Flavonoids can also modulate the activity of MAPKs as a possible mechanism for their potential antineurodegenerative action^{58,59} and protection against autoimmune, allergic, and cardiovascular diseases.^{60,61} For instance, investigations on intact antigen-presenting dendritic cells have shown that the MAP kinases involved in cell maturation (ERK, p38 kinase, JNK) can be activated by bacterial lipopolysaccharide and that this activation is strongly inhibited by pretreatment of the cells by EGCG.⁶¹ However, no evidence is provided that the mechanism actually proceeds via direct EGCG-MAPK inhibition. Interestingly, quercetin-3-*O*-glucuronide (Q3GlcU), a quercetin metabolite, is more specific than quercetin in inhibiting the activity of MAP kinases in vascular smooth muscle cells.⁶⁰ Indeed, pretreatment of the cells with Q3GlcU selectively prevents the activation of c-Jun N terminal kinase (JNK) by angiotensin II. Since JNK is responsible for c-Jun phosphorylation that produces a component of transcription factor AP-1, its inhibition blocks AP-1-mediated gene expression, which is involved in the growth of vascular smooth muscle cells. Once more, the molecular mechanism underlying the effect of the flavonoid remains unclear. Indeed, angiotensin-II-mediated JNK activation may be due to ROS produced by NADH/NADPH oxidase. Hence, its inhibition may be a combination of antioxidant action (electron transfer to ROS) and flavonoid-protein interaction (e.g., direct inhibition of JNK or NADH/NADPH oxidase). As a recent example of progress made in elucidating the molecular mechanisms by which flavonoids regulate gene expression, it has been shown that the potent inhibition by luteolin of the lipopolysaccharide-activated transcriptional activity of the nuclear factor κB in rat fibroblasts does not proceed by inhibition of the release of NF- κB from its cytoplasmic complex with inhibitor I κB nor by its translocation into the nucleus and subsequent binding to DNA.⁶² In fact, luteolin activates the PKA pathway, thereby stimulating the production of phosphorylated proteins that will compete with NF- κB for coactivator CBP. The mechanism of the luteolin-mediated PKA activation is not elucidated yet although modulation of cAMP level via inhibition of cAMP phosphodiesterase by luteolin is suggested.

Pretreatment of neurons by flavonoids (epicatechin and its 3'-*O*-methylether, kaempferol) strongly inhibits cell death induced by oxidized low-density lipoproteins (ox-LDL) without reduction of ox-LDL uptake or intracellular oxidative stress.⁵⁹ Cell protection is selectively correlated to inactivation of JNK, thus suggesting that, irrespective of their H-atom donating activity, flavonoids can selectively attenuate a pro-apoptotic signaling cascade involving MAPKs.

9.2.3.1.2 Multidrug Resistance Proteins

Cancer cells typically overexpress ATP-dependent transmembrane transporters capable of expelling a wide variety of chemically unrelated drugs used in cancer therapy. This phenomenon is known as multidrug resistance (MDR). Inhibition of MDR proteins, such as the P-glycoprotein (Pgp), to prevent drug efflux during cancer therapy has thus potential clinical value.

Quercetin was shown to efficiently inhibit the Pgp-mediated drug efflux by inhibiting the ATPase activity required for transport.⁶³ From investigations using a soluble cytosolic portion of mouse Pgp, which includes the nucleotide- and drug-binding domains, it was possible to monitor flavonoid binding by fluorescence as well as its influence on ATP binding and the efflux of the anticancer steroid drug RU 486.⁶⁴ Flavones (aglycones) bearing OH groups at positions 3 and 5 come up as efficient mouse Pgp ligands with apparent dissociation constants lower than 10 μM . By contrast, the quercetin glycoside rutin, the flavanone naringenin, and the isoflavone genistein have low affinity for Pgp. Interestingly, flavones and flavonols behave as bifunctional inhibitors whose binding site overlap the vicinal binding sites for both ATP and RU 486. Those trends were confirmed using a cytosolic portion of Pgp from the parasite *Leishmania tropica*.⁶⁵ In the presence of the most efficient Pgp inhibitors, the drug daunomycin was shown to accumulate in resistant parasites and inhibit their growth. Interestingly, the presence of a 1,1-dimethylallyl substituent at position 8 of the flavone nucleus markedly increases the affinity for Pgp (a factor ~ 20 for apigenin and kaempferide) with apparent dissociation constants of $\sim 1 \mu\text{M}$. These observations prompted the search for more active amphiphilic flavonoids bearing saturated or unsaturated (prenyl, geranyl) hydrocarbon chains.^{66,67} For instance, 4-*n*-octyloxy-2',4',6'-trihydroxychalcone displays an optimized affinity for mouse Pgp with a dissociation constant of 20 nM.

The interaction of flavonoids with MDR proteins is of interest not only in the field of cancer prevention and therapy but also in the field of flavonoid bioavailability. Indeed, using specific inhibitors of MDR proteins, it was shown that multiresistant protein 2 (MRP2), but not Pgp, is involved in the efflux of quercetin conjugates from human hepatic cells.³⁷ Similar observations were made with the cell line Caco-2 (a popular model for human intestinal absorption) where MRP2 was found responsible for the efficient efflux of chrysin after its conjugation into a mixture of glucuronide and sulfate.⁶⁸

9.2.3.2 Ligand–Receptor Interactions

9.2.3.2.1 Estrogen Receptors

Estrogen hormones influence the growth, differentiation, and functioning of many tissues from the male and female reproductive systems. They also have cardioprotective effects. Some dietary flavonoids, especially the isoflavone genistein, belong to the phytoestrogen family as they tightly bind both estrogen receptors (ER) α and β and trigger gene activation as full agonists.⁶⁹ This effect could provide a basis for interpreting the inverse relation between the risk of prostate and breast cancers and the intake of isoflavone-rich soy foods that has been put forward by epidemiological studies.

The affinity of genistein (4',5,7-trihydroxyisoflavone) for ER α and ER β is 0.7 and 13% of that for the endogenous ligand 17 β -estradiol, respectively.⁶⁹ Hence, the corresponding dissociation constants can be calculated to be as low as 7 nM (ER α) and 0.6 nM (ER β). The high affinity of isoflavones for ER α and ER β can be interpreted by the isoflavones A and C rings mimicking the estrogen A and B rings. The binding of apigenin (4',5,7-trihydroxyflavone) and kaempferol (3,4',5,7-tetrahydroxyflavone) to ER β is six to seven times weaker than that of genistein. Moreover, deletion of the 5-OH phenolic group of genistein to give daidzein weakens the binding by a factor 3 to 4 and 13 for ER α and ER β , respectively. In comparison

to their aglycones, daidzein and genistein glucuronides have an affinity for the ER β that is reduced by a factor 10 and 40, respectively.⁷⁰ Nonetheless, given the relatively high peak serum concentrations of total daidzein and genistein (0.5 to 1 μ M) reached after consumption of soy food, isoflavone glucuronide-ER β binding appears strong enough to be of biological significance. Like 17 β -estradiol binding, genistein binding promotes dimerization of the receptor and subsequent binding to DNA at the estrogen receptor element,⁷¹ thereby inducing gene activation. The estrogenic potency of genistein on ER α and ER β is 0.025 and 0.8% that of 17 β -estradiol, respectively.⁶⁹

9.2.3.2.2 GABA-A Receptor

γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. GABA-A receptors are pentameric *trans*-membrane proteins having a central chloride ion channel. The chloride flux can be regulated by a variety of neuroactive ligands including flavones that are able to bind to the benzodiazepine (BDZ) binding site at the interface between α and γ subunits. Flavones typically behave as partial agonists potentiating the GABA-activated ion current in a sub-maximal manner.⁷² As such, they are potential anxiolytic agents. Naturally occurring flavones typically bind to the BDZ receptor with moderate affinity (K_i in the range 1 to 100 μ M) and have been selected as leads for structure-affinity relationship studies including numerous synthetic flavonoid analogs. Hence, the introduction of methyl and nitro groups as well as halogen atoms on the A and B rings has emerged as a powerful way to increase the flavonoid-receptor interaction. For instance, 6-methyl-3',5-dinitroflavone binds with a record K_i value of 1.9 nM.⁷³ Moreover, recent investigations⁷⁴ have highlighted that some naturally occurring 2'-hydroxyflavones can approach such an affinity ($K_i = 6.1$ nM for 5,7,2'-trihydroxy-6,8-dimethoxyflavone extracted from a Chinese medicinal herb). It has been proposed that the critical interactions between a flavonoid ligand and the BDZ receptor include hydrogen bonding at the C ring oxygen atoms and hydrophobic interactions (possibly π -stacking interactions involving aromatic amino acid residues) developed by the A and B rings.^{72,73}

Since flavonoids are able to bind to the BDZ binding site of the GABA-A receptor, they might well interact with the BDZ binding site of the mitochondrial permeability transition protein, thereby modulating oxidative stress-induced apoptosis.⁵⁸

9.2.3.2.3 Adenosine Receptors

Adenosine receptors (subtypes A₁, A_{2A}, A_{2B}, and A₃) play a role in brain and heart protection as well as in the modulation of the immune and inflammatory systems. Hence, adenosine antagonists have potential pharmacological value. Interestingly, several flavone and flavonol aglycones have rather high affinities for adenosine receptors.⁷⁵ In addition, varying the substitution of the flavone nucleus by OH and OR groups (R = Me, Et, nPr) can produce fairly selective A₃ ligands with K_i values of the order of 1 μ M (close to that of the natural ligand adenosine). Alkylation of the OH groups typically increases both the affinity and selectivity.⁷⁶ In a functional A₃ receptor assay, the most potent ligands were also shown to reverse inhibition of adenylyl cyclase, thus demonstrating antagonism.

9.2.3.3 Redox Enzymes

Lipoxygenases (LOX), cyclooxygenases (COXs), and xanthine oxidase (XO) are metalloenzymes whose catalytic cycle involves ROS such as lipid peroxyl radicals, superoxide, and hydrogen peroxide. LOXs and COXs catalyze important steps in the biosynthesis of leukotrienes and prostaglandins from arachidonic acid, which is an important cascade in the development of inflammatory responses. XO catalyzes the ultimate step in purine biosynthesis, the conversion of xanthine into uric acid. XO inhibition is an important issue in the

treatment of gout. Flavonoids may exert part of their antioxidant and anti-inflammatory activities via direct inhibition of LOXs, COXs, and XO. Typically, interpretation of the inhibition studies are complicated because of the possible combination of distinct inhibition mechanisms: formation of noncovalent enzyme-inhibitor complexes, direct scavenging by flavonoid antioxidants of ROS inside or outside the catalytic pocket (with simultaneous oxidation of the flavonoids), chelation of the enzyme metal centers by the flavonoids, and enzyme inactivation by reactive aryloxy radicals, quinones, or quinonoid compounds produced upon flavonoid oxidation that may eventually form covalent adducts with the enzyme.

9.2.3.3.1 *Lipoxygenases and Cyclooxygenases*

Mammalian 15-lipoxygenase 1 (15-LOX1) has been proposed as an endogenous pro-oxidant enzyme capable of oxidizing LDLs, an early event in the development of atherosclerosis.⁷⁷ Hence, its inhibition by flavonoids is a potential mechanism for the prevention of cardiovascular diseases by these antioxidants. Recently, the inhibition by flavonoids of the peroxidation of linoleic acid catalyzed by rabbit reticulocyte 15-LOX1 has been investigated.⁷⁸ Flavone and flavonol aglycones come up as the most potent inhibitors and affect enzyme activity in three distinct ways: prolongation of the initial lag phase during which the accumulation of lipid hydroperoxides is very slow, lowering of the maximal peroxidation rate during the subsequent phase of hydroperoxide accumulation, and inactivation of the enzyme in a third phase due to the combined action of the flavonoid and intermediates of the catalytic cycle. In addition, the inhibition (assessed as IC_{50} values from the concentration dependence of the percentage of peroxidation rate decrease during the second phase) is insensitive to the presence of Fe(III) (an observation ruling out iron-flavonoid chelation as a possible inhibition mechanism) and stronger with flavones and flavonols having a catechol group (a critical determinant of radical scavenging efficiency) either on the A ring or on the B ring (most potent inhibitors with $IC_{50} \sim 1 \mu M$: luteolin, baicalein, fisetin). Overall, a mechanism combining direct inhibition (non-competitively to linoleic acid) and radical scavenging can be proposed. Interestingly, QGlcU, the main circulating quercetin metabolites, retain the ability to inhibit soybean LOX.³⁴ For instance, Q3'GlcU ($K_d = 6.5 \mu M$), Q4'GlcU ($K_d = 8.4 \mu M$), and Q7GlcU ($K_d = 6.0 \mu M$) display an affinity for the enzyme that is only two to three times as low as that of quercetin ($K_d = 2.8 \mu M$), whereas Q3GlcU ($K_d = 60 \mu M$) is a much poorer inhibitor.

The inhibition of the 15-LOX-induced peroxidation of LDL by quercetin and its 3-, 4', and 7-monoglucosides (QGlc) has also been addressed.⁷⁹ Quercetin, Q7Glc, and Q3Glc, which all possess a catechol group in the B ring, inhibit LDL peroxidation with IC_{50} values in the range 0.3 to 0.5 μM and efficiently spare endogenous LDL-bound α -tocopherol. By contrast, Q4'Glc, which lacks a free catechol group, is less effective ($IC_{50} = 1.2 \mu M$) and does not spare α -tocopherol. These results suggest that the inhibition of LDL peroxidation by quercetin and its glucosides mainly proceeds via peroxy radical scavenging and regeneration of α -tocopherol from the corresponding α -tocopheryl radical rather than by direct enzyme inhibition. Interestingly, the percentage of residual flavonol (initial concentration: 1 μM) after 6 h of incubation in the presence of 15-LOX and in the absence of LDL (pH 7.4, 20°C) is $\sim 0, 20, 40,$ and 90% for Q4'Glc, quercetin, Q7Glc, and Q3Glc, respectively. By contrast, in the absence of 15-LOX and in the presence of LDL, only quercetin and Q7Glc are partially consumed (residual flavonol = 60%). This unexpected result shows that 15-LOX can catalyze the autoxidation of quercetin and its monoglucosides in a way that is highly dependent on the site of glucosidation. Finally, in the presence of both 15-LOX and LDL, the flavonols are totally consumed in less than 2 h (with the exception Q3Glc consumed in 4 h) in agreement with an inhibition dominated by redox processes.

Quercetin and a selection of naturally occurring prenylated flavonoids used as anti-inflammatory agents in Chinese medicine were also tested for their ability to inhibit the

synthesis of 12- and 5-hydroxyeicosatetraenoic acids from arachidonic acid catalyzed by 12-LOX (from platelets) and 5-LOX (from polymorphonuclear leucocytes), respectively.⁸⁰ Similarly, quercetin and the prenylated flavonoids were tested for their ability to inhibit the synthesis of thromboxane B2 and prostaglandins E2 and D2 from arachidonic acid catalyzed by COX1 and COX2, respectively. Quercetin appears as a potent inhibitor of 5-LOX ($IC_{50}=0.8 \mu M$), a more modest inhibitor of 12-LOX ($IC_{50}=12 \mu M$) and COX1 ($IC_{50}=8 \mu M$), and a very poor inhibitor of COX2 ($IC_{50}=76 \mu M$). Interestingly, apigenin is inactive toward all four enzymes. In addition, some chalcones and flavanones having a lavandulyl (5-methyl-2-isopropenyl-hex-4-enyl) group at position 8 (A ring) and a 2',4'-dihydroxy substitution on the B ring are selective inhibitors of 5-LOX and COX1, and inactive toward 12-LOX and COX2.

9.2.3.3.2 Xanthine Oxidase

Regarding XO activity and the simultaneous formation of uric acid (from xanthine) and superoxide, flavonoids can act as true enzyme inhibitors (formation of enzyme-inhibitor complexes) thereby quenching both superoxide and uric acid formation or by scavenging superoxide that can be independently recorded using chemiluminescence or colorimetric methods. Hence, it is possible to rank flavonoids in distinct classes⁸¹: superoxide scavengers without inhibitory activity on XO, XO inhibitors without additional superoxide scavenging activity (IC_{50} for uric acid formation $\approx IC_{50}$ for superoxide scavenging), XO inhibitors with an additional superoxide scavenging activity (IC_{50} for uric acid formation $> IC_{50}$ for superoxide scavenging), XO inhibitors with an additional pro-oxidant effect in superoxide production (IC_{50} for uric acid formation $< IC_{50}$ for superoxide scavenging), weak XO inhibitors with an additional pro-oxidant effect in superoxide production, and flavonoids with no effect on XO and superoxide. A planar C ring (flavones, flavonols) seems required for XO inhibition (IC_{50} in the range 0.5 to 10 μM with the exception of 3-hydroxyflavone, which does not interact with XO). Hence, catechins have pure superoxide scavenging activity and do not interact with XO. Only flavonols with a catechol group on the B ring (quercetin, myricetin, fisetin) display additional superoxide scavenging activity. By contrast, some hydroxylated flavones (chrysin, apigenin, luteolin) show an underlying pro-oxidant activity. Interestingly, glycosidation of the flavonoid nucleus generally abolishes XO inhibition. For instance, the IC_{50} values of quercetin for XO inhibition and superoxide scavenging are 2.6 and 1.6 μM , respectively. By contrast, quercetin-3-*O*-rhamnoside (quercitrin) has an IC_{50} of 8.1 μM for superoxide scavenging but no longer inhibits XO ($IC_{50} > 100 \mu M$). Similarly, Q3GlcU and Q7GlcU are very poor XO inhibitors ($K_d \geq 100 \mu M$).³⁴ However, Q3'GlcU ($K_d = 1.4 \mu M$) and Q4'GlcU ($K_d = 0.25 \mu M$) are strong inhibitors of XO, the latter as potent as quercetin itself. Hence, while glucuronidation at the 3' or 4' position suppresses the free catechol moiety of the B ring and thereby most of the radical scavenging activity, the affinity for XO is spared as if flavonol-XO binding took place with marginal participation of the B ring. While epicatechin and its oligomers do not inhibit XO, oligomers of epicatechin-3-*O*-gallate (4 β -8 interflavan linkage) are inhibitors whose potency increases with the number of monomer units ($IC_{50} = 7.2$ to 4.4 μM from dimer to tetramer).⁸² Accordingly, a French maritime pine bark extract (pycnogenol) rich in procyanidins (75% weight, DP 2 to 7) was found to strongly reduce XO activity and retard the electrophoretic mobility of the protein under non-denaturing conditions only.⁸³ In addition, pure low molecular weight components of the extract are without effect. Hence, it can be concluded that XO inhibition proceeds by binding to XO of the high DP procyanidins (DP >3). Moreover, the binding is noncompetitive with respect to the substrate xanthine, abolished by polyethylene glycol or the surfactant Triton X-100 and unaffected by addition of sodium chloride or urea. Hence, it can be proposed that the binding does not take place to the xanthine binding site and primarily

involves dispersion forces and the hydrophobic effect. In the same work, the activities of catalase (from human erythrocyte), horseradish peroxidase, and soybean lipoxygenase were also shown to be inhibited by the pine bark extract. For catalase at least, this may proceed by procyanidin–protein binding since the electrophoretic mobility of catalase under nondenaturing conditions is also decreased by the extract.

Similarly, catechin polymers formed upon horseradish peroxidase-catalyzed oxidation of catechin or polycondensation of catechin with aldehydes prove much more efficient than catechin (at identical monomer concentration) at inhibiting XO and superoxide formation.^{12,13} A more detailed investigation with the catechin–acetaldehyde polycondensate (which is expected to form in wine because of the microbial oxidation of ethanol to acetaldehyde) shows that inhibition is noncompetitive to xanthine and likely occurs via binding to the FAD or Fe/S redox centers involved in electron transfers from the reduced molybdenum center to dioxygen with simultaneous production of superoxide.¹³

9.2.3.3.3 Peroxidases and Tyrosinases

These enzymes have been used to oxidize flavonoids for investigating the reactivity and potential toxicity of their aryloxy radicals (one-electron oxidation) and *o*-quinones (two-electron oxidation). For instance, 4'-hydroxyflavonoids are quickly converted into aryloxy radicals that can oxidize glutathione and NADH with concomitant reduction of dioxygen and ROS formation.^{84–86} This process provides a possible metal-independent mechanism for the pro-oxidant activity of flavonoids. Alternatively, 3',4'-dihydroxyflavonoids are oxidized into the corresponding semiquinone radicals, which quickly disproportionate. The resulting *o*-quinones can then be reduced by NADH (hydride ion transfer) or form conjugates with glutathione without dioxygen activation.^{84,85,87,88} Flavonoid-derived *o*-quinones can eventually react with Cys residues of proteins to form (reversible) covalent adducts. The latter process provides an original mechanism for the inhibition by quercetin of glutathione *S*-transferase P1-1,⁸⁹ an enzyme involved in the defense against electrophiles and in MDR of tumor cells.

9.2.3.3.4 Cytochrome P450

These heme-containing monooxygenases include several isoforms (1A1, 1A2, 1B1, 3A4, etc.) with different tissue distributions and play a key role in the metabolism of endogenous substrates (e.g., steroids) and xenobiotics (food components, drugs, carcinogens, pollutants).⁸ Although the metabolism of xenobiotics by cytochrome P450 (CYP, phase I enzymes) typically results in more hydrophilic compounds that are more readily excreted after eventual conjugation by phase II enzymes (e.g., UDP glucuronyltransferases, sulfotransferases), toxic reactive intermediates, including free radicals, can be formed. Indeed, CYPs are responsible for the conversion of some procarcinogens (e.g., polyaromatic hydrocarbons or PAHs) into carcinogens (e.g., PAH epoxides).

CYP–flavonoid interactions are a good example of the multiple ways flavonoids can affect enzymatic activities, i.e., from the regulation of gene expression to direct binding to the processed enzymes.⁸ Flavonoids can induce, or eventually inhibit, the biosynthesis of CYP 1A1 via interactions with the aryl hydrocarbon receptor (AhR), a cytosolic protein that, once activated by a ligand, translocates to the nucleus and, in association with the AhR translocator, forms a transcription factor for CYP 1A1. For instance, in human breast cancer cells, quercetin binds to AhR as an agonist (in competition with the typical AhR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin) and stimulates gene expression for CYP 1A1 with a parallel increase in CYP 1A1-mediated *O*-deethylation of 7-ethoxyresorufin.⁹⁰ This process is strikingly dependent on the hydroxylation pattern of the B ring since kaempferol (3'-deoxyquercetin) binds AhR as an antagonist (no subsequent activation of CYP 1A1). It is also highly dependent on the cell type since, in hepatic cells, quercetin binds to AhR as an antagonist,

thereby inhibiting gene expression for CYP 1A1 and benzo[*a*]pyrene activation.⁹¹ This provides a possible mechanism for the anticancer activity of quercetin.

Flavonoids, especially flavones and flavonols, also directly bind to several CYP isoforms (1A1, 1A2, 1B1, 3A4) involved in xenobiotics metabolism and inhibit enzyme activity. Structure–activity relationships^{92–95} show rather high isoform selectivities depending on the flavonoid substitution pattern and contrasted inhibition mechanisms. For instance, inhibition by flavonoids of 7-methoxyresorufin *O*-demethylation in microsomes enriched in CYP 1A1 and 1A2 reveals that galangin (3,5,7-trihydroxyflavone) is a mixed inhibitor of CYP 1A2 ($K_i = 8$ nM) and a five times less potent inhibitor of CYP 1A1. By contrast, 7-hydroxyflavone is a competitive inhibitor of CYP 1A1 ($K_i = 15$ nM) and a six times less potent inhibitor of CYP 1A2.⁹⁵ In addition, fairly selective inhibition of CYP 1B1 (specifically detected in cancer cells) by some flavonoids has been reported. For example, 5,7-dihydroxy-4'-methoxyflavone inhibits 1B1, 1A1, and 1A2 with IC_{50} values of 7, 80, and 80 nM, respectively.⁹²

Eventually, flavonoids can be hydroxylated or demethylated by CYPs. For instance, hesperetin (4'-methoxy-3',5,7-trihydroxyflavanone) is specifically demethylated by CYPs 1A1 and 1B1, but not by CYPs 1A2 and 3A4.⁹² In addition, 3,5,7-trihydroxyflavone undergoes sequential CYP 1A1-catalyzed hydroxylation at C4' and C3' to finally yield quercetin.^{96,97} These reactions may be relevant to flavonoid metabolism and cytotoxicity since the corresponding products are more reducing and thus more prone to autoxidation with simultaneous ROS production.

Finally, flavonoids are also able to inhibit CYP19 or aromatase, an enzyme catalyzing a three-step oxidation sequence resulting in aromatization of the A ring of male steroid hormones (androgens) to yield estrogens. Together with flavonoid–estrogen receptor binding, this process could be relevant to the prevention of hormone-dependent cancers by flavonoids. Binding studies^{98,99} show that flavonoids are more efficient inhibitors than isoflavonoids with the A and C rings of the former possibly mimicking the androgen D and C rings, respectively. The simultaneous presence of a 4-keto group (possibly interacting with the heme iron center as an axial ligand in agreement with the observed high spin–low spin transition upon binding) and a 7-OH group is critical for a high affinity. By contrast, hydroxylation at positions 3 and 6 is strongly destabilizing. Flavanones bind almost as tightly as flavones but isoflavones are only poor ligands⁹⁹ ($K_i = 2.6$, 5.1, and 123 μ M for 5,7-dihydroxyflavone, (\pm)-4',5,7-trihydroxyflavanone, and 4',5,7-trihydroxyisoflavone, respectively) in sharp contrast to the structural requirement for strong flavonoid–estrogen receptor binding (see above).

Interestingly, 17 β -hydroxysteroid dehydrogenase, another redox enzyme involved in steroid metabolism, is also strongly inhibited by 7-hydroxyflavonoids.¹⁰⁰ For instance, the flavone apigenin is more potent at inhibiting 17 β -hydroxysteroid dehydrogenase ($IC_{50} = 0.3$ μ M) than aromatase ($IC_{50} = 2.9$ μ M) and the isoflavone genistein, which is only a weak aromatase inhibitor, inhibits 17 β -hydroxysteroid dehydrogenase with an IC_{50} of 1 μ M.

9.2.3.4 Modulation of Antioxidant Properties and Oxidation Pathways by Binding to Proteins

In addition to enzymatic oxidation, flavonoid oxidation can take place via autoxidation (metal-catalyzed oxidation by dioxygen) and ROS scavenging. The former process can be related to flavonoid cytotoxicity (ROS production) while the latter is one of the main antioxidant mechanisms. Both processes may be modulated by flavonoid–protein binding. Although poorly documented so far, these points could be important and, for instance, albumin–flavonoid complexes with an affinity for LDL could act as the true plasma antioxidants participating in the regeneration of α -tocopherol from the α -tocopheryl radical formed

upon scavenging of LDL-bound lipid peroxy radicals. In addition, flavonoid–protein complexation can be expected to provide protection to the protein against oxidative degradation.

In principle, addition of an oxidizing agent to a mixture of flavonoid and protein can cause degradation of both partners. From the influence of the protein on the kinetics of flavonoid oxidation, it can be decided whether the bound flavonoid molecule is still accessible to the oxidizing agent, i.e., whether it is still antioxidant. On the other hand, a reliable procedure for monitoring protein oxidation (with or without cleavage of peptide bonds) is needed to assess the eventual protection of the protein by the flavonoid. For example, inhibition of the enzymatic activity of butyrylcholine esterase, a contaminant of commercially available serum albumin, is a sensitive marker of protein degradation by peroxy radicals.¹⁰¹

The influence of serum albumin on quercetin oxidation has been investigated with different one-electron or two-electron oxidizing agents: the peroxy radicals formed by thermal decomposition of diazo compound AAPH in the presence of dioxygen, sodium periodate, and potassium nitrosodisulfonate. Rather unexpectedly, quercetin–BSA binding does not affect the rate of quercetin oxidation by periodate¹⁰² and even accelerates the rate of quercetin oxidation by nitrosodisulfonate (Dufour et al., unpublished results). Hence, SA-bound quercetin remains fully accessible to these small oxidizing agents, thus suggesting that it retains its antioxidant activity. In the first step, the quercetin *o*-quinone (in fast equilibrium with a *p*-quinone methide form) that is barely detectable in the absence of BSA because of subsequent fast water addition, is strongly stabilized, a likely consequence of ligand–protein charge transfer interactions and a low local water concentration. Serum albumin was also demonstrated to protect the quercetin *p*-quinone methide–water adduct from further degradation leading to 3,4-dihydroxybenzoic acid and 2-oxo-2-(2,4,6-trihydroxyphenyl)acetic acid. Investigations with other flavonoids confirm that albumin only weakly affects the kinetics of flavonoid oxidation while leaving the product distribution essentially unchanged. Unlike quercetin and kaempferol, whose oxidation leads to C ring cleavage, oxidation of luteolin, isoquercitrin, and catechin, either in their free or BSA-bound form, preferentially leads to dimers.

Under conditions where the protein is not oxidatively degraded, its influence on the radical scavenging activity of flavonoids can be more readily assessed. For example, whereas the reaction of BSA and gelatin with the ABTS radical cation is negligible, sorghum procyanidin (15 to 17 flavanol units) can scavenge up to six ABTS radicals per monomer at pH 7.4.¹⁰³ Of the two proteins, only gelatin slightly affects the kinetics of radical scavenging. However, the overall stoichiometry remains unaffected. At pH 4.9, the inhibition of ABTS scavenging by both proteins, especially gelatin, is somehow stronger but tends to vanish with time. In the case of BSA at least, these observations could simply point to negligible protein–flavonoid binding. However, covalent coupling between the proteins and procyanidin quinones may take place during radical scavenging. Indeed, although the amount of procyanidin–protein precipitate is not affected by ABTS, procyanidin oxidation by ABTS leads to precipitates that cannot be resolubilized. Using the ABTS scavenging test, it was also possible to demonstrate that quercetin and human plasma exert nonadditive antioxidant activities,¹⁰⁴ thus suggesting that binding of quercetin to plasma proteins masks part of the electron-donating activity of quercetin. The masking effect decreases in the series quercetin > rutin > catechin. The same trend emerges when plasma is replaced by serum albumin. Interestingly, the binding affinity to serum albumin^{42,43} parallels the masking effect. However, it must be noted that ABTS is much bulkier than ROS. Hence, its scavenging by polyphenols is expected to be especially sensitive to steric hindrance brought by the protein environment.

Since lipid peroxidation is clearly related to the onset of atherosclerosis and the impairment of membrane functions, the influence of proteins on the ability of flavonoids to inhibit

lipid peroxidation deserves examination. Such investigations have been carried out with BSA and lecithin liposomes.¹⁰⁵ Whereas BSA alone already slows down the formation of lipid hydroperoxides and hexanal, its influence on the antiperoxidizing activity of the selected polyphenols is highly dependent on the polyphenolic structure. Hence, BSA lowers the inhibition of hydroperoxide formation by catechins and caffeic acid, enhances inhibition by malvidin and rutin, and leaves essentially unchanged inhibition by quercetin. No clear interpretation based on polyphenol-BSA binding can be given.

The influence of a protein environment on the antioxidant activity of flavonoids can be readily evaluated by monitoring the inhibition by flavonoids of the peroxidation of HSA-bound linoleic acid in plasma-mimicking conditions.¹⁰⁶ The AAPH-initiated peroxidation of linoleic acid leads to four isomeric hydroperoxides that further react to form the corresponding ketodienes and alcohols. As expected, the formation of the lipid peroxidation products is inhibited more efficiently by 3',4'-dihydroxyflavonoids (quercetin, quercetin-3- β -D-glucoside > catechin) than by flavonoids having a monohydroxylated B ring (kaempferol, 3'-*O*-methylquercetin, quercetin-3,4'- β -D-diglucoside). More importantly, the strong binding of quercetin to HSA (noncompetitively to linoleic acid) does not alter its antiperoxidizing activity. In contrast, α -tocopherol, although much more potent than flavonoids in the absence of HSA, and ascorbate are only weakly active. Thus, in plasma, the flavonol-albumin complex could be regarded as an antioxidant species with the flavonol molecule efficiently trapping the peroxy radicals derived from AAPH and eventually from the lipid. Similarly, HSA-bound quercetin efficiently protects the enzyme butyrylcholine esterase (a typical contaminant of commercially available HSA) from oxidative damage by AAPH-derived peroxy radicals.¹⁰¹

Oxidation of catechols in the presence of a protein may lead to extensive catechol-protein covalent coupling (Figure 9.4) as demonstrated in the case of the chlorogenic acid-BSA couple.¹⁰⁷ Autoxidation of EGCG at pH 4.9 in the presence of Zn(II) cations was shown to generate semiquinone radicals (stabilized by Zn(II) binding) mainly on the B ring moiety.¹⁰⁸ In the presence of BSA, EGCG autoxidation is accompanied by irreversible protein precipitation suggesting covalent EGCG-BSA coupling that probably involves EGCG *o*-quinones in fast disproportionation equilibrium with the semiquinone radicals. Finally, incubation of Hep G2 and Caco-2 cells with [¹⁴C]quercetin results in quercetin-protein covalent coupling (as much as 10% of the total cellular content of quercetin in the case of Caco-2 cells).¹⁰⁹ The process is insensitive to the presence of an excess ascorbate, which rules out significant autoxidation of quercetin in the buffer or cell culture medium. Hence, quercetin oxidation could involve ROS within the cells. The quercetin-derived quinone or quinone methide intermediates thus formed are then proposed to add to specific cell proteins (MW ~55–80 kDa in the case of Hep G2 cells), the major cell proteins remaining unaltered.

More generally, one-electron oxidation of protein-bound phenols to form reactive aryloxy radicals is a possible pro-oxidant mechanism since these radicals can propagate H-atom or electron transfers within the protein. In addition to phenol-protein covalent coupling, these phenol-mediated oxidative damages to proteins could be detrimental to their function as enzymes, receptors, and membrane transporters. For instance, investigations by capillary electrophoresis have shown that quercetin in concentrations lower than 25 μ M potentiates HSA degradation by AAPH-derived peroxy radicals.¹¹⁰

9.3 CONCLUSION AND PERSPECTIVES

Flavonoids, as food components or potential drugs, interact with a wide range of proteins by distinct mechanisms: weak and rather unspecific binding of tannins to proline-rich or histidine-rich random coils leading to protein precipitation, specific enzyme inhibition, and

ligand–receptor interactions mostly involving flavonoid aglycones with an unsaturated C ring, binding to transport proteins. In addition, flavonoid–protein interactions can modulate the redox properties of flavonoids that underline their antioxidant, and eventually their pro-oxidant, activity. After electrophilic activation by one-electron or two-electron oxidation, flavonoids can also form covalent bonds with proteins.

Whether these binding processes are relevant to human health is not clearly demonstrated yet. However, one of the most promising perspectives is the participation of flavonoids in the regulation of gene expression, possibly by direct interactions with specific receptors and nuclear factors. For example, quercetin has been shown to increase the intracellular glutathione level by activating the promoter of the catalytic subunit of γ -glutamylcysteine synthetase.¹¹¹ This effect is fairly specific since myricetin (5'-hydroxyquercetin) and two quercetin 3-glycosides are inactive. As a possible molecular mechanism, quercetin could help release specific nuclear factors (from inert cytosolic complexes), thereby facilitating their translocation into the nucleus. On the other hand, some flavones, isoflavones, and flavonols are also known to activate peroxisome proliferator-activated receptor- γ (PPAR- γ), thus leading to suppression of inducible COX-2 and NO synthase in mouse macrophages.¹¹² A likely mechanism consists in allosteric binding of the flavonoids to PPAR- γ and subsequent modification of the receptor conformation.

In conclusion, spectacular advances in the fields of flavonoid bioavailability and flavonoid-mediated cell effects in relation to the development of new biological tools (e.g., proteomic analysis, reporter genes) have been achieved during the last decade. A more coherent picture of the ways flavonoids combine their redox properties and affinity to specific proteins is emerging. This wealth of new chemical and biological information suggests that the elucidation of *in vivo* molecular mechanisms and receptors involved in flavonoid health effects is at hand.

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10 The Anthocyanins

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10.1 INTRODUCTION

The anthocyanins constitute a major flavonoid group that is responsible for cyanic colors ranging from salmon pink through red and violet to dark blue of most flowers, fruits, and leaves of angiosperms. They are sometimes present in other plant tissues such as roots, tubers, stems, bulbils, and are also found in various gymnosperms, ferns, and some bryophytes. As described below, the past decade has witnessed a renaissance in research activities on and general interests in these water-soluble pigments in several areas. When searching Chemical Abstracts/Medline for the word anthocyanin, the number of articles obtained was 790 in 2003 compared to 257 in 1993.

This chapter follows on from those of the four previous editions of *The Flavonoids*,¹⁻⁴ and the three review articles of Harborne and Williams.⁵⁻⁷ It will confine its attention largely to a detailed account on anthocyanin structures reported after 1992 (Section 10.2 and Table 10.2). Special effort has been made to present a comprehensive overview of all the various anthocyanins with complete structures in the literature (Section 10.2 and Appendix A). Many anthocyanins reported in checklists of previous reviews^{3,8} have been excluded from Appendix A mainly because of the lack of experimental proofs for proper determination of the linkage point(s) between one or more of the glycosidic units involved. For instance, after careful considerations of the data used as evidence for determination of the linkage position of the monosaccharides in the different anthocyanidin 5-monoglycosides presented in the various reports in the literature, we have excluded these anthocyanins apart from the deoxyanthocyanidin 5-glycosides from Appendix A.

Anthocyanin production by cell cultures (Section 10.3.1) and synthesis (Section 10.3.2), and anthocyanin localization in plant cells (Section 10.4) have been treated separately due to important progress in these fields in recent years. Motivated by the many reports on anthocyanins from various sources in the review period (Table 10.2), some chemotaxomic considerations have been included in Section 10.5. This chapter has not dealt with other articles than those written in the English or German language. Thus, this review has most probably not given the right credit, in particular, to the Japanese research groups of Tadeo Kondo and Kumi Yoshida; Norio Sait, Fumi Tatsuzawa, and Toshio Honda; and Norihiko Terahara.

10.1.1 ANTHOCYANIN STRUCTURES

The total number of different anthocyanins reported to be isolated from plants in this review is 539 (Appendix A). This number includes 277 anthocyanins that have been identified later than 1992. Several previously reported anthocyanins have for the first time received complete structural elucidation, and some structures have been revised. The majority of anthocyanins with the most complex structures and highest molecular masses have been reported in the period of this review.

Two classes of dimeric anthocyanins isolated from plants (section 10.2.6) have been identified in plants for the first time. One class includes pigments where an anthocyanin and a flavone or flavonol are linked to each end of a dicarboxylic acyl unit.⁹⁻¹² The other class includes four different catechins linked covalently to pelargonidin 3-glucoside.¹³ During the last decade, seven new desoxyanthocyanidins and a novel type of anthocyanidin called pyranoanthocyanidins have been reported (Section 10.2.2). Toward the end of the 20th century, several color-stable 4-substituted anthocyanins, pyranoanthocyanins, were discovered in small amounts in red wine and grape pomace.¹⁴⁻¹⁶ Recently, similar compounds have been isolated from extracts of petals of *Rosa hybrida* cv. "M'me Violet,"¹⁷ scales of red onion,¹⁸ and strawberries.^{13,19} About 94% of the new anthocyanins in the period of this review are based on only six anthocyanidins (Table 10.2).

The first natural C-glycosylanthocyanin has recently been isolated from flowers of *Tricyrtis formosana*.²⁰ No new monosaccharide units have been identified in anthocyanins during the last decade; however, two new disaccharides^{21–23} and one new trisaccharide^{24,25} have been reported connected to anthocyanidins (Section 10.2.4). The two anthocyanins from blue flowers of *Nymphaea caerulea*²⁶ and the two minor anthocyanins from red onions¹⁸ are, with the exception of the desoxyanthocyanins, the only anthocyanins without a sugar in the 3-positions. Among the new anthocyanins reported after 1992, around 88% contain acyl group(s). The acyl groups, *E*-3,5-dihydroxycinnamoyl in a triacylated-tetraglycosylated cyanidin derivative from *Ipomoea asarifolia*,²⁷ and tartaryl in four anthocyanins isolated from flowers of *Anemone coronaria*,^{28,29} have, for the first time, been identified as part of an anthocyanins (Section 10.2.5). The first anthocyanins found conjugated with sulfate, malvidin 3-glucoside-5-[2-(sulfato)glucoside] and malvidin 3-glucoside-5-[2-(sulfato)-6-(malonyl)glucoside], have been isolated from violet flowers of *Babiana stricta*.³⁰ Six novel anthocyanins made in transgenic plants^{31,32} and four novel anthocyanins produced in plant cell cultures^{33–36} have been included in Appendix A. Some interesting research on the complex metalloanthocyanins is outlined in Section 10.2.6.

10.1.2 NUTRITIONAL SUPPLEMENTS — HEALTH ASPECTS

There has been an explosive interest in anthocyanins as potential nutritional supplements for humans. Regular consumption of anthocyanins and other polyphenols in fruits, vegetables, wines, jams, and preserves is associated with probable reduced risks of chronic diseases such as cancer, cardiovascular diseases, virus inhibition, Alzheimer's disease. Anthocyanins and other flavonoids are regarded as important nutraceuticals mainly due to their antioxidant effects, which give them a potential role in prevention of the various diseases associated with oxidative stress. However, flavonoids have further been recognized to modulate the activity of a wide range of enzymes and cell receptors.³⁷ In spite of the voluminous literature available, Western medicine has, however, not yet used flavonoids therapeutically, even though their safety record is exceptional. Aspects related to the impact of flavonoids on human health are presented in Chapter 6. The literature on the occurrence of anthocyanins and other flavonoids in foods, their possible dietary effects, bioavailability, metabolism, pharmacokinetic data, and safety has recently been reviewed by several authors.^{33–51} The current knowledge on various molecular evidences of cancer chemoprevention by anthocyanins has been summarized by Hou.⁵² He divided the mechanisms into antioxidation, the molecular mechanisms involved in anticarcinogenesis, and the molecular mechanisms involved in the apoptosis induction of tumor cells.

10.1.3 FOOD COLORANTS

There is a worldwide interest in further use of food colorants from natural sources as a consequence of perceived consumer preferences as well as legislative action in connection with synthetic dyes. Several excellent overviews of the common anthocyanin food dyes, quantitative and qualitative aspects of anthocyanins used in food products, and physicochemical properties of anthocyanins (color characteristics and stability) have been presented in the period of this review.^{53–57} An impressive compilation of the anthocyanin content of a variety of fruits, vegetables, and grains has been published by Mazza and Miniati.⁵⁸ The flavonoid composition of foods is treated in Chapter 4. Different types of anthocyanin-derived pigments, including the pyranoanthocyanins originating by cycloaddition of diverse compounds at C-4 and the 5-hydroxyl of anthocyanidins, and compounds resulting from the condensation between anthocyanins and flavanols, either direct or mediated by acetaldehyde or other compounds, are generated in wine during storage.^{59–61} This has led to enlightenment of the color changes that take place in red wine (Chapter 5).

10.1.4 MOLECULAR BIOLOGY, BIOSYNTHESIS, AND FUNCTIONS

Advances in molecular biology, coupled with improved knowledge of anthocyanin biosynthesis, have led to increased interests in cultivars and plant mutants with new colors and shapes. A general overview of the biosynthetic pathway leading to flavonoids and recent advances in the molecular biology and biotechnology of flavonoid biosynthesis is presented in Chapter 3. Several reviews in the field of anthocyanins have recently been reported. Springob et al.⁶² covered the biochemistry, molecular biology, and regulation of anthocyanin biosynthesis, with particular emphasis on mechanistic features and late steps of anthocyanin biosynthesis, including glycosylation and vacuolar sequestration. Irani et al.⁶³ focused on molecular mechanisms of the regulation of anthocyanin biosynthesis, and the factors that influence the pigmentation properties of anthocyanins, while Ben-Meir et al.⁶⁴ outlined the biochemistry and genetics of the pathway leading to anthocyanin production, and provide an overview on the application of the generated knowledge toward molecular breeding of ornamentals. Other related excellent reviews of Forkmann and coworkers have covered classical versus molecular breeding of ornamentals,⁶⁵ metabolic engineering and applications of flavonoids,⁶⁶ and biosynthesis of flavonoids.⁶⁷

Anthocyanic coloration plays a vital role in the attraction of insects and birds, leading to pollination and seed dispersal, but their appearance in young leaves and seedlings is often transient. There is increasing evidence that anthocyanins, particularly when they are located at the upper surface of the leaf or in the epidermal cells, have a role to play in the physiological survival of plants. It has been outlined that foliar anthocyanins accumulate in young, expanding foliage, in autumnal foliage of deciduous species, in response to nutrient deficiency, temperature changes, or ultraviolet (UV) radiation exposure, and in association with damage or defense against browsing herbivores or pathogenic fungal infection.^{42,68–70} The functions of anthocyanins have in this context mainly been hypothesized as a compatible solute contributing to osmotic adjustment to drought and frost stress, an antioxidant, and a UV and visible light protectant. The flavonoid functions in plants are treated in Chapter 7.

10.1.5 ANALYTICAL METHODS AND INSTRUMENTATION

Continual improvements in methods and instrumentation (e.g., high-performance liquid chromatography [HPLC], liquid chromatography–mass spectrometry [LC–MS], and nuclear magnetic resonance [NMR] spectroscopy) used for separation and structural elucidation of anthocyanins (see Chapters 1 and 2) have made it easier to use smaller quantities of material, and to achieve results at increasing levels of precision. New anthocyanins regularly turn up in plant sources that already have been well investigated before (Table 10.2). Most anthocyanins show instability toward a variety of chemical and physical parameters, including oxygen, high temperatures, and most pH values.⁴⁹ The various anthocyanins have similar structures and may occur in complex mixtures, which makes them rather difficult to isolate. A routine analysis may involve just one HPLC injection (5 μ l) of a crude extract of dried petal (0.5 mg). However, a typical structural elucidation of a novel anthocyanin may demand more plant material (above 100 g) subjected to extraction with acidified alcoholic solvent, followed by purification and separation using various chromatographic techniques before structural elucidation by spectroscopy and sometimes chemical degradation.^{50,51} Recent MS and two-dimensional (2D) NMR techniques have, in particular, become important for the determination of many anthocyanin linkage positions and identification of aliphatic acyl groups. Analytical methods for extraction, separation, and characterization of anthocyanins have been treated in a number of recent reviews.^{72–79}

10.2 ANTHOCYANIN CHEMISTRY

10.2.1 GENERAL ASPECTS AND NOMENCLATURE

The anthocyanins consist of an aglycone (anthocyanidin), sugar(s), and, in many cases, acyl group(s). The anthocyanidins are derivatives of 2-phenylbenzopyrylium (flavylium cation) (Table 10.1). The numbering of the left structure in Table 10.1 is used for most anthocyanins. The pyranoanthocyanins are based on the skeleton represented by the structure on the right in Table 10.1. A more systematic name, e.g., 5-carboxypyranopelargonidin, can be 5-carboxy-2-(4-hydroxyphenyl)-3,8-dihydroxy-pyrano[4,3,2-*de*]-1-benzopyrylium. When a given anthocyanin is dissolved in water, a series of secondary structures are formed from the flavylium cation according to different acid–base, hydration, and tautomeric reactions.⁸⁰

While 31 monomeric anthocyanidins (Table 10.1) have been properly identified, around 90% of all anthocyanins (Appendix A) are based on only six anthocyanidins, pelargonidin (Pg), cyanidin (Cy), peonidin (Pn), delphinidin (Dp), petunidin (Pt), and malvidin (Mv). Among the 539 anthocyanins or anthocyanidins that have been identified, 97% are glycosidated (Figure 10.1). The 3-desoxyanthocyanidins, sphagnorubins and rosacyanin B (Table 10.1, Figure 10.3), are the only anthocyanidins found in their nonglycosidated form in plants. Nearly all reports on anthocyanins specifying the D or L configuration of the anthocyanin sugar moieties (monosaccharides), lack experimental evidence for this type of assignments.

10.2.2 ANTHOCYANIDINS

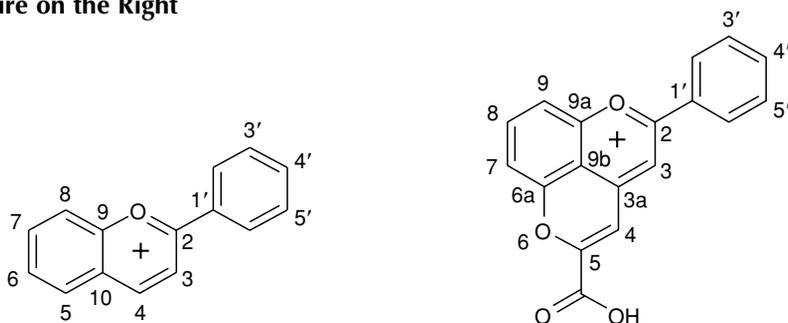
In addition to the 18 anthocyanidins listed previously,⁴ Table 10.1 contains seven new desoxyanthocyanidins and a novel type of anthocyanidin called pyranoanthocyanidins. While 31 monomeric anthocyanidins have been properly identified, most of the anthocyanins are based on cyanidin (30%), delphinidin (22%), and pelargonidin (18%), respectively (Figure 10.2). Altogether 20% of the anthocyanins are based on the three common anthocyanidins (peonidin, malvidin, and petunidin) that are methylated. Around 3, 3, and 2% of the anthocyanins or anthocyanidins are labeled as 3-desoxyanthocyanidins, rare methylated anthocyanidins, and 6-hydroxyanthocyanidins, respectively.

In bryophytes, anthocyanins are usually based on 3-desoxyanthocyanidins located in the cell wall. A new anthocyanidin, riccionidin A (Figure 10.3), has been isolated from the liverwort *Ricciocarpos natans*.⁸¹ It could be derived from 6,7,2',4',6'-pentahydroxyflavylium, having undergone ring closure of the 6'-hydroxyl at the 3-position. Its visible spectrum in methanolic HCl is at 494 nm. This pigment was accompanied by riccionidin B, which most probably is based on two molecules of riccionidin A linked via the 3'- or 5'-positions. Both pigments were also detected in the liverworts *Marchantia polymorpha*, *Riccia duplex*, and *Scapania undulata*.⁸¹ Somewhat, unexpectedly, riccionidin A has also been isolated from adventitious root cultures of *Rhus javanica* (Anacardiaceae).⁸²

A new 3-desoxyanthocyanidin, 7-*O*-methylapigeninidin, has been isolated in low concentration from grains and leaf sheaths of *Sorghum caudatum*.⁸³ Its UV–vis spectrum recorded in methanol with 0.1% HCl showed absorption maxima at 278.6 and 476.4 nm. The secondary-ion MS spectrum showed a strong [M]⁺ ion at *m/z* 269, consistent with the C₁₆H₁₃O₄ molecular formula. The ¹H and ¹³C NMR spectral data were closely related to those of apigeninidin, except for a singlet at 4.08 ppm (aromatic *O*-methyl group). This methyl group was located at C-7 after observation of DIFFNOEs between the methyl protons and both the H-8 and H-6 protons. The pigment was found in low concentration both in grains and in leaf sheaths. A similar 3-desoxyanthocyanidin has been detected in grains of *Sorghum bicolor* after incubation with the fungus *Colletotrichum sublineolum*.⁸⁴ In addition to plasma desorption MS data, bathochromic shift analyses indicated that the structure of the compound was

TABLE 10.1

The Structures of Naturally Occurring Anthocyanidin.^a The Numbering of the Structure on the Left is Used for all Anthocyanins; the Numbering for the Pyranoanthocyanins is Given in the Structure on the Right



Substitution Pattern

	3	5 (6a) ^b	6 (7) ^b	7 (8) ^b	3'	4'	5'
<i>Common anthocyanidins</i>							
Pelargonidin (Pg)	OH	OH	H	OH	H	OH	H
Cyanidin (Cy)	OH	OH	H	OH	H	OH	H
Delphinidin (Dp)	OH	OH	H	OH	OH	OH	OH
Peonidin (Pn)	OH	OH	H	OH	OMe	OH	H
Petunidin (Pt)	OH	OH	H	OH	OMe	OH	OH
Malvidin (Mv)	OH	OH	H	OH	OMe	OH	OMe
<i>Rare methylated anthocyanidins</i>							
5-MethylCy	OH	OMe	H	OH	OH	OH	H
7-MethylPn (rosinidin)	OH	OH	H	OMe	OMe	OH	H
5-MethylDp (pulchellidin)	OH	OMe	H	OH	OH	OH	OH
5-MethylPt (europinidin)	OH	OMe	H	OH	OMe	OH	OH
5-MethylMv (capensinidin)	OH	OMe	H	OH	OMe	OH	OMe
7-MethylMv (hirsutidin)	OH	OH	H	OMe	OMe	OH	OMe
<i>6-Hydroxylated anthocyanidins</i>							
6-HydroxyPg	OH	OH	OH	OH	H	OH	H
6-HydroxyCy	OH	OH	OH	OH	OH	OH	H
6-HydroxyDp	OH	OH	OH	OH	OH	OH	OH
<i>3-Desoxyanthocyanidins</i>							
Apigeninidin (Ap)	H	OH	H	OH	H	OH	H
Luteolinidin (Lt)	H	OH	H	OH	OH	OH	H
Tricetinidin (Tr)	H	OH	H	OH	OH	OH	OH
7-MethylAp ^c	H	OH	H	OMe	H	OH	H
5-MethylLt ^c	H	OMe	H	OH	OH	OH	H
5-Methyl-6-hydroxyAp (carajurone) ^c	H	OMe	OH	OH	H	OH	H
5,4'-Dimethyl-6-hydroxyAp (carajurin)	H	OMe	OH	OH	H	OMe	H
5-Methyl-6-hydroxyLt ^c	H	OMe	OH	OH	OH	OH	H
5,4'-Dimethyl-6-hydroxyLt ^c	OH	OMe	OH	OH	OH	OMe	H
Riccionidin A ^{c,d}	OH	H	OH	OH	H	OH	H

TABLE 10.1

The Structures of Naturally Occurring Anthocyanidins.^a The Numbering of the Structure on the Left is Used for all Anthocyanins; the Numbering for the Pyranoanthocyanins is Given in the Structure on the Right — *continued*

	Substitution Pattern						
	3	5 (6a) ^b	6 (7) ^b	7 (8) ^b	3'	4'	5'
<i>Pyranoanthocyanidins</i>							
5-Carboxypyranopg ^c	OH	O-	H	OH	H	OH	H
5-Carboxypyranocy ^{c,e}	OH	O-	H	OH	OH	OH	H

^aSphagnorubins A–C from peat moss, *Sphagnum*, have not been included (Figure 10.3).

^bThe numbers in parentheses correspond to the pyranoanthocyanidins.

^cNew anthocyanidins (reported between 1992 and 2004).

^dRing closure on the basis of ether linkage between the 3- and 6'-positions. Riccionidin A and its dimer, riccionidin B, have an additional OH-group in the 2'-position (Figure 10.3).

^eRosacyanin B (Figure 10.3).

consistent with that of 5-*O*-methylluteolinidin. The spectrum of this phytoalexin, which showed greater fungitoxicity than luteolinidin, revealed an absorption maximum at 495 nm.

Although a previous synthesis of the desoxyanthocyanidin carajurin, 6,7-dihydroxy-5,4'-dimethoxy-flavylium (isolated from leaves of *Arrabidaea chica*), was published in 1953,⁸⁵

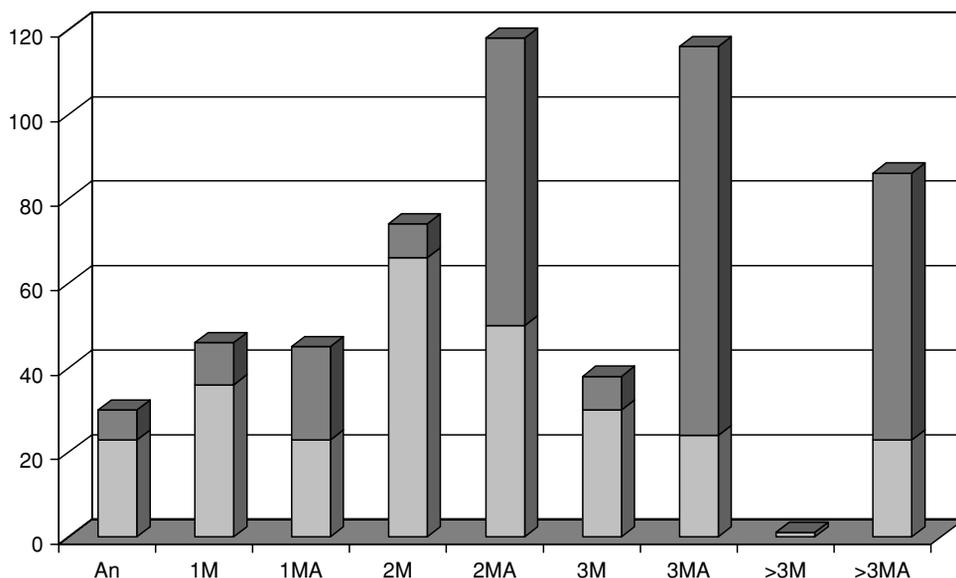


FIGURE 10.1 Anthocyanidins (An) and anthocyanins grouped according to their content of monosaccharide units and acylation. The vertical axis represents the number of pigments. The upper dark part of each bar represents the anthocyanins reported later than 1992. 1M, one monosaccharide unit; 1MA, one monosaccharide unit plus acylation; 2M, two monosaccharide units; 2MA, two monosaccharide units plus acylation; 3M, three monosaccharide units; 3MA, three monosaccharide units plus acylation; >3M, more than three monosaccharide units; >3MA, more than three monosaccharide units plus acylation.

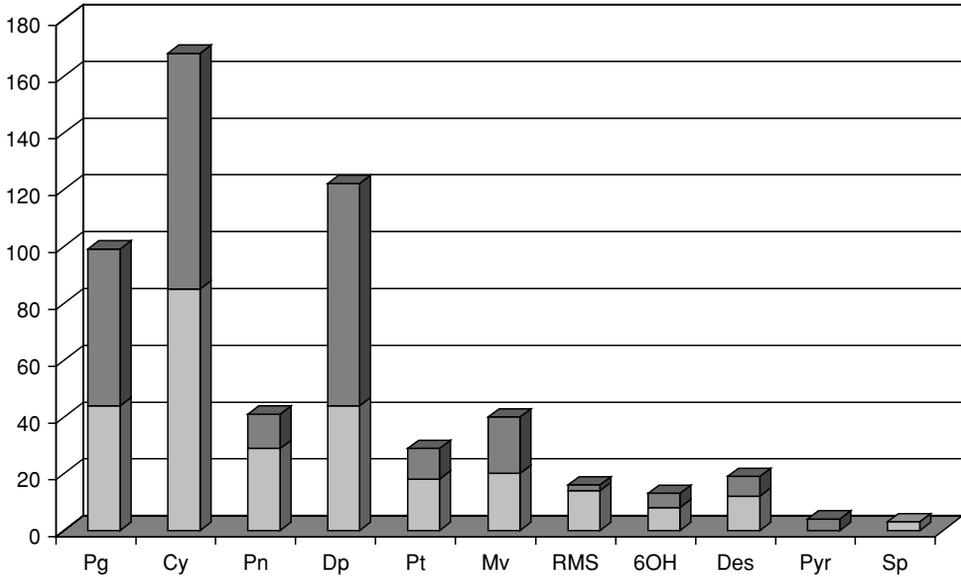


FIGURE 10.2 The number of anthocyanins based on the various anthocyanidins. The upper dark part of each bar represents the anthocyanins reported later than 1992. Pg, pelargonidin; Cy, cyanidin; Pn, peonidin, Dp, delphinidin; Pt, petunidin; Mv, malvidin; RMS, rare methylated structures; 6OH, 6-hydroxy-; Des, desoxy-; Pyr, pyrano-; Sp, sphagnorubins. See Table 10.1 for structures.

some authors have considered the structure of this pigment to be only partially described.^{1,58} More recently, two groups^{86,87} have nearly simultaneously confirmed the structure of carajurin — even with a crystal structure.⁸⁷ The structure of carajurone was also revised to be 6,7,4'-trihydroxy-5-methoxy-flavylium.⁸⁶ Additionally, two new 3-desoxyanthocyanidins,

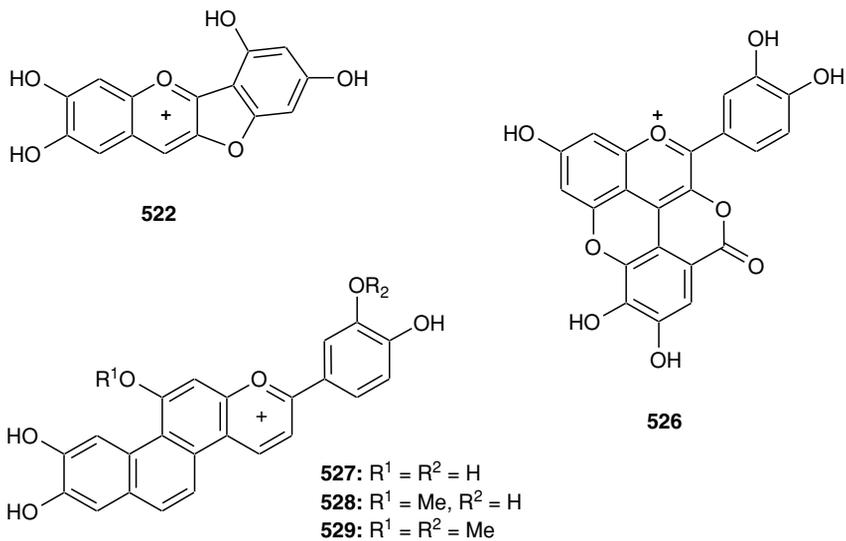


FIGURE 10.3 Some anthocyanidins with unusual structures; **522**, riccionidin A; **526**, rosacyanin B; **527–529**, sphagnorubins A–C.

6,7,3'-trihydroxy-5,4'-dimethoxy-flavylium⁸⁶ and 6,7,3',4'-tetrahydroxy-5-methoxy-flavylium,^{86,87} were isolated from these leaves, which are traditionally used by some indigenous populations of South America for body painting and for dyeing fibers.

In recent years, several color-stable 4-substituted anthocyanins have been discovered in small amounts in red wine and grape pomace (see Chapter 5). Vitisin A and acetylvitisin A were identified as the 3-glucoside and the 3-acetylglucoside of malvidin containing an additional C₃H₂O₂ unit linking the C-4 and the C-5 hydroxyl group. Vitisin B and acetylvitisin B were identified as analogous pigments having a CH=CH moiety instead of the C₃H₂O₂ unit.¹⁵ The suggested structure for carboxypyranomalvidin (vitisidin A) was later slightly revised by Fulcrand et al.,¹⁶ who proved that the C₃H₂O₂ unit was part of a pyran ring having a free acid group. They suggested that vitisin A was formed by cycloaddition of pyruvic acid involving both C-4 and the hydroxyl at C-5 of malvidin. Four reported methylpyranoanthocyanins isolated from blackcurrant seeds⁸⁸ were later shown to be the oxidative cycloaddition products of the extraction solvent (acetone) and the natural anthocyanins.⁸⁹ Pyranoanthocyanidins generated from the respective glycosides after hydrolysis were found to undergo rearrangement to form a new type of furoanthocyanidins.⁹⁰ Recently, four new pyranoanthocyanins, namely pyranocyanin C and D and pyranodelphinidin C and D, were isolated by the same group from an extract of blackcurrant seeds.⁹¹ These pigments were absent in fresh extracts, and their levels increased gradually with time. Their formation was likely to be from the reaction of the anthocyanins and *p*-coumaric acid in the extracts.

The first pyranoanthocyanidin isolated from intact plants received the trivial name rosacyanin B (Figure 10.3).¹⁷ This violet pigment was isolated in small amounts from the petals of *Rosa hybrida* cv. "M'me Violet." Its structure was revealed mainly by high resolution fast atom bombardment MS and NMR (1D and 2D). Rosacyanin B is very stable in acidic alcoholic solutions; however, under neutral or weakly acidic aqueous conditions it is precipitated before forming the colorless pseudobase. This anthocyanidin contains no sugar units. Recently, four anthocyanins with the same aglycone, 5-carboxypyranocyanidin, have been isolated from acidified, methanolic extracts of the edible scales, as well as from the dry outer scales of red onion (*Allium cepa*).¹⁸ Two of the structures were elucidated by 2D NMR spectroscopy and LC-MS as the 3-glucoside and 3-[6-(malonyl)glucoside] (**525**) of this 4-substituted aglycone. The two analog pigments methylated at either the terminal carboxyl group of the acyl moiety or at the aglycone carboxyl were most probably formed by esterification of **525** with the solvent (acidified methanol) during the isolation process. Another 3-glucoside (**523**) with the new 4-substituted aglycone, 5-carboxypyranopelargonidin, has been isolated in small amounts from the acidified, methanolic extract of strawberries (*Fragaria × ananassa*).¹⁹ By comparison of UV-vis absorption spectra, **523** showed in contrast to ordinary pelargonidin 3-glucoside (**5**) a local absorption peak around 360 nm, a hypsochromic shift (8 nm) of the visible absorption maximum, and lack of a distinct UV absorption peak around 280 nm. The similarities between the absorption spectra of **523** in various acidic and neutral buffer solutions implied restricted formation of the instable colorless equilibrium forms, which are typical for most anthocyanins in weakly acidic solutions. The molar absorptivity of **523** varied little with pH contrary to similar values of, for instance, **5**.

Each anthocyanidin is involved in a series of equilibria giving rise to different forms, which exhibit their own properties including color.⁸⁰ One- and two-dimensional NMR have been used to characterize the various forms of malvidin 3,5-diglucoside present in aqueous solution in the pH range 0.3 to 4.5 and to determine their molar fractions as a function of pH.⁹² In addition to the flavylium cation, two hemiacetal forms and both the *cis* and *trans* forms of chalcone were firmly identified. In a reexamination, the intricate pH-dependent set of chemical reactions involving synthetic flavylium compounds (e.g., 4'-hydroxyflavylium) was confirmed to be basically identical to those of natural anthocyanins (e.g., malvidin 3,5-diglucoside) in

acidic and neutral media.⁹⁴ For each process, a kinetic expression was deduced allowing calculation of all the equilibrium constants and most of the rate constants in the system. In recent years, Pina et al. have performed a systematic investigation of the photochemical and thermal reactions of synthetic flavylum compounds.⁹⁴ They have shown that 4',7-dihydroxyflavylium (AH⁺) in a water–ionic liquid biphasic system can be used as a write–read–erase system. In acid media, the *trans*-chalcone form is soluble in ionic liquids and is thermally metastable, but reacts photochemically (write) to give the yellow flavylium salt, which can be optically read without being erased. The system is prepared for a new cycle by two consecutive pH “jumps.”⁹⁵ These results are very interesting since the flavylium compounds represent examples of multistate or multifunctional chemical systems that may be used for information processing at the molecular level according to principles similar to those that govern information transfer in living organisms. In particular, flavylium compounds can behave as optical memories and logic gates systems: a write–read–erase molecular switch.

10.2.3 ANTHOCYANINS NOT BASED ON THE COMMON ANTHOCYANIDINS

Although most new anthocyanins discovered during the last decade have been based on the six common anthocyanidins (Figure 10.2), some rare exceptions with limited distribution have been reported. The major 3-deoxyanthocyanin isolated from the fern *Blechnum novae-zelandiae* was determined to be luteolinidin 5-[3-(glucosyl)-2-(acetyl)glucoside] by HPLC, NMR (1D, 2D), and electrospray MS.⁹⁶ The new 3-[6-(*p*-coumaryl)glucoside] and 3-glucoside of hirsutidin together with the known corresponding petunidin and malvidin derivatives have been identified in extracts of both cell suspensions and fresh flowers of *Catharanthus roseus*.⁹⁷ The extracts were analyzed by positive-ion electrospray ionization MS, and collision experiments were performed on molecular ions by means of ion trap facilities. Purified compounds were also analyzed by thin-layer chromatography and UV–vis spectroscopy.

The 3-rutinoside and 3-glucoside of 6-hydroxycyanidin have previously been isolated from red flowers of *Alstroemeria* cultivars,⁹⁸ whereas 6-hydroxydelphinidin 3-rutinoside, occurred in pink-purple flowers of five cultivars.⁹⁹ During the period of this review, the 3-[6-(malonyl)glucoside] of 6-hydroxycyanidin and 6-hydroxydelphinidin in addition to 6-hydroxydelphinidin 3-glucoside have been identified in various *Alstroemeria* cultivars.^{100–102} The position of the 6-hydroxyl of these anthocyanidins was unambiguously assigned by homo- and heteronuclear NMR techniques.¹⁰³ Flower color, hue, and color intensity of fresh tepals of 28 Chilean species and 183 interspecific hybrids have been described by CIELAB parameters.¹⁰⁴ Compared with flowers containing exclusively cyanidin 3-glycosides, the hues of flowers with 6-hydroxycyanidin 3-glycosides were more reddish. Substitution of the anthocyanidin A-ring with 6-hydroxyl causes a hypsochromic shift (~15 nm) in the visible spectra, which has diagnostic value.¹⁰³ The relationship between flower color and anthocyanin content has also been investigated in 45 *Alstroemeria* cultivars by Tatsuzawa et al.¹⁰⁵ The major anthocyanins of outer perianths were cyanidin 3-rutinoside and 6-hydroxycyanidin 3-rutinoside in cultivars with red flowers, 6-hydroxydelphinidin 3-rutinoside in those that were red-purple, and delphinidin 3-rutinoside in purple ones. Recently, the same group has isolated the 3-(glucoside) and 3-[6-(rhamnosyl)glucoside] of 6-hydroxypelargonidin (aurantinidin) from extracts of the orange-red flowers of the *Alstroemeria* cultivars “Oreiju,” “Mayprista,” and “Spotty-red.”¹⁰⁶ Aurantinidin has previously been reported to occur in *Impatiens aurantiaca* (Balsaminaceae).¹⁰⁷

10.2.4 GLYCOSIDES

Most anthocyanins contain two, three, or just one monosaccharide unit (Figure 10.1); however, as much as seven glucosyl units have been found in ternatin A1 (*Clitoria ternatea*)¹⁰⁸

and cyanodelphin (*Delphinium hybridum*).¹⁰⁹ Altogether 240 and 24 anthocyanins have been reported to contain a disaccharide and a trisaccharide, respectively, while no tetrasaccharide has been found yet in anthocyanins. The sugar moieties are connected to the anthocyanidins through *O*-linkages; however, recently Saito et al.²⁰ have isolated 8-*C*-glucosylcyanidin 3-[6-(malonyl)glucoside] from the purple flowers of *Tricyrtis formosana* cultivar Fujimusume (Liliaceae). Although *C*-glycosylation is common in other flavonoids, especially flavones (Chapter 13), this is the first report of a natural *C*-glycosylanthocyanin.

10.2.4.1 Monosaccharides

The anthocyanin monosaccharides are represented by glucose, galactose, rhamnose, arabinose, xylose, and glucuronic acid. There is no new monosaccharide attached to anthocyanidins reported in the period of this review. Glucosyl moieties have been identified in as much as 90% of the anthocyanins, while the rarest monosaccharide in anthocyanins, glucuronosyl, is limited to 11 anthocyanins (Figure 10.4). This latter monosaccharide has previously been identified in anthocyanins isolated from flowers of *Helenium autumnale* and *Bellis perennis* (Compositae),^{110,111} and tentatively identified as luteolinidin 4'-glucuronide in flower extracts of *Holmskioldia sanguinea*.¹¹² More recently, three delphinidin and three cyanidin derivatives based on 3-[2-(2-(caffeyl)glucosyl)galactoside]-7-[6-(caffeyl)glucoside]-3'-glucuronoside have been isolated from flowers of *Anemone coronaria*.^{28,29} Glucuronosyl units have also been found in two anthocyanin-flavonol complexes isolated from chive flowers (*Allium schoenoprasum*), however, linked to the flavonol moieties.¹¹ The acid function of the glucuronosyl of these latter complexes was considerably methylesterified during extraction with methanol containing as little as 1% trifluoroacetic acid.

10.2.4.2 Disaccharides

The following disaccharides have previously⁴ been found linked to anthocyanidins: 2-glucosylglucose (sophorose), 6-rhamnosylglucose (rutinose), 2-xylosylglucose (sambubiose), 6-glucosylglucose (gentiobiose), 6-rhamnosylgalactose (robinobiose), 2-xylosylgalactose (lathyroside), 2-rhamnosylglucose (neohesperidose), 3-glucosylglucose (laminariobiose), 6-arabinosylglucose, 2-glucuronosylglucose, 6-glucosylgalactose, and 4-arabinosylglucose (Figure 10.5). In addition, several anthocyanidin disaccharides have been detected without proper determination of the linkage points between the monosaccharides. During the period of this review, Yoshida et al.²¹ have isolated delphinidin 3-[6-(*E-p*-coumaryl)glucoside]-5-[6-(malonyl)-4-(rhamnosyl)glucoside] (muscarinin A) from purplish-blue spicate flower petals of *Muscari armeniacum* (Liliaceae), which contained an interesting 1 → 4 linkage between the rhamnose and one of the glucose units. Another new disaccharide, 2-glucosylgalactose, has been found in pelargonidin 3-[2-(2-*E*-caffeyl)glucosyl]-galactoside isolated from sepals of *Pulsatilla cernua* (Ranunculaceae),²² in the major anthocyanin, cyanidin 3-[2-(glucosyl)galactoside], isolated from scarlet fruits of *Cornus suecica* (Cornaceae),²³ and in seven exciting acylated cyanidin and delphinidin derivatives from flowers of *Anemone coronaria*^{28,29} (Table 10.2).

Among the new anthocyanins, which have been reported after 1992, 47, 22, and 36 contain sophorose, rutinose, and sambubiose, respectively (Figure 10.5). Most of the anthocyanins containing sophorose were first isolated from species belonging to Convolvulaceae (22) and Cruciferae (13) (Table 10.2). This disaccharide has also been identified in new anthocyanins isolated from *Ajuga* (Labiatae),^{36,113,114} *Consolida* (Ranunculaceae),¹¹⁵ *Begonia* (Begoniaceae),¹¹⁶ and in the flavonol unit of two covalent anthocyanin-flavonol complexes from *Allium* (Alliaceae).¹¹

Most of the novel anthocyanins containing rutinose (20) have been isolated from species belonging to the genera *Petunia* and *Solanum* in Solanaceae (Table 10.2). However,

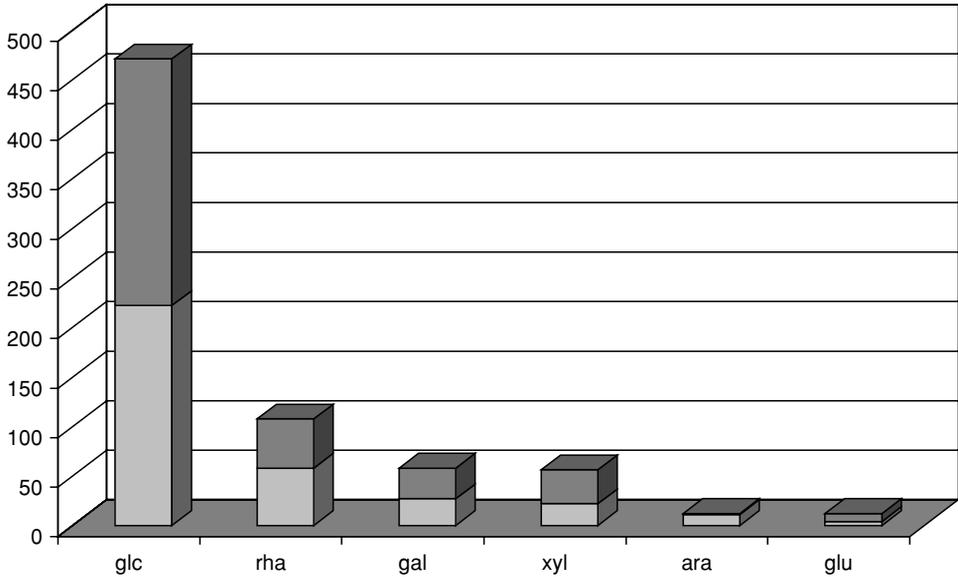


FIGURE 10.4 Numbers of anthocyanins containing the various monosaccharides identified in anthocyanins. The upper dark part of each bar represents the anthocyanins reported later than 1992. glc, glucose; rha, rhamnose; xyl, xylose; gal, galactose; ara, arabinose; glu, glucuronic acid. Some anthocyanins contain more than one type of monosaccharide.

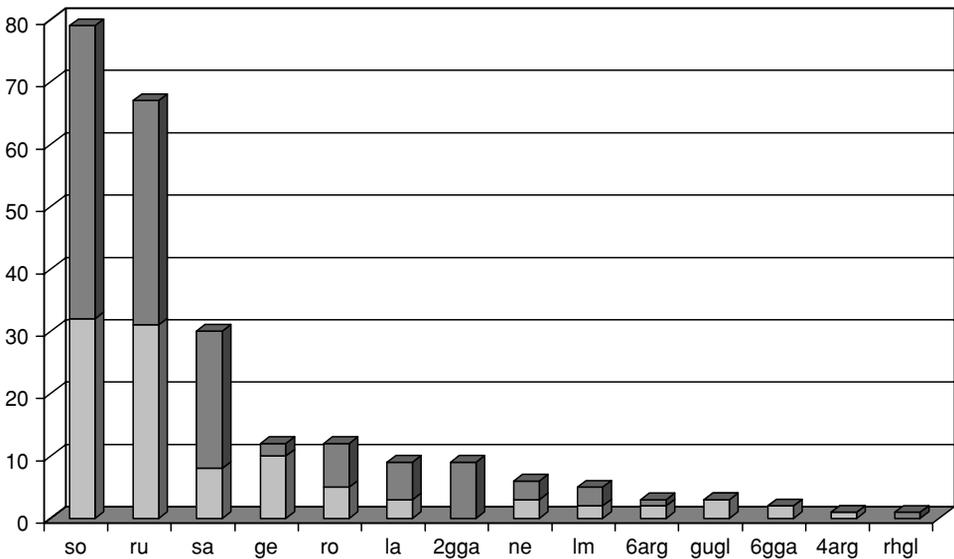


FIGURE 10.5 Numbers of anthocyanins containing the various disaccharides identified in anthocyanins. The upper dark part of each bar represents the anthocyanins reported later than 1992. so, 2-glucosylglucose; ru, 6-rhamnosylglucose; sa, 2-xylosylglucose; ge, 6-glucosylglucose; ro, 6-rhamnosylgalactose; la, 2-xylosylgalactose; 2gga, 2-glucosylgalactose; ne, 2-rhamnosylglucose; lm, 3-glucosylglucose; 6arg, 6-arabinosylglucose; gegl, 2-glucuronylglucose; 6gga, 6-glucosylgalactose; 4arg, 4-arabinosylglucose; rhgl, 4-rhamnosylglucose.

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992

Taxon	Organ	Pigment ^a	References ^b
BRYOPHYTA			
BLECHNACEAE			
<i>Blechnum novae-zelandiae</i>	Tissue	Lt5-[3-(glc)-2-(ace)glc] (514) ^c	96 (2001)
HEPATOPHYTA			
MARCHANTIACEAE			
<i>Marchantia polymorpha</i>	Cell walls	Riccionidin A (522), riccionidin B ^{d,e}	81 (1994)
RICCIACEAE			
<i>Riccia duplex</i>	Cell walls	Riccionidin A (522), riccionidin B ^{d,e}	81 (1994)
<i>Riccioarpus natans</i>			
SCAPANACEAE			
<i>Scapania undulata</i>	Cell walls	Riccionidin A (522), riccionidin B ^{d,e}	81 (1994)
GYMNOSPERMAE			
Pinaceae			
<i>Abies, Picea, Pinus, Pseudotsuga, Tsuga</i> 27 spp.	Cones	Cy3-glc, Dp3-glc, Pn3-glc, Pt3-glc	295 (2003)
<i>Pinus banksiana</i>	Needles	Cy3-glc, Dp3-glc, Pn3-glc, Pt3-glc, Mv3-glc	338 (2002)
ANGIOSPERMAE			
MONOCOTYLEDONEAE			
Palmae (= Arecaceae)			
<i>Euterpe edulis</i> ^g	Fruits	Cy3-glc, Cy3-[6-(rha)glc]	339 (1994)
<i>Pinanga polymorpha</i> ^g (Asian palm)	Fruits	Cy3-glc, Cy3-[6-(rha)glc]	339 (1994)
Commelinaceae			
<i>Tradescantia pallida</i>	Leaves	Cy3-[6-(2,5-di-(fer)ara)glc]7,3'-[6-(fer)glc] (251)	136 (1995)
Gramineae (= Poaceae)			
<i>Alopecurus, Anthoxanthum, Avenula, Bothriochloa, Dactylis, Deschampsia, Elymus, Festuca, Holcus, Hordeum, Miscanthus, Molinia, Oryza, Phalaris, Phleum, Poa, Sinarundinaria, Zea</i> , ^f 23 spp.	Grass	Cy3-[3,6-di-(mal)glc], Cy3-[6-(mal)glc], Cy3-[6-(rha)glc], Cy3-glc, Dp3-[6-(mal)glc], Dp3-glc, Pn3-[6-(mal)glc], Pn3-[di(mal)glc] ^e , Pn3-glc	296 (2001)
<i>Panicum melinis</i> ^g	Flowers	Cy3-[caf-6-(ara)glc], Cy3-[6-(ara)glc]	340 (1992)
<i>Pennisetum setaceum</i> (Rubrum Red Riding Hood)	Leaves and flowers	Cy3-[6-(rha)glc], Cy3-glc	341 (2004)
<i>Phalaris arundinacea</i>	Flower tops	Cy3-[3,6-di-(mal)glc], Cy3-[6-(mal)glc], Cy3-glc, Pn3-glc	342 (2001)
<i>Phragmites australis</i>	Flowers	Cy3-[6-(mal)glc], Cy3-[6-(suc)glc] (135), Cy3-glc	221 (1998)
<i>Sorghum bicolor</i> ^g	Grains	5MLt (519)	84 (1996) 83 (1997)
<i>Sorghum caudatum</i>	Grains	7Map (516), Ap,Lt,Lt5-glc	343 (1994) 344 (2003)

continued

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Triticum aestivum</i> [§] (cv. Katepwa)	Grains (purple wheat)	Cy3-gal, Cy3-glc, Pn3-glc	
<i>Triticum aestivum</i> [§] (cv. Purendo 38)	Grains (blue wheat)	Cy3-glc, Pn3-glc	344 (2003)
<i>Zea mays</i>	Leaves and flowers	Cy3-[3,6-di-(mal)glc], Cy3-[6-(mal)glc], Cy3-glc, Pn3-glc	342 (2001)
Iridaceae			
<i>Babiana stricta</i>	Violet flowers	Mv3-glc-5-[2-(S)-6-(mal)glc] (472), Mv3-glc-5-[2-(S)glc] (467), Mv3-glc-5-[6-(mal)glc] (469)	30 (1994)
<i>Crocus antalyensis</i>	Flowers	Dp3,5-di-glc, Dp3,7-di-glc, Dp3-glc-5-[6-(mal)glc] (338), Pt3,5-di-glc, Pt3,7-di-glc (427)	345 (1999)
<i>Crocus chrysanthus</i> (Skyline)	Flowers	Mv3,7-di[6-(mal)glc] (473), Pt3,7-di[6-(mal)glc] (439)	346 (1998)
<i>Crocus chrysanthus</i> (Eyecatcher)	Flowers	Dp3-[6-(rha)glc], Pt3-[6-(rha)glc]	346 (1998)
<i>Crocus sieberi</i> ssp. <i>sublimes</i> (Tricolor)	Flowers	Dp3,5-di-glc, Pt3,5-di-glc	346 (1998)
Liliaceae			
<i>Dianella nigra</i>	Berries	Dp3-glc-7,3',5'-tri[6-(cum)glc] (399), Dp3,7,3',5'-tetra[6-(cum)glc] (403)	146 (2001)
<i>Dianella tasmanica</i>	Berries	Dp3-glc-7,3',5'-tri[6-(cum)glc] (399), Dp3-glc-7-glc-3',5'-di[6-(cum)glc] (391)	146 (2001)
<i>Hyacinthus orientalis</i>	Red flowers	Pg3-[6-(caf)glc]-5-[6-(mal)glc], Pg3-[6-(caf)glc]-5-glc, Pg3-[6-(cum)glc]-5-[4-(mal)glc] (50), Pg3-[6-(cum)glc]-5-[6-(ace)glc] (49), Pg3-[6-(fer)glc]-5-[6-(mal)glc] (54), Pg3-[6-(fer)glc]-5-glc (46), Pg3-glc-5-[6-(mal)glc] (35), Pg3-[6-(Z-cum)glc]-5-glc (42)	201 (1995) 327 (1995)
<i>Hyacinthus orientalis</i>	Blue flowers	Cy3-[6-(cum)glc]-5-[6-(mal)glc], Dp3-[6-(caf)glc]-5-[6-(mal)glc], Dp3-[6-(cum)glc]-5-[6-(mal)glc], Dp3-[6-(cum)glc]-5-glc, Dp3-[6-(Z-cum)glc]-5-[6-(mal)glc], Pg3-[6-(cum)glc]-5-[6-(mal)glc], Pt3-[6-(cum)glc]-5-[6-(mal)glc] (443)	328 (1995)
<i>Lilium</i>	Flowers	Cy3-[6-(rha)glc]-7-glc (124), Cy3-[6-(rha)glc]	120 (1999)
<i>Muscari armeniacum</i>	Blue flowers	Dp3-[6-(cum)glc]-5-[6-(mal)-4-(rha)glc] (370), Dp3-glc, Pt3-glc, Mv3-glc	21 (2002)
<i>Tricyrtis formosana</i>	Flowers	Cy3-[6-(mal)glc]8C-glc (151)	20 (2003)
<i>Tulipa</i> (Queen Wilhelmina)	Flowers	Cy3-[6-(2-(ace)rha)glc] (144), Cy3-[6-(rha)glc], Pg3-[6-(2-(ace)rha)glc] (29) ^d , Pg3-[6-(rha)glc]	122 (1999)
<i>Tulipa gesneriana</i>	Anthers	Dp3-[6-(2-(ace)rha)glc] (332), Dp3-[6-(3-(ace)rha)glc] (333), Dp3-[6-(rha)glc]	121 (1999)
Alliaceae			
<i>Agapanthus praecox</i> ssp. <i>orientalis</i>	Flowers	Dp3-[6-(cum)glc]-7-glc[Kae3-glc-7-xy1-4'-glc suc] (538), Dp3-[6-(cum)glc]-7-glc[Kae3,7-di-glc-4'-glc suc] (539)	12 (2000)
<i>Allium cepa</i>	Bulbs	5-Carboxypyranocyanidin 3-glucoside (524), 5-Carboxypyranocyanidin 3-[6-(malonyl)glucoside] (525)	18 (2003)

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Allium cepa</i>	Bulbs	Cy3,4'-di-glc (113), Cy3,5-di-glc, Cy3-[3-(glc)-6-(mal)glc] (150), Cy3-[3-(glc)-6-(mal)glc]4'-glc (192), Cy3-[3-(glc)-6-(Me-mal)glc], Cy3-[3-(glc)glc], Cy3-[6-(mal)glc], Cy3-glc, Cy4'-glc (100), Cy7-[3-(glc)-6-(mal)glc]4'-glc (193), Pn3-[6-(mal)glc], Pn3-[6-(mal)glc]-5-glc (283)	135 (1994) 147 (2003)
<i>Allium sativum</i>	Inner scale leaves	Cy3-[(ace)glc] ^c , Cy3-[3-(mal)glc], Cy3-[3,6-di-(mal)glc], Cy3-[6-(mal)glc], Cy3-glc	204 (1997)
<i>Allium schoenoprasum</i>	Flowers	(6''-(Cy3-glc)) (2''''-(Kae3-[2''-(glc)(glc)]-7-glu)malonate (536), (6''-(Cy3-[3''-(ace)glc])) (2''''-(Kae3-[2''-(glc)(glc)]-7-glu)malonate (537), Cy3-[3,6-di-(mal)glc], Cy3-[6-(ace)glc], Cy3-[6-(mal)glc], Cy3-glc	11 (2000)
<i>Allium victorialis</i>	Stems	Cy3-[3-(mal)glc] (133), Cy3-[3,6-di-(mal)glc] (141), Cy3-[6-(mal)glc], Cy3-glc	202 (1995)
<i>Triteleia bridgesii</i>	Flowers	Cy3-[cum-glc-cum-glc]-5-[mal-glc] ^c , Dp3-[6-(4-(glc)cum)glc]-5-[6-(mal)glc] (372), Dp3-[6-(cum)glc]-5-[6-(mal)glc], Dp3-[6-(cum)glc]-5-glc, Dp3-[6-(Z-cum)glc]-5-[6-(mal)glc]	166 (1998)
Alstroemeriaceae			
<i>Alstroemeria</i> (Westland, Tiara)	Flowers/ tepals	Cy-6OH-3-[6-(mal)glc] (500), Cy-6OH-3-[6-(rha)glc], Cy-6OH-3-glc, Dp-6OH-3-[6-(mal)glc] (504), Dp-6OH-3-[6-(rha)glc], Dp-6OH-3-glc (502), Pg-6OH-3-[6-(rha)glc] (496), Pg-6OH-3-glc (495), Cy3-[6-(mal)glc], Cy3-[6-(rha)glc], Cy3-glc, Dp3-[6-(mal)glc], Dp3-[6-(rha)glc], Dp3-glc, Pg3-[6-(rha)glc]	100 (1996) 101 (2001) 102 (2002) 122 (2003)
Musaceae			
<i>Musa × paradisiaca</i> ^e	Bracts	Cy3-[6-(rha)glc], Dp3-[6-(rha)glc], Mv3-[6-(rha)glc], Pg3-[6-(rha)glc], Pn3-[6-(rha)glc]	347 (2001)
Orchidaceae			
<i>Bletilla striata</i>	Flowers	Cy3-[6-(mal)glc]-7-[6-(cum)glc]-3'-[6-(4-(6-(4-(glc)cum)glc)cum)glc] (257), Cy3-glc-7-[6-(cum)glc]-3'-[6-(4-(6-(4-(glc)cum)glc)cum)glc] (254), Cy3-[6-(mal)glc]-7-[6-(caf)glc]-3'-[6-(4-(6-(4-(glc)caf)glc)caf)glc] (258), Cy3-glc-7-[6-(caf)glc]-3'-[6-(4-(6-(4-(glc)caf)glc)caf)glc] (256)	157 (1995)
<i>Dendrobium</i> (Pompadour)	Purple flowers	Cy3-[6-(mal)glc]7,3'-di[6-(sin)glc], Cy3-glc-7,3'-di[6-(sin)glc]	181 (2002)
<i>Dendrobium phalaenopsis</i> (Pramot)	Red-purple flowers	Cy3-[6-(mal)glc]7,3'-di[6-(4-(glc)hba)glc] (253)	189 (1994)
<i>Dracula chimaera</i>	Flowers	Cy3-[6-(mal)glc], Cy3-[6-(rha)glc], Cy3-glc, Pn3-[6-(rha)glc], Pn3-[6-(mal)glc]	330 (2003)
<i>Dracula cordobae</i> × <i>Laeliocattleya</i> (minipurple)	Flowers	Cy3-[6-(mal)glc], Cy3-[6-(rha)glc], Cy3-glc, Pn3-[6-(rha)glc], Pn3-[6-(mal)glc]	330 (2003)

continued

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Laelia pumila</i> <i>Cattleya walkeriana</i>	Flowers	Cy3-[6-(mal)glc]-7-[6-(cum)glc]-3'-[6-(4-(6-(caf)glc)cum)glc] (245), Cy3-[6-(mal)glc]-7-[6-(cum)glc]-3'-[6-(4-(6-(caf)glc)caf)glc] (247), Cy3-[6-(mal)glc]-7-[6-(caf)glc]-3'-[6-(4-(6-(caf)glc)caf)glc] (249), Cy3-[6-(mal)glc]-7-[6-(fer)glc]-3'-[6-(4-(6-(fer)glc)caf)glc] ^c , Cy3-[6-(mal)glc]-7-[6-(cum)glc]-3'-[6-(4-(6-(cum)glc)cum)glc] (244)	155 (1994) 156 (1996)
<i>Phalaenopsis equestris</i> <i>P. intermedia</i> <i>P. leucorrhoda</i> <i>P. sanderiana</i> <i>P. schilleriana</i>	Flowers	Cy3-[6-(mal)glc]7,3'-di[6-(sin)glc] (230), Cy3-glc-7,3'-di[6-(sin)glc] (222)	180 (1997)
<i>Sophronitis coccinea</i>	Flowers	cumCy3-[mal-glc]7,3'-di-glc ^c , Cy3,3'-di-glc-7-[6-(caf)glc] (204), Cy3-[6-(mal)glc]-7-[6-(caf)glc]-3'-glc (210), Cy3-[6-(mal)glc]-7-[6-(fer)glc]-3'-glc (211), ferCy3,7,3'-tri-glc ^c	176 (1998)
<i>Vanda</i>	Flowers	Cy3-[6-(mal)glc]7,3'-di[6-(fer)glc] ^d , Cy3-[6-(mal)glc]7,3'-di[6-(sin)glc], Cy3-glc-7,3'-di[6-(sin)glc], Dp3-[mal-glc]7,3'-di[fer-glc] ^{d,e} , Dp3-[6-(mal)glc]7,3'-di[6-(sin)glc] (382), Dp3-glc-7,3'-di[6-(sin)glc] (376), sinferCy3-[mal-glc]7,3'-di-glc ^c , sinferDp3-[mal-glc]7,3'-di-glc ^{d,e}	182 (2004)
Pontederiaceae			
<i>Eichhornia crassipes</i>	Blue-purple flowers	(6'''-(Dp3-[6''-(glc)glc])) (6''-(Ap7-glc))malonate (534), (6'''-(Dp3-[6''-(glc)glc])) (6''-(Lt 7-glc))malonate (535)	9 (1994) 10 (2004)
DICOTYLEDONEAE			
Aceraceae			
<i>Acer platanoides</i>	Leaves	Cy3-[2-(gao)-6-(rha)glc](161), Cy3-[2-(gao)glc] (139), Cy3-[2,3di(gao)glc] (143), Cy3-[6-(rha)glc], Cy3-glc	193 (1992) 195 (1999)
Anacardiaceae			
<i>Rhus javanica</i>	Adventitious roots	Riccionidin A	82 (2000)
Araliaceae			
<i>Aralia cordata</i>	Cultured cells	Pn3-[2-(xyl)gal] (264)	133 (1994)
<i>Fatsia japonica</i>	Berries	Cy3-[2-(xyl)gal]	134 (1992)
Apocyanaceae			
<i>Catharanthus roseus</i> ^g	Cell suspension	Hi3-[6-(cum)glc] (493), Hi3-glc (492), Mv3-[6-(cum)glc] (463), Mv3-glc, Pt3-[6-(cum)glc], Pt3-glc	97 (1998) 348 (2003)
Balanophoraceae			
<i>Cynomorium coccineum</i> ^g	Floral tissue	Cy3-glc	339 (1994)
Begoniaceae			
<i>Begonia</i>	Flowers	Cy3-[2-(xyl)-6-(caf)glc] (158), Cy3-[2-(xyl)-6-(Z-caf)glc] (159) ^g , Cy3-[2-(glc)-6-(cum)glc] (167) ^g , Cy3-[2-(glc)-6-(Z-cum)glc] (168) ^g , Cy3-[2-(xyl)-6-(cum)glc] (156) ^g , Cy3-[2-(xyl)-6-(Z-cum)glc] (157) ^g	116 (1995)

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
Bignoniaceae			
<i>Arrabidaea chica</i>	Leaves	6-OH-5-Me-Ap (517), 6-OH-5-Me-Lt (520), 5,4'-Me-6-OH (521)	86 (2001) 87 (2002)
Boraginaceae			
<i>Lobostemon</i>	Flowers	Cy3,5-di-glc, Cy3-[6-(rha)glc], Cy3-glc, Dp3,5-di-glc, Dp3-[6-(rha)glc], Dp3-glc	299 (1997)
Burseraceae			
<i>Dacryodes edulis</i> ^g	Skin, pulp	Cy3-glc/gal, Pt3-glc/gal, Pn3-glc/gal	349 (2003)
Campanulaceae			
<i>Campanula isophylla</i> <i>C. carpatica</i> <i>C. poskarshyan</i> ^f	Flowers	Dp3-[6-(rha)glc]-7-[6-(4-(6-(4-(glc)hba)glc)hba)glc] (404), Dp3-[6-(rha)glc]-7-[6-(4-(6-(hba)glc)hba)glc] (387), Dp3-[6-(rha)glc]-7-glc (318), Dp3-[6-(rha)glc]-7-[6-(4-(6-(4-(6-(hba)glc)hba)glc)hba)glc]	117 (1993)
Caprifoliaceae			
<i>Lonicera caerulea</i>	Fruits	Cy3,5-di-glc, Cy3-[6-(rha)glc], Cy3-glc, Pg3-glc, Pn3-[6-(rha)glc], Pn3-glc	350 (2004)
<i>Sambucus canadensis</i>	Flowers	Cy3-[6-(Z-cum)-2-(xyl)glc]-5-glc (196), Cy3-[6-(cum)-2-(xyl)glc]-5-glc, Cy3-[2-(xyl)glc], Cy3-[2-(xyl)glc]-5-glc, Cy3,5-di-glc, Cy3-glc	125 (1995)
Caryophyllaceae			
<i>Dianthus caryophyllus</i>	Flowers	Cy3,5-glc(6'',6'''-mal diester) (152), Cy3-[6-(mly)glc]-5-glc, Pg3,5-glc(6'',6'''-mal diester) (38), Dp3,5-glc(6'',6'''-mal diester) (339) ^h , Dp3-[6-(mly)glc]-5-glc (340) ^h , Dp3,5-di[6-(mly)glc] (347) ^h	214 (1998) 215 (2000) 216 (2000) 31 (2003)
Compositae (= Asteraceae)			
<i>Cichorium intybus</i>	Flowers	Dp3,5-di[6-(mal)glc] (346), Dp3-[6-(mal)glc]-5-glc (337), Dp3glc5-[6-(mal)glc], Dp3,5-di-glc	351 (2002)
<i>Dendranthema grandiflorum</i>	Purple-red flowers	Cy3-[3,6-di(mal)glc]	206 (1997)
<i>Felicia amelloides</i>	Flowers	Dp3-[2-(rha)glc]-7-[6-(mal)glc] (357)	139 (1999)
<i>Gynura aurantiaca</i> cv.	Leaves	Cy3-[6-(mal)glc]-7-[6-(4-(6-(caf)glc)caf)glc]-3'-[6-(caf)glc] (248)	248 (1994)
<i>Helianthus annuus</i> ^g	Purple sunflower seeds	Cy3-ara, Cy3-glc, Cy3-xyl, Cy3-[di(mal)-glc], Cy3-[di(mal)-xyl] ^d , Cy3-[mal-ara], Cy3-[mal-glc], Cy3-[mal-xyl]	197 (1994)
<i>Lactuca sativa</i>	Leaves	Cy3-[6-(mal)glc]	352 (1996)
<i>Senecio cruentus</i>	Flowers	Cy3-[6-(mal)glc]-3'-[6-(caf)glc] (186), Cy3-glc-3'-[6-(caf)glc] (172), Cy3,3'-glc, Pg3-[6-(mal)glc], Pg3-[6-(mal)glc]-7-[6-(4-(6-(caf)glc)caf)glc] (85), Pg3-[6-(mal)glc]-7-[6-(caf)glc] (53)	353 (1993) 354 (1995)
<i>Evolvulus pilosus</i>	Blue flowers	Dp3-[6-(4-(6-(3-(glc)caf)glc)caf)glc]-5-[6-(mal)glc] (397), Dp3,5-di-glc, Dp3-[6-(4-(6-(3-(glc)caf)glc)caf)glc]-5-glc (392)	313 (1994) 223 (1996)

continued

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
Convolvulaceae			
<i>Ipomoea asarifolia</i>	Flowers	Cy3-[2-(6-(caf)glc)-6-(4-(6-(hca)glc)caf)glc]-5-glc (243), Cy3-[2-(6-(cum)glc)-6-(4-(6-(cum)glc)caf)glc]-5-glc (241), Cy3-[2-(6-(caf)glc)-6-(caf)glc]-5-glc, Cy3-[2-(6- (cum)glc)-6-(caf)glc]-5-glc (214)	355 (1998) 27 (2003)
<i>Ipomoea batatas (batatas)</i>	Purple tubers	Cy3-[2-(glc)glc]-5-glc, Cy3-[2-(6-(cum)glc)glc]-5-glc (199), Cy3-[6-(caf)-2-(glc)glc]-5-glc (205), Pn3-[6- (caf)-2-(glc)glc]-5-glc, Cy3-[2-(6-(hba)glc)-6-(caf)glc]- 5-glc (212), Cy3-[2-(6-(caf)glc)-6-(caf)glc]-5-glc, Cy3- [2-(6-(fer)glc)-6-(caf)glc]-5-glc (217), Pn3-[2-(6- (fer)glc)-6-(caf)glc]-5-glc, Pn3-[2-(6-(caf)glc)-6- (caf)glc]-5-glc, Pn[2-(6-(hba)glc)-6-(caf)glc]-5-glc (291)	357 (1992) 356 (1997) 188 (1999) 35 (2000)
<i>Ipomoea purpurea</i>	Brownish-red flowers	Cy3-[2-(6-(4-(6-(3-(glc)caf)glc)caf)glc)glc] (236), Cy3-[2- (glc)glc], Cy3-[2-(6-(caf)glc)glc] (174), Cy3-[2-(glc)-6- (caf)glc] (173)	358 (1998)
<i>Ipomoea purpurea</i>	Violet-blue flowers	Cy3-[2-(6-(3-(glc)caf)glc)-6-(4-(6-(caf)glc)caf)glc]-5-glc (255), Cy3-[2-(6-(3-(glc)caf)glc)-6-(caf)glc]-5-glc (235), Cy3-[2-(6-(caf)glc)-6-(caf)glc]-5-glc (215), Cy3-[2- (glc)glc]-5-glc	305 (1995)
<i>Ipomoea purpurea</i>	Red-purple flowers	Pg3-[2-(6-(3-(glc)caf)glc)-6-(4-(6-(caf)glc)caf)glc]-5-glc (93), Pg3-[2-(6-(caf)glc)-6-(4-(6-(caf)glc)caf)glc]-5-glc (91), Pg3-[2-(6-(caf)glc)-6-(caf)glc]-5-glc (76), Pg3-[2- (6-(3-(glc)caf)glc)-6-(caf)glc]-5-glc, Pg3-[2-(glc)-6- (caf)glc]-5-glc	306 (1996)
<i>Pharbitis nil/Ipomoea nil</i>	Flowers	Cy3-[2-(glc)-6-(4-(glc)caf)glc]-5-glc (232), Cy3-[6-(3- (glc)caf)glc]-5-glc (206), Pg3,5-di-glc, Pg3-[2-(6-(3- (glc)caf)glc)-6-(4-(6-(caf)glc)caf)glc]-5-glc, Pg3-[2-(6- (caf)glc)-6-(caf)glc]-5-glc, Pg3-[2-(glc)-6-(caf)glc]-5-glc (64), Pg3-[2-(6-(cum)glc)glc]-5-glc (60), Pg3-[2-(glc)-6- (4-(glc)caf)glc]-5-glc (89), Pg3-[6-(3-(glc)caf)glc] (45), Pg3-[6-(3-(glc)caf)glc]-5-glc (65), Pg3-[6-(caf)glc] (28), Pg3-glc, Pn3,5-di-glc, Pn3-[2-(glc)-2-(6-(glc)caf)glc]-5- glc, Pn3-[2-(glc)glc]-5-glc, Pn3-[6-(3-(glc)caf)glc] (285), Pn3-[6-(3-(glc)caf)glc]-5-glc (290), Pn3-glc	312 (1993) 309 (1994) 359 (1996) 310 (2001) 360 (2001)
Coriariaceae			
<i>Coriaria myrtifolia</i> ^g	Fruits	Dp-, Cy-, Pt-, Pn-, Mv3-glc, Dp-, Cy-, Pt-, Pn-, Mv3-gal	361 (2002)
Cornaceae			
<i>Cornus suecica</i>	Fruits	Cy3-[2-(glc)gal] (114), Cy3-[2-(glc)glc], Cy3-glc, Cy3-gal	23 (1998)
Crassulaceae			
<i>Crassula, Cotyledon,</i> <i>Tylecodon</i> , ^{f,g} 22 spp.	Flower	Cy3-[2-(glc)glc], Cy3-[2-(xyl)glc], Cy3-glc, Dp3-[2- (xyl)glc], Pn3-glc	298 (1995)
Cruciferae (Brassicaceae)			
<i>Brassica campestris</i>	Stem	Cy[2-(2-(sin)glc)-6-(fer)glc]-5-[6-(mal)glc] (229), Cy[2-(2- (sin)glc)-6-(cum)glc]-5-[6-(mal)glc] (227), Cy[2-(2- (sin)glc)-6-(fer)glc]-5-glc, Cy[2-(2-(sin)glc)-6- (cum)glc]-5-glc	178 (1997)

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Arabidopsis thaliana</i>	Leaves and stems	Cy3-[6-(4-(glc)cum)-2-(2-(sin)xyl)glc]-5-[6-(mal)glc] (239) ^d	167 (2002)
<i>Matthiola incana</i>	Flowers	Cy3-[2-(2-(sin)xyl)-6-(caf)glc]-5-[6-(mal)glc] (225), Cy3-[2-(2-(sin)xyl)-6-(cum)glc]-5-[6-(mal)glc] (224), Cy3-[2-(2-(sin)xyl)-6-(fer)glc]-5-[6-(mal)glc] (226), Cy3-[2-(2-(sin)xyl)-6-(fer)glc]-5-glc (218), Pg3-[2-(2-(fer)xyl)-6-(fer)glc]-5-[6-(mal)glc] (83), Pg3-[2-(2-(sin)xyl)-6-(cum)glc]-5-[6-(mal)glc] (84), Pg3-[2-(2-(sin)xyl)-6-(cum)glc]-5-glc (75), Pg3-[2-(xyl)glc]-5-glc, Pg3-[2-(xyl)-6-(cum)glc]-5-[6-(mal)glc] (68), Pg3-[2-(2-(sin)xyl)-6-(fer)glc]-5-[6-(mal)glc] (86), Pg3-[2-(2-(sin)xyl)-6-(fer)glc]-5-glc (81), Pg3-[2-(xyl)-6-(fer)glc]-5-[6-(mal)glc] (70), Pg3-glc	177 (1995) 173 (1996)
<i>Raphanus sativus</i>	Callus	Cy3-[2-(6-(fer)glc)-6-(caf)glc]-5-glc, Pg3-[2-(2-(fer)glc)-6-(fer)glc]-5-glc (80), Pg3-[2-(6-(caf)glc)-6-(caf)glc]-5-glc, Pg3-[2-(6-(caf)glc)-6-(cum)glc]-5-glc (73), Pg3-[2-(6-(caf)glc)-6-(fer)glc]-5-glc (77), Pg3-[2-(6-(fer)glc)-6-(caf)glc]-5-glc (78), Pg3-[2-(6-(fer)glc)-6-(cum)glc]-5-glc (74), Pg3-[2-(6-(fer)glc)-6-(fer)glc]-5-glc (79), Pg3-[2-(6-(fer)glc)glc]-5-glc (67), Pg3-[2-(glc)-6-(caf)glc]-5-glc, Pg3-[2-(glc)-6-(cum)glc]-5-[6-(mal)glc] (71), Pg3-[2-(glc)-6-(cum)glc]-5-glc (61), Pg3-[2-(glc)-6-(fer)glc]-5-[6-(mal)glc] (72), Pg3-[2-(glc)-6-(fer)glc]-5-glc (66)	315 (1998) 314 (2002)
Ericaceae			
<i>Vaccinium padifolium</i>	Berries	Cy3-[6-(rha)-2-(xyl)glc], Dp3-rha, Mv3-[2-(xyl)glc] (451), Mv3-[6-(rha)glc], Pn3-[2-(xyl)glc], Pn3-[6-(rha)-2-(xyl)glc] (274), Pt3-[2-(xyl)glc] (423), Pt3-[6-(rha)-2-(xyl)glc] (430)	124 (1999) 25 (2000)
Euphorbiaceae			
<i>Acalypha hispida</i>	Flowers	Cy3-[(2-(gao)-6-(rha)gal] (160) ^d , Cy3-[2-(gao)gal], Cy3-gal	130 (2003)
Gentianaceae			
<i>Eustoma grandiflorum</i> , after genetical transformation	Flowers	Cy3-[6-(rha)gal]-5-[6-(cum)glc] (180), Cy3-gal-5-[6-(cum)glc] (162), Dp3-[6-(rha)gal]-5-glc (316), Dp3-[6-(rha)gal]-5-[6-(cum)glc] (361), Dp3-[6-(rha)gal]-5-[6-(Z-cum)glc] (362), Dp3-[6-(rha)glc]-5-[6-(cum)glc] (363), Dp3-[6-(rha)glc]-5-[6-(Z-cum)glc] (364), Dp3-[6-(rha)gal]-5-[6-(fer)glc] (367), Dp3-[6-(rha)gal]-5-[6-(Z-fer)glc] (368), Dp3-gal-5-[6-(cum)glc] (342), Dp3-gal-5-[6-(Z-cum)glc] (343), Dp3-glc-5-[6-(cum)glc]	129 (1993) 123 (1996)
<i>Gentiana</i>	Blue flowers	Dp3,3'-di-glc-5-[6-(caf)glc] (369), Dp3,3'-di-glc-5-[6-(cum)glc] (366), Dp3-glc-5-,3'-di[6-(caf)glc], Dp3-glc-5-[6-(caf)glc]-3'-[6-(cum)glc] (373), Dp3-glc-5-[6-(cum)glc] (344), Dp3-glc-5-[6-(cum)glc]-3'-[6-(caf)glc] (374)	154 (1997)
<i>Gentiana</i>	Pink flowers	Cy3-glc, Cy3-glc-5,3'-di[6-(caf)glc] (216), Cy3-glc-5-[6-(caf)glc] (171), Cy3-glc-5-[6-(cum)glc] (165)	153 (1995)

continued

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
Geraniaceae			
<i>Geranium pratense</i>			
<i>G. sanguineum</i>			
<i>G. Johnson's Blue</i>	Flowers	Mv3,5-di-glc, Mv3-glc, Mv3-glc-5-[6-(ace)glc] (466), Mv5-glc	211 (1997)
<i>Geranium sylvaticum</i>	Flowers	Cy3,5-di-glc, Cy3-glc, Dp3-glc, Mv3,5-di-glc, Mv3-[6-(ace)glc]-5-glc (465)	210 (1995)
<i>Pelargonium domesticum</i> ('Dubonnet')	Flowers	Cy3-glc-5-[6-(ace)glc] (147), Cy3,5-di-glc, Dp3-glc-5-[6-(ace)glc] (334), Dp3,5-di-glc, Mv3,5-di-glc, Mv3-glc-5-[6-(ace)glc], Pg3-glc-5-[6-(ace)glc], Pg3,5-di-glc, Pn3-glc-5-[6-(ace)glc] (282), Pn3,5-di-glc, Pt3-glc-5-[6-(ace)glc] (437), Pt3,5-di-glc	317 (1998)
Grossulariaceae			
<i>Ribes nigrum</i>	Berries	Cy3-[6-(rha)glc], Dp3-glc (pyranocyanidin A, pyranocyanidin B, pyranodelphinin A, and pyranodelphinin B) ^{d,i} Cy3-[6-(cum)glc], Cy3-[ara], Cy3-glc, Dp3-[6-(cum)glc], Dp3-[6-(rha)glc], Mv3-[6-(rha)glc], Mv3-glc, Pg3-[6-(rha)glc], Pg3-glc, Pn3-[6-(rha)glc], Pn3-glc, Pt3-[6-(rha)glc], Pt3-glc (pyranocyanidin C, pyranocyanidin D, pyranodelphinin C, and pyranodelphinin D) ^d	88 (2000) 89 (2001) 365 (2002) 91 (2002)
Labiatae (=Lamiaceae)			
<i>Ajuga reptans</i>	Flowers, and cell cultures	Dp3-[2-(6-(cum)glc)-6-(cum)glc]-5-[6-(mal)glc] (379), Cy3-[2-(6-(cum)glc)-6-(cum)glc]-5-[6-(mal)glc] (223), Dp3-[2-(glc)glc]-5-glc, Cy3-[2-(glc)glc]-5-glc, Dp3-[2-(6-(fer)glc)-6-(cum)glc]-5-[6-(mal)glc] (380) ^d , Dp3-[2-(6-(fer)glc)-6-(fer)glc]-5-[6-(mal)glc] (381), Cy3-[2-(6-(cum)glc)-6-(cum)glc]-5-glc (213), Dp3-[di-fer(2glc-glc)]-5-glc ^e , Cy3-[fer-cum(2glc-glc)]-5-[mal-glc] ^e	113 (1996) 114 (2001)
<i>Lamium, Salvia, Thymus</i> , 49 spp.	Flowers	Cy3-[6-(cum)glc]-5-[4-(mal)-6-(mal)glc] (354)	200 (1992)
<i>Ocimum basilicum</i> ^g (Dark Opal, Holy Sacred Red, Opal, Osmin Purple, Purple Bush, Purple Ruffles, Red Rubin, Rubin)	Flowers and leaves	Cy3,5-di-glc, Cy3-glc, Cy3-[cum-glc], Cy3-[cum-glc]-5-glc, Pn3,5-di-glc, Pn3-[cum-glc]-5-glc	320 (1998)
<i>Salvia patens</i>	Flowers	Dp3-[6-(cum)glc]-5-[6-(mal)glc] + apigenin7,4'-di-glc + Mg (protodelphin)	226 (1994)
<i>Salvia uliginosa</i>	Flowers	Dp3-[6-(cum)glc]-5-[4-(ace)-6-(mal)glc] (353)	209 (1999)
Leguminosae (=Fabaceae)			
<i>Amphithalea, Coelidium, Hypocalyptus, Liparia</i> , ^{f,g} 10 spp.	Flowers	Cy3-glc, Cy3-[6-(ace)glc], Cy3-[6-(cum)glc], Mv3-glc, Pn3-glc, Cy3-[2-(glc)glc], Pg3-[2-(glc)glc]	326 (1995)

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Cassia auriculata</i>	Heartwood	Pg5-gal ^{d,e}	362 (1994)
<i>Clitoria ternatea</i>		Dp3-[2-(rha)-6-(mal)glc] (337), Dp3-[2-(rha)glc], Dp3-[6-(mal)glc], Dp3-[6-(mal)glc]-3'-[6-(4-(6-(cum)glc)cum)glc]5'-[6-(cum)glc] (409), Dp3-[6-(mal)glc]-3'-[6-(4-(6-(4-(glc)cum)glc)cum)glc]5'-[6-(4-(glc)cum)glc] (415), Dp3-[6-(mal)glc]-3'-[6-(4-(6-(4-(glc)cum)glc)cum)glc]5'-glc (407), Dp3-[6-(mal)glc]-3'-[6-(4-(6-(cum)glc)cum)glc]5'-[6-(4-(glc)cum)glc] (411), Dp3-[6-(mal)glc]-3'-[6-(4-(6-(4-(glc)cum)glc)cum)glc]5'-[6-(cum)glc] (410), Dp3-[6-(mal)glc]-3'-[6-(4-(6-(cum)glc)cum)glc]5'-glc (395), Dp3-[6-(mal)glc]-3'-[6-(4-(glc)cum)glc]5'-[6-(cum)glc] (396), Dp3-[6-(mal)glc]-3'-[6-(4-(glc)cum)glc]5'-glc (388), Dp3-[6-(mal)glc]-3',5'-di-[6-(cum)glc] (378), Dp3-[6-(mal)glc]-3'-[6-(cum)glc]5'-glc (371), Dp3-[6-(mal)glc]-3',5-di-glc (358), Dp3-glc, Dp3-glc-3',5'-di-[6-(4-(glc)cum)glc] (405), Dp3-glc-3'-[6-(4-(glc)cum)glc]5'-glc (386)	144 (1996) 145 (1998) 141 (2003)
<i>Glycine max</i>	Seed coats	Cy3-glc, Dp3-glc, Pt3-glc	363 (2001)
<i>Lupinus</i> (Russell hybrids)	Blue flowers	(Dp3-[6-(mal)glc]apigenin7-[6-(mal)glc]malonic residue + Fe) ^d , (Dp3-[6-(mal)glc]luteolin7-[6-(mal)glc]malonic residue + Fe) ^d	225 (1993)
<i>Lupinus</i> (Russell hybrids)	Pink flowers	Cy3-[6-(mal)glc], Pg3-[6-(mal)glc]	225 (1993)
<i>Phaseolus coccineus</i>	Seed coats	Dp3-glc	322 (1996)
<i>Phaseolus lunatus</i>	Seed coats	Pn3-glc, Pn3-[6-(rha)glc]	322 (1996)
<i>Phaseolus vulgaris</i> ^g	Seed coats	Cy3,5-di-glc, Cy3-glc, Dp3-glc, Pg3-glc, Pt3-glc, Pt3,5-di-glc, Mv3-glc, Mv3,5-di-glc	323 (1997) 363 (2001)
<i>Pisum</i> spp.	Purple pod	Dp3-[2-(xyl)gal]-5-glc (314), Dp3-[2-(xyl)gal]-5-[6-(ace)glc] (356)	131 (2000)
<i>Podalyria</i> , ^g 7 spp.	Flowers	Cy3-glc, Cy3-[6-(cum)glc]	325 (1994)
<i>Vicia villosa</i>	Blue flowers	Dp3-[6-(rha)glc]-5-glc, Pt3-[6-(rha)glc]-5-glc, Mv3-[6'(rha)glc]-5-glc	321 (1998)
<i>Vigna angularis</i>	Grains	Cy	322 (1996)
<i>Vigna subterranean</i>	Grains	Cy3-glc, Mv3-glc, Pt3-glc	324 (1997)
<i>Virgilia</i> , ^g 2 spp.	Flowers	Cy3-glc, Pn3-glc, Cy3-[6-(ace)glc], Pn3-[6-(ace)glc], Cy3-[6-(cum)glc]	325 (1994)
Linaceae			
<i>Linum grandiflorum</i>	Flowers	Cy3-[6-(rha)glc], Dp3-[2-(xyl)-6-(rha)glc] (312), Dp3-[6-(rha)glc]	24 (1995)
Lobeliaceae			
<i>Lobelia erinus</i>	Flowers	Cy3-[6-(4-(Z/E-cum)rha)glc]-5-[6-(mal)glc]-3'-[6-(caf)glc] (238)	118 (1995) 365 (1996)
Malvaceae			
<i>Lavatera maritima</i> ^g	Flowers	Mv3-[6-(mal)glc]-5-glc (468), Mv3-[6-(mal)glc] (462)	339 (1994)
Melastomataceae			
<i>Tibouchina urvilleana</i>	Flowers	Mv3-[6-(cum)glc]-5-[2-(ace)xyl] (471)	208 (1993)

continued

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
Myrtaceae			
<i>Eugenia umbelliflora</i> ^g	Berries	Cy3-glc, Dp3-glc, Mv3-glc, Pg3-glc, Pn3-glc, Pt3-glc	366 (2003)
Nymphaeaceae			
<i>Nymphaea alba</i>	Leaves	Cy3-[2-(gao)-6-(ace)gal], Cy3-[6-(ace)gal] (129), Cy3-gal, Dp3-[2-(gao)-6-(ace)gal], Dp3-[2-(gao)gal], Dp3-[6-(ace)gal], Dp3-gal	192 (2001)
<i>Nymphaea caerulea</i> (= <i>N. capensis</i>)	Flowers	Dp3'-[2-(gao)gal] (325), Dp3'-[2-(gao)-6-(ace)gal] (331)	26 (1999)
<i>Nymphaea marliacea</i> ^f	Leaves	Cy3-[2-(gao)-6-(ace)gal] (142), Dp3-gal, Dp3-[6-(ace)gal] (322), Dp3-[2''''(gao)gal]	367 (1997)
	Flowers	Dp3-[2-(gao)-6-(ace)gal] (330)	191 (1998)
Oxalidaceae			
<i>Oxalis triangularis</i> ^g	Leaves	Mv3-[6-(rha)glc]-5-glc, Mv3-mal-[6-(rha)glc]-5-glc, Mv3di-mal-[6-(rha)glc]-5-glc	368 (2001)
Papaveraceae			
<i>Meconopsis grandis</i> , ^g <i>M. horridula</i> , and <i>M. betonicifolia</i>	Flowers	Cy3-[2-(xyl)-6-(mal)glc]-7-glc (190)	127 (1996) 128 (2001)
Passifloraceae			
<i>Passiflora edulis</i>	Fruit	Cy3-[6-(mal)glc], Cy3-glc, Pg3-glc	369 (1997)
<i>Passiflora suberosa</i>	Fruit	Cy3-[6-(mal)glc], Cy3-glc, Dp3-[6-(mal)glc], Dp3-glc, Pg3-[6-(mal)glc], Pg3-glc, Pt3-[6-(mal)glc], Pt3-glc	369 (1997)
Primulaceae			
<i>Cyclamen persicum</i> (Bonfire)	Flowers	Pn3-[2-(rha)glc] (268) ^d	140 (1999)
<i>Cyclamen persicum</i> (Sierra Rose)	Flowers	Pn3,5-di-glc, Cy3,5-di-glc, Mv3,5-di-glc	140 (1999)
Ranunculaceae			
<i>Aconitum chinense</i>	Flowers	Dp3-[6-(rha)glc]-7-[6-(4-(6-(hba)glc)hba)glc]	184 (1994)
<i>Anemone coronaria</i>	Flowers	Cy3-[2-(2-(caf)glc)-6-(3-(2-tar)mal)gal]-7-[6-(caf)glc]-3'-glu (246), Cy3-[2-(2-(caf)glc)-6-(mal)glc]-7-[6-(caf)glc]-3'-glu (240), Cy3-[2-(2-(caf)glc)glc]-7-[6-(caf)glc]-3'-glu (234), Dp3-[2-(2-(caf)glc)gal-(3-(2-tar)mal)gal]-7-[6-(caf)glc] (383), Dp3-[2-(2-(caf)glc)-6-(3-(2-tar)mal)gal]-7-[6-(caf)glc]-3'-glu (401), Dp3-[2-(2-(caf)glc)-6-(mal)gal]-7-[6-(caf)glc]-3'-glu (398), Dp3-[2-(2-(caf)glc)gal]-7-[6-(caf)glc]-3'-glu (393), Pg3-[2-(xyl)-6-(mal)gal] (32), Pg3-[2-(xyl)-6-(Me-mal)gal] (33), Pg3-[2-(xyl)gal], Pg3-[2-(xyl)-6-(3-(4-(glc)caf)2-tar)mal)gal] (82)	132 (2001) 28 (2002) 29 (2003)
<i>Consolida armeniaca</i>	Flowers	Dp3-[6-(mal)glc]-7-[6-(4-(6-(hba)glc)hba)glc] (377) ^d , Dp3-[6-(mal)glc]-7-[2-(glc)-6-(4-(6-(hba)glc)hba)glc] (394) ^d , Dp3-[6-(mal)glc]-7-[2-(6-(hba)glc)-6-(4-(6-(hba)glc)hba)glc] (400) ^d , Dp3-[6-(mal)glc]-7-[glc-2-(6-(hba)glc)-6-(4-(6-(hba)glc)hba)glc] ^c	115 (1996)
<i>Delphinium hybridum</i>	Flowers	Dp3-[6-(rha)glc], Dp3-[6-(rha)glc]-7-glc, Pg3,7-di-glc, Pg3-[6-(mal)glc]-7-[6-(4-(glc)hba)glc] (69), Pg3-[6-(mal)glc]-7-glc (36), Pg3-[6-(rha)glc]-7-[6-(4-(glc)hba)glc] (87), Pg3-[6-(rha)glc]-7-[6-(hba)glc] (57), Pg3-[6-(rha)glc]-7-glc (21), Pg3-glc-7-[6-(4-(glc)hba)glc] (58)	119 (1998) 331 (1999)

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Pulsatilla cernua</i>	Flowers	Pg3-[2-(2-(caf)glc)gal] (44)	22 (1998)
<i>Ranunculus asiaticus</i>	Flower	Cy3-[2-(xyl)-6-(mal)glc] (148), Cy3-[2-(xyl)glc], Dp3-[2-(xyl)-6-(mal)glc] (335), Dp3-[2-(xyl)glc]	126 (1996)
Rhamnaceae			
<i>Ceanothus papillosus</i>	Flowers	Dp3-[6-(rha)glc]7,3'-di[6-(cum)glc] (389), Dp3-[6-(rha)glc]-7-[6-(cum)glc]-3'-glc (384)	32 (1997)
Rosaceae			
<i>Fragaria</i> × <i>ananas</i>	Berries	A5-Carboxypyranopg3-glc (523), Pg3-glc Afzelechin(4α → 8)Pg3-glc (530), Epiafzelechin (4α → 8)Pg3-glc (531), Catechin (4α → 8)Pg3-glc (532), Epicatechin (4α → 8)Pg3-glc (533)	19 (2004) 13 (2004)
<i>Prunus cerasus</i> (Balaton, Montmorency)	Fruit	Cy3-[2-(glc)-6-(rha)glc], Cy3-[6-(rha)glc], Cy3-glc	370 (1997)
<i>Rosa</i> (<i>Cinnamomeae</i> , <i>Chinenses</i> , <i>Gallicanae</i>), 44 spp.	Flowers	Cy3,5-di-glc, Cy3-[2-(glc)glc], Cy3-[6-(cum)glc], Cy3-[6-(rha)glc], Cy3-glc, Pg3,5-di-glc, Pg3-glc, Pn3,5-di-glc, Pn3-[6-(cum)glc], Pn3-[6-(rha)glc]	300 (1995) 301 (2000)
<i>Rosa hybrida</i>	Flower	Rosacyanin B (526)	17 (2002)
<i>Rubus iaciniatus</i>	Berries	Cy3-[6-di-(oxa)glc] ^{d, g}	57 (2002)
Rubiaceae			
<i>Cephaelis subcoriacea</i> ^g	Fruits	Cy3-glc	339 (1994)
Rutaceae			
<i>Citrus sinensis</i> (Florida) ^g	Juice	Cy3-[6-(mal)glc], Cy3-glc	371 (2002)
Sapindaceae			
<i>Litchi chinensis</i> Sonn.	Pericarp	Cy3-[6-(rha)glc], Cy3-glc, Cy3-gal, Pg3,7-di-glc	372 (1993) 373 (2000) 374 (2004)
Scrophulariaceae			
<i>Mimulus cardinalis</i> ^g	Flowers	Cy3-glc, Pg3-glc	375 (1997)
<i>Mimulus lewisii</i> ^g	Flowers	Cy3-glc, Pg3-glc	375 (1997)
Solanaceae			
<i>Petunia</i> ^f (<i>Baccara</i> , <i>Carpet</i> , <i>Celebrity</i> , <i>Fantasy</i> , <i>Fulcon</i> , <i>Madness</i> , <i>Prime</i> <i>Time</i>), 17 spp.	Pink flowers	Pn3-[6-(4-(4-(6-(caf)glc)cum)rha)glc]-5-glc (296), Pn3- [6-(4-(4-(4-(6-(caf)glc)cum)rha)glc]-5-glc (295), Pn3-[cum-6- (rha)glc]-5-glc, Pn3caf[6-(rha)glc]-5-glc, Pn3-[6- (rha)glc]-5-glc	165 (2004)
<i>Petunia</i> “ <i>Mitchell</i> ” (<i>P. axillaries</i> × <i>P.</i> <i>hybrida</i>) ^g	Leaves	Pt3-[cum-6-(rha)glc]-5-glc, Pt3-[6-(rha)glc]-5-glc, Pt3- [caf-6-(rha)glc]-5-glc	376 (1998)
<i>Petunia exserta</i>	Flowers	Cy3-[6-(rha)glc], Cy3-glc, Pg3-[6-(rha)glc], Pg3-glc	377 (1999)
<i>Petunia guarapuavensis</i>	Flowers	Mv3-[6-(4-(4-(6-(caf)glc)cum)rha)glc]-5-glc (482), Mv3-[6-(4-(4-(6-(caf)glc)caf)rha)glc]-5-glc (484)	162 (1997)
<i>Petunia hybrida</i>	Flowers	Mv3-[6-(4-(4-(6-(caf)glc)caf)rha)glc]-5-glc, Mv3-[6-(4- (4-(6-(caf)glc)cum)rha)glc]-5-glc, Mv3-[6-(4-(4-(6- (cum)glc)cum)rha)glc]-5-glc (481), Mv3-[6-(4-(4-(6- (fer)glc)cum)rha)glc]-5-glc (483), Mv3-[6-(4- (caf)rha)-5-glc (478), Mv3-[6-(4-(Z-cum)rha)glc]-5- glc (477), Pt3-[6-(4-(4-(6-(caf)glc)cum)rha)glc]-5-glc (445)	159 (1998) 168 (1999) 161 (2001)

continued

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
<i>Petunia integrifolia</i> subsp. <i>inflata</i>	Strains	Mv3-[6-(4-(4-(6-(caf)glc)cum)rha)glc] (480), Mv3-[6-(4-(caf)rha)glc], Mv3-[6-(4-(cum)rha)glc]	163 (1999)
<i>Petunia occidentalis</i>	Flowers	Dp3-[6-(4-(caf)rha)glc]-5-glc (365), Mv3-[caf-glc-rut] ^c , Pt3-[6-(4-(4-(glc)cum)rha)glc]-5-glc (444), Pt3-[caf-glc-caf-rut]-5-glc ^c , Pt3-[caf-glc-cum-rut]-5-glc (445) ^c , Pt3-[caf-glc-rut] ^c , Pt3-[cum-glc-rut]-5-glc ^c	160 (1999)
<i>Petunia reitzii</i>	Flowers	Dp3-[6-(4-(4-(6-(caf)glc)cum)rha)glc]-5-glc (390), Dp3-[6-(4-(4-(glc)cum)rha)glc]-5-glc (385), Dp3-[6-(rha)-2-(caf)glc]-5-glc, Dp3-[6-(rha)-2-(cum)glc]-5-glc, Dp3-[6-(rha)-2-(Z-cum)glc]-5-glc, Dp3-[6-(rha)glc], Dp3-[6-(rha)glc]-5-glc, Pt3-[6-(rha)glc]-5-glc, Pt3-[6-(rha)-2-(caf)glc]-5-glc, Pt3-[6-(rha)-2-(cum)glc]-5-glc, Pt3-[6-(rha)-2-(Z-cum)glc]-5-glc	164 (2000)
<i>Solanum melongena</i>	Skin	Dp3-[6-(4-(cum)rha)glc]-5-glc, Dp3-[6-(4-(Z-cum)rha)glc]-5-glc ^d	378 (2001)
<i>Solanum stenotomum</i> ^e	Tubers	Dp3-[cum-6-(rha)glc]-5-glc, Mv3-[cum-6-(rha)glc]-5-glc, Mv3-[fer-6-(rha)glc]-5-glc, Pn3-[caf-6-(rha)glc]-5-glc, Pn3-[cum-6-(rha)glc]-5-glc, Pn3-[fer-6-(rha)glc]-5-glc, Pt3-[caf-6-(rha)glc]-5-glc, Pt3-[cum-6-(rha)glc], Pt3-[cum-6-(rha)glc]-5-glc, Pt3-[Z-cum-6-(rha)glc]-7-glc ^c , Pt3-[fer-6-(rha)glc]-5-glc	379 (2003)
<i>Solanum andigena</i> × <i>Solanum tuberosum</i>	Tubers	Pg3-[6-(4-(fer)rha)glc]-5-glc (62), Pg3-[6-(4-(cum)rha)glc]-5-glc	171 (1998)
<i>Solanum tuberosum</i> (Congo)	Tubers and shoots	Mv3-[6-(4-(fer)rha)glc]-5-glc (479), Pt3-[6-(4-(fer)rha)glc]-5-glc (443)	172 (2000)
<i>Solanum tuberosum</i>	Tubers	Pn3-[6-(4-(caf)rha)glc]-5-glc (288), Pn3-[6-(4-(cum)rha)glc]-5-glc, Pt3-[6-(4-(caf)rha)glc]-5-glc (442), Pt3-[6-(4-(cum)rha)glc]-5-glc (441)	169 (2003)
Theaceae			
<i>Camellia sinensis</i>	Leaves	Cy3-gal, Dp3-[6-(cum)gal] (326), Dp3-gal	158 (2001)
<i>Visnea mocanera</i> ^e	Fruits	Cy3-gal, Cy3-glc, Dp3-glc, Mv3-glc, Pn3-glc, Pt3-glc	380 (1996)
Umbelliferae (=Apiaceae)			
<i>Daucus carota</i> (Nentes scarlet-104)	Cell culture	Cy3-[2-(xyl)gal], Cy3-glc	381 (2000)
<i>Glehnia littoralis</i>	Petiole-derived callus cultures	Cy3-[2-(xyl)-6-(6-(fer)glc)glc] (203)	34 (1998)
Verbenaceae			
<i>Verbena hybrida</i>	Flowers	Cy3,5-di[6-(ace)glc] (181), Cy3-[6-(mal)glc], Pg3,5-di[6-(ace)glc] (47), Pg3,5-di-glc, Pg3-[6-(ace)glc], Pg3-[6-(ace)glc]-5-glc, Pg3-[6-(mal)glc], Pg3-[6-(mal)glc]-5-[6-(ace)glc] (48), Pg3-glc-5-[6-(ace)glc] (31)	382 (1991) 383 (1995) 384 (1995)

TABLE 10.2
Occurrence of Anthocyanins Reported after 1992 — *continued*

Taxon	Organ	Pigment ^a	References ^b
Vitaceae			
<i>Vitis vinifera</i> ^g	Berries	Dp-, Cy-, Pt3-[6-(ace)glc], Pn3-[6-(ace)glc] (278), Mv3-[6-(ace)glc] (461), Dp3-[6-(cum)glc] (327), Cy-, Pt-, Pn-, Mv3-[6-(cum)glc], Dp-, Cy-, Pt-, Pn-, Mv3-glc, Dp-, Pt3,5-di-glc, Pn3-[6-(caf)glc] (281), Mv3-[6-(caf)glc]	385 (1995) 386 (2004)

Notes: ace, acetic acid; oxa, oxalic acid; mal, malonic acid; suc, succinic acid; mly, malic acid; hba, *p*-OH-benzoic acid; gao, gallic (tri-OH-benzoyl) acid; cum, *p*-coumaric acid; caf, caffeic acid; fer, ferulic acid; sin, sinapic acid; hca, 3,5-diOHcinnamic acid; tar, tartaric acid; ara, arabinose; xyl, xylose; rha, rhamnose; gal, galactose; glc, glucose; glu, glucuronic acid; 2-(xyl)glc, sambubiose; 2-(xyl)gal, lathyrose; 2-(rha)glc, neohesperidose; 6-(rha)gal, robinose; 6-(rha)glc, rutinose; 2-(glc)glc, sophorose; 3-(glc)glc, laminariobiose; 6-(glc)glc, gentiobiose.

^aSee Table 10.1 for anthocyanidin abbreviations and linkage positions.

^bNumbers in brackets refer to the year of the publication.

^cNumbers in bold represent the first report of the pigments not listed in previous editions of *The Flavonoids*.¹⁻⁴ See Appendix A.

^dPossibly new anthocyanins.

^ePigments assigned tentatively.

^fEach genus involved may include one or more samples (species or plant organs). Each sample contains one or more of the listed anthocyanins.

^gStructures are based on TLC and HPLC or MS data.

^hProduced by genetically modified violet carnations.

ⁱProduced during experimental workup.

new anthocyanins based on rutinose have also been reported from *Campanula* (Campanulaceae),¹¹⁷ *Lobelia* (Lobeliaceae),¹¹⁸ *Ceanothus* (Rhamnaceae),³² *Delphinium* (Ranunculaceae),¹¹⁹ *Lilium*,¹²⁰ *Tulipa* (Liliaceae),^{121,122} and *Alstroemeria* (Alstroemeriaceae).¹⁰⁶ It is interesting to observe that a transgenic *Eustoma* (Gentianaceae) line produced by insertion of an *Antirrhinum majus* cDNA coding for UDP-glucosyl:flavonoid-3-*O*-glucosyltransferase contained in its petals significant levels of 3-rutinoside and 3-glucoside derivatives of delphinidin, in which glucose has replaced the 3-*O*-linked galactose present in the original anthocyanins of the nontransformed plants.¹²³ After 1992, sambubiose units have mainly been found in new anthocyanins from *Matthiola* and *Arabidopsis* (Cruciferae) (Table 10.2), and in some new anthocyanins from *Begonia* (Begoniaceae),¹¹⁶ *Vaccinium* (Ericaceae),¹²⁴ *Sambucus* (Caprifoliaceae),¹²⁵ *Ranunculus* (Ranunculaceae),¹²⁶ and *Meconopsis* (Papaveraceae).^{127,128}

Among the disaccharides with more limited occurrence in anthocyanins, new reports on robinobiose include six 3-[6-(rhamnosyl)galactosides-5-glycosides of delphinidin and cyanidin isolated from purple *Lisianthus* (Gentianaceae) flowers,¹²⁹ in addition to cyanidin 3-[2-(galloyl)-6-(rhamnosyl)galactoside] from red flowers of the chenille plant *Acalypha hispida* (Euphorbiaceae).¹³⁰ Two new anthocyanins containing lathyrose, delphinidin 3-[2-(xylosyl)-galactoside]-5-[6-(acetyl)glucoside] and its deacetylated derivative, have been isolated from purple pods of pea (*Pisum* spp.).¹³¹ Both pigments showed moderate stability and anti-oxidative activity in a neutral aqueous solution. Three anthocyanins with a pelargonidin 3-lathyroside skeleton acylated with malonic acid have been isolated from scarlet flowers of *Anemone coronaria*.¹³² A minor anthocyanin accumulated in the cultured cells of *Aralia cordata* (Araliaceae) has been identified as peonidin 3-lathyroside.¹³³ This disaccharide has

previously been isolated mainly from species belonging to Umbelliferae⁴ and Araliaceae.¹³⁴ Laminariobiose seems to have an even more restricted occurrence in anthocyanins, identified in anthocyanins isolated mainly from the genus *Allium*.^{18,135} Quite extraordinary, this disaccharide has been found linked to the 5-position of the major 3-deoxyanthocyanin isolated from the fern *Blechnum novae-zelandiae*.⁹⁶

The structure of the major anthocyanin tradescantin in *Tradescantia pallida* has been determined to be cyanidin 3-[6-(2,5-di-(ferulyl)arabinosyl)glucoside]-7,3'-di-[6-(ferulyl)glucoside].¹³⁶ The same disaccharide, 6-arabinofuranosyl-glucopyranose, has previously been found in zebrinin isolated from *Zebrina pendula*,¹³⁷ which belongs to the same family (Commelinaceae) as *Tradescantia*. Tradescantin displays a very high stability,¹³⁸ most probably because of its structural conformation, which allows sandwich-type complex formation. Two malonylated 3-gentiobiosyl derivatives of delphinidin, linked covalently to different flavones, have been isolated from the flowers of *Eichhornia crassipes* (Pontederiaceae).^{9,10} Anthocyanins that contain neohesperidose have been isolated from petals of the blue marguerite daisy *Felicia amelloides* (Asteraceae) as delphinidin 3-neohesperidoside-7-[6-(malonyl)glucoside],¹³⁹ as peonidin 3-neohesperidoside from petals of two cultivars of *Cyclamen persicum* (Primulaceae),¹⁴⁰ and as the 3-[2-(rhamnosyl)-6-(malonyl)glucoside] and its deacylated form of delphinidin from petals of a mauve line of *Clitoria ternatea* (Leguminosae).¹⁴¹ Neohesperidose has previously only been identified in anthocyanins from species belonging to the gymnosperm family Podocarpaceae.¹⁴²

10.2.4.3 Trisaccharides

Altogether 19 anthocyanins based on seven trisaccharides, 2-glucosyl-6-rhamnosylglucose, 2-xylosyl-6-rhamnosylglucose, 2-xylosyl-6-glucosylgalactose, 2-xylosyl-6-glucosylglucose (new), 6-(6-glucosylglucosyl)glucose, 3-(3-glucosylglucosyl)glucose, and 3-glucosyl-6-glucosylglucose, have been identified. Among the novel anthocyanins containing a trisaccharide reported after 1992, the 3-[6-(rhamnosyl)-2-(xylosyl)glucoside] of delphinidin has been isolated as the major anthocyanin from scarlet flowers of *Linum grandiflorum*,¹⁰⁶ while the same triglycoside of petunidin and peonidin has been isolated in minor amounts from fruits of *Vaccinium padifolium*.²⁵ These latter fruits also contain three novel 3-sambubiosides of petunidin, peonidin, and malvidin,¹²⁴ in contrast to previous reports on pigments of plants in Ericaceae, which show that a variety of 3-mono-glycosides are regularly present. It is interesting to observe that cyanidin 3-[6-(6-((*E*)-sinapyl)glucosyl)-2-(xylosyl)glucoside] has been reported to be produced in *Glehnia littoralis* (Umbelliferae) callus cultures,³⁴ and not the analogous 6-rhamnosyl-2-xylosylgalactose derivative identified in its relative *Daucus carota*.³³

10.2.4.4 Glycosidic Linkages

Anthocyanins bear glycosidic moieties in the anthocyanidin 3-, 5-, 7-, 3'-, or 5'-position (Figure 10.6). Nearly all anthocyanins have a sugar located at the 3-position (Appendix A). The only exceptions are the 3'-[2-(galloyl)galactoside] and 3'-[2-(galloyl)-6-(acetyl)galactoside] of delphinidin isolated from blue flowers of the African water lily *Nymphaea caerulea*²⁶ and the 4'-glucoside and 7-[3-(glucosyl)-6-(malonyl)glucoside]-4'-glucoside of cyanidin from red onion (*Allium cepa*).¹⁸ The desoxyanthocyanins (Appendix A) including the new luteolinidin 5-[3-(glucosyl)-2-(acetyl)glucoside] recently isolated from the fern *Blechnum novae-zelandiae*,⁹⁶ of course, cannot have any sugar in their 3-positions. Several anthocyanidin 5-glycosides and anthocyanidin 7-glycosides without sugar in their 3-positions have previously been reported.⁸ However, all of these may be classified as tentative structures due to lack of data (e.g., long-range ¹H-¹³C couplings in heteronuclear NMR spectra) for absolute identification of the linkage positions.

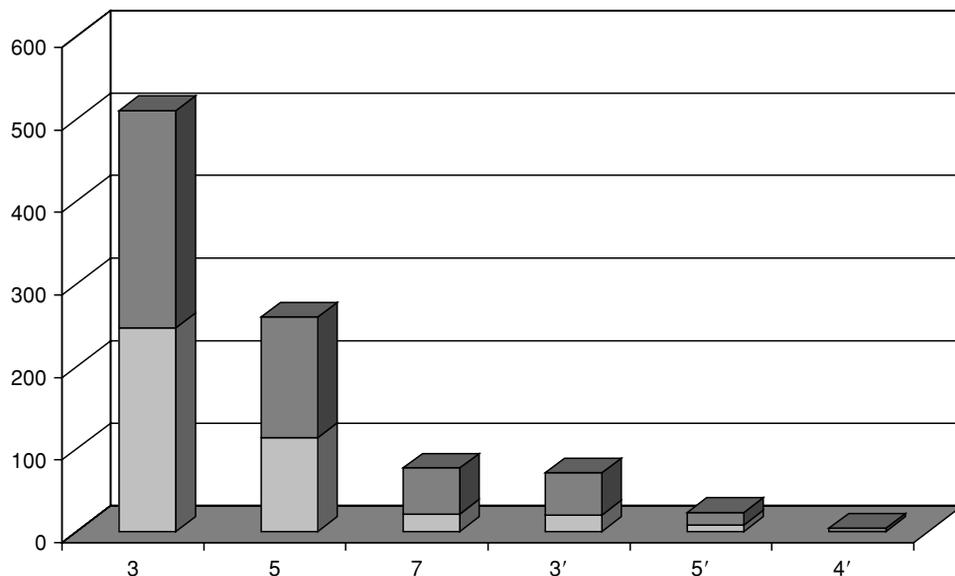


FIGURE 10.6 The number of anthocyanins with glycosyl moieties linked to the various anthocyanidin positions. The upper dark part of each bar represents the anthocyanins reported later than 1992. See Table 10.1 for structures.

About 146 and 65 novel anthocyanins reported after 1992 have a second sugar moiety located at their 5- and 7-positions, respectively (Appendix A). With the exception of two desoxyanthocyanins from the fern *Blechnum procerum*,¹⁴³ no identified anthocyanin has sugars linked to both the 5- and 7-positions. In the same period, 51 new anthocyanins with a glucosyl moiety in the 3'-position have been reported. Most of these have been isolated from species belonging to Orchidaceae (16), *Clitoria* (Leguminosae) (12), Ranunculaceae (6), and Gentianaceae (5). However, a few were found in Compositae, Liliaceae, Rhamnaceae, Nymphaeaceae, Lobeliaceae, and Commelinaceae (Table 10.2). In contrast to most anthocyanidin 3'-glycosides reported, five delphinidin and cyanidin derivatives from flowers of *Anemone coronaria* (Table 10.2) have glucuronic acid instead of glucose linked to the B-ring.^{28,29} Similarly, glucose was replaced with galactose in delphinidin 3'-[2-(galloyl)-galactoside] and its acetylated derivative isolated from extracts of *Nymphaea caerulea* flowers.²⁶

In addition to the previously identified anthocyanins with a sugar in their 5'-positions from flowers of *Clitoria ternatea* (five anthocyanins) and *Lobelia erinus* (two anthocyanins), 12 further ternatins and preternatins have been isolated from *Clitoria ternatea* (Leguminosae),^{144,145} and three anthocyanins from the genus *Dianella* (Liliaceae). The three polyacetylated delphinidin 3,7,3',5'-tetraglucosides from berries of two *Dianella* species¹⁴⁶ showed exceptional blueness at *in vivo* pH values due to effective intramolecular copigmentation involving *p*-coumarylglucose units at the 7-, 3'-, and 5'-positions of the aglycone. It has also been reported that the five new polyacylated delphinidin 3,3',5'-triglucosides from *Clitoria ternatea* flowers formed intramolecular stacking between the aglycone and the 3',5'-coumarylglucosyl side chains in solution.¹⁴⁴

Four cyanidin 4'-glucosides have recently been isolated from pigmented scales of red onion.¹⁴⁷ These structures were established by extensive use of 2D NMR spectroscopy and electrospray LC-MS. The previous report on 4'-glucosyl linkages in two anthocyanins from

*Hibiscus esculentus*¹⁴⁸ was mainly based on lack of bathochromic shifts by the addition of AlCl_3 to a solution containing these anthocyanins, which is inadequate as proof for absolute identification of the 4'-linkages. Among the desoxyanthocyanins, Nair and Mohandoss¹¹² have previously indicated the occurrence of luteolinidin 4'-glucuronide from flowers *Holmskioldia sanguinea* (Verbenaceae). Compared with spectra of cyanidin 3-glycosides, the cyanidin 4'-glucosides from red onions showed hypsochromic shifts (10 nm) of visible λ_{max} and hyperchromic effects on wavelengths around 440 nm, similar to pelargonidin 3-glycosides.¹⁴⁷ Glycosidic substitution of the other B-ring hydroxyl groups (3' and 5') causes similar hypsochromic shifts of visible λ_{max} .³

10.2.5 ANTHOCYANINS WITH ACYLATION

More than 65% of the reported anthocyanins with properly identified structures are acylated, and anthocyanin diversity is highly associated with the nature, number, and linkage positions of the acyl groups. The aromatic acyl groups of these anthocyanins include various hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic, sinapic, and 3,5-dihydroxycinnamic acids) and two hydroxybenzoic acids (*p*-hydroxybenzoic and gallic acids). Malonic acid is the most frequent aliphatic acyl group, while acetic, malic, oxalic, succinic, and tartaric acids have a more restricted distribution. As much as four different acyl groups located at four different glycosyl moieties have been identified in Lobelinin B isolated from flowers of *Lobelia erinus*.¹⁴⁹ The number of anthocyanins containing the different acyl moieties is presented in Figure 10.7.

During the period of this review, Pale et al.²⁷ have isolated a triacylated-tetraglycosylated cyanidin derivative (**243**) from flowers of *Ipomoea asarifolia*, which contained *E*-3,5-dihydroxycinnamic acid linked to the 6-position of one of the glucosyl moieties. This acyl

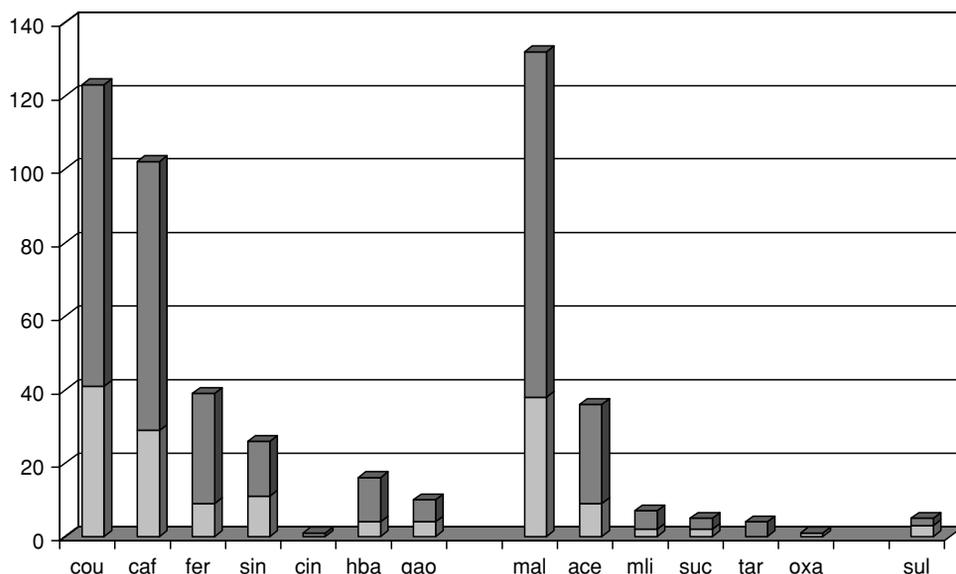


FIGURE 10.7 Numbers of anthocyanins containing the various acyl moieties identified in anthocyanins. The upper dark part of each bar represents the anthocyanins reported later than 1992. cou, *p*-coumaric acid; caf, caffeic acid; fer, ferulic acid; sin, sinapic acid; cin, 3,5-dihydroxycinnamic acid; hba, *p*-hydroxybenzoic acid; gao, gallic acid; mal, malonic acid; ace, acetic acid; mli, malic acid; suc, succinic acid; tar, tartaric acid; oxa, oxalic acid; sul, sulfate.

group has previously not been identified in any anthocyanin. Several nonnaturally occurring acids have also been identified in acylated forms in anthocyanins isolated from wild carrot suspension cultures provided with these acids in the medium.^{150,151} Among the dicarboxylic acids, tartaryl has for the first time been identified in four anthocyanins (**82**, **246**, **383**, **401**) isolated from flowers of *Anemone coronaria*.^{28,132} Interestingly, the first anthocyanins found conjugated with sulfate, malvidin 3-glucoside-5-[2-(sulfato)glucoside], and malvidin 3-glucoside-5-[2-(sulfato)-6-(malonyl)glucoside], have been isolated from violet flowers of *Babiana stricta* (Iridaceae).³⁰ Several other flavonoid classes than anthocyanins have previously been reported to contain sulfate groups.¹⁵²

10.2.5.1 Acylation with Phenolic Acids

Two hundred and seventy-nine anthocyanins with aromatic acylation have been identified (Appendix A), and 67% have been reported after 1992. Ninety-three anthocyanins are reported to be acylated with both aromatic and aliphatic acyl groups.

10.2.5.1.1 *p*-Coumaric Acid

More than 150 anthocyanins acylated with *p*-coumaric acid have been reported. Among these, most of the 132 anthocyanins, which have been assigned with a complete structure (Appendix A), have the *p*-coumaryl unit(s) in glucose 6-position(s) within a glycosidic moiety linked to the 3-position of the aglycone. In anthocyanins from the genera *Eustoma*^{123,129} and *Gentiana*^{153,154} (Gentianaceae), the *p*-coumaryl was found to be linked to the 5-glucosyl and not the 3-glucosyl. However, in Albireodelphin D (**373**), isolated from *Gentiana*,¹⁵⁴ this acyl moiety was connected to the 3'-glucosyl. This latter linkage position of the *p*-coumaryl has also been identified in delphinidin 3-[6-(rhamnosyl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(*p*-coumaryl)glucoside] isolated from flowers of *Ceanothus papillosus* (Rhamnaceae),³² and in anthocyanins from orchids (Orchidaceae),¹⁵⁵⁻¹⁵⁷ *Clitoria ternatea* (Leguminosae) (called ternatins),^{4,144,145} and *Dianella* spp. (Liliaceae) fruits.¹⁴⁶ Most of these anthocyanins from the two latter genera contained another *p*-coumaryl unit located within the 5'-glycosyl moieties. In delphinidin 3,7,3',5'-tetra[6-(*p*-coumaryl)glucoside] from *Dianella* spp., as many as four 6-*p*-coumarylglucoside units were located at different aglycone positions.¹⁴⁶

In anthocyanin 3-rutinosides acylated with *p*-coumaric acid isolated from species in Solanaceae, this acyl group was found to be linked to the rhamnose 4-position (Appendix A). A similar linkage has also been reported in anthocyanins from species belonging to *Lobelia erinus* (Lobeliaceae),^{118,149} and previously from *Silene dioica* (Caryophyllaceae), *Viola* spp. (Violaceae), and *Iris* spp. (Iridaceae). The identification by Terahara et al.¹⁵⁸ of *p*-coumaryl linked to galactose in delphinidin 3-[6-(*E-p*-coumaryl)galactoside] isolated from leaves of red flower tea, *Camellia sinensis*, is really outstanding.

In ten anthocyanins from various *Petunia* spp. (Solanaceae),¹⁵⁹⁻¹⁶⁵ the coumaryl unit had another glucosyl moiety connected to its 4-hydroxyl group, making diverse anthocyanins with 3-glucosyl-rhamnosyl-*p*-coumaryl-glucosyl-(acyl?) moieties. Similar esterification has also been found in 14 ternatins from *Clitoria ternatea* (Leguminosae),^{4,144,145} four anthocyanins from orchids (Orchidaceae),¹⁵⁵⁻¹⁵⁷ in delphinidin 3-[6-(4-(glucosyl)coumaryl)glucoside]-5-[6-(malonyl)glucoside] from flowers of *Triteleia bridgesii* (Liliaceae),¹⁶⁶ in cyanidin 3-[2-(glucosyl)-6-(4-(glucosyl)coumaryl)glucoside]-5-glucoside isolated from cabbage,¹³⁷ and in cyanidin 3-[6-(4-(glucosyl)coumaryl)-2-(2-(sinapyl)xylosyl)glucoside]-5-[6-(malonyl)glucoside] from leaves and stems of *Arabidopsis thaliana*.¹⁶⁷ In orchids, these alternating 3-glucosyl-*p*-coumaryl-glucosyl chains were characteristically located in the aglycone 3'-positions, while most of the ternatins contained this type of chain in both the aglycone 3'- and 5'-positions. The largest monomeric anthocyanin recorded to date, ternatin A1, has

been found to be built by delphinidin, seven glucosyls, four *p*-coumaryls, and one malonyl units (molecular mass: 2108.87 g mol⁻¹).¹⁰⁸

10.2.5.1.2 Caffeic Acid

Most of the 100 anthocyanins that are acylated with caffeic acid have this cinnamyl moiety linked to a glucosyl 6-position (Appendix A). Some anthocyanins isolated from species belonging to Solanaceae (**288, 365, 442, 478, 484**)^{159–164,168,169} and *Silene dioica* (Caryophyllaceae) (**166, 201**)¹⁷⁰ have a caffeoyl located on the 4-position of the rhamnosyl unit of the disaccharide rutinose. This acyl group has also been shown to be attached to the 2- and 5-hydroxyl groups of arabinose in anthocyanidin 6-arabinofuranosylglucosides (**166, 201**) isolated from *Zebrina pendula* (Commelinaceae).¹³⁷ Additionally, it has been linked to the 2-hydroxyl group of glucose in pelargonidin 3-[2-(2-(*E*-caffeoyl)glucosyl)galactoside] from flowers of *Pulsatilla cernua*,²² and to the same position of the same disaccharide in several anthocyanins (**234, 240, 246, 383, 393, 398, 401**) isolated from flowers of *Anemone coronaria*,^{28,29} which also belong to Ranunculaceae.

Rather exceptionally, caffeic acid is found to be esterified with a tartaryl hydroxyl in one end, and a glucosyl in the phenolic 4-hydroxyl in the other end in pelargonidin 3-[2-(xylosyl)-6-(3-(3-(4-(glucosyl)caffeoyl)-2-tartaryl)malonyl)galactoside] isolated from scarlet flowers of *Anemone coronaria* “St. Brigid.”¹³² Several anthocyanins from species within each of the families Compositae, Orchidaceae, Solanaceae, and Convolvulaceae have a glucosyl in the phenolic 4-hydroxyl group of caffeic acid, making a chain of alternating glucosyl–caffeoyl–glucosyl units (Table 10.2). In 15 anthocyanins from species in Convolvulaceae (Table 10.2), the last caffeoyl unit with a glucosyl linked to the phenolic end has remarkably the sugar linked to the 3-hydroxyl of caffeic acid.

10.2.5.1.3 Ferulic Acid

More than 59 different anthocyanins have been reported to be acylated with ferulic acid; however, only 39 have been assigned with a complete structure. These anthocyanins were isolated for the first time from species belonging to the following families: Cruciferae (18), Labiatae (5), Solanaceae (4), Umbelliferae (3), Convolvulaceae (2), Gentianaceae (2), Liliaceae (2), Commelinaceae (1), Lobeliaceae (1), and Orchidaceae⁴ (Table 10.2). Most of the anthocyanins have the ferulyl unit(s) in glucose 6-position(s) within a glycosidic moiety linked to the aglycone 3-position. However, this cinnamic acid has also been shown to be attached to the 4-hydroxyl group of rhamnose in three anthocyanidin 3-rutinosides (**62, 443, 479**) from red¹⁷¹ and purple¹⁷² potatoes (Solanaceae), and to the 2-hydroxyl group of xylose in pelargonidin 3-[6,2-di-(ferulyl)sambubioside]-5-[6-(malonyl)glucoside] isolated from flowers of *Matthiola incana* (Cruciferae).¹⁷³ Remarkably, this acyl group has been shown to be attached to the 2- and 5-hydroxyl groups of arabinose in cyanidin 3-[6-(2,5-di-(*E*-ferulyl)arabinosyl)-glucoside]-7, 3'-di-[6-(*E*-ferulyl)-glucoside].¹³⁶ This major anthocyanin from leaves of *Tradescantia pallida* (Comelinaceae) shows an extra absorption band at 583 nm at pH values above 4.0, which makes this pigment highly colored at these pH values.¹⁷⁴ The high stability¹³⁸ of this type of pigments with as much as four acyl units was assumed to be due to the difficulty of water molecule diffusion to the hydrophobic center formed by the acyl groups and the aglycone.¹⁷⁵

Ferulyl has been found as part of the glycosidic moiety linked to the anthocyanidin 5-position only in delphinidin 3-[6-(rhamnosyl)galactoside]-5-[6-(ferulyl)glucoside] from flowers of *Eustoma grandiflorum* (Gentianaceae),¹²⁹ to the 7-position in cyanidin 3-[6-(malonyl)glucoside]-7-[6-(*E*-ferulyl)glucoside]-3'-glucoside from *Sophronis coccinea* (Orchidaceae)¹⁷⁶ and cyanidin 3-[6-(2,5-di-(*E*-ferulyl)arabinosyl)-glucoside]-7,3'-di-[6-(*E*-ferulyl) glucoside] from *Tradescantia pallida*,¹³⁶ and to the 5'-position in Lobelinin B (**413**) from *Lobelia erinus* (Lobeliaceae).¹⁴⁹

In cyanidin 3-[6-(4-(glucosyl)ferulyl)sophoroside]-5-glucoside from red cabbage¹³⁷ and malvidin 3-[6-(4-(4-(6-ferulyl)glucosyl)-*E-p*-coumaryl)rhamnosyl]glucoside]-5-glucoside from flowers *Petunia hybrida*,¹⁶¹ the ferulyl unit has a glucosyl moiety linked to its phenolic 4-hydroxyl group.

10.2.5.1.4 Sinapic Acid

Most of the 26 anthocyanins that are acylated with sinapic acid have been isolated from cabbage (*Brassica* spp.), stock (*Matthiola incana*), mustard (*Sinapis alba*), and mustard weed (*Arabidopsis thaliana*) in Cruciferae, yam (*Dioscorea alata*) in Dioscoreaceae, and from the genera *Phalaenopsis*, *Dendrobium*, and *Vanda* in Orchidaceae⁴ (Table 10.2). In addition, the 3-[6-(6-(sinapyl)glucosyl)-2-(xylosyl)galactoside] and 3-[6-(6-(sinapyl)glucosyl)galactoside] of cyanidin have been isolated from cell suspension cultures of an Afghan cultivar of *Daucus carota* (Umbelliferae).³³

In anthocyanins, sinapyl is mainly connected to the 6-hydroxyl of glucose or 2-hydroxyl of xylose. This latter linkage was found in nine anthocyanins from stock, mustard, and mustard weed in Cruciferae.^{167,173,177} In some anthocyanins isolated from cabbage,¹⁷⁸ sinapyl has also been found in the glucose 2-position (219, 220, 227, 229), while the 3-[4-(sinapyl)-6-(glucosyl)glucosides] of cyanidin and peonidin isolated from jam¹⁷⁹ have this acyl group located in the glucose 4-position.

In anthocyanins isolated from orchids (222, 230, 376, 382), the sinapyl was part of the glycosidic moieties linked to the aglycone 7- and 3'-positions,¹⁸⁰⁻¹⁸² while the other anthocyanins mentioned above had this acyl group linked to the glycoside in the aglycone 3-position.

10.2.5.1.5 *p*-Hydroxybenzoic Acid

Most of the 16 anthocyanins that are acylated with *p*-hydroxybenzoic acid (Appendix A) have been isolated from flowers of *Delphinium hybridum*,^{109,119,183} *Aconitum chinense*,¹⁸⁴ *Consolida armeniaca* (Ranunculaceae),¹¹⁵ *Campanula* species (Campanulaceae),^{117,185,186} or roots of *Ipomoea batatas* (Convolvulaceae).^{187,188} In the examined species from Ranunculaceae and *Campanula*, one to as many as four *p*-hydroxybenzoyl moieties belong to the anthocyanidin 7-glycoside. In *p*-hydroxybenzoylated anthocyanins from sweet potatoes (*Ipomoea batatas*), this acyl group is located within the 3-glucoside. In addition, cyanidin 3-[6-(malonyl)glucoside]-7,3'-[6-(4-(glucosyl)-*p*-hydroxybenzoyl)glucoside] and cyanidin 3-[6-(6-(*p*-hydroxybenzoyl)glucosyl)-2-(xylosyl)galactoside] have been isolated from red-purple flowers of *Dendrobium* "Pramot" (Orchidaceae)¹⁸⁹ and cell suspension cultures of *Daucus carota* (Umbelliferae),³³ respectively. When determined, the *p*-hydroxybenzoyl of anthocyanins is in all cases linked to the 6-position of one glucosyl moiety.

10.2.5.1.6 Gallic Acid

Eleven anthocyanins have been reported to be galloylated. Anthocyanins with gallic acid (3,4,5-trihydroxybenzoic acid) as acyl substituent have been found in leaves of the giant water lily (*Victoria amazonica*) in Nymphaeaceae as the 3-[2-(galloyl)galactoside] of delphinidin and cyanidin.¹⁹⁰ These anthocyanins together with their 6''-acetylated analogs were also identified in *Nymphaea* × *marliacea*¹⁹¹ and *Nymphaea alba*.¹⁹² The 2''-galloyl galactoside and 2-(galloyl)-6-(acetyl)galactoside of delphinidin have been identified in the blue flowers of *Nymphaea caerulea*; however, these moieties were in this latter species located in the rare 3'-position.²⁶

The distribution of galloylated anthocyanins outside Nymphaeaceae seems to be restricted. They occur in leaves in Aceraceae as the 3-[6-(galloyl)glucoside], 3-[6-(galloyl)rutinoside], and 3-[2,3-di-(galloyl)glucoside] of cyanidin,¹⁹³⁻¹⁹⁵ and as the 3-[2-(galloyl)galactoside] and 3-[2-(galloyl)-6-(rhamnosyl)galactoside] of cyanidin in flowers of *Acalypha hispida* (Euphorbiaceae).¹³⁰ An anthocyanin from seeds of *Abrus precatorius* (Leguminosae) has tentatively been identified as delphinidin 3-*p*-coumaryl-galloylglucoside.¹⁹⁶

10.2.5.2 Acylation with Aliphatic Acids

Among the 178 anthocyanins with aliphatic acylation (Appendix A), more than 70% have been reported after 1992.

10.2.5.2.1 Malonic Acid

One or two malonyl units have been found in 132 anthocyanins from a variety of families, and 100 have been reported during the period of this review. Most of these pigments have the malonyl unit(s) in glucose 6-position(s); however, the anthocyanins containing malonyl, which have been isolated from various flowers of *Anemone coronaria* (Ranunculaceae),^{28,29,132} has this dicarboxylic acyl group linked to the galactose 6-position. In another interesting report, Mazza and Gao¹⁹⁷ have isolated the 3-malonylxyloside, 3-dimalonylxyloside, and 3-malonylarabinoside of cyanidin from seeds of *Helianthus annuus*; however, in these cases the linkage positions have not been determined.

From species in Labiatae, the 3-[6-(*p*-coumaryl)glucoside]-5-[4,6-di-(malonyl)glucoside] of cyanidin, delphinidin, and pelargonidin, and the 3-[6-(caffeyl)glucoside]-5-[4,6-di-(malonyl)glucoside] of the two latter anthocyanidins have been reported.^{203–205} The location of malonyl to the glucose 4-position has also been reported for cyanidin 3-[4-(malonyl)-2-(glucuronosyl)glucoside] and pelargonidin 3-[6-(*p*-coumaryl)glucoside]-5-[4-(malonyl)glucoside] isolated from red flowers of *Bellis perennis* (Compositae)¹¹³ and *Hyacinthus orientalis* (Liliaceae),²⁰¹ respectively. Malonyl substitution in the sugar 3-position of anthocyanins has been found in 3-[3-(malonyl)glucoside] and 3-[3,6-di-(malonyl)glucoside] of cyanidin isolated from various organs of some *Allium* species.^{202–205} This latter anthocyanin has also been identified in flowers of chrysanthemum (*Dendranthema grandiflorum*)²⁰⁶ and in many grasses.²⁰⁷ On mild hydrolysis, it was converted to the 3-[3-(malonyl)glucoside], indicating that the malonic acid linkage to the 3-position of glucose was more stable than the more common linkage at the 6-position of glucose.²⁰²

The occurrence of the diester structures of the malonic acid moiety in natural anthocyanin pigments has so far been reported in pigments from flowers of *Eichhornia crassipes*^{9,10} and chive, *Allium schoenoprasum*,¹¹ where the anthocyanin–flavone and anthocyanin–flavonol disubstituted malonate structures were exhibited, respectively (Figure 10.8 and Figure 10.9). In some anthocyanins from flowers of *Anemone coronaria*, malonic acid is esterified with galactose in one end and tartaryl in the other end.^{29,132}

Malonylation has been found in two 6-hydroxyanthocyanins from *Alstroemeria*, the 3-[6-(malonyl)glucosides] of 6-hydroxycyanidin and 6-hydroxydelphinidin,^{101,102} and in 5-carboxypyranocyanidin 3-[6-(malonyl)glucoside] from red onion.¹⁸ A pigment containing methyl-malonyl, pelargonidin 3-[2-(xylosyl)-6-(methyl-malonyl)galactoside], has been reported to have been isolated from scarlet flowers of *Anemone coronaria*.¹³² Methyl esterification of the terminal carboxyl group of malonyl units can easily occur in acidified methanolic solvents during extraction and in the isolation process.^{167,342}

10.2.5.2.2 Acetic Acid

Altogether 36 anthocyanins with one or two acetyl groups have been identified (Appendix A). The number given in parenthesis shows the number of anthocyanins reported as novel pigments in species in the various families: Verbenaceae (8), Liliaceae (5), Geraniaceae (6), Vitaceae (5), Nymphaeaceae (5), Alliaceae (1), Theaceae (1), Rutaceae (1), Melastomataceae (1), Labiatae (1), Leguminosae (1), and Blechnaceae (1).

The acetyl groups are, in general, linked to glucosyl 6-positions; however, in anthocyanins from *Nymphaea* (129, 142, 322, 330, 331) and *Tulipa* (144, 332, 333), the galactosyl and rhamnosyl moieties, respectively, are acetylated. In the latter cases, the acetyl groups are located in the rhamnosyl 2- or 3-positions. Linkage to the rare 3-position has also been

reported for the acetyl group of an anthocyanin–flavonol complex (**537**) from *Allium schoenoprasum*.¹¹ Two other outstanding acetylated pigments, malvidin 3-[6-(*p*-coumaryl)glucoside]-5-[2-(acetyl)xyloside] and delphinidin 3-[6-(*p*-coumaryl)glucoside]-5-[4-(acetyl)-6-(malonyl)glucoside], have been isolated from flowers of *Tibouchina urvilleana*²⁰⁸ and *Salvia uliginosa*,²⁰⁹ respectively. The acyl group of the two similar acetylated malvidin 3,5-diglucosides (**465**, **466**), which have been isolated from *Geranium* flowers, is reported to be attached to either the 3-glucosyl²¹⁰ or the 5-glucosyl,²¹¹ with the latter as the most probable linkage in both cases. The only report of acetylated anthocyanins outside angiosperm families is luteolinidin 5-[2-(acetyl)-3-(glucosyl)glucoside] from the fern *Blechnum novae-zelandiae*.⁹⁶

10.2.5.2.3 Malic, Succinic, Oxalic, and Tartaric Acids

Anthocyanins acylated with the dicarboxylic malic acid seem to be restricted to carnations (*Dianthus*). Wild-type carnation petals have been found to contain the 3-[6-(malyl)glucosides], 3-[6-(malyl)glucoside]-5-glucosides, and the 3-glucoside-5-glucoside (6'',6'''-malyl diesters) of pelargonidin and cyanidin.^{212–216} These two latter macrocyclic pigments^{219,221} are the only anthocyanins where the same acyl group is connected to two monosaccharides, which are attached at different locations at the anthocyanidin. The orientation of the malyl groups of these anthocyanins has been determined by long-range 2D heteronuclear NMR techniques (HMBC).²¹⁶ Recently, Fukui et al.³¹ have shown that marketed genetically modified violet carnations produce analogous delphinidin-type anthocyanins by heterologous flavonoid 3', 5'-hydroxylase gene expression.

Anthocyanins acylated with succinic acid have previously been isolated from some species in the genus *Centaurea* (Compositae), and identified as the 3-[6-(succinyl)glucoside]-5-glucosides of cyanidin and pelargonidin.^{217–219} Later, the former pigment isolated from cornflower, *Centaurea cyanus*, was shown to be part of the self-assembled supramolecule protoanin, composed of six molecules each of malonylflavone and succinylcyanin complexed with magnesium and ferric ions (Section 10.2.7).²²⁰ Succinyl has also been reported to occur in minor amounts in flowering tops of *Phragmites australis* (Gramineae) as cyanidin 3-[6-(succinyl)-glucoside],²²¹ and the structures of the two major anthocyanins (Figure 10.9) in blue *Agapanthus* (Agapanthaceae) flowers have been found to be based on a *p*-coumarylated delphinidin diglycoside attached to a flavonol triglycoside via a succinic acid diester link.¹²

Oxalic acid is another dicarboxylic acid with restricted occurrence as an acyl moiety of anthocyanins. In addition to some European orchids flowers (Section 10.5.10),²²² cyanidin 3-dioxalylglucoside has been isolated from fruits of *Rubus laciniatus*.⁵⁷ Altogether six different anthocyanins acylated with oxalic acid have been reported. With the exception of cyanidin 3-[6-(oxalyl)glucoside], evidences for proper determination of the linkage position of this acyl moiety are absent.

Tartaric acid has an even more limited distribution as acylation agent of anthocyanins, identified in only four anthocyanins (**82**, **246**, **383**, and **401**) isolated from flowers of *Anemone coronaria*.^{28,132} In these pigments, one of the tartaryl hydroxyl groups is esterified with a carboxyl group of malonic acid. In pelargonidin 3-[2-(xylosyl)-6-(3-(3-(4-(glucosyl)caffeyl)-2-tartaryl)malonyl)galactoside] (**82**), the other hydroxyl group of tartaryl is esterified with caffeyl.

10.2.6 DIMERIC FLAVONOIDS INCLUDING AN ANTHOCYANIN UNIT

Most reported anthocyanins are monomeric in nature; however, new types of flavonoids consisting of an anthocyanin moiety covalently linked to another flavonoid unit have been reported in the period of this review. One class includes one anthocyanin unit and one flavone or flavonol unit attached covalently to each end of a common dicarboxylic acid. The other class involves one anthocyanin moiety covalently linked directly to a flavanol unit.

Two dimeric pigments ((6'''-(delphinidin 3-[6''-(glucosyl)glucoside])) (6''-(apigenin 7-glucosyl))malonate (**534**) and (6'''-(delphinidin 3-[6''-(glucosyl)glucoside])) (6''-(luteolin 7-glucosyl))malonate (**535**) (Figure 10.8) have been isolated from the blue-violet flowers of *Eichhornia crassipes* (Pontederiaceae) by Toki et al.^{9,10} The major *Eichhornia* anthocyanin A has an apigenin 7-glucoside molecule (flavone) attached with an ester bond to one end of malonic acid, and delphinidin 3-gentiobioside linked with a similar bond to the other end. The minor *Eichhornia* anthocyanin B has a similar structure with apigenin 7-glucoside replaced by luteolin 7-glucoside. The three-dimensional structure of these pigments was suggested from the observation of negative Cotton effects at λ_{\max} (535 and 547 nm,

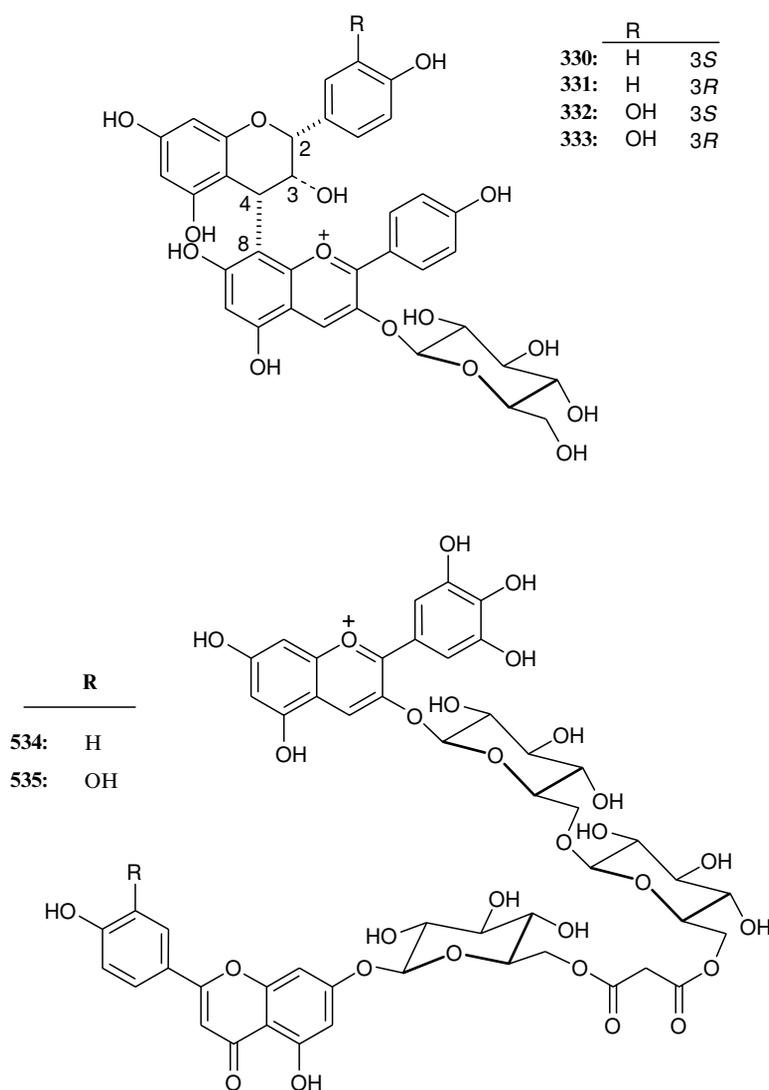


FIGURE 10.8 Afzelechin(4 α \rightarrow 8)Pg3glc (**530**), epiafzelechin (4 α \rightarrow 8)Pg3glc (**531**), catechin(4 α \rightarrow 8)Pg3glc (**532**), epicatechin(4 α \rightarrow 8)Pg3glc (**533**) isolated from extracts of strawberries, and (6'''-(delphinidin 3-[6''-(glucosyl)glucoside])) (6''-(apigenin 7-glucosyl))malonate (**534**) and (6'''-(delphinidin 3-[6''-(glucosyl)glucoside])) (6''-(luteolin 7-glucosyl))malonate (**535**) from blue-violet flowers of *Eichhornia crassipes*. Pg3glc, pelargonidin 3-glucoside.

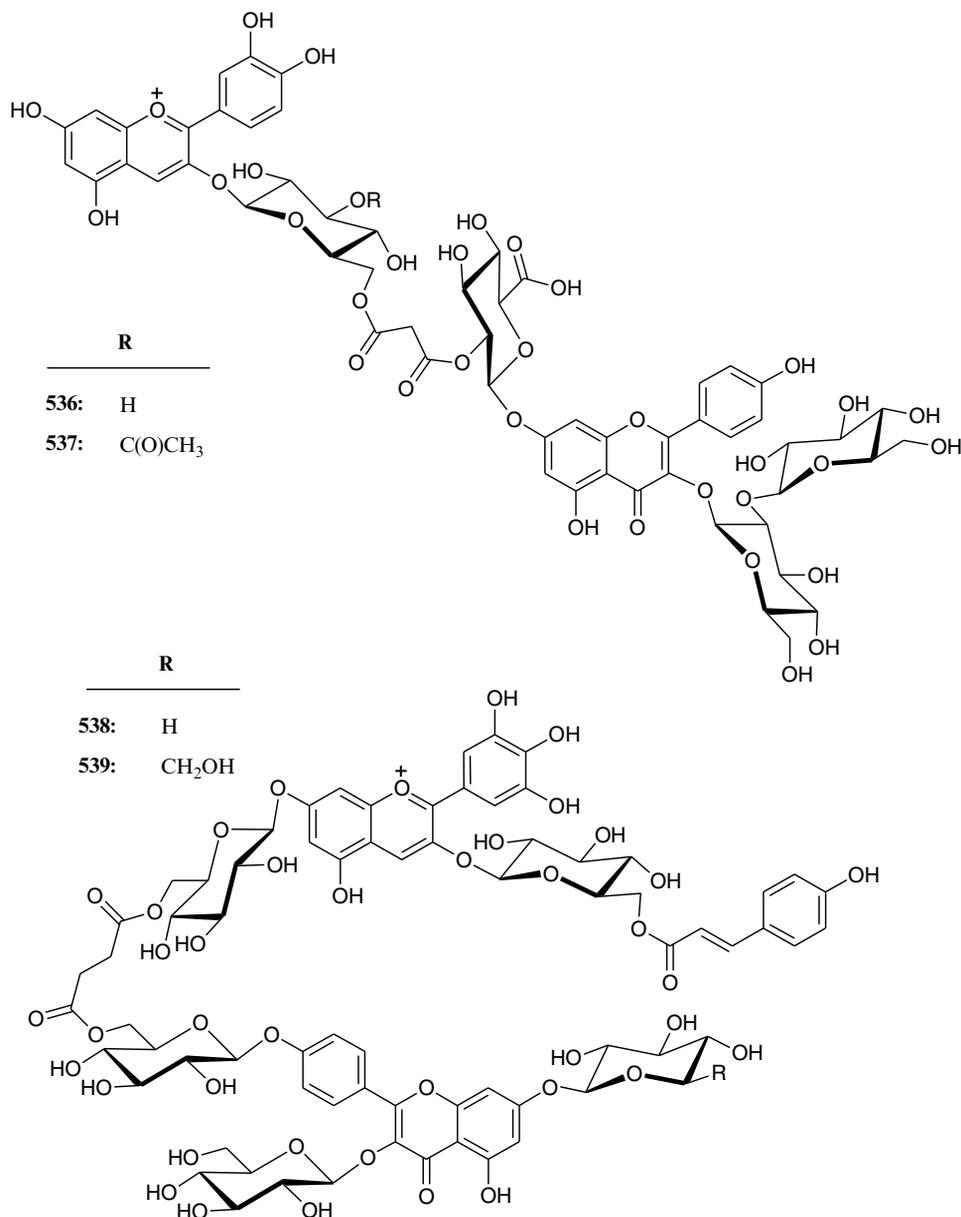


FIGURE 10.9 (6''-(cyanidin 3-glucosyl)) (2'''-(kaempferol 3-[2''-(glucosyl)(glucoside)]-7-glucuronosyl)-malonate (**536**) and (6''-(cyanidin 3-[3''-(acetyl)glucosyl]) (2'''-(kaempferol 3-[2''-(glucosyl)(glucoside)]-7-glucuronosyl))malonate (**537**) isolated from pale-purple flowers of chive (*Allium schoenoprasum*),¹¹ and (6'''-(delphinidin 3-[6''-(*p*-coumaryl)glucoside]-7-glucosyl)) (6''''-(kaempferol 3-glucoside-7-xyloside-4'-glucosyl)succinate (**538**) and (6'''-(delphinidin 3-[6''-(*p*-coumaryl)glucoside]-7-glucosyl)) (6''''-(kaempferol 3-glucoside-7-glucoside-4'-glucosyl)succinate (**539**) from blue *Agapanthus* flowers.¹²

respectively). The chromophore (delphinidin) and the copigment (flavone) occupy a folding conformation as a binary complex.^{9,10} *Eichhornia* anthocyanin A exhibited remarkable color stability in aqueous solution at mildly acidic pH values.²²³ The existence of intramolecular hydrophobic interactions between the chromophoric skeleton and the flavone group was

indicated by reduction in the hydration constant when compared with the parent delphinidin 3-glycoside. The existence of other anthocyanin–flavone complexes has previously been shown or indicated in flowers of orchids,^{222,224} lupins,²²⁵ and *Salvia patens*.²²⁶

Two anthocyanin–flavonol complexes, ((6''-(cyanidin 3-glucosyl)) (2'''-(kaempferol 3-[2''-(glucosyl)(glucoside)]-7-glucuronosyl))malonate (**536**) and (6''-(cyanidin 3-[3''-(acetyl)-glucosyl])) (2'''-(kaempferol 3-[2''-(glucosyl)(glucoside)]-7-glucuronosyl))malonate (**537**) (Figure 10.9), have been isolated from the pale-purple flowers of chive (*Allium schoenoprasum*).¹¹ These pigments, which were supplied with complete NMR assignments, were based on either cyanidin 3-glucoside or cyanidin 3-[3-(acetyl)glucoside] esterified to one end of malonic acid, and kaempferol 3-[2-(glucosyl)glucoside]-7-glucosiduronic acid connected to the other end. Compared to similar spectra of the same monomeric anthocyanins, the bathochromic shifts (9 nm) in the UV–vis spectra of the complexes revealed intramolecular association between the anthocyanin and flavonol units, which influenced the pigment color. The chemical shifts of the anthocyanidin H-4 in the two complexes were 0.3 ppm upfield for the same shifts of anthocyanins without connection to a flavonol moiety. Two similar complexes, (6'''-(delphinidin 3-[6''-(*p*-coumaryl)glucoside]-7-glucosyl)) (6'''-(kaempferol 3-glucoside-7-xyloside-4'-glucosyl))succinate (**538**) and (6'''-(delphinidin 3-[6''-(*p*-coumaryl)glucoside]-7-glucosyl)) (6'''-(kaempferol 3-glucoside-7-glucoside-4'-glucosyl))succinate (**539**) (Figure 10.9), have been isolated from the blue *Agapanthus* flowers (Agapanthaceae).¹² In these structures, succinate was involved instead of malonate to connect delphinidin 3-[6-(*p*-coumaloil)glucoside]-7-glucoside to either kaempferol 3,4'-di-glucoside-7-xyloside or kaempferol 3,7,4'-tri-glucoside.

The other class involving one anthocyanin moiety covalently linked directly to a flavanol unit has been found as minor pigments in extracts of strawberries.¹³ These purple complexes were characterized by UV–vis spectroscopy, 2D NMR techniques, and electrospray MS to be afzelechin(4 α \rightarrow 8)pelargonidin 3-*O*- β -glucopyranoside (**530**), epiafzelechin(4 α \rightarrow 8)pelargonidin 3-*O*- β -glucopyranoside (**531**), catechin(4 α \rightarrow 8)pelargonidin 3-*O*- β -glucopyranoside (**532**), and epicatechin(4 α \rightarrow 8)pelargonidin 3-*O*- β -glucopyranoside (**533**) (Figure 10.8). The stereochemistry at the 3- and 4-positions of the flavan-3-ols was elucidated after assumption of the *R*-configuration at C-2. Each of the four pigments occurred in the NMR solvent as a pair of rotamers. Other complexes based on anthocyanins or anthocyanidins directly linked to flavanol(s) have been detected in wine mainly by LC–MS techniques^{59–62} (see Chapter 5).

10.2.7 METALLOANTHOCYANINS

In the period of this review, Kondo et al. have presented several extraordinary papers in the field of metalloanthocyanins and their contribution to blue flower colors. Commelinin²²⁷ from blue flowers of *Commelina communis* has been found to consist of six molecules of delphinidin 3-[6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (malonylawobanin) copigmented with six flavone (flavocommelin) molecules complexed with two magnesium atoms (Figure 10.10). Self-association was shown to exist between the anthocyanin moieties. Intact commelinin was also obtained by reconstruction of this supramolecule from its components. Cd-commelinin, in which Mg²⁺ was replaced with Cd²⁺, has been studied with x-ray crystal structure. The blue flower color development and the stability of the color were explained by metal complexation of the anthocyanin and intermolecular hydrophobic association.²²⁷ The octaacetate derivative of the flavone part of this molecule has been determined by x-ray diffraction,²²⁸ and in the crystal the flavone molecules were arranged parallel to each other according to the periodicity of the crystal lattice. Intermolecular stacking of the flavone skeletons was, however, not observed, and the hydrophilicity of the glucose moieties was suggested as an important factor governing the self-association.

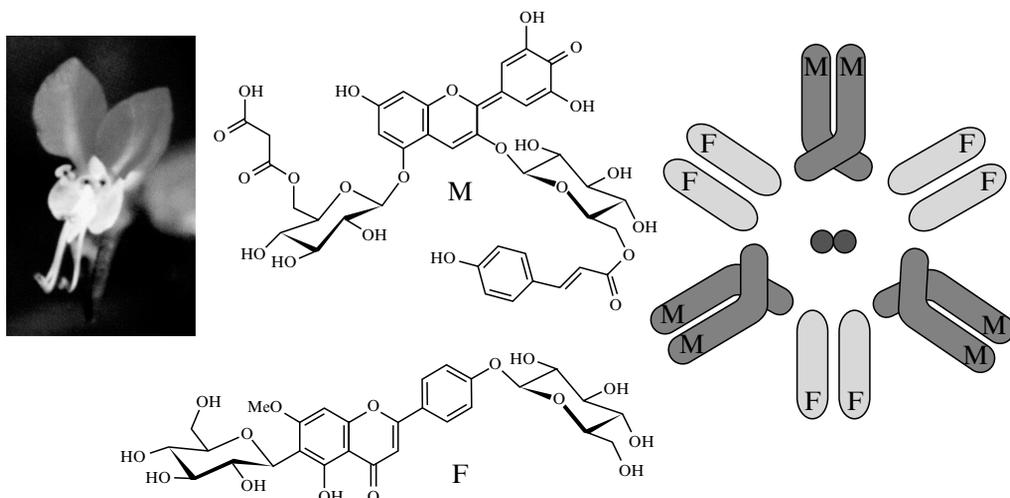


FIGURE 10.10 The illustration figure (right) indicates the mechanism known to operate for commelinin found in *Commelina communis* by Kondo et al. Commelinin consists of six molecules of delphinidin 3-[6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (malonylawobanin) (M, purple) copigmented with six flavone molecules (F, yellow) complexed with two magnesium atoms (red). (After Kondo, T. et al., *Nature*, 358, 515, 1992. With permission.)

Another metalloanthocyanin, protodelphin, similar to commelinin and protofucyanin, has been isolated from flowers of *Salvia patens*.²²⁶ Protodelphin has been shown to be composed of six molecules of delphinidin 3-[6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside], six molecules of apigenin 7,4'-diglucosides, and two magnesium ions.²²⁹ Compared to commelinin, protodelphin includes the same anthocyanin, malonylawobanin, which is, however, another flavone. Takeda et al.²²⁶ have been able to resynthesize the natural blue pigment *in vitro* by adding the three components together. Mg^{2+} could be substituted *in vitro* by other divalent metal cations (e.g., Co^{2+} , Ni^{2+} , Zn^{2+} , and Cd^{2+}). Using synthetic apigenin 7,4'-diglucosides derived from D- or L-glucose, Kondo et al.²²⁹ have shown that the sugars of the three flavone molecules in the self-association complex oriented the structure into an M helix (for D-glucose) or P helix (for L-glucose). The M helix was able to intercalate with the Mg^{2+} -malonylawobanin complex to form native pigment, whereas the P helix could not. They concluded that restricted chiral and structural recognition controlled the entire self-assembly of the metalloanthocyanin, and was responsible for the blue flower color.

In 1998, Kondo et al.²²⁰ proposed a new molecular mechanism for blue color expression with protofucyanin from cornflower, *Centaurea cyanus*. The protofucyanin structure is similar to that of commelinin, composed of six molecules each of apigenin 7-glucuronide-4'-[6-(malonyl)glucoside] and succinylcyanin (**153**), complexed with magnesium and ferric ions.²³⁰ It was proposed that the molecular stacking of the aromatic units prevented hydration of the anthocyanidin nucleus. The blue color of protofucyanin was found to be caused by ligand to metal charge transfer (LMCT) interaction between succinylcyanin and Fe^{3+} .²²⁰ This color mechanism (Figure 10.11) is different from that known to operate for commelinin.

Metal complexes involving anthocyanins have previously been proposed also in blue flowers of *Hydrangea macrophylla*.⁸⁰ Later, the color change and variation of hydrangea has been suggested to be caused by free Al^{3+} complexation of those components of the complex responding to slight vacuolar pH change.^{231,232}

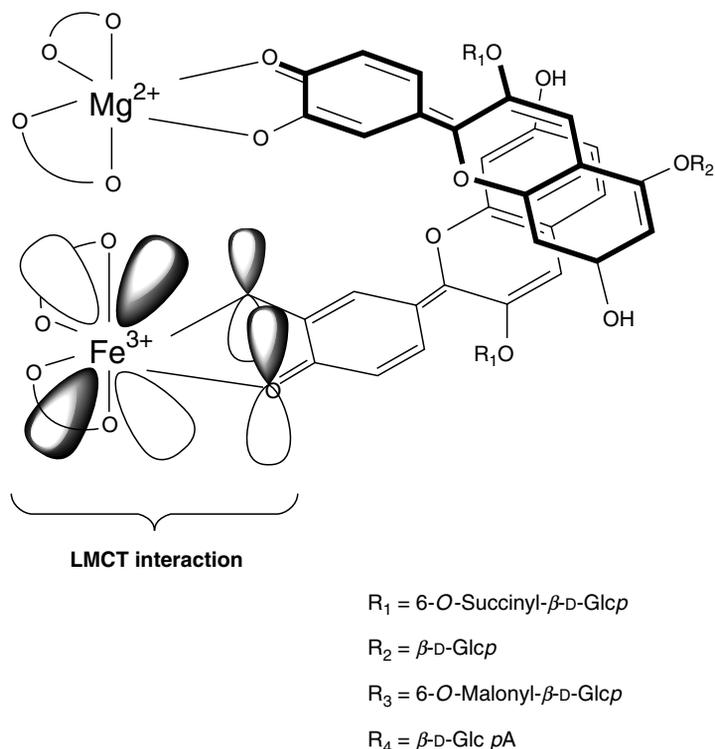


FIGURE 10.11 Schematic representation of the LMCT interaction between the anthocyanin succinylcyanin and Fe^{3+} in protocyanin. (From Kondo, T. et al., *Tetrahedron Lett.*, 39, 8307, 1998. With permission.)

It is well known that anthocyanins with hydroxyl groups in *ortho*-position to each other form complexes with aluminum ion leading to bathochromic and hyperchromic shifts in their absorption spectra. Complexation of Al^{3+} with synthetic and natural anthocyanins has been investigated in aqueous solutions within the pH range 2 to 5.^{233,234} As shown by UV-vis spectroscopic data, the complexes involved not only the colored forms but also the colorless forms of the pigment. ^1H NMR analysis in CD_3OD confirmed the conversion of the anthocyanin from the red flavylium form to the deep-purple quinonoidal forms upon coordination to Al^{3+} .²³³ From relaxation kinetics measurements (pH jump), complexation constants of Al^{3+} and several synthetic and natural anthocyanins have been calculated.²³³⁻²³⁵

10.2.8 ANTHOCYANIN COLORS AND STABILITY

One of the best-established functions of anthocyanins is in the production of flower color and the provision of colors attractive to plant pollinators. Considerable effort has been made to give explanations for the color variations expressed by anthocyanins in plants. Various factors including concentration and nature of the anthocyanidin, anthocyanidin equilibrium forms, the extent of anthocyanin glycosidation and acylation, the nature and concentration of copigmentation, metal complexes, intra- and intermolecular association mechanisms, and influence of external factors like pH, salts, etc. have been found to have impact on anthocyanin colors.⁸⁰

The role of anthocyanins and flavones in providing stable blue flower colors in the angiosperms has more recently been outlined by Harborne and Williams.⁴² It was apparent

from the data collected that delphinidin was the most common anthocyanidin in blue flowers, and that copigmentation with a specific flavone constituent was the most common mechanism for shifting the mauve colors of delphinidin glycosides toward blue. These anthocyanin–flavone complexes, where they exist, showed high flavone to anthocyanin ratios (e.g., 10:1), except when a metal cation was also present. The anthocyanin–flavone complexes in *Eichhornia crassipes*^{9,10} are unique in that the anthocyanin and the flavones were covalently linked through a central aliphatic acyl residue (Section 10.2.6). Similar bathochromic color effects have recently also been reported for dimeric anthocyanin–flavonol complexes in blue *Agapanthus* flowers (Agapanthaceae),¹² and in purple flowers of chive (*Allium schoenoprasum*).¹¹ In the latter case, the anthocyanidin is cyanidin. More about metalloanthocyanins and dimeric flavonoids containing anthocyanins is found in Sections 10.2.5 and 10.2.6, respectively.

Some very interesting macrocyclic anthocyanins, the 3-glucoside-5-glucoside (6'',6'''-malyldiesters) of cyanidin and pelargonidin, have been isolated from carnations (*Dianthus*).^{214,215} Gonnet and Fenet²¹⁵ have shown by CIELAB parameters that carnations with “cyclamen red” colors closely matched those of petals of some *Rosa* cultivars considered for comparison. However, these cyclamen colors in roses were based on the presence of cyanidin 3,5-diglucoside in contrast to the carnations, which contained pelargonidin derivatives acylated with malic acid. These macrocyclic anthocyanins were, however, relatively labile and underwent readily ring opening to furnish the corresponding 3-(6''-malyglucoside)-5-glucosides, and could only be extracted if neutral solvents were employed. *In vivo* these rare pigments appear to be stabilized by copigmentation with associated flavones. Wild-type carnations lack a flavonoid 3',5'-hydroxylase gene, which implies that they cannot produce the corresponding delphinidin derivatives. Recently, Fukui et al.³¹ showed that marketed genetically modified violet carnations produced analogous delphinidin-type anthocyanins by heterologous flavonoid 3',5'-hydroxylase gene expression. They concluded that the bluish hue of the transgenic carnation flowers was accounted for by three factors: accumulation of the delphinidin-type anthocyanins, the presence of strong copigmentation with flavone derivative, and a relatively high vacuolar pH of 5.5.

The relationship between color and substitution patterns in anthocyanidins has been investigated with the aim of developing quantitative structure–color models.²³⁶ Experimental data for the lowest UV transition in 20 substituted anthocyanidins were reviewed. While a hypsochromic effect from hydroxyl and methoxyl moieties at positions 6 and 8 was reported here,²³⁶ it is interesting to note that a C-linked sugar in the 8-position has the opposite effect producing a more bluish color.²⁰ Color and stability studies of the 3-glucosides of the six common anthocyanidins and petanin, petunidin 3-[6-(4-(*p*-coumaryl)rhamnosyl)glucoside]-5-glucoside, in aqueous solutions during several months of storage revealed especially large variation at slightly acidic to slightly alkaline pH values.^{237,238} ¹H NMR spectroscopy has been used to characterize the aggregation processes leading to color stabilization of malvidin 3-glucoside.²³⁹ The concentrations of the different forms in aqueous solution were determined as a function of pH for several values of the total anthocyanin concentration. The pH-dependent color and structural transformations in aqueous solutions of some non-acylated anthocyanins and synthetic flavylium salts have been reexamined.^{240,241}

It is known that polyacylated anthocyanins are very stable in neutral or weakly acidic aqueous solutions, whereas simple anthocyanins are quickly decolorized by hydration at the 2-position of the anthocyanidin nucleus. The shift to blue colors and increased anthocyanin stability are in many cases simply achieved by intramolecular copigmentation involving stacking between the anthocyanidin and the aromatic acyl groups.^{233,242–244} Both intra- and intermolecular interactions have been found in aromatic monoacylated anthocyanins studied by 1D and 2D proton NMR spectroscopy.²⁴⁵ These compounds formed strong intramolecular π -complexes between the cinnamic acyl group and the anthocyanidin nucleus,

the double bond of the acyl group involved as well as its aromatic ring. Upon increasing the concentration of the anthocyanins or lowering the temperature of the NMR sample, the π -complexes formed multinuclear complexes as shown by the resultant negative nuclear Overhauser effect (NOE) values. In a very detailed review, recent progress in the chemistry of polyacylated anthocyanins as flower color pigments has been outlined by Honda and Saito.²⁴⁶ It was recognized that both the bluing effect and stabilization of flower colors remarkably depended on the number of aromatic acids presented in the polyacylated anthocyanins. After classification of the polyacylated anthocyanins into seven types by the substitution pattern of the acyl functions, it was concluded that anthocyanins with the aromatic acyl groups in glycosyls in both the 7- and 3'-positions were considered to make the most stable colors in the flowers.

Red-purple colors in the flowers of orchids have been shown to be derived from altogether 15 cyanidin and peonidin glycosides, with aromatic acylated sugars attached at both the 7- and 3'-positions (Table 10.2).^{155-157,176,180-182,189} Intramolecular associations between these planar molecules in orchids provided stable colors without the need for any copigment or metal cation.²⁴⁷ Figueiredo et al.²⁴⁷ proposed that the glycosyl-acyl "side chains" attached to both positions 3' and 7 of the chromophore favored a better overlap and stronger interaction with the π -system of the central chromophore (Figure 10.12), than what was observed for other acylated anthocyanins. They supported the assessment by molecular calculations, which gave minimum energy conformation for a "sandwich" type with the 3'-chain folded "over" and the 7-chain folded "under" the chromophore (Figure 10.12). Similar acylation of glycosyls in anthocyanidin 7- and 3'-positions has also been reported for anthocyanins in Commelinaceae,¹³⁶ Compositae,²⁴⁸ Liliaceae,¹⁴⁸ and Rhamnaceae.³² The three acylated delphinidin 3,7,3',5'-tetraglucosides from berries of two *Dianella* species (Liliaceae) showed exceptional blueness at *in vivo* pH values due to effective intramolecular copigmentation involving

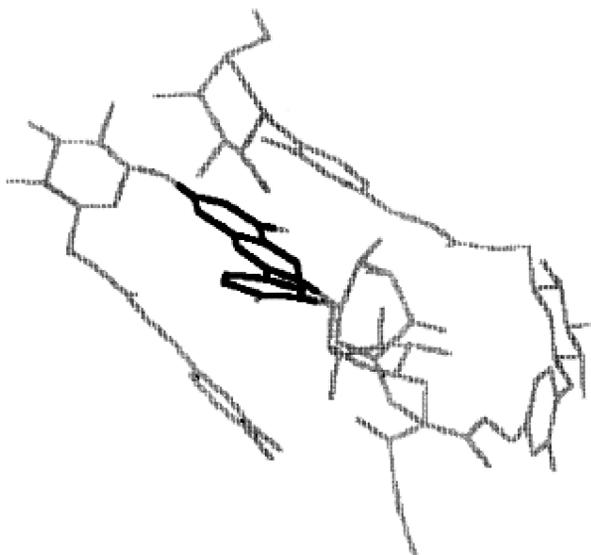


FIGURE 10.12 Structure of cyanidin 3-[6-(malonyl)glucoside]-7-[6-(caffeoyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)caffeoyl)glucosyl)caffeoyl)glucoside] (**258**) optimized by molecular calculations. The anthocyanidin is represented in black, while substituents are drawn in gray. The acylated side chains attached to the 3'- and 7-positions of the chromophore interact favorably with the π -system of the central chromophore. (From Figueiredo, P. et al., *Phytochemistry*, 51, 125, 1999. With permission.)

p-coumaryl-glucose units (GC) at the aglycone 7-, 3'-, and 5'-positions.¹⁴⁸ Evidence was presented to show that the effectiveness of the copigmentation could be ranked: 3',5'-GC > 7-GC > 3-GC. The blue flower color of *Ceanothus papillosus* (Rhamnaceae) was proposed to arise from a supramolecular complex of high stoichiometry including anthocyanins and kaempferol 3-[2-(xylosyl)rhamnoside].³² This copigmentation effect appeared to be quite specific, and did not occur to the same extent with other more common flavonols. An extraordinary, long wavelength visible absorption maximum at 680 nm was produced, which conferred additional blueness. Figueiredo et al.²⁴⁷ have also explored the role of malonic acid residues that are present in many anthocyanins. These dicarboxylic acyl groups appeared to provide color stabilization, due to an increase in acidity in the vacuolar solution of the petal. The pK_a of malonic acid was 2.83 and deprotonation of the malonyl group provided protection against alkanization of the medium and hence loss of color.

According to our data, only 17 anthocyanins acylated with hydroxycinnamic acids occur in both the *cis* (*Z*) and *trans* (*E*) configurations (Appendix A). George et al.²⁴⁹ have compared the pairs of 3-[6-(*E/Z-p*-coumaryl)glucoside]-5-[6-(malonyl)glucosides] of malvidin and delphinidin. They observed that the *cis* isomers exhibited ϵ values about 1.5 times greater than the *trans* isomers, in both pairs. It was calculated (pK'_h values) that the *cis* forms were less prone to undergo hydration reactions forming the colorless anthocyanin forms. Based on computed structures, the more coplanar arrangement allowed by the *cis* isomers was postulated as the rationale supporting the enhanced color stability.²⁴⁹ When considering the color effect of this type of intramolecular copigmentation *in vivo*, one should bear in mind that the *trans* isomer seems to predominate, and that the conversion between the two isomers is rare. When Yoshida et al.²⁵⁰ studied the *E,Z*-isomerization reaction and stability of several types of acylated anthocyanins under the influence of UV irradiation, their interest was focused on the reason why isomerization reaction of some acyl residues was prevented in living plant cells. They concluded that the stability of anthocyanins under irradiation highly depends on molecular stacking. They proposed that light energy absorbed by cinnamoyl residues might be transferred to the anthocyanidin nucleus and released without any isomerization reaction or degradation of pigments. Thus, the flower color may be stable for a long time under strong solar radiation.

10.3 ANTHOCYANIN PRODUCTION

10.3.1 CELL CULTURES

The interest in and demand for natural food colorants and pharmacologically interesting natural compounds have encouraged new research initiatives aimed at the development of more efficient means of harvesting anthocyanins. When growth procedures are optimized, cell culture systems have the potential of producing both higher anthocyanin concentrations within reduced time, and another selection of anthocyanins relative to production in whole plants. In a recent general review, Ramachandra Rao and Ravishankar²⁵¹ have dealt with the production of high-value secondary metabolites including anthocyanins through plant cell cultures, shoot cultures, root cultures, and transgenic roots obtained through biotechnological means. In an overview of the status and prospects in the commercial development of plant cell cultures for production of anthocyanin, Zhang and Furusaki²⁵² have focused on strategies for enhancement of anthocyanin biosynthesis to achieve economically viable technology.

Production of anthocyanins in plant cell and tissue cultures has been reported for more than 30 species including *Daucus carota*, *Fragaria* × *ananassa*, *Vaccinium* spp., *Vitis hybrida*, *Solanum tuberosum*, *Malus sylvestris*, *Aralia cordata*, *Perilla frutescens*, *Ipomoea batatas*, *Euphorbia millii*, *Strobilanthes dyeriana*, *Hibiscus sabariffa*, *Dioscorea cirrhosa*, etc.^{35,252,253}

The production has shown to be influenced by a variety of environmental stimuli such as light irradiation, UV light, low temperature, oxygen level, hormones, fungal elicitors, low nutrient levels.^{251–253} Increased level of oxygen supply and light irradiation have, for instance, shown independently positive influence on the production of anthocyanins in suspended cultures of *Perilla frutescens* cells in a bioreactor.²⁵⁴ However, a combination of irradiation with a higher oxygen supply reduced the production. In *Vaccinium pahalae* cell cultures, anthocyanin yield was enhanced by increasing sucrose concentration in the liquid suspension medium and by manipulating the initial inoculum density.²⁵⁵

A cell culture system has the potential advantage of facilitating selective production of certain anthocyanins. The nine acylated anthocyanins produced by flowers of *Hyacinthus orientalis* regenerated *in vitro* were identical to those of field-grown flowers.²⁵⁶ However, the concentration of cyanidin 3-[6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] was considerably higher in the regenerated flowers. Lower concentration of 2,4-dichlorophenoxyacetic acid in the medium used for strawberry suspension cultures has, for instance, limited cell growth and enhanced both anthocyanin production and anthocyanin methylation.²⁵³ The ratio of peonidin-3-glucoside to the total anthocyanin content increased significantly under these conditions. A methylated anthocyanin like peonidin 3-glucoside is normally not found in intact strawberries, and although the activity of anthocyanin methyltransferase was not measured by Nakamura et al.,²⁵³ the results indicated that lower 2,4-dichlorophenoxyacetic acid concentrations enhanced the activity of anthocyanin methyltransferase. Do and Cormier²⁵⁷ have reported that increased osmotic potential in the medium resulted in a significant intracellular accumulation of peonidin-3-glucoside in *Vitis vinifera* cells. Similarly, jasmonic acid has been reported to increase the peonidin 3-glucoside content considerably, while the other major anthocyanins only experienced smaller increments.²⁵⁸

Another example of using cell cultures has been presented by Dougall et al.¹⁵¹ To improve understanding of the ways in which cinnamic acid groups alter the color retention of anthocyanins, a series of anthocyanins that differed systematically in their acyl group was needed. When cinnamic acids were fed to wild carrot suspension cultures, the proportion of acylated to nonacylated anthocyanins increased.¹⁵¹ With high relevance for future metabolic studies, *Vitis vinifera* cells grown in a bioreactor have been used for production of isotopically ¹³C-labeled phenolic substances such as anthocyanins.^{259,260} The enrichment of labeling (between 40 and 65%) obtained for all compounds is sufficient to investigate their absorption and metabolism in humans. Similarly, ¹⁴C-L-phenylalanine has been incorporated into a range of polyphenolic compounds when fed to cell cultures.^{261,262} Experiments with *Vaccinium pahalae* berries and *Vitis vinifera* suspension cultures, using [¹⁴C]-sucrose as the carbon source, have demonstrated a 20 to 23% efficiency of ¹⁴C incorporation into the flavonoid-rich fractions.²⁶³

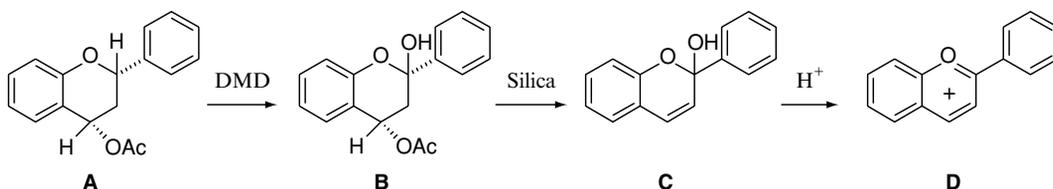
To improve production of anthocyanins, efforts have mainly been devoted to the optimization of biosynthetic pathways by both process and genetic engineering approaches. The productivity in the cultures is, however, determined by synthetic capacity, storage capacity, and the capacity to metabolize the compounds in the transport and detoxification processes.²⁶⁴ The potential of manipulation and optimization of postbiosynthetic events have recently been reviewed by Zhang et al.²⁶⁵ These events, including chemical and enzymatic modifications, transport, storage or secretion, and catabolism or degradation, were outlined with anthocyanin production in plant cell cultures as case studies.

Bioreactor-based systems for mass production of anthocyanins from cultured plant cells have been described for several species.^{254,260,266–270} A highly productive cell line of *Aralia cordata* obtained by continuous cell aggregate cloning has, for instance, been reported to yield anthocyanins in concentrations as high as 17% on a dry weight basis.²⁶⁶ However, to date economic feasibility has not been established in part because of some unique engineering challenges inherent in mass cultivation of plant cultures.

10.3.2 SYNTHESIS

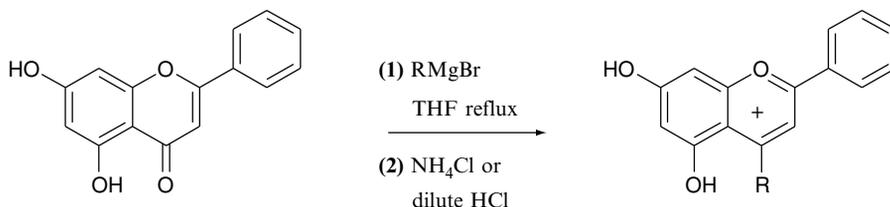
Since the early contributions of Willstatter and Robinson, several alternative approaches following mainly two routes have been considered for synthesis of anthocyanins.²⁷¹ One of the routes includes condensation reactions of 2-hydroxybenzaldehydes with acetophenones, while the other uses transformations of anthocyanidin-related compounds like flavonols, flavanones, and dihydroflavonols to yield flavylium salts. The urge for plausible sequences of biosynthetic significance has sometimes motivated this latter approach. In the period of this review, new synthetic approaches in the field have also predominantly been following the same general routes; however, some new features have been shown in synthesis of pyranoanthocyanidins.

In an interesting paper by Bernini et al.,²⁷² compounds with a flavonoid structure have been selectively oxyfunctionalized at the C-2 carbon atom by dimethyldioxirane (DMD). Products obtained in this way appeared to be useful starting materials to access anthocyanidins. An example of this route is presented in Scheme 10.1. Here, 2,4-*cis*-flavane-4-acetate (**A**) was oxidized by DMD at room temperature, affording the corresponding C-2 hydroxy derivative (**B**) as the only product (63% yield). Further treatment of **B** with silica gel eliminated acetic acid to give **C** quantitatively. Then **C** was easily transformed into the flavylium salt (**D**) by simple addition of a 37% solution of HCl in water.



Following more traditionally routes, a recent one-step synthesis giving 3-deoxyanthocyanidins (apigeninidin and luteolinidin) in high yield involved the condensation reaction between 2,4,6-triacetoxybenzaldehyde and an acetophenone derivative in anhydrous methanolic hydrogen chloride.²⁷³ The reduction of quercetin 3-*O*-(6-*O*-rhamnosylglucoside), rutin, by zinc amalgam in 3% absolute methanolic hydrochloric acid has provided pure cyanidin 3-*O*-(6-*O*-rhamnosylglucoside) in good yield.²⁷⁴

New 3-deoxyanthocyanidins have been prepared according to a Grignard reaction of some flavones with appropriate alkyl- and aryl-magnesium bromides.²⁷⁵ The reaction of 5,7-dihydroxyflavone (chrysin) with an excess of phenylmagnesium bromide in THF under reflux conditions, followed by hydrochloric acid hydrolysis, afforded **1** in Scheme 10.2. Flavylium salts bearing a substituent at the 4-position are important compounds since they are known to be less sensitive to nucleophiles, especially water, and they give only minor amounts of the colorless pseudobases.²⁷⁶



Flavylium salts with 4-methyl substitution (e.g., **2** in Scheme 10.2) might be further gently reacted with aromatic aldehydes affording anthocyanidin pigments of the pyranoanthocyanidin type.²⁷⁵

Compared to the more common flavylium salts, these latter pigments showed large bathochromic shifts (from about 100 to 200 nm), thus providing blue-violet and green colors, which are difficult to obtain with anthocyanidins in general.

The pyranoanthocyanin in red wine called vitisin A has been found by synthetic experiments to be formed by the reaction of malvidin 3-glucoside with pyruvic acid.¹⁶ Its formation resulted from cyclization between C-4 and the hydroxyl group at C-5 of the original flavylium moiety with the double bond of the enolic form of pyruvic acid, followed by dehydration and rearomatization steps. Other pyranoanthocyanins in wine formed by the reaction of malvidin 3-glucoside with vinylphenols were first observed by Fulcrand et al.,²⁷⁷ and later synthesized by nucleophilic addition of vinylphenols to malvidin 3-glucoside.²⁷⁸ The former research group has also synthesized similar orange-colored anthocyanins from monoglucosylated anthocyanins and ethanol, alpha-ketoglutaric acid, acetone, or 3-hydroxybutan-2-one.²⁷⁹ See Chapter 5 for further information about anthocyanins in wine.

Pyranoanthocyanins have also been formed by oxidative cycloaddition between anthocyanins from blackcurrant seeds and acetone,⁹⁰ which should be kept in mind when acetone is used as solvent during extraction and isolation of natural anthocyanins. When the same research group isolated four new pyranoanthocyanins (pyranocyanin C and D and pyranodelphinin C and D) from an extract of blackcurrant seeds, these structures were confirmed by chemical synthesis involving blackcurrant anthocyanins and *p*-coumaric acid.⁹¹ Recently, pyranoanthocyanins have been obtained by a simple one-step reaction involving anthocyanins and cinnamic acids bearing at least one electron-donating substituent at the *para*-position.²⁸⁰ Through this type of reaction with *p*-dimethylamino cinnamic acid, which is similar to the reaction of 4-substituted 3-desoxyanthocyanins with *p*-dimethylamino cinnamaldehyde,²⁷⁵ synthetic malvidin- and cyanidin-based anthocyanins with violet hues have been prepared.²⁸⁰

10.4 ANTHOCYANIN LOCALIZATION IN PLANT CELLS

Anthocyanins are the most common water-soluble pigments in the plant kingdom, and are normally found dissolved uniformly in vacuolar solutions of epidermal cells. However, in cases like the Sphagnorubins,²⁸¹ the pigments are so tenaciously bound to the cell wall that they are only extracted with difficulty.

In species from more than 33 families, anthocyanins have been found located in pigmented bodies in vacuoles described as anthocyanoplasts (ACPs).^{282,283} While the cells matured, these structures often disappeared. The ACPs were thought to be membranous, enclosing the site of anthocyanin biosynthesis. When the nature of ACPs from colored skin tissues of “Kyoho” grapes was investigated under a microscope, it was indicated that each ACP was surrounded by a transparent membrane, which maintained the concentration of anthocyanin within an ACP higher than that in the vacuole.²⁸⁴ Later, it was, however, reported that the spherical pigmented bodies in cells of sweet potato (*Ipomoea batatas*) in suspension culture might be protein matrices,^{285,286} and that ACPs possess neither a membrane boundary nor an internal structure.^{286–288} A protein labeled VP24 was found to accumulate in the intravacuolar pigmented globules (cyanoplasts) and not in the tonoplast.²⁸⁶ The expression of VP24 was closely correlated with the accumulation of anthocyanin in the cell lines, and this protein was suggested to play an important role in trapping of large amounts of anthocyanins that have been transported into these vacuoles. More recently, sequence analysis has indicated that mature VP24 peptide is a member of the metalloprotease family, and might be a novel vacuolar localized aminopeptidase.^{289,290} Significant evidences have also been presented that enzymes involved in anthocyanin biosynthesis were associated with the cytoplasmic face of the endoplasmic reticulum.²⁹¹ Although synthesized in the cytoplasm, the anthocya-

nins, like many other flavonoids, may be rapidly transported across the tonoplast into the cell vacuole as indicated for anthocyanins of maize tissues.²⁹² Anthocyanins thus assume their distinct color after transport to the vacuole; however, very little is still known about the trafficking mechanisms of anthocyanins and their precursor in plant cells.

Not much was documented about the chemical nature and the functional significance of these inclusions in petal cells before Markham et al.²⁹³ reported intensively colored intravascular bodies in petals of carnations (*Dianthus caryophyllus*) and lisianthus (*Eustoma grandiflorum*), which they named anthocyanic vacuolar inclusions (AVIs). The AVIs occurred predominantly in the adaxial epidermal cells, and their presence was shown to have major influence on flower color by enhancing both intensity and blueness. Electron microscopy studies on lisianthus epidermal tissue failed to detect a membrane boundary in AVI bodies, and the isolated AVIs were shown to have a protein matrix. The presence of large AVIs produced marked color intensification in the inner zone of the petal by concentrating anthocyanins above levels that would be possible in vacuolar solution. A minor subset of the total anthocyanins (four cyanidin and delphinidin acylated 3,5-diglycosides) was bound to this matrix rather than the acylated delphinidin triglycosides, which were the major forms present in the petal extracts.²⁹³ “Trapped” anthocyanins differed from solution anthocyanins only in that they lacked a terminal rhamnose on the 3-linked galactose, revealing the specificity of the interactions. Recently, Conn et al.²⁹⁴ have reported that AVIs appeared as dark red-to-purple spheres of various sizes in vacuoles in two lines of grapevine (*Vitis vinifera*) cell suspension culture due to their interaction with anthocyanins. Compared with the total anthocyanin profile, the profile of the AVI-bound anthocyanins showed an increase of ~28 to 29% in acylated (*p*-coumarylated) anthocyanins in both lines. Figure 10.13 shows AVIs from *Vitis vinifera*,²⁹⁴ and AVIs from *Eustoma grandiflorum* and *Dianthus caryophyllus*.²⁹³

10.5 CHEMOTAXONOMIC PATTERNS

The structures of nearly 540 different anthocyanins have been elucidated, and more than half of these have been reported after 1992. In the following sections, some chemotaxonomic considerations within 11 families have been included. These families have been chosen due to the fact that most of the new anthocyanins have been isolated from species belonging to these families. Each family has been represented with a general structure including all the anthocyanins we have registered in our files as identified in one or more species belonging to this family. The chemotaxonomic considerations are mainly limited to the pattern revealed by the new anthocyanins reported in these families in the period of this review. Some additional reports of chemotaxonomic relevance are mentioned below.

The anthocyanin content of male and female cones of 27 species of *Abies*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga* (Pinaceae) has been determined, and only four anthocyanins were found.²⁹⁵ The anthocyanin content of 23 grass species (Poaceae) belonging to five subfamilies has been resolved, and altogether 11 anthocyanins were identified.²⁹⁶ A comprehensive survey of the flower pigment composition of 70 species and subspecies, 43 cultivars, and six artificial hybrids of *Crocus* (Iridaceae) used for chemotaxonomic investigations has been carried out.²⁹⁷ The anthocyanin content of petals of 28 Chilean species of *Alstroemeria* (Alstroemeriaceae) and 183 interspecific hybrids has been analyzed by HPLC.¹⁰⁴ Flower color, hue, and intensity were measured by CIELAB parameters in fresh petals and compared with their anthocyanin content and the estimated flavonoid concentrations. The chemical basis of red flower pigmentation in species of *Cotyledon*, *Crassula*, and *Tylecodon* (Crassulaceae) has been outlined.²⁹⁸ Only six major anthocyanins were found in the 10 species and 22 samples investigated, and each of the genera showed a characteristic combination of compounds. The chemical basis of flower pigmentation based on six major anthocyanins in the genus

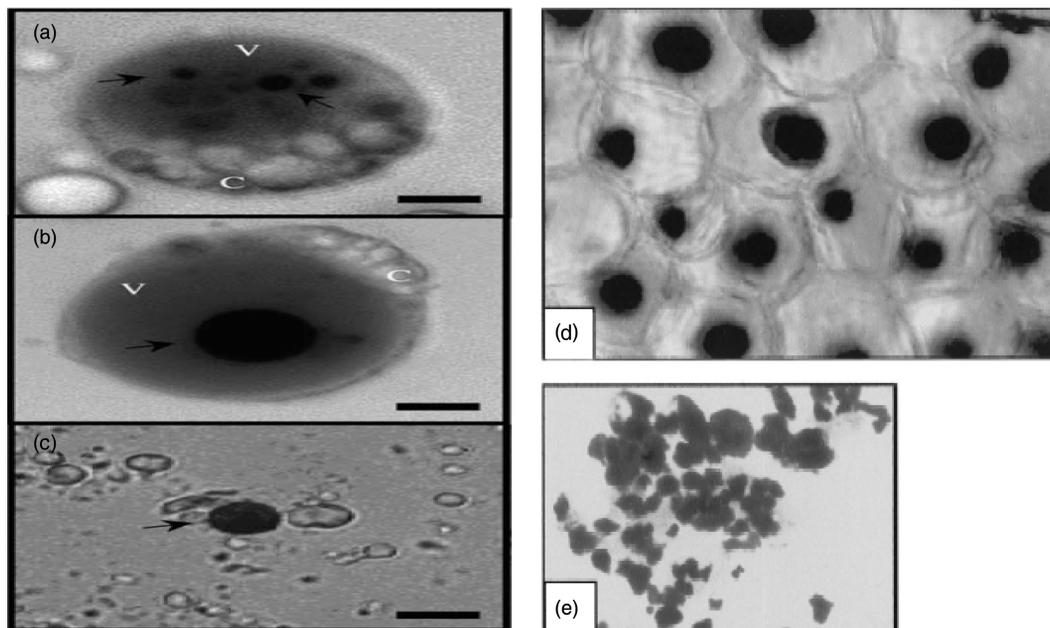


FIGURE 10.13 Left: Microscopic images of dark-grown *Vitis vinifera* protoplast of FU-2 line under bright field microscopy (a, b) before and (c) after lysis, showing a single AVI in solution. Arrows indicate AVIs, and the black bar is scaled to 10 μm . V, vacuole; C, cytoplasm. (From Conn, S. et al., *Biotechnol. Lett.*, 25, 835, 2003. With permission.) Right: (d) AVIs isolated from *Eustoma grandiflorum*, line 54, inner petal region, and (e) AVIs in an adaxial epidermal peel of *Dianthus caryophyllus*. (From Markham, K.R. et al., *Phytochemistry*, 55, 327, 2000. With permission.)

Lobostemon (Boraginaceae) has been presented.²⁹⁹ During a wide survey of flower flavonoids in a variety of sections in the genus *Rosa*, altogether 11 anthocyanins were identified.^{300,301} According to the anthocyanin distribution patterns in the genus, eight groups were classified chemotaxonomically.

10.5.1 ALLIACEAE

The genus *Allium* (Alliaceae) comprises important vegetables like onions and chive. Among the 14 anthocyanins, which have been identified in various *Allium* species (Figure 10.14), ten novel anthocyanins with characteristic structures have been reported from this genus after 1993. Several of the anthocyanins from species in *Allium* have either a glucosyl (laminariobioside), malonyl, or acetyl moiety linked distinctively to the glucosyl 3-position,^{11,135,147, 202–205,302} which is very unusual. Most of the anthocyanins in this genus are based on cyanidin. Two covalent anthocyanin–flavonol complexes (Figure 10.9) have been isolated from the flowers of chive, *Allium schoenoprasum*.¹¹

Red onion reveals one of the most characteristic anthocyanin patterns ever found. The main anthocyanins of some cultivars have been identified as the 3-[6-(malonyl)glucoside], 3-[3-(glucosyl)-6-(malonyl)glucoside], 3-[3-glucosyl]glucoside, and 3-glucoside of cyanidin.^{135,203,205} Without support from NMR or MS data, the 3-arabinoside and 3-malonylarabinoside of cyanidin have been reported to be among the main anthocyanins of Spanish red onion (cultivar “Morada de Amposta”).³⁰³ Among the minor anthocyanins the 3-[3,6-(dimalonyl)glucoside] and 3-[3-(malonyl)glucoside] of cyanidin as well as the 3,5-diglucosides of cyanidin and peonidin have been found.²⁰³ Additionally, peonidin 3-glucoside and peonidin

10.5.2 CONVOLVULACEAE

Altogether 43 different anthocyanins have been reported to occur in Convolvulaceae (Figure 10.14). Most of these pigments have a glucose unit in the 5-position, and either a mono- or diacylated glucose or sophorose unit in the 3-position of peonidin, pelargonidin, cyanidin, and rarely delphinidin. The acyl groups are mainly caffeoyl or coumaryl; however, ferulyl, *p*-hydroxybenzoyl, and malonyl have been reported. A triacylated-tetraglucosylated cyanidin derivative (**243**), which contained the novel *E*-3,5-dihydroxycinnamic acid linked to the 6-position of one of the glucosyl moieties, has recently been isolated from flowers of *Ipomoea asarifolia*.²⁷ Even though some ternatins (**414–416**, **418**) and cyanodelphin (**417**) actually have larger masses, the “Heavenly Blue Anthocyanin” (**297**), which is a peonidin 3-sophorose-5-glucoside with three caffeoylglucose residues,³⁰⁴ is among the largest monomeric anthocyanins that has been isolated ($[M]^+ = 1759$ amu).

The Morning Glory flowers (*Ipomoea/Pharbitis*) exist in a wide range of color forms. There is a good correlation between scarlet flower color and the occurrence of pelargonidin derivatives.^{305,306} Lu et al.³⁰⁷ have showed that the flower color of *Pharbitis nil* gradually shifts to the blue region with increasing numbers of caffeic acid residues in polyacylated pelargonidin glycosides. Blue and mauve flower colors, attractive to bee pollinators, are generally based on delphinidin, petunidin, or malvidin. However, exceptional cases are found, for instance, in *Ipomoea tricolor* and *Pharbitis nil* where the blue flower colors are caused by the peonidin-based “Heavenly Blue Anthocyanin.”^{308–310} Yoshida et al.³¹¹ have shown that the color change of *Ipomoea tricolor* while flowering was due to vacuolar pH changes from 6.6 to 7.7, at which range the anhydrobase anion of HBA was formed and stabilized by intramolecular stacking. A different acylated peonidin glycoside, isomeric with “Heavenly Blue Anthocyanin,”³¹² and related cyanidin derivatives have been obtained from violet-blue flowers of *Pharbitis nil*³⁰⁸ and *Ipomoea purpurea*,³⁰⁵ while related pelargonidin derivatives have been isolated from red-purple flowers.^{306,308} One blue flowered species with more expectable delphinidin chromophore has been discovered in *Evolvulus pilosus*. The pigment in this plant, **397**, which contains one malonyl unit linked to the 5-glucoside, provides a stable blue color at neutral pHs maintained by intermolecular stacking of the caffeoyl moieties between the flavylium chromophores.³¹³ None of the other anthocyanins reported from this family are malonylated.

10.5.3 CRUCIFERAE

In total, around 45 different anthocyanins have been reported to occur in various species belonging to Cruciferae (Brassicaceae), and nearly all of them share the same type of structures (Figure 10.14). They have a glucosyl at the 5-position, which in some cases is malonylated, and a disaccharide at the 3-position, which is acylated with one or two cinnamic acids. In the genera *Brassica*¹⁷⁸ and *Raphanus*,^{314,315} this disaccharide is a sophorose, while *Arabidopsis*,¹⁶⁷ *Sinapis*,³¹⁶ and *Matthiola*^{177,173} have a sambubioside in the same position. When one cinnamyl moiety occurs, it is attached to the 6-position of the inner hexose (glucose). The second cinnamic acid is linked to the 2-position of the outer hexose (xylose) when a sambubioside occurs, and at the 6-position of the outer hexose (glucose) when the corresponding sophoroside occurs. The cinnamic acids include coumaric acid, caffeic acid, ferulic acid, and sinapic acid. All anthocyanins are either based on cyanidin or pelargonidin, and 28 of these have been reported as novel in the period of this review.

10.5.4 GENTIANACEAE

Altogether 22 anthocyanins, including three produced by genetic engineering, have been reported to occur in flowers from the family Gentianaceae (Figure 10.14). Nineteen among these have been identified as novel compounds in the period of this review. The examined

species belonging to *Gentiana* contain one or more anthocyanins with a 3,5,3'-triglycosidic substitution pattern.^{153,154} The blue flowers are reported to contain delphinidin derivatives, while the pink flowers of this genus contain cyanidin derivatives.^{153,154} All the monosaccharides directly linked to the aglycone are glucosyl moieties. This is in contrast to anthocyanins isolated from *Eustoma grandiflorum*, which have a galactosyl or robinobiosyl attached to the 3-position, and no glycosyl moiety linked to the 3'-position.^{123,129} The new anthocyanins based on delphinidin isolated from flowers of *Eustoma grandiflorum* have been produced by genetic engineering after transformation with a UDP-glucose:flavonoid-3-*O*-glucosyltransferase cDNA from *Antirrhinum majus*. The galactose at the 3-position has been partly replaced in some anthocyanins by glucose during anthocyanin synthesis in the transformed plant.¹²³

10.5.5 GERANIACEAE

Thirteen different anthocyanins from species in Geraniaceae have been identified (Figure 10.14), and seven among these are novel pigments reported after 1994. The major anthocyanins of various *Pelargonium* species and cultivars were identified as the 3,5-diglucosides and 3-glucoside-5-[6-(acetyl)glucosides] of the six common anthocyanidins.³¹⁷ The major factors responsible for color variation were shown to be the types and relative levels of pigments present. Variations in pH and copigment levels were not found to contribute significantly. Flowers with colors ranging from cream and pink through to deep purple, including salmon, orange, and red, were studied. While either flavonols or carotenoids were responsible for cream or yellow coloration, all other colors resulted from anthocyanin mixtures. Similar malvidin 3,5-diglucosides have also been reported in purplish-blue *Geranium* flowers.^{210,211} Even though the acetyl in these pigments has been reported attached both to the 3-glucose and to the 5-glucose, the latter is the most probable position.²¹¹ In addition, minor amounts of the 3,5-diglucosides of malvidin and cyanidin and the 3-glucosides of cyanidin and delphinidin have been identified in purplish-blue flowers of *Geranium sylvaticum*.²¹⁰

10.5.6 LABIATAE

Around 40 anthocyanins have been identified in one or more species belonging to Labiatae (Figure 10.14), and during the last decade seven have been identified for the first time in this family. The Labiatae is a large, highly evolved plant family with a wide geographical distribution, which constitutes an appropriate group of plants for investigation of the relationship between anthocyanin structure and flower color. Studies on the distribution pattern of anthocyanins in species of *Salvia* and other genera have shown that the red, scarlet, and pink-colored flower varieties contained pelargonidin, the blue ones delphinidin, and the amethyst- and grape-violet-colored ones were based on cyanidin derivatives.^{318,200} In a survey of 49 Labiatae species and cultivars, Saito and Harborne²⁰⁰ confirm the universal occurrence of anthocyanin 3,5-diglucosides, and the widespread occurrence of both aromatic and aliphatic acylation with *p*-coumaric and caffeic acids in the majority of the species. For the more conjugated anthocyanins, the disaccharide sophorose in the 3-position is a distinct feature. Lu and Foo³¹⁹ have recently reviewed the polyphenolic content of *Salvia* including 20 anthocyanins. Phippen and Simon³²⁰ have identified anthocyanins from the herb *Ocimum basilicum* with a slightly simpler structures than the more substituted anthocyanins found in *Ajuga reptans*,^{110,111} *Ajuga pyramidalis*,³⁶ and *Salvia uliginosa*.²⁰⁹

The location of an aliphatic acyl group to the glucose 4-position as found in the 3-[6-(*p*-coumaryl)glucoside]-5-[4,6-di-(malonyl)glucosides] of cyanidin, delphinidin, and pelargonidin, the 3-[6-(caffeyl)glucoside]-5-[4,6-di-(malonyl)glucoside] of the two latter anthocyanidins,^{198–200} and delphinidin 3-[6-(*p*-coumaryl)glucoside]-5-[4-(acetyl)-6-(malonyl)glucoside] from flowers

of *Salvia uliginosa*²⁰⁹ is very characteristic for Labiatae. Protodelphin (**351**), a metalloanthocyanin similar to commelinin and protocyanin (Section 10.2.7), has been detected in flowers of *Salvia patens*.²²⁶

10.5.7 LEGUMINOSAE

More than 60 different anthocyanins have been identified in species belonging to the family Leguminosae (Figure 10.15). In the period of this review, 17 novel anthocyanins have been reported from this family, of which 13 are from *Clitoria ternatea*.^{141,144,145} Blue petals of *Clitoria ternatea* contain mainly ternatins (polyacylated delphinidin 3,3',5'-triglucosides) and preternatins. The change in flower color from blue to mauve in this species is caused by lack of (polyacylated) glucosyl substitutions at both the 3'- and 5'-positions of the ternatins.¹⁴¹

The novel delphinidin 3-xylosylgalactoside 5-acetylglucoside and its deacetylated derivative have been isolated from purple pods of pea (*Pisum* spp.).¹²⁹ Anthocyanins containing 3-galactoside (including 3-lathyroside) have previously been identified in the genus *Lathyrus*. The 3-rhamnoside-5-glucosides of petunidin (71%), delphinidin (12%), and malvidin (9%) have been isolated from the purple-blue flowers of *Vicia villosa*,³²¹ while the 3-glucosides of delphinidin, petunin, and malvidin have been mainly identified in species belonging to *Vigna*, *Phaseolus*, and *Glycine*.³²²⁻³²⁴ Other anthocyanin patterns have been shown to be of chemotaxonomic value in the tribes *Podalyrieae*³²⁵ and *Liparieae*.³²⁶ The considerable variation in flower colors in the former tribe was not reflected in the chemical variation because the flower pigments were surprisingly conservative.

10.5.8 LILIACEAE

Around 38 different anthocyanins have been reported to occur in various species belonging to Liliaceae (Figure 10.15). Seventeen have been reported after 1992, and many of these have extraordinary structures. Recently, Saito et al.²⁰ have isolated 8-*C*-glucosylcyanidin 3-[6-(malonyl)glucoside] from the purple flowers of *Tricyrtis formosana* cultivar Fujimusume, which is the first natural *C*-glycosylanthocyanin to be reported. Three acylated delphinidin 3,7,3',5'-tetraglucosides have been isolated from the remarkably colored blue berries of two *Dianella* species.¹⁴⁶ One of these pigments had as much as four 6-*p*-coumarylglucoside units located at different aglycone positions. Acetyl groups in anthocyanins are, in general, linked to glucosyl 6-positions; however, in four anthocyanins from tulips the acetyl group is located in the rhamnosyl 2- or 3-position.^{121,122} In delphinidin 3-[6-(4-(glucosyl)coumaryl)glucoside]-5-[6-(malonyl)glucoside], which is one of the major anthocyanins isolated from blue-purple flowers of *Triteleia bridgesii*, the coumaryl unit had another glucosyl moiety connected to its 4-hydroxyl group.¹⁶⁶ The other pigments in these flowers were delphinidin and cyanidin 3,5-diglucoside acylated with *p*-coumaryl and in some cases malonyl. Nearly 20 similar anthocyanidin 3,5-diglucosides with a cinnamic acid derivative located to the 6-position of the 3-sugar and possible malonyl or acetyl connected to the 5-sugar have been isolated from flowers of *Hyacinthus orientalis*.^{201,327,328} Red *Hyacinthus* petals contain pelargonidin derivatives, while the blue flowers have mainly delphinidin derivatives. Anthocyanins from flowers of *Lilium* have been reported to contain the 3-rutinoside-7-glucoside and 3-rutinoside of cyanidin.¹²⁰

10.5.9 NYMPHAEACEAE

Eleven different anthocyanins from water lilies have been identified (Figure 10.15), and six among these are novel compounds reported after 1992. One or more anthocyanins containing a galloylglucosyl moiety have been isolated from all examined species belonging to the

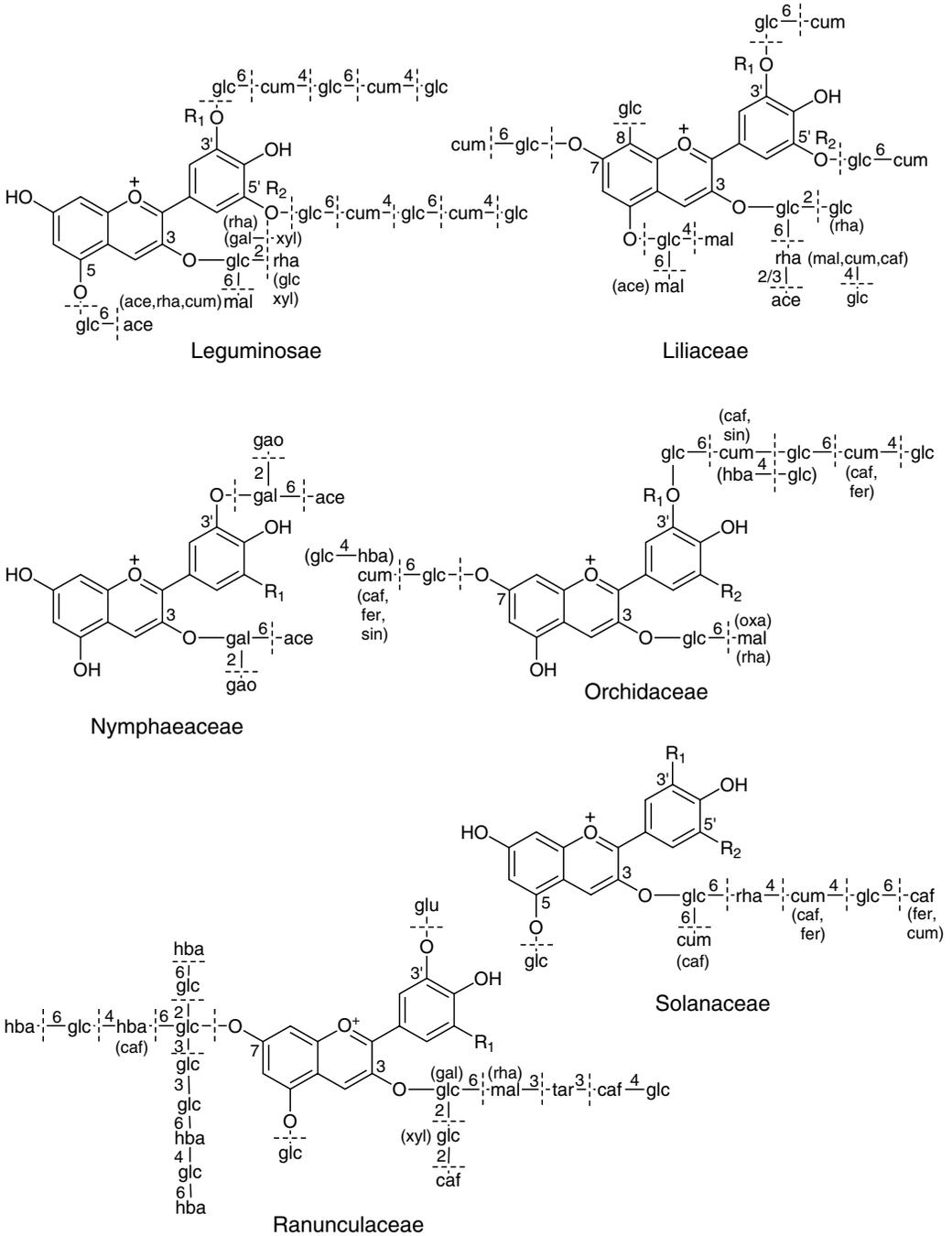


FIGURE 10.15 The structures represent a general presentation of all the anthocyanins identified in each of the families Leguminosae, Liliaceae, Nymphaeaceae, Orchidaceae, Ranunculaceae, and Solanaceae. See Table 10.2 for abbreviations.

family Nymphaeaceae.^{26,190–192} This glycosidic moiety is located at the anthocyanidin 3-position in pink or reddish flowers and leaves; however, in the blue flowers of *Nymphaea caerulea* this moiety is located in the rare 3'-position. Some of these pigments are further

acetylated on the galactosyl moiety. The pigments from *Nymphaea caerulea* are extraordinary due to their lack of glycosidic substitution at the 3-hydroxyl.¹⁹² The distribution of galloylated anthocyanins outside Nymphaeaceae is restricted to *Acalypha hispida* (Euphorbiaceae)¹³⁰ and some species in Aceraceae.^{193–195}

10.5.10 ORCHIDACEAE

Around 30 anthocyanins have been identified in orchids (Figure 10.15), and 16 among these are novel compounds reported after 1993. In orchids, cyanidin 3-oxalylglycosides have previously been reckoned to be remarkable taxonomic markers of certain European genera (e.g., *Dactylorhiza*, *Nigritella*, *Orchis*, and *Ophrys*).²²² Outside Orchidaceae, anthocyanins acylated with oxalic acid have only been reported to occur in Evergreen Blackberry (*Rubus laciniatus* Willd.).⁵⁷ More recent reports on anthocyanins from flowers of the genera *Dendrobium*,^{181,189} *Laelia*,^{155,156} *Cattleya*,^{155,156} *Bletilla*,¹⁵⁷ *Phalaenopsis*,^{180,329} *Sophronitis*,¹⁷⁶ and *Vanda*¹⁷⁹ in subfamily Epidendroideae show, however, a very different and characteristic pattern including substitution at the anthocyanidin 3,7,3'-positions. Flowers of two *Dracula* species (same subfamily) contain only various 3-glycosides of cyanidin and peonidin.³³⁰

10.5.11 RANUNCULACEAE

Nearly 35 different anthocyanins have been reported to occur in one or more species in the family Ranunculaceae (Figure 10.15), and 24 of these have been reported after 1992 as novel compounds. Flowers of species in the genera *Delphinium* (blue),¹⁰⁹ *Consolida* (blue-violet),¹¹⁵ and *Aconitum* (purplish-blue)¹⁸⁴ contain similar anthocyanins with polyacyl substitution based on *p*-hydroxybenzoylglucose residues at the 7-hydroxyl of delphinidin, in addition to a more simple glycosyl moiety at the 3-position. Red flowers of *Delphinium hybridum*^{119,331} share a similar 3,7-disubstitution pattern based on pelargonidin instead of delphinidin.

The reports on anthocyanins from *Anemone coronaria*,^{28,29,132} *Pulsatilla cernua*,²² and *Ranunculus asiaticus*¹²⁶ reveal structures that are very different from the anthocyanins described above. Many of these latter pigments are based on lathyroside or sambubioside residues located at the 3-hydroxyl of delphinidin, pelargonidin, or cyanidin (Table 10.2). Among these, four unusual anthocyanins containing the acyl moiety tartaryl have been isolated from *Anemone coronaria*.^{28,29,132} Six of the anthocyanins in this plant have a glucuronoside in the 3'-position.

10.5.12 SOLANACEAE

Altogether 50 different anthocyanins (Figure 10.15) have been reported to occur in the Solanaceae family.^{161,169} Reports on anthocyanins from the genus *Solanum*, including potatoes (*Solanum tuberosum*), reveal a dominance of one or more of the 3-[6-(4-*p*-coumaryl-rhamnosyl)-glucoside]-5-glucosides of malvidin, petunidin, delphinidin, peonidin, cyanidin, and pelargonidin. In some cases, the *p*-coumaryl moiety is replaced with sinapyl or caffeyl, and in other cases, the anthocyanins are reported without acylation. Similar anthocyanins, but pelargonidin derivatives, have also been found within the ornamental genus *Petunia*. In addition, diacylated 3-rutinoside-5-glucosides of malvidin, petunidin, delphinidin, and peonidin have also been isolated from flowers of various *Petunia* species and hybrids.^{159–165} Only some of the oldest reports on anthocyanins from species within Solanaceae present anthocyanins containing the disaccharides gentiobiose, sophorose, and a 3,7-diglucoside substitution pattern.

The inheritance and biosynthesis of anthocyanin pigmentation in *Petunia* and *Solanum* have received immense interest throughout many decades,^{332–334} and the first experiments in genetically modifying anthocyanin flower color were carried out on *Petunia hybrida*.³³⁵

This subject has been treated comprehensively in Chapter 3. Colorful potatoes have been suggested as potential sources for food colorants,^{336,337} and the major anthocyanin of the purple-fleshed potato “Congo,” petanin, shows higher color stability than simple anthocyanins at most pH values.²³⁶ The use of anthocyanins as taxonomic markers in the genus *Petunia* discussed in relation to the flower color and possible pollination vectors has been described by Ando et al.¹⁶⁰ based on the presence of at least 24 anthocyanins in the flowers of 20 native taxa.

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APPENDIX A

Checklist of all natural anthocyanidins (in bold) and anthocyanins. Anthocyanidins found in plants without sugar are labeled with numbers. For anthocyanins found after 1992 the numbers in italic correspond to the numbers in the reference list.

A1 Pelargonidin (3,5,7,4'-tetrahydroxyflavylium)

- 1 3-Arabinoside
- 2 3-Xyloside
- 3 3-Rhamnoside
- 4 3-Galactoside
- 5 3-Glucoside
- 6 3-[2-(Xylosyl)galactoside]
- 7 3-[2-(Xylosyl)glucoside]
- 8 3-Rhamnoside-5-glucoside
- 9 3-[6-(Rhamnosyl)galactoside]

- 10 3-[2-(Rhamnosyl)glucoside]
- 11 3-[6-(Rhamnosyl)glucoside]
- 12 3-Galactoside-5-glucoside
- 13 3-Glucoside-5-glucoside
- 14 3-Glucoside-7-glucoside
- 15 3-[2-(Glucosyl)glucoside]
- 16 3-[6-(Glucosyl)glucoside]
- 17 3-[6-(Rhamnosyl)-2-(xylosyl)glucoside]
- 18 3-[2-(Xylosyl)glucoside]-5-glucoside
- 19 3-[6-(Rhamnosyl)galactoside]-5-glucoside
- 20 3-[6-(Rhamnosyl)glucoside]-5-glucoside
- 21 3-[6-(Rhamnosyl)glucoside]-7-glucoside (331)
- 22 3-[6-(Rhamnosyl)-2-(glucosyl)glucoside]
- 23 3-[2-(Glucosyl)glucoside]-5-glucoside
- 24 3-[2-(Glucosyl)glucoside]-7-glucoside
- 25 3-[6-(Acetyl)glucoside]
- 26 3-[6-(Malonyl)glucoside]
- 27 3-[6-(Maly)glucoside]
- 28 3-[6-(Caffeyl)glucoside] (360)
- 29 3-[6-(2-(Acetyl)rhamnosyl)glucoside] (122)
- 30 3-[6-(Acetyl)glucoside]-5-glucoside (384)
- 31 3-Glucoside-5-[6-(acetyl)glucoside]
- 32 3-[2-(Xylosyl)-6-(malonyl)galactoside] (29)
- 33 3-[2-(Xylosyl)-6-(methyl-malonyl)galactoside] (29)
- 34 3-[6-(Malonyl)glucoside]-5-glucoside
- 35 3-Glucoside-5-[6-(malonyl)glucoside] (327)
- 36 3-[6-(Malonyl)glucoside]-7-glucoside (331)
- 37 3-[6-(Malonyl)-2-(glucosyl)glucoside]
- 38 3-Glucoside-5-glucoside (6'',6'''-maly diester) (215)
- 39 3-[6-(Succinyl)glucoside]-5-glucoside
- 40 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]
- 41 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside
- 42 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside Z (327)
- 43 3-[6-(Caffeyl)glucoside]-5-glucoside
- 44 3-[2-(2-(Caffeyl)glucosyl)galactoside] (22)
- 45 3-[6-(3-(Glucosyl)caffeyl)glucoside] (360)
- 46 3-[6-(Ferulyl)glucoside]-5-glucoside (327)
- 47 3-[6-(Acetyl)glucoside]-5-[6-(acetyl)glucoside] (382)
- 48 3-[6-(Malonyl)glucoside]-5-[6-(acetyl)glucoside] (384)
- 49 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(acetyl)glucoside] (327)
- 50 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(malonyl)glucoside] (201)
- 51 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]
- 52 3-[6-(Caffeyl)glucoside]-5-[6-(malonyl)glucoside]
- 53 3-[6-(Malonyl)glucoside]-7-[6-(caffeyl)glucoside] (354)
- 54 3-[6-(Ferulyl)glucoside]-5-[6-(malonyl)glucoside] (327)
- 55 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(malonyl)-6-(malonyl)glucoside]
- 56 3-[6-(Caffeyl)glucoside]-5-[4-(malonyl)-6-(malonyl)glucoside]
- 57 3-[6-(Rhamnosyl)glucoside]-7-[6-(*p*-hydroxybenzoyl)glucoside] (331)
- 58 3-Glucoside-7-[6-(4-(glucosyl)*p*-hydroxybenzoyl)glucoside] (331)
- 59 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside

- 60 3-[2-(6-(*p*-Coumaryl)glucosyl)glucoside]-5-glucoside (310)
 61 3-[2-(Glucosyl)-6-(*p*-coumaryl)glucoside]-5-glucoside (314)
 62 3-[6-(4-(Ferulyl)rhamnosyl)glucoside]-5-glucoside (171)
 63 3-[2-(6-(Caffeyl)glucosyl)glucoside]-5-glucoside
 64 3-[2-(Glucosyl)-6-(caffeyl)glucoside]-5-glucoside (359)
 65 3-[6-(3-(Glucosyl)caffeyl)glucoside]-5-glucoside (360)
 66 3-[2-(Glucosyl)-6-(ferulyl)glucoside]-5-glucoside (314)
 67 3-[2-(6-(Ferulyl)glucosyl)glucoside]-5-glucoside (314)
 68 3-[2-(Xylosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (173)
 69 3-[6-(Malonyl)glucoside]-7-[6-(4-(glucosyl)*p*-hydroxybenzoyl)glucoside] (331)
 70 3-[2-(Xylosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (173)
 71 3-[2-(Glucosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (314)
 72 3-[2-(Glucosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (314)
 73 3-[2-(6-(Caffeyl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-glucoside (314)
 74 3-[2-(6-(Ferulyl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-glucoside (314)
 75 3-[2-(2-(Sinapyl)xylosyl)-6-(*p*-coumaryl)glucoside]-5-glucoside (173)
 76 3-[2-(6-(Caffeyl)glucosyl)-6-(caffeyl)glucoside]-5-glucoside (306)
 77 3-[2-(6-(Caffeyl)glucosyl)-6-(ferulyl)glucoside]-5-glucoside (314)
 78 3-[2-(6-(Ferulyl)glucosyl)-6-(caffeyl)glucoside]-5-glucoside (314)
 79 3-[2-(6-(Ferulyl)glucosyl)-6-(ferulyl)glucoside]-5-glucoside (314)
 80 3-[2-(2-(Ferulyl)glucosyl)-6-(ferulyl)glucoside]-5-glucoside (315)
 81 3-[2-(2-(Sinapyl)xylosyl)-6-(ferulyl)glucoside]-5-glucoside (173)
 82 3-[2-(Xylosyl)-6-(3-(3-(4-(glucosyl)caffeyl)-2-tartaryl)malonyl)galactoside] (29)
 83 3-[2-(2-(Ferulyl)xylosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (173)
 84 3-[2-(2-(Sinapyl)xylosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (173)
 85 3-[6-(Malonyl)glucoside]-7-[6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside] (354)
 86 3-[2-(2-(Sinapyl)xylosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (173)
 87 3-[6-(Rhamnosyl)glucoside]-7-[6-(4-(glucosyl)*p*-hydroxybenzoyl)glucoside] (331)
 88 3-[2-(6-(3-(Glucosyl)caffeyl)glucosyl)glucoside]-5-glucoside
 89 3-[2-(Glucosyl)-6-(4-(glucosyl)caffeyl)glucoside]-5-glucoside (310)
 90 3-[2-(6-(3-(Glucosyl)caffeyl)glucosyl)-6-(caffeyl)glucoside]-5-glucoside
 91 3-[2-(6-(Caffeyl)glucosyl)-6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside]-5-glucoside (306)
 92 3-[6-(Rhamnosyl)glucoside]-7-[6-(4-(6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside]
 93 3-[2-(6-(3-(Glucosyl)caffeyl)glucosyl)-6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside]-5-glucoside (306)
 94 3-[2-(6-(3-(Glucosyl)caffeyl)glucosyl)-6-(4-(6-(3-(glucosyl)caffeyl)glucosyl)caffeyl)glucoside]-5-glucoside

A2 Cyanidin (3,5,7,3',4'-pentahydroxyflavylium)

- 95 3-Arabinoside
 96 3-Xyloside
 97 3-Rhamnoside
 98 3-Galactoside
 99 3-Glucoside
 100 4'-Glucoside (147)
 101 3-Arabinoside-5-glucoside
 102 3-[2-(Xylosyl)galactoside]
 103 3-[2-(Xylosyl)glucoside]

- 104 3-Rhamnoside-5-glucoside
- 105 3-Glucoside-7-rhamnoside
- 106 3-[6-(Rhamnosyl)galactoside]
- 107 3-[2-(Rhamnosyl)glucoside]
- 108 3-[6-(Rhamnosyl)glucoside]
- 109 3-Galactoside-5-glucoside
- 110 3-Glucoside-5-glucoside
- 111 3-Glucoside-7-glucoside
- 112 3-Glucoside-3'-glucoside
- 113 3-Glucoside-4'-glucoside (135)
- 114 3-[2-(Glucosyl)galactoside] (23)
- 115 3-[2-(Glucosyl)glucoside]
- 116 3-[3-(Glucosyl)glucoside]
- 117 3-[6-(Glucosyl)glucoside]
- 118 3-[2-(Glucuronosyl)glucoside]
- 119 3-[2-(Xylosyl)-6-(rhamnosyl)glucoside]
- 120 3-[2-(Xylosyl)glucoside]-5-glucoside
- 121 3-[2-(Xylosyl)-6-(glucosyl)galactoside]
- 122 3-[6-(Rhamnosyl)glucoside]-5-glucoside
- 123 3-[6-(Rhamnosyl)glucoside]-3'-glucoside
- 124 3-[6-(Rhamnosyl)glucoside]-7-glucoside (120)
- 125 3-[2-(Glucosyl)-6-(rhamnosyl)glucoside]
- 126 3-Glucoside-5-glucoside-3'-glucoside
- 127 3-Glucoside-7-glucoside-3'-glucoside
- 128 3-[2-(Glucosyl)glucoside]-5-glucoside
- 129 3-[6-(Acetyl)galactoside] (192)
- 130 3-[4-(Acetyl)glucoside]
- 131 3-[6-(Acetyl)glucoside]
- 132 3-[6-(Oxalyl)glucoside]
- 133 3-[3-(Malonyl)glucoside] (202)
- 134 3-[6-(Malonyl)glucoside]
- 135 3-[6-(Succinyl)glucoside] (221)
- 136 3-[6-(Malyl)glucoside]
- 137 3-[6-(*p*-Coumaryl)glucoside]
- 138 3-[2-(Galloyl)galactoside]
- 139 3-[2-(Galloyl)glucoside] (193)
- 140 3-[6-(Caffeyl)glucoside]
- 141 3-[3-(Malonyl)-6-(malonyl)glucoside] (202)
- 142 3-[2-(Galloyl)-6-(acetyl)galactoside] (367)
- 143 3-[2-(Galloyl)3-(galloyl)glucoside] (193)
- 144 3-[6-(2-(Acetyl)rhamnosyl)glucoside] (122)
- 145 3-[6-(4-(Acetyl)rhamnosyl)glucoside]
- 146 3-[6-(Acetyl)glucoside]-5-glucoside
- 147 3-Glucoside-5-[6-(acetyl)glucoside] (317)
- 148 3-[2-(Xylosyl)-6-(malonyl)glucoside] (126)
- 149 3-[6-(Malonyl)glucoside]-5-glucoside
- 150 3-[3-(Glucosyl)-6-(malonyl)glucoside] (135)
- 151 3-[6-(Malonyl)glucoside]6-*C*-[glucoside] (20)
- 152 3-Glucoside-5-glucoside (6'',6'''-malyl diester) (214)
- 153a 3-[6-(Succinyl)glucoside]-5-glucoside

- 153b 3-[6-(Succinyl)glucoside]-5-glucoside^a
 154 3-[4-(Malonyl)-2-(glucuronosyl)glucoside]
 155 3-[6-(Malonyl)-2-(glucuronosyl)glucoside]
 156 3-[2-(Xylosyl)-6-(*p*-coumaryl)glucoside] (116)
 157 3-[2-(Xylosyl)-6-(*p*-coumaryl)glucoside] Z (116)
 158 3-[2-(Xylosyl)-6-(caffeyl)glucoside] (116)
 159 3-[2-(Xylosyl)-6-(caffeyl)glucoside] Z (116)
 160 3-[2-(Galloyl)-6-(rhamnosyl)galactoside] (130)
 161 3-[2-(Galloyl)-6-(rhamnosyl)glucoside]
 162 3-Galactoside-5-[6-(*p*-coumaryl)glucoside] (129)
 163 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside
 164 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside Z
 165 3-Glucoside-5-[6-(*p*-coumaryl)glucoside] (153)
 166 3-[6-(4-(Caffeyl)rhamnosyl)glucoside]
 167 3-[2-(Glucosyl)-6-(*p*-coumaryl)glucoside] (116)
 168 3-[2-(Glucosyl)-6-(*p*-coumaryl)glucoside] Z (116)
 169 3-[6-(Caffeyl)glucoside]-5-glucoside
 170 3-[6-(Caffeyl)glucoside]-5-glucoside Z
 171 3-Glucoside-5-[6-(caffeyl)glucoside] (153)
 172 3-Glucoside-3'-[6-(caffeyl)glucoside] (353)
 173 3-[2-(Glucosyl)-6-(caffeyl)glucoside] (358)
 174 3-[2-(6-(Caffeyl)glucosyl)glucoside] (358)
 175 3-[6-(Ferulyl)glucoside]-5-glucoside
 176 3-[6-(6-(Ferulyl)glucosyl)galactoside]
 177 3-[6-(6-(Sinapyl)glucosyl)galactoside]
 178 3-[4-(Sinapyl)-6-(glucosyl)glucoside]
 179 3-[6-(6-(Sinapyl)glucosyl)glucoside]
 180 3-[6-(Rhamnosyl)galactoside]-5-[6-(*p*-coumaryl)glucoside] (129)
 181 3-[6-(Acetyl)glucoside]-5-[6-(acetyl)glucoside] (382)
 182 3-[6-(Malonyl)glucoside]-5-[6-(malonyl)glucoside]
 183 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]
 184 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside] Z
 185 3-[6-(Caffeyl)glucoside]-5-[6-(malonyl)glucoside]
 186 3-[6-(Malonyl)glucoside]-3'-[6-(caffeyl)glucoside] (353)
 187 3-[6-(Ferulyl)glucoside]-5-[6-(malonyl)glucoside]
 188 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(malonyl)-6-(malonyl)glucoside]
 189 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(malonyl)-6-(malonyl)glucoside] Z
 190 3-[6-(Malonyl)-2-(xylosyl)glucoside]-7-glucoside (127, 128)
 191 3-[6-(Malonyl)-2-(glucosyl)glucoside]-5-glucoside
 192 3-[3-(Glucosyl)-6-(malonyl)glucoside]-4'-glucoside (147)
 193 -7-[3-(Glucosyl)-6-(malonyl)glucoside]-4'-glucoside (147)
 194 3-[2-(Xylosyl)-6-(6-(*p*-hydroxybenzoyl)glucosyl)galactoside]
 195 3-[6-(*p*-Coumaryl)-2-(xylosyl)glucoside]-5-glucoside
 196 3-[6-(*p*-Coumaryl)-2-(xylosyl)glucoside]-5-glucoside Z (125)
 197 3-[2-(Xylosyl)-6-(6-(*p*-coumaryl)glucosyl)galactoside]
 198 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside
 199 3-[2-(6-(*p*-Coumaryl)glucosyl)glucoside]-5-glucoside (357)

^aComponent of the metalloanthocyanin protocyanin.

- 200 3-[6-(*p*-Coumaryl)-2-(glucosyl)glucoside]-5-glucoside
 201 3-[6-(4-(Caffeoyl)rhamnosyl)glucoside]-5-glucoside
 202 3-[2-(Xylosyl)-6-(6-(ferulyl)glucosyl)galactoside]
 203 3-[2-(Xylosyl)-6-(6-(ferulyl)glucosyl)glucoside] (34)
 204 3-Glucoside-3'-glucoside-7-[6-(caffeoyl)glucoside] (176)
 205 3-[6-(Caffeoyl)-2-(glucosyl)glucoside]-5-glucoside (356)
 206 3-[6-(3-(Glucosyl)caffeoyl)glucoside]-5-glucoside (312)
 207 3-[6-(Ferulyl)-2-(glucosyl)glucoside]-5-glucoside
 208 3-[2-(Xylosyl)-6-(6-(sinapyl)glucosyl)galactoside]
 209 3-[6-(Sinapyl)-2-(glucosyl)glucoside]-5-glucoside
 210 3-[6-(Malonyl)glucoside]-3'-glucoside-7-[6-(caffeoyl)glucoside] (176)
 211 3-[6-(Malonyl)glucoside]-3'-glucoside-7-[6-(ferulyl)glucoside] (176)
 212 3-[2-(6-(*p*-Hydroxybenzoyl)glucosyl)-6-(caffeoyl)glucoside]-5-glucoside (188)
 213 3-[2-(6-(*p*-Coumaryl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-glucoside (114)
 214 3-[2-(6-(*p*-Coumaryl)glucosyl)-6-(caffeoyl)glucoside]-5-glucoside (27)
 215 3-[2-(6-(Caffeoyl)glucosyl)-6-(caffeoyl)glucoside]-5-glucoside (305)
 216 3-Glucoside-5-[6-(caffeoyl)glucoside]-3'-[6-(caffeoyl)glucoside] (153)
 217 3-[2-(6-(Ferulyl)glucosyl)-6-(caffeoyl)glucoside]-5-glucoside (35)
 218 3-[6-(Ferulyl)-2-(2-(sinapyl)xylosyl)glucoside]-5-glucoside (173)
 219 3-[2-(2-(Sinapyl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-glucoside
 220 3-[2-(2-(Sinapyl)glucosyl)-6-(ferulyl)glucoside]-5-glucoside
 221 3-[2-(6-(Sinapyl)glucosyl)-6-(sinapyl)glucoside]-5-glucoside
 222 3-Glucoside-7-[6-(sinapyl)glucoside]-3'-[6-(sinapyl)glucoside] (180)
 223 3-[2-(6-(*p*-Coumaryl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (114)
 224 3-[6-(*p*-Coumaryl)-2-(2-(sinapyl)xylosyl)glucoside]-5-[6-(malonyl)glucoside] (173)
 225 3-[6-(Caffeoyl)-2-(2-(sinapyl)xylosyl)glucoside]-5-[6-(malonyl)glucoside] (177)
 226 3-[6-(Ferulyl)-2-(2-(sinapyl)xylosyl)glucoside]-5-[6-(malonyl)glucoside] (173)
 227 3-[2-(2-(Sinapyl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (178)
 228 3-[2-(6-(Ferulyl)glucosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (36)
 229 3-[2-(2-(Sinapyl)glucosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (178)
 230 3-[6-(Malonyl)glucoside]-7-[6-(sinapyl)glucoside]-3'-[6-(sinapyl)glucoside] (180)
 231 3-[2-(Glucosyl)-6-(4-(glucosyl)coumaryl)glucoside]-5-glucoside
 232 3-[2-(Glucosyl)-6-(4-(glucosyl)caffeoyl)glucoside]-5-glucoside (312)
 233 3-[2-(Glucosyl)-6-(4-(glucosyl)ferulyl)glucoside]-5-glucoside
 234 3-[2-(2-(Caffeoyl)glucosyl)galactoside]-7-[6-(caffeoyl)glucoside]-3'-glucuronoside (29)
 235 3-[2-(6-(3-(Glucosyl)caffeoyl)glucosyl)-6-(caffeoyl)glucoside]-5-glucoside (305)
 236 3-[2-(6-(4-(6-(3-(Glucosyl)caffeoyl)glucosyl)caffeoyl)glucosyl)glucoside] (358)
 237 3-[6-(6-(Sinapyl)glucosyl)glucoside]-7-[6-(sinapyl)glucoside]-3'-glucoside
 238 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-[6-(malonyl)glucoside]-3'-[6-(caffeoyl)glucoside] *Z*, *E* (118, 365)
 239 3-[6-(4-(Glucosyl)coumaryl)-2-(2-(sinapyl)xylosyl)glucoside]-5-[6-(malonyl)glucoside] (167)
 240 3-[2-(2-(Caffeoyl)glucosyl)-6-(malonyl)galactoside]-7-[6-(caffeoyl)glucoside]-3'-glucuronoside (28)
 241 3-[2-(6-(*p*-Coumaryl)glucosyl)-6-(4-(6-(*p*-coumaryl)glucosyl)caffeoyl)glucoside]-5-glucoside (27)
 242 3-[6-(2-(Caffeoyl)arabinosyl)glucoside]-7-[6-(caffeoyl)glucoside]-3'-[6-(caffeoyl)glucoside]
 243 3-[2-(6-(Caffeoyl)glucosyl)-6-(4-(6-(3,5-dihydroxycinnamyl)glucosyl)caffeoyl)glucoside]-5-glucoside (355)

- 244 3-[6-(Malonyl)glucosyl]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside] (156)
- 245 3-[6-(Malonyl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(4-(6-(caffeyl)glucosyl)coumaryl)glucoside] (155)
- 246 3-[2-(2-(Caffeyl)glucosyl)-6-[3-(2-(tartaryl)malonyl)galactoside]-7-[6-(caffeyl)glucoside]-3'-gulucuronoside] (132)
- 247 3-[6-(Malonyl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside] (156)
- 248 3-[6-(Malonyl)glucoside]-7-[6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside]-3'-[6-(caffeyl)glucoside] (248)
- 249 3-[6-(Malonyl)glucoside]-7-[6-(caffeyl)glucoside]-3'-[6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside] (156)
- 250 3-[6-(2-(Caffeyl)-5-(caffeyl)arabinosyl)glucoside]-7-[6-(caffeyl)glucoside]-3'-[6-(caffeyl)glucoside]
- 251 3-[6-(2-(Ferulyl)-5-(ferulyl)arabinosyl)glucoside]-7-[6-(ferulyl)glucoside]-3'-[6-(ferulyl)glucoside] (136)
- 252 3-[6-(3-(Glucosyl)-6-(sinapyl)glucosyl)glucoside]-7-[6-(sinapyl)glucoside]-3'-glucoside
- 253 3-[6-(Malonyl)glucoside]-7-[6-(4-(glucosyl)*p*-hydroxybenzoyl)glucoside]-3'-[6-(4-(glucosyl)*p*-hydroxybenzoyl)glucoside] (189)
- 254 3-Glucoside-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside] (157)
- 255 3-[2-(6-(3-(Glucosyl)caffeyl)glucosyl)-6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside]-5-glucoside (305)
- 256 3-Glucoside-7-[6-(caffeyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)caffeyl)glucosyl)caffeyl)glucoside] (157)
- 257 3-[6-(Malonyl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside] (157)
- 258 3-[6-(Malonyl)glucoside]-7-[6-(caffeyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)caffeyl)glucosyl)caffeyl)glucoside] (157)

A3 Peonidin (3'-methoxy-3,5,7,4'-tetrahydroxyflavylium)

- 259 3-Arabinoside
- 260 3-Rhamnoside
- 261 3-Galactoside
- 262 3-Glucoside
- 263 3-Arabinoside-5-glucoside
- 264 3-[2-(Xylosyl)galactoside] (133)
- 265 3-[2-(Xylosyl)glucoside]
- 266 3-[4-(Arabinosyl)glucoside]
- 267 3-Rhamnoside-5-glucoside
- 268 3-[2-(Rhamnosyl)glucoside] (140)
- 269 3-[6-(Rhamnosyl)glucoside]
- 270 3-Galactoside-5-glucoside
- 271 3-Glucoside-5-glucoside
- 272 3-[2-(Glucosyl)glucoside]
- 273 3-[6-(Glucosyl)glucoside] (25)
- 274 3-[6-(Rhamnosyl)-2-(xylosyl)glucoside]
- 275 3-[6-(Rhamnosyl)glucoside]-5-glucoside
- 276 3-[2-(Glucosyl)glucoside]-5-glucoside
- 277 3-[6-(6-(Glucosyl)glucosyl)glucoside]

- 278 3-[6-(Acetyl)glucoside] (385)
 279 3-[6-(Malonyl)glucoside]
 280 3-[6-(*p*-Coumaryl)glucoside]
 281 3-[6-(Caffeyl)glucoside] (386)
 282 3-Glucoside-5-[6-(acetyl)glucoside] (317)
 283 3-[6-(Malonyl)glucoside]-5-glucoside (147)
 284 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside
 285 3-[6-(3-(Glucosyl)caffeyl)glucoside] (360)
 286 3-[4-(Sinapyl)-6-(glucosyl)glucoside]
 287 3-[6-(4-(Coumaryl)rhamnosyl)glucoside]-5-glucoside
 288 3-[6-(4-(Caffeyl)rhamnosyl)glucoside]-5-glucoside (169)
 289 3-[6-(Caffeyl)-2-(glucosyl)glucoside]-5-glucoside
 290 3-[6-(3-(Glucosyl)caffeyl)glucoside]-5-glucoside (360)
 291 3-[2-(6-(*p*-Hydroxybenzoyl)glucosyl)-6-(caffeyl)glucoside]-5-glucoside (35)
 292 3-[2-(6-(Caffeyl)glucosyl)-6-(caffeyl)glucoside]-5-glucoside
 293 3-[2-(6-(Caffeyl)glucosyl)-6-(ferulyl)glucoside]-5-glucoside
 294 3-[6-(4-(Glucosyl)caffeyl)-2-(glucosyl)glucoside]-5-glucoside
 295 3-[6-(4-(4-(Glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (165)
 296 3-[6-(4-(4-(6-(Caffeyl)glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (165)
 297 3-[2-(6-(3-(Glucosyl)caffeyl)glucosyl)-6-(4-(6-(3-(glucosyl)caffeyl)glucosyl)caffeyl)glucoside]-5-glucoside

A4 Delphinidin (3,5,7,3',4',5'-hexahydroxyflavylium)

- 298 3-Arabinoside
 299 3-Rhamnoside
 300 3-Galactoside
 301 3-Glucoside
 302 3-[2-(Xylosyl)galactoside]
 303 3-[2-(Xylosyl)glucoside]
 304 3-Rhamnoside-5-glucoside
 305 3-[2-(Rhamnosyl)glucoside]
 306 3-[6-(Rhamnosyl)galactoside]
 307 3-[6-(Rhamnosyl)glucoside]
 308 3-Glucoside-5-glucoside
 309 3-Glucoside-7-glucoside
 310 3-[2-(Glucosyl)glucoside]
 311 3-[6-(Glucosyl)glucoside]
 312 3-[2-(Xylosyl)-6-(rhamnosyl)glucoside] (24)
 313 3-[6-(Rhamnosyl)galactoside]-5-rhamnoside
 314 3-[2-(Xylosyl)galactoside]-5-glucoside (131)
 315 3-[2-(Xylosyl)glucoside]-5-glucoside
 316 3-[6-(Rhamnosyl)galactoside]-5-glucoside (123)
 317 3-[6-(Rhamnosyl)glucoside]-5-glucoside
 318 3-[6-(Rhamnosyl)glucoside]-7-glucoside (117)
 319 3-Glucoside-7-glucoside-3'-glucoside
 320 3-Glucoside-3'-glucoside-5'-glucoside
 321 3-[2-(Glucosyl)glucoside]-5-glucoside
 322 3-[6-(Acetyl)galactoside] (367)
 323 3-[6-(Acetyl)glucoside]
 324 3-[6-(Malonyl)glucoside]

- 325 3'-[2-(Galloyl)galactoside] (26)
326 3-[6-(*p*-Coumaryl)galactoside] (158)
327 3-[6-(*p*-Coumaryl)glucoside] (386)
328 3-[6-(*p*-Coumaryl)glucoside] *Z*
329 3-[2-(Galloyl)galactoside]
330 3-[2-(Galloyl)-6-(acetyl)galactoside] (191)
331 3'-[2-(Galloyl)-6-(acetyl)galactoside] (26)
332 3-[6-(2-(Acetyl)rhamnosyl)glucoside] (121)
333 3-[6-(3-(Acetyl)rhamnosyl)glucoside] (121)
334 3-Glucoside-5-[6-(acetyl)glucoside] (317)
335 3-[2-(Xylosyl)-6-(malonyl)glucoside] (126)
336 3-[2-(Rhamnosyl)-6-(malonyl)glucoside]
337 3-[6-(Malonyl)glucoside]-5-glucoside (144)
338 3-Glucoside-5-[6-(malonyl)glucoside] (345)
339 3-Glucoside-5-glucoside (6'',6'''-maly diester) (215)
340 3-[6-(Maly)glucoside]-5-glucoside (31)
341 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside
342 3-Galactoside-5-[6-(*p*-coumaryl)glucoside] (123)
343 3-Galactoside-5-[6-(*p*-coumaryl)glucoside] *Z* (123)
344 3-Glucoside-5-[6-(*p*-coumaryl)glucoside] (154)
345 3-[6-(Acetyl)glucoside]-5-[6-(acetyl)glucoside]
346 3-[6-(Malonyl)glucoside]-5-[6-(malonyl)glucoside] (351)
347 3-[6-(Maly)glucoside]-5-[6-(maly)glucoside] (31)
348 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]
349 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside] *Z*
350 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]^b
351 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]^c
352 3-[6-(Caffeyl)glucoside]-5-[6-(malonyl)glucoside]
353 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(acetyl)-6-(malonyl)glucoside] (209)
354 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(malonyl)-6-(malonyl)glucoside] (200)
355 3-[6-(Caffeyl)glucoside]-5-[4-(malonyl)-6-(malonyl)glucoside]
356 3-[2-(Xylosyl)galactoside]-5-[6-(acetyl)glucoside] (131)
357 3-[2-(Rhamnosyl)glucoside]-7-[6-(malonyl)glucoside] (139)
358 3-[6-(Malonyl)glucoside]-3'-glucoside-5'-glucoside (141)
359 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside
360 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside *Z*
361 3-[6-(Rhamnosyl)galactoside]-5-[6-(*p*-coumaryl)glucoside] (123)
362 3-[6-(Rhamnosyl)galactoside]-5-[6-(*p*-coumaryl)glucoside] *Z* (123)
363 3-[6-(Rhamnosyl)glucoside]-5-[6-(*p*-coumaryl)glucoside] (123)
364 3-[6-(Rhamnosyl)glucoside]-5-[6-(*p*-coumaryl)glucoside] *Z* (123)
365 3-[6-(4-(Caffeyl)rhamnosyl)glucoside]-5-glucoside (160)
366 3-Glucoside-5-[6-(*p*-coumaryl)glucoside]-3'-glucoside (154)
367 3-[6-(Rhamnosyl)galactoside]-5-[6-(ferulyl)glucoside] (123)
368 3-[6-(Rhamnosyl)galactoside]-5-[6-(ferulyl)glucoside] *Z* (123)
369 3-Glucoside-5-[6-(caffeyl)glucoside]-3'-glucoside (154)
370 3-[6-(*p*-Coumaryl)glucoside]-5-[4-(rhamnosyl)-6-(malonyl)glucoside]

^bComponent of the metalloanthocyanin commelinin.

^cComponent of the metalloanthocyanin protodelphin.

- 371 3-[6-(Malonyl)glucoside]-3'-[6-(*p*-coumaryl)glucoside]-5'-glucoside (141)
- 372 3-[6-(4-(Glucosyl)coumaryl)glucoside]-5-[6-(malonyl)glucoside] (166)
- 373 3-Glucoside-5-[6-(caffeyl)glucoside]-3'-[6-(*p*-coumaryl)glucoside] (154)
- 374 3-Glucoside-5-[6-(*p*-coumaryl)glucoside]-3'-[6-(caffeyl)glucoside] (154)
- 375 3-Glucoside-5-[6-(caffeyl)glucoside]-3'-[6-(caffeyl)glucoside]
- 376 3-Glucoside-7-[6-(sinapyl)glucoside]-3'-[6-(sinapyl)glucoside] (182)
- 377 3-[6-(Malonyl)glucoside]-7-[6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside] (115)
- 378 3-[6-(Malonyl)glucoside]-3'-[6-(*p*-coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (141)
- 379 3-[2-(6-(*p*-Coumaryl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (113)
- 380 3-[2-(6-(Ferulyl)glucosyl)-6-(*p*-coumaryl)glucoside]-5-[6-(malonyl)glucoside] (114)
- 381 3-[2-(6-(Ferulyl)glucosyl)-6-(ferulyl)glucoside]-5-[6-(malonyl)glucoside] (114)
- 382 3-[6-(Malonyl)glucoside]-7-[6-(sinapyl)glucoside]-3'-[6-(sinapyl)glucoside] (182)
- 383 3-[2-(2-(Caffeyl)glucosyl)-6-(3-(2-tartaryl)malonyl)galactoside]-7-[6-(caffeyl)glucoside] (29)
- 384 3-[6-(Rhamnosyl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-glucoside (32)
- 385 3-[6-(4-(4-(Glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (164)
- 386 3-Glucoside-3'-[6-(4-(glucosyl)coumaryl)glucoside]-5'-glucoside (141)
- 387 3-[6-(Rhamnosyl)glucoside]-7-[6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside] (117)
- 388 3-[6-(Malonyl)glucoside]-3'-[6-(4-(glucosyl)coumaryl)glucoside]-5'-glucoside (141)
- 389 3-[6-(Rhamnosyl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(*p*-coumaryl)glucoside] (32)
- 390 3-[6-(4-(4-(6-(Caffeyl)glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (164)
- 391 3-Glucoside-7-glucoside-3'-[6-(*p*-coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (146)
- 392 3-[6-(4-(6-(3-(Glucosyl)caffeyl)glucosyl)caffeyl)glucoside]-5-glucoside (223)
- 393 3-[2-(2-(Caffeyl)glucosyl)galactoside]-7-[6-(caffeyl)glucoside]-3'-glucuronoside (29)
- 394 3-[6-(Malonyl)glucoside]-7-[2-(glucosyl)-6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside] (115)
- 395 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside]-5'-glucoside (141)
- 396 3-[6-(Malonyl)glucoside]-3'-[6-(4-(glucosyl)coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (141)
- 397 3-[6-(4-(6-(3-(Glucosyl)caffeyl)glucosyl)caffeyl)glucoside]-5-[6-(malonyl)glucoside] (313)
- 398 3-[2-(2-(Caffeyl)glucosyl)-6-(malonyl)galactoside]-7-[6-(caffeyl)glucoside]-3'-glucuronoside (29)
- 399 3-Glucoside-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(*p*-coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (146)
- 400 3-[6-(Malonyl)glucoside]-7-[2-(6-(*p*-hydroxybenzoyl)glucosyl)-6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside] (115)
- 401 3-[2-(2-(Caffeyl)glucosyl)-6-(3-(2-tartaryl)malonyl)galactoside]-7-[6-(caffeyl)glucoside]-3'-glucuronoside (29)
- 402 3-[6-(Malonyl)glucoside]-7-[6-(4-(6-(caffeyl)glucosyl)caffeyl)glucoside]-3'-[6-(caffeyl)glucoside]
- 403 3-[6-(*p*-Coumaryl)glucoside]-7-[6-(*p*-coumaryl)glucoside]-3'-[6-(*p*-coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (146)

- 404 3-[6-(Rhamnosyl)glucoside]-7-[6-(4-(6-(4-(glucosyl)*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside] (117)
- 405 3-Glucoside-3'-[6-(4-(glucosyl)coumaryl)glucoside]-5'-[6-(4-(glucosyl)coumaryl)glucoside] (141)
- 406 3-[6-(Rhamnosyl)glucoside]-7-[6-(4-(6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside]
- 407 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside]-5'-glucoside (141)
- 408 3-[6-(Malonyl)glucoside]-3'-[6-(4-(glucosyl)coumaryl)glucoside]-5'-[6-(4-(glucosyl)coumaryl)glucoside]
- 409 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (145)
- 410 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(*p*-coumaryl)glucoside] (141)
- 411 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(4-(glucosyl)coumaryl)glucoside] (141)
- 412 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-[6-(malonyl)glucoside]-3'-[6-(caffeyl)glucoside]-5'-[6-(caffeyl)glucoside]
- 413 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-[6-(malonyl)glucoside]-3'-[6-(caffeyl)glucoside]-5'-[6-(ferulyl)glucoside]
- 414 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside]
- 415 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(4-(glucosyl)coumaryl)glucoside] (141)
- 416 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(4-(6-(*p*-coumaryl)glucosyl)coumaryl)glucoside]
- 417 3-[6-(Rhamnosyl)glucoside]-7-[3-(3-(6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucosyl)glucosyl)-6-(4-(6-(*p*-hydroxybenzoyl)glucosyl)*p*-hydroxybenzoyl)glucoside]
- 418 3-[6-(Malonyl)glucoside]-3'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside]-5'-[6-(4-(6-(4-(glucosyl)coumaryl)glucosyl)coumaryl)glucoside]

A5 Petunidin (3'-methoxy-3,5,7,4',5'-pentahydroxyflavylium)

- 419 3-Arabinoside
- 420 3-Rhamnoside
- 421 3-Galactoside
- 422 3-Glucoside
- 423 3-[2-(Xylosyl)glucoside] (25)
- 424 3-Rhamnoside-5-glucoside
- 425 3-[6-(Rhamnosyl)glucoside]
- 426 3-Glucoside-5-glucoside
- 427 3-Glucoside-7-glucoside (345)
- 428 3-[2-(Glucosyl)glucoside]
- 429 3-[6-(Glucosyl)glucoside]
- 430 3-[6-(Rhamnosyl)-2-(xylosyl)glucoside] (25)
- 431 3-[6-(Rhamnosyl)glucoside]-5-glucoside
- 432 3-[6-(6-(Glucosyl)glucosyl)glucoside]
- 433 3-[2-(Glucosyl)-6-(rhamnosyl)glucoside]-3'-glucoside
- 434 3-[6-(Acetyl)glucoside]

- 435 3-[6-(Malonyl)glucoside]
 436 3-[6-(*p*-Coumaryl)glucoside]
 437 3-Glucoside-5-[6-(acetyl)glucoside] (317)
 438 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside
 439 3-[6-(Malonyl)glucoside]-7-[6-(malonyl)glucoside] (346)
 440 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]
 441 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside (169)
 442 3-[6-(4-(Caffeyl)rhamnosyl)glucoside]-5-glucoside (169)
 443 3-[6-(4-(Ferulyl)rhamnosyl)glucoside]-5-glucoside (172, 328)
 444 3-[6-(4-(4-(Glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (160)
 445 3-[6-(4-(4-(6-(Caffeyl)glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (161)

A6 Malvidin (3',5'-dimethoxy-3,5,7,4'-tetrahydroxyflavylium)

- 446 3-Arabinoside
 447 3-Rhamnoside
 448 3-Galactoside
 449 3-Glucoside
 450 3-Xyloside-5-glucoside
 451 3-[2-(Xylosyl)glucoside]
 452 3-Rhamnoside-5-glucoside
 453 3-[6-(Rhamnosyl)glucoside]
 454 3-Glucoside-5-glucoside
 455 3-Glucoside-7-glucoside
 456 3-[3-(Glucosyl)glucoside]
 457 3-[6-(Glucosyl)glucoside]
 458 3-[6-(Rhamnosyl)glucoside]-5-glucoside
 459 3-[2-(Glucosyl)glucoside]-5-glucoside
 460 3-[6-(6-(Glucosyl)glucosyl)glucoside]
 461 3-[6-(Acetyl)glucoside] (385)
 462 3-[6-(Malonyl)glucoside] (339)
 463 3-[6-(*p*-Coumaryl)glucoside] (97)
 464 3-[6-(Caffeyl)glucoside]
 465 3-[6-(Acetyl)glucoside]-5-glucoside (210)
 466 3-Glucoside-5-[6-(acetyl)glucoside] (211)
 467 3-Glucoside-5-[2-(sulfato)glucoside] (30)
 468 3-[6-(Malonyl)glucoside]-5-glucoside (339)
 469 3-Glucoside-5-[6-(malonyl)glucoside] (30)
 470 3-[6-(*p*-Coumaryl)glucoside]-5-glucoside
 471 3-[6-(*p*-Coumaryl)glucoside]-5-[2-(acetyl)xyloside] (208)
 472 3-Glucoside-5-[2-(sulfato)-6-(malonyl)glucoside] (30)
 473 3-[6-(Malonyl)glucoside]-7-[6-(malonyl)glucoside] (346)
 474 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside]
 475 3-[6-(*p*-Coumaryl)glucoside]-5-[6-(malonyl)glucoside] Z
 476 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside
 477 3-[6-(4-(*p*-Coumaryl)rhamnosyl)glucoside]-5-glucoside Z (161)
 478 3-[6-(4-(Caffeyl)rhamnosyl)glucoside]-5-glucoside (161)
 479 3-[6-(4-(Ferulyl)rhamnosyl)glucoside]-5-glucoside (172)
 480 3-[6-(4-(4-(6-(Caffeyl)glucosyl)coumaryl)rhamnosyl)glucoside] (163)
 481 3-[6-(4-(4-(6-(*p*-Coumaryl)glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (168)

- 482 3-[6-(4-(4-(6-(Caffeyl)glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (162)
483 3-[6-(4-(4-(6-(Ferulyl)glucosyl)coumaryl)rhamnosyl)glucoside]-5-glucoside (161)
484 3-[6-(4-(4-(6-(Caffeyl)glucosyl)caffeyl)rhamnosyl)glucoside]-5-glucoside (162)

A7 5-Methylcyanidin (5-methoxy-3,7,3',4'-tetrahydroxyflavylium)

- 485 3-Glucoside

A8 7-Methylpeonidin = Rosinidin (7,3'-dimethoxy-3,5,4'-trihydroxyflavylium)

- 486 3-Glucoside-5-glucoside

A9 5-Methyldelphinidin = Pulchellidin (5-methoxy-3,7,3',4',5'-pentahydroxyflavylium)

- 487 3-Rhamnoside

- 488 3-Glucoside

A10 5-Methylpetunidin = Europinidin (5,3'-dimethoxy-3,7,4',5'-tetrahydroxyflavylium)

- 489 3-Galactoside

- 490 3-Glucoside

A11 5-Methylmalvidin = Capensinidin (3,7,4'-trihydroxy-5,3',5'-trimethoxyflavylium)

- 491 3-Rhamnoside

A12 7-Methylmalvidin = Hirsutidin (3,5,4'-trihydroxy-7,3',5'-trimethoxyflavylium)

- 492 3-Glucoside (97)

- 493 3-[6-(*p*-Coumaryl)glucoside] (97)

- 494 3-Glucoside-5-glucoside

A13 6-Hydroxypelargonidin (3,5,6,7,4'-pentahydroxyflavylium)

- 495 3-Glucoside (102)

- 496 3-[6-(Rhamnosyl)glucoside] (101)

- 497 3-Glucoside-5-glucoside

- 498 3-[2-(Glucosyl)glucoside]

A14 6-Hydroxycyanidin (3,5,6,7,3',4'-hexahydroxyflavylium)

- 499 3-Glucoside

- 500 3-[6-(Malonyl)glucoside] (100)

- 501 3-[6-(Rhamnosyl)glucoside]

A15 6-Hydroxydelphinidin (3,5,6,7,3',4',5'-heptahydroxyflavylium)

- 502 3-Glucoside (101)

- 503 3-[6-(Rhamnosyl)glucoside]

- 504 3-[6-(Malonyl)glucoside] (101)

A16 Apigeninidin (5,7,4'-trihydroxyflavylium)

- 505 Apigeninidin

- 506 5-Glucoside

- 507 7-Glucoside

- 508 5-Glucoside-7-glucoside

- 509 5-[5-(Caffeyl)glucoside]

A17 Luteolinidin (5,7,3',4'-tetrahydroxyflavylium)

- 510 Luteolinidin
511 5-Glucoside
512 7-Glucoside
513 5-Glucoside-7-glucoside
514 5-[3-(Glucosyl)-2-(acetyl)glucoside] (96)

A18 Tricetinidin (5,7,3',4',5'-pentahydroxyflavylium)

- 515 Tricetinidin

A19 7-Methylapigeninidin (5,4'-dihydroxy-7-methoxyflavylium)

- 516 7-Methylapigeninidin (83)

A20 6-Hydroxy-5-methylapigeninidin = Carajuron (5-methoxy-6,7,4'-trihydroxyflavylium)

- 517 6-Hydroxy-5-methylapigeninidin (86)

A21 5,4'-Dimethyl-6-hydroxyapigeninidin = Carajurin (6,7-dihydroxy-5,4'-dimethoxyflavylium)

- 518 5,4'-Dimethyl-6-hydroxyapigeninidin

A22 5-Methyluteolinidin (5-methoxy-7,3',4'-trihydroxyflavylium)

- 519 5-Methyluteolinidin (84)

A23 6-Hydroxy-5-methyluteolinidin (5-methoxy-6,7,3',4'-tetrahydroxyflavylium)

- 520 6-Hydroxy-5-methyluteolinidin (86)

A24 5,4'-Dimethyl-6-hydroxyluteolinidin (5,4'-dimethoxy-6,7,3',4'-tetrahydroxyflavylium)

- 521 5,4'-Dimethyl-6-hydroxyluteolinidin (86)

A25 Riccionidin A (Figure 10.3)

- 522 Riccionidin A (96)

A26 5-Carboxypyranopelargonidin (Table 10.1)

- 523 5-Carboxypyranopelargonidin 3-glucoside (13)

A27 5-Carboxypyranocyanidin 3-glucoside (Table 10.1)

- 524 5-Carboxypyranocyanidin 3-glucoside (Table 10.1) (18)
525 5-Carboxypyranocyanidin 3-[6-(malonyl)glucoside] (Table 10.1) (18)

A28 Rosacyanin B (Figure 10.3)

- 526 Rosacyanin B (17)

A29 Sphagnorubin A (Figure 10.3)

- 527 Sphagnorubin A

A30 Sphagnorubin B (Figure 10.3)

- 528 Sphagnorubin B

A31 Sphagnorubin C (Figure 10.3)

- 529 Sphagnorubin C

Dimeric flavonoids including anthocyanidin

- 530 Afzelechin(4 α \rightarrow 8)pelargonidin 3-glucoside (Figure 10.8) (13)
- 531 Epiafzelechin(4 α \rightarrow 8)pelargonidin 3-glucoside (Figure 10.8) (13)
- 532 Catechin(4 α \rightarrow 8)pelargonidin 3-glucoside (Figure 10.8) (13)
- 533 Epicatechin(4 α \rightarrow 8)pelargonidin 3-glucoside (Figure 10.8) (13)
- 534 (6'''-(Delphinidin 3-[6''-(glucosyl)glucoside])) (6''-(apigenin 7-glucosyl))malonate (Figure 10.8) (9)
- 535 (6'''-(Delphinidin 3-[6''-(glucosyl)glucoside])) (6''-(luteolin 7-glucosyl))malonate (Figure 10.8) (10)
- 536 (6''-(Cyanidin 3-glucosyl)) (2''''-(kaempferol 3-[2''-(glucosyl)(glucoside)]-7-glucuronosyl))malonate (Figure 10.9) (11)
- 537 (6''-(Cyanidin 3-[3''-(acetyl)glucosyl])) (2''''-(kaempferol 3-[2''-(glucosyl)(glucoside)]-7-glucuronosyl))malonate (Figure 10.9) (11)
- 538 (6'''-(Delphinidin 3-[6''-(*p*-coumaryl)glucoside]-7-glucosyl)) (6'''-(kaempferol 3-glucoside-7-xyloside-4'-glucosyl))succinate (Figure 10.9) (12)
- 539 (6'''-(Delphinidin 3-[6''-(*p*-coumaryl)glucoside]-7-glucosyl)) (6'''-(kaempferol 3-glucoside-7-glucoside-4'-glucosyl))succinate (Figure 10.9) (12)

11 Flavans and Proanthocyanidins

Daneel Ferreira, Desmond Slade, and Jannie P.J. Marais

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11.1 INTRODUCTION

In order to ensure continuity of the excellent coverage of this topic, first by Haslam^{1,2} and later by Porter,^{3,4} this chapter will follow broadly the format of the 1988³ and 1994⁴ contributions. Complementary to these are reviews by Hemingway,⁵ Porter,⁶ and Ferreira et al.⁷⁻¹² Owing to the comprehensiveness of the Porter reviews^{3,4} and in view of the large number of compounds covered therein, the current contribution is focused on developments in the post-1992 period.

The proanthocyanidins represent a major group of phenolic compounds that occur ubiquitously in woody and some herbaceous plants.^{3-5,8,11,12} Leucocyanidins are defined as monomeric flavonoids (flavan-3,4-diols and flavan-4-ols), which produce anthocyanidins (1) by cleavage of a C—O bond on heating with mineral acid.^{2,3} The proanthocyanidins are flavan-3-ol oligomers that produce anthocyanidins by cleavage of a C—C interflavanyl bond under strongly acidic conditions. This classification is somewhat arbitrary since several biflavonoids with C—O—C interflavanyl bonds and a few trimeric analogs containing both C—C and C—O—C interflavanyl bonds have recently been identified (see Table 11.10 and Table 11.11).

Together with the bi- and triflavonoids, the proanthocyanidins constitute the two major classes of complex $C_6-C_3-C_6$ secondary metabolites. The bi- and triflavonoids^{5,13} represent products of oxidative coupling of flavones, flavonols, dihydroflavonols, flavanones, isoflavones, aurones, auronols, and chalcones and thus consistently possess a carbonyl group at C-4 or its equivalent in every constituent flavonoid unit (see Chapter 17). The proanthocyanidins, on the contrary, are usually thought to originate by ionic coupling at C-4 (C-ring) of an electrophilic flavanyl unit, generated from a flavan-4-ol⁴ or a flavan-3,4-diol,⁵ to a nucleophilic flavanyl moiety, often a flavan-3-ol. However, the limits between the bi- or triflavonoids and proanthocyanidins are of random nature in view of the growing number of “mixed” dimers, e.g., flavan-3-ol \rightarrow dihydroflavonol, and “nonproanthocyanidins” comprising oxidatively coupled flavan-3-ols.

The past 20 to 25 years have witnessed remarkable growth in our understanding of the basic structures and physicochemical properties of the proanthocyanidins. When taken in conjunction with a growing realization of their biological significance (described in, e.g., Chapters 4 to 6), such a comprehension of their chemical characteristics has highlighted the importance of this area of natural products chemistry.

11.2 NOMENCLATURE

The system of nomenclature proposed by Hemingway et al.¹⁴ and extended by Porter^{3,4} is applied consistently and is briefly summarized as follows:

1. The names of the basic flavan units are given in Table 11.1. All flavans and flavan-3-ols in this list possess 2*S* and 2*R*,3*S* absolute configuration, respectively, for example, catechin (**2**). Those with a 2*R*,3*R* configuration are prefixed with “epi,” e.g., epicatechin (**3**). Units possessing a 2*S* configuration are differentiated by the enantio (*ent*) prefix, e.g., *ent*-epicatechin (**4**) exhibiting 2*S*,3*S* absolute configuration (Figure 11.1).
2. The flavanoid skeleton is drawn and numbered in the way as illustrated for catechin (**2**).
3. The location of the interflavanyl bond in dimers and oligomers is denoted within parentheses as in carbohydrates. The orientation of the interflavanyl bond at C-4 is denoted as α or β as in the IUPAC rules. Thus, the familiar procyanidin B-1 (**5**) is named epicatechin-(4 β \rightarrow 8)-catechin, the analogous prodelfinidin (**6**) is named epigallocatechin-(4 β \rightarrow 8)-catechin, and the 2*S* analog (**7**) is named *ent*-catechin-(4 β \rightarrow 6)-*ent*-epiafzelechin.
4. A-type proanthocyanidins are often incorrectly named due to the fact that the DEF moiety in, e.g., trimeric analogs, is rotated through 180°. The proposed system^{3,4,15} cognizant of this aspect will thus be used. Proanthocyanidin A-2 (**8**) is thus named epicatechin-(2 β \rightarrow 7, 4 β \rightarrow 8)-epicatechin. The proper name for the trimeric analog **9** is epicatechin-(2 β \rightarrow 7, 4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin.
5. The rules apply equally well to the nomenclature of ether-linked proanthocyanidins, e.g., compound **10** is epioritin-(4 β \rightarrow 3)-epioritin-4 β -ol.¹⁶
6. The constituent units of proanthocyanidin oligomers may be differentiated by using ABC, DEF, etc., designations as indicated in structure **9**, or alternatively using T, M, or B designations for top, middle, or bottom units, respectively, as was proposed by Porter.⁴ These systems have the advantage that the same numbering system may be used for each monomeric unit.

With reference to Table 11.1, butiniflavan (**11**) is named from three proanthocyanidin dimers based on 4-substituted 2*S*-7,3',4'-trihydroxyflavan (2*S*-flavans unsubstituted at C-3 possess the same orientation of substituents at C-2 as 2*R*-flavan-3-ols) isolated from *Cassia petersiana*.¹⁷ The name is derived from the close structural relationship between flavan (**11**)

TABLE 11.1
Proanthocyanidin Nomenclature: Proanthocyanidin Type and Names of (2*R*,3*S*) Monomer Units (2*S*-Flavans)

Proanthocyanidin	Monomer	Hydroxylation Pattern						
		3	5	7	8	3'	4'	5'
Procassininidin	Cassiaflavan	H	H	OH	H	H	OH	H
Probutininidin	Butiniflavan	H	H	OH	H	OH	OH	H
Proapigenininidin	Apigeniflavan	H	OH	OH	H	H	OH	H
Proluteolinidin	Luteoliflavan	H	OH	OH	H	OH	OH	H
Protricitininidin	Tricetiflavan	H	OH	OH	H	OH	OH	OH
Prodisteninidin	Distenin	OH	OH	OH	H	H	H	H
Propelargonidin	Afzelechin	OH	OH	OH	H	H	OH	H
Procyanidin	Catechin	OH	OH	OH	H	OH	OH	H
Prodolphinidin	Gallocatechin	OH	OH	OH	H	OH	OH	OH
Proguibourtinidin	Guibourtinidol	OH	H	OH	H	H	OH	H
Profisetininidin	Fisetinidol	OH	H	OH	H	OH	OH	H
Prorobinetininidin	Robinetinidol	OH	H	OH	H	OH	OH	OH
Proteracacinidin	Oritin	OH	H	OH	OH	H	OH	H
Promelacacinidin	Mesquitol	OH	H	OH	OH	OH	OH	H
Propeltogynidin	Peltogynane	OCH ₂ -	H	OH	H	H	OH	OH
Promopanidin	Mopanane	OCH ₂ -	H	OH	H	OH	OH	H

and the 2*S*-7,3',4'-trihydroxyflavanone, butin. Proanthocyanidins with a butiniflavan moiety substituted at C-4 are then classified as probutinidins (see Table 11.14, Figure 11.2).

11.3 STRUCTURES AND DISTRIBUTION

The naturally occurring compounds in the flavan, flavan-3-ol, flavan-4-ol, flavan-3,4-diol, and proanthocyanidin classes, together with their plant sources, are listed in Table 11.2–Table 11.17. The lists are confined to new compounds reported in the post-1992 period or those that have been overlooked in the 1994 review, and therefore must be considered in conjunction with the corresponding tables of the Porter reviews to be comprehensive. Since many of the monomeric analogs have been published under trivial names these will be retained to facilitate electronic literature searches. Unfortunately, a considerable number of these potentially chiral compounds have been reported without assignment of absolute configuration, and are hence presented as such.

11.3.1 FLAVANS, FLAVAN-3-OLS, FLAVAN-4-OLS, AND FLAVAN-3,4-DIOLS

Owing to the purported role of the flavans and flavan-3-ols as nucleophilic chain-terminating units, and of the flavan-4-ols and flavan-3,4-diols (leucoanthocyanidins) as electrophilic chain-extension units in the biosynthesis of the proanthocyanidins,⁴ the chemistry of these four classes of compounds is intimately linked to that of the proanthocyanidins.

The most important features of the flavans and flavan-3-ols pertaining to the chemistry of the proanthocyanidins are the nucleophilicity of their A-rings, the aptitude of the heterocyclic ring of flavan-3-ols to cleavage and subsequent rearrangements, the susceptibility of analogs with pyrocatechol- or pyrogallol-type B-rings to phenol oxidative

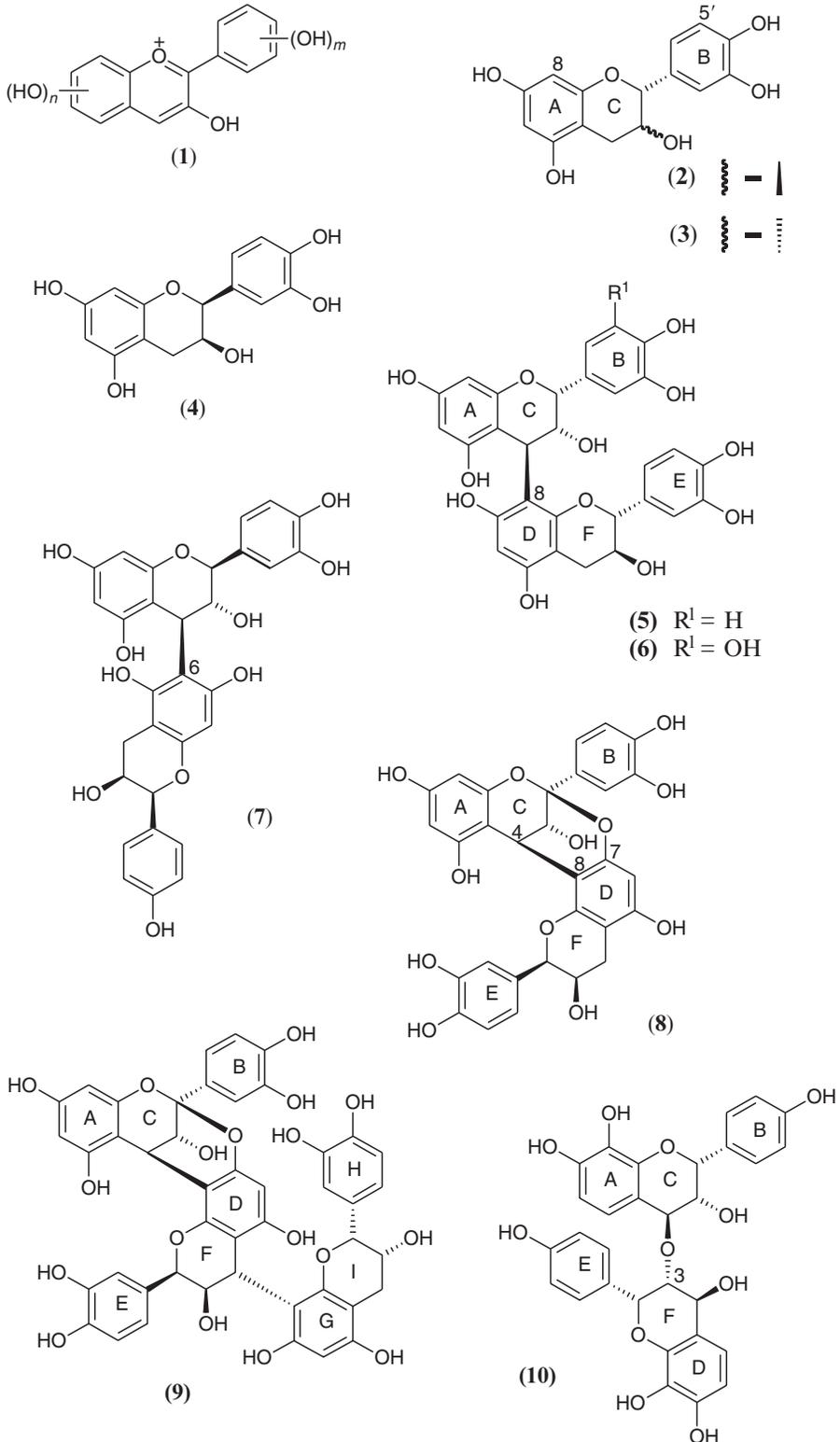


FIGURE 11.1 Structures of compounds 1–10.

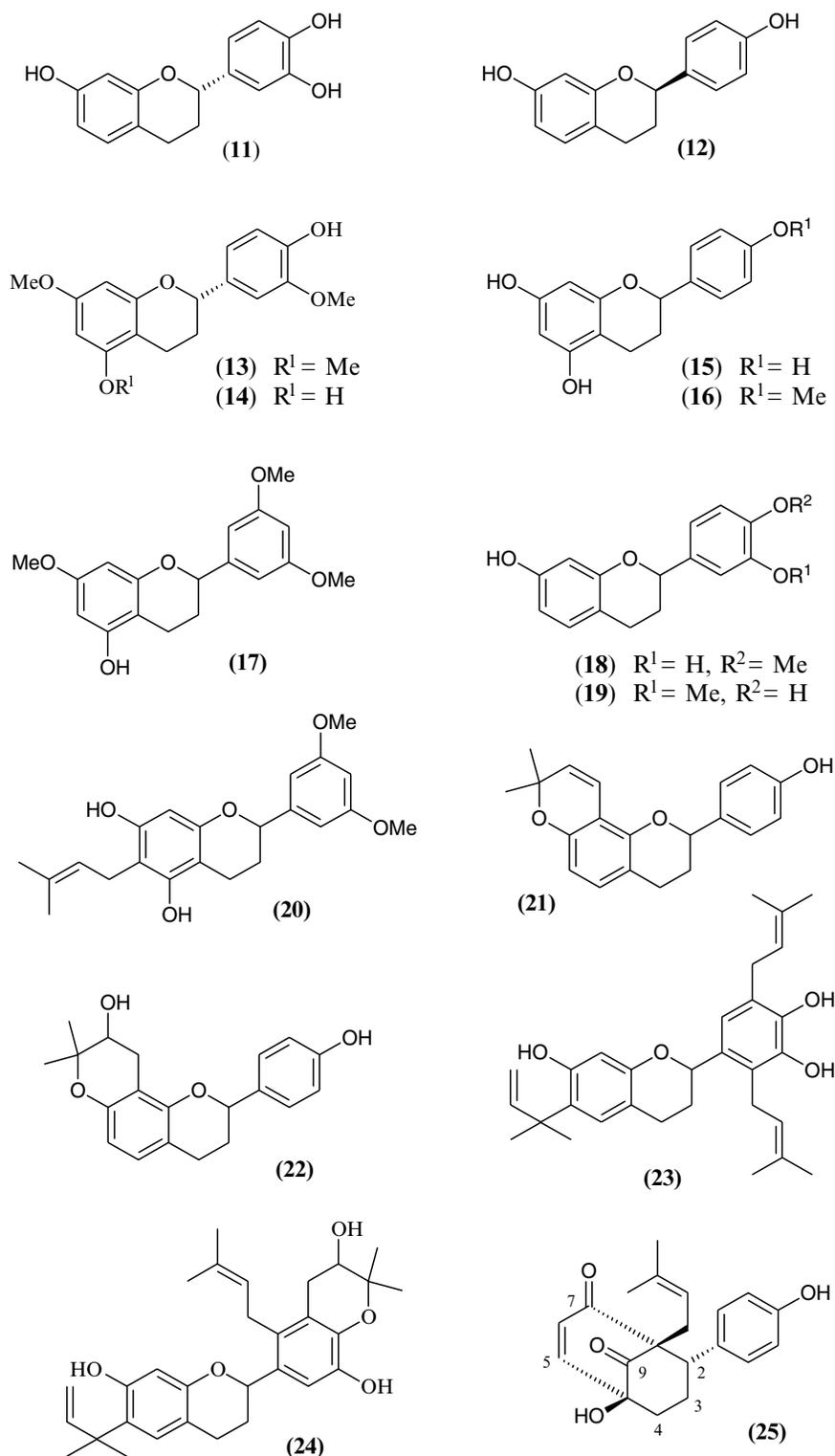


FIGURE 11.2 Structures of compounds 11–25.

TABLE 11.2
The Natural Flavans

Class	Compound	Structure	Source	Ref.
1. Simple flavans	Tupichinol C	(12)	<i>Tupistra chinensis</i>	18
	5,7,3'-Tri-OMe-4-OH	(13)	<i>Mariscus psilostachys</i>	19
	5,4'-Di-OH-7,3'-di-OH	(14)		
	5,7,4'-Tri-OH	(15)	<i>Faramea guianensis</i>	20
	5,7-Di-OH-4'-OMe	(16)		
	5-OH-7,3',5'-tri-OMe	(17)	<i>Cyperus conglomerates</i>	19
	7,3'-Di-OH-4'-OMe	(18)	<i>Terminalia argentea</i>	22
	7,4'-Di-OH-3'-OMe	(19)		
	2. Prenylated		(20)	<i>Cyperus conglomerates</i>
		(21)	<i>Brosimum acutifolium</i>	23
		(22)		
Kazinol Q		(23)	<i>Broussonetia kazinoki</i>	24
Kazinol R		(24)		
Acutifolin A		(25)	<i>Brosimum acutifolium</i>	25
Acutifolin B		(26)		
Acutifolin C		(27)		
Acutifolin D/E		(28)/(29)		
Acutifolin F		(30)		

coupling, and the conformational mobility of their benzopyran rings. The predominant feature of the flavan-4-ols and flavan-3,4-ols in the same context is their role as precursors of flavan-4-yl carbocations or A-ring quinone methide electrophiles. The stability of the carbocations and hence the ease and rate of their formation from the benzylic alcohol precursors is dependent on the degree of delocalization of the positive charge over the A-ring, and on the potential of the B-ring to contribute toward stabilization via an A-conformation.¹⁰

11.3.1.1 Flavans

In contrast to the ubiquitous distribution of flavonoids hydroxylated at C-3 or C-4 of their heterocyclic rings, the unsubstituted flavans (2-phenylchromans) are more rarely found. The flavans co-occur with chalcones, flavanones, flavan-3,4-diols, flavonols, and 1,3-diphenylpropanes.¹⁰

Several new simple flavans, including tupichinol C (12)¹⁸ with its rare (2*R*) absolute configuration, were identified. Among the remaining analogs the absolute configuration at C-2 has been established for flavans 13 and 14 only.¹⁹ Flavan 13 had 2*S* absolute configuration while 14 was obtained as a racemate.¹⁹ For the remaining simple flavans, 15, 16,²⁰ 17,²¹ and 18 and 19,²² as well as the prenylated analogs 20,²¹ 21, 22,²³ kazinols Q (23) and R (24),²⁴ and acutifolins A–F (25–30),²⁵ (Figure 11.2 and Figure 11.3) the configurational issue has simply been ignored. This is an unfortunate situation since simple techniques like measurement of optical rotation and circular dichroic (CD) data readily facilitate unequivocal assignment of absolute configuration. For example, simple flavans with 2*S* absolute configuration usually exhibit negative optical rotations,²⁶ and vice versa for 2*R* configuration.²⁷ The more sensitive CD technique exhibits a negative Cotton effect for the ¹L_b electronic transition of the aromatic chromophore in the 270 to 290 nm region of the CD spectra of 2*S*-flavans and

a positive one for 2*R*-flavans.^{28,29} Since derivatization of phenolic functionalities may sometimes influence the sign of the optical rotation, the CD method should be routinely employed to unequivocally establish the absolute configuration of nonracemic flavans.

Biogenetically, acutifolin A (**25**) may be derived from the co-occurring brosimine B (**31**) via base-catalyzed pyran-ring cleavage and oxygenation of the A-ring (Scheme 11.1). Subsequent recyclization of the B-ring quinone methide intermediate (**32**) would then lead to the formation of the unique bicyclo[3.3.1]non-3-ene-2,9-dione ring system of acutifolin A (**25**).²⁵

The flavans exhibit a wide range of biological activities. Details of these may be found in the appropriate references.

11.3.1.2 Flavan-3-ols

Two flavan-3-ols with new hydroxylation patterns were reported (Table 11.3). (2*R*,3*R*)-5,7,2',5'-Tetrahydroxyflavan-3-ol (**33**) was obtained from *Prunus prostrata*³⁰ and (+)-5,6,7,8,3',4'-hexahydroxyflavan-3-ol (elephantorrhizol) (**34**) from *Elephantorrhiza goetzei*.³¹ The absolute configuration of **33** was established by comparison of CD data with those of epicatechin, and that of **34** based on the positive optical rotation. The new epiobinetinidol (**35**) was isolated from commercial wattle (*Acacia mearnsii*) bark extract,³² *ent*-mesquitol (**36**) from *Dichrostachys cinerea*,³³ and the first guibourtinidol, (2*R*,3*S*)-guibourtinol (**37**), from *Cassia abbreviata*.³⁴ The absolute configuration of these compounds was again properly assessed via the CD for **35** and **37**, and sign of the optical rotation for **36** (Figure 11.4).

A large number and variety of new derivatives of known flavan-3-ols have been reported (Table 11.3). It should again be emphasized that in many instances the issue of absolute configuration assessment has simply been ignored. As for the flavans, this may be conveniently done by CD. The CD curves of flavan-3-ols exhibit two Cotton effects for the ¹L_a and ¹L_b electronic transitions of the A-ring aromatic chromophore in the 240 and 280 nm regions, respectively.^{35–37} Analogs with 2*R* and 2*S* absolute configurations gave negative and positive Cotton effects, respectively, in the 280 nm (¹L_b transition) region. The sign of the Cotton effect of the ¹L_a transition at ~240 nm is consistently opposite to that at longer wavelength.

In addition to identification of flavan-3-ols and derivatives from natural sources (Table 11.3, Figure 11.3–Figure 11.5, Figure 11.7, and Figure 11.8), several synthetic studies and efforts at establishing absolute configuration have been reported. The modified Mosher method has been successfully applied to configurational definition of the flavan-3-ols and 4-arylflavan-3-ols,⁶⁹ and the A-type proanthocyanidins.⁷⁰ The first stereoselective synthesis of a series of flavan-3-ol permethylaryl ethers as well as the free phenolic forms was recently developed.^{34,37,71} Substantial efforts were also devoted toward the synthesis of ¹³C- and other labeled flavan-3-ols.^{72–74} The 4β-carboxymethylepicatechin, dryopteretic acid (**46**), was recently synthesized via nucleophilic substitution at C-4 of 4β-acetoxyepicatechin.⁴⁷

The synthetic protocol (Scheme 11.2) toward the flavan-3-ol permethylaryl ethers is based upon the transformation of *retro*-chalcones into 1,3-diarylpropenes. These compounds are then subjected to asymmetric dihydroxylation to give diarylpropan-1,2-diols that are used as chiral auxiliaries for essentially enantioselective synthesis of flavan-3-ols. The protocol is demonstrated in Scheme 11.2 for the synthesis of the tetra-*O*-methyl-3-*O*-acetyl derivatives **61a**, **61b**, **62a**, and **62b** of (+)-catechin (**2**), (–)-*ent*-catechin, (–)-epicatechin (**3**), and (+)-*ent*-epicatechin (**4**).⁷¹

The (*E*)-*retro*-2-methoxymethylchalcone methyl ether (**57**) was transformed by consecutive reduction (H₂-Pd and NaBH₄) and elimination [SOCl₂ and 1,8-diazabicyclo[5.4.0]undec-7-ene(1,8-DBU)] of the ensuing alcohol (**58**), exclusively affording the (*E*)-1,3-diarylpropene

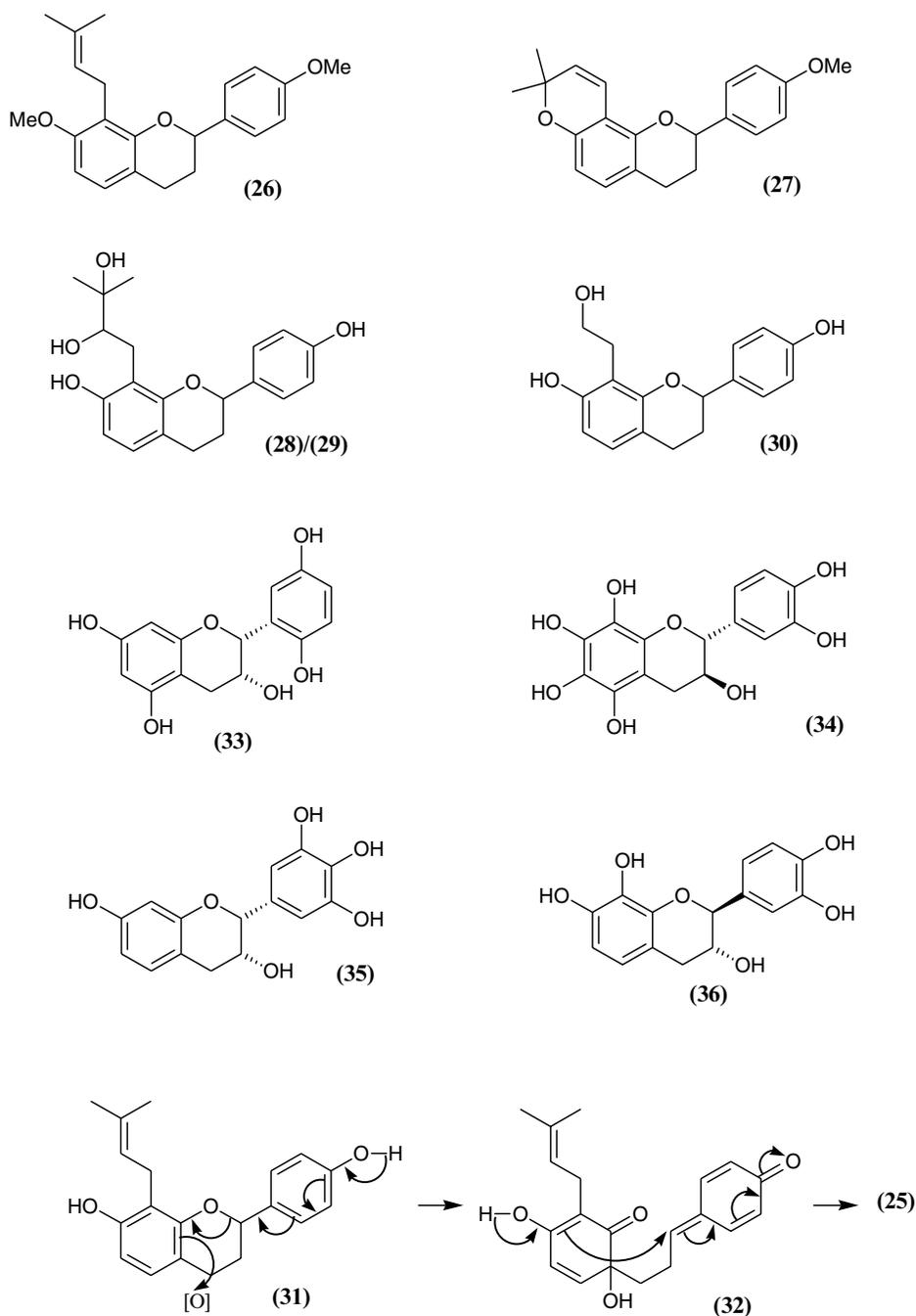


FIGURE 11.3 Structures of compounds 26–36 including Scheme 11.1: Proposed biogenetic conversion of brosimine B (31) into acutifolin A (25).

(deoxodihydrochalcone) (59). Treatment of this (*E*)-propene at 0°C with AD-mix- α (see Ref. 71 for the relevant K.B. Sharpless references) in the two-phase system Bu^tOH–H₂O (1:1) afforded the (+)-(1*S*,2*S*)-*syn*-diol (60a) in 80% yield and high optical purity (99% ee). The (–)-(1*R*,2*R*)-*syn*-diol (60b) was similarly formed by using AD-mix- β in the same two-phase

TABLE 11.3
Naturally Occurring Flavan-3-ols and Related Compounds

Class	Compound	Structure	Source	Ref.
1. Free phenolic	(2 <i>R</i> ,3 <i>R</i>)-5,7,2',5'-Tetra-OH	(33)	<i>Prunus prostrata</i>	30
	Elephantorrhizol	(34)	<i>Elephantorrhiza goetzei</i>	31
	Epirobinetinidol	(35)	Commercial wattle bark extract	32
	<i>Ent</i> -mesquitol	(36)	<i>Dichrostachys cinerea</i>	33
	Guibourtinidol	(37)	<i>Cassia abbreviata</i>	34
2. <i>O</i> -Glycosides	Catechin-3- <i>O</i> -β-D-Glp	(38)	<i>Quercus marilandica</i> Muenchh.	38
	Catechin-7- <i>O</i> -β-D-Glp-3'-Me	(39)	<i>Picea abies</i>	39
	Afzelechin-3- <i>O</i> -α-L-Rhp	(44)	<i>Cassipourea gerrardii</i>	45
	Afzelechin-4'- <i>O</i> -β-D-Glp	(45)	<i>Selliguea feei</i>	46
	Epiafzelechin-3- <i>O</i> -β-D-Alp	(47)	<i>Drynaria propinqua</i>	48
	Epicatechin-5- <i>O</i> -β-D-Glp	(48)	<i>Davallia mariesii</i> Moore	49
	Davalliosides A and B	(50), (51)		51
	Epicatechin-5- <i>O</i> -β-D-Xylp	(66)	<i>Brosimopsis acutifolium</i>	58
	Anadanthoside	(69)	<i>Anadenanthera macrocarpa</i>	60
	Epicatechin-5- <i>O</i> -β-D-Glp-3-benzoate	(71)	<i>Celastrus orbiculatus</i>	62
	Barbatoflavan	(72)	<i>Campanula barbata</i>	63
	<i>Ent</i> -afzelechin-7- <i>O</i> -β-D-Glp	(73)	<i>Daphniphyllum oldhamii</i>	64
	Catechin-5- <i>O</i> -β-D-Glp-4'-Me	(76)	<i>Celastrus angulatus</i>	65
	Catechin-7- <i>O</i> -β-D-Glp	(78)	<i>Hordeum vulgare</i> L.	67
	Catechin-3- <i>O</i> -β-D-Glp (2-cinnamoyl)	(79)	<i>Inga umbellifera</i>	68
Catechin-3- <i>O</i> -β-D-Glp (6-cinnamoyl)	(80)			
Catechin-3- <i>O</i> -β-D-Glp (2,6-bis-cinnamoyl)	(81)			
3. <i>C</i> -Glycoside	Epicatechin-8- <i>C</i> -β-D-Galp	(77)	<i>Theobroma cacao</i> L.	66
4. Simple esters	Epigallocatechin-3- <i>O</i> -Ga	(40)	<i>Cistus incanus</i>	40
	Epigallocatechin-3- <i>O</i> -Ga-5,3',5-tri-OMe	(41)	<i>Sedum sediforum</i>	41
	Epigallocatechin-3- <i>O</i> -(4-OH-Bz)	(42)	<i>Cistus salvifolius</i>	42
	Gallocatechin-3'- <i>O</i> -Ga	(53)	<i>Pithecellobium lobatum</i>	52
	Gallocatechin-4'- <i>O</i> -Ga	(54)		
	Gallocatechin-7,3'-di- <i>O</i> -Ga	(55)		
	Gallocatechin-7,4'-di- <i>O</i> -Ga	(56)		
	Epigallocatechin-3- <i>O</i> -(3,5-di-Me)-Ga	(67)	<i>Stryphnodendron adstringens</i>	59
	Epigallocatechin-3- <i>O</i> -(4-OH-3-OMe)-benzoate	(68)		
	Amurensisin (see Section 11.3.2.1.1)	(120)	<i>Vitis amurensis</i>	104
5. Simple ethers	<i>Ent</i> -gallocatechin-4'-Me	(43)	<i>Cassia trachypus</i>	43
	Gallocatechin-4'-Me		<i>Panda oleosa</i>	44
	Epicatechin-5,7,3'-tri-Me	(49)	<i>Cinnamomum camphora</i>	50
	Epicatechin-5,7-di-Me-3',4'-methylenedioxy	(52)		
	Peltogynoid analog	(63)	<i>Cassine papillosa</i>	33
	Catechin-7-Me	(64)	<i>Prunus prostrata</i>	30
	Catechin-3'-Me	(65)	<i>Pinus sylvestrus</i>	57
	Guibourtinidol-7-Me	(70)	<i>Crinum bulbispermum</i>	61
	Tupichinol A	(74)	<i>Tupistra chinensis</i>	18
	Tupichinol B	(75)		
6. C-4 Alkylated	Dryopterac acid	(46)	<i>Selliguea feei</i>	46, 47

continued

TABLE 11.3
Naturally Occurring Flavan-3-ols and Related Compounds — *continued*

Class	Compound	Structure	Source	Ref.
7. A-ring substituted	Pyranochromene	(83)	<i>Lupinus angostifolius</i>	75
	Pyranochromene	(84)		
	Cinchonain	(85)	<i>Castanopsis hystrix</i>	76
	Cinchonain	(86)		
	Apocynin A	(87)	<i>Apocynum venetum</i>	77
	Apocynin B	(88)		
	Cinchonain	(89)	<i>C. hystrix</i>	26
	Cinchonain	(90)		
	Apocynin C	(91)	<i>A. venetum</i>	77
	Apocynin D	(92)		
	Shanciol	(93)/(94)	<i>Pleione bulbocodioides</i>	78
	Shanciol A	(95)		80, 81
	Shanciol B	(96)		
	Shanciol C	(97)		

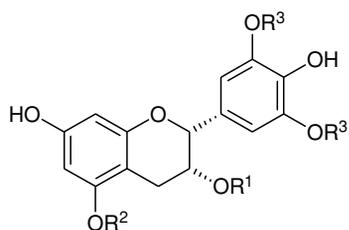
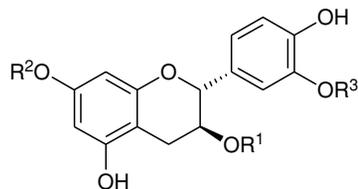
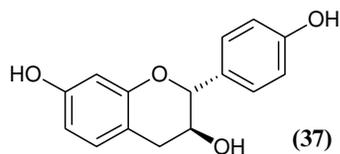
system (87% yield, 99% ee). These conversions proceeded slowly with reaction times in the range 12 to 48 h. The enantiomeric purity of the diols was determined by observing the H-1 and H-2 spin systems of the corresponding mono- or bis-MTPA esters, which show different chemical shifts in the ^1H nuclear magnetic resonance (NMR) spectra of the two diastereoisomers. The absolute configuration was tentatively assigned according to the Sharpless model (see Ref. 71 for appropriate references) for AD-mix (Figure 11.6, Scheme 11.3).

Simultaneous deprotection and cyclization of diols **60a** and **60b** with 3 M HCl in MeOH followed by acetylation yielded the 2,3-*trans*- (~50%) (**61a** and **61b**) and for the first time 2,3-*cis*-flavan-3-ol methylether acetate derivatives (~20%) (**62a** and **62b**) in excellent enantiomeric excesses (>99%). The optical purity was assessed by ^1H NMR using [Eu(hfc)₃] as chiral shift reagent. The absolute configuration of the derivatives of the *trans*- and *cis*-flavan-3-ol derivatives was assigned by comparison of CD data with those of authentic samples in the catechin or epicatechin series flavan-3-ols.^{35,36} Thus, the absolute configuration of the flavan-3-ol methyl ether acetates confirms the assigned configuration of the diols as derived from the Sharpless model.

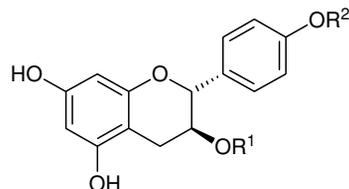
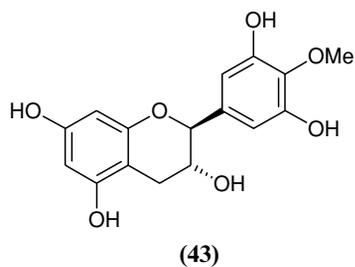
The protocol was subsequently adapted to also provide synthetic access to a variety of flavan-3-ol diastereoisomers in their free phenolic forms.^{34,37}

The co-occurrence of 4'-*O*-methylepigallocatechin and peltogynoid-type analog (**63**) in *Cassine papillosa* was demonstrated⁵³ and their biogenetic relationship established by transformation of the flavan-3-ol into **63** using acetone and toluene-*p*-sulfonic acid. This reaction occurs readily at room temperature, proceeds with retention of the C-2 and C-3 configurations, and is general for a variety of flavan-3-ols.⁵⁴ Twelve years after the latter paper was published, Fukuhara et al.^{55,56} confirmed these novel observations using a similar process to form the "planar" catechin analog (**82**) (Figure 11.7).

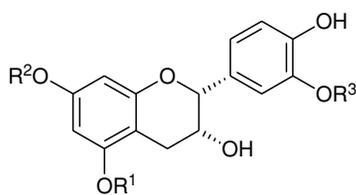
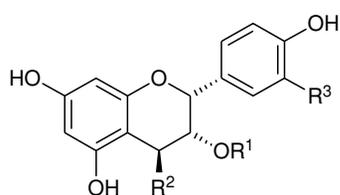
The rare series of naturally occurring flavan-3-ols with an additional pyran ring⁴ was extended by identification of the two epicatechin analogs **83** and **84**,⁷⁵ as well as four phenylpropanoid-substituted catechins, **85**, **86**, **89**, and **90**,⁷⁶ of the cinchonain type (Figure 11.8). The structure of the latter four compounds, including their absolute configurations, were elucidated on the basis of spectroscopic evidence and also by the synthesis of analogs **89**



- (40) $R^1 = 3,4,5\text{-tri-OH-benzoyl(galloyl)}, R^2 = R^3 = \text{H}$
 (41) $R^1 = \text{galloyl}, R^2 = R^3 = \text{Me}$
 (42) $R^1 = 4\text{-OH-benzoyl}, R^2 = R^3 = \text{H}$



- (45) $R^1 = \text{H}, R^2 = \beta\text{-D-glucopyranosyl}$



- (47) $R^1 = \beta\text{-D-allopyranosyl}, R^2 = \text{H}, R^3 = \text{H}$

- (49) $R^1 = R^2 = R^3 = \text{Me}$

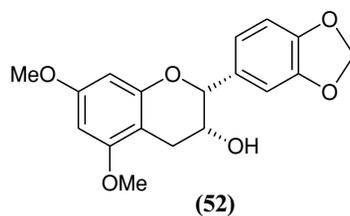
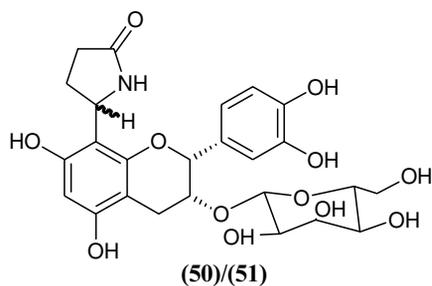


FIGURE 11.4 Structures of compounds 37–52.

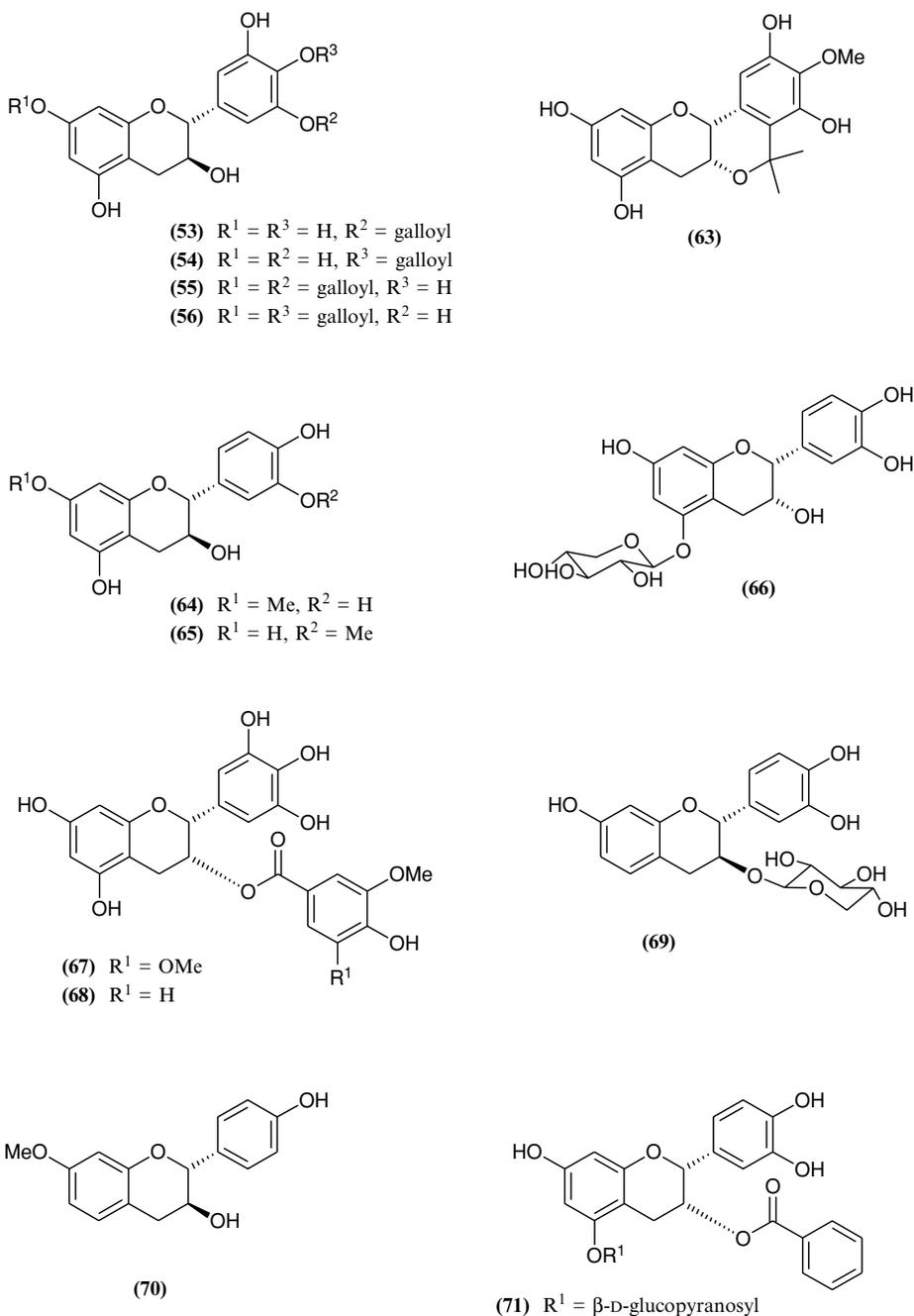
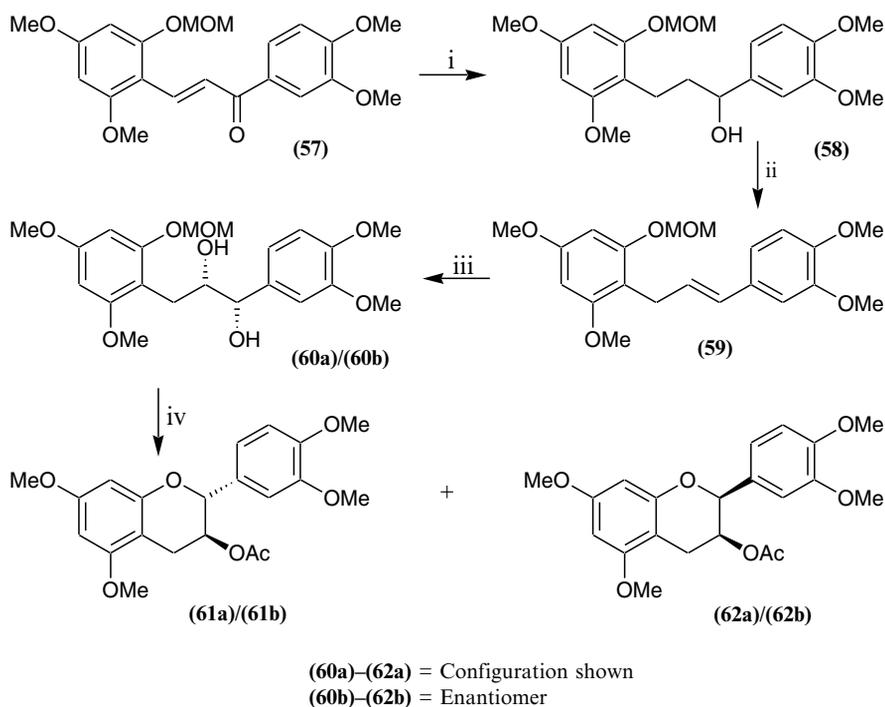
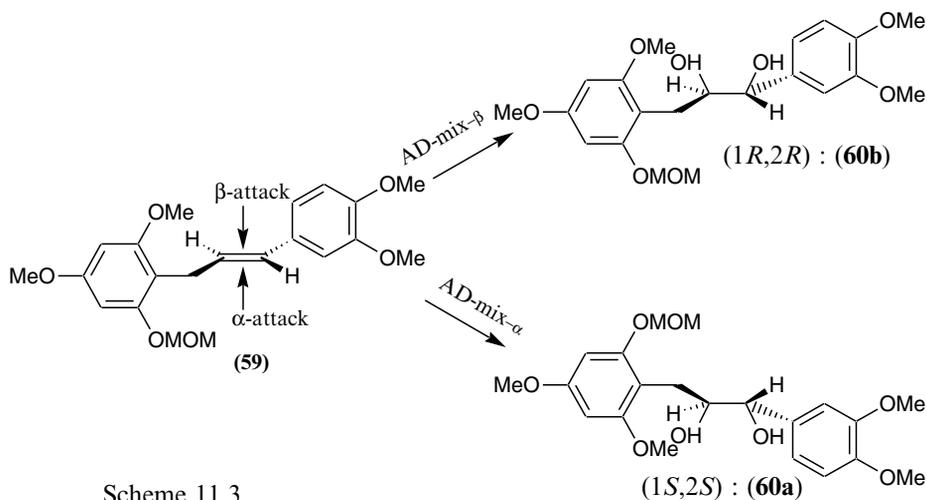


FIGURE 11.5 Structures of compounds 53–56 and 63–71.

and **90** via acid-catalyzed condensation of catechin and caffeic acid (see also Ref. 77). Comparison of the CD data of compounds **85**, **86**, **89**, and **90** with those of cinchonains 1a–1d has led to extensive revision of previous structures (see Ref. 76). The gallo catechin- and epigallo catechin-type analogs, apocynins A–D (**87**, **88**, **91**, and **92**) were identified in *Apocynum venetum*.⁷⁸



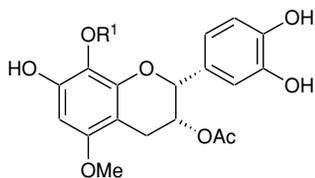
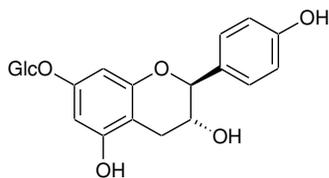
Scheme 11.2



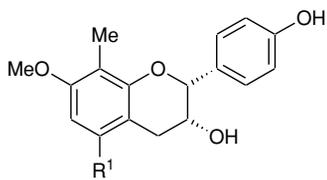
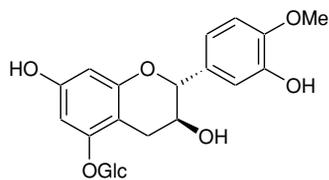
Scheme 11.3

FIGURE 11.6 Structures of compounds 57–62 including Scheme 11.2: Reagents and conditions: i, Pd-H₂, EtOH, then NaBH₄, EtOH; ii, SOCl₂, CH₂Cl₂, then 1,8-DBU, CH₂Cl₂, reflux; iii, AD-mix- α or AD-mix- β , Bu'OH-H₂O (1:1, v/v), MeSO₂NH₂, 0°C; iv, 3 M HCl, MeOH-H₂O (3:1, v/v), then Ac₂O, pyridine, and Scheme 11.3: Sharpless model for assigning absolute configuration of *syn*-diols.

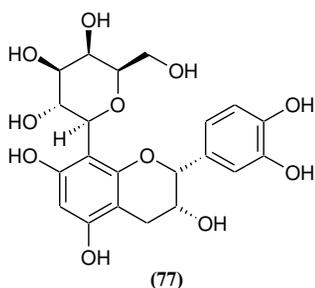
An interesting new group of flavan-3-ol analogs containing an additional C₆-C₂ unit at C-5 (A-ring) has been isolated from the tubes of *Pleione bulbocodioides*.^{79–81} Shanciols, called a dihydrophenanthropyran, was obtained in partially racemized form (93)/(94), and is accompanied by shanciols A (95), shanciols B (96), and shanciols E (97). These compounds are

(72) $R^1 = \beta\text{-D-glucopyranosyl}$ 

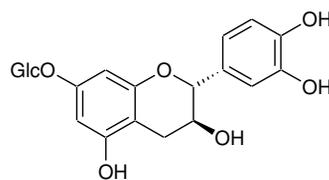
(73)

(74) $R^1 = \text{H}$ (75) $R^1 = \text{OMe}$ 

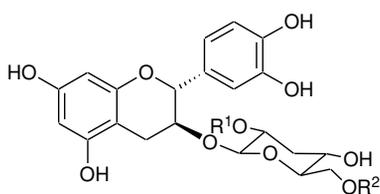
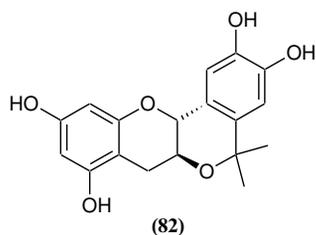
(76)



(77)



(78)

(79) $R^1 = \text{cinnamoyl}, R^2 = \text{H}$ (80) $R^1 = \text{H}, R^2 = \text{cinnamoyl}$ (81) $R^1 = R^2 = \text{cinnamoyl}$ 

(82)

FIGURE 11.7 Structures of compounds 72–81.

conspicuously accompanied by their isoflavan-4-ol isomers, e.g., shanciol C (**98**).⁸⁰ Flavan-3-ols with an A-ring phenethyl group, e.g., **95**, were implied as possible biogenetic precursors to these dihydrophenanthroprans (Figure 11.8 and Figure 11.9).⁸⁰

Nonproanthocyanidins with flavan or flavan-3-ol constituent units as well as “complex tannins,” i.e., polyphenols in which a flavan-3-ol unit is connected to a hydrolyzable tannin through a C—C linkage are discussed in Sections 11.3.3 and 11.3.4, respectively.

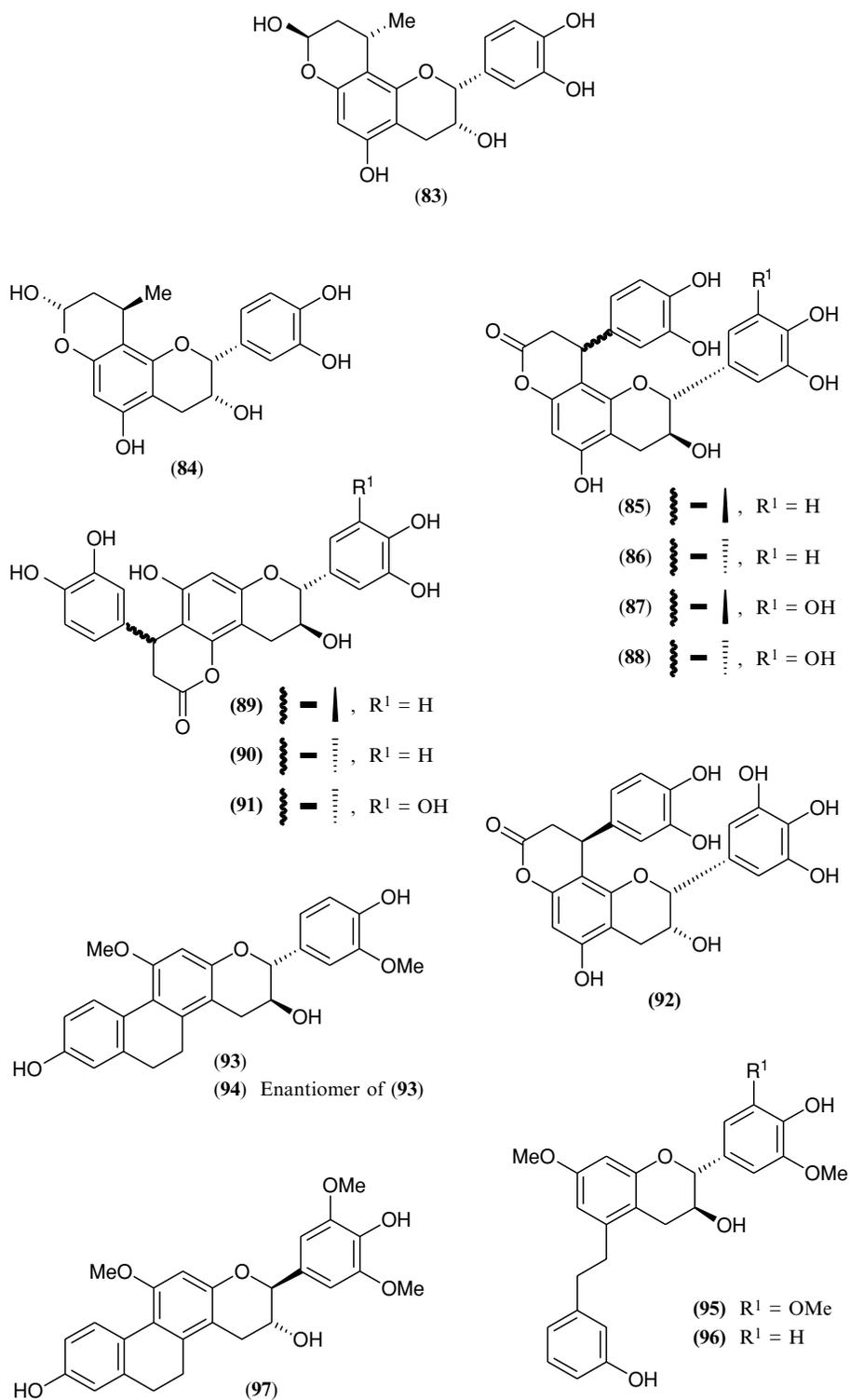


FIGURE 11.8 Structures of compounds 82–97.

11.3.1.3 Flavan-4-ols

A limited number of new flavan-4-ols has been reported since 1992 (Figure 11.9). Additions to this class of compounds comprise the 2,4-*cis*-flavan-4-ols (**99** and **100**),⁸² pneumatopterins A–D (**101–104**),⁸³ and xuulanins (**105** and **106**).⁸⁴ (2*S*)-5,7,4'-Trihydroxyflavan-4-ol (**107**) was claimed to have been identified from natural sources also.⁸⁵ This may be unlikely in view of the high reactivity of 5-oxyflavan-3,4-diols as electrophiles in weakly acidic conditions.^{7,10} The absolute configuration of compound **100** was established as 2*S*,4*S* by reference to the ORD data of its likely flavanone precursor and using the relative configuration as established by ¹H NMR coupling constants of the heterocyclic protons.⁸²

A series of flavan-4-ols, e.g., **108**, was conveniently prepared by metal hydride reduction of the corresponding flavanone.⁸⁶ The flavan-4-ols were converted into the 4-methoxyflavans, e.g., **109**, by acid-catalyzed solvolysis in methanol. Both these classes of compounds are currently evaluated as anticancer drugs. Enantiomerically enriched *cis*-flavan-4-ols have been prepared by lipase-catalyzed kinetic resolution of racemic counterparts.⁸⁷

11.3.1.4 Flavan-3,4-diols

The four flavan-3,4-diols (**110–113**) (Figure 11.9) were isolated from the seeds of *Musa sapientum*.⁸⁸ These were claimed to be the leucoguibourtinidins *ent*-epiguibourtinidol-4 α -ol (**110**), *ent*-guibourtinidol-4 α -ol (**111**), (–)-(2*S*,3*R*,4*R*)-2,3-*trans*-flavan-3,4-diol (**112**), the first flavan-3,4-diol devoid of A-ring hydroxylation, and the leucopelargonidin, *ent*-epiafzelechin-4 α -ol (**113**). The relative 2,3-*trans*-3,4-*cis* configuration of compounds **111** and **112** (³*J*_{2,3} = 10.5, ³*J*_{3,4} = 3.1 Hz and ³*J*_{2,3} = 10.5, ³*J*_{3,4} = 2.5 Hz, respectively) was convincingly deduced from ¹H NMR data of the per-*O*-acetyl derivatives. However, the proposed 2,3-*cis*-3,4-*trans* configuration of the *O*-acetyl derivatives of **110** and **113** did not appear to be supported by coupling constants (³*J*_{2,3} = 2.5, ³*J*_{3,4} = 10.5 Hz and ³*J*_{2,3} = 3.5, ³*J*_{3,4} = 9.5 Hz, respectively) and suggests that a reinterpretation of data is now required. A further feature that casts doubt on the validity of the structural claims is the apparent stability of leucopelargonidin (**113**) in contrast to the well-established reactivity of flavan-3,4-diols with 5,7-dihydroxy A-rings.¹⁰

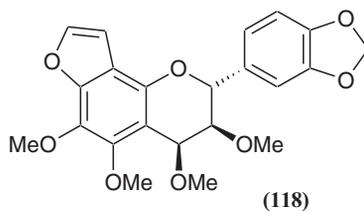
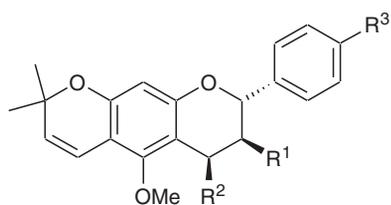
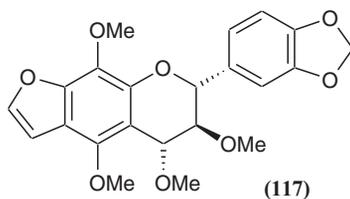
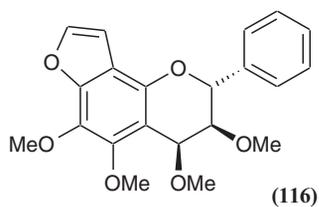
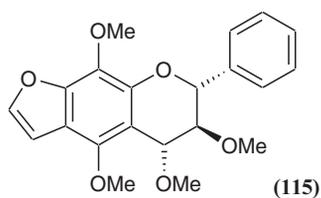
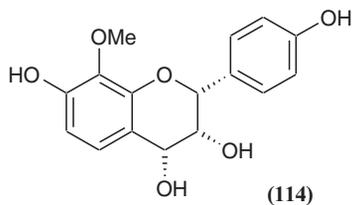
The 8-*O*-methylepioritin-4 α -ol (**114**)⁸⁹ complements the rare series of flavan-3,4-diols where *O*-methylation had occurred at one of the phenolic hydroxyl functions (Figure 11.10). Compounds **115–122** were obtained from various *Lonchocarpus* species.^{82,84,90} The enzymatic conversion of a variety of dihydroflavonols via stereospecific reduction to *cis*-flavan-3,4-diols using flower extracts of *Dianthus caryophyllus* L. was also demonstrated.⁹¹ In addition, the chiroptical data of the eight diastereomeric flavan-3,4-diols have recently been reported.⁹² These data permit unambiguous assignment of the absolute configuration at C-2 via the sign of the ¹L_b transition of the aromatic A-ring chromophore near 280 nm. A negative Cotton effect in this region invariably reflects 2*R* configuration and a positive one 2*S* configuration. The configuration at the C-3 and C-4 stereocenters then follows from the relative configurational assignment via ¹H NMR coupling constants.

For additional information regarding the chemistry of the flavans, flavan-3-ols, flavan-4-ols, and flavan-3,4-diols the reader is referred to Refs. 7–12.

11.3.2 PROANTHOCYANIDINS

11.3.2.1 B-Type Proanthocyanidins

Proanthocyanidins of the B-type are characterized by singly linked flavanyl units, usually between C-4 of the chain-extension unit and C-6 or C-8 of the chain-terminating moiety. They are classified according to the hydroxylation pattern of the chain-extension units (see Table 11.1).



(120) $R^1 = R^2 = \text{OMe}$, $R^3 = \text{OH}$

(121) $R^1 = \text{OH}$, $R^2 = \text{OMe}$, $R^3 = \text{H}$

(122) $R^1 = R^2 = \text{OH}$, $R^3 = \text{H}$

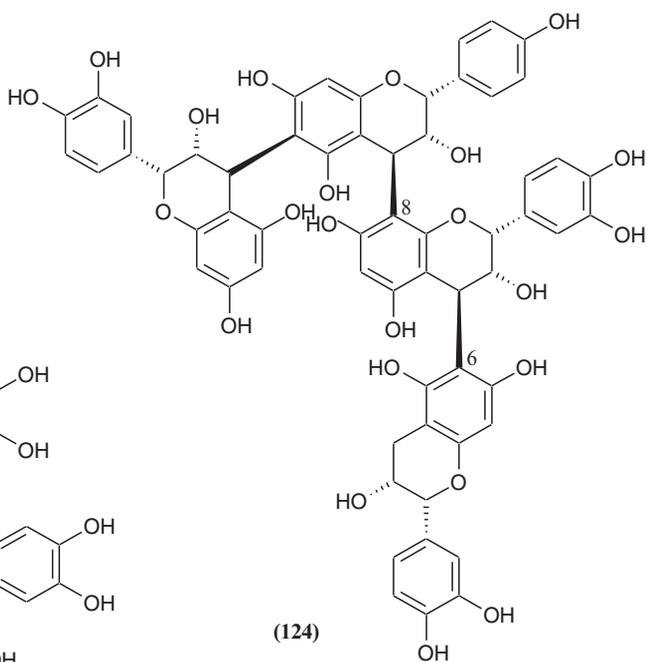
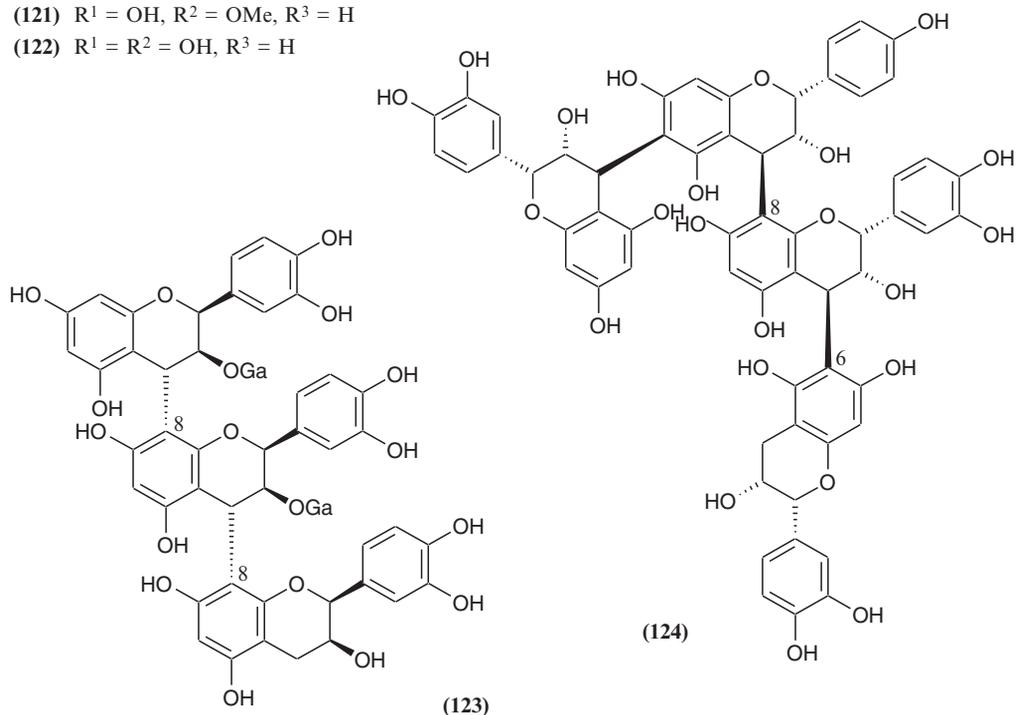


FIGURE 11.10 Structures of compounds 114–118 and 120–124.

A considerable number of new compounds have been added since 1992, such a sustained research effort motivated by the growing realization of the importance of these secondary metabolites in health, food, agriculture, and various other industrial applications. Information in this regard may be found in the cited references. A significant number of the new entries are derivatized via, e.g., galloylation and glycosylation, hence stressing the relevance of the flavan-3-ols exhibiting similar derivatization (Table 11.3).

11.3.2.1.1 Procyanidins (3,5,7,3',4'-Pentahydroxylation)

The procyanidins maintained their position as a dominant and ubiquitous group of naturally occurring proanthocyanidins. A limited number of structures will be shown, especially with a view to demonstrate perusal of the nomenclature system at the tri- and tetrameric levels, e.g., trimer (**123**) and tetramer (**124**) (Figure 11.10). New structures for di-, tri-, and tetrameric procyanidins are listed in Table 11.4. The roots of *Polygonum coriarium* afforded a “mixed” procyanidin–prodelphinidin glucosylated tetra- and hexamer, both carrying various galloyl groups.¹⁰² Possible structures were also proposed for the “mixed” procyanidin and prodelphinidin tri- and tetramers from *Geranium sanguineum*,¹⁰³ and for oligomeric proanthocyanidins D₁₄–D₁₉ from *Quercus robur*.¹⁰⁰

A number of “mixed” procyanidins or prodelphinidin analogs with exceptionally complex structures have been identified from the roots of *Clemensia semenovii*¹⁰⁵ and *Rhodiala pamiroalaica*.^{106,107} Owing to the space requirements for the naming of these macromolecules, these are listed in the text only. In addition, the authors stated that indicated configurations are relative; hence, it is unclear whether the proanthocyanidin community should indeed consider the structures of these compounds as well as those cited in Refs. 100–103, 105–107 as sufficiently defined. The analogs from *C. semenovii* are: CS-3, 7-*O*-(6-*O*-galloyl-β-D-Glcp → 6-*O*-β-D-Glcp → 6-*O*-β-D-Glcp → 6-*O*-β-D-Glcp → 6-*O*-β-D-Glcp)-(+)-catechin-(4α → 8)-(-)-epigallocatechin-(4β → 8)-(+)-catechin-(4α → 8)-(-)-epigallocatechin-(4β → 8)-(-)-epigallocatechin-(4β → 8)-(-)-epigallocatechin, and CS-4, 3-*O*-galloyl-7-*O*-[6-*O*-galloyl-β-D-Glcp → 6-*O*-β-D-Glcp → 6-*O*-β-D-Glcp]-(+)-gallocatechin-(4α → 8)-[(+)-catechin-(4α → 8)-3-*O*-galloyl-(-)-epigallocatechin]₂-(4β → 8)-(-)-epigallocatechin.

The compounds from *R. pamiroalaica* are: RP-1, 7-*O*-[6-*O*-galloyl-β-D-Glcp → *O*-β-D-Glcp → *O*-β-D-Glcp]-(+)-gallocatechin-(4α → 8)-(-)-epicatechin-(4β → 8)-(-)-epicatechin-(4β → 8)-(+)-catechin-(4α → 8)-5-*O*-[6-*O*-galloyl-β-D-Glcp → *O*-β-D-Glcp → *O*-β-D-Glcp]-(+)-catechin, RP-2, 7-*O*-[*O*-β-D-Glcp → *O*-β-D-Glcp]-(-)-epicatechin-(4β → 6)-7-*O*-β-D-Glcp-(-)-epicatechin-(4β → 6)-3-*O*-galloyl-(-)-epigallocatechin-(4β → 6)-3-*O*-galloyl-(-)-epigallocatechin-(4β → 6)-3-*O*-galloyl-5-*O*-β-D-Glcp-(-)-epicatechin, RP-3, 7-*O*-(6-*O*-galloyl-β-D-Glcp)-3-*O*-galloyl-(-)-epigallocatechin-(4β → 8)-[(-)-epicatechin-(4β → 8)-(3-*O*-galloyl-(-)-epigallocatechin)]₂-(4β → 8)-5-*O*-(β-D-Glcp-6-*O*-β-D-Glcp)-(+)-catechin, and RP-4, 7-*O*-(6-*O*-galloyl-β-D-Glcp)-3-*O*-galloyl-(-)-epigallocatechin-(4β → 8)-[3-*O*-galloyl-(-)-epicatechin]-[4β → 8)-[3-*O*-galloyl-(-)-epigallocatechin]-[4β → 8)-[3-*O*-galloyl-(-)-epicatechin]-[4β → 8)-[3-*O*-galloyl-5-(β-D-Glcp → 6-*O*-galloyl-β-D-Glcp)]-(-)-epigallocatechin.

We also need to point out the often improper use of proanthocyanidin nomenclature. In Ref. 104, both vitisinol (**125**) and amurensisin (**126**) were classified as procyanidins; per definition they do not belong to this class of compounds (Figure 11.11). Vitisinol (**125**) is rather a member of the nonproanthocyanidin class with flavan or flavan-3-ol constituent units (see Section 11.3.3), while amurensisin (**126**) is simply a gallic acid derivative of epicatechin (see Section 11.3.1.2).

In addition to the contributions dealing with the isolation and structural elucidation of procyanidins, several excellent papers describing the synthesis and chemical manipulation of the proanthocyanidins in general have been published. These may be listed as follows:

TABLE 11.4
Naturally Occurring Flavan-4-ols and Flavan-3,4-diols

Class	Compound	Structure	Source	Ref.
1. Flavan-4-ol	Furanoflavan	(99)	<i>Lonchocarpus subglaucescens</i>	82
	Furanoflavan	(100)		
	Pneumatopterin A	(101)	<i>Pneumatopteris pennigera</i>	83
	Pneumatopterin B	(102)		
	Pneumatopterin C	(103)		
	Pneumatopterin D	(104)		
	Xuulanin	(105)	<i>Lonchocarpus xuul</i>	84
2. Flavan-3,4-diol	4 β -Demethylxuulanin-4 β -ethyl ether	(106)		
	(2 <i>S</i>)-5,7,4'-Tri-OH-flavan-4-ol	(107)	<i>Streptococcus sobrinus</i>	85
	<i>Ent</i> -epiguibourtinidol-4 α -ol	(110)	<i>Musa sapientum</i>	88
	<i>Ent</i> -guibourtinidol-4 α -ol	(111)		
	2,3- <i>trans</i> -3,4- <i>cis</i> -Flavan-3,4-diol	(112)		
	<i>Ent</i> -epiafzelechin-4 α -ol	(113)		
	8- <i>O</i> -methylepioritin-4 α -ol	(114)	<i>Acacia caffra</i>	89
	Compound	(115)	<i>Lonchocarpus subglaucescens</i>	82
	Compound	(116)		
	Compound	(117)		
	Compound	(118)		
	Compound	(119)	<i>L. xuul</i>	84
Compound	(120)	<i>L. xuul/L. yucatanensis</i>	90	
Compound	(121)			
Compound	(122)			

- Continuation of the crucial role of acid-catalyzed depolymerization of proanthocyanidin polymers and subsequent capture of incipient flavanyl-4-carbocations by sulfur and oxygen nucleophiles, toward structural elucidation.^{108–113} This approach was successfully applied to demonstrate the presence of covalently bonded glycoside moieties in the chain-extension units of mangrove (*Bruguiera gymnorrhiza*) bark proanthocyanidins,¹⁰⁸ and the presence of 3-*O*-galloyl groups in the extension units of polymeric proanthocyanidins of *Hamamelis virginiana*.¹¹¹ It also highlighted the risk of using extended thiolysis to provide meaningful estimates of the molecular weight of polymeric proanthocyanidins, as well as the use of thiolysis as a means of obtaining “quantitative” information on the composition of “mixed” polymers.¹⁰⁹ The latter work was also extended to the acid-catalyzed phloroglucinolysis of Pecan (*Caraya illinoensis*) nut pith proanthocyanidins.¹¹³ A conspicuous feature of the degradation studies on the mangrove and pecan proanthocyanidins is the demonstration of the susceptibility of the C₄–C₁₀ bond of both the C-4 thio- and phloroglucinol adducts to cleavage under acidic conditions. This is demonstrated in Figure 11.11, Scheme 11.4 for an intermediate thioether (**127**). Protonation of the electron-rich A-ring leads to the weakening and subsequent cleavage of the C₄–C₁₀ bond in **128** under influence of the electron-donating benzyl sulfanyl (or phloroglucinol¹¹³) moiety. The sulfonium species (**129**) or its phloroglucinol equivalent¹¹³ is then susceptible to various rearrangements. Note that this process represents the equivalent of the cleaving of the interflavanyl

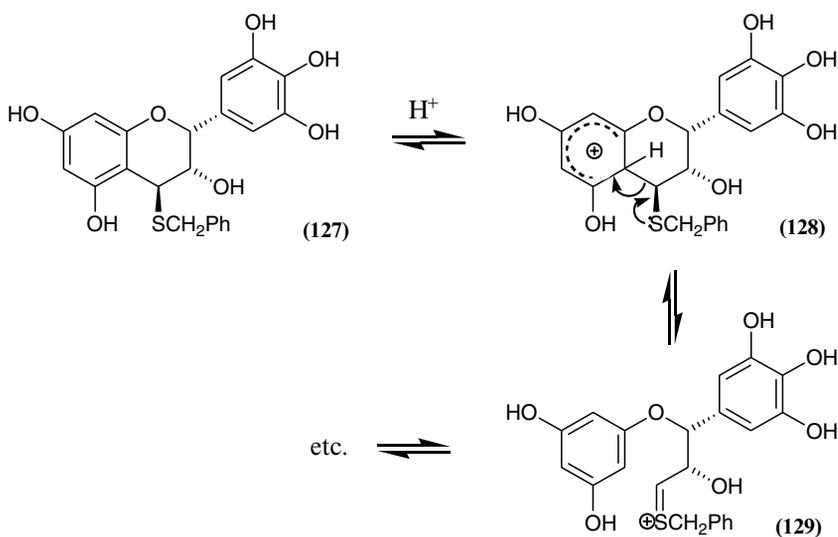
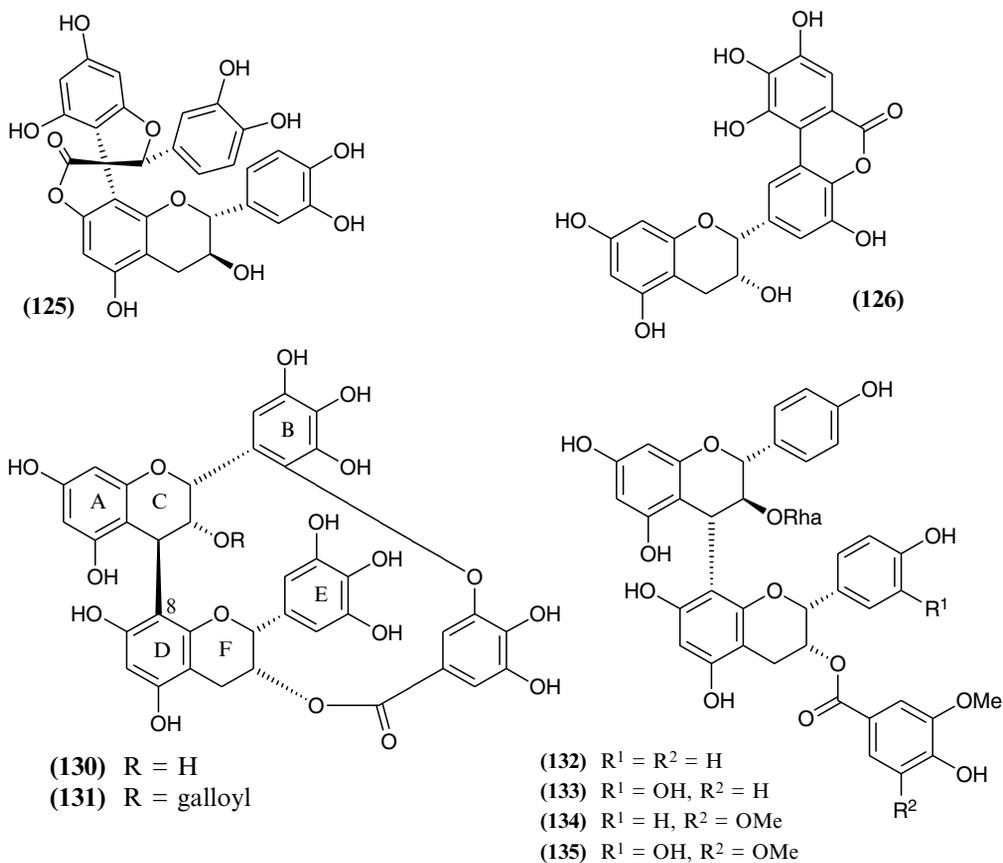


FIGURE 11.11 Structures of compounds **125–135** including Scheme 11.4: Proposed route to the acid-catalyzed cleavage of the C₄–C₁₀ bond in C-4 thiobenzylflavan-3-ols.

- bond under acidic conditions but under the influence of an external electron-donating sulfur or phloroglucinol nucleophile.
- Synthesis as a means of unequivocal structure elucidation. Synthetic studies continued to make important contributions toward the unambiguous structure elucidation of the naturally occurring proanthocyanidins. Prevalent amongst these is the synthesis of a series of di- and trimeric profisetinidins exhibiting both 2,3-*trans*- and *cis*-relative configuration of the constituent fisetinidol units,¹¹⁴ the development of a protocol to generate the interflavanyl bond of procyanidins under neutral conditions,¹¹⁵ the synthesis of ²H-,¹¹⁶ ¹³C-, and ¹⁴C-labeled catechins and procyanidins,^{117–121} the synthesis of a high molecular mass condensed tannin,¹²² synthesis of ether-linked proteracacindins or promelacacindins,¹²³ and a highlight of the review period, a series of papers dealing with synthesis of the proanthocyanidins found in cacao,¹²⁴ unequivocal proof of the 4 β -configuration in procyanidins B-2,¹²⁵ stereoselective synthesis of an unnatural procyanidin diastereoisomer, epicatechin-(4 α \rightarrow 8)-epicatechin,¹²⁶ and a process to synthesize (6 \rightarrow 6)-, (6 \rightarrow 8)-, and (8 \rightarrow 8)-linked catechin and epicatechin dimers as well as their 3,3-di-*O*-gallate esters.¹²⁷ In addition, a stereoselective route to octa-*O*-benzylprocyanidin B-3 was also described.¹²⁸
 - X-ray and CD analysis. The structure of procyanidin B-1 was unequivocally confirmed by x-ray analysis of its deca-*O*-acetyl derivative by Weinges,¹²⁹ one of the pioneers in the field of proanthocyanidin chemistry. One of the most powerful methods to establish the absolute configuration at C-4 of the T-unit in dimeric A- and B-type proanthocyanidins remains the chiroptical method via application of the aromatic quadrant rule.¹³⁰ This has been repeatedly demonstrated by the author's own work and several other contributions listed in Refs. 7–12.

11.3.2.1.2 Prodelphinidins (3,5,7,3',4',5'-Hexahydroxylation)

In addition to the "mixed" procyanidin–prodelphinidin tri- and tetramers listed in Table 11.5 and Section 11.3.2.1.1,^{105–107} a large number of new prodelphinidins have been reported. These are collated in Table 11.6. The roots of *C. semenovii* afforded a complex series of prodelphinidin oligomers.^{138,139} The structures were deduced by chemical and enzymatic degradation, as well as spectroscopic data. These are compounds CS-1, 7-*O*-[6-*O*-galloyl- β -D-Glcp-6 \rightarrow *O*- β -D-Glcp-6 \rightarrow *O*- β -D-Glcp-6 \rightarrow *O*- β -D-Glcp]-(+)-gallocatechin-(4 α \rightarrow 8)-(+)-gallocatechin-(–)-epigallocatechin-(4 β \rightarrow 8)-(-)-epigallocatechin-(4 β \rightarrow 8)-(-)-epigallocatechin-(4 β \rightarrow 8)-(+)-catechin, CS-2, 3-*O*-galloyl-7-*O*-(β -D-Glcp-6 \rightarrow *O*- β -D-Glcp-(–)-epigallocatechin-(4 α \rightarrow 8)-[3-*O*-galloyl-(–)-epicatechin]-(4 β \rightarrow 8)-[3-*O*-galloyl-(–)-epigallocatechin]-(4 β \rightarrow 8)-[3-*O*-galloyl-5-*O*-(6-*O*-galloyl-*O*- β -D-Glcp)]-(–)-epicatechin, rhodichimoside, 7-*O*-[β -D-Glcp-*O*- β -D-Glcp]₂-3-*O*-galloyl-(–)-epigallocatechin]-(4 β \rightarrow 8)-[3-*O*-galloyl-(–)-epigallocatechin]-(4 β \rightarrow 8)-3-*O*-galloyl-(–)-epigallocatechin, rhodichin, 7-*O*- β -D-Glcp-3-*O*-galloyl-(–)-epigallocatechin-(4 β \rightarrow 8)-[(–)-epigallocatechin]₂-(4 β \rightarrow 8)-epigallocatechin-(4 β \rightarrow 6)-3-*O*-galloyl-(–)-epigallocatechin.

A "mixed" propelargonidin–prodelphinidin–procyanidin pentamer, PZ-5, was identified in *Ziziphus jujuba*.¹⁴⁰ Prodelphinidin polymers of undefined structure were also obtained from white clover (*Trifolium repens* L.) flowers¹⁴¹ and *Onobrychis viciifolia* (sainfoin).¹⁴²

The two unique dimeric compounds, samarangenins A (**130**) and B (**131**),¹³³ comprise two epigallocatechin moieties possessing the characteristic C \rightarrow D-ring linkage of B-type proanthocyanidins as well as a C–O bond between a B-ring carbon and an oxygen function of the pyrogallyl-type ring of the 3-*O*-galloyl moiety at the DEF unit (Figure 11.11). Accordingly, they represent the first doubly linked proanthocyanidins that possess interflavanyl bonds originating from both two-electron (the 4 \rightarrow 8 bond) and one-electron (the carbon \rightarrow oxygen) bond processes.

TABLE 11.5
The Natural Procyanidins

Class	Compound	Structure	Source	Ref.
1. Dimers	Catechin-(4 α \rightarrow 8)-gallo catechin (tentative)		<i>Cistus incanus</i>	40
	Catechin-(4 α \rightarrow 8)-epigallocatechin		<i>Croton lechleri</i>	93
	Catechin-(4 α \rightarrow 8)-catechin-3- <i>O</i> - β - <i>D</i> -Glp		<i>Quercus marilandica</i> Muenchh.	38
	3- <i>O</i> -Ga- <i>ent</i> -epicatechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin		<i>Byrsonima crassifolia</i>	96
	<i>Ent</i> -epicatechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin-3- <i>O</i> -Ga			
	3- <i>O</i> -Ga- <i>ent</i> -epicatechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin-3- <i>O</i> -Ga			
	<i>Ent</i> -epicatechin-(4 α \rightarrow 6)- <i>ent</i> -epicatechin			
	Epicatechin-(4 β \rightarrow 8)-catechin-3- <i>O</i> -(4-OH)benzoate		<i>Hamelis virginiana</i>	97
	Epicatechin-(4 β \rightarrow 8)-epigallocatechin		<i>Ziziphus jujuba</i>	98
	Epicatechin-(4 β \rightarrow 8)-gallo catechin		<i>Alhagi sparsifolia</i>	99
	3- <i>O</i> -Ga-catechin-(4 β \rightarrow 8)-catechin-3- <i>O</i> -Ga		<i>Quercus robur</i>	100
	Epicatechin-(4 β \rightarrow 6)-epicatechin-3- <i>O</i> -Ga		<i>Vitis amurensis</i>	104
	Catechin-3- <i>O</i> - β - <i>D</i> -Glp-(4 α \rightarrow 8)-catechin-3- <i>O</i> - β - <i>D</i> -Glp(2-cinnamoyl)pyranoside		<i>Inga umbellifera</i>	68
2. Trimers	Catechin-3- <i>O</i> - β - <i>D</i> -Glp-(4 α \rightarrow 8)-epicatechin-3- <i>O</i> - β - <i>D</i> -Glp(6-cinnamoyl)pyranoside			
	Epicatechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 6)-epicatechin			
3. "Mixed" trimers	3- <i>O</i> -Ga- <i>ent</i> -epicatechin-(4 α \rightarrow 8)-3- <i>O</i> -Ga- <i>ent</i> -epicatechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin	(123)	<i>Crataegus laevigata</i> / <i>Malus pumila</i>	164, 165
	3- <i>O</i> -Ga-epicatechin-(4 β \rightarrow 8)-3- <i>O</i> -Ga- <i>ent</i> -epicatechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin		<i>Byrsonima crassifolia</i>	96
4. "Mixed" tetramers	Catechin-(4 α \rightarrow 8)-gallo catechin-(4 α \rightarrow 8)-gallo catechin		<i>Lonchocarpus</i> spp.	90
	Epiafzelechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-catechin		<i>Ziziphus jujuba</i>	98
	Epicatechin-(4 β \rightarrow 6)-epiafzelechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 6)-epicatechin (davallin)	(124)	<i>Davalia mariesii</i>	94, 95
	3- <i>O</i> -Ga-7- <i>O</i> -[<i>O</i> -(6- <i>O</i> -Ga)- β - <i>D</i> -Glp]-epigallocatechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epigallocatechin		<i>Polygonum coriarianum</i>	101
	Epicatechin-(4 β \rightarrow 8)-3- <i>O</i> -Ga-epigallocatechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin			

TABLE 11.6
The Natural Prodelphinidins

Class	Compound	Structure	Source	Ref.
1. Dimers	Gallocatechin-(4 α \rightarrow 6)-gallocatechin		<i>Cistus incanus</i>	40
	Gallocatechin-(4 α \rightarrow 6)-epigallocatechin		<i>Croton lechleri</i>	93
	Gallocatechin-(4 α \rightarrow 8)-epicatechin			
	Epigallocatechin-(4 β \rightarrow 8)-epigallocatechin		<i>Cistus salvifolius</i>	42
	Epigallocatechin-(4 β \rightarrow 8)-gallocatechin (prodelphinidin B1)		<i>Psidium guajava</i>	131,
			<i>Pithecellobium lobatum</i>	132
	Samarangenin A		(130) <i>Syzygium samarangensi</i>	133
			<i>S. aqueum</i>	
	Samarangenin B		(131)	
	Epigallocatechin-(4 β \rightarrow 6)-epigallocatechin		<i>Stryphnodendron adstringens</i>	59
	4- <i>O</i> -Methylgallocatechin-(4 α \rightarrow 8)-4'- <i>O</i> -methylgallocatechin			134
	3- <i>O</i> -Ga-Epigallocatechin-(4 β \rightarrow 6)-gallocatechin		<i>Cistus incanus</i>	135
	3- <i>O</i> -Ga-Epigallocatechin-(4 β \rightarrow 8)-gallocatechin			
	3- <i>O</i> -Ga-Epigallocatechin-(4 β \rightarrow 8)-epigallocatechin		<i>C. salvifolius</i>	42
	Epigallocatechin-(4 β \rightarrow 6)-epigallocatechin-3- <i>O</i> -Ga			
Epigallocatechin-(4 β \rightarrow 8)-epigallocatechin-3- <i>O</i> -(4-OH)-benzoate		<i>S. adstringens</i>	59	
Gallocatechin-(4 α \rightarrow 8)-epigallocatechin-3- <i>O</i> -Ga				
Gallocatechin-(4 α \rightarrow 8)-epigallocatechin-3- <i>O</i> -(4-OH)-benzoate				
2. Trimers	Gallocatechin-(4 α \rightarrow 8)-gallocatechin-(4 α \rightarrow 8)-epigallocatechin		<i>Croton lechleri</i>	93
	Gallocatechin-(4 α \rightarrow 8)-gallocatechin-(4 α \rightarrow 8)-gallocatechin		<i>Ribes nigrum</i>	136
	Epigallocatechin-(4 β \rightarrow 8)-gallocatechin-(4 α \rightarrow 8)-catechin		<i>Cistus albidus</i>	137
	Epigallocatechin-(4 β \rightarrow 8)-gallocatechin-(4 α \rightarrow 8)-gallocatechin			

11.3.2.1.3 Propelargonidins (3,5,7,4'-Tetrahydroxylation)

The limited numbers of new propelargonidins are listed in Table 11.7. This is in addition to the “mixed” procyanidin–propelargonidin tetramer, davallin (Table 11.5),^{94,95} the “mixed” propelargonidin–procyanidin trimer, epiafzelechin-(4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-catechin (Table 11.5),⁹⁸ and the “mixed” propelargonidin–prodelphinidin–procyanidin pentamer PZ-2.¹⁴⁰

11.3.2.1.4 Profisetinidins (3,7,3',4'-Tetrahydroxylation)

The profisetinidins are the most important proanthocyanidins of commerce, making up the major constituents of wattle and quebracho tannins. New entries are collated in Table 11.8.

The structures of the di- and trimeric profisetinidins from *Pithecellobium dulce* (Guamúchil) were rigorously corroborated via synthesis.¹¹⁴ The synthetic approach was additionally motivated by the precariousness of unequivocally differentiating between 2,3-*cis*-3,4-*trans*- and 2,3-*cis*-3,4-*cis*-configurations of the chain-extension units on the basis of ¹H NMR coupling constants.^{150,151} Furthermore, the powerful nuclear Overhauser effect (NOE) method for differentiating between 2,4-*cis*- and 2,4-*trans*-substitution¹⁵² is less useful at the di- and trimeric levels due to the adverse effects of dynamic rotational isomerism about the interflavanil bond(s) on ¹H NMR spectra at ambient temperatures.

TABLE 11.7
The Natural Propelargonidins

Class	Compound	Structure	Source	Ref.
1. Dimers	3- <i>O</i> -(α -L-Rhamnopyranosyl)afzelechin-(4 α \rightarrow 8)-epiafzelechin-3- <i>O</i> -vanillate	(132)	<i>Joannesia princeps</i>	143
	3- <i>O</i> -(α -L-Rhamnopyranosyl)afzelechin-(4 α \rightarrow 8)-epicatechin-3- <i>O</i> -vanillate	(133)		
	3- <i>O</i> -(α -L-Rhamnopyranosyl)afzelechin-(4 α \rightarrow 8)-epiafzelechin-3- <i>O</i> -syringate	(134)		
	3- <i>O</i> -(α -L-Rhamnopyranosyl)afzelechin-(4 α \rightarrow 8)-epicatechin-3- <i>O</i> -syringate	(135)		
	3- <i>O</i> -Ga-epiafzelechin-(4 β \rightarrow 8)-epicatechin-3- <i>O</i> -Ga ^a		Green tea	144
	3- <i>O</i> -Ga-epiafzelechin-(4 β \rightarrow 8)-epicatechin-3- <i>O</i> -Ga		Green tea	145
	3- <i>O</i> -Ga-epiafzelechin-(4 β \rightarrow 6)-epicatechin-3- <i>O</i> -Ga			
	Epiafzelechin-(4 β \rightarrow 8)-4 β -carboxymethylepiafzelechin Me ester		<i>Drynaria fortunei</i>	146
2. Trimers	Epiafzelechin-(4 β \rightarrow 8)-epiafzelechin-(4 β \rightarrow 8)-4-Me-epigallocatechin		<i>Heisteria pallida</i>	147
	Afzelechin-(4 α \rightarrow 8)-afzelechin-(4 α \rightarrow 8)-afzelechin (C-glucosylated?)		<i>Aegle marmelos</i>	148

^a(4 β \rightarrow 8) bond tentatively assigned.

The principles that govern the base-catalyzed C-ring isomerization of dimeric profisetinidins^{152–154} also control a series of similar rearrangements in the trimeric bis-fisetinidol-catechins (136–139)^{155–158} and related bis-fisetinidol-epicatechins (Figure 11.12).¹⁵⁹ Analogs possessing constituent chain-extension units with 3,4-*cis*-configuration (ABC unit in, e.g., 137) are subject to extensive 1,3-migrations in intermediate B-ring quinone methides and hence to the formation of exceptionally complex reaction mixtures.^{156–159} Compound 139 afforded no less than 16 conversion products, the structures of which were unequivocally established.¹⁵⁷ This has subsequently led to the development of a more controlled synthesis that is based upon the repetitive formation of the interflavanyl bond and pyran ring rearrangement of the chain-extension unit under mild basic conditions.¹⁶⁰ Thus, in contrast to the unrestrained course of the base-catalyzed C-ring rearrangement reactions of profisetinidin triflavonoids possessing 2,3-*trans*-3,4-*cis*-flavanyl constituent units, the stepwise construction of the dipyranochromene framework via sequential interflavanyl bond formation and pyran ring rearrangement permitted concise synthetic access to phlobatannins at the trimeric level, e.g., the hexahydrodipyrano[2,3-*f*:2',3'-*h*]chromene (140).¹⁶⁰

The readily occurring cleavage of the interflavanyl bond in proanthocyanidins that exhibit C-5 oxygenation of the A-ring of their chain-extension units, with sulfur or oxygen nucleophiles under acid catalysis, plays a crucial role in the structural elucidation of this complex group of natural products. In the 5-deoxy series of compounds, e.g., the fisetinidol-(4 α \rightarrow 8)-catechin (141), this C(sp³)-C(sp²) bond is remarkably stable under a variety of conditions and has resisted efforts at cleavage in a controlled fashion. Such a stable interflavanyl bond has adversely affected both the structural investigation of the polymeric proanthocyanidins in black wattle bark and of those from other commercial sources, as well as the establishment of the absolute configuration of the chain-terminating flavan-3-ol moieties in the 5-deoxyoligoflavanoids. It has recently been demonstrated that the interflavanyl bond

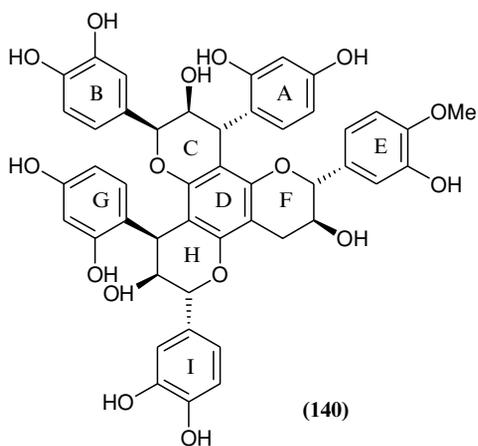
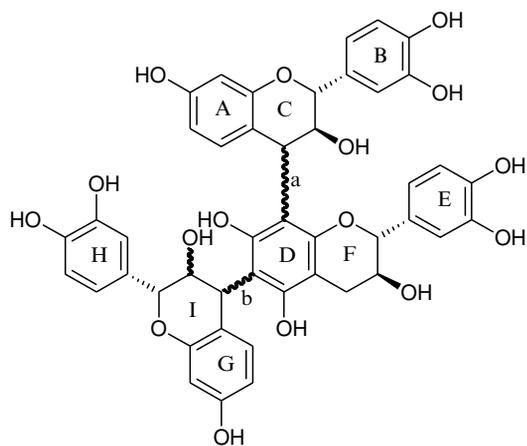
TABLE 11.8
The Natural Profisetinidins

Class	Compound	Structure	Source	Ref.
1. Dimers	Fisetinidol-(4 α \rightarrow 8)-6-Me-catechin		Commercial wattle bark extract	32
	Epifisetinidol-(4 β \rightarrow 8)-catechin		<i>Pithecellobium dulce</i>	114
2. Trimers	Epifisetinidol-(4 β \rightarrow 8)-epicatechin			
	Bis-epifisetinidol-(4 β \rightarrow 6:4 β \rightarrow 8)-catechin		<i>P. dulce</i>	114
	Bis-epifisetinidol-(4 β \rightarrow 6:4 β \rightarrow 8)-epicatechin			
	Fisetinidol-(4 α \rightarrow 8)-catechin-(6 \rightarrow 4 β)-epifisetinidol			
3. Phlobatannin related	Fisetinidol-(4 α \rightarrow 8)-epicatechin-(6 \rightarrow 4 β)-epifisetinidol			
	Bis-fisetinidol-(4 α \rightarrow 6:4 α \rightarrow 8)-catechin-3- <i>O</i> -Ga		<i>Burkea africana</i>	149
	Compound	(145)	<i>Guibourtia coleosperma</i>	155
	Compound	(146)	<i>Colophospermum mopane</i>	155
	Compound	(147)	<i>Baikiaea plurijuga</i>	156
	Compound	(148)		
	Compound	(149)	<i>C. mopane</i>	156
	Compound	(150)	<i>B. plurijuga</i>	156
Compound	(151)	<i>C. mopane/B. plurijuga</i>	159	
Compound	(152)	<i>B. plurijuga</i>		

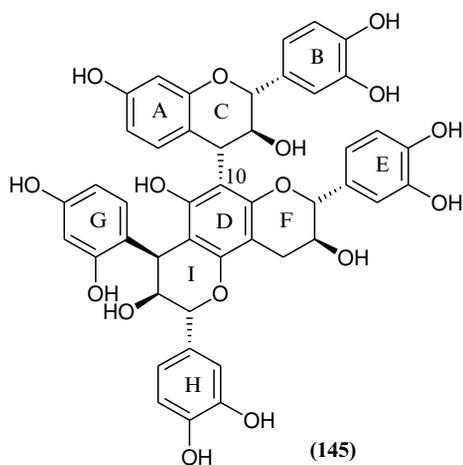
in the proanthocyanidins, including the 5-deoxy analogs and their permethylaryl ethers, is readily subject to reductive cleavage with sodium cyanoborohydride [Na(CN)BH₃] in trifluoroacetic acid (TFA) at 0°C.^{161–163} Thus, under acidic conditions profisetinidin (**141**) is protonated at C-8 (D-ring) and the interflavanyl bond in intermediate **142** cleaved by delivery of the equivalent of hydride ion either at C-4(C) to give the constituent flavan-3-ol moieties **2** and **143**, or at C-2(C) to give the 1,3-diarylpropan-2-ol (**144**) and catechin (**2**) (see Ref. 163) (Figure 11.13, Scheme 11.5). The protocol is as effective for the permethylaryl ether of **141**, for procyanidin B-3 and B-4, and also for A-type proanthocyanidins¹⁶³ (*vide infra*).

11.3.2.1.5 Prorobinetinidins (3,7,3',4',5'-Pentahydroxylation)

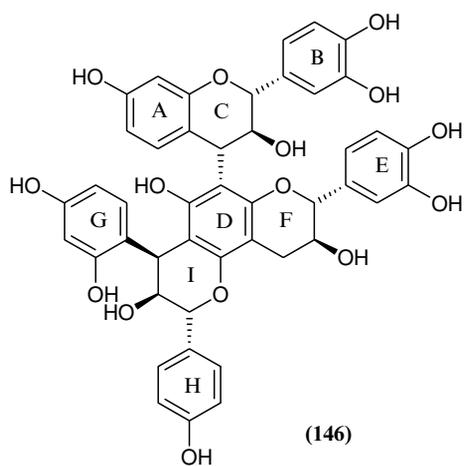
The prorobinetinidins also feature prominently in wattle bark extract.³ In the period under review, new entries, listed in Table 11.9, have been identified from wattle bark,³² *Robinia pseudacacia*,¹⁶⁶ and *Stryphnodendron adstringens*.¹⁶⁷ The recent investigation of commercial wattle bark extract³² has led to the identification of robinetinidol-(4 β \rightarrow 8)-catechin (**153**) and robinetinidol-(4 β \rightarrow 8)-gallocatechin (**154**), the first prorobinetinidins with a 3,4-*cis* C-ring configuration, as well as a prorobinetinidin-type tetrahydropyrano[2,3-*h*]chromene isomerization intermediate (**155**) (Figure 11.14). Compounds of the class **155** as well as dimeric analogs possessing the DEF-GHI core of **155** readily form from the “conventional” tri- and biflavonoids, respectively, by rearrangement of the pyran heterocycle(s) under mild basic conditions. The mechanisms explaining their intricate genesis were extensively reviewed.^{7,9,10} Such a susceptibility of the constituent flavanyl units of proanthocyanidins to intramolecular rearrangement via B-ring quinone methide intermediates under basic conditions was also demonstrated in an unusual dimerization–rearrangement reaction of catechin at pH 12 and 40°C.¹⁶⁸



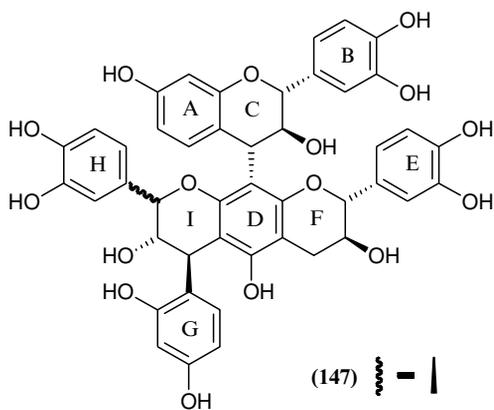
(140)



(145)



(146)



(147) -

(148) -

FIGURE 11.12 Structures of compounds 136–140 and 145–148.

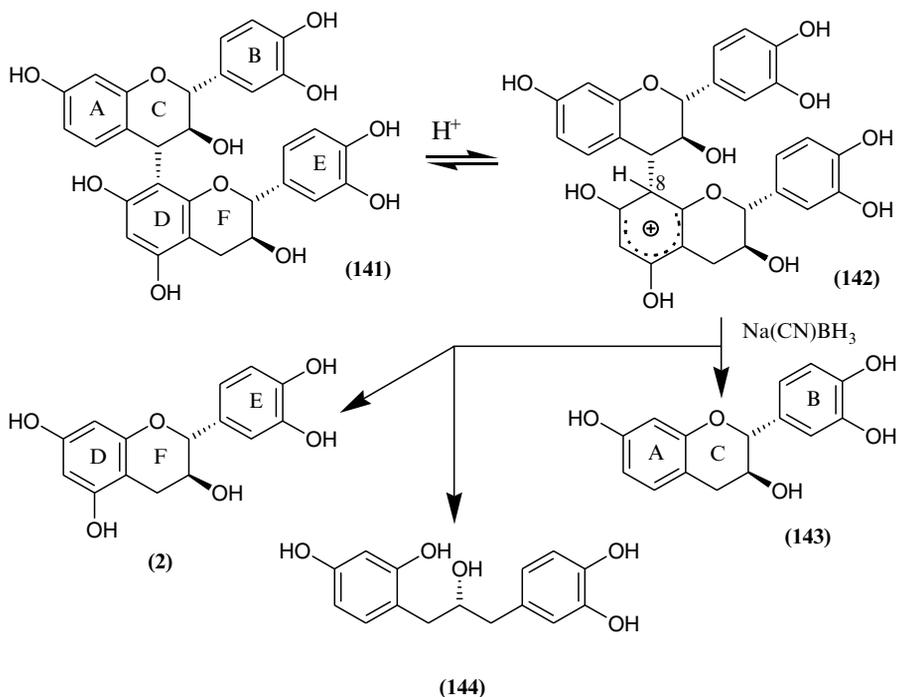


FIGURE 11.13 Structures of compounds **141–144** including Scheme 11.5: Proposed route to the reductive cleavage of the interflavanyl bond in proanthocyanidins.

In the heartwood of *R. pseudacacia*, the flavan-3,4-diol, leucorobinetidin (robinetinidol-4 α -ol), as the incipient electrophile for prorobinetidin biosynthesis, coexists with a variety of monomeric flavonoids invariably possessing C-4 oxygenation,¹⁶⁶ hence reducing the nucleophilicity of their A-rings compared to that of the corresponding functionality in the C-4 deoxy compounds, e.g., catechin. *R. pseudacacia* therefore represents a rare metabolic pool where oligomer formation has to occur via the action of a very potent electrophile on chain-terminating units apparently lacking the nucleophilicity that is associated with natural sources in which proanthocyanidin formation is paramount. The diversity of the oxidation level of the chain-terminating moieties suggests that the biflavanoids in *R. pseudacacia* may be interrelated via oxidation–reduction of these units.

11.3.2.1.6 Proteracacinidins (3,7,8,4'-Tetrahydroxylation)

In contrast to the large number of oligomeric proanthocyanidins with resorcinol- or phloroglucinol-type A-rings of the chain-extension units,^{3,4} those possessing pyrogallol-type A-rings (7,8-dihydroxylation) are rare. The first proteracacinidin analogs were identified as recently as 1994. Since then a considerable number (Table 11.10) of these compounds have been isolated from two members of the Leguminosae, *Acacia caffra* and *A. galpinii*.^{16,169–177} A conspicuous feature of the compounds listed in Table 11.10 is the heterogeneity of the interflavanyl bond in this class of naturally occurring proanthocyanidins. Both carbon and oxygen centers participate in interflavanyl bond formation and it would appear as if both one- and two-electron processes play a prominent role in establishing the interflavanyl linkage(s). This presumably reflects the poor nucleophilicity of the pyrogallol-type A-ring of the monomeric flavan-3,4-diol precursors, hence permitting alternative centers to participate in the interflavanyl bond forming process.

TABLE 11.9
The Natural Prorobinetinidins

Class	Compound	Structure	Source	Ref.
1. Dimers	Robinetinidol-(4 β \rightarrow 8)-catechin	(153)	Commercial	32
	Robinetinidol-(4 β \rightarrow 8)-gallo catechin	(154)	black wattle bark	
	Robinetinidol-(4 β \rightarrow 6)-robinetinidol-4 β -ol		<i>Robinia</i>	166
	Robinetinidol-(4 β \rightarrow 6)-robinetinidol-4 α -ol		<i>pseudacacia</i>	
	Robinetinidol-(4 α \rightarrow 2')-robinetinidol-4 β -ol			
	Robinetinidol-(4 α \rightarrow 2')-robinetinidol-4 α -ol			
	Robinetinidol-(4 α \rightarrow 2')-dihydrorobinetin			
	Robinetinidol-(4 β \rightarrow 6)-dihydrorobinetin ^a			
	Robinetinidol-(4 α \rightarrow 8)-dihydrorobinetin			
	Robinetinidol-(4 α \rightarrow 2')-robinetin ^a			
	Robinetinidol-(4 β \rightarrow 2')-7,3',4',5'-tetra-OH-flavone			
	Robinetinidol-(4 β \rightarrow 8)-epigallocatechin		<i>Stryphnodendron</i>	167
	Robinetinidol-(4 α \rightarrow 8)-epigallocatechin		<i>adstringens</i>	
	Robinetinidol-(4 β \rightarrow 8)-epigallocatechin-3- <i>O</i> -Ga			
	Robinetinidol-(4 α \rightarrow 8)-epigallocatechin-3- <i>O</i> -Ga			
	Robinetinidol-(4 α \rightarrow 6)-gallo catechin			
	Robinetinidol-(4 α \rightarrow 6)-epigallocatechin			
2. Phlobatannin related	Compound	(155)	Commercial black wattle bark	32

^aDihydrorobinetin is (2*R*,3*R*)-2,3-*trans*-7,3',4',5'-tetrahydroxydihydroflavonol and robinetin the corresponding flavonol.

Considerable effort has been devoted to confirm the constitution and absolute configuration of both the carbon–carbon and carbon–oxygen analogs via biomimetic syntheses.^{16,170–172,176} In addition, the compounds possessing carbon–oxygen linkages are susceptible to ready reductive cleavage under acidic conditions, hence permitting unequivocal structural elucidation of the constituent flavanyl moieties.¹⁷⁴ Definition of the axial chirality of analogs possessing biphenyl linkages, e.g., the four compounds from *A. galpinii*¹⁷³ and compound **156**, was unambiguously done by circular dichroism (Figure 11.15).

11.3.2.1.7 Promelacacinidins (3,7,8,3',4'-Pentahydroxylation)

The natural occurrence of promelacacinidins was until recently restricted to the heartwoods of *Prosopis glandulosa*³ and *Acacia melanoxylon*.⁴ The investigations of the heartwood constituents of *A. caffra* and *A. galpinii* also revealed the presence of a limited number of promelacacinidins (Table 11.11) as well as “mixed” di- and trimeric proteracacinidin or promelacacinidins (see Table 11.10).

Notable from Table 11.10 and Table 11.11 is the considerable number of proteracacinidins and promelacacinidins at both the di- and trimeric levels possessing 2,3-*cis*-3,4-*cis*-flavan-3,4-diol “terminating” moieties. It has been demonstrated that melacacidin (**158**) is basically inert toward solvolysis or epimerization at C-4.^{179,180} This was recently confirmed during the thiolysis of 4 β -chloroepioritin derivatives.¹⁸¹ Such stability may be ascribed to hydrogen bonding between the axial C-3(OH) and the heterocyclic oxygen, which locks the C-ring in a half-chair conformation with C_{2eq}, C_{3ax}, and C_{4eq} substituents.^{179,180} In this conformation, the appropriate C₄ σ^* orbital is at an angle of $\sim 45^\circ$ above the plane of the

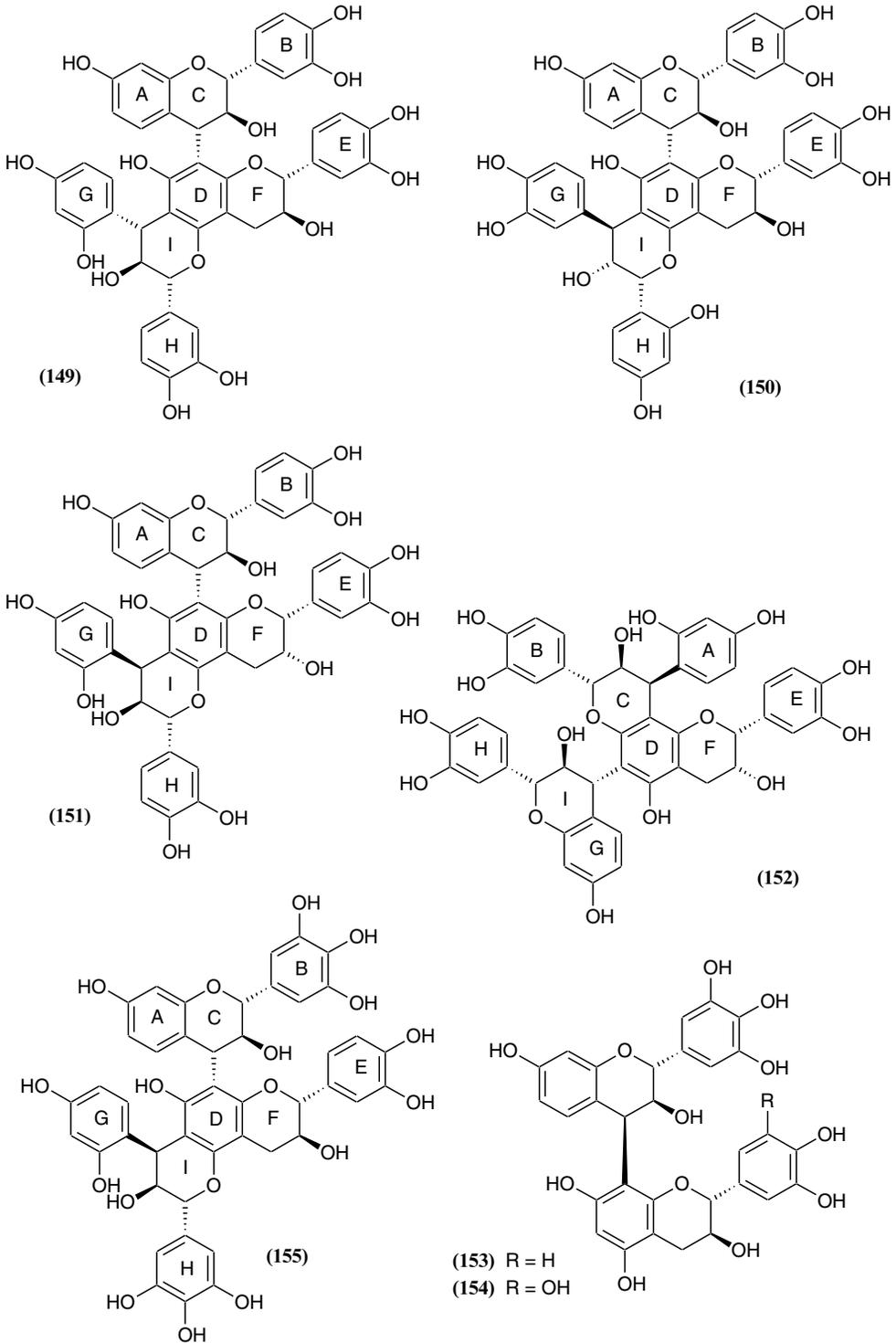


FIGURE 11.14 Structures of compounds 149–155.

TABLE 11.10
The Natural Proteracacinidins

Class	Compound	Structure	Source	Ref.
1. Dimers	<i>Ent</i> -oritin-(4 β \rightarrow 7:5 \rightarrow 6)-epioritin-4 α -ol	(156)	<i>Acacia caffra</i>	169
	<i>Ent</i> -oritin-(4 β \rightarrow 5)-epioritin-4 β -ol			89
	Eporitin-(4 β \rightarrow 3)-epioritin-4 β -ol	(10)		16
	Oritin-(4 α \rightarrow 7:5 \rightarrow 6)-epioritin-4 α -ol		<i>A. galpinii</i>	173
	Oritin-(4 β \rightarrow 7:5 \rightarrow 6)-epioritin-4 α -ol			
	Eporitin-(4 β \rightarrow 7:5 \rightarrow 6)-epioritin-4 α -ol			
	Eporitin-(4 β \rightarrow 7:5 \rightarrow 6)-oritin-4 α -ol			
	Eporitin-(4 β \rightarrow 5:3 \rightarrow 4)-oritin-4 α -ol	(157)	<i>A. caffra</i>	173
	Oritin-(4 α \rightarrow 5)-epioritin-4 β -ol		<i>A. galpinii</i>	175
	<i>Ent</i> -epioritin-(4 α \rightarrow 5)-epioritin-4 β -ol		<i>A. caffra</i>	
	Eporitin-(4 β \rightarrow 5)-epioritin-4 α -ol		<i>A. galpinii</i>	
	<i>Ent</i> -oritin-(4 β \rightarrow 5)-epioritin-4 α -ol			
	Eporitin-(4 β \rightarrow 6)-oritin-4 α -ol		<i>A. caffra</i>	176
	Eporitin-(4 β \rightarrow 6)- <i>ent</i> -oritin-4 α -ol		<i>A. galpinii</i>	
	<i>Ent</i> -oritin-(4 β \rightarrow 6)-epioritin-4 α -ol			
	<i>Ent</i> -oritin-(4 β \rightarrow 5)-oritin-4 α -ol			
	<i>Ent</i> -oritin-(4 α \rightarrow 6)-epioritin-4 α -ol		<i>A. caffra</i>	
	<i>Ent</i> -oritin-(4 α \rightarrow 6)-oritin-4 α -ol		<i>A. galpinii</i>	
	<i>Ent</i> -oritin-(4 α \rightarrow 6)-epioritin-4 β -ol		<i>A. caffra</i>	
	Eporitin-4 α -ol-(6 \rightarrow 6)-epioritin-4 β -ol		<i>A. galpinii</i>	177
(2 <i>S</i>)-7,8,4'-Trihydroxyflavan-(4 β \rightarrow 6)-epioritin-4 α -ol		<i>A. caffra</i>	182	
2. "Mixed" dimers	Epimesquitol-(4 β \rightarrow 4)-epioritin-4 β -ol		<i>A. caffra</i>	16
	Eporitin-(4 β \rightarrow 6)-epimesquitol-4 α -ol		<i>A. caffra</i>	176
	Eporitin-(4 β \rightarrow 6)-epimesquitol-4 β -ol			
3. Trimers	Epimesquitol-(4 β \rightarrow 6)-epioritin-4 α -ol			
	Eporitin-(4 β \rightarrow 6)-epioritin-(4 α \rightarrow 4)-epioritin-4 α -ol		<i>A. caffra</i>	174
	Eporitin-(4 β \rightarrow 3)-epioritin-(4 β \rightarrow 6)-epioritin-4 β -ol			
	Eporitin-(4 β \rightarrow 6)-oritin-(4 α \rightarrow 6)-epioritin-4 α -ol		<i>A. caffra</i>	177
	Oritin-(4 β \rightarrow 6)-oritin-(4 α \rightarrow 6)-epioritin-4 α -ol		<i>A. galpinii</i>	
4. "Mixed" trimers	Eporitin-(4 β \rightarrow 6)-epioritin-(4 β \rightarrow 6)-epioritin-4 α -ol		<i>A. caffra</i>	177
	Eporitin-(4 β \rightarrow 3)-epioritin-(4 β \rightarrow 6)-epimesquitol-4 α -ol		<i>A. caffra</i>	174

A-ring and "buried" in the heterocyclic ring that screens its overlap by an external nucleophile. Since a C-4 antibonding orbital orthogonal to the A-ring would permit the most effective delocalization of A-ring electron density or stabilization of electron deficiency at C-4, it is clear why an all-*cis* C-ring configuration is more common for flavan-3,4-diols with 7,8-dihydroxylated A-rings. These compounds, no doubt, will have a reduced need for delocalization of the aromatic A-ring electron density than their counterparts with more electron-rich resorcinol- and phloroglucinol-type A-rings. It may then also explain the stability and abundance of the flavan-3,4-diol, teracacidin, as well as the growing number of di- and trimers with 2,3-*cis*-3,4-*cis*-flavanyl constituent units all possessing 7,8-dihydroxy A-rings and axial C-3 hydroxyl groups.^{4,89,171,172,174,177}

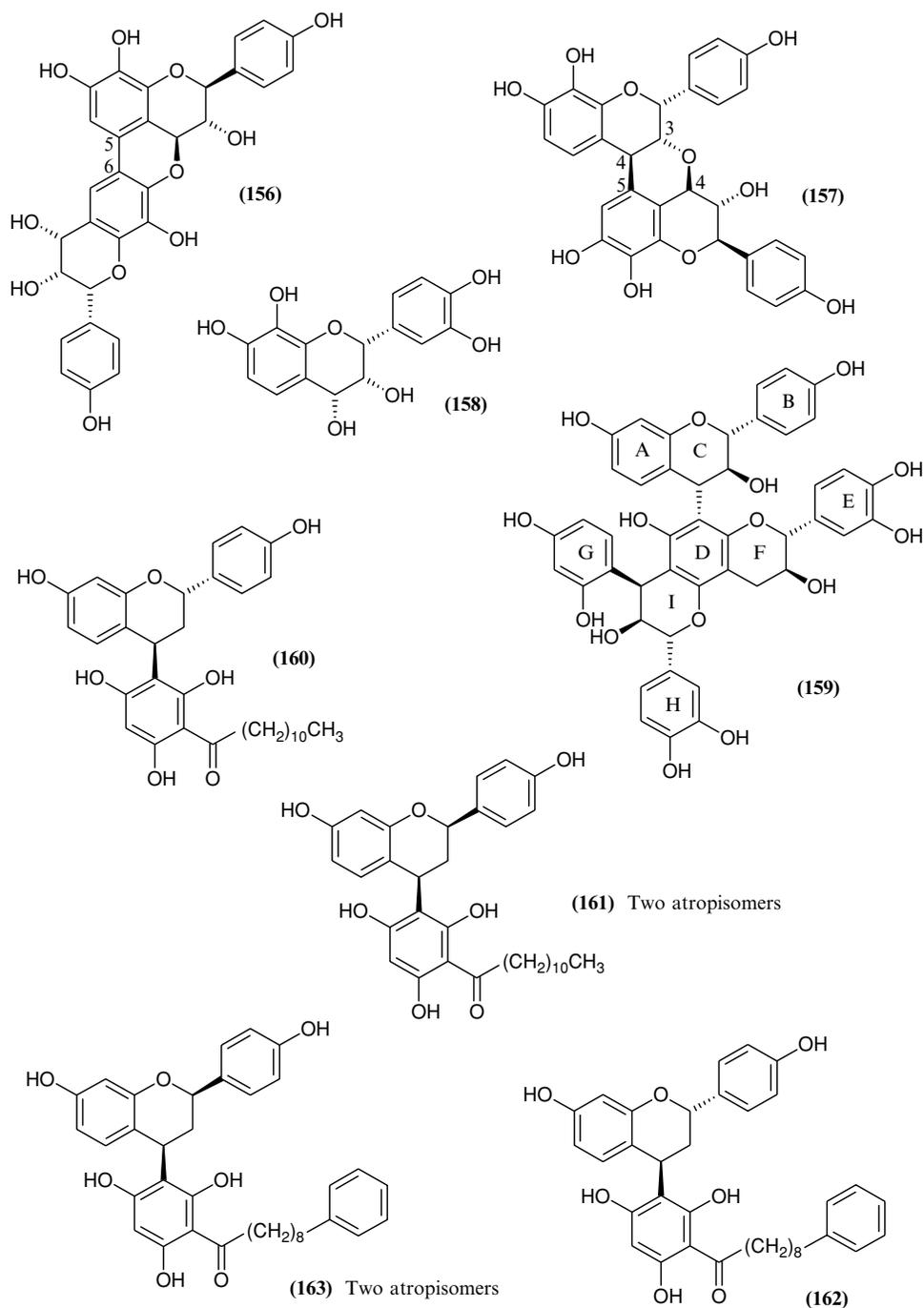


FIGURE 11.15 Structures of compounds **156–163**.

11.3.2.1.8 Proguibourtinidins (3,7,4'-Trihydroxylation)

The pro- and leucogubourtinidins with their 7,4'-dihydroxy phenolic functionality represent a rare group of proanthocyanidins. New analogs are listed in Table 11.12. The structures of the dimers from *C. abbreviata* were rigorously established using ^1H NMR and CD techniques, as

TABLE 11.11
The Natural Promelacacinidins

Class	Compound	Source	Ref.
1. Dimers	Epimesquitol-(4 β \rightarrow 6)-epimesquitol-4 β -ol	<i>Acacia caffra</i>	170
	Mesquitol-(4 β \rightarrow 6)-3,7,8,3',4'-pentahydroxyflavone	<i>A. nigrescens</i>	178
	Epimesquitol-(4 β \rightarrow 5)-3,7,8,3',4'-pentahydroxyflavone		
2. "Mixed" dimers	See Table 11.10		
3. "Mixed" trimers	See Table 11.10		

well as semisynthesis from starting materials with known absolute configuration.¹⁸³ The structure of guibourtinidol-(4 α \rightarrow 8)-afzelechin from *Ochna calodendron* was claimed on the basis of ¹H NMR evidence only,¹⁸⁴ while the known guibourtinidol-(4 α \rightarrow 8)-epiafzelechin was also identified in the stems of *Cassia biflora*.¹⁸⁵

11.3.2.1.9 Procassinidins (7,4'-Dihydroxylation)

The procassinidins with their 7,4'-dihydroxyflavan chain-extension units represent a rare group of naturally occurring proanthocyanidins and only four compounds were reported in the previous review.⁴ Seven new analogs were identified in the bark of *C. petersiana*¹⁸⁶ and are listed in Table 11.13. Synthesis as the permethylaryl acetate derivatives was done by reduction of the flavanone, including the optically pure (2*S*)-di-*O*-methylliquirtigenin, to the flavan-4-ol, which then served as electrophile in the Lewis acid (TiCl₄) catalyzed coupling with the appropriate flavan-3-ol permethylaryl ether, e.g., penta-*O*-methylepi- or -gallo catechin.¹⁸⁶ The cassiflavan-type myristinins A–F (**160–163**) (Figure 11.15) were identified in *Myristica cinnamomea*.²¹⁴

11.3.2.1.10 Probutinidins (7,3',4'-Trihydroxylation)

The probutinidins (see Section 11.2) represent a second class of proanthocyanidins with flavan chain-extension units. Only five members of this class of compounds have been identified (Table 11.14). Their structures and absolute configurations were also confirmed by synthesis via reduction of the flavanone, butin, followed by acid-catalyzed condensation with the appropriate flavan-3-ol.^{17,187} A notable feature of the synthetic studies was the apparent preference for (4 \rightarrow 8) bond formation reported by both groups of authors.

TABLE 11.12
The Natural Proguibourtinidins

Class	Compound	Structure	Source	Ref.
1. Dimers	Guibourtinidol-(4 β \rightarrow 8)-epiafzelechin		<i>Cassia abbreviata</i>	183
	Guibourtinidol-(4 β \rightarrow 8)-epicatechin			
	Guibourtinidol-(4 β \rightarrow 8)-afzelechin			
	Guibourtinidol-(4 α \rightarrow 6)-afzelechin			
	<i>Ent</i> -guibourtinidol-(4 β \rightarrow 8)-epicatechin			
	Guibourtinidol-(4 α \rightarrow 8)-afzelechin		<i>Ochna calodendron</i>	
2. Phlobatannin related	Compound	(159)	<i>Colophospermum mopane</i>	155

TABLE 11.13
The Natural Procassinidins

Class	Compound	Structure	Source	Ref.
Dimers	Cassiaflavan-(4 α \rightarrow 8)-epicatechin		<i>Cassia petersiana</i>	186
	Cassiaflavan-(4 α \rightarrow 8)-epigallocatechin			
	Cassiaflavan-(4 β \rightarrow 8)-epicatechin			
	Cassiaflavan-(4 β \rightarrow 8)-epigallocatechin			
	Cassiaflavan-(4 β \rightarrow 8)-gallocatechin			
	<i>Ent</i> -cassiaflavan-(4 β \rightarrow 8)-epicatechin			
	Cassiaflavan-(4 α \rightarrow 6)-epicatechin			214
	Cassiaflavan (myristinin A)	(160)	<i>Myristica cinnamomea</i>	
	Cassiaflavan (myristinin D)	(162)		
	<i>Ent</i> -cassiaflavan atropisomers (myristinin B and C)	(161)		
	<i>Ent</i> -cassiaflavan atropisomers (myristinin E and F)	(163)		

11.3.2.2 A-Type Proanthocyanidins

In contrast to proanthocyanidins of the B-type, where the constituent flavanyl units are linked *via* only one bond, analogs of the A-class possess an unusual second ether linkage to C-2 of the T-unit. This feature introduces a high degree of conformational stability at the interflavanyl bonding axes of dimeric analogs that culminates in high quality and unequivocal NMR spectra, conspicuously free of the effects of dynamic rotational isomerism. Compounds of this class are readily recognizable from the characteristic AB-doublet ($^3J_{3,4} = 3.4$ Hz) for both 3,4-*trans*- and 3,4-*cis*-C-ring protons³² in the heterocyclic region of their ^1H NMR spectra, and may possess either (2 α ,4 α)- or (2 β ,4 β)-double interflavanyl linkages. These alternatives are readily differentiated via CD data, which show negative and positive Cotton effects in the 220 to 240 nm region for (2 α ,4 α)- and (2 β ,4 β)-configurations, respectively.^{32,188} As a consequence of these favorable structural features and because of their considerable biological activity,^{189,190} a substantial effort has been devoted to a continued search for new analogs. The proposed and by now well-established system of nomenclature (see Section 11.2) will be used, leading in some instances to a change of published names as far as the α , β -designations are concerned. New entries are listed in Table 11.15.

The report on aesculitannins A–G from the seed shells of *Aesculus hippocastanum*¹⁹¹ demonstrates three important chemical methods to facilitate the unequivocal structural elucidation of the A-type proanthocyanidins. These protocols include thiolytic degradation using phenylmethanethiol in acidic medium, oxidative formation of the ether linkage when

TABLE 11.14
The Natural Probutinidins

Class	Compound	Source	Ref.
Dimers	Butiniflavan-(4 β \rightarrow 8)-catechin	<i>Cassia nomame</i>	187
	Butiniflavan-(4 α \rightarrow 8)-catechin		
	Butiniflavan-(4 β \rightarrow 8)-epicatechin	<i>C. petersiana</i>	17
	Butiniflavan-(4 α \rightarrow 8)-epicatechin		
	Butiniflavan-(4 β \rightarrow 8)-epigallocatechin		

the carbon–hydrogen bond at C-2 and the 4-flavanyl constituent are cofacial using hydrogen peroxide in sodium hydrogen carbonate solution, and transformation of the thermodynamically less stable 2,3-*cis*-flavan-3-ol moieties into 2,3-*trans* units via base-catalyzed epimerization at C-2.

An interesting paper reported the conversion of B- into A-type proanthocyanidins via oxidation using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals under neutral conditions.²¹² The feasibility of the method was demonstrated by transformation of procyanidins B-1 and B-2 into proanthocyanidins A-1 and A-2, respectively. Indirect evidence for the intermediacy of *p*-quinone methides of types **164** and **165** in the oxidative conversion of B- into A-type proanthocyanidins came from the oxidation of epigallocatechin with the homogenate of banana flesh polyphenol oxidase (Figure 11.16).²¹³ When the anthocyanin malvidin-3-*O*-glucoside was treated with epicatechin in ethanol at 35°C in a sealed tube, the major product was identified as the promalvidin A-type compound, malvidin-3-glucoside-(2 → 7, 4 → 8)-epicatechin (**166**).²¹⁵

The problem of assigning the absolute configuration at the stereocenters of the F-ring of the A-type proanthocyanidins was also addressed.¹⁶³ This straightforward general chemical method is based upon the consecutive reductive cleavage of the acetal functionality (cleavage a) and interflavanyl C—C bond (cleavage b). Thus, separate treatment of the hepta-*O*-methyl ethers **167** and **168** of procyanidins A-1 and A-2 with Na(CN)BH₃ in TFA at 0°C (Figure 11.17, Scheme 11.6) gave conversion into the respective monomeric units, i.e., the *ent*-catechin and catechin derivatives (**171** and **173**) from the A-1 derivative (**167**), and the *ent*-catechin and epicatechin derivatives (**171** and **172**) from the A-2 derivative (**168**).

Cleavage “a” of the carbon–oxygen bond is presumably triggered by protonation of the C-7 (D-ring) acetal oxygen and concomitant delivery of the equivalent of a hydride ion at the antibonding (σ^*) orbital of the carbon–oxygen bond in a predominant S_N2 manner. Such a transfer of hydride ion apparently occurs from a complex between the reducing agent and the axial C-3 (C-ring) oxygen lone pair, the proximity of the boron–hydrogen bonds to the backside of the acetal carbon atom being a prerequisite for reduction of the acetal bond. Reduction thus leads to “inversion” of configuration at C-2 (C-ring) of both B-type procyanidin intermediates (**169** and **170**). These intermediates are prone to facile reductive cleavage of their interflavanyl bonds (cleavage b)¹⁶² to give the *ent*-catechin derivative (**171**) from the ABC unit and, respectively, the epicatechin and catechin derivatives (**172** and **173**) from the DEF moieties. The location of the free hydroxyl group at the A-ring unambiguously defines the D-ring oxygen that is involved in the acetal functionality of the parent compounds (**167** and **168**). A similar reductive cleavage using Zn–HCl was also used to facilitate structural elucidation of the dracoflavans (Table 11.15).²⁰³

Despite the apparent clarity of the nomenclature rules, several papers in the area of the A-type proanthocyanidins still lack proper implementation of these rules. The reader must therefore ascertain the correctness of published names. In addition, the reader is also referred to the growing body of evidence of the physiological importance of these compounds, data of which can be found in several of the papers listed in the references.

11.3.3 NONPROANTHOCYANIDINS WITH FLAVAN OR FLAVAN-3-OL CONSTITUENT UNITS

In addition to the extensive range of di- and trimeric oligoflavanoids with rearranged C-rings, dubbed phlobatannins (see Section 11.3.2.1.4, Table 11.8, Table 11.9, and Table 11.12), daphnodorins A–D and larixinol were earlier reported as rearranged biflavonoid metabolites comprising either flavan or flavan-3-ol and 4,2',4',6'-tetrahydroxychalcone (chalconarin-genin) constituent units.^{3,4} Since then a considerable number of new analogs, collated in Table 11.16, have been reported.

TABLE 11.15
The Natural A-Type Proanthocyanidins

Class	Compound	Source	Ref.
I. Dimers	Procyanidins		
	Epicatechin-(2 β → 7:4 β → 6)-epicatechin (procyanidin A-6)	<i>Aesculus hippocastanum</i>	191
	Epicatechin-(2 β → 5:4 β → 6)-epicatechin (procyanidin A-7)	<i>A. hippocastanum/Theobroma cacao</i>	191, 192
	Epicatechin-(2 β → 7:4 β → 6)-catechin	<i>Arachis hypogaea</i> L.	193
	Epicatechin-(2 β → 7:4 β → 6)-ent-catechin		
	Epicatechin-(2 β → 7:4 β → 6)-ent-epicatechin	<i>Geranium niveum</i>	199
	Epicatechin-(2 β → 7:4 β → 8)-afzelechin (geranin B)	<i>Pavetta owariensis</i>	194
	Ent-epicatechin-(2 α → 7:4 α → 8)-catechin (pavetannin A-2)	<i>Theobroma cacao</i> L.	66
	3- <i>O</i> - α -L-Arabinopyranosyl-ent-epicatechin-(2 β → 7:4 α → 8)-catechin		
	Propelargonidins		
	Epiafzelechin-(2 β → 7:4 β → 8)-ent-afzelechin	<i>Cassipourea gerrardii</i>	70
	7- <i>O</i> -Me-Epiafzelechin-(2 β → 7:4 β → 8)-epiafzelechin	<i>C. gummiflua</i>	195
	7- <i>O</i> -Me-Epiafzelechin-(2 β → 7:4 β → 8)-ent-afzelechin		
	Ent-epiafzelechin-(2 α → 7:4 α → 8)-quercetin	<i>Prunus prostrata</i>	30
	Ent-epiafzelechin-3- <i>O</i> - <i>p</i> -OH-benzoate-(2 α → 7:4 α → 8)-epiafzelechin	<i>P. armeniaca</i>	196
	Ent-epiafzelechin-(2 α → 7:4 α → 8)-afzelechin	<i>Geranium niveum</i>	197
	Epiafzelechin-(2 β → 7:4 β → 8)-afzelechin (geranin A)		198, 199
	Epiafzelechin-(2 β → 7:4 β → 8)-galloocatechin (geranin C)		
	Prodelphinidins		
	Epigalloocatechin-(2 β → 7:4 β → 8)-epicatechin	<i>Dioclea lasiophylla</i>	200
Probinetimidins			
Robinetinidol-(2 β → 7:4 β → 8)-catechin	<i>Acacia mearnsii</i>	32	
Proluteolinidins			
7- <i>O</i> - β -D-Glucopyranosyl-luteolin-(2 β → 7:4 β → 8)-(2 <i>R</i>)-5,7,3',4'-tetrahydroxyflavanone (diinsiminol)	<i>Sarcophyte piriiei</i>	202	
7- <i>O</i> - β -D-Glucopyranosyl-luteolin-(2 β → 7:4 β → 8)-ent-naringenin (diinsinin)			
Prodistenidins			
6-Methyl-5- <i>O</i> -methyl-epidistenin-(2 β → 7:4 β → 8)-(2 <i>S</i>)-7-OH-5-OMe-flavan (dracoflavan B ₁)	Dragon's blood (Daemonorops)	203	
6-Methyl-5- <i>O</i> -methyl-epidistenin-(2 α → 7:4 α → 8)-(2 <i>S</i>)-7-OH-5-OMe-flavan (dracoflavan B ₂)			
Miscellaneous			
7-Hydroxy-5-methoxy-6-methylflavan-(2 β → 7:4 β → 8)-(2 <i>S</i>)-7-OH-5-OMe-flavan (dracoflavan C ₁)	Dragon's blood (Daemonorops)	203	
7-Hydroxy-5-methoxy-6-methylflavan-(2 α → 7:4 α → 8)-(2 <i>S</i>)-7-OH-5-OMe-flavan (dracoflavan C ₂)			

5,7-Di-OH-6-Me-flavan-(2 β → 7,4 β → 8)-7-OH-5-OMe-flavan (dracoflavan D ₁)			
5,7-Di-OH-6-Me-flavan-(2 α → 7,4 β → 8)-7-OH-5-OMe-flavan (dracoflavan D ₂)			
Procyanidins			
Epicatechin-(4 β → 8)-epicatechin-(2 β → 7,4 β → 8)-epicatechin (aesculitannin A)		<i>Aesculus hippocastanum</i>	191
Epicatechin-(2 β → 7,4 β → 8)- <i>ent</i> -catechin-(4 β → 8)-epicatechin (aesculitannin B) ^a			
Epicatechin-(2 β → 7,4 β → 8)-epicatechin-(2 β → 7,4 β → 8)-epicatechin (aesculitannin C)			
Epicatechin-(2 β → 7,4 β → 8)-epicatechin-(2 β → 7,4 β → 8)-catechin (aesculitannin D)			
Epicatechin-(2 β → 7,4 β → 8)-epicatechin-(4 β → 8)- <i>ent</i> -epicatechin (pavetannin B-1)		<i>Pavetta owaritensis</i>	194
Epicatechin-(2 β → 7,4 β → 8)-epicatechin-(4 β → 8)-epicatechin (pavetannin B-2)		<i>P. owaritensis/Ecdysanthera utilis</i>	194, 209
Epicatechin-(2 β → 7,4 β → 6)-epicatechin-(4 β → 8)-epicatechin (pavetannin B-3)		<i>P. owaritensis</i>	194
Epicatechin-(2 β → 7,4 β → 6)- <i>ent</i> -epicatechin-(4 α → 8)-epicatechin (pavetannin B-4)		<i>P. owaritensis</i>	204
Epicatechin-(2 β → 7,4 β → 6)-catechin-(4 α → 8)-epicatechin (pavetannin B-5)		<i>P. owaritensis</i>	194, 204
Epicatechin-(2 β → 7,4 β → 8)-epicatechin-(4 β → 8)-catechin (pavetannin B-6)			
Epicatechin-(2 β → 7,4 β → 8)- <i>ent</i> -epicatechin-(2 α → 7,4 α → 8)- <i>ent</i> -catechin (pavetannin B-7)		<i>P. owaritensis</i>	205
Epicatechin-(2 β → 7,4 β → 8)-epicatechin-(2 β → 7,4 β → 8)- <i>ent</i> -catechin (pavetannin B-8)			
Epicatechin-(2 β → 7,4 β → 8)-catechin-(4 β → 8)-catechin		<i>Aesculus hippocastanum</i>	206
Epicatechin-(4 α → 8)-epicatechin-(2 β → 7,4 β → 8)-epicatechin			
Epicatechin-(2 β → 7,4 β → 6)-epicatechin-(2 β → 7,4 β → 8)-epicatechin		<i>Parameria laevigata</i> Moldenke	207
Epicatechin-(4 β → 6)-epicatechin-(2 β → 7,4 β → 8)-epicatechin		<i>Vaccinium macrocarpon</i> Ait. (Cranberry)	189
3- <i>O</i> -Arabinopyranosylepicatechin-(2 β → 7,4 β → 8)-epicatechin-(4 β → 8)-epicatechin (3- <i>T-O</i> - α -L-arabinopyranosylcinnamtannin B ₁)		<i>Theobroma cacao</i> L.	66
3- <i>O</i> -Galactopyranosylepicatechin-(2 β → 7,4 β → 8)-epicatechin-(4 β → 8)-epicatechin (3- <i>T-O</i> - β -D-galactopyranosylcinnamtannin B ₁)			
Propelargonidins			
Epiatzelechin-(2 β → 7,4 β → 8)-epiatzelechin-(4 β → 8)-afzelechin (selligueain A)		<i>Selligaea feciei</i>	46, 208
Epiatzelechin-(2 β → 7,4 β → 8)-epiatzelechin-(4 β → 8)-afzelechin-4 β -acetic acid methyl ester [epiatzelechin-(2 β → 7,4 β → 8)-epiatzelechin-(4 β → 8)-3'-deoxydryopteriacid methyl ester] (selligueain B)			
Epiatzelechin-(2 β → 7,4 β → 8)-afzelechin-(2 β → 7,4 β → 8)-afzelechin (geranin D)		<i>Geranium niveum</i>	199
Procyanidins			
Epicatechin-(4 β → 6)-epicatechin-(2 β → 7,4 β → 8)-epicatechin-(4 β → 8)-epicatechin (pavetannin C-1)		<i>Pavetta owaritensis</i>	205, 209
Epicatechin-(2 β → 7,4 β → 8)- <i>ent</i> -epicatechin-(4 α → 8)- <i>ent</i> -epicatechin-(4 α → 8)-epicatechin (pavetannin C-2)			

continued

TABLE 11.15
The Natural A-Type Proanthocyanidins — continued

Class	Compound	Source	Ref.
	Epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin (aesculitannin E)	<i>Aesculus hippocastanum</i>	192
	Epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)- <i>ent</i> -catechin-(4 β \rightarrow 8)- ^b epicatechin-(4 β \rightarrow 8)-epicatechin (aesculitannin F)		
	Epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)- ^b epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin (aesculitannin G)	<i>Parameria laevigata</i> Moldenke	207, 211
	Epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 6)-epicatechin-(4 β \rightarrow 8)-epicatechin (parameritannin A-1)		
	Epicatechin-(2 β \rightarrow 5,4 β \rightarrow 6)-epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin (parameritannin A-2)		
	Epicatechin-(2 β \rightarrow 7,4 β \rightarrow 6)-epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin (parameritannin A-3)		
	“Mixed” propylaragonidins/procyanidins		
	Epiafzelechin-(2 β \rightarrow 7,4 β \rightarrow 8)-epicatechin-(4 β \rightarrow 8)-epicatechin (pavetannin C-3)	<i>Pavetta owarimensis</i>	210
	Epiafzelechin-(2 β \rightarrow 7,4 β \rightarrow 8)- <i>ent</i> -afzelechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin-(2 α \rightarrow 7,4 α \rightarrow 8)- <i>ent</i> -catechin (pavetannin C-4)		
	Epiafzelechin-(2 β \rightarrow 7,4 β \rightarrow 8)- <i>ent</i> -catechin-(4 α \rightarrow 8)- <i>ent</i> -epicatechin-(2 α \rightarrow 7,4 α \rightarrow 8)- <i>ent</i> -catechin (pavetannin C-5)		

^aIn the original paper aesculitannin B was named epicatechin-(2 β \rightarrow 7,4 β \rightarrow 8)-*ent*-catechin-(4 α \rightarrow 8)-epicatechin.

^bIndicated as (4 α \rightarrow 8) in the original manuscript.

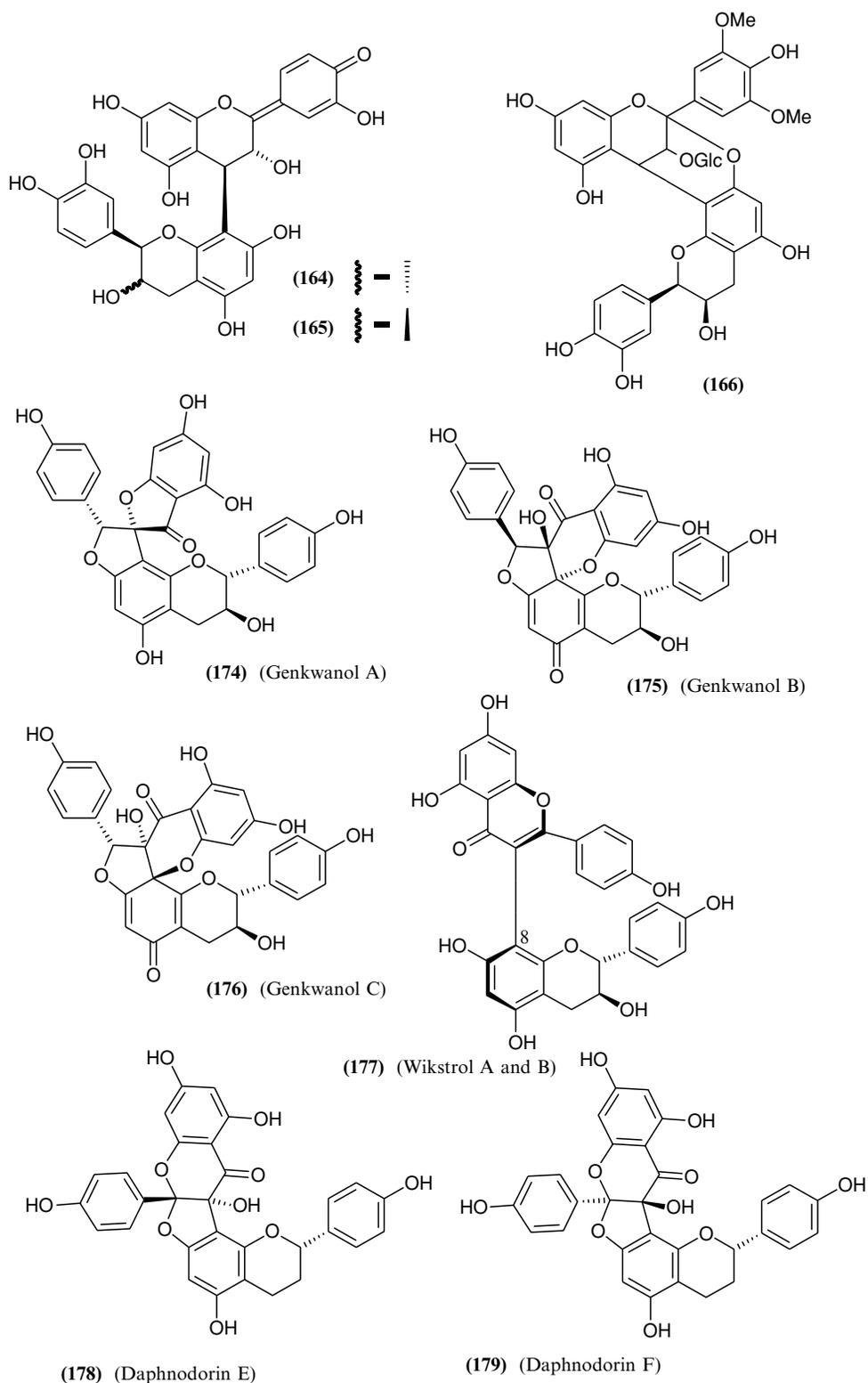


FIGURE 11.16 Structures of compounds 164–166 and 174–179.

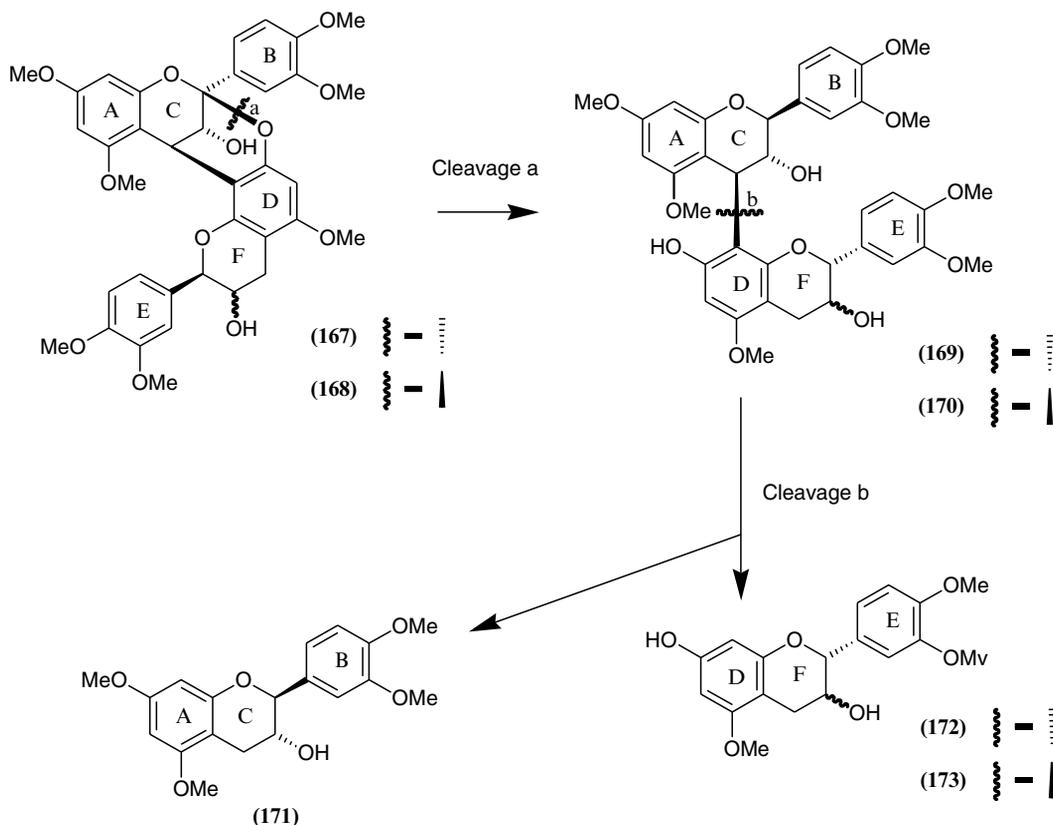


FIGURE 11.17 Scheme 11.6: Proposed route to the reductive cleavage of both interflavanyl bonds in A-type proanthocyanidins including the structures of compounds **167–173**.

The basic carbon framework of an afzelechin moiety coupled at C-8 to the α -carbon of a chalconaringenin unit is evident in the structures of the three genkwanol (**174–176**) (Figure 11.16). Their structures were meticulously corroborated by the collective utilization of ^1H and ^{13}C NMR data, x-ray analysis, and the modified Mosher method.^{216–218} Two related but nonrearranged compounds comprising 5,7,4'-trihydroxyflavone and afzelechin constituent units, the (3 \rightarrow 8)-coupled atropisomeric wikstrols A and B (**177**), were shown to arise by acid-catalyzed rearrangement of daphnodorin B.²¹⁹ Additional entries are shown in Figure 11.18.

New structures or chemistry emerging from the remarkable “black tea pool” are also listed in Table 11.16. Theaflavin (**190**) and desgalloyl theaflavin (**191**) are B,B'-linked bisflavonoids formed presumably via oxidative coupling of the flavanol glucoside, isomyricitrin, and 3-*O*-galloylepigallocatechin and epigallocatechin, respectively (Figure 11.19).²²⁵ The *R* absolute configuration of the atropisomeric biphenyl linkages of compounds **189–191** was established by comparison of CD data with those of theasinensis C and E possessing *R* and *S* axial chirality, respectively. Theadibenzotropolone A (**192**), the first theaflavin-type trimer in black tea, and theaflavin-3-gallate were formed by the reaction of (–)-epicatechin and (–)-epigallocatechin gallate with horseradish peroxidase in the presence of H_2O_2 .²²⁶ Its presence in black tea was confirmed by liquid chromatography–electrospray ionization tandem mass spectrometry (MS). An informative schematic representation of the enzymatic

TABLE 11.16
The Natural Nonproanthocyanidins with Flavan or Flavan-3-ol Constituent Units

Compound	Structure	Source	Ref.
Genkwanol A	(174)	<i>Daphne genkwa</i> Sieb. et Zucc.	216
Genkwanol B	(175)		217
Genkwanol C	(176)		218
Wikstrol A atropisomer	(177)	<i>Wikstroemia sikokiana</i>	219
Wikstrol B atropisomer	(177)		
Daphnodorin E	(178)	<i>D. odora</i> Thumb.	220
Daphnodorin F	(179)		
Daphnodorin G	(180)	<i>D. odora</i>	221
Daphnodorin H	(181)		
Daphnodorin I	(182)		
Daphnodorin J	(183)	<i>D. odora</i>	222
Daphnodorin K	(184)		
Daphnodorin L	(185)		
Daphnodorin M	(186)	<i>D. acutiloba</i>	223
Daphnodorin N	(187)		
Damalachawin	(188)	<i>Dracaena cinnabari</i>	224
Theogallinin	(189)	<i>Camellia sinensis</i> (black tea)	225
Theoflavinonin	(190)		
Desgalloyl theaflavinonin	(191)		
Theadibenzotropolone A	(192)	<i>C. sinensis</i> (black tea)	226
Theaflavate A	(193)		227
Theacitrin A	(194)		228
Theaflavate B	(195)		229
Isotheaflavin-3'- <i>O</i> -gallate	(196)		
Neotheaflavin-3- <i>O</i> -gallate	(197)		

conversion of the polyphenols in the tea leaves was also reported.²²⁵ Theaflavate A (193) formed when epicatechin-3-*O*-gallate was oxidized with $K_3Fe(CN)_6$.²²⁷ Compounds 195–197 were also produced via chemical oxidation of the appropriate flavan-3-ol or flavan-3-*O*-gallate precursors (Figure 11.20).²²⁹

11.3.4 COMPLEX TANNINS

The term, complex tannin, appears to be established as descriptor for the class of polyphenols in which a flavan-3-ol unit, representing a constituent unit of the “condensed tannins” (proanthocyanidins), is connected to a “hydrolyzable (gallo- or ellagi-) tannin” through a carbon–carbon linkage. Since the first demonstration of their natural occurrence,²³⁰ a considerable number of these unique secondary metabolites have been reported.^{3,4} New additions (Table 11.17) to this series of compounds come exclusively from the groups of Nonaka and Nishioka, and Okuda and Yoshida in Japan.

Malabathrin A (198), E (199), and F (200) (Figure 11.20 and Figure 11.21), which are composed of a C-glucosidic ellagitannin and a C—C coupled epicatechin moiety, were isolated from the leaves of *Melastoma malabatricum*.²³¹ The *S* chirality for both the hexahydroxydiphenoyl (HHDP) groups in malabathrin A (198) was deduced from its CD curve, which exhibited positive and negative Cotton effects at 233 and 262 nm, respectively. Its

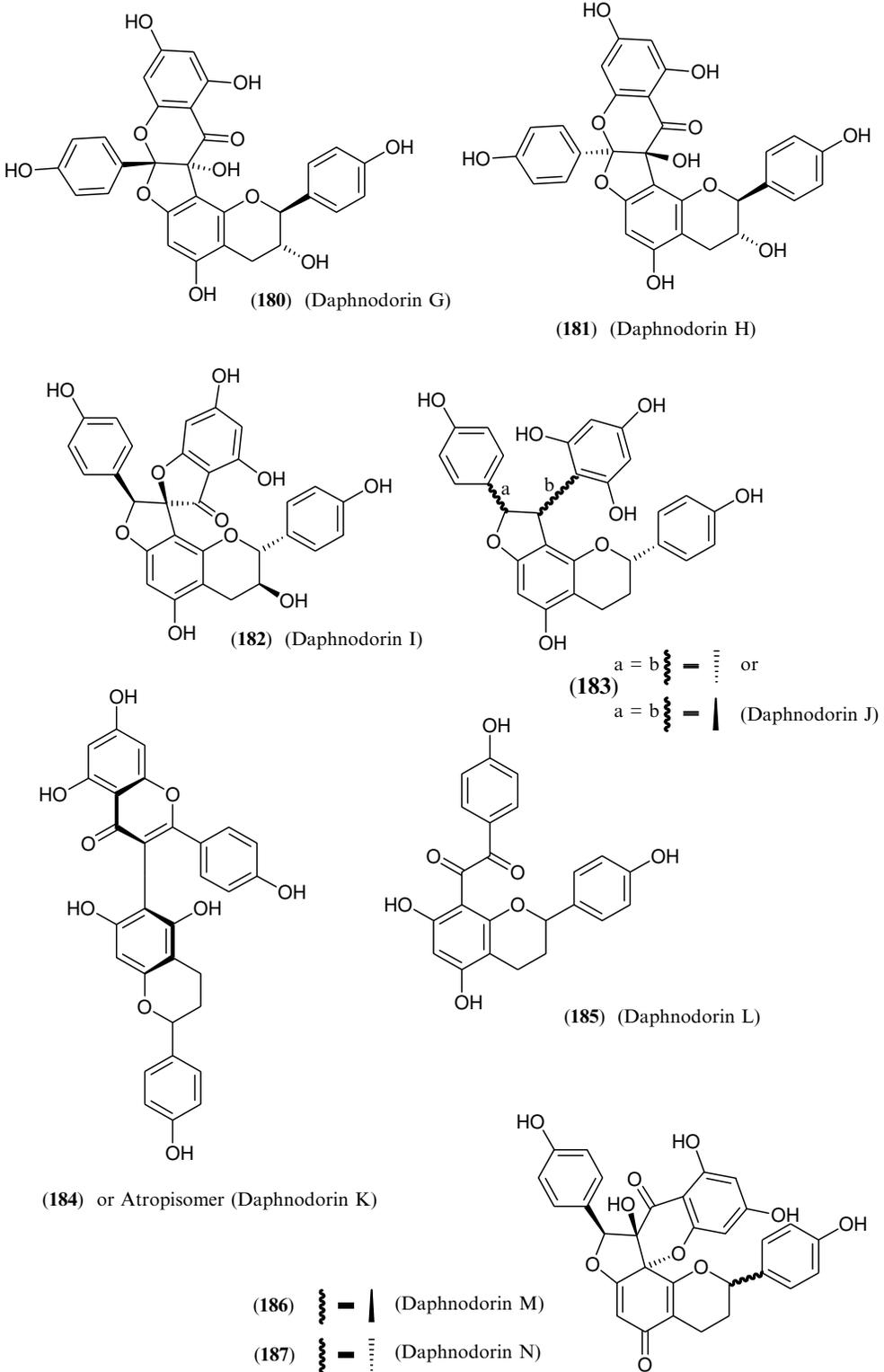
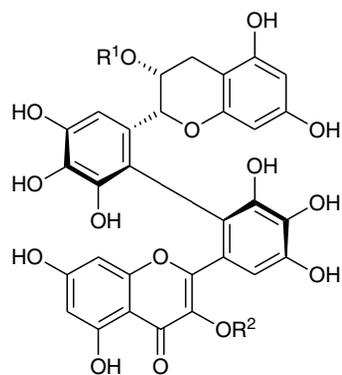
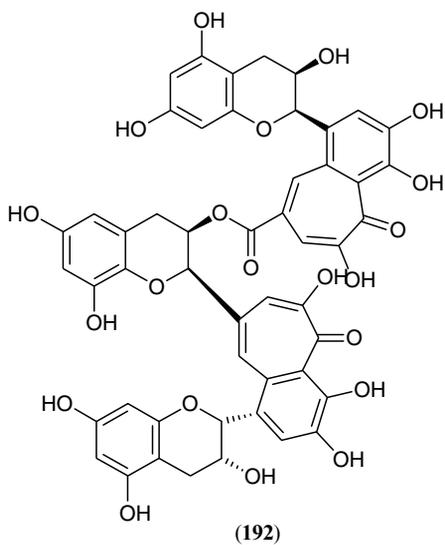
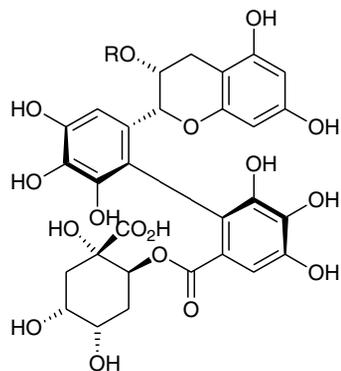
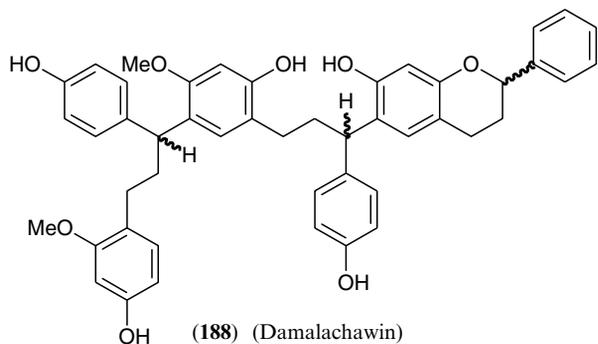


FIGURE 11.18 Structures of compounds 180–187.



(190) R¹ = galloyl, R² = β -D-glucopyranosyl (Theaflavinin)

(191) R¹ = H, R² = β -D-glucopyranosyl (Desgalloyl theaflavinin)

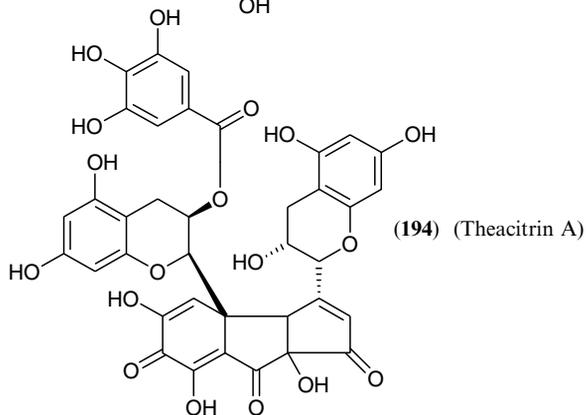
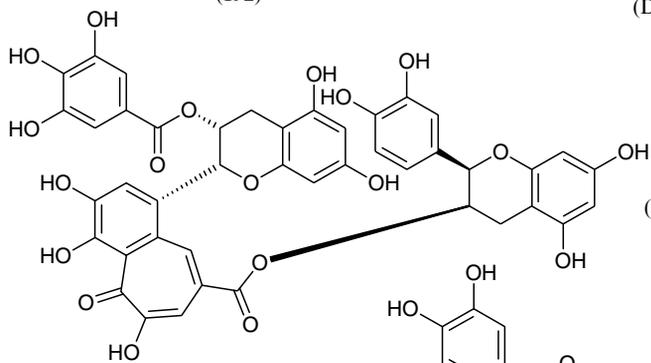


FIGURE 11.19 Structures of compounds 188–194.

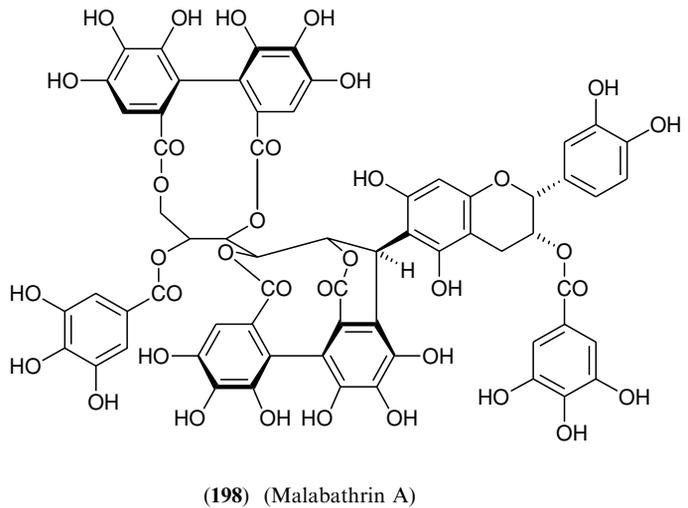
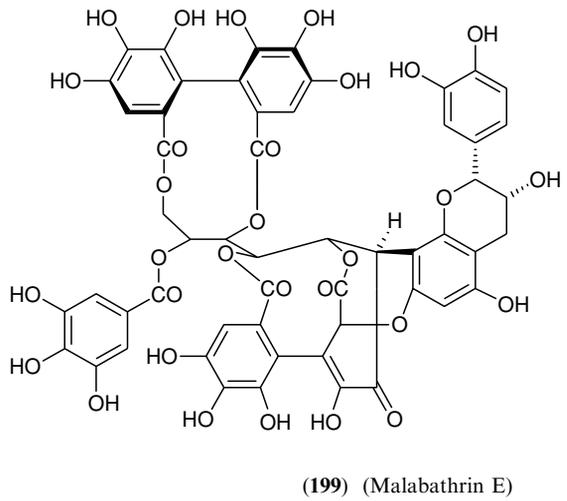
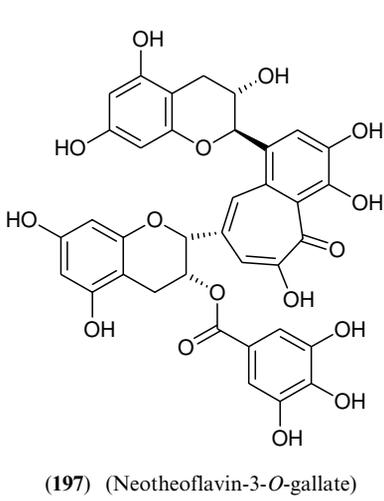
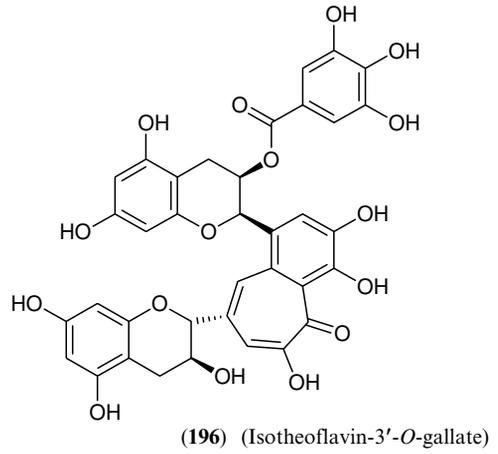
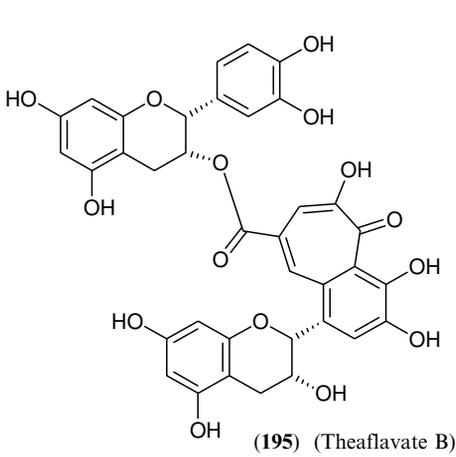


FIGURE 11.20 Structures of compounds 195–199.

TABLE 11.17
The Natural Complex Tannins

Compound	Structure	Source	Ref.
Malabathrin A	(198)	<i>Melastoma malabatricum</i>	231
Malabathrin E	(199)		
Malabathrin F	(200)		
Guajavin A	(201)	<i>Psidium guajava</i>	232
Guajavin B	(203)		
Psidin A	(205)		
Psidin B	(207)		
Psidin C	(209)		
Psiguavin	(211)		
Strobilanin	(213)	<i>Platycarya strobilacea</i> Sieb. et Zucc.	233
Camelliatannin C	(214)	<i>Camellia japonica</i>	234–236
Camelliatannin D	(216)		
Camelliatannin E	(215)		
Camelliatannin F	(217)		
Camelliatannin G	(218)		
Stachyuranin A	(219)	<i>Stachyurus praecox</i> Sieb. et Zucc.	237
Stachyuranin B	(220)		
Stachyuranin C	(221)		

structure was unequivocally confirmed by acid-catalyzed condensation of the ellagitannin, casuarinin, and epicatechin-3-*O*-gallate. The cyclopentenone moiety in malabathrins E (199) and F (200) is regarded as the product of oxidative conversion of the HHDP group at O₍₂₎–O₍₃₎ of glucose in, e.g., malabathrin A (198).²³¹

The ¹H NMR spectrum of guajavin A (201) at room temperature was complicated due to the effects of conformational isomerism, a feature that was commonly observed in complex tannins where the C-8 position of the flavan-3-ol unit is substituted.²³² Structural elucidation of guajavins A (201) and B (203) was done by comparison of their ¹³C NMR data with those of the related catechin analogs, stenophyllanin A (202) and acutissimin B (204), respectively, and by synthesis via acid-catalyzed condensation of galocatechin with the ellagitannins, stachyurin, and vescalagin, respectively. The ¹H and ¹³C NMR spectra of psidinins A (205), B (207), and C (209) similarly resembled those of their catechin analogs, mongolicains A (206) and B (208), and stenophyllin A (210), respectively, thus readily facilitating the structural elucidation of the former three compounds (Figure 11.22).

Psiguavin (211), in which the B-ring of the flavan-3-ol unit is extensively rearranged, is considered to be derived biosynthetically from eugenigrandin A (partial structure [212]) by successive oxidation of the pyrogallol B-ring, benzylic acid-type rearrangement, and decarboxylation, followed by oxidative coupling as is indicated in Figure 11.23 (Scheme 11.7).²³²

Camelliatannins C (214) and E (215) with their C-6 and C-8 substituted epicatechin moieties, respectively, represent the first examples of complex tannins lacking a C–C bond between C-1 of glucose and the HHDP group at O-2 of the glucose unit (Figure 11.24). These bonds could, however, be readily formed by treatment of analogs 214 and 215 with polyphosphoric acid, hence transforming them into camelliatannins B and A, respectively.²³⁵ Camelliatannins C (214) and E (215) may thus be considered as precursors to the “normal” type of

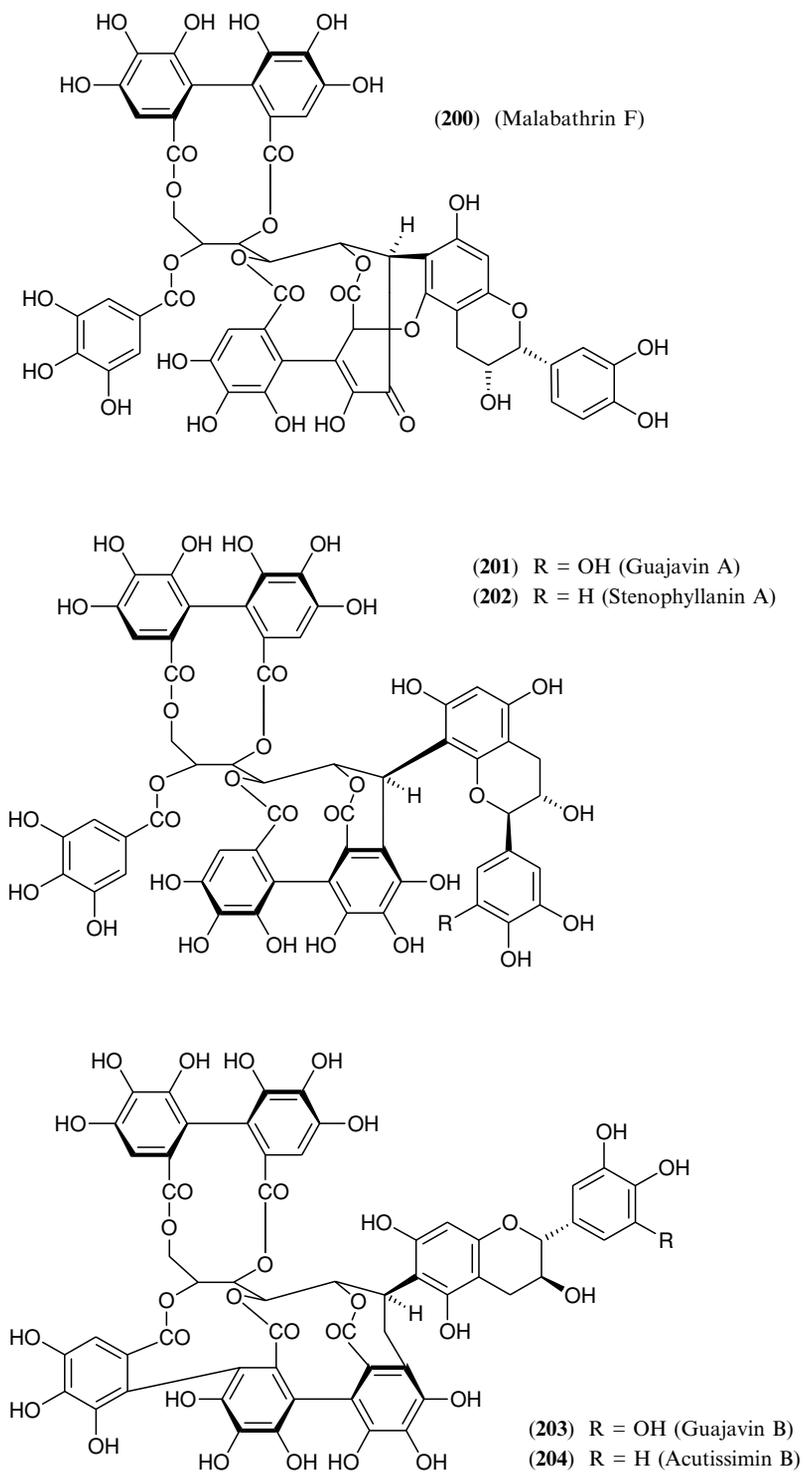


FIGURE 11.21 Structures of compounds 200–204.

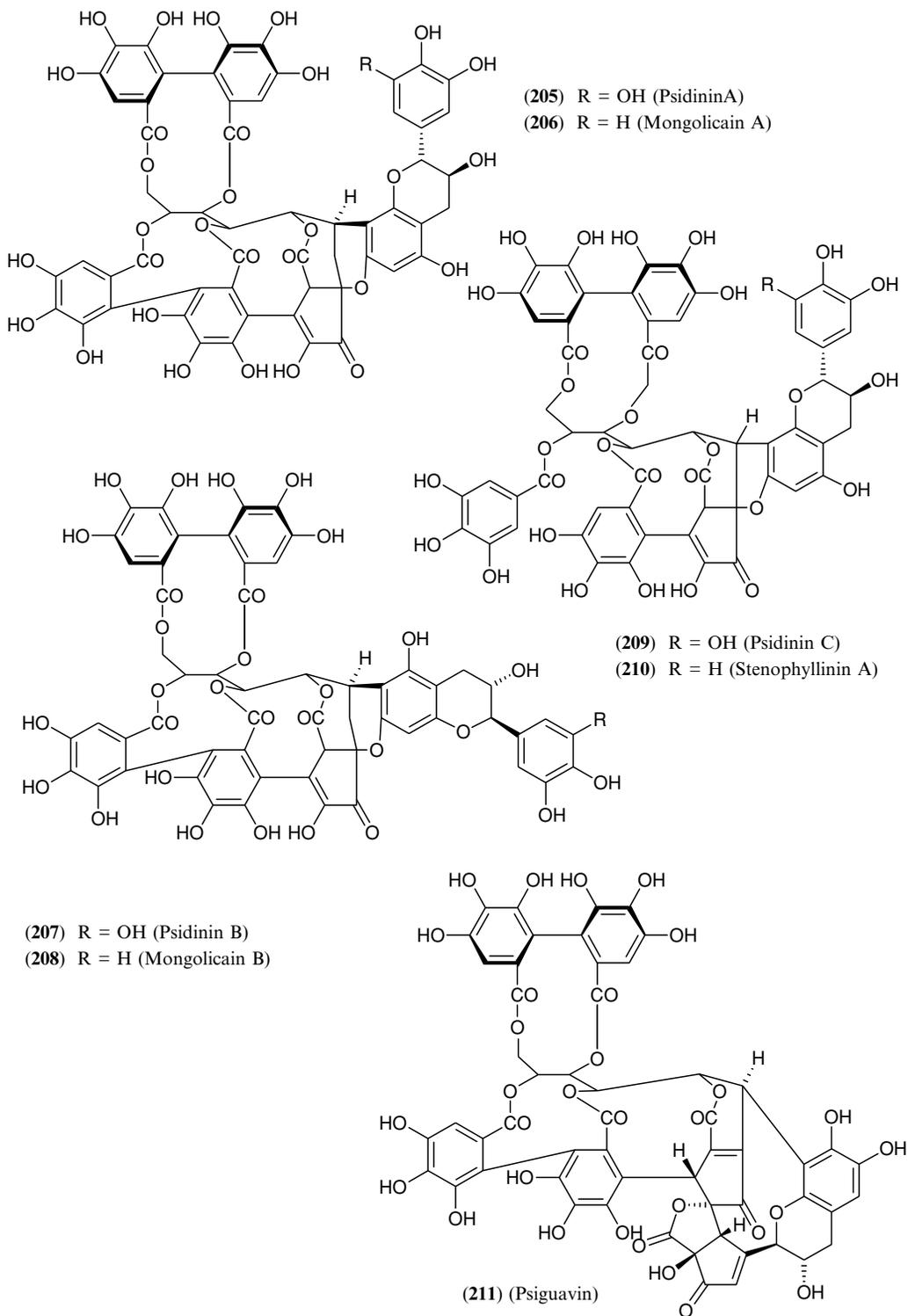


FIGURE 11.22 Structures of compounds 205–211.

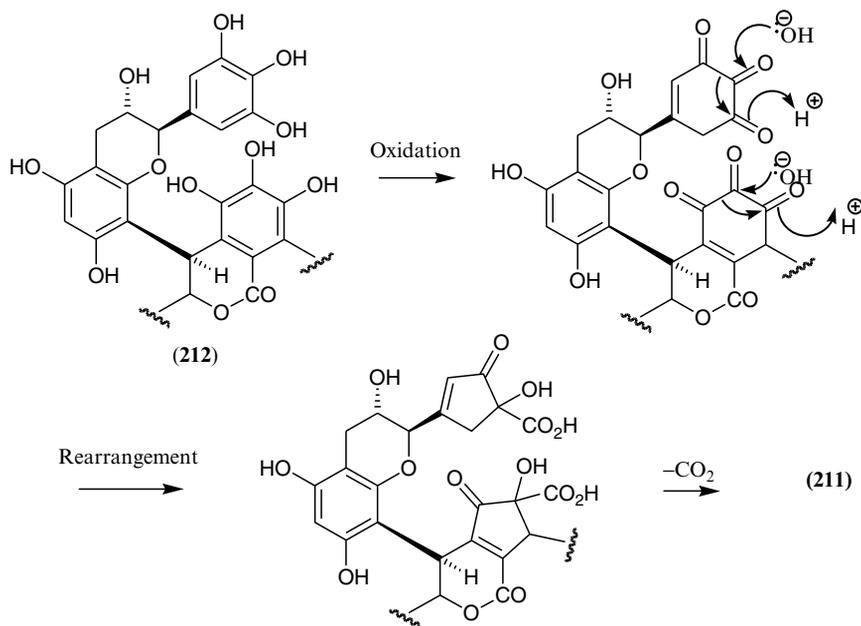


FIGURE 11.23 Scheme 11.7: Proposed route to the transformation of eugenigrandin A (**212**) into psiguavin (**211**).

complex tannins and may be anticipated to co-occur in plant sources containing the latter class of metabolites. Camelliattannin D (**216**) represents the first example composed of dimeric hydrolyzable tannin and flavan-3-ol constituent units.²³⁴ Camelliattannin A presumably serves as the biogenetic precursor to both camelliattannins F (**217**) and G (**218**).

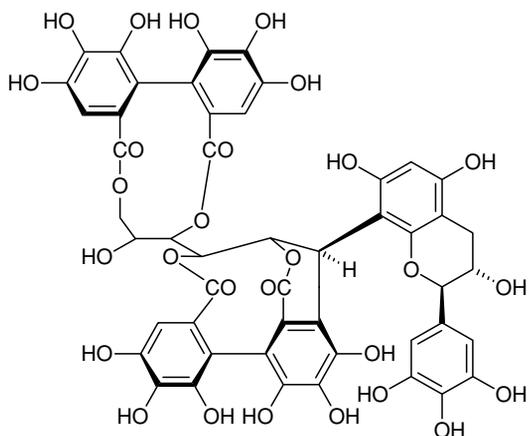
Stachyuranins A (**219**) and B (**220**) also lack the C—C linkage between C-1 of the glucose moiety and the aroyl group at O-2 of glucose (Figure 11.25). When dissolved in aqueous methanol at room temperature, stachyuranin A (**219**) is gradually converted into stenophyllanin A,²³⁰ which presumably suggests that the latter compound is produced nonenzymatically from **219** in plants.

11.3.5 MISCELLANEOUS

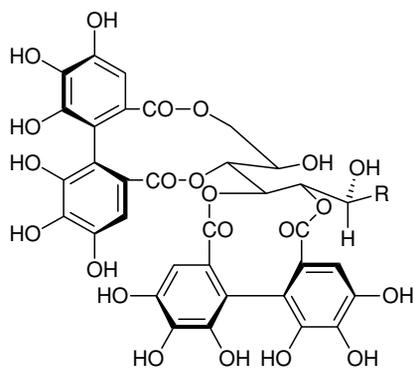
A new series of polyphenols, named castavinols, has been isolated from Bordeaux red wines.²³⁸ The three analogs (**222–224**) (Figure 11.25) (or their C-ring enantiomers) were speculated to form via [1,2]-addition of diacetyl, a C-4 yeast metabolite, to, e.g., malvidin-3-*O*-glucoside (**225**) (Figure 11.26, Scheme 11.8). The resultant intermediate (**226**) would then cyclize by addition of the vinylic double bond to the electrophilic carbonyl carbon to give a C-3 carbocation (**227**), which is reduced, presumably under enzymatic control from the yeast, to compound **222**. The remaining analogs (**223** and **224**) may similarly be derived from peonidin- and petunidin-3-glucoside, respectively.

11.3.6 NMR AND CONFORMATIONAL ANALYSIS OF PROANTHOCYANIDINS

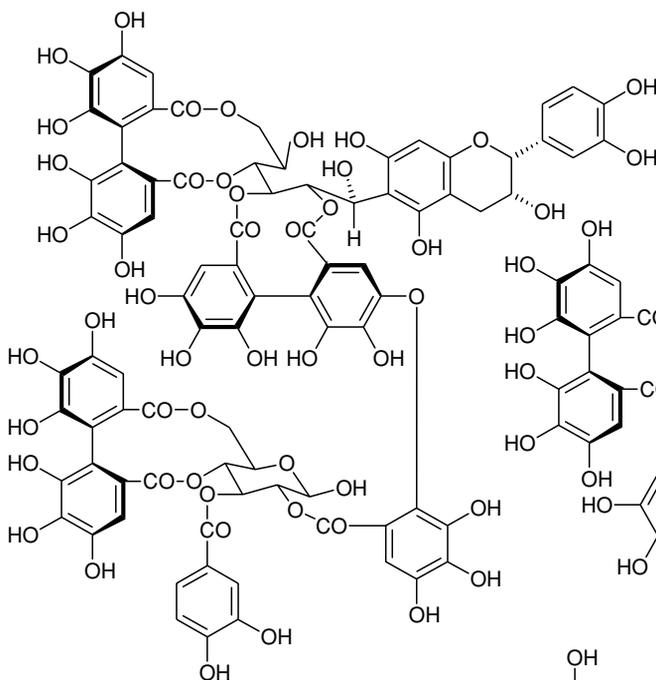
Conformational analysis of proanthocyanidins is, in principle, concerned with the conformation of the pyran heterocycle and with the phenomenon of conformational isomerism due to



(213) Strobilanin

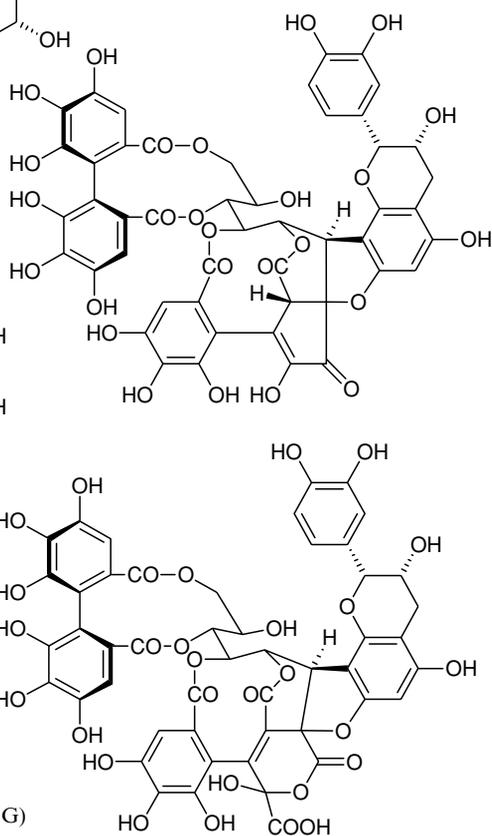


(214) R = 6-epicatechin (Camelliattannin C)
 (215) R = 8-epicatechin (Camelliattannin E)



(216) (Camelliattannin D)

(217) (Camelliattannin F)



(218) (Camelliattannin G)

FIGURE 11.24 Structures of compounds 213–218.

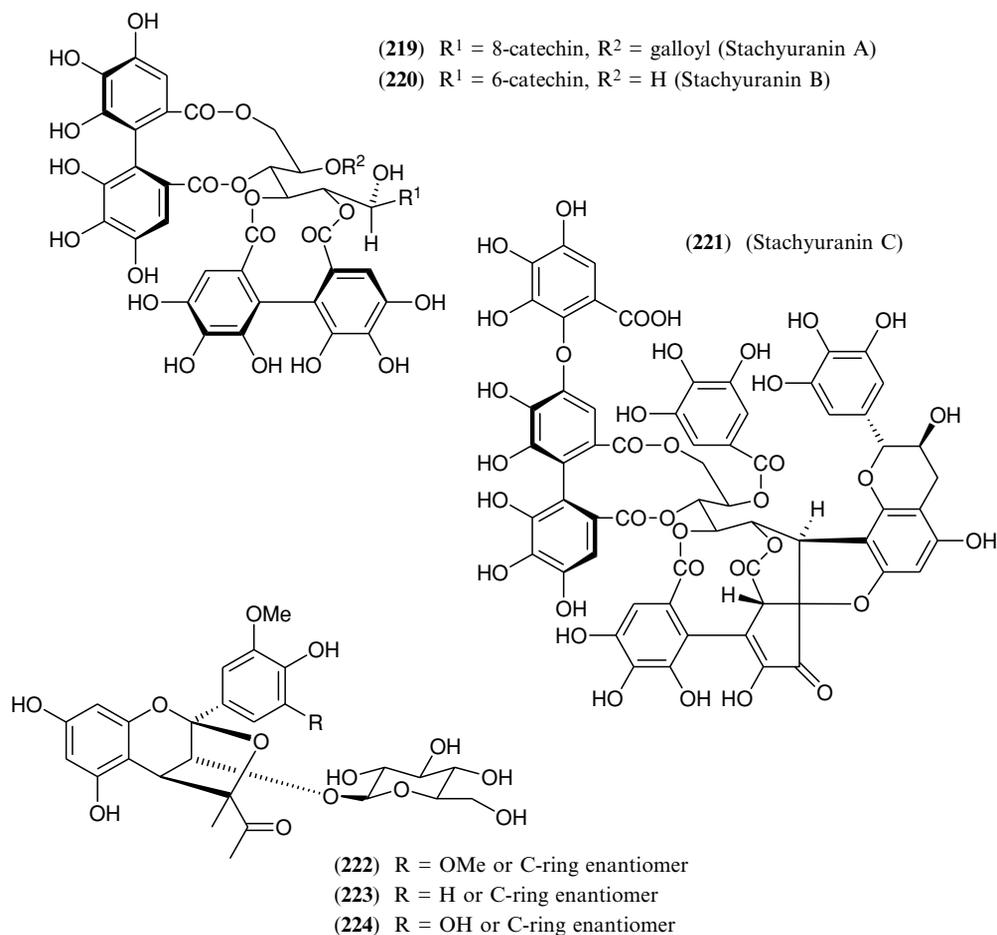


FIGURE 11.25 Structures of compounds 219–224.

restricted rotation about the interflavanyl bond axis or axes. Realization of the fact that the conformational itinerary of the heterocyclic rings involves a dynamic equilibrium between E- and A-conformers²³⁹ had a profound impact in this field. Useful discussions dealing with various aspects of the conformational behavior of the proanthocyanidins may be extracted from Refs. 4, 7–12.

The utilization of the full array of modern ^1H and ^{13}C NMR methodology has led to various contributions regarding the proton and carbon assignments as well as the conformations of di- and trimeric proanthocyanidins.^{240–249} Vercauteren and coworkers²⁴¹ differentiated C-4 \rightarrow C-8- and C-4 \rightarrow C-6-linked peracetylated procyanidins [catechin-(4 α \rightarrow 8)-catechin-(4 α \rightarrow 6)-catechin] by initial complete assignment of proton and carbon resonances of the appropriate dimers, followed by observation of differences in correlation of H-4 (C) with the quaternary carbons of the A-C- and D-F-ring junctions. The same group²⁴² also confirmed the C-8 substitution of the DEF and GHI units in the peracetate of procyanidin C-1 [catechin-(4 α \rightarrow 8)-catechin-(4 α \rightarrow 8)-catechin] in an HMBC experiment. De Bruyne et al.²⁴³ subsequently provided a full analysis of the ^1H and ^{13}C NMR data of procyanidin B-3 [catechin-(4 α \rightarrow 8)-

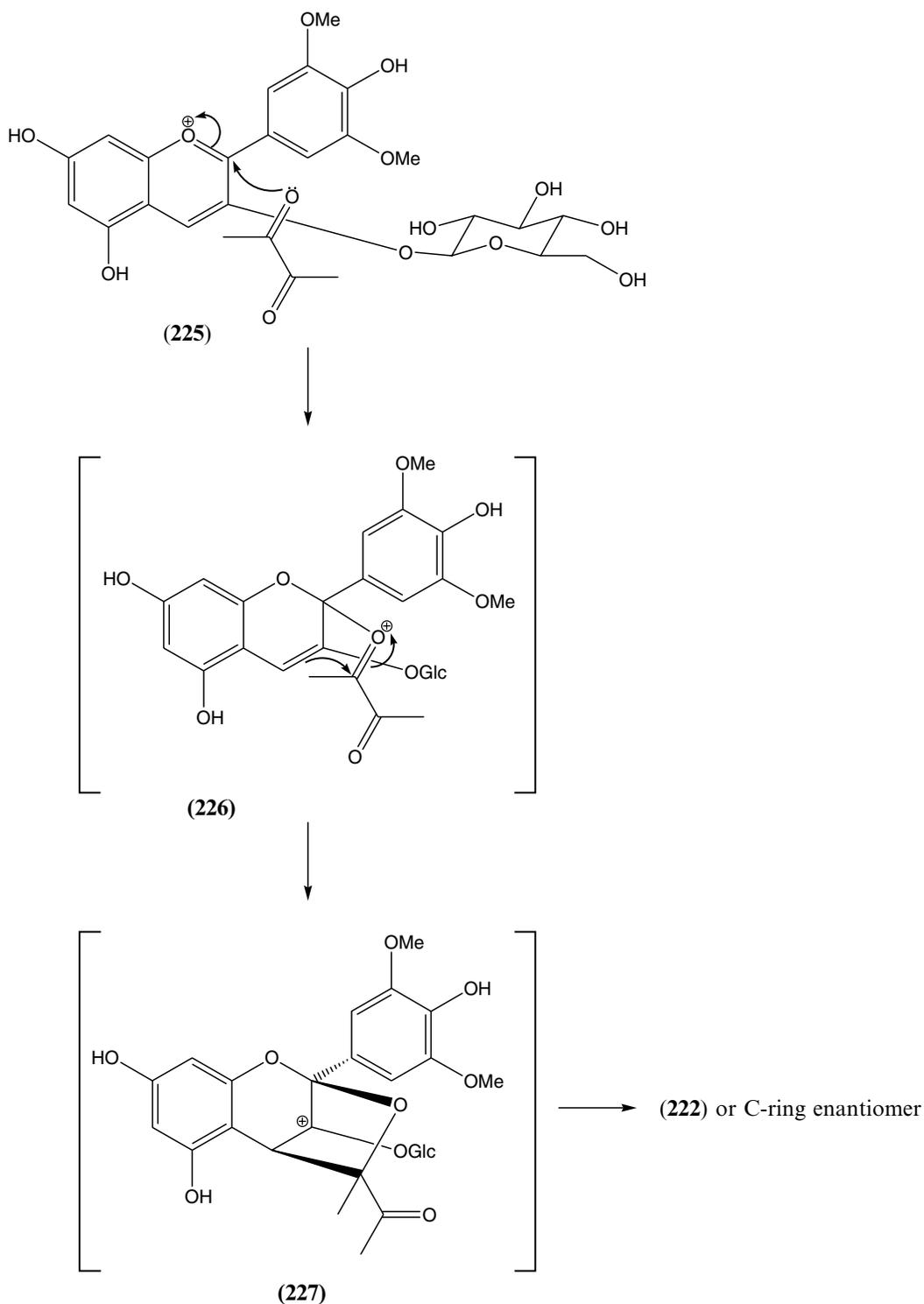


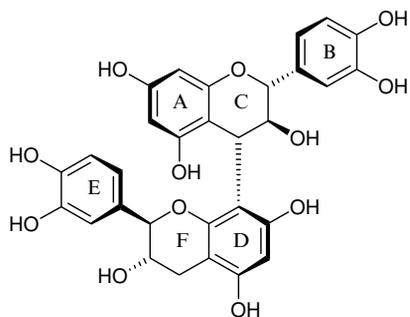
FIGURE 11.26 Scheme 11.8: Proposed route to the formation of castavinols including the structures of compounds **225–227**.

catechin]. Two-dimensional NMR sequences were also used to assign the proton and carbon resonances of procyanidin A-2 [epicatechin-(2 β \rightarrow 7, 4 β \rightarrow 8)-catechin].²⁴⁴ Rimpler and coworkers²⁴⁰ used the peracetates of tri- and tetrameric procyanidins with epicatechin constituent units to define a further diagnostic shift parameter facilitating establishment of the interflavan-yl bonding position. Williamson and coworkers²⁴⁵ similarly reported fully assigned ¹H and ¹³C NMR data of procyanidin B-2 [epicatechin-(4 β \rightarrow 8)-epicatechin] and its per-*O*-acetyl derivative. Hemingway et al.²⁴⁶ produced evidence that both H-6 and C-6 resonances of free phenolic flavan-3-ols are downfield from H-8 and C-6, hence indicating that the chemical shifts of A-ring protons are indeed reversed compared to those commonly reported. Tobiasson et al.²⁴⁷ demonstrated the temperature dependence of the pyran ring proton coupling constants of catechin. Such a temperature dependence of the coupling constants was also reproduced from the Boltzmann distribution of the conformational ensemble generated by the GMMX searching program.²⁴⁷ Additional useful information may be extracted from Ref. 248 as well as the preceding two papers in the series by the same authors. Reference 248 deals with the complete and unequivocal ¹H and ¹³C NMR assignments of a range of green tea polyphenols.

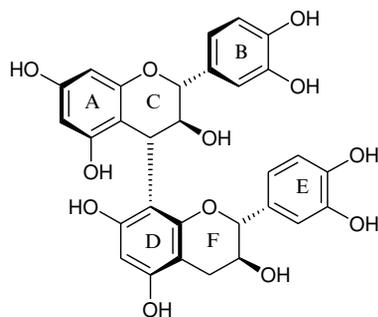
Investigations of the conformational properties of the flavan-3-ols and oligomeric proanthocyanidins have hitherto involved a variety of molecular mechanics and molecular orbital computations in combination with crystal structures, time-resolved fluorescence, as well as ¹H and ¹³C NMR methods. Representative references to all these techniques may be found in the papers listed in Refs. 241–247, 250. These “NMR papers” incidentally also represent the major contributions regarding the conformation of proanthocyanidins, and may be summarized in a conformational context by reference to the significant contributions of Hatano and Hemingway.^{251,252}

NMR analysis of procyanidin B-1 [epicatechin-(4 β \rightarrow 8)-catechin] and B-3 [catechin-(4 α \rightarrow 8)-catechin] permitted full assignment of the H- and C-resonances for both the compact (**228**) and the more extended (**229**) conformer in the free phenolic form (Figure 11.27). In organic solvents, the more extended rotamer of procyanidin B-1 is preferred over the more compact rotamer (10:7) but in D₂O the more compact rotamer dominates (10:2). When procyanidin B-3 is dissolved in organic solvents, the more compact rotamer (**228**) is slightly preferred (8:10). With D₂O as solvent only trace proportions of the more extended rotamer (**229**) are detected. In this solvent, rotational conformation exchange is detected despite the observation of two distinct and sharp sets of signals for each rotamer. The heterocyclic ring of the ABC unit exists in an approximate half-chair conformation in each rotamer for both procyanidins B-1 and B-3. The heterocyclic ring of the ABC unit exists in an approximate half-chair conformation in each rotamer for both procyanidin B-1 and B-3. Coupling constants of the protons of the pyran rings of the DEF moieties indicate substantial axial orientation of the E-ring (see compounds **230** and **231** for E- and A-conformers of the DEF unit of procyanidin B-3). Line shape analysis of H-3 (F) indicated that the “abnormal” coupling constants of the F-ring protons were reminiscent of a comparatively high-energy skewed-boat conformation for procyanidin B-1 and between a half-chair and a skewed-boat for procyanidin B-3 rather than to E \rightleftharpoons A-conformational exchange, which has hitherto been used to explain the smaller than anticipated coupling constants.

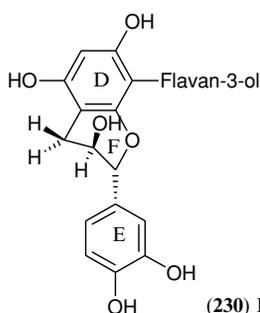
Hatano and Hemingway^{251,252} used NOE studies to assess the association of catechin and procyanidin B-3 with oligopeptides (see also relevant results in Refs. 253–256). The observed intermolecular NOEs indicating the preferred sites in the association of catechin and procyanidin B-3 with the tetrapeptide Gly–Pro–Gly–Gly are shown in **232** and **233**, respectively. The molecular shapes of both the polyphenol and polypeptide are important features as far as selectivity is concerned.



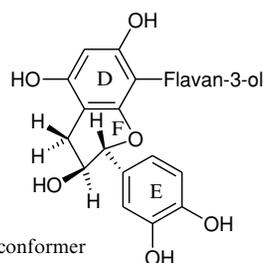
(228) (Compact rotamer of procyanidin B-3)
C₃-C₄-C₈-C₉ torsion angle, (-)



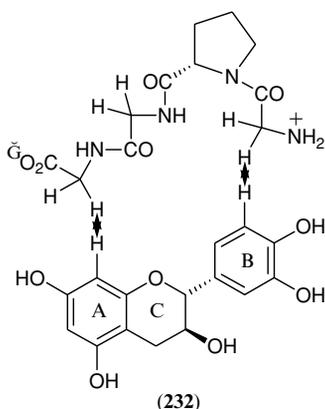
(229) (Extended rotamer of procyanidin B-3)
C₃-C₄-C₈-C₉ torsion angle, (+)



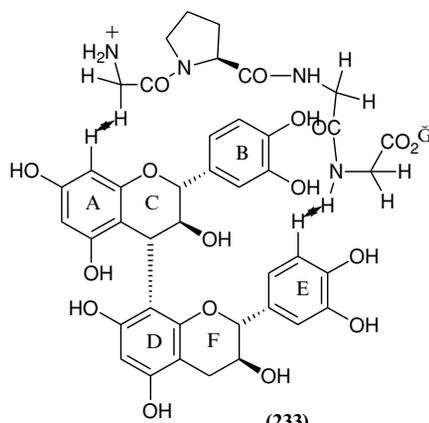
(230) E-conformer



(231) A-conformer



(232)



(233)

FIGURE 11.27 Structures of compounds 228–233.

11.3.7 HPLC–MS ANALYSIS OF PROANTHOCYANIDINS

The various MS methods to determine the molecular composition of the constituent monomeric units in proanthocyanidins oligomers are summarized in Ref. 257. Contributions focusing on proanthocyanidin analysis via the HPLC–MS protocol included a wide range of plant-derived foods and beverages, and are summarized in Refs. 12, 258–261. In addition, references to additional significant contributions in this area are readily available via several of the excellent electronic search engines that are at our disposal.

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12 Flavones and Flavonols

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12.1 INTRODUCTION

Flavonoid analyses are mostly concentrating on plants which are of either pharmaceutical interest or of commercial value. In addition, flavonoids are important factors in biological interactions between living organisms. This is best illustrated by the last review "Advances in flavonoid research since 1992" focusing on these topics.¹ In contrast, mere distribution studies or chemosystematically oriented compilations are rare (e.g., on Asteraceae).² Naturally, the presently known distribution of flavones and flavonols in plants reflects the current scientific interests, and hence the interpretation of their chemodiversity must be made with caution.

The main part of this compilation consists of extensive tables listing the flavonoids and their plant sources, which are commented accordingly. The data originate primarily from excerpts of current literature, the use of *Chemical Abstracts* and of *Current Contents* (Life Sciences and Agriculture) databases, supported by a review on prenylated flavonoids³ and data taken from the *Handbook of Natural Flavonoids*.⁴ Whenever possible, original literature was consulted to verify structures and their sources. The use of electronically available information lead to inclusion of most recent publications, but the present compilation cannot be claimed to be complete. Apologies go to colleagues whose publications may have been overlooked, and notification on reports that escaped our attention is strongly encouraged.

For compilation and arrangement of compounds, earlier reviews and surveys were taken as the basis.^{5,6} In comparison to the previously published reviews, the increasing number and complexity of structures observed is striking. Thus it became quite difficult to list all of these structures in a logical sequence, particularly prenylated derivatives with additional cyclized substituents. Substitution patterns used for grouping of the flavone and flavonol derivatives are as follows.

OH-, OMe-groups; C-methyl; methylenedioxy groups; C-prenylation; O-prenylation; (dihydro)furanosubstitution; pyranosubstitution; complex cyclosubstitution; aromatic substitution; esterification; chlorination. These residues may also occur combined in one flavonoid structure.

In many cases, abbreviation of substituents could no longer be made without ending up with hardly understandable chemical nomenclature (e.g., complex-O-cyclosubstitution). This problem was already obvious in the publication of Barron and Ibrahim³ who shifted to illustrations of such complex compounds. Consequently, figures of structures showing characteristic substitution patterns will complete the tabulated information provided here.

12.2 ORGANIZATION OF THE TABLES

All tables are organized along the same lines. For the numbering of the basic flavonoid molecule, we refrained to use the system being recommended by the Royal Society of Chemistry, in which primed numbers are mixed in with unprimed numbers. Instead, we still use the more commonly practiced system of most flavonoid scientists: the structures are arranged by number and position, in ascending order, of substituent at ring C being cited first, followed by the substituents in ring A, and then by those in ring B in primed numbers. We also prefer this convention for our tables, for reasons of increased structural information. Thus, for instance, cirsilineol is 5,4'-dihydroxy-6,7,3'-trimethoxyflavone, morin is 3,5,7,2',4'-pentahydroxyflavone, to give two examples. To further increase the value of information, compounds are listed according to increasing numbers of hydroxyl- and methoxyl-groups, and by increasing complexity of other substituents. Further columns inform about trivial names of the compounds, plant sources (species) and families (abbreviated), accumulation sites, and references.

Plant sources are not listed for widespread compounds such as apigenin or kaempferol and some further compounds as had been done previously.⁶ Instead, they are either marked

as “many records,” or, where applicable, as “widespread in Asteraceae” or other larger families. Acknowledged trivial names are cited in column 3, unreasonable names such as “dechloro-chlorapigenin” are omitted. We strictly follow the priority rule when a new trivial name had first been given to a *different* structure. In some cases, widely accepted synonyms are included (e.g., cyclomulberrin/cyclomulberrochromene³).

The listing of accumulation sites forms an essential part of the tables (under the column head “Plant organs”). In particular, attention focuses on the presence of flavonoids in exudates if indicated by the authors (marked with the abbreviation “ext.,” if not clear from a term like “bud exudate”). Critical sources are listings such as “whole plant” which could mean anything from aerial parts to inclusion of roots and flowers. Despite our efforts, we received a single reply only from the authors addressed. Hence the correctness of “whole plant” was confirmed only for two flavonols isolated from *Andrographis viscosula*.⁷ Details on specific accumulation sites and specific accumulation trends will be discussed in the text relating to the respective tables.

For easier navigation through the tabulated data, some further specifics should be pointed out to the reader. In this context, citing “last edition” refers to Wollenweber.³

- Abbreviations such as -OH = hydroxyl, -OMe = methoxy, -Me = methyl are used throughout text and tables. Further abbreviations used to describe the flavonoid structure are explained in a footnote to Table 12.3.
- Basic OH-substituted compounds not (yet) found in nature are noted in brackets, prior to their corresponding methyl derivatives.
- Compounds already listed in previous editions, but without being reported from any new source, are only cited by name.
- Products that have been reported for the first time since compilation of the previous tables are marked with an asterisk. The same applies for new trivial names.
- Names missing in previous editions have been added now (also marked by an asterisk), explaining for literature citations older than the beginning of the reporting period (e.g., Ref. 8).
- In column “Plant species,” these are grouped by families and are listed alphabetically for each flavonoid. For individual compounds of single publications, a maximum of two species per genus is noted; more than two species are abbreviated by “spp.”
- In the same column, flavonoids known so far only in glycosidic combination or in acylated form are marked “Glycoside only” and “Ester only,” respectively. No citations or sources are specified in these cases.
- Abbreviations of family names given in the following column should be generally understandable. Rare family names are written in full.
- Synthesis is noted under the head “Plant organs.” Some recently synthesized compounds that are likely to be found in nature, sooner or later, are cited in brackets, for example, the 7-methyl ether of 6-hydroxygalangin, or 8-*C*-methylapigenin. These are marked “synthesis only.”
- Some previously compiled flavonoids,⁶ which are still only known as synthetic products, have been omitted from the current tables.
- Revised structures are indicated referring to respective publications.

12.3 FLAVONES

A total of 309 entries on the distribution of flavones and their methyl ethers are summarized in Table 12.1. The substitution patterns range from unsubstituted flavone to octa-*O*-substituted flavones. As expected, the number of plant species accumulating these structures is

TABLE 12.1
Flavones and Their Methyl Ethers

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
1	Unsubstituted flavone		Flavone				
Mono-O-substituted flavones							
2	5-OH		Primuletin				
3	(6-OH)	5-OMe					
4	(7-OH)	6-OMe					
5		7-OMe					
6	2'-OH						
7	(3'-OH)	2'-OMe					
8	(4'-OH)	3'-OMe					
9		4'-OMe					
Di-O-substituted flavones							
10	(5,6-diOH)	6-OMe	Chrysin	<i>Anomianthus dulcis</i>	Annonac.	Leaf	131
11		5,6-diOMe		<i>Artemisia campestris</i> spp. <i>glutinosa</i>	Asterac.	Aerial p., ext.	132
12	5,7-diOH			<i>Baccharis viminea</i>	Asterac.	Aerial p., ext.	129
				<i>Baccharis viminea</i>	Asterac.	Aerial p., ext.	133
				<i>Mikania hirsutissima</i>	Asterac.	Aerial parts	134
				<i>Heliotropium pycnophyllum</i>	Boragin.	Aerial p., ext.	36
				<i>Eriodictyon sessilifolium</i>	Hydrophyll.	Leaf resin	135
				<i>Mimulus moschata</i>	Scrophul.	Aerial p., ext.	108
				Propolis from Egypt			136
				European Propolis			137
				Bees Wax			138

13	5-OH	7-OMe	Tectochrysin	<i>Uvaria rufas</i> <i>Baccharis viminea</i> <i>Baccharis viminea</i> <i>Lychnophora markgravii</i> <i>Godmania aesculifolia</i> <i>Heliotropium pycnophyllum</i> <i>Pelargonium crispum</i> <i>Collinsonia canadensis</i> <i>Hoslundia opposita</i> Bees wax <i>Leptospermum scoparium</i>	Annonac. Asterac. Asterac. Asterac. Bignon. Boragin. Geraniac. Lamiac. Lamiac.	Root Aerial p., ext. Aerial p., ext. Aerial parts Aerial p., ext. Aerial p., ext. Leaf exudate Aerial p., ext. Twigs	139 129 133 140 141 36 142 143 144 138 21
14		5,7-diOMe			Myrtac.	Leaf	
15	5,8-diOH		Primetin				
16	5,2'-diOH		—				
17	5-OH (6,7-diOH)	2'-OMe	—				
18	7-OH (6,3'-diOH)	6-OMe*	—				
19	(6,4'-diOH)	6,3'-diOMe					
20	6-OH (7,8-diOH)	4'-OMe		<i>Dalbergia cochinchinensis</i>	Fabac.	Stem	145
21		7,8-diOMe*		Glycoside only			
22	7,4'-diOH			<i>Godmania aesculifolia</i> <i>Dracaena cinnabari</i> <i>Glycyrrhiza eurycarpa</i> <i>Glycyrrhiza pallidiflora</i> <i>Trigonella</i> spp.	Bignon. Agavac. Fabac. Fabac. Fabac.	Leaf Resin Root Root Aerial parts	146 147 17 148 149
23	4'-OH	7-OMe	Isopratol				
24	8,2'-diOH						
25	8-OH	2'-OMe					
26	2',5'-diOH						
27	3',4'-diOH						

continued

44	5,7,2'-triOH	5-OMe*	Echiodinin	<i>Scutellaria planifolia</i>	Lamiac.	Root	55
45	7,2'-diOH	7-OMe		<i>Andrographis alata</i>	Acanthac.	Whole plant	159
46	5,2'-diOH			<i>Andrographis lineata</i>	Acanthac.	Whole plant	14
				<i>Andrographis rothii</i>	Acanthac.	Whole plant	13
47	5-OH	7,2'-diOMe*	Apigenin	<i>Andrographis viscosula</i>	Acanthac.	Whole plant	10
48		5,7,2'-triOMe*		<i>Andrographis rothii</i>	Acanthac.	Whole plant	13
49	5,7,4'-triOH			Many records	Acanthac.	Whole plant	10
50	7,4'-diOH	5-Ome	Thevetiaflavon				
51	5,4'-diOH	7-Ome	Genkwamin	Many records			
52	5,7-diOH	4'-OMe	Acacetin	Many records			
53	7-OH	5,4'-diOMe					
54	5-OH	7,4'-diOMe					
				<i>Artemisia afra</i>	Asterac.	Aerial parts	160
				<i>Artemisia diffusa</i>	Asterac.	Aerial p., ext.	161
				<i>Baccharis trinervis</i>	Asterac.	Leaf	162
				<i>Calea tenuifolia</i>	Asterac.	Aerial parts	163
				<i>Hieracium amplexicaule</i>	Asterac.	Aerial p., ext.	32
				<i>Ophryosporus charrua</i>	Asterac.	Aerial parts	164
				<i>Eucriphia</i> , 6 spp.	Cunoniac.	Leaf, bud, ext.	165
				<i>Marchesinia brachiata</i>	Hepaticae	Thallus	166
				<i>Monoclea gottschei</i>	Hepaticae	Thallus	22
				<i>Cunila angustifolia</i>	Lamiac.	Aerial parts	167
				<i>Dorystoechas hastata</i>	Lamiac.	Aerial p., ext.	168
				<i>Lycopus virginicus</i>	Lamiac.	Aerial parts	169
				<i>Petovskia</i> spp.	Lamiac.	Aerial p., ext.	170
				<i>Salvia sclarea</i>	Lamiac.	Aerial p., ext.	168
				<i>Salvia syriaca</i>	Lamiac.	Aerial parts	171
				<i>Salvia</i> , 3 spp.	Lamiac.	Aerial p., ext.	41
				<i>Sideritis</i> spp.	Lamiac.	Aerial parts	172
				<i>Teucrium marum</i> , <i>T. polium</i>	Lamiac.	Aerial p., ext.	168
				<i>Mirabilis viscosa</i>	Nyctagin.	Aerial p., ext.	43
				<i>Currantia robertiana</i>	Pteridaceae	Fruond exud.	173
				<i>Escallonia pulverulenta</i>	Saxifrag.	Aer. p., res. ex.	42

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
55		5,7,4'-triOMe		<i>Anarrhinum forskalii</i> <i>Antirrhinum</i> , 4 spp.	Scrophul. Scrophul.	Aerial p., ext. Aerial p., ext.	108 108
56	5,8,2'-triOH						
57	7,8,4'-triOH						
58	5,2',5'-triOH (6,2',3'-triOH)			Glycoside only			
59	(7,2',4'-triOH)	6,2',3'-triOMe					
60		7,2',4'-triOMe*		<i>Albizia odoratissima</i>	Mimosac.	Root bark	174
61	7,3',4'-triOH						
62	7,4'-diOH	3'-OMe	Geraldone				
63	7,3'-diOH	4'-OMe*	Farnisin*	<i>Acacia jarnesiana</i>	Mimosac.	Seed	175
64	3'-OH	7,4'-diOMe	Tithonine	<i>Albizia odoratissima</i>	Mimosac.	Root bark	174
65	3'-OH	7,4'-diOMe	Tithonine	<i>Virola michelli</i>	Myristic.	Leaf	176
66	7-OH	3',4'-diOMe*		<i>Launaea asplenifolia</i>	Asterac.	Whole plant? Synthesis	8 176
67	(3',4',5'-triOH)	7,3',4'-triOMe					
		3',4',5'-triOMe*		<i>Primula veris</i>	Primul.	Flower	177
Tetra-O-substituted flavones							
68	(5,6,7,8-tetraOH)			<i>Fissistigma lamuginosum</i> <i>Betula davurica</i>	Amnonac. Betulaceae	Leaf Leaf Synthesis	178 179 24
69	5,7-diOH	6,7-diOMe		<i>Scutellaria repens</i>	Lamiac.	Root	180
70	8-OH	6,8-diOMe*				Synthesis	24
71	5-OH	5,6,7-triOMe*	Alnetin	<i>Godmania aesculifolia</i> <i>Nothofagus cunninghamii</i> <i>Lindera lucida</i>	Bignon. Fagac. Laurac.	Synthesis Aerial p., ext. Aerial p., ext. Twigs	24 141 158 181

72		5,6,7,8-tetraOMe 5,6,7,8-tetraOMe	<i>Godmania aesculifolia</i> <i>Nothofagus cunninghamii</i>	Bignon. Fagac.	Leaf Aerial p., ext.	146 158
73	(5,6,7,2'-tetraOH)	6-OMe	<i>Centaurea jacea</i>	Asterac.	Aerial p., ext.	182
74	5,7,2'-triOH 5,6,7,4'-OH		<i>Duranta plumieri</i>	Verben.	Stem	183
75	5,7,4'-triOH	6-OMe	Many records, mostly from Asteraceae	Asterac.	Aerial p., ext.	133
76	5,6,4'-triOH	7-OMe	<i>Ambrosia ambrosioides</i> <i>Onopordon sibthorpiatum</i> <i>Mentha</i> × <i>piperita</i> <i>Thymus herba barona</i>	Asterac. Lamiac. Lamiac.	Aerial parts Aerial parts Aerial parts	184 185 186
77	5,6,7-triOH	4'-OMe	Many records, mostly from Asteraceae	Asterac.	Aerial parts	187
78	5,4'-diOH	6,7-diOMe	Many records, mostly from Asteraceae	Lamiac.	Aerial parts	187
79	5,7-diOH	6,4'-diOMe	<i>Artemisia argyri</i>	Lamiac.	Aerial parts	185
80	5,6-diOH	7,4'-diOMe	<i>Marrubium trachyticum</i> <i>Mentha</i> × <i>piperita</i> <i>Micromeria albanica</i> <i>Nepeta pungens</i> , <i>N. saturejoides</i> <i>Ocimum</i> , 5 spp. <i>Orthosiphon stamineus</i>	Lamiac. Lamiac. Lamiac. Lamiac. Lamiac. Lamiac.	Aerial parts Aerial parts Aerial parts Leaf surface Leaf surface Aerial parts	188 189 190 191
81	7-OH	5,6,4'-triOMe	<i>Salvia cyanescens</i>	Lamiac.	Aerial parts	192
82	6-OH	5,7,4'-triOMe	<i>Salvia hypoleuca</i> , <i>S. stenophylla</i>	Lamiac.	Aerial p., ext.	41
83	5-OH	6,7,4'-triOMe	<i>Salvia syriaca</i> Glycoside only	Lamiac.	Aerial p., ext.	171
84		5,6,7,4'-tetraOMe 5,6,7,4'-tetraOMe 5,6,7,4'-tetraOMe 5,6,7,4'-tetraOMe	Many records, mostly Asteraceae and Lamiaceae <i>Chromolaena odorata</i> <i>Citrus sinensis</i> <i>Orthosiphon stamineus</i> <i>Ficus altissima</i>	Lamiac. Asterac. Rutac. Lamiac. Morac.	Aerial parts Aerial p., ext. Oil Aerial parts Aerial parts	191 193 16 191 30
85	(5,7,8,2'-tetraOH)	7-OMe				
86	5,8,2'-triOH 5,7,2'-triOH	8-OMe				

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
87	5,2'-diOH	7,8-diOMe	Skullcapflavone I	<i>Andrographis affinis</i> <i>Andrographis elongata</i> <i>Andrographis lineata</i> <i>Andrographis rothii</i> <i>Scutellaria planifolia</i>	Acanthac. Acanthac. Acanthac. Acanthac. Lamiac.	Whole plant Whole plant Whole plant Whole plant Root	157 194 14 13 55
88	5,7-diOH	8,2'-diOMe		<i>Adrographis affinis</i>	Acanthac.	Whole plant	157
89	7-OH	5,8,2'-triOMe		<i>Andrographis echinoides</i>	Acanthac.	Whole plant	195
90	5-OH	7,8,2'-triOMe		<i>Andrographis paniculata</i>	Acanthac.	Whole plant	11
91	5,7,8,4'-tetraOH		Isoscutellarein	<i>Baccharis pilularis</i> <i>Odidia achlaena</i> , <i>O. angusta</i> <i>Ozothamnus scutellifolius</i>	Asterac. Asterac. Asterac.	Aerial p., ext. Aerial p., ext. Aerial p., ext.	196 197 197
92	5,8,4'-triOH	7-OMe		<i>Centaurea chilensis</i>	Asterac.	Leaf + stem	198
93	5,7,4'-triOH	8-OMe	4'-Hydroxy-wogonin	<i>Chrysothamnus nauseosus</i>	Asterac.	Aerial p., ext.	199
				<i>Madia</i> , 3 spp.	Asterac.	Aerial p., ext.	200
				<i>Zinnia acerosa</i>	Asterac.	Aerial p., ext.	133
				<i>Licania densiflora</i>	Chrysobal.	Aerial parts	202
				<i>Scutellaria repens</i>	Lamiac.	Root	180
				<i>Bupleurum scorzonerifolium</i>	Umbellif.	Root	152
				<i>Verbena litoralis</i>	Verben.	Aerial parts	203
94	5,7,8-triOH	4'-OMe	Takakin	<i>Odidia achlaena</i> , <i>O. angusta</i>	Asterac.	Aerial p., ext.	197
	5,7,8-triOH	4'-OMe	Takakin	<i>Ozothamnus scutellifolius</i>	Asterac.	Aerial p., ext.	197
95	5,4'-diOH	7,8-diOMe		<i>Helicteres isora</i>	Sterculiac.	Leaf	204
96	5,8-diOH	7,4'-diOMe*		<i>Chrysothamnus nauseosus</i>	Asterac.	Aerial p., ext.	199
97	5,7-diOH	8,4'-diOMe	Bucegin	<i>Madia sativa</i>	Asterac.	Aerial p., ext.	133
				<i>Madia</i> , 4 spp.	Asterac.	Aerial p., ext.	201
				<i>Zinnia acerosa</i>	Asterac.	Aerial p., ext.	133

98	5-OH	7,8,4'-triOMe		<i>Eucriphia jinksi</i>	Cunoniac.	Leaf, bud, ext.	165
				<i>Calceolaria irazuensis</i>	Scrophul.	Aerial p., ext.	108
				<i>Asterella blumeana</i>	Hepaticae	Thallus	205
				<i>Citrus reticulata</i>	Rutac.	Fruit peel	206
				<i>Calceolaria</i> , 3 spp.	Scrophul.	Aerial p., ext.	207
				<i>Calceolaria irazuensis</i>	Scrophul.	Aerial p., ext.	108
99		5,7,8,4'-tetraOMe		<i>Citrus sinensis</i>	Rutac.	Oil	16
100	5,7,2',5'-tetraOH*			<i>Scutellaria baicalensis</i>	Lamiac.	Root	208
101	5-OH	7,2',5'-triOMe*		<i>Andrographis neesiana</i>	Acanthac.	Whole plant	12
102	(5,6,2',6'-tetraOH)	5,7,2',5'-tetraOMe*		<i>Andrographis rolhii</i>	Acanthac.	Whole plant	13
103	5-OH	6,2',6'-triOMe	Zapotin	<i>Casimiroa tetrameria</i>	Rutac.	Leaf	209
104		5,6,2',6'-tetraOMe	Zapotin				
105	(5,6,3',5'-tetraOH)	5,6,3',5'-tetraOMe	Cerosillin	<i>Casimiroa tetrameria</i>	Rutac.	Leaf	209
106	5,7,2',3'-tetraOH		Norartocarpetin				
107	5,7,2',4'-tetraOH		Artocarpetin				
108	5,2',4'-triOH	7-OMe		<i>Scutellaria planifolia</i>	Lamiac.	Root	55
109	5-OH	7,2',4'-triOMe		<i>Andrographis viscosula</i>	Acanthac.	Whole plant	10
110		5,7,2',4'-tetraOMe		<i>Andrographis elongata</i>	Acanthac.	Whole plant	194
111	5,7,2',6'-tetraOH						
112	5,2',6'-triOH	7-OMe*		<i>Andrographis paniculata</i>	Acanthac.	Whole plant	11
113	5,7,2'-triOH	6'-OMe		Many records			
114	5-OH	7,2',6'-triOMe*					
115	5,7,3',4'-tetraOH		Luteolin	<i>Arnica longifolia</i>	Asterac.	Flower	210
116	7,3',4'-triOH	5-OMe		<i>Artemisia barrelieri</i>	Asterac.	Aerial p., ext.	132
117	5,3',4'-triOH	7-OMe		<i>Dubautia arborea</i>	Asterac.	Leaf exudate	211
				<i>Wunderlichia crulsiana</i>	Asterac.	Aerial parts	212
				<i>Heliotropium stenophyllum</i>	Boragin.	Leaf exudate	213
				<i>Nonea lutea</i> , <i>N. pulla</i>	Boragin.	Aerial p., ext.	36

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
				<i>Eucleraphia lucida</i> , <i>E. jinksii</i>	Cunoniac.	Leaf, bud, ext.	165
				<i>Salvia hypoleuca</i>	Lamiac.	Aerial p., ext.	41
				<i>Salvia sclarea</i>	Lamiac.	Aerial p., ext.	168
				<i>Sideritis</i> spp.	Lamiac.	Aerial parts	172
				<i>Teucrium marum</i>	Lamiac.	Aerial p., ext.	168
				<i>Antirrhinum</i> , 3 spp.	Scrophul.	Aerial p., ext.	108
				<i>Petunia parviflora</i>	Solanac.	Aerial p., ext.	214
				Many records			
118	5,7,4'-triOH	3'-OMe	Chrysoeriol	<i>Artemisia iwayomogi</i> , <i>A. molinieri</i>	Asterac.	Aerial p., ext.	161
119	5,7,3'-triOH	4'-OMe	Diosmetin	<i>Artemisia caerulescens</i>	Asterac.	Aerial p., ext.	132
				<i>Dubautia arborea</i>	Asterac.	Leaf exudate	211
				<i>Eupatorium altissimum</i>	Asterac.	Aerial p., ext.	215
				<i>Ozothamnus scutellifolius</i>	Asterac.	Aerial p., ext.	197
				<i>Tithonia calva</i>	Asterac.	Aerial p., ext.	182
				<i>Nonea rosea</i>	Boragin.	Aerial p., ext.	36
				<i>Aeonium glutinosum</i>	Crassul.	Aerial p., ext.	216
				<i>Cyperus alopecuroides</i>	Cyperac.	Inflo.	217
				<i>Acacia farnesiana</i>	Mimosac.	Seed	175
				<i>Hypericum perforatum</i>	Guttiferae	Callus	218
120	7,4'-diOH	5,3'-diOMe		<i>Phyllospadix japonica</i>	Zosterac.	Whole plant	219
121	7,3'-diOH	5,4'-diOMe*		<i>Artemisia caerulescens</i>	Asterac.	Aerial p., ext.	132
122	5,4'-diOH	7,3'-diOMe	Velutin	<i>Artemisia iwayomog</i>	Asterac.	Aerial p., ext.	161
				<i>Artemisia oliveriana</i>	Asterac.	Aerial p., ext.	220
				<i>Bahia glandulos</i>	Asterac.	Aerial parts	221
				<i>Bracteantha viscosa</i>	Asterac.	Aerial p., ext.	222
				<i>Haploppappus baylahuen</i>	Asterac.	Stem and leaf resin	223
				<i>Helichrysum bracteatum</i>	Asterac.	Aerial p., ext.	32
				<i>Heterotheca pilosa</i>	Asterac.	Aerial p., ext.	182
				<i>Madia sativa</i>	Asterac.	Aerial p., ext.	133
				<i>Senecio viscosa</i>	Asterac.	Aerial p., ext.	32

123	5,3'-diOH	7,4'-diOMe	Piloin	<i>Nonea lutea</i> , <i>N. pulla</i> <i>Eucryphia</i> , 4 spp. <i>Monoclea gottschei</i> <i>Eriodictyon sessilifolium</i> <i>Salvia candidissima</i> <i>Salvia chinopeplica</i> <i>Salvia sclarea</i> <i>Teucrium marum</i> <i>Kitabelia vitifolia</i> <i>Mirabilis viscosa</i> <i>Antirrhinum</i> , 3 spp. <i>Petunia parviflora</i> <i>Sabiglossis sinuata</i> <i>Leihedon tannaensis</i> <i>Lantana montevidensis</i> <i>Artemisia iwaiyomogi</i> <i>Baccharis trinervis</i> <i>Onopordon laconicum</i> <i>Godmania aesculifolia</i> <i>Eucryphia milleganii</i> <i>Lycopus virginicus</i> <i>Notholaena nivea</i> <i>Antirrhinum braun-blauquetii</i> , <i>A. graniticum</i> <i>Calea tenuifolia</i> <i>Chrysothamnus visciflorus</i> <i>Monoclea gottschei</i> , <i>M. forsteri</i> <i>Asarina barkleyana</i> <i>Baccharis trinervis</i> <i>Calea tenuifolia</i> <i>Nonea pulla</i> <i>Eucryphia lucida</i> , <i>E. milleganii</i> <i>Orthosiphon stamineus</i> <i>Sideritis</i> spp. <i>Teucrium botrys</i>	Boragin. Cunoniac. Hepaticae Hydrophyll. Lamiac. Lamiac. Lamiac. Lamiac. Malvac. Nyctagin. Scrophul. Solanac. Solanac. Thymelaeac. Verben. Asterac. Asterac. Asterac. Bignon. Cunoniac. Lamiac. Pteridaceae Scrophul. Asterac. Asterac. Hepaticae Scrophul. Asterac. Asterac. Boragin. Cunoniac. Lamiac. Lamiac. Lamiac.	Aerial p., ext. Leaf, bud, ext. Thallus Leaf resin Aerial parts Leaf Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Leaf Aerial p., ext. Aerial p., ext. Leaf Aerial parts Aerial p., ext. Leaf, bud, ext. Aerial parts Aerial parts Leaf, bud, ext. Aerial parts Aerial parts Aerial p., ext.	36 165 22 135 224 225 168 168 226 43 108 214 214 227 228 161 162 184 141 165 169 45 108 163 199 22 108 162 163 36 165 191 172 168
124	5,7-diOH	3',4'-diOMe					
125	5-OH	7,3',4'-triOMe					

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
126	(6,7,3',4'-tetraOH)	5,7,3',4'-tetraOMe		<i>Kitabelia vitifolia</i>	Malvac.	Aerial p., ext.	226
127	7,3'-diOH	6,4'-diOMe	Abrectorin	<i>Anarrhinum forskalii</i>	Scrophul.	Aerial p., ext.	108
128	(6,7,3',5'-tetraOH)	6,3',5'-triOMe*	Grantionin*	<i>Anirrhinum</i> , 5 spp.	Scrophul.	Aerial p., ext.	108
129	7-OH			<i>Lethedon tannaensis</i>	Thymelaeac.	Leaf	227
130	(7,2',4',5'-tetra-OH)	7,2',4',5'-tetraOMe*		<i>Lantana montevidensis</i>	Verben.	Aerial p., ext.	228
	7,3',4',5'-tetraOH						
	Penta-O-substituted flavones						
131	(5,6,7,8,2'-pentaOH)			<i>Inula grantioides</i>	Asterac.	Aerial parts	34
132	(5,8,2'-triOH)			<i>Calliandra californica</i>	Fabac.	Root	229
133	5,7,2'-triOH						
134	5,2'-diOH	6,7,8-triOMe	Tenaxin 1	<i>Scutellaria baicalensis</i>	Lamiac.	Root	^a
	(5,6,7,8,4'-pentaOH)	6,7-diOMe		<i>Scutellaria repens</i>	Lamiac.	Root	180
	5,8,4'-triOH	6,7-diOMe				Synthesis	24
				<i>Agastache barberi</i>	Lamiac.	Leaf surface	189
				<i>Becium grandiflorum</i>	Lamiac.	Leaf, ext.	230
				<i>Nepeta</i> spp.	Lamiac.	Leaf surface	189
				<i>Ocimum gratissimum</i>	Lamiac.	Aerial parts	231
				<i>Prunus cerasus</i>	Rosac.	Fruit	232
					—	Synthesis	24
				<i>Ambrosia trifida</i>	Asterac.	Aerial p., ext.	233
135	5,7,4'-triOH	6,8-diOMe	Desmethyl-sudachitin	<i>Madia capitata</i>	Asterac.	Aerial p., Ext.	200
				<i>Biebersteinia orphanidis</i>	Bieberstein.	Leaf surface	234
				<i>Scutellaria repens</i>	Lamiac.	Root	180

136	5,6,4'-triOH	7,8-diOMe	Thymusin	<i>Mentha</i> × <i>piperita</i> <i>Nepeta assurgens</i> <i>Origanum</i> × <i>intercedens</i> <i>Thymus herba baron</i> <i>Ocimum americanum</i> var. <i>pilosum</i>	Lamiac. Lamiac. Lamiac. Lamiac. Lamiac.	Aerial parts Leaf surface Glandul. hairs Aerial parts Leaf surface	185 189 235 186 190
137	5,7,8-triOH	6,4'-diOMe*	Pilosin *	<i>Calycadenia truncata</i>	Asterac.	Leaf exudate	236
138	5,6,8-triOH	7,4'-diOMe	Xanthomicrol	<i>Bracteantha viscosa</i>	Asterac.	Aerial p., ext.	222
139	5,6,7-triOH	8,4'-diOMe*		<i>Helichrysum</i> , 8 spp.	Asterac.	Whole plant	237
140	5,4'-diOH	6,7,8-triOMe		<i>Hymenoxis scapoza</i> <i>Varthemia iphionoides</i> <i>Ononis natrix</i> <i>Cunila angustif.</i> , <i>C. incana</i> <i>Dracunculus kotschyi</i> <i>Nepeta</i> , 5 spp.	Asterac. Asterac. Fabac. Lamiac. Lamiac. Lamiac.	Aerial parts Whole plant Aerial parts Aerial parts Leaf surface Leaf surface	238 239 240 167 189 189
141	(5,8-diOH)	6,7,4'-triOMe)	"Pedunculin"	<i>Ocimum gratissimum</i> <i>Satureja montana</i> <i>Thymus herba barona</i> <i>Tithonia</i> , 5 spp.	Lamiac. Lamiac. Lamiac. Asterac.	Aerial parts Aerial p., ext. Aerial parts Vegetative p.	231 241 186 ^b
142	5,7-diOH	6,8,4'-triOMe	Nevadensin	<i>Ocimum</i> , 4 spp. <i>Ambrosia trifida</i> <i>Baccharis grisebachii</i> <i>Madia capitata</i> <i>Tithonia calva</i> <i>Simisia cronquistii</i> <i>Viguiera rosei</i> <i>Biebersteinia orphanidis</i> <i>Ocimum</i> , 5 spp. <i>Rosa centifolia</i> cv. <i>muscosa</i> <i>Tamarix dioica</i>	Lamiac. Asterac. Asterac. Asterac. Asterac. Asterac. Bieberstein. Lamiac. Rosac. Tamaric.	Leaf surface Aerial p., ext. Res. exud. Aerial p., ext. Aerial p., ext. Aerial parts Aerial p., ext. Leaf surface Leaf surface Aerial p., ext. Aerial parts	190 233 242 200 182 243 244 234 190 18 245
143	5,6-diOH	7,8,4'-triOMe	Pebrellin*	<i>Mentha</i> × <i>piperita</i>	Lamiac.	Synthesis Aerial parts Synthesis	24 185 24

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
144	4'-OH	5,6,7,8-tetraOMe*		<i>Nothofagus menziesii</i> <i>Citrus reticulata</i>	Fagac. Rutac.	Aerial p., ext. Peel	158 206
145	7-OH	5,6,8,4'-tetraOMe		<i>Baccharis griesebachii</i>	Asterac.	Only synth.	24
146	6-OH	5,7,8,4'-tetraOMe*		<i>Biebersteinia orphanidis</i>	Bieberstein.	Res. exud.	242
	5-OH	6,7,8,4'-tetraOMe	Gardenin B	<i>Godmania aesculifolia</i>	Bignon.	Leaf surface	234
				<i>Ononis natrix</i>	Fabac.	Aerial p., ext.	141
				<i>Cumila angustifolia</i> , <i>C. fasciculata</i>	Lamiac.	Aerial parts	240
				<i>Nepeta transcaucasica</i>	Lamiac.	Aerial p., ext.	167
				<i>Ocimum</i> , 7 spp.	Lamiac.	Leaf surface	246
				<i>Satureja montana</i>	Lamiac.	Leaf surface	190
				<i>Rosa centifolia</i> cv. <i>muscosa</i>	Lamiac.	Aerial p., ext.	241
				<i>Tamarix dioica</i>	Rosac.	Aerial p., ext.	18
147		5,6,7,8,4'-pentaOMe	Tangeretin	<i>Citrus sinensis</i>	Tamaric.	Aerial parts	245
	(5,6,7,2',4'-pentaOH)	5,6,7,8,4'-pentaOMe	Tangeretin	<i>Citrus "Dancy tangerine"</i>	Rutac.	Fruit peel oil	16
148	5,7,2-triOH	6,4'-diOMe*	Tamaridone*	<i>Tamarix dioica</i>	Rutac.	Leaf	114
149	5,6,7,3',4'-pentaOH	6-Hydroxy-luteolin	6-Hydroxy-luteolin	<i>Tabeaia caraiba</i>	Tamaric.	Aerial parts	245
150	6,7,3',4'-tetraOH	5-OMe*	Carajulflavone*	<i>Arrabidaea chica</i> f. <i>cuprea</i>	Bignon.	Leaf	247
151	5,7,3',4'-tetraOH	6-OMe	Nepetin	Many records, mostly from Asteraceae	Bignon.	Leaf	248
152	5,6,3',4'-tetraOH	7-OMe	Pedalitin	<i>Leiothrix flavescens</i>	Eriocaul.	Capitula	15
153	5,6,7,4'-tetraOH	3'-OMe	Nodifloretin	<i>Monoclea gottschei</i>	Hepaticae	Thallus	22
154	5,6,7,3'-tetraOH	4'-OMe		<i>Mentha pulegium</i>	Hepaticae	Thallus	22
155	7,3',4'-triOH	5,6-diOMe		<i>Salvia blepharophylla</i>	Lamiac.	Leaf surface	249
156	5,3',4'-triOH	6,7-diOMe		<i>Mentha suaveolens</i>	Lamiac.	Leaf	250
157	5,7,4'-triOH	6,3'-diOMe		Many records, mostly Asterac. and Lamiac.	Lamiac.	Leaf surface	249
				Many records, mostly Asterac. and Lamiac.	Lamiac.	Leaf surface	249

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
164	5,7-dihydroxy	6,3',4'-trihydroxymethyl	Eupatilin	<i>Achillea santolina</i>	Asterac.	Veget. parts	261
				<i>Arnica longifolia</i>	Asterac.	Flower	210
				<i>Chromolaena arnottiana</i>	Asterac.	Aerial parts	262
				<i>Eupatorium altissimum</i>	Asterac.	Aerial p., ext.	215
				<i>Mikania minima</i>	Asterac.	Aerial parts	263
				<i>Vernonia saligna</i>	Asterac.	Aerial parts	264
				<i>Mentha × piperita</i>	Lamiac.	Aerial parts	185
				<i>Ocimum</i> , spp.	Lamiac.	Leaf surface	190
				<i>Orthosiphon stamineus</i>	Lamiac.	Aerial parts	191
				<i>Salvia macrosiphon</i> , <i>S. mirzayani</i>	Lamiac.	Aerial p., ext.	41
				<i>Salvia syriaca</i>	Lamiac.	Aerial parts	171
				<i>Sideritis</i> spp.	Lamiac.	Aerial parts	172
				<i>Trichostema lanata</i>	Lamiac.	Aerial p., ext.	41
				<i>Artemisia geraldii</i>	Asterac.	Aerial parts	265
				<i>Artemisia ludoviciana</i> var. <i>mexicana</i>	Asterac.	Aerial parts	266
				<i>Artemisia oliveriana</i>	Asterac.	Aerial p., ext.	220
				<i>Artemisia mongolica</i>	Asterac.	Aerial parts	265
<i>Artemisia umbelliformis</i>	Asterac.	Gland. trich.	267				
<i>Artemisia nitida</i> , <i>A. verlotiorum</i>	Asterac.	Aerial p., ext.	132				
<i>Baccharis gaudichaudiana</i>	Asterac.	Aerial parts	251				
<i>Inula britannica</i>	Asterac.	Aerial p., ext.	196				
<i>Tanacetum polycephalum</i>	Asterac.	Aerial p., ext.	220				
<i>Monoclea gottschei</i>	Hepaticae	Thallus	22				
<i>Salvia sclarea</i>	Lamiac.	Aerial p., ext.	168				
<i>Sideritis</i> spp.	Lamiac.	Aerial parts	172				
<i>Artemisia argyi</i>	Asterac.	Aerial parts	186				
<i>Calamintha nepeta</i>	Lamiac.	Aerial parts	252				
<i>Mentha × piperita</i>	Lamiac.	Aerial parts	185				
<i>Micromeria</i> , 3 spp.	Lamiac.	Aerial parts	252				
<i>Ocimum lamiifolium</i>	Lamiac.	Leaf surface	190				
165	5,6-dihydroxy	7,3',4'-trihydroxymethyl					

166	4'-OH	Ageconylflavon B*	<i>Origanum onites</i>	Lamiac.	Aerial parts	252
167	3'-OH	5,6,7,3'-tetraOMe*	<i>Salvia sclarea</i>	Lamiac.	Aerial p., ext.	168
168	7-OH	5,6,7,4'-tetraOMe	<i>Salvia syriaca</i>	Lamiac.	Aerial parts	171
169	6-OH	5,6,3',4'-tetraOMe	<i>Satureja thymbra</i>	Lamiac.	Aerial parts	252
170	5-OH	5,7,3',4'-tetraOMe	<i>Thymbra capitata, T. spicata</i>	Lamiac.	Aerial parts	252
		6,7,3',4'-tetraOMe	<i>Ageratum conyzoides</i>	Asterac.	Whole plant	268
			<i>Achillea conferta</i>	Asterac.	Aerial parts	269
			<i>Achillea santolina</i>	Asterac.	Aerial parts	53
			<i>Artemisia argyri</i>	Asterac.	Aerial parts	186
			<i>Artemisia austriaca</i>	Asterac.	Aerial parts	270
			<i>Artemisia girdalii</i>	Asterac.	Aerial parts	265
			<i>Artemisia sieversiana</i>	Asterac.	Aerial parts	271
			<i>Artemisia mongolica, A. verlotiorum</i>	Asterac.	Aerial p., ext.	132
			<i>Centaurea macrocephala</i>	Asterac.	Aerial p., ext.	255
			<i>Chromolaena arnottiana</i>	Asterac.	Aerial parts	262
			<i>Eupatorium altissimum</i>	Asterac.	Aerial p., ext.	215
			<i>Lagophylla glandulosa</i>	Asterac.	Leaf exudate	236
			<i>Parthenium incanum</i>	Asterac.	Aerial p., ext.	182
			<i>Cunila angustifolia, C. incana</i>	Lamiac.	Aerial parts	167
			<i>Mentha longifolium</i>	Lamiac.	Aerial parts	272
			<i>Mentha pulegium</i>	Lamiac.	Leaf surface	249
			<i>Micromeria albanica</i>	Lamiac.	Aerial parts	188
			<i>Ocimum americanum var. americanum</i>	Lamiac.	Leaf surface	190
			<i>Orthosiphon stamineus</i>	Lamiac.	Aerial parts	191
			<i>Salvia dominica</i>	Lamiac.	Aerial p., ext.	168
			<i>Salvia macrosiphon, S. mirzayani</i>	Lamiac.	Aerial p., ext.	41
			<i>Salvia syriaca</i>	Lamiac.	Aerial p., ext.	168
			<i>Sideritis</i> spp.	Lamiac.	Aerial parts	172
			<i>Teucrium abyssifolium</i>	Lamiac.	Aerial parts	257
			<i>Teucrium botrys</i>	Lamiac.	Aerial p., ext.	168
			<i>Teucrium pseudochamaepitys</i>	Lamiac.	Aerial parts	273

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
171		5,6,7,3',4'-pentaOMe	Sinensetin	<i>Ziziphora hispanica</i> <i>Citrus "Dancy tangerine"</i> <i>Chromolaena odorata</i> <i>Conoclinium coelestinum</i> <i>Eupatorium coelestinum</i> <i>Orthosiphon stamineus</i> <i>Citrus sinensis</i> <i>Citrus "Dancy tangerine"</i>	Lamiac. Rutac. Asterac. Asterac. Asterac. Lamiac. Rutac. Rutac.	Aerial p., ext. Leaf Aerial p., ext. Aerial p., ext. Aerial parts Aerial parts Fruit peel oil Leaf	170 114 193 215 274 191 16 114
172	(5,7,8,2',3'-pentaOH)	7,8-diOMe	(Norwightin)	Glycoside only			
173	5,2',3'-triOH	7,8,2'-triOMe	Wightin	<i>Mentha longifolia</i>	Lamiaceae	Aerial parts	272
174	5,3'-diOH	7,8,2',3'-tetraOMe					
175	5-OH	7,8,2',4'-tetraOMe					
176	(5,7,8,2',5'-pentaOH)	7,8-diOMe	Rehderianin I	<i>Andrographis affinis</i>		Whole plant	157
177	5,2',5'-triOH	7,8,2',5'-tetraOMe*					
178	5-OH	7,8,2',6'-tetraOMe		<i>Scutellaria baicalensis</i>	Lamiac.	Root	208
179	(5,7,8,2',6'-pentaOH)	7,8-diOMe					
180	5,2',6'-triOH	8,6'-diOMe					
181	5,2'-diOH	7,8,6'-triOMe					
182	5,7,-diOH	8,2',6'-triOMe					
183	5-OH	7,8,2',6'-tetraOMe					
184	5,7,8,3',4'-pentaOH	8-OMe		<i>Licania pyrifolia</i> <i>Centaurea chilensis</i> <i>Madiia</i> , 4 spp. <i>Onopordium laconicum</i> <i>Viguetia decurrens</i>	Chrysobal. Asterac. Asterac. Asterac. Asterac.	Aerial parts Leaf + stem Aerial p., ext. Aerial parts Aerial p., ext.	202 198 200 184 244
185	5,7,8,4'-tetraOH	3'-OMe		Glycoside only			
186	5,7,8,3'-tetraOH	4'-OMe		Glycoside only			

187	5,7,4'-triOH	8,3'-diOMe	Isosinensetin	<i>Conyza</i> spp.	Asterac.	Resin. exud.	42
188	8,3'-diOH	5,7,4'-triOMe*		<i>Hemizonia lutescens</i>	Asterac.	Aerial p., ext.	133
189	5,7'-diOH	8,3',4'-triOMe		<i>Verbena littoralis</i>	Verben.	Aerial parts	203
190	5-OH	7,8,3',4'-tetraOMe		<i>Cowanita mexicana</i> var. <i>Stansburiana</i>	Rosac.	Aerial p., ext.	19
191		5,7,8,3',4'-pentaOMe		<i>Cowanita mexicana</i> var. <i>Stansburiana</i>	Rosac.	Aerial p., ext.	19
				<i>Citrus sinensis</i>	Rutac.	Fruit peel oil	16
				<i>Ficus altissima</i>	Morac.	Aerial parts	30
192	(5,7,8,3',5'-penta-OH)	8,3',5'triOMe*		<i>Linnophila rugosa</i>	Scrophul.	Aerial parts	275
193	(5,6,2',3',4'-penta-OH)	5,6,2',3',4'-pe-OMe*		<i>Casimiroa tetrameria</i>	Rutac.	Leaf	209
194	(5,6,2',3',6'-penta-OH)	5,6,2',3',6'-pe-OMe		<i>Casimiroa tetrameria</i>	Rutac.	Leaf	209
195	(5,6,2',4',5'-penta-OH)	6,2'-diOMe*		<i>Teucrium quadrifarium</i>	Lamiac.	?	276
196	(5,6,3',4',5'-penta-OH)						
197	(5,7,2',3',4'-penta-OH)*	5,6,3',4',5'-pentaOMe	Cerosillin B				
198	5,7,2',4',5'-pentaOH	5,7,2',3',4'-penta-OMe*		<i>Andrographis lineata</i>	Acanthac.	Whole plant	14
199	5-OH	7,2',4',5'-tetraOMe		Glycoside only	Asterac.	Aerial parts	277
				<i>Artemisia campestris</i> ssp. <i>glutinosa</i>	Fabac.	Root	229
				<i>Calliandra californica</i>			
200	(5,7,2',4',6'-pentaOH)	5,7,2',4',6'-pentaOMe*		<i>Andrographis viscosula</i>	Acanthac.	Whole plant	10
201	5,7,3',4',5'-pentaOH			<i>Eucalyptus globulus</i>	Myrtac.	Pollen	20
				<i>Kunzea ericoides</i>	Myrtac.	Pollen	20
				<i>Leptospermum scoparium</i>	Myrtac.	Pollen	20
				<i>Metrosideros excelsa</i>	Myrtac.	Pollen	20
				<i>Metrosideros umbellata</i>	Myrtac.	Pollen	20
202	5,7,4',5'-tetraOH	3'-OMe	Selgin	<i>Artemisia caerulescens</i>	Asterac.	Aerial p., ext.	132
203	5,7,3',5'-tetraOH	4'-OMe		<i>Nonea lutea</i> , <i>N. pulla</i>	Borragin.	Aerial p., ext.	36
204	5,7,5'-triOH	3',4'-diOMe	Apometzgerin	<i>Asarina procumbens</i>	Scrophul.	Aerial p., ext.	278
				<i>Nonea pulla</i>	Borragin.	Aerial p., ext.	36
				<i>Asarina procumbens</i>	Scrophul.	Aerial p., ext.	278

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
205	5,7,4'-triOH	3',5'-diOMe	Tricin	<i>Epimedium brevicornum</i> <i>Agelaea pentagyna</i> <i>Castilleja fissifolia</i> <i>Xerophyta retinervis</i>	Berberid. Conmarac. Scrophul. Velloziac.	Aerial parts Leaf Aerial p., ext. Leaf	279 280 278 105
206	5,5'-diOH	7,3',4'-triOMe*	Lethedocin*	<i>Lethedon tannaensis</i>	Thymelaeac.	Leaf	227
207	5,4'-diOH	7,3',5'-triOMe*	7-Methyl-tricin*	<i>Centaurea incana</i> <i>Betonica officinalis</i> (= <i>Stachys</i>) <i>Lethedon tannaensis</i>	Asterac. Lamiac. Thymelaeac.	Aerial parts Aerial parts Leaf	281 282 227
208	5,7-diOH	3',4',5'-triOMe		<i>Nonoa pulla</i> <i>Asarina procumbens</i>	Boragin. Scrophul.	Aerial p., ext. Aerial p., ext.	36 108
209	5-OH	7,3',4',5'-tetraOMe	Corymbosin	<i>Centaurea incana</i> <i>Walsura piscidia</i>	Asterac. Meliac.	Aerial parts Aerial parts	281 283
210		5,7,3',4',5'-pentaOMe		<i>Ficus maxima</i> <i>Murraya paniculata</i> <i>Neoraputia paraensis</i> <i>Neoraputia paraensis</i>	Morac. Rutac. Rutac. Rutac.	Leaf Leaf Aerial parts Fruit	77 284 285 286
211	(5,8,3',4',5'-pentaOH)* 5,5'-diOH	8,3',4'-triOMe*		<i>Artemisia giraldii</i>	Asterac.	Aerial parts	287
212	(6,7,3',4',5'-pentaOH)	3',5'-diOMe*		<i>Artemisia giraldii</i>	Asterac.	Aerial parts	287
213	6,7,4'-triOH	3',4',5'-triOMe	Prosogerin E				
214	6,7-diOH	6,3',4',5'-tetraOMe	Prosogerin D				
215	7-OH	6,7,3',4',5'-pentaOMe	Prosogerin C				
	—						
	Hexa-O-substituted flavones						
216	(5,6,7,8,2',4'-hexaOH)	6,7,8-triOMe*	Tamadone*	<i>Tamarix dioica</i>	Tamariac.	Aerial parts	245
217	5,2',4'-triOH	6,7,8-triOMe					
218	(5,6,7,8,2',5'-hexaOH) 5,2',5'-triOH (5,6,7,8,2',6'-hexaOH) 5,6,2',6'-tetraOH	7,8-diOMe					

219	5,2',6'-triOH	6,7,8-triOMe	Skullcapflavon II	<i>Leptotrix flavescens</i>	Eriocaul.	Capitula	15
220	5,6,2'-triOH	7,8,6'-triOMe		Glycoside only	Lamiac.	Leaf surface	189
221	5,2'-diOH	6,7,8,6'-tetraOMe		<i>Nepeta</i> , 6 spp.	Asterac.	Whole plant	237
222	5,6,7,8,3',4'-hexaOH*	6-OMe		<i>Helichrysum</i> , 8 spp.	Asterac.	Whole plant	237
223	5,7,8,3',4'-pentaOH	8-OMe		<i>Madia</i> , 3 spp.	Asterac.	Aerial p., ext.	200
224	5,6,7,3',4'-pentaOH	6,7-diOMe*		<i>Mentha spicata</i>	Lamiac.	Leaf	288
225	5,8,3',4'-tetraOH	6,8-diOMe		<i>Thymus herba barona</i>	Lamiac.	Aerial parts	186
226	5,7,3',4'-tetraOH	6,8-diOMe		<i>Mentha longifolia</i>	Lamiac.	Aerial parts	272
227	5,6,7,8-tetraOH	3',4'-diOMe		<i>Tithonia calva</i>	Asterac.	Aerial p., ext.	182
228	5,3',4'-triOH	6,7,8-triOMe	Sideritiflavon	<i>Viguiera rosei</i>	Asterac.	Aerial p., ext.	244
229	5,8,4'triOH	6,7,3'triOMe	Sudachitin	<i>Biebersteinia orphanidis</i>	Bieberstein.	Leaf surface	234
230	5,7,4'-triOH	6,8,3'-triOMe		<i>Calycadenia</i> , 3 spp.	Asterac.	Leaf exudate	236
231	5,7,3'-triOH	6,8,4'-triOMe	Acerosin	<i>Biebersteinia orphanidis</i>	Bieberstein.	Leaf surface	234
232	5,6,4'-triOH	7,8,3'-triOMe	Thymonin	<i>Madia capitata</i>	Asterac.	Aerial p., ext.	200
				<i>Acinos alpinus</i> , <i>Ac. suaveolens</i>	Lamiac.	Aerial parts	252
				<i>Calamintha nepeta</i> , <i>C. sylvatica</i>	Lamiac.	Aerial parts	252
				<i>Mentha</i> , 3 spp.	Lamiac.	Aerial parts	252
				<i>Mentha</i> × <i>piperita</i>	Lamiac.	Aerial parts	185
				<i>Mentha spicata</i>	Lamiac.	Leaf	288
				<i>Micromeria</i> , 3 spp.	Lamiac.	Aerial parts	252
				<i>Origanum</i> , 9 spp.	Lamiac.	Aerial parts	252
				<i>Origanum</i> × <i>intercedens</i>	Lamiac.	Glandul. hairs	235
				<i>Satureja salzmamii</i>	Lamiac.	Aerial parts	252
				<i>Thymus</i> spp.	Lamiac.	Aerial parts	252
				<i>Vernonia saligna</i>	Asterac.	Aerial parts	264
233	8,3'-diOH	5,6,7,4'-tetraOMe*		<i>Calycadenia multiglandulosa</i>	Asterac.	Leaf exudate	236
234	5,4'-diOH	6,7,8,3'-tetraOMe		<i>Madia dissitiflora</i>	Asterac.	Aerial p., ext.	200
				<i>Cleome droserifolia</i>	Capparid.	Aerial parts	289

continued

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
235	5,3'-diOH	6,7,8,4'-tetraOMe	Gardenin D	<i>Cunila angustifolia</i>	Lamiac.	Aerial parts	167
236	5,7-diOH	6,8,3',4'-tetraOMe	Hymenoxin	<i>Micromeria albanica</i> <i>Thymus herba barona</i> <i>Calycadenia truncata</i> , <i>C. villosa</i> <i>Tithonia calva</i> <i>Viguiera rosei</i>	Lamiac. Lamiac. Lamiac. Asterac. Asterac.	Aerial parts Aerial parts Aerial parts Leaf exudate Aerial p., ext.	188 186 236 182
237	5,6-diOH	7,8,3',4'-tetraOMe		<i>Biebersteinia orphanidis</i> <i>Ononis natrix</i> <i>Ocimum × citriodorum</i> <i>Citrus "Dancy tangerine"</i> <i>Mentha spica</i> <i>Micromeria albanica</i> <i>Origanum onites</i> <i>Satureja salzmannii</i> <i>Thymus membranaceus</i>	Asterac. Fabac. Lamiac. Rutac. Lamiac. Lamiac. Lamiac. Lamiac. Lamiac.	Leaf surface Aerial parts Leaf surface Leaf Leaf Aerial parts Aerial parts Aerial parts Aerial parts	244 234 240 190 114 288 188 290 290 290
238	4'-OH	5,6,7,8,3'-pentaOMe		<i>Cunila incana</i>	Lamiac.	Aerial parts	167
239	3'-OH	5,6,7,8,4'-pentaOMe		<i>Mentha spicata</i>	Lamiac.	Leaf	288
240	5-OH	6,7,8,3',4'-pentaOMe	5-Desmethyl-nobiletin	<i>Micromeria albanica</i> <i>Ocimum americanum</i> var. <i>americanum</i> <i>Satureja montana</i> <i>Citrus sinensis</i> <i>Citrus "Dancy tangerine"</i> <i>Murraya paniculata</i>	Lamiac. Lamiac. Lamiac. Lamiac. Rutac. Rutac. Rutac.	Aerial parts Leaf surface Aerial p., ext. Fruit peel oil Leaf Leaf	188 190 241 16 114 291
241		5,6,7,8,3',4'-hexaOMe	Nobiletin	<i>Antirrhinum graniticum</i> <i>Conoclinium greggii</i> <i>Ozothamnus lycopodioides</i> <i>Viguiera rosei</i> <i>Citrus sinensis</i> <i>Citrus "Dancy tangerine"</i>	Scrophul. Asterac. Asterac. Asterac. Rutac. Rutac.	Aerial p., ext. Aerial parts Aerial p., ext. Fruit peel oil Leaf Leaf	108 292 69 244 16 114

TABLE 12.1
Flavones and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
268	(5,7,8,2',4',5'-hexaOH)	8,5'-diOMe					
269	5,7,2',4'-tetraOH (5,7,8,2',5',6'-hexaOH)	8,6'-diOMe					
270	5,7,2',5'-tetraOH (5,7,8,3',4',5'-hexaOH)	8-OMe					
271	5,7,3',4',5'-pentaOH	8,4'-diOMe					
272	5,7,3',5'-tetraOH	8,3',4'-triOMe					
273	5,7,5'-triOH	7,8,4',5'-tetraOMe					
274	5,3'-diOH	8,3',4',5'-tetraOMe					
275	5,7-dihOH	5,7,8,3',4',5'-hexaOMe		<i>Bracteantha viscosa</i>	Asterac.	Aerial p., ext.	222
276	(5,6,2',3',4',6'-hexaOH)	5,6,2',3',4',6'-hexaOMe		<i>Casimiroa tetrameria</i>	Rutac.	Leaf	209
277	(5,6,2',3',5',6'-hexOH)	5,6,2',3',5',6'-hexaOMe		<i>Casimiroa tetrameria</i>	Rutac.	Leaf	209
278	(5,7,2',3',4',5'-hexaOH)	2',3',5'-triOMe*		<i>Psidium punctulata</i>	Asterac.	Leaf	295
279	5,7,4'-triOH	2',4',5'-triOMe*		<i>Psidium arabica</i>	Asterac.	Aerial parts	9
280	5,7,3'-triOH	7,2',3',5'-tetraOMe*		<i>Psidium punctulata</i>	Asterac.	Leaf	295
281	5,4'-diOH	2',3',4',5'-tetraOMe*		<i>Psidium punctulata</i>	Asterac.	Leaf	295
282	5,7-dihOH	7,2',4',5'-tetraOMe					
283	5,3'-diOH	7,2',3',4',5'-pentaOMe		<i>Psidium punctulata</i>	Asterac.	Leaf	295
284	5-OH Hepta-O-substituted flavones (5,6,7,8,2',3',6'-heptaOH)	6,8,2'-triOMe*		<i>Scutellaria planipes</i>	Scrophul.	Root	55
285	5,7,3',6'-tetraOH	6,8,5'-triOMe					
286	(5,6,7,8,2',4',5'-heptaOH)	6,7,8,4'-tetraOMe					
287	5,7,2',4'-tetraOH	6,7,8,5'-tetraOMe					
288	5,2',5'-triOH 5,2',4'-triOH 2',4'-diOH	5,6,7,8,5'-pentaOMe	Agecorynnin-D	<i>Ononis natrix</i>	Fabac.	Aerial p., ext.	278

growing. Since the last compilation,⁶ some eight compounds (scut-6-Me, scut-6,7-diMe, scut-6,4'-diMe, scut-6,7,4'-triMe; lut-3'-Me, 6-OH-lut-6-Me, 6-OH-lut-6,7-diMe, and 6-OH-lut-6,3'-diMe) fall in the category of "widespread" in addition to apigenin, genkwanin (apigenin-7-Me), acacetin (ap-4'-Me), and luteolin. Thus, no specific sources have been listed for these compounds.

The number of newly described structures increased by about 50 entries during the reporting period. These include a series of 2'- and 5'-substituted flavones, which have been reported from several Asteraceae such as from leaves of *Psiadia punctulata*.⁹ The genus *Andrographis* (Acanthaceae) yielded several of these more complex substituted flavones, isolated from whole plants.¹⁰⁻¹⁴ In contrast to other reports on *Andrographis*,⁷ the meaning of "whole plant" could not be clarified, with root tissue probably included in the analysis as well. Since all *Andrographis* species are annuals, inclusion of root tissue probably has little influence on the flavonoid composition. Capitula of *Leiosthrix flavescens* (Eriocaulaceae) yielded a new flavone with a rare 5,6,7,8,3',4'-hexahydroxy substitution (compound 264 in Table 12.1).¹⁵ This is remarkable insofar as many of the listed flavones are (poly)methoxy derivatives and other hexahydroxyflavones are known as glycosides only.

Most of the source reports concern equally the families of the Asteraceae and Lamiaceae, followed by Rutaceae. However, it must be taken into consideration that the long list may rather be due to the number of species and not to the number of genera. The large number of results in both families may also be due to the research focus on these groups by the authors. In these families, flavone accumulation is mostly reported in leaves, aerial parts and in exudates. Species of the genus *Scutellaria* (Lamiaceae) form an exception, with analyses concentrating on roots since those are used pharmaceutically. According to the distribution of flavones in aerial parts and leaves in other Lamiaceae, similar results should also be expected from *Scutellaria*. Genera of the Rutaceae accumulate flavones primarily in aerial parts and leaves. Many of these compounds, however, were found in fruit peels of *Citrus*, particularly those with higher methylation patterns (e.g., compound 194, Table 12.1¹⁶). None of the reports, however, indicate possible external accumulation on vegetative tissue of Rutaceae.

Reports on other families are much lower in number. In Fabaceae and Mimosaceae, flavones and their methyl ethers are described from all parts of the plants, including roots of, for example, *Glycyrrhiza eurycarpa*, which primarily accumulates a series of prenylated derivatives.¹⁷ None of the reports cited here indicate external occurrence. In Rosaceae, only few reports exist on the external accumulation of higher methylated flavone aglycones.^{18,19} The occurrence of tricetin was proved for the pollen of several genera from the Myrtaceae,²⁰ with only one report concerning accumulation in leaves.²¹ Very few reports exist on families such as Solanaceae and Moraceae. This is quite in contrast to the large number of reports on prenylated flavones accumulated particularly in the Moraceae. Of the nonflowering plants, only few reports relate to the fronds of ferns. Within the mosses, apparently only the thalli of Hepaticae yielded flavones and their methyl ethers.²²

Some flavone structures have been revised during the reporting period. The structure of 5,8,2'-triOH-6,7-diOMe flavone (compound 131 in Table 12.1) had been ascribed to a product isolated from *Scutellaria baicalensis*.²³ After synthesis, it needs to be revised to 5,7,2'-triOH-6,8-diOMe flavone (compound 132, Table 12.1).²⁴ Pedunculin, earlier isolated from *Tithonia* species and claimed as 5,8-diOH-6,7,4'-triOMe-flavone (compound 141, Table 12.1),²⁵ needs to be revised, after synthesis, to 5,7-diOH-6,8,4'-triOMe flavone = nevadensin (compound 142 in Table 12.1).²⁴ In the previous review, the compound 5,6,7,4'-tetraOH-3',5'-diOMe had erroneously been cited as a component of *Artemisia assoana*.⁶ Data have now been included for the correct structure, 5,7,4'-triOH-6,3',5'-triOMe flavone (compound 251 in Table 12.1).²⁶ A further flavone reported from *Ageratum conyzoides* (compound 263 in

Table 12.1) as 5,6,8,3',4',5'-hexamethoxyflavone,²⁷ was revised to 5,6,7,3',4',5'-hexaOMe flavone (compound 261 in Table 12.1) after synthesis.²⁸

12.4 FLAVONOLS

Some 393 reports on flavonols and their distribution are listed in Table 12.2. During the reporting period, the number of new sources also increased, leading to reduction of listings in the very widespread compounds kaempferol, kaempferol-3-methyl ether, quercetin, and quercetin-3-methyl ether. About 54 compounds are reported as new structures, a number equaling that of the flavones. These include a series of polymethoxylated derivatives from species of the Asteraceae, where they are reported to occur in aerial parts as well as in leaf exudates. Species from the Rutaceae accumulate highly methoxylated flavonols in leaves as well as in fruit peels, whereas species of *Fabaceae* were found to accumulate such compounds mainly in the heartwood. A hexamethoxylated flavonol (compound 369 in Table 12.2) was isolated from *Distemonanthus benthamianus* (Fabaceae),²⁹ a species also known for accumulation of complex cycloflavonols (Table 12.4). Of the Moraceae, only one report concerns the genus *Ficus*, which produces another hexamethoxylated flavonol (compound 279 in Table 12.2) in the aerial parts.³⁰ The same applies to accumulation of flavonol aglycones in roots of *Duroia hirsuta* (Rubiaceae).³¹ The number of 2'- and 5'-substituted derivatives appears to be lower than that of the corresponding flavones.

Most of the new source reports concern species from the Asteraceae, with many of the flavonols being isolated from aerial parts, where they are accumulated externally. They range from simple to more complex structures. There appears to be a tendency towards 6-methoxylation rather than towards 8-methoxylation, in addition to possible OMe-substitution of other positions of the flavonol molecule. Flavonols with 6,8-di-*O*-methylation and additional OMe-groups are also found in several genera such as *Senecio*,³² *Psiadia*,³³ or *Imula*,³⁴ to cite but a few examples.

Aerial parts, fruits, flowers, and bark tissue of a series of Rutaceae species yielded a number of hexamethoxylated flavonols. Once more, the complexity of metabolic pathways in this family is demonstrated by the formation of such compounds. The number of entries for this family is the second largest following the Asteraceae, but it must be taken into account that only a few genera of this large family are concerned. The third largest group concerns *Heliotropium* species of the family Boraginaceae, where particularly leaf exudates yielded flavonols.^{35,36} For species of *Alkanna*, flavonols were reported for aerial parts without indicating possible external occurrence.³⁷ Interestingly, almost no flavones were reported from *Heliotropium* (see Table 12.1), and species of the genus *Nonea* were so far found to accumulate flavones only in their exudates.³⁶ Further distribution studies will have to confirm the possible chemosystematic value of these accumulation trends.

A number of new listings concern the families of Scrophulariaceae and Solanaceae. In both cases the number of reports concerning external accumulation is also increased. Thus, further research will probably reveal that this phenomenon is more widespread in these families as is obvious from the present data. In Fabaceae, most reports concern accumulation in heartwood, with a few exceptions such as leaves of *Millettia racemosa*.³⁸ However, no indication to possible external accumulation is made. Similar to flavone accumulation data, pollen of Myrtaceae were also found to accumulate flavonols.²⁰ Very few reports exist on Gymnosperms such as *Cryptomeria* (Taxodiaceae)³⁹ or *Ephedra*,⁴⁰ without indication of external accumulation. So far, no new reports on flavones are known for these taxa.

In contrast to the numerous reports on flavones in Lamiaceae, only very few genera were found to accumulate flavonols in their exudates. The accumulation of 5,6-di-*O*-methylated derivatives in species of *Salvia*⁴¹ may be of chemosystematic significance, in relation to other

TABLE 12.2
Flavonols and Their Methyl Ethers

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
	Di-O-substituted flavonols						
1	3,7-diOH			<i>Pongamia pinnata</i>	Fabac.	Root bark	297
2	(3,4'-diOH)	3,7-diOMe*					
3	3-OH	4'-OMe					
	Tri-O-substituted flavonols						
4	3,5,7-triOH		Galangin	<i>Baccharis viminea</i> <i>Cassinia quinquefaria</i> <i>Flourensia cernua</i> <i>Gnaphalium microcephalum</i> <i>Helichrysum</i> , 8 spec. <i>Helichrysum aureum</i> <i>Helichrysum aureonitens</i> <i>Heterothalamus psidioides</i> <i>Odtzia</i> , 6 spp. <i>Heliotropium filifolium</i> <i>Millettia racemosa</i> <i>Nothofagus alexsandri</i> <i>Nothofagus antarctica</i> <i>Nothofagus</i> , 6 spp. <i>Ribes viscosissimum</i> <i>Woodisia scopulina</i> Bees wax Propolis from Arizona European Propolis Propolis from Chile Propolis from Egypt <i>Helichrysum aureum</i> <i>Helichrysum picardii</i> <i>Lychmophora markgravi</i>	Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Boragin. Fabac. Fagac. Fagac. Fagac. Grossulat. Pteridac.	Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial parts Leaf Aerial p., ext. Leaf exudate Leaf Leaf Aerial p., ext. Aerial p., ext. Leaf exudate Fronds, ext.?	129 222 182 298 237 32 299 300 197 35 38 301 302 158 303 304 138 305 137 306 136 32 307 140
5	5,7-diOH	3-OMe			Asterac. Asterac. Asterac.	Aerial p., ext. Aerial p. (ext.) Aerial parts	32 307 140

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
Tetra-<i>O</i>-substituted flavonols							
13	3,5,6,7-tetraOH		6-Hydroxy-galangin	<i>Adenostoma sparsifolium</i> <i>Cassinia quinquefaria</i> <i>Platanus acerifolia</i>	Rosac. Asterac. Platanac. Asterac.	Aerial p., ext. Aerial p., ext. Bud Aerial p., ext.	19 222 a 298
14	3,5,7-triOH	6-OMe	Almusin	<i>Anaphalis margaritacea</i> <i>Cassinia quinquefaria</i> <i>Gnaphalium microcephalum</i> <i>Adenostoma sparsifolium</i>	Asterac. Asterac. Asterac. Rosac.	Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext.	222 298 298 19
15	(3,5,6-triOH	7-OMe)				Synth. only	313
16	5,7-diOH	3,6-diOMe		<i>Gomphrena boliviana</i> , <i>G. martiana</i> <i>Anaphalis margaritacea</i> <i>Gnaphalium microcephalum</i> <i>Helichrysum</i> , 8 spp. <i>Pseudognaphalium cheiranthifolium</i>	Amaranth. Asterac. Asterac. Asterac. Asterac.	Whole plant Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial parts	150 298 298 237 308
17	5,6-diOH	3,7-diOMe		<i>Gnaphalium affine</i>	Asterac.	Aerial parts	314
18	3,7-diOH	5,6-diOMe		<i>Salvia columbariae</i>	Lamiac.	Aerial p., ext.	41
19	3,7-diOH	5,6-diOMe		<i>Trichostema lanatum</i>	Lamiac.	Aerial p., ext.	41
20	3,6-diOH	5,7-diOMe					
21	3,5-diOH	6,7-diOMe					
22	5-OH	3,6,7-triOMe	Almustin			Synthesis	47
23	3,5,7,8-tetraOH	3,5,6,7-tetraOMe	8-Hydroxy-galangin				
24	5,7,8-triOH	3-OMe		<i>Nothofagus</i> , 3 spp.	Fagac.	Synthesis	47
25	5,7,8-triOH	3-OMe		<i>Ozothamnus</i> , 3 spp.	Asterac.	Aerial p., ext.	158
25	3,5,8-triOH	7-OMe		<i>Ozothamnus leafolius</i>	Asterac.	Aerial p., ext.	197
26	3,5,7-triOH	8-OMe		<i>Helichrysum aureum</i> <i>Ozothamnus expansifolius</i> <i>Nothofagus antarctica</i> <i>Nothofagus alessandri</i> <i>Nothofagus</i> , 7 spp.	Asterac. Asterac. Fagac. Fagac. Fagac.	Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Leaf Aerial p., ext.	32 197 302 301 158

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
42	3,5,7-triOH	4'-OMe	Kaempferide	<i>Heliotropium chenopodiaceum</i> var. <i>ericoideum</i>	Boragin.	Leaf exudate	35
				<i>Aeonium leucoblepharum</i> , <i>Ae.nobile</i>	Crassul.	Aerial p., ext.	216
				<i>Nothofagus cunninghamii</i>	Fagac.	Aerial p., ext.	158
				<i>Aniba</i> sp.	Laurac.	Wood, bark	316
				<i>Mirabilis viscosa</i>	Nyctagin.	Aerial p., ext.	43
				<i>Notholaena nivea</i>	Pteridac.	Fronnd exud.	45
				<i>Calceolaria irazuensis</i>	Scrophul.	Aerial p., ext.	108
				<i>Mimulus cardinalis</i>	Scrophul.	Aerial p., ext.	108
				<i>Solanum paludosum</i>	Solanac.	Aerial parts	317
				<i>Viscum cruciatum</i>	Viscac.	Cuticular wax	318
				<i>Baccharis pilularis</i>	Asterac.	Aerial p., ext.	133
				<i>Baccharis vinea</i>	Asterac.	Aerial p., ext.	129
				<i>Chrysothamnus nauseosus</i>	Asterac.	Aerial p., ext.	133
				<i>Ozothamnus scutellifolius</i>	Asterac.	Aerial p., ext.	197
				<i>Eucryphia jinksii</i>	Cunoniac.	Leaf, bud, ext.	165
				<i>Nothofagus menziesii</i> , <i>N. nervosa</i>	Fagac.	Aerial p., Ext.	158
				<i>Eriodictyon sessilifolium</i>	Hydrophyll.	Leaf resin	135
				<i>Mirabilis viscosa</i>	Nyctagin.	Aerial p., ext.	43
				<i>Currantia robertiana</i>	Pteridac.	Fronnd exud.	173
<i>Calceolaria irazuensis</i>	Scrophul.	Aerial p., ext.	108				
Brazilian propolis			319				
Propolis from Chile			306				
<i>Chrysothamnus nauseosus</i>			199				
<i>Achillea ageratum</i>			320				
<i>Alkanna orientalis</i>			37				
<i>Heliotropium chenopodiaceum</i> var. <i>ericoideum</i>			35				
<i>Heliotropium pycnophyllum</i>			36				
<i>Cleome spinosa</i>			321				
<i>Aeonium</i> spp.			216				
<i>Eucryphia lucida</i>			165				

	<i>Nothofagus cuminghamii</i>			Fagac.	Aerial p., ext.	158
	<i>Pelargonium fulgidum</i>			Geraniac.	Leaf exudate	142
	<i>Salvia cyaneascens</i>			Lamiac.	Aerial parts	192
	<i>Mirabilis viscosa</i>			Nyctagin.	Aerial p., ext.	43
	<i>Bosistoia brassii</i>			Rutac.	Leaf	322
	<i>Evodia merrillii</i>			Rutac.	Fruit	323
	<i>Calceolaria arachnoidea</i>			Scrophul.	Aerial p., ext.	108
	<i>Chamaesaracha sordida</i>			Solanac.	Aerial p., ext.	214
	<i>Solanum paludosum</i>			Solanac.	Aerial parts	317
	<i>Viscum album</i>			Viscac.	Aerial p., ext.	44
	<i>Viscum cruciatum</i>			Viscac.	Cut. wax	318
	<i>Amomum koenigii</i>			Zingib.	Fruit	324
	<i>Aeonium</i> spp.		Ermanin	Boragin.	Aerial parts	37
	<i>Eucryphia lucida</i>			Crassul.	Aerial p., ext.	216
	<i>Nothofagus menziesii</i> , <i>N. nervosa</i>			Cunoniac.	Leaf, bud, ext.	165
	<i>Fouquieria splendens</i>			Fagac.	Aerial p., ext.	158
	<i>Mirabilis viscosa</i>			Fouquieriac.	Aerial p., ext.	325
	<i>Carrania robertiana</i>			Nyctagin.	Aerial p., ext.	43
	<i>Notholaena nivea</i>			Pteridac.	Frond exud.	173
	<i>Barosma crenulata</i>			Pteridac.	Frond exud.	45
	<i>Petunia surfinia</i>			Rutac.	Aerial p., ext.	326
	<i>Viscum album</i>			Solanac.	Aerial p., ext.	214
	Brazilian Propolis			Viscac.	Cut. wax	318
	<i>Amomum koenigii</i>			Zingib.	Fruit	319
	<i>Flourensia cernua</i>	5,4'-diOMe*		Asterac.	Aerial p., ext.	324
	<i>Haplopappus hirtellus</i>	7,4'-diOMe		Asterac.	Aerial parts	182
	<i>Madia elegans</i>			Asterac.	Aerial p., ext.	327
	<i>Ozothamnus scutellifolius</i>			Asterac.	Aerial p., ext.	201
	<i>Serratula strangulata</i>			Asterac.	Whole plant	197
	<i>Stevia subpubescens</i>			Asterac.	Aerial p., ext.	328
	<i>Heliotropium stenophyllum</i>			Boragin.	Aerial p., ext.	182
	<i>Cleome spinosa</i>			Capparid.	Aerial p., ext.	36
	<i>Aeonium sedifolium</i>			Crassul.	Aerial p., ext.	321
45		5,7-diOH				216
46		3,7-diOH				
47		3,5-diOH				

continued

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
48	4'-OH	3,5,7-triOMe		<i>Salvia chinopeplica</i>	Lamiac.	Leaf	225
49	5-OH	3,7,4'-triOMe		<i>Aniba</i> spp. <i>Mirabilis viscosa</i> <i>Notholaena nivea</i> <i>Calceolaria mexicana</i>	Laurac. Nyctagin. Pteridac. Scrophul.	Wood, bark Aerial p., ext. Frond exud. Aerial p., ext.	316 43 45 108
				<i>Artemisia rupestris</i>	Asterac.	Aerial parts	132
				<i>Baccharis pilularis</i>	Asterac.	Aerial p., ext.	129
				<i>Grindelia nana</i>	Asterac.	Aerial p., ext.	133
				<i>Grindelia tenella</i>	Asterac.	Aerial p., ext.	182
				<i>Haplopappus hirtellus</i>	Asterac.	Aerial parts	327
				<i>Haplopappus sonorensis</i>	Asterac.	Aerial parts	329
				<i>Ozothamnus scutellifolius</i>	Asterac.	Aerial p., ext.	197
				<i>Senectio viscosa</i>	Asterac.	Aerial p., ext.	32
				<i>Stevia subpubescens</i>	Asterac.	Aerial p., ext.	182
				<i>Xanthocephalum gymnosperm.</i>	Asterac.	Aerial p., ext.	182
				<i>Heliotropium pycnophyllum</i>	Boragin.	Aerial p., ext.	36
				<i>Cleome spinosa</i>	Capparid.	Aerial p., ext.	321
				<i>Aeonium goochia</i> , <i>Ae. Lindleyi</i>	Crassul.	Aerial p., ext.	216
				<i>Eucryphia lucida</i> , <i>E. milliganii</i>	Cunoniac.	Leaf, bud, ext.	165
				<i>Nothofagus cunninghamii</i>	Fagac.	Aerial p., ext.	158
				<i>Dorystoechas hastata</i>	Lamiac.	Aerial parts	168
				<i>Aniba</i> spp.	Laurac.	Wood and bark	316
				<i>Mirabilis viscosa</i>	Nyctagin.	Aerial p., ext.	43
				<i>Currantia robertiana</i>	Pteridac.	Frond exud.	173
				<i>Calceolaria chelidonioides</i>	Scrophul.	Aerial p., ext.	108
				<i>Cryptomeria japonica</i>	Taxodiac.	Leaf	39
				<i>Annonum koenigii</i>	Zingib.	Fruit	324
				<i>Annonum koenigii</i>	Zingib.	Fruit	324
50							
51	3,5,8,4'-tetraOH (3,6,7,4'-tetraOH)	3,5,7,4'-tetraOMe*	Pratoletin				

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
71	3,5,7,4'-tetraOH	6-OMe		<i>Ambrosia artemisiifolia</i> <i>Ageratina espinosa</i> <i>Carthamus tinctorius</i> <i>Centaurea incana</i> <i>Chrysactinia mexicana</i> <i>Eupatorium altissimum</i> , <i>E. serotinum</i> <i>Heteranthemis viscidiflora</i> <i>Xanthium strumarium</i> <i>Aeonium</i> , 3 spp. <i>Adenostoma sparsifolium</i> Brazilian propolis	Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Crassul. Rosac.	Aerial p., ext. Aerial p., ext. Petal (nat.?) Aerial parts Aerial p., ext. Aerial p., ext. Leaf exudate Aerial p., ext. Aerial p., ext. Aerial p., ext.	182 133 339 281 182 215 340 32 216 19 341
72	3,5,6,4'-tetraOH	7-OMe		Glycoside only		Synthesis	342
73	3,5,6,7-tetraOH	4'-OMe		<i>Achillea micrantha</i>	Asterac.	Synthesis	313
74	5,7,4'-triOH	3,6-diOMe		<i>Ageratina espinosa</i> <i>Ambrosia chamissonis</i> <i>Brickellia eupatorioides</i> <i>Calycadenia multiglandulosa</i> , <i>C. villosa</i> <i>Centaurea</i> , 4 spp. <i>Eupatorium altissimum</i> <i>Flourensia cernua</i> <i>Grindelia robusta</i> <i>Grindelia squarrosa</i> <i>Heteranthemis viscidiflora</i> <i>Heterotheca villosa</i> <i>Oncosiphon grandiflorum</i> <i>Perityle lemmonii</i> <i>Psiadia dentata</i> <i>Stevia berlandieri</i>	Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac.	Aerial parts Aerial p., ext. Aerial p., ext. Aerial p., ext. Leaf exudate Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Leaf exudate Aerial p., ext. Leaf exudate Aerial p., ext. Leaf exudate Aerial p., ext. Aerial p., ext. Aerial p., ext. Leaf Aerial p., ext.	47 343 133 233 215 236 182 215 182 344 182 340 182 32 133 345 182

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
				<i>Grindelia robusta</i>	Asterac.	Leaf exudate	344
				<i>Lagophylla glandulosa</i>	Asterac.	Leaf exudate	236
				<i>Oncosiphon grandiflorum</i>	Asterac.	Aerial p., ext.	32
				<i>Tanacetum polycephalum</i>	Asterac.	Aerial p., ext.	220
				<i>Alkanna orientalis</i>	Boragin.	Aerial parts	37
				<i>Aconium</i> , 3 spp.	Crassul.	Aerial p., ext.	216
				<i>Trixis vauhierii</i>	Asterac.	Leaf	353
83	5,7-diOH	3,6,4'-triOMe	Santin	<i>Achillea latiloba</i>	Asterac.	Aerial p., ext.	354
				<i>Achillea atrata</i> ssp. <i>multifida</i>	Asterac.	Aerial parts	355
				<i>Achillea multifida</i>	Asterac.	Aerial p., ext.	196
				<i>Ageratina espinosa</i>	Asterac.	Aerial p., ext.	133
				<i>Anthemis tinctoria</i>	Asterac.	Aerial p., ext.	356
				<i>Artemisia barrelieri</i>	Asterac.	Aerial parts	132
				<i>Brickellia eupatorioides</i>	Asterac.	Aerial p., ext.	215
				<i>Eupatorium cannabinum</i>	Asterac.	Aerial p., ext.	216
				<i>Grindelia tarapacana</i>	Asterac.	Aerial p., ext.	298
				<i>Grindelia glutinosa</i>	Asterac.	Leaf exudate	344
				<i>Grindelia squarrosa</i>	Asterac.	Leaf exudate	182
				<i>Perityle lemmonii</i>	Asterac.	Aerial p., ext.	133
				<i>Stevia berlandieri</i>	Asterac.	Aerial p., ext.	182
				<i>Tanacetum microphyllum</i>	Asterac.	Aerial parts	357
				<i>Aconium</i> , 3 spp.	Crassul.	Aerial p., ext.	216
				<i>Drummondita hassellii</i>	Rutac.	Aerial parts	358
				<i>Pulicaria dysenterica</i>	Asterac.	External	347
				<i>Tanacetum parthenium</i>	Asterac.	Aerial parts	^b
84	5,6-diOH	3,7,4'-triOMe	"Tanetin"				
85	3,4'-diOH	5,6,7-triOMe	Candidol	<i>Baccharis pilularis</i>	Asterac.	Aerial p., ext.	196
86	3,5-diOH	6,7,4'-triOMe	Mikanin	<i>Achillea sibirica</i> subsp. <i>mongolica</i>	Asterac.	Aerial p., ext.	354
87	5-OH	3,6,7,4'-tetraOMe		<i>Ageratina espinosa</i>	Asterac.	Aerial p., ext.	133
				<i>Brickellia eupatorioides</i>	Asterac.	Aerial p., ext.	215

88	(3,5,6,8,4'-pentaOH) (5,4'-diOH)	3,6,8-triOMe)	"Candiron"	<i>Tephrosia candida</i>	Fabac.	Leaf exudate	344
89	(3,5,7,8,2'-pentaOH)	7-OMe	—	Ester only		Aerial p., ext.	182
90	3,5,8,2'-tetraOH	3,7,8-triOMe				Aerial p., ext.	216
91	5,2'-diOH	3,7,8,2'-tetraOMe				Aerial parts	358
92	5-OH						
93	3,5,7,8,4'-pentaOH	3-Ome	Herbacetin	<i>Ozothamnus hookeri</i>	Asterac.	Aerial p., ext.	197
94	5,7,8,4'-tetraOH			<i>Baccharis pilularis</i>	Asterac.	Aerial p., ext.	196
95	3,5,8,4'-tetraOH	7-OMe		<i>Ozothamnus</i> , 3 spp.	Asterac.	Aerial p., ext.	197
96	3,5,7,4'-tetraOH	8-OMe	Pollenitin	<i>Ephedra aphylla</i>	Ephedrac.	Aerial parts	40
97	3,5,7,8-tetraOH	4'-OMe	Sexangularetin	<i>Ozothamnus</i> , 3 spp.	Asterac.	Aerial p., ext.	197
98	3,7-diOMe	3,7-diOMe		<i>Pentagramma triangularis</i>	Pteridac.	Frond exudate	359
	5,8,4'-triOH	3,8-diOMe		<i>Ozothamnus hookeri</i>	Asterac.	Aerial p., ext.	197
	5,7,4'-triOH			<i>Brachyglottis cassinoidea</i>	Asterac.	Leaf exudate	360
				<i>Chrysothamnus nauseosus</i>	Asterac.	Aerial p., ext.	199
				<i>Haplopappus deserticola</i>	Asterac.	Resin. exud.	361
				<i>Ozothamnus</i> , 3 spp.	Asterac.	Aerial p., ext.	197
				<i>Cleome spinosa</i>	Capparid.	Aerial p., ext.	321
				<i>Calceolaria arachnoidea</i>	Scrophul.	Aerial p., ext.	108
99	5,7,8-triOH	3,4'-diOMe*		<i>Pityrogramma triangularis</i>	Pteridac.	Frond exud.	362
100	3,5,4'-triOH	7,8-diOMe		<i>Ozothamnus expansifolius</i> , <i>O. obcordatus</i>	Asterac.	Aerial p., ext.	197
101	3,5,8-triOH	7,4'-diOMe				Synthesis	313
102	3,5,7-triOH	8,4'-diOMe	Prudomestin	<i>Calceolaria irazuensis</i>	Scrophul.	Aerial p., ext.	108
103	5,4'-diOH	3,7,8-triOMe		<i>Ozothamnus</i> , 4 spp.	Asterac.	Aerial p., ext.	197
				<i>Cleome spinosa</i>	Capparid.	Aerial p., ext.	321
				<i>Calceolaria chelidonioides</i> , <i>C. triparita</i>	Scrophul.	Aerial p., ext.	108
104	5,8-diOH	3,7,4'triMe					
105	5,7-diOH	3,8,4'-triOMe		<i>Ozothamnus obcordatus</i>	Asterac.	Aerial p., ext.	197
				<i>Haplopappus deserticola</i>	Asterac.	Resin. exud.	361

continued

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	Ome-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
106	3,5-diOH	7,8,4'-triOMe	Tambulin	<i>Helichrysum foetidum</i> <i>Nothofagus</i> , 3 spp. <i>Curatania roberitiana</i> <i>Calceolaria chelidonioides</i> <i>Helianthus annuus</i> <i>Ozothamnus expansifolius</i> , <i>O. obcordatus</i> <i>Drummondita calida</i> <i>Calceolaria irazuensis</i> <i>Helichrysum foetidum</i> <i>Ozothamnus</i> , 4 spp. <i>Cleome spinosa</i> <i>Nothofagus menziesii</i> , <i>N. nervosa</i> <i>Drummondita calida</i>	Asterac. Fagac. Pteridac. Scrophul. Asterac. Asterac. Rutac. Scrophul. Asterac. Asterac. Capparid. Fagac. Rutac.	Aerial p., ext. Aerial p., ext. Frond exud. Aerial p., ext. Leaf Aerial p., ext. Aerial parts Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext.	182 158 173 108 363 197 358 108 182 197 321 158 358
107	5-OH	3,7,8,4'-tetraOMe	Flindulatin				
108	(3,6,7,8,4'-pentaOH)	3,5,7,8,4'-pentaOMe*					
109	-	3,6,7,8,4'-pentaOMe	Auranetin				
110	3,5,7,2',4'-pentaOH		Morin	<i>Milletia racemosa</i>	Fabac.	Leaf	38
111	5,7-diOH	3,2',4'-triOMe					
112	(3,5,7,2',5'-pentaOH)	7,5'-diOMe	Viscidulin I	<i>Blumea balsamifera</i>	Asterac.	Aerial parts	364
113	3,5,2'-triOH						
114	3,5,7,2',6'-pentaOH	3,5,7,2',6'-pentaOMe					
115	3,5,7,3',4'-pentaOH		Quercetin	Many records Many records			
116	5,7,3',4'-tetraOH	3-OMe					
117	3,7,3',4'-tetraOH	5-OMe	Azaleatin				
118	3,5,3',4'-tetraOH	7-OMe	Rhamnetin	<i>Artemisia campestris</i> ssp. <i>glutinosa</i> <i>Baccharis pilularis</i> <i>Baccharis pilularis</i> <i>Cassinia vauvilliersii</i> <i>Chromolaena odorata</i> <i>Chrysothamnus nauseosus</i>	Asterac. Asterac. Asterac. Asterac. Asterac. Asterac.	Aerial parts Aerial p., ext. Aerial p., ext. Aerial parts Aerial p., ext. Aerial p., ext. Aerial p., ext.	132 129 133 365 193 133

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
121	7,3',4'-triOH	3,5-diOMe	Caryatin	<i>Artemisia</i> spp.	Asterac.	Aerial parts	132
122	5,3',4'-triOH	3,7-diOMe		<i>Baccharis pilularis</i>	Asterac.	Aerial p., ext.	133
			<i>Chrysothamnus viscidiflorus</i>	Asterac.	Aerial p., ext.		199
			<i>Eirnocephala megaphylla</i>	Asterac.	Aerial parts		369
			<i>Flourensia cernua</i>	Asterac.	Aerial p., ext.		182
			<i>Grindelia tarapacana</i>	Asterac.	Aerial p., ext.		298
			<i>Haplopappus taeda</i>	Asterac.	Stems		370
			<i>Holocarpus</i> , 3 spp.	Asterac.	Leaf resin		371
			<i>Ozothamnus lycopodioides</i> , <i>O. scutellifolius</i>	Asterac.	Aerial p., ext.		197
			<i>Palafoxia sphacelata</i>	Asterac.	Aerial p., ext.		182
			<i>Siegesbeckia jorulensis</i> , <i>S. orientalis</i>	Asterac.	Aerial p., ext.		182
			<i>Heliotropium pycophyllum</i> , <i>H. stenophyllum</i>	Boragin.	Aerial p., ext.		36
			<i>Aeonium</i> spp.	Crassul.	Aerial p., ext.		216
			<i>Eucriphia milligani</i> , <i>E. moorei</i>	Cunoniac.	Leaf, bud, ext.		165
			<i>Pelargonium fulgidum</i> , <i>P. quercifolium</i>	Geraniac.	Leaf exudate		142
			<i>Mirabilis viscosa</i>	Nyctagin.	Aerial p., ext.		43
			<i>Rubus phoenicolastus</i>	Rosac.	Aerial p., ext.		18
			<i>Calceolaria</i> , 4 spp.	Scrophul.	Aerial p., ext.		108
			<i>Petunia surfinia</i>	Solanac.	Aerial p., ext.		214
			<i>Salpiglossis simata</i>	Solanac.	Aerial p., ext.		214
			<i>Lantana camara</i>	Verbenac.	Aerial p., ext.		228
			<i>Viscum album</i>	Viscac.	Aerial p., ext.		44
			<i>Viscum cruciatum</i>	Viscac.	Cut. wax		318
			<i>Anarthria scabra</i>	Anarthriac.	Leaf		554
123	5,7,4'-triOH	3,3'-diOMe	<i>Heliotropium sinuatum</i>	Boragin.	Leaf exudate		310
			<i>Heliotropium stenophyllum</i>	Boragin.	Aerial p., ext.		36
			<i>Cleome amphyocarpa</i>	Capparid.	Exudate		372
			<i>Eucriphia lucida</i>	Cunoniac.	Leaf, bud, ext.		165
			<i>Cyperus alopecuroides</i>	Cyperac.	Aerial parts		217

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
128	3,5,3'-triOH	7,4'-diOMe	Ombuin	<i>Capparis tweediana</i> <i>Aeonium</i> , 3 spp. <i>Eucryphia jinksii</i> <i>Nothofagus cunninghamii</i> <i>Kitabelia vitifolia</i> <i>Mirabilis viscosa</i> <i>Polygonum punctatum</i> <i>Notholaena nivea</i> <i>Salpiglossis sinuata</i> <i>Viscum cruciatum</i> Propolis from Arizona <i>Chromolaena odorata</i> <i>Annonum koenigii</i> <i>Chromolaena odorata</i>	Capparid. Crassul. Cunoniac. Fagac. Malvac. Nyctagin. Polygon. Pteridaceae Solamac. Viscac.	Leaf Aerial p., ext. Leaf, bud, ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial parts Frond exud. Aerial p., ext. Cut. wax	311 216 165 158 226 43 368 45 214 318 305
129	3,5,7-triOH	3,4'-diOMe	Dillenetin	<i>Chrysothamnus viscidiflorus</i> <i>Flourensia cernua</i> <i>Grindelia robusta</i> <i>Grindelia tarapacana</i> <i>Grindelia squarrosa</i> <i>Heterotheca villosa</i> <i>Senecio viscosa</i> <i>Senecio viscosissimus</i> <i>Xanthocephalum gymnospermoides</i> <i>Heliotropium sinuatum</i> <i>Nothofagus cunninghamii</i> <i>Mirabilis viscosa</i> <i>Bosistoa floydii</i> , <i>B. medicinalis</i> <i>Melicope elleryana</i> <i>Melicope ternata</i>	Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Boragin. Fagac. Nyctagin. Rutac. Rutac. Rutac.	Aerial p., ext. Aerial p., ext. Leaf exudate Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. External Aerial p., ext. Leaf exudate Leaf exudate Aerial p., ext. Aerial p., ext. Leaf Fruit Bark	199 182 344 352 182 182 32 378 182 310 158 43 322 379 380
130	7,4'-diOH	3,5,3'-triOMe					
131	5,4'-diOH	3,7,3'-triOMe	Pachypodol				

132	5,3'-diOH	3,3',4'-triOMe	Ayanin	<i>Euodia merrillii</i> <i>Euodia viticina</i> <i>Adenosma capitatum</i> <i>Mimulus lewisii</i> <i>Petunia surfina</i> <i>Salpiglossis sinuata</i> <i>Viscum album</i> <i>Viscum cruciatum</i> <i>Bahia glandulosa</i> <i>Grindelia squarrosa, tenella</i> <i>Haplopappus hirtellus</i> <i>Ozothamnus scutellifolius</i> <i>Pisadia dentata</i> <i>Siegesbeckia jorullensis, orientalis</i> <i>Stevia subpubescens</i> <i>Heliotropium chenopodiaceum</i> var. <i>ericoides</i> <i>Heliotropium pycnophyllum</i> <i>Eucryphia lucida, E. milliganii</i> <i>Petunia surfina</i> <i>Lantana camara</i> <i>Anomum koenigii</i> <i>Flourensia cernua</i> <i>Grindelia nana</i> <i>Grindelia robusta</i> <i>Siegesbeckia jorullensis</i> <i>Eucryphia lucida, E. milliganii</i> <i>Petunia surfina</i> <i>Barbacia rubro-virens</i> <i>Anomum koenigii</i> <i>Chrysothamnus viscidiflorus</i> <i>Chromolaena odorata</i> <i>Aeonium arboreum</i> <i>Kitabelia vitifolia</i>	Rutac. Rutac. Scrophul. Scrophul. Solamac. Solamac. Viscac. Viscac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Boragin. Boragin. Cunoniac. Solamac. Verbenac. Zingib. Asterac. Asterac. Asterac. Asterac. Cunoniac. Scrophul. Solamac. Velloziac. Zingib. Asterac. Asterac. Crassul. Malvac.	Fruit Fruit External? Leaf exudate Aerial p., ext. Aerial p., ext. Aerial p., ext. Cut. wax Aerial parts Aerial p., ext. Aerial parts Aerial p., ext. Leaf Aerial p., ext. Aerial p., ext. Exudate Aerial p., ext. Leaf, bud, ext. Aerial p., ext. Aerial p., ext. Fruit Aerial p., ext. Aerial p., ext. Leaf exudate Aerial p., ext. Leaf, bud, ext. Leaf exudate Aerial p., ext. Leaf surface Fruit Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Fruit Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext.	323 381 382 375 214 214 44 318 221 182 327 197 345 182 182 35 36 165 214 228 324 182 133 344 182 165 375 214 105 324 199 193 216 226
133	5,7-diOH	3,3',4'-triOMe					
134	3,7-diOH	5,3',4'-triOMe*					
135	3,5-diOH	7,3',4'-triOMe					

continued

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
136	4'-OH	3,5,7,3'-tetraOMe		<i>Mirabilis viscosa</i> <i>Amonum koenigii</i>	Nyctagin. Zingib.	Aerial p., ext. Fruit	43 324
137	5-OH	3,7,3',4'-tetraOMe	Retusin	<i>Distemonanthus benthamianus</i> <i>Artemisia rupestris</i> <i>Brickellia eupatorioides</i> <i>Grindelia nana</i> <i>Grindelia tenella</i> <i>Stiegesbeckia jorullensis</i> , <i>S. orientalis</i> <i>Urolepis hecatantha</i> <i>Xanthocephalum gymnospermoides</i> <i>Aeonium lindleyi</i> <i>Eucryphia lucida</i> , <i>E. milliganii</i> <i>Bridelia ferruginea</i> <i>Nothofagus cunninghamii</i> <i>Mirabilis viscosa</i> <i>Evodia merrillii</i> <i>Solanum plusiodum</i> <i>Cryptomeria japonica</i> <i>Amonum koenigii</i>	Fabac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Asterac. Crassul. Cunoniac. Euphorb. Fagac. Nyctagin. Rutac. Solanac. Taxodiac. Zingib.	Heartwood Aerial parts Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial p., ext. Aerial parts Aerial p., ext. Aerial p., ext. Leaf, bud, ext. Stem bark Aerial p., ext. Aerial p., ext. Leaf, bud, ext. Stem bark Aerial p., ext. Fruit Aerial parts Leaf Fruit	383 132 215 133 182 182 262 182 216 165 384 158 43 323 317 39 324
138	3-OH	5,7,3',4'-tetraOMe		<i>Amonum koenigii</i>	Zingib.	Fruit	324
139	(3,5,7,3',5'-pentaOH)	3,5,7,3',4'-pentaOMe					
140	3,5,7-triOH	3',5'-diOMe	Morelosin				
141	3,6,7,3',4'-pentaOH		Rhynchosin				
142	6,7,3',4'-tetraOH	3-OMe*		<i>Graziella mollissima</i>	Asterac.	Aerial parts	330
143	3,6,3',4'-tetraOH	7-OMe*		<i>Dalbergia odorifera</i>	Fabac.	Heartwood	333
144	(7-OH)	3,6,3',4'-tetraOMe)	"Santoflavone"	<i>Achillea santolina</i>	Asterac.	Aerial parts	d 332
145	3,7,8,3',4'-pentaOH		Melanoxetin	<i>Acacia karroo</i> , <i>A. montana</i>	Mimosac.	Heartwood	332
146	7,8,3',4'-tetraOH	3-OMe	Transilitin	<i>Acacia nigrescens</i>	Mimosac.	Heartwood	385

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
167		3,5,6,7,8,4'-hexaOMe*		<i>Drummondia calida</i>	Rutac.	Aerial parts Synthesis	358 336
168	(3,5,6,7,2',3'-hexaOH) 5,2',3'-triOH	3,6,7-triOMe*		<i>Vitex rotundifolia</i>	Verbenac.	Fruit	388
169	(3,5,6,7,2',4'-hexaOH) 5,4'-diOH	3,6,7,2'-tetraOMe	Chrysozolin				
170	5-OH	3,6,7,2',5'OMe*	Grantioidin				
171	3,5,6,7,3',4'-hexaOH		Quercetagenin				
172	5,6,7,3',4'-pentaOH	3-OMe					
173	3,6,7,3',4'-pentaOH	5-OMe	“Allopatuletin”	<i>Tagetes patula</i>	Asterac.	Synthesis	47
174	3,5,7,3',4'-pentaOH	6-OMe	Patuletin	<i>Anthemis tinctoria</i>	Asterac.	Synthesis	28 ^e
				<i>Artemisia barrelieri</i>	Asterac.	Petals	389
				<i>Centaurea incana</i>	Asterac.	Flower	132
				<i>Chrysactinia mexicana</i>	Asterac.	Aerial parts	281
				<i>Eriophyllum confertum</i>	Asterac.	Aerial p., ext.	182
				<i>Pallenia spinosa</i>	Asterac.	Aerial p., ext.	133
					Asterac.	Aerial parts	390
					Asterac.	Synthesis	342
					Asterac.	Synthesis	47
					Asterac.	Synthesis	47
					Asterac.	Synthesis	47
175	3,5,6,3',4'-pentaOH	7-OMe			Asterac.	Aerial p., ext.	233
176	3,5,6,7,4'-pentaOH	3'-OMe			Asterac.	Aerial parts	132
177	3,5,6,7,3'-pentaOH	4'-OMe			Asterac.	Aerial p., ext.	32
178	5,7,3',4'-tetraOH	3,6-diOMe	Axillarin	<i>Ambrosia chamissonis</i>	Asterac.	Aerial p., ext.	391
				<i>Artemisia australis</i>	Asterac.	Aerial parts	236
				<i>Asteriscus sericeus</i>	Asterac.	Aerial p., ext.	133
				<i>Bahia pringlei</i>	Asterac.	Aerial parts	
				<i>Calycadenia</i> , 3 spp.	Asterac.	Leaf exudate	
				<i>Eriophyllum confertum</i>	Asterac.	Aerial p., ext.	
				<i>Eriophyllum staechadifolium</i>	Asterac.	Aerial p., ext.	133
				<i>Gymnosperma glutinosa</i>	Asterac.	Aerial p., ext.	196

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
186	5,7,4'-triOH	3,6,3'-triOMe	Jaceidin	<i>Inula britannica</i>	Asterac.	Aerial p., ext.	32
				<i>Inula germanica</i>	Asterac.	Aerial p., ext.	32
				<i>Madia sativa</i>	Asterac.	Leaf exudate	236
				<i>Oncosiphon grandiflorum</i>	Asterac.	Aerial p., ext.	32
				<i>Pulicaria gnaphalodes</i>	Asterac.	Aerial p., ext.	220
				<i>Tanacetum polycephalum</i>	Asterac.	Aerial p., ext.	220
				<i>Rosa centifolia</i> cv. <i>muscosa</i>	Rosac.	Aerial p., ext.	18
				<i>Achillea clusiana</i>	Asterac.	Aerial p., ext.	196
				<i>Achillea micrantha</i>	Asterac.	Aerial parts	343
				<i>Asteriscus graveolens</i>	Asterac.	Aerial parts	111
				<i>Asteriscus sericeus</i>	Asterac.	Aerial p., ext.	32
				<i>Bahia pringlei</i>	Asterac.	Aerial parts	391
				<i>Centaurea</i> , 3 spp.	Asterac.	Aerial p., ext.	182
				<i>Eriophyllum staechadifolium</i>	Asterac.	Aerial p., ext.	133
				<i>Eupatorium buniifolium</i>	Asterac.	Aerial parts	393
				<i>Flourensia cernua</i>	Asterac.	Aerial p., ext.	182
187	5,7,3'-triOH	3,6,4'-triOMe	Centaureidin	<i>Inula britannica</i>	Asterac.	Aerial p., ext.	196
				<i>Lagophylla glandulosa</i>	Asterac.	Leaf exudate	236
				<i>Pulicaria gnaphalodes</i>	Asterac.	Aerial p., ext.	220
				<i>Tanacetum parthenium</i>	Asterac.	Leaf	49
				<i>Alkanna orientalis</i>	Boragin.	Aerial parts	37
				<i>Mirabilis viscosa</i>	Nyctagin.	Aerial p., ext.	43
				<i>Melicope coodeana</i>	Rutac.	Leaf	374
				<i>Achillea atrata</i> ssp. <i>multifida</i>	Asterac.	Aerial parts	355
				<i>Achillea multifida</i>	Asterac.	Aerial p., ext.	196
				<i>Ambrosia chamissonis</i>	Asterac.	Aerial p., ext.	233
				<i>Artemisia tinctoria</i>	Asterac.	Aerial p., ext.	356
<i>Artemisia abrotanum</i>	Asterac.	Aerial parts	392				
<i>Artemisia barrelieri</i>	Asterac.	Aerial parts	132				
<i>Baccharis saligna</i>	Asterac.	Aerial parts	394				

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
196	5,3'-diOH	3,6,7,4'-tetraOMe	Casticin	<i>Achillea sibirica</i> subsp. <i>mongolica</i> <i>Artemisia abrotanum</i> <i>Lagophylla glandulosa</i> <i>Parthenium incanum</i> <i>Tanacetum polycephalum</i>	Asterac. Asterac. Asterac. Asterac. Asterac.	Aerial p., ext. Aerial parts Leaf exudate Aerial p., ext. Aerial parts	354 392 236 182 220
197	5,7-diOH	3,6,3',4'-tetraOMe	Bonanzin	<i>Vitex rotundifolia</i>	Verbenac.	Fruit	388
198	5,6-diOH	3,7,3',4'-tetraOMe		<i>Bahia xylopoda</i>	Asterac.	Aerial parts	391
199	3,3'-diOH	5,6,7,4'-tetraOMe	Eupatoretin	<i>Pulicaria dysenterica</i>	Asterac.	Aerial p., ext.	32
200	3,5'-diOH	6,7,3',4'-tetraOMe		<i>Artemisia annua</i>	Asterac.	Aerial parts	397
201	3,5'-diOH	6,7,3',4'-tetraOMe		<i>Baccharis saligna</i>	Asterac.	Aerial parts	394
202	4'-OH	3,5,6,7,3'-pentaOMe		<i>Parthenium incanum</i>	Asterac.	Aerial p., ext.	182
203	7-OH	3,5,6,3',4'-pentaOMe*		<i>Pallemis spinosa</i>	Asterac.	Aerial parts	390
204	5-OH	3,6,7,3',4'-pentaOMe	Artemetin	<i>Vigua spiralis</i> <i>Citrus sinensis</i> <i>Achillea conferta</i> <i>Achillea sibirica</i> subsp. <i>mongolica</i> <i>Artemisia annua</i>	Fabac. Rutac. Asterac. Asterac. Asterac.	Leaf and stem Fruit peel oil Aerial parts Aerial p., ext. Aerial parts	398 16 269 354 397
205	3-OH	5,6,7,3',4'-pentaOMe*	Marionol*	<i>Artemisia mongolica</i> , <i>A. verlotiorum</i> <i>Inula britannica</i>	Asterac. Asterac.	Aerial parts Aerial p., ext.	132 196
206	— (3,5,6,8,3',4'-hexaOH) (3,5,7,8,2,3'-hexaOH)	3,5,6,7,3',4'-hexaOMe (7-chloro-derivative)		<i>Inula britannica</i> <i>Parthenium incanum</i> <i>Ficus altissima</i> <i>Adenosma capitatum</i> <i>Vitex rotundifolia</i>	Asterac. Asterac. Morac. Scrophul. Verbenac.	Aerial p., ext. Aerial p., ext. Aerial parts External? Fruit	32 182 30 382 388
207	5,2',3'-triOH	3,7,8-triOMe		<i>Chromolaena odorata</i> <i>Pallemis spinosa</i>	Asterac. Asterac.	Aerial p., ext. Aerial parts	399 390

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
232	5,4'-diOH	3,7,8,3'-tetraOMe	Terminin	<i>Euodia viticina</i> <i>Melicope elleryana</i> <i>Melicope simplex</i> , <i>M. ternata</i> <i>Boronia coerulescens</i>	Rutac. Rutac. Rutac. Rutac.	Fruit Fruit Bark Aerial parts	403 379 380
233	5,3'-diOH	3,7,8,4'-tetraOMe		<i>Calceolaria tenella</i>	Serophul.	Aerial p., ext.	108
234	5,8-diOH	3,7,3',4'-tetraOMe		<i>Solanum paludosum</i>	Solanac.	Aerial parts	317
235	5,7-diOH	3,8,3',4'-tetraOMe					
236	7-OH	3,5,8,3',4'-pentaOMe					
237	5-OH	3,7,8,3',4'-pentaOMe					
238	—	3,5,7,8,3',4'-hexaOMe					
239	3,6,7,8,3',4'-hexaOH					Synthesis	402
240	3,5,7,2',3',4'-hexaOH					Synthesis	402
241	5,2',3'-triOH		Apuleidin	<i>Helichrysum foetidum</i>	Asterac.	Aerial p., ext.	182
242	3,5,7,2',4',5'-hexaOH		5'-Hydroxy-morin	<i>Ozothamnus lycopodioides</i>	Asterac.	Aerial p., ext.	197
243	5,7,4',5'-tetraOH			<i>Murraya paniculata</i>	Rutac.	Fruit	404
244	5,7,2',5'-tetraOH			<i>Solanum paludosum</i>	Solanac.	Aerial parts	317
245	5,2',5'-triOH			<i>Murraya paniculata</i>	Rutac.	Fruit	404
246	3,5,7-triOH						
247	2',5'-diOH						
248	5,2'-diOH						
249	5'-OH						
250	2'-OH						
251	5-OH						
252	—	3,5,7,2',4',5'-hexaOMe					
253	(3,6,7,2',4',5'-hexaOH) 2',5'-diOH	3,6,7,4'-tetraOMe	Oxyyanin-A	<i>Psidium punctulata</i>	Asterac.	Aerial parts	33

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
279		3,5,7,3',4',5'-hexaOMe*		<i>Ficus altissima</i> <i>Murraya paniculata</i>	Morac. Rutac.	Aerial parts Flower	30 61
280	3,5,8,3',4',5'-hexaOH (3,6,7,3',4',5'-hexaOH)						
281	3,7,4',5'-tetraOH (3,7,8,2',4',5'-hexaOH)	6,3'-diOMe*	Inucritimin*	<i>Inula crithmoides</i>	Asterac.	Aerial parts	407
282	2'-OH	3,7,8,4',5'-pentaOMe*		<i>Parkia clappertoniana</i>	Fabac.	Leaf	331
Hepta-O-substituted flavonols							
	(3,5,6,7,8,2',4'-OH)						
283	5,2',4'-triOH	3,6,7,8-tetraOMe					
284	5,4'-diOH	3,6,7,8,2'-pentaOMe					
285	5,2'-diOH	3,6,7,8,4'-pentaOMe					
286	5-OH (3,5,6,7,8,2',5'-OH)	3,6,7,8,2',4'-hexaOMe					
287	5-OH	3,6,7,8,2,5'-hexa-OMe*	Grantiodinin*	<i>Inula grantioides</i>	Asterac.	Aerial parts	34
289	3,5,6,7,8,3',4'-heptaOH						
290	5,7,8,3',4'-pentaOH	3,6-diOMe				Synthesis	335
291	3,5,7,3',4'-pentaOH	6,8-diOMe		<i>Calycadenia</i> , 3 spp.	Asterac.	Leaf exudate	236
292	5,7,3',4'-tetraOH	3,6,8-triOMe		<i>Madia</i> , 3 spp.	Asterac.	Aerial p., ext. Synthesis	200 335
293	5,6,3',4'-tetraOH	3,7,8-triOMe					
294	3,5,3',4'-tetraOH	6,7,8-triOMe				Synthesis	335
295	3,5,7,4'-tetraOH	6,8,3'-triOMe	Limocitrol			Synthesis	335
296	3,5,7,3'-tetraOH	6,8,4'-triOMe	Isolimocitrol			Aerial parts, ext.	237
297	5,3',4'-triOH	3,6,7,8-tetraOMe		Glycoside only <i>Helichrysum</i> , 8 spp. <i>Madia</i> , 3 spp.	Asterac. Asterac.	Aerial p., ext. Aerial p., ext.	200
298	5,7,4'-triOH	3,6,8,3'-tetraOMe		<i>Rosa centifolia</i> cv. <i>Muscosa</i>	Rosac.	Synthesis	18
	5,7,4'-triOH	3,6,8,3'-tetraOMe				Synthesis	335
299	5,7,3'-triOH	3,6,8,4'-tetraOMe				Synthesis	335

352	5,7,2',4'-tetraOH	3,8,5'-triOMe	Hibiscetin	Glycoside only	Polygon.	Whole plant	7
353	5,3',4'-triOH	3,7,8,5'-tetraOMe		<i>Chorizanthe diffusa</i>			
354	5,7,3'-triOH	3,8,4',5'-tetraOMe		Glycoside only			
355	3,5,7,8,3',4',5'-heptaOH						
356	3,5,7,3',4',5'-hexaOH	8-OMe					
357	5,8,3',4',5'-pentaOMe	3,7-diOMe*					
358	3,7,3',4'-pentaOH	8,5'-diOMe					
359	5,8,3',5'-tetraOH	3,7,4'-triOMe					
360	5,7,3',5'-tetraOH	3,8,4'-triOMe					
361	3,5,7,4'-tetraOH	8,3',5'-triOMe					
362	5,3',5'-triOH	3,7,8,4'-tetraOMe					
363	5,7,3'-triOH	3,8,4',5'-tetraOMe					
364	5,7-diOH	3,8,3',4',5'-pentaOMe	Conyzatin				
365	3,5-diOH	7,8,3',4',5'-pentaOMe					
366	8-OH	3,5,7,3',4',5'-hexaOMe*					
367	5-OH	3,7,8,3',4',5'-hexaOMe*		<i>Murraya paniculata</i>	Rutac.	Fruit	404
368	(3,5,7,2',3',4',6'-OH)	3,5,7,8,3',4',5'-heptaOMe		<i>Murraya paniculata</i>	Rutac.	Fruit	404
369	5-OH	3,7,2',3',4',6'-hexaOMe*		<i>Distemonanthus benthamianus</i>	Fabac.	Heartwood	29
Octa-O-substituted flavonols							
370	(3,5,6,7,8,2',4',5'-octaOH)	3,6,8,2'-tetraOMe					
371	5,7,4',5'-tetraOH	3,6,8,4'-tetraOMe					
372	5,7,2',5'-tetraOH	3,6,8,5'-tetraOMe					
373	5,7,2',4'-tetraOH	3,7,8,4'-tetraOMe					
374	5,6,2',5'-tetraOH	3,6,7,8,2'-pentaOMe					
375	5,4',5'-triOH	3,6,8,2',4'-pentaOMe					
376	5,7,5'-triOH	3,6,8,4',5'-pentaOMe					
377	5,7,2'-triOH	3,6,7,8,4',5'-hexaOMe					
378	5,2'-diOH	3,6,8,2',4',5'-hexaOMe					
379	5,7-OH	3,5,6,7,8,2',4',5'-octaOMe	Purpurascenin (6,8-Dihydroxy-myricetin)				
380	(3,5,6,7,8,3',4',5'-octaOH)	—					
381	5,7,3',4',5'-pentaOH	3,6,8-triOMe					
	5,7,3',5'-tetraOH	3,6,8,4'-tetraOMe		<i>Zieridium pseudobotsifolium</i>	Rutac.	Leaf	408

continued

TABLE 12.2
Flavonols and Their Methyl Ethers — continued

No.	OH-Substitution	OMe-Substitution	Trivial Name	Plant Species	Family	Plant Organ	Ref.
382	5,7,3',4'-tetraOH	3,6,8,5'-tetraOMe					
383	5,6,3',5'-tetraOH	3,7,8,4'-tetraOMe					
384	5,3',5'-triOH	3,6,7,8,4'-pentaOMe					
385	5,7,4'-triOH	3,6,8,3',5'-pentaOMe					
386	5,7,3'-triOH	3,6,8,4',5'-pentaOMe					
387	3,5,3'-triOH	6,7,8,4',5'-pentaOMe*	Desmethyl-digititrin	<i>Zieridium pseudobutusifolium</i>	Rutac.	Leaf	408
388	3',5'-diOH	3,5,6,7,8,4'-hexaOMe					
389	5,3'-diOH	3,6,7,8,4',5'-hexaOMe	Digititrin	<i>Zieridium pseudobutusifolium</i>	Rutac.	Leaf	408
390	5,7'-diOH	3,6,8,3',4',5'-hexaOMe		<i>Gymnosperma glutinosum</i>	Asterac.	Aerial p., ext.	129
391	3'-OH	3,5,6,7,8,4',5'-heptaOMe					
392	5-OH	3,6,7,8,3',4',5'-heptaOMe					
393		3,5,6,7,8,3',4',5'-octaOMe	Exoticin				

*For explanation, please see text.

^aRevised to 3,5,7,8-tetraOH, see text.

^bRevised to 3,6,4'-triMe, see text.

^cRevised to 3,6,7-triMe, see text.

^dRevised to 5-OH-6,7,3',4'-tetraOMe, see text.

^eRevised to Queg-7-Me, see text.

species of this genus. Single reports exist for families such as the Saxifragaceae⁴² or Nyctaginaceae⁴³. New results on Rosaceae and Viscaceae deserve special consideration. A quite complex derivative (compound 298 in Table 12.2) was isolated from several Rosaceae. By contrast, rather simple derivatives were found in the leaf wax of *Viscum* spp.⁴⁴ Particularly with the Rosaceae, more results in this direction are to be expected when more material is analyzed. Frond exudates of several ferns proved to be a rich source for various flavonol derivatives, which outnumber the few corresponding flavones.⁴⁵

Several compounds were structurally revised. 6-Hydroxygalangin (compound 13 in Table 12.2), as reported from *Platanus* buds,⁴⁶ was revised to 8-hydroxygalangin after synthesis (compound 23, Table 12.2).⁴⁷ 6-Hydroxykaempferol-3,7,4'-triMe had been reported as "tane-tin" (compound 84, Table 12.2) from *Tanacetum parthenium*.⁴⁸ Its structure was later revised to 6-hydroxykaempferol-3,6,4'-triMe = santin (compound 83, Table 12.2).⁴⁹ The name "tane-tin" is hence obsolete. 5,4-diOH-3,6,8-triOMe-flavone had been isolated from *Tephrosia candida* and named "candiron" (compound 88, Table 12.2).⁵⁰ Synthesis revealed that the structure must be revised to 5,4'-diOH-3,6,7-triOMe-flavone = penduletin (compound 82, Table 12.2).⁵¹ The name "candiron" must not be used, therefore. "Santoflavone," a compound isolated from *Achillea santolina* and claimed to be 7-OH,3,6,3',4'-tetramethoxyflavone (compound 144, Table 12.2),⁵² was later revised to 5-hydroxy-6,7,3',4'-tetraOMe flavone (compound 170, Table 12.1).⁵³ Bhardwaj et al. had reported "alopatuletin" to be a 3,6,7,3',4'-pentahydroxy-5-methoxyflavone (compound 173, Table 12.2), from *Tagetes pen-dula*.⁵⁴ After synthesis, revision of this structure to quercetagetin-7-Me is required (compound 175, Table 12.2).⁴⁷ Zhang et al. reported "viscidulin III" from the roots of *Scutellaria planipes*.⁵⁵ Unfortunately, it remains dubious whether the authors used the name in its initial meaning, that is, as 3,5,7,3'-tetraOH-2',4'-diOMe flavone or as its revised structure 5,7,3',6'-tetraOH-8,2'-diOMe (for which the name ganhuangenin would apply).⁵⁶ Since the authors did not answer relevant requests, this report has not been included in our tables.

12.5 FLAVONES WITH OTHER SUBSTITUENTS

As already mentioned in Section 12.1, several biosynthetic trends are included in this section. These concern *C*-methylated as well as other *C*-substituted flavones, further methylenedioxy derivatives and structures resulting from prenylation and cyclization processes. Most of the prenyl side chains (C5, C10, or C15) are linked directly to the flavone molecule; rarely *O*-prenylation occurs. Extensive modification of the terpenoid side chain may occur by further oxidation, reduction, dehydration, and cyclization. In addition, cyclization of the terpenoid chains with an *ortho*-phenolic OH-group to give pyrano- or furano-derivatives is quite common. Apart from the flavanones, the flavones are the second most abundant class of isoprenylated flavonoids.³ Compounds which have not been included here are those resulting from Diels–Alder reaction, forming adducts (e.g., Brosimone D) or dimeric flavones (for these structures, see Ref. 3). Most reports concern a few genera of the Moraceae and Fabaceae, exhibiting quite diverse biosynthetic capacities in terms of complex substitution patterns, a phenomenon earlier also addressed by Barron and Ibrahim³. Thus, flavonoid profiles of some of these genera will be discussed separately.

12.5.1 *C*-METHYLFLAVONES AND *C*2/*C*3-SUBSTITUTED FLAVONES

Direct methylation through *C*-bonds appears to be common in the positions 6 and 8 of the flavonoid molecule. Other positions are rarely *C*-methylated (C7, saltillin; C3, a glycoside only; compound 23, Table 12.3). Most reports concentrate on species from the family Myrtaceae, where *C*-methylflavones also occur externally.⁵⁷ *Desmos cochinchinensis* (Annonaceae) was

TABLE 12.3
Flavones with Other Substituents

No.	OH- and OMe-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
C-Methyl- and C2/C3-substituted flavones							
1	5,7-diOH	6-Me	Strobocrysin	<i>Matteucia orientalis</i>	Aspid.	Rhizome	415
2	5,7-diOH	6,8-diMe*	Matteuorien*	<i>Leptospermum scoparium</i>	Myrtac.	Leaf	416
3	5-OH, 7-OMe	6-Me		<i>Leptospermum scoparium</i>	Myrtac.	Leaf	59
4	5-OH, 7-OMe	6,8-diMe*		<i>Desmos cochinchinensis</i>	Annon.		58
5	5-OH, 7-OMe	6,8-diMe*	Desmosflavone	<i>Desmos chinensis</i>	Annon.	Seeds	60
6	5,7-diOMe	6-Me*		<i>Leptospermum scoparium</i>	Myrtac.	Leaf	417
7	5-OH, 4'-OMe	7-Me	Saltillin				
8	5,7-diOH, 8-OMe	6-Me		<i>Trianthema portulacastrum</i>	Aizoaoac.	Whole plant	418
9	5,2'-diOH, 7-OMe	6,8-diMe*		<i>Valeriana wallichii</i>	Valerian.	Aerial parts	419
10	5,7,4'-triOH	6-Me*	6-Methyl-apigenin			Synthesis	420
11	5,7,4'-triOH	8-Me*	8-Methyl-apigenin			Synthesis	420
12	5,7,4'-triOH	3-Me	Glycoside only	<i>Callistemon</i> , 5 spp.	Myrtac.	External	57
13	5,7,4'-triOH	6,8-diMe*	6,8-Dimethyl-apigenin	<i>Pancreatium maritimum</i>	Amaryllid.	Bulb	421
			Syzaltein*	<i>Syzygium alternifolium</i>	Myrtac.	Leaf	422
14	5,4'-diOH, 7-OMe	6-Me	8-Desmethyl-sideroxylin	<i>Callistemon</i> , 8 spp.	Myrtac.	External	57
				<i>Eucalyptus saligna</i>	Myrtac.	Leaf wax	423
15	5,4'-diOH, 7-OMe	6,8-diMe	Sideroxylin	<i>Callistemon</i> , 9 spp.	Myrtac.	Synthesis	420
				<i>Eucalyptus saligna</i>	Myrtac.	External	57
				<i>Leptospermum laevigatum</i>	Myrtac.	Leaf wax	423
16	5,7-diOH, 4'-OMe	6-Me*				Leaf wax	424
17	5,7-diOH, 4'-OMe	8-Me*				Synthesis only	420
18	5-OH, 7,4'-diOMe	6-Me	8-Desmethyl-eucalyptin	<i>Callistemon</i> , 5 spp.	Myrtac.	Synthesis only	420
19	5-OH, 7,4'-diOMe	6,8-diMe	Eucalyptin	<i>Callistemon lanceolatus</i>	Myrtac.	External	57
				<i>Callistemon</i> , 5 spp.	Myrtac.	Leaf	425
20	5,7,3',4'-tetraOH	3-Me	Glycoside only			External	57
21	5,7,3',4'-tetraOH	6-Me*	6-Methyl-luteolin*	<i>Salvia nemorosa</i>	Lam.	Aerial parts	426

22	5,3',4'-triOH-7-OMe	6-Me*	Dasytrichone	<i>Hydrastis canadensis</i>	Ranunculac.	Root	427
23	5,3',4'-triOH-7-OMe	6,8-diMe*	Unonal	<i>Hydrastis canadensis</i>	Ranunculac.	Root	427
24	5-OH	6-Me, 8-diMe, 7=O		<i>Dasydaschalon trichophorum</i>	Annonac.	Stems, leaves	63
25	5,7-diOH	8-Me, 6-CHO		<i>Dasydaschalon rostratum</i>	Annonac.	Stems	64
26	5,7-diOH	6-Me, 8-CHO	Isounonal	<i>Desmos chinensis</i>	Annonac.	Seeds	60
27	5-OH, 7-OMe	8-Me, 6-CHO	Unonal-7-Me	<i>Desmos chinensis</i>	Annonac.	Root	62
28	5,3',4'-triOH-7-OMe	6,8-diCH ₃ -5'-C5*	Muxiangrine III*	<i>Desmos cochinchinensis</i>	Annonac.	Stem	64
29	5,5'-diOH, 7-OMe	6-CH ₃ , 4',3'-ODmp*	Muxiangrine II*	<i>Dasydaschalon rostratum</i>	Annonac.	Seeds	60
30	5,3'-diOH, 7-OMe	6,8-diCH ₃ , 4',3'-ODmp*	Muxiangrine I*	<i>Desmos chinensis</i>	Annonac.	Seeds	60
31	5,7,4'-triOH	6-C2*	Drymariatin; glycoside only	<i>Desmos chinensis</i>	Annonac.	Seeds	60
32	5-OH, 7-OMe	6-CH ₂ CHO, see Figure 1	Hoslundal	<i>Elsoltzia stauntonii</i>	Lam.	Aerial parts	65
33	5,7,3',4'-tetraOH	6-Acrylic acid*, see Figure 1	DeMe-torosaflavone D*	<i>Elsoltzia stauntonii</i>	Lam.	Aerial parts	65
34	5,7,3'-triOH, 4'-OMe	6-Acrylic acid *, see Figure 1	Torosaflavone D*	<i>Elsoltzia stauntonii</i>	Lam.	Aerial parts	65
Methylendioxy-flavones							
35	5-OH	6,7-OCH ₂ O		<i>Cassia nonane</i>	Fabac.	Aerial parts	68
36	7-OMe	3',4'-OCH ₂ O*		<i>Cassia torosa</i>	Fabac.	Leaf	67
37	7-OMe	3',4'-OCH ₂ O*		<i>Millettia erythrocalyx</i>	Fabac.	Leaf	428
38	7-OMe	5,6-/3',4'-diOCH ₂ O		<i>Millettia leucantha</i>	Fabac.	Stem bark	429
39	5,6-diOMe	3',4'-OCH ₂ O		<i>Millettia leucantha</i>	Fabac.	Stem bark	429
40	5-OH-7-OMe	3',4'-OCH ₂ O*		<i>Neoraputia magnifica</i>	Rutac.	Fruit	286
41	5,7-diOMe	3',4'-OCH ₂ O*	Prosogerin-A	<i>Millettia erythrocalyx</i>	Fabac.	Stem bark	428
42	7-OH, 6-OMe	3',4'-OCH ₂ O	Milletin C	<i>Millettia erythrocalyx</i>	Fabac.	Stem bark	428
43	6,7-diOMe	3',4'-OCH ₂ O	Kanzakiflavon-2	<i>Albizia odoratissima</i>	Fabac. (Mim.)	Root bark	174
44	5,4'-diOH	6,7-OCH ₂ O	Millettocalyxin A*	<i>Limnophila indica</i>	Scroph.	Whole plant	430
45	7,2-diOMe	4',5'-OCH ₂ O*		<i>Ageratum conyzoides</i>	Ast.	Whole plant	268
46	7,8-diOMe	3',4'-OCH ₂ O*					
47	5-OH, 6,8-OMe	3',4'-OCH ₂ O*	Ageonyflavon A*				
48	5,6,7-triOMe	3',4'-OCH ₂ O*					

continued

TABLE 12.3
Flavones with Other Substituents — continued

No.	OH- and OMe-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
49	5,7,4'-triOMe	3',4'-OCH ₂ O*		<i>Neoraputia magnifica</i>	Rutac.	Fruit	286
50	5,7,5'-triOMe	3',4'-OCH ₂ O		<i>Neoraputia paraensis</i>	Rutac.	Aerial parts	285
51	5,8-diOH, 4'-OMe	6,7-O ₂ CH ₂	Kanzakiflavon-1				
52	5,7-diOH, 6,8-diOMe	3',4'-OCH ₂ O	Linderoflavone A				
53	5,6,7,8-tetraOMe	3',4'-OCH ₂ O	Linderoflavone B	<i>Ozothamnus lycopodioides</i>	Ast.	Leaf exudate	69
54	5,6,7,5'-tetraOMe	3',4'-OCH ₂ O		<i>Ficus maxima</i>	Morac.	Leaf	77
55	5,3',4',5'-tetraOMe	6,7-O ₂ CH ₂		<i>Neoraputia paraensis</i>	Rutac.	Aerial parts	285
56	7-OH, 5,6,8,5'-OMe	3',4'-OCH ₂ O		<i>Ageratum conyzoides</i>	Ast.	Aerial parts	27
57	5,6,7,8,5'-pentaOMe	3',4'-OCH ₂ O	Eupalestin	<i>Ageratum tomentosum</i> var. <i>bracteatum</i>	Ast.	Leaf + flower	431
				<i>Ozothamnus lycopodioides</i> #	Ast.	Leaf exudate	69
C-Prenylflavones							
58	5-OMe	7,8-di-C5*	5-Methoxy-7,8-diprenylflavone	<i>Tephrosia barbiger</i>	Fabac.	Seeds	4
59	7-OMe	8-C5-OH	<i>trans</i> -Lanceolatin; Lanceolatin A				
60	5,7-diOH	6-C5	6-Prenylchrysin			Synthesis	432
61	5,7-diOH	8-C5	8-Prenylchrysin			Synthesis	432
62	5-OH, 7-OMe	8-C5	Tephirone				
63	5,7-diOMe	8-C5-OH	<i>cis</i> -Tephrostachin				
64	5,7-diOMe	8-C5-OH	<i>trans</i> -Tephrostachin				
65	5,7-diOMe	8-C5	<i>trans</i> -Anhydrotephr				
66	7,4'-diOH	6-C5*	Licoflavon A*	<i>Glycyrrhiza eurycarpa</i>	Fabac.	Root	17
67	7,4'-diOH	8-C5		<i>Glycyrrhiza echinata</i>	Fabac.	Cell culture	3
68	7,4'-diOH	8-C5(OH) ₂ *	Brosimacutin F	<i>Brosimum acutifolium</i>	Morac.	Bark	433
69	7,4'-diOH	3'-C5*	Kanzonol D	<i>Glycyrrhiza eurycarpa</i>	Fabac.	Root	17
						Synthesis	434
70	7,4'-diOH	6,3'-diC5*	Licoflavone B	<i>Glycyrrhiza inflata</i>	Fabac.	Root	74
71	7,4'-diOH	6,3'-diC5	(Prenyllicoflavone A)	<i>Glycyrrhiza glabra</i>	Fabac.	Root	75
72	5,7-OH, 6OMe	3-C5		<i>Ehretia ovalifolia</i>	Borag.	Leaves	78

73	5,7,4'-triOH	6-C5*	6-Prenyl-apigenin	<i>Cudrania cochinchinensis</i> <i>Dorstenia ciliata</i> <i>Dorstenia kameruniana</i> <i>Maclura pomifera</i> <i>Dorstenia dinklagii</i> <i>Maclura pomifera</i>	Morac. Morac. Morac. Morac. Morac. Morac.	Root Aerial parts Leaf Fruit Twigs Stem, leaf	435 436 437 438 439 440
74	5,7,4'-triOH	6-C5-OH*	Dinklagin C*	<i>Cudrania cochinchinensis</i> <i>Dorstenia ciliata</i> <i>Dorstenia poinsettifolia</i> <i>Glycyrrhiza inflata</i>	Morac. Morac. Morac. Fabac.	Root Aerial parts Twigs Leaf	435 436 441 74
75	5,7,4'-triOH	8-C5*	Licoflavone C*, 8-prenylapigenin	<i>Cudrania cochinchinensis</i>	Morac.	Root	435
76	5,7,4'-triOH	8-C5	8-Prenylapigenin	<i>Genista ephedroides</i>	Fabac.	Synthesis	442
77	5,7,4'-OH	8-C5-OH*	Ephedroidin	<i>Epimedium sagittatum</i>	Berberidac.	Aerial parts	443
78	5,7,4'-triOH	3'-C5	Yinyanghuo D	<i>Yanconuvertia hexandra</i>	Berberidac.	Leaf	444
79	5,7,4'-triOH	3'-C5-OH	Albanin D, revised	<i>Morus alba</i>	Berberidac.	Underground parts	553
80	5,7,4'-OH	6-C10	Kuwanon S	<i>Artocarpus integrifolia</i>	Morac.	Synthesis	71
81	5,7,4'-triOH	3'-C10	Isoartocarpin	<i>Yanconuvertia hexandra</i>	Morac.	Root bark	445
82	5,7,4'-triOH	6,2'-diC5	Gancanin Q	—	Morac.	Heartwood	3
83	5,7,4'-triOH	6,3'-diC5	Honyucitrin	<i>Epimedium sagittatum</i>	Berberidac.	Leaf	444
84	5,7,4'-triOH	6,3'-diC5-OH	Yinyanghuo B*	<i>Artocarpus heterophyllus</i>	Morac.	Bark	446
85	5,7,4'-triOH	8,3'-diC5	Artonin U	—	Morac.		
86	5,7,4'-triOH	3',5'-diC5	Rubraflavone A	<i>Cudrania cochinchinensis</i>	Morac.	Root	435
87	5,7,4'-triOH	3'-C5, 5'-C5-OH*	Albanin A	<i>Artocarpus elasticus</i>	Morac.	Wood	447
88	5,4'-diOH, 7-OMe	8-C5	Artocarpesin	<i>Artocarpus heterophyllus</i>	Morac.	Heartwood	448
89	5,7-dihOH, 4'-OMe	8,3'-diC5	Oxidihydroartocarpesin	<i>Maclura pomifera</i>	Morac.	Fruit	438
90	5,7-dihOH, 4'-OMe	3-C10	Albanin E, revised	—	Morac.	Synthesis	71
91	7,2',4'-triOH	3-C5	Moralbanone*	<i>Morus alba</i>	Morac.	Root bark	445
92	5,7,2',4'-tetraOH	6-C5					
93	5,7,2',4'-tetraOH	6-C5					
94	5,7,2',4'-tetraOH	6-C5-OH					
95	5,7,2',4'-tetraOH	6-C10					
96	5,7,2',4'-tetraOH	8-C15					

continued

TABLE 12.3
Flavones with Other Substituents — continued

No.	OH- and OMe-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
97	5,7,2',4'-tetraOH	3,6-diC5	Cudraflavone C	<i>Cudrania tricuspidata</i>	Morac.	Root bark	449
98	5,7,2',4'-tetraOH	3,8-diC5	Mulberrin, Kuwanon C	<i>Morus australis</i>	Morac.	Root bark	450
99	5,7,2',4'-tetraOH	3-C10, 6-C5	Rubraflavone C				
100	5,7,2',4'-tetraOH	8-C5, 3-C10*	Artocommunol CD*	<i>Artocarpus communis</i>	Morac.	Root cortex	451
101	5,7,2',4'-tetraOH	6,5'-diC5	Cudraflavone D	<i>Cudrania tricuspidata</i>	Morac.	Root bark	449
102	5,7,2',4'-tetraOH	3,3'-diC5	Kuwanon T	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	452
103	5,7,2',4'-tetraOH	3,6,8-triC5*	Artelasticin*	<i>Artocarpus elasticus</i>	Morac.	Wood	447
104	5,7,2',4'-tetraOH	3,6,8-triC5	Dorsilurin D	<i>Dorstenia psilurus</i>	Morac.	Root	453
105	5,7,2',4'-tetraOH	6,8,3'-triC5*	Dorsilurin A*	<i>Dorstenia psilurus</i>	Morac.	Root	454
106	5,2',4'-triOH, 7-OMe	3-C5	Integrin	—	—	—	—
107	5,2',4'-triOH, 7-OMe	8-C5	Artocarpetin A	<i>Artocarpus heterophyllus</i>	Morac.	Root	455
108	5,2',4'-triOH, 7-OMe	3,6-diC5	Artocarpin	<i>Clarisia racemosa</i>	Morac.	?	456
109	5,2',4'-triOH, 7-OMe	3-C5, 8-C10	Brosimone H	—	—	—	—
110	5,3',4'-triOH-7-OMe	6,8-diCH ₃ , 5'-C5*	Muxiangrine III*	<i>Elsholtzia stauntonii</i>	Lam.	Aerial parts	65
111	5,7,2'-triOH, 4'-OMe	6,8-diC5	Artocarpetin B	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	452
112	5,4'-diOH, 7,2'-OMe	8-C5*	Gancaonin O* 6-prenyluteolin	<i>Glycyrrhiza uralensis</i>	Fabac.	Aerial parts	457
113	5,7,3',4'-tetraOH	6-C5*	8-Prenyluteolin	<i>Hypericum perforatum</i>	Hypericaceae	Callus	218
114	5,7,3',4'-tetraOH	6-C5*	Epimedokoreanin B	<i>Epimedium koreanum</i>	Berberidac.	Aerial parts	458
115	5,7,3',4'-tetraOH	8-C5	6-Prenylchrysoeriol	<i>Dorstenia mannii</i>	Morac.	Aerial parts	459
116	5,7,3',4'-tetraOH	8,5'-diC5	Cannflavin A				
117	5,7,4'-triOH, 3'-OMe	6-C5*	Cannflavin B				
118	5,7,4'-triOH, 3'-OMe	6-C10	Heteroartoinin A*	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	452
119	5,3'-diOH, 7,4'-diOMe	6-C5	Artoindonesianin Q*	<i>Artocarpus champeden</i>	Morac.	Heartwood	460
120	5,7,2',5'-OH-4'-OMe	3,3'-diC5*	Artoindonesianin R*	<i>Artocarpus champeden</i>	Morac.	Heartwood	460
121	5,2',5'-OH-7,4'-OMe	3-C5*	Asplenetin				
122	5,7,5'-OH-2',4'-OMe	3-C5*	Baohuosu				
123	5,7,3',4',5'-pentaOH	3-C5					
124	5,7,4'-OH-3',5'-OMe	8-C5					
O-Prenylflavones							
125	4'-OH, 5-OMe	7-O-C5 (epoxy)		<i>Achyrocline flaccida</i>	Ast.	Aerial parts	76
126	4'-OH, 7-OMe	7-O-C5 (epoxy)*		<i>Achyrocline flaccida</i>	Ast.	Aerial parts	76

127	6,7,4'-triOMe	5-O-allyl	Millettocalyxin B*	<i>Ficus maxima</i>	Morac.	Leaf	77
128	5,6,7,3',5'-OMe	4'-O-C5*	Ugonine C	<i>Millettia erythrocalyx</i>	Fabac.	Stem bark	461
129	7-OMe-6-OC5	4',5'-OCH ₂ O*	Ovalifolin	<i>Helminthostachys zeylanica</i>	Ophioglossac.	Rhizome	462
130	5,4'-diOH, 6-OMe	7,8-fur		<i>Pongamia pinnata</i>	Fabac.	Root	297
131	6-O-C5	7,8-fur*		<i>Millettia erythrocalyx</i>	Fabac.	Leaf	461
Pyranoflavones							
132		7,8-ODmp*		<i>Dahlstedtia pentaphylla</i>	Fabac.	Root	463
				<i>Lonchocarpus subglaucesc.</i>	Fabac.	Root	464
133	5-OH	7,8-ODmp		<i>Tephrosia praecans</i>	Fabac.	Seed	465
134	5-OMe	7,6-ODmp		<i>Pongamia pinnata</i>	Fabac.	Root	297
135	5-OMe	7,8-ODmp	Isopongaflavon (Candidin)	<i>Tephrosia tunicata</i>	Fabac.	Root	466
				<i>Pongamia pinnata</i> (syn. <i>P. glabra</i>)	Fabac.	Synthesis	467
136	5-OMe	7,8-ODmp-diOAc, see Figure 12.2				Stem bark	468
137	6-OMe	7,8-ODmp*		<i>Lonchocarpus subglaucescens</i>	Fabac.	Root	464
138	2'-OH	4,3/6,5/7,8-triODmp, 4' = O see Figure 12.2	Dorsilurin E	<i>Dorstenia psilurus</i>	Morac.	Root	453
139	5'-OMe	7,8-ODmp		<i>Epimedium sagittatum</i>	Berberidac.	Leaf	444
140	5,7-diOH	4',3'-ODmp*	Yinyanghuo C*	<i>Vancouveria hexandra</i>	Berberidac.	Underground parts	553
						Synthesis	434
141	5,4'-diOH	7,6-ODmp	Carpachromene*	<i>Lonchocarpus xauil</i> , <i>L. yucatanensis</i>	Fabac.	Leaf	469
				<i>Dorstenia kameruniana</i>	Morac.	Leaf	437
				<i>Maclura pomifera</i>	Morac.	Stem and leaf	440
142	5,4'-diOMe	7,6-ODmp-OH*	Dinklagin B*	<i>Atalantia monophylla</i>	Rutac.	Leaf	470
143	5,4'-diOMe	7,6-ODmp	Atalantoflavon	<i>Dorstenia dinklagii</i>	Morac.	Twig	439
144	5,4'-diOH	7,8-ODmp		<i>Lonchocarpus xauil</i> , <i>L. yucatanensis</i>	Fabac.	Leaf	469
145	5,4'-diOH	7,6-/3,6'-ODmp	Isocyclomorusin	<i>Artocarpus altilis</i>	Morac.	Stem	471
146	5,4'-diOH	7,8-/3,6'-ODmp	Cyclomorusin = cyclomulberochromene	<i>Morus alba</i>	Morac.	Root bark	445
				<i>Artocarpus communis</i>	Morac.	Root cortex	451

continued

TABLE 12.3
Flavones with Other Substituents — continued

No.	OH- and OMe-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
147	5,7,4'-triOH	3,6'-ODmp*	Cyclocommunol*	<i>Artocarpus communis</i>	Morac.	Root bark	472
148	5,7,5'-triOH	4',3'-ODmp*	Yinyanghuo E	<i>Epimediium sagittatum</i>	Berberidac.	Leaf	444
149	5,2',4'-triOH	7,6-ODmp	Cycloartocarpesin	<i>Cudrania tricuspidata</i>	Morac.	Root bark	449
				<i>Maclura pomifera</i>	Morac.	Cell culture	473
150	7,4'-diOH,3'-OMe	5,6-ODmp	Ciliatin B	<i>Dorstenia ciliata</i>	Morac.	Aerial parts	436
151	5,4'-diOH,3'-OMe	7,6-ODmp		<i>Lonchocarpus xuiul</i> , <i>L. yucatanensis</i>	Fabac.	Leaf	469
152	5,4'-diOH, 3'-OMe	7,8-ODmp	Racemoflavon				
153	5,7,3',4'-tetraOH	2',3'-ODmp*	Cyclochampedol*	<i>Artocarpus champeden</i>	Morac.	Bark	474
154	5,4'-diOH,3',5'-diOMe-	7,6-ODmp		<i>Neorapuitia paraensis</i>	Rutac.	Aerial parts	475
155	5-OH, 7,8,3',4'-OMe	5,6-ODmp					
156	5-OH-7,3',4',5'-OMe	7,6-ODmp*		<i>Neorapuitia paraensis</i>	Rutac.	Aerial parts	285
157	5,4'-diOH,8,3',5'-triOMe	7,6-ODmp*					
158	5-OH, 8,3',4',5'-tetra-OMe	7,6-ODmp		<i>Neorapuitia alba</i>	Rutac.	Leaf	476
159	7,8,3',4',5'-pentaOMe	5,6-ODmp	Australon A*	<i>Morus australis</i>	Morac.	Root bark	450
160	5,2',4'-triOH	7,6-ODmp-C5*	Brosimone G	<i>Brosimopsis oblongifolia</i>	Morac.	Root	477
161	5,2',4'-triOH	7,8-ODmp-C5					
Furanoflavones							
162		7,8-fur	Lanceolatin B	<i>Millettia peguensis</i>	Fabac.	Leaf, st. bark	478
				<i>Millettia sanagana</i>	Fabac.	Root bark	79
				<i>Pongamia pinnata</i>	Fabac.	Root	297
163	5-OH	7,8-fur	Pongagablol	<i>Millettia peguensis</i>	Fabac.	Leaf, st. bark	478
				<i>Tephrosia purpurea</i>	Fabac.	Aerial parts	479
164	5-OMe	7,6-fur	Pinnatin	<i>Millettia sanagana</i>	Fabac.	Root bark	79
165	6-OMe	7,8-fur	Kanjone*	<i>Pongamia glabra</i>	Fabac.	Seed	480
				<i>Millettia erythrocalyx</i>	Fabac.	Root	461
				<i>Millettia peguensis</i>	Fabac.	Leaf, st. bark	478
166	8-OMe	7,6-fur		<i>Millettia sanagana</i>	Fabac.	Root bark	79
167	2-OMe	7,8-fur		<i>Pongamia glabra</i>	Fabac.	Leaf	480
				<i>Pongamia glabra</i>	Fabac.	Seed	481

168	2'-OMe	7,8-fur	Pongol-Me	<i>Millettia erythrocalyx</i>	Fabac.	Leaf	461
169	2'-OMe	7,8-fur	Pongone	<i>Pongamia glabra</i>	Fabac.	Flower	482
170	3'-OH	7,8-fur	Isopongaglabol	<i>Derris mollis</i>	Fabac.	Root	482
171	3'-OMe	7,6-fur	Glabone	<i>Dorstenia ciliata</i>	Morac.	Aerial parts	436
172	4'-OH	7,8-fur		<i>Maclura pomifera</i>	Morac.	Stem and leaf	440
173	4'-OMe	7,6-fur		<i>Maclura pomifera</i>	Morac.	Stem and leaf	440
174	4'-OMe	7,8-fur		<i>Epimedium koreanum</i>	Berberidac.	Aerial parts	484
175	5,4'-diOH	7,6-dihydrofur-C3, see Figure 12.3	Ciliatin A*	<i>Artocarpus elasticus</i>	Morac.	Wood	485
176	5,4'-diOH	7,6-dihydrofur-C3-OH, see Figure 12.3	"Compound 8"	<i>Millettia erythrocalyx</i>	Morac.	Leaf	461
177	5,4'-diOH	7,6-dihydrofurODmp-OH*	Epimedokoreanin A*	<i>Cassia nomane</i>	Fabac.	Aerial parts	68
178	5,3'-diOH	7,8-dihydrofur-2''-C3/4, 5'-dihfur-OH-5''-C3-OH; see Figure 12.3		<i>Cassia torosa</i>	Fabac.	Leaf	67
179	5,4'-diOH	7,8-dihydrofurODmp-OH, see Figure 12.6	Artelastofuran*	<i>Millettia sanagana</i> *	Fabac.	Root bark	79
180	4'-OH, 6-OMe	7,8-fur	6-OMe-isopongaglabol				
181	2',5'-diOMe	7,8-fur*	Millettocalyxin C*				
182	5,3',4'-triOH	7,6-bisfuranone — see Figure 12.3	Demethyltorosaflavone C				
183	5,3'-diOH, 4'-OMe	7,6- bisfuranone — see Figure 12.3	Torosaflavone C				
Furano- and pyranosubstitution							
184		6,5-ODmp, 7,8-fur*	Samaganone*				
185	5,3',6'-triOH	7,6-ODmp, 4',5'-dihydrofur-2''-C3	Dihydrofur-arto-bilichromene b1				
186	5,3',6'-triOH	7,6-ODmp, 4',5'-dihydrofur-2''-C3	Dihydrofur-arto-bilichromene b2				
187	5,3',4'-triOH	7,6-ODmp, 6',5'-dihydrofur-2''-C3	Dihydrofur-arto-bilichromene a				
C-prenyl- and C-pyranosubstitution							
188	5'-OH	6-C5, 7,8-ODmp	Fulvinervin B	<i>Tephrosia fulvinervis</i>	Fabac.	Pods	486
189	5'-OH	6-C5-OH, 7,8-ODmp	Fulvinervin C	<i>Tephrosia fulvinervis</i>	Fabac.	Seed	487
190	7'-OH	6-C5, 3',4'-ODmp*	Kanzonol E*	<i>Glycyrrhiza eurycarpa</i>	Fabac.	Root	17
191	5,7-diOH	5'-C5-OH, 4',3'-ODmp	Yinyanghuo A*	<i>Epimedium sagittatum</i>	Berberidac.	Synthesis	434
192	5,2'-diOH	6-C5, 7,8/4',5'-diODmp		<i>Euchresta formosana</i>	Fabac.	Leaf	444
193	5,2'-diOH	8-C5, 7,6/4',5'-diODmp		<i>Euchresta formosana</i>	Fabac.	Root	488
194	5,4'-diOH	3-C5-OH, 7,8-ODmp	Artocommunol CC*	<i>Artocarpus communis</i>	Morac.	Root cortex	451
195	5,4'-diOH*	6-C5, 7,8-ODmp*	Laxifolin*	<i>Derris laxiflora</i>	Fabac.	Root	489
				<i>Derris laxiflora</i>	Fabac.	Root	490

continued

TABLE 12.3
Flavones with Other Substituents — continued

No.	OH- and OMe-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
196	5,4'-diOH	8-C5, 7,6-ODmp*	Isolaxifolin*	<i>Derris laxiflora</i> <i>Derris laxiflora</i>	Fabac. Fabac.	Root Root	489 490
197	5,7,2'-triOH	3-C5, 3',4'-ODmp	Kuwanon B				
198	5,7,4'-triOH	8-C5, 2',3-ODmp	Cyclomulberrin				
199	5,7-diOH	3'-C5, 4',5'-ODmp					
200	5,2',4'-triOH	3-C5, 7,8-ODmp	Morusin; Mulberrochromene	<i>Vancouveria hexandra</i>	Berberidac.	Underground parts	553
201	5,2',4'-triOH	3-C5-OH, 7,8-ODmp	Oxydihydro-morusin	<i>Morus australis</i>	Morac.	Root bark	450
202	5,2',4'-triOH	3-C10, 7,6-ODmp	Rubraflavone D	<i>Morus alba</i>	Morac.	Root bark	445
203	5,2',4'-triOH	3,6-diC5, 7,8-ODmpOH*	Dorsilurin B*	<i>Dorstenia psiluris</i>	Morac.	Root	454
204	5,2',4'-triOH	7,6-ODmp, 3-C5-OH	Morusignin L	<i>Morus insignis</i>	Morac.	Root bark	491
205	5,2',4',5'-tetraOH	3-C5, 7,8-ODmp	Artotonin E; KB-3	<i>Artocarpus communis</i>	Morac.	Shoot bark	492
206	5,2',4',5'-tetraOH	3-C5, 7,8-ODmp	KB-2	<i>Artocarpus kemando</i>	Morac.	Stem bark	493
207	5,2',4',5'-tetraOH	3,8-diC5; 7,6-ODmp, see Figure 12.2	Heterophyllin	<i>Artocarpus communis</i> <i>Artocarpus heterophyllus</i>	Morac. Fabac.	Shoot bark Root bark	494 452
208	5,2',4',5'-tetraOH	3'-C5, 7,6-ODmp	Artobilochromene				
209	5,7,2'-triOH	3-C5, 2',3'-ODmp-C ₅	Sangganon K	<i>Morus</i> sp.	Morac.	Root bark	495
210	5,7,2'-triOH	3-C5, 3',4'-ODmp-C ₅	Sangganon J	<i>Morus</i> sp.	Morac.	Root bark	495
C-linked aromatic substituents							
211	5,7-diOH	6-C-cinnamyl		chinese propolis			81
212	5,7,4'-triOH	8-C- <i>p</i> -OH-benzyl [†] *		<i>Thymus hirtus</i>	Lam.	Aerial parts	80
213	5,7,3',4'-tetraOH	8-C- <i>p</i> -OH-benzyl [†] *	<i>p</i> -Hydroxybenzyl-luteolin	<i>Thymus hirtus</i>	Lam.	Aerial parts	80
214	5,7,3'-OH-4'-OMe	8-C- <i>p</i> -OH-benzyl [†] *	<i>p</i> -Hydroxybenzyl-diosmetin	<i>Thymus hirtus</i>	Lam.	Aerial parts	80
C-linked ketopyrano substituents							
215	5-OH,7-OMe	6,5''-Ketopyrano...3''-OH*, see Figure 12.4	Hosloppin (3''-O-demethylhoslundin)*	<i>Hoslundia opposita</i>	Lam.	Leaf	496
216	5-OH, 7-OMe	6,5''-Ketopyrano...3''-Me, see Figure 12.4	Hoslundin				
217	5,7-diOMe	6,5''-Ketopyrano...3''-OH, see Figure 12.4	5-OMe-hoslundin	<i>Hoslundia opposita</i>	Lam.	Twigs	144

218	5,7-diOMe	6,6'-Ketopyrano...3''-OH*, see Figure 12.4	Oppositin	<i>Bidens pilosa</i> <i>Hoslunda opposita</i>	Ast. Lam.	Aerial parts Twigs	497 144
<i>Tephrosia flavones</i>							
219		7,8-bisfur —	Glabrathephrinol	<i>Tephrosia apollinea</i>	Fabac.	Seed	498
220		7,8-bisfur —	Glabrathephrin	<i>Tephrosia semiglabra</i>	Fabac.	Aerial parts and root	499
221		7,8-bisfur —	Semiglabrinol	<i>Tephrosia semiglabra</i>	Fabac.	Aerial part. roots	500
222		7,8-bisfur —	Semiglabrin	<i>Tephrosia semiglabra</i>	Fabac.	Aerial part. roots	500
223		7,8-bisfur-OH	Pseudosemiglabrinol	<i>Tephrosia purpurea</i>	Fabac.	Aerial parts	479
224		7,8-bisfur-Oac —	Pseudosemiglabrin	<i>Tephrosia apollinea</i>	Fabac.	Aerial parts	501
225	5-OMe	7,8-bisfur —*, see Figure 12.5	Enantiomultijugin	<i>Tephrosia semiglabra</i>	Fabac.	Aerial parts	502
226	5-OMe	7,8-bisfur —	Multijugin	<i>Tephrosia vicoides</i>	Fabac.	Aerial parts	503
227	5-OMe	7,8-bisfur —	Multijuginol	<i>Tephrosia multijuga</i>	Fabac.	Aerial parts and root	504
228	5-OMe	7,8-pyr-fur (3''-oxo)	Stachyoidin	<i>Tephrosia polystachyaoides</i>	Fabac.	Aerial parts and root	504
229	5-OMe	7,8-pyr-fur (3''-oxo-4''-OAc)	Tephrocin	<i>Tephrosia polystachyaoides</i>	Fabac.	Not mentioned	3
230	5-OMe	7,8-fur-C4-diOAc	Polystachin	<i>Tephrosia polystachyaoides</i>	Fabac.	Not mentioned	3
231	5,7-diOMe	8-diMe-oxo-furano*, see Figure 12.5	Hookerianin*	<i>Tephrosia polystachyaoides</i>	Fabac.	Aerial parts	555
232	5-OMe	7,8-oxofuryl*, see Figure 12.5	Tephrorianin*	<i>Tephrosia hookeriana</i>	Fabac.	Root	505
233	7-OMe	8-furyl (2'',4''-diOH)	Tepurindol	<i>Tephrosia hookeriana</i>	Fabac.	Pods	82
234	7-OMe	8-furyl (4''-oxo)	Tephroglabrine	<i>Tephrosia purpurea</i>	Fabac.	Root	500
235	7-OMe	8-furyl (2'' = oxo), see Figure 12.5	Apollinine	<i>Tephrosia purpurea</i>	Fabac.	Root	500
236	5,7-diOMe	8-furyl (2''-oxo)	Tachrosin	<i>Tephrosia apollinea</i>	Fabac.	Seed	500
<i>Artocarpus flavones</i>							
237	5,7,5'-triOH,4'-OMe	3,6'-cyclo C6-C3; 6-C5	Cycloaitilisin	<i>Artocarpus altilis</i>	Morac.	Stem	471
238	5,2',5'-triOH,7,4'-diOMe	3,6'-cyclo C6-C3*	Artoindonesianin S*	<i>Artocarpus champeden</i>	Morac.	Heartwood	460
239	5,7,2',5'-tetraOH, 4'-OMe	3,6'-cyclo C6-C3*	Artoindonesianin T*	<i>Artocarpus champeden</i>	Morac.	Heartwood	460
240	5,7,2',4',tetraOH	3,6'cyclo-C6-diMe-fur*	Artoindonesianin P*	<i>Artocarpus lanceifolius</i>	Morac.	Tree bark	506
241	5,4'-diOH, 7,2'-diOMe	3,6'-cyclo C6-5'-fur*	Artonin L*	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	507
242	5,2',4'-triOH, 7-OMe	3,6'-cyclo C6-5'-fur*, see Figure 12.6	Artonin K*	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	492
243	5,7,2',4'-tetraOH-	3,6'-cyclo-C6-5'-fur; 4'-C5*	Artonin J*	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	507
244	5,2',4'-triOH,7-OMe	3,6'-cyclo-C6-5'-fur; 4'-C5*	Artonin T*	<i>Artocarpus heterophyllus</i>	Morac.	Root bark	446

continued

TABLE 12.3
Flavones with Other Substituents — continued

No.	OH- and OMe-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
245	5,2',4',5'-tetra-OH	7,8-ODmp, 3,6'-cyclo-C6	KB-1	<i>Artocarpus communis</i>	Morac.	Shoot bark	494
246	5-OH, 4'-OMe	7,8-ODmp; 3,6'-cyclo-C6-C3	Artumoxanthentrione	<i>Artocarpus communis</i>	Morac.	Root bark	508
247	5,2',4',5'-tetraOH	7,8-ODmp, 3,6'-cyclo-C6-C3	Artibioxanthone	<i>Artocarpus nobilis</i>	Morac.	Bark	509
				<i>Artocarpus communis</i>	Morac.	Bark	492
248	5,2',5'-triOH, 4'-OMe	7,8-ODmp, 3,6'-cyclo C6	Artumoxanthone	<i>Artocarpus communis</i>	Morac.	Root bark	3
249	5,2',4',triOH	7,8-ODmp, 3,6'-cyclo-C6-diMe-fur	Cycloartbioxanthone	<i>Artocarpus nobilis</i>	Morac.	Bark	509
				<i>Artocarpus communis</i>	Morac.	Bark	492
250	5,2',4'-triOH	7,6-ODmp; 3,6'-cyclo C6-5'-fur	Artonin M	<i>Artocarpus rigida</i>	Morac.	Bark	552
251	5,2',4'-triOH	8-C10, 7,6-ODmp-3,6' cyclo-C6-diMe-fur*	Artoindonesianin A*	<i>Artocarpus champeden</i>	Morac.	Root	510
252	5,2'-diOH, 4'-OMe	7,8-ODmp, 3,6'-cyclo C6-5'-fur*	Cycloartumoxanthone*	<i>Artocarpus communis</i>	Morac.	Root bark	511
253	5,2'-diOH, 4'-OMe	6-C5; 7,8-ODmp, 3,6'-cyclo C6-5'-fur*	Artonin F*	<i>Artocarpus communis</i>	Morac.	Bark	512
254	5,2',5'-triOH, 7-OMe	3',4'-ODmp, 3,6'-cyclo-C6-C3*, see Figure 12.6	Artonol E*	<i>Artocarpus communis</i>	Morac.	Bark	492
255	5,7,2',4'-tetraOH	6-C5, 3',4'-ODmp, 3,6'-cyclo-C6-C3*	Artonin N*	<i>Artocarpus rigida</i>	Morac.	Bark	552
256	5,2',5'-triOH	7,8-ODmp, 3',4'-ODmp, 3,6'-cyclo-C6-C3*	Artonol C*	<i>Artocarpus communis</i>	Morac.	Bark	492
257	5,7,4'-triOH, 3',6'-di-oxo	6,5'-C5; 3,6'-cyclo-C6-C3*	Artonin O*	<i>Artocarpus rigida</i>	Morac.	Bark	552
				<i>Artocarpus kemando</i>	Morac.	Stem bark	493
258	5-OH; 2',5'-di-oxo	7,8-ODmp, 3',4'-ODmp, 3,6'-cyclo-C6-C3*, see Figure 12.6	Artonol D*	<i>Artocarpus communis</i>	Morac.	Bark	492
259	5,4'-diOH, 2',5'-di-oxo	7,8-ODmp; 3,6'-cyclo-C6-C3, 2',5'-epoxy*	Artonin P*	<i>Artocarpus rigida</i>	Morac.	Bark	552
260	5OH,7OMe	6-C5, 3,6'-cyclo O-C ₆ -C ₃ *	Artoindonesianin B*	<i>Artocarpus champeden</i>	Morac.	Root	510
261	5,4'-diOH	8-C5, 7,6-ODmp, 3,6'-cyclo-OC5*	Artelastochromene*	<i>Artocarpus elasticus</i>	Morac.	Wood	447
262	5,4'diOH#	8-C5-7,6-fur-C3-OH, 3,6'-cyclo-O-C6-C3-OH*	Carpelastofuran*	<i>Artocarpus elasticus</i>	Morac.	Wood	513
263	5-OH,4'OMe	7,8-ODmp, C5-O-C5; 3,6'-cyclo-O-C5*, see Figure 12.6	Artocommunol CA*	<i>Artocarpus communis</i>	Morac.	Root cortex	451
264	5,7,4'-triOH	6-C5; 3,6'-cyclo-O-C5*	Cyclocommunin = (isocyclomulberriin*)	<i>Artocarpus altifolius</i>	Morac.	Stem	471
265	5,7,4'-triOH	6-C5, 3,6'-cyclo-O-C5	Brosimone I	<i>Artocarpus communis</i>	Morac.	Root bark	472
266	5,7,4'-triOH	6,8-diC5; 3,6'-cyclo-O-C5	Artelastin*	<i>Artocarpus elasticus</i>	Morac.	Wood	485
				<i>Brosimopsis oblongifolia</i>	Morac.	Root	477
				<i>Artocarpus elasticus</i>	Morac.	Wood	447

267	5,7,4'-triOH	6,8-diC5, 3,6'-cyclo-O-C6-C3-OH, see Figure 12.6	Artelastocarpin	<i>Artocarpus elasticus</i>	Morac.	Wood	485
268	5,4'-diOH, 7-OMe	3,6'-cyclo O-C6*	Oxyisocyclointegrin*	<i>Artocarpus elasticus</i>	Morac.	Wood	513
269	5,4'-diOH, 7-OMe	3,6'-cyclo O-C7*	Cyclointegrin*	<i>Artocarpus integrifolia</i>	Morac.	Heartwood	3
270	5,4'-diOH, 7-OMe	6-C5, 3,6'-cyclo O-C6-C3	Artonin S	<i>Artocarpus integrifolia</i>	Morac.	Heartwood	3
271	5,4'-diOH, 7-OMe	6-C5, 3,6'-cyclo-O-C5	Cycloartocarpin	<i>Artocarpus heterophyllus</i>	Morac.	Bark; shoot	446
272	5,4',5'-triOH	8-C5, 7,6-ODmp, 3,6'-cyclo-O-C5	Cycloheterophyllin				
273	5,5'-diOH, 4'-OMe	7,8-ODmp, 3,6'-cyclo-O-C5	Cycloartomunin	<i>Artocarpus communis</i>	Morac.	Root bark	511
274	5,3',4'-triOH,7-OMe	8-C5, 3,6'-cyclo-O-C5-ODmp*	Dihydro-isocycloartomunin*	<i>Artocarpus communis</i>	Morac.	Root bark	511
Flavone-coumarin hybrids							
275	5,7,4'-triOH	6-(8''-umbelliferyl)-*, see Figure 12.7		<i>Gnidia soccotrana</i>	Thymeleac.		83
276	5,7,4'-triOH	8-(6''-umbelliferyl)-*, see Figure 12.7		<i>Gnidia soccotrana</i>	Thymeleac.		83

Notes: C5 means, e.g., Me₂CH-CH=CH- or Me₂C=CH-CH₂-; C5-OH means, e.g., Me₂C(OH)CH₂-CH₂-; C10 means, e.g., geranyl- or lavandulyl-. For further possibilities see Barron and Ibrahim.³ The pyrano ring is indicated by -ODmp for oxygen-linked dimethylallyl unit. For the meaning of C3, cyclo-C6, etc. see examples in figures, as indicated for related structures.

*For explanation, please see text.

reported as a new source for desmosflavone (5-OH-7OMe-6,8-diMe flavone),⁵⁸ a compound already known from *Leptospermum scoparium* (Myrtaceae).⁵⁹

C-Formylflavones, being substituted at the 6 and 8 position (unonal and related compounds), have been reported for species of the genus *Desmos* (Annonaceae).⁶⁰⁻⁶² Another derivative with unusual substitution, dasytrichone, was isolated from two species of *Dasy-mascholon* (Annonaceae).^{63,64} Thus, the accumulation of these types of compounds in Annonaceae may be of chemosystematic significance. Muxiangrines I and II, comprising a pyrano structure and C-methyl substitution (compounds 28, 29, Table 12.3), have been isolated along with muxiangrine III (C-methyl- and prenyl-substitution; compound 30, Table 12.3) from the aerial parts of *Elsholtzia stauntonii* (Lamiaceae).⁶⁵

Drymariatin, a C-2-substituted derivative, is known as glycoside only (*Drymaria diandra*, Caryophyllaceae).⁶⁶ Another C-2-substituted flavone, hoslundal, was earlier reported from *Hoslunda* (Lamiaceae).⁶ From species of *Cassia* (Fabaceae), flavones with a C-6-acrylic acid substituent were isolated.^{67,68} This substitution pattern appears to be quite unique. Hoslundal, torosaflavone D, and its dimethyl derivative (for formulae see Figure 12.1) were all detected in either leaves or aerial parts, but not indicated as exudate compounds.

12.5.2 METHYLENEDIOXYFLAVONES

Predominantly, this substitution occurs in the 3',4'-position of ring B, rarely between neighboring OH-groups in ring A. Such compounds were reported from Fabaceae, Rutaceae, and some Asteraceae, from all parts of the plants. Only in rare cases, their accumulation as exudate constituents was documented (*Ozothamnus*, Asteraceae).^{69,70}

12.5.3 C-PRENYLFLAVONES

Linear substituted prenyl flavones exhibit a tendency towards prenylation at positions 3,6- and 8- of the flavonoid molecule. 3'-Prenylation of ring B occurs occasionally. The prenyl residue is mostly of the 3,3-dimethylallyl structure or the OH-equivalent of it. The prenyl residue "1,1-dimethylallyl" is rare. Geranylated flavones are also not very

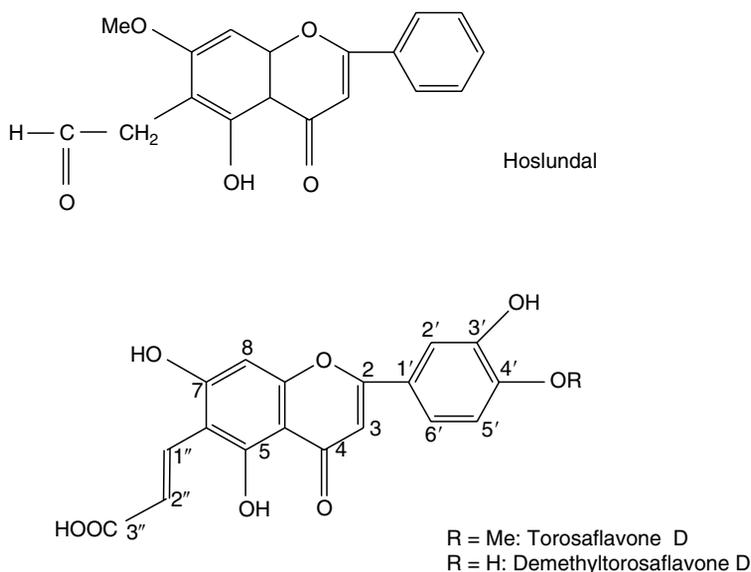


FIGURE 12.1 C₂/C₃-substituted flavones.

common (e.g., albanin D). For further types of substituents the reader may consult the review of Barron and Ibrahim.³ The 7,6-chromenoflavone carpachromene affords an example of a more widespread occurrence in species of Fabaceae, Moraceae, and Rutaceae (Table 12.3). Similar results may be expected for other complex flavones in the future.

Some problematical structures and names should be mentioned here. Revised structures concern albanins D (compound 80, Table 12.3) and E (compound 95, Table 12.3) from *Morus alba*, which are not 8-geranyl-, but 6-geranyl-derivatives of 5,7,4'-triOH and 5,7,2',4'-tetraOH flavone, respectively.⁷¹ The flavone lanceolatin A (compound 59, Table 12.3) is definitely a C-prenylated flavone, isolated originally from the stems of *Tephrosia lanceolata*.⁷² Thus, this name must not be used for a biflavone from *Lophira lanceolata* as Pegnyemb et al. have done later.⁷³ The compound 7,4'-diOH-6,3'-diC₅ flavone, isolated from the roots of *Glycyrrhiza inflata* and named "licoflavone B" by Kajiyamam et al.,⁷⁴ was later isolated from the roots of *G. glabra* and named prenyllicoflavone A (compound 70, Table 12.3).⁷⁵ Consequently, the latter name falls into the category of synonyms.

12.5.4 O-PRENYLFLAVONES

Only very few flavones of this type exist, in most cases showing various other types of substituents as well. O-prenylation is known to occur at position 6, 7, or 4'-OH. Most of the substituents are 3,3-dimethylallyl structures. Whereas epoxyprenyl derivatives have been reported from the aerial parts of *Achyrocline flaccida* (Asteraceae),⁷⁶ a new compound with 4'-O-dimethylallyl substitution was later reported from the leaves of *Ficus maxima* (compound 128, Table 12.3).⁷⁷ There is no indication of external accumulation in any of the plants listed. Millettocalyxin B (compound 129, Table 12.3) from the stem bark of *Millettia erythrocalyx* represents an example of a mixed structure (methylenedioxy- and O-prenylsubstitution). Similarly, ovalifolin (compound 131, Table 12.3) has a furano-substituent in addition and is being reported for two new sources of Fabaceae. The name ovalifolin (published 1974) has priority for this furanoflavone; its use for a structurally different compound from *Ehretia ovalifolia*⁷⁸ is, therefore, obsolete.

12.5.5 PYRANOFLAVONES

These types of flavones are characterized by cyclization between an OH-group with a prenyl residue to result in chromene or chromane structures, abbreviated as O-Dmp in Table 12.3. This section contains quite a large number of compounds and sources. Most cyclizations take place between 7-OH and 6- or 8-position of ring A, particularly observed in species of Rutaceae and Fabaceae. Additional cyclization between 2'-OH and C-3 is mainly found in members of the Moraceae, whereas cyclization between 3'-OH or 4'-OH with the neighboring prenyl is reported from Berberidaceae (yinyanghuo C, E; compounds 140, 148, Table 12.3). Pyranoflavones are reported to occur in all parts of the plants, but there are no reports explicitly citing their external accumulation. Some special structures are illustrated in Figure 12.2, such as a flavone acetylated at the chromene residue from *Pongamia pinnata* (compound 136, Table 12.3) or the tri-ODmp-substituted dorsilurin E (compound 138, Table 12.3).

12.5.6 FURANOFLAVONES

Cyclization resulting in furano-substitution is quite frequent between 7-OH and the neighboring 6 or 8-position of ring A. Most reports concentrate on Fabaceae and Moraceae, again from all parts of the plants, except external accumulation. The number of compounds known is smaller than that of the pyranoderivatives. Furano-substitution at ring B is rarely observed (e.g. epimedokoreanin A; compound 178, Table 12.3 and in Figure 12.3) from *Epimedium*

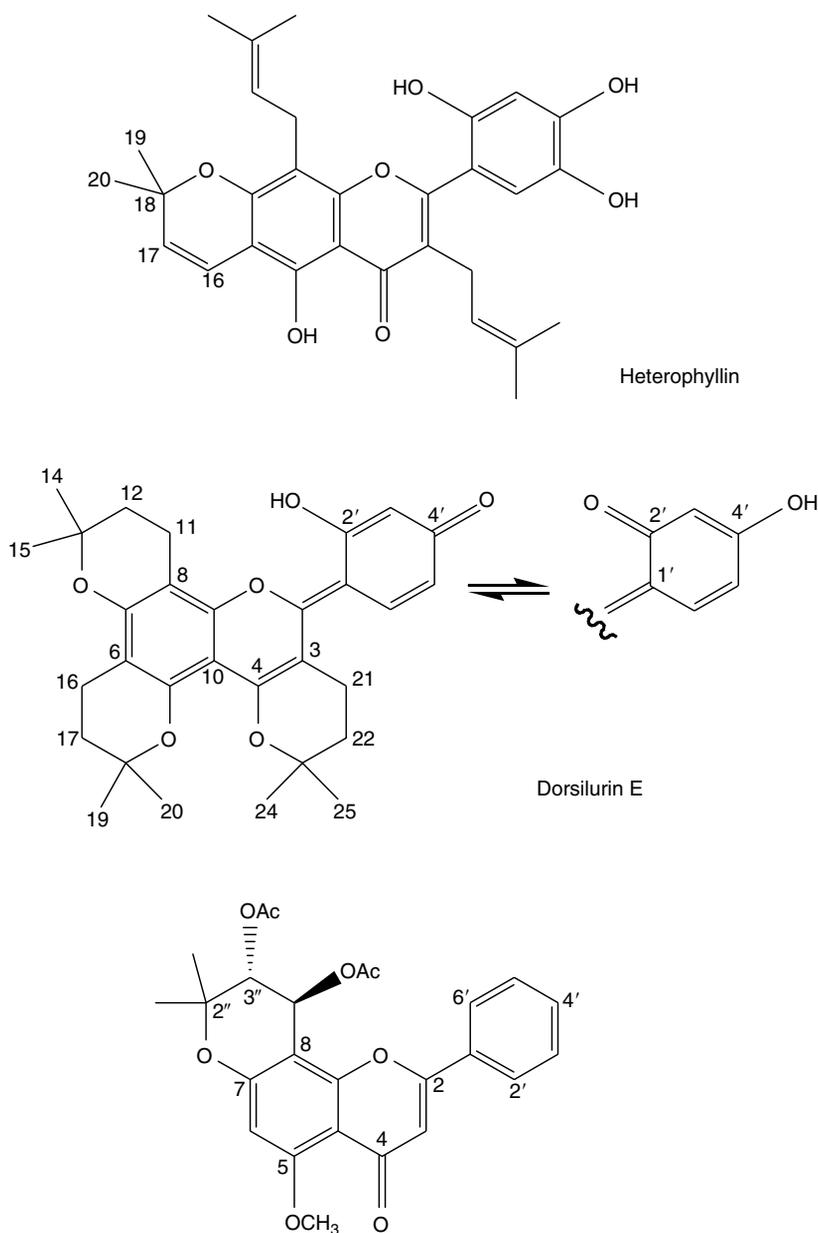


FIGURE 12.2 (Dihydro-) pyranoflavones.

(Berberidaceae). This compound exhibits a more complex furano-substitution, similar to ciliatin A (Figure 12.3) and “compound 8” (Figure 12.3) from species of Moraceae. Torosafavone C and its demethyl derivative, both isolated from *Cassia* species^{67,68} are bisfuran-substituted (compounds 182 and 183, Table 12.3 and in Figure 12.3), similar to compounds found in *Tephrosia* species (see Section 12.5.10). In *Epimedium*, there appears to be a strong tendency towards cyclization between 3'-position and 4'-OH, both in pyrano- and furano-derivatives. Further distribution studies are needed to confirm these biosynthetic trends and their possible chemosystematic significance.

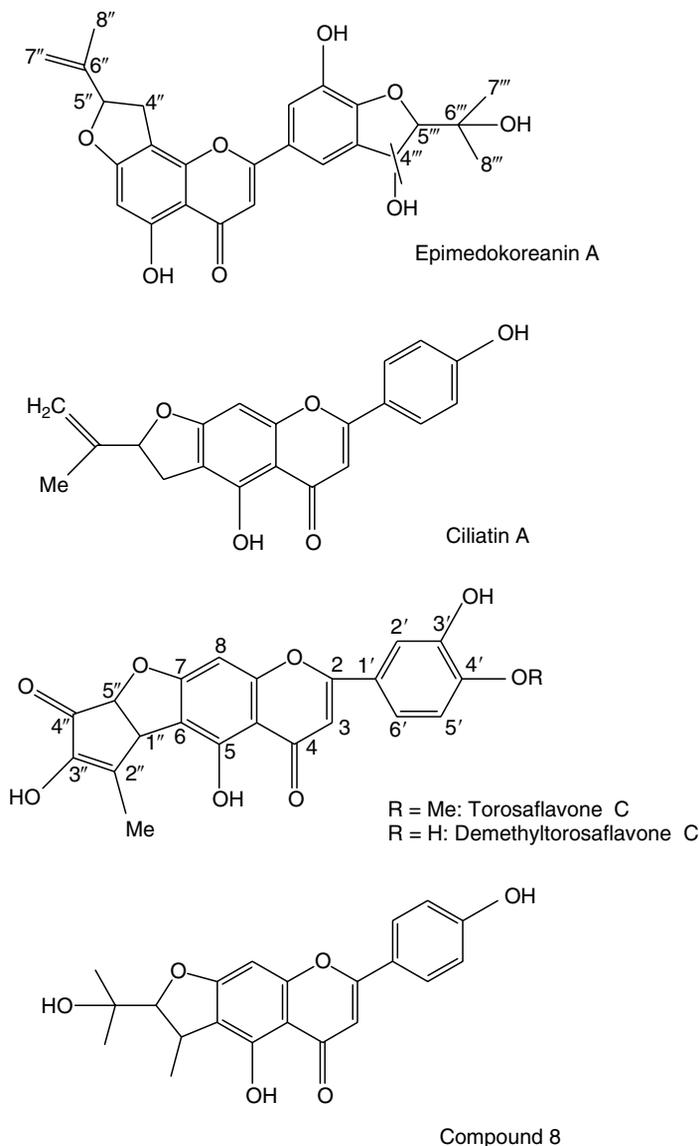


FIGURE 12.3 Furanoflavones.

12.5.7 FURANO- AND PYRANO-SUBSTITUTION

With the exception of sanaganone, all other compounds listed in this section exhibit a chromeno-structure between 7-OH and C-6 of ring A, whereas the furano-substitution occurs on ring B. During the reporting period, only sanaganone is a newly described compound, occurring in the root bark of *Millettia* (Fabaceae).⁷⁹

12.5.8 C-PRENYL- AND PYRANO-SUBSTITUTION

This section comprises a series of flavones, mostly reported from genera of the Moraceae (*Morus*, *Dorstenia*, *Artocarpus*) and the Fabaceae (*Derris*, *Tephrosia*, *Euchresta*). Very few reports exist on *Vancouveria* and *Epimedium* (Berberidaceae). In contrast to most of the other

reported structures, these Berberidaceae flavones are again B-ring-substituted only (e.g., Yinyanghuo A, compound 191, Table 12.3). The majority of the other flavones are cyclized to chromene structures between 7,6- or 7,8 of ring A, and C-5-substitution concentrates on positions 3, 6, and 8 (see heterophyllin, Figure 12.2). They have been reported from all parts of the plants, but there is a strong indication for roots and root barks as major sources. No reference exists on external accumulation of such compounds.

12.5.9 C-LINKED AROMATIC- AND KETOPYRANO-SUBSTITUTION

C-Linked aromatic substituents are reported to occur in aerial parts of *Thymus hirtus*,⁸⁰ along with one report on Chinese propolis.⁸¹ In aerial parts of some members of Asteraceae and Lamiaceae, flavones with a C-6-ketopyrano-substitution were reported (e.g., hosloppin, oppositin; see Figure 12.4). These aromatic substituents are positioned at the 6-, and rarely, at the 8-position of ring A. They are all reported from aerial parts, but no reference is made to their possible occurrence as exudates constituents.

12.5.10 TEPHROSIA FLAVONES

The genus *Tephrosia* (Fabaceae) was selected to demonstrate the biosynthetic capacity of flavone substitution. In particular, there is a strong tendency towards formation of furano-residues, linked through C-bonds on position 8 of the flavone nucleus (e.g., apollinine, hookerianin; Figure 12.5). The basic flavone structure is mostly 5- and 7-O-methylated. These compounds have been exclusively reported to occur in roots, leaf and stem as well as

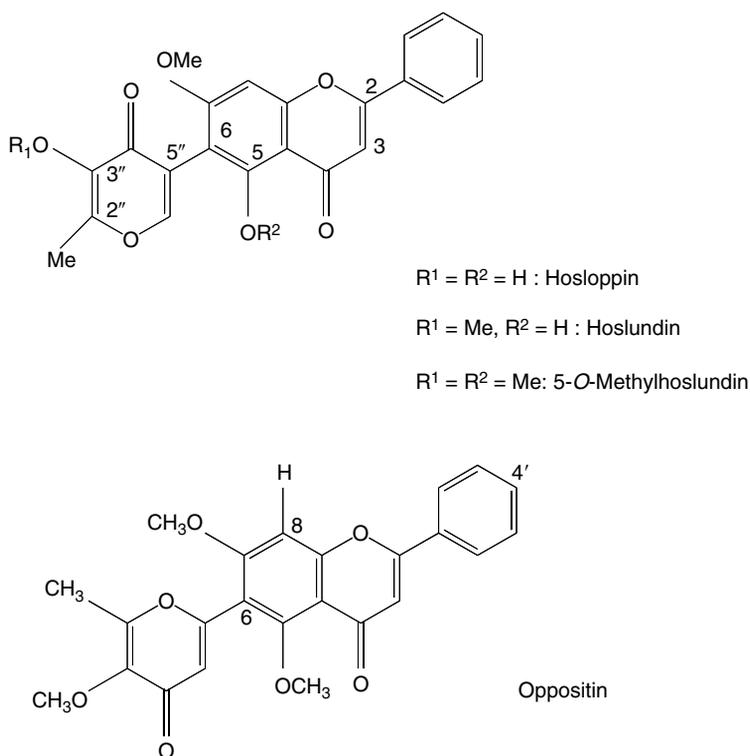


FIGURE 12.4 Flavones with ketopyrano substitution.

in seeds of *Tephrosia* species. Several compounds are 7,8-disubstituted (furanogroup between 7-OH and C-8; e.g., tephrorianin; Figure 12.5) isolated from pods.⁸² Aerial parts of *Tephrosia* spp. yielded primarily bisfuran structures, which are also 7,8-disubstituted, such as semi-glabin, multijugin, and enantiomultijugin (Figure 12.5). There is also a tendency observed towards acetylation on the bisfuran moiety. Even more complex structures arise by addition of bicyclic substituents, being combined from furano and pyrano residues (stachyoidin, tephrocin, compounds 228 and 229, Table 12.3). These structures have been known for a long time, their accumulation site, however, being not indicated.³ Only two pyranoflavones are accumulated: isopongaflavone and 5-OMe-7,6-(2,2-dimethylchromeno)-flavone (compounds 135 and 136, Table 12.3). Further compounds include C-prenylpyranoflavones such

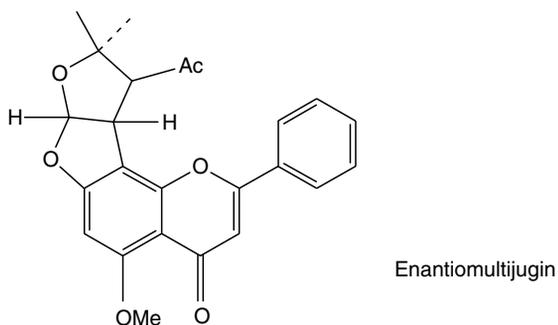
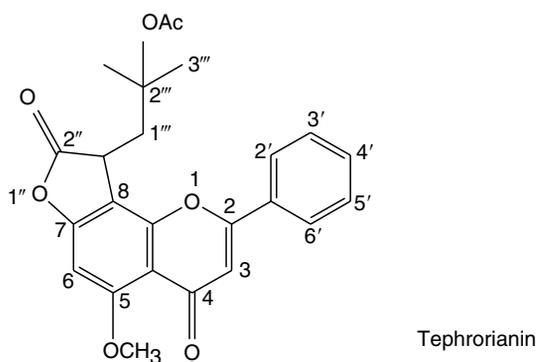
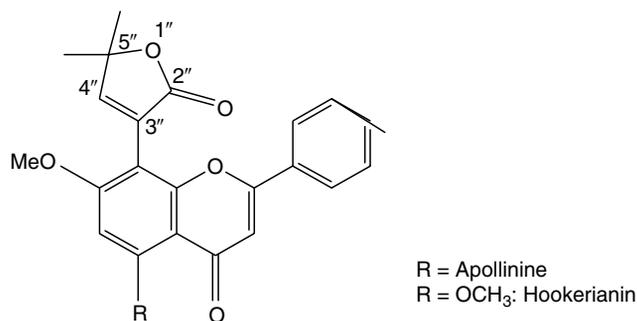


FIGURE 12.5 Furanoflavones from *Tephrosia*.

as fulvinervins (compounds 188 and 189, Table 12.3). Generally, the trend to 7-OH-C-8-substitution is quite prominent in this genus. Altogether, the biosynthetic capacity of *Tephrosia* is remarkable.

12.5.11 ARTOCARPUS FLAVONES

The genus *Artocarpus* (Moraceae) affords a prime example of biosynthetic activities to produce complex cyclized flavone structures, being primarily accumulated in addition to *C*-prenylated and pyrano-substituted flavones (see Table 12.3; Section 12.5.2 and Section 12.5.8). Apart from the pyrano substitution also encountered in other plants (see Section 12.5.5, Table 12.3), cyclization occurs between the 6'- and 3-position of the flavone molecule to yield xanthonoid structures (e.g., artonol E, D; Figure 12.6). A further and even more prominent tendency is represented by cyclization between the 2'-OH-group and the position 6 of the flavonoid nucleus. In this case, cyclization may lead to either six- or seven-membered rings including oxygen (see structures of artocommunol CA and artelastocarpin in Figure 12.6). Artonin K serves as another example of complex cyclization (Figure 12.6).

Generally, cyclization between 7-OH and C-8 occurs in various flavone derivatives of *Artocarpus* (e.g., artonol D; artocommunol CA, artelastofuran; Figure 12.6.). However, *A. rigida* yielded both 7, 6- and 7, 8-cyclized compounds (artonin M, P; compounds 250 and 259, Table 12.3). In this section, compounds with pyranosubstitution at both ring A and B are listed, for example, artonol D (Figure 12.6). All of these compounds appear to be mainly accumulated in rather lignified parts of the plant, such as heartwood, bark, and shoot. It is hardly perceivable that such complex compounds could occur in exudates of aerial parts.

12.5.12 FLAVONE-COUMARIN HYBRIDS

Only two flavones of this type are known so far. They were reported from *Gnidia soccotrana* (Thymeleaceae).⁸³ For formula see Figure 12.7. Similarly, only a few flavonols are known as hybrid structures, but none of them with coumarins (see Table 12.4).

12.6 FLAVONOLS WITH OTHER SUBSTITUENTS

Data on this type of flavonols are summarized in Table 12.4. In contrast to the corresponding flavones, the number and complexity of derivatives is smaller. This concerns particularly the formation of furano-, pyrano- and other cyclic flavonols. There is a remarkable number of *O*-prenylated flavonols known to date, contrasting to only very few flavones exhibiting this substitution pattern (see Table 12.3). Similar trends have been earlier documented in the review of Barron and Ibrahim.³ The occurrence of a series of glycosides based on *C*-prenylated structures is considerable.³ This substitution trend concerns also some of the dihydroflavonols, thus indicating specific enzyme activities probably dependent on the presence of a 3-OH group.

12.6.1 C-METHYLFLAVONOLS

In this section, reports concentrate on genera from the families Caesalpiniaceae, Myrtaceae, and Velloziaceae. Apart from the Myrtaceae, no *C*-methylflavones have been reported to occur in the other two families as yet (see Table 12.3). In Myrtaceae, *C*-methylflavonols have been found also in exudates.⁵⁷ Most of the other species listed here accumulate these flavonols in the leaves without further specification. Fungal sources include two species of *Colletotrichum*, where *C*-methylflavonols have been found in the culture filtrate.⁸⁴ So far, no C2- or C3- *C*-linked flavonols have been reported as was the case with the flavones.

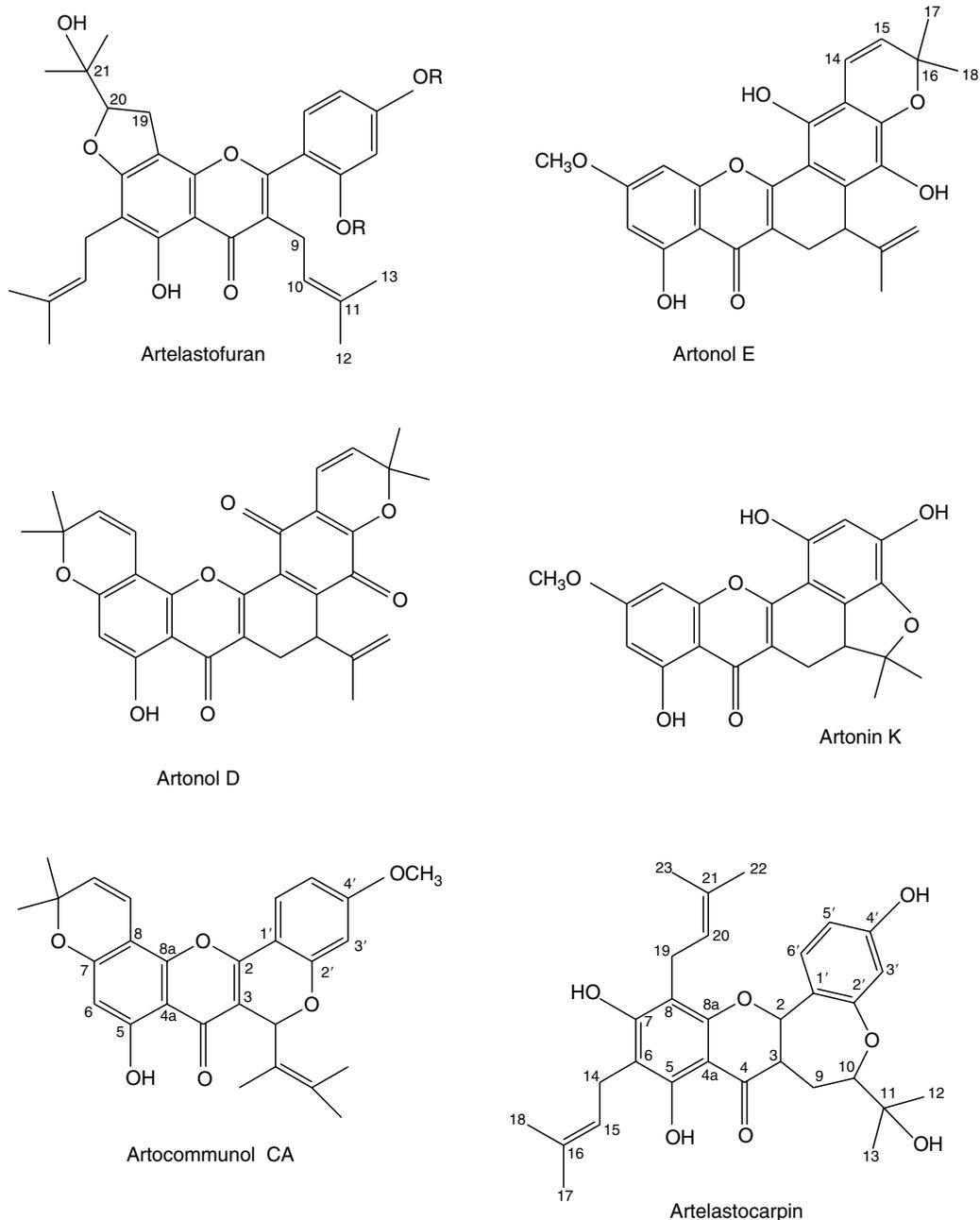
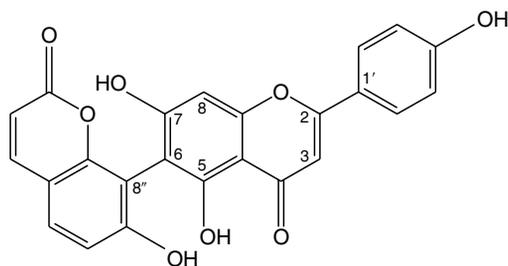


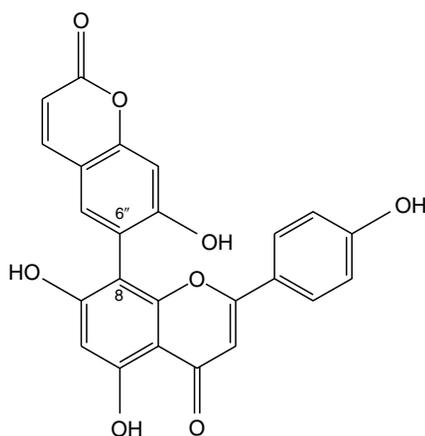
FIGURE 12.6 Flavones from *Artocarpus* spp.

12.6.2 METHYLENEDIOXYFLAVONOLS

The Rutaceae appear to be a rich source of flavonols with methylenedioxy substitution. Their main accumulation site is apparently bark tissue, followed by leaves. It may be assumed that some of these leaf constituents are accumulated externally as was also the case with the corresponding flavone derivatives of other plant families. In general, the Rutaceae exhibit a



6-(8''-Umbelliferyl)-apigenin



8-(6''-Umbelliferyl)-apigenin

FIGURE 12.7 Flavone–coumarin hybrids.

trend rather to produce flavonols of this type, whereas one corresponding flavone only (ageconylflavon A) is so far known from this family. Very few reports concern the genus *Millettia* (Fabaceae; stem bark)⁸⁵ and some Amaranthaceae (aerial parts, whole plants).⁸⁶

12.6.3 C-PRENYLFLAVONOLS

Within the complex flavonols, C-5 substitution is by far the most prominent trend in terms of numbers of compounds and sources. Also, the number of structurally different C-5-residues is remarkable. Apart from the common “3,3-dimethylallyl” and the rarer “1,1-dimethylallyl,” several hydroxylated C-5-residues such as in topazolin hydrate, isolated from roots of *Lupinus luteus* (Fabaceae; compound 94 in Table 12.4). Another source of such compounds is the whole plant of *Duranta repens* (Verbenaceae).^{87,88} Plant organs accumulating C-prenylflavonols range from buds to leaves and from aerial parts to roots. Only the bud constituents may be considered as exudate compounds. From *Lilium candidum*, a more complex flavonol was reported (Figure 12.8).⁸⁹ This genus also accumulates N-containing flavonoid derivatives discussed earlier.⁶

Some remarks should be made regarding structures revised during the reporting period. A flavonol isolated from *Glycyrrhiza lepidota*, named glepidotin, had been ascribed the structure of 8-C-prenylgalangin.⁹⁰ Comparison with the synthetic product and its isomer revealed that it is, in fact, 6-C-prenylgalangin (compound 78, Table 12.4).⁹¹ Noricaritin

TABLE 12.4
Flavonols with Other Substituents

No.	O-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
	C-Methylflavonols						
1	3,5,7-triOH	8-Me	8-Methylgalangin				
2	3,5,7-triOH	6,8-diMe					
3	5,7-diOH, 3-OMe	8-Me					
4	5,7-diOH, 3-OMe	6,8-diMe					
5	3,5-diOH, 7-OMe	8-Me					
6	3,5-diOH, 7-OMe	6,8-diMe					
7	3,5,6,7-tetraOH	8-Me	Isoplatanin				
8	3,5,7,8-tetraOH	6-Me	Platanin				
9	5,7,8-triOH, 3-OMe	6-Me					
10	3,5,7-triOH, 8-OMe	6-Me	Pityrogrammin				
11	5,6,4'-triOH, 3-OMe	8-Me	Sylpin				
12	3,5,7,4'-tetraOH	6-Me	6-Methylkaempferol				
13	5,7,4'-triOH, 3-OMe	6-Me					
14	5,7,4'-triOH, 3-OMe	6,8-diMe					
15	5,4'-diOH-3,7-diOMe	6-Me	8-Desmethyllatifolin	<i>Pilosstigma thomningii</i>	Caesalpini.	Leaf	514
16	5,4'-diOH, 3,7-diOMe	6,8-diMe	Latifolin	<i>Callistemon</i> , 4 spp.	Myrt.	External	57
17	5-OH, 3,7,4'-triOMe	6-Me	8-Desmethyalkalmiatin	<i>Leptospermum laevigatum</i>	Myrt.	Leaf wax	424
18	5-OH, 3,7,4'-triOMe	6,8-diMe	Kalmiatin	<i>Pilosstigma thomningii</i>	Caesalpini.	Leaf	514
19	5,4'-diOH-3,6,7-triOMe	6-Me*		<i>Colleotrichum denatium</i>	Fungus!	(cult. filtr.)	84
20	5,4'-diOH-3,6,7-triOMe	8-Me*		<i>Vellozia laevis</i>	Velloz.	Leaf surface	105
				<i>Vellozia namuzae</i>	Velloz.	Leaf	515
21	3,5,8,4'-OH, 7-OMe	6-Me					
22	5,8,4'-triOH, 3,7-OMe	6-Me					
23	5,4'-diOH-3,7,8-triOMe	6-Me*		<i>Colleotrichum denatium</i>	Fungus!	(cult. filtr.)	84
24	3,5,7,3',4'-pentaOH	6-Me	Pinoquercetin				
25	(3,5,7,3',4'-pentaOH)	8-Me	Glycoside only				

continued

TABLE 12.4
Flavonols with Other Substituents — continued

No.	O-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
26	3,5,7,3',4'-pentaOH	6,8-diMe		<i>Ptilostigma thonningii</i>	Caesalpin.	Leaf	514
27	5,7,3',4'-OH, 3-OMe	6-Me		<i>Vellozia phalocarpus</i>	Velloz.	Leaf	105
				<i>Vellozia phalocarpus</i>	Velloz.	Leaf	516
				<i>Xerophyta retinervis</i>	Velloz.	Leaf	105
28	5,7,3',4'-tetraOH-3-OMe	6,8-diMe*		<i>Ptilostigma thonningii</i>	Caesalpin.	Leaf	514
29	5,3',4'-triOH-3,7-diOMe	6-Me*		<i>Ptilostigma thonningii</i>	Caesalpin.	Leaf	514
30	5,3',4'-triOH-3,7-diOMe	6,8-diMe*		<i>Ptilostigma thonningii</i>	Caesalpin.	Leaf	514
31	5,7,4'-OH, 3,3'-diOMe	6-Me					
32	5,7,4'-OH, 3,3'-diOMe	6,8-diMe					
33	5,4'-diOH, 3,7,3'-OMe	6-Me		<i>Callistemon salignus</i>	Myrt.	External	57
				<i>Leptospermum laevigatum</i>	Myrt.	Leaf wax	424
				<i>Ptilostigma thonningii</i>	Caesalpin.	Leaf	514
34	5,4'-diOH, 3,7,3'-OMe	6,8-diMe					
35	5-OH-3,7,3',4'-OMe	6-Me*		<i>Leptospermum laevigatum</i>	Myrt.	Leaf wax	424
36	5,3',4'-OH-3,6,7-OMe	8-Me*		<i>Vellozia epidendroides, V. lilacina*</i>	Velloz.	Leaf	517
37	5,7,4'-OH-3,6,3'-OMe	8-Me*		<i>Vellozia nanuzae</i>	Velloz.	Leaf	515
38	5,4'-OH, 3,6,7,3'-OMe*	8-Me*		<i>Vellozia stipitata</i>	Velloz.	Whole plant	104
				<i>Vellozia nanuzae</i>	Velloz.	Leaf	515
				<i>Vellozia epidendroides, V. lilacina*</i>	Velloz.	Leaf	517
				<i>Vellozia laevis</i>	Velloz.	Leaf	516
				<i>Vellozia laevis, V. phalocarpa</i>	Velloz.	Leaf	105
39	5,3'-OH, 3,6,7,4'-OMe	8-Me*					
40	3,5,7,3',4',5'-hexaOH	6-Me	Alluaudiol				
41	5,7,3',4',5'-OH, 3-OMe	6-Me					
42	5,7,3',4',5'-OH, 3-OMe	6,8-diMe					
43	3,5,7,3',5'-OH, 4'-OMe	6-Me	Dumosol				
44	3,5,7,3',5'-OH, 4'-OMe	6,8-diMe					
45	5,7,3',5'-OH, 3,4'-OMe	6-Me					
46	5,7,3',5'-OH, 3,4'-OMe	6,8-diMe					

Methylendioxyflavonols												
47	3,5-diOMe	6,7-OCH ₂ O	Melitermin	<i>Gomphrena martiana</i> , <i>G. boliviana</i>	Amaranth.	Whole plant	150					
48	3,5-diOMe	6,7-/3',4'-diOCH ₂ O	Melitermin	<i>Melicope simplex</i> , <i>M. ternata</i>	Rut.	Bark	380					
49	3,7-diOMe	3',4'-OCH ₂ O	Desmethykanugin	<i>Comptonella microcarpa</i>	Rut.	Leaf (ext.?)	518					
50	5-OH,3,7-diOMe	3',4'-OCH ₂ O	Desmethykanugin	<i>Milletia leucantha</i>	Fab.	Stem bark	85					
51	3,5,7-triOMe	3',4'-OCH ₂ O	Isokanugin	<i>Melicope simplex</i> , <i>M. ternata</i>	Rut.	Bark	380					
52	3,5,8-triOH	6,7-/3',4'-diOCH ₂ O	Isokanugin	<i>Comptonella microcarpa</i>	Rut.	Leaf (ext.?)	518					
53	3,5,8-triOMe	6,7-OCH ₂ O										
54	3,5,8-triOMe	3',4'-OCH ₂ O										
55	3,5,8-triOMe	6,7-/3',4'-diOCH ₂ O										
56	3,5,3'-triOMe	6,7-/4',5'-diOCH ₂ O										
57	3,5,4'-triOH	6,7-OCH ₂ O	Gomphrenol									
58	3,7,3'-triOMe	3',4'-OCH ₂ O	Kanugin									
59	3,7-diOH,5,6-diOMe	3',4'-OCH ₂ O	Kanugin									
60	5-OH,3,6,7-triOMe	3',4'-OCH ₂ O	Melitimmin	<i>Melicope simplex</i> , <i>M. ternata</i>	Rut.	Bark	380					
61	3,5,6,7-tetraOMe	3',4'-OCH ₂ O	Melitimmin	<i>Melicope ternata</i>	Rut.	Bark	380					
62	5,7-diOH,3,8-diOMe	3',4'-OCH ₂ O*	Melitimmin	<i>Melicope coodeana</i>	Rut.	Leaf	374					
63	5-OH,3,7,8-triOMe	3',4'-OCH ₂ O	5-Desmethylnelitermin	<i>Melicope simplex</i>	Rut.	Bark	380					
64	7-OH,3,5,8-triOMe	3',4'-OCH ₂ O	5-Desmethylnelitermin									
65	3,5,7,8-tetraOMe	3',4'-OCH ₂ O	Melitermin	<i>Comptonella microcarpa</i>	Rut.	Leaf (ext.?)	518					
66	3,5,8,3'-tetraOMe	6,7-/3',4'-diOCH ₂ O	Melitermin	<i>Melicope ternata</i>	Rut.	Bark	380					
67	5,3',4'-OH,3-OMe	6,7-OCH ₂ O										
68	3,5,3'-triOH,4'-OMe	6,7-OCH ₂ O*										
69	5,3',4'-triOH,3-OMe	7,8-OCH ₂ O	Wharangin	<i>Blutaparon portulacoides</i>	Amaranth.	Aerial parts	519					
70	5,4'-OH,3,3'-diOMe	6,7-OCH ₂ O	Melinervin									
71	3,5,7-triOH,6,8-diOMe	3',4'-OCH ₂ O	Melinervin									
72	5,7-diOH,3,6,8-triOMe	3',4'-OCH ₂ O*										
73	3,5-diOH,6,7,8-triOMe	3',4'-OCH ₂ O										
74	7-OH,3,5,6,8-tetraOMe	3',4'-OCH ₂ O*										
75	5-OH,3,6,7,8-tetraOMe	3',4'-OCH ₂ O										
76	3,5,6,7,8-pentaOMe	3',4'-OCH ₂ O	5-Desmethylnelitermin	<i>Melicope ternata</i>	Rut.	Bark	380					
77	3,5,8,3',4'-pentaOMe	6,7-OCH ₂ O	Melitimmin Melicophyllin	<i>Melicope ternata</i>	Rut.	Bark	380					

continued

TABLE 12.4
Flavonols with Other Substituents — continued

No.	O-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
C-Prenylflavonols							
78	3,5,7-triOH	6-C5	Glepidotin A; 6-prenylgalangin; revised	<i>Platanus acerifolius</i>	Synthesis	Bud	91
79	3,5,7-triOH	8-C5	8-(1,1-dimethylallyl)-galangin		Platanac.	Bud	522
80	3,7,4'-triOH	8-C5-OH					
81	3,5,7,8-tetraOH	6-C5	Platanetin	<i>Platanus acerifolia</i>	Platanac.	Bud	46
82	3,5,7,4'-tetraOH	6-C5*	Licoflavonol*	<i>Glycyrrhiza</i> spp.	Fab.	Root	523
83	3,5,7,4'-tetraOH	6-C5-OH	Macarangin*	<i>Macaranga vedeliana</i>	Synthesis	—	91
84	3,5,7,4'-tetraOH	6-C10*		<i>Monotes africanus</i>	Euphorb.	Leaf	524
85	3,5,7,4'-tetraOH	8-C5	Noranhydroicaritin	<i>Macaranga denticulata</i>	Dipteroc.	Leaf	525
86	3,5,7,4'-tetraOH	6-C5-OH	8-(1,1-dimethylallyl)-kaempferol	<i>Epimedium koreanum</i>	Euphorb.	Leaf	526
87	3,5,7,4'-tetraOH	8-C-(3-methyl-succinoyl), see Figure 12.8	Noricaritin, revised	<i>Platanus acerifolia</i>	Berb.	Aerial parts	527
88	3,5,7,4'-tetraOH	8-C10*	Isomacarangin*	—	Platanac.	Bud	46
89	3,5,7,4'-tetraOH	3-C5	Isolicoflavonol	<i>Lilium candidum</i>	—	—	91
90	3,5,7,4'-tetraOH	6,8-diC5	6,8-Diprenylkaempferol	<i>Lilium candidum</i>	Liliac.	—	89
91	3,5,7,4'-tetraOH	6,3'-diC5	Glyasperin A; 6,3'-diprenylkaempferol	<i>Macaranga schweinfurthii</i>	Euphorb.	Leaf	528
92	3,5,7,4'-tetraOH	8,3'-diC5	Broussonetol F	<i>Glycyrrhiza spec.</i>	Fab.	Liquorice	334
93	5,7,4'-triOH, 3-OMe	6-C5	Topazolin	<i>Platanus acerifolia</i>	Platanac.	Bud	46
94	5,7,4'-triOH, 3-OMe	6-C5-OH	Topazolin hydrate	<i>Monotes africanus</i>	Dipteroc.	Leaf	525
95	3,7,4'-triOH, 5-OMe	8-C5	Sophoflavescenol	<i>Glycyrrhiza aspera</i>	Fab.	Root	529
96	3,5,4'-triOH, 7-OMe	8-C5	Isoanhydroicaritin	<i>Broussonetia papyrifera</i>	Morac.	Root bark	93
97	3,5,7-triOH, 4'-OMe	8-C5	Anhydroicaritin	<i>Lupinus luteus</i>	Fab.	Root	530
98	3,5,7-triOH, 4'-OMe	8-C5-OH	Icaritin	<i>Lupinus luteus</i>	Fab.	Root	530
99	3,5,7-triOH, 4'-OMe	8-C5-OMe*	Brevicornin*	<i>Sophora flavescens</i>	Fab.	Roots	531
100	5,7,4'-triOH, 3,6-diOMe	3'-C5-OH	Aliarin	<i>Epimedium koreanum</i>	Berb.	Aerial parts	527
				<i>Epimedium brevicornum</i>	Berb.	Aerial parts	279

101	3,7,4'-triOH, 5,6-diOMe	3'-C5-OH	Viscosol	<i>Duranta repens</i>	Verben.	Whole plant	87
102	5,7-diOH, 3,6,4'-triOMe	3-C5		<i>Duranta repens</i>	Verben.	Whole plant	88
103	5,7-diOH,3,6,4'-triOMe	3'-C5-OH*		<i>Duranta repens</i>	Verben.	Whole plant	87
104	3,7-diOH,5,6,4'-triOMe	3'-C5-OH					
105	3,5,7,2',4'-pentaOH	8-C10	Kushenol C	<i>Glycyrrhiza uralensis</i>	Fab.	Aerial parts	457
106	3,5,7,2',4'-pentaOH	8-C10-OH	Kushenol G	<i>Dorstenia ciliata</i>	Morac.	Aerial parts	436
107	3,5,7,3',4'-pentaOH	6-C5*	Gancaonin P*	<i>Glycyrrhiza uralensis</i>	Fab.		532
108	3,5,7,4'-tetraOH, 3'-OMe	6-C5	Gancanonin P -3'-Me	Glycoside only	—	—	—
109	3,5,7,3',4'-pentaOH	6-C5-OH		<i>Glycyrrhiza uralensis</i>	Fab.	Leaves	533
110	3,5,7,3',4'-pentaOH	5'-C5	Uralenol	<i>Glycyrrhiza uralensis</i>	Fab.	Leaves	534
111	3,5,7,3',4'-pentaOH	6,5'-diC5*	Broussonol E*	<i>Broussonetia papyrifera</i>	Morac.		
112	3,5,7,3',4'-pentaOH	8,5'-diC5*	Broussonol D*	<i>Broussonetia kazinoki</i>	Morac.	Leaf	98
113	3,5,7,3',4'-penta-OH	8,2',6'-triC5	8,2',6'-Triprenylquercetin	<i>Broussonetia kazinoki</i>	Morac.	Leaf	98
114	3,5,7,3',4'-pentaOH	8,2',3'-triC5*	Broussonol G*	<i>Petalostemum purpureum</i>	Fab.	Root, may be artifact	535
115	3,5,7,3',4'-pentaOH	8,2',6'-triC5	Broussonol C	renamed from Broussonol "E"			94
116	5,7,3',4'-OH-3-OMe	6-C5*		<i>Velozia coronata, V. namuzae</i>	Velloz.	Leaf surface	515
117	5,7,3',4'-tetraOH, 3-OMe	5'-C5	Uralenol-3-Me	<i>Glycyrrhiza uralensis</i>	Fab.	Leaves	536
118	5,7,3',4'-OH, 3-OMe	6,8-diC5	Broussonol B				
119	3,5,7,4'-tetraOH,3'-OMe	6,8-diC5*	Dorsmanin D*	<i>Dorstenia mannii</i>	Morac.	Twigs	454
120	5,3',4'-OH-3,7-OMe	6-C5*		<i>Velozia coronata</i>	Velloz.	Leaf surface	515
121	5,7,4'-triOH-3,8-diOMe	6-C5*	6-Prenylherbaectin*	<i>Velozia scoparia</i>	Velloz.	Leaf surface	105
122	3,5,3'-OH, 7,4'-diOMe	6-C5*	Isorhynchosperrin	<i>Artemisia campestris glutinosa</i>	Ast.	Aerial parts	277
123	3,5,3'-OH, 7,4'-diOMe	8-C5	Rhynchosperrin				
124	5,4'-diOH, 3,7,3'-OMe	8-C5	8-Prenylpachypodol	<i>Glycyrrhiza uralensis</i>	Fab.	Leaves	533
125	3,6,7,3',4'-pentaOH	2'-C5	Neouralenol	<i>Glycyrrhiza uralensis</i>	Fab.	Leaves	536
126	5,6,4',5'-tetraOH-3OMe	2'-C5	Uralene				
	O-Prenylflavonols						
127	5,7-diOH,3-OMe	4'-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
128	5,7-diOH, 3-OMe	4'-O-C5		<i>Bosstoa brassii</i>	Rut.	Leaf	537
129	5-OH, 3,8-diOMe	7-O-C5 (epoxy)	7-Epoxyprenylgnaphaliin				

continued

TABLE 12.4
Flavonols with Other Substituents — continued

No.	O-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
130	5,7-diOH,8-OMe*	3,4'-di-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
131	3,5,7-OH,6-OMe	4'-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
132	5,7-OH,3,6-OMe	4'-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
133	3,5,7-triOH, 8-OMe*	4'-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
134	5,7-diOH,3,8-diOMe*	4'-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
135	5,4'-diOH, 3,3'-diMe	7-O-C5		<i>Euodia glabra</i>	Rut.	Shoot bark	538
				<i>Melicope elleryana</i>	Rut.	Fruit	379
136	5,4'-diOH, 3,3'-diOMe	7-O-C5 (epoxy)					
137	5,7-diOH, 3,3'-diOMe	4'-O-C5		<i>Bosistoa medicinalis</i>	Rut.	Leaf	322
138	5,3'-diOH, 3,4'-diOMe	7-O-C5		<i>Melicope elleryana</i>	Rut.	Fruit	379
139	4'-OH, 3,5,3'-triOMe	7-O-C5					
140	3,5,3'-triOH,6,7-diOMe	4'-O-C10-OH	Geranioloxylatum flavone	<i>Zanthoxylum alatum</i>	Rut.	Seed	401
141	5,7-diOH, 3,8,3'-OMe*	4'-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
142	3,5,4'-triOH,8,3'-diOMe*	7-O-C5*		<i>Melicope micrococca</i>	Rut.	Aerial parts	96
143	5,4'-diOH, 3,8,3'-OMe	7-O-C5*		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
144	5,4'-diOH, 3,8,3'-OMe	7-O-C5		<i>Melicope elleryana</i>	Rut.	Fruit	379
145	4'-OH, 3,5,8,3'-tetraOMe	7-O-C5*		<i>Melicope triphylla</i>	Rut.	Leaf	521
146	3,5,8,3',4'-pentaOMe	7-O-C5		<i>Melicope triphylla</i>	Rut.	Leaf + bark	539
147	5,4'-OH, 3,6,8,3'-OMe	7-O-C5		<i>Boronia coerulescens</i>	Rut.	Aerial parts	95
148	3,5,8,4'-OH-7,3'-OMe	6-O-C5		<i>Melicope micrococca</i>	Rut.	Aerial parts	96
149	5-OH,3,8-diOMe,7-O-C5	3',4'-OCH ₂ O		<i>Melicope triphylla</i>	Rut.	Leaf	521
150	3,5,8-triOMe, 7-O-C5	3',4'-OCH ₂ O		<i>Achyrocline flaccida</i>	Ast.	Aerial parts	540
				<i>Melicope triphylla</i>	Rut.	Leaf + bark	539
152	3,5,6,8-tetraOMe-7O-C5	3',4'-OCH ₂ O		<i>Comptonella microcarpa</i>	Rut.	Leaf (ext.?)	518
				<i>Comptonella microcarpa</i>	Rut.	Leaf (ext.?)	518
Pyranoflavonols							
153	3,5,4'-triOH	7,6-ODmp	Desmethyl-anhydroicaritin				91
154	3-OMe	7,8-ODmp	Karanjachromene				541
155	3,5-diOMe	7,8-ODmp*		<i>Lonchocarpus latifolius</i>	Fab.	Root	541
156	3,6-diOMe	7,8-ODmp		<i>Lonchocarpus latifolius</i>	Fab.	Root	541

TABLE 12.4
Flavonols with Other Substituents — continued

No.	O-Substitution	Other Substituents	Trivial Name	Plant Species	Family	Plant Organ	Ref.
Flavonols with aromatic substituents							
184	3,5,7,4'-tetraOH	8-C-p-OH-benzyl [*]	<i>p</i> -Hydroxybenzyl-kaempferol [*]	<i>Thymus hirtus</i>	Lam.	Aerial parts	80
185	3,5,7,3',4'-pentaOH	8-C-p-OH-benzyl [*]	<i>p</i> -Hydroxybenzyl-querceetin [*]	<i>Thymus hirtus</i>	Lam.	Aerial parts	80
186	5,7-diOH, 3,4'-diOMe	8-C-p-OH-phenylethyl	Haplopappin	<i>Haplopappus foliosus</i>	Ast.		100
Various cyclo-flavonols							
187	5,7,4,5'-tetraOH	3,2'-O-CH ₂ -		<i>Acacia crombei</i> , <i>A. carnei</i>	Mimosac.		
188	5,7,3',4'-tetraOH-6OMe	3,2'-O-CH ₂ -	Benthiaminin	<i>Distemonanthus benthamianus</i>	Fab.	Heartwood	
189	5,7,3',4'-tetraOH-6OMe	3,2'-O-CH ₂ -	Distemonanthin	<i>Distemonanthus benthamianus</i>	Fab.	Heartwood	
190	7,4,5'-triOH	3,2'-O-CH ₂ -OH, see Figure 12.9	Fasciculiferin	<i>Acacia fasciculifera</i>	Mimosac.	Heartwood	547
191	7,4,5'-triOH	3,2'-O-CH ₂ -	Peltogynin	<i>Acacia peuce</i> , <i>A. crombei</i> , <i>A. fasciculifera</i>	Mimosac.	Heartwood	548
192	7,3',4'-triOH	3,2'-O-CH ₂ -	Moparin	<i>Colophospermum mopane</i>	Caesalpin.		549
193	5-OH, 7-OMe, 3',4'-O ₂ CH ₂	3,2'-O-CH ₂ -	Pulcherrimin	<i>Caesalpinia pulcherrima</i>	Caesalpin.	Stem	550
194	5-OH, 6,7-diOMe, 3',4'-O ₂ CH ₂	3,2'-O-CH ₂ -	6-OMe-pulcherrimin	<i>Caesalpinia pulcherrima</i>	Caesalpin.	Stem	550
195	3,5-diOH	7,8-O-cycl-phenylethyl		<i>Pityrogramma calomelanos</i>	Pteridaceae	Frond exudate	121
196	3,5,4'-triOH	7,8-O-cycl-phenylethyl	Calomelanol D	<i>Pityrogramma calomelanos</i>	Pteridaceae	Frond exudate	122
Hybrid structures							
197	3,5,7,4'-tetraOH	Diterpene-flavonol, see Figure 12.9	Denticulaflavonol	<i>Macaranga denitculata</i>	Euphorb.	Leaves	526
198	5'-OH, 3,5,2'-OMe	Flavono-lignoid		<i>Distemonanthus benthamianus</i>	Fab.	Heartwood	102

^{*}For explanation, please see text.

For abbreviations see footnote to Table 12.3.

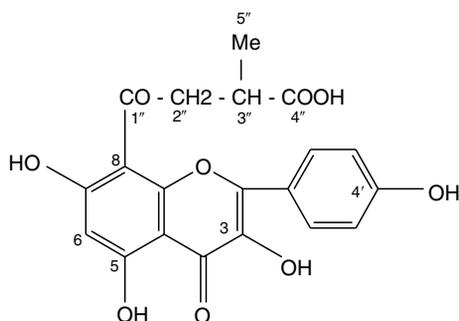


FIGURE 12.8 Flavonol from *Lilium candidum*.

(compound 86, Table 12.4), originally isolated from *Bursera leptophloeos* as 3,5,7,4'-tetraOH-8-C-hydroxyprenylflavone,⁹² needs to be revised to the corresponding 6-C-derivative.⁹¹ Finally, the structure of brousoflavonol E (3,5,7,3',4'-pentaOH-8,2',6'-triprenylflavone) from *Broussonetia papyrifera*⁹³ was later revised to 3,5,7,3',4'-pentaOH-8,2',3'-triprenylflavone and renamed brousoflavonol G (compound 114, Table 12.4).⁹⁴

12.6.4 O-PRENYLFLAVONOLS

These compounds are almost exclusively accumulated in aerial parts and leaves from genera of the Rutaceae such as *Bosistoa*, *Boronia*, and *Melicope*, and rarely in *Euodia* and *Zanthoxylum*. There is a strong tendency towards prenylation at the 7-OH or 4'-OH group. From aerial parts of *Boronia coerulescens*, a derivative with 3,4'-O-prenylation was also described (compound 130, Table 12.4).⁹⁵ Similarly, only one C-6-O-derivative was reported from the aerial parts of *Melicope*.⁹⁶ Two 7-epoxyderivatives have already been listed in the previous survey.⁶ Combination with methylenedioxy substitution is less frequent, concerning a few sources of Rutaceae and Asteraceae only. Probably, these constituents are partly accumulated externally.

12.6.5 PYRANOFLAVONOLS

In contrast to corresponding flavones, only a few structures are reported with a pyrano-substitution, mostly of the chromeno-type between the 7-OH and the neighboring C-8. Major sources are roots, aerial parts, and leaves from Rutaceae, Moraceae, and Fabaceae. The earlier reported *Asclepias syriaca* (Asclepiadaceae) affords a rare source of such structures. So far, the corresponding 7,6-chromeno structures (sarthranol) are known only from whole plants of *Hypericum japonicum* (Hypericaceae).⁹⁷

Poinsettifolin A serves as an example of a recently isolated flavonol with a C-5-unit attached to the chromene structure (Figure 12.9). Desmethylanhydroicaritin, isolated from *Bursera leptophloeos* as 3,5,4'-trihydroxy-7,8-pyrano-flavone,⁹² needs to be revised to 3,5,4'-triOH-7,6-pyrano-flavone (compound 157, Table 12.4).⁹¹

12.6.6 FURANOFLAVONOLS

Major sources of the few flavonols with furano-substitution are roots of various Fabaceae genera. With exception of ponganone XI (7,6-furano-), all compounds listed here are furano-substituted between 7-OH and C-8 of ring A. Similarly, compounds exhibiting

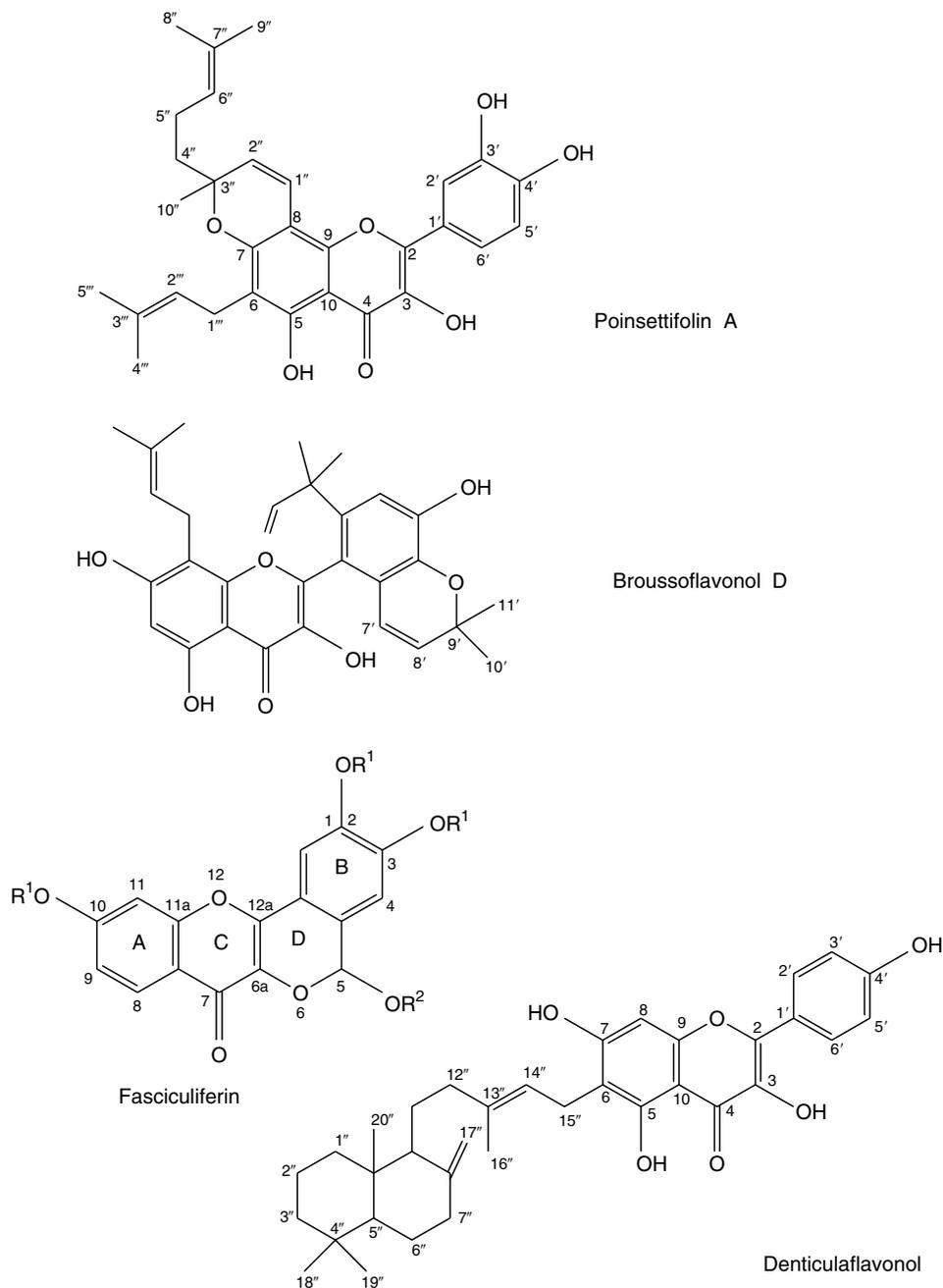


FIGURE 12.9 Complex flavonol structures.

additional substitution, such as methylenedioxy-, pyrano-, or *C*-prenylmoieties, are of limited number. During the reporting period, roots of *Lonchocarpus latifolius* (Fabaceae) were described as new source for pongapin, a methylenedioxyfuranoflavonol. Leaves of *Broussonetia kazinoki* (Moraceae) represent a new source for mixed furanoflavonols such as broussonol C and B, respectively, with no indication as to possible external accumulation.⁹⁸

12.6.7 C-PRENYL- AND PYRANO-SUBSTITUTION

Flavonols with this substitution pattern occur mainly in species of Moraceae. A related structure, petalopurpureanol was found in roots of *Petalostemon purpureus* (Fabaceae).⁹⁹ Cyclization into chromene structures may occur between 7-OH and C-6 or 7-OH and C-8, but substitution between 3'-OH and C-4' is also encountered (e.g., broussofflavonol D; Figure 12.9).

12.6.8 FLAVONOLS WITH AROMATIC SUBSTITUENTS

As with the corresponding flavones, aerial parts of *Thymus hirtus* (Lamiaceae) afforded *p*-OH-benzyl derivatives of kaempferol and quercetin, respectively.⁸⁰ Earlier, *Haplopappus foliosus* (Asteraceae) was reported to accumulate haplopappin, a phenylethyl substituted quercetin derivative.¹⁰⁰ Similar substituted flavones have also been found mainly to occur in members of Lamiaceae and Asteraceae (see Table 12.3), thus being probably chemosystematically significant accumulation trends.

Frond exudates of the genus *Pityrogramma* (Pteridaceae) afforded calomelanol D and a related structure (compounds 195, 196, Table 12.4), being cyclized between 7-OH and C-8, whereby a phenylethyl unit is further attached to the 7,8-chromene unit. A corresponding flavone derivative was also isolated.¹⁰¹ By comparison with flavanone and chalcone analogues, Iinuma et al. suggested nonenzymatical processes upon which linear and angular calomelanols should be formed and hence coined the term "tertiary metabolites" for such compounds.¹⁰¹ This could possibly apply also to some of the other complex flavone and flavonol derivatives presented in this chapter.

12.6.9 VARIOUS CYCLOFLAVONOLS

This section comprises a series of structurally different flavonols, with specific cyclization not falling in any of the other categories listed. These include the so-called "peltogynoids," which are cyclized between 3-OH and C-2' of ring B (as in fasciculiferin, Figure 12.9). They were reported from heartwood and stems of some Fabaceae and Caesalpiniaceae, respectively. 6-Methoxypulcherrimin bears a methylenedioxy group in addition. No new source has been published during the reporting period.

12.6.10 HYBRID STRUCTURES

Only two compounds are listed here, in which a flavonol molecule is linked to a biosynthetically different product such as a terpenoid (denticulaflavonol; Figure 12.9) and a flavonol-lignoid structure isolated from heartwood of *Distemonanthus benthamianus* (Fabaceae).¹⁰² In addition, the latter taxon accumulates derivatives with a 3-OH-2'-cyclization as mentioned before (see previous section). Earlier, similar substituted flavone-lignoid derivatives named scutellaprostins were reported from *Scutellaria* spp. (for references see Wollenweber⁶).

12.6.11 FURANOFLAVONOLS OF VELLOZIA

Species of the genus *Vellozia* have been extensively studied for their flavonoid complement in relation to chemosystematics.¹⁰³⁻¹⁰⁷ In addition to a series of C-methylflavonols and two C-prenylated flavonols, derivatives of vellokaempferol and velloquercetin are accumulated in whole plants, leaves, and leaf exudates. The basic structure of these compounds is characterized by 7,6-isopropenylfuran substitution, based upon kaempferol, quercetin, and their O-methyl ethers. In addition, 8-C-methyl derivatives of these compounds were also identified from leaves of *V. stipitata*.¹⁰⁴ So far, species of this genus are the only reported sources of these compounds, which in parts have been proved to be accumulated externally.¹⁰⁴⁻¹⁰⁶ Structures are exemplified by Figure 12.10.

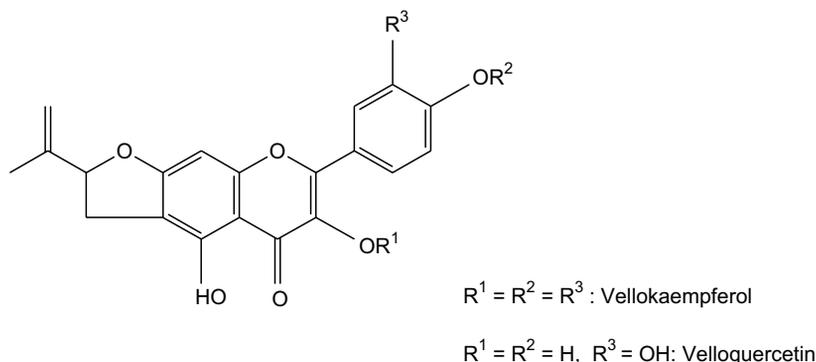


FIGURE 12.10 Furanoflavonols from *Vellozia*.

12.7 FLAVONE AND FLAVONOL ESTERS

The occurrence of acylated flavones and flavonols still appears interesting enough to justify a short paragraph on this subject (for compilation see Table 12.5). Of the flavones, only three compounds are known to date, with one newly reported isobutyrate flavone from leaf exudates of *Asarina procumbens* (Scrophulariaceae).¹⁰⁸ One further compound, the 5'-benzoate of 8,2',5'-trihydroxyflavone, was isolated recently from the exudate of *Primula palinuri* (Inuma and Wollenweber, unpublished).

By comparison, a series of mostly monoacylated flavonols is known to date and recent reports increased the number slightly. Four new products came from *Pseudognaphalium robustum* and *Tanacetum microphyllum* (both Asteraceae), and from *Adina cordifolia* (Rubiaceae). A diacetylated compound (3,5-diacetyltambulin) was recently isolated from the bark of *Zanthoxylum integrifolium* (Rutaceae).¹⁰⁹ Since most of the flavonols are monoacylated, the accumulation of quercetin tetraacetate in *Adina cordifolia*¹¹⁰ is a remarkable result. Altogether, the newly reported compounds occur scattered in the plant kingdom; their occurrence is so far of little chemosystematic value. Aerial parts of *Tanacetum microphyllum* (Asteraceae) yielded a derivative, which is structurally not an ester. It is, indeed, a carbomethoxy derivative of 6-hydroxyluteolin-4'-methyl ether (compound 34 in Table 12.5). No other flavonoid of this type is known so far.

Several of these compounds are accumulated externally as was proven for the farinose exudates of ferns, the flavonoid aglycones from *Primula* species, or those washed from the surface leaves and stems from Asteraceae. Heartwood (*Adina cordifolia*) also is a well-known accumulation site for lipophilic products. In this case, the occurrence of a natural tetraacetylflavonol is of particular interest. However, the accumulation site of the angelate of tri-*O*-methylgossypetin from *Polygonum flaccidum*¹¹¹ is unclear. Since it was isolated along with caryophyllenepoxide, borneol, sitosterin, and stigmaterol, it might well be present in some lipophilic epicuticular material. Generally speaking, the production of acylated flavonoids still is a rare phenomenon, and so far Cheilantheid ferns are the most important source of such products.

12.8 CHLORINATED FLAVONOIDS

These are extremely rare natural products. Among the flavones, 6-chloroapigenin is the only compound of this type occurring naturally. It had been isolated in 1980 from an *Equisetum* species.¹¹² The flavonol chlorflavonin (5,2'-diOH-3,7,8-triOMe-3'-chloroflavone) had been isolated from *Aspergillus candidus* already in 1969.¹¹³ Since then, only the 7-chloroderivatives

TABLE 12.5
Flavone and Flavonol Esters

No.	OH-Substitution	OMe-Substitution	Acyl Moiety	Plant Species	Family	Plant Organ	Ref.
Flavon-ester							
1	5-diOH		7- <i>O</i> -benzoate				
2	2'-diOH		5'- <i>O</i> -acetate				
3	5,4'-diOH	7,8-diOMe	6- <i>O</i> -isobutyrate*	<i>Asarina procumbens</i>	Scroph.	Leaf exudate	108
Flavonol-ester							
4	5,7-diOH	3-OMe	8- <i>O</i> -butyrate*	<i>Pseudognaphalium robustum</i>	Ast.	Resinous exudate	551
5	5,7-diOH	3-OMe	8-Me-butenolate				
6	3,5-diOH	7-OMe	8- <i>O</i> -acetate				
7	3,5-diOH	7-OMe	8- <i>O</i> -butyrate				
8	5-OH	3,7-diOMe	8- <i>O</i> -acetate				
9	5-OH	3,7-diOMe	8- <i>O</i> -butyrate				
10	5,7-diOH	3,6-diOMe	8- <i>O</i> -butyrate*	<i>Pseudognaphalium robustum</i>	Ast.	Resinous exudate	551
11	3,5,2'-triOH	7-OMe	8- <i>O</i> -acetate				
12	3,5,4'-triOH	7-OMe	8- <i>O</i> -acetate				
13	3,5,4'-triOH	7-OMe	8- <i>O</i> -butyrate				
14	3,5-diOH	7,4'-diOMe	8- <i>O</i> -acetate				
15	3,5-diOH	7,4'-diOMe	8- <i>O</i> -butyrate				
16		7,8,4'-triOMe	3,5-di- <i>O</i> -acetate*	<i>Zanthoxylum integrifolium</i>	Rutac.	Fruit	109
17	5,7,3',4'-tetraOH	(Quercetin)	3- <i>O</i> -isobutyrate				
18	3,5,7,4'-tetraOH	(Quercetin)	3'- <i>O</i> -isobut.				
19	3,5,7,3'-tetraOH	(Quercetin)	4'- <i>O</i> -isobut.				
20	3'-OH	(Quercetin)	3,5,7,4'-tetra- <i>O</i> -acetate*	<i>Adina cordifolia</i>	Naucleac.	Heartwood	110
21	5,7-diOH	3,3'-diOMe	4'- <i>O</i> -Me-butyrate				
22	5,7-diOH	3,3'-diOMe	4'- <i>O</i> -isovalerate				
23	5,7-diOH	3,6,4'-triOMe	8- <i>O</i> -tiglate				
24	5,4'-diOH	3,6,7-triOMe	8- <i>O</i> -Me-butyrate				
25	3,5,6,3'-tetraOH	4'-OMe	7- <i>O</i> -acetate*	<i>Tanacetum microphyllum</i>	Ast.	Aerial parts	395
26	5,5'-diOH	3,7,8-triOMe	2'- <i>O</i> -acetate				
27	3,5,3'-triOH	7,4'-diOMe	8- <i>O</i> -butyrate				
28	3,5,3'-triOH	7,4'-diOMe	8- <i>O</i> -butyrate				
29	5,4'-diOH	3,7,3'-triOMe	8- <i>O</i> -acetate				
30	5,7-diOH	8,3',4'-triOMe	3- <i>O</i> -angelate				
31	5,7,2'-triOH	3,4'-diOMe	5'- <i>O</i> -acetate				
32	5,7,3'-triOH	3,4'-diOMe	5'- <i>O</i> -acetate				
33	5-OH	3,7,2',3',4'-pentaOMe	8- <i>O</i> -acetate				
Carbomethoxy-flavonol							
34	3,5,3'-OH	4'-OMe	7-COOCH ₃ *	<i>Tanacetum microphyllum</i>	Ast.	Aerial parts	395

of 3,5,6,8,4'-pentamethoxyflavone and of 3,5,6,8,3',4'-hexamethoxyflavone, both isolated from leaves of *Citrus* "Dancy tangerine,"¹¹⁴ were reported as new chloroflavonols. The earlier expectation that such compounds might be found in Asteraceae, which are known for the production of other chlorinated natural products, was not fulfilled.

12.9 FLAVONOIDS OF *HELMINTHOSTACHYS*

Rhizomes of *Helminthostachys zeylanica* (Ophioglossaceae) yielded a series of complex flavone and flavonol derivatives with singular structures. They were named ugonins A–I. Whereas some flavones of this medicinally used plant are known for a long time,¹¹⁵ new data include also a series of flavonols.¹¹⁶ The complexity of these compounds is remarkable. Some of the structures are depicted in Figure 12.11.

12.10 COMMENTS ON DISTRIBUTION AND ACCUMULATION

In a compilation such as the present one, it is tempting to interpret the data regarding substitution patterns and distribution within the plant kingdom. However, the data presented here only comprise those not included in previous editions (e.g., Wollenweber⁶), hence the distribution picture sure is somewhat distorted. The new entries concern not only several families of the Angiosperms, but include also results on ferns and mosses. Although not from a plant source in the strict sense, the *C*-methylflavonols from fungi such as *Colletotrichum dematium*⁸⁴ were also included (Table 12.4). This organism is pathogenic to *Epilobium angustifolium*. The possible uptake of these compounds by the fungus from the plant can be excluded since substitution patterns of the fungal flavonols and the flavonoids from *Epilobium* do not coincide.¹¹⁷ Another fungus, *Aspergillus flavus*, proved to be able to synthesize prenylated naringenin derivatives from supplied flavanones.¹¹⁸ Apparently, these fungi are able to metabolize flavonoids from precursors, but nothing is known about the basic biosynthesis of flavonoids in these organisms.

One of the hypotheses regarding evolutionary aspects of flavonoid diversification concerns the concept of flavonol accumulation in basal Angiosperms versus flavone accumulation in advanced families. Recently, some further efforts have been made towards defining the flavone/flavonols ratio in Dicotyledonae and their relation to lignification,¹¹⁹ indicating an increased tendency towards flavonol accumulation in lignified plants, whereas herbaceous species tend to accumulate more of the flavones. From the presented entries, it appears that flavone derivatives are more abundant in Lamiaceae than flavonols. In the Asteraceae, however, more data concern the flavonols. Both families are more or less herbaceous and members of the more advanced Angiosperms.

It might be of more value to check the substitution patterns for their chemosystematic significance, as had been done earlier in frequency analysis.⁵⁶ According to current data, 6-substitution, both —OH and —OMe, appears to be more frequent than the corresponding 8-substitution in flavones. The number of their 6,8-diOMe derivatives is quite considerable though. By comparison, the number of the related 6,8-OH-flavones is restricted to a few compounds reported from natural sources (compounds 136, 222, 227, and 262 in Table 12.1). All of the other polyhydroxylated structures have so far not been found as natural products. A similar ratio between 6- and 8-substitution was found with the flavonols, but the number of naturally occurring 6,8-diOH flavonols is limited to two compounds only (compounds 239 and 289 in Table 12.2). Further accumulation trends of possible chemosystematic relevance have been discussed in the respective sections.

Aspects on substitution patterns of prenylated flavonoids and their derivatives including the frequency of occurrence have been discussed in detail.³ Thus, these aspects will not be

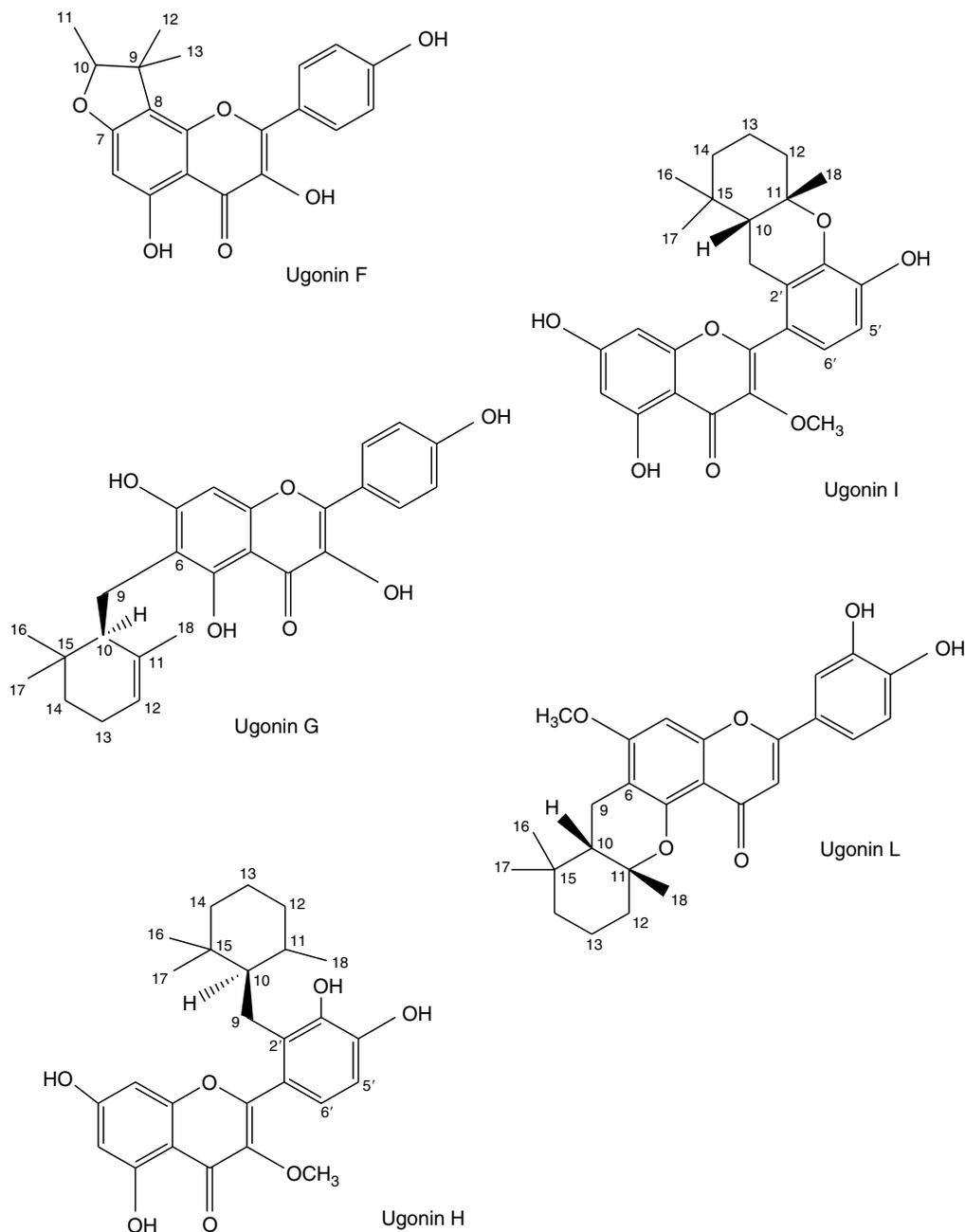


FIGURE 12.11 Flavonoids from *Helminthostachys*.

covered here. From the present data, the tendency to produce complex flavones is predominant. Such products are of limited distribution in few genera of some families only, as exemplified in the respective sections of this chapter (e.g., *Artocarpus-Moraceae*; *Tephrosia-Fabaceae*). The degree of complexity differs between flavones and flavonols. Thus, the flavones outnumber the flavonols, both in number and complexity. Interestingly, the number of *O*-prenylated flavonols is much higher than that of the corresponding flavones. The

occurrence of complex flavones and flavonols in various *Pteridophyta* and *Hepaticae* is further remarkable. Apparently, various evolutionary independent groups in the plant kingdom are able to synthesize biosynthetically complex products.

Some general comments on the external accumulation and excretion of flavonoid aglycones should be made. In most cases, flavone and flavonol methyl ethers contribute to the exudates, sometimes including methylenedioxy derivatives, *C*-methyl derivatives, and flavone and flavonol esters. Complex structures, however, are rarely reported to occur in exudates, as for example, *C*-prenylflavonols in Velloziaceae,¹⁰⁵ or 7,8-cycloflavonols from frond exudates of Pteridaceae.^{120–122} The most complex structures found in *Artocarpus* spp. are not known to be excreted, for reasons unknown. Maybe the responsible enzymes cannot be compartmented in the cells of, for example, glandular hairs, which are frequently the accumulation site of lipophilic material. For flavonoid aglycones, this was established for instance in a study on *Mentha*.¹²³ Several other studies are similarly conclusive (e.g., Heinrich et al.¹²⁴). The presence of the basic enzymes for flavonoid production in head cells of glands was reported for *Primula kewensis*.¹²⁵ *Primula* species are widely known for their production of flavonoid exudates. Afolayan and Meyer¹²⁶ hypothesized that exudate flavonoids of *Helichrysum aureonitens* were probably produced by the endoplasmatic reticulum in the secreting trichomes. There would be many more interesting aspects to be discussed, such as ecological significance (e.g., Tattini et al.¹²⁷) or ontological differentiation during plant development,¹²⁸ which would be beyond the scope of this review. Ecological and other aspects not fully discussed here have been addressed in previous publications.^{6,129,130} It is hoped that the aspects discussed here will stimulate further research outside the flavonoid community as well.

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APPENDIX

Trivial Name

Trivial Name	Substitution
Abrectorin	7,3'-diOH, 6,4'-diOMe — flavone
Acacetin	5,7-diOH, 4'-OMe — flavone
Acerosin	5,7,3'-triOH, 6,8,4'-triOMe — flavone
Ageconylflavon A	5,6,7-triOMe, 3',4'-OCH ₂ O — flavone
Ageconylflavon B	4'-OH, 5,6,7,3'-tetraOMe — flavone
Ageconylflavon C	4'-OH, 5,6,7,3',5'-OMe — flavone
Agecorynin F	5-OH, 6,7,2',3',4',5'-hexaOMe — flavone
Agecorynin G	3'-OH, 5,6,7,2',4',5'-hexaOMe — flavone
Agecorynin-C	5,6,7,8,2',4',5'-heptaOMe — flavone
Agecorynin-D	5,2',4'-triOH, 6,7,8,5'-tetraOMe — flavone
Agehoustin A	5,6,7,8,2',3',4',5'-octaOMe — flavone
Agehoustin B	5,6,7,2',3',4',5'-heptaOMe — flavone
Agehoustin C	3'-OH, 5,6,7,8,2',4',5'-heptaOMe — flavone
Agehoustin D	5,3'-diOH, 6,7,8,2',4',5'-hexaOMe — flavone
Agehoustin E	5-OH, 6,7,8,2',4',5'-hexaOMe — flavone
Agehoustin F	5,2'-diOH, 6,7,8,4',5'-pentaOMe — flavone
Albanin A	5,7,2',4'-tetraOH, 3-C5 — flavone
Albanin D, revised	5,7,4'-OH, 6-C10 — flavone
Albanin E, revised	5,7,2',4'-tetraOH, 6-C10 — flavone
Aliarin	5,7,4'-triOH, 3,6-diOMe, 3'-C5-OH — flavonol
“Allopatuletin” — obsolete	
Alluaudiol	5,7,3',4',5'-OH, 3-OMe, 6-Me — flavonol
Alnetin	5-OH, 6,7,8-triOMe — flavone
Alnusin	3,5,7-triOH, 6-OMe — flavonol
Alnustin	5-OH, 3,6,7-triOMe — flavonol
Altisin	5-OH, 7,8,2',6'-tetraOMe — flavone
Anhydroicaritin	3,5,7-triOH, 4'-OMe, 8-C5 — flavonol
Annulatin	5,7,3',4',5'-pentaOH, 3-OMe — flavonol
Apigenin	5,7,4'-triOH — flavone
Apollinine	7-OMe, 8-furyl (2'' = oxo) — flavone, see Figure 12.5
Apometzgerin	5,7,5'-triOH, 3',4'-diOMe — flavone
Apuleidin	5,2',3'-triOH, 3,7,4'-triOMe — flavonol
Apulein	2',5'-diOH, 3,5,6,7,4'-pentaOMe — flavonol
Apuleirin	7,5'-diOH, 3,5,7,3',4'-pentaOMe — flavonol
Apuleisin	5,6,2',3'-tetraOH, 3,7,4'-triOMe — flavonol
Apuleitrin	5,6,5'-triOH, 3,7,3',4'-tetraOMe — flavonol
Araneol	5,7-diOH, 3,6,8-triOMe — flavonol
Araneosol	5,7-diOH, 3,6,8,4'-tetraOMe — flavonol
Aracapillin	5,2',5'-triOH, 6,7,5'-triOMe — flavone
Arteanoflavon	5,7-diOH, 6,3',4',5'-tetraOMe — flavone
Artelasticin	5,7,2',4'-tetraOH, 3,6,8-triC5 — flavone
Artelastin	5,7,4'-triOH, 6,8-diC5, 3,6'-cycl-OC5 — flavone
Artelastocarpin	5,7,4'-triOH, 6,8-diC5, 3,6'-cyclo-O-C6-C3-OH — flavone, see Figure 12.6
Artelastochromene	5,4'-diOH, 8-C5, 7,6-ODmp, 3,6'-cycl-OC5 — flavone
Artelastofuran	5,4'-diOH, 7,8-dihydrofuroDmp-OH — flavone, see Figure 12.6
Artemetin	5-OH, 3,6,7,3',4'-pentaOMe — flavonol
Artobilochromene	5,2',4',5'-tetraOH, 3'-C5, 7,6-ODmp — flavone

continued

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Artobiloxanthone	5,2',4',5'-tetraOH, 7,8-ODmp, 3,6'-cyclo-C6-C3 — flavone
Artocarpesin	5,7,2',4'-tetraOH, 6-C5 — flavone
Artocarpetin	5,2',4'-triOH, 7-OMe — flavone
Artocarpetin A	5,2',4'-triOH,7-OMe, 8-C5 — flavone
Artocarpetin B	5,4'-diOH, 7,2'-OMe, 8-C5 — flavone
Artocarpin	5,2',4'-triOH, 7-OMe, 3,6-diC5 — flavone
Artocommunol CA	5-OH,4'OMe, 7,8-ODmp, C5-O-C5; 3,6'-cyclo-O-C5 — flavone, see Figure 12.6
Artocommunol CC	5,4'-diOH, 3-C5-OH, 7,8-ODmp — flavone
Artocommunol CD	5,7,2',4'-tetraOH, 8-C5, 3-C10 — flavone
Artoindonesianin A	5,2',4'-triOH, 8-C10, 7,6-ODmp-3,6' cyclo-C6-diMe-fur — flavone
Artoindonesianin B	5-OH, 7OMe, 6-C5, 3,6'-cyclo O-C6-C3 — flavone
Artoindonesianin P	5,7,2',4'-tetraOH, 3,6'cyclo-C6-diMe-fur — flavone
Artoindonesianin Q	5,2',5'-OH, 7,4'-OMe, 3-C5 — flavone
Artoindonesianin R	5,7,5', OH-2',4'-OMe, 3-C5 — flavone
Artoindonesianin S	5,2',5'-triOH, 7,4'-diOMe, 3,6'-cyclo C6-C3 — flavone
Artoindonesianin T	5,7,2',5'-tetraOH, 4'-OMe, 3,6'-cyclo C6-C3 — flavone
Artomunoxanthentrione	5-OH, 4'-OMe, 7,8-ODmp; 3,6'-cycloC6-C3 — flavone
Artomunoxanthone	5,2',5'-triOH, 4'-OMe, 7,8-ODmp, 3,6'-cyclo C6 — flavone
Artonin E (“KB-3”)	5,2',4',5'-tetraOH, 3-C5, 7,8-ODmp — flavone
Artonin F	5,2'-diOH, 4'-OMe, 6-C5;7,8-ODmp, 3,6'-cyclo C6-5'-fur — flavone
Artonin J	5,7,2',4'-tetraOH, 3,6'-cycloC6-5'-fur; 4'-C5 — flavone
Artonin K	5,2',4'-triOH, 7-OMe, 3,6'-cyclo C6-5'-fur — flavone, see Figure 12.6
Artonin L	5,4'-diOH, 7,2'-diOMe, 3,6'-cyclo C6-5'-fur — flavone
Artonin M	5,2',4'-triOH, 7,6-ODmp; 3,6'-cyclo C6-5'-fur — flavone
Artonin N	5,7,2',4'-tetraOH, 6-C5, 3',4'-ODmp, 3,6'-cycloC6-C3 — flavone
Artonin O	5,7,4'-triOH, 3',6'-di-oxo, 6,5'-C5; 3,6'-cycloC6-C3 — flavone
Artonin P	5,4'-dOH, 2',5'-di-oxo, 7,8-ODmp; 3,6'-cycloC6-C3, 2',5'-epoxy — flavone
Artonin S	5,4'-diOH, 7-OMe, 6-C5, 3,6'-cyclo O-C6-C3 — flavone
Artonin T	5,2',4'-triOH, 7-OMe, 3,6'-cycloC6-5'-fur; 4'-C5 — flavone
Artonin U	5,4'-diOH, 7-OMe, 8-C5 — flavone
Artonol C	5,2',5'-triOH, 7,8-ODmp, 3',4'-ODmp, 3,6'-cyclo-C6-C3 — flavone
Artonol D	5-OH, 2',5'-di-oxo, 7,8-ODmp, 3',4'-ODmp, 3,6'-cyclo-C6-C3 — flavone, see Figure 12.6
Artonol E	5,2',5'-triOH, 7-OMe, 3',4'-ODmp, 3,6'-cyclo-C6-C3 — flavone, see Figure 12.6
Asplenetin	5,7,3',4',5'-pentaOH, 3-C5 — flavone
Atalantoflavon	5,4'-diOH, 7,8-ODmp — flavone
Auranetin	3,6,7,8,4'-pentaOMe — flavonol
Australon A	5,2',4'-triOH, 7,6-ODmp-C5 — flavone
Axillarin	5,7,3',4'-tetraOH, 3,6-diOMe — flavonol
Ayanin	5,3'-diOH, 3,7,4'-triOMe — flavonol
Azaleatin	3,7,3',4'-tetraOH, 5-OMe — flavonol
Baicalein	5,6,7-triOH — flavone
Baohuosu	5,7,4'-OH. 3',5'-OMe, 8-C5 — flavone
Benthamianin	5,7,3',4'-tetraOH, 6OMe, 3,2'-O-CH2 — flavonol
Betuletol	3,5,7-triOH, 6,4'-diOMe — flavonol
Bonanzin	5,7-diOH, 3,6,3',4'-tetraOMe — flavonol
Brevicornin	3,5,7-triOH, 4'-OMe, 8-C5-OMe — flavone
Brickellin (revised)	5,2'-diOH, 3,6,7,4',5'-pentaOMe — flavonol
Brosimacutin F	7,4'-diOH, 8-C5(OH)2 — flavone

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Brosimone G	5,2',4'-triOH, 7,8-ODmp-C5 — flavone
Brosimone H	5,2',4'-triOH, 7-OMe, 3-C5, 8-C10 — flavone
Brosimone I	5,7,4'-triOH, 6-C5, 3,6'-cycl-O-C5 — flavone
Broussoflavonol A	5,3',4'-OH, 3-OMe, 8-C5, 7,6-ODmp — flavonol
Broussoflavonol B	5,7,3',4'-OH, 3-OMe, 6,8-diC5 — flavonol
Broussoflavonol C	3,5,7,3',4'-pentaOH, 8,2',6'-triC5 — flavonol
Broussoflavonol D	3,5,7,5'-tetraOH, 8,2'-diC5, 3',4'-ODmp — flavonol, see Figure 12.9
Broussoflavonol E	3,5,7,5'-tetraOH, 8,2'-diC5, 3',4'-ODmp — flavonol
Broussoflavonol F	3,5,7,4'-tetraOH, 8,3'-diC5 — flavonol
Broussoflavonol G	3,5,7,3',4'-pentaOH, 8,2',3'-triC5 — flavonol
Broussoflavonol A	3,5,7,3'tetraOH, 4',5'ODmp-8-C5 — flavonol
Broussoflavonol B	3,5,3'triOH, 4',5'-ODmp, 7,8-triMe-fur — flavonol
Broussoflavonol C	3,5,3',4'-tetraOH, 5'-C5; 7,8-triMe-fur — flavonol
Broussoflavonol D	3,5,7,3',4'-pentaOH, 8,5'-diC5 — flavonol
Broussoflavonol E	3,5,7,3',4'-pentaOH, 6,5'-diC5 — flavonol
Bucegin	5,7-diOH, 8,4'-diOMe — flavone
Calomelanol D	3,5,4'-triOH, 7,8-O-cycl-phenylethyl — flavonol
Calycopterin	5,4'-diOH, 3,6,7,8-tetraOMe — flavonol
Candidin (syn.: Isopongaflavone)	
Candidol	3,4'-diOH, 5,6,7-triOMe — flavonol
“Candiron” — obsolete	
Cannflavin A	5,7,4'-triOH, 3'-OMe, 6-C10 — flavone
Cannflavin B	5,3'-diOH, 7,4'-diOMe, 6-C5 — flavone
Carajuflavone	6,7,3',4'-tetraOH, 5-OMe — flavone
Carpachromene	5,4'-diOH, 7,6-ODmp — flavone
Carpelastofuran	5,4'diOH, 8-C5-7,6-fur-C3-OH, 3,6'-cyclo-O-C6-C3-OH — flavone
Caryatin	7,3',4'-triOH, 3,5-diOMe — flavonol
Casticin	5,3'-diOH, 3,6,7,4'-tetraOMe — flavonol
Centaureidin	5,7,3'-triOH, 3,6,4'-triOMe — flavonol
Cerosillin	5,6,3',5'-tetraOMe — flavone
Cerosillin B	5,6,3',4',5'-pentaOMe — flavone
Chlorflavonin	5,2'-diOH, 3,7,8-triOMe, 3'-chloro — flavonol
6-Chloroapigenin	5,7,4'-triOH, 6-chloro — flavone
Chrysin	5,7-diOH — flavon
Chrysoeriol	5,7,4'-triOH, 3'-OMe — flavone
Chrysofenetin	5,4'-diOH, 3,6,7,3'-tetraOMe — flavonol
Chrysofenol-C	5,6,4'-triOH, 3,7,3'-triOMe — flavonol
Chrysofenol-D	5,3',4'-triOH, 3,6,7-triOMe — flavonol
Chrysofelin	5,4'-diOH, 3,6,7,2'-tetraOMe — flavonol
Ciliatin A	5,4'-diOH, 7,6-dihydrofur-C3 — flavone, see Figure 12.3
Ciliatin B	7,4'-diOH, 3'-OMe, 5,6-ODmp — flavone
Cirsilineol	5,4'-diOH, 6,7,3'-triOMe — flavone
Cirsiliol	5,3',4'-triOH, 6,7-diOMe — flavone
Cirsimaritin	5,4'-diOH, 6,7-diOMe — flavone
Citrusinol	3,5,4'-triOH, 7,8-ODmp — flavonol
Combretol	5-OH, 3,7,3'4'5'-pentaOMe — flavonol

continued

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Conyzatin	5,7-diOH, 3,8,3',4',5'-pentaOMe — flavonol
Corymbosin	5-OH, 7,3',4',5'-tetraOMe — flavone
Cudraflavone C	5,7,2',4'-tetraOH, 3,6-diC5 — flavone
Cudraflavone D	5,7,2',4'-tetraOH, 6,5'-diC5 — flavone
Cycloaitilisin	5,7,5'-triOH,4'-OMe, 3,6'-cyclo C6-C3; 6-C5 — flavone
Cycloartobiloxanthone	5,2',4',triOH, 7,8-ODmp, 3,6'cyclo-C6-diMe-fur — flavone
Cycloartocarpesin	5,2',4'-triOH, 7,6-ODmp — flavone
Cycloartocarpin	5,4'-diOH, 7-OMe, 6-C5, 3,6'-cycl-O-C5 — flavone
Cycloartomunin	5,5'-diOH, 4'-OMe, 7,8-ODmp, 3,6'-cycl-O-C5 — flavone
Cycloartomunoxanthone	5,2'-diOH, 4'-OMe, 7,8-ODmp, 3,6'-cyclo C6-5'-fur — flavone
Cyclochampedol	5,7,3',4'-tetraOH, 2',3-ODmp — flavone
Cyclocommunin (syn.: Isocyclomulberrin)	5,7,4'-triOH, 6-C5, 3,6'-cycl-O-C5 — flavone
Cyclocommunol	5,7,4'-triOH, 3,6'-ODmp — flavone
Cycloheterophyllin	5,4',5'-triOH, 8-C5, 7,6-ODmp, 3,6'-cycl-O-C5 — flavone
Cyclointegrin	5,4'-diOH, 7-OMe, 3,6'-cyclo O-C7 D202 — flavone
Cyclomorusin (syn.: Cyclomulberrochromene)	5,4'-diOH, 7,8-/3,6'-ODmp — flavone
Cyclomulberrin	5,7,4'-triOH, 8-C5, 2',3-ODmp — flavon
Cyclomulberrochromene (syn.: Cyclomorusin)	
Dasytrichone	5-OH, 6-Me, 8-diMe, 7=O — flavone
Datin	3,5,2'-triOH, 7-OMe — flavonol
Datisectin	3,5,7,2'-tetraOH — flavonol
Demethyltorosaflavone C	5,3',4'-triOH, 7,6- bisfurano — flavone, see Figure 12.3
Demethyltorosaflavone D	5,7,3',4'-tetraOH, 6-acrylic acid — flavone, see Figure 12.1
Denticulaflavonol	3,5,7,4'-tetraOH, diterpene-flavonol — flavonol, see Figure 12.9
Desmethylcentaureidin	5,7,3'-triOH, 6,4'-diOMe — flavone
Desmethyلسudachitin	5,7,4'-triOH, 6,8-diOMe — flavone
Desmethylanhydroicaritin	3,5,4'-triOH, 7,6-ODmp — flavonol
Desmethyldigicitrin	3,5,3'-triOH, 6,7,8,4',5'-pentaOMe — flavonol
8-Desmethyleucalyptin	5-OH, 7,4'-diOMe, 6-Me — flavone
8-Desmethyلكalmiatin	5-OH, 3,7,4'-triOMe, 6-Me — flavonol
Desmethyلكanugin	3,7-diOMe, 3',4'-OCH2O — flavonol
8-Desmethyllatifolin	5,4'-diOH, 3,7-diOMe, 6-Me — flavonol
5-Desmethyلمelibentoin	5-OH, 3,6,7,8-tetraOMe, 3',4'-O2CH2 — flavonol
5-Desmethyلمeliterin	5-OH, 3,7,8-triOMe, 3',4'-OCH2O — flavonol
5-Desmethyلمobiletin	5-OH, 6,7,8,3',4'-pentaOMe — flavone
8-Desmethyلسideroxylin	5,4'-diOH, 7-OMe, 6-Me — flavone
Desmosflavone	5-OH, 7-OMe, 6,8-diMe — flavone
Digicitrin	5,3'-diOH, 3,6,7,8,4',5'-hexaOMe — flavonol
Dihydrofuranoartobilichromene a	5,3',4'-triOH, 7,6-ODmp, 6',5'-dihydrofur-2''-C3 — flavone
Dihydrofuranoartobilichromene b1	5,3',6'-triOH, 7,6-ODmp, 4',5'-dihydrofur-2''-C3 — flavone
Dihydrofuranoartobilichromene b2	5,3',6'-triOH, 7,6-ODmp, 4',5'-dihydrofur-2''-C3 — flavone
Dillenetin	3,5,7-triOH, 3',4'-diOMe — flavonol
5,6-Dimethoxypongapin	3,5,6-triOMe, 3',4'-O2CH2, 7,8-fur — flavonol
Dihydroisocycloartomunin	5,3',4'-triOH,7-OMe, 8-C5, 3,6'-cycl.-O-C5-ODmp — flavone
8-(1,1-Dimethylallyl)-galangin	3,5,7-triOH, 8-C5 — flavonol
8-(1,1-Dimethylallyl)-kaempferol	3,5,7,4'-tetraOH, 8-C5 — flavonol
6,8-Dimethylapigenin	5,7,4'-triOH, 6,8-diMe — flavone
Diosmetin	5,7,3'-triOH, 4'-OMe — flavone

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
6,8-Diprenylkaempferol	see Glyasperin A
Dinklagin B	5,4'-diOMe, 7,6-ODmp-OH — flavone
Distemonanthin	5,7,3',4'-tetraOH, 6-OMe, 3,2'-O-CH ₂ — flavonol
Dorsilurin A	5,7,2',4'-tetraOH, 6,8,3'-triC ₅ — flavone
Dorsilurin B	5,2',4'-triOH, 3,6-diC ₅ , 7,8-ODmpOH — flavone
Dorsilurin C	3,5,7-triOH, 6,8-diC ₅ -4',3'-ODMp — flavonol
Dorsilurin D	5,7,2',4'-tetraOH, 3,6,8-triC ₅ — flavone
Dorsilurin E	2'-OH, 4,3/6,5/7,8-triODmp, 4' = O... — flavone, see Figure 12.2
Dorsmanin C	3,5,3',4'-tetraOH, 7,8-ODmp-6-C ₁₀ — flavonol
Dorsmanin D	3,5,7,4'-tetraOH, 3'-OMe, 6,8-diC ₅ — flavonol
Dumosol	3,5,7,3',5'-pentaOH, 4'-OMe, 6-Me — flavonol
Echioidinin	5,2'-diOH, 7-OMe — flavone
Emmaosunin	5-OH, 3,6,7,8,3'-pentaOMe — flavonol
Enantiomultijugin	5-OMe, 7,8-bisfur — flavone, see Figure 12.5
Ephedroidin	5,7,4'-OH, 8-C ₅ -OH — flavone
Epimedokoreanin A	5,3'-diOH, 7,8-dihydrofur-2''-C ₃ /4',5'-dihfur-OH-5''-C ₃ -OH... — flavone, see Figure 12.3
Epimedokoreanin B	5,7,3',4'-tetraOH, 8,5'-diC ₅ — flavone
7-Epoxyrenylgnaphaliin	5-OH, 3,8-diOMe, 7-O-C ₅ (epoxy) — flavonol
Eriostemin	3,8-diOH, 5,6,7,4'-tetraOMe — flavonol
Ermanin	5,7-diOH, 3,4'-diOMe — flavonol
Eucalyptin	5-OH, 7,4'-diOMe, 6,8-diMe — flavone
Eupalestin	5,6,7,8,5'-pentaOMe, 3',4'-OCH ₂ O — flavone
Eupalitin	3,5,4'-triOH, 6,7-diOMe — flavonol
Eupatilin	5,7-diOH, 6,3',4'-triOMe — flavone
Eupatin	3,5,3'-triOH, 6,7,4'-triOMe — flavonol
Eupatolitin	3,5,3',4'-tetraOH, 6,7-diOMe — flavonol
Eupatoretin	3,3'-diOH, 5,6,7,4'-tetraOMe — flavonol
Eupatorin	5,3'-diOH, 6,7,4'-triOMe — flavone
Europetin	3,5,3',4',5'-pentaOH, 7-OMe — flavonol
Exoticin	3,5,6,7,8,3',4',5'-octaOMe — flavonol
Farnisin	7,3'-diOH, 4'-OMe — flavone
Fasciculiferin	7,4,5'-triOH, 3,2'-O-CH ₂ -OH — flavonol, see Figure 12.9
Ferrugin	5,7-diOH, 3',4',5'-triOMe — flavonol
Fisetin	3,7,3',4'-tetraOH — flavonol
Flindulatin	5-OH, 3,7,8,4'-tetraOMe — flavonol
Fulvinervin B	5-OH, 6-C ₅ , 7,8-ODmp — flavone
Fulvinervin C	5-OH, 6-C ₅ -OH, 7,8-ODmp — flavone
Galangin	3,5,7-triOH — flavonol
Gancaonin O	5,7,3',4'-tetraOH, 6-C ₅ — flavone
Gancaonin P	3,5,7,3',4'-pentaOH, 6-C ₅ — flavonol
Gancaonin Q	5,7,4'-triOH, 6,3'-diC ₅ — flavone
Ganhuangenin	5,7,3',6'-tetraOH, 8,2'-diOMe — flavone
Gardenin A	5-OH, 6,7,8,3',4',5'-hexaOMe — flavone
Gardenin B	5-OH, 6,7,8,4'-tetraOMe — flavone
Gardenin C	5,3'-diOH, 6,7,8,4',5'-pentaOMe — flavone
Gardenin D	5,3'-diOH, 6,7,8,4'-tetraOMe — flavone

continued

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Gardenin E	5,3',5'-triOH, 6,7,8,4'-tetraOMe — flavone
Genkwanin	5,4'-diOH, 7-OMe — flavone
Geraldol	3,7,4'-triOH, 3'-OMe — flavonol
Geraldone	7,4'-diOH, 3'-OMe — flavone
Geranioloxylatum flavone	3,5,3'-triOH-6,7-diOMe, 4'-O-C10-OH — flavonol
Glabone	4'-OMe, 7,6-fur — flavone
Glabratephrin	7,8-bisfur — flavone
Glabratephrinol	7,8-bisfur — flavone
Glepidotin A	3,5,7-triOH, 6-C5 — flavonol
Glyasperin A	3,5,7,4'-tetraOH, 6,3'-diC5 — flavonol
Glycyrrhiza-flavonol A	3,5,7-triOH, 4',3'-cycl-OC5-OH — flavonol
Gnaphalin	5,7-diOH, 3,8-diOMe — flavonol
Gomphrenol	3,5,4'-triOH, 6,7-OCH2O — flavonol
Gossypetin	3,5,7,8,3',4'-hexaOH — flavonol
Grantiodinin	5-OH, 3,6,7,8,2,5'-hexa-OMe — flavonol
Grantioidin	5-OH, 3,6,7,2'5'-pentaOMe — flavonol
Grantionin	7-OH, 6,3',5'-triOMe — flavone
Haplopappin	5,7-diOH, 3,4'-diOMe, 8-C-p-OH-phenylethyl — flavonol
Herbacetin	3,5,7,8,4'-pentaOH — flavonol
Heteroartoinin A	5,7,2',5'-OH-4', OMe, 3,3'-diC5 — flavone
Heterophyllin	5,2',4',5'-tetraOH, 3,8-diC5; 7,6-ODmp — flavone, see Figure 12.2
Hibiscetin	3,5,7,8,3',4',5'-heptaOH — flavonol
Hispidulin	5,7,4'-triOH, 6-OMe — flavone
Honyucitrin	5,7,4'-triOH, 3',5'-diC5 — flavone
Hookerianin	5,7-diOMe, 8-diMe-oxo-furano — flavone, see Figure 5
Hosloppin	5-OH,7-OMe, 6,5''-Ketopyrano... 3''-OH — flavone, see Figure 12.4
Hoslundin	5-OH, 7-OMe, 6,5''-Ketopyrano... 3''-Me — flavone, see Figure 12.4
5-Hydroxyauranetin	5-OH, 3,6,7,8,4'-pentaOMe — flavonol
6-Hydroxygalangin	3,5,6,7-tetraOH — flavonol
8-Hydroxygalangin	3,5,7,8-tetraOH — flavonol
5'-Hydroxymorin	3,5,7,2',4',5'-hexaOH — flavonol
6-Hydroxykaempferol	3,5,6,7,4'-pentaOH — flavonol
6-Hydroxyluteolin	5,6,7,3',4'-pentaOH — flavone
6-Hydroxymyricetin	3,5,6,7,3',4',5'-OH — flavonol
4'-Hydroxywogonin	5,7,4'-triOH, 8-OMe — flavone
Hymenoxin	5,7-diOH, 6,8,3',4'-tetraOMe — flavonol
Hypolaetin	5,7,8,3',4'-pentaOH, — flavone
Icaritin	3,5,7-triOH, 4'-OMe, 8-C5-OH — flavonol
Integrin	5,2',4'-triOH, 7-OMe, 3-C5 — flavone
Inucrithmin	3,7,4',5'-tetraOH, 6,3'-diOMe — flavonol
Isoanhydroicaritin	3,5,4'-triOH, 7-OMe, 8-C5 — flavonol
Isoartocarpin	5,7,4'-triOH, 6,2'-diC5 — flavone
Isocyclomorusin	5,4'-diOH, 7,6-/3,6'-ODmp — flavone
Isocyclomulberrin (syn.: Cyclocommunin)	
Isoetin	5,7,2',4',5'-pentaOH — flavone
Isognaphalin	5,8-diOH, 3,7-diOMe — flavonol
Isokaempferide	5,7,4'-triOH, 3-OMe — flavonol
Isokanugin	3,5,7-triOMe, 3',4'-OCH2O — flavonol

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Isolaxifolin	5,4'-diOH, 8-C5, 7,6-ODmp — flavone
Isolicoflavonol	3,5,7,4'-tetraOH, 3'-C5 — flavonol
Isolimocitrol	3,5,7,3'-tetraOH, 6,8,4'-triOMe — flavonol
Isomacarangin	3,5,7,4'-tetraOH, 8-C10 — flavonol
Isoplatanin	3,5,6,7-tetraOH, 8-Me — flavonol
Isopongaflavone (syn.: Candidin)	5-OMe, 7,8-ODmp — flavone
Isopongaglabol	4'-OH, 7,8-fur — flavone
Isoprato	4'-OH, 7-OMe — flavone
Isorhamnetin	3,5,7,4'-tetraOH, 3'-OMe — flavonol
Isorhynchosperrin	3,5,3'-OH, 7,4'-diOMe, 6-C5+D348 — flavonol
Isoscutellarein	5,7,8,4'-tetraOH — flavone
Isosinensetin	5,7,8,3',4'-pentaOMe — flavone
Isothymusin	5,8,4'-triOH, 6,7-diOMe — flavone
Isounonal	5,7-diOH, 6-Me, 8-CHO — flavone
Izalpinin	3,5-diOH, 7-OMe — flavonol
Jaceidin	5,7,4'-triOH, 3,6,3'-triOMe — flavonol
Jaceosidin	5,7,4'-triOH, 6,3'-diOMe — flavone
Kaempferide	3,5,7-triOH, 4'-OMe — flavonol
Kaempferol	3,5,7,4'-tetraOH — flavonol
Kalmiatin	5-OH, 3,7,4'-triOMe, 6,8-diMe — flavonol
Kanjone	6-OMe, 7,8-fur — flavone
Kanugin	3,7,3'-triOMe, 3',4'-OCH ₂ O — flavonol
Kanzakiflavon-1	5,8-diOH, 4'-OMe, 6,7-O ₂ CH ₂ — flavone
Kanzakiflavon-2	5,4'-diOH, 6,7-OCH ₂ O — flavone
Kanzonol D	7,4'-diOH, 3'-C5 — flavone
Kanzonol E	7-OH, 6-C5, 3',4'-ODmp — flavone
Karanjachromene	3-OMe, 7,8-ODmp — flavonol
Karanjin	3-OMe, 7,8-fur — flavonol
KB-1	5,2',4',5'-tetra-OH, 7,8-ODmp, 3,6'-cyclo C ₆ — flavone
KB-2	5,2',4',5'-tetraOH, 3-C5, 7,8-ODmp — flavone
Kumatakenin	5,4'-diOH, 3,7-diOMe — flavonol
Kushenol C	3,5,7,2',4'-pentaOH, 8-C10 — flavonol
Kushenol G	3,5,7,2',4'-pentaOH, 8-C10-OH — flavonol
Kuwanon B	5,7,2'-triOH, 3-C5, 3',4'-ODmp — flavone
Kuwanon C (syn.: Mulberrin)	
Kuwanon S	5,7,4'-triOH, 3'-C10 — flavone
Kuwanon T	5,7,2',4'-tetraOH, 3,3'-diC ₅ — flavone
Laciniatin	3,5,7,3'-tetraOH, 6,4'-diOMe — flavonol
Ladanein	5,6-diOH, 7,4'-diOMe — flavone
Lanceolatin A	7-OMe, 8-C5-OH — flavone
Lanceolatin B	7,8-fur — flavone
Laricitrin	3,5,7,4',5'-pentaOH, 3'-OMe — flavonol
Latifolin	5,4'-diOH, 3,7-diOMe, 6,8-diMe — flavonol
Laurentinol	3,7,4'-triOH, 3',5'diOMe — flavone
Laxifolin	5,4'-diOH, 6-C5, 7,8-ODmp — flavone
Lethedocin	5,5'-diOH, 7,3',4'-triOMe — flavone

continued

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Licoflavone A	7,4'-diOH, 6-C5 — flavone
Licoflavone B	7,4'-diOH, 6,3'-diC5 — flavone
Licoflavone C	5,7,4'-triOH, 8-C5 — flavone
Licoflavonol	3,5,7,4'-tetraOH, 6-C5 — flavonol
Limocitrin	3,5,7,4'-tetraOH, 8,3'-diOMe — flavonol
Limocitrol	3,5,7,4'-tetraOH, 6,8,3'-triOMe — flavonol
Linderoflavone A	5,7-diOH, 6,8-diOMe, 3',4'-OCH ₂ O — flavone
Linderoflavone B	5,6,7,8-tetraOMe, 3',4'-OCH ₂ O — flavone
Luteolin	5,7,3',4'-tetraOH — flavone
Macaflavone I	3,3',4'-triOH, 7,6-ODmp, 8-C5 — flavonol
Macaflavone II	3',4'-diOH, 3-OMe, 7,6-ODmp, 8-C5 — flavonol
Macarangin	3,5,7,4'-tetraOH, 6-C10 — flavonol
Marionol	3-OH, 5,6,7,3',4'-pentaOMe — flavonol
Matteuorin	5,7-diOH, 6,8-diMe — flavon
Mearnsetin	3,5,7,3',5'-pentaOH, 4'-OMe — flavonol
Melanoxetin	3,7,8,3',4'-pentaOH — flavonol
Melibentin	3,5,6,7,8-pentaOMe, 3',4'-OCH ₂ O — flavonol
Melicophyllin	3,5,8,3',4'-pentaOMe, 6,7-OCH ₂ O — flavonol
Melinervin	3,5,7-triOH, 6,8-diOMe, 3',4'-OCH ₂ O — flavonol
Melisimplexin	3,5,6,7-tetraOMe, 3',4'-OCH ₂ O — flavonol
Melisimplin	5-OH, 3,6,7-triOMe, 3',4'-OCH ₂ O — flavonol
Melitermatin	3,5-diOMe, 6,7-/3',4'-diOCH ₂ O — flavonol
Melitermin	3,5,7,8-tetraOMe, 3',4'-OCH ₂ O — flavonol
6-Methylapigenin	5,7,4'-triOH, 6-Me — flavone
8-Methylapigenin	5,7,4'-triOH, 8-Me — flavone
8-Methylgalangin	3,5,7-triOH, 8-Me — flavonol
Methylgnaphalin	5-OH, 3,7,8-triOMe — flavonol
6-Methylkaempferol	3,5,7,4'-tetraOH, 6-Me — flavonol
6-Methyluteolin	5,7,3',4'-tetraOH, 6-Me — flavone
7-Methyltricin	5,4'-diOH, 7,3',5'-triOMe — flavone
7-Methylwogonin	5-OH, 7,8-diOMe — flavone
Mikanin	3,5-diOH, 6,7,4'-triOMe — flavonol
Milletenin C	6,7-diOMe, 3',4'-OCH ₂ O — flavone
Millettocalyxin A	7,2'diOMe, 4', 5'-OCH ₂ O — flavone
Millettocalyxin B	7-OMe-6-OC5, 4',5'-OCH ₂ O — flavone
Millettocalyxin C	2',5'-diOMe, 7,8-fur+D426 — flavone
Moparin	7,3',4'-triOH, 3,2'-O-CH ₂ — flavonol
Moralbanone	5,7,2',4'-tetraOH, 8-C15 — flavone
Morelosin	3,5,7-triOH, 3',5'-diOMe — flavonol
Morin	3,5,7,2',4'-pentaOH — flavonol
Morusignin L	5,2',4'-triOH, 7,6-ODmp, 3-C5OH — flavone
Morusin (syn.: Mulberrochromene)	5,2',4'-triOH, 3-C5, 7,8-ODmp — flavone
Mosloflavone	5-OH, 6,7-diOMe — flavone
Moslosooflavone	5-OH, 7,8-diOMe — flavone
Mulberrin (syn.: Kuwanon C)	5,7,2',4'-tetraOH, 3,8-diC5 — flavone
Mulberrochromene (syn.: Morusin)	
Multijugin	5-OMe, 7,8-bisfur — flavone
Multijuginol	5-OMe, 7,8-bisfur — flavone

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Murrayanol	5,4'-diOH, 3,6,7,3',5'-pentaOMe — flavonol
Muxiangrine I	5,3'-diOH, 7-OMe, 6,8-diCH ₃ , 4',3'-ODmp — flavone
Muxiangrine II	5,5'-diOH, 7-OMe, 6-CH ₃ , 4',3'-ODmp — flavone
Muxiangrine III	5,3',4'-triOH-7-OMe, 6,8-diCH ₃ -5'-C ₅ — flavone
Myricetin	3,5,7,3',4',5'-hexaOH — flavonol
Natsudaidain	3-OH, 5,6,7,8,3',4'-hexaOMe — flavonol
Negletein	5,6-diOH, 7-OMe — flavone
Neouralenol	3,6,7,3',4'-pentaOH, 2'-C ₅ — flavonol
Nepetin	5,7,3',4'-tetraOH, 6-OMe — flavone
Nevadensin	5,7-diOH, 6,8,4'triOMe — flavone
Nobiletin	6,6,7,8,3',4'-hexaOMe — flavone
Nodifloretin	5,6,7,4'-tetraOH, 3'OMe — flavone
Noranhydro-icaritin	3,5,7,4'-tetraOH, 8-C ₅ — flavonol
Norartocarpetin	5,7,2'4'-tetraOH — flavone
Norartocarpin (syn.: Mulberrin)	
Noricaritin (revised)	3,5,7,4'-tetraOH, 6-C ₅ -OH — flavonol
Norwightin	5,7,8,2',3'-pentaOH — flavone
Norwogonin	5,7,8-triOH — flavone
Ombuin	3,5,3'-triOH, 7,4'-diOMe — flavonol
Onopordin	5,7,3',4'-tetraOH, 8-OMe — flavone
Oppositin	5,7-diOMe, 6,6''-Ketopyrano... 3''-OH — flavone, see Figure 12.4
Oroxilin	5,7-diOH, 6-OMe — flavone
Ovalifolin	6-O-C ₅ , 7,8-fur — flavone
Oxidihydroartocarpesin	5,7,2',4'-tetraOH, 6-C ₅ -OH — flavone
Oxyayanin-A	5,2',5'-triOH, 3,7,4'-triOMe — flavonol
Oxyayanin-B	5,6,3'-triOH, 3,7,4'-triOMe — flavonol
Oxydihydromorusin	5,2',4'-triOH, 3-C ₅ -OH, 7,8-ODmp — flavone
Oxyisocyclointegrin	5,4'-diOH, 7-OMe, 3,6'-cyclo O-C ₆ — flavone
Pachypodol	5,4'-diOH, 3,7,3'-triOMe — flavone
Patuletin	3,5,7,3',4'-pentaOH, 6-OMe — flavone
Pectolinarigenin	5,7-diOH, 6,4'-diOMe — flavone
Pedalitin	5,6,3',4'-tetraOH, 7-OMe — flavone
Pediflavone	5,8-diOH, 7-OMe — flavone
“Pedunculin” — obsolete	
Peltogynin	7,4,5'-triOH, 3,2'-O-CH ₂ — flavonol
Penduletin	5,4'-diOH, 3,6,7-triOMe — flavone
Petalopurpureol	3,5,7,4'-tetraOH, 3',2'-ODmp-C ₆ — flavonol
<i>p</i> -Hydroxybenzyluteolin	5,7,3',4'-tetraOH, 8-C- <i>p</i> -OH-benzyl — flavone
<i>p</i> -Hydroxybenzyldiosmetin	5,7,3'-OH, 4'-OMe, 8-C- <i>p</i> -OH-benzyl — flavone
<i>p</i> -Hydroxybenzylkaempferol	3,5,7,4'-tetraOH, 8-C- <i>p</i> -OH-benzyl — flavonol
<i>p</i> -Hydroxybenzylquercetin	3,5,7,3',4'-pentaOH, 8-C- <i>p</i> -OH-benzyl — flavonol
Pilloin	5,3'-diOH, 7,4'-diOMe — flavone
Pilosin	5,7,8-triOH, 6,4'-diOMe — flavone
Pinnatin	5-OMe, 7,6-fur — flavone
Pinoquercetin	3,5,7,3',4'-pentaOH, 6-Me — flavonol
Pityrogrammin	3,5,7-triOH, 8-OMe, 6-Me — flavonol

continued

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Platanetin	3,5,7,8-tetraOH, 6-C5 — flavonol
Platanin	3,5,7,8-tetraOH, 6-Me — flavonol
Poinsettifolin A	3,5,3',4'-tetraOH, 7,8-ODmp, 6-C5 — flavonol, see Figure 12.9
Pollenitin	3,5,8,4'-tetraOH, 7-OMe — flavonol
Polystachin	5-OMe, 7,8-fur-C4-diOAc — flavone
Pongachromene	3,5-diOMe, 3',4'-OCH ₂ O, 7,8-ODmp — flavonol
Pongaglabol	5-OH, 7,8-fur — flavone
Ponganone XI	3-OMe, 7,6-fur — flavonol
Pongapin	3-OMe, 3',4'-OCH ₂ O, 7,8-fur — flavonol
Pongone	3'-OMe, 7,6-fur — flavone
Pratensin A	5,7-diOH, 3,6,4'-triOMe, 8-tigliat — flavonol
Pratensin B	5,4'-diOH, 3,6,7-triOMe, 8-Me-but — flavonol
Pratoletin	3,5,8,4'-tetraOH — flavonol
6-Prenylapigenin	5,7,4'-triOH, 6-C-5 — flavone
8-Prenylapigenin, see Licoflavone C	
6-Prenylchrysin	5,7-diOH, 6-C5 — flavone
8-Prenylchrysin	5,7-diOH, 8-C5 — flavone
6-Prenylchrysoeriol	5,7,4'-triOH, 3'-OMe, 6-C5 — flavone
6-Prenylgalangin, see Glepidotin A	
6-Prenylherbacetin	5,7,4'-triOH, 3,8-diOMe, 6-C5 — flavonol
Prenyllicoflavone A (syn.: licoflavone B)	
6-Prenylluteolin, see Gancaonin O	
8-Prenylluteolin	5,7,3',4'-tetraOH, 8-C5 — flavone
8-Prenylpachypodol	5,4'-diOH, 3,7,3'-OMe, 8-C5 — flavonol
Primetin	5,8-diOH — flavone
Primuletin	5-OH — flavone
Prosogerin A	7-OH, 6-OMe, 3',4'-OCH ₂ O — flavone
Prosogerin C	6,7,3',4',5'-pentaOMe — flavone
Prosogerin D	7-OH, 6,3',4',5'-tetraOMe — flavone
Prosogerin E	6,7-diOH, 3',4',5'-triOMe — flavone
Prudomestin	3,5,7-triOH, 8,4'-diOMe — flavonol
Pseudosemiglabin	7,8-bisfur -OAc — flavone
Pseudosemiglabinol	7,8-bisfur -OH — flavone
Psiadiarabicin	5,3'-diOH, 6,7,2',4',5'-pentaOMe — flavone
Ptaeroxyol	3,5,7-triOH, 2'-OMe — flavonol
Pulcherrimin	5-OH, 7-OMe, 3',4'-O ₂ CH ₂ , 3,2'-O-CH ₂ — flavonol
Purpurascenin	3,5,6,7,8,2',4',5'-octaOMe — flavonol
Quercetagetin	3,5,6,7,3',4'-hexaOH — flavonol
Quercetin	3,5,7,3',4'-pentaOH — flavonol
Racemoflavon	5,4'-diOH, 3'-OMe, 7,8-ODmp — flavone
Rehderianin I	5,2',5'-triOH, 7,8-diOMe — flavone
Retusin	5-OH, 3,7,3',4'-tetraOMe — flavonol
Rhamnazin	3,5,4'-triOH, 7,3'-diOMe — flavonol
Rhamnetin	3,5,3',4'-tetraOH, 7-OMe — flavonol
Rhamnocitrin	3,5,4'-triOH, 7-OMe — flavonol
Rhynchosin	3,6,7,3',4'-pentaOH — flavonol
Rhynchospermin	3,5,3'-OH, 7,4'-diOMe, 8-C5 — flavonol
Rivularin	5,2'-diOH, 7,8,6'-triOMe — flavone

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Robinetin	3,7,3',4',5'-pentaOH — flavonol
Rubraflavone A	7,2',4'-triOH, 3-C10 — flavone
Rubraflavone C	5,7,2',4'-tetraOH, 3-C10, 6-C5 — flavone
Rubraflavone D	5,2',4'-triOH, 3-C10, 7,6-ODmp — flavone
Saltillin	5-OH, 4'-OMe, 7-Me — flavone
Salvigenin	5-OH, 6,7,4'-triOMe — flavone
Sanaganone	6,5-ODmp, 7,8-fur — flavone
Sanggenon J	5,7,2'-triOH, 3-C5, 3',4'-ODmp-C5 — flavone
Sanggenon K	5,7,2'-triOH, 3-C5, 2',3'-ODmp-C5 — flavone
Santin	5,7-diOH, 3,6,4'-triOMe — flavonol
“Santoflavone” — obsolete	
Sarothranol	5,3',4'-OH, 3-OMe, 7,6-ODmp — flavonol
Sarothrin	5,7,4'-triOH, 3,6,8-triOMe — flavonol
Scaposin	5,7,5'-triOH, 6,8,3',4'-tetraOMe — flavone
Scutellarein	5,6,7,4'-OH — flavone
Scutevulin	5,7,2'-triOH, 8-OMe — flavone
Selgin	5,7,4',5'-tetraOH, 3'-OMe — flavone
Semiglabin	7,8- bisfur — flavone
Semiglabinol	7,8- bisfur — flavone
Sericetin	3,5-diOH, 8-C5, 7,6-ODmp — flavonol
Serpyllin	5-OH, 7,8,2',3',4'-pentaOMe — flavone
Sexangularetin	3,5,7,4'-tetraOH, 8-OMe — flavonol
Sideritiflavon	5,3',4'-triOH, 6,7,8-triOMe — flavone
Sideroxylin	5,4'-diOH, 7-OMe, 6,8-diMe — flavone
Sinensetin	5,6,7,3',4'-pentaOMe — flavone
Skullkapflavone I	5,2'diOH, 7,8-diOMe — flavone
Skullkapflavone II	5,2'-diOH, 6,7,8,6'-tetraOMe — flavone
Sophoflavescenol	3,7,4'-triOH, 5-OMe, 8-C5 — flavonol
Sorbifolin	5,6,4'-triOH, 7-OMe — flavone
Spinacetin	3,5,7,4'-tetraOH, 6,3'-diOMe — flavonol
Stachyoidin	5-OMe, 7,8-pyr-fur (3''-oxo) — flavone
Strobochrysin	5,7-diOH, 6-Me — flavone
Sudachitin	5,7,4'-triOH, 6,8,3'-triOMe — flavone
Sylpin	5,6,4'-triOH, 3-OMe, 8-Me — flavonol
Syringetin	3,5,7,4'-tetraOH, 3',5'-diOMe — flavonol
Syzalterin	5,7,4'triOH, 6,8-diMe — flavone
Tabularin	5,7-diOH, 6,2',4',5'-tetraOMe — flavone
Tachrosin	5,7-diOMe, 8-furyl (2''-oxo) — flavon
Takakin	5,7,8-triOH, 4'-OMe — flavone
Tamadone	5,2',4'-triOH, 6,7,8-triOMe — flavon
Tamaridone	5,7,2'-triOH, 6,4'-diOMe — flavone
Tamarixetin	3,5,7,3'-tetraOH, 4'-OMe — flavonol
Tambulin	3,5-diOH, 7,8,4'-triOMe — flavonol
“Tanetin” — obsolete	
Tangeretin	5,6,7,8,4'-pentaOMe — flavone
Tectochrysin	5-OH, 7-OMe — flavone

continued

APPENDIX

Trivial Name — *continued*

Trivial Name	Substitution
Tenaxin 1	5,2'-diOH, 6,7,8-triOMe — flavone
Tephriinone	5-OH, 7-OMe, 8-C5 — flavone
Tephrodin	5-OMe, 7,8-pyr-fur (3''-oxo-4''-OAc) — flavone
Tephroglabrine	7-OMe, 8-furyl (4''-oxo) — flavone
Tephrorianin	5-OMe, 7,8-oxofuryl — flavone, see Figure 5
<i>cis</i> -Tephrostachin	5,7-diOMe, 8-C5-OH — flavone
Tepurindol	7-OMe, 8-furyl (2'',4''-diOH) — flavone
Ternatin	5,4'-diOH, 3,7,8,3'-tetraOMe — flavonol
Thevetiaflavon	7,4'-diOH, 5-OMe — flavone
Thymonin	5,6,4'-triOH, 7,8,3'-triOMe — flavone
Thymusin	5,6,4'-triOH, 7,8-diOMe — flavone
Tithonine	3'-OH, 7,4'-diOMe — flavone
Tomentin	5,6,3',4'-tetraOH, 3,7-diOMe — flavonol
Topazolin	5,7,4'-triOH, 3-OMe, 6-C5 — flavonol
Topazolin hydrate	5,7,4'-triOH, 3-OMe, 6-C5-OH — flavonol
Torosaflavone C	5,3'-diOH, 4'-OMe, 7,6- bisfurano — flavone, see Figure 12.3
Torosaflavone D	5,7,3'-triOH, 4'-OMe, 6-acrylic acid — flavone, see Figure 12.1
<i>trans</i> -Anhydrotephronin	5,7-diOMe, 8-C5 — flavone
Transilitin	7,8,3',4'-tetraOH, 3-OMe — flavonol
<i>trans</i> -Lanceolatin (syn.: Lanceolatin A)	
<i>trans</i> -Tephrostachin	5,7-diOMe, 8-C5-OH — flavone
Tricetin	5,7,3',4',5'-pentaOH — flavone
Tricin	5,7,4'-triOH, 3',5'-diOMe — flavone
Trimethylwogonin	5,6,7-triOH, 8,3',4',5'-tetraOMe — flavone
8,2',6'-Triprenylquercetin	3,5,7,3',4'-penta-OH, 8,2',6'-triC5 — flavonol
Ugonine C	5, 4'-diOH, 6-OMe, 7,8-fur — flavone
Umhugengerin	5-OH, 6,7,3',4',5'-pentaOMe — flavone
Unonal	5,7-diOH, 8-Me, 6-CHO — flavone
Uralene	5,6,4',5'-tetraOH, 3OMe, 2'-C5 — flavonol
Uralenol	3,5,7,3',4'-pentaOH, 5'-C5 — flavonol
Velutin	5,4'-diOH, 7,3'-diOMe — flavone
Veronicafolin	3,5,4'-triOH, 6,7,3'-triOMe — flavonol
Viscidulin I	3,5,7,2',6'-pentaOH — flavonol
Viscidulin III	3,5,7,3'-tetraOH, 2',4'-diOMe — flavon
Viscosol	5,7-diOH, 3,6,4'-triOMe, 3'-C5 — flavonol
Wharangin	5,3',4'-triOH, 3-OMe, 7,8-OCH ₂ O — flavonol
Wightin	5,3'-diOH, 7,8,2'-triOMe — flavone
Wogonin	5,7-diOH, 8-OMe — flavone
Xanthomicrol	5,4'-diOH, 6,7,8-triOMe — flavone
Yinyanghuo A	5,7-diOH, 5'-C5-OH, 4',3'-ODmp — flavone
Yinyanghuo B	5,7,4'-triOH, 3'-C5, 5'-C5-OH — flavone
Yinyanghuo C	5,7-diOH, 4',3'-ODmp — flavone
Yinyanghuo D	5,7,4'-triOH, 3'-C5 — flavone
Yinyanghuo E	5,7,5'-triOH, 4',3'-ODmp — flavone
Zapotin	5,6,2',6'-tetraOMe — flavone
Zapotinin	5-OH, 6,2',6'-triOMe — flavone

13 Flavone and Flavonol *O*-Glycosides

Christine A. Williams

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13.1 INTRODUCTION

Flavone and flavonol *O*-glycosides make up one of the largest classes of flavonoid constituents with over 2000 known structures. There are 279 glycosidic combinations of the most common flavonol aglycone, quercetin, and 347 kaempferol *O*-glycosides listed in the check list (Appendix B) for the period ending December 2003. The group includes any bound form of flavone or flavonol such as acylated and sulfated derivatives and not only those with just sugar. Thus, the number of possible combinations is enormous because of the wide structural variation, i.e., in (1) the hydroxylation and methoxylation pattern of the aglycone; (2) the number and nature of sugars and their position of attachment through hydroxyl groups to the aglycone; (3) the nature of the sugar linkage to the aglycone, different interglycosidic linkages and whether the sugars are in the pyranose or furanose form; (4) the nature, number, and position of attachment of aliphatic or aromatic acyl groups to one or more sugars or directly through a hydroxyl group to the aglycone; and (5) the presence and position of attachment of one or more sulfate groups through a hydroxyl group of the aglycone or that of a sugar. Sugars can also be attached through a carbon bond but these compounds, the *C*-glycosylflavonoids, are dealt within Chapter 14.

The monosaccharides most frequently found in O-combination with flavone and flavonols are glucose and rhamnose and less frequently arabinose, xylose, and glucuronic acid. These sugars are usually present in the expected pyranose form and with the appropriate linkage, i.e., β for glucose, galactose, xylose, and glucuronic acid and α for rhamnose. Arabinose can occur in either the pyranose or furanose form and with an α - or β -linkage. Seven other monosaccharides have been found occasionally linked to flavones or flavonols (see Section 13.3.1). The most usual place of sugar attachment in flavonols is at the 3-hydroxyl and at the 7-hydroxyl in flavones but sugars have been found at all the other possible positions.

In the first edition of *The Flavonoids*,¹ published in 1975, 134 flavone glycosides and 252 flavonol glycosides were listed. The original intent of this first edition was to compile an updated version of the classic monograph edited by the late Ted Geissman, entitled *The Chemistry of Flavonoid Compounds*, which was published in 1962, in which only 30 flavone and 54 flavonol glycosides were described.² The glycosides listed in the monograph included the best-known flavonol glycoside, quercetin 3-rhamnosyl(1 \rightarrow 6)glucoside (rutin) and the common flavone glycoside, luteolin 7-glucoside (glucoluteolin), but did not include any acylated or sulfated derivatives. Thirteen years later, in the first edition of *The Flavonoids*,¹ nine of the listed 134 flavone glycosides and 18 of the 247 recorded flavonol glycosides were found to be acylated with acids such as *p*-coumaric, caffeic, ferulic, sinapic, gallic, benzoic, *p*-hydroxybenzoic, acetic, and malonic. However, the most exciting find of this review period was the discovery that flavonoid conjugates are not such a rarity in the plant kingdom as once thought and that they are in fact frequent constituents of many salt-tolerant and water-stress resilient plants. At this stage, only 11 had been fully characterized but many more had been detected and flavonoid sulfates were regularly recorded on a presence or absence basis. The second edition of *The Flavonoids*, which covered the new compounds found between 1975 and 1980,³ showed a large increase in the number of both acylated and sulfated glycosides bringing the total number of flavone glycosides in that check list to 271 and flavonol glycosides to 486. Both the third edition, covering the years 1981 to 1985,⁴ and the fourth edition⁵ (1986 to 1991) of *The Flavonoids* showed a steady increase in the number of flavone and flavonol glycosides to 345 and 647 and 463 and 906, respectively, but with comparatively small increases in the number of new acylated and sulfated glycosides. No trisaccharides were reported in association with flavones or flavonols in Geissman's monograph² and only a small number of flavonol triosides, some with linear and some with branched sugars, were recorded in book one¹ of *The Flavonoids*. It is not until book four⁵ that there is a marked increase in the number of trisaccharides with 4 new linear and 11 new branched structures recorded. Flavone triosides continued to be of rare occurrence in books three⁴ and four.⁵ However, the first known tetrasaccharide, [rhamnosyl(1 \rightarrow 4)glucosyl]sophorose, was recorded in 1987 (book four)⁵ in combination with the flavone acacetin (apigenin 4'-methyl ether) at the 7-position with an acetyl group at the 6''-position of the sophorose in leaves of *Peganum harmala* (Zygophyllaceae).⁶

The main remit of this chapter is to provide reference and plant source details of new flavone and flavonol O-glycosides discovered since 1991, i.e., covering the years 1992 to 2003. A checklist of all (as far as possible) known structures is also included in Appendices A and B. A series of reviews, which include most of the data on new O-glycosylflavones and flavonols presented here, have appeared in *Natural Product Reports* and cover the years 1992 to 1994,⁷ 1995 to 1997,⁸ and 1998 to 2000,⁹ and with a fourth (2001 to 2003)¹⁰ in press. Other useful sources of data are *The Phytochemical Dictionary*,¹¹ *The Handbook of Natural Flavonoids*,¹² and for general background reading Jeffrey Harborne's *Comparative Biochemistry of the Flavonoids*.¹³

13.2 SEPARATION, PURIFICATION, AND IDENTIFICATION

The traditional methods of separation, purification, and identification of flavone and flavonol *O*-glycosides, including paper, column, and thin layer chromatography and UV spectral analysis of pure compounds, acid, alkaline, and enzyme hydrolysis, and identification of the resulting aglycones and sugars are well described by Mabry et al.,¹⁴ by Markham,^{15,16} and by Harborne.¹⁷ High-performance liquid chromatography (HPLC) is now a standard technique used in all phytochemistry laboratories. HPLC with UV detection is an established method for separating and detecting flavonoid glycosides in complex mixtures, for comparing flavonoid profiles of related plant taxa and for identifying known compounds. However, it has been recently superseded by HPLC–mass spectrometry (MS) techniques such as liquid chromatography (LC)–MS, which gives the molecular weight of the glycoside and LC–MS–MS, which also gives the mass spectrum of the fragmentation ions. Atmospheric pressure chemical isolation (APCI) is especially useful for obtaining the molecular weight of the glycoside, together with the molecular ions for the aglycone and any intermediate sugars or acylated sugars from very small amounts (~0.1 mg) of pure compound. In their paper in *Phytochemical Analysis*, Grayer et al.¹⁸ describe the application of APCI to a chemotaxonomic study of the flavonoids in the genus *Ocimum*. Fast atom bombardment MS gives a strong molecular ion, which indicates the number and type of sugar units present and useful fragmentation patterns with obvious loss of sugars and any methoxyls from the aglycone. This method has tended to be replaced by electrospray MS, which is less costly but does not give so much fragmentation data, although MS–MS on the resulting product ions can give further useful fragmentation ions. However, the techniques of choice for flavonoid glycoside identification are ¹H NMR, ¹³C NMR, and two-dimensional nuclear magnetic resonance (NMR), which allow complete characterization including details of form and linkage of the sugars, the nature and position of acyl groups, and number of sulfate groups. For more detailed information on HPLC, MS, NMR, and other recent separation, purification, and identification techniques see Chapters 1 and 2.

13.3 NEW FLAVONE AND FLAVONOL *O*-GLYCOSIDES

Some 228 new flavone *O*-glycosides and over 500 new flavonol *O*-glycosides have been reported in the period 1992 to 2003. This brings the number of known structures listed in the check lists (Appendix A) to 679 flavone and 1331 flavonol glycosides. (Appendix B) The new glycosides are listed in Table 13.1^{19–164} and Table 13.2,^{23,107,165–479} respectively. In all these entries and in later tables the sugars are assumed to be in the pyranose form and to have the appropriate linkage, i.e., β for glucose, α for rhamnose, etc. except where otherwise stated. Reports of new monosaccharides, disaccharides, trisaccharides, tetrasaccharides, acylating agents, and sulfate conjugates will be considered first.

13.3.1 MONOSACCHARIDES

A list of all the monosaccharides that have been found in *O*-combination with flavones or flavonols are given in Table 13.3. These include five sugars, fructose, allulose, lyxose, fucose, and glucosamine, which have been recorded since 1992. α -D-Fructofuranose has been reported from leaves of *Crataegus pinnatifida* (Rosaceae)⁴¹ linked to both the C-8 position and the 7-hydroxyl of apigenin. Here, the *C*- and *O*-glycosidic linkages to the sugar form a unique ring structure (Pinnatifinoside A, **13.1**). There has been one previous report of fructose, as a tricin fructosylglucoside in *Hyacinthus orientalis* (Liliaceae),⁴⁸⁰ but the structure of this glycoside was never confirmed. An acetylated derivative, Pinnatifinoside B (**13.2**), and

TABLE 13.1
New Flavone Glycosides

Glycoside	Source	Family	Ref.
5,7-Dihydroxyflavone (chrysin) 7-(4''-Acetylglucoside)	<i>Calicotome villosa</i> Aerial parts	Leguminosae	19
7-(6''-Acetylglucoside) 5,6,7-Trihydroxyflavone (baicalein) 7-(6''-Malonylglucoside)	<i>Cephalocereus senilis</i> suspension cultures	Cactaceae	20
Baicalein 5-methyl ether 7-Glucoside	<i>Cephalocereus senilis</i> chitin-treated cell suspension cultures	Cactaceae	21
Baicalein 6-methyl ether (oxoxylin A) 5-Rhamnoside	<i>Trichosanthes anguina</i> seeds	Cucurbitaceae	22
7-Glucosyl(1 → 3)rhamnoside	<i>Eupatorium africanum</i> whole plant	Compositae	23
Baicalein 7-methyl ether (negletein) 6-Xyloside	<i>Bauhinia purpurea</i> stems	Leguminosae	24
6-Glucoside	<i>Colebrookea oppositifolia</i> bark	Labiatae	25
6-Rhamnoside(1 → 2)fucoside	<i>Origanum vulgare</i>	Labiatae	26
5,7,8-Trihydroxyflavone (norwogonin) 5-Glucoside	<i>Pyracantha coccinea</i> roots	Rosaceae	27
7-Galactoside	<i>Scutellaria ocellata</i> and <i>S. nepetoides</i>	Labiatae	28
7-Hydroxy-5,8-dimethoxyflavone 7-Glucoside	<i>Scutellaria immaculate</i> aerial parts	Labiatae	29
7-Glucuronide	<i>Scutellaria rivularis</i> roots	Labiatae	30
5,7,2'-Trihydroxyflavone 7-Glucoside	<i>Scutellaria ramosissima</i> aerial parts	Labiatae	31
2'-Glucoside 5,2'-Dihydroxy-7-methoxyflavone (echioidinin) 5-Glucoside	<i>Andrographis alata</i> whole plant	Acanthaceae	32
2'-(6''-Acetylglucoside)	<i>Andrographis affinis</i> whole plant	Acanthaceae	33
5,7,4'-Trihydroxyflavone (apigenin) 7-Apiofuranosyl(1 → 6)glucoside	<i>Gonocaryum calleryanum</i> leaves	Icacinaeae	34
7-Cellobioside	<i>Salvia uliginosa</i> petals	Labiatae	35
7-Sophorotrioside	<i>Leptostomum macrocarpon</i> gametophyte	Bryales	36
7-(2 ^G -Rhamnosyl)rutinoside	<i>Ligustrum vulgare</i> leaves	Oleaceae	37
7-(2 ^G -Rhamnosyl)gentiobioside	<i>Lonicera gracilepes</i> var. <i>glandulosa</i> leaves	Caprifoliaceae	38
7-Rhamnoside-4'-glucosylrhamnoside	<i>Asplenium normale</i>	Aspleniaceae	39
7-Cellobioside-4'-glucoside	<i>Salvia uliginosa</i> petals	Labiatae	35
7-Glucosyl(1 → 2)glucuronide-4'-glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	40
Pinnatifinoside A (13.1) Pinnatifinoside B (13.2) Pinnatifinoside C (13.3) Pinnatifinoside D (13.4)	<i>Crataegus pinnatifida</i> var. <i>major</i> leaves	Rosaceae	41
7-(2''-E-p-Coumaroylglucoside)	<i>Echinops echinatus</i> flowers	Compositae	42
7-(3''-p-Coumaroylglucoside)	<i>Stachys aegyptiaca</i> aerial parts	Labiatae	43
7-(6''-E-p-Coumarylgalactoside)	<i>Lagopsis supina</i> whole plant	Labiatae	44
7-(6''-E-Caffeoylglucoside)	<i>Bellis perennis</i> flowers	Compositae	45

TABLE 13.1
New Flavone Glycosides — continued

Glycoside	Source	Family	Ref.
7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Chamaemelum nobile</i> flowers	Compositae	46
7-(3'',6''-Di- <i>E-p</i> -coumaroylgalactoside)	<i>Lagopsis supina</i> whole plant	Labiatae	44
7-(3''-Acetyl-6''- <i>E-p</i> -coumaroylglucoside)	<i>Blepharis ciliaris</i> aerial parts	Acanthaceae	47
7-Rhamnosyl(1 → 6)(4''- <i>E-p</i> -methoxycinnamoylglucoside)	<i>Chrozophora oblongifolia</i> aerial parts	Euphorbiaceae	48
4'-(2''-Feruloylglucuronosyl)(1 → 2)glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	49
7-Glucuronosyl(1 → 3)[(2''- <i>p</i> -coumaroylglucuronosyl)(1 → 2)glucuronide]	<i>Medicago sativa</i> aerial parts	Leguminosae	40
7-Glucuronosyl(1 → 3)[(2'''-feruloylglucuronosyl)(1 → 2) glucuronide]			
7-(2''-Feruloylglucuronosyl)(1 → 2) glucuronide-4'-glucuronide			
7-Glucuronide-4'-(2''- <i>E-p</i> -coumaroylglucuronosyl)(1 → 2)glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	49
7-Glucuronide-4'-(2'''-feruloylglucuronosyl)(1 → 2) glucuronide			
Apigenin 7-methyl ether (genkwanin)			
4'- α -L-Arabinopyranosyl(1 → 6)galactoside	<i>Salvia moorcroftiana</i> whole plant	Labiatae	50
4'-Rhamnosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]			
Apigenin 4'-methyl ether (acacetin)			
7-Rhamnoside	<i>Peganum harmalai</i>	Zygophyllaceae	51
7-Glucosyl(1 → 4)xyloside	<i>Centratherum anthelminticum</i> seeds	Compositae	52
7-Apiosyl(1 → 6)glucoside	<i>Crotalaria podocarpa</i> aerial parts	Leguminosae	53
7-(2 ^G -Rhamnosyl)rutinoside	<i>Buddleia officinalis</i> flowers	Loganiaceae	54
7-Rhamnosyl(1 → 2)glucosyl(1 → 2)glucoside	<i>Peganum harmala</i> leaves	Zygophyllaceae	51
7-Rhamnosyl(1 → 2)glucosyl(1 → 2)glucosyl(1 → 2)glucosyl(1 → 2)glucoside	<i>Peganum harmala</i> leaves	Zygophyllaceae	55
7-(4'''-Acetyl)rutinoside)	<i>Thalictrum przewalskii</i> aerial parts	Ranunculaceae	56
7-Rhamnosyl(1 → 6)[2''-acetylglucosyl(1 → 2)glucoside]	<i>Dendranthema lavandulifolium</i> whole plant	Compositae	57
7-[6'''-Acetylglucosyl(1 → 2)]rhamnosyl(1 → 6)glucoside	<i>Calamintha glandulosa</i> leaves	Labiatae	58
7-Glucosyl(1 → 6)[3'''-acetylramnosyl(1 → 2)glucoside]	<i>Peganum harmala</i> leaves	Zygophyllaceae	51
7-(4''''-Acetylramnosyl)(1 → 6) glucosyl(1 → 3)(6''-acetylglucoside)	<i>Thalictrum przewalskii</i> aerial parts	Ranunculaceae	56
6-Hydroxyapigenin (scutellarein)			
7-Xylosyl(1 → 2)xyloside	<i>Hebe stenophyllum</i> leaves	Scrophulariaceae	59
7-Xylosyl(1 → 2)glucoside			
7-Xylosyl(1 → 6)galactoside	<i>Semecarpus kurzii</i> leaves	Anacardiaceae	60
7-Glucuronosyl(1 → 2)glucuronide	<i>Perilla ocimoides</i> leaves	Labiatae	61
7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Frullania muscicola</i> whole plant	Frullaniaceae	62
Scutellarein 6-methyl ether (hispidulin)			
7-Rhamnoside	<i>Picnemon acarna</i> aerial parts	Compositae	63
7-Methylglucuronide	<i>Centaurea furfuracea</i> aerial parts	Compositae	64

continued

TABLE 13.1
New Flavone Glycosides — continued

Glycoside	Source	Family	Ref.
4'-Glucoside	<i>Cirsium oligophyllum</i> leaves	Compositae	65
7-Xylosyl(1 → 2)xyloside	<i>Chirita fimbriepala</i> roots	Gesneriaceae	66
7-Neohesperidoside	<i>Ipomoea purpurea</i> flowers	Convolvulaceae	67
7-(6''- <i>E-p</i> -Coumaroylglucoside)	<i>Eriocaulon buergerianum</i> capitula	Eriocaulaceae	68
Scutellarein 4'-methyl ether			
7-Rutinoside	<i>Teucrium parvifolium</i> leaves, stems, and fruits	Labiatae	69
7-(2'',6''-Diacetylalloside)	<i>Sideritis perfoliata</i>	Labiatae	70
Scutellarein 5,4'-dimethyl ether			
7-Glucoside	<i>Striga passargei</i> whole plant	Scrophulariaceae	71
7-(4 ^{Rha} -Acetylrutinoside)			
Scutellarein 6,7-dimethyl ether			
4'-Glucuronide	<i>Conyza linifolia</i>	Compositae	72
Scutellarein 6,4'-dimethyl ether (pectolinarigenin)			
7-(6''-Acetylglucoside)	<i>Lantana camara</i> aerial parts	Verbenaceae	73
7-(2'''-Acetylrutinoside)	<i>Linaria japonica</i> whole plant	Scrophulariaceae	74
7-(3'''-Acetylrutinoside)			
7-(4'''-Acetylrutinoside)	<i>Linaria haelava</i> whole plant	Scrophulariaceae	75
Scutellarein 7,4'-dimethyl ether			
6-Xylosyl(1 → 2)glucoside	<i>Gelonium multiflorum</i> seeds	Euphorbiaceae	76
6-Neohesperidoside			
Scutellarein 6,7,4'-trimethyl ether (salvigenin)			
5-[6''-Acetylglucosyl(1 → 3)galactoside]	<i>Striga aspera</i>	Scrophulariaceae	77
8-Hydroxyapigenin (isoscutelellarein)			
7-Glucosyl(1 → 2)xyloside	<i>Sideritis</i> spp. aerial parts	Labiatae	78
8-Sophoroside	<i>Gratiola officinalis</i> leaves	Scrophulariaceae	79
8-(6''- <i>E-p</i> -Coumaroylglucoside)	<i>Stachys aegyptiaca</i> whole plant	Labiatae	80
8-(2''-Sulfatoglucuronide)	<i>Helicteres angustifolia</i> root bark	Sterculiaceae	81
8-(2'',4''-Disulfatoglucuronide)	<i>Helicteres isora</i> fruit	Sterculiaceae	82
Isoscutelellarein 4'-methyl ether			
8-Glucoside	<i>Glossostemon bruguieri</i> roots	Sterculiaceae	83
8-(6''- <i>n</i> -butylglucuronide)	<i>Helicteres isora</i> fruit	Sterculiaceae	82
7-Allosyl(1 → 2)glucoside	<i>Sideritis javalambrensis</i> aerial parts	Labiatae	84
8-(2''-Sulfatoglucuronide)	<i>Helicteres angustifolia</i> root bark	Sterculiaceae	81
8-(2'',4''-Disulfatoglucuronide)	<i>Helicteres isora</i> fruit	Sterculiaceae	82
6,8-Dihydroxy-7,4'-dimethoxyflavone			
6-Rutinoside	<i>Dicliptera riparia</i> whole plant	Acanthaceae	85
6-(4''-Acetylramnosyl)(1 → 6)glucoside			
5,7,2'-Trihydroxy-6-methoxyflavone			
7-Glucoside	<i>Scutellaria amoena</i> roots	Labiatae	86
7-Methylglucuronide			
5,7,2',6'-Tetrahydroxyflavone			
2'-Glucoside	<i>Scutellaria baicalensis</i> hairy root cultures	Labiatae	87

TABLE 13.1
New Flavone Glycosides — continued

Glycoside	Source	Family	Ref.
5,2',6'-Trihydroxy-7-methoxyflavone 2'-Glucoside	<i>Andrographis alata</i> whole plant	Acanthaceae	88
5,7,8,2'-Tetrahydroxyflavone 7-Glucuronide	<i>Scutellaria rivularis</i> roots	Labiatae	30
5,2'-Dihydroxy-7,8-dimethoxyflavone (skullcapflavone 1) 2'-Glucoside	<i>Andrographis paniculata</i> roots	Acanthaceae	89
2'-(2''-E-Cinnamoylglucoside)	<i>Andrographis serpyllifolia</i> whole plant	Acanthaceae	90
2'-(3''-E-Cinnamoylglucoside)			
2'-(4''-E-Cinnamoylglucoside)	<i>Andrographis elongata</i> whole plant	Labiatae	91
5,7,3',4'-Tetrahydroxyflavone (luteolin) 5-Glucuronide-6'-methyl ester	<i>Dumortiera hirsuta</i> gametophytes	Hepaticae	92
5-Rutinoside	<i>Salvia lavandulifolia</i> ssp. <i>oxyodon</i> aerial parts	Labiatae	93
7-Glucosyl(1 → 4)-α-L-arabinopyranoside	<i>Cassia glauca</i> seeds	Leguminosae	94
7-Xylosyl(1 → 6)glucoside (primeveroside)	<i>Halenia corniculata</i> whole plant	Gentianaceae	95
7-Apiosyl(1 → 6)glucoside	<i>Phlomis nissolii</i> aerial parts	Labiatae	96
7-Rhamnosyl(1 → 6)galactoside (7-robinobioside)	<i>Pteris cretica</i> fronds	Adiantaceae	97
7-Sophoroside	<i>Pteris cretica</i> aerial parts	Adiantaceae	98
7-Galactosyl(1 → 6)galactoside	<i>Anogeissus latifolia</i>	Combretaceae	99
7-Galactosylglucuronide	<i>Andryala reguisina</i> aerial parts	Compositae	100
7-Glucoside-3'-glucuronide	<i>Melissa officinalis</i> leaves	Labiatae	101
3'-Xylosyl(1 → 2)glucoside	<i>Viburnum grandiflorum</i> leaves	Caprifoliaceae	102
4'-Rutinoside	<i>Dalbergia stipulacea</i> leaves	Leguminosae	103
7-Sophorotrioside	<i>Leptostomum macrocarpon</i> gametophytes	Bryales	36
7-(6''-p-Benzoylglucoside)	<i>Vitex agnus-castus</i> root bark	Verbenaceae	104
7-[6''-(2-Methylbutyryl)glucoside]	<i>Arnica chamissonis</i> flowers	Compositae	105
7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Frullania muscicola</i> whole plant	Frullaniaceae	62
3'-(3''-Acetylglucuronide)	<i>Rosmarinus officinalis</i> leaves	Labiatae	106
3'-(4''-Acetylglucuronide)			
7-Glucosyl(1 → 6)(4'''-caffeoylglucoside)	<i>Lonicera implexa</i> leaves	Caprifoliaceae	107
7-Glucoside-4'-(Z-2-methyl-2-butenolate) (7-glucoside-4'-angelate)	<i>Polygonum aviculare</i> whole plant	Polygonaceae	108
7-Apiosyl(1 → 2)[glucosyl (1 → 4)(6-malonylglucoside)]	<i>Capsicum anuum</i> fruit	Solanaceae	109
7-(Acetylsophorotrioside)	<i>Leptostomum macrocarpon</i> gametophytes	Bryales	36
7-(6'''-Acetylallosyl)(1 → 3)glucosyl (1 → 2)glucoside (veronicoside A)	<i>Veronica didyma</i>	Scrophulariaceae	110
7-(2''-Feruloylglucuronosyl) (1 → 2)glucuronide-4'-glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	40
7-(2''-Sulfatoglucoside)	<i>Thalassia testudinum</i> leaves	Hydrocharitaceae	111
Luteolin 5-methyl ether			
7-Xylosyl(1 → 6)glucoside	<i>Dirca palustris</i> twigs	Thymelaeaceae	112
Luteolin 7-methyl ether			

continued

TABLE 13.1
New Flavone Glycosides — *continued*

Glycoside	Source	Family	Ref.
3'-Glucoside	<i>Avicennia marina</i> aerial parts	Avicenniaceae	113
3'-Galactoside			
Luteolin 3'-methyl ether (chrysoeriol)			
7- α -L-Arabinofuranosyl(1 \rightarrow 6)glucoside	<i>Tagetes patula</i> whole plant	Compositae	114
7-Apiosyl(1 \rightarrow 6)glucoside	<i>Phlomis nissolii</i> aerial parts	Labiatae	96
7-Neohesperidoside	<i>Morinda morindoides</i> leaves	Rubiaceae	115
7,4'-Diglucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	116
7-(3''-Z-p-coumaroylglucoside)	<i>Ballota acetabulosa</i> flowering aerial parts	Labiatae	117
7-(3'',6''-di-E-p-coumaroylglucoside)	<i>Marrubium velutinum</i> aerial parts	Labiatae	118
7-(2'''-Feruloylglucuronosyl) (1 \rightarrow 2)glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	116
7-[Glucuronosyl(1 \rightarrow 3)(2'''-feruloylglucuronosyl)] (1 \rightarrow 2)glucuronide			
Luteolin 4'-methyl ether (diosmetin)			
3'-Glucoside	<i>Cassia torosa</i> leaves	Leguminosae	119
7-Arabinosyl(1 \rightarrow 6)glucoside	<i>Galium palustre</i>	Rubiaceae	120
7-Xylosyl(1 \rightarrow 6)glucoside (as a mixture)			
7-Xylosyl(1 \rightarrow 6)glucoside	<i>Hebe parviflora</i> and <i>H. traversii</i> leaves	Scrophulariaceae	59
7-Neohesperidoside (neodiosmin)	<i>Citrus aurantium</i> leaves	Rutaceae	121
7-(2'',6''-Dirhamnosyl)glucoside	<i>Buddleia madagascariensis</i> leaves	Loganiaceae	122
7-Apiosyl(1 \rightarrow 2)(6''-acetylglucoside)	<i>Paullinia pinnata</i> leaves	Sapindaceae	123
Luteolin 5,3'-dimethyl ether			
7-Glucoside	<i>Pyrus serotina</i> leaves	Rosaceae	124
4'-Glucoside			
Luteolin 5,4'-dimethyl ether			
7-Xylosyl(1 \rightarrow 6)glucoside	<i>Dirca palustris</i> dried twigs	Thymelaeaceae	112
Luteolin 7,3'-dimethyl ether			
4'-Apiosyl(1 \rightarrow 2)glucoside	<i>Viscum alniformosanae</i> leaves and stems	Loranthaceae	125
Luteolin 5,3',4'-trimethyl ether			
7-Xylosyl(1 \rightarrow 6)glucoside	<i>Dirca palustris</i> dried twigs	Thymelaeaceae	112
7-Rutinoside			
Luteolin 7,3',4'-trimethyl ether			
5-Glucoside	<i>Lethedon tannaensis</i> leaves	Thymelaeaceae	126
5-Xylosyl(1 \rightarrow 6)glucoside			
6-Hydroxyluteolin			
6-Rhamnaside	<i>Erythroxylum leal-costae</i> leaves	Erythroxylaceae	127
7-Xylosyl(1 \rightarrow 2)xyloside	<i>Hebe stenophylla</i> aerial parts	Scrophulariaceae	128
7-Xylosyl(1 \rightarrow 6)glucoside	<i>Hebe stenophylla</i> aerial parts	Scrophulariaceae	59
7-Sambubioside	<i>Hebe stricta</i> leaves	Scrophulariaceae	129
7-[3''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Frullania muscicola</i> whole plant	Frullaniaceae	62
7-[4''-(3-Hydroxy-3-methylglutaryl)glucoside]			
7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]			
7-(6''-E-Caffeoylglucoside)	<i>Veronica liwanensis</i> and <i>V. longifolia</i> aerial parts	Scrophulariaceae	130

TABLE 13.1
New Flavone Glycosides — continued

Glycoside	Source	Family	Ref.
6-Glucoside-7-[6''-(3-hydroxy-3-methylglutaryl)glucoside]	<i>Frullania teneriffae</i>	Hepaticae	131
7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]-3-glucuronide	<i>Frullania cesatiana</i>	Hepaticae	131
6-Methoxyluteolin (nepetin, eupafolin)			
7-Glucuronide	<i>Digitalis lanata</i> leaves	Scrophulariaceae	132
7-Methylglucuronide			
4'-Glucoside	<i>Cirsium oligophyllum</i> leaves	Compositae	65
7-Rhamnoside-3'-xyloside	<i>Chenopodium ambrosioides</i>	Chenopodiaceae	133
7-[6''-(2-Methylbutyryl)glucoside]	<i>Arnica chamissonis</i> flowers	Compositae	105
6-Hydroxyluteolin 3'-methyl ether (nodifloretin)			
7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Frullania polysticta</i> whole plant	Frullaniaceae	62
6-Hydroxyluteolin 4'-methyl ether			
7-Rhamnosyl(1 → 2)(6''-acetylglucoside)	<i>Veronica liwanensis</i> and <i>V. longifolia</i> aerial parts	Scrophulariaceae	130
6-Hydroxyluteolin 6,3'-dimethyl ether			
5-Rhamnoside	<i>Tridax procumbens</i> leaves	Compositae	134
7-Rutinoside	<i>Kichxia elatine</i> aerial parts	Scrophulariaceae	135
6-Hydroxyluteolin 5,6,3',4'-tetramethyl ether			
7-Cellobioside	<i>Sphaeranthus indicus</i> stems	Compositae	136
8-Hydroxyluteolin (hypolaetin)			
8-Rhamnoside	<i>Erythroxyllum leal-costae</i> leaves	Erythroxylaceae	127
7-Sophoroside	<i>Gratiola officinalis</i> leaves	Scrophulariaceae	79
8-Glucoside-3'-rutinoside	<i>Cornulaca monacantha</i> aerial parts	Chenopodiaceae	137
7-Sulfatoglucoside	<i>Leptocarpus elegans</i> culm	Restionaceae	138
7-Sulfatogalactoside			
7-Sulfatoglucuronide	<i>Meeboldina thysanantha</i> culm	Restionaceae	138
7-Sulfate-8-glucoside	<i>Hypolaena fastigiata</i> culm	Restionaceae	138
Hypolaetin 7-methyl ether			
3'-Sulfatogalactoside	<i>Leptocarpus tenax</i> culm	Restionaceae	138
3'-Sulfatoglucuronide	<i>Leptocarpus elegans</i> culm	Restionaceae	138
Hypolaetin 3'-methyl ether			
8-Glucuronide	<i>Gratiola officinalis</i> leaves	Scrophulariaceae	79
7-Sophoroside			
Hypolaetin 4'-methyl ether			
7-(6''-Acetylallosyl)(1 → 2)(6''-acetylglucoside)	<i>Sideritis syriaca</i> and <i>S. scardica</i>	Labiatae	139
Hypolaetin 7,3'-dimethyl ether			
4'-Glucoside	<i>Leptocarpus elegans</i> culm	Restionaceae	138
5,6,4'-Trihydroxy-7,8-dimethoxy flavone (thymusin)			
6-Isobutyrate	<i>Asarina procumbens</i> aerial parts	Scrophulariaceae	140
5,8,4'-Trihydroxy-6,7-dimethoxy flavone (isothymusin)			
8-Glucoside	<i>Becium grandiflorum</i> leaves	Labiatae	141
5,7-Dihydroxy-6,8,4'-trimethoxy flavone (nevadensin)			

continued

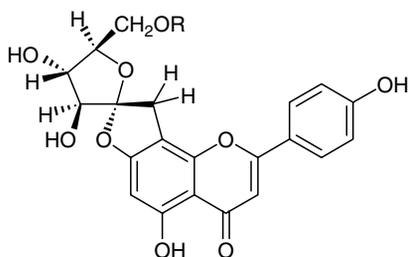
TABLE 13.1
New Flavone Glycosides — *continued*

Glycoside	Source	Family	Ref.
5-Glucoside	<i>Lysionotus pauciflorus</i> aerial parts	Gesneriaceae	142
7-Glucoside	<i>Lysionotus pauciflorus</i> aerial parts	Gesneriaceae	143
5-Gentiobioside	<i>Lysionotus pauciflorus</i> aerial parts	Gesneriaceae	142
7-Rutinoside	<i>Lysionotus pauciflorus</i> aerial parts	Gesneriaceae	143
5,8-Dihydroxy-6,7,4'-trimethoxyflavone (8-hydroxysalvigenin)			
8-Glucoside	<i>Isodon erianderianus</i> leaves	Labiatae	144
5,6,7,8,3',4'-Hexahydroxyflavone			
7-Glucoside	<i>Juniperus zeravschanica</i> fruits	Cupressaceae	145
5,6,7,3',4'-Pentahydroxy-8-methoxyflavone			
7-Glucoside	<i>Vellozia nanuzae</i> leaves	Velloziaceae	146
5,6,3',4'-Tetrahydroxy-7,8-dimethoxyflavone (pleurostimin 7-methyl ether)			
6-Glucoside	<i>Vellozia nanuzae</i> leaves	Velloziaceae	146
5,2',6'-Trihydroxy-6,7-dimethoxyflavone			
2'-Glucoside	<i>Scutellaria baicalensis</i> roots	Labiatae	147
5,2',6'-Trihydroxy-7,8-dimethoxyflavone			
2'-Glucuronide	<i>Scutellaria rivularis</i> roots	Labiatae	30
5,2'-Dihydroxy-7,8,6'-trimethoxyflavone			
2'-Glucuronide	<i>Scutellaria rivularis</i> roots	Labiatae	30
5,7,2',4',5'-Pentahydroxyflavone (isoetin)			
4'-Glucuronide	<i>Adonis aleppica</i> whole plant	Ranunculaceae	148
5,7,3',4',5'-Pentahydroxyflavone (tricetin)			
3'-Rhamnosyl(1 → 4)rhamnoside	<i>Mentha longifolia</i> aerial parts	Labiatae	149
3'-Glucoside-5'-rhamnoside			
7-Glucoside-3'-[6''-(3-hydroxy-3-methylglutaryl) glucoside]	<i>Frullania polysticta</i> whole plant	Frullaniaceae	62
Tricetin 7-methyl ether			
3'-Glucoside-5'-rhamnoside	<i>Mentha longifolia</i> aerial parts	Labiatae	149
Tricetin 3'-methyl ether			
7-Glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	116
Tricetin 4'-methyl ether			
7-Apiosyl(1 → 2)(6''-acetylglucoside)	<i>Paullinia pinnata</i> leaves	Sapindaceae	123
Tricetin 3',5'-dimethyl ether (tricin)			
7-β-D-Arabinopyranoside (setaricin)	<i>Setaria italica</i> leaves	Gramineae	150
4'-Apioside	<i>Salsola collina</i> aerial parts	Chenopodiaceae	151
7-(2''-p-Coumaroylglucuronosyl) (1 → 2)glucuronide	<i>Medicago sativa</i> aerial parts	Leguminosae	116
7-(2''-Feruloylglucuronosyl)(1 → 2)glucuronide			
7-(2''-Sinapoylglucuronosyl)(1 → 2)glucuronide			
7-[Glucuronosyl(1 → 3)(2''-feruloylglucuronosyl) (1 → 2)glucuronide]			
7-[X''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Frullania polysticta</i> whole plants	Frullaniaceae	62
Tricetin 7,3',4'-trimethyl ether			
5-Glucoside	<i>Lethedon tannaensis</i> leaves	Thymelaeaceae	126
Tricetin 7,3',4',5'-tetramethyl ether			
5-Glucoside	<i>Lethedon tannaensis</i> leaves	Thymelaeaceae	126

TABLE 13.1
New Flavone Glycosides — continued

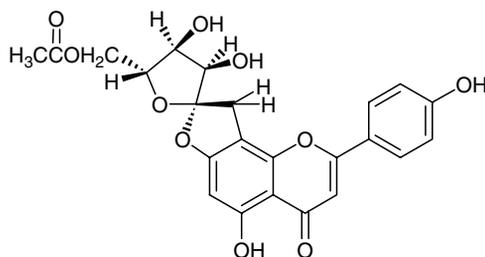
Glycoside	Source	Family	Ref.
5-Xylosyl(1 → 2)rhamnoside	<i>Bauhinia variegata</i> seeds	Leguminosae	152
5-Xylosyl(1 → 6)glucoside	<i>Lethedon tannaensis</i> leaves	Thymelaeaceae	126
6-Hydroxytricetin 6,3',5'-trimethyl ether			
7- α -L-Arabinosyl(1 → 6)glucoside	<i>Mimosa rubicaulis</i> roots	Leguminosae	153
6-Hydroxytricetin 6,4',5'-trimethyl ether			
3'-Rhamnoside	<i>Mimosa rubicaulis</i> roots	Leguminosae	154
6-Hydroxytricetin 6,7,3',5'-tetramethyl ether			
5-Robinoside	<i>Aloe barbadensis</i> leaves	Liliaceae	155
8-Hydroxytricetin			
5-Rhamnoside	<i>Argyrea speciosa</i> leaves	Convolvulaceae	156
5-Glucoside			
5,6,7,8,3',4'- Hexahydroxyflavone			
7-Glucoside	<i>Juniperus seravschanica</i> fruits	Cupressaceae	157
5,7,2',5'-Tetrahydroxy-8,6'-tetrahydroxy-8,6'-dimethoxyflavone (viscidulin III)			
2'-Glucoside	<i>Scutellaria baicalensis</i> roots	Labiatae	158
5,2',6'-Trihydroxy-6,7,8-trimethoxyflavone			
2'-Glucoside	<i>Scutellaria baicalensis</i> roots	Labiatae	147
5,7-Dihydroxy-6-C-methylflavone			
7-Xylosyl(1 → 3)xyloside	<i>Mosla chinensis</i>	Labiatae	159
5,7-Dihydroxy-6,8-di-C-methylflavone (matteuorien)			
7-[6'-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Matteuccia orientalis</i> rhizomes	Aspleniaceae	160
Stachysetin (13.5)	<i>Stachys aegyptiaca</i> aerial parts	Labiatae	161
8-Prenylapigenin			
4'-Rutinoside	<i>Desmodium gangeticum</i> stems	Leguminosae	162
3'-Prenylapigenin			
7-Rutinoside	<i>Pithecellobium dulce</i> stems	Leguminosae	163
8-C-Prenyl-5,7,4'-trihydroxy-3'-methoxyflavone (8-C-prenylchrysoeriol)			
7-Glucosyl(1 → 3)- α -L-arabinopyranoside	<i>Erythrina indica</i> seeds	Leguminosae	164

two related nonacetylated glycosides, Pinnatifinosides C and D (13.3 and 13.4), in which the fructose has been replaced by the sugars β -D-allulofuranose and α -D-allulofuranose, respectively, co-occurred with Pinnatifinoside A.⁴¹ A second pentose sugar, α -D-lyxose, was found attached to the 8-hydroxyl of gossypetin (8-hydroxyquercetin) in the aerial parts of *Orostachys japonicus*,⁴²² a member of the Crassulaceae. This is an unexpected discovery as the 2-epimer of xylose is very rare in nature. Fucose, a characteristic constituent of algal and plant polysaccharides, has been found in combination with 5,6-dihydroxy-7-methoxyflavone (negletein) as the 6-rhamnosyl(1 → 2)fucoside in *Origanum vulgare*.²⁶ Glucosamine (2-amino-2-deoxyglucose) is the only amino sugar to have been found in combination with flavones or flavonols. This sugar is important in animal physiology as a component of chitin, mucoproteins, and mucopolysaccharides. The presence of α -D-glucosamine in the aerial parts of *Halocnemum strobilaceum* (Chenopodiaceae)³⁷⁷ at the 7-hydroxyl of isorhamnetin is totally unexpected and should be further investigated to establish its exact location in the plant and to screen related plants for similar structures.

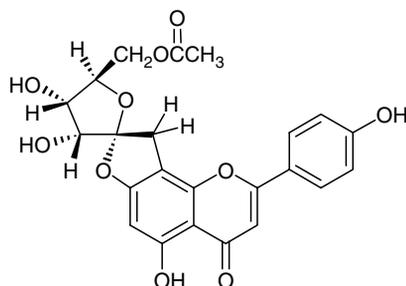


13.1 R = H

13.2 R = Acetyl



13.3



13.4

13.3.2 DISACCHARIDES

Twenty-one new disaccharides have been found in combination with flavones or flavonols since 1992. These are listed with previously known disaccharides in Table 13.4. Four of the new structures are novel sugar combinations: xylose–xylose, rhamnose–fucose, apiosyl–rhamnose, and glucose–arabinose. Both xylosyl(1 → 2)xylose and xylosyl(1 → 3)xylose have been found, the former at the 7-hydroxyl of scutellarein 6-methyl ether (patuletin) from roots of *Chirita fimbrisepala* (Gesneriaceae)⁶⁶ and its 1 → 3 isomer from *Mosla chinensis* (Labiatae)¹⁵⁹ at the 7-hydroxyl of 5,7,-dihydroxy-6-C-methylflavone. Rhamnosyl(1 → 2)fucose has already been dealt with under monosaccharides above. Apiofuranosyl(1 → 4)rhamnose has been recorded from *Chenopodium murale*¹⁹⁸ in combination with kaempferol at the 3-position and with rhamnose at the 7-hydroxyl. Five new glucosylarabinose isomers have been recorded during the review period. This is not surprising as arabinose can occur in either the pyranose or furanose form, with α or β linkage and with five possible linkage positions, which can all now be easily distinguished by modern NMR techniques. Glucosyl(1 → 4)- α -L-arabinopyranose was found at the 7-position of the flavone luteolin in seed of *Cassia glauca*⁹⁴ and its 1 → 5 linked furanose isomer in aerial parts of the legume, *Retama sphaerocarpa*,⁴⁰² at the 3-hydroxyl of quercetin 7,3'-dimethyl ether (rhamnazin). Glucosyl(1 → 3)- α -L-arabinopyranose occurs at the 7-hydroxyl of 8-prenylchrysoeriol in seeds of *Erythrina indica*¹⁶⁴. The other two isomers were present only in acylated form. Thus, glucosyl(1 → 2)- β -arabinopyranose was recorded at the 3-hydroxyl of quercetin with glucose at the 7-hydroxyl and a feruloyl group at the 6-position of the glucose in a whole plant extract of *Carrichtera annua* (Cruciferae),³⁵⁷ while the remaining isomer, glucosyl(1 → 2)- α -L-arabinofuranose, from *Euphorbia pachyrhiza*,³⁴⁰ was found attached to the 3-hydroxyl of quercetin with a galloyl group at the 2-hydroxyl of the glucose.

TABLE 13.2
New Flavonol Glycosides

Glycoside	Source	Family	Ref.
3,5,7-Trihydroxyflavone (galangin)			
3-Glucoside-8-sulfate	<i>Phyllanthus virgatus</i> whole plant	Euphorbiaceae	165
3,7-Dihydroxy-8-methoxyflavone			
7-Rhamnoside	<i>Butea superba</i> stems	Leguminosae	166
7-Rhamnosyl(1 → 4) rhamnosyl(1 → 6)glucoside	<i>Shorea robusta</i> seeds	Dipterocarpaceae	167
5,7-Dihydroxy-3,6-dimethoxyflavone			
5- α -L-Arabinosyl(1 → 6)glucoside	<i>Acacia catechu</i> stems	Leguminosae	168
3,6,7-Trihydroxy-4'-methoxyflavone			
7-Rhamnoside	<i>Setaria italica</i> leaves	Gramineae	169
Kaempferol			
3- α -D-Arabinopyranoside	<i>Persea americana</i> leaves	Lauraceae	170
5-Glucuronide	<i>Leucanthemum vulgare</i> leaves	Compositae	171
7-Alloside	<i>Indigofera hebeptala</i> leaves	Leguminosae	172
3-Rhamnosyl(1 → 2)- α -L-arabinofuranoside (arapetaloside B)	<i>Artabotrys hexapetalus</i> leaves	Annonaceae	173
3-Xylosyl(1 → 2)glucoside	<i>Galium sinaicum</i> aerial parts	Rubiaceae	174
3-Rhamnoside(1 → 2)rhamnoside	<i>Cassia hirsuta</i> flowers	Leguminosae	175
3-Glucosyl(1 → 2)rhamnoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	176, 177
7-Glucosyl(1 → 4)xyloside	<i>Crotalaria laburnifolia</i>	Leguminosae	178
7-Neohesperidoside	<i>Caralluma tuberculata</i>	Asclepiadaceae	179
7-Glucosyl(1 → 3)rhamnoside	<i>Rhodiola crenulata</i> roots	Crassulaceae	180
7-Sophoroside	<i>Crocus sativus</i> stamens (saffron)	Iridaceae	181
3,5-Diglucoside	<i>Dryopteris dickinsii</i> fronds	Aspleniaceae	182
3,7-Diarabinoside	<i>Indigofera hebeptala</i> leaves	Leguminosae	172
3,4'-Diglucoside	<i>Picea abies</i> needles	Pinaceae	183
7-Rhamnoside-4'-glucoside	<i>Pteridium aquilinum</i> aerial parts	Dennstaedtiaceae	184
7,4'-Diglucoside	<i>Cassia javanica</i>	Leguminosae	185
3-Xylosyl(1 → 3)rhamnosyl(1 → 6)galactoside	<i>Astragalus caprinus</i> leaves	Leguminosae	186
3-Xylosyl(1 → 6)glucosyl(1 → 2)rhamnoside	<i>Helicia nilagirica</i> leaves	Proteaceae	187
3-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)glucoside	<i>Camellia sinensis</i> green tea	Theaceae	188
3-Rhamnosyl(1 → 2)glucosyl(1 → 6)galactoside	<i>Cassia marginata</i> stems	Leguminosae	189
3-Rhamnosyl(1 → 6)glucosyl(1 → 6)galactoside	<i>Albizia lebbeck</i> leaves	Leguminosae	190
3-Glucosyl(1 → 4)rhamnosyl(1 → 2)glucoside	<i>Allium neapolitanum</i> whole plant	Liliaceae	191
3-Glucosyl(1 → 2)galactosyl(1 → 2)glucoside	<i>Nigella sativa</i> seeds	Ranunculaceae	192
3-Rhamnosyl(1 → 2)[glucosyl(1 → 3)glucoside]	<i>Impatiens balsamina</i> petals	Balsaminaceae	193
3-Rhamnosyl(1 → 2)[glucosyl(1 → 4)glucoside]	<i>Allium neapolitanum</i> whole plant	Liliaceae	194
3-Glucosyl(1 → 2)[glucosyl(1 → 3)rhamnoside]	<i>Crocus speciosus</i> and <i>C. antalyensis</i> flowers	Iridaceae	195
7-(3 ^G -Glucosylgentiobioside)	<i>Brassica juncea</i> leaves	Cruciferae	196
3-Rhamnosyl(1 → 2)galactoside-7- α -L-arabinofuranoside			
3-Robinoside-7- α -L-arabinofuranoside	<i>Indigo hebeptala</i> flowers	Leguminosae	197
3-Xylosyl(1 → 4)rhamnoside-7-rhamnoside	<i>Chenopodium murale</i> whole plant	Chenopodiaceae	198
3-Rhamnoside-7-xylosyl(1 → 2)rhamnoside	<i>Chenopodium murale</i> aerial parts	Chenopodiaceae	199
3-Apiosyl(1 → 4)rhamnoside-7-rhamnoside	<i>Chenopodium murale</i> whole plant	Chenopodiaceae	198

continued

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-Neohesperidoside-7-rhamnoside	<i>Sedum telephium</i> subsp. <i>maximum</i> leaves	Crassulaceae	200
3-Rhamnoside-7-glucosyl(1 → 2)rhamnoside	<i>Siraita grosvenori</i> fresh fruit	Cucurbitaceae	201
3-Glucosyl(1 → 4)galactoside-7- α -L-arabinofuranoside	<i>Corchorus depressus</i> whole plant	Tiliaceae	202
3-Glucosyl(1 → 6)galactoside-7- α -L-arabinofuranoside			
3-Apioside-7-rhamnosyl(1 → 6)galactoside	<i>Silphium perfoliatum</i> leaves	Compositae	203
3-Glucosyl(1 → 2)rhamnoside-7-glucoside	<i>Crocus chrysanthus-biflorus</i> cvs “eye-catcher” and “spring pearl” flowers	Iridaceae	204
3-Gentiobioside-7-rhamnoside	<i>Arabidopsis thaliana</i> leaves	Cruciferae	205
3-Glucosyl(1 → 2)galactoside-7-glucoside	<i>Nicotiana</i> spp. flowers	Solanaceae	206
3-Sophoroside-7-glucuronide	<i>Allium cepa</i> guard cells	Alliaceae	207
3-Neohesperidoside-4'-glucoside	<i>Pseuderucaria clavata</i> aerial parts	Cruciferae	208
3-Neohesperidoside-7,4'-diglucoside	Minus flowers		
3-Gentiobioside-4'-glucoside	<i>Asplenium incisum</i> fronds	Aspleniaceae	209
3-Galactoside-3',4'-dirhamnoside	<i>Astragalus tana</i> aerial parts	Leguminosae	210
3-Rhamnoside-7,4'-digalactoside	<i>Warburgia ugandensis</i> leaves	Rubiaceae	211
4'-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)galactoside	<i>Rhamnus thymifolius</i> fruits	Rhamnaceae	212
3-Rhamnosyl(1 → 2)[xylosyl (1 → 3)rhamnosyl(1 → 6)galactoside]	<i>Astragalus caprinus</i> leaves	Leguminosae	213
3-Glucosyl(1 → 3)rhamnosyl(1 → 2) [rhamnosyl(1 → 6) galactoside]	<i>Maytenus aquifolium</i> leaves	Celestraceae	214
3-Rhamnosyl(1 → 4)rhamnosyl (1 → 6)galactoside-7-rhamnoside (3-isorhamminoside-7-rhamnoside)	<i>Vigna</i> spp. whole plant	Leguminosae	215
3-Rutinoside-7-sophoroside	<i>Equisetum</i> spp.	Equisetaceae	216
3-Sophoroside-7-cellobioside	<i>Brassica oleracea</i> leaves	Cruciferae	217
3-(2 ^G -Glucosylrutinoside)-7-rhamnoside	<i>Sophora japonica</i> seeds	Leguminosae	218
3-(2 ^G -Rhamnosylrutinoside)-7-glucoside (mauritanian 7-glucoside)	<i>Alangium premnifolium</i> leaves	Alangiaceae	219
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2) galactoside]-7-rhamnoside	<i>Astragalus shikokianus</i> aerial parts	Leguminosae	220
3-Glucosyl(1 → 2)[rhamnosyl(1 → 6) galactoside]-7-rhamnoside	<i>Cephalocereus senilis</i> whole young plants	Cactaceae	221
3-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]	<i>Citrus aurantifolia</i> callus cultures	Rutaceae	222
3-(6''-p-Hydroxybenzoyl)galactoside)	<i>Persicaria lapathifolia</i> whole plants	Polygonaceae	223
3-(2''-Galloylarabinoside)	<i>Eucalyptus rostrata</i> leaves	Myrtaceae	224
3-(6''-Galloyl)galactoside)	<i>Pemphis acidula</i> leaves	Lythraceae	225
3-(2'',6''-Digalloyl)glucoside)	<i>Loropetalum chinense</i> leaves	Hamamelidaceae	226
3-(2''-E-p-Coumaroyl- α -L-arabinofuranoside)	<i>Prunus spinosa</i> flowers	Rosaceae	227
3-(2''-E-p-Coumaroyl)rhamnoside)	<i>Platanus orientalis</i> buds	Platanaceae	228
	<i>Platanus acerifolia</i> buds	Platanaceae	229
3-(2''-Z-p-Coumaroyl)rhamnoside)	<i>Platanus acerifolia</i> buds	Platanaceae	229
3-(2''-Z-p-Coumaroyl)glucoside)	<i>Eryngium campestre</i> aerial parts	Umbelliferae	230
3-(4''-p-Coumaroyl)glucoside)	<i>Elaeagnus bockii</i> whole plant	Elaeagnaceae	231

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(6''-Caffeoylglucoside)	<i>Pteridium aquilinum</i> aerial parts	Dennstaedtiaceae	232
3-(5''-Feruloylapioside)	<i>Pteridium aquilinum</i> aerial parts	Dennstaedtiaceae	233
3-(6''-Feruloylglucoside)	<i>Polylepis incana</i> leaves	Rosaceae	234
3-(6''-Acetylglucoside)	<i>Picea abies</i> needles	Pinaceae	235
7-(6''-p-Coumaroylglucoside)	<i>Buddleia coriacea</i> aerial parts	Loganiaceae	236
3-(2'',3''-di-E-p-Coumaroylrhamnoside)	<i>Platanus orientalis</i> buds	Platanaceae	228
3-(2'',4''-di-E-p-Coumaroylrhamnoside)	<i>Pentachondra pumila</i> leaves and stems	Epacridaceae	237
3-(2'',4''-di-Z-p-Coumaroylrhamnoside)	<i>Laurus nobilis</i> leaves	Lauraceae	238
3-(2'',6''-di-E-p-Coumaroylglucoside)	<i>Quercus canariensis</i>	Fagaceae	239
3-(2''-Z-p-Coumaroyl-6''-E-p-coumaroylglucoside)			
3-(3'',6''-di-Z-p-Coumaroylglucoside) (stenopalustroside A)	<i>Stenochlaena palustris</i> leaves	Pteridaceae	240
3-(3''-Z-p-Coumaroyl-6''-E-feruloylglucoside) (stenopalustroside B)			
3-(3''-Z-p-Coumaroyl-6''-E-p-coumaroylglucoside) (stenopalustroside C)			
3-(3''-E-p-Coumaroyl-6''-Z-p-coumaroylglucoside) (stenopalustroside D) (isolated as a mixture)			
3-(3''-E-p-Coumaroyl-[6''-(4-O-(4-hydroxy-3-methoxyphenyl)-1,3-dihydroxyisopropyl-feruloyl)] glucoside (stenopalustroside E)			
3-(2''-E-p-Coumaroyl-6''-acetylglucoside)	<i>Quercus dentata</i> leaves	Fagaceae	241
3-(3''-Acetyl-6''-p-coumaroylglucoside)	<i>Anaphalis aurea-punctata</i> whole plant	Compositae	242
3-(3'',4''-Diacylglucoside)	<i>Minthostachys spicata</i> aerial parts	Labiatae	243
3-(6 ^G -Malonylneohesperidoside)	<i>Clitoria teratea</i> petals	Leguminosae	244
3-(2''-E-Feruloylgalactosyl(1 → 4)glucoside)	<i>Allium porrum</i> bulbs	Alliaceae	245
3-(2''-E-Feruloylgalactosyl(1 → 6)glucoside)			
3-(2 ^G -E-p-Coumaroylrutinoside)	<i>Alibertia sessilis</i> leaves	Rubiaceae	246
3-(6'''-Caffeoylglucosyl)(1 → 4)rhamnoside	<i>Rorippa indica</i> whole plant	Cruciferae	247
3-(6''-E-Feruloylglucosyl)(1 → 2)galactoside	<i>Hedyotis diffusa</i> whole plant	Rubiaceae	248
3-(6'''-Sinapoylglucosyl)(1 → 2)galactoside	<i>Thevetia peruviana</i> leaves	Apocynaceae	249
3-(3'''-Acetyl-α-L-arabinopyranosyl)(1 → 6)glucoside	<i>Thalictrum atriplex</i> aerial parts	Ranunculaceae	250
3-(6'''-Acetylglucosyl)(1 → 3)galactoside	<i>Ricinus communis</i> roots	Euphorbiaceae	251
3-(2''-Feruloylglucosyl)(1 → 2)(6''-malonylglucoside)	<i>Petunia</i> cv "Mitchell" and its LC transgenic leaves	Solanaceae	252
3-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]-7-glucoside	<i>Citrus aurantifolia</i> callus cultures	Rutaceae	222
3-(6''-Malonylglucoside)-7-glucoside	<i>Equisetum</i> spp.	Equisetaceae	216
3-(2''-E-p-Coumaroyl-α-L-arabinofuranoside)-7-rhamnoside	<i>Prunus spinosa</i> leaves	Rosaceae	253
3-(3''-p-Coumaroylrhamnoside)-7-rhamnoside	<i>Cheilanthes fragrans</i> aerial parts	Sinopteridaceae	254
3-(6''-E-p-Coumaroylglucoside)-7-glucoside	<i>Lotus polyphyllus</i> whole plant	Leguminosae	255
3-(2'',3''-Diacylrhamnoside)-7-rhamnoside	<i>Dryopteris crassirhizoma</i> rhizomes	Filicales	256
3-(2'',4''-Diacylrhamnoside)-7-rhamnoside			
3-(3'',4''-Diacylrhamnoside)-7-rhamnoside			

continued

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(4'',6''-Diacylglycoside)-7-rhamnoside	<i>Delphinium formosum</i> flowers	Ranunculaceae	257
3-(2''',3''',4'''-Triacetyl- α -L-arabinopyranosyl)(1 \rightarrow 6) glucoside	<i>Calluna vulgaris</i> flowers	Ericaceae	258
3-[6''-(7''''-Glucosyl- <i>p</i> -coumaroyl)glucosyl](1 \rightarrow 2)rhamnoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	177
3-Rhamnosyl(1 \rightarrow 3)(4'''-acetylramnosyl)(1 \rightarrow 6)glucoside	<i>Camellia sinensis</i> green tea	Theaceae	259
3-[2 ^{Gal} -(6'''-Feruloylglucosyl)robinobioside]	<i>Brunfelsia grandiflora</i> ssp. <i>grandiflora</i> aerial parts	Solanaceae	260
3-Rhamnosyl(1 \rightarrow 3)(4'''-acetylramnosyl)(1 \rightarrow 6)galactoside]	<i>Rhamnus thymifolius</i> fruits	Rhamnaceae	212
3-Glucosyl(1 \rightarrow 4)[(6'''-sinapoylglucosyl)(1 \rightarrow 2)galactoside]	<i>Thevetia peruviana</i> leaves	Apocynaceae	249
3-(2'''-Sinapoylglucosyl(1 \rightarrow 4)[(6'''-sinapoylglucosyl)(1 \rightarrow 2)galactoside]			
3-[2 ^{Gal} (4'''-Acetylramnosyl)robinobioside]	<i>Galega officinalis</i> aerial parts	Leguminosae	261
3-[2''-(4'''-Acetylramnosyl)sophoroside]	<i>Ammi majus</i> aerial parts	Umbelliferae	262
3-Neohesperidoside-7-(6''-malonylglucoside)	<i>Crocus chrysanthus biflorus</i> cvs "eye catcher" and "spring pearl" flowers	Iridaceae	204
3-Neohesperidoside-7-(6''-acetylglucoside)			
3-Neohesperidoside-7-(2''- <i>E-p</i> -coumaroylglucoside)	<i>Allium ursinum</i> whole plant	Liliaceae	263
3-Neohesperidoside-7-(2''- <i>E-feruloylglucoside)</i>			
3-(4'''- <i>p</i> -Coumaroylglucosyl)(1 \rightarrow 2)rhamnoside-7-glucoside	<i>Mentha lavandulacea</i> aerial parts	Labiatae	264
3-(6'''- <i>p</i> -Coumaroylglucosyl)(1 \rightarrow 2)rhamnoside-7-glucoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	265
3-(6'''- <i>p</i> -Coumaroylglucosyl)(1 \rightarrow 2)rhamnoside-7-glucoside	<i>Reseda muricata</i> leaves	Resedaceae	266
3-Glucosyl(1 \rightarrow 2)rhamnoside-7-(6''- <i>E-p</i> -coumaroylglucoside)	<i>Aconitum napellus</i> ssp. <i>tauricum</i> flowers	Ranunculaceae	267
3-(6'''- <i>E-p</i> -Coumaroylglucosyl)(1 \rightarrow 2)glucoside-7-rhamnoside			
3-Glucoside-7-(6'''- <i>E-p</i> -coumaroylglucosyl)(1 \rightarrow 3) rhamnoside	<i>Aconitum napellus</i> ssp. <i>neomontanum</i>	Ranunculaceae	268
3-Glucoside-7-(6'''- <i>E-caffeoylglucosyl)(1 \rightarrow 3)rhamnoside</i>			
3-(2'''- <i>E-p</i> -Coumaroylsophoroside)-7-glucoside	<i>Brassica oleracea</i> leaves	Cruciferae	269
3-(2'''- <i>E-caffeoylsophoroside)-7-glucoside</i>			
3-(2'''- <i>E-Feruloylsophoroside)-7-glucoside</i>			
3-Apioside-7-rhamnosyl(1 \rightarrow 6)(2''- <i>E-caffeoylgalactoside)</i>	<i>Silphium perfoliatum</i> leaves	Compositae	203
3-Xylosyl(1 \rightarrow 2)rhamnoside-7-(4''-acetylramnoside)	<i>Kalanchoe streptantha</i> leaves	Crassulaceae	270
3-Glucosyl(1 \rightarrow 2)(6''-acetylgalactoside)-7-glucoside	<i>Trigonella foenum graecum</i>	Leguminosae	271
3-Rhamnosyl(1 \rightarrow 2)[glucosyl(1 \rightarrow 3)(4'''- <i>p</i> -coumaroylramnosyl)(1 \rightarrow 6)galactoside]	<i>Lysimachia capillipes</i> whole plant	Primulaceae	272
3-Rhamnosyl(1 \rightarrow 2)[xylosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6) (3''- <i>p</i> -coumaroylgalactoside)]	<i>Astragalus caprinus</i> leaves	Leguminosae	213
3-Rhamnosyl(1 \rightarrow 2)[xylosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6) (4''- <i>p</i> -coumaroylgalactoside)]			
3-Rhamnosyl(1 \rightarrow 2)[xylosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6) (3''-feruloylgalactoside)]			
3-Rhamnosyl(1 \rightarrow 2)[xylosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6) (4''-feruloylgalactoside)]			
3-Neohesperidoside-7-[2''- <i>E-p</i> -coumaroyllaminaribioside]	<i>Allium ursinum</i> whole plant	Liliaceae	263

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(2''- <i>E</i> -Caffeoylglucosyl)(1 → 2) glucoside-7-cellobioside	<i>Brassica oleracea</i> leaves	Cruciferae	217
3-(2''- <i>E</i> -Feruloylglucosyl)(1 → 2) glucoside-7-cellobioside			
3-(2''- <i>E</i> -Sinapoylglucosyl)(1 → 2) glucoside-7-cellobioside			
3-Glucosyl(1 → 6)[rhamnosyl(1 → 3)(2''- <i>E</i> - <i>p</i> -coumaroylglucoside)]-7-rhamnosyl(1 → 3) rhamnosyl(1 → 3)(4''- <i>E</i> - <i>p</i> -coumaroylrhamnoside)	<i>Planchonia grandis</i>	Lecythidaceae	273
3-Glucosyl(1 → 6)[rhamnosyl(1 → 3)(2''- <i>E</i> - <i>p</i> -coumaroylglucoside)]-7-rhamnosyl(1 → 3) rhamnosyl(1 → 3)(4''- <i>Z</i> - <i>p</i> -coumaroylrhamnoside)			
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 3)(2''- <i>E</i> - <i>p</i> -coumaroylglucoside)]-7-rhamnosyl(1 → 3) rhamnosyl(1 → 3)rhamnosyl(1 → 3) (4''- <i>E</i> - <i>p</i> -coumaroylrhamnoside)			
3-Sulfate-7- α -arabinopyranoside	<i>Atriplex hortensis</i> leaves	Chenopodiaceae	274
8-C-Sulfate	<i>Phyllanthus virgatus</i> whole plant	Euphorbiaceae	165
Kaempferol 3-methyl ether			
7-Glucuronide	<i>Centaurea bracteata</i> aerial parts	Compositae	275
Kaempferol 7-methyl ether (rhamnocitrin)			
4'-Glucoside	<i>Cotoneaster simonsii</i> leaves	Rosaceae	276
3-Xylosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]	<i>Cestrum nocturnum</i> leaves	Solanaceae	277
3-Rhamnosyl(1 → 3)[apiosyl(1 → 6)glucoside]	<i>Mosla soochouensis</i> stem wood	Labiatae	278
3-Apiosyl(1 → 5)apioside-4'-glucoside	<i>Mosla chinensis</i>	Labiatae	279
3-Neohesperidoside-4'-glucoside	<i>Cadaba glandulosa</i> aerial parts	Capparidaceae	280
3-Apiosyl(1 → 5)apiosyl(1 → 2) [rhamnosyl(1 → 6)glucoside]	<i>Viscum angulatum</i> whole plant	Viscaceae	281
3-[3-Hydroxy-3-methylglutaryl(1 → 6)][apiosyl (1 → 2)galactoside]	<i>Astragalus caprinus</i> leaves	Leguminosae	186
3-[5''- <i>p</i> -Coumaroylapiosyl(1 → 2)glucoside]	<i>Astragalus complanatus</i> seeds	Leguminosae	282
3-[5''-Feruloylapiosyl(1 → 2)glucoside]			
3-glucoside-4'-(3'''-dihydrophaseoylglucoside)			
3-(6- <i>E</i> -3,5-Dimethoxy-4-hydroxycinnamoylglucosyl) (1 → 2)[rhamnosyl(1 → 6)glucoside]	<i>Cestrum nocturnum</i> leaves	Solanaceae	277
Kaempferol 4'-methylether (kaempferide)			
3-Rhamnoside	<i>Agrimonia eupatoria</i> aerial parts	Rosaceae	283
3-Neohesperidoside	<i>Costus spicatus</i> leaves	Costaceae	284
3-Rhamnoside-7-xyloside	<i>Cassia biflora</i> leaves	Leguminosae	285
3-(4 ^{Rha} -Rhamnosylrutinoside)	<i>Sageretia filiformis</i> leaves	Rhamnaceae	286
3-(2 ^{Glc} -Glucosylrutinoside)	<i>Dianthus caryophyllus</i> cv. "Novada" leafy stems and roots	Caryophyllaceae	287
3-[6''-Acetyl(4''- α -methylsinapoylneohesperido-side)]	<i>Aerva tomentosa</i>	Amaranthaceae	288
Kaempferol 3,5-dimethyl ether			
7-Glucoside	<i>Nitraria tangutorum</i> leaves	Nitrariaceae	289

continued

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
Kaempferol 3,7-dimethyl ether 4'-Glucoside	<i>Lantana camara</i> leaves	Verbenaceae	290
Kaempferol 7,4'-dimethyl ether 3-Glucoside	<i>Gymnotheca involucrata</i> whole plant	Saururaceae	291
3-Neohesperidoside	<i>Costus spiralis</i> leaves	Costaceae	292
6-Hydroxykaempferol 3-Glucoside	<i>Carthamus tinctorius</i> petals	Compositae	293
7-Alloside	<i>Tagetes erecta</i> flowers	Compositae	294
3-Rutinoside	<i>Daphniphyllum calycinum</i> leaves	Daphniphyllaceae	295
3,6-Diglucoside	<i>Carthamus tinctorius</i> petals	Compositae	293
3,6,7-Triglucoside			
3-Rutinoside-6-glucoside 7-(6''-Caffeoylglucoside)	<i>Eupatorium glandulosum</i> leaves	Compositae	296
6-Hydroxykaempferol 6-methyl ether (eupafolin) 3-(6''-p-Coumaroylglucoside)	<i>Paepalanthus polyanthus</i> , <i>P. hilairei</i> , <i>P. robustus</i> , <i>P. ramosus</i> , and <i>P. denudatus capitulae</i>	Eriocaulaceae	297
6-Hydroxykaempferol 4'-methyl ether 7-Glucoside	<i>Serratula strangulata</i> whole plant	Compositae	298
7-Galactoside			
6-Hydroxykaempferol 6,4'-dimethyl ether 3-Glucoside	<i>Arnica montana</i> flowers	Compositae	299
6-Hydroxykaempferol 3,5,7,4'-tetramethyl ether 6-Rhamnoside	<i>Pterocarpus marsupium</i> roots	Leguminosae	300
8-Hydroxykaempferol (herbacetin) 3-β-D-Glucofuranoside	<i>Jungia paniculata</i> whole plant	Compositae	301
3-Rhamnoside-8-glucoside	<i>Ephedra aphylla</i> aerial parts	Ephedraceae	302
Herbacetin 7-methyl ether 8-Sophoroside	<i>Ranunculus sardous</i> pollen	Ranunculaceae	303
3-(2''-E-Feruloylglucoside)	<i>Ranunculus sardous</i> pollen	Ranunculaceae	304
Herbacetin 8-methyl ether (sexangularetin) 3-Neohesperidoside	<i>Crateagus monogyna</i> pollen	Rosaceae	305
Herbacetin 7,8,4'-trimethyl ether (tambulin) 3,5-Diacetate	<i>Zanthoxylum integrifolium</i> fruits	Rutaceae	306
5,7,8-Trihydroxy-3-methoxyflavone 8-(E-2-Methylbut-2-enoate)	<i>Pseudognaphalium robustum</i> and <i>P. cheirantifolium</i> whole plant	Compositae	307
5,7,8-TriOH-3,6-dimethoxyflavone 8-(E-2-Methylbut-2-enoate)	<i>Pseudognaphalium robustum</i> and <i>P. cheirantifolium</i> whole plant	Compositae	307
6,8-Dihydroxykaempferol 3-Rutinoside	<i>Withania somnifera</i> leaves	Solanaceae	308
Quercetin			
3-α-D-Arabinopyranoside	<i>Persea americana</i> leaves	Lauraceae	170
5-Glucuronide	<i>Leucantheum vulgare</i> leaves	Compositae	171
4'-Galactoside	<i>Cornulaca monacantha</i> aerial parts	Chenopodiaceae	309

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
4'-Glucuronide	<i>Psidium guajava</i> leaves	Myrtaceae	310
3-Rhamnosyl(1 → 2)-α-L-arabinofuranoside (arapetaloside A)	<i>Artabotrys hexapetalus</i> leaves	Annonaceae	173
3-α-L-Arabinofuranosyl(1 → 2)glucoside	<i>Prunus spinosa</i> leaves	Rosaceae	253
3-Xylosyl(1 → 6)glucoside	<i>Cistus ladanifer</i> pollen	Cistaceae	311
3-Rhamnosyl(1 → 2)rhamnoside	<i>Centaurea horrida</i> aerial parts	Compositae	312
3-Glucosyl(1 → 2)rhamnoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	177
3-Galactosyl(1 → 2)rhamnoside	<i>Embelia schimperi</i> leaves	Myrsinaceae	313
3-Laminaribioside	<i>Pteridium aquilinum</i> aerial parts	Dennstaedtiaceae	314
3-Glucosyl(1 → 3)galactoside	<i>Filipendula formosa</i> aerial parts	Rosaceae	315
3-Glucosyl(1 → 4)galactoside	<i>Rumex chalepensis</i> leaves	Polygonaceae	316
3-Glucosyl(1 → 2)glucuronide	<i>Cordia macleodii</i> leaves and flowers	Boraginaceae	317
3-Rhamnoside-3'-glucoside	<i>Myrsine seguinii</i> leaves	Myrsinaceae	318
3-Xylosyl(1 → 2)rhamnosyl(1 → 6)glucoside	<i>Camellia saluensis</i> leaves	Theaceae	319
3-Xylosyl(1 → 2)rhamnoside	<i>Helicia nilagirica</i> leaves	Proteaceae	187
3-Apiosyl(1 → 2)rhamnosyl(1 → 6)glucoside	<i>Baccharis thesioides</i> aerial parts	Compositae	320
3-(6''-Rhamnosylgentiobioside)	<i>Capparis spinosa</i> aerial parts	Capparidaceae	321
3-Rhamnosyl(1 → 2)glucosyl(1 → 6)galactoside	<i>Cassia marginata</i> stems	Leguminosae	189
3-Rhamnosyl(1 → 6)glucosyl(1 → 6)galactoside	<i>Albizia lebbeck</i> leaves	Leguminosae	190
3-Glucosyl(1 → 2)galactosyl(1 → 2)glucoside	<i>Nigella sativa</i> seeds	Ranunculaceae	192
7-(2 ^G -Xylosylrutinoside)	<i>Bidens andicola</i> aerial parts	Compositae	322
3-Glucosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]	<i>Thevetia peruviana</i> leaves	Apocynaceae	323
3-Rhamnosyl(1 → 2)-α-L-arabinopyranoside-7-glucoside	<i>Putoria calabrica</i> aerial parts	Rubiaceae	324
3-Xylosyl(1 → 2)glucoside-7-rhamnoside	<i>Lathyrus chrysanthus</i> and <i>L. chloranthus</i> flowers	Leguminosae	325
3-Galactoside-7-glucosyl(1 → 4)rhamnoside			
3-Neohesperidoside-7-rhamnoside	<i>Sedum telephium</i> subsp. <i>maximum</i> leaves	Crassulaceae	200
3-Rhamnosyl(1 → 4)rhamnoside-7-galactoside	<i>Maesa lanceolata</i> leaves	Myrsinaceae	326
3-Neohesperidoside-7-glucoside	<i>Nicotiana</i> spp. flowers	Solanaceae	206
3-Glucosyl(1 → 2)rhamnoside-7-glucoside	<i>Crocus chrysanthus-biflorus</i> cvs "eye catcher" and "spring pearl" flowers	Iridaceae	204
3-Glucosyl(1 → 2)galactoside-7-glucoside	<i>Trigonella foenum-graecum</i> stems	Leguminosae	271
3-Sophoroside-7-glucuronide	<i>Allium cepa</i> guard cells	Alliaceae	207
3-Rutinoside-3'-apioside	<i>Plantago ovata</i> and <i>P. psyllium</i> seeds	Plantaginaceae	327
3,3',4'-Triglucoside	<i>Eruca sativa</i> leaves	Cruciferae	328
3-Xylosyl(1 → 4)[xylosyl(1 → 6)glucosyl(1 → 2)rhamnoside]	<i>Helicia nilagirica</i> leaves	Protaceae	187
3-Xylosyl(1 → 3)rhamnosyl(1 → 6)[apiosyl(1 → 2)galactoside]	<i>Astragalus caprinus</i> leaves	Leguminosae	213
3-Glucosyl(1 → 4)rhamnoside-7-rutinoside	<i>Myrsine africana</i> leaves	Myrsinaceae	329
3-Rhamnosyl(1 → 6)[glucosyl(1 → 2)glucoside]-7-rhamnoside	<i>Warburgia ugandensis</i> leaves	Rubiaceae	211
7-[Xylosyl(1 → 2)rhamnosyl(1 → 2)rhamnosyl](1 → 6)glucoside	<i>Bidens andicola</i> aerial parts	Compositae	322

continued

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(4''-Malonylrhamnoside)	<i>Ribes alpinum</i> leaves	Grossulariaceae	330
3-(2''-Caffeoylglucuronide)	<i>Scolymus hispanicus</i>	Compositae	331
3-(6''-Feruloylgalactoside)	<i>Persicaria lapathifolia</i> aerial parts	Polygonaceae	332
3-(2''-Acetylrhamnoside)	<i>Nymphaea caerulea</i> blue flowers	Nymphaeaceae	333
3-(2''-Acetylgalactoside)	<i>Hypericum perforatum</i> dried crude drug	Guttiferae	334
3-(6''- <i>n</i> -Butylglucuronide) (parthenosin)	<i>Parthenocissus tricuspidata</i> leaves	Vitaceae	335
7-(6''-Galloylglucoside)	<i>Acacia farnesiana</i> pods	Leguminosae	336
7-(6''-Acetylgalactoside)	<i>Carthamus tinctorius</i> leaves	Compositae	337
4'-(6''-Galloylglucoside)	<i>Eucalyptus rostrata</i> leaves	Myrtaceae	224
3-(2'',6''-Digalloylgalactoside)	<i>Acer okamotoanum</i> leaves	Aceraceae	338
3-(3'',6''-Diacetylgalactoside)	<i>Tagetes elliptica</i> aerial parts	Compositae	339
3-(2'',3'',4''-triacylgalactoside)			
3-(2'''-Galloylglucosyl)(1 → 2)- α -L-arabinofuranoside	<i>Euphorbia pachyrrhiza</i>	Euphorbiaceae	340
3-(2''-Galloylglucoside)-4'-vinylpropionate	<i>Psidium guajava</i> seeds	Myrtaceae	341
3-(2''-Galloylrutinoside)	<i>Euphorbia ebractedata</i> aerial parts	Euphorbiaceae	342
3-(6 ^G -Malonylneohesperidoside)	<i>Clitoria ternatea</i> petals	Leguminosae	244
3- α -L-Arabinopyranosyl(1 → 6) (2''- <i>E-p</i> -coumaroylglucoside)	<i>Vicia angustifolia</i> leaves and stems	Leguminosae	343
3- α -L-Arabinopyranosyl(1 → 6) (2''- <i>E-p</i> -coumaroylgalactoside)			
3-(2 ^G - <i>E-p</i> -Coumaroylrutinoside)	<i>Alibertia sessilis</i> leaves	Rubiaceae	246
3-(2'''- <i>E</i> -Caffeoyl- α -L-arabinopyranosyl)(1 → 6)glucoside	<i>Morina nepalensis</i> var. <i>alba</i> whole plant	Morinaceae	344
3-(2'''- <i>E</i> -Caffeoyl- α -L-arabinopyranosyl)(1 → 6)galactoside			
3-(6''-Caffeoylgentiobioside)	<i>Lonicera implexa</i> leaves	Caprifoliaceae	107
3-(6''-Caffeoylsophoroside)	<i>Bassia muricata</i> aerial parts	Chenopodiaceae	345
3-(6''-Feruloylsophoroside)			
3-(2'''-Feruloylsophoroside)	<i>Petunia</i> cv “Mitchell” and its LC transgenic leaves	Solanaceae	252
3-(6'''-Sinapoylglucosyl)(1 → 2)galactoside	<i>Thevetia peruviana</i> leaves	Apocynaceae	249
3-Rhamnosyl(1 → 6)(2''-acetylgalactoside)	<i>Prumus mume</i> flowers	Rosaceae	346
3-(6''-Acetylgalactosyl)(1 → 3)galactoside (euphorbianin)	<i>Euphorbia hirta</i> leaves	Euphorbiaceae	347
3-(2''-Caffeoylglucoside)(1 → 2) (6''-malonylglucoside)	<i>Petunia</i> cv. “Mitchell” and its LC transgenic leaves	Solanaceae	252
3-(3'',4''-Diacetylrhamnosyl)(1 → 6)glucoside	<i>Tordylium apulum</i> aerial parts	Umbelliferae	348
3-[2'',3'',4''-Triacetyl- α -L-arabinopyranosyl(1 → 6)glucoside]	<i>Calluna vulgaris</i> flowers	Ericaceae	349
3-[2'',3'',4''-Triacetyl- α -L-arabinopyranosyl(1 → 6)galactoside]	<i>Calluna vulgaris</i> flowers	Ericaceae	350
3-[2'',3'',5''-Triacetyl- α -L-arabinopyranosyl(1 → 6)glucoside]	<i>Calluna vulgaris</i> flowers	Ericaceae	258
3-[2'',6''-{ <i>p</i> -(7'''-Glucosyl)coumaroyl}glucosyl]rhamnoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	177

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(6''-Malonylglucoside)-7-glucoside	<i>Ranunculus fluitans</i> leaves	Ranunculaceae	351
3-(6''-E-p-Coumaroylglucoside)-7-glucoside	<i>Lotus polyphyllus</i> whole plant	Leguminosae	255
3-(6'''-p-Coumaroylsophorotrioside)	<i>Pisum sativum</i> shoots	Leguminosae	352
3-(6'''-Caffeoylsophorotrioside)			
3-(6'''-Feruloylsophorotrioside)			
3-(6'''-Sinapoylsophorotrioside)			
3-(6'''-Feruloylglucosyl)(1 → 2)galactosyl(1 → 2)glucoside	<i>Nigella sativa</i> seeds	Ranunculaceae	192
3-Rutinoside-7-(6''-benzoylglucoside)	<i>Canthium dicocum</i> leaves	Rubiaceae	353
3-(p-Coumaroylsambubioside)-7-glucoside	<i>Ranunculus</i> spp. leaves	Ranunculaceae	354
3-(6''-p-Coumaroylglucosyl)(1 → 2)rhamnoside-7-glucoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	177
3-(6''-E-p-Coumaroylsophoroside)-7-rhamnoside	<i>Aconitum napellus</i> ssp. <i>tauricum</i> flowers	Ranunculaceae	267
3-(p-Coumaroylsophoroside)-7-glucoside	<i>Ranunculus</i> spp. leaves	Ranunculaceae	354
3-(Caffeoylarabinosylglucoside)-7-glucoside			
3-(2'''-Caffeoylsambubioside)-7-glucoside			
3-(Feruloylsambubioside)-7-glucoside			
3-(4'''-Caffeoylrhamnosyl)(1 → 2)-α-L-arabinopyranoside-7-glucoside	<i>Putoria calabrica</i> aerial parts	Rubiaceae	324
3-Glucosyl-7-(6''-E-caffeoylglucosyl)(1 → 3)rhamnoside	<i>Aconitum napellus</i> ssp. <i>neomontanum</i> flowers	Ranunculaceae	268
3-(6''-Caffeoylsophoroside)-7-rhamnoside	<i>Aconitum baicalense</i> aerial parts	Ranunculaceae	355
3-Sophoroside-7-(6''-trans-caffeoylglucoside)	<i>Symplocarpus renifolius</i> leaves	Araceae	356
3-(2'''-E-Caffeoylsophoroside)-7-glucoside	<i>Brassica oleracea</i> leaves	Cruciferae	269
3-(2'''-E-Feruloylsophoroside)-7-glucoside			
3-[(6''-Feruloylglucosyl)(1 → 2)-β-arabinopyranoside]-7-glucoside	<i>Carrichtera annua</i> whole plant	Cruciferae	357
3-(6''-E-Sinapoylsophoroside)-7-rhamnoside	<i>Elaeagnus bockii</i> leaves	Elaeagnaceae	358
3-Caffeoylsophoroside-7-caffeoylglucoside	<i>Ranunculus fluitans</i> leaves	Ranunculaceae	351
3-Caffeoylsophoroside-7-feruloylglucoside			
3-(2''-Sinapoylglucoside)-3'-(6''-sinapoylglucoside)-4'-glucoside	<i>Eruca sativa</i> leaves	Cruciferae	328
3,4'-Diglucoside-3'-(6''-sinapoylglucoside)			
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(3''-E-p-coumaroylgalactoside)]-7-rhamnoside	<i>Rhazya orientalis</i> aerial parts	Apocynaceae	359
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(4''-E-p-coumaroylgalactoside)]-7-rhamnoside			
3-Rhamnosyl(1 → 2)[glucosyl(1 → 3)(4'''-p-coumaroylrhamnosyl)(1 → 6)galactoside]	<i>Lysimachia capillipes</i> whole plant	Primulaceae	272
Diquercetin 3-galactoside ester of tetrahydroxy-μ-truxinic acid (monochaetin, 13.6)	<i>Monochaetum multiflorum</i> leaves	Melastomataceae	360
3-Sulfate-7-α-arabinopyranoside	<i>Atriplex hortensis</i> leaves	Chenopodiaceae	274
3-Rhamnoside-3'-sulfate	<i>Leea guineensis</i> leaves	Leeaceae	361
3-Glucoside-3'-sulfate	<i>Centaurea bracteata</i> aerial parts	Compositae	275

continued

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
7,4'-Disulfate	<i>Alchornea laxiflora</i> whole plant	Euphorbiaceae	362
Quercetin 3-methyl ether			
5-Glucoside	<i>Asplenium trichomanes-ramosum</i> fronds	Aspleniaceae	363
7- α -L-Arabinofuranosyl(1 \rightarrow 6)glucoside	<i>Lepisorus ussuriensis</i> whole plant	Polypodiaceae	364
7-Rutinoside	<i>Bidens leucantha</i> leaves	Compositae	365
7-Gentiobioside	<i>Lonicera implexa</i> leaves	Caprifoliaceae	107
7-Galactosyl(1 \rightarrow 4)glucoside	<i>Acacia catechu</i> stems	Leguminosae	366
5-Glucoside-3'-sulfate	<i>Calorophus elongatus</i> culms	Restionaceae	367
Quercetin 7-methyl ether (rhamnetin)			
3- α -L-Arabinopyranosyl(1 \rightarrow 3)galactoside	<i>Pongamia pinnata</i> seeds	Leguminosae	368
3-Robinobioside	<i>Cassia siamea</i> stem bark	Leguminosae	369
3-Laminaribioside	<i>Pteridium aquilinum</i> aerial parts	Dennstaedtiaceae	370
3-Gentiobioside	<i>Cassia fistula</i> roots	Leguminosae	371
3- α -L-Arabinopyranosyl(1 \rightarrow 3) [galactosyl(1 \rightarrow 6)galactoside]	<i>Pongamia pinnata</i> seeds	Leguminosae	368
3-[3-Hydroxy-3-methylglutaryl(1 \rightarrow 6)] [apiosyl (1 \rightarrow 2)galactoside]	<i>Astragalus caprinus</i> leaves	Leguminosae	186
3-(3''''- <i>p</i> -Coumaroylrhamnoside)	<i>Rhamnus petiolaris</i> fruit	Rhamnaceae	372
3,3'-Disulfate	<i>Argyrea mollis</i> roots	Convolvulaceae	373
3,3',4'-Trisulfate	<i>Tamarix amplexicaulis</i> leaves	Tamaricaceae	374
Quercetin 3'-methyl ether			
3-Rhamnoside	<i>Oxytropis lanata</i> aerial parts	Leguminosae	375
5-Galactoside	<i>Pyrus bourgaeana</i> aerial parts	Rosaceae	376
7- α -D-Glucosaminopyranoside	<i>Haloenemum strobilaceum</i> aerial parts	Chenopodiaceae	377
3- α -Arabinopyranosyl(1 \rightarrow 6)galactoside	<i>Trillium apeton</i> and <i>T. kamtechaticum</i> leaves	Liliaceae	378
3-Xylosyl(1 \rightarrow 2)glucoside	<i>Lathyrus chrysanthus</i> and <i>L. chloranthus</i> flowers	Leguminosae	325
3-Xylosyl(1 \rightarrow 6)glucoside	<i>Cistus ladanifer</i> pollen	Cistaceae	311
3-Xylosyl(1 \rightarrow 2)galactoside	<i>Asclepias syriaca</i> flowers	Asclepiadaceae	379
3-Apiosyl(1 \rightarrow 2)glucoside	<i>Vernonia galamensis</i> ssp. <i>galamensis</i> var. <i>petitiana</i> whole plant	Compositae	380
3-Apiosyl(1 \rightarrow 2)galactoside	<i>Vernonia galamensis</i> spp. <i>nairobiensis</i> leaves	Compositae	381
3-Laminaribioside	<i>Pteridium aquilinum</i> aerial parts	Dennstaedtiaceae	314
3-Glucosyl(1 \rightarrow 3)galactoside	<i>Achlys triphylla</i> underground parts	Berberidaceae	382
3-Galactoside-7-rhamnoside	<i>Lathyrus chrysanthus</i> and <i>L. chloranthus</i> flowers	Leguminosae	325
4'-Rhamnosyl(1 \rightarrow 2)glucoside (crosatoside A)	<i>Crocus sativus</i> pollen	Iridaceae	383
3-Xylosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6)glucoside	<i>Hamada scoparia</i> leaves	Chenopodiaceae	384
3-Xylosylrobinobioside	<i>Nitraria retusa</i> leaves and young stems	Nitrariaceae	385
3-Apiosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)glucoside]	<i>Pituranthos tortuosus</i> shoots	Umbelliferae	386
3-Rhamnosyl(1 \rightarrow 2)[glucosyl(1 \rightarrow 6)glucoside]	<i>Allium neapolitanum</i> whole plant	Liliaceae	191
3-(4 ^R _{ha} -Galactosylrobinobioside)	<i>Nitraria retusa</i> leaves and young stems	Nitrariaceae	385
3-Galactosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)glucoside]	<i>Calotropis gigantean</i> aerial parts	Asclepiadaceae	387
3-Xylosyl(1 \rightarrow 2)glucoside-7-rhamnoside	<i>Lathyrus chrysanthus</i> and <i>L. chloranthus</i> flowers	Leguminosae	325

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-Glucosyl(1 → 6)galactoside-7-glucoside	<i>Heterotropa aspera</i> leaves	Aristolochiaceae	388
3-Rhamnosyl(1 → 2)gentiobiosyl (1 → 6)glucoside	<i>Allium neopolitanum</i> whole plant	Liliaceae	191
3-Rhamnosyl(1 → 2)gentiobioside-7-glucoside			
3-(2 ^G -Rhamnosylrutinoside)-7-rhamnoside	<i>Coleogyne ramosissima</i> aerial parts	Rosaceae	389
3-[6''-(2- <i>E</i> -Butenoyl)glucoside]	<i>Zygophyllum simplex</i> aerial parts	Zygophyllaceae	390
3-(2'',3'',4''-Triacetylglucoside)	<i>Warburgia stuhlmanii</i> leaves	Cannellaceae	391
7-(6''- <i>p</i> -Coumaroylglucoside)	<i>Buddleia coriacea</i> aerial parts	Loganiaceae	236
3-(6''- <i>p</i> -Coumaroylglucosyl)(1 → 2)rhamnoside	<i>Ginkgo biloba</i> leaves	Ginkgoaceae	265
3-(3''-Feruloylrhamnosyl)(1 → 6)galactoside	<i>Allium neopolitanum</i> whole plant	Liliaceae	191
3-(6''- <i>E</i> -Sinapoylsphoroside)	<i>Cassia marginata</i> stems	Leguminosae	189
3-(2''-Acetyl- α -arabinopyranosyl)(1 → 6) galactoside	<i>Trillium apetalon</i> and <i>T. kamtschaticum</i> leaves	Liliaceae	378
3-Rhamnosyl(1 → 6)(2''-acetylglucoside)	<i>Prunus mume</i> flowers	Rosaceae	346
3-(6''-Acetylglucosyl)(1 → 3)galactoside	<i>Achlys triphylla</i> underground parts	Berberidaceae	382
3-(4'',6''-Diacetylglucosyl)(1 → 3)galactoside			
3-(6''- <i>E-p</i> -Coumaroylglucoside)-7-glucoside	<i>Lotus polyphyllus</i> whole plant	Leguminosae	255
3-[2''-(4''-Acetyl-rhamnosyl)gentiobioside]	<i>Ammi majus</i> aerial parts	Umbelliferae	262
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2) (3''- <i>E-p</i> -coumaroylgalactoside)]-7-rhamnoside	<i>Rhazya orientalis</i> aerial parts	Apocynaceae	359
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2) (4''- <i>p</i> -coumarylgalactoside)]-7-rhamnoside			
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2) (4''- <i>Z-p</i> -coumaroylgalactoside)]			
3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2) (4''- <i>E</i> -feruloylgalactoside)]-7-rhamnoside			
3-(4''-Sulfatorutinoside)	<i>Zygophyllum dumosum</i> aerial parts	Zygophyllaceae	392
Quercetin 4'-methyl ether (tamarixetin)			
3-Galactoside	<i>Cynanchum thesioides</i> whole plant	Asclepiadaceae	393
3-Neohesperidoside	<i>Costus spicatus</i> leaves	Costaceae	284
3-Glucosyl(1 → 2)galactoside	<i>Cynanchum thesioides</i> whole plant	Asclepiadaceae	393
3,7-Diglucoside	<i>Zanthoxylum bungeanum</i> pericarps	Rutaceae	394
3-Rutinoside-7-rhamnoside	<i>Cassia italica</i> aerial parts	Leguminosae	395
3-Glucoside-7-sulfate	<i>Polygonum hydropiper</i> leaves	Polygonaceae	396
Quercetin 3,5-dimethyl ether (caryatin)			
7-Glucoside	<i>Eucryphia glutinosa</i> twigs	Eucryphiaceae	397
Quercetin 3,7-dimethyl ether			
3'-Neohesperidoside	<i>Dasymaschalon sootepense</i> leaves	Annonaceae	398
3'-(6 ^G -Rhamnosylneohesperidoside)			
4'-Sulfate	<i>Ipomoea regnelli</i>	Convolvulaceae	373
Quercetin 3,3'-dimethyl ether			
7-Rutinoside	<i>Bidens pilosa</i> roots	Compositae	399
Quercetin 3,4'-dimethyl ether			
7-Glucoside	<i>Zanthoxylum bungeanum</i> pericarps	Rutaceae	394
7- α -L-Arabinofuranosyl(1 → 6)glucoside	<i>Punica granatum</i> bark	Punicaceae	400
7-Rutinoside	<i>Bidens pilosa</i> var. <i>radiata</i> aerial parts	Compositae	401
7-Rutinoside	<i>Bidens leucantha</i> leaves	Compositae	365

continued

TABLE 13.2
New Flavonol Glycosides — *continued*

Glycoside	Source	Family	Ref.
7-(2 ^G -Rhamnosylrutinoside)			
7-(2 ^G -Glucosylrutinoside)			
Quercetin 7,3'-dimethyl ether (rhamnazin)			
3-Glucosyl(1 → 5)-α-L-arabinofuranoside	<i>Retama sphaerocarpa</i> aerial parts	Leguminosae	402
3-Xylosyl(1 → 2)glucoside	<i>Albizzia julibrissin</i> seeds	Leguminosae	403
3-Glucosyl(1 → 5)[apiosyl(1 → 2)-α-L-arabinofuranoside]	<i>Retama sphaerocarpa</i> aerial parts	Leguminosae	404
Quercetin 7,4'-dimethyl ether (ombuin)			
3-Arabinofuranoside	<i>Coccinia indica</i> roots	Cucurbitaceae	405
3-Glucoside	<i>Gynostemma yixingense</i>	Cucurbitaceae	406
Quercetin 3',4'-dimethyl ether			
3-Neohesperidoside	<i>Crotalaria verrucosa</i> stems	Leguminosae	407
3,7-Diglucoside	<i>Calamintha grandiflora</i> leaves and flowers	Labiatae	408
Quercetin 3,7,4'-trimethyl ether			
3-Sulfate	<i>Ipomoea regnelli</i>	Convolvulaceae	373
Quercetin 5,3',4'-trimethyl ether			
3-Galactosyl(1 → 2)rhamnoside-7-rhamnoside	<i>Alhagi persarum</i> aerial parts	Leguminosae	409
Quercetin 5,7,3',4'-tetramethyl ether			
3-Galactoside	<i>Sesbania aculeata</i>	Leguminosae	410
6-Hydroxyquercetin (quercetagenin)			
6-Glucoside	<i>Tagetes mandonii</i> aerial parts	Compositae	411
7-(6''-Isobutyryl)glucoside)	<i>Buphthalmum salicifolium</i> flowers	Compositae	412
7-(6''-Isovaleryl)glucoside)			
7-[6''-(2-Methylbutyryl)glucoside]			
7-(6''- <i>E</i> -Caffeoyl)glucoside)	<i>Eupatorium glandulosum</i> leaves	Compositae	413
7-(6''-Acetyl)glucoside)			
Quercetagenin 6-methyl ether (patuletin)			
3,7-Diglucoside	<i>Arnica montana</i> flowers	Compositae	299
3-(6''- <i>p</i> -Coumaroyl)glucoside)	<i>Paepalanthus polyanthus</i> , <i>P. hilairei</i> , <i>P. robustus</i> , <i>P. ramosus</i> , and <i>P. denudatus</i> capitulae	Eriocaulaceae	297
3-(6''- <i>E</i> -Feruloyl)glucoside)	<i>Paepalanthus polyanthus</i> aerial parts	Eriocaulaceae	414
7-(6''-Isobutyryl)glucoside)	<i>Buphthalmum salicifolium</i> flowers	Compositae	412
	<i>Inula britannica</i> flowers	Compositae	415
7-[6''-(2-Methylbutyryl)glucoside]	<i>Inula britannica</i> flowers	Compositae	415
7-(6''-Isovaleryl)glucoside)			
3-Rhamnoside-7-(2''-acetyl)rhannoside)	<i>Kalanchoe brasiliensis</i> stems and leaves	Crassulaceae	416
3-(4''-Acetyl)rhannoside)-7-rhamnoside			
3-(4''-Acetyl)rhannoside)-7-(2'''-acetyl)rhannoside)			
3-(2''-Feruloyl)glucosyl(1 → 6) [apiosyl(1 → 2)glucoside]	<i>Spinacia oleracea</i> leaves	Chenopodiaceae	417
Quercetagenin 7-methyl ether			
6-Glucoside	<i>Tagetes mandonii</i> aerial parts	Compositae	418
4'-Glucoside	<i>Paepalanthus latipes</i> leaves and scapes	Eriocaulaceae	419

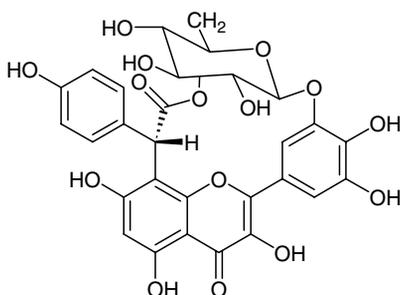
TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-Neohesperidoside	<i>Paepalanthus vellozioides</i> leaves and scapes	Eriocaulaceae	419
3-Cellobioside	<i>Paepalanthus latipes</i> and <i>P. vellozioides</i> leaves and scapes	Eriocaulaceae	419
3-(2''-Caffeoylglucosyl)(1 → 2)glucuronide	<i>Paepalanthus latipes</i> leaves and scapes	Eriocaulaceae	419
Quercetagenin 3,6-dimethyl ether (axillarin)			
5- α -L-Arabinosyl(1 → 6)glucoside	<i>Acacia catechu</i> stems	Leguminosae	420
7-Sulfate	<i>Centaurea bracteata</i> roots	Compositae	421
Quercetagenin 6,3'-dimethyl ether (spinacetin)			
3-(2''-Apiosylgentiobioside)	<i>Spinacia oleracea</i> leaves	Chenopodiaceae	417
3-(2''-Feruloylgentiobioside)			
3-(2''- <i>p</i> -Coumaroylglucosyl)(1 → 6) [apiosyl(1 → 2)glucoside]			
3-(2''-Feruloylglucosyl)(1 → 6) [apiosyl(1 → 2)glucoside]			
Quercetagenin 7,3'-dimethyl ether			
6-Glucoside	<i>Tagetes mandonii</i> aerial parts	Compositae	418
Quercetagenin 3,6,3'-trimethyl ether (jaceidin)			
5-Glucoside	<i>Eucryphia glutinosa</i> twigs	Eucryphiaceae	397
Quercetagenin 6,7,3'-trimethyl ether (veronicafolin)			
3-Glucosyl(1 → 3)galactoside	<i>Eupatorium africanum</i>	Compositae	23
Quercetagenin 6,3',4'-trimethyl ether			
3-Glucoside	<i>Arnica montana</i>	Compositae	299
8-Hydroxyquercetin (gossypetin)			
8- α -D-Lyxopyranoside	<i>Orostachys japonicus</i> aerial parts	Crassulaceae	422
Gossypetin 8-methyl ether			
3-Xylosyl(1 → 2)rhamnoside	<i>Butea superba</i> stems	Leguminosae	423
Gossypetin 3,8-dimethyl ether			
5-Glucoside	<i>Eugenia edulis</i> leaves	Myrtaceae	424
Gossypetin 7,8-dimethyl ether			
3-Glucoside	<i>Erica cinerea</i> flowers	Ericaceae	425
4'-Glucoside			
3,3'-Disulfate	<i>Erica cinerea</i> flowers	Ericaceae	426
Gossypetin 8,3'-dimethyl ether (limocitrin)			
3-Rutinoside-7-glucoside	<i>Coleogyne ramosissima</i> aerial parts	Rosaceae	389
3,5,7,3',4',5'-Hexahydroxyflavone (myricetin)			
3'-Rhamnoside	<i>Davilla flexuosa</i> leaves	Dilleniaceae	427
3-Xylosyl(1 → 3)rhamnoside	<i>Maesa lanceolata</i> leaves	Myrsinaceae	326
3-Rhamnosyl(1 → 2)rhamnoside	<i>Licania densiflora</i> leaves	Chrysobalanaceae	428
3-Neohesperidoside	<i>Physalis angulata</i> leaves	Solanaceae	429
3-Robinobioside	<i>Nymphaea</i> × <i>Marliacea</i> leaves	Nymphaeaceae	430
3-Rhamnoside-3'-glucoside	<i>Myrsine seguinii</i> leaves	Myrsinaceae	318
3-Galactoside-3'-rhamnoside	<i>Buchanania lanzan</i> leaves	Anacardiaceae	431
3,4'-Dirhamnoside	<i>Myrsine seguinii</i> leaves	Myrsinaceae	318
3,4'-Diglucoside	<i>Picea abies</i> needles	Pinaceae	432
3-Rhamnosyl(1 → 3)glucosyl(1 → 6)glucoside	<i>Oxytropis glabra</i>	Leguminosae	433

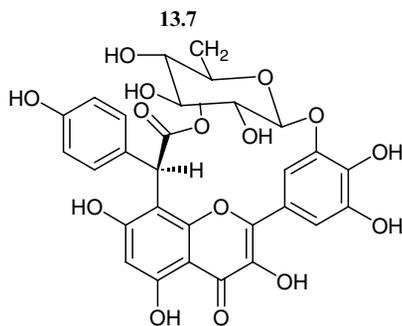
continued

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(2 ^G -Rhamnosylrutinoside)	<i>Clitoria ternatea</i> petals	Leguminosae	244
3-Glucosyl(1 → 2)rhamnoside-7-glucoside	<i>Crocus chrysanthus-biflorus</i> cvs “eye catcher” and “spring pearl” flowers	Iridaceae	204
3-(4''-Malonylrhamnoside)	<i>Ribes alpinum</i> leaves	Grossulariaceae	330
3-(2''-p-Hydroxybenzoylrhamnoside)	<i>Limonium sinense</i> aerial parts	Plumbaginaceae	434
3-(3''-Galloylrhamnoside)	<i>Myrica esculenta</i> bark	Myricaceae	435
3-(3''-Galloylgalactoside)			
3-(2''-Galloylglucoside)	<i>Geranium pratense</i> aerial parts	Geraniaceae	436
3-(6''-p-Coumaroylglucoside)	<i>Nymphaea lotus</i> leaves	Nymphaeaceae	437
Nympholide A			



Nympholide B



13.8

3-(2''-Acetylrhamnoside)	<i>Nymphaea caerulea</i> blue flowers	Nymphaeaceae	333
3-(4''-Acetylrhamnoside)	<i>Eugenia jambola</i> leaves	Myrtaceae	438
7-(6''-Galloylglucoside)	<i>Acacia farnesiana</i> pods	Leguminosae	336
3-(2'',3''-Digalloylrhamnoside)	<i>Acacia confusa</i> leaves	Leguminosae	439
3-(3'',4''-Diacetylrhamnoside)	<i>Myrsine africana</i> leaves	Myrsinaceae	440
3-(2'',3'',4''-Triacetylxylloside)	<i>Maesa lanceolata</i> leaves	Myrsinaceae	326
3-(4''-Acetyl-2''-galloylrhamnoside)	<i>Eugenia jambolana</i> leaves	Myrtaceae	441
3-(3'''-6'''-Diacetylglucosyl)(1 → 4) (2'',3''-diacetylrhamnoside)	<i>Maesa lanceolata</i> leaves	Myrsinaceae	326
Myricetin 7-methyl ether			
3-(2''-Galloylrhamnoside)	<i>Acacia confusa</i> leaves	Leguminosae	439
3-(3''-Galloylrhamnoside)			
Myricetin 3'-methyl ether (larycitrin)			
3-α-L-Arabinofuranoside	<i>Lysimachia congestiflora</i> whole plant	Primulaceae	442

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
3-(4''-Malonylrhamnoside)	<i>Ribes alpinum</i> leaves	Grossulariaceae	330
Myricetin 4'-methyl ether			
3-Galactoside	<i>Licania heteromorpha</i> var. <i>heteromorpha</i> aerial parts	Chrysobalanaceae	443
3-(4''-Acetylrhamnoside)	<i>Eugenia jambolana</i> leaves	Myrtaceae	441
Myricetin 3',4'-dimethyl ether			
3-Rhamnoside	<i>Clausena excavata</i> aerial parts	Rutaceae	444
3-Glucoside	<i>Licania densiflora</i>	Chrysobalanaceae	429
Myricetin 3',5'-dimethyl ether (syringetin)			
3-Rhamnosyl(1 → 5)-α-L-arabinofuranoside	<i>Lysimachia congestiflora</i> whole plant	Primulaceae	442
3-Robinoside	<i>Catharanthus roseus</i> stems	Apocynaceae	445
3-(2'',3''-Diacetylglucoside)	<i>Warburgia stuhlmannii</i> leaves	Canellaceae	391
3-(6''-Acetylglucosyl)(1 → 3)galactoside	<i>Achlys triphylla</i> underground parts	Berberidaceae	382
8-Hydroxymyricetin 8-methyl ether			
3-Rhamnoside	<i>Erica verticillata</i> aerial parts	Ericaceae	446
8-Hydroxymyricetin 8,5'-dimethyl ether			
3-Rhamnoside	<i>Erica verticillata</i> aerial parts	Ericaceae	446
8-Hydroxymyricetin 8,3',5'-trimethyl ether			
3-Rhamnoside	<i>Erica verticillata</i> aerial parts	Ericaceae	446
3,7,3',4',5'-Pentalhydroxyflavone (5-deoxymyricetin, robinetin)			
7-Glucoside	<i>Alternanthera sessilis</i>	Amaranthaceae	447
3-Rutinoside	<i>Ateleia Herbert-smithii</i> leaves	Leguminosae	448
3,4'-Dihydroxy-7,3',5'-trimethoxyflavone			
3-Galactosyl(1 → 4)xyloside	<i>Abrus precatorius</i> seeds	Leguminosae	449
5,7,8-Trihydroxy-3,6,4'-trimethoxyflavone			
8-Tiglate (pratensin A)	<i>Galeana pratensis</i> aerial parts	Compositae	450
3,5,2'-Trihydroxy-7,8,4'-trimethoxyflavone			
5-Glucosyl(1 → 2)galactoside	<i>Cassia occidentalis</i> whole plant	Leguminosae	451
3,5,6,7,8,4'-Hexahydroxy-3'-methoxyflavone			
3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)glucoside	<i>Eschsholtzia californica</i> aerial parts	Papaveraceae	452
3,5,7,4'-Tetrahydroxy-6,8,3'-trimethoxyflavone			
3-α-L-Arabinopyranosyl(1 → 3)galactoside	<i>Pongamia pinnata</i> heartwood	Leguminosae	453
3-α-L-Arabinopyranosyl(1 → 3)[galactosyl (1 → 6)galactoside]			
3,6,7,8,3',4'-Hexahydroxy-5'-methoxyflavone			
7-Neohesperidoside	<i>Hibiscus vitifolius</i>	Malvaceae	454
5,7,2',3',4'-Pentahydroxy-3,6-dimethoxyflavone			
7-Glucoside	<i>Tridax procumbens</i> aerial parts	Compositae	455
5,2'-Dihydroxy-3,6,7,4',5'-pentamethoxyflavone (brickellin)			
2'-Glucoside	<i>Chrysosplenium grayanum</i>	Saxifragaceae	456
3,5,7,2',6'-Pentahydroxyflavone			
2'-Glucoside	<i>Scutellaria amoena</i> roots	Labiatae	457
5,7-Dihydroxy-3,6,8,4'-tetramethoxyflavone			
7-Glucosyl(1 → 3)galactoside	<i>Aspilia africana</i> whole plant	Compositae	458
7,4'-Dihydroxy-3,5,6,8-tetramethoxyflavone			
4'-Glucosyl(1 → 3)galactoside	<i>Centaurea senegalensis</i> whole plant	Compositae	459

continued

TABLE 13.2
New Flavonol Glycosides — *continued*

Glycoside	Source	Family	Ref.
5,8-Dihydroxy-3,6,7,4'-tetramethoxyflavone 8-Neohesperidoside	<i>Peperomia pellucida</i>	Piperaceae	460
5,4'-Dihydroxy-6,7,8,3'-tetramethoxyflavone (africanutin) 4'-Galactoside	<i>Eupatorium africanum</i> whole plant	Compositae	23
3,5,7,2',3',4'-Hexahydroxyflavone 3-Glucoside	<i>Eupatorium sternbergianum</i> whole plant	Compositae	461
5,7,2'-Trihydroxy-3,6,4'-trimethoxyflavone 7-Glucoside	<i>Tridax procumbens</i> whole plant	Compositae	462
5,2',4'-Trihydroxy-3,7,5'-trimethoxyflavone 2'-Galactosyl(1 → 4)glucoside	<i>Albizia procera</i> stems	Leguminosae	463
Methylenedioxyflavonol glycosides			
3-Methoxy-5-hydroxy-6,7-methylenedioxyflavone 4'-Glucuronide	<i>Spinacia oleracea</i> leaves	Chenopodiaceae	464
3-Hydroxy-5,4'-dimethoxy-6,7-methylenedioxyflavone 3-Xyloside (viviparum A)	<i>Polygonum viviparum</i>	Polygonaceae	465
3,3'-Dihydroxy-5,4'-dimethoxy-6,7- methylenedioxyflavone 3-Xyloside (viviparum B)	<i>Polygonum viviparum</i>	Polygonaceae	465
Prenyl- and pyranoflavonol glycosides			
8-Prenylkaempferol[noranhydroicaritin, 3,5,7,4'- tetrahydroxy-8-(3'',3''-dimethylallyl)flavone] 3,7-Diglucoside	<i>Vancouveria hexandra</i> underground and aerial parts	Berberidaceae	466
8-Prenylkaempferol 7-methyl ether 3-Rhamnosyl(1 → 3)[apiosyl(1 → 6)glucoside]	<i>Mosla soochouensis</i> stem wood	Labiatae	278
8-Prenylkaempferol 4'-methyl ether (anhydroicaritin) 7-Glucosyl(1 → 4)glucoside (cuhuoside, 7-cellobioside)	<i>Epimedium acuminatum</i>	Berberidaceae	467
3-Rhamnosyl(1 → 6)galactoside-7-galactoside	<i>Sesbania grandiflora</i> bark	Leguminosae	468
3-Glucosyl(1 → 3)rhamnoside-7-glucoside	<i>Vancouveria hexandra</i> underground and aerial parts	Berberidaceae	466
3-Rhamnosyl(1 → 2)rhamnoside-7-sophoroside (acuminatoside)	<i>Epimedium acuminatum</i> aerial parts	Berberidaceae	469
3-[4'',6'''-Diacylglycosyl(1 → 3)-4''-acetyl]rhamnoside]	<i>Berberis dictyota</i> aerial parts	Berberidaceae	470
3-[2'',6'''-Diacylglycosyl(1 → 3)-4''-acetyl]rhamnoside]- 7-glucoside (epimedin K)	<i>Epimedium koreanum</i> aerial parts	Berberidaceae	471
3''-[4'',6'''-Diacylglycosyl(1 → 3)-4''-acetyl]rhamnoside]- 7-glucoside	<i>Epimedium koreanum</i> aerial parts	Berberidaceae	472
8-(3''-Hydroxy-3''-methylbutyl)kaempferol 4'-methyl ether (icaritin) 3-Rhamnosyl(1 → 2)rhamnoside (wanepimedeside A)	<i>Epimedium wanshanense</i> whole plant	Berberidaceae	473
8-(γ-Methoxy-γγ-dimethyl)propylkaempferol 4'-methyl ether 7-Glucoside (cahuoside D)	<i>Epimedium koreanum</i> aerial parts	Berberidaceae	474
8-Prenylquercetin 4'-methyl ether 3-Rhamnoside (cahuoside C)	<i>Epimedium koreanum</i> aerial parts	Berberidaceae	475

TABLE 13.2
New Flavonol Glycosides — continued

Glycoside	Source	Family	Ref.
8-Prenylquercetin 7,4'-dimethyl ether			
3-Rhamnosyl(1 → 4)rhamnoside	<i>Butea monosperma</i> stems	Leguminosae	476
6'',6''-Dimethylpyrano(2'',3'':7,8)-4'-methoxykaempferol			
3-Rhamnoside	<i>Epimedium acuminatum</i>	Berberidaceae	477
C-Methylated flavonol glycosides			
5,7-Dihydroxy-6,8-di-C-methyl-3-methoxyflavone			
7-Galactosyl(1 → 2)rhamnoside	<i>Cotula anthemoides</i> seeds		478
2'-C-Methylmyricetin			
3-Rhamnoside-5'-gallate	<i>Syzygium samarangense</i> leaves	Myrtaceae	479

All the other new disaccharides are new isomers of known sugar combinations. Among the pentose–pentose sugars are three rhamnosylarabinoses. A rhamnosyl(1 → 2)arabinose was listed in the fourth edition of *The Flavonoids*⁵ but no details of form or stereochemistry were given. The new sugars include a rhamnosyl(1 → 2)- α -L-arabinopyranose, which was identified in the aerial parts of *Putoria calabrica* (Rubiaceae)³²⁴ in combination with quercetin at the 3-hydroxyl and its furanose isomer at the 3-hydroxyl of kaempferol in leaves of *Artabotrys hexapetalus* (Annonaceae).¹⁷³ The report of myricetin 3',5'-dimethyl ether (syringetin) 3-rhamnosyl(1 → 5)arabinofuranoside from the whole plant of *Lysimachia congestiflora*⁴⁴² provides the third new isomer. Xylosyl(1 → 4)rhamnose, found at the 3-position of kaempferol with rhamnose at the 7-hydroxyl in *Chenopodium murale*,¹⁹⁸ is an expectable new isomer of the known 1 → 2 and 1 → 3 linked sugars.

There are three new isomers of pentose–hexose disaccharides in Table 13.4. These include α -L-arabinopyranosyl(1 → 3)galactose found in combination with 3,5,7,4'-tetrahydroxy-6,8,3'-flavone at the 3-hydroxyl in the heartwood of the legume, *Pongamia pinnata*.⁴⁵³ Lathyrose, xylosyl(1 → 6)galactose was found in a member of the Anacardiaceae, *Semecarpus kurzii*,⁶⁰ at the 7-hydroxyl of scutellarein (6-hydroxyapigenin). The third new isomer in this

TABLE 13.3
Monosaccharides of Flavone and Flavonol Glycosides

Pentoses	Hexoses	Uronic Acids
D-Apiose	D-Allose	D-Galacturonic acid ^a
L-Arabinose ^b	D-Allulose ^{c,d}	D-Glucuronic acid ^a
D-Fructose ^c	D-Fucose ^c	
D-Lyxose ^c	D-Galactose	
L-Rhamnose	D-Glucosamine ^c	
D-Xylose	D-Glucose	
	D-Mannose	

^aAlso reported to occur as the methyl and ethyl ethers.

^bKnown to occur in both pyranose and furanose forms; all other sugars (except apiose, fructose, and allulose) are normally in the pyranose form.

^cNewly reported since 1992.

^dRecorded as D-allulose but preferred name is D-psicose or D-ribo-2-hexulose.

group is apiosyl(1 → 6)glucose found at the 7-position of three different flavones: luteolin and its 3'-methyl ether (chrysoeriol) in *Phlomis nissolii* (Labiatae)⁹⁶ and acacetin (apigenin 4'-methyl ether) in *Crotalaria podocarpa* (Leguminosae).⁵³ There are two further new hexose-pentoses: glucosyl(1 → 4)xylose has been isolated at the 7-hydroxyl of kaempferol from *Crotalaria laburnifolia*¹⁷⁸ and galactosyl(1 → 2)rhamnose at the 3-position of quercetin from *Embelia schimperi* (Myrsinaceae).³¹³ Noteworthy is the first record of the hexose-hexose, cellobiose (glucosyl(1 → 4)glucose) from *Epimedium acuminatum*,⁴⁶⁷ which was present at the 7-hydroxyl of 8-prenylkaempferol 4'-methyl ether. Cellobiose has the same sugar linkage as cellulose and has been found in a further four families in combination with two flavones and two flavonols during the review period. Thus, 6-hydroxyluteolin 5,6,3',4'-tetramethyl ether 7-cellobioside has been isolated from stems of the Composite *Sphaeranthus indicus*,¹³⁶ and apigenin 7-cellobioside and 7-cellobioside-4'-glucoside from petals of *Salvia uliginosa* (Labiatae).³⁵ Quercetageitin 3-cellobioside has been reported in stems and scapes of *Paepalanthus latipes* and *P. vellozioides*,⁴¹⁹ from the monocot family Velloziaceae. Kaempferol 3-sophoroside-7-cellobioside and three acylated kaempferol 3-diglycosides, the 3-(2-*E*-caffeoylsophoroside)-7-cellobioside and the corresponding feruoyl and sinapoyl isomers, were found in leaves of *Brassica oleracea* (Cruciferae).²¹⁷ Two further glucosylgalactose isomers are listed in Table 13.4. Glucosyl(1 → 3)galactose was present in underground parts of *Achlys triphylla* (Berberidaceae),³⁸² in acetylated form attached to the 3-hydroxyls of both isorhamnetin and syringetin, while its 1 → 4 isomer was found at the 3-hydroxyl of quercetin in leaves of *Rumex chalepensis* (Polygonaceae).³¹⁶

TABLE 13.4
Disaccharides of Flavone and Flavonol Glycosides

Structure	Trivial Name
<i>Pentose-pentose</i>	
<i>O</i> -β-D-Xylosyl(1 → 2)xylose ^{a,66}	
<i>O</i> -β-D-Xylosyl(1 → 3)xylose ^{a,159}	
<i>O</i> -α-L-Apiofuranosyl(1 → 2)xylose	
<i>O</i> -α-L-Apiofuranosyl(1 → 4)rhamnose ^{a,198}	
<i>O</i> -α-L-Rhamnosyl(1 → 5)arabinofuranose ^{a,442}	
<i>O</i> -α-L-Rhamnosyl(1 → 2)-α-L-arabinopyranose ^{a,324}	
<i>O</i> -α-L-Rhamnosyl(1 → 2)-α-L-arabinofuranose ^{a,b,173}	
<i>O</i> -α-L-Rhamnosyl(1 → 2)rhamnose	
<i>O</i> -α-L-Rhamnosyl(1 → 3)rhamnose	
<i>O</i> -α-L-Rhamnosyl(1 → 4)rhamnose	
<i>O</i> -α-L-Rhamnosyl(1 → 4)xylose	
<i>O</i> -β-D-Xylosyl(1 → 2)rhamnose	
<i>O</i> -β-D-Xylosyl(1 → 3)rhamnose	
<i>O</i> -β-D-Xylosyl(1 → 4)rhamnose ^{a,198}	
<i>Pentose-hexose</i>	
<i>O</i> -α-L-Arabinosyl(1 → 6)glucose	Vicianose
<i>O</i> -α-L-Arabinopyranosyl(1 → 3)galactose ^{a,453}	
<i>O</i> -α-D-Arabinosyl(1 → 6)galactose	
<i>O</i> -β-D-Xylosyl(1 → 2)glucose	Sambubiose
<i>O</i> -β-D-Xylosyl(1 → 6)glucose	
<i>O</i> -β-D-Xylosyl(1 → 2)galactose	
<i>O</i> -β-D-Xylosyl(1 → 6)galactose ^{a,60}	Lathyrose
<i>O</i> -β-D-Apiosyl(1 → 2)glucose	

TABLE 13.4
Disaccharides of Flavone and Flavonol Glycosides — *continued*

Structure	Trivial Name
<i>O</i> -β-D-Apiosyl(1 → 6)glucose ^{a,53,96}	
<i>O</i> -β-D-Apiosyl(1 → 2)galactose	
<i>O</i> -α-L-Rhamnosyl(1 → 2)glucose	Neohesperidose
<i>O</i> -α-L-Rhamnosyl(1 → 3)glucose	Rungiose
<i>O</i> -α-L-Rhamnosyl(1 → 6)glucose	Rutinose
<i>O</i> -α-L-Rhamnosyl(1 → 2)galactose	
<i>O</i> -α-L-Rhamnosyl(1 → 6)galactose	Robinobiose
<i>O</i> -α-L-Rhamnosyl(1 → 2)fucose ^{a,26}	
<i>Hexose–pentose</i>	
<i>O</i> -β-D-Glucosyl(1 → 2)-β-arabinopyranose ^{a,357}	
<i>O</i> -β-D-Glucosyl(1 → 2)-α-L-arabinofuranose ^{a,c,340}	
<i>O</i> -β-D-Glucosyl(1 → 3)-α-L-arabinopyranose ^{a,164}	
<i>O</i> -β-D-Glucosyl(1 → 4)-α-L-arabinopyranose ^{a,94}	
<i>O</i> -β-D-Glucosyl(1 → 5)-α-L-arabinofuranose ^{a,402}	
<i>O</i> -β-D-Glucosyl(1 → 2)rhamnose	
<i>O</i> -β-D-Glucosyl(1 → 3)rhamnose	
<i>O</i> -α-L-Glucosyl(1 → 4)rhamnose	
<i>O</i> -β-D-Glucosyl(1 → 2)xylose	
<i>O</i> -β-D-Glucosyl(1 → 4)xylose ^{a,178}	
<i>O</i> -β-D-Galactosyl(1 → 2)rhamnose ^{a,313}	
<i>O</i> -β-D-Galactosyl(1 → 3)rhamnose	
<i>O</i> -β-D-Galactosyl(1 → 4)rhamnose	
<i>Hexose–hexose</i>	
<i>O</i> -β-D-Glucosyl(1 → 2)glucose	Sophorose
<i>O</i> -β-D-Glucosyl(1 → 3)glucose	Laminaribiose
<i>O</i> -β-D-Glucosyl(1 → 4)glucose ^{a,467}	Cellobiose
<i>O</i> -β-D-Glucosyl(1 → 6)glucose	Gentiobiose
<i>O</i> -β-D-Glucosyl(1 → 2)galactose	
<i>O</i> -β-D-Glucosyl(1 → 3)galactose ^{a,382}	
<i>O</i> -β-D-Glucosyl(1 → 4)galactose ^{a,316}	
<i>O</i> -β-D-Galactosyl(1 → 4)glucose	Lactose
<i>O</i> -β-D-Galactosyl(1 → 6)glucose	
<i>O</i> -β-D-Galactosyl(1 → 4)galactose	
<i>O</i> -β-D-Galactosyl(1 → 6)galactose	
<i>O</i> -β-D-Allosyl(1 → 2)glucose	
<i>O</i> -β-D-Mannosyl(1 → 2)allose	
<i>Pentose–uronic acid</i>	
<i>O</i> -α-L-Rhamnosyl(1 → 2)galacturonic acid	
<i>Uronic acid–uronic acid</i>	
<i>O</i> -β-D-Glucuronosyl(1 → 2)glucuronic acid	

^aDisaccharides newly reported since 1992 with reference number. Except where otherwise stated, sugars are assumed to be in the pyranose form and to have the appropriate linkage, i.e., β for glucosides, α for rhamnosides, etc.

^bReported only at the 3-hydroxyl of quercetin with glucose at the 7-position.

^cReported only in acylated form.

TABLE 13.5
Trisaccharides of Flavonol Glycosides

Structure	Trivial Name
<i>Linear</i>	
<i>O</i> -β-Glucosyl(1 → 4)- <i>O</i> -α-arabinofuranosyl(1 → 2)arabinopyranose	Primflasin
<i>O</i> -β-D-Xylosyl(1 → 2)- <i>O</i> -α-L-rhamnosyl(1 → 6)glucose ^{a,319}	
<i>O</i> -β-D-Xylosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 6)glucose ^{a,384}	
<i>O</i> -β-D-Xylosyl(1 → 6)- <i>O</i> -β-D-glucosyl(1 → 2)rhamnose ^{a,187}	
<i>O</i> -β-D-Xylosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 6)galactose ^{a,186}	
<i>O</i> -α-L-Rhamnosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 3)rhamnose ^{a,b,273}	
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -α-L-rhamnosyl(1 → 6)glucose	2'-Rhamnosylrutinoside
<i>O</i> -α-L-Rhamnosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 6)glucose ^{a,188}	
<i>O</i> -α-L-Rhamnosyl(1 → 4)- <i>O</i> -α-L-rhamnosyl(1 → 6)glucose ^c	
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -β-D-glucosyl(1 → 3)glucose	3'-Rhamnosyllaminaribiose
<i>O</i> -α-L-Rhamnosyl(1 → 3)- <i>O</i> -β-D-glucosyl(1 → 6)glucose ^{a,433}	
<i>O</i> -β-D-Glucosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 6)glucose	3'-Glucosylrutinoside
<i>O</i> -β-D-Glucosyl(1 → 4)- <i>O</i> -α-L-rhamnosyl(1 → 2)glucose ^{a,191}	
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -β-D-glucosyl(1 → 2)rhamnose	
<i>O</i> -β-D-Glucosyl(1 → 6)- <i>O</i> -β-D-glucosyl(1 → 4)rhamnose	
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -β-D-glucosyl(1 → 2)glucose	Sophorotriose
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -β-D-glucosyl(1 → 6)glucose	2'-Glucosylgentiobiose
<i>O</i> -β-D-Glucosyl(1 → 4)- <i>O</i> -β-D-glucosyl(1 → 6)glucose	6'-Maltosylglucose
<i>O</i> -β-D-Glucosyl(1 → 6)- <i>O</i> -β-D-glucosyl(1 → 4)glucose	Sorborose
<i>O</i> -α-L-Rhamnosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 6)galactose	Rhamnose
<i>O</i> -α-L-Rhamnosyl(1 → 4)- <i>O</i> -α-L-rhamnosyl(1 → 6)galactose	Isorhamnose
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -β-D-glucosyl(1 → 6)galactose ^{a,189}	
<i>O</i> -α-L-Rhamnosyl(1 → 6)- <i>O</i> -β-D-glucosyl(1 → 6)galactose ^{a,190}	
<i>O</i> -β-D-Glucosyl(1 → 3)- <i>O</i> -α-L-rhamnosyl(1 → 6)galactose ^d	Sugar of faralatoside
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -β-D-galactosyl(1 → 2)glucose ^{a,192}	
<i>Branched</i>	
<i>O</i> -α-L-Arabinopyranosyl(1 → 3)- <i>O</i> -[β-D-galactosyl(1 → 6)galactose] ^{a,453}	
<i>O</i> -β-D-Apiosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 4)glucose]	2 ^G -Apiosylrutinose
<i>O</i> -β-D-Apiosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)galactose]	2 ^{Gal} -Apiosylrobinobiose
<i>O</i> -β-D-Glucosyl(1 → 5)- <i>O</i> -[β-D-aposyl(1 → 2)-α-L-arabinofuranose] ^{a,404}	
<i>O</i> -α-L-Rhamnosyl(1 → 3)- <i>O</i> -[β-D-aposyl(1 → 6)glucose] ^{a,278}	
<i>O</i> -β-D-Xylosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)glucose]	2 ^G -Xylosylrutinose
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)glucose]	2 ^G -Rhamnosylrutinose
<i>O</i> -α-L-Rhamnosyl(1 → 4)- <i>O</i> -[α-L-rhamnosyl(1 → 2)glucose]	4 ^G -Rhamnosylneohesperidose
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -[β-D-aposyl(1 → 2)glucose]	
<i>O</i> -α-L-Rhamnosyl(1 → 6)- <i>O</i> -[β-D-glucosyl(1 → 2)glucose]	6 ^G -Rhamnosylsophorose
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -[β-D-glucosyl(1 → 4)glucose] ^{a,194}	2 ^G -Rhamnosylcellobiose
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -[β-D-glucosyl(1 → 6)glucose]	2 ^G -Rhamnosylgentiobiose
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)glucose]	2 ^G -Glucosylrutinose
<i>O</i> -β-D-Glucosyl(1 → 3)- <i>O</i> -[α-L-rhamnosyl(1 → 2)glucose]	3 ^G -Glucosylneohesperidose
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -[β-D-glucosyl(1 → 3)rhamnose] ^{a,195}	
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -[β-D-glucosyl(1 → 6)galactose]	
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)galactose]	2 ^G -Glucosylrobinobiose
<i>O</i> -β-D-Galactosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)glucose]	2 ^G -Galactosylrutinose
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -[β-D-glucosyl(1 → 6)glucose]	2 ^G -Glucosylgentiobiose

TABLE 13.5
Trisaccharides of Flavonol Glycosides — continued

Structure	Trivial Name
<i>O</i> -β-D-Glucosyl(1 → 3)- <i>O</i> -[β-D-glucosyl(1 → 6)glucose] ^{a,196}	3 ^G -Glucosylgentiobiose
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)galactose]	2 ^{Gal} -Rhamnosylrobinobiose
<i>O</i> -α-L-Rhamnosyl(1 → 4)- <i>O</i> -[α-L-rhamnosyl(1 → 6)galactose]	4 ^{Gal} -Rhamnosylrobinobiose
<i>O</i> -α-L-Rhamnosyl(1 → 6)- <i>O</i> -[α-L-rhamnosyl(1 → 2)galactose] ^{a,220}	
<i>O</i> -β-D-Glucosyl(1 → 4)- <i>O</i> -[β-D-glucosyl(1 → 2)galactose] ^{a,e,249}	

^aNewly reported since 1992 with reference number.

^bPresent at the 7-position of kaempferol with a *p*-coumaric acid attached at the 4-hydroxyl of the first rhamnose and a known acylated branched trisaccharide at the 3-hydroxyl of the aglycone.

^cOnly present with a caffeyl or *p*-coumaryl group at the 6-hydroxyl of the second glucose.

^dOnly present with an acetyl group at the 4-hydroxyl of the rhamnose.

^ePresent at the 3-hydroxyl of kaempferol in mono- or diacylated form with sinapic acid.

13.3.3 TRISACCHARIDES

Some 11 new linear and 8 new branched trisaccharides have been discovered in combination with flavonols since 1992. These are presented in Table 13.5 together with previously known trisaccharides. Among the linear trisaccharides are seven novel sugar combinations. The most interesting are four structures with xylose as the terminal sugar. The only previously known trisaccharide containing xylose is the branched sugar, 2^G-xylosylrutinose. The linear trisaccharide, xylosyl(1 → 2)rhamnosyl(1 → 6)glucose was found attached to the 3-hydroxyl of quercetin in leaves of *Camellia saluensis* (Theaceae),³¹⁹ while its isomer, xylosyl(1 → 3)rhamnosyl(1 → 6)glucose, was found at the 3-position of isorhamnetin in *Hamada scoparia* (Chenopodiaceae).³⁸⁴ The other two structures were both found in combination with kaempferol at the 3-position, xylosyl(1 → 6)glucosyl(1 → 2)rhamnose from *Helicia nilagirica* (Proteaceae)¹⁸⁷ and xylosyl(1 → 3)rhamnosyl(1 → 6)galactose from the legume *Astragalus caprinus*.¹⁸⁶ The former was also present in similar combination with quercetin. The first linear trirhamnose, rhamnosyl(1 → 3)rhamnosyl(1 → 3)rhamnose, has been recorded from

TABLE 13.6
Trisaccharides of Flavone Glycosides

Structure	Trivial Name
<i>Linear</i>	
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -β-D-glucosyl(1 → 2)glucose ^{a,51}	
<i>O</i> -β-D-Allosyl(1 → 3)- <i>O</i> -β-D-glucosyl(1 → 2)glucose ^{a,110}	
<i>Branched</i>	
<i>O</i> -α-L-Rhamnosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)galactose] ^b	2 ^{Gal} -Rhamnosylrobinobiose
<i>O</i> -β-D-Glucosyl(1 → 2)- <i>O</i> -[α-L-rhamnosyl(1 → 6)glucose] ^{b,c}	2 ^G -Glucosylrutinose
<i>O</i> -β-D-Apiosyl(1 → 2)- <i>O</i> -[β-D-glucosyl(1 → 4)glucose] ^{a,c,109}	2 ^G -Apiosylcellobiose
<i>O</i> -β-D-Glucuronosyl(1 → 3)- <i>O</i> -[β-D-glucuronosyl(1 → 2)glucuronic acid] ^{a,c,40,116}	

^aNewly reported trisaccharides with reference number.

^bFound previously only attached to flavonols.

^cPresent only in acylated form (see Table 13.1)

Planchonia grandis (Lecythidaceae).²⁷³ This sugar occurred at the 7-hydroxyl of kaempferol acylated with *p*-coumaric acid at the 4-position of the first rhamnose and with a known acylated branched trisaccharide at the 3-hydroxyl. Two further acylated kaempferol glycosides with known trisaccharides at both the 3- and 7-hydroxyls were also present in this plant. This is the first record of flavonol (or flavone) glycosides containing six sugars. The three other new linear combinations were isolated as kaempferol 3-rhamnosyl(1 → 2)glucosyl(1 → 6)galactoside from *Cassia marginata*,¹⁸⁹ kaempferol and quercetin 3-rhamnosyl(1 → 6)glucosyl(1 → 6)galactoside from *Albizia lebbek*¹⁹⁰ (both legumes), and kaempferol and quercetin 3-glucosyl(1 → 2)galactosyl(1 → 2)glucosides from seeds of *Nigella sativa* (Ranunculaceae).¹⁹² The remaining new linear trisaccharides in Table 13.2: rhamnosyl(1 → 3) rhamnosyl(1 → 6)glucose,¹⁹² rhamnosyl(1 → 3)glucosyl(1 → 6)glucose,⁴³³ and glucosyl(1 → 4)rhamnosyl(1 → 2)glucose¹⁹¹ are all new isomers of known structures. Details will not be given here but these sugars are marked as new in Table 13.6 and with a reference number, which relates both to the main reference list and to the reference numbers in Table 13.2.

Among the eight new branched trisaccharides are five new sugar combinations, including the first to contain arabinose. Thus, α-L-arabinopyranosyl(1 → 3)[galactosyl(1 → 6)galactose] was found at the 3-hydroxyl of 3,5,7,4'-tetrahydroxy-6,8,3'-trimethoxyflavone in the heartwood of *Pongamia pinnata* (Leguminosae)⁴⁵³ and glucosyl(1 → 5)[apiosyl(1 → 2)-α-L-arabinofuranose] at the 3-hydroxyl of quercetin 7,3'-dimethyl ether in another legume, *Retama sphaerocarpa*.⁴⁰⁴ Another novel apiose containing sugar, rhamnosyl(1 → 3)[apiosyl(1 → 6)glucose], present at the 3-hydroxyls of kaempferol 7-methyl ether and 8-prenylrhamnetin, was isolated from stemwood of the Labiate, *Mosla soochouensis*.²⁷⁸ The remaining new combinations include kaempferol 3-glucosyl(1 → 2)[glucosyl(1 → 3)rhamnoside] from flowers of *Crocus speciosus* and *C. antalyensis*¹⁹⁵ and glucosyl(1 → 4)[glucosyl(1 → 2)galactose], which was found attached to the 3-position of kaempferol in mono- and diacylated forms with sinapic acid, in *Thevetia peruviana* (Apocynaceae).²⁴⁹ Details of three new isomers of known sugar combinations, rhamnosyl(1 → 2)[glucosyl(1 → 4)glucose],¹⁹⁴ glucosyl(1 → 3)[glucosyl(1 → 6)glucose],¹⁹⁶ and rhamnosyl(1 → 6)[rhamnosyl(1 → 2)galactose]²²⁰ are given in Table 13.2 and Table 13.5.

It is of some note that trisaccharides have been found in combination with flavones for the first time. Six structures have been identified since 1992; two linear and four branched trisaccharides are listed in Table 13.6. However, flavone trioses are still of rare occurrence compared with the large number of known flavonol trisaccharide combinations. Rhamnosyl(1 → 2)glucosyl(1 → 2)glucose was found at the 7-hydroxyl of acacetin in aerial parts of *Peganum harmala* (Zygophyllaceae)⁵¹ and allosyl(1 → 3)glucosyl(1 → 2)glucose, acetylated at the 6-position of the allose and attached to luteolin at the 7-hydroxyl, in *Veronica didyma*.¹¹⁰ These are both new trisaccharides that have not been found in association with flavonols. Two of the branched sugars are also novel structures, apiosyl(1 → 2)[glucosyl(1 → 4)glucose] and glucuronosyl(1 → 3)[glucuronosyl(1 → 2)glucuronic acid]. The former was discovered in fruits of *Capsicum annum* (Solanaceae)¹⁰⁹ at the 7-hydroxyl of luteolin and the latter, in acylated form, attached to the 7-hydroxyls of apigenin,⁴⁰ chrysoeriol,¹¹⁶ and tricrin,¹¹⁶ in aerial parts of *Medicago sativa* (Leguminosae). The remaining two branched trisaccharides have been found previously in combination with flavonols.

13.3.4 TETRASACCHARIDES

Only one branched tetrasaccharide was listed in the last edition of *The Flavonoids*:⁵ [rhamnosyl(1 → 4)glucosyl(1 → 6)]sophorose, which was present at the 7-hydroxyl of acacetin and acetylated at the 6''-position of the sophorose in leaves of *Peganum harmala* (Zygophyllaceae).⁶ Since then some eight new branched tetrasaccharides have been reported, all attached

to flavonols and all with unique sugar combinations. Details of the new structures together with the known tetrasaccharide are given in Table 13.7. No linear tetrasaccharide has yet been recorded. Apiosyl(1 → 5)apiosyl[rhamnosyl(1 → 6)glucose], which was found at the 7-hydroxyl of kaempferol 7-methyl ether in the mistletoe, *Viscum angulatum*,²⁸¹ is the first report of any sugar to contain two linked apiose moieties. There are three new structures with xylose as the terminal sugar. Two were found in combination with quercetin at the 3-position. Thus, xylosyl(1 → 3)rhamnosyl(1 → 6)[apiosyl(1 → 2)galactose] was present in leaves of *Astragalus caprinus*²¹³ and xylosyl(1 → 4)[xylosyl(1 → 6)glucosyl(1 → 2)rhamnose] in leaves of *Helicia nilagirica*.¹⁸⁷ The third sugar, xylosyl(1 → 2)[rhamnosyl(1 → 2)rhamnosyl(1 → 6)glucose], was attached to the 7-hydroxyl of both quercetin 3-methyl ether and 7-methyl ether in *Bidens andicola* (Compositae).³²² Three of the remaining structures were found attached to the 3-hydroxyl of kaempferol. These are: glucosyl(1 → 3)rhamnosyl(1 → 2)[rhamnosyl(1 → 6)galactose], which was isolated from leaves of *Maytenus aquifolium*,²¹⁴ rhamnosyl(1 → 2)[xylosyl(1 → 3)rhamnosyl(1 → 6)galactose] from leaves of *Astragalus caprinus*,²¹³ and rhamnosyl(1 → 2)[glucosyl(1 → 3)rhamnosyl(1 → 6)galactose] present in acylated form, together with the corresponding quercetin glycoside, in *Lysimachia capillipes* (Primulaceae).²⁷² The eighth new sugar, rhamnosyl(1 → 2)[gentiobiosyl(1 → 6)glucose] was discovered at the 3-hydroxyl of isorhamnetin in *Allium neapolitanum*.¹⁹¹

13.3.5 SULFATE CONJUGATES

Only a comparatively small number of new flavonoid sulfate conjugates have been recorded between 1993 and 2003. Eleven flavone derivatives are recorded in Table 13.1 and 15 flavonol derivatives in Table 13.2, mostly from plants that grow in water-stress conditions. Amongst the flavones the most notable are six sulfate conjugates discovered in some Australian species of the monocot family, the Restionaceae. These plants are unusual in having no true leaves so that the compounds were isolated from culm tissue. Four are hypolaetin (8-hydroxyluteolin) derivatives: the 7-sulfatoglucoside and 7-sulfatoglucuronide from *Leptocarpus elegans*,¹³⁸ the 7-sulfatoglucuronide from *Meeboldina thysanantha*,¹³⁸ and the 7-sulfate-8-glucoside from *Hypolaena fastigiata*.¹³⁸ The other two flavone sulfates are hypolaetin 7-methyl ether 3'-sulfatogalactoside from *Leptocarpus tenax*¹³⁸ and the corresponding 3'-sulfatoglucuronide

TABLE 13.7
Tetrasaccharides of Flavone and Flavonol O-Glycosides

Structure	Ref.
<i>Flavone tetrasaccharide</i>	
[O-α-L-Rhamnosyl(1 → 4)-O-β-D-glucosyl(1 → 6)]-O-sophorose	6
<i>Flavonol tetrasaccharides</i>	
O-β-D-Apiosyl(1 → 5)-O-β-D- <i>apiosyl</i> (1 → 2)-O-[α-L-rhamnosyl(1 → 6)glucose] ^a	281
O-β-D-Xylosyl(1 → 3)-O-α-L-rhamnosyl(1 → 6)-O-[β-D- <i>apiosyl</i> (1 → 2)galactose] ^a	213
O-β-D-Xylosyl(1 → 4)-O-[β-D-xylosyl(1 → 6)-O-β-D-glucosyl(1 → 2)rhamnose] ^a	187
O-β-D-Xylosyl(1 → 2)-O-[α-L-rhamnosyl(1 → 2)-O-α-L-rhamnosyl(1 → 6)glucose] ^a	322
O-α-L-Rhamnosyl(1 → 2)-O-[β-D-xylosyl(1 → 3)-O-α-L-rhamnosyl(1 → 6)galactose] ^{a,b}	213
O-α-L-Rhamnosyl(1 → 2)-O-[β-D-glucosyl(1 → 3)-O-α-L-rhamnosyl(1 → 6)galactose] ^{a,b}	272
O-β-D-Glucosyl(1 → 3)-O-α-L-rhamnosyl(1 → 2)-O-[α-L-rhamnosyl(1 → 6)galactose] ^a	214
O-α-L-Rhamnosyl(1 → 2)[gentiobiosyl(1 → 6)glucose] ^a	191

^aNewly reported since 1992.

^bPresent only in acylated form.

from *L. elegans*.¹³⁸ Flavonoid sulfates were found to be characteristic constituents of the Restionaceae being detected in 27% of the 115 taxa surveyed. The first report of a sulfate 2''-linked to glucose is from the water plant, *Thalassia testudinum*,¹¹¹ another monocot, where it is found in association with luteolin at the 7-position. The other flavone sulfates are 8-hydroxyapigenin (isoscuteallarein) 8-(2''-sulfatoglucuronide)⁸¹ and 8-(2'',4''-disulfatoglucuronide) and the corresponding isoscuteallarein 4'-methyl ether conjugates from fruits of *Helicteres isora* (Sterculiaceae).⁸²

Among the more interesting new flavonol conjugates are three sulfates linked directly to the aglycone via a carbon rather than an oxygen atom. Compounds such as these have previously only been recorded from synthesis. Thus, galangin 3-glucoside-8-C-sulfate and 8-C-sulfate and kaempferol 8-C-sulfate have been characterized from a whole plant extract of *Phyllanthus virgatus*,¹⁶⁵ a member of the Euphorbiaceae. Two new sulfated flavonols recorded from *Centaurea bracteata*²⁷⁵ are quercetin 3-glucoside-3'-sulfate and quercetagenin (6-hydroxyquercetin) 3,6-dimethyl ether 7-sulfate. Another quercetin conjugate, the 3-rhamnoside-3'-sulfate, has been isolated from leaves of *Leea guineensis* (Leeaceae),³⁶¹ a monogeneric family close to the vines (Vitaceae). Quercetin 7-methyl ether 3,3'-disulfate from roots of *Argyrea mollis*³⁷³ and quercetin 3,7-dimethyl ether 4'-sulfate and quercetin 3,7,4'-trimethyl ether 3-sulfate from aerial parts of *Ipomoea regnelli*³⁷³ are the first sulfated flavonoids to be isolated from Convolvulaceae species. Most of the other new flavonol sulfates are all single family occurrences: quercetin 7,4'-disulfate from *Alchornea laxiflora*,³⁶² another member of the Euphorbiaceae, and the 3,3',4'-trisulfate from leaves of *Tamarix amplexicaulis* (Tamarixaceae),³⁷⁴ isorhamnetin 3-(4''-sulfatorutinoside) from aerial parts of *Zygophyllum dumosum* (Zygophyllaceae),³⁹² quercetin 4'-methyl ether 3-glucoside-7-sulfate from *Polygonum hydro-piper* (Polygonaceae)³⁹⁶ leaves, and gossypetin 7,8-dimethyl ether 3,3'-disulfate from flowers of the bell heather, *Erica cinerea* (Ericaceae).⁴²⁶ The structure of quercetin 5-glucoside-3'-

TABLE 13.8
Acylating Acids and Alcohol and a Lignan Found in Flavone and Flavonol Derivatives

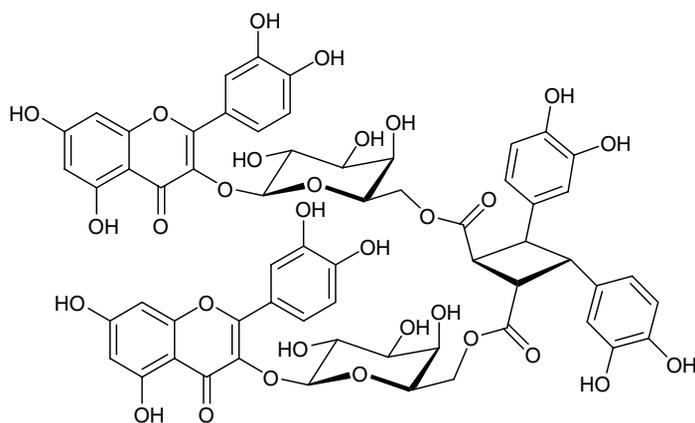
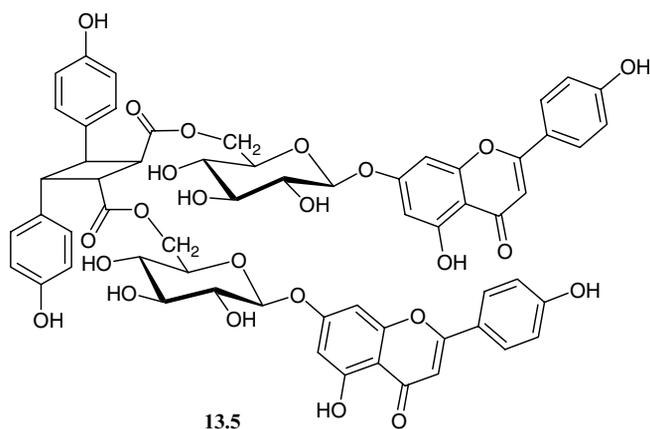
Aliphatic Acids and Alcohol	Aromatic Acids	Sesquiterpene Acid	Lignans
Acetic	Benzoic	Dihydrophaseic	μ -Truxillic acid ³⁶⁰
Malonic	<i>p</i> -Hydroxybenzoic		<i>p,p</i> -Dihydroxytruxillic acid ¹⁶¹
Lactic (2-hydroxypropionic)	Gallic		
Vinylpropionic ^{a,341}	Cinnamic		
Succinic	<i>p</i> -Coumaric		
Butyric	Caffeic		
Isobutyric	Ferulic		
3-Methylbutyric	Isoferulic		
Crotonic (<i>E</i> -2-butenic)	Sinapic		
2-Methyl-2-butenic	α -Methylsinapic ^{a,288}		
<i>n</i> -Butanoic ^{a,335}			
Isovaleric (isopentanoic) ^{a,412,417}			
Tiglic (<i>E</i> -2-methyl-2-butenic)			
3-Hydroxy-3-methylglutaric			
Quinic			
4-Hydroxy-3-methoxyphenyl-1, 3-dihydroxypropan-2-ol ^{a,240}			

^aNewly reported since 1992 with reference numbers.

sulfate from another restionad, *Calorophus elongatus*,³⁶⁷ has not been completely established. However, the possibility that it might be the corresponding 5-sulfate-3'-glucoside seems unlikely since it co-occurs with quercetin 5-glucoside.

13.3.6 ACYLATED DERIVATIVES

Some 77 new acylated flavone and 224 new acylated flavonol derivatives are included in Table 13.1 and Table 13.2, respectively. Only one new acylating acid has been found in combination with flavones and three new aliphatic acids, a new aliphatic alcohol, a lignan, and a new aromatic acid have been discovered in association with flavonols. These are listed with previously recorded structures in Table 13.8. Thus, the lignan, *p,p*-dihydroxytruxillic acid has been found linked to two molecules of apigenin 7-glucoside through the 6-positions of the two sugar moieties in the biflavone glycoside, stachysetin (13.5), from *Stachys aegyptiaca*.¹⁶¹ μ -Truxillic acid has been identified more recently in the biflavonol glycoside, monochaetin (13.6). Here, two molecules of quercetin 3-galactoside are attached through the 6-hydroxyls of the two galactoses to the carboxyl groups of the μ -truxillic acid. Monochaetin was isolated from a leaf extract of the Columbian species *Monochaetum multiflorum* (Melastomataceae).³⁶⁰



The aliphatic acid, vinylpropionic, has been found in seeds of *Psidium guajava* (Myrtaceae)³⁴¹ directly attached to the 4'-hydroxyl of quercetin 3-(3''-galloylglucoside) and *n*-butanoic acid as quercetin 3-(6''-*n*-butylglucuronide) in leaves of the vine, *Parthenocissus tricuspidata*.³³⁵ There are two independent reports of isovaleric (isopentanoic) acid, the first as quercetagenin 7-(6''-isovalerylglucoside) in flowers of *Bupthalmum salicifolium*⁴¹² and the corresponding patuletin glycoside in flowers of another Composite, *Inula Britannica*.⁴¹⁵ The new aliphatic alcohol, 4-hydroxyl-3-methoxyphenyl-1,3-dihydroxypropan-2-ol, has been found, in combination with two additional aromatic acylating acids, *p*-coumaric and ferulic, attached to kaempferol 3-glucoside (stenopalustroside E) in the fern, *Stenochlaena palustris*.²⁴⁰ Three new related kaempferol glycosides, the 3-(3''-*Z*-*p*-coumaroyl-6''-feruloylglucoside) (stenopalustroside B), 3-(3-*Z*-*p*-coumaroyl-6''-*E*-*p*-coumaroylglucoside) (stenopalustroside C), and its stereoisomer, stenopalustroside D, were also present in this plant. The only new aromatic acid, α -methylsinapic, was found in *Aerva tomentosa* (Amaranthaceae),²⁸⁸ attached to kaempferol 4'-methyl ether 3-(6'''-acetyl neohesperidoside) at the 4-position of the glucose moiety. Among the new reports of previously known acylating agents attached to flavonols, *p*-coumaric and acetic acids are the most common with 61 and 50 entries, respectively. Malonic acid, a frequent acylating agent in anthocyanins (see Chapter 10), is rarely found in association with flavones and flavonols. The one new flavone entry is of luteolin 7-apiosyl(1 \rightarrow 2)[glucosyl(1 \rightarrow 4)(malonylglucoside)] from fruits of *Capsicum annuum*¹⁰⁹ and there are some six malonated flavonol glycosides listed in Table 13.2.^{7,38,51,82,168,185}

Reports of unusual known acylating acids include 12 new entries for 3-hydroxy-3-methylglutaric acid, ten are in combination with glycosides of scutellarein, tricetin, luteolin, 6-hydroxyluteolin, and its 3-methyl ether, from four species of the liverwort genus, *Frullania*.^{62,131} The other reports are of the 7-[6''-(3-hydroxy-3-methylglutaryl)glucoside] of apigenin from *Chamaemelum nobile* (Compositae)⁴⁶ and of 5,7-dihydroxy-6,8-di-*C*-methylflavone from the fern, *Matteuccia orientalis*.¹⁶⁰ There are also new reports of 2-methyl butyric acid and 2-methyl-butenoic (angelic) acid, the former occurs as luteolin 7-[6''-(2-methylbutryl)glucoside] in flowers of *Arnica chamissonis* (Compositae)¹⁰⁵ and the latter as luteolin 7-glucoside-4'-angelate in *Polygonum aviculare*.¹⁰⁸ Tiglic acid (*E*-2-methyl-2-butenoic acid) has been found, attached to the 8-hydroxyl of 5,7,8-trihydroxy-3,6,4'-trimethoxyflavone, in *Galeana pratensis*,⁴⁵⁰ another Composite.

13.3.7 NEW FLAVONE GLYCOSIDES — FURTHER CONSIDERATIONS

Some 228 new flavone glycosides are listed in Table 13.1 with details of plant source and references. This increases the total of known glycosides by some third to 700 and includes 26 new apigenin, 25 new luteolin, 8 new chrysoeriol, and 2 new tricetin glycosides bringing their totals to, 99, 111, 44, and 44, respectively. A complete check list of all the known flavone glycosides is given in Appendix A. There are a small number of new monoglycosides still discovered. Among the most interesting finds are three 5-glycosides of simple flavones, i.e., baicalein 6-methyl ether 5-rhamnoside from seeds of *Trichosanthes anguina* (Cucurbitaceae),²² and the 5-glucosides of 5,7,8-trihydroxyflavone (norwogonin) and 5,2'-dihydroxy-7-methoxyflavone from *Pyracantha coccinea* (Rosaceae)²⁷ and *Andrographis alata* (Acanthaceae),³² respectively. The new structures in Table 13.1 also include 32 flavone aglycones that have been found in glycosidic combination for the first time. For example, scutellarein 5,4'-dimethyl ether as the 7-glucoside and 7-(4^R_{ha}-acetylrutinoside) from *Striga passargei* (Scrophulariaceae)⁷¹ and four new luteolin methyl ethers, the 5,3'-dimethyl ether as the 7-glucoside and 4'-glucoside from *Pyrus serotina*,¹²⁴ the 5,4'-di- and 5,3',4'-trimethyl ether as their 7-xylosyl(1 \rightarrow 6)glucosides from *Dirca palustris* (Thymelaeaceae),¹¹² and the 7,3',4'-trimethyl ether as the 5-glucoside and 5-xylosyl(1 \rightarrow 6)glucoside in *Lethedon tannaensis*,¹²⁶ another

member of the Thymelaeaceae. Among the 8-hydroxyluteolin (hypolaetin) derivatives are three glycosides from the Restionaceae: hypolaetin 7-methyl ether 3'-sulfatoglucuronide and 3'-sulfatogalactoside and hypolaetin 7,3'-dimethyl ether 4'-glucoside from three *Leptocarpus* species.¹³⁸ Four glycosides, the 5- and 7-glucosides, the 5-gentiobioside, and the 7-rutinoside, of the trimethylated flavone, nevadensin (5,7-dihydroxy-6,8,4'-trimethoxyflavone), have been reported from *Lysionotus pauciflorus* (Gesneriaceae).^{142,143} 2'-Methylation and 2'-glycosylation are characteristic features of *Scutellaria* and other Labiate species. Therefore, it is not surprising to find reports of further 2',6'-hydroxylated flavones in glycosidic combination in *Scutellaria baicalensis*¹⁴⁷ and *S. rivularis*.³⁰ Glycosides of several new methyl ethers of tricetin and 6-hydroxytricetin have also been discovered (see Table 13.1). These include further glycosides from *Lethedon tannaensis*,^{126,127} namely, tricetin 7,3',4'-trimethyl ether 5-glucoside and tricetin 7,3',4',5'-tetramethyl ether 5-glucoside and 5-xylosyl(1 → 6)glucoside. Two C-methylated flavones have been found in glycosidic combination for the first time bringing the total number of known structures to five. One of the novel glycosides, 5,7-dihydroxy-6,8-di-C-methylflavone 7-[6''-(3-methylglutaryl)glucoside], was isolated from the rhizomes of the fern, *Matteuccia orientalis*¹⁶⁰ and the other, 5,7-dihydroxy-6-C-methylflavone 7-xylosyl(1 → 3)xyloside, from the Labiate, *Mosla chinensis*.¹⁵⁹

13.3.8 NEW FLAVONOL GLYCOSIDES — FURTHER CONSIDERATIONS

There has been a very large increase in the number of flavonol glycosides discovered, especially kaempferol derivatives. Thus, over 500 new structures have been listed in Table 13.2 bringing the total number of known flavonol glycosides to 1333. These are included with previously known flavonol glycosides in Appendix B. There are some 140 new kaempferol, 107 quercetin, and 28 new myricetin glycosides. Half of all the new flavonol glycosides are acylated, often with two or more acyl groups (see Section 13.3.6). A number of new acylated kaempferol glycosides have been isolated from ferns, for example, the 3-(6''-caffeoylglucoside) and 3-(5''-feruloylapioside) from *Pteridium aquilinum*^{232,233} and three 3-(diacetyl-rhamnoside)-7-rhamnoside isomers (2',3'-, 2',4'-, and 3',4'-diacetyl) from *Dryopteris crassirhizoma*.²⁵⁶ Thirty-four known flavonol aglycones have been found in combination with sugars for the first time. These include three interesting new methylated 8-hydroxymyricetin derivatives, the 3-rhamnosides of the 8-mono-, 8,5'-di-, and 8,3',5'-trimethyl ethers, from *Erica verticillata*.⁴⁴⁶ Also two glycosides of 5-deoxymyricetin (robinetin) have been reported, the 7-glucoside from *Alternanthera sessilis* (Amaranthaceae)⁴⁴⁷ and the 3-rutinoside from *Ateleia herbert-smithii*,⁴⁴⁸ a member of the Leguminosae, a family rich in 5-deoxy and 5-methylated flavonoids. The first reported glycosides of gossypetin 3,8-dimethyl ether and its 7,8-isomer have been identified in *Eugenia edulis* (Myrtaceae)⁴²⁴ and *Erica cinerea*,^{425,426} respectively. There are six new records of 2'- or 2',6'-hydroxylated flavonols in glycosidic combination including one from the roots of another *Scutellaria* species, *S. amoena*,⁴⁵⁷ but most of the reports are from members of the Compositae. Two further C-methylated flavonols have been found in glycosidic combination, the 3-rhamnoside-5'-gallate of 2'-C-methyl myricetin from *Syzygium samarangense* (Myrtaceae)⁴⁷⁹ and the 7-galactosyl(1 → 2)rhamnoside of 5,7-dihydroxy-6,8-di-C-methyl-3-methoxyflavone from *Cotula anthemoides* (Compositae).⁴⁷⁸ The only previous entry was of 8-C-methylkaempferol 7-glucoside from roots of *Sophora leachiana* (Leguminosae).⁴⁸¹

13.3.9 GLYCOSIDES OF PRENYLATED FLAVONES AND FLAVONOLS, AND OF PYRANO AND METHYLENEDIOXYFLAVONOLS

No prenylated flavone glycosides were recorded in the last edition of *The Flavonoids*. Therefore, it is significant that the present list in Table 13.1 should contain three such compounds, all isolated from members of the Leguminosae. They include 8-C-prenylapigenin 4'-rutinoside

from *Desmodium gangeticum*,¹⁶² 3'-*C*-prenylapigenin 7-rutinoside from *Pithecellobium dulce*¹⁶³ (both from stem tissue), and 8-*C*-prenylchrysoeriol 7-glucosyl(1 → 3)- α -L-arabinopyranoside from seeds of *Erythrina indica*.¹⁶⁴

Thirteen new prenylated flavonol glycosides have been discovered during the review period, all but three of them from species of the Berberidaceae. There are four new aglycone sugar combinations including the first prenylated quercetin glycosides, i.e., 8-prenylquercetin 4'-methyl ether 3-rhamnoside from *Epimedium koreanum* (Berberidaceae)⁴⁷⁵ and 8-prenylquercetin 7,4'-dimethyl ether 3-rhamnosyl(1 → 4)rhamnoside from *Butea monosperma* (Leguminosae).⁴⁷⁶ 6,6''-Dimethylpyrano(2'',3'':7,8)-4'-methoxykaempferol 3-rhamnoside was also present in *Epimedium acuminatum*.⁴⁷⁷ This is only the second occurrence of a pyranoflavonol glycoside. Three new methylenedioxyflavonol glycosides have been reported bringing the total number of known structures to four. They include two glycosides, viviparum A and B from *Polygonum viviparum*⁴⁶⁵ and 3-methoxy-5-hydroxy-6,7-methylenedioxyflavone 4'-glucuronide from spinach, *Spinacia oleracea* (Chenopodiaceae).⁴⁶⁴

13.4 DISTRIBUTION PATTERNS

Flavone and flavonol *O*-glycosides are widely distributed in the angiosperms and gymnosperms, mosses, liverworts, and ferns. However, in some monocot families they are largely replaced by or co-occur with flavone *C*-glycosides, for example, in the grasses, palms, Cyperaceae, and Iridaceae. In the dicots the more evolutionary advanced families tend to accumulate flavone *O*-glycosides and complex methylated or extra hydroxylated flavonoid aglycones, while the more primitive families produce flavonol *O*-glycosides, especially myricetin derivatives, together with proanthocyanins. Gymnosperms are characterized by the presence of flavonol *O*-glycosides, flavone *C*-glycosides, and biflavonoids. Flavonoid *O*- and *C*-glycosides also occur in mosses, liverworts, and ferns. Biflavonoids are important constituents of the bryophytes but are rare in ferns, where flavonol and flavone *O*-glycosides, glycoflavones, and dihydroflavonols are the most frequent components. There have been no major reviews of the distribution of flavonoids in lower plants, gymnosperms, or the dicotyledons since those by Markham, Niemann, and Giannasi in the third edition of *The Flavonoids*,⁴ Chapters 12 to 14, respectively. However, the chapter by Williams and Harborne on the distribution of flavonoids in the monocotyledons was updated in 1994.⁴⁸² Undoubtedly, the current emphasis on molecular taxonomy has led to a reduction in research in chemotaxonomy with most flavonoid projects now based on a search for biologically active or medicinally useful secondary constituents. Space does not allow a complete review of flavonoid distribution here but some of the more interesting findings and the more extensive surveys will be mentioned.

Among the bryophytes, ten new flavone glycosides, all acylated with 3-hydroxy-3-methylglutaric acid, have been reported from three *Frullania* species.^{62,131} In another liverwort, *Dumortiera hirsuta*,⁹² luteolin 5-glucuronide-6''-methyl ether has been identified and apigenin and luteolin 7-sophorotrioside and luteolin 7-(acetylsophorotrioside) have been characterized from gametophytes of the moss, *Leptostomum macrocarpon*.³⁶ There are reports of new flavone *O*-glycosides from two ferns, luteolin 7-robinobioside⁹⁷ and 7-sophoroside⁹⁸ from *Pteris cretica* and 5,7-dihydroxy-6,8-di-*C*-methylflavone 7-[6''-(3-hydroxy-3-methylglutaryl)-glucose] from *Matteuccia orientalis*.¹⁶⁰ The other novel fern glycosides are all flavonol and include the unusual quercetin 3-methyl ether 5-glucoside from *Asplenium trichomanes-ramosum*,³⁶³ four acylated kaempferol glycosides, stenopalustrosides B–E from *Stenochlaena palustris*,²⁴⁰ which were discussed in Section 13.3.6 and quercetin 3-methyl ether 7- α -L-arabinofuranosyl(1 → 6)glucoside from *Lepisorus ussuriensis*.³⁶⁴ Six further flavonol glycosides have been recorded for brachen, *Pteridium aquilinum*^{184,232,233,314} (Table 13.2).

Reports of flavonoid O-glycosides from the gymnosperms include the unusual 5,6,7,8,3',4'-hexahydroxyflavone 7-glucoside from fruits of *Juniperus zeravschanica* (Cupressaceae)¹⁴⁵ and kaempferol 3,4'-diglucoside from needles of the common spruce, *Picea abies*.¹⁸³ Five further glycosides have been identified in leaves of the Maidenhair tree, *Ginkgo biloba*,^{176,177,265} including the unusual acylated glycosides, kaempferol and quercetin 3-[2''6'''-{-p-(7'''-glucosyl)coumaroyl}glucosyl]rhamnosides,¹⁷⁷ in which the p-coumaric acid moiety is linked in a linear fashion to the 6'''- and 4'''-hydroxyls of the two glucose molecules.

There have been three major flavonoid surveys of monocot families since 1991. Thus, 115 Restionaceae species⁴⁸³ endemic to Australia were analyzed for their culm flavonoids. The data are mainly of flavonoid aglycones but a variety of new glycosides were also characterized, including six new flavone sulfate conjugates (discussed in Section 13.3.5). The aglycones were determined after acid hydrolysis and hypolaetin (found in 23 of 34 genera), luteolin (in 25 genera), flavone C-glycosides (in 13 genera), and sulfates (in 15 genera) were found to be the most typical flavonoid constituents. Gossypetin (in seven genera), tricetin (in seven genera), and myricetin (in two genera) were relatively rare in these plants. A survey of three families related to Restionaceae, the Anarthriaceae, Ecdiaceae, and Lygineaceae,⁴⁸⁴ which are endemic to South Western Australia, showed the regular presence of myricetin, quercetin, and isorhamnetin with only traces of kaempferol. These flavonols were present mainly as 3-O-glycosides but some unknown conjugates were found to be characteristic of the genus *Anarthria*. In a further flavonoid survey of Velloziaceae taxa two new unusual flavone glycosides, 5,6,7,3',4'-pentahydroxy-8-methoxyflavone 7-glucoside and 5,6,3',4'-tetrahydroxy-7,8-dimethoxyflavone 6-glucoside, have been identified in *Vellozia nanza*.¹⁴⁶ However, most genera of the Velloziaceae are characterized by the presence of lipophilic, prenyl-, pyran-, or C-methylated flavonols, with some simple flavones and flavanones. Flavonol O-glycosides are common in *Aylthonia*, *Barbacenia*, and *Xerophyta* but most *Vellozia* species accumulate flavone C-glycosides. The lipophilic and vacuolar flavonoid data for *Vellozia* species are summarized by Harborne et al.⁴⁸⁵ and the data for all the genera of the Velloziaceae by Williams et al.⁴⁸⁶ A flavonoid survey of *Iris* species⁴⁸⁷ showed the characteristic constituents were glycoflavones but here they co-occur with isoflavones and the xanthone mangiferin and its derivatives.

Among the dicotyledons three of the larger families will be considered, Compositae, Labiatae, and Leguminosae. The Compositae is very rich in flavonoids and there have been a number of recent surveys of tribes or genera but nearly all are confined to or concentrate on the lipophilic surface constituents. There is one useful new book by Bohm and Stuessy entitled *Flavonoids of the Sunflower Family (Asteraceae)*, published in 2001,⁴⁸⁸ which after a general introduction to the family and to flavonoids, summarizes all the then known flavonoid data for each tribe and considers the efficacy of flavonoids at different taxonomic levels. However, it is not always reader friendly with many back references to previous sections or chapters so that access to the reference information is not as easy as it might be. The genus *Tanacetum*⁴⁸⁹ and other members of the tribe Anthemideae¹⁷¹ have been surveyed for both surface lipophilic and vacuolar flavonoids. In *Tanacetum* species, apigenin and luteolin 7-glucuronides are the characteristic vacuolar flavonoids. However, 6-hydroxyluteolin 7-glucoside was found in *T. corymbosum*, chrysoeriol 7-glucuronide in *T. parthenium*, *T. macrophyllum*, and *T. cinerariifolium*, and quercetin 7-glucuronide in *T. parthenium*, *T. corymbosum*, and *T. cinerariifolium*.⁴⁸⁹ The lipophilic flavonoids are based mainly on 6-hydroxykaempferol 3,6,4'-trimethyl ether and quercetagenin 3,6,3'-trimethyl ether with methyl ethers of scutellarein and 6-hydroxyluteolin in some species. Both lipophilic and polar flavonoids were isolated from leaf, ray, and disc florets of other Anthemideae, *Anthemis*, *Chrysanthemum*, *Cotula*, *Ismelia*, *Leucanthemum*, and *Tripleuropernum*.¹⁷¹ *Anthemis* species characteristically produced flavonol glycosides in the leaves while in the other taxa

flavone *O*-glycosides were more usual. Two flavonol glycosides, quercetin and kaempferol 5-glucuronides, were identified in leaves of *Leucanthemum vulgare*.¹⁷¹ In most of these plants ray and disc florets had noticeably different flavonoid patterns, the former based on apigenin or luteolin 7-glucoside or glucuronide and the latter having additional flavonol glycosides such as quercetin and patuletin 7-glucosides and quercetin 7-glucuronide. A similar flavonoid survey of *Pulicaria* species (tribe Inulae)⁴⁹⁰ has also been published.

There have been a considerable number of chemotaxonomic studies of Labiate taxa since 1991. These include a survey of flavonoid aglycones and glycosides in *Sideritis* species⁴⁹¹ from the Canary Islands and Madeira and the discovery of a new flavone glycoside, isoscutellarein 7-glucosyl(1 → 2)xyloside, from *Sideritis luteola* and 15 other Spanish *Sideritis* species.⁷⁸ A review of the polyphenolics of the genus *Salvia*⁴⁹² includes flavonoid *O*-glycoside data and Tomás-Barberán et al.⁴⁹³ have determined the distribution of flavonoid *p*-coumaroylglucoside and 8-hydroxyflavone allosylglucosides in the Labiatae. Five other studies concern leaf flavonoid glycosides as taxonomic characters in the genera *Ocimum*,⁴⁹⁴ *Calamintha* and *Micromeria*,⁴⁹⁵ *Teucrium* and *Tripura*,⁴⁹⁶ *Oxera* and *Faradaya*,⁴⁹⁷ and *Lavandula* and *Sabaudia*.⁴⁹⁸

The Leguminosae is phytochemically one of the most diverse families and contains a wealth of flavonoid constituents. A review of the seed polysaccharides and flavonoids, which includes flavonoid *O*-glycosides, provides a useful chemical overview of the family.⁴⁹⁹ However, the definitive publications on the chemistry of the family are undoubtedly the three latest Leguminosae volumes (11a–11c) of Robert Hegnauer's *Chemotaxonomie der Pflanzen*.⁵⁰⁰ A more recent review of the phytochemistry of the large and taxonomically difficult genus *Acacia* has been published by Seigler.⁵⁰¹

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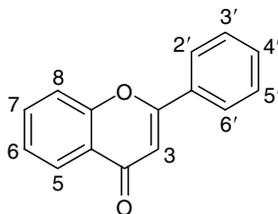
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APPENDIX A

CHECKLIST OF KNOWN FLAVONE GLYCOSIDES



13.9

5,7-Dihydroxyflavone (chrysin)

1. 5-Xyloside
2. 5-Glucoside (toringin)
3. 7-Glucoside
4. 7-Galactoside
5. 7-Glucuronide
6. 7-Rutinoside
7. 7-Gentiobioside
8. 7-Benzoate
9. 7-(4''-Acetylglucoside)*
10. 7-(6''-Acetylglucoside)*

6-Hydroxy-4''-methoxyflavone

11. 6-Arabinoside

7,2'-Dihydroxyflavone

12. 7-Glucoside

7,4'-Dihydroxyflavone

13. 7-Glucoside
14. 4'-Glucoside
15. 7-Rutinoside

3',4'-Dihydroxyflavone

16. 4'-Glucoside

2',5'-Dihydroxyflavone

17. 5'-Acetate

5,6,7-Trihydroxyflavone (baicalein)

18. 6-Glucoside
19. 6-Glucuronide
20. 7-Rhamnoside
21. 7-Glucuronide
22. 7-(6''-Malonylglucoside)*

- Baicalein 5-methyl ether
 - 23. 7-Glucoside*
- Baicalein 6-methyl ether (oroxylin A)
 - 24. 5-Rhamnoside*
 - 25. 7-Glucoside
 - 26. 7-Glucuronide
 - 27. 7-Glucosyl(1 → 3)rhamnoside*
- Baicalein 7-methyl ether (negletein)
 - 28. 5-Glucuronide
 - 29. 5-Glucuronosylglucoside
 - 30. 6-Xyloside*
 - 31. 6-Glucoside*
 - 32. 6-Rhamnosyl(1 → 2)fucoside*
- Baicalein 5,6-dimethyl ether
 - 33. 7-Glucoside
- 5,7,8-Trihydroxyflavone (norwogonin)
 - 34. 5-Glucoside*
 - 35. 7-Galactoside*
 - 36. 7-Glucuronide
 - 37. 8-Glucuronide
- 5,7-Dihydroxy-8-methoxyflavone (wogonin)
 - 38. 5-Glucoside
 - 39. 7-Glucoside
 - 40. 7-Glucuronide
- 7-Hydroxy-5,8-dimethoxyflavone
 - 41. 7-Glucoside*
 - 42. 7-Glucuronide*
- 5-Hydroxy-7,8-dimethoxyflavone
 - 43. 5-Glucoside
- 7,3',4'-Trihydroxyflavone
 - 44. 7-Glucoside
 - 45. 7-Galactoside
 - 46. 7-Rutinoside
- 7,4'-Dihydroxy-3'-methoxyflavone
 - 47. 7-Glucoside
- 5,7,2'-Trihydroxyflavone
 - 48. 7-Glucoside*
 - 49. 7-Glucuronide
 - 50. 2'-Glucoside*
- 5,2'-Dihydroxy-7-methoxyflavone (echioidin)
 - 51. 5-Glucoside*
 - 52. 2'-Glucoside (echioidin)
 - 53. 2'-(6''-Acetylglucoside)*
- 7,8,4'-Trihydroxyflavone
 - 54. 8-Neohesperidoside
- 5,7,4'-Trihydroxyflavone (apigenin)
 - 55. 5-Glucoside
 - 56. 5-Galactoside
 - 57. 7-Arabinoside
 - 58. 7-Xyloside

59. 7-Rhamnoside
60. 7-Glucoside (cosmosiin)
61. 7-Galactoside
62. 7-Glucuronide
63. 7-Galacturonide
64. 7-Methylglucuronide
65. 7-Methylgalacturonide
66. 7-(6''-Ethylglucuronide)
67. 4'-Arabinoside
68. 4'-Glucoside
69. 4'-Glucuronide
70. 7-Arabinofuranosyl(1 → 6)glucoside
71. 7-Arabinopyranosyl(1 → 6)glucoside
72. 7-Xylosyl(1 → 2)glucoside
73. 7-Xylosyl(1 → 6)glucoside
74. 7-Apiofuranosyl(1 → 6)glucoside (apiin)*
75. 7-Rutinoside
76. 7-Neohesperidoside
77. 7-Rhamnosylglucuronide
78. 7-Dirhamnoside
79. 7-Glucosylrhamnoside
80. 7-Cellobioside*
81. 7-Allosyl(1 → 2)glucoside
82. 7-Galactosyl(1 → 4)mannoside
83. 7-Xylosylglucuronide
84. 7-Rhamnosylglucuronide
85. 7-Rhamnosyl(1 → 2)galacturonide
86. 7-Digalacturonide
87. 7-Galacturonylglucoside
88. 7-Glucuronosyl(1 → 2)glucuronide
89. 7,4'-Diglucoside
90. 7,4'-Dialloside
91. 7,4'-Diglucuronide
92. 7-Glucuronide-4'-rhamnoside
93. 4'-Diglucoside
94. 7-Sophorotrioside*
95. 7-(2^G-Rhamnosyl)rutinoside*
96. 7-(2^G-Rhamnosyl)gentiobioside*
97. 7-Rhamnoside-4'-glucosylrhamnoside*
98. 7-Rutinoside-4'-glucoside
99. 7-Neohesperidoside-4'-glucoside
100. 7-Cellobioside-4'-glucoside*
101. 7-Rhamnoside-4'-rutinoside
102. 7-Neohesperidoside-4'-sophoroside
103. 7-Glucosyl(1 → 2)glucuronide-4'-glucuronide*
104. 7-Digalacturonide-4'-glucoside
105. 7-Diglucuronide-4'-glucuronide
106. Pinnatifinoside A (13.1)
107. Pinnatifinoside B (13.2)
108. Pinnatifinoside C (13.3)

109. Pinnatifinoside D (13.4)
110. 7-(2''-*E-p*-Coumaroylglucoside)*
111. 7-(3''-*p*-Coumaroylglucoside)*
112. 7-(4''-*Z-p*-Coumaroylglucoside)
113. 7-(4''-*E-p*-Coumaroylglucoside)
114. 7-(6''-*p*-Coumaroylglucoside)
115. 7-(6''-*E-p*-Coumaroylgalactoside)*
116. 7-(6''-*E*-Caffeoylglucoside)*
117. 5-(6''-Malonylglucoside)
118. 7-(6''-Malonylglucoside)
119. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*
120. 7-(2''-Acetylglucoside)
121. 7-(6''-Acetylglucoside)
122. 7-(6''-Crotonylglucoside)
123. 7-(2''-Acetyl-6''-methylglucuronide)
124. 7-Lactate
125. 7-(2''-Glucosyllactate)
126. 7-(2''-Glucuronosyllactate)
127. 7-Glucoside-4'-*p*-coumarate
128. 7-Glucoside-4'-caffeate
129. 7-(2'',6''-Di-*p*-coumaroylglucoside)
130. 7-(3'',6''-Di-*p*-coumaroylglucoside)
131. 7-(3'',6''-Di-*E-p*-coumaroylgalactoside)*
132. 7-(4'',6''-Di-*p*-coumaroylglucoside)
133. 7-(2'',3''-Diacetylglucoside)
134. 7-(3'',4''-Diacetylglucoside)
135. 7-(3''-Acetyl-6''-*E-p*-coumaroylglucoside)*
136. 5-Rhamnosyl(1 → 2)(6''-acetylglucoside)
137. 7-Rhamnosyl(1 → 6)(4''-*E-p*-methoxycinnamoylglucoside)*
138. 4'-(2''-Feruloylglucuronosyl(1 → 2)glucuronide)*
139. 7-(6'''-Acetylallosyl)(1 → 2)glucoside
140. 7-(6'''-Malonylneohesperidoside)
141. 7-(Malonylapiosyl)glucoside
142. 7-Rutinoside-4'-caffeate
143. 7-(6''-Acetylalloside)-4'-alloside
144. 7-(4'',6''-Diacetylalloside)-4'-alloside
145. 7-Glucuronide-4'-(6''-malonylglucoside)
146. 7-Glucuronosyl(1 → 3)[(2''-*p*-coumaroylglucuronosyl)(1 → 2)glucuronide]*
147. 7-Glucuronosyl(1 → 3)[(2''-feruloylglucuronosyl)(1 → 2)glucuronide]*
148. 7-(2''-Ferulylglucuronosyl)(1 → 2)glucuronide-4'-glucuronide*
149. 7-Glucuronide-4'-(2'''-*E-p*-coumaroylglucuronosyl)(1 → 2)glucuronide*
150. 7-Glucuronide-4'-(2'''-feruloylglucuronosyl)(1 → 2)glucuronide*
151. 7-Sulfatoglucoside
152. 7-Sulfatogalactoside
153. 7-Sulfatoglucuronide
154. 7-Sulfate
- Apigenin 7-methyl ether (genkwanin)
155. 5-Glucoside
156. 4'-Glucoside
157. 5-Xylosylglucoside

158. 4'- α -L-Arabinopyranosyl(1 \rightarrow 6)galactoside*
159. 4'-Glucosylrhamnoside
160. 4'-Rhamnosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)galactoside]*
161. 5-(6''-Malonylglucoside)
- Apigenin 4'-methyl ether (acacetin)
 162. 7-Rhamnoside*
 163. 7-Glucoside (tilianine)
 164. 7-Galactoside
 165. 7-Glucuronide
 166. 7-(6''-Methylglucuronide)
 167. 7-Arabinosylrhamnoside
 168. 7-Glucosyl(1 \rightarrow 4)xyloside*
 169. 7-Apiosyl(1 \rightarrow 6)glucoside*
 170. 7-Rutinoside (linarin)
 171. 7-Neohesperidoside (fortunellin)
 172. 7-Diglucoside
 173. 7-Rhamnosylgalacturonide
 174. 7-Glucuronosyl(1 \rightarrow 2)glucuronide
 175. 7-(2^G-Rhamnosylrutinoside)*
 176. 7-Rhamnosyl(1 \rightarrow 2)glucosyl(1 \rightarrow 2)glucoside*
 177. 7-Rhamnosyl(1 \rightarrow 2)glucosyl(1 \rightarrow 2)glucosyl(1 \rightarrow 2)glucoside*
 178. 7-(2''-Acetylglucoside)
 179. 7-(6''-Acetylglucoside)
 180. 7-(4''-Acetylrutinoside)
 181. 7-(4'''-Acetylrutinoside)*
 182. 7-[2'''-(2-Methylbutyryl)rutinoside]
 183. 7-[3'''-(2-Methylbutyryl)rutinoside]
 184. 7-Rhamnosyl(1 \rightarrow 6)[2''-acetylglucosyl(1 \rightarrow 2)glucoside]*
 185. 7-[6'''-Acetylglucosyl(1 \rightarrow 2)][rhamnosyl(1 \rightarrow 6)glucoside]*
 186. 7-Glucosyl(1 \rightarrow 6)[3'''-acetylrhamnosyl(1 \rightarrow 2)glucoside]*
 187. 7-(4'''-Acetylrhamnosyl)(1 \rightarrow 6)glucosyl(1 \rightarrow 3)(6''-acetylglucoside)*
 188. 7-[Rhamnosyl(1 \rightarrow 4)glucosyl(1 \rightarrow 6)](6'''-acetylsophoroside)
 189. Di-6''-(acacetin-7-glucosyl)malonate
- Apigenin 5,7-dimethyl ether
 190. 4'-Galactoside
- Apigenin 7,4'-dimethyl ether
 191. 5-Xylosylglucoside
- 6-Hydroxyapigenin (scutellarein)
 192. 5-Glucuronide
 193. 6-Xyloside
 194. 6-Glucoside
 195. 7-Rhamnoside
 196. 7-Glucoside
 197. 7-Glucuronide
 198. 4'-Arabinoside
 199. 7-Xylosyl(1 \rightarrow 2)xyloside*
 200. 7-Xylosyl(1 \rightarrow 4)rhamnoside
 201. 7-Xylosyl(1 \rightarrow 2)glucoside*
 202. 7-Xylosyl(1 \rightarrow 6)galactoside*
 203. 7-Glucosyl(1 \rightarrow 4)rhamnoside

204. 7-Rutinoside
205. 7-Neohesperidoside
206. 7-Rhamnosyl(1 → 2)galactoside
207. 7-Diglucoside
208. 7-Glucuronosyl(1 → 2)glucuronide*
209. 6-Xyloside-7-rhamnoside
210. 7,4'-Dirhamnoside
211. 7-(6''-Malonylglucoside)
212. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*
213. 7-(6''-Feruloylglucuronide)
214. 7-(Sinapoylglucuronide)
- Scutellarein 6-methyl ether (Hispidulin)
 215. 7-Rhamnoside*
 216. 7-Glucoside (homoplantaginin)
 217. 7-Glucuronide
 218. 7-Methylglucuronide*
 219. 4'-Glucoside*
 220. 7-Xylosyl(1 → 2)glucoside*
 221. 7-Rutinoside
 222. 7-Neohesperidoside*
 223. 7-(6''-*E-p*-Coumaroylglucoside)*
 224. 7-Sulfate
 225. 4'-Sulfate
 226. 7,4'-Disulfate
- Scutellarein 7-methyl ether
 227. 6-Glucoside
 228. 6-Galactoside
 229. 7-Glucoside
 230. 6-Rhamnosylxyloside
- Scutellarein 4'-methyl ether
 231. 6-Glucoside
 232. 7-Glucoside
 233. 7-Glucuronide
 234. 7-Rutinoside*
 235. 7-Sophoroside
 236. 7-(2'',6''-Diacetylalloside)*
 237. 7-(*p*-Coumaroylglucosyl)(1 → 2)mannoside
- Scutellarein 5,4'-dimethyl ether
 238. 7-Glucoside*
 239. 7-(4^{Rha}-Acetylrutinoside)*
- Scutellarein 6,7-dimethyl ether
 240. 4'-Glucoside
 241. 4'-Glucuronide*
 242. 4'-Rutinoside
- Scutellarein 6,4'-dimethyl ether (pectolarigenin)
 243. 7-Rhamnoside
 244. 7-Glucoside
 245. 7-Glucuronide
 246. 7-Glucuronic acid methyl ether
 247. 7-Rutinoside

- 248. 7-(6''-Acetylglucoside)*
- 249. 7-(2'''-Acetylrutinoside)*
- 250. 7-(3'''-Acetylrutinoside)*
- 251. 7-(4'''-Acetylrutinoside)*
- Scutellarein 7,4'-dimethyl ether
- 252. 6-Glucoside
- 253. 6-Xylosyl(1 → 2)glucoside*
- 254. 6-Neohesperidoside*
- Scutellarein 6,7,4'-trimethyl ether (salvigenin)
- 255. 5-Glucoside
- 256. 5-(6''-Acetylglucosyl)(1 → 3)galactoside*
- 8-Hydroxyapigenin (isoscuteallarein)
- 257. 7-Xyloside
- 258. 7-Glucoside
- 259. 7-Glucosyl(1 → 2)xyloside*
- 260. 8-Glucuronide
- 261. 7-Neohesperidoside
- 262. 7-Allosyl(1 → 2)glucoside
- 263. 8-Sophoroside*
- 264. 8-(6''-*E-p*-Coumaroylglucoside)*
- 265. 7-(6''-Acetylallosyl)(1 → 2)glucoside
- 266. 7-[6'''-Acetylallosyl(1 → 2)6''-acetylglucoside]
- 267. 8-(2''-Sulfatoglucuronide)*
- 268. 8-(2'',4''-Disulfatoglucuronide)*
- 8-Hydroxyapigenin 4'-methyl ether
- 269. 8-Glucoside*
- 270. 8-Glucuronide
- 271. 8-(6''-*n*-butylglucuronide)*
- 272. 7-Allosyl(1 → 2)glucoside*
- 273. 8-Xylosylglucoside
- 274. 7-(6'''-Acetylallosyl)(1 → 2)glucoside
- 275. 8-(2''-Sulfatoglucoside)
- 276. 8-(2''Sulfatoglucuronide)*
- 277. 8-(2'',4''-Disulfatoglucuronide)*
- 8-Hydroxyapigenin 8,4'-dimethyl ether
- 278. 7-Glucuronide
- 6,8-Dihydroxy-7,4'-dimethoxyflavone
- 279. 6-Rutinoside*
- 280. 6-(4''-Acetylramnosyl)(1 → 6)glucoside*
- 7,3',4',5'-Tetrahydroxyflavone
- 281. 7-Rhamnoside
- 282. 7-Glucoside
- 5,6,7,2'-Tetrahydroxyflavone
- 283. 7-Glucuronide
- 5,7,2'-Trihydroxy-6-methoxyflavone
- 284. 7-Glucoside*
- 285. 7-Methylglucuronide*
- 5,7,2',6'-Tetrahydroxyflavone
- 286. 2'-Glucoside*
- 5,2',6'-Trihydroxy-7-methoxyflavone

287. 2'-Glucoside*
- 5,7,8,2'-Tetrahydroxyflavone
288. 7-Glucuronide*
- 5,7,2'-Trihydroxy-8-methoxyflavone
289. 7-Glucuronide
- 5,2'-Dihydroxy-7,8-dimethoxyflavone (skullcapflavone 1)
290. 2'-Glucoside*
291. 2'-(2''-*E*-Cinnamoylglucoside)*
292. 2'-(3''-*E*-Cinnamoylglucoside)*
293. 2'-(4''-*E*-Cinnamoylglucoside)*
- 5,7-Dihydroxy-8,2'-dimethoxyflavone
294. 7-Glucuronide
- 5-Hydroxy-7,8,2'-trimethoxyflavone
295. 5-Glucoside
- 5,7,3',4'-Tetrahydroxyflavone (luteolin)
296. 5-Glucoside (galuteolin)
297. 5-Galactoside
298. 5-Glucuronide
299. 5-Glucuronide-6''-methyl ester*
300. 7-Xyloside
301. 7-Rhamnoside
302. 7-Glucoside
303. 7-Galactoside
304. 7-Glucuronide
305. 7-Galacturonide
306. 7-Methylglucuronide
307. 3'-Xyloside
308. 3'-Rhamnoside
309. 3'-Glucoside
310. 3'-Glucuronide
311. 3'-Galacturonide
312. 4'-Arabinoside
313. 4'-Glucoside
314. 4'-Glucuronide
315. 5-Rutinoside*
316. 7-Dirhamnoside
317. 7-Arabinofuranosyl(1 → 6)glucoside
318. 7-Arabinopyranosyl(1 → 6)glucoside
319. 7-Glucosyl(1 → 4) α -L-arabinopyranoside*
320. 7-Xylosyl(1 → 6)glucoside (primeveroside)*
321. 7-Apiosyl(1 → 6)glucoside*
322. 7-Sambubioside
323. 7-Apiosylglucoside
324. 7-Rutinoside
325. 7-Neohesperidoside (veronicastrósíde)
326. 7-Glucosylrhamnoside
327. 7-Robinoside*
328. 7-Sophoroside*
329. 7-Gentiobioside
330. 7-Laminaribioside

331. 7-Glucosylgalactoside
332. 7-Galactosyl(1 → 6)galactoside*
333. 7-Allosyl(1 → 2)glucoside
334. 7-Glucosylglucuronide
335. 7-Galactosylglucuronide*
336. 7-Glucuronosyl(1 → 2)glucuronide
337. 7-Glucoside-3'-xyloside
338. 7,3'-Diglucoside
339. 7-Glucoside-3'-glucuronide*
340. 7-Glucuronide-3'-glucoside
341. 7,3'-Diglucuronide
342. 7,3'-Digalacturonide
343. 7,4'-Diglucoside
344. 7-Galactoside-4'-glucoside
345. 7-Glucuronide-4'-rhamnoside
346. 7-Galacturonide-4'-glucoside
347. 7,4'-Diglucuronide
348. 3'-Xylosyl(1 → 2)glucoside*
349. 4'-Rutinoside*
350. 4'-Neohesperidoside
351. 3',4'-Diglucoside
352. 3',4'-Diglucuronide
353. 3',4'-Digalacturonide
354. 7-Rhamnosyldiglucoside
355. 7-Sophorotrioside*
356. 7-Glucosylarabinoside-4'-glucoside
357. 7-Rutinoside-3'-glucoside
358. 7-Rutinoside-4'-glucoside
359. 7-Neohesperidoside-4'-glucoside
360. 7-Glucoside-4'-neohesperidoside
361. 7-Gentiobioside-4'-glucoside
362. 7-Glucuronide-3',4'-dirhamnoside
363. 7,4'-Diglucuronide-3'-glucoside
364. 7-Glucuronosyl(1 → 2)glucuronide-4'-glucuronide
365. 7,3',4'-Triglucuronide
366. 7-Neohesperidoside-4'-sophoroside
367. 7-(6''-*E*-Cinnamoylglucoside)
368. 7-(2''-*p*-Coumaroylglucoside)
369. 7-(6''-*p*-Coumaroylglucoside)
370. 7-Caffeoylglucoside
371. 7-(6''-Feruloylglucoside)
372. 7-(6''-*p*-Benzoylglucoside)*
373. 5-(6''-Malonylglucoside)
374. 7-(6''-Malonylglucoside)
375. 7-[6''-(2-Methylbutyryl)glucoside]*
376. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*
377. 3'-Acetylglucuronide
378. 7-(6''-Acetylglucoside)
379. 3'-(3''-Acetylglucuronide)*
380. 3'-(4''-Acetylglucuronide)*

381. 7-Glucosyl(1 → 6)(4'''-caffeoylglucoside)*
382. 7-Glucoside-4'-(*Z*-2-methyl-2-butenoate)*
383. 7-Glucuronide-3'-feruloylglucoside
384. 7-Neohesperidoside-6''-malonate
385. 7-(3''-Acetylapiosyl)(1 → 2)xyloside
386. 7-(6'''-Acetylallosyl)(1 → 2)glucoside
387. 7-(6'''-Acetylsophoroside)*
388. 7-Apiosyl(1 → 2)[glucosyl(1 → 4)(6-malonylglucoside)]*
389. 7-(Acetylsophorotrioside)*
390. 7-(6''''-Acetylallosyl)(1 → 3)glucosyl(1 → 2)glucoside*
391. 7-(2''-Feruloylglucuronosyl)(1 → 2)glucuronide-4'-glucuronide*
392. 7,4'-Diglucuronide-3'-feruloylglucoside
393. 7-Lactate
394. 7-(2''-Glucosyllactate)
395. 7-(2''-Glucuronosyllactate)
396. 7-Sulfatoglucoside
397. 7-(2''-Sulfatoglucoside)*
398. 7-Sulfatoglucuronide
399. 7-Sulfate-3'-glucoside
400. 7-Sulfatorutinoside
401. 7-Sulfate-3'-rutinoside
402. 7-Sulfate
403. 3'-Sulfate
404. 4'-Sulfate
405. 7-Disulfatoglucoside
406. 7,3'-Disulfate
- Luteolin 5-methyl ether
407. 7-Glucoside
408. 7-Xylosyl(1 → 6)glucoside*
- Luteolin 7-methyl ether
409. 5-Glucoside
410. 3'-Glucoside*
411. 3'-Galactoside*
412. 4'-Rhamnoside
413. 5-Xylosylglucoside
414. 4'-Gentiobioside
- Luteolin 3'-methyl ether (chrysoeriol)
415. 5-Glucoside
416. 7-Xyloside
417. 7-Rhamnoside
418. 7-Glucoside
419. 7-Glucuronide
420. 4'-Glucoside
421. 5-Diglucoside
422. 7-Arabinofuranosyl(1 → 2)glucoside
423. 7- α -L-Arabinofuranosyl(1 → 6)galactoside*
424. 7-Apiosyl(1 → 6)glucoside*
425. 7-Rutinoside
426. 7-Neohesperidoside*
427. 7-Rhamnosylgalactoside

428. 7-Rhamnosylglucuronide
429. 7-Digalactoside
430. 7-Mannosyl(1 → 2)alloside
431. 7-Allosyl(1 → 2)glucoside
432. 7-Glucuronosyl(1 → 2)glucuronide
433. 5,4'-Diglucoside
434. 7,4'-Dixyloside
435. 7,4'-Diglucoside
436. 7-Glucuronide-4'-rhamnoside
437. 7,4'-Diglucuronide*
438. 7-Sophorotrioside
439. 7-(6''-Crotonylglucoside)
440. 7-(Malonylglucoside)
441. 7-(3''-Z-p-Coumaroylglucoside)*
442. 7-(3'',6''-Di-E-p-coumaroylglucoside)*
443. 7-p-Coumaroylglucosylglucuronide
444. 7-(2'''-Feruloylglucuronosyl(1 → 2)glucuronide)*
445. 7-(6'''-Acetylglucosyl)(1 → 2)mannoside
446. 7-[Glucuronosyl(1 → 3)(2'''-feruloylglucuronosyl)](1 → 2)glucuronide*
447. 7-Sulfatoglucoside
448. 7-Disulfatoglucoside
449. 7-Sulfate
- Luteolin 4'-methyl ether (diosmetin)
450. 7-Glucoside
451. 7-Glucuronide
452. 3'-Glucoside*
453. 7-Arabinosyl(1 → 6)glucoside*
454. 7- α -Glucosyl(1 → 6)glucoside*
455. 7- β -Glucosyl(1 → 6)arabinoside
456. 7-Xylosyl(1 → 6)glucoside*
457. 7-Rutinoside (diosmin)
458. 7-Neohesperidoside (neodiosmin)*
459. 7-Diglucoside
460. 7-(2'',6''-Dirhamnosyl)glucoside*
461. 7-(6''-Malonylglucoside)
462. 7-Apiosyl(1 → 2)(6''-Acetylglucoside)*
463. 7-Sulfate
464. 3'-Sulfate
465. 7,3'-Disulfate
- Luteolin 5,3'-dimethyl ether
466. 7-Glucoside*
467. 4'-Glucoside*
- Luteolin 5,4'-dimethyl ether
468. 7-Xylosyl(1 → 6)glucoside*
- Luteolin 7,3'-dimethyl ether
469. 5-Rhamnoside
470. 5-Glucoside
471. 4'-Glucoside
472. 4'-Apiosyl(1 → 2)glucoside*

Luteolin 7,4'-dimethyl ether

473. 3'-Glucoside

474. 5-Xylosylglucoside

Luteolin 3',4'-dimethyl ether

475. 7-Rhamnoside

476. 7-Glucuronide

Luteolin 5,3',4'-trimethyl ether

477. 7-Xylosyl(1 → 6)glucoside*

478. 7-Rutinoside*

Luteolin 7,3',4'-trimethyl ether

479. 5-Glucoside*

480. 5-Xylosyl(1 → 6)glucoside*

6-Hydroxyluteolin

481. 5-Glucoside

482. 6-Xyloside

483. 6-Rhamnoside*

484. 6-Glucoside

485. 6-Glucuronide

486. 7-Arabinoside

487. 7-Xyloside

488. 7-Rhamnoside

489. 7-Apioside

490. 7-Glucoside

491. 7-Galactoside

492. 7-Glucuronide

493. 7-Xylosyl(1 → 2)xyloside*

494. 7-Xylosyl(1 → 6)glucoside*

495. 7-Rhamnosyl(1 → 4)xyloside

496. 7-Sambubioside*

497. 6-Glucoside-3'-rhamnoside

498. 7-Rutinoside

499. 7-Sophoroside

500. 7-Gentiobioside

501. 7-Arabinoside-4'-rhamnoside

502. 7-(6''-Malonylglucoside)

503. 7-[3''-(3-Hydroxy-3-methylglutaryl)glucoside]*

504. 7-[4''-(3-Hydroxy-3-methylglutaryl)glucoside]*

505. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*

506. 7-(6''-*E*-Caffeoylglucoside)*507. 7-(6'''-*p*-Coumaroylsophoroside)

508. 7-(6'''-Caffeoylsophoroside)

509. 6-Glucoside-7-[6'''-(3-hydroxy-3-methylglutaryl)glucoside]*

510. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]-3'-glucuronide*

511. 6-Sulfate

512. 7-Sulfate

513. 6,7-Disulfate

6-Methoxyluteolin

514. 7-Glucoside

515. 7-Glucuronide*

516. 7-Methylglucuronide*

517. 4'-Glucoside*

- 518. 7-Rutinoside
- 519. 7-Rhamnosyl-3'-xyloside*
- 520. 7-[6''-(2-Methylbutyryl)glucoside]*
- 521. 7-Sulfate
- 522. 3',4'-Disulfate
- 6-Hydroxyluteolin 7-methyl ether (pedalitin)
 - 523. 6-Glucoside (pedaliin)
 - 524. 6-Galactoside
 - 525. 7-Glucuronide
 - 526. 7-Methylglucuronide
 - 527. 6-Galactosylglucoside
- 6-Hydroxyluteolin 3'-methyl ether (nodifloretin)
 - 528. 7-Diglucoside
 - 529. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*
 - 530. 7-Sulfate
 - 531. 6,7-Disulfate
- 6-Hydroxyluteolin 4'-methyl ether
 - 532. 7-Allosyl(1 → 2)glucoside
 - 533. 7-Rhamnosyl(1 → 2)(6''-acetylglucoside)*
 - 534. 7-[6'''-Acetylallosyl(1 → 2)6''-acetylglucoside]
- 6-Hydroxyluteolin 6,7-dimethyl ether (cirsiliol)
 - 535. 4'-Glucoside
- 6-Hydroxyluteolin 6,3'-dimethyl ether
 - 536. 5-Rhamnoside*
 - 537. 7-Rhamnoside
 - 538. 7-Glucoside
 - 539. 7-Rutinoside*
 - 540. 7-Sulfate
 - 541. 7,4-Disulfate
- 6-Hydroxyluteolin 6,4'-dimethyl ether
 - 542. 7-Glucoside
 - 543. 7-Rutinoside
- 6-Hydroxyluteolin 7,3'-dimethyl ether
 - 544. 6-Glucoside
- 6-Hydroxyluteolin 6,7,3'-trimethyl ether (cirsilineol)
 - 545. 4'-Glucoside
- 6-Hydroxyluteolin 5,6,3',4'-tetramethyl ether
 - 546. 7-Cellobioside*
- 8-Hydroxyluteolin (hypolaetin)
 - 547. 7-Xyloside
 - 548. 7-Glucoside
 - 549. 8-Rhamnoside*
 - 550. 8-Glucoside
 - 551. 8-Glucuronide
 - 552. 7-Sophoroside*
 - 553. 7-Allosyl(1 → 2)glucoside
 - 554. 8-Gentiobioside
 - 555. 8,4'-Diglucuronide
 - 556. 8-Glucoside-3'-rutinoside*
 - 557. 7-(6'''-Acetylallosyl)(1 → 2)glucoside

558. 7-[6'''-Acetylallosyl(1 → 2)6''-acetylglucoside]
559. 7-[6'''-Acetylallosyl(1 → 2)3''-acetylglucoside]
560. 7-Sulfatoglucoside*
561. 7-Sulfatogalactoside*
562. 7-Sulfatoglucuronide*
563. 7-Sulfate-8-glucoside*
564. 8-Glucoside-3'-sulfate
565. 8-Sulfate
- Hypolaetin 7-methyl ether
566. 3'-Sulfatogalactoside*
567. 3'-Sulfatoglucuronide*
- Hypolaetin 3'-methyl ether
568. 7-Glucoside
569. 8-Glucuronide*
570. 7-Sophoroside*
571. 7-Allosyl(1 → 2)glucoside
572. 7-Mannosyl(1 → 2)glucoside
- Hypolaetin 4'-methyl ether
573. 8-Glucoside
574. 8-Glucuronide
575. 7-Allosyl(1 → 2)glucoside
576. 7-(6'''-Acetylallosyl)(1 → 2)glucoside
577. 7-[6'''-Acetylallosyl(1 → 2)6''-acetylglucoside]*
578. 8-Glucoside-3'-sulfate
- Hypolaetin 7,3'-dimethyl ether
579. 4'-Glucoside*
- Hypolaetin 8,3'-dimethyl ether
580. 7-Glucoside
- 5,6,4'-Trihydroxy-7,8-dimethoxyflavone (thymusin)
581. 6-Isobutyrate*
5,8,4'-Trihydroxy-6,7-dimethoxyflavone (isothymusin)
582. 8-Glucoside*
5,7,-Dihydroxy-6,8,4'-trimethoxyflavone (nevadensin)
583. 5-Glucoside*
584. 7-Glucoside*
585. 5-Gentiobioside*
586. 7-Rutinoside*
- 5,8-Dihydroxy-6,7,4'-trimethoxyflavone
587. 8-Glucoside*
- 5,6,7,8,3',4'-Hexahydroxyflavone
588. 7-Glucoside*
- 5,6,7,3',4'-Pentahydroxy-8-methoxyflavone (pleurostimin)
589. 7-Apioside
590. 7-Glucoside*
- 5,6,3',4'-Tetrahydroxy-7,8-dimethoxyflavone (pleurostimin 7-methyl ether)
591. 6-Glucoside*
- 5,7,3',4'-Tetrahydroxy-6,8-dimethoxyflavone
592. 7-Glucoside
- 5,8,3',4'-Tetrahydroxy-6,7-dimethoxyflavone
593. 8-Glucoside

- 5,7,3'-Trihydroxy-6,8,4'-trimethoxyflavone (acerosin)
594. 5-(6''-Acetylglucoside)
- 5,7,4'-Trihydroxy-6,8,3'-trimethoxyflavone (sudachitin)
595. 7-Glucoside
596. 4'-Glucoside
597. 7-(3-Hydroxy-3-methylglutarate)-4'-glucoside
598. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]
599. 4'-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]
600. Sudachitin D
- 5,2',3'-Trihydroxy-7,8-dimethoxyflavone
601. 3'-Glucoside
- 5,2',6'-Trihydroxy-6,7-dimethoxyflavone
602. 2'-Glucoside*
- 5,2',6'-Trihydroxy-7,8-dimethoxyflavone
603. 2'-Glucuronide*
- 5,2'-Dihydroxy-7,8,6'-trimethoxyflavone
604. 2'-Glucuronide*
- 5-Hydroxy-7,8,2',3'-tetramethoxyflavone
605. 5-Glucoside
- 5,7,2',4',5'-Pentahydroxyflavone (isoetin)
606. 7-Glucoside
607. 7-Arabinoside
608. 2'-Xyloside
609. 4'-Glucuronide*
610. 5'-Glucoside
611. 7-Xylosylarabinosylglucoside
612. 7-Glucoside-2'-xyloside
613. 2'-(4''-Acetylxyloside)
614. 7-Glucoside-2'-(4''-acetylxyloside)
- 5,7,3',4',5'-Pentahydroxyflavone (tricetin)
615. 7-Glucoside
616. 3'-Xyloside
617. 3'-Glucoside
618. 3'-Rhamnosyl(1 → 4)rhamnoside*
619. 7,3'-Diglucuronide
620. 3'-Glucoside-5'-rhamnoside*
621. 7-Glucoside-3'-[6''-(3-hydroxy-3-methylglutaryl)glucoside]*
622. 7-Diglucoside
623. 3',5'-Diglucoside
624. 3'-Sulfate
625. 3'-Disulfate
- Tricetin 7-methyl ether
626. 3'-Glucoside-5'-rhamnoside*
- Tricetin 3'-methyl ether
627. 7-Glucoside
628. 7-Glucuronide*
629. 7,5'-Diglucuronide
- Tricetin 4'-methyl ether
630. 7-Apiosyl(1 → 2)(6''-acetylglucoside)*

Tricetin 3',4'-dimethyl ether

631. 7-Glucuronide

Tricetin 3',5'-dimethyl ether (Tricin)

632. 5-Glucoside

633. 7- β -D-Arabinopyranoside*

634. 7-Xyloside

635. 7-Glucoside

636. 7-Glucuronide

637. 4'-Apioside*

638. 4'-Glucoside

639. 5-Diglucoside

640. 7-Rutinoside

641. 7-Neohesperidoside

642. 7-Diglucoside

643. 7-Fructosylglucoside

644. 7-Rhamnosylglucuronide

645. 7-Rhamnosyl(1 \rightarrow 2)galacturonide

646. 7-Diglucuronide

647. 5,7-Diglucoside

648. 7-Rutinoside-4'-glucoside

649. 7-(2''-*p*-Coumaroylglucuronosyl)(1 \rightarrow 2)glucuronide*650. 7-(2''-Feruloylglucuronosyl)(1 \rightarrow 2)glucuronide*651. 7-(2''-Sinapoylglucuronosyl)(1 \rightarrow 2)glucuronide*652. 7-[Glucuronosyl(1 \rightarrow 3)(2'''-feruloylglucuronosyl)](1 \rightarrow 2)glucuronide*

653. 7-[X'-(3-Hydroxy-3-methylglutaryl)glucoside]*

654. 7-Sulfatoglucoside

655. 7-Sulfatoglucuronide

656. 7-Disulfatoglucuronide

3-(3-Methylbutyl)tricetin

657. 5-Neohesperidoside

Tricetin 7,3',4'-trimethyl ether

658. 5-Glucoside*

Tricetin 7,3',4',5'-tetramethyl ether

659. 5-Glucoside*

660. 5-Xylosyl(1 \rightarrow 2)rhamnoside*661. 5-Xylosyl(1 \rightarrow 6)glucoside*

6-Hydroxytricetin 6,3',5'-trimethyl ether

662. 7- α -L-Arabinosyl(1 \rightarrow 6)glucoside*

6-Hydroxytricetin 6,4',5'-trimethyl ether

663. 3'-Rhamnoside*

6-Hydroxytricetin 6,7,3',5'-tetramethyl ether

664. 5-Robinoside*

8-Hydroxytricetin

665. 5-Rhamnoside*

666. 5-Glucoside*

667. 7-Glucuronide

5,6,7,8,3',4'-Hexahydroxyflavone

688. 7-Glucoside*

5,7,2',5'-Tetrahydroxy-8,6'-dimethoxyflavone (viscidulin III)

669. 2'-Glucoside*

5,2',6'-Trihydroxy-6,7,8-trimethoxyflavone

670. 2'-Glucoside*

5,4'-Dihydroxy-7,8,2',3'-tetramethoxyflavone

671. 5-Glucoside

C-Methylflavones

5,7-Dihydroxy-6-C-methylflavone

672. 7-Xylosyl(1 → 3)xyloside*

3-C-Methylapigenin

673. 5-Rhamnoside

5,7,4'-Trihydroxy-3'-C-methylflavone

674. 4'-Rhamnoside

5,7-Dihydroxy-6,8-di-C-methylflavone (matteuorien)

675. 7-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*

3-C-Methylfuteolin

676. 5-Rhamnoside

677. Stachysetin (13.5)*

Prenylated flavones

8-Prenylapigenin

678. 4'-Rutinoside*

3'-Prenylapigenin

679. 7-Rutinoside*

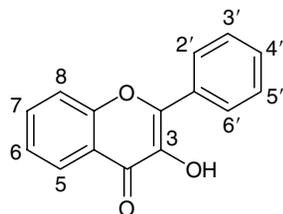
8-C-Prenyl-5,7,4'-trihydroxy-3'-methoxyflavone (8-C-Prenylchrysoeriol)

680. 7-Glucosyl(1 → 3)- α -L-arabinopyranoside*

*Flavone glycosides newly reported since 1992.

APPENDIX B

CHECKLIST OF KNOWN FLAVONOL GLYCOSIDES



13.10

3,5,7-Trihydroxyflavone (galangin)

1. 3-Glucoside

2. 7-Glucoside

3. 3-Rutinoside

4. 3-Galactosyl(1 → 4)rhamnoside

5. 8-Glucoside-8-sulfate*

6. 8-Sulfate*

3,7-Dihydroxy-8-methoxyflavone

7. 7-Rhamnoside*

8. 7-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)glucoside*

3,7,4'-Trihydroxyflavone

9. 3-Glucoside

10. 7-Glucoside
11. 4'-Glucoside
12. 7-Rutinoside
- 5,7-Dihydroxy-3,6-dimethoxyflavone
 13. 5- α -L-Arabinosyl(1 \rightarrow 6)glucoside*
- 3,6,7-Trihydroxy-4'-methoxyflavone
 14. 7-Rhamnoside*
- 8-Hydroxygalangin 3-methyl ether
 15. 8-(*Z*-2-Methyl-2-butenate)
 16. 8-(2-Methylbutyrate)
- 8-Hydroxygalangin 7-methyl ether
 17. 8-Acetate
 18. 8-Butyrate
- 3,7,3',4'-Tetrahydroxyflavone (fisetin)
 19. 3-Glucoside
 20. 7-Glucoside
 21. 4'-Glucoside
 22. 7-Rutinoside
- 3,7,4'-Trihydroxy-3'-methoxyflavone (geraldol)
 23. 4'-Glucoside
- 3,5,7,4'-Tetrahydroxyflavone (kaempferol)
 24. 3- α -D-Arabinopyranoside*
 25. 3-Arabinofuranoside (juglanin)
 26. 3-Xyloside
 27. 3-Rhamnoside (afzelin)
 28. 3-Glucoside (astragalin)
 29. 3- α -D-Galactoside
 30. 3- β -D-Galactoside (trifolin)
 31. 3-Alloside (asiaticalin)
 32. 3-Glucuronide
 33. 3-(6''-Ethylglucuronide)
 34. 5-Rhamnoside
 35. 5-Glucoside
 36. 5-Glucuronide*
 37. 7-Arabinoside
 38. 7-Xyloside
 39. 7-Rhamnoside
 40. 7-Glucoside (populnin)
 41. 7-Alloside*
 42. 4'-Rhamnoside
 43. 4'-Glucoside
 44. 3-Rhamnosyl(1 \rightarrow 2)- α -L-arabinofuranoside (arapetaloside B)*
 45. 3-Xylosyl(1 \rightarrow 2)rhamnoside
 46. 3-Rhamnosylxyloside
 47. 3-Arabinosyl(1 \rightarrow 6)galactoside
 48. 3-Xylosyl(1 \rightarrow 2)glucoside*
 49. 3-Xylosyl(1 \rightarrow 2)galactoside
 50. 3-Rhamnosyl(1 \rightarrow 2)rhamnoside*
 51. 3-Apiosyl(1 \rightarrow 2)glucoside
 52. 3-Apiosyl(1 \rightarrow 2)galactoside
 53. 3-Glucosyl(1 \rightarrow 2)rhamnoside*

54. 3-Glucosyl(1 → 4)rhamnoside
55. 3-Rutinoside
56. 3-Neohesperidoside
57. 3-Rhamnosyl(1 → 3)glucoside (runggioside)
58. 3-Robinoside
59. 3-Rhamnosyl(1 → 2)galactoside
60. 3-Sambubioside
61. 3-Gentiobioside
62. 3-Sophoroside
63. 3-Glucosyl(1 → 6)galactoside
64. 3-Glucosyl(1 → 2)galactoside
65. 3-Galactosylglucoside
66. 3-Digalactoside
67. 7-Glucosyl(1 → 4)xyloside*
68. 7-Neohesperidoside*
69. 7-Glucosyl(1 → 3)rhamnoside*
70. 7-Galactosyl(1 → 4)rhamnoside
71. 7-Sophoroside
72. 3,5-Diglucoside
73. 3,5-Digalactoside
74. 3,7-Diarabinoside
75. 3-Arabinoside-7-rhamnoside
76. 3- α -L-Arabinofuranoside-7- α -L-rhamnopyranoside
77. 3-Rhamnoside-7-arabinoside
78. 3-Glucoside-7-arabinoside
79. 3-Xyloside-7-rhamnoside
80. 3-Rhamnoside-7-xyloside
81. 3-Xyloside-7-glucoside
82. 3-Glucoside-7-xyloside
83. 3,7-Dirhamnoside
84. 3-Rhamnoside-7-glucoside
85. 3- α -D-Glucoside-7- α -L-rhamnoside
86. 3- β -D-Glucoside-7-rhamnoside
87. 3-Galactoside-7-rhamnoside
88. 3-Glucoside-7-galactoside
89. 3,7-Diglucoside
90. 3-Rhamnoside-7-galacturonide
91. 3-Glucoside-7-glucuronide
92. 3-Glucuronide-7-glucoside
93. 3,4'-Dixyloside
94. 3,4'-Diglucoside*
95. 3-Rhamnoside-4'-arabinoside
96. 3-Rhamnoside-4'-xyloside
97. 3-Galactoside-4'-glucoside
98. 7,4'-Dirhamnoside
99. 7-Rhamnoside-4'-glucoside*
100. 7,4'-Diglucoside*
101. 3-Glucosyl- β -(1 → 4)arabinofuranosyl- α -(1 → 2)arabinopyranoside (primflasin)
102. 3-Xylosylrutinoside
103. 3-Xylosyl(1 → 3)rhamnosyl(1 → 6)galactoside*

104. 3-Xylosyl(1 → 6)glucosyl(1 → 2)rhamnoside*
105. 3-Rhamnosyl(1 → 2)rhamnosyl(1 → 6)glucoside
106. 3-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)glucoside*
107. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)glucoside
108. 3-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)galactoside (rhamninoside)
109. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)galactoside (isorhamninoside)
110. 3-Rhamnosyl(1 → 2)glucosyl(1 → 6)galactoside*
111. 3-Rhamnosyl(1 → 6)glucosyl(1 → 6)galactoside*
112. 3-Glucosyl(1 → 4)rhamnosyl(1 → 2)glucoside*
113. 3-Glucosyl(1 → 3)rhamnosyl(1 → 6)galactoside
114. 3-Glucosyl(1 → 2)gentiobioside
115. 3-Sophorotrioside
116. 3-β-Maltosyl(1 → 6)glucoside
117. 3-Glucosyl(1 → 2)galactosyl(1 → 2)glucoside*
118. 3-Xylosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]
119. 3-Rhamnosyl(1 → 2)[rhamnosyl(1 → 6)glucoside] (mauritianin)
120. 3-Rhamnosyl(1 → 2)[glucosyl(1 → 3)glucoside]*
121. 3-Rhamnosyl(1 → 2)[glucosyl(1 → 4)glucoside]*
122. 3-Rhamnosyl(1 → 6)[glucosyl(1 → 2)glucoside]
123. 3-Glucosyl(1 → 2)[glucosyl(1 → 3)rhamnoside]*
124. 3-Glucosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]
125. 3-Galactosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]
126. 3-(2^G-Rhamnosylrutinoside)
127. 3-(2^G-Rhamnosylgentiobioside)
128. 3-(3^R-Glucosylrutinoside)
129. 3-(2^G-Glucosylrutinoside)
130. 3-(2'-Rhamnosyllaminaribioside)
131. 3-(2^G-Glucosylgentiobioside)
132. 3-Apiosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]
133. 7-(3^G-Glucosylgentiobioside)*
134. 4'-Rhamnosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]
135. 3-Rhamnosylarabinoside-7-rhamnoside
136. 3-Rhamnosyl(1 → 2)galactoside-7-α-L-arabinofuranoside*
137. 3-Robinoside-7-α-L-arabinofuranoside*
138. 3-Glucosylxyloside-7-xyloside
139. 3-β-D-Apiofuranosyl(1 → 2)-α-L-arabinofuranosyl-7-α-L-rhamnoside
140. 3-Xylosyl(1 → 2)rhamnoside-7-rhamnoside (sagittatin A)
141. 3-Xylosyl(1 → 4)rhamnoside-7-rhamnoside*
142. 3-Rhamnoside-7-xylosyl(1 → 2)rhamnoside*
143. 3-Apiosyl(1 → 4)rhamnoside-7-rhamnoside*
144. 3-Rhamnosyl(1 → 4)rhamnoside-7-rhamnoside
145. 3-Rhamnosylxyloside-7-glucoside
146. 3-Neohesperidoside-7-rhamnoside*
147. 3-Glucosylrhamnoside-7-rhamnoside
148. 3-Rutinoside-7-rhamnoside
149. 3-Rhamnoside-7-rhamnosylglucoside
150. 3-Rhamnoside-7-glucosyl(1 → 2)rhamnoside*
151. 3-Glucosyl(1 → 3)rhamnoside-7-rhamnoside
152. 3-Rhamnosyl(1 → 2)galactoside-7-rhamnoside
153. 3-Robinoside-7-rhamnoside (robinin)

154. 3-Rhamnosyl(1 → 2)galactoside-7-glucoside
155. 3-Sophoroside-7- α -L-arabinoside
156. 3-Glucosyl(1 → 4)galactoside-7- α -L-arabinofuranoside*
157. 3-Glucosyl(1 → 6)galactoside-7- α -L-arabinofuranoside*
158. 3-Apioside-7-rhamnosyl(1 → 6)galactoside*
159. 3-Robinobioside-7-glucoside
160. 3-Lathyroside-7-rhamnoside
161. 3-Sambubioside-7-glucoside
162. 3-Rutinoside-7-glucoside
163. 3-Neohesperidoside-7-glucoside
164. 3-Glucosyl(1 → 2)rhamnoside-7-glucoside*
165. 3-Rutinoside-7-galactoside
166. 3-Rutinoside-7-glucuronide
167. 3-Sophoroside-7-rhamnoside
168. 3-Laminaribioside-7-rhamnoside
169. 3-Gentiobioside-7-rhamnoside*
170. 3-Sophoroside-7-glucoside
171. 3-Glucoside-7-sophoroside
172. 3-Gentiobioside-7-glucoside
173. 3-Glucoside-7-gentiobioside
174. 3-Glucosyl(1 → 2)galactoside-7-glucoside*
175. 3-Sophoroside-7-glucuronide*
176. 3-Gentiobioside-7-glucuronide
177. 3-Rutinoside-4'-glucoside
178. 3-Neohesperidoside-4'-glucoside*
179. 3-Neohesperidoside-7,4'-diglucoside*
180. 3-Sophoroside-4'-glucoside
181. 3-Gentiobioside-4'-glucoside*
182. 3-Glucoside-7,4'-dirhamnoside
183. 3-Galactoside-3,4'-dirhamnoside*
184. 3-Rhamnoside-7,4'-digalactoside*
185. 4'-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)galactoside*
186. 3,7,4'-Triglucoside
187. 3-Rhamnosyl(1 → 2)[xylosyl(1 → 3)rhamnosyl(1 → 6)galactoside]*
188. 3-Glucosyl(1 → 3)rhamnosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]*
189. 3-Xylosylrutinoside-7-glucoside
190. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)galactoside-7-rhamnoside*
191. 3-Sophorotrioside-7-rhamnoside
192. 3-Rutinoside-7-sophoroside*
193. 3-(2^G-Glucosylrutinoside)-7-rhamnoside*
194. 3-(2^G-Rhamnosylrutinoside)-7-glucoside* (mauritanin 7-glucoside)
195. 3-Galactosyl(1 → 6)glucoside-7-dirhamnoside (malvitin)
196. 3-Sophorotrioside-7-glucoside
197. 3-Sophoroside-7-cellobioside*
198. 3-Rhamnosyl(1 → 6)[glucosyl(1 → 2)glucoside]-7-glucoside
199. 3-(2^G-Glucosylrutinoside)-7-glucoside
200. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)galactoside]-7-rhamnoside* (astrasikokioside)
201. 3-Glucosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]-7-rhamnoside*
202. 3-Rutinoside-4'-diglucoside
203. 3-Gentiobioside-7,4'-bisglucoside

204. 3-*Z/E-p*-Coumarate
205. 3-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]*
206. 3-(*p*-Hydroxybenzoylglucoside)
207. 3-(6''-*p*-Hydroxybenzoylgalactoside)*
208. 3-Benzoylglucoside
209. 3-(6''-Succinylglucoside)
210. 3-(6''-Malonylglucoside)
211. 3-(6''-Malonylgalactoside)
212. 3-(2''-Galloylarabinoside)*
213. 3-(2''-Galloylglucoside)
214. 3-(6''-Galloylglucoside)
215. 3-(6''-Galloylgalactoside)*
216. 3-(2'',6''-Digalloylglucoside)*
217. 3-(6''-*Z*-Cinnamoylglucoside)
218. 3-(2''-*E-p*-Coumaroyl- α -L-arabinofuranoside)*
219. 3-(2''-*E-p*-Coumaroylrhamnoside)*
220. 3-(2''-*Z-p*-Coumaroylrhamnoside)*
221. 3-(X''-*p*-Coumaroylglucoside) (tiliroside)
222. 3-(2''-*Z-p*-Coumaroylglucoside)
223. 3-(3''-*p*-Coumaroylglucoside)
224. 3-(4''-*p*-Coumaroylglucoside)*
225. 3-(6''-*p*-Coumaroylglucoside) (tribuloside)
226. 3-(6''-*p*-Coumaroylgalactoside)
227. 3-(6''-Caffeoylglucoside)*
228. 3-(5''-Feruloylapioside)*
229. 3-(6''-Feruloylglucoside)*
230. 3-(2''-Acetylramnoside)
231. 3-(3''-Acetylramnoside)
232. 3-(4''-Acetylramnoside)
233. 3-(6''-Acetylglucoside)*
234. 3-(3'',4''-Diacetylglucoside)*
235. 7-(6''-Succinylglucoside)
236. 7-Galloylglucoside
237. 7-(6''-*p*-Coumaroylglucoside)*
238. 3-(2'',3''-Di-*E-p*-coumaroylrhamnoside)*
239. 3-(2'',4''-Di-*E-p*-coumaroylrhamnoside)*
240. 3-(2'',4''-Di-*Z-p*-coumaroylrhamnoside)*
241. 3-(2'',4''-Di-*p*-coumaroylglucoside)
242. 3-(2'',6''-Di-*E-p*-coumaroylglucoside)
243. 3-(2'',6''-Di-*Z-p*-coumaroylglucoside)*
244. 3-(3'',6''-Di-*Z-p*-coumaroylglucoside) (stenopalustroside A)*
245. 3-(6''-*p*-Coumaroylacetylglucoside)
246. 3-(3''-*Z-p*-Coumaroyl-6''-feruloylglucoside) (stenopalustroside B)*
247. 3-(4''-Acetyl-6''-*p*-coumaroylglucoside)
248. 3-(3''-*Z-p*-Coumaroyl-6''-*E-p*-coumaroylglucoside) (stenopalustroside C)*
249. 3-(3''-*E-p*-Coumaroyl-6''-*Z-p*-coumaroylglucoside) (stenopalustroside D)*
250. 3-(3''-*E-p*-Coumaroyl-[6''-(4-*O*-{4-hydroxy-3-methoxyphenyl}-1,3-dihydroxyisopropyl-feruloyl)]glucoside (stenopalustroside E)*
251. 3-(2''-*E-p*-Coumaroyl-6''-acetylglucoside)*
252. 3-(3''-Acetyl-6''-*p*-coumaroylglucoside)*

253. 3-(3'',4''-Diacetylglucoside)*
254. 3-(2'',3''-Diacetyl-4'-*p*-coumaroylrhamnoside)
255. 3-(2'',3''-Diacetyl-4''-*Z-p*-coumaroyl)-6''-(*E-p*-coumaroylglucoside)
256. 3-(3'',4''-Diacetyl-2'',6''-di-*E-p*-coumaroylglucoside)
257. 3-Apiosylmalonylglucoside
258. 3-(6^G-Malonylneohesperidoside)*
259. 3-(2''-*E*-Feruloylgalactosyl)(1 → 4)glucoside*
260. 3-(2''-*E*-Feruloylgalactosyl)(1 → 6)glucoside*
261. 3-(2^G-*E-p*-Coumaroylrutinoside)*
262. 3-(4''-*E-p*-Coumaroylrobinobioside)
263. 3-(6'''-*p*-Coumaroylglucosyl)(1 → 2)rhamnoside
264. 3-(4''-*Z-p*-Coumaroylrobinobioside)
265. 3-(6''-Caffeoylglucosyl)(1 → 4)rhamnoside*
266. 3-(Feruloysophoroside) (petunoside)
267. 3-(6''-*E*-Feruloylglucosyl)(1 → 2)galactoside*
268. 3-(6'''-Sinapoylglucosyl)(1 → 2)galactoside*
269. 3-(3'''-Acetyl- α -L-arabinopyranosyl)(1 → 6)glucoside*
270. 3-(2'''-Acetylarabinosyl)(1 → 6)galactoside
271. 3-(6'''-Acetylglucosyl)(1 → 3)galactoside*
272. 3-[2'''-Feruloylglucosyl(1 → 2)6''-malonylglucoside]*
273. 3-Benzoylglucoside-7-glucoside
274. 3-*p*-Hydroxybenzoylglucoside-7-glucoside
275. 3-[6''-(3-Hydroxy-3-methylglutaryl)glucoside]-7-glucoside*
276. 3-(6''-Malonylglucoside)-7-glucoside*
277. 3-(2''-*E-p*-Coumaroyl- α -L-arabinofuranoside)-7-rhamnoside*
278. 3-(3''-*p*-Coumaroylrhamnoside)-7-rhamnoside*
279. 3-(6''-*E-p*-Coumaroylglucoside)-7-glucoside*
280. 3-Glucoside-7-(*p*-coumaroylglucoside)
281. 3-Caffeoylsophoroside
282. 3-(6'''-Caffeoylglucosyl)(1 → 2)galactoside
283. 3-(2''-Caffeoylglucoside)-7-rhamnoside
284. 3-(Caffeoylglucoside)-7-glucoside
285. 3-Feruloylglucoside-7-glucoside
286. 3-(3''-Acetylarabinofuranoside)-7-glucoside
287. 3-(4''-Acetylrhamnoside)-7-rhamnoside (sutchuenoside A)
288. 3-(6''-Acetylglucoside)-7-rhamnoside
289. 3-(6''-Acetylglactoside)-7-rhamnoside
290. 3-(6''-Acetylglucoside)-7-glucoside
291. 3-(2'',3''-Diacetylrhamnoside)-7-rhamnoside*
292. 3-(2'',4''-Diacetylrhamnoside)-7-rhamnoside*
293. 3-(3'',4''-Diacetylrhamnoside)-7-rhamnoside*
294. 3-(4'',6''-Diacetylrhamnoside)-7-rhamnoside*
295. 3-(2''',3''',4'''-Triacetyl- α -L-arabinopyranosyl)(1 → 6)glucoside*
296. 3-(2''',3''',5'''-Triacetylarabinofuranosyl)(1 → 6)glucoside
297. 3-[6'''-(7'''-Glucosyl-*p*-coumaroyl)glucosyl](1 → 2)rhamnoside*
298. 3-(*p*-Coumaroylsophoroside)
299. 3-(*p*-Coumarylglucoside)-4'-glucoside
300. 3-(2-Hydroxypropionylglucoside)-4'-glucoside
301. 3-[2^{Gal}-(6''-Feruloylglucosyl)robinobioside]*
302. 3-(*p*-Coumaroylsophorotrioside)

303. 3-(Feruloylsophorotrioside)
304. 3-Glucosyl(1 → 4)[(6'''-sinapoylglucosyl)(1 → 2)galactoside]*
305. 3-(2'''-Sinapoylglucosyl)(1 → 4)[(6'''-sinapoylglucosyl)(1 → 2)galactoside]*
306. 3-Rhamnosyl(1 → 4)(3'''-acetylramnosyl)(1 → 6)galactoside
307. 3-Rhamnosyl(1 → 3)(4'''-acetylramnosyl)(1 → 6)glucoside*
308. 3-Rhamnosyl(1 → 3)(2'''-acetylramnosyl)(1 → 6)galactoside*
309. 3-Glucosyl(1 → 3)(4'''-acetylramnosyl)(1 → 6)galactoside
310. 3-[2^{Gal}-(6'''-Feruloylglucosyl)robinobioside]*
311. 3-[2^{Gal}-(4'''-Acetylramnosyl)robinobioside]*
312. 3-[2''-(4'''-Acetylramnosyl)sophoroside]*
313. 3-Neohesperidoside-7-(6''-malonylglucoside)*
314. 3-(4''-*E-p*-Coumaroylrobinobioside)-7-rhamnoside
315. 3-(4''-*Z-p*-Coumaroylrobinobioside)-7-rhamnoside (variabiloside D)
316. 3-(*p*-Coumaroylrutinoside)-7-glucoside
317. 3-Neohesperidoside-7-(2''-*E-p*-coumaroylglucoside)*
318. 3-(4''-*p*-Coumaroylglucosyl)(1 → 2)rhamnoside-7-glucoside*
319. 3-(6''-*p*-Coumaroylglucosyl)(1 → 2)rhamnoside-7-glucoside*
320. 3-Glucosyl(1 → 2)rhamnoside-7-(6''-*E-p*-coumaroylglucoside)*
321. 3-(6''-*E-p*-Coumaroylglucosyl)(1 → 2)glucoside-7-rhamnoside*
322. 3-Glucoside-7-(6''-*E-p*-coumaroylglucosyl)(1 → 3)rhamnoside*
323. 3-(2'''-*E-p*-Coumaroylsophoroside)-7-glucoside*
324. 3-Apioside-7-rhamnosyl(1 → 6)(2''-*E*-caffeoylgalactoside)*
325. 3-(Caffeoylrobinobioside)-7-rhamnoside
326. 3-(6''-*E*-Caffeoylglucosyl)(1 → 2)glucoside-7-rhamnoside*
327. 3-Glucoside-7-(6''-*E*-caffeoylglucosyl)(1 → 3)rhamnoside*
328. 3-(2''-Caffeoyllamaribioside)-7-rhamnoside
329. 3-(4''-Caffeoyllamaribioside)-7-rhamnoside
330. 3-(2'''-*E-p*-Coumaroylsophoroside)-7-glucoside*
331. 3-(2'''-*E*-Caffeoylsophoroside)-7-glucoside*
332. 3-(Feruloylrobinobioside)-7-rhamnoside
333. 3-Neohesperidoside-7-(2''-*E*-feruloylglucoside)*
334. 3-(2'''-*E*-Feruloylsophoroside)-7-glucoside*
335. 3-Sophoroside-7-(2''-feruloylglucoside)
336. 3-(Sinapoylsophoroside)-7-glucoside
337. 3-Xylosyl(1 → 3)(4''-acetylramnoside)-7-rhamnoside
338. 3-Xylosyl(1 → 2)rhamnoside-7-(4'''-acetylramnoside)*
339. 3-Neohesperidoside-7-(6''-acetylglucoside)*
340. 3-Glucosyl(1 → 2)(6''-acetylgalactoside)-7-glucoside*
341. 3,4'-Diglucoside-7-(2''-feruloylglucoside)
342. 3-(*p*-Coumaroylglucoside)-7,4'-diglucoside
343. 3-(2''-Feruloylglucoside)-7,4'-diglucoside
344. 3-(Sinapoylglucoside)-7-sophoroside
345. 3-(*p*-Coumaroylferuloyldiglucoside)-7-rhamnoside
346. 3-Rhamnosyl(1 → 2)[glucosyl(1 → 3)(4'''-*p*-coumaroylramnosyl)(1 → 6)galactoside]*
347. 3-Rhamnosyl(1 → 2)[xylosyl(1 → 3)rhamnosyl(1 → 6)(3'''-*p*-coumaroylgalactoside)]*
348. 3-Rhamnosyl(1 → 2)[xylosyl(1 → 3)rhamnosyl(1 → 6)(4'''-*p*-coumaroylgalactoside)]*
349. 3-Rhamnosyl(1 → 2)[xylosyl(1 → 3)rhamnosyl(1 → 6)(3'''-feruloylgalactoside)]*
350. 3-Rhamnosyl(1 → 2)[xylosyl(1 → 3)rhamnosyl(1 → 6)(4'''-feruloylgalactoside)]*
351. 3-Gentiobioside-7-(caffeoylarabinosylramnoside)
352. 3-Neohesperidoside-7-[2''-*E-p*-coumaroyllamaribioside]*

353. 3-(2'''-*E*-Caffeoylglucosyl)(1 → 2)glucoside-7-cellobioside*
354. 3-(2'''-*E*-Feruloylglucosyl)(1 → 2)glucoside-7-cellobioside*
355. 3-(2'''-*E*-Sinapoylglucosyl)(1 → 2)glucoside-7-cellobioside*
356. 3-Glucosyl(1 → 6)[rhamnosyl(1 → 3)(2''-*E-p*-coumaroylglucoside)]-7-rhamnosyl(1 → 3)rhamnosyl(1 → 3)(4''-*E-p*-coumaroylrhamnoside)*
357. 3-Glucosyl(1 → 6)[rhamnosyl(1 → 3)(2''-*E-p*-coumaroylglucoside)]-7-rhamnosyl(1 → 3)rhamnosyl(1 → 3)(4''-*Z-p*-coumaroylrhamnoside)*
358. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 3)(2''-*E-p*-coumaroylglucoside)]-7-rhamnosyl(1 → 3)rhamnosyl(1 → 3)(4''-*E-p*-coumaroylrhamnoside)*
359. 3-Sulfatorhamnoside
360. 3- α -(6''-Sulfatoglucoside)
361. 3- β -(3''-Sulfatoglucoside)
362. 3- β -(6''-Sulfatoglucoside)
363. 3-Glucuronide-7-sulfate
364. 3-Sulfatorutinoside
365. 3-(6''-Sulfatogentiobioside)
366. 3-Sulfate-7- α -arabinopyranoside*
367. 3-Sulfate
368. 7-Sulfate
369. 8-Sulfate*
370. 3,7-Disulfate
371. 3,7,4'-Trisulfate
- Kaempferol 3-methyl ether
372. 7-Rhamnoside
373. 7-Glucoside
374. 7-Glucuronide*
375. 7-Rutinoside
- Kaempferol 5-methyl ether
376. 3-Galactoside
- Kaempferol 7-methyl ether (rhamnocitrin)
377. 3-Rhamnoside
378. 3-Alloside
379. 3-Glucoside
380. 3-Galactoside
381. 3-Glucuronide
382. 5-Glucoside
383. 4'-Glucoside*
384. 3-Rutinoside
385. 3-Neohesperidoside
386. 3-Galactoside-4'-glucoside
387. 3-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)galactoside (3-rhamninoside, alaternin, catharticin)
388. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)galactoside (3-isorhamninoside)
389. 3-Xylosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]*
390. 3-Rhamnosyl(1 → 3)[apiosyl(1 → 6)glucoside]*
391. 3-Apiosyl(1 → 5)apioside-4'-glucoside*
392. 3-Neohesperidoside-4'-glucoside*
393. 3-Apiosyl(1 → 5)apiosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]*
394. 3-[3-Hydroxy-3-methylglutaryl(1 → 6)][apiosyl(1 → 2)galactoside]*
395. 3-(5'''-*p*-Coumaroylapiosyl)(1 → 2)glucoside*

396. 3-(5''-Feruloyllapiosyl)(1 → 2)glucoside*
397. 3-(6''-*E*-Sinapoylglucosyl)(1 → 2)rutinoside*
398. 3-Glucoside-4'-(2''-dihydrophaseoylglucoside)
399. 3-Glucoside-4'-(3'''-dihydrophaseoylglucoside)*
400. 3-(6-*E*-3,5-Dimethoxy-4-hydroxycinnamoylglucosyl)(1 → 2)[rhamnosyl(1 → 6)glucoside]*
401. 3-Sulfate
- Kaempferol 4'-methyl ether (kaempferide)
402. 3-Rhamnoside*
403. 3-Galactoside
404. 3-Glucuronide
405. 3-Neohesperidoside*
406. 3-Diglucoside
407. 3-Rhamnoside-7-xyloside*
408. 3,7-Dirhamnoside
409. 3-Rhamnoside-7-glucoside
410. 3-Glucoside-7-rhamnoside
411. 3,7-Diglucoside
412. 3-(4^{Rha}-Rhamnosylrutinoside)*
413. 3-(2^{Glc}-Glucosylrutinoside)*
414. 3-[6'''-Acetyl(4'-α-methylsinapoylneohesperidoside)]*
415. 3-Rhamnoside-7-(6''-succinylglucoside)
416. 3-Sulfate
- Kaempferol 3,5-dimethyl ether
417. 7-Glucoside*
- Kaempferol 3,7-dimethyl ether
418. 4'-Glucoside*
- Kaempferol 3,4'-dimethyl ether
419. 7-Glucoside
- Kaempferol 7,4'-dimethyl ether
420. 3-Glucoside*
421. 3-Neohesperidoside*
422. 3-(6''-*p*-Coumaroylglucoside)
423. 3-Sulfate
- 6-*C*-Methylkaempferol
424. 3-Glucoside
- 6-Hydroxykaempferol
425. 3-Glucoside*
426. 7-Glucoside
427. 7-Alloside*
428. 3-Rutinoside*
429. 7-Rutinoside
430. 3,6-Diglucoside*
431. 3-Rutinoside-6-glucoside*
432. 3,6,7-Triglucoside*
433. 7'-(6''-Caffeoylglucoside)*
434. 7-Acetylglucoside
- 6-Hydroxykaempferol 3-methyl ether
435. 6-Glucoside
436. 7-Glucoside

- 437. 7-Sulfate
- 6-Hydroxykaempferol 5-methyl ether
- 438. 4'-Rhamnoside
- 439. 3-Arabinosylrhamnoside
- 6-Hydroxykaempferol 6-methyl ether (eupafolin)
- 440. 3-Rhamnoside
- 441. 3-Glucoside
- 442. 3-Galactoside
- 443. 3-Glucuronide
- 444. 7-Glucoside
- 445. 4'-Rhamnoside
- 446. 3-Rutinoside
- 447. 3-Robinobioside
- 448. 3,7-Dirhamnoside
- 449. 3-(6''-*p*-Coumaroylglucoside)*
- 450. 3-Rhamnoside-7-(4'''-acetylramnoside)
- 451. 3-(3''-Acetylramnoside)-7-(3''-acetylramnoside)
- 452. 3-(6''-Acetylglucoside)
- 453. 3-Sulfate
- 6-Hydroxykaempferol 7-methyl ether
- 454. 6-Rhamnosyl(1 → 4)xyloside
- 6-Hydroxykaempferol 4'-methyl ether
- 455. 7-Glucoside*
- 456. 7-Galactoside*
- 457. 3,7-Dirhamnoside
- 6-Hydroxykaempferol 3,6-dimethyl ether
- 458. 7-Glucoside
- 6-Hydroxykaempferol 6,7-dimethyl ether (eupalitin)
- 459. 3-Rhamnoside (eupalin)
- 460. 3-Galactoside
- 461. 5-Rhamnoside
- 462. 3-Galactosylrhamnoside
- 463. 3-Diglucoside
- 464. 3-Glucosylgalactoside
- 465. 3-Sulfate
- 6-Hydroxykaempferol 6,4'-dimethyl ether
- 466. 3-Glucoside*
- 467. 3-Galactoside
- 6-Hydroxykaempferol 7,4'-dimethyl ether
- 468. 3-Glucoside
- 469. 3-Sulfate
- 6-Hydroxykaempferol 3,6,7-trimethyl ether
- 470. 4'-Glucoside
- 6-Hydroxykaempferol 6,7,4'-trimethyl ether (mikanin)
- 471. 3-Glucoside
- 472. 3-Galactoside
- 6-Hydroxykaempferol 3,5,7,4'-tetramethyl ether
- 473. 6-Rhamnoside*
- 8-Hydroxykaempferol (herbacetin)

- 474. 3-Glucoside
- 475. 3- β -D-Glucofuranoside*
- 476. 7-Arabinoside
- 477. 7-Rhamnoside
- 478. 7-Glucoside
- 479. 8- α -L-Arabinopyranoside
- 480. 8-Xyloside
- 481. 8-Rhamnoside
- 482. 8-Glucoside
- 483. 4'-Glucoside
- 484. 3-Rhamnoside-8-glucoside*
- 485. 3-Glucuronide-8-glucoside
- 486. 7-Glucosyl(1 \rightarrow 3)rhamnoside
- 487. 8-Rutinoside
- 488. 8-Gentiobioside
- 489. 3-Glucoside-8-xyloside
- 490. 7-Rhamnoside-8-glucoside
- 491. 8,4'-Dixyloside
- 492. 8-Arabinoside-4'-xyloside
- 493. 3-Sophoroside-8-glucoside
- 494. 7-(6''-Quinylglucoside)
- 495. 8-(3''-Acetyl- α -L-arabinopyranoside)
- 496. 8-(3''-Acetylxyloside)
- 497. 8-(2'',3''-Diacetylxyloside)
- 498. 8-(Diacetylglucoside)
- 499. 8-(2'',3'',4''-Triacetylxyloside)
- 500. 8-Acetate
- 501. 8-Butyrate
- Herbacetin 7-methyl ether
- 502. 8-Sophoroside*
- 503. 3-(2''-*E*-Feruloylglucoside)*
- 504. 8-Acetate
- 505. 8-Butyrate
- Herbacetin 8-methyl ether (sexangularetin)
- 506. 3-Glucoside
- 507. 3-Galactoside
- 508. 3-Rutinoside
- 509. 3-Neohesperidoside*
- 510. 3-Sophoroside*
- 511. 3-Glucoside-7-rhamnoside
- 512. 3,7-Diglucoside
- 513. 3-Rhamnosylglucoside-7-rhamnoside
- 514. 3-Rutinoside-7-glucoside
- 515. 3-Glucoside-7-rutinoside
- Herbacetin 7,8-dimethyl ether
- 516. 3-Rhamnoside
- Herbacetin 7,4'-dimethyl ether
- 517. 8-Acetate
- 518. 8-Butyrate
- Herbacetin 7,8,4'-trimethyl ether (tambulin)
- 519. 3,5-Diacetate*

- 5,7,8-Trihydroxy-3-methoxyflavone
520. 8-(*E*-2-Methylbut-2-enoate)*
- 5,7,8-Trihydroxy-3,6-dimethoxyflavone
521. 8-(*E*-2-Methylbut-2-enoate)*
- 6,8-Dihydroxykaempferol
522. 3-Rutinoside*
- 3,5,7,3',4'-Pentahydroxyflavone (quercetin)
523. 3- α -L-Arabinofuranoside (avicularin)
524. 3- α -L-Arabinopyranoside (guaijaverin, foeniculin)
525. 3- α -D-Arabinopyranoside*
526. 3- β -L-Arabinoside (polystachioside)
527. 3-Xyloside (reynoutrin)
528. 3-Rhamnoside (quercitrin)
529. 3-Glucoside (isoquercitrin)
530. 3-Galactoside (hyperin)
531. 3-Alloside
532. 3-Glucuronide (miquelianin)
533. 3-Galacturonide
534. 3-(6''-Methylglucuronide)
535. 3-(6''-Ethylglucuronide)
536. 5-Glucoside
537. 5-Glucuronide*
538. 7-Arabinoside
539. 7-Xyloside
540. 7-Rhamnoside
541. 7-Glucoside (quercimeritrin)
542. 7- α -Galactoside
543. 3'-Xyloside
544. 3'-Glucoside
545. 4'-Glucoside (spiraeoside)
546. 4'-Galactoside*
547. 4'-Glucuronide*
548. 3-Diarabinoside
549. 3-Arabinosylxyloside
550. 3-Rhamnosyl(1 \rightarrow 2)- α -L-arabinofuranoside (arapetaloside A)*
551. 3-Rhamnosyl(1 \rightarrow 2)arabinoside
552. 3- α -L-Arabinofuranosyl(1 \rightarrow 2)glucoside*
553. 3-Arabinosyl(1 \rightarrow 6)glucoside (vicianoside)
554. 3-Arabinosyl(1 \rightarrow 6)galactoside
555. 3-Galactosylarabinoside
556. 3-Dixyloside
557. 3-Xylosyl(1 \rightarrow 2)rhamnoside
558. 3-Xylosyl(1 \rightarrow 2)glucoside (3-sambubioside)
559. 3-Xylosyl(1 \rightarrow 6)glucoside*
560. 3-Glucosyl(1 \rightarrow 2)xyloside
561. 3-Xylosyl(1 \rightarrow 2)galactoside
562. 3-Apiofuranosyl(1 \rightarrow 2)arabinoside
563. 3-Apiofuranosyl(1 \rightarrow 2)xyloside
564. 3-Rhamnosylxyloside
565. 3-Rhamnosyl(1 \rightarrow 2)rhamnoside*

566. 3-Rhamnosyl(1 → 2)galactoside
567. 3-Apiofuranosyl(1 → 2)glucoside
568. 3-Apiofuranosyl(1 → 2)galactoside
569. 3-Rutinoside (rutin)
570. 3-Neohesperidoside
571. 3-Glucosyl(1 → 2)rhamnoside*
572. 3-Glucosyl(1 → 4)rhamnoside
573. 3-Galactosyl(1 → 2)rhamnoside*
574. 3-Galactosyl(1 → 4)rhamnoside
575. 3-Rhamnosyl(1 → 6)galactoside
576. 3-Laminaribioside*
577. 3-Sophoroside
578. 3-Gentiobioside
579. 3-Galactosylglucoside
580. 3-Glucosyl(1 → 2)galactoside
581. 3-Glucosyl(1 → 3)galactoside*
582. 3-Glucosyl(1 → 4)galactoside*
583. 3-Glucosyl(1 → 6)galactoside
584. 3-Digalactoside
585. 3-Glucosylmannoside
586. 3-Glucosyl(1 → 2)glucuronide*
587. 3-Galactosylglucuronide
588. 7-Rutinoside
589. 7-Glucosylrhamnoside
590. 3,5-Digalactoside
591. 3-Arabinoside-7-glucoside
592. 3-Glucoside-7-arabinoside
593. 3-Xyloside-7-glucoside
594. 3-Galactoside-7-xyloside
595. 3,7-Dirhamnoside
596. 3-Rhamnoside-7-glucoside
597. 3-Glucoside-7-rhamnoside
598. 3-Galactoside-7-rhamnoside
599. 3,7-Diglucoside
600. 3-Galactoside-7-glucoside
601. 3-Glucoside-7-glucuronide
602. 3-Glucuronide-7-glucoside
603. 3,7-Diglucuronide
604. 3,3'-Diglucoside
605. 3-Rhamnoside-3'-glucoside*
606. 3,4'-Diglucoside
607. 7,4'-Diglucoside
608. 3-Xylosyl(1 → 2)rhamnosyl(1 → 6)glucoside*
609. 3-Xylosyl(1 → 6)glucosyl(1 → 2)rhamnoside*
610. 3-Rhamnosyl(1 → 2)rutinoside
611. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)glucoside
612. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)galactoside
613. 3-Apiosyl(1 → 2)rhamnosyl(1 → 6)glucoside*
614. 3-(6'''-Rhamnosylgentiobioside)*
615. 3-Rhamnosyl(1 → 2)glucosyl(1 → 6)galactoside*

616. 3-Rhamnosyl(1 → 6)glucosyl(1 → 6)galactoside*
617. 3-Glucosyl(1 → 4)galactosylrhamnoside
618. 3-Glucosyl(1 → 3)rhamnosyl(1 → 6)galactoside
619. 3-Sophorotrioside
620. 3-Glucosyl(1 → 2)galactosyl(1 → 2)glucoside*
621. 3-(2^G-Rhamnosylrutinoside)
622. 3-(2^G-Apiosylrutinoside)
623. 3-Rhamnosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]
624. 3-Xylosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]
625. 3-(2^G-Glucosylrutinoside)
626. 3-(3^R-Glucosylrutinoside)
627. 3-(2^G-Rhamnosylgentiobioside)
628. 3-Rhamnosyl(1 → 2)[glucosyl(1 → 6)galactoside]
629. 3-Glucosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]*
630. 3-(2^G-Glucosylgentiobioside)
631. 7-(2^G-Xylosylrutinoside)*
632. 3-Rhamnosyl(1 → 2)-α-L-arabinopyranoside-7-glucoside*
633. 3-Dixyloside-7-glucoside
634. 3-Xylosyl(1 → 2)glucoside-7-rhamnoside*
635. 3-Xyloside-7-xylosylglucoside
636. 3-Xylosyl(1 → 2)glucoside-7-glucoside (3-sambubioside-7-glucoside)
637. 3-Rutinoside-7-rhamnoside
638. 3-Neohesperidoside-7-rhamnoside*
639. 3-Glucosyl(1 → 2)rhamnoside-7-rhamnoside
640. 3-Rhamnosyl(1 → 4)rhamnoside-7-galactoside*
641. 3-Robinobioside-7-rhamnoside
642. 3-Rutinoside-7-glucoside
643. 3-Neohesperidoside-7-glucoside*
644. 3-Glucosyl(1 → 2)rhamnoside-7-glucoside*
645. 3-Glucoside-7-rutinoside
646. 3-Rhamnosyl(1 → 2)galactoside-7-glucoside
647. 3-Galactosyl-7-glucosyl(1 → 4)rhamnoside*
648. 3-Glucoside-7-neohesperidoside
649. 3-Rutinoside-7-galactoside
650. 3-Galactoside-7-neohesperidoside
651. 3-Robinobioside-7-glucoside
652. 3-Rutinoside-7-glucuronide
653. 3-Gentiobioside-7-glucoside
654. 3-Sophoroside-7-glucoside
655. 3-Glucosyl(1 → 2)galactoside-7-glucoside*
656. 3-Sophoroside-7-glucuronide*
657. 3-Gentiobioside-7-glucuronide
658. 3-Sambubioside-3'-glucoside
659. 3-Rutinoside-3'-apioside*
660. 3-Xylosyl(1 → 2)rhamnoside-4'-rhamnoside
661. 3-Rutinoside-4'-glucoside
662. 3,7,4'-Triglucoside
663. 3,3',4'-Triglucoside*
664. 3-Xylosyl(1 → 4)[xylosyl(1 → 6)glucosyl(1 → 2)rhamnoside]*
665. 3-Xylosyl(1 → 3)rhamnosyl(1 → 6)[apiosyl(1 → 2)galactoside]*

666. 3-Rhamnosylglucoside-7-xylosylglucoside
667. 3-(2^G-Rhamnosylrutinoside)-7-glucoside
668. 3-Glucosyl(1 → 4)rhamnoside-7-rutinoside*
669. 3-Rhamnosyl(1 → 6)[glucosyl(1 → 2)glucoside]-7-rhamnoside*
670. 3-Rhamnosyldiglucoiside-7-glucoside
671. 7-[Xylosyl(1 → 2)rhamnosyl(1 → 2)rhamnosyl](1 → 6)glucoside*
672. 3-Rutinoside-7,3'-bisglucoside
673. 3-Rutinoside-4'-diglucoiside
674. 3-Isobutyrate
675. 3'-Isobutyrate
676. 4'-Isobutyrate
677. 3-[6''-(3-Hydroxy-3-methylglutaryl)galactoside]
678. 3-(6''-*p*-Hydroxybenzoylgalactoside)
679. 3-(4''-Malonylrhamnoside)*
680. 3-(6''-Malonylglucoside)
681. 3-(6''-Malonylgalactoside)
682. 3-(2''-Galloyl- α -arabinopyranoside)
683. 3-(2''-Galloylrhamnoside)
684. 3-(2''-Galloylglucoside)
685. 3-(6''-Galloylglucoside)
686. 3-(2''-Galloylgalactoside)
687. 3-(6''-Galloylgalactoside)
688. 3-(2''-*p*-Coumarylglucoside)
689. 3-(3''-*p*-Coumarylglucoside)
690. 3-(6''-*p*-Coumarylglucoside) (helichryoside)
691. 3-(6''-Caffeoylgalactoside)
692. 3-(2''-Caffeoylglucuronide)*
693. 3-(6''-Feruloylgalactoside)*
694. 3-Isoferuloylglucuronide
695. 3-(2''-Acetylrhamnoside)*
696. 3-(3''-Acetylrhamnoside)
697. 3-(4''-Acetylrhamnoside)
698. 3-(6''-Acetylglucoside)
699. 3-(2''-Acetylgalactoside)*
700. 3-(3''-Acetylgalactoside)
701. 3-(6''-Acetylgalactoside)
702. 3-(6''-*n*-Butylglucuronide) (parthenosin)*
703. 7-(6''-Galloylglucoside)*
704. 7-(6''-Acetylglucoside)*
705. 4'-(6''-Galloylglucoside)*
706. 3-Diacetylglucoside
707. 7-Acetyl-3'-glucoside
708. 3- α -(2''-*p*-Hydroxybenzoyl)-4'-*p*-coumarylrhamnoside
709. 3-(2'',6''-Digalloylgalactoside)*
710. 3-(3'',6''-Di-*p*-coumarylglucoside)
711. 3-(3'',6''-Diacetylgalactoside)*
712. 3-(2'',3'',4''-Triacetylgalactoside)*
713. 3-(2'''-Galloylglucosyl)(1 → 2)- α -L-arabinofuranoside*
714. 3-(2''-Galloylrutinoside)*
715. 3-(6^G-Malonylneohesperidoside)*

716. 3-(X''-Benzoyl-X''-xylosylglucoside)
717. 3-(X''' or X''''-Benzoyl-X''-glucosylglucoside)
718. 3- α -L-Arabinopyranosyl(1 \rightarrow 6)(2''-*E-p*-coumaroylglucoside)*
719. 3- α -L-Arabinopyranosyl(1 \rightarrow 6)(2''-*E-p*-coumaroylgalactoside)*
720. 3-(2^G-*E-p*-Coumaroylrutinoside)*
721. 3-(4''-*E-p*-Coumaroylrhobioside)
722. 3- α -(6''-*p*-Coumaroylglucosyl)(1 \rightarrow 4)rhamnoside
723. 3-(2'''-*E*-Caffeoyl- α -L-arabinopyranosyl)(1 \rightarrow 6)glucoside)*
724. 3-(2''-*E*-Caffeoyl- α -L-arabinopyranosyl)(1 \rightarrow 6)galactoside)*
725. 3-(6''-Caffeoylsphoroside)*
726. 3-(6''-Caffeoylgentiobioside)*
727. 3-(Acetylrutinoside)
728. 3-(2''-Feruloylsphoroside)*
729. 3-(6''-Feruloylsphoroside)*
730. 3-(6'''-Sinapoylglucosyl)(1 \rightarrow 2)galactoside*
731. 3-Rhamnosyl(1 \rightarrow 6)(2''-acetylglucoside)*
732. 3-(6''-Acetylglucosyl)(1 \rightarrow 3)galactoside (euphorbianin)
733. 3-(2'''-Caffeoylglucoside)(1 \rightarrow 2)(6''-malonylglucoside)*
734. 3-(3'',4''-Diacetylramnosyl)(1 \rightarrow 6)glucoside*
735. 3-[2''',3''',4'''-Triacetyl- α -L-arabinopyranosyl(1 \rightarrow 6)glucoside]*
736. 3-[2''',3''',4'''-Triacetyl- α -L-arabinopyranosyl(1 \rightarrow 6)galactoside]*
737. 3-[2''',3''',5'''-Triacetyl- α -L-arabinopyranosyl(1 \rightarrow 6)glucoside]*
738. 3-(6''-Malonylglucoside)-7-glucoside*
739. 3-(6''-*E-p*-Coumaroylglucoside)-7-glucoside*
740. 3-Feruloylglucoside-7-glucoside
741. 3-(4''-Acetylramnoside)-7-rhamnoside
742. 3-(6''-Acetylgalactoside)-7-rhamnoside
743. 3-(2''-Galloylglucoside)-4'-vinylpropionate*
744. 3-Malonylglucoside-4'-glucoside
745. 3-Feruloylglucoside-4'-glucoside
746. 3-[2'',6''-{*p*-(7'''-Glucosyl)coumaroyl}glucosyl]rhamnoside*
747. 3-(6'''-*p*-Coumaroylsphorotrioside)*
748. 3-(6''''-Caffeoylsphorotrioside)*
749. 3-(6''''-Feruloylsphorotrioside)*
750. 3-(6''''-Feruloylglucosyl)(1 \rightarrow 2)galactosyl(1 \rightarrow 2)glucoside*
751. 3-(6''''-Sinapoylsphorotrioside)*
752. 3-Glucosyl(1 \rightarrow 3)(4'''-acetylramnosyl)(1 \rightarrow 6)-galactoside
753. 3-(3'''-Benzoylsphoroside)-7-rhamnoside
754. 3-Rutinoside-7-(6''-benzoylglucoside)*
755. 3-(*p*-Coumaroylsambubioside)-7-glucoside*
756. 3-(4''-*E-p*-Coumaroylrhobioside)-7-rhamnoside
757. 3-(6'''-*p*-Coumaroylglucosyl)(1 \rightarrow 2)rhamnoside-7-glucoside*
758. 3-(4''-*E-p*-Coumaroylrhobioside)-7-glucoside (variabiloside A)
759. 3-(4''-*Z-p*-Coumaroylrhobioside)-7-glucoside (variabiloside B)
760. 3-(6''-*E-p*-Coumaroylsphoroside)-7-rhamnoside*
761. 3-(*p*-Coumaroylsphoroside)-7-glucoside*
762. 3-(6''-*p*-Coumaroylgentiobioside)-7-rhamnoside
763. 3-(Caffeoylarabinosylglucoside)-7-glucoside*
764. 3-(4'''-Caffeoylramnosyl)(1 \rightarrow 2)- α -L-arabinopyranoside-7-glucoside*
765. 3-(2'''-Caffeoylsambubioside)-7-glucoside*

766. 3-Glucosyl-7-(6''-*E*-caffeoylglucosyl)(1 → 3)rhamnoside*
767. 3-(6''-*E*-Caffeoylsophoroside)-7-rhamnoside*
768. 3-(2''-*E*-Caffeoylsophoroside)-7-glucoside*
769. 3-Sophoroside-7-(6''-*E*-caffeoylglucoside)*
770. 3-(6''-*E*-Feruloylglucosyl)(1 → 2)-β-arabinopyranoside-7-glucoside*
771. 3-(Feruloylsambubioside)-7-glucoside*
772. 3-(2''-*E*-Feruloylsophoroside)-7-glucoside*
773. 3-(6''-*E*-Sinapoylsophoroside)-7-rhamnoside*
774. 3-(Caffeoylsophoroside)-7-(caffeoylglucoside)*
775. 3-(Caffeoylsophoroside)-7-(feruloylglucoside)*
776. 3-Acetylsophoroside-7-rhamnoside
777. 3-Rhamnoside-7-glucoside-4'-(caffeoylgalactoside)
778. 3-Ferulylglucoside-7,4'-diglucoside
779. 3-(2''-Sinapoylglucoside)-3'-(6''-sinapoylglucoside)-4'-glucoside*
780. 3,4'-Diglucoside-3'-(6''-sinapoylglucoside)*
781. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(3''-*E*-*p*-coumaroylgalactoside)]-7-rhamnoside*
782. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(4''-*E*-*p*-coumaroylgalactoside)]-7-rhamnoside*
783. 3-Rhamnosyl(1 → 2)[glucosyl(1 → 3)(4'''-*p*-coumaroylrhamnosyl)(1 → 6)galactoside]*
784. Diquercetin 3-galactoside ester of tetrahydroxy-μ-truxinic acid (13.6)*
785. 3-Sulfatorhamnoside
786. 3-(3''-Sulfatoglucoside)
787. 3-Sulfate-7-α-arabinopyranoside*
788. 3-Glucuronide-7-sulfate
789. 3-Rhamnoside-3'-sulfate*
790. 3-Glucoside-3'-sulfate*
791. 3-Acetyl-7,3',4'-trisulfate
792. 3-Sulfate
793. 3'-Sulfate
794. 3,7-Disulfate
795. 3,3'-Disulfate
796. 7,4'-Disulfate*
797. 3',4'-Disulfate
798. 3,7,3'-Trisulfate
799. 3,7,4'-Trisulfate
800. 3,7,3',4'-Tetrasulfate
801. 3-Sulfatoglucoside
802. 3-Glucuronide-3'-sulfate
- Quercetin 3-methyl ether
803. 5-Glucoside*
804. 7-α-L-Arabinofuranosyl(1 → 6)glucoside*
805. 7-Rhamnoside
806. 7-Glucoside
807. 3'-Xyloside
808. 4'-Glucoside
809. 7-Rutinoside*
810. 7-Gentiobioside*
811. 7-Galactosyl(1 → 4)glucoside*
812. 7-Rhamnosyl-3'-xyloside
813. 7-Diglucoside-4'-glucoside
814. 5-Glucoside-3'-sulfate*

Quercetin 5-methyl ether (azaleatin)

- 815. 3-Arabinoside
- 816. 3-Rhamnoside (azalein)
- 817. 3-Galactoside
- 818. 3-Glucuronide
- 819. 3-Xylosylarabinoside
- 820. 3-Rhamnosylarabinoside
- 821. 3-Arabinosylgalactoside
- 822. 3-Rutinoside
- 823. 3-Diglucoside

Quercetin 7-methyl ether (rhamnetin)

- 824. 3- α -L-Arabinofuranoside
- 825. 3- α -L-Arabinopyranoside
- 826. 3-Rhamnoside
- 827. 3-Glucoside
- 828. 3-Galactoside
- 829. 5-Glucoside
- 830. 3'-Glucuronide
- 831. 3- α -Diarabinoside
- 832. 3- β -Diarabinoside
- 833. 3- α -L-Arabinopyranosyl(1 \rightarrow 3)galactoside*
- 834. 3-Rhamnosyl(1 \rightarrow 4)rhamnoside
- 835. 3-Rutinoside
- 836. 3-Neohesperidoside
- 837. 3-Robinobioside*
- 838. 3-Laminaribioside*
- 839. 3-Gentiobioside*
- 840. 3-Galactosyl(1 \rightarrow 4)galactoside
- 841. 3-Galactosyl(1 \rightarrow 6)galactoside
- 842. 3-Mannosyl(1 \rightarrow 2)alloside
- 843. 3-Galactoside-3'-rhamnoside
- 844. 3-Rhamnosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6)galactoside (3-rhamninoside, xanthorhamnin A and B)
- 845. 3- α -L-Arabinopyranosyl(1 \rightarrow 3)[galactosyl(1 \rightarrow 6)galactoside]*
- 846. 3-Arabinoside-3',4'-diglucoside
- 847. 3,3',4'-Triglucoside
- 848. 3-Galactoside-3',4'-diglucoside
- 849. 3-Rhamnosyl(1 \rightarrow 3)(4''-acetylramnosyl)(1 \rightarrow 6)galactoside
- 850. 3-[3-Hydroxy-3-methylglutaryl(1 \rightarrow 6)][apiosyl(1 \rightarrow 2)galactoside]*
- 851. 3-(3''''-*p*-Coumaroylrhamninoside*)
- 852. 3-Sulfate
- 853. 3,3'-Disulfate*
- 854. 3,3',4'-Trisulfate*
- 855. 3,5,4'-Trisulfate-3'-glucuronide

Quercetin 3'-methyl ether (isorhamnetin)

- 856. 3- α -L-Arabinofuranoside
- 857. 3- α -L-Arabinopyranoside (distinchin)
- 858. 3-Xyloside
- 859. 3-Rhamnoside*
- 860. 3-Glucoside

861. 3-Galactoside
862. 3-Glucuronide
863. 5-Glucoside
864. 5-Galactoside*
865. 7-Rhamnoside
866. 7-Glucoside
867. 7- α -D-Glucosaminopyranoside*
868. 3-Arabinosyl(1 \rightarrow 2)rhamnoside
869. 3-Arabinosyl(1 \rightarrow 6)glucoside
870. 3- α -Arabinopyranosyl(1 \rightarrow 6)galactoside*
871. 3-Xylosyl(1 \rightarrow 2)glucoside*
872. 3-Xylosyl(1 \rightarrow 6)glucoside*
873. 3-Xylosyl(1 \rightarrow 2)galactoside*
874. 3-Apiosyl(1 \rightarrow 2)glucoside*
875. 3-Apiosyl(1 \rightarrow 2)galactoside*
876. 3-Rhamnosyl(1 \rightarrow 2)rhamnoside
877. 3-Rutinoside (narcissin)
878. 3-Neohesperidoside
879. 3-Rhamnosyl(1 \rightarrow 2)galactoside
880. 3-Robinobioside
881. 3-Laminaribioside*
882. 3-Sophoroside
883. 3-Gentiobioside
884. 3-Lactoside
885. 3-Glucosyl(1 \rightarrow 2)galactoside
886. 3-Glucosyl(1 \rightarrow 3)galactoside*
887. 4'-Rhamnosyl(1 \rightarrow 2)glucoside (crosatoside A)*
888. 3-Arabinoside-7-rhamnoside
889. 3-Arabinoside-7-glucoside
890. 3-Glucoside-7-arabinoside
891. 3-Glucoside-7-xyloside
892. 3,7-Dirhamnoside
893. 3-Rhamnoside-7-glucoside
894. 3-Glucoside-7-rhamnoside
895. 3-Galactoside-7-rhamnoside*
896. 3,7-Diglucoside
897. 3-Galactoside-7-glucoside
898. 3-Glucoside-4'-rhamnoside
899. 3,4'-Diglucoside (dactylin)
900. 3-Galactoside-4'-glucoside
901. 7-Sophoroside
902. 3-Xylosyl(1 \rightarrow 3)rhamnosyl(1 \rightarrow 6)glucoside*
903. 3-Xylosylrutinoside
904. 3-Xylosylrobinobioside*
905. 3-Rhamnosyl(1 \rightarrow 4)rhamnosyl(1 \rightarrow 6)glucoside
906. 3-Apiosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)glucoside]*
907. 3-Rutinosylglucoside
908. 3-(2^G-Rhamnosylrutinoside) (typhaneoside)
909. 3-Rhamnosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)galactoside]
910. 3-Rhamnosyl(1 \rightarrow 2)[glucosyl(1 \rightarrow 6)glucoside]*

911. 3-Glucosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]
912. 3-Galactosyl(1 → 2)[rhamnosyl(1 → 6)glucoside]*
913. 3-(4^{Rha}-Galactosylrobinobioside)*
914. 3-Xylosyl(1 → 2)glucoside-7-rhamnoside*
915. 3-Rutinoside-7-rhamnoside
916. 3-Rhamnosyl(1 → 2)galactoside-7-glucoside
917. 3-Robinobioside-7-rhamnoside
918. 3-Rutinoside-7-glucoside
919. 3-Sophoroside-7-rhamnoside
920. 3-Rhamnoside-7-sophoroside
921. 3-Sophoroside-7-glucoside
922. 3-Glucoside-7-gentiobioside
923. 3-Gentiobioside-7-glucoside
924. 3-Glucosyl(1 → 6)galactoside-7-glucoside*
925. 3-Rutinoside-4'-rhamnoside
926. 3-Rutinoside-4'-glucoside
927. 3-Gentiotrioside-7-glucoside
928. 3-Rhamnosyl(1 → 2)[gentiobiosyl(1 → 6)glucoside]*
929. 3-Rhamnosyl(1 → 2)[glucosyl(1 → 6)glucoside]-7-glucoside*
930. 3-(2^G-Rhamnosylrutinoside)-7-rhamnoside*
931. 3-Rhamnosyl(1 → 2)[rhamnosyl(1 → 6)galactoside]-7-rhamnoside
932. 3-(6''-Malonylglucoside)
933. 3-(6''-Galloylglucoside)
934. 3-(6''-*p*-Coumaroylglucoside)
935. 3-(2''-Acetylglucoside)
936. 3-(6''-Acetylglucoside)
937. 3-(6''-Acetylgalactoside)
938. 3-[6''-(2-*E*-Butenoyl)glucoside]*
939. 3-(2'',3'',4''-Triacetylglucoside)*
940. 7-(6''-*p*-Coumaroylglucoside)*
941. 3-(3-Methylbutyrylrutinoside)
942. 3-(3'',6''-Di-*p*-Coumaroylglucoside)
943. 3-(6''-*p*-Coumaroylglucosyl)(1 → 2)rhamnoside*
944. 3-(4'''-*p*-Coumaroylrobinobioside)
945. 3-(3'''-Feruloylrhamnosyl)(1 → 6)galactoside*
946. 3-(6''-*E*-Sinapoylsophoroside)*
947. 3-(2'''-Acetyl- α -arabinopyranosyl)(1 → 6)galactoside*
948. 3-Rhamnosyl(1 → 6)(2''-acetylglucoside)*
949. 3-(6''-Acetylglucosyl)(1 → 3)galactoside*
950. 3-(4'',6''-Diacetylglucosyl)(1 → 3)galactoside*
951. 3-(6''-*E-p*-Coumaroylglucoside)-7-glucoside*
952. 3-Feruloyl-7-rhamnosylglucoside
953. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(4''-*Z-p*-coumaroylgalactoside)]*
954. 3-[2''-(4'''-Acetyl-rhamnosyl)gentiobioside]*
955. 3-(*p*-Coumaroylrhamnosylgalactoside)-7-rhamnoside
956. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(3'''-*E-p*-coumaroylgalactoside)]-7-rhamnoside*
957. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(4''-*p*-coumaroylgalactoside)]-7-rhamnoside*
958. 3-Rhamnosyl(1 → 6)[rhamnosyl(1 → 2)(4''-*E*-feruloylgalactoside)]-7-rhamnoside*
959. 3-Sulfatorutinoside

- 960. 3-Glucuronide-7-sulfate
- 961. 3-Sulfate
- 962. 7-Sulfate
- 963. 3,7-Disulfate
- 964. 3,4'-Disulfate
- 965. 3,7,4'-Trisulfate
- 966. 3-(4''-Sulfatorutinoside)*
- Quercetin 4'-methyl ether (tamarixetin)
 - 967. 3-Rhamnoside
 - 968. 3-Glucoside
 - 969. 3-Galactoside*
 - 970. 3-Neohesperidoside*
 - 971. 3-Robinobioside
 - 972. 3-Glucosyl(1 → 2)galactoside*
 - 973. 3-Digalactoside
 - 974. 3,7-Diglucoside*
 - 975. 5-Glucoside-7-glucuronide
 - 976. 3-Rutinoside-7-rhamnoside*
 - 977. 3-Sulfate
 - 978. 3-Glucoside-7-sulfate*
- Quercetin 3,5-dimethyl ether (caryatin)
 - 979. 3'-(or 4'-)Glucoside
 - 980. 7-Glucoside*
- Quercetin 3,7-dimethyl ether
 - 981. 5-Glucoside
 - 982. 3'-Neohesperidoside*
 - 983. 3'-(6^G-Rhamnosylneohesperidoside)*
 - 984. 4'-Sulfate*
- Quercetin 3,3'-dimethyl ether
 - 985. 7-Glucoside
 - 986. 4'-Glucoside
 - 987. 7-Rutinoside*
- Quercetin 3,4'-dimethyl ether
 - 988. 7-Glucoside*
 - 989. 7- α -L-Arabinofuranosyl(1 → 6)glucoside*
 - 990. 7-Rutinoside*
 - 991. 7-(2^G-Rhamnosylrutinoside)*
 - 992. 7-(2^G-Glucosylrutinoside)*
- Quercetin 5,3'-dimethyl ether
 - 993. 3-Glucoside
- Quercetin 7,3'-dimethyl ether (rhamnazin)
 - 994. 3-Glucoside
 - 995. 3-Galactoside
 - 996. 3-Rhamnoside
 - 997. 4'-Glucoside
 - 998. 3-Glucosyl(1 → 5)- α -L-arabinofuranoside*
 - 999. 3-Xylosyl(1 → 2)glucoside*
- 1000. 3-Rutinoside
- 1001. 3-Neohesperidoside
- 1002. 3-Galactoside-4'-glucoside

1003. 3-Rhamnosyl(1 → 3)rhamnosyl(1 → 6)galactoside (xanthorhamnin C)
1004. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)galactoside (3-isorhamninolide)
1005. 3-Glucosyl(1 → 5)[apiosyl(1 → 2)- α -L-arabinofuranoside]*
1006. 3-Sulfate
Quercetin 7,4'-dimethyl ether (ombuin)
1007. 3-Arabinofuranoside*
1008. 3-Glucoside*
1009. 3-Galactoside
1010. 3-Rutinoside (ombuoside)
1011. 3,5-Diglucoside
1012. 3-Rutinoside-5-glucoside
1013. 3-Sulfate
Quercetin 3',4'-dimethyl ether
1014. 3-Rutinoside
1015. 3-Neohesperidoside*
1016. 3,7-Diglucoside*
1017. 5-Glucoside-7-glucuronide
Quercetin 3,7,4'-trimethyl ether
1018. 3-Sulfate*
Quercetin 5,3',4'-trimethyl ether
1019. 3-Galactosyl(1 → 2)rhamnoside-7-rhamnoside*
Quercetin 7,3',4'-trimethyl ether
1020. 3-Arabinoside
1021. 3-Digalactoside
Quercetin 5,7,3',4'-tetramethyl ether
1022. 3-Arabinoside
1023. 3-Galactoside*
1024. 3-Rutinoside
3,5,7-Trihydroxy-3',5'-dimethoxyflavone
1025. 7-Glucoside (lagotiside)
3,5,8,5'-Tetrahydroxy-7-methoxyflavone
1026. 8-Acetate*
6-Hydroxyquercetin (quercetagenin)
1027. 3-Rhamnoside
1028. 3-Glucoside (tagetiin)
1029. 6-Glucoside*
1030. 7-Glucoside
1031. 3,7-Diglucoside
1032. 7-(6''-Isobutyrylglucoside)*
1033. 7-(6''-Isovalerylglucoside)*
1034. 7-[6''-(2-Methylbutyryl)glucoside]*
1035. 7-(6''-E-Caffeoylglucoside)*
1036. 7-(6''-Acetylglucoside)*
Quercetagenin 3-methyl ether
1037. 7-Glucoside
1038. 7-Sulfate
Quercetagenin 6-methyl ether (patuletin)
1039. 3-Xyloside
1040. 3-Rhamnoside
1041. 3-Glucoside

1042. 3-Galactoside
1043. 3-Glucuronide
1044. 5-Glucoside
1045. 7-Glucoside
1046. 3-Rutinoside
1047. 3-Robinoside
1048. 3-Galactosylrhamnoside
1049. 3-Gentiobioside
1050. 3-Digalactoside
1051. 3,7-Dirhamnoside
1052. 3,7-Diglucoside*
1053. 3-Digalactosylrhamnoside
1054. 3-Glucosyl(1 → 6)[apiosyl(1 → 2)glucoside]
1055. 3-(6''-*p*-Coumaroylglucoside)*
1056. 3-(6''-*E*-Feruloylglucoside)*
1057. 3-(6''-Acetylglucoside)
1058. 7-(6''-Isobutyrylglucoside)*
1059. 7-[6''-(2-Methylbutyryl)glucoside]*
1060. 7-(6''-Isovalerylglucoside)*
1061. 3-Rhamnoside-7-(2''-acetylramnoside)*
1062. 3-Rhamnoside-7-(3'''-acetylramnoside)
1063. 3-Rhamnoside-7-(4'''-acetylramnoside)
1064. 3-(4''-Acetylramnoside)-7-rhamnoside*
1065. 3-Rhamnoside-7-(3''',4'''-diacetylramnoside)
1066. 3-(3''-Acetylramnoside)-7-(3'''-acetylramnoside)
1067. 3-(4''-Acetylramnoside)-7-(2'''-acetylramnoside)*
1068. 3-(4''-Acetylramnoside)-7-(3'''-acetylramnoside)
1069. 3-(4''-Acetylramnoside)-7-(2''',4'''-diacetylramnoside)
1070. 3-(4''-Acetylramnoside)-7-(2''',4'''-diacetylramnoside)
1071. 3-(2''-Feruloylglucosyl)(1 → 6)[apiosyl(1 → 2)glucoside]*
1072. 3-Sulfate
1073. 7-Sulfate
1074. 3,3'-Disulfate
1075. 3-Glucoside-7-sulfate
- Quercetagenin 7-methyl ether
1076. 3-Glucoside
1077. 6-Glucoside*
1078. 4'-Glucoside*
1079. 3-Neohesperidoside*
1080. 3-Cellobioside*
1081. 3-(2'''-Caffeoylglucosyl)(1 → 2)glucuronide*
- Quercetagenin 3'-methyl ether
1082. 3-Glucoside
1083. 3-Galactoside
1084. 7-Glucoside
- Quercetagenin 4'-methyl ether
1085. 3-Arabinoside
- Quercetagenin 3,6-dimethyl ether (axillarin)
1086. 7-Glucoside (axillaroside)
1087. 4'-Glucuronide

1088. 5- α -L-Arabinosyl(1 \rightarrow 6)glucoside*
1089. 7-Sulfate*
Quercetagenin 3,7-dimethyl ether
1090. 6-Glucoside
1091. 6-Galactoside
1092. 4'-Glucoside
Quercetagenin 6,7-dimethyl ether
1093. 3-Rhamnoside (eupatolin)
1094. 3-Apioside
1095. 3-Glucoside
1096. 3-Galactoside
1097. 3-Glucosylgalactoside
1098. 3-Sulfate
Quercetagenin 3,3'-dimethyl ether
1099. 7-Glucoside
Quercetagenin 6,3'-dimethyl ether (spinacetin)
1100. 3-Glucoside
1101. 7-Glucoside
1102. 3-Rutinoside
1103. 3-Gentiobioside
1104. 3-(2''-Apiosylgentiobioside)*
1105. 3-(2'''-Feruloylgentiobioside)*
1106. 3-(2''-*p*-Coumaroylglucosyl)(1 \rightarrow 6)[apiosyl(1 \rightarrow 2)glucoside]*
1107. 3-(2''-Feruloylglucosyl)(1 \rightarrow 6)[apiosyl(1 \rightarrow 2)glucoside]*
1108. 3-Sulfate
Quercetagenin 7,3'-dimethyl ether
1109. 6-Glucoside*
Quercetagenin 3,6,7-trimethyl ether
1110. 4'-Glucoside
Quercetagenin 3,6,3'-trimethyl ether (jaceidin)
1111. 5-Glucoside*
1112. 7-Glucoside (jacein)
1113. 7-Neohesperidoside
1114. 4'-Sulfate
Quercetagenin 3,6,4'-trimethyl ether
1115. 7-Glucoside (centaurein)
Quercetagenin 3,7,4'-trimethyl ether
1116. 6-Glucoside
1117. 3'-Glucoside
Quercetagenin 6,7,3'-trimethyl ether (veronicafolin)
1118. 3-Rutinoside
1119. 3-Glucosyl(1 \rightarrow 3)galactoside*
1120. 3-Digalactoside
1121. 3-Sulfate
Quercetagenin 6,7,4'-trimethyl ether (eupatin)
1122. 3-Sulfate
Quercetagenin 6,3',4'-trimethyl ether
1123. 3-Glucoside*
1124. 3-Sulfate
Quercetagenin 7,3',4'-trimethyl ether

1125. 3-Rhamnoside
Quercetagenin 3,6,7,3'-tetramethyl ether
1126. 4'-Glucoside (chryso splenin)
1127. 4'-Galactoside (galactobuxin)
Quercetagenin 3,6,7,3',4'-pentamethyl ether (artemetin)
1128. 5-Glucosylrhamnoside
8-Hydroxyquercetin (gossypetin)
1129. 3-Glucoside
1130. 3-Galactoside
1131. 3-Glucuronide
1132. 7-Rhamnoside
1133. 7-Glucoside
1134. 8- α -D-Lyxopyranoside*
1135. 8-Rhamnoside
1136. 8-Glucoside
1137. 8-Glucuronide
1138. 3-Glucoside-8-glucuronide
1139. 3-Glucuronide-8-glucoside
1140. 7-Rhamnoside-8-glucoside
1141. 3-Gentiotrioside
1142. 3-Sophoroside-8-glucoside
1143. 8-Glucuronide-3-sulfate
1144. 3-Sulfate
Gossypetin 7-methyl ether
1145. 3-Arabinoside
1146. 3-Rhamnoside
1147. 3-Galactoside
1148. 8-Glucoside
1149. 3-Rutinoside
1150. 3-Galactoside-8-glucoside
Gossypetin 8-methyl ether (corniculatusin)
1151. 3- α -L-Arabinofuranoside
1152. 3-Glucoside
1153. 3-Galactoside
1154. 7-Glucoside
1155. 3-Xylosyl(1 \rightarrow 2)rhamnoside*
1156. 3-Robinoside
Gossypetin 3'-methyl ether
1157. 7-Glucoside
1158. 3-Rutinoside
1159. 7-Neohesperidoside (haploside F)
1160. 7-(6''-Acetylglucoside)
1161. 7-(6''-Acetylrhamnosyl)(1 \rightarrow 2)glucoside
Gossypetin 3,8-dimethyl ether
1162. 5-Glucoside*
Gossypetin 7,8-dimethyl ether
1163. 3-Glucoside*
1164. 4'-Glucoside*
1165. 3,3'-Disulfate*
Gossypetin 7,4'-dimethyl ether

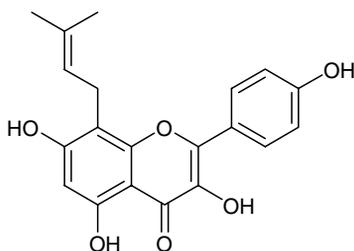
- 1166. 8-Glucoside
- 1167. 8-Acetate
- 1168. 8-Butyrate
- Gossypetin 8,3'-dimethyl ether (limocitrin)
- 1169. 3-Rhamnoside
- 1170. 3-Glucoside
- 1171. 3-Galactoside
- 1172. 3-Rutinoside
- 1173. 7-Glucoside
- 1174. 7-Neohesperidoside
- 1175. 3-Sophoroside
- 1176. 3,7-Diglucoside
- 1177. 3-Rutinoside-7-glucoside*
- 1178. 7-(6''-Acetylglucoside)
- 1179. 7-(6''-Acetylneohesperidoside)
- 3,5,7,3',4',5'-Hexahydroxyflavone (myricetin)
- 1180. 3-Arabinoside
- 1181. 3- α -Arabinofuranoside
- 1182. 3-Xyloside
- 1183. 3-Rhamnoside (myricitrin)
- 1184. 3-Glucoside
- 1185. 3-Galactoside
- 1186. 7-Arabinoside
- 1187. 7-Glucoside
- 1188. 3'-Arabinoside
- 1189. 3'-Xyloside
- 1190. 3'-Rhamnoside*
- 1191. 3'-Glucoside
- 1192. 3-Dixyloside
- 1193. 3-Dirhamnoside
- 1194. 3-Xylosyl(1 \rightarrow 2)rhamnoside
- 1195. 3-Xylosyl(1 \rightarrow 3)rhamnoside*
- 1196. 3-Xylosylglucoside
- 1197. 3-Rhamnosyl(1 \rightarrow 2)rhamnoside*
- 1198. 3-Rutinoside
- 1199. 3-Neohesperidoside*
- 1200. 3-Robinobioside*
- 1201. 3-Diglucoside
- 1202. 3-Galactosylglucoside
- 1203. 3-Digalactoside
- 1204. 3-Rhamnoside-7-glucoside
- 1205. 3-Rhamnoside-3'-glucoside*
- 1206. 3-Galactoside-3'-rhamnoside*
- 1207. 3,3'-Digalactoside
- 1208. 3,4'-Dirhamnoside*
- 1209. 3,4'-Diglucoside*
- 1210. 3-Rhamnosyl(1 \rightarrow 3)glucosyl(1 \rightarrow 6)glucoside*
- 1211. 3-(2^G-Rhamnosylrutinoside)*
- 1212. 3-Glucosylrutinoside
- 1213. 3-Triglucoside

1214. 3-Rutinoside-7-rhamnoside
1215. 3-Robinoside-7-rhamnoside
1216. 3-Rutinoside-7-glucoside
1217. 3-Glucosyl(1 → 2)rhamnoside-7-glucoside*
1218. 3-(2''-*p*-Hydroxybenzoylrhamnoside)*
1219. 3-(4''-Malonylrhamnoside)*
1220. 3-(2''-Galloylrhamnoside)
1221. 3-(3''-Galloylrhamnoside)*
1222. 3-(4''-Galloylrhamnoside)
1223. 3-(2''-Galloylglucoside)*
1224. 3-(6''-Galloylglucoside)
1225. 3-(3''-Galloylgalactoside)*
1226. 3-(6''-Galloylgalactoside)
1227. 3-(6''-*p*-Coumarylglucoside)*
1228. Nympholide A* (13.7)
1229. Nympholide B* (13.8)
1230. 3-(2''-Acetylrhamnoside)*
1231. 3-(4''-Acetylrhamnoside)*
1232. 7-(6''-Galloylglucoside)*
1233. 3-(2'',3''-Digalloylrhamnoside)*
1234. 3-(3'',4''-Diacetylrhamnoside)*
1235. 3-(2'',3'',4''-Triacetylxyloside)*
1236. 3-(4''-Acetyl-2''-galloylrhamnoside)*
1237. 3-[3''',6'''-Diacetylglucosyl(1 → 4)2'',3''-diacetylrhamnoside]*
1238. 3-(*p*-Coumarylrhamnosylgalactoside)
1239. 3-Glucosyl(1 → 2)(6'''-caffeylglucosyl)(1 → 2)rhamnoside-4'-rhamnosyl(1 → 4)xyloside (montbretin A)
1240. 3-Glucosyl(1 → 2)(6'''-*p*-coumaroylglucosyl)(1 → 2)rhamnoside-4'-rhamnosyl(1 → 4)xyloside (montbretin B)
1241. 3-Sulfatorhamnoside
- Myricetin 3-methyl ether
1242. 7-Rhamnoside
1243. 3'-Xyloside
1244. 3'-Glucoside
1245. 7-Rhamnoside-3'-xyloside
- Myricetin 5-methyl ether
1246. 3-Rhamnoside
1247. 3-Galactoside
- Myricetin 7-methyl ether (europetin)
1248. 3-Rhamnoside
1249. 3-(2''-Galloylrhamnoside)*
1250. 3-(3''-Galloylrhamnoside)*
- Myricetin 3'-methyl ether (larycitrin)
1251. 3- α -L-Arabinofuranoside*
1252. 3-Rhamnoside
1253. 3-Glucoside
1254. 3-Galactoside
1255. 7-Glucoside
1256. 5'-Glucoside
1257. 3-Rutinoside

1258. 3,5'-Diglucoside
1259. 3-Rhamnosylrutinoside
1260. 3-Rutinoside-7-glucoside
1261. 3,7,5'-Triglucoside
1262. 3-(4''-Malonylrhamnoside)*
1263. 3-*p*-Coumarylglucoside
Myricetin 4'-methyl ether
1264. 3-Rhamnoside (mearnsitrin)
1265. 3-Galactoside*
1266. 3-Galactosyl(1 → 4)galactoside
1267. 3,7-Dirhamnoside
1268. 3-(4''-Acetylrhamnoside)*
Myricetin 3,4'-dimethyl ether
1269. 3'-Xyloside
1270. 7-Rhamnoside-3'-xyloside
Myricetin 7,4'-dimethyl ether
1271. 3-Galactoside
Myricetin 3',4'-dimethyl ether
1272. 3-Rhamnoside*
1273. 3-Glucoside*
Myricetin 3',5'-dimethyl ether (syringetin)
1274. 3-Arabinoside
1275. 3-Rhamnoside
1276. 3-Xyloside
1277. 3-Glucoside
1278. 3-Galactoside
1279. 3-Rhamnosyl(1 → 5)- α -L-arabinofuranoside*
1280. 3-Rutinoside
1281. 3-Robinoside*
1282. 3-Rhamnosylrutinoside
1283. 3-Rutinoside-7-glucoside
1284. 3-(*p*-Coumarylglucoside)
1285. 3-(2'',3''-Diacetylglucoside)*
1286. 3-(6''-Acetylglucosyl)(1 → 3)galactoside*
6-Hydroxymyricetin 6,3',5'-trimethyl ether
1287. 3-Glucoside
6-Hydroxymyricetin 3,6,3',5'-tetramethyl ether
1288. 7-Glucoside
8-Hydroxymyricetin (hibiscetin)
1289. 3-Glucoside
1290. 8-Glucosylxyloside
8-Hydroxymyricetin 8-methyl ether
1291. 3-Rhamnoside*
8-Hydroxymyricetin 8,5-dimethyl ether
1292. 3-Rhamnoside*
8-Hydroxymyricetin 8,3',5'-trimethyl ether
1293. 3-Rhamnoside*
3,5,7,2'-Tetrahydroxyflavone (datiscetin)
1294. 3-Glucoside
1295. 3-Rutinoside

- 3,7,2',3',4'-Pentahydroxyflavone
1296. 3-Neohesperidoside
3,7,3',4',5'-Pentahydroxyflavone (5-deoxymyricetin, robinetin)
1297. 7-Glucoside*
1298. 3-Rutinoside*
3,4'-Dihydroxy-7,3',5'-trimethoxyflavone
1299. 3-Galactosyl(1 → 4)xyloside*
3,5,7,2',6'-Pentahydroxyflavone
1300. 2'-Glucoside*
5,7-Dihydroxy-3,6,8,4'-tetramethoxyflavone
1301. 7-Glucosyl(1 → 3)galactoside*
7,4'-Dihydroxy-3,5,6,8-tetramethoxyflavone
1302. 4'-Glucosyl(1 → 3)galactoside*
5,8-Dihydroxy-3,6,7,4'-tetramethoxyflavone
1303. 8-Neohesperidoside*
5,4'-Dihydroxy-6,7,8,3'-tetramethoxyflavone (africanutin)
1304. 4'-Galactoside*
3,5,7,2',3',4'-Hexahydroxyflavone
1305. 3-Glucoside*
5,7,2'-Trihydroxy-3,6,4'-trimethoxyflavone
1306. 7-Glucoside*
5,2',5'-Trihydroxy-3,7,8-trimethoxyflavone
1307. 2'-Acetate
5,2'-Dihydroxy-3,7,4'-trimethoxyflavone
1308. 2'-Glucoside
5,2',4'-Trihydroxy-3,7,5'-trimethoxyflavone
1309. 2'-Galactosyl(1 → 4)glucoside*
5,2'-5'-Trihydroxy-3,7,4'-trimethoxyflavone
1310. 2'-Glucoside
5,6',5'-Trihydroxy-3,7,4'-trimethoxyflavone
1311. 5'-Glucoside
5,2'-Dihydroxy-3,7,4',5'-tetramethoxyflavone
1312. 2'-Glucoside
5,5'-Dihydroxy-3,6,7,4'-tetramethoxyflavone
1313. 5'-Glucoside
5,7,8-Trihydroxy-3,6,4'-trimethoxyflavone
1314. 8-Tiglate*
5,8,4'-Trihydroxyflavone-3,7,3'-trimethoxyflavone
1315. 8-Acetate
3,5,2'-Trihydroxy-7,8,4'-trimethoxyflavone
1316. 5-Glucosyl(1 → 2)galactoside*
3,5,6,7,8,4'-Hexahydroxy-3'-methoxyflavone
1317. 3-Rhamnosyl(1 → 4)rhamnosyl(1 → 6)glucoside*
3,5,7,3',4'-Pentahydroxy-6,8-dimethoxyflavone
1318. 3-Arabinoside
3,5,7,4'-Tetrahydroxy-6,8,3'-trimethoxyflavone
1319. 3- α -L-Arabinopyranosyl(1 → 3)galactoside*
1320. 3-Rhamnosyl(1 → 2)glucoside
1321. 3- α -L-Arabinopyranosyl(1 → 3)[galactosyl(1 → 6)galactoside]*
3,6,7,8,3',4'-Hexahydroxy-5'-methoxyflavone

1322. 7-Neohesperidoside*
 5,7,2',3',4'-Pentahydroxy-3,6-dimethoxyflavone
 1323. 7-Glucoside*
 5,2',5'-Trihydroxy-3,6,7,4'-tetramethoxyflavone
 1324. 5'-Glucoside
 5,2'-Dihydroxy-3,6,7,4',5'-pentamethoxyflavone (brickellin)
 1325. 2'-Glucoside*
 5,5'-Dihydroxy-3,6,7,2',4'-pentamethoxyflavone
 1326. 5'-Glucoside
 5,8-Dihydroxy-3,7,2',3',4'-pentamethoxyflavone
 1327. 8-Acetate
 5,7,3',5'-Tetrahydroxy-3,6,8,4'-tetramethoxyflavone
 1328. 3'-Glucoside
 C-Methylated flavonol glycosides
 5,7-Dihydroxy-6,8-di-C-methyl-3-methoxyflavone
 1329. 7-Galactosyl(1 → 2)rhamnoside*
 3,5,7,4'-Tetrahydroxy-8-C-methyl flavone (8-C-Methylkaempferol)
 1330. 7-Glucoside
 3,5,7,3',4',5'-Hexahydroxy-2'-C-methyl flavone (2'-C-methylmyricetin)
 1331. 3-Rhamnoside-5'-gallate*
 Prenylated, Pyrano and Methylenedioxy Flavonol Glycosides



13.11

8-Prenylkaempferol (noranhydroicaritin, 3,5,7,4'-tetrahydroxy-8-(3,3'-dimethylallyl)flavone)

1. 3-Rhamnoside (ikaroside A)
 2. 3-Rhamnosyl(1 → 2)rhamnoside
 3. 3-Xylosyl(1 → 2)rhamnoside (ikaroside D)
 4. 3-Glucosyl(1 → 2)rhamnoside (ikaroside B)
 5. 3-Rhamnoside-7-glucoside (epimedeside A)
 6. 3,7-Diglucoside*
 7. 3-Rhamnosyl(1 → 2)xyloside-7-glucoside (epimedeside E)
 8. 3-Glucosyl(1 → 2)rhamnoside-7-glucoside (Ikaroside C, diphyllaside A)
 9. 3-Rhamnosyl(1 → 2)rhamnoside-7-glucoside (diphyllaside B)
 10. 3-Rhamnosyl(1 → 2)glucoside-7-glucoside
 11. 3-Glucosyl(1 → 2)rhamnoside-7-glucosyl(1 → 2)glucoside (diphyllaside C)
 12. 3-Xylosyl(1 → 2)rhamnoside-7-glucosyl(1 → 2)glucoside (hexandroside C)
 13. 3-(4''-Acetyl)rhamnoside (ikaroside F)
- 8-Prenylkaempferol 7-methyl ether
14. 3-Rhamnosyl(1 → 3)[apiosyl(1 → 6)glucoside]*

- 8-Prenylkaempferol 4'-methyl ether (anhydroicaritin)
15. 3-Rhamnoside
 16. 3-Glucoside
 17. 7-Glucoside (icaraside I)
 18. 3-Rhamnosyl(1 → 2)rhamnoside*
 19. 3-Xylosyl(1 → 2)rhamnoside (sagittatoside B)
 20. 3-Rutinoside
 21. 3-Glucosyl(1 → 2)rhamnoside (sagittatoside A)
 22. 3-Rhamnoside-7-glucoside (icariin)
 23. 7-Cellobioside (cuhuoside)*
 24. 3-Xylosyl(1 → 2)rhamnoside-7-glucoside (epimedin B)
 25. 3-Xylosyl(1 → 2)rhamnoside-7-glucoside
 26. 3-Rhamnosyl(1 → 2)rhamnoside-7-glucoside (epimedin C)
 27. 3-Rhamnosyl(1 → 3)rhamnoside-7-glucoside (hexandroside D)
 28. 3-Glucosyl(1 → 2)rhamnoside-7-glucoside (epimedin A)
 29. 3-Glucosyl(1 → 3)rhamnoside-7-glucoside*
 30. 3-Galactosyl(1 → 3)rhamnoside-7-glucoside
 31. 3-Rhamnosyl(1 → 6)galactoside-7-galactoside*
 32. 3-Rhamnosyl(1 → 2)rhamnoside-7-sophoroside* (acuminatoside)
 33. 3-[3'''-Acetylxylosyl(1 → 3)4''-acetylramnoside] (sempervirenoside)
 34. 3-Glucosyl(1 → 2)(3''-acetylramnoside) (sagittatoside C)
 35. 3-Glucosyl(1 → 3)(4''-acetylramnoside) (epimedokoreanoside II)
 36. 3-[4'''',6'''-Diacetylglucosyl(1 → 3)4''-acetylramnoside]*
 37. 3-[2'''',6'''-Diacetylglucosyl(1 → 3)4''-acetylramnoside]-7-glucoside (epimedin K)
 38. 3-Xylosyl(1 → 3)(4''-acetylramnoside)-7-glucoside
 39. 3-[6'''-Acetylglucosyl(1 → 3)4''-acetylramnoside]-7-glucoside (epimedokoreanoside I)
 40. 3-[4'''',6'''-Diacetylglucosyl(1 → 3)4''-acetylramnoside]-7-glucoside*
 41. 3-(6'''-Acetylgalactosyl)(1 → 3)rhamnoside-7-glucoside
 42. 3-[3'''-Acetylxylosyl(1 → 3)4''-acetylramnoside]-7-glucoside
- 8-(3''-Hydroxy-3''-methylbutyl)kaempferol 4'-methyl ether (icaritin)
43. 3-Rhamnoside
 44. 3-Rhamnosyl(1 → 2)rhamnoside (wanepimedeside A)*
- 8-(γ -Methoxy- $\gamma\gamma$ -dimethyl)propylkaempferol 4'-methyl ether
45. 7-glucoside* (caohuoside D)
- 8-Prenylquercetin 4'-methyl ether
46. 3-Rhamnoside* (caohuoside C)
- 8-Prenylquercetin 7,4'-dimethyl ether
47. 3-Rhamnosyl(1 → 4)rhamnoside*
- 6'',6'''-Dimethylpyrano(2'',3''':7,8)kaempferol
48. 3-Rhamnoside
- 6'',6'''-Dimethylpyrano(2'',3''':7,8)-4'-methoxykaempferol
49. 3-Rhamnoside*
- 3-Methoxy-5-hydroxy-6,7-methylenedioxyflavone
50. 4'-Glucuronide*

3,5,4'-Trihydroxy-6,7-methylenedioxyflavone

51. 3-Glucoside

3-Hydroxy-5,4'-dimethoxy-6,7-methylenedioxyflavone

52. 3-Xyloside (viviparum A)*

3,3'-Dihydroxy-5,4'-dimethoxy-6,7-methylenedioxyflavone

53. 3-Xyloside (viviparum B)*

*Flavonol glycosides newly reported since 1992.

14 C-Glycosylflavonoids

Maurice Jay, Marie-Rose Viricel, and Jean-François Gonnet

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14.1 NATURAL SOURCES AND SOME TAXONOMIC IMPLICATIONS

The natural sources of C-glycosylflavonoids reported for the last 10 years are listed in Table 14.1. The number of species possessing C-glycosylflavonoids is given in parentheses for each genus, except in the case where a unique species was under investigation.

14.1.1 PLANT SPECIES RICH IN C-GLYCOSYLFLAVONOIDS

In Bryophyta, the presence of C-glycosylflavones was reported for the first time in the genera *Plagiochila* and *Plagiochasma*. It is the same situation in Gymnospermae for the genera *Cycas* and *Abies*. In ferns, the confirmation of the presence of C-glycosylflavonoids was provided for three families: Aspleniaceae (*Asplenium* sp.), Athyriaceae, and Hymenophyllaceae (*Trichomanes* sp.). In monocots, several investigations have concerned two species, *Zea mays* and *Hordeum vulgare*, and two families, Restionaceae and Velloziaceae. In dicots, the first citations for C-glycosylflavones concern the families Araceae, Betulaceae, Brassicaceae, Capparaceae, Chenopodiaceae, Droseraceae, Geraniaceae, Illecebraceae, Mimosaceae, Oleaceae, Orchidaceae, Oxalidaceae, Pistaciaceae, Plumbaginaceae, Polygalaceae, Sapindaceae, Solanaceae, Sterculiaceae, Turneraceae, Urticaceae, and Violaceae; important additions to the occurrence of C-glycosylflavones were given for Cucurbitaceae, Rosaceae, and Theaceae.

14.1.2 C-GLYCOSYLFLAVONOIDS AND TAXONOMIC ASPECTS

14.1.2.1 Ferns

The Athyriaceae and Aspleniaceae are two large families of leptosporangiate ferns. However, until the second half of the 20th century, they were united in one family because of the superficial resemblance of their sori. This similarity having been analyzed as the result of convergence rather than of close phylogenetic relationship, the two subfamilies were raised to the family rank. The investigation of Umikalsom et al.³⁶² provided additional chemical data for characterizing and separating the two taxa. A large collection of 15 species of *Asplenium*, four species of *Athyrium*, 12 species of *Diplazium*, and two species of *Deparia* were compared for their flavonoid contents based on proanthocyanidins, flavonol-O-glycosides, flavone-O-glycosides, and flavone-C-glycosides. Nearly all species representatives of these four genera showed their own specific flavonoid pattern. A large range of flavone C- and O-glycosides was found in *Asplenium*: the flavone C-glycosides were based on both apigenin and luteolin,

TABLE 14.1
Natural Sources of C-Glycosylflavonoids
(Since 1991). The Number of Species Under
Investigation Given in Parentheses

BRYOPHYTA

*Frullania polysticha*¹⁸⁶
*Frullania cesatiana*¹⁸⁷
*Frullania tamatisci*³²⁹
*Plagiochila jamesonii*³¹⁸
*Plagioclasma rupestre*³¹⁸
Plagiomnium sp.^{16,399}

PTERIDOPHYTA

Aspleniaceae

*Asplenium viviparum*¹⁵⁶
Asplenium (3)³⁶³
Asplenium (4 hybrids)²⁴⁹
 Aspleniaceae (15)³⁶²

Athyriaceae

Athyriaceae (18)³⁶²

Hymenophyllaceae

Trichomanes (23)³⁷⁹

GYMNOSPERMAE

Ephedraceae

*Ephedra aphylla*¹⁵³

Cycadaceae

*Cycas panzihuaensis*⁴¹⁶

Pinaceae

Abies (7)³³¹

ANGIOSPERMAE MONOCOTYLEDONAE

Eriocaulaceae

Eriocaulaceae³¹¹
Syngonanthus (22)³⁰⁹

Gramineae

Bambusa (13)³⁵¹
*Deschampsia antarctica*³⁸³
Hordeum vulgare^{239,258,260,278,279}
*Hyparrhenia hirta*³⁷
*Sasa borealis*⁴⁰⁵
Triticum aestivum^{129,255}
Zea mays^{333-336,345,346}

Iridaceae

Iris (2)¹⁴

Restionaceae

Restionaceae (115)³⁸⁷

Velloziaceae

Velloziaceae (4 genus)³⁸⁶
Vellozia (10)^{126,127}

ANGIOSPERMAE DICOTYLEDONAE

Acanthaceae

*Climacanthus nutans*³⁵²

continued

TABLE 14.1
Natural Sources of C-Glycosylflavonoids
(Since 1991). The Number of Species Under
Investigation Given in Parentheses — *continued*

Amaranthaceae
<i>Alternanthera maritima</i> ³¹⁶
Araceae
<i>Arum palaestinum</i> ^{8,9}
<i>Arum dracunculus</i> ³⁰⁰
<i>Xanthosoma violaceum</i> ²⁹⁸
Betulaceae
<i>Betula platyphylla</i> ²⁰⁴
Bombacaceae
<i>Bombax ceiba</i> ^{99,100}
Brassicaceae
<i>Barbarea vulgaris</i> ³²⁴
Capparaceae
<i>Cleome</i> (4), <i>Capparis</i> (3) ³²⁶
Caryophyllaceae
<i>Silene conoidea</i> ¹⁵
<i>Stellaria media</i> ³⁰⁴
Chenopodiaceae
<i>Beta vulgaris</i> ¹⁰⁹
Combretaceae
<i>Combretum quadrangulare</i> ²⁴
<i>Terminalia catappa</i> ²¹³
Compositae
<i>Achillea nobilis</i> ^{192,234}
<i>Achillea setacea</i> ²³⁵
<i>Achillea</i> sp. ³⁶⁵
<i>Atractylis carduus</i> ²⁵²
<i>Centaurea</i> (2) ^{115–117}
<i>Felicia amelloides</i> ³⁴
<i>Otanthus maritimus</i> ⁹²
Crucifereae
<i>Boreava orientalis</i> ³¹²
Cucurbitaceae
<i>Bryonia</i> (2) ^{188,189}
<i>Citrullus colocynthis</i> ²²³
<i>Cucumis sativus</i> ^{3,13,191,228}
<i>Lagenaria siceraria</i> ^{188,190}
Droseraceae
<i>Drosophyllum lusitanicum</i> ⁴³
Euphorbiaceae
<i>Aleurites moluccana</i> ^{253,254,268}
<i>Glochidion zeylanica</i> ²⁸⁸
<i>Jatropha polhiana</i> ⁴⁰⁰
Gentianaceae
<i>Gentiana arisanensis</i> ^{198,211}
<i>Gentianella azurea</i> ⁴¹⁴

TABLE 14.1
Natural Sources of C-Glycosylflavonoids
(Since 1991). The Number of Species Under
Investigation Given in Parentheses — *continued*

*Tripterospermum japonicum*²⁸⁹

Geraniaceae

*Pelargonium reniforme*²⁰⁰

Pelargonium (58)³⁹²

Guttiferae

*Chusia sandiensis*⁷⁴

Illecebraceae

Scleranthus uncinatus^{403,404}

Labiatae

Faradaya (4)¹²⁰

Ocimum (9)^{121,122}

*Otostegia fruticosa*²

Oxera (20)¹²⁰

*Salvia officinalis*²¹⁸

*Schnabelia tetradonta*⁷⁶

*Scutellaria alba*³³⁰

*Scutellaria amoena*⁴¹⁷

Scutellaria baicalensis^{412,413}

*Scutellaria pontica*²⁸²

Leguminosae

*Abrus precatorius*²²²

Acacia saligna^{89,94}

*Acacia leucophloea*³⁶⁶

Bocoa (2)¹⁸⁰

*Cassia nomame*¹⁷⁸

*Cassia occidentalis*¹³⁰

*Crotalaria thebaica*¹⁵⁴

*Cyclopia intermedia*¹⁶⁸

*Desmodium tortuosum*²⁰⁷

*Glycyrrhiza glabra*²¹⁰

*Glycyrrhiza eurycarpa*²¹⁶

*Herminiera elaphroxylon*⁹³

*Lagonychium farcatum*⁹³

*Lupinus hartwegii*¹⁶⁹

*Lupinus luteus*⁴⁰⁶

*Mohgania macrophylla*³⁹⁸

*Phaseolus radiatus*¹⁶⁵

Phaseolineae³⁹³

*Prosopis chilensis*⁸⁸

Rhynchosia (2)⁴²¹

*Vigna radiata*¹⁹⁹

Mimosaceae

*Mimosa pudica*²¹⁷

Myrtaceae

*Eucalyptus globulus*²³²

continued

TABLE 14.1
Natural Sources of C-Glycosylflavonoids
(Since 1991). The Number of Species Under
Investigation Given in Parentheses — *continued*

Oleaceae*Ligustrum vulgare*³⁵³**Orchidaceae**Ornithocephalinae (15)³⁸⁵*Tylostylis discolor*²³¹**Oxalidaceae***Biophytum sensitivum*⁴²**Passifloraceae***Passiflora incarnata*^{56,208,305,373}*Passiflora* (3)²⁹⁵*Passiflora* (2)²⁹⁴*Passiflora* sp.¹**Pistaciaceae***Pistacia atlantica*²⁶⁹**Plumbaginaceae***Plumbago zeylanica*²¹²**Polygalaceae***Polygala telephioides*¹⁹⁵**Polygonaceae***Rumex* (8)³¹⁴*Polygonum perfoliatum*⁴¹⁸**Ranunculaceae***Trollius lebedouri*⁴²⁰**Rhamnaceae***Ziziphus jujuba*⁵⁴*Rhamnella inaequilatera*³⁵⁰**Rosaceae***Cotoneaster thymaefolia*²⁹¹*Cotoneaster wilsonii*⁴⁹*Crataegus monogyna*³⁰⁸*Crataegus pinnatifida*^{175,411}*Crataegus sinica*⁸⁷*Cydonia oblonga*¹⁰²*Eriobotrya japonica*¹⁵⁹**Rutaceae***Citrus* (review)²³³*Citrus* (2)¹²⁴*Citrus* sp.^{23,111,259–261,361}*Feronia elephantum*⁸⁵*Raputia paraensis*²²**Sapindaceae***Allophylus edulis*¹⁴³**Saxifragaceae***Itea/Pterostemon*³⁵**Scrophulariaceae***Gratiola officinalis*¹²³

TABLE 14.1
Natural Sources of C-Glycosylflavonoids
(Since 1991). The Number of Species Under
Investigation Given in Parentheses — *continued*

Solanaceae
<i>Capsicum annuum</i> ²⁴⁶
Sterculiaceae
<i>Theobroma cacao</i> ³¹⁷
Theaceae
<i>Thea</i> sp. ^{96,375}
<i>Thea</i> (90 beverages) ⁹⁷
Thymeleaceae
<i>Daphne laureola</i> ³⁵⁹
Turneraceae
<i>Turnera diffusa</i> ²⁹⁷
Urticaceae
<i>Cecropia lyratifolia</i> ²⁸⁵
Verbenaceae
<i>Verbena pinnatifida</i> ⁸⁵
<i>Vitex polygama</i> ²⁰⁵
Violaceae
<i>Viola arvensis</i> ⁴⁶
<i>Viola yedoensis</i> ⁴⁰¹
Vitidaceae
<i>Vitis</i> (22) ²⁶³
<i>Tetragymma hemsleyanum</i> ²¹⁵

both mono-*C*- and di-*C*-glycosylated; some of them were further *O*-glycosylated. In Athyriaceae (the other three genera), it was only apigenin-based *C*-glycosides that were found; *O*-glycosyl-*C*-glycosylflavones and *O*-glycosylflavones were absent. Accordingly, the Athyriaceae were clearly distinguished and considered as more primitive than the Aspleniaceae.

In the genus *Asplenium*, Matsumoto et al.²⁴⁹ studied the flavonoid composition of four natural hybrids in natural populations where the hybrids could be collected with individuals of parental genotypes: *Asplenium normale* × *A. boreale*, *A. normale* × *A. shimurae*, *A. normale* × *A. oligophlebium*, and *A. boreale* × *A. oligophlebium*. The phenolic patterns consisted of *O*-glycosylflavones and di-*C*-glycosylflavones. Interestingly, the flavonoid composition of the hybrids was shown to be total addition of the parental attributes.

14.1.2.2 Angiospermae Monocotyledonae

14.1.2.2.1 Family Eriocaulaceae

A recent study by Salatino et al.³¹¹ concerned the distribution of flavonoids in four genera of Eriocaulaceae: *Eriocaulon*, *Leiothrix*, *Paepalanthus*, and *Syngonanthus*. The authors compared the flavonoid patterns with the results of cladistic analyses based on 49 predominantly morphological characters. This morphometric analysis suggests that *Paepalanthus* is polyphyletic, *Eriocaulon* is closely related to some small subgroups of *Paepalanthus*, while *Leiothrix* and *Syngonanthus* appear as more advanced sister groups. Moreover, at the sectional level within *Syngonanthus*, the *Eulepis* and *Thysanocephalus* sections seemed to constitute a more advanced monophyletic group than the *Carpocephalus* and *Syngonanthus* sections.

The structural aspects of flavonoids seem to have paralleled the evolution of Eriocaulaceae. The 6-oxygenation has been superseded during evolution: in the primitive side, *Paepalanthus* and *Eriocaulon* accumulate 6-OH flavonols; in the more advanced groups, *Leiothrix*, *Syngonanthus-Carpocephalus*, *Syngonanthus-Syngonanthus*, 6-OH flavones are found; finally, in highly advanced sections, *Syngonanthus-Eulepis* and *Syngonanthus-Thysanoccephalus*, 6-oxygenated derivatives are lacking while there is a prevalence of *C*-glycosylflavones.

14.1.2.2 Family Velloziaceae

In another group of monocots, the Velloziaceae family, chemical studies^{127,386} clarified the delineation of subfamilies and genera, which had been the subject of much dispute. The flavonoid patterns were described for about 100 species representative of the subfamilies Vellozioidae (*Vellozia*, *Nanuza*, *Barbaceniopsis*, *Xerophyta*, *Talbotia*), Barbacenioidae (*Aylthonia*, *Barbacenia*, *Burlemarxia*, *Pleurostigma*). Flavone *C*-glycosides have been identified in both subfamilies; however, the Vellozioidae could be distinguished from the Barbacenioidae by the accumulation of flavone mono-*C*-glycosides rather than di-*C*-glycosides. It was apparent that *Barbaceniopsis*, *Xerophyta* (Madagascan species), and *Talbotia* differ from most other members of the subfamily in the absence of *C*-glycosylflavones, and predominance of flavonol glycosides. The species *Nanuza plicata* remains unique within Vellozioidae in the accumulation of biflavonoids. And finally the genus *Pleurostigma* was unique within the Barbacenioidae in accumulating 6-OH flavonoids instead of *C*-glycosylflavones.

14.1.2.3 Angiospermae Dicotyledonae

14.1.2.3.1 Tribe Phasaeolinae

The *C*-glycosylflavones clarify the position of the genus *Dysalobium* within the tribe Phasaeolinae (Leguminosae Fam.).³⁹³ The Phasaeolinae is a taxonomically very complex group because of the limited number of useful morphological characters available to distinguish generic limits. The main taxa are *Phaseolus*, *Dysalobium*, *Macroptilium*, *Strophostyles*, and *Vigna*. The flavonoid profiles of 49 representative species of these five genera were compared and about 35 flavonoids were identified. A statistical procedure using binary presence-absence data for the leaf flavonoids, and based on Sneath's simple matching coefficient, indicated four main groupings, one of them restricted to *Dysalobium*, which is clearly separated from all others while it alone produces only *C*-glycosylflavones.

14.1.2.3.2 Tribe Ornithocephalinae

Ornithocephalinae,³⁸⁵ a small subtribe of the family Orchidaceae, is traditionally recognized as one of the most advanced in this family. The complexity and diversity of floral and vegetative morphologies of the species of this subtribe seem to have reached a level that is apparently only paralleled by some members of the Oncidiinae usually interpreted as a putative close relative. The results of the leaf flavonoid analyses of 15 species representative of the genera, *Zygostates*, *Ornithocephalus*, *Chytroglossa*, *Phymatidium*, and *Rauhiella*, showed the presence of 16 different *C*-glycosylflavones. These taxa could be distinguished from each other by occurrence of different isomers; these were all apigenin-based structures, with a large representation of different apigenin 7,4'-dimethylether 6-*C*-glycosyl *X''*-*O*-glycosides, and apigenin 7-methylether 6-*C*-glycosyl-*X''*-*O*-glycosides (*O*-glycosidic linkages were not precisely determined). All these flavonoids are unusual and quite rare in the Angiosperms.

Methylated flavonoids, considered to be advanced chemical characters, suggested that *Zygostates*, *Ornithocephalus*, *Chytroglossa*, and *Phymatidium* might be highly evolved genera, while the presence of isovitexin and absence of methylated derivatives in *Rauhiella* clearly separated this genus from the other members.

As already mentioned, subtribe Oncidiinae has been considered as a probable sister group of the Ornithocephalinae; *Oncidium* species so far surveyed lack flavone *C*-glycosides, and possess new and unusual 6-hydroxyflavone glycosides. Thus, different flavonoid patterns did not support a close association of these two subtribes.

14.1.2.3.3 Genus *Pelargonium*

The first example concerns the chemical survey of 56 *Pelargonium* species (representative of 19 sections).³⁹² Their flavonoid composition revealed a large chemical diversity: flavonols, flavones, *C*-glycosylflavones, proanthocyanidins, and ellagitannins. No individual phenolic compound or group of compounds provides taxonomic markers at the sectional level. However, the data indicated which sections are homogenous in their phenolic profiles and which not and in this way could support or refute the current classification. *C*-Glycosylflavones were well represented in the sections Subsucculentia, Chorisma, Perista, Reniformia, and Jenkinsonia, for example, while they were not found in the *Pelargonium*, *Otidia*, and *Cortusina* sections. Interestingly, most species possessing high levels of *C*-glycosylflavones showed a strong correlation between these compounds and the presence of ellagitannins. In other sections lacking *C*-glycosylflavones, a correlation exists between proanthocyanidins and the flavonol myricetin. Thanks to these correlations, it was possible to detect in each section well placed and misplaced species. Thus in *Reniformia*, three species, *P. reniforme*, *P. exstipulatum*, and *P. album*, have the basic flavone-*C*-glycoside or ellagitannin profile but *P. odoratissimum* has a very different myricetin or proanthocyanidin pattern, suggesting it may need to be moved.

14.1.2.3.4 Genus *Itea* and *Pterostemon*

Itea and *Pterostemon*, sister taxa close to *Ribes* in the Saxifragaceae family, were studied³⁵ for chemical data in comparison to gene sequence data. Recent phylogenetic analyses of *rbcL*, 18S rDNA, *matK*, and *atpB* sequences all concur in suggesting that *Itea* and *Pterostemon* are sister taxa, close to *Ribes* in the Saxifragaceae as a part of a larger Saxifragales clade that also includes Hamamelidaceae, Crassulaceae, Penthoraceae, etc. The flavonoid profile of *Pterostemon* comprises *O*-glycosides of quercetin and *C*-glucosylflavones (vitexin, isovitexin, orientin and their *O*-glycoside derivatives, especially *X''*-*O*-xylosides). This *C*-glycosylflavone pattern resembles very closely that observed in *Itea*, and provides support for the closeness of their relationship recently demonstrated on the basis of gene sequence data. But this finding indicates that the flavonoid profiles of *Itea* and *Pterostemon* are dramatically different from the overall profile of a large number of species of Saxifragaceae, which is mainly based upon various *O*-glycosylflavonols but with no trace of *C*-glycosylflavones. If the DNA studies indicate that *Itea* and *Pterostemon* are sister taxa within the Saxifragaceae, the phenolic contents demonstrate that the two genera possess unusual and non-saxifragoid flavonoid chemistry. On the evolutionary sequence of flavonoid production, it appears that these genera represent a derived clade with a replacement of flavonols by *C*-glycosylflavones, and that *Pterostemon*, due to *O*-glycosylflavonols, possibly represents an intermediate form between *Itea* and the true Saxifragaceae.

14.1.2.3.5 Genus *Centaurea*

Based on glycosylflavones within blue flowering alpine cornflowers, *Centaurea montana* L. and *Centaurea triumfetti* All. (Compositae), a major discussion concerned the influence of microscale environmental conditions and the role of reproductive modes on the distribution of *C*-glycosylflavones. Twenty-one out of the 48 flavonoid glycosides detected in plants from different origins are basic 6- and 8-*C*-monoglucosides of apigenin, luteolin, and chrysoeriol, 6,8-di-*C*-glucosyl apigenin, 2''-*O*-glucosides and arabinosides of the 6-*C*-glucosides, and caffeoyl derivatives of 2''-*O*-glucosides along with some other incompletely

identified *C*-glycosides. The other 27 are *O*-glycosides, mono- and di-glycosides, most of which were based on the above three aglycones.¹¹⁵

In individual plants of *Centaurea montana* originating from different restricted areas in the French southern Alps, in addition to practically pure *O*-glycosidic patterns, these molecules are arranged in many diverse assemblages of simple or complex *C*-glycoside derivatives — along with *O*-glycosides in some. This results in an extraordinary diversity of flavonoid patterns of this species, which comprise from five to more than 20 compounds.

This flavonoid variation is first correlated with the phytosociological origin of *Centaurea montana* plants: those displaying *C*-glycosidic patterns (all types) are largely predominant in meadows of *Trisetum-Polygonion* while in tall grass prairies under *Larix* or *Adenostylyon*, *O*-glycosidic types are the most frequent. In some meadows, the individuals with different types of *C*-glycosidic patterns are distributed according to microstational parameters as detected by aerial infrared remote sensing.

The origin of the huge chemical diversity in *Centaurea montana* was shown to result from its reproductive mode combining vegetative reproduction and strictly allogamous pollination (completely preventing autogamy and strongly limiting fertility between genetically related partners), both confirmed by flavonoid analysis of wild and experimental plants from breeding experiences. Clonal ramets are readily identifiable by identical flavonoid profiles of closely collected individuals. Interindividual diversity is also consistently observed in the progeny of most of the experimental crosses, revealing a generalized heterozygosis of wild individuals, an expectable consequence of the obligate allogamy of this species.

For instance, in a breeding experience involving two wild partners with *O*-glycosidic dominant profiles (more than 75% of the total flavonoids) and collected in a single meadow, one out the three descendants displayed a *C*-glycosidic pattern featuring 2''-*O*-glycosides of mono-*C*-glucosides (35% of the total) and their caffeoyl derivatives (45% of the total) along with the three basic *C*-glycosides (10%). By contrast, in the lineage of combinations of other individuals featuring *O*-glycosidic patterns also, only *O*-glycosidic phenotypes were observed, in which the traces of *C*-glycosides present in the parental fingerprints (10 to 20% of the total) completely disappeared. Consequently, *C*-glycosidic phenotypes seemed to proceed from a hypostatic determinism. This was extensively confirmed by many experiments with individuals displaying the two extreme phenotypes detected in *Centaurea montana*. Thus, when crossing plants with *O*-glycosidic (but *C*-glycosides — all types — remaining present, each 2 to 5% of the total) and *C*-glycosidic (blend of “complex” [85%] and “simple” [5%] types; *O*-glycosides: <10% of the total) patterns, 80% of the progeny displayed phenotypes close to that of the *O*-glycosidic parental type. Only one *C*-glycosidic pattern was observed in this progeny, in which the accumulation of caffeoyl conjugates was noticeably reduced.

In addition to a hypostatic determinism of *C*-glycosidic pathways vs. *O*-glycosidic ones, the variations observed suggest that each of the biosynthetic steps of the *C*-glycosidic metabolism is independently affected by heterogeneity. Regarding the genetic control involved, the loss or gain of molecules or their quantitative variations suggests the existence of heterozygosis of dominant or recessive characters in a polygenic system.¹¹⁷

Flavonoid glycoside variations of comparable amplitude are also observable in the individual fingerprints of sister species *Centaurea triumfetti*.¹¹⁶ All the features of variation in *Centaurea triumfetti* closely compare to those observed in *Centaurea montana* and its origin probably rests on the same biological parameters, mainly obligate allogamy coupled to vegetative multiplication. The main difference between these two species is *Centaurea triumfetti*, which completely lacks the three caffeoyl derivatives of *O*-glucosyl-*C*-glucosyl flavones. Based on reports of the flavonoid chemistry of tetraploids (Mears, 1980), this

feature can be regarded as a clue to the existence of relationships between these species; e.g., *Centaurea montana* ($2n=44$) could be the tetraploid of *Centaurea triumfetti* ($2n=22$). In this framework, the gain in the flavonoid profiles of tetraploid cytotypes of “new” compounds (complex C-glycosides, here) structurally based on those present in the profiles of diploid cytotypes (basic mono-C-glycosides and their O-glucosidic derivatives) is interpretable as functional (de)repression of existing structural genes (present but inactive in the diploid cytotypes) during the polyploidization process. Consequently, the large presence of simple C-glycosides in the fingerprints of *Centaurea triumfetti* individuals (diploids), which are the biosynthetic precursors of most of the major substituted molecules in the profiles of *Centaurea montana* (tetraploids), can be regarded as a sign of the existence of relationships between these two species, in the context of a polyploidization process.

14.2 NATURALLY OCCURRING C-GLYCOSYLFLAVONOIDS

Natural C-glycosylflavonoids are presented in four groups: the mono-C-glycosylflavonoids (Table 14.2), the di-C-glycosylflavonoids (Table 14.3), the O-glycosyl-C-glycosylflavonoids (Table 14.4), and the O-acyl-C-glycosylflavonoids (Table 14.5).

For each compound, the first line or reference indicates the first mention of this molecule in the phytochemical literature. Additional lines (when present) give information on other citations during the last 12 years. New C-glycosylflavonoids described since 1992 are specifically marked by an asterisk for their first mention in the plant kingdom.

14.2.1 MONO-C-GLYCOSYLFLAVONOIDS

For the period under review, 17 new mono-C-glycosylflavonoids were described. Two interesting features are evident:

- Seven new aglycones support the C-glycosidic bond in this class: 5,7-dihydroxyflavone (chrysin) in *Scutellaria*,^{257,413} 5-hydroxy 7-methoxyflavone (tectochrysin) in *Piper*,²⁶⁵ 5,7,2',4',5'-pentahydroxyflavone (isoetin) in *Hordeum*,²⁷⁹ 5,7,2',3',5',6'-hexahydroxyflavone in *Polygala*,¹⁹⁵ 3,5,7,4'-tetrahydroxy 3',5'-dimethoxyflavone (myricitrin) in *Moghania*,³⁹⁸ 5,7,2',4',5'-pentahydroxyflavonol (5'-OH morin) in *Bombax*,⁹⁹ and 5,7-dihydroxy 6,2',4',5'-tetramethoxyisoflavone in *Dalbergia*.³⁰⁶
- Three original C-substitutions other than sugar have been observed: 4-hydroxy-1-ethyl benzene in Cucumerin A and B isolated from *Cucumis*,²²⁸ p-hydroxybenzyl in *Citrus*,²²³ and 1,5,8-trihydroxy 3-methoxyxanthone in *Swertia*.³⁸¹ (Figure 14.1).

14.2.2 DI- AND TRI-C-GLYCOSYLFLAVONOIDS

Thirteen new compounds were found for the first time during the last 12 years:

- Two new sugars were linked by a C-glycosidic bond: β -D-ribose in *Passiflora*⁵⁶ and β -D-6-deoxyglucopyranoside in *Viola*.⁴⁶ A 3,6,8-tri-C-xylosylflavone was isolated from *Asplenium*.¹⁵⁶
- Several isomers were detected under the di-C-arabinosylflavone patterns: di-C- α -L-arabinopyranosyl in *Schnabellia*,⁷⁶ *Plagiochila*, and *Plagiochasma*,³¹⁸ 6-C- α -L-arabinopyranosyl-8-C- β -L-arabinopyranosyl in *Viola*,⁴⁰¹ and 6-C- β -L-arabinopyranosyl-8-C- α -L-arabinopyranosyl in *Schnabellia*.⁷⁶

TABLE 14.2
Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*])

Compounds	Sources	Ref.
C-GLYCOSYLFLAVONES		
(*) 8-C- β -D-Glucosylchrysin (5,7-diOH 8-Gl)	<i>Scutellaria baicalensis</i> (Lab.)	Miyaichi and Tomimori (1994) Zhang et al. (1997)
	<i>Scutellaria amoena</i> (Lab.)	Zhou and Yang (2000)
(*) 6-C- β -D-Glucosylchrysin (5,7-diOH 6-Gl)	<i>Scutellaria baicalensis</i> (Lab.)	Zhang et al. (1997)
(*) Kaplanin (5-OH 7-OMe 8-Glc) Bayin	<i>Piper ihotzkyanum</i> (Pip.)	Moreira et al. (2000)
(7,4'-diOH 8-Glc) Isovitexin (saponaretin)	<i>Castanospermum australe</i> (Leg.)	Eade et al. (1962, 1966)
(5,7,4'-triOH 6-Glc)	<i>Vitex lucens</i> (Verb.) Many sources	Horowitz and Gentili (1964)
(*) Cucumerin B 8-C-(4-OH-1-ethyl benzene) isovitexin	<i>Cucumis sativus</i> (Cucur.)	McNally et al. (2003)
(*) 8-C- <i>p</i> -OH benzyl isovitexin (5,7,4'-triOH 6-Glc 8-OH-benzyl) Vitexin	<i>Citrullus colocynthis</i> (Cucur.)	Maatooq et al. (1997)
(5,7,4'-triOH 8-Glc)	<i>Vitex lucens</i> (Verb.) Many sources	Horowitz and Gentili (1964)
(*) Cucumerin A 6-C-(4-OH-1-ethyl benzene) vitexin	<i>Cucumis sativus</i> (Cucur.)	McNally et al. (2003)
(*) 6-C- <i>p</i> -OH benzyl vitexin (5,7,4'-triOH 8-Glc 6-OH-benzyl) 8-C- β -D-Galactopyranosylapigenin	<i>Citrullus colocynthis</i> (Cucur.)	Maatooq et al. (1996)
(5,7,4'-triOH 8-Gal) Cerarvensin	<i>Briza media</i> (Gram.)	Castledine and Harborne (1976)
(5,7,4'-triOH 6-Xyl) Isomollupentin	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1982)
(5,7,4'-triOH 6-Ara) Mollupentin	<i>Spergularia rubra</i> (Caryo.)	Bouillant et al. (1979)
(5,7,4'-triOH 8-Ara) Isofurcatain	<i>Mollugo pentaphylla</i> (Mollug.)	Chopin et al. (1979)
(5,7,4'-triOH 6-Rha)	<i>Metzgeria furcata</i> (Bryo.) <i>Otanthus maritimus</i> (Comp.)	Markham et al. (1982) El-Sayed et al. (1992)
3'-Deoxyderhamnosylmaysin (5,7,4'-triOH 6-(6-deoxy-xylo-hexos-4-ulosyl))	<i>Zea mays</i> (Gram.)	Elliger et al. (1980)
6-C- β -D-Galactopyranosylapigenin (5,7,4'-triOH 6-Gal)	<i>Semecarpus kurzii</i> (Anacard.)	Jain et al. (1990)
Torosaflavone A (5,7,4'-triOH 6-Olio)	<i>Cassia torosa</i> (Leg.)	Kitanaka et al. (1989)
Swertisin (5,4'-diOH 7-OMe 6-Glc)	<i>Swertia japonica</i> (Gent.) <i>Iris germanica</i> (Irid.)	Komatsu and Tomimori (1966) Ali et al. (1993)

TABLE 14.2
Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
	<i>Passiflora incarnata</i> (Passifl.)	Rahman et al. (1997)
	<i>Zizyphus jujuba</i> (Rham.)	Cheng et al. (2000)
Isoswertisin (5,4'-diOH 7-OMe 8-Glc)	<i>Centaurea cyanus</i> (Comp.)	Asen and Jurd (1967)
	<i>Deschampsia antarctica</i> (Gram.)	Webby and Markham (1994)
8-C-Rhamnosylgenkwanin (5,4'-diOH 7-OMe 8-Rha)	<i>Adina cordifolia</i> (Rub.)	Srivastava and Srivastava (1986)
Isomolludistin (5,4'-diOH 7-OMe 6-Ara)	<i>Mollugo distica</i> (Mollug.)	Chopin et al. (1978)
Molludistin (5,4'-diOH 7-OMe 8-Ara)	<i>Mollugo distica</i> (Mollug.)	Chopin et al. (1978)
Isocytisoides (5,7-diOH 4'-OMe 6-Glc)	<i>Fortunella margarita</i> (Rut.)	Horowitz et al. (1974)
	<i>Combretum quadrangulare</i> (Comb.)	Banskota et al. (2000)
Cytisoides (5,7-diOH 4'-OMe 8-Glc)	<i>Cytisus laburnum</i> (Leg.)	Paris (1957)
8-C-Rhamnosyl-5-O-methylacetin (7-OH 5,4'-diOMe 8-Rha)	<i>Adina cordifolia</i> (Rub.)	Srivastava and Srivastava (1986)
Embigenin (5-OH 7,4'-diOMe 6-Glc)	<i>Iris tectorum</i> (Irid.)	Hirose et al. (1962)
Isoembigenin (5-OH 7,4'-diOMe 8-Glc)	<i>Siphonoglossa sessilis</i> (Acan.)	Hilsenbeck and Mabry (1983)
	<i>Ornithocephalinae</i> (Orch.)	Williams et al. (1994a)
7,4-Di-O-methylisomollupentin (5-OH 7,4'-diOMe 6-Ara)	<i>Asterostigma riedelianum</i> (Arac.)	Markham and Williams (1980)
8-O-methylswertisin (preparatorin I) (5,4'-diOH 7,8-diOMe 6-Glc)	<i>Siphonoglossa</i> sp. (Acan.)	Hilsenbeck and Mabry (1990)
	<i>Abrus precatorius</i> (Leg.)	Ma et al. (1998)
6-C-Galactosylisoscuteallarein (5,7,8,4'-tetraOH 6-Gal)	<i>Stellaria dichotoma</i> (Caryo.)	Yasukawa et al. (1982)
8-C-Glucosyl-6,7-di-O-methyl-scutellarein (abrusin) (5,4'-diOH 6,7-diOMe 8-Glc)	<i>Siphonoglossa</i> sp. (Acan.)	Hilsenbeck and Mabry (1990)
	<i>Abrus precatorius</i> (Leg.)	Markham et al. (1989)
Isoorientin (5,7,3',4'-tetraOH 6-Glc)	<i>Polygonum Orientale</i> (Polyg.)	Hörhammer et al. (1958)
	Many sources	
Orientin (5,7,3',4'-tetraOH 8-Glc)	<i>Polygonum Orientale</i> (Polyg.)	Hörhammer et al. (1958)
	Many sources	
6-C-β-D-Xylopyranosylluteolin (5,7,3',4'-tetraOH 6-Xyl)	<i>Phlox drummondii</i> (Polem.)	Mabry et al. (1971)
Derhamnosylmaysin (5,7,3',4'-tetraOH 6- (6-deoxyxylo-hexos-4-ulosyl))	<i>Zea mays</i> (Gram.)	Elliger et al. (1980)

continued

TABLE 14.2
Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
6-C-Galactosylluteolin (5,7,3',4'-tetraOH 6-Gal)	<i>Muhlenbergia</i> sp. (Gram.) <i>Zea mays</i> (Gram.)	Peterson and Rieseberg (1987) Snook et al. (1994)
8-C-Galactosylluteolin (5,7,3',4'-tetraOH 8-Gal)	<i>Parkinsonia aculeata</i> (Leg.)	El-Sayed et al. (1990)
6-C- α -L-Arabinosylluteolin (5,7,3',4'-tetraOH 6-Ara)	<i>Muhlenbergia</i> sp. (Gram.) <i>Sasa borealis</i> (Gram.)	Herrera and Bain (1991) Yoon et al. (2000)
8-C- α -L-Arabinosylluteolin (5,7,3',4'-tetraOH 8-Ara)	<i>Mucuna sempervirens</i> (Leg.)	Ishikura and Yoshitama (1988)
6-C-Quinovopyranosylluteolin (5,7,3',4'-tetraOH 6-Chino)	<i>Passiflora edulis</i> (Pass.)	Mareck et al. (1991)
6-C-Fucopyranosylluteolin (5,7,3',4'-tetraOH 6-Fuco)	<i>Passiflora edulis</i> (Pass.)	Mareck et al. (1991)
(*) Demethyltorosaflavone C Parkinsonin A (7,3',4'-triOH 5-OMe 8-Glc)	<i>Cassia nomame</i> (Leg.) <i>Parkinsonia aculeata</i> (Leg.)	Kitanaka and Takido (1992) Bhatia et al. (1966)
Swertiajaponin (5,3',4'-triOH 7-OMe 6-Glc)	<i>Swertia japonica</i> (Gent.) <i>Deschampsia antarctica</i> (Gram.)	Komatsu and Tomimori (1966) Webby and Markham (1994)
Isoswertiajaponin (5,3',4'-triOH 7-OMe 8-Glc)	<i>Gnetum gnemon</i> (Gnet.) <i>Deschampsia antarctica</i> (Gram.)	Wallace and Morris (1978) Webby and Markham (1994)
(+)Isoscoparin (5,7,4'-triOH 3'-OMe 6-Glc)	<i>Hordeum vulgare</i> (Gram.) <i>Barbarea vulgaris</i> (Brass.) <i>Centaurea triumfetti</i> (Comp.) <i>Citrullus colocynthis</i> (Cucur.) <i>Vellozia</i> sp. (Velloz.)	Seikel et al. (1962) Senatore et al. (2000) Gonnet (1993) Maatooq et al. (1997) Williams et al. (1994b)
(-)Isoscoparin (5,7,4'-triOH 3'-OMe 6-Glc)	<i>Arenaria kansuensis</i> (Caryo.)	Wu et al. (1990)
Scoparin (5,7,4'-triOH 3'-OMe 8-Glc)	<i>Sarothamnus scoparius</i> (Leg.)	Chopin et al. (1968)
(*) 6-C- β -Fucopyranosylchrysoeriol (5,7,4'-triOH 3'-OMe 6-Fuc)	<i>Zea mays</i> (Gram.)	Suzuki et al. (2003b)
6-C- β -L-Boivinopyranosyl- Chrysoeriol: alternanthin (5,7,4'-triOH 3'-OMe 6-Boiv)	<i>Alternanthera philoxeroides</i> (Amar.) <i>Zea mays</i> (Gram.)	Zhou et al. (1988) Suzuki et al. (2003a)
3'-O-Methyl-derhamnosylmaysin (5,7,4'-triOH 3'-OMe 6- (6-deoxyxylo-hexos-4-ulosyl)) 6-C- β -D-Glucopyranosyldiosmetin	<i>Zea mays</i> (Gram.)	Elliger et al. (1980)

TABLE 14.2
Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
(5,7,3'-triOH 4'-OMe 6-Glc)	<i>Citrus limon</i> (Rut.)	Gentili and Horowitz (1968)
8-C-β-D-Glucopyranosyldiosmetin		
(5,7,3'-triOH 4'-OMe 8-Glc)	<i>Citrus limon</i> (Rut.)	Gentili and Horowitz (1968)
Torosaflavone B		
(5,7,3'-triOH 4'-OMe 6-Olio)	<i>Cassia torosa</i> (Leg.)	Kitanaka et al. (1989)
	<i>Cassia occidentalis</i> (Leg.)	Hatano et al. (1999)
Parkinsonin B		
(3',4'-diOH 5,7-diOMe 8-Glc)	<i>Parkinsonia aculeata</i> (Leg.)	Bhatia et al. (1966)
7,3'-Di-O-methylisoorientin		
(5,4'-diOH 7,3'-diOMe 6-Glc)	<i>Achillea cretica</i> (Comp.)	Valant et al. (1980)
7,3'-Di-O-methylorientin		
(5,4'-diOH 7,3'-diOMe 8-Glc)	<i>Saccharum</i> sp. (Gram.)	Mabry et al. (1984)
6-C-β-D-Glucopyranosylpilloin		
(5,3'-diOH 7,4'-diOMe 6-Glc)	<i>Parkinsonia aculeata</i> (Leg.)	El-Sayed et al. (1991)
7,3',4'-Tri-O-methylisoorientin		
(5-OH 7,3',4'-triOMe 6-Glc)	<i>Linum maritimum</i> (Lin.)	Volk and Sinn (1968)
Isoaffinetin		
(5,7,3',4',5'-pentaOH 6-Glc)	<i>Polygonum affine</i> (Polyg.)	Krause (1976a,b)
	<i>Plumbago zeylanica</i> (Plumb.)	Lin and Chou (2003)
	<i>Frullania</i> sp. (Bryo.)	Kraut et al. (1993)
Affinetin		
(5,7,3',4',5'-pentaOH 8-Glc)	<i>Trichomanes venosum</i> (Pterido.)	Markham and Wallace (1980)
Isopyrenin		
(5,7,4'-triOH 3',5'-diOMe 6-Glc)	<i>Gentiana pyrenaica</i> (Gent.)	Marston et al. (1976)
6-C-Glucosyl-5,7-dihydroxy-8,3',4',5'-tetramethoxyflavone	<i>Vitex negundo</i> (Verb.)	Subramanian and Misra (1979)
(*) 6-C-β-D-Glucopyranosyl		
5,7,2',4',5'-Pentahydroxyflavone	<i>Hordeum vulgare</i> (Gram.)	Norbaek et al. (2000)
(*) Telephiodin		
(5,7,2',3',5',6'-hexahydroxy-6-C-β-D-Glc)	<i>Polygala telephioides</i> (Poly.)	Kumar et al. (1999)
C-GLYCOSYLFLAVONOLS		
8-C-Glucosyl-5-deoxykämpferol		
(3,7,4'-triOH 8-Glc)	<i>Pterocarpus marsupium</i> (Leg.)	Bezuidenhoudt et al. (1987)
8-C-Glucosylfisetin		
(3,7,3',4'-tetraOH 8-Glc)	<i>Pterocarpus marsupium</i> (Leg.)	Bezuidenhoudt et al. (1987)
6-C-Glucosylkämpferol		
(3,5,7,4'-tetraOH 6-Glc)	<i>Zelkova</i> sp. (Ulm.)	Hayashi et al. (1987)
	<i>Cyclopia intermedia</i> (Leg.)	Kamara et al. (2003)
(*) 8-C-β-D-Glucopyranosylkämpferol		
(3,5,7,4'-tetraOH 8-Glc)	<i>Cyclopia intermedia</i> (Leg.)	Kamara et al. (2003)
Keyakinin		
(3,5,4'-triOH 7-OMe 6-Glc)	<i>Zelkova serrata</i> (Ulm.)	Funaoka (1956)
6-C-Glucosylquercetin		

continued

TABLE 14.2
Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
(3,5,7,3',4'-pentaOH 6-Glc)	<i>Ageratina calophylla</i> (Comp.)	Fang et al. (1986)
Keyakinin B		
(3,5,3',4'-tetraOH 7-OMe 6-Glc)	<i>Zelkova serrata</i> (Ulm.)	Hillis and Horn (1966)
8-C-Rhamnosyl-5,7,3'-trihydroxy-3,4'-dimethoxy flavone	<i>Adina cordifolia</i> (Rub.)	Srivastava and Srivastava (1986)
8-C-Rhamnosyleuropetin		
(3,5,3',4',5'-pentaOH 7-OMe 8-Rha)	<i>Cassia sophora</i> (Leg.)	Tiwari and Bajpai (1981)
(*) Moghanin A		
(3,5,7,4'-tetraOH 3',5'-diOMe 6-C- β -D-Glc)	<i>Moghania macrophylla</i> (Leg.)	Wu et al. (1997)
(*) Shamimin		
3,5,7,2',4',5'-hexaOH 6-C-Glc	<i>Bombax ceiba</i> (Bomb.)	Faizi and Ali (1999)
C-GLYCOSYLFLAVANONES		
Aervanone		
(7,4-diOH 8-Gal)	<i>Aervia persica</i> (Amar.)	Garg et al. (1980)
Hemiphloin		
(5,7,4'-triOH 6-Glc)	<i>Eucalyptus hemiphloia</i> (Myrt.) <i>Acacia saligna</i> (Leg.) <i>Betula platyphylla</i> (Bet.)	Hillis and Carle (1963) El-Shafae et al. (1998) Lee (1994)
Isohemiphloin		
(5,7,4'-triOH 8-Glc)	<i>Eucalyptus hemiphloia</i> (Myrt.)	Hillis and Horn (1965)
Palodulcin B		
(7,3',4'-triOH 8-Gal)	<i>Eysenhardtia polystachia</i> (Leg.)	Vita-Finzi et al. (1980)
C-GLYCOSYLFLAVANONOLS		
(*) 8-C-Glucosyldihydrokaempferol		
(3,5,7,4'-tetraOH 8-Glc)	<i>Acacia saligna</i> (Leg.)	El-Sawi (2001)
6-C-Glucosyldihydrokaempferol		
(3,5,7,4'-tetraOH 6-Glc)	<i>Zelkova</i> sp. (Ulm.) <i>Betula platyphylla</i> (Bet.)	Hayashi et al. (1987) Lee (1994)
Keyakinol		
(3,5,4'-triOH 7-OMe 6-Glc)	<i>Zelkova serrata</i> (Ulm.)	Funaoka (1956)
6-C-Glucosyldihydroquercetin		
(3,5,7,3',4'-pentaOH 6-Glc)	<i>Zelkova</i> sp. (Ulm.)	Hayashi et al. (1987)
C-GLYCOSYLCHALCONES		
3'-C-Glucosylisoliquiritigenin		
(2',4,4'-triOH 3'-Glc)	<i>Cladrastis platycarpa</i> (Leg.)	Ohashi et al. (1977)
C-GLYCOSYLDIHYDROCHALCONES		
Nothofagin		
(2',4',6',4'-tetraOH C-Gly)	<i>Nothofagus fusca</i> (Fag.)	Hillis and Inoue (1967)
Konnarin		
(2',4',6',3,4-pentaOH C-Gly)	<i>Nothofagus fusca</i> (Fag.)	Hillis and Inoue (1967)
Aspalathin		
(2',4',6',3,4-pentaOH 3'-Glc)	<i>Aspalathus linearis</i> (Leg.)	Dahlgren (1963)

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Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*])
 — *continued*

Compounds	Sources	Ref.
C-GLYCOSYL- α - HYDROXYDIHYDROCHALCONES		
Coatline A (α -2',4',4-tetraOH 3'-Glc)	<i>Eysenhardtia polystachya</i> (Leg.)	Beltrami et al. (1982)
Coatline B (α -2',4',3,4-pentaOH 3'-Glc)	<i>Eysenhardtia polystachya</i> (Leg.)	Beltrami et al. (1982)
C-GLYCOSYL- β - HYDROXYDIHYDROCHALCONES		
Pterosupin (β -2,2',4',4-tetraOH 3'-Glc)	<i>Pterocarpus marsupium</i> (Leg.)	Adinarayana et al. (1982)
C-GLYCOSYLFLAVANOLS		
6-C-Glucosyl(-)-epicatechin (3,5,7,3',4'-pentaOH 6-Glc)	<i>Cinnamomum cassia</i> (Laur.)	Morimoto et al. (1986a)
8-C-Glucosyl(-)-epicatechin (3,5,7,3',4'-pentaOH 8-Glc)	<i>Cinnamomum cassia</i> (Laur.)	Morimoto et al. (1986a)
C-GLYCOSYLPROANTHOCYANIDINS		
6-C-Glucosylprocyanidin B2	<i>Cinnamomum cassia</i> (Laur.)	Morimoto et al. (1986b)
8-C-Glucosylprocyanidin B2	<i>Cinnamomum cassia</i> (Laur.)	Morimoto et al. (1986b)
C-GLYCOSYLQUINOCHALCONE		
Carthamin	<i>Carthamus tinctorius</i> (Comp.)	Takahashi et al. (1982)
C-GLYCOSYLISOFLAVONES		
Puerarin (7,4'-diOH 8-Glc)	<i>Pueraria thunbergiana</i> (Leg.) <i>Zizyphus jujuba</i> (Rham.)	Murakami et al. (1960) Cheng et al. (2000)
8-C- β -D-Glucopyranosylgenistein (5,7,4'-triOH 8-Glc)	<i>Lupinus luteus</i> (Leg.) <i>Lupinus luteus</i> (Leg.)	Zapesochnaya and Laman (1977) Zavodnik et al. (2000)
8-C-Glucosylprunetin (5,4'-diOH 7-OMe 8-Glc)	<i>Dalbergia paniculata</i> (Leg.)	Parthasarathy et al. (1974)
Isovolubilin (5-OH 7,4'-diOMe 6-Rha)	<i>Dalbergia volubilis</i> (Leg.)	Chawla et al. (1974)
Volubilin (5-OH 7,4'-diOMe 8-Rha)	<i>Dalbergia volubilis</i> (Leg.)	Chawla et al. (1974)
6-C-Glucosylorobol (5,7,3',4'-tetraOH 6-Glc)	<i>Dalbergia monetaria</i> (Leg.)	Nunes et al. (1989)
8-C-Glucosylorobol (5,7,3',4'-tetraOH 8-Glc)	<i>Lupinus luteus</i> (Leg.)	Zapesochnaya and Laman (1977)
Dalpanitin (5,7,4'-triOH 3'-OMe 8-Glc)	<i>Dalbergia paniculata</i> (Leg.)	Adinarayana and Rao (1972)
Volubilin (5,7-diOH 6,4'-diOMe 8-Glc)	<i>Dalbergia volubilis</i> (Leg.)	Chawla et al. (1976)
(*) Dalpaniculin		

(5,7-diOH 2',4',5',6-tetraOMe 8-Glc)

Dalbergia paniculata (Leg.)

Rao and Rao (1991)

TABLE 14.2
Naturally Occurring Mono-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
C-GLYCOSYLISOFLAVANONES		
Dalpanin	<i>Dalbergia paniculata</i> (Leg.)	Adinarayana and Rao (1975)
Macrocarposide		
6-C-Glucosyldalbergioidin (5,7,2',4'-tetraOH 6-Glc)	<i>Pterocarpus macrocarpus</i> (Leg.)	Verma et al. (1986)
C-GLYCOSYLCHROMONES		
Aloeresin B		
(7-OH 5-Me 2-Acetyl 8-Glc)	<i>Aloe</i> sp. (Lil.)	Haynes and Holsworth (1970)
7-O-Methyl-5-methyl 2-(2-hydroxy) propyl 8-C-glucoside	<i>Aloe</i> sp. (Lil.)	Speranza et al. (1986)
C-GLYCOSYLFLAVONE-XANTHONES		
(*) Swertifrancheside		
5,7,3',4'-tetraOH 6-C-β-D Glucopyranosyl 8-C-(1''',5''',8'''-triOH 3'''- OMe Xanthonyl) flavone	<i>Swertia franchetiana</i> (Gent.)	Wang et al. (1994)

14.2.3 O-GLYCOSYL-C-GLYCOSYLFLAVONOIDS

Forty-six new compounds were described during the period for review.

A large number of new flavonoid aglycones in mono-C-glycosides have been described as supports for O-glycosidic bonds:

- On apigenin base: 8-C-*p*-hydroxybenzylvitexin in *Citrullus*,²²³ apigenin-6-C-(6''-O-galactosyl) galactoside in *Cecropia*,²⁸⁵ mollupentin in *Allophylus*,¹⁴³ 8-methoxyswertisin in *Abrus*,²²² and 6-C-(6-deoxy-ribo-hexos-3-ulosyl)apigenin in *Cassia*.¹³⁰
- On luteolin base: 6-C-fucosylluteolin and 6-C-quinovosylluteolin in *Zea*,³³⁵ 6-C-(6-deoxy-ribo-hexos-3-ulosyl)luteolin in *Cassia*,¹³⁰ and 8-quinovosylluteolin in *Turnera*.²⁹⁷
- On 7-O-methyluteolin base: isoswertiajaponin in *Deschampsia*.³⁸³
- On chrysoeriol base: 6-C-boivinosylchrysoeriol, 6-C-fucosylchrysoeriol, and 6-C-(6-deoxy-ribo-hexos-4-ulosyl)chrysoeriol in *Zea*^{333,335,345,346} and 8-C-xylosylchrysoeriol in *Scleranthus*.⁴⁰³
- On diosmetin base: torosaflavone B and 6-C-(6-deoxy-ribo-hexos-3-ulosyl)diosmetin in *Cassia*.¹³⁰
- On flavonol base: 6-C-glucosyl-3-O-glucosyl kaempferol in *Cyclopia*,¹⁶⁸ 6-C-rhamnosylrhamnetin in *Frullania*,³²⁹ and 8-C-glucosylquercetin in *Eucalyptus*.²³²

In addition, four groups of closely related molecules were described:

- Series of cassiaoccidentalins built on a 6-C-(6-deoxy-ribo-hexos-3-ulosyl)flavone general structure, and isolated from *Cassia*¹³⁰ — cassiaoccidentalins A: 6-C-glycosyl (2''-O-rhamnosyl)apigenin; B: 6-C-glycosyl (2''-O-rhamnosyl)luteolin; and C: 6-C-glycosyl (2''-O-rhamnosyl)diosmetin.

TABLE 14.3
Naturally Occurring Di-C-Glycosylflavonoids and Tri-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*])

Compounds	Sources	Ref.
DI-C-GLYCOSYLFLAVONES		
6-C-Glucopyranosyl-8-C-arabinopyranosylchrysin (5,7-diOH 6-Glc 8-Ara)	<i>Scutellaria baicalensis</i> (Lab.) <i>Scutellaria amoena</i> (Lab.)	Takagi et al. (1981) Zhou and Yang (2000)
6-C-Arabinopyranosyl-8-C-glucopyranosylchrysin (5,7-diOH 6-Ara 8-Glc)	<i>Scutellaria baicalensis</i> (Lab.)	Takagi et al. (1981)
Vicenin-2 (5,7,4'-triOH 6,8-diGlc)	<i>Citrus lemon</i> (Rut.) Many sources	Chopin et al. (1964)
3,6-Di-C-glucosylapigenin (5,7,4'-triOH 3,6-diGlc)	<i>Citrus unshiu</i> (Rut.)	Matsubara et al. (1985a)
3,8-Di-C-glucosylapigenin (5,7,4'-triOH 3,8-diGlc)	<i>Citrus sudachi</i> (Rut.)	Matsubara et al. (1985b)
6,8-Di-C-galactopyranosylapigenin (5,7,4'-triOH 6,8-diGal)	<i>Stellaria dichotoma</i> (Caryo.)	Yasukawa et al. (1982)
6-C-Glucopyranosyl-8-C-galactopyranosylapigenin (5,7,4'-triOH 6-Glc-8-Gal)	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1984)
Vicenin-3 (5,7,4'-triOH 6-Glc-8-Xyl)	<i>Vitex lucens</i> (Verb.)	Seikel et al. (1966)
Vicenin-1 (5,7,4'-triOH 6-Xyl-8-Glc)	<i>Vitex lucens</i> (Verb.) <i>Athyriaceae/Aspleniaceae</i> (Pterid.) <i>Cotoneaster wilsonii</i> (Ros.) <i>Prosopis chilensis</i> (Leg.)	Seikel et al. (1966) Umikalsen et al. (1994) Chang and Jeon (2003) Elrady and Saad (1994)
Violanthin (5,7,4'-triOH 6-Glc-8-Rha)	<i>Viola tricolor</i> (Viol.) <i>Viola arvensis</i> (Viol.)	Hörhammer et al. (1965) Carnat et al. (1998)
Isoviolanthin (5,7,4'-triOH 6-Rha-8-Glc)	<i>Angiopteris evecta</i> (Pterid.) <i>Athyriaceae/Aspleniaceae</i> (Pterid.)	Wallace et al. (1979) Umikalsen et al. (1994)
(*) 6-C-β-D-Glucopyranosyl-8-C-β-D-apiofuranosylapigenin (5,7,4'-triOH 6-Glc-8-Apio)	<i>Xanthosoma violaceum</i> (Arac.)	Picerno et al. (2003)
(*) 6-C-β-D-Glucopyranosyl-8-C-β-D-ribofuranosylapigenin (5,7,4'-triOH 6-Glc-8-Rib)	<i>Passiflora incarnata</i> (Passif.)	Chimichi et al. (1998)
Schaftoside (5,7,4'-triOH 6-Glc-8-Ara)	<i>Silene schafta</i> (Caryo.) Many sources	Chopin et al. (1974)
Isoschaftoside (5,7,4'-triOH 6-Ara-8-Glc)	<i>Flourensia cernua</i> (Comp.) Many sources	Dillon et al. (1976)
Neoschaftoside (5,7,4'-triOH 6-Glc-8-Ara)	<i>Catananche coerulea</i> (Comp.)	Proliac et al. (1973)

continued

TABLE 14.3
Naturally Occurring Di-C-Glycosylflavonoids and Tri-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — *continued*

Compounds	Sources	Ref.
	<i>Atractylis carduus</i> (Comp.)	Melek et al. (1992)
	<i>Capsicum annuum</i> (Solan.)	Materska et al. (2003)
	<i>Viola yedoensis</i> (Viol.)	Xie et al. (2003)
Neoisoschaftoside (5,7,4'-triOH 6-Ara-8-Glc)	<i>Minum undulatum</i> (Bryo.)	Osterdahl (1979)
Isocorymboside (5,7,4'-triOH 6-Gal-8-Ara)	<i>Polygonatum multiflorum</i> (Lil.)	Chopin et al. (1977b)
Corymboside (5,7,4'-triOH 6-Ara-8-Glc)	<i>Carlina corymbosa</i> (Comp.)	Besson et al. (1979)
6,8-Di-C-arabinosylapigenin (5,7,4'-triOH 6,8-diAra)	<i>Melilotus alba</i> (Leg.)	Specht et al. (1976)
(*) 6,8-Di-C- α -L-arabinopyranosylapigenin (5,7,4'-triOH 6,8-diAra)	<i>Schnabelia tetradonta</i> (Lab.)	Dou et al. (2002)
(*) 6-C- α -L-Arabinopyranosyl-8-C- β -L-arabinopyranosylapigenin (5,7,4'-triOH 6,8-diAra)	<i>Viola yedoensis</i> (Viol.)	Xie et al. (2003)
(*) 6-C- β -L-Arabinopyranosyl-8-C- α -L-arabinopyranosylapigenin (5,7,4'-triOH 6,8-diAra)	<i>Schnabelia tetradonta</i> (Lab.)	Dou et al. (2002)
6-C- β -D-Xylopyranosyl-8-C- α -L-arabinopyranosylapigenin (5,7,4'-triOH 6-Xyl-8-Ara)	<i>Mollugo pentaphylla</i> (Mollug.) <i>Viola yedoensis</i> (Viol.)	Chopin et al. (1982) Xie et al. (2003)
6-C-Arabinosyl-8-C-xylosylapigenin (5,7,4'-triOH 6-Ara-8-Xyl)	<i>Mollugo pentaphylla</i> (Mollug.) <i>Viola yedoensis</i> (Viol.)	Chopin et al. (1982) Xie et al. (2003)
Neocorymboside (5,7,4'-triOH 6-Ara-8-Gal)	<i>Atractylis gummifera</i> (Comp.)	Chaboud et al. (1988)
(*) 6-C- β -D-Glucopyranosyl-8-C- β -D-6-deoxygulopyranosylapigenin (5,7,4'-triOH 6-Glc-8-Deoxygul)	<i>Viola arvensis</i> (Viol.)	Carnat et al. (1998)
6-C-Glucosyl-8-C-galactosylgenkwanin (5,4'-diOH 7-OMe 6-Glc-8-Gal)	<i>Glycine max</i> (Leg.)	Jay et al. (1984)
6-C-Glucosyl-8-C-arabinosylgenkwanin (5,4'-diOH 7-OMe 6-Glc-8-Ara)	<i>Almeidea guyanensis</i> (Rut.)	Wirasutisna et al. (1986)
Almeidein (5,4'-diOH 7-OMe 6,8-diAra)	<i>Almeida guyanensis</i> (Rut.)	Jay et al. (1979)
6,8-Di-C-glucosylgenkwanin (5,4'-diOH 7-OMe 6,8-diGlc)	<i>Galipea trifoliata</i> (Rut.)	Baktiar et al. (1990)
3,6-Di-C-glucosylacetin (5,7-diOH 4'-OMe 3,6-diGlc)	<i>Fortunella japonica</i> (Rut.)	Kumamoto et al. (1985b)
7,4'-Di-O-methyl-6,8-di-C-arabinosylapigenin		

(5-OH 7,4'-diOMe 6,8-diAra)

Asterostigma riedelianum (Arac.) Markham and Williams (1980)

TABLE 14.3
Naturally Occurring Di-C-Glycosylflavonoids and Tri-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
Lucenin-2 (5,7,3',4'-tetraOH 6,8-diGlc)	<i>Vitex lucens</i> (Verb.) Many sources	Seikel et al. (1966)
Lucenin-3 (5,7,3',4'-tetraOH 6-Glc-8-Xyl)	<i>Vitex lucens</i> (Verb.)	Seikel et al. (1966)
Lucenin-1 (5,7,3',4'-tetraOH 6-Xyl-8-Glc)	<i>Vitex lucens</i> (Verb.)	Seikel et al. (1966)
Carlinoside (5,7,3',4'-tetraOH 6-Glc-8-Ara)	<i>Carlina vulgaris</i> (Comp.) <i>Hordeum vulgare</i> (Gram.)	Raynaud and Rasolojaona (1976) Norbaek et al. (2000)
Isocarlinoside (5,7,3',4'-tetraOH 6-Ara-8-Glc)	<i>Lepedeza capitata</i> (Leg.) <i>Capsicum annuum</i> (Solan.) <i>Viola yedoensis</i> (Viol.)	Linard et al. (1982) Materska et al. (2003) Xie et al. (2003)
Neocarlinoside (5,7,3',4'-tetraOH 6-Glc-8-Ara)	<i>Lepedeza capitata</i> (Leg.)	Linard et al. (1982)
(*) 6-C-β-D-Glucopyranosyl-8-C-α-L-rhamnopyranosylluteolin (Elatin)		
(5,7,3',4'-tetraOH 6-Glc-8-Rha)	<i>Plagiomnium elatum</i> (Bryo.)	Anhut et al. (1992)
(*) 6,8-Di-C-α-L-arabinosylluteolin		
(5,7,4'-triOH 6,8-diAra)	<i>Plagiochasma rupestre</i> (Bryo.)	Schoeneborn and Mues (1993)
(*) 6-C-hexosyl-8-C-rhamnosylchrysoeriol		
(5,7,4'-triOH 3'-OMe 6-hex-8-rha)	<i>Plagiomnium elatum</i> (Bryo.)	Anhut et al. (1992)
6,8-Di-C-Glucosylchrysoeriol (Stellarin-2)		
(5,7,4'-triOH 3'-OMe 6,8-diGlc)	<i>Stellaria holostea</i> (Caryo.) <i>Cydonia oblonga</i> (Ros.) <i>Citrus limon</i> (Rut.)	Zoll and Nouvel (1974) Ferreret et al. (2003) Gil Izquierdo et al. (2004)
6-C-Glucosyl-8-C-arabinosylchrysoeriol		
(5,7,4'-triOH 3'-OMe 6-Glc-8-Ara)	<i>Trichophorum cespitosum</i> (Cyp.)	Salmenkallio et al. (1982)
(*) 6-C-β-D-Glucosyl-8-C-β-D-xylosylchrysoeriol		
(5,7,4'-triOH 3'-OMe 6-Glc-8-Xyl)	<i>Raputia paraensis</i> (Rut.)	Bakhtiar et al. (1991)
(*) 6-C-Xylosyl-8-C-glucosylchrysoeriol		
(5,7,4'-triOH 3'-OMe 6-Xyl-8-Glc)	<i>Raputia paraensis</i> (Rut.)	Bakhtiar et al. (1991)
6-C-Arabinosyl-8-C-glucosylchrysoeriol		
(5,7,4'-triOH 3'-OMe 6-Ara-8-Glc)	<i>Trichophorum cespitosum</i> (Cyp.)	Salmenkallio et al. (1982)
6,8-Di-C-glucosyldiosmetin		
(5,7,3'-triOH 4'-OMe 6,8-diGlc)	<i>Citrus limon</i> (Rut.) <i>Citrus</i> sp. (Rut.)	Kumamoto et al. (1985a) Tsiklauri and Shalashvili (1995)
3,8-Di-C-glucosyldiosmetin		
(5,7,3'-triOH 4'-OMe 3,8-diGlc)	<i>Citrus sudachi</i> (Rut.)	Matsubara et al. (1985b)
6,8-Di-C-glucosyltricetin		
(5,7,3',4',5'-pentaOH 6,8-diGlc)	<i>Plagiochila asplenoides</i> (Bryo.) <i>Frullania polysticha</i> (Bryo.)	Mues and Zinsmeister (1976) Kraut et al. (1993)

continued

TABLE 14.3

Naturally Occurring Di-C-Glycosylflavonoids and Tri-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — *continued*

Compounds	Sources	Ref.
6-C- β -D-Glucosyl-8-C- α -L-arabinosyltricetin (5,7,3',4',5'-pentaOH 6-Glc-8-Ara)	<i>Plagiochila jamesonii</i> (Bryo.)	Schoeneborn and Mues (1993)
6-C-Arabinosyl-8-C-glucosyltricetin (5,7,3',4',5'-pentaOH 6-Ara-8-Glc)	<i>Radula complanata</i> (Bryo.)	Markham and Mues (1984)
(*) 6,8-Di-C- α -L-arabinopyranosyltricetin (5,7,3',4',5'-pentaOH 6,8-diAra)	<i>Plagiochila jamesonii</i> (Bryo.)	Schoeneborn and Mues (1993)
6,8-Di-C-glucosyltricetin (5,7,4'-triOH 3',5'-diOMe 6,8-diGlc)	<i>Apometzgeria pubescens</i> (Bryo.)	Theodor et al. (1980, 1981a)
6-C-Glucosyl-8-C-arabinosyltricetin (5,7,4'-triOH 3',5'-diOMe 6-Glc-8-Ara)	<i>Apometzgeria pubescens</i> (Bryo.)	Theodor et al. (1980, 1981a)
6-C-Arabinosyl-8-C-glucosyltricetin (5,7,4'-triOH 3',5'-diOMe 6-Ara-8-Glc)	<i>Apometzgeria pubescens</i> (Bryo.)	Theodor et al. (1980, 1981a)
6,8-Di-C-arabinosyltricetin (5,7,4'-triOH 3',5'-diOMe 6,8-diAra)	<i>Apometzgeria pubescens</i> (Bryo.)	Theodor et al. (1980, 1981a)
6,8-Di-C-arabinosylapometzgerin (5,7,5'-triOH 3',4'-diOMe 6,8-diAra)	<i>Apometzgeria pubescens</i> (Bryo.)	Theodor et al. (1980, 1981a)
DI-C-GLYCOSYLFLAVANONES		
6,8-Di-C-glucosylnaringenin (5,7,4'-triOH 6,8-diGlc)	<i>Zizyphus jujuba</i> (Rham.)	Okamura et al. (1981)
DI-C-GLYCOSYLISOFLAVONES		
Paniculatin (5,7,4'-triOH 6,8-diGlc)	<i>Dalbergia paniculata</i> (Leg.)	Narayanan and Seshadri (1971)
6,8-Di-C-glucosylorobol (5,7,3',4'-tetraOH 6,8-diGlc)	<i>Dalbergia nitidula</i> (Leg.)	Van Heerden et al. (1980)
DI-C-GLYCOSYLQUINOCHALCONES		
Safflor yellow A	<i>Carthamus tinctoria</i> (Comp.)	Takahashi et al. (1982)
Safflor yellow B	<i>Carthamus tinctoria</i> (Comp.)	Takahashi et al. (1982)
TRI-C-GLYCOSYLFLAVONES		
(*) 3,6,8-Tri-C-xylosylapigenin (5,7,4'-triOH 3,6,8-triXyl)	<i>Asplenium viviparum</i> (Pteri.)	Imperato (1993)

- Series of rhamnellaflavosides based on 6-C-glycosyl (4'-O-glucosyl)apigenin, with three new compounds characterized in *Rhamnella*³⁵⁰ — ramnellaflavoside A: 6-C- β -D-oliosylpyranosyl; B: 6-C- β -D-boivinopyranosyl; and C: 6-C- β -D-4-epioliosyl.
- Series of maysins (6-C-glycosyl, 2''-O-rhamnosylflavone) isolated from *Zea*,^{333,335,345,346} with the following basic structures: 6-C-(6-deoxy-ribo-hexos-4-ulosyl)apigenin, 6-C-fucosylluteolin, 6-C-quinovosylluteolin, 6-C- β -fucosylchrysoeriol, 6-C- β -boivinosylchrysoeriol, and 6-C-(6-deoxy-ribo-hexos-4-ulosyl)chrysoeriol.
- Series of preclatorins isolated from *Abrus*²²² with preclatorin II: 2''-O-apiofuranosyl 8-O-methylswertisin; III: 2''-O-apiofuranosylswertisin (preclatorin I: 8-O-methylswertisin).

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*])

Compounds	Sources	Ref.
MONO-C-GLYCOSYLFLAVONES		
Bayin		
2''-O-Rhamnoside (sophoraflavone A)	<i>Sophora subprostata</i> (Leg.)	Shirataki et al. (1986)
Isovitexin		
X''-Rhamnoside	<i>Bocoa</i> sp. (Leg.)	Kite and Ireland (2002)
X''-Xyloside	<i>Itea/Pterostemom</i> (Saxif.)	Bohm et al. (1999)
7-O-Glucoside (saponarin)	<i>Saponaria officinalis</i> (Caryo.) Many sources	Barger (1906)
7-O-Galactoside (neosaponarin)	<i>Melandrium album</i> (Caryo.)	Wagner et al. (1979)
7-O-Rhamnoside	<i>Yeatesia viridiflora</i> (Acan.)	Hilsenbeck et al. (1984)
7-O-Xyloside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1972)
7-O-Rhamnosylglucoside	<i>Passiflora platyloba</i> (Pass.)	Ayanoglu et al. (1982)
7,2''-Di-O-glucoside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1974)
7,2''-Di-O-galactoside	<i>Gentiana depressa</i> (Gent.)	Chulia (1984)
7-O-Glucoside 2''-O-arabinoside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1972)
7-O-Glucoside 2''-O-rhamnoside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1972)
7-O-Galactoside 2''-O-glucoside	<i>Melandrium album</i> (Caryo.)	Wagner et al. (1979)
7-O-Galactoside 2''-O-rhamnoside	<i>Melandrium album</i> (Caryo.)	Wagner et al. (1979)
7-O-Galactoside 2''-O-arabinoside	<i>Silene pratensis</i> (Caryo.)	Steyns et al. (1983)
7-O-Xyloside 2''-O-glucoside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1974)
7-O-Xyloside 2''-O-rhamnoside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1974)
7-O-Xyloside 2''-O-arabinoside	<i>Melandrium album</i> (Caryo.)	Van Brederode and Nigtevecht (1974)
7-O-Arabinoside 2''-O-glucoside	<i>Silene dioica</i> (Caryo.)	Mastenbroek et al. (1983)
4'-O-Glucoside (isosaponarin)	<i>Spirodela oligorrhiza</i> (Lemn.)	Jurd et al. (1957)
4'-O-Arabinoside	<i>Leptodactylon</i> sp. (Polem.)	Smith et al. (1982)
4'-X-O-Diglucoside	<i>Cucumis sativus</i> (Cucurb.)	Krauze and Cisowski (2001)
4',2''-Di-O-glucoside	<i>Gentiana asclepiadea</i> (Gent.)	Goetz and Jacot-Guillarmod (1977)
4'-O-Glucoside 2''-O-arabinoside	<i>Vaccaria segetalis</i> (Caryo.)	Baeva et al. (1974)
(*) 4'-O-Rhamnoside	<i>Xanthosoma violaceum</i> (Arac.)	Picerno et al. (2003)
2''-O-Arabinoside	<i>Avena sativa</i> (Gram.) <i>Centaurea triumphetti</i> (Comp.)	Chopin et al. (1977a) Gonnet (1993)
2''-O-Galactoside	<i>Secale cereale</i> (Gram.)	Dellamonica et al. (1983)
2''-O-Glucoside	<i>Oxalis acetosella</i> (Oxal.) <i>Centaurea triumphetti</i> (Comp.) <i>Cucumis sativus</i> (Cucurb.) <i>Passiflora incarnata</i> (Passif.) <i>Zizyphus jujuba</i> (Rham.)	Tschesche and Struckmeyer (1976) Gonnet (1993) Krauze and Cisowski (2001) Li et al. (1991), Rahman et al. (1997) Cheng et al. (2000)
2''-O-Ramnoside	<i>Crataegus monogyna</i> (Ros.) <i>Allophylus edulis</i> (Sapin.) <i>Biophytum sensitivum</i> (Oxal.) <i>Tripterospermum japonicum</i> (Gent.)	Nikolov et al. (1976) Hoffmann et al. (1992) Bucar et al. (1998) Otsuka and Kijima (2001)
2''-O-Xyloside	<i>Desmodium canadense</i> (Leg.) <i>Tripterospermum japonicum</i> (Gent.)	Chernobrovaya (1973) Otsuka and Kijima (2001)
6''-O-Arabinoside	<i>Swertia perennis</i> (Gent.)	Hostettmann and Jacot-Guillarmod (1976)

continued

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — *continued*

Compounds	Sources	Ref.
(*) 6''-O-Glucoside	<i>Gentiana arisanensis</i> (Gent.)	Lin et al. (1997)
(*) 6''-O-Rhamnoside	<i>Vitis</i> sp. (Vitid.) <i>Vigna radiata</i> (Legum.)	Moore and Giannasi (1994) Larsen et al. (1995)
8-C-<i>p</i>-Hydroxybenzylisovitexin		
(*) 4'-O-Glucoside	<i>Citrullus colocynthis</i> (Cucurb.)	Maatooq et al. (1997)
Vitexin		
X''-O-Rhamnoside	<i>Bocoa</i> sp. (Leg.)	Kite and Ireland (2002)
X''-O-Xyloside	<i>Itea/Pterostemom</i> (Saxif.)	Bohm et al. (1999)
7-O-Glucoside	<i>Trigonella fenumgreacum</i> (Leg.)	Adamska and Lutomski (1971)
7-O-Rhamnosylglucoside	<i>Phoenix</i> sp. (Palm.)	Williams et al. (1973)
4'-O-Glucoside	<i>Brisa media</i> (Gram.)	Williams and Murray (1972)
4'-O-Galactoside	<i>Crotalaria retusa</i> (Leg.)	Srinivasan and Subramanian (1983)
4'-O-Rhamnoglucoside	<i>Crataegus oxyacantha</i> (Ros.)	Lewak (1966)
4'-O-Glucoside 2''-O-rhamnoside	<i>Passiflora coactilis</i> (Pass.)	Escobar et al. (1983)
(*) 2''-O- α -D-Arabinofuranoside	<i>Cotoneaster thymaefolia</i> (Ros.)	Palme et al. (1994)
2''-O-Glucoside	<i>Polygonatum odoratum</i> (Lil.) <i>Alternanthera maritima</i> (Amaranth.)	Morita et al. (1976) Salvador and Dias (2004)
2''-O-Rhamnoside	<i>Crataegus monogyna</i> (Ros.) <i>Alternanthera maritima</i> (Amaranth.) <i>Allophylus edulis</i> (Sapind.) <i>Clusia sandiensis</i> (Gutt.) <i>Cotoneaster thymaefolia</i> (Ros.) <i>Crataegus monogyna</i> (Ros.) <i>Crataegus sinaica</i> (Ros.) <i>Crataegus pinnatifida</i> (Ros.) <i>Rhamnella inaequilatera</i> (Rham.) <i>Turnera diffusa</i> (Turn.)	Nikolov et al. (1976) Salvador and Dias (2004) Hoffmann et al. (1992) Delle Monache (1991) Palme et al. (1994) Rehwald et al. (1994) Kim and Kim (1993) Zhang and Xu (2002) Takeda et al. (2003) Piacente et al. (2002)
2''-O-Xyloside	<i>Vitex lucens</i> (Verb.) <i>Beta vulgaris</i> (Chenop.) <i>Citrus</i> sp. (Rut.)	Seikel et al. (1959) Gil et al. (1998) Tsiklauri and Shalashvili (1995), Manthey et al. (2001)
2''-O-Sophoroside	<i>Polygonatum odoratum</i> (Lil.)	Morita et al. (1976)
4''-O-Rhamnoside	<i>Crataegus curvisepala</i> (Ros.) <i>Silene conoidea</i> (Caryo.)	Batyuk et al. (1966) Ali et al. (1999)
6''-O-Gentiobioside (marginatoside)	<i>Piper marginatum</i> (Pip.)	Tillequin et al. (1978)
(*) 6''-O-Glucoside	<i>Xanthosoma violaceum</i> (Arac.)	Picerno et al. (2003)
6''-O-Rhamnoside	<i>Larix sibirica</i> (Gymno.)	Medvedeva et al. (1974)
6''-O-Xyloside	<i>Gypsophila paniculata</i> (Caryo.)	Darmograi et al. (1968)
(*) 6''-O-Rhamnosyl-4'''-O-glucosyl-2'''-O-galactosyl (panzhihuacyside)	<i>Cycas panzhihuaensis</i> (Cyc.)	Zhou et al. (2002)
6-C-Xylosylapigenin (cerarvensin)		
7-O-Glucoside	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1980)

2''-O-Rhamnoside

Phlox drummondii (Polem.)

Bouillant et al. (1978)

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
	<i>Allophylus edulis</i> (Sapind.)	Hoffmann et al. (1992)
	<i>Rhamnella inaequilatera</i> (Rham.)	Takeda et al. (2004)
6-C-Arabinosylapigenin (isomollupentin)		
7-O-Glucoside	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1985)
	<i>Climacanthus nutans</i> (Acant.)	Teshima et al. (1997)
7-O-Rhamnosylglucoside	<i>Passiflora platyloba</i> (Pass.)	Ayanoglu et al. (1982)
7,2''-Di-O-glucoside	<i>Spergularia rubra</i> (Caryo.)	Bouillant et al. (1979)
7-O-Glucoside 2''-O-arabinoside	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1983)
7-O-Glucoside 2''-O-xyloside	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1983)
4'-O-Glucoside	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1985)
2''-O-Glucoside	<i>Cerastium arvense</i> (Caryo.)	Dubois et al. (1985)
4''-O-Rhamnoside (hemsleyanoside)	<i>Tetrastigma hemsleyanum</i> (Vitid.)	Liu et al. (2002)
6-C-β-D-Oliopyranosylapigenin		
(*) 4'-O-β-D-Glucopyranoside (rhamnellaflavoside A)	<i>Rhamnella inaequilatera</i> (Rham.)	Takeda et al. (2004)
6-C-β-D-Boivinopyranosylapigenin		
(*) 4'-O-β-D-Glucopyranoside (rhamnellaflavoside B)	<i>Rhamnella inaequilatera</i> (Rham.)	Takeda et al. (2004)
6-C-β-D-4-Epioliosylapigenin		
(*) 4'-O-β-D-Glucopyranoside (rhamnellaflavoside C)	<i>Rhamnella inaequilatera</i> (Rham.)	Takeda et al. (2004)
6-C-Rhamnosylapigenin (isofurcatain)		
7-O-Glucoside	<i>Metzgeria furcata</i> (Bryo.)	Markham et al. (1982)
6-C-Galactosylapigenin		
(*) 6''-O-Galactoside	<i>Cecropia lyratifolia</i> (Urt.)	Oliveira et al. (2003)
6-C-(6-Deoxy-xylo-hexos-4-ulosyl)apigenin		
2''-O-Rhamnoside (apimaysin)	<i>Zea mays</i> (Gram.)	Elliger et al. (1980)
6-C-(6-Deoxy-ribo-hexos-3-ulosyl)apigenin		
(*) 2''-O-Rhamnoside (cassiaoccidentalinalin A)	<i>Cassia occidentalis</i> (Leg.)	Hatano et al. (1999)
8-C-Arabinosylapigenin (mollupentin)		
(*) 2''-O-Rhamnoside	<i>Allophylus edulis</i> (Sapind.)	Hoffmann et al. (1992)
(*) 4''-O-Rhamnoside (isohemsleyanoside)	<i>Tetrastigma hemsleyanum</i> (Vitid.)	Liu et al. (2002)
Swertisin		
5-O-Glucoside	<i>Enicostemma hyssopifolium</i> (Gent.)	Ghosal and Jaiswal (1980)
4'-O-Glucoside		
4'-O-Glucoside (flavocommelinin)	<i>Commelina</i> sp.(Comm.)	Komatsu et al. (1968)
4'-O-Rhamnoside	<i>Passiflora biflora</i> (Pass.)	McCormick and Mabry (1983)
(*) 4'-O-Glucoside 2''-O-rhamnoside	<i>Felicia amelloides</i> (Comp.)	Bloor (1999)
(*) 2''-O-Apiofuranoside (preparatorin III)	<i>Abrus precatorius</i> (Leg.)	Ma et al. (1998)
2''-O-Arabinoside	<i>Achillea fragrantissima</i> (Comp.)	Ahmed et al. (1988)
2''-O-Glucoside (spinosin)	<i>Zizyphus vulgaris</i> (Rham.)	Woo et al. (1979)
	<i>Desmodium tortuosum</i> (Leg.)	Lewis et al. (2000)
	<i>Zizyphus jujuba</i> (Rham.)	Cheng et al. (2000)
2''-O-Rhamnoside	<i>Gemmingia chinensis</i> (Irid.)	Shirane et al. (1982)
	<i>Aleurites moluccana</i> (Euphorb.)	Meyre-Silva et al. (1999)

continued

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Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — *continued*

Compounds	Sources	Ref.
4''-O-Glucoside (zivulgarin)	<i>Zizyphus spinosus</i> (Rham.)	Zeng et al. (1987)
6''-O-Rhamnoside (fagovatin)	<i>Fagraea obovata</i> (Logan.)	Qasim et al. (1987)
8-O-Methylswertisin		
(*) 2''-O-Apiofuranoside (preparatorin II)	<i>Abrus precatorius</i> (Leg.)	Ma et al. (1998)
Isoswertisin		
5-O-Glucoside	<i>Enicostemma hyssopifolium</i> (Gent.)	Ghosal and Jaiswal (1980)
4'-O-Glucoside	<i>Triticum aestivum</i> (Gram.)	Julian et al. (1971)
(*) 2''-O-β-Arabinoside	<i>Deschampsia antarctica</i> (Gram.)	Webby and Markham (1994)
2''-O-Glucoside (isospinosin)	<i>Gnetum</i> sp. (Gymn.)	Ouabonzi et al. (1983)
	<i>Zizyphus jujuba</i> (Rham.)	Cheng et al. (2000)
2''-O-Rhamnoside	<i>Avena sativa</i> (Gram.)	Chopin et al. (1977a)
2''-O-Xyloside	<i>Gnetum</i> sp. (Gymn.)	Ouabonzi et al. (1983)
Isomolludistin		
2''-O-Glucoside	<i>Asterostigma riedelianum</i> (Arac.)	Markham and Williams (1980)
Molludistin		
2''-O-Glucoside	<i>Almeidea guyanensis</i> (Rut.)	Jay et al. (1979)
2''-O-Xyloside	<i>Almeidea guyanensis</i> (Rut.)	Wirasutisna et al. (1986)
2''-O-Rhamnoside	<i>Mollugo distica</i> (Mollug.)	Chopin et al. (1978)
Isocytoside		
7-O-Glucoside	<i>Gentiana pyrenaica</i> (Gent.)	Marston et al. (1976)
2''-O-Glucoside	<i>Securigera coronilla</i> (Leg.)	Jay et al. (1980)
2''-O-Rhamnoside (isomargariten)	<i>Fortunella margarita</i> (Rut.)	Horowitz et al. (1974)
	<i>Ornithocephalinae</i> (Orch.)	Williams et al. (1994 a)
(*) 3''-O-α-L-Rhamnopyranoside	<i>Anthurium versicolor</i> (Arac.)	Aquino et al. (2001)
(*) 3''-O-β-D-Xylopyranoside	<i>Anthurium versicolor</i> (Arac.)	Aquino et al. (2001)
(*) 6''-O-β-D-Apiofuranoside	<i>Anthurium versicolor</i> (Arac.)	Aquino et al. (2001)
Cytoside		
7-O-Glucoside	<i>Trema aspera</i> (Ulm.)	Oelrichs et al. (1968)
2''-O-Rhamnoside (margariten)	<i>Fortunella margarita</i> (Rut.)	Horowitz et al. (1974)
(*) 3''-O-β-D-Rhamnopyranoside	<i>Anthurium versicolor</i> (Arac.)	Aquino et al. (2001)
Embigenin		
2''-O-Glucoside (embinoidin)	<i>Siphonoglossa sessilis</i> (Acan.)	Hilsenbeck and Mabry (1983)
2''-O-Rhamnoside (embinin)	<i>Iris tectorum</i> (Irid.)	Hirose et al. (1962)
	<i>Ornithocephalinae</i> (Orch.)	Williams et al. (1994a)
7-4'-Di-O-methylisomollupentin		
2''-O-Glucoside	<i>Asterostigma riedelianum</i> (Arac.)	Markham and Williams (1980)
Abrusin		
2''-O-β-apiofuranoside	<i>Abrus precatorius</i> (Leg.)	Markham et al. (1989)
	<i>Abrus precatorius</i> (Leg.)	Ma et al. (1998)
Isorientin		
X''-O-Xyloside	<i>Itea/Pterostemom</i> (Saxif.)	Bohm et al. (1999)
7-O-Apioside	<i>Vellozia</i> sp. (Vell.)	Williams et al. (1991)
	<i>Vellozia</i> sp. (Velloz.)	Harborne et al. (1993)
	(Velloz.)(Velloz.)	Williams et al. (1994b)

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
7- <i>O</i> -Galactoside	<i>Eminium spiculatum</i> (Epacr.)	Shammas and Couladi (1988)
7- <i>O</i> -Glucoside (lutonarin)	<i>Hordeum vulgare</i> (Gram.) <i>Biophytum sensitivum</i> (Oxal.) <i>Bryonia</i> sp. (Cucurb.) <i>Hordeum vulgare</i> (Gram.) <i>Lagenaria siceraria</i> (Cucurb.) <i>Plagiomnium</i> sp. (Bryo.) <i>Vellozia</i> sp. (Velloz.) (Velloz.)	Seikel and Bushnell (1959) Bucar et al. (1998) Krauze and Cisowski (1994) Markham and Mitchell (2003) Krauze and Cisowski (1994) Anhut et al. (1992) Harborne et al. (1993, 1994) Williams et al. (1994b)
(*) 7- <i>O</i> -Di-glucoside	<i>Vellozia</i> sp. (Vell.)	Williams et al. (1991)
(*) 7- <i>O</i> -Rhamnoside	<i>Hyparrhenia hirta</i> (Gram.)	Bouaziz et al. (2001)
7- <i>O</i> -Rhamnosylglucoside	<i>Triticum aestivum</i> (Gram.)	Julian et al. (1971)
(*) 7- <i>O</i> -Xyloside	<i>Vellozia</i> sp. (Velloz.) (Velloz.)	Harborne et al. (1993, 1994) Williams et al. (1994b)
3'- <i>O</i> -Glucoside	<i>Gentiana nivalis</i> (Gent.)	Hostettmann andet Jacot-Guillarmod (1974)
3'- <i>O</i> -Glucuronide	<i>Rynchospora eximia</i> (Cyp.)	Williams and Harborne (1977)
3'- <i>O</i> -Neohesperidoside	<i>Plagiomnium affine</i> (Bryo.)	Freitag et al. (1986)
3'- <i>O</i> -Sophoroside	<i>Plagiomnium affine</i> (Bryo.)	Freitag et al. (1986)
3',6''-Di- <i>O</i> -glucoside	<i>Gentiana pedicellata</i> (Gent.)	Chulia and Mariotte (1985)
4'- <i>O</i> -Glucoside	<i>Briza media</i> (Gram.)	Williams and Murray (1972)
4',2''-Di- <i>O</i> -glucoside	<i>Gentiana asclepiadea</i> (Gent.)	Goetz and Jacot-Guillarmod (1977)
(*) 2''- <i>O</i> -Apiofuranoside	<i>Achillea nobilis</i> (Comp.)	Marchart et al. (2003), Krenn et al. (2003)
2''- <i>O</i> -Arabinoside	<i>Avena sativa</i> (Gram.) <i>Centaurea triumfetti</i> (Comp.)	Chopin et al. (1977a) Gonnet (1993)
2''- <i>O</i> -β-L-Arabinofuranoside	<i>Trichomanes venosum</i> (Pterido.)	Markham and Wallace (1980)
2''- <i>O</i> -Glucoside	<i>Gentiana verna</i> (Gent.) <i>Centaurea triumfetti</i> (Comp.) <i>Passiflora incarnata</i> (Passif.)	Hostettmann and Jacot-Guillarmod (1975) Gonnet (1993) Qimin et al. (1991) Rahman et al. (1997)
2''- <i>O</i> -Mannoside	<i>Poa annua</i> (Gram.)	Rofi and Pomilio (1987)
2''- <i>O</i> -Rhamnoside	<i>Coronilla varia</i> (Leg.) <i>Biophytum sensitivum</i> (Oxal.)	Sherwood et al. (1973) Bucar et al. (1998)
2''- <i>O</i> -Xyloside	<i>Desmodium canadense</i> (Leg.)	Chernobrovaya (1973)
6''- <i>O</i> -Arabinoside	<i>Swertia perennis</i> (Gent.)	Hostettmann and Jacot-Guillarmod (1976)
6''- <i>O</i> -Glucoside	<i>Gentiana pedicellata</i> (Gent.) <i>Gentiana arisanensis</i> (Gent.)	Chulia and Mariotte (1985), Chulia et al. (1986) Lin et al. (1997)
6''- <i>O</i> -Di-glucoside	<i>Triticum</i> sp. (Gram.)	Harborne et al. (1986a)
6''- <i>O</i> -Rhamnoside	<i>Triticum</i> sp. (Gram.)	Harborne et al. (1986)
Orientin		
7- <i>O</i> -Glucoside	<i>Phenix canariensis</i> (Palm.) <i>Vellozia</i> sp. (Vell.)	Harborne et al. (1974) Williams et al. (1994b)
7- <i>O</i> -Rhamnoside	<i>Linum usitatissimum</i> (Lin.)	Ibrahim and Shaw (1970)
4'- <i>O</i> -Glucoside	<i>Briza media</i> (Gram.)	Williams and Murray (1972)
4'- <i>O</i> -Glucoside 2''- <i>O</i> -rhamnoside	<i>Passiflora coactilis</i> (Pass.)	Escobar et al. (1983)

continued

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — *continued*

Compounds	Sources	Ref.
2''-O-β-L-Arabinofuranoside	<i>Trichomanes venosum</i> (Pterido.)	Markham and Wallace (1980)
(*) 2''-O-α-L-Arabinopyranoside	<i>Deschampsia antarctica</i> (Gram.)	Webby and Markham (1994)
2''-O-Glucoside	<i>Cannabis sativa</i> (Cannab.)	Segelman et al. (1978)
2''-O-Rhamnoside	<i>Crataegus monogyna</i> (Ros.) <i>Allophylus edulis</i> (Sapind.) <i>Turnera diffusa</i> (Turn.)	Nikolov et al. (1976) Hoffmann et al. (1992) Piacente et al. (2002)
2''-O-Xyloside (adonivernith)	<i>Adonis vernalis</i> (Ranun.)	Hörhammer et al. (1960)
6-C-(6-Deoxy-xylo-hexos-4-ulosyl)luteolin		
2''-O-α-L-Rhamnoside (maysin)	<i>Zea mays</i> (Gram.)	Waisse et al. (1979)
6-C-Fucosylluteolin		
(*) 2''-O-α-L-Rhamnoside (ax-4''-OH maysin)	<i>Zea mays</i> (Gram.) <i>Mimosa pudica</i> (Mim.)	Snook et al. (1995) Lobstein et al. (2002)
6-C-Quinovopyranosylluteolin		
(*) 2''-O-α-L-Rhamnoside (eq-4''-OH maysin)	<i>Zea mays</i> (Gram.)	Snook et al. (1995)
6-C-(6-Deoxy-ribo-hexos-3-ulosyl)luteolin		
(*) 2''-O-Rhamnoside (cassiaoccidentalinalin B)	<i>Cassia occidentalis</i> (Leg.) <i>Mimosa pudica</i> (Leg.)	Hatano et al. (1999) Lobstein et al. (2002)
6-C-Xylosylluteolin		
2''-O-Rhamnoside	<i>Phlox drummondii</i> (Polem.)	Bouillant et al. (1984)
8-C-Quinovopyranosylluteolin		
(*) 2''-O-α-L-Rhamnopyranoside	<i>Turnera diffusa</i> (Turn.)	Piacente et al. (2002)
Swertiajaponin		
3'-O-Gentiobioside	<i>Phragmites australis</i> (Gram.)	Nawwar et al. (1980)
3'-O-Glucoside	<i>Phragmites australis</i> (Gram.)	Nawwar et al. (1980)
(*) 4'-O-Di-glucoside	<i>Cucumis sativus</i> (Cucurb.)	Krauze and Cisowski (2001)
4'-O-Rhamnoside	<i>Passiflora biflora</i> (Pass.)	McCormick and Mabry (1983)
2''-O-Glucoside (luteoayamenin)	<i>Iris nertshinskia</i> (Irid.)	Hirose et al. (1981)
2''-O-Rhamnoside	<i>Securigera coronilla</i> (Leg.)	Jay et al. (1980)
Isoswertiajaponin		
(*) 2''-O-Arabinopyranoside	<i>Deschampsia antarctica</i> (Gram.)	Webby and Markham (1994)
6-C-β-Boivinopyranosylchrysoeriol		
(*) 7-O-β-Glucopyranoside	<i>Zea mays</i> (Gram.)	Suzuki et al. (2003b)
6-C-β-Fucosylchrysoeriol		
(*) 2''-O-Rhamnoside (ax-4''-OH 3'-OMe maysin)	<i>Zea mays</i> (Gram.)	Snook et al. (1995)
6-C-(6-Deoxy-xylo-hexos-4-ulosyl)chrysoeriol		
(*) 2''-O-α-L-Rhamnoside (3'-OMe maysin)	<i>Zea mays</i> (Gram.)	Snook et al. (1993)
8-C-Xylopyranosylchrysoeriol		
(*) 2''-O-Glucoside	<i>Scleranthus uncinatus</i> (Caryo.)	Yayli et al. (2001)
8-C-β-L-Xylofuranosylchrysoeriol		

(*) 2''-O-Glucoside

Scleranthus uncinatus (Caryo.)

Yayli et al. (2001)

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
Isoscoparin		
7-O-Glucoside	<i>Hordeum vulgare</i> (Gram.)	Seikel et al. (1962)
	<i>Hordeum vulgare</i> (Gram.)	Norbaek et al. (2000)
	<i>Plagiomnium</i> sp. (Bryo.)	Anhut et al. (1992)
2''-O-Glucoside	<i>Oryza sativa</i> (Gram.)	Besson et al. (1985)
	<i>Centaurea trimufetti</i> (Comp.)	Gonnet (1993)
	<i>Passiflora incarnata</i> (Pass.)	Rahman et al. (1997)
2''-O-Rhamnoside	<i>Silene alba</i> (Caryo.)	Van Brederode and Kamps-Heinsbroek (1981)
Scoparin		
2''-O-Glucoside	<i>Setaria italica</i> (Gram.)	Gluchoff-Fiasson et al. (1989)
2''-O-Rhamnoside	<i>Passiflora coactilis</i> (Pass.)	Escobar et al. (1983)
2''-O-Xyloside	<i>Setaria italica</i> (Gram.)	Gluchoff-Fiasson et al. (1989)
(*) 6''-O-Glucoside	<i>Turnera diffusa</i> (Turn.)	Piacente et al. (2002)
Episcoparin		
7-O-Glucoside (knautoside)	<i>Knautia montana</i> (Dips.)	Zemtsova and Bandyukova (1974)
Torosafllavone B		
(*) 3'-O-β-D-Glucoside	<i>Cassia torosa</i> (Leg.)	Kitanaka and Takido (1992)
	<i>Cassia occidentalis</i> (Leg.)	Hatano et al. (1999)
8-C-Glucosyldiosmetin		
2''-O-Rhamnoside	<i>Fortunella japonica</i> (Rut.)	Kumamoto et al. (1985b)
(*) 4''-O-Rhamnopyranoside	<i>Silene conoidea</i> (Caryo.)	Ali et al. (1999)
6-C-(6-Deoxy-ribo-hexos-3-ulosyl)diomestin		
(*) 2''-O-Rhamnoside (cassiaoccidentalinalin C)	<i>Cassia occidentalis</i> (Leg.)	Hatano et al. (1999)
7,3',4'-Tri-O-methylisoorientin		
2''-O-Rhamnoside (linoside B)	<i>Linum maritimum</i> (Lin.)	Volk and Sinn (1968)
Isopyrenin		
7-O-Glucoside	<i>Gentiana pyrenaica</i> (Gent.)	Marston et al. (1976)
6-C-Glucosyl-5,7-dihydroxy-8,3',4',5'-tetramethoxyflavone		
5-O-Rhamnoside	<i>Vitex negundo</i> (Verb.)	Subramanian and Misra (1979)
MONO-C-GLYCOSYLFLAVONOLS		
6-C-β-D-Glucopyranosylkaempferol		
(*) 3-O-β-D-Glucopyranoside	<i>Cyclopia intermedia</i> (Leg.)	Kamara et al. (2003)
6-C-Rhamnopyranosylrhamnetin		
(*) 3-O-Glucopyranoside	<i>Frullania tamarisei</i> (Bryo.)	Singh and Singh (1991)
8-C-Glucosylquercetin		
(*) 2''-O-Rhamnoside	<i>Eucalyptus globulus</i> (Myrt.)	Manguro et al. (1995)
MONO-C-GLYCOSYLISOFLAVONES		
Puerarin		
6''-O-β-Apiofuranoside (mirificin)	<i>Pueraria mirifica</i> (Leg.)	Ingham et al. (1986)
6''-O-Xyloside	<i>Pueraria lobata</i> (Leg.)	Kinjo et al. (1987)

continued

TABLE 14.4
Naturally Occurring C-Glycosylflavonoids O-Glycosides. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — *continued*

Compounds	Sources	Ref.
8-C-Glucosylgenistein		
6''-O-Apiosyl	<i>Pueraria lobata</i> (Leg.)	Kinjo et al. (1987)
MONO-C-GLYCOSYLCHROMONES		
Chromones		
Aloeresin B		
7-O-Glucoside	<i>Aloe</i> sp. (Lil.)	Speranza et al. (1985)
7-Hydroxy-5-methyl-2-acetyl-6-C-glucopyranosylchromone 2''-O-glucoside	<i>Chrozophora prostrata</i> (Euph.)	Agrawal and Singh (1988)
DI-C-GLYCOSYLFLAVONES		
Vicenin-2		
6''-O-Glucoside	<i>Stellaria holostea</i> (Caryo.)	Bouillant et al. (1984)
(*) 2''-2'''-Di-O-β-Glucopyranoside	<i>Ephedra aphylla</i> (Ephed.)	Hussein et al. (1997)
Schaftoside		
6''-O-Glucoside	<i>Stellaria holostea</i> (Caryo.)	Bouillant et al. (1984)
6,8-Di-C-glycosylgenkwamin		
2'''-O-Xyloside	<i>Galipea trifoliata</i> (Rut.)	Bakhtiar et al. (1990)
Lucenin-2		
3'-O-Glucoside	<i>Pleurozia</i> sp. (Bryo.)	Mues et al. (1991)

Finally, in two molecules the flavone structure was linked to four sugar residues: 2'', 2'''-di-O-β-glucopyranosylvicenin-2 with two C-glycosides and two O-glycosides, isolated from *Ephedra*,¹⁵³ and 6''-O-rhamnosyl 4'''-O-glucosyl 2'''-O-galactosylvitexin with one C-glycoside and three O-glycosides, named panzhihuacacaside, and isolated from *Cycas*⁴¹⁶ (Figure 14.2).

14.2.4 O-ACYL-C-GLYCOSYLFLAVONIDS

Forty new compounds have been reported in this class during the last 12 years. Many references concerned the well-known O-acetyl, O-caffeoyl, O-p-coumaroyl, and O-feruloyl esters. The p-hydroxybenzoyl and p-methoxybenzoyl groups gave rise to two new natural 6''-O-acylated isoorientin derivatives in *Polygonum*⁴¹⁸: perfoliatumin A and B.

However, the true novelty comes with four O-galloyl esters identified in *Terminalia*²¹³ and *Pelargonium*,²⁰⁰ six O-methylbutyryl esters in *Trollius*,⁴²⁰ one O-malonyl ester in *Beta*,¹⁰⁹ and one O-(3-hydroxy-3-methyl)glutaroyl ester in *Glycyrrhiza*.²¹⁶

14.3 SEPARATION AND IDENTIFICATION OF C-GLYCOSYLFLAVONIDS

14.3.1 CAPILLARY ZONE ELECTROPHORESIS SEPARATION

For the separation of C-glycosylflavones, high-performance liquid chromatography (HPLC) has been mainly used in recent works. However, we have to underline a few interesting contributions on the performance of capillary zone electrophoresis (CZE) in this field.

TABLE 14.5
Naturally Occurring Acyl-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*])

Compounds	Sources	Ref.
MONO-C-GLYCOSYLFLAVONES		
Isovitexin		
(*) 7- <i>O</i> -(6'''-Caffeoyl)- β -D-glucopyranoside	<i>Bryonia dioica</i> (Cucurb.)	Krauze and Cisowski (1995a)
(*) 7- <i>O</i> -(6'''- <i>O</i> - <i>E</i> - <i>p</i> -Coumaroyl)glucoside	<i>Hordeum vulgare</i> (Gram.)	Norbaek et al. (2003)
7- <i>O</i> -Feruloylglucoside	<i>Silene pratense</i> (Caryo.) <i>Hordeum vulgare</i> (Gram.)	Niemann (1981, 1982) Norbaek et al. (2003)
(*) 7- <i>O</i> -(6'''- <i>O</i> - <i>E</i> -Feruloyl)glucoside	<i>Hordeum vulgare</i> (Gram.)	Norbaek et al. (2003)
7-Sulfate	(Palm.)	Williams et al. (1973)
(*) 2'',6''-Di- <i>O</i> -acetyl	<i>Crotalaria thebaica</i> (Leg.)	Ibraheim (1994)
(*) 2''- <i>O</i> -(6'''-(<i>E</i>)- <i>p</i> -Coumaroyl)glucoside	<i>Cucumis sativus</i> (Cucurb.)	Aboud-Zaid et al. (2001)
(*) 2''- <i>O</i> -(6'''-(<i>E</i>)- <i>p</i> -Coumaroyl) Glucoside	<i>Cucumis sativus</i> (Cucurb.)	Aboud-Zaid et al. (2001)
4'- <i>O</i> -glucoside	<i>Gentiana punctata</i> (Gent.)	Luong and Jacot-Guillarmod (1977)
2''- <i>O</i> -Feruloyl		
(*) 2''- <i>O</i> -Feruloylglucoside	<i>Bryonia</i> sp. (Cucurb.) <i>Cucumis sativus</i> (Cucurb.)	Krauze and Cisowski (1994) Aboud-Zaid et al. (2001)
2''- <i>O</i> -(<i>E</i>)-Feruloyl 4'- <i>O</i> -glucoside	<i>Gentiana punctata</i> (Gent.)	Luong and Jacot-Guillarmod (1977)
(*) 2''- <i>O</i> -(6'''-(<i>E</i>)-Feruloyl)glucoside		
4'- <i>O</i> -Glucoside	<i>Cucumis sativus</i> (Cucurb.)	Aboud-Zaid et al. (2001)
(*) 2''- <i>O</i> -Galloyl	<i>Terminalia catappa</i> (Combr.) <i>Pelargonium reniforme</i> (Ger.)	Lin et al. (2000) Latte et al. (2002)
(*) 6''- <i>O</i> -Acetyl	<i>Crotalaria thebaica</i> (Leg.)	Ibraheim (1994)
Vitexin		
<i>X</i> - <i>O</i> -Acetyl 2''- <i>O</i> -rhamnoside	<i>Crataegus sinaica</i> (Ros.)	El-Mousallamy (1998)
7-Sulfate	(Palm.)	Williams et al. (1973)
7- <i>O</i> -Rutinoside sulfate	(Palm.)	Williams et al. (1973)
2''- <i>O</i> -Acetyl	<i>Crataegus sanguinea</i> (Ros.) <i>Crataegus pinnatifida</i> (Ros.)	Kashnikova et al. (1984) Zang and Xu (2002)
(*) 2''- <i>O</i> -Acetyl 4''- <i>O</i> -rhamnoside	<i>Crataegus monogyna</i> (Ros.)	Rehwald et al. (1994)
(*) 2''- <i>O</i> -(2''-Methylbutyryl)	<i>Trollius ledebouri</i> (Ranun.)	Zou et al. (2004)
(*) 2''- <i>O</i> -(3'',4''-Dimethylbutyryl)	<i>Trollius ledebouri</i> (Ranun.)	Zou et al. (2004)
2''- <i>O</i> - <i>p</i> -Coumaroyl	<i>Trigonella fenum-graecum</i> (Leg.)	Sood et al. (1976)
2''- <i>O</i> - <i>p</i> -Coumaroyl 7- <i>O</i> -glucoside	<i>Mollugo oppositifolia</i> (Mollug.)	Chopin et al. (1984)
(*) 2''- <i>O</i> -Galloyl	<i>Terminalia catappa</i> (Combr.) <i>Pelargonium reniforme</i> (Ger.)	Lin et al. (2000) Latte et al. (2002)
2''- <i>O</i> - <i>p</i> -Hydroxybenzoyl	<i>Vitex lucens</i> (Verb.)	Horowitz and Gentili (1966)
(*) 3''- <i>O</i> -Acetyl	<i>Crataegus pinnatifida</i> (Ros.)	Zang and Xu (2002)
(*) 6''- <i>O</i> -Acetyl	<i>Crataegus pinnatifida</i> (Ros.)	Zang and Xu (2002)
6''- <i>O</i> -Acetyl 4'- <i>O</i> -rhamnoside		
(Cratenacin)	<i>Crataegus curvisepala</i> (Ros.)	Batyuk et al. (1966)
(*) 6''- <i>O</i> -Acetyl 2''- <i>O</i> -rhamnoside	<i>Clusia sandiensis</i> (Gutt.)	Delle Monache (1991)
(*) 6''- <i>O</i> -Malonyl 2''- <i>O</i> -xyloside	<i>Beta vulgaris</i> (Chenop.)	Gil et al. (1998)

continued

TABLE 14.5
Naturally Occurring Acyl-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
(*) 2''-O-Acetyl		
2''-O- α -L-Rhamnopyranosyl	<i>Tripterospermum japonicum</i> (Gent.)	Otsuka and Kijima (2001)
(*) 3''',4'''-Di-O-acetyl 2''-O-rhamnoside	<i>Crataegus sinaica</i> (Ros.)	El-Mousallamy (1998)
4'''-O-Acetyl 2''-O-rhamnoside	<i>Crataegus monogyna</i> (Ros.)	Nikolov et al. (1976)
	<i>Crataegus sinaica</i> (Ros.)	El-Mousallamy (1998)
	<i>Tripterospermum japonicum</i> (Gent.)	Otsuka and Kijima (2001)
8-C-β-D-Glucofuranosylapigenin		
(*) 2''-O-Acetyl	<i>Crataegus pinnatifida</i> (Ros.)	Zang and Xu (2002)
8-C-Galactosylapigenin		
6''-O-Acetyl	<i>Briza media</i> (Gram.)	Chari et al. (1980)
Swertisin		
6'''-O- <i>p</i> -Coumaroyl 2''-O-glucoside	<i>Zizyphus jujuba</i> (Rham.)	Woo et al. (1980)
6'''-O-Feruloyl 2''-O-glucoside	<i>Zizyphus jujuba</i> (Rham.)	Woo et al. (1980)
6'''-O-Sinapoyl 2''-O-glucoside	<i>Zizyphus jujuba</i> (Rham.)	Woo et al. (1980)
Isoswertisin		
2''-O-Acetyl	<i>Brackenridgea zanguebaria</i> (Ochn.)	Bombardelli et al. (1974)
(*) 2''-O-(2''-Methylbutyryl)	<i>Trollius ledebouri</i> (Ranun.)	Zou et al. (2004)
(*) 3''-O-(2''-Methylbutyryl)	<i>Trollius ledebouri</i> (Ranun.)	Zou et al. (2004)
(*) 6'''-O-Feruloyl 2''-O-glucoside	<i>Zizyphus jujuba</i> (Rham.)	Cheng et al. (2000)
Cytisoides		
O-Acetyl 7-O-glucoside		
O-Acetyl 7-O-glucoside (tremasperin)	<i>Trema aspera</i> (Ulm.)	Oelrichs et al. (1968)
Embigenin		
2'''-O-Acetyl 2''-O-rhamnoside	<i>Iris lactea</i> (Irid.)	Pryakhina et al. (1984)
7,4'-Di-O-methylisomollupentin		
2''-O-Caffeoylglucoside	<i>Asterostigma riedelianum</i> (Arac.)	Markham and Williams (1980)
Isorientin		
(*) 7-O-(6'''-O- <i>E</i> -Feruloyl)glucoside	<i>Hordeum vulgare</i> (Gram.)	Norbaek et al. (2003)
2''-O-Acetyl	<i>Rumex acetosa</i> (Polyg.)	Kato and Morita (1990)
2'',6''-Di-O-acetyl	<i>Rumex acetosa</i> (Polyg.)	Kato and Morita (1990)
2''-O-(<i>E</i>)-Caffeoyl	<i>Gentiana burseri</i> (Gent.)	Jacot-Guillarmod et al. (1975)
2''-O-(<i>E</i>)-Caffeoyl glucoside	<i>Cucumis melo</i> (Cucurb.)	Monties et al. (1976)
2''-O-(<i>E</i>)-Caffeoyl 4'-O-glucoside	<i>Gentiana punctata</i> (Gent.)	Luong and Jacot-Guillarmod (1977)
2''-O-(<i>E</i>)- <i>p</i> -Coumaroyl	<i>Gentiana</i> sp. (Gent.)	Luong et al. (1980)
2''-O-(<i>E</i>)-Feruloyl	<i>Gentiana burseri</i> (Gent.)	Jacot-Guillarmod et al. (1975)
2''-O-(<i>E</i>)-Feruloyl 4'-O-glucoside	<i>Gentiana burseri</i> (Gent.)	Jacot-Guillarmod et al. (1975)
(*) 2''-O-Galloyl	<i>Pelargonium reniforme</i> (Ger.)	Latte et al. (2002)
2''-O- β -Glucosyl-(<i>E</i>)-caffeoyl 4'-O-glucoside	<i>Gentiana burseri</i> (Gent.)	Jacot-Guillarmod et al. (1975)
2''-O-(4-O-Glucosyl-2,4,5-trihydroxy-(<i>E</i>)-cinnamoyl) 4'-O-glucoside	<i>Gentiana</i> sp. (Gent.)	Luong et al. (1981)
2''-O- <i>p</i> -Hydroxybenzoyl	<i>Gentiana asclepiadea</i> (Gent.)	Goetz and Jacot-Guillarmod (1978)
2''-O- <i>p</i> -Hydroxybenzoyl 4'-O-glucoside	<i>Gentiana asclepiadea</i> (Gent.)	Goetz and Jacot-Guillarmod (1978)

TABLE 14.5
Naturally Occurring Acyl-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
(*) 6''-O-Acetyl	<i>Crotalaria thebaica</i> (Leg.)	Ibraheim (1994)
(*) 6''-O-Caffeoyl	<i>Gentiana arisensis</i> (Gent.)	Kuo et al. (1996)
6''-O-Caffeoyl 7-sulfate	(Palm.)	Williams et al. (1973)
(*) 6''-O- <i>p</i> -Hydroxybenzoyl (perfoliatumin A)	<i>Polygonum perfoliatum</i> (Polyg.)	Zhu et al. (2000)
(*) 6''-O- <i>p</i> -Methoxybenzoyl (perfoliatumin B)	<i>Polygonum perfoliatum</i> (Polyg.)	Zhu et al. (2000)
Orientin		
(*) 7-O-Caffeoyl	<i>Vellozia</i> sp. (Velloz.)	Harborne et al. (1994)
	(Velloz.)	Williams et al. (1994b)
7-O-Glucoside sulfate	(Palm.)	Williams et al. (1973)
7-Sulfate	(Palm.)	Williams et al. (1973)
2''-O-Acetyl	<i>Hypericum hirsutum</i> (Hyper.)	Kitanov et al. (1979)
2'',6''-Di- <i>O</i> -acetyl	<i>Rumex acetosa</i> (Polyg.)	Kato and Morita (1990)
(*) 2''-O-Caffeoyl	<i>Vitex polygama</i> (Verb.)	Leitao and Delle Monache (1998)
(*) 2''-O-Galloyl	<i>Pelargonium reniforme</i> (Ger.)	Latte et al. (2002)
(*) 2''-O-(2''-Methylbutyryl)	<i>Trollius ledebouri</i> (Ranun.)	Zou et al. (2004)
(*) 2''-O-(3''',4'''-Dimethylbutyryl)	<i>Trollius ledebouri</i> (Ranun.)	Zou et al. (2004)
6''-O-Feruloyl 2''-O-xyloside	<i>Setaria italica</i> (Gram.)	Gluchoff-Fiasson et al. (1989)
Isoscoparin		
(*) 2''-O-Feruloyl	<i>Bryonia</i> sp. (Cucurb.)	Krauze and Cisowski (1994)
6'''-O- <i>p</i> -Coumaroyl 2''-O-glucoside	<i>Oryza sativa</i> (Gram.)	Besson et al. (1985)
	<i>Cucumis sativus</i> (Cucurb.)	Aboud-Zaid et al. (2001)
(*) 6'''-O- <i>p</i> -Coumaroyl 2''-O-glucosyl		
4'-O-Glucoside	<i>Cucumis sativus</i> (Cucurb.)	Aboud-Zaid et al. (2001)
6'''-O-Feruloyl 2''-O-glucoside	<i>Oryza sativa</i> (Gram.)	Besson et al. (1985)
Scoparin		
6''-O-Acetyl	<i>Sarothamnus scoparius</i> (Leg.)	Prum-Bousquet et al. (1977)
7,3',4'-Tri-O-methylisorientin		
6''-O-Acetyl 2''-O-rhamnoside (linoside A)	<i>Linum maritimum</i> (Lin.)	Chari et al. (1978)
8-C-β-D-Xylopyranosylchrysoeriol		
(*) 4'-O-Acetyl 2''-O-glucoside	<i>Scleranthus uncinatus</i> (Caryo.)	Yayli et al. (2001)
MONO-C-GLYCOSYLISOFLAVONES		
Puerarin		
4',6''-Di- <i>O</i> -acetyl	<i>Pueraria tuberosa</i> (Leg.)	Bhutani et al. (1969)
8-C-Glucoylorobol		
6''-O-Acetyl	<i>Dalbergia monetaria</i> (Leg.)	Nunes et al. (1989)
8-C-GLUCOSYLCHROMONES		
2'-O- <i>p</i> -Coumaroyl 5-methyl-7-hydroxy 2-acetonyl		
Aloeresin A	<i>Aloe</i> sp. (Lil.)	Gramatica et al. (1982)
2'-O- <i>p</i> -Coumaroyl 5-methyl-7-O-glucosyl 2-acetonyl		

continued

TABLE 14.5
Naturally Occurring Acyl-C-Glycosylflavonoids. (Hypothetic Structures Previously Mentioned in the Last Edition Have Been Removed If Not Confirmed in Their True Structure; the New Compounds for the Period 1992 to 2004 Are Indicated by an Asterisk [*]) — continued

Compounds	Sources	Ref.
Aloeresin C	<i>Aloe</i> sp. (Lil.)	Speranza et al. (1985)
2'- <i>O-p</i> -Coumaroyl 5-methyl-7-methoxyl 2-(2-hydroxy)propyl	<i>Aloe ferox</i> (Lil.)	Speranza et al. (1986)
Aloeresin D		
2'- <i>O-p</i> -Methoxycoumaroyl 5-methyl-7- hydroxy 2-acetonyl	<i>Aloe excelsa</i> (Lil.)	Mebe (1987)
DI-C-GLYCOSYLFLAVONES		
Vicenin-1		
(*) 6''- <i>O</i> -Acetyl	<i>Prosopis chilensis</i> (Leg.)	Elrady and Saad (1994)
6-C-α-L-Rhamnosylapigenin		
(*) 8-C-(6'''-3-Hydroxy-3-methylglutaroyl)glucoside	<i>Glycyrrhiza eurycarpa</i> (Leg.)	Liu et al. (1994)
Isoschaftoside		
2'''- <i>O</i> -Feruloyl	<i>Metzgeria</i> sp. (Bryo.)	Theodor et al. (1981b)

14.3.1.1 Qualitative Aspects

The first application of CZE concerns a mixture of *C*-glycosylflavones from different market samples of *Passiflora incarnata*.³⁷³ Factors mainly responsible for differences in electrophoretic mobility (EM) of *C*-glycosylflavones are identical to those defined from the separation of flavonoid-*O*-glycosides: number and position of the free hydroxy groups on the flavone skeleton, number and type of attached sugar groups. The best technical compromise between resolution efficiency and retention time was obtained using an operating buffer of 5% methanol in 75 nM borate buffer at pH 10, with the capillary temperature maintained at 50°C, the applied voltage at 18 kV, and an operating current of 40 μ A. From *Passiflora*

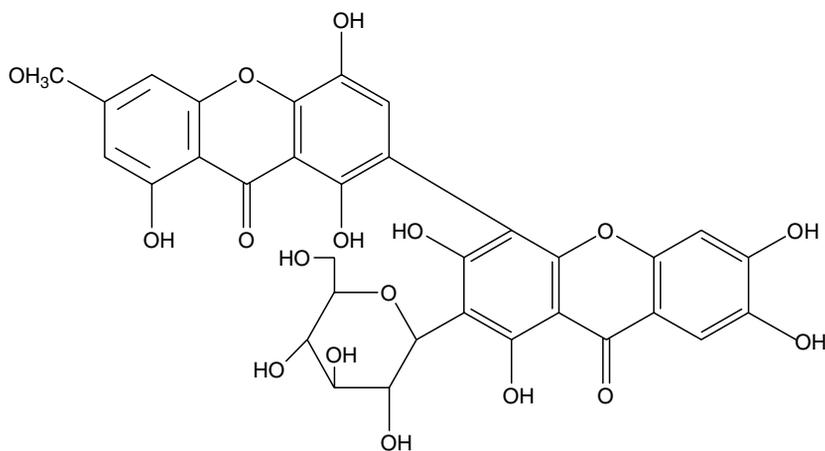


FIGURE 14.1 The structure of the flavone-xanthone dimer, swertifrancheside, isolated from *Swertia franchetiana*. (From Wang, J. et al., *J. Nat. Prod.*, 57, 211, 1994. With permission.)

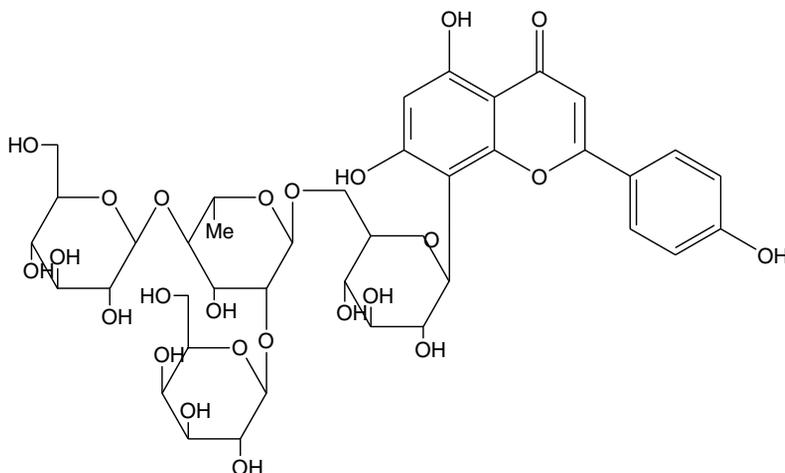


FIGURE 14.2 The structure of panzhihuacycaside isolated from *Cycas panzhihuaensis*. (From Zhou, Y. et al., *Zhiwu Xuebao*, 44, 101, 2002. With permission.)

incarnata extracts, 11 different *C*-glycosylflavones were separated on the electrophoregram. This technical approach permitted qualitative comparison of the chemical contents of different geographic samples of *Passiflora*.

Moreover, typical features of the electrophoretic behavior of *C*-glycosylflavones were discussed in relation to their structural characteristics. The EM values of flavone 6-*C*-glycosides are always lower than for flavone 8-*C*-glycoside isomers. For the flavone diglycoside derivatives of the same molecular weight, lucenin-2 and isoorientin 2''-*O*-glucoside, vicenin-2 and isovitexin 2''-*O*-glucoside, the EM values of *O*-glycosyl derivatives are higher than those of corresponding di-*C*-glycosyl flavones. The *C*-7 substitution on ring A of the flavone has a strong influence on EM values. Thus, glycosylation or methylation results in the loss of the most acidic hydroxyl group and consequently in a lower EM. Similarly, the increase of molecular size also results in a lower EM; e.g., isovitexin is higher than isoorientin, vicenin-2 is higher than lucenin-2, and schaftoside and isoschaftoside are higher than vicenin-2.

14.3.1.2 Quantitative Aspects

The CZE technique was also applied to complex mixtures of *C*-glycosylflavones from leaves of *Achillea setacea*, in order to quantify each compound.²³⁵ The optimum separation was obtained using 25 mM sodium tetraborate with 20% methanol (pH 9.3). Six *C*-glycosylflavones, five *O*-glycosylflavonoids, and three caffeoyl derivatives were efficiently separated. For the quantification, calibration curves were established. A linear correlation from 20 to 200 $\mu\text{g/ml}$ was found for vitexin with a coefficient R^2 very close to 1.0. The detection limit of the CZE method was about 9.3 $\mu\text{g/ml}$, corresponding to 0.023% in the drug. Concerning the reproducibility of extractions, the relative standard deviation was less than 5%, with the migration time coefficients of variation ranging from 0.34 to 0.45%. The accuracy of the method was examined by recovery studies: the results are around 98 to 99% with a relative standard deviation of about 3 to 5%.

14.3.2 HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY SEPARATION

Separation of *C*-glycosylflavones by using high-speed countercurrent chromatography (HSCCC) was described by Oliveira et al.²⁸⁵ using an ethylacetate extract of *Cecropia*

lyratifolia. The different components were separated in two steps: initially the mixture chloroform–methanol–water (46:25:29) was used as solvent system with the upper phase as the stationary phase. The flavonoid fraction was purified and concentrated in this stationary phase. Thereafter, the flavonoid block was submitted to a further HSCCC procedure using ethylacetate–butanol–methanol–water mixture (35:10:11:44) with the upper phase as the stationary phase. HPLC analysis of the collected fractions revealed the separation of 6-*C*-(6''-*O*-galactoside)-galactoside apigenin (20 mg), isoorientin (45 mg), and a mixture of orientin and isovitexin (74 mg). The HSCCC technique has been found to be the most effective method to resolve this flavonoid mixture, since it does not display the undesirable adsorption phenomenon and sample loss that are associated with other chromatographic techniques.

14.3.3 NEW POLYMERS FOR COLUMN CHROMATOGRAPHY

An unusual chromatographic support, chitin, was tried for separation of swertisin and 2''-*O*-rhamnosylswertisin in a methanolic extract of leaves of *Aleurites moluccana*.²⁶⁸ The chitin is a straight copolymer composed of β -(1,4)-linked GlcNAc units (85%) and β -(1,4)-2-amino-2-deoxy-D-glucose units (15%) with a three-dimensional α -helical configuration stabilized by intramolecular hydrogen bonding. Chitin is an important natural polysaccharide with a great variety of functional groups: —OH, —NH₂, —NHCOCH₃. From chitin, a new polymer was prepared, chitin-100 or fully *N*-acetylated chitin, under heterogeneous conditions using a binary mixture of methanol–formamide and acetic anhydride. After processing, the *N*-acetylation degree was 99.2%. The ethyl acetate fraction of *Aleurites moluccana* (150 mg) was chromatographed on chitin, chitin-100, and silica gel; quantitative results showed that chitin-100 is the best sorbent for separation of swertisin and 2''-*O*-rhamnosylswertisin (12.8 and 15.8 mg, respectively) from the ethyl acetate extract. In comparison, on silica gel only 3.0 mg of swertisin and 9.2 mg of 2''-*O*-rhamnosylswertisin were eluted.

14.3.4 MASS SPECTROMETRY AND STRUCTURAL IDENTIFICATION

The development of mass spectrometry (MS) procedures for the study of *C*-glycosylflavonoids is particularly significant because of the resistance of the *C*-glycosidic bond to hydrolysis. It is important, from the structural point of view, to determine the molecular mass and to localize the sugar substituents on the aglycone moiety. This has been achieved in the past by electron impact EI-MS of permethylated derivatives.

14.3.4.1 Fast Atom Bombardment and Tandem Mass Spectrometry

More recently, fast atom bombardment (FAB), which enables direct analysis on underivatized *O*- and *C*-glycosylflavones, in combination with collisionally activated dissociation (CAD) and tandem MS, has been shown to be useful for structural characterization of various kinds of compounds. The advantages of using FAB in combination with CAD and tandem MS have been documented for *O*- and *C*-glycosylflavones. The characteristic fragment ions of $(M - H)^-$ ions allow the differentiation between *C*-glycosylation at the 6- and 8-positions.^{27,69} An application of liquid secondary ion mass spectrometry, which is a variant of FAB, in combination with CAD and linked scanning at constant B/E, to obtain daughter ion spectra of $(M + H)^+$ and $(M - H)^-$ ions was investigated for di-*C*-glycosylflavones and *O*-glycosyl-*C*-glycosylflavones.^{208,209} Low-energy collisions allow the determination of the type of the terminal *O*-linked sugar residues, while high-energy collisions enable identification of the type and the linkage position of the *C*-linked sugars. The results illustrate that the daughter ion spectra of both $(M + H)^+$ and $(M - H)^-$ ions show diagnostic ions, which allow

differentiation between the isomeric 6,8-di-*C*-glycosides and provide proof of sugar linkage in *O*-glycosyl-*C*-glycosylflavones.

14.3.4.2 Liquid Chromatography–Mass Spectrometry: Thermospray and Atmospheric Pressure Ionization

Much data on the structure of flavonoids in crude or semipurified plant extracts have been obtained by HPLC coupled with MS, in order to obtain information on sugar and acyl moieties not revealed by ultraviolet spectrum, without the need to isolate and hydrolyze the compounds. In the last decade, soft ionization MS techniques have been used in this respect, e.g., thermospray (TSP) and atmospheric pressure ionization (API). However, the most used methods for the determination of phenols in crude plant extracts were the coupling of liquid chromatography (LC) and MS with API techniques such as electrospray ionization (ESI) MS and atmospheric pressure chemical ionization (APCI) MS. ESI and APCI are soft ionization techniques that generate mainly protonated molecules for relatively small metabolites such as flavonoids.

TSP was used in a so-called buffer ionization mode requiring the addition of a volatile buffer before vaporizing the HPLC eluate, and the discharge ionization mode that is based on a discharge electrode that produces a plasma of the HPLC eluent. LC–TSP–MS allowed the identification of several compounds from *Tea C*-glycosylflavone extracts¹⁷⁴ and *Citrus* di-*C*-glycosylflavone mixtures.²³

The coupling of LC and MS with API techniques was applied to the structural characterization of *Ocimum* mono-*C* and di-*C*-glycosylflavones¹²¹ by LC–APCI–MS and *Theobroma* mono-*C*-glycosylflavones by LC–ESI–MS.³¹⁷

14.3.4.3 LC–API–MS–MS

Molecular mass information alone, however, is not sufficient for the online (LC–MS) structural elucidation of natural compounds, and fragment information generated by collision-induced dissociation (CID) MS–MS becomes necessary for partial online identification. An interesting application of the low-energy CID–MS–MS spectra on various *C*-mono and *C*-diglycosylflavones was made in LC–APCI–MS–MS and LC–ESI–MS–MS modes.³⁸² These procedures were studied on two types of MS instruments: a quadrupole time-of-flight one and an ion trap one. It has been demonstrated that fragment ions provide important structural information for the nature and site of attachment of sugars in *O*-glycosyl *C*-glycosylflavones, and on the distribution of substituents between the A and B rings of the flavone skeleton.

14.3.5 NMR SPECTROSCOPY AND FINE STRUCTURE ELUCIDATION

14.3.5.1 Improved NMR Techniques

¹H and ¹³C NMR spectroscopies were systematically used to elucidate the structure of *C*-glycosylflavonoids; the assignment of signals was based upon various experiments, HMBC, HMQC, COSY, etc., as shown in the following examples:

In the case of isorientin 6''-*O*-caffeate isolated from *Gentiana arisanensis*,¹⁹⁸ the ¹³C NMR spectrum was assigned by ¹H-decoupled spectra, DEPT pulse sequence, ¹H–¹³C COSY spectrum, long-range ¹³C–¹H COSY, and NOESY experiments; the ¹H NMR spectrum was analyzed with the aid of ¹H–¹H COSY and ¹H–¹³C COSY.

For the new *C*-hydroxybenzyl glycoflavones from *Citrullus colocynthis*,²²³ the complete ¹³C NMR data were assigned and additionally confirmed by 2D-COSY, HETCOR, and selective INEPT experiments.

The structure of shamimin^{99,100} isolated from the leaves of *Bombax ceiba* was determined by studies of the one-bond ^1H - ^{13}C connectivities from the HMQC/HeteroCOSY spectrum and by long-range correlations from HMBC or COLOC experiments. The ^1H COSY-45 and ^1H - ^1H COSY spectrum revealed the vicinal couplings in the sugar part; the *J*-resolved spectrum confirmed the assignment of the sugar protons.

In cassiaoccidentalins A, B, and C,¹³⁰ ^1H - ^1H COSY spectrum and HMBC correlations substantiated the 3-keto structures and indicated that the rhamnose residue is attached to *O*-2'' of the 3-keto sugar residue. NOESY spectrum was used to assign the structure of 6-deoxyribo-hexos-3-ulose.

In kaplanin, the β configuration of the sugar in the 5-hydroxy-7-methoxy-8-*C*- β -glucosylflavone²⁶⁵ was indicated by ^1H NMR and supported by ^1H - ^1H COSY. ^1H and ^{13}C NMR and HETCOR spectra gave the precise location of the glucose moiety.

The complex structure isolated from *Scleranthus uncinatus*,^{403,404} 5,7-dihydroxy-3'-methoxy-4'-acetoxyflavone-8-*C*- β -*D*-xylopyranoside-2''-*O*-glucoside, was determined by using 1D NMR (^1H , ^{13}C , DEPT) and 2D NMR (H-COSY, TOCSY, HMQC, HMBC, NOESY) data; sequence and linkage of the sugar chain and acylation site were confirmed by observation of inter-residue NOEs in the NOESY spectrum.

For the five new acetylated flavone *C*-glycosides (such as isovitexin 2''-*O*-(6'''-(*E*)-*p*-coumaroyl)glucoside-4'-*O*-glucoside) isolated from *Cucumis sativus*,³ the structures were elucidated using 1D NMR (^1H , XSROESY, ^{13}C) and DEPT, DQF-COSY, HSQC, and HMBC experiments. The precise nature of linkages between the residues was determined using ^1H - ^1H dipolar connectivities detected in 1D XSROESY experiments.

4-Hydroxy-1-ethylbenzene derivatives of vitexin and isovitexin (cucumerin A and B, two regioisomers)²²⁸ were studied by ^1H and ^{13}C NMR analysis with DEPT, TOCSY, and COSY experiments; HMBC and NOESY correlations were also used for assignment of the hydroxyethylbenzene residue.

14.3.5.2 NMR and Rotational Isomers

Another aspect often underlined in the NMR studies on *C*-glycosylflavonoids is the broadening or the doubling of the signals due to the existence of rotational isomers. In the 1960s, the broadening of NMR signals was reported in *C*-glycosylflavones and was explained by the existence of rotational isomers. Kato and Morita¹⁷³ observed the doubling of signals in ^1H and ^{13}C NMR of peracetylated *C*-glycosylflavones due to the restricted rotation of the acetylated glucosyl moieties; the conformations of rotational isomers of hepta-*O*-acetyl vitexin and octa-*O*-acetyl orientin were assigned as *+sp* (major) and *-sc* (minor) for both compounds after COSY and NOESY experiments on NMR spectra in CDCl_3 .

During the structural elucidation of the first flavone-xanthone dimer (swertifranche-side)³⁸¹ through a series of 1D or 2D NMR techniques including COSY, phase-sensitive ROESY, reversed-detected HMQC, HMBC, and selective INEPT experiments, ^1H and ^{13}C signals appeared as two peaks indicating two conformers; and classically, the coalescence of the split signal was observed as the temperature was increased.

The fine structure of the three cassiaoccidentalins¹³⁰ with a 6-*C* (2''-*O*-rhamnosyl) 6-deoxy-ribo-hexos-3-ulosyl moiety was established on the basis of spectroscopic evidence, as already mentioned; the ^1H and ^{13}C NMR spectra showed signals of two conformers due to hindered rotation around the *C*-6-glycosidic linkage, with duplication or broadening of the signals remaining even at elevated temperatures. The NOESY spectrum showed a cross-peak between H-8 of the flavone nucleus and H-6''' of the rhamnose for the major conformer and a cross-peak between OH-5 and rhamnose H-2''' for the minor conformer.

An initial study of the ^1H NMR of spinosin,^{54,207} 6-*C*-(2''-*O*-glucosyl)-glucosyl-7-*O*-methyl apigenin, revealed a doubling of many of the signals and it was noted that the paired signals were in a nearly 1:1 ratio; the ^{13}C NMR data showed similar duplication of signals. HMBC data permitted the matching of each signal of a duplicated pair of carbon signals of the flavone nucleus to the corresponding signal of the pair given by its attached proton. A HSBC experiment was necessary in order to achieve the carbon and proton chemical shifts assignments for the sugar moiety. The 2D NMR experiments allowed the complete assignment of the ^1H and ^{13}C data to each rotamer for the disaccharide part of the molecule.

The pattern of duplication of signals suggested that at 303 K there were two rotamers of about equal energies. At 338 K, the signals were still detected independently although the two signals had broadened and moved closer together. The two signals coalesced at 363 K for the entire ^1H spectrum and the free energy of activation for rotation was calculated using the Eyring equation: 75 kJ mol^{-1} . On the basis of these observations, it was proposed that spinosin showed rotational isomers separated by an energy barrier about the *C*-glycosidic bond sufficiently high to prevent fast exchange between the two conformers at room temperature. The data from T-ROESY experiments were in accordance with this conclusion. Hence, the H-1'' proton is oriented *syn* to the methoxy group in one rotamer while in the other the H-2'' proton is *syn* to it. Grid Scan procedure enables systematic exploration of conformations by stepwise alteration of selected torsion angles over specified range of values.

It was noteworthy that the ^1H NMR spectra of compounds 2''-*O*-galloyl-vitexin and 2''-*O*-galloyl-orientin at room temperatures displayed severe line broadening of the well-separated 2''-H signal of the glycosyl unit in each instance.²⁰⁰ Such a phenomenon presumably reflected restricted conformational flexibility at the C6–C1'' bond associated with the presence of the 2''-*O*-galloyl group, and a limitation in the dynamic rotational isomerism. Under the same conditions, ^1H NMR spectra of 2''-*O*-galloyl-isovitexin and 2''-*O*-galloyl-isorientin displayed a typical duplication of most of the signals; the two rotamers coexisted in the ratio of 8:1 in each case. Interestingly, this ratio was solvent-dependent: with acetone- d_6 the signal intensity of the minor rotamer was enhanced, culminating in a 3:1 relative abundance of the two conformers. The authors underlined that when the 2''-position is occupied by a rhamnosyl instead of a galloyl residue, no doubling of signals is found, suggesting that the presence of an additional sugar at the 2''-position locks the 8-glucosyl unit in a position precluding its interaction with the aromatic B-ring. In the *C*-6 glycosylated compounds the preferred conformations are those that are stabilized via attractive *p*-stacking between the A-ring and the galloyl aromatic ring. This conformation is obviously stable enough to retard free rotation about the *C*-6-glycosyl bond to such an extent that broadened ^1H NMR resonances are observed. In the *C*-8 glycosylated analogs the conformation is stabilized by *p*-stacking between the aromatic ring of the ester and the B-ring of the flavone. This interaction produces a more pronounced stabilizing effect with the rotation being slowed down to such an extent that two distinct rotamers are observed, hence resulting in duplication of ^1H and ^{13}C NMR resonances.

The ability to show several conformers was used for the 2'-hydroxyisorientin isolated from barley leaves, as a new method to measure NOE signals of *C*-glycoside flavonoids.²⁷⁹ When NMR measurements were performed very close to the freezing temperature of the dimethyl sulfoxide (DMSO) solvent, the molecular rotation and exchange among the protons were highly hindered so that the rotamers could be observed separately because of the slow correlation time. Therefore, it became possible to observe clear NOE signals between spatially close protons; by preirradiation of a signal for a longer time than the correlation time, but shorter than the chemical exchange time for difference NOE. The residue signals differed from each other. Also, NOE signals between protons of the hydroxyl groups and the chromophore could be observed due to slow proton exchange among phenolic hydroxyl groups in DMSO at low temperature. A negative NOE produced by irradiation of H-1''

was observed at OH-5 and OH-7, and by irradiation of H-3 of the chromophore, a negative NOE was produced in the signals of H-6' and OH-2'.

14.4 SYNTHESIS OF C-GLYCOSYLFLAVONOIDS

The synthesis of *C*-glycosylflavones has been achieved mainly by Friedel–Crafts-type reactions or Koenigs–Knorr glycosylations. However, yields were poor due to steric hindrance of the substrates. Several other procedures have been proposed in the last decade.

14.4.1 *O*–*C* GLYCOSIDE REARRANGEMENT

An aryl *C*-glycosylation method via *O*–*C* glycoside rearrangement was developed by Kumazawa et al.¹⁹⁶ The glycosylation using phloroglucinol diacetate as an acceptor and 2,3,4,6-tetra-*O*-benzyl- α -D-glucopyranosyl fluoride as donor afforded the *O*-glucoside with a yield of 95%. When phloroglucinol dimethyl ether replaced the phloroglucinol diacetate, the reaction afforded both *O*- and *C*-glucosides in a total yield of 84% (30 and 54, respectively). Using the same glycosyl donor and several 2-acetyl phloroglucinol derivatives (di-methyl, di-benzyl, methyl benzyl so that there was a free hydroxyl group *ortho* to the acetyl group) as glycosyl acceptors, the glycosylation reaction gave the *C*-glucoside predominantly. This method was successfully applied to the synthesis of *C*-glucosyl chalcone.

More recently, Kumazawa et al.¹⁹⁷ reported on the synthesis of several 8-*C*-glucosylflavones from phloracetophenone derivatives via the same stereoselective *O*–*C* glycosyl rearrangement.

By selective protection of the phloracetophenone with methoxymethyl chloride, and then regioselective benzylation with benzyl chloride, the 2,4-dibenzylphloracetophenone was synthesized. By reacting with benzyl-protected glucopyranosyl fluoride in the presence of boron trifluoride diethyl etherate, this compound led to a *C*-glucosyl derivative via a highly regio- and stereoselective *O*–*C* glycosyl rearrangement. Its aldol condensation with 3,4-*bis*-benzyloxybenzaldehyde afforded a *C*-glucosyl chalcone derivative. This chalcone was oxidized in the presence of selenium dioxide to give the partially benzyl-protected orientin. All benzyl-protected groups on this flavone were removed by hydrogenolysis to afford orientin. If the partial benzyl-protection orientin was previously methylated with Me₂SO₄, the deprotection by hydrogenolysis led to parkinsonin A (5-methyl orientin). Alternatively, if the 2-benzyl-4-methyl phloracetophenone is used as the *C*-glucosylation acceptor, the aldol condensation produces isoswertiajaponin, and after the methylation process parkinsonin B (5,7-dimethyl orientin) is obtained.

14.4.2 FRIES-TYPE REARRANGEMENT

Another strategy was developed^{95,230} for the synthesis of phenolic *C*-glycosyl derivatives via glycosylation of partially *O*-unprotected phenols followed by Fries-type rearrangement of the *O*-glycoside intermediate. Thus, with *O*-glycosyl trichloroacetimidates as donors, only catalytic amounts of trimethylsilyl triflate as promoters are required for reaction with phenol derivatives as acceptors. The studies have involved mono-*C*-glycosylation and di-*C*-glycosylation of partially *O*-protected phenols.

For the mono-*C*-glycosylphenol, the commercially available 2,4,6-trihydroxyacetophenone was chosen and selectively methylated at *C*-2 and *C*-4. The partially protected phenol was glycosylated with the *O*-benzyl-protected glucosyl trichloroacetimidate in the presence of trimethylsilyl triflate as promoter to give directly a *C*-(benzyl protected)glycosylphenol. The unprotected hydroxyl group of this compound was converted with benzoyl chloride into a fully protected *C*-glycoside phenol. Treatment of the benzoate derivative with sodium hydroxide in

DMSO resulted in an intramolecular ester condensation (Baker–Venkataraman-type rearrangement) to give a C15 phenol the cyclization of which furnished the *O*-benzyl-protected flavone-*C*-glucoside. After deprotection, isoembigenin was obtained.

For a di-*C*-glycosyl compound, the *O*-benzyl-protected *O*-glucosyl trichloroacetimidate was reacted with 3,5-dimethoxyphloroglucinol in the presence of trimethylsilyl triflate to lead to 2-*C*-(tetra-*O*-benzylglucosyl) 3,5-dimethoxyphenol. Further reaction of this last compound with a second donor molecule under similar reaction conditions afforded the 2,6-di-*C*-glucosyl 3,5-dimethoxyphloroglucinol in very high yield (93%). Similarly, when the first intermediate 3,5-dimethoxy 2-*C*-(tetra-*O*-benzylglucosyl)phenol was reacted with *O*-(acetyl)-galactosyl trichloroacetimidate, another di-*C*-glycosyl phenol was obtained in good yield: 3,5-dimethoxy-6-*C*-(tetra-*O*-acetyl-galactosyl)-2-*C*-(tetra-*O*-benzylglucosyl)phenol.

14.4.3 VIA C- β -D-GLUCOPYRANOSYL 2,6-DIMETHOXYBENZENE

Very recently, a new strategy was developed²⁰¹ for the synthesis of a *C*-glycosylisoflavone, puerarin. The key intermediate is the β -D-glucopyranosyl-2,6-dimethoxybenzene. This was obtained by coupling a lithiated aromatic reagent (2,6-dimethoxybenzene, for example) with a benzyl protected glycopyranolactone, followed by hydride reduction. The *C*-(benzyl protected) glucoside was obtained in 56% overall yield. Hydrogenolysis of this compound proceeded smoothly to give the *C*-glucosylated 2,6-dimethoxybenzene, the acetylation of which gave a tetraacetate derivative. Reaction of this last compound in anhydrous AlCl₃ gave *C*-(tetraacetylglucosyl) 6-methoxy 2-hydroxy-3-acetyl benzene. After removal of acetyl groups on the glucose, the compound was condensed with *p*-methoxybenzaldehyde to give a chalcone. Oxidative rearrangement of the acetyl-protected chalcone in a mixed solvent of methanol and trimethylorthofolate followed by refluxing in methanol with HCl gave 7,4'-di-*O*-methylpuerarin in 84% yield. After demethylation, puerarin was obtained in 35% yield. The overall yield of the synthesis of puerarin was about 10%.

14.5 C-GLYCOSYLFLAVONOIDS AND VACUOLAR STORAGE

A number of different functions have been attributed to plant secondary substances, especially *C*-glycosylflavonoids; in many cases these functions require rather high local concentration (millimolar range) and many of these compounds are harmful to the plant producing these compounds. Therefore, the presence and synthesis require a strict compartmentalization of the sites of production and storage. For many metabolites, it has been shown that they are efficiently stored within the vacuoles. Consequently, transport mechanisms for vacuole deposition of glycosylated compounds are important. This raises the question of which structural features determine the specificity of glycosylated compounds for their recognition either by a glucoside pump or by a secondary energized glucoside transporter. This question was considered by Frangne et al.¹⁰³ and Klein et al.¹⁸¹: in *Hordeum vulgare* the twofold glycosylated saponarin (apigenin 6-*C*-glucosyl-7-*O*-glucoside) accumulates as the major compound while apigenin 6-*C*-glucoside, the precursor, is present only in trace amounts. Saponarin is taken up by a proton antiport system into barley vacuoles and the transport activity is strongly reduced in a barley mutant impaired in flavonoid biosynthesis. The isovitexin was a competitive inhibitor of saponarin uptake, arguing for the fact that the same vacuolar transporter accepts both flavone glycosides. Interestingly, the saponarin was taken up by an ABC-type transporter into vacuoles of another species (*Arabidopsis*) that does not synthesize this class of flavonoids. Thus, specific proton antiport systems would be responsible for the vacuolar transport of endogenous glucosides, whereas vacuolar ABC-type transporters would be involved for exogenous compounds, which would be considered as foreign substances to be detoxified.

14.6 BIOLOGICAL PROPERTIES OF C-GLYCOSYLFLAVONOIDS

14.6.1 ANTIOXIDANT ACTIVITY OF C-GLYCOSYLFLAVONOIDS

Antioxidants are scavengers of oxygen radicals or hydroxy radicals that attack polyunsaturated fatty acids in cell membranes giving rise to lipid peroxidation. Lipid peroxidation is strongly associated with aging and carcinogenesis. Synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been widely used as antioxidants for foods, but the uses have begun to be restricted because of their toxicity. Therefore, the interest for natural antioxidants has increased greatly. Many natural resources have been tested for their antioxidant effect and some of them accumulated large amounts of C-glycosylflavones.

Xanthosoma violaceum, Araceae, widely distributed in Dominican Republic, Puerto Rico, Guatemala, and Equator, was investigated for *in vitro* antioxidant and free-radical scavenger activities.²⁹⁸ The fraction rich in C-glycosylflavones showed with 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) test an EC₅₀ of 11.6 µg/ml, compared with quercetin (2.3 µg/ml) and α-tocopherol (10.1 µg/ml).

Terminalia catappa, Combretaceae, commonly used in the folk medicine in Taiwan, contains several C-glycosylflavones, two of them galloyl esters of vitexin and isovitexin. The isolated compounds were tested for antioxidative activity on Cu²⁺/O₂-induced low-density lipoprotein peroxidation.²¹³ IC₅₀ values were 2.1 µM for vitexin derivatives and 4.5 µM for isovitexin derivatives compared to 4.0 µM for probucol chosen as positive control.

Genistein 8-C-glucoside, isolated from *Lupinus luteus*, develops a clear-cut antioxidant effect in liver homogenates and microsomes preventing the destruction of cytochrome P450 and its conversion to an inactive form cytochrome P420. Moreover, oxidative damage caused by tertbutyl hydroperoxide and hypochloric acid on red blood cells was inhibited by about 50% on pretreatment with this C-glucosyl isoflavone in a concentration range of 3 to 5 mM.^{44,406}

Other reports have concerned the antioxidant effect of C-glycosylflavones from *Carthamus tinctorius*,²⁰³ *Anthurium versicolor*,¹⁷ Lemon fruits,^{259,260} and *Hordeum vulgare*.^{239,258,286}

14.6.2 C-GLYCOSYLFLAVONOIDS AND PLANT-INSECT RELATIONSHIPS

Natural resistance in crops is an important aspect of any integrated pest management program, and it has been a major target in response to environmental concerns and increased resistance of insects to traditional insecticides.

14.6.2.1 *Zea mays* and *Helicoverpa zea*

The development of natural resistance in corn (*Zea mays*) to the corn earworm (CEW) (*Helicoverpa zea*) received many contributions. The CEW is a major insect pest of maize and other crops (cotton, soybeans, etc.); the eggs are laid on the silks, and the larvae access the ear by feeding through the silk channel.

Host-plant resistance to CEW by antibiosis is caused by the presence of several C-glycoflavones: maysin, apimaysin, methoxymaysin³³³; maysin seemed to be one of the most important natural resistance factors in corn silk.³³⁴ Maysin is the predominant compound in most genotypes and a highly significant relationship was found between silk maysin concentration in fresh organs and weights of corn earworm larvae fed diets containing silk extracts. It has been shown that corn silk with maysin levels higher than 0.2% fresh weight reduced larval weights by 50% of controls, while silk maysin higher than 0.4% reduced

weights by about 70%. Upon ingestion by CEW, the flavone molecules are oxidized to quinines, which bind amino acids making them unavailable and thus inhibiting larval growth.

The results of flavonoid analyses^{70,384} showed that there is a wide range in silk maysin levels, from 0.01 to 0.5% fresh weight. A significant proportion (one fifth) of these sources presented silk maysin levels above the 0.2% fresh weight threshold, considered to be significant for cornworm antibiosis.

The presence of *o*-dihydroxy substituent on the B-ring of the C-glycosylflavones is important for antibiosis activity, while the nature of the sugar is not important. Consequently, the discovery of other *o*-dihydroxyflavonoids in corn silks would be advantageous in breeding resistance with a broad and diverse chemical basis. In the course of the survey of many corn inbreds, populations, and lines for flavonoid contents, several reduced maysin analogs were found, e.g., equatorial 4''-OH-maysin, axial 4''-OH-maysin, axial 4''-OH-3'-methoxymaysin.³³⁵ The laboratory bioassays for CEW larval growth inhibition showed that the 4''-OH-maysins are almost as active as maysin in reducing larval growth. Genetic control of these particular C-glycosylflavones received much attention during the last 10 years.^{45,202,227,347,410}

14.6.2.2 *Oryza sativa* and *Nilaparvata lugens*

Another example concerns the Brown planthopper (*Nilaparvata lugens*), a serious rice pest in Asia. It has been shown that the resistance factors to the insect are located in the phloem.¹¹⁹ The chemical analysis of the sap revealed the presence of three main C-glycosyl flavones: schaftoside, isoschaftoside, and probably neoschaftoside. When sucrose was fed together with these C-glycosylflavones in concentrations similar to those in the stems, the mean duration of the ingestion pattern was reduced by 75% as compared with sucrose alone, evidencing the deterrent role of these compounds in the phloem of resistant rice varieties. Simultaneously, a large increase in the mean probing frequency was recorded from 2.3 to 6.6 over 2 h. It is likely that whenever the insects encounter a deterrent substance, they react by increasing their probing frequency in order to find a more acceptable food source.

14.6.3 C-GLYCOSYLFLAVONOIDS AND ANTIMOLLUSCIDAL ACTIVITY

El-Sawi⁸⁹ reported the molluscicidal activity of alcoholic extracts from aerial parts of *Acacia saligna*, which exhibited strong activity against *Biomphalaria alexandrina*, the intermediate host of *Schistosoma mansoni*. These extracts were particularly rich in flavonoids and more especially in a new compound identified as 8-C-glucosyldihydrokaempferol.

14.6.4 C-GLYCOSYLFLAVONOIDS AND PLANT–MICROORGANISM RELATIONSHIPS

14.6.4.1 Arbuscular Mycorrhiza: *Cucumis melo* and *Glomus caledonium*

Arbuscular mycorrhiza is the most widespread form of symbiotic association between soil-borne fungi and plant roots. This symbiosis confers benefits to the host-plant growth and development through the acquisition of phosphate and minerals from the soil by the fungi. Moreover, it enhances the plant resistance to pathogens and environmental stresses. It has been calculated that the arbuscular mycorrhizal fungi are able to colonize the roots of 80% of terrestrial plants. Despite the ubiquitous occurrence of this symbiosis and its importance in sustainable agriculture, the mechanisms for the formation of a functional symbiosis are almost entirely unknown. The role of C-glycosylflavones has been underlined¹³ in the case of the arbuscular mycorrhizal colonization by *Glomus caledonium* in melon roots (*Cucumis melo*). The accumulation of 2''-O-glucosylvitexin was caused by a phosphate deficiency. This

compound stimulated arbuscular mycorrhiza formation in melon roots. At low phosphate levels, the degree of colonization in control roots (22%) was much greater than when grown under high phosphate conditions (8.8%). With a treatment of roots by 2'-*O*-glucosylvitexin, the degree of colonization was markedly increased by up to 35% on low phosphate and 25% on high phosphate levels.

14.6.4.2 Disease: *Cucumis sativus* and *Podosphaera xanthii*

The phenolic compositions of cucumber leaves (*Cucumis sativus* var. *corona*) were analyzed under different selective pressures of powdery mildew fungi.²²⁸ Cucumber plants were tested under two treatments: plants infected with *Podosphaera xanthii* and rendered resistant with bioprotectant treatment elicitor of phytoalexin production, and control plants that consisted of infected, nonelicited susceptible plants. The leaf tissue of cucumber expressing induced resistance against powdery mildew fungi was characterized by high accumulation of two major *C*-glycosylflavones, cucumerin A and cucumerin B, which are new compounds with an original substitution, 4-hydroxy-1-ethylbenzene, and six known *C*-glycosylflavones. All these compounds were found in much higher concentrations within leaves of elicited cucumber plants while they were near absent within susceptible control plants. A role for these compounds in this species was suspected as phytoalexins.

14.6.5 ANTIBACTERIAL ROLE OF C-GLYCOSYLFLAVONOIDS

Due to the antimicrobial activity of many of the phenolic compounds against different bacterial and fungal strains, several reports about the antibacterial effects of *C*-glycosylflavones have appeared.

The extracts of *Triticum aestivum* were studied on six different bacteria. The activity was measured by using the agar-diffusion method. The acetone extracts rich in di-*C*-glycosyl and *C*-diglycosylflavones showed antibacterial effects on *Sarcina lutea* and *Bacillus subtilis* (two Gram-positive bacteria), while the ethanolic extracts rich in mono-*C*-glycosylflavones showed an antibacterial effect on *Escherichia coli* (a Gram-negative bacteria). The extracts did not have any effect on *Bacillus pumilus*, *Brodetella brochiseptica*, and *Staphylococcus aureus*.²⁵⁵

C-Glycosylflavones of *Arum palaestinum* showed antimicrobial activity against *E. coli* and *Staphylococcus aureus*, which were the most susceptible bacteria to vitexin and isoorientin.⁹

Antimicrobial activity was also described for *C*-glycosylflavones from *Otostegia fruticosa*: vicenin-2.^{2,99}

14.6.6 C-GLYCOSYLFLAVONOIDS AND GENERAL ANIMAL PHYSIOLOGY

14.6.6.1 Antinociceptive Activity

The leaves of *Aleurites moluccana* contain 2'-*O*-rhamnosylswertisin and swertisin. The antinociceptive effect of both compounds was evaluated by the writhing test in mice.²⁵⁴ The results indicated that the first derivative inhibits, dose dependently, the abdominal constrictions caused by acetic acid with an ID₅₀ value of 6.9 to 10.2 $\mu\text{M}/\text{kg}$ and maximal inhibition of 92%. When compared with aspirin ID₅₀ = 133 $\mu\text{M}/\text{kg}$, the *C*-glycosylflavone was about 16-fold more potent. On the other hand, the swertisin alone did not show any effect.

14.6.6.2 Antispasmodic Activity

Arum palaestinum contains isoorientin and vitexin, which were studied for their antispasmodic activity on isolated organs.⁸ Isoorientin in concentrations ranging from 10⁻⁷ to 6 \times 10⁻⁴ *M* decreased the frequency and the amplitude of the phasic contractions of uterine

segments isolated from rats and guinea pigs; the EC_{50} values on the amplitude of contractions were 2.05×10^{-4} M in rats and 5.66×10^{-5} M in guinea pigs. The myolytic activity of isoorientin on uterine smooth muscle could be explained as due to inhibition of phosphodiesterases and consequently to an increase in the cellular concentration of cyclic nucleotides.

14.6.6.3 Sedative Activity

The seeds of *Ziziphus jujuba* var. *spinosa* (Bunge) are used as a sedative in Chinese medicine. They accumulate eight C-glycosylflavonoids based on the aglycones apigenin and genistein. Among them, spinosin and swertisin possess significant sedative activities. The oral administration of these compounds (4×10^{-5} M/kg) prolonged pentobarbital-induced sleeping time by about 30% compared to the control group.⁵⁴

14.6.6.4 Antihepatotoxic Activity

The leaves of *Allophylus edulis* are used for the treatment of liver ailments, such as jaundice, in the traditional medicine of Paraguay. Ten C-glycosylflavones were isolated from this plant: schaftoside, vicenin-2, lucenin-2, isovitexin 2''-O-rhamnoside, cerarvensin 2''-O-rhamnoside, mollupentin 2''-O-rhamnoside, etc. Monitoring of antihepatotoxic activity of these compounds was achieved against CCl_4 toxicity in primary cultured rat hepatocytes. The protection against CCl_4 toxicity was 35% with schaftoside, 50% with isovitexin 2''-O-rhamnoside, and 45% for cerarvensin 2''-O-rhamnoside. The C-8 isomers are less efficient than C-6 isomers and if a rhamnosyl residue is attached to the C-bound sugar, an enhancement in antihepatotoxic activity can be registered. If the free hydroxy group at position 7 of 6-C-glycosylflavones is replaced by O-glycosyl or O-methyl, the hepatoprotective activity is markedly enhanced.¹⁴³

Green tea has a preventive effect on D-galactosamine (D-GalN)-induced liver injury in rats. The rats were given free access to the experimental diets for 10 days; at the 11th day, the D-galactosamine was injected intraperitoneally, and the activities of alanine aminotransferase and aspartate aminotransferase were measured from the plasma 22 h after the beginning of the treatment. When the experimental diet contained isoschaftoside isolated from green tea, the increase of plasma enzyme activities was restricted to about 30%.³⁷⁵

Extracts of leaves of *Combretum quadrangulare* showed promising hepatoprotective effect on D-GalN or tumor necrosis factor- α (TNF- α)-induced cell death in primary cultured mouse hepatocytes. The hepatoprotective effect of C-glycosylflavones from *C. quadrangulare* was determined by evaluation of the serum enzyme level in comparison with the D-GalN or TNF- α treated control.²⁴ At the concentration of 200 μ g/ml, orientin and isoscoparin possessed an inhibitory effect on TNF- α -induced cell death of about 20%, while vitexin at 100 μ g/ml produces a 95% inhibitory effect.

14.6.6.5 Antiinflammatory Activity

Much of the activity of *Citrus* flavonoids (flavanone glycosides, flavone O- and C-glycosides, polymethoxyflavones) appears to impact blood leukocytes and microvascular endothelial cells, and it is not surprising that two of the main areas of research on the biological actions of *Citrus* flavonoids have been inflammation and cancer. Inflammation is typically characterized by increased permeability of endothelial tissue and influxes of blood leukocytes into the interstitium, resulting in edema. Many properties of *Citrus* flavonoids could be linked to the abilities of these compounds to inhibit enzymes involved in cell activation: phosphodiesterases, kinases, topoisomerases, etc. Signal transduction in early stages of inflammation involves phosphodiesterases and among them, phosphodiesterase-4 isozyme influences the expression of the TNF- α and other proinflammatory protein cytokines. So, inhibition of this

enzyme is a target for the potential treatment of inflammatory diseases. Vitexin, isovitexin, and 2''-*O*-rhamnosylvitexin appeared as inhibitors of this enzyme at IC₅₀ of 7.1, 25, and 25 μ M, respectively. Comparatively, the assays with polymethoxyflavones such as nobiletin or with the flavanone naringin gave IC₅₀ of 3.2 and 10 μ M, respectively. These results could be attributed to the abilities of *Citrus* flavonoids to interact with the nucleotide binding sites of phosphodiesterases because of similarities in the ring structures of flavonoids and the adenosine of ATP.²³³

Others reports describe the antiinflammatory activity of *C*-glycosylflavones from *Swertia franchetiana*³⁸¹ and *Pinelliae tuber*.²⁴⁴

14.6.6.6 Antidiabetic Activity

Related to the common uses of corn silk in Chinese folk medicine, *C*-glycosylflavones from corn silk (chrysoeriol 6-*C*-fucopyranoside, chrysoeriol 6-*C*-boivinopyranoside, chrysoeriol 6-*C*-boivinopyranosyl-7-*O*-glucopyranoside, 4''-OH-3'-methoxymaysin) were tested for the prevention of diabetic complications. These complications arise when reducing sugars react nonenzymatically with amino groups in proteins, lipids, etc., through a series of reactions forming Schiff bases and Amadori products. This leads to the accumulation of some kinds of aggregate or advanced glycation end products (AGEs) such as pentosidine or *N-E*-(carboxymethyl)lysine (CML). This process is known as glycation and the formation of these AGEs in the human body is associated with the inducement of diabetic complications.

The CML is the most characterized AGE and is referred to as a glycoxidation product. The inhibitory effects of *C*-glycosylflavones on the CML formation were tested by enzyme-linked immunosorbent assay in kidney diabetic subjects.^{345,346} The results showed that the percent inhibition was about 53% for chrysoeriol 6-*C*-boivinosyl 7-*O*-glucoside, 64% for chrysoeriol 6-*C*-boivinosyl, 80% chrysoeriol 6-*C*-fucosyl, and only 2% for 4''-OH-3'-methoxymaysin versus 60% for the standard glycation inhibitor, aminoguanidine.

14.6.6.7 Antihypertensive Activity

The effects of crude flavonoids of lemon juice on blood pressure were examined using spontaneously hypertensive rats. The systolic blood pressure of the animals fed a diet with lemon crude flavonoids for 16 weeks was significantly lower than the control group. When the animals were fed a diet containing a purified fraction of *Citrus* flavonoids (6,8-di-*C*-glycosylflavones), the same result was reached after 4 weeks. The flavonoid glycosides had an inhibitory effect on angiotensin I converting enzyme.²⁶¹

Use of *Bombax ceiba* leaves to reduce blood sugar levels is common in North Pakistan; Saleem et al.³¹³ tried an evaluation of the hypotensive activities (and hypoglycemic effect) of the shamimin, a new *C*-glycosylflavone isolated from this plant. This compound caused 81, 67, and 51% falls in blood pressure at the doses of 15, 3, and 1 mg/kg, respectively. The effect lasted for 2 to 4 min. Shamimin also produced a significant hypoglycemic effect in Sprague–Dawley rats at the dose of 500 mg/kg; the decrease remained constant for the next 4 h. Plasma sugar levels showed a decline of 26%.

14.7 MANUFACTURE AND CULTIVATION PROCESS AND C-GLYCOSYLFLAVONOIDS

The influence of a process on the flavonoid content was studied with *Pennisetum americanum*, the pearl millet that is largely grown as a food grain in Africa and Asia. Traditional processing of millet for food involves the removal of some of the outer layers of the kernel by

pounding the grain in a mortar. The decorticated grain is steeped overnight in water containing tamarind bean extract or sour milk to bleach pigments and finally the bleached flour is then cooked. According to the variety, the content of C-glycosylflavones is 137 to 275 mg/100 g with a mean at 157 mg/100 g. A decortication level of 20% alters the C-glycosylflavone content to a range of 31 to 95 mg/100 g with a mean at 64. The cooking significantly reduced the concentration of C-glycosylflavones by about 30%. This result is important since millet diets were linked with the high incidence of endemic goiter in millet-consuming populations. Animal feeding studies¹² showed that the process in which 25% of the grain was removed as bran successfully removed the antithyroid properties of pearl millet as demonstrated by patterns of serum thyroid hormones and thyroid histopathology.

Among Mediterranean fruits orange juice must be highlighted, as this product is a major source of flavonoid intake in the diet of developed countries. *Citrus* flavonoids possess health-promoting properties thanks to flavanones, polymethoxyflavones, and C-glycosylflavones. An important question is the process applied to natural resources and its role on natural compounds. The flavonoid content of orange juices¹⁰ produced by different processing techniques (hand squeezed, pasteurized, mildly pasteurized, etc.) was compared. For C-glycosylflavones, freshly prepared hand-squeezed navel orange juice contained 80 mg/l of vicenin-2, the pasteurized juice only 27 to 51 mg/l, and the mild pasteurized form 40 to 80 mg/l, indicating that the pasteurization process at higher temperature leads to a strong decrease in juice C-glycosylflavones.

A very recent report¹¹ concerns the cultivation process with the effect of grafting on the chemical quality of *Citrus* lemon juice. *Citrus aurantium* and *C. macrophylla* trees were selected as rootstock, and seven interstocks were also used (from different cultivars of *C. sinensis*, *C. aurantifolia*, and *C. reticulata*). The rootstock grafting is an agronomical technique able to improve production and quality of fruit, while the interstock grafting increases longevity of trees and decreases the thickness of the trunk at the grafting point. The rootstock was a more important factor than interstock on the total flavonoid content of lemon juice. Regarding the individual flavonoids, di-C-glucosyldiosmetin was the flavonoid most affected by the type of rootstock used (range: 400 to 1000 mg/l according to the variety).

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15 Flavanones and Dihydroflavonols

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15.1 GENERAL INTRODUCTION

In three of the volumes of *The Flavonoids, Advances in Research*, published between 1975 and 1993, flavanones and dihydroflavonols were part of the chapter on “Minor Flavonoids,” expertly written by Professor Bruce Bohm.^{1–4} These “Minor Flavonoid” chapters also included chalcones, dihydrochalcones, and aurones. The term “Minor Flavonoids” was first used by Harborne in 1967 to encompass not only flavanones, chalcones, and aurones, but also isoflavonoids, biflavonyls, and leucoanthocyanidins, because so few compounds belonging to each of these flavonoid classes were known at that time.⁵ For example, only about 30 flavanone and dihydroflavonol aglycones, 19 chalcones, and 7 aurones were known in 1967. The number of known “minor flavonoids” increased considerably in the next two decades, so that when the checklist for *The Flavonoids, Advances in Research Since 1980* was published in 1988, 429 known flavanones and dihydroflavonols (including glycosides) were listed, 268 chalcones and dihydrochalcones, and 29 aurones.⁶ In the last 15 years, the total number of known compounds in these flavonoid classes has more than doubled, so that the term “minor flavonoids” is no longer appropriate. Consequently, it has been decided that separate chapters should be devoted to the flavanones and dihydroflavonols (this chapter), and chalcones, dihydrochalcones, and aurones (Chapter 16).

The general structures and atom numbering of flavanones and dihydroflavonols are given in Figure 15.1. Flavanones (also called dihydroflavones) and dihydroflavonols (also called 3-hydroxyflavanones or flavanonols) lack the double bond between carbons 2 and 3 in the C-ring of the flavanoid skeleton, which is present in flavones and flavonols. Thus, in flavanones, C-2 bears one hydrogen atom in addition to the phenolic B-ring, and C-3 two hydrogen atoms. Two stereoisomeric forms of each flavanone structure are possible, since C-2 is a center of asymmetry (epimeric center). Consequently, the B-ring can be either in the (2*S*)- or (2*R*)-configuration (see Figure 15.1). The great majority of the flavanones isolated from plants are laevorotatory (–) or (2*S*)-flavanones, because the enzymatic reaction catalyzing the conversion of chalcones to flavanones is stereospecific. The C-3 atom of dihydroflavonols bears both a hydrogen atom and a hydroxyl group, and is therefore an additional center of asymmetry (see Figure 15.1). Thus, four stereoisomers are possible for each dihydroflavonol structure, (2*R*,3*R*), (2*R*,3*S*), (2*S*,3*R*), and (2*S*,3*S*). All four configurations have been found in naturally occurring dihydroflavonols, but the (2*R*,3*R*)-configuration is by far the most common.

As in all other flavonoids, there is structural variation in flavanones and dihydroflavonols because of variation in hydroxylation, methoxylation, methylation, prenylation, benzylation, glycosylation, etc. of suitable carbon atoms in the skeleton, i.e., C-5, C-6, C-7, and C-8 of the A-ring, C-2', C-3', C-4', C-5', and C-6' of the B-ring, and C-2 of the C-ring in both flavanones

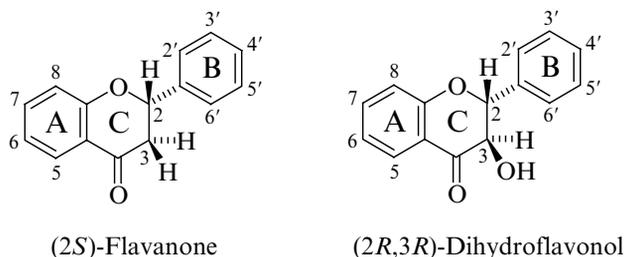


FIGURE 15.1 Skeletons of a (2*S*)-flavanone and a (2*R*,3*R*)-dihydroflavonol, showing the numbering of the carbons and naming of the rings.

and dihydroflavonols. In addition, the hydroxyl group at C-3 in dihydroflavonols can be methylated, glycosylated, or esterified.

Biogenetically, chalcones are the immediate precursors of flavanones, and some flavanones isomerize by ring opening into chalcones during isolation from plants or after chemical treatment with alkali. In turn, flavanones are intermediates in the biosynthesis of most other flavonoid groups, including flavones, flavonols, and isoflavonoids. For more information on the biosynthesis of flavonoids and flavanones in particular, the reader is referred to Chapter 3 and reviews by Heller and Forkmann.⁷⁻⁹

This chapter deals mainly with the flavanones and dihydroflavonols that have been newly reported from 1992 to 2003. Most of the literature references for these compounds have been obtained by searching *Chemical Abstracts*, *The Combined Chemical Dictionary Database of Natural Products*, and reviews published in *Natural Product Reports*.¹⁰⁻¹³ For the compilation of the checklist of all known flavanones and dihydroflavonols (see appendix), the information given in the four volumes of *The Flavonoids, Advances in Research*,¹⁻⁴ and that in the two volumes of *The Handbook of Natural Flavonoids*^{14,15} were used for the compounds recorded before 1992. Biflavonoids containing one or two flavanone or dihydroflavonol subunits have been omitted from the present chapter and checklist, since so many of these dimers and heterodimers are known that they warrant a separate chapter. However, flavanone-chalcone heterodimers and Diels-Alder adducts of chalcones and flavanones are discussed in Chapter 16 (see Table 16.4 and Table 16.5). The same applies to a number of flavanone-aurone and flavanone-auronol heterodimers (see Table 16.15). In this chapter, the newly reported flavanones and dihydroflavonols are presented and discussed in separate sections on aglycones and glycosides. The aglycones are further subdivided into different groups based on their substituents. The newly reported compounds are arranged in ten tables. The following data are supplied for each compound in the tables: *O*-substitution and *C*-substitution patterns, which provide their semisystematic name; molecular formula and relative molecular mass; trivial name where given; the plant species, family, and organ from which the compound was isolated; and the literature reference from which these details were obtained. In some more complex compounds, further modifications of the flavonoid skeleton or side groups are such that it is difficult or impossible to describe them in terms of their *O*- and *C*-substituents alone; in these cases the trivial name is used and the structure presented in one of the figures. Although most flavanones discussed in this chapter are in the (2*S*)-configuration, it is not always clear from papers describing new compounds whether the stereochemistry has been verified. In cases where the authors have determined the configuration as a (2*S*)-flavanone, the corresponding entry in each table has been annotated with an asterisk after the compound number; in the few flavanones where the configuration has been determined to be (2*R*), this has also been indicated. For dihydroflavonols, an asterisk indicates that the compound is in the (2*R*,3*R*)-configuration.

In the text, the most interesting compounds in each section are discussed with regard to their different substitution patterns, their distribution in the plant kingdom, and their biological activities if these have been studied. Furthermore, some aspects of the biosynthesis of selected compounds or groups of compounds are described. Methods and techniques for the detection, separation, purification, and identification of flavanones and dihydroflavonols are not presented in this chapter, but can be found in Chapters 1 and 2 of this book and elsewhere.¹⁶ However, it is worth pointing out here that these compounds can easily be distinguished from other groups of flavonoids when analyzed by high-performance liquid chromatography with diode array or ultraviolet (UV) detection, since most flavanones and dihydroflavonols exhibit a characteristic maximum at a wavelength of ca. 290 nm, accompanied by a small shoulder at ca. 330 to 360 nm. In the case of a glycoside acylated with a phenolic acid, the UV spectrum of the acid is superimposed on that of the flavanone or dihydroflavonol.

15.2 FLAVANONES

15.2.1 SIMPLE FLAVANONES

15.2.1.1 Simple Flavanones with *O*-Substitution Only

Flavanones substituted by hydroxy, methoxy, methylenedioxy, and *C*-methyl or related groups could conveniently be called “simple flavanones,” in contrast to flavanones bearing more complex substituents such as prenyl and benzyl groups. Simple flavanones without *C*-substitution, newly reported between 1992 and 2003, are presented in Table 15.1 (compounds **1–35**).^{17–49} Table 15.1 has been subdivided into groups according to the number of *O*-containing substituents, and within the groups the compounds have been arranged according to their number of hydroxy and methoxy substituents and relative molecular mass. As can be seen, no new mono-*O*- or di-*O*-substituted simple flavanones have been discovered in the review period and only one new tri-*O*-substituted flavanone has been isolated in the last 12 years, 5,2'-dihydroxy-7-methoxyflavanone (dihydroechioidin, **1**) from *Andrographis echioides* (Acanthaceae).¹⁷ In contrast, 17 new tetra-*O*-substituted flavanones have been reported in this period, which increases the number of known compounds in this group by 50%. For the new penta-*O*- and hexa-*O*-substituted flavanones there are nine and five newly discovered compounds, respectively, and the latter are the first five hexa-oxygenated “simple” flavanones recorded from plants.

An interesting new compound in the tetra-*O*-substituted group is 2,5-dihydroxy-6,7-dimethoxyflavanone (mosloflavanone, **7**, Figure 15.2), because accumulation of 2-hydroxylated flavanones is rather rare in plants. This flavanone was isolated independently from two species of Lamiaceae, *Mosla soochouensis*²² and *Collinsia canadensis*.²³ Mosloflavanone was shown to have antifungal activity against *Cladosporium cucumerinum* (thin-layer chromatography assay), and radical scavenging activities in the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) spectrophotometric assay.²² Alyssifolinone (**4**) from another species of Lamiaceae, *Teucrium alyssifolium*, has a 3',5'-di-*O*-substitution pattern in the B-ring, which is also quite rare.²⁰

A striking feature of about half of the new flavanones is that they contain a 2'-hydroxyl or 2'-methoxyl group in the B-ring, most notably in the genus *Andrographis* (Acanthaceae), which has yielded many new 2'-oxygenated flavanones. These include compound **1** mentioned above, compound **9** from *A. lineata*,²⁵ compound **16** from *A. viscosula*,³² compound **18** from *A. rothii*,³⁴ and compound **27** from *A. affinis*.⁴² The rhizomes of *Iris tenuifolia* (Iridaceae) are the source of the largest number of new 2'-*O*-substituted simple flavanones in a single species, namely **2**, **5**, **12**, and **19**.¹⁸ Additionally, the 2'-oxygenated dihydroflavonols corresponding to flavanones **5** and **12** were found in the same species¹⁸ (see Table 15.9). Two new 2'-*O*-substituted flavanones have also been reported from a representative of the family Moraceae, *Artocarpus heterophyllus*, flavanones **10**²⁶ and **23**, which is 5-hydroxy-7,2',4',6'-tetramethoxyflavanone.³⁹ The 8-prenyl derivative of **23** and related prenylated flavanones also occur in *A. heterophyllus*.³⁹ Prenylated derivatives of the unmethylated parent compound of **23**, 5,7,2',4',6'-pentahydroxyflavanone, are abundant in species of *Echinosophora* and *Sophora* (Leguminosae) (see Table 15.3), but the corresponding nonprenylated pentahydroxyflavanone has yet not been recorded. New 2'-*O*-substituted flavanones have also been reported from species in the families Asteraceae (**11**),²⁷ Leguminosae (**21**),^{36,37} and Boraginaceae (**22**).³⁸ The hexa-*O*-substituted (2*S*)-5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavanone (**30**) from *Scutellaria oxystegia* (Lamiaceae)⁴⁵ has a 2',6'-dioxygenation pattern in the B-ring, in common with several previously reported flavanones from the genus *Scutellaria*.¹⁵ This oxygenation pattern is rare in other plant taxa.

An interesting new penta-*O*-substituted flavanone is 8-hydroxyhesperetin (5,7,8,3'-tetrahydroxy-4'-methoxyflavanone, **20**), which was produced by the fungus *Aspergillus saitoi*

TABLE 15.1
Flavanones with Hydroxy, Methoxy, and Methylendioxy Substituents Reported from 1992 to 2003

No.	O-Substitution of Flavanone	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^a	Ref.
Tri-O-substituted								
1*	5,2'-DiOH-7-OMe	C ₁₆ H ₁₄ O ₅	286	Dihydroeochiodin	<i>Andrographis echioides</i>	Acanthaceae	Whole	17
Tetra-O-substituted								
2*	5,2'-DiOH-6,7-methylenedioxy	C ₁₆ H ₁₂ O ₆	300		<i>Iris tenuifolia</i>	Iridaceae	Root	18
3	5,7,8-TriOH-4'-OMe	C ₁₆ H ₁₄ O ₆	302		<i>Licania densiflora</i>	Chrysobalanaceae	Leaf	19
4	5,7,3'-TriOH-5'-OMe	C ₁₆ H ₁₄ O ₆	302	Alyssifolinone	<i>Teucrium alyssifolium</i>	Lamiaceae	Aerial	20
5*	5,2',3'-TriOH-7-OMe	C ₁₆ H ₁₄ O ₆	302		<i>Iris tenuifolia</i>	Iridaceae	Root	18
6	6,7,8-TriOH-5-OMe	C ₁₆ H ₁₄ O ₆	302	Oresbusin	<i>Isodon oreshius</i>	Lamiaceae	Whole	21
7*	2,5-DiOH-6,7-diOMe	C ₁₇ H ₁₆ O ₆	316	Mosloflavanone (Figure 15.2)	<i>Mosla soochouensis</i>	Lamiaceae	Whole	22
8	5,7-DiOH-8,4'-diOMe	C ₁₇ H ₁₆ O ₆	316		<i>Collinsia canadensis</i>	Lamiaceae	Aerial	23
9	5,2'-DiOH-7,8-diOMe	C ₁₇ H ₁₆ O ₆	316		<i>Chromolaena subscandens</i>	Asteraceae	Leaf	24
10	5,2'-DiOH-7,4'-diOMe	C ₁₇ H ₁₆ O ₆	316	Dihydroskullcap flavone I	<i>Andrographis lineata</i>	Acanthaceae	Whole	25
11	5,2'-DiOH-7,5'-diOMe	C ₁₇ H ₁₆ O ₆	316		<i>Artocarpus heterophyllus</i>	Moraceae	Root	26
12*	5,3'-DiOH-7,2'-diOMe	C ₁₇ H ₁₆ O ₆	316		<i>Eupatorium odoratum</i>	Asteraceae	Aerial	27
13	7,8-DiOH-6,4'-diOMe	C ₁₇ H ₁₆ O ₆	316		<i>Iris tenuifolia</i>	Iridaceae	Root	18
14	7,4'-DiOH-8,3'-diOMe	C ₁₇ H ₁₆ O ₆	316		<i>Tecoma stans</i>	Bignoniaceae	Flower	28
15*	5,7-DiOMe-3,4'-methylenedioxy	C ₁₈ H ₁₆ O ₆	328		<i>Wedelia asperina</i>	Asteraceae	Aerial	29
16	(2R)-5-OH-7,2',3'-triOMe	C ₁₈ H ₁₈ O ₆	330		<i>Caesalpinia pulcherrima</i>	Leguminosae	Aerial	30
17	8-OH-5,6,7-triOMe	C ₁₈ H ₁₈ O ₆	330	Kwangstenin A	<i>Bauhinia variegata</i>	Leguminosae	RootB	31
18	5,7,2',5'-TetraOMe	C ₁₉ H ₂₀ O ₆	344		<i>Andrographis viscosa</i>	Acanthaceae	Whole	32
Penta-O-substituted								
19*	5,2',3'-TriOH-6,7-methylenedioxy	C ₁₆ H ₁₂ O ₇	316		<i>Iris tenuifolia</i>	Iridaceae	Root	18
20	5,7,8,3'-TetraOH-4'-OMe	C ₁₆ H ₁₄ O ₇	318	8-Hydroxyhesperetin	Biotransformation product of hesperidin from citrus by <i>Aspergillus saitoi</i>	—	—	35
21	5,2',5'-TriOH-6,7-diOMe	C ₁₇ H ₁₆ O ₇	332	Dioclein	<i>Dioclea grandiflora</i> <i>Acacia longifolia</i>	Leguminosae Leguminosae	Root Root	36 37

continued

TABLE 15.1
Flavanones with Hydroxy, Methoxy, and Methylenedioxy Substituents Reported from 1992 to 2003 — continued

No.	O-Substitution of Flavanone	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^a	Ref.
22*	5,2'-DiOH-7,4',5'-triOMe	C ₁₈ H ₁₈ O ₇	346		<i>Onosma hispidum</i>	Boraginaceae		38
23	5-OH-7,2',4',6'-TetraOMe	C ₁₉ H ₂₀ O ₇	360	Heteroflavanone A	<i>Artocarpus heterophyllus</i>	Moraceae	RootB	39
24	4'-OH-5,6,7,3'-TetraOMe	C ₁₉ H ₂₀ O ₇	360	Agcorynin E	<i>Ageratum corymbosum</i>	Asteraceae	Aerial	40
25*	5,6,7,8,4'-PentaOMe	C ₂₀ H ₂₂ O ₇	374		<i>Citrus kinokuni</i>	Rutaceae	Fruit	41
26*	5,6,7,3',4'-PentaOMe	C ₂₀ H ₂₂ O ₇	374		<i>Citrus kinokuni</i>	Rutaceae	Fruit	41
27*	5,7,2',3',4'-PentaOMe	C ₂₀ H ₂₂ O ₇	374		<i>Andrographis affinis</i>	Acanthaceae	Whole	42
Hexa-O-substituted								
28	5,7-DiOH-6,3'-diOMe-4',5'-methylenedioxy	C ₁₈ H ₁₆ O ₈	360	Agamanone	<i>Agave americana</i>	Agavaceae	Aerial	43
29	5,7,3'-TriOH-6,4',5'-triOMe	C ₁₈ H ₁₈ O ₈	362		<i>Greigia sphacelata</i>	Bromeliaceae	Aerial	44
30*	5,2'-DiOH-6,7,8,6'-tetraOMe	C ₁₉ H ₂₀ O ₈	376		<i>Scutellaria oxystegia</i>	Lamiaceae	Root	45
31	5,3'-DiOH-6,7,4',5'-tetraOMe	C ₁₉ H ₂₀ O ₈	376		<i>Greigia sphacelata</i>	Bromeliaceae	Aerial	44
32	5,6,7,3',4',5'-HexaOMe	C ₂₁ H ₂₄ O ₈	404		<i>Neoraputia magnifica</i>	Rutaceae	Stem	46
Flavanone esters								
33	5,7-DiOH-flavanone (pinocembrin) 7-O-benzoate	C ₂₂ H ₁₆ O ₅	360		<i>Lophopappus tarapacanus</i>	Asteraceae	Aerial	47
34	5,7,4'-TriOH-3'-OMe-flavanone 4'-O-isobutyrate	C ₂₀ H ₂₀ O ₇	372		<i>Eriodictyon californicum</i>	Hydrophyllaceae	Leaf	48
35	5,7,4'-TriOH-flavanone (naringenin) 7-sulfate	C ₁₅ H ₁₂ O ₈ S	352		Fermentation product of naringenin using <i>Cunninghamella elegans</i>	—	—	49

* (2S)-Flavanones.

^aWhole, whole plant; Aerial, aerial parts; Root, roots or other underground parts; RootB, root bark; StemB, stem bark.

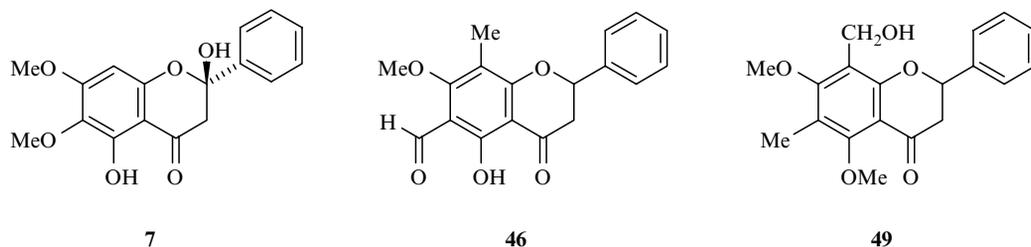


FIGURE 15.2 Flavanones with simple patterns of substitution.

when incubated with the flavanone glycoside hesperidin (hesperetin 7-rutinoside), which is common in *Citrus* fruits. The fungus also produced the known 6- and 8-hydroxylated derivatives of naringenin (carthamidin and isocarthamidin, respectively) from another common citrus flavanone glycoside, naringin (naringenin 7-neohesperidoside).³⁵ These three flavanones, produced by *A. saitoi*, were found to be potent radical scavengers and antioxidants using the DPPH test and methyl linoleate oxidation system, and had a greater activity than the original glycosides. The activities of the two 8-hydroxyflavanones were comparable to that of α -tocopherol, but that of the 6-hydroxy derivative was weaker.³⁵

Two new pentamethoxyflavanones (**25** and **26**) have been reported from *Citrus kinokuni* (Rutaceae)⁴¹ and the new hexamethoxyflavanone (**32**) from another member of the Rutaceae, *Neoraputia magnifica*.⁴⁶ The oxygenation pattern of **32** (5,6,7,3',4',5'-hexa-*O*) is also found in the new hexa-*O*-substituted flavanones **29** and **31** from *Greigia sphacelata* (Bromeliaceae)⁴⁴ and the methylenedioxyflavanone **28** from *Agave americana* (Agavaceae).⁴³

Three new esters of flavanone aglycones are listed in Table 15.1. These are the 7-*O*-benzoate of pinocembrin (**33**) from *Lophopappus tarapacanus* (Asteraceae),⁴⁷ the 4'-*O*-isobutyrate of eriodictyol 3'-methyl ether (**34**) from *Eriodictyon californicum* (Hydrophyllaceae), which is a potential cancer chemopreventive agent,⁴⁸ and the 7-sulfate of naringenin (**35**), which was obtained as a fermentation product of naringenin using the fungus *Cunninghamella elegans* NRRL 1392 in 23% yield.⁴⁹

During the last 12 years, several publications have dealt with the biological activities of some known "simple" flavonoids. For example, the antifungal activity of the previously known mono-oxygenated 7-hydroxyflavanone, isolated from the roots of *Virola surinamensis* (Myristicaceae), was tested against *Cladosporium cladosporoides*, and the compound had a tenfold higher activity than the positive control nystatin.⁵⁰ The 7-methyl ether of naringenin, sakuranetin (5,4'-dihydroxy-7-methoxyflavanone), named after the Japanese name for cherry tree, sakura, from which it was first isolated nearly 100 years ago, also has antifungal activity. Sakuranetin was isolated as a phytoalexin (an antifungal compound not present in healthy plants, but produced in large quantities after infection or physical damage of the plant) from UV-irradiated rice leaves⁵¹ and later also from rice leaves infected with the rice blast fungus.⁵² The ED₅₀ value of this flavanone against spore germination of *Pyricularia oryzae*, the rice blast pathogen, was ca. 15 ppm. The sakuranetin content of resistant cultivars of rice after infection with *P. oryzae* was generally much higher than that of susceptible cultivars,^{51,52} so that the production of this phytoalexin may be involved in the resistance of rice plants against the blast pathogen. Sakuranetin has also been found as an induced compound in rice plants after infection by the stem nematode *Ditylenchus angustus*.⁵³ Infection of bark of the Weymouth pine (*Pinus strobus*, Pinaceae) with the pinewood nematode, *Bursaphelenchus xylophilus*, induced the production of a related flavanone, pinocembrin (5,7-dihydroxyflavanone).⁵⁴ Sakuranetin and the corresponding dihydroflavonol, aromadendrin 7-methyl ether, isolated from the leaves of *Trixis vauthieri* (Asteraceae), were active against the trypanostigote forms

of *Trypanosoma cruzi*, the protozoan that causes Chagas' disease, which affects 18 million people in Latin America. The trypanocidal activity of sakuranetin at 0.5 mg/ml was 100%, and that of aromadendrin 7-methyl ether at the same concentration was $86 \pm 13\%$.⁵⁵

15.2.1.2 Simple Flavanones with Both *O*- and *C*-Substitution

Fourteen new simple flavanones with *C*-methyl, *C*-hydroxymethyl, and *C*-formyl substituents reported between 1992 and 2003 are listed in Table 15.2 (compounds **36–49**).^{56–66} For each compound the *O*- and *C*-substituents are given in separate columns. Two interesting new compounds are the related *C*-methylated 2-hydroxyflavanones, 2,5-dihydroxy-7-methoxy-6-*C*-methylflavanone (**36**) and 2,5-dihydroxy-7-methoxy-8-*C*-methylflavanone (**37**), which have been reported from the stem-bark of *Friesodielsia enghiana* (Annonaceae).⁵⁶ The same compounds were later isolated from the leaves of *Leptospermum polygalifolium* ssp. *polygalifolium* (Myrtaceae), together with the 6-*C*- and 8-*C*-regioisomers of 2,5,7,4'-tetrahydroxydihydroflavonol (see Table 15.9).⁶⁷ *C*-Methylflavonoids are quite common in the family Myrtaceae, and a new 8-*C*-methylated flavanone (**40**) was found in another species from this family, *Callistemon coccineus*.⁵⁹ The 6-*C*-regioisomer of the latter compound, **39**, has been reported from the conifer *Pseudotsuga wilsoniana* (Pinaceae).⁵⁸ From the related *Pseudotsuga sinensis* the 6-*C*-methylated flavanone **41** was reported,⁶⁰ with the same unusual 3',5'-*O*-substitution of the flavanone B-ring as alyssifolinone (**4**). Another unusual B-ring oxygenation pattern, based on 2',4'-di-*O*-substitution, is present in flavanone **43** from the roots of *Terminalia alata* (Combretaceae),⁶² and also in 2'-hydroxymatteucinol (**45**) from the fern *Matteucia orientalis*.⁶⁴ The 6,8-di-*C*-methylated flavanone, isomatteucinol (**44**), which bears a sole 3'-methoxy group in the B-ring, was isolated from the same species.⁶³ The only monocot from which *C*-methylflavanones have been reported in the last 12 years is *Vellozia nanuzae* (Velloziaceae), from which 6-*C*-methyleryodictyol 7,3',4'-trimethyl ether (**42**) was obtained in addition to a range of isoprenylated flavonoids.⁶¹ The 8-*C*-methyl derivative of naringenin 4'-methyl ether, 8-methylisosakuranetin (**38**), has been reported from *Amaranthus caudatus* (Amaranthaceae), a member of the plant order Caryophyllales.⁵⁷ Unusual flavanones and chalcones with both *C*-methyl and *C*-formyl or *C*-hydroxymethyl substituents have been discovered in another member of the Caryophyllales, *Petiveria alliacea* (Phytolaccaceae). These include 5-hydroxy-7-methoxy-6-*C*-formyl-8-*C*-methylflavanone (leridal, **46**, Figure 15.2), 5-hydroxy-7-methoxy-6-*C*-hydroxymethyl-8-*C*-methylflavanone (leridol, **48**), and 5,7-dimethoxy-6-*C*-hydroxymethyl-8-*C*-methylflavanone (5-*O*-methylleridol, **49**, Figure 15.2).⁶⁵ However, the related 5,7-dihydroxy-6-*C*-formyl-8-*C*-methylflavanone (7-demethylleridal) reported as new from the same species⁶⁸ had already been recorded previously as lawinal from *Unona lawii* (Annonaceae).¹⁵ Lawinal isolated from species of *Desmos* (also Annonaceae) exhibited a potent anti-HIV activity with EC₅₀ values of 0.022 μg/ml and a therapeutic index of 489. Thus, the compound was considered to be an excellent lead for the development of anti-HIV drugs.⁶⁹ A new *C*-formylflavanone, 7-hydroxy-5-methoxy-6-*C*-methyl-8-*C*-formylflavanone (desmosflavanone II, **47**), has been obtained from another species of this genus, *D. cochinchinensis*.⁶⁶

15.2.2 ISOPRENYLATED FLAVANONES

15.2.2.1 Introduction

In the first volume of *The Flavonoids*, published in 1975, only eight isoprenylated flavanones were described.¹ Since then many more have been isolated and characterized, and at present the number of known isoprenylated flavanones (excluding dihydroflavonols) is nearly 300. Most of these substances have been obtained from species of Leguminosae, although their distribution is not quite so restricted as thought previously, as isoprenylated flavanones have

TABLE 15.2
Flavanones with C-Methyl, C-Hydroxymethyl, and C-Formyl Substituents Reported from 1992 to 2003

No.	O-Substitution	C-Substitution ^a	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^a	Ref.
36	2,5-DiOH-7-OMe	6-Me	C ₁₇ H ₁₆ O ₅	300		<i>Friesodielsia enghiana</i>	Annonaceae	StemB	56
37	2,5-DiOH-7-OMe	8-Me	C ₁₇ H ₁₆ O ₅	300		<i>Friesodielsia enghiana</i>	Annonaceae	StemB	56
38	5,7-DiOH-4-OMe	8-Me	C ₁₇ H ₁₆ O ₅	300	8-Methylisosakuranetin	<i>Amaranthus caudatus</i>	Amaranthaceae	Flower	57
39	5,4'-DiOH-7-OMe	6-Me	C ₁₇ H ₁₆ O ₅	300	6-Methylsakuranetin	<i>Pseudotsuga wilsoniana</i>	Pinaceae	Heartwood	58
40	5,4'-DiOH-7-OMe	8-Me	C ₁₇ H ₁₆ O ₅	300	8-Methylsakuranetin	<i>Callistemon coccineus</i>	Myrtaceae	Leaf wax	59
41	5,7,3',5'-TetraOH	6-Me	C ₁₆ H ₁₄ O ₆	302		<i>Pseudotsuga sinensis</i>	Pinaceae	Leaf	60
42	5-OH-7,3',4'-TriOMe	6-Me	C ₁₉ H ₂₀ O ₆	344	6-Methyleriodictyol 7,3',4'-trimethyl ether	<i>Vellozia namuzae</i>	Velloziaceae	Leaf	61
43	5,7,2',4'-TetraOMe	8-Me	C ₂₀ H ₂₂ O ₆	358		<i>Terminalia alata</i>	Combretaceae	Root	62
44	5,7-DiOH-3'-OMe	6,8-diMe	C ₁₈ H ₁₈ O ₅	314	Isomatteucinol	<i>Matteucia orientalis</i>	Aspleniaceae, Pteridophyta		63
45	5,7,2'-TriOH-4'-OMe	6,8-diMe	C ₁₈ H ₁₈ O ₆	330	2'-Hydroxymatteucinol	<i>Matteucia orientalis</i>	Aspleniaceae, Pteridophyta		64
46	5-OH-7-OMe	6-Formyl, 8-Me	C ₁₈ H ₁₆ O ₅	312	Leridal (Figure 15.2)	<i>Petiveria alliacea</i>	Phytolaccaceae	Leaf	65
47	7-OH-5-OMe	6-Me, 8-formyl	C ₁₈ H ₁₆ O ₅	312	Desmosflavanone II	<i>Desmos cochinchinensis</i>	Annonaceae	Root	66
48	5-OH-7-OMe	6-CH ₂ OH, 8-Me	C ₁₈ H ₁₈ O ₅	314	Leridal	<i>Petiveria alliacea</i>	Phytolaccaceae	Leaf	65
49	5,7-DiOMe	6-CH ₂ OH, 8-Me	C ₁₉ H ₂₀ O ₅	328	5-O-Methyleriodol (Figure 15.2)	<i>Petiveria alliacea</i>	Phytolaccaceae	Leaf	65

^a(2S)-Flavanones.

^aWhole, whole plant; Aerial, aerial parts; Root, roots or other underground parts; StemB, stem bark.

been isolated from 14 different plant families in the last 12 years. A useful review on isoprenylated flavonoids up to 1995 is that by Barron and Ibrahim.⁷⁰

Until a decade ago, the mevalonate pathway was assumed to be the general biosynthetic route leading to isoprenoid substituents, but then a new mevalonate-independent route was discovered, initially in bacteria but later also in higher plants, the glyceraldehyde pyruvate pathway.⁷¹ To investigate whether the mevalonate or the glyceraldehyde pyruvate pathway is used for the biosynthesis of isoprenylated flavanones, [1-¹³C]-glucose was administered to *Glycyrrhiza glabra* roots, and it was found that the prenyl groups of glabrol (7,4'-dihydroxy-8,3'-di-C-prenylflavanone) were synthesized via the latter pathway.⁷²

A wide range of different isoprenoid substituents have been found conjugated to flavanones from plants. The most common of these is the 3-methylbut-2-enyl group, also described as 3,3-dimethylallyl or isopentenyl, but for convenience often called "prenyl" group, a term which is used in this chapter. In some flavonoids, the monoprenyl group is rearranged into a 1,1-dimethylallyl substituent. When two isoprenoid units are linked, a geranyl, neryl, or lavandulyl moiety may be formed, and linkage of three isoprenoid units may result in a farnesyl group. The isoprenylated side chains may be modified by reduction, oxidation, and dehydration. Furthermore, cyclization of the isoprenoid substituent with an *ortho*-hydroxyl group on the phenolic A- or B-ring may result in furano- or dimethylpyrano ring structures. For convenience, the isoprenylated flavanones in this section have been divided into three groups, (1) those with noncyclic isoprenoid groups, (2) flavanones with furano or dihydrofurano rings, and (3) flavanones with dimethylpyrano or dimethyldihydropyrano rings. This division is rather artificial from a biogenetic point of view, as these three types of moieties are closely related and representatives of all three groups may be found in the same plant species, although the isoprenylated flavanones of the Moraceae and Rutaceae tend to bear cyclic rather than noncyclic isoprenoid substituents.

15.2.2.2 Flavanones with Noncyclic Isoprenoid Substituents

Flavanones with noncyclic isoprenoid substituents reported during 1992 to 2003 are listed in Table 15.3 (compounds **50–140**).^{39,61,73–127} They have been divided into six groups according to their isoprenoid side chains: prenyl, 1,1-dimethylallyl, geranyl, lavandulyl, farnesyl, and oxygen-containing isoprenyl substituents. Within these groups the compounds have been arranged according to the number of prenyl groups (mono-, di-, or triprenylated) and then according to the number of oxygens in the molecular formula and by relative molecular mass. For each compound, the *O*- and *C*-substituents are given in separate columns. More than two thirds of the 90 flavanones listed in Table 15.3 have been isolated from representatives of the family Leguminosae, and more than 30 from species of the genus *Sophora* or the related *Echinosophora*, notably by Iinuma and coworkers.^{82,85,87,90,102,108,112,114,116} Most of the isoprenoid flavanones have been obtained from root tissue, but others have been found in a wide variety of other organs, such as stems, leaves, tubers, flowers, and seeds.

Most of the isoprenyl groups are *C*-linked to the flavanone skeleton, but in some compounds they are *O*-linked, for example, in 4'-*O*-prenylsakuranetin (**58**) and 4'-*O*-geranyl-naringenin (**98**) from *Boronia coerulescens* ssp. *spinescens* (Rutaceae),⁸⁰ ponganone V (**67**) from *Pongamia pinnata* (Leguminosae),⁸⁸ monotesone A (**62**) from *Monotes engleri* (Dipterocarpaceae),⁸³ and 5-hydroxy-7-nerylxyflavanone (**76**, Figure 15.3) from *Helichrysum rugulosum* (Asteraceae).⁹³ The *C*-prenyl groups are usually attached to C-6 or C-8 of the A-ring of the flavonoid skeleton, but may also be attached to C-3', C-5', or C-6' of the B-ring. Examples are the 3',5'-di-*C*-prenylated abyssinone V 4'-methyl ether (**79**) from *Erythrina burttii*⁹⁷ and *E. abyssinica* (Leguminosae),⁹⁸ the 5',6'-di-*C*-prenylated abyssinin III (**84**) from *E. abyssinica*,⁸⁶ and the 6,8,5'-tri-*C*-prenylated isoamoritin (**88**) from *Amorpha fruticosa* (Leguminosae).¹⁰³

TABLE 15.3
Flavanones with Noncyclic Isoprenoid Substituents Reported from 1992 to 2003

No.	O-Substitution	C-Substitution ^a	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^b	Ref.
PRENYLFLAVANONES									
Di-O-substituted monoprenyl									
—	5,4'-DiOH	6-Pr	C ₂₀ H ₂₀ O ₄	324	Crotaromin ^c	<i>Crotalaria ramosissima</i>	Leguminosae	Root	73
50	5,7-DiOH	6-Me-8-Pr	C ₂₁ H ₂₂ O ₄	338		<i>Dalea caerulea</i>	Leguminosae		74
51*	4'-OH-7-OMe	8-Pr	C ₂₁ H ₂₂ O ₄	338	Mundulea flavanone A	<i>Mundulea suberosa</i>	Leguminosae	StemB	75
Tri-O-substituted monoprenyl									
52*	5,7,2'-TriOH	8-Pr	C ₂₀ H ₂₀ O ₅	340	Kushenol S	<i>Sophora flavescens</i>	Leguminosae	Root	76
53*	5,7,4'-TriOH	6-Me-8-Pr	C ₂₁ H ₂₂ O ₅	354		<i>Eysenhardtia texana</i>	Leguminosae	Aerial	77
54*	5,7,4'-TriOH	8-Me-6-Pr	C ₂₁ H ₂₂ O ₅	354		<i>Eysenhardtia texana</i>	Leguminosae	Aerial	77
55	5,7-DiOH-6-OMe	8-Pr	C ₂₁ H ₂₂ O ₅	354	Agrandol	<i>Dioclea grandiflora</i>	Leguminosae	RootB	78
56	(2 <i>R</i>)-5,7-DiOH-8-OMe	6-Pr	C ₂₁ H ₂₂ O ₅	354	Microfolione	<i>Cedrelopsis microfoliata</i>	Ptaeroxylaceae	StemB	79
57*	5,4'-DiOH-7-OMe	8-Pr	C ₂₁ H ₂₂ O ₅	354	Mundulea flavanone B	<i>Mundulea suberosa</i>	Leguminosae	StemB	75
58	5-OH-7-OMe-4'-OPr	8-Pr	C ₂₁ H ₂₂ O ₅	354	4'-O-Prenylsakuranetin	<i>Boronia coerulescens</i> ssp. <i>spinescens</i>	Rutaceae	Aerial	80
59	5-OH-7,4'-diOMe	8-Pr	C ₂₂ H ₂₄ O ₅	368	Flowerine	<i>Azadirachta indica</i>	Meliaceae	Flower	81
Tetra-O-substituted monoprenyl									
60	5,7,8,4'-TetraOH	3-(3-Me-but-3-enyl)	C ₂₀ H ₂₀ O ₆	356	Flowerone	<i>Azadirachta indica</i>	Meliaceae	Flower	81
61*	5,7,2,4'-TetraOH	8-Pr	C ₂₀ H ₂₀ O ₆	356	Leachianone G	<i>Sophora leachiana</i>	Leguminosae	Root	82
62	5,7,3'-TriOH-4'-OPr	6-Pr	C ₂₀ H ₂₀ O ₆	356	Monotesone A	<i>Monotes engleri</i>	Dipterocarpaceae		83
63	(2 <i>R</i>)-5,7,2'-TriOH-8-OMe	6-Pr	C ₂₁ H ₂₂ O ₆	370	Dioflorin	<i>Dioclea grandiflora</i>	Leguminosae	RootB	84
64	5,7,4'-TriOH-3'-OMe	8-Pr	C ₂₁ H ₂₂ O ₆	370	Exiguaflavanone K	<i>Sophora exigua</i>	Leguminosae	Root	85
65	5,7,4'-TriOH-3'-OMe	5'-Pr	C ₂₁ H ₂₂ O ₆	370	Abyssinin II	<i>Erythrina abyssinica</i>	Leguminosae	StemB	86
66*	5,2,4'-TriOH-7-OMe	8-Pr	C ₂₁ H ₂₂ O ₆	370	Kenusanone I	<i>Echinophora koreensis</i>	Leguminosae	Stem	87
67	7-OMe-3',4'-methylenedioxy-6-O-Pr	6-Pr	C ₂₃ H ₂₂ O ₆	382	Ponganone V	<i>Pongamia pinnata</i>	Leguminosae	RootB	88
68	5,7-DiOH-3',4'-diOMe	5'-Pr	C ₂₂ H ₂₄ O ₆	384		<i>Melilotus alba</i>	Leguminosae	Leaf, flower	89
Penta-O-substituted monoprenyl									
69*	5,7,2,4'-TetraOH-5'-OMe	6-Pr	C ₂₁ H ₂₂ O ₇	386	Kushenol V	<i>Sophora flavescens</i>	Leguminosae	Root	76

continued

TABLE 15.3
Flavanones with Noncyclic Isoprenoid Substituents Reported from 1992 to 2003 — continued

No.	O-Substitution	C-Substitution ^a	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^b	Ref.
70	5,7,2',4'-TetraOH-5'-OMe	8-Pr	C ₂₁ H ₂₂ O ₇	386	Kushenol W	<i>Sophora flavescens</i>	Leguminosae	Root	76
71*	5,7,2',6'-TetraOH-4'-OMe	8-Pr	C ₂₁ H ₂₂ O ₇	386	Kenusanone D	<i>Echinosophora koreensis</i>	Leguminosae	Root	90
72*	5,2',6'-TriOH-7,4'-diOMe	8-Pr	C ₂₂ H ₂₄ O ₇	400	Kenusanone E	<i>Echinosophora koreensis</i>	Leguminosae	Root	90
73	5,7-DiOH-2',4',6'-triOMe	8-Pr	C ₂₃ H ₂₆ O ₇	414	Heteroflavanone C	<i>Artocarpus heterophyllus</i>	Moraceae	RootB	91
74	5-OH-7,2',4',6'-tetraOMe	8-Pr	C ₂₄ H ₂₈ O ₇	428	Heteroflavanone B	<i>Artocarpus heterophyllus</i>	Moraceae	RootB	39
75*	5,7,4'-TriOH-3'-OMe	6-(β-Hydroxyethyl)-8-Pr	C ₂₃ H ₂₆ O ₇	414	Laxiflorin	<i>Derris laxiflora</i>	Leguminosae	Aerial	92
Di-O-substituted diprenyl									
76	5-OH-7-O-neryl		C ₂₅ H ₂₈ O ₄	392	(Figure 15.3)	<i>Helichrysum rugulosum</i>	Asteraceae		93
Tri-O-substituted diprenyl									
77	5,7,4'-TriOH	6,3'-diPr	C ₂₅ H ₂₈ O ₅	408	Paratocarpin L or Macaranga flavanone B	<i>Paratocarpus venosa</i>	Moraceae	Leaf	94
78	5,7-DiOH-4'-OMe	3'-(3-Me-but-1,3-dienyl)-5'-Pr	C ₂₆ H ₂₈ O ₅	420	Burtinonedehydrate	<i>Macaranga pleiostenma</i> <i>Erythrina burttii</i>	Euphorbiaceae Leguminosae	StemB	95 96
79	5,7-DiOH-4'-OMe	3',5'-diPr	C ₂₆ H ₃₀ O ₅	422	Abyssinone V 4'-methyl ether	<i>Erythrina burttii</i>	Leguminosae	StemB	97
79	5,7-DiOH-4'-OMe	3',5'-diPr	C ₂₆ H ₃₀ O ₅	422	Abyssinone V 4'-methyl ether	<i>Erythrina abyssinica</i>	Leguminosae	StemB	98
80*	5,7-DiOH-4'-OMe	6-Me-8,3'-diPr	C ₂₇ H ₃₂ O ₅	436	Lespedezaflavanone F	<i>Lespedeza formosa</i>	Leguminosae	RootB	99
81*	5,7-DiOH-4'-OMe	8-Me-6,3'-diPr	C ₂₇ H ₃₂ O ₅	436	Lespedezaflavanone G	<i>Lespedeza formosa</i>	Leguminosae	RootB	99
Tetra-O-substituted diprenyl									
82	5,7,3',4'-TetraOH	6,8-diPr	C ₂₅ H ₂₈ O ₆	424	6,8-Diprenyleiodictyol	<i>Vellozia namizae</i>	Velloziaceae	Leaf	61
83	5,7,3',4'-TetraOH	6,5'-diPr	C ₂₅ H ₂₈ O ₆	424		<i>Schoenus nigricans</i>	Cyperaceae	Tuber	100
84	5,7,3',4'-TetraOH	5',6'-diPr	C ₂₅ H ₂₈ O ₆	424	Abyssinin III	<i>Erythrina abyssinica</i>	Leguminosae	StemB	86
85	5,7,3',5'-TetraOH	6,8-diPr	C ₂₅ H ₂₈ O ₆	424	Monotesone B	<i>Monotes engleri</i>	Dipterocarpaceae		83
86	5,2',4'-TriOH-7-OMe	8,5'-diPr	C ₂₆ H ₃₀ O ₆	438	Maackiaflavanone	<i>Maackia amurensis</i> ssp. <i>buergeri</i>	Leguminosae	Root	101
Penta-O-substituted diprenyl									
87*	5,7,2',4',6'-PentaOH	6,8-diPr	C ₂₅ H ₂₈ O ₇	440	Kenusanone B	<i>Echinosophora koreensis</i>	Leguminosae	Root	102

88	Tetra-O-substituted triprenyl 5,7,3'-TriOH-4'-OMe 1,1-DIMETHYLALLYL- FLAVANONES	6,8,5'-triPr	C ₃₁ H ₃₈ O ₆	506	Isoamoritin	<i>Amorpha fruticosa</i>	Leguminosae	Root	103
89*	5,7,4'-TriOH	6-(1,1-DMA)	C ₂₀ H ₂₀ O ₅	340	(Figure 15.3)	<i>Monotes engleri</i>	Dipterocarpaceae	Leaf	104
90	5,7,4'-TriOH	8-(1,1-DMA)	C ₂₀ H ₂₀ O ₅	340	Ugonin E	<i>Helminthostachys zeylanica</i>	Ophioglossaceae/ Pteridophyta	Rhiz	105
91*	5,7,3',4'-TetraOH	6-(1,1-DMA)	C ₂₀ H ₂₀ O ₆	356	(Figure 15.3)	<i>Monotes engleri</i>	Dipterocarpaceae	Leaf	104
92*	5,7,4'-TriOH-3'-OMe	6-(1,1-DMA)	C ₂₁ H ₂₂ O ₆	370	(Figure 15.3)	<i>Monotes engleri</i>	Dipterocarpaceae	Leaf	104
93	5,7,2',4'-TetraOH	6-Pr-5'-(1,1-DMA)	C ₂₅ H ₂₈ O ₆	424		<i>Dalea elegans</i>	Leguminosae	Root	106
94*	5,7,2',4'-TetraOH	8-Pr-5'-(1,1-DMA)	C ₂₅ H ₂₈ O ₆	424		<i>Dalea scandens</i> var. <i>paucifolia</i>	Leguminosae	Root	107
95*	5,7,4'-TriOH-2'-OMe	8-Pr-5'-(1,1-DMA)	C ₂₆ H ₃₀ O ₆	438		<i>Dalea scandens</i> var. <i>paucifolia</i>	Leguminosae	Root	107
GERANYL-FLAVANONES									
96*	7,4'-DiOH	8-Ger	C ₂₅ H ₂₈ O ₄	392	Prostratol F	<i>Sophora prostrata</i>	Leguminosae	Root	108
97	5,7,4'-TriOH	3'-Ger	C ₂₅ H ₂₈ O ₅	408	Macaranga flavanone A	<i>Macaranga pleiostemona</i>	Euphorbiaceae	Leaf	95
98	5,7-DiOH-4'-OGer		C ₂₅ H ₂₈ O ₅	408	4'-O-Geranylningenin	<i>Boronia caerulescens</i> ssp. <i>spinescens</i>	Rutaceae	Aerial	80
99	5,7,2',4'-TetraOH	8-Ger	C ₂₅ H ₂₈ O ₆	424	Sophoraflavanone C	<i>Echinosophora koreensis</i>	Leguminosae	Stem	87
100	5,7,2',4'-TetraOH	3'-Ger	C ₂₅ H ₂₈ O ₆	424	Sanggenol A	<i>Morus cathayana</i>	Moraceae	Root/B	109
101	5,7,2',4'-TetraOH	6-Ger-8-Pr	C ₃₀ H ₃₆ O ₆	492	Macrourone C	<i>Morus macroura</i>	Moraceae	Stem/B	110
102*	5,7,2',4',6'-PentaOH	6-Ger	C ₂₅ H ₂₈ O ₇	440	Sophoraflavanone D	<i>Echinosophora koreensis</i>	Leguminosae	Root	102
103*	5,7,2',4',6'-PentaOH	8-Ger	C ₂₅ H ₂₈ O ₇	440	Sophoraflavanone E	<i>Echinosophora koreensis</i>	Leguminosae	Root	102
104	5,7,2',4',6'-PentaOH LAVANDULYL- FLAVANONES	6-Ger-8-Pr	C ₃₀ H ₃₆ O ₇	508	Tomentosanol E (Figure 15.3)	<i>Sophora tomentosa</i>	Leguminosae	Root	111
105	5,7,4'-TriOH	8-Lav	C ₂₅ H ₂₈ O ₅	408	Leachianone E	<i>Sophora leachiana</i>	Leguminosae	Root	112
106	5,7,4'-TriOH	8-(2-isopropyl)-5- Me-5-hexenyl)	C ₂₅ H ₂₈ O ₅	408	Remangiflavanone A	<i>Physena madagascariensis</i>	Capparaceae	Leaf	113
107*	7,2'-DiOH-5-OMe	8-Lav	C ₂₆ H ₃₀ O ₅	422	Kushenol R (Figure 15.3)	<i>Sophora flavescens</i>	Leguminosae	Root	76
108*	7,4'-DiOH-5-OMe	8-Lav	C ₂₆ H ₃₀ O ₅	422	Kushenol U	<i>Sophora flavescens</i>	Leguminosae	Root	76
109	7,4'-DiOH-2'-OMe	8-Lav	C ₂₆ H ₃₀ O ₅	422	Alopecurone G	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
110	5,7,2',4'-TetraOH	8-(2-isopropyl)-5- Me-5-hexenyl)	C ₂₅ H ₂₈ O ₆	424	Remangiflavanone B	<i>Physena madagascariensis</i>	Capparaceae	Leaf	113

continued

TABLE 15.3
Flavanones with Noncyclic Isoprenoid Substituents Reported from 1992 to 2003 — continued

No.	O-Substitution	C-Substitution ^a	Mol.		Trivial Name	Plant Source	Family	Organ ^b	Ref.
			Formula	M _r					
111	5,7,2',6'-TetraOH	8-Lav	C ₂₅ H ₂₈ O ₆	424	Exiguatflavanone A	<i>Sophora exigua</i>	Leguminosae	Root	115
112*	5,2',5'-TriOH-7-OMe	8-Lav	C ₂₆ H ₃₀ O ₆	438	Exiguatflavanone F	<i>Sophora exigua</i>	Leguminosae	Root	116
113	5,2',6'-TriOH-7-OMe	8-Lav	C ₂₆ H ₃₀ O ₆	438	Exiguatflavanone B	<i>Sophora exigua</i>	Leguminosae	Root	115
114	7,2',4'-TriOH-5-OMe	8-Lav	C ₂₆ H ₃₀ O ₆	438	Kurarinone	<i>Sophora flavescens</i>	Leguminosae	Root	117
115	7,4'-DiOH-5,2'-diOMe	8-Lav	C ₂₇ H ₃₂ O ₆	452	Kurarinone	<i>Gentiana macrophylla</i>	Gentianaceae	Root	118
					2'-methyl ether	<i>Sophora flavescens</i>	Leguminosae	Root	117
116*	5,7,2',4',6'-PentOH	6-Lav	C ₂₅ H ₂₈ O ₇	440	Exiguatflavanone C	<i>Sophora exigua</i>	Leguminosae	Root	116
117*	5,7,2',4',6'-PentOH	8-Lav	C ₂₅ H ₂₈ O ₇	440	Exiguatflavanone G	<i>Sophora exigua</i>	Leguminosae	Root	85
118*	5,2',4'-TriOH-7,5'-diOMe	8-Lav	C ₂₇ H ₃₂ O ₇	468	Exiguatflavanone E	<i>Sophora exigua</i>	Leguminosae	Root	116
119	5,7,2',4',6'-PentOH	6-Pr-8-Lav	C ₃₀ H ₃₆ O ₇	508	Exiguatflavanone J	<i>Sophora exigua</i>	Leguminosae	Root	85
120*	5,7,2',6'-TetraOH-4'-OMe	6-Pr-8-Lav	C ₃₁ H ₃₈ O ₇	522	Exiguatflavanone D	<i>Sophora exigua</i>	Leguminosae	Root	116
121	5,7,3',4'-TetraOH FLAVANONES BEARING HYDROXY- OR EPOXY- PRENYL GROUPS	6-Farnesyl	C ₃₀ H ₃₆ O ₆	492	(Figure 15.3)	<i>Boronia ramosa</i>	Rutaceae	Aerial	119
122	5,7,4'-TriOH	8-(2-OH-3-Me-but-3-enyl)	C ₂₀ H ₂₀ O ₆	356	Tomentosanol D	<i>Sophora tomentosa</i>	Leguminosae	Root	111
123	5,7-DiOH-4'-OMe	8-(2-OH-3-Me-but-3-enyl)	C ₂₁ H ₂₂ O ₆	370		<i>Bosistoa brassii</i>	Rutaceae	Leaf	120
124	(2R)-7,4'-DiOH-5-OMe	8-[4-OH-3-Me-(2Z)-butenyl]	C ₂₁ H ₂₂ O ₆	370		d d	—	—	121
125	(2R)-7,4'-DiOH-5-OMe	8-[5-OH-3-Me-(2E)-butenyl]	C ₂₁ H ₂₂ O ₆	370		dd	—	—	121
126	5,7,4'-TriOH	8-Pr-6-(2-OH-3-Me-but-3-enyl)	C ₂₅ H ₂₈ O ₆	424	Lupiniols A1 and A2	<i>Lupinus luteus</i>	Leguminosae	Root	122

127	5,7,4'-TriOH	8-Pr-3'-(2-OH-3-Me-but-3-enyl)	C ₂₅ H ₂₈ O ₆	424	Lupinoli B	<i>Lupinus luteus</i>	Leguminosae	Root	122
128*	5,7,2'-TriOH	8-(5-OH-2-isopropenyl-5-Me-hexyl)	C ₂₅ H ₃₀ O ₆	426	Kushenol T	<i>Sophora flavescens</i>	Leguminosae	Root	76
129	5,7-DiOH-4'-OMe	3'-Pr-5'-(3-OH-3-Me-buten-1-yl)	C ₂₆ H ₃₀ O ₆	438	Buritinone	<i>Erythrina burttii</i>	Leguminosae	StemB	97
130	5,7-DiOH-4'-OMe	8-Pr-3'-(3-OH-3-Me-butyl)	C ₂₆ H ₃₂ O ₆	440	Licoleafol	<i>Azadirachta indica</i>	Meliaceae	Aerial	123
131*	5,7,3',4'-TetraOH	8-[(E)-3-Hydroxymethyl-2-butenyl]	C ₂₀ H ₂₀ O ₇	372	(Figure 15.3)	<i>Glycyrrhiza uralensis</i>	Leguminosae	Leaf	124
132	5,7,3',4'-TetraOH	6-(3-OH-3-methylbutyl)	C ₂₀ H ₂₂ O ₇	374	6-(3-Hydroxy-isopentanyl)-eriodictyol	<i>Vellozia nanuzae</i>	Velloziaceae	Leaf	61
133*	5,7-DiOH-2',4'-diOMe	8-(2,3-epoxy-3-Me-butyl)	C ₂₂ H ₂₄ O ₇	400		<i>Atylosia scarabaeoides</i>	Leguminosae	Root	125
134	5,7,3',4'-TetraOH	6-Pr-8-(2-OH-3-Me-but-3-enyl)	C ₂₅ H ₂₈ O ₇	440	Dorsmanin H	<i>Dorstenia mannii</i>	Moraceae	Twig	126
135	5,7,3',4'-TetraOH	8-Pr-6-(2-OH-3-Me-but-3-enyl)	C ₂₅ H ₂₈ O ₇	440	(Figure 15.3)	<i>Monotes engleri</i>	Dipterocarpaceae	Leaf	83
136*	5,7,2',4'-TetraOH	8-[2-(2-OH-isopropyl)-5-Me-4-hexenyl]	C ₂₅ H ₃₀ O ₇	442	Kushenol Q	<i>Sophora flavescens</i>	Leguminosae	Root	76
137	5,7,2',6'-TetraOH	8-[2-(2-OH-isopropyl)-5-Me-4-hexenyl]	C ₂₅ H ₃₀ O ₇	442	Exiguaflavanone M	<i>Sophora exigua</i>	Leguminosae	Root	85
138	5,7,4'-TriOH-2'-OMe	8-(5-OH-5-Me-2-isopropenyl- <i>trans</i> -hex-3-enyl)	C ₂₆ H ₃₀ O ₇	454	Leachianone D	<i>Sophora leachiana</i>	Leguminosae	Root	112
139*	5,7,4'-TriOH-2'-OMe	8-(5-OH-5-Me-2-isopropenyl-hexyl)	C ₂₆ H ₃₂ O ₇	456	Kushenol P1	<i>Sophora flavescens</i>	Leguminosae	Root	76
140	5,7,3',4'-TetraOH	8-(4-OAc-3-Me-but-2-enyl)	C ₂₂ H ₂₂ O ₈	414	Kanzonol S	<i>Glycyrriza eurycarpa</i>	Leguminosae	Aerial	127

*(2S)-Flavanones.

^aPr, prenyl (3,3-dimethylallyl = 3-methylbut-2-enyl); Ger, geranyl; Lav, lavandulyl; DMA, dimethylallyl.

^bWhole, whole plant; Root, roots or other underground parts; RootB, root bark; Aerial, aerial parts; Bark, stem bark.

^cStructure has been revised to a dihydrochalcone (see Chapter 16).

^dMicrobial metabolite of xanthohumol using culture broth of *Cunninghamella echinulata* NRRL 3655.

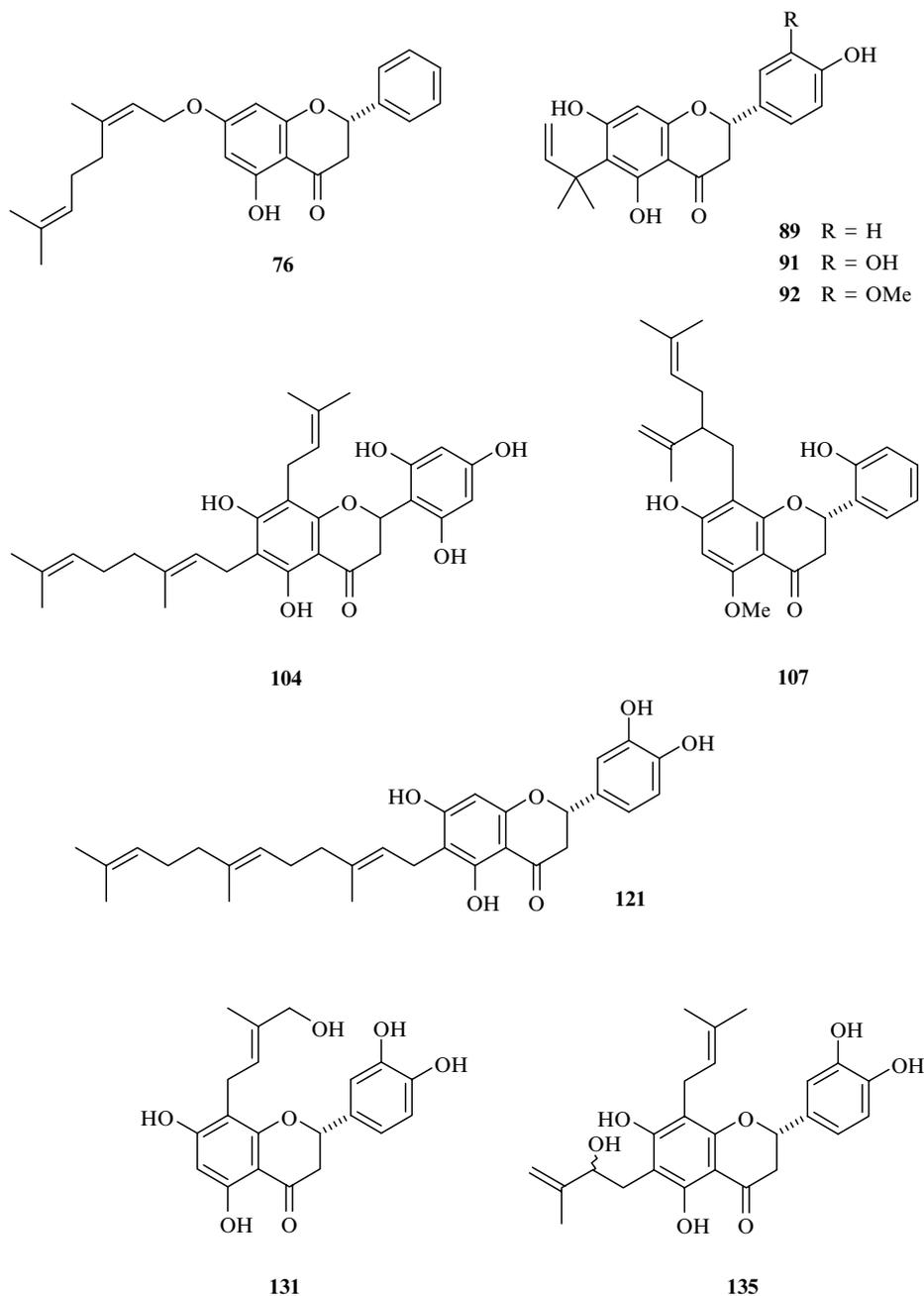


FIGURE 15.3 Examples of flavanones bearing noncyclic isoprenoid substituents.

A related compound to **79**, but containing two double bonds in one of the prenyl groups, is 5,7-dihydroxy-4'-methoxy-3'-C-(3-methylbut-1,5-dienyl)-5'-C-prenylflavanone (burttinone-dehydrate, **78**), also isolated from *E. burtii*.⁹⁶

Some of the compounds listed in Table 15.3 not only bear prenyl substituents, but also C-methyl or related groups, e.g., flavanone **50** from *Dalea caerulea* (Leguminosae),⁷⁴ the regioisomers **53** and **54** from *Eysenhardtia texana* (Leguminosae),⁷⁷ and the regioisomers

lespedezaflavanones F (**80**) and G (**81**), from *Lespedeza formosa* (Leguminosae).⁹⁹ Flavanones **53** and **54** from *E. texana* showed antibacterial activity as inhibitors of the growth of *Staphylococcus aureus* at a concentration of 0.1 mg/ml. Compound **54** also inhibited the growth of *Candida albicans* in an agar-gel diffusion assay⁷⁷ and thus showed antifungal activity. An unusual C-hydroxyethyl group is present in laxiflorin (5,7,4'-trihydroxy-3'-methoxy-6-C-(β -hydroxyethyl)-8-C-prenylflavanone, **75**) from *Derris laxiflora* (Leguminosae).⁹² Laxiflorin showed significant inhibitory activity against protein-tyrosine kinase.⁹²

As mentioned above, more than 30 of the newly reported noncyclic isoprenylated flavanones have been isolated from the genus *Sophora* (six species) and the related *Echinosophora koreensis* (Leguminosae), almost all from roots. Most of these isoprenylated flavanones are characterized by 2'-substitution of the B-ring. The 2',4'- and 2',4',6'-oxygenation patterns are found most frequently, but 2'-O-, 2',6'-di-O-, and 2',4',5'-tri-O-substitution also occurs. Examples of isoprenylated 2',4',6'-tri-oxygenated flavanones are kenusanone D (5,7,2',6'-tetrahydroxy-4'-methoxy-8-C-prenylflavanone, **71**) and its 7-methyl ether, kenusanone E (**72**) from *Echinosophora koreensis*.⁹⁰ In 2',6'-dioxygenated flavanones, such as **71** and **72**, the resonances of H-2 and H-3 show unusual chemical shift values in the ¹H nuclear magnetic resonance (NMR) spectrum. Oxygenation at C-2' causes a slight downfield shift of H-2, while oxygenation at both C-2' and C-6' causes a conspicuous downfield shift of H-2 by ca. 0.3 ppm in most solvents. When flavanones are oxygenated at both C-2' and C-6', H-3_{eq} is shifted upfield (ca. 0.3 to 0.4 ppm), whereas H-3_{ax} is shifted downfield (0.5 to 0.8 ppm) in acetone-*d*₆ and DMSO-*d*₆, but not in CDCl₃. These shifts can be used as a diagnostic tool for the structural determination of both 2',6'-dioxygenated flavanones and dihydroflavonols.⁹⁰

Another feature of the *Sophora* flavanones is substitution with a ten-carbon geranyl or lavandulyl side group, which is sometimes hydroxylated. Eight new flavanones from *S. exigua* are lavandulylated, exiguaflavanones A–G, and J (**111–113** and **116–120**).^{85,116} Lavandulyl groups are also present in the newly reported leachianone E (**105**) from *S. leachiana*¹¹² and kushenols R (**107**, Figure 15.3) and U (**108**) from *S. flavescens*.⁷⁶ In all but one compound, the lavandulyl substituent is attached at C-8 (see Table 15.3). Geranyl rather than lavandulyl side chains are found in the newly reported sophoraflavanones C–E (**99**, **102**, **103**) from *Echinosophora koreensis*,^{87,102} prostratol F (**96**) from *S. prostrata*,¹⁰⁸ and tomentosanol E (**104**, Figure 15.3) from *S. tomentosa*.¹¹¹ A hydroxylated prenyl group is present in tomentosanol D (**122**) from the same species,¹¹¹ and hydroxylated lavandulyl groups (5-hydroxy-2-isopropenyl-5-methylhexyl) in kushenols P₁ (**139**), Q (**136**), and T (**128**) from *S. flavescens*⁷⁶ and leachianone D (**138**) from *S. leachiana*.¹¹² Again, the modified lavandulyl group was attached at C-8 of the flavanone skeleton. Biosynthetic studies of the ten-carbon lavandulyl side chain were carried out using cell suspension cultures of *S. flavescens* in which 8-prenyltransferase activity had been detected.¹²⁸ When naringenin was used as a prenyl acceptor, only 8-prenylnaringenin was formed. However, when 2'-hydroxynaringenin was added as a prenyl acceptor, the 6-prenyl- and 8-lavandulylflavanones were the main products and very little of the 8-prenyl derivative was produced. These results suggest that the 2'-hydroxy group of naringenin may play an important role in the formation of the lavandulyl group,¹²⁸ and that it may not be a coincidence that the majority of the lavandulylflavanones have a 2'-oxygenated B-ring and that the lavandulyl group is usually attached to C-8.

Some of the prenylated and lavandulylated flavanones from *S. flavescens*, kushenols P–S, exhibited significant antibacterial activities against the Gram-positive bacteria, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis*, and *Propionibacterium acnes*. They also exhibited antiandrogenic activities.⁷⁶ Kurarinone (**114**), 2'-O-methylkurarinone (**115**), and the known sophoraflavanone G and leachianone A from the roots of *S. flavescens*, which all have 8-lavandulyl substitution and 2',4'-di-O-substitution of the B-ring, exhibited cytotoxic activity

against human myeloid leukemia HC-60 cells with IC_{50} values of 13.7, 18.5, 12.5, and 11.3 μM , respectively.¹¹⁷

As is clear from Table 15.3, most of the newly reported flavanones with noncyclic isoprenoid groups have been isolated from members of the Leguminosae. However, one or more new prenylated flavanones have also been reported from the families Ptaeroxylaceae, Rutaceae, Meliaceae, Asteraceae, Euphorbiaceae, Velloziaceae, Cyperaceae, Ophioglossaceae, Capparaceae, Gentianaceae, and especially Moraceae and Dipterocarpaceae. For example, two highly methoxylated 5,7,2',4',6'-penta-*O*-substituted prenylated flavanones, showing the same unusual B-ring substitution as found in many prenylflavanones from *Sophora*, have been reported from the root bark of *Artocarpus heterophyllus* (Moraceae) as heteroflavanones C (73)⁹¹ and B (74).³⁹

From a chemosystematic point of view, it is interesting to note that prenylated flavonoids such as microfolione (56) have been found in a species of the family Ptaeroxylaceae,⁷⁹ because the relationships of this family with other families were disputed in the past. Most taxonomists considered the Ptaeroxylaceae closely related to families in the order Rutales to which the Rutaceae and Meliaceae belong, whereas others considered it related to the Sapindaceae. Flavonoid chemistry supports a close relationship to the Rutaceae and Meliaceae, as isoprenylated flavanones also occur in these families, e.g., 58 and 98 in *Boronia coerulescens* ssp. *spinescens*,⁸⁰ the farnesyl-bearing 121 (Figure 15.3) in *B. ramosa* (Rutaceae),¹¹⁹ and flowerine (59) and flowerone (60) in *Azadirachta indica* (Meliaceae).⁸¹ Microfolione is one of the few new flavanones for which the (2*R*)-configuration has been determined.

The occurrence of prenyloxyflavanones in *Monotes engleri* (Dipterocarpaceae) has already been discussed, but it is worth adding here that five further new isoprenylated flavanones have been reported from this species, including three 6-*C*-1,1-dimethylallyl (1,1-DMA)-substituted flavanones (89, 91, and 92, Figure 15.3).⁸³ These compounds and the known 6,8-diprenyleriodictyol and its 3'-methyl ether, hirvanone, from the same plant displayed cytotoxic activity against several human cancer cell lines, whereas another novel isoprenylated flavanone from *M. engleri*, bearing a hydroxylated prenyl group (135, Figure 15.3), was nontoxic.⁸³ Two further 1,1-DMA-substituted flavanones, 94 and 95 from *Dalea scandens* var. *paucifolia* (Leguminosae), showed significant activity against methicillin-susceptible and -resistant strains of *Staphylococcus aureus*.¹⁰⁷

A species of the family Euphorbiaceae, *Macaranga pleiostemma*, also yielded biologically active isoprenylated flavanones, macaranga flavanones A (97) and B (77), which showed significant antibacterial activity against *Escherichia coli*.⁹⁵ Flavanone 77 was also reported as paratocarpin L from *Parartocarpus* (incorrectly spelled as *Paratocarpus*) *venenosa* (Moraceae).⁹⁴ Two more antibacterial flavanones bearing an 8-*C*-(2-isopropyl-5-methyl-5-hexenyl) side chain, which is similar to a lavandulyl group, but which has a double bond between C-5 and C-6 of the isoprenoid chain instead of between C-4 and C-5, are remangiflavanones A (106) and B (110). These were isolated from *Physena madagascariensis*, family Capparaceae. The compounds were bacteriocidal at 9 and 4 μM , respectively, against *Staphylococcus aureus*, and also bacteriocidal or antibacterial against seven other bacteria, with minimum effective concentrations of 0.3 to 10 μM . This species also produces a flavanone dimer, remangiflavanone C, which was not active.¹¹³

Vellozia and *Schoenus* are the only genera of Monocotyledoneae from which new isoprenylated flavanones have been reported, 6,8-diprenyleriodictyol (82) from the leaves of *Vellozia nanuzae* (Velloziaceae)⁶¹ and 5,7,3',4'-tetrahydroxy-6,5'-di-*C*-prenylflavanone (83) from the tubers of *Schoenus nigricans* (Cyperaceae).¹⁰⁰ The known regioisomer of 83, 5,7,3',4'-tetrahydroxy-8,5'-di-*C*-prenylflavanone, was also reported from the latter species.¹⁰⁰

Several new flavanones were produced as biotransformation products of the prenylchalcone, xanthohumol, when this compound was incubated with the microorganism

Cunninghamella echinulata NRRL 3655. Xanthohumol is an important constituent of beer, as it is a major constituent of hops (*Humulus lupulus*, Cannabinaceae) used in the brewing industry. Microbial transformation studies of xanthohumol were carried out to generate compounds similar to mammalian metabolites of the constituents of hops. Two flavanones bearing the same hydroxylated prenyl side chain, the *E,Z*-isomers **124** and **125**, were some of the transformation products obtained. These and other transformation compounds from xanthohumol were tested for anticancer activity, but did not show cytotoxicity at 25 $\mu\text{l/ml}$ against a range of human cancer cell lines as well as noncancerous Vero cells. However, antimalarial activity was exhibited by **125** (but not by **124**) against D6 (chloroquine-sensitive) strains of the malaria parasite, *Plasmodium falciparum* (IC_{50} , 2 $\mu\text{g/ml}$).¹²¹ The known flavanone 8-prenylaringenin is present in low concentrations in beer as an isomerization product of the chalcone desmethylxanthohumol (another hop constituent) and is the most potent phytoestrogen so far discovered.¹²⁹ 8-Prenylaringenin was also tested for antifungal activity together with semisynthetic 6- and 3'-prenylaringenin, using *Cladosporium herbarum* as the test organism. The 8-prenylflavanone was more fungitoxic than the corresponding 3'-prenylated isomer, whereas 6-prenylaringenin was inactive, indicating a strong regiospecific influence of the prenyl side chain on fungitoxicity.¹²²

During a survey of species and hybrids of *Glycyrrhiza* (Leguminosae) from Central Asia, licoleafol (**131**, Figure 15.3), a new flavanone that contains a hydroxylated prenyl side chain, was found to be a chemotaxonomic marker to distinguish *G. uralensis*, in which the compound occurred in high levels, from *G. glabra*, in which it could not be detected. A morphologically intermediate plant, which was thought to be a hybrid between *G. uralensis* and *G. glabra*, produced small amounts of licoleafol.¹²⁴

The compound listed in Table 15.3 as crotamosmin from *Crotalaria ramosissima* (Leguminosae) was reported as the new 5,4'-dihydroxy-6-*C*-prenylflavanone.⁷³ This structure was reassigned on the basis of detailed spectroscopic studies as 2',4'-dihydroxy-6'',6''-dimethylpyrano[2'',3'':4',3']-dihydrochalcone (compound **286** in Chapter 16).¹³⁰

15.2.2.3 Flavanones with Furano Rings

When a *C*-prenyl group attached to a phenolic ring of a flavonoid forms a linkage with an *ortho*-hydroxyl, the result may be a heterocyclic isoprop(en)ylfurano or dimethylpyrano ring, or dihydro derivatives of these. Dimethylpyranoflavanones are discussed in the next section. Further modification of the furano ring may lead to hydroxylation or loss of the isopropyl side chain. Figure 15.4 shows representatives of flavanones with various types of furano substituents and gives the numbering of the atoms used in this chapter, since various different numbering conventions are presented in the literature.

More than 20 furanoflavanones have been reported during the period under review, whereas only one furanoflavanone was known previously. The newly reported structures are listed in Table 15.4 (compounds **141–161**).^{61,98,104,122,126,131–140} The compounds are arranged according to the number of oxygens in the molecular formula and the relative molecular masses. More than half of the furanoflavanones contain two isoprenoid substituents as they also bear an additional *C*-prenyl group.

In the new compounds, the furano ring is usually attached to C-7 and C-8 (or C-6) of the flavanone, and incorporates the oxygen at C-7. However, in abyssinoflavanone IV (**150**) from *Erythrina abyssinica* (Leguminosae), C-5' and the oxygen at C-4' are involved in the ring structure,⁹⁸ and in flavanones **151** and **161** from *Paramignya griffithii* (Rutaceae), C-6 and the oxygen at C-5 are part of the ring.¹³⁹

Flavanones bearing a simple furano ring without an isopropyl side chain include compounds **141** from *Millettia erythrocalyx*,¹³¹ **143** from *Lonchocarpus latifolius*,¹³³ **144** from

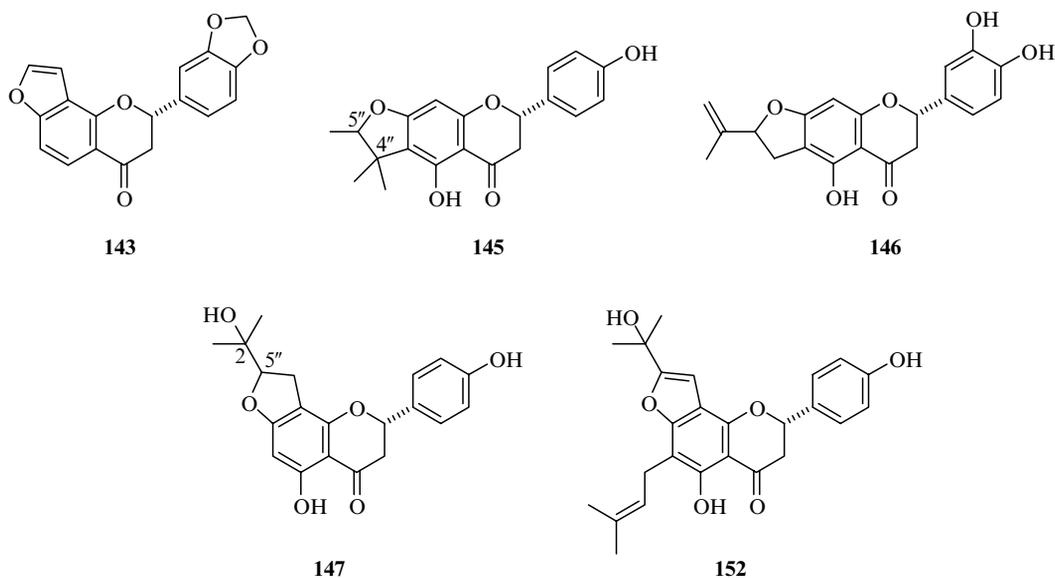


FIGURE 15.4 Examples of furanoflavanones.

L. subglaucescens,¹³⁴ and **150** from *Erythrina abyssinica*.⁹⁸ All these species belong to the Leguminosae. Although the majority of the furanoflavanones have been found in members of the Leguminosae, as is the case with most other classes of prenylated flavonoids, the families Moraceae and Rutaceae are also good sources. From another family rich in isoprenylated flavonoids, Velloziaceae, a flavanone having an isopropenyl group at C-5'' of the dihydrofurano ring has been isolated, velloeriodictyol (**146**).⁶¹ The same 5''-isopropenyldihydrofurano substituent is present in emoroidenone (**142**) from *Tephrosia emoroides* (Leguminosae). This flavanone has a strong antifeedant activity against the larvae of the insect *Chilo portellus*.¹³² The furano or dihydrofurano rings of most of the remaining flavanones in Table 15.4 bear hydroxylated isopropyl groups, e.g., compound **147** found in *Macaranga conifera* (Euphorbiaceae),¹³⁵ phellodensin D (**148**) from both *Phellodendron chinense* var. *glabriusculum* (Rutaceae)¹³⁷ and *Broussonetia papyrifera* (Moraceae),¹³⁶ flavanones **152–156** from *Lupinus luteus* (Leguminosae),¹²² and compounds **157–160** from *Dorstenia mannii* (Moraceae).^{126,140} Compound **152**, lupinenol, is distinguished from the other new flavanones from *L. luteus* in having a furano rather than a dihydrofurano ring. It should be noted that C-5'' of the dihydrofurano ring is an asymmetric center, and therefore two stereoisomers are possible. Although the dihydrofuranoflavanones lonchocarpols C and D were known compounds, the two epimers of each had not been reported previously. They have now been isolated from *L. luteus* and separated by high-performance liquid chromatography to yield lonchocarpol C₁ (**153**) and its 5''-epimer lonchocarpol C₂ (**154**), and lonchocarpol D₁ (**155**) and its 5''-epimer lonchocarpol D₂ (**156**).¹²² The 5''-epimers of dorsmanins F (**157**) and G (**158**) from *Dorstenia mannii* have also been reported, as epidorsmanins F (**159**) and G (**160**), respectively.¹⁴⁰

Furanoflavanones **152–156** from *L. luteus* were tested for antifungal activity using *Cladosporium herbarum* as the test organism. Lonchocarpol D₁ showed the highest fungitoxicity and there was evidence for stereospecific activity, as lonchocarpol D₂ was much less active. Lonchocarpols C₁ and C₂ were very weakly antifungal.¹²² Flavanone **148** (discussed above) and the known (2*S*)-abyssinone II from *Broussonetia papyrifera* (Moraceae) are very potent inhibitors of aromatase, which catalyzes the final step in estrogen biosynthesis, with IC₅₀ values of 0.1 and 0.4 μ M, respectively.¹³⁶

TABLE 15.4
Furanoflavanones Reported from 1992 to 2003

No.	OH-, OMe-, and Methyleneedioxy Substituents	Furano and Prenyl Substituents ^a	Mol. Formula	Mr	Trivial Name	Plant Source	Family	Organ ^b	Ref.
141	6-Ome	Furano[2'',3'':7,8]	C ₁₇ H ₁₄ O ₄	282		<i>Milletia erythracalyx</i>	Leguminosae	Root	131
142	5-Ome	5'-Isopropenyl(dihydrofuranol[2'',3'':7,8])	C ₂₁ H ₂₀ O ₄	336	Emoroidenone	<i>Tephrosia emoroides</i>	Leguminosae		132
143	3',4'-Methyleneedioxy	Furano[2'',3'':7,8]	C ₁₈ H ₁₂ O ₅	308	Figure 15.4	<i>Lonchocarpus latifolius</i>	Leguminosae	Root	133
144*	5,6-DiOMe	Furano[2'',3'':7,8]	C ₁₉ H ₁₆ O ₅	324		<i>Lonchocarpus subglaucescens</i>	Leguminosae		134
145	5,4'-DiOH	4'',4''-Dimethyl-5''-methyl(dihydrofuranol[2'',3'':7,6])	C ₂₀ H ₂₀ O ₅	340	Figure 15.4	<i>Monotes engleri</i>	Dipterocarpaceae	Leaf	104
146	5,3',4'-TriOH	5''-Isopropenyl(dihydrofuranol[2'',3'':7,6])	C ₂₀ H ₁₈ O ₆	354	Velloeriodietylol, (Figure 15.4)	<i>Vellozia glabra</i>	Velloziaceae	Leaf	61
147	5,4'-DiOH	5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,8]	C ₂₀ H ₂₀ O ₆	356	Figure 15.4	<i>Macaranga conifera</i>	Euphorbiaceae	Leaf	135
148	2,4'-DiOH	5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,8]	C ₂₀ H ₂₀ O ₆	356	Phellodensin D	<i>Broussonetia papyrifera</i>	Moraceae	Whole	136
						<i>Phellodendron chinense</i> var. <i>glabriusculum</i>	Rutaceae	Leaf	137
149	4'-OH-5-OMe	(E)-5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,8]	C ₂₁ H ₂₂ O ₆	370			Leguminosae		138
150	5,7,3'-TriOH	2'-Pr-furano[2'',3'':4',5']	C ₂₂ H ₂₂ O ₆	382	Abyssinoflavanone IV	<i>Erythrina abyssinica</i>	Leguminosae		98
151	3',4'-DiOH-7-OMe	8-Pr-furano[2'',3'':5,6]	C ₂₃ H ₂₂ O ₆	394		<i>Paramignya griffithii</i>	Rutaceae	Stem	139
152	5,4'-DiOH	6-Pr-5''-(2-OH-isopropenyl)furanol[2'',3'':7,8]	C ₂₅ H ₂₆ O ₆	422	Lupinolenol (Figure 15.4)	<i>Lupinus luteus</i>	Leguminosae	Root	122
153	5,4'-DiOH	6-Pr-5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,8]	C ₂₅ H ₂₆ O ₆	424	Lonchocarpol C ₁	<i>Lupinus luteus</i>	Leguminosae	Root	122
154	5,4'-DiOH	5''-Epimer of 153	C ₂₅ H ₂₆ O ₆	424	Lonchocarpol C ₂	<i>Lupinus luteus</i>	Leguminosae	Root	122
155	5,4'-DiOH	8-Pr-5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,6]	C ₂₅ H ₂₆ O ₆	424	Lonchocarpol D ₁	<i>Lupinus luteus</i>	Leguminosae	Root	122
156	5,4'-DiOH	5''-Epimer of 155	C ₂₅ H ₂₆ O ₆	424	Lonchocarpol D ₂	<i>Lupinus luteus</i>	Leguminosae	Root	122
157	5,3',4'-TriOH	6-Pr-5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,8]	C ₂₅ H ₂₆ O ₇	440	Dorsmanin F	<i>Dorstenia mamii</i>	Moraceae	Twig	126
158	5,3',4'-TriOH	8-Pr-5''-(2-OH-isopropenyl)dihydrofuranol[2'',3'':7,6]	C ₂₅ H ₂₆ O ₇	440	Dorsmanin G	<i>Dorstenia mamii</i>	Moraceae	Twig	126
159	5,3',4'-TriOH	5''-Epimer of 157	C ₂₅ H ₂₆ O ₇	440	Epidsorsmannin F	<i>Dorstenia mamii</i>	Moraceae	Twig	140
160	5,3',4'-TriOH	5''-Epimer of 158	C ₂₅ H ₂₆ O ₇	440	Epidsorsmannin G	<i>Dorstenia mamii</i>	Moraceae	Twig	140
161	3',4'-DiOH-7-OMe	8-Pr-5''-(2-OH-isopropenyl)furanol[2'',3'':5,6]	C ₂₆ H ₂₈ O ₇	452		<i>Paramignya griffithii</i>	Rutaceae	Stem	139

^aPr, prenyl.

^bWhole, whole plant.

^cMicrobial metabolite of xanthohumol using a culture broth of *Pichia membranifaciens*.

In the previous section, the microbial transformation of the isoprenylated chalcone, xanthohumol, was discussed. When a culture broth of a different fungus, *Pichia membranifaciens*, was used, the new furanoflavonone **149** was produced from xanthohumol.¹³⁸

15.2.2.4 Flavanones with Pyrano Rings

Some 50 new dimethylpyrano (DMP), dimethyldihydropyrano (DMDHP), and related pyranoflavanones have been reported from 1992 to 2003, more than doubling the number of known pyranoflavanones. The newly reported compounds are presented in Table 15.5 (compounds **162–211**).^{82,85–88,94,98,126,134,135,140–162} They are firstly arranged according to the number of oxygen atoms in the compounds, and within these groups according to their relative molecular masses. There is no free hydroxyl group in maximaflavanone A (**162**) from *Tephrosia maxima* (Leguminosae),¹⁴¹ but there is of course an oxygen atom incorporated in the DMP group. Similarly, there is no free hydroxyl in dorsmanin B (**168**) from *Dorstenia mannii* (Moraceae),¹⁴⁷ although it is di-*O*-substituted, since two *O*-containing DMDHP groups are attached to this flavanone. Figure 15.5 shows representatives of DMP and DMDHP flavanones, and the numbering system used in this chapter for pyranoflavanones. The place of attachment of the pyrano ring structure to the flavonoid is more varied than that of the furanoflavanones discussed above. Most commonly the structures are characterized by ring closure of a prenyl group at C-8 (or C-6) with the oxygen at C-7; less commonly C-3' (or C-5') and an oxygen at C-4' are involved in the pyrano ring, and rarely C-6 and the oxygen at C-5. The newly reported DMP flavanones have been isolated from species belonging to the families Leguminosae, Moraceae, Rutaceae, Asteraceae, and Euphorbiaceae, and the new DMDHP flavanones from the same families apart from the Rutaceae. There does not seem to be a chemosystematic difference between the occurrence of these two groups, since all genera in which new DMDHP flavanones have been recorded also produced new DMP flavanones, except *Echinosophora koreensis*, but this species is closely related to the genus *Sophora* in which both types were present.

Many of the new pyranoflavanones show the common 4'-*O*- or 3',4'-di-*O*-substitution patterns of the B-ring, but in most of the compounds from *Sophora* and *Echinosophora* (Leguminosae) C-2' is oxygenated in addition to or instead of C-4' and sometimes C-6', e.g., in kusanone J (**181**) from *E. koreensis*,⁸⁷ leachianone F (**193**) from *S. leachiana*,⁸² and exiguaf flavanones L (**194**), I (**208**), and H (**209**) from *S. exigua*.⁸⁵ The latter two compounds are also special because they additionally contain a lavandulyl group. These *Sophora* and *Echinosophora* DMP-flavanones are closely related to the noncyclic isoprenoid-containing kenusanones, leachianones, and exiguaf flavanones discussed previously.^{82,85,87,90,102,112,115,116}

2',4'-Di-*O*-substitution of the B-ring is also found in three new pyranoflavanones from *Morus* species (Moraceae), sanggenol L (**189**, Figure 15.5) from *M. mongolica*,¹⁵⁶ and sanggenols N (**188**) and O (**187**) from *M. australis*.¹⁵⁵ In the latter compound, both the 2'-*O*- and 4'-*O*-functionalities are part of DMP rings. In sanggenol L (**189**), the pyrano ring seems to have been formed from a geranyl unit rather than the usual prenyl unit, since C-6'' bears only one methyl group, and an additional 6-carbon isohexenyl chain (4-methylpent-3-enyl) (Figure 15.5).

More than half of the compounds in this section bear a second *C*-isoprenoid group in addition to the dimethylpyrano group. This is either a prenyl, geranyl, or lavandulyl group, or a second dimethylpyrano group. A 2'-*C*-geranyl group is found in tanariflavanone A (**201**, Figure 15.5), isolated from *Macaranga tanarius* (Euphorbiaceae).¹⁵⁸ In the related tanariflavanone B (**200**, Figure 15.5) from *M. tanarius*, the 2'-*C*-geranyl has formed a ring with the hydroxyl on C-3' to form the same 6''-methyl-6''-(4-methylpent-3-enyl)pyrano ring as found

TABLE 15.5
Dimethylpyrano- and Dimethylidihydropranoflavanones Reported from 1992 to 2003

No.	OH-, OMe-, OPn-, and Methyleneedioxy-Substituents ^a	Dimethylpyrano-, Dimethylidihydroprano-, and Other C-substituents ^a	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^b	Ref.
162	—	6-Pr-6'',6''-DMP[2'',3'':7,8]	C ₂₅ H ₂₆ O ₃	374	Maximaflavanone A	<i>Tephrosia maxima</i>	Leguminosae	Root	141
163	4'-OMe	6',6''-DMDHP[2'',3'':7,8]	C ₂₁ H ₂₀ O ₄	336	Dorspoinsettifolin	<i>Dorstenia poinsettifolia</i>	Moraceae	Twig	142
164*	5-OH	6-(3-Me-but-1,3-dienyl)-6',6''-DMP[2'',3'':7,8]	C ₂₅ H ₂₄ O ₄	388	Spinoflavanone A	<i>Tephrosia spinosa</i>	Leguminosae	Whole	143
165	7-OH	6-Pr-6'',6''-DMP[2'',3'':4,3]	C ₂₅ H ₂₆ O ₄	390	Dinklagin A	<i>Dorstenia dinklagei</i>	Moraceae	Twig	144
166	4'-OH	3'-Pr-6'',6''-DMP[2'',3'':7,8]	C ₂₅ H ₂₆ O ₄	390	Shinflavanone	<i>Glycyrrhiza glabra</i>	Leguminosae	Root	145
167*	7-OH	8-Pr-6'',6''-DMDHP[2'',3'':4,3]	C ₂₅ H ₂₈ O ₄	392	Euchrenone a ₁₇	<i>Euchresta formosana</i>	Asteraceae	Root	146
168*	—	Bis(6'',6''-DMDHP[2'',3'':7,6][2'',3'':4,3])	C ₂₅ H ₂₈ O ₄	392	Dorsmanin B	<i>Dorstenia mami</i>	Moraceae	Twig	147
169	5,4-DiOH	6',6''-DMP[2'',3'':7,6]	C ₂₀ H ₁₈ O ₅	338	Paratocarpin K	<i>Parartocarpus venenosa</i>	Moraceae	Root	94
170	5-OH-4'-OMe	6',6''-DMP-[2'',3'':7,8]	C ₂₁ H ₂₀ O ₅	352 (Figure 15.5)	(Figure 15.5)	<i>Macaranga confiera</i>	Euphorbiaceae	Leaf	135
171	5,4'-DiOMe	6',6''-DMP[2'',3'':7,8]	C ₂₂ H ₂₂ O ₅	366	Glyflavanone A	<i>Glycosmis citrifolia</i>	Rutaceae	Leaf	148
172*	3,4'-DiOMe	6',6''-DMP[2'',3'':7,8]	C ₂₂ H ₂₂ O ₅	366	Ponganone III	<i>Pongania pinnata</i>	Leguminosae	RootB	88
						<i>Lonchocarpus subglaucescens</i>	Leguminosae	Root	134
173	5,7-DiOH	6-Pr-6'',6''-DMP[2'',3'':4,3]	C ₂₅ H ₂₆ O ₅	406	Paratocarpin H	<i>Parartocarpus venenosa</i>	Moraceae	Root	94
174	5,4'-DiOH	3'-Pr-6'',6''-DMP[2'',3'':7,6]	C ₂₅ H ₂₆ O ₅	406	Paratocarpin I	<i>Parartocarpus venenosa</i>	Moraceae	Root	94
175	5,4'-DiOH	6''-Me,6''-(4-methylpent-3-enyl)-pyrano[2'',3'':7,8]	C ₂₅ H ₂₆ O ₅	406	Cycloaltitisin 7	<i>Artocarpus altitilis</i>	Moraceae	Bud covers	149
176	5-OH	Bis(6'',6''-DMDHP[2'',3'':7,6][2'',3'':4,3])	C ₂₅ H ₂₈ O ₅	408	Paratocarpin J (Figure 15.5)	<i>Parartocarpus venenosa</i>	Moraceae	Root	94
177	5,7-DiOH	8-Pr-6'',6''-DMDHP[2'',3'':4,3]	C ₂₅ H ₂₈ O ₅	408	Euchrenone a ₁₆	<i>Euchresta formosana</i>	Leguminosae	Root	150
178	3,4'-DiOMe	6-Pr-6'',6''-DMP[2'',3'':7,8]	C ₂₇ H ₃₀ O ₅	434	Cubé resin ^c	<i>Cubé resin^c</i>	Leguminosae	Root	151
179	5-OH	6-Pr-bis(6'',6''-DMP [2'',3'':7,8][2'',3'':4,3])	C ₃₀ H ₃₂ O ₅	472	Euchrenone a ₁₅	<i>Euchresta tubulosa</i>	Leguminosae	Root	152
180	5-OH	8-Pr-bis(6'',6''-DMP [2'',3'':7,6][2'',3'':4,3])	C ₃₀ H ₃₂ O ₅	472	Euchrenone a ₁₄	<i>Euchresta tubulosa</i>	Leguminosae	Root	152
181	5,2,4'-TriOH	6',6''-DMDHP[2'',3'':7,8]	C ₂₀ H ₂₀ O ₆	356	Kenusanone J	<i>Echinosophora koreensis</i>	Leguminosae	Stem	87
182*	5,7-DiOH-3'-OMe	6',6''-DMP[2'',3'':4,5]	C ₂₁ H ₂₀ O ₆	368	Abyssinin I	<i>Erythrina abyssinica</i>	Leguminosae	StemB	86

continued

TABLE 15.5
Dimethylpyrano- and Dimethylidihydropranoflavanones Reported from 1992 to 2003 — continued

No.	OH-, OMe-, OPr-, and Methylenedioxy-Substituents ^a	Dimethylpyrano-, Dimethylidihydroprano-, and Other C- substituents ^a	Mol. Formula	M _r	Trivial Name	Plant Source	Family	Organ ^b	Ref.
183*	5,3'-DiOH-4'-OMe	6'',6''-DMP[2'',3'':7,8]	C ₂₁ H ₂₀ O ₆	368		<i>Feronia limonia</i>	Rutaceae	StemB	153
184*	5,4'-DiOH	8-Hydroxymethyl-6'',6''-DMP[2'',3'':7,6]	C ₂₁ H ₂₀ O ₆	368	Figure 15.5	<i>Derris reticulata</i>	Leguminosae	Stem	154
185	5,3',4'-TriOMe	6'',6''-DMP[2'',3'':7,8]	C ₂₃ H ₂₄ O ₆	396	Glyflavanone B	<i>Glycosmis citrifolia</i>	Rutaceae	Leaf	148
186	6,3',4'-TriOMe	6'',6''-DMP[2'',3'':7,8]	C ₂₃ H ₂₄ O ₆	396	Ponganone IV	<i>Pongamia pinnata</i>	Leguminosae	RootB	88
187	5,7-DiOH	Bis(6'',6''-DMP [2'',3'':2'',3'':4'',5])	C ₂₅ H ₂₄ O ₆	420	Sanggenol O	<i>Morus australis</i>	Moraceae	RootB	155
188	5,7,2'-TriOH	5'-Pr-6'',6''-DMP[2'',3'':4'',3]	C ₂₅ H ₂₆ O ₆	422	Sanggenol N	<i>Morus australis</i>	Moraceae	RootB	155
189	5,2',4'-TriOH	6''-Me-6''-(4-methylpent-3-enyl)-pyrano[2'',3'':7,8]	C ₂₅ H ₂₆ O ₆	422	Sanggenol L (Figure 15.5)	<i>Morus mongolica</i>	Moraceae	StemB	156
190	5,3',4'-TriOH	8-Pr-6'',6''-DMP[2'',3'':7,6]	C ₂₅ H ₂₆ O ₆	422	Dorsmanin I	<i>Dorstenia mannii</i>	Moraceae	Twig	140
191	5,4'-DiOH	8-(2,3-Epoxy-3-Me-butyl)-6'',6''-DMP[2'',3'':7,6]	C ₂₅ H ₂₆ O ₆	422	2'',3''-Epoxyilupinifolin	<i>Derris reticulata</i>	Leguminosae	Stem	157
192	5,4'-DiOH	8-(2-OH-3-Me-but-3-enyl)-6'',6''-DMP[2'',3'':7,6]	C ₂₅ H ₂₆ O ₆	422	Dereticulatin	<i>Derris reticulata</i>	Leguminosae	Stem	157
193	5,2',4'-TriOH	5''-Pr-6'',6''-DMDHP[2'',3'':7,8]	C ₂₅ H ₂₈ O ₆	424	Leachianone F	<i>Sophora leachiana</i>	Leguminosae	Root	82
194	5,2',6'-TriOH	5''-Pr-6'',6''-DMDHP[2'',3'':7,8]	C ₂₅ H ₂₈ O ₆	424	Exiguafavanone L	<i>Sophora exigua</i>	Leguminosae	Root	85
195	5,3',4'-TriOH	8-Pr-6'',6''-DMDHP[2'',3'':7,6]	C ₂₅ H ₂₈ O ₆	424	Dorsmanin J	<i>Dorstenia mannii</i>	Moraceae	Twig	140
196	3',4'-DiOH	Bis(6'',6''-DMDHP [2'',3'':5,6][2'',3'':7,8])	C ₂₅ H ₂₈ O ₆	424	Dorsmanin E	<i>Dorstenia mannii</i>	Moraceae	Twig	126
197	5,3'-DiOH-4'-OMe	6-Pr-6'',6''-DMP[2'',3'':7,8]	C ₂₆ H ₂₈ O ₆	436		Cubé resin ^c	Leguminosae	Root	151
198	5,4'-DiOH-3'-OMe	6-Pr-6'',6''-DMP[2'',3'':7,8]	C ₂₆ H ₂₈ O ₆	436		Cubé resin ^c	Leguminosae	Root	151

199	5-OH-3',4'-DiOMe	6-Pr-6',6''-DMP[2'',3'',7,8]	C ₂₇ H ₃₀ O ₆	450	Tanarilavanone B	Leguminosae	Cubé resin ^c	Root	151
200	5,7,4'-TriOH	6-Pr-6'-Me,6''-(4-methylpent-3-enyl)-pyrano[2'',3'',3',2']	C ₃₀ H ₃₄ O ₆	490	Tanarilavanone B (Figure 15.5)	Euphorbiaceae	<i>Macaranga tanarius</i>	Leaf (fallen)	158
201	5,3',4'-TriOH	2'-Ger-(5''-OH-6''-6''-DMDHP[2'',3'',7,6])	C ₃₀ H ₃₆ O ₆	492	Tanarilavanone A (Figure 15.5)	Euphorbiaceae	<i>Macaranga tanarius</i>	Leaf (fallen)	158
202	5,4'-DiOH	8-(1-OH-2,3-epoxy-3-Me-butyl)-6',6''-DMP[2'',3'',7,6]	C ₂₅ H ₂₆ O ₇	438	1''-OH-2''',3'''-Epoxy-lupinifolin (Figure 15.5)	Leguminosae	<i>Derris reticulata</i>	Stem	159
203	5,7,3'-TriOH	2'-Pr-(5''-OH-6',6''-DMDHP[2'',3'',4,5'])	C ₂₅ H ₂₈ O ₇	440	Abyssinoflavanone V	Leguminosae	<i>Erythrina abyssinica</i>		98
204	5,7-DiOH	(5''-OH-6',6''-DMP[2'',3'',7,6])-(6''',6''-DMDHP[2'',3'',4,3])	C ₂₅ H ₂₈ O ₇	440	Abyssinoflavanone VI	Leguminosae	<i>Erythrina abyssinica</i>		98
205*	5,4'-DiOH	8-(2,3-dioH-3-Me-butyl)-6',6''-DMP[2'',3'',7,6]	C ₂₅ H ₂₈ O ₇	440	2'',3''-Dihydroxylupinifolin (Figure 15.5)	Leguminosae	<i>Derris reticulata</i>	Stem	154
206	5,4'-DiOH	See Figure 15.5	C ₂₆ H ₂₈ O ₇	452	Derriflavanone	Leguminosae	<i>Derris laxiflora</i>	Root	160
207	5,4'-DiOH	See Figure 15.5	C ₂₆ H ₂₈ O ₇	452	Epi-derriflavanone	Leguminosae	<i>Derris laxiflora</i>	Root	160
208	5,2',4',6'-TetraOH-	6-Lav-6',6''-DMP[2'',3'',7,8]	C ₃₀ H ₃₄ O ₇	506	Exiguafilavanone I	Leguminosae	<i>Sophora exigua</i>	Root	85
209	5,2',4',6'-TetraOH	8-Lav-6',6''-DMP[2'',3'',7,6]	C ₃₀ H ₃₄ O ₇	506	Exiguafilavanone H	Leguminosae	<i>Sophora exigua</i>	Root	85
210	5,7,3'-TriOH	(4'',5''-dioH-6',6''-DMDHP[2'',3'',4,5'])	C ₃₀ H ₃₀ O ₈	388	Sigmoidin G	Leguminosae	<i>Erythrina sigmoidea</i>	StemB	161
211	2',3',6'-TriOH	8-Pr-5''-(2,4-dioH-phenyl)-6',6''-DMP[2'',3'',7,6]	C ₃₂ H ₃₀ H ₈	528	Eriosemaone C	Leguminosae	<i>Eriosema tuberosum</i>	Root	162

*(2S)-Flavanones.

^aPr, prenyl; Ger, geranyl; Lav, lavandulyl; DMP, dimethylpyrano; DMDHP, dimethyldihydropyrano.

^bRhiz, rhizomes; Tissue, plant tissue culture; RootB, root bark; Aerial, aerial parts; StemB, stem bark.

^cCubé resin is an extract of the roots of *Lonchocarpus utilis* and *L. urticu* (Leguminosae).

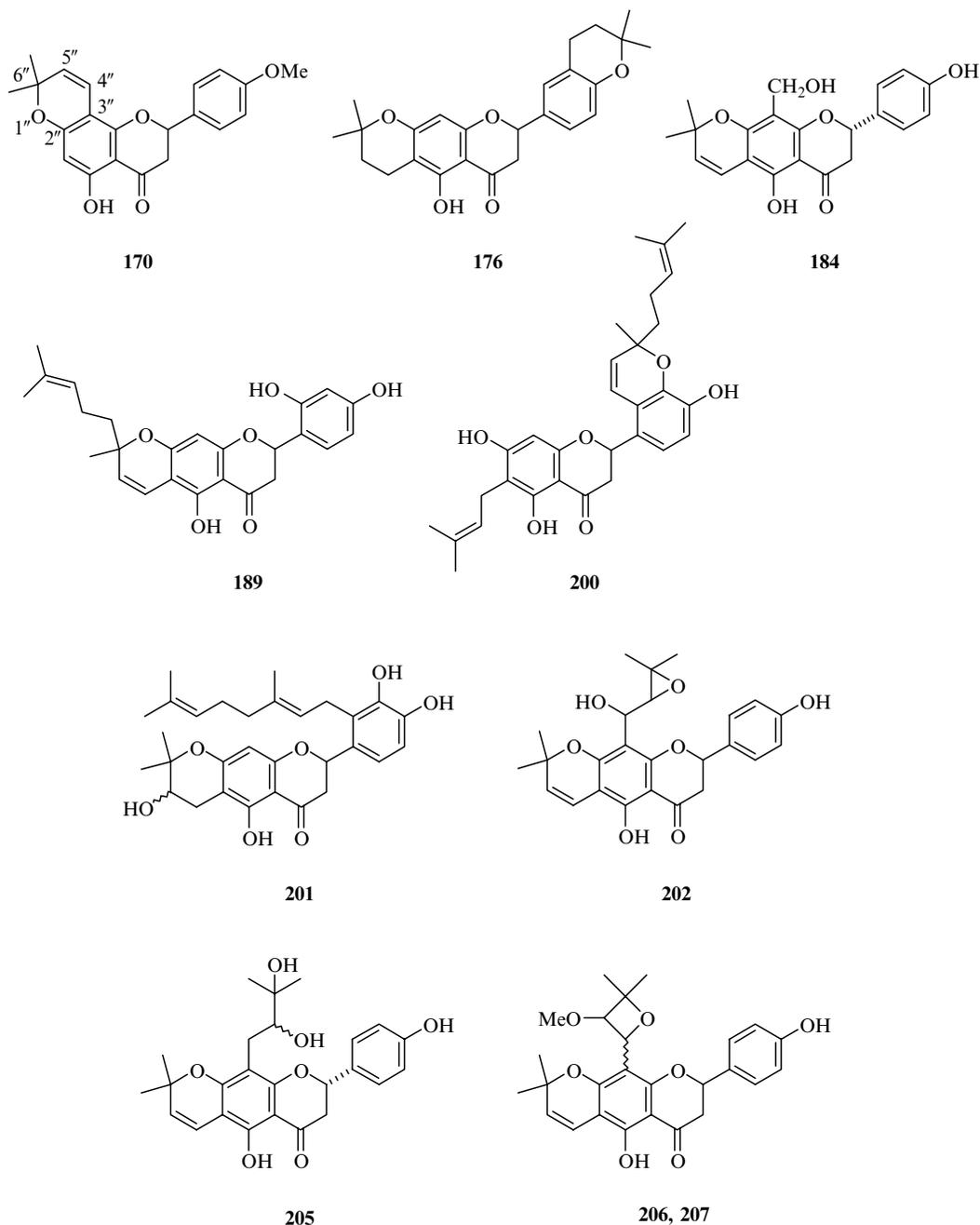


FIGURE 15.5 Examples of pyranoflavanones.

in sanggenol L (**189**, see above). Flavanones **200** and **201** were obtained from fallen leaves and showed allelopathic activity (inhibition of radicle growth of lettuce seedlings at 200 ppm).¹⁵⁸ Two DMP rings are present in euchrenones a₁₄ and a₁₅ (**180** and **179**) from *Euchresta tubulosa* (Leguminosae)¹⁵² and in sanggenol O (**187**) from *Morus australis* (Moraceae),¹⁵⁵ whereas two DMDHP rings are present in dorsmanins B (**168**)¹⁴⁷ and E (**196**)¹²⁶ from *Dorstenia mannii*

(Moraceae) and paratocarpin J (**176**, Figure 15.5) from *Parartocarpus venenosa* (Moraceae).⁹⁴ Abyssinoflavanone VI (**204**) from *Erythrina abyssinica* (Leguminosae) contains both DMP and DMDHP ring structures.⁹⁸

Seven new flavanones have been reported from species of *Derris* (Leguminosae), which in addition to a DMP ring have unusual substituents. For example, flavanone **184** (Figure 15.5) from *D. reticulata* bears an 8-*C*-hydroxymethyl substituent, and 2'',3''-dihydroxylupinifolin (**205**, Figure 15.5) from the same species bears an 8-*C*-isopentyl group hydroxylated in the 2- and 3-positions.¹⁵⁴ In the related 2'',3''-epoxylupinifolin (**191**), also from *D. reticulata*, there is an epoxy group between C-2 and C-3 of the 8-*C*-isopentyl chain;¹⁵⁷ 1''-hydroxy-2'',3''-epoxylupinifolin (**202**, Figure 15.5) is similar to **191**, but there is an additional hydroxyl at C-1 in the 8-*C*-isopentyl group.¹⁵⁹ In dereticulatin (**192**), there is an 8-*C*-pentenyl group that is hydroxylated at C-2.¹⁵⁷ The close biogenetic relationships among compounds **191**, **192**, **202**, and **205** are obvious, but **184** seem different because there is no isoprenyl group attached to C-8. However, it was suggested that **184** could be derived from the co-occurring 1''-OH-2'',3''-epoxylupinifolin by acid-catalyzed opening of the epoxide ring, followed by carbon-carbon bond cleavage to produce a precursor of **184**.¹⁵⁴ *In vitro* bioassay evaluation of **184** and **191** revealed cytotoxic activity in the P-388 cell line with IC₅₀ values of 6.4 and 1.3 μm/ml, respectively, but they were inactive against the KB cell line. Even more unusual are the diastereoisomeric flavanones derriflavanone (**206**) and epi-derriflavanone (**207**) from *Derris laxiflora*. These contain a four-membered heterocyclic 3-methoxy-2,2-dimethyl-oxetane ring, which is C-C linked at C-4 to C-8 of 5,4'-dihydroxy-6'',6''-DMP[2'',3'':7,6]flavanone (Figure 15.5).¹⁶⁰ The two compounds are C-4-epimers. Four new bioactive pyranoflavanones, **178** and **197–199**, have been reported from cubé resin, which is an extract of the roots of *Lonchocarpus utilis* and *L. urucu* (Leguminosae) used as an insecticide and piscicide.¹⁵¹ The active principles of cubé resin are rotenone (44%) and deguelin (22%), but many other flavonoids are present as minor components. A number of these minor constituents, including the four pyranoflavanones, were tested for other activities, including the following: (1) inhibition of NADH:ubiquinone oxidoreductase *in vitro*; (2) inhibition of phorbol ester-induced ornithine decarboxylase activity in cultured MCF-7 cells; and (3) cytotoxicity in MCF-7 and Hepa lcl7 cells. All four pyranoflavanones were active in these tests, but compound **198** was the most potent. This flavanone differs from the other three in having a free 4'-hydroxyl substituent, a feature that may be important for the activity. The well-known isoflavone genistein and stilbene *trans*-resveratrol, also obtained from the same plant, were much less active in these bioassays than the pyranoflavanones.¹⁵¹

Pyranoflavanone **183** from *Feronia limonia* (Rutaceae) was active against both Gram-positive and Gram-negative bacteria (at 100 μg/ml against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella erogenes*), but did not show any antifungal activity.¹⁵³

15.2.3 COMPLEX FLAVANONES

15.2.3.1 Benzylated Flavanones

Six new flavanones having one or more *C*-benzyl groups attached to C-6 or C-8, or both, have been reported from members of the Annonaceae, a family from which many *C*-benzylated flavanones and dihydrochalcones had previously been described.¹⁵ The new compounds (**212–217**) are presented in Table 15.6.^{163–166} Macrophyllol (**212**) and macrophyllol A (**213**, Figure 15.6) have been isolated from the roots of *Uvaria macrophylla*. These consist of 5-hydroxy-6,7-dimethoxyflavanone with one 2-hydroxy-5-methoxybenzyl unit attached to C-8 and C-6, respectively.^{163,164} The roots of *Xylopia africana* yielded isouvarinol (**214**, Figure 15.6), in which one 2-hydroxybenzyl unit is attached to C-6 and two units to C-8. Its

TABLE 15.6
C-Benzylated Flavanonones Reported from 1992 to 2003

No.	OH- and OMe-Substitution	C-Benzyl (Bn) Substitution	Formula	M_r	Trivial Name	Plant Source	Family	Organ	Ref.
212	5-OH-6,7-diOMe	8-(2-OH-5-OMe-Bn)	$C_{25}H_{24}O_7$	436	Macrophyllol	<i>Uvaria macrophylla</i>	Annonaceae	Root	163
213	5-OH-7,8-diOMe	6-(2-OH-5-OMe-Bn)	$C_{25}H_{24}O_7$	436	Macrophyllol A (Figure 15.6)	<i>Uvaria macrophylla</i>	Annonaceae	Root	164
214	5,7-DiOH	6-(2-OH-Bn)-8-(2 × 2-OH-Bn)	$C_{36}H_{30}O_7$	574	Isouvarinol (Figure 15.6)	<i>Xylopia africana</i>	Annonaceae	Root	165
215	5,7-DiOH	6-(3 × 2-OH-Bn)-8-(2-OH-Bn)	$C_{43}H_{36}O_8$	680	2'''-OH-3''''-Benzylisouvarinol	<i>Xylopia africana</i>	Annonaceae	Root	166
216	5,7-DiOH	6-(2-OH-Bn)-8-(3 × 2-OH-Bn)	$C_{43}H_{36}O_8$	680	2''''-OH-5''''-Benzylisouvarinol A	<i>Xylopia africana</i>	Annonaceae	Root	166
217	5,7-DiOH	6-(2 × 2-OH-Bn)-8-(2 × 2-OH-Bn)	$C_{43}H_{36}O_8$	680	2''-OH-5''-Benzylisouvarinol B	<i>Xylopia africana</i>	Annonaceae	Root	166

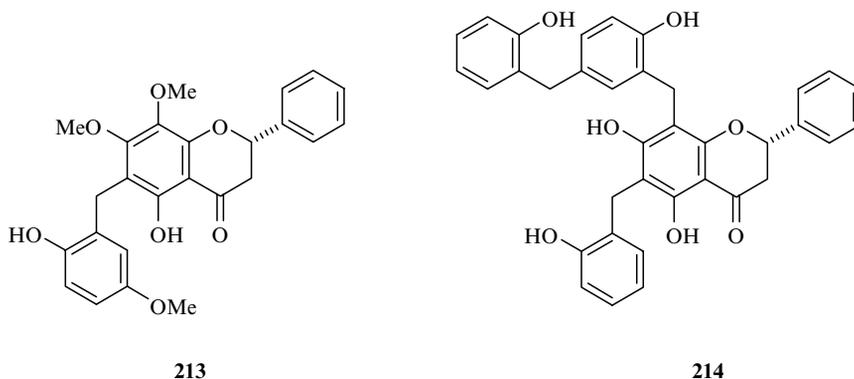


FIGURE 15.6 C-Benzyl-substituted flavanones.

known regioisomer, the cytotoxic and antibacterial uvarinol, was also produced by the same plant.¹⁶⁵ In addition, three tetra-(2-hydroxybenzyl)flavanones have been reported from *X. africana*, with the cumbersome names 2''''-hydroxy-3''''-benzyluvarinol (**215**), in which C-6 bears three 2-hydroxybenzyl units and C-8 one such unit; 2''''-hydroxy-5''''-benzylisouvarinol (**216**), with one 2-hydroxybenzyl unit at C-6 and three units at C-8; and 2''''-hydroxy-5''''-benzylisouvarinol (**217**) with two units at both C-6 and C-8.¹⁶⁶

15.2.3.2 Flavanone-Stilbenes

The roots of several species of *Sophora* (Leguminosae) produce flavanones that have a stilbene, usually resveratrol, condensed to one of the phenolic rings. Six such flavanone-stilbenes, alopecurones A–F, have been reported from *S. alopecurooides* (**218–223**, Table 15.7).¹¹⁴ In these compounds, resveratrol is condensed to the A-ring of the flavanone (Figure 15.7). In alopecurones A, B, D, and E, the A-ring additionally bears a 8-*C*-lavandulyl substituent, and the B-ring is 2',4'-dioxxygenated, so that they have a similar substitution in this respect to alopecurone G (**109**), a compound discussed previously (see Table 15.3). Alopecurones C and F are characterized by an 8-*C*-prenyl side chain.¹¹⁴ In another new flavonostilbene, leachianone I (**224**, Figure 15.7) from *S. leachiana*, the flavanone part is also 5,7,2',4'-tetraoxygenated, but it bears a hydroxylated prenyl group at C-8 and the resveratrol unit is condensed to the B-ring of the flavanone.¹⁶⁷

15.2.3.3 Anastatins

Flavanones with a novel carbon skeleton, anastatins A (**225**) and B (**226**, Table 15.7), have been isolated from *Anastatica hierochuntica* (Brassicaceae), an Egyptian medicinal herb used to treat fatigue and uterine haemorrhage.¹⁶⁸ In these flavanones, a 3,4-dihydroxyphenyl group can be considered to form a benzofuran ring with the 7-hydroxyl of naringenin. In anastatin A, C-7 and C-6 of naringenin are part of the furan ring, whereas in anastatin B the furan ring involves C-7 and C-8 (Figure 15.8). The hepatoprotective effects of anastatins A and B on D-galactosamine-induced cytotoxicity in primary cultured mouse hepatocytes have been determined. The activities found were compared with those of the common flavanones and dihydroflavonols, naringenin, eriodictyol, aromadendrin, and taxifolin, from the same species, and with a commercial sample of the flavanone mixture silybin, which is a known hepatoprotective agent. The hepatoprotective activities of anastatins A and B appeared to be stronger than those of all the other compounds tested, including silybin.¹⁶⁸

TABLE 15.7
Complex Flavonones Reported from 1992 to 2003

No.	Flavanone	Formula	M_r	Plant Source	Family	Organ ^a	Ref.
Flavanone-stilbenes (Figure 15.7)							
218	Alopecurone A	C ₃₉ H ₃₈ O ₉	650	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
219	Alopecurone B	C ₃₉ H ₃₈ O ₉	650	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
220	Alopecurone C	C ₃₄ H ₃₀ O ₈	566	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
221	Alopecurone D	C ₄₀ H ₄₀ O ₉	664	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
222	Alopecurone E	C ₄₀ H ₄₀ O ₉	664	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
223	Alopecurone F	C ₃₄ H ₃₀ O ₉	582	<i>Sophora alopecuroides</i>	Leguminosae	Root	114
224	Leachianone I	C ₃₄ H ₃₀ O ₁₀	598	<i>Sophora leachiana</i>	Leguminosae	Root	167
Anastatins (Figure 15.8)							
225	Anastatin A	C ₂₁ H ₁₄ O ₇	378	<i>Anastatica hierochuntica</i>	Brassicaceae	Whole	168
226	Anastatin B	C ₂₁ H ₁₄ O ₇	378	<i>Anastatica hierochuntica</i>	Brassicaceae	Whole	168
Complex Myrtaceae flavanones (Figure 15.9)							
227	Baeckea flavanone	C ₃₀ H ₃₄ O ₇	506	<i>Baeckea frutescens</i>	Myrtaceae	Aerial	169
228	BF-4	C ₃₀ H ₃₂ O ₆	488	<i>Baeckea frutescens</i>	Myrtaceae	Leaf	170
229	BF-5	C ₃₀ H ₃₂ O ₆	488	<i>Baeckea frutescens</i>	Myrtaceae	Leaf	170
230	BF-6	C ₃₃ H ₃₀ O ₆	522	<i>Baeckea frutescens</i>	Myrtaceae	Leaf	170
231	Leucadenone A	C ₃₃ H ₃₂ O ₇	540	<i>Melaleuca leucadendron</i>	Myrtaceae	Leaf	171
232	Leucadenone B	C ₃₃ H ₃₂ O ₇	540	<i>Melaleuca leucadendron</i>	Myrtaceae	Leaf	171
233	Leucadenone C	C ₃₃ H ₃₂ O ₇	540	<i>Melaleuca leucadendron</i>	Myrtaceae	Leaf	171
234	Leucadenone D	C ₃₃ H ₃₂ O ₇	540	<i>Melaleuca leucadendron</i>	Myrtaceae	Leaf	171
235	Lumaflavanone A (2 <i>S</i>)	C ₃₀ H ₃₂ O ₆	488	<i>Luma checken</i>	Myrtaceae	Leaf	172
236	Lumaflavanone B (2 <i>R</i>)	C ₃₀ H ₃₂ O ₆	488	<i>Luma checken</i>	Myrtaceae	Leaf	172
237	Lumaflavanone C	C ₃₀ H ₃₄ O ₇	506	<i>Luma checken</i>	Myrtaceae	Leaf	172
Calomelanols (Figure 15.10)							
238	Calomelanol G	C ₂₅ H ₂₀ O ₇	432	<i>Pityrogramma calomelanos</i>	Adiantaceae, Pteridophyta	Fronde	173
239	Calomelanol H	C ₂₄ H ₁₈ O ₆	402	<i>Pityrogramma calomelanos</i>	Adiantaceae, Pteridophyta	Fronde	173
240	Calomelanol I	C ₂₄ H ₁₈ O ₆	402	<i>Pityrogramma calomelanos</i>	Adiantaceae, Pteridophyta	Fronde	173
241	Calomelanol J	C ₂₄ H ₁₈ O ₅	386	<i>Pityrogramma calomelanos</i>	Adiantaceae, Pteridophyta	Fronde	173
Diarylheptanoids (Figure 15.11)							
242	Calyxin C	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
243	Calyxin D	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
244	Calyxin G	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
245	Calyxin J	C ₄₂ H ₃₈ O ₉	686	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
246	Calyxin K	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
247	Calyxin M	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
248	Epicalyxin C	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
249	Epicalyxin D	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
250	Epicalyxin G	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
251	Epicalyxin J	C ₄₂ H ₃₈ O ₉	686	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
252	Epicalyxin K	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174
253	Epicalyxin M	C ₃₅ H ₃₄ O ₈	582	<i>Alpinia blepharocalyx</i>	Zingiberaceae	Seed	174

TABLE 15.7
Complex Flavanones Reported from 1992 to 2003 — continued

No.	Flavanone	Formula	M_r	Plant Source	Family	Organ ^a	Ref.
Miscellaneous (Figure 15.12)							
254	Kurziflavolactone A (2 <i>R</i>)	C ₃₂ H ₃₀ O ₇	526	<i>Cryptocarya kurzii</i>	Lauraceae	Leaf	175
255	Kurziflavolactone B (2 <i>S</i>)	C ₃₂ H ₃₀ O ₇	526	<i>Cryptocarya kurzii</i>	Lauraceae	Leaf	175
256	Kurziflavolactone C (2 <i>S</i>)	C ₃₂ H ₃₀ O ₇	526	<i>Cryptocarya kurzii</i>	Lauraceae	Leaf	175
257	Kurziflavolactone D (2 <i>R</i>)	C ₃₂ H ₃₀ O ₇	526	<i>Cryptocarya kurzii</i>	Lauraceae	Leaf	175
258	Tephrocin A	C ₂₄ H ₂₆ O ₇	426	<i>Tephrosia purpurea</i>	Leguminosae		176
259	Tephrocin B	C ₃₀ H ₂₈ O ₆	484	<i>Tephrosia purpurea</i>	Leguminosae		176

^aRootB, root bark; Aerial, aerial parts; Whole, whole plant.

15.2.3.4 Complex Myrtaceae Flavanones

A series of very unusual flavanones has been detected in the genera *Baeckea*, *Luma*, and *Melaleuca*, all belonging to the family Myrtaceae (Table 15.7).^{169–172} The compounds are based on 6-*C*-methylpinocembrin with an unusual substituent at C-8, including a methylated phloroglucinol-based ring structure fused by a 6-carbon heterocyclic ring (which bears either

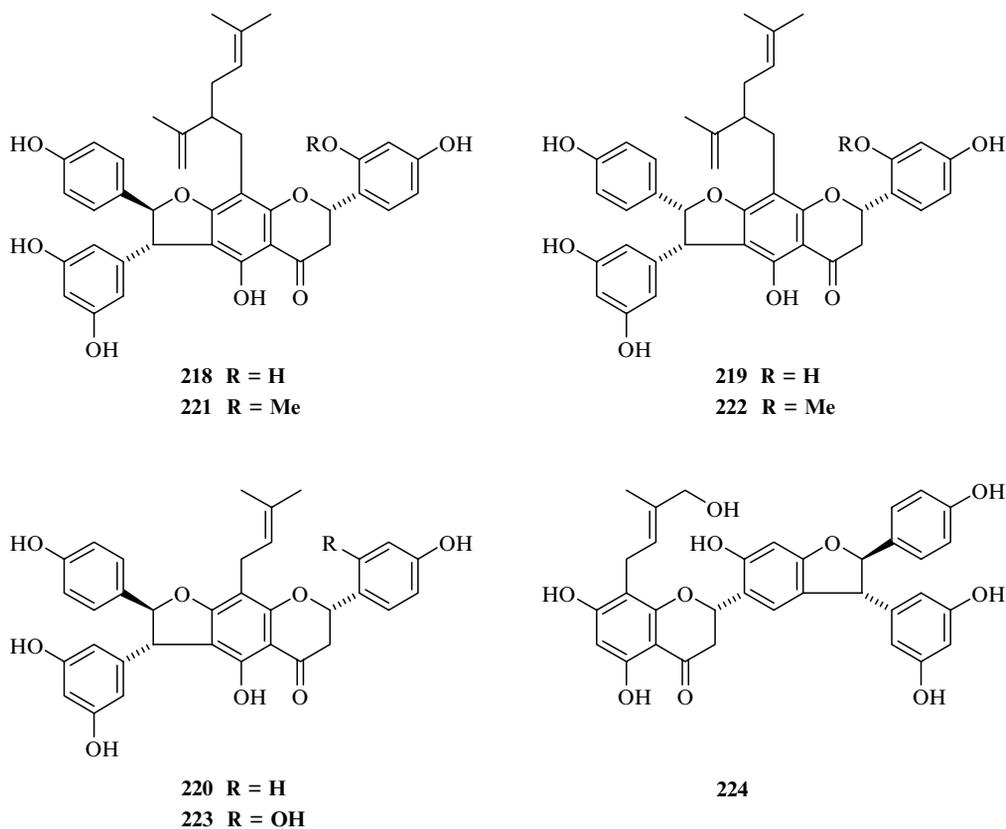


FIGURE 15.7 Flavanone-stilbenes.

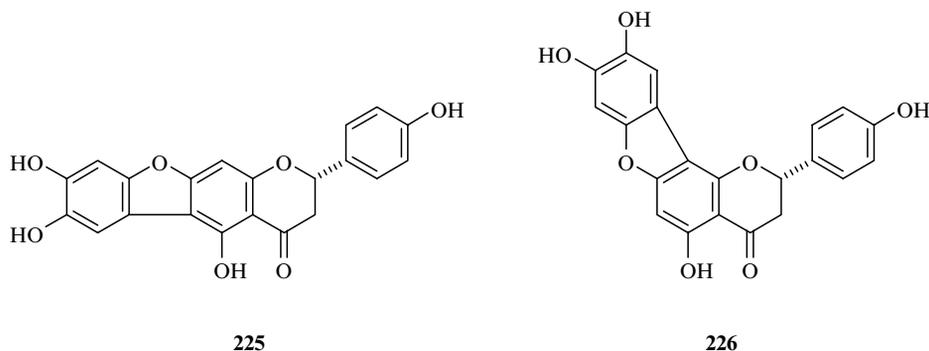


FIGURE 15.8 Anastatins.

an isopropyl or a phenyl group) to the A-ring of the flavanone (Figure 15.9). Four such compounds have been isolated from the leaves of *Baeckea frutescens*, a southeastern-Asian shrub used for treating rheumatism, fever, and snake bites. In the flavanone **227**, the phloroglucinol-like ring is connected via an isobutyl chain to C-8 of the flavanone.¹⁶⁹ Two more flavanones, BF-4 (**228**) and BF-5 (**229**), are C-2 epimers and contain an isopropyl side chain, whereas a third, BF-6 (**230**), is slightly different since it bears a phenyl side group and has two ketone functions in the phloroglucinol-like ring.¹⁷⁰ BF-4 and BF-5 showed strong cytotoxic activity against leukemia cells (L1210) in tissue culture ($IC_{50} = 0.2$ to 0.5 $\mu\text{g/ml}$).¹⁷⁰ Structures very similar to BF-6 (bearing two ketones and a phenyl rather than an isopropyl side group) were obtained from the leaves of *Melaleuca leucadendron*. There are four epimeric centers in the molecule, so that many different stereoisomers are possible. The four obtained so far are known as leucadenones A–D (**231–234**).¹⁷¹ For the absolute configurations of these compounds, see Figure 15.9.

Bioassay-guided fractionation of an extract of *Luma checken* leaves led to the isolation of three further related compounds, lumaflavanones A (**235**), B (**236**), and C (**237**),¹⁷² which are structurally similar to both the *Baeckea* and *Melaleuca* flavanones (Figure 15.9). The lumaflavanones were active in the brine shrimp test, they showed insect-antifeedant activity against *Spodoptera littoralis*, and exhibited antifungal activity against *Botrytis cinerea*.¹⁷² All the active flavanones of *Baeckea* and *Luma* bear an isopropyl rather than phenyl side group, so that the isopropyl chain may be important for these bioactivities. These unusual flavanones may also have chemosystematic importance, as they are all found in genera from the same family.

15.2.3.5 Calomelanols

The farinose exudate of the frond of the fern *Pityrogramma calomelanos* (Adiantaceae) has been the source of complex flavonoids characterized by a novel $C_6-C_3-C_6-C_3-C_6$ skeleton (Table 15.7).¹⁷³ This group includes four flavanones, calomelanols G (**238**), H (**239**), I (**240**), and J (**241**) (Figure 15.10). In these compounds, a molecule of *p*-coumaric or cinnamic acid appears to be fused with the A-ring of the flavanone. Biosynthetic pathways for these complex flavanones and related flavones, chalcones, and dihydrochalcones in *P. calomelanos* and other *Pityrogramma* species have been proposed by the authors.¹⁷³

15.2.3.6 Diarylheptanoid Flavonones

Diarylheptanoids are characteristic phenolics found in the family Zingiberaceae, e.g., curcumin, the bioactive yellow pigment from the spice turmeric (roots of *Curcuma longa*) and similar compounds from ginger (*Zingiber officinalis*). A series of novel diarylheptanoids

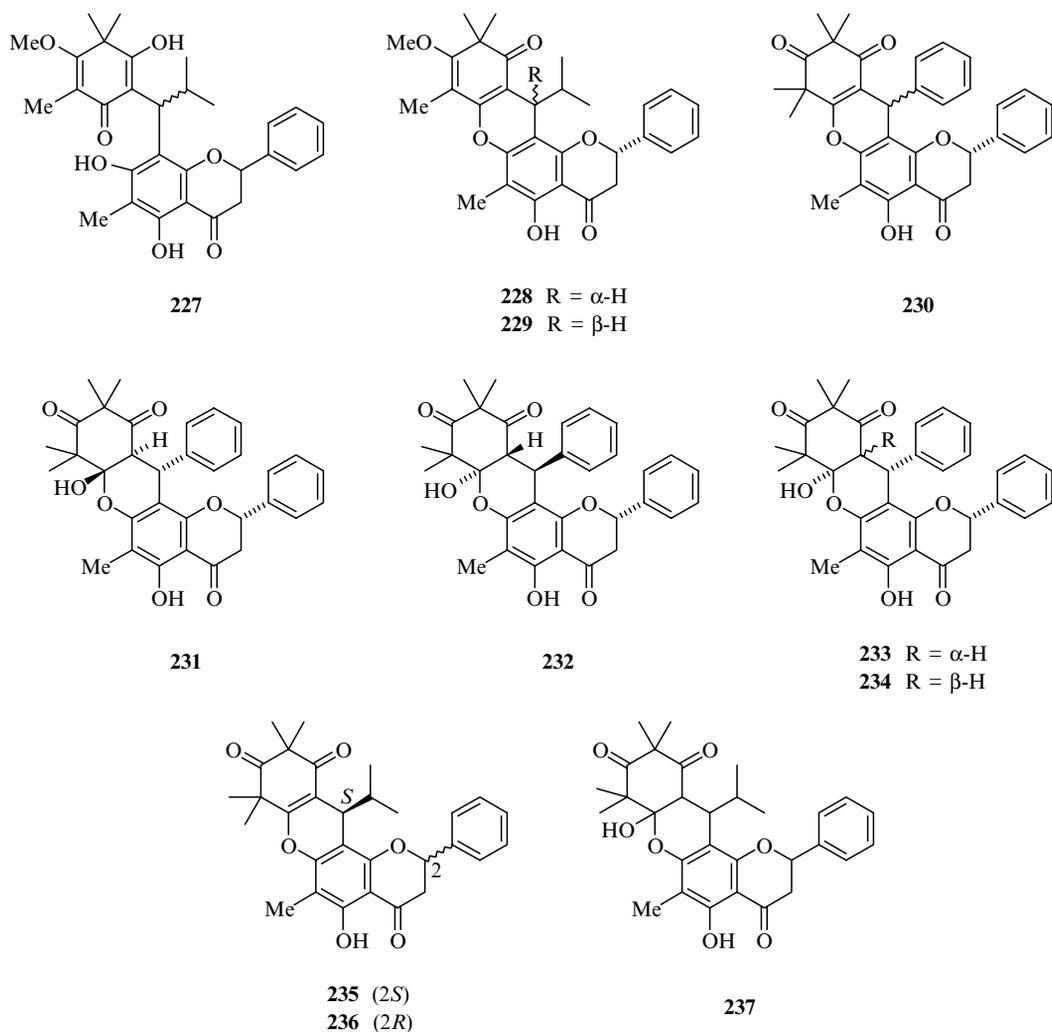


FIGURE 15.9 Complex Myrtaceae flavanones.

conjugated with chalcones and flavanones has been discovered in the seeds of another Zingiberaceae species, *Alpinia blepharocalyx*, which is used in Chinese traditional medicine for the treatment of stomach disorders (Table 15.7).¹⁷⁴ The series includes 12 diarylheptanoid flavanones, calyxins C, D, G, J, K, and M (242–247) and the corresponding epicalyxins (248–253). In all these compounds the flavonoid moiety is 5-O-methylnaringenin, with C-5 or C-7 of the diarylheptanoid attached to C-8 of the flavanone via a C–C linkage (Figure 15.11).¹⁷⁴ The calyxins and epicalyxins differ from each other in their configuration of the 2-position of the flavanone, but the absolute configurations in this position have not been determined. The compounds can be arranged into four groups according to structural features of the diarylheptanoid moiety. In calyxins and epicalyxins C and D, the heptanoid chain is acyclic and the flavanone is attached to C-7 of the diarylheptanoid (Figure 15.11). This C-7 atom is an asymmetric center, and calyxins C (242) and D (243) are thus 7-epimers. Epicalyxins C (248) and D (249) are also 7-epimers, and so are the pairs calyxins G (244) and K (246), and epicalyxins G (250) and K (252). However, in calyxins and epicalyxins

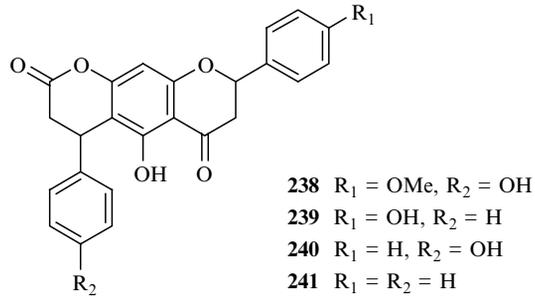


FIGURE 15.10 Calomelanols.

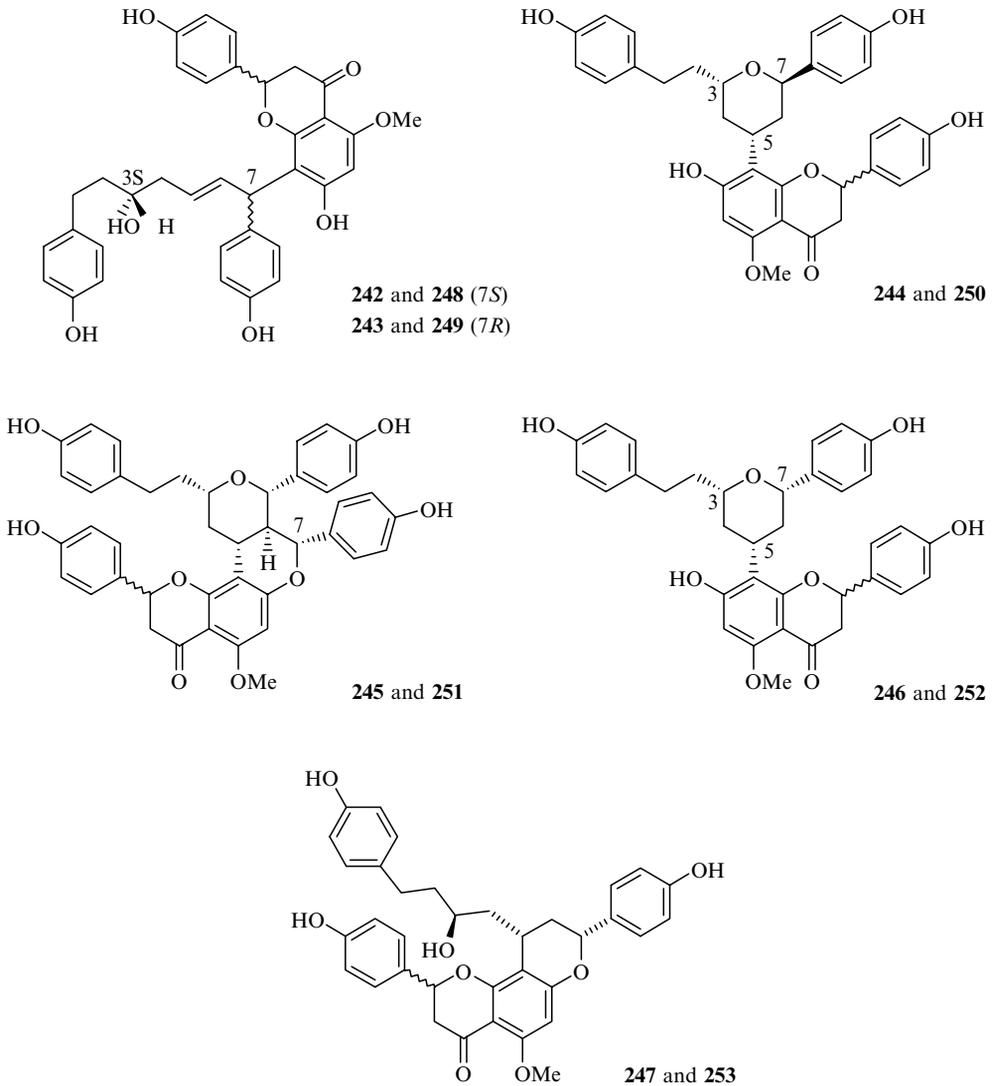


FIGURE 15.11 Diarylheptanoid flavanones.

G and K the heptanoid chain has formed a ring and the flavanone is attached to C-5 of the diarylheptanoid. Calyxin J (**245**) and epicalyxin J (**251**) also contain a cyclic heptanoid, which forms a second tetrahydropyrano ring with the 7-hydroxyl group of the flavanone, and in addition they contain a *p*-hydroxybenzyl group (Figure 15.11). Finally, in calyxin M (**247**) and epicalyxin M (**253**), C-5 and C-7 of the heptanoid chain are attached to C-8 and the 7-hydroxyl of the flavanone, respectively, to form a tetrahydropyrano ring. In a range of bioactivity tests, it was found that the epimeric mixture of calyxin and epicalyxin J (**245** and **251**) exhibited a strong antiproliferative activity against human HT-1080 fibrosarcoma (IC_{50} 0.3 μM) and had some activity against murine colon 26-L5 carcinoma (IC_{50} 13.7). The other flavanone-diaryl heptanoids were much less active in these tests¹⁷⁴ (see also Section 16.2.6).

15.2.3.7 Miscellaneous Complex Flavanones

Flavonoids containing an unusual 17-carbon substituent fused to the A-ring have been found in *Cryptocarya kurzii* (Lauraceae), including four flavanones, kurziflavolactones A–D (**254**–**257**, Table 15.7).¹⁷⁵ The substituent consists of a phenylpropenyl part and an aliphatic part including a tetrahydropyran ring. In kurziflavanones A (**254**) and B (**255**), which are C-2 epimers, the side chain forms a ring with the 7-hydroxyl and C-8 of the flavanone to form an additional ring. In the C-2 epimers **256** and **257**, the side chain is attached to the 7-hydroxyl and C-6 of the flavanone (Figure 15.12). Kurziflavanone B showed slight cytotoxicity against KB cells, with an IC_{50} value of 4 $\mu g/ml$.¹⁷⁵

Tephrosia purpurea (Leguminosae) is the source of two flavanones bearing a novel tetrahydrofuran ring as a side chain at the C-8 position, (+)-tephrorins A (**258**) and B (**259**).¹⁷⁶ The compounds differ from each other in the substitution of the flavanone A-ring and that of

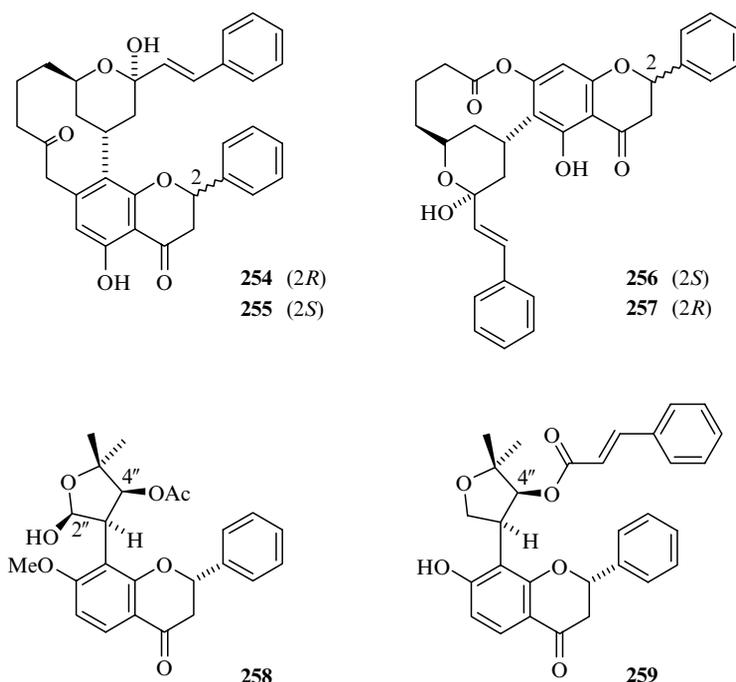


FIGURE 15.12 Kurziflavolactones (**254**–**257**) and tephrorins (**258** and **259**).

C-2'' and C-4'' of the side group; **258** bears a methoxy group at C-7, a hydroxyl at C-2'' and the C-4'' hydroxyl is acylated with acetic acid, whereas **259** bears a hydroxyl at C-7, C-2'' is not hydroxylated and the C-4'' hydroxyl is acylated with (*E*)-cinnamic acid (Figure 15.12). The two flavanones were evaluated for their potential as quinone reductase inducers in cultured mouse Hepa 1c1c7 cells. Tephrosin A significantly reduced quinone reductase activity, whereas tephrosin B was inactive. It was thought that the presence of the bulky cinnamic acid group at C-4'' may affect its biological activity.¹⁷⁶

15.2.4 FLAVANONE GLYCOSIDES

Table 15.8 presents a list of 69 flavanone glycosides reported between 1992 and 2003 (compounds **260–328**).^{137,145,177–228} The new glycosides are based on 35 different aglycones, most of which are “simple flavanones” bearing only hydroxy and methoxy substituents, and these are arranged according to their *O*-substitution pattern in the same manner as in Table 15.1. Four of the aglycones are *C*-methylated and four are prenylated. In Table 15.8, glycosides of the same aglycone are grouped together, and glycosides based on *C*-methyl and prenylated aglycones follow those with only *O*-substituents. The linkages between aglycones and sugars, and between two sugars or a sugar and an acyl group are *C–O–C* in all flavanones, the only exception being a *C–C* linkage in (*2R*)- and (*2S*)-eriodictyol 6-*C*-glucosides (**298** and **299**) from rooibos tea (prepared from *Aspalathus linearis*, Leguminosae), but these are artifacts and do not occur in unprocessed plant material. Rooibos leaves are processed, like those of *Camellia sinensis* tea, by a fermentation and drying process, which may cause the natural dihydrochalcone *C*-glycosides present in the plant to be converted into flavanone *C*-glycosides.²⁰⁹

In approximately one third of the new glycosides only one sugar is attached to the flavanone (monosides) and in a further third two sugars are attached as a disaccharide to one hydroxyl of the flavanone, e.g., homoesperetin 7-rhamnosyl(1 → 6)glucoside (**311**) from *Vernonia diffusa* (Asteraceae).²¹⁷ The correct term for this type of glycoside is bioside and not diglycoside, because there is only one glycosidic linkage with the flavonoid.⁵ Five compounds are diglycosides, i.e., that they have one or more sugars attached to two different hydroxyls (two glycosidic linkages with the flavanone),⁵ e.g., carthamidin 6,7-diglucoside (**293**) from *Carthamus tinctorius* (Asteraceae).²⁰⁴ There are six triosides (a combination of three sugars attached to one hydroxyl),⁵ and in two of those compounds the sugar chains are branched. These are the 7-(2,6-dirhamnosyl)glucosides of naringenin (**285**) and hesperetin (**307**). Both compounds were obtained from the fruits of *Citrus junos* (Rutaceae)¹⁹⁶ and **308** was first described from leaves of *Buddleja madagascariensis* (Buddlejaceae).²¹⁵ The hesperetin glycoside inhibits the influenza A virus.¹⁹⁶

Glucose is by far the most common sugar in the new flavanone glycosides, either as a monoside or as one or more of the sugars in the biosides, triosides, diglycosides, or acylated glycosides; glucose is lacking in only eight of the compounds. The second most common sugar in the newly reported glycosides is apiose, which was found in 15 different glycosides. This is a little surprising, since apiose is not considered to be a common sugar in flavonoid glycosides in general. However, most of the new apiose-containing glycosides have been reported from only a few genera, especially *Glycyrrhiza*, in which nine such compounds were found, e.g., the diglycoside liquiritigenin 7-apiofuranoside-4'-glucoside (**265**) from *Glycyrrhiza inflata* (Leguminosae).¹⁸⁴ Rhamnose is the third most common sugar (in 14 glycosides), glucuronic acid is present in three of the glycosides, and arabinose and xylose in two. Galactose is only present in alhagidin from *Alhagi pseudalhagi* (Leguminosae), which is hesperetin 7-galactosyl(1 → 2)[rhamnosyl(1 → 6)]glucoside (**309**)¹⁹⁹ and the unusual sugar fucose has been reported to be present in isosakuranetin 7-fucopyranosyl(1 → 6)glucoside (longitin, **286**)

TABLE 15.8
New Flavanone Glycosides Reported from 1992 to 2003

No.	Flavanone Glycoside	Formula	M _r	Trivial Name	Plant Source	Family	Organ ^a	Ref.
	<i>7-OH-Flavanone</i>							
260	7-Glucoside	C ₂₁ H ₂₂ O ₈	402		Clerodendrum phlomidis Cochlospermum regium	Lamiaceae Bixaceae	Leaf Leaf	177 178
	<i>5,7-DiOH-Flavanone (pinoembrin)</i>							
261	7-Glucoside (2S)	C ₂₁ H ₂₂ O ₉	418	Pinoembrin	<i>Glycyrrhiza glabra</i> <i>Penthorum chinense</i> <i>Onychium japonicum</i>	Leguminosae Saxifragaceae Adiantaceae, Pteridophyta	Aerial	179 180 181
262	7-Rhamnosylglucoside	C ₂₇ H ₃₂ O ₁₃	564					
263	7-Apiosyl(1 → 5)apiosyl(1 → 2)glucoside <i>5-OH-7-OMe-Flavanone (pinoestrobin)</i>	C ₃₁ H ₃₈ O ₁₇	682		<i>Viscum angulatum</i>	Viscaceae	Whole	182
264	5-Glucoside <i>7,4'-diOH-Flavanone (lquitritigenin)</i>	C ₂₂ H ₂₄ O ₉	432		<i>Pyracantha coccinea</i>	Rosaceae	Root	183
265	7-Apiofuranoside-4'-glucoside	C ₂₆ H ₃₀ O ₁₃	550		<i>Glycyrrhiza inflata</i>	Leguminosae	Root	184
266	7-(3-Acetylapioside)-4'-glucoside	C ₂₈ H ₃₂ O ₁₄	592		<i>Glycyrrhiza inflata</i>	Leguminosae	Root	184
267	4'-[3-Acetylapiosyl(1 → 2)glucoside]	C ₂₈ H ₃₂ O ₁₄	592		<i>Glycyrrhiza uralensis</i>	Leguminosae	Root	185
268	7-Glucoside-4'-apiosyl(1 → 2)glucoside	C ₃₂ H ₄₀ O ₁₈	712	Glucoliquiritin apioside	<i>Glycyrrhiza glabra</i>	Leguminosae	Root	145
269	Licorice glycoside E	C ₃₅ H ₃₅ O ₁₄ N	693	Figure. 15.13	<i>Glycyrrhiza uralensis</i>	Leguminosae	Root	186
270	4'-[4- <i>p</i> -Coumaroylapiosyl(1 → 2) glucoside] (2R)	C ₃₅ H ₃₆ O ₁₅	696	Licorice glycoside D ₁	<i>Glycyrrhiza uralensis</i>	Leguminosae	Root	186
271	4'-[4- <i>p</i> -Coumaroylapiosyl(1 → 2) glucoside] (2S)	C ₃₅ H ₃₆ O ₁₅	696	Licorice glycoside D ₂	<i>Glycyrrhiza uralensis</i>	Leguminosae	Root	186
272	4'-[4-Feruloylapiosyl(1 → 2)glucoside] (2R)	C ₃₆ H ₃₈ O ₁₆	726	Licorice glycoside C ₁	<i>Glycyrrhiza uralensis</i>	Leguminosae	Root	186
273	4'-[4-Feruloylapiosyl(1 → 2)glucoside] (2S) <i>5,6,7-TriOH-Flavanone (dihydrobaicalin)</i>	C ₃₆ H ₃₈ O ₁₆	726	Licorice glycoside C ₂	<i>Glycyrrhiza uralensis</i>	Leguminosae	Root	186
274	7-Glucoside (2S) <i>5,7,8-TriOH-Flavanone (dihydronorwogonin)</i>	C ₂₁ H ₂₂ O ₁₀	434		<i>Cephalocereus senilis</i>	Cactaceae	Cells ^b	187
275	5-Glucoside <i>5,7,2'-TriOH-Flavanone</i>	C ₂₁ H ₂₂ O ₁₀	434		<i>Pyracantha coccinea</i>	Rosaceae	Root	183
276	7-Glucoside (2S)	C ₂₁ H ₂₂ O ₁₀	434		<i>Scutellaria ramosissima</i>	Lamiaceae	Aerial	188
277	7-O-(Methyl-β-D-glucuronate) (2S)	C ₂₂ H ₂₂ O ₁₁	462		<i>Scutellaria ramosissima</i>	Lamiaceae	Aerial	189

continued

TABLE 15.8
New Flavanone Glycosides Reported from 1992 to 2003 — continued

No.	Flavanone Glycoside	Formula	M_r	Trivial Name	Plant Source	Family	Organ ^a	Ref.
278	7- <i>O</i> -(Ethyl- β -D-glucuronate) (2S) 5,7,4'- <i>TriOH</i> -Flavanone (<i>naringenin</i>)	C ₂₃ H ₂₄ O ₁₁	476		<i>Scutellaria ramosissima</i>	Lamiaceae	Aerial	189
279	4'-Rhamnoside	C ₂₁ H ₂₂ O ₉	418		<i>Crotalaria striata</i>	Leguminosae	Stem	190
280	7-(6-Acetylglucoside)	C ₂₃ H ₂₄ O ₁₁	476		<i>Acacia saligna</i>	Leguminosae	Flower	191
281	7-(2- <i>p</i> -Coumaroylglucoside)	C ₃₀ H ₂₈ O ₁₂	580		<i>Ricinus communis</i>	Euphorbiaceae	Seed	192
282	7-[β -Acetyl-6- <i>p</i> -coumaroylglucoside]	C ₃₂ H ₃₀ O ₁₃	622		<i>Blepharis ciliaris</i>	Acanthaceae	Aerial	193
283	7-(4,6-Digalloylglucoside)	C ₃₅ H ₃₀ O ₁₈	738		<i>Acacia farnesiana</i>	Leguminosae	Fruit	194
284	7-Rhamnosyl(1 \rightarrow 2)(4- <i>O</i> -methyl- β -D-glucoside)	C ₂₈ H ₃₄ O ₁₄	594	Fumotonaringenin	<i>Microleptia marginata</i>	Dennstaedtiaceae, Pteridophytaceae		195
285	7-(2,6-Dirhamnosylglucoside) 5,7- <i>DiOH</i> -4'- <i>OMe</i> -Flavanone (<i>isosakuranetin</i>)	C ₃₃ H ₄₂ O ₁₈	726		<i>Citrus junos</i>	Rutaceae	Fruit	196
286	7-Fucopyranosyl(1 \rightarrow 6)glucoside	C ₂₇ H ₃₂ O ₁₄	580	Longitin	<i>Mentha longifolia</i>	Lamiaceae	Aerial	197
287	7- α -L-Arabinofuranosyl(1 \rightarrow 6)glucoside 7,4'- <i>DiOH</i> -5'- <i>OMe</i> -Flavanone (5'- <i>O</i> -methylnaringenin)	C ₂₇ H ₃₂ O ₁₄	580		<i>Punica granatum</i>	Punicaceae	StemB	198
288	4'-Glucoside	C ₂₂ H ₂₄ O ₁₀	448	Alhagitin	<i>Alhagi pseudalhagi</i>	Leguminosae	Whole	199
289	7-Neohesperidoside-4'-glucoside 5- <i>OH</i> -7,8- <i>diOMe</i>	C ₃₄ H ₄₄ O ₁₉	756		<i>Clerodendrum phlomidoides</i>	Lamiaceae	Root	200
290	5-Rhamnoside 4'- <i>OH</i> -5,7- <i>diOMe</i> -Flavanone	C ₂₃ H ₂₆ O ₉	446		<i>Albizia procera</i>	Leguminosae	Stem	201
291	4-[2-(5-Cinnamoyl)- β -D- <i>apiofuranosyl</i>]glucoside 2,5,7,4'- <i>TetraOH</i> -Flavanone (2-hydroxynaringenin)	C ₃₇ H ₄₀ O ₁₅	724		<i>Viscum album</i> ssp. <i>album</i>	Viscaceae		202
292	7-Glucoside 5,6,7,4'- <i>TetraOH</i> -Flavanone (<i>carthamidin</i>)	C ₂₁ H ₂₂ O ₁₁	450		<i>Chaenomeles sinensis</i>	Rosaceae	Fruit	203
293	6,7-Diglucoiside 5,7,8,4'- <i>TetraOH</i> -Flavanone (<i>isocarthamidin</i>)	C ₂₇ H ₃₂ O ₁₆	612		<i>Carthamus tinctorius</i>	Asteraceae		204
294	7-Rhamnoside	C ₂₁ H ₂₂ O ₁₀	434		<i>Spartium junceum</i>	Leguminosae	Aerial	205
295	8-Glucoside 5,7,2',4'- <i>TetraOH</i> -Flavanone (<i>steppogenin</i>)	C ₂₁ H ₂₂ O ₁₁	450	3-Desoxycallunin	<i>Calluna vulgaris</i>	Ericaceae	Flower	206
296	4'-Glucoside 5,7,2',5'- <i>TetraOH</i> -Flavanone	C ₂₁ H ₂₂ O ₁₁	450		<i>Maclura tinctoria</i>	Moraceae	StemB	207
297	7-Glucoside	C ₂₁ H ₂₂ O ₁₁	450	Coccinoside B	<i>Pyracantha coccinea</i>	Rosaceae	Leaf	208

298	5,7,3',4'- <i>TetraOH-Flavanone</i> (<i>eriodictyol</i>)								
299	6-C-Glucoside (2R)	C ₂₁ H ₂₂ O ₁₁	450	Coccinose A	<i>Aspalathus linearis</i>	Leguminosae	Leaf, stem ^c	209	
300	6-C-Glucoside (2S)	C ₂₁ H ₂₂ O ₁₁	450		<i>Aspalathus linearis</i>	Leguminosae	Leaf, stem ^c	209	
301	7-Glucuronide (2S)	C ₂₁ H ₂₀ O ₁₂	464		<i>Chrysanthemum indicum</i>	Asteraceae	Flower	210	
302	7-(6-Acetylglucoside)	C ₂₃ H ₂₄ O ₁₂	492		<i>Pyracantha coccinea</i>	Rosaceae	Leaf	208	
303	7- α -L-Arabinofuranosyl(1 \rightarrow 6)glucoside	C ₂₆ H ₃₀ O ₁₅	582		<i>Punica granatum</i>	Punicaceae	StemB	198	
304	7-(6- <i>p</i> -Coumaroylglucoside) (2S)	C ₃₀ H ₂₈ O ₁₃	596		<i>Phyllanthus emblica</i>	Euphorbiaceae	Aerial	211	
305	3-(6- <i>p</i> -Coumaroylglucoside)	C ₃₀ H ₂₈ O ₁₃	596		<i>Malus</i> \times <i>domestica</i>	Rosaceae	Leaf ^d	212	
306	7-(6-Galloylglucoside) (2S)	C ₂₈ H ₂₆ O ₁₅	602		<i>Phyllanthus emblica</i>	Euphorbiaceae	Aerial	211	
307	7-(2,4,6-Triacetylglucoside)	C ₂₇ H ₂₈ O ₁₄	576		<i>Bidens pilosa</i>	Asteraceae	Aerial	213	
308	5,6,7-TriOH-4'-O-Me-Flavanone (4'-methylcarthamidin)								
309	7-(2- <i>p</i> -Coumaroylglucoside)	C ₃₁ H ₃₀ O ₁₃	610		<i>Crotalaria prostrata</i>	Leguminosae	Leaf	214	
310	5,7,3'-TriOH-4'-OMe-Flavanone (<i>hesperetin</i>)								
311	7-(2,6-Dirhamnosylglucoside)	C ₃₄ H ₄₄ O ₁₉	756		<i>Buddleja madagascariensis</i>	Buddlejaceae	Leaf	215	
312	7-(2,6-Dirhamnosylglucoside)	C ₃₄ H ₄₄ O ₁₉	756		<i>Citrus junos</i>	Rutaceae	Fruit	196	
313	7-Galactosyl(1 \rightarrow 2)[rhamnosyl(1 \rightarrow 6)] glucoside	C ₃₄ H ₄₄ O ₂₀	810	Alhagidin	<i>Alhagi pseudalhagi</i>	Leguminosae	Whole	199	
314	5,7,4'-TriOH-3'-OMe-Flavanone								
315	7- β -L-Rhamnosyl(1 \rightarrow 6)glucoside	C ₂₈ H ₃₄ O ₁₅	610		<i>Clematis armandii</i>	Ranunculaceae	Aerial	216	
316	5,7-DiOH-3',4'-diOMe-Flavanone (<i>homoesperetin</i>)								
317	7-Rhamnosyl(1 \rightarrow 6)glucoside	C ₂₉ H ₃₆ O ₁₅	624		<i>Vernonia diffusa</i>	Asteraceae	Wood	217	
318	5,3'-DiOH-7,4'-diOMe-Flavanone (<i>persicogenin</i>)								
319	3'-Glucoside	C ₂₃ O ₂₆ O ₁₁	478		<i>Prunus amygdalus</i>	Rosaceae	StemB	218	
320	5,4'-DiOH-7,3'-diOMe-Flavanone								
321	4'-Apiosyl(1 \rightarrow 2)glucoside	C ₂₈ H ₃₄ O ₁₅	610		<i>Viscum alniformosanae</i>	Viscaceae	Aerial	219	
322	4'-OH-5,7,2'-triOMe-Flavanone								
323	4-Rhamnosyl(1 \rightarrow 6)glucoside	C ₃₀ H ₃₈ O ₁₅	638		<i>Terminalia alata</i>	Combretaceae	Root	220	
324	5,7,4'-TriOH-3',5'-OMe-Flavanone								
325	5-Glucoside (2R)	C ₂₃ O ₂₆ O ₁₂	494	Peruvianoside I	<i>Thevetia peruviana</i>	Apocynaceae	Leaf	221	
326	5-Glucoside (2S)	C ₂₃ O ₂₆ O ₁₂	494	Peruvianoside II	<i>Thevetia peruviana</i>	Apocynaceae	Leaf	221	
327	5,7-diOH-6-C-Me-Flavanone (<i>strobopinin</i>)								
328	7-Xylosyl(1 \rightarrow 3)xyloside	C ₂₆ H ₃₀ O ₁₂	534		<i>Mosla chinensis</i>	Lamiaceae		222	
329	5,7-diOH-6,8-di-C-Me-Flavanone								

continued

TABLE 15.8
New Flavanone Glycosides Reported from 1992 to 2003 — continued

No.	Flavanone Glycoside	Formula	M _r	Trivial Name	Plant Source	Family	Organ ^a	Ref.
318	7-[6-(3-OH-3-Methylglutaryl)glucoside]	C ₂₉ H ₃₄ O ₁₃	590	Matteuoniate B (Figure 15.13)	<i>Matteucia orientalis</i>	Aspleniaceae, Pteridophyta	Rhiz	223
319	5,7,4'-TriOH-6,8-di-C-Me-Flavanone (<i>farrerol</i>) 7-β-D-Apiofuranosyl(1 → 6)glucoside	C ₂₈ H ₃₄ O ₁₄	594	Miconioside B	<i>Miconia trailii</i>	Melastomataceae	Leaf, stem	224
320	7-Apioosyl(1 → 6)glucoside	C ₂₉ H ₃₆ H ₁₄	608		<i>Rhododendron simsii</i>	Ericaceae	Leaf	225
321	7-α-L-Arabinopyranosyl(1 → 6)glucoside	C ₂₉ H ₃₆ H ₁₄	608	Miconioside A	<i>Miconia trailii</i>	Melastomataceae	Aerial	224
322	7-(4,6-(S)-Hexahydroxydiphenylglucoside)	C ₃₈ H ₃₄ O ₁₈	778		<i>Miconia myriantha</i>	Melastomataceae	Aerial	226
323	7-(4,6-Digalloylglucoside)	C ₃₈ H ₃₆ O ₁₈	780		<i>Miconia myriantha</i>	Melastomataceae	Aerial	226
324	7-[6-(3-OH-3-Methylglutaryl)glucoside]	C ₃₀ H ₃₆ O ₁₄	620	Matteuoniate A (Figure 15.13)	<i>Matteucia orientalis</i>	Aspleniaceae, Pteridophyta	Rhiz	223
325	5,4'-DiOH-6-C-Prenylflavanone 4'-Xylosyl(1 → 2)rhamnoside	C ₃₁ H ₃₈ O ₁₂	602		<i>Gliricidia maculata</i>	Leguminosae	Seed	227
326	5,7,4'-TriOH-8-C-Prenylflavanone 7-Glucoside	C ₂₆ H ₃₀ O ₁₀	502	Phellodensin F	<i>Phellodendron chinense</i> var. <i>glabriusculum</i>	Rutaceae	Leaf	137
327	5,7,4'-TriOH-8-C-(3-Hydroxymethyl-2-butenyl)flavanone 7-Glucoside	C ₂₆ H ₃₀ O ₁₁	518	Phellodensin E	<i>Phellodendron chinense</i> var. <i>glabriusculum</i>	Rutaceae	Leaf	137
328	<i>Sophora flavanone I</i> 7-Glucoside	C ₄₅ H ₄₈ O ₁₄	812	Figure. 15.13	<i>Sophora stenophylla</i>	Leguminosae	Root	228

Notes: If the configuration and form of the sugars has not been specified, rhamnoside, α-L-rhamnopyranoside; apioside, β-apiofuranoside; galactoside, β-D-galactopyranoside; xyloside, β-D-xylopyranoside; glucuronide, β-D-glucuronopyranoside. Cinnamic acids are assumed in the *E*-form.

^aAerial, aerial parts; Whole, whole plant; StemB, stem bark; Rhiz, rhizome.

^bChitin-treated cell suspension cultures.

^cFermented leaves and stem.

^dLeaves treated with prohexadione-Ca.

from *Mentha longifolia* (Lamiaceae).¹⁹⁷ However, the identification of fucose was not fully supported by a complete set of ¹H and ¹³C resonance assignments for the sugar in the NMR spectra of this compound and should therefore be regarded as tentative.

More than 20 of the new glycosides are acylated, *p*-coumaroyl and acetyl being the most common acyl groups. Diacylation is present in naringenin 7-[3-acetyl-6-(*E*)-*p*-coumaroylglucoside] (**282**) from *Blepharis ciliaris* (Acanthaceae),¹⁹³ and in the 7-(4,6-digalloylglucosides) of naringenin (**283**) from *Acacia farnesiana* (Leguminosae)¹⁹⁵ and matteucinol (**323**) from *Miconia myriantha* (Melastomataceae).²²⁶ Another glycoside from *M. myriantha* (**322**) is very similar to **323**, but here the two gallic acid molecules are C–C linked to form one acyl group, the two carboxyl groups of which are esterified with the 4- and 6-hydroxyls of the glucose, resulting in matteucinol 7-(4,6-hexahydroxydiphenylglucoside), in which the sugar and acyl groups form a ring.²²⁶ Triacylation is found in only one new glycoside, iso-okanin 7-(2,4,6-triacetylglucoside) (**306**) from *Bidens pilosa* (Asteraceae).²¹³

In two thirds of the newly reported flavanone glycosides the sugars are attached to the 7-hydroxyl of the flavanone moiety. Linkage to the 4'-hydroxyl is also very common, however, as this is found in 17 structures listed in Table 15.8. Examples include an interesting group of new flavanone glycosides reported from *Glycyrrhiza uralensis*, which all contain liquiritigenin as the aglycone and apiose and glucose in the sugar moiety.¹⁸⁶ In licorice glycosides C₁ (**272**) and C₂ (**273**), which are epimers at C-2, the apiose is acylated with ferulic acid, whereas in licorice glycosides D₁ (**270**) and D₂ (**271**) (also epimers at C-2), the acyl group is *p*-coumaric acid. Licorice glycoside E (**269**) has the same basic structure, but the hydroxycinnamoyl has been replaced by an indole-2-carboxyl group (Figure 15.13). It is the only nitrogen-containing flavanone glycoside.¹⁸⁶

There are five new 5-*O*-glycosides, flavanones **264**, **275**, **290**, **315**, and **316**, but only one 8-*O*-glycoside, isocarthamidin 8-glucoside (3-desoxycallunin, **294**) from *Calluna vulgaris* (Ericaceae).²⁰⁶

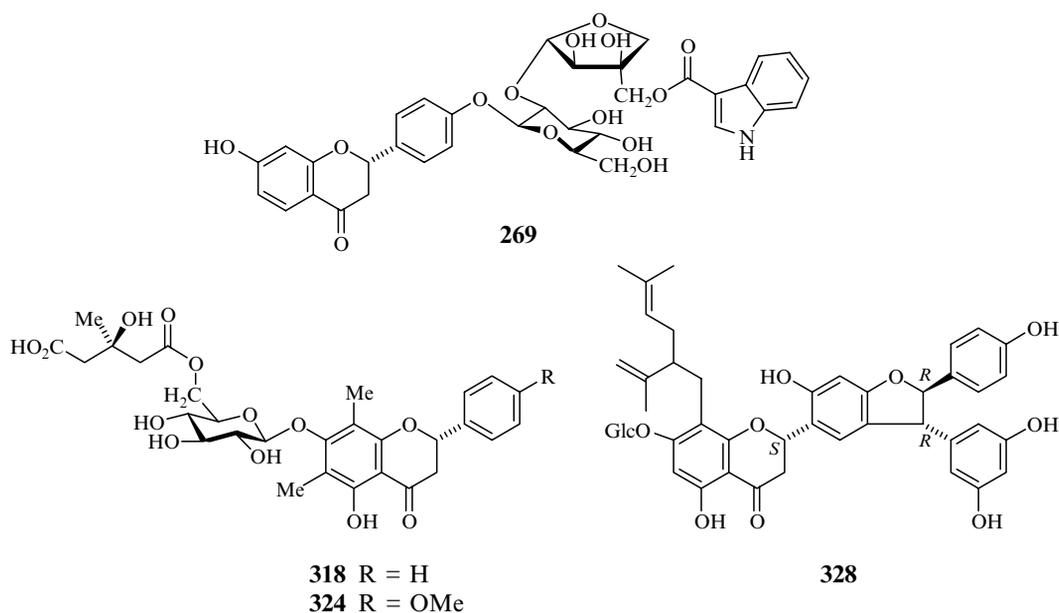


FIGURE 15.13 Examples of flavanone glycosides.

In Table 15.8, the configurations of the sugars are assumed to be β -D for glucose and glucuronic acid, and α -L for rhamnose, but the 7- β -L-rhamnosyl(1 \rightarrow 6)glucoside of 5,7,4'-trihydroxy-3'-methoxyflavanone (**310**) has been reported from *Clematis armandi* (Ranunculaceae).²¹⁶ The β -configuration of the rhamnose is not supported by NMR data, which indicates that the α -rhamnosyl sugar is present as expected.

New glycosides of C-methylflavanones have been isolated from species of just four different families, the Aspleniaceae, Ericaceae, Lamiaceae, and Melastomataceae. The most unusual glycosides of these are the acylated matteuorientates A (**324**) and B (**318**) from the fern *Matteucia orientalis* (Aspleniaceae). They are the 3-methylglutaric acid esters of matteucinol and farrerol 7-glucoside, respectively (Figure 15.13). Both matteuorientates have been found to be potent lens aldose reductase inhibitors.²²³ Glycosides of prenylated flavanones have been found in species of Leguminosae and Rutaceae, which are well-known sources of isoprenylated flavanones in general. The aglycone of glycoside **328**, sophoraflavanone I, is a flavanonostilbene very similar to the alopecurones discussed in Section 15.2.3.2 (see Figure 15.13).

Some new flavanone glycosides that are interesting from an ecological or bioactivity point of view and have not yet been mentioned include (2*S*)-dihydrobaicalein 7-*O*-glucoside (**274**), a compound induced by chitin in cell suspension cultures of "old man" cactus, *Cephalocereus senilis* (Cactaceae).¹⁸⁷ A rhamnosylglucoside of pinocembrin (**262**) has been isolated from aerial parts of the fern *Onichium japonicum* (Adiantaceae), in which it occurs in high levels (0.13% of the dried weight). This glycoside showed moderate activity against P-388 lymphocytic leukemia with an IC₅₀ of 2.58 μ g/ml, but did not exhibit any activity against Hela cells up to 20 μ g/ml.¹⁸¹ Unfortunately, the authors did not specify the linkage between rhamnose and glucose in the sugar moiety. (2*S*)-Eriodictyol 7-glucuronide (**300**) and its known (2*R*)-epimer from *Chrysanthemum indicum* (Asteraceae) showed inhibitory activity toward rat lens aldose reductase,²¹⁰ and finally, 4'-hydroxy-5,7,2'-trimethoxyflavanone 4'-rhamnosyl(1 \rightarrow 6)glucoside (**314**) from *Terminalia alata* (Combretaceae) displayed antifungal activity.²²⁰

15.3 DIHYDROFLAVONOLS

15.3.1 DIHYDROFLAVONOL AGLYCONES

The number of new dihydroflavonols or flavanonols reported during the 1992 to 2003 period is much smaller than that of the respective flavanones, and they are collated in a single table (Table 15.9, compounds **329–396**).^{18,57,67,76,78,80,90,109,133,134,161,229–264} Within this table, the compounds are arranged in the same manner as the flavanones, into "simple dihydroflavonols," C-methylated and isoprenylated dihydroflavonols, etc.

15.3.1.1 Simple Dihydroflavonols with *O*-Substitution Only

Only 15 newly reported "simple dihydroflavonols" are listed in Table 15.9. Several of the compounds are just simply the C-3 epimers of known (2*R*,3*R*)-dihydroflavonols, e.g., (2*R*,3*S*)-3-hydroxy-5,7-dimethoxyflavanone (**329**) from the fern *Woodsia scopulina* (Dryopteridaceae),²²⁹ (2*R*,3*S*)-3,5,3',4'-tetrahydroxy-7-methoxyflavanone (3-epipadmatin, **333**) from *Inula graveolens* (Asteraceae),²³² (2*R*,3*S*)-3,5,3'-trihydroxy-7,4'-dimethoxyflavanone (**337**) from *Lansea coromandelica* (Anacardiaceae),²³¹ and (2*R*,3*S*)-3,5,7,3',4',5'-hexahydroxyflavanone (hovenitin III, **338**) from the seeds and fruits of *Hovenia dulcis* (Rhamnaceae).²³⁵ The previously known (2*R*,3*R*)-stereoisomer of hovenitin III has the trivial name ampelopsin.¹⁵ The same plant source has yielded the (2*R*,3*R*)- and (2*R*,3*S*)-epimers of 3,5,7,4',5'-pentahydroxy-3'-methoxyflavanone, which are known as hovenitins I (**340**) and II (**341**), respectively.²³⁵

TABLE 15.9
Dihydroflavonols (Flavanonols) Reported from 1992 to 2003

No.	O-Substitution	Other Substituents ^a	Formula	M _r	Trivial Name	Plant Source	Family	Organ ^b	Ref.
Simple dihydroflavonols									
<i>Tri-O-substituted</i>									
329 (2R,3S)	3-OH-5,7-diOMe		C ₁₇ H ₁₆ O ₃	300		<i>Woodisia scopulina</i>	Dryopteridaceae, Pteridophyta	FronD	229
<i>Tetra-O-substituted</i>									
330*	3,5,8-TriOH-7-OMe		C ₁₆ H ₁₄ O ₆	302		<i>Muntingia calabura</i>	Elaeocarpaceae	Leaf	230
331*	3-OH-5,7,4'-triOMe		C ₁₈ H ₁₈ O ₆	330		<i>Lanmea coromandelica</i>	Anacardiaceae	StemB	231
<i>Penta-O-substituted</i>									
332	3,5,2',3'-TetraOH-7-OMe		C ₁₆ H ₁₄ O ₇	318		<i>Iris tenuifolia</i>	Iridaceae	Root	18
333 (2R,3S)	3,5,3',4'-TetraOH-7-OMe		C ₁₆ H ₁₄ O ₇	318	3-Epipadmatin	<i>Inula graveolens</i>	Asteraceae	Aerial	232
334	3,5,7-TriOH-6,4'-diOMe		C ₁₇ H ₁₆ O ₇	332		<i>Prunus domestica</i>	Rosaceae	Wood	233
335*	3,5,2'-TriOH-7,5'-diOMe		C ₁₇ H ₁₆ O ₇	332		<i>Blumea balsamifera</i>	Asteraceae	Aerial	234
336	3,5,3'-TriOH-7,2'-diOMe		C ₁₇ H ₁₆ O ₇	332		<i>Iris tenuifolia</i>	Iridaceae	Root	18
337 (2R,3S)	3,5,3'-TriOH-7,4'-diOMe		C ₁₇ H ₁₆ O ₇	332		<i>Lanmea coromandelica</i>	Anacardiaceae	StemB	231
<i>Hexa-O-substituted</i>									
338 (2R,3S)	3,5,7,3',4',5'-HexaOH		C ₁₅ H ₁₂ O ₈	320	Hovenitin III	<i>Hovenia dulcis</i>	Rhamnaceae	Seed, fruit	235
339	3,5,7,2',5'-PentaOH-6-OMe		C ₁₆ H ₁₄ O ₈	334	Diosalol	<i>Dioclea grandiflora</i>	Leguminosae	RootB	78
340*	3,5,7,4',5'-PentaOH-3'-OMe		C ₁₆ H ₁₄ O ₈	334	Hovenitin I	<i>Hovenia dulcis</i>	Rhamnaceae	Seed, fruit	235
341 (2R,3S)	3,5,7,4',5'-PentaOH-3'-OMe		C ₁₆ H ₁₄ O ₈	334	Hovenitin II	<i>Hovenia dulcis</i>	Rhamnaceae	Seed, fruit	235
342*	3,5,3',4'-TetraOH-7,8-diOMe		C ₁₇ H ₁₆ O ₈	348		<i>Erica cinerea</i>	Ericaceae	Flower	236
<i>Hepta-O-substituted</i>									
343*	3,5,3'-TriOH-8,5'-diOMe-6,7-methylenedioxy		C ₁₈ H ₁₆ O ₉	376	Plumbaginol	<i>Plumbago indica</i>	Plumbaginaceae	Aerial	237
<i>Esters</i>									
344 (2R,3S)	5,7-diOH-3-O-acetate		C ₁₇ H ₁₄ O ₆	314	<i>cis</i> -Pinobanksin 3-O-acetate	<i>Woodisia scopulina</i>	Dryopteridaceae, Pteridophyta	FronD	229
345	5,7,3'-TriOH-3-O-isobutyrate		C ₁₉ H ₁₈ O ₇	358		<i>Flourensia retinophylla</i>	Asteraceae	Aerial	238
346	5,7-DiOH-4'-OMe-3-O-acetate		C ₁₈ H ₁₆ O ₇	344		<i>Afromomum hanburyi</i>	Zingiberaceae		239
347 (2R,3S)	5,4'-DiOH-7-OMe-3-O-acetate		C ₁₈ H ₁₆ O ₇	344		<i>Inula graveolens</i>	Asteraceae	Aerial	232
348 (2R,3R)	5,2'-DiOH-7,8-diOMe-3-O-acetate		C ₁₉ H ₁₈ O ₈	374		<i>Notholaena sulphurea</i>	Pteridaceae, Pteridophyta	FronD	240

continued

TABLE 15.9
Dihydroflavonols (Flavanonols) Reported from 1992 to 2003 — continued

No.	O-Substitution	Other Substituents ^a	Formula	M _r	Trivial Name	Plant Source	Family	Organ ^b	Ref.
	<i>C-Methyl-substituted</i>								
349	3,5,7-TriOH-4'-OMe	6-Me	C ₁₇ H ₁₆ O ₆	316		<i>Amaranthus caudatus</i>	Amaranthaceae	Flower	57
350	2,3,5,7,4'-PentaOH	6-Me	C ₁₆ H ₁₄ O ₇	318		<i>Leptospermum polygalifolium</i> ssp. <i>polygalifolium</i>	Myrtaceae	Leaf	67
351	2,3,5,7,4'-PentaOH	8-Me	C ₁₆ H ₁₄ O ₇	318		<i>Leptospermum polygalifolium</i> ssp. <i>polygalifolium</i>	Myrtaceae	Leaf	67
352*	3,5,7,3',4'-PentaOH	6'-CH ₂ OH	C ₁₆ H ₁₄ O ₈	334		<i>Trifolium alexandrinum</i>	Leguminosae	Seed	241
	Prenyl-, geranyl-, and lavandulyl-substituted dihydroflavonols								
353*	3,5,7-TriOH-8-OMe	6-Pr	C ₂₁ H ₂₂ O ₆	370	Dioclenol	<i>Dioclea grandiflora</i>	Leguminosae	Root/B	242
354*	3,5,3',4'-TetraOH-7-OPr		C ₂₀ H ₂₀ O ₇	372		<i>Pterocaulon alopecuroides</i>	Asteraceae	Aerial	243
355*	3,5,7,4'-TetraOH-6-OMe	8-Pr	C ₂₁ H ₂₂ O ₇	386	Floranol	<i>Dioclea grandiflora</i>	Leguminosae	Root	244
356*	3,5,4'-TriOH-7,3'-diOMe	6-Pr	C ₂₂ H ₂₄ O ₇	400		<i>Rhynchosia densiflora</i>	Leguminosae	Leaf	245
357*	3,5,4'-TriOH-7,3'-diOMe	8-Pr	C ₂₃ H ₂₄ O ₇	400	Scariosin	<i>Paracalyx scariosa</i>	Leguminosae	Leaf	246
358	3,5,7,2,5'-PentaOH-6-OMe	8-Pr	C ₂₁ H ₂₂ O ₈	402	Paraibanol	<i>Dioclea grandiflora</i>	Leguminosae	Root/B	78
359	3,5,7,4'-TetraOH	6,8-diPr	C ₂₃ H ₂₈ O ₆	424	6,8-Diprenyl-aromadendrin	<i>Monotes africanus</i>	Dipterocarpaceae	Leaf	247
360*	3,5,4'-TriOH-7-O-Ger		C ₂₅ H ₂₈ O ₆	424		<i>Boronia caerulea</i> ssp. <i>spicata</i>	Rutaceae	Aerial	80
361	3,5,7,4'-TetraOH	8-Pr-3'-Ger	C ₃₀ H ₃₆ O ₆	492	Sanggenol C	<i>Morus cathayana</i>	Moraceae	Root/B	109
362*	3,5,7,2',4'-PentaOH	8-Lav	C ₂₅ H ₂₈ O ₇	440	Kushenol X	<i>Sophora flavescens</i>	Leguminosae	Root	76
363	3,5,7,3',4'-PentaOH	8,2',6'-triPr	C ₃₀ H ₃₆ O ₇	508	Petalostemumol	<i>Petalostemum purpureum</i>	Leguminosae	Whole	248
364	3,5,7,2',4'-PentaOH	6-Pr-3'-Ger	C ₃₀ H ₃₆ O ₇	508	Sanggenol K	<i>Morus cathayana</i>	Moraceae	Root/B	249
365	3,5,7,2',4'-PentaOH	3'-Ger-5'-Pr	C ₃₀ H ₃₆ O ₇	508	Sanggenol D	<i>Morus cathayana</i>	Moraceae	Root/B	109
366	3,5,7,2',4'-PentaOH	8,5'-diPr-3'-Ger	C ₃₃ H ₄₄ O ₇	576	Sanggenol E	<i>Morus cathayana</i>	Moraceae	Root/B	109
367	3,5,7,2',4'-PentaOH	6-(3-OH-3-Me-butyl)-8-Lav	C ₃₀ H ₃₆ O ₈	524	K osamol A	<i>Sophora flavescens</i>	Leguminosae	Root	250
368*	3,5,7,2',6'-PentaOH-4'-OMe	6-Ger-8-Pr	C ₃₁ H ₃₈ O ₈	538	K emusanone C	<i>Echinophora koreensis</i>	Leguminosae	Root	90
369	3-OMe	[2'',3'':7,8]Furanoflavanone	C ₁₈ H ₁₄ O ₄	294		<i>Lonchocarpus latifolius</i>	Leguminosae	Root	133
370*	3,5,6-TriOMe	[2'',3'':7,8]Furanoflavanone	C ₂₀ H ₁₈ O ₆	354		<i>Lonchocarpus subglaucescens</i>	Leguminosae	Root	134
	Dimethylpyrano-dihydroflavonols								
371	3-OH	Bis(6'',6''-DMPI[2'',3'':5,6] [2'',3'':7,8])	C ₂₅ H ₂₄ O ₅	404	MS II	<i>Mandaleia suberosa</i>	Leguminosae	Root	251
372*	3,4-DiOH	3'-Pr-6'',6''-DMPI[2'',3'':7,8]	C ₂₅ H ₂₆ O ₅	406	Kanzonol Z	<i>Glycyrrhiza glabra</i>	Leguminosae	Root	252
373	3,5,2'-TriOH	8-Pr-6'',6''-DMPI[2'',3'':7,6]	C ₂₅ H ₂₆ O ₆	422	Jayacanol	<i>Lonchocarpus oxacensis</i>	Leguminosae	Root	253

374	3,4'-DIOH-5-OMe	8-Pr-6''-6''-DMP[2',3'':7,6]	C ₂₀ H ₂₈ O ₆	436	Eriotriol	<i>Lonchocarpus atropurpureus</i>	Leguminosae	Root	254
375	5,2'-DIOH-3-OMe	8-Pr-6''-6''-DMP[2',3'':7,6]	C ₂₆ H ₂₈ O ₆	436		<i>Mundulea suberosa</i>	Leguminosae	StemB	255
376*	3,5,4'-TriOH-3'-OMe	6''-6''-DMP[2',3'':7,6]	C ₂₁ H ₂₀ O ₇	384		<i>Lonchocarpus atropurpureus</i>	Leguminosae	Root	254
						<i>Erythrina eriотricha</i>	Leguminosae	StemB	161
Isoprenylated C-3-(C-2-ether linked dihydroflavonols (Figure 15.14))									
377	3,5,7,4'-TetraOH	2,6-diPr	C ₂₅ H ₂₆ O ₇	438	Sangganol F	<i>Morus cathayana</i>	Moraceae	RootB	249
378	3,5,4'-TriOH	2,3'-diPr-6''-6''-DMP[2',3'':7,6]	C ₃₀ H ₃₂ O ₇	504	Sorocein D	<i>Sorocea bonplandii</i>	Moraceae	RootB	256
379	3,5,4'-TriOH	2,3'-diPr-6''-6''-DMP[2',3'':7,8]	C ₃₀ H ₃₂ O ₇	504	Sorocein E	<i>Sorocea ilicifolia</i>	Moraceae	RootB	257
380	3,5,7,4'-TetraOH	2,6,8-triPr	C ₃₀ H ₃₄ O ₇	506	Sorocein G	<i>Sorocea ilicifolia</i>	Moraceae	RootB	257
381	3,5,7,4'-TetraOH	2,6,3'-triPr	C ₃₀ H ₃₄ O ₇	506	Sorocein F	<i>Sorocea ilicifolia</i>	Moraceae	RootB	257
382	3,5,7,4'-TetraOH	2-Pr-8-Ger	C ₃₀ H ₃₄ O ₇	506	Sangganol I	<i>Morus cathayana</i>	Moraceae	RootB	249
383	3,5,7,4'-TetraOH	2-Pr-3'-Ger	C ₃₀ H ₃₄ O ₇	506	Sangganol G	<i>Morus cathayana</i>	Moraceae	RootB	249
384	3,5,7,4'-TetraOH	2-Farnesyl	C ₃₀ H ₃₄ O ₇	506	Sangganol H	<i>Morus cathayana</i>	Moraceae	RootB	249
C-Benzyl-substituted (Figure 15.15)									
385	3,5,7,4'-TetraOH	6-(4-OH-Benzyl)	C ₂₂ H ₁₈ O ₇	394	Gericudranin E	<i>Cudrania tricuspidata</i>	Moraceae	StemB	258
386	3,5,7,3',4'-PentaOH	6-(4-OH-Benzyl)	C ₂₂ H ₁₈ O ₈	410	Gericudranin B	<i>Cudrania tricuspidata</i>	Moraceae	StemB	259
387	3,5,7,3',4'-PentaOH	8-(4-OH-Benzyl)	C ₂₃ H ₁₈ O ₈	410	Gericudranin C	<i>Cudrania tricuspidata</i>	Moraceae	StemB	259
388	3,5,7,4'-TetraOH	6,8-Di-(4-OH-benzyl)	C ₂₉ H ₂₄ O ₈	500	Gericudranin D	<i>Cudrania tricuspidata</i>	Moraceae	StemB	258
389	3,5,7,3',4'-PentaOH	6,8-Di-(4-OH-benzyl)	C ₂₉ H ₂₄ O ₉	516	Gericudranin A	<i>Cudrania tricuspidata</i>	Moraceae	StemB	259
Miscellaneous substitutions (Figure 15.16)									
390	3,5,7,3',4'-PentaOH	6-(3-Oxobutyl)	C ₁₉ H ₁₈ O ₈	374		<i>Bauhinia purpurea</i>	Leguminosae	Heart-wood	260
391	3,5,7,4'-TetraOH	3'-(4-OH-Benzaldehyde)	C ₂₃ H ₁₆ O ₈	408	Hypnogenol F	<i>Hypnum cupressiforme</i>	Hypnaceae, Musci	Gam.	261
392	3,5,7,4'-TetraOH	3'-(4-OH-Benzoic acid)	C ₂₃ H ₁₆ O ₉	424	Hypnum acid	<i>Hypnum cupressiforme</i>	Hypnaceae, Musci	Gam.	262
393	3,5,7,4'-TetraOH	3'-(4-OH-Benzoic acid methyl ester)	C ₂₃ H ₁₈ O ₉	438	Hypnum acid methyl ester	<i>Hypnum cupressiforme</i>	Hypnaceae, Musci	Gam.	262
394	<i>rel</i> -5-Hydroxy-7,4'-dimethoxy-2''-S-(2,4,5-trimethoxy- <i>E</i> -styryl) tetrahydrofuro[4'' <i>R</i> ,5'' <i>R</i> :2,3] flavanonol		C ₃₀ H ₃₀ O ₁₀	550		<i>Alpinia flabellata</i>	Zingiberaceae	Leaf	263
395	<i>rel</i> -5-Hydroxy-7,4'-dimethoxy-3''-S-(2,4,5-trimethoxy- <i>E</i> -styryl) tetrahydrofuro[4'' <i>R</i> ,5'' <i>R</i> :2,3] flavanonol		C ₃₀ H ₃₀ O ₁₀	550		<i>Alpinia flabellata</i>	Zingiberaceae	Leaf	263
396	5,7,4'-TriOH-3- <i>O</i> -(1,8,14-trimethylhexadecanyl)		C ₃₄ H ₅₀ O ₆	554	Muscanone	<i>Commiphora wightii</i>	Burseraceae	Trunk	264

* Dihydroflavonols for which the (2*R*,3*R*)-configuration has been confirmed.

^aPr, prenyl; Ger, geranyl; Lav, lavandulyl; DMP, dimethylpyrano; DMDHP, dimethyldihydropyrano.

^bStemB, stem bark; RootB, root bark; Whole, whole plant; Gam, gametophyte.

Dihydroflavonols **332** and **336** from *Iris tenuifolia* (Iridaceae) show 2',3'-dioxygenation of the B-ring.¹⁸ Flavanones with this unusual B-ring substitution pattern have also been found in this species (see Section 15.2.1.1). An equally unusual 2',5'-dioxygenation pattern for the B-ring is present in flavanonol **335** from *Blumea balsamifera* (Asteraceae).²³⁴ A bioactive dihydroflavonol with 8-*O*-substitution of the A-ring, (2*R*,3*R*)-3,5,8-dihydroxy-7-methoxyflavanone (**330**), has been isolated from the leaves of *Muntingia calabura* (Elaeocarpaceae).²³⁰ This compound and chalcones and isoflavones from the same species were active in a quinone reductase induction assay with cultured Hepa lcl (mouse hepatoma cells).²³⁰ Flowers of the heather *Erica cinerea* also contain a novel 8-oxygenated dihydroflavonol, 3,5,3',4'-tetrahydroxy-7,8-dimethoxyflavanone (**342**).²³⁶ In plumbaginol (**343**) from *Plumbago indica* (Plumbaginaceae), all the carbons of the A-ring are *O*-substituted and the B-ring has the unusual 3',5'-dioxygenation pattern. The compound also bears a methylenedioxy substituent at C-6 and C-7.²³⁷

Two known dihydroflavonols, 3-hydroxy-5-methoxy-6,7-methylenedioxyflavanone and 3,5,7,4'-tetrahydroxy-3'-methoxyflavanone, are produced as phytoalexins by sugarbeet roots (*Beta vulgaris*, Chenopodiaceae) when inoculated with *Rhizoctonia solani*. They had not been previously reported for *B. vulgaris*.²⁶⁵

The 3-hydroxyl of dihydroflavonols frequently forms an ester with various acids and previously 18 dihydroflavonols-esters have been described, notably acetates.¹⁵ In the last decade or so, several new acetates have been added to this list (compounds **344–348**). (2*R*,3*R*)-3,5,2'-Trihydroxy-7,8-dimethoxyflavanone 3-*O*-acetate (**348**), which has an unusual oxygenation pattern in both the A- and B-rings, has been isolated from the frond of the fern *Notholaena sulphurea* (Pteridaceae),²⁴⁰ where it is present in the yellow farinose coating on the lower leaf surface. This acetylated dihydroflavonol is characteristic of the chemotype of *N. sulphurea* that has a yellow exudate. In contrast, the white form of this species mainly produces methylated dihydrochalcones.²⁴⁰ Isobutyric acid represents the acyl group in the dihydroflavonol **345** from *Flourensia retinophylla* (Asteraceae).²³⁸

15.3.1.2 Simple Dihydroflavonols with *O*- and *C*-Substitution

Three new *C*-methyl-substituted dihydroflavonols are listed in Table 15.9 (compounds **349–351**), together with one *C*-hydroxymethylflavanonol (**352**) from *Trifolium alexandrinum* (Leguminosae), in which the hydroxymethyl group is attached to C-6'.²⁴¹ The flowers of *Amaranthus caudatus* (Amaranthaceae) were the source of the 6-methyl-substituted dihydroflavonol **349**.⁵⁷ The 2-hydroxylated methylflavanonol, 2,3,5,7,4'-pentahydroxy-6-*C*-methylflavanone (**350**), has been isolated from *Leptospermum polygalifolium* ssp. *polygalifolium*, together with its 8-*C*-methyl regioisomer **351**.⁶⁷ Both *A. caudatus* and *L. polygalifolium* ssp. *polygalifolium* also contain related *C*-methylflavanones^{57,67} (see Section 15.2.1.2).

15.3.1.3 Dihydroflavonols with Noncyclic Isoprenoid Substituents

Table 15.9 also presents the newly reported dihydroflavonols with prenyl, geranyl, and lavandulyl side chains (compounds **353–368**), which have been mainly found in species of the family Leguminosae. For example, the root bark of *Dioclea grandiflora* has been the source of three new prenylated dihydroflavonols, dioclenol (**353**),²⁴² floranol (**355**),²⁴⁴ and paraibanol (**358**).⁷⁸ Paraibanol is the 8-prenyl derivative of the dihydroflavonol diosalol (3,5,7,2',5'-pentahydroxy-6-methoxyflavanone, **339**), which was isolated from the same plant.⁷⁸ Other newly reported prenylated dihydroflavonols from this family include the monoprenylated flavanones **356** from *Rhynchosia densiflora*²⁴⁵ and scariosin (**357**) from

Paracalyx scariosa.²⁴⁶ Lavandulyl groups are present in both kushenol X (**362**)⁷⁶ and kosamol A (**367**)²⁵⁰ from *Sophora flavescens*. Petalostemumol (**364**) from *Petalostemum purpureum* is 8,2',6'-triprenylated; its structure was determined by a single-crystal x-ray diffraction analysis.²⁴⁸ The compound showed good activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* ATCC 6633, with MIC values of 3.12 and 0.78 µg/ml, respectively. Activity against the Gram-negative bacterium *Escherichia coli* and against the yeast *Candida albicans* was only moderate in comparison (MIC 6.25 and 12.5 µg/ml, respectively).²⁴⁸

The family Moraceae is another good source of dihydroflavonols bearing noncyclic isoprenylated substituents. From the root bark of *Morus cathayana* four flavanonols with both prenyl and geranyl groups have been isolated, sanggenols C–E and K (**361**, **365**, **366**, and **364**).^{109,249} Only a few new prenylated dihydroflavonols have been reported from families other than the Leguminosae and Moraceae, e.g., the 7-*O*-geranyl derivative of aromadendrin (**360**) from *Boronia caeruleascens* ssp. *spicata* (Rutaceae).⁸⁰ From a species of Dipterocarpaceae, *Monotes africanus*, the new 6,8-diprenylaromadendrin (**359**) has been obtained together with a range of prenylated flavonoids belonging to other classes. These compounds were tested for HIV-inhibitory activity in the XTT-based whole cell screen²⁴⁷ and all 6,8-diprenylated flavonoids isolated, including the dihydroflavonol **359**, the flavanone 6,8-diprenylaringenin (lonchocarpol A), and the flavanonol 6,8-diprenylkaempferol, showed activity, with IC₅₀ values of 4.7, 2.7, and 5.8 µg/ml, respectively.²⁴⁷

The known dihydroflavonol kushenol I (3,7,2',4'-tetrahydroxy-5-methoxy-8-*C*-lavandulylflavanone) was isolated, together with the corresponding flavanone kurarinone, from the roots of *Gentiana macrophylla* (Gentianaceae) and shown to be active against the plant pathogenic fungus *Cladosporium cucumerinum*. Kurarinone also inhibited the growth of the human pathogenic yeast *Candida albicans*.¹¹⁸ It is interesting that a dihydroflavonol and flavanone with substitutions typical for root flavonoids in species of the Leguminosae (lavandulyl side chain and 2',4'-di-*O*-substitution of the B-ring) have been found in the roots of a totally unrelated species. The flavonoids in plant roots have not been studied nearly as well as those of leaves. Perhaps these unusually substituted flavonoids, which are often associated with antifungal activity and may protect the roots against attacks by fungi and parasites, are much more widespread in plant roots than are presently realized.

15.3.1.4 Dihydroflavonols with Furano or Pyrano Rings

Two new dihydroflavonols with furano rings and six with pyrano rings are listed in Table 15.9 (compounds **369**–**376**). They were all isolated from members of the Leguminosae. Both furanoflavanonols have been obtained from the genus *Lonchocarpus*, which is also a source of furanoflavanones. Dihydroflavonol **369** was found in the roots of *L. latifolius*¹³³ and **370** in *L. subglaucescens*.¹³⁴ In these compounds, the furano ring does not bear an isopropenyl side chain and no free hydroxyl groups are present, only methoxyl groups.

Two of the new dimethylpyranoflavanonols have also been found in the genus *Lonchocarpus*; jayacanol (**373**), in the roots of *L. oaxacensis*,²⁵³ and compound **375** in *L. atropurpureus*,²⁵⁴ which also contained jayacanol. These substances are characterized by 2'-hydroxylation of the B-ring. Jayacanol (**373**) and its known 2'-deoxy derivative, mundulinol, were tested for their activity against the wood-rotting fungus *Postia placenta*. Only the 2'-hydroxylated jayacanol was active.²⁵³ Two further dimethylpyranoflavanonols are constituents of *Mundulea suberosa*, MS II (**371**)²⁵¹ and compound **374**.²⁵⁵ The remaining two new dihydroflavonols in this class are kanzonol Z (**372**) from *Glycyrrhiza glabra*²⁵² and eriotrinol (**376**) from *Erythrina eriotricha*.¹⁶¹

15.3.1.5 Isoprenylated Dihydroflavonols with a C-3-C-2' Ether Linkage

In the 1980s, several unusual dihydroflavonoids were reported from the root bark of *Morus mongolica* (Moraceae), characterized by an ether linkage between C-3 and C-2' of the flavonoid, e.g., sanggenons A and M.²⁶⁶ They were thought to bear a prenyl group at C-3 and a hydroxyl group at C-2. More recently, similar compounds, soroceins D–G, were discovered in the root bark of species belonging to the related genus *Sorocea* (*S. bonplandii* and *S. ilicifolia*, Moraceae), but these were identified as bearing a prenyl group at C-2 and hydroxyl at C-3.^{256,257} This led to a structure revision of some of the sanggenons, so that they are now also described as 2-prenylated dihydroflavonols rather than 2-hydroxylated 3-prenylflavanones.²⁶⁷ Several related dihydroflavonols have recently been discovered in root bark extracts of *Morus cathayana* (sanggenols F–I).²⁴⁹ These soroceins and sanggenols are hydroxylated at C-3, C-5, C-7, and C-4' (see Table 15.9, compounds 377–384, and Figure 15.14). In sanggenol F (377), there is an additional prenyl group at C-8,²⁴⁹ whereas in soroceins F (381) and G (380) there are two additional prenyl groups, at C-6 and C-3' in 381 and C-6 and C-8 in 380.²⁵⁷ In soroceins D (378) and E (379), a dimethylpyrano group is attached to the A-ring and an additional prenyl group to C-3'.^{256,257} In sanggenol G (383), there is a geranyl substituent at C-3' in addition to the prenyl at C-2, whereas in sanggenol I (382), the geranyl group is attached to C-8. In sanggenol H (384), there is a 15-carbon farnesyl chain at C-2 instead of a prenyl group and the molecule bears no further isoprenoid substituents.²⁴⁹

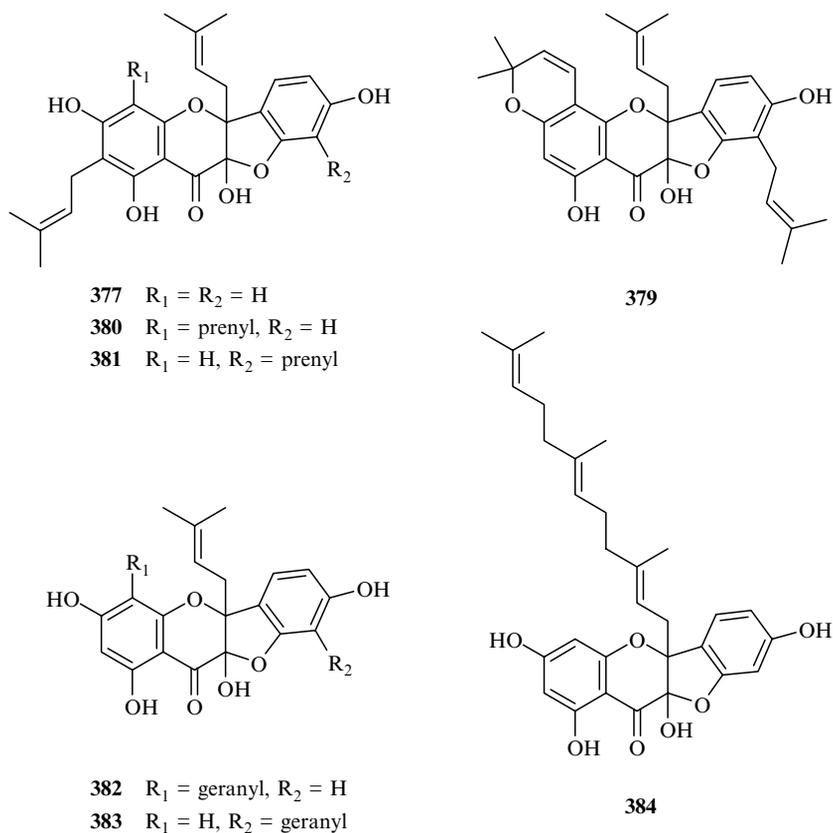


FIGURE 15.14 C-2-Isoprenylated C-3–C-2'-ether-linked dihydroflavonols.

15.3.1.6 Benzylated Dihydroflavonols

The first C-benzylated dihydroflavonols, gericudranins A (**389**), B (**386**), C (**387**), D (**388**), and E (**385**), have been discovered in stem bark extracts of *Cudrania tricuspidata* (Moraceae) (Table 15.9; Figure 15.15).^{258,259} Gericudranins A–C are based on taxifolin (5,7,3',4'-tetrahydroxydihydroflavonol), whereas gericudranins D and E are based on aromadendrin (5,7,4'-trihydroxydihydroflavonol), and they all contain a 4-hydroxybenzyl moiety that is attached to C-6 or C-8 (or both) of the flavanone. The antitumor activity of gericudranins A–C was tested, indicating cytotoxicity to the human tumor cell lines CRL 1579 (skin), LOX-IMVI (skin), MOLT-4F (leukemia), KM 12 (colon), and UO-31 (renal) in culture, with ED₅₀ values of 2.7 to 31.3 μg/ml.²⁵⁹

15.3.1.7 Dihydroflavonols with Miscellaneous Substituents

There are a few newly reported dihydroflavonols with unusual side groups or skeletons that cannot easily be arranged into any of the groups discussed so far and are therefore treated together in a “miscellaneous” group (Table 15.9). These include 6-(3-oxobutyl)taxifolin (**390**, Figure 15.16) from *Bauhinia purpurea* (Leguminosae), which bears an uncommon C–C-linked side chain.²⁶⁰ The gametophyte of the moss *Hypnum cupressiforme* (Hypnaceae) produces a range of dihydroflavonols with C–C-linked attachments, all at C-3'. In hypnogenol F (**391**, Figure 15.16), C-3' of aromadendrin (3,5,7,4'-tetrahydroxyflavanone) is linked to C-3 of 4-hydroxybenzaldehyde,²⁶¹ whereas in hypnum acid (**392**) and hypnum acid methyl ester (**393**), C-3' of aromadendrin is linked to C-3 of 4-hydroxybenzoic acid and its methyl ester, respectively.²⁶² This species also produces a wide variety of biflavonoids.^{261,262} From the rare Japanese species *Alpinia flabellata* (Zingiberaceae), two flavanol–phenylbutadiene adducts have been isolated, **394** and **395** (Figure 15.16). In each of these compounds, the substitution of the phenolic ring in the phenylbutanoid moiety is 2,4,5-trimethoxy, and the butenoid chain forms a tetrahydrofurano ring with the dihydroflavonol.²⁶³ An antifungal aromadendrin derivative, muscanone (**396**, Figure 15.16), was obtained from the trunk of *Commiphora wightii* (Burseraceae). This dihydroflavonol has a 1,8,14-trimethylhexadecanyl side chain ether-linked to the 3-hydroxyl of aromadendrin. The compound was active against *Candida albicans* at 250 μg/ml.²⁶⁴

15.3.2 DIHYDROFLAVONOL GLYCOSIDES

Relatively few dihydroflavonol glycosides have been reported during the period between 1992 and 2003; 16 are listed in Table 15.10 (compounds **397–412**) based on ten different aglycones,

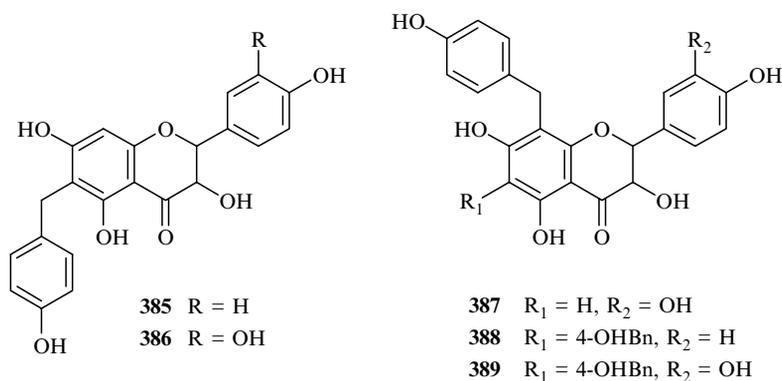


FIGURE 15.15 C-Benzyl-substituted dihydroflavonols (Bn, benzyl).

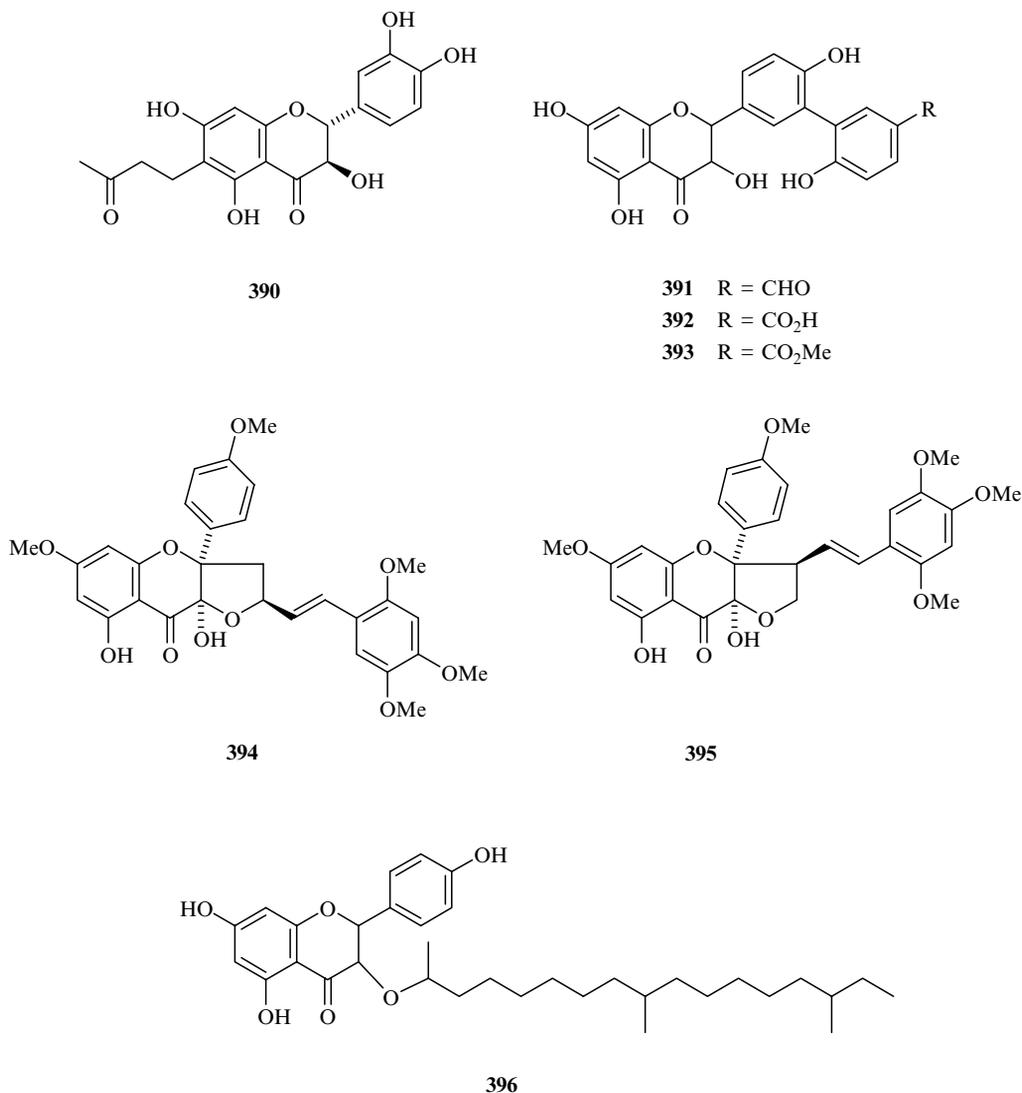


FIGURE 15.16 Dihydroflavonols with miscellaneous substituents.

including two that are prenylated.^{206,268–281} The conventions used in this table are the same as those described for the flavanone glycosides in Section 15.2.4. Rhamnose and glucose are the most frequent sugar moieties, appearing in seven and six of the dihydroflavonol glycosides, respectively. Most are monosides; only two biosides, aromadendrin 7-rhamnosyl(1 → 4)galactoside (**397**) from *Crotalaria laburnifolia* (Leguminosae)²⁶⁸ and taxifolin 7-rhamnosyl(1 → 6)glucoside (**406**) from *Platycodon grandiflorum* (Campanulaceae),²⁷⁵ are among the new compounds, and one diglycoside (taxifolin 3,7-dirhamnoside, **405**) from *Hypericum japonicum* (Clusiaceae = Guttiferae).²⁷⁴ Some of the new dihydroflavonol glycosides are stereoisomers, e.g., the 3-rhamnosides of (2*R*,3*R*)-, (2*R*,3*S*)-, and (2*S*,3*S*)-3,5,7,3',5'-pentahydroxyflavanone (**408**, **409**, and **410**, respectively), **408** from *Excoecaria agallocha* (Euphorbiaceae),²⁷⁷ and **409** (smitilbin) and **410** (neosmitilbin) from *Smilax glabra* (Smilacaceae).^{278,279}

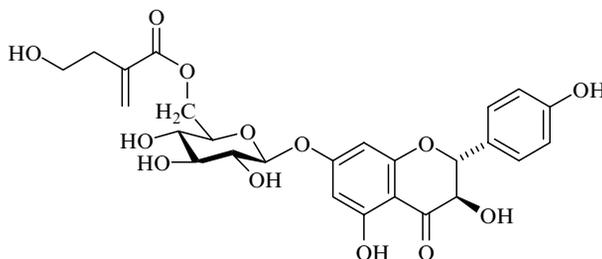
The new glycosides include arabinose in different forms and configurations, e.g., as β-D-arabinopyranose in dihydrorhamnetin 3-β-D-arabinopyranoside (**407**) from *Pyrola*

TABLE 15.10
Dihydroflavonol Glycosides Reported from 1992 to 2003

No.	Dihydroflavonol Glycoside ^a	Formula	M _r	Plant Source	Family	Organ ^b	Ref.
397	3,5,7,4'-TetraOH-Flavanone (<i>aromadendrin</i>)	C ₂₇ H ₃₂ O ₁₅	596	<i>Crotalaria laburnifolia</i>	Leguminosae	Seed	268
398 (2R,3R)	7-Rhamnosyl(1 → 4)galactoside	C ₂₆ H ₃₈ O ₁₃	548	<i>Azizia bella</i>	Leguminosae	StemB	269
399	3,5,7,8,4'-PentaOH-Flavanone	C ₂₃ H ₂₄ O ₁₃	508	<i>Calluna vulgaris</i>	Ericaceae	Flower	206
400	8-(2-Acetylglucoside) (2''-acetylcallunin)	C ₂₁ H ₂₂ O ₁₁	450	<i>Plinia pinnata</i>	Myrtaceae	Aerial	270
401 (2R,3R)	3,7,3',4'-PentaOH-Flavanone (<i>taxifolin</i>)	C ₂₀ H ₂₀ O ₁₁	436	<i>Rhododendron ferrugineum</i>	Ericaceae	Leaf, flower	271
402 (2R,3S)	3-α-Arabinopyranoside	C ₂₀ H ₂₀ O ₁₁	436	<i>Rhododendron ferrugineum</i>	Ericaceae	Leaf, flower	271
403	3-α-L-Arabinofuranoside	C ₃₀ H ₂₀ O ₁₁	436	<i>Fragaria × ananassa</i>	Rosaceae	Root	272
404	3-(3-Cinnamoylrhamnoside)	C ₃₀ H ₂₈ O ₁₂	580	<i>Andira inermis</i>	Leguminosae	Leaf	273
405	3,7-Dirhamnoside	C ₂₇ H ₃₂ O ₁₅	596	<i>Hypericum japonicum</i>	Clusiaceae	Aerial	274
406 (2R,3R)	7-Rhamnosyl(1 → 6)glucoside (flavoplatycoside)	C ₂₇ H ₃₂ O ₁₆	612	<i>Platycodon grandiflorum</i>	Campanulaceae	Seed	275
407	3,5,3',4'-Tetra-7-OMe-Flavanone (<i>padmatin</i> , <i>dihydroorhammetin</i>)	C ₂₁ H ₂₂ O ₁₁	450	<i>Pyrola elliptica</i>	Pyrolaceae	Whole	276
408 (2R,3R)	3-β-D-Arabinopyranoside	C ₂₁ H ₂₂ O ₁₁	450	<i>Excoecaria agallocha</i>	Euphorbiaceae	Stem	277
409 (2R,3S)	3-Rhamnoside (smitilbin)	C ₂₁ H ₂₂ O ₁₁	450	<i>Smitax glabra</i>	Smilacaceae	Rhiz	278
410 (2S,3S)	3-Rhamnoside (neosmitilbin)	C ₂₁ H ₂₂ O ₁₁	450	<i>Smitax glabra</i>	Smilacaceae	Rhiz	279
411	3,5,7,4'-TetraOH-3'-Pr-Flavanone	C ₂₆ H ₃₀ O ₁₁	518	<i>Phellodendron chinense</i>	Rutaceae	Leaf	280
412	7-Glucoside (phellochimim)	C ₂₆ H ₃₀ O ₁₂	534	<i>Ochna integerrima</i>	Ochnaceae	Leaf	281
	3,5,7,3',4'-PentaOH-6-Pr-Flavanone (<i>6-prenyltaxifolin</i>)						
	7-Glucoside						

^aAll sugars are *O*-substituted unless specified as *C*-glycosides; rhamnoside, α-L-rhamnopyranoside; glucoside, β-D-glucopyranoside; arabinose as is specified. Pr, prenyl.

^bStemB, stem bark; Aerial, aerial parts; Whole, whole plant; Rhiz, rhizome.



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FIGURE 15.17 Acylated dihydroflavonol glycoside.

elliptica (Pyrolaceae),²⁷⁶ and as α -L-arabinofuranose in taxifolin 3- α -L-arabinofuranoside (**403**) from strawberry roots (Rosaceae).²⁷² Furthermore, the 3- α -arabinopyranosides of the epimers (2*R*,3*R*)- and (2*R*,3*S*)-taxifolin (**401** and **402**) have been reported from leaves and flowers of *Rhododendron ferrugineum* (Ericaceae).²⁷¹ The Ericaceae are a well-known source of dihydroflavonols, and the new acylated 2''-acetylcallunin (**399**) has been reported from the flowers of the common heather, *Calluna vulgaris*.²⁰⁶ In this compound, the sugar moiety is attached to the 8-hydroxyl of the dihydroflavanol; in all other new glycosides it is attached to the 3- or 7-hydroxyl. Two more new acylated dihydroflavonols have been described, both from legumes, (2*R*,3*R*)-aromadendrin 7-(6-[4-hydroxy-2-methylenebutanoyl]glucoside) (**398**, Figure 15.17) from the stem bark of *Afzelia bella*²⁶⁹ and taxifolin 3-(3-cinnamoylramnoside) (**404**, Figure 15.17) from the leaves of *Andira inermis*.²⁷³ The latter compound exhibited antiplasmodial activity with an IC₅₀ value of 10.4 μ g/ml against the *Plasmodium falciparum* chloroquinone-sensitive strain PoW, and an IC₅₀ of 4.2 μ g/ml against the resistant strain Dd2.²⁷³

Two new prenylated dihydroflavonol glycosides are listed in Table 15.10, 6-prenyltaxifolin 7-glucoside (**412**) from *Ochna integerrima* (Ochnaceae)²⁸¹ and phellochinin (**411**) from *Phellodendron chinense* (Rutaceae),²⁸⁰ which is the 7-glucoside of 3'-prenylaromadendrin. The presence of prenylated dihydroflavonol glycosides appears to be a chemosystematic marker for *Phellodendron*, since previously four related compounds have been isolated from four different species of this genus, *P. amurense*, *P. japonicum*, *P. lavalleyi*, and *P. sachalinense*.¹⁵

15.4 ACKNOWLEDGMENTS

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APPENDIXChecklist of Known Flavanones and Dihydroflavonols^a**FLAVANONES****Flavanone aglycones bearing hydroxy, methoxy, and methylenedioxy substituents only**Mono-*O*-substituted

1. 7-OH (H2/1011)
2. 7-OMe (H2/1012)

Di-*O*-substituted

3. 5,7-DiOH (pinocembrin, H2/1022)
4. 7,4'-DiOH (liquiritigenin, H2/1082)
5. 5-OH-7-OMe (pinostrobin, H2/1031)
6. 7-OH-5-OMe (alpinetin, H2/1030)
7. 7-OH-8-OMe (isolarrein, H2/1013)
8. 7-OH-4'-OMe (H2/1342)
9. 4'-OH-7-OMe (H2/1090)
10. 5,7-DiOMe (H2/1032)
11. 7,8-DiOMe (H2/1015)

Tri-*O*-substituted

12. 2,5,7-TriOH (H2/1319)
13. 5,6,7-TriOH (dihydrobaicalein, H2/1033)
14. 5,7,8-TriOH (dihydronorwogonin, H2/1038)
15. 5,7,2'-TriOH (H2/1392)
16. 5,7,3'-TriOH (H2/1393)
17. 5,7,4'-TriOH (naringenin, H2/1098)
18. 5,8,4'-TriOH (H2/1304)
19. 7,8,4'-TriOH (H2/1081)
20. 7,3',4'-TriOH (butin, H2/1092)
21. 5,6-DiOH-4'-OMe (H2/1303)
22. 5,7-DiOH-6-OMe (dihydrooroxylin, H2/1035)
23. 5,7-DiOH-8-OMe (dihydrowogonin, H2/1043)
24. 5,7-DiOH-4'-OMe (isosakuranetin, H2/1119)
25. 5,8-DiOH-7-OMe (H2/1357)
26. 5,2'-DiOH-7-OMe (dihydroechioidin, **1**)
27. 5,4'-DiOH-7-OMe (sakuranetin, H2/1117)

^aThis checklist of flavanones and dihydroflavonols contains compounds of these flavonoid classes reported in the literature as natural products to the end of 2003. Compounds recorded before 1992 are cross-referenced to numbered entries in volume 2 of the *Handbook of Natural Flavonoids*, using the prefix "H2."¹⁵ Compounds reported from 1992 to 2003 are shown by numbers in bold typeface, corresponding to the entries in Table 15.1 to Table 15.10. The compounds are arranged into flavanone aglycones, flavanone glycosides, dihydroflavonol aglycones, and dihydroflavonol glycosides, and within these classes as (i) derivatives with hydroxy, methoxy, and methylenedioxy substituents; (ii) derivatives with methyl, hydroxymethyl, and formyl substituents; (iii) derivatives with prenyl, geranyl, and lavandulyl substituents; (iv) derivatives with isoprenyl ring structures; (v) benzylated derivatives; (vi) miscellaneous structures. Within these groups the compounds are generally arranged according to the number of *O*-substituents on the flavonoid skeleton and compounds with the same number of *O*-substituents are grouped numerically according to the numbering of the A- and B-rings. The unprimed numbers (A-ring) precede the primed numbers (B-ring), and fully hydroxylated compounds come before those with methoxy groups. Only the (2*R*)-configuration of flavanones and not the more common (2*S*)-configuration has been indicated in the checklist. Similarly, the common (2*R*,3*R*)-configuration has generally not been given in the checklist for dihydroflavonols, and only in cases where several different configurations of the same dihydroflavonol have been found is this indicated in the table. Sugars are assumed to be *O*-linked unless specified as *C*-linked.

28. 7,4'-DiOH-5-OMe (H2/1126)
29. 5-OH-6,7-diOMe (onysilin, H2/1036)
30. 5-OH-7,8-diOMe (H2/1041)
31. 5-OH-7,4'-diOMe (H2/1127)
32. 6-OH-5,7-diOMe (H2/1037)
33. 7-OH-5,4'-diOMe (tsugafolin, H2/1349)
34. 8-OH-5,7-diOMe (H2/1042)
35. 3'-OH-7,4'-diOMe (tithonin, H2/1093)
36. 4'-OH-5,7-diOMe (H2/1128)
37. 5,7,8-TriOMe (H2/1045)
38. 5,7,4'-TriOMe (H2/1131)
39. 6,3',4'-TriOMe (H2/1178)

Tetra-*O*-substituted

40. 5,6,7,4'-TetraOH (carthamidin, H2/1132)
41. 5,7,8,4'-TetraOH (isocarthamidin, H2/1134)
42. 5,7,2',4'-TetraOH (steppogenin, H2/1277)
43. 5,7,2',5'-TetraOH (H2/1264)
44. 5,7,2',6'-TetraOH (H2/1248)
45. 5,7,3',4'-TetraOH (eriodictyol, H2/1193)
46. 6,7,3',4'-TetraOH (plathymenin, H2/1184)
47. 7,8,3',4'-TetraOH (isookanin, H2/1185)
48. 5,7,8-TriOH-4'-OMe (**3**)
49. 5,7,3'-TriOH-4'-OMe (hesperetin, H2/1203)
50. 5,7,3'-TriOH-5'-OMe (alyssifolinone, **4**)
51. 5,7,4'-TriOH-6-OMe (H2/1135)
52. 5,7,4'-TriOH-8-OMe (H2/1358)
53. 5,7,4'-TriOH-3'-OMe (homoeriodictyol, H2/1202)
54. 5,8,2'-TriOH-7-OMe (H2/1359)
55. 5,2',3'-TriOH-7-OMe (**5**)
56. 5,2',4'-TriOH-7-OMe (artocarpanone, H2/1272)
57. 5,2',6'-TriOH-7-OMe (scutamoenin, H2/1324)
58. 5,3',4'-TriOH-7-OMe (sternbin, H2/1214)
59. 6,7,8-TriOH-5-OMe (oresbusin, **6**)
60. 7,2',6'-TriOH-5-OMe (H2/1249)
61. 7,3',4'-TriOH-8-OMe (8-methoxybutin, H2/1188)
62. 2,5-DiOH-6,7-diOMe (mosloflavanone, **7**)
63. 5,7-DiOH-8,2'-diOMe (H2/1162)
64. 5,7-DiOH-8,4'-diOMe (**8**)
65. 5,8-DiOH-6,7-diOMe (didymocarpin A, H2/1046)
66. 5,2'-DiOH-7,8-diOMe (dihydroskullcap flavone I, **9**)
67. 5,2'-DiOH-7,4'-diOMe (**10**)
68. 5,2'-DiOH-7,5'-diOMe (**11**)
69. 5,3'-DiOH-7,2'-diOMe (**12**)
70. 5,3'-DiOH-7,4'-diOMe (persicogenin, H2/1210)
71. 5,4'-DiOH-6,7-diOMe (H2/1155)
72. 5,4'-DiOH-7,8-diOMe (H2/1157)
73. 5,4'-DiOH-7,3'-diOMe (H2/1209)
74. 7,8-DiOH-6,4'-diOMe (**13**)
75. 7,4'-DiOH-8,3'-diOMe (**14**)
76. 5-OH-6,7,8-triOMe (H2/1048)

77. 5-OH-6,7,4'-triOMe (H2/1156)
 78. 5-OH-7,8,4'-triOMe (H2/1158)
 79. 5-OH-7,2',3'-triOMe (**16**)
 80. 5-OH-7,3',4'-triOMe (H2/1259)
 81. 6-OH-5,7,8-triOMe (isopedicin, H2/1047)
 82. 7-OH-5,8,2'-triOMe (H2/1163)
 83. 7-OH-5,2',4'-triOMe (cerasinone, H2/1265)
 84. 8-OH-5,6,7-triOMe (kwang sienin A, **17**; H2/1400)
 85. 4'-OH-5,6,7-triOMe (H2/1180)
 86. 5,6,7,8-TetraOMe (kanakugin, H2/1049)
 87. 5,6,7,4'-TetraOMe (H2/1136)
 88. 5,7,2',4'-TetraOMe (arjunone, H2/1266)
 89. 5,7,2',5'-TetraOMe (**18**)
- Penta-*O*-substituted
90. 5,7,3',4',5'-PentaOH (H2/1252)
 91. 5,7,8,3'-TetraOH-4'-OMe (**20**)
 92. 5,7,2',5'-TetraOH-6-OMe (H2/1247)
 93. 5,7,3',4'-TetraOH-6-OMe (H2/1327)
 94. 5,7,3',4'-TetraOH-8-OMe (H2/1360)
 95. 5,7,4'-TriOH-8,3'-diOMe (H2/1216)
 96. 5,2',5'-TriOH-6,7-diOMe (dioclein, **21**)
 97. 5,2',5'-TriOH-7,8-diOMe (H2/1323)
 98. 5,3',4'-TriOH-7,5'-diOMe (H2/1254)
 99. 5,6-DiOH-7,8,4'-triOMe (H2/1159)
 100. 5,2'-DiOH-6,7,6'-triOMe (H2/1250)
 101. 5,2'-DiOH-7,8,6'-triOMe (H2/1251)
 102. 5,2'-DiOH-7,4',5'-triOMe (**22**)
 103. 5,3'-DiOH-6,7,4'-triOMe (H2/1217)
 104. 5,3'-DiOH-7,8,4'-triOMe (H2/1218)
 105. 5,4'-DiOH-6,7,8-triOMe (H2/1160)
 106. 6,4'-DiOH-5,7,3'-triOMe (agestricin D, H2/1219)
 107. 5-OH-6,7,8,4'-tetraOMe (H2/1179)
 108. 5-OH-6,7,3',4'-tetraOMe (H2/1328)
 109. 5-OH-7,2',4',6'-tetraOMe (heteroflavanone A, **23**; H2/1448)
 110. 5-OH-7,3',4',5'-tetraOMe (H2/1270)
 111. 6-OH-5,7,3',4'-tetraOMe (agestricin C, H2/1220)
 112. 4'-OH-5,6,7,3'-tetraOMe (agecorynin A, **24**)
 113. 5,6,7,8,4'-PentaOMe (**25**)
 114. 5,6,7,3',4'-PentaOMe (**26**)
 115. 5,7,2',3',4'-PentaOMe (**27**)
- Hexa-*O*-substituted
116. 5,7,3'-TriOH-6,4',5'-triOMe (**29**)
 117. 5,2'-DiOH-6,7,8,6'-tetraOMe (**30**; H2/1403)
 118. 5,3'-DiOH-6,7,4',5'-tetraOMe (**31**)
 119. 5,6,7,3',4',5'-HexaOMe (**32**)
- Hepta-*O*-substituted
120. 5,6,7,8,3',4',5'-HeptaOMe (H2/1329)
 121. 5,6,7,2',3',4',5'-HeptaOMe (H2/1258)
- Methylene dioxy-substituted
122. 5,2'-DiOH-6,7-methylenedioxy (**2**)

123. 5,7-DiOMe-3',4'-methylenedioxy (**15**)
124. 5,2'-DiOMe-6,7-methylenedioxy (betagarin, H2/1301)
125. 5,4'-DiOMe-6,7-methylenedioxy (H2/1161)
126. 6,7-DiOMe-3',4'-methylenedioxy (H2/1310)
127. 7,2'-DiOMe-4',5'-methylenedioxy (H2/1307)
128. 5,2',3'-TriOH-6,7-methylenedioxy (**19**)
129. 6-OH-5,7-diOMe-3',4'-methylenedioxy (agestricin B, H2/1221)
130. 5,7-DiOH-6,3'-diOMe-4',5'-methylenedioxy (agamanone, **28**; H2/1404)
131. 5,6,7,3'-TetraOMe-4',5'-methylenedioxy (agecorynin A, H2/1256)
132. 5,6,7,8,3'-PentaOH-4',5'-methylenedioxy (agecorynin B, H2/1257)

Esters

133. 5,7-DiOH-7-*O*-benzoate (**33**; H2/1394)
134. 5,8-DiOH-7-OMe-8-*O*-acetate (H2/1040)
135. 5,7,4'-TriOH-3'-OMe-4'-*O*-isobutyrate (**34**)

Flavanone aglycones with C-methyl, C-hydroxymethyl, and C-formyl substituentsDi-*O*-substituted

136. 5,7-DiOH-6-Me (strobopinin, H2/1050)
137. 5,7-DiOH-8-Me (cryptostrobin, H2/1054)
138. 5-OH-7-OMe-6-Me (H2/1053)
139. 5-OH-7-OMe-8-Me (H2/1055)
140. 7-OH-5-OMe-6-Me (comptonin, H2/1052)
141. 7,4'-DiOMe-6-Me (H2/1091)
142. 5,7-DiOMe-6-Me (H2/1332)
143. 5,7-DiOH-6,8-diMe (desmethoxymatteucinol, H2/1056)
144. 5-OH-7-OMe-6,8-diMe (H2/1057)
145. 7-OH-5-OMe-6,8-diMe (H2/1069)

Tri-*O*-substituted

146. 5,7,4'-TriOH-3-Me (3-methylnaringenin, H2/1320)
147. 5,7,4'-TriOH-3-Me-8-Cl (H2/1321)
148. 5,7,4'-TriOH-6-Me (poriol, H2/1139)
149. 2,5-DiOH-7-OMe-6-Me (**36**)
150. 2,5-DiOH-7-OMe-8-Me (**37**)
151. 5,7-DiOH-4'-OMe-8-Me (**38**)
152. 5,4'-DiOH-7-OMe-6-Me (**39**)
153. 5,4'-DiOH-7-OMe-8-Me (**40**)
154. 5,7-DiOH-6-formyl-8-Me (lawinal, H2/1058)
155. 5-OH-7-OMe-6-formyl-8-Me (leridal, **46**; H2/1395)
156. 7-OH-5-OMe-6-Me-8-formyl (desmosflavanone II, **47**)
157. 5-OH-7-OMe-6-hydroxyMe-8-Me (leridol, **48**; H2/1396)
158. 5,7-DiOMe-6-hydroxyMe-8-Me (**49**; H2/1397)
159. 5,7,4'-TriOH-6,8-diMe (farrerol, H2/1141)
160. 5,7-DiOH-3'-OMe-6,8-diMe (isomatteucinol, **44**)
161. 5,7-DiOH-4'-OMe-6,8-diMe (matteucinol, H2/1145)
162. 5,4'-DiOH-7-OMe-6,8-diMe (angophorol, H2/1144)

Tetra-*O*-substituted

163. 5,7,3',5'-TetraOH-6-Me (**41**)
164. 5-OH-7,3',4'-triOMe-6-Me (**42**; H2/1402)
165. 3'-OH-5,7,4'-triOMe-8-Me (H2/1224)
166. 5,7,2',4'-TetraOMe-8-Me (**43**)
167. 5,7,3',4'-TetraOH-6,8-diMe (cyrtominetin, H2/1262)

168. 5,7,2'-TriOH-4'-OMe-6,8-diMe (**45**)
 169. 5,7,4'-TriOH-3'-OMe-6,8-diMe (H2/1225)
 Penta-*O*-substituted
 170. 5,7,3'-TriOH-4',5'-diOMe-6,8-diMe (H2/1255)

Flavanone aglycones bearing noncyclic isoprenyl substituents^a

Mono-*O*-substituted

171. 7-OH-8-Pr (ovaliflavanone B, H2/1021)
 172. 7-OH-6,8-diPr (ovaliflavanone A, H2/1020)
 173. 6-OMe-5-(1,1-diMe-2-OH-prop-2-enyl) (falciformin, H2/1014)
 174. 7-OMe-8-Pr (isoderricin A, H2/1305)
 175. 7-OPr (isoderricidin, H2/1016)
 176. 7-OPr-8-(3-OH-3-Me-*trans*-buten-1-yl) (H2/1074)

Di-*O*-substituted

177. 5,7-DiOH-6-Me-8-Pr (**50**)
 178. 5,7-DiOH-6-Pr (H2/1059)
 179. 5,7-DiOH-6,8-diPr (H2/1077)
 180. 5,7-DiOH-8-Ger (H2/1065)
 181. 5,7-DiOH-8-Me-6-Pr (H2/1076)
 182. 5,7-DiOH-8-Pr (glabranin, H2/1060)
 183. 5,7-DiOH-8-(γ -Me- γ -formylallyl) (H2/1064)
 184. 5,7-DiOH-8-(4-OH-3-Me-2-butenyl) (H2/1063)
 185. 7,4'-DiOH-6-Pr (bavachin, H2/1150)
 186. 7,4'-DiOH-8-Pr (isobavachin, H2/1088)
 187. 7,4'-DiOH-3'-Pr (abyssinone II, H2/1094)
 188. 7,4'-DiOH-6,8-diPr (H2/1087)
 189. 7,4'-DiOH-8,3'-diPr (glabrol, H2/1372)
 190. 7,4'-DiOH-8-Ger (prostratol F, **96**)
 191. 7,4'-DiOH-3',5'-diPr (abyssinone IV, H2/1095)
 192. 7-4'-DiOH-8,3', 5'-tripr((-)-sophoranone (H2/1089)
 193. 5-OH-7-OMe-6-Pr (H2/1061)
 194. 5-OH-7-OMe-8-Pr (tephrinone, H2/1066)
 195. 5-OH-7-OMe-8-(3-OH-3-Me-*trans*-but-1-enyl) (tephroleocarpin A, H2/1440)
 196. 5-OH-7-OMe-8-(3-OH-3-Me-butyl) (tephrowatsin C, H2/1075)
 197. 5-OH-7-O-(3-Me-2,3-epoxybutoxy) (H2/1051)
 198. 5-OH-7-OPr (7-*O*-prenylpinocembrin, H2/1078)
 199. 5-OH-7-OPr-8-Me (7-*O*-prenylcryptostrobin, H2/1079)
 200. 5-OH-7-O-neryl (**76**)
 201. 5-OH-7-OPr-8-Pr (H2/1071)
 202. 4'-OH-7-OMe-6-Pr (bavachinin, H2/1151)
 203. 4'-OH-7-OMe-8-Pr (mundulea flavanone A, **51**)
 204. 5-OMe-7-OPr (H2/1072)
 205. 5-OMe-7-OPr-8-Pr (H2/1073)
 206. 5,7-DiOMe-8-Pr (candidone, H2/1296)
 207. 5,7-DiOMe-8-(2,3-epoxy-3-Me-butyl) (epoxycandidone, H2/1368)
 208. 5,7-DiOMe-8-(3-OH-3-Me-buten-1-yl) (quercetol C, H2/1335)

Tri-*O*-substituted

209. 5,7,2'-TriOH-8-Pr (kushenol S, **52**)
 210. 5,7,2'-TriOH-8-Lav (kushenol A, H2/1173)
 211. 5,7,2'-TriOH-8-(5-OH-2-isopropenyl-5-Me-hexyl) (kushenol T, **128**)

212. 5,7,4'-TriOH-6-(1,1-DMA) (**89**)
213. 5,7,4'-TriOH-6-Pr (H2/1147)
214. 5,7,4'-TriOH-6-Pr-8-Me (**54**)
215. 5,7,4'-TriOH-6-Me-8-Pr (**53**)
216. 5,7,4'-TriOH-6-Ger (bonannione A, H2/1176)
217. 5,7,4'-TriOH-6-Pr-8-(2,3-diOH-3-Me-butyl) (lonchocarpol B, H2/1337)
218. 5,7,4'-TriOH-6,8-diPr (lonchocarpol A, H2/1336)
219. 5,7,4'-TriOH-6,3'-diPr (paratocarpin L; macaranga flavanone B, **77**)
220. 5,7,4'-TriOH-6,8,3'-triPr (amorilin, H2/1172)
221. 5,7,4'-TriOH-8-(1,1-DMA) (ugonin E, **90**)
222. 5,7,4'-TriOH-8-Pr (sophoraflavanone B, H2/1148)
223. 5,7,4'-TriOH-8-Ger (sophoraflavanone A, H2/1177)
224. 5,7,4'-TriOH-8-Lav (leachianone E, **105**; H2/1435)
225. 5,7,4'-TriOH-8-(2-isopropyl-5-Me-5-hexenyl) (remangiflavanone A, **106**)
226. 5,7,4'-TriOH-8-(2-OH-3-Me-but-3-enyl) (tomentosanol D, **122**)
227. 5,7,4'-TriOH-8-Pr-6-(2-OH-3-Me-but-3-enyl) (lupiniols A1 and A2, **126**; H2/1424)
228. 5,7,4'-TriOH-8-Pr-3'-(2-OH-3-Me-but-3-enyl) (lupiniol B, **127**; H2/1425)
229. 5,7,4'-TriOH-8-Pr-3'-(3-Me-2,3-epoxybutenyl) (flemiflavanone D, H2/1175)
230. 5,7,4'-TriOH-8,3'-diPr (euchrestaflavanone A, H2/1169)
231. 5,7,4'-TriOH-8,3',5'-triPr (hydroxysophoranone, H2/1171)
232. 5,7,4'-TriOH-3'-Pr (licoflavanone H2/1364)
233. 5,7,4'-TriOH-3'-Ger (macaranga flavanone A, **97**)
234. 5,7,4'-TriOH-3',5'-diPr (abyssinone V, H2/1375)
235. 7,2',4'-TriOH-8-Pr (euchrenone a₇, H2/1365)
236. 7,2',4'-TriOH-8-Lav (lehmannin, H2/1312)
237. 5,7-DiOH-6-OMe-8-Pr (agrandol, **55**)
238. 5,7-DiOH-8-OMe-6-Pr (microfolione, **56**)
239. 5,7-DiOH-4'-OPr (selinone, H2/1137)
240. 5,7-DiOH-4'-OGer (**98**; H2/1406)
241. 5,7-DiOH-4'-OMe-8-Pr (H2/1366)
242. 5,7-DiOH-4'-OMe-8-(2-OH-3-Me-3-butenyl) (**123**; H2/1420)
243. 5,7-DiOH-4'-OMe-8-Pr-3'-(3-OH-3-Me-butyl) (**130**; H2/1421)
244. 5,7-DiOH-4'-OMe-8,3'-diPr (H2/1170)
245. 5,7-DiOH-4'-OMe-6-Me-8,3'-diPr (lespedezaflavanone G, **80**; H2/1409)
246. 5,7-DiOH-4'-OMe-8-Me-6,3'-diPr (lespedezaflavanone F, **81**; H2/1408)
247. 5,7-DiOH-4'-OMe-3'-(3-Me-but-1,3-dienyl)-5'-Pr (burttinonedehydrate, **78**)
248. 5,7-DiOH-4'-OMe-3'-Pr-5'-(3-OH-3-Me-buten-1-yl) (burttinone, **114**)
249. 5,7-DiOH-4'-OMe-3',5'-diPr (**79**)
250. 5,4'-DiOH-7-OMe-6-Pr (H2/1166)
251. 5,4'-DiOH-7-OMe-8-Pr (mundulea flavanone B, **57**)
252. 5,4'-DiOH-7-OPr (H2/1168)
253. 7,2'-DiOH-5-OMe-8-Lav (kushenol R, **107**)
254. 7,2'-DiOH-4'-OMe-8,5'-diPr (euchrenone a₈, H2/1376)
255. 7,4'-DiOH-5-OMe-8-Pr (isoxanthohumol, H2/1149)
256. 7,4'-DiOH-5-OMe-8-Lav (kushenol U, **108**)
257. (2*R*)-7,4'-DiOH-5-OMe-8-[4-OH-3-Me-(2*Z*)-butenyl] (**124**)
258. (2*R*)-7,4'-DiOH-5-OMe-8-[5-OH-3-Me-(2*E*)-butenyl] (**125**)
259. 7,4'-DiOH-2'-OMe-8-Lav (alopecurone G, **109**)
260. 5-OH-7-OMe-4'-OPr (**58**; H2/1407)
261. 5-OH-7,4'-diOMe-8-Pr (flowerine, **59**)

262. 5-OH-8-OMe-7-OPr (H2/1361)
263. 7-OH-3',4'-methylenedioxy-8-Pr (ovaliflavanone C, H2/1189)
264. 7-OH-3',4'-methylenedioxy-6,8-diPr (ovaliflavanone D, H2/1190)
265. 7-OMe-3',4'-methylenedioxy-8-Pr (H2/1306)
Tetra-*O*-substituted
266. 5,7,8,4'-TetraOH-3'-(3-methylbut-3-enyl) (flowerone, **60**)
267. 5,7,2',4'-TetraOH-6-Lav (kushenol F, H2/1244)
268. 5,7,2',4'-TetraOH-6,8-diPr (kushenol E, H2/1380)
269. 5,7,2',4'-TetraOH-6-Pr-8-Lav (kushenol B, H2/1246)
270. 5,7,2',4'-TetraOH-6-Ger-8-Pr (**101**)
271. 5,7,2',4'-TetraOH-6-Pr-5'-(1,1-DMA) (**93**; H2/1458)
272. 5,7,2',4'-TetraOH-6,8,3'-triPr (lespedezaflavanone E, H2/1391)
273. 5,7,2',4'-TetraOH-6,8,5'-triPr (amorisin, H2/1233)
274. 5,7,2',4'-TetraOH-8-Pr (leachianone G, **61**)
275. 5,7,2',4'-TetraOH-8-Ger (sophoraflavanone C, **99**; H2/1412)
276. 5,7,2',4'-TetraOH-8-Lav (sophoraflavanone G, H2/1274)
277. 5,7,2',4'-TetraOH-8-(2-isopropyl-5-Me-5-hexenyl) (remangiflavanone B, **110**)
278. 5,7,2',4'-TetraOH-8-(2-(2-OH-isopropyl)-5-Me-4-hexenyl) (kushenol Q, **136**)
279. 5,7,2',4'-TetraOH-8,5'-diPr (lespedezaflavanone D, H2/1381)
280. 5,7,2',4'-TetraOH-8-Pr-5'-(1,1-DMA) (**94**)
281. 5,7,2',4'-TetraOH-3'-Ger (sanggenol A, **100**)
282. 5,7,2',4'-TetraOH-5'-Ger (kuwanon E, H2/1238)
283. 5,7,2',6'-TetraOH-8-Lav (exiguaflavanone A, **111**; H2/1432)
284. 5,7,2',6'-TetraOH-8-[2-(2-OH-isopropyl)-5-Me-4-hexenyl] (exiguaflavanone M, **137**; H2/1455)
285. 5,7,3',4'-TetraOH-6-Pr (6-prenyleriodictyol, H2/1226)
286. 5,7,3',4'-TetraOH-6-(1,1-DMA) (**91**)
287. 5,7,3',4'-TetraOH-6-Ger (diplacone, H2/1236)
288. 5,7,3',4'-TetraOH-6-farnesyl (**121**)
289. 5,7,3',4'-TetraOH-6-(2-OH-3-Me-but-3-enyl)-8-Pr (**135**)
290. 5,7,3',4'-TetraOH-6-(3-OH-3-Me-butyl) (**132**; H2/1422)
291. 5,7,3',4'-TetraOH-6-Pr-8-(2-OH-3-Me-but-3-enyl) (dorsmanin H, **134**)
292. 5,7,3',4'-TetraOH-6,8-diPr (**82**; H2/1410)
293. 5,7,3',4'-TetraOH-6,5'-diPr (**83**; H2/1457)
294. 5,7,3',4'-TetraOH-8-Pr (8-prenyleriodictyol, H2/1227)
295. 5,7,3',4'-TetraOH-8-[(*E*)-3-hydroxymethyl-2-butenyl] (licoleafol, **131**)
296. 5,7,3',4'-TetraOH-8-(4-OAc-3-Me-but-2-enyl) (kanzonol S, **140**)
297. 5,7,3',4'-TetraOH-8,5'-diPr (gancaonin E, H2/1382)
298. 5,7,3',4'-TetraOH-2'-Pr (2'-prenyleriodictyol, H2/1367)
299. 5,7,3',4'-TetraOH-2',5'-diPr (sigmoidin A, H2/1229)
300. 5,7,3',4'-TetraOH-5'-Pr (sigmoidin B, H2/1228)
301. 5,7,3',4'-TetraOH-5',6'-diPr (abyssinin III, **84**)
302. 5,7,3',5'-TetraOH-6,8-diPr (monotesone B, **85**)
303. (2*R*)-5,7,2'-TriOH-8-OMe-6-Pr (dioflorin, **63**)
304. 5,7,2'-TriOH-4'-OMe-6,8-diPr (flemiflavanone A, H2/1268)
305. 5,7,3'-TriOH-4'-OMe-6-Ger (4'-*O*-methyl-diplacone, H2/1237)
306. 5,7,3'-TriOH-4'-OMe-6,8,5'-triPr (isoamoritin, **88**)
307. 5,7,3'-TriOH-4'-OMe-5'-Pr (4'-methylsigmoidin B, H2/1369)
308. 5,7,3'-TriOH-4'-OPr (monotesone A, **62**)

309. 5,7,4'-TriOH-2'-OMe-8-(5-OH-5-Me-2-isopropenyl-*trans*-hex-3-enyl) (leachianone D, **138**; H2/1434)
310. 5,7,4'-TriOH-2'-OMe-8-(5-OH-5-Me-2-isopropenyl-hexyl) (kushenol P1, **139**)
311. 5,7,4'-TriOH-3'-OMe-6-(1,1-DMA) (**92**)
312. 5,7,4'-TriOH-3'-OMe-6-(β -OH-ethyl)-8-Pr (laxiflorin, **75**)
313. 5,7,4'-TriOH-3'-OMe-6,8,5'-triPr (amoritin, H2/1234)
314. 5,7,4'-TriOH-3'-OMe-8-Pr (exiguaflavanone K, **64**; H2/1453)
315. 5,7,4'-TriOH-3'-OMe-5'-Pr (abyssinin II, **65**)
316. 5,7,4'-TriOH-2'-OMe-6-Lav (isokurarinone, H2/1245)
317. 5,7,4'-TriOH-2'-OMe-8-Lav (leachianone A, H2/1346)
318. 5,7,4'-TriOH-2'-OMe-8-Pr-5'-(1,1-DMA) (**95**)
319. 5,7,4'-TriOH-3'-OMe-6,8-diPr (hiravanone, H2/1383)
320. 5,2',4'-TriOH-7-OMe-8-Pr (kenusanone I, **66**; H2/1411)
321. 5,2',4'-TriOH-7-OMe-8,5'-diPr (maackiaflavanone, **86**; H2/1456)
322. 5,2',5'-TriOH-7-OMe-8-Lav (exiguaflavanone F, **112**; H2/1447)
323. 5,2',6'-TriOH-7-OMe-8-Lav (exiguaflavanone B, **113**; H2/1433)
324. 5,3',4'-TriOH-7-OMe-8-Pr (H2/1267)
325. 5,3',4'-TriOH-7-OMe-6,8-diPr (amoradecin, H2/1231)
326. 7,2',4'-TriOH-5-OMe-8-Lav (kurarinone, **114**)
327. 5,7-DiOH-2',4'-diOMe-8-(2,3-epoxy-3-Me-butyl) (**133**)
328. 5,7-DiOH-3',4'-diOMe-5'-Pr (**68**)
329. 5,4'-DiOH-7,3'-diOMe-6,8-diPr (amoradinin, H2/1232)
330. 7,4'-DiOH-5,2'-diOMe-8-Lav (**115**)
331. 5-OH-7,3'-diOMe-4'-OPr (H2/1260)
332. 7-OMe-3',4'-Methylenedioxy-6-OPr (ponganone V, **67**)
- Penta-O-substituted**
333. 5,7,2',4',6'-PentaOH-6-Ger (sophoraflavanone D, **102**; H2/1413)
334. 5,7,2',4',6'-PentaOH-6-Lav (exiguaflavanone C, **116**; H2/1444)
335. 5,7,2',4',6'-PentaOH-6,8-diPr (kenusanone B, **87**; H2/1415)
336. 5,7,2',4',6'-PentaOH-6-Ger-8-Pr (tomentosanol E, **104**)
337. 5,7,2',4',6'-PentaOH-6-Pr-8-Lav (exiguaflavanone J, **119**; H2/1452)
338. 5,7,2',4',6'-PentaOH-8-Ger (sophoraflavanone E, **103**; H2/1414)
339. 5,7,2',4',6'-PentaOH-8-Lav (exiguaflavanone G, **117**; H2/1449)
340. 5,7,2',4'-TetraOH-5'-OMe-6-Pr (kushenol V, **69**)
341. 5,7,2',4'-TetraOH-5'-OMe-8-Pr (kushenol W, **70**)
342. 5,7,2',6'-TetraOH-4'-OMe-6-Pr-8-Lav (exiguaflavanone D, **120**; H2/1445)
343. 5,7,2',6'-TetraOH-4'-OMe-8-Pr (kenusanone D, **71**; H2/1417)
344. 5,2',4'-TriOH-7,5'-diOMe-8-Lav (exiguaflavanone E, **118**; H2/1446)
345. 5,2',6'-TriOH-7,4'-diOMe-8-Pr (kenusanone E, **72**; H2/1416)
346. 5,7-DiOH-2',4',6'-triOMe-8-Pr (heteroflavanone C, **73**; H2/1418)
347. 5-OH-7,2',4',6'-tetraOMe-8-Pr (heteroflavanone B, **74**; H2/1419)
- Flavanone aglycones bearing furano substituents^a**
- Di-O-substituted**
348. 5-OMe-5''-isoprenyldihydrofurano[2'',3'':7,8] (emoroidenone, **142**)
349. 6-OMe-furano[2'',3'':7,8] (**141**)
- Tri-O-substituted**
350. 5,6-DiOMe-furano[2'',3'':7,8] (**144**)
351. 5,4'-DiOH-6-Pr-5''-(2-OH-isopropyl)furano[2'',3'':7,8] (lupinenol, **152**; H2/1426)
352. 5,4'-DiOH-6-Pr-5''-(2-OH-isopropyl)dihydrofurano[2'',3'':7,8] (lonchocarpol C₁, **153**)

353. 5''-Epimer of lonchocarpol C₁ (lonchocarpol C₂, **154**)
 354. 5,4'-DiOH-8-Pr-5''-(2-OH-isopropyl)furanol[2'',3'':7,6] (lonchocarpol D₁, **155**)
 355. 5''-Epimer of lonchocarpol D₁ (lonchocarpol D₂, **156**)
 356. 5,4'-DiOH-4'',4''-dimethyl-5''-methyldihydrofuranol[2'',3'':7,6] (**145**)
 357. 5,4'-DiOH-[5''-(2-OH-isopropyl)dihydrofuranol][2'',3'':7,8] (**147**)
 358. 2',4'-DiOH-[5''-(2-OH-isopropyl)dihydrofuranol][2'',3'':7,8] (phellodensin D, **148**)
 359. 4'-OH-5-OMe-(E)-5''-(2-OH-isopropyl)dihydrofuranol[2'',3'':7,8] (**149**)
 360. 4'-OH-bis(5''-(2-OH-isopropyl)dihydrofuranol[2'',3'':7,8][2'',3'':5,6]) (lonchocarpol E, H2/1340)
 361. 3',4'-Methylenedioxyfuranol[2'',3'':7,8] (**143**)
 Tetra-*O*-substituted
 362. 5,7,3'-TriOH-2'-Pr-furanol[2'',3'':4',5'] (abyssinoflavanone IV, **150**)
 363. 5,3',4'-TriOH-6-Pr-(2-OH-isopropyl)furanol[2'',3'':7,8] (dorsmanin F, **157**)
 364. 5''-Epimer of dorsmanin F (epidorsmanin F, **159**)
 365. 5,3',4'-TriOH-8-Pr-(2-OH-isopropyl)furanol[2'',3'':7,6] (dorsmanin G, **158**)
 366. 5''-Epimer of dorsmanin G (epidorsmanin G, **160**)
 367. 5,3',4'-TriOH-5''-isopropenyldihydrofuranol[2'',3'':7,6] (velloeriodictyol, **146**; H2/1423)
 368. 3',4'-DiOH-7-OMe-8-Pr-furanol[2'',3'':5,6] (**151**)
 369. 3',4'-DiOH-7-OMe-8-Pr-5''-(2-OH-isopropyl)furanol[2'',3'':5,6] (**161**)
 Penta-*O*-substituted
 370. 5-OH-6-OMe-3',4'-methylenedioxyfuranol[2'',3'':7,8] (H2/1222)
Flavanone aglycones bearing pyrano substituents^a
 Mono-*O*-substituted
 371. 6'',6''-DMP[2'',3'':7,8] (isolonchocarpin, H2/1017)
 372. 6-Pr-6'',6''-DMP[2'',3'':7,8] (maximaflavanone A, **162**)
 373. 8-Pr-6'',6''-DMP[2'',3'':7,6] (H2/1018)
 Di-*O*-substituted
 374. 5-OH-6-(3-Me-but-1,3-dienyl)-6'',6''-DMP[2'',3'':7,8] (spinoflavanone A, **164**)
 375. 5-OH-6-Pr-6'',6''-DMP[2'',3'':7,8] (fulvinervin A, H2/1080)
 376. 5-OH-6'',6''-DMP[2'',3'':7,8] (obovatin, H2/1344)
 377. 7-OH-6-Pr-6'',6''-DMP[2'',3'':4',3'] (dinklugin A, **165**)
 378. 7-OH-8-Pr-6'',6''-DMP[2'',3'':4',3'] (euchrenone a₅, H2/1371)
 379. 7-OH-8-Pr-6'',6''-DMDHP[2'',3'':4',3'] (euchrenone a₁₇, **167**)
 380. 7-OH-8,5'-diPr-6'',6''-DMP[2'',3'':4',3'] (sophoranochromene, H2/1154)
 381. 7-OH-5'-Pr-6'',6''-DMP[2'',3'':4',3'] (abyssinone III, H2/1097)
 382. 7-OH-6'',6''-DMP[2'',3'':4',3'] (abyssinone I, H2/1096)
 383. 4'-OH-3'-Pr-6'',6''-DMP[2'',3'':7,8] (shinflavanone, **166**)
 384. 4'-OH-6'',6''-DMP[2'',3'':7,8] (4'-hydroxyisolonchocarpin, H2/1311)
 385. 5-OMe-6'',6''-DMP[2'',3'':7,8] (obovatin methyl ether, H2/1062)
 386. 6-OMe-6'',6''-DMP[2'',3'':7,8] (ovalichromene, H2/1019)
 387. 4'-OMe-6'',6''-DMP[2'',3'':7,8] (dorspoinsettifolin, **163**)
 388. Bis(6'',6''-DMP[2'',3'':7,8][2'',3'':4',3']) (xambioona, H2/1345)
 389. Bis(6'',6''-DMDHP[2'',3'':7,6][2'',3'':4',3']) (dormanin B, **168**)
 Tri-*O*-substituted
 390. 5,7-DiOH-6-Pr-6'',6''-DMP[2'',3'':4',3'] (paratocarpin H, **173**)
 391. 5,7-DiOH-6,8-diPr-6'',6''-DMP[2'',3'':4',3'] (euchrenone a₄, H2/1384)
 392. 5,7-DiOH-8-Pr-6'',6''-DMDHP[2'',3'':4',3'] (euchrenone a₁₆, **177**)
 393. 5,2'-DiOH-8-Pr-6'',6''-DMP[2'',3'':7,6] (minimiflorin, H2/1164)
 394. 5,4'-DiOH-6-Pr-6'',6''-DMP[2'',3'':7,8] (cajaflavanone, H2/1152)
 395. 5,4'-DiOH-8-Pr-6'',6''-DMP[2'',3'':7,6] (lupinifolin, H2/1165)

396. 5,4'-DiOH-8-hydroxymethyl-6'',6''-DMP[2'',3'':7,6] (**184**)
397. 5,4'-DiOH-8-(1-OH-2,3-epoxy-3-Me-butyl)-6'',6''-DMP[2'',3'':7,6] (1'''-OH-2''',3'''-epoxylupinifolin, **202**)
398. 5,4'-DiOH-8-(2,3-diOH-3-Me-butyl)-6'',6''-DMP[2'',3'':7,6] (2'',3''-dihydroxylupinifolin, **205**)
399. 5,4'-DiOH-8-(2,3-epoxy-3-Me-butyl)-6'',6''-DMP[2'',3'':7,6] (2''',3'''-epoxylupinifolin, **191**)
400. 5,4'-DiOH-8-(2-OH-3-Me-but-3-enyl)-6'',6''-DMP[2'',3'':7,6] (dereticulatin, **192**)
401. 5,4'-DiOH-3'-Pr-6'',6''-DMP[2'',3'':7,6] (paratocarpin I, **174**)
402. 5,4'-DiOH-3'-Pr-6'',6''-DMP[2'',3'':7,8] (euchrenone a₂, H2/1374)
403. 5,4'-DiOH-6'',6''-DMP[2'',3'':7,6] (paratocarpin K, **169**)
404. 5,4'-DiOH-6'',6''-DMP[2'',3'':7,8] (citflavanone, H2/1363)
405. 5,4'-DiOH-6''-Me,6'',(4-methylpent-3-enyl)pyrano[2'',3'':7,8] (cycloaltisin 7, **175**)
406. 5-OH-4'-OMe-6'',6''-DMP[2'',3'':7,8] (**170**)
407. 5,4'-DiOMe-6'',6''-DMP[2'',3'':7,8] (glyflavanone A, **171**)
408. 3',4'-DiOMe-6-Pr-6'',6''-DMP[2'',3'':7,8] (**178**)
409. 3',4'-DiOMe-6'',6''-DMP[2'',3'':7,8] (ponganone III, **172**)
410. 3',4'-Methylenedioxy-6'',6''-DMP[2'',3'':7,8] (ovalichromene B, H2/1191)
411. 5-OH-bis(6'',6''-DMP[2'',3'':7,8][2'',3'':4',3']) (euchrenone a₁, H2/1373)
412. 5-OH-bis(6'',6''-DMDHP[2'',3'':7,6][2'',3'':4',3']) (paratocarpin J, **176**)
413. 5-OH-6-Pr-bis(6'',6''-DMP[2'',3'':7,8][2'',3'':4',3']) (euchrenone a₁₅, **179**)
414. 5-OH-8-Pr-bis(6'',6''-DMP[2'',3'':7,6][2'',3'':4',3']) (euchrenone a₁₄, **180**)
- Tetra-*O*-substituted
415. 5,7,2'-TriOH-6-Pr-6'',6''-DMP[2'',3'':4',5'] (cudraflavanone A, H2/1242)
416. 5,7,2'-TriOH-6,8-diPr-6'',6''-DMP[2'',3'':4',5'] (euchrenone a₆, H2/1388)
417. 5,7,2'-TriOH-8-Pr-6'',6''-DMP[2'',3'':4',5'] (euchrestaflavanone C, H2/1241)
418. 5,7,2'-TriOH-5'-Pr-6'',6''-DMP[2'',3'':4',3'] (sanggenol N, **188**)
419. 5,7,2'-TriOH-6'',6''-DMP[2'',3'':4',3'] (sanggenon F, H2/1239)
420. 5,7,3'-TriOH-6,8-diPr-6'',6''-DMP[2'',3'':4',5'] (amorinin, H2/1235)
421. 5,7,3'-TriOH-2'-Pr-(5''-OH-6'',6''-DMDHP[2'',3'':4',5']) (abyssinoflavanone V, **203**)
422. 5,7,3'-TriOH-2'-Pr-6'',6''-DMP[2'',3'':4',5'] (sigmoidin F, H2/1379)
423. 5,7,3'-TriOH-(4''-5''-diOH-6'',6''-DMDHP[2'',3'':4',5']) (sigmoidin G, **210**, H2/1429)
424. 5,7,3'-TriOH-(5''-OH-6'',6''-DMDHP[2'',3'':4',5']) (sigmoidin D, H2/1370)
425. 5,7,3'-TriOH-6'',6''-DMP[2'',3'':4',5'] (sigmoidin C, H2/1230)
426. 5,7,4'-TriOH-6-Pr-6''-Me,6''-(4-Me-pent-3-enyl)pyrano[2'',3'':3',2'] (tanariflavanone B, **200**)
427. 5,7,4'-TriOH-6'',6''-DMP[2'',3'':2',3'] (sanggenon H, H2/1240)
428. 5,2',4'-TriOH-6-Pr-6'',6''-DMP[2'',3'':7,8] (euchrenone a₉, H2/1377)
429. 5,2',4'-TriOH-8-Pr-6'',6''-DMP[2'',3'':7,6] (flemichin D, H2/1269)
430. 5,2',4'-TriOH-(5''-Pr-6'',6''-DMDHP[2'',3'':7,8]) (leachianone F, **193**)
431. 5,2',4'-TriOH-6'',6''-DMDHP[2'',3'':7,8] (kenusanone J, **181**)
432. 5,2',4'-TriOH-6''-Me,6''-(4''-Me-pent-3-enyl)pyrano[2'',3'':7,6] (kuwanol C, H2/1341)
433. 5,2',4'-TriOH-6''-Me,6''-(4''-Me-pent-3-enyl)pyrano[2'',3'':7,8] (sanggenol L, **189**)
434. 5,2',6'-TriOH-8-Pr-6'',6''-DMP[2'',3'':7,6] (orotinin, H2/1378)
435. 5,2',6'-TriOH-5''-Pr-6'',6''-DMP[2'',3'':7,8] (exiguaflavanone L, **194**)
436. 5,3',4'-TriOH-8-Pr-6'',6''-DMP[2'',3'':7,6] (dorsmanin I, **190**)
437. 5,3',4'-TriOH-8-Pr-6'',6''-DMDHP[2'',3'':7,6] (dorsmanin J, **195**)
438. 5,3',4'-TriOH-8,5''-diPr-6'',6''-DMP[2'',3'':7,6] (amoridin, H2/1389)
439. 5,3',4'-TriOH-2'-Ger-(5''-OH-6'',6''-DMDHP[2'',3'':7,6]) (tanariflavanone A, **201**)
440. 2',3',6'-TriOH-8-Pr-5''-(2,4-diOH-phenyl)-6'',6''-DMP[2'',3'':7,6] (eriosemaone C, **211**)

441. 5,7-DiOH-3'-OMe-6'',6''-DMP[2'',3'':4',5'] (abyssinin I, **182**)
442. 5,7-DiOH-bis(6'',6''-DMP[2'',3'':2',3']][2'',3'':4',5']) (sanggenol O, **187**)
443. 5,7-DiOH-(5''-OH-6'',6''-DMP[2'',3'':7,6])-6'',6''-DMDHP[2'',3'':4',3'] (abyssinoflavanone VI, **204**)
444. 5,2'-DiOH-6-Pr-bis(6'',6''-DMP[2'',3'':7,8][2'',3'':4',5']) (euchrenone a₁₂, H2/1386)
445. 5,2'-DiOH-8-Pr-bis(6'',6''-DMP[2'',3'':7,6][2'',3'':4',5']) (euchrenone a₁₁, H2/1385)
446. 5,2'-DiOH-8-Pr-6''DMP6''[2'',3'':7,8],(5''-OH-6'',6''-DMDHP[2'',3'':4',3']) (flemichin E, H2/1280)
447. 5,2'-DiOH-4'-OMe-6-Pr-6'',6''-DMP[2'',3'':7,8] (flemione, H2/1243)
448. 5,2'-DiOH-6''-Me,6''-(4-Me-pent-3-enyl)pyrano[2'',3'':7,8],6'',6''-DMP[2'',3'':4',3'] (flemichin A, H2/1279)
449. 5,3'-DiOH-8-Pr-bis(6'',6''-DMP[2'',3'':7,6][2'',3'':4',5']) (amorin, H2/1387)
450. 5,3'-DiOH-4'-OMe-6'',6''-DMP[2'',3'':7,8] (**183**)
451. 5,3'-DiOH-4'-OMe-6-Pr-6'',6''-DMP[2'',3'':7,8] (**197**)
452. 5,4'-DiOH-2'-OMe-5''-Pr-6'',6''-DMDHP[2'',3'':7,8] (leachianone B, H2/1436)
453. 5,4'-DiOH-3'-OMe-6-Pr-6'',6''-DMP[2'',3'':7,8] (**198**)
454. 5,4'-DiOH-3'-OMe-8-Pr-6'',6''-DMP[2'',3'':7,6] (3'-methoxyxylupinifolin, H2/1427)
455. 5,4'-DiOH-3'-OMe-8,5''-diPr-6'',6''-DMP[2'',3'':7,6] (amoricin, H2/1390)
456. 3',4'-DiOH-bis(6'',6''-DMDHP[2'',3'':5,6][2'',3'':7,8]) (dorsmanin E, **196**)
457. 5-OH-3',4'-DiOMe-6-Pr-6'',6''-DMP[2'',3'':7,8] (**199**)
458. 5,3',4'-TriOMe-6'',6''-DMP[2'',3'':7,8] (glyflavanone B, **185**)
459. 6,3',4'-TriOMe-6'',6''-DMP[2'',3'':7,8] (ponganone IV, **186**)
460. 5-OMe-3',4'-methylenedioxy-6'',6''-DMP[2'',3'':7,8] (isoglabrachromene, H2/1442)
461. 6-OMe-3',4'-methylenedioxy-6'',6''-DMP[2'',3'':7,8] (ovalichromene A, H2/1192)
- Penta-*O*-substituted
462. 5,2',4',6'-TetraOH-6-Lav-6'',6''-DMP[2'',3'':7,8] (exiguaflavanone I, **208**)
463. 5,2',4',6'-TetraOH-8-Lav-6'',6''-DMP[2'',3'':7,6] (exiguaflavanone H, **209**)
- Flavanone aglycones bearing benzyl substituents**
- Mono-*C*-benzyl
464. 5,7-DiOH-6-(2-OH-benzyl) (isochamanetin, H2/1067)
465. 5,7-DiOH-8-(2-OH-benzyl) (chamanetin, H2/1068)
466. 7-OH-5-OMe-8-(2-OH-benzyl) (5-*O*-methylchamanetin, H2/1070)
467. 5-OH-6,7-diOMe-8-(2-OH-5-OMe-benzyl) (macrophyllol, **212**)
468. 5-OH-7,8-diOMe-6-(2-OH-5-OMe-benzyl) (macrophyllol A, **213**)
- Di-*C*-benzyl
469. 5,7-DiOH-6,8-di(2-OH-benzyl) (dichamanetin, H2/1302)
- Tri-*C*-benzyl
470. 5,7-DiOH-6-(2 × 2-OH-benzyl)-8-(2-OH-benzyl) (uvarinol, H2/1284)
471. 5,7-DiOH-6-(2-OH-benzyl)-8-(2 × 2-OH-benzyl) (isouvarinol, **214**)
- Tetra-*C*-benzyl
472. 5,7-DiOH-6-(3 × 2-OH-benzyl)-8-(2-OH-benzyl) (2''''-OH-3''''-benzyluvarinol, **215**)
473. 5,7-DiOH-6-(2-OH-benzyl)-8-(2 × 2-OH-benzyl) (2''''-OH-5''''-benzyluvarinol A, **216**)
474. 5,7-DiOH-6-(2 × 2-OH-benzyl)-8-(2 × 2-OH-benzyl) (2''''-OH-5''''-benzyluvarinol B, **217**)
- Complex flavanone aglycones (arranged alphabetically)**
475. Alopecurone A (**218**)
476. Alopecurone B (**219**)
477. Alopecurone C (**220**)
478. Alopecurone D (**221**)
479. Alopecurone E (**222**)
480. Alopecurone F (**223**)

481. Anastatin A (**225**)
482. Anastatin B (**226**)
483. Baeckea flavanone (**227**)
484. BF-4 (**228**)
485. BF-5 (**229**)
486. BF-6 (**230**)
487. Breverin (H2/1273)
488. Calomelanol G (**238**)
489. Calomelanol H (**239**)
490. Calomelanol I (**240**)
491. Calomelanol J (**241**)
492. Calyxin C (**242**)
493. Calyxin D (**243**)
494. Calyxin G (**244**)
495. Calyxin J (**245**)
496. Calyxin K (**246**)
497. Calyxin M (**247**)
498. Derriflavanone (**206**)
499. Epicalyxin C (**248**)
500. Epicalyxin D (**249**)
501. Epicalyxin G (**250**)
502. Epicalyxin J (**251**)
503. Epicalyxin K (**252**)
504. Epicalyxin M (**253**)
505. Epiderriflavanone (**207**)
506. Kurziflavalactone A (2*R*) (**254**)
507. Kurziflavalactone B (2*S*) (**255**)
508. Kurziflavalactone C (2*S*) (**256**)
509. Kurziflavalactone D (2*R*) (**257**)
510. Kuwanon D (H2/1291)
511. Kuwanon F (H2/1290)
512. Kuwanon L (H2/1295)
513. Leachianone C (H2/1437)
514. Leachianone I (**224**)
515. Lepidissipyronone (H2/1317)
516. Leucadenone A (**231**)
517. Leucadenone B (**232**)
518. Leucadenone C (**233**)
519. Leucadenone D (**234**)
520. Linderatone (H2/1315)
521. Louisfieserone A (H2/1281)
522. Louisfieserone B (H2/1282)
523. Lumaflavanone A (**235**)
524. Lumaflavanone B (**236**)
525. Lumaflavanone C (**237**)
526. Neolinderatone (H2/1316)
527. Neosilyhermin A (H2/1288)
528. Neosilyhermin B (H2/1289)
529. 8-Prenyllepidissipyronone (H2/1318)
530. Protofarrerol (H2/1334)

531. Purpurin (H2/1283)
532. Remerin (H2/1275)
533. Scaberin (H2/1276)
534. Silandrin (H2/1285)
535. Silymonin (H2/1286)
536. Silyhermin (H2/1287)
537. Sophoraflavanone I (H2/1438)
538. Tephroleocarpin B (H2/1441)
539. Tephrorin A (**258**)
540. Tephrorin B (**259**)
- Flavanone glycosides^a**
7-OH-flavanone
541. 7-Glucoside (**260**)
5,7-DiOH-flavanone (pinocembrin)
542. 5-Glucoside (H2/1023)
543. 7-Apiosyl(1 → 5)apiosyl(1 → 2)glucoside (**263**)
544. 7-Glucoside (**261**)
545. 7-Neohesperidoside 2''-acetate (H2/1026)
546. 7-Neohesperidoside 3''-acetate (H2/1027)
547. 7-Neohesperidoside 4''-acetate (H2/1028)
548. 7-Neohesperidoside 6''-acetate (H2/1029)
549. 7-Rhamnoside (H2/1024)
550. 7-Rhamnosyl(1 → 2)glucoside (sarotanoside, onychin, H2/1025)
551. 7-Rhamnosyl(1 → 6)glucoside (**262**)
5-OH-7-OMe-flavanone (pinostrobin)
552. 5-Glucoside (**264**; H2/1398)
7,4'-DiOH-flavanone (liquiritigenin)
553. 7-(3-Acetylapioside)-4'-glucoside (**266**)
554. 7-Apioside-4'-glucoside (**265**)
555. 7-Glucoside (neoliquiritin, H2/1084)
556. 7-Glucoside-4'-apiosyl(1 → 2)glucoside (glucoliquiritin apioside, **268**)
557. 7-Rhamnosylglucoside (rhamnoliquiritin, H2/1086)
558. 4'-[3-Acetylapiosyl(1 → 2)glucoside] (**267**)
559. 4'-Apiosyl(1 → 2)glucoside (H2/1085)
560. 4'-[4-*p*-Coumaroylapiosyl(1 → 2)glucoside] (2*S*) (licorice glycoside D₂, **271**)
561. 4'-[4-*p*-Coumaroylapiosyl(1 → 2)glucoside] (2*R*) (licorice glycoside D₁, **270**)
562. 4'-[4-Feruloylapiosyl(1 → 2)glucoside] (2*S*) (licorice glycoside C₂, **272**)
563. 4'-[4-Feruloylapiosyl(1 → 2)glucoside] (2*R*) (licorice glycoside C₁, **273**)
564. 4'-Glucoside (liquiritin, H2/1083)
565. Licorice glycoside E (**269**)
5,6,7-TriOH-flavanone (dihydrobaicalein)
566. 7-Glucoside (**274**)
567. 7-Glucuronide (H2/1034)
5,7,8-TriOH-flavanone (dihydronorwogonin)
568. 5-Glucoside (**275**)
569. 7-Glucuronide (H2/1039)
5,7,2'-TriOH-flavanone
570. 7-Glucoside (**276**, H2/1326)
571. 7-*O*-(Ethyl-β-D-glucopyranosiduronate) (**278**)
572. 7-*O*-(Methyl-β-D-glucopyranosiduronate) (**277**)

5,7,4'-TriOH-flavanone (naringenin)

573. 5-Glucoside (floribundoside, salipurposide, H2/1099)
574. 5-Rhamnoside (H2/1100)
575. 5-Rhamnosyl(1 → 2)glucoside (= 5-neohesperidoside) (H2/1101)
576. 5,7-Diglucoside (H2/1110)
577. 7-[3-Acetyl-6-(*E*)-*p*-coumaroylglucoside] (**282**)
578. 7-(6-Acetylglucoside) (**280**)
579. 7-Arabinopyranosyl(1 → 6)glucoside (H2/1113)
580. 7-(2-*p*-Coumaroylglucoside) (**281**)
581. 7-(3-*p*-Coumaroylglucoside) (H2/1108)
582. 7-(6-*p*-Coumaroylglucoside) (H2/1107)
583. 7-(2,6-Dirhamnosylglucoside) (**285**)
584. 7-(3,6-Di-*p*-coumaroylglucoside) (H2/1109)
585. 7-(4,6-Digalloylglucoside) (**283**)
586. 7-Galactosyl(1→4)glucoside (H2/1114)
587. 7-(6-Galloylglucoside) (H2/1106)
588. 7-Glucoside (prunin, H2/1102)
589. 7-Neohesperidoside (naringin) (H2/1112)
590. 7-Neohesperidoside-4'-glucoside (H2/1298)
591. 7-Neohesperidoside-6''-malonate (H2/1443)
592. 7-Rhamnoside (naringerin, H2/1103)
593. 7-Rhamnosyl(1 → 2)(4-*O*-methyl-β-D-glucoside) (fumotonaringenin) (**284**)
594. 7-Rhamnosyl(1 → 6)glucoside (=7-rutinoside) (narirutin, H2/1111)
595. 7-Rutinoside-4'-glucoside (H2/1297)
596. 7-Xylosylglucoside (H2/1116)
597. 4'-Galactoside (H2/1105)
598. 4'-Glucoside (H2/1104)
599. 4'-Rhamnoside (**279**)
600. 4'-Rutinoside (H2/1115)
- 7,3',4'-TriOH-flavanone (butin)*
601. 7-Glucoside (isocoreopsin 2, H2/1182)
602. 7,3'-Diglucoside (butrin, H2/1183)
603. 3'-Glucoside (isomonospermoside, H2/1181)
- 5,7-DiOH-8-OMe-flavanone (dihydrowogonin)*
604. 7-Glucoside (H2/1044)
- 5,7-DiOH-4'-OMe-flavanone (isosakuranetin)*
605. 5-Glucoside (H2/1120)
606. 7-α-L-Arabinofuranosyl(1 → 6)glucoside (**287**)
607. 7-Fucopyranosyl(1 → 6)glucoside (longitin, **286**)
608. 7-Galactoside (puddumin B, H2/1348)
609. 7-Glucoside (H2/1122)
610. 7-Glucosyl(1 → 4)rhamnoside (acinoside, H2/1125)
611. 7-Neohesperidoside (poncirin, citrifolioside, H2/1124)
612. 7-Rhamnoside (isosakuranin, H2/1347)
613. 7-Rutinoside (didymin, neoponcirin, H2/1123)
614. 7-Xyloside (H2/1121)
- 5,2'-DiOH-7-OMe-flavanone*
615. 2'-Glucoside (haplanthin, H2/1138)
- 5,4'-DiOH-7-OMe-flavanone (sakuranetin)*
616. 5-Glucoside (sakuranin, H2/1118)

- 6,7-DiOH-5-OMe-flavanone
617. 7-Glucoside (H2/1399)
7,4'-DiOH-5-OMe-flavanone (*5-O-methyl-naringenin*)
618. 7-Glucoside (puddumin A, H2/1314)
619. 7-Neohesperidoside-4'-glucoside (**289**)
620. 4'-Glucoside (alhagitin, **288**)
621. 4'-Rhamnosylglucoside (H2/1130)
622. 4'-Xylosyl(1 → 4)arabinoside (H2/1129)
5-OH-7,8-diOMe
623. 5-Rhamnoside (**290**)
624. 5-Glucoside (andrographidin, H2/1362)
4'-OH-5,7-diOMe-flavanone (*5,7-di-O-methyl-naringenin*)
625. 4'-[(5-Cinnamoyl)-β-D-apiofuranosyl(1 → 2)glucoside] (**291**)
2,5,7,4'-TetraOH-flavanone (*2-hydroxynaringenin*)
626. 7-Glucoside (**292**)
5,6,7,4'-TetraOH-flavanone (*carthamidin*)
627. 5-Glucoside (H2/1133)
628. 6,7-Diglucoside (**293**)
629. 7-Rhamnoside (H21401/)
5,7,8,4'-TetraOH-flavanone (*isocarthamidin*)
630. 7-Rhamnoside (**294**)
631. 8-Glucoside (3-desoxycallunin, **295**)
5,7,2',4'-TetraOH-flavanone (*steppogenin*)
632. 7-Glucoside (stepposide, H2/1278)
633. 4'-Glucoside (**296**)
5,7,2',5'-TetraOH-flavanone
634. 7-Glucoside (coccinoside B, **297**)
635. 7-Rutinoside (H2/1322)
5,7,3',4'-TetraOH-flavanone (*eriodictyol*)
636. 5-Glucoside (H2/1195)
637. 5-Rhamnoside (H2/1194)
638. 6-C-Glucoside (2*S*) (**298**)
639. 6-C-Glucoside (2*R*) (**299**)
640. 7-(6-Acetylglucoside) (coccinoside A, **301**)
641. 7-α-L-Arabinofuranosyl(1 → 6)glucoside (**302**)
642. 7-(6-*p*-Coumaroylglucoside) (**303**)
643. 7-(6-Galloylglucoside) (**305**)
644. 7-Glucoside (H2/1196)
645. 7-Glucoside 3',4',2'',3'',4'',6''-hexaacetate (hexaacetylpyracanthoside, H2/1439)
646. 7-Glucuronide (2*S*) (**300**)
647. 7-Glucuronide (2*R*) (H2/1350)
648. 7-Neohesperidoside (neoeriocitrin, H2/1201)
649. 7-Rhamnoside (eriodictin, H2/1197)
650. 7-Rutinoside (eriocitrin, H2/1200)
651. 3'-(6-*p*-Coumaroylglucoside) (**304**)
652. 3'-Glucoside (H2/1198)
653. 5,3'-Diglucoside (H2/1199)
7,8,3',4'-TetraOH-flavanone (*iso-okanin*)
654. 7-Glucoside (H2/1186)
655. 7-Rhamnoside (H2/1187)

656. 7-(2,4,6-Triacetylglucoside) (**306**)
5,6,7-TriOH-4'-OMe-flavanone (4'-methylcarthamidin)
657. 7-(2-*p*-Coumaroylglucoside) **307**
5,7,3'-TriOH-4'-OMe-flavanone (hesperetin)
658. 5-Glucoside (H2/1204)
659. 7-(2,6-Dirhamnosylglucoside) (**308**)
660. 7-Galactosyl(1 → 3)[rhamnosyl(1 → 6)]glucoside] (alhagidin, **309**)
661. 7-Glucoside (H2/1205)
662. 7-Neohesperidoside (neohesperidin, H2/1208)
663. 7-Rhamnoside (H2/1206)
664. 7-Rutinoside (hesperidin, H2/1207)
5,7,4'-TriOH-3'-OMe-flavanone
665. 7-(6-Acetylglucoside) (viscumneoside VI, H2/1355)
666. 7-Apiosyl(1 → 5)apiosyl(1 → 2)glucoside (viscumneoside V, H2/1356)
667. 7-Glucoside (viscumside A, H2/1354)
668. 7-Glucoside-4'-apioside (viscumneoside I, H2/1353)
669. 7-Rutinoside (**310**)
5,3',4'-TriOH-7-OMe-flavanone
670. 3'-Glucoside (H2/1352)
7,3',4'-TriOH-5-OMe-flavanone
671. 7-Xylosyl(1 → 4)arabinoside (H2/1213)
5,7-DiOH-3',4'-diOMe-flavanone (homoesperetin)
672. 5-Glucoside (H2/1212)
673. 7-Rutinoside (**311**)
5,3'-DiOH-7,4'-diOMe-flavanone (persicogenin)
674. 5-Glucoside (persicoside, H2/1211)
675. 3'-Glucoside (**312**)
5,4'-DiOH-7,3'-diOMe-flavanone
676. 4'-Apiosyl(1 → 2)glucoside (**313**)
7,3'-DiOH-5,4'-diOMe-flavanone
677. 7-Glucoside (H2/1215)
4'-OH-5,7,2'-triOMe-flavanone
678. 4'-Rhamnosyl(1 → 6)glucoside (**314**)
2,5,7,3',4'-PentaOH-flavanone
679. 5-Glucoside (H2/1263)
5,7,3',4',5'-PentaOH-flavanone
680. 3'-Glucoside (plantagoside, H2/1253)
5,7,4'-TriOH-3',5'-OMe-flavanone
681. 5-Glucoside (2*R*) (peruvianoside I, **315**)
682. 5-Glucoside (2*S*) (peruvianoside II, **316**)
7,8,4'-TriOH-3',5'-OMe-flavanone
683. 4'-Glucoside (H2/1308)
5,2'-DiOH-7,8,6'-triOMe-flavanone
684. 2'-Glucuronide (H2/1325)
5-OH-6,7,3',4',5'-OMe-flavanone
685. 5-Rhamnoside (H2/1271)
7-OH-6,8-diMe-flavanone
686. 7-Arabinoside (H2/1309)
5,7-diOH-6-Me-flavanone (strobopinin)
687. 7-Galactoside (H2/1330)

688. 7-Glucoside (H2/1331)
 689. 7-Xylosyl(1 → 3)xyloside (**317**)
5,7-diOH-6,8-diMe-flavanone
 690. 7-[6-(3-OH-3-Methylglutaryl)glucoside] (matteuorientate B, **318**)
5,7,4'-TriOH-6-Me-flavanone (poriol)
 691. 7-Glucoside (poriolin, H2/1140)
5,7,4'-TriOH-6,8-diMe-flavanone (farrerol)
 692. 5,7-Diglucoside (H2/1143)
 693. 7-β-D-Apiosyl(1 → 6)glucoside (miconioside B, **319**)
 694. 7-Glucoside (cyrtopterin, H2/1142)
5,7-diOH-4'-OMe-6,8-diMe-flavanone (matteucinol)
 695. 7-β-L-Apiosyl(1 → 6)glucoside (**320**)
 696. 7-α-L-Arabinopyranosyl(1 → 6)glucoside (miconioside A, **321**)
 697. 7-(4,6-Digalloylglucoside) (**323**)
 698. 7-Glucoside (H2/1146)
 699. 7-[4,6-(S)-Hexahydroxydiphenylglucoside] (**322**)
 700. 7-[6-(3-OH-3-Methylglutaryl)glucoside] (matteuorientate A, **324**)
5,7,3',4'-Tetra-OH-6-Me-flavanone
 701. 7-Glucoside (H2/1261)
5,7,3',4'-Tetra-OH-5'-Me-flavanone
 702. 3'-Galactosyl(1 → 4)rhamnoside (mesuein, H2/1333)
7,3',4'-TriOH-5-OMe-6-Me-flavanone
 703. 7-Glucoside (H2/1223)
5,4'-DiOH-6-Pr-flavanone
 704. 4'-Xylosyl(1 → 2)rhamnoside (**325**)
5,7,4'-TriOH-8-Pr-flavanone
 705. 7-Glucoside (phellodensin F, **326**)
 706. 7,4'-Diglucoside (flavaprenin 7,4'-diglucoside)
5,6,7,4'-TetraOH-8-Pr-flavanone
 707. 5-Rutinoside (nirurin, H2/1174)
5,7,4'-TriOH-8-(3-hydroxymethyl-2-butenyl)flavanone
 708. 7-Glucoside (phellodensin E, **327**)
5-OH-7,4'-diOMe-6,8-diMe-flavanone
 709. 5-Galactoside (H2/1167)
Sophora flavanone I
 710. 7-Glucoside (**328**)

DIHYDROFLAVONOLS

Dihydroflavonol aglycones bearing hydroxy, methoxy, and methylenedioxy substituents only

- Di-O-substituted
 711. 3,7-DiOH-flavanone (7-OH-flavanonol, H2/1459)
 Tri-O-substituted
 712. 3,5,7-TriOH-flavanone (pinobanksin, H2/1460)
 713. 3,7,4'-TriOH-flavanone (garbanzol, H2/1466)
 714. 3,5-DiOH-7-OMe-flavanone (alpinone, H2/1463)
 715. 3,7-DiOH-5-OMe-flavanone (H2/1462)
 716. 3,7-DiOH-6-OMe-flavanone (H2/1465)
 717. 5,7-DiOH-3-OMe-flavanone (H2/1461)
 718. 3-OH-5,7-diOMe-(2R,3R)-flavanone (H2/1464)
 719. 3-OH-5,7-diOMe-(2R,3S)-flavanone (**329**)

Tetra-*O*-substituted

720. 3,5,7,2'-TetraOH-flavanone (H2/1468)
721. 3,5,7,4'-TetraOH-flavanone (aromadendrin, H2/1469)
722. 3,7,3',4'-TetraOH-(2*R*,3*R*)-flavanone ((2*R*,3*R*)-fustin, H2/1477)
723. 3,7,3',4'-TetraOH-(2*S*,3*S*)-flavanone ((2*S*,3*S*)-fustin, H2/1478)
724. 3,5,7-TriOH-6-OMe-flavanone (alnustinol, H2/1467)
725. 3,5,7-TriOH-4'-OMe-flavanone (H2/1473)
726. 3,5,8-TriOH-7-OMe-flavanone (**330**)
727. 3,5,4'-TriOH-7-OMe-flavanone (H2/1472)
728. 3,7,4'-TriOH-5-OMe-flavanone (H2/1471)
729. 5,7,4'-TriOH-3-OMe-flavanone (H2/1470)
730. 7,3',4'-TriOH-3-OMe-(2*R*,3*R*)-flavanone (H2/1479)
731. 3,5-DiOH-6,7-methylenedioxy-flavanone (H2/1519)
732. 3,5-DiOH-7,4'-diOMe-flavanone (H2/1475)
733. 3,4'-DiOH-5,7-diOMe-flavanone (H2/1474)
734. 3',4'-DiOH-3,7-diOMe-flavanone (H2/1480)
735. 3-OH-5-OMe-6,7-methylenedioxy-flavanone (H2/1520)
736. 3-OH-5,7,4'-triOMe-flavanone (**331**)
737. 7-OH-3,5,4'-triOMe-flavanone (H2/1476)

Penta-*O*-substituted

738. 3,5,7,2',4'-PentaOH-flavanone (dihydromorin, H2/1487)
739. 3,5,7,2',5'-PentaOH-flavanone (H2/1488)
740. 3,5,7,2',6'-PentaOH-flavanone (H2/1491)
741. 3,5,7,3',4'-PentaOH-(2*R*,3*R*)-flavanone ((2*R*,3*R*)-taxifolin, H2/1492)
742. 3,5,7,3',4'-PentaOH-(2*S*,3*S*)-flavanone ((2*S*,3*S*)-taxifolin, H2/1493)
743. 3,7,8,3',4'-PentaOH-(2*R*,3*R*)-flavanone ((2*R*,3*R*)-8-hydroxyfustin, H2/1502)
744. 3,7,8,3',4'-PentaOH-(2*R*,3*S*)-flavanone ((2*R*,3*S*)-8-hydroxyfustin, H2/1503)
745. 3,7,3',4',5'-PentaOH-flavanone (dihydrorobinetin, H2/1505)
746. 3,5,7,2'-TetraOH-5'-OMe-flavanone (H2/1489)
747. 3,5,7,3'-TetraOH-4'-OMe-flavanone (H2/1499)
748. 3,5,7,4'-TetraOH-6-OMe-flavanone (H2/1481)
749. 3,5,7,4'-TetraOH-8-OMe-flavanone (H2/1485)
750. 3,5,7,4'-TetraOH-3'-OMe-flavanone (dihydroisorhamnetin, H2/1498)
751. 3,5,2',3'-TetraOH-7-OMe-flavanone (**332**)
752. 3,5,3',4'-TetraOH-7-OMe-(2*R*,3*R*)-flavanone (padmatin, H2/1496)
753. 3,5,3',4'-TetraOH-7-OMe-(2*R*,3*S*)-flavanone (epipadmatin, **333**; H2/1497)
754. 3,7,3',4'-TetraOH-5-OMe-flavanone (H2/1495)
755. 3,7,3',4'-TetraOH-8-OMe-flavanone (8-methoxyfustin, H2/1504)
756. 3,7,3',5'-TetraOH-4'-OMe-flavanone (sepinol, H2/1506)
757. 5,7,3',4'-TetraOH-3-OMe-flavanone (H2/1494)
758. 3,5,7-TriOH-6,4'-diOMe-flavanone (**334**; H2/1483)
759. 3,5,7-TriOH-8,4'-diOMe-flavanone (H2/1486)
760. 3,5,2'-TriOH-7,8-diOMe-flavanone (H2/1484)
761. 3,5,2'-TriOH-7,5'-diOMe-flavanone (**335**, H2/1490)
762. 3,5,3'-TriOH-7,2'-diOMe-flavanone (**336**)
763. 3,5,3'-TriOH-7,4'-diOMe-(2*R*,3*R*)-flavanone (H2/1501)
764. 3,5,3'-TriOH-7,4'-diOMe-(2*R*,3*S*)-flavanone (**337**)
765. 3,5,4'-TriOH-6,7-diOMe-flavanone (H2/1482)
766. 3,5,4'-TriOH-7,3'-diOMe-flavanone (H2/1500)

Hexa-*O*-substituted

767. 3,5,7,3',4',5'-HexaOH-(2*R*,3*R*)-flavanone (ampelopsin, H2/1510)
 768. 3,5,7,3',4',5'-HexaOH-(2*R*,3*S*)-flavanone (hovenitin III, **338**)
 769. 3,5,7,2',5'-PentaOH-6-OMe-flavanone (diosalol, **339**)
 770. 3,5,7,3',4'-PentaOH-6-OMe-flavanone (H2/1507)
 771. 3,5,7,3',5'-PentaOH-4'-OMe-flavanone (pallasiin, H2/1511)
 772. 3,5,7,4',5'-PentaOH-3'-OMe-(2*R*,3*R*)-flavanone (hovenitin I, **340**)
 773. 3,5,7,4',5'-PentaOH-3'-OMe-(2*R*,3*S*)-flavanone (hovenitin II, **341**)
 774. 3,5,7,3'-TetraOH-8,4'-diOMe-flavanone (H2/1509)
 775. 3,5,7,4'-TetraOH-3',5'-diOMe-flavanone (dihydrosyringetin, H2/1512)
 776. 3,5,3',4'-TetraOH-7,8-diOMe-flavanone (**342**; H2/1508)
 Hepta-*O*-substituted
 777. 3,5,3'-TriOH-8,5'-diOMe-6,7-methylenedioxy-flavanone (plumbaginol, **343**)
Dihydroflavonol aglycones with C-methyl and C-hydroxymethyl substituents
 Tri-*O*-substituted
 778. 3,5,7-TriOH-6-Me-flavanone (H2/1513)
 Tetra-*O*-substituted
 779. 3,5,7-TriOH-4'-OMe-6-Me-flavanone (**349**)
 780. 3,7,4'-TriOH-5-OMe-6-Me-flavanone (H2/1514)
 Penta-*O*-substituted
 781. 2,3,5,7,4'-PentaOH-6-Me-flavanone (**350**)
 782. 2,3,5,7,4'-PentaOH-8-Me-flavanone (**351**)
 783. 3,5,7,3',4'-PentaOH-6-Me-flavanone (cededarin, H2/1515)
 784. 3,5,7,3',4'-PentaOH-8-Me-flavanone (deodarin, H2/1516)
 785. 3,5,7,3',4'-PentaOH-6'-hydroxymethyl-flavanone (**352**)
 Hexa-*O*-substituted
 786. 3,5,7,3',4',5'-HexaOH-6-Me-flavanone (cedrin, H2/1517)
 787. 3,5,7,3',4',5'-HexaOH-6,8-diMe-flavanone (H2/1518)
Dihydroflavonol aglycones with prenyl, geranyl, and lavandulyl substituents^a
 Tri-*O*-substituted
 788. 3,5,7-TriOH-6-Ger-(2*R*,3*S*)-flavanone (H2/1567)
 789. 3,5,7-TriOH-6-Pr-(2*R*,3*R*)-flavanone (glepidotin B, H2/1528)
 790. 3,5,7-TriOH-6-Pr-(2*R*,3*S*)-flavanone (H2/1529)
 791. 3,7,4'-TriOH-8,3'-diPr-flavanone (3-hydroxyglabrol, H2/1532)
 792. 3,7,4'-TriOH-8,3',5'-triPr (H2/1533)
 793. 3,5-DiOH-7-OMe-8-Pr-flavanone (leaserone, H2/1531)
 Tetra-*O*-substituted
 794. 3,5,7,4'-TetraOH-6-Ger-flavanone (bonanniol A, H2/1568)
 795. 3,5,7,4'-TetraOH-6-Pr-flavanone (shuterin, H2/1534)
 796. 3,5,7,4'-TetraOH-6,8-diPr-flavanone (6,8-diprenylaromadendrin, **359**)
 797. 3,5,7,4'-TetraOH-8-Pr-flavanone (H2/1537)
 798. 3,5,7,4'-TetraOH-8-(3-OH-3-Me-butyl)-flavanone (H2/1538)
 799. 3,5,7,4'-TetraOH-8,3'-diPr-flavanone (lespedezaflavanone C, H2/1543)
 800. 3,5,7,4'-TetraOH-8-Pr-3'-Ger-flavanone (sanggenol C, **361**)
 801. 3,5,7-TriOH-8-OMe-6-Pr-flavanone (dioclenol, **353**)
 802. 3,5,4'-TriOH-7-OGer-flavanone (**360**; H2/1569)
 803. 3,5,4'-TriOH-7-OPr-flavanone (H2/1536)
 804. 3,7,4'-TriOH-5-OMe-6-Ger-flavanone (bonanniol B, H2/1570)
 805. 3,7,4'-TriOH-5-OMe-8-Pr-flavanone (H2/1545)
 Penta-*O*-substituted
 806. 3,5,7,2',4'-PentaOH-6-Pr-8-Lav (kushenol M, H2/1565)

807. 3,5,7,2',4'-PentaOH-6-Pr-3'-Ger-flavanone (sanggenol K, **364**)
808. 3,5,7,2',4'-PentaOH-6-(3-OH-3-Me-butyl)-8-Lav-flavanone (kosamol A, **367**)
809. 3,5,7,2',4'-PentaOH-6,8-diPr-flavanone (kushenol L, H2/1548)
810. 3,5,7,2',4'-PentaOH-8-Lav-flavanone (kushenol X, **362**)
811. 3,5,7,2',4'-PentaOH-8-(2-isopropenyl-5-OH-5-Me-hexyl)flavanone (kushenol G, H2/1571)
812. 3,5,7,2',4'-PentaOH-8,5'-diPr-3'-Ger-flavanone (sanggenol E, **366**)
813. 3,5,7,2',4'-PentaOH-3'-Ger-5'-Pr-flavanone (sanggenol D, **365**)
814. 3,5,7,3',4'-PentaOH-8,2',6'-triPr-flavanone (petalostemumol, **363**; H2/1559)
815. 3,5,7,3',4'-PentaOH-6-Ger-flavanone (diplacol, H2/1576)
816. 3,5,7,3'-TetraOH-4'-OMe-6-Ger-flavanone (diplacol 4'-methyl ether, H2/1577)
817. 3,5,7,4'-TetraOH-6-OMe-8-Pr-flavanone (floranol, **355**)
818. 3,5,7,4'-TetraOH-3'-OMe-8-Pr-flavanone (H2/1561)
819. 3,5,3',4'-TetraOH-7-OPr-flavanone (**354**)
820. 3,7,2',4'-TetraOH-5-OMe-8-(2-isopropenyl-5-OH-5-Me-hexyl)(2*R*,3*R*)-flavanone (kushenol H, H2/1574)
821. 3,7,2',4'-TetraOH-5-OMe-8-(2-isopropenyl-5-OH-5-Me-hexyl)(2*R*,3*S*)-flavanone (kushenol K, H2/1575)
822. 3,7,2',4'-TetraOH-5-OMe-8-Lav-(2*R*,3*R*)-flavanone (kushenol I, H2/1572)
823. 3,7,2',4'-TetraOH-5-OMe-8-Lav-(2*R*,3*S*)-flavanone (kushenol N, H2/1573)
824. 3,5,3'-TriOH-7,4'-diOMe-6-Pr-flavanone (isotirumalin, H2/1563)
825. 3,5,3'-TriOH-7,4'-diOMe-8-Pr-flavanone (tirumalin, H2/1564)
826. 3,5,4'-TriOH-7,3'-diOMe-6-Pr-flavanone (**356**)
827. 3,5,4'-TriOH-7,3'-diOMe-8-Pr-flavanone (scariosin, **357**; H2/1562)
- Hexa-*O*-substituted
828. 3,5,7,2',5'-PentaOH-6-OMe-8-Pr-flavanone (paraibanol, **358**)
829. 3,5,7,2',6'-PentaOH-4'-OMe-6-Ger-8-Pr-flavanone (kenusanone C, **368**; H2/1566)
- Dihydroflavonol aglycones bearing furano substituents**
830. 3-OMe-[2'',3'':7,8]furanoflavanone (**369**)
831. 3,5,6-TriOMe-[2'',3'':7,8]furanoflavanone (**370**)
832. 3,5,4'-TriOH-5''-isopropenyldihydrofuranol[2'',3'':7,6]-(2*R*,3*R*)-flavanone (shuterol, H2/1535)
833. 3,5,4'-TriOH-5''-isopropenyldihydrofuranol[2'',3'':7,8]flavanone (H2/1539)
834. 3,5,2',4'-TetraOH-5''-isopropenyldihydrofuranol[2'',3'':7,8]-(2*R*,3*R*)flavanone (shuterone A, H2/1546)
835. 3,5,2',4'-TetraOH-5''-isopropenyldihydrofuranol[2'',3'':7,8]-(2*S*,3*R*)-flavanone (shuterone B, H2/1547)
836. 3,5,6-TriOMe-3',4'-methylenedioxyfuranol[2'',3'':7,8]flavanone (H2/1521)
- Dihydroflavonol aglycones bearing pyrano substituents^a**
837. 3-OH-6'',6''-DMP[2'',3'':7,8]flavanone (3-hydroxyisolonchocarpin, H2/1527)
838. 3-OH-bis(6'',6''-DMP[2'',3'':5,6][2'',3'':7,8])flavanone (MS II, **371**)
839. 3,5-DiOH-8-Pr-6'',6''-DMP[2'',3'':7,6]flavanone (mundulinol, H2/1530)
840. 3,4'-DiOH-3'-Pr-6'',6''-DMP[2'',3'':7,8]flavanone (kazonol Z, **372**)
841. 3,5,2'-TriOH-8-Pr-6'',6''-DMP[2'',3'':7,6]flavanone (jayacanol, **373**)
842. 3,5,4'-TriOH-8-Pr-6'',6''-DMP[2'',3'':7,6]flavanone (lupinifolinol, H2/1542)
843. 3,5,4'-TriOH-6'',6''-DMP[2'',3'':7,8]flavanone (H2/1540)
844. 3,5,4'-TriOH-6'',6''-DHDMP[2'',3'':7,8]flavanone (H2/1541)
845. 3,4'-DiOH-5-OMe-8-Pr-6'',6''-DMP[2'',3'':7,6]flavanone (**374**)
846. 5,2'-DiOH-3-OMe-8-Pr-6'',6''-DMP[2'',3'':7,6]flavanone (**375**)

847. 5,4'-DiOH-3-OMe-8-Pr-6'',6''-DMP[2'',3'':7,6]flavanone (3-*O*-methylpinifolinol, H2/1544)

848. 3,5,7-TriOH-2'-OMe-6'',6''-DMP[2'',3'':4',3'](*2S,3S*)-flavanone (H2/1556)

849. 3,5,4'-TriOH-3'-OMe-6'',6''-DMP[2'',3'':7,6]flavanone (eriotrinol, **376**; H2/1560)

Dihydroflavonol aglycones having a C-3–C-2' ether link and an isoprenylated C-2

850. Sanggenol F (**377**)

851. Sanggenol G (**383**)

852. Sanggenol H (**384**)

853. Sanggenol I (**382**)

854. Sorocein D (**378**)

855. Sorocein E (**379**)

856. Sorocein F (**381**)

857. Sorocein G (**380**)

Dihydroflavonol aglycones bearing benzyl substituents

858. 3,5,7,4'-TetraOH-6-(4-OH-benzyl)-flavanone (gericudranin E, **385**, H2/1579)

859. 3,5,7,4'-TetraOH-6,8-di(4-OH-benzyl)-flavanone (gericudranin D, **388**, H2/1580)

860. 3,5,7,3',4'-PentaOH-6-(4-OH-benzyl)-flavanone (gericudranin C, **386**, H2/1581)

861. 3,5,7,3',4'-PentaOH-6,8-di(4-OH-benzyl)flavanone (gericudranin A, **389**, H2/1583)

862. 3,5,7,3',4'-PentaOH-8-(4-OH-benzyl)-flavanone (gericudranin B, **387**, H2/1582)

Dihydroflavonol aglycones bearing miscellaneous substituents

863. Crombeone (H2/1524)

864. 7,8-Dihydrooxepinodihydroquercetin (H2/1558)

865. *rel*-5-Hydroxy-7,4'-dimethoxy-2''*S*-(2,4,5-trimethoxy-*E*-styryl)tetrahydrofuro[4''*R*,5''*R*:2,3] (**394**)

866. *rel*-5-Hydroxy-7,4'-dimethoxy-3''*S*-(2,4,5-trimethoxy-*E*-styryl)tetrahydrofuro[4''*R*,5''*R*:2,3] (**395**)

867. Isosilybin (H2/1585)

868. Isosilychristin (H2/1587)

869. 5-Methoxypeltogynone (H2/1525)

870. 5-Methoxymopanone (H2/1526)

871. Mopanone (H2/1523)

872. Peltogynone (H2/1522)

873. 3,5,7,3',4'-PentaOH-6-(3-oxobutyl)-flavanone (**390**)

874. Pseudotsuganol (H2/1590)

875. Silybin (H2/1584)

876. Silychristin (H2/1586)

877. Silydianin (H2/1588)

878. 3,5,7,4'-TetraOH-3'-(4-OH-benzaldehyde) (hypnogenol F, **391**)

879. 3,5,7,4'-TetraOH-3'-(4-OH-benzoic acid) (hypnum acid, **392**)

880. 3,5,7,4'-TetraOH-3'-(4-OH-benzoic acid methyl ester) (hypnum acid methyl ester, **393**)

881. 3,5,7,4'-TetraOH-3'-(5''-formyl-2''-hydroxyphenyl)flavanone (H2/1578)

882. 5,7,4'-TriOH-3-*O*-(1,8,14-trimethylhexadecanyl)flavanone (muscanone, **396**)

Dihydroflavonol esters

883. 3,5,7-TriOH-(*2R,3R*)-flavanone 3-acetate ((*2R,3R*)-pinobanksin 3-acetate, H2/1658)

884. 3,5,7-TriOH-(*2R,3S*)-flavanone 3-acetate ((*2R,3S*)-pinobanksin 3-acetate, **344**)

885. 3,5,7-TriOH-flavanone 3-propionate (pinobanksin 3-propionate, H2/1660)

886. 3,5,7-TriOH-flavanone 3-benzoate (pinobanksin 3-benzoate, H2/1661)

887. 3,5,7-TriOH-flavanone 3-cinnamate (pinobanksin 3-cinnamate, H2/1662)

888. 3,5-DiOH-7-OMe-flavanone 3-acetate (3-acetylalpinone, H2/1663)

889. 3,5,7,3'-TetraOH-flavanone 3-isobutyrate (**345**)

890. 3,5,7,4'-TetraOH-flavanone 3-acetate (aromadendrin 3-acetate, H2/1664)
 891. 3,5,7-TriOH-4'-OMe-flavanone 3-acetate (**346**)
 892. 3,5,4'-TriOH-7-OMe-(2*R*,3*R*)-flavanone 3-acetate (H2/1665)
 893. 3,5,4'-TriOH-7-OMe-(2*R*,3*S*)-flavanone 3-acetate (**347**)
 894. 3,5,7,3',4'-PentaOH-flavanone 3-acetate (taxifolin 3-acetate, H2/1670)
 895. 3,5,7,4'-TetraOH-6-OMe-flavanone 3-acetate (H2/1666)
 896. 3,5,7,4'-TetraOH-8-OMe-flavanone 3-acetate (H2/1668)
 897. 3,5,7,4'-TetraOH-8-OMe-flavanone 3-angelate (H2/1669)
 898. 3,5,7,4'-TetraOH-3'-OMe-flavanone 3-acetate (H2/1672)
 899. 3,5,3',4'-TetraOH-7-OMe-flavanone 3-acetate (padmatin 3-acetate, H2/1671)
 900. 3,5,2'-TriOH-7,8-diOMe-flavanone 3-acetate (**348**)
 901. 3,5,2'-TriOH-7,8-diOMe-flavanone 2'-acetate (H2/1667)
 902. 3,5,7,3',4'-PentaOH-6-OMe-flavanone 3-acetate (H2/1673)
 903. 3,5,7,3',4',5'-HexaOH-flavanone 3-gallate-3'-sulfate (myricatin, H2/1674)
 904. 3,5,7-TriOH-6-Me 3-acetate (H2/1675)
 905. 3,5,7-TriOH-6-Pr 3-acetate (H2/1676)

Dihydroflavonol glycosides^a

- 3,5,7-TriOH-flavanone (*pinobanksin*)
 906. 5-Galactosyl(1 → 4)glucoside (H2/1591)
 3,7,4'-TriOH-flavanone (*garbanzol*)
 907. 3-Glucoside (lecontin, H2/1592)
 3,7-DiOH-4'-OMe-flavanone
 908. 7-Xylosyl(1 → 6)glucoside (kushenol J, H2/1593)
 3,5,7,4'-TetraOH-flavanone (*aromadendrin*)
 909. 3-β-L-Arabinopyranoside (H2/1594)
 910. 3-Galactoside (H2/1595)
 911. 3-Glucoside (H2/1596)
 912. 3-Rhamnoside (2*R*,3*R*) (engeletin, H2/1597)
 913. 3-Rhamnoside (2*R*,3*S*) (isoengeletin, H2/1598)
 914. 3-Rhamnoside-5-glucoside (H2/1603)
 915. 7-Glucoside (sinensin, H2/1599)
 916. 7-[6-(4-Hydroxy-2-methylenebutanoyl)glucoside] (**398**)
 917. 7-Rhamnoside (H2/1600)
 918. 7-Rhamnosyl(1 → 4)galactoside (**397**; H2/1604)
 919. 4'-Glucoside (H2/1602)
 920. 4'-Xyloside (H2/1601)
 3,7,3',4'-TetraOH-flavanone (*fustin*)
 921. 3-Glucoside (H2/1611)
 922. 3,7-Diglucoside (H2/1613)
 923. 7-Rhamnoside (H2/1612)
 3,5,7-TriOH-4'-OMe-flavanone (*dihydrokaempferide*)
 924. 3-Glucuronide (H2/1607)
 925. 7-Rhamnoside (H2/1608)
 3,5,4'-TriOH-7-OMe-flavanone
 926. 5-Glucoside (H2/1605)
 927. 5,4'-Diglucoside (micrantoside, H2/1606)
 3,5-DiOH-7,4'-diOMe-flavanone
 928. 3-Rhamnoside (aurapin, H2/1609)
 929. 5-Glucoside (H2/1610)

3,5,7,8,4'-PentaOH-flavanone

930. 8-Glucoside (callunin, H2/1614)
931. 8-(2-Acetylglucoside) (2''-acetylcallunin, **399**; H2/1615)

3,5,7,2',5'-PentaOH-flavanone

932. 3-Rhamnoside (**400**; H2/1617)

3,5,7,3',4'-PentaOH-flavanone (taxifolin)

933. 3-Apioside (H2/1618)

934. 3- α -Arabinofuranoside (**403**; H2/1619)

935. 3- α -Arabinopyranoside (2*R*,3*R*) (**401**)

936. 3- α -Arabinopyranoside (2*R*,3*S*) (**402**)

937. 3-Galactoside (dihydrohyperin, H2/1624)

938. 3-Galactosyl(1 \rightarrow 6)glucoside (H2/1640)

939. 3-Glucoside (2*R*,3*R*) (glucodistylin, H2/1625)

940. 3-Glucoside (2*R*,3*S*) (isoglucodistylin, H2/1626)

941. 3-Glucoside (2*S*,3*S*) (H2/1627)

942. 3-Glucosyl(1 \rightarrow 3)rhamnoside (huangqioid E, H2/1641)

943. 3-Glucosyl(1 \rightarrow 4)rhamnoside (H2/1642)

944. 3-Rhamnoside (2*R*,3*R*) (astilbin, H2/1629)

945. 3-Rhamnoside (2*R*,3*S*) (isoastilbin, H2/1630)

946. 3-Rhamnoside (2*S*,3*R*) (neoisoastilbin, H2/1631)

947. 3-Rhamnoside (2*S*,3*S*) (neoastilbin, H2/1632)

948. 3-Xyloside (2*R*,3*R*) (H2/1620)

949. 3-Xyloside (2*R*,3*S*) (H2/1621)

950. 3-Xyloside (2*S*,3*R*) (H2/1622)

951. 3-Xyloside (2*S*,3*S*) (H2/1623)

952. 3,5-Dirhamnoside (H2/1643)

953. 3,7-Dirhamnoside (**405**)

954. 3-(3-Cinnamoylrhamnoside) (**404**)

955. 3-(6-Galloylglucoside) (taxillusin, H2/1628)

956. 5-Galactoside (H2/1633)

957. 7-Galactoside (H2/1634)

958. 7-Glucoside (H2/1635)

959. 7-Rhamnoside (H2/1636)

960. 7-Rhamnosyl(1 \rightarrow 6)glucoside (flavoplatycoside, **406**; H2/1644)

961. 3'-Glucoside (H2/1637)

962. 3'-(6-Phenylacetylglucoside) (H2/1638)

963. 4'-Glucoside (H2/1639)

3,5,7,3',5'-PentaOH-flavanone

964. 3-Rhamnoside (2*R*,3*R*) (**408**)

965. 3-Rhamnoside (2*R*,3*S*) (smitilbin, **409**)

966. 3-Rhamnoside (2*S*,3*S*) (neosmitilbin, **410**)

3,5,7,3'-TetraOH-4'-OMe-flavanone

967. 3-Rhamnosyl(1 \rightarrow 6)glucoside (anacheiloid, H2/1647)

3,5,3',4'-TetraOH-7-OMe-flavanone (padmatin, dihydrorhamnetin)

968. 3 β -(-D-Arabinopyranoside) (**407**)

969. 3-Glucoside (H2/1645)

970. 5-Glucoside (H2/1646)

3,5,7-TriOH-8,4'-diOMe-flavanone

971. 7-Glucoside (dihydroprudomenin, H2/1616)

- 3,5,7,3',4',5'-HexaOH-flavanone (ampelopsin)*
972. 3-Rhamnoside (H2/1648)
973. 7-Glucoside (dihydroprudomenin, 2/1649)
974. 3'-Glucoside (H2/1650)
3,5,7,3',4'-PentaOH-5'-OMe-flavanone (5'-O-methylampelopsin)
975. 4'-Rhamnoside (H2/1651)
3,5,7,4'-TetraOH-6-Me-flavanone
976. 7-Glucoside (H2/1652)
3,5,7,3',4',5'-HexaOH-6-Me-flavanone
977. 3'-Glucoside (cedrinoside, H2/1653)
3,5,7,4'-TetraOH-8-Pr-flavanone
978. 7-Glucoside (phellamurin, H2/1655)
3,5,7,4'-TetraOH-3'-Pr-flavanone
979. 7-Glucoside (phellochinin, **411**)
3,5,4'-TriOH-6'',6''-DMDHP[2'',3'':7,8]flavanone
980. 3-Glucoside (phellodendroside, H2/1657)
3,5,7,4'-TetraOH-6-(3-OH-3-Me-butyl)-flavanone
981. 7-Glucoside (phellavin, H2/1654)
3,5,7,4'-TetraOH-8-(3-OH-3-Me-butyl)-flavanone
982. 7,3''-Diglucoside (dihydrophelloside, H2/1656)
3,5,7,3',4'-PentaOH-6-Pr-flavanone
983. 7-Glucoside (**412**)

³Pr, prenyl; Ger, geranyl; Lav, lavandulyl; DMP, dimethylpyrano; DMDHP, dimethyldihydropyrano. Rhamnoside, α -L-rhamnopyranoside; apioside, β -apiofuranoside; glucoside, β -D-glucopyranoside; galactoside, β -D-galactopyranoside; xyloside, β -D-xylopyranoside; glucuronide, β -D-glucuronopyranoside. Cinnamic acids are assumed to be in the *E*-form.

16 Chalcones, Dihydrochalcones, and Aurones

Nigel C. Veitch and Renée J. Grayer

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16.1 GENERAL INTRODUCTION

The chalcones, dihydrochalcones, and aurones are three distinctive classes of compound that comprise more than 900 of all the naturally occurring flavonoids reported in the literature to

the end of 2003. Viewed from a historical perspective, the chalcones and aurones are best known as the yellow to orange colored flower pigments of some species of *Coreopsis* and other Asteraceae taxa. The distribution of these compounds is not restricted to flowers, however, and examples of all three classes can be found in many different plant tissues. The chalcones are structurally one of most diverse groups of flavonoids, as witnessed by the formation of a wide range of dimers, oligomers, Diels–Alder adducts, and conjugates of various kinds. At the same time, they are of great significance biosynthetically as the immediate precursors of all other classes of flavonoid. Underlying these important attributes is the unique feature that distinguishes chalcones and dihydrochalcones from other flavonoids, the open-chain three-carbon structure linking the A- and B-rings in place of a heterocyclic C-ring (Figure 16.1). In plants, chalcones are converted to the corresponding (2*S*)-flavanones in a stereospecific reaction catalyzed by the enzyme chalcone isomerase. This close structural and biogenetic relationship between chalcones and flavanones explains why they often co-occur as natural products. It is also the reason why chalcones, dihydrochalcones, and aurones are sometimes described together with flavanones and dihydroflavonols.^{1–3} Whether this group should continue to be known as the “minor flavonoids” is a matter for debate; however, the significant increase in the number of new examples of each of these flavonoid classes in the recent literature suggests that this title may no longer be appropriate. For this reason, the chalcones, dihydrochalcones, and aurones are treated separately from the flavanones and dihydroflavonols (Chapter 15) in this volume. The main purpose of this chapter is to review the scientific literature on all chalcones, dihydrochalcones, and aurones reported as new natural products during the period from 1992 to 2003. This continues the tradition set by the four volumes of the *Advances in Flavonoids* series,^{1–4} which provide the most comprehensive treatment available of the scientific literature relating to flavonoids up to the end of 1991. It is not the purpose of this review to describe general methods for the detection, isolation, and characterization of chalcones, dihydrochalcones, and aurones, as these have been discussed extensively not only in the “Advances” series^{1–4} but also in more general texts on flavonoids.^{5–10} However, points of interest relating to the source, identification, and biological activity of new compounds are covered, as well as wider issues such as biosynthesis and chemosystematic or ecological significance. The chemical synthesis of these three flavonoid classes (a major subject in its own right) is not treated here, although synthetic proced-

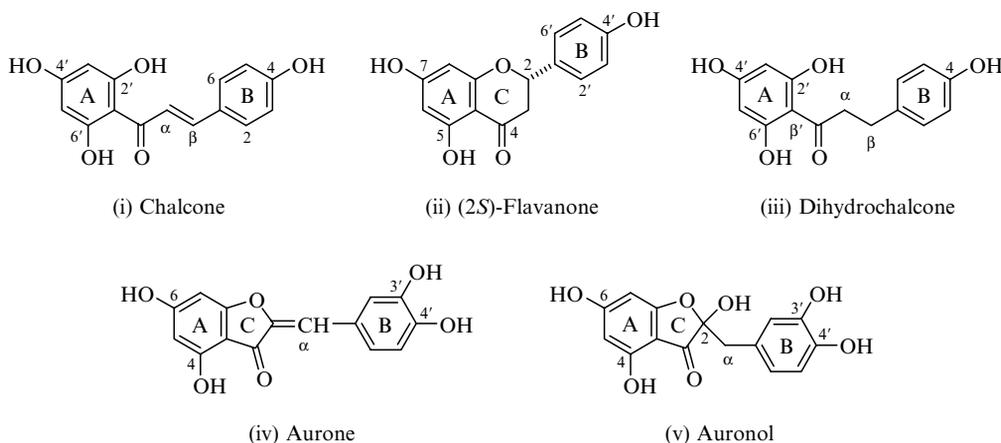


FIGURE 16.1 Guide to the structures, ring labeling, and atom numbering of chalcones, dihydrochalcones, aurones, and auronols. The corresponding flavanone structure is shown for reference.

ures that are specific to new compounds will be highlighted. The total number of flavonoids presented in this chapter as new natural products is 377, comprising 248 chalcones, 91 dihydrochalcones, and 38 aurones. Among these are a small number of known compounds for which new or revised structures have been proposed. It should be noted that new sources of well-known compounds have not been documented in this survey.

16.1.1 NOMENCLATURE

The nomenclature and in particular the atom numbering of chalcones, dihydrochalcones, and aurones remain a potential source of confusion when compared to that of other classes of flavonoid. The A- and B-rings of all the flavonoids have the same origin in biosynthetic terms, with the A-rings derived from the acetate pathway and the B-rings from the shikimate pathway. Similarly, all the structures are written by convention with the A-ring to the left (although this convention may break down for the more complex chalcones, such as dimers, oligomers, and Diels–Alder adducts). The crucial difference is in the style of atom numbering, in which primed numbers are used to refer to the A-ring of chalcones and dihydrochalcones, but to the B-ring of other flavonoid classes, including the aurones. Similarly, the B-rings of chalcones and dihydrochalcones carry the nonprimed numbers instead of the A-ring. The numbering scheme followed for chalcones and dihydrochalcones is also different, because the C₃ unit linking the A- and B-rings is referred to only in terms of carbonyl (β'), α - and β -carbons, whereas the equivalent carbon atoms of the heterocyclic C-rings of other flavonoids are numbered together with the rest of the molecule. An example of the two systems is shown in Figure 16.1 for clarification. Notice that the aurone numbering system is anomalous because of the five-membered C-ring. The result is that the A-ring positions equivalent to other flavonoids (excluding chalcones and dihydrochalcones) bear a number one less in value. Aurones are also referred to as 2-benzylidencoumaranones, although the correct systematic name is 2-benzylidene-3(2*H*)-benzofuranone. Similarly, auronols may also be found described as 2-hydroxy-2-benzylcoumaranones.

The IUPAC-approved systematic name for chalcone of 1,3-diphenyl-2-propen-1-one is generally thought too cumbersome for routine use, even for simple naturally occurring derivatives such as the commonly found 2',4',4'-trihydroxychalcone (isoliquiritigenin), which bears the systematic name 1-(2,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one. For this reason, the use of semi-systematic (2',4',4'-trihydroxychalcone) and trivial (isoliquiritigenin) names is widespread. So far as this chapter is concerned, semi-systematic names are given where possible, although the complexity of some of the compounds precludes the use of any but trivial names. The new compounds are arranged sequentially in 15 tables following the order chalcones (Table 16.1–Table 16.7), dihydrochalcones (Table 16.8–Table 16.12), and aurones (Table 16.13–Table 16.15). Within each class, the compounds are introduced as (i) simple derivatives (i.e., hydroxy, methoxy, methylenedioxy, and methyl substituted), (ii) isoprenylated derivatives, (iii) glycosides, and (iv) dimers, oligomers, adducts, and other conjugates or special groups. In tables of types (i) to (iii), the compounds are arranged in order of increasing *O*-substitution. Within each group the precedence of A- over B-ring over α - and β -carbons is observed for the individual substitution patterns, thus (2',4',6')-tri-*O*-substituted chalcones will be found listed before their (2',4',4)-tri-*O*-substituted analogs. Within the subgroups compounds with the greatest number of free hydroxyl groups take precedence, thus 2',4',2-trihydroxy-6'-methoxychalcone **6** appears before 2'-hydroxy-4',6',2-trimethoxychalcone (**7**), although both are examples of (2',4',6',2)-tetra-*O*-substituted chalcones. The purpose of this system is to allow groups of compounds with a particular substitution pattern to be identified easily for comparison with previous listings.^{1–4} Furthermore, this arrangement of compounds on a structural basis enables trends of potential

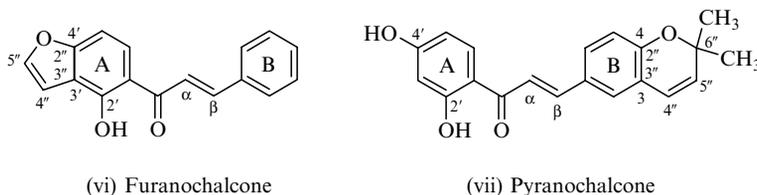


FIGURE 16.2 Atom numbering in furano- and pyranochalcones.

biosynthetic or chemosystematic interest to be recognized more easily. With regard to isoprenylated derivatives, the isoprenoid-derived groups are treated as substituents to the flavonoid skeleton, for example, as prenyl-, geranyl-, furano-, and pyranochalcones. The numbering system used to identify the location of the fused-ring substituents is indicated in Figure 16.2. In tables listing chalcone, dihydrochalcone, and aurone glycosides, the sugars can be assumed to be pyranosides unless otherwise stated. The absolute configurations of *D* and *L* for the sugars have been omitted as most were not determined experimentally. A checklist of all new chalcones, dihydrochalcones, and aurones (1–377) arranged by molecular formula is given in Appendix A. This is followed by a list containing entries for all known compounds of these flavonoid classes published to the end of 2003 (Appendix B).

16.1.2 OVERVIEW OF CHALCONE BIOSYNTHESIS

Much has been written about the biosynthesis of flavonoids, and several recent review articles are available.^{11–13} Only a few essential points relating to chalcone biosynthesis will be mentioned here. An outline of the most important enzyme-catalyzed reactions leading to the production of chalcones and 6'-deoxychalcones is given in Figure 16.3. Central to this scheme is the biosynthesis of 2',4',6',4-tetrahydroxychalcone, which is also known by the trivial names of chalconaringenin or naringenin chalcone. This compound is formed by sequential condensation of three molecules of malonyl coenzyme A (malonyl-CoA) with one of *p*-coumaroyl-CoA, a reaction catalyzed by chalcone synthase.¹⁴ The final step is believed to be the cyclization of a tetraketide precursor (Figure 16.3). The production of chalcones as natural products represents the convergence of two biosynthetic pathways, the acetate (leading to the A-ring) and the shikimate (leading to the B-ring), respectively, as mentioned previously. It might be supposed that the extent of hydroxyl substitution in the B-ring could be controlled by introduction of different shikimate-derived cinnamoyl-CoA precursors. Thus, cinnamoyl-, *p*-coumaroyl-, caffeoyl-, and 3,4,5-trihydroxycinnamoyl-CoA would give an unsubstituted B-ring, 4-hydroxy, 3,4-dihydroxy, and 3,4,5-trihydroxy substitution patterns, respectively. However, many studies indicate that the extent of B-ring hydroxyl substitution in flavonoids is controlled at the C₁₅ level, rather than by incorporation of specific cinnamoyl-CoA derivatives.¹¹ Thus, further elaboration of the B-ring resulting from *p*-coumaroyl-CoA (4-hydroxyl) appears to be achieved through specific hydroxylase and methyl transferase enzymes. Some evidence has been presented for the existence of a specific 3-hydroxylase for chalcones that is distinct from the general flavonoid 3'-hydroxylase,¹⁵ although both enzymes are known to be cytochrome P450-dependent monooxygenases. How the number of hydroxyl groups in the A-ring of chalcones is controlled is also an interesting question because of the existence of many 6'-deoxychalcone derivatives. A typical example is isoliquiritigenin (2',4',4-trihydroxychalcone), a common constituent of

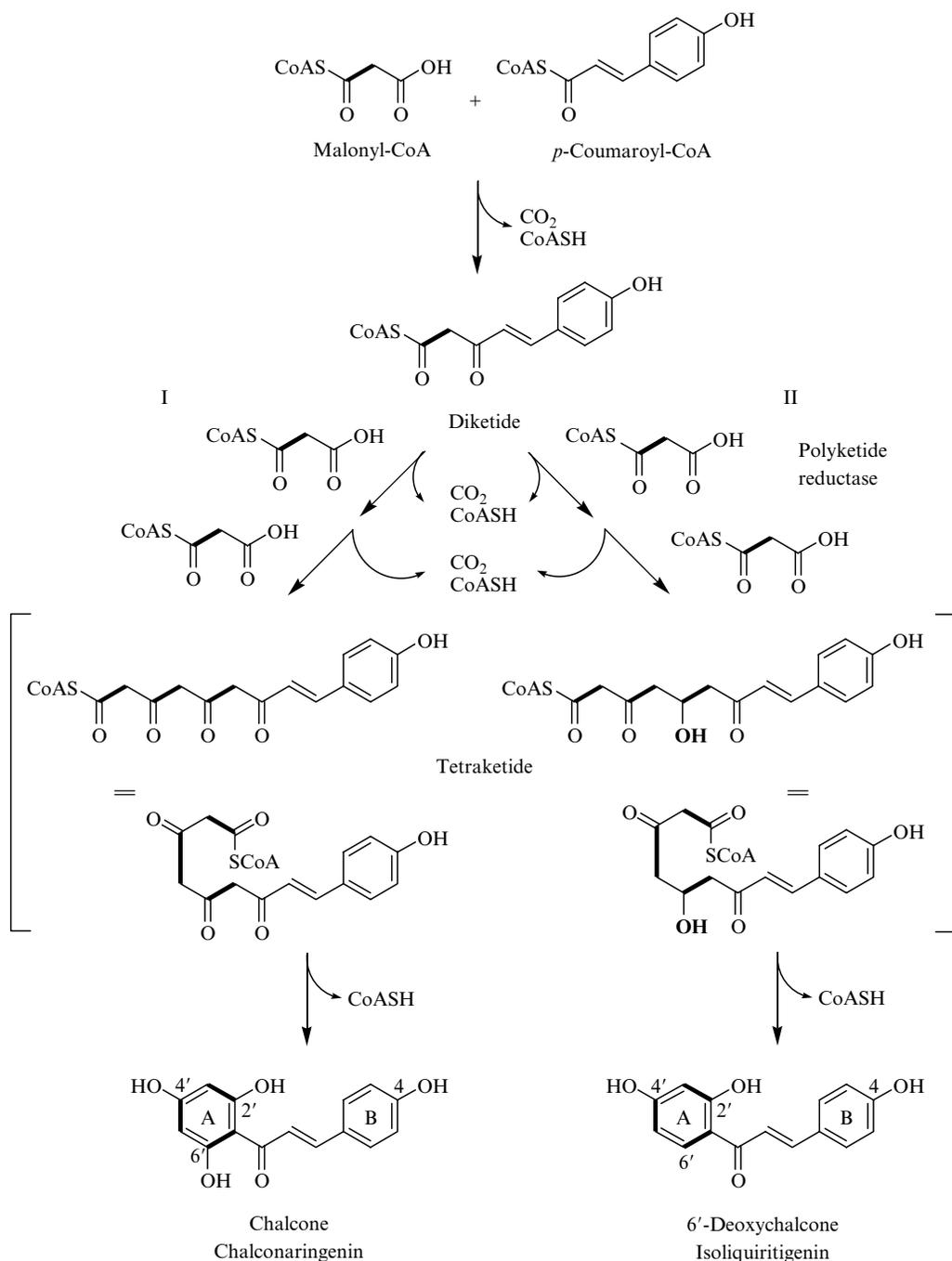


FIGURE 16.3 Overview of the biosynthesis of (I) chalcones and (II) 6'-deoxychalcones. The sequential condensation of three molecules of malonyl-CoA (acetate pathway) and *p*-coumaroyl-CoA (shikimate pathway) is catalyzed by the enzyme chalcone synthase.¹¹ The production of 6'-deoxychalcones is thought to involve an additional reduction step at the tri- or tetraketide level, catalyzed by polyketide reductase.^{14,16} The origin of the A-ring carbons derived from the acetate pathway is indicated in bold. CoA, coenzyme A.

the Leguminosae. The most likely explanation for their formation is a reduction step at the polyketide level, as shown in Figure 16.3. This type of reaction is catalyzed by a NADPH-dependent monomeric enzyme referred to in the literature as either chalcone ketide reductase or polyketide reductase.^{14,16} An insight into the biosynthesis of the less common *C*-methyl chalcones has resulted from the identification of a chalcone synthase-related protein in *Pinus strobus* seedlings that catalyzes a chain extension reaction with methylmalonyl-CoA.¹⁷ Incorporation is predicted to occur at the diketide stage in order to produce 2',4',6'-trihydroxy-3'-methylchalcone, the immediate precursor of the flavanones cryptostrobin (5,7-dihydroxy-8-methylflavanone) and strobopinin (5,7-dihydroxy-6-methylflavanone) found in this species.¹⁷

A small group of chalcone derivatives for which the typical *O*-substitution patterns of the A- and B-rings are apparently reversed are known as retrochalcones.²⁻⁴ A typical example is echinatin (4',4'-dihydroxy-2-methoxychalcone), a compound isolated from tissue culture of *Glycyrrhiza echinata* (Leguminosae),¹⁸ which is the retrochalcone equivalent of isoliquiritigenin 2'-methyl ether (4',4'-dihydroxy-2'-methoxychalcone). The first studies on the biosynthesis of echinatin by Saitoh and colleagues show that in contrast to normal chalcones, the A-ring is shikimate-derived and the B-ring acetate-derived.¹⁹ The co-occurrence of echinatin, licodione, and licodione 2'-methyl ether in *G. echinata* cell cultures suggested that retrochalcones might be formed from chalcones with dibenzoylmethanes (the keto tautomers of β -hydroxychalcones) as intermediates.²⁰⁻²² The possibility of 2-hydroxyflavanones as precursors to dibenzoylmethanes was also considered.²² Additional support for this hypothesis came from the observation that isoliquiritigenin, liquiritigenin (7,4'-dihydroxyflavanone), 7,4'-dihydroxyflavone, licodione, licodione 2'-methyl ether, and echinatin were all constituents of *G. pallidiflora*.²³ More recent work on the enzymology of cultured licorice cells (*G. echinata*) confirms the intermediacy of both 2-hydroxyflavanones and dibenzoylmethanes in retrochalcone synthesis,^{24,25} as summarized in Figure 16.4. Some new examples of both retrochalcones and retrodihydrochalcones can be found in Table 16.1 and Table 16.8, respectively.

Little is known about the biosynthesis of dihydrochalcones from chalcones, but an enzyme involved in aurone biosynthesis has recently been identified for the first time.²⁶ This important breakthrough came from the work of Nakayama and colleagues on the origins of the yellow flower color of the snapdragon, *Antirrhinum majus* (Scrophulariaceae). This is due to aurone glycosides located in vacuoles of the epidermal cells of the flowers, such as the 6-*O*-glucosides of aureusidin (4,6,3',4'-tetrahydroxyaurone) and bracteatin (4,6,3',4',5'-pentahydroxyaurone). The aglycones of these aurones are now known to be formed through the action of aureusidin synthase on the chalcone precursors, chalconarinigenin (2',4',6',4'-tetrahydroxychalcone) and 2',4',6',3,4-pentahydroxychalcone (Figure 16.5).²⁷⁻²⁹ This enzyme has been characterized as a monomeric 39-kDa glycoprotein with a binuclear copper center. Sequence homology analysis indicates that it belongs to the family of plant polyphenol oxidases,²⁶ enzymes that catalyze the conversion of monophenols to *ortho*-diphenols and *ortho*-quinones. Two important features of the mechanism involving aureusidin synthase (Figure 16.5) are the *ortho*-hydroxylation of the chalcone B-ring and the oxidative cyclization step to give the aurone C-ring.^{28,29} However, aureusidin can also be formed from 2',4',6',3,4-pentahydroxychalcone, presumably by the initial transformation of the *o*-dihydroxy B-ring of the latter to an *o*-diquinone. The biosynthetic origin of aurones with only one or no hydroxyl groups in the B-ring is unknown at present.

For details of the enzymes that further modify chalcone and other flavonoid structures by hydroxylation, methylation, glycosylation, and other processes, the review by Forkmann and Heller can be recommended.¹¹

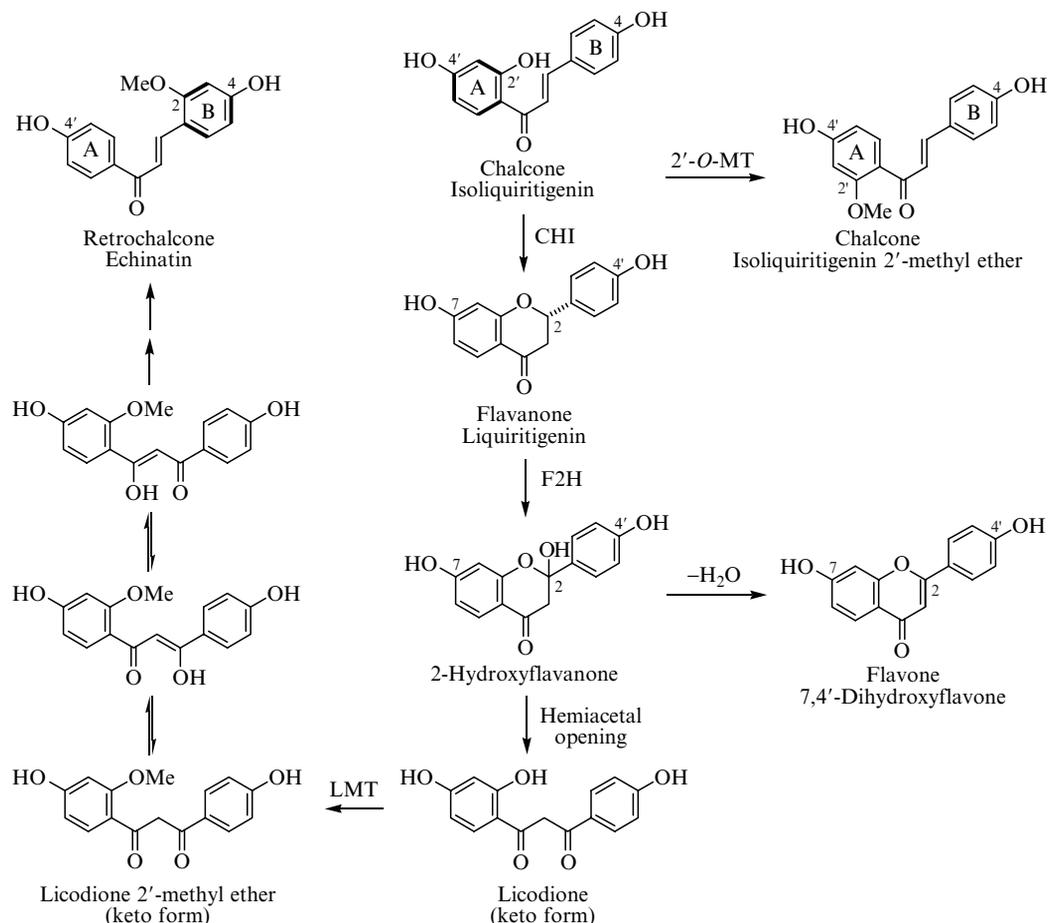


FIGURE 16.4 An outline of the biosynthesis of retrochalcones based on the production of echinatin in cell cultures of *Glycyrrhiza echinata* (Leguminosae).^{19–22,24,25} The transformation of isoliquiritigenin to its 2'-methyl ether is a reaction catalyzed by 2'-O-methyltransferase (2'-O-MT) in *Medicago sativa* (Leguminosae) cell cultures.¹¹ Enzymes involved in the biosynthesis of echinatin include chalcone isomerase (CHI), flavanone 2-hydroxylase (F2H), and licodione methyl transferase (LMT). The order of the final sequence of reactions leading to echinatin remains to be confirmed.

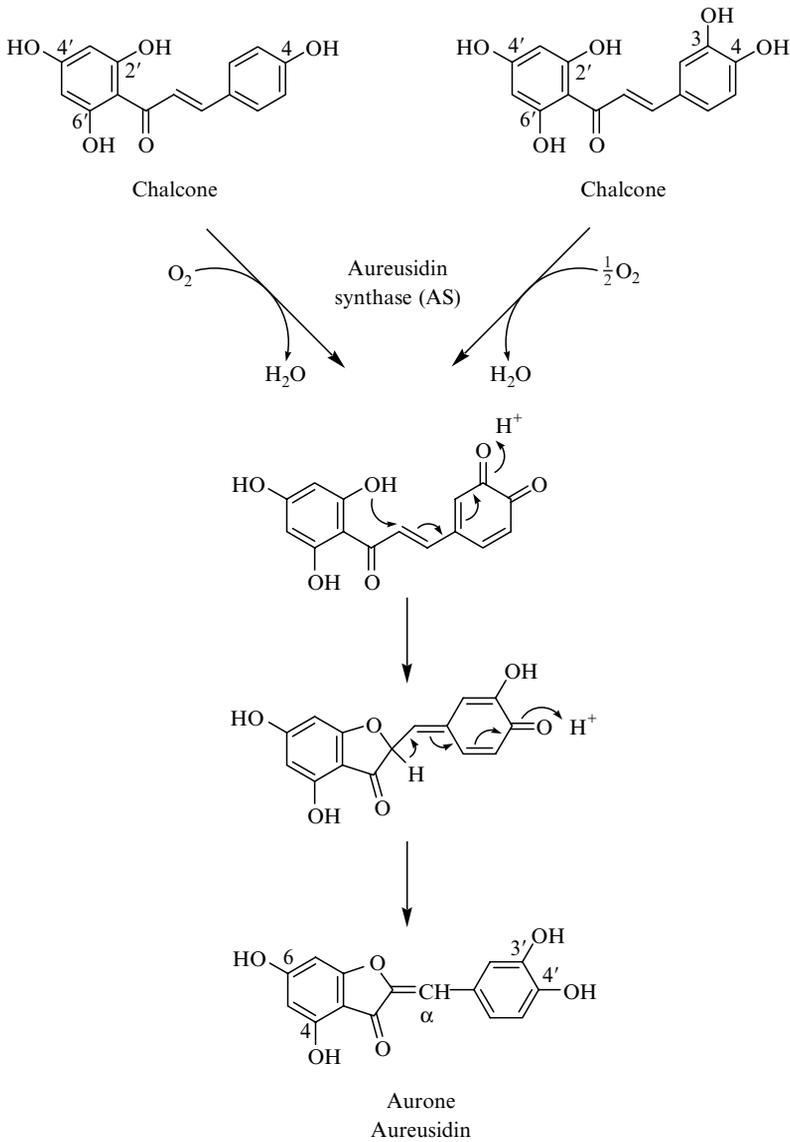
16.2 CHALCONES

16.2.1 CHALCONES WITH SIMPLE PATTERNS OF O-SUBSTITUTION

16.2.1.1 Structures and Synthetic Derivatives

New chalcone aglycones reported in the literature between 1992 and 2003 are shown in Table 16.1, with some examples illustrated in Figure 16.6. This listing excludes the larger group of isoprenylated chalcones that are found in Table 16.2, but does include a small number of examples with C-methyl, formyl, and halogen substituents. Most of the patterns of O-substitution (hydroxy, methoxy, and methylenedioxy) represented by the chalcones in Table 16.1 are well known,^{1–4,10} although both new and rare combinations continue to be discovered. Among these are a di-O-substituted chalcone (**1**) isolated from the whole plant of *Primula macrophylla* (= *P. stuartii*) (Primulaceae) and known previously only as a synthetic

A



B

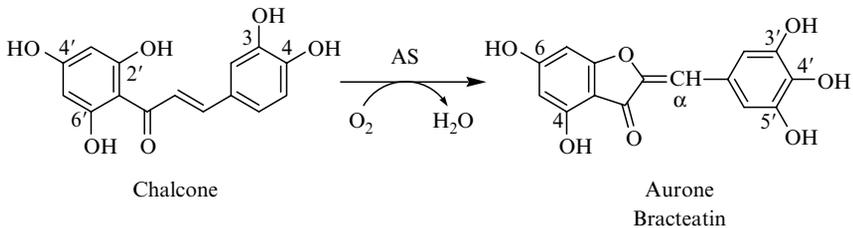


FIGURE 16.5 Reactions catalyzed by the plant polyphenol oxidase, aureusidin synthase (AS), in the transformation of chalcones to aurones.^{26–29} (A) The biosynthesis of aureusidin from either tetra- or pentahydroxychalcone precursors through an *ortho*-diquinone intermediate. (B) The aureusidin synthase-catalyzed formation of bracteatin from a pentahydroxychalcone precursor.

TABLE 16.1
New Chalcones with Simple Patterns of O-Substitution Reported in the Literature from 1992 to 2003

No.	OH	OME	Other	Mol. Formula	Trivial Name	Source	Family	Ref.
Di O-substituted								
	(3',3')							
1	3',3	—	—	C ₁₅ H ₁₂ O ₃		<i>Primula macrophylla</i>	Primulaceae	30
Tri O-substituted								
	(2',4',6')							
2	2',6'	4'	3'-CHO, 5'-Me	C ₁₈ H ₁₆ O ₅	Leridalchalcone	<i>Petiveria alliacea</i>	Phytolaccaceae	31
	(2',5',4)							
3	2',5',4	—	—	C ₁₅ H ₁₂ O ₄		<i>Platymiscium yucatanum</i>	Leguminosae	32
	(4',2,4)							
4	4	4',2	—	C ₁₇ H ₁₆ O ₄	Glypallichalcone	<i>Glycyrrhiza pallidiflora</i>	Leguminosae	33
Tetra O-substituted								
	(2',3',4',4)							
5	2',4	3',4'	—	C ₁₇ H ₁₆ O ₅	Heliannone A	<i>Helianthus annuus</i>	Asteraceae	34
	(2',4',6',2)							
6	2',4',2	6'	—	C ₁₆ H ₁₄ O ₅		<i>Scutellaria strigilosa</i>	Lamiaceae	35
	(2',4',6',4)							
7	2'	4',6',2	—	C ₁₈ H ₁₈ O ₅		<i>Andrographis lineata</i>	Acanthaceae	36
	(2',4',3,4)							
8	2',4'	6',4	—	C ₁₇ H ₁₆ O ₅		<i>Vitex leptobotrys</i>	Verbenaceae	37
	(2',3,4)							
9	2'	4',6',4	5'-Br	C ₁₈ H ₁₇ O ₅ Br		<i>Garcinia nervosa</i>	Guttiferae	38
	(2',3,4,5)							
10	4'	2',6',4	—	C ₁₈ H ₁₈ O ₅		<i>Vitex leptobotrys</i>	Verbenaceae	37
	(2',3,4,6)							
11	2',3,4	4'	—	C ₁₆ H ₁₄ O ₅	Calythroprosin	<i>Calythropsis aurea</i>	Myrtaceae	39
	(2',3,4,5)							
12	—	2',4'	3,4-OCH ₂ O—	C ₁₈ H ₁₆ O ₅		<i>Millettia erythrocalyx</i>	Leguminosae	40
	(2',3,4,6)							
13	2'	3,4,5	—	C ₁₈ H ₁₈ O ₅	Crotaoprostrin	<i>Crotalaria prostrata</i>	Leguminosae	41
	(2',3,4,6)							
14	2	3,4,6	—	C ₁₈ H ₁₈ O ₅	Tepanone	<i>Elliptea cuneifolia</i>	Annonaceae	42

continued

TABLE 16.1
New Chalcones with Simple Patterns of O-Substitution Reported in the Literature from 1992 to 2003 — continued

No.	OH	OMe	Other	Mol. Formula	Trivial Name	Source	Family	Ref.
Penta O-substituted								
	(2',3',4',3,4)							
15	2',4',4	3',3	—	C ₁₇ H ₁₆ O ₆		<i>Wedelia asperima</i>	Asteraceae	43
	(2',4',6',2,3)							
16	2'	4',6',2,3	—	C ₁₉ H ₂₀ O ₆		<i>Caesalpinia pulcherrima</i>	Leguminosae	44
	(2',4',6',3,4)							
17	—	2',4',6'	3,4-OCH ₂ O-	C ₁₉ H ₁₈ O ₆		<i>Millettia leucantha</i>	Leguminosae	45
	(3',4',2,3,4)							
18	3',4',3,4	2	—	C ₁₆ H ₁₄ O ₆		<i>Glycyrrhiza uralensis</i>	Leguminosae	46
Hexa O-substituted								
	(2',3',4',5',6',4)							
19	3',4	2',4',5',6'	—	C ₁₉ H ₂₀ O ₇		<i>Didymocarpus leucocalyx</i>	Gesneriaceae	47
	(2',3',4',6',3,4)							
20	6',3,4	2',3',4'	—	C ₁₈ H ₁₈ O ₇	Hamilcone	<i>Uvaria hamiltonii</i>	Annonaceae	48
	(2',4',6',2,3,4)							
21	2'	3',4',6',3,4	—	C ₂₀ H ₂₂ O ₇		<i>Citrus kinokuni</i>	Rutaceae	49
	(2',4',6',2,3,4)							
22	—	2',4',6',2,3,4	—	C ₂₁ H ₂₄ O ₇		<i>Andrographis neesiana</i>	Acanthaceae	50
β-Hydroxy substituted								
	(2',4',6',β)							
23	2',β	4',6'	3'-Me	C ₁₈ H ₁₈ O ₅		<i>Leptospermum scoparium</i>	Myrtaceae	51
	(3',4',3,4,β)							
24	β	—	3',4':3,4-bis (-OCH ₂ O-)	C ₁₇ H ₁₂ O ₆	Galiposin	<i>Gatipea granulosa</i>	Rutaceae	52
	(2',4',6',3,4,β)							
25	β	2',4',6'	3,4-OCH ₂ O-	C ₁₉ H ₁₈ O ₇	Ponganone X	<i>Pongamia pinnata</i>	Leguminosae	53
	(3',4',2,4,6,β)							
26	—	2,4,6,β	3',4'-OCH ₂ O-	C ₂₀ H ₂₀ O ₇		<i>Millettia leucantha</i>	Leguminosae	45

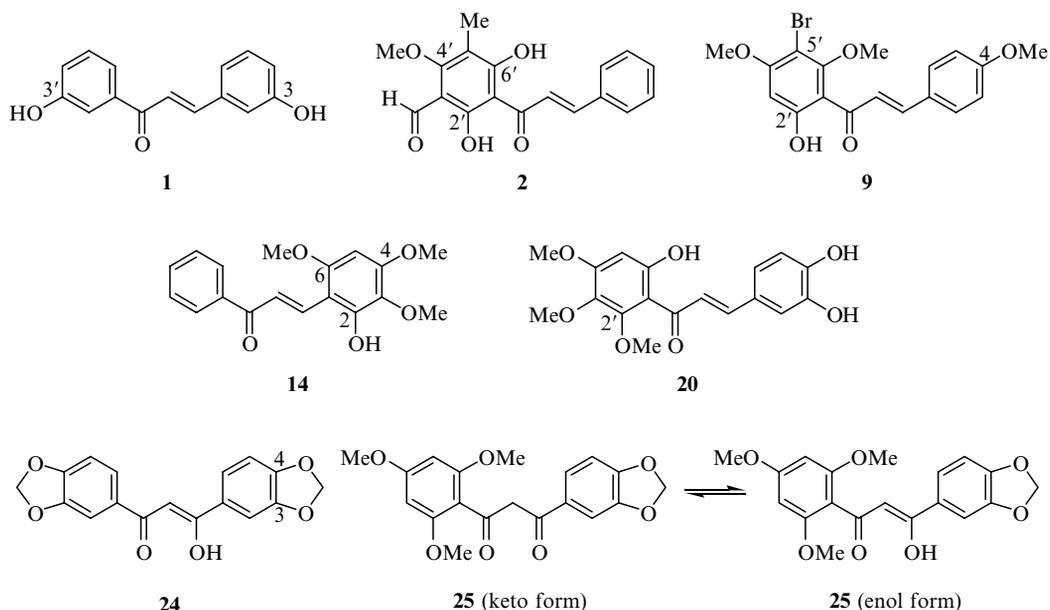


FIGURE 16.6 Chalcones with simple patterns of *O*-substitution (see Table 16.1).

product.³⁰ Other di-*O*-substituted chalcones found previously in this genus are 2',2'-dihydroxychalcone from *P. denticulata* and 2', β -dihydroxychalcone from *P. pulverulenta*.^{54,55} An extract of the heartwood of *Platymiscium yucatanum* (Leguminosae), a tropical wood that is highly resistant to the fungi *Coriolus versicolor* and *Lenzites trabea*, yielded an antifungal chalcone (**3**) with the rare (2',5',4)-*O*-substitution pattern.³² Several chalcones produced by synthesis have now been found as natural products, including **12**, **13**, and **16**.^{40,41,44} The structure of 2',4',4'-trihydroxy-3,3'-dimethoxychalcone (**15**), a constituent of the aerial parts of *Wedelia asperima* (Asteraceae), was confirmed by chemical synthesis as well as by spectroscopic methods.⁴³ Compounds **13** and **14** are both examples of retrochalcones, in which the typical patterns of *O*-substitution in the A- and B-rings appear to be reversed. The methyl ether of tepanone (**14**), 2,3,4,6-tetramethoxychalcone, prepared either by methylation of the natural product or by Claisen–Schmidt condensation of acetophenone with 2,3,4,6-tetramethoxybenzaldehyde, showed *trans*–*cis* isomerization.⁴² The instability of the parent compound (tepanone) may be due to a similar isomerization step, as the *cis*-isomer readily converts to a 2-hydroxyflav-3-ene derivative (or a colored flavylum salt in the presence of acid). Among the more highly substituted chalcones listed in Table 16.1 is a new example from leaves of *Didymocarpus leucocalyx* (Gesneriaceae) with a fully substituted A-ring (**19**).⁴⁷ Several other compounds of this type were obtained previously from *D. pedicellata*, although their distribution is not exclusive to this genus.¹⁰

Leridalchalcone (**2**) is a rare example of a chalcone with both *C*-methyl and formyl substituents isolated from the aerial parts of *Petiveria alliacea* (Phytolaccaceae).³¹ Perhaps more striking is a report from Ilyas et al. describing the first halogenated chalcone to be obtained as a natural product.³⁸ This compound, 5'-bromo-2'-hydroxy-4',6',4'-trimethoxychalcone (**9**), was obtained from the leaves of *Garcinia nervosa* (Guttiferae) together with the known derivatives 2'-hydroxy-4',4'-dimethoxychalcone and 2'-hydroxy-4',6',3,4-tetramethoxychalcone. The presence of bromine as a substituent was confirmed by mass spectrometry and chemical analysis. The plant material used for the isolation work was collected

TABLE 16.2
New Isoprenylated Chalcones Reported in the Literature from 1992 to 2003

No.	O-Substituents	Other Substituents	Mol. Formula.	Trivial Name	Source	Family	Ref.
Di O-substituted							
<i>(2',4')</i>							
27	2',4'-diOH	3',5'-Diprenyl	C ₂₅ H ₂₈ O ₃	Spinochalcone A	<i>Tephrosia spinosa</i>	Leguminosae	63
28	2'-OH	6''-(4-Methylpent-3-enyl)-6''-methylpyrano[2'',3'':4',3']	C ₂₅ H ₂₆ O ₃	Spinochalcone B	<i>Tephrosia spinosa</i>	Leguminosae	63
29	2'-OH	3'-Prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5']	C ₂₅ H ₂₆ O ₃	Spinochalcone C	<i>Tephrosia spinosa</i>	Leguminosae	64
30	2'-OH	Complex (FIGURE 16.7)	C ₂₁ H ₂₀ O ₅	(+)-Tephrone	<i>Tephrosia purpurea</i>	Leguminosae	65
Tri O-substituted							
<i>(2',4',6')</i>							
31	2',4',6'-triOH	3'-Neryl	C ₂₅ H ₂₈ O ₄		<i>Helichrysum retrosum</i>	Asteraceae	66
32	2',4'-diOH	3'-Prenyl, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofuranol[2'',3'':6',5']	C ₂₅ H ₂₈ O ₅	Cedrediprenone	<i>Cedrelopsis grevei</i>	Pteroxylaceae	67
33	4',6'-diOH	6'',6''-Dimethyl-5''-hydroxy-4'',5''-dihdropyranol[2'',3'':2',3']	C ₂₀ H ₂₀ O ₅		<i>Helichrysum aphelxioides</i>	Asteraceae	66
34	2'-OH, 6'-OMe	5''-Isopropenyl-4'',5''-dihydrofuranol[2'',3'':4',3']	C ₂₁ H ₂₀ O ₄	Crassichalcone	<i>Tephrosia crassifolia</i>	Leguminosae	68
35	2'-OH, 6'-OMe	4'',5''-Epoxy(6'',6''-dimethyl-4'',5''-dihdropyranol[2'',3'':4',3'])	C ₂₁ H ₂₀ O ₅	Epoxyobovatachalcone	<i>Tephrosia carrollii</i>	Leguminosae	69
36	2'-OH, 6'-OMe	6'',6''-Dimethyl-5''-hydroxy-4'',5''-dihdropyranol[2'',3'':4',3']	C ₂₁ H ₂₂ O ₅	6''-Methoxy-helikrauschalcone	<i>Cedrelopsis grevei</i>	Pteroxylaceae	67
37	2'-OH, 6'-OMe	Complex (FIGURE 16.7)	C ₂₄ H ₂₄ O ₇	(+)-Tephropurpurin	<i>Tephrosia purpurea</i>	Leguminosae	70
38	4'-OH, 2'-OMe	6'',6''-Dimethylpyrano[2'',3'':6',5']	C ₂₁ H ₂₀ O ₄	Cedreprenone	<i>Cedrelopsis grevei</i>	Pteroxylaceae	67
39	4'-OH, 6'-OMe	6'',6''-Dimethyl-5''-hydroxy-4'',5''-dihdropyranol[2'',3'':2',3']	C ₂₁ H ₂₂ O ₅		<i>Helichrysum aphelxioides</i>	Asteraceae	66
40	6'-OH, 4'-OMe	Complex-C ₁₀ (FIGURE 16.7)	C ₂₆ H ₃₀ O ₅	Linderol A	<i>Lindera umbellata</i>	Lauraceae	71
<i>(2',4',4')</i>							
41	2',4',4'-triOH	3'-(2-Hydroxy-3-methylbut-3-enyl)	C ₂₀ H ₂₀ O ₅		<i>Maclura tinctoria</i>	Moraceae	72
42	2',4',4'-triOH	3'-(4-Coumaroyloxy-3-methyl-but-2(E)-enyl)	C ₂₉ H ₂₆ O ₇	Isogemichalcone B	<i>Hypericum geminiflorum</i>	Guttiferae	73
43	2',4',4'-triOH	3'-(4-Coumaroyloxy-3-methyl-but-2(Z)-enyl)	C ₂₉ H ₂₆ O ₇	Gemichalcone B	<i>Hypericum geminiflorum</i>	Guttiferae	73
44	2',4',4'-triOH	3'-(4-Feruloyloxy-3-methyl-but-2(Z)-enyl)	C ₃₀ H ₂₈ O ₈	Gemichalcone A	<i>Hypericum geminiflorum</i>	Guttiferae	73
45	2',4',4'-triOH	3',3'-Diprenyl	C ₂₅ H ₂₈ O ₄	Kanzonol C	<i>Glycyrrhiza</i> sp.	Leguminosae	74
<i>(2',4',4',6')</i>							
46	2',4',4'-triOH	3'-Prenyl, 3'-(2-hydroxy-3-methylbut-3-enyl)	C ₂₅ H ₂₈ O ₅	Paratocarpin D	<i>Glycyrrhiza eurycarpa</i>	Moraceae	75
47	2',4',4'-triOH	3'-(2-Hydroxy-3-methylbut-3-enyl), 3'-prenyl	C ₂₅ H ₂₈ O ₅	Paratocarpin E	<i>Parartocarpus venenosa</i>	Moraceae	76
48	2',4',4'-triOH	5',3'-Diprenyl	C ₂₅ H ₂₈ O ₄	Stipulin	<i>Dalbergia stipulacea</i>	Leguminosae	77

49	2',4',4-triOH	3-Prenyl		C ₂₀ H ₂₀ O ₄	Licoagrochalcone A	<i>Glycyrrhiza glabra</i>	Leguminosae	78
50	2',4',4-triOH	3-(2-Hydroxy-3-methylbut-3-enyl), 5-prenyl		C ₂₅ H ₂₈ O ₅	Anthyllin	<i>Anthyllis hermantiata</i>	Leguminosae	79
51	2',4'-diOH	6',6'-Dimethylpyrano[2'',3'':4,3]		C ₂₀ H ₁₈ O ₄	Kanzonol B	<i>Glycyrrhiza eurycarpa</i>	Leguminosae	75
52	2',4'-diOH	3'-Prenyl, 6'',6''-dimethylpyrano[2'',3'':4,3]		C ₂₅ H ₂₆ O ₄	Paratocarpin C	<i>Parartocarpus venenosa</i>	Moraceae	76
53	2',4'-diOH	3'-Prenyl, 5''-(2-hydroxyisopropyl)-4''-hydroxy-4'',5''-dihydrofuranol[2'',3'':4,3]		C ₂₅ H ₂₈ O ₆	Paratocarpin G	<i>Parartocarpus venenosa</i>	Moraceae	80
54	2',4'-diOH	5-Prenyl, 6',6'-dimethylpyrano[2'',3'':4,3]		C ₂₅ H ₂₆ O ₄	Anthyllisone	<i>Anthyllis hermantiata</i>	Leguminosae	79
55	2',4'-diOH, 4'-OMe	3-Ceranyl		C ₂₆ H ₃₀ O ₄	Xanthoangelol F	<i>Angelica keiskei</i>	Umbelliferae	81
56	2',4'-diOH, 4'-OMe	3'-(3,7-Dimethyl-6-hydroxyocta-2,7-dienyl)		C ₂₆ H ₃₀ O ₅	Xanthoangelol G	<i>Angelica keiskei</i>	Umbelliferae	81
57	2',4'-diOH	6',6'-Dimethyl-4'',5''-dihydropyrano[2'',3'':4,3]		C ₂₀ H ₂₀ O ₄	Dorsmanin A	<i>Dorstenia mannii</i>	Moraceae	82
58	2',4'-diOH	6',6'-Dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':4,3]		C ₂₀ H ₂₀ O ₅		<i>Dorstenia zenkeri</i>	Moraceae	83
59	2',4'-diOH	3-Prenyl, 6',6'-dimethylpyrano[2'',3'':4,3]		C ₂₅ H ₂₆ O ₄	Paratocarpin B	<i>Parartocarpus venenosa</i>	Moraceae	76
60	2'-OH	Bis(6',6''-dimethylpyrano)[2'',3'':4,3][2'',3'':4,3]		C ₂₅ H ₂₄ O ₄	Paratocarpin A	<i>Parartocarpus venenosa</i>	Moraceae	76
61	2'-OH	5''-(2-Hydroxyisopropyl)-4'',5''-dihydrofuranol[2'',3'':4,3], 6'',6''-dimethylpyrano[2'',3'':4,3]		C ₂₅ H ₂₆ O ₅	Paratocarpin F	<i>Parartocarpus venenosa</i>	Moraceae	80
62	2'-OH	Bis(6',6''-dimethyl-4'',5''-dihydropyrano)[2'',3'':4,3][2'',3'':4,3]		C ₂₅ H ₂₈ O ₄	Artoindonesiamin J	<i>Artocarpus bracteata</i>	Moraceae	84
63	2'-OH	Bis(6',6''-dimethyl-4'',5''-dihydropyrano)[2'',3'':4,5][2'',3'':4,3]		C ₂₅ H ₂₈ O ₄		<i>Dorstenia kameruniana</i>	Moraceae	85
64	4-OH, 4'-OMe (4',2,4)	6',6''-Dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':2,3']		C ₂₁ H ₂₂ O ₅	Xanthoangelol H	<i>Angelica keiskei</i>	Umbelliferae	81
65	4',4'-diOH, 2'-OMe	3-Prenyl		C ₂₁ H ₂₂ O ₄	Licochalcone C	<i>Glycyrrhiza inflata</i>	Leguminosae	86
66	2,4'-diOH	6',6''-Dimethylpyrano[2'',3'':4,3]		C ₂₀ H ₁₈ O ₄	Munsericin	<i>Mundulea sericea</i>	Leguminosae	87
67	4'-OH, 2'-OMe	6',6''-Dimethylpyrano[2'',3'':4,3]		C ₂₁ H ₂₀ O ₄	Licoagrochalcone B	<i>Glycyrrhiza glabra</i>	Leguminosae	88
68	4'-OH, 2'-OMe	5''-(2-Hydroxyisopropyl)-4'',5''-dihydrofuranol[2'',3'':4,3]		C ₂₁ H ₂₂ O ₅	Licoagrochalcone D	<i>Glycyrrhiza glabra</i>	Leguminosae	88
		Tetra O-substituted						
		(2',4',6',4)						
69	2',4',6',4-tetraOH	3'-Geranyl		C ₂₅ H ₂₈ O ₅	3'-Geranylchalconaringenin	<i>Humulus lupulus</i>	Cannabaceae	89
70	2',4',4-triOH, 6'-OMe	3'-(2-Hydroxy-3-methylbut-3-enyl)		C ₂₁ H ₂₂ O ₆	Xanthohumol D	<i>Humulus lupulus</i>	Cannabaceae	90
71	2',4',4-triOH, 6'-OMe	3',5'-Diprenyl		C ₂₆ H ₃₀ O ₅	5'-Prenyl-xanthohumol	<i>Humulus lupulus</i>	Cannabaceae	89
72	2',4',4-triOH	3'-Prenyl, 6',6''-dimethylpyrano[2'',3'':6',5']		C ₂₅ H ₂₆ O ₅	Xanthohumol E	<i>Humulus lupulus</i>	Cannabaceae	90

continued

TABLE 16.2
New Isoprenylated Chalcones Reported in the Literature from 1992 to 2003 — continued

No.	O-substituents	Other Substituents	Mol. Formula.	Trivial Name	Source	Family	Ref.
73	2',6',4-triOH, 4'-OMe	3'-Prenyl	C ₂₁ H ₂₂ O ₅	Xanthogalenol	<i>Humulus lupulus</i>	Cannabinaaceae	90
74	2',4-diOH, 6'-OMe	6'',6''-Dimethylpyrano[2'',3'':4',3']	C ₂₁ H ₂₀ O ₅	Xanthohumol C, dehydrocyclooxanthohumol	<i>Humulus lupulus</i>	Cannabinaaceae	89
75	2',4-diOH, 6'-OMe	6'',6''-Dimethyl-4''-hydroxy-4''-5''-dihydropyrano[2'',3'':4',3']	C ₂₁ H ₂₂ O ₆	Isodehydrocyclooxanthohumol hydrate	<i>Humulus lupulus</i>	Cannabinaaceae	91
76	2',4-diOH, 6'-OMe	6'',6''-Dimethyl-5''-hydroxy-4''-5''-dihydropyrano[2'',3'':4',3']	C ₂₁ H ₂₂ O ₆	Xanthohumol B, dehydrocyclooxanthohumol hydrate	<i>Humulus lupulus</i>	Cannabinaaceae	89
77	2',4-diOH	Bis(6'',6''-dimethylpyrano)[2'',3'':4',3']-[2'',3'':6',5']	C ₂₅ H ₂₄ O ₅	Laxichalcone	<i>Derris laxiflora</i>	Leguminosae	92
78	2',4-diOH	6'',6''-Dimethylpyrano[2'',3'':4',3'], 6'',6''-dimethyl-4''-hydroxy-5''-methoxy-4'',5''-dihydropyrano[2'',3'':6',5']	C ₂₆ H ₂₈ O ₇	Derrichalcone	<i>Derris laxiflora</i>	Leguminosae	92
79	2'-OH, 4',4-diOMe	6'',6''-Dimethylpyrano[2'',3'':6',5']	C ₂₂ H ₂₂ O ₅	Glychalcone A	<i>Neoraputia magnifica</i>	Rutaceae	93
80	2'-OH, 6',4-diOMe (2',4',2,4)	6'',6''-Dimethylpyrano[2'',3'':4',3']	C ₂₂ H ₂₂ O ₅	Glychalcone A	<i>Glycosmis citrifolia</i>	Rutaceae	94
81	2',4',2,4-tetraOH	3'-(4-Coumaroyloxy-3-methyl-but-2(E)-enyl)	C ₂₉ H ₂₆ O ₈	Demethoxyisogemichalcone C	<i>Broussonetia papyrifera</i>	Moraceae	95
82	2',4',2,4-tetraOH	3'-(4-Feruloyloxy-3-methyl-but-2(E)-enyl)	C ₃₀ H ₂₈ O ₉	Isogemichalcone C	<i>Broussonetia papyrifera</i>	Moraceae	95
83	2',4',2,4-tetraOH (2',4',3,4)	3'-(4-Feruloyloxy-3-methyl-but-2(Z)-enyl)	C ₃₀ H ₂₈ O ₉	Gemichalcone C	<i>Hypericum geminiflorum</i>	Guttiferae	96
84	2',4',3,4-tetraOH	3'-Geranyl	C ₂₅ H ₂₈ O ₅		<i>Artocarpus incisus</i>	Moraceae	97
85	2',4',3,4-tetraOH	3',5-Digeranyl	C ₃₅ H ₄₄ O ₅		<i>Dorstenia prorpens</i>	Moraceae	83
86	2',4',3-triOH	3'-Prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5']	C ₂₅ H ₂₆ O ₅		<i>Glycyrrhiza</i> sp.	Leguminosae	74
87	2',4',4-triOH	3'-Prenyl, 6''-(4-methylpent-3-enyl), 6''-methylpyrano[2'',3'':3,2]	C ₃₀ H ₃₄ O ₅	Poinsettifolin B	<i>Dorstenia poinsettifolia</i>	Moraceae	99
88	2',3-diOH	Bis(6'',6''-dimethylpyrano)[2'',3'':4',3']-[2'',3'':4,5]	C ₂₅ H ₂₄ O ₅	Glyinflanin G	<i>Glycyrrhiza inflata</i>	Leguminosae	100
89	2'-OH, 3,4,-diOMe	6'',6''-Dimethylpyrano[2'',3'':4',3']	C ₂₂ H ₂₂ O ₅	3,4-Dimethoxylonchocarpin	<i>Lonchocarpus subglaucescens</i>	Leguminosae	101

90	(3',4',2,4) 3',4',4-triOH, 2-OMe (4',2,3,4)	3'-Prenyl	C ₂₁ H ₂₂ O ₅	Licoagrochalcone C	<i>Glycyrrhiza glabra</i>	Leguminosae	88
91	4',3,4-triOH, 2-OMe Penta O-substituted (2',4',5',3,4)	3'-Prenyl	C ₂₁ H ₂₂ O ₅	Licochalcone D	<i>Glycyrrhiza inflata</i>	Leguminosae	86
92	2'-OH, 5',3,4-triOMe (2',4',6',3,4)	6'',6'-Dimethylpyrano[2'',3'':4',3']	C ₂₃ H ₂₄ O ₆	Ponganone VI	<i>Pongamia pinnata</i>	Leguminosae	53
93	2'-OH, 4',3,4-triOMe	6'',6'-Dimethylpyrano[2'',3'':6',5']	C ₂₃ H ₂₄ O ₆		<i>Neoraputia magnifica</i>	Rutaceae	93
94	2'-OH, 6',3,4-triOMe (2',4',2,4,5)	6'',6'-Dimethylpyrano[2'',3'':4',3']	C ₂₃ H ₂₄ O ₆	Glychalcone B	<i>Glycosmis citrifolia</i>	Rutaceae	94
95	2',4',2,4,5-pentaoH Hexa O-substituted (2',3',4',6',3,4)	3'-Prenyl	C ₂₀ H ₂₀ O ₆	Ramosisin	<i>Crotalaria ramosissima</i>	Leguminosae	102
96	2'-OH, 3',6'-diOMe, 3,4-OCH ₂ O- (2',4',6',3,4,5)	Furan[2'',3'':4',5']	C ₂₀ H ₁₆ O ₇		<i>Lonchocarpus subglaucescens</i>	Leguminosae	101
97	6'-OH, 4',3,4,5-tetraOMe β-Hydroxy substituted (2',4',6',β)	6'',6'-Dimethylpyrano[2'',3'':2',3']	C ₂₄ H ₂₆ O ₇		<i>Neoraputia magnifica</i>	Rutaceae	103
98	2',6',β-triOH, 4'-OMe	3'-Prenyl	C ₂₁ H ₂₂ O ₅		<i>Tephrosia major</i>	Leguminosae	104
99	2',6',β-triOMe (2',4',4,β)	6'',6'-Dimethylpyrano[2'',3'':4',3']	C ₂₃ H ₂₄ O ₅	7-Methoxypraecansone B	<i>Pongamia pinnata</i>	Leguminosae	105
100	2',4',4,β-tetraOH	5',3-Diprenyl	C ₂₅ H ₂₈ O ₅	Glycyrdione A (glyinflarin A)	<i>Glycyrrhiza inflata</i>	Leguminosae	106
101	2',4',4,β-tetraOH	5'-Prenyl, 3-(2-hydroxy-3-methylbut-3-enyl)	C ₂₅ H ₂₈ O ₆	Glyinflarin E	<i>Glycyrrhiza inflata</i>	Leguminosae	100
102	2',4',4,β-tetraOH	3'-Prenyl	C ₂₀ H ₂₀ O ₅	Kanzonol A	<i>Glycyrrhiza eurycarpa</i>	Leguminosae	75
103	2',4',β-triOH	5'-Prenyl, 6'',6'-dimethylpyrano[2'',3'':4',3]	C ₂₅ H ₂₆ O ₅	Glycyrdione B	<i>Glycyrrhiza inflata</i>	Leguminosae	106
104	2',4,β-triOH	6'',6'-Dimethylpyrano[2'',3'':4',5']	C ₂₀ H ₁₈ O ₅	Glyinflarin B	<i>Glycyrrhiza inflata</i>	Leguminosae	107
105	2',4,β-triOH	3'-Prenyl, 6'',6'-dimethylpyrano[2'',3'':4',5']	C ₂₅ H ₂₆ O ₅	Glyinflarin C (glycyrdione C)	<i>Glycyrrhiza inflata</i>	Leguminosae	107, 108
106	2',4,β-triOH	3'-Prenyl, 5''-(2-hydroxyisopropyl)-4'',5''- dihydrofuran[2'',3'':4',5']	C ₂₅ H ₂₈ O ₆	Glyinflarin F	<i>Glycyrrhiza inflata</i>	Leguminosae	100
107	2',β-diOH (2',4',6',3,4,β)	Bis(6'',6'-dimethylpyrano[2'',3'':4',5']-[2'',3'':4',3])	C ₂₅ H ₂₄ O ₅	Glyinflarin D	<i>Glycyrrhiza inflata</i>	Leguminosae	107
108	β-OH, 2',6'-diOMe, 3,4-OCH ₂ O-	6'',6'-Dimethylpyrano[2'',3'':4',3']	C ₂₃ H ₂₂ O ₇	Pongapinone A	<i>Pongamia pinnata</i>	Leguminosae	109

from a site on contaminated land, which may explain the unexpected occurrence of the bromochalcone.

16.2.1.2 Biological Activity

Bioassay data are available for some of the new chalcones listed in Table 16.1. For example, Maćias et al. assessed the allelopathic activity of heliannone A (**5**) and other flavonoids from *Helianthus annuus* cultivars, including the known chalcone, kukulkanin B (2',4',4-trihydroxy-3'-methoxychalcone).³⁴ Despite the structural similarity of these compounds, heliannone A inhibited germination of both tomato and barley and shoot growth of barley, whereas kukulkanin B inhibited only the shoot growth of tomato. Calythropsin (**11**) was obtained together with its dihydrochalcone analog (**263**) during cytotoxicity-guided fractionation of an extract of the roots of *Calythropsis aurea* (Myrtaceae).³⁹ It showed weak cytotoxicity in the United States National Cancer Institute panel of 60 cell lines, a small effect on tubulin polymerization and weak antimitotic activity. Dihydrocalythropsin (**263**) was 10-fold less potent in the cell line panel compared to calythropsin, but is more abundant as a constituent of the original extract.³⁹ Moderate cytotoxicity was also reported for 2',4',6'-trimethoxy-3,4-methylenedioxychalcone (**17**), one of several chalcones and dihydrochalcones isolated from the stem bark of *Millettia leucantha* (Leguminosae).⁴⁵ Hamilcone (**20**) was tested for both cytotoxicity and DNA strand-scission activity but was only weakly active in these assays.⁴⁸ The penta-*O*-substituted chalcone **18** was one of several phenolic constituents isolated in a study of a commercial licorice sourced from *Glycyrrhiza uralensis* (Leguminosae) and used in northeastern China.⁴⁶ This compound shows a potent scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical and forms a stable radical in solution that can be detected using electron paramagnetic resonance (EPR) spectroscopy. The authors speculate that the stability of this radical may be responsible for the good activity shown by the chalcone in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay for antioxidant activity.⁴⁶

16.2.1.3 β -Hydroxychalcones

The β -hydroxychalcones are a relatively small group of chalcones that occur as the enol-tautomers of dibenzoylmethane derivatives (see Figure 16.6, **25**). Four new examples are listed in Table 16.1, and some additional isoprenylated derivatives will be found in Table 16.2. The extent of keto-enol tautomerism is largely solvent dependent, and nuclear magnetic resonance (NMR) spectroscopy provides one of the best methods to determine the ratio of the tautomers present. In ¹H NMR spectra recorded in CDCl₃, the exchangeable proton of the β -OH of the enol tautomer appears as a 1H singlet at ca. 16 ppm, whereas the α -CH₂ protons of the keto tautomer appear as a 2H singlet at ca. 4.50 ppm. Another diagnostic resonance is the 1H methine singlet of the enol tautomer (α -CH), which is found at ca. 6.50 ppm, with its corresponding C- α resonance at 90 to 92 ppm in ¹³C NMR spectra. For example, galiposin (**24**), a constituent of the bark of *Galipea granulosa* (Rutaceae), exists entirely in the enol form in solution; a sharp 1H singlet appears at 16.94 ppm (β -OH) and no resonance is found in the spectral region corresponding to the α -CH₂ protons of the keto form.⁵² The ¹H and ¹³C resonances of the enol methine (α -CH) are found at 6.62 and 91.6 ppm, respectively. This compound is also remarkable as the first bis(methylenedioxy)chalcone to be reported in the literature. The ¹H NMR spectrum of ponganone X (**25**) in CDCl₃ indicated a keto-enol tautomer ratio of 1:4, based on the relative intensities of the corresponding α -CH₂ and β -OH resonances at 4.31 ppm (2H, s) and 16.35 ppm (1H, s), respectively.⁵³ Similarly, the *C*-methylated derivative, 2', β -dihydroxy-4',6'-dimethoxy-3'-methylchalcone (**23**), from the aerial parts of *Leptospermum scoparium* (a plant from the

Myrtaceae used in Australian traditional medicine), exists in a keto–enol tautomer ratio of 3:2.⁵¹ This compound was known previously as a synthetic product but only limited physical (melting point) and no spectroscopic data were available.⁵⁶ The β -hydroxychalcone constituent obtained from *Millettia leucantha* (**26**) is a further example of a retrochalcone.⁴⁵

16.2.2 ISOPRENYLATED CHALCONES

16.2.2.1 Structural Diversity and Chemosystematic Trends

Table 16.2 lists more than 80 examples of new isoprenylated chalcones reported in the period 1992 to 2003 (see also Figure 16.7). Almost half of the compounds described here are from the Leguminosae, a trend that is also evident in earlier surveys.^{1–4} Other plant families that are well represented in Table 16.2 are the Moraceae and the Cannabinaceae. The literature on isoprenylated flavonoids in general has been reviewed by Barron and Ibrahim to the end of 1994.⁵⁷ The phenolic constituents of *Glycyrrhiza* species (licorice), among which are many isoprenylated chalcones, were the subject of an extensive review that includes literature published up to the end of 1996.⁵⁸ Nomura and Hano have reviewed the literature on isoprenylated phenolic compounds of the Moraceae to the end of 1993.⁵⁹ More recent descriptions of isoprenylated flavonoids are available for the hop plant, *Humulus lupulus* (Cannabinaceae),⁶⁰ and the Moraceae genera *Artocarpus*⁶¹ and *Dorstenia*.⁶²

The arrangement of the Table 16.2 entries follows the scheme adopted in Table 16.1, in which compounds are listed in order of increasing *O*-substitution. Within each heading, specific patterns of A- and B-ring substitution are noted. This system of structure classification allows types of chalcone that are specific to a particular species, genus, or family to be recognized more clearly. For example, isoprenylated (2',4')-di-*O*-substituted (**27–30**)^{63–65} and (4',2,4)-tri-*O*-substituted (**65–68**)^{86–88} chalcones reported in Table 16.2 are restricted to the Leguminosae. The compounds within these groupings are often closely related, such as spinochalcones A–C (**27–29**) obtained from the roots of *Tephrosia spinosa* (Leguminosae).^{63,64} In contrast, (+)-tephrosone (**30**), a constituent of *T. purpurea*, has the unusual feature of two fused furan rings,⁶⁵ as was also found in (+)-tephropurpurin (**37**), isolated from the same species (Figure 16.7).⁷⁰ The absolute configurations of the stereogenic centers of **30** were determined on the basis of ¹H NMR analysis of Mosher esters and by comparison with the recently determined absolute configuration of the related compound, (+)-purpurin, a flavanone first isolated in 1980 from seeds of *T. purpurea*.^{65,110,111} Isoprenylated chalcones of the Moraceae are predominantly isoliquiritigenin (2',4',4-trihydroxychalcone) derivatives, such as paratocarpins A–G (**46, 47, 52, 53, 59–61**) from *Parartocarpus venenosa* (cited incorrectly as *Paratocarpus venenosa*).^{76,80} Other typical substitution patterns for the Moraceae are represented in Table 16.2 by isoprenylated 2',4',2,4- (**81, 82**)⁹⁵ and 2',4',3,4-tetrahydroxychalcones (**84, 85, 87**).^{83,97,99} Compounds **81** and **82** represent a particularly unusual type of chalcone in which a prenyl side-chain is esterified by a hydroxycinnamic acid.⁹⁵ Four similar compounds (**42–44, 83**) have been isolated from the heartwood and root of *Hypericum geminiflorum* (Guttiferae).^{73,96}

Further investigation of the chemical constituents of the hop plant, *Humulus lupulus* (Cannabinaceae), has revealed a series of isoprenylated chalconaringenin derivatives (**69–76**), most of which are minor components related to xanthohumol (2',4',4-trihydroxy-6'-methoxy-3'-prenylchalcone), the major chalcone of this species.^{89–91} The new compounds, which are also components of the resin secreted by glandular trichomes (lupulin glands) on female inflorescences (“hop cones”) and the undersides of young leaves, occur at concentrations that are 10- to 100-fold less than xanthohumol.⁶⁰ Liquid chromatography coupled to mass spectrometry was used to detect the presence of 3',5'-diprenylchalconaringenin in hop extracts, a compound identified by comparison with a synthetic sample.⁹⁰ This isoprenylated

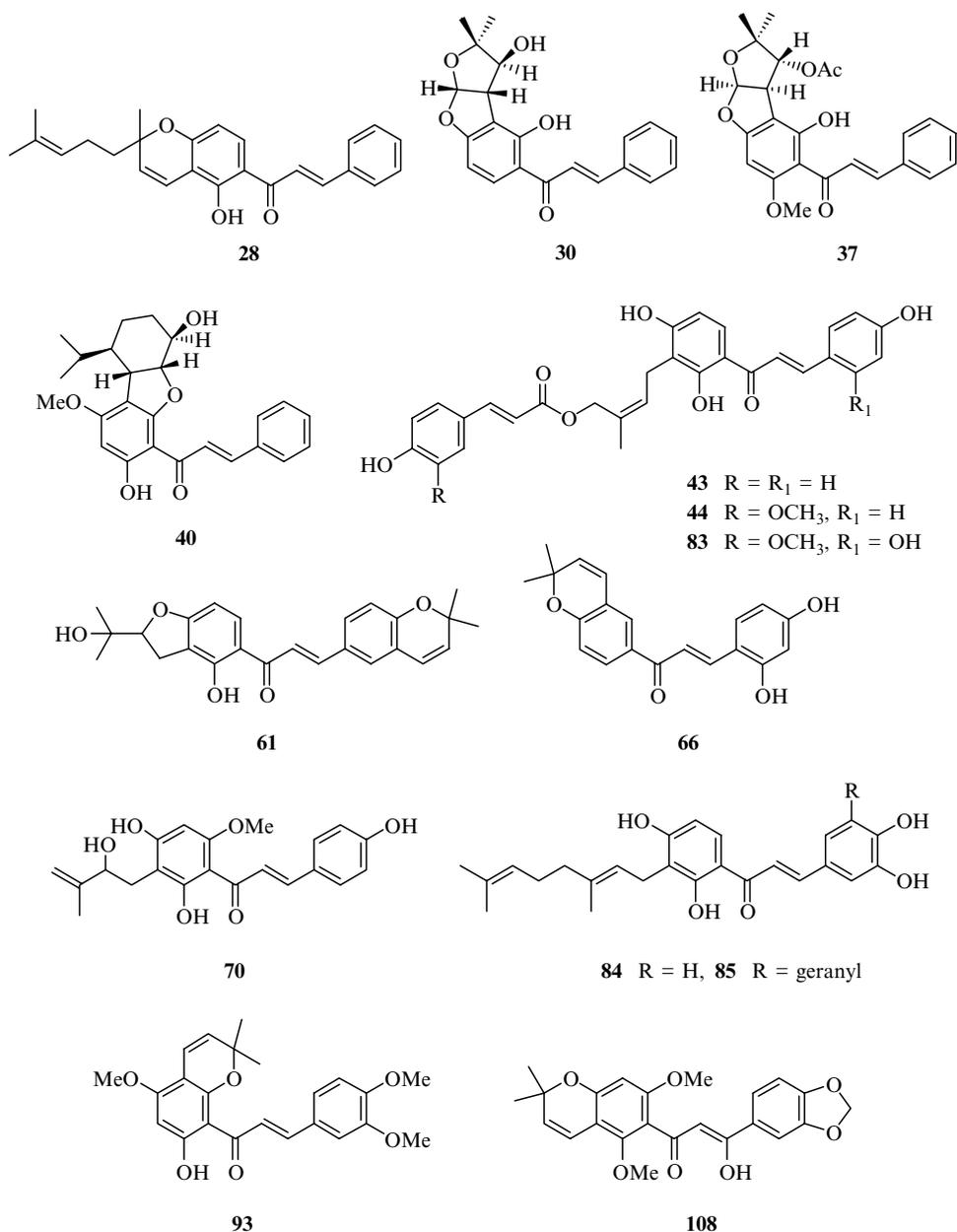


FIGURE 16.7 Isoprenylated chalcones (see Table 16.2).

chalcone still awaits isolation and characterization from a natural source. An extensive survey of the distribution of chalcones in 120 accessions of *H. lupulus* varieties and cultivars from three main geographical regions (Europe, Japan, and North America) has been made by Stevens and colleagues.⁹⁰ The compounds with the greatest diagnostic value for distinguishing regional chemotypes and possible evolutionary lineages are the 4'-*O*-methylchalcones, xanthohumol (73), xanthohumol 4'-methyl ether, and chalconaringenin 4',6'-dimethyl ether. The characteristic isoprenylated chalcones of *H. lupulus* were found to be absent from *H. japonicus*, a species from eastern Asia.⁹⁰

A survey of lipophilic phenolic compounds of *Helichrysum* endemics (Asteraceae) of Madagascar reveals that the constituent chalcones are largely characterized by unsubstituted B-rings and either *C*- or *O*-isoprenylation in the A-ring.⁶⁶ Three new chalcones (**31**, **33**, **39**) were obtained in the study, including one with the less commonly observed neryl substituent (**31**). It is interesting to note from a taxonomic viewpoint that this profile of chalcones and other phenolic substituents was not substantially different from that recorded for other African species of *Helichrysum*, indicating that these compounds represent chemical characters that were conserved during the origin of the endemic taxa of Madagascar.⁶⁶ For a comprehensive treatment of the flavonoids of the Asteraceae and their distribution, the monograph by Bohm and Stuessy is recommended.¹¹²

The Lauraceae is a family from which relatively few isoprenylated chalcones have been reported, and only one new example, linderol A (**40**),⁷¹ from the bark of *Lindera umbellata*, is listed in Table 16.2. In this compound, the B-ring is unsubstituted (Figure 16.7), a common feature of Lauraceae chalcones.¹⁰ Several other unusual monoterpene-substituted chalcones have been reported previously from the Lauraceae genera *Aniba* and *Lindera*.¹⁰ The unusual structure of linderol A (**40**), in which the A-ring is functionalized by a cyclized monoterpényl substituent, prompted the first total synthesis of this molecule.¹¹³

16.2.2.2 Biological Activity

The cancer chemopreventive properties of (+)-tephrosone (**30**) and (+)-tephropurpurin (**37**) from *Tephrosia purpurea* (Leguminosae) have been assessed in a cell-based quinone reductase induction assay.^{65,70} The induction of quinone reductase, a phase II drug-metabolizing enzyme, is believed to be an important protection mechanism against tumor initiation. Chang et al. demonstrated that (+)-tephropurpurin (**37**) was three times more active than sulforaphane, the positive control used in the assay, and had low cytotoxicity.⁷⁰ This compound may thus be a useful lead for development as a cancer chemopreventive agent. In a similar search for cancer chemopreventive agents from natural sources, munsericin (**66**) was found to inhibit phorbol ester-induced ornithine decarboxylase activity in cell culture.⁸⁷ The potential health-promoting effects of xanthohumol and other prenylated flavonoids from hops (*Humulus lupulus*) have been of great interest due to the presence of these compounds in beer. Stevens and Page characterize xanthohumol as a “broad spectrum” cancer chemopreventive agent with three important properties: (i) inhibition of the metabolic activation of procarcinogens, (ii) induction of carcinogen-detoxifying enzymes, and (iii) early stage inhibition of tumor growth.⁶⁰ Much of the literature on the biological activity of xanthohumol and other hop flavonoids, including the new isoprenylated chalcones listed in Table 16.2 (**69–76**), has been reviewed recently.⁶⁰

Kanzonol C (**45**), obtained by bioassay-guided fractionation of licorice roots (*Glycyrrhiza* sp.), showed potent antileishmanial activity *in vitro*, using an assay based on the inhibition of thymidine uptake in proliferating promastigotes of *Leishmania donovani*.⁷⁴ This compound had been synthesized previously as a potential antiulcer drug,¹¹⁴ but was not known as a natural product. A second chalcone (**86**) obtained from licorice roots showed relatively poor activity in the same assay.⁷⁴ The biological activity of many chalcones and other flavonoids from *Glycyrrhiza* species was reviewed in 1998 by Nomura and Fukai.⁵⁸

The biological activity of isoprenylated chalcones from the Moraceae has been described both in original papers and reviews.^{61,62} Of particular interest is the potent 5- α -reductase inhibition shown by a geranylated chalcone (**84**) isolated from leaves of *Artocarpus incisus*.⁹⁷ The inhibitory effect is decreased by a factor of 2 when the geranyl substituent is lacking, as in butein (2',4',3,4-tetrahydroxychalcone). Compound **41**, which was obtained from the leaves of *Maclura tinctoria* together with four known isoprenylated flavonoids, showed inhibitory

activity toward the AIDS-related opportunistic pathogens, *Candida albicans* and *Cryptococcus neoformans*.⁷² Among the reports of biological activity of chalcones from plant families less well represented in Table 16.2 are the superoxide scavenging properties of cedrediprenone (**32**), a compound obtained from an extract of the fruits and seeds of *Cedrelopsis grevei* (Ptaeroxylaceae),⁶⁷ and the potent inhibitory activity of linderol A (**40**) on melanin biosynthesis in cultured B-16 melanoma cells.⁷¹ The fruits of *Neoraputia magnifica* (Rutaceae) yielded a pyranochalcone (**93**), with weak inhibitory activity toward glycosomal glyceraldehyde-3-phosphate dehydrogenase from *Trypanosoma cruzi*, the causative agent of Chagas' disease.⁹³ Two polymethoxylated flavones isolated from the same source showed potent inhibition in this assay.

16.2.2.3 β -Hydroxychalcones

The 11 new isoprenylated β -hydroxychalcones (**98–108**) listed in Table 16.2 are restricted to three genera of the Leguminosae, *Glycyrrhiza*, *Pongamia*, and *Tephrosia*. The distribution of the tautomeric dibenzoylmethane (keto) and β -hydroxychalcone (enol) forms of the compounds can be assessed under solution conditions by NMR spectroscopy, as outlined in Section 16.2.1.3. For example, kanzonol A (**102**) was found to adopt an equilibrium mixture of keto–enol tautomers of ca. 2:3,⁷⁵ whereas the corresponding ratio for glycyrdiones A (**100**) and B (**103**) was close to 2:1.¹⁰⁶ Pongapinone A (**108**), which was obtained by bioassay-guided fractionation of extracts of the bark of *Pongamia pinnata*, inhibited the production of interleukin-1 with an IC_{50} value of 2.5 $\mu\text{g/ml}$.¹⁰⁹ In a related study, activity-guided fractionation of extracts of stem bark of the same species yielded 7-methoxypraecansone B (**99**), a compound active in the quinone reductase induction assay.¹⁰⁵

16.2.3 CHALCONE GLYCOSIDES

The structures of the 25 new chalcone glycosides reported from 1992 to 2003 (Table 16.3 and Figure 16.8) reflect trends already evident from analysis of ca. 60 such compounds described prior to 1992.¹⁰ Thus, isoliquiritigenin glycosides (**110–112**) appear to be typical of the Leguminosae (although not exclusive to this family), while glycosides of okanin and its methyl ethers (**125–131**) are characteristic of the Asteraceae and, in particular, the genus *Bidens*. The glycosidic profile of chalcone glycosides is relatively conservative compared to that of the glycosides of flavonols and flavones. Almost all chalcone monoglycosides are β -glucopyranosides, and only a few disaccharides are encountered with any frequency. No tri- or higher oligosaccharides have yet been reported as glycosidic components of chalcones. Only one new disaccharide is listed among the new chalcone glycosides in Table 16.3, the 4'-*O*- β -xylopyranosyl(1''' \rightarrow 6'')- β -glucopyranoside (primveroside) of okanin 4-methyl ether (**130**) isolated from aerial parts of *Bidens campylothea*.¹²⁹ The new bisdesmosidic triglycoside **110**, isolated from the roots of *Glycyrrhiza aspera* (Leguminosae), is only the second chalcone glycoside with three sugars to be reported in the literature.¹¹⁶ Its structure was determined by spectroscopic methods and confirmed by partial enzymatic hydrolysis to the known compound, isoliquiritin apioside. The disaccharide component of **110**, β -apiofuranosyl(1 \rightarrow 2)- β -glucopyranoside, is found in several chalcone glycosides of the Leguminosae (**110–112**)^{116,117} and Loranthaceae (**123**).¹²⁶ A further bisdesmosidic chalcone triglycoside (**120**) has been reported from the leaves of *Asarum canadense* (Aristolochiaceae).¹²² Variation in the types of organic acids attached to the sugars of chalcone glycosides by ester linkages is limited compared to other acylated flavonoid glycosides; for example, prior to 1992 only acetic, malonic, and *p*-coumaric acids had been reported.¹⁰ Among the new chalcone glycosides in Table 16.3 are the first examples with cinnamic (**123**),¹²⁶ caffeic (**126**, **127**, **129**),¹²⁸ and ferulic (**112**)¹¹⁷ acids as acylating groups.

The majority of the chalcone glycosides listed in Table 16.3 are based on well-known aglycones such as isoliquiritigenin, chalconaringenin, and okanin. However, the aglycone of 2',3',4-trihydroxychalcone 4-*O*-glucoside (**109**)¹¹⁵ is unknown as a natural product. The first isoprenylated chalcone glycoside (**113**) has now been obtained from the stem bark of *Maclura tinctoria* (Moraceae) together with the acylated chalcone glycoside **117** and several known chalcone glycosides and flavanones.¹¹⁸ Measurement of the free radical scavenging potential of these compounds in two different antioxidant assays showed **113** to have the greatest activity. The characterization of a new chalcone glycoside isolated from the flowers of *Clerodendron phlomidis* (Verbenaceae) as a di-*O*- α -glucopyranoside (**122**)¹²⁵ calls for close scrutiny as it is the β -anomer of this sugar that is invariably found in flavonoid glycosides.¹³² The determination of the configuration of the α -glucopyranose sugars was supported by the measurement of $^3J_{\text{H-1,H-2}}$ coupling constants of 3 Hz for the anomeric protons in the ^1H NMR spectrum of this compound (the corresponding coupling constant for the β -anomer is typically 7 Hz);¹³² however, this structure deserves further investigation.

16.2.4 CHALCONE DIMERS AND OLIGOMERS

The number of biflavonoid and oligomeric flavonoid structures in which chalcones are incorporated has increased significantly in the period 1992 to 2003, as Table 16.4 indicates. Not only have both dimers and heterodimers of chalcones been reported, but also oligomers comprising up to six chalcone-derived structural units (Figure 16.9; see also Chapter 17). The dimers and oligomers are found most commonly in the Ochnaceae, and in particular from species in the genera *Lophira* and *Ochna*. Chalcone dimers are also well represented in the Anacardiaceae. For example, rhuschalcone VI (**143**) is one of six bichalcones obtained from either twigs or stem bark of *Rhus pyroides*, a shrub commonly found in eastern Botswana.^{134,135} It is the only example described to date of a dimer in which two chalcones are linked by a single C–C bond, in this case between C-5' of the A-ring and C-3 of the B-ring of two molecules of isoliquiritigenin (2',4',4-trihydroxychalcone). A second unsymmetrical dimer, rhuschalcone V (**142**), is characterized by a C–C bond between C-5' of the A-ring of isoliquiritigenin and C-3 of the B-ring of the equivalent dihydrochalcone, davidigenin (2',4',4-trihydroxydihydrochalcone).¹³⁵ Rhuschalcones I–IV (**138–141**) are unsymmetrical *O*-linked chalcone dimers comprising combinations of isoliquiritigenin and its 4'-methyl ether.¹³⁵ Three of these (**138–140**, Figure 16.9) are characterized by C–O–C bonds from C-5' (A-ring) to C-4 (B-ring OH of the second molecule) and one (**141**) by a similar linkage from C-5' (A-ring of isoliquiritigenin 4'-methyl ether) to C-4' (A-ring OH of isoliquiritigenin). The structures of rhuschalcones I–III (**138–140**) were confirmed by total synthesis based on Ullmann coupling of 4-hydroxy-2', 4'-dimethoxychalcone and 5'-bromo-2',4',4-trimethoxychalcone.¹³⁵ Rhuschalcones I–VI (**138–143**) were screened for cytotoxicity in the United States National Cancer Institute panel of 60 different human tumor cell lines. The most potent activity was shown by rhuschalcone IV on melanoma cell lines. A general observation was that rhuschalcones as a group of compounds show activity on colon cancer cell lines.¹³⁵ The four chalcone dimers (**134–137**) obtained from *Myracrodruon urundeuva* (Anacardiaceae)¹³³ are structurally more complex than the rhuschalcones, but similar to licobichalcone (**146**), a novel biflavonoid from the roots of *Glycyrrhiza uralensis* (Leguminosae).¹³⁷ A biosynthetic pathway proposed for the formation of licobichalcone is based on the coupling of radicals of the constituent monomer, licochalcone B (4',3,4-trihydroxy-2-methoxychalcone). Subsequent rearrangements result in a condensed bichalcone structure with an additional six-membered ring. On this basis, the monomeric precursors of the condensed structures of urundeuvine A (**134**), B (**135**), and matosine (**137**) are revealed as butein (2',4',3,4-tetrahydroxychalcone) and isoli-

TABLE 16.3
New Chalcone Glycosides Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
	<i>2',3',4-Trihydroxychalcone</i>				
109	4- <i>O</i> -Glucoside	C ₂₁ H ₂₂ O ₉	<i>Ammi majus</i>	Umbelliferae	115
	<i>2',4',4-Trihydroxychalcone (isoliquiritigenin)</i>				
110	4'- <i>O</i> -Glucoside 4- <i>O</i> -apiofuranosyl(1''' → 2'')-glucoside	C ₃₂ H ₄₀ O ₁₈	<i>Glycyrrhiza aspera</i>	Leguminosae	116
111	4- <i>O</i> -(5'''- <i>O</i> - <i>p</i> -Coumaroyl)-apiofuranosyl(1''' → 2'')-glucoside	C ₃₅ H ₃₆ O ₁₅	<i>Glycyrrhiza uralensis</i>	Leguminosae	117
112	4- <i>O</i> -(5'''- <i>O</i> -Feruloyl)-apiofuranosyl(1''' → 2'')-glucoside	C ₃₆ H ₃₈ O ₁₆	<i>Glycyrrhiza uralensis</i>	Leguminosae	117
	<i>2',4',4-Trihydroxy-3'-prenylchalcone</i>				
113	4'- <i>O</i> -Glucoside	C ₂₆ H ₃₀ O ₉	<i>Maclura tinctoria</i>	Moraceae	118
	<i>2',3',4',4-Tetrahydroxychalcone</i>				
114	4'- <i>O</i> -(2''- <i>O</i> - <i>p</i> -Coumaroyl)glucoside	C ₃₀ H ₂₈ O ₁₂	<i>Maclura tinctoria</i>	Moraceae	118
115	4'- <i>O</i> -(6''- <i>O</i> - <i>p</i> -Coumaroyl)glucoside	C ₃₀ H ₂₈ O ₁₂	<i>Bidens leucantha</i>	Asteraceae	119
116	4'- <i>O</i> -(2''- <i>O</i> -Acetyl-6''- <i>O</i> -cinnamoyl)glucoside	C ₃₂ H ₃₀ O ₁₂	<i>Bidens andicola</i>	Asteraceae	120
117	4'- <i>O</i> -(2''- <i>O</i> - <i>p</i> -Coumaroyl-6''- <i>O</i> -acetyl)glucoside	C ₃₂ H ₃₀ O ₁₃	<i>Maclura tinctoria</i>	Moraceae	118
	<i>2',6',2-Trihydroxy-4'-methoxychalcone</i>				
118	2'- <i>O</i> -Glucoside (androechin)	C ₂₂ H ₂₄ O ₁₀	<i>Andrographis echiodes</i>	Acanthaceae	121
	<i>2',4',6',4-Tetrahydroxychalcone (chalconaringenin)</i>				
119	2',4'-Di- <i>O</i> -glucoside	C ₂₇ H ₃₂ O ₁₅	<i>Asarum canadense</i> <i>Asarum macranthum</i>	Aristolochiaceae	122 123
120	2'- <i>O</i> -Glucoside 4'- <i>O</i> -gentobioside	C ₃₃ H ₄₂ O ₂₀	<i>Asarum canadense</i>	Aristolochiaceae	122
	<i>2',4',4-Trihydroxy-6'-methoxychalcone (helichrysetin)</i>				
121	4- <i>O</i> -Glucoside	C ₂₂ H ₂₄ O ₁₀	<i>Pyracantha coccinea</i>	Rosaceae	124
122	4',4-Di- <i>O</i> -α-glucoside	C ₂₈ H ₃₄ O ₁₅	<i>Clerodendron phlomidis</i>	Verbenaceae	125
	<i>2',4-Dihydroxy-4',6'-dimethoxychalcone (flavokawin C)</i>				
123	4- <i>O</i> -(5'''- <i>O</i> - <i>p</i> -Cinnamoyl)-apiofuranosyl(1''' → 2'')-glucoside	C ₃₇ H ₄₀ O ₁₅	<i>Viscum album</i> ssp. <i>album</i>	Loranthaceae	126
	<i>2',4',4-Trihydroxy-3-methoxychalcone (homobutein)</i>				
124	4'- <i>O</i> -Glucoside	C ₂₂ H ₂₄ O ₁₀	<i>Wedelia asperrima</i>	Asteraceae	43
	<i>2',3',4',3,4-Pentahydroxychalcone (okanin)</i>				
125	4'- <i>O</i> -(4'',6''-Di- <i>O</i> -acetyl)glucoside	C ₂₅ H ₂₆ O ₁₃	<i>Bidens pilosa</i> var. <i>radiata</i>	Asteraceae	127
126	4'- <i>O</i> -(2''- <i>O</i> -Caffeoyl-6''- <i>O</i> -acetyl)glucoside	C ₃₂ H ₃₀ O ₁₅	<i>Bidens frondosa</i>	Asteraceae	128
127	4'- <i>O</i> -(2''- <i>O</i> -Caffeoyl-6''- <i>O</i> - <i>p</i> -coumaroyl)glucoside	C ₃₉ H ₃₄ O ₁₆	<i>Bidens frondosa</i>	Asteraceae	128
	<i>2',3',4',3-Tetrahydroxy-4-methoxychalcone (okanin 4-methyl ether)</i>				
128	4'- <i>O</i> -(6''- <i>O</i> - <i>p</i> -Coumaroyl)glucoside	C ₃₁ H ₃₀ O ₁₃	<i>Bidens frondosa</i>	Asteraceae	128
129	4'- <i>O</i> -(2''- <i>O</i> -Caffeoyl-6''- <i>O</i> -acetyl)glucoside	C ₃₃ H ₃₂ O ₁₅	<i>Bidens frondosa</i>	Asteraceae	128
130	4'- <i>O</i> -Primveroside	C ₂₇ H ₃₂ O ₁₅	<i>Bidens campylothea</i>	Asteraceae	129
	<i>2',4',4-Trihydroxy-3',3-dimethoxychalcone</i>				
131	4'- <i>O</i> -Glucoside	C ₂₃ H ₂₆ O ₁₁	<i>Wedelia asperrima</i>	Asteraceae	43
	<i>2'-Hydroxy-4',6',2,4-tetramethoxychalcone</i>				
132	2'- <i>O</i> -Glucoside	C ₂₅ H ₃₀ O ₁₁	<i>Terminalia alata</i>	Combretaceae	130
	<i>4',6',3-Trihydroxy-2',4-dimethoxychalcone</i>				
133	4'- <i>O</i> -Rutinoside	C ₂₉ H ₃₆ O ₁₅	<i>Myrtus communis</i>	Myrtaceae	131

quiritigenin (2',4',4-trihydroxychalcone), while urundevine C (**136**) is a heterodimer based on okanin (2',3',4',3,4-pentahydroxychalcone) and isoliquiritigenin.¹³⁷

The chalcone dimers of the Ochnaceae differ structurally to those of the other plant families represented in Table 16.4. Compounds **147–149** and **151** are dimers in which combinations of isoliquiritigenin and davidigenin (2',4',4-trihydroxydihydrochalcone) are coupled through a dihydrofurano ring formed between the B-ring of one molecule (at C-3 and 4-OH) with the α- and β-carbons of the other (Figure 16.9).^{138–140} Similarly, the structures of

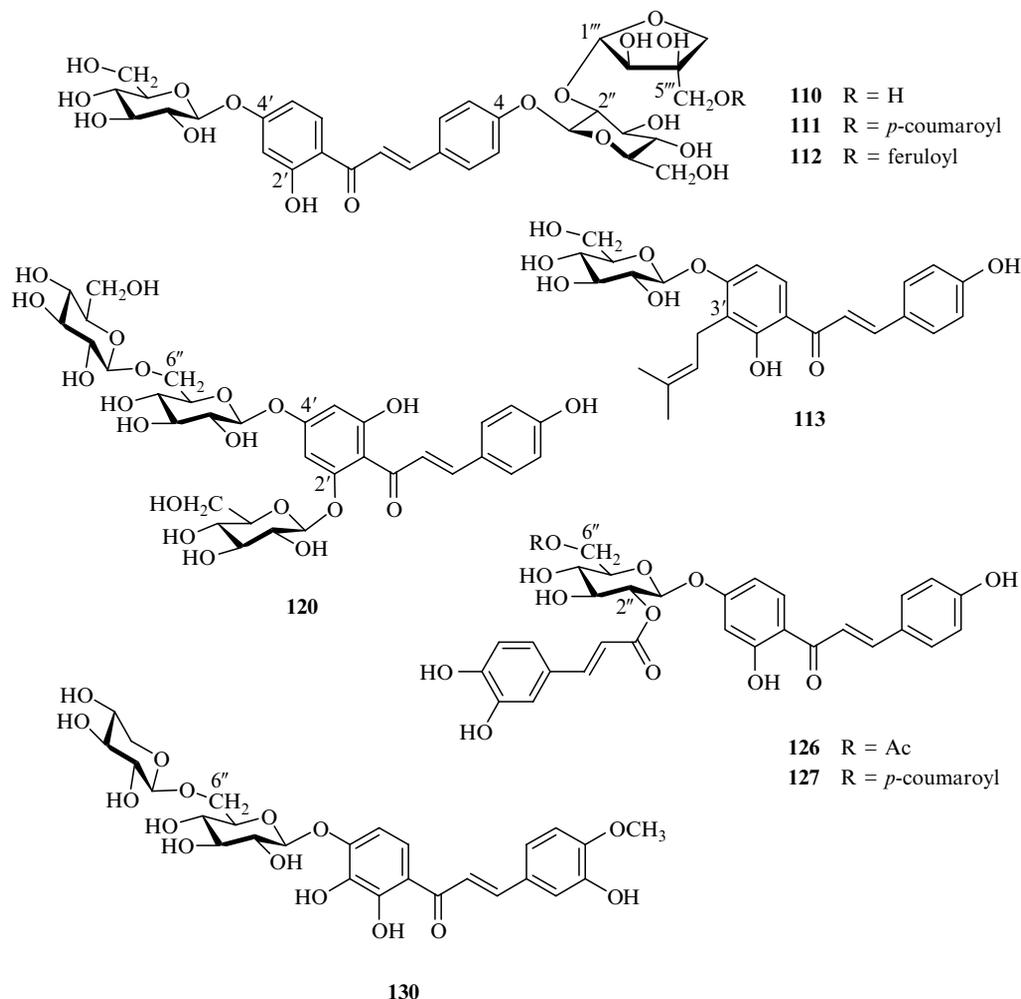


FIGURE 16.8 Chalcone glycosides (see Table 16.3).

calodenin A (**150**) and flavumone A (**152**) appear to result from condensation of the A-ring of phloretin (2',4',6',4-tetrahydroxydihydrochalcone) and 2',3',4',6',4-pentahydroxychalcone, respectively, with the α,β -carbons of isoliquiritigenin.^{140,141} Azobechalcone A (**147**) showed potent inhibition of Epstein–Barr virus early antigen induction caused by the tumor promoter teleocidin B-4.¹³⁸ Perhaps the most unusual of the chalcone dimer structures are two compounds (**144**, **145**) isolated from kamala, a red colored exudate found on glandular trichomes of the surface of the fruits of *Mallotus philippensis* (Euphorbiaceae).¹³⁶ Kamalachalcone A (**144**) is formed by the condensation of two molecules of 2',4'-dihydroxy-3'-methyl-6'',6''-dimethylpyrano[2'',3'':6',5']chalcone, a known constituent of the same plant species.¹⁵⁸ Similarly, kamalachalcone B (**145**) is a dimer comprised of the same chalcone and rottlerin, a benzylated pyranochalcone from *Rottlera tinctoria* (Euphorbiaceae).^{10,159} A cyclobutane chalcone dimer based on the 2 + 2 cycloaddition of two molecules of chalconaringenin 6',4-dimethyl ether (**8**) was isolated during a study of the constituents of *Goniothalamus gardneri* (Annonaceae), but is considered to be an artifact.¹⁶⁰

TABLE 16.4
Chalcone Dimers, Heterodimers, and Oligomers Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
Dimers (chalcone)					
134	Urundevine A	C ₃₀ H ₂₂ O ₉	<i>Myracrodruon urundeuva</i>	Anacardiaceae	133
135	Urundevine B	C ₃₀ H ₂₀ O ₉	<i>Myracrodruon urundeuva</i>	Anacardiaceae	133
136	Urundevine C	C ₃₀ H ₂₂ O ₁₀	<i>Myracrodruon urundeuva</i>	Anacardiaceae	133
137	Matosine	C ₃₀ H ₂₄ O ₁₀	<i>Myracrodruon urundeuva</i>	Anacardiaceae	133
138	Rhuschalcone I	C ₃₂ H ₂₆ O ₈	<i>Rhus pyroides</i>	Anacardiaceae	134
139	Rhuschalcone II	C ₃₀ H ₂₂ O ₈	<i>Rhus pyroides</i>	Anacardiaceae	135
140	Rhuschalcone III	C ₃₁ H ₂₄ O ₈	<i>Rhus pyroides</i>	Anacardiaceae	135
141	Rhuschalcone IV	C ₃₁ H ₂₄ O ₈	<i>Rhus pyroides</i>	Anacardiaceae	135
142	Rhuschalcone V	C ₃₀ H ₂₄ O ₈	<i>Rhus pyroides</i>	Anacardiaceae	135
143	Rhuschalcone VI	C ₃₀ H ₂₂ O ₈	<i>Rhus pyroides</i>	Anacardiaceae	135
144	Kamalachalcone A	C ₄₂ H ₄₀ O ₈	<i>Mallotus philippensis</i>	Euphorbiaceae	136
145	Kamalachalcone B	C ₅₁ H ₄₈ O ₁₂	<i>Mallotus philippensis</i>	Euphorbiaceae	136
146	Licobichalcone	C ₃₂ H ₂₆ O ₁₀	<i>Glycyrrhiza uralensis</i>	Leguminosae	137
147	Azobechalcone A	C ₃₁ H ₂₆ O ₈	<i>Lophira alata</i>	Ochnaceae	138
148	Isolophirone C	C ₃₀ H ₂₂ O ₈	<i>Ochna afzelii</i>	Ochnaceae	139
149	Dihydrolophirone C	C ₃₀ H ₂₄ O ₈	<i>Ochna afzelii</i>	Ochnaceae	139
150	Calodenin A	C ₃₀ H ₂₂ O ₉	<i>Ochna calodendron</i>	Ochnaceae	140
151	Lophirone K	C ₃₀ H ₂₂ O ₉	<i>Ochna calodendron</i>	Ochnaceae	140
152	Flavumone A	C ₃₀ H ₂₀ O ₁₀	<i>Ouretea flava</i>	Ochnaceae	141
Dimers (chalcone-flavan)					
153	Daphnodorin J	C ₃₀ H ₂₄ O ₉	<i>Daphne odora</i>	Thymelaeaceae	142
154	Daphnodorin M	C ₃₀ H ₂₂ O ₁₀	<i>Daphne acutiloba</i>	Thymelaeaceae	143
155	Daphnodorin N	C ₃₀ H ₂₂ O ₁₀	<i>Daphne acutiloba</i>	Thymelaeaceae	143
Dimers (chalcone-flavan-3-ol)					
156	Daphnodorin I	C ₃₀ H ₂₂ O ₁₀	<i>Daphne odora</i>	Thymelaeaceae	144
157	Genkwanol B	C ₃₀ H ₂₂ O ₁₁	<i>Daphne genkwa</i>	Thymelaeaceae	145
158	Genkwanol C	C ₃₀ H ₂₂ O ₁₁	<i>Daphne genkwa</i>	Thymelaeaceae	146
Dimers (chalcone-flavanone)					
159–162	Chalcocaryanones A–D	C ₃₄ H ₂₈ O ₈	<i>Cryptocarya infectoria</i>	Lauraceae	147
163	6'''-Hydroxylophirone B	C ₃₀ H ₂₂ O ₉	<i>Ochna integerrima</i>	Ochnaceae	148
164	6'''-Hydroxylophirone B 4'''-O-glucoside	C ₃₆ H ₃₂ O ₁₄	<i>Ochna integerrima</i>	Ochnaceae	148
165	Flavumone B	C ₃₀ H ₂₀ O ₉	<i>Ouretea flava</i>	Ochnaceae	141
Dimers (chalcone-flavone)					
166–169	<i>Aristolochia</i> dimers (isomers)	C ₃₃ H ₂₄ O ₁₁	<i>Aristolochia ridicula</i>	Aristolochiaceae	149
170	Cissampeloflavone	C ₃₄ H ₂₆ O ₁₁	<i>Cissampelos pareira</i>	Menispermaceae	150
171	Calodenone	C ₃₁ H ₂₄ O ₈	<i>Ochna calodendron</i>	Ochnaceae	151
Trimers					
172	Caloflavan A	C ₄₅ H ₃₈ O ₁₃	<i>Ochna calodendron</i>	Ochnaceae	152
173	Caloflavan B	C ₄₅ H ₃₈ O ₁₃	<i>Ochna calodendron</i>	Ochnaceae	152
Tetramers					
174	<i>Aristolochia</i> tetraflavonoid	C ₆₆ H ₄₆ O ₂₁	<i>Aristolochia ridicula</i>	Aristolochiaceae	149
175	Alatachalcone	C ₆₀ H ₄₈ O ₁₅	<i>Lophira alata</i>	Ochnaceae	153
176	Isolophirachalcone	C ₆₀ H ₄₈ O ₁₅	<i>Lophira alata</i>	Ochnaceae	138
177	Lophiroflavan A	C ₆₀ H ₄₈ O ₁₅	<i>Lophira alata</i>	Ochnaceae	154
178	Lophiroflavan B	C ₆₀ H ₅₀ O ₁₅	<i>Lophira alata</i>	Ochnaceae	155
179	Lophiroflavan C	C ₆₀ H ₅₀ O ₁₅	<i>Lophira alata</i>	Ochnaceae	155
Pentamer					
180	Ochnachalcone	C ₇₅ H ₆₂ O ₂₁	<i>Ochna calodendron</i>	Ochnaceae	156
Hexamer					
181	Azobechalcone	C ₉₀ H ₇₀ O ₂₂	<i>Lophira alata</i>	Ochnaceae	157

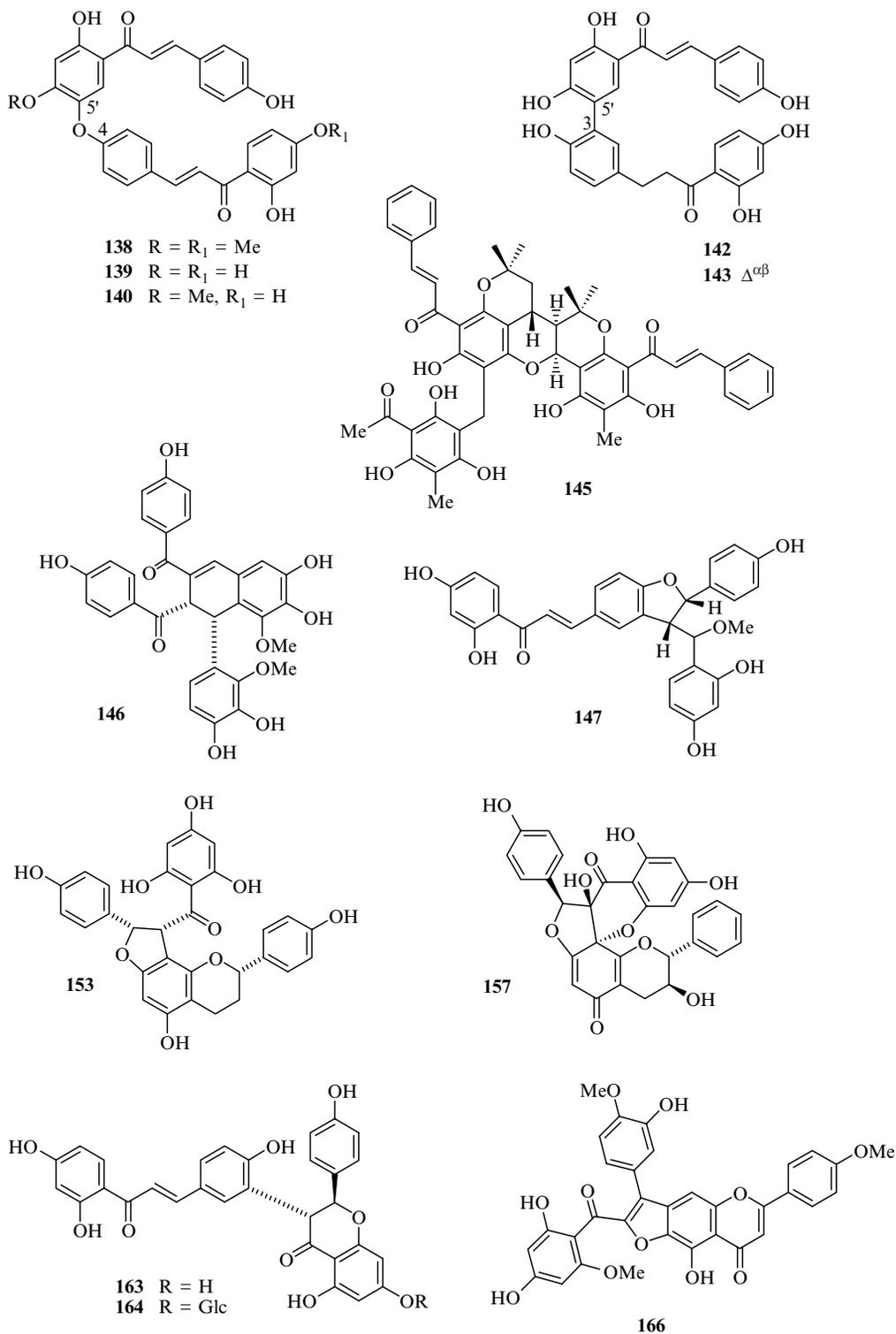


FIGURE 16.9 Chalcone dimers, heterodimers, and oligomers (see Table 16.4). — *continued*

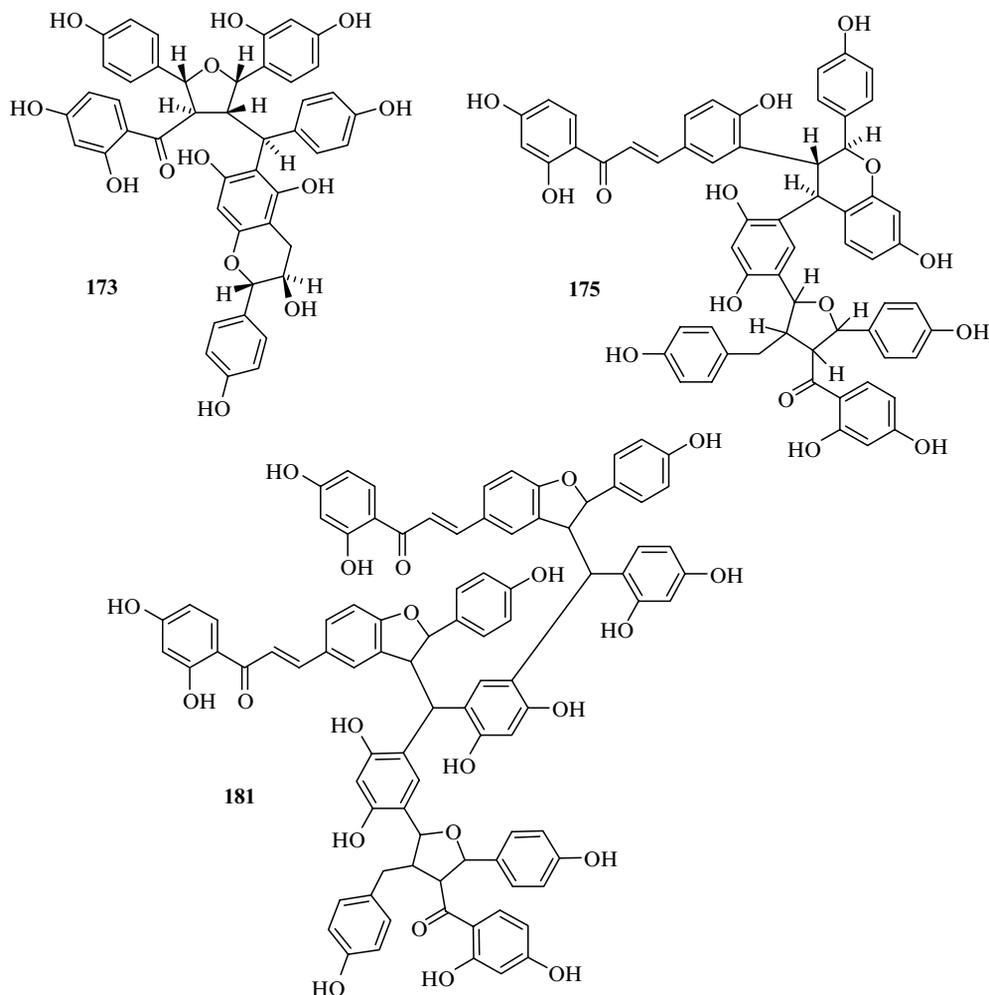


FIGURE 16.9 Chalcone dimers, heterodimers, and oligomers (see Table 16.4).

Rare dimers comprising a chalcone and a second class of flavonoid continue to be reported from a small number of plant species, as indicated in Table 16.4 and Figure 16.9. These “mixed” dimers include two series of compounds derived from the condensation of chalconaringenin with either apigeniflavan (5,7,4'-trihydroxyflavan) (**153–155**)^{142,143} or afzelechin (3,5,7,4'-tetrahydroxyflavan) (**156–158**),^{144–146} followed by internal cyclization reactions. Dimers of flavanones or flavones with flavans or flavan-3-ols may also be produced as an outcome of similar cyclization and rearrangement processes.^{144,161,162} The compounds are characteristic constituents of the roots and bark of some species of *Daphne* (Thymelaeaceae),^{142–146} although genkwanol B (**157**) has also been reported from the roots of *Wikstroemia sikokiana* (Thymelaeaceae), together with many other related biflavonoids.¹⁶¹ The absolute configuration of genkwanol B was obtained by Mosher's method and x-ray crystallography of a permethylated derivative.¹⁶³ The stem bark of *Ochna integerrima* (Ochnaceae) yielded the chalcone–flavanone dimer, 6'''-hydroxylophirone C (**163**), characterized by a C–C bond between C-3 of the B-ring of isoliquiritigenin to C-3 of the C-ring of naringenin.¹⁴⁸ A second derivative (**164**) represents a similarly linked dimer of isoliquiritigenin and naringenin

7-*O*- β -glucopyranoside (prunin). The absolute configuration of the flavanone components of these compounds was determined to be (2*S*,3*R*) from circular dichroism spectra.¹⁴⁸ The remarkable chalcone–flavanone dimers, chalcocaryanones A–D (**159–162**), were obtained from an extract of the trunk bark of *Cryptocarya infectoria* (Lauraceae).¹⁴⁷ Both the chalcone and flavanone components of these dimers show the unusual feature of reduced A-rings (See Section 17.3.1.27). The constituent chalcone monomer is cryptocaryone, whose absolute configuration was determined by x-ray crystallographic analysis of a synthetic bromo derivative.¹⁴⁷ Four chalcone–flavone dimers (**166–169**) have been reported from the stems of *Aristolochia ridicula* (Aristolochiaceae), together with a novel tetramer (**174**).¹⁴⁹ The chalcone component is the rare chalconaringenin 6',4-dimethyl ether (**8**), 2',4',3-trihydroxy-6',4-dimethoxychalcone, or 2',4',4-trihydroxy-6',3-dimethoxychalcone. The latter two compounds have not been reported as natural products. The flavone components of these dimers, 5,6-dihydroxy-4'-methoxyflavone and 5,6,4'-trihydroxy-3'-methoxyflavone, are also notable for the unusual substitution pattern of the A-ring. In structural terms, the monomers are linked through the creation of an additional furan ring based on 6-OH and C-7 of the flavone A-ring and the α,β -carbons of the chalcone (Figure 16.9 and Section 17.3.1.19). The tetramer **174** comprises a biflavone *O*-linked to a chalcone–flavone dimer based on chalconaringenin 6'-methyl ether and 5,6-dihydroxy-3',4'-dimethoxyflavone, and is probably the result of oxidative coupling of the biflavonoid units see Section 17.3.3.¹⁴⁹ Cissampeloflavone (**170**) is a chalcone–flavone dimer with a structure similar to those of the *Aristolochia* biflavonoids (**166–169**).¹⁵⁰ It showed good activity in antiprotozoal assays against *Trypanosoma cruzi* and *T. brucei rhodiense* and low cytotoxicity in the human KB cell line. The constituent monomers of cissampeloflavone are the more common flavokawin A (2'-hydroxy-4',6',4-trimethoxychalcone) and chrysoeriol (5,7,4'-trihydroxy-3'-methoxyflavone). Calodenone (**171**), isolated from the stem bark of *Ochna calodendron* (Ochnaceae), appears to be a rearranged biflavonoid based on chalcone (isoliquiritigenin 4'-methyl ether) and flavone components, but nothing is known of its biosynthesis.¹⁵¹ A closely related derivative (lophirone A) with isoliquiritigenin as the chalcone component is a known constituent of *Lophira lanceolata* (Ochnaceae).¹⁶⁴

Caloflavans A and B are trimers isolated from the leaves of *Ochna calodendron* that differ only in the attachment of the chalcone dimer, isombamichalcone,¹⁶⁵ to either C-8 or C-6 of the A-ring of afzelechin, respectively.¹⁵² The chalcone tetramers and higher oligomers listed in Table 16.4 are based on the condensation of isoliquiritigenin, with the exception of the *Aristolochia* tetramer (**174**) discussed above. Some are of interest as antitumor promoters, such as alatachalcone (**175**)¹⁵³ and isolophirachalcone (**176**),¹⁴⁸ constituents of the bark of *Lophira alata* (Ochnaceae), a rich source of bi- and tetraflavonoids. Tih et al. consider that lophiroflavans A and B (**177**, **178**) arise from coupling of the biflavonoids, isombamichalcone, and lophirone H, whereas the immediate precursors of lophiroflavan C (**179**) appear to be lophirone H and mbamichalcone.^{154,155} Ochnachalcone (**180**), a pentaflavonoid from the stem bark of *Ochna calodendron*, has a most unusual structure (see Section 17.3.4) in which two molecules of the chalcone dimer isombamichalcone are linked by C–C bonds to C-6 and C-8 of the A-ring of (2*R*,3*S*)-3,5,7,4'-tetrahydroxyflavan (afzelechin).¹⁵⁶ The extraordinary hexaflavonoid, azobealchalcone (**181**), which should not be confused with the chalcone dimer azobealchalcone A (**147**), has a structure based on the condensation of six molecules of isoliquiritigenin (Figure 16.9).¹⁵⁷ In biosynthetic terms, its immediate precursors are considered to be the bi- and tetraflavonoids, lophirone C and lophirachalcone,¹⁶⁵ respectively. The structural determination and NMR spectral assignment of these oligomers is a challenging task requiring extensive use of two-dimensional NMR (COSY, HMQC, HMBC) and measurement of “through-space” proton–proton connectivities by NOE and ROE experiments. These methods have also been used to investigate the relative configuration and stereochemistry of other chalcone oligomer substructures such as those of ochnachalcone (**180**).¹⁵⁶

16.2.5 DIELS–ALDER ADDUCTS OF CHALCONES

One of the most characteristic features of the structural chemistry of chalcones is their ability to act as dienophiles in enzyme-catalyzed Diels–Alder reactions (Figure 16.10). The dienes that participate in the formation of the so-called Diels–Alder adducts range from simple isoprene and monoterpene compounds to coumarins and other classes of flavonoid. A full list of Diels–Alder adducts of chalcones reported in the literature from 1992 to 2003 is given in Table 16.5, which is arranged according to the diene component of the compounds. New or revised structures for some adducts described in the literature before 1992 are also included here. An important point that is evident from Table 16.5 is the predominance of Diels–Alder adducts of chalcones in the Moraceae, and in particular, the genus *Morus* (mulberry trees). Other plant families are represented only within the chalcone–monoterpene group of adducts, which are found mainly in the Annonaceae. A commentary on some aspects of the chemistry and biosynthesis of Diels–Alder adducts from *Morus* was published in 1999;¹⁸⁵ however, this continues to be an area of active research interest. Some representative examples of the Diels–Alder adducts of chalcones listed in Table 16.5 are illustrated in Figure 16.11.

Sanggenon R (**182**) is a good example of a Diels–Alder adduct whose chalcone origins may not be immediately obvious (Figure 16.10). According to the biosynthetic scheme proposed by Hano et al., the initial adduct formed by Diels–Alder reaction of 2',4',2,4-tetrahydrochalcone and isoprene is subject to several further rearrangement and oxidation steps to produce sanggenon R.¹⁶⁶ This compound is one of many Diels–Alder adducts and isoprenylated flavonoids obtained from the root bark of *Morus* sp., the source of the Chinese herbal medicine “Sang-Bai-Pi.” Four pairs of regioisomeric Diels–Alder adducts formed by reaction of chalcones with the monoterpene myrcene (7-methyl-3-methylene-1,6-octadiene)

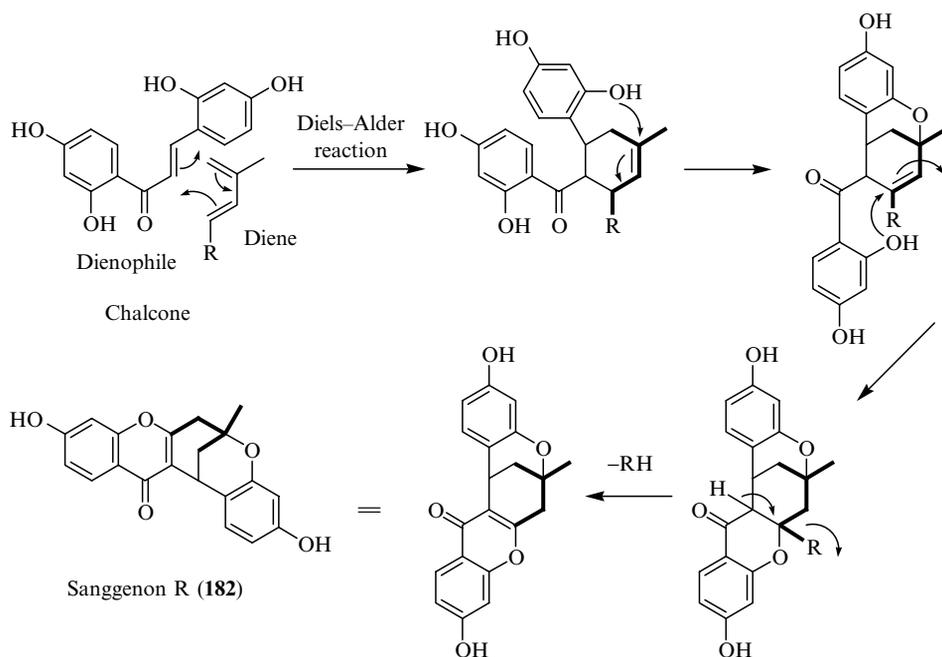


FIGURE 16.10 A biosynthetic pathway proposed for the formation of the chalcone–isoprene Diels–Alder adduct, sanggenon R (**182**), after Hano et al.¹⁶⁶ The fate of the diene is indicated in bold type.

have been characterized from the Annonaceae taxa, *Cyathocalyx crinatus* and *Fissistigma lanuginosum*.^{167,168} Of these, crinatusins A₁ (**183**) and A₂ (**184**) originate from the reaction of 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone and myrcene. The remaining pairs of regioisomers, crinatusins B₁ (**185**) and B₂ (**186**), C₁ (**187**) and C₂ (**188**), and fissistin (**189**) and isofissistin (**190**), are based on stercurensin (2',4'-dihydroxy-6'-methoxy-3'-methylchalcone), cardamonin (2',4'-dihydroxy-6'-methoxychalcone), and 2',5'-dihydroxy-3',4',6'-trimethoxychalcone, respectively. The crinatusins showed lethal toxicity in the brine shrimp bioassay.¹⁶⁷ Cytotoxicity toward KB cells was recorded for both fissistin and isofissistin (IC₅₀ = 0.15 μg/ml).¹⁶⁸ Two adducts (**192**, **193**) isolated from the rhizomes of *Kaempferia pandurata* (Zingiberaceae) are regioisomers resulting from the Diels–Alder reaction of cardamonin with β-ocimene (3,7-dimethyl-1,3,6-octatriene).¹⁷⁰ A closely related Diels–Alder adduct (**191**) derived from 2',4',6'-trihydroxychalcone and β-ocimene and found in the rhizomes of *Boesenbergia pandurata* (Zingiberaceae) was reported first by Trakoontivakorn et al.,¹⁶⁹ who noted its similarity to panduratin A, a known Diels–Alder adduct of 2',6'-dihydroxy-4'-methoxychalcone and β-ocimene.¹⁸⁶ These authors assigned the incorrect trivial name of “4-hydroxypanduratin A” to **191**. In a subsequent paper, Tuchinda et al. described an anti-inflammatory compound identical to **191** and from the same source, as a new chalcone derivative, giving it the incorrect trivial name “(-)-hydroxypanduratin A.”¹⁸⁷ The correct trivial name for this Diels–Alder adduct treated as a derivative of panduratin A is 4'-demethylpanduratin A. The racemic nicolaioidesin A (**194**) is diastereoisomeric to panduratin A, to which (±)-nicolaioidesin B (**195**) is a positional isomer.¹⁷¹

Palodesangrens A–E (**197–201**), obtained from the bark of the Amazonian tree, *Brosimum rubescens* (Moraceae), are Diels–Alder adducts of chalcones and an isoprenylated coumarin.¹⁷² Three of the compounds (**199–201**) were effective inhibitors toward the formation of a 5α-dihydrotestosterone–androgen receptor complex implicated in androgen-dependent diseases. Palodesangretins A and B (**202**, **203**), isolated from the same source, are considered to originate from the Diels–Alder reaction of chalcones and 8-hydroxy-7-methoxy-6-(3-methylbut-1,3-dienyl)coumarin followed by a five-membered ring closure.¹⁷³ The leaves of *Morus insignis* yielded mulberrofuran U (**204**), a new example of a Diels–Alder adduct of 2',4',2,4-tetrahydroxychalcone (a typical chalcone of the Moraceae) and an isoprenylated arylbenzofuran.¹⁷⁴ Several related compounds have been reported prior to 1992, also from species of *Morus*.¹⁰ It is perhaps not surprising that Diels–Alder adducts can be formed solely from isoprenylated chalcones and several new examples (**205–207**) are listed in Table 16.5. For example, the formation of dorstenone (**206**) proceeds by Diels–Alder reaction of 2',4',4-trihydroxy-3'-prenylchalcone (dienophile) and the corresponding dehydro derivative, 2',4',4-trihydroxy-3'-(3-methylbut-1,3-dienyl)chalcone (diene).¹⁷⁶ Similarly, artonin X (**205**), a constituent of the bark of *Artocarpus heterophylla* (cited incorrectly as *A. heterophyllus*), is an adduct of 2',4',2,4-tetrahydroxy-3'-prenylchalcone (dienophile) and 2',4',4-trihydroxy-3'-(3-methylbut-1,3-dienyl)chalcone (diene).¹⁷⁵ A more complex bichalcone (**207**) isolated from twigs of *Dorstenia zenkeri* is considered to be an elaborated form of the Diels–Alder adduct, **206**, modified by subsequent cyclization reactions (Figure 16.10).⁸³

Among the most intensively investigated of all the chalcone Diels–Alder adducts are a group obtained solely from *Morus* species in which the diene component of the reaction is a dehydroprenylflavanone. The structures of several such compounds published prior to 1992 have now been revised on the basis of new spectroscopic and chemical data. Among the most important of the techniques used were two-dimensional NMR and circular dichroism spectroscopy. The revised structures listed in Table 16.5 are those of sanggenons C (**210**), D (**211**), E (**212**), and O (**213**).^{179,180} In these compounds, the flavanones show the common feature of 3-hydroxy-2-prenyl substitution with an ether linkage between C-3 and C-2' of the B-ring. A method for determining the absolute configurations at C-2 and C-3 has now been

TABLE 16.5
Diels–Alder Adducts of Chalcones Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
	Chalcone–isoprene				
182	Sanggenon R	C ₂₀ H ₁₆ O ₅	<i>Morus</i> sp.	Moraceae	166
	Chalcone–monoterpene				
183	Crinatusin A ₁	C ₂₈ H ₃₄ O ₄	<i>Cyathocalyx crinatus</i>	Annonaceae	167
184	Crinatusin A ₂	C ₂₈ H ₃₄ O ₄	<i>Cyathocalyx crinatus</i>	Annonaceae	167
185	Crinatusin B ₁	C ₂₇ H ₃₂ O ₄	<i>Cyathocalyx crinatus</i>	Annonaceae	167
186	Crinatusin B ₂	C ₂₇ H ₃₂ O ₄	<i>Cyathocalyx crinatus</i>	Annonaceae	167
187	Crinatusin C ₁	C ₂₆ H ₃₀ O ₄	<i>Cyathocalyx crinatus</i>	Annonaceae	167
188	Crinatusin C ₂	C ₂₆ H ₃₀ O ₄	<i>Cyathocalyx crinatus</i>	Annonaceae	167
189	Fissistinin	C ₂₈ H ₃₄ O ₆	<i>Fissistigma lanuginosum</i>	Annonaceae	168
190	Isofissistinin	C ₂₈ H ₃₄ O ₆	<i>Fissistigma lanuginosum</i>	Annonaceae	168
191	<i>Boesenbergia</i> adduct	C ₂₅ H ₂₈ O ₄	<i>Boesenbergia pandurata</i>	Zingiberaceae	169
192–193	<i>Kaempferia</i> adducts	C ₂₆ H ₃₀ O ₄	<i>Kaempferia pandurata</i>	Zingiberaceae	170
194–196	(±)-Nicolaoidesins A–C	C ₂₆ H ₃₀ O ₄	<i>Renalmia nicolaoides</i>	Zingiberaceae	171
	Chalcone–coumarin				
197	Palodesangren A	C ₃₀ H ₂₆ O ₇	<i>Brosimum rubescens</i>	Moraceae	172
198	Palodesangren B	C ₃₀ H ₂₆ O ₇	<i>Brosimum rubescens</i>	Moraceae	172
199	Palodesangren C	C ₃₀ H ₂₆ O ₆	<i>Brosimum rubescens</i>	Moraceae	172
200	Palodesangren D	C ₃₀ H ₂₆ O ₆	<i>Brosimum rubescens</i>	Moraceae	172
201	Palodesangren E	C ₃₁ H ₂₈ O ₇	<i>Brosimum rubescens</i>	Moraceae	172
202	Palodesangretin A	C ₃₁ H ₂₈ O ₈	<i>Brosimum rubescens</i>	Moraceae	173
203	Palodesangretin B	C ₃₁ H ₂₈ O ₈	<i>Brosimum rubescens</i>	Moraceae	173
	Chalcone–arylbenzofuran				
204	Mulberrofuran U	C ₃₉ H ₃₆ O ₉	<i>Morus insignis</i>	Moraceae	174
	Chalcone–chalcone				
205	Artonin X	C ₄₀ H ₃₈ O ₉	<i>Artocarpus heterophylla</i>	Moraceae	175
206	Dorstenone	C ₄₀ H ₃₈ O ₈	<i>Dorstenia barteri</i>	Moraceae	176
207	<i>Dorstenia</i> chalcone dimer	C ₄₀ H ₃₈ O ₈	<i>Dorstenia zenkeri</i>	Moraceae	83
	Chalcone–flavanone				
208	Sanggenol J	C ₄₅ H ₄₄ O ₁₂	<i>Morus cathayana</i>	Moraceae	177
209	Sanggenol M	C ₄₅ H ₄₆ O ₁₁	<i>Morus mongolica</i>	Moraceae	178
210	Sanggenon C ^a	C ₄₀ H ₃₆ O ₁₂	<i>Morus mongolica</i>	Moraceae	179
211	Sanggenon D ^a	C ₄₀ H ₃₆ O ₁₂	<i>Morus mongolica</i>	Moraceae	179
212	Sanggenon E ^a	C ₄₅ H ₄₄ O ₁₂	<i>Morus mongolica</i>	Moraceae	179
213	Sanggenon O ^a	C ₄₀ H ₃₆ O ₁₂	<i>Morus mongolica</i>	Moraceae	180
214	Sanggenon S	C ₄₀ H ₃₄ O ₁₂	<i>Morus</i> sp.	Moraceae	166
215	Sanggenon T	C ₄₀ H ₄₀ O ₁₂	<i>Morus</i> sp.	Moraceae	166
216	Cathayanon A	C ₄₀ H ₃₆ O ₁₂	<i>Morus cathayana</i>	Moraceae	181
217	Cathayanon B	C ₄₀ H ₃₆ O ₁₂	<i>Morus cathayana</i>	Moraceae	181
	Chalcone–flavone				
218	Artonin I	C ₄₀ H ₃₆ O ₁₁	<i>Artocarpus heterophylla</i>	Moraceae	182
219	Multicaulisin	C ₄₀ H ₃₆ O ₁₁	<i>Morus multicaulis</i>	Moraceae	183
	Miscellaneous				
220	Sorocenol B	C ₃₁ H ₂₈ O ₇	<i>Sorocea bonplandii</i>	Moraceae	184

^a Indicates revised structure.

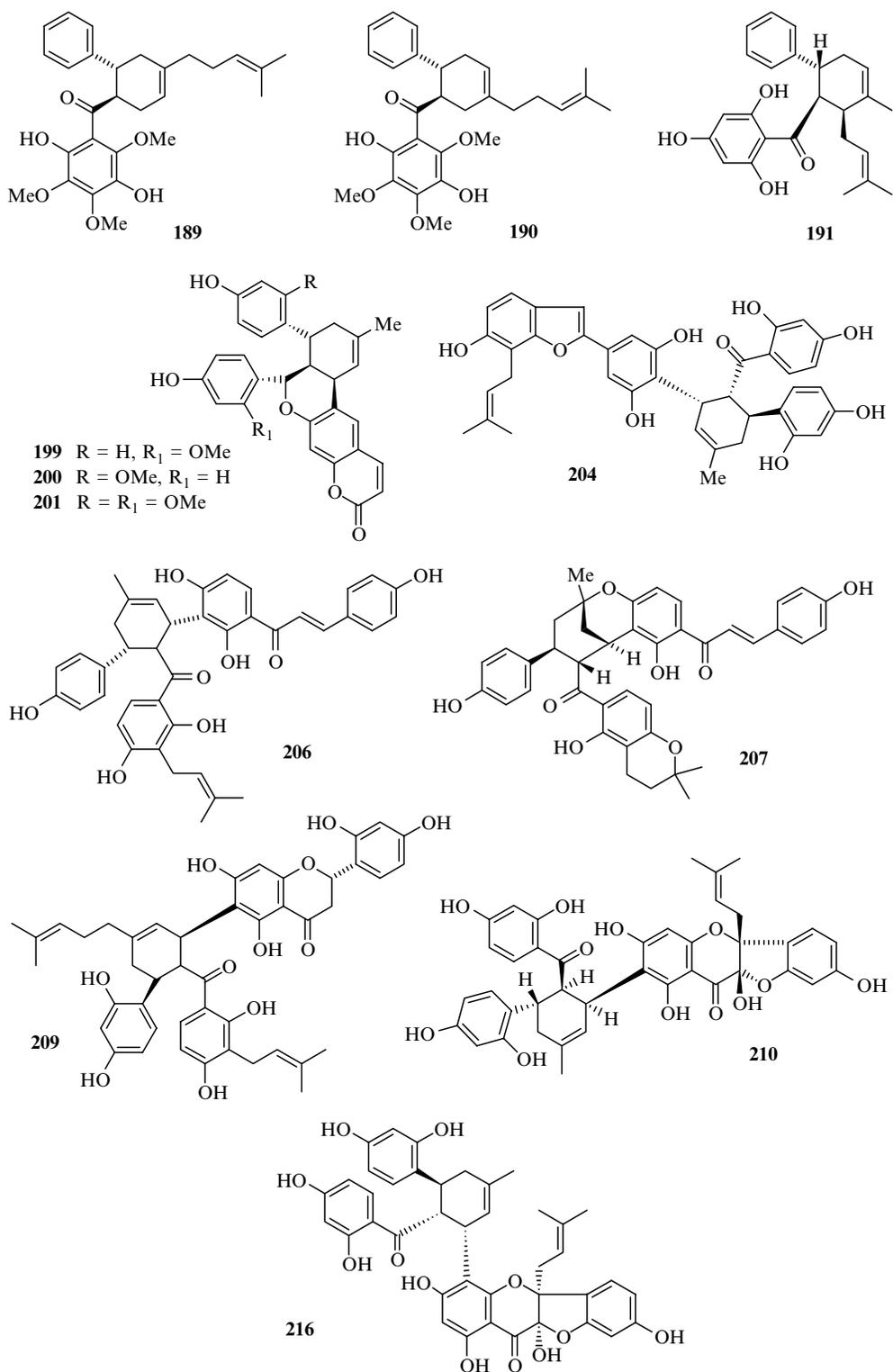


FIGURE 16.11 Diels–Alder adducts of chalcones (see Table 16.5).

described.¹⁸⁸ New Diels–Alder adducts incorporating this type of flavanone include sanggenol J (**208**)¹⁷⁷ and cathayanons A and B (**216**, **217**).¹⁸¹ The structure of cathayanon A was confirmed by x-ray crystallography. In sanggenons C (**210**), D (**211**), and O (**213**) and cathayanons A (**216**) and B (**217**), the chalcone dienophile is the commonly found 2',4',2,4-tetrahydrochalcone, whereas in sanggenon E it is the 3'-prenyl derivative of the same chalcone. In contrast, the more unusual 2',2,4-trihydroxy-6'',6''-dimethyl-4'',5''-dihydropyrano[2'':3'':4',3']chalcone is the dienophile component of sanggenol J (**208**).¹⁷⁷ Sanggenol M (**209**) is a Diels–Alder adduct of 2',4',2,4-tetrahydroxy-3'-prenylchalcone and 6-dehydrogeranyl-5,7,2',4'-tetrahydroxyflavanone.¹⁷⁸ It showed the greatest cytotoxicity toward human oral tumor cell lines of a series of isoprenylated flavanones and chalcone–flavanone Diels–Alder adducts isolated from *Morus mongolica*.¹⁷⁸ Sanggenon T (**215**) is also an example of a Diels–Alder adduct in which the diene component is a dehydrogeranylflavanone derivative.¹⁶⁶ In this respect both sanggenol M (**209**) and sanggenon T (**215**) resemble the known derivative sanggenon G.¹⁸⁹ The structural origins of sanggenon S (**214**) are less easy to discern, but it appears to be an adduct of the sanggenon C-type that has undergone further cyclization and rearrangement.¹⁶⁶ Two Diels–Alder adducts in which the diene is an isoprenylated flavone have also been characterized, artonin I (**218**), from *Artocarpus heterophylla*,¹⁸² and multicaulisin (**219**),¹⁸³ a constituent of the roots of *Morus multicaulis*. Partial enzymatic synthesis of artonin I by introduction of artocarpesin (5,7,2',4'-tetrahydroxy-6-prenylflavone), a known constituent of *A. heterophylla*, into a *Morus alba* cell culture was used to confirm that the diene component was 6- rather than 8-isoprenylated.¹⁸²

16.2.6 CHALCONE CONJUGATES

The seeds of *Alpinia blepharocalyx* (Zingiberaceae) contain a novel series of diarylheptanoids, some of which are characterized by conjugation to either chalcones (Table 16.6) or flavanones. This plant is used in Chinese traditional medicine to treat stomach disorders, and extracts of the seeds show both hepatoprotective and antiproliferative activities.²⁰⁰ The structural determination, stereochemical assignment, biosynthesis, and biological activity of these *Alpinia* diarylheptanoids have been described in detail in a recent review,²⁰⁰ where the following classification of chalcone-bearing diarylheptanoids was proposed (see Figure 16.12 for examples):

- (i) Acyclic diarylheptanoids with a chalcone at C-7: calyxin B (**223**), epicalyxin B (**224**), calyxin H (**228**), and epicalyxin H (**229**)
- (ii) Acyclic diarylheptanoids with a chalcone at C-5: calyxin A (**221**) and deoxycalyxin A (**222**)
- (iii) Cyclic diarylheptanoids with a chalcone at C-5: calyxin F (**225**), epicalyxin F (**226**), and 6-hydroxycalyxin F (**227**)
- (iv) Cyclic diarylheptanoids with a chalcone and an additional *p*-hydroxybenzyl group: calyxin I (**230**) and epicalyxin I (**231**)
- (v) Diarylheptanoids with an ether bond between C-7 and the C-5-linked chalcone: calyxin L (**232**)
- (vi) Dimeric diarylheptanoids conjugated to a chalcone: blepharocalyxins A, B, and E (**233–235**)

Many of the chalcone–diarylheptanoid conjugates occur as pairs of epimers, as illustrated by calyxin B and epicalyxin B (Figure 16.12). These can be separated by high-performance liquid chromatography (HPLC) using chiral column packings.¹⁹² The absolute configurations of chiral carbons with secondary hydroxyl functions were determined using Mosher's

method.¹⁹² Similarly, the relative stereochemistry of other chiral centers, for example, in ring structures, was assigned on the basis of correlations observed in ROE experiments. The chalcone component of all the conjugates (**221–235**) is 2',4',4-trihydroxy-6'-methoxychalcone (helichrysetin). In several of the compounds (**221–229**, **235**), the chalcone is linked only by a C–C bond from C-3' of the A-ring. A shared tetrahydropyran ring based on C-3' and O-2' of the chalcone A-ring to C-5 and C-7 of the diarylheptanoid, respectively, characterizes calyxin I (**230**), epicalyxin I (**231**), and calyxin L (**232**).¹⁹¹ In blepharocalyxins A (**233**) and B (**234**), the chalcone is linked to two diarylheptanoid units, one by a C–C bond from C-3' of the A-ring and the other at the α,β -carbons through a tetrahydropyran ring.¹⁹⁴ Some potential pathways for the biosynthesis of these molecules have been proposed.²⁰⁰ The most promising biological activities uncovered in a series of assays are the potent inhibition of nitric oxide (NO) formation by blepharocalyxin B (**234**)²⁰¹ and the antiproliferative effect of calyxin B (**223**) and epicalyxin F (**226**) toward the human HT-1080 fibrosarcoma and colon 26-L5 carcinoma cell lines, respectively.²⁰²

Table 16.6 also includes several chalcone conjugates that do not share an obvious structural relationship with other more clearly defined groups of compounds. Some of these conjugates are illustrated in Figure 16.12. Among the most interesting is a labdane diterpenoid linked by a C–C bond to C-3' of the A-ring of 2',4'-dihydroxy-6'-methoxychalcone (cardamonin) (**237**),¹⁹⁸ and didymocalyxin B (**239**), a compound thought to be formed by the oxidative coupling of an enolic tautomer of cinnamoylacetic acid with 2',6'-dihydroxy-3',4'-dimethoxychalcone (pashanone).⁴⁷ The genus *Cryptocarya* (Lauraceae) is the source of several unusual chalcone derivatives including the chalcone–flavone dimers **159–162**.¹⁴⁷ To their number must be added the unique conjugate, kurzichalcolactone (**238**),¹⁹⁹ and infectocaryone (**240**),¹⁴⁷ a chalcone with a reduced A-ring that is related structurally to cryptocaryone.

16.2.7 QUINOCHALCONES

A small group of chalcones characterized as either chalcoquinones or chalcoquinone glycosides are shown in Table 16.7 and Figure 16.13. Desmosdumotin C (**241**) is a constituent of the roots of *Desmos dumosus* (Asteraceae), which showed significant and selective cytotoxicity when evaluated against a panel of cancer cell lines.²⁰³ The structure of this compound was confirmed by x-ray crystallography.²⁰³ The *gem*-diprenylquinochalcone, tunicatachalcone (**243**), was obtained from the roots of *Tephrosia tunicata* (Leguminosae) together with several other isoprenylated flavonoids.²⁰⁵ It is closely related in structure to munchiwarin (**242**), an orange pigment isolated from the roots of *Crotalaria trifoliastrum* (Leguminosae) for which the crystal structure has been determined.²⁰⁴ This indicates that the conjugated quinochalcone skeleton is essentially planar, with the *gem*-diprenyl substituents directed above and below the plane of the molecule and perpendicular to it. The third prenyl substituent of the quinochalcone A-ring lies in the plane of the molecule.

Of all the quinochalcones studied to date, the red and yellow glycosidic pigments obtained from the flowers of *Carthamus tinctorius* (the safflower) are probably the most fascinating. Their use in coloring and flavoring foods, as medicines, and for making dyes, has been known since ancient times. The results of the first studies on the chemical properties of the pigments were published by Johann Beckmann in 1773,²¹² while professor at the University of Göttingen. During the first half of the nineteenth century a number of scientists continued to investigate and debate the chemical composition of the pigments.^{213–216} It was not until 1979 that the structure of the red pigment (carthamin) was solved by Obara and Onodera.²¹⁷ The absolute configuration of this unusual C-glycosylquinochalcone dimer has been obtained more recently from the analysis of circular dichroism spectra of synthetic model

TABLE 16.6
Chalcone Conjugates Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
Chalcone–diarylheptanoid					
221	Calyxin A	C ₃₅ H ₃₄ O ₉	<i>Alpinia blepharocalyx</i>	Zingiberaceae	190
222	Deoxycalyxin A	C ₃₅ H ₃₄ O ₈	<i>Alpinia blepharocalyx</i>	Zingiberaceae	191
223	Calyxin B	C ₃₅ H ₃₄ O ₈	<i>Alpinia blepharocalyx</i>	Zingiberaceae	192
224	Epicalyxin B	C ₃₅ H ₃₄ O ₈	<i>Alpinia blepharocalyx</i>	Zingiberaceae	192
225	Calyxin F	C ₃₅ H ₃₄ O ₈	<i>Alpinia blepharocalyx</i>	Zingiberaceae	190
226	Epicalyxin F	C ₃₅ H ₃₄ O ₈	<i>Alpinia blepharocalyx</i>	Zingiberaceae	191
227	6-Hydroxycalyxin F	C ₃₅ H ₃₄ O ₉	<i>Alpinia blepharocalyx</i>	Zingiberaceae	190
228	Calyxin H	C ₃₅ H ₃₄ O ₇	<i>Alpinia blepharocalyx</i>	Zingiberaceae	193
229	Epicalyxin H	C ₃₅ H ₃₄ O ₇	<i>Alpinia blepharocalyx</i>	Zingiberaceae	193
230	Calyxin I	C ₄₂ H ₃₈ O ₉	<i>Alpinia blepharocalyx</i>	Zingiberaceae	191
231	Epicalyxin I	C ₄₂ H ₃₈ O ₉	<i>Alpinia blepharocalyx</i>	Zingiberaceae	191
232	Calyxin L	C ₃₅ H ₃₄ O ₈	<i>Alpinia blepharocalyx</i>	Zingiberaceae	191
Chalcone–Bis(diarylheptanoid)					
233	Blepharocalyxin A	C ₅₄ H ₅₄ O ₁₁	<i>Alpinia blepharocalyx</i>	Zingiberaceae	193, 194
234	Blepharocalyxin B	C ₅₄ H ₅₄ O ₁₁	<i>Alpinia blepharocalyx</i>	Zingiberaceae	193, 194
235	Blepharocalyxin E	C ₅₄ H ₅₄ O ₁₁	<i>Alpinia blepharocalyx</i>	Zingiberaceae	195, 196
Miscellaneous					
236	2',4'-Dihydroxy-3'-C-(2,6-dihydroxybenzyl)-6'-methoxychalcone	C ₂₃ H ₂₀ O ₆	<i>Desmos chinensis</i>	Annonaceae	197
237	2',4'-Dihydroxy-6'-methoxy-3'-(8,17-epoxy-16-oxo-12,14-labdadien-15-yl)chalcone	C ₃₆ H ₄₂ O ₆	<i>Alpinia katsumadai</i>	Zingiberaceae	198
238	Kurzichalcolactone	C ₃₂ H ₃₀ O ₇	<i>Cryptocarya kurzii</i>	Lauraceae	199
239	Didymocalyxin B	C ₂₈ H ₂₂ O ₇	<i>Didymocarpus leucocalyx</i>	Gesneriaceae	47
240	Infectocaryone	C ₁₈ H ₁₈ O ₄	<i>Cryptocarya infectoria</i>	Lauraceae	147

compounds.²¹⁸ The flowers of safflower are yellow on first opening and turn red after a few days. Two groups have now solved the structure of one of the yellow pigment precursors of carthamin, naming it precarthamin (**247**).^{209,210} It differs from carthamin only by the presence of an additional carboxyl group at the bridging carbon between the two C-glycosylquinochalcone monomers. Analysis of the flavonoid pigments of the orange flowers of *C. tinctorius* cv. Ken-ba revealed the presence of another yellow pigment and carthamin precursor, anhydrosafflor yellow B (**244**).²⁰⁶ The techniques used to obtain the structure of this compound included circular dichroism spectroscopy, NOE and ROE measurements by ¹H NMR, and molecular mechanics calculations. In the same study, the flavonoid and quinochalcone constituents of three distinct *C. tinctorius* cultivars were compared by HPLC and a scheme for the biosynthesis of quinochalcone pigments from chalcone precursors was proposed.²⁰⁶ Several other yellow pigments that are C-glycosylquinochalcone monomers have been reported, including hydroxysafflor yellow A (**246**),²⁰⁸ tinctormine (**248**),^{208,211} and

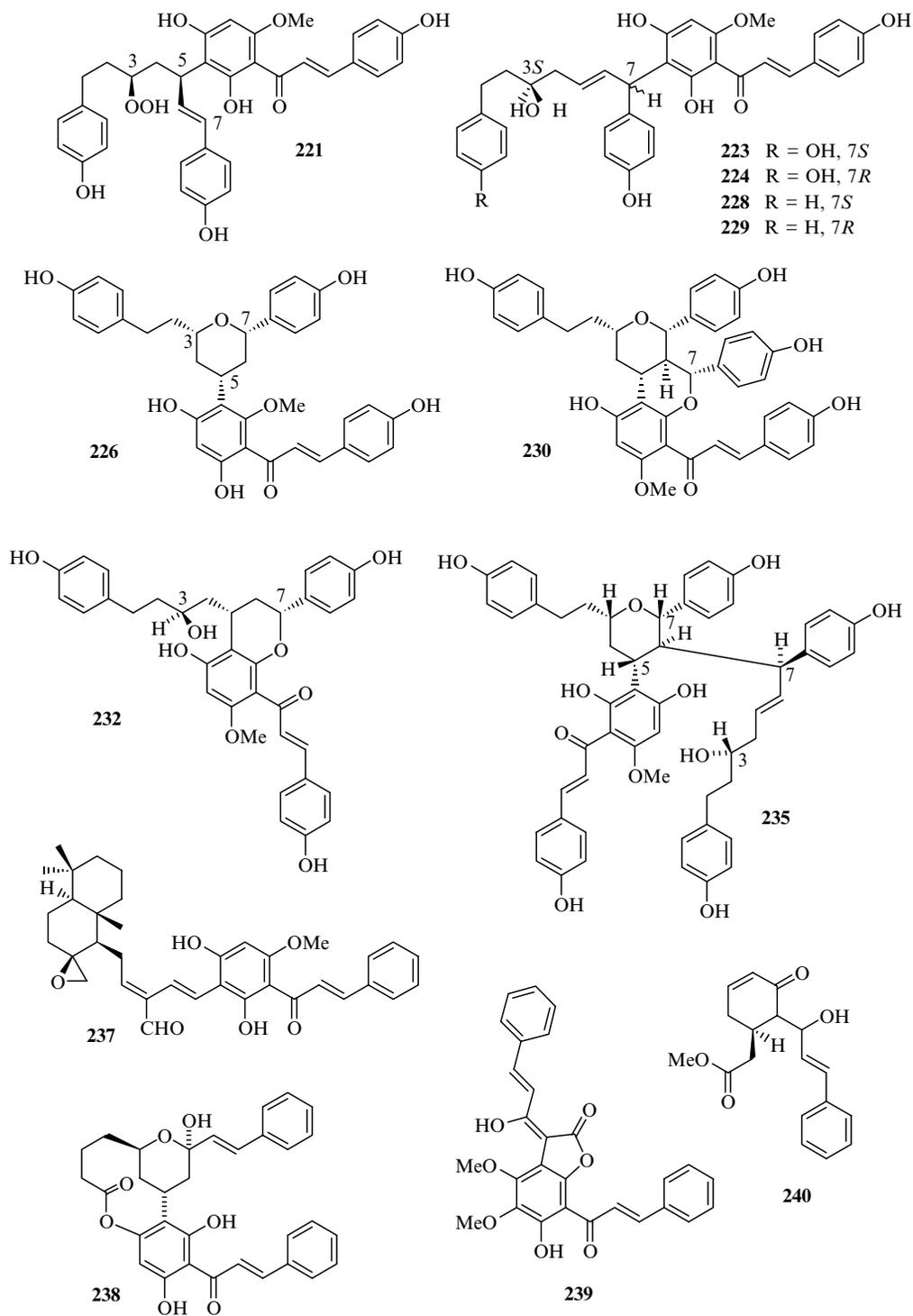


FIGURE 16.12 Chalcone conjugates (see Table 16.6).

TABLE 16.7
Quinochalcons Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
Aglycones					
241	Desmosdumotin C	C ₁₉ H ₂₀ O ₄	<i>Desmos dumosus</i>	Asteraceae	203
242	Munchiwarin	C ₃₀ H ₃₆ O ₄	<i>Crotalaria trifoliastrum</i>	Leguminosae	204
243	Tunicatachalcone	C ₂₆ H ₃₀ O ₄	<i>Tephrosia tunicata</i>	Leguminosae	205
C-Glycosides					
244	Anhydrosafflor yellow B	C ₄₈ H ₅₂ O ₂₆	<i>Carthamus tinctorius</i>	Asteraceae	206
245	Cartormin	C ₂₇ H ₂₉ O ₁₃ N	<i>Carthamus tinctorius</i>	Asteraceae	207
246	Hydroxysafflor yellow A	C ₂₇ H ₃₂ O ₁₆	<i>Carthamus tinctorius</i>	Asteraceae	208
247	Precarthamin	C ₄₄ H ₄₄ O ₂₄	<i>Carthamus tinctorius</i>	Asteraceae	209, 210
248	Tinctormine	C ₂₇ H ₃₁ O ₁₄ N	<i>Carthamus tinctorius</i>	Asteraceae	208, 211

cartormin (**245**).²⁰⁷ Tinctormine (**248**), a potent Ca²⁺ antagonist, is structurally remarkable for its unusual C-linked polyhydroxylated pyrrolidine ring (Figure 16.13). The structure of cartormin (**245**), a quinochalcone C-glucoside with a novel C-glycosylpyrrole substituent, was confirmed by x-ray crystallography.²⁰⁷

16.3 DIHYDROCHALCONES

16.3.1 DIHYDROCHALCONES WITH SIMPLE PATTERNS OF O-SUBSTITUTION

16.3.1.1 Structure, Chemosystematic Trends, and Biological Activity

New dihydrochalcones reported in the literature from 1992 to 2003 are listed in Table 16.8 and some examples are shown in Figure 16.14. The two methyl ethers (**249**, **250**) of the simplest of the O-substituted dihydrochalcone found in plants, 2',4'-dihydroxydihydrochalcone, have now been isolated from leaf surface extracts of *Empetrum nigrum* (Empetraceae) together with the parent compound.²¹⁹ The latter was cited as new, although it had been reported previously from *Ceratiola ericoides*,²³⁵ another species of the same family. The three-dimensional structure of the 2'-methyl ether (**250**) was solved using x-ray crystallography by Krasnov et al.²³⁶ As expected, the molecule is essentially planar apart from the unsubstituted B-ring, and the dihedral angle between the planes of the two phenyl rings is close to 80°. A di-O-substituted retrodihydrochalcone obtained from the red-colored resin of *Dracaena cinnabari*, a tree endemic to the island of Socotra, was characterized as 4-hydroxy-2-methoxydihydrochalcone (**251**).²²⁰ This pattern of substitution has not been reported previously. The resin, which is more commonly known as dragon's blood, can also be obtained from *D. draco*, the source of 4',2,4-trihydroxydihydrochalcone (**256**).²²⁴ Three further retrodihydrochalcones obtained from species of *Dracaena* are **257**, **265**, and **266** (Table 16.8). One of these (**266**), a constituent of the stem bark of *D. loureiri*, showed estrogenic activity comparable to that of the isoflavones daidzein and genistein.²³⁰ It is interesting to note that most of the retrodihydrochalcones cited in the pre-1992 literature were also found in *Dracaena*.¹⁰ However, compounds **258** and **268**, which are examples of the (2',3',4',6')-O-substitution pattern and its retrodihydrochalcone equivalent, respectively, were both obtained from species of *Uvaria* (Annonaceae).^{226,231} Lusianin (**260**) and two known chalcones isolated from the orchid *Lusia volucris* are cited by Majumder et al.²²⁸ as the first examples of this class of compound to be

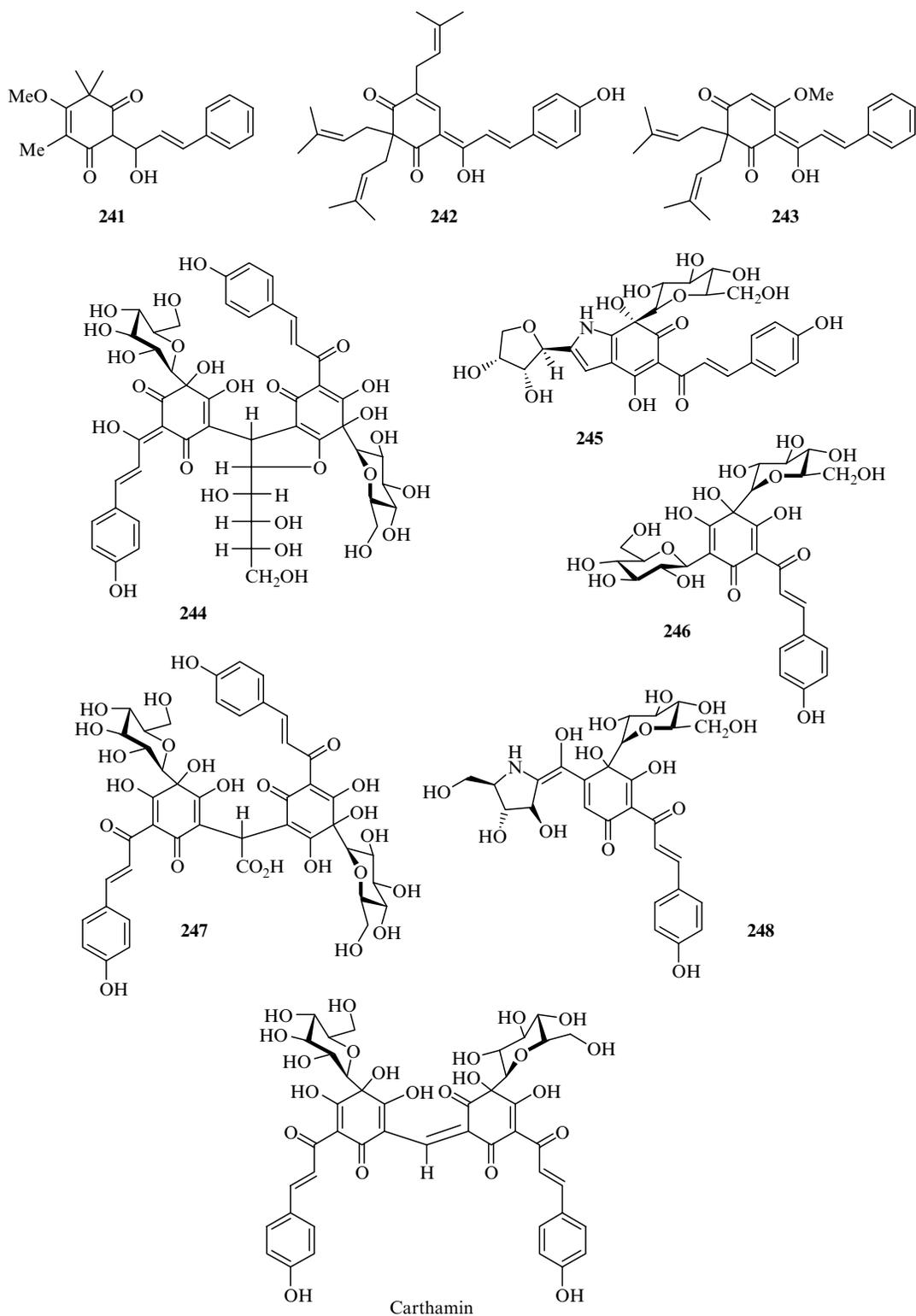


FIGURE 16.13 Quinochalcones and quinochalcone glycosides (see Table 16.7).

TABLE 16.8
New Dihydrochalcones with Simple Patterns of O-Substitution Reported in the Literature from 1992 to 2003

No.	OH	OMe	Other	Mol. Formula	Trivial Name	Source	Family	Ref.
Di O-substituted								
	(2',4')							
249	2'	4'	—	C ₁₆ H ₁₆ O ₃		<i>Empetrum nigrum</i>	Empetraceae	219
250	4'	2'	—	C ₁₆ H ₁₆ O ₃		<i>Empetrum nigrum</i>	Empetraceae	219
	(2,4)							
251	4	2	—	C ₁₆ H ₁₆ O ₃		<i>Dracaena cinnabari</i>	Dracaenaceae	220
Tri O-substituted								
	(2',4',6')							
252	2',4',6'	—	3'-Me	C ₁₆ H ₁₆ O ₄		<i>Leptospermum recurvum</i>	Myrtaceae	221
253	2',4'	6'	3'-Me	C ₁₇ H ₁₈ O ₄	Myrigalone H	<i>Myrica gale</i>	Myricaceae	222
254	2',6'	4'	3'-Me	C ₁₇ H ₁₈ O ₄	Myrigalone G	<i>Myrica gale</i>	Myricaceae	222
	(2',4',4)							
255	4	2',4'	—	C ₁₇ H ₁₈ O ₄		<i>Crinum bulbispermum</i>	Amaryllidaceae	223
	(4',2,4)							
256	4',2,4	—	—	C ₁₅ H ₁₄ O ₄		<i>Dracaena draco</i>	Dracaenaceae	224
	(4',2,6)							
257	4'	2,6	—	C ₁₇ H ₁₈ O ₄		<i>Dracaena cochinchinensis</i>	Dracaenaceae	225
Tetra O-substituted								
	(2',3',4',6')							
258	2',3'	4',6'	—	C ₁₇ H ₁₈ O ₅		<i>Uvaria dulcis</i>	Annonaceae	226
259	2'	3',4',6'	—	C ₁₈ H ₂₀ O ₅		<i>Fissistigma bracteolatum</i>	Annonaceae	227
	(2',3',4',4)							
260	2',4'	3',4	—	C ₁₇ H ₁₈ O ₅	Lusianin	<i>Lusit volucris</i>	Orchidaceae	228
	(2',4',6',4)							
261	2',4	4',6'	—	C ₁₇ H ₁₈ O ₅		<i>Iryanthera lancifolia</i>	Myricaceae	229
262	2'	4',6',4	—	C ₁₈ H ₂₀ O ₅		<i>Goniothalamus gardneri</i>	Annonaceae	160

263	(2',4',3,4) 2',3,4	4'	—	C ₁₆ H ₁₆ O ₅	Dihydrocalythrospins	<i>Calythrospis aurea</i>	Myrtaceae	39	
264	—	2',4'	3,4-OCH ₂ O-	C ₁₈ H ₁₈ O ₅	Ponganone VII	<i>Pongamia pinnata</i>	Leguminosae	53	
265	(4',2,4,6)	4,6	—	C ₁₇ H ₁₈ O ₅		<i>Dracaena loureiri</i>	Dracaenaceae	230	
266	4',4	2,6	—	C ₁₇ H ₁₈ O ₅		<i>Dracaena loureiri</i>	Dracaenaceae	230	
267	(2,3,4,6)	3,4,6	—	C ₁₈ H ₂₀ O ₅		<i>Fissistigma bracteolatum</i>	Annonaceae	227	
268	6	2,3,4	—	C ₁₈ H ₂₀ O ₅		<i>Uvaria mocoi</i>	Annonaceae	231	
Penta O-substituted									
269	(2',3',4',5',6')	2',5'	—	C ₁₈ H ₂₀ O ₆	Dihydropedicin	<i>Fissistigma lamuginosum</i>	Annonaceae	168	
270	3',5'	2',4',6'	—	C ₁₈ H ₂₀ O ₆		<i>Lindera lucida</i>	Lauraceae	232	
271	(2',4',6',3,4)	4',3,4	—	C ₁₈ H ₂₀ O ₆		<i>Pityrogramma tartarea</i>	Hemionitidaceae	233	
272	2',6'	2',4',6'	3,4-OCH ₂ O-	C ₁₉ H ₂₀ O ₆		<i>Milletia leucantha</i>	Leguminosae	45	
α-Hydroxy-substituted									
273	(2',4',3,4,α)	—	—	C ₁₅ H ₁₄ O ₆		<i>Eysenhardtia polystachya</i>	Leguminosae	234	
β-Hydroxy-substituted									
274	(2',4',6',4,β)	6'	—	C ₁₆ H ₁₆ O ₆		<i>Vitex leptobotrys</i>	Verbenaceae	37	

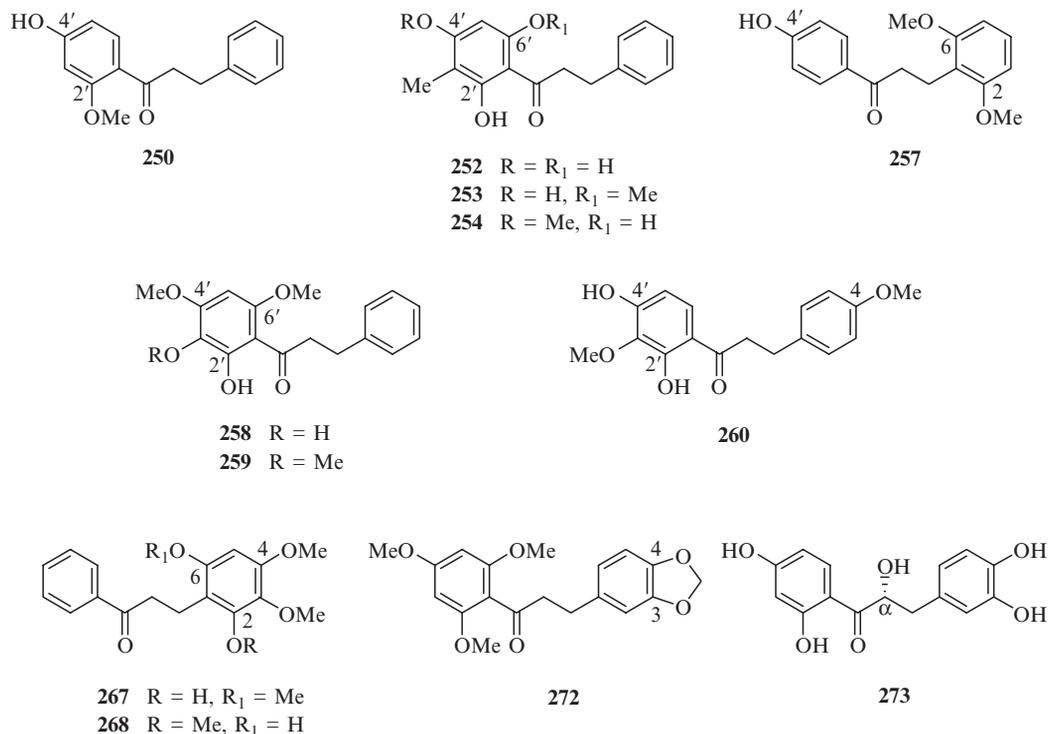


FIGURE 16.14 Dihydrochalcones with simple patterns of *O*-substitution (see Table 16.8).

found in the Orchidaceae. The (2',3',4',4)-*O*-substitution pattern of lusianin has not been found previously in dihydrochalcones although several chalcone equivalents are known from the Leguminosae.¹⁰ Two new phloretin (2',4',6',4-tetrahydroxydihydrochalcone) methyl ethers (**261**, **262**) were isolated from extracts of the pericarps of *Iryanthera lancifolia* (Myristicaceae) and the aerial parts of *Goniothalamus gardneri* (Annonaceae), respectively.^{160,229} Phloretin 4',6'-dimethyl ether (**261**) was obtained both as the free dihydrochalcone and as the diastereoisomeric lignan conjugates, iryantherins K (**338**) and L (**339**) (see Table 16.12).²²⁹ The compounds showed greater antioxidant activity than either α -tocopherol or quercetin in an assay based on the inhibition of lipid peroxidation. The phloretin trimethyl ether derivative, **262**, was previously known as a synthetic product.¹⁶⁰ Moderate antiherpes simplex virus activity has been measured for 2',4',6'-trimethoxy-3,4-methylenedioxydihydrochalcone (**272**) and the known *O*-methyl dihydromillettene. These compounds were obtained from the stem bark of *Millettia leucantha* together with the chalcones **17** and **26** (Table 16.1).⁴⁵

Myriganones G (**254**) and H (**253**), constituents of the fruits of *Myrica gale* (Myricaceae),²²² are two examples of a small group of *C*-methylated dihydrochalcones all of which show 3'-methyl substitution of the A-ring (Table 16.8). Myriganone G (**254**) was also obtained together with 2',4',6'-trihydroxy-3'-methyl dihydrochalcone (**252**) from the dried foliage of *Leptospermum recurvum* (Myrtaceae),²²¹ a plant endemic to Mt. Kinabalu in Sabah, Malaysia. In assays for antiviral activity (herpes-simplex virus) only myriganone G (**254**) yielded positive results; however, **252** showed both cytotoxicity and antimicrobial activity. Synthesis of **252** by a standard three-step procedure from phloroglucinol yielded 2',4',6'-trihydroxy-3'-formyl dihydrochalcone as an intermediate and precursor to the final product.²²¹ This formyl derivative was reported previously as a natural product from *Psidium acutangulum*

(Myrtaceae),²³⁷ from which the related 2',4',6'-trihydroxy-3'-formyl-5'-methyl-dihydrochalcone was also obtained.²³⁸ The NMR spectra of the synthetic formyl derivative show evidence for exchange broadening, which was attributed to conformational exchange between two stable hydrogen-bonded rotamers from molecular mechanics and *ab initio* calculations.²²¹

16.3.1.2 α - and β -Hydroxydihydrochalcones

The α -hydroxydihydrochalcones are a small subclass of compounds found mainly in the Leguminosae. To their number can be added the new derivative **273**, a constituent of extracts of the bark and wood of *Eysenhardtia polystachya*.²³⁴ This species is already known as the source of the α -hydroxydihydrochalcone C-glucosides, coatline A and B.²³⁹ Comparison of the circular dichroism spectrum of **273** with that of related compounds allowed the configuration at C- α to be deduced as *R* (see Figure 16.14). Most known examples of β -hydroxydihydrochalcones are also constituents of the Leguminosae, thus the isolation of 2',4',4', β -tetrahydroxy-6'-methoxydihydrochalcone (**274**) from the aerial parts of *Vitex leptobotrys* represents the first report of this type of compound in the Verbenaceae.³⁷ Nel et al. have reported an improved method for the enantioselective synthesis of β -hydroxydihydrochalcones.³¹²

16.3.2 ISOPRENYLATED DIHYDROCHALCONES

A comparison of Table 16.2 and Table 16.9 indicates that the number of new isoprenylated dihydrochalcones reported in the literature from 1992 to 2003 is far fewer than the equivalent number of chalcones; nevertheless, 28 examples are listed in the latter. Some of the most interesting compounds are illustrated in Figure 16.15. Many of the dihydrochalcones are characterized by prenyl, geranyl, furano, and pyrano substituents, which are unexceptional in structural terms. However, a group of isoprenylated (2',4',6')-*O*-substituted dihydrochalcones (**275–285**) found in the Annonaceae and Piperaceae calls for special comment. Two derivatives (**275**, **276**)²⁴⁰ isolated from extracts of the stem bark of *Mitrella kentii* (Annonaceae) were characterized as enantiomers of the known compounds, neolinderatin and linderatin, obtained previously from *Lindera umbellata* (Lauraceae).²⁵¹ For example, the ¹H and ¹³C NMR spectra of **276** were superimposable with those of (+)-neolinderatin, but the optical rotation values of the compounds were of opposite sign and their circular dichroism spectra showed an inverse relationship.²⁴⁰ Compounds **275** and **276** were, therefore, assigned as (–)-linderatin and (–)-neolinderatin, respectively. Both are unusual dihydrochalcone derivatives substituted by *p*-menthenyl groups at either C-3' (**275**) or C-3' and C-5' (**276**) of the A-ring. *In vitro* testing of each enantiomer of the two pairs against a non-small-cell bronchopulmonary lung carcinoma cell line uncovered promising activity for (–)-linderatin (IC₅₀ = 3.8 μ g/ml), whereas the other enantiomers proved to be inactive (IC₅₀ > 30 μ g/ml).²⁴⁰ A compound isolated from leaves of *Piper aduncum* (Piperaceae) with identical physical and spectroscopic data to (+)-methyllinderatin,²⁵² but with the opposite sign of optical rotation, was characterized as (–)-methyllinderatin (**277**).²⁴¹ Five 6'-*O*-linked *p*-menthenyl derivatives of 2',6'-dihydroxy-4'-methoxydihydrochalcone, adunctins A–E (**281–285**), were also obtained from the same source.²⁴¹ The *p*-menthenyl substituent is cyclized to the dihydrochalcone A-ring through an additional C–C bond at C-5' in all but adunctin A (**281**). Adunctins B–D (**282–284**) and (–)-methyllinderatin (**277**) showed antibacterial activity toward *Micrococcus luteus*. Cytotoxicity toward a KB nasopharyngeal carcinoma cell line was shown by (–)-methyllinderatin (**277**), whereas adunctins A–E were inactive (**281–285**).²⁴¹ Two isoprenylated derivatives of 2',6'-dihydroxy-4'-methoxydihydrochalcone with additional methyl *p*-hydroxybenzoate substitution were obtained by bioassay-guided fractionation in a later

TABLE 16.9
New Isoprenylated Dihydrochalcones Reported in the Literature from 1992 to 2003

No.	O-Substituents	Other Substituents	Mol. Formula	Trivial Name	Source	Family	Ref.
Tri O-substituted							
	(2',4',6')						
275	2',4',6'-triOH	3'-C ₁₀	C ₂₅ H ₃₀ O ₄	(-)-Linderatin	<i>Mitrella kentii</i>	Annonaceae	240
276	2',4',6'-triOH	3',5'-di-C ₁₀	C ₃₅ H ₄₆ O ₄	(-)-Neolinderatin	<i>Mitrella kentii</i>	Annonaceae	240
277	2',6'-diOH, 4'-OMe	3'-C ₁₀	C ₂₆ H ₃₂ O ₄	(-)-Methylinderatin	<i>Piper aduncum</i>	Piperaceae	241
278	2',6'-diOH, 4'-OMe	5'-(1''-Aryl)prenyl	C ₂₉ H ₃₀ O ₇	Piperaduncin A	<i>Piper aduncum</i>	Piperaceae	242
279	2'-OH, 4'-OMe	5''-Arylfuranol[2'',3'',6'',5']	C ₂₆ H ₂₂ O ₇	Longicaudatin	<i>Piper longicaudatum</i>	Piperaceae	243
280	2'-OH, 4'-OMe	4''-Aryl-5''-(2-hydroxyisopropyl) dihydrofuranol[2'',3'',6'',5']	C ₂₉ H ₃₀ O ₈	Piperaduncin B	<i>Piper aduncum</i>	Piperaceae	242
281	2'-OH, 4'-OMe, 6'-O-C ₁₀	—	C ₂₆ H ₃₂ O ₄	Adunctin A	<i>Piper aduncum</i>	Piperaceae	241
282	2'-OH, 4'-OMe	[5',6']-C ₁₀	C ₂₆ H ₃₀ O ₄	Adunctin B	<i>Piper aduncum</i>	Piperaceae	241
283	2'-OH, 4'-OMe	[5',6']-C ₁₀	C ₂₆ H ₃₀ O ₄	Adunctin C	<i>Piper aduncum</i>	Piperaceae	241
284	2'-OH, 4'-OMe	[5',6']-C ₁₀	C ₂₆ H ₃₀ O ₄	Adunctin D	<i>Piper aduncum</i>	Piperaceae	241
285	2'-OH, 4'-OMe	[5',6']-C ₁₀	C ₂₆ H ₃₂ O ₅	Adunctin E	<i>Piper aduncum</i>	Piperaceae	241
	(2',4')						
286	2',4'-diOH	6'',6''-Dimethylpyrano[2'',3'',4',3']	C ₂₀ H ₂₀ O ₄	Crotaramosmin	<i>Crotalaria ramosissima</i>	Leguminosae	244
287	2'-OH, 4'-OMe	6'',6''-Dimethylpyrano[2'',3'',4',3']	C ₂₁ H ₂₂ O ₄	Crotaramin	<i>Crotalaria ramosissima</i>	Leguminosae	245
Tetra O-substituted							
	(2',3',4',6')						
288	4',6'-diOH, 3'-OMe, 2'-oxo	3'-Prenyl	C ₂₁ H ₂₄ O ₅		<i>Helichrysum aphelexioides</i>	Asteraceae	66
	(2',4',6',4)						
289	2',4',6',4-tetraOH	3,5-Diprenyl	C ₂₅ H ₃₀ O ₅		<i>Boronita inconspicua</i>	Rutaceae	246
290	2',4',6',4-tetraOH	3-Geranyl, 5-prenyl	C ₃₀ H ₃₈ O ₅		<i>Boronita inconspicua</i>	Rutaceae	246
291	2',4',4-triOH, 6'-OMe	3'-Prenyl	C ₂₁ H ₂₄ O ₅	α,β-Dihydroxanthohumol	<i>Humulus lupulus</i>	Cannabinaeae	91
	(2',4',3,4)						
292	2',3,4-triOH	6'',6''-Dimethylpyrano[2'',3'',4',3']	C ₂₀ H ₂₀ O ₅	Crotin	<i>Crotalaria ramosissima</i>	Leguminosae	245
293	2'-OMe, 3,4-OCH ₂ O-	Furanol[2',3',4',3']	C ₁₉ H ₁₆ O ₅		<i>Lonchocarpus subglaucescens</i>	Leguminosae	101
	(2',4',α)						
294	2',4',4,α-tetraOH	5',3-Diprenyl	C ₂₅ H ₃₀ O ₅	Kanzonol Y	<i>Glycyrrhiza glabra</i>	Leguminosae	247

295	(2',4',4,α) continued 2',4,α-triOH, 4'-geranyloxy	—	C ₂₅ H ₃₀ O ₅	<i>Millettia usaramensis</i> ssp. <i>usaramensis</i>	Leguminosae	248
Penta O-substituted						
	(2',4',6',3,4)					
296	2',4',6',3,4-pentaoH	3',5-Diprenyl	C ₂₅ H ₃₀ O ₆	<i>Esenbeckia grandiflora</i> ssp. <i>grandiflora</i>	Rutaceae	249
297	2',4',6',3,4-pentaoH	3'-Geranyl, 5-prenyl	C ₃₀ H ₃₈ O ₆	<i>Esenbeckia grandiflora</i> ssp. <i>grandiflora</i>	Rutaceae	249
298	2',4',6',3-tetraOH	3'-Geranyl, 6'',6''- dimethylpyrano[2'',3'',4,5]	C ₃₀ H ₃₆ O ₆	<i>Esenbeckia grandiflora</i> ssp. <i>grandiflora</i>	Rutaceae	249
299	2',4',6',3-tetraOH, 4-OMe	3',5-Diprenyl	C ₂₆ H ₃₂ O ₆	<i>Metrodorea nigra</i>	Rutaceae	250
300	2',6',3-triOH, 4-OMe	5-Prenyl, 6'',6''-dimethyl-4'',5''- dihydropyrano[2'',3'',4',3']	C ₂₆ H ₃₂ O ₆	<i>Metrodorea nigra</i>	Rutaceae	250
301	(2',4',3,4,β) 2',β-diOMe, 3,4-OCH ₂ O-	Furan[2'',3'',4',3']	C ₂₀ H ₁₈ O ₆	<i>Pongamia pinnata</i>	Leguminosae	53
Hexa O-substituted						
	(2',4',5',3,4,β)					
302	2',5',β-triOMe, 3,4-OCH ₂ O-	6'',6''-Dimethylpyrano[2'',3'',4',3']	C ₂₄ H ₂₆ O ₇	<i>Pongamia pinnata</i>	Leguminosae	53

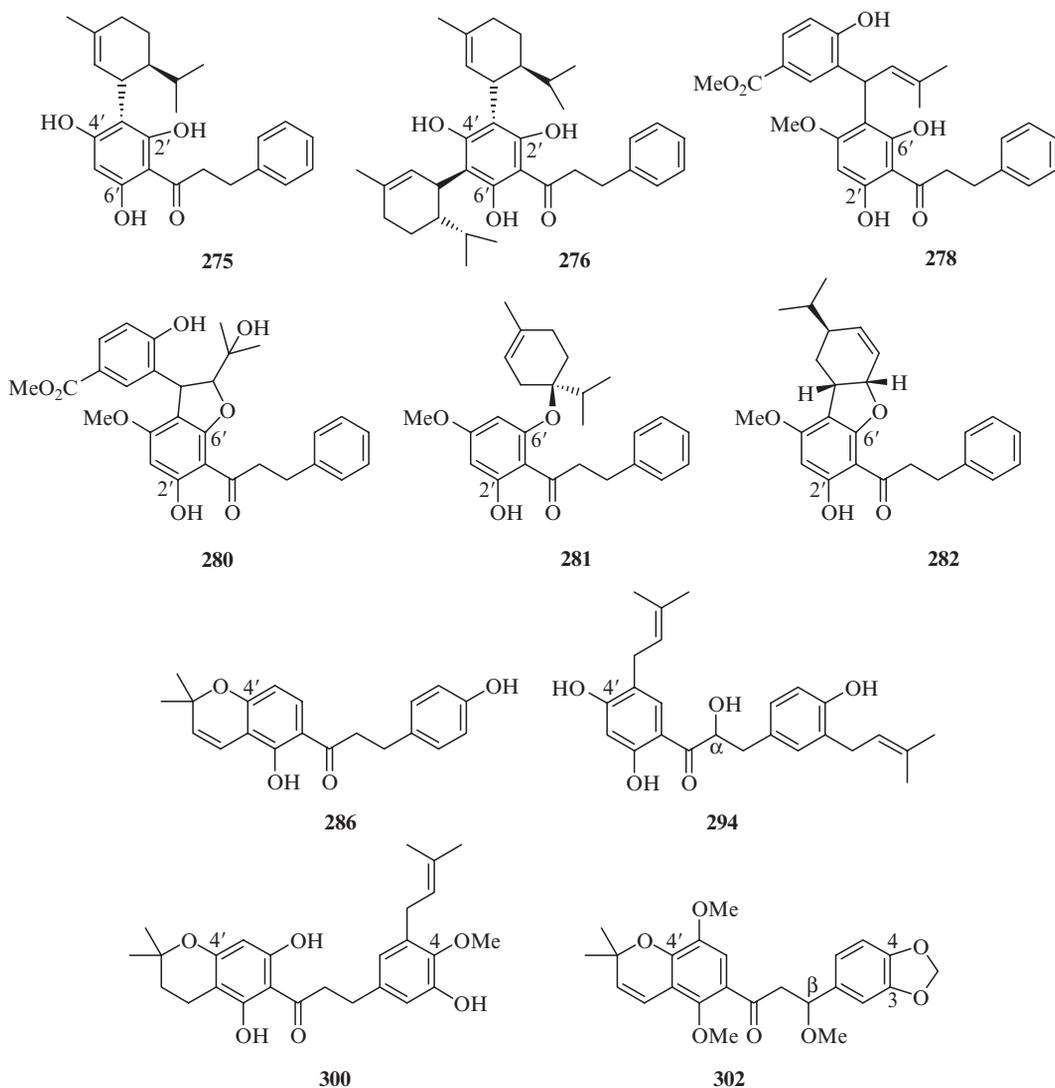


FIGURE 16.15 Isoprenylated dihydrochalcones (see Table 16.9).

study of *P. aduncum* constituents.²⁴² The compounds piperaduncins A (**278**) and B (**280**) showed antibacterial activity toward both *Bacillus subtilis* and *Micrococcus luteus* and were cytotoxic toward a KB nasopharyngeal carcinoma cell line. Bioassay-guided fractionation of an extract of dried leaves and twigs of *Piper longicaudatum* (Piperaceae) gave piperaduncin B (**280**) and a new constituent, longicaudatin (**279**), a furanodihydrochalcone with the furan ring substituted by methyl *p*-hydroxybenzoate.²⁴³

Rao et al. revised the structure of crotagamosmin (**286**), formerly described as a prenylflavanone, to 2',4-dihydroxy-6'',6''-dimethylpyrano[2'',3'';4',3']dihydrochalcone.²⁴⁴ Two derivatives of crotagamosmin, crotagamin (**287**) and crotin (**292**), were later isolated from the same source, *Crotalaria ramosissima* (cited incorrectly as *Crotolaria ramosissima*).²⁴⁵ Kanzonol Y (**294**) is an uncommon diprenylated α -hydroxydihydrochalcone found in cultivated licorice (*Glycyrrhiza glabra*)²⁴⁷ for which the absolute configuration was determined by

Mosher's method as $\alpha(R)$.²⁵³ All the new isoprenylated α - and β -hydroxydihydrochalcones reported in Table 16.9 are constituents of the Leguminosae, a general trend observed both in Table 16.8 and the earlier literature.¹⁰ Another apparent trend is the predominance of isoprenylated (2',4',6',3,4)-penta-*O*-substituted dihydrochalcones in species of the Rutaceae (296–300).^{249,250} Only one other example of this type of compound has been recorded in the literature, a geranylated derivative from *Helichrysum monticola* (Asteraceae).²⁵⁴

16.3.3 DIHYDROCHALCONE GLYCOSIDES

Relatively few dihydrochalcone glycosides have been reported in the literature, with only 11 *O*-glycosides and 3 *C*-glycosides published prior to 1992, according to the *Handbook of Natural Flavonoids*.^{9,10} The *O*-glycosides are mainly 2'- and 4'-*O*-glucosides of simple dihydrochalcones while only one higher glycoside, the incompletely characterized 2'-*O*-xylosylglucoside of phloretin (2',4',6',4-tetrahydroxydihydrochalcone), has been described.²⁵⁵ Table 16.10 lists new dihydrochalcone glycosides published in the period 1992 to 2003, and some examples are shown in Figure 16.16. Among the new compounds are the first acylated dihydrochalcone glycosides, including two acetylated glucosides of phloretin (306, 307).^{258,259} Of particular interest are thoningianins A and B (303, 304), unusual galloylated glucosides of 2',4',6'-trihydroxydihydrochalcone isolated from the roots of the African medicinal herb *Thonningia sanguinea* (Balanophoraceae).²⁵⁶ Both structures feature a 4'-*O*-glucopyranosyl moiety acylated at 4-OH and 6-OH by a C–C linked digallic acid. The compounds are effective scavengers of the DPPH radical, according to analysis by EPR spectroscopy.²⁵⁶ In contrast, salicifolioside A, a 4'-*O*-gentobioside of 2',4'-dihydroxy-3',6'-dimethoxydihydrochalcone isolated from aerial parts of *Polygonum salicifolium* (Polygonaceae), was not active in the DPPH assay.²⁵⁷ The β -Glc-(1 \rightarrow 6)- β -Glc interglycosidic linkage of this compound was confirmed from HMBC data.

The first di-*C*-glycoside of a dihydrochalcone has been reported as a characteristic constituent of species of *Fortunella* (Rutaceae).²⁶⁰ The compound, phloretin 3',5'-di-*C*-glucoside (308), accumulates in the fruits and leaves. Only two of 28 species of *Citrus* (*C. halimii* and *C. madurensis*) surveyed contain this glycoside and it was absent from *Poncirus trifoliata*. In contrast, large amounts were detected in many *Fortunella*–*Citrus* hybrids. The authors suggest that accumulation of phloretin 3',5'-di-*C*-glucoside is a generic trait of *Fortunella*, and that inheritance of the trait among intergeneric hybrids is under the control of a dominant allele.²⁶⁰ A 3'-*C*-xyloside of 2',4',3,4, $\alpha(R)$ -penta-hydroxydihydrochalcone (314), the corresponding aglycone (273), and a compound described as the 3'-*O*-xyloside of the same dihydrochalcone were obtained from *Eysenhardtia polystachya* (Leguminosae).²³⁴ However, the NMR data presented for the latter compound do not support its identification as an *O*-glycoside. Two 3'-*C*-glucosides of α -hydroxydihydrochalcones were isolated previously from *E. polystachya* (coatline A and B)²³⁹ and *C*-glucosides of both α -hydroxy- (coatline A)²⁶⁶ and β -hydroxydihydrochalcones have been reported from *Pterocarpus marsupium* (Leguminosae).^{267,268}

16.3.4 DIHYDROCHALCONE DIMERS

The only example of a dihydrochalcone dimer reported in the literature prior to 1992 is brackenin, a $C\alpha$ – $C\alpha$ linked dimer of davidigenin from *Brackenridgea zanguebarica* (Ochnaceae).²⁶⁹ Several new examples of this rare class of dihydrochalcones cited between 1992 and 2003 are shown in Table 16.11 and Figure 16.17 (note that the “mixed” chalcone–dihydrochalcone dimers 142 and 148–150 are described in Table 16.4 and Section 16.2.4). The leaves and inflorescences of *Iryanthera sagotiana* (Myristicaceae) yielded a dimer (317) comprising

TABLE 16.10
New Dihydrochalcone Glycosides Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
303	2',4',6'-Trihydroxydihydrochalcone 4'-O-(3''-O-Galloyl-4'',6''-O,O- hexahydroxydiphenoylglucoside) (thonningianin A)	C ₄₂ H ₃₄ O ₂₁	<i>Thonningia sanguinea</i>	Balanophoraceae	256
304	4'-O-(4'',6''-O,O- Hexahydroxydiphenoylglucoside) (thonningianin B)	C ₃₅ H ₃₀ O ₁₇	<i>Thonningia sanguinea</i>	Balanophoraceae	256
305	2',4'-Dihydroxy-3',6'-dimethoxydihydrochalcone 4'-O-Glucosyl-(1''' → 6'')glucoside (salicifolioside A)	C ₂₉ H ₃₈ O ₁₅	<i>Polygonum salicifolium</i>	Polygonaceae	257
306	2',4',6',4'-Tetrahydroxydihydrochalcone (<i>phloretin</i>) 2'-O-(6''-O-Acetylglucoside)	C ₂₃ H ₂₆ O ₁₁	<i>Loiseleuria procumbens</i>	Ericaceae	258
307	4'-O-(2''-O-Acetylglucoside)	C ₂₃ H ₂₆ O ₁₁	<i>Lithocarpus pachyphyllus</i>	Fagaceae	259
308	3',5'-Di-C-glucoside	C ₂₇ H ₃₄ O ₁₅	<i>Fortunella</i> spp.	Rutaceae	260
309	2',4',6'-Trihydroxy-4-methoxydihydrochalcone 2'-O-Glucoside	C ₂₂ H ₂₆ O ₁₀	<i>Iryanthera sagotiana</i>	Myristicaceae	261
310	2',4-Dihydroxy-4',6'-diacetoxydihydrochalcone 2'-O-Glucoside (<i>zosterin</i>)	C ₂₅ H ₂₈ O ₁₂	<i>Zostera</i> sp.	Zosteraceae	262
311	4-Hydroxy-2',4',6'-trimethoxydihydrochalcone 4-O-Glucoside (<i>bidenoside B</i>)	C ₂₄ H ₃₀ O ₁₀	<i>Bidens bipinnata</i>	Asteraceae	263
312	2',4',4,α-Tetrahydroxydihydrochalcone α-O-Glucoside (<i>licoagroside F</i>)	C ₂₁ H ₂₄ O ₁₀	<i>Glycyrrhiza pallidiflora</i>	Leguminosae	264
313	2',4',4,β-Tetrahydroxydihydrochalcone 2'-O-Glucoside (<i>rocymosin B</i>)	C ₂₁ H ₂₄ O ₁₀	<i>Rosa cymosa</i>	Rosaceae	265
314	2',4',3,4,α-Pentahydroxydihydrochalcone 3'-C-Xyloside	C ₂₀ H ₂₂ O ₁₀	<i>Eysenhardtia polystachya</i>	Leguminosae	234

two molecules of 2',4',6'-trihydroxy-4-methoxydihydrochalcone linked by a C–C bond between C-3' of each A-ring.²⁶¹ Cycloaltisin 6 (**319**) is the only known example of an isoprenylated dihydrochalcone dimer and a constituent of the bud covers of *Artocarpus altilis* (Moraceae).²⁷² This compound, which comprises two molecules of 2',4',3,4-tetrahydroxy-2'-geranyldihydrochalcone linked by a C–C bond between the B-rings (Figure 16.17), is a potent inhibitor of cathepsin K, a novel cysteine protease implicated in osteoporosis. A novel feature of the structure of piperaduncin C (**318**) is a methylene bridge connecting C-3' of the A-rings of two molecules of 2',6'-dihydroxy-4'-methoxydihydrochalcone.²⁴² Littorachalcone (**315**)²⁷⁰ and verbenachalcone (**316**)²⁷¹ are the first examples of ether-linked dihydrochalcone dimers and were isolated from the aerial parts of *Verbena littoralis* (Verbenaceae). Both compounds enhanced the effect of neural growth factor on stimulating neurite outgrowth in PC12D cells, with littorachalcone (**315**) showing greater potency. A characteristic structural feature of both littorachalcone (**315**) and verbenachalcone (**316**) is an ether bridge between the

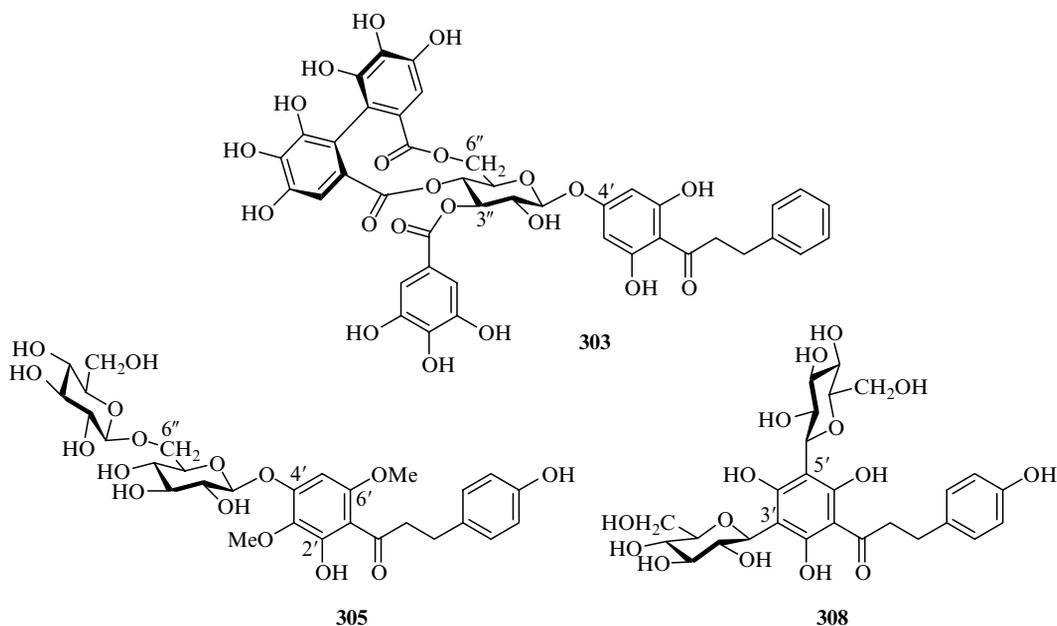


FIGURE 16.16 Dihydrochalcone glycosides (see Table 16.10).

B-rings of their constituent dihydrochalcone monomers. A concise synthesis of verbenachalcone (**316**) by catalytic copper-mediated coupling of phenol and aryl halides has been reported by Xing et al., who also prepared two further derivatives for preliminary structure–activity studies.²⁷⁷ One of the latter, the corresponding bichalcone (deoxo) derivative of verbenachalcone showed no activity in the neural outgrowth stimulation bioassay mentioned above.

A resin from *Dracaena cinnabari* known as “dragon’s blood,” mentioned previously as the source of the dihydrochalcone **251** (Section 16.3.1), also yielded cinnabarone (**321**), a dihydrochalcone linked by a C–C bond from C-5 of its B-ring to the deoxy-carbon of a deoxote-tetrahydrochalcone.²²⁰ The closely related compound cochinchinenin (**320**) is characterized by the same linkage, and was isolated from “Chinese dragon’s blood,” the resin of *D. cochinchinensis*.²⁷³ It is interesting to note that both cochinchinenin and cinnabarone are dimers derived from retrodihydrochalcones, given the prevalence of this type of compound in species of *Dracaena* (Table 16.8).

Trianguletin (**322**) is a novel dihydrochalcone–flavonol dimer in which the A-rings of the two flavonoids are linked by a methylene bridge similar to that of piperaduncin C (**318**).²⁷⁴ In the case of trianguletin, this bridge connects C-3' of 2',6'-dihydroxy-4'-methoxy-5'-methyl-dihydrochalcone with C-8 of 3,5,7-trihydroxy-4'-methoxyflavone (kaempferide). The structure of this compound, which is a constituent of the farinose exudate of the fronds of the fern, *Pentagramma triangularis* ssp. *triangularis*, was confirmed by x-ray crystallography.²⁷⁴ Four structurally related dimers also obtained from farinose exudates of *P. triangularis* comprise slightly different combinations of dihydrochalcones and flavonols; for example, trianguletin “B” (**323**) contains 5,7-dihydroxy-3-methoxyflavone (galangin 3-methyl ether) instead of kaempferide.²⁷⁵ Similarly, trianguletins “C” to “E” (**324–326**) comprise 2',6',4-trihydroxy-4'-methoxy-5'-methyl-dihydrochalcone with kaempferide, ermanin (kaempferol 3,4'-dimethyl ether), and galangin 3-methyl ether, respectively.²⁷⁶

TABLE 16.11
Dihydrochalcone Dimers and Heterodimers Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
Dihydrochalcone–dihydrochalcone					
315	Littorachalcone	C ₃₀ H ₂₆ O ₈	<i>Verbena littoralis</i>	Verbenaceae	270
316	Verbenachalcone	C ₃₁ H ₂₈ O ₉	<i>Verbena littoralis</i>	Verbenaceae	271
317	3',3'-Bis(2',4',6'-trihydroxy-4-methoxydihydrochalcone)	C ₃₂ H ₃₀ O ₁₀	<i>Iryanthera sagotiana</i>	Myristicaceae	261
318	Piperaduncin C	C ₃₃ H ₃₂ O ₈	<i>Piper aduncum</i>	Piperaceae	242
319	Cycloaltilisins 6	C ₅₀ H ₅₈ O ₁₀	<i>Artocarpus altilis</i>	Moraceae	272
Dihydrochalcone–deoxotetrahydrochalcone					
320	Cochinchinenin	C ₃₁ H ₃₀ O ₇	<i>Dracaena cochinchinensis</i>	Liliaceae	273
321	Cinnabarone	C ₃₂ H ₃₂ O ₇	<i>Dracaena cinnabari</i>	Liliaceae	220
Dihydrochalcone–flavonol					
322	Trianguletin	C ₃₄ H ₃₀ O ₁₀	<i>Pentagramma triangularis</i> ssp. <i>triangularis</i>	Adiantaceae	274
323	Trianguletin "B"	C ₃₄ H ₃₀ O ₉	<i>Pentagramma triangularis</i>	Adiantaceae	275
324	Trianguletin "C"	C ₃₄ H ₃₀ O ₁₁	<i>Pentagramma triangularis</i>	Adiantaceae	276
325	Trianguletin "D"	C ₃₅ H ₃₂ O ₁₁	<i>Pentagramma triangularis</i>	Adiantaceae	276
326	Trianguletin "E"	C ₃₄ H ₃₀ O ₁₀	<i>Pentagramma triangularis</i>	Adiantaceae	276

16.3.5 BENZYLATED DIHYDROCHALCONES

New reports of *C*-benzylated dihydrochalcones are summarized in Table 16.12. Examples of this unique group of dihydrochalcones were known previously only from *Xylopiya africana* and species of *Uvaria* (Annonaceae),^{10,278} and these taxa continue to be the only recorded sources of the compounds. Those listed in Table 16.12 were obtained from either the root bark of *Uvaria leptocladon* or the dried roots of *Xylopiya africana*, and are based on uvangelin (2',4'-dihydroxy-6'-methoxydihydrochalcone), with the exception of **327**, which is a derivative of 2',6'-dihydroxy-4'-methoxydihydrochalcone.²⁷⁹ Some examples of this group of dihydrochalcones are shown in Figure 16.18. The 2-hydroxybenzyl substituents (2-OHBn) can be at either C-3' or C-5' of the A-ring or at both positions. Many of the compounds have chains of 2-hydroxybenzyl unit linked successively from C-5_n to C-1_{n+1}, which may also incorporate a C-3_n to C-1_{n+1} linkage. For example, compounds **332** and **333** feature chains of four 2-hydroxybenzyl groups as substituents to the dihydrochalcone A-ring.²⁸³ Preliminary data on the antibacterial activity of some of the compounds (**330–333**) have been published.^{282,283}

16.3.6 DIHYDROCHALCONE–LIGNAN CONJUGATES

Iryantherins G–L (**334–339**) are flavonolignans thought to arise from the oxidative coupling of dihydrochalcones and lignans (Figure 16.18). They represent further examples of a series of compounds reported exclusively from species of *Iryanthera* (Myristicaceae). The diastereoisomeric pairs iryantherins G and H (**334**, **335**) and I and J (**336**, **337**) were isolated from the fruits of *I. grandis*, a known source of dihydrochalcones and lignans.²⁸⁴ The relative configurations of the lignan components of iryantherins G–J were deduced from NOE

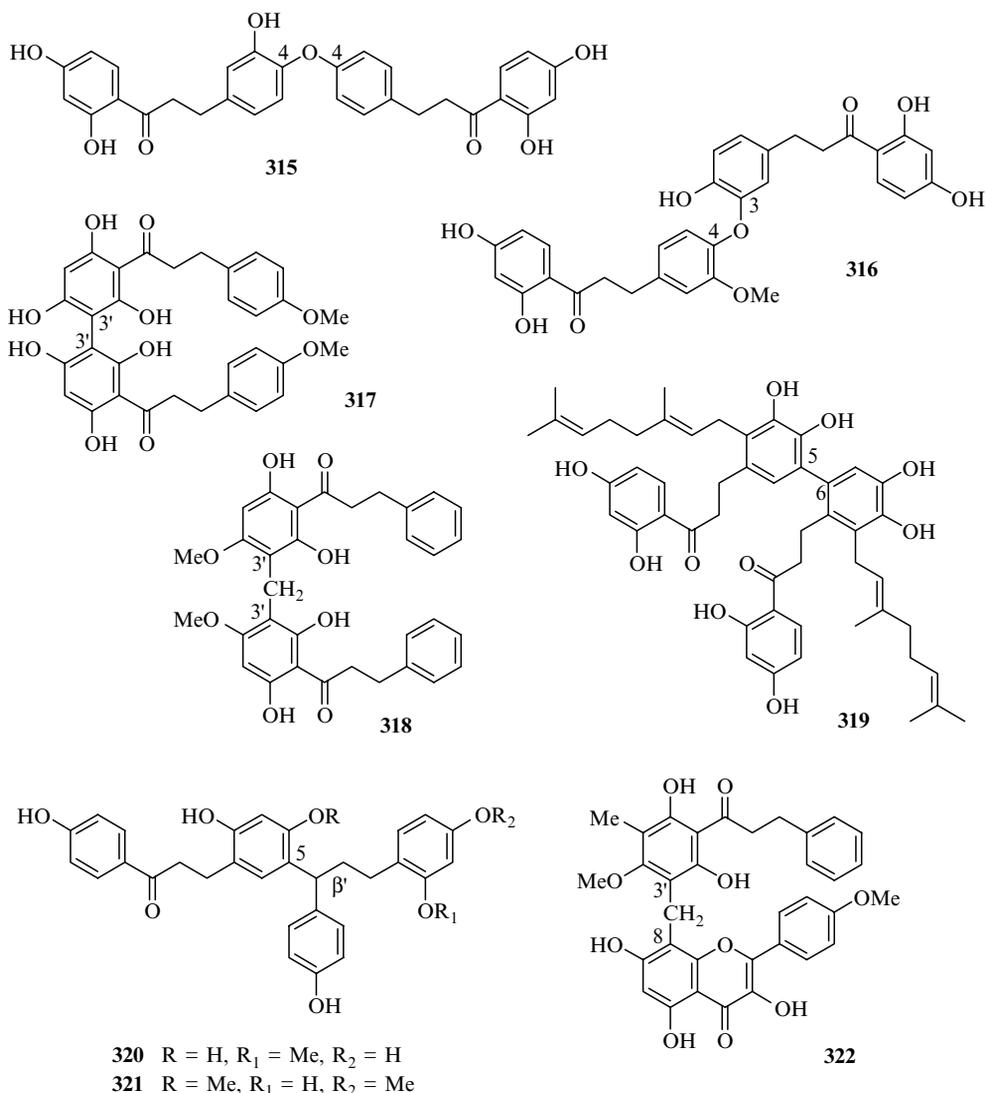


FIGURE 16.17 Dihydrochalcone dimers and heterodimers (see Table 16.11).

measurements and coupling constant data. The lignans are linked to C-3' of the A-rings of either 2',4',6'-trihydroxy-4-methoxy- (G and H) or 2',4',6'-trihydroxy-3,4-methylenedioxydihydrochalcone (I and J). An additional pair of flavonolignan diastereoisomers (iryrantherins K and L) was isolated subsequently by Silva et al. from the pericarps of *I. lancifolia*.²²⁹ Both compounds showed moderate antioxidant activity. Iryrantherin L (339) appears to be synonymous with iryantherin B, a compound found previously in both *I. laevis* and *I. ulei* for which the relative stereochemistry was not defined.²⁸⁵ Iryrantherins K (338) and L (339) are also known as constituents of the stem bark of *I. megistophylla*, and were assayed for antibacterial, antifungal, antiviral, and antiacetylcholinesterase activity.²⁸⁶ Iryrantherin K (338) showed high levels of inhibition against potato virus, but only moderate inhibition of acetylcholinesterase.

TABLE 16.12
Miscellaneous Dihydrochalcones Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
C-Benzylated Dihydrochalcones^a					
327	2',6'-DiOH, 4'-OMe, 3'-(2-OHBn)	C ₂₃ H ₂₂ O ₅	<i>Xylopia africana</i>	Annonaceae	279
328	2',4'-DiOH, 6'-OMe, 3'-(2-OHBn), 5'-(2 × 2-OHBn) (triuvaretin)	C ₃₇ H ₃₄ O ₇	<i>Uvaria leptoclodon</i> <i>Xylopia africana</i>	Annonaceae	280 281
329	2',4'-DiOH, 6'-OMe, 3'-(2 × 2-OHBn), 5'-(2-OHBn) (isotriuvaretin)	C ₃₇ H ₃₄ O ₇	<i>Uvaria leptoclodon</i> <i>Xylopia africana</i>	Annonaceae	280 281
330	2',4'-DiOH, 6'-OMe, 3'-(2-OHBn), 5'-(3 × 2-OHBn)	C ₄₄ H ₄₀ O ₈	<i>Xylopia africana</i>	Annonaceae	282
331	2',4'-DiOH, 6'-OMe, 3'-(3 × 2-OHBn), 5'-(2-OHBn)	C ₄₄ H ₄₀ O ₈	<i>Xylopia africana</i>	Annonaceae	282
332	2',4'-DiOH, 6'-OMe, 3'-(2-OHBn), 5'-(4 × 2-OHBn)	C ₅₁ H ₄₆ O ₉	<i>Xylopia africana</i>	Annonaceae	283
333	2',4'-DiOH, 6'-OMe, 3'-(4 × 2-OHBn), 5'-(2-OHBn)	C ₅₁ H ₄₆ O ₉	<i>Xylopia africana</i>	Annonaceae	283
Dihydrochalcone-lignans					
334	Iryantherin G	C ₃₄ H ₃₆ O ₇	<i>Iryanthera grandis</i>	Myristicaceae	284
335	Iryantherin H	C ₃₄ H ₃₆ O ₇	<i>Iryanthera grandis</i>	Myristicaceae	284
336	Iryantherin I	C ₃₄ H ₃₄ O ₈	<i>Iryanthera grandis</i>	Myristicaceae	284
337	Iryantherin J	C ₃₄ H ₃₄ O ₈	<i>Iryanthera grandis</i>	Myristicaceae	284
338	Iryantherin K	C ₃₅ H ₃₈ O ₇	<i>Iryanthera lancifolia</i> (<i>I. ulei</i>)	Myristicaceae	229
339	Iryantherin L	C ₃₅ H ₃₈ O ₇	<i>Iryanthera lancifolia</i> (<i>I. ulei</i>)	Myristicaceae	229

^aBn, benzyl.

16.4 AURONES

16.4.1 AURONES AND AURONOLS

The number of new aurone and auronol aglycones reported in the literature between 1992 and 2003 is relatively small, as Table 16.13 indicates. Some examples of these compounds are illustrated in Figure 16.19. Only six of the compounds listed in Table 16.13 represent new combinations of hydroxy and methoxy substituents. Among these are two examples from Asteraceae taxa, 5-hydroxy-4,6,4'-trimethoxyaurone (**342**) from the flowers of cultivated sunflowers (*Helianthus annuus*)²⁸⁸ and 4,6,7,4'-tetrahydroxyaurone (**343**) from *Helminthia echioides* (= *Picris echioides*).^{289,290} Hamiltrone (3',4'-dihydroxy-4,5,6-trimethoxyaurone) (**350**) showed the highest DNA strand-scission activity of several constituents isolated from a combined leaf and stem extract of *Uvaria hamiltonii* (Annonaceae).⁴⁸ The structural similarity of this aurone to combretastatin A-4, a tumor vascular targeting agent, prompted Lawrence et al. to develop a total synthesis for the compound.²⁹⁵ This was achieved in four steps and 37% overall yield from benzofuranone and benzaldehyde precursors and facilitated the preparation of a series of substituted aurone analogs. Synthetic hamiltrone showed only poor cell-growth inhibition against a K562 cell line (IC₅₀ = 12 μM), but the activity of 3'-hydroxy-5,6,7,4'-tetramethoxyaurone was more than 200-fold greater (IC₅₀ = 50 nM).²⁹⁵ A general observation was that 5,6,7-trimethoxyaurones showed greater cell-growth inhibition

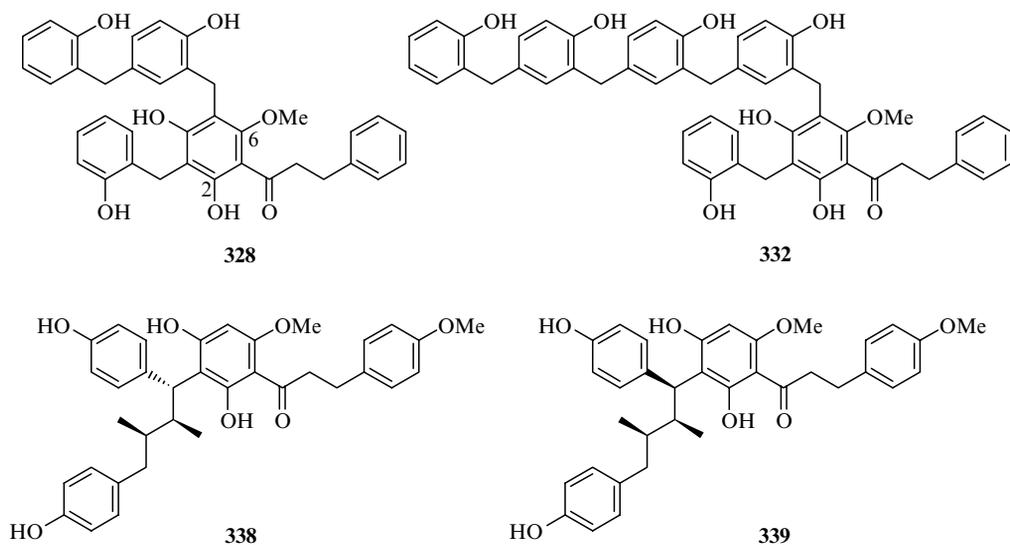


FIGURE 16.18 C-Benzylated dihydrochalcones and dihydrochalcone-lignan conjugates (see Table 16.12).

than the corresponding 4,5,6-trimethoxyaurones. The two new auroneols from the bark of *Pseudolarix amabilis* (Pinaceae), amaronols A (**351**) and B (**352**), were considered to be enantiomeric pairs because of the reversible hemiketal at C-2.²⁹³ These compounds were inactive in a series of antifungal and antibacterial assays against test organisms.

The ground rhizomes and roots of *Cyperus capitatus* (Cyperaceae) are the only known source of aurone aglycones with C-methyl substituents (**344–348**),^{291,292} although two aurone glycosides with C-7 methyl groups were described in 1989 from *Pterocarpus marsupium* (Leguminosae).^{296,297} Two new examples of isoprenylated aurones based on sulfuretin (6,3',4'-trihydroxyaurone) have been found in the cortex of *Broussonetia papyrifera* (Moraceae) and hairy root cultures of *Glycyrrhiza glabra* (Leguminosae), and assigned the trivial names of brouossoaurone A (**340**)²⁸⁷ and licoagroaurone, respectively (**341**).⁸⁸ Licoagroaurone is the first aurone to be isolated from species of *Glycyrrhiza* (licorice), from which more than 100 flavonoids have already been described.²⁵³ Perhaps the most unusual of the aurones in Table 16.13 are the chlorinated derivatives **353** and **354**, obtained from the marine brown alga *Spatoglossum variabile*.²⁹⁴ These are the first halogenated aurones to be described from a natural source. The auroneol 4'-chloro-2-hydroxyaurone (**354**) was found as a racemic mixture of (*R*)- and (*S*)-epimers at C-2.

16.4.2 AURONE GLYCOSIDES

Nine aurone and auroneol glycosides reported in the literature between 1992 and 2003 are listed in Table 16.14. Structures of some of these compounds are given in Figure 16.20. Dalmaisione D (**355**) from the roots of *Polygala dalmaisia* (Polygalaceae) is an unusual example of an aurone glycoside for which the corresponding aglycone, 2'-hydroxyaurone, is unknown.²⁹⁸ Indeed, no other 2'-*O*-substituted aurones have been reported in the literature. Species from the genus *Bidens* (Asteraceae) are a well-documented source of glucosides and acylated glucosides of maritimetin (6,7,3',4'-tetrahydroxyaurone)¹⁰ and two new di- and triacetylated 6-*O*-glucosides (**358**, **359**) have now been described from the aerial parts of *B. bipinnata* and *B. pilosa* var. *radiata*, respectively.^{263,127} Caulesauroneside (**356**), an aurone

TABLE 16.13
New Aurones and Auronols Reported in the Literature from 1992 to 2003

No.	OH	OMe	Other	Mol. Formula	Trivial Name	Source	Family	Ref.
Tri O-substituted								
	(6,3',4')							
340	6,3',4'	—	5-Pr	C ₂₀ H ₁₈ O ₅	Broussourone A	<i>Broussonetia papyrifera</i>	Moraceae	287
341	6,3',4'	—	7-Pr	C ₂₀ H ₁₈ O ₅	Licoagroaurone	<i>Glycyrrhiza glabra</i>	Leguminosae	88
Tetra O-substituted								
	(4,5,6,4')							
342	5	4,6,4'	—	C ₁₈ H ₁₆ O ₆		<i>Helianthus annuus</i>	Asteraceae	288
	(4,6,7,4')							
343	4,6,7,4'	—	—	C ₁₅ H ₁₀ O ₆		<i>Helminthia echioides</i>	Asteraceae	289, 290
	(4,6,3',4')							
344	4,6,3',4'	—	5-Me	C ₁₆ H ₁₂ O ₆		<i>Cyperus capitatus</i>	Cyperaceae	291
345	4,6,3',4'	—	7-Me	C ₁₆ H ₁₂ O ₆		<i>Cyperus capitatus</i>	Cyperaceae	291
346	6,3',4'	4	5-Me	C ₁₇ H ₁₄ O ₆		<i>Cyperus capitatus</i>	Cyperaceae	291
347	6,3',4'	4	7-Me	C ₁₇ H ₁₄ O ₆		<i>Cyperus capitatus</i>	Cyperaceae	291
348	6,3'	4,4'	5-Me	C ₁₈ H ₁₆ O ₆		<i>Cyperus capitatus</i>	Cyperaceae	291
349	—	4,6,3',4'	—	C ₁₉ H ₁₈ O ₆		<i>Cyperus capitatus</i>	Cyperaceae	292
Penta O-substituted								
	(4,5,6,3',4')							
350	3',4'	4,5,6	—	C ₁₈ H ₁₆ O ₇	Hamiltonone	<i>Uvaria hamiltonii</i>	Annonaceae	48
Auronols								
	(2,4,6,3',4',5')							
351	2,4,6,3',4',5'	—	—	C ₁₅ H ₁₂ O ₈	Amaronol A	<i>Pseudolarix amabilis</i>	Pinaceae	293
352	2,4,6,3',5'	4'	—	C ₁₆ H ₁₄ O ₈	Amaronol B	<i>Pseudolarix amabilis</i>	Pinaceae	293
Halogenated								
	—							
353	—	—	4'-Cl	C ₁₅ H ₉ ClO ₂		<i>Spataglossum variabile</i>	Dictyotaceae	294
354	2	—	4'-Cl	C ₁₅ H ₁₁ ClO ₃		<i>Spataglossum variabile</i>	Dictyotaceae	294

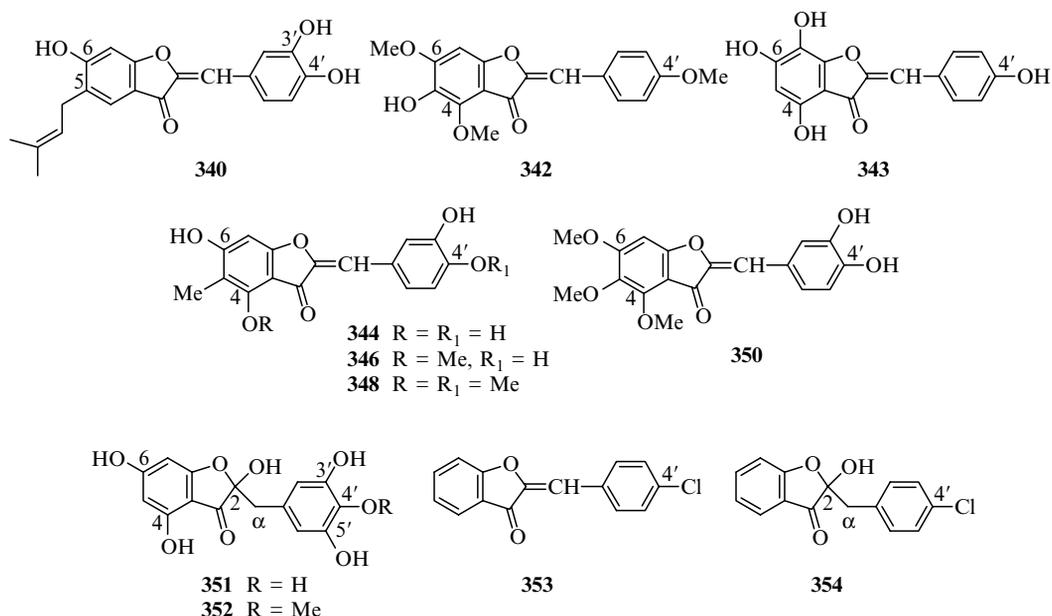


FIGURE 16.19 Aurones and auronols (see Table 16.13).

diglucoside from the roots and rhizomes of *Asarum longirhizomatosum*, is the first aurone to be found in the Aristolochiaceae.²⁹⁹ A *C*-methylated aurone rhamnoside (**360**)³⁰⁰ from the heartwood of *Pterocarpus santalinus* (Leguminosae) is an analog of similar structures described previously from *P. marsupium*.^{296,297} The characterization of the glycosidic component of a second aurone glycoside (**357**) from *P. santalinus* as neohesperidoside must be regarded as preliminary due to the incomplete nature of the supporting spectroscopic data.³⁰⁰

The first auronol glycosides to be described are all from genera of the Rhamnaceae and based on maesopsin (2,4,6,4'-tetrahydroxy-2-benzylcoumaranone). Hovetrichosides C (**361**) and D (**363**) were obtained from the bark of *Hovenia trichocarea* together with maesopsin and neolignan and phenylpropanoid glycosides.³⁰¹ Similarly, maesopsin 6-*O*-glucoside (**362**) was obtained together with maesopsin from root bark of *Ceanothus americanus*.³⁰² These compounds showed poor activity as inhibitors of the growth of both Gram-negative, anaerobic periodontal pathogens and Gram-positive carcinogenic bacteria.³⁰²

16.4.3 AURONE AND AURONOL DIMERS

The first biaurone to be reported in the literature has been isolated from the gametophytes of two species of moss, *Aulacomnium androgynum* and *A. palustre*, and named aulacomnium-biaureusidin (**364**).³⁰³ As the trivial name suggests, it is a dimer of the well-known compound aureusidin (4,6,3',4'-tetrahydroxyaurone), and is characterized by a C–C bond from C-5' of the B-ring of one aurone monomer to C-5 of the A-ring of the other (Figure 16.21). Two further biaurones have now been discovered as indicated in Table 16.15. One is a dimer of sulfuretin (6,3',4'-trihydroxyaurone) based on a C–C linkage between C α atoms (**365**)³⁰⁴ while licoagrone (**366**), the first isoprenylated biaurone, features a C–C bond from C-2' of 7-prenylsulfuretin to C-2 of a 5,3'-diprenylhispidol analog.³⁰⁵ Geiger and Markham have described the first aurone heterodimer, campylopusaurone (**367**), from the mosses *Campylopus clavatus* and *C. holomitrium*.³⁰⁶ In this unique compound, a C–C bond links C-5' of the

TABLE 16.14
New Aurone and Auronol Glycosides Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
355	2'-Hydroxyaurone 2'- <i>O</i> -Glucosyl-(1''' → 6'')- glucoside (dalmaisione D)	C ₂₇ H ₃₀ O ₁₃	<i>Polygala dalmaisiana</i>	Polygalaceae	298
356	4,6,4'-Trihydroxyaurone 4,6-Di- <i>O</i> -glucoside (caulesauroneside)	C ₂₇ H ₃₀ O ₁₅	<i>Asarum longirhizomatosum</i>	Aristolochiaceae	299
357	4- <i>O</i> -Rhamnosyl-(1''' → 2'')-glucoside 6,7,3',4'-Tetrahydroxyaurone (<i>maritimetin</i>)	C ₂₇ H ₃₀ O ₁₄	<i>Pterocarpus santalinus</i>	Leguminosae	300
358	6- <i>O</i> -(3'',6''-Di- <i>O</i> -acetylglucoside) (bidenoside A)	C ₂₅ H ₂₄ O ₁₃	<i>Bidens bipinnata</i>	Asteraceae	263
359	6- <i>O</i> -(3'',4'',6''-Tri- <i>O</i> -acetylglucoside) 4,6-Dihydroxy,3',4',5'-Trimethoxy-7- -methylaurone	C ₂₇ H ₂₆ O ₁₄	<i>Bidens pilosa</i> var. <i>radiata</i>	Asteraceae	127
360	4- <i>O</i> -Rhamnoside 2,4,6,4'-Tetrahydroxy-2- benzylcoumaranone (<i>maesopsin</i>)	C ₂₅ H ₂₈ O ₁₂	<i>Pterocarpus santalinus</i>	Leguminosae	300
361	4- <i>O</i> -Glucoside (hovetrichoside C)	C ₂₁ H ₂₂ O ₁₁	<i>Hovenia trichocarea</i>	Rhamnaceae	301
362	6- <i>O</i> -Glucoside	C ₂₁ H ₂₂ O ₁₁	<i>Ceanothus americanus</i>	Rhamnaceae	302
363	4- <i>O</i> -Glucoside 4'- <i>O</i> -rhamnoside (hovetrichoside D)	C ₂₇ H ₃₂ O ₁₅	<i>Hovenia trichocarea</i>	Rhamnaceae	301

B-ring of the aurone, aureusidin, to C-6 of the A-ring of the flavanone, eriodictyol (5,7,3',4'-tetrahydroxyflavanone).

An extraordinary series of rare auronol dimers and heterodimers (**368–377**) has been described in several papers by Bekker and colleagues.^{307–310} Some examples of the structures of these compounds are shown in Figure 16.21. The source of the dimers is the heartwood of

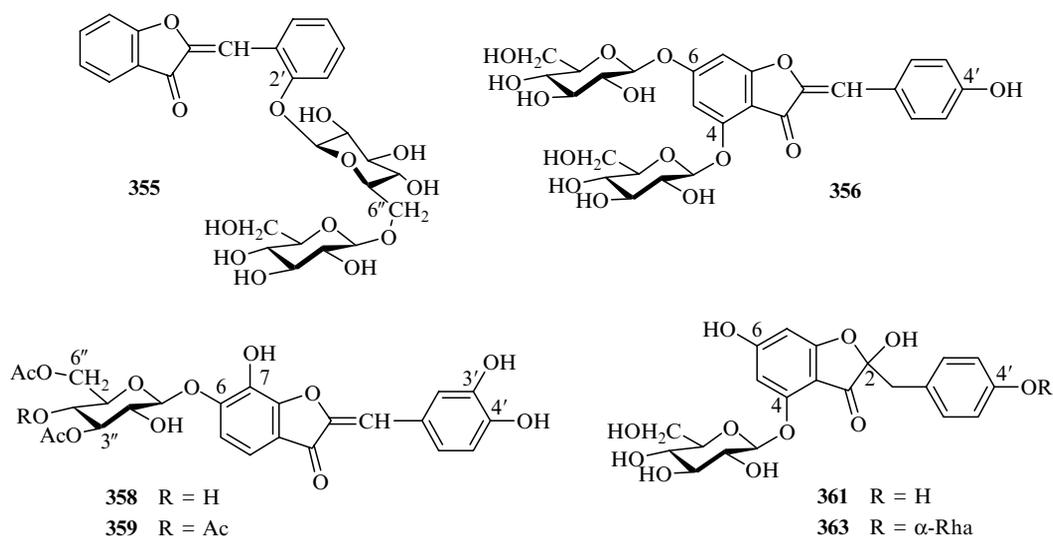


FIGURE 16.20 Aurone and auronol glycosides (see Table 16.14).

TABLE 16.15
Aurone and Auronol Dimers and Heterodimers Reported from 1992 to 2003

No.	Compound	Mol. Formula	Source	Family	Ref.
Aurone–aurone					
364	Aulacomniumbiaureusidin	C ₃₀ H ₁₈ O ₁₂	<i>Aulacomnium androgynum</i> <i>Aulacomnium palustre</i>	Aulacomniaceae	303
365	Disulfuretin	C ₃₀ H ₁₈ O ₁₀	<i>Cotinus coggygria</i>	Anacardiaceae	304
366	Licoagrone	C ₄₅ H ₄₂ O ₁₀	<i>Glycyrrhiza glabra</i>	Leguminosae	305
Aurone–flavanone					
367	Campylopousaurone	C ₃₀ H ₂₀ O ₁₂	<i>Campylopus clavatus</i> <i>Campylopus holomitrum</i>	Dicranaceae	306
Auronol–auronol					
368	(2 <i>S</i>)-2-Deoxymaesopsin-(2 → 7)-(2 <i>R</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	307
369	(2 <i>R</i>)-2-Deoxymaesopsin-(2 → 7)-(2 <i>S</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	307
370	(2 <i>R</i>)-2-Deoxymaesopsin-(2 → 7)-(2 <i>R</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	307
371	(2 <i>S</i>)-2-Deoxymaesopsin-(2 → 7)-(2 <i>S</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	307
Auronol–flavanone					
372	(2 <i>R</i> ,3 <i>S</i>)-Naringenin-(3α → 5)-(2 <i>R</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	308
373	(2 <i>R</i> ,3 <i>S</i>)-Naringenin-(3α → 5)-(2 <i>S</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	308
374	(2 <i>R</i> ,3 <i>S</i>)-Naringenin-(3α → 7)-(2 <i>R</i>)-maesopsin (zeyherin) ^a	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	309
375	(2 <i>R</i> ,3 <i>S</i>)-Naringenin-(3α → 7)-(2 <i>S</i>)-maesopsin ^a	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	309
Auronol–isoflavanone					
376	(2 <i>S</i> ,3 <i>R</i>)-Dihydrogenistein-(2α → 7)-(2 <i>R</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	308
377	(2 <i>S</i> ,3 <i>R</i>)-Dihydrogenistein-(2α → 7)-(2 <i>S</i>)-maesopsin	C ₃₀ H ₂₂ O ₁₁	<i>Berchemia zeyheri</i>	Rhamnaceae	308

^a Indicates revised structure

Berchemia zeyheri (Rhamnaceae), a tree native to southern Africa which is prized for its beautiful wood, known as “pink ivory” or “red ivory.” The complexity of the phenolic compounds present in heartwood extracts prompted their analysis as permethylated derivatives. Stereochemical features were determined by using both NMR and circular dichroism spectroscopy of the parent compounds and their degradation products. These methods were used successfully to obtain a full stereochemical description of the zeyherin epimers **374** and **375**,³⁰⁹ which were first isolated in 1971 but not fully characterized at that time.³¹¹ Subsequent work has led to the discovery of further aurone dimers and novel heterodimers with flavanone or isoflavanone constituents as summarized in Table 16.15.^{307,308,310}

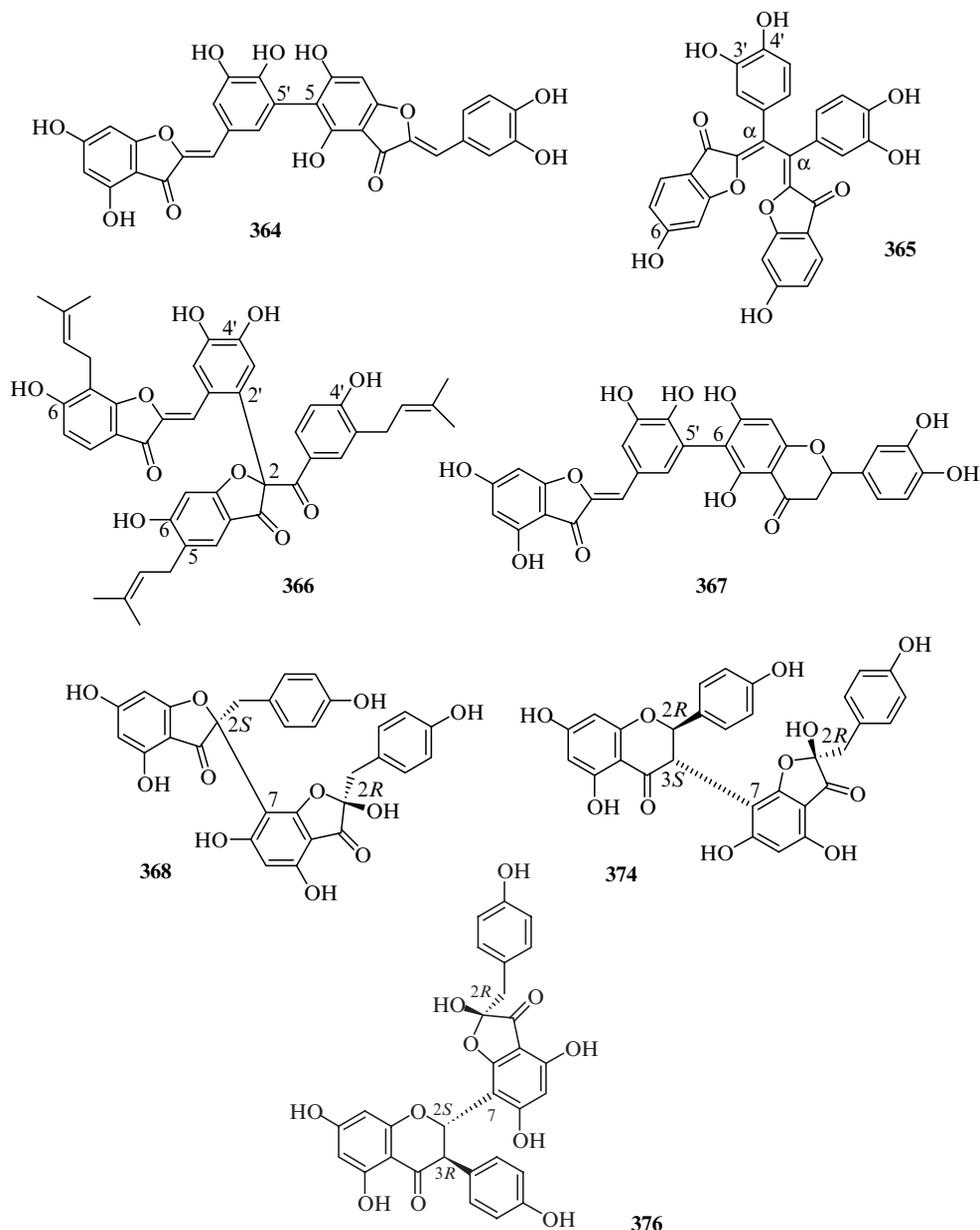


FIGURE 16.21 Aurone and auronol dimers and heterodimers (see Table 16.15).

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APPENDIX A Index of Molecular Formulae

Chalcones, dihydrochalcones, and aurones described in the literature from 1992 to 2003

Formula	Compound Number
C ₁₅ H ₉ ClO ₂	353
C ₁₅ H ₁₀ O ₆	343
C ₁₅ H ₁₁ ClO ₃	354
C ₁₅ H ₁₂ O ₃	1
C ₁₅ H ₁₂ O ₄	3
C ₁₅ H ₁₂ O ₈	351
C ₁₅ H ₁₄ O ₄	256
C ₁₅ H ₁₄ O ₆	273
C ₁₆ H ₁₂ O ₆	344, 345
C ₁₆ H ₁₄ O ₅	6, 11
C ₁₆ H ₁₄ O ₆	18
C ₁₆ H ₁₄ O ₈	352
C ₁₆ H ₁₆ O ₃	249, 250, 251
C ₁₆ H ₁₆ O ₄	252
C ₁₆ H ₁₆ O ₅	263
C ₁₆ H ₁₆ O ₆	274
C ₁₇ H ₁₂ O ₆	24
C ₁₇ H ₁₄ O ₆	346, 347
C ₁₇ H ₁₆ O ₄	4
C ₁₇ H ₁₆ O ₅	5, 8
C ₁₇ H ₁₆ O ₆	15
C ₁₇ H ₁₈ O ₄	253, 254, 255, 257
C ₁₇ H ₁₈ O ₅	258, 260, 261, 265, 266
C ₁₈ H ₁₆ O ₅	2, 12
C ₁₈ H ₁₆ O ₆	342, 348
C ₁₈ H ₁₆ O ₇	350
C ₁₈ H ₁₇ BrO ₅	9
C ₁₈ H ₁₈ O ₄	240
C ₁₈ H ₁₈ O ₅	7, 10, 13, 14, 23, 264
C ₁₈ H ₁₈ O ₇	20
C ₁₈ H ₂₀ O ₅	259, 262, 267, 268

APPENDIX A Index of Molecular Formulae — *continued*

Formula	Compound Number
$C_{18}H_{20}O_6$	269, 270, 271
$C_{19}H_{16}O_5$	293
$C_{19}H_{18}O_6$	17, 349
$C_{19}H_{18}O_7$	25
$C_{19}H_{20}O_4$	241
$C_{19}H_{20}O_6$	16, 272
$C_{19}H_{20}O_7$	19
$C_{20}H_{16}O_5$	182
$C_{20}H_{16}O_7$	96
$C_{20}H_{18}O_4$	51, 66
$C_{20}H_{18}O_5$	104, 340, 341
$C_{20}H_{18}O_6$	301
$C_{20}H_{20}O_4$	49, 57, 286
$C_{20}H_{20}O_5$	33, 41, 58, 102, 292
$C_{20}H_{20}O_6$	95
$C_{20}H_{20}O_7$	26
$C_{20}H_{22}O_7$	21
$C_{20}H_{22}O_{10}$	314
$C_{21}H_{20}O_4$	34, 38, 67
$C_{21}H_{20}O_5$	30, 35, 74
$C_{21}H_{22}O_4$	65, 287
$C_{21}H_{22}O_5$	36, 39, 64, 68, 73, 90, 91, 98
$C_{21}H_{22}O_6$	70, 75, 76
$C_{21}H_{22}O_9$	109
$C_{21}H_{22}O_{11}$	361, 362
$C_{21}H_{24}O_5$	288, 291
$C_{21}H_{24}O_7$	22
$C_{21}H_{24}O_{10}$	312, 313
$C_{22}H_{22}O_5$	79, 80, 89
$C_{22}H_{24}O_{10}$	118, 121, 124
$C_{22}H_{26}O_{10}$	309
$C_{23}H_{20}O_6$	236
$C_{23}H_{22}O_5$	327
$C_{23}H_{22}O_7$	108
$C_{23}H_{24}O_5$	99
$C_{23}H_{24}O_6$	92, 93, 94
$C_{23}H_{26}O_{11}$	131, 306, 307
$C_{24}H_{24}O_7$	37
$C_{24}H_{26}O_7$	97, 302
$C_{24}H_{30}O_{10}$	311
$C_{25}H_{24}O_4$	60
$C_{25}H_{24}O_5$	77, 88, 107
$C_{25}H_{24}O_{13}$	358
$C_{25}H_{26}O_3$	28, 29

APPENDIX A Index of Molecular Formulae — *continued*

Formula	Compound Number
$C_{25}H_{26}O_4$	52, 54, 59
$C_{25}H_{26}O_5$	61, 72, 86, 103, 105
$C_{25}H_{26}O_{13}$	125
$C_{25}H_{28}O_3$	27
$C_{25}H_{28}O_4$	31, 45, 48, 62, 63, 191
$C_{25}H_{28}O_5$	32, 46, 47, 50, 69, 84, 100
$C_{25}H_{28}O_6$	53, 101, 106
$C_{25}H_{28}O_{12}$	310, 360
$C_{25}H_{30}O_4$	275
$C_{25}H_{30}O_5$	289, 294, 295
$C_{25}H_{30}O_6$	296
$C_{25}H_{30}O_{11}$	132
$C_{26}H_{22}O_7$	279
$C_{26}H_{28}O_7$	78
$C_{26}H_{30}O_4$	55, 187, 188, 192, 193, 194, 195, 196, 243, 282, 283, 284
$C_{26}H_{30}O_5$	40, 56, 71
$C_{26}H_{30}O_9$	113
$C_{26}H_{32}O_4$	277, 281
$C_{26}H_{32}O_5$	285
$C_{26}H_{32}O_6$	299, 300
$C_{27}H_{26}O_{14}$	359
$C_{27}H_{29}O_{13}N$	245
$C_{27}H_{30}O_{13}$	355
$C_{27}H_{30}O_{14}$	357
$C_{27}H_{30}O_{15}$	356
$C_{27}H_{31}O_{14}N$	248
$C_{27}H_{32}O_4$	185, 186
$C_{27}H_{32}O_{15}$	119, 130, 363
$C_{27}H_{32}O_{16}$	246
$C_{27}H_{34}O_{15}$	308
$C_{28}H_{22}O_7$	239
$C_{28}H_{34}O_4$	183, 184
$C_{28}H_{34}O_6$	189, 190
$C_{28}H_{34}O_{15}$	122
$C_{29}H_{26}O_7$	42, 43
$C_{29}H_{26}O_8$	81
$C_{29}H_{30}O_7$	278
$C_{29}H_{30}O_8$	280
$C_{29}H_{36}O_{15}$	133
$C_{29}H_{38}O_{15}$	305
$C_{30}H_{18}O_{10}$	365
$C_{30}H_{18}O_{12}$	364
$C_{30}H_{20}O_9$	135, 165
$C_{30}H_{20}O_{10}$	152

APPENDIX A Index of Molecular Formulae — *continued*

Formula	Compound Number
$C_{30}H_{20}O_{12}$	367
$C_{30}H_{22}O_8$	139, 143, 148
$C_{30}H_{22}O_9$	134, 150, 151, 163
$C_{30}H_{22}O_{10}$	136, 154, 155, 156
$C_{30}H_{22}O_{11}$	157, 158, 368–377
$C_{30}H_{24}O_8$	142, 149
$C_{30}H_{24}O_9$	153
$C_{30}H_{24}O_{10}$	137
$C_{30}H_{26}O_6$	199, 200
$C_{30}H_{26}O_7$	197, 198
$C_{30}H_{26}O_8$	315
$C_{30}H_{28}O_8$	44
$C_{30}H_{28}O_9$	82, 83
$C_{30}H_{28}O_{12}$	114, 115
$C_{30}H_{34}O_5$	87
$C_{30}H_{36}O_4$	242
$C_{30}H_{36}O_6$	298
$C_{30}H_{38}O_5$	290
$C_{30}H_{38}O_6$	297
$C_{31}H_{24}O_8$	140, 141, 171
$C_{31}H_{26}O_8$	147
$C_{31}H_{28}O_7$	201, 220
$C_{31}H_{28}O_8$	202, 203
$C_{31}H_{28}O_9$	316
$C_{31}H_{30}O_7$	320
$C_{31}H_{30}O_{13}$	128
$C_{32}H_{26}O_8$	138
$C_{32}H_{26}O_{10}$	146
$C_{32}H_{30}O_7$	238
$C_{32}H_{30}O_{10}$	317
$C_{32}H_{30}O_{12}$	116
$C_{32}H_{30}O_{13}$	117
$C_{32}H_{30}O_{15}$	126
$C_{32}H_{32}O_7$	321
$C_{32}H_{40}O_{18}$	110
$C_{33}H_{24}O_{11}$	166–169
$C_{33}H_{32}O_8$	318
$C_{33}H_{32}O_{15}$	129
$C_{33}H_{42}O_{20}$	120
$C_{34}H_{26}O_{11}$	170
$C_{34}H_{28}O_8$	159–162
$C_{34}H_{30}O_9$	323
$C_{34}H_{30}O_{10}$	322, 326
$C_{34}H_{30}O_{11}$	324

APPENDIX A Index of Molecular Formulae — *continued*

Formula	Compound Number
$C_{34}H_{34}O_8$	336, 337
$C_{34}H_{36}O_7$	334, 335
$C_{35}H_{30}O_{17}$	304
$C_{35}H_{32}O_{11}$	325
$C_{35}H_{34}O_7$	228, 229
$C_{35}H_{34}O_8$	222–226, 232
$C_{35}H_{34}O_9$	221, 227
$C_{35}H_{36}O_{15}$	111
$C_{35}H_{38}O_7$	338, 339
$C_{35}H_{44}O_5$	85
$C_{35}H_{46}O_4$	276
$C_{36}H_{32}O_{14}$	164
$C_{36}H_{38}O_{16}$	112
$C_{36}H_{42}O_6$	237
$C_{37}H_{34}O_7$	328, 329
$C_{37}H_{40}O_{15}$	123
$C_{39}H_{34}O_{16}$	127
$C_{39}H_{36}O_9$	204
$C_{40}H_{34}O_{12}$	214
$C_{40}H_{36}O_{11}$	218, 219
$C_{40}H_{36}O_{12}$	210, 211, 213, 216, 217
$C_{40}H_{38}O_8$	206, 207
$C_{40}H_{38}O_9$	205
$C_{40}H_{40}O_{12}$	215
$C_{42}H_{34}O_{21}$	303
$C_{42}H_{38}O_9$	230, 231
$C_{42}H_{40}O_8$	144
$C_{44}H_{40}O_8$	330, 331
$C_{44}H_{44}O_{24}$	247
$C_{45}H_{38}O_{13}$	172, 173
$C_{45}H_{42}O_{10}$	366
$C_{45}H_{44}O_{12}$	208, 212
$C_{45}H_{46}O_{11}$	209
$C_{48}H_{52}O_{26}$	244
$C_{50}H_{58}O_{10}$	319
$C_{51}H_{46}O_9$	332, 333
$C_{51}H_{48}O_{12}$	145
$C_{54}H_{54}O_{11}$	233–235
$C_{60}H_{48}O_{15}$	175, 176, 177
$C_{60}H_{50}O_{15}$	178, 179
$C_{66}H_{46}O_{21}$	174
$C_{75}H_{62}O_{21}$	180
$C_{90}H_{70}O_{22}$	181

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones^a**CHALCONES****Chalcone aglycones**Mono-*O*-substituted*(4')*

1. 4'-OMe (H2/454)

Di-*O*-substituted*(2',4')*

2. 2',4'-diOH (H2/457)
3. 2',4'-diOH, 3'-prenyl (isocordoin, H2/582)
4. 2',4'-diOH, 3'-(1,1-dimethylallyl) (ψ -isocordoin, H2/583)
5. 2',4'-diOH, 3',5'-diprenyl (spinochalcone A, **27**)
6. 2'-OH, 4'-OMe, 3'-prenyl (derricin, H2/589)
7. 2'-OH, 4'-prenyloxy (derricidin, cordoin, H2/590)
8. 2'-OH, furano[2'',3'':4',3'] (H2/556)
9. 2'-OH, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'':4',3'] (flemistrictin B, H2/586)
10. 2'-OH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (lonchocarpin, H2/587)
11. 2'-OH, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':4',3'] (flemistrictin C, H2/588)
12. 2'-OH, 6''-(4-methylpent-3-enyl),6''-methylpyrano[2'',3'':4',3'] (spinochalcone B, **28**)
13. 2'-OH, 3'-prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5'] (spinochalcone C, **29**)
14. 2'-OH, complex substituent ((+)-tephrosone, **30**)
15. 4'-OH, 2'-OMe (H2/458)
16. 4'-OH, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'':2',3'] (flemistrictin E, H2/584)
17. 4'-OH, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':2',3'] (flemistrictin F, H2/585)
18. 2'-OMe, furano[2'',3'':4',3'] (ovalitenin A, H2/557)
19. 2'-OMe, 4-Me, furano[2'',3'':4',3'] (purpuritenin A, H2/559)
20. 2'-OMe, α -Me, furano[2'',3'':4',3'] (purpuritenin B, H2/560)

(2',2')

21. 2',2-diOH (H2/455)

(2', β)

22. 2', β -diOH (H2/530)

(3',3')

23. 3',3-diOH (**1**)

(4',4')

24. 4',4-diOH (H2/456)

Tri-*O*-substituted*(2',3',4')*

^a This checklist of chalcones, dihydrochalcones, and aurones contains compounds of these classes reported in the literature as natural products to the end of 2003. Compounds published before 1992 are cross-referenced to numbered entries in volumes 1 and 2 of the *Handbook of Natural Flavonoids*^{9,10} using the abbreviations H1 and H2, respectively. Compounds published from 1992 to 2003 are cross-referenced to Table 16.1–Table 16.15 using numbers in bold type. The compounds are listed according to the system outlined in Section 16.1.1, with the exception that isoprenylated derivatives are included under the heading of aglycones. Bn, benzyl.

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

25. 2',4'-diOH, 3'-OMe (larrein, H2/468)
(2',3',2)
26. 2',3',2-triOH, 4'-geranyl (fleimiwallichin E, H2/661)
(2',4',5')
27. 2',4'-diOH, 5'-OMe (flemichapparin, H2/469)
28. 2',5'-diOH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (flemichapparin A, H2/616)
29. 2',5'-diOH, (6''-(4-hydroxy-4-methylpent-2-enyl),6''-methyl)pyrano[2'',3'':4',3'] (fleimiwallichin F, H2/666)
(2',4',6')
30. 2',4',6'-triOH (H2/470)
31. 2',4',6'-triOH, 3'-prenyl (desmethylisoxanthohumol, H2/617)
32. 2',4',6'-triOH, 3'-geranyl (H2/667)
33. 2',4',6'-triOH, 3'-neryl (**31**)
34. 2',4',6'-triOH, 3'-C₁₀ (linderachalcone, H2/668)
35. 2',4',6'-triOH, 3',5'-diC₁₀ (neolinderachalcone, H2/674)
36. 2',4'-diOH, 6'-OMe (cardamonin, H2/472)
37. 2',4'-diOH, 6'-OMe, 3'-Me (stercurensin, H2/579)
38. 2',4'-diOH, 6'-OMe, 3',5'-diMe (H2/581)
39. 2',4'-diOH, 6'-OMe, 3'-prenyl (H2/622)
40. 2',4'-diOH, 3'-Me, 6'',6''-dimethylpyrano[2'',3'':6',5'] (H2/659)
41. 2',4'-diOH, 3'-prenyl, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'':6',5'] (cedrediprenone, **32**)
42. 2',4'-diOH, 6'',6''-dimethylpyrano[2'',3'':6',5'], 3'-(3-acetyl-5-methyl-2,4,6-trihydroxybenzyl) (rottlerin, H2/699)
43. 2',6'-diOH, 4'-OMe (H2/471)
44. 2',6'-diOH, 4'-OMe, 3'-CHO, 5'-Me (leridalchalcone, **2**)
45. 2',6'-diOH, 4'-OMe, 3'-C₁₀ (H2/669)
46. 2',6'-diOH, 4'-prenyloxy (H2/625)
47. 2',6'-diOH, 4'-OMe, 3'-Me (triangularin, H2/578)
48. 2',6'-diOH, 4'-OMe, 3'-prenyl (isoxanthohumol, H2/620)
49. 2',6'-diOH, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':4',3'] (helikrausichalcone, H2/618)
50. 4',6'-diOH, 2'-OMe, 3'-Me (aurentiacin A, H2/577)
51. 4',6'-diOH, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':2',3'] (**33**)
52. 2'-OH, 4',6'-diOMe (flavokawin B, H2/473)
53. 2'-OH, 4',6'-diOMe, 3'-Me (aurentiacin, H2/580)
54. 2'-OH, 4',6'-diOMe, 3'-prenyl (ovalichalcone, H2/624)
55. 2'-OH, 6'-OMe, 4'-prenyloxy (H2/626)
56. 2'-OH, 6'-OMe, 5''-isopropenyl-4'',5''-dihydrofurano[2'',3'':4',3'] (crassichalcone, **34**)
57. 2'-OH, 6'-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (pongachalcone I, oaxacacin (revised structure)⁶⁹ H2/623)
58. 2'-OH, 6'-OMe, 4'',5''-epoxy(6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':4',3']) (epoxyobovatachalcone, **35**)
59. 2'-OH, 6'-OMe, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':4',3'] (6'-methoxyhelikrausichalcone, **36**)
60. 2'-OH, 6'-OMe, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (boesenbergin B, H2/671)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

61. 2'-OH, 6'-OMe, complex substituent ((+)-tephropurpurin, **37**)
62. 2'-OH, bis(6'',6''-dimethylpyrano)[2'',3'':4',3'],[2'',3'':6',5'] (flemiculosin, H2/627)
63. 2'-OH, complex (4',6'-di-*O*-,5'-*C*-) substituent, 3'-prenyl (3'-prenylrubranine, H2/697)
64. 4'-OH, 2'-OMe, 6'',6''-dimethylpyrano[2'',3'':6',5'] (cedreprenone, **38**)
65. 4'-OH, 6'-OMe, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':2',3'] (**39**)
66. 6'-OH, 4'-OMe, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':2',3'] (helichromanochalcone, H2/621)
67. 6'-OH, 4'-OMe, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':2',3'] (boesenbergin A, H2/670)
68. 6'-OH, 4'-OMe, complex substituent (linderol A, **40**)
69. 6'-OH, complex (2',4'-di-*O*-,3'-*C*-) substituent (rubranine, H2/672) (2',4',4)
70. 2',4',4-triOH (isoliquiritigenin, H2/462)
71. 2',4',4-triOH, 3'-prenyl (isobavachalcone, H2/597)
72. 2',4',4-triOH, 3'-(2-hydroxy-3-methylbut-3-enyl) (**41**)
73. 2',4',4-triOH, 3'-geranyl (xanthoangelol, H2/663)
74. 2',4',4-triOH, 3'-(3,7-dimethyl-6-hydroxy-2,7-octadienyl) (xanthoangelol B, H2/664-structure drawing incorrect)
75. 2',4',4-triOH, 3'-(3-methyl-6-oxo-2-hexenyl) (xanthoangelol C, H2/698)
76. 2',4',4-triOH, 3'-(4-coumaroyloxy-3-methyl-but-2(*E*)-enyl) (isogemichalcone B, **42**)
77. 2',4',4-triOH, 3'-(4-coumaroyloxy-3-methyl-but-2(*Z*)-enyl) (gemichalcone B, **43**)
78. 2',4',4-triOH, 3'-(4-feruloyloxy-3-methyl-but-2(*Z*)-enyl) (gemichalcone A, **44**)
79. 2',4',4-triOH, 3',5'-diprenyl (H2/612)
80. 2',4',4-triOH, 3',3-diprenyl (kanzonol C, **45**)
81. 2',4',4-triOH, 3'-prenyl, 3-(2-hydroxy-3-methylbut-3-enyl) (paratocarpin D, **46**)
82. 2',4',4-triOH, 3'-(2-hydroxy-3-methylbut-3-enyl), 3-prenyl (paratocarpin E, **47**)
83. 2',4',4-triOH, 3',3,5-triprenyl (sophoradin, H2/613)
84. 2',4',4-triOH, 5'-prenyl (brousochalcone B, H2/606)
85. 2',4',4-triOH, 5',3-diprenyl (stipulin, **48**)
86. 2',4',4-triOH, 3-prenyl (licoagrochalcone A, **49**)
87. 2',4',4-triOH, 3,5-diprenyl (abyssinone VI, H2/610)
88. 2',4',4-triOH, 3-(2-hydroxy-3-methylbut-3-enyl), 5-prenyl (anthyllin, **50**)
89. 2',4'-diOH, 4-OMe (H2/463)
90. 2',4'-diOH, 6'',6''-dimethylpyrano[2'',3'':4,3] (kanzonol B, **51**)
91. 2',4'-diOH, 3'-prenyl, 6'',6''-dimethylpyrano[2'',3'':4,3] (paratocarpin C, **52**)
92. 2',4'-diOH, 3'-prenyl, 5''-(2-hydroxyisopropyl)-4''-hydroxy-4'',5''-dihydrofurano[2'',3'':4,3] (paratocarpin G, **53**)
93. 2',4'-diOH, 3',5-diprenyl, 6'',6''-dimethylpyrano[2'',3'':4,3](sophoradochromene, H2/614)
94. 2',4'-diOH, 5-prenyl, 6'',6''-dimethylpyrano[2'',3'':4,3] (anthyllisone, **54**)
95. 2',4-diOH, 4'-OMe (H2/465)
96. 2',4-diOH, 4'-OMe, 5'-CHO (neobavachalcone, H2/576)
97. 2',4-diOH, 4'-OMe, 3'-prenyl (H2/602)
98. 2',4-diOH, 4'-OMe, 3'-(2-hydroxy-3-methylbut-3-enyl) (xanthoangelol D, H2/603)
99. 2',4-diOH, 4'-OMe, 3'-(2-hydroperoxy-3-methylbut-3-enyl) (xanthoangelol E, H2/604)
100. 2',4-diOH, 4'-OMe, 3'-geranyl (xanthoangelol F, **55**)
101. 2',4-diOH, 4'-OMe, 3'-(3,7-dimethyl-6-hydroxy-2,7-octadienyl) (xanthoangelol G, **56**)
102. 2',4-diOH, 4'-OMe, 5'-prenyl (bavachalcone, H2/608)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

103. 2',4-diOH, 4'-prenyloxy (H2/605)
104. 2',4-diOH, 4'-geranyloxy (H2/662)
105. 2',4-diOH, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'',4',3'] (bakuchalcone, H2/598)
106. 2',4-diOH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (isobavachromene, H2/599)
107. 2',4-diOH, 6'',6''-dimethyl-4'',5''-dihidropyrano[2'',3'':4',3'] (dorsmanin A, **57**)
108. 2',4-diOH, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihidropyrano[2'',3'':4',3'] (**58**)
109. 2',4-diOH, 3-prenyl, 6'',6''-dimethylpyrano[2'',3'':4',3'] (paratocarpin B, **59**)
110. 2',4-diOH, 6'',6''-dimethylpyrano[2'',3'':4',5'] (bavachromene, H2/607)
111. 2',4-diOH, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (lespeol, H2/665)
112. 4',4-diOH, 2'-OMe (H2/464)
113. 4',4-diOH, 2'-OMe, 5'-CHO (isoneobavachalcone, H2/575)
114. 4',4-diOH, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihidropyrano[2'',3'':2',3'] (bavachromanol, H2/600)
115. 2'-OH, 4',4-diOMe (H2/466)
116. 2'-OH, 4',4-diOMe, 5'-prenyl (H2/609)
117. 2'-OH, 4-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (H2/601)
118. 2'-OH, bis(6'',6''-dimethylpyrano)[2'',3'':4',3'],[2'',3'':4,3] (paratocarpin A, **60**)
119. 2'-OH, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'':4',3'], 6'',6''-dimethylpyrano[2'',3'':4,3] (paratocarpin F, **61**)
120. 2'-OH, bis(6'',6''-dimethyl-4'',5''-dihidropyrano)[2'',3'':4',3'],[2'',3'':4,3] (artoindonesianin J, **62**)
121. 2'-OH, bis(6'',6''-dimethyl-4'',5''-dihidropyrano)[2'',3'':4',5'],[2'',3'':4,3] (**63**)
122. 4-OH, 4'-OMe, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihidropyrano[2'',3'':2',3'] (xanthoangelol H, **64**)
(2',4', β)
123. β -OH, 2',4'-diOMe, 5'-prenyl (pongagallone A, H2/540)
124. β -OH, 2'-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (H2/539)
125. β -OH, 2'-OMe, furano[2'',3'':4',3'] (pongamol, H2/555)
126. 2', β -diOMe, furano[2'',3'':4',3'] (H2/558)
(2',5',4)
127. 2',5',4-triOH (**3**)
128. 2',5'-diOH, 4-OMe (H2/467)
(2',3,4)
129. 2',3,4-triOH (H2/461)
(3',4',4)
130. 4',4-diOH, 6'',6''-dimethyl-4'',5''-dihidropyrano[2'',3'':3',2'] (crotmadine, H2/615)
(4',2,4)
131. 4',4-diOH, 2-OMe (echinatin, H2/459)
132. 4',4-diOH, 2-OMe, 3-prenyl (licoachalcone C, **65**)
133. 4',4-diOH, 2-OMe, 5-(1,1-dimethylallyl) (licoachalcone A, H2/594)
134. 2,4-diOH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (munsericin, **66**)
135. 4'-OH, 2-OMe, 6'',6''-dimethylpyrano[2'',3'':4,3] (licoagrochalcone B, **67**)
136. 4'-OH, 2-OMe, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'':4,3] (licoagrochalcone D, **68**)
137. 4-OH, 4',2-diOMe (glypallichalcone, **4**)
(2,4,6)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

138. 4,6-diOH, 2-OMe, 3-CHO, 5-Me (H2/574)
Tetra-*O*-substituted
(2',3',4',6')
139. 2',3',6'-triOH, 6'',6''-dimethylpyrano[2'',3'':4',5'] (mallotus A, H2/647)
140. 2',3'-diOH, 4',6'-diOMe (H2/498)
141. 2',4'-diOH, 3',6'-diOMe (H2/497)
142. 2',4'-diOH, 6'-OMe, 3'-angeloyloxy (H2/644)
143. 2',4'-diOH, 6'-OMe, 3'-(2-methylbutyryloxy) (H2/645)
144. 2',4'-diOH, 6'-OMe, 3'-isovaleryloxy (H2/646)
145. 2',6'-diOH, 3',4'-diOMe (pashanone, H2/496)
146. 2'-OH, 3',4',6'-triOMe (H2/500)
147. 6'-OH, 2',3',4'-triOMe (helilandin B, H2/499)
148. 2',6'-diOMe, 3',4'-OCH₂O- (helilandin A, H2/563)
149. 2',3',4',6'-tetraOMe (H2/501)
(2',3',4',4')
150. 2',3',4',4'-tetraOH (H2/485)
151. 2',4',4'-triOH, 3'-OMe (kukulkanin B, H2/486)
152. 2',4'-diOH, 3',4'-diOMe (kukulkanin A, H2/487)
153. 2',4'-diOH, 3',4'-diOMe (heliannone A, **5**)
(2',4',5',2')
154. 2',4',2'-triOH, 5'-OMe, 3'-geranyl (H2/681)
155. 2',5',2'-triOH, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (flemingin A, H2/680)
(2',4',5',4')
156. 2',5',4'-triOH, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (flemingin D, H2/682)
157. 2',5',4'-triOH, (6''-(4-hydroxy-4-methylpent-2-enyl),6''-methyl)pyrano[2'',3'':4',3'] (flemingin E, H2/683)
158. 2',5',4'-triOH, (6''-(3-hydroxy-4-methylpent-4-enyl),6''-methyl)pyrano[2'',3'':4',3'] (flemingin F, H2/684)
(2',4',6',2')
159. 2',4',2'-triOH, 6'-OMe (**6**)
160. 2'-OH, 4',6',2'-triOMe (**7**)
(2',4',6',4')
161. 2',4',6',4'-tetraOH (chalconaringenin, H2/488)
162. 2',4',6',4'-tetraOH, 3'-geranyl (**69**)
163. 2',4',6'-triOH, 4-OMe (H2/489)
164. 2',4',4'-triOH, 6'-OMe (helichrysetin, H2/491)
165. 2',4',4'-triOH, 6'-OMe, 3'-prenyl (xanthohumol, H2/638)
166. 2',4',4'-triOH, 6'-OMe, 3'-(2-hydroxy-3-methylbut-3-enyl) (xanthohumol D, **70**)
167. 2',4',4'-triOH, 6'-OMe, 3',5'-diprenyl (5'-prenylxanthohumol, **71**)
168. 2',4',4'-triOH, 3'-prenyl, 6'',6''-dimethylpyrano[2'',3'':6',5'] (xanthohumol E, **72**)
169. 2',4',4'-triOH, 3'-(3-acetyl-5-methyl-2,4,6-trihydroxybenzyl), 6'',6''-dimethylpyrano[2'',3'':6',5'] (4-hydroxyrottlerin, H2/701)
170. 2',6',4'-triOH, 4'-OMe (neosakuranetin, H2/490)
171. 2',6',4'-triOH, 4'-OMe, 3'-prenyl (xanthogalenol, **73**)
172. 2',6',4'-triOH, 4'-prenyloxy (H2/640)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

173. 2',6',4-triOH, 3'-prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5'] (sericone, H2/641)
174. 2',4'-diOH, 6',4-diOMe (**8**)
175. 2',6'-diOH, 4',4-diOMe (gymnogrammene, H2/492)
176. 2',4-diOH, 4',6'-diOMe (flavokawin C, H2/494)
177. 2',4-diOH, 4',6'-diOMe, 3'-prenyl (H2/639)
178. 2',4-diOH, 6'-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (xanthohumol C, **74**)
179. 2',4-diOH, 6'-OMe, 6'',6''-dimethyl-4''-hydroxy-4'',5''-dihydropyrano[2'',3'':4',3'] (isodehydrocycloxanthohumol hydrate, **75**)
180. 2',4-diOH, 6'-OMe, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano[2'',3'':4',3'] (xanthohumol B, **76**)
181. 2',4-diOH, bis(6'',6''-dimethylpyrano[2'',3'':4',3'],[2'',3'':6',5']) (laxichalcone, **77**)
182. 2',4-diOH, 6'',6''-dimethylpyrano[2'',3'':4',3'], 6'',6''-dimethyl-4''-hydroxy-5''-methoxy-4'',5''-dihydropyrano[2'',3'':6',5'] (derrichalcone, **78**)
183. 4',4-diOH, 2',6'-diOMe (H2/493)
184. 6',4-diOH, 4'-OMe, 6'',6''-dimethylpyrano[2'',3'':2',3'] (citrunobin, H2/637)
185. 2'-OH, 4',6',4-triOMe (flavokawin A, H2/495)
186. 2'-OH, 4',6',4-triOMe, 5'-Br (**9**)
187. 2'-OH, 4',4-diOMe, 6'',6''-dimethylpyrano[2'',3'':6',5'] (**79**)
188. 2'-OH, 6',4-diOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (glychalcone A, **80**)
189. 4'-OH, 2',6',4-triOMe (**10**)
(2',4',6', β)
190. 2',4',6', β -tetraOH, 3'-CHO, 5'-Me (H2/537)
191. 2',4', β -triOH, 6'-OMe (H2/534)
192. 2',6', β -triOH, 4'-OMe (H2/533)
193. 2',6', β -triOH, 4'-OMe, 3'-CHO, 5'-Me (H2/536)
194. 2',6', β -triOH, 4'-OMe, 3'-prenyl (**98**)
195. 2', β -diOH, 4',6'-diOMe, 3'-Me (**23**)
196. 2', β -diOH, 6'-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (demethylpraecansone B, H2/538)
197. β -OH, 2',6'-diOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (praecansone B, H2/549)
198. 2',6', β -triOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (**99**)
(2',4',2,4)
199. 2',4',2,4-tetraOH (H2/477)
200. 2',4',2,4-tetraOH, 3'-prenyl (morachalcone A, H2/630)
201. 2',4',2,4-tetraOH, 3'-lavandulyl (ammothamnidin, H2/677)
202. 2',4',2,4-tetraOH, 3'-(4-coumaroyloxy-3-methyl-but-2(*E*)-enyl)
(demethoxyisogemichalcone C, **81**)
203. 2',4',2,4-tetraOH, 3'-(4-feruloyloxy-3-methyl-but-2(*E*)-enyl) (isogemichalcone C, **82**)
204. 2',4',2,4-tetraOH, 3'-(4-feruloyloxy-3-methyl-but-2(*Z*)-enyl) (gemichalcone C, **83**)
205. 2',4',2,4-tetraOH, 5-geranyl (kuwanol D, H2/676)
206. 2',2,4-triOH, 3'-prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5'] (H2/631)
(2',4',2,5)
207. 2',2,5-triOH (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (fleimiwallichin D, H2/679)
(2',4',3,4)
208. 2',4',3,4-tetraOH (butein, H2/478)
209. 2',4',3,4-tetraOH, 3'-geranyl (**84**)
210. 2',4',3,4-tetraOH, 3',5-digeranyl (**85**)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

211. 2',4',3,4-tetraOH, 5'-prenyl (brousochalcone A, H2/634)
212. 2',4',3-triOH, 4-OMe (H2/480)
213. 2',4',3-triOH, 3'-prenyl 6'',6''-dimethylpyrano[2'',3'':4,5] (**86**)
214. 2',4',4-triOH, 3-OMe (homobutein, H2/479)
215. 2',4',4-triOH, 3'-prenyl, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':3,2] (poinsettifolin B, **87**)
216. 2',3,4-triOH, 4'-OMe (calythropsin, **11**)
217. 2',3,4-triOH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (H2/632)
218. 4',3,4-triOH, 2'-OMe (sappanchalcone, H2/481)
219. 2',4'-diOH, 3,4-diOMe (H2/483)
220. 2',3-diOH, bis(6'',6''-dimethylpyrano)[2'',3'':4',3'],[2'',3'':4,5] (glyinflanin G, **88**)
221. 2',4-diOH, 3'-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (pongachalcone II, H2/633)
222. 2'-OH, 3,4-diOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (3,4-dimethoxylonchocarpin, **89**)
223. 2'-OH, 3,4-OCH₂O-, 6'',6''-dimethylpyrano[2'',3'':4',3'] (glabrachromene II, H2/571)
224. 2'-OMe, 3,4-OCH₂O-, furano[2'',3'':4',3'] (ovalitenin C, H2/561)
225. 2',4'-diOMe, 3,4-OCH₂O- (**12**)
(2',4',3,5)
226. 2',4',3,5-tetraOH (pseudosindorin, H2/484)
(2',4',4,β)
227. 2',4',4,β-tetraOH (licodione, H2/531)
228. 2',4',4,β-tetraOH, 5'-prenyl (H2/541)
229. 2',4',4,β-tetraOH, 5',3-diprenyl (glycyrdione A, **100**)
230. 2',4',4,β-tetraOH, 5'-prenyl, 3-(2-hydroxy-3-methylbut-3-enyl) (glyinflanin E, **101**)
231. 2',4',4,β-tetraOH, 3-prenyl (kanzonol A, **102**)
232. 2',4',β-triOH, 5'-prenyl, 6'',6''-dimethylpyrano[2'',3'':4,3] (glycyrdione B, **103**)
233. 2',4',β-triOH, 6'',6''-dimethylpyrano[2'',3'':4',5'] (glyinflanin B, **104**)
234. 2',4',β-triOH, 3-prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5'] (glyinflanin C, **105**)
235. 2',4',β-triOH, 3-prenyl, 5''-(2-hydroxyisopropyl)-4'',5''-dihydrofurano[2'',3'':4',5'] (glyinflanin F, **106**)
236. 4',4,β-triOH, 2'-OMe (H2/532)
237. 2',β-diOH, bis(6'',6''-dimethylpyrano)[2'',3'':4',5'],[2'',3'':4,3] (glyinflanin D, **107**)
(2',3,4,5)
238. 2'-OH, 3,4,5-triOMe (crotaoprostrin, **13**)
(3',4',2,4)
239. 3',4',4-triOH, 2-OMe, 3-prenyl (licoagrochalcone C, **90**)
(4',2,3,4)
240. 4',3,4-triOH, 2-OMe (licoachalcone B, H2/475)
241. 4',3,4-triOH, 2-OMe, 3'-prenyl (licoachalcone D, **91**)
(4',2,4,6)
242. 2,4-diOH, 4',6-diOMe (H2/476)
(2,3,4,6)
243. 2-OH, 3,4,6-triOMe (tepanone, **14**)
(2,4,6,β)
244. 2,6,β-triOMe, 6'',6''-dimethylpyrano[2'',3'':4,3] (praecansone A, H2/629)
- Penta-*O*-substituted
(2',3',4',5',6')
245. 2',4'-diOH, 3',5',6'-triOMe (isodidymocarpin, H2/520)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

246. 2',5'-diOH, 3',4',6'-triOMe (pedicin, H2/519)
247. 4',6'-diOH, 2',5'-diOMe, 3'-angeloyloxy (H2/656)
248. 4',6'-diOH, 2',5'-diOMe, 3'-(2-methylbutyryloxy) (melafolone, H2/657)
249. 4',6'-diOH, 2',5'-diOMe, 3'-isovaleryloxy (valafolone, H2/658)
250. 2'-OH, 3',4',5',6'-tetraOMe (kanakugiol, H2/522)
251. 3'-OH, 2',4',5',6'-tetraOMe (H2/521)
252. 2',3',4',5',6'-pentaOMe (pedicellin, H2/523)
(2',3',4',6',2)
253. 2',4'-diOH, 3',6',2-triOMe (H2/504)
(2',3',4',6',4)
254. 2',4'-diOH, 3',4',6'-triOMe (H2/517)
255. 2',4'-diOH, 6'-OMe, 3',4'-OCH₂O- (H2/564)
256. 6',4'-diOH, 2',3',4'-triOMe (H2/516)
257. 6'-OH, 2',3',4',4'-tetraOMe (H2/518)
(2',3',4',3,4)
258. 2',3',4',3,4-pentaOH (okanin, H2/506)
259. 2',4',3,4-tetraOH, 3'-OMe (lanceoletin, H2/507)
260. 2',4',4-triOH, 3',3-diOMe (**15**)
261. 2'-OH, 3',4',3,4-tetraOMe (H2/508)
(2',4',5',2,5)
262. 2',4',2,5-tetraOH, 5'-OMe, 3'-geranyl (homoflemingin, H2/694)
263. 2',5',2,5-tetraOH (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (flemingin C, H2/691)
(2',4',5',2,6)
264. 2',5',2,6-tetraOH (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (flemingin B, H2/692)
(2',4',5',3,4)
265. 2',4',5',3,4-pentaOH (neoplathymenin, H2/509)
266. 2',4'-diOH, 5'-OMe, 3,4-OCH₂O- (prosogerin B, H2/565)
267. 2'-OH, 5',3,4-triOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (ponganone VI, **92**)
268. 5'-OH, 2'-OMe, 3,4-OCH₂O-, furano[2'',3'':4',3'] (H2/562)
(2',4',6',2,3)
269. 2'-OH, 4',6',2,3-tetraOMe (**16**)
(2',4',6',2,4)
270. 2',4',2,4-tetraOH, 6'-OMe, 3'-lavandulyl (kuraridin, H2/687)
271. 2',4',2,4-tetraOH, 6'-OMe, 3'-(5-hydroxy-2-isopropenyl-5-methylhexyl) (kuraridinol, H2/688)
272. 2',4',4-triOH, 6',2-diOMe 3'-lavandulyl (kushenol D, H2/678)
273. 2',4'-diOH, 6',2,4-triOMe (cerasin, H2/502)
274. 2'-OH, 4',6',2,4-tetraOMe (cerasidin, H2/503)
(2',4',6',2,5)
275. 2',4',2,5-tetraOH, 6'-OMe, 3'-geranyl (fleмиwallichin C, H2/690)
276. 2',6',2,5-tetraOH (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (fleмиwallichin B, H2/689)
(2',4',6',2,6)
277. 2',6',2,6-tetraOH (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':4',3'] (fleмиwallichin A, H2/693)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

278. 2',2,6-triOH, 6'-OMe, 3'-prenyl, 6'',6''-dimethylpyrano[2'',3'':4',5'] (orotinchalcone, H2/649)
(2',4',6',3,4)
279. 2',4',6',3,4-pentaOH (H2/510)
280. 2',4',6',3-tetraOH, 4-OMe, 3',2-diprenyl (antiarone C, H2/655)
281. 2',4',6',3-tetraOH, 4-OMe, 2,5-diprenyl (antiarone E, H2/652)
282. 2',4',6',3-tetraOH, 4-OMe, (2,β)-C₅ (antiarone J, H2/653)
283. 2',4',6',4-tetraOH, 3-OMe (H2/511)
284. 2',4',6',4-tetraOH, 3-OMe, 3',2-diprenyl (antiarone D, H2/654)
285. 2',4',3,4-tetraOH, 6'',6''-dimethylpyrano[2'',3'':6',5'], 3'-(3-acetyl-5-methyl-2,4,6-trihydroxybenzyl) (3,4-dihydroxyrottlerin, H2/700)
286. 2',6',3,4-tetraOH, 3',4'-dihydrooxepino (H2/651)
287. 2',4',6'-triOH, 3,4-diOMe, (2,β)-C₅ (antiarone K, H2/650)
288. 2',6',4-triOH, 4',3-diOMe (H2/512)
289. 2',3-diOH, 4',6',4-triOMe (H2/513)
290. 2'-OH, 4',6',3,4-tetraOMe (H2/514)
291. 2'-OH, 4',3,4-triOMe, 6'',6''-dimethylpyrano[2'',3'':6',5'] (**93**)
292. 2'-OH, 6',3,4-triOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (glychalcone B, **94**)
293. 2'-OH, 4',6'-diOMe, 3,4-OCH₂O- (tephrone, H2/566)
294. 2'-OH, 4',6'-diOMe, 3,4-OCH₂O-, 3'-prenyl (ovalichalcone A, H2/572)
295. 2'-OH, 6'-OMe, 3,4-OCH₂O-, 6'',6''-dimethylpyrano[2'',3'':4',3'] (glabrachromene I, H2/573)
296. 2',4',6'-triOMe, 3,4-OCH₂O- (**17**)
(2',4',2,4,5)
297. 2',4',2,4,5-pentaOH, 3'-prenyl (ramosismin, **95**)
298. 2'-OH, 2,4,5-triOMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (glabrachalcone, H2/648)
(2',4',3,4,5)
299. 2',4',3,4,5-pentaOH (robtein, H2/505)
(2',4',3,4,α)
300. 2',4',3,4,α-pentaOH (H2/515)
301. 2',4',3,4-tetraOH, *cyclo*[α-OCH₂-2] (mopachalcone, H2/720)
(2',4',3,4,β)
302. β-OH, 2',4'-diOMe, 3,4-OCH₂O- (milletenone, H2/552)
303. β-OH, 2',4'-diOMe, 3,4-OCH₂O-, 5'-prenyl (pongagallone B, H2/550)
304. β-OH, 2',4'-diOMe, 3,4-OCH₂O-, α-Me (tinospirone, H2/553)
305. β-OH, 2'-OMe, 3,4-OCH₂O-, furano[2'',3'':4',3'] (ovalitenone, H2/554)
(2',4',4,5,α)
306. 2',4',4,5-tetraOH, *cyclo*[α-OCH₂-2] (peltochalcone, H2/719)
(3',4',2,3,4)
307. 3',4',3,4-tetraOH, 2-OMe (**18**)
(3',4',3,4,β)
308. β-OH, 3',4':3,4-bis(-OCH₂O-) (galiposin, **24**)
- Hexa-*O*-substituted
(2',3',4',5',6',4)
309. 2',5'-diOH, 3',4',6',4-tetraOMe (H2/527)
310. 3',4-diOH, 2',4',5',6'-tetraOMe (**19**)
(2',3',4',6',3,4)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

311. 6',3,4-triOH, 2',3',4'-triOMe (hamilcone, **20**)
312. 3',6'-diOH, 2',4'-diOMe, 3,4-OCH₂O- (agestricin, H2/567)
313. 2'-OH, 3',4',6',3,4-pentaOMe (**21**)
314. 2'-OH, 3',6'-diOMe, 3,4-OCH₂O-, furano[2'',3'':4',5'] (**96**)
315. 6'-OH, 2',3',4',3,4-pentaOMe (H2/526)
(2',4',6',2,3,4)
316. 2',4',6',2,3,4-hexaOMe (**22**)
(2',4',6',2,4,5)
317. 2'-OH, 4',6',2,4,5-pentaOMe (rubone, H2/524)
(2',4',6',3,4,5)
318. 2'-OH, 4',6',3,4,5-pentaOMe (H2/525)
319. 6'-OH, 4',3,4,5-tetraOMe, 6'',6''-dimethylpyrano[2'',3'':2',3'] (**97**)
(2',4',6',3,4,β)
320. β-OH, 2',4',6'-triOMe, 3,4-OCH₂O- (ponganone X, **25**)
321. β-OH, 2',6'-diOMe, 3,4-OCH₂O-, 6'',6''-dimethylpyrano[2'',3'':4',3'] (pongapinone A, **108**)
(3',4',2,4,6,β)
322. 2,4,6,β-tetraOMe, 3',4'-OCH₂O- (**26**)
- Octa-*O*-substituted
(2',3',4',6',2,3,4,5)
323. 2',2-diOH, 6',3,4,5-tetraOMe, 3',4'-OCH₂O- (H2/568)
324. 6'-OH, 2',3',4',2,3,4,5-heptaOMe (H2/528)
325. 6'-OH, 2',2,3,4,5-pentaOMe, 3',4'-OCH₂O- (H2/569)
326. 2',3',4',6',2,3,4,5-octaOMe (H2/529)
327. 2',6',2,3,4,5-hexaOMe, 3',4'-OCH₂O- (H2/570)
(2',4',5',2,3,4,5,β)
328. β-OH, 2',4',5',2,3,4,5-heptaOMe (H2/535)
- Chalcone glycosides**
- 4'-Hydroxychalcone*
329. 4'-*O*-Glucoside (H2/721)
- 2',4-Dihydroxychalcone*
330. 4'-*O*-Glucoside (H2/723)
- 3,4-Dihydroxychalcone*
331. 4-*O*-β-Arabinosyl-(1''' → 4'')-galactoside (H2/722)
- 2',3',4-Trihydroxychalcone*
332. 4'-*O*-Glucoside (**109**)
- 2',4',4-Trihydroxychalcone (isoliquiritigenin)*
333. 2'-*O*-Glucosyl-(1''' → 4'')-rhamnoside (H2/729)
334. 4'-*O*-Glucoside (neoisoliquiritin, H2/725)
335. 4'-*O*-Glucosylglucoside (H2/730)
336. 4',4-Di-*O*-glucoside (H2/728)
337. 4'-*O*-Glucoside 4-*O*-apiofuranosyl-(1''' → 2'')-glucoside (**110**)
338. 4'-*O*-Glucosylglucoside 4-*O*-glucoside (H2/731)
339. 4-*O*-Glucoside (isoliquiritin, H2/724)
340. 4-*O*-Apiofuranosyl-(1''' → 2'')-glucoside (licuroside, H2/726)
341. 4-*O*-(5'''-*O*-*p*-Coumaroyl)apiofuranosyl-(1''' → 2'')-glucoside (**111**)
342. 4-*O*-(5'''-*O*-Feruloyl)apiofuranosyl-(1''' → 2'')-glucoside (**112**)
343. 4-*O*-Rhamnosylglucoside (H2/727)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

344. 3'-*C*-Glucoside (H1/2597)
 2',4',4'-Trihydroxy-3'-prenylchalcone (3'-prenylisoliquiritigenin)
345. 4'-*O*-Glucoside (113)
 2',3',4',4'-Tetrahydroxychalcone
346. 4'-*O*-(2''-*O*-*p*-Coumaroyl)glucoside (114)
347. 4'-*O*-(6''-*O*-*p*-Coumaroyl)glucoside (115)
348. 4'-*O*-(2''-*O*-Acetyl-6''-*O*-cinnamoyl)glucoside (116)
349. 4'-*O*-(2''-*O*-*p*-Coumaroyl-6''-*O*-acetyl)glucoside (117)
 2',6',2'-Trihydroxy-4'-methoxychalcone
350. 2'-*O*-Glucoside (androechin, 118)
 2',4',6',4'-Tetrahydroxychalcone (chalconaringenin)
351. 2'-*O*-Glucoside (isosalipurposide, H2/744)
352. 2'-*O*-(6''-*O*-*p*-Coumaroyl)glucoside (H2/742)
353. 2'-*O*-Xyloside (H2/743)
354. 2'-*O*-Rhamnosyl-(1''' → 4'')-glucoside (H2/747)
355. 2'-*O*-Rhamnosyl-(1''' → 4'')-xyloside (H2/746)
356. 2',4'-Di-*O*-glucoside (119)
357. 2'-*O*-Glucoside 4'-*O*-gentobioside (120)
358. 4'-*O*-Glucoside (H2/745)
359. 4-*O*-Glucoside (H2/741)
 2',4',4'-Trihydroxy-6'-methoxychalcone (helichrysetin)
360. 4'-*O*-Glucoside (helichrysin, H2/749)
361. 4-*O*-Glucoside (121)
362. 4',4-Di-*O*-α-glucoside (122)
 2',6',4'-Trihydroxy-4'-methoxychalcone (neosakuranetin)
363. 2'-*O*-Glucoside (neosakuranin, H2/748)
 2',4-Dihydroxy-4',6'-dimethoxychalcone (flavokawin C)
364. 4-*O*-Glucoside (H2/751)
365. 4-*O*-Apiosyl-(1''' → 2'')-glucoside (H2/752)
366. 4-*O*-(5'''-*O*-*p*-Cinnamoyl)apiofuranosyl-(1''' → 2'')-glucoside (123)
 2',4',6',β-Tetrahydroxychalcone
367. 2'-*O*-Glucoside (H2/753)
368. 4'-*O*-Glucoside (H2/754)
 2',4',3,4-Tetrahydroxychalcone (butein)
369. 2',3-Di-*O*-glucoside (H2/735)
370. 4'-*O*-Glucoside (coreopsin, H2/733)
371. 4'-*O*-Malonylglucoside (H2/734)
372. 4'-*O*-Arabinosyl-(1''' → 4'')-galactoside (H2/737)
373. 4'-*O*-Glucosylglucoside (H2/738)
374. 4'-*O*-Malonylsphoroside (H2/739)
375. 4',3-Di-*O*-glucoside (isobutrin, H2/736)
376. 3-*O*-Glucoside (monospermoside, H2/732)
 2',4',4'-Trihydroxy-3-methoxychalcone (homobutein)
377. 4'-*O*-Glucoside (124)
378. 4-*O*-Glucoside (H2/740)
 2',4-Dihydroxy-4',6',3-trimethoxychalcone

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

379. 4-*O*-Glucoside (H2/780)
2',3',4',3,4-Pentahydroxychalcone (okanin)
380. 3'-*O*-Glucoside (H2/755)
381. 3',4'-Di-*O*-glucoside (H2/764)
382. 4'-*O*-Glucoside (marein, H2/756)
383. 4'-*O*-(6''-*O*-Acetyl)glucoside (H2/757)
384. 4'-*O*-(6''-*O*-*p*-Coumaroyl)glucoside (H2/758)
385. 4'-*O*-(4'',6''-Di-*O*-acetylglucoside) (**125**)
386. 4'-*O*-(4''-*O*-Acetyl-6''-*O*-*p*-coumaroyl)glucoside (H2/759)
387. 4'-*O*-(2''-*O*-Caffeoyl-6''-*O*-acetylglucoside) (**126**)
388. 4'-*O*-(2''-*O*-Caffeoyl-6''-*O*-*p*-coumaroyl)glucoside) (**127**)
389. 4'-*O*-(2'',4'',6''-Tri-*O*-acetyl)glucoside (H2/760)
390. 4'-*O*-(2'',4''-Di-*O*-acetyl-6''-*O*-*p*-coumaroyl)glucoside (H2/762)
391. 4'-*O*-(3'',4'',6''-Tri-*O*-acetyl)glucoside (H2/761)
392. 4'-*O*-(3'',4''-Di-*O*-acetyl-6''-*O*-*p*-coumaroyl)glucoside (H2/763)
393. 4'-*O*- α -Arabinofuranosyl-(1''' \rightarrow 4'')-glucoside (H2/765)
394. 4'-*O*-Glucosyl-(1''' \rightarrow 6'')-glucoside (H2/766)
2',3',4',3-Tetrahydroxy-4-methoxychalcone (okanin 4-methyl ether)
395. 3'-*O*-Glucoside (H2/767)
396. 3'-*O*-(6''-*O*-Acetyl)glucoside (H2/768)
397. 4'-*O*-Glucoside (H2/769)
398. 4'-*O*-(6''-*O*-Acetyl)glucoside (H2/770)
399. 4'-*O*-(6''-*O*-*p*-Coumaroyl)glucoside) (**128**)
400. 4'-*O*-(2''-*O*-Caffeoyl-6''-*O*-acetylglucoside) (**129**)
401. 4'-*O*-Primveroside (**130**)
2',4',3,4-Tetrahydroxy-3'-methoxychalcone (lanceoletin)
402. 4'-*O*-Glucoside (lanceolin, H2/772)
2',3',4'-Trihydroxy-3,4-dimethoxychalcone (okanin 3,4-dimethyl ether)
403. 4'-*O*-Glucoside (H2/773)
2',4',4-Trihydroxy-3',3-dimethoxychalcone (okanin 3',3-dimethyl ether)
404. 4'-*O*-Glucoside (**131**)
2',4'-Dihydroxy-3',3,4-trimethoxychalcone (okanin 3',3,4-trimethyl ether)
405. 4'-*O*-Glucoside (H2/774)
2',4',5',3,4-Pentahydroxychalcone
406. 4'-*O*-Glucoside (stillopsin, H2/776)
2'-Hydroxy-4',6',2,4-tetramethoxychalcone
407. 2'-*O*-Glucoside (**132**)
2',4',6',3,4-Pentahydroxychalcone
408. 2'-*O*-Glucoside (H2/777)
409. 4'-*O*-Glucoside (H2/778)
2',4',6',4-Tetrahydroxy-3-methoxychalcone
410. 2'-*O*-Glucoside (H2/779)
4',6',3-Trihydroxy-2',4-dimethoxychalcone
411. 4'-*O*-Rutinoside (**133**)
2',4',6',3,4,5-Hexahydroxychalcone
412. 2'-*O*-Glucoside (H2/781)
2',4',6',3,4, β -Hexahydroxychalcone

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

413. 2'-*O*-Glucoside (H2/782)
2',3',4',6',3,4, α -Heptahydroxychalcone
414. 2'-*O*-Glucoside (H2/783)

Chalcone dimers and oligomers*Dimers (chalcone)*

415. Azobechalcone A (147)
416. *Brackenridgea* orange pigment (H1/3090)
417. Calodenin A (150)
418. Calodenin B (H1/3089)
419. α -Diceroptene (H1/3145)
420. Dihydrolophirone C (149)
421. α -Diohobanin (H1/3144)
422. Flavumone A (152)
423. Isolophirone C (148)
424. Isombamichalcone (H1/3097)
425. Kamalachalcone A (144)
426. Kamalachalcone B (145)
427. Licobichalcone (146)
428. Lophirone C (H1/3092)
429. Lophirone F (H1/3098)
430. Lophirone G (H1/3099)
431. Lophirone K (151)
432. Matosine (137)
433. Mbamichalcone (H1/3096)
434. Rhuschalcone I (138)
435. Rhuschalcone II (139)
436. Rhuschalcone III (140)
437. Rhuschalcone IV (141)
438. Rhuschalcone V (142)
439. Rhuschalcone VI (143)
440. Urundeuvine A (134)
441. Urundeuvine B (135)
442. Urundeuvine C (136)

Dimers (chalcone-flavan)

443. Daphnodorin A (H2/1727)
444. Daphnodorin C (H2/1728)
445. Daphnodorin J (153)
446. Daphnodorin M (154)
447. Daphnodorin N (155)

Dimers (chalcone-flavan-3-ol)

448. Daphnodorin B (H2/1895)
449. Daphnodorin I (156)
450. Dihydrodaphnodorin B (H2/1896)
451. Genkwanol A¹⁴⁵
452. Genkwanol B (157)
453. Genkwanol C (158)
454. Larixinol (H2/1897)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued**Dimers (chalcone–flavanone)*

- 455. Chalcocaryanone A (159)
- 456. Chalcocaryanone B (160)
- 457. Chalcocaryanone C (161)
- 458. Chalcocaryanone D (162)
- 459. Flavumone B (165)
- 460. 6'''-Hydroxylophirone B (163)
- 461. 6'''-Hydroxylophirone B 4'''-*O*-glucoside (164)
- 462. Lophirone B (H1/3086)
- 463. Lophirone H (H1/3087)
- 464. Occidentoside (H1/2946)

Dimers (chalcone–flavene)

- 465. Bongosin (H1/3085)

Dimers (chalcone–flavone)

- 466. *Aristolochia* dimer “A” (166)
- 467. *Aristolochia* dimer “B” (167)
- 468. *Aristolochia* dimer “C” (168)
- 469. *Aristolochia* dimer “D” (169)
- 470. Calodenone (171)
- 471. Chamaechromone (H1/3127)
- 472. Cissampeloflavone (170)
- 473. Lophirone A (H1/3125)

Trimers

- 474. Caloflavan A (172)
- 475. Caloflavan B (173)

Tetramers

- 476. Alatachalcone (175)
- 477. *Aristolochia* tetraflavonoid (174)
- 478. Isolophirachalcone (176)
- 479. Lophirochalcone (H1/3133)
- 480. Lophiroflavan A (177)
- 481. Lophiroflavan B (178)
- 482. Lophiroflavan C (179)

Pentamer

- 483. Ochnachalcone (180)

Hexamer

- 484. Azobebechalcone (181)

Chalcone Diels–Alder adducts*Chalcone–isoprene*

- 485. Sanggenon R (182)

Chalcone–monoterpene

- 486. *Boesenbergia* adduct (191)
- 487. Crinatusin A₁ (183)
- 488. Crinatusin A₂ (184)
- 489. Crinatusin B₁ (185)
- 490. Crinatusin B₂ (186)
- 491. Crinatusin C₁ (187)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

- 492. Crinatusin C₂ (**188**)
- 493. Fissistin (**189**)
- 494. Isofissistin (**190**)
- 495. Isoschefflerin (H2/685)
- 496. *Kaempferia* adduct “A” (**192**)
- 497. *Kaempferia* adduct “B” (**193**)
- 498. (±)-Nicolaioidesin A (**194**)
- 499. (±)-Nicolaioidesin B (**195**)
- 500. (±)-Nicolaioidesin C (**196**)
- 501. Panduratin A (H2/673)
- 502. Panduratin B (H2/675)
- 503. Schefflerin (H2/686)

Chalcone-coumarin

- 504. Palodesangren A (**197**)
- 505. Palodesangren B (**198**)
- 506. Palodesangren C (**199**)
- 507. Palodesangren D (**200**)
- 508. Palodesangren E (**201**)
- 509. Palodesangretin A (**202**)
- 510. Palodesangretin B (**203**)

Chalcone-arylbenzofuran

- 511. Albafuran C (H2/954)
- 512. Albanol A (mulberrofuran G) (H2/957)
- 513. Albanol B (H2/958, possible artifact)
- 514. Chalcomoracin (H2/966)
- 515. Mulberrofuran C (H2/983)
- 516. Mulberrofuran E (H2/984)
- 517. Mulberrofuran J (H2/985)
- 518. Mulberrofuran O (H2/986)
- 519. Mulberrofuran T (H2/987)
- 520. Mulberrofuran U (**204**)

Chalcone-chalcone

- 521. Artonin C (H2/959)
- 522. Artonin D (H2/960)
- 523. Artonin X (**205**)
- 524. Brosimone A (H2/963)
- 525. Brosimone D (H2/964)
- 526. *Dorstenia* chalcone dimer (**207**)
- 527. Dorstenone (**206**)
- 528. Kuwanon I (H2/968)
- 529. Kuwanon J (H2/969)
- 530. Kuwanon Q (H2/974)
- 531. Kuwanon R (H2/975)
- 532. Kuwanon V (H2/976)
- 533. Sorocein B (H2/995)

Chalcone-flavanone

- 534. Kuwanon K (H2/970)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

- 535. Kuwanon N (H2/971)
- 536. Kuwanon O (H2/972)
- 537. Sanggenol J (**208**)
- 538. Sanggenol M (**209**)
- 539. Sanggenon C (**210**)
- 540. Sanggenon D (**211**)
- 541. Sanggenon E (**212**)
- 542. Sanggenon O (**213**)
- 543. Sanggenon P (H2/993)
- 544. Sanggenon Q (H2/994)
- 545. Sanggenon S (**214**)
- 546. Sanggenon T (**215**)
- 547. Cathayanon A (**216**)
- 548. Cathayanon B (**217**)

Chalcone-flavone

- 549. Albanin F (kuwanon G, moracenin B) (H2/955)
- 550. Albanin G (kuwanon H, moracenin A) (H2/956)
- 551. Artonin I (**218**)
- 552. Brosimone B (H2/965)
- 553. Kuwanon W (H2/977)
- 554. Moracenin C (H2/981)
- 555. Moracenin D (H2/982)
- 556. Multicaulisin (**219**)

Chalcone-stilbene

- 557. Kuwanol E (H2/967)
- 558. Kuwanon P (H2/973)
- 559. Kuwanon X (H2/978)
- 560. Kuwanon Y (H2/979)
- 561. Kuwanon Z (H2/980)

Miscellaneous

- 562. Sorocenol B (**220**)

Chalcone conjugates*Chalcone-diarylheptanoid*

- 563. Calyxin A (**221**)
- 564. Calyxin B (**223**)
- 565. Calyxin F (**225**)
- 566. Calyxin H (**228**)
- 567. Calyxin I (**230**)
- 568. Calyxin L (**232**)
- 569. Deoxycalyxin A (**222**)
- 570. Epicalyxin B (**224**)
- 571. Epicalyxin F (**226**)
- 572. Epicalyxin H (**229**)
- 573. Epicalyxin I (**231**)
- 574. 6-Hydroxycalyxin F (**227**)

Chalcone-bis(diarylheptanoid)

- 575. Blepharocalyxin A (**233**)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

576. Blepharocalyxin B (**234**)
 577. Blepharocalyxin E (**235**)
Miscellaneous
 578. 8-Caffeoyl-3,4-dihydro-5,7-dihydroxy-4-phenylcoumarin (H2/704)
 579. 8-Cinnamoyl-3,4-dihydro-5,7-dihydroxy-4-phenylcoumarin (H2/702)
 580. 8-*p*-Coumaroyl-3,4-dihydro-5,7-dihydroxy-4-phenylcoumarin (H2/703)
 581. Cryptocaryone (H2/716)
 582. Didymocalyxin B (**239**)
 583. 2',4'-Dihydroxy-3'-*C*-(2,6-dihydroxybenzyl)-6'-methoxychalcone (**236**)
 584. 2',4'-Dihydroxy-6'-methoxy-3'-(8,17-epoxy-16-oxo-12,14-labdadien-15-yl)chalcone (**237**)
 585. Lophirone D (H2/705)
 586. Lophirone E (H2/706)
 587. Infectocaryone (**240**)
 588. Kurzichalcolactone (**238**)

Quinochalcones

Aglycones

589. Ceroptene (H2/713)
 590. Desmosdumotin C (**241**)
 591. 2-Hydroxy-7,8-dehydrograndiflorone (H2/712)
 592. Methylpedicinin (H2/715)
 593. Munchiwarin (**242**)
 594. Pedicinin (H2/714)
 595. Ohobanin (H2/711)
 596. Tunicatachalcone (**243**)

C-Glycosides

597. Anhydrosafflor yellow B (**244**)
 598. Carthamin (H1/2845)
 599. Cartormin (**245**)
 600. Hydroxysafflor yellow A (**246**)
 601. Precarthamin (**247**)
 602. Safflomin A (H1/2944)
 603. Safflomin C (H1/2945)
 604. Safflor yellow A (H1/2734)
 605. Safflor yellow B (H1/2846)
 606. Tinctormine (**248**)

DIHYDROCHALCONES

Dihydrochalcone aglycones

607. Dihydrochalcone (H2/784)

Di-*O*-substituted

(2',4')

608. 2',4'-diOH (H2/785)
 609. 2'-OH, 4'-OMe (**249**)
 610. 2'-OH, 4'-prenyloxy (dihydrocordoin, H2/875)
 611. 4'-OH, 2'-OMe (**250**)
 (2,4)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

612. 4-OH, 2-OMe (**251**)

Tri-*O*-substituted

(2',4',6')

613. 2',4',6'-triOH (H2/791)

614. 2',4',6'-triOH, 3'-Me (**252**)

615. 2',4',6'-triOH, 3'-formyl (H2/868)

616. 2',4',6'-triOH, 3'-prenyl (H2/877)

617. 2',4',6'-triOH, 3'-C₁₀ ((+)-linderatin, H2/889)

618. 2',4',6'-triOH, 3'-C₁₀ ((-)-linderatin, **275**)

619. 2',4',6'-triOH, 3',5'-diprenyl (H2/882)

620. 2',4',6'-triOH, 3',5'-diC₁₀ ((+)-neolinderatin, H2/891)

621. 2',4',6'-triOH, 3',5'-diC₁₀ ((-)-neolinderatin, **276**)

622. 2',4'-diOH, 6'-OMe (uvangoletin, H2/793)

623. 2',4'-diOH, 6'-OMe, 3'-Me (myrigalone H, **253**)

624. 2',4'-diOH, 6'-OMe, 3',5'-diMe (angoletin, H2/872)

625. 2',4'-diOH, 3'-Me, 6'',6''-dimethyl-4''-hydroxy-4'',5''-dihydropyrano[2'',3'':6',5'] (H2/886)

626. 2',4'-diOH, 3'-Me, 6'',6''-dimethyl-5''-hydroxy-4'',5''-dihydropyrano [2'',3'':6',5'] (H2/887)

627. 2',6'-diOH, 4'-OMe (H2/792)

628. 2',6'-diOH, 4'-prenyloxy (H2/880)

629. 2',6'-diOH, 3',4'-dihydrooxepino (H2/878)

630. 2',6'-diOH, 4'-OMe, 3'-Me (myrigalone G, **254**)

631. 2',6'-diOH, 4'-OMe, 3',5'-diMe (myrigalon B, H2/871)

632. 2',6'-diOH, 4'-OMe, 3'-prenyl (H2/879)

633. 2',6'-diOH, 4'-OMe, 3'-C₁₀ ((+)-methyllinderatin, H2/890)

634. 2',6'-diOH, 4'-OMe, 3'-C₁₀ ((-)-methyllinderatin, **277**)

635. 2',6'-diOH, 4'-OMe, 5'-(1''-aryl)prenyl (piperaduncin A, **278**)

636. 2'-OH, 4',6'-diOMe (dihydroflavokawin B, H2/794)

637. 2'-OH, 4',6'-diOMe, 3'-Me (H2/869)

638. 2'-OH, 4',6'-diOMe, 3'-formyl, 5'-Me (H2/870)

639. 2'-OH, 4'-OMe, 5''-arylfurano[2'',3'':6',5'] (longicaudatin, **279**)

640. 2'-OH, 4'-OMe, 4''-aryl-5''-(2-hydroxy isopropyl)dihydrofuran[2'',3'':6',5'] (piperaduncin B, **280**)

641. 2'-OH, 4'-OMe, 6'-*O*-C₁₀ (adunctin A, **281**)

642. 2'-OH, 4'-OMe, [5',6']-C₁₀ (adunctin B, **282**)

643. 2'-OH, 4'-OMe, [5',6']-C₁₀ (adunctin C, **283**)

644. 2'-OH, 4'-OMe, [5',6']-C₁₀ (adunctin D, **284**)

645. 2'-OH, 4'-OMe, [5',6']-C₁₀ (adunctin E, **285**)

646. 2'-OH, 6'-OMe, 4'-prenyloxy (H2/881)

(2',4',4')

647. 2',4',4'-triOH (davidigenin, H2/789)

648. 2',4',4'-triOH, 3',5'-diprenyl (gancaonin J, H2/876)

649. 2',4',4'-triOH, 3-geranyl (H2/888)

650. 2',4-diOH, 4'-OMe (H2/790)

651. 2',4-diOH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (crotamosmin, **286**)

652. 2'-OH, 4-OMe, 6'',6''-dimethylpyrano[2'',3'':4',3'] (crotamin, **287**)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

653. 4-OH, 2',4'-diOMe (**255**)
(2',4', α)
654. 2', α -diOH, furano[2'',3'':4',3'] (castillene E, H2/826)
(2',4', β)
655. 2', β -diOMe, furano[2'',3'':4',3'] (ovalitenin B, H2/827)
(4',2,4)
656. 4',2,4-triOH (**256**)
657. 4',2-diOH, 4-OMe (H2/787)
658. 4',4-diOH, 2-OMe (loureirin C, H2/786)
659. 4'-OH, 2,4-diOMe (loureirin A, H2/788)
(4',2,6)
660. 4'-OH, 2,6-diOMe (**257**)
Tetra-*O*-substituted
(2',3',4',6')
661. 2',4',6'-triOH, 3'-OMe, 5'-prenyl (H2/885)
662. 2',3'-diOH, 4',6'-diOMe (**258**)
663. 2',6'-diOH, 3',4'-diOMe (dihdropashanone, H2/805)
664. 2',6'-diOMe, 3',4'-OCH₂O- (H2/820)
665. 3',6'-diOMe, 2'-OMe, 6'',6''-dimethylpyrano[2'',3'':4',5'] (flemistrictin D, H2/884)
666. 2'-OH, 3',4',6'-triOMe (**259**)
(2',3',4',4)
667. 2',4'-diOH, 3',4-diOMe (lusianin, **260**)
(2',4',6',4)
668. 2',4',6',4-tetraOH (phloretin, H2/799)
669. 2',4',6',4-tetraOH, 3,5-diprenyl (**289**)
670. 2',4',6',4-tetraOH, 3-geranyl, 5-prenyl (**290**)
671. 2',4',6'-triOH, 4-OMe (H2/800)
672. 2',4',4-triOH, 6'-OMe (H2/802)
673. 2',4',4-triOH, 6'-OMe, 3'-prenyl (α,β -dihydroxanthohumol, **291**)
674. 2',6',4-triOH, 4'-OMe (asebogenin, H2/801)
675. 2',6',4-triOH, 4'-OMe, 3'-Me (H2/873)
676. 2',6',4-triOH, 4'-OMe, 3',5'-diMe (H2/874)
677. 2',4'-diOH, 6',4-diOMe (H2/804)
678. 2',6'-diOH, 4',4-diOMe (calomelanone, H2/803)
679. 2',4-diOH, 4',6'-diOMe (**261**)
680. 2'-OH, 4',6',4-triOMe (**262**)
(2',4',3,4)
681. 2',4',3,4-tetraOH, 2-geranyl (H2/892)
682. 2',3,4-triOH, 4'-OMe (dihydrocalythrospin, **263**)
683. 2',4',4-triOH, 3-OMe (H2/797)
684. 2',4',4-triOH, (6''-(4-methylpent-3-enyl),6''-methyl)pyrano[2'',3'':3,2]
(H2/893)
685. 2',3,4-triOH, 6'',6''-dimethylpyrano[2'',3'':4',3'] (crocin, **292**)
686. 2',4'-diOH, 3,4-OCH₂O- (H2/819)
687. 2'-OMe, 3,4-OCH₂O-, furano[2'',3'':4',3'] (**293**)
688. 2',4'-diOMe, 3,4-OCH₂O- (ponganone VII, **264**)
(2',4',4, α)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

689. 2',4',4, α -tetraOH (H2/811)
690. 2',4',4, α -tetraOH, 5',3-diprenyl (kazonol Y, **294**)
691. 2',4', α -triOH, 4-OMe (H2/812)
692. 2',4, α -triOH, 4'-geranyloxy (**295**)
693. 4',4, α -triOH, 2'-OMe (H2/813)
694. 2', α -diOH, 4',4-diOMe (odoratol, H2/814)
695. α -OH, 2',4',4-triOMe (H2/815)
(4',2,4,6)
696. 4',2,4-triOH, 6-OMe (loureirin D, H2/795)
697. 4',2-diOH, 4,6-diOMe (**265**)
698. 4',4-diOH, 2,6-diOMe (**266**)
699. 4'-OH, 2,4,6-triOMe (loureirin B, H2/796)
(2,3,4,6)
700. 2-OH, 3,4,6-triOMe (**267**)
701. 6-OH, 2,3,4-triOMe (**268**)
- Penta-*O*-substituted
(2',3',4',5',6')
702. 2',5'-diOH, 3',4',6'-triOMe (dihydropedicin, **269**)
703. 3',5'-diOH, 2',4',6'-triOMe (**270**)
704. 2'-OH, 3',4',5',6'-tetraOMe (dihydrokanakugiol, H2/810)
(2',3',4',6',4)
705. 2',3',4',6',4-pentaOH, 5'-geranyl (H2/896)
706. 2',3',4',6',4-pentaOH, 5'-neryl (H2/897)
(2',3',4',6', β)
707. β -OH, 2',6'-diOMe, 3',4'-OCH₂O- (H2/825)
(2',4',6',3,4)
708. 2',4',6',3,4-pentaOH (H2/806)
709. 2',4',6',3,4-pentaOH, 3',5-diprenyl (**296**)
710. 2',4',6',3,4-pentaOH, 3'-geranyl, 5-prenyl (**297**)
711. 2',4',6',3-tetraOH, 3'-geranyl, 6'',6''-dimethylpyrano[2'',3'':4,5] (**298**)
712. 2',4',6',3-tetraOH, 4-OMe, 3',5-diprenyl (**299**)
713. 2',4',3,4-tetraOH, 6'-OMe, 3'-geranyl (H2/895)
714. 2',6',3-triOH, 4-OMe, 5-prenyl, 6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':4',5'] (**300**)
715. 2',4'-diOH, 6',3,4-triOMe (H2/808)
716. 2',4'-diOH, 6'-OMe, 3,4-OCH₂O- (H2/821)
717. 2',6'-diOH, 4',3,4-triOMe (**271**)
718. 2',4',6'-triOMe, 3,4-OCH₂O- (**272**)
(2',4',6',4, α)
719. 2',4',6',4, α -pentaOH (nubigenol, H2/817)
(2',4',6',4, β)
720. 2',4',4, β -tetraOH, 6'-OMe (**274**)
(2',4',3,4, α)
721. 2',4',3,4, α -pentaOH (**273**)
722. 4',3,4, α -tetraOH, 2'-OMe (H2/816)
(2',4',3,4, β)
723. 2',4', β -triOMe, 3,4-OCH₂O- (dihydromillettone methyl ether, H2/824)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

724. 2', β -diOMe, 3,4-OCH₂O-, furano[2'',3'':4',3'] (ponganone IX, **301**)
(3',4',2,4, β)
725. 2,4, β -triOMe, 3',4'-OCH₂O- (dihydroisomilletinone methyl ether, H2/823)
Hexa-*O*-substituted
(2',3',4',5',6',4)
726. 2',6'-diOH, 3',4-diOMe, 4',5'-OCH₂O- (H2/822)
(2',3',4',6',3,4)
727. 6'-OH, 2',3'-diOMe, 3,4-OCH₂O-, furano[2'',3'':4',5'] (H2/828)
(2',4',5',3,4, β)
728. 2',5', β -triOMe, 3,4-OCH₂O-, 6'',6''-dimethylpyrano[2'',3'',4',3']
(ponganone VIII, **302**)
(2',4',3,4,5, β)
729. 2',4',3,5, β -pentaOH, 4-OMe (gliricidol, H2/818)
- Dihydrochalcone glycosides**
- 2',4',6'-Trihydroxydihydrochalcone*
730. 4'-*O*-(3''-*O*-Galloyl-4'',6''-*O,O*-hexahydroxydiphenylglucoside) (thonningianin A, **303**)
731. 4'-*O*-(4'',6''-*O,O*-Hexahydroxydiphenylglucoside) (thonningianin B, **304**)
2',4',2-Trihydroxydihydrochalcone (davidigenin)
732. 2'-*O*-Glucoside (davidioside, H2/857)
733. 4'-*O*-Glucoside (confusoside, H2/858)
2',4-Dihydroxy-4'-methoxydihydrochalcone
734. 2'-*O*-Glucoside (H2/859)
2',4'-Dihydroxy-3',6'-dimethoxydihydrochalcone
735. 4'-*O*-Glucosyl-(1''' \rightarrow 6'')-glucoside (salicifolioside A, **305**)
2',4',6',4-Tetrahydroxydihydrochalcone (phloretin)
736. 2'-*O*-Glucoside (phloridzin, H2/860)
737. 2'-*O*-(6''-*O*-Acetyl)glucoside (**306**)
738. 2'-*O*-Rhamnoside (glycyphyllin, H2/861)
739. 2'-*O*-Xylosylglucoside (H2/863)
740. 4'-*O*-Glucoside (trilobatin, H2/862)
741. 4'-*O*-(2''-*O*-Acetyl)glucoside (**307**)
742. 3',5'-Di-*C*-glucoside (**308**)
2',4',6'-Trihydroxy-4-methoxydihydrochalcone
743. 2'-*O*-Glucoside (**309**)
2',6',4-Trihydroxy-4'-methoxydihydrochalcone (asebogenin)
744. 2'-*O*-Glucoside (asebotin, H2/864)
2',4'-Dihydroxy-4',6'-diacetyoxydihydrochalcone
745. 2'-*O*-Glucoside (zosterin, **310**)
4-Hydroxy-2',4',6'-trimethoxydihydrochalcone
746. 4-*O*-Glucoside (bidenoside B, **311**)
2',4',4, α -Tetrahydroxydihydrochalcone
747. α -*O*-Glucoside (licoagroside F, **312**)
748. 3'-*C*-Glucoside (coatline A, H1/2598)
2',4',4, β -Tetrahydroxydihydrochalcone
749. 2'-*O*-Glucoside (rocymosin B, **313**)
750. 3'-*C*-Glucoside (pterosupin, H1/2600)
2',4',6',3,4-Pentahydroxydihydrochalcone

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

751. 2'-*O*-Galactoside (H2/865)
 752. 2'-*O*-Glucoside (H2/866)
 753. 4'-*O*-Glucoside (sieboldin, H2/867)
 754. 3'-*C*-Glucoside (aspalathin, H1/2579)
 2',4',3,4, α -Pentahydroxydihydrochalcone
 755. 3'-*C*-Glucoside (coatline B, H1/2599)
 756. 3'-*C*-Xyloside (**314**)

Dihydrochalcone dimers

Dihydrochalcone–dihydrochalcone

757. Brackenin (H1/3095)
 758. Cycloaltisin 6 (**319**)
 759. Iryantherin F (H2/846)
 760. Littorachalcone (**315**)
 761. Piperaduncin C (**318**)
 762. 3',3'-bis(2',4',6'-Trihydroxy-4-methoxydihydrochalcone) (**317**)
 763. Verbenachalcone (**316**)

Dihydrochalcone–deoxotetrahydrochalcone

764. Cinnabarone (**321**)
 765. Cochinchinenin (**320**)

Dihydrochalcone–flavonol

766. Trianguletin (**322**)
 767. Trianguletin 'B' (**323**)
 768. Trianguletin 'C' (**324**)
 769. Trianguletin 'D' (**325**)
 770. Trianguletin 'E' (**326**)

Dihydrochalcone conjugates

C-Benzyl derivatives

771. 2',4'-diOH, 6'-OMe, 3'-(2-OHBn) (uvaretin, H2/830)
 772. 2',4'-diOH, 6'-OMe, 3'-(2 \times 2-OHBn) (angoluarin, H2/832)
 773. 2',4'-diOH, 6'-OMe, 3'-(2-OHBn), 5'-Me (anguvetin, H2/831)
 774. 2',4'-diOH, 6'-OMe, 3',5'-di(2-OHBn) (diuvaretin, H2/833)
 775. 2',4'-DiOH, 6'-OMe, 3'-(2-OHBn), 5'-(2 \times 2-OHBn) (triuvaretin, **328**)
 776. 2',4'-DiOH, 6'-OMe, 3'-(2-OHBn), 5'-(3 \times 2-OHBn) (**330**)
 777. 2',4'-DiOH, 6'-OMe, 3'-(2-OHBn), 5'-(4 \times 2-OHBn) (**332**)
 778. 2',4'-DiOH, 6'-OMe, 3'-(2 \times 2-OHBn), 5'-(2-OHBn) (isotriuvaretin, **329**)
 779. 2',4'-DiOH, 6'-OMe, 3'-(3 \times 2-OHBn), 5'-(2-OHBn) (**331**)
 780. 2',4'-DiOH, 6'-OMe, 3'-(4 \times 2-OHBn), 5'-(2-OHBn) (**333**)
 781. 2',6'-diOH, 4'-OMe, 3'-(2-OHBn) (**327**)
 782. 4',6'-diOH, 2'-OMe, 3'-(2-OHBn) (isouvaretin, H2/829)
 783. Chamuvaretin (H2/834)

Dihydrochalcone–lignans

784. Iryantherin A (H2/841)
 785. Iryantherin B (H2/842)
 786. Iryantherin C (H2/843)
 787. Iryantherin D (H2/844)
 788. Iryantherin E (H2/845)
 789. Iryantherin G (**334**)

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

790. Iryantherin H (335)
 791. Iryantherin I (336)
 792. Iryantherin J (337)
 793. Iryantherin K (338)
 794. Iryantherin L (339)

Miscellaneous

795. Calomelanol A (H2/836)
 796. Calomelanol B (H2/837)
 797. Calomelanol C (H2/838)
 798. Calomelanol D-1 (H2/835)

Quinodihydrochalcones

799. Ceratiolin (H2/850)
 800. Grandiflorone (H2/849)
 801. Grenoblone (H2/854)
 802. *Helichrysum aphelexiodes* quinodihydrochalcone (288)
 803. *Helichrysum forskahlii* quinodihydrochalcone (H2/852)
 804. Helihumulone (H2/853)
 805. Helilupolone (H2/856)
 806. 4-Hydroxygrenoblone (H2/855)
 807. *Myrica gale* quinodihydrochalcone (H2/848)
 808. Syzygiol (H2/851)

AURONES

Aurone aglycones

Mono-*O*-substituted

(6)

809. Furano[2'',3''':6,7] (H2/910)

Di-*O*-substituted

(4,6)

810. 4-OH, Furano[2'',3''':6,7] (H2/911)
 811. 4-OMe, Furano[2'',3''':6,7] (H2/912)

(6,4')

812. 6,4'-diOH (hispidol, H2/898)

Tri-*O*-substituted

(4,5,6)

813. 6-OH, 4,5-OCH₂O- (H2/909)

(4,6,4')

814. 4,6,4'-triOH (H2/899)

(6,3',4')

815. 6,3',4'-triOH (sulfuretin, H2/900)

816. 6,3',4'-triOH, 5-prenyl (broussoaurone A, 340)

817. 6,3',4'-triOH, 7-prenyl (licoagroaurone, 341)

818. 3',4'-OCH₂O-, furano[2'',3''':6,7] (H2/913)

Tetra-*O*-substituted

(4,5,6,4')

819. 5-OH, 4,6,4'-triOMe (342)

(4,6,7,4')

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

820. 4,6,7,4'-tetraOH (**343**)
(4,6,3',4')
821. 4,6,3',4'-tetraOH (aureusidin, H2/901)
822. 4,6,3',4'-tetraOH, 5-Me (**344**)
823. 4,6,3',4'-tetraOH, 7-Me (**345**)
824. 4,6,3',4'-tetraOH, 5,2'-diprenyl (antiarone A, H2/907)
825. 4,6,3',4'-tetraOH, 2',5'-diprenyl (antiarone B, H2/908)
826. 6,3',4'-triOH, 4-OMe (rengasin, H2/902)
827. 6,3',4'-triOH, 4-OMe, 5-Me (**346**)
828. 6,3',4'-triOH, 4-OMe, 7-Me (**347**)
829. 6,3'-diOH, 4,4'-diOMe, 5-Me (**348**)
830. 4,6,3',4'-tetraOMe (**349**)
(6,7,3',4')
831. 6,7,3',4'-tetraOH (maritimetin, H2/903)
832. 6,3',4'-triOH, 7-OMe (leptosidin, H2/904)
- Penta-*O*-substituted
(4,5,6,3',4')
833. 3',4'-diOH, 4,5,6-triOMe (hamiltrone, **350**)
(4,6,3',4',5')
834. 4,6,3',4',5'-pentaOH (bracteatin, H2/905)
- Halogenated
835. 4'-Cl (**353**)
- Miscellaneous
836. Derriobtusone A (H2/914)
837. Derriobtusone B (H2/915)
- Auronol aglycones**
- Di-*O*-substituted
(2,6)
838. 2-OMe, furano[2'',3'':6,7] (castillene A, H2/949)
- Tetra-*O*-substituted
(2,4,6,4')
839. 2,4,6,4'-tetraOH (maesopsin, H2/943)
840. 2,6,4'-triOH, 4-OMe (carpusin, H2/944)
(2,6,3',4')
841. 2,6,3',4'-tetraOH (H2/945)
842. 2,6,3'-triOH, 4'-OMe (H2/946)
843. 2-OMe, 3',4'-OCH₂O-, furano[2'',3'':6,7] (castillene D, H2/952)
- Penta-*O*-substituted
(2,4,6,3',4')
844. 2,4,6,3',4'-pentaOH (alphitonin, H2/947)
(2,6,7,3',4')
845. 2,6,7,3',4'-pentaOH (nigrescin, H2/948)
- Hexa-*O*-substituted
(2,4,6,3',4',5')
846. 2,4,6,3',4',5'-hexaOH (amaronol A, **351**)
847. 2,4,6,3',5'-pentaOH, 4'-OMe (amaronol B, **352**)
- Halogenated

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — continued

848. 2-OH, 4'-Cl (354)

Miscellaneous

849. Castillene B (H2/950)

850. Castillene C (H2/951)

851. Crombenin (H2/953)

Aurone and auronol glycosides*2'-Hydroxyaurone*

852. 2'-O-Glucosyl-(1''' → 6'')-glucoside (dalmaisione D, 355)

6,4'-Dihydroxyaurone (hispidol)

853. 6-O-Glucoside (H2/916)

6,4'-Dihydroxy-7-methylaurone

854. 6-O-Rhamnoside (H2/941)

4,6,4'-Trihydroxyaurone

855. 4-O-Rhamnosyl-(1''' → 2'')-glucoside (357)

856. 4,6-di-O-Glucoside (caulesauroneside, 356)

857. 6-O-Rhamnoside (H2/917)

4,6,4'-Trihydroxy-7-methylaurone

858. 4-O-Rhamnoside (H2/942)

6,3',4'-Trihydroxyaurone (sulfuretin)

859. 6-O-Glucoside (sulfurein, H2/918)

860. 6-O-Glucosylglucoside (H2/919)

861. 6,3'-Di-O-glucoside (palasitrin, H2/920)

4,6,3',4'-Tetrahydroxyaurone (aureusidin)

862. 4-O-Glucoside (cernuoside, H2/921)

863. 4,6-Di-O-glucoside (H2/925)

864. 6-O-Glucoside (aureusin, H2/922)

865. 6-O-Glucuronide (H2/924)

866. 6-O-Rhamnoside (H2/923)

6,7,3',4'-Tetrahydroxyaurone (maritimetin)

867. 6-O-Glucoside (maritimein, H2/926)

868. 6-O-(6''-O-Acetyl)glucoside (H2/927)

869. 6-O-(6''-O-p-Coumaroyl)glucoside (H2/928)

870. 6-O-(3'',6''-Di-O-acetyl)glucoside (bidenoside A, 358)

871. 6-O-(4'',6''-Di-O-acetyl)glucoside (H2/929)

872. 6-O-(2'',4'',6''-Tri-O-acetyl)glucoside (H2/930)

873. 6-O-(3'',4'',6''-Tri-O-acetyl)glucoside (359)

874. 6-O-Glucosylglucoside (H2/932)

875. 7-O-Glucoside (H2/931)

6,3',4'-Trihydroxy-7-methoxyaurone (leptosidin)

876. 6-O-Glucoside (leptosin, H2/933)

877. 6-O-Glucosyl-(1''' → 4'')-rhamnoside (H2/935)

878. 6-O-Xylosyl-(1''' → 4'')-arabinoside (H2/934)

4,6,3',4',5'-Pentahydroxyaurone (bracteatin)

879. 4-O-Glucoside (bractein, H2/936)

880. 6-O-Glucoside (H2/937)

4,6-Dihydroxy-3',4',5'-trimethoxy-7-methylaurone

APPENDIX B Checklist of Known Chalcones, Dihydrochalcones, and Aurones — *continued*

881. 4-*O*-Rhamnoside (**360**)

6,3',4',5'-Tetrahydroxy-4-methoxyaurone (bracteatin 4-methyl ether)

882. 6-*O*-Rhamnosyl-(1''' → 4'')-glucoside (subulin, H2/938)

2,4,6,4'-Tetrahydroxy-2-benzylcoumaranone (maesopsin)

883. 4-*O*-Glucoside (hovetrichoside C, **361**)

884. 6-*O*-Glucoside (**362**)

885. 4-*O*-Glucoside 4'-*O*-rhamnoside (hovetrichoside D, **363**)

Miscellaneous

886. Neoraufuracin (H2/939)

887. Ambofuracin (H2/940)

Aurone and auronol dimers

Aurone–aurone

888. Aulacomniumbiaureusidin (**364**)

889. Disulfuretin (**365**)

890. Licoagron (**366**)

Aurone–flavanone

891. Campylopusaurone (**367**)

Auronol–auronol

892. (2*S*)-2-Deoxymaesopsin-(2 → 7)-(2*R*)-maesopsin (**368**)

893. (2*R*)-2-Deoxymaesopsin-(2 → 7)-(2*S*)-maesopsin (**369**)

894. (2*R*)-2-Deoxymaesopsin-(2 → 7)-(2*R*)-maesopsin (**370**)

895. (2*S*)-2-Deoxymaesopsin-(2 → 7)-(2*S*)-maesopsin (**371**)

Auronol–flavanone

896. (2*R*,3*S*)-Naringenin-(3α → 5)-(2*R*)-maesopsin (**372**)

897. (2*R*,3*S*)-Naringenin-(3α → 5)-(2*S*)-maesopsin (**373**)

898. (2*R*,3*S*)-Naringenin-(3α → 7)-(2*R*)-maesopsin (zeyherin epimer, **374**)

899. (2*R*,3*S*)-Naringenin-(3α → 7)-(2*S*)-maesopsin (zeyherin epimer, **375**)

Auronol–isoflavanone

900. (2*S*,3*R*)-Dihydrogenistein-(2α → 7)-(2*R*)-maesopsin (**376**)

901. (2*S*,3*R*)-Dihydrogenistein-(2α → 7)-(2*S*)-maesopsin (**377**)

17 Bi-, Tri-, Tetra-, Penta-, and Hexaflavonoids

Daneel Ferreira, Desmond Slade, and Jannie P.J. Marais

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17.1 INTRODUCTION

Several comprehensive reviews dealing with bi- and triflavonoids have been published.^{1–7} This chapter focuses on compounds that have been reported since the last review by Geiger was published in 1994.⁷ Since that time tetra-, penta-, and hexaflavonoids have also been identified, hence necessitating a change in the title of this chapter compared to the previous review.⁷

Together with the proanthocyanidins, the bi- and triflavonoids constitute the two major classes of complex C6–C3–C6 secondary metabolites. These compounds represent products of phenol oxidative coupling of flavones, flavonols, dihydroflavonols, flavanones, isoflavones, aurones, auronols, and chalcones, and thus predominantly possess a carbonyl group at C-4 or its equivalent in every constituent unit. However, there are now several examples where the presence of a C-4 carbonyl group is not evident, e.g., the flavanone–isoflavan, licoagrodin (**91**),⁸⁸ where the carbonyl group was subject to secondary modifications, e.g., the calycopteronones (**127–129**),^{102,104} or where the carbon framework is obscured by, e.g., a dienone–phenol rearrangement as in licobichalcone (**35**).⁶⁸ It should also be emphasized that the terms bi- and triflavonoids are used loosely. A multitude of compounds that “do not arise” via phenol oxidative coupling of the aforementioned classes of monomeric flavonoids possessing C-4 carbonyl functional groups are also being reported as “bi- or triflavonoids.” This is exemplified by bichalcone (**139**), a “biflavonoid” generated via an intermolecular Diels–Alder process.¹⁰⁵ Several additional examples can be found in Chapter 11 or by simply entering “biflavonoid” in one of the several powerful electronic search engines that cover the primary literature.

Essentially all the biflavonoid classes covered in the Geiger review⁷ were supplemented since 1992. In addition, several new classes have been reported, e.g., the bi-4-aryldihydrocoumarins (neoflavones) (**16** and **17**),²⁹ the biauronols (**19–22**),³⁸ the isoflavanone–auronols (**100** and **101**),^{35,36} a number of *O*-linked bichalcones (see Section 17.3.1.5), and the first (I-6,O,II-8)- (**42**),⁸⁹ (I-2',II-8)- (**43**),⁸⁷ and (I-3,II-6)- (**49–52**)^{45,84} biflavones. Such a rapid growth not only in the number of new compounds but also in the extended diversity in the location of the interflavonoid bond is discernable in terms of their genesis via phenol oxidative coupling reactions.⁸ Notable in these radical couplings is the exclusive formation of carbon–carbon and carbon–oxygen bonds but the conspicuous absence of the generation of oxygen–oxygen bonds.

17.2 NOMENCLATURE

There is no commonly accepted trivial nomenclature for the bi- and triflavonoids and higher oligomeric forms. Their full systematic names, not to mention their often complex common names, are extremely cumbersome. Geiger and Quinn have proposed a system^{3–5,7} that requires frequent reference for understanding and has become increasingly difficult to implement as the number of new compounds has grown. No doubt, the nomenclature of this class of compounds is in disarray and it depends on active researchers in the field to select a simple but logical system and then to use it consistently.

Locksley² has proposed a system in which the position of substitution of the upper (I) and lower (II) units in biflavonoids is defined from the usual numbering of the parent flavonoid, and each unit is also described from the parent monomer, e.g., apigenin, luteolin, naringenin, genistein, etc. Thus, the well-known biflavones, amentoflavone is named apigeninyl-(I-3', II-8)-apigenin, the tri-*O*-methyl derivative (**11**) 7,4'-di-*O*-methylnaringeninyl-(I-3', II-8)-4'-*O*-methylnaringenin, and the flavanone–auronol (**74**) (2*S*)-naringeninyl-(I-3 α , II-5)-(2*R*)-mae-

sopsin. The consistent implementation of this method for naming the many straightforward examples should be strongly encouraged.

17.3 STRUCTURE AND DISTRIBUTION

The naturally occurring bi- and triflavonoids, together with their plant sources are listed in the following sections. The entries listed are confined to new compounds reported in the post-1992 period or those that have not been dealt with in Geiger's 1994 review.⁷ In order to be comprehensive, the listed compounds must be considered in conjunction with the tables in the Geiger and Quinn^{3-5,7} and Hemingway⁶ reviews. Since many of the analogs have been reported under trivial names these will be retained to facilitate future electronic literature searches.

The first compounds belonging to the tetra-, penta-, and hexaflavonoid classes have also been reported. These complex structures are covered in Sections 17.3.3–17.3.5. As with all published complex structures the reader must avail himself or herself of the correctness of the proposed structure based upon the supporting experimental data.

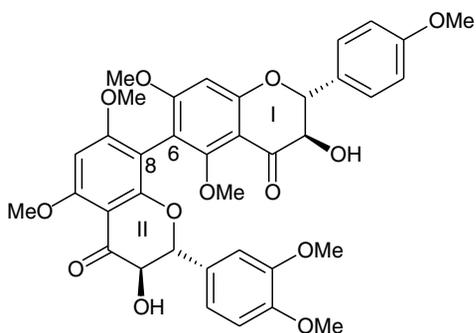
With a few exceptions the issue of absolute configuration of optically active analogs is often completely ignored. In a large number of cases absolute configuration is readily accessible from chiroptical data. The utility of the circular dichroism method in this regard has been amply demonstrated in several reports. Useful information may be extracted from papers dealing with the chiroptical properties of monomeric constituent units like flavanones and 3-hydroxyflavanones (dihydroflavonols),¹¹⁰ auronols,¹¹⁴ a summary of the various classes of stereogenic monomers (Ref. 113 and references cited therein), dimeric compounds like the flavanone- and isoflavanone-auronol- and bis-auronol-type biflavonoids,³⁵⁻³⁸ and several other classes of biflavonoids.^{23,74,111,112}

In addition to the multitude of biological activities reported in the references listed with the compound or natural source, supplementary information can also be found in Refs. 90 and 97. Representative contributions regarding synthesis of the biflavonoids are available in Refs. 91–93, 96, and 99, while useful NMR and x-ray data may be retrieved from Refs. 94 and 98, respectively.

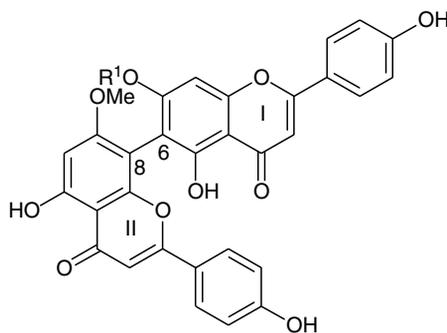
A comprehensive listing of chalcone dimers and oligomers, dihydrochalcone dimers, and aurone and auronol dimers may also be found in Chapter 16.

17.3.1 BIFLAVONOIDS

17.3.1.1 Agathisflavones [(I-6,II-8)-Coupling]

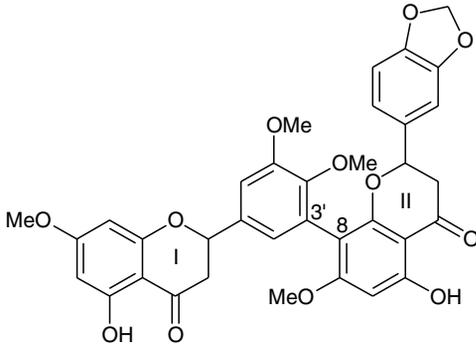


(1) *Ouratea multiflora*, relative configuration, Ref. 25

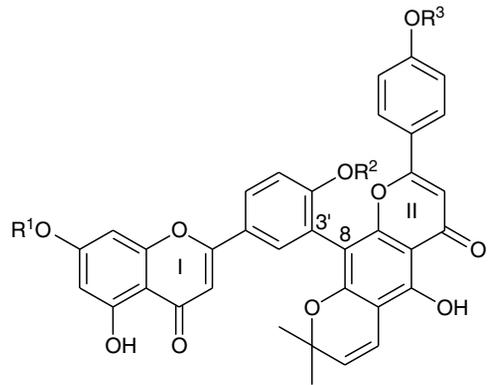


(2) R¹ = Me — *Ouratea spectabilis*, Ref. 28
(3) R¹ = H — *Ouratea hexasperma*, Ref. 33

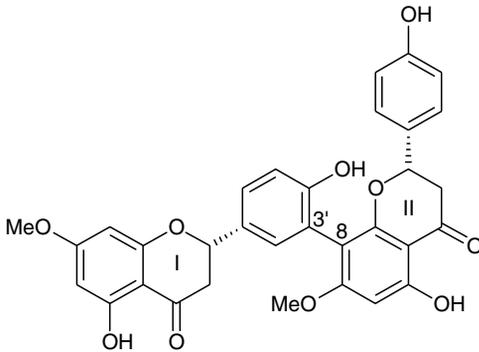
17.3.1.2 Amentoflavones [(I-3',II-8)-Coupling]



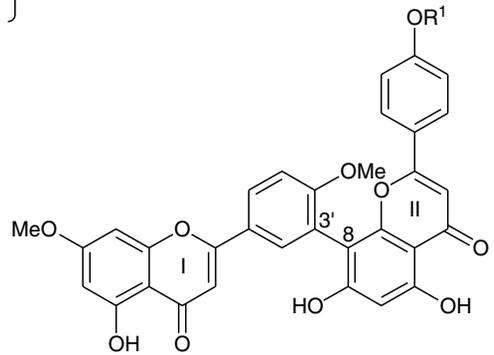
(4) Anacarduflavanone — *Semecarpus anacardium* Linn., rel. config., Ref. 18



- (5) $R^1 = R^2 = R^3 = H$ — Pyranoamentoflavone — *Calophyllum inophylloide*, Ref. 19
 (6) $R^1 = Me, R^2 = R^3 = H$
 (7) $R^1 = R^3 = H, R^2 = Me$
 (8) $R^1 = R^3 = Me, R^2 = H$
 (9) $R^1 = R^2 = Me, R^3 = H$ } — *Calophyllum venulosum*, Refs. 20,21

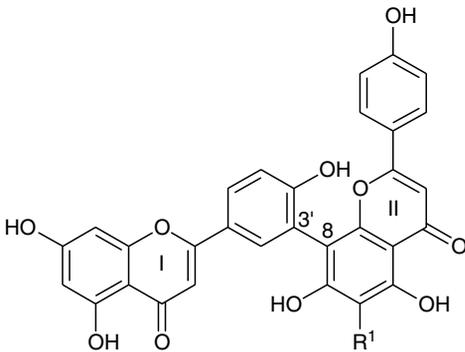


(12) (I-2*S*,II-2*S*)-I-7,II-7-Di-*O*-methyltetrahydroamentoflavone — *Rhus retinorrhoea*, abs. config., Ref. 23

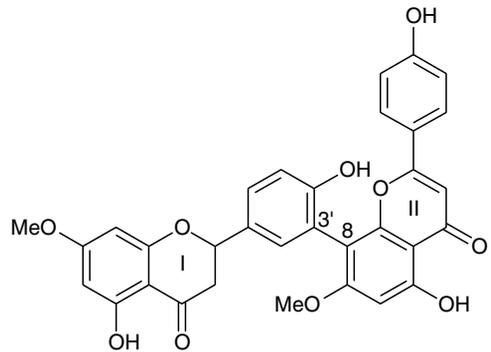


- (10) $R^1 = H$
 (11) $R^1 = Me$ } — *Taxus baccata*, Ref. 22

Amentoflavone is the free phenolic form of compound (11)

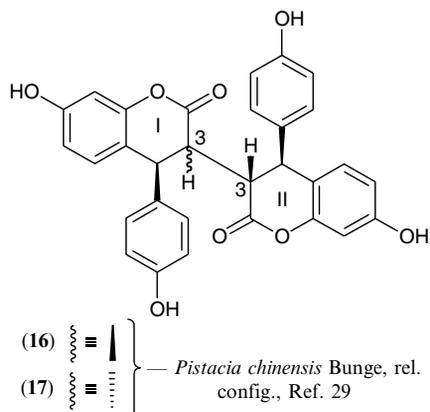


- (13) $R^1 = \text{---CH=CH}_2$
 (14) $R^1 = \text{---CH(OH)-CH}_2$ } — *Calophyllum venulosum*, Ref. 21

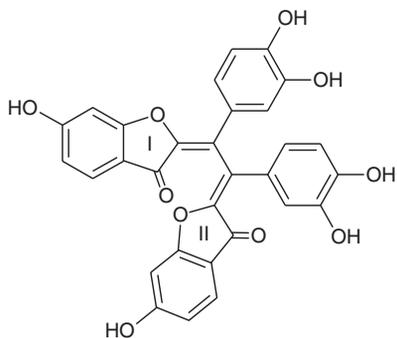
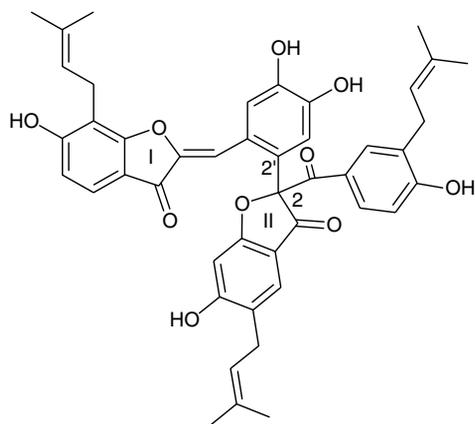
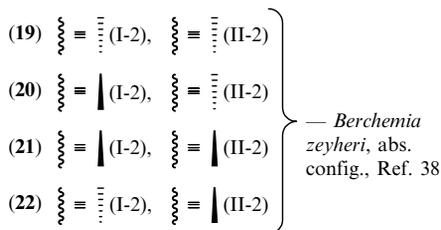
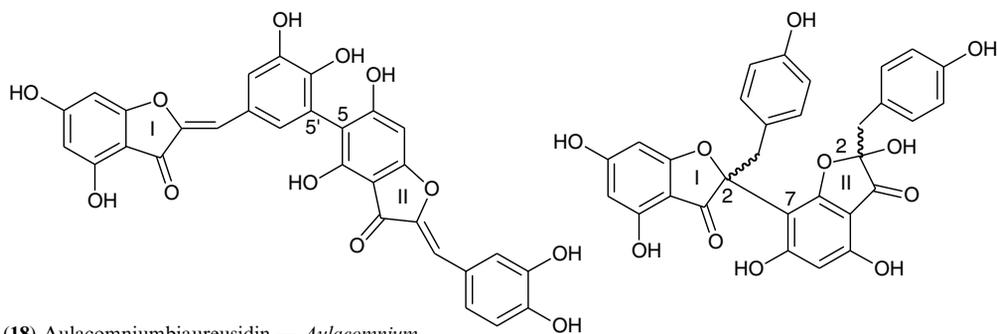


(15) *Amentotaxus yunnanensis*, rel. config., Ref. 44

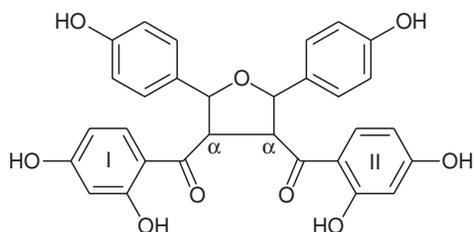
17.3.1.3 Bi-4-aryldihydrocoumarins (Bineoflavones)



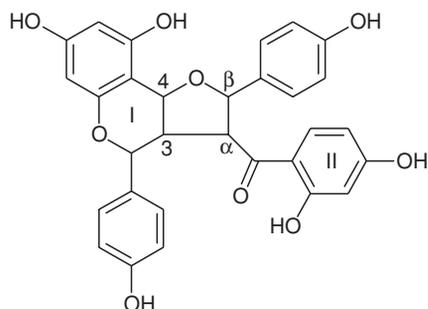
17.3.1.4 Biauronols and Biaurones



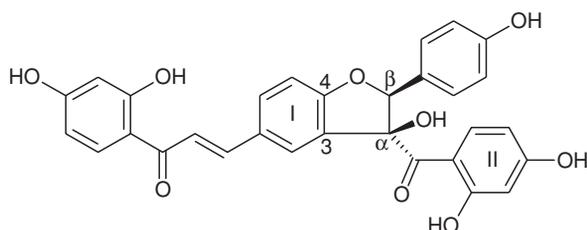
17.3.1.5 Bichalcones



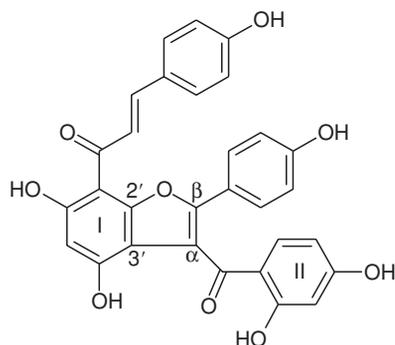
(25) Cordigone — *Cordia goetzei*, no config. indicated, Ref. 31



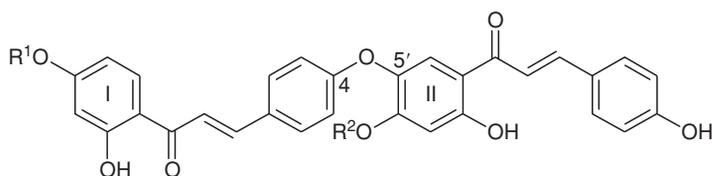
(26) Cordigol — *Cordia goetzei*, no config. indicated, Ref. 31



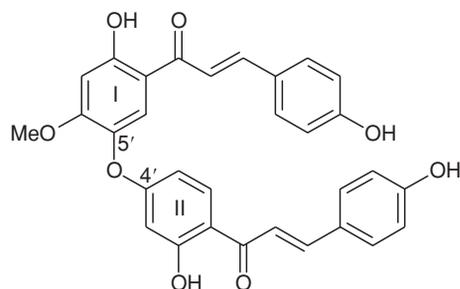
(27) Lophirone K — *Ochna calodendron*, rel. config., Ref. 32



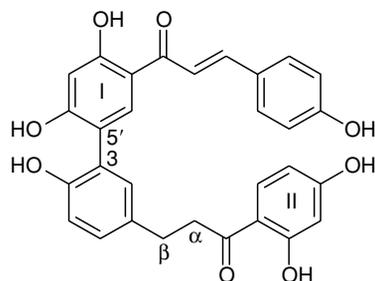
(28) Calodenin A — *Ochna calodendron*, Ref. 32



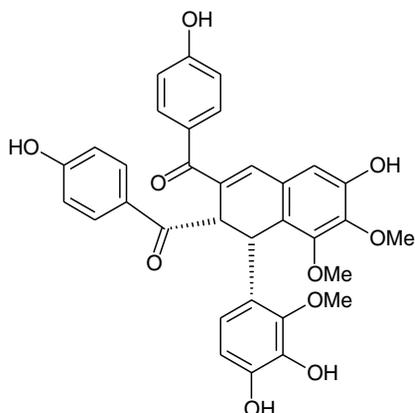
(29) $R^1 = R^2 = \text{Me}$ — Rhuschalcone I
 (30) $R^1 = R^2 = \text{H}$ — Rhuschalcone II
 (31) $R^1 = \text{Me}, R^2 = \text{H}$ — Rhuschalcone III } — *Rhus pyroides* Burch. } Ref. 58
 } Ref. 67



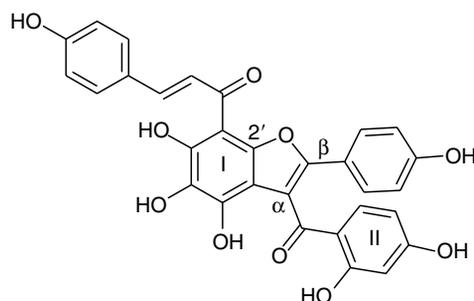
(32) Rhuschalcone IV — *Rhus pyroides* Burch., Ref. 67



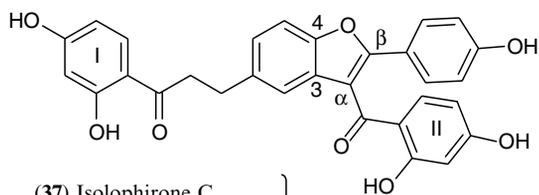
(33) Rhuschalcone V
 (34) α, β -Double bond, } — *Rhus pyroides*
 rhuschalcone VI } Burch., Ref. 67



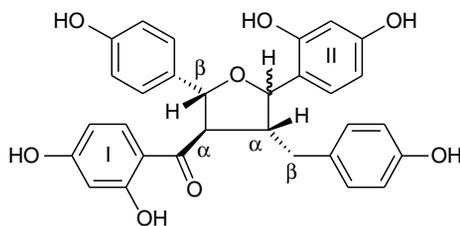
(35) Licobichalcone — *Glycyrrhiza uralensis*, rel. config., Ref. 68



(36) Flavumone A — *Oureatea flava*, Ref. 64

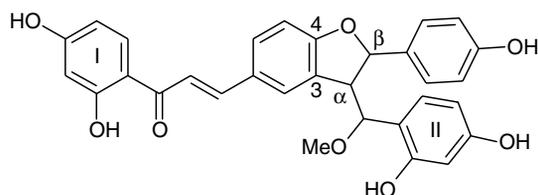


(37) Isolophirone C
 (38) Dihydrolophirone C, α,β -dihydro analog of isolophirone C } — *Ochna afzelii*, Ref. 66



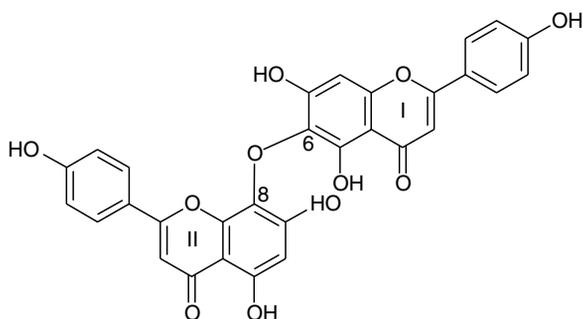
(39) ≡ ≡ ≡, Mbamichalcone — *Lophira alata*, rel. config., Ref. 61

(40) ≡ ≡ ≡, Isombamichalcone — *Lophira lanceolata*, rel. config., Ref. 62



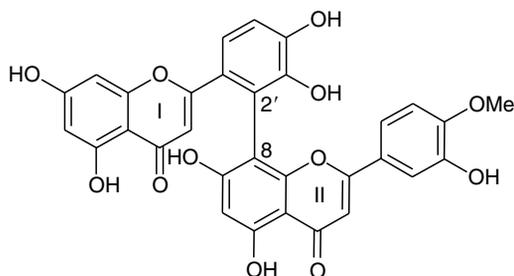
(41) Azobeichalcone — *Lophira alata*, rel. config., Ref. 50

17.3.1.6 (I-6,O,II-8)-Biflavones

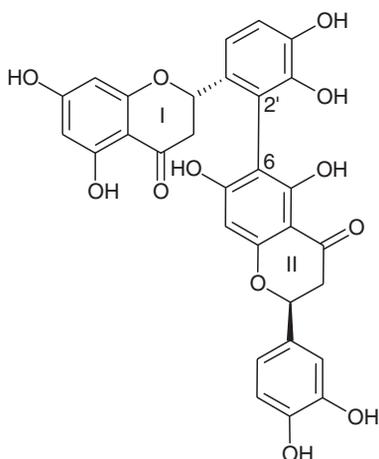
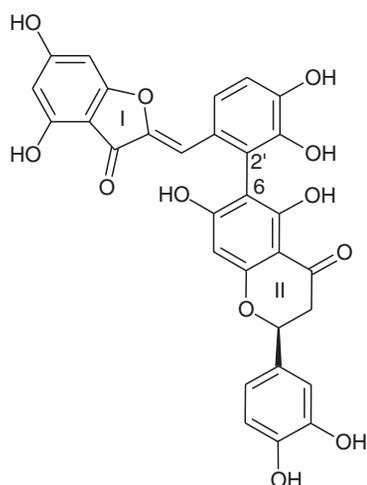
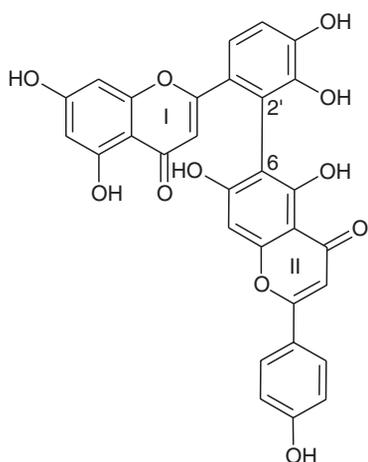
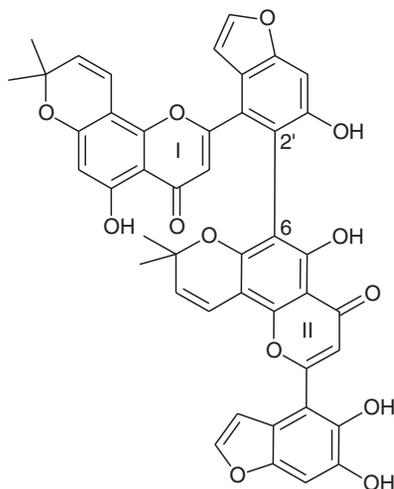


(42) *Viburnum cotinifolium*, Ref. 89

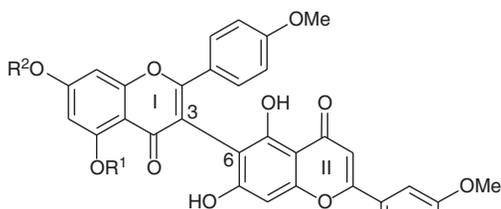
17.3.1.7 (I-2',II-8)-Biflavones

(43) Philonotisflavone-II-4'-methyl ether — *Mnium hornum*, Ref. 87

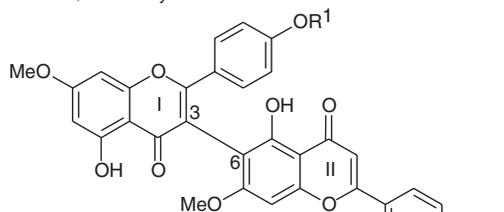
17.3.1.8 (I-2',II-6)-Biflavones and Aurone-Flavones

(44) Tetrahydrodicranolomin — *Pilotrichella flexilis*, abs. config., Ref. 71(45) Pilotrichellaaurone — *Pilotrichella flexilis*, abs. config., Ref. 71(46) 3''-Desoxydicranolomin } — *Plagiommium undulatum*, Ref. 85
(47) I-2,3-Dihydro derivative }(48) Leucaediflavone — *Leucaena diversifolia*, Ref. 107

17.3.1.9 (I-3,II-6)-Biflavones

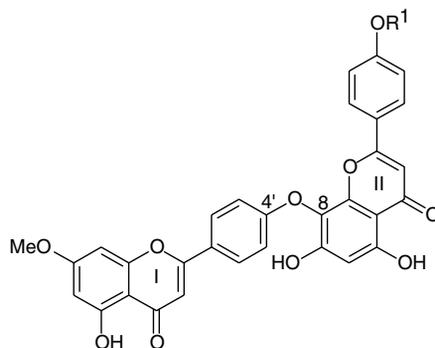


(49) $R^1 = H, R^2 = Me$ } — *Aristolochia ridicula*, Ref. 45
 (50) $R^1 = Me, R^2 = H$ }



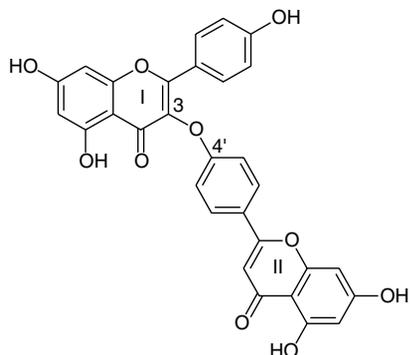
(51) $R^1 = H$, Stephaflavone A } — *Stephania tetrandra*, Ref. 84
 (52) $R^1 = Me$, Stephaflavone B }

17.3.1.10 (I-4',O,II-8)-Biflavones



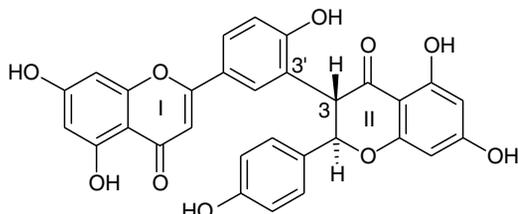
(53) $R^1 = H$ } — *Ouratea semiserrata*, Ref. 26
 (54) $R^1 = Me$ }

17.3.1.11 (I-3,O,II-4')-Biflavones

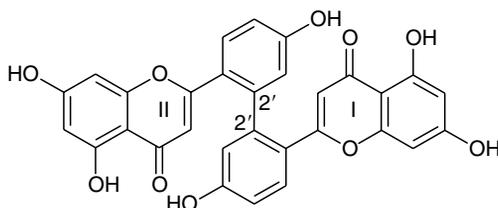


(55) Delicaflavone — *Selaginella delicatula*, Ref. 10

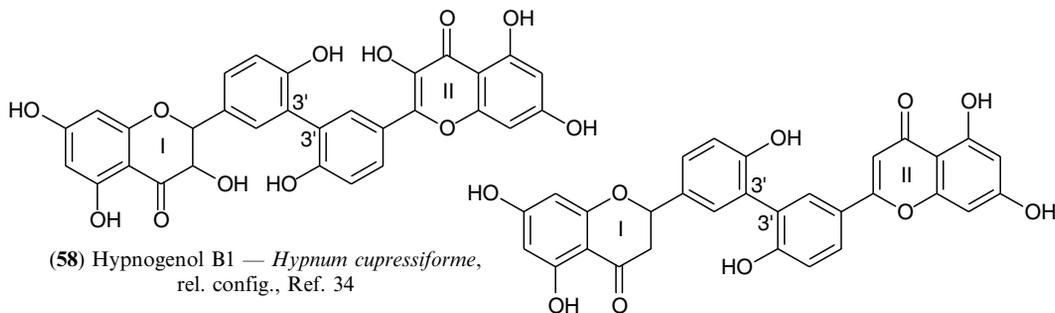
17.3.1.12 (I-3',II-3)-Biflavones

(56) Lanceolatin A — *Lophira lanceolatum*, rel. config., Ref. 83

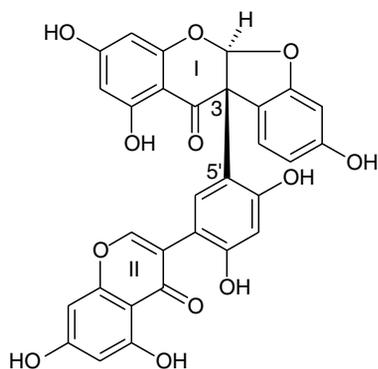
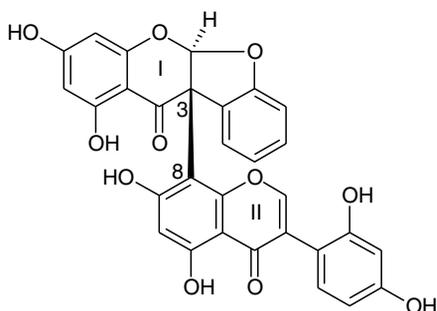
17.3.1.13 (I-2',II-2')-Biflavonols

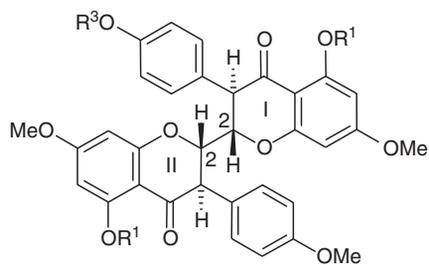
(57) *Garcinia nervosa*, Ref. 108

17.3.1.14 (I-3',II-3')-Biflavones

(58) Hypnogenol B1 — *Hypnum cupressiforme*,
rel. config., Ref. 34(59) 2,3-Dihydroapigeninyl-(I-3',II-3')-apigenin
— *Homalothecium lutescens*, rel. config., Ref. 40

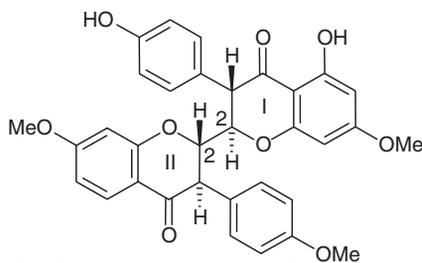
17.3.1.15 Bi-Isoflavonoids

(60) *Lupinus albus L.*, rel. config., Ref. 12(61) *Lupinus albus L.*, rel. config., Ref. 12

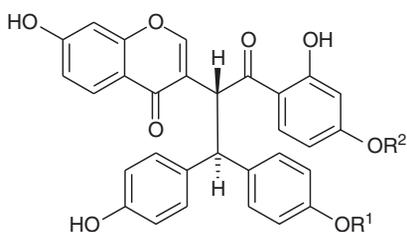


- (62) $R^1 = R^2 = H, R^3 = Me$, Hexaspermone A
 (63) $R^1 = R^3 = H, R^2 = Me$, Hexaspermone B
 (64) $R^1 = R^2 = R^3 = H$, Hexaspermone C

— *Oureatea hexasperma*, rel. config., Ref. 30

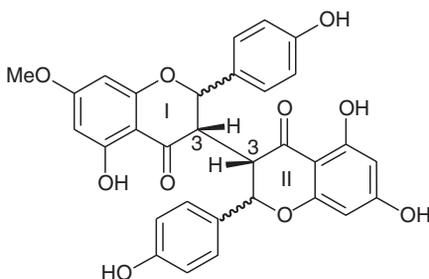


- (65) Dehydroxyhexaspermone C
 — *Ochna macrocalyx*, rel. config., Ref. 13

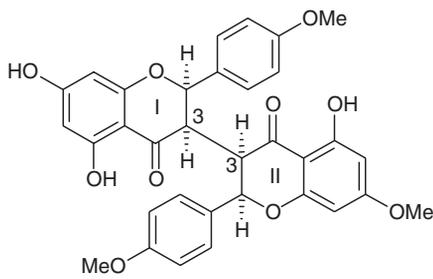


- (66) $R^1 = R^2 = Me$, Afzelone D — *Ochna afzelii*, rel. config., Ref. 16
 (67) $R^1 = H, R^2 = Me$, Calodendrone — *Ochna calodendron*, rel. config., Ref. 42
 (68) $R^1 = R^2 = H$, Lophirone A — *Lophira lanceolata*, rel. config., Ref. 65

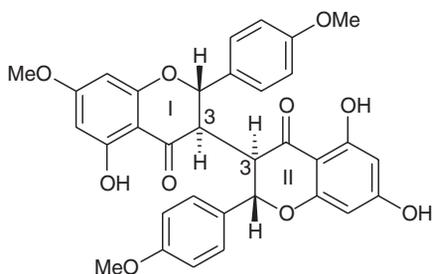
17.3.1.16 Chamaejasmins [(I-3,II-3)-Coupling]



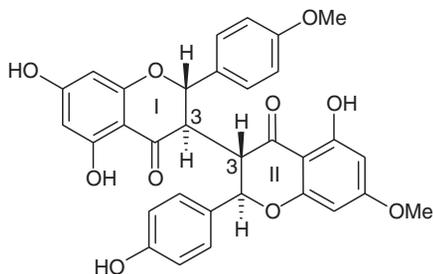
- (69) *Stellera chamaejasme* L., rel. config., Ref. 27



- (70) Ruixianglangdusu A — *Stellera chamaejasme*,
 rel. config., Ref. 81

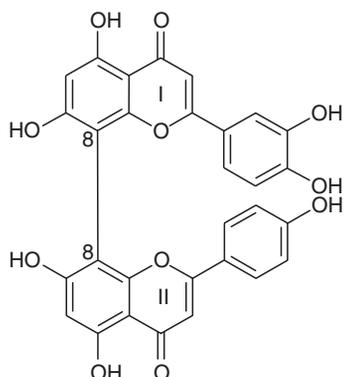


- (71) Ruixianglangdusu B — *Stellera chamaejasme*,
 rel. config., Ref. 81



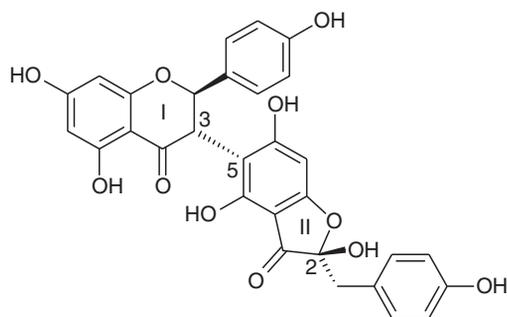
- (72) Sikokianin — *Wikstroemia sikokiana*,
 rel. config., Ref. 82
 Also named Sikokianin C — *Wikstroemia indica*, Ref. 109

17.3.1.17 Cupressuflavones [(I-8,II-8)-Coupling]

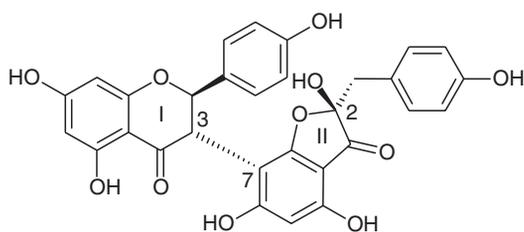


(73) Mogathin (I-3'-hydroxycupressuflavone)
— *Glossostemon bruguieri* (Desf.), Ref. 9

17.3.1.18 Flavanone–Auronols

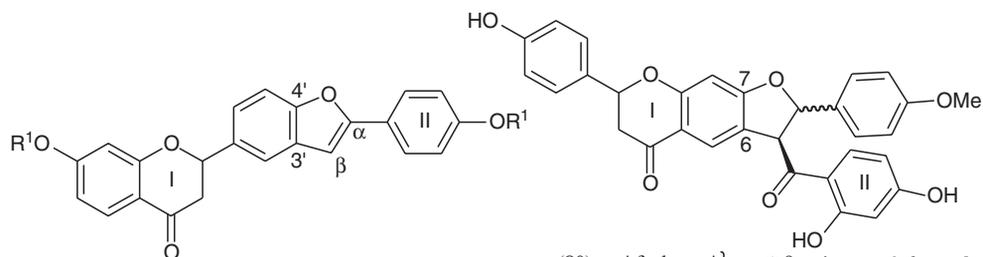


(74) Diastereoisomer shown } — *Berberia zeyheri*, abs. config., Refs. 35, 36
(75) (II-2)-*S*-epimer

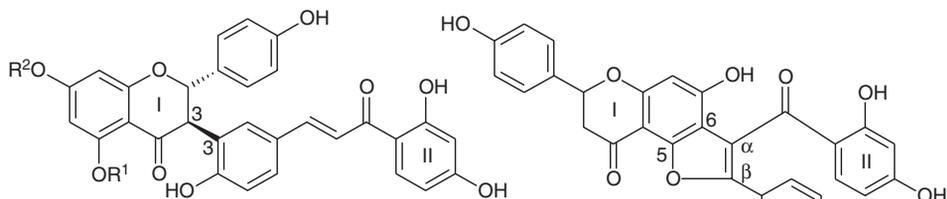


(76) Diastereoisomer shown } — *Berberia zeyheri*,
(77) (II-2)-*S*-epimer } abs. config., Ref. 37

17.3.1.19 Flavanone and Flavone–Chalcones

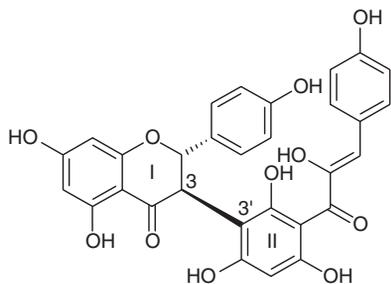


(78) $R^1 = \text{H}$, Lophirone I } — “Cleaved” chalcones — *Lophira*
(79) $R^1 = \text{Me}$, Lophirone J } — *lanceolata*, rel. config., Ref. 48
(80) — Afzelone A } — C- β -epimers, *Ochna afzelii*,
(81) Afzelone B } — rel. config., Ref.

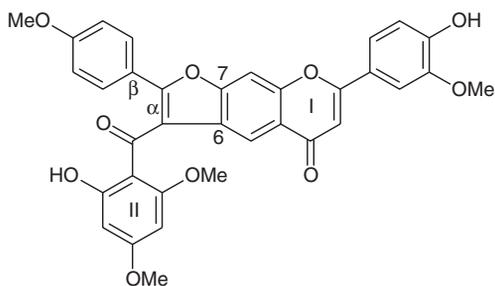


(83) $R^1 = R^2 = \text{H}$, 6''-Hydroxylophirone B } — *Ochna integerrima*,
 (84) $R^1 = \text{H}$, $R^2 = \beta\text{-D-Glc}$ } — abs. config., Ref. 52

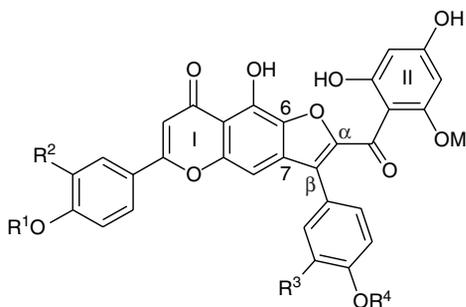
(82) Flavanone B — *Ouratea flava*, rel. config., Ref. 64



(85) Flavanone- α -hydroxychalcone — *Berchemia zeyheri*, abs. config., Ref. 37

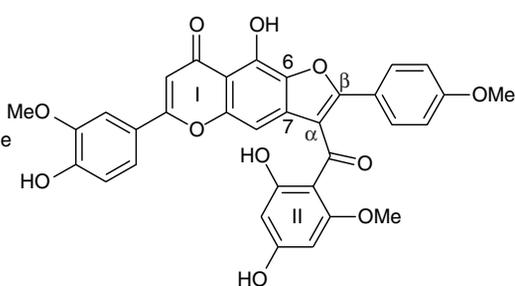


(86) Cissampeloflavone — *Cissampelos pareira*, Ref. 53



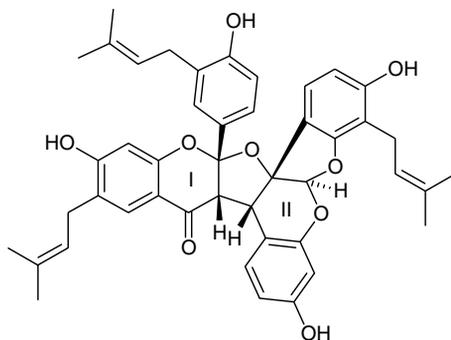
(87) $R^1 = \text{Me}$, $R^2 = R^4 = \text{H}$, $R^3 = \text{OMe}$ }
 (88) $R^1 = R^3 = \text{H}$, $R^2 = \text{OMe}$, $R^4 = \text{Me}$ } — *Aristolochia ridiculosa*, Ref. 45
 (89) $R^1 = R^4 = \text{Me}$, $R^2 = \text{H}$, $R^3 = \text{OH}$ }

Note the odd I-A hydroxylation pattern.



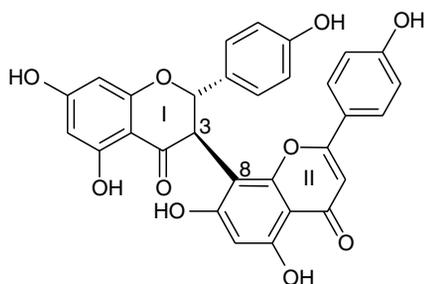
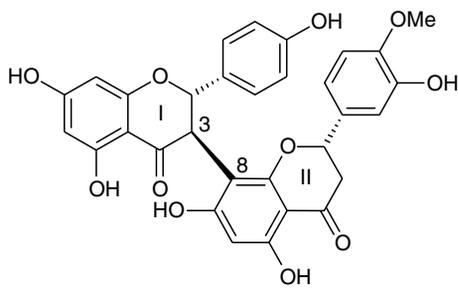
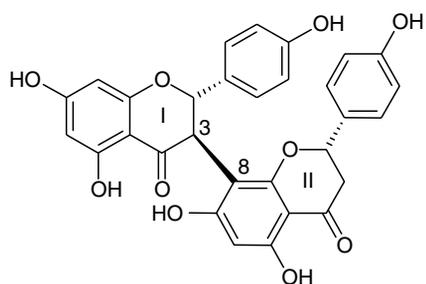
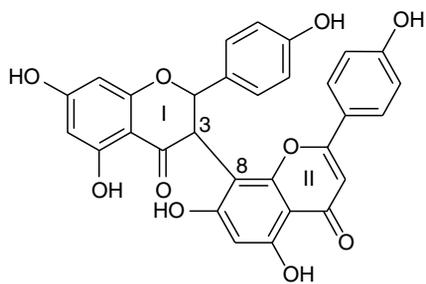
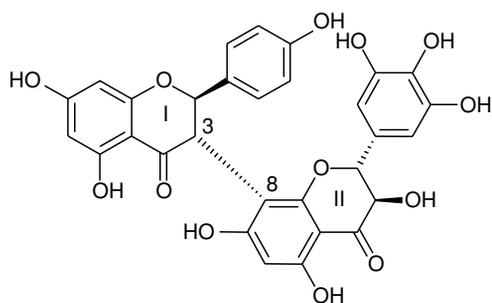
(90) *Aristolochia ridiculosa*, Ref. 45
 Note the odd I-A hydroxylation pattern.

17.3.1.20 Flavanone-Isflavans

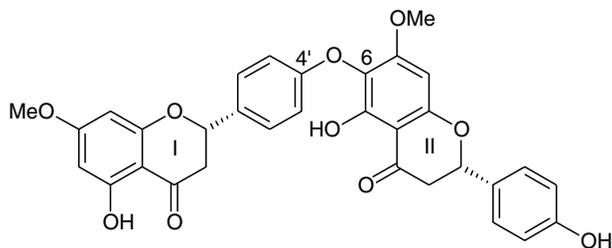


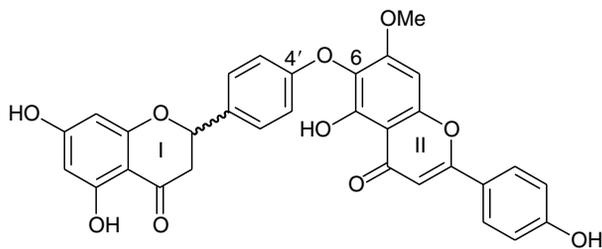
(91) Licoagrodin — *Glycyrrhiza glabra*, rel. config., Ref. 88

17.3.1.21 GB-flavones [(I-3,II-8)-Coupling]

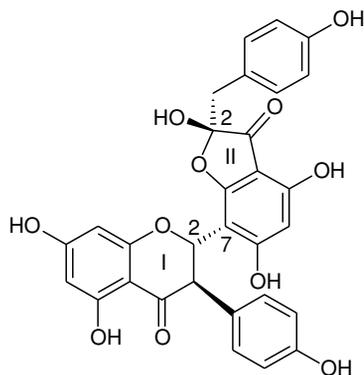
(92) Garcinianin atropisomers — *Garcinia kola*,
rel. config., Ref. 69(93) GB-2a-II-4'-OMe — *Rheedia gardneriana*,
rel. config., Ref. 70(94) (+)-GB-lb — *Garcinia kola*, abs. config., Ref. 72(95) Pancibiflavonol — *Callophyllum paniciflorum*,
rel. config., Ref. 73(96) GB-4, (I-2*R*,3*S*; II-2*R*,3*R*) } — *Gnidia involucrata*,
(97) GB-4a, (I-2*S*,3*R*; II-2*R*,3*R*) } abs. config., Ref. 74

17.3.1.22 Hinokiflavones [(I-4',O,II-6)-Coupling]

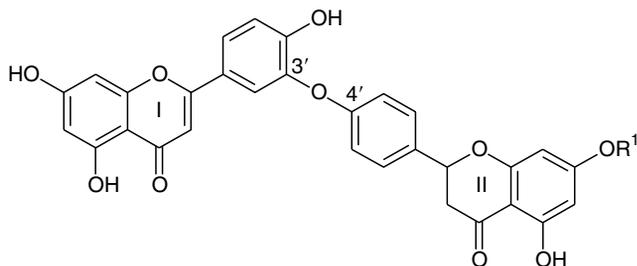
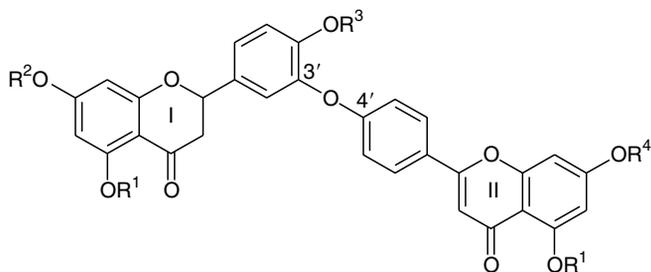
(98) I-7,II-7-Di-O-methyltetrahydrohinokiflavone — *Cycas beddomei*, abs. config., Ref. 80

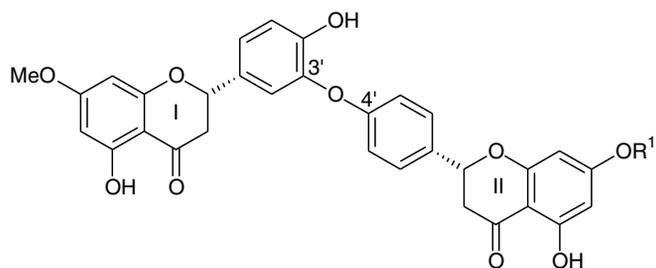
(99) 2,3-Dihydroisocryptomerin — *Selaginella delicatula*, rel. config., Ref. 10

17.3.1.23 Isoflavanone–Auronols [(I-2,II-7)-Coupling]

(100) Diastereoisomer shown } — *Berchemia zeyheri*, abs.
(101) (II-2)-*S*-epimer } config., Refs. 35, 36

17.3.1.24 Ochnaflavones [(I-3',O,II-4')-Coupling]

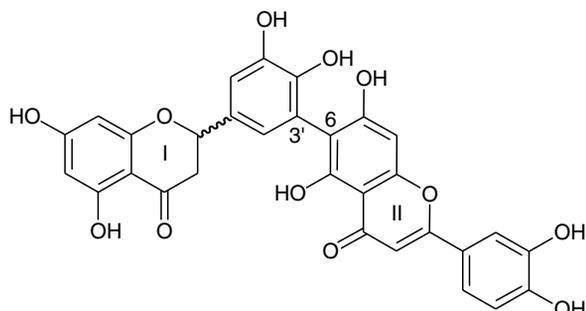
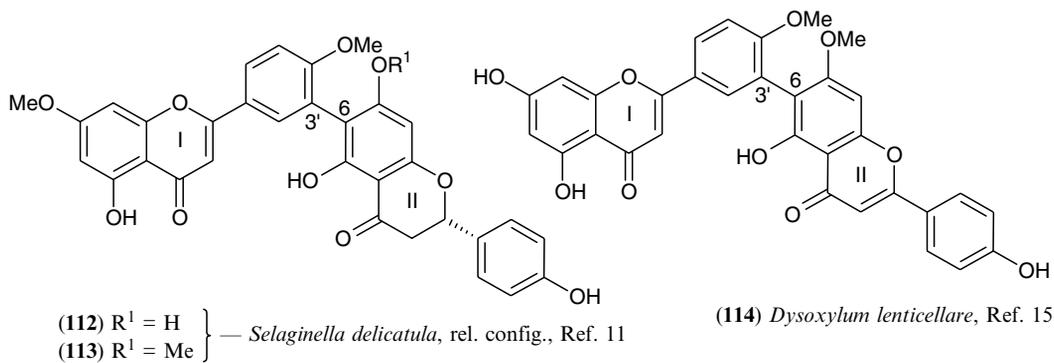
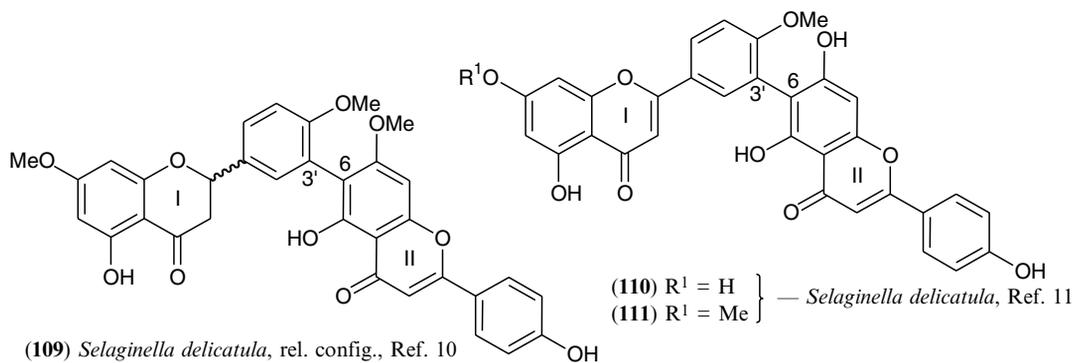
(102) $R^1 = H$ — *Luxemburgia nobilis* (EICHL), rel. config., Refs. 14, 77(103) $R^1 = Me$ — *Ochna integerrima*, rel. config., Ref. 77(104) $R^1 = R^2 = R^3 = R^4 = H$ } — *Ochna obtusata*, rel. config., Ref. 75(105) $R^1 = R^3 = R^4 = H, R^2 = Me$ }
(106) $R^1 = H, R^2 = R^3 = R^4 = Me$ — *Ochna beddomei*, rel. config., Ref. 78



(107) $R^1 = H$ — *Ochna beddomei*, abs. config., Ref. 76

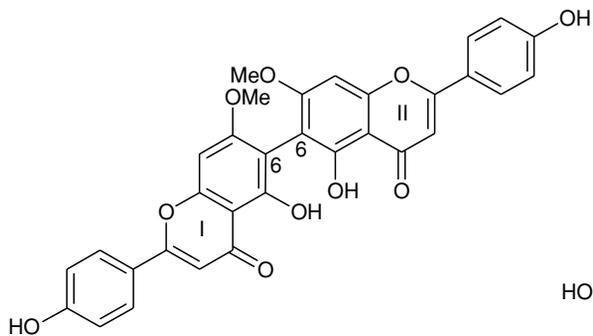
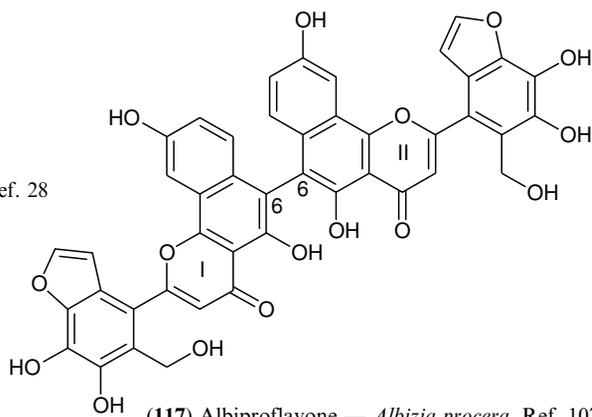
(108) $R^1 = Me$ — *Quintinia acutifolia*, rel. config., Ref. 79

17.3.1.25 Robustaflavones [(I-3',II-6)-Coupling]

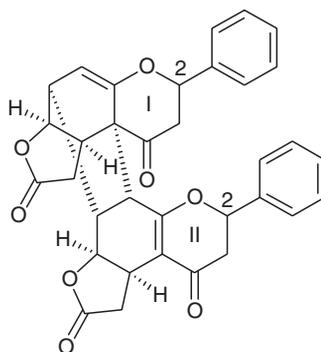
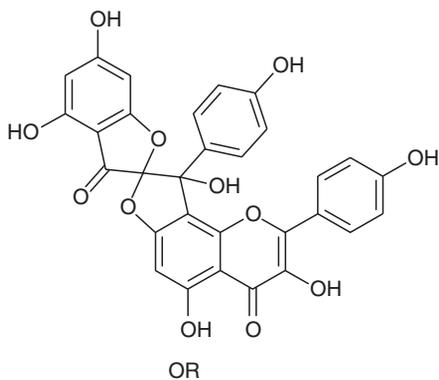


(115) *Plagiomnium undulatum*, rel. config., Ref. 85

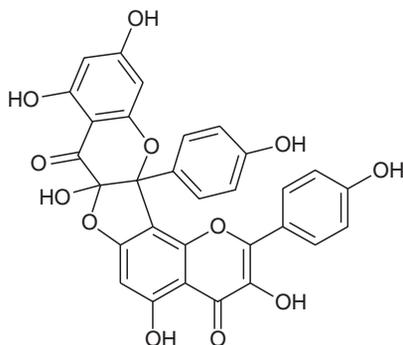
17.3.1.26 Succedaneoflavones [(I-6,II-6)-Coupling]

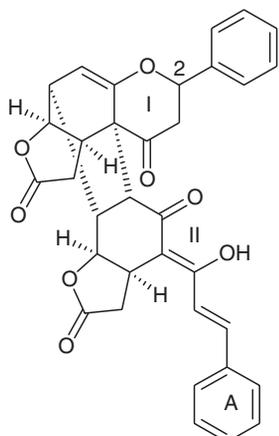
(116) 6,6''-Bigenkwanin — *Ouratea spectabilis*, Ref. 28(117) Albiproflavone — *Albizia procera*, Ref. 107
Note the naphthopyrano functionalities.

17.3.1.27 "Unusual" Biflavonoids



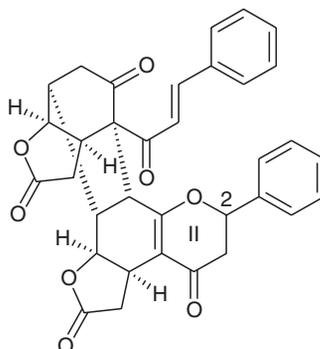
(119) Bicaryone A (I-2S, II-2S)
 (120) Bicaryone B (I-2S, II-2R)
 (121) Bicaryone C (I-2R, II-2S)
 (122) Bicaryone D (I-2R, II-2R)

— *Cryptocarya infectoria*,
Ref. 101(118) VC-15B (vahlia biflavone) — *Vahlia capensis*, Ref. 100



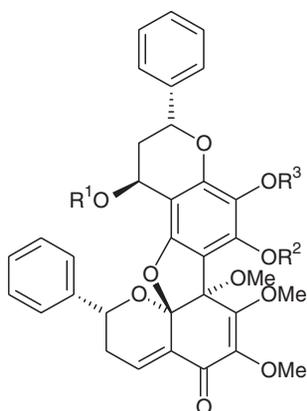
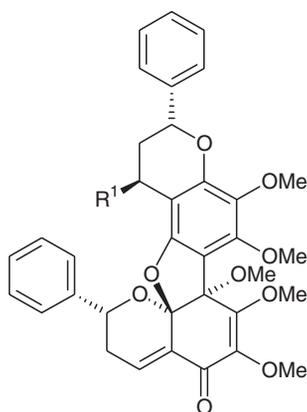
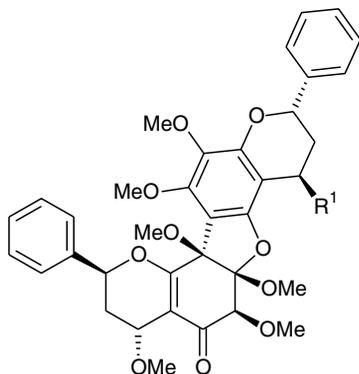
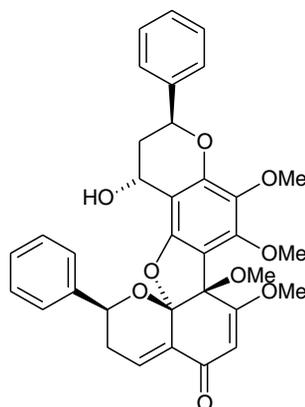
(123) Chalcocaryanone A (I-2R)

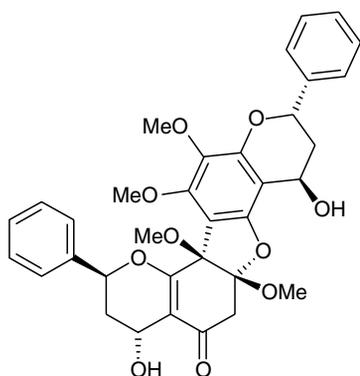
(124) Chalcocaryone B (I-2S)

Cryptocarya infectoria, Ref. 101

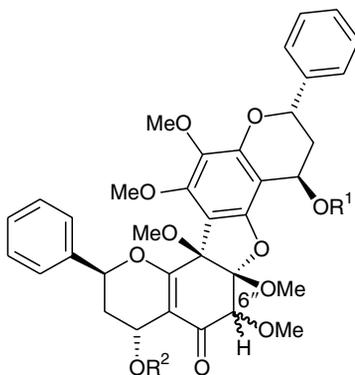
(125) Chalcocaryanone C (I-2S)

(126) Chalcocaryone D (I-2R)

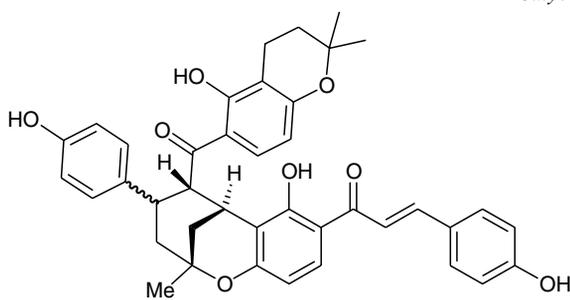
Cryptocarya infectoria, Ref. 101(127) $R^1 = R^3 = \text{Me}$, $R^2 = \text{H}$, Calycopteronone(128) $R^1 = R^2 = \text{Me}$, $R^3 = \text{H}$, Isocalycopteronone(129) $R^1 = R^2 = \text{H}$, $R^3 = \text{Me}$, 4-Demethylcalycopteronone*Calycopterus floribunda* Lamk., Ref. 102(130) $R^1 = \text{OH}$, Neocalycopteronone(131) $R^1 = \text{OMe}$, Neocalycopteronone-4-Me*Calycopterus floribunda*, Ref. 102(132) $R^1 = \text{OMe}$, Calyflorenone A(133) $R^1 = \text{OH}$, Calyflorenone B*Calycopterus floribunda*, Ref. 103(134) 6''-Demethoxynecalycopteronone
Calycopterus floribunda, abs. config., Ref. 104



(135) Calyflorenone D — *Calycopteris floribunda*,
abs. config., Ref. 104



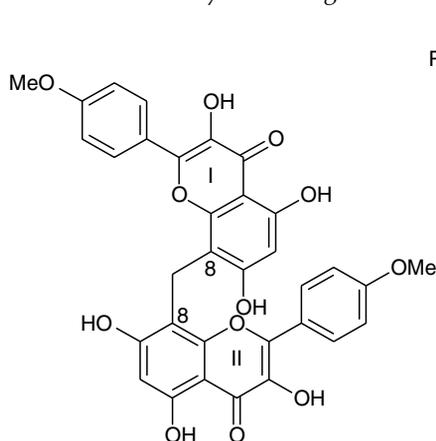
(136) $R^1 = R^2 = H$, 6'' β -OMe, Calyflorenone C
 (137) $R^1 = H$, $R^2 = Me$, 6'' α -OMe, 6-*epi*-Calyflorenone B
 (138) $R^1 = R^2 = H$, 6'' α -OMe, 6-*epi*-Calyflorenone C
Calycopteris floribunda, abs. config., Ref. 104



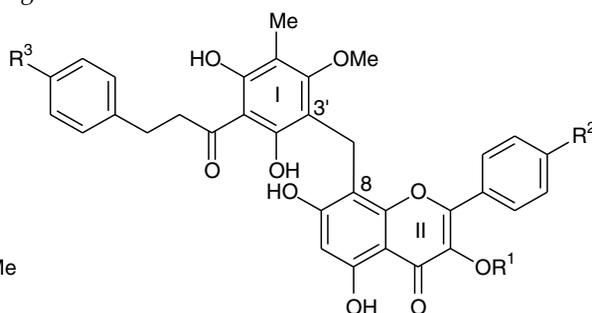
(139) *Dorstenia zenkeri*, rel. config., Ref. 105

17.3.1.28 Miscellaneous Biflavonoids

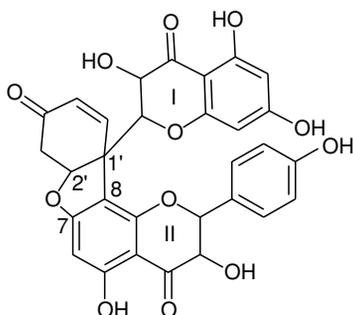
17.3.1.28.1 Methylene-Bridged Analogs



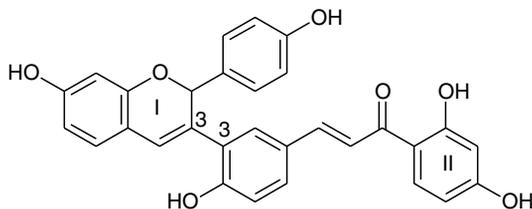
(140) Pentagrammetin — *Pentagramma triangularis*
spp. *triangularis*, Ref. 41



(141) $R^1 = R^3 = H$, $R^2 = OMe$, Trianguletin
— *Pentagramma triangularis* spp. *triangularis*, Ref. 41
 (142) $R^1 = Me$, $R^2 = R^3 = H$
— *Pentagramma triangularis*, Ref. 43
 (143) $R^1 = H$, $R^2 = OMe$, $R^3 = OH$
 (144) $R^1 = Me$, $R^2 = OMe$, $R^3 = OH$
 (145) $R^1 = Me$, $R^2 = H$, $R^3 = OH$ } — *Pentagramma triangularis*, Ref.

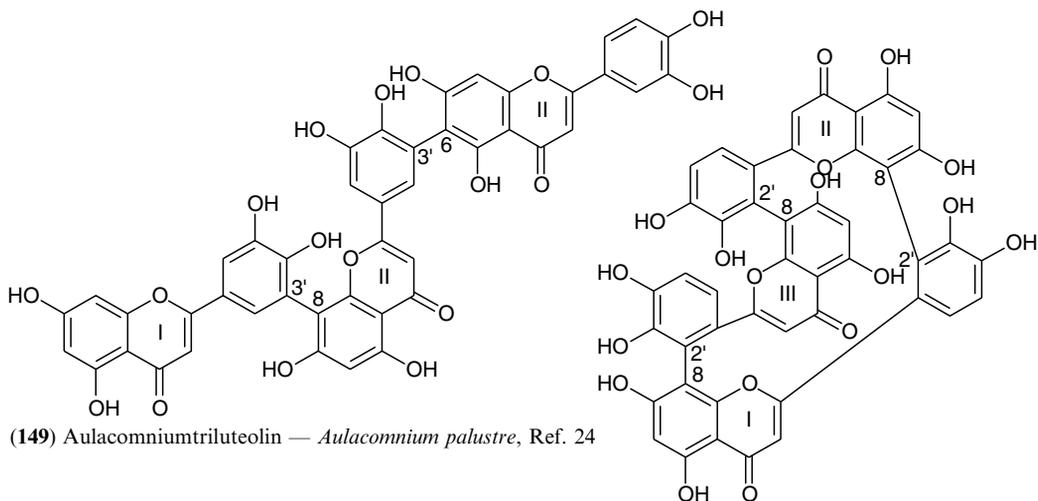
17.3.1.28.2 *Hypnumbiflavonoid A*

(146) Hypnumbiflavonoid A — *Hypnum cupressiforme*, rel. config., Ref. 34
See also the structures of two phenylacetic acid-substituted aromadendrin analogs from the same source.

17.3.1.28.3 *Bongosin*

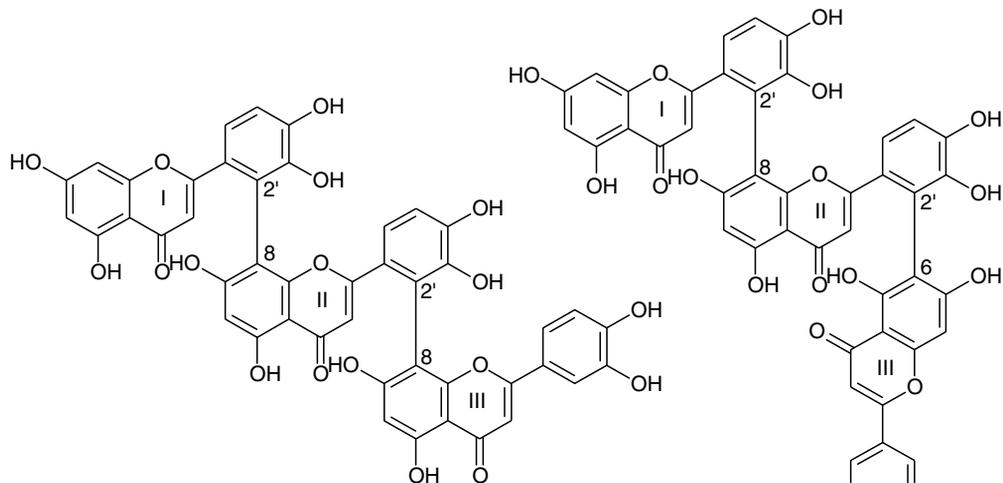
(147) Bongosin — *Lophira alata*, Ref. 59

17.3.2 TRIFLAVONOIDS



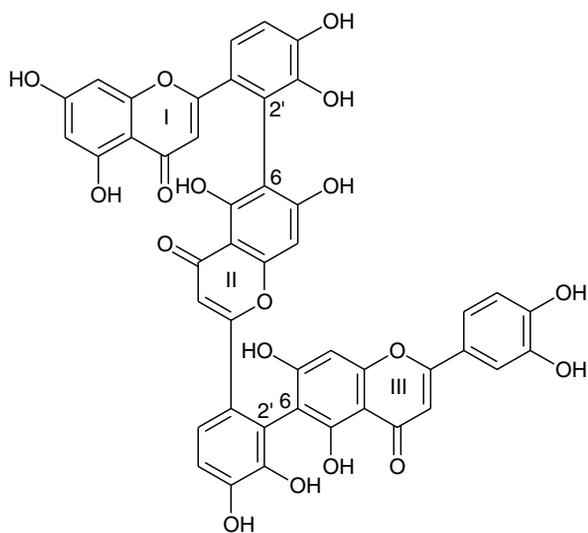
(149) Aulacomniumtriluteolin — *Aulacomnium palustre*, Ref. 24

(148) Cyclobartramiatriluteolin — *Bartramia stricta*, Ref. 17

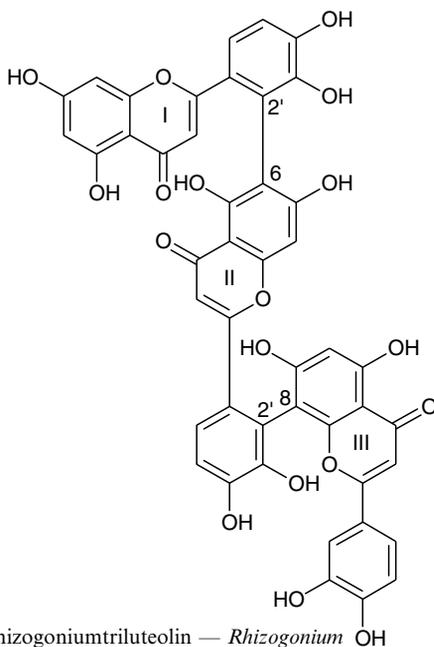


(150) Epibartamiatrilineol atropisomers
Bartramia pomiformis and *B. stricta*, Ref. 55

(151) Strictatrilineol atropisomers — *Bartramia pomiformis* and *B. stricta*, Ref. 55

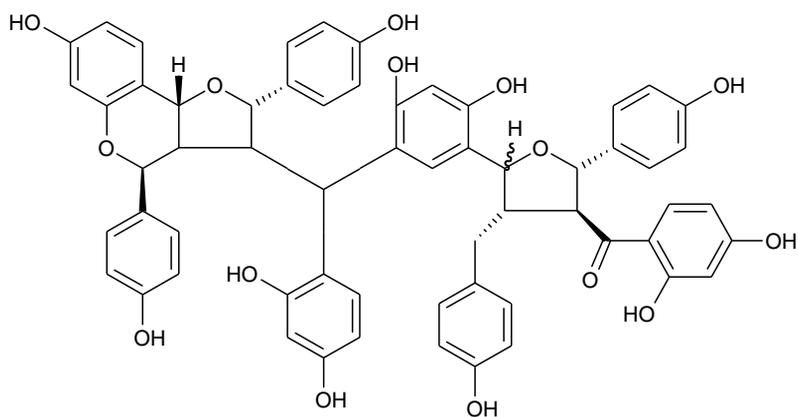
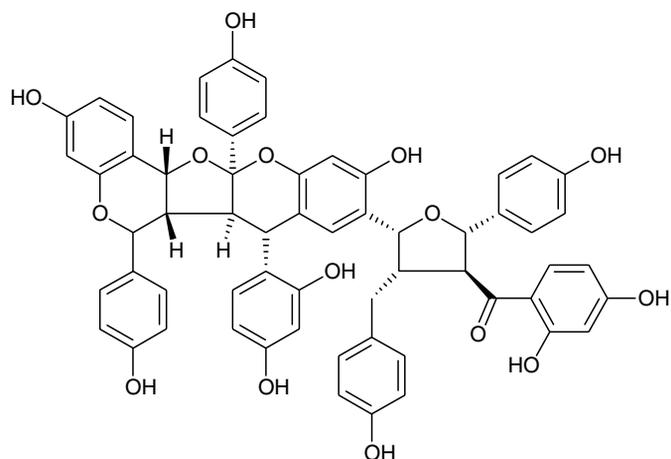
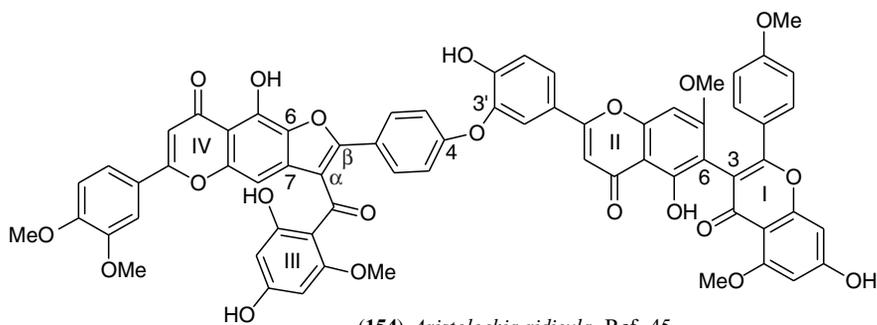


(152) Distichumtrilineol — *Rhizogonium distichum*, Ref. 86

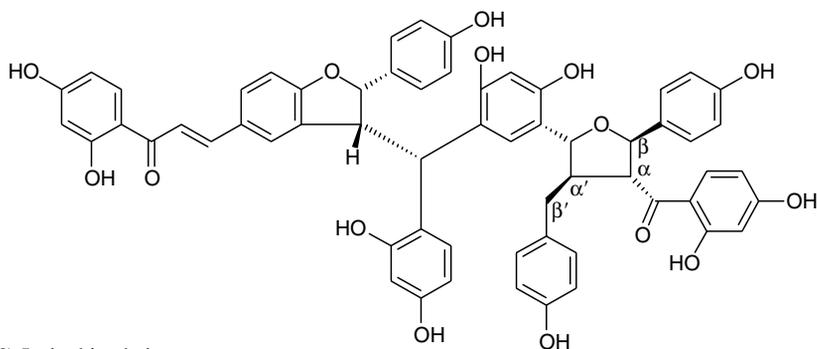


(153) Rhizogoniumtrilineol — *Rhizogonium distichum*, Ref. 86

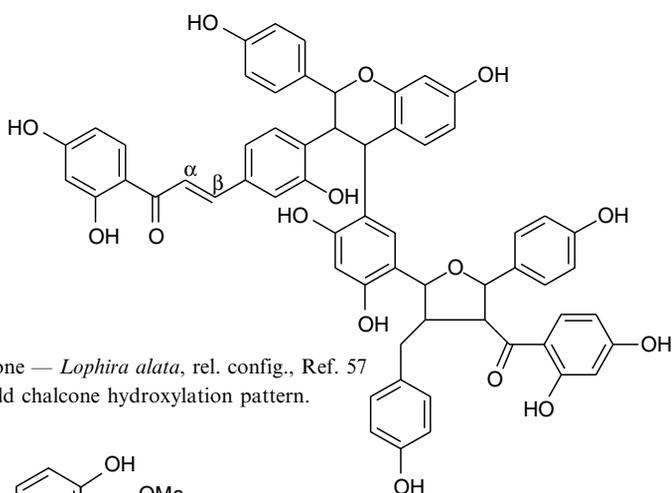
17.3.3 TETRAFLAVONOIDS



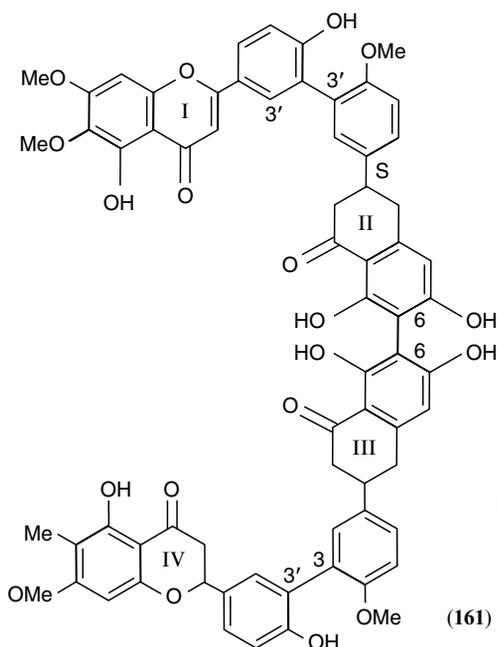
— *Lophira alata*, rel. config., Ref. 47



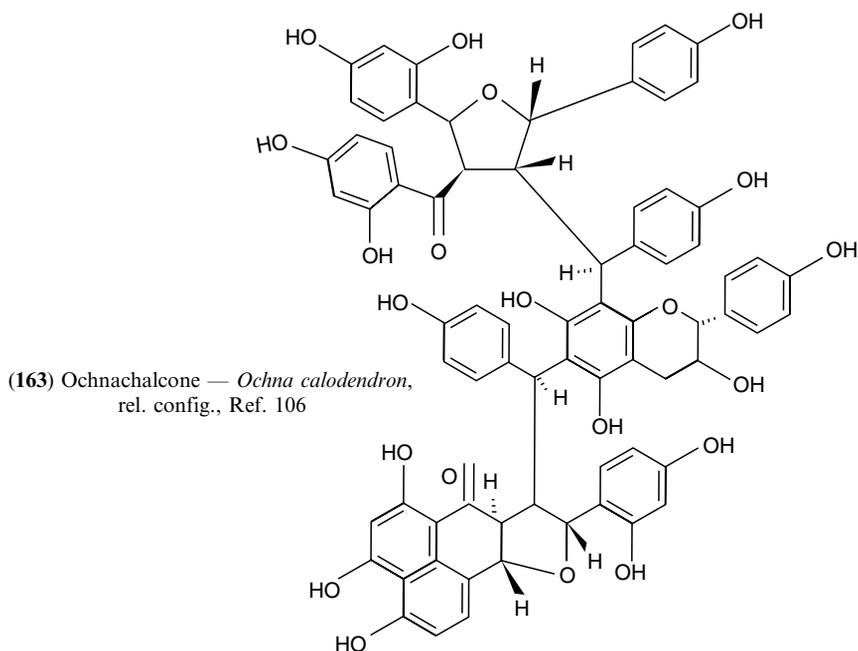
(158) Isolophirachalcone

(159) (C- α,α',β -enantiomer) Lophirachalcone} — *Lophira alata*, rel. config., Refs. 50, 51(160) Alatachalcone — *Lophira alata*, rel. config., Ref. 57

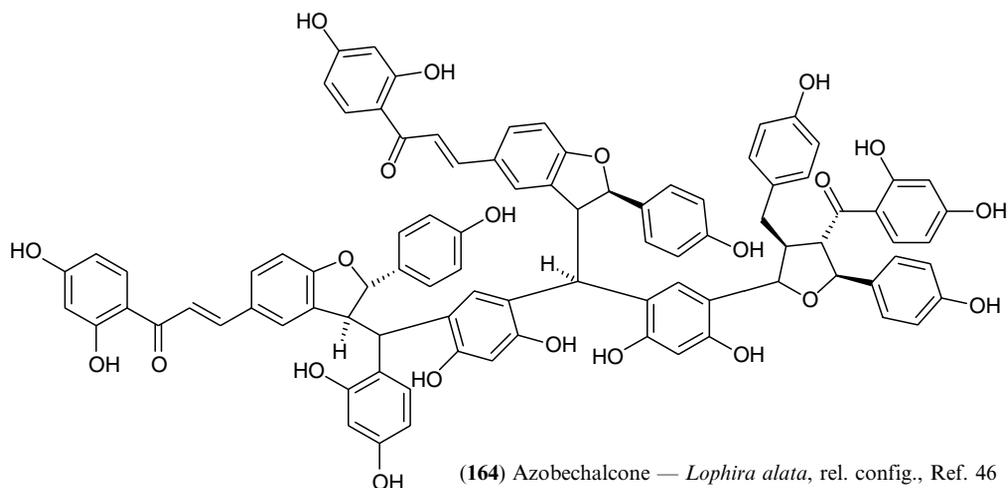
Note the odd chalcone hydroxylation pattern.

(161) Lophirochalcone — *Lophira lanceolata*, rel. config., Ref. 62(162) Taiwanhomoflavone C — *Cephalotaxus wilsoniana*, Ref. 60

17.3.4 PENTAFLAVONOIDS



17.3.5 HEXAFLAVONOIDS



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APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004

	Name	Trivial Name	Mass	Formula	Ref.
	ISOFLAVANES				
1	7,4'-Dihydroxyisoflavan	Equol	242.28	C ₁₅ H ₁₄ O ₃	2719
2	7,2',4'-Trihydroxyisoflavan	Demethylvestitol	258.28	C ₁₅ H ₁₄ O ₄	2720
3	2',4'-Dihydroxy-7-methoxyisoflavan	Neovestitol	272.30	C ₁₆ H ₁₆ O ₄	2723
4	7,2'-Dihydroxy-4'-methoxyisoflavan	Vestitol	272.30	C ₁₆ H ₁₆ O ₄	2721
5	7,4'-Dihydroxy-2'-methoxyisoflavan	Isovestitol	272.30	C ₁₆ H ₁₆ O ₄	2722
6	7,4'-Dihydroxy-3'-methoxyisoflavan		272.30	C ₁₆ H ₁₆ O ₄	F90
7	2',5'-Diketo-7-hydroxy-4'-methoxyisoflavan	Clauesquinone	286.29	C ₁₆ H ₁₄ O ₅	2758
8	2'-Hydroxy-7,4'-dimethoxyisoflavan	Isosativan	286.33	C ₁₇ H ₁₈ O ₄	2725
9	4'-Hydroxy-7,2'-dimethoxyisoflavan	Arvensan	286.33	C ₁₇ H ₁₈ O ₄	2726
10	7-Hydroxy-2',4'-dimethoxyisoflavan	Sativan	286.33	C ₁₇ H ₁₈ O ₄	2724
11	3,7,2'-Trihydroxy-4'-methoxyisoflavan		288.30	C ₁₆ H ₁₆ O ₅	F28
12	(3 <i>R</i>)-7,2',3'-Trihydroxy-4'-methoxyisoflavan	Arizonicanol A	288.30	C ₁₆ H ₁₆ O ₅	F255
13	7,2',4'-Trihydroxy-5'-methoxyisoflavan	Lespedezol GI	288.30	C ₁₆ H ₁₆ O ₅	F171
14	7-Hydroxy-2'-methoxy-4',5'-methylenedioxyisoflavan	Astraciceran	300.31	C ₁₇ H ₁₆ O ₅	2727
15	2',4'-Dihydroxy-5,7-dimethoxyisoflavan	Lotisoflavan	302.33	C ₁₇ H ₁₈ O ₅	2732
16	(3 <i>R</i>)-7,2'-Dihydroxy-4',5'-dimethoxyisoflavan	Methoxyvestitol	302.33	C ₁₇ H ₁₈ O ₅	3059
17	(3 <i>R</i>)-8,2'-Dihydroxy-7,4'-dimethoxyisoflavan		302.33	C ₁₇ H ₁₈ O ₅	F255
18	(3 <i>S</i>)-7,2'-Dihydroxy-3',4'-dimethoxyisoflavan	Isomucronulatol	302.33	C ₁₇ H ₁₈ O ₅	2728
19	(3 <i>S</i>)-7,2'-Dihydroxy-8,4'-dimethoxyisoflavan	8-Methoxyvestitol	302.33	C ₁₇ H ₁₈ O ₅	F63
20	7,2'-Dihydroxy-5,4'-dimethoxyisoflavan	5-Methoxyvestitol	302.33	C ₁₇ H ₁₈ O ₅	2731
21	7,3'-Dihydroxy-2',4'-dimethoxyisoflavan	Mucronulatol	302.33	C ₁₇ H ₁₈ O ₅	2729
22	7,4'-Dihydroxy-2',3'-dimethoxyisoflavan	Sphaerosin	302.33	C ₁₇ H ₁₈ O ₅	2730
23	(3 <i>R</i>)-7,8,2',3'-Tetrahydroxy-4'-methoxyisoflavan	(3 <i>R</i>)-8,3'-Dihydroxyvestitol	304.30	C ₁₆ H ₁₆ O ₆	3062
24	(3 <i>R</i>)-2',5'-Diketo-3-hydroxy-7,8-dimethoxyisoflavan	Astragalquinone	316.31	C ₁₇ H ₁₆ O ₆	F63
25	2',5'-Diketo-7-hydroxy-3',4'-dimethoxyisoflavan	Pendulone	316.31	C ₁₇ H ₁₆ O ₆	2759
26	(3 <i>R</i>)-2',5'-Diketo-7-hydroxy-8,4'-dimethoxyisoflavan	Mucroquinone	316.31	C ₁₇ H ₁₆ O ₆	2760a
27	(3 <i>S</i>)-2',5'-Diketo-7-hydroxy-8,4'-dimethoxyisoflavan	Mucroquinone	316.31	C ₁₇ H ₁₆ O ₆	2760b
28	(3 <i>R</i>)-2'-Hydroxy-7,3',4'-trimethoxyisoflavan	7- <i>O</i> -Methylisomucronulatol	316.35	C ₁₈ H ₂₀ O ₅	F261

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
29	(3 <i>R</i>)-7-Hydroxy-2',3',4'-trimethoxyisoflavan		316.35	C18H20O5	F257
30	(3 <i>R</i> ,4 <i>S</i>)-4,2',3',4'-Tetrahydroxy-6,7-methylenedioxyisoflavan	Bolusanthol A	318.28	C16H14O7	F30
31	6,7,3'-Trihydroxy-2',4'-dimethoxyisoflavan	Bryaflavan	318.33	C17H18O6	2733
32	7,8,3'-Trihydroxy-2',4'-dimethoxyisoflavan	8-Demetylduartin	318.33	C17H18O6	2734
33	2',4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan	Glabridin	324.37	C20H20O4	2740
34	(3 <i>R</i>)-2',4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavan	Eryarin C	324.37	C20H20O4	F282
35	(3 <i>R</i>)-7,4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':2,3]isoflavan	Erythbidin A	324.37	C20H20O4	F273
36	7,2'-Dihydroxy-5''-(1-methylethenyl)-4'',5''-dihydrofuran[2'',3'':4'',5'']isoflavan	Crotmarine	324.37	C20H20O4	3061
37	7,2'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':4'',3'']isoflavan	Phaseollinisoflavan	324.37	C20H20O4	2739
38	3-Hydroxy-2',4'-dimethoxyfuran[2'',3'':8,7]isoflavan	Smiranin	326.34	C19H18O5	F220
39	(3 <i>R</i>)-2',4'-Dihydroxy-5'',4'',4'',4''-trimethyl-4'',5''-dihydrofuran[2'',3'':7,6]isoflavan	Cyclomilanol	326.39	C20H22O4	3064
40	(3 <i>R</i>)-7,2',4'-Trihydroxy-5''-(1,1-dimethyl-2-propenyl)isoflavan	Manuifolin K	326.39	C20H22O4	F347
41	(3 <i>R</i>)-7,2',4'-Trihydroxy-6-prenylisoflavan	Manuifolin H	326.39	C20H22O4	F347
42	7,2',4'-Trihydroxy-6-(1,2-dimethyl-2-propenyl)isoflavan	Neomilanol	326.39	C20H22O4	F204
43	(3 <i>R</i>)-2',5'-Diketo-7,3',4'-trimethoxyisoflavan	Colutequinone	330.34	C18H18O6	F82
44	(3 <i>R</i>)-2',5'-Diketo-7,4',6'-trimethoxyisoflavan	Colutequinone B	330.34	C18H18O6	F83
45	8-Carboxyaldehyde-7,3'-dihydroxy-2',4'-dimethoxyisoflavan	Sphaerosin s3	330.34	C18H18O6	2729
46	(3 <i>R</i>)-2',5'-Dihydroxy-7,3',4'-trimethoxyisoflavan	Colutehydroquinone	332.36	C18H20O6	F82
47	(3 <i>R</i>)-3',5'-Dihydroxy-7,2',4'-trimethoxyisoflavan	Coluteol	332.36	C18H20O6	F83
48	(3 <i>R</i>)-7,2'-Dihydroxy-8,3',4'-trimethoxyisoflavan	Isoduartin	332.36	C18H20O6	3060
49	(3 <i>R</i>)-7,5'-Dihydroxy-2',3',4'-trimethoxyisoflavan		332.36	C18H20O6	F257
50	7,3'-Dihydroxy-8,2',4'-trimethoxyisoflavan	Duartin	332.36	C18H20O6	2736
51	7,4'-Dihydroxy-2',3',6'-trimethoxyisoflavan	Lonchocarpan	332.36	C18H20O6	2735
52	2'-Hydroxy-4'-methoxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavan	Gancaonin X	338.40	C21H22O4	F77
53	2'-Hydroxy-4'-methoxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan	4- <i>O</i> -Methylglabridin	338.40	C21H22O4	2742
54	(6 <i>aR</i> ,11 <i>aR</i>)-9-Hydroxy-3-methoxy-10-prenylpteroocarpan	2'- <i>O</i> -Methylphaseollinisoflavan	338.40	C21H22O4	F168
55	7-Hydroxy-2'-methoxy-6'',6''-dimethylpyrano[2'',3'':4'',3'']isoflavan	Gancaonin Y	338.40	C21H22O4	2741
56	7-Hydroxy-4'-methoxy-6'',6''-dimethylpyrano[2'',3'':2'',3'']isoflavan	Gancaonin Z	338.40	C21H22O4	F77
57	(3 <i>R</i>)-7,2'-Dihydroxy-4'-methoxy-3'-prenylisoflavan		340.42	C21H24O4	F77
58	(3 <i>R</i>)-7,2'-Dihydroxy-4'-methoxy-6-(1,1-dimethyl-2-propenyl)isoflavan	(<i>R</i>)-Isomilanol B	340.42	C21H24O4	F212

59	(3R)-7,4'-Dihydroxy-2'-methoxy-6-(1,1-dimethyl-2-propenyl)isoflavan				
60	(3S)-7,2'-Dihydroxy-4'-methoxy-8-prenylisoflavan				
61	7,4'-Dihydroxy-2'-methoxy-3'-prenylisoflavan				
62	7,2',4'-Trihydroxy-6-(1-hydroxymethyl-1-methyl-2-propenyl)isoflavan				
63	2',5'-Diketo-7-hydroxy-8,3',4'-trimethoxyisoflavan				
64	3',6'-Diketo-7-hydroxy-8,2',4'-trimethoxyisoflavan				
65	6,8,2'-Trihydroxy-7,3',4'-trimethoxyisoflavan				
66	2'-Hydroxy-4',5'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan				
67	2',3'-Dihydroxy-4'-methoxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavan				
68	2',3'-Dihydroxy-4'-methoxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan				
69	2',4'-Dihydroxy-3'-methoxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan				
70	2',4'-Dihydroxy-5-methoxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavan				
71	7,4'-Dihydroxy-5-methoxy-6'',6''-dimethylpyrano[2'',3'':2,3]isoflavan				
72	(3R)-7,2',4'-Trihydroxy-5-methoxy-6-prenylisoflavan				
73	7,2',3'-Trihydroxy-4'-methoxy-5'-(1,1-dimethyl-2-propenyl)isoflavan				
74	7,3',4'-Trihydroxy-2'-methoxy-5'-(1,1-dimethyl-2-propenyl)isoflavan				
75	2',5'-Diketo-6,7,3',4'-tetramethoxyisoflavan				
76	(3S)-7,3'-Dihydroxy-8,2',4',5'-tetramethoxyisoflavan				
77	6,2'-Dihydroxy-7,8,3',4'-tetramethoxyisoflavan				
78	6,2'-Dihydroxy-4',5'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan				
79	(3R)-3'-Hydroxy-2',4'-dimethoxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavan				
80	4'-Hydroxy-2',3'-dimethoxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavan				
81	(3R)-2',4'-Dihydroxy-5,7-dimethoxy-6-prenylisoflavan				
82	(3R)-7,2'-Dihydroxy-5,4'-dimethoxy-3'-prenylisoflavan				
83	7,3'-Dihydroxy-2',4'-dimethoxy-5'-(1,1-dimethyl-2-propenyl)isoflavan				
84	7,3'-Dihydroxy-2',4'-dimethoxy-8-prenylisoflavan				
85	(3S)-7-Hydroxy-8,2',3',4',5'-pentamethoxyisoflavan				
86	2',5'-Diketo-6-hydroxy-7,8,3',4'-tetramethoxyisoflavan				
87	(3S)-2',5'-Diketo-7-hydroxy-6,8,3',4'-tetramethoxyisoflavan				
88	(3S)-2',5'-Diketo-8-hydroxy-6,7,3',4'-tetramethoxyisoflavan				
89	(6aR,11aR)-9-Acetoxy-3-methoxy-2-prenylpterocarpin				
90	(3R)-8-Carboxyaldehyde-7,4'-dihydroxy-5-methoxy-6'',6''-dimethylpyrano[2'',3'':2,3]isoflavan				
91	(3R)-8-Carboxyaldehyde-7,2',4'-trihydroxy-5-methoxy-3'-prenylisoflavan				
92	2',5'-Diketo-6,7,8,3',4'-pentamethoxyisoflavan				
	Millinol B	340.42	C21H24O4	3063	
	4'-O-Methylpreglabridin	340.42	C21H24O4	3065	
	2'-O-Methylphaseollidinisoflavan	340.42	C21H24O4	2743	
	Millinolol	342.39	C20H22O5	F204	
	Amorphaquinone	346.34	C18H18O7	2761	
	Laurentiquinone	346.34	C18H18O7	F117	
	Machaerol C	348.35	C18H20O7	2737	
	Letocin	352.38	C21H20O5	2744	
	Arizonicanol B	354.40	C21H22O5	F289	
	3'-Hydroxy-4'-O-methylglabridin	354.40	C21H22O5	F121	
	3'-Methoxyglabridin	354.40	C21H22O5	2746	
	Neorauflavane	354.40	C21H22O5	2747	
	Gancaanol C	354.40	C21H22O5	F70	
	Glyasperin C	356.41	C21H24O5	F350	
	Secundiflorol G	356.41	C21H24O5	F289	
	a,a-Dimethylallyl-cyclolobin	356.41	C21H24O5	2748	
	Abruquinone A	360.37	C19H20O7	2762	
		362.38	C29H22O7	F10	
	Machaerol B	362.38	C19H22O7	2738	
	Letocinol	368.39	C21H20O6	2745	
	Glyasperin H	368.43	C22H24O5	F151	
	Sphaerosinin	368.43	C22H24O5	2749	
	Glyasperin D	370.45	C22H26O5	F350	
	Kanzanol R	370.45	C22H26O5	F76	
	Unanisoflavan	370.45	C22H26O5	2750	
	Sphaerosinol	370.45	C22H26O5	F151	
		376.40	C20H24O7	F10	
	Abruquinone C	376.37	C19H20O8	2763	
	Abruquinone D	376.37	C19H20O8	F133	
	Abruquinone F	376.37	C19H20O8	F133	
		380.43	C23H24O5	F168	
	Kanzanol O	382.41	C22H22O6	F76	
	Kanzanol N	384.42	C22H24O6	F76	
	Abruquinone B	390.39	C20H22O8	2764	

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
93	2'-Hydroxy-(2'',3'',7,8),(2'',3'',4',3')-bis(6,6-dimethylpyrano)isoflavan	Hispaglabridin B	390.48	C ₂₅ H ₂₆ O ₄	2751
94	4'-Hydroxy-(2'',3'',7,6),(2'',3'',2',3')-bis(6,6-dimethylpyrano)isoflavan	Glymiflanin J	390.48	C ₂₅ H ₂₆ O ₄	F75
95	4'-Hydroxy-(2'',3'',7,8),(2'',3'',2',3')-bis(6,6-dimethylpyrano)isoflavan	Glymiflanin K	390.48	C ₂₅ H ₂₆ O ₄	F75
96	2',4'-Dihydroxy-3'-prenyl-6'',6''-dimethylpyranol[2'',3'',7,8]isoflavan	Hispaglabridin A	392.50	C ₂₅ H ₂₈ O ₄	2752
97	2',4'-Dihydroxy-8'-prenyl-6'',6''-dimethylpyranol[2'',3'',7,6]isoflavan	Eryzerin D	392.50	C ₂₅ H ₂₈ O ₄	F277
98	(3R)-7',4'-Dihydroxy-6'-prenyl-6'',6''-dimethylpyranol[2'',3'',2',3']isoflavan	Glymiflanin I	392.50	C ₂₅ H ₂₈ O ₄	F75
99	7',2'-Dihydroxy-8'-prenyl-6'',6''-dimethylpyranol[2'',3'',4',3']isoflavan	Manuifolin D	392.50	C ₂₅ H ₂₈ O ₄	F121
100	(3R)-7',2'-Dihydroxy-5'-(1,1-dimethyl-2-propenyl)-4'-prenylisoflavan	Manuifolin F	394.50	C ₂₅ H ₃₀ O ₄	F346
101	(3R)-7',2',4'-Trihydroxy-5'-(1-isopropylethenyl)-8'-prenylisoflavan	Manuifolin E	394.50	C ₂₅ H ₃₀ O ₄	F346
102	(3R)-7',2',4'-Trihydroxy-6,5'-bis(1,1-dimethyl-2-propenyl)isoflavan	Kanzonol X	394.50	C ₂₅ H ₃₀ O ₄	F73
103	(3R)-7',2',4'-Trihydroxy-8,3'-diprenylisoflavan	Kanzonol M	398.46	C ₂₃ H ₂₆ O ₆	F76
104	(3R)-8-Carboxyaldehyde-7,2'-dihydroxy-5,4'-dimethoxy-3'-prenylisoflavan	Heminitidulan	406.51	C ₂₆ H ₃₀ O ₄	2753
105	2'-Hydroxy-4'-methoxy-6''-methyl-6''-(4-methyl-3-pentenyl)pyranol[2'',3'',7,8]isoflavan	Eryzerin C	406.51	C ₂₆ H ₃₀ O ₄	F277
106	(3R)-7',2',4'-Trihydroxy-6,8-diprenylisoflavan	Tenuifolin A	408.53	C ₂₆ H ₃₂ O ₄	F348
107	(3R)-7',4'-Dihydroxy-2'-methoxy-5'-(1,1-dimethyl-2-propenyl)-8'-prenylisoflavan	Manuifolin G	412.52	C ₂₅ H ₃₂ O ₅	F347
108	(3R)-7',2',4'-Trihydroxy-5'-(1,1-dimethyl-2-propenyl)-8-(3-hydroxy-3-methylbutyl)isoflavan	Abruinone E	420.41	C ₂₁ H ₂₄ O ₉	F133
109	(3S)-2',5'-Diketo-6,7,8,3',4',6'-hexamethoxyisoflavan	Nitidulan	420.50	C ₂₆ H ₂₈ O ₅	2754
110	2'-Hydroxy-5',6'-methylenedioxy-6''-methyl-6''-(4-methyl-3-pentenyl)pyranol[2'',3'',7,8]isoflavan	Nitidulan	422.51	C ₂₆ H ₃₀ O ₅	2755
111	2',3'-Dihydroxy-4'-methoxy-6''-methyl-6''-(4-methyl-3-pentenyl)pyranol[2'',3'',7,8]isoflavan	Kanzonol J	422.51	C ₂₆ H ₃₀ O ₅	F74
112	(3R)-4'-Hydroxy-5-methoxy-6'',6''-6''-tetramethylpyranol[2'',3'',2',3',4',5'',-dihydropyrano[2'',3'',7,6]isoflavan	Kanzonol H	424.54	C ₂₆ H ₃₂ O ₅	F74
113	(3R)-2',4'-Dihydroxy-5-methoxy-6'',6''-dimethyl-3'-prenyl-4'',5''-dihydropyranol[2'',3'',7,6]isoflavan	Licoricidin	424.54	C ₂₆ H ₃₂ O ₅	2756
114	5,2',4'-Trihydroxy-7-methoxy-6,3'-diprenylisoflavan	Kanzonol I	424.54	C ₂₆ H ₃₂ O ₅	F168
115	7,2',4'-Trihydroxy-5-methoxy-6,3'-diprenylisoflavan	7-O-Methyllicoricidin	436.54	C ₂₇ H ₃₂ O ₅	F74
116	(3R)-4'-Hydroxy-5,7-dimethoxy-6'-prenyl-6'',6''-dimethylpyranol[2'',3'',2',3']isoflavan	Manuifolin Q	438.57	C ₂₇ H ₃₄ O ₅	3066
117	(+)-2',4'-Dihydroxy-5,7-dimethoxy-6,3-diprenylisoflavan		448.51	C ₂₇ H ₂₈ O ₆	F349
118	(3R,4R)-7,2'-Dihydroxy-4'-methoxy-4-(2,4-dihydroxy-5'-(1,1-dimethyl-2-propenyl)phenyl)isoflavan				
119	2'-Hydroxy-4'-methoxy-4''-phenylpyranol[2'',3'',7,6]-6''-ylidene-5''-hydroxy-2''-methoxy-2''',5''-cyclohexadien-1''',one-isoflavan	Neocandemonin	522.54	C ₃₂ H ₂₆ O ₇	F21

Appendix — Checklist for Isoflavonoids

Øyvind M. Andersen

The following checklist of isoflavonoids contains compounds reported in the literature as natural products to the end of 2004. Compounds published before 1991 are referenced to numbered entries in Volume 2 of the *Handbook of Natural Flavonoids* (J.B. Harborne and H. Baxter), John Wiley & Sons, Chichester, 1999, using a number consisting of four digits. Compounds published in the period 1991–2004 are referenced with numbers having F as prefix before the number of the publication found in the reference list. The various isoflavonoid classes are shown in Figure A1.

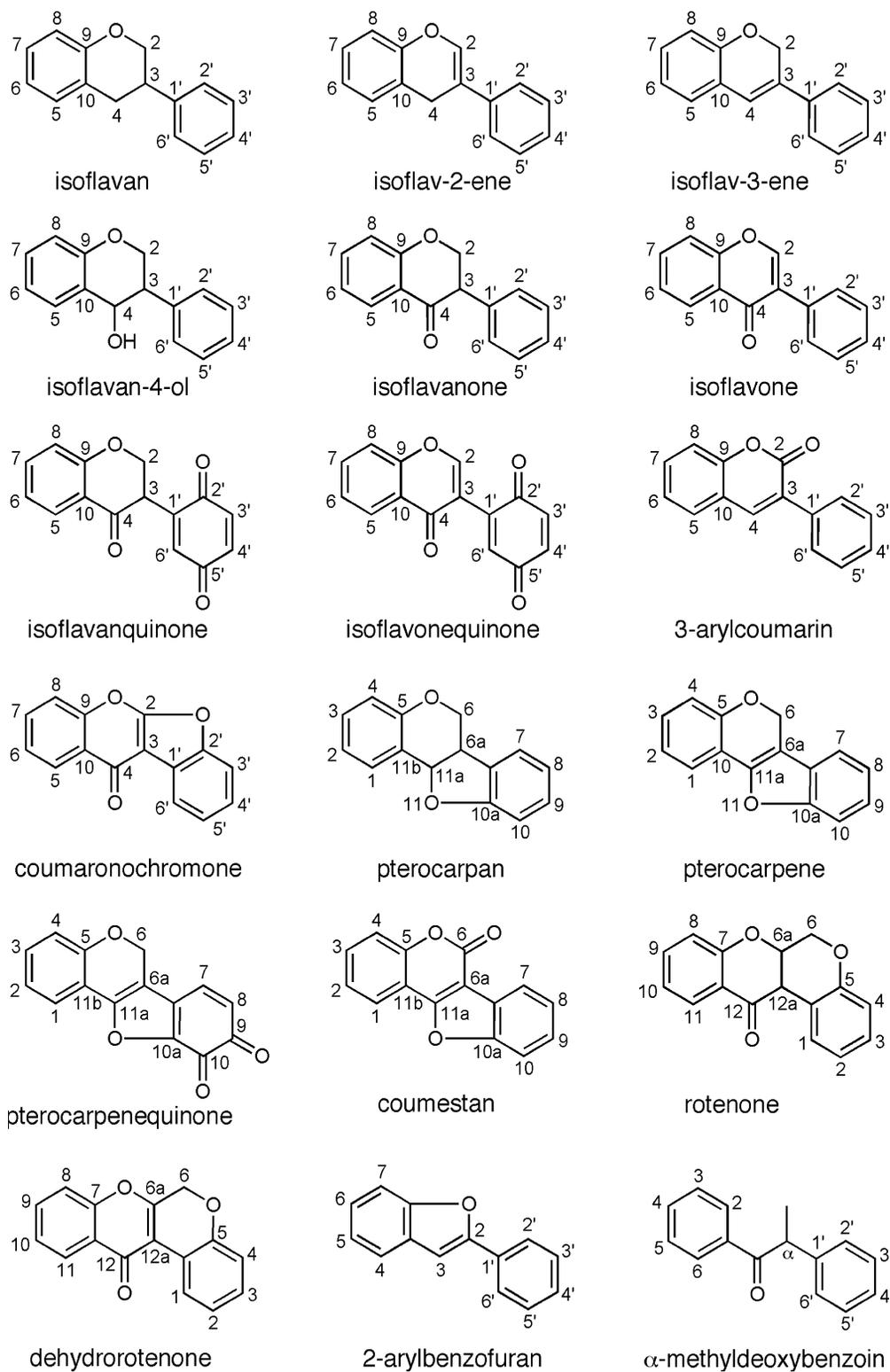


FIGURE A1 Isoflavonoid classes.

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
	ISOFLAVAN-4-OLS				
148	5-Hydroxy-8-methoxy-6,7-methylenedioxyisoflavan-4-ol	Laphatinol	316.31	C17H16O6	3069
149	2-Methoxy-4',5'-methylenedioxyfuranol[2'',3'':7,6]isoflavan-4-ol	Ambanol	340.33	C19H16O6	2757
	ISOFLAVANONES				
150	7,4'-Dihydroxyisoflavanone	Dihydrodaidzein	256.26	C15H12O4	2514
151	7-Hydroxy-4'-methoxyisoflavanone	Dihydroformononetin	270.29	C16H14O4	2515
152	7,2',4'-Trihydroxyisoflavanone	2'-Hydroxydihydrodaidzein	272.26	C15H12O5	2516
153	5,7-Dihydroxy-4'-methoxyisoflavanone	Dihydrobiochanin A	286.29	C16H14O5	2536
154	7,2'-Dihydroxy-4'-methoxyisoflavanone	Vestitone	286.29	C16H14O5	2517
155	7,3'-Dihydroxy-4'-methoxyisoflavanone	3'-Hydroxydihydroformononetin	286.29	C16H14O5	2518
156	5,7,2',4'-Tetrahydroxy-4'-isoflavanone	Dalbergoidin	288.26	C15H12O6	2537
157	7,2'-Dihydroxy-4',5'-methylenedioxyisoflavanone	Sophorol	300.27	C16H12O6	2521
158	2'-Hydroxy-7,4'-dimethoxyisoflavanone	Isosativanone	300.31	C17H16O5	2522
159	7-Hydroxy-2',4'-dimethoxyisoflavanone	Sativanone	300.31	C17H16O5	2519
160	7-Hydroxy-3',4'-dimethoxyisoflavanone	3'-Methoxydihydroformononetin	300.31	C17H16O5	2520
161	3,7,2'-Trihydroxy-4'-methoxyisoflavanone		302.29	C16H14O6	F132
162	(3R)-4'-Methoxy-3,7,2'-trihydroxyisoflavanone		302.29	C16H14O6	F35
163	(3R)-7,2',3'-Trihydroxy-4'-methoxyisoflavanone		302.29	C16H14O6	2986
164	5,2',4'-Trihydroxy-7-methoxyisoflavanone	Dihydrocajanin	302.29	C16H14O6	F192
165	5,7,2'-Trihydroxy-4'-methoxyisoflavanone	Ferreirin	302.29	C16H14O6	2538
166	5,7,3'-Trihydroxy-4'-methoxyisoflavanone	Kenusanone G	302.29	C16H14O6	F100
167	5,7,4'-Trihydroxy-2'-methoxyisoflavanone	Isoferreirin	302.29	C16H14O6	2539
168	7,2',4'-Trihydroxy-5'-methoxyisoflavanone	Lespedol D	302.29	C16H14O6	F171
169	7-Hydroxy-2'-methoxy-4',5'-methylenedioxyisoflavanone	Onogenin	314.29	C17H14O6	2523
170	5,2',4'-Trihydroxy-7-methoxy-6-methylisoflavanone	Ougenin	316.31	C17H16O6	2543
171	5,4'-Dihydroxy-5,2'-dimethoxyisoflavanone		316.31	C17H16O6	2980
172	5,4'-Dihydroxy-7,2'-dimethoxyisoflavanone	Cajanol	316.31	C17H16O6	2541
173	5,7-Dihydroxy-2',4'-dimethoxyisoflavanone	Homoferreirin	316.31	C17H16O6	2540
174	7,3'-Dihydroxy-2',4'-dimethoxyisoflavanone	Violanone	316.31	C17H16O6	2524

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
209	7,2',4'-Trihydroxy-8-(4-hydroxy-3-dimethyl-2-butenyl)isoflavanone	5-Deoxykievitol	356.37	C ₂₀ H ₂₀ O ₆	2991
210	(S)-5,7,2',4'-Tetrahydroxy-3'-prenylisoflavanone	Dihydrolicoisoflavone	356.37	C ₂₀ H ₂₀ O ₆	F59
211	7,2',4'-Trihydroxy-8-(3-hydroxy-3-methylbutyl)isoflavanone	5-Deoxykievitone hydrate	358.39	C ₂₀ H ₂₂ O ₆	2982
212	5,7,3'-Trihydroxy-6,4',5'-trimethoxyisoflavanone	2,3-Dihydroirigenin	362.33	C ₁₈ H ₁₈ O ₈	F112
213	5,4'-Dihydroxy-2'-methoxy-5''-(1-methylphenyl)-4'',5''-dihydrofurano-(2'',3'',7,6)-isoflavanone	Uncinaneone C	368.39	C ₂₁ H ₂₀ O ₆	F297
214	5,7-Dihydroxy-2'-methoxy-6'',6''-dimethylpyranol[2'',3'',4',3]isoflavanone	Isosaphoronol	368.39	C ₂₁ H ₂₀ O ₆	2547
215	7,2'-Dihydroxy-5-methoxy-6'',6''-dimethylpyranol[2'',3'',4',3]isoflavanone	Glyasperin M	368.39	C ₂₁ H ₂₀ O ₆	F72
216	7,3'-Dihydroxy-5-methoxy-6'',6''-dimethylpyranol[2'',3'',4',5]isoflavanone	Glycyrrhisoflavanone	368.39	C ₂₁ H ₂₀ O ₆	2990
217	(3R)-7,2',3'-Trihydroxy-4'-methoxy-5''-(1,1-dimethyl-2-propenyl)isoflavanone	3'-O-Demethylpervilleanone	370.40	C ₂₁ H ₂₂ O ₆	F80
218	(3R)-7,2',3'-Trihydroxy-4'-methoxy-5'-prenylisoflavanone		370.40	C ₂₁ H ₂₂ O ₆	F303
219	(3S)-5,7,3'-Trihydroxy-4'-methoxy-5'-prenylisoflavanone	Glyasperin B	370.40	C ₂₁ H ₂₂ O ₆	2989
220	5,2',4'-Trihydroxy-7-methoxy-6-prenylisoflavanone	Vogelin A	370.40	C ₂₁ H ₂₂ O ₆	F350
221	5,7,2'-Trihydroxy-4'-methoxy-5'-prenylisoflavanone		370.40	C ₂₁ H ₂₂ O ₆	F14
222	5,7,2'-Trihydroxy-4'-methoxy-6-prenylisoflavanone	Diphysolidone	370.40	C ₂₁ H ₂₂ O ₆	2984
223	5,7,2'-Trihydroxy-4'-methoxy-8-prenylisoflavanone	4-O-Methylkievitone	370.40	C ₂₁ H ₂₂ O ₆	2985
224	5,7,3'-Trihydroxy-4'-methoxy-2'-prenylisoflavanone	Arizoneanol D	370.40	C ₂₁ H ₂₂ O ₆	F289
225	5,7,4'-Trihydroxy-2'-methoxy-3'-prenylisoflavanone	Sophoraisoflavanone A	370.40	C ₂₁ H ₂₂ O ₆	2548
226	5,7,4'-Trihydroxy-2'-methoxy-5''-(1,1-dimethyl-2-propenyl)isoflavanone	Fraserinone A	370.40	C ₂₁ H ₂₂ O ₆	F102
227	5,7,4'-Trihydroxy-2'-methoxy-5'-prenylisoflavanone	Erypoeigin C	370.40	C ₂₁ H ₂₂ O ₆	F278
228	5,7,2',4'-Tetrahydroxy-8-(4-hydroxy-3-dimethyl-2-butenyl)isoflavanone	Kievitol	372.37	C ₂₀ H ₂₀ O ₇	2993
229	5,7,3',4'-Tetrahydroxy-5''-(2-epoxy-3-methylbutyl)isoflavanone	Erypoeigin G	372.37	C ₂₀ H ₂₀ O ₇	F28
230	5-Hydroxy-7,2'-dimethoxy-6'',6''-dimethylpyranol[2'',3'',4',5]isoflavanone	Sophoronol	382.41	C ₂₂ H ₂₂ O ₆	F272
231	3,5,7'-Trihydroxy-2'-methoxy-6'',6''-dimethylpyranol[2'',3'',4',3]isoflavanone		384.39	C ₂₁ H ₂₀ O ₇	2999
232	(3R)-7,2'-Dihydroxy-3',4'-dimethoxy-5''-(1,1-dimethyl-2-propenyl)isoflavanone	Pervilleanone	384.42	C ₂₂ H ₂₄ O ₆	F80
233	5,4'-Dihydroxy-7,2'-dimethoxy-5''-(1,1-dimethyl-2-propenyl)isoflavanone	Echinoisophoranone	384.42	C ₂₂ H ₂₄ O ₆	2988
234	5,4'-Dihydroxy-7,2'-dimethoxy-5'-prenylisoflavanone	Erypoeigin D	384.42	C ₂₂ H ₂₄ O ₆	F278
235	7,4'-Dihydroxy-2',5'-dimethoxy-6-prenylisoflavanone	Sigmoidin J	384.42	C ₂₂ H ₂₄ O ₆	F181
236	7,4'-Dihydroxy-2',5'-dimethoxy-8-prenylisoflavanone	Eryvarin N	384.42	C ₂₂ H ₂₄ O ₆	F280
237	(R)-5,2',4'-Trihydroxy-7-methoxy-6-methyl-8-prenylisoflavanone	Desmodianone B	384.42	C ₂₂ H ₂₄ O ₆	F51
238	(S)-5,2'-Dihydroxy-7,4'-dimethoxy-6-prenylisoflavanone	Glyasperin K	384.42	C ₂₂ H ₂₄ O ₆	F72
239	3,5,7,4'-Tetrahydroxy-2'-methoxy-3'-prenylisoflavanone	Kenusanone F	386.40	C ₂₁ H ₂₂ O ₇	F100

240	3,7,2,3'-Tetrahydroxy-4'-methoxy-5'-(1,1-dimethyl-2-propenyl)isoflavanone	Secundifloran	386.40	C21H22O7	2535
241	(3 <i>R</i>)-5,7,2,3'-Tetrahydroxy-4'-methoxy-5'-prenylisoflavanone		386.40	C21H22O7	F303
242	(5 <i>S</i>)-3,7,2,3'-Tetrahydroxy-4'-methoxy-5'-prenylisoflavanone		386.40	C21H22O7	F303
243	7,4'-Dihydroxy-3'-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienylisoflavanone	Tetrapterol E	392.50	C25H28O4	F101
244	7,4'-Dihydroxy-4'-methoxy-6,3'-diprenylisoflavanone	Tetrapterol I	392.50	C25H28O4	F241
245	(+)-3,5,7-Trihydroxy-2',4'-dimethoxy-3'-prenylisoflavanone	(+)-Echinoisoflavanone	400.43	C22H24O7	2998
246	5,7,3'-Trihydroxy-2',4'-dimethoxy-5'-(1,1-dimethyl-2-propenyl)isoflavanone	Secundiflorol E	400.43	C22H24O7	F242
247	5,7,3'-Trihydroxy-2',4'-dimethoxy-6-prenylisoflavanone	Arizonicanol C	400.43	C22H24O7	F289
248	3,5,7,2,3'-Tetrahydroxy-4'-methoxy-3'-(1,1-dimethyl-2-propenyl)isoflavanone	Secundiflorol A	402.39	C21H22O8	F103
249	2',4'-Dihydroxy-6-prenyl-6'',6''-dimethylpyranol[2'',3'':7,8]isoflavanone	Orientalol F	406.47	C25H26O5	F281
250	2',4'-Dihydroxy-6''-(4-methyl-3-pentenyl)-6''-methylpyranol[2'',3'':7,6]isoflavanone		406.47	C25H26O5	F167
251	2',4'-Dihydroxy-8-prenyl-6'',6''-dimethylpyranol[2'',3'':7,6]isoflavanone	Bidwillon B	406.47	C25H26O5	F97
252	5,7,4'-Trihydroxy-3'-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienylisoflavanone	Tetrapterol D	408.50	C25H28O5	F101
253	5,7,4'-Trihydroxy-6,3'-diprenylisoflavanone	Bolusanthol C	408.50	C25H28O5	F28
254	7,2',4'-Trihydroxy-5'-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienylisoflavanone	Prostratol A	408.50	C25H28O5	F106
255	7,2',4'-Trihydroxy-6,3'-diprenylisoflavanone		408.50	C25H28O5	F168
256	7,2',4'-Trihydroxy-6,8-diprenylisoflavanone	Eriotrichin B	408.50	C25H28O5	F186
257	7,2',4'-Trihydroxy-6,8-diprenylisoflavanone	Bidwillon A	408.50	C25H28O5	F97
258	(±)-7,2',4'-Trihydroxy-8,3'-diprenylisoflavanone	Eryzerin A	408.50	C25H28O5	F277
259	(3 <i>R</i>)-5-Hydroxy-2',4',5'-trimethoxy-6'',6''-dimethylpyranol[2'',3'':4'',5''-(1''-methylphenyl)]isoflavanone	Sacленone	412.44	C23H24O7	F338
260	5,7,2'-Trihydroxy-6'',6''-dimethylpyranol[2'',3'':4'',5''-(1''-methylphenyl)]isoflavanone	Tetrapterol A	418.45	C25H22O6	F101
261	5,2,4'-Trihydroxy-3'-prenyl-6'',6''-dimethylpyranol[2'',3'':7,6]isoflavanone	Lespedeol A	422.48	C25H26O6	2550
262	5,2,4'-Trihydroxy-3'-prenyl-6'',6''-dimethylpyranol[2'',3'':7,6]isoflavanone	Cajanone	422.48	C25H26O6	2549
263	5,2,4'-Trihydroxy-8-prenyl-6'',6''-dimethylpyranol[2'',3'':7,6]isoflavanone	2,3-Dihydroauriculatin	422.48	C25H26O6	2997
264	(3 <i>R</i>)-5,7-Dihydroxy-4'-methoxy-6,3'-diprenylisoflavanone	Vogelin D	422.51	C26H30O5	F209
265	(3 <i>R</i>)-7,4'-Dihydroxy-2'-methoxy-6,8-diprenylisoflavanone	Eryzerin B	422.51	C26H30O5	F277
266	7,4'-Dihydroxy-2'-methoxy-6-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienylisoflavanone		422.51	C26H30O5	F167
267	3,7,2,4'-Tetrahydroxy-6,8-diprenylisoflavanone	Orientalol D	424.50	C25H28O6	F281
268	5,7,2,4'-Tetrahydroxy-5'-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienylisoflavanone	Kenusanone A	424.50	C25H28O6	F98
269	5,7,2,4'-Tetrahydroxy-6-geranylisoflavanone	Lespedeol B	424.50	C25H28O6	2551
270	5,7,2,4'-Tetrahydroxy-6,3'-diprenylisoflavanone	Glisoflavanone	424.50	C25H28O6	F88
271	5,7,2,4'-Tetrahydroxy-6,5'-diprenylisoflavanone	Tetrapterol G	424.50	C25H28O6	F241
272	5,7,2,4'-Tetrahydroxy-6,8-diprenylisoflavanone	Orientalol E	424.50	C25H28O6	F281
273	5,7,2,4'-Tetrahydroxy-8-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienylisoflavanone	Kenusanone H	424.50	C25H28O6	F100

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
274	5,7,2',4'-Tetrahydroxy-8-prenyl-3'-(1,1-dimethyl-2-propenyl)isoflavanone	Dalversinol A	424.50	C ₂₅ H ₂₈ O ₆	F26
275	5,7,2',4'-Tetrahydroxy-8,3'-diprenylisoflavanone	3'-Dimethylallylkievitone	424.50	C ₂₅ H ₂₈ O ₆	2996
276	5,7,2'-Trihydroxy-6-methyl-6'',6''-dimethyl-4''-5''-dihydropyrano[2'',3'':4'',5'']-3''-methylbenzo[1''',6''':5'',4''']isoflavanone	Methyltetrapterol A	432.47	C ₂₆ H ₂₄ O ₆	F31
277	5,7-Dihydroxy-2'-methoxy-3'-prenyl-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavanone	2'-O-Methylcajanone	436.50	C ₂₆ H ₂₈ O ₆	2552
278	5,7-Dihydroxy-6-methyl-3-(1a,2,3,3a,8b,8c-hexahydro-6-hydroxy-1,1,3a-trimethyl-1H-4-oxabenzof[icyclobutylidene-7-yl])-2,3-dihydro 4H-1-benzopyran-4-one	Desmodianone D	436.50	C ₂₆ H ₂₈ O ₆	F31
279	5,7,2'-Trihydroxy-6-methyl-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':4'',5'']-2''-methylcyclohexene[5'',6''':5'',4''']isoflavanone	Desmodianone E	436.50	C ₂₆ H ₂₈ O ₆	F31
280	(R)-5,7,2'-Trihydroxy-6,6''-dimethyl-6''-(4-methylpent-3-enyl)pyrano(2'',3'':4'',5)isoflavanone	Desmodianone A	436.50	C ₂₆ H ₂₈ O ₆	F51
281	(3S)-5,2',4'-Trihydroxy-7-methoxy-6,3'-diprenylisoflavanone	Kanzonol G	438.51	C ₂₆ H ₃₀ O ₆	F74
282	5,7,2'-Trihydroxy-4'-methoxy-6,3'-diprenylisoflavanone	Sophoraisoflavanone B	438.51	C ₂₆ H ₃₀ O ₆	2554
283	5,7,2'-Trihydroxy-4'-methoxy-8,5'-diprenylisoflavanone	Tetrapterol H	438.51	C ₂₆ H ₃₀ O ₆	F241
284	5,7,4'-Trihydroxy-2'-methoxy-6,3'-diprenylisoflavanone	Isosophoranone	438.51	C ₂₆ H ₃₀ O ₆	2553
285	5,7,4'-Trihydroxy-2'-methoxy-8-(2E;6E)-3,7-dimethyl-2,6-octadienylisoflavanone	Tetrapterol C	438.51	C ₂₆ H ₃₀ O ₆	F101
286	5,7,4'-Trihydroxy-2'-methoxy-8,3'-diprenylisoflavanone	Phyllanone B	438.51	C ₂₆ H ₃₀ O ₆	F219
287	(R)-5,7,2',4'-Tetrahydroxy-6-methyl-5''-(3,7-dimethylocta-2(E),6-dienyl)isoflavanone	Desmodianone C	438.51	C ₂₆ H ₃₀ O ₆	F51
288	3,5,7,2',4'-Pentahydroxy-8,3'-diprenylisoflavanone	Bolusanthin II	440.49	C ₂₅ H ₂₈ O ₇	F29
289	5,7,2',4',5'-Pentahydroxy-6,8-diprenylisoflavanone	Erysgalensein B	440.49	C ₂₅ H ₂₈ O ₇	F306
290	3,5,7-Trihydroxy-2'-methoxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'':4'',3]isoflavanone	Phyllanone A	452.50	C ₂₆ H ₂₈ O ₇	F219
291	5,2',4'-Trihydroxy-5'-methoxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavanone	Erysgalensein B	452.50	C ₂₆ H ₂₈ O ₇	F306
292	5,7,4'-Trihydroxy-3'-geranyl-6-prenylisoflavanone	Sophoraisoflavanone C	476.62	C ₃₀ H ₃₆ O ₅	2994
293	5,7,2',4'-Tetrahydroxy-5'-geranyl-6-prenylisoflavanone	Sophoraisoflavanone D	492.62	C ₃₀ H ₃₆ O ₅	2995
Isoflavanone glycosides					
294	7,4'-Dihydroxyisoflavanone 7-O-glucoside	Dihydrodaidzin	418.39	C ₂₁ H ₂₂ O ₉	F92
295	7-Hydroxy-4'-methoxyisoflavanone 7-O-glucoside	Dihydroformonetin 7-O-glucoside	432.43	C ₂₂ H ₂₄ O ₉	3219
296	5,7,4'-Trihydroxyisoflavanone 7-O-glucoside	Dihydrogenistin	434.39	C ₂₁ H ₂₂ O ₁₀	F92
297	5,7,2',4'-Tetrahydroxyisoflavanone 4'-O-glucoside	Dalbergioidin 4'-O-glucoside	450.39	C ₂₁ H ₂₂ O ₁₁	F99
298	7-Hydroxy-2'-methoxy-4',5'-methylenedioxyisoflavanone 7-O-glucoside	Onogenin 7-O-glucoside	476.44	C ₂₃ H ₂₄ O ₁₁	3220

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299	5,8-Dihydroxy-7-methoxyisoflavone		238.24	C15H10O3 F243
300	7-Hydroxy-2-methylisoflavone		252.27	C16H12O3 2339
301	5,7-Dihydroxyisoflavone		254.24	C15H10O4 2839
302	7,4'-Dihydroxyisoflavone	Daidzein	254.24	C15H10O4 2340
303	8,4'-Dihydroxyisoflavone		254.24	C15H10O4 2870
304	7-Methoxy-2-methylisoflavone		266.30	C17H14O3 2341
305	4'-Hydroxy-7-methoxyisoflavone	Isoformononetin	268.27	C16H12O4 2343
306	5-Hydroxy-4'-methoxyisoflavone	Pallidiflorin	268.27	C16H12O4 2867
307	5-Hydroxy-7-methoxyisoflavone		268.27	C16H12O4 2416
308	7-Hydroxy-4'-methoxyisoflavone	Formononetin	268.27	C16H12O4 2342
309	5,7,3'-Trihydroxyisoflavone		270.24	C15H10O5 2840
310	5,7,4'-Trihydroxyisoflavone	Genistein	270.24	C15H10O5 2417
311	6,7,4'-Trihydroxyisoflavone	Demethyltaxin	270.24	C15H10O5 2346
312	7,2',4'-Trihydroxyisoflavone	2'-Hydroxydaidzein	270.24	C15H10O5 2344
313	7,3',4'-Trihydroxyisoflavone	3'-Hydroxydaidzein	270.24	C15H10O5 2345
314	7,8,4'-Trihydroxyisoflavone	8-Hydroxydaidzein	270.24	C15H10O5 2877
315	3-Carboxyaldehyde-7,4'-dihydroxyisoflavone	Corylinal	282.25	C16H10O5 2347
316	7-Hydroxy-3',4'-methylenedioxyisoflavone	Pseudobaptigenin	282.25	C16H10O5 2348
317	5,7-Dimethoxyisoflavone		282.30	C17H14O4 2418
318	7,4'-Dimethoxyisoflavone	7,4'-Di-O-methyl daidzein	282.30	C17H14O4 2349
319	2',4'-Dihydroxy-7-methoxyisoflavone	2'-Methoxyformononetin	284.27	C16H12O5 F113
320	5,4'-Dihydroxy-7-methoxyisoflavone	Prunetin	284.27	C16H12O5 2420
321	5,7-Dihydroxy-3'-methoxyisoflavone	Mutabilein	284.27	C16H12O5 F55
322	5,7-Dihydroxy-4'-methoxyisoflavone	Biochanin A	284.27	C16H12O5 2419
323	5,7,2'-Trihydroxy-6-methylisoflavone	Abronisoflavone	284.27	C16H12O5 F321
324	6,4'-Dihydroxy-7-methoxyisoflavone	Kakkatin	284.27	C16H12O5 2355
325	6,7-Dihydroxy-4'-methoxyisoflavone	Texasin	284.27	C16H12O5 2354
326	6',7-Dihydroxy 3'-methoxyisoflavone		284.27	C16H12O5 F344
327	7,2'-Dihydroxy-4'-methoxyisoflavone	2'-Hydroxyformononetin	284.27	C16H12O5 2350
328	7,2'-Dihydroxy-5'-methoxyisoflavone		284.27	C16H12O5 F343
329	7,2'-Dihydroxy-6-methoxyisoflavone		284.27	C16H12O5 2843
330	7,3'-Dihydroxy-4'-methoxyisoflavone	3'-Hydroxyformononetin	284.27	C16H12O5 2352
331	7,3',4',5'-Tetrahydroxyisoflavone	Baptigenin	284.27	C15H10O6 2358

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
332	7,4'-Dihydroxy-2'-methoxyisoflavone	Theralin	284.27	C ₁₆ H ₁₂ O ₅	2351
333	7,4'-Dihydroxy-3'-methoxyisoflavone	3-Methoxydaidzein	284.27	C ₁₆ H ₁₂ O ₅	2353
334	7,4'-Dihydroxy-5-methoxyisoflavone	5- <i>O</i> -Methylgenistein	284.27	C ₁₆ H ₁₂ O ₅	2421
335	7,4'-Dihydroxy-6-methoxyisoflavone	Glycitein	284.27	C ₁₆ H ₁₂ O ₅	2356
336	7,8-Dihydroxy-4'-methoxyisoflavone	Retusin	284.27	C ₁₆ H ₁₂ O ₅	2357
337	8,4'-Dihydroxy-7-methoxyisoflavone		284.27	C ₁₆ H ₁₂ O ₅	F95
338	5,6,7,4'-Tetrahydroxyisoflavone	6-Hydroxygenistein	286.24	C ₁₅ H ₁₀ O ₆	2424
339	5,7,2',4'-Tetrahydroxyisoflavone	2'-Hydroxygenistein	286.24	C ₁₅ H ₁₀ O ₆	2422
340	5,7,3',4'-Tetrahydroxyisoflavone	Orobol	286.24	C ₁₅ H ₁₀ O ₆	2423
341	7,8,2',4'-Tetrahydroxyisoflavone		286.24	C ₁₅ H ₁₀ O ₆	2878
342	7-Acetoxy-2-methylisoflavone	Glyzarin	294.31	C ₁₈ H ₁₄ O ₄	2359
343	8-Acetyl-7-hydroxy-2-methylisoflavone	Maximaisoflavone H	294.31	C ₁₈ H ₁₄ O ₄	2360
344	4-Methoxy-7,8-methylenedioxyisoflavone	Pseudobaptigenin methyl ether	296.28	C ₁₇ H ₁₂ O ₅	2845
345	7-Methoxy-3',4'-methylenedioxyisoflavone	Dihydroisoderrondiol	296.28	C ₁₇ H ₁₂ O ₅	2361
346	(4''S,5''R)-5,7,4'',5'-Tetrahydroxy-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':4',3']isoflavone		296.32	C ₂₀ H ₁₈ O ₇	F201
347	7,2'-Dimethoxy-8-methylisoflavone		296.32	C ₁₈ H ₁₆ O ₄	F27
348	5,2'-Dihydroxy-6,7-methylenedioxyisoflavone	Irisonone B	298.25	C ₁₆ H ₁₀ O ₆	2881
349	5,4'-Dihydroxy-6,7-methylenedioxyisoflavone	Irilone	298.25	C ₁₆ H ₁₀ O ₆	2427
350	5,7-Dihydroxy-3',4'-methylenedioxyisoflavone	5-Hydroxypseudobaptigenin	298.25	C ₁₆ H ₁₀ O ₆	2425
351	7,2'-Dihydroxy-3,4'-methylenedioxyisoflavone	Glyzaglabrin	298.25	C ₁₆ H ₁₀ O ₆	2363
352	2,5'-Diketo-7-hydroxy-4'-methoxyisoflavone	Bowdichione	298.25	C ₁₆ H ₁₀ O ₆	2511
353	2-Hydroxy-7,4'-dimethoxyisoflavone		298.30	C ₁₇ H ₁₄ O ₅	F191
354	4-Hydroxy-6,7-methoxyisoflavone		298.30	C ₁₇ H ₁₄ O ₅	F159
355	4-Hydroxy-7,3'-dimethoxyisoflavone	Sayanedine	298.30	C ₁₇ H ₁₄ O ₅	2364
356	5-Hydroxy-7,4'-dimethoxyisoflavone		298.30	C ₁₇ H ₁₄ O ₅	F352
357	5,7,4'-Trihydroxy-6,8-dimethylisoflavone		298.30	C ₁₇ H ₁₄ O ₅	F33
358	6-Hydroxy-7,4'-dimethoxyisoflavone	Alfalone	298.30	C ₁₇ H ₁₄ O ₅	2875
359	7-Hydroxy-2',4'-dimethoxyisoflavone	2-Methoxyformononetin	298.30	C ₁₇ H ₁₄ O ₅	F113
360	7-Hydroxy-3',4'-dimethoxyisoflavone	Cladrin	298.30	C ₁₇ H ₁₄ O ₅	2362
361	7-Hydroxy-3',5'-dimethoxyisoflavone		298.30	C ₁₇ H ₁₄ O ₅	F1
362	7-Hydroxy-5,4'-dimethoxyisoflavone	5- <i>O</i> -Methylbiochanin A	298.30	C ₁₇ H ₁₄ O ₅	2426

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
397	5,4'-Dihydroxy-7,3'-dimethoxyisoflavone	7,3'-Dimethylorobol	314.29	C17H14O6	2872
398	5,7-Dihydroxy-2,4'-dimethoxyisoflavone	2'-Methoxybiochanin A	314.29	C17H14O6	2437
399	5,7-Dihydroxy-3',4'-dimethoxyisoflavone	Pratensein 3'-O-methyl ether	314.29	C17H14O6	2841
400	5,7-Dihydroxy-6,2'-dimethoxyisoflavone		314.29	C17H14O6	2850
401	5,7-Dihydroxy-6,4'-dimethoxyisoflavone	Irissolidone	314.29	C17H14O6	2438
402	5,7-Dihydroxy-8,4'-dimethoxyisoflavone		314.29	C17H14O6	F23
403	6,4'-Dihydroxy-5,7-dimethoxyisoflavone	Muningin	314.29	C17H14O6	2440
404	7,2'-Dihydroxy-4',5'-dimethoxyisoflavone		314.29	C17H14O6	F35
405	7,2'-Dihydroxy-6,4'-dimethoxyisoflavone		314.29	C17H14O6	2876
406	7,3'-Dihydroxy-6,4'-dimethoxyisoflavone	Odoratin	314.29	C17H14O6	2373
407	7,3'-Dihydroxy-8,4'-dimethoxyisoflavone	3'-Hydroxy-8-O-methylretusin	314.29	C17H14O6	2374
408	7,4'-Dihydroxy-3',5'-dimethoxyisoflavone		314.29	C17H14O6	F37
409	7,4'-Dihydroxy-5,3'-dimethoxyisoflavone	Gerontoisoflavone A	314.29	C17H14O6	F36
410	7,8-Dihydroxy-6,4'-dimethoxyisoflavone	Dipteryxin	314.29	C17H14O6	2375
411	5,6,7,2'-Tetrahydroxy-3'-methoxyisoflavone		316.27	C16H12O7	2852
412	5,6,7,3'-Tetrahydroxy-4'-methoxyisoflavone		316.27	C16H12O7	F112
413	5,6,7,4'-Tetrahydroxy-8-methoxyisoflavone		316.27	C16H12O7	2855
414	5,7,2',4'-Tetrahydroxy-5'-methoxyisoflavone		316.27	C16H12O7	F176
415	5,7,3',4'-Tetrahydroxy-6-methoxyisoflavone	Piscerygenin	316.27	C16H12O7	F42
416	5,7,3',5'-Tetrahydroxy-4'-methoxyisoflavone	Irlin D	316.27	C16H12O7	F42
417	4'-Hydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone	Junipegenin A	316.27	C16H12O7	2441
418	4'-Hydroxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavone	Erythrinin A	320.34	C20H16O4	2397
419	6-Hydroxy-6'',6''-dimethylpyrano[2'',3'':4',4',3]isoflavone	Bidwillon C	320.34	C20H16O4	F104
420	7-Hydroxy-6'',6''-dimethylpyrano[2'',3'':4',3]isoflavone	Corylin	320.34	C20H16O4	F4
421	7-Hydroxy-4'-prenyloxyisoflavone	Nordurtlettone	322.36	C20H18O4	2396
422	7,4'-Dihydroxy-3'-prenyloxyisoflavone	Neobavaisoflavone	322.36	C20H18O4	2868
423	7,4'-Dihydroxy-8-prenyloxyisoflavone	8-Prenyldaidzein	322.36	C20H18O4	2398
424	7-Acetoxy-2'-methoxy-8-methylisoflavone		324.34	C19H16O5	F85
425	3',4'-Dimethoxy-6,7-methylenedioxyisoflavone		326.31	C18H14O6	F27
426	3',4'-Dimethoxy-7,8-methylenedioxyisoflavone	Maximaisoflavone D	326.31	C18H14O6	2378
427	5,2'-Dimethoxy-6,7-methylenedioxyisoflavone	Tlatlancuayin	326.31	C18H14O6	2848
					2443

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
462	7,8,3'-Trihydroxy-6,4'-dimethoxyisoflavone		330.29	C17H14O7	F253
463	4'-Methoxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavone	Calopogoniumisoflavone A	334.37	C21H18O4	2399
464	2'-Methoxy-4',5'-methylenedioxyfuran[2'',3'':7,6]isoflavone	Dehydronectone	336.30	C19H12O6	2402
465	3',4'-Methylenoxyfuran[2'',3'':7,8]isoflavone	Garhwalin	336.30	C19H12O6	2934
466	5,4'-Dihydroxy-5''-(1-methylethenyl)-4'',5''-dihydrofuran[2'',3'':7,6]isoflavone	Licoagroisoflavone	336.34	C20H16O5	F141
467	5,4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone	Alpinumisoflavone 5	336.34	C20H16O5	2468
468	5,4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavone		336.34	C20H16O5	2469
469	5,7'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':4',3']isoflavone	Derrone	336.34	C20H16O5	2898
470	5,7'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':4',3']isoflavone	Isoderrone	336.34	C20H16O5	2898
471	7,2'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':4',5']isoflavone	Eurycarpin B	336.34	C20H16O5	F353
472	7,2'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':4',3']isoflavone	Puerarone	336.34	C20H16O5	2896
473	7,4'-Methoxy-4'-prenyloxyisoflavone	Glabron	336.34	C20H16O5	2400
474	4'-Methoxy-7-prenyloxyisoflavone	Durlettone	336.38	C21H20O4	2401
475	5,4'-Dihydroxy-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'':7,6]isoflavone	Maximaisoflavone J	336.39	C21H20O4	2835
476	5,4'-Dihydroxy-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'':7,8]isoflavone	Erythravarone A	338.36	C20H18O5	F94
477	5,7,4'-Trihydroxy-3'-prenylisoflavone	Crotalarin	338.36	C20H18O5	2933
478	5,7,4'-Trihydroxy-6-(1,1-dimethyl-2-propenyl)isoflavone	Isowighteone	338.36	C20H18O5	2856
479	5,7,4'-Trihydroxy-6-prenylisoflavone	6-(1,1-Dimethylallyl)genistein	338.36	C20H18O5	2470
480	5,7,4'-Trihydroxy-8-(1,1-dimethylprop-2-enyl)isoflavone	Wighteone	338.36	C20H18O5	2471
481	5,7,4'-Trihydroxy-8-prenylisoflavone	Lupiwighteone	338.36	C20H18O5	F210
482	7,2',4'-Trihydroxy-3'-prenylisoflavone	Eurycarpin A	338.36	C20H18O5	2921
483	7,5''-Dihydroxy-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'':4',3']isoflavone	Psoralenol	338.36	C20H18O5	F354
484	3'-Methoxy-6,7,4',5'-bis(methylenedioxy)isoflavone		338.36	C20H18O5	2403
485	5-Methoxy-6,7,3',4'-bis(methylenedioxy)isoflavone		340.28	C18H12O7	F299
486	5,7,8-Trimethoxy-3',4'-methylenedioxyisoflavone		340.28	C18H12O7	F299
487	3'-Hydroxy-5,4'-dimethoxy-6,7-methylenedioxyisoflavone	Iriskumaonin	340.33	C19H16O6	F180
488	3'-Hydroxy-5,5'-dimethoxy-6,7-methylenedioxyisoflavone	Isoriskashmirianin	342.30	C18H14O7	2453
489	4'-Hydroxy-3',5'-dimethoxy-6,7-methylenedioxyisoflavone	Kashmigenin	342.30	C18H14O7	2888
490	4'-Hydroxy-5,3'-dimethoxy-6,7-methylenedioxyisoflavone	Iriskashmirianin	342.30	C18H14O7	F217
491	5-Hydroxy-3',4'-dimethoxy-6,7-methylenedioxyisoflavone	Squarrosin	342.30	C18H14O7	2887
492	5-Hydroxy-7,3'-dimethoxy-4,5-methylenedioxyisoflavone	Sanchemarroquin	342.30	C18H14O7	2886
					F56

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Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
526	5,4'-Dihydroxy-6''-hydroxymethyl-6''-methylpyrano[2'',3'':7,6]isoflavone	Erysubin B	352.34	C ₂₀ H ₁₆ O ₆	F283
527	5,7,2'-Trihydroxy-6'',6''-dimethylpyran[2'',3'':4',3]isoflavone	Licoisoflavone B	352.34	C ₂₀ H ₁₆ O ₆	2474
528	5,7,3'-Trihydroxy-6'',6''-dimethylpyran[2'',3'':4',5]isoflavone	Semilicoisoflavone B	352.34	C ₂₀ H ₁₆ O ₆	2905
529	5,7,4'-Trihydroxy-5''-(1-methylethenyl)-4'',5''-dihydrofuran[2'',3'':2',3]isoflavone	Crotarin	352.34	C ₂₀ H ₁₆ O ₆	2900
530	5,7,4'-Trihydroxy-6'',6''-dimethylpyran[2'',3'':2',3]isoflavone	Sophoraisoflavone A	352.34	C ₂₀ H ₁₆ O ₆	2899
531	3'-Hydroxy-4'-methoxy-7-prenyloxyisoflavone	Gancaonin G	352.38	C ₂₁ H ₂₀ O ₅	2836
532	5,4'-Dihydroxy-7'-methoxy-6-prenylisoflavone		352.38	C ₂₁ H ₂₀ O ₅	2913
533	5,7-Dihydroxy-4'-methoxy-3'-prenylisoflavone		352.38	C ₂₁ H ₂₀ O ₅	2913
534	5,7-Dihydroxy-4'-methoxy-6-prenylisoflavone		352.38	C ₂₁ H ₂₀ O ₅	F336
535	5,7-Dihydroxy-4'-methoxy-8-prenylisoflavone	Gancaonin A	352.38	C ₂₁ H ₂₀ O ₅	2912
536	7,4'-Dihydroxy-5-methoxy-8-prenylisoflavone	Gancaonin M	352.38	C ₂₁ H ₂₀ O ₅	2922
537	(2E)-5,7,4'-Trihydroxy-3'-(4-hydroxy-3-methyl-2-butenyl)isoflavone	5-Methylupiwighteone	352.38	C ₂₁ H ₂₀ O ₅	2923
538	(2E)-5,7,4'-Trihydroxy-6'-(4-hydroxy-3-methyl-2-butenyl)isoflavone	Vogelin E	354.36	C ₂₀ H ₁₈ O ₆	F209
539	5,2',4'-Trihydroxy-4'',4'',5''-trimethyl-4'',5''-dihydrofuran(2'',3'':7,6)isoflavone	Glabrisoflavone	354.36	C ₂₀ H ₁₈ O ₆	F345
540	5,2',4'-Trihydroxy-7-prenyloxyisoflavone		354.36	C ₂₀ H ₁₈ O ₆	F210
541	5,4'-Dihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuran[2'',3'':7,6]isoflavone	Erythrinin C	354.36	C ₂₀ H ₁₈ O ₆	F210
542	5,7-Dihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuran[2'',3'':4',3]isoflavone	Lupinisoflavone C	354.36	C ₂₀ H ₁₈ O ₆	2479
543	5,7,2',4'-Tetrahydroxy-3'-prenylisoflavone	Licoisoflavone A	354.36	C ₂₀ H ₁₈ O ₆	2857
544	5,7,2',4'-Tetrahydroxy-5''-(1-dimethyl-2-propenyl)isoflavone	Fremontin	354.36	C ₂₀ H ₁₈ O ₆	2477
545	5,7,2',4'-Tetrahydroxy-5'-prenylisoflavone	Alloicoisoflavone A	354.36	C ₂₀ H ₁₈ O ₆	2901
546	5,7,2',4'-Tetrahydroxy-6-prenylisoflavone	Luteone	354.36	C ₂₀ H ₁₈ O ₆	F176
547	5,7,2',4'-Tetrahydroxy-8-(1,1-dimethylprop-2-enyl)isoflavone		354.36	C ₂₀ H ₁₈ O ₆	2478
548	5,7,2',4'-Tetrahydroxy-8-prenylisoflavone	2,3-Dehydrokieveitone	354.36	C ₂₀ H ₁₈ O ₆	F210
549	5,7,3',4'-Tetrahydroxy-6-prenylisoflavone		354.36	C ₂₀ H ₁₈ O ₆	2480
550	5,7,3',4'-Tetrahydroxy-8-prenylisoflavone	Gancaonin L	354.36	C ₂₀ H ₁₈ O ₆	2915
551	5,7,4'-Trihydroxy-6-(2-hydroxy-3-methyl-3-butenyl)isoflavone	Laburnetin	354.36	C ₂₀ H ₁₈ O ₆	2925
552	5,7,4'-Trihydroxy-6-(4-hydroxy-3-methyl-2-butenyl)isoflavone	Hydroxywighteone	354.36	C ₂₀ H ₁₈ O ₆	F222
553	5,7,4'-Trihydroxy-8-(4-hydroxy-3-methyl-2-butenyl)isoflavone	Gancaonin C	354.36	C ₂₀ H ₁₈ O ₆	2917
554	5,7,5''(S)-Trihydroxy-6'',6''-dimethyl-4'',5''-dihdropyran[2'',3'':4',3]isoflavone	Ficuisoflavone	354.36	C ₂₀ H ₁₈ O ₆	2927
555	5,2',5'-Trimethoxy-6,7-methylenedioxyisoflavone	Hemerocallone	354.36	C ₂₀ H ₁₈ O ₆	F142
556	5,3',4'-Trimethoxy-6,7-methylenedioxyisoflavone	Iriskumaonin methyl ether	356.33	C ₁₉ H ₁₆ O ₇	2844

557	5,6,7-Trimethoxy-3',4'-methylenedioxyisoflavone				
558	6,7,2'-Trimethoxy-4',5'-methylenedioxyisoflavone				
559	6,7,3'-Trimethoxy-4',5'-methylenedioxyisoflavone				
560	6,7,8-Trimethoxy-3',4'-methylenedioxyisoflavone				
561	7,8,2'-Trimethoxy-3',4'-methylenedioxyisoflavone				
562	7,8,2'-Trimethoxy-4',5'-methylenedioxyisoflavone				
563	8,3',4'-Trimethoxy-6,7-methylenedioxyisoflavone				
564	5,3'-Dihydroxy-4',5'-dimethoxy-6,7-methylenedioxyisoflavone				
565	5,7-Dihydroxy-6,2'-dimethoxy-4',5'-methylenedioxyisoflavone				
566	3'-Hydroxy-5,6,7,2'-tetramethoxyisoflavone				
567	5-Hydroxy-6,7,3',4'-tetramethoxyisoflavone				
568	5-Hydroxy-7,2',4',5'-tetramethoxyisoflavone				
569	6-Hydroxy-2',4',5',7'-tetramethoxyisoflavone				
570	7-Hydroxy-6,2',4',5'-trimethoxyisoflavone				
571	7,4'-Dihydroxy-5,3',5'-trimethoxy-6-methylisoflavone				
572	5,7-Dihydroxy-4'-(<i>p</i> -methylbenzyl)isoflavone				
573	5,7,3'-Trihydroxy-6,4',5'-trimethoxyisoflavone				
574	5,7,4'-Trihydroxy-2',3',6'-trimethoxyisoflavone				
575	6,4',5'-Trihydroxy-5,7,3'-trimethoxyisoflavone				
576	7,8,5'-Trihydroxy-6,3',4'-trimethoxyisoflavone				
577	3-Hydroxy-4',5'-methylenedioxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
578	3-Carboxyaldehyde-5,4'-dihydroxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
579	3'-Hydroxy-4,5-methylenedioxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
580	5-Hydroxy-3',4'-methylenedioxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
581	5-Hydroxy-3',4'-methylenedioxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
582	5,4'-Dimethoxy-5''-(1-methylethenyl)-4'',5''-dihydrofuranol[2'',3'';7'',6]isoflavone				
583	5,4'-Dimethoxy-6'',6''-dimethylpyranol[2'',3'';7'',6]isoflavone				
584	6,4'-Dimethoxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
585	2',4'-Dihydroxy-3',4'-methylenedioxy-7-prenyloxyisoflavone				
586	2',4'-Dihydroxy-5-methoxy-6'',6''-dimethylpyranol[2'',3'';7'',8]isoflavone				
587	5,3'-Dihydroxy-4'-methoxy-6'',6''-dimethylpyranol[2'',3'';7'',6]isoflavone				
588	5,7-Dihydroxy-3',4'-methylenedioxy-6-prenylisoflavone				
589	7,2'-Dihydroxy-5-methoxy-6'',6''-dimethylpyranol[2'',3'';4'',5]isoflavone				
	Odoratine	356.33	C19H16O7	2458	
		356.33	C19H16O7	2387	
		356.33	C19H16O7	2388	
		356.33	C19H16O7	2390	
	Petalostetin	356.33	C19H16O7	F205	
	Maximaisoflavone L	356.33	C19H16O7	2389	
		356.33	C19H16O7	2391	
		358.30	C18H14O8	2854	
	Dalpalatin	358.30	C18H14O8	2889	
		358.35	C19H18O7	F3	
	Belamcandin	358.35	C19H18O7	2885	
	Robustigenin	358.35	C19H18O7	2460	
		358.35	C19H18O7	F130	
		358.35	C19H18O7	2392	
		358.35	C19H18O7	F221	
	Brachyrachisin	358.39	C23H18O4	F178	
		360.32	C18H16O8	2461	
	Irigenin	360.32	C18H16O8	F107	
	Nervosin	360.32	C18H16O8	F15	
	Soforanarin B	360.32	C18H16O8	F208	
		364.35	C21H16O6	F333	
	Norisojamicin	364.35	C21H16O6	F43	
	Scandenal	364.35	C21H16O6	F342	
	Norisojamicin	364.35	C21H16O6	2481	
	Robustone	364.35	C21H16O6	2932	
		364.40	C22H20O5	F13	
	Thonninginisoflavone	364.40	C22H20O5	2482	
	Alpinumisoflavone dimethyl ether	364.40	C22H20O5	F340	
	6-Methoxycalopogonium	366.36	C21H18O6	F205	
	Maximaisoflavone K	366.36	C21H18O6	2931	
	Barpisoflavone C	366.36	C21H18O6	2484	
	3'-Hydroxylpinumisoflavone 4-methyl ether	366.36	C21H18O6	2483	
	Derrubone	366.36	C21H18O6	2483	
	Glycyrrhiza isoflavone B	366.36	C21H18O6	F89	

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
590	7,2'-Dihydroxy-5'-methoxy-6'',6''-dimethylpyrano[2'',3'',4'',3'']isoflavone	Piscisoflavone B	366.36	C ₂₁ H ₁₈ O ₆	F176
591	7,4'-Dihydroxy-5'-methoxy-6'',6''-dimethylpyrano[2'',3'',3'':3'':2'']isoflavone	Piscisoflavone D	366.36	C ₂₁ H ₁₈ O ₆	F264
592	3',4'-Dihydroxy-7-prenylloxysiflavone		366.41	C ₂₂ H ₂₂ O ₅	2837
593	5,2',4'-Trihydroxy-7-methoxy-6-prenylisoflavone	7-O-Methyluteone	368.39	C ₂₁ H ₂₀ O ₆	F339
594	5,7'-Dihydroxy-6-methoxy-4'-prenylloxysiflavone	Isaurmillone	368.39	C ₂₁ H ₂₀ O ₆	2851
595	5,7'-Dihydroxy-8-methoxy-4'-prenylloxysiflavone	Aurmillone	368.39	C ₂₁ H ₂₀ O ₆	2485
596	5,7,2'-Trihydroxy-4'-methoxy-5'-prenylisoflavone	Lysisteisoflanone	368.39	C ₂₁ H ₂₀ O ₆	F62
597	5,7,2'-Trihydroxy-4'-methoxy-6-prenylisoflavone	Gancaonin N	368.39	C ₂₁ H ₂₀ O ₆	2914
598	5,7,3'-Trihydroxy-4'-methoxy-5'-prenylisoflavone		368.39	C ₂₁ H ₂₀ O ₆	F336
599	5,7,3'-Trihydroxy-4'-methoxy-6-prenylisoflavone	Gancaonin B	368.39	C ₂₁ H ₂₀ O ₆	2916
600	5,7,4'-Trihydroxy-2-methoxy-5'-prenylisoflavone	Vogelin F	368.39	C ₂₁ H ₂₀ O ₆	F209
601	5,7,4'-Trihydroxy-3'-methoxy-5'-prenylisoflavone	2'-Deoxyiscerythron	368.39	C ₂₁ H ₂₀ O ₆	2859
602	5,7,4'-Trihydroxy-3'-methoxy-8-prenylisoflavone		368.39	C ₂₁ H ₂₀ O ₆	2926
603	5,7,4'-Trihydroxy-5'-methoxy-3'-prenylisoflavone		368.39	C ₂₁ H ₂₀ O ₆	F264
604	7,2'-Dihydroxy-6'-methoxy-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'',4'',5'']isoflavone	2'-Deoxyiscerythron	368.39	C ₂₁ H ₂₀ O ₆	F89
605	7,2',3'-Trihydroxy-4'-methoxy-3'-(1,1-dimethyl-2-propenyl)isoflavone	Secundiflorol C	368.39	C ₂₁ H ₂₀ O ₆	F103
606	7,2',4'-Trihydroxy-5'-methoxy-8-prenylisoflavone	Barpisoflavone B	368.39	C ₂₁ H ₂₀ O ₆	2924
607	7,2',4'-Trihydroxy-5'-methoxy-3'-prenylisoflavone	Piscisoflavone A	368.39	C ₂₁ H ₂₀ O ₆	F176
608	7,3',4'-Trihydroxy-5'-methoxy-5'-prenylisoflavone	Glisoflavone	368.39	C ₂₁ H ₂₀ O ₆	2902
609	7,4',6'-Trihydroxy-3'-methoxy-2'-prenylisoflavone	K wakhurin	368.39	C ₂₁ H ₂₀ O ₆	2897
610	5,2'-Dimethoxy-6,7,4',5'-bis(methylenedioxy)isoflavone	Lupinisoflavone B	370.32	C ₁₉ H ₁₄ O ₈	F299
611	5,3'-Dimethoxy-6,7,4',5'-bis(methylenedioxy)isoflavone	Lupinisoflavone D	370.32	C ₁₉ H ₁₄ O ₈	F299
612	5,2',4'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofurano[2'',3'',7,6]isoflavone		370.36	C ₂₀ H ₁₈ O ₇	2863
613	5,2',4'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofurano[2'',3'',7,8]isoflavone	Lunatone	370.36	C ₂₀ H ₁₈ O ₇	2935
614	5,7,2'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofurano[2'',3'',4',3]isoflavone	Lupinisoflavone D	370.36	C ₂₀ H ₁₈ O ₇	2858
615	5-Hydroxy-3',4',5'-trimethoxy-6,7-methylenedioxyisoflavone	2,3-Dehydrokivitol	372.33	C ₂₀ H ₁₆ O ₈	F322
616	5,6,7,3',4'-Pentamethoxyisoflavone		372.37	C ₂₀ H ₂₀ O ₇	F299
618	5,7,2',4'-Tetrahydroxy-8-(3-hydroxy-3-methyl-2-butyl)isoflavone	2,3-Dehydrokivitol hydrate	372.37	C ₂₀ H ₂₀ O ₇	2930
619	5,7,2',4',5'-Pentamethoxyisoflavone	Robustigenin methyl ether	372.37	C ₂₀ H ₂₀ O ₇	2462
620	6,7,2',3',4'-Tetramethoxyisoflavone		372.37	C ₂₀ H ₂₀ O ₇	2393

621	6,7,2',4',5'-Tetramethoxyisoflavone	372.37	C20H20O7	2394
622	6,7,3',4',5'-Tetramethoxyisoflavone	372.37	C20H20O7	2395
623	7,8,3',4',5'-Pentamethoxyisoflavone	372.37	C20H20O7	F40
624	5,3'-Dihydroxy-6,7,8,2'-tetramethoxyisoflavone	374.35	C19H18O8	2894
625	5,7-Dihydroxy-6,2',3',4'-tetramethoxyisoflavone	374.35	C19H18O8	F169
626	5,7-Dihydroxy-6,2',4',5'-tetramethoxyisoflavone	374.35	C19H18O8	2463
627	5,7-Dihydroxy-6,3',4',5'-tetramethoxyisoflavone	374.35	C19H18O8	2464
628	5,7-Dihydroxy-8,2',4',5'-tetramethoxyisoflavone	374.35	C19H18O8	2465
629	5-Hydroxy-5'-methoxy-2'-prenyloxazol[2',3':4',3']isoflavone	377.39	C22H19O5N1	F177
630	7-Hydroxy-5'-methoxy-2'-prenyloxazol[2',3':4',3']isoflavone	377.39	C22H19O5N1	F264
631	2-Methoxy-4',5'-methylenedioxy-6',6'-dimethylpyranol[2',3':7,8]isoflavone	378.38	C22H18O6	2406
632	3-Methoxy-4',5'-methylenedioxy-6',6'-dimethylpyranol[2',3':7,8]isoflavone	378.38	C22H18O6	2911
633	5-Methoxy-3',4'-methylenedioxy-5''-(1-methylethenyl)-4'',5''-dihydrofuranol[2',3':7,6]isoflavone	378.38	C22H18O6	2487
634	5-Methoxy-3',4'-methylenedioxy-6',6''-dimethylpyranol[2',3':7,6]isoflavone	378.38	C22H18O6	2486
635	6-Methoxy-3',4'-methylenedioxy-6',6''-dimethylpyranol[2',3':7,8]isoflavone	378.38	C22H18O6	2407
636	2'-Hydroxy-5,4'-dimethoxy-6',6''-dimethylpyranol[2',3':7,6]isoflavone	380.40	C22H20O6	F184
637	2'-Methoxy-4',5'-methylenedioxy-7-prenyloxyisoflavone	380.40	C22H20O6	2408
638	6-Hydroxy-3',4'-dimethoxy-6',6''-dimethylpyranol[2',3':7,8]isoflavone	380.40	C22H20O6	F341
639	7-Hydroxy-6-methoxy-3',4'-methylenedioxy-8-prenylisoflavone	380.40	C22H20O6	2936
640	8-Methoxy-3',4'-methylenedioxy-7-prenyloxyisoflavone	380.40	C22H20O6	2847
641	5,5',7,5''-Tetrahydroxy-5-methoxy-6',6''-dimethyl-4'',5''-dihydropyranol[2',3':7,6]isoflavone	382.27	C21H18O7	F47
642	5,7,3',5''-Tetrahydroxy-5-methoxy-6',6''-dimethyl-4'',5''-dihydropyranol[2',3':4',5']isoflavone	382.37	C20H18O7	F89
643	5,7-Dihydroxy-3',4'-dimethoxy-5'-prenylisoflavone	382.41	C22H22O6	2903
644	7,3'-Dihydroxy-5,4'-dimethoxy-5'-prenylisoflavone	382.41	C22H22O6	2904
645	7,6-Dihydroxy-2',4'-dimethoxy-3'-prenylisoflavone	382.41	C22H22O6	2409
646	5,4'-Dihydroxy-5''-(1-hydroxy-1-methylethyl)-4''-methoxyfuranol[2',3':7,6]isoflavone	382.41	C22H22O6	F138
647	5,4',5''-Trihydroxy-4''-methoxy-6',6''-dimethyl-4'',5''-dihydrofuranol[2',3':4',5']isoflavone	384.39	C21H20O7	F222
648	5,7,2'-Trihydroxy-3'-methoxy-5''-(1-hydroxy-1-methylethyl)-4''-5''-dihydrofuranol[2',3':4',5']isoflavone	384.39	C21H20O7	F264
649	5,7,2'-Trihydroxy-4'-methoxy-8-(4-hydroxy-3-methyl-2-butenyl)isoflavone	384.39	C21H20O7	2929
650	5,7,2',3'-Tetrahydroxy-4'-methoxy-3''-(1,1-dimethyl-2-propenyl)isoflavone	384.39	C21H20O7	F103
651	5,7,2',4'-Tetrahydroxy-5'-methoxy-3'-prenylisoflavone	384.39	C21H20O7	2488
652	5,7,2',4'-Tetrahydroxy-5'-methoxy-6-prenylisoflavone	384.39	C21H20O7	F176
653	5,7,3',4'-Tetrahydroxy-5'-methoxy-2'-prenylisoflavone	384.39	C21H20O7	F268

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
654	5,7,4'-Trihydroxy-3'-methoxy-6'',6''-dimethylpyranol[2'',3'';5',6]isoflavone	Piscidanone	384.39	C ₂₁ H ₂₀ O ₇	F176
655	5,2,3',6'-Tetrahydroxy-6,7-methylenedioxyisoflavone	Madhushazone	386.36	C ₂₀ H ₁₈ O ₈	F245
656	5,3',4',5'-Tetramethoxy-6,7-methylenedioxyisoflavone	Irisflorentin	386.36	C ₂₀ H ₁₈ O ₈	2466
657	5,6,7,8-Tetramethoxy-3',4'-methylenedioxyisoflavone	Kwakhurin hydrate	386.36	C ₂₀ H ₁₈ O ₈	2467
658	7,4',6'-Trihydroxy-3'-methoxy-2-(3-hydroxy-3-methylbutyl)isoflavone		386.40	C ₂₁ H ₂₂ O ₇	2895
659	6,8,3'-Trichloro-5,4'-dihydroxy-7-methoxyisoflavone		387.60	C ₁₆ H ₉ O ₅ Cl ₃	F301
660	4'-Prenyloxy-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone		388.46	C ₂₅ H ₂₄ O ₄	F341
661	7,3-Dihydroxy-8,2-diprenylisoflavone	Erysubin F	390.48	C ₂₅ H ₂₆ O ₄	F275
662	5-Hydroxy-8-methoxy-3',4'-methylenedioxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone		394.38	C ₂₂ H ₁₈ O ₇	F156
663	6-Hydroxy-5-methoxy-3,4'-methylenedioxy-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	Griffonianone B	394.38	C ₂₂ H ₁₈ O ₇	F331
664	2',4',5'-Trimethoxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone		394.42	C ₂₃ H ₂₂ O ₆	F118
665	2',4',5'-Trimethoxy-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	Barbigeron	394.42	C ₂₃ H ₂₂ O ₆	2410
666	6,3',4'-Trimethoxy-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	Duralone	394.42	C ₂₃ H ₂₂ O ₆	F341
667	4'-Hydroxy-2',5'-dimethoxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Elongatin	396.40	C ₂₂ H ₂₀ O ₇	2490
668	5,4'-Dihydroxy-2,5'-dimethoxy-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	4'-Demethyltoxical	396.40	C ₂₂ H ₂₀ O ₇	F49
669	5,4'-Dihydroxy-3,5'-dimethoxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Pumilaisoflavone D	396.40	C ₂₂ H ₂₀ O ₇	2918
670	7-Hydroxy-6,3',4'-trimethoxy-8-prenylisoflavone	Predurallone	396.44	C ₂₃ H ₂₄ O ₆	F341
671	5,7,4'-Trihydroxy-5'-methoxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuranol[2'',3'';2',3]isoflavone	Piscerisoflavone E	398.36	C ₂₁ H ₁₈ O ₈	F264
672	5,7-Dihydroxy-4',5'-dimethoxy-3-(1 <i>E</i>)-3-hydroxy-3-methyl-1-butenyl)isoflavone	Piscerynetin	398.41	C ₂₂ H ₂₂ O ₇	F264
673	5,7,2'-Trihydroxy-4',5'-dimethoxy-3-prenylisoflavone	2'-Hydroxypiscerythrinetin	398.41	C ₂₂ H ₂₂ O ₇	2906
674	5,7,4'-Trihydroxy-2',5'-dimethoxy-6-prenylisoflavone	Viridiflorin	398.41	C ₂₂ H ₂₂ O ₇	2860
675	5,7,2'-Trihydroxy-5'-methoxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuranol[2'',3'';4',3]isoflavone	Piscerisoflavone B	400.38	C ₂₁ H ₂₀ O ₈	F264
676	5,7,2',4'-Tetrahydroxy-5'-methoxy-3-(2 <i>E</i>)-4-hydroxy-3-methyl-2-butenyl)isoflavone	Piscerisoflavone D	400.38	C ₂₁ H ₂₀ O ₈	F264
677	5,7,2',5'-Tetrahydroxy-5'-methoxy-6'',6''-dimethyl-4'',5''-dihydrodipyrano[2'',3'';4',3]isoflavone	Piscerisoflavone A	400.38	C ₂₁ H ₂₀ O ₈	F264
678	5,7,4'-Trihydroxy-5'-methoxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuranol[2'',3'';2',3]isoflavone	Piscerisoflavone C	400.38	C ₂₁ H ₂₀ O ₈	F264
679	5,7,4',5'-Tetrahydroxy-5'-methoxy-6'',6''-dimethyl-4'',5''-dihydrodipyrano[2'',3'';2',3]isoflavone		400.38	C ₂₁ H ₂₀ O ₈	F264
680	5,7,4',5'-Tetrahydroxy-5'-methoxy-6'',6''-dimethyl-4'',5''-dihydrodipyrano[2'',3'';3',2]isoflavone	Piscidanol	402.39	C ₂₁ H ₂₂ O ₈	F40
681	6,7,8,3',4',5'-Hexamethoxyisoflavone	Ulexin B	402.45	C ₂₅ H ₂₂ O ₅	F165
682	5-Hydroxy-(2'',3'';7,6)(2'',3'';4',3')-bis(6,6-dimethylpyranol)isoflavone	Ulexone B	402.45	C ₂₅ H ₂₂ O ₅	2962
683	5-Hydroxy-(2'',3'';7,8)(2'',3'';4',3')-bis(6,6-dimethylpyranol)isoflavone	Genistein-4'-(6''-methyl)salicylate	404.37	C ₂₃ H ₁₆ O ₇	F159
684	4'-(6''-Methyl)salicylate-5,7-dihydroxyisoflavone				

685	5,7-Dihydroxy-6,2',3',4',5'-pentamethoxyisoflavone	404.37	C20H20O9	F169
686	5-Hydroxy-4'-prenyloxy-6'',6''-dimethylpyrano[2'',3'';7'',6]isoflavone	404.46	C25H24O5	2861
687	5,4'-Dihydroxy-3'-prenyl-6'',6''-dimethylpyrano[2'',3'';7'',6]isoflavone	404.46	C25H24O5	2491
688	5,4'-Dihydroxy-3'-prenyl-6'',6''-dimethylpyrano[2'',3'';7'',8]isoflavone	404.46	C25H24O5	F157
689	5,4'-Dihydroxy-6-prenyl-6'',6''-dimethylpyrano[2'',3'';7'',6]isoflavone	404.46	C25H24O5	2492
690	5,4'-Dihydroxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'';7'',6]isoflavone	404.46	C25H24O5	2493
691	5,7-Dihydroxy-6-prenyl-6'',6''-dimethylpyrano[2'',3'';4'',3]isoflavone	404.46	C25H24O5	2940
692	5,7-Dihydroxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'';4'',3]isoflavone	404.46	C25H24O5	2960
693	5,7,4'-Trihydroxy-8-(1 <i>R</i> ,6 <i>R</i>)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl)isoflavone	404.46	C25H24O5	F5
694	4'-Methoxy-7-geranyloxyisoflavone	404.51	C26H28O4	2869
695	3',4'-Dihydroxy-7-(3,7-dimethyl-1,2(<i>E</i>),6-octadienyl)oxyisoflavone	406.47	C25H26O5	F332
696	4'-Hydroxy-(2'',3'',5,6),(2'',3'',7,8)-bis(4,5-dihydro-6,6-dimethylpyrano)isoflavone	406.47	C25H26O5	F94
697	5,7,2',4'-Tetrahydroxy-3'-prenyl-5-(1,1-dimethyl-2-propenyl)isoflavone	406.47	C25H26O5	2908
698	5,7,4'-Trihydroxy-3',5'-diprenylisoflavone	406.47	C25H26O5	2907
699	5,7,4'-Trihydroxy-6,3'-diprenylisoflavone	406.47	C25H26O5	2494
700	5,7,4'-Trihydroxy-6,8-diprenylisoflavone	406.47	C25H26O5	2495
701	5,7,4'-Trihydroxy-8-(3,7-dimethyl-2,6-octadienyl)isoflavone	406.47	C25H26O5	F171
702	5,7,4'-Trihydroxy-8,3'-diprenylisoflavone	406.47	C25H26O5	F267
703	7-Hydroxy-4'-geranyloxyisoflavone	406.47	C25H26O5	F69
704	2',6'-Dimethoxy-3',4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'';7'',8]isoflavone	408.41	C23H20O7	2411
705	5,6-Dimethoxy-3',4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'';7'',8]isoflavone	408.41	C23H20O7	2938
706	6,2'-Dimethoxy-4',5'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'';7'',8]isoflavone	408.41	C23H20O7	2412
707	5-Hydroxy-2',4',5'-trimethoxy-6'',6''-dimethylpyrano[2'',3'';7'',8]isoflavone	410.42	C23H22O7	2496
708	7-Hydroxy-2',5'-dimethoxy-3',4'-methylenedioxy-8-prenylisoflavone	410.42	C23H22O7	2910
709	7-Hydroxy-5,6-dimethoxy-3',4'-methylenedioxy-8-prenylisoflavone	410.42	C23H22O7	2937
710	7,2',4',5'-Tetramethoxy-8-prenylisoflavone	410.47	C24H26O6	2909
711	5,7-Dihydroxy-4',5'-dimethoxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuran[2'',3'';2'',3]isoflavone	414.41	C22H22O8	F264
712	5,7,5''-Trihydroxy-4',5'-dimethoxy-6'',6''-dimethyl-4'',5''-dihydroprano[2'',3'';2'',3]isoflavone	414.41	C22H22O8	F264
713	5,7,2'-Trihydroxy-4',5'-dimethoxy-3''-(3-hydroxy-3-methyl-butyl)isoflavone	416.42	C22H24O8	F264
714	2'-Methoxy-(2'',3'';7,6),(2'',3'';4'',3)-bis(6,6-dimethylpyrano)isoflavone	416.48	C26H24O5	2413
715	5-Hydroxy-5''-(1-hydroxy-1-methylethyl)furan[2'',3'';7,6]-6'',6''-dimethylpyrano[2'',3'';4'',3]isoflavone	418.45	C25H24O6	F164
716	5,2'-Dihydroxy-(2'',3'';7,6),(2'',3'';4'',3)-bis(6,6-dimethylpyrano)isoflavone	418.45	C25H22O6	F58
717	5,7-Dihydroxy-6''-hydroxymethyl-6'',6''-trimethyl-(2'',3'';7,8),(2'',3'';4'',3)-bis(pyran)isoflavone	418.45	C25H22O6	2963

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
718	3',4'-Methylenedioxy-7-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienyloxy)isoflavone	7- <i>O</i> -Geranylpsudobaptigenin	418.49	C ₂₆ H ₂₆ O ₅	F333
719	4'-Hydroxy-5-methoxy-6-prenyl-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	Scandinone	418.49	C ₂₆ H ₂₆ O ₅	2497
720	5-Methoxy-4'-prenyloxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone		418.49	C ₂₆ H ₂₆ O ₅	F13
721	5-Hydroxy-5''-(1-hydroxy-1-methylethyl)-4',5''-dihydrofuranol[2'',3'';7,8]-6''',6'''-dimethylpyranol[2''',3''',4',3']isoflavone	Ulexone C	420.46	C ₂₅ H ₂₄ O ₆	2964
722	5-Hydroxy-5''-(1-hydroxy-1-methylethyl)-4''',5'''-dihydrofuranol[2''',3''',7,6]-6''',6'''-dimethylpyranol[2''',3''',4',3']isoflavone	Ulexin D	420.46	C ₂₅ H ₂₄ O ₆	F164
723	5,2-Dihydroxy-4'-prenyloxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Isoauriculatin	420.46	C ₂₅ H ₂₄ O ₆	2498
724	5,2,4'-Trihydroxy-3'-prenyl-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Angustone C	420.46	C ₂₅ H ₂₄ O ₆	2954
725	5,2,4'-Trihydroxy-8-prenyl-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Auriculatin	420.46	C ₂₅ H ₂₄ O ₆	2501
726	5,3'-Dihydroxy-4'-prenyloxy-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Isoauriculasin	420.46	C ₂₅ H ₂₄ O ₆	2499
727	5,3',4'-Trihydroxy-6-prenyl-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	Pomiferin	420.46	C ₂₅ H ₂₄ O ₆	2500
728	5,3',4'-Trihydroxy-8-prenyl-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Auriculasin	420.46	C ₂₅ H ₂₄ O ₆	2502
729	5,4'-Dihydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethylpyranol[2'',3'';7,8]isoflavone	Euchrenone b9	420.46	C ₂₅ H ₂₄ O ₆	2973
730	5,4'-Dihydroxy-8-(2-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Erysenegalsein M	420.46	C ₂₅ H ₂₄ O ₆	F309
731	5,4'-Dihydroxy-8-(2-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Euchrenone b8	420.46	C ₂₅ H ₂₄ O ₆	2968
732	5,4'-Dihydroxy-8-(2,3-epoxy-3-methylbutyl)-6'',6''-dimethylpyranol[2'',3'';7,6]isoflavone	Erysenegalsein G	420.46	C ₂₅ H ₂₄ O ₆	F310
733	5,4',7''-Trihydroxy-7''-methyl-4''-(1-methylethyl)-3'',4'',5'',6'',7'',7''a-hexahydrobenzofuranol[2'',3'';7,8]isoflavone	Ficusin B	420.46	C ₂₅ H ₂₄ O ₆	F5
734	5,7-Dihydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethylpyranol[2'',3'';4',3']isoflavone	Ulexin A	420.46	C ₂₅ H ₂₄ O ₆	F165
735	5,7,2'-Trihydroxy-6-prenyl-6'',6''-dimethylpyranol[2'',3'';4',3']isoflavone	Angustone B	420.46	C ₂₅ H ₂₄ O ₆	2944
736	5,7,2'-Trihydroxy-6-prenyl-6'',6''-dimethylpyranol[2'',3'';4',5']isoflavone	Kraussanone 2	420.46	C ₂₅ H ₂₄ O ₆	F58
737	5,7,3'-Trihydroxy-6-prenyl-6'',6''-dimethylpyranol[2'',3'';4',5']isoflavone	Gancaonin H	420.46	C ₂₅ H ₂₄ O ₆	2948
737b	5,7,3'-Trihydroxy-2'-prenyl-6'',6''-dimethylpyranol[2'',3'';4',5']isoflavone		420.46	C ₂₅ H ₂₄ O ₆	F4b
738	5,7,4'-Trihydroxy-8-prenyl-6'',6''-dimethylpyranol[2'',3'';2',3']isoflavone	Glyasperin N	420.46	C ₂₅ H ₂₄ O ₆	F72
739	4'-Methoxy-7-(8-hydroxy-3,7-dimethyl-2(<i>E</i>),6(<i>Z</i>)-octadienyloxy)isoflavone	7- <i>O</i> -Methylisupalbigenin	420.50	C ₂₆ H ₂₈ O ₅	F332
740	5,4'-Dihydroxy-7-methoxy-8,3'-diprenylisoflavone	Olibergin A	420.50	C ₂₆ H ₂₈ O ₅	F164
741	5,7-Dihydroxy-4'-methoxy-8-geranylisoflavone	Conrautnone C	420.50	C ₂₆ H ₂₈ O ₅	F111
742	7-Hydroxy-5-methoxy-4'-geranyloxyisoflavone		420.50	C ₂₆ H ₂₈ O ₅	F69
743	7,4'-Dihydroxy-5-methoxy-6,8-diprenylisoflavone	Derritisoflavone A	420.50	C ₂₆ H ₂₈ O ₅	F232
744	3,5,7,4'-Tetrahydroxy-6,3'-diprenylisoflavone	Glyasperin A	422.48	C ₂₅ H ₂₆ O ₆	F350

APPENDIX

Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
774	5,2',5''-Trihydroxy-4'',5''-dihydroxy-(2'',3'',7'',6'')(2'',3'',4'',3'')-bis(6,6-dimethylpyrano)isoflavone	Kraussianone 6	436.46	C ₂₅ H ₂₄ O ₇	F57
775	5,7,2'-Trihydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethylpyrano[2'',3'',4'',3']isoflavone	Kraussianone 7	436.46	C ₂₅ H ₂₄ O ₇	F57
776	2-Hydroxy-4'-methoxy-(2'',3'',5'',6'')(2'',3'',7'',8'')-bis(4,5-dihydro-6,6-dimethylpyrano)isoflavone	1'',2''-Dihydro-2'-hydroxycycloosajin	436.50	C ₂₆ H ₂₈ O ₆	2506
777	4'-Hydroxy-3'-methoxy-(2'',3'',5'',6'')(2'',3'',7'',8'')-bis(4,5-dihydro-6,6-dimethylpyrano)isoflavone	1'',2''-Dihydro-O-methylcyclopomiferin	436.50	C ₂₆ H ₂₈ O ₆	2507
778	4'-Hydroxy-5-methoxy-5''-(1-hydroxy-1-methylethyl)-6-prenyl-4'',5''-dihydrofuran[2'',3'',7'',8']isoflavone	Derrisoflavone C	436.50	C ₂₆ H ₂₈ O ₆	F232
779	5,4'-Dihydroxy-8-(3-methoxy-3-methylbutyl)-6'',6''-dimethylpyrano(2'',3'',7'',6'')isoflavone	Eturmagarone	436.50	C ₂₆ H ₂₈ O ₆	F215
780	5,7,3'-Trihydroxy-4'-methoxy-6,8-diprenylisoflavone	6,8-Diprenylpratensein	436.50	C ₂₆ H ₂₈ O ₆	2866
781	5,7,4'-Trihydroxy-2'-methoxy-6,3'-diprenylisoflavone	2'-Methoxylupalbigenin	436.50	C ₂₆ H ₂₈ O ₆	2864
782	5,7,4'-Trihydroxy-2'-methoxy-6,8-diprenylisoflavone	Euchrenone b15	436.50	C ₂₆ H ₂₈ O ₆	F175
783	5,7,4'-Trihydroxy-3'-methoxy-2',5'-diprenylisoflavone	Millewanin A	436.50	C ₂₆ H ₂₈ O ₆	F110
784	7,4'-Dihydroxy-5-methoxy-6-(2-hydroxy-3-methyl-3-butenyl)-8-prenylisoflavone	Derrisoflavone D	436.50	C ₂₆ H ₂₈ O ₆	F232
785	7,4'-Dihydroxy-5-methoxy-8-(2-hydroxy-3-methyl-3-butenyl)-6-prenylisoflavone	Derrisoflavone E	436.50	C ₂₆ H ₂₈ O ₆	F232
786	4-Amino-5,7,3'-trihydroxy-5'-methoxy-2',6'-diprenylisoflavone	Piscerythramine	437.52	C ₂₆ H ₂₉ O ₆ N1	2978
787	5,6,2'-Trimethoxy-4',5'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'',7'',8']isoflavone	Conrauinone A	438.44	C ₂₄ H ₂₂ O ₈	F68
788	5-Hydroxy-(2'',3'',7'',6'')(2'',3'',4'',3'')-bis(5-(1-hydroxy-1-methylethyl)-4,5-dihydrofuran)isoflavone	Isolupinisoflavone E	438.48	C ₂₅ H ₂₆ O ₇	F142
789	5,2',4'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-3'-prenyl-4'',5''-dihydrofuran[2'',3'',7'',6']isoflavone	Lupinisoflavone H	438.48	C ₂₅ H ₂₆ O ₇	2958
790	5,2',4'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-8-prenyl-4'',5''-dihydrofuran[2'',3'',7'',6']isoflavone	Erysenegalensein H	438.48	C ₂₅ H ₂₆ O ₇	F308
791	5,2',4'-Trihydroxy-8-prenyl-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'',7'',6']isoflavone	Erysenegalensein I	438.48	C ₂₅ H ₂₆ O ₇	F308
792	5,2',4',5''-Tetrahydroxy-6-prenyl-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'',7'',8']isoflavone	Erysenegalensein O	438.48	C ₂₅ H ₂₆ O ₇	F190
793	5,4'-Dihydroxy-8-(2-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'',4'',3']isoflavone	Millewanin E	438.48	C ₂₅ H ₂₆ O ₇	F110
794	5,7,2'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-6-prenyl-4'',5''-dihydrofuran[2'',3'',4'',3']isoflavone	Lupinisoflavone I	438.48	C ₂₅ H ₂₆ O ₇	2946
795	5,7,2'-Trihydroxy-6-(3-hydroxy-3-methyl-3-butenyl)-6'',6''-dimethylpyrano[2'',3'',4'',3']isoflavone	Kraussianone 3	438.48	C ₂₅ H ₂₆ O ₇	F58
796	5,7,2'-Trihydroxy-6-(3-hydroxy-3-methylbutyl)-6'',6''-dimethylpyrano[2'',3'',4'',3']isoflavone	Kanzonol T	438.48	C ₂₅ H ₂₆ O ₇	F79
797	5,7,2',4'-Tetrahydroxy-3-(2-hydroxy-3-methyl-3-butenyl)-6-prenylisoflavone	Lupinisol C	438.48	C ₂₅ H ₂₆ O ₇	2941
798	5,7,2',4'-Tetrahydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-3'-prenylisoflavone	Lupinisol B	438.48	C ₂₅ H ₂₆ O ₇	2951
799	5,7,2',4'-Tetrahydroxy-6-(2-hydroxy-3-methyl-3-butenyl)-8-prenylisoflavone	Erysenegalensein N	438.48	C ₂₅ H ₂₆ O ₇	F190
800	5,7,2',4'-Tetrahydroxy-8-(2-hydroxy-3-methyl-3-butenyl)-6-prenylisoflavone	Erysenegalensein D	438.48	C ₂₅ H ₂₆ O ₇	F307
801	5,7,2',5''-Tetrahydroxy-6-prenyl-6'',6''-dimethyl-4'',5''-dihydrofuran[2'',3'',4'',3']isoflavone	Lupinisolone B	438.48	C ₂₅ H ₂₆ O ₇	2945
802	5,7,4'-Trihydroxy-5''-(1-hydroxy-1-methylethyl)-6-prenyl-4'',5''-dihydrofuran[2'',3'',2'',3']isoflavone	Lupinisoflavone J	438.48	C ₂₅ H ₂₆ O ₇	2943

803	5,7,4',5'-Tetrahydroxy-6-prenyl-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':2'',3'']isoflavone	438.48	C25H26O7	2942
804	4-Methoxy-7-(2 <i>E</i>)-6,7-dihydroxy-3,7-dimethyl-2-octaenyloxyisoflavanone	438.51	C26H30O6	F330
805	5,7-Dihydroxy-8,2,4',5'-tetramethoxy-8-prenylisoflavone	442.47	C24H26O8	2939
806	7-Keto-6-methoxy-3',4'-methylenedioxy-8,8-diprenylisoflavone	448.51	C27H28O6	F331
807	2-Hydroxy-4',6'-dimethoxy-3'-prenyl-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone	450.49	C26H26O7	2414
808	5,7,2'-Trihydroxy-4',5'-methylenedioxy-6,8-diprenylisoflavone	450.49	C26H26O7	2966
809	5,7-Dihydroxy-3',4'-dimethoxy-6,8-diprenylisoflavone	450.52	C27H30O6	F41
810	5,7-Dimethoxy-3',4'-diprenyloxyisoflavone	450.52	C27H30O6	2508
811	4-Amino-5,7,3'-trihydroxy-5'-methoxy-8,2'-diprenylisoflavone	451.51	C26H29O6N1	F177
811b	4-Sulfate-2'-hydroxy-6,3'-dimethoxy-5'-(2-propenoic acid)isoflavone	452.43	C20H20O10S1	F11b
812	5,7,3',4'-Tetrahydroxy-5'-methoxy-2',6'-diprenylisoflavone	452.50	C26H28O7	F263
813	5,7-Dihydroxy-6-(2,3-dihydroxy-3-methylbutyl)-5'-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuranol[2'',3'':4',3']isoflavone	456.49	C25H28O8	2952
814	5-Hydroxy-2'-methoxy-4',5'-methylenedioxy-6-prenyl-6'',6''-dimethylpyrano[2'',3'':7,8]isoflavone	462.50	C27H26O7	2972
815	5-Hydroxy-3',5'-dimethoxy-4-(1,1,-dimethyl-2-propenyloxy)-5'-(1-methylethenyl)-4'',5''-dihydrofuranol[2'',3'':7,6]isoflavone	464.52	C27H28O7	2920
816	5-Hydroxy-3',5'-dimethoxy-4-(1,1,-dimethyl-2-propenyloxy)-6'',6''-dimethylpyrano[2'',3'':7,6]isoflavone	464.52	C27H28O7	2919
817	5,7-Dihydroxy-2'-methoxy-4',5'-methylenedioxy-6,8-diprenylisoflavone	464.52	C27H28O7	2967
818	6-Methoxy-3',4'-methylenedioxy-7-(7-hydroxy-3,7-dimethyl-2(<i>E</i>),5''-octadienyloxy)isoflavone	464.52	C27H28O7	F68
819	5,4'-Dihydroxy-5'-(1-hydroxyethyl-1-methylethyl)-8-prenyl-4'',5''-dihydrofuranol[2'',3'':7,6]isoflavone	466.53	C27H30O7	2971
820	5,7,4'-Trihydroxy-3',5'-dimethoxy-6,2'-diprenylisoflavone	466.53	C27H30O7	2949
821	5,7,4'-Trihydroxy-5'-methoxy-5''-(1-hydroxy-1-methylethyl)-6'-prenyl-4'',5''-dihydrofuranol[2'',3'':3',2']isoflavone	468.50	C26H28O8	F264
822	5,7,4'-Trihydroxy-5'-methoxy-5''-(1-hydroxy-1-methylethyl)-6'-prenyl-4'',5''-dihydrofuranol[2'',3'':3',2']isoflavone	468.50	C26H28O8	F264
823	5,7,4'-Trihydroxy-5'-methoxy-6'-prenyl-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':3',2']isoflavone	468.50	C26H28O8	F264
824	5,7,4'-Trihydroxy-5'-methoxy-6'-prenyl-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'':3',2']isoflavone	468.50	C26H28O8	F264
825	5-Hydroxy-2',4'-dimethoxy-8-(3-hydroxy-3-methylbutyl)-6'',6''-dimethyl-4'',5''-dihydrofuranol[2'',3'':7,6]isoflavone	468.55	C27H32O7	2509
826	5-Hydroxy-3',4'-dimethoxy-8-(3-hydroxy-3-methylbutyl)-6'',6''-dimethyl-4'',5''-dihydrofuranol[2'',3'':7,6]isoflavone	468.55	C27H32O7	2510
827	5,7,2'-Trihydroxy-6-(2,3-dihydroxy-3-methylbutyl)-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuranol[2'',3'':4',3']isoflavone	472.49	C25H28O9	2953
828	5,7,4'-Trihydroxy-5'-(2 <i>E</i>)-3,7-dimethyl-2,6-octadienyl)-2'-prenylisoflavone	474.60	C30H34O5	F110
829	5,7,4'-Trihydroxy-6,8,3'-triprenylisoflavone	474.60	C30H34O5	2976
830	5,7,4'-Trihydroxy-8,3',5'-triprenylisoflavone	474.60	C30H34O5	2865

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
831	5,2,4'-Trihydroxy-6,3'-diprenyl-6'',6''-dimethylpyranof[2'',3'':7,8]isoflavone	Euchrenone b16	488.57	C30H32O6	F175
832	5,3,4'-Trihydroxy-8-prenyl-6'',6''-dimethyl-5''-(1,1-dimethyl-2-propenyl)pyranof[2'',3'':7,6]isoflavone	Flemphilippinin A	488.57	C30H32O6	F41
833	5,7,3',4'-Tetrahydroxy-5'-(2E)-3,7-dimethyl-2,6-octadienyl)-2'-prenylisoflavone	Millewanin C	490.59	C30H34O6	F110
834	5,7,2',4'-Tetrahydroxy-6,8,3'-triprenylisoflavone	Euchrenone b2	490.60	C30H34O6	2977
835	5,4'-Dihydroxy-2'-methoxy-6,3'-diprenyl-6'',6''-dimethylpyranof[2'',3'':7,8]isoflavone	Euchrenone b13	502.60	C31H34O6	F175
836	5,4'-Dihydroxy-2'-methoxy-8,3'-diprenyl-6'',6''-dimethylpyranof[2'',3'':7,6]isoflavone	Euchrenone b12	502.60	C31H34O6	F175
837	5,7,4'-Trihydroxy-2'-methoxy-6,8,3'-triprenylisoflavone	Euchrenone b14	504.61	C31H36O6	F175
838	5,7,4'-Trihydroxy-3'-methoxy-5'-(2E)-3,7-dimethyl-2,6-octadienyl)-2'-prenylisoflavone	Millewanin B	504.61	C31H36O6	F110
839	5,5'-Dihydroxy-2'-methoxy-6-prenyl-(2'',3'':7,8),(2'',3'':4',3')-bis(6,6-dimethylpyranof[2'',3'':7,8])isoflavone	Euchrenone b11	516.48	C31H32O7	F175
840	5,5'-Dihydroxy-2'-methoxy-8-prenyl-(2'',3'':7,6),(2'',3'':4',3')-bis(6,6-dimethylpyranof[2'',3'':7,6])isoflavone	Euchrenone b10	516.48	C31H32O7	F175
841	5,7-Dihydroxy-8-(2-hydroxy-3-methyl-3-butenyl)-4-(1-(hydroxymethyl)pentacosyloxy)-6-prenylisoflavone	Indicanin D	803.16	C51H78O7	F184
Isoflavone glycosides					
842	7,4'-Dihydroxyisoflavone 7-O-(2-O-methylrhamnoside)	Daidzein G 3	414.41	C22H22O8	F93
843	5,7,4'-Trihydroxyisoflavone 5-O-rhamnoside	Genestein G 1	416.39	C21H20O9	F93
844	5,7,4'-Trihydroxyisoflavone 7-O-rhamnoside	Genistein 4'-O-rhamnoside	416.39	C21H20O9	F224
845	7,4'-Dihydroxyisoflavone 4'-O-glucoside	Daidzein 4'-O-glucoside	416.39	C21H20O9	3110
846	7,4'-Dihydroxyisoflavone 7-O-glucoside	Daidzein 7-O-glucoside	416.39	C21H20O9	3108
847	7,8-Dihydroxy-4'-methoxyisoflavone 7-O-arabinoside	Retusin 7-O-arabinoside	416.39	C21H20O9	3215
848	4'-Hydroxy-7-methoxyisoflavone-4'-O-glucoside	Isoononin	430.41	C22H22O9	F351
849	5,7-Dihydroxy-6-methoxyisoflavone 7-O-rhamnoside		430.41	C22H22O9	3191
850	7-Hydroxy-4'-methoxyisoflavone 7-O-glucoside	Formononetin 7-O-glucoside	430.41	C22H22O9	3113
851	5,7,2'-Trihydroxyisoflavone 7-O-glucoside	Isogenistein 7-O-glucoside	432.39	C21H20O10	3148
852	5,7,4'-Trihydroxyisoflavone 4'-O-glucoside	Genistein 4'-O-glucoside	432.39	C21H20O10	3141
853	5,7,4'-Trihydroxyisoflavone 5-O-glucoside	Genistein 5-O-glucoside	432.39	C21H20O10	3207
854	5,7,4'-Trihydroxyisoflavone 7-O-glucoside	Genistein 7-O-glucoside	432.39	C21H20O10	3139
855	6,7,4'-Trihydroxyisoflavone 4'-O-glucoside	Demethyltaxasin 4'-O-glucoside	432.39	C21H20O10	3117
856	7,2,4'-Trihydroxyisoflavone-4'-O-glucoside		432.39	C21H20O10	F179
857	7-Hydroxy-3',4'-methylenedioxyisoflavone 7-O-glucoside	Pseudobaptigenin 7-O-glucoside	444.40	C22H20O10	3118

858	7,4'-Dihydroxy-6-methoxyisoflavone 7-O-(2-O-methylrhamnoside)	Daidzein G 2	444.43	C23H24O9	F93
859	5,4'-Dihydroxy-7-methoxyisoflavone 4-O-galactoside	Prunetin 4'-O-galactoside	446.41	C22H22O10	3213
860	5,4'-Dihydroxy-7-methoxyisoflavone 4-O-glucoside	Prunetin 4'-O-glucoside	446.41	C22H22O10	3155
861	5,4'-Dihydroxy-7-methoxyisoflavone 5-O-glucoside	Prunetin 5-O-glucoside	446.41	C22H22O10	3212
862	5,7-Dihydroxy-3'-methoxyisoflavone 7-O-glucoside	Mutabilin	446.41	C22H22O10	F55
863	5,7-Dihydroxy-4'-methoxyisoflavone 7-O-glucoside	Biochanin A 7-O-glucoside	446.41	C22H22O10	3149
864	5,7-Dihydroxy-4'-methoxyisoflavone 8-C-glucoside	4-O-Methyl-genistein-8-C-glucoside	446.41	C22H22O10	F2
865	6,7-Dihydroxy-4'-methoxyisoflavone 7-O-glucoside	Texasin 7-O-glucoside	446.41	C22H22O10	3123
866	7,3'-Dihydroxy-4'-methoxyisoflavone 7-O-galactoside	Calycosin 7-O-galactoside	446.41	C22H22O10	3205
867	7,3'-Dihydroxy-4'-methoxyisoflavone 7-O-glucoside	Calycosin 7-O-glucoside	446.41	C22H22O10	3121
868	7,4'-Dihydroxy-5-methoxyisoflavone 4-O-glucoside	Genistein 5-methyl ether 4'-glucoside	446.41	C22H22O10	F194
869	7,4'-Dihydroxy-5-methoxyisoflavone 7-O-glucoside	7-O-Methylgenistein 7-O-glucoside	446.41	C22H22O10	3211
870	7,4'-Dihydroxy-6-methoxyisoflavone 7-O-glucoside	Glycetein 7-O-glucoside	446.41	C22H22O10	3124
871	7,5'-Hydroxy-3'-methoxyisoflavone-7-O-glucoside	Retusin 7-O-glucoside	446.41	C22H22O10	3190
872	7,8-Dihydroxy-4'-methoxyisoflavone 7-O-glucoside	Derriscandenoside A	446.41	C22H22O10	F34
873	7,8-Dihydroxy-4'-methoxyisoflavone 8-O-glucoside	2-Hydroxygenistein 4'-O-glucoside	448.39	C21H20O11	F240
874	5,7,2',4'-Tetrahydroxyisoflavone 4-O-glucoside	2-Hydroxygenistein 7-O-glucoside	448.39	C21H20O11	F240
875	5,7,2',4'-Tetrahydroxyisoflavone 7-O-glucoside	Orobol 7-O-glucoside	448.39	C21H20O11	3156
876	5,7,3',4'-Tetrahydroxyisoflavone 8-C-glucoside	Daidzin 6'-O-acetate	458.42	C23H22O10	3109
877	5,7,3',4'-Tetrahydroxyisoflavone 7-O-glucoside	Irilone 4'-O-glucoside	458.47	C24H26O9	3197
878	7,4'-Dihydroxyisoflavone 7-O-(6'-acetylglucoside)	6-Hydroxypseudobaptigenin 7-O-glucoside	460.39	C22H20O11	3161
879	7-Hydroxy-5,4'-dimethoxy-8-methylisoflavone 7-O-rhamnoside	6-Hydroxypseudobaptigenin 7-O-glucoside	460.39	C22H20O11	3159
880	5,4'-Dihydroxy-6,7-methylenedioxyisoflavone 4-O-glucoside	460.44	C23H24O10	F61	
881	5,7-Dihydroxy-3',4'-methylenedioxyisoflavone 7-O-glucoside	460.44	C23H24O10	F226	
882	5,7-Dihydroxy-3',4'-dimethoxyisoflavone 7-O-rhamnoside	460.44	C23H24O10	3192	
883	5,7-Dihydroxy-6,2'-dimethoxyisoflavone 7-O-rhamnoside	Irisolidone 7-O-rhamnoside	460.44	C23H24O10	3126
884	5,7-Dihydroxy-6,4'-dimethoxyisoflavone 7-O-rhamnoside	Cladrin 7-O-glucoside	460.44	C23H24O10	3126
885	7-Hydroxy-3',4'-dimethoxyisoflavone 7-O-glucoside	Aformosin 7-O-galactoside	460.44	C23H24O10	F198
886	7-Hydroxy-6,4'-dimethoxyisoflavone 7-O-galactoside	Aformosin 7-O-glucoside	460.44	C23H24O10	3128
887	7-Hydroxy-6,4'-dimethoxyisoflavone 7-O-glucoside	8-O-Methylretusin 7-O-glucoside	460.44	C23H24O10	3131
888	7-Hydroxy-8,4'-dimethoxyisoflavone 7-O-glucoside	462.41	C22H22O10	F129	
889	5,3',4'-Trihydroxy-7-methoxyisoflavone 3'-O-glucoside	Pratensein 7-O-glucoside	462.41	C22H22O10	3162
890	5,7,3'-Trihydroxy-4'-methoxyisoflavone 7-O-glucoside		462.41	C22H22O10	F61
891	5,7,3',4'-Tetrahydroxy-8-methylisoflavone 7-O-glucoside				

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
892	5,7,4'-Trihydroxy-3'-methoxyisoflavone 7- <i>O</i> -glucoside	3'- <i>O</i> -Methylorobol 7- <i>O</i> -glucoside	462.41	C22H22O11	3163
893	5,7,4'-Trihydroxy-6-methoxyisoflavone 4'- <i>O</i> -glucoside	Tectorigenin 4'-glucoside	462.41	C22H22O11	F236
894	5,7,4'-Trihydroxy-6-methoxyisoflavone 7- <i>O</i> -glucoside	Tectorigenin 7- <i>O</i> -glucoside	462.41	C22H22O11	3164
895	5,7,4'-Trihydroxy-8-methoxyisoflavone 7- <i>O</i> -glucoside	Isotectorigenin 7- <i>O</i> -glucoside	462.41	C22H22O11	3217
896	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-acetylglucoside)	Formononetin 7- <i>O</i> -(6''-acetylglucoside)	472.45	C24H24O10	3201
897	4'-Hydroxy-5-methoxy-6,7-methylenedioxyisoflavone 4'- <i>O</i> -glucoside	Germanaism B	474.42	C23H22O11	F17
898	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-acetylglucoside)	Genistin 6''- <i>O</i> -acetate	474.42	C23H22O11	3140
899	6,3'-Dihydroxy-7-methoxy-4',5'-methylenedioxyisoflavone 6- <i>O</i> -rhamnoside		474.42	C23H22O11	F356
900	6,7-Dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6- <i>O</i> -rhamnoside		474.42	C23H22O11	F312
901	7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -glucoside	Fujikinetin 7- <i>O</i> -glucoside	474.42	C23H22O11	3133
902	7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -glucoside	6'-Methoxy pseudobaptigenin 7- <i>O</i> -glucoside	474.42	C23H22O11	F137
903	5,7,2'-Trihydroxy-4',5'-methylenedioxyisoflavone 2'- <i>O</i> -glucoside		476.39	C22H20O12	F197
904	5,4'-Dihydroxy-6,7-dimethoxyisoflavone 4'- <i>O</i> -galactoside		476.44	C23H24O11	3216
905	5,4'-Dihydroxy-6,7-dimethoxyisoflavone 4'- <i>O</i> -glucoside	7- <i>O</i> -Methyltectorigenin 4'- <i>O</i> -galactoside	476.44	C23H24O11	3169
906	5,7-Dihydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -galactoside	7- <i>O</i> -Methyltectorigenin 4'- <i>O</i> -glucoside	476.44	C23H24O11	F231
907	5,7-Dihydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -glucoside	Irisolidone 7- <i>O</i> -glucoside	476.44	C23H24O11	3167
908	5,7-Dihydroxy-8,4'-dimethoxyisoflavone 7- <i>O</i> -glucoside		476.44	C23H24O11	F7
909	7,2'-Dihydroxy-3',4'-dimethoxyisoflavone 7- <i>O</i> -glucoside		476.44	C23H24O11	3206
910	7,3'-Dihydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -glucoside		476.44	C23H24O11	F302
911	5,6,7,4'-Tetrahydroxy-8-methoxyisoflavone 7- <i>O</i> -glucoside	Odoratin-7- <i>O</i> -glucoside	478.41	C22H22O12	F225
912	7,4'-Dihydroxy-6-methoxyisoflavone 7- <i>O</i> -(6''-acetylglucoside)	Glycitein 7- <i>O</i> -(6''-acetylglucoside)	488.45	C24H24O11	F131
913	5,4'-Dihydroxy-2'-methoxy-6,7-methylenedioxyisoflavone 4'- <i>O</i> -glucoside	Iriflogenin 4'- <i>O</i> -glucoside	490.32	C23H22O12	3172
914	5,7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -galactoside	Dalispinin 7- <i>O</i> -galactoside	490.32	C23H22O12	3195
915	6,3'-Dihydroxy-7-methoxy-4',5'-methylenedioxyisoflavone 6- <i>O</i> -glucoside		490.32	C23H22O12	F356
916	6,7-Dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6- <i>O</i> -glucoside		490.32	C23H22O12	F312
917	7-Hydroxy-2',4',5'-trimethoxyisoflavone 7- <i>O</i> -glucoside	5-Methoxyafformosin 7- <i>O</i> -glucoside	490.47	C24H26O11	3135
918	7-Hydroxy-5,6,4'-trimethoxyisoflavone 7- <i>O</i> -glucoside	Cladrastin 7- <i>O</i> -glucoside	490.47	C24H26O11	3173
919	7-Hydroxy-6,3',5'-trimethoxyisoflavone 7- <i>O</i> -glucoside		490.47	C24H26O11	3136
920	5,7,2'-Trihydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -glucoside	Iristectorigenin A 7- <i>O</i> -glucoside	492.44	C23H24O12	3175
921	5,7,4'-Trihydroxy-6,3'-dimethoxyisoflavone 7- <i>O</i> -glucoside	Iristectorigenin B 7- <i>O</i> -glucoside	492.44	C23H24O12	3176
922	5,7,4'-Trihydroxy-8,3'-dimethoxyisoflavone 7- <i>O</i> -glucoside	Homotectorigenin 7- <i>O</i> -glucoside	492.44	C23H24O12	3177

922b	7,2',5'-Trihydroxy-6,4'-dimethoxyisoflavone 2'- <i>O</i> -glucoside	Licoagroside A	492.44	C23H24O12	F141b
923	5,7,4'-Trihydroxy-6-prenylisoflavone 7- <i>O</i> -glucoside	Genisteone	500.49	C26H28O10	F202
924	7,4'-Dihydroxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Daidzin 6''- <i>O</i> -malonate	502.43	C24H22O12	F135
925	7,4'-Dihydroxyisoflavone 8- <i>C</i> -(6''-malonylglucoside)	Puerarin 6''- <i>O</i> -malonate	502.43	C24H22O12	F135
926	4-Hydroxy-5,3'-dimethoxy-6,7-methylenedioxyisoflavone 4'- <i>O</i> -glucoside	Germanaisin A	504.45	C24H24O12	F17
927	7-Hydroxy-2',6-dimethoxy-4',5'-methylenedioxyisoflavone 7- <i>O</i> -glucoside	Dalpatenin 7- <i>O</i> -glucoside	504.45	C24H24O12	3138
928	7-Hydroxy-5,6-dimethoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -glucoside	Isoplatycarpanetin 7- <i>O</i> -glucoside	504.45	C24H24O12	3178
929	7-Hydroxy-5,8-dimethoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -glucoside	Platycarpanetin 7- <i>O</i> -glucoside	504.45	C24H24O12	3179
930	5,2',3'-Trihydroxy-7-methoxy-2-hydroxymethyl-6-methylisoflavone 3'- <i>O</i> -glucoside	Mirabilajone C	506.46	C24H26O12	F316
931	5,3'-Dihydroxy-7,4',5'-trimethoxyisoflavone 3'- <i>O</i> -glucoside	Vavain 3'- <i>O</i> -glucoside	506.46	C24H26O12	F189
932	5,7'-Dihydroxy-6,3',4'-trimethoxyisoflavone 7- <i>O</i> -glucoside	Junipeginin 7- <i>O</i> -glucoside	506.46	C24H26O12	3194
933	6,8,3',5'-Tetrahydroxy-7,4'-dimethoxyisoflavone 6- <i>O</i> -glucoside		508.43	C23H24O13	F208
934	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(2'',6''-diacetylglucoside)	2'',6''- <i>O</i> -Diacetyloninin	514.48	C26H26O11	F92
935	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-succinylglucoside)	Genistein 7- <i>O</i> -(6''-succinylglucoside)	516.45	C25H24O12	F294
936	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Formononetin 7- <i>O</i> -(6''-malonylglucoside)	516.46	C25H24O12	3116
937	5,7,2',4'-Tetrahydroxy-3'-prenylisoflavone 4'- <i>O</i> -glucoside	licoisoflavone A 4- <i>O</i> -glucoside	516.49	C26H28O11	F240
938	5,7,2',4'-Tetrahydroxy-3'-prenylisoflavone 7- <i>O</i> -glucoside	licoisoflavone A 7- <i>O</i> -glucoside	516.49	C26H28O11	F240
939	5,7,2',4'-Tetrahydroxy-6-prenylisoflavone 7- <i>O</i> -glucoside	luteone 7- <i>O</i> -glucoside	516.49	C26H28O11	F240
940	5,7,4'-Trihydroxyisoflavone 4'- <i>O</i> -(6''-malonylglucoside)	Genistein 7- <i>O</i> -(6''-malonylglucoside)	518.43	C24H22O13	F240
941	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Genistein 7- <i>O</i> -(6''-malonylglucoside)	518.43	C24H22O13	3147
942	5,7,4'-Trihydroxyisoflavone 8- <i>C</i> -(6''-malonylglucoside)	Genistein 8- <i>C</i> -glucoside 6''- <i>O</i> -malonate	518.43	C24H22O13	F135
943	5,7,3'-Trihydroxy-6,4',5'-trimethoxyisoflavone 7- <i>O</i> -glucoside	Irigenin 7- <i>O</i> -glucoside	522.46	C24H26O13	3181
944	5,7,4'-Trihydroxy-6,3',5'-trimethoxyisoflavone 7- <i>O</i> -glucoside		522.46	C24H26O13	3196
945	5,7-Dihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Biochanin A 7- <i>O</i> -(6''-malonylglucoside)	532.46	C25H24O13	3154
946	7,4'-Dihydroxyisoflavone 7- <i>O</i> -(6''-succinylglucoside)	Daidzein 7- <i>O</i> -(6''-succinylglucoside)	532.46	C25H24O13	F294
947	5,7,2',4'-Tetrahydroxyisoflavone 4'- <i>O</i> -(6''-malonylglucoside)	2'-Hydroxygenistein 7- <i>O</i> -(6''-malonylglucoside)	534.43	C24H22O14	F240
948	5,7,2',4'-Tetrahydroxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	2'-Hydroxygenistein 7- <i>O</i> -(6''-malonylglucoside)	534.43	C24H22O14	F240
949	5,7,3',4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Obrol 7- <i>O</i> -(6''-malonylglucoside)	534.43	C24H22O14	3187
950	5,7-Dihydroxy-6,2',4',5'-tetramethoxyisoflavone 7- <i>O</i> -glucoside	Caviunin 7- <i>O</i> -glucoside	536.49	C25H28O13	3182
951	5,7-Dihydroxy-6,2',4',5'-tetramethoxyisoflavone 8- <i>C</i> -glucoside	Dalpaniculin	536.49	C25H28O13	F174
952	5,7-Dihydroxy-8,2',4',5'-tetramethoxyisoflavone 7- <i>O</i> -glucoside	Isocaviunin 7- <i>O</i> -glucoside	536.49	C25H28O13	3184
953	7,5'-Dihydroxy-5,4'-dimethoxy-3'-prenylisoflavone 7- <i>O</i> -galactoside		544.55	C28H32O11	F328
954	7-Hydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Aformosin 7- <i>O</i> -(6''-malonylglucoside)	546.48	C26H26O13	3214
955	7,4'-Dihydroxy-6-methoxyisoflavone 7- <i>O</i> -(6''-succinylglucoside)	Glycitein 7- <i>O</i> -(6''-succinylglucoside)	546.48	C26H26O13	F294

continued

APPENDIX

Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
956	5,7,3'-Trihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-malonylglucoside)	Pratensein 7- <i>O</i> -(6''-malonylglucoside)	548.46	C ₂₅ H ₂₄ O ₁₄	3189
957	7,4'-Dihydroxyisoflavone 7- <i>O</i> -(6''-apiosylglucoside)	Daidzein 7- <i>O</i> -(6''-apiosylglucoside)	548.50	C ₂₆ H ₂₈ O ₁₃	3200
958	7,4'-Dihydroxyisoflavone 7- <i>O</i> -glucoside-4'- <i>O</i> -apioside	Daidzein 7- <i>O</i> -glucoside-4'- <i>O</i> -apioside	548.50	C ₂₆ H ₂₈ O ₁₃	3199
959	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(2''- <i>p</i> -hydroxybenzoylglucoside)	Formononetin 7- <i>O</i> -(2''- <i>p</i> -hydroxybenzoylglucoside)	550.52	C ₂₉ H ₂₆ O ₁₁	3204
960	5,7,4'-Trihydroxyisoflavone 7,4'-bis(3-cymaroside)	Genistein 4',7- <i>O</i> -bis(3-cymaroside)	558.57	C ₂₉ H ₃₄ O ₁₁	F159
961	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(2''-apiosylglucoside)	Formononetin 7- <i>O</i> -(6''-apiosylglucoside)	562.53	C ₂₇ H ₃₀ O ₁₃	3202
962	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-xylosylglucoside)	Formononetin 7- <i>O</i> -(6''-xylosylglucoside)	562.53	C ₂₇ H ₃₀ O ₁₃	3203
963	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(rhamnosylglucoside)	Daidzein 7- <i>O</i> -rhamnosylglucoside	562.53	C ₂₇ H ₃₀ O ₁₃	3112
964	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-apiosylglucoside)	Genistein 7- <i>O</i> -(6''-apiosylglucoside)	564.50	C ₂₆ H ₂₈ O ₁₄	3208
965	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-arabinosylglucoside)	Genistin 6''- <i>O</i> -arabinosyl	564.50	C ₂₆ H ₂₈ O ₁₄	F323
966	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-xylosylglucoside)	Genistin 6''- <i>O</i> -xylosyl	564.50	C ₂₆ H ₂₈ O ₁₄	F323
967	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -glucoside-4'- <i>O</i> -apioside	Genistein 7- <i>O</i> -glucoside-4'- <i>O</i> -apioside	564.50	C ₂₆ H ₂₈ O ₁₄	3210
968	5,7,4'-Trihydroxyisoflavone 7,4'- <i>O</i> -di(2- <i>O</i> -methylrhamnoside)	Daidzein G 1	574.58	C ₂₉ H ₃₄ O ₁₂	F93
969	5,7,4'-Trihydroxyisoflavone 5- <i>O</i> -rhamnoside-7-(2- <i>O</i> -methylrhamnoside)	Genestein G 2	576.56	C ₂₈ H ₃₂ O ₁₃	F93
970	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-rhamnosylglucoside)	Formononetin 7- <i>O</i> -rutinoside	576.56	C ₂₈ H ₃₂ O ₁₃	3114
971	4',7-Dihydroxy-6-methoxyisoflavone 7- <i>O</i> -(6''-xylosylglucoside)		578.53	C ₂₇ H ₃₀ O ₁₄	F195
972	5,7-Dihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(2''-apiosylglucoside)	Coromandelin	578.53	C ₂₇ H ₃₀ O ₁₄	F211
973	5,7-Dihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-apiosylglucoside)	Biochanin A 7- <i>O</i> -(6''-apiosylglucoside)	578.53	C ₂₇ H ₃₀ O ₁₄	3153
974	5,7-Dihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-xylosylglucoside)	Biochanin A 7- <i>O</i> -(6''-xylosylglucoside)	578.53	C ₂₇ H ₃₀ O ₁₄	3152
975	5,7,4'-Trihydroxyisoflavone 4'- <i>O</i> -(2''-rhamnosylglucoside)	Genistein 7- <i>O</i> -neohesperidostide	578.53	C ₂₇ H ₃₀ O ₁₄	3145
976	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(2''- <i>p</i> -coumaroylglucoside)	Genistein 7- <i>O</i> -(2''- <i>p</i> -coumaroylglucoside)	578.53	C ₃₀ H ₂₆ O ₁₂	3209
977	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -(6''-rhamnosylglucoside)	Genistein 7- <i>O</i> -rutinoside	578.53	C ₂₇ H ₃₀ O ₁₄	3144
978	7,4'-Dihydroxy-6-methoxyisoflavone 7-(6''-xylosylglucoside)	Tectorigenin 7- <i>O</i> -(6''-xylosylglucoside)	578.53	C ₂₇ H ₃₀ O ₁₄	F188
979	7,4'-Dihydroxyisoflavone 7- <i>O</i> -glucoside-4'- <i>O</i> -glucoside	Daidzein 7,4'- <i>O</i> -diglucoside	578.53	C ₂₇ H ₃₀ O ₁₄	3111
980	5,2'-Dihydroxy-8-prenyl-6'',6''-dimethylpyrano[2',3':7,6]isoflavone 4'- <i>O</i> -glucoside	Auriculatin 4'- <i>O</i> -glucoside	582.60	C ₃₁ H ₃₄ O ₁₁	F183
981	7-Hydroxy-3,4'-methylenedioxyisoflavone 7- <i>O</i> -(rhamnosylglucoside)	Pseudobaptigenin 7- <i>O</i> -rhamnosylglucoside	590.54	C ₂₈ H ₃₀ O ₁₄	3119
982	4',5-Dihydroxy-7-methoxyisoflavone 3'- <i>O</i> -(3''- <i>E</i> -cinnamoylglucoside)		590.55	C ₃₁ H ₂₈ O ₁₂	F128
983	5,7-Dihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-rhamnosylglucoside)	Biochanin A 7- <i>O</i> -rutinoside	592.56	C ₂₈ H ₃₂ O ₁₄	3150
984	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(3''-glucosylglucoside)	Formononetin 7- <i>O</i> -laminaribioside	592.56	C ₂₈ H ₃₂ O ₁₄	3115
985	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-glucosylglucoside)	Formononetin 7- <i>O</i> -gentiobioside	592.56	C ₂₈ H ₃₂ O ₁₄	F96
986	7-Hydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -(2''-apiosylglucoside)	Afromosin 7- <i>O</i> -(2''-apiosylglucoside)	592.56	C ₂₈ H ₃₂ O ₁₄	F296

987	7,3'-Dihydroxy-4'-methoxyisoflavone 7-O-(rhamnonylglucoside)	592.56	C28H32O14	3122
988	7,8-Dihydroxy-4'-methoxyisoflavone 7-O-(2''-rhamnonylglucoside)	592.56	C28H32O14	3125
989	7,8-Dihydroxy-4'-methoxyisoflavone 7-O-(6''-rhamnonylglucoside)	592.56	C28H32O14	F218
990	4',7-Dihydroxy-6-methoxyisoflavone 7-O-(6''-xylosylglucoside)	594.53	C27H30O15	F195
991	5,6,7,4'-Tetrahydroxyisoflavone 7-O-(rhamnonylglucoside)	594.53	C27H30O15	3158
992	5,7,2',4'-Tetrahydroxyisoflavone 6-C-(2''-rhamnonylglucoside)	594.53	C27H30O15	F108
993	5,7,3',4'-Tetrahydroxyisoflavone 7-O-(rhamnonylglucoside)	594.53	C27H30O15	3157
994	5,7,4'-Trihydroxyisoflavone 7-O-(6''-apiosylglucoside)	594.53	C27H30O15	F65
995	5,7,4'-Trihydroxyisoflavone 7-O-(glucosylglucoside)	594.53	C27H30O15	3142
996	5,7,4'-Trihydroxyisoflavone 7-O-glucoside-4'-O-glucoside	594.53	C27H30O15	3143
997	5,7,4'-Trihydroxyisoflavone 8-C-glucoside-4'-O-glucoside	594.53	C27H30O15	F319
998	7,2',4'-Trihydroxyisoflavone 7-O-glucoside-4'-O-glucoside	594.53	C27H30O15	3186
999	7-Hydroxy-3',4'-methylenedioxyisoflavone 7-O-(3''-glucosylglucoside)	606.54	C28H30O15	3120
1000	7-Hydroxy-6,4'-dimethoxyisoflavone 7-O-(6''-rhamnonylglucoside)	606.58	C29H34O14	F218
1001	7-Hydroxy-8,4'-dimethoxyisoflavone 7-O-(6''-rhamnonylglucoside)	606.58	C29H34O14	F140
1002	8-Hydroxy-7,4'-dimethoxyisoflavone 8-O-(6''-rhamnonylglucoside)	606.58	C29H34O14	F218
1003	5,4'-Dihydroxy-6,7-dimethoxyisoflavone 4'-O-(6''-apiosylglucoside)	608.54	C28H32O15	F160
1004	5,7-Dihydroxy-4'-methoxyisoflavone 7-O-(6''-glucosylglucoside)	608.54	C28H32O15	3151
1005	5,7-Dihydroxy-6,4'-dimethoxyisoflavone 7-O-(2''-apiosylglucoside)	608.54	C28H32O15	F295
1006	5,7-Dihydroxy-6,4'-dimethoxyisoflavone 7-O-(xylosylglucoside)	608.54	C28H32O15	3168
1007	7,4'-Dihydroxy-5-methoxyisoflavone 7,4'-O-diglucoiside	608.54	C28H32O15	F319
1008	5,6,7,4'-Tetrahydroxyisoflavone 4'-O-(6''-glucosylglucoside)	610.53	C27H30O16	F16
1009	5,7,2',4'-Tetrahydroxyisoflavone 7,4'-O-diglucoiside	610.53	C27H30O16	F319
1010	5,7,3',4'-Tetrahydroxyisoflavone 7-O-(2''-glucosylglucoside)	610.53	C27H30O16	3188
1011	5,7-Dihydroxy-3',4'-methylenedioxyisoflavone 7-O-(glucosylglucoside)	622.54	C28H30O16	3160
1012	6,3'-Dihydroxy-7-methoxy-4',5'-methylenedioxyisoflavone 6-O-(6''-xylosylglucoside)	622.54	C28H30O16	F356
1013	6,7-Dihydroxy-3'-methoxy-4',5'-methylenedioxyisoflavone 6-O-(6''-xylosylglucoside)	622.54	C28H30O16	F312
1014	5,4'-Dihydroxy-6,7-dimethoxyisoflavone 4'-O-(rhamnonylglucoside)	622.58	C29H34O15	3170
1015	5,7-Dihydroxy-6,4'-dimethoxyisoflavone 7-O-(6''-rhamnonylglucoside)	622.58	C29H34O15	F218
1016	7-Hydroxy-3',4'-dimethoxyisoflavone 7-O-(3''-glucosylglucoside)	622.58	C29H34O15	3127
1017	7-Hydroxy-6,4'-dimethoxyisoflavone 7-O-(3''-glucosylglucoside)	622.58	C29H34O15	3130
1018	7-Hydroxy-8,4'-dimethoxyisoflavone 7-O-(3''-glucosylglucoside)	622.58	C29H34O15	3132
1019	5,7,4'-Trihydroxy-6-methoxyisoflavone 4'-O-(6''-glucosylglucoside)	624.56	C28H32O16	F66
1020	5,7,4'-Trihydroxy-6-methoxyisoflavone 7-O-(6''-glucosylglucoside)	624.56	C28H32O16	3165
	Calycosin 7-O-rhamnonylglucoside			
	Retusin 7-O-neohesperidose			
	Derriscandenoside B			
	6-Hydroxygenistein 7-O-rhamnonylglucoside			
	Nodosin			
	Orobol 7-O-rhamnonylglucoside			
	Tectorigenin 7-O-(6''-apiosylglucoside)			
	Genistein 7-O-glucosylglucoside			
	Genistein 7,4'-O-glucoside			
	Genistein 8-C-glucoside-4'-O-glucoside			
	2'-Hydroxydaidzein 7,4'-O-diglucoiside			
	Pseudobaptigenin 7-O-laminariobioside			
	Derriscandenoside D			
	Derriscandenoside B			
	Derriscandenoside C			
	Biochanin A 7-O-gentiobioside			
	Pubescidin			
	Irisolidone 7-O-xylosylglucoside			
	Isoprunein 7,4'-O-diglucoiside			
	Germanaism D			
	2'-Hydroxygenistein 7,4'-O-diglucoiside			
	Orobol 7-O-sophoroside			
	7-O-Methyltectorigenin 4'-O-rhamnonylglucoside			
	Derriscandenoside E			
	Cladrin 7-O-laminariobioside			
	Afrormosin 7-O-laminariobioside			
	8-O-Methylretusin 7-O-laminariobioside			
	Tectorigenin 4'-O-(6''-glucosylglucoside)			
	Tectorigenin 7-O-gentiobioside			

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1021	5,7,4'-Trihydroxy-6-methoxyisoflavone 7- <i>O</i> -glucoside-4'- <i>O</i> -glucoside	Tectorigenin 7,4'- <i>O</i> -diglucoside	624.56	C ₂₈ H ₃₂ O ₁₆	F250
1022	4'-Hydroxy-5-methoxy-6,7-methylenedioxyisoflavone 4'- <i>O</i> -(6''-glucosylglucoside)	Germanaism E	636.56	C ₂₉ H ₃₂ O ₁₆	F16
1023	7-Hydroxy-6-methoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -(3''-glucosylglucoside)	Fujikinetin 7- <i>O</i> -laminariobioside	636.56	C ₂₉ H ₃₂ O ₁₆	3134
1024	5,3'-Dihydroxy-7,4',5'-trimethoxyisoflavone 3'- <i>O</i> -(6''-arabinosylglucoside)		638.58	C ₂₉ H ₃₄ O ₁₆	F298
1025	5,4'-Dihydroxy-6,7-dimethoxyisoflavone 4'- <i>O</i> -(6''-glucosylglucoside)	7- <i>O</i> -Methyltectorigenin 4'- <i>O</i> -geniobioside	638.58	C ₂₉ H ₃₄ O ₁₆	3171
1026	5,7,3'-Trihydroxy-6,4',5'-trimethoxyisoflavone 7-(6''-(4-hydroxybenzoyl)glucoside)	6''- <i>O</i> - <i>p</i> -Hydroxybenzoyliridin	642.56	C ₃₁ H ₃₀ O ₁₅	F112
1027	7-Hydroxy-4'-methoxy-3'-prenylisoflavone 7- <i>O</i> -(2''- <i>p</i> -coumaroyl)glucoside)	4'- <i>O</i> -Methylneobavaisoflavone 7- <i>O</i> -(2''- <i>p</i> -coumaroyl)glucoside)	644.68	C ₃₆ H ₃₆ O ₁₁	3218
1028	5-Hydroxy-7,3',4'-trimethoxy-8-methylisoflavone 5- <i>O</i> -(2''-rhamnopylglucoside)		650.64	C ₃₁ H ₃₈ O ₁₅	3198
1029	5,7-Dihydroxy-6,3',4'-trimethoxyisoflavone 7- <i>O</i> -(6''-rhamnopylglucoside)		652.61	C ₃₀ H ₃₆ O ₁₆	F326
1030	7-Hydroxy-5,6,4'-trimethoxyisoflavone 7- <i>O</i> -(3''-glucosylglucoside)	5-Methoxyafnormosin 7- <i>O</i> -laminariobioside	652.61	C ₃₀ H ₃₆ O ₁₆	3174
1031	7-Hydroxy-6,3',4'-trimethoxyisoflavone 7- <i>O</i> -(3''-glucosylglucoside)	Cladrastin 7- <i>O</i> -laminariobioside	652.61	C ₃₀ H ₃₆ O ₁₆	3137
1032	5,7,2'-Trihydroxy-6,4'-dimethoxyisoflavone 7- <i>O</i> -(6''-glucosylglucoside)	Iristectorigenin 7- <i>O</i> -gentiobioside	654.58	C ₂₉ H ₃₄ O ₁₇	3193
1033	5,7,4'-Trihydroxy-6,3'-dimethoxyisoflavone 7- <i>O</i> -(6''-glucosylglucoside)	Iristectorigenin B 7- <i>O</i> -(6''-glucosylglucoside)	654.58	C ₂₉ H ₃₄ O ₁₇	F66
1034	7-Hydroxy-5,8-dimethoxy-3',4'-methylenedioxyisoflavone 7- <i>O</i> -(3''-glucosylglucoside)	Platycarpanetin 7- <i>O</i> -laminariobioside	666.59	C ₃₀ H ₃₄ O ₁₇	3180
1035	5,7,3'-Trihydroxy-6,4',5'-trimethoxyisoflavone 7-(6''- <i>O</i> -(4-hydroxy-3-methoxybenzoyl)glucoside)	Shigansu A	672.59	C ₃₂ H ₃₂ O ₁₆	F357
1036	7-Hydroxy-5,6,2'-trimethoxyisoflavone 7- <i>O</i> -(2''- <i>p</i> -coumaroyl)glucoside)		680.61	C ₃₄ H ₃₂ O ₁₅	F329
1037	5,7-Dihydroxy-6,2',4',5'-tetrahydroxyisoflavone 7- <i>O</i> -(rhamnopylglucoside)	Cavinunin 7- <i>O</i> -rhamnopylglucoside	682.64	C ₃₁ H ₃₈ O ₁₇	3183
1038	5,7,3'-Trihydroxy-6,4',5'-trimethoxyisoflavone 7- <i>O</i> -(6''-glucosylglucoside)	Germanaism C	684.60	C ₃₀ H ₃₆ O ₁₈	F16
1039	5,7-Dihydroxy-8,2',4',5'-tetramethoxyisoflavone 7- <i>O</i> -(6''-glucosylglucoside)	Isocavinunin 7- <i>O</i> -gentiobioside	698.64	C ₃₁ H ₃₈ O ₁₈	3185
1040	5,7-Dihydroxy-4'-methoxyisoflavone 7- <i>O</i> -(6''-(5''-(apiosyl)-apiosyl)-glucoside)	Biochanin A 7- <i>O</i> -(6''-(5''-(apiosyl)apiosyl)glucoside)	710.63	C ₃₂ H ₃₈ O ₁₈	F45
1041	5,7,2',4'-Tetrahydroxy-6,3'-diprenylisoflavone 5- <i>O</i> -(4''-rhamnopyl)glucoside)		714.75	C ₃₇ H ₄₆ O ₁₄	F327
1042	7-Hydroxy-4'-methoxyisoflavone 7- <i>O</i> -(2''-6'-di- <i>O</i> - <i>E</i> - <i>p</i> -coumaroyl)-glucoside	Formononetin 7- <i>O</i> -(2''-6''-di- <i>O</i> -(<i>E</i> - <i>p</i> -coumaroyl)glucoside)	722.69	C ₄₀ H ₃₄ O ₁₃	F251
1043	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -rhamnopylglucoside-4'- <i>O</i> -(2''-rhamnopylglucoside)		724.66	C ₃₃ H ₄₀ O ₁₈	F290
1044	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -glucoside-4'- <i>O</i> -(2''-rhamnopylglucoside)		740.66	C ₃₃ H ₄₀ O ₁₉	F290
1045	5,7,4'-Trihydroxyisoflavone 7- <i>O</i> -rhamnopylglucoside-4'- <i>O</i> -(2''-glucosylglucoside)		740.66	C ₃₃ H ₄₀ O ₁₉	F290

1046	5,7,4'-Trihydroxyisoflavone 7-O-glucoside-4'-O-(2''-glucosylglucoside)	756.66	C33H40O20	F290
1047	4'-Hydroxy-5-methoxy-6,7-methylenedioxyisoflavone 4'-O-(2''-glucosyl-6''-rhamnosylglucoside)	782.70	C35H42O20	F16
1048	3',4'-Dihydroxy-5-methoxy-6,7-methylenedioxyisoflavone 3'-O-glucoside-4'-O-(2''-O-(4''-acetyl-2''''-methoxyphenyl)glucoside)	800.71	C38H40O19	F16
1049	5,7,4'-Trihydroxyisoflavone 7-O-(4''-glucosylapioside)-4'-O-(4''-glucosylapioside)	858.76	C37H46O23	3146
3-ARYLCOUMARINS				
1050	7,2'-Dihydroxy-4'-methoxy-3-phenylcoumarin	284.27	C16H12O5	2802
1051	7,2'-Dihydroxy-4',5'-methylenedioxy-3-phenylcoumarin	298.25	C16H10O6	2803
1052	4'-Hydroxy-4,7,2'-trimethoxy-3-phenylcoumarin	328.32	C18H16O6	F153
1053	2-Methoxy-4',5'-methylenedioxyfuranol[2'',3'':7,6]-3-phenylcoumarin	336.30	C19H12O6	2804
1054	2',4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]-3-phenylcoumarin	336.34	C20H16O5	F73
1055	2',4',5'-Trimethoxyfuranol[2'',3'':7,6]-3-phenylcoumarin	352.34	C20H16O6	F154
1056	7,4'-Dihydroxy-2'-methoxy-3'-prenyl-3-phenylcoumarin	352.38	C21H20O5	F280
1057	4,5,7-Trimethoxy-4',5'-methylenedioxy-3-phenylcoumarin	356.33	C19H16O7	2807
1058	2',4'-Dimethoxy-6'',6''-dimethylpyrano[2'',3'':7,8]-3-phenylcoumarin	364.40	C22H20O5	F122
1059	8,2'-Dimethoxy-4',5'-methylenedioxyfuranol[2'',3'':7,6]-3-phenylcoumarin	366.33	C20H14O7	2805
1060	4,4'-Dihydroxy-5-methoxy-6'',6''-dimethylpyrano[2'',3'':7,6]-3-phenylcoumarin	366.36	C21H18O6	F305
1061	7,2'-Dihydroxy-5-methoxy-6'',6''-dimethylpyrano[2'',3'':4',3']-3-phenylcoumarin	366.36	C21H18O6	F72
1062	2',4'-Dihydroxy-5-methoxy-6'',6''-dimethyl-4',5''-dihydropyrano[2'',3'':7,6]-3-phenylcoumarin	368.39	C21H20O6	3071
1063	7,2',4'-Trihydroxy-5-methoxy-3'-prenyl-3-phenylcoumarin	368.39	C21H20O6	F78
1064	7,2',4'-Trihydroxy-5-methoxy-6-prenyl-3-phenylcoumarin	368.39	C21H20O6	3070
1065	7,2',4'-Trihydroxy-5-methoxy-8-(1,1-dimethyl-2-propenyl)-3-phenylcoumarin	368.39	C21H20O6	3073
1066	4-Hydroxy-5,6,7-trimethoxy-4',5'-methylenedioxy-3-phenylcoumarin	372.33	C19H16O8	F332
1067	2-Methoxy-3',4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,6]-3-phenylcoumarin	378.38	C22H18O6	F193
1068	4-Hydroxy-5,4'-dimethoxy-6'',6''-dimethylpyrano[2'',3'':7,6]-3-phenylcoumarin	380.40	C22H20O6	2808
1069	2',4'-Dihydroxy-5,7-dimethoxy-6-prenyl-3-phenylcoumarin	382.41	C22H22O6	2806
1070	4-Hydroxy-5,4'-dimethoxy-5''-(1-methylethenyl)-4',5''-dihydrofuranol[2'',3'':7,6]-3-phenylcoumarin	382.41	C22H20O6	F185
1071	7-Hydroxy-5,2'-dimethoxy-6'',6''-dimethyl-4',5''-dihydropyrano[2'',3'':4',3']-3-phenylcoumarin	382.41	C22H22O6	F70
1072	2,4'-Dihydroxy-5-methoxy-5''-(1-hydroxy-1-methylethyl)-4',5''-dihydrofuranol[2'',3'':7,6]-3-phenylcoumarin	384.39	C21H20O7	F88
1073	2',4'-Dihydroxy-5-methoxy-6''-(hydroxymethyl)-6''-methyl-4',5''-dihydropyrano[2'',3'':7,6]-3-phenylcoumarin	384.39	C21H20O7	3072
	Germanaisol F			
	Germanaisol G			
	Genistein 7,4'-O-bis(glucosylapioside)			
	Melissanol B			
	Pachyrhizin			
	Kanzanol W			
	Eryvarin O			
	Derrussin			
	Neofolin			
	Indicanine B			
	Glyasperin L			
	Isoglycycomarin			
	Gancaonin W			
	Glycycomarin			
	Licoarylcomarin			
	Pervilleainin			
	Robustic acid			
	Glycyrin			
	Indicanine A			
	Gancaonol A			
	Licofuranocoumarin			
	Licopyranocoumarin			

continued

APPENDIX Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1074	4-Hydroxy-5-methoxy-3,4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,6]3-phenylcoumarin	Robustin	394.38	C22H18O7	2810
1075	4-Hydroxy-5-methoxy-3,4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,8]3-phenylcoumarin	Isorobustin	394.38	C22H18O7	3076
1076	4,5,4'-Trimethoxy-6'',6''-dimethylpyrano[2'',3'':7,6]3-phenylcoumarin	Robustic acid methyl ether	394.42	C23H22O6	2809
1077	7,2,4'-Trihydroxy-8,3'-diprenyl-3-phenylcoumarin	Licoumarin A	406.47	C25H26O5	F19
1078	4-Hydroxy-5,8,4'-trimethoxy-5''-(1-methylethenyl)furan[2'',3'':7,6]3-phenylcoumarin	Thonningine B	408.41	C23H20O7	3075
1079	4,5-Dimethoxy-3,4'-methylenedioxy-5''-(1-methylethenyl)-4'',5''-dihydrofuran[2'',3'':7,6]3-phenylcoumarin	Glabrescin	408.41	C23H20O7	2812
1080	4,5-Dimethoxy-3,4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,6]3-phenylcoumarin	Robustin methyl ether	408.41	C23H20O7	2811
1081	4,5-Dimethoxy-3,4'-methylenedioxy-6'',6''-dimethylpyrano[2'',3'':7,8]3-phenylcoumarin	Isorobustin 4-methyl ether	408.41	C23H20O7	3077
1082	4-Hydroxy-5,8-dimethoxy-3,4'-methylenedioxy-5''-(1-methylethenyl)furan[2'',3'':7,6]3-phenylcoumarin	Thonningine A	422.39	C23H18O8	3074
1083	4,4'-Dihydroxy-5-methoxy-6-prenyl-6'',6''-dimethylpyrano[2'',3'':7,8]3-phenylcoumarin	Scandenin	434.49	C26H26O6	2813
1084	4,4'-Dihydroxy-5-methoxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'':7,6]3-phenylcoumarin	Lonchocarpic acid	434.49	C26H26O6	2814
1085	4-Hydroxy-5,3,4'-trimethoxy-5''-(1-methylethenyl)-4'',5''-dihydrofuran[2'',3'':7,6]3-phenylcoumarin	Thonningin C	438.43	C24H22O8	F12
1086	4-Hydroxy-5,4'-dimethoxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'':7,6]3-phenylcoumarin	Lonchocarpenin	448.51	C27H28O6	2815
1087	4-Keto-4'-hydroxy-5-methoxy-6-prenyl-3-(2-oxopropyl)-6'',6''-dimethylpyrano[2'',3'':7,8]3-phenylcoumarin		490.54	C29H30O7	F155
COUMARONOCHROMONES					
1088	Coumaronochromone	Wrightiadione	248.23	C16H8O3	F144
1089	5,7,3'-Trihydroxycoumaronochromone	Cocconeone A	284.23	C15H8O6	F67
1090	5,7,4'-Trihydroxycoumaronochromone	Lupinalbin A	284.23	C15H8O6	3093
1091	5,4'-Dihydroxy-7-methoxycoumaronochromone	Sophorophenolone	298.25	C16H10O6	F291
1092	7,4'-Dihydroxy-5'-methoxycoumaronochromone	Desmoxiphyllin B	298.25	C16H10O6	F172
1093	5,7,4'-Trihydroxy-5'-methoxycoumaronochromone	Desmoxiphyllin A	314.25	C16H10O7	F172
1094	5,7,5'-Trihydroxy-4'-methoxycoumaronochromone	Oblonginol	314.25	C16H10O7	F145
1095	5,4'-Dihydroxy-6'',6''-dimethylpyrano[2'',3'':7,6]coumaronochromone	Lupinalbin H	350.32	C20H14O6	F267
1096	5,7,4'-Trihydroxy-3'-prenylcoumaronochromone	Lupinalbin D	352.34	C20H16O6	3096
1097	5,7,4'-Trihydroxy-6'-prenylcoumaronochromone	Lupinalbin B	352.34	C20H16O6	3096
1098	5,4'-Dihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuran[2'',3'':7,6]coumaronochromone	Lupinalbin C	368.34	C20H16O7	3097
1099	5,7-Dihydroxy-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuran[2'',3'':4,3']coumaronochromone	Lupinalbin E	368.34	C20H16O7	3095
1100	3,2,4'-Trihydroxy-8-prenylfuran[2'',3'':7,6]coumaronochromone	Erysenegalensein K	378.38	C22H18O6	F311

1101	5,7,4'-Trihydroxy-3'-methoxy-5'-prenylcoumaronochromone	Lisetin	382.27	C21H18O7	2512
1102	5,7,5''-Trihydroxy-4'-methoxy-6''-dimethyl-4'',5''-dihydropyrano[2'',3'';3',2']coumaronochromone	Lisetinone	398.36	C21H18O8	F264
1103	5,4'-Dihydroxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'';7,6]coumaronochromone	Millettin	418.45	C25H22O6	2513
1104	5,7,4'-Trihydroxy-6,3'-diprenylcoumaronochromone	Lupinalbin F	420.46	C25H24O6	3098
1105	5,7,5'-Trihydroxy-6,8-diprenyl-6'',6''-dimethylpyrano[2'',3'';4',3]coumaronochromone	Euchretin A	420.46	C25H24O6	3102
1106	7,4',5'-Trihydroxy-3-(3,7-dimethyl-2,6-octadienyl)coumaronochromone	Lespedezol C1	420.46	C25H24O6	F170
1107	5,4',5'-Trihydroxy-6-prenyl-6'',6''-dimethylpyrano[2'',3'';7,8]coumaronochromone	Euchretin G	434.44	C25H22O7	F173
1108	5,5'-Dihydroxy-6'',6'',6''-tetramethyl-4'',5''-dihydropyrano[2'',3'';7,8]-pyrano[2'',3'';4',3]coumaronochromone	Formosanatin D	434.44	C25H22O7	F148
1109	5,7,5'-Trihydroxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'';4',3]coumaronochromone	Euchretin H	434.44	C25H22O7	F173
1110	3,5,6'-Trihydroxy-8-prenyl-6'',6''-dimethylpyrano[2'',3'';7,6]coumaronochromone	Erysenegalensein J	436.46	C25H24O7	F311
1111	5,7,4',5'-Tetrahydroxy-6,8-diprenylcoumaronochromone	Euchretin F	436.46	C25H24O7	F173
1112	5,7,4',5'-Tetrahydroxy-8,3'-diprenylcoumaronochromone	Euchretin B	436.46	C25H24O7	3099
1113	5,7,5'-Trihydroxy-8-prenyl-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'';4',3]coumaronochromone	Euchretin L	436.46	C25H24O7	F149
1114	5,7,4'-Trihydroxy-5'-methoxy-8,3'-diprenylcoumaronochromone	8-Dimethylallylisetin	450.49	C26H26O7	F100
1115	7,4'-Dihydroxy-5'-methoxycoumaronochromone 7-O-glucoside	Desmoxypyhyllin B 7-O-glucoside	460.39	C22H20O11	F172
1116	5,7,4'-Trihydroxy-5'-methoxycoumaronochromone 7-O-glucoside	Desmoxypyhyllin A 7-O-glucoside	476.39	C22H20O12	F172
1117	5,5'-Dihydroxy-6-prenyl-(2'',3'';7,8),(2'',3'';4',3)-bis(6,6-dimethylpyrano)coumaronochromone	Euchretin E	500.54	C30H28O7	F174
1118	5,5'-Dihydroxy-8-prenyl-(2'',3'';7,6),(2'',3'';4',3)-bis(6,6-dimethylpyrano)coumaronochromone	Euchretin D	500.54	C30H28O7	F174
1119	5,5'-Dihydroxy-8-prenyl-6'',6'',6''-tetramethyl-4'',5''-dihydropyrano[2'',3'';7,6]pyrano[2'',3'';4',3]coumaronochromone	Formosanatin C	502.56	C30H30O7	F148
1120	7,5'-Dihydroxy-8-prenyl-6'',6'',6''-tetramethyl-4'',5''-dihydropyrano[2'',3'';5,6]pyrano[2'',3'';4',3]coumaronochromone	Euchretin J	502.56	C30H30O7	F149
1121	5,7,4',5'-Tetrahydroxy-6,8,3'-triprenylcoumaronochromone	Euchretin C	504.58	C30H32O7	3101
1122	7,5'-Dihydroxy-8-prenyl-(2'',3'';5,6),(2'',3'';4',3)-bis(6,6-dimethyl-4,5-dihydropyrano)coumaronochromone	Euchretin K	504.58	C30H32O7	F149
1123	5,7,4'-Trihydroxy-5'-methoxy-6,8,3'-triprenylcoumaronochromone	Euchretin N	518.60	C31H34O7	F149
1124	5,7,5'-Trihydroxy-8-prenyl-6-(3-hydroxy-3-methylbutyl)-6'',6''-dimethylpyrano[2'',3'';7,8]-pyrano[2'',3'';4',3]coumaronochromone	Formosanatin A	520.57	C30H32O8	F148
1125	5,7,4',5'-Tetrahydroxy-6-(3-hydroxy-3-methylbutyl)-8,3'-diprenylcoumaronochromone	Euchretin M	522.59	C30H34O8	F149
1126	5,7,5',4''(S),5''(R)-Pentahydroxy-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'';4',3]coumaronochromone	Formosanatin B	536.57	C30H32O9	F148
COUMARANOCROMANONES					
1127	(2R,3S)-3,5,7,4'-Tetrahydroxy-6-prenylcoumaranochroman-4-one	Lupinol C	370.35	C20H18O7	F266
1128	(2S,3S)-3,5,7,4'-Tetrahydroxy-5'-methoxy-3'-prenylcoumaranochroman-4-one	Piscerythrol	400.38	C21H20O8	F266

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1129	(2 <i>S</i> ,3 <i>S</i>)-3,5,7,4'-Tetrahydroxy-6,3'-diprenylcoumaranochroman-4-one	Lupinol A	438.48	C ₂₅ H ₂₆ O ₇	F266
1130	(2 <i>S</i> ,3 <i>S</i>)-5,7,4'-Trihydroxy-3-methoxy-6,3'-diprenylcoumaranochroman-4-one	Lupinol B	452.50	C ₂₆ H ₂₈ O ₇	F266
PTEROCARPANES					
1131	(6 <i>aS</i> ,11 <i>aS</i>)-4,9-Dihydroxy-3,8-dimethoxypterocarpan	Derricarpin	316.31	C ₁₇ H ₁₆ O ₆	F147
1132	3,9-Dihydroxypterocarpan	Demethylmedicarpin	256.26	C ₁₅ H ₁₂ O ₄	2608
1133	3-Hydroxy-9-methoxypterocarpan	(-)-Medicarpin	270.29	C ₁₆ H ₁₄ O ₄	2609
1134	9-Hydroxy-3-methoxypterocarpan	Isomedicarpin	270.29	C ₁₆ H ₁₄ O ₄	2610
1135	3,4,9-Trihydroxypterocarpan	4-Hydroxydemethylmedicarpin	272.26	C ₁₅ H ₁₂ O ₅	2611
1136	3,6 <i>a</i> ,9-Trihydroxypterocarpan	Glycinol	272.26	C ₁₅ H ₁₂ O ₅	2680
1137	9-Hydroxyfuranol[2',3':3,2]pterocarpan	Neodunol	280.28	C ₁₇ H ₁₂ O ₄	2640
1138	3-Hydroxy-8,9-methylenedioxypterocarpan	(-)-Maackiaian	284.27	C ₁₆ H ₁₂ O ₅	2612
1139	3,9-Dimethoxypterocarpan	(-)-Homopterocarpan	284.31	C ₁₇ H ₁₆ O ₄	2613
1140	1,9-Dihydroxy-3-methoxypterocarpan	Desmocarpin	286.29	C ₁₆ H ₁₄ O ₅	3021
1141	3,10-Dihydroxy-9-methoxypterocarpan	Vesticarpin	286.29	C ₁₆ H ₁₄ O ₅	2615
1142	3,4-Dihydroxy-9-methoxypterocarpan	4-Hydroxymedicarpin	286.29	C ₁₆ H ₁₄ O ₅	2616
1143	3,6 <i>a</i> -Dihydroxy-9-methoxypterocarpan	6 <i>a</i> -Hydroxymedicarpin	286.29	C ₁₆ H ₁₄ O ₅	2681
1144	3,7-Dihydroxy-9-methoxypterocarpan	Nissicarpin	286.29	C ₁₆ H ₁₄ O ₅	3018
1145	3,9-Dihydroxy-10-methoxypterocarpan	Nissolin	286.29	C ₁₆ H ₁₄ O ₅	2614
1146	3,9-Dihydroxy-8-methoxypterocarpan	Kushenin	286.29	C ₁₆ H ₁₄ O ₅	3020
1147	4,9-Dihydroxy-3-methoxypterocarpan	Melitocarpin B	286.29	C ₁₆ H ₁₄ O ₅	3023
1148	6 <i>a</i> ,9-Dihydroxy-3-methoxypterocarpan	6 <i>a</i> -Hydroxysomedicarpin	286.29	C ₁₆ H ₁₄ O ₅	2682
1149	(6 <i>aR</i> ,11 <i>aR</i>)-3,8-Dihydroxy-9-methoxypterocarpan	Lespedezol D1	286.29	C ₁₆ H ₁₄ O ₅	3034
1150	(6 <i>aR</i> ,11 <i>aR</i>)-3,9-Dihydroxy-8-methoxypterocarpan	11 <i>b</i> -Hydroxydihydrodicarpin	286.29	C ₁₆ H ₁₄ O ₅	F171
1151	(6 <i>aR</i> ,11 <i>aS</i>)-3-Keto-11 <i>b</i> -hydroxy-9-methoxy-1,2-dihydropterocarpan	9- <i>O</i> -Methylnesodunol	288.30	C ₁₆ H ₁₆ O ₅	F22
1152	9-Methoxyfuranol[2',3':3,2]pterocarpan	Methyl-oroxylopterocarpan	294.31	C ₁₈ H ₁₄ O ₄	3030
1153	(6 <i>aR</i> ,11 <i>aR</i>)-5',5'-Dimethyl-4',5'-dihydrofuranol[2',3':3,2]pterocarpan	Pterocarpin	294.34	C ₁₉ H ₁₈ O ₃	F8
1154	3-Methoxy-8,9-methylenedioxypterocarpan	Erysubin C	298.30	C ₁₇ H ₁₄ O ₅	2617
1155	(6 <i>aR</i> ,11 <i>aR</i>)-2-Carboxyaldehyde-9-hydroxy-3-methoxypterocarpan	2-Hydroxymaackiaian	298.30	C ₁₇ H ₁₄ O ₅	F275
1156	2,3-Dihydroxy-8,9-methylenedioxypterocarpan		300.27	C ₁₆ H ₁₂ O ₆	3036

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1191	3,9-Dihydroxy-7,10-dimethoxypterocarpan	Phileopteran	316.31	C17H16O6	2629
1192	4,10-Dihydroxy-3,9-dimethoxypterocarpan	Melilotocarpan D	316.31	C17H16O6	3024
1193	(6a <i>R</i> ,11a <i>R</i>)-4,9-Dihydroxy-3,10-dimethoxypterocarpan	Melilotocarpan E	316.31	C17H16O6	3025
1194	(6 <i>S</i> ,6a <i>S</i> ,11a <i>R</i>)-3-Keto-11b-hydroxy-6,9-dimethoxy-1,2-dihydropterocarpan	Kushecarpin A	318.33	C17H18O6	F134
1195	(6a <i>R</i> ,11a <i>R</i>)-3-Hydroxy-5-(1-methylethyl)furanol[2,3':9,10]pterocarpan	Crotafuran A	320.34	C20H16O4	F320
1196	(6a <i>R</i> ,11a <i>R</i>)-5-Acetyl-3-hydroxyfuranol[2,3':9,10]pterocarpan	Crotafuran B	322.31	C19H14O5	F320
1197	3-Hydroxy-6',6'-dimethylpyranol[2,3':9,10]pterocarpan	Phaseollin	322.36	C20H18O4	2642
1198	3-Hydroxy-6',6'-dimethylpyranol[2,3':9,8]pterocarpan	Isonorauteonol	322.36	C20H18O4	3028
1199	(6a <i>R</i> ,11a <i>R</i>)-3-Hydroxy-5-(1-methylethyl)furanol[2,3':9,10]pterocarpan	Barbacarpan	322.36	C20H18O4	F20
1200	9-Hydroxy-6',6'-dimethylpyranol[2,3':3,2]pterocarpan	Neorauteonol	322.36	C20H18O4	2643
1201	6a-Hydroxy-8,9-methyleneoxyfuranol[2,3':3,2]pterocarpan	Neobanol	324.29	C18H12O6	2691
1202	9,10-Dimethoxyfuranol[2,3':3,2]pterocarpan	Ambonane	324.34	C19H16O5	2646
1203	3,9-Dihydroxy-10-prenylpterocarpan	Phaseollidin	324.37	C20H20O4	2645
1204	3,9-Dihydroxy-2-prenylpterocarpan	Calocarpin	324.37	C20H20O4	2647
1205	3,9-Dihydroxy-8-prenylpterocarpan	Sophorapterocarpan A	324.37	C20H20O4	2644
1206	(6a <i>R</i> ,11a <i>R</i>)-3,9-Dihydroxy-6a-prenylpterocarpan	Lepedezol D2	324.37	C20H20O4	F171
1207	6a-Hydroxy-(3,4:8,9)-bis(methyleneedioxy)pterocarpan	Acanthocarpan	328.28	C17H12O7	2688
1208	2,3-Dimethoxy-8,9-methyleneedioxypterocarpan	2-Methoxypterocarpin	328.32	C18H16O6	2632
1209	3,4-Dimethoxy-8,9-methyleneedioxypterocarpan	4-Methoxypterocarpin	328.32	C18H16O6	2633
1210	(6 <i>S</i> ,6a <i>S</i> ,11a <i>R</i>)-3,6-Dimethoxy-8,9-methyleneedioxypterocarpan	6-Methoxypterocarpin	328.32	C18H16O6	3033
1211	1,3-Dihydroxy-2-methoxy-8,9-methyleneedioxypterocarpan	Trifolien	330.29	C17H14O7	2635
1212	3,6a-Dihydroxy-2-methoxy-8,9-methyleneedioxypterocarpan	Hildecarpin	330.29	C17H14O7	3047
1213	3,6a-Dihydroxy-4-methoxy-8,9-methyleneedioxypterocarpan	Tephrocarpin	330.29	C17H14O7	2689
1214	(6a <i>R</i> ,11a <i>R</i>)-2,6a-Dihydroxy-3-methoxy-8,9-methyleneedioxypterocarpan	2-Hydroxyptisatin	330.29	C17H14O7	F126
1215	3-Hydroxy-7,9,10-trimethoxypterocarpan	9- <i>O</i> -Methylphileopteran	330.34	C18H18O6	2634
1216	4-Hydroxy-2,3,9-trimethoxypterocarpan	Melilotocarpan C	330.34	C18H18O6	2636
1217	4-Hydroxy-3,9,10-trimethoxypterocarpan	Odotocarpan	330.34	C18H18O6	3026
1218	(6a <i>R</i> ,11a <i>R</i>)-10-Hydroxy-3,4,9-trimethoxypterocarpan	Kushecarpin	330.34	C18H18O6	3027
1219	(6 <i>S</i> ,6a <i>S</i> ,11a <i>R</i> ,11b <i>S</i>)-3-Keto-11b-hydroxy-6-methoxy-8,9-methyleneedioxy-1,2-dihydropterocarpan	Kushecarpin C	332.30	C17H16O7	F134
1220	6a-Hydroxy-5'-(1-methylethyl)furanol[2,3':3,4]pterocarpan	Clandestacarpin	336.34	C20H16O5	2692
1221	6a,9-Dihydroxy-5'-(1-methylethyl)furanol[2,3':3,4]pterocarpan	Clandestacarpin	336.34	C20H16O5	3048

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1256	(6 <i>aR</i> ,11 <i>aR</i>)-3-Hydroxy-8,9-methylenedioxy-4-prenylpterocarpan	4'-Dehydroxycabeneigrin A-I	352.38	C ₂₁ H ₂₀ O ₅	F46
1257	(6 <i>aR</i> ,11 <i>aR</i>)-8-Hydroxy-9-methoxy-5'-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':3,2]pterocarpan	Pervillinin	352.38	C ₂₁ H ₂₀ O ₅	F193
1258	8,9-Methylenedioxy-4',5'-dihydro-6',6'-dimethylpyranol[2',3':3,2]pterocarpan	Neorautane	352.38	C ₂₁ H ₂₀ O ₅	2657
1259	9-Hydroxy-1-methoxy-6',6'-dimethylpyranol[2',3':3,2]pterocarpan	Edulelanol	352.38	C ₂₁ H ₂₀ O ₅	2658
1260	(6 <i>aR</i> ,11 <i>aR</i>)-3,9-Dimethoxy-4-prenylpterocarpan	Bitucarpin A	352.42	C ₂₂ H ₂₄ O ₄	F203
1261	6 <i>a</i> ,9-Dihydroxy-5'-(hydroxymethylethyl)furanol[2',3':3,2]pterocarpan	Glycoofuran	354.36	C ₂₀ H ₁₈ O ₆	2703
1262	3-Hydroxy-9-methoxy-6',6'-dimethyl-4',5'-dihydropyranol[2',3':1,2]pterocarpan	Bolucarpan C	354.40	C ₂₁ H ₂₂ O ₅	F29
1263	3,10-Dihydroxy-9-methoxy-8-(1,1-dimethyl-2-propenyl)pterocarpan	Arizoncanol E	354.40	C ₂₁ H ₂₂ O ₅	F289
1264	3,6 <i>a</i> -Dihydroxy-9-methoxy-10-prenylpterocarpan	Cristacarpin	354.40	C ₂₁ H ₂₂ O ₅	2701
1265	3,9-Dihydroxy-1-methoxy-10-prenylpterocarpan	1-Methoxyphascollidin	354.40	C ₂₁ H ₂₂ O ₅	2659
1266	3,9-Dihydroxy-1-methoxy-2-prenylpterocarpan	Eduliol	354.40	C ₂₁ H ₂₂ O ₅	2660
1267	3,9-Dihydroxy-8-methoxy-4-prenylpterocarpan	Eryvarin K	354.40	C ₂₁ H ₂₂ O ₅	F276
1268	6 <i>a</i> ,9-Dihydroxy-3-methoxy-2-prenylpterocarpan	Glyceollin IV	354.40	C ₂₁ H ₂₂ O ₅	2702
1269	(6 <i>aR</i> ,11 <i>aR</i>)-1,9-Dihydroxy-3-methoxy-2-prenylpterocarpan	Lespedezol D4	354.40	C ₂₁ H ₂₂ O ₅	F229
1270	(6 <i>aR</i> ,11 <i>aR</i>)-3,8-Dihydroxy-9-methoxy-6 <i>a</i> -prenylpterocarpan	Asperopterocarpin	354.40	C ₂₁ H ₂₂ O ₅	F171
1271	(6 <i>aR</i> ,11 <i>aR</i>)-9-Hydroxy-1-methoxy-3-prenyloxypterocarpan		354.40	C ₂₁ H ₂₂ O ₅	F124
1272	3-Hydroxy-9-methoxy-10-(3-hydroxy-3-methylbutyl)pterocarpan		356.41	C ₂₁ H ₂₄ O ₅	F120
1273	(6 <i>aR</i> ,11 <i>aR</i>)-2,3,4-Trimethoxy-9,8-methylenedioxypterocarpan		358.35	C ₁₉ H ₁₈ O ₇	F166
1274	8-Hydroxy-3,4,9,10-tetramethoxypterocarpan		360.37	C ₁₉ H ₂₀ O ₇	2638
1275	3-Sulfate-8,9-methylenedioxypterocarpan	(-)-Maackiain sulfate	364.33	C ₁₆ H ₁₂ O ₈ S	F196
1276	1-Hydroxy-8,9-methylenedioxy-6',6'-dimethylpyranol[2',3':3,2]pterocarpan	Neorautenanol	366.36	C ₂₁ H ₁₈ O ₆	2661
1277	3-Hydroxy-8,9-methylenedioxy-6',6'-dimethylpyranol[2',3':1,2]pterocarpan	Bolucarpan B	366.36	C ₂₁ H ₁₈ O ₆	F29
1278	1,9-Dimethoxy-6',6'-dimethylpyranol[2',3':3,2]pterocarpan	Edulelane	366.41	C ₂₂ H ₂₂ O ₅	2662
1279	(6 <i>aR</i> ,11 <i>aR</i>)-8-Hydroxy-5'-hexylethyl-4',5'-dihydrofuranol[2',3':3,2]pterocarpan	Hexyl-oroxylopterocarpan	366.45	C ₂₃ H ₂₆ O ₄	F8
1280	3-Hydroxy-8,9-methylenedioxy-4-(4-hydroxy-3-methyl-2-butenyl)pterocarpan	Cabeneigrin A-II	368.39	C ₂₁ H ₂₀ O ₆	3031
1281	3-Hydroxy-8,9-methylenedioxy-6',6'-dimethyl-3',4'-dihydropyranol[2',3':1,2]pterocarpan	Bolucarpan A	368.39	C ₂₁ H ₂₀ O ₆	F29
1282	5'-Hydroxy-8,9-methylenedioxy-6',6'-dimethyl-4',5'-dihydropyranol[2',3':3,2]pterocarpan	Neorautanol	368.39	C ₂₁ H ₂₀ O ₆	2663
1283	6 <i>a</i> -Hydroxy-9-methoxy-5'-(hydroxymethylethyl)furanol[2',3':3,2]pterocarpan	9- <i>O</i> -Methylglycofuran	368.39	C ₂₁ H ₂₀ O ₆	2704
1284	1,9-Dimethoxy-6',6'-dimethyl-4',5'-dihydropyranol[2',3':3,2]pterocarpan	Edulane	368.43	C ₂₂ H ₂₄ O ₅	2665
1285	3-Hydroxy-1,9-dimethoxy-2-prenylpterocarpan	Edulelanol	368.43	C ₂₂ H ₂₄ O ₅	2664

1286	(6aR,11aR)-9-Hydroxy-1,3-dimethoxy-2-prenylpterocarpan	Kanzonol P	368.43	C22H24O5	F76
1287	3-Hydroxy-8,9-methylenedioxy-2-(4-hydroxy-3-methylbutyl)pterocarpan	Cabegrin A-II	370.40	C21H22O6	3029
1288	(6aS,11aR,11bS)-3-Keto-6a,11b-dihydroxy-9-methoxy-10-prenylpterocarpan	Hydroxyeristacarpone	370.40	C21H22O6	F286
1289	(6aS,11aS,5'S)-3,6a,5'-Trihydroxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':9,10]pterocarpan	Eryvarin A	370.40	C21H22O6	F284
1290	(6aS,11aS)-3,6a-Dihydroxy-9-methoxy-10-(2-keto-3-methylbutyl)-pterocarpan	Erypoeigin I	370.40	C21H22O6	F272
1291	(6aS,11aS)-3,6a,8-Trihydroxy-9-methoxy-10-prenylpterocarpan	Sphenostylin B	370.40	C21H22O6	3049
1292	2,8-Dihydroxy-3,4,9,10-tetramethoxypterocarpan		376.37	C19H20O8	2639
1293	(6aR,11aR)-8-Hydroxy-5'-heptylethyl-4',5'-dihydrofuran[2',3':3,2]pterocarpan	Heptyl-oroxylopterocarpan	380.48	C24H28O4	F8
1294	(6aS,11aS)-6a-Hydroxy-8,9-methylenedioxy-5'-(1-hydroxymethyl-ethenyl)-4',5'-dihydrofuran[2',3':3,2]pterocarpan	Hildecarpidin	382.37	C21H18O7	3053
1295	1-Methoxy-8,9-methylenedioxy-4',5'-dihydro-6',6'-dimethylpyrano[2',3':3,2]pterocarpan	Neorautanin	382.41	C22H22O6	2667
1296	3-Hydroxy-4-methoxy-8,9-methylenedioxy-2-prenylpterocarpan	Neoraucarpanol	382.41	C22H22O6	2668
1297	9-Hydroxy-1,8-dimethoxy-6',6'-dimethylpyrano[2',3':3,2]pterocarpan	Desmodin	382.41	C22H22O6	2666
1298	(6aS,11aS)-3,6a-Dihydroxy-9-methoxy-10-prenylpterocarpan	Orientanol A	388.42	C21H24O7	F285
1299	(6aS,11aS)-3,6a,8-Trihydroxy-9-methoxy-10-(3-hydroxy-3-methylbutyl)pterocarpan	Sphenostylin C	388.42	C21H24O7	3051
1300	3-Hydroxy-2-prenyl-6',6'-dimethylpyrano[2',3':9,10]pterocarpan	Folitenol	390.48	C25H26O4	2669
1301	6',6'-Dimethyl-4',5'-dihydropyrano[2',3':3,2]-6',6'-dimethylpyrano[2'',3'':9,10]pterocarpan	Folinin	390.48	C25H26O4	2670
1302	(6aR,11aR)-3-Hydroxy-4-prenyl-6',6'-dimethylpyrano[2',3':9,10]pterocarpan	Erybraedin B	390.48	C25H26O4	3045
1303	(6aR,11aR)-3-Hydroxy-4-prenyl-6',6'-dimethylpyrano[2',3':9,8]pterocarpan	Erybraedin D	390.48	C25H26O4	3043
1304	(6aR,11aR)-9-Hydroxy-10-prenyl-6',6'-dimethylpyrano[2',3':3,2]pterocarpan	Orientanol C	390.48	C25H26O4	F288
1305	3,9-Dihydroxy-10-geranylpterocarpan	Lespedezin	392.50	C25H28O4	2672
1306	3,9-Dihydroxy-2,10-diprenylpterocarpan	Erythrabyssin II	392.50	C25H28O4	2674
1307	3,9-Dihydroxy-2,4-diprenylpterocarpan	Eryvarin J	392.50	C25H28O4	F276
1308	3,9-Dihydroxy-2,8-diprenylpterocarpan	Ficifolinol	392.50	C25H28O4	2673
1309	3,9-Dihydroxy-6a,10-diprenylpterocarpan	Lespein	392.50	C25H28O4	2671
1310	(6aR,11aR)-3,9-Dihydroxy-2-(1,1-dimethyl-2-propenyl)-10-prenylpterocarpan	Striatin	392.50	C25H28O4	F158
1311	(6aR,11aR)-3,9-Dihydroxy-4,10-diprenylpterocarpan	Erybraedin A	392.50	C25H28O4	3044
1312	(6aR,11aR)-3,9-Dihydroxy-4,8-diprenylpterocarpan	Erybraedin C	392.50	C25H28O4	3042
1313	3,4-Dimethoxy-8,9-methylenedioxy-2-prenylpterocarpan	Neoraucarpanol	396.44	C23H24O6	2675
1314	(6aS,11aS)-6a-Hydroxy-3,8,9-trimethoxy-10-prenylpterocarpan	Sphenostylin A	398.46	C23H26O6	3050
1315	1,3-Dihydroxy-6',6'-dimethylpyrano[2',3':9,8]([1''-methylphenyl])[3',4'':4',5']pterocarpan	Tetrapterol B	402.45	C25H22O5	F101
1316	(6aS,11aS)-3,6a-Dihydroxy-8,9-dimethoxy-10-(3-hydroxy-3-methylbutyl)pterocarpan	Sphenostylin D	402.45	C22H26O7	3052
1317	3-Hydroxy-9-methoxy-2,10-diprenylpterocarpan	Eryvarin E	404.51	C26H28O4	F282

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1318	(6aR,11aR)-1-Hydroxy-2-prenyl-6',6'-dimethyl-4'',5''-dihydropyranol[2',3':9,10]pterocarpan dimethylpyranol[2',3':3,2]pterocarpan		406.47	C ₂₅ H ₂₆ O ₅	F230
1319	(6aS,11aS)-3,6a-Dihydroxy-2-prenyl-6',6'-dimethylpyranol[2',3':9,10]pterocarpan	Erysubin E	406.47	C ₂₅ H ₂₆ O ₅	F275
1320	(6aR,11aR)-3-Hydroxy-9-methoxy-2,10-diprenylpterocarpan	Erycrisin	406.51	C ₂₆ H ₃₀ O ₄	3040
1321	(6aR,11aR)-3-Hydroxy-9-methoxy-8-(2E)-3,7-dimethyl-2,6-octadienylpterocarpan	Prostratol E	406.51	C ₂₆ H ₃₀ O ₄	F105
1322	3,6a,9-Trihydroxy-2,10-diprenylpterocarpan	2-Prenyl-6a-hydroxyphaseollidin	408.50	C ₂₅ H ₂₈ O ₅	3054
1323	(6aR,11aR)-3,5'-Dihydroxy-2-prenyl-6',6'-dimethyl-4'',5''-dihydropyranol[2',3':9,10]pterocarpan	Erysubin D	408.50	C ₂₅ H ₂₈ O ₅	F275
1324	1-Methoxy-(2',3':3,2),(2'',3':9,10)-bis(6,6-dimethylpyranol)pterocarpan	Gangetinin	418.49	C ₂₆ H ₂₆ O ₅	2677
1325	8,9-Methylenedioxy-6'-methyl-6'-prenylpyranol[2',3':3,2]pterocarpan	Nitiducarpin	418.49	C ₂₆ H ₂₆ O ₅	2676
1326	3-Hydroxy-8,9-methylenedioxy-4-geranylpterocarpan	Nitiducol	420.50	C ₂₆ H ₂₈ O ₅	2678
1327	(6aR,11aR)-3-Hydroxy-1-methoxy-2-prenyl-6',6'-dimethylpyranol[2',3':9,8]pterocarpan	Kanzonol F	420.50	C ₂₆ H ₂₈ O ₅	F74
1328	(6aS,11aS)-6a-Hydroxy-9-methoxy-10-prenyl-6',6'-dimethylpyranol[2',3':3,2]pterocarpan	Erypogein J	420.50	C ₂₆ H ₂₈ O ₅	F272
1329	9-Hydroxy-1-methoxy-10-prenyl-6',6'-dimethylpyranol[2',3':3,2]pterocarpan	Gangetin	420.50	C ₂₆ H ₂₈ O ₅	2679
1330	3,9-Dihydroxy-1-methoxy-2,8-diprenylpterocarpan	1-Methoxyficifolinol	422.51	C ₂₆ H ₃₀ O ₅	3046
1331	(6aS,11aS)-3,11a-Dihydroxy-9-methoxy-2,10-diprenylpterocarpan	Erystagillin A	422.51	C ₂₆ H ₃₀ O ₅	F287
1332	(6aS,11aS)-3,6a-Dihydroxy-9-methoxy-4,10-diprenylpterocarpan	Eryzerin E	422.51	C ₂₆ H ₃₀ O ₅	F277
1333	(6aR,11aR)-3,8,5'-Trihydroxy-6'-methyl-6'-(4-methyl-3-pentenyl)-4'',5''-dihydropyranol[2',3':9,10]pterocarpan	Lespedezol D5	424.50	C ₂₅ H ₂₈ O ₆	F171
1334	(6aS,11aS)-3,9,6a-Trihydroxy-1-methoxy-2,10-diprenylpterocarpan	Erystagillin B	438.51	C ₂₆ H ₃₀ O ₆	F287
1335	(6aR,11aR)-5'-Dodecanyl-5'-methyl-4'',5''-dihydrofuranol[2',3':3,2]pterocarpan	Dodecanyl-oroxylopterocarpan	448.64	C ₃₀ H ₄₀ O ₃	F8
Pterocarpan glycosides					
1336	(6aR,11aR)-3-Hydroxy-8,9-methylenedioxypterocarpan 3-O-rhamnoside		430.41	C ₂₂ H ₂₂ O ₉	F228
1337	3-Hydroxy-9-methoxypterocarpan 3-O-glucoside	Medicarpin 3-O-glucoside	432.43	C ₂₂ H ₂₄ O ₉	3229
1338	3-Hydroxy-8,9-methylenedioxypterocarpan 3-O-galactoside	Maackain 3-O-galactoside	446.41	C ₂₂ H ₂₂ O ₁₀	3233
1339	3-Hydroxy-8,9-methylenedioxypterocarpan 3-O-glucoside	(+)-Maackain 3-O-glucoside	446.41	C ₂₂ H ₂₂ O ₁₀	3231
1340	3-Hydroxy-8,9-methylenedioxypterocarpan 3-O-glucoside	(-)-Maackain 3-O-glucoside	446.41	C ₂₂ H ₂₂ O ₁₀	3232
1341	3-Hydroxy-9,10-dimethoxypterocarpan 3-O-glucoside	Methylnissolin 3-O-glucoside	462.45	C ₂₃ H ₂₆ O ₁₀	3235
1342	3-Hydroxy-9,10-methylenedioxypterocarpan 3-O-(6'-acetylglucoside)	Trifolirhizin 6'-monoacetate	488.45	C ₂₄ H ₂₄ O ₁₁	3234
1343	3-Hydroxy-9-methoxypterocarpan 3-O-(6'-malonylglucoside)	Medicarpin 3-O-(6'-malonylglucoside)	518.48	C ₂₅ H ₂₆ O ₁₂	3230
1344	3,4-Dihydroxy-9-methoxypterocarpan 3,4-di-O-glucoside	4-Hydroxymedicarpin 3,4-O-diglucoside	610.56	C ₂₈ H ₃₄ O ₁₅	F235

PTEROCARPENE				
1345	3,9-Dihydroxypteroicarpene	Anhydroglycinol	254.24	C15H10O5
1346	9-Hydroxy-3-methoxypteroicarpene	Lespedezol A1	268.26	C16H12O4
1347	9-Hydroxyfuranol[2',3',3',2]pteroicarpene	Neorauteen	278.27	C17H10O4
1348	3-Hydroxy-8,9-methylenedioxypteroicarpene		282.25	C16H10O5
1349	3,9-Dimethoxypteroicarpene	Anhydroglycinol	282.30	C17H14O4
1350	8,9-Dihydroxy-3-methoxypteroicarpene	Lespedezol A4	284.26	C16H12O5
1351	3-Methoxy-8,9-methylenedioxypteroicarpene	Anhydroptisatin	296.28	C17H12O5
1352	8,9-Methylenoxyfuranol[2',3',3',2]pteroicarpene	Neoduleen	306.28	C18H10O5
1353	3-Hydroxy-4-methoxy-8,9-methylenedioxypteroicarpene		312.28	C17H12O6
1354	3-Hydroxy-8,9-methylenedioxy-6-hydroxymethylpteroicarpene		312.28	C17H12O6
1355	3,9,10-Trimethoxypteroicarpene	Andriol B	312.32	C18H16O5
1356	1,7-Dihydroxy-3,9-dimethoxypteroicarpene	Anhydroglycinol	314.29	C17H14O6
1357	3-Hydroxy-6,6'-dimethylpyrano[2',3',9,8]pteroicarpene	Anhydrotuberosin	320.34	C20H16O4
1358	3,9-Dihydroxy-10-prenylpteroicarpene	Erypogin H	322.36	C20H18O4
1359	3,9-Dihydroxy-4-prenylpteroicarpene	Erypogin E	322.36	C20H18O4
1360	10-Hydroxy-3,8,9-trimethoxypteroicarpene	Bryacarpene 2	328.32	C18H16O6
1361	4-Hydroxy-3,9,10-trimethoxypteroicarpene	Bryacarpene 4	328.32	C18H16O6
1362	3-Methoxy-6,6'-dimethylpyrano[2',3',9,8]pteroicarpene	3-O-Methylanhydrotuberosin	334.37	C21H18O4
1363	2-Hydroxy-1,3-dimethoxy-8,9-methylenedioxypteroicarpene	Leicocalycin	342.30	C18H14O7
1364	3-Hydroxy-7-methoxy-8,9-methylenedioxy-6-hydroxymethylpteroicarpene	Andriol A	342.30	C18H14O7
1365	3,8,9,10-Tetramethoxypteroicarpene	Bryacarpene 3	342.35	C19H18O6
1366	4,10-Dihydroxy-3,8,9-trimethoxypteroicarpene	Bryacarpene 1	344.32	C18H16O7
1367	1,3-Dihydroxy-9-methoxy-10-prenylpteroicarpene	Hedysarimpteroicarpene B	352.38	C21H20O5
1368	3,9-Dihydroxy-8-methoxy-7-prenylpteroicarpene	Puemicarpene	352.38	C21H20O5
1369	3,9-Dihydroxy-2,10-diprenylpteroicarpene	Erycristagallin	390.48	C25H26O4
1370	3,8-Dihydroxy-6'-methyl-6-(4-methyl-3-pentenyl)pyrano[2',3',9,10]pteroicarpene	Lespedezol A3	404.46	C25H24O5
1371	3,8,9-Trihydroxy-10-(3,7-dimethyl-2,6-octadienyl)pteroicarpene	Lespedezol A2	406.47	C25H26O5
1372	3,8,9-Trihydroxy-7-(methylinethanoate)-10-(3,7-dimethyl-2,6-octadienyl)pteroicarpene	Lespedezol A5	464.52	C27H28O7
PTEROCARPENEQUINONES				
1373	9,10-Diketo-3,7-dimethoxypteroicarpene	4-Deoxybryaquinone	312.28	C17H12O6
1374	9,10-Diketo-4-hydroxy-3,7-dimethoxypteroicarpene	Bryaquinone	328.28	C17H12O7

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
	Coumestanes				
1375	3,9-Dihydroxycoumestran	Coumestrol	268.23	C ₁₅ H ₈ O ₅	2774
1376	3-Hydroxy-9-methoxycoumestran	9- <i>O</i> -Methylcoumestrol	282.25	C ₁₆ H ₁₀ O ₅	2775
1377	8,9-Dihydroxy-1-methylcoumestran	Mutisifurocoumarin	282.25	C ₁₆ H ₁₀ O ₅	3090
1378	1,3,9-Trihydroxycoumestran	Aureol	284.23	C ₁₅ H ₈ O ₆	3078
1379	2,3,9-Trihydroxycoumestran	Lucernol	284.23	C ₁₅ H ₈ O ₆	2777
1380	3,4,9-Trihydroxycoumestran	4-Hydroxycoumestrol	284.23	C ₁₅ H ₈ O ₆	F182
1381	3,7,9-Trihydroxycoumestran	Repensol	284.23	C ₁₅ H ₈ O ₆	2776
1382	3-Hydroxy-8,9-methyldioxycoumestran	Medicagol	296.24	C ₁₆ H ₈ O ₆	2778
1383	3,9-Dimethoxycoumestran	Coumestrol dimethyl ether	296.28	C ₁₇ H ₁₂ O ₅	2779
1384	2,9-Dihydroxy-3-methoxycoumestran	Melimessanol A	298.25	C ₁₆ H ₁₀ O ₆	F153
1385	3,7-Dihydroxy-9-methoxycoumestran	Trifolol	298.25	C ₁₆ H ₁₀ O ₆	2780
1386	3,9-Dihydroxy-1-methoxycoumestran	Isotrifolol	298.25	C ₁₆ H ₁₀ O ₆	F88
1387	3,9-Dihydroxy-4-methoxycoumestran	Pongacoumestran	298.25	C ₁₆ H ₁₀ O ₆	F324
1388	3,9-Dihydroxy-8-methoxycoumestran	8-Methoxycoumestrol	298.25	C ₁₆ H ₁₀ O ₆	2781
1389	4,9-Dihydroxy-3-methoxycoumestran	Sativol	298.25	C ₁₆ H ₁₀ O ₆	2782
1390	1,3,8,9-Tetrahydroxy-2-prenylcoumestran		300.23	C ₁₅ H ₈ O ₇	F32
1391	1,3,8,9-Tetrahydroxycoumestran	Demethylweddelolactone	300.23	C ₁₅ H ₈ O ₇	2783
1392	3-Methoxy-8,9-methyldioxycoumestran	Flemichapparin C	310.26	C ₁₇ H ₁₀ O ₆	2784
1393	3-Hydroxy-7,9-dimethoxycoumestran	Wairol	312.28	C ₁₇ H ₁₂ O ₆	2785
1394	3-Hydroxy-8,9-dimethoxycoumestran	3-hydroxy-8,9-Dimethoxycoumestran	312.28	C ₁₇ H ₁₂ O ₆	2786
1395	1,8,9-Trihydroxy-3-methoxycoumestran	Weddelolactone	314.25	C ₁₆ H ₁₀ O ₇	2787
1396	3-Carboxylic acid-5,6-dihydroxy-2-(2',4',6'-trihydroxyphenyl)benzofuran	Norwedelic acid	318.24	C ₁₅ H ₁₀ O ₈	3082
1397	8,9-Methyldioxyfuran[2',3':3,2]coumestran	Erosnin	320.26	C ₁₈ H ₈ O ₆	2792
1398	2-Hydroxy-3-methoxy-8,9-methyldioxycoumestran	2-Hydroxyflemichapparin C	326.26	C ₁₇ H ₁₀ O ₇	2788
1399	3-Hydroxy-2-methoxy-8,9-methyldioxycoumestran	Tephrosol	326.26	C ₁₇ H ₁₀ O ₇	2789
1400	3-Hydroxy-4-methoxy-8,9-methyldioxycoumestran	Sophoracoumestran B	326.26	C ₁₇ H ₁₀ O ₇	2790
1401	1,9-Dihydroxy-3,8-dimethoxy-2-prenylcoumestran		328.28	C ₁₇ H ₁₂ O ₇	F32
1402	3,9-Dihydroxy-1,8-dimethoxy-2-prenylcoumestran		328.28	C ₁₇ H ₁₂ O ₇	F32
1403	3-Hydroxy-6'-6'-dimethylpyranol[2',3':9,8]coumestran	Sophoracoumestran A	334.33	C ₂₀ H ₁₄ O ₅	2793

1404	9-Hydroxy-6',6'-dimethylpyrano[2',3':3,4]coumestan	Plicadin	334.33	C20H14O5	F216
1405	3-Hydroxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':9,8]coumestan	Sojagol	336.34	C20H16O5	2794
1406	3,9-Dihydroxy-10-prenylcoumestan	Isosojagol	336.34	C20H16O5	3080
1407	3,9-Dihydroxy-2-prenylcoumestan	Psoralidin	336.34	C20H16O5	2795
1408	3,9-Dihydroxy-4-prenylcoumestan	Phaseol	336.34	C20H16O5	3081
1409	9-Hydroxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':3,2]coumestan	Isopsoralidin	336.34	C20H16O5	2796
1410	9-Hydroxy-1,3,8-trimethoxy-2-prenylcoumestan		342.30	C18H14O7	F32
1411	3-Methoxy-6',6'-dimethylpyrano[2',3':9,8]coumestan	Tuberostan	348.36	C21H16O5	3079
1412	3-Hydroxy-5'-(1-hydroxy-1-methylethyl)-4',5'-dihydrofuran[2',3':9,8]coumestan	Bavacoumestan B	352.34	C20H16O6	3084
1413	3,3'-Dihydroxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':9,8]coumestan	Bavacoumestan A	352.34	C20H16O6	3083
1414	3,9-Dihydroxy-2-(3-methyl-2,3-epoxybutyl)coumestan	Psoralidin oxide	352.34	C20H16O6	2797
1415	2-Hydroxy-1,3-dimethoxy-8,9-methylethoxycoumestan		356.29	C18H12O8	2791
1416	1,3,8,9-Tetramethoxy-2-prenylcoumestan		356.33	C19H16O7	F32
1417	8,9-Methylenedioxy-5'-(1-methylethyl)-4',5'-dihydrofuran[2',3':3,2]coumestan	Tephcalostan	362.33	C21H14O6	F123
1418	7-Hydroxy-9-methoxy-6',6'-dimethylpyrano[2',3':3,4]coumestan	Gancaonol B	364.36	C21H16O6	F70
1419	9-Hydroxy-1-methoxy-6',6'-dimethylpyrano[2',3':3,2]coumestan	Sojagol	364.36	C21H16O6	3092
1420	1,9-Dihydroxy-3-methoxy-2-prenylcoumestan	Glycyrol	366.36	C21H18O6	2798
1421	3,9-Dihydroxy-1-methoxy-8-prenylcoumestan		366.36	C21H18O6	3088
1422	3,9-Dihydroxy-4-methoxy-8-prenylcoumestan	Puerarostan	366.36	C21H18O6	3089
1423	3,9-Dihydroxy-8-methoxy-7-prenylcoumestan	Mirificoumestan	366.36	C21H18O6	3086
1424	9-Hydroxy-3-methoxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':1,2]coumestan	Isoglycyrol	366.36	C21H18O6	2799
1425	9,4',5'-Trihydroxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':3,2]coumestan	Corylidin	368.34	C20H16O7	2800
1426	9-Hydroxy-1,3-dimethoxy-2-prenylcoumestan	1-O-Methylglycyrol	380.40	C22H20O6	2801
1427	3,9-Dihydroxy-8-methoxy-7-(3-hydroxy-3-methylbutyl)coumestan	Mirificoumestan hydrate	384.39	C21H20O7	3085
1428	3,9-Dihydroxy-8-methoxy-7-(2,3-dihydroxy-3-methylbutyl)coumestan	Mirificoumestan glycol	400.39	C21H20O8	3087
1429	3,9-Dihydroxy-2-geranylcoumestan	Puerarol	404.46	C25H24O5	3090
1430	3,9-Dihydroxy-2,10-diprenylcoumestan	Sigmoidin K	404.46	C25H24O5	F181
1431	3,8,9-Trihydroxy-10-(3,7-dimethyl-2,6-octadienyl)coumestan	Lespedezol A6	420.46	C25H24O6	F171
Coumestan glycosides					
1432	3-Hydroxy-9-methoxycoumestan 3-O-glucoside	Licoagroside C	444.39	C22H20O10	F141
1433	1,3,8,9-Tetrahydroxycoumestan 3-O-glucoside	3-Demethylweddelactone-O-glucoside	462.37	C21H18O12	3238
1434	3,9-Dihydroxycoumestan 3-O-glucoside	Coumestrol 3-O-glucoside	430.37	C21H18O10	3237

continued

APPENDIX

Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
ROTONONES					
1435	6,9,11-Trihydroxyrotenone	Coccineone B	298.25	C ₁₆ H ₁₀ O ₆	F67
1436	(-)-4,9,11,12a-Tetrahydroxyrotenone		316.27	C ₁₆ H ₁₂ O ₇	3008
1437	(-)-4,11,12a-Trihydroxy-9-methoxyrotenone		330.29	C ₁₇ H ₁₄ O ₇	3009
1438	2,3-Methylenedioxyfuranol[2',3':9,10]rotenone	Dolineone	336.30	C ₁₉ H ₁₂ O ₆	2557
1439	2,3,9-Trimethoxyrotenone	Munduserone	342.35	C ₁₉ H ₁₈ O ₆	2555
1440	(-)-4,11,12a-Trihydroxy-9-methoxy-10-methylrotenone	Boeravinone C	344.32	C ₁₈ H ₁₆ O ₇	3010
1441	(6a <i>R</i> ,12a <i>S</i>)-11,12a-Dihydroxy-9,10-dimethoxyrotenone	9-Methoxyirispirinol	344.32	C ₁₈ H ₁₆ O ₇	F236
1442	12a-Hydroxy-2,3-methylenedioxyfuranol[2',3':9,10]rotenone	12a-Hydroxydolineone	352.30	C ₁₉ H ₁₂ O ₇	2577
1443	2,3-Dimethoxyfuranol[2',3':9,10]rotenone	Erosone	352.34	C ₂₀ H ₁₆ O ₆	2559
1444	2,3-Dimethoxyfuranol[2',3':9,8]rotenone	Elliptone	352.34	C ₂₀ H ₁₆ O ₆	2558
1445	12-Hydroxy-2,3-dimethoxyfuranol[2',3':9,8]rotenone	Elliptinol	354.36	C ₂₀ H ₁₈ O ₆	3005
1446	(6a <i>R</i> ,12a <i>R</i>)-12a-Hydroxy-2,3-dimethoxyfuranol[2',3':9,8]rotenone	12a-Hydroxyelliptone	354.36	C ₂₀ H ₁₈ O ₆	F293
1447	(6a <i>R</i> ,12a <i>S</i>)-12a-Hydroxy-2,3,8,9-bis(methylenedioxy)rotenone	Usarotenoid A	356.29	C ₁₈ H ₁₂ O ₈	F342
1448	(6a <i>R</i> ,12 <i>R</i> ,12a <i>R</i>)-12,12a-Dihydroxy-12-dihydro-2,3,8,9-bis(methylenedioxy)rotenone	12-Dihydrousarotenoid A	358.30	C ₁₈ H ₁₄ O ₈	F342
1449	11-Hydroxy-2,3,9-trimethoxyrotenone	Sermundone	358.35	C ₁₉ H ₁₈ O ₇	2556
1450	12a-Hydroxy-2,3,9-trimethoxyrotenone	12a-Hydroxymunduserone	358.35	C ₁₉ H ₁₈ O ₇	2575
1451	(6a <i>R</i> ,12a <i>S</i>)-4,11,12a-Trihydroxy-9-methoxy-8,10-dimethylrotenone	Mirabijalone A	358.35	C ₁₉ H ₁₈ O ₇	F316
1452	12a-Methoxy-2,3-methylenedioxyfuranol[2',3':9,10]rotenone	12a-Methoxydolineone	366.33	C ₂₀ H ₁₄ O ₇	2578
1453	8-Methoxy-2,3-methylenedioxyfuranol[2',3':9,10]rotenone	Pachyrizone	366.33	C ₂₀ H ₁₄ O ₇	2560
1454	11-Hydroxy-2,3-dimethoxyfuranol[2',3':9,8]rotenone	Malacol	368.34	C ₂₀ H ₁₆ O ₇	2561
1455	12a-Hydroxy-2,3-dimethoxyfuranol[2',3':9,10]rotenone	12a-Hydroxyerosone	368.34	C ₂₀ H ₁₆ O ₇	2579
1456	(6a <i>S</i> ,12a <i>S</i>)-12a-Hydroxy-2,3-dimethoxyfuranol[2',3':9,8]rotenone	12a-Hydroxyelliptone	368.34	C ₂₀ H ₁₆ O ₇	F109
1457	(6a <i>R</i> ,12a <i>S</i>)-12a-Hydroxy-8,9-dimethoxy-2,3-methylenedioxyrotenone	Usarotenoid B	372.33	C ₁₉ H ₁₆ O ₈	F342
1458	6,11-Dihydroxy-2,3,9-trimethoxyrotenone	Dihydrostemonal	374.35	C ₁₉ H ₁₈ O ₈	3001
1459	(6a <i>R</i> ,12a <i>R</i>)-11,12a-Dihydroxy-2,3,9-trimethoxyrotenone	6-Deoxyclitoriacetal	374.35	C ₁₉ H ₁₈ O ₈	F143
1460	(6a <i>S</i> ,12a <i>R</i>)-6,12a-Dihydroxy-2,3,9-trimethoxyrotenone	11-Deoxyclitoriacetal	374.35	C ₁₉ H ₁₈ O ₈	F162
1461	(6 <i>R</i> ,6a <i>S</i> ,12a <i>R</i>)-6,9,11,12a-Tetrahydroxy-2,3-dimethoxyrotenone	9-Deethylclitoriacetal	376.31	C ₁₈ H ₁₆ O ₉	F246
1462	2,3-Methylenedioxy-5'-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Isomillettone	378.38	C ₂₂ H ₁₈ O ₆	2563
1463	2,3-Methylenedioxy-6'-6'-dimethylpyranol[2',3':9,8]rotenone	Millettone	378.38	C ₂₂ H ₁₈ O ₆	2562
1464	12a-Hydroxy-8-methoxy-2,3-methylenedioxyfuranol[2',3':9,10]rotenone	12a-Hydroxypachyrizone	382.33	C ₂₀ H ₁₄ O ₈	2581

1465	2,3,1,2a-Trimethoxyfuranol[2',3':9,10]rotenone	Neobanone	382.37	C20H18O7	2580
1466	6,11,12a-Trihydroxy-2,3,9-trimethoxyrotenone	Clitoriacetal	390.35	C19H18O9	2576
1467	12a-Hydroxy-2,3-methylenedioxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	12a-Hydroxymillettone	394.38	C22H18O7	2583
1468	12a-Hydroxy-2,3-methylenedioxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Millettosin	394.38	C22H18O7	2582
1469	(6aR,12aS)-12a-Hydroxy-2,3-methylenedioxy-6,6'-dimethoxypyryl[2',3':9,8]rotenone	12a-Epimillettosin	394.38	C22H18O7	F342
1470	2,3-Dimethoxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Rotenone	394.42	C23H22O6	2565
1471	2,3-Dimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Deguelin	394.42	C23H22O6	2564
1472	9-Hydroxy-2,3-dimethoxy-10-(1,4-pentadienyl)rotenone	Myriconol	394.42	C23H22O6	2566
1473	3-Hydroxy-2-methoxy-5'-(1-hydroxymethyl-1-ethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	3-O-Demethylamorphigenin	396.40	C22H20O7	3000
1474	9-Hydroxy-2,3-dimethoxy-8-prenylrotenone	Rotenonic acid	396.44	C23H24O6	2567
1475	11-Hydroxy-2,3-dimethoxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Sumatrol	410.42	C23H22O7	2570
1475b	(12R)-12-Hydroxy-2,3-dimethoxy-5-(1'-hydroxymethylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Dalcochinin	412.44	C23H24O7	F261b
1476	11-Hydroxy-2,3-dimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Toxicarol	410.42	C23H22O7	2569
1477	12a-Hydroxy-2,3-dimethoxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	12a-Hydroxyrotenone	410.42	C23H22O7	2585
1478	12a-Hydroxy-2,3-dimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Tephrosin	410.42	C23H22O7	2584
1479	2,3-Dimethoxy-5-(1-hydroxymethylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Amorphigenin	410.42	C23H22O7	2568
1480	6-Hydroxy-2,3-dimethoxy-5-(1-methyl-1-ethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	6-Hydroxyrotenone	410.42	C23H22O7	3003
1481	(6aR,12aS)-12a-Hydroxy-9-methoxy-2,3-methylenedioxy-8-prenylrotenone	Usaratenoid C	410.42	C23H22O7	F334
1482	(6R,6aS,12aS)-6-Hydroxy-2,3-dimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Hydroxydeguelin	410.42	C23H22O7	F150
1483	2,3-Dimethoxy-5-(1-hydroxy-1-methylethyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Daupanol	412.44	C23H24O7	2572
1484	2,3-Dimethoxy-5-(1-hydroxymethylethyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Dihydroamorphigenin	412.44	C23H24O7	2571
1485	<i>cis</i> -9,12a-Dihydroxy-2,3-dimethoxy-8-prenylrotenone	<i>cis</i> -12a-Hydroxyrot-2'-enonic acid	412.44	C23H24O7	3006
1486	3,12a-Dihydroxy-2-methoxy-5-(2-hydroxy-1-methylethyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Volubinol	414.41	C22H22O8	3007
1487	6-Acetoxy-11-hydroxy-2,3,9-trimethoxyrotenone	6-Acetyldihydrostemonal	416.39	C21H20O9	3002
1488	12a-Methoxy-2,3-dimethoxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	12a-Methoxyrotenone	424.45	C24H24O7	2586
1489	(+)-2,3,10-Trimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Erythronone	424.45	C24H24O7	3004
1490	11,12a-Dihydroxy-2,3-dimethoxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Villosinol	426.42	C23H22O8	2589
1491	11,12a-Dihydroxy-2,3-dimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	12a-Hydroxytephrosin	426.42	C23H22O8	2588
1492	12a-Hydroxy-2,3-dimethoxy-5-(1-hydroxymethylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Dalbinal	426.42	C23H22O8	2587
1493	6,11-Dihydroxy-2,3-dimethoxy-5-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Villosin	426.42	C23H22O8	2573
1494	(6aR,12aR)-11,12a-Dihydroxy-2,3-dimethoxy-6,6'-dimethylpyranol[2',3':9,10]rotenone	12a-hydroxy-b-toxicarol	426.42	C23H22O8	F11
1495	(6R,6aS,12aR)-6,12a-Dihydroxy-2,3-dimethoxy-6,6'-dimethylpyranol[2',3':9,8]rotenone	Hydroxytephrosin	426.42	C23H22O8	F150
1496	12,12a-Dihydroxy-2,3-dimethoxy-5-(1-hydroxymethylethenyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	12-Dihydrodalbinol	428.44	C23H24O8	2590
1497	2,3-Dimethoxy-5-(1-hydroxy-1-hydroxymethylethyl)-4',5'-dihydrofuranol[2',3':9,8]rotenone	Amorphigenol	428.44	C23H24O8	2574

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1498	(6a <i>S</i> ,12a <i>S</i> ,4 <i>S</i> ,5 <i>R</i>)-4',5'-Dihydroxy-2,3,4'-dimethoxy-6',6'-dimethyl-4,5-dihydropyrano[2',3':9,8]rotenone	Derrisin	428.44	C ₂₃ H ₂₄ O ₈	F269
1499	(+)-12a-Hydroxy-2,3,10-trimethoxy-6',6'-dimethylpyrano[2',3':9,8]rotenone	(+)-12a-Hydroxyerythronone	440.45	C ₂₄ H ₂₄ O ₈	3011
1500	6,11,12a-Trihydroxy-2,3-dimethoxy-5-(1-methyllethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone	Villoil	442.42	C ₂₃ H ₂₂ O ₉	2591
1501	(6a <i>R</i> ,12a <i>R</i>)-11,12a-Dihydroxy-2,3,4'-trimethoxy-6',6'-dimethyl-4',5'-dihydropyrano[2',3':9,8]rotenone		474.46	C ₂₄ H ₂₆ O ₁₀	F115
1502	(6a <i>S</i> ,12a <i>S</i>)-12-Keto-12a-hydroxy-3,4-dimethoxy-9-((2 <i>E</i> ,5 <i>E</i>)-7-hydroxy-3,7-dimethyl-2,5-octadienyloxy) rotenone	Griffonianone A	496.55	C ₂₈ H ₃₂ O ₈	F331
Rotenone glycosides					
1503	(6a <i>R</i> ,12a <i>R</i>)-11,12a-Dihydroxy-2,3,9-trimethoxyrotenone 11- <i>O</i> -glucoside	6-Deoxyclitoriacetal 11- <i>O</i> -glucoside	536.49	C ₂₅ H ₂₈ O ₁₃	F200
1504	(6 <i>S</i> ,6a <i>S</i> ,12a <i>R</i>)-6,12a-Dihydroxy-2,3,9-trimethoxyrotenone 11- <i>O</i> -glucoside	Rotenoid 11a- <i>O</i> -glucoside	536.49	C ₂₅ H ₂₈ O ₁₃	F161
1505	(6 <i>R</i> ,6a <i>S</i> ,12a <i>R</i>)-6,11,12a-Trihydroxy-2,3,9-trimethoxyrotenone 11- <i>O</i> -glucoside	Clitoriacetal 11- <i>O</i> -glucoside	552.48	C ₂₅ H ₂₈ O ₁₄	F44
1506	2,3-Dimethoxy-5-(1''-hydroxymethyllethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -glucoside	Amorphigenin 7- <i>O</i> -glucoside	572.57	C ₂₉ H ₃₂ O ₁₂	3221
1507	(6a <i>R</i> ,12a <i>R</i>)-11,12a-Dihydroxy-2,3-dimethoxy 5'-(1-hydroxy-1-methylethyl)furan[2',3':9,8]rotenone 1''- <i>O</i> -glucoside	Dehydrotalpanol <i>O</i> -glucoside	572.57	C ₂₉ H ₃₂ O ₁₂	F214
1507b	(12 <i>R</i>)-12-Hydroxy-2,3-dimethoxy-5-(1''-hydroxymethylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -glucoside	Dalcochinin <i>O</i> -glucoside	574.58	C ₂₉ H ₃₄ O ₁₂	F261b
1508	2,3-Dimethoxy-5-(1''-hydroxy-1''-methylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -glucoside	Dalpanol <i>O</i> -glucoside	574.58	C ₂₉ H ₃₄ O ₁₂	3223
1509	12a-Hydroxy-2,3-dimethoxy-5-(1''-hydroxymethylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -glucoside	Dalbinol <i>O</i> -glucoside	588.57	C ₂₉ H ₃₂ O ₁₃	3226
1510	12-Hydroxy-2,3-dimethoxy-5-(1''-hydroxymethylethyl)-4',5'-dihydrofuran[2',3':9,8]-12-dihydrorotenone 1''- <i>O</i> -glucoside	12-Dihydrodalbinol <i>O</i> -glucoside	590.58	C ₂₉ H ₃₄ O ₁₃	3228
1511	2,3-Dimethoxy-5-(1''-hydroxy-1''-hydroxymethylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -glucoside	Amorphigenol <i>O</i> -glucoside	590.58	C ₂₉ H ₃₄ O ₁₃	3224
1512	2,3-Dimethoxy-5-(1''-hydroxymethylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -(6'''-arabinosylglucoside)	Amorphigenin 7- <i>O</i> -vicianoside	704.69	C ₃₄ H ₄₀ O ₁₆	3222
1513	2,3-Dimethoxy-5-(1''-2''-dihydroxy-1''-methylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -(6'''-arabinosylglucoside)	Amorphigenol <i>O</i> -vicianoside	704.69	C ₃₄ H ₄₀ O ₁₆	3225
1514	12a-Hydroxy-2,3-dimethoxy-5-(1''-hydroxymethylethyl)-4',5'-dihydrofuran[2',3':9,8]rotenone 1''- <i>O</i> -(6'''-arabinosylglucoside)	Dalbinol <i>O</i> -vicianoside	720.68	C ₃₄ H ₄₀ O ₁₇	3227

DEHYDROROTENONES					
1515	Furanol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1516	6,9,11-Trihydroxy-10-methyl-6a,12a-didehydrodrorotenone				
1517	1,11-Dihydroxy-9,10-methylenedioxy-6a,12a-didehydrodrorotenone				
1518	6-Oxo-3,9,11-Trihydroxy-10-methyl-6a,12a-didehydrodrorotenone				
1519	9,11-Dihydroxy-6-methoxy-10-methyl-6a,12a-didehydrodrorotenone				
1520	3,6,9,11-Tetrahydroxy-10-methyl-6a,12a-didehydrodrorotenone				
1521	2,3-Methylenedioxyfuranol[2',3':9,10]-6a,12a-didehydrodrorotenone				
1522	3,6,11-Trihydroxy-9-methoxy-10-methyl-6a,12a-didehydrodrorotenone				
1523	3,9,11-Trihydroxy-6-methoxy-10-methyl-6a,12a-didehydrodrorotenone				
1524	4,6,9,11-Tetrahydroxy-8,10-dimethyl-6a,12a-didehydrodrorotenone				
1525	6-Acetoxy-9,10,11-trihydroxy-6a,12a-didehydrodrorotenone				
1526	8-Methoxy-2,3-methylenedioxyfuranol[2',3':9,10]-6a,12a-didehydrodrorotenone				
1527	6-Oxo-11-hydroxy-2,3,9-trimethoxy-6a,12a-didehydrodrorotenone				
1528	6-Acetoxy-3,9,10,11-tetrahydroxy-6a,12a-didehydrodrorotenone				
1529	6,11-Dihydroxy-2,3,9-trimethoxy-6a,12a-didehydrodrorotenone				
1530	2,3-Methylenedioxy-6',6'-dimethylpyranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1531	2,3-Dimethoxy-5'-(1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1532	2,3-Dimethoxy-6',6'-dimethylpyranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1533	2,3-Dimethoxy-5'-(1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1534	6-Pentanoate-4,9-dihydroxy-10-methyl-6a,12a-didehydrodrorotenone				
1535	11-Dihydroxy-6-ethoxy-2,3,9-trimethoxy-6a,12a-didehydrodrorotenone				
1536	6-Oxo-2,3-dimethoxy-5'-(1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1537	11-Hydroxy-2,3-dimethoxy-5'-(1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1538	11-Hydroxy-2,3-dimethoxy-6',6'-dimethylpyranol[2',3':9,10]-6a,12a-didehydrodrorotenone				
1539	11-Hydroxy-2,3-dimethoxy-6',6'-dimethylpyranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1540	2,3-Dimethoxy-5'-(1-hydroxymethyléthényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1541	6-Hydroxy-2,3-dimethoxy-5'-(1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1542	2,3-Dimethoxy-5'-(1-hydroxy-1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1543	6-Oxo-11-hydroxy-2,3-dimethoxy-5'-(1-hydroxyméthylethényl)furanol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1544	6-Oxo-11-hydroxy-2,3-dimethoxy-5'-(1-methylethényl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1545	6-Oxo-11-hydroxy-2,3-dimethoxy-6',6'-dimethoxyfuranol[2',3':9,8]-6a,12a-didehydrodrorotenone				
1546	(5'R,6S)-6,11-Dihydroxy-2,3-dimethoxy-5'-(1-methylethényl)furanol[2',3':9,8]-6a,12a-didehydrodrorotenone				
		Pongarotene	290.27	C18H10O4	F249
		Boeravinone B	312.28	C17H12O6	3015
			326.26	C17H10O7	F250
		Boeravinone F	326.26	C17H10O7	F136
		Boeravinone A	326.31	C18H14O6	3016
		Boeravinone E	328.28	C17H12O7	F136
		Dehydrodolineone	334.29	C19H10O6	2595
		Mirabjalone D	342.30	C18H14O7	F316
		Boeravinone D	342.30	C18H14O7	F136
		Mirabjalone B	342.30	C18H14O7	F316
		Repenone	356.29	C18H12O8	3013
		Dehydropacchyrhizone	364.31	C20H12O7	2596
		Stemonone	370.32	C19H14O8	2592
		Repenol	372.29	C18H12O9	3014
		Stemonal	372.33	C19H16O8	2593
		Dehydromillettone	376.37	C22H16O6	2597
		Dehydrorotene	392.41	C23H20O6	2599
		Dehydroguelin	392.41	C23H20O6	2598
		Dehydrodihydrodrorotenone	394.42	C23H22O6	3017
		Diffusarotenoid	396.40	C22H20O7	F84
		Stemomalacetal	400.38	C21H20O8	2594
		Rotenonone	406.39	C23H18O7	2600
		Villosol	408.41	C23H20O7	2604
		6a,12a-Dehydro-a-toxicarol	408.41	C23H20O7	F147
		Dehydrotoxicarol	408.41	C23H20O7	2603
		Dehydroamorphigenin	408.41	C23H20O7	2602
		Amorpholone	408.41	C23H20O7	2601
		Dehydroalpanol	410.42	C23H22O7	2605
		6-K-etodehydroamorphigenin	422.39	C23H18O8	F258
		Villosone	422.39	C23H18O8	2606
		6-Oxo-6a,12a-didehydro-a-toxicarol	422.39	C23H18O8	F146
		6a,12a-Dehydrovillosin	424.41	C23H20O8	F207

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1547	(+)-6,11-Dihydroxy-2,3-dimethoxy-6',6'-dimethylpyranol[2',3':9,8]-6a,12a-didehydrorotenone	6-Hydroxy-6a,12a-dehydro-a-toxicarol	424.41	C ₂₃ H ₂₀ O ₈	3012x
1548	2,3-Dimethoxy-6-methoxy-5'-(1-methylethenyl)-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrorotenone	Villinol	438.44	C ₂₄ H ₂₂ O ₈	2607
1549	9,11-Dihydroxy-2,3,6-trimethoxy-8-prenyl-6a,12a-didehydrorotenone		440.45	C ₂₄ H ₂₄ O ₈	F206
1550	11,5'-Dihydroxy-2,3,4'-trimethoxy-6',6'-dimethyl-4',5'-dihydrofuranol[2',3':9,8]-6a,12a-didehydrorotenone		456.45	C ₂₄ H ₂₄ O ₉	F114
	Dehydrorotenone glycosides				
1551	6,9-Dihydroxy-2,3,10-trimethoxy-6a,12a-didehydrorotenone 9-O-glucoside		534.47	C ₂₅ H ₂₆ O ₁₃	F248
	2-ARYLBENZOFURAN				
1552	6,2',4'-Trihydroxy-2-arylbenzofuran	Corsifuran B	242.23	C ₁₄ H ₁₀ O ₄	2821
1553	5-Hydroxy-4'-methoxy-2,3-dihydro-2-arylbenzofuran	Parvifuran	242.27	C ₁₅ H ₁₄ O ₃	F304
1554	5-Hydroxy-6-methoxy-3-methyl-2-arylbenzofuran	Corsifuran C	254.29	C ₁₆ H ₁₄ O ₃	2822
1555	5,4'-Dimethoxy-2-arylbenzofuran	Centroblofuran	254.29	C ₁₆ H ₁₄ O ₃	F304
1556	6,2'-Dihydroxy-4'-methoxy-2-arylbenzofuran	6-Demethylvignafuran	256.26	C ₁₅ H ₁₂ O ₄	3103
1557	6,4'-Dihydroxy-2'-methoxy-2-arylbenzofuran	Corsifuran A	256.26	C ₁₅ H ₁₂ O ₄	2823
1558	5,4'-Dimethoxy-2,3-dihydro-2-arylbenzofuran		256.30	C ₁₆ H ₁₆ O ₃	F304
1559	2',4'-Dihydroxy-5,6-methylenedioxy-2-arylbenzofuran		270.24	C ₁₅ H ₁₀ O ₅	2825
1560	4'-Hydroxy-2',6'-dimethoxy-2-arylbenzofuran	Vignafuran	270.29	C ₁₆ H ₁₄ O ₄	2824
1561	2'-Hydroxy-4'-methoxy-5,6-methylenedioxy-2-arylbenzofuran		284.27	C ₁₆ H ₁₂ O ₅	2826
1562	6-Hydroxy-2'-methoxy-4',5'-methylenedioxy-2-arylbenzofuran	Cicerfuran	284.27	C ₁₆ H ₁₂ O ₅	F259
1563	5,2'-Dihydroxy-6,4'-dimethoxy-2-arylbenzofuran	Sainfuran	286.29	C ₁₆ H ₁₄ O ₅	3104
1564	5,6-Dihydroxy-2',4'-dimethoxy-2-arylbenzofuran		286.29	C ₁₆ H ₁₄ O ₅	2829
1565	6,3'-Dihydroxy-2',4'-dimethoxy-2-arylbenzofuran	Pterofuran	286.29	C ₁₆ H ₁₄ O ₅	2827
1566	6,4'-Dihydroxy-2',3'-dimethoxy-2-arylbenzofuran	Isopterofuran	286.29	C ₁₆ H ₁₄ O ₅	2828
1567	6,4'-Dihydroxy-2',5'-dimethoxy-2-arylbenzofuran	Eryvarin L	286.29	C ₁₆ H ₁₄ O ₅	F276
1568	3-Carboxyaldehyde-6'-hydroxy-6,4'-dimethoxy-2-arylbenzofuran	Melimessanol C	298.29	C ₁₇ H ₁₅ O ₅	F153
1569	2',4'-Dihydroxy-3'-methoxy-5,6-methylenedioxy-2-arylbenzofuran	Sophorafuran A	300.27	C ₁₆ H ₁₂ O ₆	2830
1570	5-Hydroxy-6,2',4'-trimethoxy-2-arylbenzofuran	Methylsainfuran	300.31	C ₁₇ H ₁₆ O ₅	3105
1571	5,4'-Dihydroxy-6',6'-dimethylpyranol[2',3':2',3']-2-arylbenzofuran	Kanzonol U	308.33	C ₁₉ H ₁₆ O ₄	F73

1572	6,2'-Dihydroxy-6',6''-dimethylpyrano[2'',3'';4',3,7]-2-arylbenzofuran	308.33	C19H16O4	F75
1573	3-Carboxyaldehyde-6,4'-dihydroxy-2',5'-dimethoxy-2-arylbenzofuran	314.29	C17H14O6	F280
1574	3-Carboxylic acid methyl ester-4,6,3',4'-tetrahydroxy-2-arylbenzofuran	316.27	C16H12O7	F87
1575	6,3'-Dihydroxy-5'-methoxy-4'-prenyl-2-arylbenzofuran	324.37	C20H20O4	F86
1576	6,4'-Dihydroxy-2'-methoxy-3'-prenyl-2-arylbenzofuran	324.37	C20H20O4	F104
1577	6,4'-Dihydroxy-2'-methoxy-6-(1,1-dimethyl-2-propenyl)-2-arylbenzofuran	324.37	C20H20O4	F337
1578	5,3',5'-Trihydroxy-6-(4-hydroxy-3-methyl-2(E)-butenyl)-2-arylbenzofuran	326.34	C19H18O5	F163
1578b	3-Carboxyaldehyde-4,3',4'-trihydroxy-6,2'-dimethoxy-2-arylbenzofuran	330.29	C17H14O7	F127b
1579	2,4'-Dihydroxy-4-methoxy-6'',6''-dimethylpyrano[2'',3'';6,5]-2-arylbenzofuran	338.36	C20H18O5	2832
1580	6,2',4'-Trihydroxy-4-methoxy-5-prenyl-2-arylbenzofuran	340.38	C20H20O5	3106
1580b	3-Carboxyaldehyde-4,3'-dihydroxy-6,2',4'-trimethoxy-2-arylbenzofuran	344.32	C18H16O7	F127b
1581	3-Carboxyaldehyde-2',4'-dihydroxy-6-methoxy-2-arylbenzofuran	352.38	C21H20O5	F272
1582	2,4'-Dihydroxy-4,6-dimethoxy-5-prenyl-2-arylbenzofuran	354.40	C21H22O5	3107
1583	6,2'-Dihydroxy-3,4'-dimethoxy-5-prenyl-2-arylbenzofuran	354.40	C21H22O5	2833
1583b	3-Carboxyaldehyde-4,3'-dihydroxy-2',4'-dimethoxy-2'',5''-dihydrofuranol[3'',4'';5,6]-2-arylbenzofuran	356.33	C19H16O7	F127b
1584	3'-Hydroxy-4,6,2',4'-tetramethoxy-3-hydroxymethyl-2-arylbenzofuran	360.37	C19H20O7	F127
1585	4,3'-Dihydroxy-5,7,2',4'-tetramethoxy-2-arylbenzofuran	374.35	C19H18O8	2831
1586	5,4'-Dihydroxy-6-prenyl-6'',6''-dimethylpyrano[2'',3'';2',3]-2-arylbenzofuran	376.44	C24H24O4	F73
1587	6,3',5'-Trihydroxy-2',6'-diprenyl-2-arylbenzofuran	378.46	C24H26O4	F71
1588	3-Carboxyaldehyde-6,2',4'-trihydroxy-7,5'-diprenyl-2-arylbenzofuran	406.47	C25H26O5	F280
1589	5,6,2',4'-Tetrahydroxy-3-methyl-7-(3,7-dimethyl-2,6-octadienyl)-2-arylbenzofuran	408.50	C25H28O5	F170
1590	6,3',5'-Trihydroxy-5-methoxy-4-(2(E)-3,7-dimethyl-2,6-octadienyl)-2-arylbenzofuran	408.50	C25H28O5	F238
1591	6,3',5'-Trihydroxy-5-methoxy-2-arylbenzofuran 3'-O-glucoside	434.39	C21H22O10	F119
1592	3',5'-Dihydroxy-2',6'-diprenyl-6'',6''-dimethylpyrano[2'',3'';6,7]-2-arylbenzofuran	444.56	C29H32O4	F254
1593	6,3',5'-Trihydroxy-2-(2(E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienyl)-2-arylbenzofuran	446.58	C29H34O4	F238
1594	6,3',5'-Trihydroxy-7,2',6'-triprenyl-2-arylbenzofuran	446.58	C29H34O4	F254
1595	6,5'-Dihydroxy-3'-methoxy-7-[(2(E)-3,7-dimethyl-2,6-octadienyl]-2'-prenyl-2-arylbenzofuran	460.60	C30H36O4	F239
1596	(2',R)-6,5'-Dihydroxy-7-(2(E)-3,7-dimethyl-2,6-octadienyl)-5''-(1-hydroxy-1-methylethyl)-4'',5''-dihydrofuranol[2'',3'';3,2]-2-arylbenzofuran	462.58	C29H34O5	F238
1597	6,3',5'-Trihydroxy-5-methoxy-4-(2(E)-3,7-dimethyl-2,6-octadienyl)-2'-prenyl-2-arylbenzofuran	476.62	C30H36O5	F238
1598	6,3',5'-Trihydroxy-7-prenyl-4-(1(S,5,5,6,5)-6-(2,4-dihydroxyphenyl)methanonyl)-5-(2,4-dihydroxyphenyl)-3-methyl-2-cyclohexen-1-yl)-2-arylbenzofuran	648.70	C39H36O9	F24
2-Arylbenzofuran glycosides				
1599	6,3',5'-Trihydroxy-2-arylbenzofuran 3'-O-glucoside	404.37	C20H20O9	F24
1600	3',5',5''-Trihydroxy-6'',6''-dimethyl-4'',5''-dihydropyrano[2'',3'';3',6,5]-2-arylbenzofuran 3'-O-glucoside	458.47	C24H26O9	F262

continued

APPENDIX
Checklist for Isoflavonoids Described in the Literature During the Period 1991–2004 — continued

	Name	Trivial Name	Mass	Formula	Ref.
1601	2,5-DIARYLBENZOFURAN (2 <i>R</i> ,3 <i>S</i> ,5' <i>S</i> ,5' <i>S</i> ,3',3''-Dihydroxy-2,3-dihydro-2,5'-diaryl-(cyclopentan[1'',2'':2,3]benzofuran)	Ferrugin	434.49	C ₂₆ H ₂₆ O ₆	F50
	3-ARYL-2,3-DIHYDROBENZOFURANS				
1602	3-Carboxyaldehyde-6,4'-dihydroxy-2',5'-dimethoxy-3-aryl-2,3-dihydrobenzofuran	Eryvarin R	316.31	C ₁₇ H ₁₆ O ₆	F280
1603	(2 <i>S</i> ,3 <i>S</i>)-7,4'-Dihydroxy-3'-methoxy-2-hydroxyethyl-5-hydroxypropyl-2-aryl-2,3-dihydrobenzofuran 4'- <i>O</i> -rhamnoside	Mulberrofuran Z	476.62	C ₃₀ H ₃₆ O ₅	F237
1604	2-ARYLBENZOFURANQUINONE 3-Carboxyaldehyde-4,7-diketo-3'-hydroxy-6,2',4'-trimethoxy-5-prenyl-2-arylbenzofuranquinone	Bryebinalquinone	358.30	C ₁₈ H ₁₄ O ₈	2834
	α-METHYLDEOXYBENZOINS				
1605	2,4-Dihydroxy-4'-methoxy-α-methyldeoxybenzoin	Angolensin	272.30	C ₁₆ H ₁₆ O ₄	2816
1606	2-Hydroxy-4,4'-dimethoxy-''α-methyldeoxybenzoin	4- <i>O</i> -Methylangolensin	286.33	C ₁₇ H ₁₈ O ₄	2818
1607	4-Hydroxy-2,4'-dimethoxy-''α-methyldeoxybenzoin	2- <i>O</i> -Methylangolensin	286.33	C ₁₇ H ₁₈ O ₄	2817
1608	2-Hydroxy-4'-methoxy-4-[1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenyl]oxy[α-methyldeoxybenzoin	4- <i>O</i> -α-Cadinylangolensin	476.66	C ₃₁ H ₄₀ O ₄	2819
1609	6-Hydroxy-4'-methoxy-4-[1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenyl]oxy[α-methyldeoxybenzoin	4- <i>O</i> -T-Cadinylangolensin	476.66	C ₃₁ H ₄₀ O ₄	2820

This checklist of isoflavonoids contains various compounds reported in the literature as natural products to the end of 2004. Compounds published before 1991 are referenced to numbered entries in Volume 2 of the Handbook of Natural Flavonoids (J.B. Harborne and H. Baxter), John Wiley & Sons, Chichester 1999, using a number consisting of four digits. Compounds published in the period 1991–2004 are referenced with numbers having F as prefix before the number of the publication found in the reference list.

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