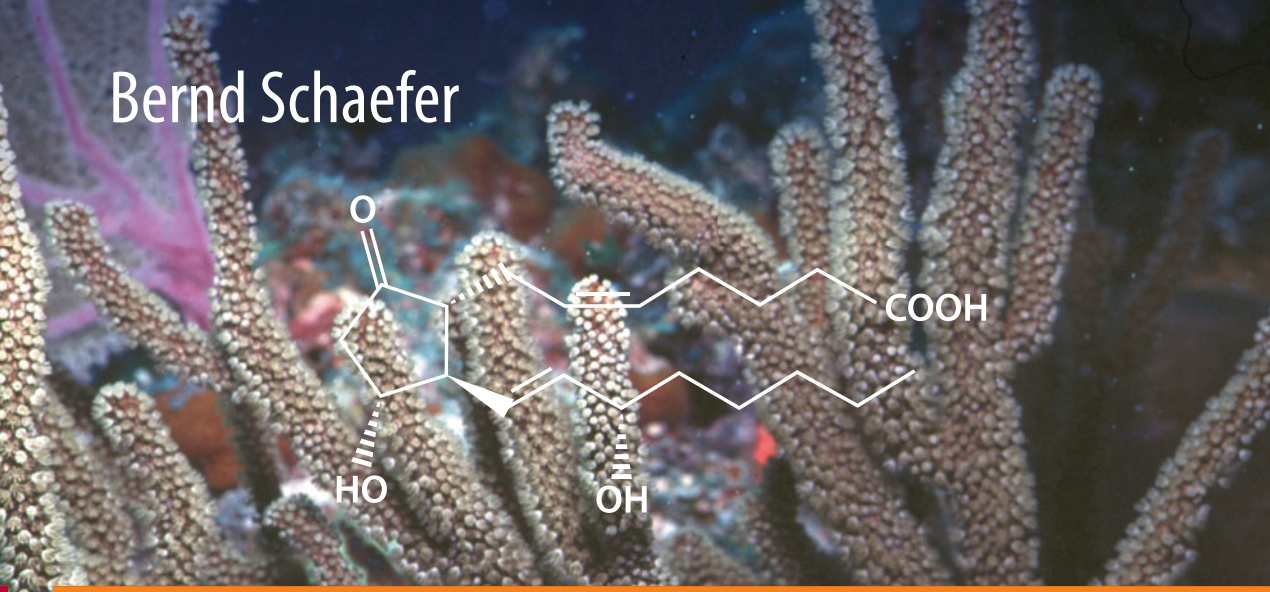
Bernd Schaefer



Natural Products in the Chemical Industry

Springer

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Bernd Schaefer

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Extended and translated edition of the German textbook
Naturstoffe der chemischen Industrie
Translators
David Smith and Bernd Janssen

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To

Kathrin, Anselm, Heike,

Clara and Miriam

Preface to the second edition

Every year, more than a million visitors travel to Washington, DC end of March/early April to celebrate the National Cherry Blossom Festival (Fig. 0.1). Are you surprised that the roots of this spectacular event are closely linked to a discovery in natural products chemistry? You can find out more about this topic in Chapter 6.3.

0.1 *In March 1912, Tokyo Mayor Yukio Ozaki gave the gift of 3,020 Japanese cherry trees to the city of Washington. They symbolize friendship between nations, the renewal of spring, and the ephemeral nature of life.*

During my career in the chemical industry, I was always fascinated by natural products for two reasons:

1. They are a crucial factor in securing and enriching our livelihood not only with food and medicine, but also by stimulating our senses with colour, scent and flavour.
2. When industrial chemistry was called upon to satisfy an ever growing demand for such products, this became a challenging incentive for developing efficient and scalable processes to generate often rather complex molecules. Here, the ingenuity and creativity of chemists in academia and industry is displayed most prominently with admirable achievements.

My curiosity and my excitement for natural products from an industrial perspective led to questions about their discovery, early application and production, their economic and sometimes social ramifications. When asked to prepare a lecture course on "Natural Products in the Chemical Industry" at the University of Heidelberg, I intended to share this multifaceted view on the subject with my students, instead of conveying only the bare bones of chemical technology. Over

the past 12 years, the course has indeed sustained remarkable interest and subsequently resulted in a German print edition of "Naturstoffe der chemischen Industrie". The current English version is based on its largely revised German counterpart and aims at addressing a wider audience.

This is not a classical textbook, and even less a reference work. Whether you are a teacher or a scholar, an undergraduate or graduate student, a professional chemist in industry or academia, or someone just interested in natural sciences, you will find plenty of food for thought – facts and information along with enjoyable anecdotes, historical, political, biological and social considerations.

Of course, a large portion of this book is dedicated to the art of natural products synthesis. Among many other conditions, it requires conceptual vision, as well as profound knowledge of highly diverse compound classes and reaction mechanisms, but also persistence and often good luck to design a successful route and construct the target molecules. Interdisciplinary know-how is necessary to find adequate solutions, *e.g.* by embarking on biosynthetic strategies Nature itself has employed or by using enzymatic transformations. Insight into physiology and pharmacology may lead the way to simpler structures with the same or even superior effects than its natural prototype.

The task to adopt a small-scale reaction to an economically viable and ecologically responsible manufacturing process, which yields large quantities of pure material, poses particular challenges to chemists, engineers and experts from many other disciplines. In this regard it is as well intriguing to witness the advancements and elegance of modern chemical technologies.

Do you have to read this book to advance your professional career? Probably not. But if you are looking for fun with science, you may want to consider placing it on your night stand.

Most of the reaction schemes are presented in colour, which should facilitate the easy recognition of building block fragments in the final products. From the colour of the bonds, however, it is not always possible to draw conclusions on the reaction mechanism. While this is in many cases rather complex, the reader is therefore referred to the primary literature sources. A comprehensive index section will assist in navigating through this book when searching for specific topics, and the rich illustrations – many of them in colour – are meant to familiarise with the origin and application of natural products, but also as a tool to make a somewhat "arid subject" better digestible.

I am in particular grateful to three colleagues and friends of mine for their assistance in this book project: Dr. Richard Riggs, Dr. David Smith and Dr. Bernd Janssen. Without their commitment and their sound knowledge of the subject, this book would not have found its way into print.

Richard Riggs helped me to identify a freelance translator of my manuscript and he read carefully the entire book. My warm thanks go to him for many valuable suggestions and improvements.

David Smith, a chemist from Saint Andrews University and Richard's former PhD supervisor actually accepted the burden and challenges to provide a drafted translation of my revised German textbook. I'm very grateful for David's hard work over some 18 months.

Last but not least I would like to express my deep gratitude to Bernd Janssen, owner of the Massachusetts based Science&Management Consulting operation, former colleague at BASF Pharma and friend for many years. Bernd had already supported the earlier German edition of my book, and his unique diligence, accuracy, perseverance and enthusiasm proved invaluable in compiling its current English version. His careful proof-reading, suggestions for enhancements and translation of parts of this book are especially acknowledged.

I warmly thank my editor Mrs. Elizabeth Hawkins of Springer Verlag, Heidelberg for her patience as well as the enjoyable and productive collaboration. Mrs. Hawkins has supported the concept of this book from its early stages, and has helpfully guided the development process through all phases.

My thanks go in particular to Fotosatz-Service Köhler GmbH in Würzburg, Germany for their excellent design of the layout. They incorporated my numerous requests for changing texts and illustrations in a very professional and diligent way. This was a marvellous collaboration.

I owe particular thanks to all those, who have made available to me excellent illustration material, mostly even free of charge. I am delighted about such a "worldwide" backing of my endeavour, which I would like to recognise by specifically naming the corresponding individuals and companies in the directory of illustrations.

My final and most heartfelt thanks I want to give to my wife Wilgard, who has encouraged and supported me wonderfully in so many ways during those countless evenings and weekends I had been engaged in this book project.

Bernd Schaefer
Dierbach, November 2014

Content

1 | Introduction

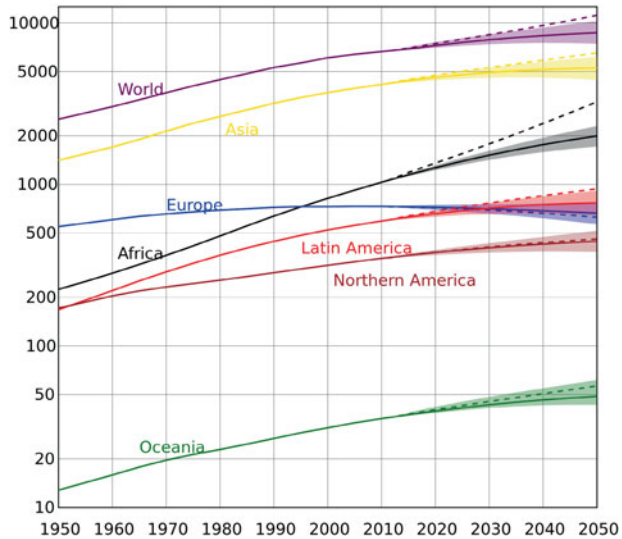
Natural products in the chemical industry – should we recognise that as a contradiction, a rhetorical combination of two mutually exclusive terms, an oxymoron? On the one hand, Nature, with its materials harmonically regulated in cycles; and on the other hand, mankind, producing, consuming and disposing? There is a seemingly unbridgeable divide between the natural and the unnatural, the artificial – how readily we use the term 'chemical' – and we have become accustomed to thinking in terms of such dichotomies. [1] While on the contrary, from its very beginnings, natural product chemistry has given major impulse to the chemical industry. Natural products sustainably contributed in many ways to master global challenges, and to satisfying our daily needs. A few examples may serve as illustrations.

1.1 Important Fields of Application for Natural Product Chemistry

1.1.1 Population Growth

On 12th October 1999, a boy born shortly after midnight in Sarajevo was declared by the United Nations to be the 6-billionth human being; on 31th October 2011 we counted 7 billion [2], and population growth continues unabated, so that by the year 2050 we can expect 9 to 10 billion humans on the planet (Fig. 1.1).

Born in 1960, the author himself calculated to be the 3,030,056,466th.



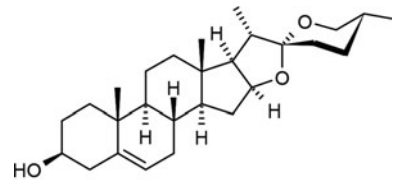
1.1 *Projected growth of the World population. The annual number of births worldwide stands today at 130 million, that of deaths at around 52 million. The developing countries account for around 98 % of this increase in population (x-axis: year; y-axis: population (millions)).*

B. Schaefer, *Natural Products in the Chemical Industry*,
DOI 10.1007/978-3-642-54461-3_1, © Springer-Verlag Berlin Heidelberg 2014

1.2 *The land area of the Earth measures 149 million km², but it is certainly not all habitable, as for example the African desert or Antarctica. This picture was taken on 7th December 1972 from Apollo 17 on its flight to the Moon.*

With 7 billion human beings, we are already approaching the boundaries in respect of living space. The land area of the Earth is 149 million km² (Fig. 1.2). If one distributes the population equally over this area, the space available for each person would be only 2.1 ha: this corresponds to a square with sides of about 150 m. Thus, from the statistical viewpoint, we are in pretty close proximity to any neighbour!

The limitation of human population growth is among the greatest challenges of our time. To cope with this question, we do not lack the necessary technical means, and natural product chemistry has an important part in this: Starting from an extract of yam root, the American chemist Russell Marker created the chemical basis for the development of hormonal contraceptives. Diosgenin was the starting material for the preparation of the first semi-synthetic steroid hormones (*cf.* Section 6.1).



Diosgenin

1.1.2 Nutrition

The World population of more than 7 billion must be supplied with water and food. Current estimates are that around 800 million people are undernourished. In the past, through massive use of pesticides and fertilisers in agriculture, it became possible to treble the yield of crops per hectare; however, it is rather unlikely that this will happen again. [3]

A massive problem is caused by, for example, insects. Harmful insects feed on our foodstuffs and natural fibres; they destroy what we have built (houses, furniture), and they transmit a whole range of serious diseases. Every person invests on average 1.40 US dollars per annum on insecticides; nevertheless, five dollars are lost on feeding the surviving insects with our foodstuffs. The overall annual damage amounts to 30 billion dollars.

In the 1960s and 1970s, from ingredients of chrysanthemums, an important group of insecticides, the pyrethroids, was developed: these are derived from chrysanthemic acid. The pyrethroids and the phosphate esters replaced the previously intensively applied chlorinated hydrocarbons such as, for example, DDT.

Chrysanthemic acid

It is noteworthy that the insecticidal effect of pyrethroids depends strongly on their absolute configuration (*cf.* Section 8.3). Thus, for this class of compounds one had to attempt, for the first time in the history of plant protection, to prepare enantiomerically pure agrochemicals.

1.1.3 Healthcare

Everyone on the planet has a right to adequate healthcare. When in 1882 Robert Koch discovered the bacterial cause of tuberculosis (*Mycobacterium tuberculosis*), in Germany one person in every seven died of this disease. At that time, tuberculosis was the most frequent cause of death in Europe. [4] Through other bacterial infections such as plague (*Yersinia pestis*) and cholera (*Vibrio cholerae*), whole regions had earlier been depopulated. We can scarcely imagine the sorrow of those who were so afflicted, and the following table of prominent victims of tuberculosis is a mere reflection of what this disease may bring about (Tab. 1.1).

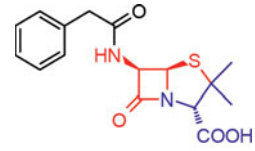
Tab. 1.1 *Prominent victims of tuberculosis. How much more enriched could mankind be today, with concerts, scientific discoveries, poems and paintings, if these individuals had been allowed to live only a few years longer?*

Name	Profession	Year of birth	Age
Emily Brontë	Novelist	1818	22
Giovanni B. Pergolesi	Composer	1710	26
John Keats	Poet	1795	26
Anne Brontë	Novelist	1820	29
Tristan Corbière	Poet	1845	29
Ernest Duchesne	Physician	1874	37
Carl Maria von Weber	Composer	1786	39
Joseph von Fraunhofer	Physicist	1787	39
Frederic Chopin	Composer	1810	39
Franz Kafka	Novelist	1883	41
Madame de Pompadour	Mistress	1721	43
Francis of Assisi	Monk	1181	44
Anton P. Chekhov	Playwright, Physician	1860	44
Auguste Laurent	Chemist	1807	45
David Herbert Lawrence	Novelist	1885	45
Friedrich Schiller	Playwright	1759	46
George Orwell	Novelist	1902	47

With the discovery of antibiotics, it became possible for the first time to set up a defence against the causes of this disease. In 1943, Selman A. Waksman discovered streptomycin, an active ingredient of the mould *Streptomyces griseus*; this shows exceptionally good activity against the tuberculosis-causing bacteria, and its use provided the means to combat the disease.

At the same time, there was intensive activity on the preparation of another antibiotic, which was effective against meningitis, scarlet fever, pneumonia, gonorrhoea and blood poisoning (sepsis): this was penicillin (*cf.* Section 5.2). The Scottish bacteriologist Alexander Fleming had already discovered this material in 1928; however, its preparation proved very difficult. In

Despite all the research into healthcare, today there is still a range of "neglected diseases", which occur predominantly in developing countries and for which suitable medication is still missing. These diseases include those caused by water-borne nematodes (ascariasis), parasitic microorganisms (trachoma, leishmaniasis, Chagas' disease, African sleeping sickness), and viral infections (dengue and yellow fever).



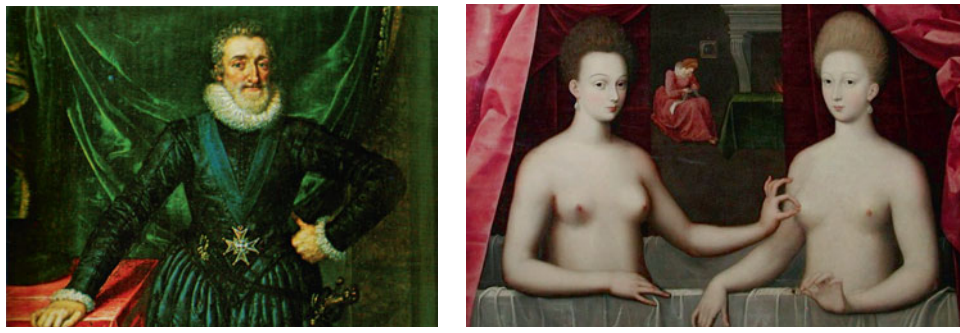
Penicillin G

Britain and the USA, it was possible for the first time at the end of the Second World War to obtain penicillin on a larger scale. Production in Germany was begun in 1944 by Hoechst and in 1946 by Schering. Also, directly after the end of the war, "penicillin wound-powder" was being produced by the Microbiological Institute in Jena, which became VEB Jenapharm (*Volks*eigener *B*etrieb, people-owned enterprise; abb.: VEB) in 1950.

1.1.4 Fragrances

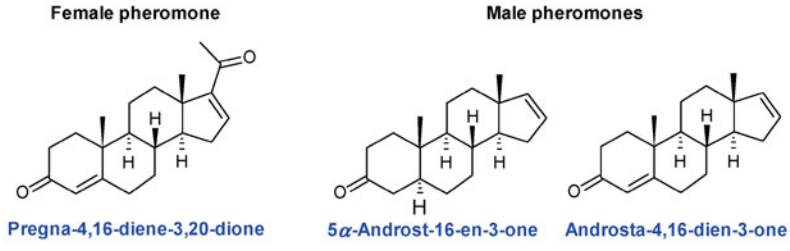
While products such as plastics, pharmaceuticals and agrochemicals may convey the impression that chemistry is a rather purposive science, so fragrances represent more a category of dispensable affluence. Such affluence, on the other hand, allowed mankind also to create a culture. Fragrances and flavourings have accompanied us throughout our entire cultural history.

Henry IV (1553–1610), the first Bourbon on the French throne, is believed to have written as follows to his mistress, Gabrielle d'Estrées (Fig. 1.3): Surtout ne te lave pas, j'arrive! (Please refrain from washing, my darling; I shall be back… (in a week.)) Apparently he was longing for the distinctive scent of his beloved; from what we know nowadays, we may guess that he was probably missing a whiff of pregna-4,16-diene-3,20-dione!



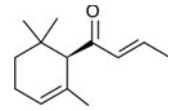
1.3 *The nobility also possessed a body odour: Henry IV and his mistress, Gabrielle d'Estrées (right).*

But men also have their chemical appeal. Their 5α-androst-16-en-3-one and androsta-4,16-dien-3-one act as attractants for women. Tests have been carried out, whereby it was observed that women – without being aware of their selection – prefer to sit on chairs, which have been doped with these substances.



In the very recent past, the sensational discovery of the human vomeronasal organ (VNO, pheromone-sensitive organ), and its fascinating properties, have provided interesting starting points for intervention in our nonverbal communication. For this purpose, delicate perfumery uses nowadays a broad range of natural products.

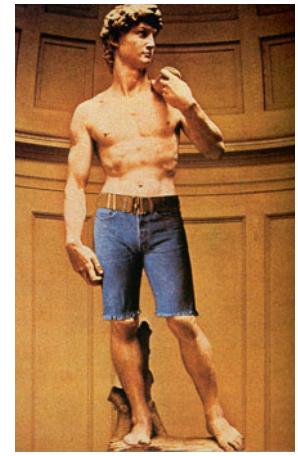
Another fascinating compound, although for entirely different reasons, is α-damascone, which is responsible, at least in part, for the scent of roses. α-Damascone may be prepared on an industrial scale by means of an enantioselective protonation (*cf.* Section 3.1), a new stereoselective synthetic method.



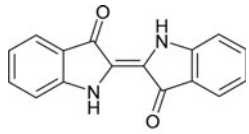
α-Damascone

1.1.5 Colourants

Just like perfumes, textile dyes are associated with a luxury attribute of mankind's possessions. In addition to its functional purpose, *i.e.* to warm and protect the body, clothing has always possessed an aesthetic and decorative component. Gowns either emphasise or cover the proportions of the human body. They convey class differences, a scale of values, and are an expression of our attitude towards life. In this, colours play an important part. For example, with natural indigo (*cf.* Section 2.1), coloured clothing in earlier times was a symbol of authority; nowadays synthetic indigo is an essential colourant for our "leisure-look" (Fig. 1.4).



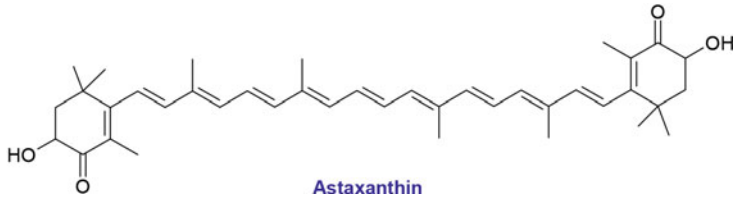
1.4 *Indigo-coloured blue jeans have become an essential item of clothing. ('David' by Michelangelo (1475–1564), Florence, Galleria dell'Accademia).*



Indigo

However, we have also some other colouristic needs – from a completely different area. Worldwide, 2,400,000 tonnes of salmon are 'grown' annually in wire cages (Fig. 1.5). In order to ensure that fillets from this 'farmed' salmon have the same colour as those from wild salmon, the farmed fish are fed the naturally occurring carotenoid dyestuff astaxanthin, in the form of amorphous nanoparticles, as part of their regular diet.

1.5 *The salmon fillets on our plate are always the same colour.*



Astaxanthin

1.6 *The consumer's nationality may be recognised by the colour of their breakfast egg's yolk.*

The same also holds for the carotenoid content of poultry food (Fig. 1.6). In this connection it is important to attain the yolk colours expected by the consumer: pale, almost colourless yolks in Norway, strong yellow yolks in Germany, and orange-red duck egg yolks in Thailand (*cf.* Section 7.1).

1.1.6 Inspiration for Innovations

Apart from the immediate exploitation of natural products to satisfy our needs, there are several intellectual reasons for the employment of natural product chemistry.

Whereas Man has carried out organic chemistry in the modern sense for around 200 years, Nature has already mastered this business for several billion years. In the last 50 years, biochemistry has revealed a fascinating variety of reaction pathways and manufacturing processes. In this sense, we are just beginning to understand Nature.

In many cases, she already provides us with solutions, or at least points us into that direction. In general, Nature has optimised an active substance, dedicated for a particular animal or plant, but not for the treatment of a human illness or for pest control. Structure variation however offers us the chance to optimise this activity further to meet our desired applications and our needs. Natural products are often complex, but they may lend themselves in many cases to structural simplification while still retaining their activity.

A further aspect of natural product chemistry is now becoming more and more important. Nature provides not only interesting lead structures for research in plant protection and pharmaceuticals, but also the "genetic code" for the biosynthesis of these materials. This is an important gateway for modern gene- and biotechnology, for example for research into resistance, or to the generation of genetically modified crops possessing pest resistance. Meanwhile, we obtain from Nature not merely products, but also the corresponding "recipes".

28% of the pharmaceuticals which have been approved in the USA, Europe or Japan over the past 25 years are derived from natural products. [5, 6]

1.2 Natural Product Chemistry in the Chemical Industry

We now turn to the question of what special features characterise the production of natural compounds or their derivatives on a technical scale. Common to all of these products is that their utility resulted from incidental discoveries or as the result of a targeted search. Such property or activity of a natural product was often used in the past without isolating the pure material – and of course mostly without any idea of its chemical structure. Often centuries, or at least decades, passed between the first use of the natural product and the determination of its chemical structure. Once the structure was known, by means of targeted modifications it became possible to develop analogues with new or improved properties: new synthetic methods and reaction conditions could then be developed. Introduc-

tion of such new products to the market, and feedback from a breadth of applications, lead then to further product improvements and corresponding inventions.

Nowadays, the development of new syntheses is a key aspect and an important prerequisite for the industrial production of natural products. In many cases, the zest lies in preparing complex molecules using the simplest possible methods.

This involves making the most economical use of all resources (Fig. 1.7). Processes are all the better, the fewer stages and shorter procedures they comprise, the fewer chemicals and less energy they require, and the fewer the by-products, which have to be avoided. Also reduction of technical and organisational expenditure, *e.g.* the use of special equipment or the avoidance of labour-intensive and time-consuming reaction control, is of major significance. [7, 8]

In 1975, James B. Hendrickson defined the "ideal synthesis" as one which: creates a complex molecule … in a sequence of only construction reactions involving no intermediary refunctionalisations, and leading directly to the target, not only its skeleton but also its correctly placed functionality. [9]

Barry Trost spoke of "atom efficiency", by which he meant the use of as many atoms as possible from the reagents for the construction of the target molecule. Later on, chemists developed the economies of synthesis, which comprises also step economy (Paul Wender) and redox economy (Phil Baran, Reinhard Hoffmann). Nowadays, we are aiming for the ideal synthesis, but there is still an urgent need improving elegance in organic chemistry. [10]

Roger Sheldon defined the E-factor as the quotient of the amount of by-products and the desired product. He further introduced a term called the Q-factor, which recognises the quality of the by-products. The Q-factor is a measure of environment-unfriendliness, which is defined in part by social and political criteria. The product of both values defines the environment-friendliness of the synthesis. The smaller the number, the better and more elegant the process. [11, 12]

E = [by-products]/[desired product]
Q = environment-unfriendliness factor
Elegance Product = E × Q
For example: NaCl: Q = 1; Chromium salts: Q = 100 or 1000?

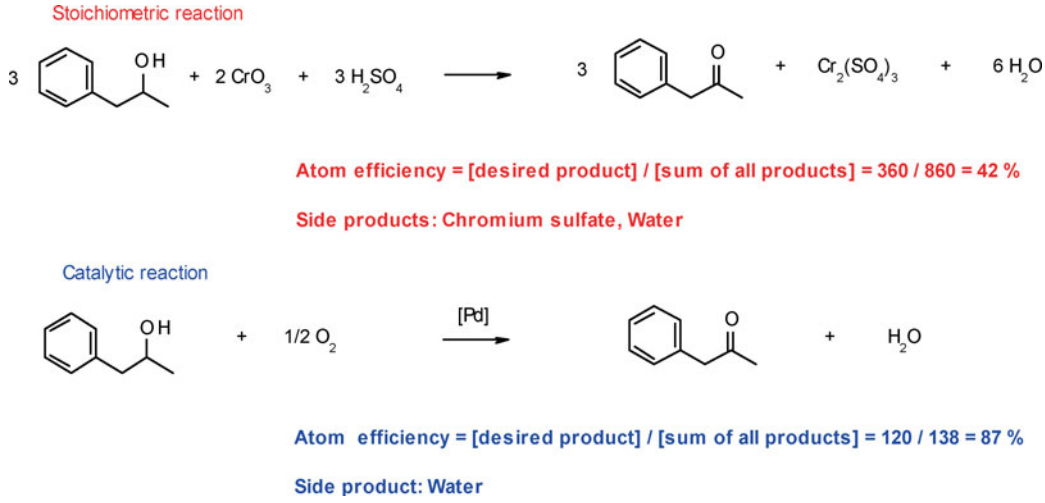
If we take as an example the oxidation of alcohols, chromium salts are in fact selective and have a variety of uses, but the atom efficiency is conceivably bad. [13] In this sense, catalytic oxidation with oxygen is far superior to stoichiometric oxidation with chromium salts. [14, 15] (*cf.* next page)

With new technical processes, the determination of the process mass intensity (PMI = total mass in a process (kg)/mass of product (kg)) and considerations of possible adverse effects on the environment usually form part of the process evaluation (Green Chemistry). On grounds of modern environmental protection legislation, in many cases environmentally friendly procedures are reckoned better than older processes, which have a greater polluting effect on the environment (Fig. 1.8). [16, 17]

i Discoveries apply to objects or facts (chemical compounds, organisms, countries, continents, or stars), which exist independently of any human involvement. The latter, however, is indispensible in the case of inventions. Therefore, whereas natural products can be *discovered*, methods for their preparation are acts of *invention*.

i Measured by the quantity of their main products most organic chemists are genuinely inorganic chemists!

1.7 *The Franciscan monk William of Ockham (1286–1347), in the context of philosophical, theological argumentation, developed the theory of thrift. He is well known for the principle named after him (Ockham's razor), with which one cuts off, in the figurative sense, all superfluous argumentation. He put it aptly: "It is futile to do with more what can be done with fewer." – Sketch labelled 'frater Occham iste' is taken from a manuscript of Ockham's Summa Logicae, MS Gonville and Caius College, Cambridge, 464/571, fol. 69r, 1341.*



1.8 *If we talk today of "Green Chemistry", many may also think of the colour of the American one-dollar banknote ("green buck"). The introduction of environmentally friendly processes often yields benefit in the long term!*

The ideal synthesis is highly convergent; it consists only of additions or rearrangements, and reaches a high degree of complexity only at the end of the complete synthesis. Convergent syntheses have thus not only the advantage that they lead to higher overall yields, but they also avoid the accumulation of functional groups, which play no part in the intended reactions and which add to the complexity of the process towards the end of the complete synthesis.

At least equally important as atom-efficiency are reaction- and stereochemical efficiency of a complete synthesis. The elegance of a synthesis – indeed as well in an aesthetic sense – is enhanced if it succeeds in

- replacing stoichiometric reactions by catalytic procedures;
- employing a stereoselective synthesis instead of unselective methods;
- avoiding protecting groups;
- combining of reaction steps;
- improving material and energy balances.

The wolf-cabbage-goat problem – a protecting-group parable

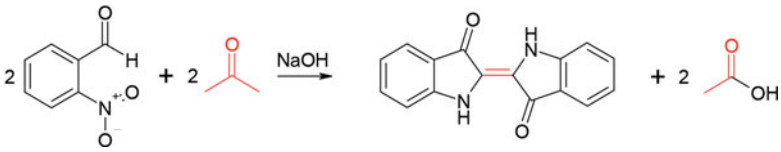
A farmer is on the road with a cart, on which are a wolf, a cabbage and a goat. As long as the farmer can prevent it, none may eat up the other. Then they come to a river, through which the farmer can carry only the wolf, the cabbage or the goat, one at a time. The problem lies in determining the order in which the farmer should move his property to the opposite bank. [18]

For the transfer of a laboratory synthesis on to a production scale, process chemists from AstraZeneca, Pfizer and GlaxoSmithKline have together created the acronym SELECT (Safety, Environment, Legal, Economics, Control and Throughput) for those criteria, which allow potential processes to be compared with one another and assessed; thereby setting out and summarising further requirements for an elegant manufacturing process. A similar evaluation procedure was also published by chemists from Boehringer Ingelheim. [19]

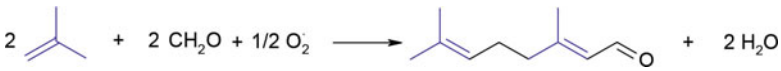
Of particular significance for a reaction in question is the selection of chemicals used, both in terms of material safety and energy balance. The selected process must not contravene laws or regulations, and must be very tightly controlled in terms of product quality and product safety, for example by using cGMP (current Good Manufacturing Practice) in accordance with the ICH guidelines for the production of pharmaceuticals. Of course, it must also guarantee a reasonable throughput and be altogether economic.

1.2.1 A Short Selection of Elegant Syntheses

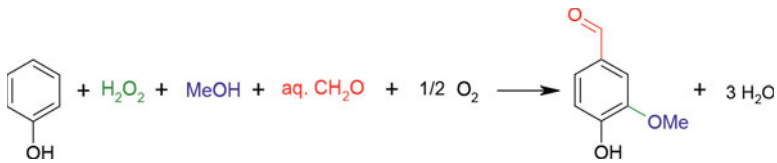
In many areas of industrial natural product synthesis, there are examples which are "elegant" in the meaning above. Adolf von Baeyer, for instance, developed in partnership with BASF a synthesis of indigo, starting from *o*-nitrobenzaldehyde, acetone and sodium hydroxide (*cf.* Section 2.1.4). Though, at that time its technical realisation failed due to the inaccessibility of *o*-nitrobenzaldehyde.



For both, the perfumery market and also as a building block in carotenoid synthesis, BASF currently prepares citral, starting from isobutene and formaldehyde. The only side-product is water (*cf.* Section 3.2).

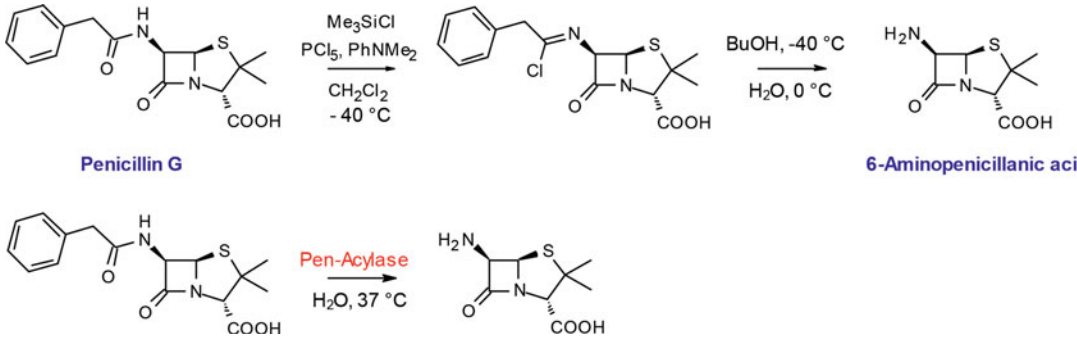


Rhône-Poulenc has developed an elegant process for the synthesis of vanillin, the most important "aroma chemical" apart from menthol (*cf.* Sections 3.4 and 3.5). The four-stage process starts from phenol, and again produces water as the only side-product. [20]

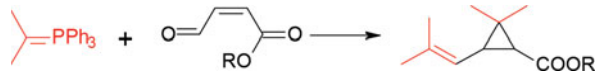


Degussa manufactures methionine by a process, in which practically no salts accumulate as waste, and all the side-products are recyclable (*cf.* Chapter 4).

DSM has succeeded in simplifying considerably the synthesis of the antibiotic intermediate, 6-aminopenicillanic acid (*cf.* Section 5.2). A process using an immobilised enzyme has superseded the costly chemical synthesis. [21]



Finally, the "one-pot" synthesis of chrysanthemic acid developed by Roussel Uclaf belongs in this list of elegant syntheses (*cf.* Section 8.3). Treatment of maleic aldehyde ester with two equivalents of isopropylidenetriphenylphosphorane produces an interesting building block for the synthesis of pyrethroids.



Summary in Bullet Points

- For industrial-scale natural product chemistry there is a host of motives: control of population growth, securing nutrition, improving healthcare, supply with luxury items.
- Nature already offers us a wealth of problem solutions.
- The spectrum of applications extends from colourants and amino acids over a broad range of pharmaceuticals, fragrances, vitamins and hormones to plant protection agents.
- In the synthesis of complex molecules, the challenges are always atom economy, eco-efficiency, and simply elegance in chemistry.

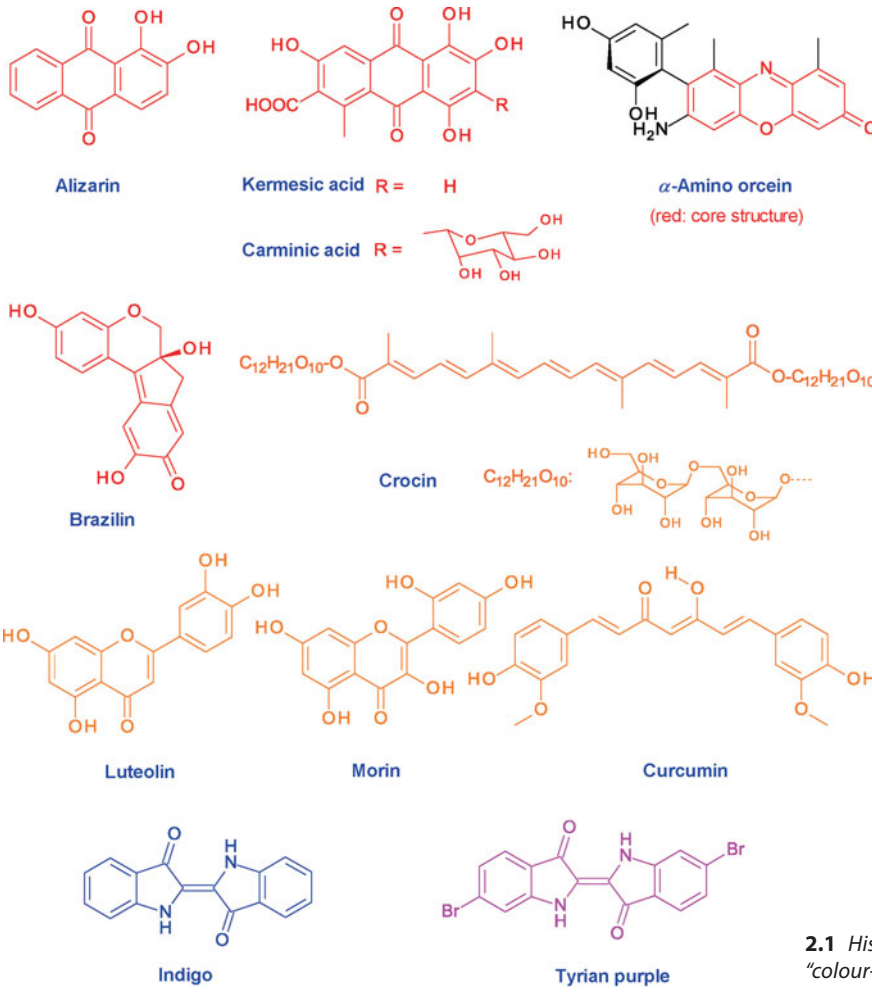
References

1. H. Markl, Wissenschaft im Widerstreit, VCH, Weinheim, 1990, 23.
2. Bibliographisches Institut & F. A. Brockhaus AG, Electronic Release, 2001; http://www.worldometers.info/world-population/; http://www.bbc.co.uk/news/world-15391515.
3. Quadbeck-Seeger, Chemierekorde, Wiley-VCH, Weinheim, 2nd Edn., 1999, 122.
4. www.cell.de/gesundheitspark/tuberkulose.html.
5. M. S. Butler, *Nat. Prod. Rep.* **22** (2005) 162.
6. D. J. Newman, G. M. Cragg, K. M. Snader, *J. Nat. Prod.* **66** (2003) 1022.
7. R. A. Sheldon, *Pure Appl. Chem.* **72** (2000) 1233.
8. R. Hoffmann, V. I. Minkin, B. K. Carpenter, *Bull. Soc. Chim. Fr.* **133** (1996) 117.
9. J. B. Hendrickson, *J. Am. Chem. Soc.* **97** (1975) 5784.

10. T. Newhouse, P. S. Baran, R. W. Hoffmann, *Chem. Soc. Rev.* **38** (2009) 3010; T. Gaich, P. S. Baran, *J. Org. Chem.* **75** (2010) 4657.
11. R. A. Sheldon, *Chemtech*. (1994) 38; R. A. Sheldon, *J. Mol. Catal*. **107** (1996) 75.
12. R. A. Sheldon, *Green Chem*. **9** (2007) 1273.
13. R. A. Sheldon, J. Chem. Tech. *Biotechnol*. **68** (1997) 381.
14. G.-J. ten Brink, I. W. C. E. Arends, R. A. Sheldon, *Science* **287** (2000) 1626.
15. L. Jaenicke, *Chemie in unserer Zeit* **34** (2000) 180.
16. E. Gärtner, *Nachr. Chem. Labor, Tech*., **48** (2000) 1357.
17. P. Dunn, J. L. Leazer, K. Lorenz, *Org. Proc. Res. Dev*. **12** (2008) 807; C. Jimenez-Gonzalez, C. S. Ponder, Q.B. Broxterman, J. B. Manley, *Org. Proc. Res. Dev*. **15** (2011) 912.
18. First solution: Goat across, cabbage across, goat back, wolf across, goat across. Second solution: Goat across, wolf across, goat back, cabbage across, goat across.
19. M. Butters, D. Catterick, A. Craig, A. Curzons, D. Dale, A. Gillmore, S. P. Green, I. Marziano, J.-P. Sherlock, W. White, *Chem. Rev*. **106** (2006) 3002; R. Dach, J. J. Song, F. Roschangar, W. Samstag, C. H. Senanayake, *Org. Proc. Res. Dev.* **16** (2012) 1697.
20. S. Ratton, *Chem. Today*, March/April 1998, 33.
21. Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, 5th Edn., Vol. B8, 302.

2 | Colourants

For thousands of years Man has made use of dried materials from plant or animal sources. In Chinese as well as European culture, these "drugs" were used – sometimes unaltered and sometimes as extracts – as remedies as well as for technical purposes. For example, kermes served both as a red dyestuff and as a drug for the treatment of heart disease (Fig. 2.1). [1]



2.1 *Historically important "colour-drugs".*

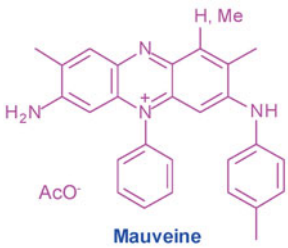
B. Schaefer, *Natural Products in the Chemical Industry*,
DOI 10.1007/978-3-642-54461-3_2, © Springer-Verlag Berlin Heidelberg 2014

Madder, kermes, carmine, archil and redwood were all used as red dyestuffs. Alizarin (*cf.* Section 2.3) provides the core structure for the dye, which was obtained from the root of madder (*Rubia tinctorum* L.), and also for kermes and carmine, which are both extracted from scale insects (*Kermococcus ilicis* L. and *Dactylopius coccus* L., respectively). These are mordant dyes, which form with transition metal salts, depending on the individual metal, diversely coloured complexes on textiles. Archil is a colour preparation from lichens, which grow on many sea coasts, and are used for direct dyeing of wool and silk. The alcoholic extract of these composite organisms is known as orcein. It contains a series of different colouring dyes based on the phenoxazone chromophore. Brazilin is the colouring component of South American redwoods, and serves as mordant dye for colourising wool, silk and cotton.

Chemically related to brazilin are the yellow mordant dyes, luteolin and morin. Both are flavone derivatives; whereas the former is obtained from yellow weed or weld (*Reseda luteola* L.), which grows widely across Europe, the latter comes from dyer's mulberry (*Maclura tinctoria*, syn.: *Morus tinctorius* L.). Alum- and tin-based mordants from luteolin produce a beautiful true yellow on all textile materials. Apart from weld, saffron (*Crocus sativus* L.) used to be the most important yellow colourant. The ancient Greeks and Romans used the dried stigmas from this species of crocus to obtain crocin, which was absorbed onto textiles with an alum mordant. Like saffron, curcuma (also known as turmeric) serves as a colourant for foodstuffs (*e.g.* for mustard and curry), and as a somewhat acrid spice in Oriental and South-east Asian cuisine. Curcuma is obtained from the root nodules of, for instance, *Curcuma longa* L., which is cultivated in plantations in China and the East Indies.

The most important source of blue colourants in Europe was woad (*Isatis tinctoria*), from which indigo could be produced (*cf.* Section 2.1). Chemically related are the purple dyes obtained in ancient times from sea-snails of the genus *Murex* (*cf.* Section 2.2). Depending on the particular species of snail, violet to reddish-purple textile dyes can be obtained. Tyrian purple and indigo are both vat dyes.

Apart from a few inorganic pigments, these represent the important dyestuffs, which determined the colours available for many centuries. In the course of the first Industrial Revolution, with the invention of power looms and steam engines, the textile industry blossomed. There was a growing need for artificial bleaching agents. Initially, dilute sulfuric acid, prepared by the lead chamber process (John Roebuck, *ca.* 1750), was used for this purpose. Since this however caused damage to the fabric, hypochlorite became the preferred bleach (Charles Tennant, 1799). At the same time, there was an increasing need for textile dyes. When in 1856 William Henry Perkin, in an attempt to prepare quinine, found instead the first coal-tar dye, mauveine, no one foresaw that he had thereby laid a crucial cornerstone for an entirely new industry branch. In fact, the most important roots of the chemical industry lie in the production of colourants and the preparation of pharmaceuticals, fragrances and explosives (Tab. 2.1).



Mauveine

Tab. 2.1 *The original activities of a few major chemical companies*

Company	Founded in (year)	Initial area of activity
Geigy	1758	Colourants
Ciba	1859	Colourants (fuchsine)
Bayer	1863	Colourants (ultramarine)
Hoechst	1863	Colourants
BASF	1865	Colourants
Sandoz	1886	Colourants
Merck	1827	Pharmaceuticals (morphine)
Eli Lilly	1876	Pharmaceuticals
Boehringer Ingelheim	1885	Pharmaceuticals
BMS	1887	Pharmaceuticals
Hoffmann La Roche	1896	Pharmaceuticals
Haarmann & Reimer	1874	Flavourings (vanillin)
Dragoco	1919	Aroma chemicals
Dupont	1802	Explosives
Nobel Industries	1894	Explosives

Towards the end of the 19th century, many of the newly established firms developed aniline- and triphenylmethane dyestuffs. In 1859, Ciba began the production of fuchsine, which enabled the dyeing of silk. In 1877, BASF was granted the first German patent for a coal tar dye entitled "Preparation of Blue Dyestuffs from Dimethylaniline" (*viz*. Methylene Blue) (Fig. 2.2).

In the same year, Otto Fischer synthesised Malachite Green. A year later, Adolf von Baeyer elucidated the structure of indigo. Several firms manufactured alizarin, but the first industrial-scale synthesis of indigo followed only in 1897. Around the same time, several firms developed and produced the first azo dyes, after Paul Böttiger had improved the preparation of Congo Red from benzidine in 1884. René Bohn synthesised the first anthraquinone dye, Indanthrene Blue RS in 1901 – a vat dye, like indigo, but much more light- and waterfast. In 1927, Henri de Diesbach and Edmond von der Weid prepared the first copper-phthalocyanine pigment, a material which was marketed a few years later by several firms. After the Second World War, phthalocyanines developed into an important class of colourant in their own right. The first perylene pigments became technically accessible in 1950: their most important application is currently in premium automotive finish. In 1952, the first reactive dyes with vinyl sulfone groups came on to the market. Quinacridone pigments for high-quality colour printing, automotive enamels and powder coating for plastics followed in 1958. In the 1960s, dyes for the colouring of synthetic fibres grew in importance. One of the more recently introduced organic pigment classes are the diketopyrrolopyrroles (DPP). These red pigments were developed by Ciba in the 1980s and 1990s. They are used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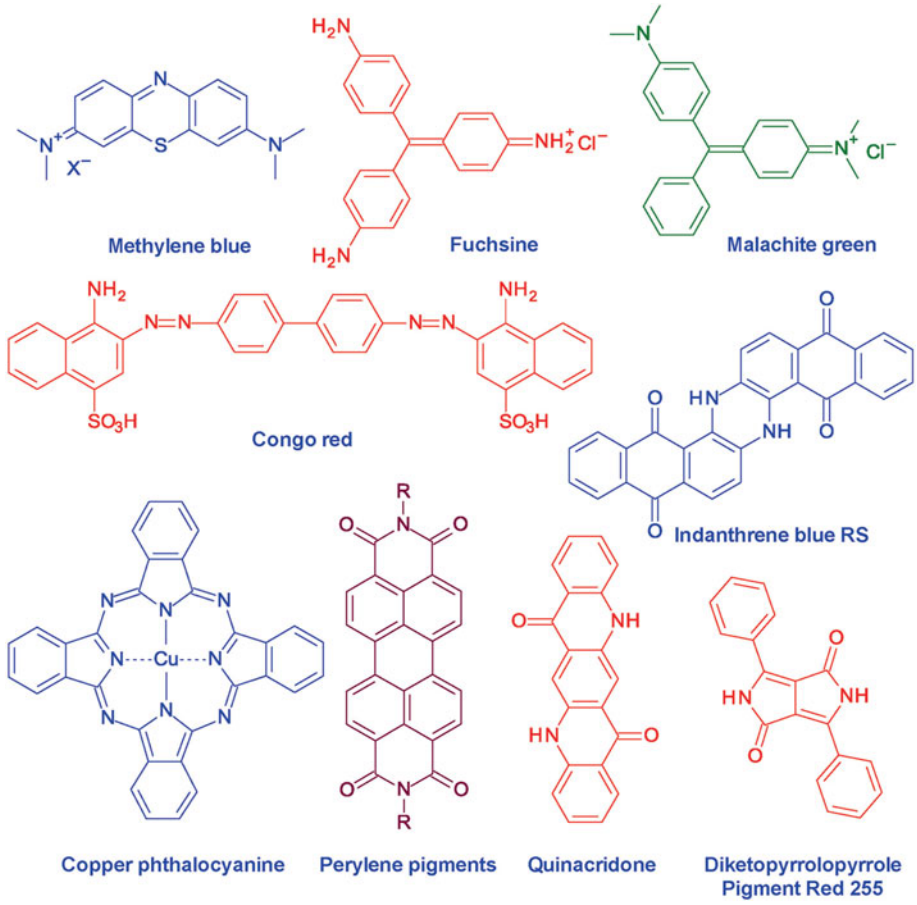
2.2 *Patent document for the preparation of Methylene Blue*.



2.3 *Selected synthetic colourants of the last hundred years.*

predominantly in high performance plastics and automotive paints, and are known for their outstanding brightness and weather stability.

Modern developments in the dyestuff and pigment sector are luster pigments, IR-reflectors for house façades and windows, light-harvesting systems for solar collectors and light-emitting diodes in the electronic industry (Fig. 2.3).

The following sections of this chapter deal with indigo, Tyrian purple and alizarin. By example of these three important colourants, we can familiarise with the historic processes which had a lasting impact on shaping the chemical industry. Alizarin was the first natural dyestuff to be prepared synthetically on a large scale, and along with indigo it can be considered as a trigger for the development of modern industrial production processes for a number of basic inorganic chemicals, such as chlorine, ammonia and sulfuric acid. Dyeing with Tyrian purple had already in the ancient world an industrial dimension. However, the demand for mauveine stimulated the advancement of industrial chemistry to a different level. The market was conquered by this

synthetic product, before Tyrian purple from synthetic origin could even claim a position. Thus, there was never an industrial manufacturing process developed for Tyrian purple.

Summary in Bullet Points

- Man has used natural colourants for thousands of years.
- With the synthesis of the first coal tar dyestuff, mauveine, W. H. Perkin laid an important cornerstone for modern chemical industry.
- More recent developments in colour chemistry focus on luster pigments, IR-reflectors, light harvesting systems and light-emitting diodes.

2.1 Indigo

2.1.1 Introduction

Among the earliest preserved evidences of our culture are cave paintings of the Cro-Magnon Man (European Early Modern Humans, EEMH, *Homo sapiens sapiens*), which were produced 10 000 to 30 000 years ago in places like the caves at Lascaux, located in France's Dordogne region. The pictures display a broad colour palette with subtle shading – what they are lacking is blue (and green, which could have been produced by mixing with yellow ochre). Yet, blue tones are not uncommon in Nature, where they are however frequently caused by interference phenomena, not only in media like water and air, but also in the feathers of birds and wings of butterflies and beetles. The colour of the rose chafer (*Cetonia aurata*) for example, changes from bronze to green depending on the observation angle (Fig. 2.4).

2.4 *Cave painting from Lascaux and a rose chafer.*

Apart from ultramarine (see marginal note on next page) [2], indigo was one of the few colourants to close this "blue gap" in the colour palette. In ancient times, it was reckoned as valuable on account of its beauty and lightfastness, which lent itself to wider applications, not only for the colouring of precious textiles but also for artwork.

2.5 *During the years 1858–1861, relics of the Roman Iron Age were found in the peat of Thorsberg Moor near Süderbrarup, Schleswig-Holstein, Germany, where a Germanic tribe, the Angles, deposited votive offerings for approximately four centuries. Among them was the so-called Thorsberg cape (probably dating to 3rd/4th century AD). Its blue colour resulted from dyeing with woad.*

It is also known from grave-finds that Egyptians liked to colour their linen with indigo. From the early Iron Age, the Teutons used woad to produce blue textiles, as in the magnificent (reconstructed) cape from Thorsberg (Fig. 2.5). The ancient Greeks and Romans already knew the indigo which came from India, as we have learned from the writings of Dioscurides and Vitruvius. However, it was used mainly as an artist's pigment; for the dyeing of textiles they used also woad. Pliny the Elder (23 or 24 AD – August 25, 79 AD, while attempting the rescue of a friend and his family by ship from the eruption of Mount Vesuvius) describes in his *Naturalis Historia* the high value of indigo. He also mentions that dishonest dealers tried to eke out the expensive product by colouring chalk with woad.

In *De Bello Gallico*, Julius Caesar (100–44 BC) describes another interesting use of indigo by the British Celts. Indigo serves as a component of make-up for war-paint. (see marginal note) [3]

In the High Gothic period, there developed a diverse colour symbolism. By virtue of the price of clear deep blue shades, artists confined themselves to use blue only to depict the saints. In this way, blue emerged as the symbolic colour for the Virgin Mary (Fig. 2.6).

The word "indigo" is derived from the river Indus, where traces of the Indian Harappan civilisation were found: this was the first such culture to use indigo for colouring. [4] In the whole of the Mediterranean region there were no indigenous blue plant colourants. Marco Polo (1254–1324) was the first to describe the production of indigo from the indigo plant in India. In 1498, indigo from India was imported for the first time on a larger scale. [5] Thus, from the Old Indic word "nilaa" meaning "dark blue" came the Portuguese term "anil" for indigo (and thereby "aniline", the name of a decomposition product from indigo, obtained by Carl Julius Fritzsche in 1841 by oxidation of indigo in molten potash [6]).

Apart from the tropical and subtropical indigo plant, *Indigofera tinctoria* (Fig. 2.7), the dyer's knotweed (*Polygonum tinctorium*, syn.: *Persicaria tinctoria*) was cultivated in southern China and Japan, and woad (*Isatis tinctoria*) in northern Europe. In the 13th century the cultivation extended to the whole of central Europe, *e.g.* to England (Somerset and Lincolnshire), France (Normandy, Somme, and Languedoc), Germany (the Jülich area and Thuringia) and Italy (Piemont and Tuscany): in corresponding areas of France and Germany (*e.g.* in Thuringia) it generated considerable wealth. Nowadays, these plants can still be found growing wild along the river Rhine. [7, 8]

In the 18th century, the spread of woad cultivation contracted, since British-Indian companies had established large *Indigofera* plantations, particularly in Eastern India. The yield of the colourant from *Indigofera* was around 30 times higher. From 100 kg of leaves one could obtain 2 kg of indigo. [9] So in 1897,

The name 'ultramarine' for the semi-precious stone, Lapis lazuli ("Blue Stone of Heaven"), which occurs in Nature as a deep-blue coloured mineral (lazurite), relates to the overseas origin of this material, which was brought over the sea to Italy: *ultra* (Latin) = on the other side, *mare* (Latin) = sea. The best Lapis lazuli comes from Afghanistan. It had already been imported into ancient Egypt, where *e.g.* the death mask of Tutankhamun is richly decorated with this matter.

Omnes vero se Britanni vitro inficiunt, quod caeruleum efficit colorem, atque hoc horridiores sunt in pugna aspectu.
All the Britains, indeed, dye themselves with woad, which occasions a bluish colour, and thereby have a more horrible appearance in fight.

6,000 tonnes of natural indigo were produced. In the year 1853, at the time of the Californian gold-rush, an immigrant from Bavaria to the USA, by the name of Levi Strauss, set up a wholesale business of haberdashery in San Francisco. Along with a tailor, Jacob Davis, he produced canvas pants for the gold-diggers, which were reinforced at their stress points with copper rivets (Fig. 2.8). In 1873, Levi Strauss obtained a patent for these. He got the material for the blue, white and brown sailcloth trousers from the Amoskeag mill in New Hampshire. In particular, the indigo-coloured denim enjoyed great popularity and morphed over the years into "blue jeans".

Even before the outbreak of the First World War, synthetic indigo had almost completely superseded the natural product. In India alone, the area of the plantations had diminished from 700,000 ha in 1897 to 121,000 ha in 1914. On account of the naval blockade during the First World War, natural indigo became scarce in the short term; and its final end came after the war, when dye manufacturers in Switzerland, the USA, Japan and England set up their own indigo production.

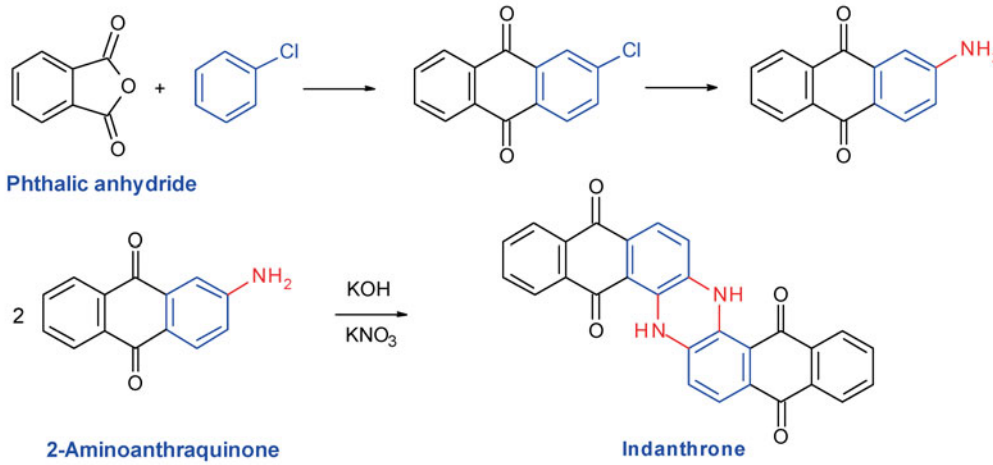
2.6 *The "Madonna in the Rose Bower" by Stephan Lochner (ca. 1410–1451), the most important late Gothic painter of the Cologne School (Wallraf-Richartz Museum, Cologne).*

2.7 *Indigofera tinctoria: Coloured copperplate print by Nicolaus Friedrich Eisenberger, taken from the herbal of Elisabeth Blackwell, Nuremberg, 1765 and a photograph of the flowering plant. Woad (Isatis tinctoria) can be seen on the image to the right.*

2.8 *Levi Strauss (left), the "father" of indigo-coloured "blue jeans", which scored their first success at the time of the Californian gold-rush.*

Nevertheless, with the arrival of synthetic indigo in the 20th century, more beautiful and water-fast cotton dyes than ever before could be produced, *e.g.* Indanthrene Blue. Arising out of the chemistry of alizarin, work began on anthraquinone dyestuffs. In 1901, René Bohn at BASF made a remarkable dis-

covery: melting 2-aminoanthraquinone with potassium hydroxide and potassium nitrate gave a blue dye, which, on account of its origin and its colour, Bohn called "Indanthrone".

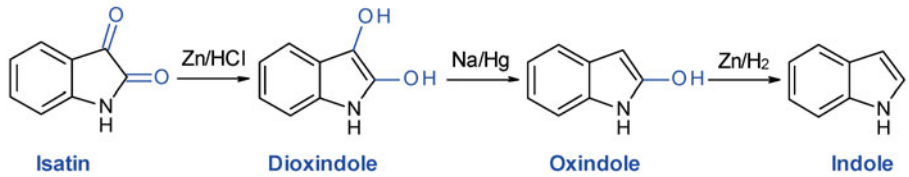


Only in the 1960s, indigo experienced an unexpected comeback. It was James Dean's blue jeans and leather jacket which became initially a symbol of protest, and then the uniform for a whole generation. Indigo, the king of colourants, and once reserved for the establishment, became the colour of the non-establishment and the label for a particular life style.

2.1.2 Structure Determination

More than half a century passes from initial research the pharmacist Otto Paul Unverdorben (1806–1873) had conducted in 1826 to elucidate the structure, when Adolf von Baeyer was able to dispose of the final uncertainties regarding indigo's chemical constitution in 1883.

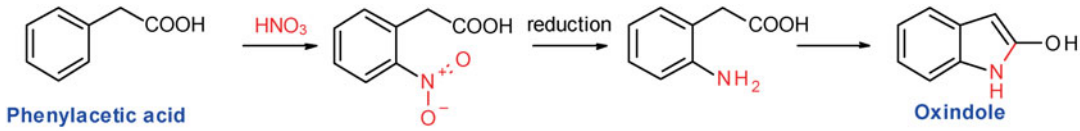
By attempted distillation of indigo with lime, Unverdorben obtained aniline. Under milder conditions, anthranilic acid was produced. The most important degradation reaction of indigo is oxidation with nitric acid, as performed in 1841 by Otto Linné Erdmann (1804–1869) and Auguste Laurent (1807–1853), which resulted in isatin. This was, more or less, the state of knowledge when Adolf von Baeyer began in Strasbourg his work on indigo in 1865. One of the critical questions was, at which position in the five-



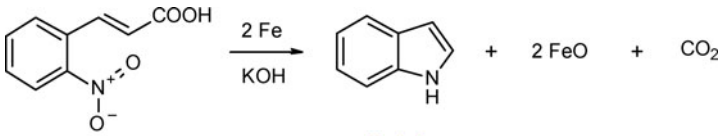
membered ring of indigo is the oxygen atom attached? For this purpose Baeyer reduced isatin stepwise to indole. Zinc/hydrochloric acid reduction rendered first dioxindole, which he reduced further with sodium amalgam to oxindole; finally, he obtained indole by distilling oxindole with zinc dust in a stream of hydrogen.

Baeyer confirmed his results by the total synthesis of oxindole, isatin and indole. Nitration of phenylacetic acid, isolation of the *o*-nitro isomer, and reduction of the latter followed by ring closure gave oxindole. Reaction with "nitrous acid" (*i.e.* potassium nitrate and sulfuric acid) gave isatin oxime, from which by reduction, dehydrogenation with iron(III) chloride and final hydrolysis, isatin itself was obtained.

Baeyer had tried out zinc dust distillation for the first time as a synthetic route to indole. In connection with the structure determination of many natural products, this later proved a valuable method for the transformation of phenols into hydrocarbons.

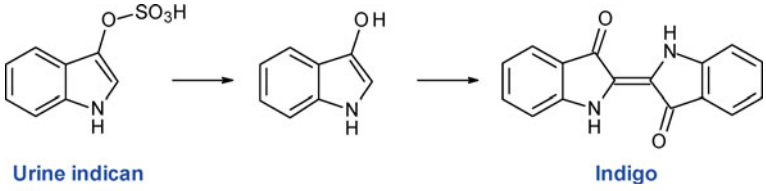


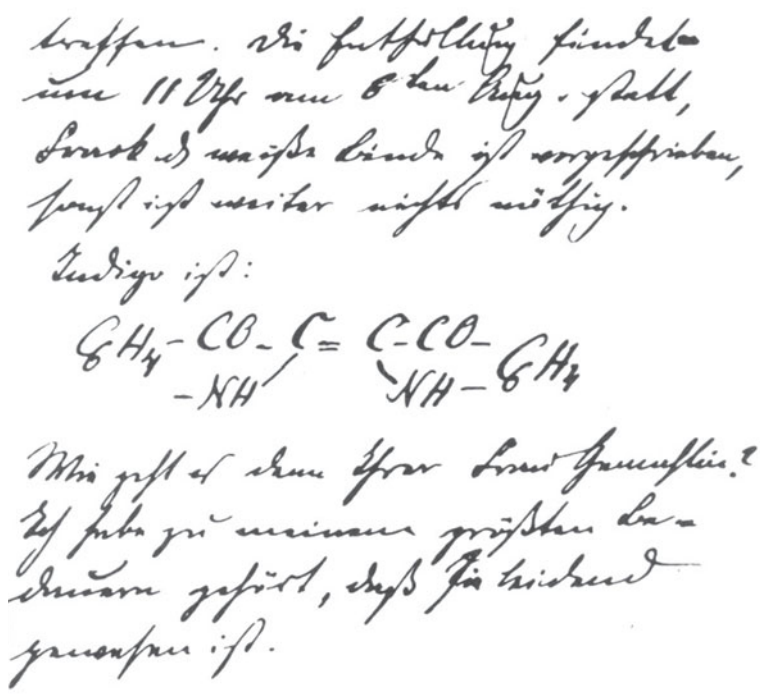
Baeyer synthesised indole by reduction of *o*-nitrocinnamic acid with iron and potassium hydroxide (Baeyer-Emmerling indole synthesis).



All this work was novel, but did not answer the question concerning the structure of indigo. As we shall see, with oxindole, Bayer had chosen the wrong substitution pattern.

As it often happens, a decisive piece of information came from an entirely different quarter. In 1879, Baumann and Tiemann had isolated urinary indican. This is the potassium salt of indoxyl sulfate, which arises in the urine under



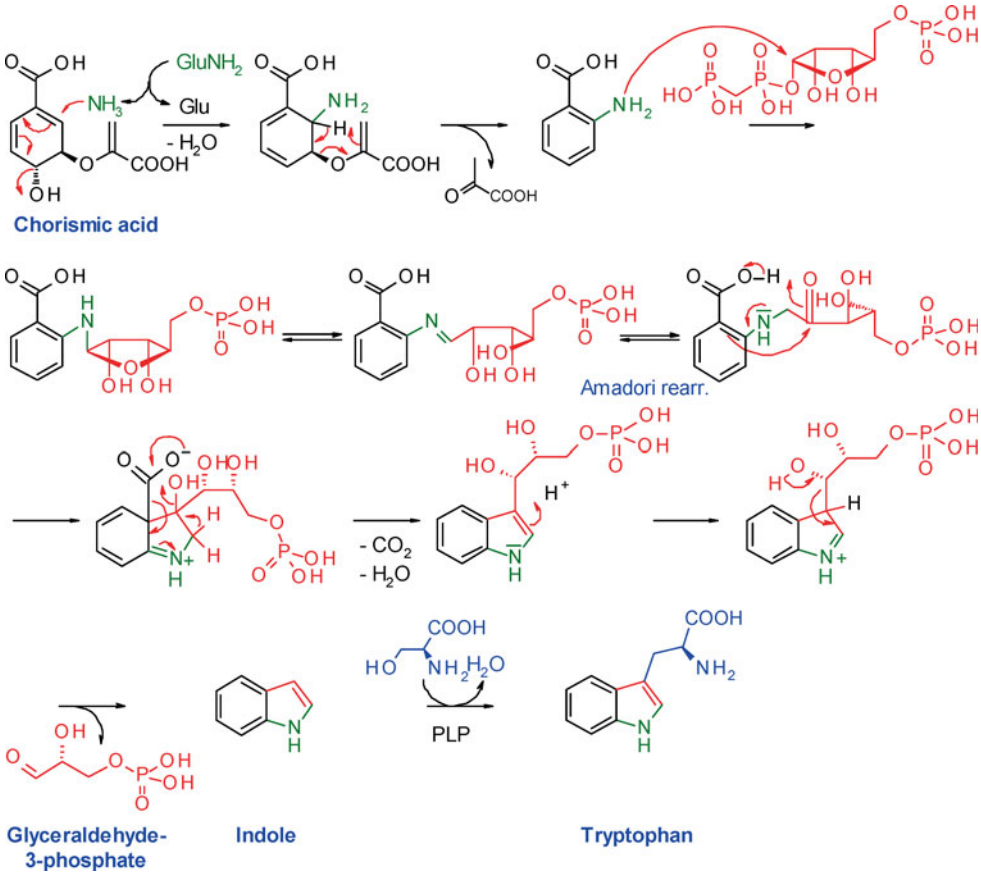


2.9 *On August 3rd 1883, Baeyer, wrote in a letter to his friend Heinrich Caro, Head of Research at BASF, mainly about the dress code for the unveiling of Liebig's statue in Munich, and mentioned along with other private matters the structure of indigo in passing. "The unveiling will take place at 11 o'clock on August 6th. White tie and tails are obligatory, but nothing else is important. The structure of indigo is: (showing the formula). How is your spouse doing? I was very sorry to hear that she had been in poor health."*

constipation, along with Hartnup disease or renal insufficiency [10] and is apparently a metabolite of tryptophan. When it is hydrolysed in acid, it gives indoxyl, which is spontaneously oxidised to indigo upon exposure to air. Thereby the puzzle was solved (Fig. 2.9), [11] although the *cis*/*trans*-isomer question was only resolved in 1928 by X-ray crystallography. [12]

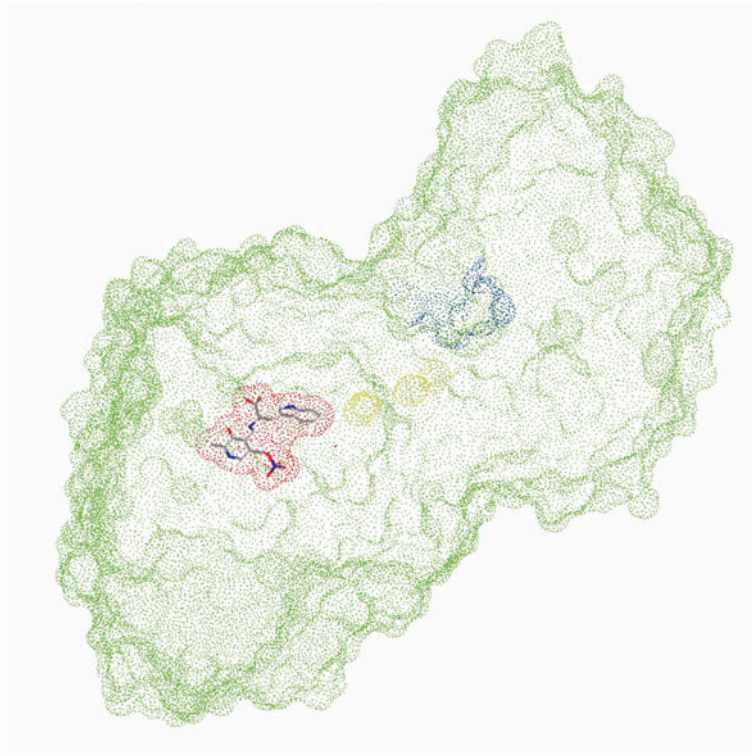
2.1.3 Biosynthesis

Interestingly, indigo itself is not found as a constituent of plants, but its colourless precursor, indoxyl. The biogenesis of indoxyl, as described in the literature, shows apparent contradictions. Tryptophan was initially regarded as the direct biosynthetic precursor of indoxyl (as for the indican in urine). [13] Starting from chorismic acid – the background of which is dealt with in Chapter 4.3, Amino acids –, amination (glutamine providing the source of ammonia) followed by elimination of pyruvic acid and water, gives anthranilic acid. This in turn reacts with 5-phosphoribosyl-1-diphosphate, and the resulting sequence of steps (ring-opening to give a Schiff base; two 1,3-hydrogen shifts (which are called Amadori rearrangement), and decarboxylative heterocyclisation) gives indole-3-glyceryl phosphate. Loss of glyceraldehyde-3-phosphate (giving indole) and reaction of the indole with serine finally gives tryptophan. This last step is pyridoxal-dependent, and may be regarded most simply as an electrophilic substitution at C-3 of the indole, the electrophile being the enamine derived from serine and pyridoxal phosphate (PLP). [14, 15]

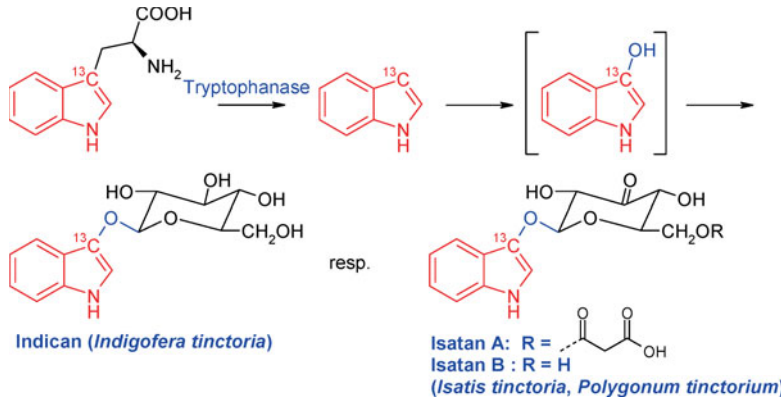


The final two steps in the biosynthesis of tryptophan are both catalysed by the enzyme tryptophan synthase (Fig. 2.10). Indole is formed in the α-segments of the enzyme (blue reaction centre) and (*L*)-tryptophan, bonded to PLP, is produced in the β-segments (red reaction centre). The reaction centres are linked with each other through a channel of 25–30 Å in length (shown in yellow), and the indole is transported through this channel from one reaction centre to the other. This clearly increases the rate of the reaction because of the high local concentration of the reaction partners. Glyceraldehyde-3-phosphate leaves the enzyme along the way to which indole-3-glyceryl phosphate is bonded. The same applies correspondingly to serine and tryptophan.

In 1992, Xia and Zenk could show in "feeding studies" with ^{13}C-labelled indole and 3-^{13}C-labelled (*S*)-tryptophan on various plants, including *Polygonum tinctorium*, *Indigofera tinctoria* and *Isatis tinctoria*, that the metabolism does not involve tryptophan directly, but proceeded *via* indole. [16] In the biosynthesis of indoxyl, tryptophan is first degraded, by means of a tryptophanase, to indole, and this is then hydroxylated. [17] Finally, indoxyl binds to various sugars.



2.10 *Tryptophan synthase from Salmonella typhimurium is a symmetric α-β-β-α complex, 15 nm in length. The X-ray structure analysis shows half of this bifunctional enzyme.*



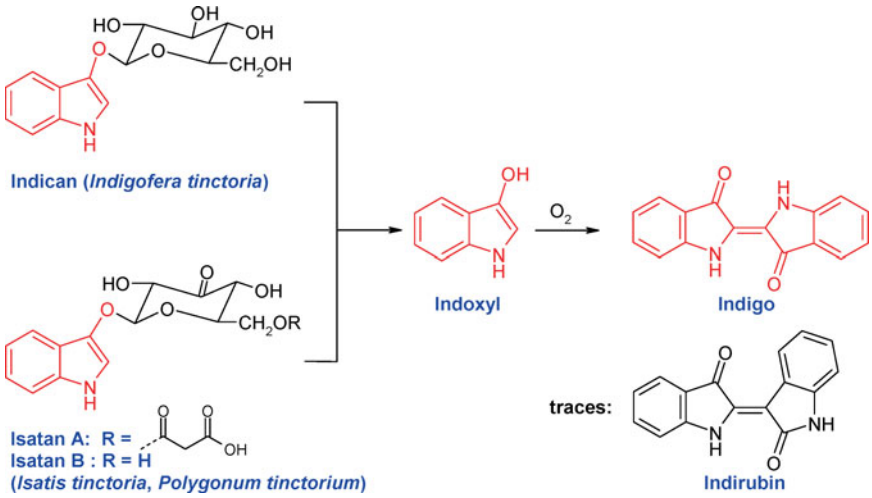
In order to isolate indigo itself, batches of the above plant species were subjected to fermentation. The following set of instructions comes from the *Papyrus Graecus Holmiensis* (Stockholm Papyrus) which dates from the 3rd century AD:

Measure out around 25 kg of uniformly dried woad into a vat which stands open to sunlight and holds at least 600 litres. Pile the woad up evenly, and pour in sufficient urine to cover the entire solid. Allow the mixture to warm up in the sun. The following day, macerate it in the sun by mashing it up with your feet

until it is thoroughly soaked; this must be repeated on three consecutive days. Then stir the woad and the whole of the supernatant urine vigorously through … [18]

Thereby indican (1*H*-indol-3-yl *β*-(*D*)-glucoside) from *Indigofera tinctoria*), and respectively isatan A and B (1*H*-indol-3-yl 6'-*O*-malonyl-(*D*)-ribohex-3'-ulopyranoside and 1*H*-indol-3-yl *β*-(*D*)-ribohex-3'-ulopyranoside from *Isatis tinctoria* and *Polygonum tinctorium*), are all cleaved enzymatically by *β*-glucosidases. [13, 19]

Indoxyl is spontaneously oxidised by atmospheric oxygen to indigo. For the dyeing process, the fermentation broth is normally decanted from the solid plant material; the textiles to be dyed are then soaked in the vat (a wooden tub), and then hung up to dry in the air. The indigo so obtained is sparingly soluble in water and absorbed onto the fibres as small pigment particles.

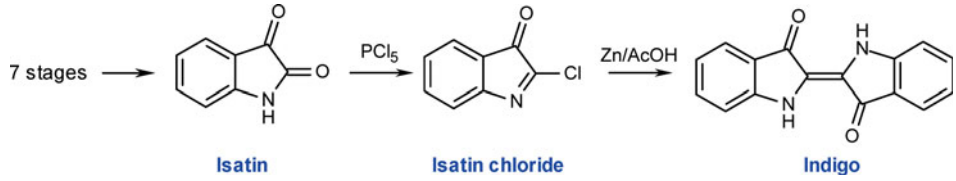


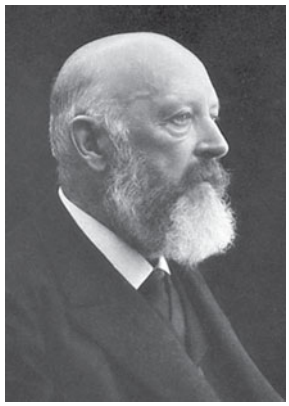
Both, natural and synthetic indigo contain as a by-product the isomer indirubin, the proportion of which depends on the origin of the colourant and the particular oxidation process. Bengal indigo contains around 2–4 %, and Java indigo, produced from *Indigofera arrecta*, up to 15 %. Indirubin is formed especially readily, if the oxidation utilises a fermentation vat containing insufficient alkali. [8]

Dyeing with indigo, together with alcoholic fermentation, baking of bread with sour dough, leather tanning and cheese-making, counts among mankind's earliest excursions into biotechnology, as may be demonstrated for all civilisations, except that of Australia.

2.1.4 Industrial Synthesis

Adolf von Baeyer's first synthetic efforts on the preparation of indigo – for which the question of its correct structure remained unanswered – were not exploitable on an industrial scale due to the inaccessibility of the starting materials. Already in 1870, following his seven-stage isatin synthesis, Baeyer (Fig. 2.11) had succeeded in converting this material with phosphorus pentachloride into isatin chloride: reductive dehalogenation of the latter with zinc yielded indigo directly.



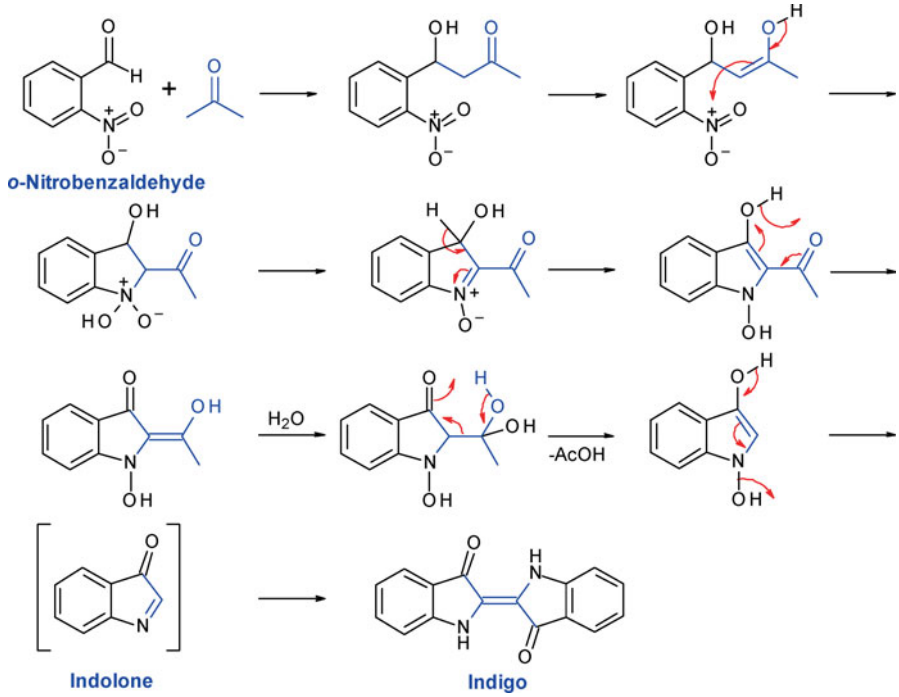


2.11 *Adolf von Baeyer.*

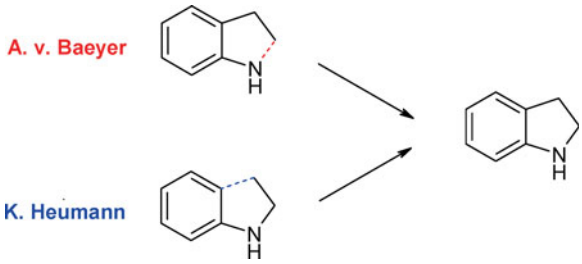
Baeyer's second indigo synthesis started from *o*-nitrobenzaldehyde, and seemed attractively simple and elegant (the Baeyer-Drewsen reaction). An aldol condensation of the aldehyde with acetone in weakly basic media gives 1-hydroxy-1-(*o*-nitrophenyl)butan-3-one. Cyclisation accompanied by loss of acetic acid and water may be regarded as producing the unstable indolone, of which indigo, formally at least, is the dimer. [20] The reaction mechanism is really unusual: there are only a few examples in which an enol reacts as a nucleophile with a nitro group. [21, 22]

The accessibility of *o*-nitrobenzaldehyde was the problem. Toluene, which served as a starting material and could be isolated from coal tar, was restricted in availability, and its chlorination and nitration were considered unsatisfactory on the grounds of selectivity and yield.

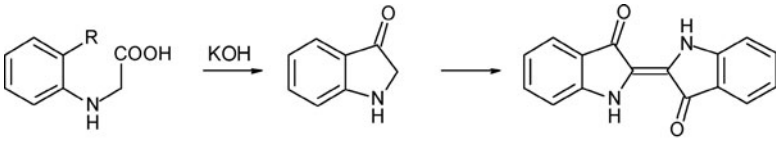
In the meantime, Hoechst and BASF had teamed up to form what nowadays would be described as a "joint venture" – they had acquired the patent rights from Baeyer and set up enormous research activities. In the end, those efforts remained unsuccessful.



The breakthrough came through the work of the Zurich Professor Karl Heumann (1850–1894), who in 1890 succeeded in obtaining indigo from phenylglycine in an alkali melt. While Adolf von Baeyer had thought that construction of the indole skeleton required the formation of a C-N bond, the paradigm change of Karl Heumann to bring about ring closure by means of a C-C bond formation, led to success. It is noteworthy, as neither von Baeyer nor Heumann could have known, that this route corresponds formally to the biosynthetic pathway.



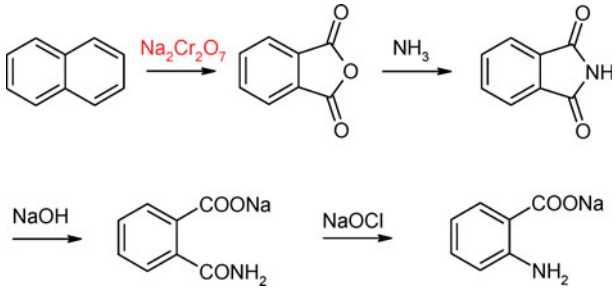
Starting from aniline and chloroacetic acid, phenylgycine was accessible, and this, when melted with solid potassium hydroxide, gave indigo.



R = H: 1st Heumann Indigo synthesis
R = COOH: 2nd Heumann Indigo synthesis

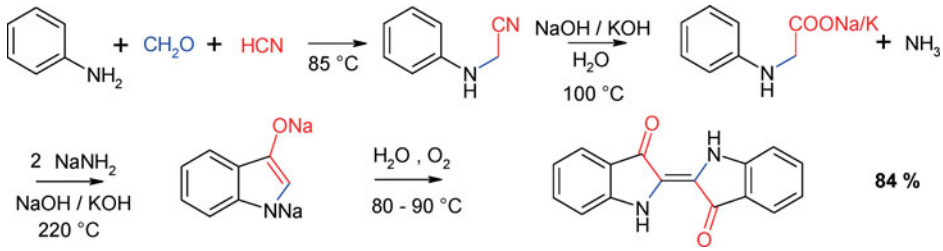
Unfortunately it very quickly became clear, that even under optimised reaction conditions, the yield was only 10 %.

The so-called "second Heumann indigo synthesis" started from anthranilic acid rather than aniline, and gave substantially better yields. Anthranilic acid was accessible by oxidation of naphthalene to phthalic anhydride, after Hoogewerf and van Dorp had shown that Hofmann degradation of the half-amide of phthalic acid leads to the desired product.



The key step was the oxidation of naphthalene: the dichromate could however not readily be regenerated from the chromium(III) liquor. BASF and Hoechst then followed separate paths again. While Hoechst relied on an existing procedure using electrochemical oxidation, BASF revisited the previously unsuccessful attempts to oxidise naphthalene with oleum. In the course of these investigations, a careless laboratory worker smashed a mercury thermometer – and discovered that just that experiment produced the best result. Because of this fortunate incident, and the fact that Rudolf Knietsch had at that time worked out the Contact process for oleum in connection with the alizarin synthesis, BASF established a significant lead on the long road towards the "King of Colourants". Nevertheless, the industrial realisation of an indigo synthesis required altogether enormous efforts (17 years of development time and a cost of 18 million gold marks) and a readiness to take risks, before BASF could bring the first synthetic indigo to the market in 1897. [23, 24]

A considerable improvement in indigo synthesis resulted from an invention by Degussa. It was Johannes Pfleger, who in 1901 succeeded in boosting the yield of indigo by the addition of a new reagent, sodamide, to the alkali melt of phenylglycine. Hoechst acquired the relevant patent and founded together with Degussa the company "Indigo GmbH". Thus, five years after BASF, Hoechst became the second worldwide supplier of synthetic indigo.

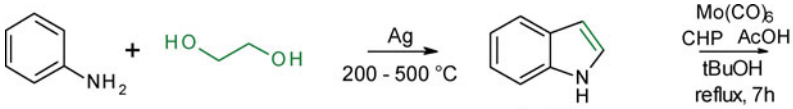


Under competitive pressure, BASF rationalised in 1925 its production process. As starting materials for phenylglycine serve aniline, formaldehyde and hydrogen cyanide: these are converted into *N*-phenylglycinonitrile at 85 °C. Hydrolysis takes place in aqueous alkali at 100 °C. The sodamide for the next stage is obtained by passing ammonia over molten sodium. The dried salt of phenylglycine is dissolved in a eutectic, and therefore stirrable, mixture of sodium and potassium hydroxides at 220 °C and portions of molten sodamide are added. The reaction leads to sodium indoxylate, which undergoes oxidative hydrolysis to give, finally, indigo in an overall yield of around 84 %. This procedure is carried out unchanged right up to the present day.

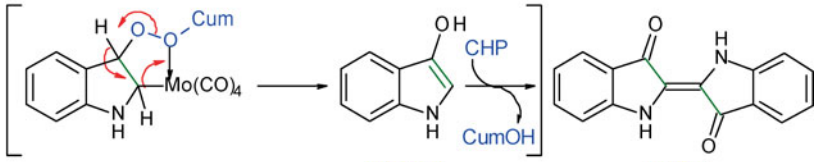
2.1.5 Modern Lab-scale Indigo Synthesis

Most recently, Yoshihiro Yamamoto published a novel, outstanding one-pot synthesis of indigo from indole *via* a molybdenum-catalysed oxidation. [25] The

raw material, which used to be obtained from a fraction of coal tar, is nowadays easily available on industrial scale from aniline by a silver-catalysed reaction with ethylene glycol. Indole is oxidized in position 3 by cumene hydroperoxide (CHP) to give indoxyl, which forms directly indigo under the reaction conditions. The product is obtained just by filtration as a deep-blue solid in 81 % yield.



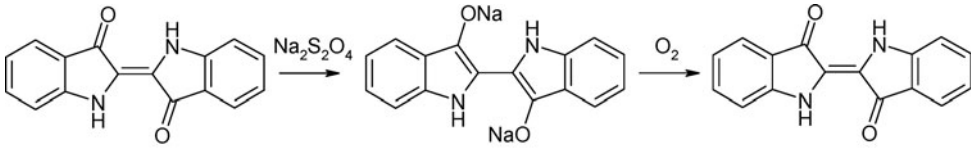
Indole



Indoxyl Indigo

2.1.6 Industrial Dyeing Process

For the dyeing of cotton, indigo is reduced with sodium dithionite in alkaline solution. The dyer speaks of "vatting". When this reaction is carried out in dyeworks, large quantities of waste water containing sulfite and sulfate are produced.



Indigo white

Therefore, in order to improve the ecological efficiency of the process, BASF made an alteration to the vatting step. At the end of the synthesis, the indigo is converted directly by catalytic hydrogenation [26] into the leuco-form, and stored under nitrogen. In presence of molasses, the solution can be evaporated, and is marketed as granules containing 60 % of dyestuff. The yellowish indigo white (leucoindigo) can be used directly by the dyer. It is water-soluble and has particular properties: It adheres to the cellulose fibre, but – unlike other vat dyes – it does not penetrate deep into the fibre due to the high polarity of the di-sodium salt. By "hanging" of the fabric in air (*i.e.* re-oxidation) finely dispersed indigo is deposited, effectively as a pigment, on the surface. Several repetitions of the procedure are necessary in order to attain sufficient colour strength. [27]

Blue jeans acquire their typical appearance only if they are manufactured out of indigo-dyed denim. A particular weave and the poor fastness to rubbing of the indigo dyeing are critical here. Before the weaving, only the warp threads

are dyed, whereas the weft threads remain plain white. Thus, the fabric consists of these blue warp threads and white weft threads. The threads of the warp lie mainly on the surface of the material, and so under abrasive conditions, such as in "stone-washed" jeans, the inner undyed threads in the fabric become visible and result in the "Blue-Jeans-Look".

2.1.7 Outlook

Current sales of jeans amount to approximately 1 billion pairs per annum. The dyeing of one pair requires around 10 grams of indigo. In Europe DyStar (formerly part of BASF) is the largest producer and the indigo which it makes comes exclusively from a single production plant in Ludwigshafen. The worldwide annual production is about 30 000 tonnes. As measured by the quantity produced, indigo is the most important textile dye.

Starting in the 1980s, scientists in Amgen Inc. discovered that a genetically modified strain of bacteria can produce indigo from naphthalene. [28] Recombinant *Escherichia coli* are able to produce indigo from glucose. [17, 29]

Most recently, it has also been shown that a strain of bacteria isolated from a dye vat – for which the name *Clostridium isatidis* has been suggested – reduces indigo to leucoindigo. [30] While the process has not yet been proven to be economically viable, this example clearly shows how production processes are constantly changing. Even for commodity products, their mature production methods are revisited and possibly transformed by modern biotechnology.

Summary in Bullet Points

- The importance of indigo as a highly valued colourant stretches from ancient times right up to the modern day.
- The development of industrial indigo syntheses laid the foundation for the production of a whole range of basic chemicals.
- Elucidation of the biosynthetic pathway offers the prospect of a biotechnological production of indigo.

2.2 Tyrian Purple

2.2.1 Introduction

According to the "Onomasticon" (dictionary) of the Roman mythographer Julius Pollux, Heracles was the first to dye a dress with purple, as a gift of love to the nymph Tyros (Fig. 2.12). In fact, it was assumed, that the Phoenicians discovered in the 13th century BC that various sea snails of the genus *Murex* [31] and *Purpura* secreted a colourless slime, which gave a deep violet colour of rela-

2.12 *The painting "The Discovery of Purple Snails" is by Theodore von Tulden (1606–1676). Heracles observed how the mouth of his dog turned red after it had chewed a purple snail.*

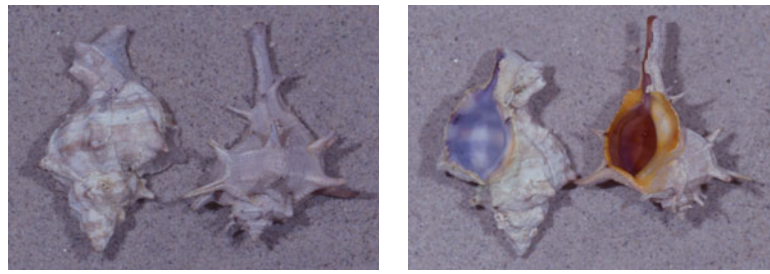
tively high fastness when applied to textile fabrics and exposed to air and light. However, recently the archaeological discovery of substantial numbers of *Murex* shells and collocated pottery on Crete suggests that the Minoans already may have produced the dye centuries earlier during the Middle Minoan period in the 20th–18th century BC. [31]

Also in Jewish culture, located right adjacent to the Phoenician Empire, purple was a greatly prized colourant. In the book of Exodus, Chapter 28, verses 4–8, (English Standard Version) can be found the following description:

4 These are the garments that they shall make: a breastpiece, an ephod, a robe, a coat of checker work, a turban, and a sash. They shall make holy garments for Aaron your brother and his sons to serve me as priests. **5** They shall receive gold, blue and purple and scarlet yarns, and fine twined linen. **6** And they shall make the ephod of gold, of blue and purple and scarlet yarns, and of fine twined linen skilfully worked. **7** It shall have two shoulder pieces attached to its two edges, so that it may be joined together. **8** And the skilfully woven band on it shall be made like it and be of one piece with it, of gold, blue and purple and scarlet yarns, and fine twined linen.

The distinction between "blue and purple and scarlet" ("blue" and "red" purple in the German Luther Bible) refers to the different shades, which range from reddish-violet to bluish-violet according to the species of snail (Fig.

Purple snails occur in the Mediterranean, on the west coast of Africa, the coast of Brittany, Central America, Ecuador and Peru.



2.13 *Purple snails: besides Murex brandaris (for reddish-purple, each on the right), Trunculariopsis trunculus [31] (for bluish-purple: each on the left), Murex erinaceus and Purpura haemostoma may also be used for the preparation of Tyrian purple. By the South Harbour of Saida in Lebanon (Sidon in the ancient world) the beach, 25 metres in breadth and hundreds of metres long, is covered to a depth of several metres with shells of Trunculariopsis trunculus.*

Agamemnon
In the play *Agamemnon* by Aeschylus (525–456 BC) is described the return of the victor (Agamemnon) from Troy to Argos (Mycenae). The people receive Agamemnon with the highest honour and spread a crimson tapestry before him. However, Agamemnon hesitates. "Only gods are honoured so; how could I, a mere mortal, dare without shame to set foot on this splendid creation?" In truth, Agamemnon had a bad conscience. Only a few heroes returned with him and – worse still – he had offered up his own daughter Iphigenia as a human sacrifice. In spite of his scruples, his wife Clytemnestra forced him to walk across the red tapestry. Later she avenged her child by murdering Agamemnon in his bath. To Aeschylus, purple symbolises metaphoric significance; it is a sign of human hybris and sinful pride, which must inevitably be punished. [35]

A modern example of the esteem associated with coloured paper is the salmon colour of the *Financial Times*. Since 1893 it appeared in pink and was more readily recognised on the newsstand than white newspapers, which resulted in a significant rise of its print run.

2.13). [32–34] Many species of snail produce 6-bromoindigo and indigo along with purple.

The leuco-form of purple is light-sensitive. This property is associated with stepwise debromination, which means that the fraction of indigo and thereby the blue component can be distinctively different, depending on the dyeing process.

Through several papyrus discoveries in the lower Nile valley, and a cuneiform tablet with instructions for dyeing wool with Tyrian purple, found in Sippar on the east bank of the Euphrates river and dated back to the neo-babylonian period (600–500 BC), as well as the writings of Vitruvius and Pliny the Elder, we now know very well how Tyrian purple was manufactured and used in ancient times for dyeing – and how it was eked out and was the object of forgeries (Fig. 2.14). [36]

To obtain the colourant, the snails were killed, and by means of fermentation and decay the organic material was separated off. This produced a hideous stench that was actually mentioned by ancient authors. [31] Sidon and Tyre were centres of the antique industrial purple production.

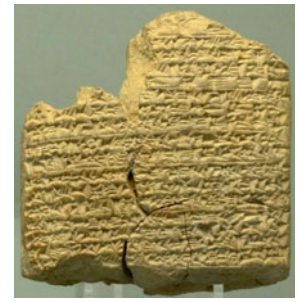
Although Tyrian purple was reserved for only the highest dignitaries, in ancient times it must also have given rise to an active trade in the dyestuff. In the Acts of the Apostles, Chapter 16, verse 14, we read (from *The New English Bible*):

One of (the women) named Lydia, a dealer in Tyrian purple fabric from the city of Thyatira, who was a worshipper of God, was listening, and the Lord opened her heart to respond to what Paul said.

Subsequently, she invited the Apostle Paul, on his missionary journey from Philippi to Thessalonica, into her own house. At that time, Thyatira was famous for Tyrian purple dyeworks and for dealing in purple. [37]

From ancient times up to the Middle Ages, Tyrian purple dyeing blossomed in Byzantium. However, at the end of the Fourth Crusade and the conquest of Constantinople in 1204, the production of Tyrian purple for the Byzantine court came to an abrupt end. The new rulers were unable to raise sufficient funds required for the continuation of murex purple production. [31]

Apart from textile dyeing, Tyrian purple gained early importance also in book illustration. Purple, as well as cheaper colours, were used, for instance Litmus (an extract from several lichens, especially *Roccella tinctoria*), in order to ground parchment sheets before they were artfully sketched and painted (Fig. 2.15).



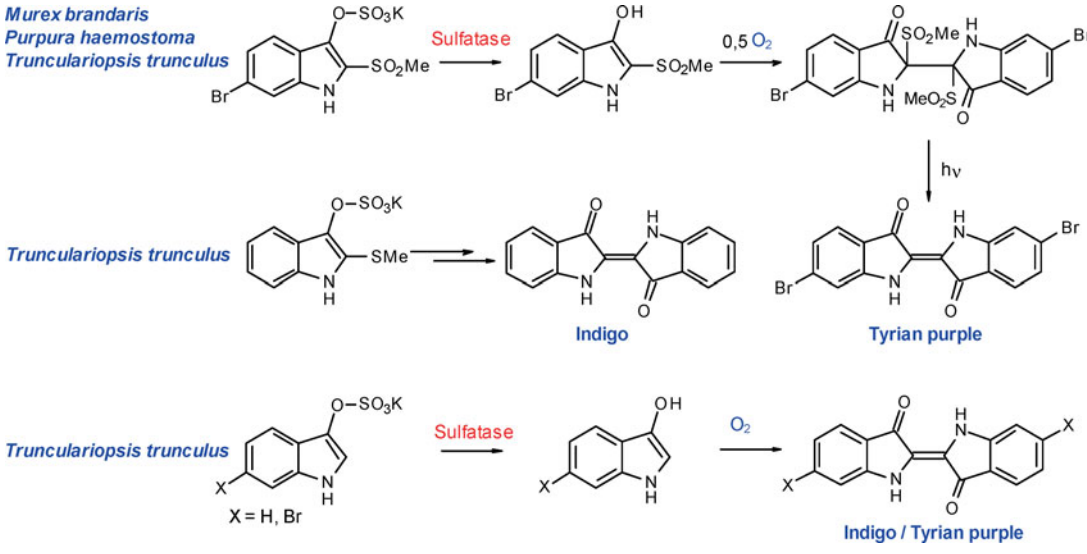
2.14 *A cuneiform tablet with instructions for dyeing wool with Tyrian purple.*

2.15 *The "Codex Purpureus Rossanensis", written and painted around the year 600, is kept today at the Diocesan Museum in Rossano (Calabria).*

2.2.2 Biosynthesis

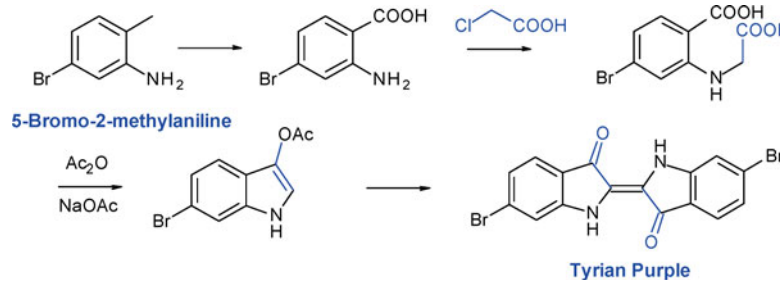
In 1685, the physician William Cole had observed that the fresh hypobronchial secretion of purple snails changed its appearance upon exposure to light from bright green to dark green, deep sea-green, bright blue, reddish-purple and finally dark purple. Based on biochemical studies, it is now recognised that the biosynthesis of the chromogens of indigo and purple are similar. Many purple snails, like *Murex brandaris* and *Purpura haemostoma*, produce only one chromogen for pure purple, whereas others, such as *Trunculariopsis trunculus,* synthesise different chromogens leading to a mixture of indigo and purple. This explains why natural purple can display various shades. [35, 38]

In the hypobronchial gland of *Trunculariopsis trunculus*, four distinct chromogens were detected, which lead *via* different routes to purple and indigo. [39] In all cases, the first step is a sulfatase-mediated liberation of the indoxyl derivatives. In the case of the 2-unsubstituted derivatives, the presence of oxygen leads directly to indigo and purple. In the case of methylsulfanyl- or methanesulfonyl- substituted chromogens, the colourants are generated only after the impact of light.

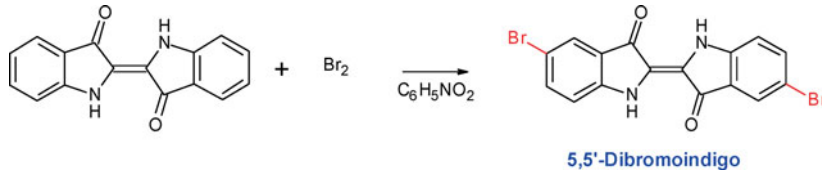


2.2.3 Structure Determination

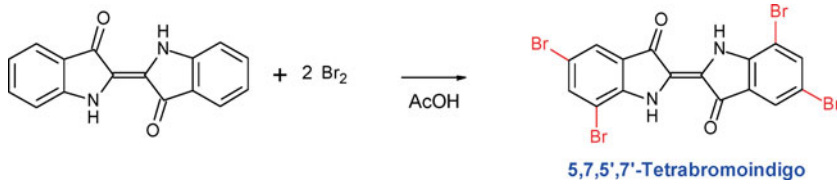
The similarity of the dyeing processes fuelled the suspicion that there exists a structural relationship between Tyrian purple and indigo. In 1907, Paul Friedländer (1857–1923) bought 12,000 purple snails of the genus *Murex brandaris* from the market of Trieste, since these corresponded best with those described by Pliny. He was able to isolate 1.4 grams of Tyrian purple from their hypobronchial glands [40], an amount sufficient to elucidate the structure. Given the analytical possibilities available at the time, this was a tremendous achievement. Friedländer found, by elemental analysis and total synthesis of the possible constitutional isomers, that Tyrian purple has the structure of 6,6'-dibromoindigo.



In hindsight, this is remarkable in several respects. For one, purple stands out as one of the first known examples of natural brominated organic compounds. For another, the substitution pattern is unusual: bromination of indigo in nitrobenzene gives 5,5'-, not 6,6'-dibromoindigo.

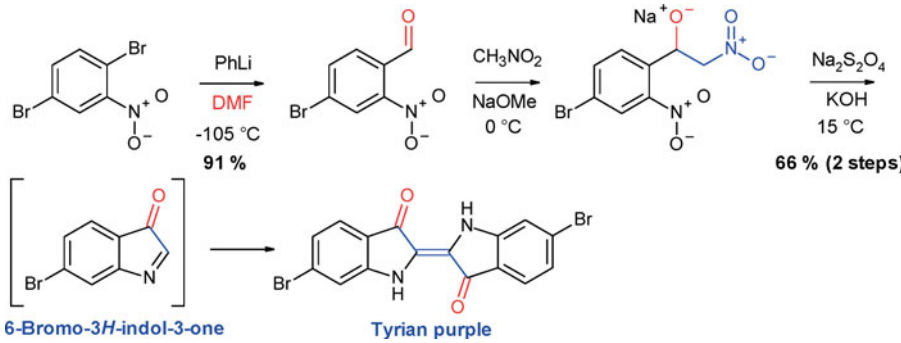


On account of its neutral blue colour, 5,7,5',7'-tetrabromoindigo, which is obtained by bromination in acetic acid, is also of a certain industrial interest. [41]



An elegant three-stage laboratory synthesis of ancient purple was published in 1989 by Hans Jakob Gerlach at the University of Bayreuth. [42] By halogen/metal exchange and reaction with DMF, 2,5-dibromonitrobenzene is converted regioselectively into 4-bromo-2-nitrobenzaldehyde. From this, by following a

route analogous to Adolf von Baeyer's second indigo synthesis, Tyrian purple is obtained in 42 % yield. The yield is substantially higher, if 4-bromo-2-nitrobenzaldehyde is condensed with nitromethane and the product reduced with sodium dithionite. Through several intermediate stages, in analogy of the Baeyer-Drewsen reaction, this leads presumably to 6-bromo-3*H*-indol-3-one, which rapidly dimerises.



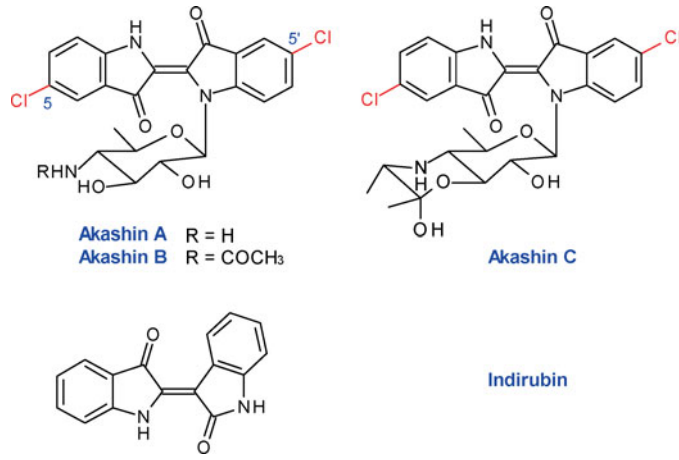
2.2.4 Technical Application

As described above, there was an industrial application of Tyrian purple back in ancient times. Dyeing with alum mordant, like madder and kermes, however competed with the more expensive purple. The structure elucidation of the latter by Friedländer, which could have formed the basis for development of an industrial synthesis, came too late. By that time, synthetic and simply-prepared dyestuffs had already been found, which covered this segment of the colour range. Perkin's mauveine and other synthetic dyes imitated the colour tone of purple, so that, in contrast to indigo, the economic incentive to engage in a large-scale production of this dye did no longer exist. In addition, the dyeing properties of Tyrian purple are inadequate to meet modern-day standards of colour fastness.

2.2.5 Newer Developments

Natural colourants often played an important role as pharmaceuticals. Thus, complex mixtures containing indigo powder have a firm place in traditional Chinese medicine. [43] That halogenated derivatives of indigo are still of biological interest, is demonstrated by the following example:

Recently, in the terrestrial *Streptomycete*s strain GW48/1497, there were discovered for the first time derivatives of 5,5'-dichloroindigo, a previously unknown natural product; these derivatives were named akashin A, B and C (Nepalese, *akash* = heaven). [44] In these, 5,5'-dichloroindigo is unsymmetrically bonded to various sugars (Fig. 2.16).



2.16 *Contrary to indigo, akashins, along with indirubin [45], a component of the Chinese drug Danggui Longhui Wan, are effective against various human tumour cell lines, such as specific colon cancers, melanoma, lung cancer, types of breast cancer and kidney tumours.*

Summary in Bullet Points

- As a dyestuff, Tyrian purple is of historical importance only.
- When its industrial manufacturing became possible, other dyestuffs had already captured this segment of the market.
- Related compounds have recently drawn attention as tools in oncological research.

2.3 Alizarin

2.3.1 Introduction

Common madder (*Rubia tinctorum*) [46] is a plant already known in the Middle East back in ancient times. From its roots a red extract, called lake, could be isolated (Fig. 2.17). It was already described by Pliny the Elder. The Arabic name for the red root is "alizari".

2.17 *Rubia tinctorum: Coloured copperplate print by Nicolaus Friedrich Eisenberger, from the Herbal of Elisabeth Blackwell, Nuremberg (1765) and a photograph of the plant.*

In his Estates decree *Capitulare de villis*, Charles the Great (Charlemagne) issued regulations for the cultivation of madder by farms in all the Carolingian, or Feudal estates. In the Middle Ages, up to the Renaissance, madder was produced in many regions of central Europe for dyeing of textiles. In 1467, Pope Paul II introduced red garments as the insignia of a Cardinal – the "purpurati" (Fig. 2.18). [47] Titian (1488/90 –1576) used madder lake in his paintings.

In the 16th century, the process for the preparation of madder lake reached France. In 1868, 50,000 tonnes of madder roots with a dye content of 1 % were harvested.

Depending on the laking medium, the colour of the lake obtained varied from scarlet (tin-containing lake) through carmine-red (aluminium lake, "Turkey red") and brown-violet (chromium-containing mordant) to blue or even violet (iron-containing complexes).

Insects provided another important source of red colours, Kermes, a mordant dye like alizarin, was the most important red dyestuff in Europe from antiquity. Reference to the dyeing of robes and tapestries may be found in the Old Testament.

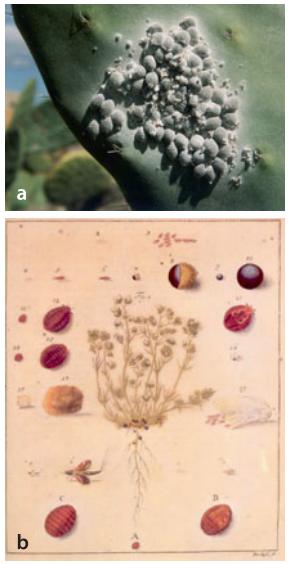
The dyestuff, which is also called scarlet or carmine, may be obtained from the dried female kermes scale-insects (various species, *e.g. Kermes vermilio* and *Kermococcus ilicis* L.) by extraction with ethanol (Fig. 2.19). Kermesic acid serves as an ant repellent to these insects living on Kermes oaks (*Quercus coccifera*) in the Mediterranean. [50] The Polish cochineal (*Porphyrophora polonica* L.), which lives on the roots of a member of the carnation family (Perennial knawel, *Scleranthus perennis*), was first mentioned in 812 in Charlemagne's *Capitulare.* It was also known as "Saint John's blood", because harvesting began by tradition on the feast-day of St John the Baptist (June 24) (Fig. 2.20).

2.18 *Raphael (1483–1520) painted Pope Leo X around 1517. The painting is to be found today in the Uffizi Gallery in Florence.*

Purpurati
In contrast to what the title implies, a cardinal's cassock was not dyed with Tyrian purple but with scarlet, obtained from the cochineal and kermes scale insects. [48] In the end, this may have been not least because of simple monetary reasons. Colouring with this mordant dye required alum, which since the 12th century had been imported from Syria, Egypt, Greece and Asia Minor. However, in the 15th century alum was discovered in Italy (near Tolfa), and the Pope obtained a monopoly over the alum from the mines there. [49]

2.19 *The Coronation robe of the Emperors of the Holy Roman Empire (Imperium Romanum Sacrum) was manufactured around 1134 in the Royal silk factory in Palermo for the Norman king, Roger II. The beautiful red colour came from Kermes vermilio.*

The most important suppliers of natural cochineal are still the Canary Islands, such as Lanzarote and Fuerteventura, and also Mexico and Peru.



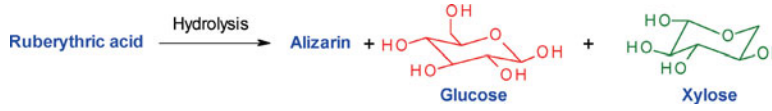
2.20 *The female cochineal scale insects attach themselves to and feed from the cactus; previously they had been misconceived as fruits of the plants (a). – The larvae of Porphyrophora polonica are sessile parasites living on the roots of the perennial knawel (b), growing on sandy soils of Central Europe.*

With the conquest of Mexico by the Spaniards, dyeing with cochineal came to Europe in 1530 (Fig. 2.20). The dyestuff was obtained from the American cochineal scale insect (*Dactylopius coccus* Costa), which had already been bred in a big way by the Aztecs on cactus plants (*Opuntia monacantha*, *Opuntia vulgaris* and *Nopalea cochenillifera*). Due to the higher yield of dyestuff, cochineal displaced kermes almost completely. In the heyday of cochineal production, around 1870, the Canary Islands annually exported 3,000 tonnes of cochineal (Fig. 2.21).

However, great cuts in the export of cochineal were experienced a little later, with the growth in production of fuchsine and azo dyestuffs. [51] Nowadays, cochineal is used for colouring of Easter eggs, cosmetics, pills and drinks (natural food dye E 120), although, also here the synthetic dyestuff Cochineal red A (Ponceau 4R, E 124) is coming into use, *e.g.* for the colouring of jelly babies. [52–54]

2.3.2 Structure Determination

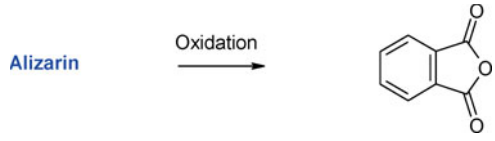
The most important dyestuff in the madder root is alizarin. In the plant it occurs as a glycoside, bonded to glucose and xylose (ruberythric acid or alizarin-2-*O*-*β*-primeveroside). The structure was elucidated in 1868 by Carl Graebe and Carl Liebermann (two students of Adolf von Baeyer) at the Berliner Gewerbeakademie (Berlin Trade Academy).



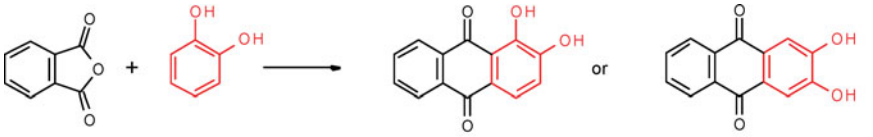
By the hydrolysis of ruberythric acid, it was concluded that alizarin must be a dihydroxy-compound. Distillation over zinc dust gave anthracene.

2.21 *Up until 1954, the ceremonial uniforms of the British Guards regiments were dyed with natural cochineal.*

Based on alizarin's orange-red colour, Graebe and Liebermann presumed that alizarin must be a derivative of anthraquinone, although the position of the two hydroxy-groups was still unclear. Since oxidation of alizarin gave phthalic acid, this suggested that one benzenoid ring had to be unsubstituted.

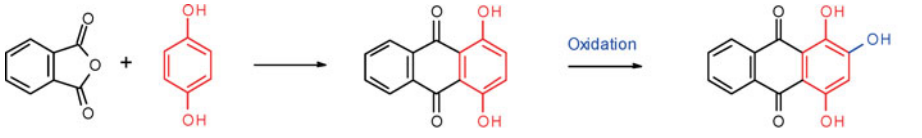


On the other hand, alizarin could be prepared from phthalic anhydride and catechol, therefore both hydroxy groups must be *ortho*- to each other, which left the two remaining options for its structure:



1,2-Dihydroxyanthraquinone 2,3-Dihydroxyanthraquinone

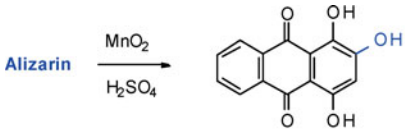
Correspondingly, the reaction of phthalic anhydride with hydroquinone gave quinizarin, which can be further oxidised to purpurin, a dyestuff which occurs along with alizarin.

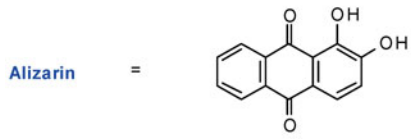


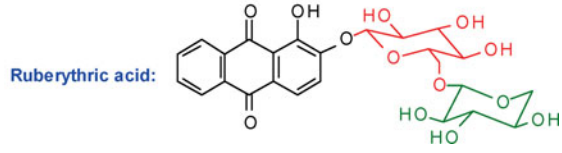
Quinizarin (1,4-dihydroxyanthra-9,10-quinone) Purpurin (1,2,4-trihydroxyanthra-9,10-quinone)

Purpurin is therefore a trihydroxyanthraquinone, with two of the hydroxy-groups *para* to each other. On the other hand, oxidation of purpurin also gave phthalic anhydride, which showed conclusively that also in purpurin one benzenoid ring was unsubstituted.

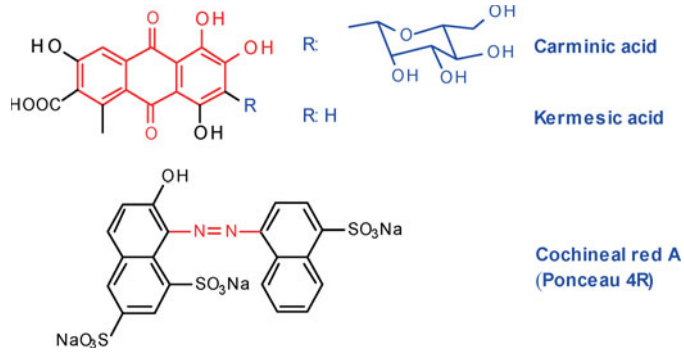
Since oxidation of alizarin with manganese dioxide and sulfuric acid also gave purpurin, only one possibility remained for the structure of alizarin. Further work allowed then also to assign the structure of ruberythric acid.







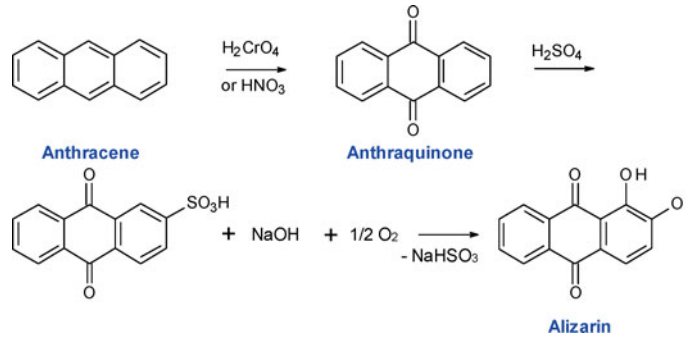
Carminic acid and kermesic acid can be regarded as more highly substituted derivatives of alizarin. In 1858, carminic acid was isolated in pure form, and in 1916, its structure was determined by Otto Dimroth (1872–1940). [55] The dyestuff, carminic acid, is distinguished from kermesic acid by a C-glucosyl residue in the former (Fig. 2.22).



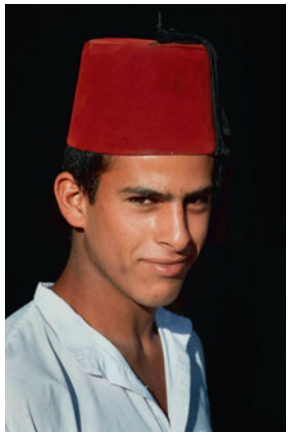
2.22 *The poor availability of carminic acid triggered the search for a substitute. Cochineal red A is a synthetic azo dye used for drinks, sweets, fruit preserves and fish.*

2.3.3 Industrial Synthesis

One year after the structure elucidation of alizarin, Heinrich Caro at BASF developed in collaboration with Graebe and Liebermann the following successful synthesis. Sulfonation of anthraquinone with oleum [56] gives in the absence of catalysts, on steric grounds, anthraquinone-2-sulfonic acid [57] as the main product. This intermediate is then subjected to an alkali melt under oxidative conditions.



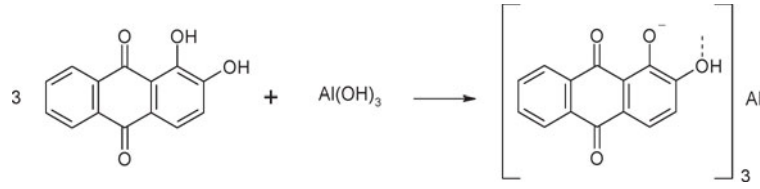
The felt of a Turkish fez was mordanted with alum/potassium bitartrate, by addition of a small amount of curcuma, followed by dyeing with madder and finally with kermes (Fig. 2.23). [51]



2.23 *A young Bedouin man wearing a fez.*

2.3.4 Industrial Dyeing Process

The dyeing process comprises mordanting of the fabric and the actual dyeing procedure. Thus, wool or cotton are first steeped in potassium alum ($KAl(SO_4)_2 \times 12\ H_2O$) and then treated with steam. Thereby, finely dispersed aluminium hydroxide is precipitated onto the surface of the fibres. For the dyeing step, the alum-mordanted wool or cotton is boiled with a fine suspension of alizarin for a longer period. [58]

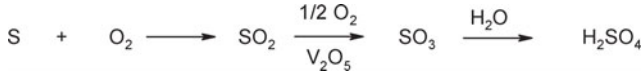


2.3.5 Impact on the Development of the "Young" Chemical Industry

Alizarin was the first large-scale natural product, previously obtained from biological sources, to be fully replaced by a wholly synthetic compound, and may therefore be considered the first industrially synthesised natural product.

For the production of alizarin, BASF required increasing quantities of sulfuric acid. The most important suppliers were Bohemian (*i.e.* Czech) "vitriol distilleries", which were soon unable to meet the growing demand. BASF itself used the Lead Chamber process for the production of dilute sulfuric acid, which had finally to be concentrated in a platinum kettle. Since this device had cost 30,000 gulden, it was regularly locked away at night in the gatehouse. Arising out of such completely inadequate conditions, Rudolf Knietsch developed in 1888 the Contact process, by which sulfuric acid could be obtained catalytically; this enabled BASF to become the largest producer of sulfuric acid in the world. [59] This process was the first industrially applied large-scale catalysis, which, in a modified form, is still used today. [60]

In the end, the first industrial natural product synthesis provided the trigger for a whole range of inventions in the field of basic chemicals, and was therefore decisive for the upstream integration of the young chemical industry at the beginning of the 20th century.

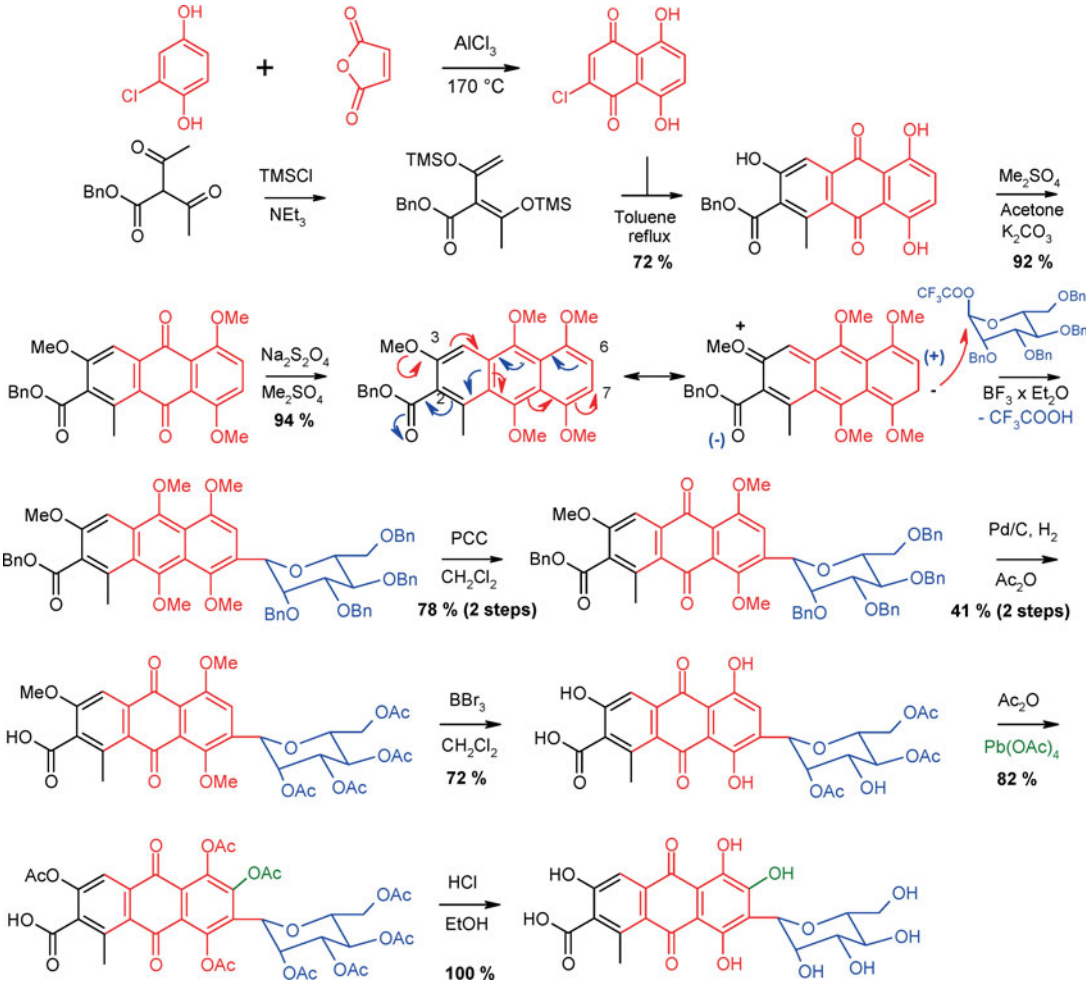


2.3.6 Modern Developments

Even if alizarin had served its time as a dyestuff for textiles, natural cochineal still finds broad application as a colourant for foodstuffs. Given the high expenditure required for isolation of the natural product, a fully synthetic preparation of "nature-identical" kermesic and carminic acids can be attractive if one takes into account that higher prices can be realised for food colourants than for textile dyes. In this connection, the first total synthesis of carminic acid has been described by John Tyman and Pietro Allevi. [62, 63]

The anthraquinone skeleton of carminic acid is constructed by Friedel-Crafts acylation [64] and a Diels-Alder reaction. For the *C*-glycosidation, the quinonoid system is reduced with sodium dithionite and stepwise permethylated. The substitution takes place stereospecifically at C-7, since the methoxy-group at C-3 induces a negative partial charge there (red arrows), whereas C-6 is deactivated through the ester group (blue arrows). After selective oxidation with pyridinium chlorochromate, the benzyl protecting groups are removed by hydrogenation and the hydroxy-groups acetylated. The demethylation is performed with boron tribromide, and the acetoxy-group in position 6 is introduced by the use of lead tetraacetate / acetic anhydride. All esters are finally hydrolysed.

In 1862, Gaspare Campari (1828–1882) prepared in Milan the world-famous drink bearing his name, the precise recipe for which is a family secret. According to the firm's tradition, Campari is prepared exclusively from natural ingredients: 86 herbs, roots, spices and citrus fruits, which have been macerated in distilled water, and mixed with pure ethanol. The alcohol content amounts to 25 %. The bitter taste arises from quinine, and the carmine-red colour from the cochineal scale insect (Fig. 2.24). [61]



2.24 *A bottle of Campari®.*

This is a synthesis, which for sure cannot yet be scaled up to industrial quantities without further enhancement: for example, the handling of pyridinium chlorochromate and lead tetraacetate may be problematic. Also the protecting group strategy would have to be improved.

Summary in Bullet Points

- Alizarin was the first natural product to be prepared industrially on a large scale.
- The synthesis also required the development of efficient processes for the manufacture of basic chemicals.
- Whereas alizarin has been replaced commercially by modern dyestuffs, related anthraquinone dyestuffs offer at least potentially attractive fields of application.

References

References to Introduction

1. E. S. B. Ferreira, A. N. Hulme, H. McNab, A. Quye, *Chem. Soc. Rev.* **33** (2004), 329.

References to 2.1 Indigo

2. W. Gerstner, *Die BASF* (1977) 2, 60; H. Berke, *Chem. Soc. Rev*. **36** (2007) 15.
3. G. Julius Caesar, *Commentarii de Bello Gallico*: English translations by, among others, S. A. Handford ("The Conquest of Gaul") and K. Gilliver ("Caesar's Gallic Wars, 58–50 BC"); see also online synopses.
4. G. Paul, BASF-Information, 9.8.1995.
5. J. Altmann, K. Elbl-Weiser, *Chem. Ind. (London)* (1997) 654.
6. W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 21st Edn., 1969, 148.
7. Etappen der BASF-Forschung, BASF-Druckschrift (1985) 17.
8. H. Schweppe, Handbuch der Naturfarbstoffe, Ecomed Verlagsgesellschaft, Landsberg, 1993, 290, 295.
9. L. Meinzer, 125 Jahre BASF: Stationen ihrer Geschichte, *BASF* (1990) 18.
10. T. Niwa, *Nagoya J. Med. Sci.* **72** (2010) 1.
11. For the history of the Indigo synthesis: A. v. Baeyer, *Ber. deut. chem. Ges*. **33** (1900) Sonderheft, Anlage IV.
12. A. Reis, W. Schneider, *Z. Kristallographie* **68** (1928) 543.
13. D. Gröger, *Phytochemistry*. **29** (1990) 817.
14. E. W. Miles, *Adv. Enzymol*. **64** (1991) 93.
15. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 726.
16. Z.-Q. Xia, M. H. Zenk, *Phytochemistry* **31** (1992) 2695.
17. A. Berry, T. C. Dodge, M. Pepsin, W. Weyler, *J. Ind. Microbiol. Biotechnol*. **28** (2002) 127.
18. W. Gerstner, *Die BASF* (1977) 2, 60.
19. S. Struckmeier, *Chemie in unserer Zeit* **37** (2003) 402; C. Oberthür, B. Schneider, H. Graf, M. Hamburger, *Chem. Biodivers*. **1** (2004) 174 (revised structures for Isatan A and B).
20. A. v. Baeyer, V. Drewsen, *Ber. Deut. Chem. Ges.* **15** (1882) 2856; *ibid.* **16** (1883) 2205.
21. J. R. McKee, *J. Chem. Ed*. **68** (1991) A242.
22. H. Krauch, W. Kunz, Reaktionen der Organischen Chemie, Dr. Alfred Hüthig Verlag, Heidelberg, 5th. Edn., 1976, 469.
23. H. Brunck, *Ber. Deut. Chem. Ges.* **33** (1900), Sonderheft, Anlage V.
24. H. Schmidt, *Chemie in unserer Zeit* **31** (1997) 121.
25. Y. Yamamoto, Y. Inoue, U. Takaki, H. Suzuki, *Bull. Chem. Soc. Jpn*. **84** (2011) 82.
26. M. Gäng, R. Krüger, P. Miederer, DE 19831291 (1998).
27. E. Steingruber in: Winnacker–Küchler, Chemische Technik, Vol. 7, Wiley-VCH, Weinheim, 2004, 434.
28. J. Hamilton, *Business Week*, New York, 24.3.1997.
29. D. Murdock, B. D. Ensley, C. Serdar, M. Thalen, *Biotechnol.* **11** (1993) 381.
30. A. N. Padden, V. M. Dillon, P. John, J. Edmonds, M. D. Collins, N. Alvarez, *Nature* **396** (1998) 225.

References to 2.2 Tyrian Purple

31. http://en.wikipedia.org/wiki/Tyrian_purple; *Trunculariopsis trunculus* is used synonymously with *Murex trunculus* and *Hexaplex trunculus*.
32. P. Friedländer, *Ber. Dtsch. Chem. Ges.* **42** (1909) 765.
33. S. Struckmeier, *Chemie in unserer Zeit* **37** (2003) 402.
34. F. P. Möhres, *Die BASF* (1962) 163.

35. R. R. Melzer, P. Brandhuber, T. Zimmermann, U. Smola, *Biologie in unserer Zeit* **31** (2001) 30.
36. E. Ploss, *Die BASF* (1962) 168.
37. C. Schuster, Vom Farbenhandel zur Farbenindustrie, BASF Firmenschrift, 1973, 37.
38. H. Schweppe, Handbuch der Naturfarbstoffe, ecomed Verlagsgesellschft, Landsberg, 1993, 31 and 282 ff.
39. K. Benkendorff, J. B. Bremner, A. R. Davis, *Molecules* **6** (2001) 70.
40. P. Friedländer, *Monatsh. Chem.* **28** (1907) 991.
41. E. Steingruber in Ullmanns Encyclopedia of Industrial Chemistry, 6th Edn., 2000, Electronic Release, Indigo and Indigo Colourants.
42. G. Voß, H. J. Gerlach, *Chem. Ber.* **122** (1989) 1199; H. J. Gerlach, DE 3910648 (1989).
43. C.-X. Liu, W.-G. He, *J. Ethnopharm.* **34** (1991) 83.
44. R. P. Maskey, I. Grün-Wollny, H. H. Fiebig, H. Laatsch, *Angew. Chem. Int. Ed.* **41** (2002) 597; R. P. Maskey, I. Grün-Wollny, H. Laatsch, *Nat. Prod. Res.* **19** (2005) 137.
45. R. Jantelat, T. Brumby, M. Schäfer, H. Briem, G. Eisenbrand, S. Schwahn, M. Krüger, U. Lücking, O. Prien, G. Siemeister, *ChemBioChem* **6** (2005) 531; T. C. Wang, J. Z. Wei, C. S. Guo, H. B. Zhang. H. X. Fan, *Chin. Chem. Lett.* **21** (2010) 1407; G. Karapetyan, K. Chakrabarty, M. Hein. P. Langer, *ChemMedChem* **6** (2011) 25.

References to 2.3 Alizarin

46. W. Gerstner, *Die BASF* (1977) 2, 60.
47. M. C. Whiting, *Chemie in unserer Zeit* **15** (1981) 179.
48. R. R. Melzer, P. Brandhuber, T. Zimmermann, U. Smola, *Biologie in unserer Zeit* **31** (2001) 30.
49. S. Struckmeier, *Chemie in unserer Zeit* **37** (2003) 402.
50. Römpp, Chemie Lexikon, Thieme Verlag, Stuttgart, 1995, Electronic Release.
51. H. Schweppe, Handbuch der Naturfarbstoffe, ecomed Verlagsgesellschft, Landsberg, 1993, 259.
52. Cochineal and Natural dyes, Encyclopedia Britannica, Inc., Electronic Release, 1994–2001.
53. http://www.seilnacht.com/Lexikon/Cochenil.htm
54. http://www.kremer-pigmente.de/kermes.htm; http://www.kremer-pigmente.de/§36040.htm
55. O. Dimroth, R. Fick, *Liebigs Ann. Chem*. **411** (1916) 315.
56. Etappen der BASF-Forschung, BASF-Druckschrift (1985) 5.
57. In presence of mercury, one obtains the kinetically-controlled anthraquinone-1-sulfonic acid: W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 21st. Edn., (1969) 173.
58. W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 21st. Edn., (1969) 353, 372.
59. L. Meinzer, 125 Jahre BASF – Stationen ihrer Geschichte, *BASF*, (1990) 10, 11, 22.
60. Holleman, Wiberg, Lehrbuch der Anorganischen Chemie, Walter de Gruyter, Berlin-New York (1985) 507, 510.
61. http://www.strawberryfeels.de/campari.htm
62. P. Allevi, M. Anastasia, P. Ciuffreda, A. Fiecchi, A. Scala, S. Bingham, M. Muir, J. Tyman, *J. Chem. Soc., Chem. Commun.* (1991) 1319; J. H. P. Tyman, A. Fiecchi, US 5424421 (1991).
63. P. Allevi, M. Anastasia, S. Bingham, P. Ciuffreda, A. Fiecchi, G. Cighetti, M. Muir, A. Scala, J. Tyman, *J. Chem. Soc., Perkin Trans.1* (1998) 575.
64. A. Bekaert, J. Andrieux, M. Plat, *Bull. Soc. Chim. Fr.* (1986) 314.

3 | Flavours and Fragrances

Flavours and fragrances constitute some of the most beautiful and amazing facets of chemistry. Whereas in many respects chemistry is a very purpose-oriented science, perfumes (and to a lesser extent flavours) may be regarded as luxury items which conspicuously link science and culture.

Günther Ohloff (1924–2005), one of the most prominent chemists at Firmenich, has to be quoted here: Perfumes are like symphonies and the perfumers are their composers.

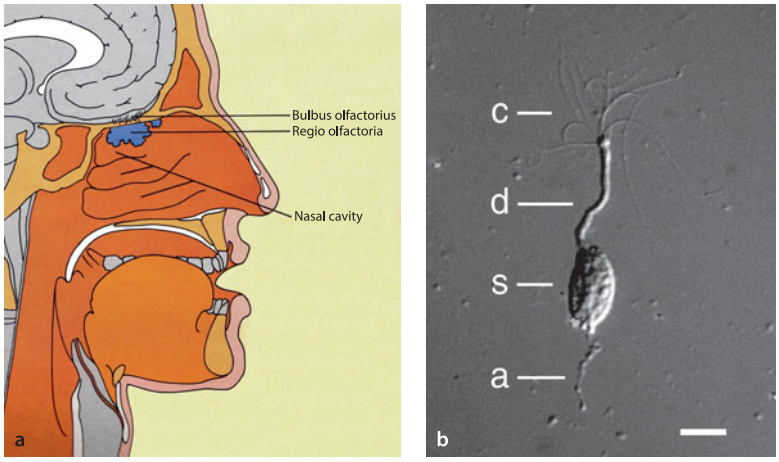
Scents

The process of smelling begins when scents in the inhaled air approach the olfactory epithelium (*Regio olfactoria*) (Fig. 3.1 and 3.2). Richard Axel and Linda B. Buck (Nobel Prize laureates for Pharmacy or Medicine in 2004) identified 339 intact, trans-membrane olfactory receptor proteins, to which transport protein-associated scent molecules can bind.

In general, scent molecules bind to various receptor proteins, and on each receptor protein bind different scent molecules. Thus, for each scent results a characteristic binding profile. [1]

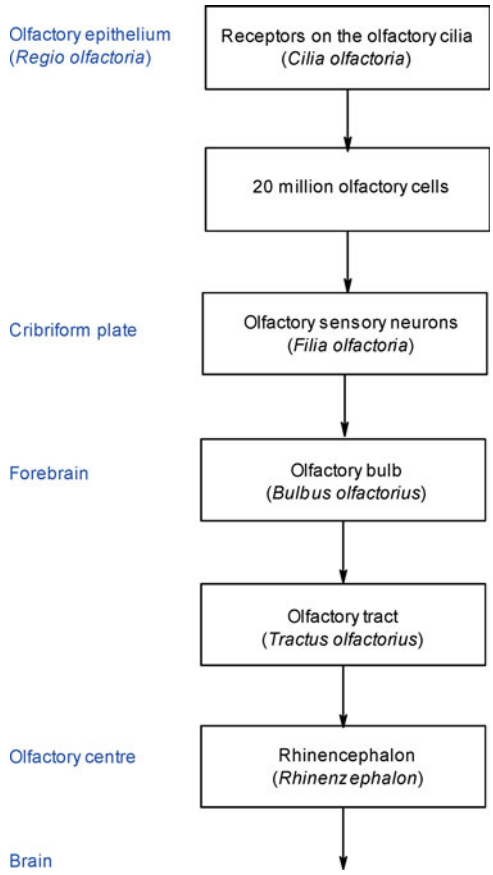
The olfactory receptors are arranged on whip-like scent hairs, held together in bundles of up to ten cilia; these are found on the 20 million olfactory cells. [2] Each of these cells expresses only one scent receptor. By binding of the scent molecule to the receptor, the quaternary structure of the receptor protein is

Humans use around 1 % of their genome in order to code the scent receptor proteins, which belong to the family of rhodopsin-like G-protein-coupled receptors (GPCRs).

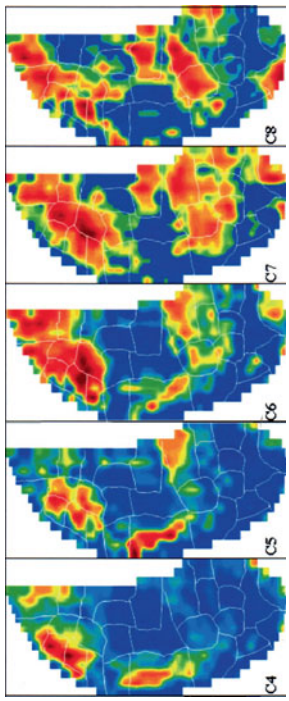


3.1 *Cross-section of the human skull (a) and a single olfactory receptor neuron of a frog (Rana pipiens) (b) (c: cilia; d: dendrite; s: perikaryon; a: axonal segment; calibration bar is 10 µm).*

B. Schaefer, *Natural Products in the Chemical Industry*,
DOI 10.1007/978-3-642-54461-3_3, © Springer-Verlag Berlin Heidelberg 2014



3.2 *The smelling process of humans.*



3.3 *The excitation pattern, which a scent releases in the olfactory bulb, can be visualised in living mice using functional magnetic resonance imaging (fMRI). Shown are the patterns for butanal (C4), pentanal (C5), hexanal (C6), heptanal (C7) and octanal (C8) (high activity, red; slight activity, blue).*

altered, which leads to a depolarisation of the olfactory membrane and eventually to the collapse of the electrical potential of the cell. This triggers an electrical impulse, which is conducted by olfactory sensory neurons (*Filia olfactoria*) through the cribriform plate in the upper nasal cavity into the olfactory bulb in the forebrain. There are to be found clusters of neurons called *glomeruli*, each of which at any one time is assigned to one receptor protein. Each scent produces a characteristic glomeruli-excitation pattern (Fig. 3.3). [3]

The excitation pattern represents all olfactory properties of the scent molecule, which are forwarded *via* the olfactory tract (*Tractus olfactorius*) into the rhinencephalon and other parts of the brain. The perception is compared with characteristic olfactory impressions in the memory, which finally enables humans to distinguish a diversity of approximately 10,000 scents (Fig. 3.4). [4]

Human Scent

The human body has two to four million sweat glands (or sudoriferous glands, from *sudor* (lat.) sweat), which are devided into two main groups: The eccrine and the apocrine sweat glands. The eccrine sweat glands are distributed almost all over the human body and are utilised for cooling. The apocrine sweat glands

3.4 *The perfumer classifies scents into eight categories. The scents are distinguished according to their first and dominant impact (top notes), their principal characteristic odour (middle notes or heart notes), and their persistence after several hours (base notes). By the use of a fixative, one attempts to slow down the evaporation of the more volatile components, so that the characteristic odour persists for a longer period.*

are larger, have a different secretion mechanism, are mostly limited to the armpits and to the genital area and contribute little to cooling. They produce an odourless oily milky secretion evolved not to evaporate but coat and stick to hair so that odour-causing bacteria can grow on it. The resulting body odour is dependent on the hormonal balance, the metabolism, the diet, psychic and social influences and the genome. Corresponding to the human genetic diversity there are great differences between various ethnic groups: for example, Koreans have almost no apocrine sweat glands, and Chinese and Japanese only a few. On the other hand, white people clearly have more, and the dark-skinned have the most glands.

Human body odour may be described as a mixture of animal musk with a strong sandalwood-like component, sweat with a urine-like character, and a smell of fatty acids. Since body odour is also a part of one's private life, it means that to speak of it is to break a taboo. Normally people observe a distance to one another and apply – just in case – a decently dosed perfume.

To Japanese, the smell of fatty acids is very unpleasant; therefore Americans and Europeans were sometimes referred to by the term *bata-kusai* (butter-stink).

Human Pheromones

Are there human pheromones? Fifty years ago, Peter Karlson (1918–2001) from the Max-Planck-Institute for Biochemistry in Munich and Martin Lüscher (1917–1979) from the University of Bern introduced this concept for substances, which are sent out by an animal in order to achieve a change in behaviour or a physiological response in another animal of the same species. [5] Mammals pick up the pheromones in special receptors in the vomeronasal organ (VNO, *Vomer* – an unpaired facial bone in the skull) [6] located in the nasal cavity. [7] The information is transmitted to the hypothalamus, which influences the endocrine system. Until a few years ago, it was believed that in adult men the vomeronasal organ is atrophied. Then an intact VNO was discovered in the proximity of the nasal cavity's partition wall (*Septum nasi).* [8, 9]

In men, lutropin (LH = luteinising hormone) triggers androgen synthesis and the release of testosterone. Follitropin (FSH = follicle-stimulating hormone) stimulates spermatogenesis. During pregnancy, women have permanently a high progesterone level (= pregn-4-ene-3,20-dione).

Kathleen Stern and Martha K. McClintock at the University of Chicago could show that a woman's follicular or ovulatory phase of her menstrual cycle may be extended or shortened if the woman had odourless axillary (underarm) sweat from other women applied to her upper lip over several cycles, until eventually the ovulations were synchronised. [10, 11] This phenomenon had been known earlier in women living in closed communities, *e.g.* barracks, brothels, convents or prisons. Behavioural scientists recognised it as a relic of ancient times, when the simultaneous birth of many young creatures produced a surplus, which offered a better chance of surviving predators.

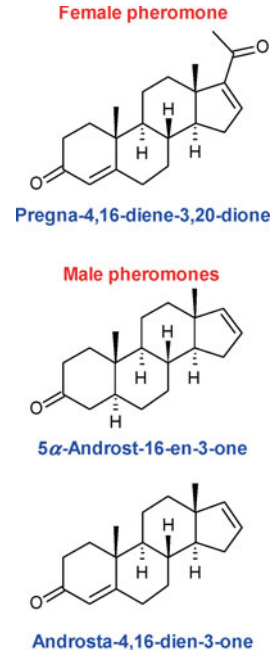
When men detect pregna-4,16-diene-3,20-dione at a nanomolar concentration, this leads to a statistically significant reduction in plasma lutropin and follitropin level. The breathing rate is reduced and the heart rate increases. The electrical conductivity of the skin and the EEG pattern are changed (Fig. 3.5). On the other hand, when women detect pregna-4,16-diene-3,20-dione, no hormonal effects are observed. [12]

Truffles are highly esteemed, subterranean mushrooms, which can be retrieved by sexually mature sows, because they emit traces of 5α-androst-16-en-3-one. [15]

Men also have chemical stimulants. [14] Prelog and Ružička resolved the structure of 5α-androst-16-en-3-one, the sex pheromone of the boar (*Sus scrofa domestica*), [15] and, as it became clear later, also of men. [16] It is a metabolite of testosterone and its odour was described as a "scent of receptacles", which had been used over a longer period to store urine. In waiting rooms, women preferred to sit rather on androstenone-impregnated chairs than on those which were untreated. Also, photographs of men, animals and houses primed with traces of 5α-androst-16-en-3-one were considered more warm and friendly than those which lacked the boar pheromone.

The so-called "*pheroboar*®" (referring to an aerosol spray) – a pheromone preparation consisting of a mixture of 5α-androst-16-en-3-one and 5α-androst-16-en-3-ol – is used in veterinary medicine and by pig farmers to stimulate sows prior to artificial insemination. The pheromone induces the release of oxytocin, which leads to contraction of the Fallopian tube and the uterus, and thereby increases the conception rate. [12]

3.5 *The reason why human scents have been preserved during evolution might be based upon a connection between the perception of odour and the major histocompatibility complex (MHC). [13]*



More recently it was found that also androsta-4,16-dien-3-one, a metabolite of testosterone as well, has strong pheromone-like activities in humans. It affects significantly the mood of heterosexual women and homosexual men, but it does not alter behavior overtly, although it may have more subtle effects on attention. Androstadienone is commonly sold in male fragrances, in order to increase sexual attraction. [17]

History of Fragrances

Fragrances and flavours have accompanied humankind throughout its entire cultural history. [18] Claudius Galenus (129– 200 AD) (better known as Galen of Pergamon), the founder of Galenics, and physician to Marcus Aurelius (121–180 AD), discovered the olfactory nerves, which Leonardo da Vinci (1452–1519) described accurately in his anatomical drawings. He also knew of their connection with the olfactory bulb. Titus Lucretius Carus (97–55 BC), the Roman poet and natural philosopher, developed the first structure-activity relationships of fragrances. In his work De *rerum naturae* he assigned to pleasant scents a rounded form, and to stinking substances a sharp, spiky form. An odour impression arises, when these particles pass through slits with a complementary structure. Thus, 2000 years before Emil Fischer (1852–1919), Carus anticipated the lock-and-key theory for substrate-enzyme (or ligand-protein) interactions. Linus Pauling (1901–1994) applied this idea to olfaction, which was ultimately the basis for John E. Amoore's "stereochemical theory of olfaction". [19] According to John Amoore (1930–1998), molecules with similar surfaces have similar scents.

In the Old Testament we read from the book of Proverbs:

I have sprinkled my bed with myrrh, my clothes with aloes and cassia (*i.e.* cinnamon). Come, let us drown ourselves in pleasure, let us spend a whole night of love, until the day appears. [20]

The word "perfume" (parfum in French, Parfüm in German) is derived from the Latin *per fumum*, meaning sacrificial smoke. Burnt offerings constitute one of the oldest forms of worship. The smoke rises on high and spreads through the resulting fragrance an air of wellbeing.

This is where the beginning of scent culture is to be found – long before recorded history and therefore at the very beginning of human history. Egyptians, Persians and Scythians used resins and fragrant plant oils for embalming the dead. Soon scents were also used for cosmetic and other purposes.

The Romans took up the use of scented compounds from the Etruscans, who knew myrtle, labdanum, broom, pine resin and Arabian frankincense. [21, 22] Also the luxurious lifestyle of the Greeks had a strong influence on the young Roman Empire. In the days of the Emperors, Rome fell into a veritable scent craze. Pliny records that the annual cost of importing scents from India and Arabia climbed to more than 100 million sesterces (2.5 million dollars). For ointment preparation, India provided cardamom, nutmeg, ginger, cinnamon, pepper, costus roots, spikenard, aloe and sandalwood. Additionally, native species used were rose, iris, quince, narcissus, jasmine and wild vine. Among animal-based fragrances, the only one of firmly established use was produced from the dried scent glands of a widespread cuttlefish: when powdered, this exhales a pleasant musk-like scent.

Patrick Süskind's novel, *Perfume* tells the horrific story of an 18th century serial killer of young ladies, who attempts to prepare the most unique perfume from them. This plot is fictional, but the techniques of preparing fragrances are described in detail and rather accurately.

The original meaning of the word "perfume" connects two features of mankind's existence: the use of fire and the affinity to spirituality.

In the "Capitulars" (collections of laws and decrees) of Charlemagne (741–814) can be found written comments by the Benedictine monk Ansegisus (770–834) about rosemary, thyme, sage, dill, parsley, marjoram, mint and rue; these were cultivated in the monastery garden for use as spices and drugs. Hildegard of Bingen (1098–1179) dedicated a separate paper, *De Lavendula*, to lavender. With the homecoming of the Crusaders and the spread of the teaching of celebrated Arabian physicians, the Arabs' extensive knowledge of spices and scents was taken up in northern Europe. Here, an important role was played by the Medical School of Salerno, the Translation School of Toledo and the Trading Centre Venice.

The small town of Grasse, in the south of France, developed into a centre for perfumery, after Catherine de Medici (1519–1589) set up a laboratory there for the apothecary and alchemist Francesco Tombarelli. At the University of Montpellier she had research carried out into new methods of isolating scented materials from plants. At that time, the most popular perfume was *Frangipani,* an alcoholic extract of iris powder, musk and civet, invented by Maurice Frangipani, a descendant of that mighty noble Roman dynasty.

It was also Italians who introduced perfumery into Germany, for instance the family of Giovanni Paolo de Feminis, who immigrated around the year 1690. In Cologne (Köln) they produced *Aqua mirabilis* along native recipes, for which they obtained a certificate from the University of Cologne together with instructions on how to employ this product.

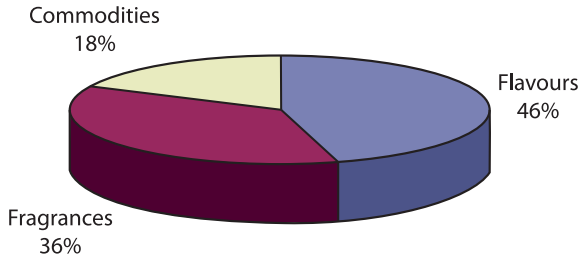
In 1709, the family of Giovanni Maria Farina (1685–1766) from Milan started similarly the production of *Kölnisch Wasser* (which is known in the English-speaking world by the French label *Eau de Cologne*).

A Carthusian monk of the Farina family (Franz Maria Carl Gereon Farina) sent Wilhelm Mülhens (1762–1841), as a present on his wedding day (8th October 1792), the recipe for a perfume. Mülhens quickly recognised the value of the gift, and set up a business for the production of Eau de Cologne. Four years later, Cologne had in the meantime been occupied by French Revolutionary troops, following a decree of General Charles Daurier (1768–1833), all the houses in the city were consecutively numbered to make it easier for the soldiers to find their quarters.

3.6 *In 1875, Ferdinand Mülhens had registered the trademark "4711". His marketing strategy was clearly intended to distinguish his product from the multitude of other "Colognes".*

The Mülhens family's house in the *Glockengasse* was given the number 4711; thereby, a distinct name for their new brand was born. The industrially manufactured product was a huge success right from its beginning (Fig. 3.6). Up to now, the precise recipe for *4711*® has remained a secret, in spite of a decree from Napoleon Bonaparte, which was issued in the year 1810. Though, a few constituents are known, like the essential oils from orange peel, orange blossom, lemon, rosemary, lavender and bergamot.

In 1889, a turning point in perfume preparation had come, when Aimé Guerlain introduced his perfume *Jicky*®, in which, for the first time, only synthetically generated vanillin, coumarin and heliotropin (3,4-methylenedioxybenzaldehyde; also known as piperonal) were used. In the course of his work on highly nitrated benzene derivatives, which would become very important as explosives, the French chemist Albert Baur found by chance a group of compounds with a musk odour. Ferdinand Tiemann in Berlin, looking for a syn-



3.7 *The present-day scents and flavours market has a total volume of 11 billion Euro.*

thetic route to the scents of iris root, serendipitously found similarly scented ionone derivatives, the first representatives with the scent of violets. Among the group of salicylates, not only by *Aspirin*® achieved high importance, but also isoamyl salicylate, the scent of which is reminiscent of blooming clover, and imparting its typical note to *Tréfle incarnat* (1889). In 1921, Coco Chanel (Gabrielle Bonheur Chanel, 1883–1971) scored an unusual success with the unconventionally composed *Chanel No. 5*®. The aversion to synthetic scents was thereby broken (Tab. 3.1). Today, the 150 natural essential oils are faced with some 3000 synthetic fragances. The worldwide turnover of scented compounds amounts to 11 billion Euro (Fig. 3.7).

The fragance market with a volume of 4 billion Euro is divided into 35 % for soaps and detergents, and 30 % for cosmetics and personal hygiene; whereof 20 % account for fine perfumery and another 10 % for industrial products. Important firms in the scent and flavours area are IFF, Givaudan, Symrise and Firmenich.

Around 75 % of all the raw materials for perfumery are produced by chemical synthesis. The remaining 25 % are obtained from biological sources like aromatic plants, which are cultivated in the Mediterranean region, South-East Asia and Latin America. Of animal origin are musk (from musk deer), civet (from

Tab. 3.1 *Important compounds, which became accessible during the 19th century through new synthetic methods.*

1855	Benzyl alcohol	Cannizzaro
1855	Phenylacetic acid	Cannizzaro
1866	Coumarin	Perkin
1876	Salicylaldehyde	Reimer
1876	Vanillin	Reimer, Tiemann
1878	Cinnamic acid	Perkin
1883	Phenylacetaldehyde	Erlenmeyer, Lipp
1885	*α*-Terpineol	Wallach
1890	Heliotropin (piperonal)	Ciamician, Silber
1891	Nitro musks*	Baur
1893	Ionone	Tiemann, Krüger

* Di- and tri-nitroarenes *cf.* section 3.6.4.

civets), castoreum (from the North American beaver), and ambergris (from the sperm whale. [23]

Summary in Bullet Points

- Smell is our chemical sense. With it we perceive scents and aromas.
- Every human being has its individual scent, which is closely connected to the major histocompatibility complex.
- Scents have accompanied mankind throughout its entire cultural history.

3.1 Damascone

Around 800 BC, in the *Iliad* and *Odyssey*, Homer describes the popularity of roses. Virgil (79–19 BC) mentions the Damask rose, which originated in Persia (Fig. 3.8) as the "Rose from Paestum", and Pliny reports on the especial significance of *Rosa gallica* and the Rose of Miletus (an ancient Greek city on the west coast of Anatolia, now part of Turkey). For the traditional rose festivals (*rosalia*), Nero (37–68 AD) acquired rose petals, worth of 4 million sesterces (100,000 US dollars). Caligula (12–41 AD) and Vespasian (9–79 AD) also loved fragrances. On the other hand, Julius Caesar had expressed earlier a preference for garlic. He said to his perfumed generals, "I wished that you would stink of garlic". [24]

3.8 *The Damask rose (Rosa damascena) is also known as the "Rose of Kazanlak" (a town in Bulgaria), and since the 17th century it has been the source of Bulgarian rose oil.*

Es ist ein' Ros' entsprungen,
Aus einer Wurzel zart.
Wie uns die Alten sungen,
Von Jesse war die Art.
Und hat ein Blüm'lein 'bracht;
Mitten im kalten Winter,
Wohl zu der halben Nacht.

A spotless rose is growing,
Sprung from a tender root,
Of ancient seers' foreshowing,
Of Jesse promised fruit.
Its fairest bud unfolds to light
Amid the cold, cold winter,
And in the dark midnight.

In Christian mysticism, the rose has acquired great significance. It symbolised the blood of Christ and also of the Virgin Mary. This is reflected in the famous Christmas carol by Michael Praetorius (1571/72–1621): Es ist ein' Ros' entsprungen. The most familiar English-language version, shown alongside the German original, is the work of the 19th-century scholar, Catherine Winkworth (1827–1878) (see marginal note).

In the sagas of the *Nibelungen* and also in the Icelandic equivalent *Edda*, the rose is as well revered. The first evidence of rose cultivation north of the Alps dates from the time of Charlemagne, in the year 812. The returning Crusaders brought back *Eau de Chypre* and rose water into northern Europe

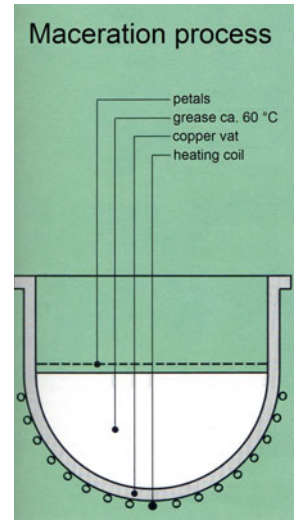
from the perfumery centre on Cyprus (which is Chypre in French). In 1250, the Bishop of Regensburg described the Rose de Mai (*Rosa centifolia* L.), the Eglantine rose (*Rosa rubiginosa*), the Dog rose (*Rosa canina*) and the Field rose (*Rosa arvensis*).

3.1.1 Rose Scent from Rose Flowers

In ancient times, the usual method for obtaining fragrant ointments and oils was maceration (Latin: *macere* – to soak), whereby the plants, or the relevant parts of plants, were treated with heated oils and fats. The scented (organic) compounds were thereby extracted into the oil or fat (Fig. 3.9).

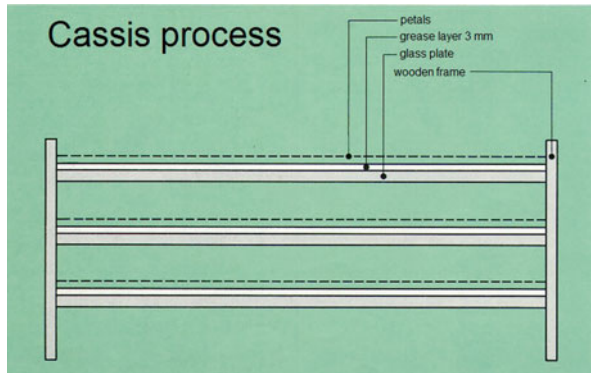
The isolation of pure rose oil was only achieved in the 9th century, after the Arabs had invented steam distillation (Fig. 3.10). [25] Rose water was inevitably obtained as a by-product.

Another method for obtaining raw materials for perfumery is *enfleurage* following the Cassis procedure (Fig. 3.11). In this, freshly picked petals are stuffed onto frames covered with beef fat and pork lard, whereby the scented compounds are absorbed into the fat. This procedure is repeated until saturation is reached, and the *pomade* thus obtained is then extracted with 96 % ethanol



3.9 *Oleum rosarium was obtained from rose blooms via maceration.*

3.10 *Production of rose oil by steam distillation in Turkey.*



3.11 *Nowadays, the Cassis procedure is also used to obtain the absolue from jasmine, gardenia, tuberose, narcissus and jonquil.*

A *concrète* (engl.: concrete) is obtained from parts of a plant by extraction with organic solvents, which are subsequently evaporated. Dissolution of the residue in ethanol and filtration delivers upon re-evaporation also the oily *absolue*.

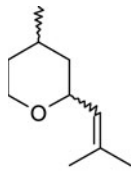
(*lavage*). Filtration and vaporisation of the alcohol leads to the *absolue* (engl.: absolute). Since the 17th century, *enfleurage* has been carried out on an industrial scale in Grasse, in the south of France.

Today, the production of rose oil utilises mainly the Damask rose and the Rose de Mai, the primary sources of which are the south of France, Liguria and Calabria in Italy, and Morocco. The annual world production of rose petals amounts to 52,000 tonnes; this yields 15 tonnes of rose oil, one of the most expensive essential oils for the fine perfume industry.

Chemists have been engaged early on in the analysis of rose oil and ways to produce its synthetic equivalent. At the beginning of the 20th century, five compounds were known – citronellol, geraniol, nerol, linalool and β-phenylethanol – which together account for 80 % (by weight) of rose oil. Nevertheless, it is relatively easy to distinguish this synthetic mixture from genuine rose oil.

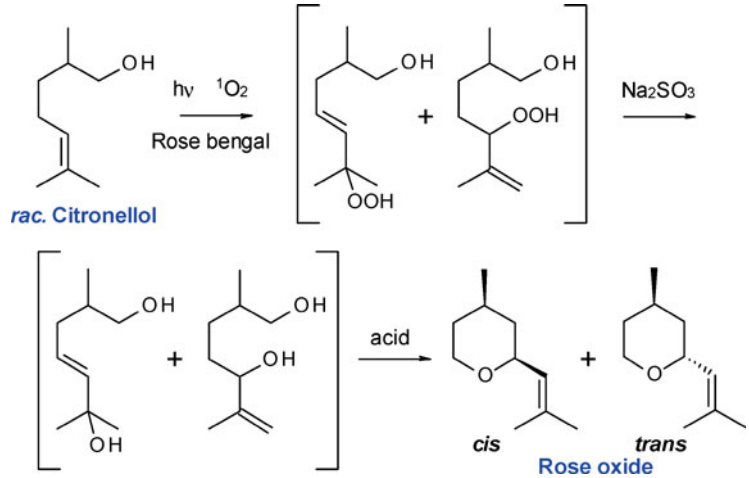
Due to its good solubility in water, β-phenylethanol is the main component of rose water. The alcohol has a slight, but very characteristic, scent of roses. Because of its adaptability, β-phenylethanol is found in more than 80 % of all perfumes. The technical synthesis goes back to Louis Bouveault (1864–1909) and his student Gustave Blanc (1872–1927), who discovered in 1903 that esters can be reduced to the corresponding alcohols by sodium in ethanol. [26]

At the beginning of the 1960s, Ervin Kováts (1927–2012) found that rose oil consists of at least 275 components (which led to certain disillusionment). These substances, characteristic of rose scent, were still unknown, and might, in an unfavourable case, be present in only trace amounts. The first important component to be discovered was rose oxide [27], a cyclic monoterpene ether, at a concentration of 0.5 %. Rose oxide has an unpleasant smell, reminiscent of mineral oil. At high dilution however, it exudes a scent of beautiful freshness and a nuance reminiscent of green leaves.

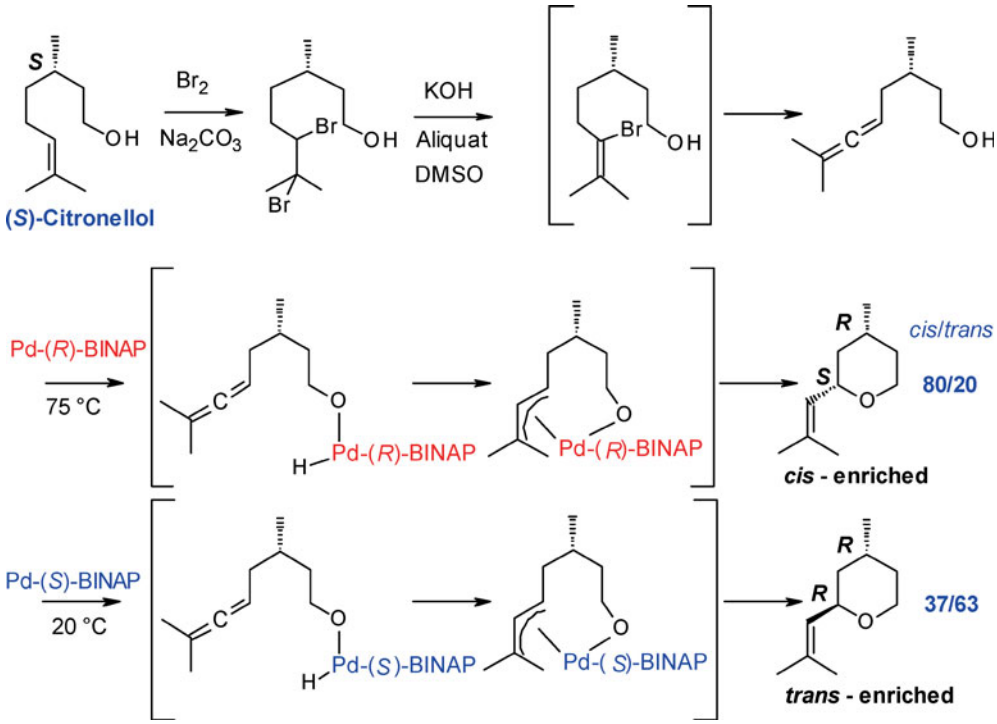


Rose oxide

Numerous syntheses of racemic rose oxide have been developed. One, which is especially noteworthy, originates at Dragoco. [28] It starts from (+/–)-citronellol; by photooxidation and acid-catalysed cyclisation, this gives rose oxide as a 91 : 9 mixture of *cis* and *trans* isomers. At present, the production is carried out by Symrise on an annual scale of greater than 100 tonnes.



Enantiomerically enriched rose oxide is obtained according to a procedure of Takasago, by a palladium-BINAP-catalysed cyclisation of dehydrocitronellol. By using (*S*)- and (*R*)-citronellol and (*R*)- and (*S*)-BINAP, all four diastereomers, which are distinguishable by their smell, may be obtained in enriched form. [29] The (4*R*)-*cis* isomer has the lowest scent threshold (5 ppb) and a sharp, pure, metallic rose scent. [30]



The decisive breakthrough was achieved in 1970 with the discovery of the rose ketones, which were named *damascones,* after *Rosa damascena.* Since scent thresholds can vary over multiple decimal powers. Günther Ohloff (1924–2005) calculated the relative scent contribution of the various components in Bulgarian rose oil according to the formula:

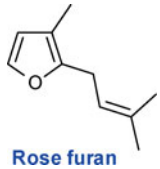
scent unit = content of component (ppb) × 100 / scent threshold (ppb)

Thereby one can see that the main olfactory components of Bulgarian rose oil are (–)-citronellol, (–)-rose oxide, β-damascenone and β-ionone (Tab. 3.2). [31]

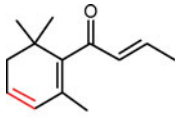
The scent threshold is the concentration of a substance, which a human can barely smell under standardised conditions.

3.1.2 Biogenesis of Damascone

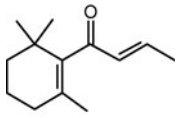
The biogenesis of damascone starts from β-carotene, which is enzymatically degraded. [32–34] The oxidative degradation of carotenoids [37] leads, for



Rose furan



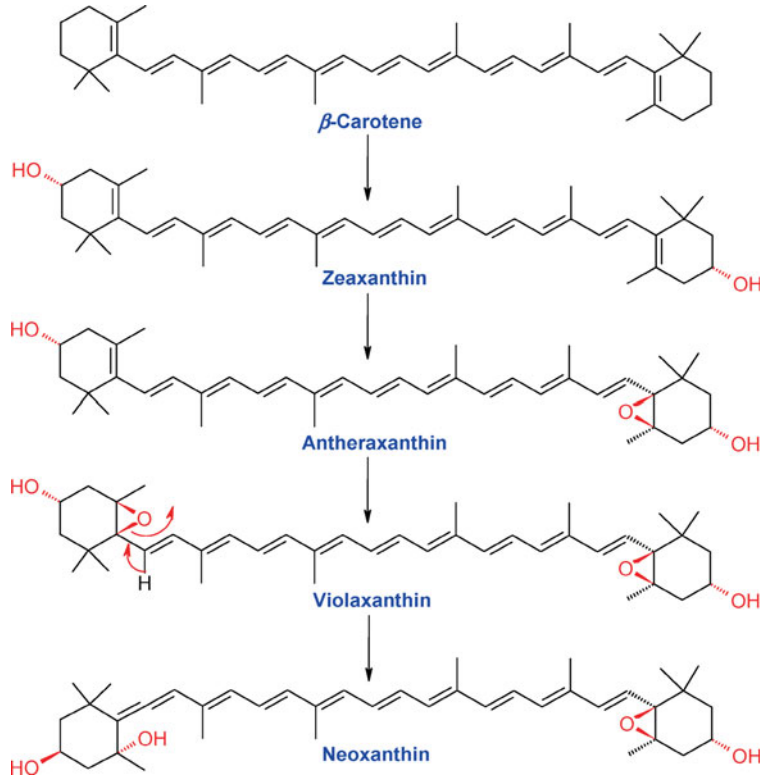
β-Damascenone



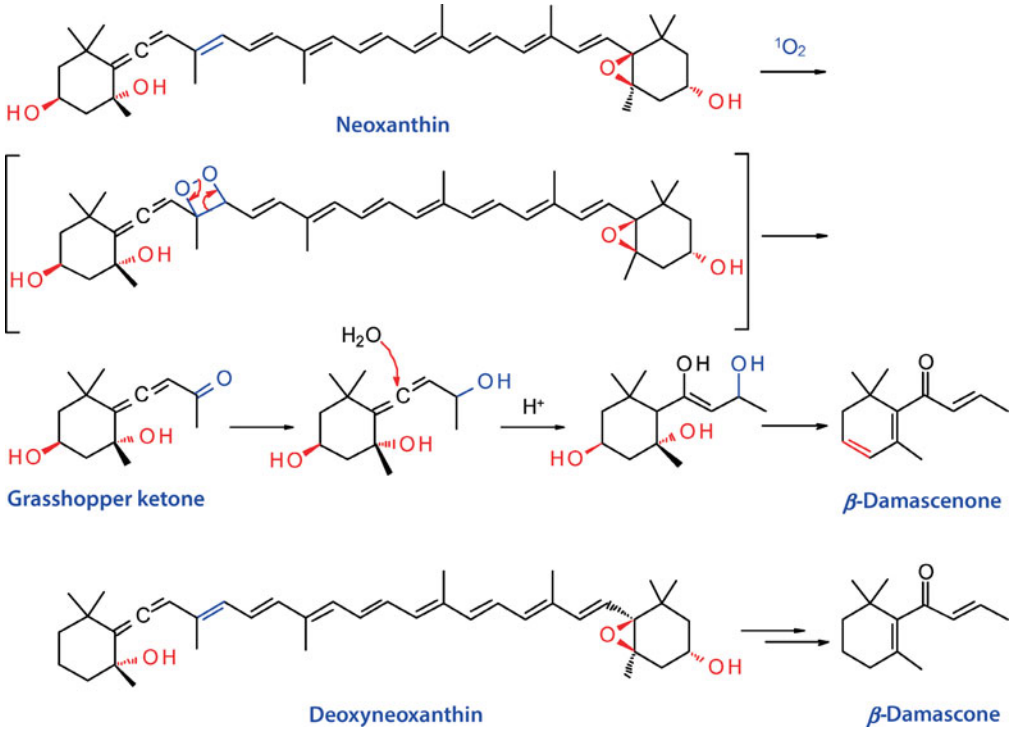
β-Damascone

Tab. 3.2 *Components of Bulgarian rose oil.*

Component	Proportion in rose oil (%))	Scent threshold (ppb)	Units of scent	Relative proportion of scent units (%)
(–)-Citronellol	38	40	9,500,000	4.3
C_{14}–C_{16} alkanes	16	0	_	_
Geraniol	14	75	1,866,667	0.8
Nerol	7	300	233,333	0.1
2-Phenylethanol	2.8	750	37,333	0.0
Eugenol methyl ether	2.4	820	29,268	0.0
Eugenol	1.2	30	400,000	0.2
Farnesol	1.2	20	600,000	0.3
Linalool	1.4	6	2,333,333	1.0
(–)-Rose oxide	0.46	0.5	9,200,000	4.1
(–)-Carvone	0.41	50	82,000	0.0
Rose furan	0.16	200	8,000	0.0
β-Damascenone	0.14	0.009	155,555,556	69.8
β-Ionone	0.03	0.007	42,857,143	19.2

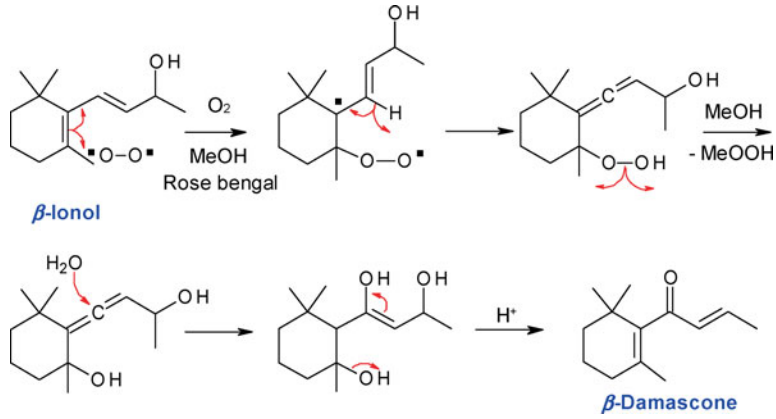


The grasshopper *Romalea microptera*, produces a foam which contains "grasshopper ketone" as a repellant towards its enemies such as ants.



example, from β-carotene by way of neoxanthin to the so-called "grasshopper ketone" [35], which after reduction, addition of water and dehydration gives damascenone. β-Damascone correspondingly derives from deoxyneoxanthin.

Since damascone is practically always detected together with ionones, the following genesis is also conceivable; both, *in vitro* and, by analogy, *in vivo*. [36, 37] Laboratory experiments suggest the following pathway:



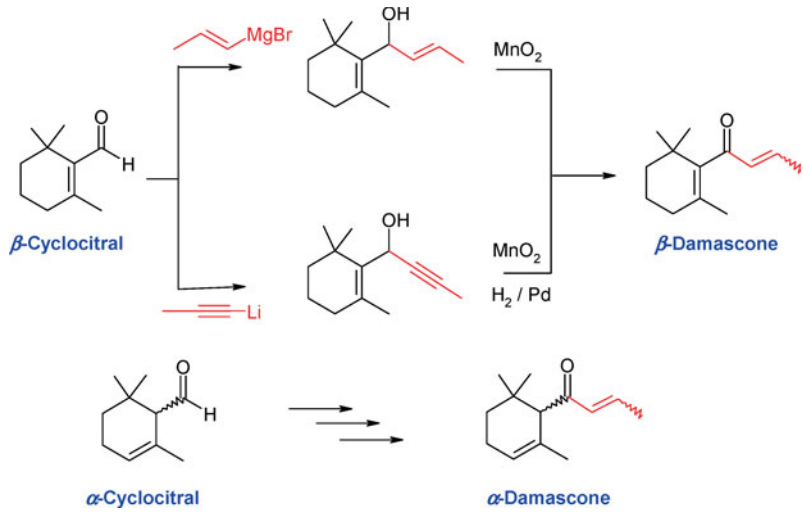
In contrast to the laboratory experiment, Nature uses iron-protoporphyrin IX for the photochemical excitation of oxygen.

Damascones have a heavy, narcotically spicy scent, with undertones reminiscent of blackcurrants and prunes. The two enantiomers together amount at most to 0.15 % by weight in rose oil, but they contribute decisively to its base note. Interesting is damascone's very low scent threshold of 0.009 ppb. Rose oxide and the rose ketones have subsequently been discovered in a whole range of aromas, for example in the bouquet of several wine varieties.

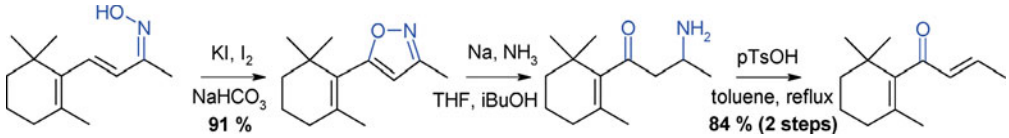
3.1.3 Synthesis of Damascone

Racemic Synthesis

The first syntheses of damascones started from cyclocitral or ionone. Cyclocitral is treated with either propenylmagnesium bromide or propynyllithium, then oxidised with manganese dioxide and, if necessary, the product is catalytically hydrogenated. In case of α-damascone, the products are racemic. [38]

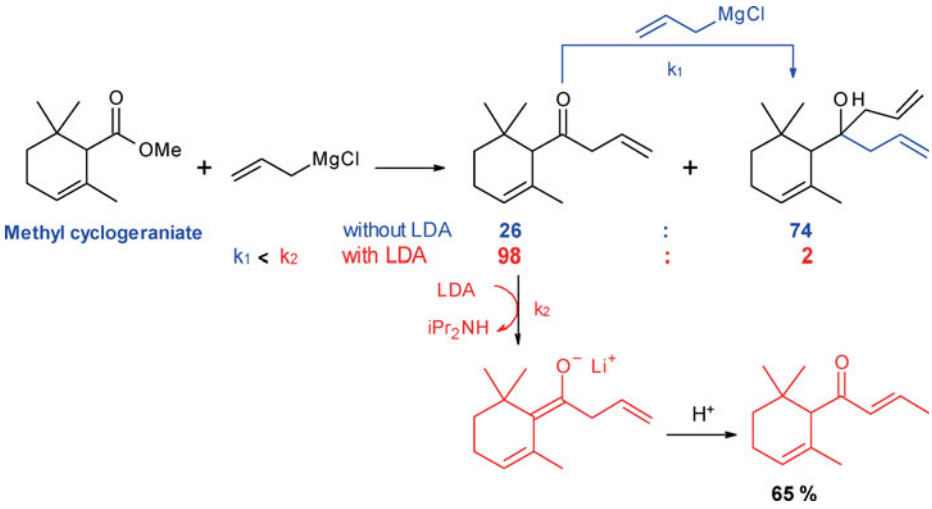


George Büchi (1921–1998) and John C. Vederas developed a synthesis, in which β-ionone oxime is cyclised to an isoxazole under conditions similar to those for iodolactonisation; Birch reduction of the isoxazole cleaves the N-O bond and produces a β-aminoketone; acid-catalysed elimination of ammonia then yields β-damascone. [39]

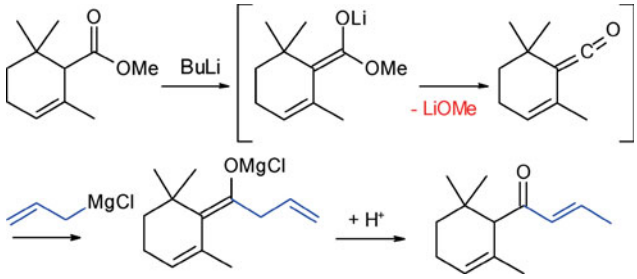


The increasing demand for rose ketones led in the 1980s to the development of industrial syntheses of these scented compounds by Firmenich. The starting

material is methyl cyclogeraniate. Whereas a normal Grignard reaction with allylmagnesium chloride leads to partial diallylation and the production of a tertiary alcohol, the presence of LDA suppresses the secondary reaction almost completely. At low temperature, the ketone intermediate is deprotonated by LDA to give the enolate, so that a second Grignard reaction is avoided. Interestingly, deprotonation with MeOMgCl (and allylMgCl) is much slower than with LDA. The acid-catalysed isomerisation of the double bond leads to racemic α-damascone in satisfactory yields. [40]

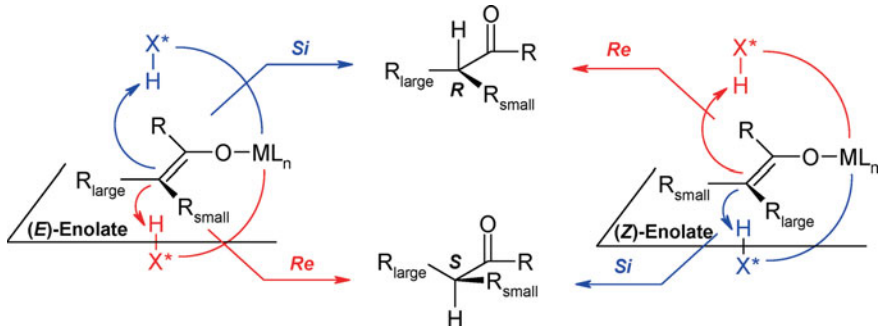


At elevated temperature, a second competing mechanism is the decomposition of the ester enolate to a ketene, followed by a Grignard reaction. [41]



Enantioselective Protonation

Although the perfume industry, unlike the pharmaceutical industry, is not subject to such strict regulations regarding enantiomeric purity, the scent quality of the enantiomers is often sufficiently distinguishable to make an enantioselective synthesis desirable. Whereas, for example, (*S*)-α-damascone smells of rose petals, the (*R*)-enantiomer has a distinct apple character and an undesirable smell of cork. However, the (*R*)-enantiomer has a scent threshold 70 times higher, so that small amounts of the unwanted enantiomer do not cause a major problem; nonetheless, the synthesis of the pure (*S*)-enantiomer, or of a mixture



3.12 *Prochiral enolates possess two enantiotopic sides, so that the kinetically controlled proton transfer leads selectively to a single enantiomer (priority for (R)/(S) and (E)/(Z): $R_{large} > R_{small}$ > rest of the molecule).*

strongly enriched in this compound, was still desirable; and this led to one of the most exciting fields of work in modern organic chemistry: the enantioselective protonation (Fig. 3.12). [42–44]

When a prochiral (*E*)-enolate is selectively (*Si*)-facially protonated, the result is the (*R*)-enantiomer. (*Re*)-Facial protonation leads to the (*S*)-enantiomer. From the (*Z*)-enolate, the direct opposite is obtained. If it is not possible to control the (*E*)/(*Z*)-configuration of the enolate, in order to obtain good selectivity, one needs then an enantiomerically pure acid, whose protonation preference is dependent on the enolate configuration, *i.e.* for example, it transfers a proton (*Si*)-facially to the (*E*)-enolate, but (*Re*)-facially to the (*Z*)-enolate. In many successful cases the enantiomerically pure acid is bonded to the metal of the enolate; therefore, at the same time it acts also as a Lewis base. In addition, at least from a theoretical point of view, enantioselective inter- and intra-molecular protonations with achiral acids are conceivable, in which another ligand of the enolate complex is enantionmerically pure.

Along with steric aspects, the kinetics of the enantioselective protonation plays a crucial role. Here it is important that proton exchange reactions between electronegative atoms are usually very fast, since there is a threat that the reactions become diffusion-controlled. Thermodynamic control then leads to the racemic product.

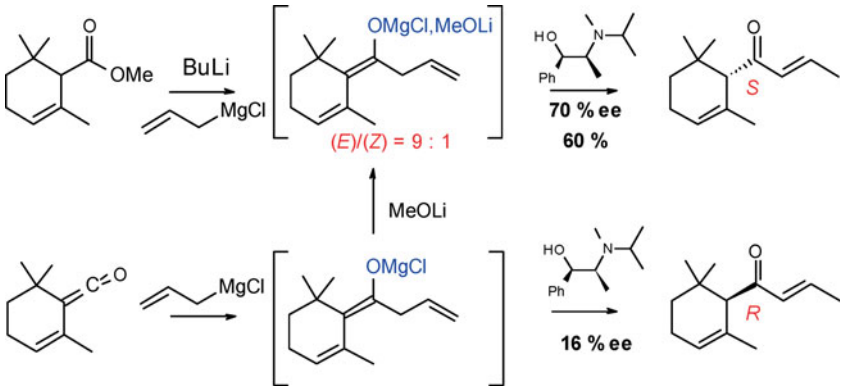
The protonation must take place irreversibly at the prochiral C-atom; *O*-protonation likewise leads to the racemic product. Unfortunately, many enantioselective proton transfers are often strongly influenced by solvation, aggregation and complexation, which are less well understood. [45]

3.1.4 Industrial Process

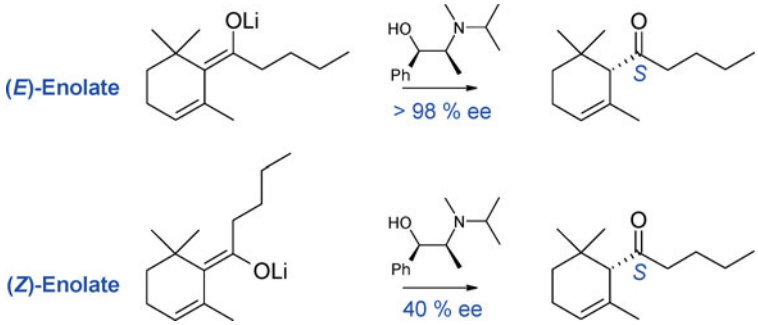
The starting point for the preparation of enantiomerically-enriched α-damascone was the synthesis of the racemate already described above, and the idea of carrying out enantioselective protonation using ephedrine derivatives. The Grignard reaction of methyl cyclogeraniate gave the keto-enolate with an

(*E*)/(*Z*)-ratio of 9 : 1. Protonation of the mixed lithium/magenesium enolate with (–)-isopropylephedrine at -10 °C gave (*S*)-α-damascone with an enantiomeric excess (ee) of 70 %.

Treatment of the ketene from cyclogeranic acid with allylmagnesium bromide gives the pure magnesium enolate. Its protonation using isopropylephedrine however gives (*R*)-α-damascone with an ee of only 16 %. In contrast, repetition of the process in presence of one equivalent of lithium methoxide leads again to (*S*)-α-damascone with 70 % ee.



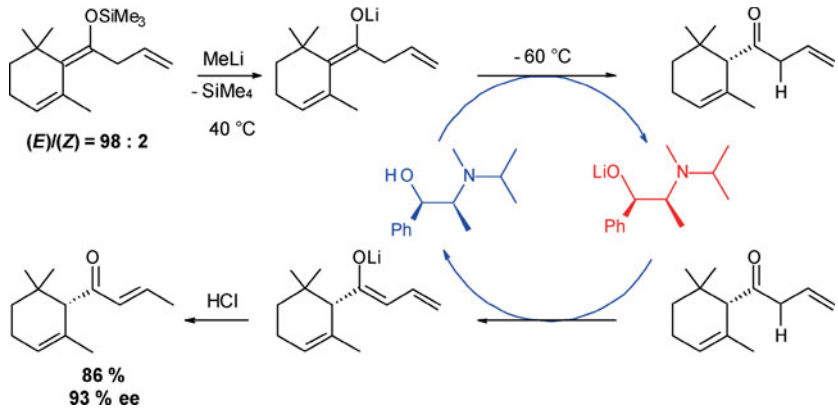
The choice of lithium and/or magnesium alkoxides plays obviously a defining role in the enantioselective protonation. The question arises as to whether pure lithium salts would result in better selectivity, and what influence the (*E*/*Z*)-configuration might have. This was examined using the pure lithium enolate derived from *n*-butyl-(2,6,6-trimethyl-cyclohex-2-enyl) ketone.



Apparently, the enantioselective protonation gives the same stereochemical preference, but with clearly varied enantiofacial differentiation.

These experiments also enabled the discovery of catalytic enantioselective protonation. [46] Highly (*E*)- enriched, pure lithium enolate (98 : 2) was obtained by a Grignard reaction, addition of chlorotrimethylsilane, fractional distillation and treatment with methyllithium. For the stereoselective protonation, 0.2–0.3 equivalents of isopropylephedrine were sufficient, because this was re-

protonated by the allyl α-hydrogen. After protolysis, (*S*)-α-damascone was obtained in 86 % yield and 93 % ee.



This is certainly the simplest and most elegant synthesis of (*S*)-α-damascone on industrial scale. Because of the sub-stoichiometric use of *N*-isopropylephedrine, the process is especially attractive.

Summary in Bullet Points

- Rose-scented compounds class among the high- valued components in fine perfumery, and continue to be isolated in part from natural sources.
- Damascone and damascenone are ranked among the most important olfactory components. In plants, they are produced by the metabolic degradation of carotenoids.
- The key step in the industrial synthesis of enantiomerically enriched α-damascone is the enantioselective protonation of prochiral enolates, a method specifically developed for this compound.

3.2 Ionone

Greeks and Romans highly appreciated the perfume of violets. Perfumers of ancient times produced ointments smelling of violets. The Persian (*i.e.* Iranian) poet, Khwāja Shamsu d-Dīn Muhammad Hāfez-e Shīrāzī (1325–1389), wrote in his *Poems from the Divan*: [47]

کنون که در چمن آمد گل از عدم به وجود
بنفشه در قدم او نهاد سر به سجود

بنوش جام صبوحی به ناله دف و چنگ
ببوس غبغب ساقی به نغمه نی و عود

When now the rose upon the meadow
from Nothing into Being springs,
When at her feet the humble violet with
her head low in worship clings,
Take from thy morn-filled cup refreshment
while tabors and the harp inspire,
Nor fail to kiss the chin of Saki while the
flute warbles and the lyre.

Two centuries later, in the mid 1590s William Shakespeare (1564–1616) wrote in *A Midsummer Night's Dream*:

> I know a bank where the wild thyme blows,
> Where oxlips and the nodding violet grows,
> Quite over-canopied with luscious woodbine,
> With sweet musk-roses and with eglantine

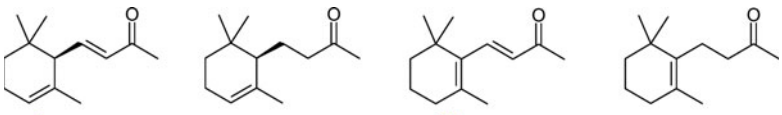
Later, the violet was also stylised in Johann Wolfgang Goethe's poem *Das Veilchen* ("The Violet") of 1774 ("Ein Veilchen auf der Wiese stand") as a symbol of modesty and faithfulness; this poem was famously set to music by Wolfgang Amadeus Mozart in 1785 (K476). [47]

In the 19th century, the scent of violets enjoyed a renaissance. By *enfleurage* of the English violet (or wood violet, *Viola odorata* L.) (Fig. 3.13), the readily volatile components were extracted and thus made accessible for fine perfumery. The price for one kilogram of violet oil in 1904 was estimated at 80,000 German Goldmarks, which corresponds to about 400,000 Euro. [48]

3.13 *The English violet (Viola odorata).*

3.2.1 Violet and Iris Oil

The characteristic scent of violets comes from their flowers. Violet flower oil contains around 22 % of enantiomerically pure (*R*)-α-ionone and β-ionone, as well as their dihydro derivatives (Fig. 3.14). Their discovery was based upon an inaccurate structure determination. In 1893, Ferdinand Tiemann believed, that with the ionones he had identified the odouriferous principle of iris oil. As became apparent some 50 years later, this consisted however of a mixture of structurally related irones, which are methyl-substituted ionones.

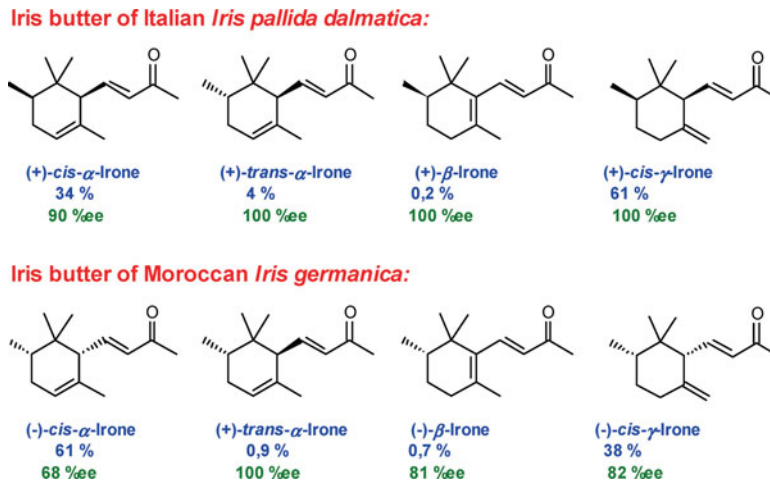


3.14 *Constituents of violet flower oil. Undiluted, α-ionone smells of cedar wood; by dilution with alcohol, the violet fragrance clearly emerges. The scent threshold in air is approximately 10^{-7} mg / litre.*

3.15 *The German iris (Iris germanica).*

Iris oil is isolated from the roots (rhizomes) of the blue- and violet-flowered "bearded" irises, *Iris pallida* (the Dalmatian iris) and *Iris germanica* (the German iris), which are grown mainly around Florence and in Morocco (Fig. 3.15). The essential oil is isolated from the powdered rhizomes by warming with diluted sulfuric acid and steam distillation. The special cultivation in iris plantations, the three-year storage of the rhizomes and the complicated work-up procedure make iris oil one of the most expensive ingredients in perfumery. One kilogram of iris *absolue*, which is obtained from the high-value iris butter, costs 40,000–50,000 Euro. [49, 50]

The main constituents of iris oil from *Iris pallida* are (+)-*cis*-α-irone, (+)-*trans*-α-irone, (+)-β-irone and (+)-*cis*-γ-irone. *Iris germanica* contains virtually the same irones, but with predominantly the opposite chirality and to some extent also different enantiomeric purity: (–)-cis-α-irone, (+)-trans-α-irone, (–)-β-irone and (–)-cis-γ-irone (Fig. 3.16).



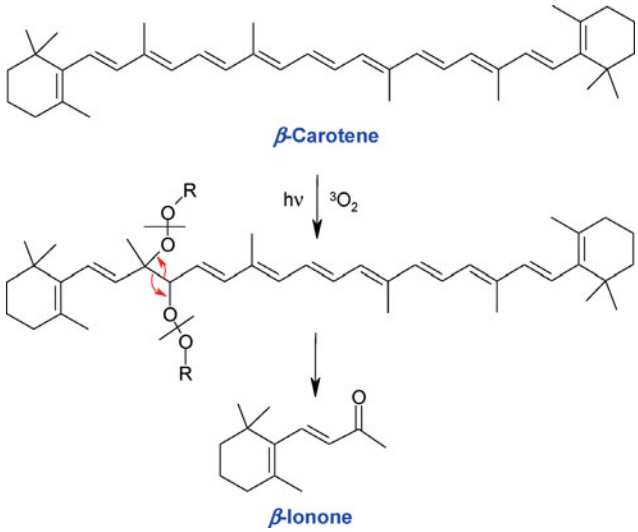
3.16 *Constituents of iris oil.*

(+)-*cis*-α-irone and (–)-*trans*-α-irone possess a sweet iris scent, but the distinctive character of iris butter is attributed to (–)-*cis*-α-irone.

3.2.2 Biosynthesis

α- and β-ionone have been discovered not only in violets but also in raspberries, blackberries (brambles), tobacco, black tea, and yellow passion fruit. They are found in various kinds of whisky and brandy, α-ionone in grapes, and β-ionone in orange and celery oil. Also the smell of carrots contains ionones and their oxidation products (epoxides). [51, 52]

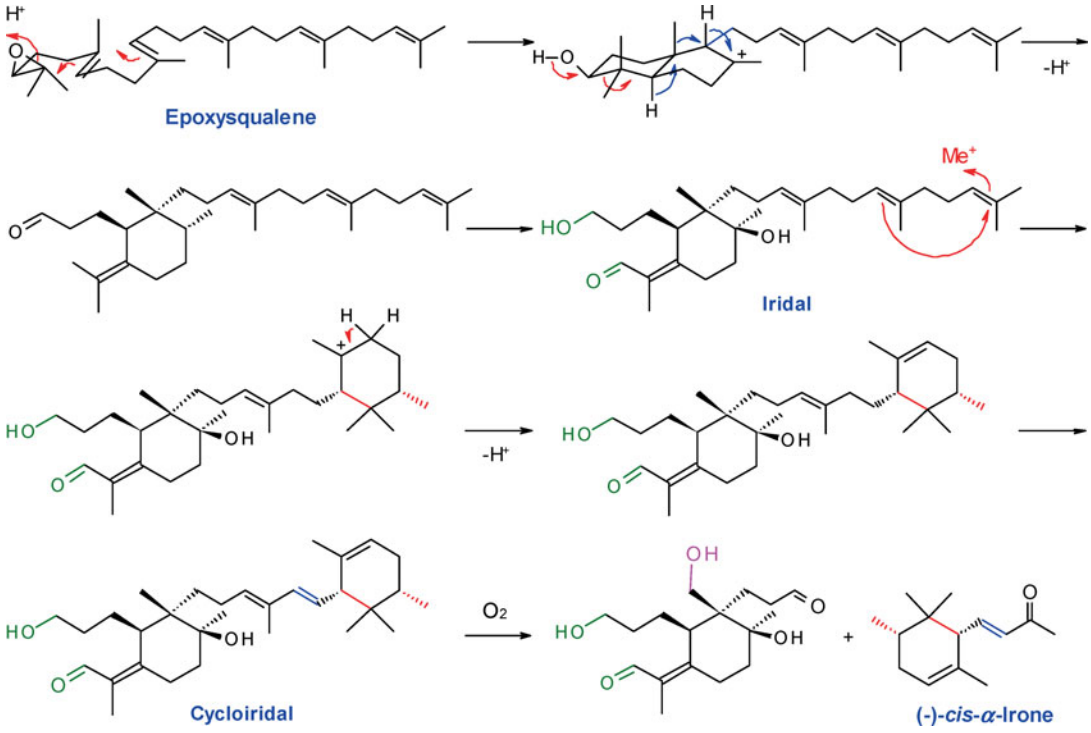
Ionones are metabolites of the corresponding carotenoids. As long ago as 1910, Richard Willstätter [53] observed the oxidation of carotene. It could be shown experimentally that carotene reacted photochemically with oxygen in the absence of a sensitiser to produce β-ionone and other oxidation products. [54] Also, thermolysis of β-carotene produces β-ionone in significant amounts. [55]



Later it has been proven that β-ionone could also be produced enzymatically from carotene. [56] For example, a portion of carotene in green tea leaves was transformed during fermentation (to produce black tea) into β-ionone.

The irones are oxidative degradation products of cycloiridal, which is derived itself from squalene. Key steps in the biosynthesis of cycloiridal are an enantioselective epoxidation for the construction of one ring, and a stereoselective methylation for the other ring system.

The in comparison to steroids atypical epoxysqualene cyclisation, followed by a series of sequential rearrangements within the A,B-ring system, produces first the monocyclic iridal. Addition of a methyl group from *S*-adenosylmethionine at the terminal double bond of the side-chain initiates the second cyclisation. Cycloiridal results from dehydrogenation of the side-chain. Oxidative cleavage of the central double bond finally produces the desired scent molecule. [57]

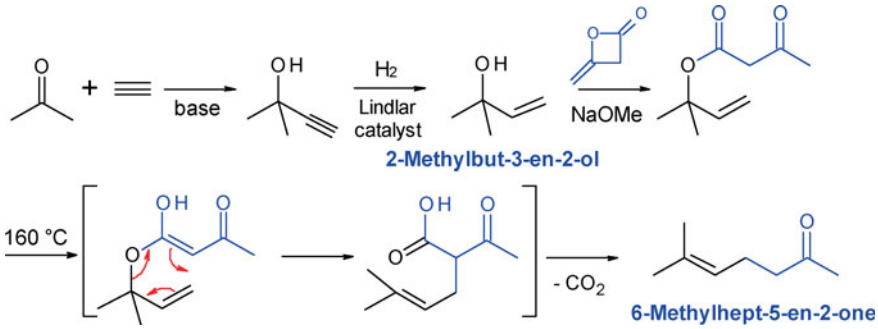


3.2.3 Industrial Syntheses

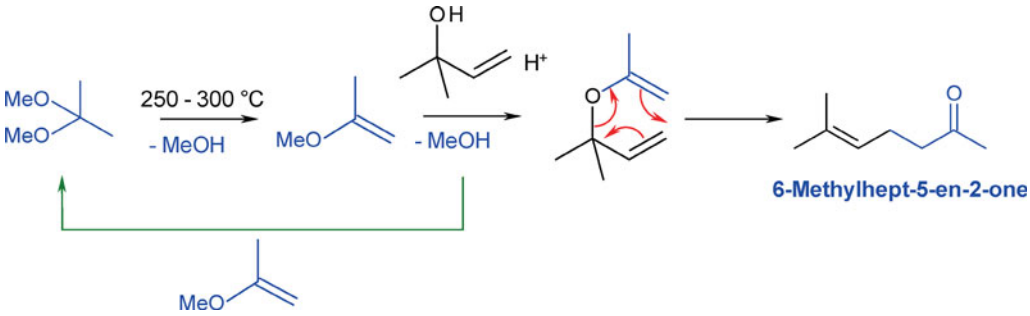
Ionones

The industrial syntheses of ionones have beginnings, which are identical to those involved in the production of carotenoids, and in fact, the two processes have been developed in parallel. The tonnages are correspondingly large. The most important producers are Hoffmann-la-Roche (the Vitamin Division of which now belongs to DSM), BASF, and Rhône-Poulenc (now Rhodia, respectively Adisseo). The following description uses the names of the firms at the time, when these processes were being developed. Each firm had its syntheses based on its own starting material: Hoffmann-la-Roche on acetylene, BASF on isobutene and Rhône-Poulenc on isoprene. All of the syntheses had the same initial target: 6-methylhept-5-en-2-one.

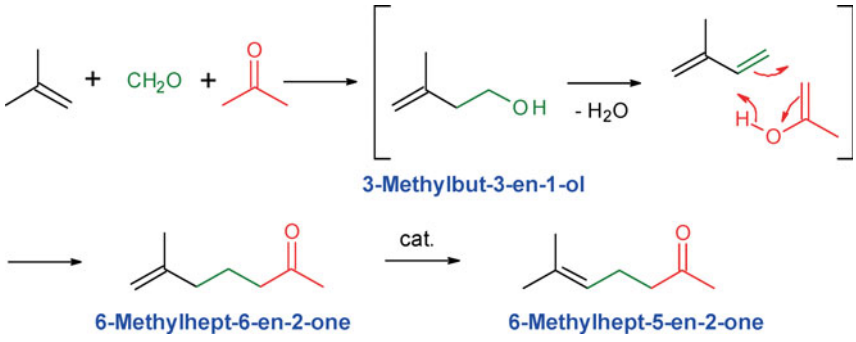
According to a Hoffmann-la-Roche patent procedure, acetylene is added to acetone in the presence of a base. Reduction with a Lindlar catalyst gives 2-methylbut-3-en-2-ol. Treatment of this with diketene and sodium methoxide renders an isolable acetoacetate ester, which undergoes at 160 °C a Carroll reaction [58] (a Claisen rearrangement with loss of carbon dioxide) to yield the desired intermediate product.



The reaction, which is in fact practised industrially, is the Saucy-Marbet reaction, where 2- methoxypropene, obtained by thermolysis of 2,2-dimethoxypropane, is converted into 2-methylbut-3-en-2-ol. The intermediate, which is not isolated, undergoes a sigmatropic rearrangement (Claisen rearrangement) to give 6-methylhept-5-en-2-one.

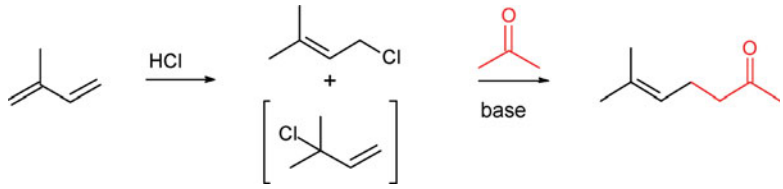


According to a procedure claimed by BASF, isobutene, formaldeyde and acetone are transformed in a continuous process at 290 °C under pressure, where a Prins reaction leads to 3-methylbut-3-en-1-ol. This in turn undergoes an ene-like reaction to give 6-methylhept-6-en-2-one, the terminal double bond of which is isomerised in a subsequent step.



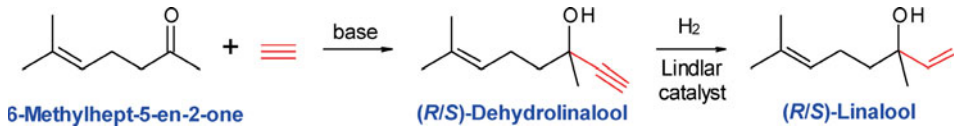
This process is however not economic and was therefore abandoned in favour of the procedure leading directly to citral (a down-stream product of 6-methylhept-6-en-2-one) (see next page).

A procedure, which was worked on by Rhône-Poulenc, involves the 1,4-addition of HCl to isoprene in a two-phase system. In presence of potassium carbonate or amines, the chloro-compound reacts with acetone to give the desired intermediate product.

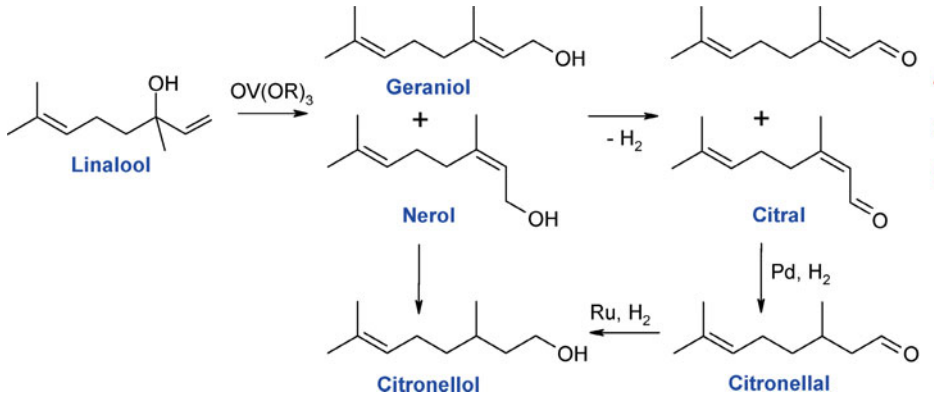


However, not only because of the unselective nature of the HCl addition, but also because of the (stoichiometric) quantity of salt produced, this procedure is no longer attractive to have nowadays a chance of industrial realisation.

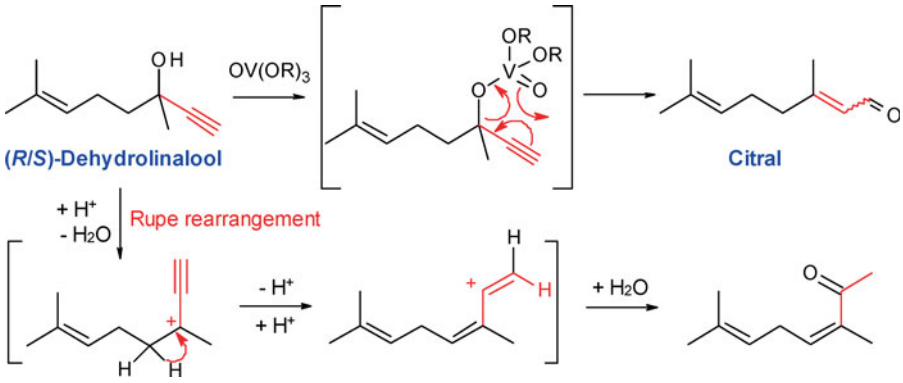
The annual world production of methylheptenone amounts to around 20,000 tonnes. It is the most important starting material for manufacturing linalool from petrochemical sources. The synthesis formally constitutes a repetition of the Hoffmann-la-Roche method for the preparation of methylheptenone.



The vanadate-catalysed rearrangement of linalool produces a mixture of geraniol and nerol. Upon dehydrogenation over a copper catalyst, this gives citral, as the thermodynamic (4 : 1)-mixture of geranial and neral. Partial hydrogenation of geraniol and nerol gives citronellol. Citral, in turn, can undergo selective reduction of the enone double bond to give citronellal, and this may then be reduced further to citronellol.



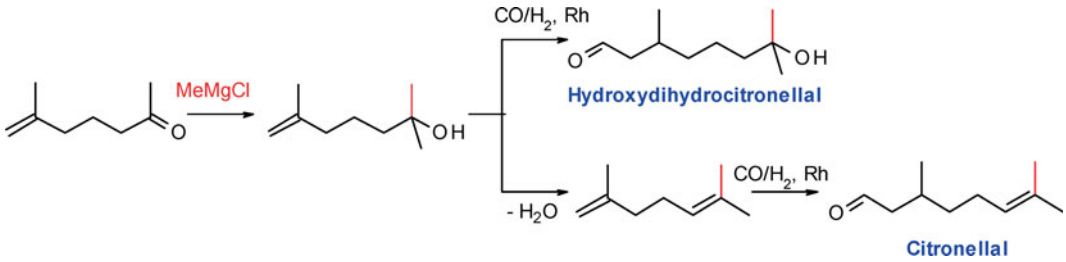
(*R/S*)-Dehydrolinalool may also be converted directly into citral by a vanadate-catalysed rearrangement (formally a Meyer-Schuster reaction), although, because of the concurrent Rupe rearrangement [59], this route has never reached industrial maturity.



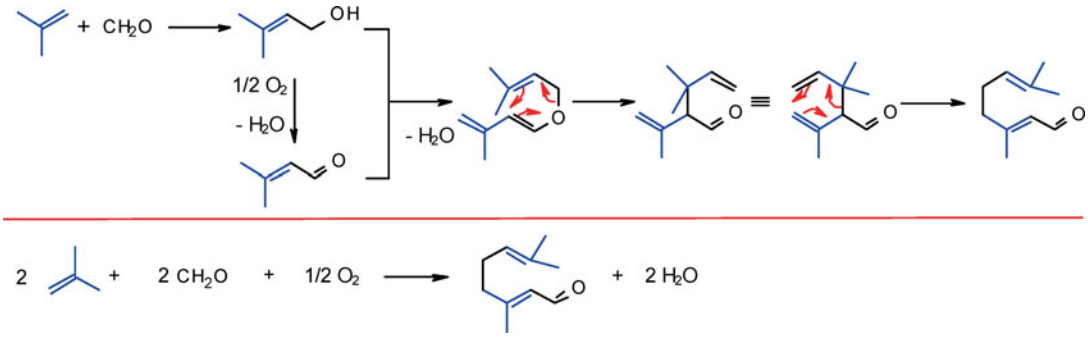
Excursus: Hydroxydihydrocitronellal

The scent from lily of the valley (*Convallaria majalis*) is, in chemical terms, (*R*)-hydroxydihydrocitronellal. This compound was introduced to the market already in 1908 under the label *Cyclosia Base*® by Firmenich. Java-citronellal served as the starting material.

BASF has developed an original procedure for the preparation of racemic hydroxydihydrocitronellal, which may also be used to produce citronellal. The starting material is 6-methylhept-6-en-2-one (see above), which is reacted with methylmagnesium chloride. The desired products are obtained by hydroformylation, either directly or after elimination of water. [60, 61] The stereoselective synthesis of (*R*)-hydroxydihydrocitronellal is described in the section on menthol (section 3.4).



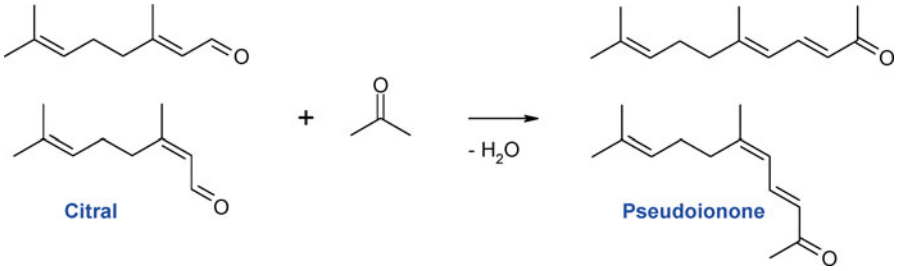
BASF carries out a synthesis of citral which requires, apart from isobutene and formaldehyde, only air for the oxidation. The only by-product is water. With the domino Claisen-Cope rearrangement one can obtain a value-added product in only a single step.

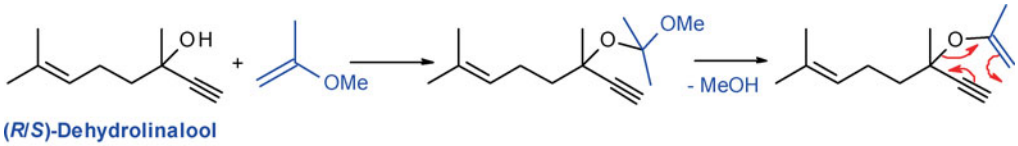


3.17 *BASF's citral plant has an annual production capacity of 40,000 tonnes.*

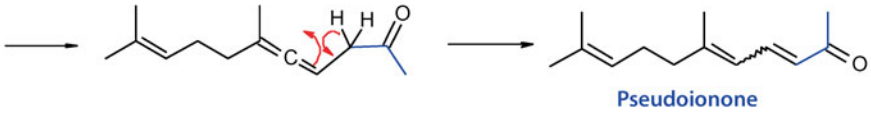
Citral is a central C10-building block for the synthesis of a whole host of products, not only for the synthesis of ionone, but also for carotenoids, and is therefore produced on a large scale (Fig. 3.17). Thanks to its strong lemon smell, citral serves also as the citrus scent in perfumery.

All industrial syntheses of ionones proceed by way of pseudoionones. These may be obtained either by the still employed Tiemann synthesis, *i.e.* aldol condensation of citral with acetone, or by the Saucy-Marbet reaction starting from dehydrolinalool.

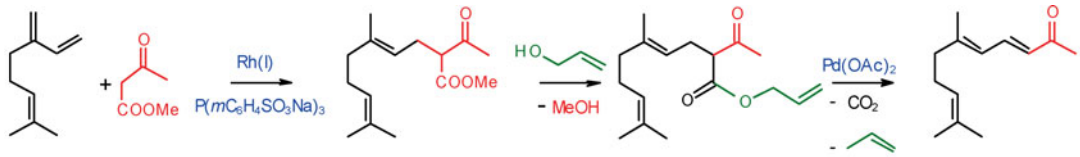


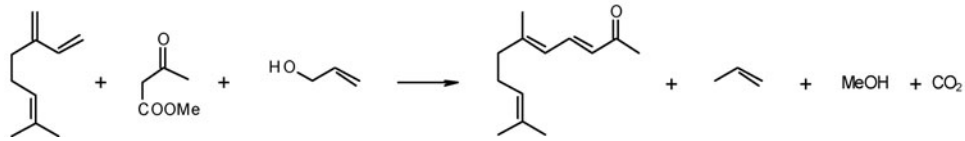


(*R*/*S*)-Dehydrolinalool

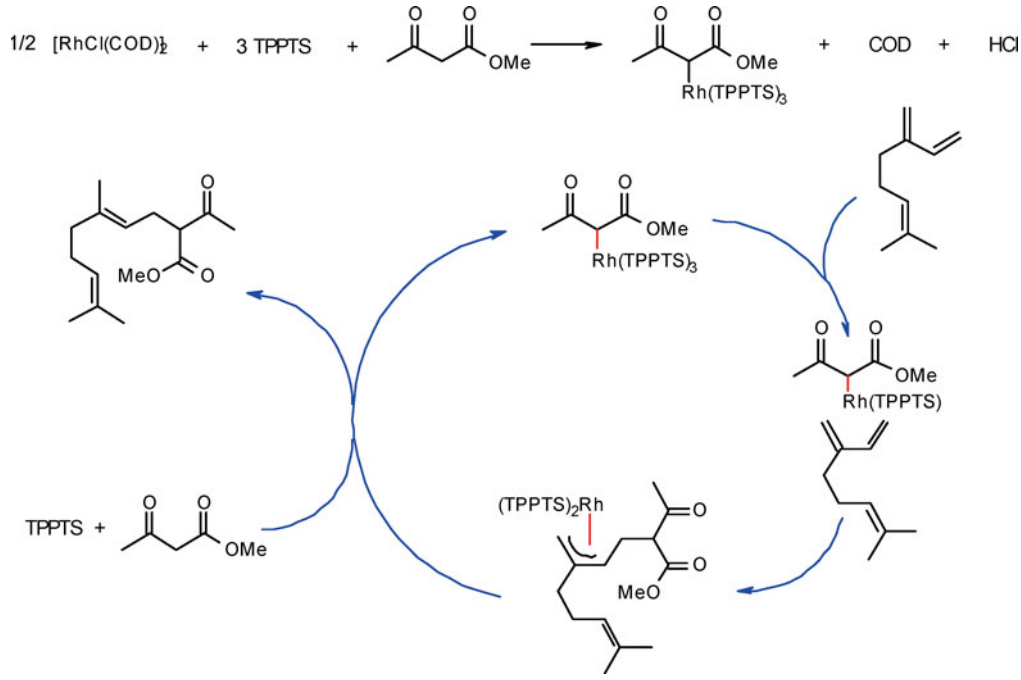


Rhône-Poulenc has developed a route to pseudoionone along a three-stage synthesis based on myrcene, which itself is obtainable in one step from isoprene (*cf.* section 3.4 Menthol). Myrcene is treated with methyl acetoacetate in presence of a rhodium catalyst: thereby the terminal double bond is isomerised into the chain. After transesterification with allyl alcohol, there follows a palladium-catalysed decarboxylation. All by-products are readily volatile. [62]

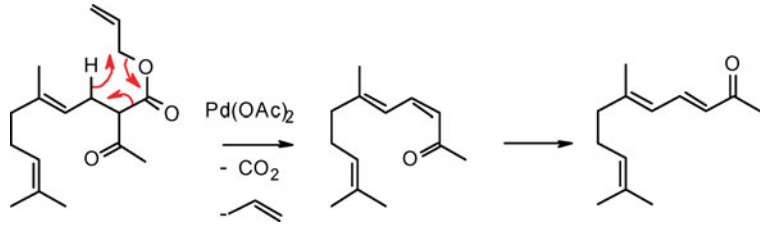




For the rhodium catalysis the following mechanism is suggested:

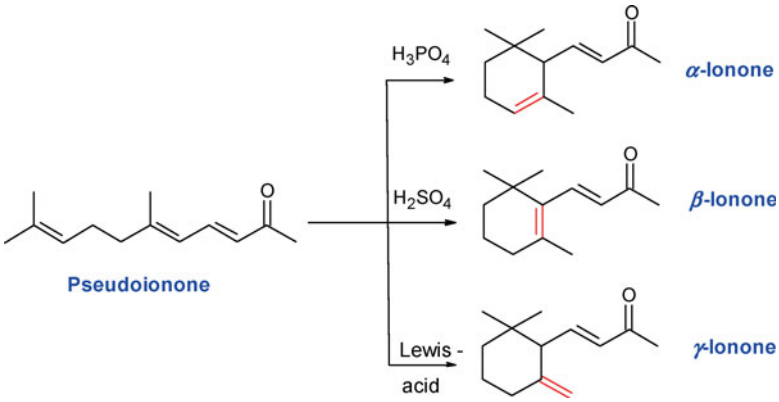


The palladium-catalysed double bond migration leads finally to elimination of propene and carbon dioxide, whereby the conjugated system is formed.



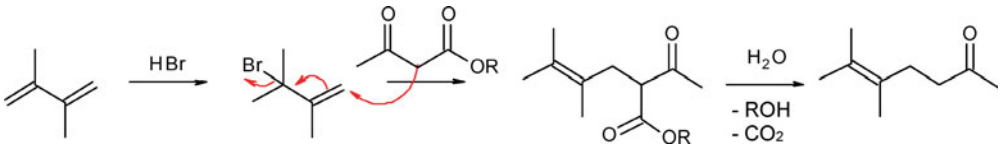
However, the process is uneconomic, not least because of the precious metal catalysts required; thus, it has not been executed industrially.

Finally, the various ionones may be obtained by Brønsted- or Lewis-acid-catalysed cyclisations. Phosphoric acid gives preferentially α-ionone, sulfuric acid β-ionone, and Lewis acids γ-ionone. [63]

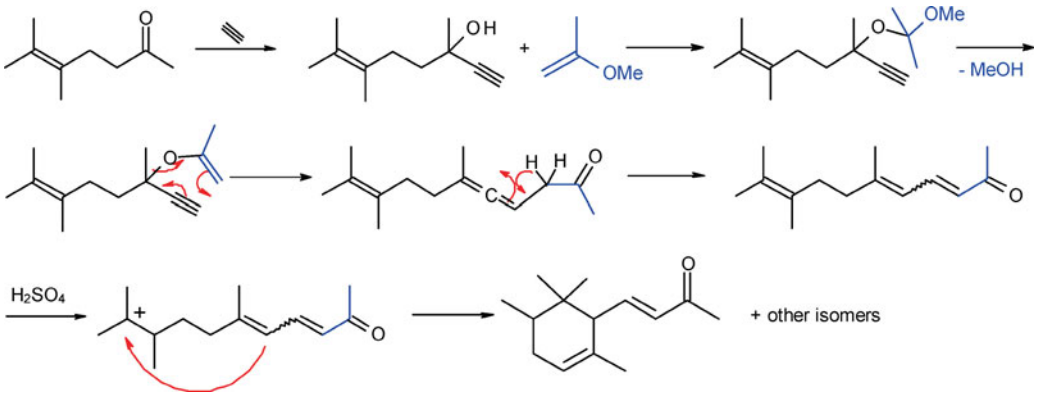


Irone

For the synthesis of diastereomeric irones, in principle the same synthetic strategy is pursued as for ionones. Hydrogen bromide is added to dimethylbutadiene, and the product is substituted at the allylic position with an alkyl acetoacetate. During the subsequent hydrolysis, the ketoester undergoes decarboxylation.



The following synthetic sequence corresponds to the ionone syntheses. Depending on the cyclisation conditions, isomeric double bonds and diastereomers are formed. The racemic *cis*- and *trans*-α-irone, for example, are commercially available from Givaudan under the name *Irone alpha*®.

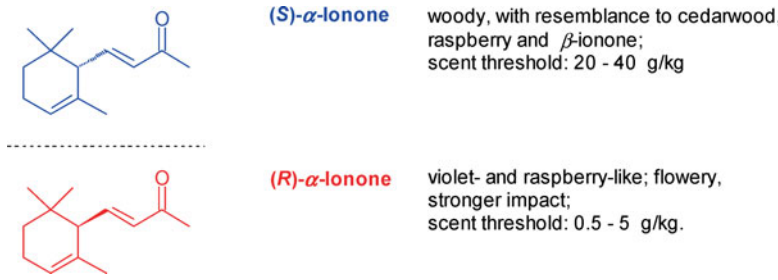


3.2.4 Enantiomerically Pure Products

Enantiomerically Pure α-Ionone

Whereas racemic α-ionone is accessible in large amounts, there are only a few methods for the targeted preparation of the individual enantiomers. There is

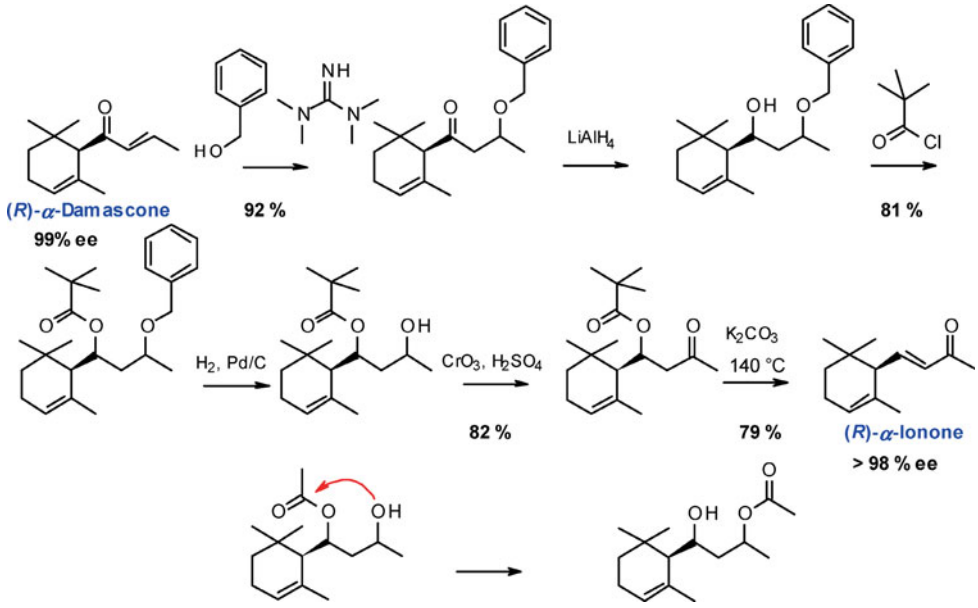
however significant practical interest in these compounds, for example as building blocks for the preparation of (+)-(*R*)-β-carotene, or as components of perfumes. Natural α-ionone from violets is the (*R*)-enantiomer; its olfactory characteristics are clearly distinguished from those of its (*S*)-antipode (Fig. 3.18). [64]:



3.18 *The enantiomers of α-ionone have distinctly different scents.*

R. B. Woodward was the first to separate the enantiomers of α-ionone. He converted racemic α-ionone into its menthylhydrazone and separated the diastereomers by fractional crystallisation. 20 recrystallisations were necessary in order to obtain the (*R*)-enantiomer in 1 % yield. [65]

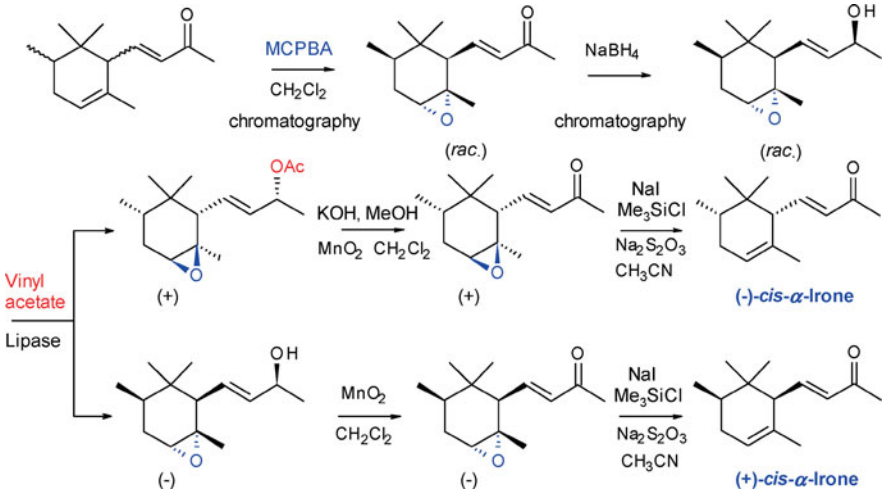
The best approach at present starts from (*R*)-α-damascone, and uses an enone transposition developed especially for ionone. [64] In the first step, benzyl alcohol is added (in effect a 1,4-addition) to the enone. After reduction with lithium aluminium hydride, the alcohol is esterified with pivaloyl chloride and the benzyl group reductively removed. If the esterificaton is carried out with acetic anhydride, surprisingly a 9 : 1 mixture of 7- and 9-acetoxy isomers is obtained; this presumably results from a 1,3-transposition of the acetyl group. Jones oxidation and base-catalysed thermal elimination give (*R*)-α-ionone in enantiomerical pure form and with an over-all yield of 48 %.



The enantiomerically pure α-ionones are also available by enzymatic resolution of ionols, as described below for α-irones. [66, 67]

Enantiomerically Pure α-Irone

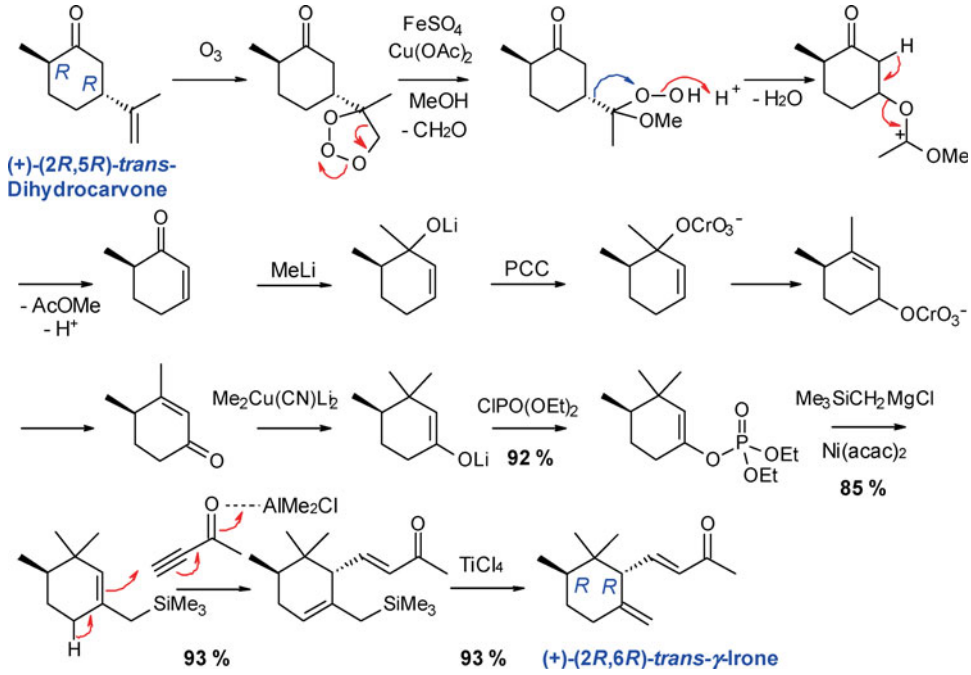
Like the α-ionones, the enantiomers of α-irone are distinguishable by their olfactory properties. The commercially available racemic mixture does not fulfil the demands of fine perfumery. Enantiomerically pure α-irones may be obtained by means of a twice-repeated chromatographic separation of the epoxides starting from *Irone alpha*®, and enzymatic kinetic resolution. The alcohols are reoxidised with manganese dioxide and the epoxides reconverted into the alkenes by sodium iodide and chlorotrimethylsilane under mild conditions.



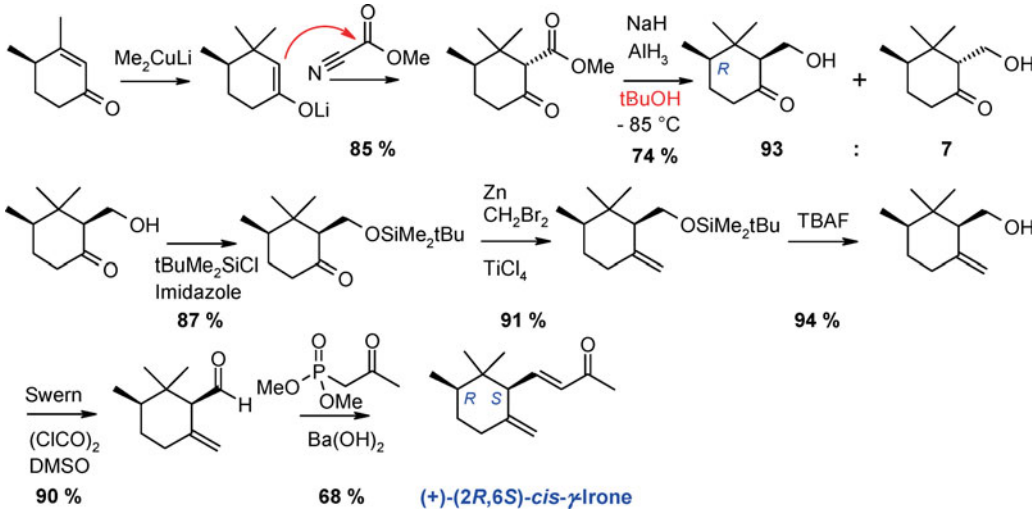
Both (+)- and (–)-*trans*-α-irone may be obtained by the same method. [49, 68, 69]

Enantiomerically Pure γ-Irone

One of the most modern syntheses which leads to α-irone, is owed to Honoré Monti. [70] Starting from (+)-(2*R*,5*R*)-*trans*-dihydrocarvone, one may obtain (+)-(2*R*,6*R*)-*trans*-γ-irone in 9 stages. After degradation of the side-chain by ozonolysis, with the introduction of a double bond, the resulting enantiomerically pure cyclohexenone is reacted with methyllithium and then directly with pyridinium chlorochromate. Isomerisation of the chromic ester (allylic rearrangement) leads to the sterically less demanding allyl alcohol, which is then oxidised (oxidative 1,3-transposition of the hydroxyl function). [71] A Michael-like 1,4-addition, using a cuprate, introduces a second methyl group, and the resulting alkoxide is esterified with diethyl chlorophosphate. Finally, the enol phosphate reacts with Grignard reagents in presence of nickel salts in the manner of a cross-coupling reaction with preservation of the stereochemistry. [72, 73] Key step in the synthesis is the diastereoselective ene-reaction catalysed by dimethylaluminium chloride.

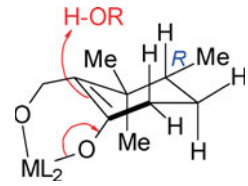


Monti has also developed an elegant synthesis of (+)-(2*R*,6*S*)-*cis*-γ-irone, where he used an intermediate of the previous reaction sequence. [74] In this case, the key step is a diastereoselective protonation.



Starting from 3,4-dimethylcyclohex-2-enone, a methyl group and the ester function are introduced in a single step. Prior to the reduction of the ester with aluminium hydride, the keto-function is protected as an enolate by deprotonation with sodium hydride. The aluminate thus produced is protonated diastereose-

lectively, with *t*-butanol being the acid of choice. Quantum mechanical MP3-calculations show in accordance with the experimental findings, that the lowest-energy conformation, in which all the substituents are equatorial, is the one which is protonated. The protonation takes place on the side, which is sterically less hindered.



After protection of the alcohol function, the carbonyl group is converted into a methylene group by means of the Tebbe reagent, followed by a Swern oxidation and a Horner-Wadsworth-Emmons reaction.

3.2.5 Importance for the Fragrance Industry

Iris oil is a constituent of, for example, *Chanel No. 19*® (1971) and *Silence*® (Jacomo, 1979) and *So pretty*® (Cartier,1995). α- and β-ionones, with a few homologues, are among the most regular constituents of modern perfumes. However, the use of irones is on the margin in terms of cost: α-ionone costs *ca.* 30 Euro/kg, and α-irone 20 times as much. β-Ionone is an important synthetic building block for Vitamin A, and as such the cheapest of the violet scents.

Summary in Bullet Points

- The ionones are metabolites of the corresponding carotenoids.
- The synthetic routes from citral (BASF), dehydrolinalool (Hoffmann-la-Roche) or myrcene (Rhône-Poulenc) are particularly elegant.
- β-Ionone is obtainable in large quantities, because it also serves as an important building block for carotenoid chemistry and for Vitamin A synthesis.
- On the other hand, it is a fact that enantiomerically pure α-ionone and the structurally related irones are definite rarities in fine perfumery.

3.3 Jasmonoids

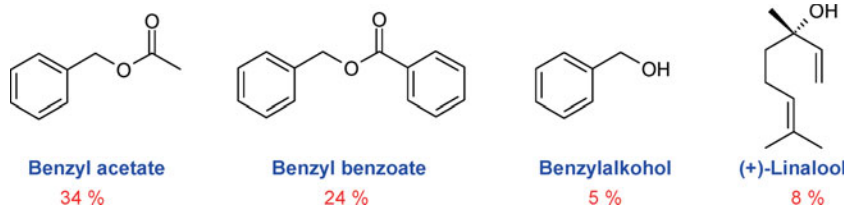
The so called Spanish or Catalonian jasmine originates from the lower valleys of the Himalayas. It consists mainly of *Jasminum grandiflorum* L., which is grafted on to the wild *Jasminum officinale* L. in order to make it frost-resistant (Fig. 3.19). The Moors brought jasmine to Spain. From there it spread during the 16th and 17th centuries over the entire Mediterranean region. Jasmine was cultivated in Grasse from 1860 onwards on a grand scale for the production of the scent from its blossom. [75]

It is not possible to obtain the essential oil directly from jasmine flowers. The scented material is isolated by *enfleurage* and subsequent extraction with hexane, benzene or ethanol. Jasmine *absolue* is a red-brown liquid, which darkens further on storage and possesses a delicate jasmine odour. From a tonne of hand-picked petals, one may obtain 1–3 kilograms of jasmine *absolue*. The most important sources at the present time are Egypt, Italy, Morocco, India and

3.19 *Jasmine (Jasminum officinale L.) is native to the Caucasus, northern Iran, Afghanistan, the Himalayas and western China. The genus name is derived from the Persian Yasameen ("gift from God").*

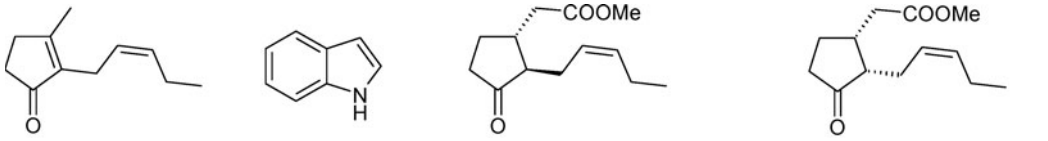
China. The annual production amounts to approximately 10 tonnes. Jasmine *absolue* is nowadays one of the most valuable flower scents in fine perfumery. [76]

Being of high value, jasmine scent excited the interest of chemists at a very early stage. Between 1899 and 1904, Albert Hesse and Friedrich Müller identified more than 70 % by mass of the scented components, including the floral-scented benzyl alcohol, its acetate and benzoate esters, and the lavender perfume, linalool. The characteristic scent of jasmine, however, could not be completely explained (Fig. 3.20).



3.20 *Principal constituents of jasmine scent.*

In 1933, Leopold Ružička and M. Pfeiffer in Zürich and W. Treff and H. Werner in Leipzig got closer to the objective in determining the structure of jasmone. Also of great importance for the scent profile is indole, the odour of which resembles that of human faeces. However, at high dilution it develops a powerful floral component with unusually interesting scent properties. In 1962, Edouard Demole and Edgar Lederer achieved the critical isolation and structure determination of a third characteristically scented component of jasmine *absolue*, namely methyl (*Z*)-(3*R*,7*R*)-jasmonate and methyl (*Z*)-(3*R*,7*S*)-jasmonate (Fig. 3.21). [77] Today we know however that jasmine *absolue* contains more than 100 further compounds.



(*Z*)-Jasmone 3 %

Indole 2,5 %

Methyl (*Z*)-(3*R*,7*R*)-jasmonate 1,7 %

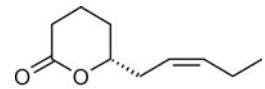
Methyl (*Z*)-(3*R*,7*S*)-jasmonate 0,2 %

3.21 *Characteristic components of jasmine absolue.*

3.3.1 Occurrence in Nature

Jasmonoids are widely distributed in Nature. (*Z*)-Jasmone is found, for example, in the oils of orange blossom, narcissus, bergamot, lavender and peppermint, and as well in Ceylon tea (*Thea chinensis*). Jasmone has also been detected in the pheromone of the butterfly *Amauris ochlea*.

Methyl (*Z*)-(–)-jasmonate has been identified as a component in the scent of Tunisian rosemary, in the stems and leaves of wormwood (*Artemisia absinthium* L.), and in the *absolue* of tuberose and gardenia flowers. Methyl (*Z*)-(–)-(3*R*,7*S*)-jasmonate has been discovered in the oil of lemon peel and in the pheromone of the oriental fruit moth (*Grapholitha molesta* B.) The culture filtrate of the fungus *Lasiodiplodia theobromae* contains the free acid. In the oils from the blooms of osmanthus, gardenia and mimosa, (–)-jasmolactone has been found, which is also an important component of tea aroma. [75] On the other hand, the oil from tuberose flowers contains (+)-jasmolactone.



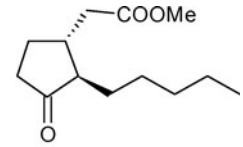
(-)-(*R*)-Jasmolactone

3.3.2 Use in Perfumery

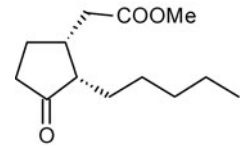
"No perfume without jasmine" indicates the extraordinary significance of jasmine scent in fine perfumery. Jasmine *absolue* goes very well with other floral scents, and confers on them a roundedness and elegance. Due to the comparatively straightforward synthetic access, synthetic jasmine scents are used in perfumes and especially in soaps. By skilful composition of synthetic scented compounds, it is now possible to faithfully imitate the scent of natural jasmine.

In this context, *Hedione*®, a mixture of methyl *cis*- and *trans*- dihydrojasmonates, deserves attention, since it imparts the perfume with a floral, jasmine-like, transparent but nevertheless warm character. This fresh floral note embodies the creation of a new category of scents, that of "Eaux fraîches" ("fresh waters").

"Eaux fraîches" form the basis of various perfumes with a variable proportion of *Hedione*®, ranging from 2 % in Christian Dior's *Eau Sauvage*® (1966) to 63 % in *Odeur 53*® by Comme des Garçons (1998). [77, 78]

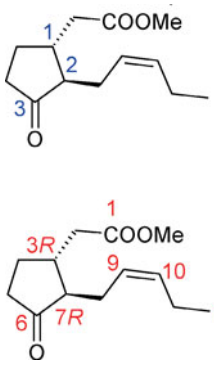


(+/-)-Methyl dihydrojasmonate



(+/-)-Methyl dihydroepijasmonate

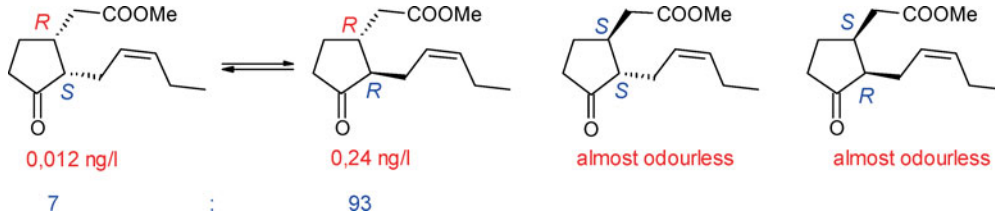
3.3.3 Nomenclature and Structure-Activity Relationships



In older literature, a cyclopentane-based nomenclature was often used, where, the side-chains were located at C-1 and C-2, and the carbonyl carbon atom was assigned the number 3. From time to time, the absolute configuration at C-1 was wrongly denoted as (*S*). Newer literature treats jasmonates as derivatives of fatty acids, and numbers the carbon atoms accordingly. The correct absolute configuration of the most stable methyl jasmonate is (3*R*,7*R*). [79]

The primary product of biosynthesis is the (3*R*,7*S*)-jasmonate. The thermodynamic equilibrium lies at 7 : 93 in favour of the *trans* isomers, which results very easily from keto-enol tautomerism. It is not clear whether epimerisation takes already place in the plant or first occurs during the extraction process. The four diastereomers of methyl (*Z*)-jasmonate can be separated by preparative HPLC.

Remarkably, only the (3*R*)-diastereomers are scented: they are described by the perfumer as floral, jasmine-like, and mildly fruity. Both of the other diastereomers are almost odourless. The scent thresholds of the (3*R*,7*S*)- and (3*R*,7*R*) diastereomers differ from each other by a factor of approximately 20 (Fig. 3.22). [80]

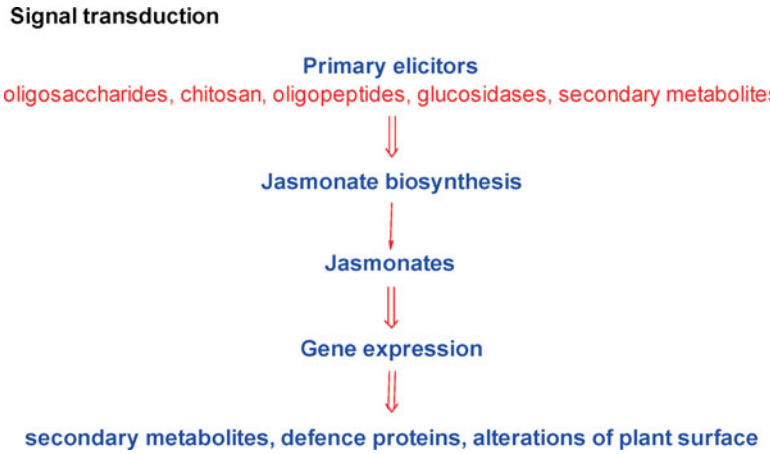


3.22 *The diastereomers of methyl (Z)-jasmonate are distinguished by their scent.*

3.3.4 Biological Properties

The normal concentration of jasmonates in plants lies at around 1–50 ng/g of drained weight. In the case of damage or a microbial infection (the presence of elicitors) the concentration in the entire plant may rise by a factor of a thousand. For tomato and tobacco plants it could be shown that damage causes the initiation of jasmonate biosynthesis and that gene expression of protease inhibitors and secondary metabolites is triggered (Fig. 3.23). If undamaged plants are treated only with jasmonate, one finds the same gene expression pattern, which means that jasmonates are plant hormones signalling damage. [81] Similar results were found with soya beans and barley. Tobacco accumulates an increased amount of nicotine in the leaves. Maize (known in some English-speaking countries as corn) produces a higher proportion of volatile compounds for protection against herbivorous pests.

If maize is for instance infested by caterpillars of the butterfly *Spodoptera exigua,* then the volicitin (*N*-(17-hydroxylinolenyl)-(*L*)-glutamine) from its



3.23 *Thus, in respect of signal transduction, jasmonates fulfil a function in plants similar to that of the structurally related prostaglandins in mammals. Furthermore, jasmonates are also involved in the aging process of plants. For example, they inhibit growth, promote the curling of tendrils of climbing plants, and induce tuber formation in potatoes.*

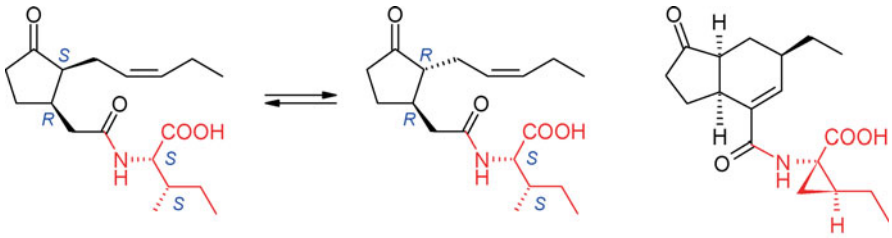
salivary secretion triggers the release of a bouquet of terpenoid compounds and indole, which in turn attracts a predator of this caterpillar, the wasp *Cotesia marginiventris*.

The damage to leaf surfaces in tomato plants leads to the biosynthesis of protease inhibitors, which interfere with the digestive system of the infesting insects and restrict the availability of essential amino acids, eventually retarding growth and development of the pests.

Most of the experiments were carried out with racemic methyl jasmonate, so that for a long time it was not clear which enantiomer has the higher biological activity. In addition, hydrolysis and epimerisation could not be excluded. [79]

Dosage/response experiments then showed that the biological activity resides mainly with the primary product, namely the (3*R*,7*S*)-jasmonic acid to a lesser extent with its methyl ester. Jasmonic acid does not bind as the free acid, but as a conjugate with isoleucine to the same receptor as coronatine, a phytotoxin produced by *Pseudomonas syringae*. In this conjugate, epimerisation at C-7, which is associated with a dramatic loss in activity, is used by the plants to regulate hormone activity. [82]

Interestingly, methyl jasmonate also possesses cytotoxic properties towards a range of human breast, prostate and cervical cancer cell lines. [82]



3.3.5 Biosynthesis

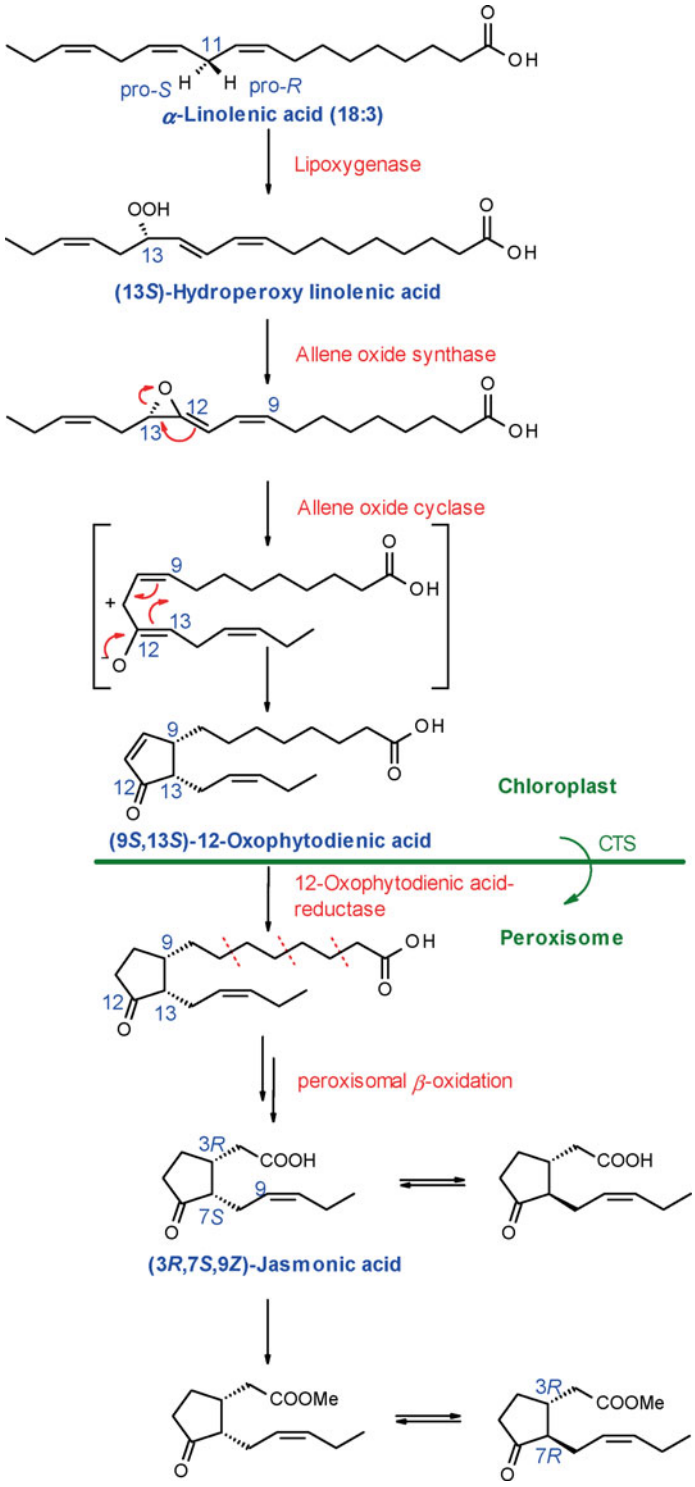
The biosynthesis of jasmonoids in plants and fungi shows striking similarity to that of prostaglandins and leukotrienes in mammals. In response to stress or injury, a lipase releases α-linolenic acid (18:3) from the cell membrane of chloroplasts, and thereby initiates jasmonate biosynthesis. The first step is the production of a hydroperoxide with the aid of a lipoxygenase (LOX). This enzyme contains an iron atom in its active site, which stereospecifically abstracts the (11-pro-*S*)-hydrogen atom. In case of the isozyme LOX-1, for example from soya beans, the reaction with oxygen then gives stereo- and regio-specifically the (13*S*)-hydroperoxide. In the case of isozymes LOX-2 and LOX-3, these produce also in approximately equal amounts the 9-hydroperoxide from the pentadienyl radical.

Conversion of the (13*S*)-hydroperoxide into the unstable allene oxide is catalysed by allene oxide synthase. This is a cytochrome P450-type enzyme, although it does not act as an oxygenase. The allene oxide may be isolated, but is spontaneously hydrolysed in the presence of water to α- and β-ketols, or else instantly decomposes into racemic 12-oxophytodienic acid. In the presence of allene oxide cyclase, an enzyme-controlled, conrotatory, electrocyclic reaction takes place, which leads stereoselectively to the *cis*-configured (9*S*,13*S*)-12-oxophytodienic acid. [83] In view of the high reactivity of the allene oxide, the principal function of the allene oxide cyclase is not to reduce the activation energy, but to control the stereochemical course of the rearrangement by steric interactions within a rigid hydrophobic barrel-like binding pocket.

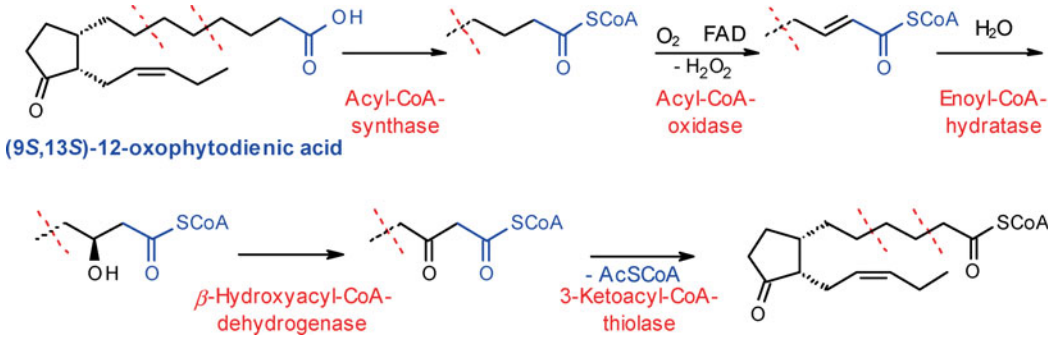
The remainder of the biosynthesis takes place in the peroxisomes. The substrate is imported by the cassette-transporter comatose (CTS). The hydrogenation of the endocyclic double bond is carried out by 12-oxophytodienic acid reductase; this enzyme belongs to a small group of flavin-dependent oxidoreductases. In accord with the reaction mechanism, which was suggested for the related Old Yellow Enzyme of yeast, two hydrogen bridges from histidine (His-186 and His-189 in the 12-oxophytodienic acid reductase from mouse-ear cress (*Arabidopsis thaliana*)) towards the carbonyl group polarise the double bond, so that a hydride of the reduced flavin cofactor can be transferred to C-10. The carbanion in the 11-position is then protonated by Tyr-191.

The hydrogenation is followed by the peroxisomal fatty acid degradation. For this, (9*S*,13*S*)-12-oxophytodienic acid is first activated by esterification with Coenzyme A, and then desaturated at the α-position by the acyl-CoA-oxidase. This process depends on FAD. Hydrogen peroxide is produced, which disproportionates into water and oxygen under the influence of the peroxisomal enzyme catalase. Both of the following steps are associated with a single enzyme: first addition of water to the double bond and then oxidation. In the last step, the peroxisomal thiolase cleaves off acetyl-CoA to give the di-nor-fatty acid Coenzyme A ester.

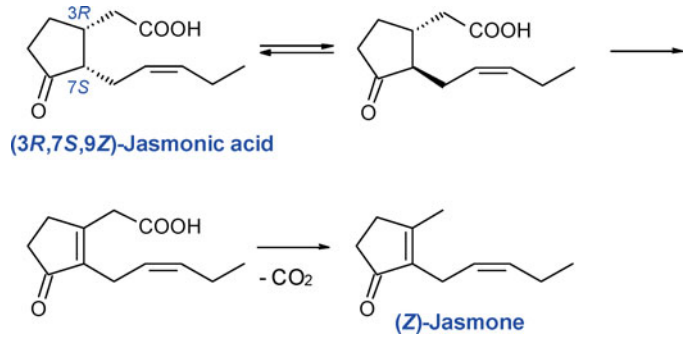
The overall reaction sequence can also be carried through, starting from hexadecatrienoic acid (16 : 3), which is two carbon atoms shorter. Here, the fatty


11
pro-S H
H pro-R
α-Linolenic acid (18:3)
Lipoxygenase
OOH
13
(13S)-Hydroperoxy linolenic acid
Allene oxide synthase
12
9
13
Allene oxide cyclase
9
13
12
9
12
13
(9S,13S)-12-Oxophytodienic acid
Chloroplast
CTS
12-Oxophytodienic acid-reductase
Peroxisome
peroxisomal β-oxidation
3R
COOH
9
7S
(3R,7S,9Z)-Jasmonic acid
COOMe
3R
7R

acid degradation results in the cleavage of only two acetyl-CoA units instead of three. [83] Finally follows a re-esterification to give the methyl ester and epimerisation to the *trans*-compound.



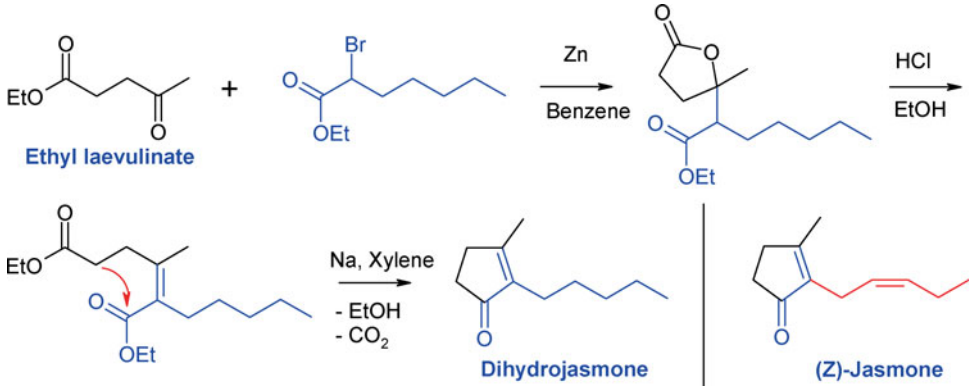
By means of deuterium-labelled jasmonate in Lima beans (*Phaseolus lunatus*), it could be demonstrated that an important biosynthetic pathway to deactivate the plant hormone is its transformation into the more volatile (*Z*)-jasmone. Therefore, first the corresponding enone is formed through oxidation, which then readily undergoes decarboxylation.



3.3.6 Chemical Syntheses

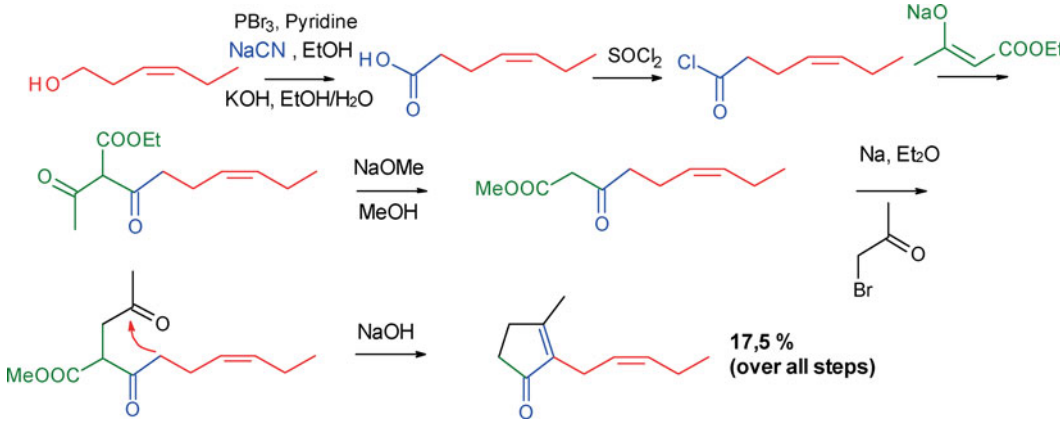
The first synthesis of a jasmonoid, namely dihydrojasmone, took place nine years before the structure of jasmone itself was established. This goal was achieved in 1924 by Hermann Staudinger and Leopold Ružička in the course of their work on pyrethroids. [84] They carried out a Reformatzky reaction between ethyl laevulinate and ethyl 2-bromoheptanoate, and obtained the desired product, although in poor yield, by a Dieckmann cyclisation, followed by hydrolysis and decarbethoxylation (see next page).

Once the structure of jasmone had been established, Treff and Werner were able to prepare this compound by the same method, starting from "leaf alcohol", (*Z*)-hex-3-en-1-ol. [85] The (*Z*)-configuration of the double bond was however not confirmed until 20 years later, through the work of Harper and Smith. [86]



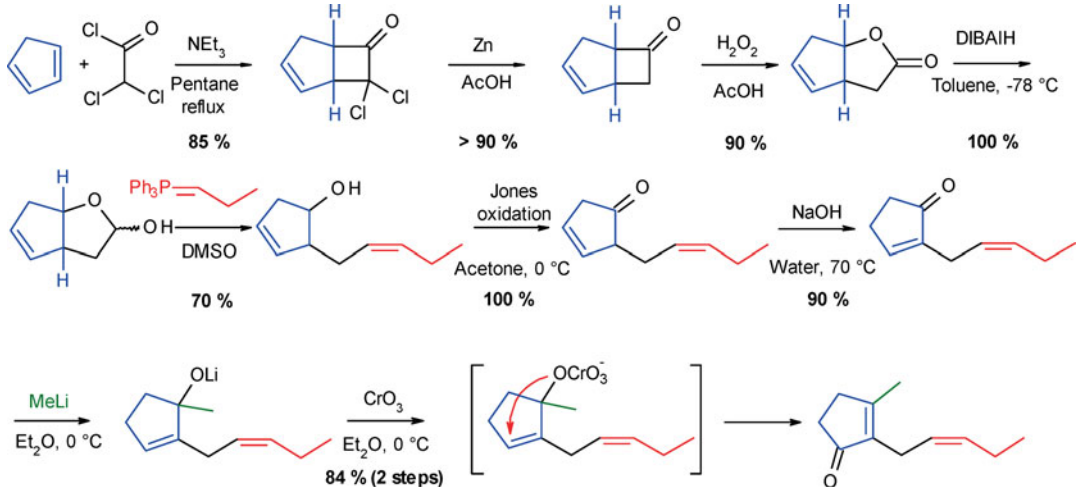
Leaf alcohol was first isolated from hornbeam leaves (*Carpinus betulus*), although it can be found in the leaves of many plants. Diluted, it smells of freshly cut grass and green leaves, and is thus used in perfumery as the embodiment of the green scents. It is accessible synthetically from but-1-yne and ethylene oxide. [87]

The first practical synthesis of the cyclopentenone was published in 1942 by Heinz Hunsdiecker. [88] In case of (*Z*)-jasmone, leaf alcohol, which was obtained from Japanese peppermint oil, served again as starting material. The central synthetic building block is a 1,4-diketone, which in aqueous alkali undergoes an intramolecular aldol condensation to close the five-membered ring; the desired product is then obtained by hydrolysis and decarboxylation.

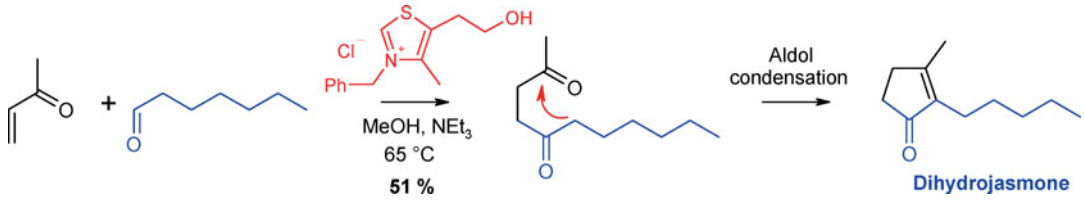


An attractive jasmone synthesis comes from Grieco. [89] A bicyclic ketone is obtained from the [2 + 2]-cycloaddition of dichloroketene to cyclopentadiene, followed by reduction with zinc. After Baeyer-Villiger oxidation, reduction to

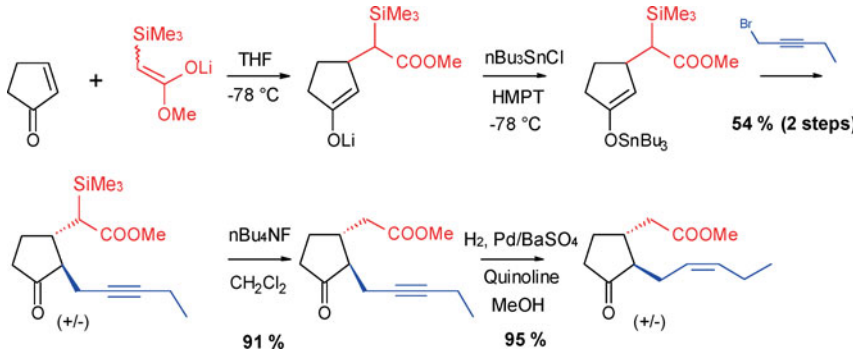
the lactol and a *cis*-selective Wittig reaction, the conjugated ketone results from Jones oxidation, followed by isomerisation of the double bond. Methyllithium adds directly to the keto-function; by oxidation with chromium trioxide and rearrangement of the chromium salt, one may obtain (*Z*)-jasmone in an overall yield of just under 40 %.



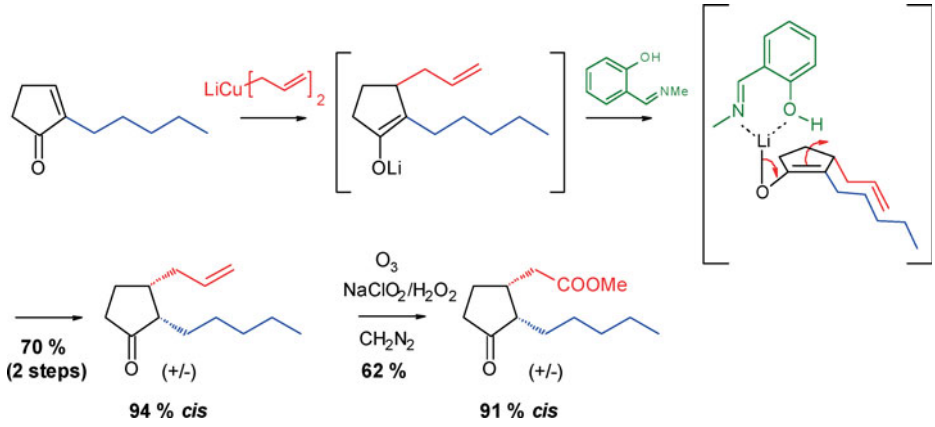
Dihydrojasmone is also much valued as a scented compound because of its floral and fruity character. A convenient synthetic route is provided by the Stetter reaction (a reaction of aldehydes with Michael acceptors in the presence of thiazolium salts, developed by Hermann Stetter (1917–1993)) of methyl vinyl ketone and heptanal [90], followed by an intramolecular aldol condensation.



The literature contains a whole host of syntheses of racemic methyl jasmonate, due to its significance for the fragrances industry. [91] As a representative example may serve the conjugate addition/alkylation to cyclopentenone, which formally corresponds to the Noyori three-component synthesis of prostaglandins (*cf.* section 5.6). The ester function is introduced first by means of a Michael reaction with an ester enolate; it is noteworthy that this lithium ester enolate adds very selectively in a 1,4-fashion, and not partially in the 1,2- (Peterson olefination) position, as could have been expected. The resulting lithium enolate is trapped by tributyltin chloride, and the stannyl enolate is then alkylated with 1-bromopent-2-yne. After removal of the trimethylsilyl group, a Lindlar reduction of the triple bond leads to racemic methyl jasmonate. [92]

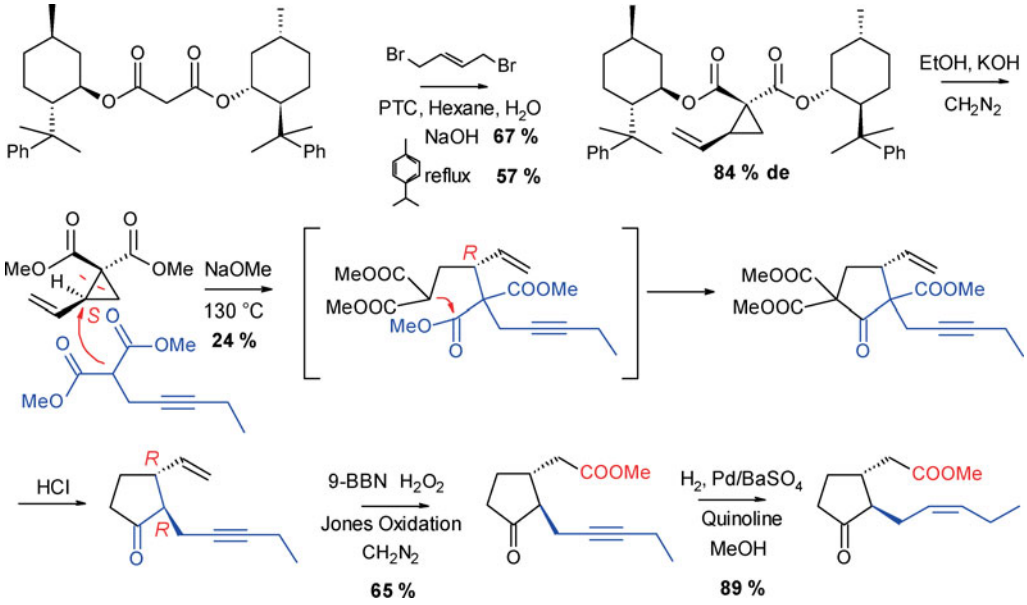


An interesting synthesis of almost pure methyl *cis*-dihydrojasmonate is provided by Ebert and Krause. [93] The key step is a diastereoselective protonation of an enolate, generated from 2-pentylcyclopent-2-enone and lithium diallylcuprate. Ozonolysis, oxidation and esterification give methyl dihydrojasmonate, which is 91 % *cis* configured.

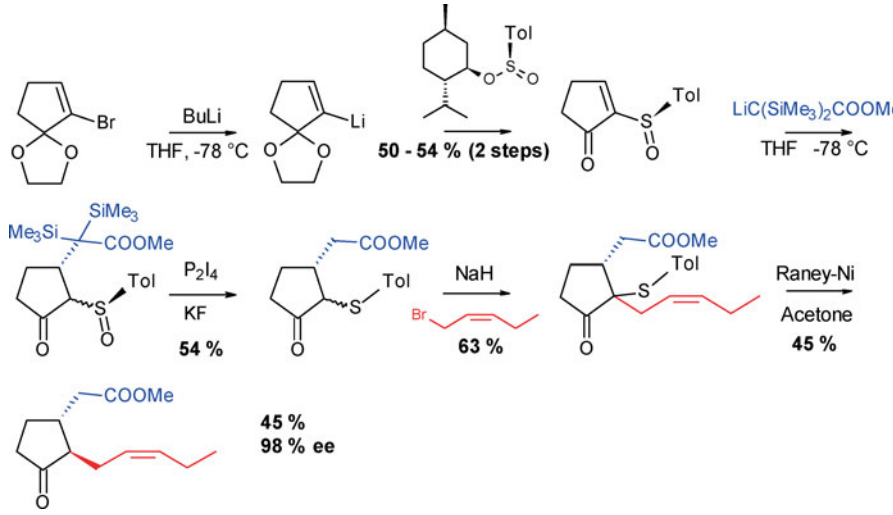


3.3.7 Stereoselective Syntheses

The first diastereoselective synthesis of methyl (3*R*,7*R*)-jasmonate is attributed to Gerhard Quinkert. [94] In the initial step, starting from bis-(8-phenylmenthyl) malonate, a vinylcyclopropane is constructed. After removal of the chiral auxiliary, the cyclopentanone is built up by means of a "domino" homo-Michael reaction / Dieckmann cyclisation. Attack of dimethyl pent-2-ynylmalonate leads to inversion at the stereogenic centre at the vinyl cyclopropane. After decarboxylation, the vinyl residue is transformed into an ester group and the triple bond hydrogenated with a Lindlar catalyst.

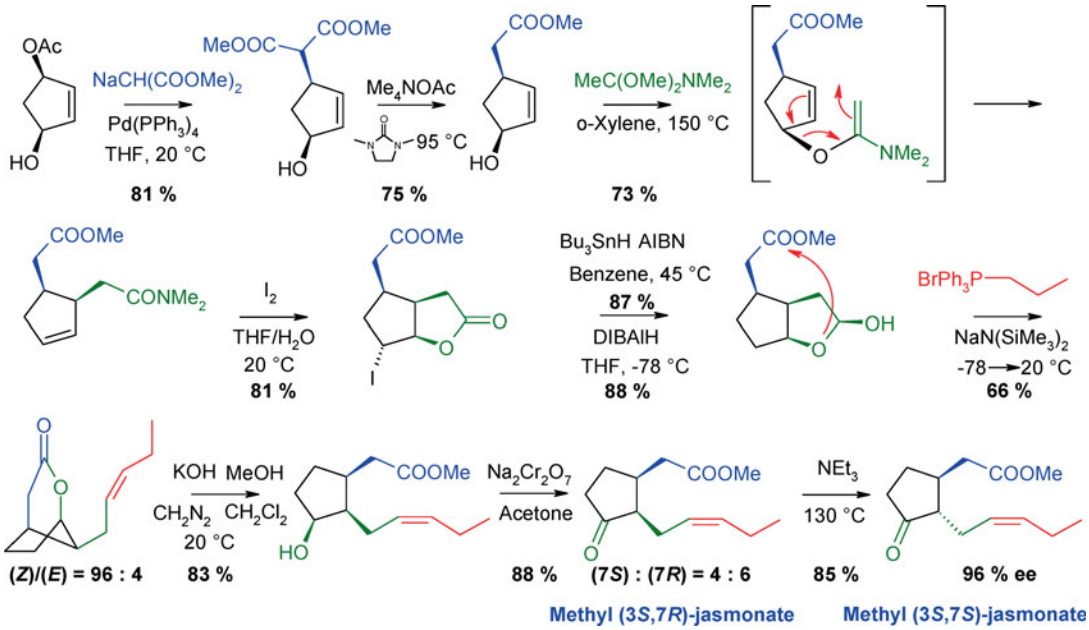


A comparatively short stereoselective synthesis comes form the work of Posner and Asivatham. [95, 96] Here the key step is a diastereoselective Michael reaction of (*R*)-(*p*-tolylsulfinyl)-cyclopentenone. Subsequently, the sulfinyl residue is reduced and the silyl groups are cleaved off. After introduction of the C_5 side-chain, the sulfide residue is reduced off with Raney nickel.

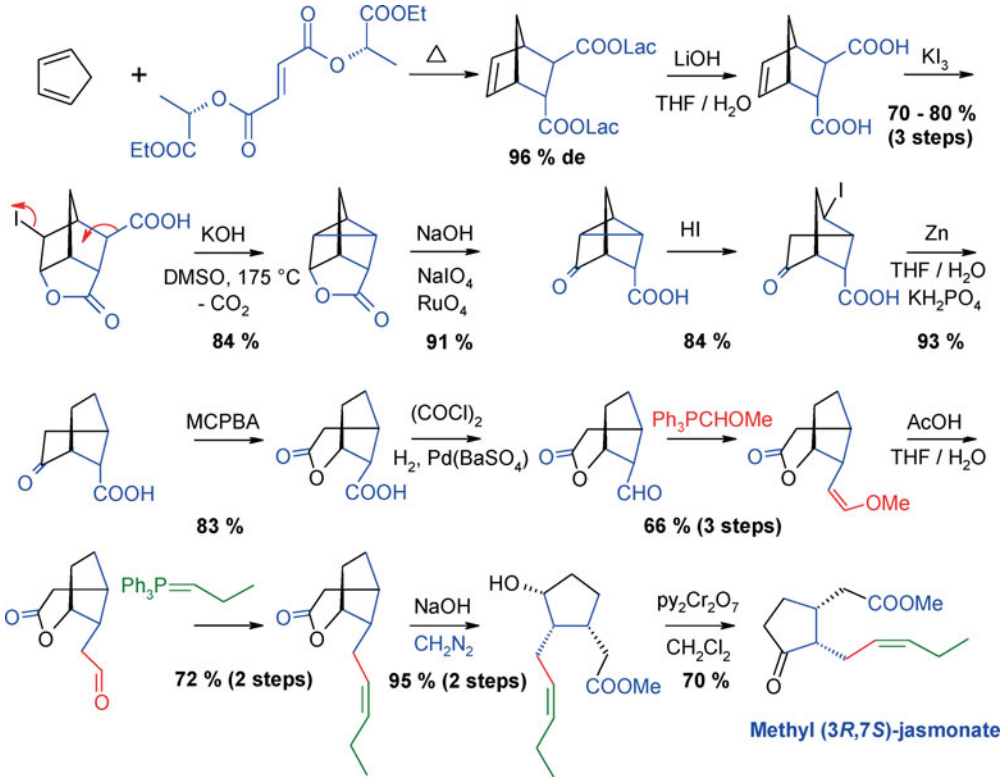


Monfort's synthesis of methyl (3*S*,7*S*)-jasmonate starts from an enantiomerically pure acetoxycyclopentenol, one which also plays an important role in prostaglandin syntheses (see section 5.6). [97] In the first step, which involves a double inversion, the acetoxy group undergoes a palladium-catalysed substitution by malonate. Following mono-demethoxycarbonylation, the alcohol is treated with

N,*N*-dimethylacetamide dimethyl acetal. Under the control of the hydroxy-group, the Claisen rearrangement yields a *cis*-disubstituted cyclopentene. Iodo-lactonisation takes place selectively with the amide function; after deiodination and reduction of the lactone to the lactol, re-lactonisation and a *cis*-selective Wittig reaction lead to the desired intermediate, which must then merely under-go re-esterification, oxidation and epimerisation.

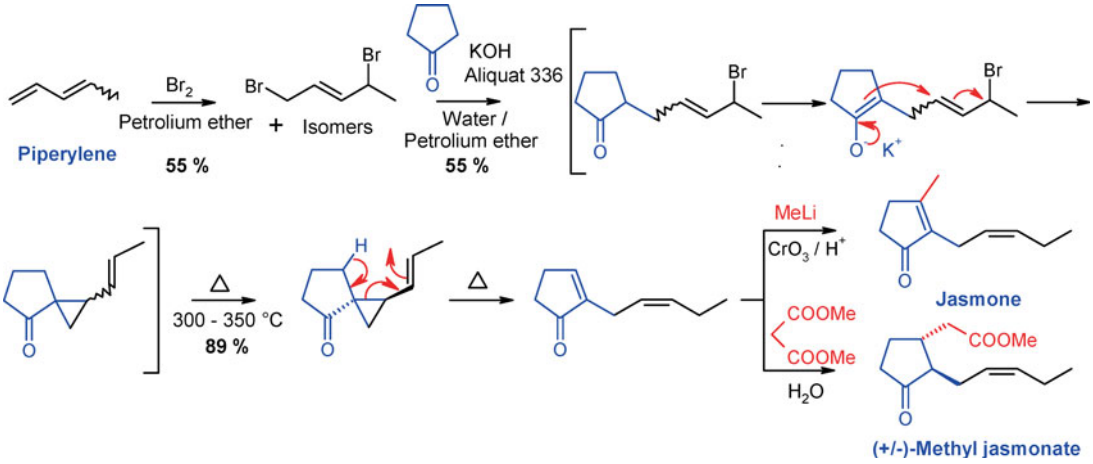


The first synthesis of enantiomerically pure methyl (3*R*,7*S*)-jasmonate comes from Günter Helmchen and co-workers. [98] The stereo-differentiating step is a diastereoselective Diels-Alder reaction of cyclopentadiene and diethyl bis-(*S*)-lactylfumarate. Cleavage of the chiral auxiliary is followed by an iodolactonisation with the *endo*-carboxylic acid. After formation of a cyclo-propane, hydrolysis of the lactone and oxidation of the alcohol function, regioselective opening of the three-membered ring with hydrogen iodide gives an iodo-keto-acid, containing the basic norbornane framework. In the next but one step, a Baeyer-Villiger oxidation leads to the key building block for *cis*-substituted jasmonates. Conversion of the carboxyl function into the C_5 side-chain requires five further steps, the most important among these being a Rosenmund reduction and a "Li-salt-free" Wittig reaction, in order to ensure the (*Z*)-configuration of the double bond. The final sequence comprises hydrolysis of the lactone, esterification and oxidation of the alcohol function.

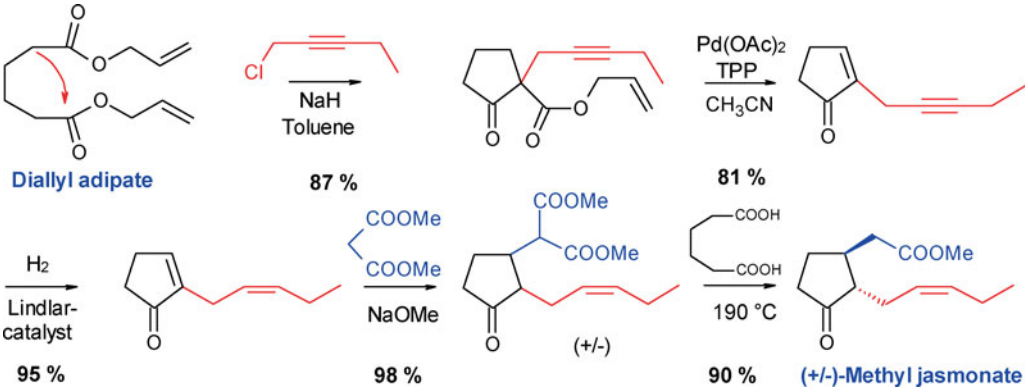


3.3.8 Industrial Syntheses

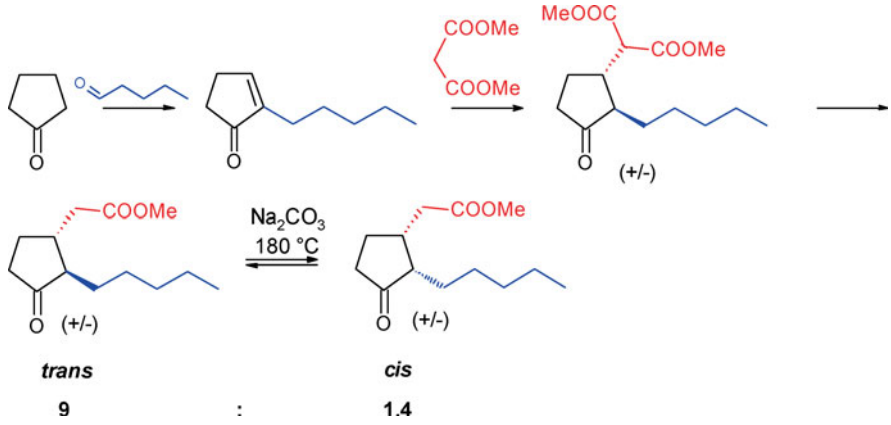
In 1978, Firmenich published an industrial synthesis of (*Z*)-jasmone and racemic methyl jasmonate. [99] The starting compound is piperylene, which is brominated. This produces a number of isomers, the crude mixture of which is reacted with cyclopentanone in a two-phase system with a phase-transfer catalyst. A double substitution leads to a *spiro*-compound, which is afterwards thermolysed in the gas phase. After epimerisation of the *syn-spiro*-compound, a homo-[1.5]-hydrogen shift produces remarkably high yields of (*Z*)-pentenyl-cyclopentenone. This may be converted by methylation and oxidation into jasmone, or into racemic methyl jasmonate by reaction with dimethyl malonate and decarboxylation.



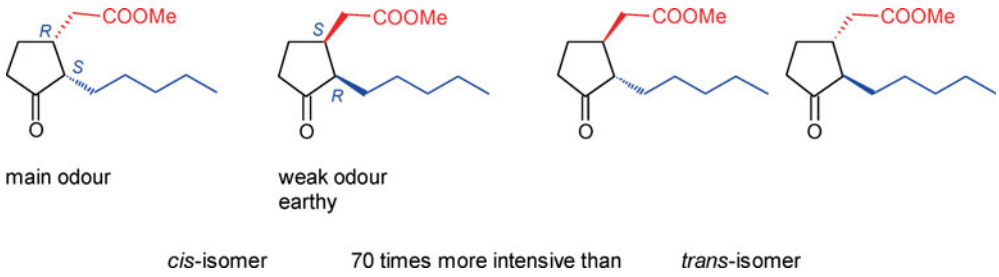
A few years later, Nippon Zeon published another interesting synthesis of racemic methyl jasmonate. [100] The starting material is diallyl adipate, which undergoes Dieckmann cyclisation and alkylation in one step. The subsequent palladium-catalysed decarboxylation/dehydrogenation gives pentynylcyclopentenone, which is then hydrogenated in the presence of a Lindlar catalyst. Michael addition of dimethyl malonate and decarboxylation finally give methyl jasmonate.



The industrial synthesis of *Hedione*® starts from cyclopentanone: this is reacted with pentanal in an aldol condensation. A Michael reaction with dimethyl malonate, followed by hydrolysis and decarboxylation, generates at the thermodynamic equilibrium a *cis/trans*-isomeric mixture of methyl dihydrojasmonates in a ratio of 9 : 1.4. [101]



However, the scent is primarily defined by the *cis*-isomers, and in particular the (3*R*,7*S*)-enantiomer. The *cis*-isomers can be separated by fractional distillation, and the equilibrium re-established by heating in the presence of sodium carbonate (Fig. 3.24).



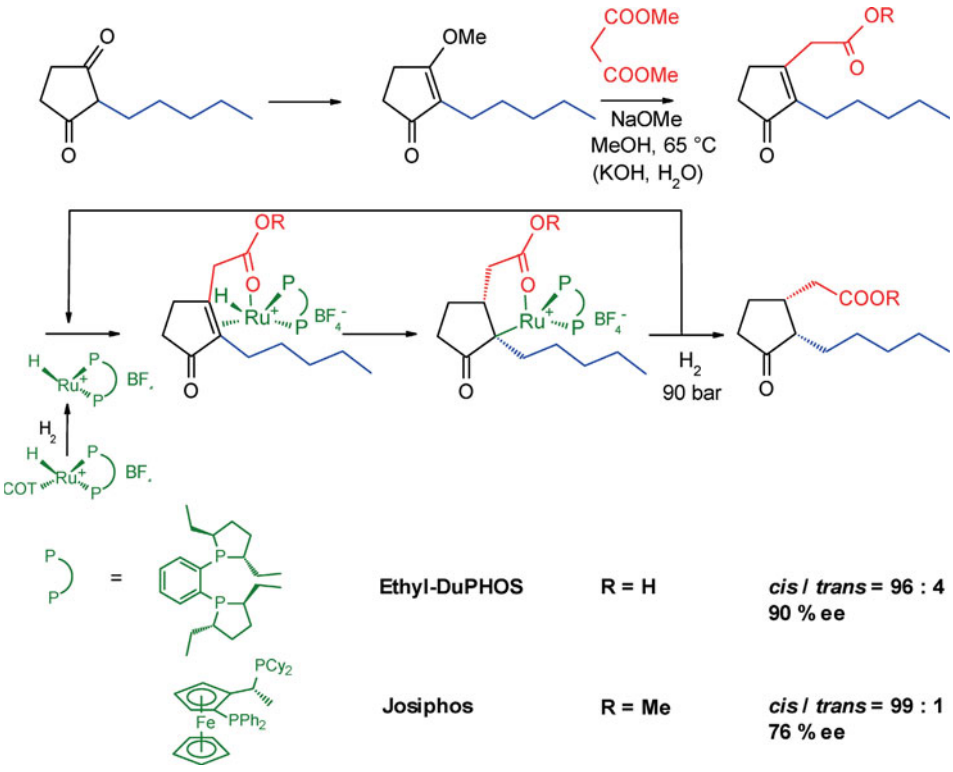
3.24 *The diastereomers of Hedione® are distinguishable by their scent.*

Cepionate® was the first product, in which the *cis*-isomers were enriched to around 30 %, followed by Firmenich's *Kharismal®*, and finally *Hedione HC®*, a principal constituent of *CK one®* (Calvin Klein, 1994) and *Pleasures®* (Estée Lauder, 1995) (Fig. 3.24 and Tab. 3.3). An even higher enrichment of the *cis*-isomers was limited by the rapid epimerisation, especially above or below the narrow pH range of 5.5–6.5.

Tab. 3.3 *The trans/cis ratio of methyl dihydrojasmonate in various products.*

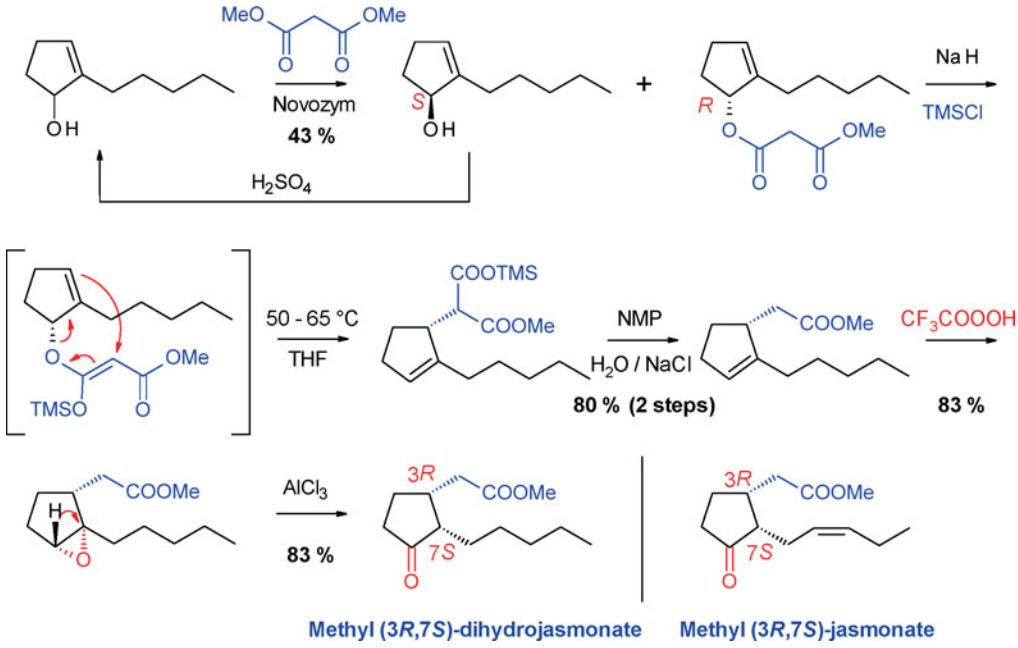
Product	trans/cis	Scent threshold (ng/l)
Hedione®	90/10	0.3
Cepionate®	70/30	0.1
Kharismal®	40/60	0.05
Super Cepionate®	30/70	0.04
Hedione HC®	25/75	0.03

Essential progress could be achieved, when methyl dihydrojasmonate was prepared by enantioselective hydrogenation of the corresponding cyclopentenone. [102] However, the high degree of substitution at the double bond caused initially a problem, since all the catalyst systems known so far failed. Only the use of a very electrophilic, coordinatively unsaturated ruthenium complex yielded the first success. The electrophilicity was increased by adding HBF_4 and turning it into a cationic complex. The corresponding base and the solvent dichloromethane form only a weak complex. This is however stabilised by a bidentate phosphane: suitable ligands are, for example, ethyl-DuPHOS and Josiphos. [103, 104] Thus, it became possible to prepare methyl (3*R*,7*S*)-dihydrojasmonate (*Paradisone*®) on an industrial scale and in the quality required for the scent industry.



An attractive alternative synthesis was likewise developed by Firmenich. [105, 106] Pentylcyclopentenol, is enzymatically transesterified with dimethyl malonate, whereby the (*S*)-enantiomer remains unaltered, but this can be racemised in the presence of sulfuric acid. The malonate is converted into an allylsilylketene acetal, which is then subjected to a Claisen rearrangement; this proceeds under the control of the allyl alcohol at relatively low temperatures and with high stereoselectivity. After decarboxylation, there follows the key step in the synthesis: a *syn*-selective epoxidation with highly electrophilic peroxy-acids, such as peroxytrifluoroacetic acid. The high *syn*-selectivity arises from electrostatic effects between the more electron-rich π-side of the douple bond and the partial

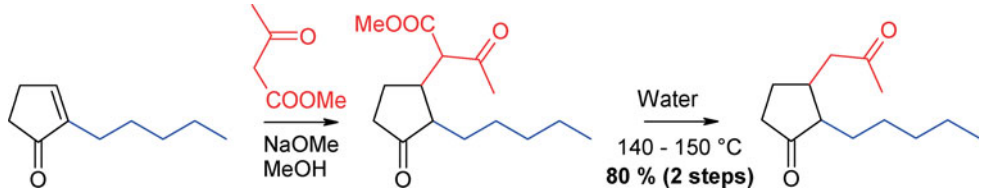
positive charge carried by the peroxy-trifluoroacetic acid. [107] The aluminium chloride-induced epoxide ring-opening proceeds in the manner of a suprafacial 1,2-hydrogen migration with inversion of the absolute configuration at C-7.



The advantage of this process is that methyl (3*R*,7*S*)-jasmonate is obtainable by an entirely analogous route: this compound is not accessible by enantioselective hydrogenation.

3.3.9 *Magnolione*®

Closely related to methyl dihydrojasmonate, both in terms of structure and olfactory effect, is *Magnolione*®, which carries an acetyl residue instead of an ester function. Its scent is reminiscent of magnolia; its synthesis is analogous to that of *Hedione*®, with the difference that methyl acetoacetate is used in place of dimethyl malonate. [108]



This modification produces a scent of stronger intensity, better stability and a rather distinctive floral jasmine note. *Magnolione*® is found in a subtype of the

Eau de Chypre family, for example *Coriandre*® (Couturier, 1973) and in *Eden*® (Cacharel, 1994).

Summary in Bullet Points

- Jasmine oil belongs to the most expensive ingredients of valuable perfumes.
- The jasmonoids are secondary metabolites of the fatty acid metabolism. Jasmonates play a role in signal transduction in plants, similar to that of the structurally related prostaglandins in mammals.
- An especially elegant industrial-scale access to enantiomerically enriched methyl dihydrojasmonate is illustrated by the enantioselective ruthenium-catalysed hydrogenation of the corresponding cyclopentenone.

3.4 Menthol

(–)-Menthol is the principal component of Japanese peppermint oil. In Japan it has been obtained since the 17th century from corn mint (*Mentha arvensis* L.) (Fig. 3.25). Important areas of cultivation are at present in India, China, Japan, Brazil and Taiwan.

Peppermint (*Mentha piperita* L.) is a hybrid of spearmint (green mint, *Mentha spicata*) and water mint (*Mentha aquatica*), which was bred in England (Mitcham, Surrey – now part of Greater London) around 1750. The plant also

3.25 *Corn mint (Mentha arvensis L.).*

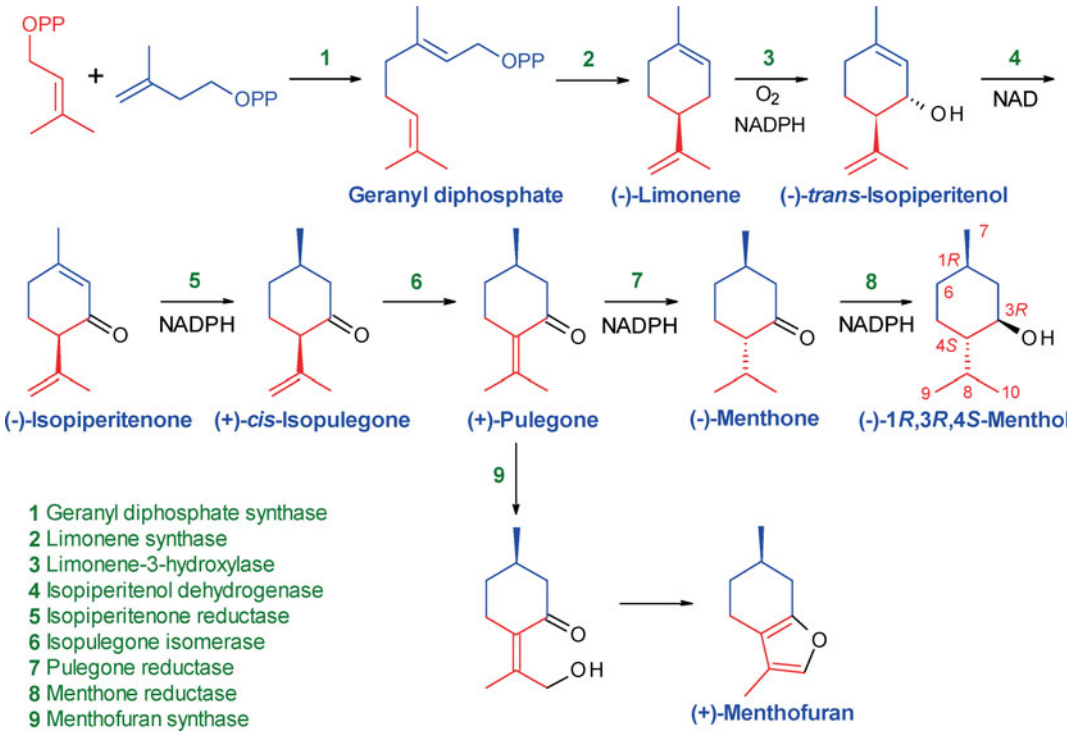
3.26 *Peppermint (Mentha piperita L.).*

contains menthol (Fig. 3.26), and is cultivated mainly in the north-western USA, in China and in Europe, as a source of peppermint oil, to aromatise certain medicines beverages and foodstuffs. The leaves are used for peppermint tea. [109]

3.4.1 Biosynthesis

The biosynthetic route to menthol, starts from isopentenyl- and dimethylallyl diphosphate, which in the case of mint derive from the triose-pyruvate pathway, and consists of eight discrete steps (see also section 7.1.2). This route was established by feeding experiments with radio-labelled intermediates and cell-free enzyme studies. [110] Condensation of isopentenyl- and dimethylallyl diphosphate gives geranyl diphosphate, which is cyclised to (–)-limonene. Both steps are Mg^{2+}-dependent. By-products of the cyclisation are around 2 % of myrcene and both, α- and β-pinene. The limonene synthases in *Mentha piperita* and *Mentha spicata* are identical, which shows how closely related to each other the species are.

Limonene is hydroxylated at C-3, and the alcohol function then oxidised. Three stereogenic centres are generated by subsequent reduction steps. In this context it is noteworthy that isomerisation of the double bond of isopulegone to pulegone, and reduction of the double bond result in inversion of the stereocentre at C-4.

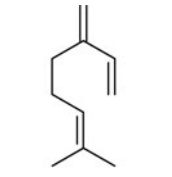


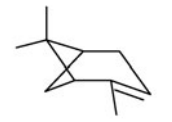
An important junction in the menthol biosynthesis is the formation of (+)-menthofuran from (+)-pulegone, which is brought about by menthofuran synthase. This path is promoted by short daylight hours or low-intensity daylight, warm overnight temperatures, and shortage of water or nutrients, and results in a considerable quality loss of the peppermint oil.

Once the understanding of the biosynthetic pathway to menthol was complete, this enabled the development of transgenic cell lines, which, by overexpression of certain enzymes on the triose-pyruvate pathway, produced up to 40 % higher yields of peppermint oil. In addition, the suppression of the menthofuran formation improved the product quality under stress conditions.

Moreover, peppermint plants were produced, exhibiting resistance to broad-spectrum herbicides like glyphosate and glufosinate; this greatly simplified mint cultivation (*cf.* section 8.1 Amino acid herbicides).

(–)-Menthol from natural sources is obtained predominantly from the essential oil of corn mint (*Mentha arvensis*), which is produced by steam distillation or a distillation/extraction procedure using supercritical carbon dioxide (*cf.* section 5.11 Caffeine). The menthol is frozen out and the crystalline mass centrifuged. Traces of impurities confer a slightly minty aroma to natural menthol.


Myrcene

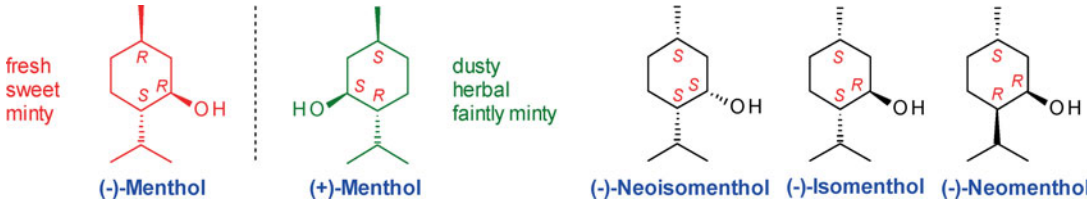

(-)-(1*S*,5*S*)-*α*-Pinene


(-)-(1*S*,5*S*)-*β*-Pinene

3.4.2 Structure and Activity

There is evidence that menthol was known in Japan for more than 2000 years. However, the "Japanese camphor" was isolated in pure form only in 1771 by the Heidelberg-born chemist and physician, Hieronymus David Gaubius (1705–1780). The first studies on its characterisation and structure determination were published by Oppenheim, Beckett, Moriya and Atkinson in the years 1862–1882. [111]

Menthol possesses three stereogenic centres and therefore four enantiomeric pairs are possible. The diastereomers of menthol are named neomenthol, isomenthol and neoisomenthol (Fig. 3.27). Whereas laevorotatory menthol displays a fresh, sweet, minty odour, its antipode smells dusty, herbal and only faintly minty. The synthesis of enantiomerically pure (–)-menthol is therefore essential.



3.27 *The diastereomers of menthol and their enantiomers have distinctive olfactory properties.*

"Cool" with and without Menthol

Humans have at their disposal thermoreceptors, as part of the peripheral nervous system: in the skin, in the cornea, on the tongue and in the bladder. One can differentiate warm and cold receptors, which allow, quite precisely, for the detection of small temperature differences, without being able to estimate the absolute temperature with nearly the same accuracy. In the hypothalamus, the temperature signals are processed, compared with the body's normal temperature, and the actual body temperature is correspondingly regulated. Small temperature differences feel pleasant or unpleasant. Temperatures, which are potentially harmful to the body, mostly those below 0 °C or above 45 °C, cause a feeling of pain.

Capsaicin, the active principle of chilli peppers, binds to the warm receptor TRPV1. That is why chilli is not only "spicy" but also "hot" (*cf.* section 3.5.2).

The cooling effect of menthol was discovered by Alfred Goldscheider (1858–1935) in Leipzig as long ago as 1886. [112] Then, in 1951, the effect of menthol on temperature perception was described somewhat more precisely by the Heidelberg physiologist Herbert Hensel (1920–1983) and the Stockholm electrophysiologist Yngve Zotterman (1898–1982). [113] Their instrumental experiments on the trigeminal nerve of cats showed that menthol heightened the sensitivity of the nerve endings for stimulation by cold. They suspected that there was a menthol-sensitive protein on the sensory endings of the trigeminus, which functioned as a cold receptor. Surprisingly, it still required more than 50 years before the effect was completely understood.

It was only in 2002 that two research groups, working independently of each other, were able to describe the first cold receptor as a Transient Receptor Poten-

tial cation channel, subfamily M, member 8 (TRPM8): this is synonymous with Cold and Menthol Receptor 1 (CMR1). [114, 115] The receptor functions as an ion channel, which opens at low temperatures and allows sodium and calcium ions to flow into the cell. [116] The depolarisation results in an action potential.

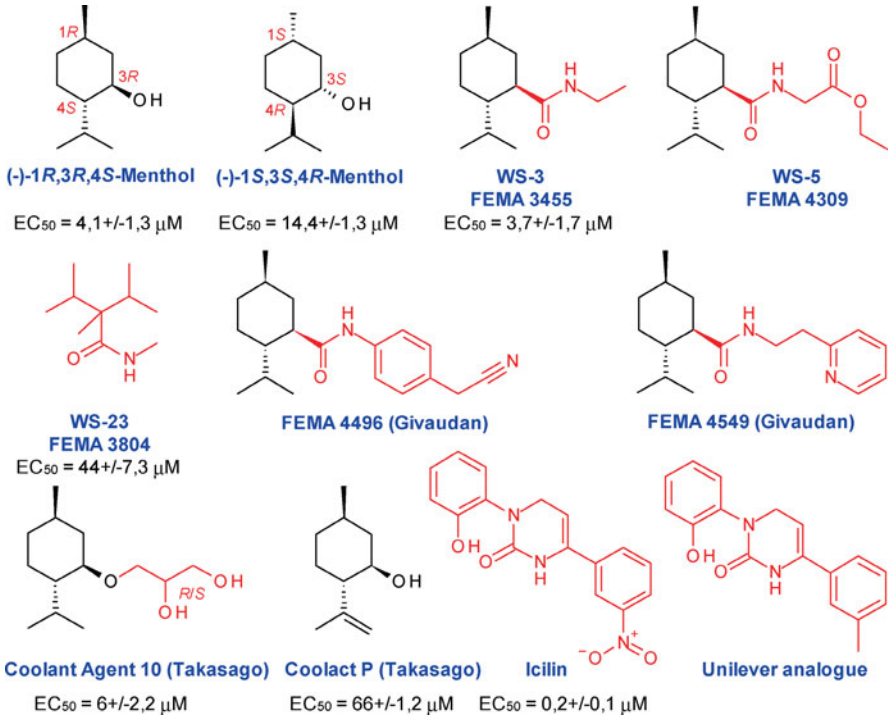
Interestingly, not only low temperatures cause the opening of this ion channel, but also substances like (–)-menthol. This binds to the TRPM8, opens the ion channel, and thereby simulates coldness, although there is no change in temperature. It is noteworthy that (+)-menthol binds to the receptor as well, but its cooling effect reaches only 25 % compared to the enantiomer.

Back in the 1970s, Wilkinson Sword Ltd. launched an extensive research programme, seeking substances, which were toxicologically safe and, as far as possible, tasteless and odourless, and which had a longer-lasting cooling effect compared with (–)-menthol. [117] Out of around 1200 substances tested, three finally reached the market: WS-3 (*N*-ethyl-*p*-menthane-3-carboxamide), WS-5 (ethyl 3-(*p*-menthane-3-carboxamido)acetate) and WS-23 (2-isopropyl-*N*,2,3-trimethylbutyramide). Later Givaudan marketed two other related menthanecarboxamides: FEMA 4496 (*N*-(4-cyanomethylphenyl)-*p*-menthanecarboxamide) and FEMA 4549 ((1*R*,2*S*,5*R*)-2-isopropyl-5-methyl-*N*-[2-(2-pyridinyl) ethyl]cyclohexanecarboxamide).

WS-3 is a colourless, crystalline, almost odourless substance with a strong cooling effect without any burning, stinging or tingling. The cooling effect is noticeable at concentrations above 200 ppb, when tested in the mouth with strips of paper doped with WS-3. WS-5 is around three times stronger than WS-3. Structurally WS-23 is considerably different from WS-3 and WS-5, but astonishingly, it possesses very similar properties. According to Givaudan, FEMA 4496 shows approximately 10 times, and FEMA 4549 around 100 times the cooling effect of menthol.

Coolant Agent 10® (*R*/*S*)-(–)-menthoxypropane-1,2-diol) is a cooling substance, which was brought to the market by Takasago. It consists of a diastereomeric mixture, with a cooling effect around one-quarter that of menthol. In addition, Takasago discovered that highly purified enantiomerically pure (–)-isopulegol is odourless, and imparts a feeling of freshness, crispness, and coolness to citrus-type fragrances. (–)-Isopulegol is sold by Takasago under the name *Coolact P*®.

The cooling effect of icilin (AG-3-5, 3-(2-hydroxyphenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidin-2-one) was discovered by chance in 1983, when the substance came accidentally into contact with the nasal mucous membrane, the lips and the eyes of a researcher. It originated from a research programme into CNS-active substances at Delmar Chemicals Ltd. in Quebec, where it was strikingly discovered, that the compound produced a change in the behaviour of mice, rats, rabbits, cats and dogs which was named "wet dog shakes", similar to the shaking of dogs after a bath. [118] Icilin is nearly 200 times more potent than the reference cold thermosensory agonist, menthol. David A. Andersson and co-workers at Novartis have shown that the activation of TRPM8 cold receptor by icilin and cold, but not by menthol, is modulated by the intracellular pH. Their data suggest that, compared to menthol, the activation by icilin and cold involves a different mechanism. [119] Unilever Home and Personal Care USA,



3.28 *Cool substances.*

a division of Conopco Inc., have synthesised several series of icilin analogues which reveal cooling potencies similar to menthol (Fig. 3.28).

In the year 2004, H.-J. Behrendt published a study comparing the binding affinities of various coolant substances. [120] With a few exceptions, these data are in agreement, at least qualitatively, with the sensory results. With this test system, a new way is opened up to search for even more effective substances.

Typical applications of these cool substances include their use as coolants in medicinal preparations, shower gels, oral care products and confectionery products. Surprisingly, in 2004 Markus Gautschi and Philippe Blondeau at Givaudan discovered that cool substances such as WS-3 also have a repellant effect on cockroaches. [121]

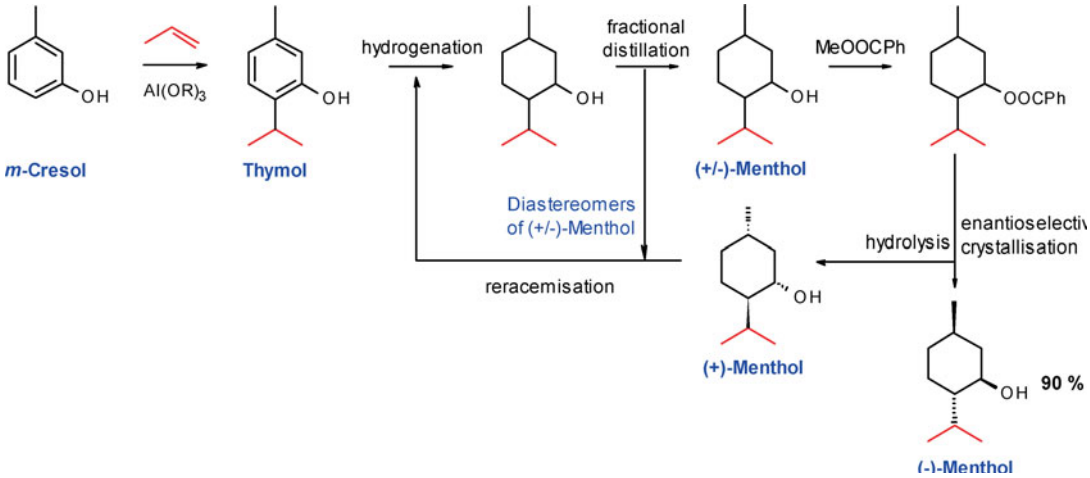
3.4.3 Industrial Syntheses

Since several decades, for the manufacturing of synthetic menthol, two industrial processes are employed: the Haarmann-Reimer Process and the Takasago Process. BASF has developed a third one, which has recently gone into use.

Haarmann-Reimer Process

The starting material for the Haarmann-Reimer process is *m*-cresol, which is alkylated with propene. Hydrogenation then produces the racemates of menthol, neomenthol, isomenthol and neoisomenthol. Although the boiling points lie

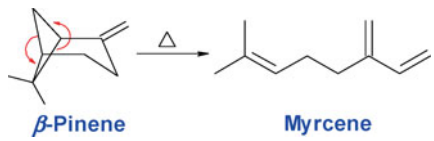
close to one another, (+/-)-menthol may be separated by fractional distillation. Following transesterification using methyl benzoate, the mixture of enantiomers is resolved by fractional crystallisation after seeding with the benzoate of enantiopure (–)-menthol. The menthol itself is obtained by hydrolysis of the benzoate and recrystallisation. The (+)-menthyl benzoate is similarly hydrolysed and recycled, along with the other diastereomers, into the hydrogenation step of the process. In this way an overall yield in the range of 90 % is obtained. [122, 123]



Takasago Process

The starting material for the Takasago process is *N*,*N*-diethylgeranylamine, which is obtained either from isoprene or from myrcene.

The natural source of myrcene is β-pinene, and thus goes back ultimately to pine resin (the annual worldwide production of which amounts to 1.5 million tonnes). The resin is obtained mostly from living trees and less often from resin-saturated stumps or roots. Half of the production comes from the USA, followed by China, Russia and the Mediterranean region. By steam distillation, the readily volatile turpentine oil (see marginal note on next page) is separated from the involatile resin (also known as colophony). A significant part of the turpentine oil arises in the course of the wood pulping. The composition of the turpentine oil depends on the nature of the conifer source, the extraction process, and the region from which the tree originates. Whereas Greek turpentine oil consists of 96.5 % pure α-pinene, the American oil contains 28.1 % of β-pinene. Also the optical purity is dependent on the country of origin. Myrcene is obtained by the thermal "cracking" of β-pinene. Quite interestingly, all asymmetry of the molecule is eliminated, in contrast to the usual synthetic strategy; one merely takes advantage of the carbon skeleton and introduces the chirality from scratch by a catalytic process.

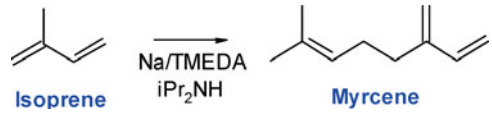


Excursus

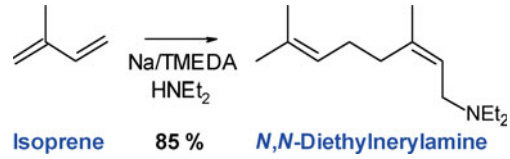
From a historical perspective, the Monsanto process for the preparation of (*L*)-DOPA in 1974 laid the foundation stone for industrial enantioselective catalysis. Since then it has been joined by a number of other asymmetric methods, such as enantioselective Sharpless epoxidation (glycidol (ARCO) and disparlure (Baker)), and cyclopropanation (cilastatin (Merck, Sumitomo) and pyrethroids (Sumitomo)). Nevertheless, besides the enantioselective hydrogenation of an imine for the production of (*S*)-metolachlor (a herbicide from Syngenta), the Takasago process for the production of (–)-menthol remains since 1984 as the largest worldwide industrial application of homogeneous asymmetric catalysis. [124]

Inhaling turpentine oil has a range of harmful effects, and causes an occupational toxic solvent syndrome, known as "painter's disease", which may be recognised by a violet-like odour of the urine.

Myrcene is also accessible from isoprene, which derives from the steamcracker's C5-cracking fraction by dehydrogenation. In contrast, construction of its carbon skeleton, for example from acetylene and acetone or by metathesis of isobutene and but-2-ene, are declining in importance. [125] The sodium-catalysed dimerisation in the presence of diisopropylamine leads to myrcene.

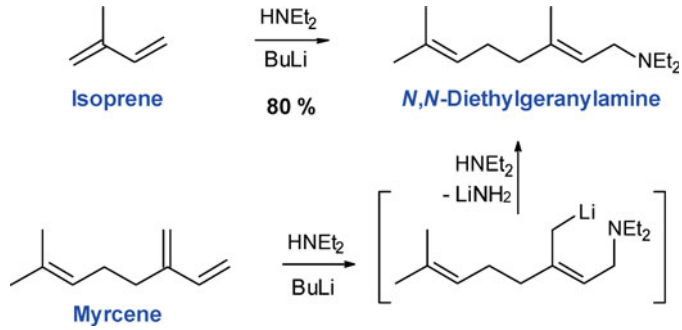


On the other hand, the head-to-tail telomerisation of isoprene in the presence of diethylamine delivers *N*,*N*-diethylnerylamine in 85 % yield and with high selectivity.

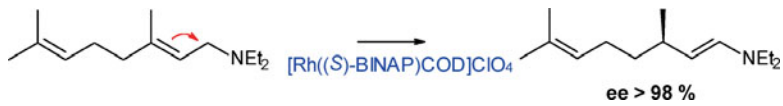


With lithium bases isoprene or myrcene gives preferentially *N*,*N*-diethylgeranylamine. With *n*-butyllithium as catalyst, the reaction proceeds both regio- and stereoselectively.

The annual worldwide production of turpentine oil amounts to 260,000 tonnes. This is a vanishingly small part of the biologically generated material, if one considers that annually 438 million tonnes of monoterpenes evaporate into the atmosphere.

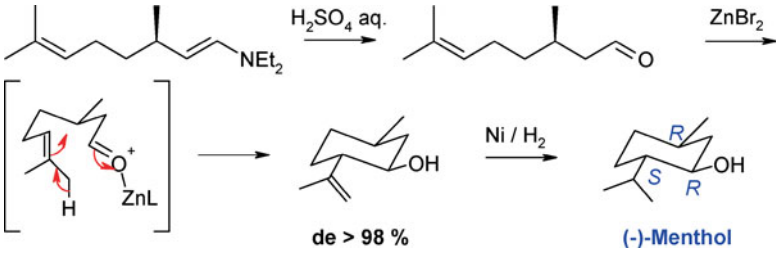


In the next step, diethylgeranylamine is treated with an enantiomerically pure rhodium-BINAP catalyst. The product, an enamine of citronellal, is obtained in quantitative yield and in high optical purity.



The catalyst/substrate ratio is *ca.* 1 : 9000. The enamine is produced on a 9-tonne scale. At the end of the reaction, the enamine is separated off by distillation and the catalyst recycled. After hydrolysis in acid, there follows a diastereoselective Lewis acid-catalysed cyclisation, with zinc bromide or zinc chloride, in the man-

ner of a carbonyl-ene reaction; this gives isopulegol, which is finally hydrogenated over Raney nickel to produce (–)-menthol.



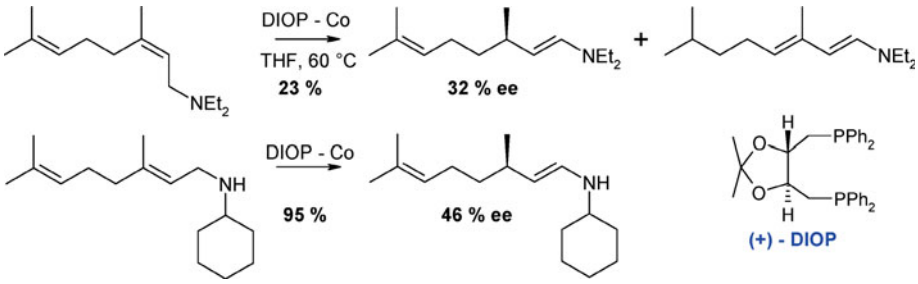
The stereochemistry of menthol results ultimately from a transition state in the chair conformation, in which all the substituents are arranged equatorially; the diastereoselectivity is greater than 98 %.

Catalyst Development

The double bond migration, formally a [1,3]-H-shift, is a comparatively minor structural alteration, but is actually the crucial step in the generation of three stereogenic centres.

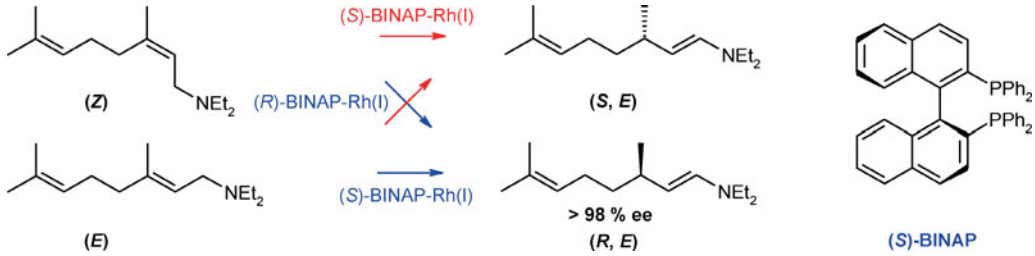
In terms of catalyst development, studies by Otsuka and Tani led the way with a (+)-DIOP-modified cobalt catalyst (Kagan, 1972). [126] Diethylnerylamine could be converted, in 23 % yield and with an enantiomeric excess of 32 %, to the corresponding enamine of citronellal. However, concomitantly a dienamine was produced in quantities which were not insignificant.

Under milder conditions cyclohexylgeranylamine gave with the same catalyst the desired product in very good yield. The stereoselectivity was also improved: the ee obtained was 46 %.



The breakthrough came in 1980, when Ryoji Noyori (Fig. 3.29) showed that rhodium(I)-catalysed double bond migrations could proceed stereoselectively at or below room temperature. BINAP served as the ligand. [127] It is obtainable in both enantiomeric forms. The rhodium complex catalyses very selectively the double bond migration. Here occurs an interesting stereochemical connection; the (*S*)-BINAP-rhodium complex converts diethylnerylamine into the (*S*)-enantiomer, but converts diethylgeranylamine into its (*R*)-enantiomer. The (*R*)-BINAP complex reacts *vice versa*. Obviously, the catalyst very efficiently differentiates between the enantiofacial sides of the $\Delta^{2,3}$-double bond, and thereby between the two enantiotopic H-atoms at C-1.

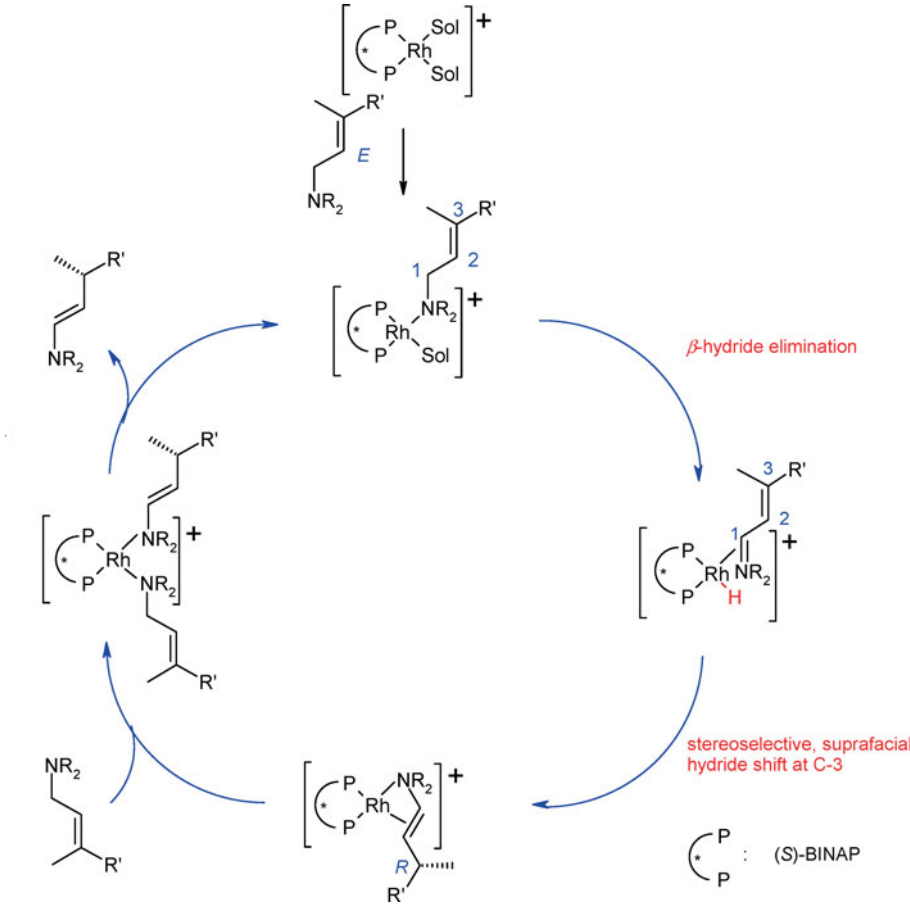
3.29 *Ryoji Noyori (*1938).*



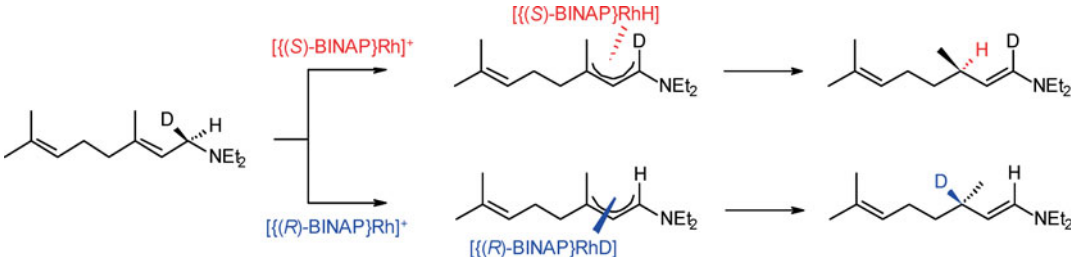
This is very advantageous, since according to the starting material, both educt and product may be independently determined by the choice of the appropriate ligand. A prerequisite of stereoselectivity is, of course, that the raw materials, diethylnerylamine or diethylgeranylamine, have to be pure.

Mechanism

The critical steps in the catalytic cycle are the β-hydride elimination from C-1 with the formation of a π-iminium-rhodium hydride species, and the stereoselective suprafacial hydride addition at C-3. The η^3-enamine-complex can be detected by NMR spectroscopy. The next step is rate-determining. The catalytic cycle is complete with the release of the product.

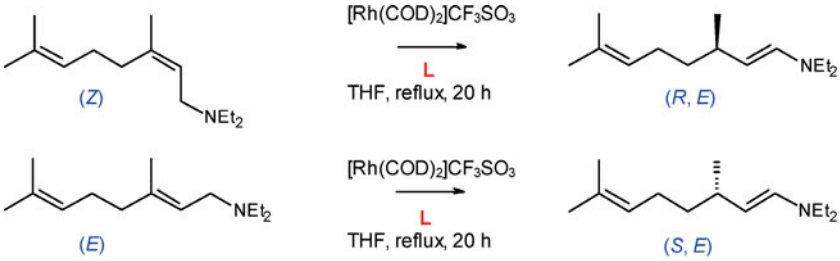


The (*S*)-BINAP-rhodium complex transfers the (pro-*S*) hydrogen atom from C-1 to C-3, and the (*R*)-BINAP-rhodium complex transfers the (pro-*R*) hydrogen. On account of the selective suprafacial hydrogen transfer, opposite absolute configurations of the products are obtained depending on the (*E*/*Z*)-configuration of the starting material. For steric reasons, the product in all cases has the (*E*)-configuration. [128]



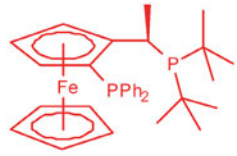
Recently it has become known from work at Firmenich, that BINAP can be effectively replaced by the Josiphos and Daniphos ligands. [129] From diethylgeranylamine one obtains after hydrolysis (*S*)-citronellal, and from diethylnerylamine (*R*)-citronellal (analogous to (*R*)-BINAP). By attaching a linker to the unsubstituted cyclopentadiene ring, the catalyst can be immobilised on various carriers. In some cases this reduces the activity, but the stereoselectivity remains comparable.

The "Josiphos ligands" were developed in the 1990s by Antonio Togni at the ETH and termed after Josi Puleo, the technician who prepared the first one. In analogy to "Josiphos", Albrecht Salzer at the RWTH Aachen named in 2003 the "Daniphos ligands" after his coworker Daniela Vasen. [129]



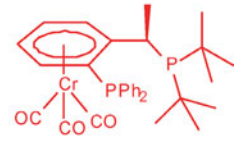
Josiphos type

L:



	Conversion	ee
from (*E*)	99 %	92 %
from (*Z*)	100 %	97 %

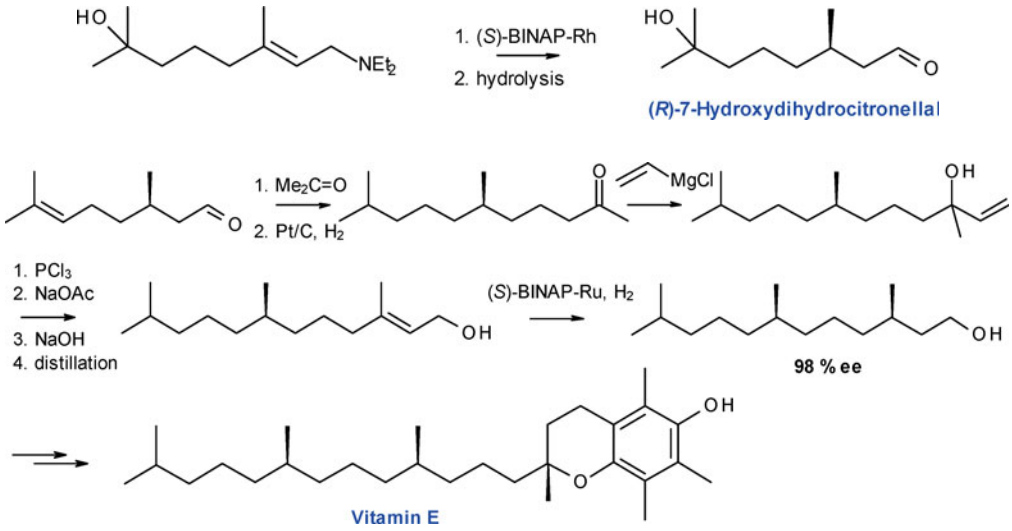
Daniphos type



Conversion	ee
99 %	96 %

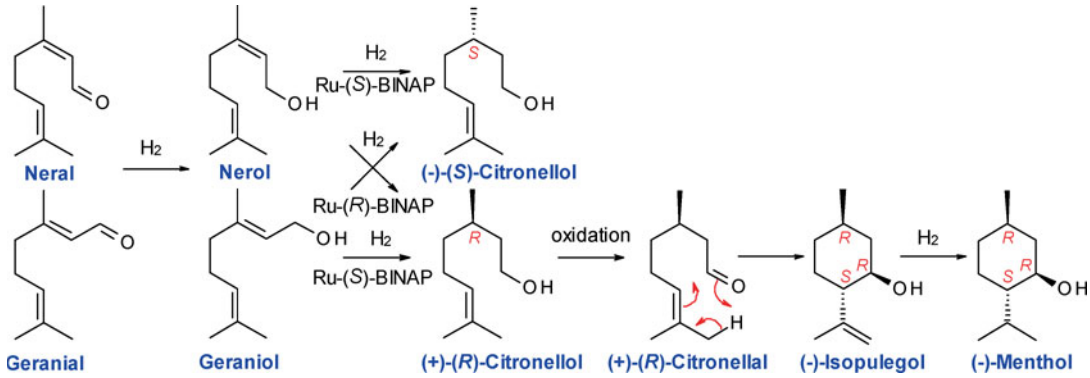
Excursus

The rhodium-catalysed enantioselective double bond migration has found further interesting applications apart from menthol synthesis. (*R*)-7-Hydroxydihydrocitronellal, which provides the scent in lily of the valley (*Convallaria majalis*) is accessible in this way. When combined with an enantioselective hydrogenation, this catalyst can also be used to build up the side-chain of α-tocopherol (Vitamin E).



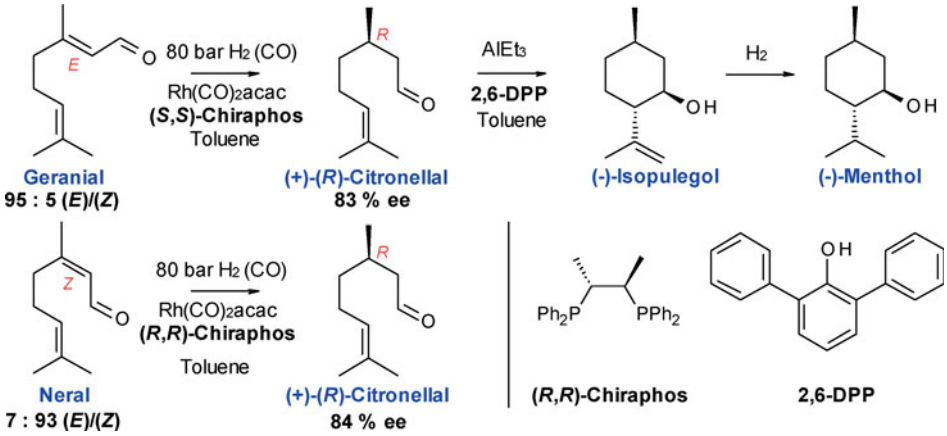
BASF Process

The most recent route to menthol comes from BASF. [130] The main starting material is citral (*cf.* section 3.2.3), which first is hydrogenated to produce a 1 : 4 mixture of nerol and geraniol. This mixture is then separated by distillation. The enantioselective hydrogenation with a ruthenium-BINAP catalyst and subsequent oxidation leads to (+)-(*R*)-citronellal, which may be converted in two stages by known methods into (–)-menthol.



More advantageous, due to fewer reaction steps, is the direct enantioselective hydrogenation of geranial or neral to (+)-(*R*)-citronellal with an enantiomerically pure rhodium-Chiraphos ligand. It is also attractive to apply the carbonyl-ene reaction in the presence of a sterically demanding aluminium complex, in order to suppress largely the formation of isopulegol diastereomers. [129, 131]

Most recently, Takasago published the synthesis of (*R*)-citronellal by enantioselective hydrogenation of the mixture of geranial and neral in the presence of a dual catalyst ($Pd/BaSO_4$ and an enantiopure 2-diarylmethylpyrrolidine). [132]



3.4.4 Uses of Menthol

In quantitative terms, menthol ranks as one of the major aroma chemicals. Apart from its use in perfumery / shower and shaving products (7 %), menthol finds use in the manufacture of toothpaste, (28 %), pharmaceuticals (27 %; cough medicine and nasal sprays), cigarettes (25 %), confectionery and chewing gum (11 %), liqueurs – and in chemistry as a chiral auxiliary.

The worldwide annual production is in excess of 19,000 tones, of which 13,000 tonnes come from natural sources, mainly from India and China. [133] The price fluctuates appreciably, usually in the range of 20–60 dollars per kilogram.

Fishermen's Friend
In the year 1865, the young pharmacist James Lofthouse developed strong menthol pastilles to ease the respiratory illnesses of deep-sea fishermen. Soon the seafarers referred to it as their friend, from which the brand name arose. The menthol pastilles are produced in Fleetwood, Lancashire, in twelve flavours. Each pastille contains 10 mg of menthol (Fig. 3.30).

3.30 *Fishermen's Friend tin.*

Summary in Bullet Points

- Menthol is an oxidation product of the terpene limonene.
- (–)-Menthol is obtained industrially starting from cresol according to the Haarmann-Reimer process, which involves a classical resolution of the racemate.
- The Takasago process is one of the most important enantioselective processes which are practised industrially. The key step is a rhodium-catalysed double bond migration.

Excursus
Food residues containing cocoa were also found in vessels dating from the preclassical Maya period (900 BC–250 AD). The vessels were burial artefacts, which were found in the city of Colha (52 km north of Belize City). Cocoa (*Theobroma cacao*) is the only Central American plant which contains theobromine. Therefore, it is the indicator substance to prove the prior existence of cocoa at those archaeological sites; its detection involves HPLC coupled mass spectrometry. [135]

3.31 *Hernán Cortés was the first to bring cocoa and vanilla to Spain, and from there these aromatic substances spread right across Europe.*

The vanillin content of Limousin oak wood, which is used for wine-casks, contributes to the aromatic character of barrique wines.

3.5 Vanillin

Montezuma II (about 1466–1520) drank Xocolatl, [134] a chocolate drink made from cocoa, before he paid court to his wives, and Giacomo Girolamo Casanova (1725–1798) was convinced that chocolate possesses aphrodisiac properties. The Aztecs used vanilla to enhance the aroma of chocolate, centuries before in 1520 the Spanish Conquistador Hernán Cortés (1485–1547) tasted it at the court of Montezuma (Fig. 3.31).

Because of its taste, its aroma and its texture, eating chocolate is a hedonistic experience for many people, one, which cheers them up. Scientifically it can be shown, that the flavonoids in cocoa are powerful antioxidants, which serve to protect against age-related diseases, *e.g.* of the heart, and also against cancer.

3.5.1 Occurrence

Vanillin is found in *Styrax* species, in clove oil, and in the flowers of black salsify (*Scorzonera*), *Spiraea* and potato. In addition, various foodstuffs, such as milk, wine and rice wine contain vanillin. The smell of old, yellowed paper with a high wood content can be attributed to this compound as well. It is also found in smaller concentrations in the woody part of many plants. Vanillin is found in tobacco to a considerable extent, and it is also contained in the bark of the Ponderosa pine (*Pinus ponderosa*). [136] Moreover, male bugs of the species *Eurygaster integriceps* secrete vanillin as an attractant. [137] The most important vanilla plant is the climbing orchid, *Vanilla planifolia*, the fruits of which are harvested nine months before ripeness (Fig. 3.32).

3.32 *The fruit capsules of the yellowish-white flowering vanilla plant Vanilla planifolia, which wind themselves, liana-like, around the tree branches, are harvested while still unripe.*

Fresh vanilla beans have no aroma. By drying in the sun their milky juice is converted through fermentation into a brownish-black balsamic mass with an intensive odour (Fig. 3.33). The glucosidically-bonded vanillin (Vanillosid) is thus released and the pods acquire a sweet taste. By further curing they become covered with a white film, which consists of crystalline vanillin. The vanillin content of the pods may be up to 2 %. Vanilla extract is obtained from the dried crushed pods by extraction with 35 % ethanol (Tab. 3.4). The full aroma of genuine vanilla extract derives from more than 120 natural flavouring compounds.

For many centuries, Central America provided the only source of vanilla, because in other places the plant's natural pollinator, the melipona bees and hummingbirds, are not indigenous. From 1841 onwards, however, artificial pollination was developed, so that vanilla could also be cultivated in Africa and the West Indies.

Vanillosid
β-(*D*)-Glucoside of Vanillin

3.33 *After sorting, the vanilla pods are blanched in hot water, and finally dried in the afternoon sun (a). Thereby fermentation sets in. The pods turn a brownish-black colour and produce a unique aroma, which can be extracted with ethanol (b).*

Tab. 3.4 *The most important constituents of vanilla extract.*

Constituent	Concentration (mg / 100 ml)
Vanillin	135–175
4-Hydroxybenzaldehyde	10–12
Vanillic acid	7–8.5
4 -Hydroxybenzoic acid	1.5–3.3

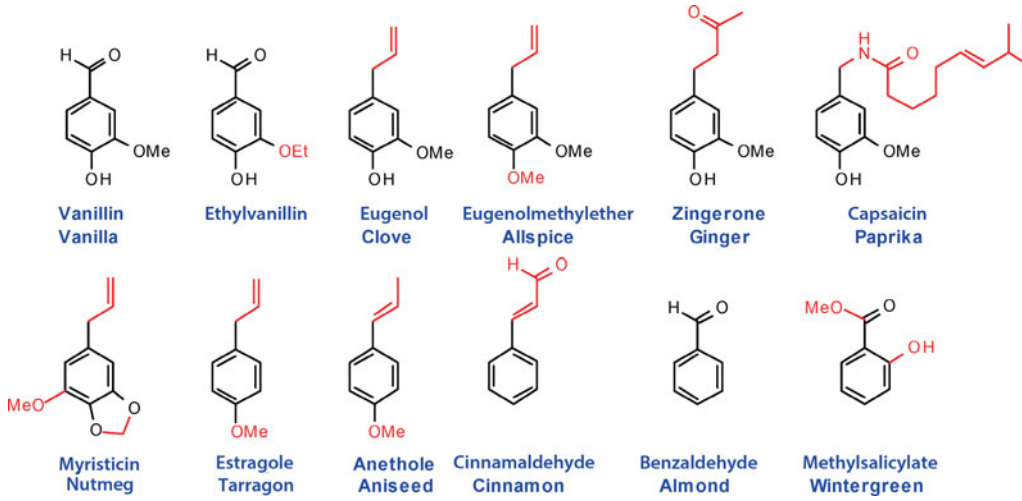
The best variety is Bourbon vanilla or Mexican vanilla, which is native to Central and South America. Next to this is Tahiti vanilla (*Vanilla tahitensis*), which grows in Oceania. The most important sources of vanilla are Madagascar, the Comoro Islands, Réunion, Mexico, Uganda and French Polynesia. [138]

3.5.2 Odour and Taste

Structurally, vanillin belongs to a large group of benzenoid plant constituents with similar substitution patterns (Fig. 3.34): these produce the distinctive aro-

Capsaicin is also used in repellent sprays: at 2 % concentration for use against humans, 3.5–5 % against dogs and 10 % against bears (but without guarantee!)

mas which are characteristic of numerous spices. It is impressive that minor structural alterations cause a drastic change in smell and taste. Ethylvanillin, a commercial synthetic aroma chemical, has an aroma three-and-a-half times stronger than vanillin itself. Where the methoxy- and hydroxy-groups are lacking, the compounds have a pleasant smell and taste of almonds or cinnamon. With an increasing number of substituents, the flavour changes to bitter and sharp. Capsaicin is the sharp-tasting component of chilli peppers (*Capsicum annuum*), and is one thousand times sharper than zingerone. [139]



3.34 *Plant constituents related to vanillin (ethylvanillin is a synthetic analogue).*

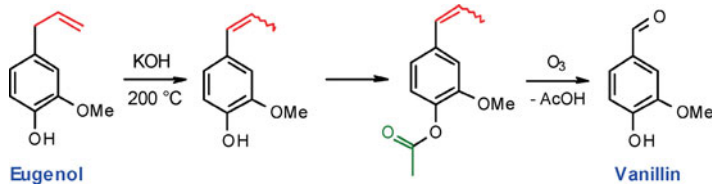
From the biogenetic viewpoint, these are all phenylpropanoids, which are derived from the amino acid phenylalanine.

3.5.3 Chemical Syntheses

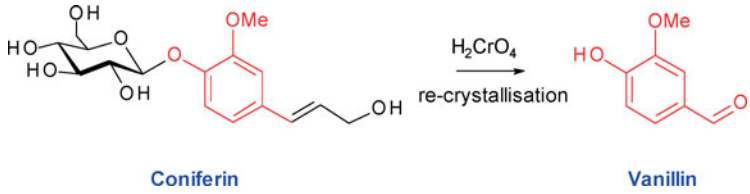
First Syntheses from Eugenol

Vanillin was isolated for the first time from vanilla pods by Nicolas-Theodore Gobley in 1858, and by 1874 its structure was elucidated by Ferdinand Tiemann and Wilhelm Haarmann. Because of its high price and a considerably variable product yield, dependent on weather conditions, there was a strong incentive to prepare vanillin synthetically. This was the time, when the scent and flavours industry was born.

In the 1870s, synthetic vanillin was already available in France and the USA for 175 dollars per kilogram. The starting material was eugenol, the most important constituent (85–95 %) of the oil from cloves (*Eugenia caryophyllata*). Isomerisation of the double bond by potassium hydroxide at 200 °C gives isoeugenol, which, after neutralisation with sulfuric acid, is extracted with benzene. The hydroxy-group is acetylated and finally the double bond is cleaved with ozone, potassium permanganate or potassium dichromate.



At the same time, Wilhelm Haarmann in Holzminden (Germany) prepared vanillin directly by oxidation of coniferin, which is a glucoside obtained from the cambial sap of various conifers (Fig. 3.35). [140]

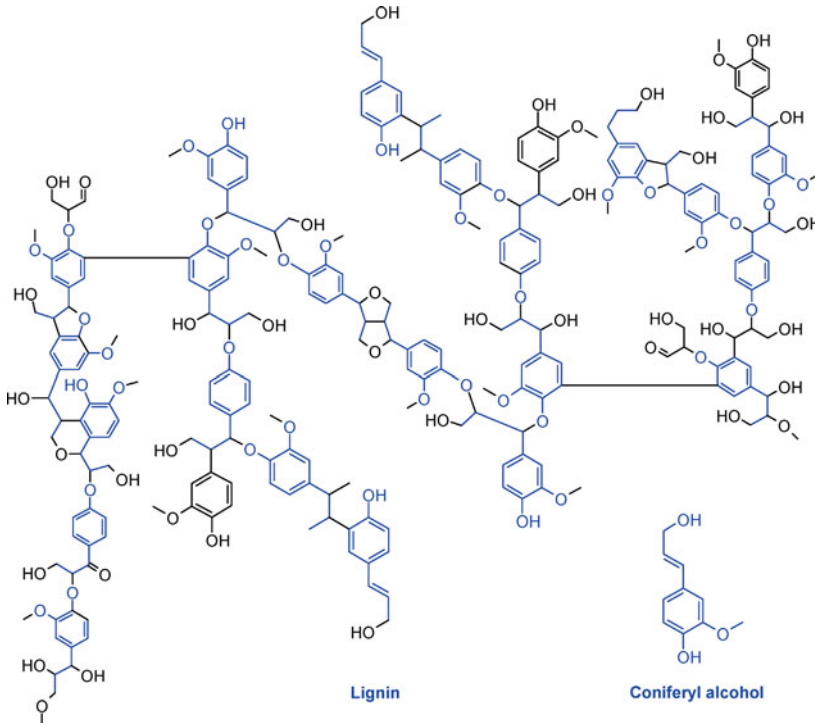


At that time, the price of synthetic vanillin was approximately in the same range, between 180 and 800 dollars per kilogram, as the natural product from vanilla pods. Up to the end of the 1920s, eugenol from clove oil served as the starting material for vanillin production.

3.35 *Wilhelm Haarmann's patent for the preparation of vanillin.*

Synthesis from Lignin Sulfonates

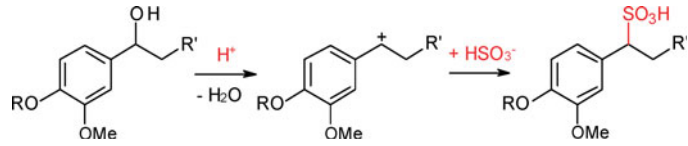
Apart from cellulose, wood contains incrustations of lignin, gums (*e.g.* xylans) and resins (Fig. 3.36).



3.36 *Lignin consists of macromolecular branched-chain compounds, the building blocks of which are dihydroxyphenylpropanes.*

In 1956, Karl Freudenberg at the University of Heidelberg had already succeeded in using an enzyme (phenol-oxidoreductase) from button mushrooms (*Agaricus bisporus*) to simulate the oligomerisation of coniferyl alcohol and thereby the biosynthesis of lignin. [141]

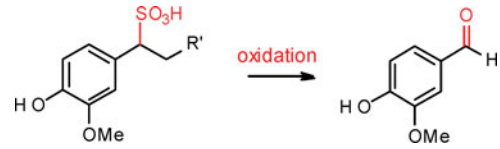
For the preparation of paper, the incrustations must be removed. To accomplish this, there are two main processes: decomposition with sodium hydroxide ("soda pulp") or with calcium hydrogen sulfite ("sulfite pulp"). The soda pulp process generates water-soluble phenolates of lignin. In a remarkable manner, the hydrogen sulfite process yields benzylsulfonic acids (Holmberg decomposition).



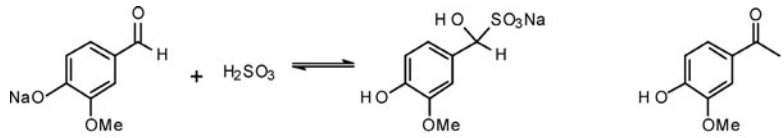
The sulfite pulp procedure was invented by the American brevetted Brigadier General Benjamin Chew Tilghman (1821–1901) in 1867.

In the Howard process, the lignin sulfonate is precipitated by an excess of lime (aqueous solution of calcium hydroxide). Depending on the reaction conditions, one may obtain lignin sulfonates of variable quality; the greatest influences being the temperature (125–130 °C), the duration of the reaction (7 or 6 hours) and the Ca^{++}/SO_2 ratio.

The first indication that vanillin could be obtained out of the sulfite washings from paper production came in the year 1875, and was due to the vanillin-like smell of these solutions. In order to obtain the vanillin, the sulfonated lignin fragments were oxidised under alkaline conditions: often the oxidants used are atmospheric oxygen or persulfate in the presence of a copper salt. Ceric or cobalt salts are also efficient catalysts. The mechanism proceeds *via* free benzyl radicals. The yields of vanillin range between 4–6.2 %.



Purification of the vanillin is effected by re-extraction with sodium hydroxide and treatment of the extract with sulfurous acid: this gives the water-soluble bisulfite adduct. Filtration then separates out acetovanillone (also known as apocynin) and other phenols. For use in foodstuffs, vanillin must be neutralised, vacuum distilled and recrystallised once more.

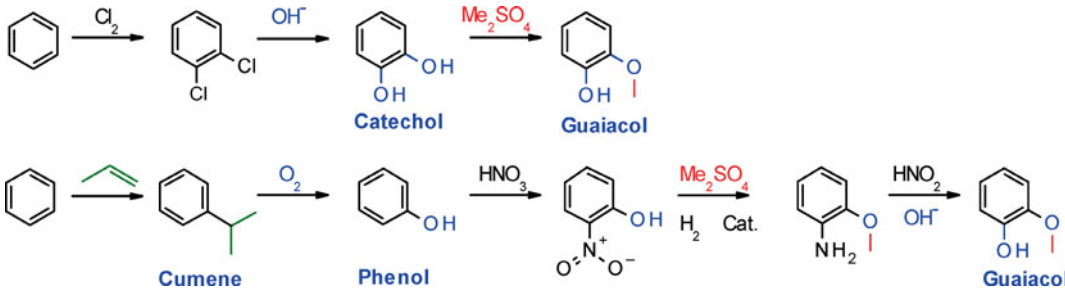


Acetovanillone

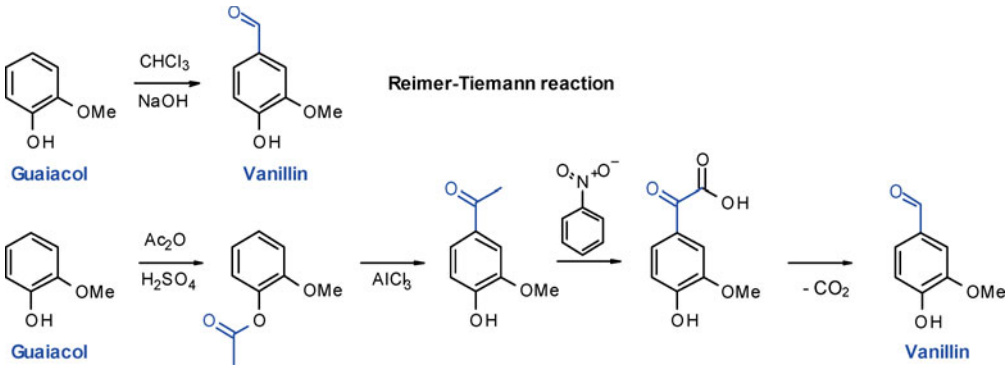
Only Borregaard in Sarpsborg, Norway, still prepares vanillin from lignin. Under licence from Monsanto, Borregaard uses a process which differs from the previously used procedures by ultrafiltration of the sulfite washings. Thereby, low-molecular-weight components, which cannot be oxidised to vanillin, can be separated out.

Synthesis from Guaiacol

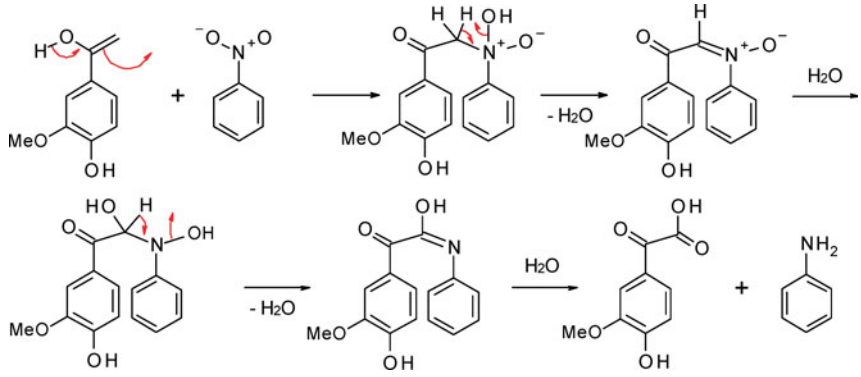
Nowadays, synthetic vanillin is obtained predominantly from petrochemical sources. For vanillin, the key compound is guaiacol (*o*-methoxyphenol). This is obtained initially from wood tar, coal tar, wood distillate or lignite distillate. Targeted syntheses start from benzene; *via* dichlorobenzene, catechol may be obtained, which is further monomethylated. The second route begins with the oxidation of cumene (the so called "Hock synthesis") to give phenol, and continues with nitration, methylation, reduction, diazotisation and heating of the diazonium salt solution. [142]



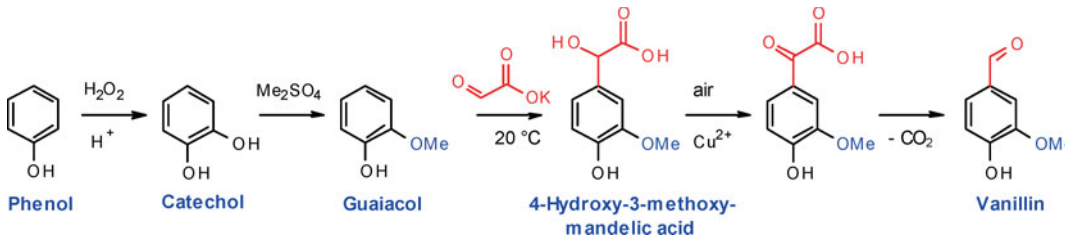
For the introduction of the aldehyde group there is also a range of possibilities. The synthesis of vanillin was one of the first industrial applications of the Reimer-Tiemann reaction. [143] The Fries rearrangement of guaiacol acetate gives acetovanillone, which must then be degraded to vanillin. Oxidation of the rearrangement product with nitrobenzene leads to vanilloylformic acid, which is decarboxylated. The disadvantages of this sequence are the number of stages, and also the stoichiometric amounts of the co-product, aniline, and other reduction products of nitrobenzene.



The mechanism of the oxidation with nitrobenzene in basic media is not certain. [144] It is interesting to note the similarity with the second indigo synthesis of Adolf von Baeyer (*cf.* section 2.1.4).

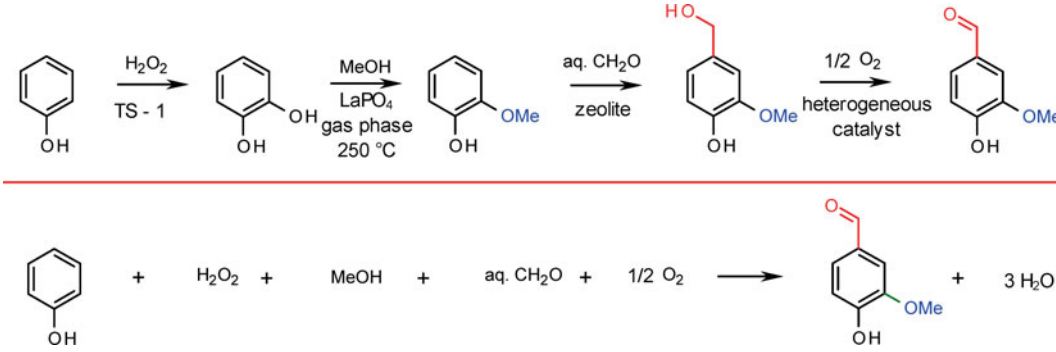


Since the 1970s, Rhône-Poulenc has used a process in which phenol is hydroxylated to catechol by hydrogen peroxide in acidic media. The introduction of the formyl group involves a modified so called Riedel process using glyoxylic acid, which itself results as a by-product in the preparation of glyoxal from acetaldehyde, or is obtainable directly by nitric acid oxidation of glyoxal. After methylation of the catechol, the product is treated in dilute alkali with glyoxylate at room temperature. The potassium salt is being preferred to the sodium salt for its geater solubility. Also, in order to avoid double alkylation, an excess of guaiacol is used and the surplus recycled. Oxidation of the 4-hydroxy-3-methoxymandelic acid uses air and a copper(II) catalyst. [145, 146] Impure vanillin is obtained by acidification and spontaneous decarboxylation. Commercial quality material is obtained by vacuum distillation and recrystallisation.



The advantages of this process are the avoidance of co-products and the high regioselectivity of the alkylation, *para*- to the hydroxy-group, so that costly separation operations can be avoided.

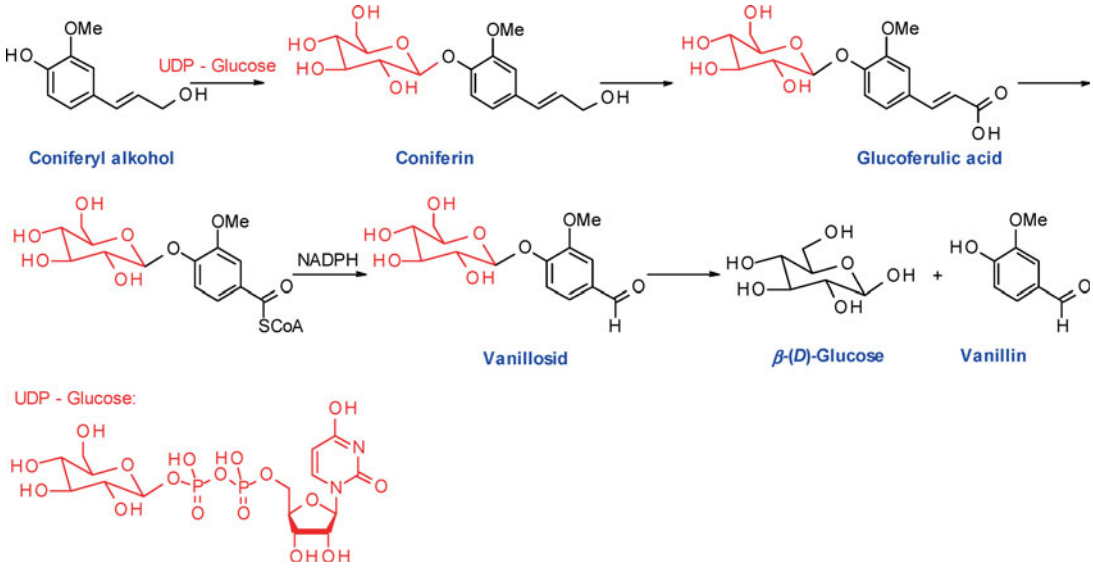
Rhône Poulenc developed the process further, with the aim of improving the atom efficiency even more, and especially to use alternatives to dimethyl sulfate and glyoxylic acid. This involved a gas-phase methylation with a lanthanum salt as catalyst, and a zeolite-catalysed hydroxymethylation with formaldehyde. [147, 148]



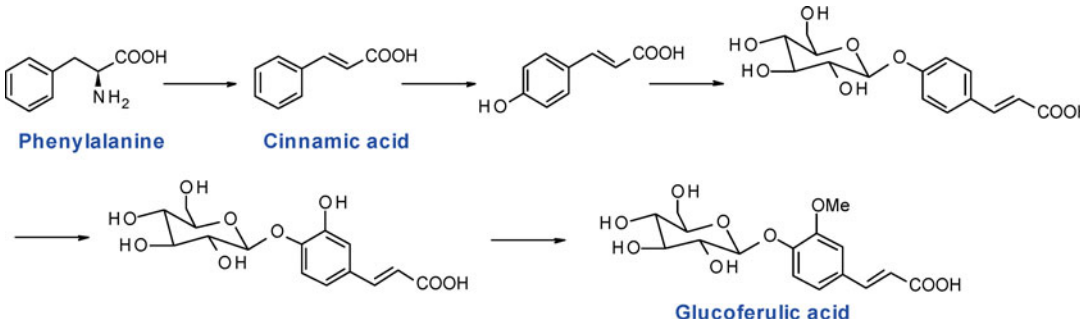
3.5.4 Modern Developments

More recent reports in the literature indicate that vanillin may be produced in the future by biotechnological processes. Important criteria will be the price of the starting materials, the space/time yields, and the disposal of effluent and waste by-products.

The biosynthesis of vanillin is fairly complex. In 1965, on the basis of the observation that ferulic acid was better incorporated into the biosynthetic pathway than vanillic acid, Meinhart Hans Zenk (1933–2011) proposed the following synthetic route: Coniferyl alcohol is bonded to glucose by means of the enzyme coniferylalcohol-glucosyltransferase. Oxidation of the alcohol function gives the glucoside of ferulic acid, and hence vanillosid by β-oxidation, ester cleavage from the β-dicarbonyl compound, and NADPH reduction; this is cleaved by a β-glucosidase during the ripening process of vanilla pods. [149]

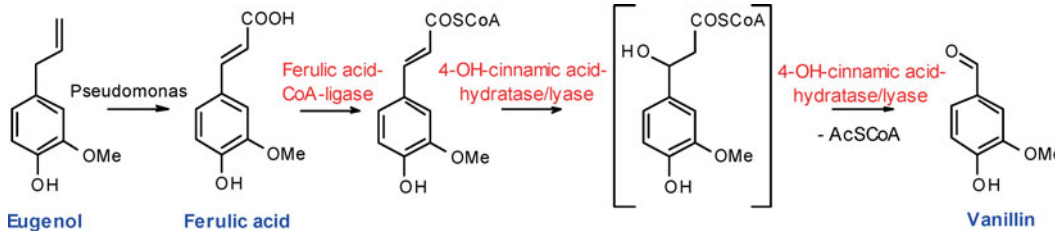


Since the work of Zenk, a series of contributions has appeared, containing other biosynthetic proposals. Ferulic acid is an important intermediate on the biosynthetic pathway to phenylpropanoids. Starting from phenylalanine, this compound is formed on membrane-associated multi-enzyme complexes. Practically at every intermediate stage further routes may branch off, leading to derivatives of benzaldehyde and finally to vanillin. [150, 151]

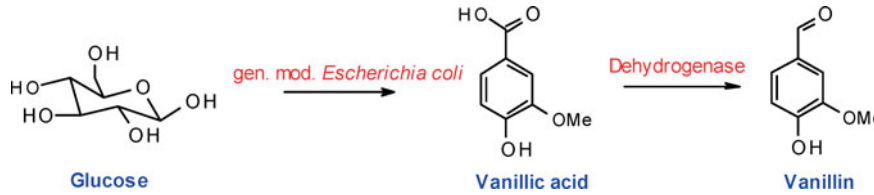


However, ferulic acid is also obtainable from eugenol in an aerobic process, in the presence of *Pseudomonas* strains. In Asia, ferulic acid is obtained form rice bran.

Givaudan developed a strain of *Streptomyces*, which produces vanillin from ferulic acid. *Pseudomonas* strains possess this ability too, and fermentation with *Amycolatopsis* leads similarly to vanillin. The most important producers of vanillin from ferulic acid are Symrise and Rhodia (*Rhovanil Natural*®). [152]



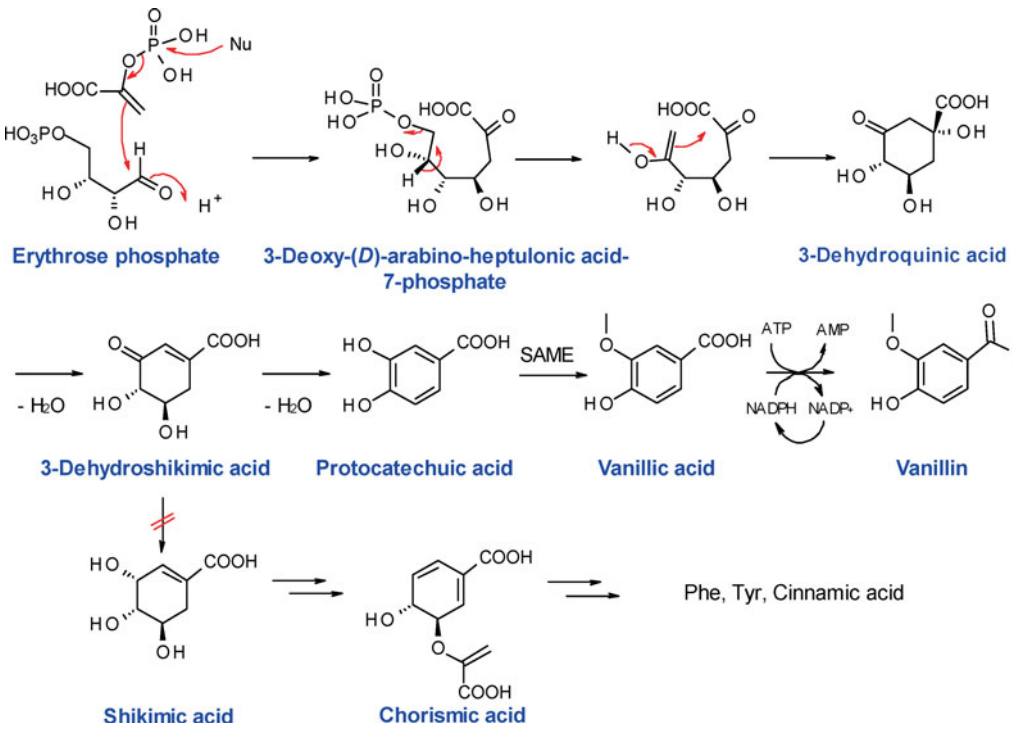
Frost developed another fermentative process for the synthesis of vanillin. [153] In this case, the starting material is the inexpensive glucose, which is converted into vanillin with genetically modified *Escherichia coli*. The reduction to vanillin is catalysed by an acetaldehyde-dehydrogenase enzyme from the fungus *Neurospora crassa.*



The starting compounds for the biosynthesis of vanillic acid in genetically modified *Escherichia coli* are erythrose 4-phosphate and phosphoenol pyruvate. The erythrose is an intermediate product in carbohydrate metabolism (Calvin cycle, "dark reaction" of photosynthesis). [154, 155] Phosphoenol pyruvate is produced in several steps from 3-phosphoglyceric acid, or from a technical point of view, from succinic acid *via* the citric acid cycle. [156]

The enzyme-catalysed condensation of erythrose and phosphoenol pyruvate leads to 3-deoxy-(*D*)-arabino-heptulosonic acid 7-phosphate: this loses phosphate to give an enol, which cyclises to 3-dehydroquinic acid. Aromatisation goes along with the loss of two molecules of water. Finally, a catechol-*O*-methyltransferase from *S*-adenosylmethionine (SAME) brings about methylation of the hydroxy-function. Vanillic acid is the end-product obtained from the microorganism.

For the synthesis of vanillin itself, there follows, in a separate step, a further enzymatic reduction of the carboxylic function. To recover the $NADP^+$, the reaction product is stirred for 7 hours at 30 °C together with glucose in the presence of the arylaldehyde-dehydrogenase from *Neurospora crassa* and glucose phosphate dehydrogenase.

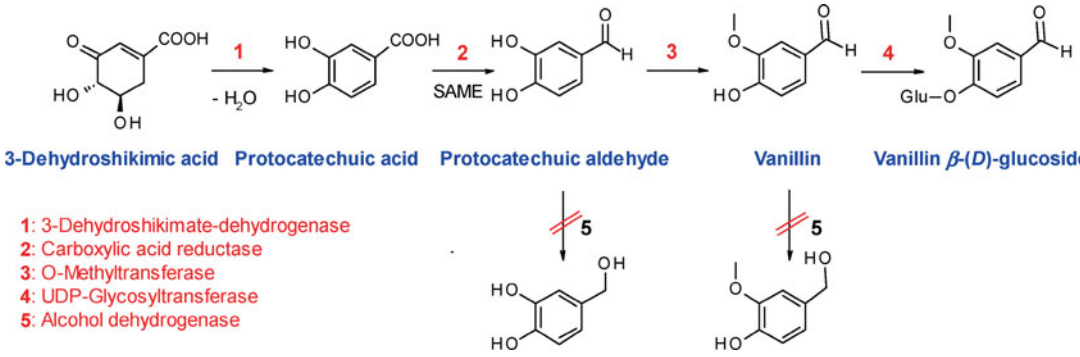


Because of the lack of shikimic acid dehydrogenase, 3-dehydroshikimic acid accumulates. Catechol-*O*-methyltransferase is not very selective. Apart from methylation in the *meta*-position, the *para*-hydroxy group is also methylated to an extent of *ca*. 20 mole %. The addition of methionine increases significantly the vanillic acid content in the product mixture.

Of prime importance for the industrial production process is an improvement in the selectivity of the methylation. Catechol-*O*-methyltransferases are widely distributed, so that there is a good chance that such an improvement may be attained with an appropriate isozyme.

The separate reduction of vanillic acid with an enzyme from *Neurospora crassa* poses a major drawback to the whole process. For an industrially viable process, it is essential to carry through all the steps of the biotechnological synthesis of vanillin with a single intact organism.

Recently, Danish researchers have used gene technology to modify African beer yeast (*Schizosaccharomyces pombe*) so that it produces the glucoside of vanillin directly. [157] The genetic modifications comprise the incorporation of a 3-dehydroshikimate-dehydrogenase from the dung mould *Podospora pauciseta*, an aromatic carboxylic acid reductase from *Nocardia* sp., and an *O*-methyltransferase from *Homo sapiens*. To ensure that vanillin is not reduced to vanillyl alcohol, the alcohol dehydrogenase ADH6 was deactivated. The biosynthesis was further improved by introduction of a UDP-glucosyltransferase from *Arabidopsis thaliana*: this converts vanillin into its *β*-(*D*)-glucoside, which is not toxic to the yeast cells and may therefore accumulate in larger amounts. It was possible so far to achieve a productivity of 45–65 mg per litre of the fermentation broth. For an industrial application this certainly is still much too low.



The advantages of biotechnological syntheses of vanillin are evident, in that toxic and mutagenic compounds such as phenol and dimethyl sulfate are avoided, and also that corrosive substances like hydrogen peroxide are not necessary. Finally, glucose is an inexpensive renewable raw material, which is converted into vanillin by way of well-defined intermediates.

The fermentative production of vanillin is elegant, and with future optimisation and improvements at various stages, it may still have the chance to become the basic pillar of a fourth generation of industrial vanillin syntheses.

3.5.5 Applications

The most successful perfume of all times is *Chanel No. 5*®, which dates back to the year 1921. It consists of aliphatic aldehydes, with scents reminiscent of rancid

butter and candles, also of exotic blossom scents, civet, musk and vanilla. *Chanel No.5*® not only represented the beginning of a new epoch of designing perfumes, but also paved the way for new synthetic scented materials. Musk and vanilla are the characteristic components of these perfumes with an oriental character. An example of a modern product in this category is *Un Bois Vanille*® (Serge Lutens) (Fig. 3.37).

In terms of production quantity, vanillin is one of the most important scent and aroma chemicals. It is the main component of natural vanilla flavour, which has been used as a spice for centuries. Nowadays, vanilla is used as a flavour in many desserts, drinks, chocolate, confectionery, ice cream and bakery products, as well as in perfumery.

The world-wide demand for vanillin amounts to 15,000–16,000 tonnes annually. Its main customer is the flavours industry, which accounts for 84 %; 13 % find application in drug preparation (*e.g.* (*L*)-DOPA and papaverine), but only 3 % in perfumery. With a price of 10–15 Euro per kilogram, the market is worth around 180 million Euro per annum. Only 20–40 tonnes of vanillin come directly from natural sources. [153, 157] This corresponds approximately to 1,500–2,000 tonnes of dried vanilla pods.

3.37 *Un Bois Vanille*® *is a valuable perfume from the absolue of Mexican vanilla.*

Summary in Bullet Points

- Vanillin is an important aroma compound, which in part is brought to the market in the form of vanilla.
- The majority is produced by chemical synthesis according to a process of Rhône-Poulenc.
- Biotechnological processes have a good chance of replacing the chemical syntheses.

3.6 Muscone

For thousands of years, animal secretions, glands and organ parts with intense scent properties have had considerable significance for mankind. Animal-based preparations have been used for religious purposes, as pharmaceuticals and as scents. Right up to the present time, ambergris, musk, civet and castoreum find widespread use in fine perfumery. Just as with rose ketones, also here it holds true: less is often more. Fresh musk glands have a urine-like, sweetish smell and the gland secretion of the civets has an unpleasant, sweetish-faecal smell as well, which is caused by traces of skatole (3-methylindole). [158, 159] In high dilution, however, it develops a wonderful flowery scent.

3.6.1 Natural Sources

"Musk" refers to the exocrine scent glands (called musk pods) of the musk deer (*Moschus moschiferus*) (Fig. 3.38), which are externally visible in the vicinity of

The word "musk" is due to an anatomical error. It comes originally from a Sanskrit word *Muṣká* meaning "testicle".

the male sex organs. During the rut, these are the size of hens' eggs. The secretion serves for territorial marking and the attraction of females (*i.e.* as a pheromone). The musk deer lives in the high valleys of the Himalayas, in Nepal, Myanmar, Tibet, the Tongkin area (the northernmost part of Vietnam) and Siberia. The first authentic descriptions of these scent glands in Europe come from the travel reports of Marco Polo (1254–1324). Their use as drugs, especially against the plague, goes back to the School of Medicine in Salerno. It was only in 1891 that musk disappeared from the pharmacopoeia. At that time, the highly-prized Tongkin musk was worth twice the price of gold. Around 1920 well over 100,000 animals were slaughtered in China, to an extent, that this species became almost extinct. The Chinese way of species protection at that time – cutting off both hands of the poachers – prevented this!

3.38 *Since 1973, the musk deer has been a protected species in all parts of East Asia, and the black market has shrunk to a minimum. In recent times, attempts have been made to obtain musk by curettage (scraping out of the scent glands) of live animals raised on farms.*

Civet is a yellowish viscous secretion of the Large Indian civet (*Viverra zibetha*) and African civet (*Civettictis civetta*) (Fig. 3.39). Both sexes use it for the marking of their roaming area. For centuries the scented material is obtained by curettage of the perineal glands, located near the sexual organs of the civets.

The muskrat (*Ondatra zibethicus*) is a rodent, around 30 cm long, belonging to the family of voles; it is valued especially for its fur (Fig. 3.40). Originally native to North America, the muskrat is also found nowadays in central Europe. The muskrat has "pockets" near its sexual organs, which produce a musk-like scent. A chemical process of extracting it was developed in the 1940s, but it did not prove commercially worthwhile. [158]

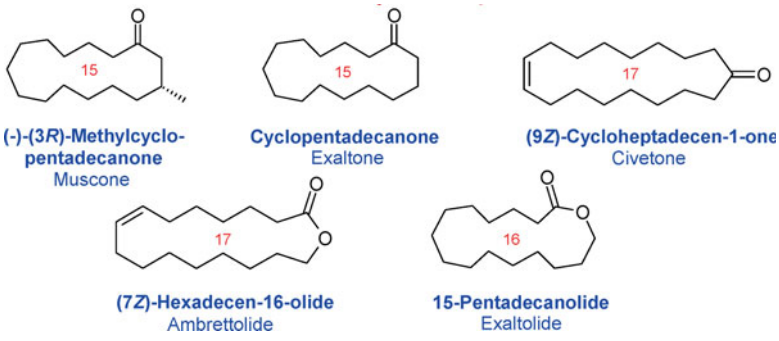
3.39 *Viverra zibetha (a) and Civettictis civetta (b) are among the largest representatives of the family of Viverridae. African civets have been kept in captivity and milked for their civet which is diluted into perfumes. They can secrete three to four grams of civet per week. [158]*

3.40 *Since the 17th century it has been known that muskrats (Ondatra zibethicus) secrete a glandular substance with a musky odour in order to mark their territories.*

3.6.2 Structure Determination

In 1906, Heinrich Walbaum isolated muscone from 7 kilograms of Tongkin musk by extraction with ether, steam distillation of the residue and renewed fractionation. [159] With good-quality starting material the yield was around 1.2 %.

Civetone, the most important scent-bearing compound in natural civet, was discovered in 1915 by Erwin Sack. [160] At present, Ethiopia still exports around 2 tonnes of civet each year. In a Chinese variant, 14 other structurally similar compounds and traces of muscone were found. In 1926, Leopold Ružička determined the structures of muscone and civetone, and recognised their structural relationship. [161, 162]



3.41 *The Musk mallow (left) is an aromatic and medicinal plant, which is native to India. – The Garden angelica was cultivated as a vegetable and a medicinal plant since the 10th century and achieved popularity especially in Scandinavia.*

The scent from the muskrat consists of eleven ketones, the most abundant of which are cycloheptadecanone (41 %) and cyclopentadecanone (21 %).

This structure determination was not only a great moment in the chemistry of fragrances, but also a pioneering achievement for organic chemistry as a whole, since the very existence of macrocycles had long been deemed impossible on theoretical grounds (Adolf von Baeyer, 1885).

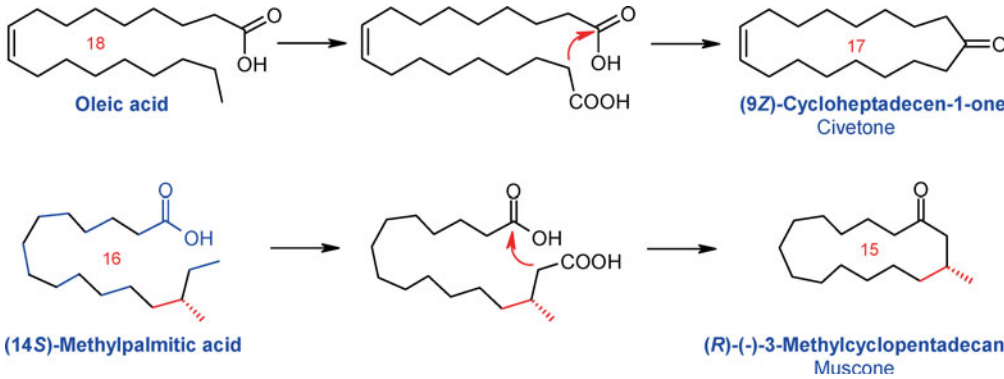
Whereas the musk scents from animals mostly originate from macrocyclic ketones, the corresponding lactones could be isolated from plants. In 1927, Max Kerschbaum at Haarmann & Reimer discovered ambrettolide, a seventeen-membered unsaturated lactone, in the seed oil from the Indonesian musk mallow (*Abelmoschus moschatus*; formerly called *Hibiscus abelmoschus*) (Fig. 3.41). [163]

The scented material from Garden angelica root (*Archangelica officinalis* Hoffm.) is a mixture of ambrettolide and exaltolide (Fig. 3.41). This latter compound is also responsible for the musky character of Oriental tobacco.

Exaltone came to the market at the end of the 1920s, and exaltolide followed in 1933. The sales price for both these fragrances was initially exorbitant (50,000 and 20,000 Swiss francs per kilogram respectively), which – in addition to their fascinating structures – was good enough reason to engage in their total synthesis not only scientifically.

3.6.3 Biosynthesis

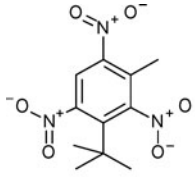
Relatively little is known about the biosynthesis of civetone and muscone. Ružička had recognised already the structural relationship of civetone and oleic acid, and so it could be imagined that civetone might be derived from oleic acid by terminal oxidation and Dieckmann cyclisation.

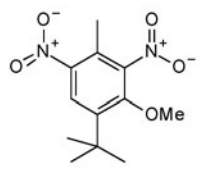


The construction of muscone can be explained in this way: the multienzyme complex of fatty acid biosynthesis is initiated with acetyl-CoA. Then one propionyl-CoA unit is incorporated, followed by six further acetyl-CoA units, resulting in the mono-branched (14*S*)-methylpalmitic acid. Ring closure forms an analogue of civetone. [164]

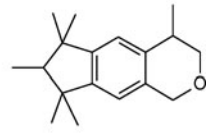
3.6.4 Surrogates

In 1888, Albert Baur at the Chemistry School of Mulhouse (Alsace) discovered by chance, while looking for new explosive materials, that many polynitrated toluene derivatives possess a musk-like odour. [166–168] The current annual production of these compounds amounts to *ca*. 300 tonnes. Their main use is in the soap and cleaning industry. Some of these nitro-compounds are certainly not entirely safe, either ecologically or toxicologically, and their production is therefore in sharp decline. In the meantime, other aromatic compounds, smelling of musk, but lacking nitro-groups, have been recognised; these include, for example, galaxolide, which is derived from tetralin and is produced in quantities of around 4,000 tonnes annually.


Musk Baur



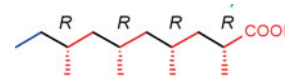
Musk ambrette

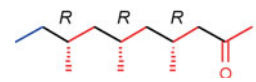


Galaxolide

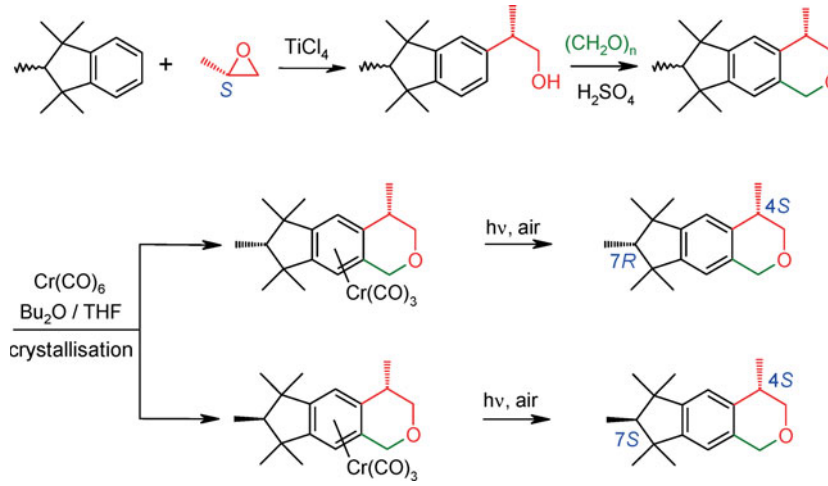
Only recently, the diastereoselective synthesis and preparative separation of the enantiomers of galaxolide (Givaudan) have been described. [169] The titanium tetrachloride-catalysed Friedel-Crafts alkylation of 1,1,2,3,3-pentamethylindane with (*S*)-propylene oxide produces two epimeric alcohols (whereby no racemisation is observed); with paraformaldehyde and catalytic amounts of sulfuric acid, these are converted into the desired isochroman diastereomers. The separation of the epimers is accomplished by means of the corresponding chro-

Geese (*Anser anser domesticus*) produce an exceptionally highly-branched fatty acid in their uropygial gland (lat. *Glandula uropygialis*). A related ketone is the major component in the aggregation pheromone of the storage mite (*Chortoglyphus arcuatus*). Both compounds are considered to be produced by the same biosynthetic pathway. [165]





mium tricarbonyl complexes. The diastereomeric chromium complexes may be separated by flash chromatography, and the decomposition of the metal complexes is achieved using the Jones reagent or by UV-irradiation in the presence of air.

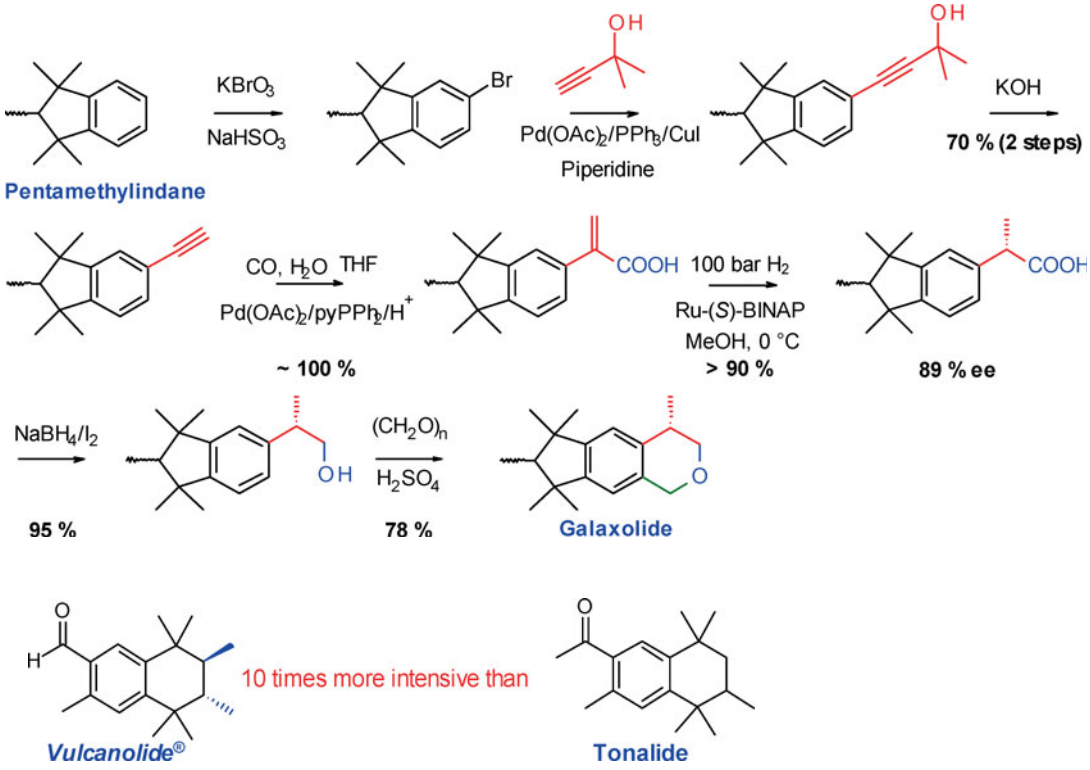


Galaxolide may affect the androgen, progesterone and oestrogen receptors in humans.

Because of the differences in the olfactory properties of the enantiomers (Tab. 3.5), one of the first diastereoselective syntheses of galaxolide realised at industrial scale, aimed to control the centre of chirality at C-4. [170] Bromination of the pentamethylindanes with potassium bromate / sodium hydrogen sulfite avoids the technically far-from-simple procedure using elemental bromine. A Sonogashira reaction with the readily obtainable 2-methylbutyn-2-ol connects the latter moiety directly, and is followed by the cleavage of the protecting group (acetone) prior to work-up. After a palladium-catalysed hydrocarboxylation, the key step of the synthesis is an enantioselective hydrogenation in the presence of a ruthenium-BINAP complex. Reduction of this structural moiety is of more general interest, for example in the preparation of "profens", which are important NSAIDs (*non-steroidal anti-inflammatory drugs*). The carboxylic acid is reduced under mild conditions, and the pyran ring closed using paraformaldehyde.

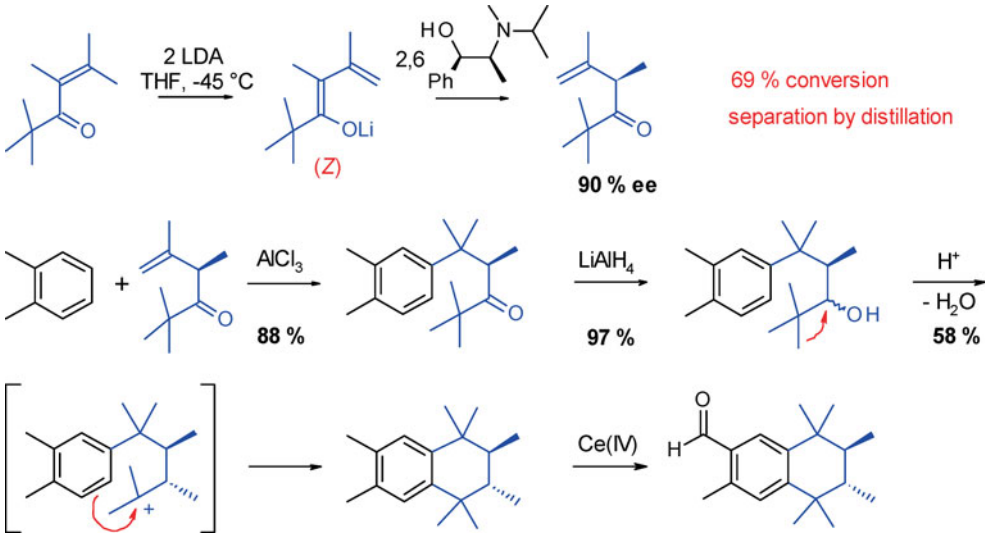
Tab. 3.5 *Olfactory differences of various isomers of Galaxolide.*

Isomer	Scent threshold (ng/l)	Description
(4*S*,7*R*)	0.63	pure musk scent
(4*S*,7*S*)	1.0	similar to (4*S*,7*R*), but dry character
(4*R*,7*S*)	130	Uncharacteristic
(4*R*,7*R*)	440	fruity, almost odourless



The variety of uses of (–)-isopropylephedrine as a chiral auxiliary in enantioselective protonation was demonstrated by Charles Fehr, not only for damascone but also for tetralin-musk-scented compounds (*e.g. Vulcanolide*®). Deprotonation of 2,2,4,5-tetramethylhex-4-en-3-one with LDA gives the dienolate in an (*E*/*Z*)-ratio of 1 : 9. This can be protonated very selectively with iso-

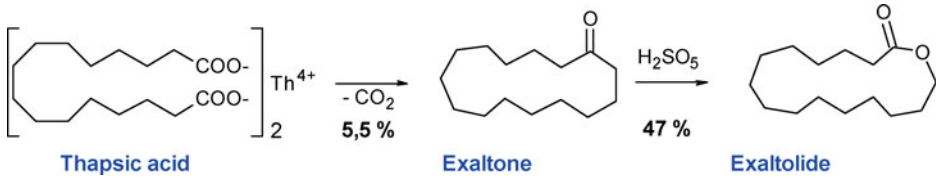
Another important tetralin-musk scent is tonalide. Its worldwide annual production amounts to more than 3.000 tonnes.



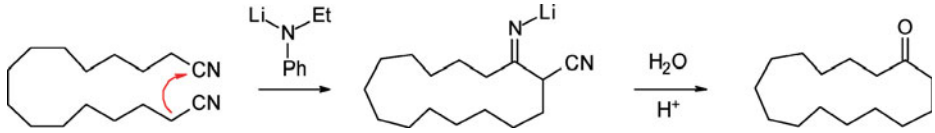
propylephedrine. After distillative removal of unreacted starting material, there follows a Friedel-Crafts alkylation with *o*-xylene. Surprisingly, no considerable racemisation is observed, either at this stage or during the subsequent acid-catalysed cyclisation. The tetralin-musk is finally generated by selective oxidation of one methyl group of the *meso*-compound. [171]

3.6.5 Historical Synthesis of Macrocycles

The first syntheses [172] of macrocyclic scented compound were based on the Piria cyclisation (Raffaele Michele Rocco Piria (1814–1865), Italian chemist) of terminally functionalised aliphatic precursors. Thus, pyrolysis of the thorium salt of thapsic acid (Fig. 3.42) and purification *via* the semicarbazone gave exaltone in a yield of 5.5 % (Ružička cyclisation). The mechanism of this reaction has not been finally established, although it is accepted that free radicals are involved in the cyclisation. [173] Oxidation using Caro's acid then gives exaltolide in 47 % yield.

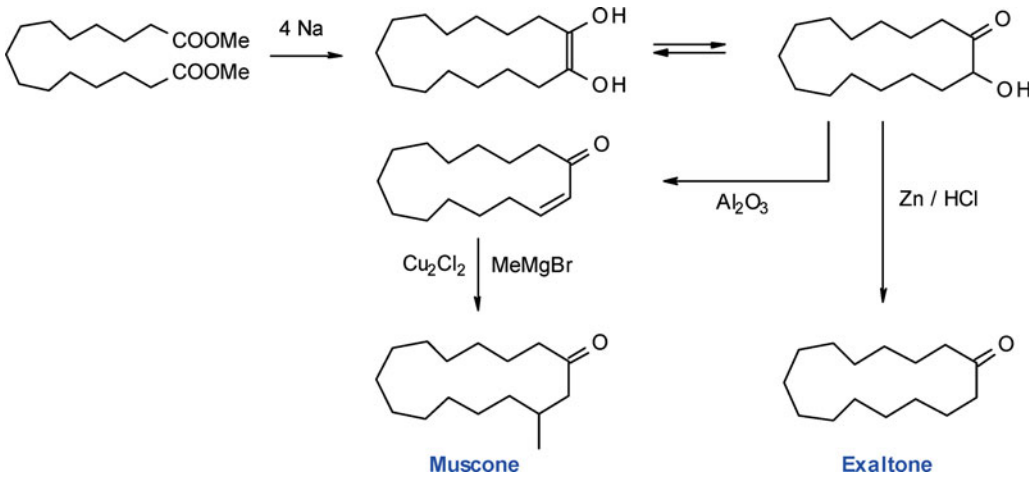


The intramolecular Thorpe-Ziegler reaction of hexadecadinitrile with lithium *N*-ethylanilide according to the Ruggli-Ziegler dilution principle provided a technical advancement. At a concentration of 0.067 mole/l the yield ranged around 60–70 %.



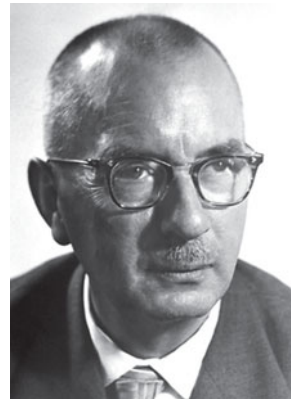
The technical breakthrough was however only attained with the Stoll-Hansley-Prelog procedure for the synthesis of exaltone. By reaction with sodium, dimethyl hexadecanedioate was converted into the acyloin, [174] the reduction of which with zinc and hydrochloric acid (Clemmensen reduction) gave exaltone. On the other hand, dehydration of the acyloin over hot aluminium oxide gave exaltenone, which could be converted by a copper(I)-catalysed reaction with methylmagnesium bromide into (+/–)-muscone.

3.42 *Thapsic acid (hexadecanedioic acid) may be isolated from the dried roots of Thapsia garganica, a Mediterranean, umbelliferous plant, which was known already in ancient times to be poisonous (due to thapsigargin and other components). In Arabian caravans it was called "deadly carrot," because camels would eat it and die quickly.*



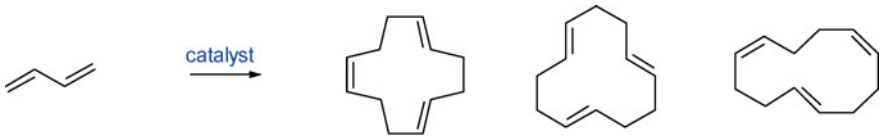
3.6.6 Syntheses of Exaltone

A substantial improvement in the availability of the raw materials came from polymer research. In 1953, Karl Ziegler had discovered the polymerisation of ethylene at normal pressure: he succeeded in polymerising ethylene to polyethylene in a 5-litre preserving jar with a mixture of titanium tetrachloride and diethylaluminium chloride (Fig. 3.43). At the end of the 1950s, Günther Wilke intended to prepare butadiene from acetylene and ethylene with Ziegler catalysts, but preliminary experiments showed that the selected catalysts reacted violently with butadiene, and that the product was not a polymer but cyclododecatriene. Wilke later found, by using Ni(0)-complexes (which he called "naked nickel"), that the isomer ratio was clearly in favour of the all-*trans* isomer.



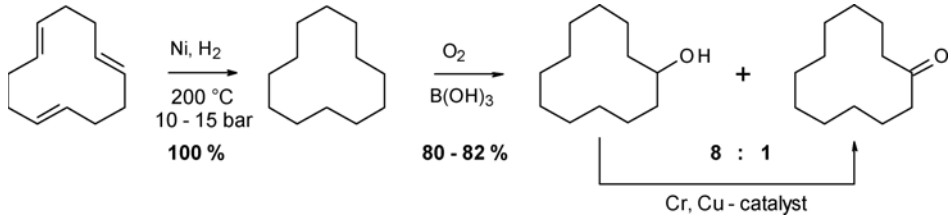
3.43 *Karl Waldemar Ziegler (1898–1973)*

Butadiene trimerisation is carried out nowadays on an industrial scale by Evonik, Arkema, Ems-Chemie and Ube. Cyclododecatriene is the starting material for the synthesis of high-value polyamides (*Vestamid*®). The worldwide production has a volume of around 100,000 tonnes.

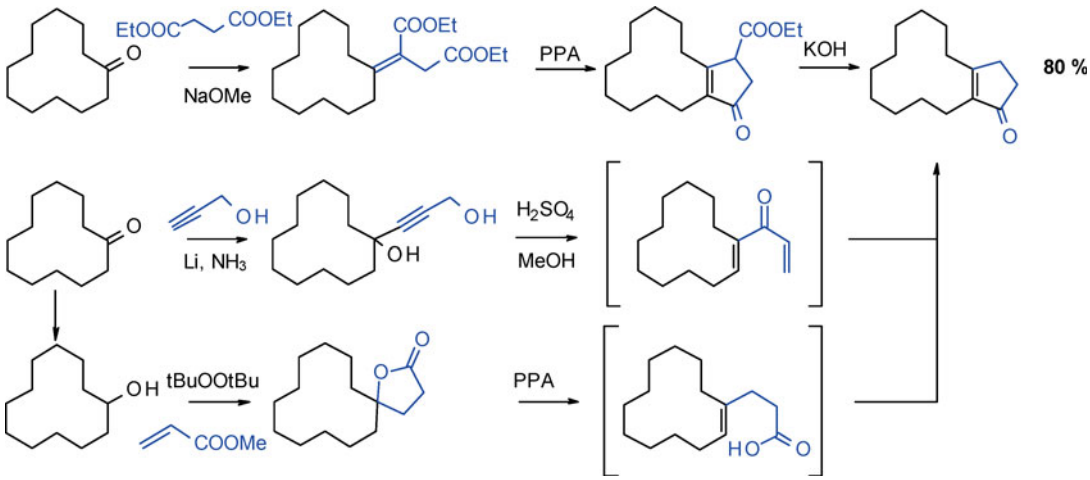


catalyst:	$TiCl_4$, Et_2AlCl	95	5	0
	$Ni(COD)_2$	5	85	10

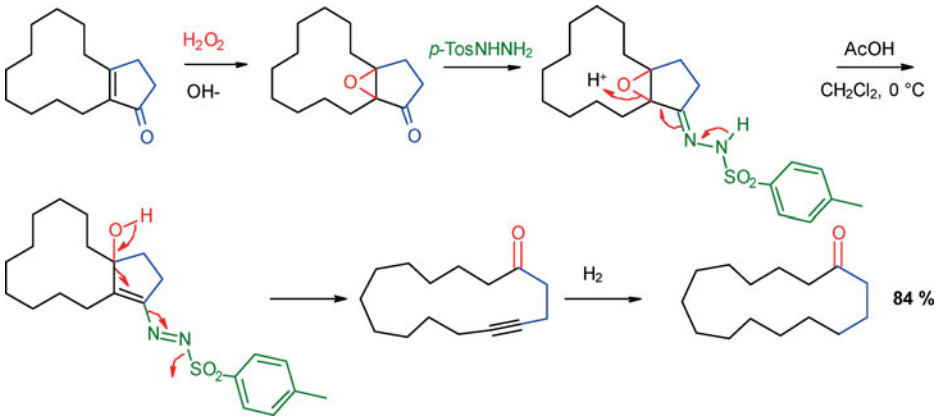
Via hydrogenation and oxidation, cyclododecanone is accessible in large quantities, and serves like cyclohexanone as an intermediate for the preparation of polyamides and polyesters. [175]



Ring expansion by three carbon atoms [176] is achieved by the Stobbe condensation with diethyl succinate [177], treatment with polyphosphoric acid, and decarboxylative hydrolysis; this sequence leads initially to an unsaturated bicyclic ketone. Alternatively, cyclododecanone may be treated with propargyl alcohol; a Meyer-Schuster rearrangement then yields a cross-conjugated dienone, which finally undergoes a Nazarov cyclisation (Ivan Nikolaevich Nazarov (1906–1957), Russian chemist) to produce the desired bicycle. An elegant option is the radical-induced reaction of cyclododecanol with methyl acrylate to give a *spiro*-lactone, which is rearranged to the desired product with polyphosphoric acid.

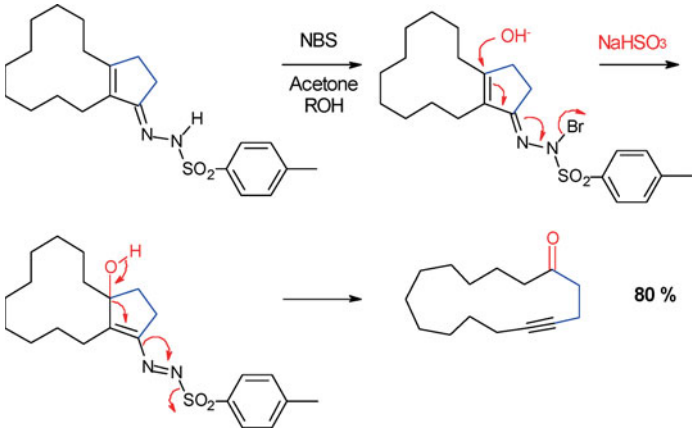


For the ring expansion, a particular method has been developed, which became known as the "Eschenmoser fragmentation". [178, 179] After a Weitz-Schaefer epoxidation and the formation of a tosylhydrazone, its decomposition is carried out at 0 °C in weakly acidic media (acetic acid diluted with dichloromethane), which contrasts the otherwise similar Wolff-Kishner reduction.

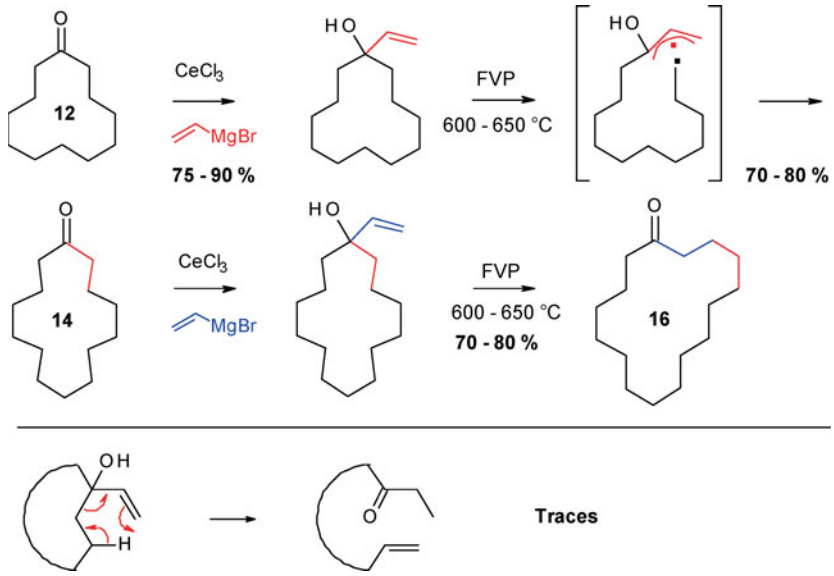


The epoxidation of the unsaturated ketone limits the scope of the reaction and impacts the yield, since a Baeyer-Villiger oxidation is competing. However, at a 100-kilogram scale, the yield ranges around 84 %, based on 50 % conversion of starting material. Another way around this problem is peracid epoxidation of the corresponding allyl alcohol, and oxidation with chromium trioxide: the conversion is then quantitative.

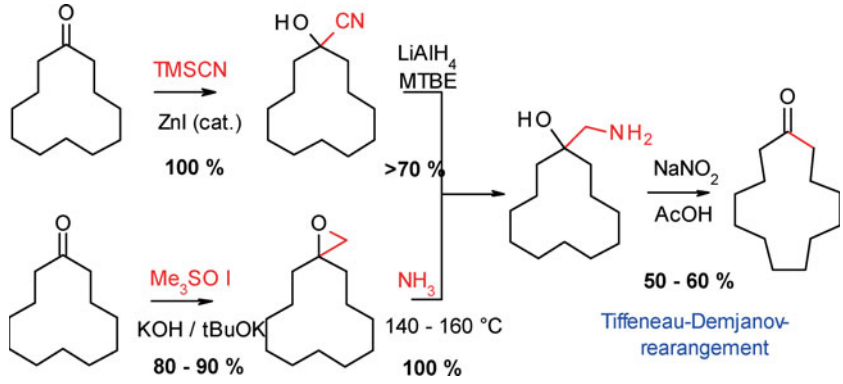
The epoxidation may be avoided at all by bromination of the tosylhydrazone with *N*-bromosuccinimide, followed by cleavage in weak base. The yield is then 80 %. [180]



A modern method of expanding medium and large rings by two carbon atoms uses flash vacuum pyrolysis (FVP) of allyl alcohols. Under the reaction conditions, only traces of the expected [1,5]-hydrogen migration are observed: the main product arises presumably from a [1,3]-carbon shift, a process which involves a diradical intermediate. This ring-expansion sequence may also be repeated successfully. [181]

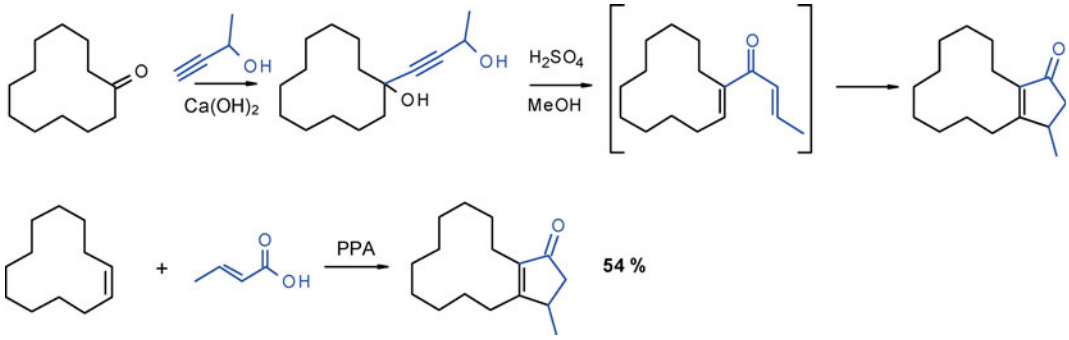


Ring expansion by one carbon atom, for instance by the use of a Tiffeneau-Demjanov rearrangement, provides access to the series of odd-numbered macrocyclic systems. The precursor is readily available through cyanhydrin formation and hydrogenation, or Corey epoxidation and epoxide ring-opening with ammonia.

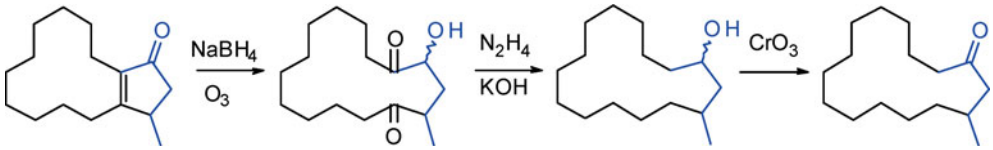


3.6.7 Syntheses of Racemic Muscone

One of the first industrial muscone syntheses stems from work at BASF. [182] Analogous to the synthesis of exaltone, cyclododecanone is converted, by reaction with but-3-yn-2-ol, into a diol, which is further transformed by a Meyer-Schuster rearrangement and Nazarov cyclisation into a methyl-substituted bicycle. A very short route to the same product was described by Mitsui Petrochemical Industries a few years later: this involved the direct conversion of cyclododecene with crotonic acid in the presence of polyphosphoric acid, and resulted in a remarkable yield of 54 %. [183]

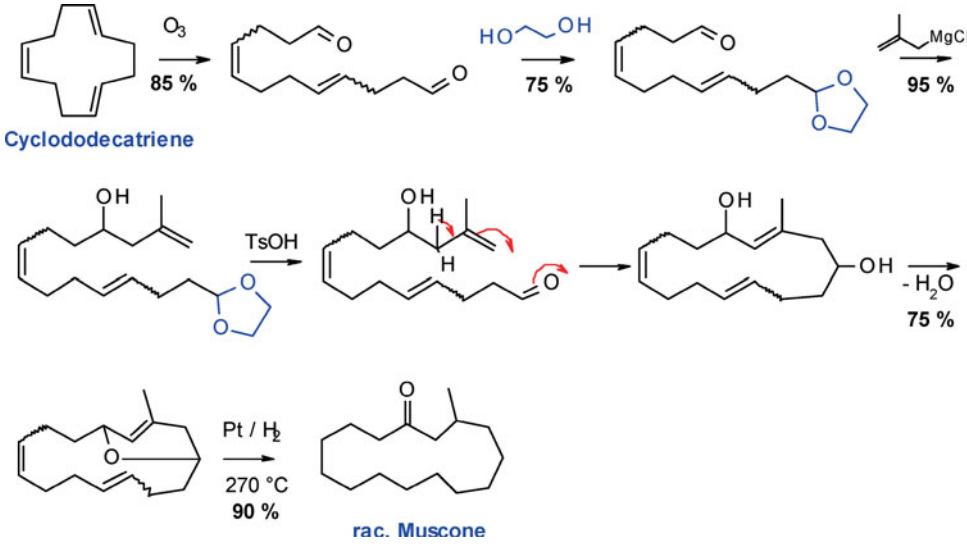


Muscone is then prepared by the following sequence: reduction to the allyl alcohol, ozonolysis of the double bond, Wolff-Kishner reduction of both carbonyl groups, and Jones oxidation. The overall yield is around 40 % based on cyclododecanone.



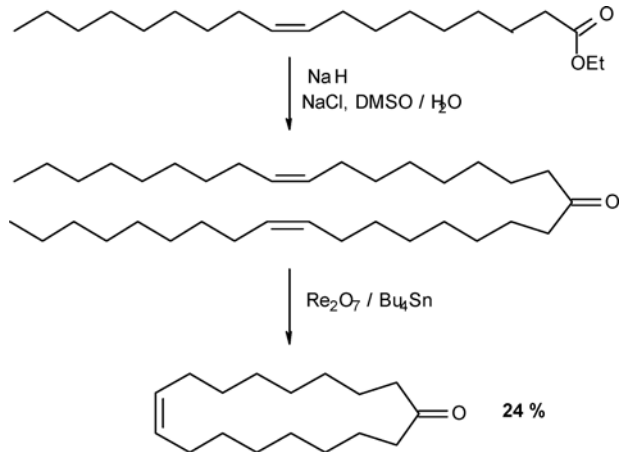
Although the process is industrially feasible, the ozonolysis requires special equipment, and poses potential hazard.

Another efficient muscone synthesis is possible *via* an intramolecular Prins reaction. [184] The starting point is cyclododecatriene, which is subjected to partial ozonolysis. With one aldehyde group protected, the other is reacted with isobutenylmagnesium chloride, and the protecting acetal group is hydrolysed off. Then follows what is formally a Prins reaction, and etherification results in cyclisation to the bicyclic dihydropyran. Hydrogenation (at an unusually high temperature) finally gives racemic muscone.

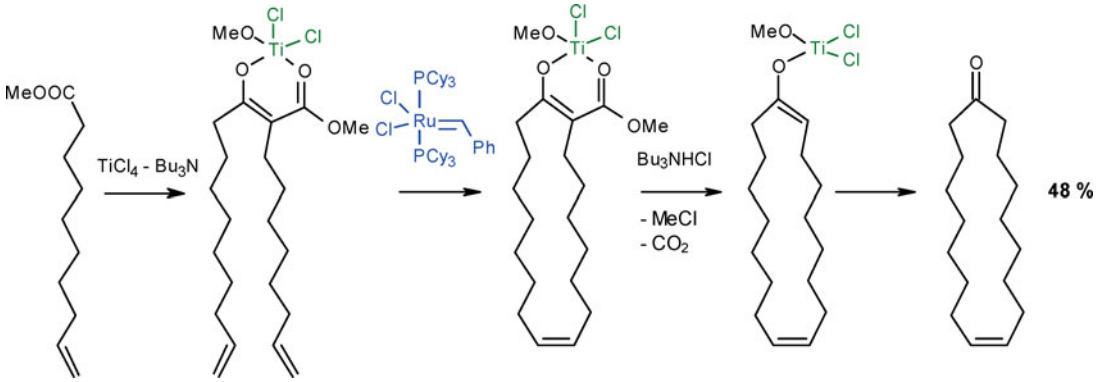


3.6.8 Syntheses of Civetone

After the development of the modern metathesis catalysts, [185] the synthesis of civetone was one of the first applications of ring-closing metathesis. [186] A Claisen condensation followed by metathesis can be used to prepare civetone, but even under extreme dilution (10^{-2}–10^{-4} molar), the maximum yield is only 24 %, because of considerable polymer formation.



Recently Y. Tanabe published a "one-pot" synthesis starting from methyl dec-9-enoate. [187, 188] Key steps are a Lewis acid-catalysed Claisen condensation and a ring-closing metathesis. It is noteworthy that the metathesis proceeds in presence of a titanium enolate complex. Decarboxylation follows spontaneously and gives the product in 48 % yield.

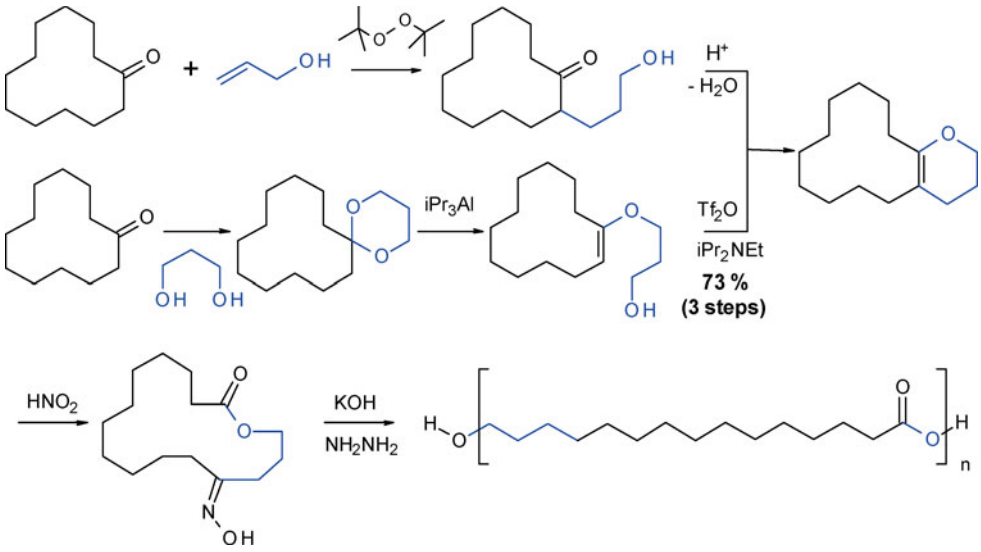


When the synthesis is carried out in a stepwise manner, civetone is obtained with a yield of even 74 %.

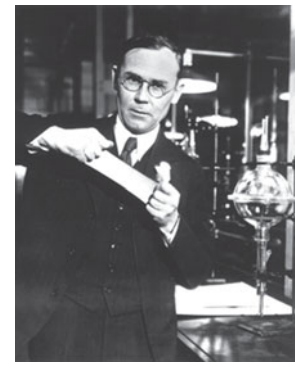
3.6.9 Syntheses of Exaltolide

The industrial syntheses of exaltolide start in part likewise from cyclododecanone. Key reactions are ring expansions and depolymerisation of the polyester of ω-hydroxypentadecanoic acid. Of course, ring-closing metathesis also offers an attractive path to macrocyclic lactones.

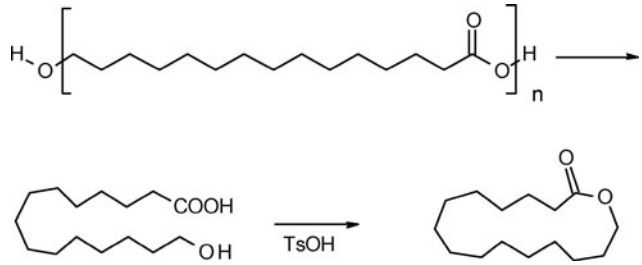
Radical addition of allyl alcohol to cyclododecanone and dehydrative cyclisation gives a bicyclic dihydropyran. The ring-opening of the cyclic acetal of cyclododecanone with triisopropylaluminium and reclosure using trifluoromethanesulfonyl anhydride provides an interesting alternative. The 15-hydroxypentadecanoic acid is then accessible by means of nitrosation, followed by Wolff-Kishner reduction. Apart from the reduction with hydrazine, catalytic, electrochemical and Clemmensen reduction are also well-established.



The $MgCl_2\,6H_2O$-mediated depolymerisation (in a more narrow sense: a decondensation) [189] of the polyester to give 15-hydroxypentadecanoic acid, and the acid-catalysed lactonisation (called 'Carothers synthesis' after Wallace Hume Carothers (1896–1937), an American chemist and inventor of Nylon and Neoprene (Fig. 3.44)) brought about the hoped-for economic breakthrough for exaltolide. The consequence was, that prices tumbled a tenfold, while production rose by three orders of magnitude. Nowadays, a whole range of "depolymerisation catalysts" [*e.g.* PbO, $Al(OMe)_3$, $Ti(OBu)_4$, $Zn(OAc)_2(H_2O)_2$, Bu_2SnO] and procedures is known.

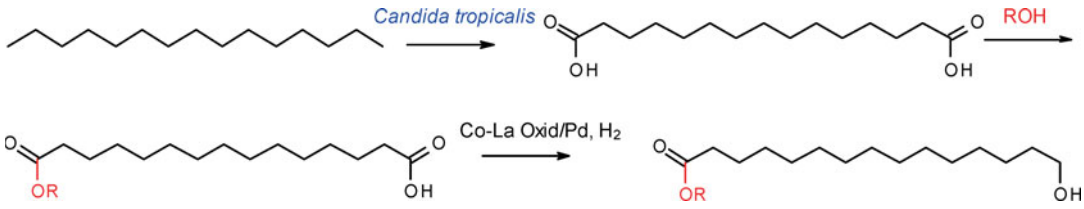


3.44 *Wallace Hume Carothers (1896–1937) was a highly gifted chemist at DuPont, who suffered from mental depression since his youth. Despite his success with nylon, he felt that he had not accomplished much. At the age of 41 he committed suicide with KCN.*

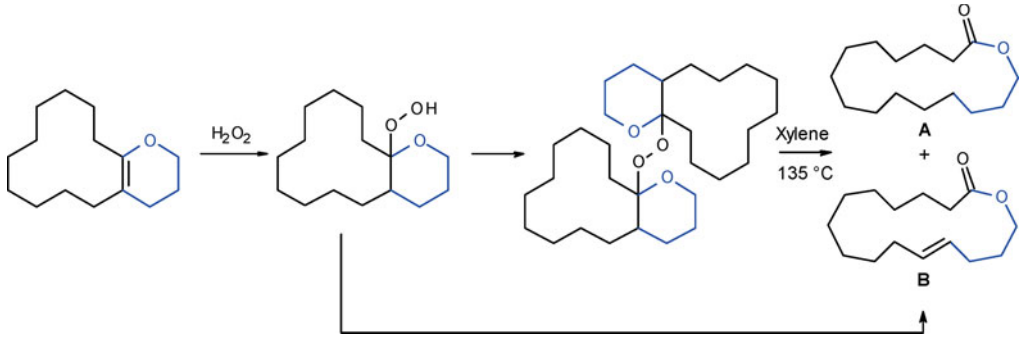


This targeted oxidation of unfunctionalised hydrocarbons is one of the most fascinating reactions, where our capabilities still lag far behind Nature.

A modern approach to 15-hydroxypentadecanoic acid comprises fermentation of pentadecane with, for example, *Candida tropicalis or Cryptococcus neoformans*, to yield pentadecanedioic acid [172, 190], the mono-alkyl ester of which may be reduced catalytically under high pressure to the corresponding hydroxy-ester.



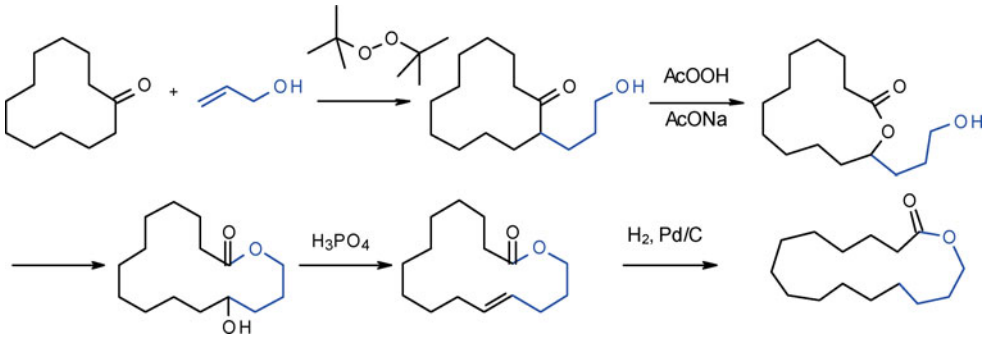
As an alternative to depolymerisation, ring expansions, starting from a common intermediate, have been developed. Hydrogen peroxide adds regiospecifically to the bicyclic dihydropyran. The hydroperoxide reacts with a second molecule of the educt. Thermolysis leads eventually to exaltolide, with xylene serving as the hydrogen donor. Small amounts of 15-pentadec-11-enolide are formed as a by-product (Tab. 3.6).



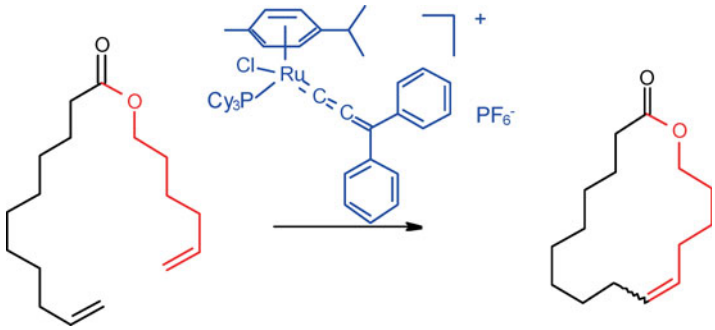
Tab. 3.6 *Control of the product distribution with various reagents.*

Reagent	A : B	Yield (%)
$Na_2S_2O_5$, Na_2SO_3	2 : 1	67
[Fe/Cu]	1 : 9	72
heat, xylene	9 : 1	73

Intramolecular translactonisation is another variant for ring expansion; Baeyer-Villiger oxidation with peracetic acid in a buffered system gives a macrocyclic lactone, which undergoes transformation on thermodynamic grounds into the more stable C_{15} lactone. Dehydration to a mixture of olefins, and subsequent hydrogenation finally gives exaltolide.

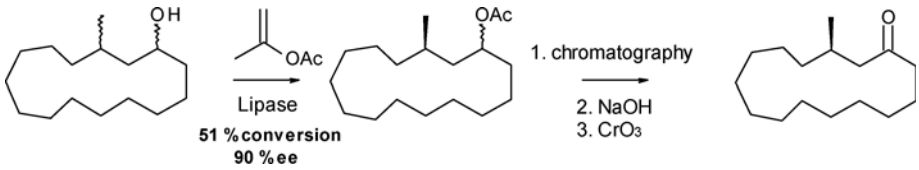


Alois Fürstner used ring-closing metathesis for what is the most elegant synthesis of exaltolide to date. [191–193] Starting from hex-5-enyl undec-10-oate, pentadec-10-enolide was obtained in 90 % yield. Simple hydrogenation of the double bond then gave the desired compound. Since the double bond in a different position would also undergo hydrogenation, a whole range of other starting materials is conceivable.

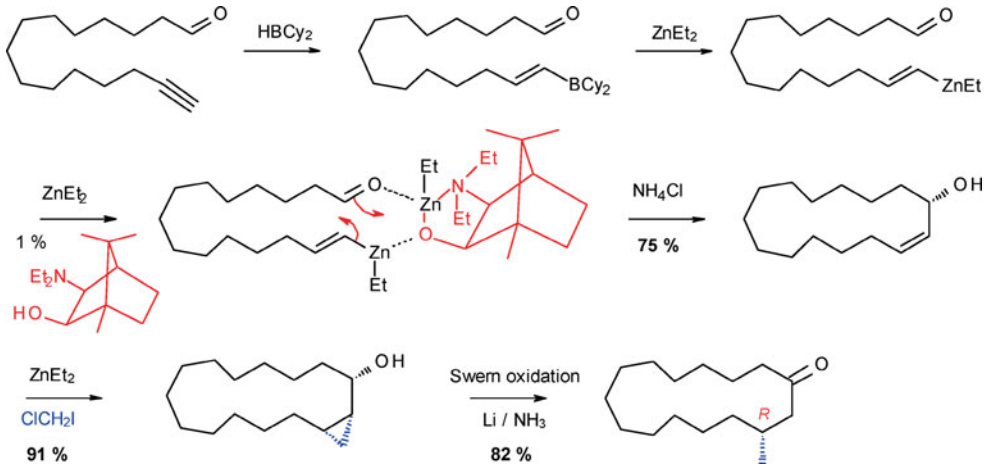


3.6.10 Enantioselective Syntheses of Muscone

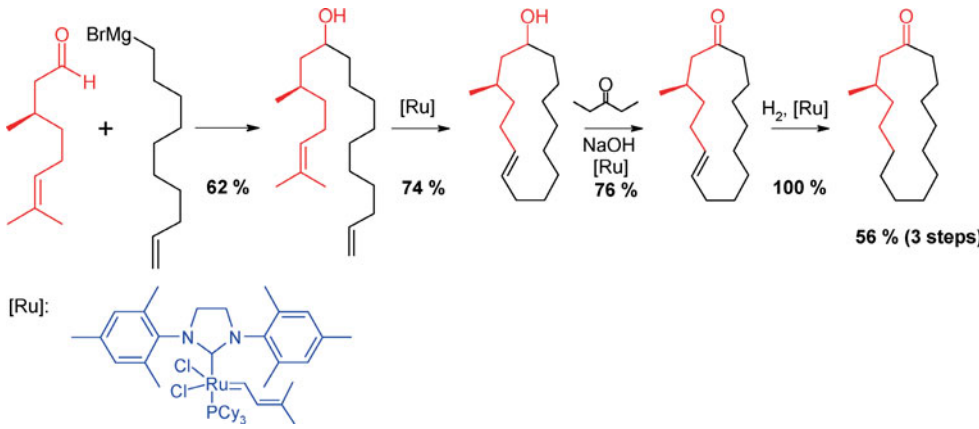
Enzymatic resolution of the mixture of 3-methylcyclopentadecanol diastereomers gives selectively the acetates of the (3*R*)-diastereomers. After chromatographic separation from the unreacted (3*S*)-alcohols, followed by hydrolysis and oxidation, *R*-(–)-muscone is obtained with an enantiomeric excess of 90 %. [194]



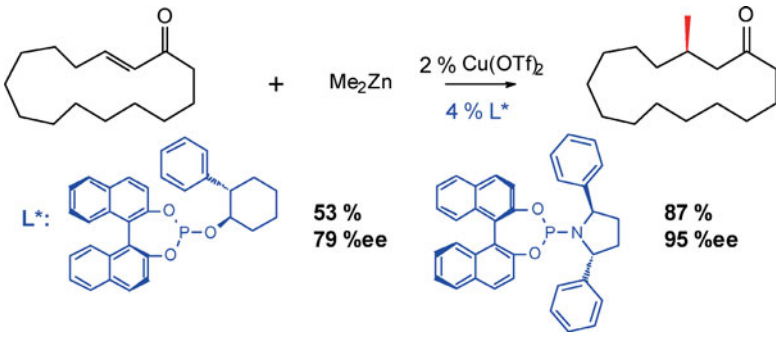
Wolfgang Oppolzer's muscone synthesis is the first enantioselective macrocyclisation. [195] It starts with pentadec-14-ynal, which is converted by hydroboration and transmetallation into the corresponding organozinc compound. The ring closure takes place in the presence of catalytic amounts of a diethylzinc/(-)*exo*-3-(diethylamino)borneol adduct. After work-up, the cyclic allyl alcohol is obtained in 75 % yield and with an ee of 92 %. The hydroxy-group directs the diastereoselective cyclopropanation (Simmons-Smith reaction). The final steps are a Swern oxidation and selective ring-opening of the cyclopropane under Birch conditions.



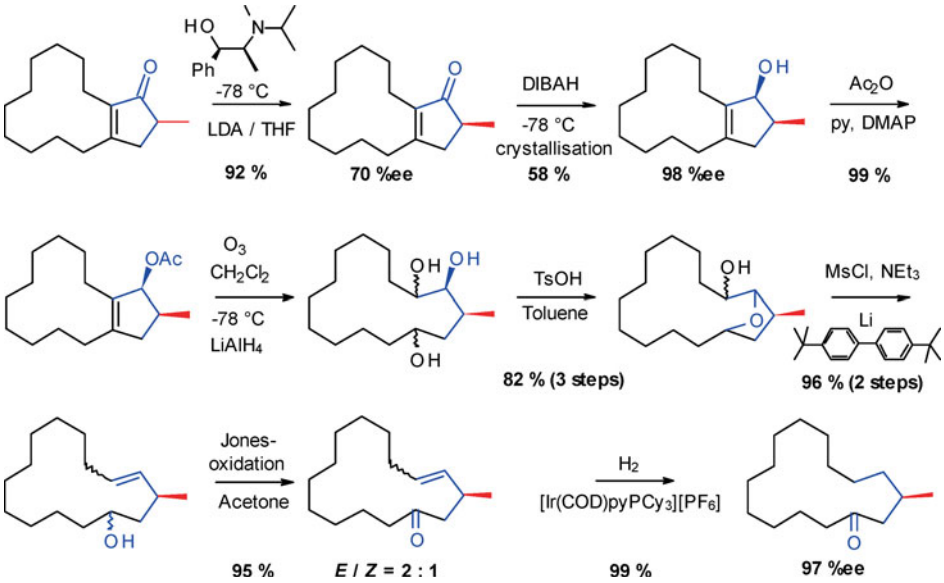
Starting from (*R*)-(+)-citronellal, a diene is obtainable by means of a Grignard reaction: this diene yields (*R*)-(–)-muscone *via* ring-closing metathesis and hydrogenation. [196, 197] With the newer, more reactive imidazol-2-yl-ruthenium metathesis catalysts, Robert Grubbs was able to develop a "one-pot" synthesis starting from the enantiomerically pure alcohol: The ring-closing metathesis is followed by a transfer dehydrogenation (initiated by the addition of sodium hydroxide and pentan-3-one), and a final hydrogenation of the double bond (by pressurizing the reaction mixture with hydrogen). All of the steps are catalysed by the initially added ruthenium complex. [198]



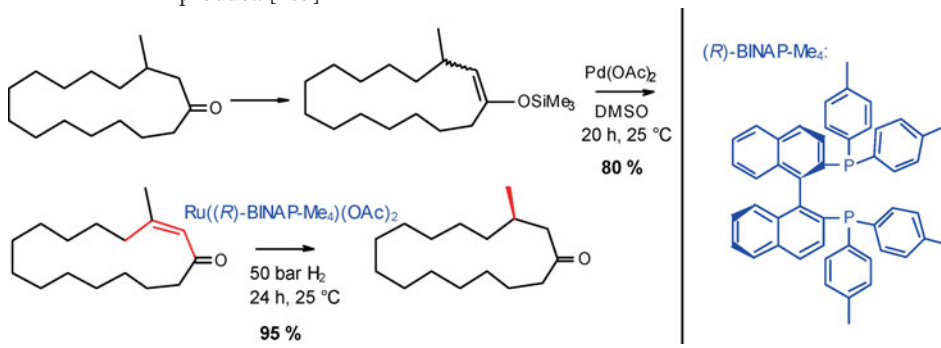
If exaltenone, generated for example *via* the Stoll-Hansley-Prelog procedure (*cf.* section 3.6.5), is treated, not with Cu_2Cl_2/methylmagnesium bromide, but with dimethylzinc in the presence of an enantiomerically pure copper complex, then, starting from the (*E*)-enone, the desired product is obtained in excellent yields and high optical purity. [199, 200]



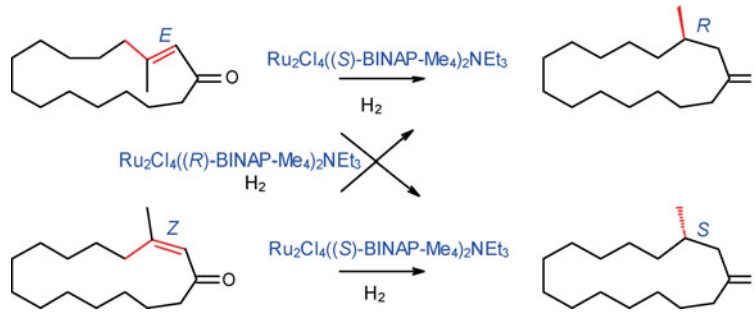
In 2004, Firmenich published a synthesis of enantomerically pure (*R*)-muscone, the key step of which is the enantioselective protonation of an enolate. [201] The starting material is obtained by methylation of bicyclo[10.3.0]pentadec-1(12)-en-13-one. [172] Isopropylephedrine proved to be the best chiral acid for the enantioselective protonation, as in case of damascone (*cf.* section 3.1.3). Reduction of the ketone with DIBAlH gives the corresponding alcohol, which can be enantiomerically enriched to 98 % ee by crystallisation from heptane. After protection of the alcohol with acetic anhydride, and ring opening by ozonolysis, the reductive deprotection of the acetate gives a triol, which is converted in four subsequent steps into (*E/Z*)-muscenone. For the final hydrogenation of the double bond, the "Crabtree catalyst" is particularly suitable. The overall yield, based on the enantiomerically pure alcohol, is around 73 %.



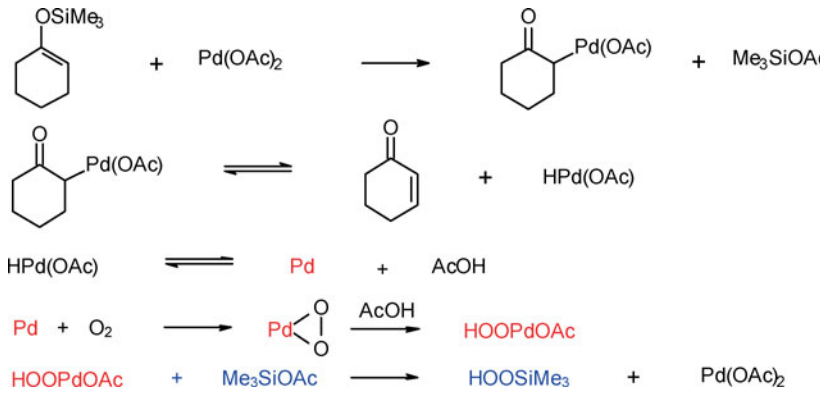
Takasago Perfumery Ltd. manufactures optically pure (*R*)-muscone from the racemic compound by way of its silyl enol ether which is dehydrosilylated with palladium acetate to the pure (*Z*)-enone. [202] The enantioselective hydrogenation with ruthenium-BINAP catalysts finally gives the enantiomerically pure product. [203]



The above example shows one of the hitherto very few successful enantioselective hydrogenations of enones. The enantiomeric excess lies in the range of 94–98 %. The direction of induction, just as in the synthesis of menthol (*cf.* section 3.4.2), is strongly dependent on the configuration of the double bond and that of the catalyst. [204]



For the transformation of the silyl enol ether into the α,β-unsaturated ketone, Richard Larock has suggested the following mechanism [205]:



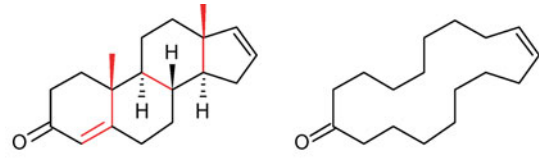
3.45 *Aimé, the son of Pierre-François-Pascal Guerlain (1798-1864), the company's founder, created the perfume Jicky® in 1889, and thereby set down a milestone in the history of perfumes. – Tabac®, designed for male customers, was created by Mäurer & Wirtz. It is said to have floral and spicy herbal fragrances, accented with tobacco, oakmoss and vanilla and rounded off with a blend of musk and ambergris.*

After the reductive elimination, the palladium(0) is oxidised by atmospheric oxygen to Pd(II). DMSO is apparently essential for this process. Noteworthy, and decisive for the Takasago muscone process, is its high (*E*)/(*Z*) selectivity, which can also be found in open-chain and macrocyclic systems. [206]

3.6.11 Animal Fragrances

Animal scents are essential constituents of many valuable perfumes. Their individual scent defines the perfume's basic character. They are very adaptable, and impart a soft, velvety tone to the other scented components. In many cases they are also used as fixatives.

Ambergris is the most subtle of the animal perfumes. It is used to make floral scents appear richer and to "round off" perfumes with aldehydic character such as *Chanel No.5*®. Civetone contributes a typical animal-like note, as in *Jicky*® from Guerlain (Fig. 3.45). In *Musk for Men Old Spice*®, dating from 1974, musk provides the only dominant basic scent. Combinations of musk, ambergris and oak moss are found in more than 50 % of all men's toiletries: a typical representative is *Tabac*® from the year 1959. Perfumes for ladies also make use of musk scents, as for example in *Narciso Rodriguez*®. Synthetic muscone is used, since this possesses a smoother, less penetrant scent than animal musk.



Androsta-4,16-dien-3-one Civetone

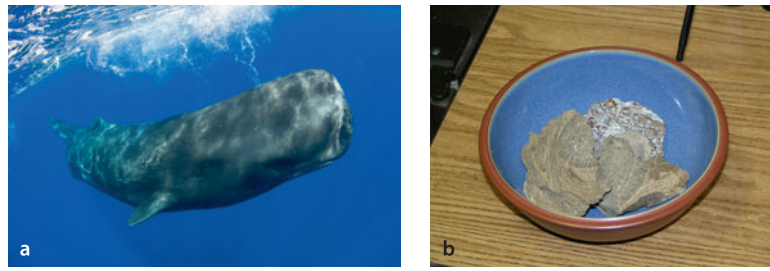
The putative human pheromone androsta-4,16-dien-3-one and civetone are structurally closely related.

Summary in Bullet Points

- Musk-scented materials are in part still obtained from natural sources.
- Considering this high priced product family, there was – and is – a large market for surrogates.
- The particular structural characteristics of natural musk-scented compounds (macrocyclic ketones and lactones) provided the opportunity to develop a host of elegant synthetic methods.

3.7 *Ambrox*®

It is to metabolic, or digestive disorders of one of the largest living mammals, the sperm whale (*Physeter catodon* or *Physeter macrocephalus*), that we owe one of the most valuable scents in fine perfumery: Ambergris (Fig. 3.46). [207]



3.46 *The sperm whale (Physeter catodon or Physeter macrocephalus) and Ambergris.*

According to whaling reports, around 1 % of the animals (principally males) suffer from this disease. However, the amount of ambergris in hunted whales can be considerable. In the case of one sperm whale, which died in the Antarctic in 1953, this came to 421 kg (Fig. 3.47). The ambergris from slaughtered animals is of inferior quality, because it has not been ripened in sea-water. Nowadays, sperm whales are a protected species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (also known as the Washington Convention), and the scented materials are obtained by chemical synthesis.

3.47 *Large lump of ambergris from a sperm whale shot in the Antarctic in 1953. [208]*

Sperm whales grow up to 18 metres in length and 50 tonnes in weight. The intestinal tract of a sperm whale can reach a length of up to 250 metres. – Sperm whales are smaller than blue whales (*Balaenoptera musculus*, up to 30 metres and 180 tonnes).

Ambergris consists primarily of a soft, waxy mass which builds up in the intestine of the sperm whale, in response to mechanical injury from horny jawbones of cuttlefish and squid, its preferred food. Ambergris acts as an antibiotic wound closure, in which indigestible food components are embedded. The concretions fall into the sea through vomit, faecal discharge, or the natural death of the animals. The almost black lumps weigh anywhere from a few grams up to 100 kg, and smell initially of faeces. They drift on the surface due to their low density (0.78–0.93 g/cm^3), and over the course of years or decades, they are converted

by photochemical degradation and oxidation into the highly prized raw material for perfumery. The ultimately stone-like, light grey, fragrant pieces of ambergris are either recovered by fishing boats or washed ashore. The character of the scent lies between woody, dry, balsamic, somewhat tobacco-like, and a bouquet with an aphrodisiac note. Ambergris is found in the Atlantic and Indian Oceans, on the coasts of Brazil, Africa, Madagascar, the Maldives, China, Japan, New Zealand and in the Caribbean.

3.7.1 History of the Scent

Back in ancient times, ambergris was already a much sought-after scent and a valuable commodity. The Egyptians burned ambergris as a sacrificial offering, and the fragrance was also cherished in Jewish culture, as referenced in the Song of Solomon, and it was known in the coastal regions of the Indian Ocean.

Mixed with wine it was considered an aphrodisiac; it served as defence against the plague and as a medicament for treatment of headaches, chills, epilepsy and other illnesses. Louis XV of France is said to have seasoned his favourite food with ambergris, and Elizabeth I of England used ambergris to perfume her gloves.

In Egypt, ambergris is still used for aromatisation of cigarettes.

The first evidence of the use of ambergris in fine perfumery comes from the Arabs who ruled southern Spain during the 10th century. Ambergris was imported for this purpose from the Sunda Islands (in the Far East) and from the Maghreb region of North Africa. Right up to the beginning of modern times, ambergris was traded right across Europe, and in many cases it was considered in value like gold. Al-Hasan ibn Mohammed al-Wassan (also named Johannes Leo Africanus; born around 1490 in Granada; died after 1550 in Tunis) wrote that the price of one pound (454 g) of ambergris in the market of Fez (Morocco) was around 60 ducats, equivalent to the purchase of three slaves.

For a long time, there was considerable uncertainty regarding the origin of ambergris. The Chinese called it "lung sien hiang" (龍涎香). They envisaged dragons which rested on the coastal rocks and dribbled into the sea while they slept. From the spittle, they believed, the scent was exuded. In Japanese culture people spoke of "kunsurano fuu", *i.e.* of whale excrement. The Arab traveller and historian Al-Mas'udi (who died in 957 in al-Fustal, Egypt) reported on dealers and seafarers, who believed that ambergris grew like a fungus on the sea-bed and was occasionally washed up to the shore by storms. Marco Polo (1254–1324) was the first Western traveller to report, that the sperm whale was hunted, for its ambergris, by Yemeni seafarers of the island of Socotra (small archipelago of four islands in the Indian Ocean).

In terms of cultural history and etymology, ambergris is often confused with amber resin (genuine yellow or Prussian amber, Latin: *Succinum,* Greek: *Electron*). Similarly, *ambra* or *amber* is the English or old German word for the resin. In order to make the distinction, the excretion product of the sperm whale is now referred to as *ambergris*, which is derived from the French *ambre gris* and ultimately from the Arabic *anbar*.

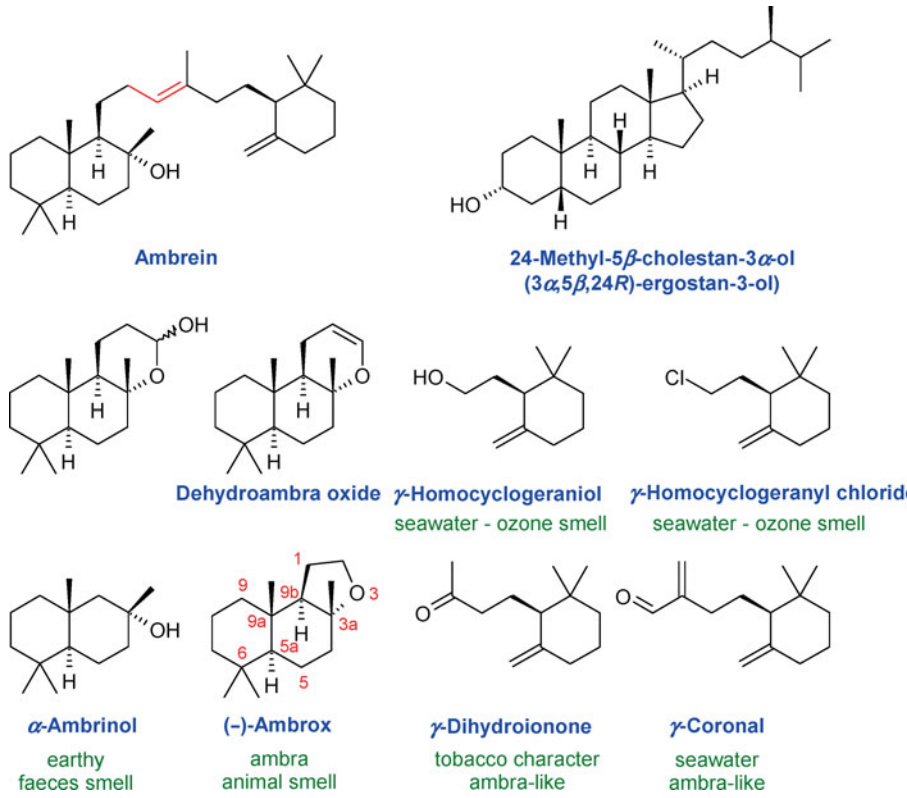
However, certainty on the source of ambergris was first obtained with the blossoming of the American whaling industry in Nantucket (Massachusetts) in the 18th century. More and more sperm whales were killed, in the intestines of which lumps of ambergris were found.

3.7.2 Constituents of Ambergris

The initial chemical analysis of ambergris goes back to the French chemists Joseph-Bienaimé Caventou (1795–1877) and Pierre-Joseph Pelletier (1788–1842). In 1820, they first isolated and characterised the odourless triterpene, ambrein. [209] The second main component of fresh ambergris is 24-methyl-5β-cholestan-3α-ol; this together with ambrein accounts for around 70–90 % of the total mass.

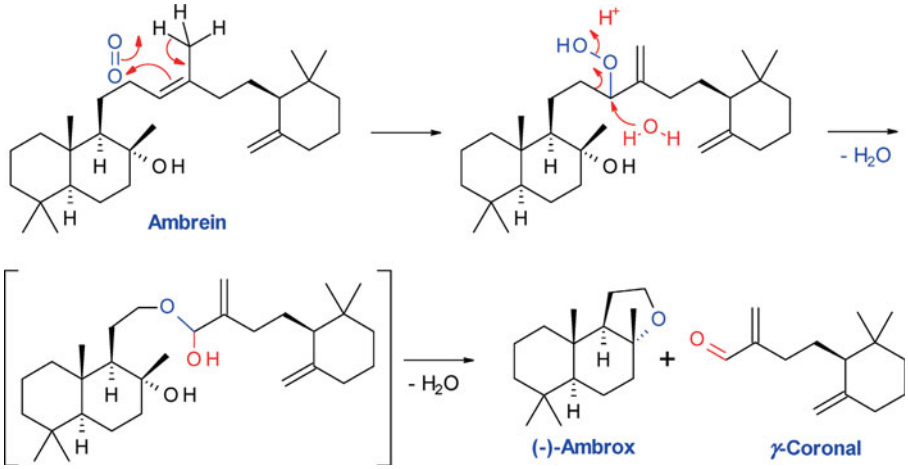
The odourless ambrein is used in the perfumery industry as a fixative.

The important olfactory components arise during the period of maturation in the sea. Oxidative degradation of ambrein around its central double bond produces volatile mono- and carbobicyclic compounds, some of which, because of the salt content of seawater, are chlorinated (Fig. 3.48). [210]



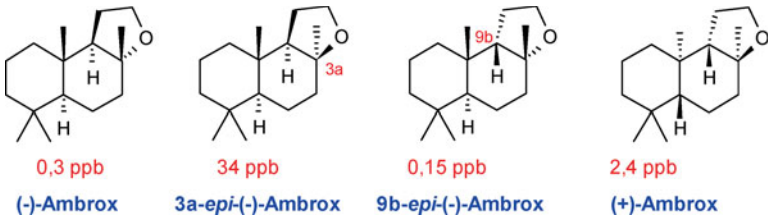
3.48 *Constituents of fresh ambergris.*

The key reaction in the degradation is a photooxidation by singlet oxygen, followed by a Hock rearrangement. Haemocyanin, a blue, copper-containing and oxygen-carrying metalloprotein in some octopuses, which is also found in ambergris, can serve as a sensitizer in the oxidation step. In model reactions with pure ambrein and singlet oxygen, beside of *Ambrox*® and γ-coronal, dehydroambra oxide and α-ambrinol are also obtained. [211]



The most important olfactory constituent in ambergris is (–)-*Ambrox*® ((3a*R*,5a*S*,9a*S*,9b*R*)-3a,6,6,9a-tetramethyldodecahydronaphtho[2,1-*b*]furan). A 3 % ambergris tincture in 90 % alcohol, which is allowed to mature for a while with occasional shaking, embodies the first four tonalities of a damp, mossy forest floor, a strong scent of tobacco and balsamic sandalwood, mixed with the character of a warm animal musk.

The various diastereomers of (–)-*Ambrox*® have similar scents, but are distinguished in part by their scent thresholds. [212, 213] That of 3a-*epi*-(–)-*Ambrox*® is by a factor of 100 higher than that of *Ambrox*®. The 9b-*epi*-(–)-*Ambrox*® has a scent character similar to that of *Ambrox*®, but the scent threshold is only half as high. (+)-*Ambrox*® is eight times weaker than its natural enantiomer. The racemate has a scent threshold of 0.5 ppb, and is hardly distinguishable in character from that of *Ambrox*® alone (Fig. 3.49).



3.49 *The scent thresholds of the stereoisomers of Ambrox*®.

(–)-Ambrox® is found not only in natural ambergris but also in the *absolue* of tobacco (*Nicotiana tabacum*), and it is detected in the essential oils from the clary sage (*Salvia sclarea*), the rock rose (*Cistus labdaniferus*) and the Mediterranean cypress (*Cupressus sempervirens*) (Fig. 3.50).

3.50 *The rock rose (Cistus labdaniferus) and the Mediterranean cypress (Cupressus sempervirens).*

The word *Ambrox*® is used to describe the enantiomerically pure compound. The racemate is called *Cetalox*® (Lat. *Cetus* = whale). Both are trade names of Firmenich. *Ambroxan*® and *Amberlyn*® are synonymous with *Ambrox*®: these are the trade names of Cognis / BASF (acquired from Henkel) and Quest.

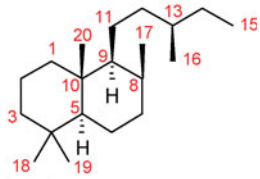
3.7.3 Partial Syntheses

The first chemical synthesis of (–)-*Ambrox*® appeared in 1950 from the work of Max Hinder and Max Stoll at the Firmenich company. [214] The starting material for the synthesis of this natural product was the diterpene sclareol, which is obtained from the clary sage (Fig. 3.51).

Legend has it that the first to use clary sage "industrially" were dishonest German wine merchants, who thereby "magically" created "genuine" Muscatel wine out of their mediocre Rhine wines. Nowadays, clary sage oil is used in the preparation of Eaux de Cologne, Eaux de toilette, and for the aromatisation of herbal liquors.

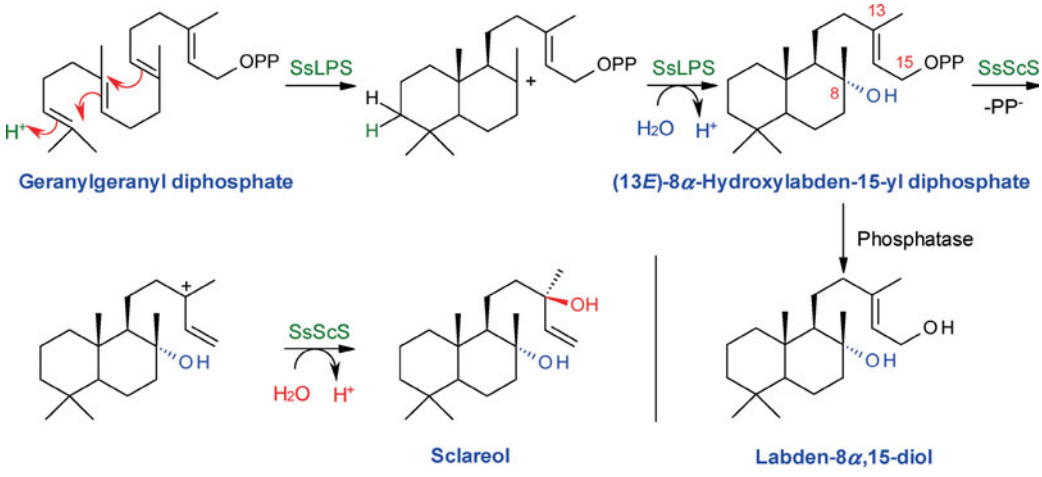
3.51 *The clary sage (Salvia sclarea), also known as muscatel sage, Roman sage, or in some parts of Europe, Scharlei, is grown mainly in the Mediterranean region, in the Balkans, in the territory of the former Soviet Union, and the USA. The clary sage is an evergreen biennial or perennial plant. The fully-grown plants reach a height of 50–220 cm. Clary sage has been cultivated at least since the 9th century. It finds application in cosmetics, perfumery, as an herbal medicine and as a spice.*

Sclareol belongs to the sizable class of labdanoid diterpenes. The name is derived from *labdanum*, a resin found in certain rock rose (*Cistus*) species, from which the first member with this core structure was isolated.



Biosynthesis of Sclareol

A huge varity of natural diterpenes, such as sclareol, derives from cyclisation reactions of geranylgeranyl diphosphate, catalyzed by diterpene synthases. These cyclisation reactions can be initiated either by activation of the diphosphate moiety or by protonation of the terminal double bond. However, in the biosynthesis of many polycyclic diterpenes, both types of activation are applied in two successive enzymatic reactions. In the case of the biosynthesis of sclareol in clary sage, and this is in contrast to previous biochemical experiments, two distinct synthases are involved: the (13*E*)-8α-hydroxylabden-15-yl diphosphate synthase (SsLPS) and the sclareol synthase (SsScS). [215]



In general, clary sage is harvested in July and August. In the case of exceptionally good weather, a second crop of inferior quality can be collected later in the year. Steam distillation of 100 kg of the inflorescence and shoot tips produces around 800 g of clary sage oil (*oleum salviae sclareae*). The main constituents of the clear colourless essential oil are linalyl acetate, linalool, nerolidol, nerolidyl acetate and sclareol; their amount however can vary strongly, depending on the region of cultivation and climate conditions.

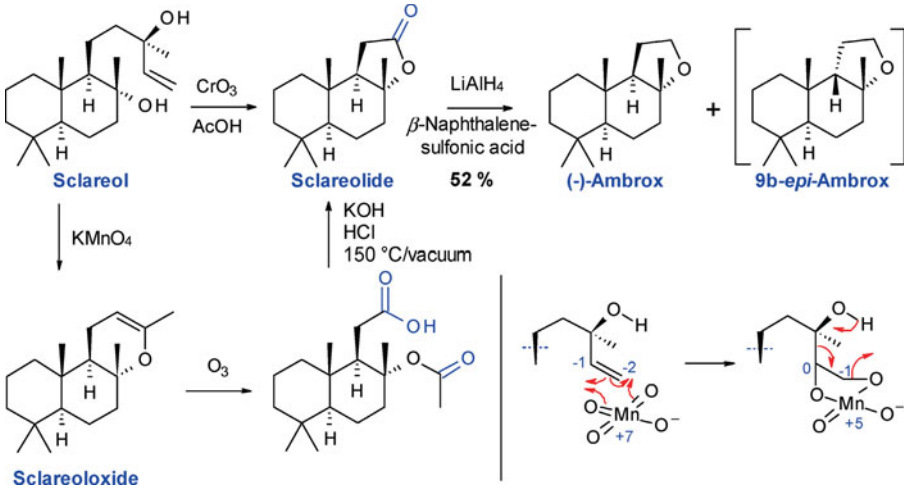
A higher proportion of sclareol is present in the clary sage concrète, which is obtained by extraction of the blooming plants with hexane; its content may amount to around 70 %. After counter-extraction with 87 % methanol and evaporation of the solvent, a syrupy residue remains, which crystallises and has a sclareol content of over 90 %. [216, 217] The worldwide annual production is estimated at 50–150 tonnes. [218]

Most recently, Michel Schalk and Laurent Daviet at Firmenich reconstructed the sclareol biosynthesis in genetically engineered *Escherichia coli*. [215] Unexpectedly, they had realized earlier that sclareol, an in general strong fungitoxic compound, excerts almost no toxicity towards this bacterium. Under optimized reaction conditions they reached sclareol titers of about 1.5 g/l after 48 h under high cell density fermentation conditions. Interestingly, in the fermentation broth there was also found a small amount of labden-8α,15-diol, which might originate from excretion of the dephosphorylation product of (13*E*)-8α-hydroxylabden-15-yl diphosphate into the medium. This intermediate might subsequently be converted into (–)-*Ambrox*® as well.

Partial Synthesis

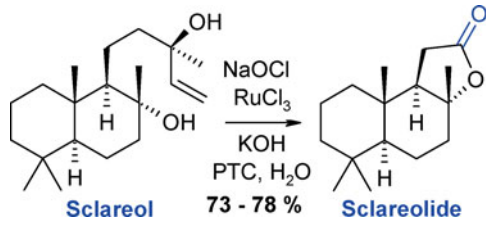
The key step in the industrial partial synthesis of (–)-*Ambrox*® is the oxidative cleavage of the side-chain of sclareol, either by oxidation with chromium trioxide or by potassium permanganate, followed by ozonolysis.

Reduction of the lactone with lithium aluminium hydride gives the diol, which is cyclised in acid to the desired ether. This step requires however special care, since under the reaction conditions the thermodynamically more stable 9b-*epi*-*Ambrox*® is formed.



At the beginning of the 1980s, the commercial use of *Ambrox*® was no longer protected by patents, so that a variety of other industrial synthesis methods could be developed by companies and universities. Thus, Sir Derek Barton published a cleavage procedure of sclareol, for which a patent application was filed by Quest, whereby sodium periodate replaced potassium permanganate. [219, 220]

Henkel developed a very attractive process for the preparation of sclareolide from sclareol by oxidation with sodium hypochlorite in the presence of ruthenium chloride and a phase-transfer catalyst. [221]



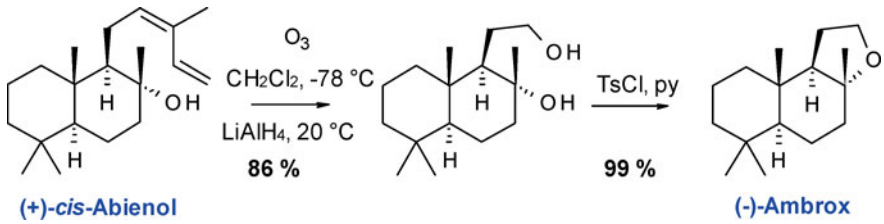
3.52 *The balsam fir (Abies balsamea) is a medium-sized tree which grows to a height of up to 20 m (rarely more than 30 m) and has a trunk diameter of up to 50 cm. It can reach an age of up to 200 years.*

Especially noteworthy is the research at Fritsche Dodge and Olcott and also International Flavors & Fragrances (IFF) in this area, where they showed that the side-chain could be cleaved through fermentation with *Hyphozyma roseoniger* or *Cryptococcus albidus. [*222, 223]

Apart from the use of chromium trioxide or potassium permanganate as oxidising agents, other drawbacks in this synthesis lie in the unreliability of supply with sclareol, and in widely fluctuating prices in the past. [212]

(+)-*cis*-Abienol – obtained from Canada balsam – may also be used as an alternative to sclareol for the preparation of *Ambrox*®. The resin is obtained by scratching the bark of the balsam fir (*Abies balsamea*; Fig. 3.52), which grows in the north of the USA and Canada. By steam distillation, extraction and crystallisation, (+)-*cis*-abienol may be obtained.

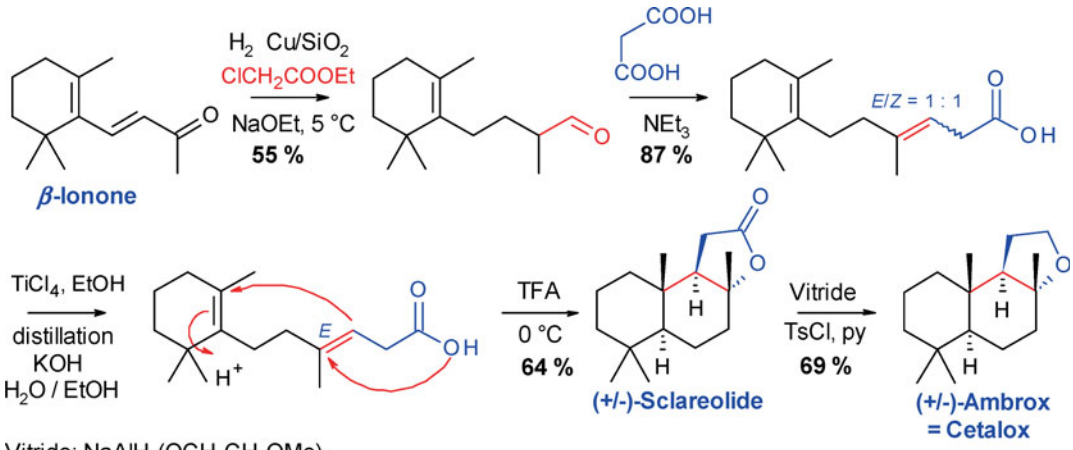
Ozonolysis of (+)-*cis*-abienol and reductive work-up leads to a diol, which can be cyclised to *Ambrox*® with tosyl chloride in pyridine in excellent yield. [224]



Apart from these particularly elegant cleavage reactons, (–)-*Ambrox*® may also be synthesised from a range of other natural products, such as (–)-drimenol, [225] (–)-thujone, [226] or (+)-carvone. [227]

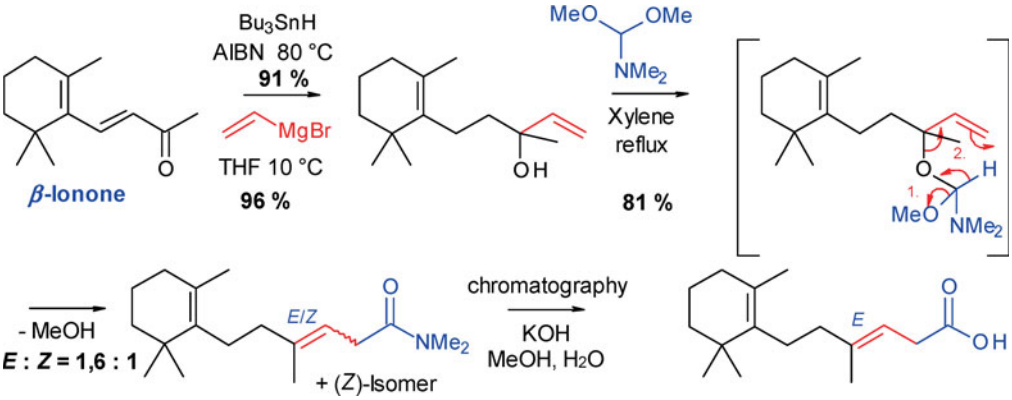
3.7.4 Total Syntheses

The first total synthesis of *Cetalox®* is that of Masanao Matsui. [228] The starting material is *β*-ionone [229]; its side-chain double bond can be hydrogenated regioselectively on a copper catalyst. The side-chain is then extended by three carbon atoms by means of a Darzens reaction [230], followed by a Knoevenagel condensation. The (*E*/*Z*)-isomeric mixture (1 : 1) of acids is separated by fractional distillation of their ethyl esters; the (*E*)-isomer is hydrolysed and cyclised with trifluoroacetic acid to sclareolide. Reduction of the latter with *Vitride®* (sodium bis(2-methoxyethoxy)aluminium hydride) and ether formation then produces *Cetalox®*.

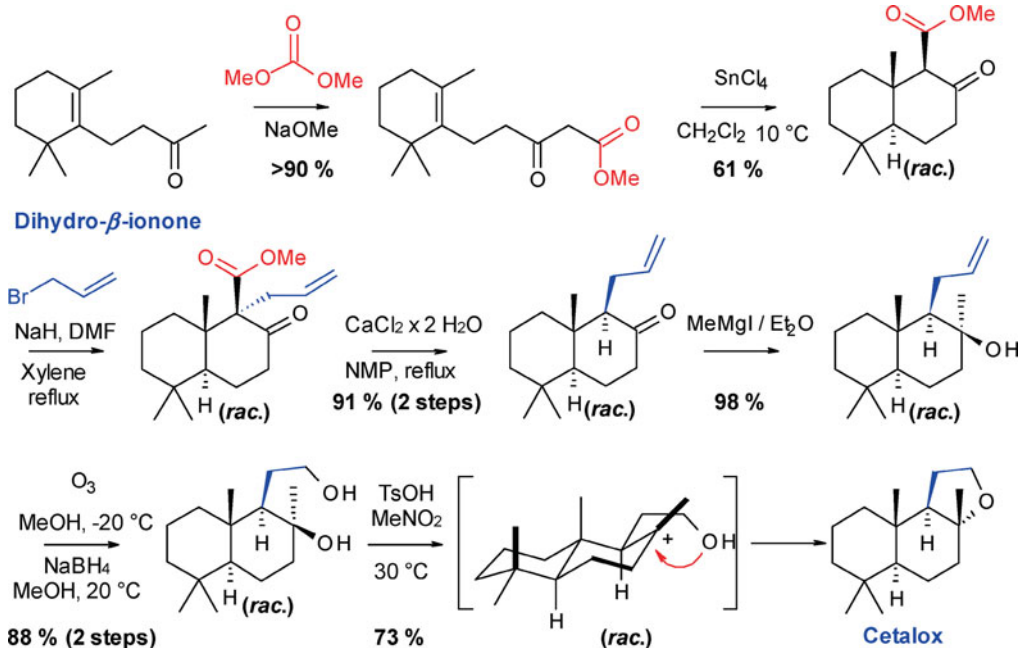


Vitride: $NaAlH_2(OCH_2CH_2OMe)_2$

An attractive alternative for the synthesis of the sclareolide precursor comprises hydrogenation of *β*-ionone with tributylstannane, followed by a Grignard reaction with vinylmagnesium bromide, and finally treatment with dimethylformamide dimethyl acetal; this generates an intermediate, which undergoes a [2,3]-sigmatropic rearrangement to an isomeric mixture of carboxamides; these (*E*)- and (*Z*)-isomers may be separated by chromatography. [231, 232]



In 1989, George Büchi and Hans Wüst at MIT, published a synthesis of *Cetalox*®, which was taken up on an industrial scale by Firmenich and improved in several important aspects. [233] The starting material is likewise dihydro-*β*-ionone, which is converted by reaction with dimethyl carbonate into the corresponding *β*-keto ester, and cyclised with stannic chloride. Direct alkylation of the product at the *α*-position could not be achieve, though *O*-alkylation followed by a Claisen rearrangement succeeded in producing the *α*-allyl compound. While the subsequent demethoxycarbonylation may be carried out in DMSO, the use of NMP as solvent gives better yields. After a Grignard reaction to introduce the methyl group, the side-chain is degraded by ozonolysis, and a following reduction produces the required alcohol. The most difficult step of the whole synthesis is the ring closure to the *trans*-fused tetrahydrofuran under kinetic control, since the thermodynamically-favoured product is 9b-*epi-Cetalox*®. Under the chosen conditions, however, the reaction proceeds very selectively, and the amount of 9b-*epi-Cetalox*® is less than 1 %.

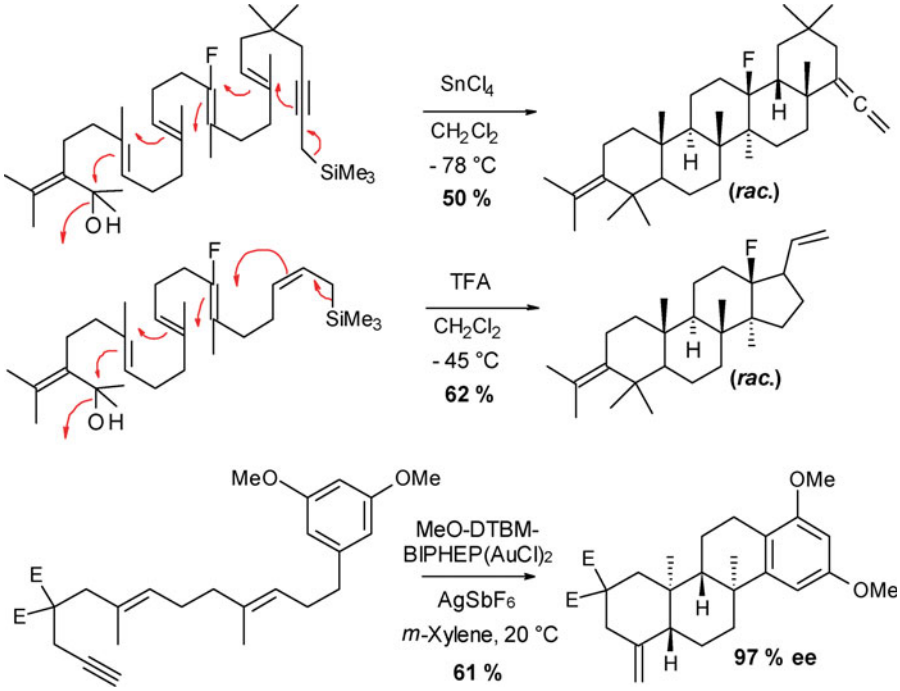


3.7.5 Polyene Cyclisations

Of particular interest and of industrial relevance are the attempts to make *Cetalox*® and *Ambrox*® accessible using biomimetic approaches.

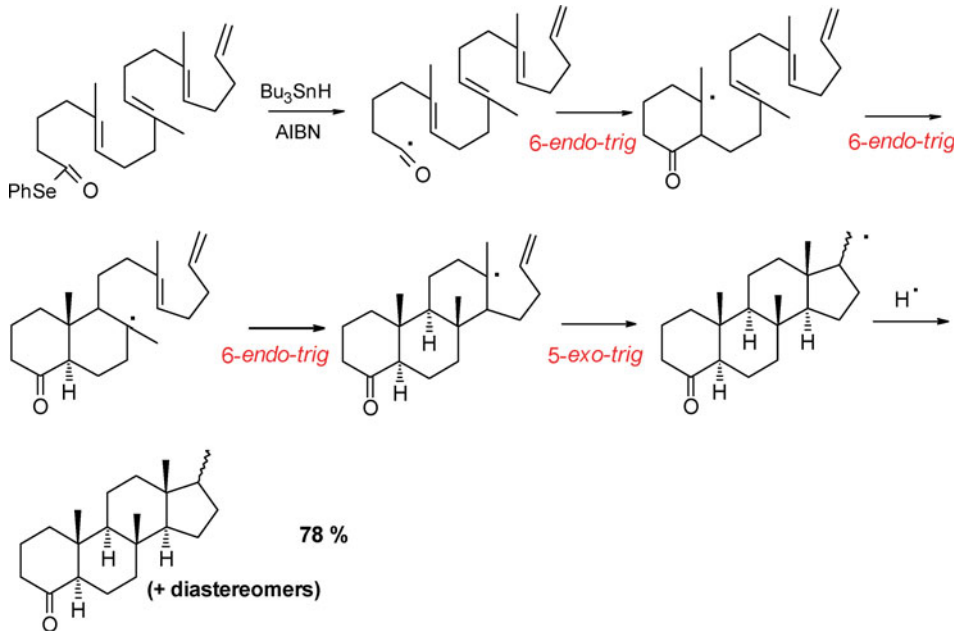
Basics

The fundamental systematic studies on synthetic polyene cyclisation was carried out in the 1950s and 1960s by the groups of Gilbert Stork, Albert Eschenmoser and Eugene Earle van Tamelen (1925–2009), who examined the acid-catalysed cyclisation of farnesylic acid and squalene oxide. [234–236] However, the first published work on the acid-catalysed cyclisation of homofarnesylic acid to a mixture of sclareolide diastereomers in the year 1960 came from Günther Lucius at VEB Chemische Fabrik Miltitz, Leipzig. [237] Over recent years, there have appeared further very elegant examples of the "domino synthesis", in particular to build the steroid scaffold (*cf.* section 6.1 Steroids and Hormonal Contraceptives). The biomimetic tetra- and pentacyclisations of William S. Johnson are initiated by Lewis acids or trifluoroacetic acid; [238] F. Dean Toste, in a more recent example, used a gold catalyst for the enantioselective polycyclisation. [239]



Radical polycyclisations are known as well. The polyene cyclisations of Gerald Pattenden [240–242] and David MacMillan (organo-SOMO catalysis) [243] rank among the most prominent of these: they may serve as teaching examples for the Baldwin rules. The polycyclisation proceeds *via* free radicals, and runs through a cascade ("domino reaction") of 6-*endo-trig* cyclisations to end with a final 5-*exo-trig* reaction. In order to avoid side-reactions, the critical issue is the

skilful construction of the proper polyene, which in favourable cases can be cyclised to a steroidal scaffold in enormously high yields.

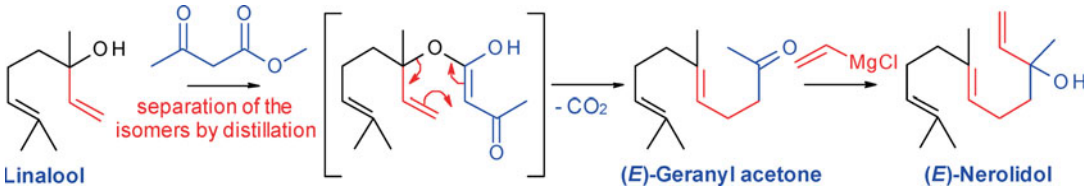


Cabreúva oil is obtained by steam distillation from the tropical Brazilian wood *Myrocarpus fastigiatus*. (Cabreúva is the name of a district in Brazil, near Sao Paulo.) The main constituent of the oil is the sesquiterpene nerolidol.

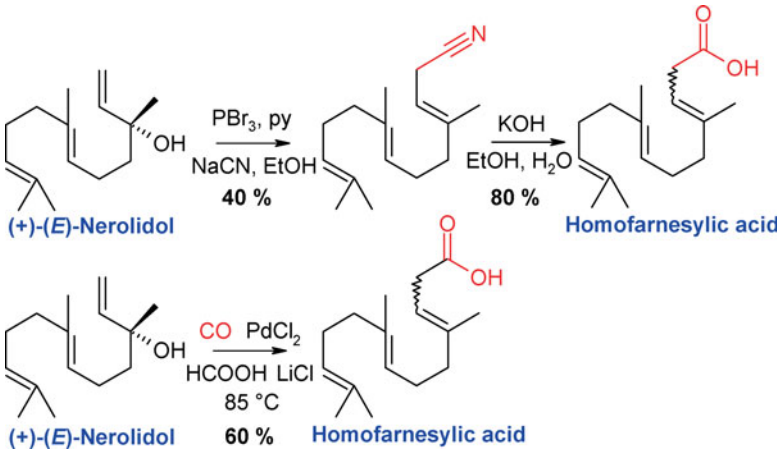
Starting Materials for Polyene Cyclisations

The central starting material for the preparation of homofarnesic acid and homofarnesol is (+)-(*E*)-nerolidol, which can be obtained from Cabreúva oil by fractional distillation.

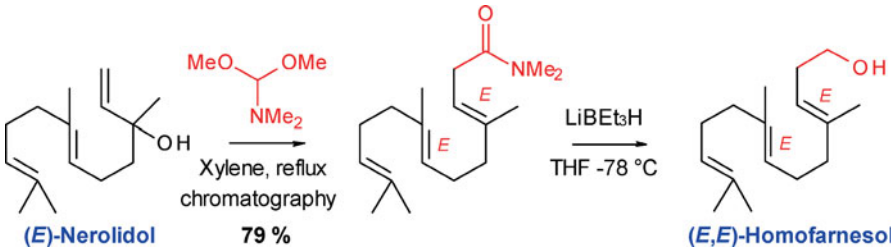
Racemic (*E*)-nerolidol is obtained, equally easily, from linalool using diketene or methyl acetoacetate by a Carroll reaction and treatment with vinylmagnesium chloride. Alternatively, linalool can be treated with diketene or isopropenyl methyl ether (Saucy-Marbet reaction); instead of the Grignard reaction, the intermediate undergoes an addition of acetylene, followed by a Lindlar hydrogenation. [244]



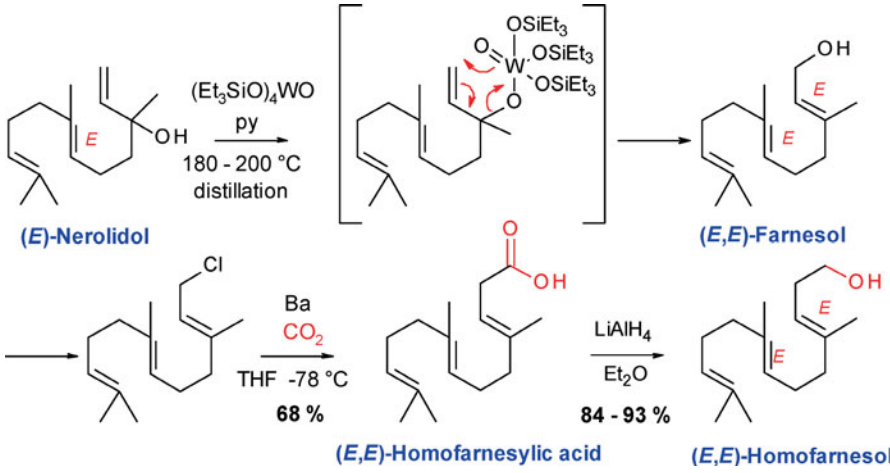
The conversion of nerolidol into homofarnesylic acid, either *via* the corresponding bromide, its substitution by cyanide and hydrolysis, or by direct carbonylation, [245] produces a mixture of (3*E*/*Z*,7*E*)-isomers.



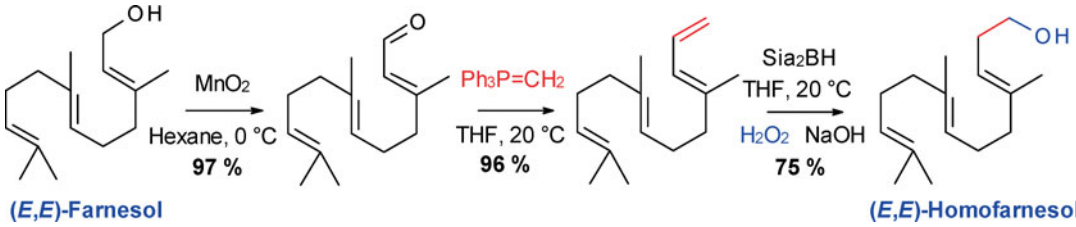
(*E,E*)-Homofarnesol is obtained from nerolidol by a [2,3]-sigmatropic rearrangement, chromatographic or distillative separation of the (2 : 1)-mixture of homofarnesylamides, and reduction with lithium triethylborohydride. [231]



Alternatively, nerolidol can be isomerised into farnesol, *via* a trisilylated tungstate (formally a metalla-Claisen rearrangement). The product is purified by distillation. [246] After its conversion into farnesyl chloride, this is treated with carbon dioxide and freshly precipitated barium, giving (*E,E*)-homofarnesylic acid, which is finally reduced with lithium aluminium hydride. [247, 248]

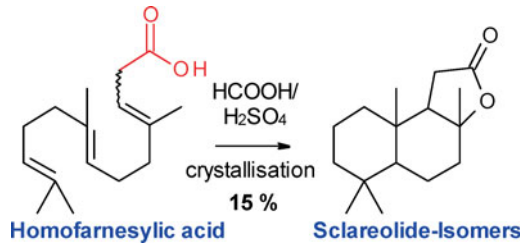


Farnesol may be also converted into (*E,E*)-homofarnesol by the following sequence: oxidation with manganese dioxide, a Wittig reaction, and hydroboration using disiamylborane, which is prepared *in situ* from the borane-THF complex and 2-methylbut-2-ene. The overall yield is 70 %. [248]



Sclareolide and *Ambrox*®

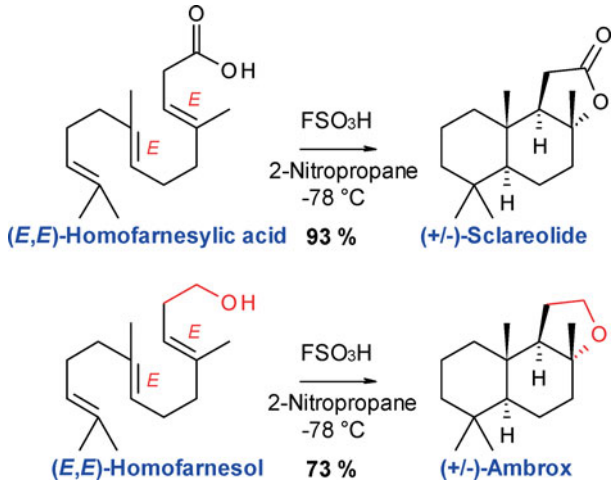
Günther Lucius succeeded in cyclising homofarnesylic acid into a mixture of sclareolide diastereomers. These could be separated by crystallisation, although the yield was only around 15 %, while the mixture did not contain racemic sclareolide. [237]



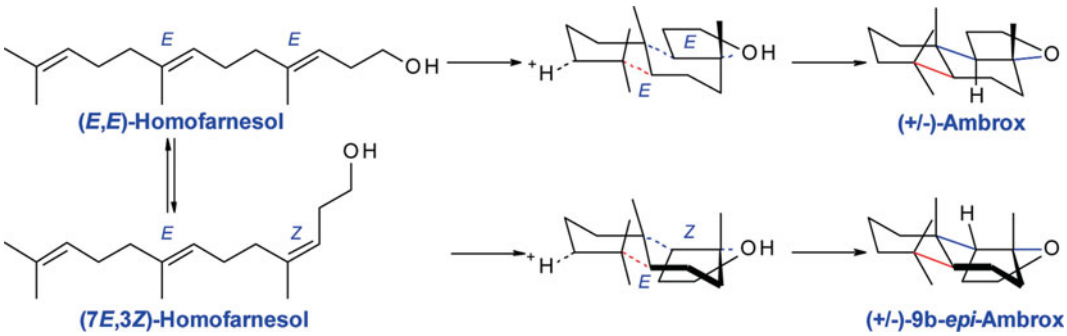
As a consequence, a series of other Brønsted and Lewis acids were probed for their impact on the stereoselectivity of cyclisations. Thus, the sclareolide isomers were obtained from homofarnesylic acid in 91 % yield, when the reaction was carried out with stannic chloride in dichloromethane at –78 °C. [249] On the other hand, the use of boron trifluoride as catalyst produced racemic sclareolide in a yield of only 38 %. [250] The company Henkel patented a procedure, in which an (*E*/*Z*)-mixture of homofarnesylic acid isomers was treated at –10 °C with methanesulfonic acid in dichloromethane, although, this yielded only a mixture of sclareolide isomers. [251]

The breakthrough was eventually achieved by Pavel F. Vlad at the Chemical Institute of the University of Kishinev, Moldova, using superacids such as fluorosulfonic acid in 2-nitropropane at low temperatures. [252] Interestingly, starting from (*E,E*)-homofarnesol, he obtained racemic *Ambrox*® under very similar reaction conditions in a yield of 73 % and with a high degree of diastereoselectivity. [231, 247]

A critical comment is necessary here: the yields obtained by Firmenich under very similar conditions were only around 40 %, and reactions with chlorosulfonic acid in 1-nitropropane gave likewise yields in this range. [231, 253]

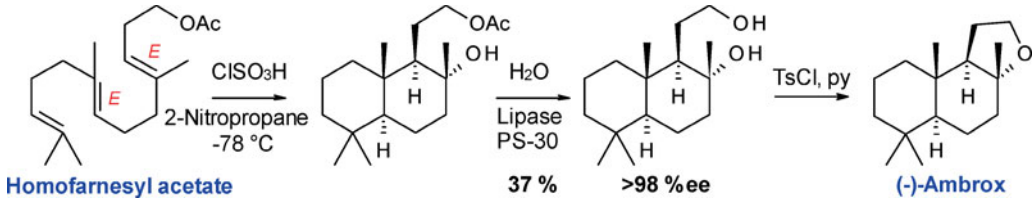


Very detailed mechanistic studies on polyene cyclisation have been pursued by Roger Snowden at Firmenich. [254] Cyclisation of the tricyclic system is initiated by terminal protonation, and *anti*-addition to the double bonds of the nascent six-membered rings occurs from their chair conformations. Whether or not the cyclisation proceeds in a concerted manner, according to the Stork-Eschenmoser hypothesis, cannot be resolved from the experimental data. A stepwise process, in which the bond indicated in red is formed first, is equally conceivable. The following ring closure giving *Ambrox®* or 9b-*epi*-*Ambrox®* is faster than the conformational change of the cyclohexyl carbocation, so that a *trans*-decalin system results. Isomerisation of the central double bond in homofarnesol is very slow as compared to the terminal double bond, and therefore the proportion of other diastereomers is correspondingly low.



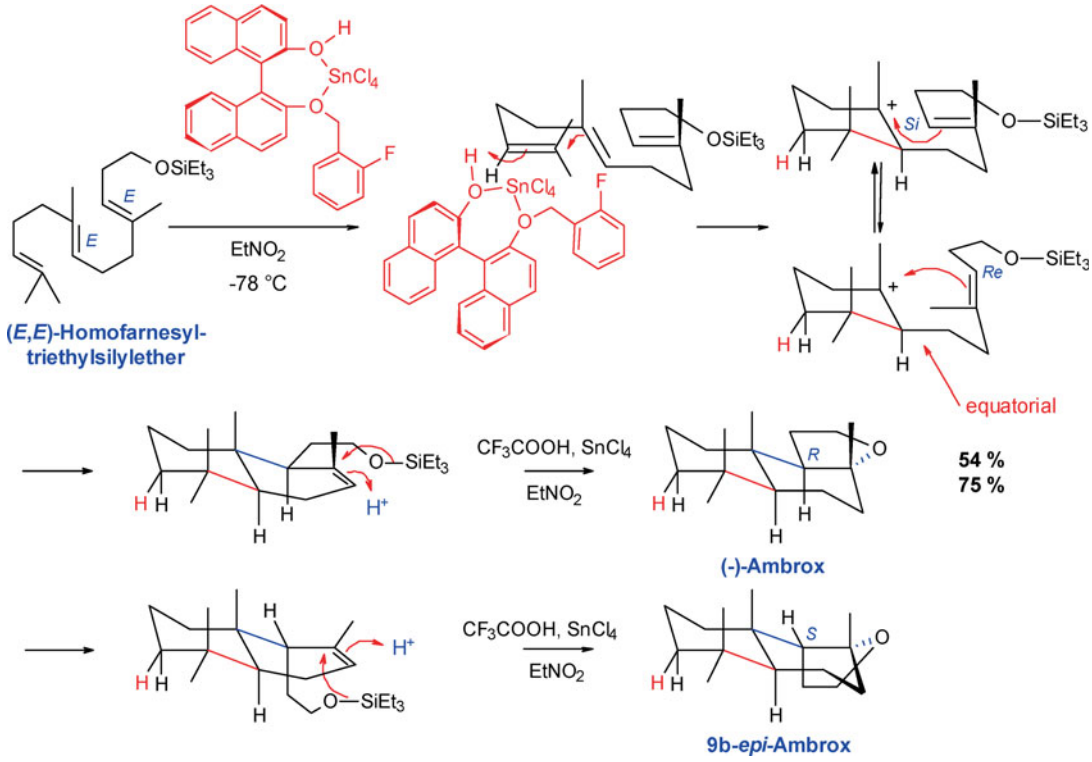
Enantiomerically pure *Ambrox®* is accessible by total synthesis, if homofarnesyl acetate is cyclised with chlorosulfonic acid and the product deacetylated using a *Pseudomonas* lipase. Cyclisation of the diol to a tetrahydrofuran gives the desired product. [255]

The unquestionably most elegant total synthesis of *Ambrox®* is achieved by an enantioselective polyene cyclisation. For this reaction, Hisashi Yamamoto used an enantiomerically pure Lewis acid / Brønsted acid from *O*-(*o*-fluoro-

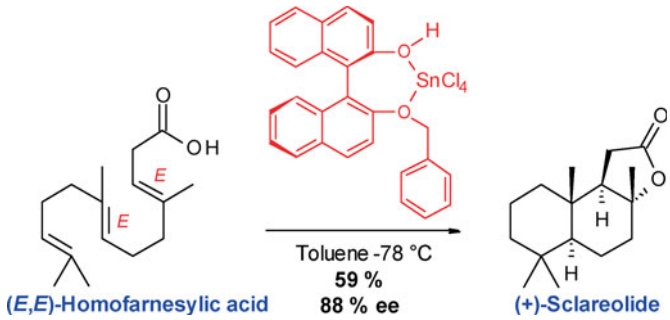


benzyl)-binol and stannic chloride, and thereby obtained (–)-*Ambrox*® in 75 % ee and in a yield of still 54 %. [248]

(*E,E*)-homofarnesyl triethylsilyl ether served as the starting material. In this way, the cyclisation stops after the enantioselective protonation at the decalin stage. The following cyclisation proceeds with higher diastereoselectivity, if it is carried out in strong acid. The balance between the stability of the Si-O bond and the Brønsted acidity in the enantiomerically pure Lewis/Brønsted acid couple is of critical importance for a high diastereoselectivity. The *Si*-facial ring closure of the cyclohexyl-cation with the equatorial side-chain leads to *Ambrox*®, and the *Re*-facial attack to 9b-*epi-Ambrox*®. Ring closure of the cyclohexyl-cation with the axial side-chain gives two other diastereomers, 5a-*epi-Ambrox*® and 5a-*epi*-9b-*epi-Ambrox*®. Thus, the mechanism for the formation of the diastereomers involves not only the (*E/Z*)-isomerisation at the homofarnesol stage, but also the (*Re/Si*)-facial cyclisation options, and the inversion of the conformation of the six-membered ring.



Under very similar conditions, although without the addition of the trifluoroacetic acid / stannic chloride complex, (+)-sclareolide may be obtained in approximately the same yields with an ee of 88 %. [232]

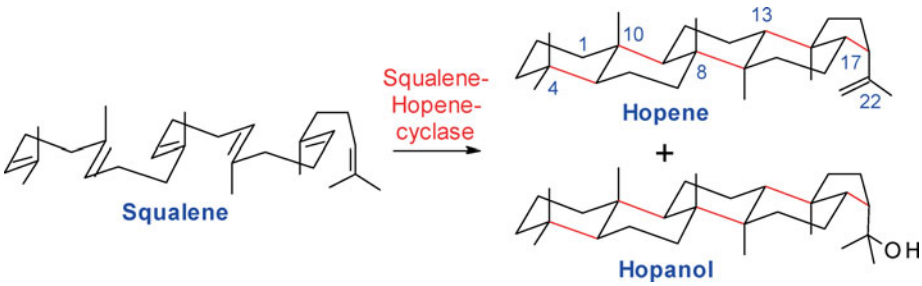


3.7.6 Enzymatic Polyene Cyclisation

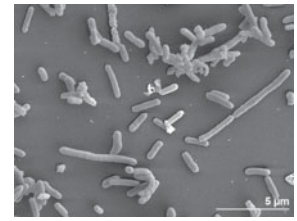
The enantioselective polyene cyclisation of squalene to the pentacyclic hopene and hopanol ranks as one of the most complicated biochemical reactions. In a single step, nine stereogenic centres are formed. Hopene is one of 512 (2^9) possible stereoisomers.

The cyclisation is mediated by squalene-hopene cyclase from prokaryotic species, and is analogous in certain aspects to the squalene oxide – lanosterol cyclase from eukaryotic sources. [257]

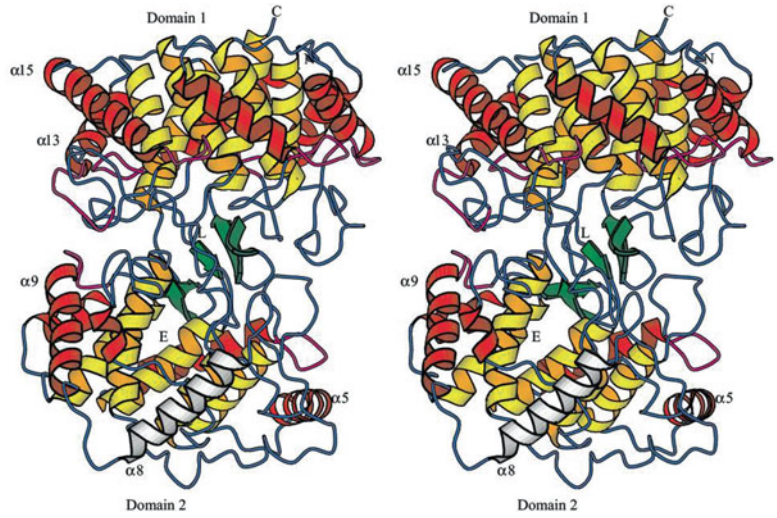
Hopanoids are important components of the bacterial cell wall. They may be found today in large quantities in up to 500 million years old oil shale; estimates go as high as 10^{12} tonnes. Therefore, in terms of quantity, hopanoids represent, next to cellulose, the most abundant organic materials on Earth. [256]



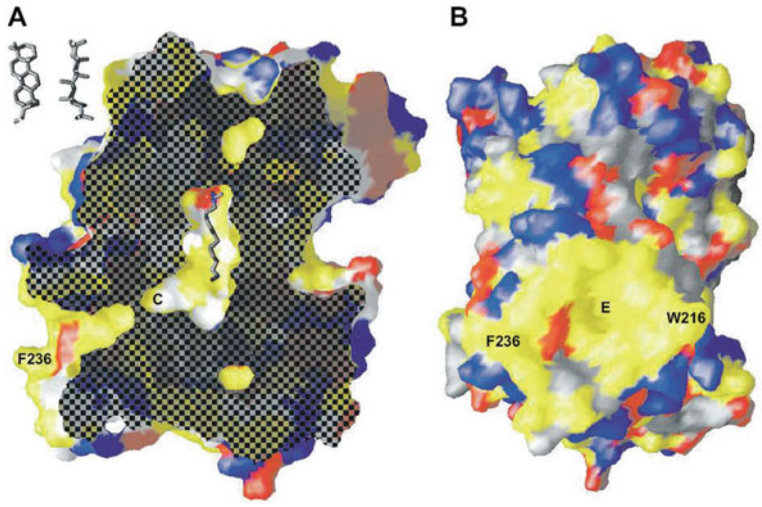
In 1997, Georg E. Schulz in Freiburg succeeded in determining the structure of the membrane-bound squalene-hopene cyclase from the thermophilic microorganism *Alicyclobacillus acidocaldarius* (Fig. 3.53 and Fig. 3.54). [258] The enzyme consists of two domains of approximately equal size. Between the two is a cavity with a volume of 1200 Å^3, which contains the active site: this is accessible through a non-polar channel in domain 2 (Fig. 3.55).



3.53 *Alicyclobacillus acidocaldarius*

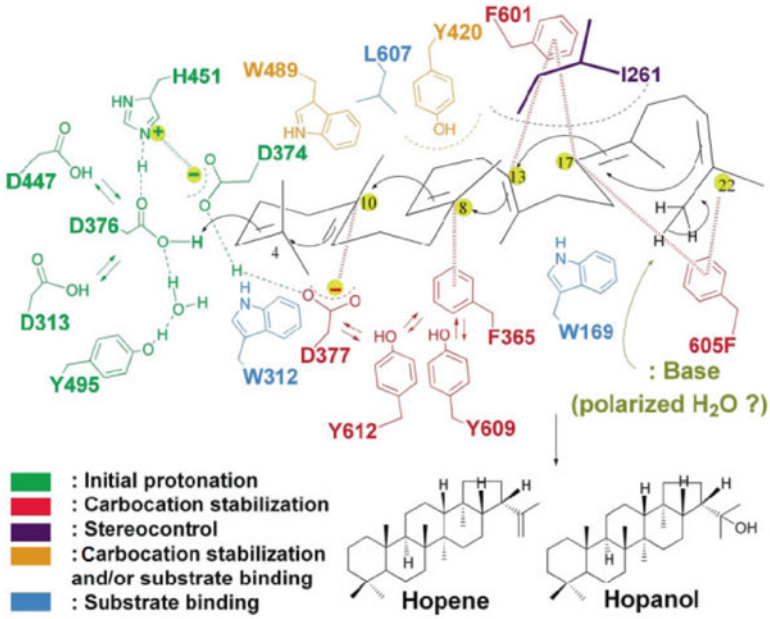


3.54 *Three-dimensional view of squalene-hopene cyclase.*



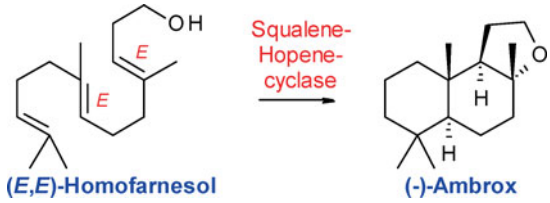
3.55 *The binding pocket of squalene-hopene cyclase. Non-polar surface areas are shown in yellow, positive in blue and negative in red. View (A) shows the enzyme "cut open". The cross-section is in the area of the large binding pocket, in which the competitive inhibitor N,N-dimethyldodecylamine-N-oxide (LDAO) is bound instead of hopene (upper left, two views, shown at scale). A non-polar channel leads leftwards to a similarly unoccupied opening. View (B) shows the channel entrance of the enzyme from outside. It is surrounded by a non-polar surface region of approximately 1600 Å^2.*

Squalene is bound into the active site in a chair-chair conformation. [259] The cyclisation begins with a protonation at C-3 of the future hopene by aspartic acid D376. Carbocation π-interactions with various aromatic amino acids, such as tyrosine, phenylalanine and tryptophan, stabilise the cationic intermediates, until eventually deprotonation produces hopene and hydroxylation gives hopanol (Fig. 3.56).



3.56 *Polyene cyclisation in squalene-hopene cyclase. (D: aspartic acid, F: Phenylalanine, H: Histidine, L: Leucine, W: Tryptophan, Y: Tyrosine).*

Stefan Neumann and Helmut Simon (from the Technical University of Munich) had discovered already in 1986 that the squalene-hopene cyclase from *Alicyclobacillus acidocaldarius* [260] carries out the cyclisation, not only of squalene to hopene and hopanol, but also of homofarnesol to *Ambrox®*. [261] It may therefore be assumed, that this latter cyclisation proceeds in a very similar way as in case of squalene.



Studies on this topic have recently been picked up by the Japanese company Kao Corporation. [262] Unfortunately, none of their publications contains details on selectivity or yield.

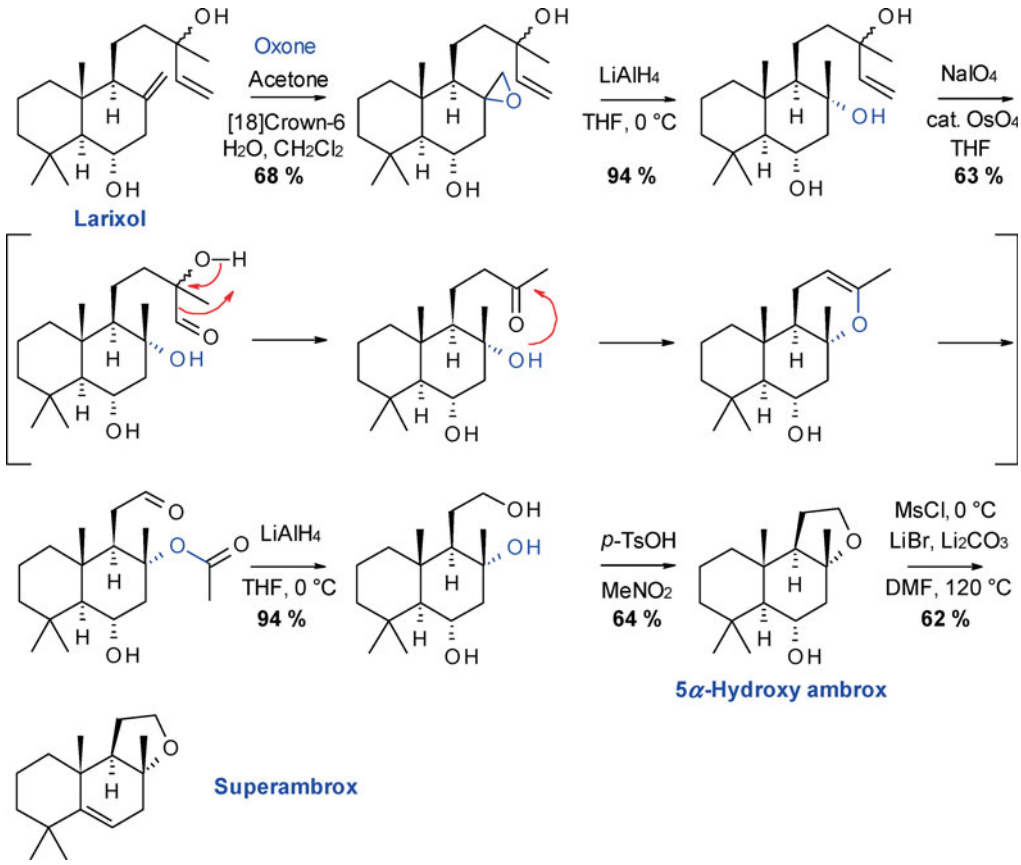
3.7.7 Superambrox

In 1993, chemists at Firmenich described the scent of Superambrox (5,5a-dehydroambrox). [263] The excellent scent qualities are characterised by a vibrant and beautiful ambergris tone with a full body and strong top notes.

An interesting partial synthesis was described by Aede de Groot in 2001. [264] The starting material is larixol, which is obtained from the turpentine of larches (Fig. 3.57) by extraction and crystallisation.

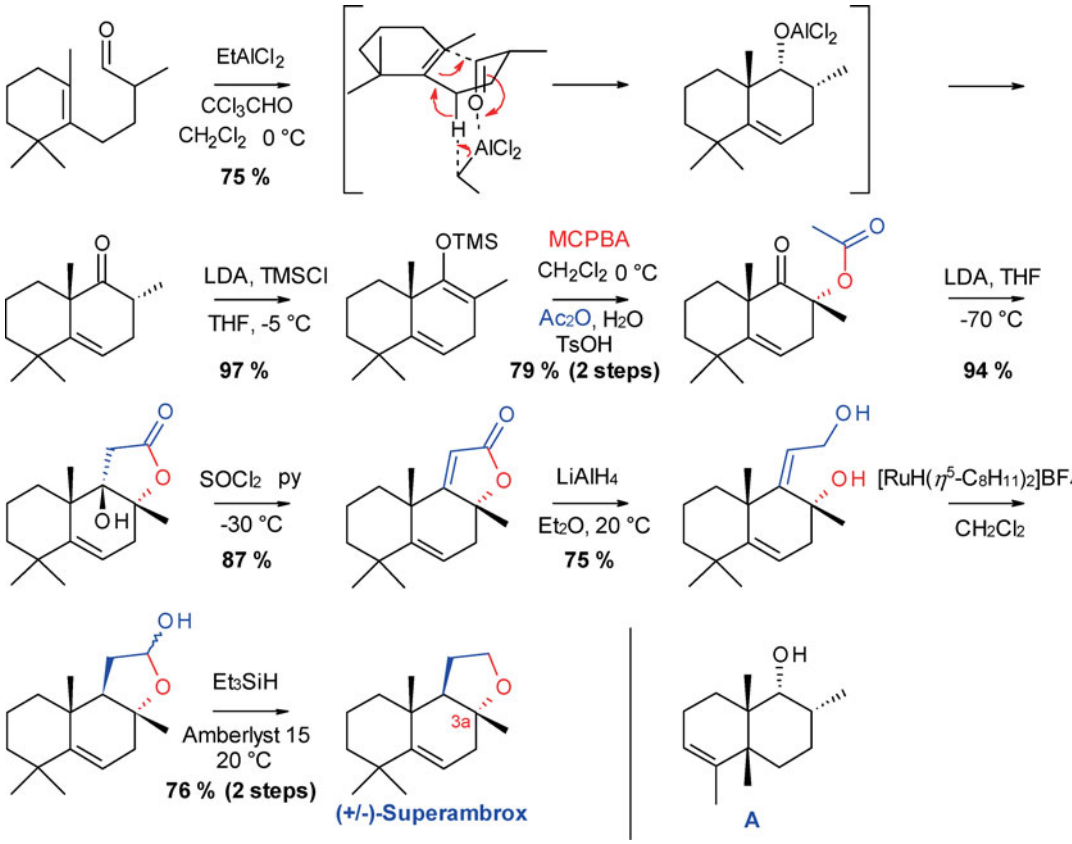
3.57 *The European larch (Larix europaea, Pinus larix, Larix decidua)*

Oxone (2 $KHSO_5$, $KHSO_4$, K_2SO_4) epoxidises preferably the exocyclic double bond; the diepoxide by-product is found in about 19 % yield. Reductive ring opening with lithium aluminium hydride produces a triol. Its side-chain is then degraded by periodate oxidation (Lemieux-Johnson oxidation). Subsequent reduction and ring closure with *p*-toluenesulfonic acid in nitromethane gives 5*α*-hydroxyambrox, which is dehydrated (*via* its mesylate) to Superambrox.



A particularly elegant stereoselective total synthesis of Superambrox has been reported by Charles Fehr's group. [265] Whereas Brønsted-acid-catalysed ene reactions of the racemic dihydro-C_{14} aldehyde proceed with migration of a methyl group to give the decalinol **A** in yields of *ca.* 40 %, the Lewis-acid-catalysed ene reaction with ethylaluminium chloride gives the desired bicyclic product. This step is presumably concerted, involving an eight-membered cyclic transition state. The primary aluminium alkoxide product can then be subjected directly to an Oppenauer oxidation with chloral, and the decalinone is isolated without any further treatment. Its silyl enol ether derivative is then oxidised with *m*-chloroperbenzoic acid in a diastereoselective manner on the side opposite to the axial methyl group (Rubottom oxidation); the *α*-siloxyketone is converted into the corresponding acetate, and deprotonation with LDA leads to an intramolecular aldol reaction under formation of a tricyclic lactone. Dehydration is

followed by reduction with lithium aluminium hydride to open the lactone ring. In presence of the Chaudret ruthenium hydrogenation catalyst, the intended hydrogenation does not occur. Instead, a double bond migration, stereochemically controlled by the OH group at C-3a, produces a lactol, which is finally reduced with triethylsilane to racemic Superambrox. The total yield over all the steps amounts to 27 %.



If the enantiomers are separated at the stage of the racemic dihydro-C14-aldehyde, this method can be applied to produce optically pure Superambrox as well.

3.7.8 Commercial Use

Cetalox® and *Ambrox®* find application in premium perfumes for both, women, *e.g.* in *Chanel No.5®*, and for men, *e.g. Davidoff Cool Water®*, or in *Azzaro pour Homme®* (Fig. 3.58).

Apart from their outstanding olfactory qualities, the scented compounds readily find application as fixatives. The price of *Ambrox®* is in the range of 1000 dollars per kilogram; the worldwide annual production of *Ambrox®* and its analogues are in excess of 30 tonnes. [208]

3.58 *Typical retail products with an ambergris character.*

Summary in Bullet Points

- For thousands of years, ambergris has been a highly prized scent, originating from the digestive tract of the sperm whale.
- The most important olfactory constituent of ambergris is (–)-*Ambrox*®, which may be obtained by partial synthesis from sclareol, isolated from clary sage.
- Polyene cyclisations of homofarnesol and homofarnesylic acid constitute especially elegant total syntheses.
- 5,5a-Dehydroambrox is a new scent with outstanding fragrance properties and intriguing examples of its smart total syntheses.

References

References to Introduction

1. T. Dingermann, I. Zündorf, *Pharm. unserer Zeit* **33** (2004) 432; U. J. Meierhenrich, J. Golebiowski, X. Fernandez, D. Cabrol-Bass, *Angew. Chem. Internat. Edn.* **43** (2004) 6410.
2. S. J. Kleene, R. C. Gesteland, *Brain Res.* **229** (1981) 536.
3. F. Xu, N. Liu, I. Kida, D. L. Rothman, F. Hyder, G. M. Shepherd, *PNAS* **100** (2003) 11029.
4. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin, Heidelberg, New York, 1990, 1.
5. P. Karlson, M. Lüscher, *Nature* **183** (1959) 55.
6. A. Faller, Der Körper des Menschen, Georg Thieme Verlag, Stuttgart, 7th Edn., 1976, 98.
7. *Naturwissenschaftl. Rundschau* **53** (2000) 35.
8. L. Monti-Boch, C. Jennings-White, D. S. Dolberg, D. L. Berliner, *Psychoneuroendocrinology* **19** (1994) 673.
9. A. Faller, Der Körper des Menschen, Georg Thieme Verlag, Stuttgart, 7th Edn., 1976, 226.
10. K. Stern, M. K. McClintock, *Nature* **392** (1998) 177.

11. A. Weller, *Nature* **392** (1998) 126.
12. D. L. Berliner, L. Monti-Bloch, C. Jennings-White, V. Diaz-Sanchez, *J. Steroid Biochem. Molec. Biol*. **58** (1996) 259; O. Vostrowsky, http://www.organik.uni-erlangen.de/vostrowsky/natstoff/naturstoffe.html.
13. http://en.wikipedia.org/wiki/Major_histocompatibility_complex_and_sexual_selection.
14. A. Comfort, *Nature* **230** (1971) 432.
15. V. Prelog, L. Ružička, *Helv. Chim. Acta* **27** (1944) 61; V. Prelog, L. Ružička, P. Wieland, *Helv. Chim. Acta* **27** (1944) 66; http://de.wikipedia.org/wiki/Androstenon.
16. R. Claus, W. Alsing, *J. Endocr*. **68** (1976) 483.
17. C. Wyart, W. W. Webster, J. H. Chen, S. R. Wilson, A. McClary, R. M. Khan, N. Sobel, J. *Neuroscience* **27** (2007) 1261; J. N. Lundström, M. J. Olsson, Biol. Psychol. 70 (2005) 197; T. A. Hummer, M. K. McClintock, *Horm. Behav.* **55** (2009) 548; http://en.wikipedia.org/wiki/Androstadienone.
18. G. Ohloff, Irdische Düfte himmlische Lust, Birkhäuser, Basel, Boston, Berlin, 1992, 21.
19. J. E. Amoore, *Nature* **198** (1963) 271; G. Ohloff, Riechstoffe und Geruchssinn, Springer, Berlin Heidelberg, New York, 1990, 16.
20. Proverbs, Chapter 7, verses 17 and 18, in Martin Luther's translation (23rd Edn., 1905); also in *The New English Bible*, Oxford and Cambridge University Presses, 1970).
21. G. Ohloff, Irdische Düfte himmlische Lust, Birkhäuser, Basel, Boston, Berlin, 1992, 106.
22. Myrtle (*Myrtus communis*) is an evergreen shrub, the essential oil of which is much prized in perfumery. On the other hand, myrrh (Hebrew: *murr* = bitter) refers to the resin of the tree *Commiphora myrrha*, from the balsam family, which is used in embalming.
23. M. Mattner, in Winnacker, Küchler, Chemische Technik, Vol. 8, Wiley-VCH, Weinheim, 2005, 519; D. J. Rowe, Chemistry and Technology of Flavors and Fragrances. Blackwell Publishing Ltd., Oxford, 2005.

References to 3.1 Damascone

24. G. Ohloff, Irdische Düfte himmlische Lust, Birkhäuser Verlag, Basel, Boston, Berlin, 1992, 108.
25. G. Ohloff, Irdische Düfte himmlische Lust, Birkhäuser Verlag, Basel, Boston, Berlin, 1992, 111.
26. L. Bouveault, G. Blanc, *Compt. Rend*. **136** (1903) 1676.
27. C. Seidel, M. Stoll, *Helv. Chim. Acta* **42** (1959) 1830; M. Wüst, *Chemie in unserer Zeit* **37** (2003) 8.
28. W. Pickenhagen, D. Schatkowski, DE 19645922 (1998); M. Oelgemöller, C. Jung, J. Mattay, *Pure Appl. Chem* **79** (2007) 1939.
29. M. Al-Masum, Y. Yamamoto, *J. Am. Chem. Soc.* **120** (1998) 3809; T. Yamamoto, H. Matsuda, Y. Utsumi, T. Hagiwara, T Kanisawa, *Tetrahedron Lett*. **43** (2002) 9077.
30. H. Matsuda, T. Yamamoto, T. Kanisawa, Flavours, Fragrances and Essential Oils, Proceedings 13th Int. Congress of Flavours, Fragrances and Essential Oils, Istanbul, Turkey, 15–19 Oct. 1995, Vol. 3, 85.
31. http:/www.leffingwell.com/rose.htm.
32. G. Ohloff, V. Rautenstrauch, K. H. Schulte-Elte, *Helv. Chim. Acta* **56** (1973) 1503.
33. S. Isoe, S. Katsumura, T. Sakan, *Helv. Chim. Acta* 5**6** (1973) 1514.
34. G. Ohloff, *Prog. Chem. Org. Nat. Prod*. **35** (1978) 431.
35. J. Meinwald, R. Erickson, M. Hartshom, Y. C. Meinwald, T. Eisner, *Tetrahedron Lett*. (1968) 2959; S. W. Russell, B. C. L. Weedon, *Chem. Commun*. (1969) 85.

36. S. Isoe, S. B. Hyeon, H. Ichikawa, S. Katsumura, T. Sakan, *Tetrahedron Lett*. (1968) 5561.
37. S. Isoe, S. Katsumura, S. B. Hyeon, T. Sakan, *Tetrahedron Lett*. (1971) 1089.
38. E. Demole, P. Enggist, U. Säuberli, M. Stoll, E. sz. Kováts, *Helv. Chim. Acta* **53** (1970) 541; G. Ohloff, G. Uhde, *Helv. Chim. Acta* **53** (1970) 531.
39. G. Büchi, J. C. Vederas, *J. Am. Chem. Soc*. **94** (1972) 9128.
40. C. Fehr, J. Galindo, *Helv. Chim. Acta* **69** (1986) 228.
41. C. Fehr, J. Galindo, *J. Org. Chem*. **53** (1988) 1828.
42. C. Fehr, *Angew. Chem. Internat. Edn*. **35** (1996) 2566.
43. C. Fehr, Chiriality in Industry II, Wiley, Chichester, 1997, 335.
44. L. Duhamel, P. Duhamel, L.-C. Plaquevent, *Tetrahedron: Asymmetry* **15** (2004) 3653.
45. B. Schäfer, *Chemie in unserer Zeit* **36** (2002) 382
46. C. Fehr, J. Galindo, *Angew. Chem. Internat. Edn*. **33** (1994) 1888.

References to 3.2 Ionone

47. Ghazal of Hafez Shirazi, in Persian, compiled and corrected by Dr. Behrouz Homayoun Far (far@acm.org), poem 219, engl. translation: http://www.fordham.edu/halsall/source/1370hafiz.asp, poem: LXXI; Appreciation is gratefully acknowledged to Vajihe Safavi, Berlin, for her assistance to find an appropiate translation; G. Ohloff, Irdische Düfte himmlische Lust, Birkhäuser Verlag, Basel, Boston, Berlin (1992), 118; H. Brunner, *Nachr. Chem*. **52** (2004) 445.
48. G. Uhde, G. Ohloff, *Helv. Chim. Acta* **44** (1972) 2621; Purchasing power of the German Goldmark currency: Hamburger Staatsarchiv und Statistischem Bundesamt.
49. E. Brenna, C. Fuganti, S. Serra, *Compt. Rend. Chimie* **6** (2003) 529; A. Abate, E. Brenna, C. Fuganti, F. G. Gatti, S. Serra, *Chem. Biodiv.* **1** (2004) 1888.
50. F.-J. Marner, T. Runge, W. A. König, *Helv. Chim. Acta* **73** (1990) 2165.
51. Y. R. Naves, *J. Soc. Cosmet. Chem.* **22** (1971) 439.
52. G. Ohloff, *Prog. Chem. Org, Nat. Prod.* **35** (1978) 431.
53. R. Willstätter, H. H. Escher, *Z. physiol. Chem*. **64** (1910) 47.
54. S Isoe, S. Be Hyeon, T. Sakan, *Tetrahedron Lett*. **4** (1969) 279.
55. P. Schreier, F. Drawert, S. Bhiwapurkar, *Chem. Mikrobiol. Technol. Lebensm*. **6** (1979) 90.
56. G. W. Sanderson, H. Co, J. G. Gonzalez, *J. Food Sci.* **36** (1971) 231; S. Baldermann, M. Kato, M. Kurosawa, Y. Kurobayashi, A. Fujita, P. Fleischmann, N. Watanabe, *J. Exp. Bot.* **61** (2010) 2967.
57. F.-J. Marner, *Curr. Org. Chem.* **1** (1997) 153.
58. M. F. Carroll, *J. Chem. Soc.* (1940) 704.
59. A. Hassner, C. Stumer, Organic Syntheses Based on Name Reactions and unnamed Reactions, Pergamon, Oxford (1995) 328.
60. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin Heidelberg, New York (1990), 113.
61. H. Siegel, W. Himmele, *Angew. Chem. Internat. Edn*. **13** (1980) 178.
62. C. Mercier, P. Chabardes, *Pure Appl. Chem*. **66** (1994) 1509.
63. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin, Heidelberg, New York, 1990, 117; M. N. Kashid, I. Yuranov, P. Raspail, P. Prechtl, J. Membrez, A. Renken, L. Kiwi-Minsker, *Ind. Eng. Chem. Res.* **50** (2011) 7920 (reaction mechanism and kinetics).
64. C. Fehr, O. Guntern, *Helv. Chim. Acta* **75** (1992) 1023.
65. R. B. Woodward, T. P. Kohmann, G. C. Harris, *J. Am. Chem. Soc*. **63** (1941) 120.
66. E. Brenna, C. Fuganti, P. Grasselli, M. Redaelli, S. Serra, *J. Chem. Soc., Perkin Trans. 1* (1998) 4129.

67. J. Aleu, E. Brenna, C. Fuganti, S. Serra, *J. Chem. Soc., Perkin Trans. 1* (1999) 271.
68. E. Brenna, C. Fuganti, G. Fronza, L. Malpezzi, A. Righetti, S. Serra, *Helv. Chim. Acta* **82** (1999) 2246.
69. E. Brenna, C. Fuganti, S. Serra, *Chem. Soc. Rev*. **37** (2008) 2443.
70. H. Monti, G. Audran, J.-P. Monti, G. Leandri, *J. Org. Chem*. **61** (1996) 6021.
71. W. G. Dauben, D. M. Michno, *J. Org. Chem*. **42** (1977) 682.
72. T. Hayashi, T. Fujiwa, Y. Okamoto, Y. Katsuro, M. Kumada, *Synthesis* (1981) 1001.
73. K. Takai, K. Oshima, H. Nozaki, *Tetrahedron Lett*. **21** (1980) 2531.
74. G. Laval, G. Audran, J.-M. Galano, H. Monti, *J. Org. Chem*. **65** (2000) 3551.

References to 3.3 Jasmonoids

75. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin, 1990, 149; http://en.wikipedia.org/wiki/Jasmine.
76. Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edn., 1998, Electronic Release, Flavors and Fragrances.
77. E. P. Demole, The Fragrance of Jasmine, in Fragrance Chemistry, Ed.: E. Theimer, Academic Press, New York, 1982, 349; C. Chapuis, *Helv. Chim. Acta* **95** (2012) 1479.
78. P. Kraft, J. A. Bajgrowicz, C. Denis, G. Fráter, *Angew. Chem. Internat. Edn*. **39** (2000) 2980.
79. M. H. Beale, J. L. Ward, Nat. Prod. Rep. (1998) 533; F. Schröder, *Angew. Chem. Internat. Edn*. **37** (1998) 1213.
80. P. Kraft, G. Fráter, *Chirality* **13** (2001) 388.
81. B. Schulze, Christian Kost, G.-A. Arimura, W. Boland, *Chemie in unserer Zeit* **40** (2006) 366.
82. S. Fonseca, A. Chini, M. Hamberg, B. Adie, A. Porzel, R. Kramell, O. Miersch, C. Wasternack, R. Solano, *Nature Chem. Bio*. **5** (2009) 344; A. J. K. Koo, G. A. Howe, *Phytochem.* **70** (2009) 1600.
83. L. Crombie, D. O. Morgan, *J. Chem. Soc., Chem. Commun*. (1988) 558; A. Schaller, A. Stintzi, *Phytochem.* **70** (2009) 1532; D. Voet, J. A. Voet, Biochemie, VCH, Weinheim, 1994, 632.
84. H. Staudinger, L. Ružička, *Helv. Chim. Acta* **7** (1924) 245.
85. W. Treff, H. Werner, *Ber. Dtsch. Chem. Ges. B* **68** (1935) 640.
86. S. H. Harper, R. J. D. Smith, *J. Chem. Soc*. (1955) 1512.
87. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin, 1990, 161.
88. H. Hunsdiecker, *Ber. Dtsch. Chem. Ges*. **75** (1942) 460.
89. P. A. Grieco, *J. Org. Chem*. **37** (1972) 2363.
90. H. Stetter, H. Kuhlmann (Bayer AG) DE 2437219 (1974).
91. T. K. Sarkar, B. K. Ghorai, *J. Indian Chem. Soc*. **76** (1999) 693 (Review).
92. H. Nishiyama, K. Sakuta, K. Itoh, *Tetrahedron Lett*. **25** (1984) 2487.
93. N. Krause, S. Ebert, A. Haubrich, *Liebigs Ann. / Recueil* (1997) 2409.
94. G. Quinkert, F. Adam, G. Durner, *Angew. Chem. Internat. Edn.* **21** (1982) 856.
95. M. Hulce, J. P. Mallomo, L. L. Frye, T. P. Kogan, G. H. Posner, *Org. Synth. Coll. Vol. VII* (1990) 495.
96. G. H. Posner, E. Asivatham, *J. Org. Chem*. **50** (1985) 2589.
97. F.-P. Montforts, I. Gesing-Zibulak, W. Grammenos, M. Schneider, K. Laumen, *Helv. Chim. Acta* **72** (1989) 1852.
98. G. Helmchen, A. Goeke, G. Lauer, M. Urmann, J. Fries, *Angew. Chem. Internat. Edn.* **29** (1990) 1024.
99. F. Näf, R. Decorzant, *Helv. Chim. Acta* **61** (1978) 2524.
100. H. Kataoka, T. Yamada, K. Goto, J. Tsuji, *Tetrahedron* **43** (1987) 4107.
101. G. Fráter, J. A. Bajgrowicz, P. Kraft, *Tetrahedron* **54** (1998) 7633.
102. P. Oberhänsli (Givaudan), DE 2008833 (1969).

103. J.-P. Genêt, *Acc. Chem. Res*. **36** (2003) 908.
104. D. A. Dobbs, K. P. M. Vanhessche, E. Brazi, V. Rautenstrauch, J.-Y. Lenoir, J.-P. Genêt, J. Wiles, S. H. Bergens, *Angew. Chem. Internat. Edn.* **39** (2000) 1992; J. A. Wiles, S. H. Bergens, K. P. M. Vanhessche, D. A. Dobbs, V. Rautenstrauch, *Angew. Chem. Internat. Edn*. **40** (2001) 914.
105. C. Fehr, J. Galindo, O. Etter, E. Ohleyer, *Chimia* **53** (1999) 376.
106. C. Fehr, J. Galindo, *Angew. Chem. Internat. Edn*. **39** (2000) 569.
107. C. Fehr, *Angew. Chem. Internat. Edn*. **37**(1998) 2407.
108. C. Celli, DE 224263 (1971).

References to 3.4 Menthol

109. S. Gamez, A. Petitjean, *Le Bup* 98 (2004) 1411.
110. R. B. Croteau, E. M. Davis, K. L. Ringer, M. R. Wildung, *Naturwissenschaften* **92** (2005) 562.
111. Hieronymus David Gaubius, Adversoriorum varii argumentii, Book 1, Leiden, 1771, 99; A. Oppenheim, *J. Chem. Soc*. **15** (1862) 24; G. H. Beckett, C. R. Alder Wright, *J. Chem. Soc*. **29** (1876) 1; M. Moriya, *J. Chem. Soc*. **39** (1881) 77; R. W. Atkinson, H. Yoshida, *J. Chem. Soc*. **41** (1882) 49.
112. A. Goldscheider, *Arch. Anat. Physiol. Abt. Leipzig* (1886) 555.
113. H. Hensel, Y. Zotterman, *Acta Physiol. Scand*. **24** (1951) 27.
114. D. D. McKemy, W. M. Neuhausser, D. Julius, *Nature* **416** (2002) 52.
115. A. M. Peier, A. Moqrich, A. V. Hergarden, A. J. Reeve, D. A. Andersson, G. M. Story, T. J. Rearley, I. Dragoni, P. McIntyre, S. Bevan, A. Patapoutian, *Cell* **108** (2002) 705.
116. G. Reid, M.-L. Flonta, *Nature* **413** (2001) 480; G. Reid, M.-L. Flonta, *Neurosci. Lett*. **297** (2001) 171; G. Reid, B. Amuzescu, E. Zech, M.-L. Flonta, *J. Neurosci. Methods* **111** (2001) 1.
117. http://www.leffingwell.com/cooler_than_menthol.htm.
118. E. T. Wei, D. A. Seid, *J. Pharm. Pharmacol*. **35** (1983) 110; C. Pdoesva, J. M. D. Nascimento, US 3821221 (1974).
119. D. A. Andersson, H. W. N. Chase, S. Bevan, *J. Neurosci*. **24** (2004) 5364.
120. H.-J. Behrendt, T. Germann, C. Gillen, H. Hatt, R. Jostock, *Br. J. Pharmacol.* **141** (2004) 737.
121. P. Gautschi, M. Blondeau, US 2004/0028714 (2004).
122. E. Brenna, C. Fuganti, S. Serra, *Compt. Rend. Chimie* **6** (2003) 529.
123. D. H. Pybus, C. S. Sell, The Chemistry of Fragrances, The Royal Society of Chemistry, Cambridge (1999) 70.
124. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 343.
125. A. Behr, L. Johnen, *ChemSusChem* **2** (2009) 1072; K. Weissermel, H.-J. Arpe, Industrielle Organische Chemie, VCH, Weinheim, 4th Edn., 1994, 125.
126. H. B. Kagan, T.-P. Dang, *J. Am. Chem. Soc*. **94** (1972) 6429.
127. A. Miyashita, A. Yasuda, H. Takaya, K. Toriumi, T. Ito, T. Souchi, R. Noyori, *J. Am. Chem. Soc*. **102** (1980) 7932.
128. S. Otsuka, K. Tani, Asymmetric Synthesis, Academic Press, Orlando, Vol. 5 (1985) 171.
129. C. Chapuis, M. Barthe, J.-Y. de Saint Laumer, *Helv. Chim. Acta* **84** (2001) 230, footnote 4; C. Jäkel, R. Paciello, in H.-U. Blaser, H.-J. Federsel, Asymmetric Catalysis on Industrial Scale, Wiley-VCH, Weinheim, 2010, 187; http://en.wikipedia.org/wiki/Josiphos ligands; A. Togni, *Chimia* **50** (1996) 86; A. Salzer, *Coord. Chem. Rev.* **242** (2003) 59.
130. http://www.leffingwell.com/menthol13/menthol_basf.htm; for asymmetric transfer hydrogenation: R. Wu, M. G. Beauchamps, J. M. Laquidara, J. R. Sowa Jr., *Angew. Chem.* **124** (2012) 2148.

131. C. Jäkel, R. Paciello, US 7534921 (2009); M. Friedrich, K. Ebel, N. Götz, US 7550633 (2009); M. Friedrich, K. Ebel, N. Götz, W. Krause, C. Zahm, US 7608742 (2009).
132. H. Maeda, S. Yamada, H. Itoh, Y. Hori, *Chem. Commun.* **48** (2012) 1772.
133. T. Yamamoto, in K. A. D. Swift, Current Topics in Flavours and Fragrances, Kluwer Acad. Publ., Dordrecht, 1999, 33; G. S. Clark, *Perfumer & Flavorist* **32** (2007) 38–47; http://www.leffingwell.com/menthol1/menthol1.htm.

References to 3.5 Vanillin

134. H. Schmitz, *Chem. Ind.* (*London*) (2001) 803.
135. J. Hurst, S. M. Tarka jr., T. G. Powis, F. Valdez jr, T. R. Hester, *Nature* **418** (2002) 289.
136. M. B. Hocking, *J. Chem. Educ*. **74** (1997) 1055.
137. CD Römpp Chemie Lexikon, Version 1.0, Georg Thieme Verlag, Stuttgart/New York, 1995.
138. A. S. Ranadive, in G. Charalambous, Spices, Herbs and Edible Fungi, Elsevier Science B.V., 1994, 517 (Review on Vanilla).
139. W. Freist, *Chemie in unserer Zeit* **25** (1991) 135; K. Roth, *Chemie in unserer Zeit* **44** (2010) 414.
140. W. Haarmann, Fortschritte der Theerfarbenfabrikation, Springer Verlag, Berlin, 1888, 583.
141. K. Freudenberg, *Angew. Chem*. **68** (1956) 508.
142. Organikum, VEB Deutscher Verlag der Wissenschaften, 15th Edn., Berlin, 1981, 661.
143. K. Reimer, *Ber. Dtsch. Chem. Ges.* **9** (1876) 423; F. Tiemann, P. Koppe, *Ber. Dtsch. Chem. Ges*. **14** (1881), 2015; M. C. Traub, DE 80195 (1894).
144. A. v. Wacek, K. Kratzl, *Ber. Dtsch. Chem. Ges*. **76** (1943) 891.
145. Ullmann's Encyclopedia of Industrial Chemistry, 6th Edn., 1999, (Electronic Release, Glyoxylic Acid).
146. K. Bauer, W. Steuer, DE 2115551 (1971).
147. S. Ratton, *Chem. Today*, March/April 1998, 33.
148. C. Moreau, Catalysis of Organic Reactions, F. E. Herkes, 1998, 51.
149. M. H. Zenk, *Z. Pflanzenphysiol*. **35** (1965) 404.
150. B. Winkel-Shirley, *Physiol. Plantarum* **107** (1999) 142.
151. N. J. Walton, M. J. Mayer, A. Narbad, *Phytochem.* **63** (2003) 505.
152. J.-R. Desmurs, D. Giannotta, M. Gelo-Pujic, C. Role, P. Lancelin, *Parfumer & Flavorist* **29** (2004) 32.
153. K. Li, J. W. Frost, *J. Am. Chem. Soc*. **120** (1998) 10545.
154. P. Karlson, Biochemie, Georg Thieme Verlag, Stuttgart, 7th. Edn. 1970, 256.
155. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 608.
156. K. Li, J. W. Frost, *J. Am. Chem. Soc*. **121** (1999) 9461.
157. E. H. Hansen, B. L. Moller, G. R. Kock, C. M. Bünner, C. Kristensen, O. R. Jensen, F. T. Okkels, C. E. Olsen, M. S. Motawia, J. Hansen, *Appl. Environ. Microbiol.* **75** (2009) 2765.

References to 3.6 Muscone

158. http://en.wikipedia.org/wiki/Musk; http://en.wikipedia.org/wiki/African_Civet.
159. H. Walbaum, *Ber. Dtsch. Chem. Ges.* **33** (1900) 1903; H. Walbaum, *J. prakt. Chem.* **73** (1906) 488.
160. E. Sack, *Chem. Ztg*. **39** (1915) 538.
161. L. Ružička, *Helv. Chim. Acta* **9** (1926) 715.
162. L. Ružička, *Helv. Chim. Acta* **9** (1926) 230.
163. M. Kerschbaum, *Ber. Dtsch. Chem. Ges*. **60** (1927) 902.

164. R. W. Moncrieff, *Soap, Perfumery & Cosmetics* (1947) 261.
165. G. Helmchen, Biosynthese-Vorlesungsmanuskript, University Heidelberg, 2003, 102; S. Schulz, J. Fuhlendorff, J. L. M. Steidle, J. Collatz, J.-T. Franz, *ChemBioChem* **5** (2004) 1500.
166. A. Baur, *Chem. Ztg.* **14** (1890) 1094; A. Baur, *Ber. Dtsch. Chem. Ges.* **24** (1891) 2832; J. Streith, personal communication (2011).
167. A. Baur, *Ber. Dtsch. Chem. Ges.* **27** (1894) 1614.
168. A. Baur, *Ber. Dtsch. Chem. Ges.* **31** (1898) 1344.
169. G. Fráter, U. Müller, P. Kraft, *Helv. Chim. Acta* **82** (1999) 1656.
170. A. Ciappa, U. Matteoli, A. Scrivanti, *Tetrahedron: Asymmetry* **13** (2002) 2193.
171. C. Fehr in A. N. Collins, G. Sheldrake, J. Crosby (Ed.), Chirality in Industry II, Wiley, Chichester, 1997, 335.
172. A. S. Williams, *Synthesis* (1999) 1707.
173. R. A. Hites, K. Biemann, *J. Am. Chem. Soc.* **94** (1972) 5772.
174. Organikum, VEB Deutscher Verlag der Wissenschaften, Berlin 1981, 547 (Mechanism).
175. K. Weissermel, H.-J. Arpe, Industrielle Organische Chemie, VCH Weinheim, 4th Edn., 1994, 264.
176. G. Ohloff, J. Becker, K. H. Schulte-Elte, *Helv. Chim. Acta* **50** (1967) 705.
177. K. Biemann, G. Büchi, B. H. Walker, *J. Am. Chem. Soc.* **79** (1957) 5558.
178. A. Eschenmoser, D. Felix, G. Ohloff, *Helv. Chim. Acta* **50** (1967) 708.
179. D. Felix, J. Schreiber, G. Ohloff, A. Eschenmoser, *Helv. Chim. Acta* **54** (1971) 2896.
180. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin, Heidelberg, New York, 1990, 204; C. Fehr, G. Ohloff, G. Büchi, *Helv. Chim. Acta* **62** (1979) 2655.
181. M. Nagel, H.-J. Hansen, G. Frater, *Synlett* (2002) 280.
182. M. Baumann, W. Hoffmann, N. Müller, *Tetrahedron Lett.* **40** (1976) 3585.
183. K. Yokoyama, H. Nanbu, JP 54092947 (1979).
184. K. H. Schulte-Elte, A. Hauser, G. Ohloff, *Helv. Chim. Acta* **62** (1979) 2673.
185. R. H. Grubbs, *Tetrahedron* **60** (2004) 7117.
186. M. F. C. Plugge, J. C. Mol, *Synlett* (1991) 507.
187. R. Hamasaki, S. Funakoshi, T. Misaki, Y. Tanabe, *Tetrahedron* **56** (2000) 7423.
188. J. C. Mol, *Green Chemistry* **4** (2002) 5.
189. E. W. Spanagel, W. H. Carothers, *J. Am. Chem. Soc.* **58** (1936) 654.
190. E.-C. Chan, J. Kuo, *Enzyme Microb. Technol.* **20** (1997) 585.
191. A. Fürstner, D. Koch, K. Langemann, W. Leitner, C. Six, *Angew. Chem. Internat. Edn.* **36** (1997) 2466.
192. A. Fürstner, M. Picquet, C. Bruneau, P. H. Dixneuf, *Chem. Commun.* (1998) 1315.
193. A. Fürstner, *Chem. Commun.* (1999) 95; A. Fürstner, *Science* **341** (2013) 1229713.
194. Y. Matsumura, H. Fukawa, Y. Terao, *Chem. Pharm. Bull.* **46** (1998) 1484.
195. W. Oppolzer, R. R. N. Radinow, *J. Am. Chem. Soc.* **115** (1993) 1593.
196. V. P. Kamat, H. Hagiwara, T. Suzuki, M. Ando, *J. Chem. Soc. Perkin Trans. 1* (1998) 2253; V. P. Kamat, H. Hagiwara, T. Katsumi, T. Hoshi, T. Suzuki, M. Ando, *Tetrahedron* **56** (2000) 4397.
197. T. Weskamp, W. C. Schattenmann, M. Spiegler, W. A. Herrmann, *Angew. Chem Internat. Edn.* **37** (1998) 2490; A. Fürstner, W. A. Herrmann, *Tetrahedron Lett.* **40** (1999) 4787.
198. J. Louie, C. W. Bielawski, R. H. Grubbs, *J. Am. Chem. Soc.* **123** (2001) 11312.
199. A. Alexakis, C. Benhaim, X. Fournioux, A. van den Heuvel, J.-M. Levêque, S. March, S. Rosset, *Synlett* (1999) 1811.
200. Y. H. Choi, J. Y. Choi, H. Y. Yang, Y. H. Kim, *Tetrahedron: Asymmetry* **13** (2002) 801.

201. C. Fehr, J. Galindo, I. Farris, A. Cuenca, *Helv. Chim. Acta* **87** (2004) 1737.
202. S. Ogura, H. Matsuda, T. Yamamoto, JP 07267968 (1995).
203. S. Ogura, T. Yamamoto, JP 06192161 (1994).
204. T. Yamamoto, M. Ogura, T. Kanisawa, *Tetrahedron* **58** (2002) 9209.
205. R. C. Larock, T. R. Hightower, G. A. Kraus, P. Hahn, D. Zheng, *Tetrahedron Lett*. **36** (1995) 2423.
206. Y. Ho, T. Hirao, T. Saegusa, *J. Org. Chem*. **43** (1978) 1011.

References to 3.7 *Ambrox*®

207. G. Ohloff, Irdische Düfte – Himmlische Lust, Birkhäuser Verlag, Basel 1992, 136.
208. C. Allemann, Synthesis and Application of an Electronically Chiral Mimic of CpFe, Dissertation, Université de Fribourg (Switzerland), 2002.
209. Total synthesis of ambrein: K. Mori, H. Tamura, *Liebigs Ann. Chem*. (1990) 361.
210. C. S. Sell, A Fragrant Introduction to Terpenoid Chemistry, Royal Society of Chemistry, Cambridge, 2003, 229.
211. G. Ohloff, Riechstoffe und Geruchssinn, Springer Verlag, Berlin, 1990, page 209.
212. G. Ohloff, W. Giersch, W. Pickenhagen, A. Furrer, B. Frei, *Helv. Chim. Acta* **68** (1985) 2022.
213. G. Fráter, J. A. Bajgrowicz, P. Kraft, *Tetrahedron* **54** (1998) 7633.
214. M. Hinder, M. Stoll, *Helv. Chim. Acta* **33** (1950) 1251; M. Hinder, M. Stoll, *Helv. Chim. Acta* **33** (1950) 1308.
215. M. Schalk, L. Pastore, M. A. Mirata, S. Khim, M. Schouwey, F. Deguerry, V. Pineda, L. Rocci, L. Daviet, *J. Am. Chem. Soc.* **134** (2012) 18900; D. V. Banthorpe, J. T. Brown, G. S. Morris, *Phytochemistry* **31** (1992) 3391; Z. Guo, R. F. Severson, G. J. Wagner, *Biochem. Biophys.* **308** (1994) 103.
216. M.p. of sclareol: 95–100 °C
217. C. E. Teague Jr., T. C. James, C. D. Mays, US 3060172 (1962).
218. Ullmann Encyclopedia, Electronic Release, Flavors and Fragrances, Clary sage oil.
219. D. H. R. Barton, D. K. Taylor, C.-I. Tse, *Tetrahedron Lett.* **35** (1994) 9505.
220. D. H. R. Barton, D. K. Taylor, C.-L. Tse, US 5473085 (1994).
221. M. Schneider, T. Stalberg, T. Gerke, WO 93/21174 (1993).
222. M. I. Farbood, B. J. Willis, US 4798799 (1989).
223. M. I. Farbood, J. A. Morris, A. E. Downey, US 4970163 (1990).
224. A. F. Barrero, E. J. Alvarez-Manzaneda, J. Altarejos, S. Salido, J. M. Ramos, *Tetrahedron* **49** (1993) 10405.
225. H. Akita, M. Nozawa, A. Mitsuda, H. Ohsawa, *Tetrahedron: Asymmetry* **11** (2000) 1375.
226. J. P. Kutney, C. Cirera, *Can. J. Chem*. **75** (1997) 1136.
227. A. A. Verstqen-Haaksma, H. J. Swarts, B. J. M. Jansen, A. de Groot, *Tetrahedron* **50** (1994) 10095.
228. T. Kawanobe, K. Kogami, M. Matsui, *Agric. Biol. Chem*. **50** (1986) 1475.
229. N. Ravasio, M. Antenori, M. Gargano, P. Mastrorilli, *Tetrahedron Lett.* **37** (1996) 3529.
230. Analogue of the C14-aldehyde synthesis for the preparation of Vitamin A acetate according to Hoffmann-la-Roche.
231. A. F. Barrero, J. Altarejos, E. J. Alvarez-Manzaneda, J. M. Ramos, S. Salido, *J. Org. Chem*. **61** (1996) 2215.
232. K. B. Upar, S. J. Mishra, S. P. Nalawade, S. A. Singh, R. P. Khandare, S. V. Bhat, *Tetrahedron: Asymmetry* **20** (2009) 1637.
233. G. Büchi, H. Wüst, *Helv. Chim. Acta* **72** (1989) 996.
234. G. Stork, A. W. Burgstrahler, *J. Am. Chem. Soc*. **77** (1955) 5068.

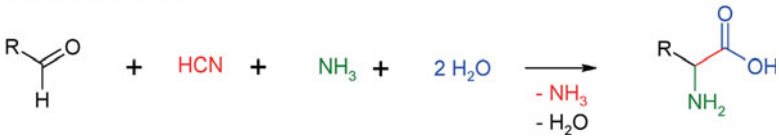
235. A. Eschenmoser, L. Ružička, O. Jeger, D. Arigoni, *Helv. Chim. Acta* **38** (1955) 1890.
236. E. E. van Tamelen, *Acc. Chem. Res*. **1** (1968) 111.
237. G. Lucius, *Chem. Ber*. **93** (1960) 2663.
238. P. V. Fish, W. S. Johnson, *Tetrahedron Lett*. **35** (1994) 1469; W. S. Johnson, W. R. Bartlett, B. A. Czeskis, A. Gautier, C. H. Lee, R. Lemoine, E. J. Leopold, G. R. Luedtke, K. J. Bancroft, *J. Org. Chem*. **64** (1999) 9587.
239. S. G. Sethofer, T. Mayer, F. D. Toste, *J. Am. Chem. Soc.* **132** (2010) 8276.
240. A. Batsanov, L. Chen, G. B. Gill, G. Pattenden, *J. Chem. Soc., Perkin Trans. 1* (1996) 45.
241. S. Handa, G. Pattenden, W.-S. Li, *Chem. Commun*. (1998) 311; S. Handa, P. S. Nair, G. Pattenden, *Helv. Chim. Acta* **83** (2000) 2629.
242. E. E. Korshin, G. H. Posner, *Chemtracts – Organic Chemistry* **12** (1999) 45.
243. S. Rendler, D. W. C. MacMillan, *J. Am. Chem. Soc.* **132** (2010) 5027.
244. Ullmann Encyclopedia, Electronic Release, Flavours and Fragrances.
245. J. M. Cassel, S. M. Hogagland, J. M. Renga, WO 92/06063 (1992).
246. T. Hosogai, Y. Fujita, Y. Ninagawa, T. Nishida, *Chem. Lett*. (1982) 357.
247. P. F. Vlad, N. D. Ungur, V. B. Perutskii, *Chem. Heterocycl. Compds, Engl. Transl.* **26** (1990) 746.
248. K. Ishihara, H. Ishibashi, H. Yamamoto, *J. Am. Chem. Soc*. **124** (2002) 3647.
249. G. Staiger, A. Macri, DE3240054 (1984).
250. A. Saito, H. Matsushita, H. Kaneko, *Chem. Lett.* (1984) 591.
251. A. Bomhard, J. Cassel, A. Olivero, WO94/17053 (1994).
252. P. F. Vlad, N. D. Ungur, V. B. Perutskii, *Chem. Heterocycl. Compds., Engl. Transl.* **27** (1991) 246.
253. R. L. Snowden, *Chem. Biodivers*. **5** (2008) 958.
254. R. L. Snowden, J.-C. Eichenberger, S. M. Linder, P. Sonnay, C. Vial, K. H. Schulte-Elte, *J. Org. Chem.* **57** (1992) 955.
255. H. Tanimoto, T. Oritani, *Tetrahedron: Asymmetry* **7** (1996) 1695.
256. Römpp Online, Thieme Verlag, Hopanoide (2010).
257. T. Hoshino, Y. Kumai, I. Kudo, S. Nakano, S. Ohashi, *Org. Biomol. Chem*. **2** (2004) 2650.
258. K. U. Wendt, K. Poralla, G. E. Schulz, *Science* **277** (1997) 1811; D. J. Reinert, G. Balliano, G. E. Schulz, *Chem. Biol.* **11** (2004) 121.
259. T. Hoshino, T. Sato, *Chem. Commun*. (2002) 291.
260. M. Breuer, personal communication.
261. S. Neumann, H. Simon, *Biol. Chem. Hoppe-Seyler* **367** (1986) 723.
262. A. Hayase, K. Igarashi, JP 2009-60799 (2009).
263. R. L. Snowden, S. Escher, EP 558928 (1993).
264. M. G. Bolster, B. J. M. Jansen, A. de Groot, *Tetrahedron* **57** (2001) 5663.
265. C. Fehr, I. Farris, *Angew. Chem. Internat. Edn*. **45** (2006) 6904.

4 | Amino acids

The history of amino acids begins four billion years ago. The Earth's atmosphere then consisted of water vapour, carbon dioxide, nitrogen, carbon monoxide, hydrogen, methane and ammonia. It was hot, and for millions of years lightning flashes discharged across the sky (Fig. 4.1). Under these conditions initially aldehydes and hydrogen cyanide originated, and therefrom amino acids were produced (by Strecker reaction).

4.1 *Electrostatic discharges led to the first organic compounds.*

Strecker reaction:



Such a scenario was simulated in 1953 at the University of Chicago in legendary experiments by the young American chemist Stanley Lloyd Miller (1930–2007), who could recreate these processes in his laboratory within a week (Fig. 4.2). [1] Already in the first experiments he found glycine, alanine, β-alanine, aspartic acid and α-aminobutyric acid. [2] Miller's reports caused a sensation – up until that time, it was believed, that the building stones of life could not emerge under "inorganic" conditions. Later it was discovered, that amino acids are even converted into short peptides by iron/nickel sulfides in the presence of carbon monoxide and hydrogen sulfide. [3, 4]

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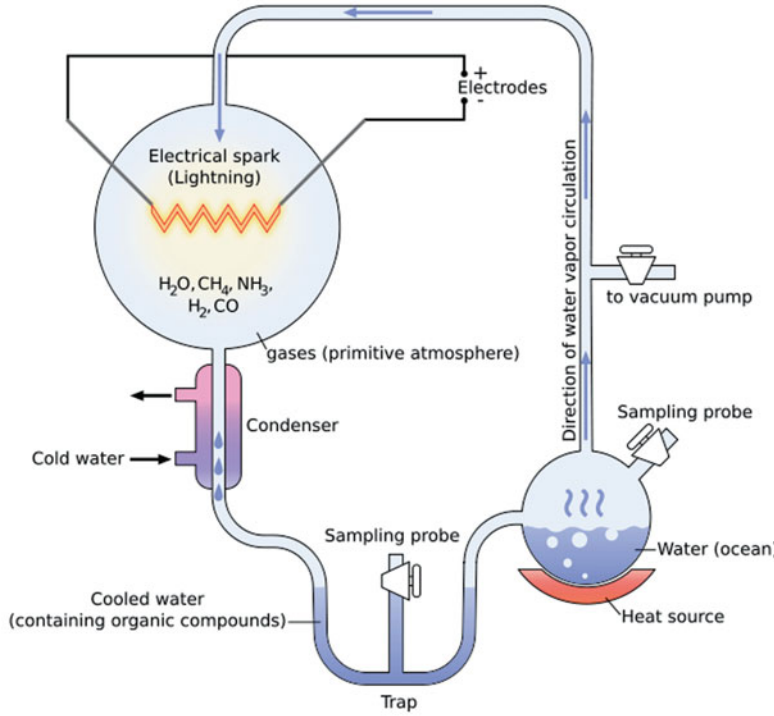
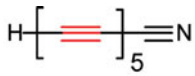


4.2 *By using Stanley Miller's apparatus, it became possible to prove that the building blocks of life had their origin in the prebiotic atmosphere.*

Perhaps life also came out of the depths of the universe, in the form of cosmic dust or from meteorites. Following on from these first random discoveries, by around 1970, astrophysicists began employing spectroscopic methods to search systematically for organic compounds in the huge interstellar dust clouds. [5, 6] The result was overwhelming in terms of both, the number of compounds discovered (>170), and also their structure. By microwave spectroscopy of the dust clouds in the centre of our galaxy and in constellations of Orion and Taurus (Taurus dark nebula) there were found, for example, common chemicals like ethanol, methanethiol, hydrogen cyanide, methyl formate, methylamine, formaldehyde and ketene, but also some extremely unusual compounds, such as 2,4,6-heptatriynonitrile or 2,4,6,8,10-undecapentaynonitrile (Fig. 4.3). [7] Interestingly enough, the Cassini-Huygens space probe revealed similar compounds also in our planetary neighbourhood, in the athmosphere of Saturn's moon Titan. [8] In 2002, at the National Astronomy Observatory in Arizona, the first amino acid (glycine) was detected with a 12-metre telescope in the molecular clouds of Sagittarius B2 (the archer), Orion KL and W51, by observation of a characteristic set of 27 lines in the rotation/vibration spectrum. [9, 10]



2,4,6,8,10-Undecapentaynonitrile

Even more convincing than the spectroscopic proof of amino acids in the universe is the chemical analysis of chondrites (meteorites). Seventeen amino acids were discovered in the Murchison chondrite, which was found in Australia; ten of these do not occur in Nature (on Earth). In terms of the total amino acid content of the Murchison chondrite, about one third consists of glycine,

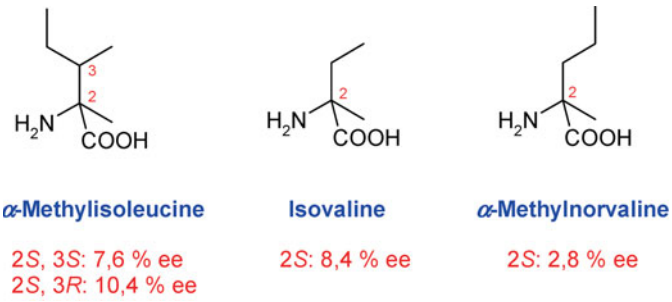
4.3 *The Great Orion Nebula M42 is located in the so-called "sword" area underneath the star Alnitak in Orion's Belt. The distance of M42 from the Earth is 1,344 light years. The Orion nebula contains dust, and it may be assumed that new stars can be formed in this area.*

followed by alanine, aspartic acid and valine. This or similar proportions have been found in all those chondrites, in which amino acids have been observed. It is impressive, not only that these amino acids are produced preferentially in Miller's experiments, but also that glycine, alanine, aspartic acid and valine are the most frequently-occurring proteinogenic amino acids in the biosphere. [11]

In the case of a few α-methylated amino acids (α-methylisoleucine, isovaline, α-methylnorvaline), it is established, that an enantiomeric excess favours the (*L*)-enantiomer by up to 10 %. [12–14] Obviously, enantiomerically enriched amino acids may also result under conditions in outer space (Fig. 4.4). This was surprising, since Miller's experiments always produced racemic amino acids.

The loss of symmetry [15–18] in the formation of the first amino acids can have various causes. Apart from the chance of a local enantiomeric excess

In the morning of September 28, 1969, a carbon-containing chondrite fell to Earth in the neighbourhood of Murchison, Victoria (Australia). This actual meteorite disintegrated on entry into the Earth's atmosphere, and scattered its fragments over an area of five square miles.



4.4 *Enantiomerically enriched amino acids in the Universe and parts of some carbonaceous chondrites from Pueblito de Allende (Mexico), Yukon (Canada) and Murchison (Australia) (left to right).*

resulting through enantioselective adsorption on chiral crystals, *e.g.* (*D*)-alanine on α-quartz, or spontaneous crystallisation in chiral crystal forms [19], extraterrestrial influences have been discussed, whereby the young Earth may have been inoculated with enantiomerically enriched material. Circularly polarised light from the universe could have led to the loss of parity, as well as the more recently discussed weak interaction.

Enantioselective enriching processes lead to homochirality [20] in amino acids, which is not a consequence of, but a prerequisite for the origin of life. Mutation and selection formed the first self-replicating, but still prebiotic, molecular systems of catalytically highly active peptides and oligoribonucleotides. Ultimately, macroscopic asymmetry derived *a posteriori* from non-linear amplification mechanisms. [21, 22]

Most recently, there are among artificial systems some very impressive illustrations of enantiomeric enrichment by means of enantioselective autocatalysis. [23–25]

All proteinogenic amino acids now are α-amino acids, which, except for glycine, are all chiral and possess the (*L*)-configuration.

4.1 Biological Nitrogen Fixation

The biosynthesis of amino acids first requires access to a nitrogen source. In 1888, Hermann Hellriegel (1831–1895) and Hermann Wilfarth (1853–1904) discovered the process of nitrogen fixation by nodular bacteria in the root nodules of the *Leguminosae*. [26]

This was one of the far-reaching discoveries in biochemistry, since nitrogen fixation is of similar importance to life on Earth as photosynthesis. Although the atmosphere consists of 78 % of molecular nitrogen, its access for chemical conversion is not simple.

Nitrogen fixation takes place in bacteria living in symbiosis, such as *Rhizobium meliloti* in *Leguminosae*, but also in free living bacteria like *Azotobacter vinelandii* (aerobic soil bacteria) or *Clostridium pasteurianum* (anaerobic soil bacteria). In this way, at ambient temperature and pressure, Nature produces around 1.7×10^8 tonnes of ammonia per annum. [27]

$$N_2 + 8\,H^+ + 8\,e^- \xrightarrow[\text{FeMo-Nitrogenase}]{} 2\,NH_3 + H_2$$

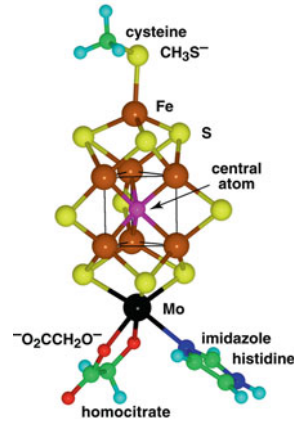
In 1930, Hermann Bortels (1902–1979) recognised that nitrogen fixation is a molybdenum-dependent process. Obviously, the nitrogenases from *Rhizobium meliloti*, *Azotobacter vinelandii* and *Clostridium pasteurianum* have a similar constitution. In 1966, Leonard E. Mortenson identified for the first time an "Fe-" and a "MoFe-protein" as parts of the nitrogenase enzyme system. The exact structure of the nitrogenase-molybdenum-iron protein from *Azotobacter vinelandii* [28] was clarified in 1992, and that from *Clostridium pasteurianum* [29] in 1993, both by Douglas C. Rees. [30] The Fe-protein is a γ_2-dimer with a molar mass of some 60,000 Daltons, and the MoFe-protein is an $\alpha_2\beta_2$-tetramer of *ca.* 240,000 Daltons. [31, 32]

1 Dalton = 1 (g/mole)/Avogadro number (1/mole) = 1.66×10^{-24} g.

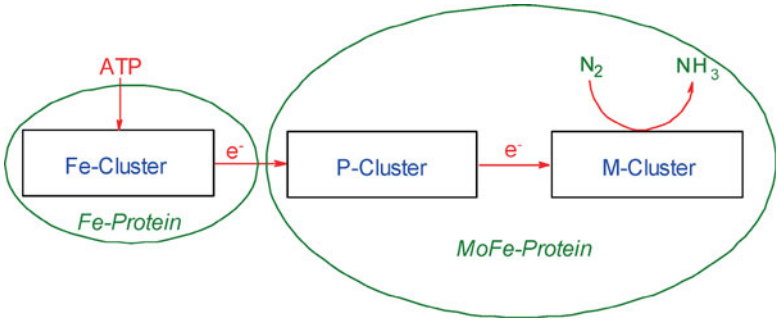
The Fe-protein binds over two cysteine residues a cube-like $[Fe_4S_4]$-cluster, ADP and two $[Mg(H_2O)_4]^{++}$. The MoFe-protein contains as cofactor a so-called P-cluster, from a Fe_4S_4-cube and an Fe_4S_3-fragment. Beside the P-cluster, the

MoFe-Protein contains a second cluster, the M-cluster. [33] This is covalently bonded to cysteine and histidine of the α-subunit. A homocitrate anion ((*R*)-2-Hydroxybutane-1,2,4-tricarboxylic acid) is complexed as a bidentate ligand with molybdenum (Fig. 4.5). On the basis of better resolved X-ray structural analysis and quantum mechanical calculations, initially it was thought, a nitrogen atom is located in the centre of the Fe/Mo complex, [34] but more recent results gave evidence for an interstitial carbido ligand [35, 36], which is unprecedent in bioinorganic chemistry.

In an ATP-dependent reaction, the Fe-protein transfers electrons *via* the P-cluster to the M-cluster, where the nitrogen is finally reduced (Fig. 4.6). How this occurs in particular cases, is still subject of intensive research. [37, 38]



4.5 *The M-cluster complexes molecular nitrogen and reduces it to ammonia.*



4.6 *The energy for reduction of nitrogen is transferred through different iron-sulfur clusters.*

It is interesting, that the substrate specificity of nitrogenases is low in comparison with other enzymes. They also reduce acetylene (to ethylene), hydrazine [39], cyanide and azide. [40] From this, and from the presumably very high biological age of the enzyme system, W. S. Silver and John R. Postgate [41] concluded that the enzyme's original purpose was not nitrogen fixation, but cyanide detoxification in the biosphere of Precambrian bacteria.

4.2 Artificial Nitrogen Fixation

In 1840, Justus von Liebig (1803–1873) discovered that the growth of plants is dependent on a range of elements in available form (nutrients). Liebig's Law of the Minimum states, that the yield of a crop is proportional to the amount of the most limiting nutrient, whichever nutrient it may be. [42] Plants require principally potassium, calcium, magnesium, iron, carbon, nitrogen, oxygen, phosphorus, sulfur and hydrogen along with very small amounts of a range of metals, mainly from the 3rd row. Liebig recognised that the supply of nitrogen for growth is often limited, and he developed the first chemical fertiliser. Thereby it became possible to increase agricultural yields significantly and improve the human food supply substantially.

"Saltpeter" denominates historically the salts of nitric acid. For agriculture, "ammonsaltpeter" (ammonium nitrate, NH_4NO_3) is of great importance.

4.7 *Fritz Haber (1868–1934) discovered the osmium-catalysed synthesis of ammonia.*

Saltpeter was also needed for the preparation of "black powder". It was so valuable, that wars were incited over natural deposits (the "Nitrate War", 1879–1882, between Bolivia, Peru and Chile).

For both, the peaceful and the violent purposes – which demonstrates the ambivalence of scientific knowledge – the Haber-Bosch process provided the necessary starting material: ammonia (Fig. 4.7).

In 1913, a cyclic process was started at BASF: from nitrogen and hydrogen, at a temperature of 500 °C and a pressure of 200 bar over an α-iron catalyst [43], ammonia was produced with a turnover of 17 % (Fig. 4.8 and Fig. 4.9).

$$N_2 + H_2 \xrightarrow[\alpha\text{-Fe}]{500\ ^\circ C,\ 200\ bar} NH_3$$

In the most modern production plants of the Kellogg Advanced Ammonia Process, there have now been staged reactors installed. The first stage is charged with an iron catalyst and the following three with ruthenium catalysts. Thereby, the working pressure may be lowered to around 90 bar. To some extent, this advantage is offset by the higher cost of the ruthenium catalyst. [44]

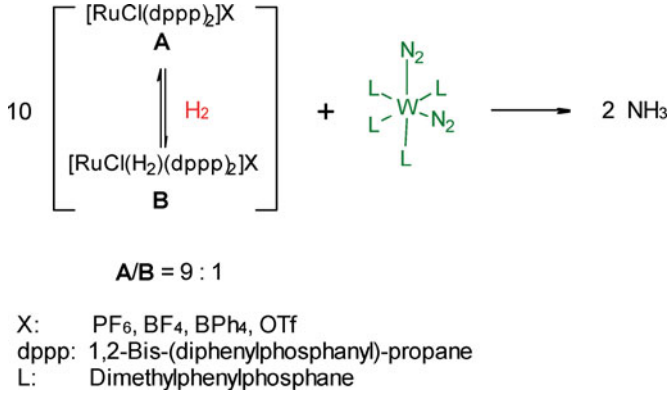
Most recently, the synthesis of ammonia without high pressure has been achieved at a laboratory scale by an electrochemical route at temperatures of up to 450–570 °C. [45, 46]

Compared to the efficiency of Nature with its nodular bacterial system to bind and metabolise nitrogen from the atmosphere under ambient conditions, Man-designed technology has so far to be rated as only modest. [48]

The isolation of the first N_2 complex [49] $[Ru(NH_3)_5(N_2)]^{2+}$ by Albert D. Allen and Caesar V. Senoff at the University of Toronto in 1965 was considered as a sensation. In the meantime, a series of other nitrogen complexes have become known, but they are still unsatisfactory for the synthesis of ammonia. The stage for new developments was set in 1985 by Joseph Chatt, who could show that the protolysis of *cis*-$[W(N_2)_2(PMe_2Ph)_4]$ gives ammonia. Ten years later, Christopher Picket and Jean Talermin developed an electrolytic synthesis of ammonia. [50] More recently, with novel catalytic systems it became possible to improve the yields of nitrogen reduction to ammonia.

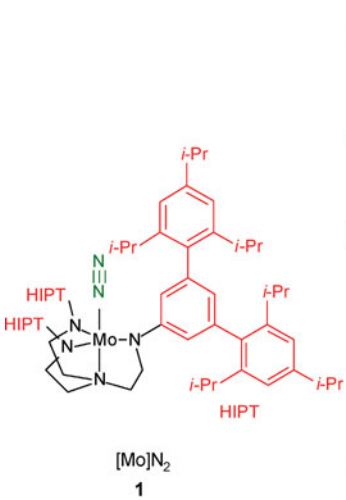
In a hydrogen atmosphere, a ruthenium complex **A** absorbs hydrogen, whereby 10 % of the complex **B** is formed, which is the reducing species for nitrogen, bound to a tungsten complex, to form ammonia. This reaction is however not catalytic. Under optimal conditions, the best yield reaches around 55 %. [51]

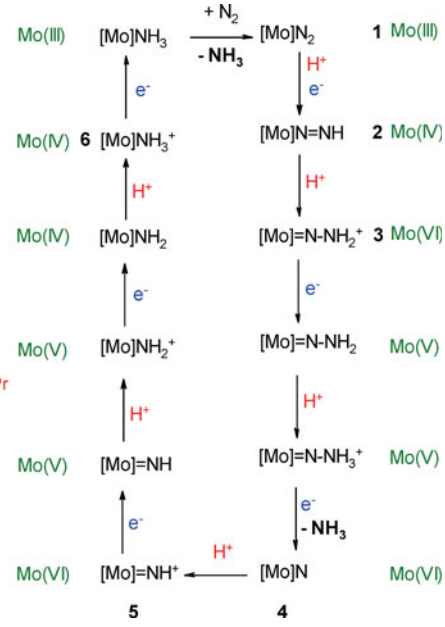
Up to the early 20th century, osmium was very expensive, because the World's inventory amounted to only around 100 kg; in addition, the material was hardly suited for industrial application. This prompted Alwin Mittasch (1869–1953) at BASF to explore more convenient catalysts. For this, he used around 30 experimental kilns, and after more than 20,000 experiments, he found the optimal catalyst: α-iron. Mittasch's work constitutes an early example of catalyst high-throughput screening, a technique which is no longer obscure to modern research.

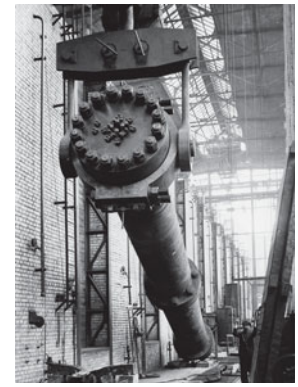


4.8 *The structure of α-iron is body-centred cubic. α-Iron is ferromagnetic. The Atomium, the symbol of the Brussels World Fair of 1958, is a 102 m high construction in the form of a 165-billion-times enlarged unit cell of an α-iron crystal. [43]*

Richard Schrock published for the first time the structure of a molybdenum complex, with which catalytic reduction of molecular nitrogen to ammonia was successfully achieved. [52, 53] The sterically extraordinarily demanding tetradentate ligand inhibits the formation of a binuclear, unreactive, end-on-bridged dinitrogen-molybdenum complex, and creates a cavity, in which the reaction proceeds. Decamethylchromocene (Cp^*_2Cr) serves as the reducing agent and 2,6-lutidinium tetra-(3,5-bistrifluoromethylphenyl)-borate $\{(2,6\text{-}Me_2C_5H_3NH)^+ B[3,5\text{-}(CF_3)_2C_6H_3]_4^-\}$ as the proton source. It is noteworthy, that Schrock also prepared several of the intermediates (**2**–**6**) of the postulated catalytic cycle, and was able to convert one into another. Under strictly controlled reaction conditions (slow addition of Cp^*_2Cr), a turnover number of four has been attained thus far.





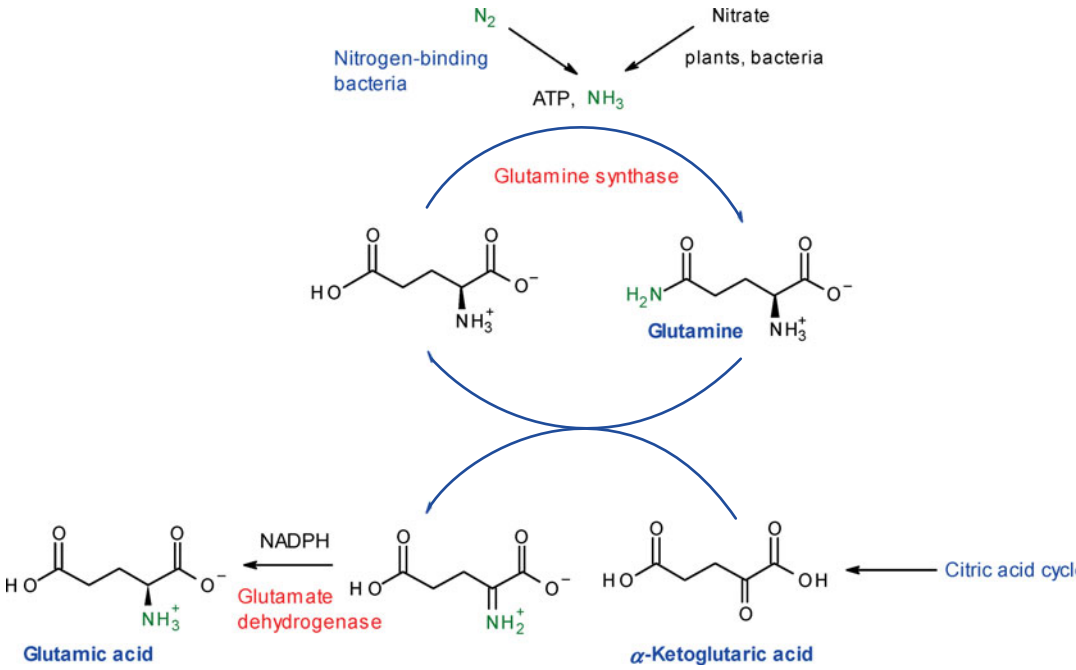


4.9 *Ammonia reactor during construction of the plant. The worldwide annual production of ammonia is in the region of 150 million tonnes. [44] 40 % of the nitrogen content of every European and US citizen's body has already seen the inside of an ammonia plant. For the Chinese, this value is even at 66 %, due to differences in diet. [47]*

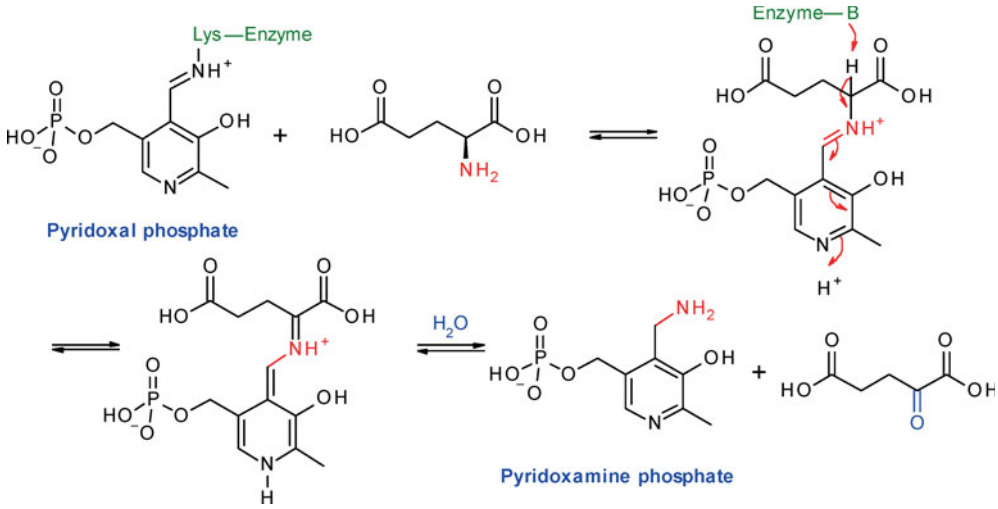
4.3 Biosynthesis

There are many facets to the biosynthesis of amino acids, merely to generate the different residues [54, 55] of the 22 proteinogenic amino acids. Only those selected aspects will be described here, which are also of relevance to the later consideration of industrial syntheses.

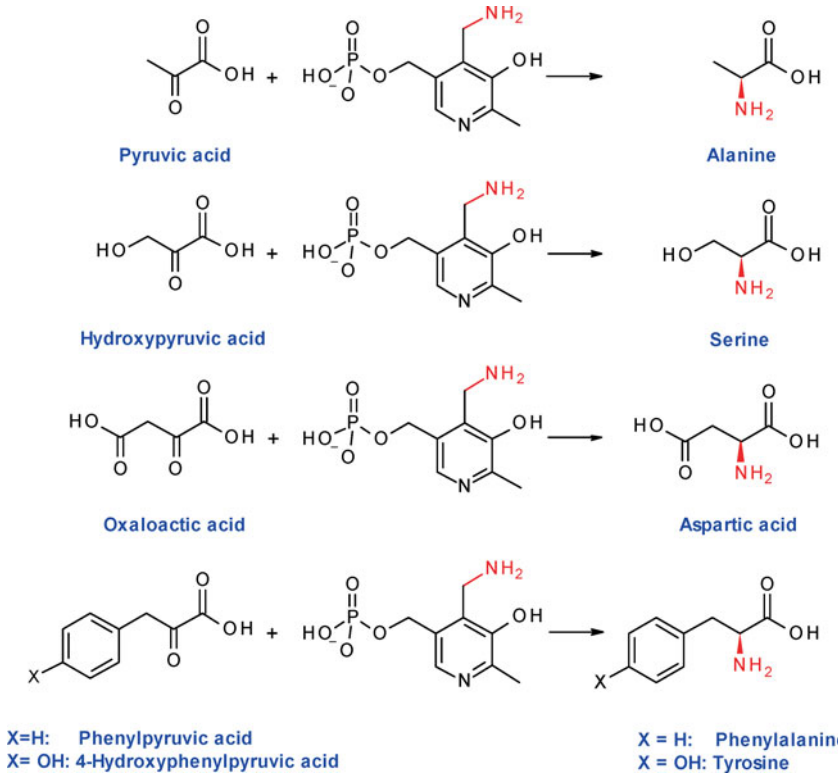
In a first step ammonia is bound to glutamic acid by glutamine synthase. Glutamine serves then as the source of ammonia for the transformation of ketoglutaric acid. The ketoglutaric acid, which is generated in the citric acid cycle, reacts with glutamine to produce an imine, which is reduced to glutamic acid by the NADPH-dependent glutamate dehydrogenase.



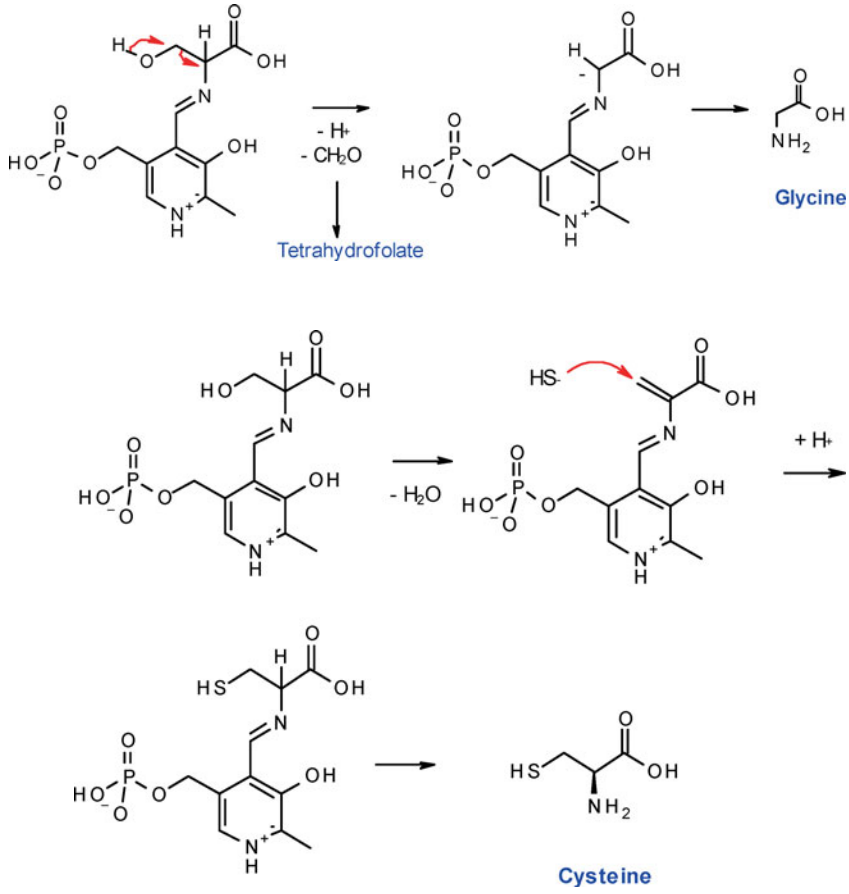
By transamination, a whole series of other amino acids becomes accessible. Pyridoxal phosphate functions as a coenzyme, which is primarily bonded to a lysine residue of the enzyme. The attack of glutamic acid leads to the corresponding imine. Tautomerisation (proton transfer) and hydrolysis give pyridoxamine phosphate and α-ketoglutaric acid. Pyridoxamine phosphate then reacts with other α-ketoacids, following the corresponding mechanism.



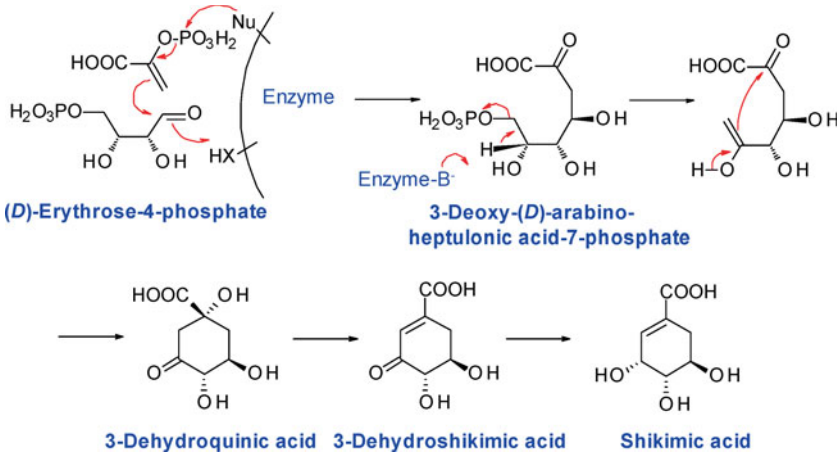
In this way, a variety of further amino acids are produced, *e.g.* alanine from pyruvic acid, serine from hydroxyl pyruvic acid and aspartic acid from oxaloacetic acid, as well as phenylalanine and tyrosine from phenylpyruvic acid and 4-hydroxyphenylpyruvic acid respectively.



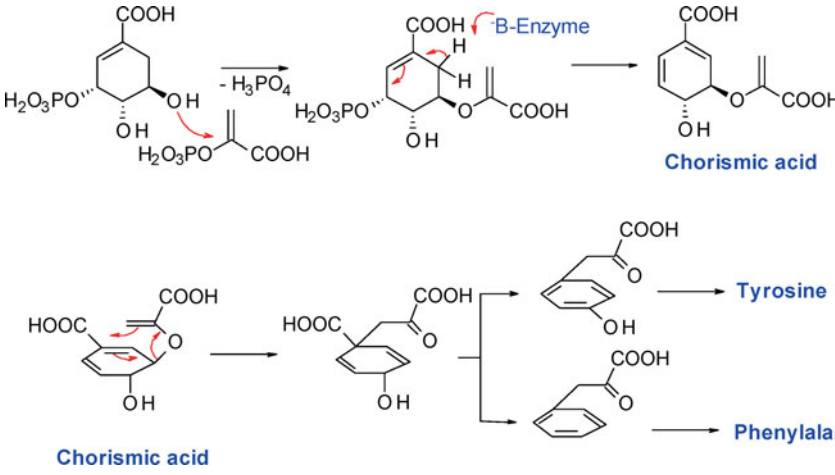
Serine is an important precursor for other amino acids. Thus, a retro-aldol reaction gives glycine, while elimination of water and addition of hydrogen sulfide produces cysteine.



The biosynthesis of the aromatic amino acids proceeds *via* shikimic acid. [56] The starting point is erythrose-4-phosphate, which is produced in the Calvin cycle. The enzyme-catalysed aldol condensation with phosphoenol pyruvate leads to a heptulose, 3-deoxy-(*D*)-arabino-heptulonic acid 7-phosphate. Elimination of phosphate produces an enol, which is converted by a further aldol condensation into 3-dehydroquinic acid. Elimination of water and reduction then yield shikimic acid.



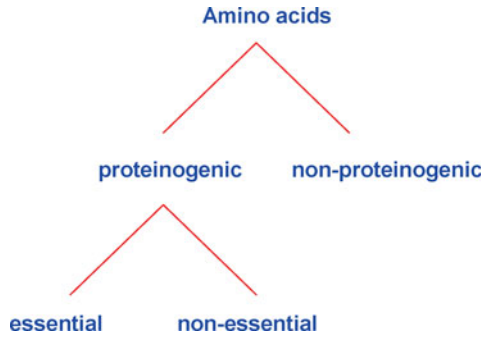
Shikimic acid, phosphorylated at the C-3 hydroxyl group, is reacted again with phosphoenol pyruvate, and, following cleavage of phosphoric acid, chorismic acid is formed. After a subsequent Claisen-type electrocyclic rearrangement, which is a fairly rare biochemical process, the biosynthetic pathway branches off. Decarboxylation and aromatisation (oxidation) give 4-hydroxyphenylpyruvic acid, the precursor of tyrosine (Tyr). If aromatisation takes place through cleavage of water, this generates phenylpyruvic acid, the educt for phenylalanine (Phe).



The biosynthesis of tryptophan branches off from chorismic acid, and was already described in the section on indigo (*cf.* section 2.1 – Indigo).

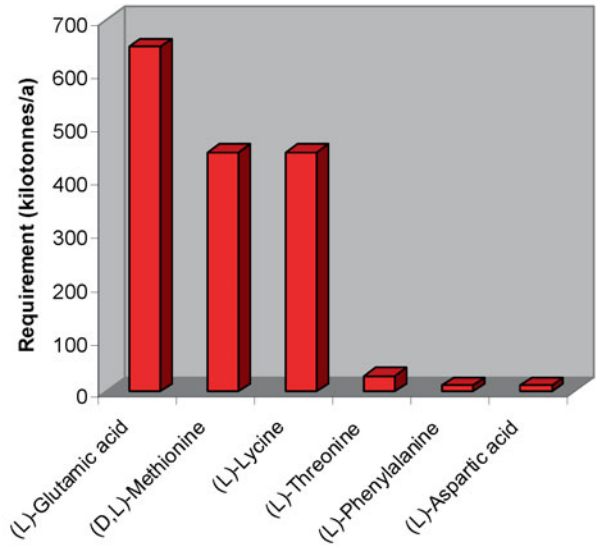
4.4 Industrial Synthesis

The first amino acids were discovered at the beginning of the 19th century: asparagine in 1806 by Nicolas Vauquelin (1763–1829) and Pierre Robiquet (1780-1840) in asparagus (*Asparagus officinalis*), leucine in 1818 by Joseph Proust (1754–1826) in curds, and glycine in 1820 by Henri Braconnot (1780–1855) in gelatine (Fig. 4.10). The industrial preparation of amino acids began in Japan in 1908 with the isolation of monosodium glutamate from the hydrolysis of wheat glue (gluten) with hydrochloric acid.



4.10 *Nowadays, amino acids are divided into proteinogenic and non-proteinogenic, and the former into essential (e.g. Leu, Lys, Val, Trp, Met) and non-essential amino acids. There are 22 proteinogenic, but more than 300 non-proteinogenic amino acids known in Nature.*

The present-day worldwide annual production of monosodium glutamate adds up to more than 650,000 tonnes. The annual demand for (*D*,*L*)-methionine and that for (*L*)-lysine come to 450,000 tonnes each, that for (*L*)-threonine to 30,000 tonnes and that for (*L*)-phenylalanine and (*L*)-aspartic acid to some 12,000 tonnes each (Fig. 4.11). [57, 58, 63]



4.11 *The combined production of all amino acids in recent years totals to more than 1.6 million tonnes annually. This corresponds to a market volume of ca. 3.5 billion Euro, 55 % of which is allotted to the animal feed segment.*

In human nutrition, free amino acids play an important role in aromatisation, as flavour enhancers, and as sweeteners. Monosodium glutamate, in concentrations of 0.1–0.4 %, is probably the most prominent flavour enhancer for spices, soups, sauces, meat and fish. (*L*)-Cysteine amplifies the flavour of onions. Glycine is used to mask the aftertaste of saccharin. Whereas (*L*)-amino acids may taste slightly bitter, the (*D*)-enantiomers have a sweet taste. This is in general also true for the corresponding di- and oligopeptides – except for the methyl ester of (*L*)-aspartyl-(*L*)-phenylalanine (Aspartame).

Important amino acids to supplement animal nutrition are methionine (mainly for poultry), lysine (mainly for pigs), threonine and tryptophan. [59] Accordingly, what's valid for plants (Liebig's law) applies also to animals: The least quantity of available amino acids in feed limits the generation of animal protein. The amino acid supplementation of maize, rice, soya- and fish-meal serves the purpose of producing more meat with less feedstuff, of making better use of inferior fodder, and of conserving natural resources. Thus, one kilogram of (*D*,*L*)-methionine replaces 50 kilograms of fish meal, which would have to be processed from 230 kilograms of fish. Through a demand-regulated amino acid supply, the quantity of slurry is reduced as well, something which is in the end also beneficial for the environment.

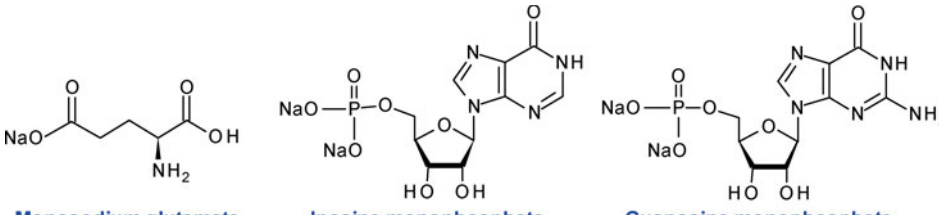
For pharmaceutical purposes 2000–3000 tonnes of amino acids are required annually worldwide. More than half of this amount ends up in infusion solutions for artificial nutrition. Many amino acid derivatives are pharmacologically important: acetylcysteine is mucolytic, (*L*)-DOPA an active agent to combat Parkinson's disease, and Oxitriptan ((*S*)-5-hydroxytryptophan) is an antidepressant. Specifically substituted, (*D*)-configured, α- and β-amino acids find widespread application as building blocks for drug synthesis.

Amino acids find use in cosmetics, in plant protection agents [60] (*e.g.* *Roundup*®, *Basta*® – *cf.* section 8.1), as dispersion aids, stabilisers for PVC, vulcanisation accelerators, corrosion inhibitors, and as additives in electroplating and photography.

For modern industrial amino acid production, a broad range of procedures has been developed: Extracts of protein hydrolysates, chemical syntheses from petrochemical precursors, microbial fermentation, as well as enzymatic transformations. The selection of the most advantageous procedure depends on the particular amino acid product, its quality specification and market price, the supply situation for raw materials, and available technical knowhow.

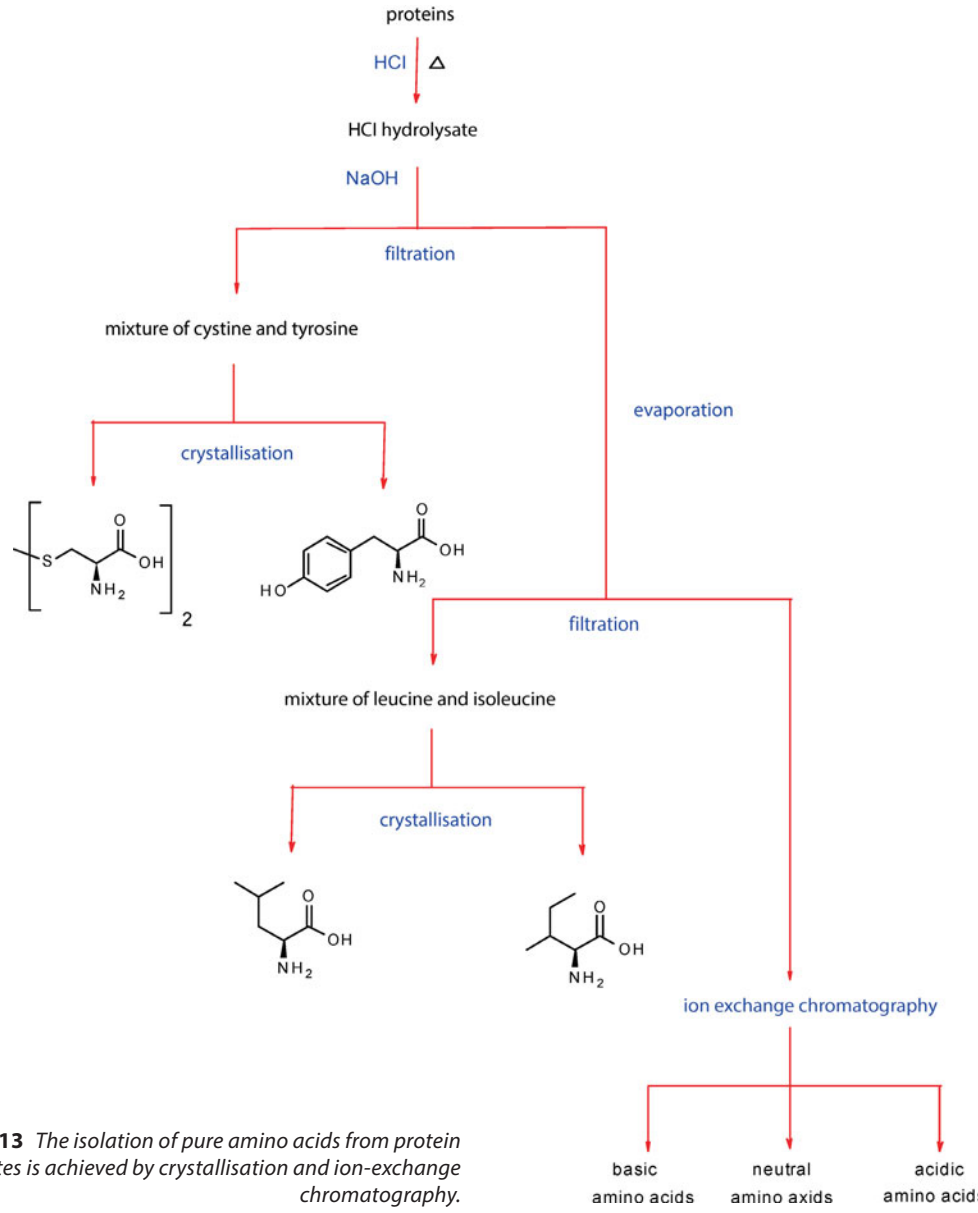
In 1908, 2250 years after Aristotle (384 BC–322 BC) had described four tastes, sweet, sour, bitter and briny, the Japanese chemist Kikunae Ikeda (1864–1936) discovered a fifth one, which he named "umami". Ikeda realized that it is triggered by glutamate. Larger amounts of glutamate are found in cheese (Parmigiano reggiano: about 1.7 w%), matured tomatoes (> 2 % of its dry weight), seaweed broth, soy sauce and also human breast milk (20 mg/100 ml of milk, corresponding to about 50 w% of the free amino acid content). Two other umami compounds were detected in 1913 by S. Kodoma (inosine monophosphate from dried bonito) and in 1956 by A. Kuninaka (guanosine monophosphate from dried shiitake mushroom) (Fig. 4.12). [58]

4.12 *Kikunae Ikeda and umami compounds.*



4.4.1 Protein Hydrolysis

Keratins, collagens and plant-derived proteins are hydrolysed in boiling hydrochloric acid, whereby the peptide bonds are cleaved. Following neutralisation, a sparingly water-soluble fraction, rich in cystine and tyrosine, is precipitated and can be filtered off; (*L*)-cystine and (*L*)-tyrosine may then be separated by fractional crystallisation. Partial evaporation provides a fraction rich in (*L*)-leucine and (*L*)-isoleucine. The remaining amino acids are separated by organic ion-exchange chromatography. In this way, cystine, tyrosine and proline are isolated (Fig. 4.13). [61]



4.13 *The isolation of pure amino acids from protein hydrolysates is achieved by crystallisation and ion-exchange chromatography.*

Acidic and basic amino acids, like glutaric acid, aspartic acid and lysine, are also isolated from protein hydrolysates for artificial nutrition. The fractions are filtered off under sterile conditions and further purified by crystallisation. Since the BSE crisis, the supply with amino acids has come under scrutiny; manufacturers had to assure regulators and customers likewise, that their starting material does not originate from cattle. Mostly used are poultry feathers and pork gelatine. [62]

4.4.2 Chemical Synthesis

As a rule, the chemical synthesis of amino acids has to address sooner or later the issue of optical purity. Methionine for supplemental feedstuff purposes is an exception, since both enantiomers have almost equal nutritional value. Poultry and pigs possess special enzymes, which are able to convert the (*D*)-form into the (*L*)-form. [63]

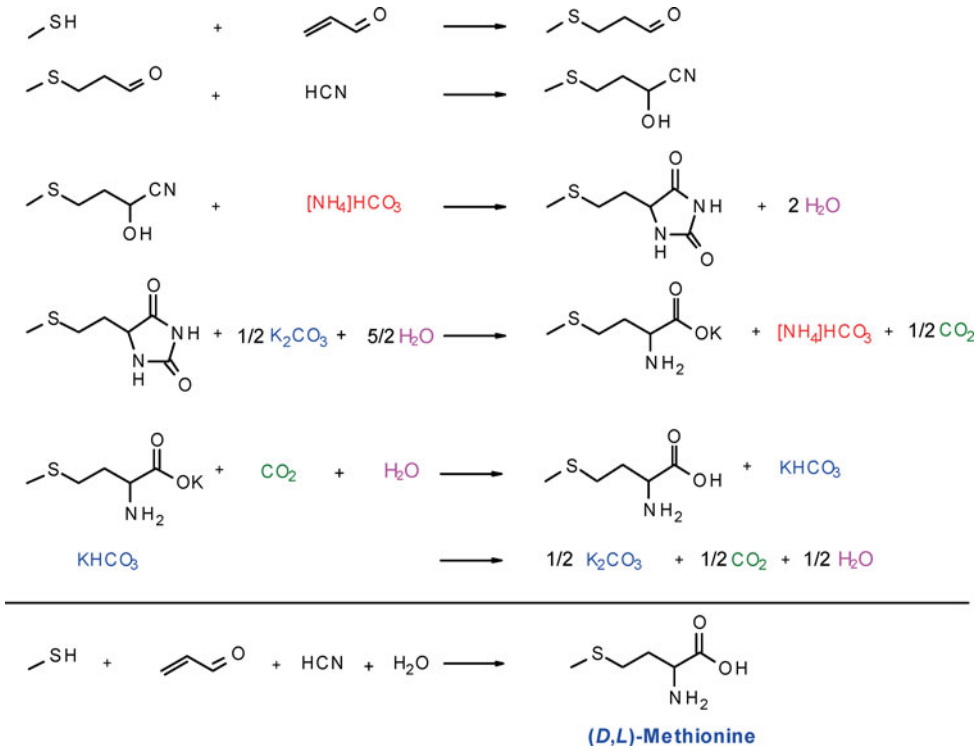
The starting material for Degussa's methionine synthesis is acrolein, to which methanethiol (methyl mercaptan) is added. Then follows a cyanohydrin synthesis, and subsequent addition of carbon dioxide and ammonia gives the corresponding hydantoin, which is then hydrolysed with potassium carbonate (Bucherer-Bergs reaction). Methionine is released through pressurising with carbon dioxide. The product is then filtered off and the mother-liquor evaporated. Thereby, potassium hydrogen carbonate is converted into potassium carbonate, which can be recycled. Carbon dioxide and ammonia are recycled as well; thus, the salt accumulation is minimised, resulting in significant environmental benefit.

Since 2007 Degussa is part of Evonik Industries.

The Degussa methionine process exemplifies, how many industrial procedures gain attraction through the elegance of how they are conducted. In summary, methionine is formed from equimolar portions of acrolein, methanethiol, hydrogen cyanide and water. The yields of all the reaction steps exceed 90 % (Fig. 4.14 and Fig. 4.15).

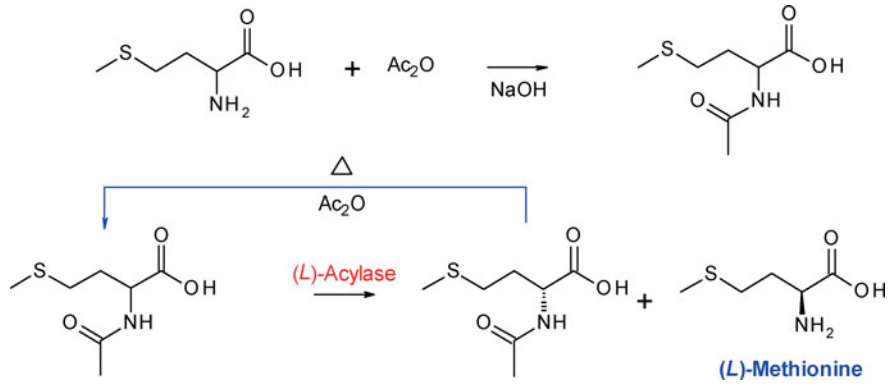
4.14 *Degussa's methionine plant in Antwerp.*

4.15 *Drumming of methionine powder at the Degussa plant in 1952.*



4.4.3 Enzymatic Methods

Enzymatic methods offer in principle the possibility of a direct enantioselective synthesis of amino acids. Enzymes are often used for separation of racemic mixtures, as examplified in the case of methionine. Although racemic methionine is adequate for the animal feed sector, other applications require the enantiomerically pure (*L*)-form. For the resolution, (*L*)-acylases from *Aspergillus* sp. are often used, since they can accept a broad spectrum of substrates, are highly active, and very stable under the production conditions. [62]

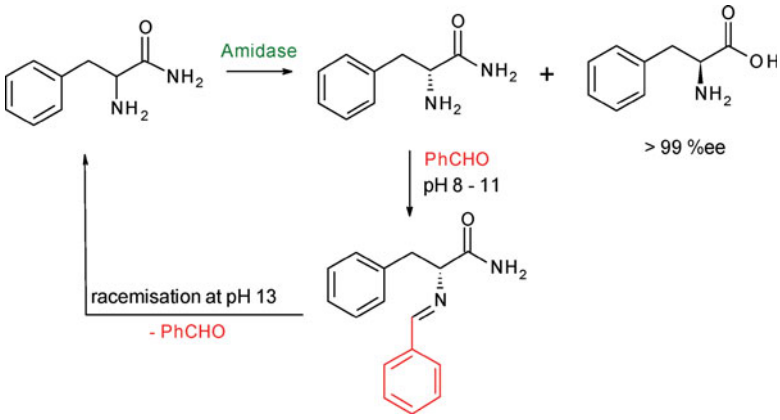


After methionine is *N*-acetylated, the (*L*)-enantiomer is enzymatically hydrolysed in a stereoselective manner. The economy of the process depends on whether the (*D*)-enantiomer can then be racemised and recycled, for example by heating with acetic anhydride.

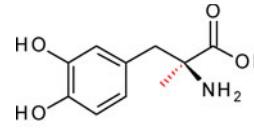
The work-up of batch processes, run in stirred vessels, had often faced the challenge to efficiently separate and recover the enzyme used. Meanwhile, there is abundant know-how available to immobilise enzymes on different carriers, though some issues need always to be considered: maintained activity of the enzyme, its stability towards solvents and the operating temperature used in a reaction. Enzyme immobilisation allows for continuous reactions carried out in columns or in a sequence of continuous stirred-tank reactors. Certain advantages are offered by Degussa's "enzyme-membrane-reactor" (EMR), where the enzyme is surrounded by a hollow-fibre membrane, that is permeable to substrate and product.

In this way, (*L*)-valine, (*L*)-alanine, (*L*)-phenylalanine and (*L*)-tryptophan, but also rare amino acids like (*L*)-propargylglycine, (*L*)-*p*-fluorophenylalanine or (*L*)-3-(1'-naphthyl)alanine are prepared. (*D*)-acylases are used to obtain (*D*)-propargylglycine, (*D*)-tryptophan or (*D*)-*p*-chlorophenylalanine. [62]

DSM has developed an industrial process for the preparation of (*D*)- and (*L*)-amino acids, which is based on the enantioselective hydrolysis of racemic amino acid amides using amidases, for example from *Pseudomonas putida*. It is often not necessary to isolate the pure enzyme; standardised whole-cell or crude enzyme preparations can be used instead. It is noteworthy that in some cases the enzyme activity can be increased up to ten-fold by the addition of magnesium salts. The enzymes accommodate a broad spectrum of substrates with considerable selectivity. Typical products are (*L*)-phenylalanine and (*L*)-homophenylalanine.

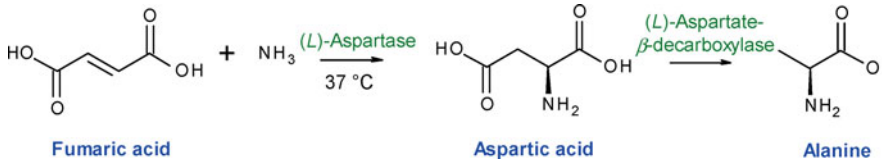


DSM has also succeeded in reacting α-branched amino acid amides with peptidases from *Mycobacterium neoaurum*. By this method, compounds such as the antihypertonic (*L*)-α-methyl-3,4-dihydroxyphenylalanine ((*L*)-α-methyl-DOPA) are accessible. For very similar transformations, Ube (Japan) has used amidases from *Pseudomonas fluorescens*. [64]



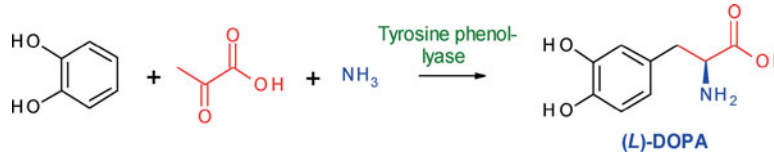
(*L*)-α-Methyl-DOPA

(*L*)-Aspartases from *Escherichia coli* and *Brevibacterium flavum* catalyse the stereospecific addition of ammonia to fumaric acid. Nanning Only-Time in China, Kyowa Hakko Kogyo and Tanabe Seiyaku in Japan produce aspartic acid accordingly. Using an (*L*)-aspartate-*β*-decarboxylase, alanine can be prepared in a subsequent step as well. [62]

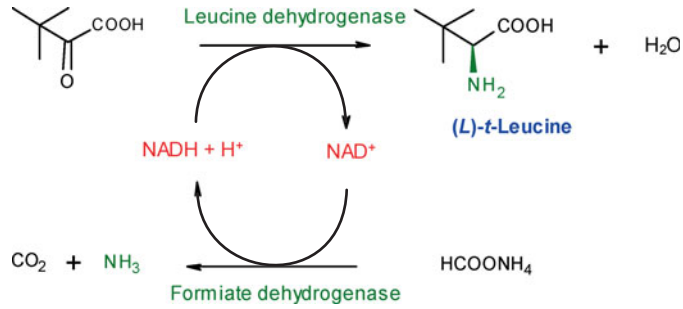


(*L*)-Phenylalanine may be generated similarly from (*E*)-cinnamic acid with an ammonia lyase (for instance from *Rhodococcus rubra*). This procedure is however not used industrially, due to the availability of a more economic fermentative process. [58]

However, of industrial importance is the manufacturing of (*L*)-3,4-dihydroxyphenylalanine ((*L*)-DOPA), an agent against Parkinson's disease, using a tyrosine phenol-lyase. Ajinomoto employs a whole-cell preparation from *Erwinia herbicola* to obtain the target compound in a three-component reaction, starting from catechol, pyruvic acid and ammonia. The annual capacity amounts to around 250 tonnes. [64]

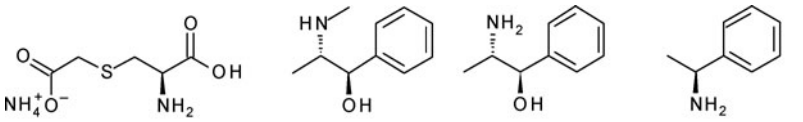


Another chemically appealing process is the enantioselective, reductive amination with an amino acid dehydrogenase, for example from *Bacillus sphaericus*. This reaction requires stoichiometric amounts of NADH, which has to be prepared simultaneously, using a formate dehydrogenase (*e.g.* from *Candida boidinii*). By means of a leucine dehydrogenase, Degussa/Rexim produces (*L*)-*t*-leucine on a tonne scale from trimethylpyruvic acid. [62] With the same enzymes, (*L*)-neopentylglycine can be prepared as well. The method has proven its general applicability for amino acids with sterically demanding side-chains.



4.4.4 Separation of Enantiomers by Crystallisation

Even for industrial-scale production of optically active amino acids, classical crystallisation technologies may offer an appropriate alternative to enzymatic methods. Either the seeding with one of the enantiomers (*e.g.* from *S*-(carboxymethyl)-(*L*)-cysteine), or the crystallisation of diastereomeric salts with bases like derivatives of ephedrine or phenylethylamine may be suitable (Fig. 4.16).



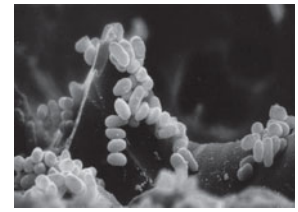
4.16 *Reagents for the resolution of amino acids.*

In this context, chemists at DSM have made interesting observations. They could demonstrate better yields and selectivities for the crystallisation of substance families, rather than pure substances (Dutch Resolution). [65–69]

A primary disadvantage, namely the maximum yield of 50 %, can in many cases be compensated by subsequent racemisation. Since the development of a diastereoselective crystallisation method is frequently straight forward, this technology still claims its competitive and viable role.

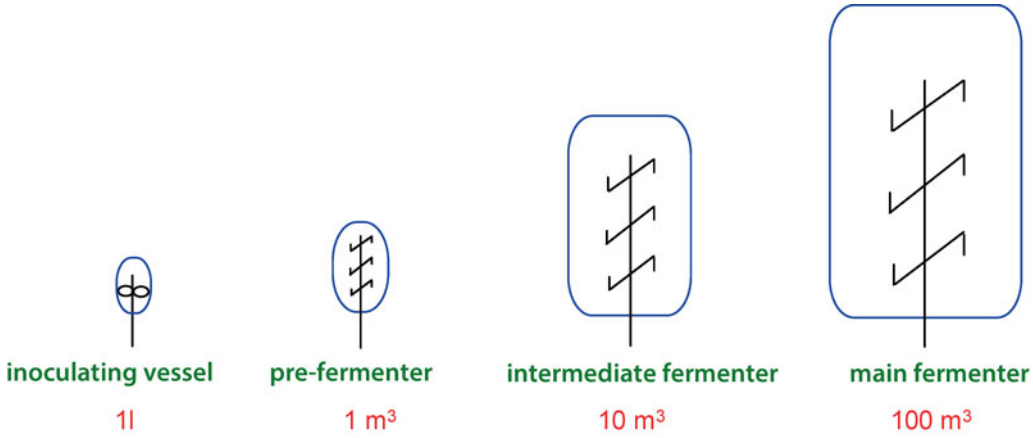
4.4.5 Fermentation Methods

Fermentation is a process, by which amino acids are generated by virtue of the natural metabolism of microorganisms. *Brevibacterium flavum* and *Corynebacterium glutamicum* (Fig. 4.17) possess a really large enzymatic capacity to produce (*L*)-glutamic acid from (*D*)-glucose. With modern production methods, from one kilogram of glucose around 0.5 kilograms of glutamic acid can be produced, and amino acid concentrations of up to 160 grams per litre can be attained. [58] The transformations follow along the biosynthetic pathways as described above. For the industrial synthesis of glutamic acid, cheap molasses, a by-product from sugar production, is used.



4.17 *Electron microscopic photograph of Corynebacterium glutamicum.*

Cultivating and expanding the fermentation broth is similar to the preparation of bread from sour dough. A small amount of a freeze-dried microorganism culture is combined, typically in a one-litre agitator vessel, under sterile conditions with nutrient solution and allowed to incubate. The next up-scaling steps in the preliminary and intermediate fermenters are typically run at 1 and 10 cubic metres volume. The latter material is used to inoculate the main fermenter (Fig. 4.18). The fermentation broth contains vitamins, minerals and other nutrients, while air and ammonia are supplied under appropriate stirring; antifrothing agents will suppress foam formation. The reaction is usually carried out at 35 °C, and nutrient content, as well as product formation and other parameters, like pH, are carefully monitored analytically. Once the fermentation has reached its optimum, the reaction broth is submitted, as necessary, to a sophisticated and sometimes laborious work-up.



4.18 *The scale-up of fermentation procedures.*

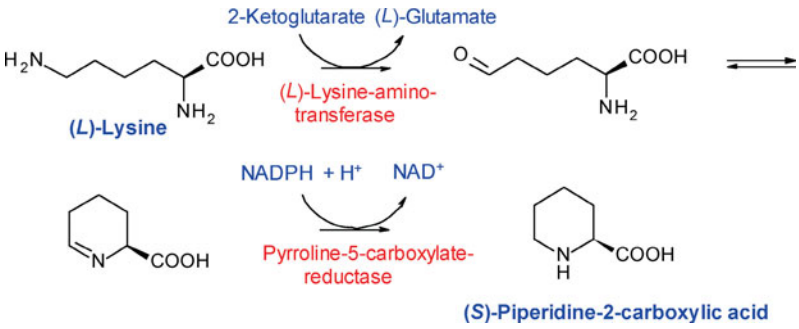
By use of wild type microorganisms, alanine and valine may also be prepared in this way. Cysteine is produced by Wacker, *via* fermentation with a recombinant bacterial cell line. The preparation of lysine, phenylalanine, tyrosine [70] and tryptophan [71] succeed with a genetically modified high-yielding strain of *Corynebacterium glutamicum*.

The goal of re-engineering the genetic makeup of the microorganism is, to alter or tune its metabolism and maximise the yield of the desired product, without harming or killing the bacteria. In case of lysine, starting from molasses, within 60 hours the fermentation broth contains more than 70–80 grams per litre of lysine. The sugar-based yields (g Lys×HCl / g sugar) lie above 40 %. Water is removed from the broth through a falling film evaporator, and the concentrate is subsequently dried in a spray granulator. This procedure benefits in the end from the fact, that the employed microorganism is quite safe for humans, animals and the environment, and does not require to be separated off from the valuable product.

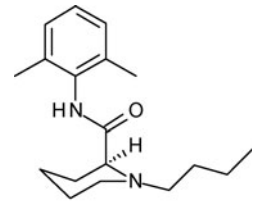
Degussa/Rexim, partly in collaboration with Chinese manufacturers, produce (*L*)-threonine, (*L*)-valine and (*L*)-isoleucine by fermentation. [62] With genetically modified strains from *Echerichia coli* and *Serratia marcescens*, they are able to achieve end concentrations of 100 grams per litre.

The potential of biotransformations with genetically modified microorganisms can be illustrated by the following example, where the chain of added-value to amino acid products is also recognisable: The Mercian Company (Japan) uses a recombinant *E. coli* strain to prepare (*S*)-piperidine-2-carboxylic acid from (*L*)-lysine. In this strain, the (*L*)-lysine-permease transport system is overexpressed, so that in this pathway lysine is produced efficiently in the cells. The bacteria possess additionally a (*L*)-lysine-aminotransferase from *Flavobacterium lutescens*, which brings about the deamination of lysine. The thus generated aldehyde is in equilibrium with its intramolecular imine, which, in presence of the *E. coli*-specific pyrroline-5-carboxylate reductase, is reduced with NADPH to (*S*)-piperidine-2-carboxylic acid. The turnover

exceeds 90 %. The product end-concentration amounts to around 50 grams per litre. [58]



(*S*)-Piperidine-2-carboxylic acid is an important building block for pharmaceuticals, for example local anaesthetics like *Levobupivacaine*® from Abbott.

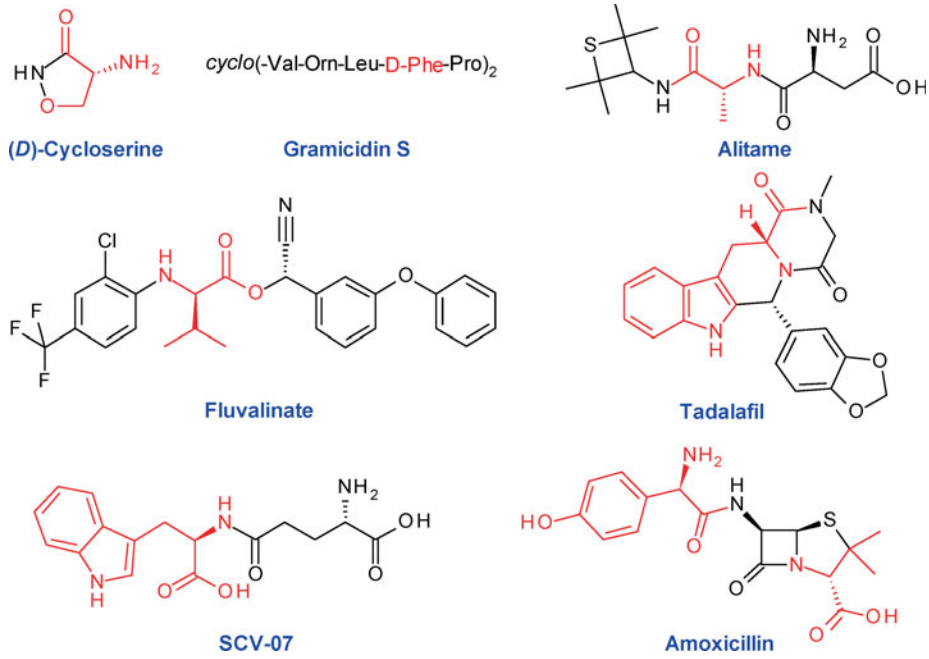


Levobupivacaine

4.5 (*D*)-Amino Acids

Contrary to the superficial perception that (*D*)-amino acids are unnatural amino acids, for many organisms they instead play a vital role. Thus, there exists indeed a whole arsenal of synthetic methods and enzymes to generate these unusual amino acids. [72] For instance, (*D*)-alanine and (*D*)-glutamic acid are essential for the formation of bacterial peptidoglycans. But (*D*)-amino acids have also been discovered in fungi, algae, higher plants, amphibians and even in mammals.

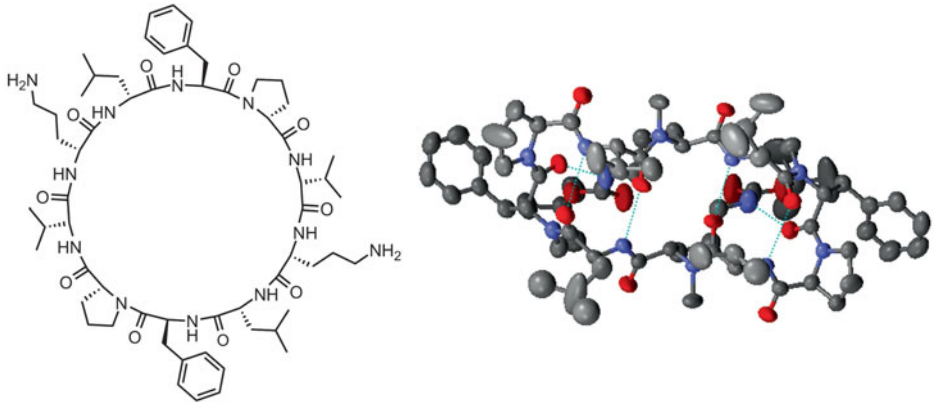
They also assume important functions in a whole range of pharmacologically active compounds and effect materials (Fig. 4.19): (*D*)-cycloserine can be used for the treatment of severe cases of tuberculosis; Gramicidin A, B, C and S are small peptides with spermicidal, antibiotic and antiviral properties (especially against HIV and *Herpes simplex*); and Alitame is a sweetener. Fluvalinate acts like a pyrethroid (*cf.* section 8.3), while Tadalafil and SCV-07 are physiologically active compounds for the treatment of erectile dysfunction and in chronic hepatitis C respectively. However, the greatest commercial importance of (*D*)-amino acids lies in the preparation of β-lactam antibiotics: the quantities of (*D*)-phenylglycine and (*D*)-*p*-hydroxyphenylglycine required annually for manufacturing Ampicillin and Amoxicillin surpass now 1,000 tonnes.



4.19
Active chemicals with (D)-amino acids.

Gramicidin S

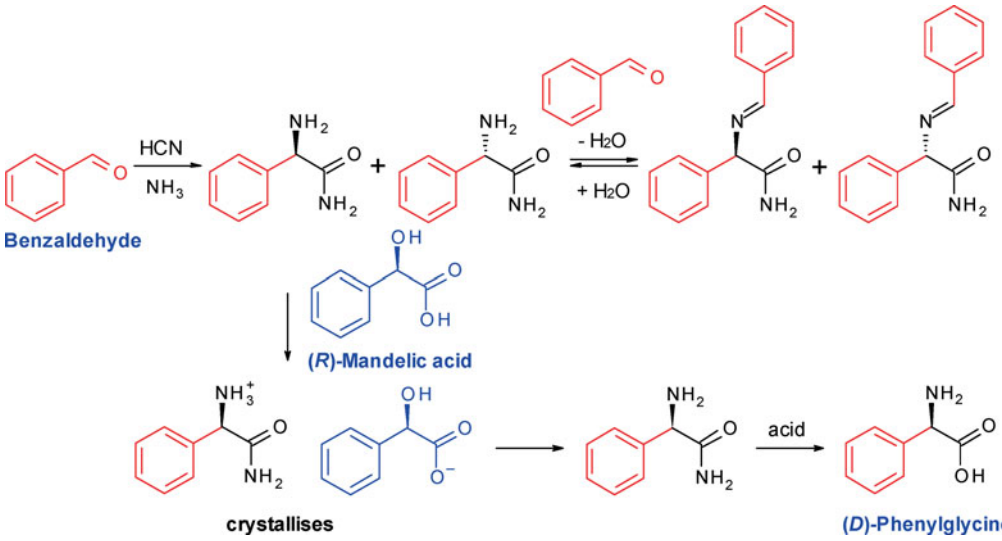
Gramicidin S is a peptide antibiotic from the bacterium *Bacillus brevis*. It was discovered in 1942 by the Russian microbiologist Georgyi Frantsevitch Gause (1910–1986) and his wife Maria. With the help of the International Red Cross, the Health Minister of the USSR sent a sample to Britain in 1944 for structure determination. For examining this peptide, Richard Synge (1914–1994) used his newly-developed paper chromatography, and Dorothy Mary Crowfoot Hodgkin (1910–1994) finally solved the structure of the decapeptide by X-ray crystallography (Fig. 4.20). During and after the World War II, Gramicidin S achieved such an importance for the USSR that Gause was spared from the persecution many scientists faced during the Lyssenkoism (pseudo-scientific doctrine named after Trofim Denissowitsch Lyssenko (1898–1976)).



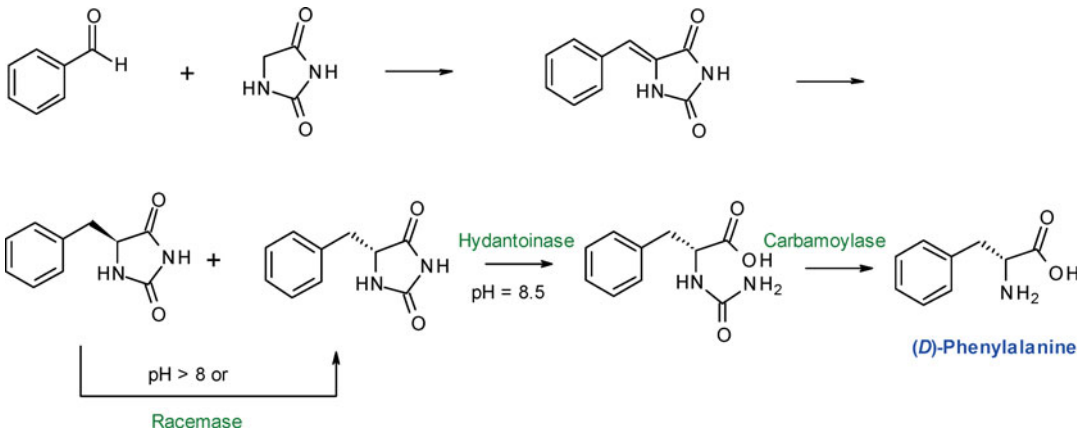
4.20
Crystal structure of modified Gramicidin S.

Preparation of (*D*)-Amino Acids

Remarkable is DSM's process for the preparation of (*D*)-phenylglycine by classical resolution with *in situ* racemisation. The imine of phenylglycinamide, prepared in a Strecker reaction, is readily racemised, so that in the presence of (*R*)-mandelic acid, the (*D*)-phenylglycinamide crystallises out almost quantitatively. In acidic medium, the mandelic acid is separated off and recycled; the remaining amide is cleaved to yield free (*D*)-phenylglycine.



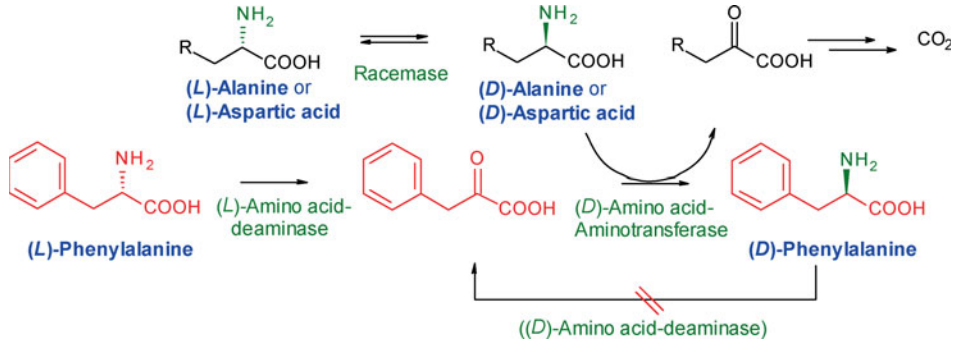
There are still other opportunities in using elegant enzymatic synthesis methods, for instance, the hydrolysis of *N*-acetyl-(*D*)-amino acids from racemic mixtures by (*L*)-acylase cleavage, by dynamic kinetic resolution, or by employing a racemase and a hydantoinase.



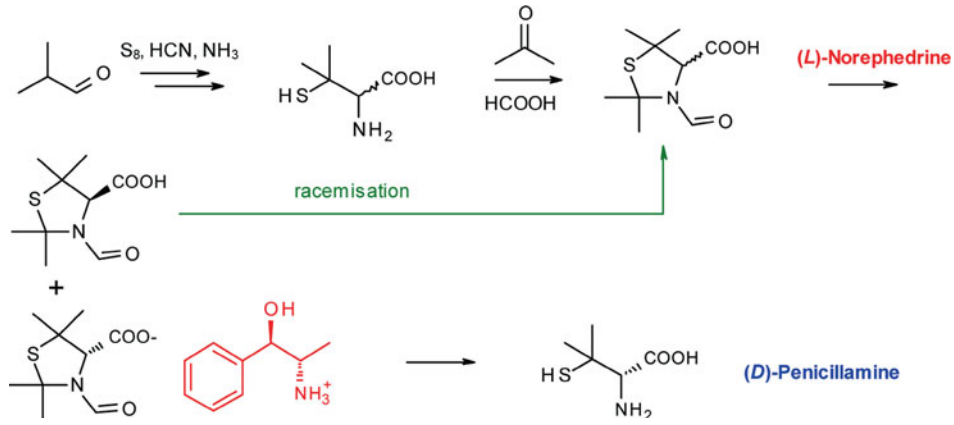
In 1946, Friedrich Asinger, a research chemist at Leuna GmbH, Merseburg and honorary lecturer at the University of Halle-Wittenberg, Germany, was deported for eight years to Dzerzhinsk, near Nischni Nowgorod, Russia, where he discovered a multi-component reaction, the Asinger reaction, for the synthesis of thiazoline derivatives from aldehydes or ketones, ammonia or amines and sufur.

A range of (*D*)-amino acids is also accessible by a fermentation route. The microorganisms are for this purpose modified, so that they no longer express functional (*D*)-amino acid deaminases. However, they contain instead genes for (*L*)-amino acid deaminases, (*D*)-amino acid aminotransferases and a special racemase. Monsanto, for example, has patented such an approach for the preparation of (*D*)-phenylalanine.

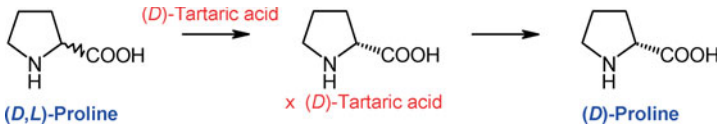
A racemase brings about inversion of the relatively cheap (*L*)-isomers of alanine or aspartic acid, but not of (*D*)-phenylalanine. Only (*L*)-phenylalanine is deaminated by an (*L*)-amino acid deaminase, whereas (*D*)-phenylalanine is not. The latter is generated by ammonia transfer from (*D*)-alanine or (*D*)-aspartic acid with a (*D*)-amino acid aminotransferase. The equilibria are moved in favour of the product, either by the metabolism of pyruvic acid or oxosuccinic acid. Since (*L*)-amino acid deaminases, like (*D*)-amino acid aminotransferases, are non-specific, they also permit the preparation of a variety of other (*D*)-amino acids. [58]



(*D*)-Amino acids are also prepared through crystallisation of their corresponding diastereomeric salts. An important application of this truly competitive method was developed by Friedrich Asinger (1907 - 1999, Austrian chemist) and the product successfully commercialised by Degussa: Separation of the (*D*,*L*)-penicillamine-acetone adduct with (*L*)-norephedrine was conducted at a ten-tonne scale. The unwanted (*L*)-penicillamine-acetone adduct is racemised and recycled.



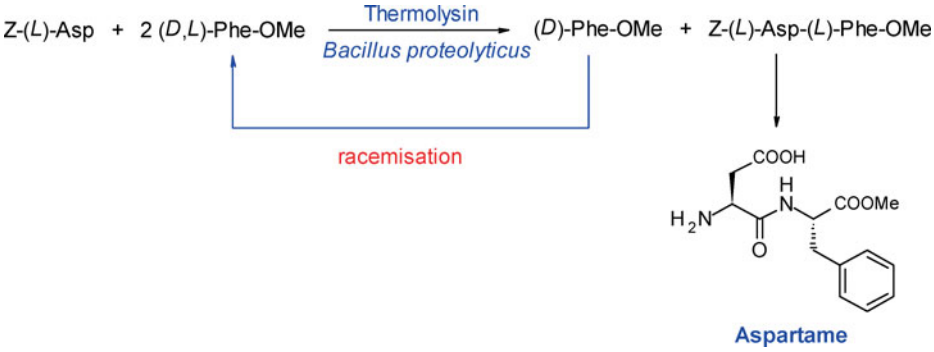
(*D*)-Proline has gained significant importance in recent years as a building block for the preparation of pharmaceuticals. Its resolution is effected by (*D*)-tartaric acid. The products are recovered using an ion-exchanger. [62]



4.6 Aspartame

The widely used sugar substitute aspartame, a dipeptide of two (*L*)-amino acids, is about 200 times sweeter than sucrose. This is certainly surprising, since (*L*)-amino acids, as mentioned above, have in general a tendency to taste bitter.

In 1965, James M. Schlatter, a chemist working for Searle, accidentally discovered aspartame, when he licked his aspartame-contaminated finger to lift up a piece of paper.



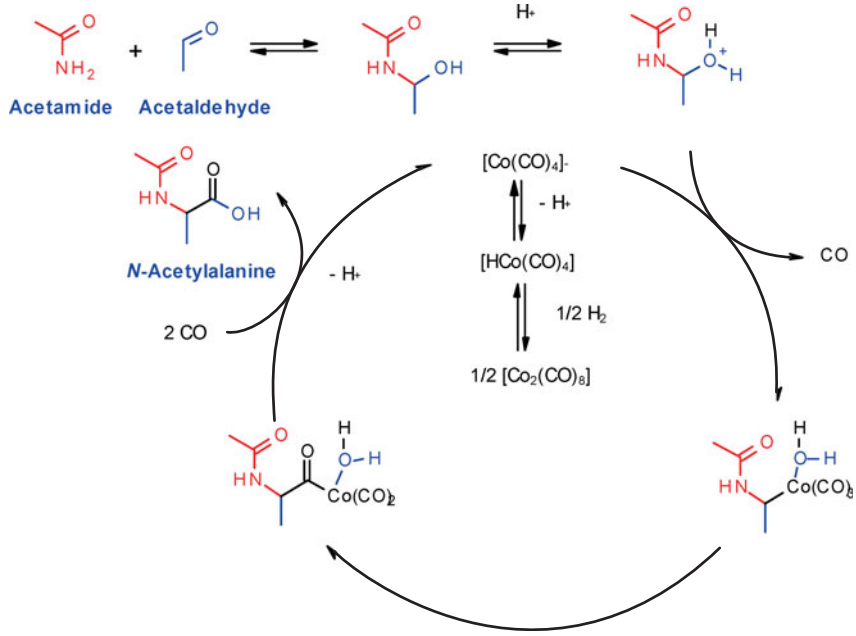
DSM uses an enzymatic procedure for the preparation of this product (capacity > 2,000 tonnes). Z-protected (*L*)-aspartic acid is reacted enantioselectively with racemic phenylalanine methyl ester, using thermolysin from *Bacillus proteolyticus*. Unreacted (*D*)-phenylalanine methyl ester is separated off, racemised and recycled. The remaining step is cleavage of the Z-protecting group to get to the desired product.

4.7 New Developments

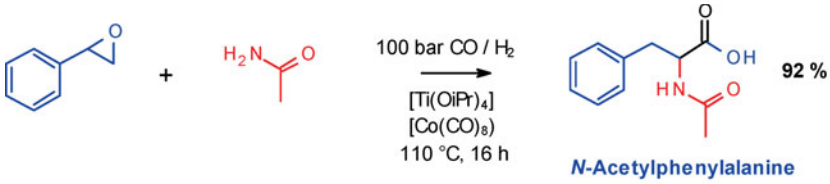
4.7.1 Amidocarbonylation

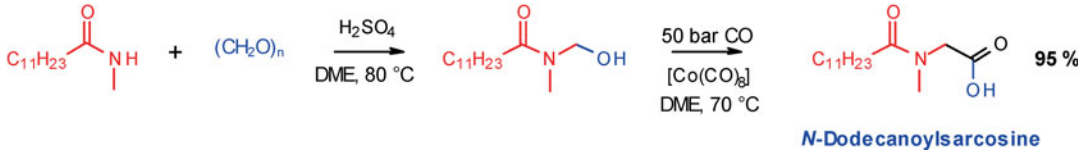
Amidocarbonylation is the only transition metal-catalysed multi-component reaction, by which the amino acid framework is constructed directly from simple building blocks. [73] In the early 1970s, Hachiro Wakamatsu at Ajinomoto discovered by chance the cobalt-catalysed amidocarbonylation during investigation of the Oxo process with acrylonitrile, where he also found traces of α-aminobutyric acid. To determine the mechanism of this side-reaction, he

treated (*inter alia*) acetaldehyde with acetamide and synthesis gas in presence of a cobalt catalyst, and obtained *N*-acetylalanine in good yields.



Industrial applications of this reaction, which have in part been tested already on a pilot plant scale, are for example the synthesis of phenylalanine for Aspartame and of long-chain *N*-acyl derivatives of sarcosine (*N*-methylglycine) for anionic surfactants.





In the classical sarcosinate procedure, the appropriate alkanoyl chloride is treated with sodium sarcosinate (worldwide capacity amounts to more than 10,000 tonnes annually). In contrast, amidocarbonylation offers the advantage that no salt is formed as a by-product.

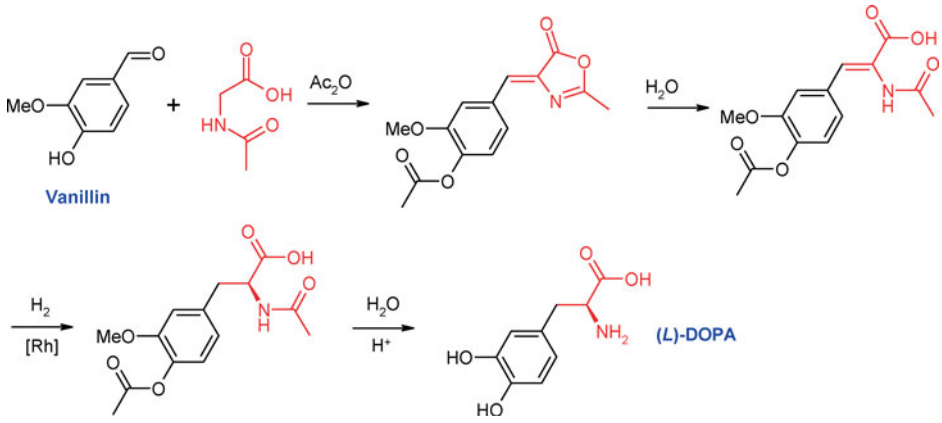
In 1987, while searching for patent-free catalysts for amidocarbonylation, Erhard Jägers at Hoechst AG found that palladium complexes are efficient catalysts for this reaction as well. These are commonly around 10–100 times more active than the corresponding cobalt systems. Also the reaction pressure and temperature are lower. An even broader industrial application of transition metal-catalysed amidocarbonylations can be expected over time.

4.7.2 Enantioselective Synthesis

The DOPA Process

The classic example of enantioselective synthesis of amino acids concerns the enantioselective hydrogenation of an aminocinnamic acid derivative for the preparation of the unnatural amino acid (*L*)-3,4-dihydroxyphenylalanine (*L*-DOPA). This is a very convincing and instructive case, because for the first time a particularly high enantiomeric excess was achieved, and here some fundamental aspects of enantioselective catalysis can be demonstrated.

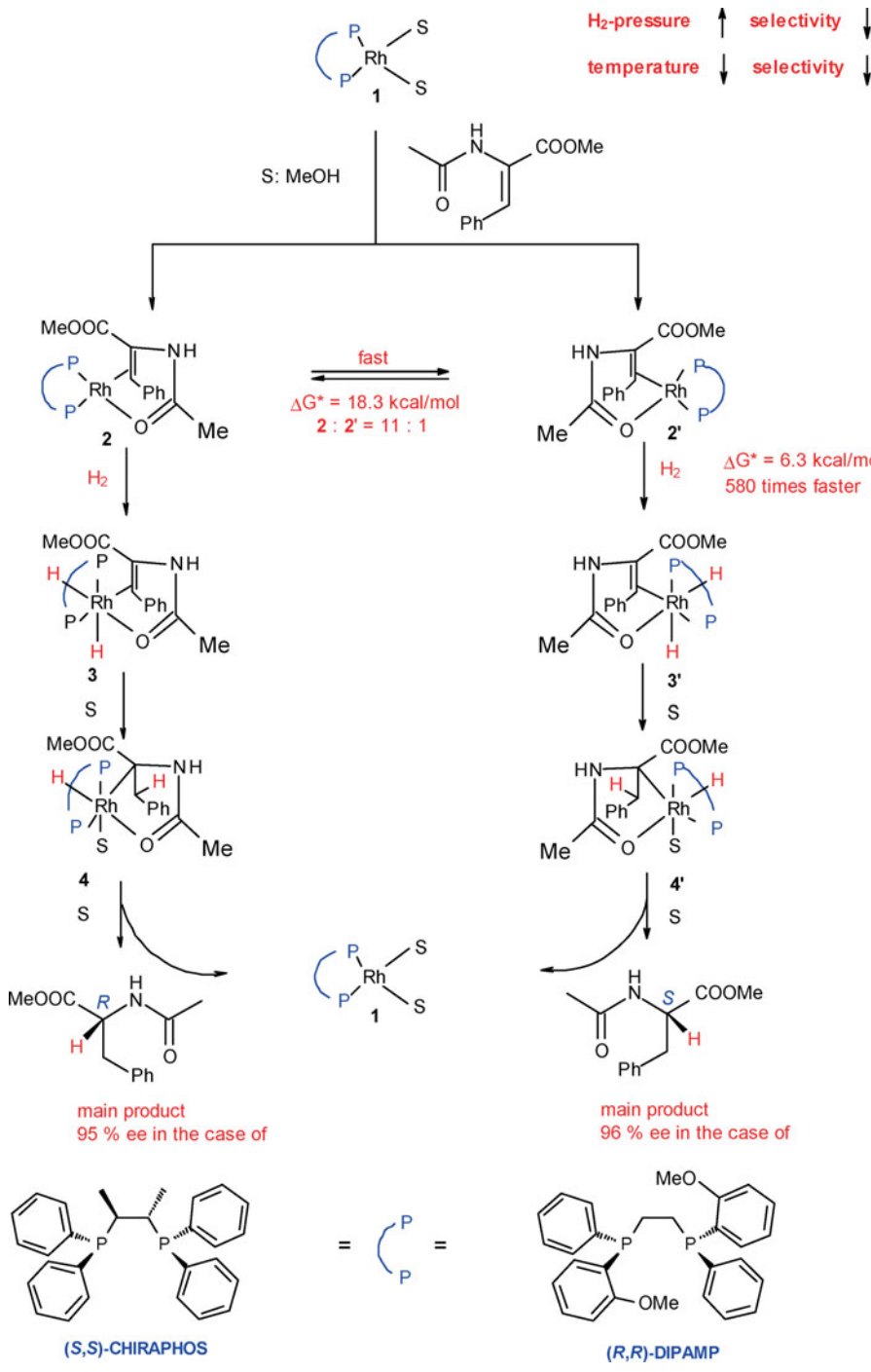
The starting material for the enantioselective hydrogenation is obtained very elegantly by an Erlenmeyer-Plöchl reaction of vanillin, *N*-acetylglycine and acetic anhydride. The azlactone is carefully hydrolysed with water and the enamide purified by crystallisation.



More than 40 years ago, William S. Knowles (1917–2012) and Leopold Horner (1911–2005) developed the use of chiral phosphorus ligands for enantioselective hydrogenation. [74, 75] Monsanto transferred the process on to an industrial scale. [76]

In the meantime, the mechanistic steps in enantioselective hydrogenation have been well investigated and are understood. [77–81] Out of the enantiomerically pure solvent complex **1**, both diastereomeric π-complexes **2** and **2'** are formed. On the basis of the X-ray structure analysis it is clear that, apart from the double bond, the amidocarbonyl group also interacts with the rhodium. The chelate formation is responsible for the configurational stability. In case of (*R,R*)-DIPAMP, an 11 : 1 ratio of the complexes **2** : **2'** is observed using NMR

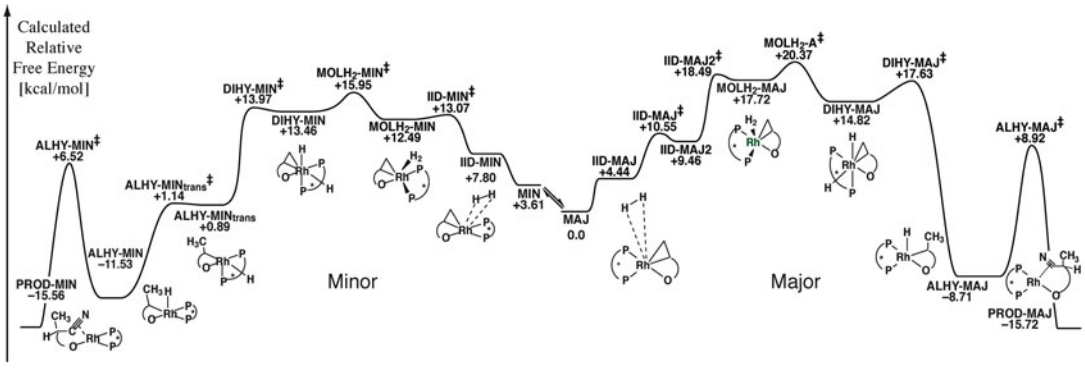
spectroscopy; nevertheless, the main product is (*S*)-configured with 96 % ee. If (*S,S*)-CHIRAPHOS is used instead of (*R,R*)-DIPAMP, similar ratios are observed for the (*R*)-enantiomer.



Therefore, the concentration of **2** is not product-determining; it is rather the addition of hydrogen to the rhodium complexes **2** and **2'**. The reaction rate for **2'** to **3'** is 580 times higher than that of **2** to **3**. [82] A difference in concentration by a factor of 10 is therefore no longer of any consequence. The equilibration of the balance between **2** and **2'** is very fast. The conversion proceeds dissociatively by cleavage of the metal-olefin bond. By increasing the hydrogen pressure, the dissociation of **2** or **2'** is clearly slower than the hydrogen addition, so that the selectivity drops.

The activation enthalpy of the dissociation amounts to 18.3 kcal mole^{-1}, that of the hydrogen addition to 6.3 kcal mole^{-1}. With a drop in the reaction temperature, the equilibration is frozen first, which leads to the unusual observation that lowering of the temperature drives the selectivity down.

It has been possible to calculate the reaction pathways by quantum mechanical methods (Fig. 4.21). In good agreement with the experimental data, all intermediate stages and reactions can be simulated and the selectivity explained. [83]



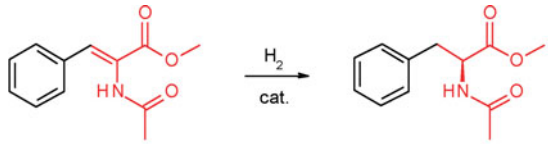
4.21 *Quantum mechanical calculation of the enantioselective hydrogenation of cinnamic acid derivatives.*

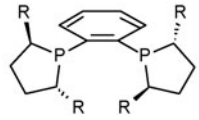
A disadvantage of the first-generation enantioselective hydrogenation catalysts was that pure (*Z*)-enamide had to be used. The (*E*)-enamide showed a poor turnover and the enantioselectivity was rather low. [84]

The problem was solved by using DuPhos ligands. With comparable substrates, Me-DuPhos and Et-DuPhos give, irrespective of the configuration of the double bond, enantiomeric excesses of 95–99 %.

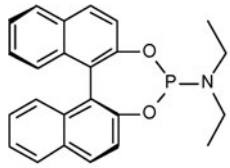
The hydrogenation with monodentate phosphorus ligands like Binol-phosphoramidites (MonoPhos) stand out, inasmuch as these are stable on air and comparatively simple to prepare. The enantioselectivity of the hydrogenation is practically independent of the hydrogen pressure, but the choice of solvent is certainly important. Non-protic solvents like dichloromethane or ethyl acetate are much more suitable than protic, *e.g.* methanol. [85]

For rhodium-catalysed hydrogenations, two equivalents of ligand (with reference to the metal) are generally required, whereas for iridium-catalysed hydrogenations only one equivalent of the phosphoramidite is required. Another essential advantage is that, by comparison with rhodium, the price of iridium is distinctly lower. [86]

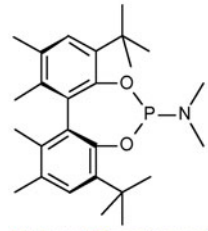




Rh+ (*E*)-Enamide >95 %ee
(*Z*)-Enamide >95 %ee



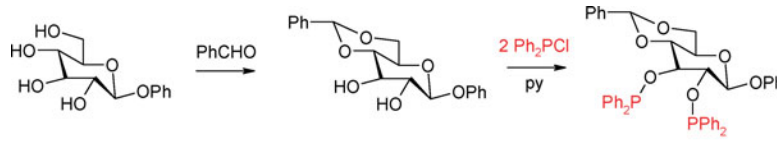
Rh+ MeOH 70 %ee
CH_2Cl_2 >95 %ee



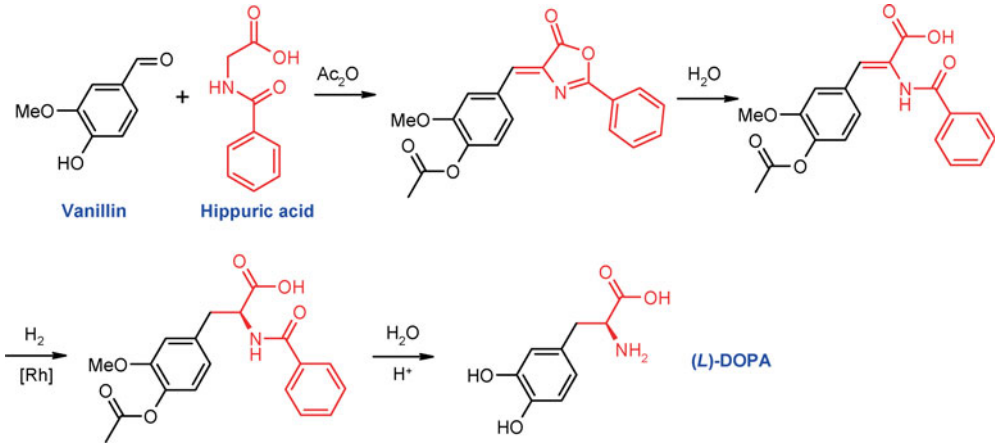
Ir+ CH_2Cl_2 98 %ee

The Other DOPA Process

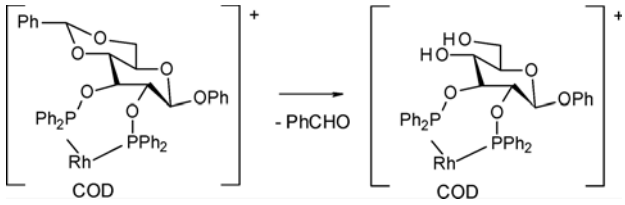
Not least due to the award of the 2001 Nobel Prize for Chemistry to William S. Knowles, Ryoji Noyori and Karl Barry Sharpless, the DOPA process from Monsanto and the story of its development achieved renewed general attention, and is today referenced in many textbooks. Less known is another story – presumably because it was set behind the Iron Curtain in the GDR (the former East Germany) – which led several years later to an alternative catalyst system. [87] In the 1960s and 70s Horst Pracejus (1927–1987) was working in Rostock on cellulose derivatives for rhodium-catalysed, enantioselective hydrogenations. In 1978, Rüdiger Selke and Horst Pracejus at the Volkseigener Betrieb (VEB) (the former state-owned enterprise) ISIS-Chemie in Zwickau suggested "Ph-*β*-glup" [88] as a ligand for the preparation of (*L*)-DOPA. This was readily accessible from phenyl *β*-(*D*)-glucoside.



In contrast to the Monsanto process, it was decided to use hippuric acid in the Erlenmeyer-Plöchl condensation for the VEB ISIS-Chemie Zwickau procedure.



An interesting observation was made with regard to the catalyst system. Probably under the reaction conditions for the enantioselective hydrogenation, large amounts of the pre-catalyst were hydrolysed, forming a complex with even higher selectivity.



The enantioselectivity is practically unaffected, whether the reaction is carried out at normal pressure or at 10 bar. Also the temperature, in the region of 40–60 °C, has no influence. Methanol proves to be especially advantageous as a solvent. Substrate/catalyst ratios of up to 20,000 : 1 are achievable.

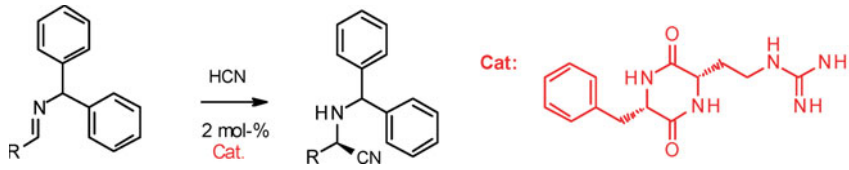
In 1985, ISIS-Chemie took up the production of (*L*)-DOPA. The reaction was carried out in 80- litre glass reactors, regardless of the fact, that larger reactors would have been more economical. There was a fear for the loss of valuable material, in case of batch failures. The annual production ran to approximately 1 tonne. In 1990, the production was terminated, one year after the fall of the Berlin Wall and one month after a decision to expand the production.

4.7.3 Enantioselective Strecker Synthesis

One of the most interesting developments in the field of stereoselective synthesis of amino acids is unquestionably the enantioselective Strecker reaction. Being a fairly recent discovery, the reaction has not yet been advanced to the industrial

scale. Nevertheless, this method has already spurred traction among research laboratories in industry, who are interested in its large scale application. [89]

Already in 1963, Kaoru Harada had pioneered asymmetric Strecker reactions using (*S*)-phenylethylamine in place of ammonia. In 1996, Mark Lipton reported for the first time that addition of hydrogen cyanide to imines of aromatic aldehydes, in the presence of 2 mole % of an enantiomerically pure diketopiperazine, leads with high enantioselectivity to the corresponding amino-nitriles. [90]

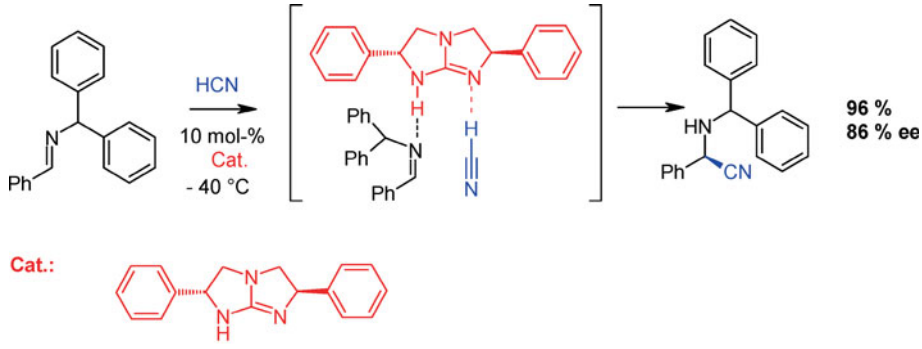


Unfortunately, with nitro-substituted benzaldehydes, heterocyclic and aliphatic aldehydes the selectivity completely breaks down. A low temperature is required, in order to suppress the non-catalysed Strecker reaction (Tab. 4.1).

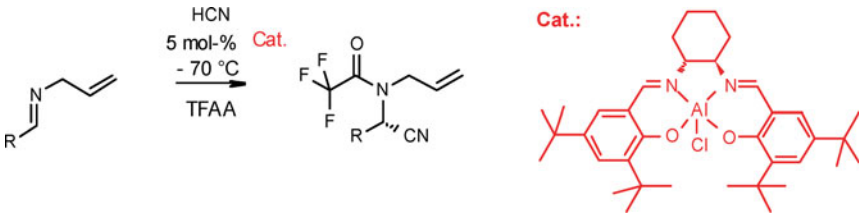
Tab. 4.1 *Enantioselective synthesis of amino-nitriles according to Lipton*

R	Temp. (°C)	Yield (%)	ee (%)
Ph	–25	97	> 99
4-Cl-C_6H_4	–75	94	> 99
4-MeO-C_6H_4	–75	90	96
3-Cl-C_6H_4	–75	80	> 99
3-MeO-C_6H_4	–75	82	> 99
3-NO_2-C_6H_4	–75	71	< 10
2-Furyl	–75	94	32
t-Bu	–75	80	17

Elias J. Corey used a bicyclic, enantiomerically pure guanidine as catalyst, and in the case of benzaldehyde, he achieved an enantiomeric excess of 86 %. Crucial to the enantioselectivity are the van der Waals forces and the π-stacking interactions between imine and catalyst. [91]



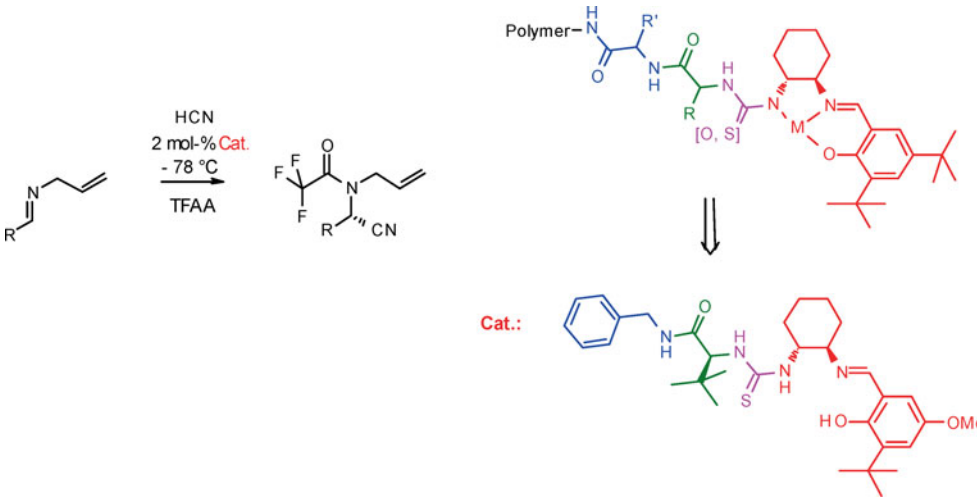
Eric Jacobsen's first catalyst for the enantioselective Strecker reaction was an aluminium-salen complex (Tab. 4.2). [92]



Tab. 4.2 *Enantioselective synthesis of amino-nitriles with an aluminium-Salen complex*

R	Yield (%)	ee (%)
Ph	91	95
4-Cl-C_6H_4	92	81
4-MeO-C_6H_4	93	91
t-Bu	69	37

For optimisation of the catalyst, Jacobsen used solid-phase parallel synthesis. He retained the motif of the Salen complex, however replaced one salicylaldehyde by a linker to the solid phase. This consists of a urea or thiourea moiety, and to increase diversity, additionally one or two amino acids. [93]



A careful screening of metals and of amino acids in the complex showed, that the best results were achieved in the absence of metal salts. The variation in the amino acids revealed that *t*-leucine is an advantageous residue. The second amino acid and the polymer can be replaced by a simple benzyl group (Tab. 4.3).

Tab. 4.3 *Enantioselective Synthesis of amino-nitriles according to Jacobsen*

R	Yield (%)	ee (%)
Ph	78	91
4-Br-C_6H_4	65	86
4-MeO-C_6H_4	92	70
t-Bu	70	85

In 2012, Choong Eui Song published the use of a simple BINOL-derived catalyst for enantioselective, organocatalytic Stecker reactions. [97]

The enantioselective Strecker reaction also succeeds with ketoimines. Thus, α-branched amino-nitriles [94] and also unnatural amino acids [95] can be obtained by this method. The precise mechanism of the catalysis is unclear. Kinetic investigations have previously shown, that the turnover reflects a Michaelis-Menton relationship. The imine is bonded reversibly to the catalyst. The addition of hydrogen cyanide is rate-determining. [96]

4.8 The Maillard Reaction

At the end of this chapter, the Maillard reaction and the Amadori rearrangement should be mentioned – two named reactions, without which life would be grim and dull (Fig. 4.22). Many meals would be relatively tasteless without these reactions. So it was (naturally!) a Frenchman, Louis-Camille Maillard (Fig. 4.23), who discovered in 1912, that reducing sugars and amino acids under heating, produced brown products, the so-called melanoidins. [98, 99]

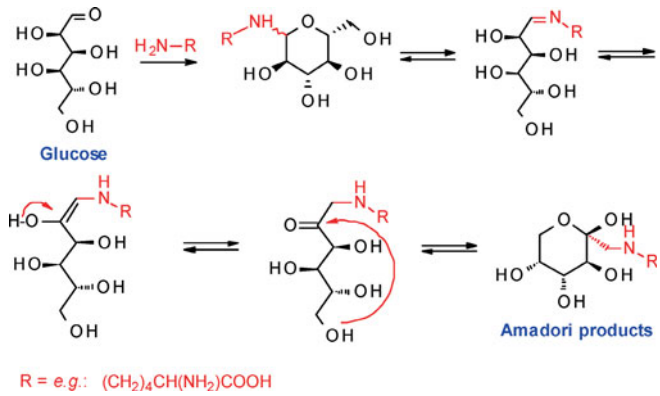
A hundred thousand years ago, Man had begun to prepare food with the aid of fire, and started chemistry while cooking. With help from the Maillard reaction, the taste of his food became more sophisticated and pleasant. The typical smell of warm, crusty bread, the marvellous taste of a roast, the fine odour of roasted coffee, the spicy aroma and the colour of beer, result all from this little-known named reaction. Precise analysis of food ingredients and the development of highly specialised processing technologies allow for the industrial-scale preparation of tasty, appealing and readily available meals and snacks, which are

4.22 *Maillard reaction: Huge food related industries (sausages, bakery products, coffee, beer, whisky) make, often unwittingly, use of this named reaction. The value of the worldwide food and beverage market has been estimated at 2,000 billion US dollars, with an annual growth rate of around 2 %.*

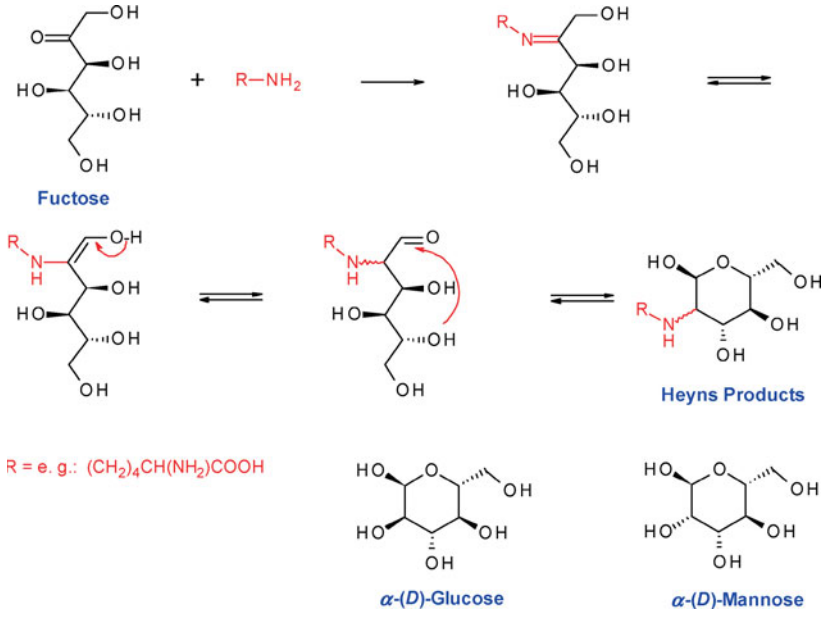
tailored to present-day lifestyles and eating patterns. The aroma industry provides essences, which mediate impressions of taste and smell of meat, poultry, fish and vegetables. In the future, an even higher attention will be paid to functional foods, which are labelled as especially health-promoting.

By heating of foodstuffs, aldoses and amino acids form *N*-glycosides, which lead to Amadori products by an Amadori rearrangement (Mario Amadori (1886–1941), Italian chemist). The mechanism is analogous to that of the Lobry de Bruyn-van Ekenstein rearrangement (Cornelis Adriaan Lobry van Troostenburg de Bruyn (1857–1904) and Willem Alberda van Ekenstein (1858–1937) were chemists from the Netherlands).

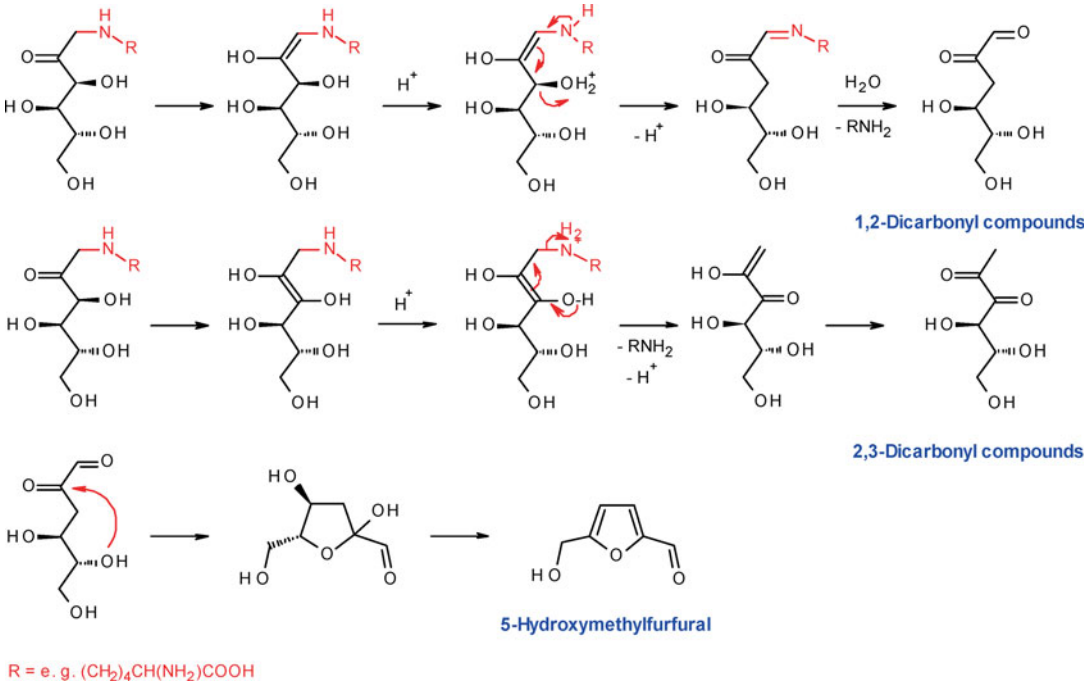
4.23 *Louis-Camille Maillard (1878–1936).*



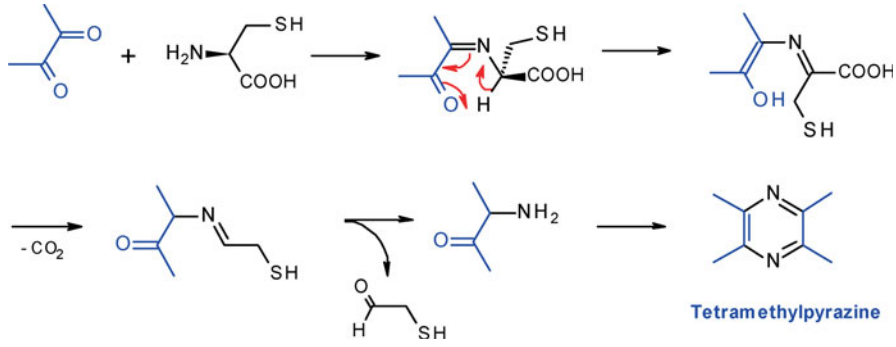
Ketoses react correspondingly to form Heyns products (Kurt Heyns (1908–2005), German chemist). Isomerisation of the double bond leads unselectively to a new stereogenic centre. Consequently, the products have a glucose or mannose configuration.



The Amadori products decompose to characteristic α-dicarbonyl compounds and 5-hydroxymethylfurfural (bread and coffee aroma). In the end, like their aldimines or ketimines, they lead in follow-on reactions to melanoidins (high molecular-weight compounds), which, together with caramel products, create the brown colour of grilled or toasted food.



The α-dicarbonyl compounds can also react with amino acids along a Strecker decomposition, whereby the α-amino-ketones dimerise to pyrazines.



The evolution of aromas is largely associated with sulfur-containing amino acids, which are decomposed to mercaptoaldehydes. Disulfides and thiols in high dilution are critical for the aroma of onions, tomatoes, potatoes, mushrooms, meat, beer, bread, coffee and tea.

The Maillard reaction is responsible for the smell, taste and colour of cooked foodstuffs (Fig. 4.24). It affects their storability, but is also the source of thereby formed mutagenic substances, and it contributes to a lower nutritional value. The Maillard reaction is connected as well to the origin of various diseases, such as *Diabetes mellitus,* and it is suspected to impact the aging process. [100]

4.24 *The pleasure of eating a sausage is rarely augmented by knowing how it is made. – But the Maillard reaction is indispensable for enhancing that experience.*

Summary in Bullet Points

- The biosynthesis of amino acids reaches far back to the origin of life. Up to present days, biological nitrogen fixation and homochirality of amino acids are attractive areas for research.
- The understanding of the biosynthesis of amino acids provides the basis for numerous biotechnological processes.
- All three access routes to amino acids are important: isolation procedures from natural sources, chemical syntheses and enzymatic methods.
- There exists a range of options for enantioselective chemical synthesis.
- Amino acids are of high value for the pharmaceutical industry, for animal nutrition and for the food industry.

References

1. S. Miller, *J. Am. Chem. Soc.* **77** (1955) 2351.
2. H. J. Bogen, Knaurs Buch der modernen Biologie, Droemer Knaur, München/Zürich 1967, 291.
3. K. Severin, *Angew. Chem. Internat. Edn.* **39** (2000) 3589.
4. C. Huber, G. Wächtershäuser, *Science* **281** (1998) 670.
5. T. Hennings, F. Salama, *Science* **282** (1998) 2204; C. Giri, F. Goesmann, C. Meinert, A. C. Evans, U. J. Meierhenrich, *Top. Curr. Chem.* **333** (2013) 41 and **333** (2013) 307.
6. F. Salama, *Origins of Life and Evolution of the Biosphere* **28** (1998) 349.
7. M. Winnewisser, *Chemie in unserer Zeit* **18** (1984) 1; M. Winnewisser, *Chemie in unserer Zeit* **18** (1984) 55; N. Kerisit, L. Toupet, Y. Trolez, J.-C. Guillemin, *Chem. Eur. J.* **19** (2013) 17683.
8. R. I. Kaiser, A. M. Mebel, *Chem. Soc. Rev.* **41** (2012) 5490.
9. Y.-J. Kuan, S. B. Charnley, H.-C. Huang, W. L. Tseng, Z. Kisiel, *Astrophys. J.* **593** (2003) 848; the results were doubted in L. E. Snyder, F. J. Lovas, J. M. Hollis, D. N. Friedel, P. R. Jewell, A. Remijan, V. V. Ilyushin, E. A. Alekseev, S. F. Dyubko, *Astrophys. J.* **619** (2005) 914.
10. J. Herrmann, Welcher Stern ist das?, Franckh-Kosmos Verlag, Stuttgart, 26th Edn., 1998, 150.
11. H. v. Ditfurth, Wir sind nicht nur von dieser Welt, 10th Edn., dtv, München, 1994, 64; S. Pizzarello, *Chemistry & Biodiversity* **4** (2007) 680; A. S. Burton, J. C. Stern, J. E. Elsila, D. P. Glavin, J. P. Dworkin, *Chem. Soc. Rev.* **41** (2012) 5459; E. I. Klabunovskii, *Russ. J. Org. Chem.* **48** (2012) 881.
12. J. R. Cronin, S. Pizzarello, *Science* **275** (1997) 951.
13. J. Podlech, *Angew. Chem. Internat. Edn.* **38** (1999) 477.
14. M. H. Engel, A. Macko, *Nature* **389** (1997) 265.
15. H. Buschmann, R. Thede, D. Heller, *Angew. Chem. Internat. Edn.* **39** (2000) 4033.
16. B. L. Feringa, R. A. van Delden, *Angew. Chem. Internat. Edn.* **38** (1999) 3418.

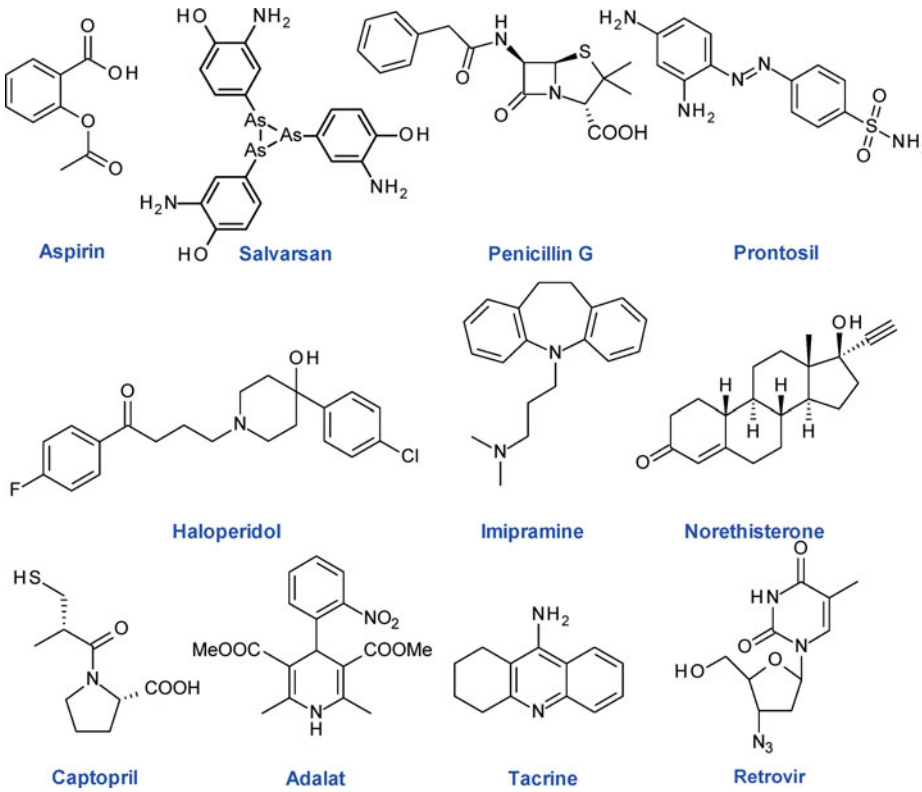
17. M. Avalos, R. Babiano, P. Cintas, J. L. Jiménez, J. C. Palacios, *Chem. Commun*. (2000) 887.
18. M. Avalos, R. Babiano, P. Cintas, J. L. Jiménez, J. C. Palacios, *Tetrahedron: Asymmetry* **11** (2000) 2874.
19. D. K. Kondepudi, *Int. J. Quant. Chem*. **98** (2004) 222.
20. P. Cintas, *Angew. Chem. Internat. Edn.* **41** (2002) 1139.
21. M. Eigen, Das Spiel, Piper, 3rd Edn., München/Zürich, 1979, 144; W. A. Bonner, *Top. Stereochem.* **18** (1988) 1.
22. A. Brack, *Chemistry & Biodiversity* **4** (2007) 665.
23. M. H. Todd, *Chem. Soc. Rev.* **31** (2002) 211.
24. K. Soai, I. Sato, T. Shibata, *ACS Symp. Ser.* **880** (2004) 85.
25. M. Reggelin, *Nachr. Chem. Tech. Lab.* **45** (1997) 622; T. Gehring, M. Busch, M. Schlageter, D. Weingand, *Chirality* **22** (2010) E173.
26. J. Erfkamp, A. Müller, *Chemie in unserer Zeit* **24** (1990) 267.
27. M. Dörr, J. Käßbohrer, R. Grunert, G. Kreisel, W. A. Brand, R. A. Werner, H. Geilmann, C. Apfel, C. Robl, W. Weigand, *Angew. Chem. Internat. Edn*. **42** (2003) 1540.
28. J. Kim, D. C. Rees, *Nature* **360** (1992) 553.
29. J. Kim. D. Woo, D. C. Rees, *Biochemistry* **32** (1993) 7104.
30. J. B. Howard, D. C. Rees, *Chem. Rev*. **96** (1996) 2965.
31. J. W. Peters, M. H. Stowell, S. M. Soltis, M. G. Finnegan, M. K. Johnson, D. C. Rees, *Biochemistry* **36** (1997) 1181.
32. A. Müller, E. Krahn, *Angew. Chem. Internat. Edn.* **34** (1995) 1071.
33. P. C. Do Santos, D. R. Dean, Y. Hu, M. W. Ribbe, *Chem. Rev*. **104** (2004) 1159.
34. O. Einsle, F. A. Tezcan, S. L. A. Andrade, B. Schmid, M. Yoshida, J. B. Howard, D. C. Rees, *Science* **297** (2002) 1696; B. Hinnemann, J. K. Norskov, *J. Am. Chem. Soc*. **125** (2003) 1466; I. Dance, *Chem. Commun*. **2003**, 324.
35. K. M. Lancaster, M. Roemelt, P. Ettenhuber, Y. Hu, M. W. Ribbe, F. Neese, U. Bergmann, S. DeBeer, *Science* **334** (2011) 974.
36. T. Spatzal, M. Aksoyoglu, L.-P. Zhang, S. L. A. Andrade, E. Schleicher, S. Weber, D. C. Rees, O. Einsle, *Science* **334** (2011) 940.
37. B. K. Burgess, D. J. Lowe, *Chem. Rev*. **96** (1996) 2983; F. Osterloh, Y. Sanakis, R. J. Staples, E. Münck, R. H. Holm, *Angew. Chem. Internat. Edn*. **38** (1999) 2066 (artificial complexes).
38. B. M. Hoffman, D. R. Dean, L. C. Seefeldt, *Acc. Chem. Res*. **42** (2009) 609.
39. F. A. Cotton, G. Wilkinson, Anorganische Chemie, 4th Edn., Wiley-Interscience, New York, 1980, 1341.
40. G. N. Schrauzer, G. W. Kiefer, P. A. Doemeny, H. Kisch, *J. Am. Chem. Soc*. **95** (1973) 5582.
41. W. S. Silver, J. R. Postgate, *J. theor. Biol.* **40** (1973) 1.
42. J. v. Liebig, Agriculturchemie, 1840.
43. Bibliographisches Institut & F. A. Brockhaus AG, 2001.
44. L. Forni, *Chim. Ind*. **108** (2009) 108; S. E. Nielsen, ACS Symposium Series, (2009) 15; A. F. Holleman, N. Wiberg, Lehrbuch der Anorganischen Chemie, Walter de Gruyter, Berlin, New York (1985) 507, 1133.
45. G. Marnellos, M. Stoukides, *Science* **282** (1998) 98.
46. T. Murakami, T. Nishikiori, T. Nohira, Y. Ito, *J. Am. Chem. Soc.* **125** (2003) 334.
47. V. Smil, Enriching the Earth: Fritz Haber, Carl Bosch, and the Transformation of World Food Production, MIT Press, Cambridge, MA, 2001; R. Hoffmann, P. Laszlo, *Angew. Chem. Int. Edn*. **40** (2001) 4599.
48. M. P. Shaver, M. D. Fryzuk, *Adv. Synth. Chem*. **345** (2003) 1061; N. Hazari, *Chem. Soc. Rev.* **39** (2010) 4044.
49. A. D. Allen, C. V. Senoff, *J. Chem. Soc. Chem. Commun*. **1965**, 621; C. V. Senoff, *J. Chem. Educ.* **67** (1990) 368.

50. J. Chatt, A. J. Pearman, R. L. Richards, *Nature* **253** (1975) 39; J. Chatt, J. R. Dilworth, R. L. Richards, *Chem. Rev.* **78** (1978) 589; C. J. Pickett, J. Talermin, *Nature* **317** (1985) 652; C. J. Pickett, *J. Biol. Inorg. Chem*. **1** (1996) 601; F. Tuczek, *Angew. Chem. Internat. Edn*. **37** (1998) 2636; A. Dreher, G. Stephan, F. Tuczek, *Adv. Inorg. Chem*. **61** (2009) 367.
51. M. Hidai, *Science* **279** (1998) 540; M. Hidai, *Coord. Chem. Rev*. **185–186** (1999) 99.
52. D. V. Yandulov, R. R. Schrock, *Science* **301** (2003) 76; R. R. Schrock, *Chem. Commun*. **2003**, 2389.
53. J. Sgrignani, D. Franco, A. Magistrato, *Molecules* **16** (2011) 442.
54. B. Hao, W. Gong, T. K. Ferguson, C. M. James, J. A. Krzycki, M. K. Chan, *Science* **296** (2002) 1462.
55. K. Roth, *Chemie in unserer Zeit* **41** (2007) 448.
56. K. M. Draths, J. W. Frost, *J. Am. Chem. Soc.* **113** (1991) 9361.
57. K. Weissermel, H.-J. Arpe, Industrielle Organische Chemie, VCH, Weinheim, 4th Edn. 1994, 284, 312.
58. M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer, T. Zelinski, *Angew. Chem. Internat. Edn*. **43** (2004) 788; E. Nakamura, *Chem. Asian J.* **6** (2011) 1659; D. K. Rassin, J. A. Sturman, G. E. Gaull, *Early Human Dev.* **2** (1978) 1.
59. R. Dittmeyer, W. Keim, G. Kreysa, A. Oberholz, Chemische Technik, Vol. 8, Wiley-VCH, Weinheim, 2005, 717.
60. K.-H. König, *Chemie in unserer Zeit* **24** (1990) 217; C. Lamberth, *Tetrahedron* **66** (2010) 7239.
61. B. Hoppe, J. Martens, *Chemie in unserer Zeit* **18** (1984) 73.
62. K. Drauz, S. Eils, M. Schwarm, *Chimica Oggi,* Jan./Feb. 2002, 15.
63. M. Kircher, W. Leuchtenberger, *Biologie in unserer Zeit* **28** (1998) 281.
64. H. Gröger, K. Drauz in H. U. Blaser, E. Schmidt, Asymmetric Catalysis on the Industrial Scale: Challenges, Approaches and Solutions, Wiley-VCH, Weinheim, 2004, 131.
65. J. Dalmolen, T. D. Tiemersma-Wegman, J.-W. Nieuwenhuijzen, M. van der Sluis, E. van Echten, T. R. Vries, B. Kaptein, Q. B. Broxterman, R. M. Kellogg, *Chem. Eur. J.* **11** (2005) 5619.
66. A. Collet, *Angew. Chem. Internat. Edn*. **37** (1998) 3239.
67. Q. B. Broxterman, *Chimica Oggi* **16** (1998) 9, 34.
68. B. Kaptein, T. R. Vries, J. W. Nieuwenhuijzen, R. M. Kellogg, R. F. P. Grimbergen, Q. B. Broxterman, *Pharma Chem*. **2** (2003) 17.
69. R. M. Kellogg, J. W. Nieuwenhuijzen, K. Puower, T. R. Vries, Q. B. Broxterman, R. F. P. Grimbergen, R. M. La Crois, E. de Wever, K. Zwaagstra, A. C. van der Laan, *Synthesis* (2003) 1626.
70. M. Ikeda, R. Katsumata, *App. Environ. Microbiol*. **58** (1992) 781.
71. R. Katsumata, M. Ikeda, *Biotechnol.* **11** (1993) 921.
72. S. Martínez-Rodríguez, A. I. Martínez-Gómez, F. Rodríguez-Vico, J. M. Clemente-Jiménez, F. J. Las Heras-Vázquez, *Chem. Biodiv.* **7** (2010) 1531.
73. M. Beller, M. Eckert, *Angew. Chem. Internat. Edn*. **39** (2000) 1010.
74. W. S. Knowles, M. J. Sabacky, *J. Chem. Soc., Chem. Commun*. 1968, 1445.
75. L. Horner, H. Siegel, H. Buthe, *Angew. Chem. Internat. Edn*. **7** (1968) 942.
76. B. D. Vineyard, W. S. Knowles, M. J. Sabacky, G. L. Bachman, D. J. Weinkauff, *J. Am. Chem. Soc.* **99** (1977) 5946.
77. J. Halpern, *Science* **217** (1982) 401.
78. K. E. Koenig, M. J. Sabacky, G. L. Bachman, W. C. Christopfel, H. D. Barnstorff, R. B. Friedman, W. S. Knowles, B. R. Stults, B. D. Vineyard, D. J. Weinkauff, *Ann. N. Y. Acad. Sci.* **333** (1980) 16.
79. W. S. Knowles, *Acc. Chem. Res*. **16** (1983) 106.

80. K. E. Koenig, Catalysis of Organic Reactions, Marcel Dekker, New York, 1984, 63.
81. K. E. Koenig, in Asymmetric Synthesis, ed. J. D. Morrison, Academic Press, Orlando **5** (1985) 71.
82. C. R. Landis, J. Halpern, *J. Am. Chem. Soc.* **109** (1987) 1746.
83. C. R. Landis, S. Feldgus, *Angew. Chem. Internat. Edn.* **39** (2000) 2863.
84. D. J. Ager, L. Lefort, J. G. de Vries, *ACS Symposium Series* **1009** (2009) 239.
85. M. van den Berg, A. J. Minnaard, E. P. Schudde, J. van Esch, A. H. M. de Vries, J. G. de Vries, B. L. Feringa, *J. Am. Chem. Soc.* **122** (2000) 11539.
86. F. Giacomina, A. Meetsma, L. Panella, L. Lefort, A. H. M. de Vries, J. G. de Vries, *Angew. Chem. Internat. Edn.* **46** (2007) 1497.
87. R. Selke in H. U. Blaser, E. Schmidt, Asymmetric Catalysis on Industrial Scale: Challenges, Approaches and Solutions, Wiley-VCH, Weinheim, 2004, 39.
88. M. Diéguez, O. Pàmies, C. Claver, *Chem. Rev.* **104** (2004) 3189.
89. H. Gröger, *Chem. Rev.* **103** (2003) 2795; M. Shibasaki, M. Kanai, T. Mita, *Org. React.* **70** (2008) 1.
90. K. Harada, *Nature* **200** (1963) 1201; M. S. Iyer, K. M. Gigstad, N. D. Namdev, M. Lipton, *J. Am. Chem. Soc.* **118** (1996) 4910.
91. E. J. Corey, *Org. Lett.* **1** (1999) 157.
92. M. S. Sigman, E. N. Jacobsen, *J. Am. Chem. Soc.* **120** (1998) 5315.
93. M. S. Sigman, E. N. Jacobsen, *J. Am. Chem. Soc.* **120** (1998) 4901.
94. P. Vachal, E. N. Jacobsen, *Org. Lett.* **2** (2000) 867; P. Vachal, E. N. Jacobsen, *J. Am. Chem. Soc.* **124** (2002) 10012.
95. S. J. Zuend, M. P. Coughlin, M. P. Lalonde, E. N. Jacobson, *Nature* **461** (2009) 968.
96. P. Karlson, Kurzes Lehrbuch der Biochemie, Georg Thieme Verlag, Stuttgart, 1970, 68; M. S. Sigman, P. Vachal, E. N. Jacobsen, *Angew. Chem. Internat. Edn.* **39** (2000) 1279.
97. H. Yan, J. Suk Oh, J. W. Lee, C. Eui Song, *Nature Commun.* **3** (2012) 1212.
98. E. Lück, *Chemie in unserer Zeit* **19** (1985) 156.
99. F. Ledl, E. Schleicher, *Angew. Chem. Internat. Edn.* **29** (1990) 565.
100. J. A. Gerrard, *Aust. J. Chem.* **55** (2002) 299.

5 | Pharmaceuticals

A boy who is born in Germany today has a probable life expectancy of around 77 years, and a girl one of 82 years – twice as long as in the year 1880. This increase is a consequence of both, better living circumstances – working conditions, food, clothing, housing –, and also a result of more comprehensive medical care. Thus, a whole range of infectious diseases has been wiped out, or brought at least under control, and the mortality rate for civilisation- and age-related diseases has dropped. Vaccinations and drugs play a key role in this outcome. The 20th century is characterised especially by the worldwide blossoming of the pharmaceutical industry. More and more, the targeted search for and development of new drugs have replaced serendipitous discoveries (Fig. 5.1).



5.1 *Pharmaceuticals of the last century.*

B. Schaefer, *Natural Products in the Chemical Industry*,
DOI 10.1007/978-3-642-54461-3_5, © Springer-Verlag Berlin Heidelberg 2014

An early landmark in this development is the analgesic *Aspirin®*, which was launched by Bayer in 1899. Nowadays, we can hardly imagine our pharmaceutical armoury without this drug, which has become a household brand like *Coca Cola®*, *Pampers®* or *Apple®*. Also, wherever we travel, even to the moon, *Aspirin®* is a regular component of every first-aid kit.

In 1910, *Salvarsan®* (arsphenamine) followed. This arsenic-containing compound, discovered by Sahachirō Hata (1873–1938) at Paul Ehrlich's lab in Frankfurt am Main, Germany, was the first effective medicament for the scourge of the population, syphilis. Paul Ehrlich is now reckoned as being the founder of chemotherapy against microbial diseases.

The incidental discovery of penicillin by Sir Alexander Fleming in 1928 turned out to become an epoch-defining event. However, it was not until the end of the Second World War that this medication finally reached the market, since in the beginning, the drug could not be produced in sufficient quantities. Only thanks to new fermentation procedures, its large-scale production became feasible. Penicillin still saves millions of lives every year.

During the first third of the 20th century, infectious diseases were the most frequent cause of death. Physicians were largely helpless against pneumonia, blood poisoning, meningitis and scarlet fever. In 1935, an effective treatment of these infectious diseases became available with *Prontosil®*, an antibacterial compound of the sulfonamide class developed by Gerhard Domagk, Fritz Mietzsch and Josef Klarer. *Prontosil®* had its chemical roots in the previously well-developed field of azo dyestuff research. Along procedures, which had emerged in that arena, azo-coupling permitted the preparation of a large number of compounds, which could then be examined for their antibacterial effect.

The determination of the DNA structure in 1953 by James Watson and Francis Crick laid the foundation stone of genetic engineering. In the meantime, the genetic code of many living organisms, including Man, has been deciphered. With the pioneering work of Watson and Crick, there began a scientific era with a rapidly growing body of knowledge and novel techniques in molecular biology, the fruits of which are now being harvested in the form of new types of medicine, diagnostic capabilities and treatment options.

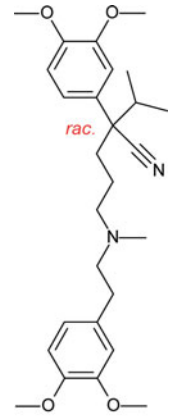
In the 1950s, the first medicines for treatment of mental illness reached the market. Paul Janssen had developed haloperidol for treatment of schizophrenia, and Geigy introduced imipramine as a therapy for depression. Thereby, for the first time these widespread and serious illnesses could be managed successfully. Patients, who had previously been admitted to mental institutions on a routine basis, were now able to live on in their familiar surroundings.

That infantile paralysis (poliomyelitis), a previously dreaded infectious disease, has been *de facto* eliminated in Germany and many other countries, is thanks to the vaccine developed by Jonas E. Salk in 1954. In 1961, West Germany had still 4.461 recorded cases of polio following the outbreak of an epidemic. However, one year later, after an important campaign under the slogan "Oral vaccination is sweet, polio is cruel", only 300 new cases of polio were registered.

Towards the end of the 1950s, the first of the nowadays called lifestyle drugs, the "Pill" received market approval. Gregory Pincus (1903–1967) and John Rock

(1890–1984) enhanced the physiological compatibility and ovulation inhibition properties of norethisterone, originally synthesised by Carl Djerassi and Luis Ernesto Miramontes Cárdenas (1925–2004). This compound was used initially to treat menstruation problems, and due to its contraceptive side-effect, it soon became a means of birth control. By 1965, the "sexual revolution" had broken further ground, while several million women were taking the "Pill". Thereafter, reproduction and sex life became uncoupled.

In our times, high blood pressure is one of the leading diseases of the cardiovascular system. Hypertension is still, ahead of cancer, the most frequent cause of death in industrial countries. After the development of Verapamil (*Isoptin*®) by Knoll AG in 1963, *Adalat*® – from the work of Friedrich Bossert and Wulf Vater at Bayer – was marketed in 1975 as another and even stronger calcium antagonist. Apart from ACE-inhibitors (*e.g.* Captopril from Squibb), this class of drugs provides enhanced blood flow through the coronary arteries. Up to now, these therapeutics are still in use against hypertension, and they reduce significantly the risk of heart attacks and stroke.



Verapamil

Since 1922, diabetes has been treated with insulin, which was isolated from the pancreas of pigs or cattle. Because of the continually increasing number of diabetics, the World Health Organisation issued a warning in the 1970s about a supply bottleneck. Ultimately, it was the work of Watson and Crick, which enabled human insulin as the first genetically engineered therapeutic to be brought to the market in 1982. Nowadays, the market share of human insulin lies at around 80 %.

In 1906, the Bavarian psychiatrist Alois Alzheimer described a peculiar disease of the cerebral cortex. He did not anticipate at the time, that by the end of the century this illness would have increased so dramatically to become one of the main problems in neuropathology. 20 % of all men over the age of 80 suffer from progressive Alzheimer's disease, which is accompanied by a complete loss of personality. In 1993, the reversible acetylcholinesterase inhibitor Tacrine was approved in the USA; for the first time, significant responses were seen in the treatment of this devastating illness.

A therapeutic challenge, not unlike syphilis at the beginning of the 20th century, was posed towards the end of the century by AIDS, a pandemic viral disease of the immune system. In an attempt to control the disease, in the late eighties, the first reverse-transcriptase inhibitors (*e.g.Retrovir*®) were developed to the market. Within two years of these drugs in use, the death rate from AIDS in New York had dropped from 7,046 to 4,994 cases. Nowadays, the reverse-transcriptase inhibitors are considered one of the most benign and therapeutically efficacious groups of drugs in medical history.

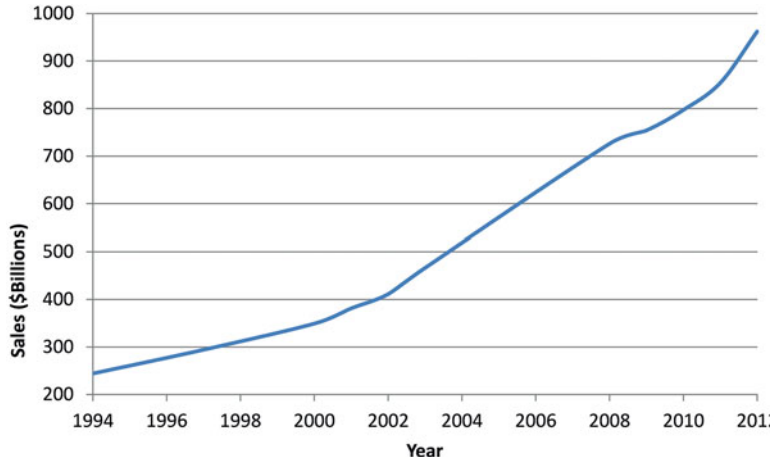
Certainly most of the HIV patients today reside in developing countries, where they don't have access to effective medication to fight this cruel disease. The probably biggest challenge for the 21st century lies in providing new and innovative medicines to those 90 % of the world's population, who live in the poor regions of our world. [1]

The pharmaceutical industry can be rightfully proud of its contributions to medical progress in the century that just ended. While at the beginning of this era, there were individual scientists detecting new pharmacologically active

compounds, today, mostly interdisciplinary teams at large industrial and national organisations are required to develop new pharmaceuticals and to secure their safety and quality.

An overview of the drug market in Germany is offered by the "Rote Liste" (red list). For 2013, there are registered 6,482 approved pharmaceutical preparations, containing about 2,200 active pharmaceutical ingredients (APIs) from 457 manufacturers.

The world drug market is estimated at more than 900 billion dollars, and an annual growth rate exceeding 8 % (Fig. 5.2) – indicative of a huge industry.

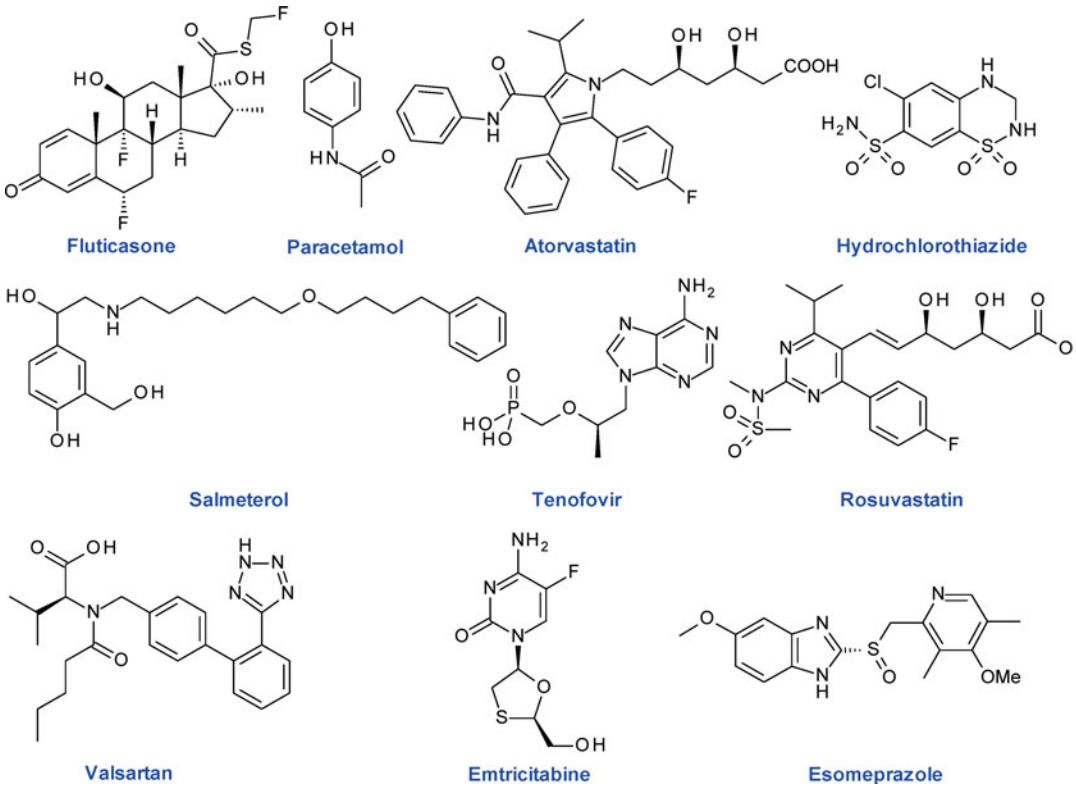


5.2 *The World pharmaceuticals market from 1994 to 2012.*

Tab. 5.1 *Worldwide sales of the top ten active pharmaceutical ingredients (APIs) in 2012 (Total "small molecules" market volume: 828 billion Dollars).*

Compound (INN)	Company	Sales ($10^9$$)	Consumption (t/a)	Mode of action	Indication
Fluticasone	GSK	11.7	3	Antiinflammatory, immunosuppressive	Asthma
Paracetamol	many worldwide	10.8	53,200	Prostaglandin synthesis inhibitor	Fever, pain-killer
Atorvastatin	Pfizer	10.3	306	HMG-CoA reductase inhibitor	Dyslipidemia
Hydrochloro-thiazide	Merck	10.1	492	NaCl-transport inhibitor	High blood pressure
Salmeterol	GSK	9.5	0.5	β_2-sympathomimetic drug	Asthma
Tenofovir	Gilead Sciences	9.3	129	Reverse transcriptase inhibitor	HIV, hepatitis B
Rosuvastatin	AstraZeneca	9.0	74	HMG-CoA reductase inhibitor	Dyslipidemia
Valsartan	Novartis	8.4	1,039	Angiotensin II receptor antagonist	High blood pressure
Emtricitabine	Gilead Sciences	8.4	59	Reverse transcriptase inhibitor	HIV, hepatitis B
Esomeprazole	AstraZeneca	8.3	175	Proton pump inhibitor	Dyspepsia, Peptic ulcer

The USA still maintains its leading position in this market, followed by Japan, Germany and France. The sales figures achieved by individual compounds are also considerable (Tab. 5.1 and Fig. 5.3).



5.3 *The structures of the pharmaceuticals with the largest turnover in the year 2012.*

In terms of therapeutic indications, it is evident that these can be almost entirely attributed to diseases of civilisation and to the physical deficiencies of an ageing population. Thus, a large portion of medicaments is predominantly employed by the geriatric society of industrialised countries. In Germany, 30 % within the age group of 40–49 take tablets regularly; for the 50–59 years old, this fraction is 42 %, and for those aged 60 and above, the number rises to more than 70 %.

According to the WHO (World Health Organisation), there are around 30,000 known diseases worldwide. While approximately one-third of these are amenable to medical treatment, the objective of pharmaceutical research is to increase this proportion. Prevention and treatment of illness by medication often proves more cost-effective than admission to a hospital or spa. For numerous widespread serious illnesses like cancer, rheumatism, viral diseases and psychosis, however, a convincing therapeutic concept is still in demand.

Worldwide pharmaceutical research endeavours are underway to meet these deficits with new therapeutic approaches. However, this is extraordinarily costly. From a statistical perspective, testing of more than 10,000 compounds is needed before one of them will reach the market. In spite of many scientific and technical innovations, like rational drug design, combinatorial chemistry and

automated mass screening, the development of an active compound from the bench to the pharmacy still requires eight to ten years and costs more than 500 million dollars. Particularly high expectations are placed at present on knowledge arising from the deciphering of the human genome. In the future, this will perhaps enable accelerated identification of "target genes", which could facilitate the search for new and better efficacious drugs. [2]

Summary in Bullet Points

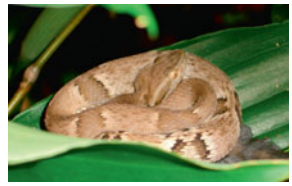
- The 20th century has seen industrial growth worldwide, especially the blossoming of the pharmaceutical industry.
- The drug market reflects – with its turnover of more than 800 billion dollars worldwide –the success of one of the largest industries.
- Revealingly, most of the medications find predominant use in the geriatric society of industrialised countries.

5.1 ACE Inhibitors

Snakes have always held a fascination for man. To the Egyptians, the snake was the envoy of the Sun-god Amon-Ra. A death by snakebite carried the promise of immortality. Cleopatra committed suicide with a viper. The Uraeus snake was the symbol of the Pharaohs. Adam and Eve were led astray by the serpent. Hercules slew the nine-headed Hydra in the marsh at Lerna, near Argolis. In Germanic mythology, the Earth is surrounded by an ocean in which the Midgard snake lives between the Niflheim to the north, and the Muspelheim to the south. The Aesculap rod with the snake is the symbol of the healing art, and all chemists since August Kekulé think of the benzene formula, the snake biting its tail, as a symbol of perpetual circulation.

The history of ACE (*angiotensin-converting enzyme*) inhibitors for the treatment of cardiovascular illnesses begins in the banana plantations of south-western Brazil. There were repeated instances reported of workers suffering sudden collapse and death. The cause was a bite from a speckled olive-green to grey-brown snake of around 1.2 m in length: the Brazilian lancehead snake (*Bothrops jararaca*) (Fig. 5.4).

Most snake venom contains proteins, which act either as a neurotoxin or a cardiotoxin. They paralyse the muscular system and lead to cardiac arrest. Apart from this, various enzymes destroy cells and tissue. The toxin from *Bothrops jararaca* and several other snakes, [3] such as the pit viper *Agkistrodon halys blomhoffii*, leads to a dramatic drop in blood pressure through vasodilation, which paralyses the victim. In addition, it causes massive bleeding due to the inhibition of thrombocyte aggregation and increased permeability of the blood vessels. Certain parts of the smooth muscle system contract. The toxin quickly spreads; the victim is scarcely capable of movement, and death rapidly follows.



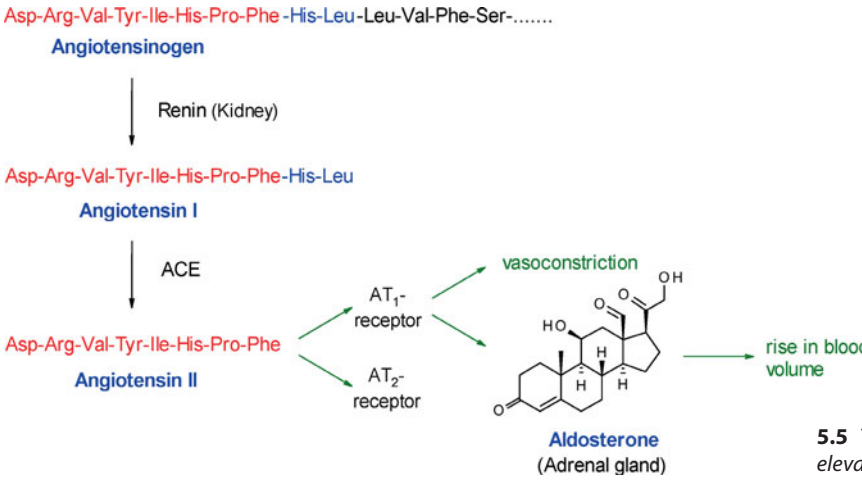
5.4 *The Brazilian lancehead snake (Bothrops jararaca). Its common name derives from the Tupi words yarará and ca, which means "large snake."*

5.1.1 Physiological Fundamentals

In 1898, there was published, just on a single page, the will of Alfred Nobel, who had died two years earlier from heart failure, and also a 48-page work by Robert Tigerstedt and Per Bergmann from the Karolinska Institute (Stockholm) on the

discovery of renin. Whereas the latter work gathered dust for the following 36 years in the Scandinavian Archives of Physiology, Nobel's testament enjoyed worldwide attention from the very beginning.

What had Tigerstedt and Bergmann found out? They injected the extract from a kidney into a rabbit and observed a dramatic rise in blood pressure. They suspected that the cause of this was a hormone, which they named *renin*. In 1934, Harry Goldblatt, a physician from Cleveland, was the first to pick up these results and to develop an animal model for high blood pressure. By using a dog, he clamped off the blood supply to the kidneys and observed the same symptom Tigerstedt and Bergmann had previously obtained in the rabbit. Subsequently it was recognised that such renal ischaemia leads *via* a cascade of events to the release of a substance, which causes increased blood pressure. Further research showed that renin is not a hormone, but a 37 kDa protease, which cleaves the serum globulin angiotensinogen, secreted by the liver, to the decapeptide angiotensin I. Leonard T. Skeggs showed in 1956, that this decapeptide does not affect the blood pressure, but once shortened by two amino acids, the resulting angiotensin II acts as a strong vasoconstrictor. This transformation is carried out by the dipeptidylcarboxypeptidase ACE (Angiotensin Converting Enzyme), primarily located in the lung capillaries. In addition to its direct vasoactive properties, this octapeptide acts also on the adreanal glands to release the steroid hormone aldosterone, which causes increased re-absorption of water by the kidneys, leading to an augmented blood volume and thus higher blood pressure. Both, the direct and the indirect pharmacological effect are mediated by interaction of angiotensin II with its AT_1 (angiotensin II type 1) receptor (Fig. 5.5).



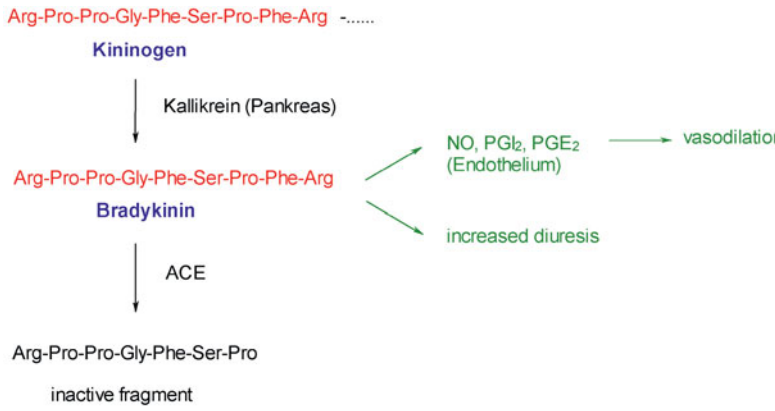
5.5 *The blood pressure elevating system.*

High blood pressure damages the blood vessels, and can lead to stroke or heart attacks (infarctions), as well as heart and kidney failure (Fig. 5.6).

To ensure the stability of the circulation, the blood pressure elevating system is counterbalanced by a corresponding blood pressure lowering system (Fig. 5.7). Kallikrein, an enzyme in the pancreas, is responsible for the production of bradykinin from kininogen. Bradykinin exerts its multiple tissue dependent

5.6 *Franklin D. Roosevelt (centre, 1882–1945) was diagnosed with high blood pressure in 1937. He was prescribed bed rest, a low-salt diet and Phenobarbital, though this did not improve his condition. In 1940, his heart failed several times, whereupon he received digitalis. By the time of the Yalta conference in February 1945, his blood pressure had risen to 250/150. A few months later, he died of a massive cerebral haemorrhage.*

pharmacological effects by interaction with B_1 and B_2 receptors, whereupon vascular endothelial cells are stimulated to release not only prostacyclin PGI_2 and prostaglandin PGE_2, but also nitric oxide (Fig. 5.8), the most powerful endogenous vasodilator. Interestingly, a crosstalk between the renin/angiotensin and the kallikrein/bradykinin system becomes evident in that ACE inactivates bradykinin.



5.7 *The blood pressure lowering system.*

In 1948, bradykinin was discovered by Mauricio Rocha e Silva (1910–1983), Wilson T. Beraldo (1917–1998) and Gastão Rosenfeld (1912–1990) at the Instituto Biológico in São Paulo, Brazil, when they added venom from *Bothrops jararaca* to the blood plasma of animals.

5.8 *Alfred Nobel (1833–1896) suffered from Angina pectoris. Shortly before his death, he wrote: "It sounds like the irony of fate that I should be ordered by my doctor to take nitroglycerin internally." Indeed, nitroglycerin is a highly efficient drug for the treatment of this illness, because NO is formed in course of its degradation. However, it's mode of action has been discovered only in the 1970s. [4]*

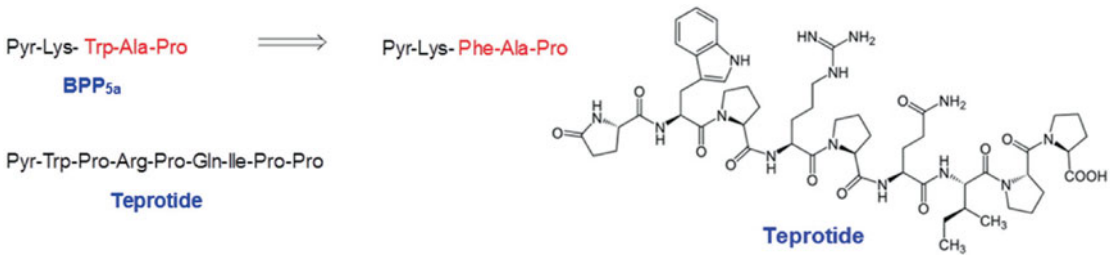
Robert Bruce Merrifield, who is famous for having developed a solid-phase peptide coupling technique, succeeded in the synthesis of bradykinin, with an 85 % yield in just 27 hours. [6]

ACE is therefore a key enzyme for the regulation of blood pressure. Inhibition of this enzyme leads at the same time to a decreased conversion of angiotensin and a decreased degradation of bradykinin. However, ACE has still more functions. It is also involved in the metabolism of a whole range of other endogenous peptides, for example endorphins (*cf.* Opiates – section 5.3). [5]

5.1.2 History of Discovery

Since 1948, it has been known that the vasodilatory effect of the snake venom from the Brazilian lancehead snake is connected to bradykinin. Important contributions towards clarifying the mode of action of the toxic principle are due to the research of the Brazilian pharmacologist Sérgio Henrique Ferreira. One of

the first peptides, which he isolated and identified in 1970, was the pentapeptide BPP_{5a} (bradykinin-potentiating peptide: IC_{50} 1.2 μM) (Fig. 5.9). [3] He later found in the venom of *Bothrops jararaca* a series of other peptides, which enhance the effect of bradykinin. To enrich their menu, snakes have developed a broad spectrum of poisonous peptides, in order to match the genetic diversity of their prey. [3]



5.9 *BPP_{5a} was the first peptide to be isolated from the venom of the Brazilian lancehead snake. Its pharmacophore and that of a more stable analogue are highlighted in red. Teprotide is a further optimised nonapeptide with blood pressure lowering properties.*

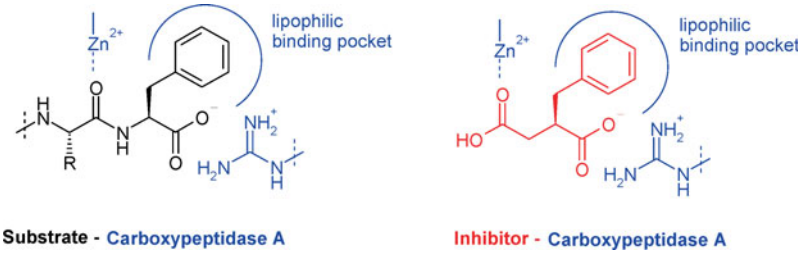
While Ferreira continued to focus on bradykinin, Sir John R. Vane (1927–2004) and Yeshwant S. Bakhle recognised that the snake venom also had an effect on the renin-angiotensin system. Miguel Ondetti (1930–2004) and David Cushman (1939–2000) at Squibb (now Bristol-Myers-Squibb) isolated and characterised six other ACE-inhibitory peptides, among them teprotide (IC_{50}: 250 nM). [7, 8]

After the development of a simple *in vitro* test system for ACE activity, more than 2,000 peptides, both natural and synthetic, were tested. The sequence Trp-Ala-Pro, contained in BPP_{5a}, proved to be optimal for binding to the active site of ACE, although it was subject to rapid proteolytic deactivation. A pentapeptide with the partial structure Phe-Ala-Pro possessed greater stability. Nevertheless, in spite of a completely different amino acid sequence, the best results in humans were achieved with teprotide. Presumably, the four proline residues in this peptide render it less sensitive towards proteolysis. Though after oral application, no activity is observed, because teprotide is due to its size not absorbed. [9] However, the intravenous administration poses for a daily used drug a serious convenience problem. In 1973, Squibb gave up further development of teprotide for lack of commercial interest.

In the same year, there appeared a paper by Larry D. Byers and Richard V. Wolfenden, in which they described that (*L*)-benzylsuccinic acid is an inhibitor of the digestive enzyme carboxypeptidase A, which splits off phenylalanine from the C-terminal end of proteins. [10] Of particular related importance was, that a few years earlier, William Lipscomb had elucidated the structure of this enzyme by X-ray analysis. It was thus possible to build a spatial model showing the interaction between the enzyme and its substrate. Cushman and Ondetti developed the idea that in the active site of the enzyme there was a pocket housing the phenylalanine residue, and that the succinyl residue was complexed by zinc. (*L*)-Benzylsuccinic acid binds in a similar mode and thereby inhibits carboxypeptidase A (Fig. 5.10).

Later it was discovered that the venom of various scorpions, like *Tityus serrulatus* and *Buthus occitanus*, as well as spiders, like the Southern or Mediterranean Black Widow (*Latrodectus mactans tredecimguttatus*), also contains bradykinin-potentiating peptides. [3]

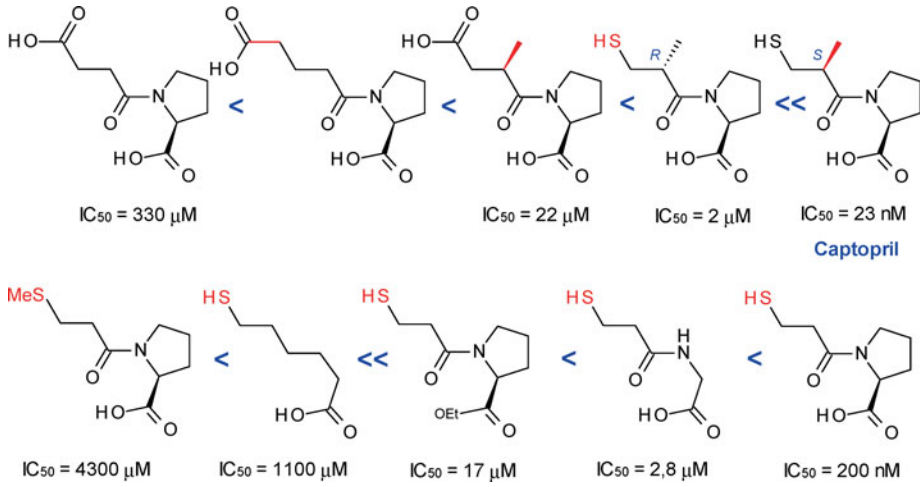
IC_{50}: the concentration of an inhibitor, by which the enzyme activity is reduced by 50 %.



5.10 *The example of Carboxypeptidase A demonstrates how novel technologies enable to study the interaction between an enzyme and its substrate or inhibitor.*

Whereas carboxypeptidase A "cuts off" only one amino acid from the C-terminal end, ACE splits off two amino acids. Consequently, Cushman and Ondetti concluded, that a succinoylated amino acid ought to be a good inhibitor for ACE. They selected proline as the proper amino acid, because it forms the C-terminus in BPP_{5a}. And indeed, *N*-succinoylproline possesses some weak activity, and served as a starting point for further structure optimisation.

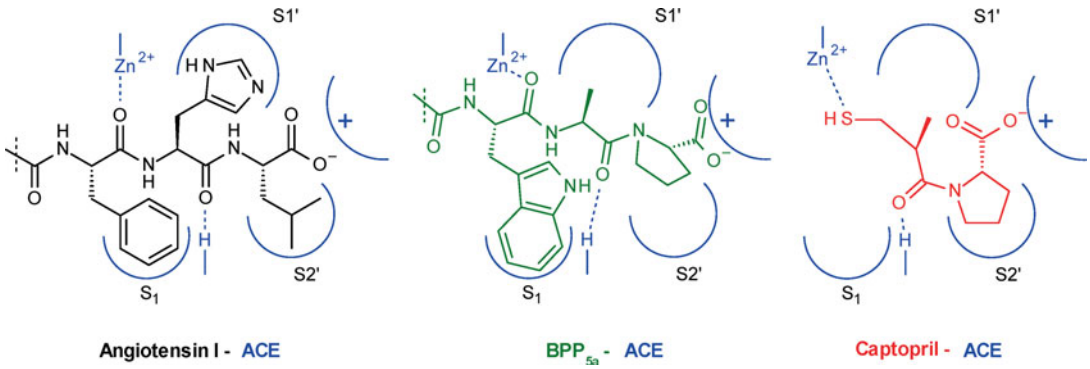
Since variation on the proline moiety did not improve the activity, the C-terminal end was retained. Glutaryl-(*L*)-proline showed a higher affinity already, which could still be further enhanced by a methylsuccinoyl residue. The decisive breakthrough came, when the carboxylic function was replaced by a thiol group, whereby the affinity rose by three orders of magnitude. Further structural variations around captopril demonstrated, that an actual optimum had been reached (Fig. 5.11). Binding to ACE requires both, a free thiol group and an acid function. The (*S*)-configured methyl group improves the affinity by a factor of around 10, compared with *N*-(3-mercaptopropionyl)-proline. [11] The affinity of (*S*,*S*)-Captopril is around 100 times higher than of its (*R*,*S*)-configured isomer. [12]



5.11 *An essential part of rational drug design is the establishing of structure-activity relationships.*

Although Cushman and Ondetti had no support from an X-ray structure analysis of ACE, they only needed 60 compounds for their structure optimisation. Nowadays, one can envisage the binding mode of angiotensin I, the pentapeptide from *Bothrops jararaca*, and of captopril as follows:

The side-chains of the amino acids bind into the lipophilic binding pockets of the enzyme. The Lewis-acidic zinc ion is complexed by the carbonyl or the thiol function respectively. A hydrogen bond to the oxygen of the penultimate carboxylic acid, and an ionic interaction of the C-terminus contribute to the binding of the substrate (Fig. 5.12).



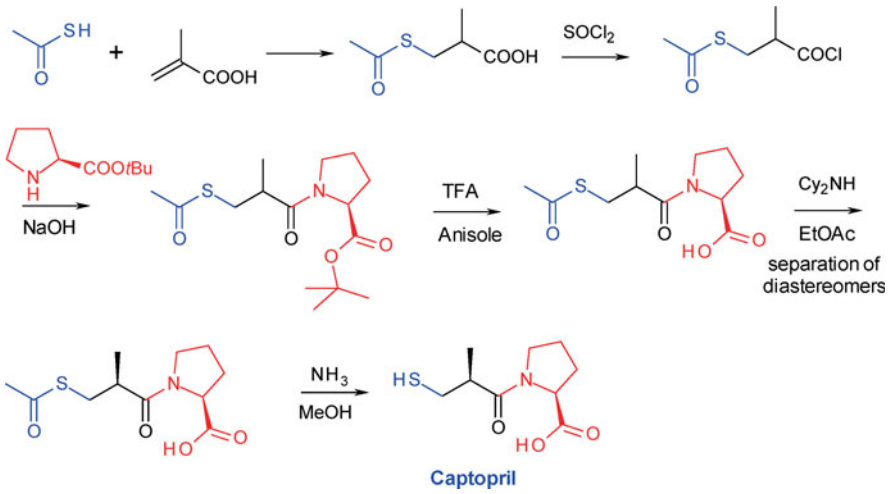
5.12 *The snake venom peptide BPP_{5a} and captopril bind at the active site of ACE in a competitive manner, thereby-replacing angiotensin I and bradykinin.*

Captopril represents the first highly specific enzyme inhibitor, which did not result from an accidental discovery, but on the basis of rational drug design. This pioneering methodology led the way to many other structure optimisations. Nowadays, all larger pharmaceutical companies take advantage of improved X-ray analytical methods and more powerful computers with faster calculating programmes in order to optimise their "lead structures".

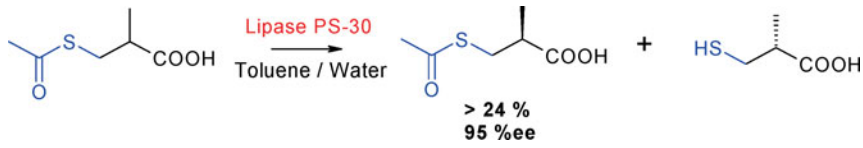
It is interesting that Nature's evolution has not yet provided the Brazilian lancehead snake with venom peptides, which contain thiol groups as higher affinity ligands for complexing zinc.

5.1.3 Syntheses

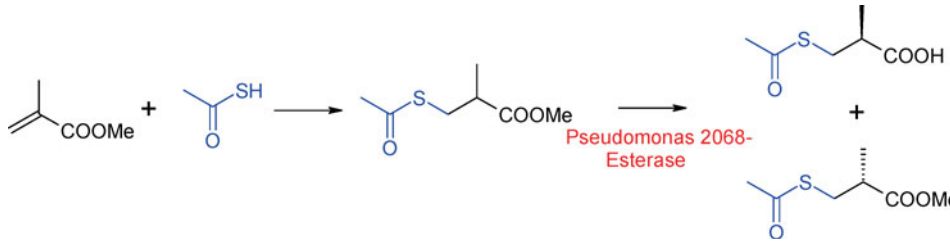
The first syntheses of captopril started from methacrylic acid, to which was added thioacetic acid. After coupling with (*S*)-proline *t*-butyl ester and hydrolysis of the ester, the diastereomers were separated *via* their dicyclohexylammonium salts. Treatment with ammonia finally generated captopril in enantiomerically pure form. [12, 13]



The synthesis may be improved by separating the enantiomers already at the 3-(acetylsulfanyl)-2-methylpropionic acid stage, using a lipase.

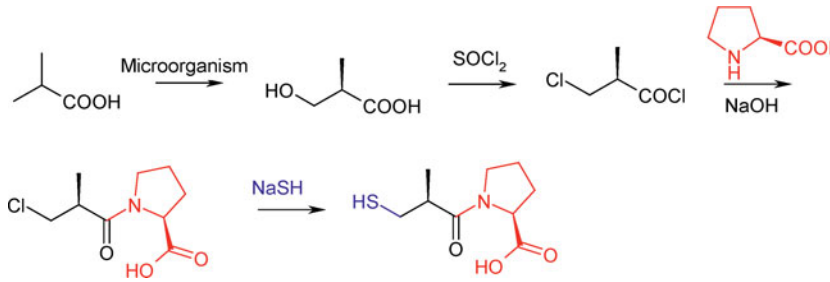


Optionally, the reaction sequence may start from methyl methacrylate, by hydrolysing the methyl ester selectively with a *Pseudomonas* esterase.



"*Meso-trick*" is a term used to describe the desymmetrisation of a prochiral- or *meso*-compound in order to obtain an enantiopure product in theoretically 100 % yield.

Especially elegant is Kaneka's enantioselective microbial hydroxylation of isobutyric acid by applying the "*meso-trick*". Suitable microorganisms are, for example, *Candida rugosa*/FO-0750, *Candida rugosa*/FO-0591 or *Candida utilis*/FO-0396. The hydroxy-group is substituted, and in the same sequence, the acid also converted into the acid chloride by reaction with thionyl chloride. The last steps are coupling with (*S*)-proline and substitution of the chlorine with sodium hydrogen sulfide.



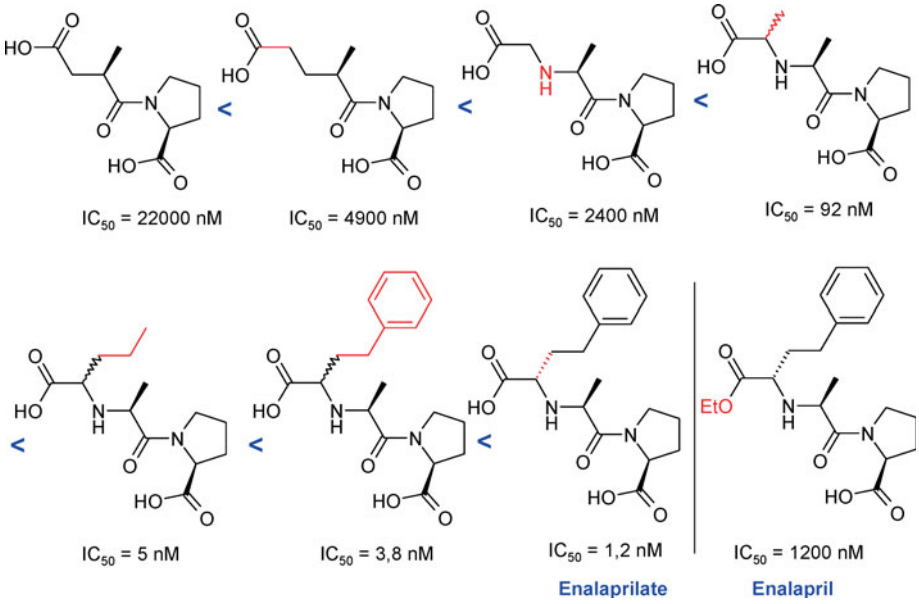
Captopril was approved in 1982 by the FDA for the treatment of high blood pressure. One year later followed the approval for treatment of heart failure, and finally in 1994, the drug received approval for treatment of kidney disease caused by *Diabetes mellitus*.

5.1.4 Second-Generation ACE Inhibitors

Since 1975, researchers at the American company Merck had been working on improvements to captopril, which is associated with a number of undesirable side-effects; these are attributed in part to the free mercapto group: haemato-

logical effects, proteinuria, skin rashes and taste alterations. Therefore, in subsequent products this part of the pharmacophore was altered back to a carboxylic acid moiety. A common characteristic of all the newer members of this drug class is their structure optimisation at the S_1-, $S_{1'}$- and $S_{2'}$-binding pockets of the ACE receptors. Typical representatives are enalapril, lisinopril and trandolapril.

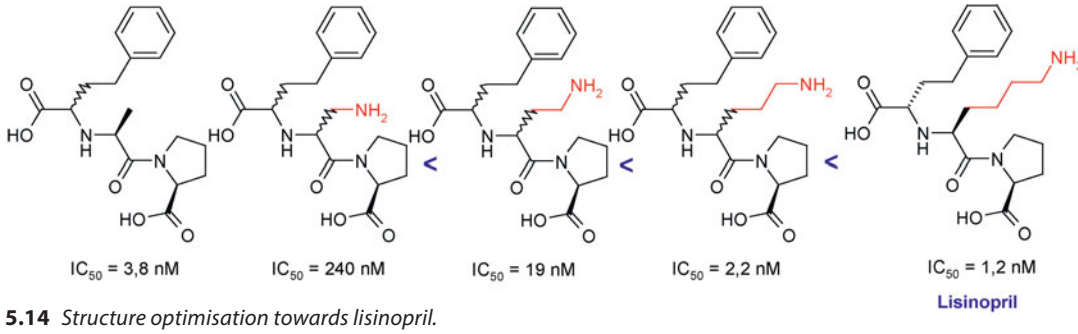
While Ondetti and Cushman used carboxypeptidase A as their model enzyme, the Merck group employed the as well zinc-containing endopeptidase thermolysin. Starting points for their development work were methylsuccinyl-proline, and the working hypothesis to generate a dipeptide structure. In fact, replacing the succinyl residue by a glutaryl group, and incorporating a nitrogen atom into the basic scaffold, led to a tenfold increase in affinity. Structural variations of the Ala-Pro motif eventually yielded enalaprilate (Fig. 5.13). Affinity studies showed, that in respect of the S_1-binding pocket, thermolysin represents the better model.



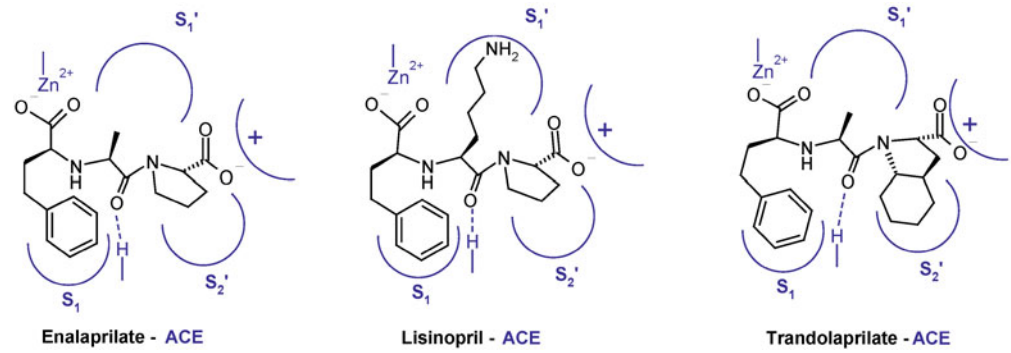
5.13 *Structure optimisation towards enalaprilate.*

However, it turned out that enalaprilate possessed a poor oral bioavailability in rats, dogs and humans. Only the development of a pro-drug, the ethyl ester, solved this problem. After enalapril is absorbed, esterases then release the active agent. Enalapril itself binds insufficiently to the enzyme. [14]

With lisinopril, the problem of poor oral bioavailability was solved by changing the alanine moiety to lysine (Fig. 5.14). Systematic investigation of the $S_{1'}$-binding pocket with enalaprilate derivatives showed, that highly potent inhibitors result from replacing alanine by an aminoalkyl-glycine. The optimum is attained with lysine (Fig. 5.15). Though, inhibitors with even longer side-chains exhibit lower affinity.



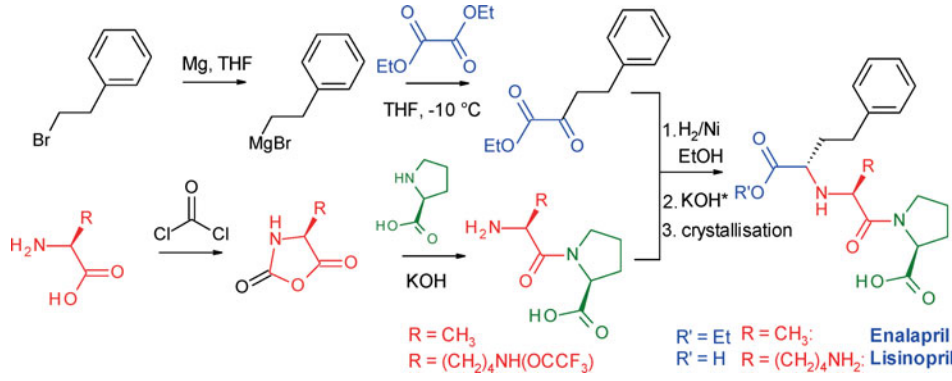
5.14 *Structure optimisation towards lisinopril.*



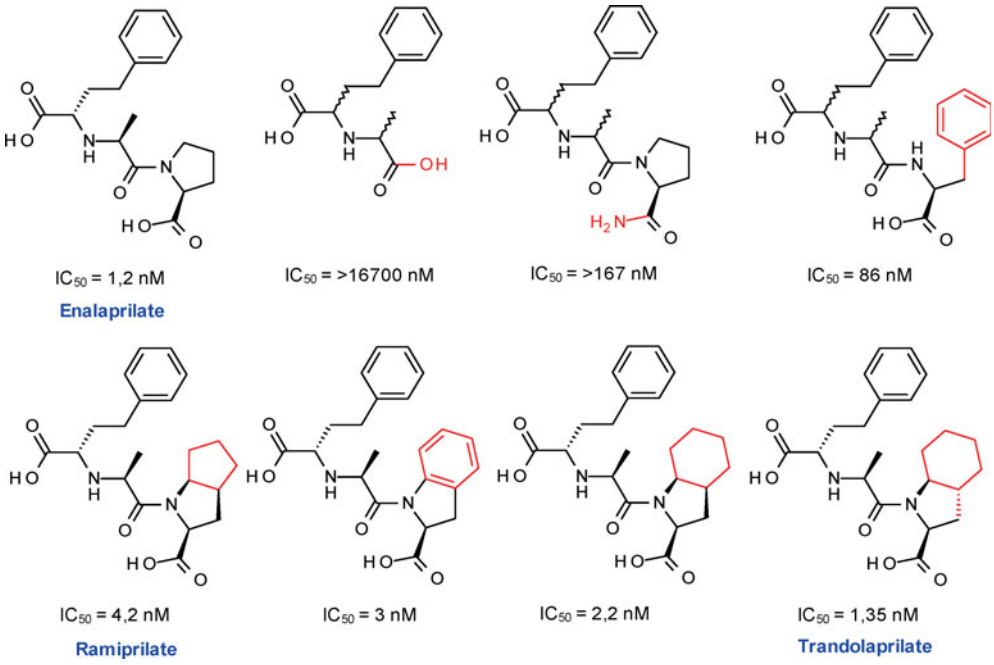
5.15 *Enalaprilate, lisinopril and trandolapril in the active site of ACE.*

Whereas lisinopril is more slowly and to a lesser extent absorbed than enalapril, it offers the advantage that no metabolic activation is required. [14]

The convergent synthesis of these compounds is rather simple. On the one side, the required α-keto ester may be synthesised from diethyl oxalate by a Grignard reaction. On the other side, alanine or trifluoroacetyllysine is coupled with proline *via* the oxazolidine-2,5-dione (the so-called "Leuchs' anhydride"). The two "halves" are combined by reductive amination. The diastereoselectivity of the hydrogenation is dependent on the choice of catalyst. With Raney nickel, the diastereoselectivity lies in case of enalapril at 87 : 13 and for lisinopril at 95 : 5 in favour of the desired diastereomers. In the case of lisinopril, there follows a final hydrolysis of the ester and amide function. The active material is ultimately purified by crystallisation.

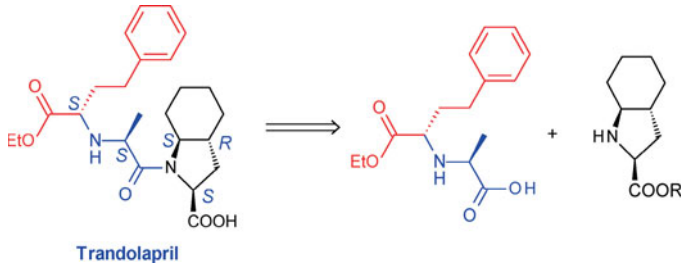


The importance of proline as the C-terminal amino acid in ACE inhibitors is best seen, if this building block is removed from enalaprilate: that leads to a complete loss of activity. If the terminal carboxylic acid function is converted into the corresponding amide, or if proline is replaced by phenylalanine, this results in relatively poor affinity as well. However, good activity is found with bicyclic proline derivatives, which are optimised for their interactions in the S_2'-pocket (Fig. 5.15). [14, 15] Many of the active compounds following on from enalapril possess this structural motif, *e.g.* ramiprilate (Hoechst) and trandolaprilate (Roussel) (Fig. 5.16). Like the majority of ACE inhibitors approved up to 2003, these compounds are administered as pro-drugs (ethyl esters).

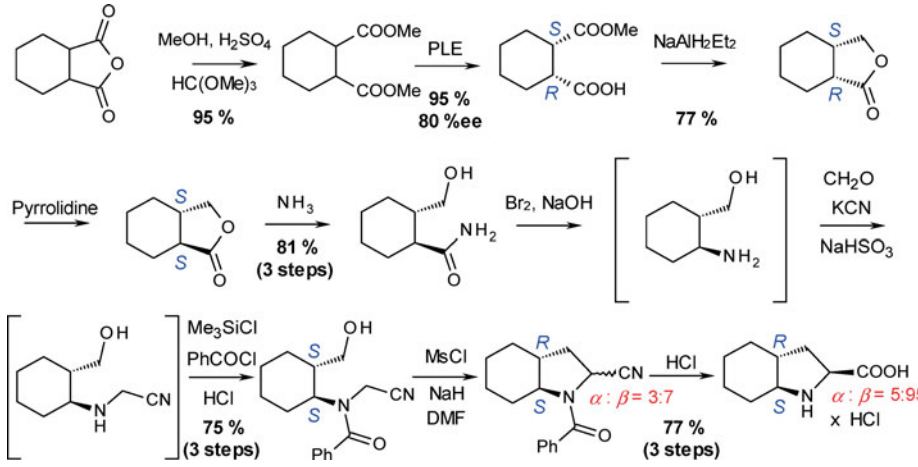


5.16 *Structure optimisation for trandolaprilate.*

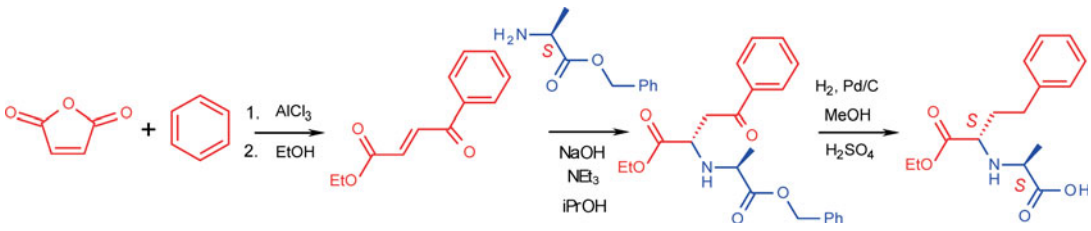
For the synthesis of trandolapril, Roussel Uclaf developed a convergent route, based on the retrosynthetic disconnection between the octahydroindolecarboxylic acid and the remainder of the molecule. The synthetic challenge lies predominantly in the indole component. Firstly, there is the question of a *trans*-fused bicyclic system, which is not simply accessible by hydrogenation, and secondly, the carboxylic function at C-2 tends to promote epimerisation.



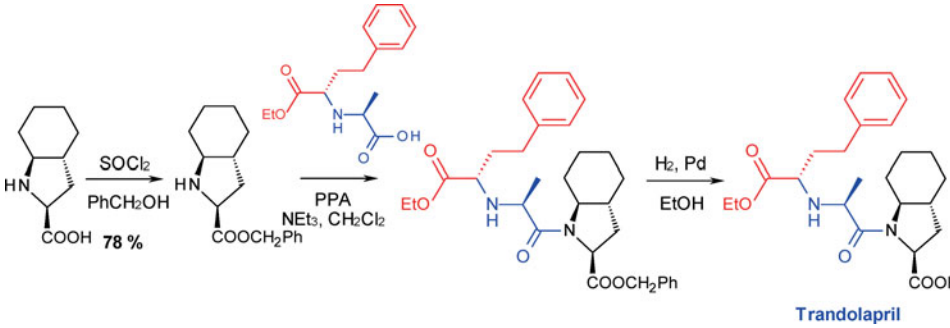
The enantiomerically pure starting material for octahydroindolecarboxylic acid is obtained by enzymatic resolution of methyl *cis*-cyclohexanedicarboxylate with pig liver esterase (PLE). The chemoselective reduction with sodium diethylaluminium hydride leads to a *cis*-lactone, which can be epimerised into the *trans*-lactone with pyrrolidine. Ammonolysis gives an amide, which is subjected to a Hofmann rearrangement ("Hofmann degradation"). This is followed, without work-up, by a Strecker reaction with formaldehyde, and the first product isolated is the *N*-benzoylated amino-nitrile (the alcohol function had also been temporarily protected). After mesylation of the hydroxy-group, the ring is closed by treatment with sodium hydride in DMF. By acid hydrolysis and crystallisation, the almost epimerically pure carboxylic acid is finally obtained. [16]



The starting material for the second building block is benzene, which is subjected to Friedel-Crafts acylation with maleic anhydride. The stereoselective addition of alanine benzyl ester to the α,β-unsaturated ketone, hydrogenation of the keto-group and reductive cleavage of the benzyl protecting group leads to the second building block. [17]



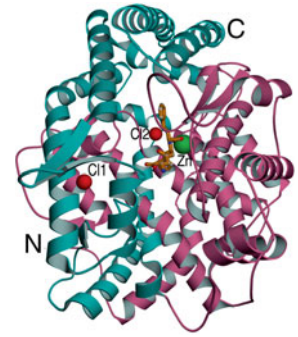
The concluding sequence involves an amino acid coupling with propanephosphonic anhydride (PPA) and also the reductive removal of the benzyl protecting group. The API is obtained in pharmaceutical quality by recrystallisation.



5.1.5 X-ray Structure Analysis of ACE

For the first time, it was possible in 2003 to determine the structure of human testicular ACE by means of high-resolution X-ray analysis (Fig. 5.17). [18, 19] There are two isoforms of ACE: the somatic form, a glycoprotein with a chain of 1,277 amino acids, and the ACE of germ cells; this is smaller and comprises 701 amino acids. The somatic ACE contains two homologous domains, the N- and C-domains, the latter of which being identical with the testicular ACE. This domain is responsible for the regulation of blood pressure.

It is noteworthy that there is only a slight similarity between this structure and carboxypeptidase A, the original enzyme, on which the rational drug design of ACE inhibitors was based. Instead, there are found structural similarities to neurolysin and *Pyrococcus furiosus* carboxypeptidases, which in turn show practically no similarities at the level of the amino acid sequence. Obviously, Nature is able to construct three-dimensionally similar structures with entirely different amino acid sequences. This has far-reaching consequences for drug research, because the similarity of receptors cannot be deduced from the amino acid sequence, which is comparatively easy to obtain, but is only revealed by the X-ray analysis based structure of the protein. If this is known, then it provides good prerequisites for the development and optimisation of new drugs.



5.17 *The ACE-lisinopril complex. The structure consists of two sub-domains (shown in turquoise and violet); it contains two chloride ions (red), and in the catalytic centre the zinc ion (green) and the inhibitor lisinopril (brown).*

5.1.6 Economic Relevance

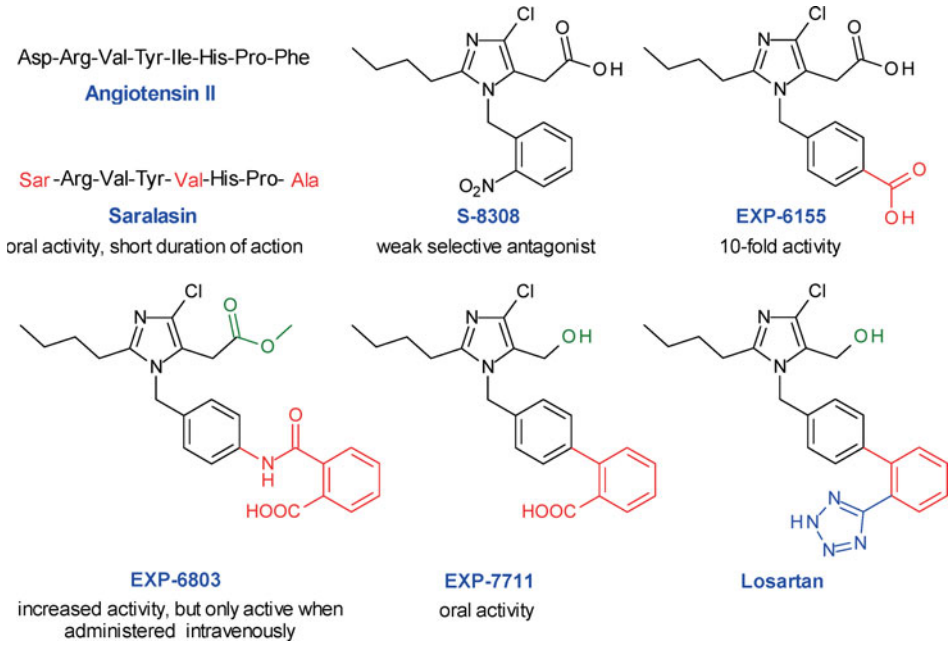
ACE inhibitors are nowadays among the pharmaceuticals with the highest turnover. In Germany, 20 % of the population suffer from high blood pressure. Around one-third of them take ACE inhibitors as a monotherapy. More than half treat this disease with a combination of an ACE inhibitor and a second drug. In the German market, which is dominated by generic pharmaceuticals, enalapril is the most important drug at present.

5.1.7 Recent Developments

Sartans

At the same time as Squibb and Merck developed the first ACE inhibitors, medicinal chemists at DuPont and Takeda focused on angiotensin II receptor

blockers (Fig. 5.18). [20] Initially, angiotensin II analogues like, for example, Saralasin (**sar**cosine-**ala**nine-angioten**sin**), were synthesised. They were potent inhibitors in-vitro, but lack of oral bioavailablity was a major issue. In the early 1980s, Takeda discovered 1-benzylimidazole-5-acetic acids, such as S-8308, with moderate potency, short duration of action and limited oral bioavailability. Nevertheless, members of this compound class were selective and competitive AT_1 receptor antagonists, and served as lead structures for further optimisation. The group at DuPont put forward the hypothesis that angiotensin II and Takeda's compounds interact at the same binding site. They used NMR studies and computer modelling for their design of new structures, based on a comparison of angiotensin II and S-8308. Introducing a carboxylic acid moiety, as in EXP-6155, leads to a 10-fold higher binding affinity. Replacing the carboxyl group by a more bulky 2-carboxy-benzamido-moiety, as in EXP-6803, enhances binding even further; though, this leads to complete loss of oral availability. In EXP-7711, the introduction of a lipophilic biphenyl unit brought back good oral activity, but at the expense of slightly lower affinity to the AT_1 receptor. Finally, exchanging the carboxylic acid function by tetrazole, a more lipophilic but still acidic bioisoster, increases the bioavailability and duration of action. This compound, named losartan, became the first successful angiotensin II receptor antagonist approved by the FDA in 1995 and marketed by MSD. Since then, several other sartans have been launched, such as valsartan (Novartis), irbesartan (Sanofi), candesartan cilexetil (Takeda), telmisartan (Boehringer Ingelheim) and olmesartan medoxomil (Sankyo).



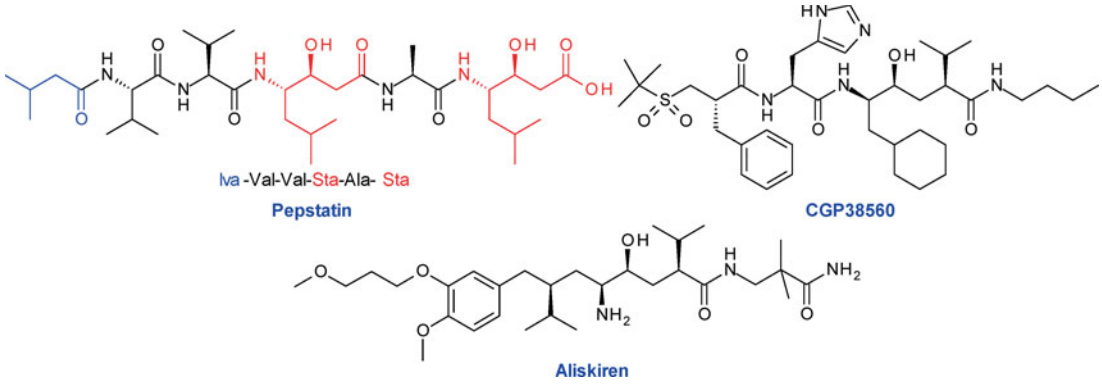
5.18 *Angiotensin II receptor blockers: sartans.*

Renin Inhibitors

An alternative option for the treatment of hypertension was pursued with direct renin inhibitors. [21] Monoclonal antibodies, directed against renin, had shown blood pressure lowering properties in animal models. However, due to their immunogenicity and mandatory parenteral administration, they proved not suitable for long term treatment.

The first low-molecular weight renin inhibitors were aspartyl protease inhibitors from *Actinomycetes*, such as pepstatin from *Streptomyces testaceus*. Pepstatin, originally discovered as a picomolar inhibitor of pepsin, is a hexa-peptide containing the unusual amino acid statine (**Sta**, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid). [22] Pepstatin is a transition-state inhibitor, suffering the same major drawback as antibodies, namely a lack of oral bioavailability.

The synthesis of less peptide-like analogues at Ciba-Geigy led to CGP38560. Markus Grütter and John Priestle succeeded in solving the co-crystal structure of CGP38560 bound to renin. This was a major breakthrough, since it opened the possibility to optimise ligands at each of the binding pockets in the protease by the use of molecular modelling techniques. [23] The result of this optimisation process was aliskiren, a completely non-peptidic and orally active transition-state renin inhibitor of low-molecular weight, which was launched by Novartis in 2007 (Fig. 5.19). [24]



5.19 *Aliskiren (Rasilez®, Tekturna®) is the first direct renin inhibitor, which was brought on to the market by Novartis in 2007 for the treatment of essential hypertension.*

Summary in Bullet Points

- In understanding the physiological role of ACE and bradykinin, it became possible to develop new and efficient drugs for the treatment of high blood pressure.
- Captopril was the first oral ACE inhibitor on the market. The active compound was developed with the help of rational drug design techniques.
- All other follow-on drugs share a common pattern, in being variants of the lead motif Phe-Ala-Pro.

5.2 β-Lactam Antibiotics

5.2.1 Introduction

5.20 *The Third Man is the story of the end of a youthful friendship, of unscrupulous machinations, and the abysses of the human soul. It is a work of fiction, but one which is based on actual events.*

After the Second World War, penicillin was a very scarce drug, which could be prepared only in quantities insufficient to meet the demand, and was therefore a much sought-after item on the black market.

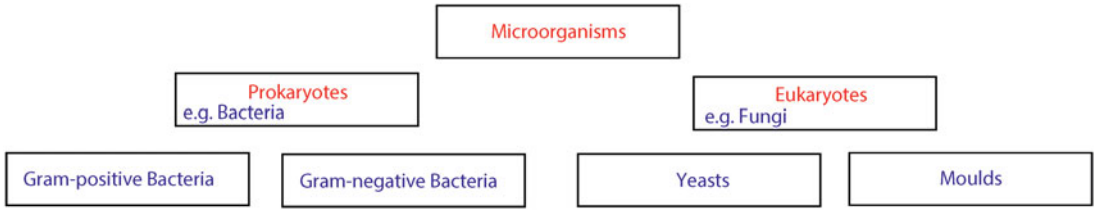
Graham Greene has described this scenario in his novel *The Third Man,* with the character of Harry Lime as one of the profiteering masterminds in post-war Vienna (Fig. 5.20).

"At that time, penicillin was supplied in Austria only to the military hospitals; no civilian doctor, not even a civilian hospital, could obtain it by legal means… Penicillin would be stolen by medical orderlies and sold to Austrian doctors for very high sums… When… they wanted more money and quicker money, they began to dilute the penicillin with coloured water, and, in the case of penicillin dust, with sand… men have lost their arms and legs that way – and their lives. But perhaps what horrified me most was visiting the children's hospital here. They had bought some of this penicillin for use against meningitis. A number of children simply died, and a number went off their heads. You can see them now in the mental ward." [25] (Graham Greene, *The Third Man*)

Nowadays, we have a wide range of antibiotics at our disposal. In general, these may be grouped into the following classes of compounds: amino-glycosides, β-lactams, chloramphenicol (first isolated from *Streptomyces venezuelae*), glycopeptides (*e.g.* vancomycin), lincomycin, macrolides, polyethers and tetracyclines.

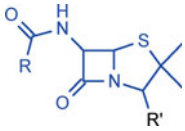
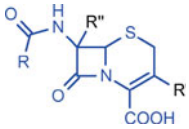
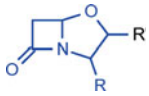
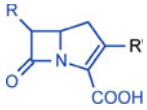
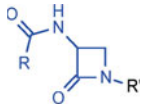
5.2.2 Classification

Both, eukaryotic and prokaryotic microorganisms are able to produce β-lactam antibiotics (Fig. 5.21).They may be divided into five classes corresponding to their core structures (Tab. 5.2). [26] Tab. 5.3 lists some of the most important intermediates and active compounds from the penicillin and cephalosporin series.

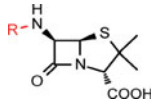
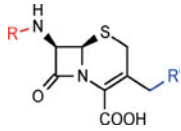


5.21 *Eukaryotes, in contrast to prokaryotes, possess a cell nucleus and organelles, e.g. mitochondria or microbodies. Bacteria are prokaryotes, the Gram-staining characteristics of which provide information about the construction of the bacterial cell wall. Fungi are eukaryotes, which grow as single cells and are then termed yeasts, or moulds, which exhibit a filamentous structure (hyphae).*

Tab. 5.2 *Fungi and bacteria as sources of β-lactam antibiotics*

Structure	β-Lactam	Fungus	Gram (+) bacteria	Gram (–) bacteria
	Penam	*Aspergillus* *Acremonium* *Penicillium* *Epidermomyces* *Trichophyton* *Polypaecilum* *Malbranchea*		
	Cephem (core structure without double bond = cepham)	*Acremonium* *Anixiopsis* *Arachnomyces* *Spiroidium* *Scopulariopsis* *Diheterospora*	*Streptomyces* *Nocardia*	*Flavobacterium* *Xanthomonas* *Lysobacter*
	Clavam		*Streptomyces*	
	Carbapenem		*Streptomyces*	*Seratia* *Erwinia*
	Monolactam		*Nocardia*	*Pseudomonas* *Gluconobacter* *Chromobacter* *Agrobacter* *Acetobacter* *Flexibacter*

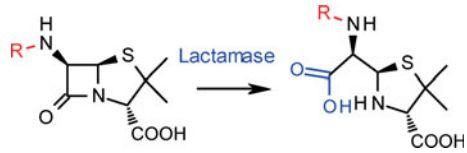
Tab. 5.3 *Fermentation products, intermediates and active compounds in the β-lactam series*

Penicillins			
Fermentation products	$PhCH_2C(O)$		Penicillin G
	$PhOCH_2C(O)$		Penicillin V
Intermediate	H		6-Aminopenicillanic acid
Antibiotics	(*D*)-4-HO-$C_6H_4CH(NH_2)C(O)$		Amoxicillin
	(*D*)-$PhCH(NH_2)C(O)$		Ampicillin
Cephalosporins			
Fermentation products	$HO_2CCH(NH_2)(CH_2)_3C(O)$	OAc	Cephalosporin C
	$HO_2C(CH_2)_4C(O)$	H	Adipoyl-7-aminodesacetoxycephalosporanic acid
Intermediates	H	OAc	7-Aminocephalosporanic acid
	H	H	7-Aminodesacetoxycephalosporanic acid
Antibiotics	(*D*)-4-HO-$C_6H_4CH(NH_2)C(O)$	H	Cefadroxil
	(*D*)-$PhCH(NH_2)C(O)$	H	Cephalexin

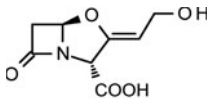
5.2.3 Mode of Action

5.22 *The Danish bacteriologist Christian Gram (1853–1938) gained international reputation by discovering in 1884 a technique of staining the heat-fixed smear of a bacterial culture with crystal violet and iodine.*

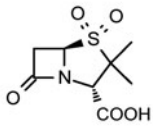
The cell wall of Gram-positive and Gram-negative bacteria (Fig. 5.22) consists of covalently linked sugar and peptide moieties (peptidoglycans), which are called murein (lat. *murus*: wall). The fraction of murein in the cell wall amounts to *ca.* 50 % in Gram-positive bacteria, and 5–10 % in Gram-negative germs. [27] To sustain growth, bacteria must be able to build up and break down their cell walls. Penicillin inhibits the enzymes involved, what ultimately causes the bacteria to die off. Since penicillin does not have high affinity to the enzymes of the human organism, its toxicity is low. Bacteria can develop resistance, by producing a lactamase, which cleaves the β-lactam ring. However, by modification of the side-chain R, the resistance can be overcome. [28, 29]



The resistance problem may be circumvented, if the antibiotic is administered along with a β-lactamase inhibitor. Clavulanic acid (produced from *Streptomycetes*) and Sulbactam themselves are only weak antibiotics, but inhibit the β-lactam ring-cleaving enzymes very efficiently.



Clavulanic acid



Sulbactam

β-Lactam antibiotics may also trigger allergic reactions. This is caused as well by cleavage of the lactam ring and an irreversible reaction with the body's own proteins. Thus, complete antigens (haptens) are formed, which are recognized by the immune system as foreign, initiating the defence mechanism of the host. This can lead to sensitisation and allergic reactions. Depending on the individual disposition, the route of application and the type of penicillin used, the spectrum of allergic reactions ranges from mild skin rashes to anaphylactic shock with fatal outcome.

5.2.4 The Discovery of Penicillin

The application of antibiotics for the treatment of diseases is not new. [30] In Chinese folk medicine, the healing properties of curd cheese with mouldy beans had been known for at least 2,500 years. From records we know that the Romans and Egyptians used mouldy bread for the treatment of diseases. The peoples of Sudan and Nubia also used antibiotics from a very early date on. Fluorescence spectra of bones from those times show strong agreement with the spectra from bones of patients, who have been treated with tetracyclines.

In 1877, Louis Pasteur discovered that bacteria were able to inhibit the growth of other germs. For this phenomenon, the French Army doctor Jean Antoine Villemin (1828–1892) coined the term "Antibios".

Already in1889, the German bacteriologist Rudolf Emmerich (1852–1914) and the German chemist Oscar Löw (1844–1941) had isolated a bactericidal

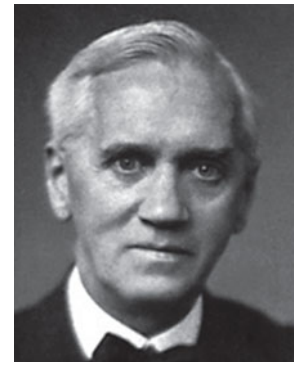
liquid from a culture of *Bacterium pyocyaneus* (*Pseudomonas pyocyanea*), which they called "pyocyanase" and used to treat infectious diseases. In1906, the Sächsisches Serumwerk Dresden (Saxony Serum Factory, now part of GlaxoSmith-Kline) marketed "neo-pyocyanase" as a pharmaceutical.

In 1897, the young French physician Ernest Duchesne (1874–1912) described in his PhD thesis that certain moulds kill bacteria (Fig. 5.23). At *l'Ecole du Service de Santé Militaire de Lyon* (the Military Health Service School of Lyon), he had observed that the Arab stable boys kept their saddles in a dark and damp room. They told Duchesne that the mould growing on the leather helped to heal the saddle sores on the horses. Encouraged by this observation, he successfully tested these moulds in ill guinea pigs. Later, he was also able to show that *Penicillium glaucum* is effective against typhoid (caused by *Salmonella typhi*). Unfortunately, Ernest Duchesne was completely unknown at that time, so that the *Institut Pasteur* did not even acknowledge the receipt of his dissertation, and all his achievements remained unrecognised for several decades.

5.23 *Ernest Duchesne (1874–1912) contracted a serious chest disease (probably tuberculosis) in 1904, from which he died at the age of 37.*

As a result, one of mankind's greatest discoveries, which has saved up to the present the lives of more than 100 million people, occurred only by chance not until 1928 – through the carelessness of the Scottish pharmacologist Alexander Fleming (Fig. 5.24). A fungal spore had contaminated an unattended and almost discarded culture of *Staphylococcus aureus* in a Petri dish, and had created a zone of inhibition to bacterial growth.

The real question as to the source of the fungal spore was never answered in the story of penicillin. The Royal Society of Chemistry is of the opinion that it was from a cup of coffee residues, which Fleming had left for several weeks in his laboratory. Caffeine acts as an antimycotic, so that only selected species could thrive in this biotope, those, which spread after a longer period through airborne spores. [31] Fleming identified the fungus as *Penicillium notatum* and named the substance, which killed the *staphylococci*, "penicillin". He recognised that even at a hundred-fold dilution this material was effective against *pneumococci* and *streptococci*, the cause of serious diseases. [32]



5.24 *Sir Alexander Fleming (1881–1955) received the 1945 Nobel Prize for Medicine along with Sir Howard Walter Florey (1898–1968) and Sir Ernst Boris Chain (1906–1979).*

Fleming could show that penicillin was non-toxic in animal experiments and not damaging towards human cells. Initially, it was impossible to isolate the active compound in pure form and to determine its structure. Up to the outbreak of the Second World War, no significant progress was made, despite considerable effort.

Only towards the end of the 1930s, the Australian-born pathologist Howard W. Florey and the German-Jewish chemist Ernst Boris Chain in Oxford purified penicillin from fungal cultures. They prepared the compound in sufficient quantities to treat for the first time humans. During the war, the experimental conditions were conceivably bad. In order to obtain penicillin, use was made of urine bottles, bedpans and biscuit tins (Fig. 5.25). The compound was so scarce, that the urine of already-treated patients was worked up in order to recover and recycle the excreted penicillin.

Florey sought collaboration with American laboratories to be able to produce penicillin on a larger scale. He was lucky with the Northern Regional Research Laboratory of the U.S. Department of Agriculture in Peoria, Illinois. It was shown, that the yield rose by a factor of ten if the fermentation broth of

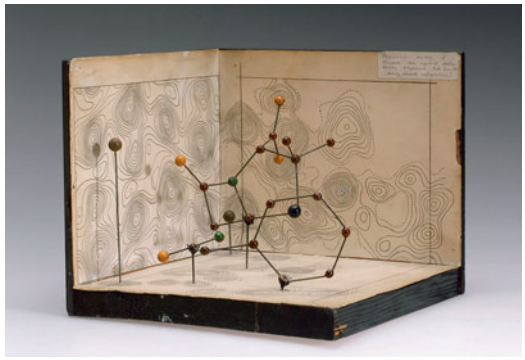
The first patient to be treated with penicillin, on February 12, 1941, was a policeman aged 43 with a staphylococcal infection on the upper lip. Tragically the patient died shortly afterwards from sepsis, because no more of the drug was available. [30]

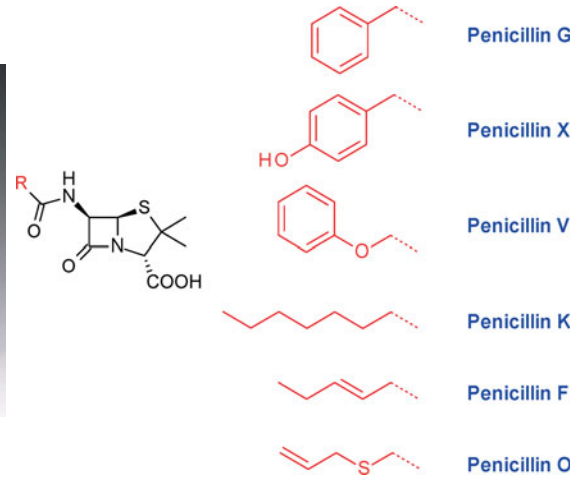
5.25 *After the Second World War, penicillin was prepared batch-wise in flat glass bottles, which were stacked up in large racks.*

Penicillium notatum was mixed with a by-product of maize processing, the so-called "corn steep liquor". In the search for even more productive strains, hundreds of moulds were tested. In 1943, Mary Hunt (nicknamed "Mouldy Mary"), a lab technician from the Northern Regional Research Laboratory, discovered the best mould on a cantaloupe (a type of melon) in the weekly market. With this fungus, the overall yield of penicillin increased around twenty-fold. By the end of 1944, it was possible to produce enough penicillin to treat 500,000 people (Fig. 5.26).

In spite of the greatest efforts on the part of the USA and Britain, the aim of developing a viable chemical synthesis was still not reached before the end of the war. Only in 1945 (published in 1949), Dorothy Crowfoot Hodgkin (1910–1994) in Oxford succeeded in determining the structure of penicillin G by using X-ray structure analysis. [33] Over the years it was discovered, that Nature produces a whole range of discrete penicillins, which differ merely in the side-chain. Beyond that, a multitude of synthetic penicillins arose also from industrial laboratories (Fig. 5.27).

5.26 *Nowadays, a whole range of high-performance bacterial strains is known. One of the most important is Penicillium chrysogenum.*

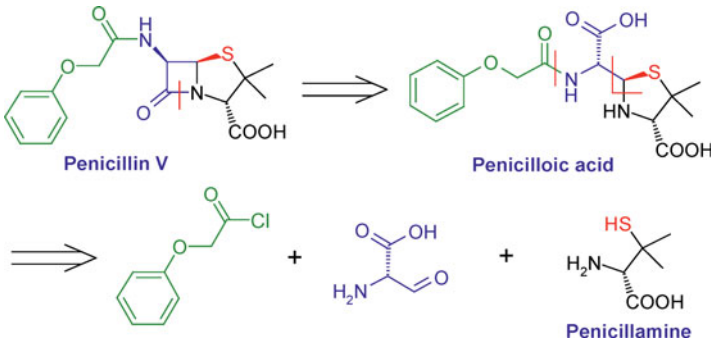




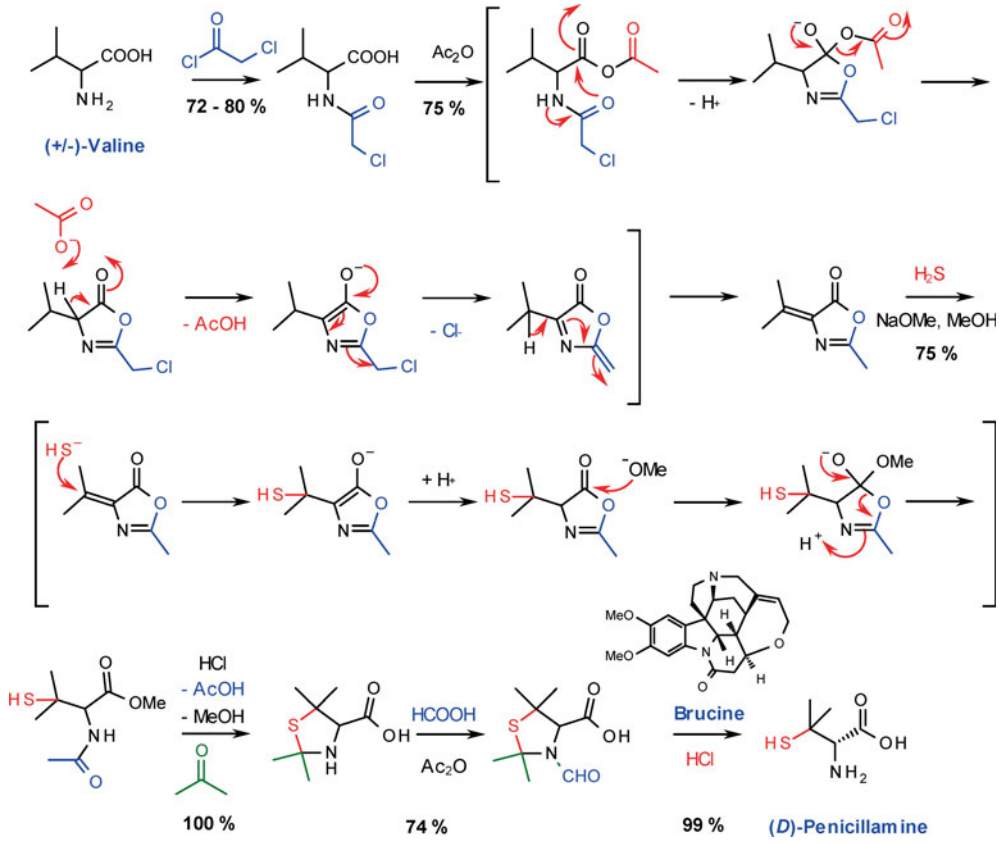
5.27 *X-ray structure of penicillin G by Dorothy Crowfoot Hodgkin. Through variation of the side-chain, many hundreds of synthetic penicillins now exist.*

5.2.5 First Total Synthesis

The challenges to chemical synthesis are associated with the high reactivity of the β-lactam system. R. B. Woodward recognised that in contrast to open-chain-amides, the β-lactam lacks resonance stabilisation due to the ring strain in the bicyclic framework, which imparts to this system the reactivity of an acid chloride. It was not before 1957 that John Clark Sheehan (1915–1992) at the Massachusetts Institute of Technology achieved the first total synthesis of Penicillin V. He compared this synthesis to the task of exchanging the main spring of a wristwatch with the tools of a blacksmith. [33] Sheehan's retrosynthetic analysis of the penicillin synthesis shows, that in acknowledgement of the high reactivity of the β-lactam ring, he had deliberately chosen the four-membered ring cyclisation as the last step of the overall synthesis. The synthesis concentrates in principle on the preparation of penicilloic acid, which is constructed from the building blocks phenoxyacetyl chloride, an aminomalonaldehyde monoester and β-mercaptovaline. The latter is also called penicillamine, and its preparation is the most elaborate part.

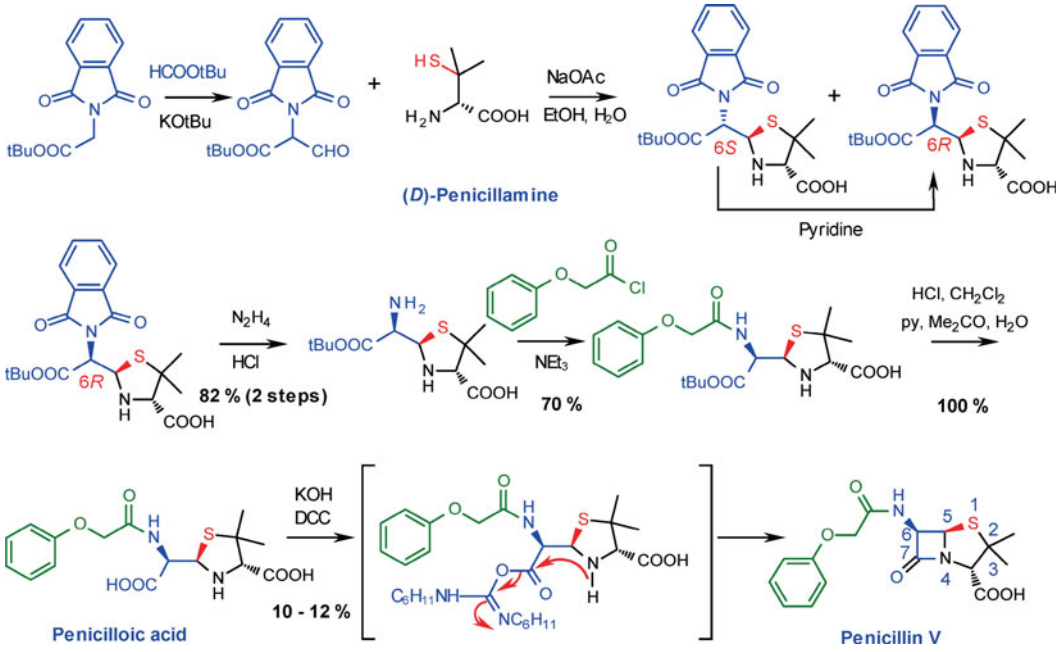


Sheehan used racemic valine as the starting material. In the first step, this is *N*-acylated with chloroacetyl chloride. Reaction with acetic anhydride gives, after isomerisation, an isopropylidene oxazolinone (isopropylidene azlactone), which is a good Michael acceptor for hydrogen sulfide. Attack by sodium methoxide cleaves the oxazolinone, and results in an *N*- and *O*-protected penicillamine. Both protecting groups can be removed by acid hydrolysis, and the amino- and mercapto-groups protected as the thiazolidine by reaction with acetone. Treatment with formic acid and acetic anhydride gives racemic *N*-formylisopropylidene penicillamine, which is resolved into its enantiomers with brucine. The protecting groups are then removed by hydrochloric acid, and enantiomerically pure (*D*)-penicillamine results.

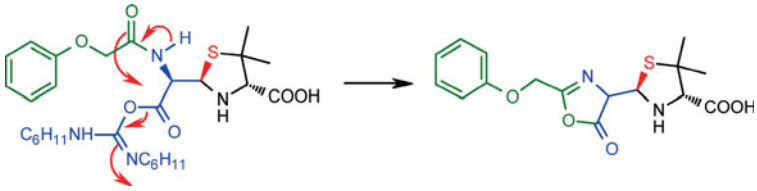


t-Butyl phthalimidoacetate is accessible from glycine and may be converted with *t*-butylformate and potassium *t*-butoxide into phthalimidomalonaldehyde *t*-butyl ester. Condensation of the racemic aldehyde with enantiomerically pure (*D*)-penicillamine leads to two new diastereomers, rather than the theoretically expected four, because of a highly selective stereo-directing effect of the carboxyl function. The second stereogenic centre in the thiazolidine ring has the (*R*)-configuration, so that ultimately only two diastereomers, epimers at C-6, are found experimentally in a ratio of ca. 1 : 1.

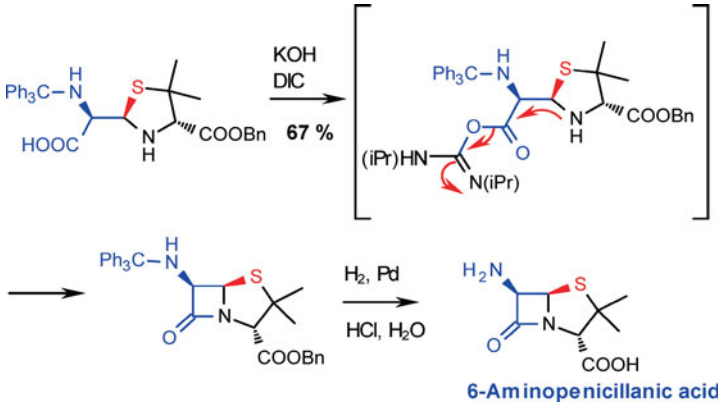
Interestingly, the unwanted (6*S*)-diastereomer is epimerised by heating in pyridine, and the (6*R*)-diastereomer crystallises out selectively upon cooling, what improves the yield considerably. In the following step, the phthaloyl residue is cleaved by hydrazinolysis; the *t*-butyl group survives the weakly acidic work-up. Finally, the phenoxyacetyl group is introduced. Cleavage of the *t*-butyl esters with Brønsted acids in anhydrous media, now a common method for deprotection, was developed by Sheehan at the beginning of the penicillin synthesis programme. The penicilloic acid is recrystallised from a pyridine/acetone/water mixture. Sheehan is also the originator of an amide forming method with DCC under dehydration in presence of water-containing solvents. The application of this protocol for the lactamisation of penicilloic acid produces penicillin V in yields of 10–12 %.



One reason for the poor yield in the last step is a side-reaction caused by the side-chain. By attack of the carboxyamide function of the phenoxyacetyl residue on the carboxy centre, which is activated as the isourea, an azlactone is formed.



In subsequent studies, Sheehan could show that in case of the synthesis of 6-amino-penicillanic acid with carbodiimides and a trityl protecting group, lactamisation works much better, because the formation of an azlactone is excluded by steric hindrance.



5.2.6 The Discovery of Cephalosporins

At the beginning of the 1950s, resistance towards penicillin appeared for the first time on a larger scale. In particular, *Staphylococci* were more prone to develop resistance. Their penicillinases caused deactivation of many penam antibiotics. Cepham antibiotics with modified basic skeletons and altered side-chains offer an interesting starting point to address this issue. [26] Although a solution to this problem had already been discovered, it required several more years before effective remedies could be provided.

In 1948, Giuseppe Brotzu (1895–1976), a bacteriologist from Sardinia, had isolated an antibiotic from the fungus *Cephalosporium acremonium* (also called *Acremonium chrysogenum*), which he found at a site, where the sewage waters from the town of Cagliari were discharged into the Mediterranean Sea (Fig. 5.28). The objective of his investigations was to determine the mechanisms of self-purification of sewage. Since the extracts of liquid cultures with *Cephalosporium acremonium* exhibit strong antibiotic activity, Brotzu, while bypassing animal experiments, concentrated and injected these locally and systemically with significant success to patients, who were suffering from infections, *e.g.* typhoid fever.

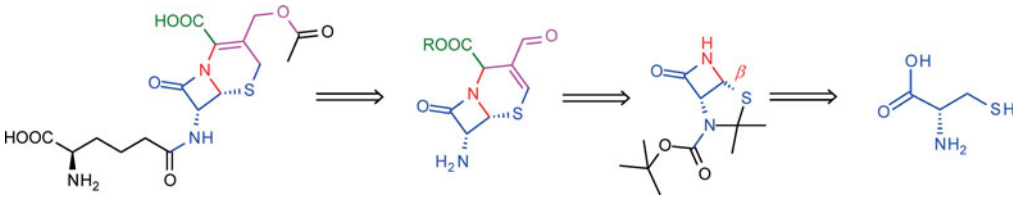
5.28 *At that time, many young people were used to swimming in the polluted waters, but against all expectations they did not became ill. Giuseppe Brotzu managed to isolate an antimicrobial agent Cephalosporium acremonium from the sewage fluids and was able to demonstrate the inhibitory effect on Gram-negative bacteria, such as Salmonella typhi, Vibrio cholerae and Brucella melitensis.*

5.29 *The structure of cephalosporin C was also confirmed by a simultaneous publication of the X-ray crystal analysis by Dorothy Crowfoot Hodgkin (1910–1994). [35]*

Brotzu didn't win much interest in his discovery from pharmaceutical firms, and so he published his findings in the Italian-language journal *Lavori dell'Instituto di Igiene di Cagliari* ("Papers from the Hygiene Institute of Cagliari"). It was the only issue of this journal, and it was only *via* Blyth Brooke, a medical officer of the allied forces in Sardinia, that Brotzu's results and a sample of his preparation reached Sir Edward Penley Abraham at the Oxford School of Pathology. [34] Abraham succeeded in growing a larger scale culture of the fungus, in isolating and purifying the extract components, and finally in determining the structure of its most active ingredient in 1961 (Fig. 5.29).

5.2.7 First Total Synthesis

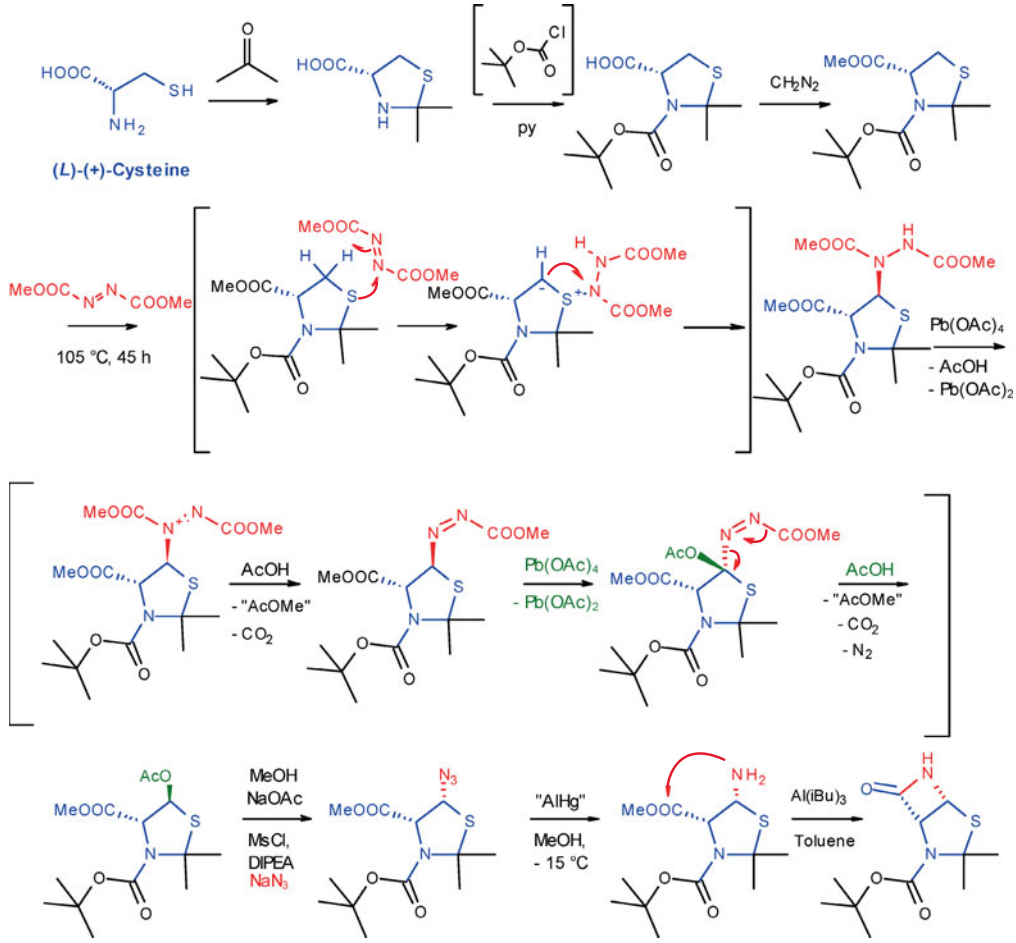
The first total synthesis came from R. B. Woodward (Fig. 5.30). [36] The basic idea of this synthesis was to start from (*L*)-(+)-cysteine, in order to produce directly the optically pure cephalosporin C. By the introduction of a Boc-protecting group and reaction with acetone, an attempt was made to reduce the number of inherent reaction sites of cysteine. The formation of the comparatively rigid thiazolidine ring offered hope, to introduce an amino-group at the *β*-position in the most stereoselective manner. Ring closure, giving the *β*-lactam, should then be conformationally favoured. The nitrogen in *β*-lactams is sufficiently activated to react under mild conditions with strong electrophiles, like alkenes with several acceptor substituents. Skilful choice of these substituents should, after cyclisation and rearrangement, produce a suitable precursor molecule, which, following attachment of the side-chain and cleavage of the protecting groups, would finally yield cephalosporin C.



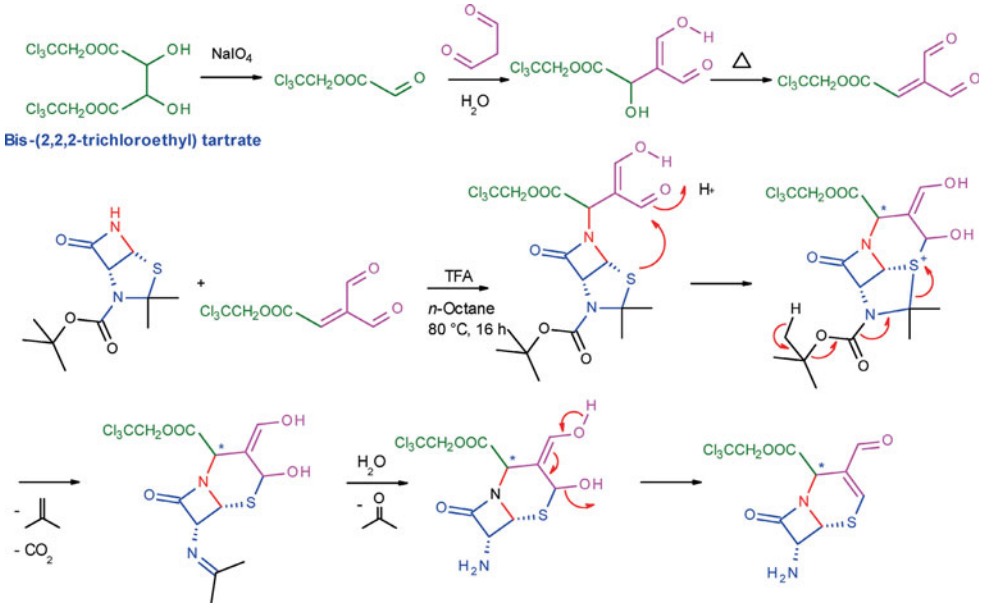
The initial stages comprise protection of (*L*)-(+)-cysteine with acetone, *t*-butoxycarbonyl chloride (from *t*-butanol and phosgene) and diazomethane. The first key step was treatment with dimethyl azodicarboxylate, a new reaction, which Woodward found after several attempts (see next page).

Presumably, the reaction begins with the attack of sulfur on the azo-group, which leads to a hydrogen transfer and ends with a 1,2-shift of hydrazine. There is some similarity to the first steps of the Mitsunobu reaction, which was discovered in 1967 (here, instead of sulfur, the azo-ester is attacked by triphenylphosphine). The reaction proceeds stereoselectively, although leading regrettably to the *trans*-product, which requires an inversion of the absolute configuration. For this purpose, the compound is oxidised with two equivalents of lead tetraacetate, and subsequently heated in methanol with anhydrous sodium acetate. It may be assumed that the hydrazine is oxidised. The resulting cation promotes cleavage of the ester and decarboxylation. Oxidation with a second equivalent of lead tetraacetate leads to loss of carbon dioxide and nitrogen, and introduction of the acetoxy-group. The reaction sequence is completed by base-catalysed methanolysis of the acetoxy-group. Introduction of the acetoxy-group involves a Walden inversion, so that the *trans*-*β*-hydroxy-ester is finally obtained. This is mesylated, substituted by azide and reduced with aluminium amalgam. Another Walden inversion at the stereogenic centre in the *β*-position produces a *cis*-amino-ester, the configuration of which favours the ring closure with triisobutylaluminium.

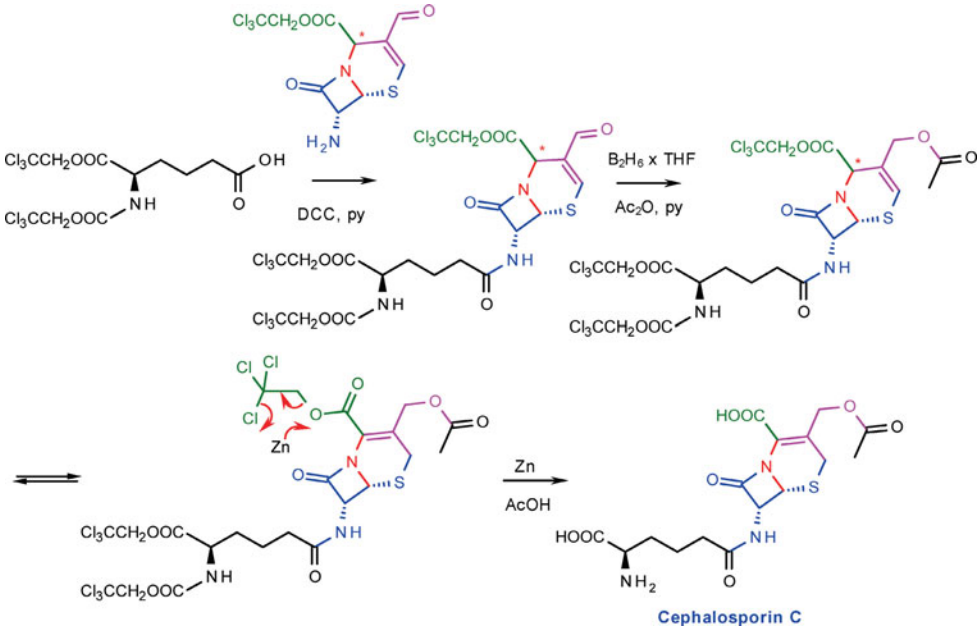
5.30 *Robert Burns Woodward (1917–1979).*



In the second main section of the synthesis, bis-(2,2,2-trichloroethyl) tartrate is oxidatively cleaved by sodium periodate, and the glyoxylate ester condensed with malondialdehyde. The initially-obtained hydrate can lose water by heating in octane. The lactam from the previous stage is sufficiently reactive for the transformation with the strong electrophile; in the presence of trifluoroacetic acid, nucleophilic attack by the sulfur on the carbonyl centre leads to the formation of the six-membered ring. For this, several mechanisms are conceivable. Noteworthy is the elegant protecting-group strategy. Exactly at the right moment, the Boc- and acetone protecting groups disappear, almost on their own. The resulting mixture of diastereomers does not require separation, because of the subsequent double bond migration.



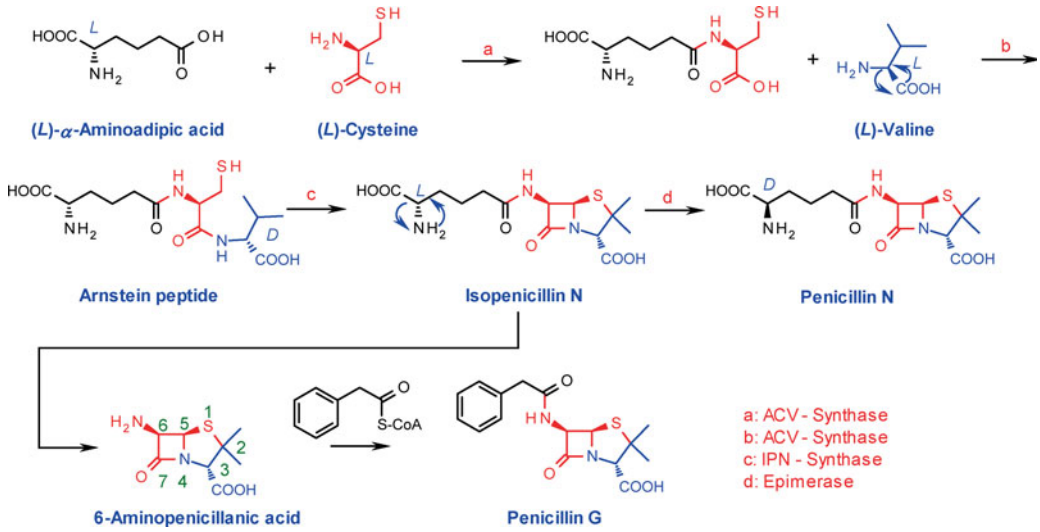
The closing synthetic sequence includes the DCC-mediated formation of an amide with a protected amino-adipic acid. The aldehyde function is reduced to the alcohol with diborane in THF, and the alcohol esterified by acetic anhydride. Over the course of three days, the non-conjugated ester forms a thermodynamic equilibrium with the conjugated ester by dissolving in pyridine. After chromatographic separation and purification, the protecting groups are reductively removed with zinc dust. The cephalosporin C obtained in this way is identical with the natural product in all spectroscopical, chromatographical and biological properties.



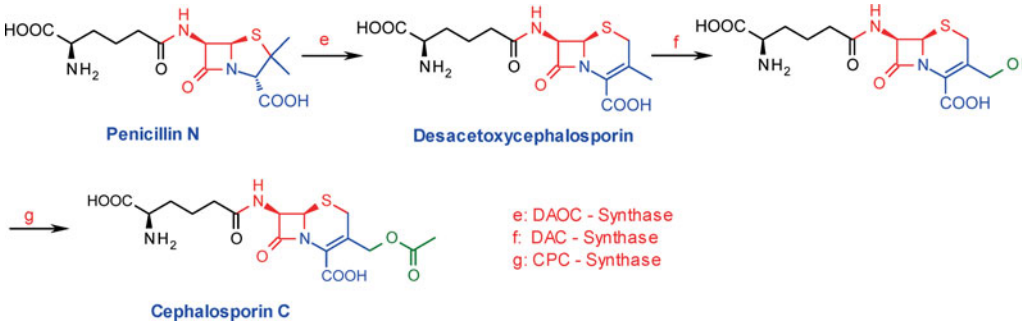
The total synthesis of cephalosporin C by Woodward was just one of the great achievements of this genial chemist. However, this route was much too complicated for an industrial synthesis. The yield of cephalosporin from its original source, *Cephalosporium acremonium,* was also too low to allow sufficient drug supply for medicinal use. Fortunately, various far more productive mutants were found. In addition, chemical modification of the natural product enabled the production of new semi-synthetic antibiotics.

5.2.8 Biosynthesis

In the first step of the biosynthesis [37] of penicillins and cephalosporins (corresponding to the industrial synthesis), (*L*)-aminoadipic acid and cysteine are converted into a dipeptide by means of the so-called ACV-synthase. The same enzyme condenses this dipeptide product with valine and inverts its stereogenic centre to give the so-called Arnstein peptide (steps a and b). The reaction is Mg^{++}- and ATP-dependent. Only (*L*)-valine is incorporated into the peptide; (*D*)-valine inhibits the biosynthesis. A non-haem-iron enzyme in presence of oxygen and ascorbate or α-ketoglutarate brings about the ring closure to isopenicillin (step c). Isopenicillin epimerase inverts the stereogenic centre in the side-chain to penicillin N (step d). In *Aspergillus nidulans* and *Penicillium chrysogenum,* for the formation of penicillin G from isopenicillin N, the (*L*)-α-aminoadipoyl residue is cleaved off and the 6-aminopenicillanic acid then reacted with phenylacetic acid, activated by means of Coenzyme A. Both reaction steps are catalysed by a bifunctional enzyme.

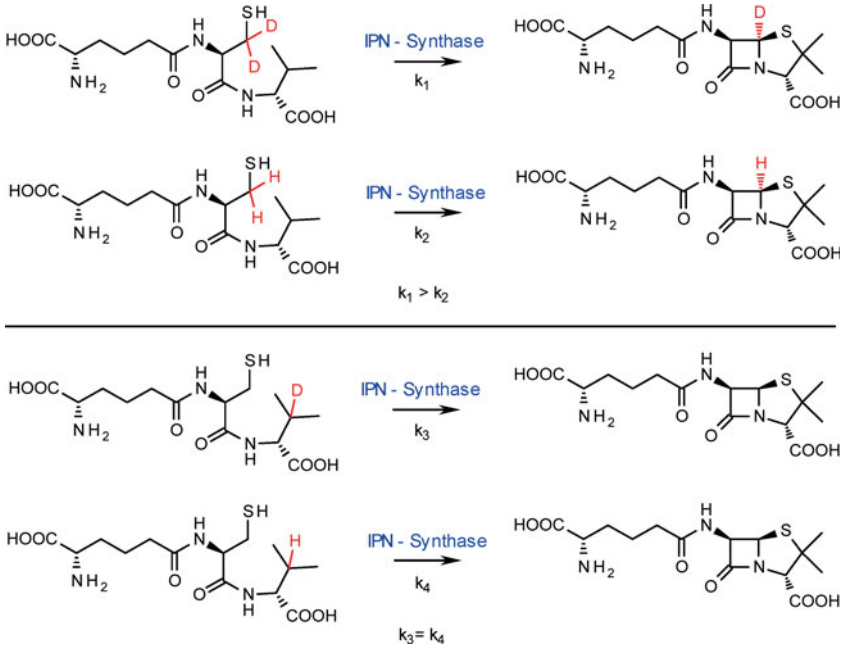


Cephalosporin C is formed from penicillin N. The ring expansion by DAOC-synthase, in a likewise oxygen- and Fe^{++}-dependent reaction, gives desacetoxy cephalosporin (step e). Afterwards, the methyl group is hydroxylated and then acetylated (steps f and g).



One of the most fascinating transformations in the biosynthesis of β-lactam antibiotics is the key step of oxidative cyclisation by isopenicillin-N-synthase. Despite intensive searches for intermediates in the ring-closure reaction, enzyme-free monocyclic intermediates have never been found. It has been concluded, that both cyclisations occur within the same enzyme-substrate complex. Structural variations in the aminoadipoyl and (*D*)-valine moieties are well tolerated by the enzyme. On the other hand, cysteine cannot be altered. If the isopropyl group in valine is exchanged for an allyl or cyclopropylmethyl group, rearrangement products are found, which are typical of radical reactions. This indicates that an isopropyl radical is involved in the formation of the thiazolidine ring.

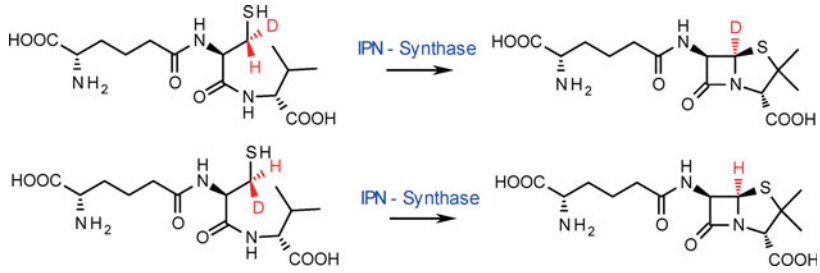
Important contributions towards elucidating the biosynthesis of penicillin, up to the structure determination of isopenicillin-N-synthase [38] in the 1980s and 1990s, originate from Sir Jack Edward Baldwin at MIT and University of Oxford. [39] Skilfully planned labelling experiments provided insight into the mechanism of the cyclisation. Thus, a large kinetic isotope effect was observed,



Baldwin is probably best known for "Baldwin's rules" on the preferred ring closure of alicyclic compounds.

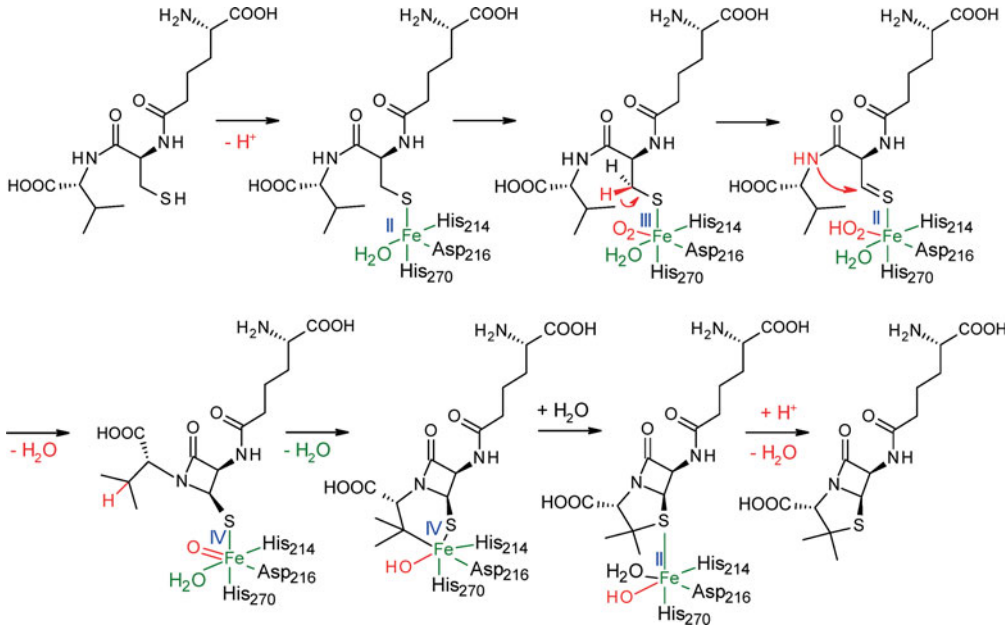
when cysteine in the 3-position was doubly deuterated, and reacted in a mixture with unlabelled Arnstein peptide. Deuteration of valine in the 3-position, on the other hand, showed no difference in the reaction rate. This leads to the conclusion that formation of the β-lactam ring is rate-determining, and takes place prior to the thiazolidine ring formation.

Stereospecific labelling of the cysteine teaches, that both, hydrogen abstraction and ring-closure occur with retention of absolute configuration, which is conceivable in terms of an enzyme-bound thioaldehyde.

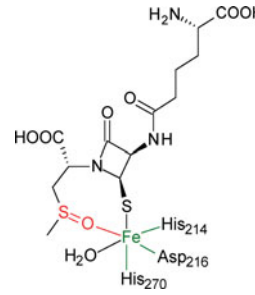


With the X-ray analysis of isopenicillin N-synthase and of the intact enzyme-substrate complex under anaerobic conditions, Baldwin succeeded in elucidating the mechanism of biosynthesis of isopenicillin N, finally and unequivocally.

The lack of oxygen in the reaction mixture prevented the enzyme from causing uncontrolled transformation of the tripeptide. By adding on nitric oxide, the analogous nitrosyl complex was obtained, which can be considered as an unreactive oxygen addition product. Treatment of an enzyme-substrate complex with a small amount of oxygen gave on the other hand isopenicillin N, which remained partially bound to the active site.



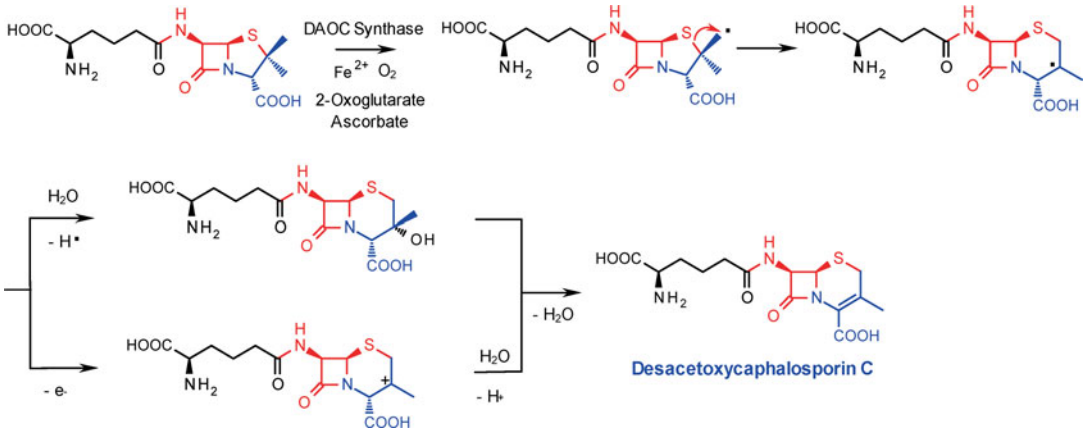
Altogether, the obtained data indicate the following synthetic route. The Arnstein peptide attaches in extended form to the active site of IPN synthase. First, the thiol function of the cysteine, and then oxygen, bind to an iron complex. Intramolecular transfer of a hydrogen radical generates a thioaldehyde function and reduces the iron again to oxidation state +2. Nucleophilic attack of the amidic nitrogen on the thioaldehyde group closes the β-lactam ring with loss of a water molecule. Thus, the isopropyl group approaches the highly electrophilic iron(IV)-oxo-ligand. [40] Thereby another hydrogen transfer is facilitated, which probably leads to an isopropyl radical. The latter attacks the thiol function, and finally generates isopenicillin N by reductive elimination.



In another series of experiments, Baldwin reacted in presence of IPN-synthase a tripeptide, in which methylcysteine replaced valine, with small amounts of oxygen, and could thus detect a monocyclic β-lactam product, which was attached to the enzyme's active site *via* the cysteine sulfur atom and a methylsulfoxid group. This probably originates from the iron(IV)-oxo-species.

The ring expansion of penicillin N to desacetoxycephalosporin is interesting as well. In spite of intensive efforts, this biosynthesis is still not completely clear. On the basis of substrate studies and kinetic investigations, it is assumed, that the chemistry of isopenicillin N-synthase and expandase are alike and that the ring expansion also proceeds *via* a ferryl-oxo-intermediate, [41] and that a radical mechanism is involved as well. [42]

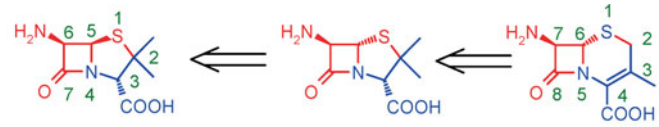
Labelling experiments demonstrate that a free radical is first generated on the (*Si*)-facial methyl group, which rearranges to the cepham radical. This then either undergoes formal addition of a hydroxy-radical and dehydration, or transfer of one electron to the enzyme along a cationic mechanism, to produce desacetoxycaphalosporin C.



It was also shown, that the sulfoxide from penicillin N, as well as penicillin N bearing an acetoxy-substituted β-methyl group, are not substrates for DAOC-synthase.

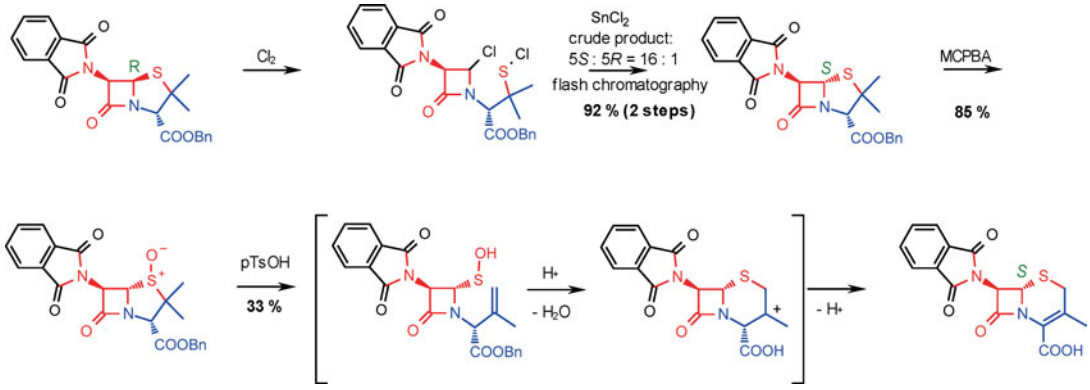
Methods for Epimerisation and Ring Expansion

The elegance of the biogenesis of cephalosporins is unsurpassed. By comparison, the synthetic efforts, especially for the preparation of the (6*S*)-epimers, appear clumsy and immature. [43] Starting from 6-aminopenicillanic acid, the synthetic route involves inversion of the stereogenic centre at the 5-position and then expansion of the thiazolidine ring.



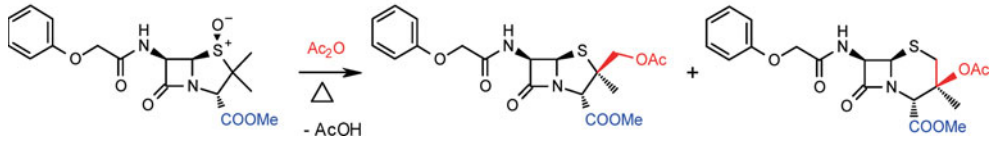
6-Aminopenicillanic acid (6*S*)-7-Aminodesacetoxycephalosporanic acid

The method of choice for the epimerisation of 6-aminopenicillanic acid at the 5-position was developed by Stjepan P. Kukolja at Eli Lilly. [44, 45] It consists of treating adequately protected derivatives with chlorine or sulfuryl chloride, with the objective of selectively cleaving the bond between C-5 and S. Tin(II) chloride is then used for recyclisation. The mixture of epimers can be purified by flash chromatography. The ring expansion follows according to the so-called Morin rearrangement. [46, 47] First, the sulfur is oxidised to sulfoxide with *m*-chloroperbenzoic acid, and this is then heated in DMF to 100 °C in presence of catalytic amounts of *p*-toluene-sulfonic acid.



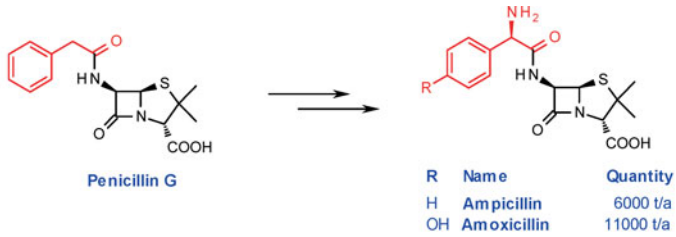
The mechanism of the reaction is analogous to the Pummerer reaction, leading to two acetoxy-substituted derivatives.

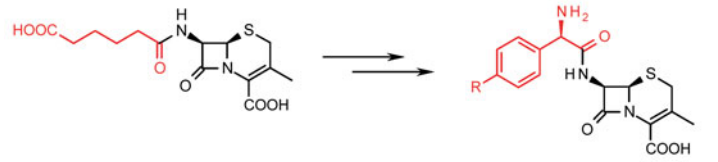
Pummerer-type reaction:



5.2.9 Biotechnological Syntheses

The starting material for the most important semi-synthetic β-lactam antibiotics is penicillin G or adipoyl-7-aminodesacetoxycephalosporanic acid. From these, ampicillin, amoxicillin, cephalexin and cefadroxil are obtained in considerable tonnage. [48] The essential intermediates for this are 6-aminopenicillanic acid or 7-aminodesacetoxycephalosporanic acid.

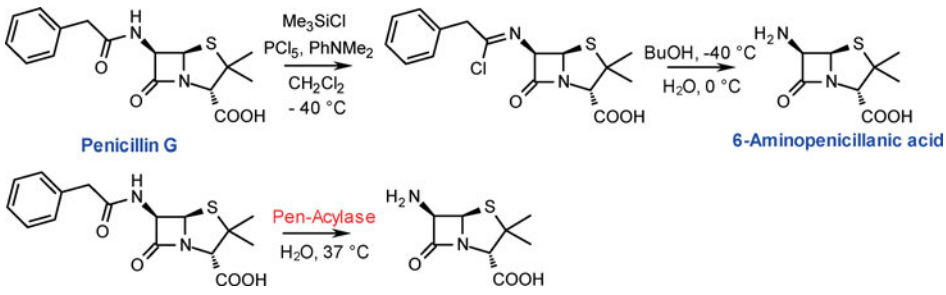




6-Aminopenicillanic acid

6-Aminopenicillanic acid is prepared from penicillin G by cleavage of the phenylacetyl group. In the early 1960s, penicillin G-acylase was used for this. However, the process was inefficient by present-day criteria, since the space/time yield was low and the enzyme could not be reused. Therefore, a "chemical solution" to the problem was sought.

The synthetic challenge centred around the issue that a β-lactam ring is more easily hydrolysed than a normal amide. For a selective hydrolysis, special reaction conditions are required, which differentiate between secondary and tertiary amides. Additionally, the carboxy group must be protected before hand. DSM developed a particular process for this, calling it "Delft cleavage". Nowadays, the reaction can be carried out in an intrinsically more elegant way with the known penicillin-acylase, which however, in contrast to earlier methods, is now immobilised (Tab. 5.4). [49]



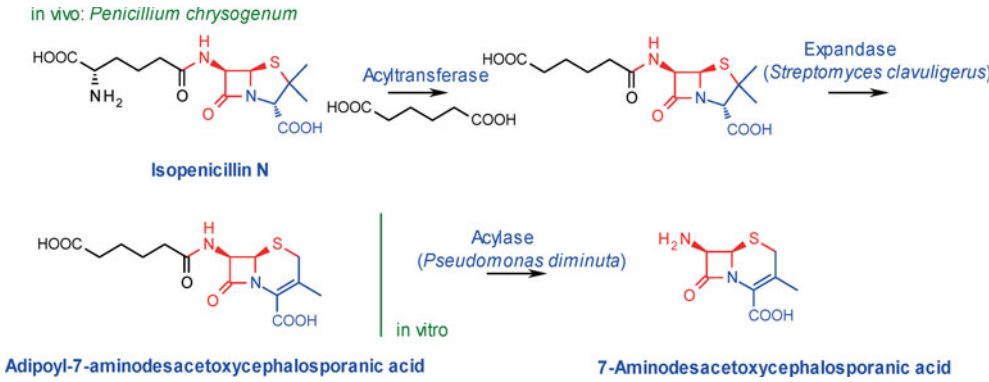
This switch from a chemical to an enzymatic process marks in the 1990s the beginning of biocatalysis on an industrial scale for the synthesis of fine chemicals. Similar processes were also developed for cephalosporin production.

Tab. 5.4 *Comparison of reaction conditions for the cleavage of the side-chain of penicillin G.*

Process	Chemical	Enzymatic
Reagents	Chlorotrimethylsilane Phosphorus pentachloride *N,N*-Dimethylaniline Butanol	Pen-acylase
Solvent	Dichloromethane	Water
Reaction temperature	–40 °C	+ 37°C

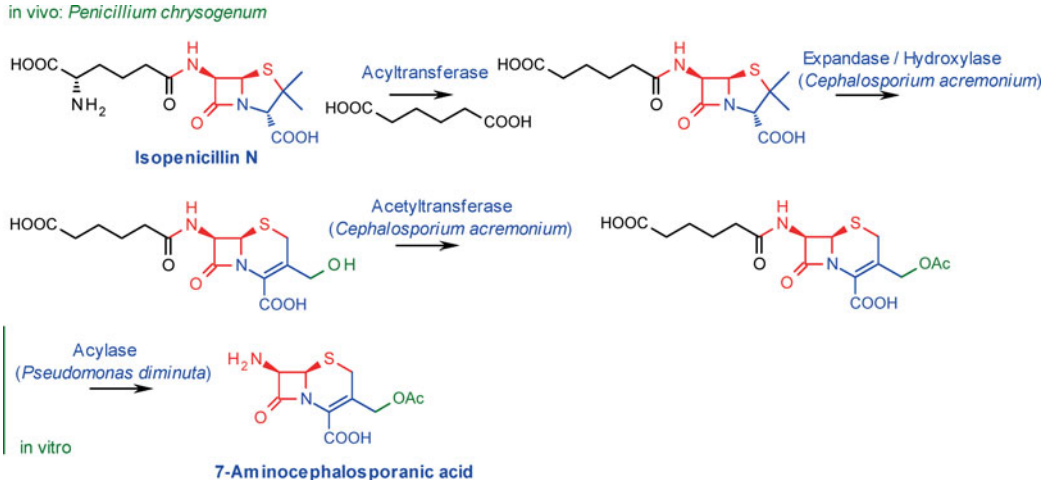
7-Aminodesacetoxycephalosporanic acid

The most essential starting compounds for semi-synthetic antibiotics are obtained nowadays with genetically modified strains of *Penicillium chrysogenum*. By addition of adipic acid to the culture medium, isopenicillin N is processed into adipoyl-6-aminopenicillanic acid and not penicillin G, which would be a dead end for further transformations by this organism. In case the engineered fungus *Penicillium chrysogenum* contains an expandase from the bacterium *Streptomyces clavuligerus*, adipoyl-7-aminodesacetoxycephalosporanic acid is formed. After hydrolysis of the latter in a cell-free system (*in vitro*) with an acylase from *Pseudomonas diminuta,* 7-aminodesacetoxycephalosporanic acid is obtained.

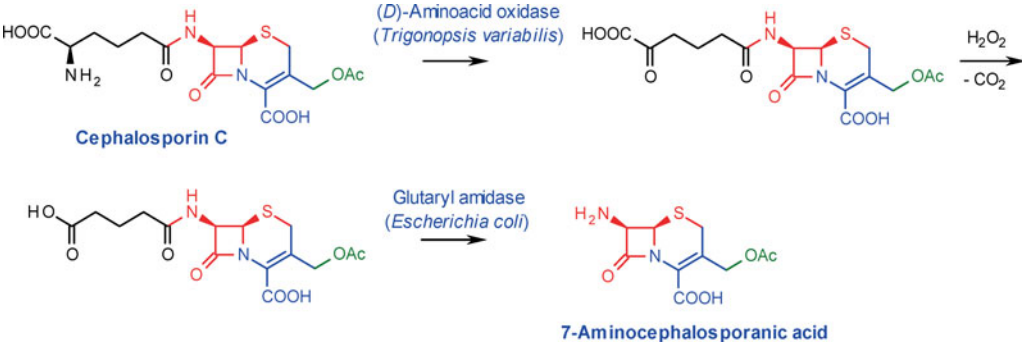


7-Aminocephalosporanic acid

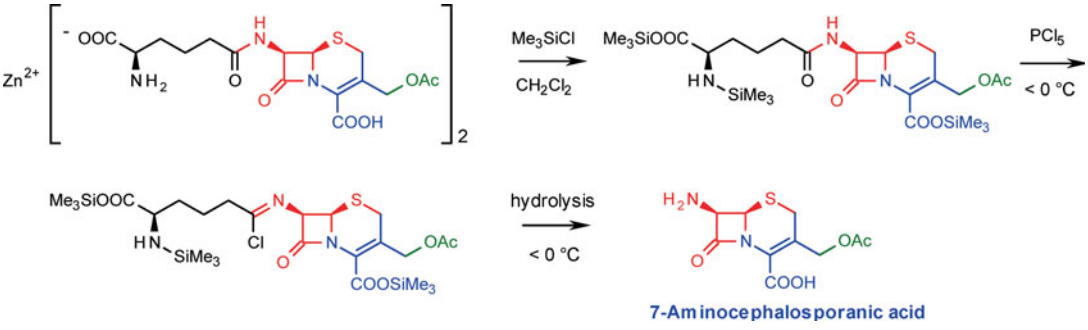
When *Penicillium chrysogenum* contains an expandase/hydroxylase as well as an acetyltransferase from *Acremonium chrysogenum*, then 7-aminocephalosporanic acid is generated correspondingly.



Starting from cephalosporin C, 7-aminocephalosporanic acid may also be obtained on an industrial scale by a combined *in vivo/in vitro* procedure. Cephalosporin C is produced using *Acremonium chrysogenum*, and oxidised by a (*D*)-amino acid oxidase from the yeast *Trigonopsis variabilis* to α-ketoadipoyl-7-aminocephalosporanic acid. In a "chemical step" it is decarboxyated. The last stage, an enzymatic again, involves a glutarylamidase from *Escherichia coli*.



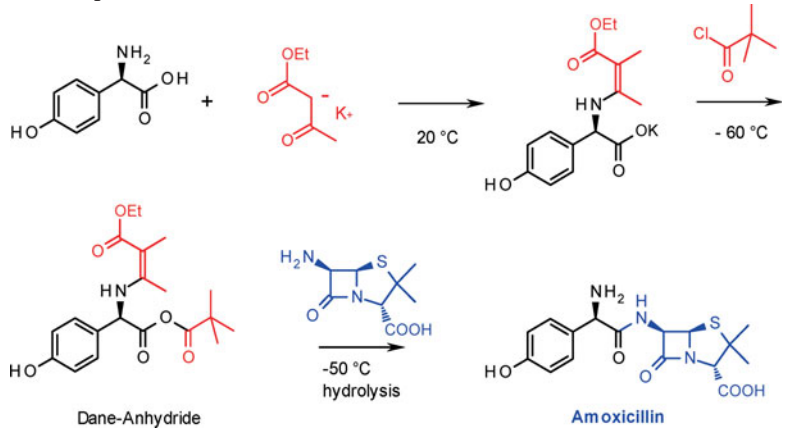
A corresponding chemical process starts with the zinc salt of cephalosporin C and follows the same strategy as for 6-aminocephalosporanic acid.



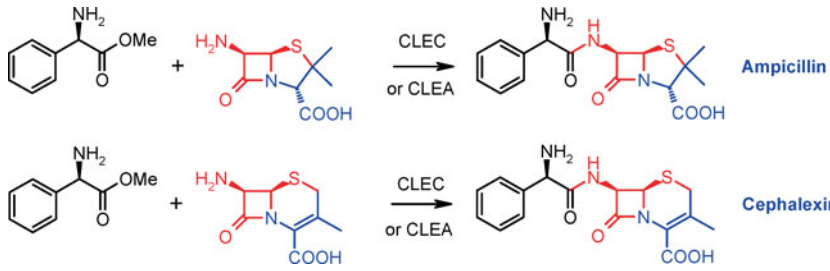
The advantage of the enzymatic process is, that for every tonne of product, only 0.3 tonnes of waste are produced. In the case of the chemical process, the waste amounts to 31 tonnes.

Ampicillin, Cephalexin, Amoxicillin

The so-called Dane-anhydride route for the synthesis of semi-synthetic β-lactam antibiotics can be used practically for all members of this class of compounds, and may be exemplified here for amoxicillin. (*D*)-4-Hydroxyphenylglycine is treated first with the potassium salt of ethyl acetoacetate and then with pivaloyl chloride. This results in the Dane-anhydride, which may easily be coupled with 6-aminopenicillanic acid.



For the enzymatic synthesis of the active compounds, one immobilises, for example, penicillin G-acylase in the form of *cross-linked enzyme crystals* (CLECs) or as *cross-linked enzyme aggregates* (CLEAs). [50] These biocatalysts combine high activity, high purity and high stability towards solvents and temperature. CLECs are obtained by connecting the enzyme crystals *via* bi- or multi-functional linkers, like glutaraldehyde, on a suitable matrix. Conversely, CLEAs, which are less expensive to produce, result through aggregation of proteins in the presence of salts (*e.g.* ammonium sulfate) and non-ionic polymers, *e.g.* polyethylene glycol (PEG 8000). In both cases, these are heterogeneous catalysts with the advantage of their facile separation, once the reaction is completed.

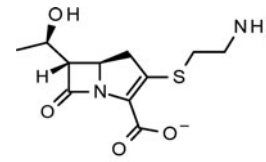


Here also biochemical processes are increasingly displacing the purely chemical procedures. Because of the enormous quantities of waste, which are often 30–40 times larger in relation to the product, the enzymatic and fermentative processes

are more elegant than the chemical syntheses. [48] Apart from that, there are fully synthetic active compounds, for the synthesis of which no enzymes are available.

5.2.10 Carbapenem Antibiotics

Thienamycin



Thienamycin

Looking at the structure of thienamycin [51], the impression arises that it may result from a structure-activity optimisation programme, which has been carried out by a large pharma company, starting from penicillin. Compared with the penicillins, in thienamycin the side-chains are modified and the position of the heteroatoms is altered.

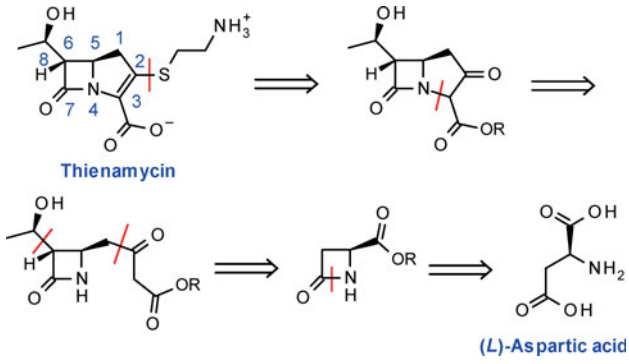
However, this impression is misleading. In 1976, scientists at Merck Sharp&Dohme discovered this substance in the fermentation broth of the soil bacterium *Streptomyces cattleya*. It showed good activity against *Pseudomonas* and β-lactamase-producing species. The structure determination revealed that this represented a new class of β-lactam antibiotics, the carbapenems.

Since thienamycin is stable only in a narrow pH range around neutrality, its isolation from the fermentation broth presented greater difficulties. Also, it was not possible to improve the fermentation yield significantly by strain development. Thus, over the next few years, Merck developed several chemical syntheses, one of which we should take a closer look at.

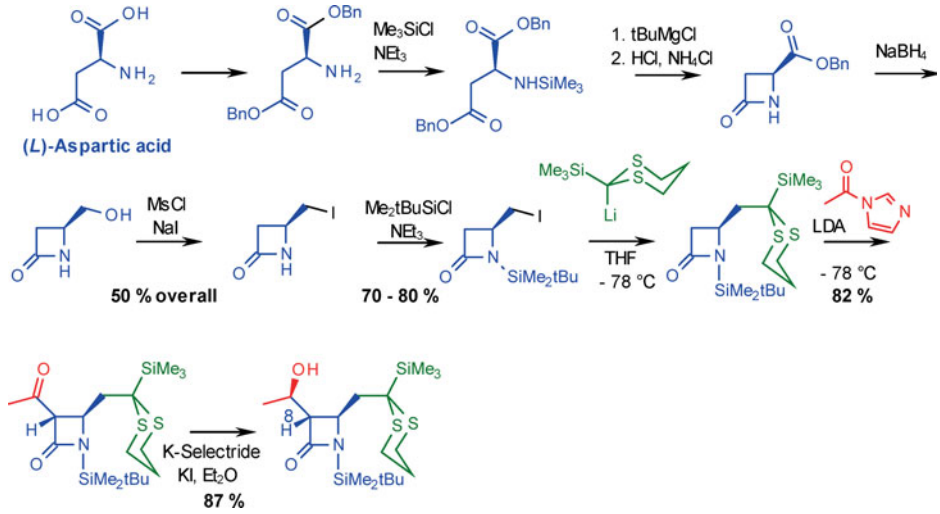
Important aspects of this synthesis are:

1. postponement of constructing the basic carbapenem scaffold to a late stage, due to its high reactivity;
2. introduction of the side-chains, hydroxyethyl and cysteamino (2-aminoethylsulfanyl), at the most appropriate stages of the synthesis;
3. for stereoselective synthesis, starting out from a simple and readily accessible enantiomerically pure building block.

The retrosynthesis of thienamycin follows really practical considerations. First, the cysteamino side-chain is removed, which simplifies the work-up and isolation of the intermediate product. Opening of the pyrrolidinone ring between positions 3 and 4, leaves a highly substituted azetidinone, the side-chains of which can be removed stepwise. In this way, one finally arrives at (*L*)-aspartic acid.



In Merck's total synthesis, (*L*)-aspartic acid is converted into its dibenzyl ester and *N*-protected with chlorotrimethylsilane. Ring closure to benzyl azetidinone carboxylate is carried out with *t*-butylmagnesium chloride. Reduction with sodium borohydride, mesylation and reaction with sodium iodide lead to the corresponding iodide. Introduction of a protected carboxylic acid function, by means of 2-trimethylsilyldithiane (thereby using the *Umpolung* principle), succeeds in extending the side-chain by one carbon atom. In the next step, the C_2 side-chain is introduced. The direct route should be reaction of the lactam enolate with acetaldehyde. Both main products have actually the desired *trans*-configuration at position 6, but consist of an approximately 1 : 1 mixture of epimers at position 8. More advantageous is the reaction with *N*-acetylimidazole and subsequent reduction with potassium tri-*sec.*-butylborohydride in the presence of potassium iodide. The epimeric ratio in respect of position 8 is then around 9 : 1. The undesired epimer can be separated off and recycled by oxidation. Thereby, all the stereocentres of thienamycin are generated, and attention can be turned to the construction of the bicyclic system and introduction of the second side-chain.

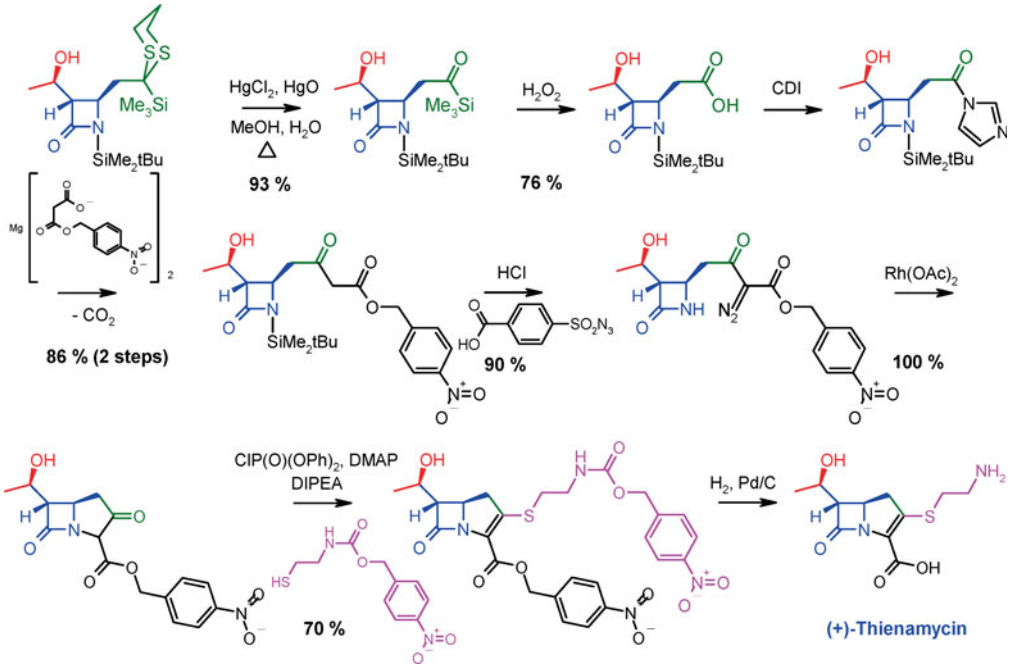


Next are removal of the dithiane system with mercury (II) chloride and mercuric oxide, and oxidative cleavage of the silyl group. Following Masamune's procedure, the carboxylic acid is then activated with 1,1'-carbonyldiimidazole and the side-chain undergoes a two carbon extension by reaction with the magnesium salt of a monoalkyl malonate. Following removal of the silyl protecting group with HCl, a diazo-group is introduced by Regitz' diazo transfer, producing an α-diazo-β-ketoester.

By warming of the diazo-compound with traces of rhodium acetate in toluene at 80°C, the carbenoid inserts smoothly into the neighbouring N-H bond. This reaction step is of particular importance, since it constitutes a new methodology for the construction of the carbapenem skeleton. Although investigations on comparable bicyclic β-ketoesters showed that these exist almost entirely in their keto-form, reaction with diphenyl chlorophosphonate and Hünig's Base leads to the enol phosphate.

Noteworthy is the high regioselectivity, so that the alcohol function at C-8 must not be protected. The protected cysteamine adds in a heteroanalogous Michael fashion, and diethyl phosphate is cleaved off.

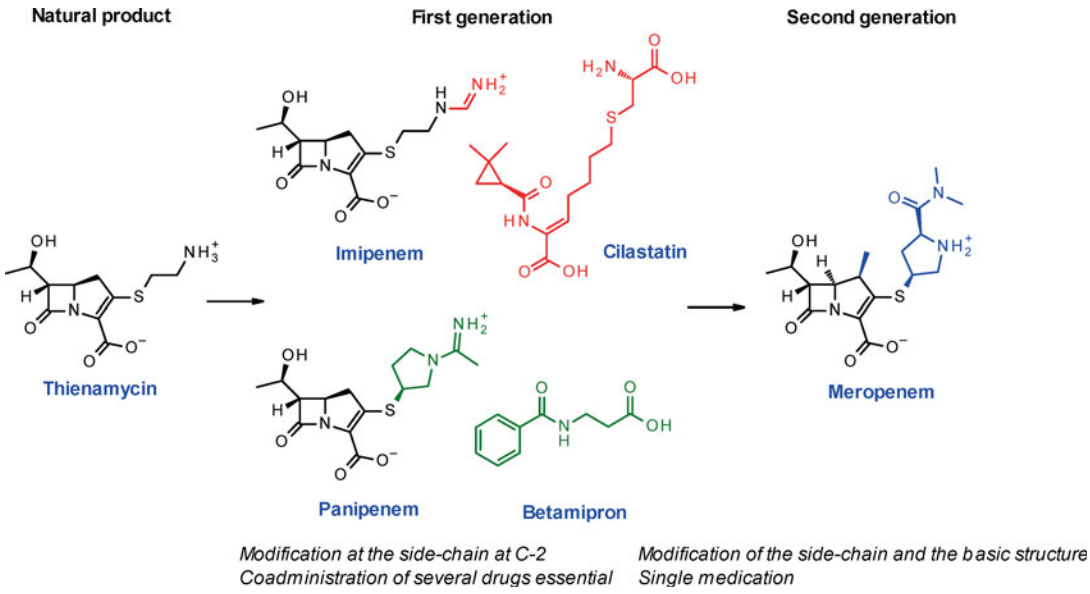
The total synthesis of thienamycin is completed by reductive cleavage of the protecting groups. The synthetically produced drug is comparable with the natural product in all its analytical and biological properties.



Meropenem

The Route from Thienamycin to Meropenem

The discovery of thienamycin had a trend-setting influence on the development of new antibiotics. [52, 53] The basic structure of carbapenem provided new starting points in the search for β-lactam antibiotics, which were effective against resistant bacterial strains. Nevertheless, it was clear that thienamycin could not be developed on account of its chemical and biological instability, as well as its nephrotoxic (Greek, *nephros*: kidney) and neurotoxic properties. This kindled an intensive search for improved carbapenem drugs. Thus, in the past 25 years more than 50 natural products with the carbapenem motif have been isolated from microorganisms. However, none possesses a better activity profile than thienamycin. Another line of development followed the so-called lead structure optimisation, starting from thienamycin. This led to extensive investigations of the structure-activity relationships of carbapenems, their chemical and biological stability, as well as the nephro-and neuro-toxicity of this new drug class. Out of these development programmes, in which many pharmaceutical companies participated, three active compounds had been moved into the clinic by the turn of the century (Fig. 5.31).



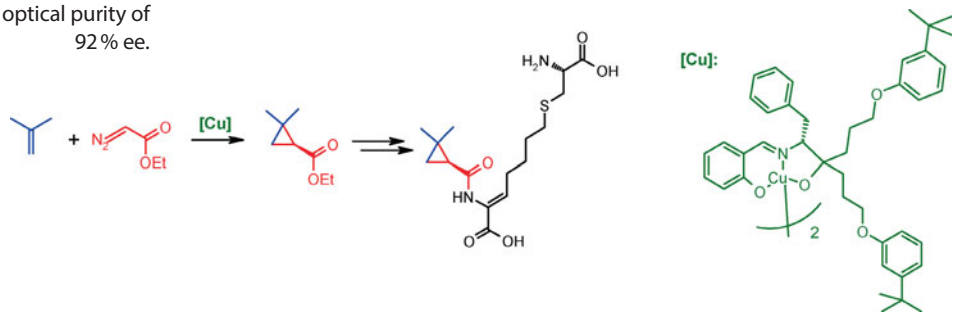
5.31 *Development phases of carbapenems.*

The synthesis of cilastatin constitutes one of the first enantioselective syntheses on an industrial scale. In 1966, H. Nozaki and R. Noyori investigated the enantioselective [2 + 1]-cycloaddition of diazoacetic esters to olefins. From this, Sumitomo developed industrial processes for the preparation of pyrethroids and cilastatin. In the key step, ethyl diazoacetate is decomposed in presence of isobutene at a dimeric, enantiomerically pure copper complex. The resulting ethyl dimethylcyclopropanecarboxylate has an optical purity of 92 % ee.

In 1984, Merck launched imipenem (*N*-formimidoylthienamycin). Imipenem acts as a nephrotoxin by interaction with the renal dehydropeptidase-1 (an enzyme of the kidney, which cleaves dipeptides and metabolises imipenem). For this reason, imipenem must be administered together with cilastatin [54, 55], which blocks dehydropeptidase-1.

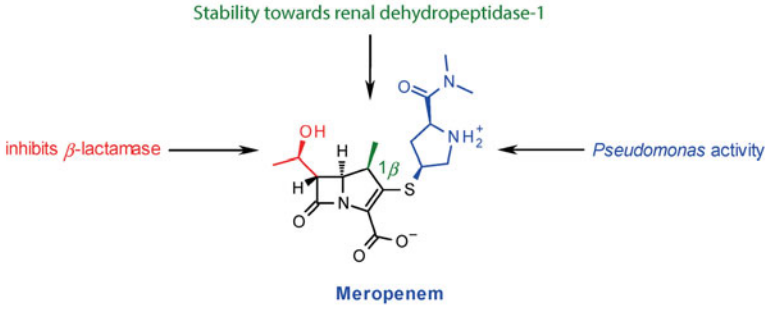
In 1993, panipenem from Sankyo obtained approval in Japan. This drug is also nephrotoxic. Concurrent administration of betamipron blocks the transport of organic anions, whereby the toxicity of panipenem is reduced. Both first-generation carbapenem drugs possess the regular carbapenem scaffold, and a strongly basic residue in the side-chain at C-2.

The first second-generation carbapenem drug, meropenem, which obtained approval in 1994, originates from Sumitomo. Meropenem is substantially more active than imipenem against Gram-negative bacteria and somewhat weaker against Gram-positive bacteria. What is special about meropenem, is its low metabolism by renal dehydropeptidase-1, so that co-medication can be spared

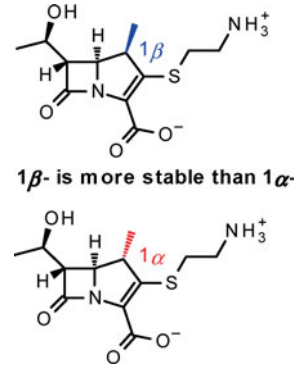


(Fig. 5.32). The pharmacokinetics of meropenem closely resemble that of the imipenem/cilastatin combination, and its nephro-and neuro-toxicity is correspondingly low.

Meropenem differs structurally from its earlier generation family members by a far less basic side-chain and the 1β-methyl group, which are both responsible for the increased biological stability, without suffering loss of activity (Fig. 5.33). [52]



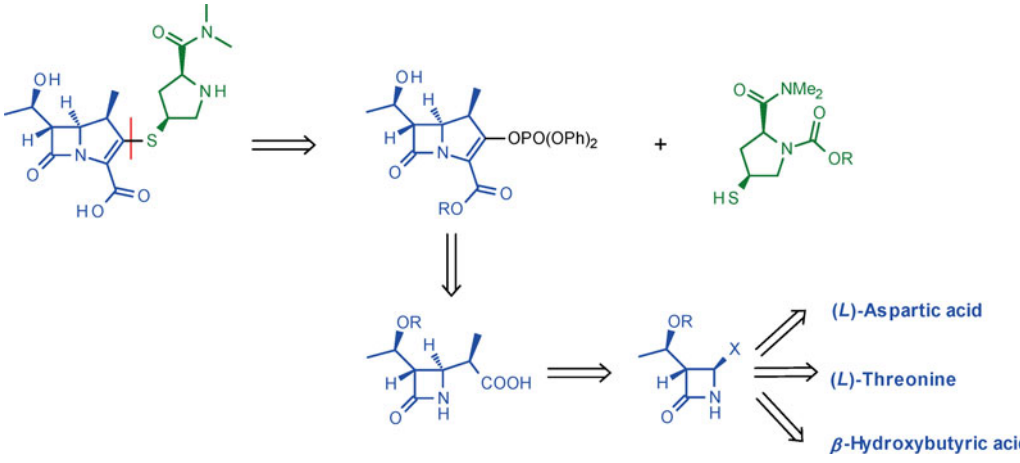
5.33 *By studying a great number of meropenem analogues, it became possible to gain an understanding, which role various parts of the molecule play.*



5.32 *Exemplified by methylthienamycin, the Merck group had already recognised in 1983, that the metabolic stability is dependent on the absolute configuration of the methyl group.*

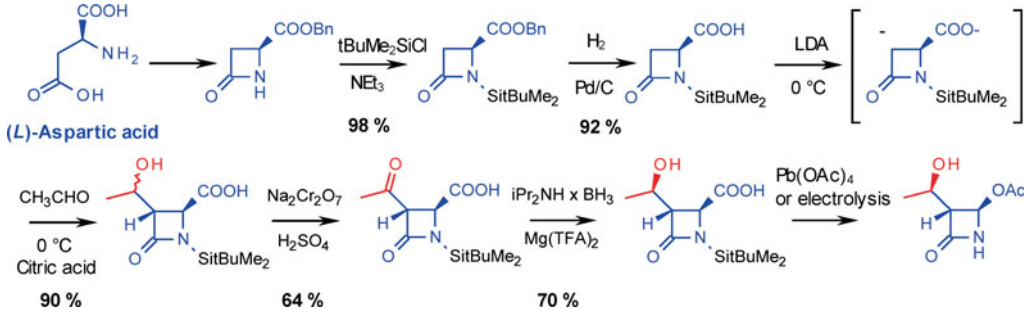
Industrial Syntheses

The synthesis of meropenem is highly convergent. The two large building blocks are put together only at the penultimate stage. The penem fragment can in the end be traced back to (*L*)-aspartic acid, (*L*)-threonine or both enantiomers of β-hydroxybutyric acid. [56]

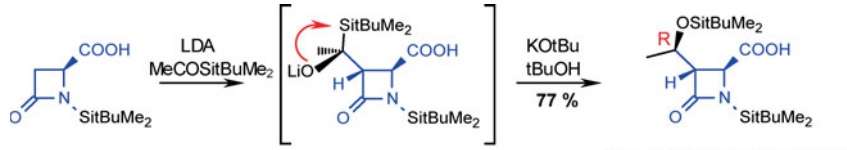


The construction of the β-lactam by Merck starts, as for thienamycin, from the inexpensive aspartic acid. [57] The silyl-protected benzyl azetidinone carboxylate, [58] after cleavage of the benzyl protecting group, is treated at 0°C with LDA and acetaldehyde. The dianion protects the stereogenic centre from racemisation. The mixture of epimers is oxidised, then stereospecifically reduced by

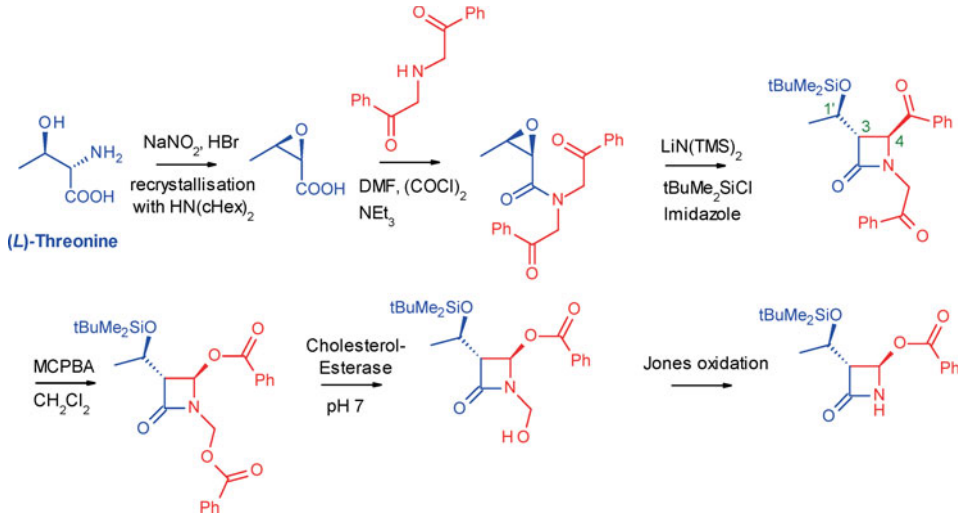
diisopropylamine-borane, to give the desired alcohol, which can be easily transformed into the required synthetic building block.



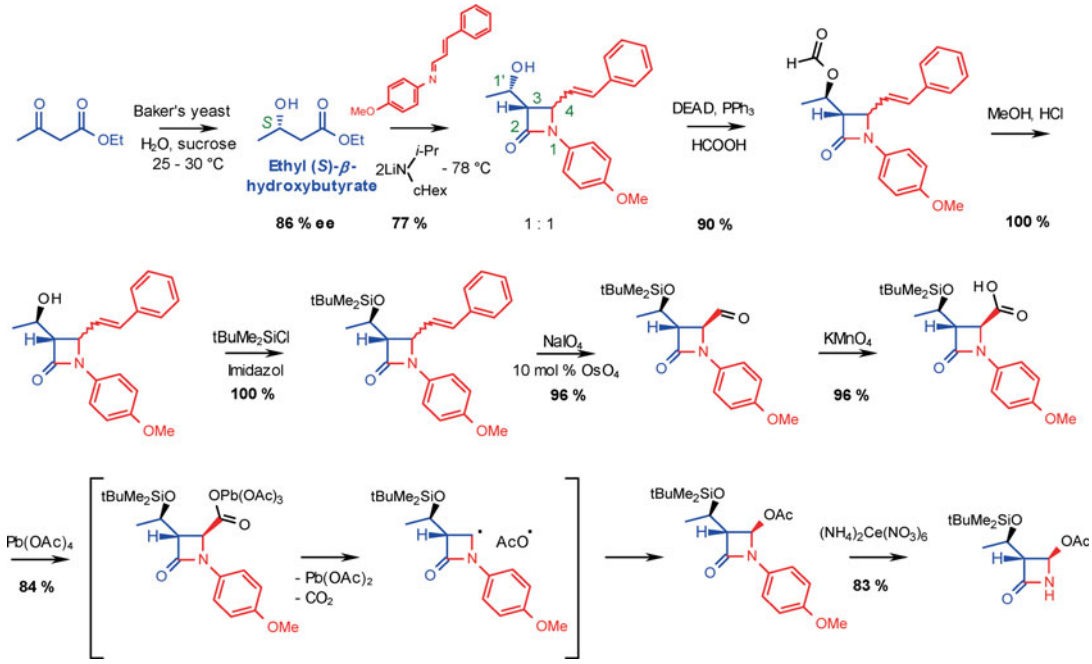
The synthetic route can be shortened, if a sterically demanding silyl ketone is used as the synthetic equivalent of acetaldehyde, according to Bouffard and Salzmann. The lithium alkoxide is then transformed stereoselectively in a Brook rearrangement into the silyl ether. This is of advantage, since during the downstream-processing no separate protection step is needed. [59]



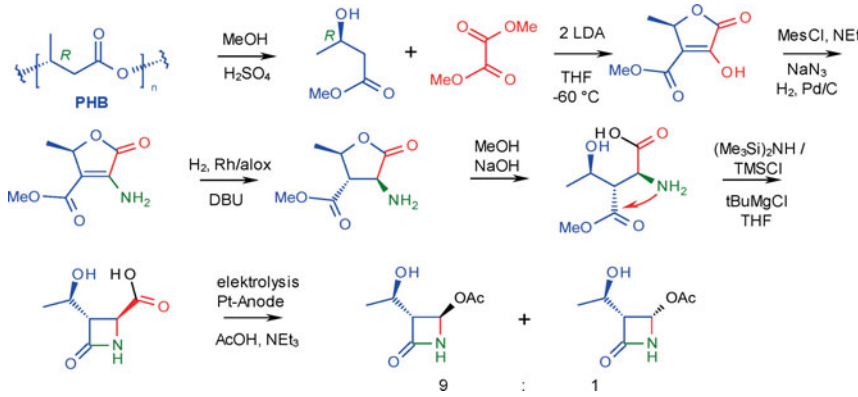
Ciba-Geigy's route towards the desired β-lactam building block starts from (2*S*,3*R*)-threonine. [56] Both stereocentres of threonine are used to generate the absolute configuration at position 3 and 1'. The third stereocentre is directed to the *trans*-configuration in course of the formation of the lactam ring. The double Baeyer-Villiger oxidation leads to a dibenzoate, which is selectively deprotected by enzymatic ester cleavage. The final Jones oxidation is "environmentally problematic" and therefore challenges this approach.



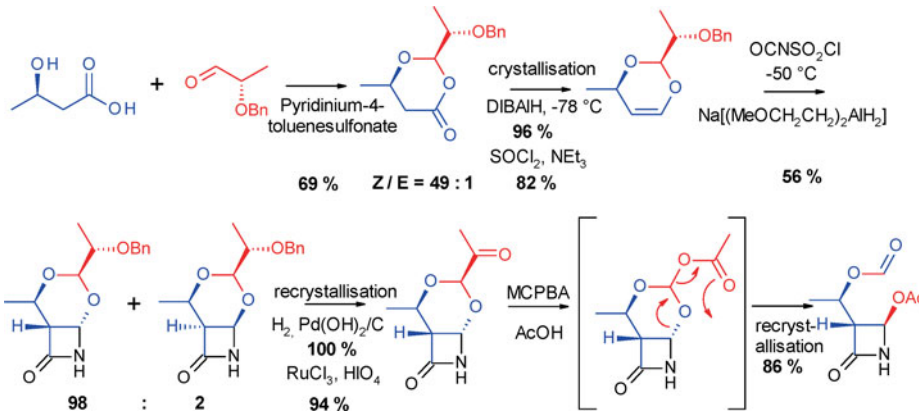
In 1983/84, D. J. Hart and G. I. Georg published a lab synthesis of the desired azetidinone, starting from ethyl (*S*)-β-hydroxybutyrate, which can be easily prepared by a baker's yeast reduction of ethyl acetylacetate. [56] The (*S*)-enantiomer is mandatory in order to obtain the correct absolute configuration at position 3 in course of the enolate imine cyclisation reaction. Consequently, the configuration at C-1' must be inverted by a Mitsunobu reaction in the following step. After protection of the hydroxyl group, the product is obtained by a sequence of oxidation reactions.



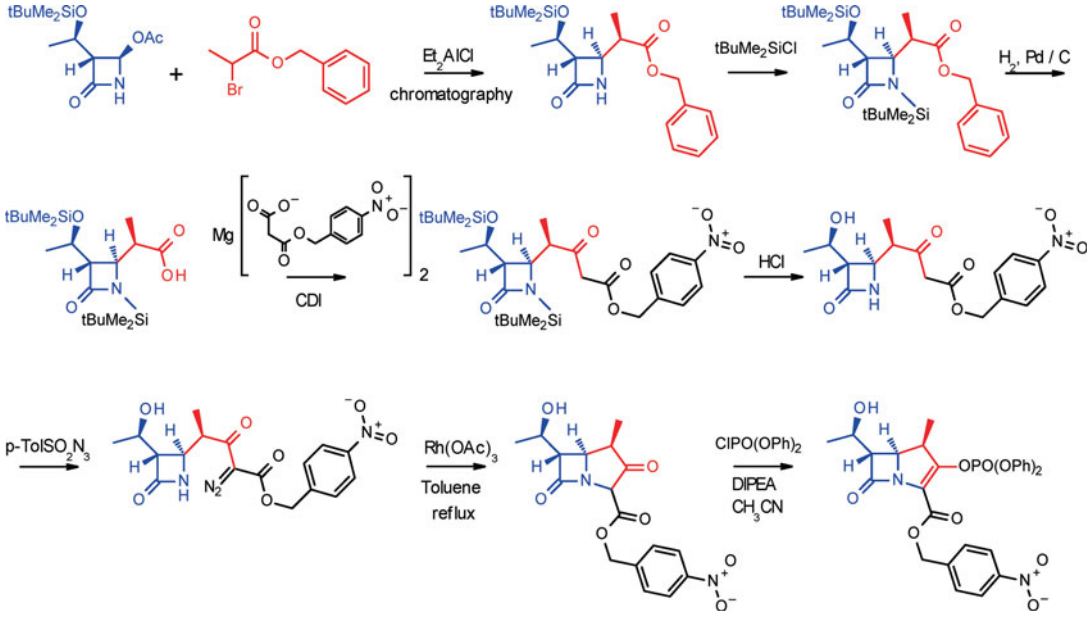
Ciba-Geigy developed a more concise approach, starting with ethyl (*R*)-β-hydroxybutyrate, which derives from ICI's low-cost biopolymer PHB, a fermentation product of *Alcaligenes eutrophus*. [56] After depolymerisation and a low-temperature condensation with methyl oxalate, the amino group is introduced by an azide substitution and reduction. The correct absolute configuration at position 3 and 4 is achieved by DBU-epimerisation to the most stable all-*trans*-diastereomer. Prior to the Breckpot-Grignard β-lactam cyclisation, the intermediate is persilylated. In the final step, the carboxylate is degradated to an acetate by electrolysis.



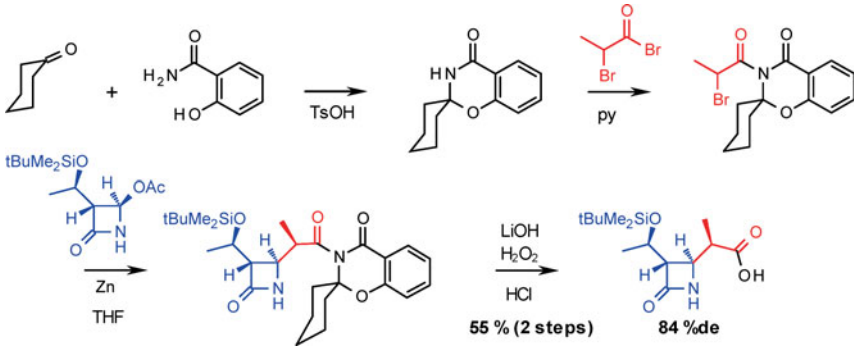
A highly attractive enol ether/chlorosulfonylisocyanate-cycloaddition approach was developed by Sagami Ltd. [56] The stereoselectivity of the cycloaddition is controlled by double chiral induction with (*S*)-benzyloxypropanal. In course of the following steps, the small amount of the undesired diastereomer is removed by crystallisation. Finally, after an elegant Baeyer-Villiger oxidation and rearrangement, the chiral auxiliary remains in the molecule as an acetate group.



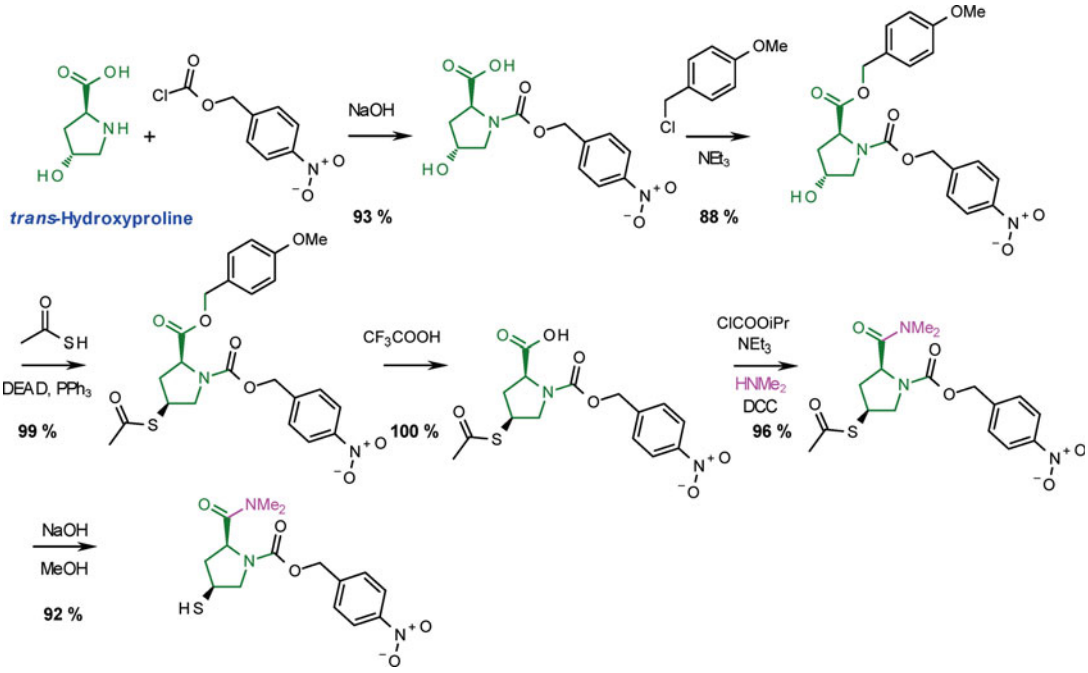
In another penem building block synthesis, a functionalised azetidinone is reacted with benzyl 2-bromopropionate in the presence of diethylaluminium chloride in hexane/THF. A mixture of epimers is obtained, which has to be separated chromatographically. Afterwards, the lactam nitrogen is protected, and the benzyl residue reductively cleaved. The next steps bear a close resemblance to those of the thienamycin synthesis: construction of a β-ketoester, diazo transfer and rhodium-catalysed insertion of a carbenoid into the lactam N-H bond. Finally, the β-keto-ester is activated with diphenyl chlorophosphonate. [60]



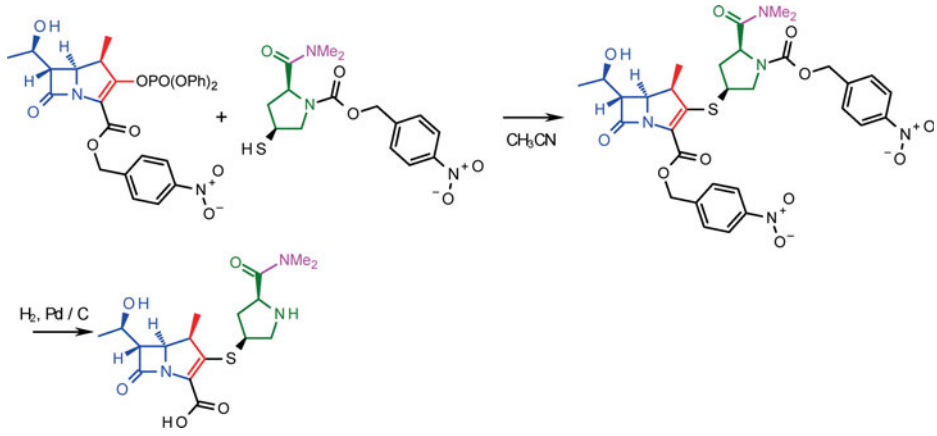
An improved variant uses a bromopropionamide for the construction of the side-chain carboxylic acid; this amide is simply accessible in two stages from cyclohexanone, salicylamide and bromopropionyl bromide. Then follows a Reformatzky reaction with remarkable diastereoselectivity under formal retention of the absolute configuration. [61] Chromatographic purification is not necessary.



The synthesis of the second building block [62] for meropenem starts from *trans*-hydroxyproline. First, the amino acid is protected on both, the amino and the carboxyl group. By reaction with thioacetic acid in a Mitsunobu esterification, the thiol function is introduced, and the carboxyl group is then selectively deprotected with trifluoroacetic acid. The dimethylamino-group is introduced with the aid of isopropyl chloroformate, and the thiol acetate is finally hydrolysed with aqueous sodium hydroxide.



The convergent synthesis ends with the reaction of both building blocks in acetonitrile and cleavage of the protecting groups by hydrogenolysis.

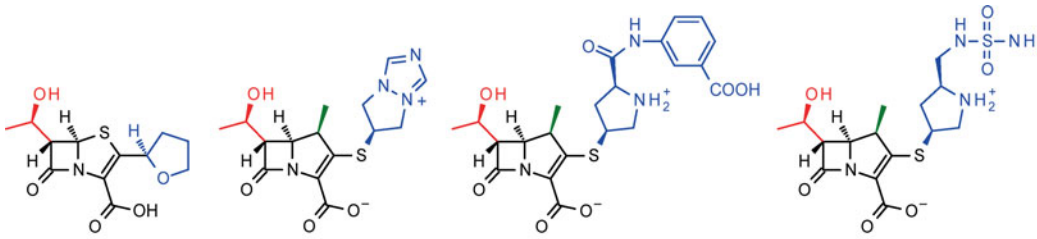


5.2.11 Newer β-Lactam Antibiotics

Among the newer β-lactam antibiotics is faropenem; with its unsaturated five-membered ring, it is structurally positioned between penicillins and cephalosporins. Like the carbapenem drugs, it possesses a hydroxyethyl side-chain with inherent stability against lactamases. Faropenem, market since 1997 by the

Japanese firm Suntory, is orally available and active against a broad spectrum of Gram-positive and Gram-negative bacteria.

Following meropenem, a series of further carbapenems have meanwhile reached the market. They differ from the former merely in their side-chains at C-2 . These are "well-behaved" antibiotics, highly active for the treatment of complicated infections of the skin, soft tissue, abdomen and urinary tract. Doripenem acquired considerable importance for the treatment of nosocomial pneumonia. [63]

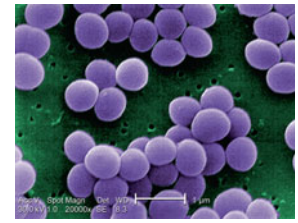


Faropenem (Suntory, 1997) **Biapenem** (Lederle, 2001) **Ertapenem** (AstraZeneca, 2002) **Doripenem** (Shionogi, 2005)

5.2.12 Outlook

Nowadays, antibiotics belong to everyday life, and in prosperous parts of the world, there is hardly a single person, who has not come into contact with these drugs in the course of his/her life. The generous use of antibiotics often brings about a deceptive sense of security against the danger of a bacterial infection, because over the course of time, many of the once sensitive pathogens have developed resistances towards antibiotics.

While in the 1960s it was still believed, that the 20th century would see infectious diseases to be wiped out, in the 21st century we are however still afar off. [64, 65] Under favourable conditions, the doubling time of a bacterial culture lies between 20–30 minutes, and the mutants found per millilitre of culture medium ranges from 100,000 up to a million. If only a few mutated bacteria survive under treatment with an antibiotic, this escape initiates a new resistance (Fig. 5.34).



5.34 *Staphylococcus aureus is a Gram-positive bacterium. In 1940, 90 % of the Staphylococcus aureus strains were sensitive to penicillin. By 1950, already 50 % of the strains had developed resistance, and today this rate exceeds 90 %. [66]*

In the USA, more than 22,000 tonnes of antibiotics are produced annually. Around 40 % thereof are used as growth promoters in the livestock and aquaculture industry. In the meantime, an estimated 80 % of all pigs, cattle and poultry are treated with antibiotics.

In Denmark, there were in 1994 some 24 tonnes of vancomycin derivatives used in animal health, and only 24 kilograms for the treatment of human diseases. Later, the Danish Government prohibited the use of vancomycin derivatives as feedstock additives; the European Union followed suit in 1998. All classes of antibiotics, which find use in human medicine, are no longer allowed as growth promoters in livestock.

Another problem can be attributed to inappropriate personal behaviour. Broad-spectrum antibiotics are almost universally applicable, and may frequent-

ly lead to self-medication. A false diagnosis is often followed by inadequate therapy. Early signs of improved disease symptoms may lead to insufficient compliance; and even doctors contribute to the spread of resistance: Approximately 50 % of all prescribed antibiotics are provided to patients with viral infections.

Up to the present, the perfect antibiotic has not been found. [67] The problem is, that our armamentarium of drugs is facing a multitude of diverse pathogens, which have perfected their survival strategies under permanent selection pressure over millions of years. Microorganisms are able to adapt to changing conditions of their environment by rapid mutations. For each new antibiotic, it is only a matter of time, before resistance ruins its antimicrobial efficacy. There is a continuous race between the innovation cycle for new drugs and the re-occurrence of bacterial resistance. While the time it takes to develop a new pharmaceutical compound cannot be shortened at will, every effort should be made to ensure an extended therapeutic benefit of currently available antibiotics.

Summary in Bullet Points

- β-Lactam antibiotics irreversibly inhibit the remodelling of bacterial cell walls by acylation of essential enzymes involved.
- In spite of elegant total syntheses, biotechnological production of penicillins and cephalosporins is clearly superior to chemical synthesis. For derivatisation, enzymatic procedures are often used.
- Carbapenem antibiotics, on the other hand, are prepared by chemical synthesis.

5.3 Opiates

"Opioids" is a general term, which covers opiates and all other compounds, which act in a similar way. Opiates are compounds, the structures of which are derived from that of morphine.

"We have won!" may have been the last words of the Marathon runner Pheidippides, who brought the news to Athens of the battle between the Athenians under Miltiades and the Persians at Marathon in 490 BC. He had run around 42 kilometres as fast as he could, before he collapsed and died (Fig. 5.35).

By endurance sporting activities such as jogging and marathon running, opioid peptides are released in the brain; these dampen sensitivity to pain and can trigger a sense of euphoria. [68]

5.35 *The stela of the first "Marathon runner" is also known as the 'Hoplitodromos', the original of which is in the National Archaeological Museum of Athens. The wall relief actually dates from the year 510 BC, but is regarded nowadays as a memorial to this famous hoplite.*

5.3.1 History of the Poppy

The earliest evidence for the deliberate use of poppies are the poppy seeds and poppy capsules, which have been found from archaeological unearthing of buildings on stilts, dating back to the early Stone Age (3000–2500 BC) in the shallow lakes of Switzerland (cantons Zurich, Thurgau, Bern). [69] Similar discoveries have been made in the provinces of Milan, Savoy and Provence. The poppy was presumably used as a spice in foodstuffs and for the preparation of vegetable oils.

Early indications of the use of poppies as raw materials for drugs are found in Greek mythology and in ancient scientific works. Hippocrates (460–377 BC) describes in the collection of medical writings, known as the *Corpus Hippocraticum*, the juice "Mekonion", which was obtained by squeezing the poppy plant, and he praises its narcotic and constipating effect. Diagoras of Melos (5th century BC) was the first to mention that administration of opium (Greek, *opion*: poppy juice) caused addiction and robbed people of the sense of reality. Pliny the Elder and Dioscurides describe in detail the production and use of the poppy and opium. Poppy seeds are roasted and mixed with honey as a dessert, or used in bakeries for the preparation of coarse rye bread. Opium was also diluted with other latices, because it had become scarce and very valuable. Abū Alī al-Husain ibn Abdullāh ibn Sīnā(Avicenna, 980–1036), the most important physician, physicist and mathematician of Arab culture, describes in his main work *Qanun*, the Canon of Medicine, the isolation and use of opium. Theophrastus Bombastus von Hohenheim (known under the nom de plume Paracelsus, 1493–1541) (Fig. 5.36) propagated the simplified and uniform preparation of theriac (Fig. 5.37), an opium-

5.36 *Von Hohenheim used the nom de plume Paracelsus in order to protect himself from the Fugger family, by whom he felt threatened. They imported guaiacum wood for treatment of syphilis. Paracelsus spoiled their business, because he exposed the ineffectiveness of guaiacum wood extracts.*

5.37 *Theriac, a mixture of up to 300 components, was produced in huge quantities from wine all over Europe. The inscription on the barrel in the Museum of Pharmacy at Kraków reads as follows: "Vinum Creticum Generosum ad Theriaces" (Cretan noble wine for Theriac).*

To ensure standardisation and quality in Venice, the Theriac was prepared under the supervision of the Doge and the highest authorities. Already then, public surveillance of the production of pharmaceuticals became common practice. Today, the Food and Drug Administration (FDA) monitors the production of pharmaceuticals worldwide.

5.38 *Tschibuk rauchender Orientale auf einem Diwan ("Waterpipe-smoking Oriental on a divan"), 19th century painting from Carl Spitzweg (WV 656, 1856).*

containing medication, which was regarded in the Middle Ages as a fabulous panacea, and of which he wrote: je länger geschrifft, je kleiner der verstandt (the longer the recipe, the worse its meaning, or: the shorter the better).

When smoking of opium became fashionable in China during the second half of the 17th century – presumably in response of the ban on tobacco smoking under Emperor Tsung Cheng–, the East India Company cultivated the poppy in Bengal as a source of opium (Fig. 5.38).

In 1820, the Chinese authorities enacted a ban on the import of opium, presumably because of the drug problem. As a result, opium smuggling took place to an enormous extent. The destruction of 20,000 cases of opium, with a value of £4 million sterling, led to the opium war between China and Britain during the years 1840–1842.

The coming of industrialisation, and the increase in female labour in factories had the result, that children spent longer times alone at home and had to be "sedated". For this, a diluted form of laudanum (an opium tincture) was a proven method.

Up to the early 20th century, it was not only the poorer segments of the populace, who consumed drugs; well-to-do citizens and intellectuals also indulged themselves in search for new adventure and the pursuit of the ultimate 'high'. Many famous personalities fell victim to opium addiction (Tab. 5.5).

On an annual average, 1,000 deaths from narcotic poisoning are registered in Germany. In 80 % of these cases, they were due to overdosing with heroin or intoxication with mixtures of compounds. [70] An estimated number of 77 million Americans have used an illegal drug at least once in their lives; some 22 million take illegal drugs or abuse a psychotherapeutic medication constantly. [71]

The French Romantic composer Hector Berlioz (1803–1869) used opium for inspiration, subsequently producing his *Symphonie Fantastique*. [70]

Tab. 5.5 *Opium use and misuse extend from ancient times up to the present.*

Person	Date of birth/death	Occupation	Reason
Aurelius, Marcus	121–180	Roman Emperor	Egypt. beans: Immunisation
Baudelaire, Charles	1821–1867	Author	Laudanum: addiction
Berlioz, Hector	1803–1869	Composer	opium for inspiration
Byron, George Gordon Noel (Lord)	1788–1824	Author, poet	Opium: addiction
Cocteau, Jean	1889–1963	Director, poet	Opium: addiction
Coleridge, Samuel Taylor	1772–1834	Poet, *"Kubla Khan"*	Opium: addiction
Fallada, Hans	1893–1947	Author	Morphine: addiction
Grabbe, Christian Dietrich	1801–1836	Jurist, author	Opium: to counter alcoholism
George V	1865–1936	King of Britain	Morphine: aid to death
Goering, Hermann	1893–1946	Nazi politician, war criminal	Morphine: pain management, addiction
Hadrian, Publius Aelius	76–138	Roman Emperor	Opium: control of grief
Hall, Robert	1764–1831	Preacher	Laudanum: addiction
Halsted, William Steward	1852–1922	Physician	Opiate: addiction
Heine, Heinrich	1797–1856	Author, poet	Opium: addiction
Hendrix, Jimmy	1942–1970	Singer	Opioids: addiction
Hoffman, Philip Seymour	1967–2014	Actor	Heroin: addiction
Hoffmann, E. T. A.	1776–1822	Musician, poet	Opium: to counter alcoholism
Joplin, Janis	1943–1970	Singer	Heroin: addiction
Keats, John	1795–1821	Poet	Opium: addiction
Lincoln, Mary Todd	1818–1882	First Lady of the US	Laudanum: addiction
Maximilian of Mexico	1832–1867	Emperor	Morphine: addiction
Novalis	1772–1801	Poet	Opium: addiction
Piaf, Edith	1915–1963	Singer	Morphine: addiction
Poe, Edgar Allan	1809–1849	Poet, *"Ligeia"*	Laudanum: suicide attempts
Presley, Elvis	1935–1977	Singer	Opioids: addiction
Quincey, Thomas de	1785–1859	Author, *"Confessions of an English Opium Eater"*	Opium: addiction
Richelieu, Louis F. A. du Plessis de	1696–1788	Cardinal	Opium: addiction
Scott, Sir Walter	1771–1832	Jurist, poet, novelist	Laudanum: addiction
Thompson, Francis	1859–1907	Poet	Opium: addiction experiences
Verne, Jules	1828–1905	Author	Morphine: pain relief
Voltaire, Francois-Marie Arouet	1694–1778	Philosopher	Opium: pain relief
Wilberforce, William	1759–1833	British politician, leader of the movement to abolish the slave trade	Laudanum: addiction

5.3.2 Physiology and Pharmacology

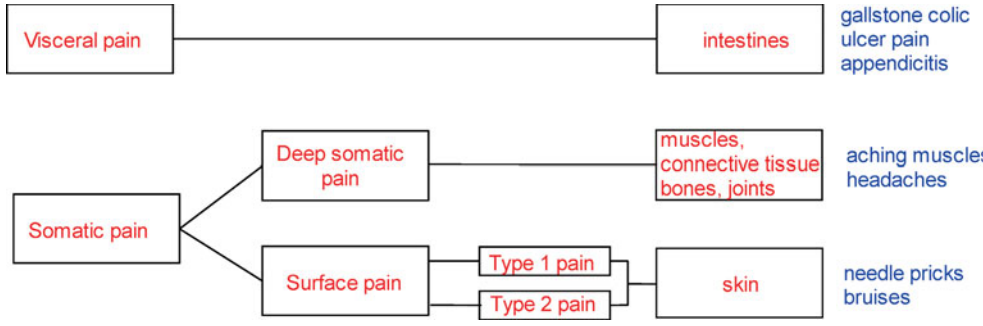
5.39 *One of the first scholars to write about sensitivity to pain (nociception) was René Descartes (1596–1650). His book "Traité de l'homme" ("Treatise of Man") was only published posthumously, out of fear of the inquisition.*

Experience of pain is the most frequent symptom of a noxa (illness, injury, damage or strong irrritation; Latin, *noxa*: harm). [72–74] Pain has both, a warning and a protective function (Fig. 5.39 and Fig. 5.40). Those areas sensitive to pain are the entire outer skin, a large part of the mucous membranes and numerous tissues and organs in the body's interior.

A distinction can be made between visceral pains, which originate in the intestines, and somatic pains, which can be localised on the skin, in muscles, connective tissue, bones and joints. Visceral pain is dull and resembles those reactions, which accompany deep pain.

Somatic pain is subdivided into deep pain, which often cannot be precisely localised and spreads into the surroundings, and surface pain, which can generally be well localised. The latter may be further subdivided into the initial pain, which normally induces a reflexive flight reaction (like the pulling away of a finger from a hot cooker plate), is easily localised, and rapidly abates after the end of the stimulation, whereas the second type of surface pain appears after a short interval as dull and burning; it is more difficult to localise and subsides only more slowly.

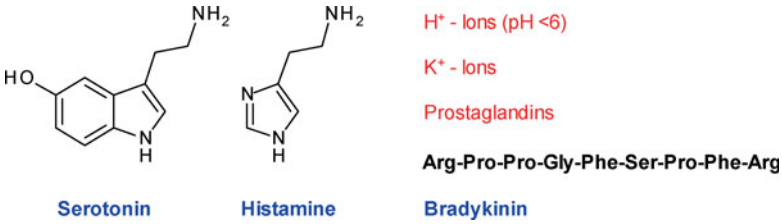
By "acute" pain one understands a pain of limited duration, which rapidly subsides after removal of the cause. Chronic pains last longer, as with back pains and tumour pains, or are recurring pains, like migraine-type headaches or heart pains of *Angina pectoris*.



5.40 *Pain can be subdivided according to its quality and localisation.*

As a rule, one refers to chronic pains, if they exceed six months' duration.They may dominate the symptoms of the underlying illness, and establish an independent disease syndrome.

The Pain-mediating System



The triggering, transmission and the central mechanisms of processing pain is termed nociception (Latin, *nocere*: pain; *receptere*: to receive). Pain is triggered by pain-causing compounds:

- Hydrogen and potassium ions are of low potency in this respect. By lowering of the pH value to less than 6, or by raising the potassium ion concentration in the interstitium above 20 mmole/l, a feeling of pain can be triggered.
- Histamine is a strong elicitor of pain at concentrations $>10^{-8}$g/l.
- Acetylcholine sensitises pain receptors at low concentration. At higher concentrations it can trigger pain on its own.
- Prostaglandins are likewise sensitisers, and are involved decisively in long-lasting pain.
- Serotonin is a highly potent pain-causing substance.
- Kinins, especially bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), are among the compounds, which are most powerful in causing pain.

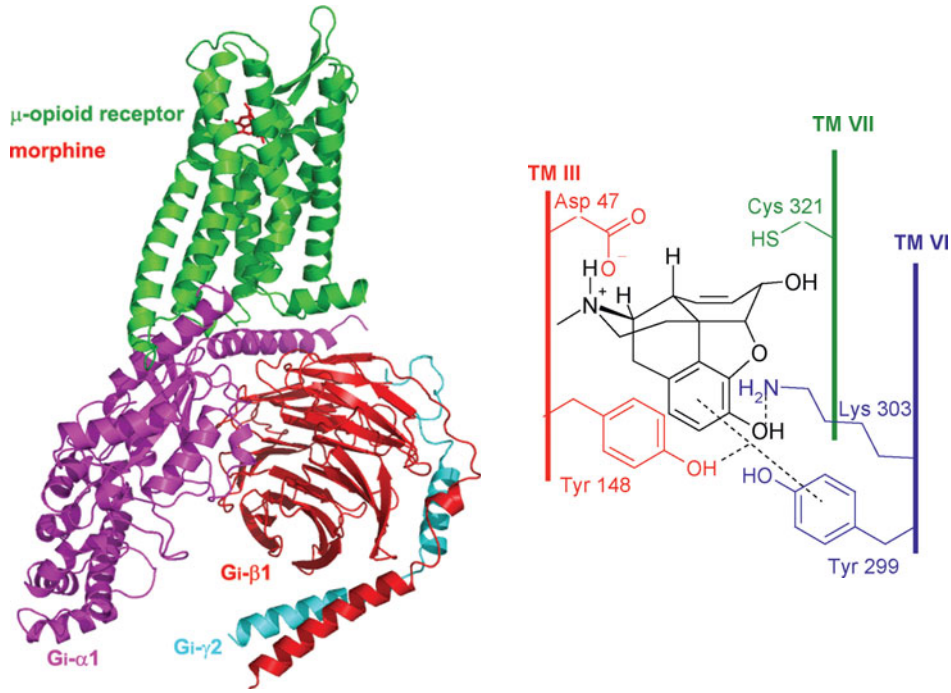
Pain stimuli are picked up by pain receptors on free nerve endings. Depending on their site of origin, these signals are transmitted through various nerve fibres into the spinal cord, from where they reach the thalamus and the cerebral cortex. In addition, during the sensation of pain, the limbic system as well as the hypothalamus contribute to the emotional and vegetative reactions respectively.

With pain, catecholamines (dopamine, noradrenaline, adrenaline) are released, heart-rate and blood pressure increase, the pupils are dilated, and possibly motor reactions work to counteract pain generation. With visceral pain, it can bring about nausea, vomiting, sweating and a fall in blood pressure. The emotional processing of pain can be individually quite unique and situation-dependent.

The Pain-relieving System

Apart from the pain-mediating system, there also exists an endogenous pain-relieving system, primarily in the brain stem and spinal cord, which attenuates the transmission of pain signals. This maintains in the interim the capacity of the body to act, even with strong and paralysing pain. The pain-relieving system possesses opiate receptors, which are divided into three groups, denoted by the Greek letters μ, δ and κ (Fig. 5.41). [75, 76] Activation of the μ-receptors has an analgesic effect and creates euphoria; however, it also causes dependence and

respiratory depression. On the other hand, activation of the κ-receptors in the spinal cord leads to decreased hyperalgesia. However, other serious side-effects may occur, such as drowsiness, dysphoria, hallucinations and disturbance of spatial perception. The importance of the δ-receptors in analgesia is a matter of debate.



5.41 *Opioid receptors are G-protein-coupled pre- and post-synaptic receptors, with seven transmembrane domains (TM), to which opioids bind.*

Opioids

It is common to all receptors that, in case of binding to agonistic opioids, they reduce the neurotransmitter concentration in the synaptic gap of the pain-mediating nerve tract.

Among the body's own opioids are β-endorphin (*endorphin* = *end*ogenic-m*orphine*), its *N*-terminal pentapeptide methionine-enkephalin, and the very similar leucine-enkephalin (Fig. 5.42). Closely analogous are dynorphin A, dynorphin B and *α*-neoendorphin. [77] They originate from the corresponding precursor peptides in the brain, the pituitary gland and the adrenal medulla. According to more recent knowledge, endogenous non-peptidic morphine derivatives also serve to control pain.

The structural commonality between the peptidic opioids and the opiates themselves is tyrosine, which is still recognisable in the morphine structure. What significance this moiety has for interaction with the opioid receptors, may be concluded from the fact that endorphins, which bear no tyrosine at the *N*-terminus, are not active (Fig. 5.43). [78]

Rats, which suffer from arthritis have an elevated morphine concentration in their spinal cord and urine. It is presumed, that also the human body produces endogenous morphine for pain regulation during the course of particular illnesses.

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH

β-Endorphin

μ-selective

Tyr-Gly-Gly-Phe-Met

Methionine-Enkephalin

δ-selective

Tyr-Gly-Gly-Phe-Leu

Leucine-Enkephalin

δ-selective

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys

Dynorphin A

κ-selective

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Leu-Leu-Thr

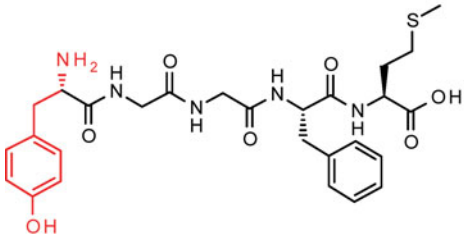
Dynorphin B

κ-selective

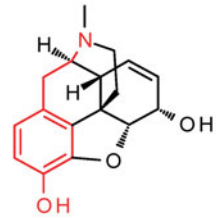
Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys

α-Neoendorphin

κ-selective

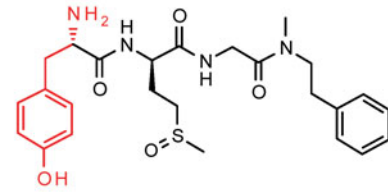


Tyr-Gly-Gly-Phe-Met



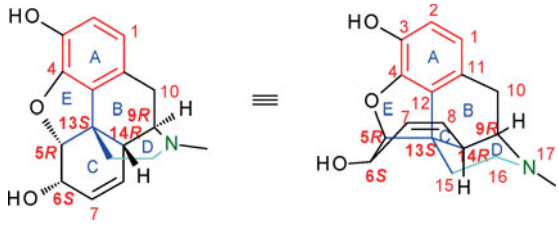
(-)-Morphine

μ-selective



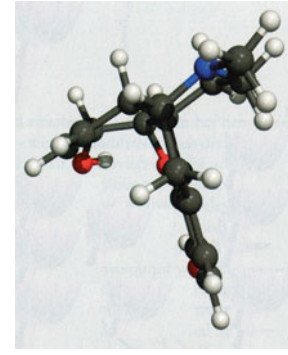
Syndyphalin-33

5.42 *Hitherto, enkephalins have found no medicinal use as analgesics, since they are rapidly degraded in the body, cannot cross the blood-brain barrier, and moreover cause addiction. Targeted structure modification leads to metabolically stable tripeptides, the syndyphalins; these surpass morphine in their analgesic potency by a factor of around 20,000.*



5.43 *The "analgesic core structure" of opiates is characterised by a flat aromatic system next to an (S)-configured (mostly) quaternary carbon atom, which generates a T-shaped structure, and by a basic nitrogen atom, located two carbons away from the quaternary centre.*

Crystal structure analysis and quantum mechanical calculations confirm the T-shape of morphine (Fig. 5.44). This corresponds optimally to the structure of the opioid receptors in the brain and spinal cord.



5.44 *Morphine has a T-shaped structure.*

For reasons of uniformity, the projection formula of the morphine skeleton, as proposed by Sir Robert Robinson in 1925, is hereafter used exclusively.

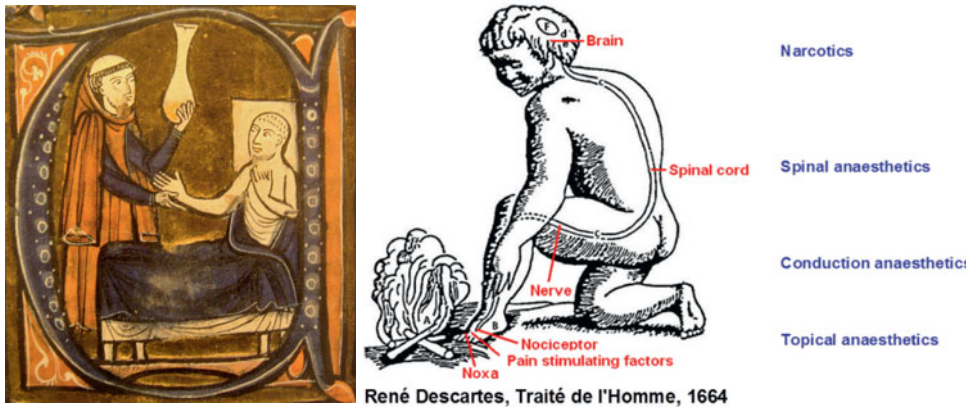
Because of the similarity in several structural characteristics, one can understand why opiates bind to the same receptors as opioid peptides. Nevertheless, there are differences in pharmacokinetics, since due to their peptidic structure, endorphins can be rapidly hydrolysed by proteases, whereas opiates cannot.

Each opioid has an individual, characteristic receptor binding profile. Apart from controlling pain, opioid peptides also have an impact on emotional life. Pain relief using acupuncture or from placebo preparations is attributable to these compounds in the same way as is euphoria while running a marathon.

Analgesics

Based on our nowadays understanding of pains, it has been realised, that these can be controlled in a number of different ways by pain-killers, called analgesics (Fig. 5.45). Analgesics are substances, which, at the proper therapeutic dose, reduce or suppress the sensation of pain without commonly exhibiting a narcotic effect. As to their site of action, two groups of agents are differentiated:

- Hypnoanalgesics with mainly central action (especially opioids).
- Non-opioid analgesics with predominantly peripheral action, mostly inhibiting prostaglandin synthesis (conductive and surface anaesthetics).



5.45 *The transmission of pain and the possibilities for anaesthesia.*

The Persian physician, Abu Bakr Muhammad b. Zakariya ar-Razi, دمحم ركب وبأ ايزارلا ايركز (864 –925) was one of the first to describe the use of opium as an anaesthetic. Later, at the famous translation school in Toledo, Gerard of Cremona (1114–1187) translated Razi's "Book of Medicine" and thereby made the extensive knowledge of Arabian physicians accessible to Western medicine.

Morphine, the prototype of opioid analgesics, acts at all locations of the spinal cord and in the brain, where the signal transduction of pain takes place. The compound acts as a selective agonist at the μ-receptors and thus reduces the experience of pain and its emotional assessment. This occurs by reduction of the mental activity (sedation) and elimination of the feelings of conflict and anxiety (tranquillisation). Moreover, morphine raises the release of dopamine in the brain, which is the cause of an euphoric feeling during pain treatment. Another central effect concerns a lower breathing rate, caused by a reduced sensitivity of the respiratory centre towards carbon dioxide, (breathing depression) and suppression of coughing (antitussive activity). A noticeable effect of μ-receptor agonists is also contraction of the pupils. Peripheral signs are a delayed emptying of the stomach, severe constipation

(spastic obstruction), overfilling of the bladder, and a disturbance of bile flow.

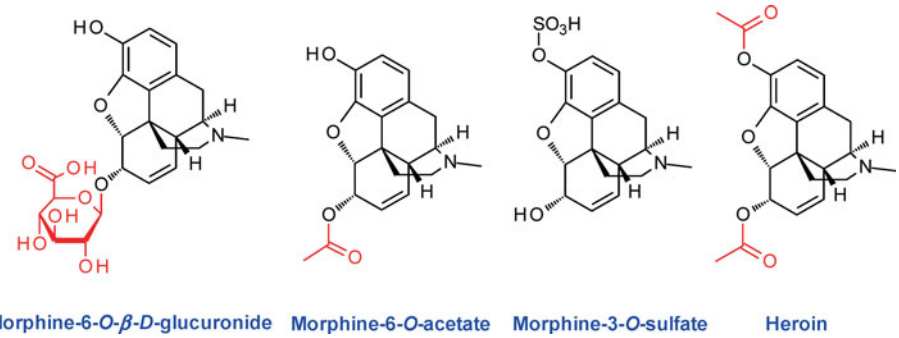
Tolerance development and physical and mental dependence are among the most well-known of the undesirable effects of morphine. "Tolerance development" refers to the phenomenon that prolonged medication to attain a particular effect requires higher and higher doses. For morphine, such dose escalations amount to a 10- or 20-fold increase.

"Physical dependence" refers to the fact that opioids become indispensible for certain bodily functions. After the cessation of treatment, withdrawal symptoms may appear (motor unrest, fever, pupil dilation, vomiting, rise in blood pressure and heart rate, muscle cramps).

The term "mental dependence" is based on the euphoric effect of opioids. The loss of euphoria, concurrent with the appearance of physical withdrawal symptoms, makes it even more difficult for patients to overcome their addiction. For the therapeutic treatment of addiction, as well as for acute opiate poisoning, opiate antagonists (*e.g.* Naloxone) have been developed.

The bioavailability of morphine (pKa= 8.1), because of its high degree of ionisation in blood (pH = 7.4), lies at only around 20–30 %. However, morphine is glycosylated in the liver at C-3 and C-6 with glucuronic acid. Whereas the 3-glucuronide has no opiod activity, the 6-glucuronide is able to cross the blood-brain barrier more easily than morphine, and shows a stronger and longer-lasting effect. Morphine 6-*O*-acetate and morphine 3-*O*-sulfate, which could be detected as metabolites in humans and other mammals, proved as well more potent than morphine. The same is true for heroin as well (Fig. 5.46). Because of its higher lipophilicity, heroin crosses the blood-brain barrier around 100 times better than morphine and is *ca.* 6 times more effective.

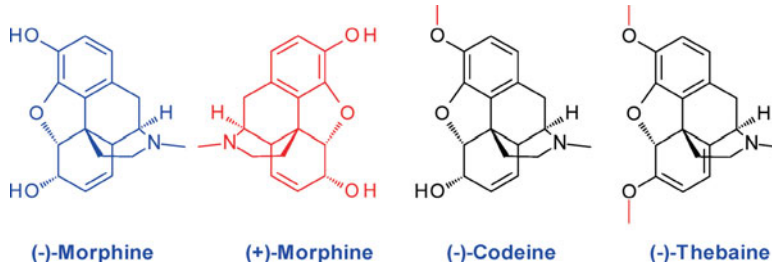
The disruption of breathing is a common symptom and the cause of death from an overdose of opiates.



5.46 *Metabolites and derivatives of morphine.*

(–)-Morphine is also still in use today for extreme pain, as in cases of severe burns or open fractures, where its application is limited to emergencies, because of the significant risk of addiction. Conversely (+)-morphine, like (+)-codeine or (+)-heroin, possesses no analgesic effect. By comparison with (–)-morphine, (–)-codeine is much less effective; however, it inhibits selectively the cough centre, and has a substantially lower potential for addiction. It is therefore the main component of many cough syrups. Thebaine, on the other hand, produces like strychnine severe convulsions, and finds therefore no therapeutic applica-

tion, while it is, along with morphine, the most important starting material for semi-synthetic opiates (Fig. 5.47). [79]



5.47 *Opioid receptors are highly selective. Already small structural modifications may alter the activity spectrum of the drug.*

5.3.3 Botany and Cultivation

Nowadays, around 200 species of the poppy genus (Latin, *Papaver*) are known. The poppy is found across almost the whole land area of the Earth, initially excepting South America. During the First World War, the American Mafia feared being cut off from supplies of the raw material by the intensified war at sea, and therefore had poppy fields planted, first in Mexico and later in Colombia.

Familiar species include the Oriental poppy (*Papaver orientale*), the Corn or Field poppy (*Papaver rhoeas*), the Long-headed poppy or Blindeyes (*Papaver dubium*), the Alpine poppy (*Papaver alpinum*) and the Opium poppy (*Papaver somniferum*; *Somnus*: Roman God of sleep) (Fig. 5.48). The latter one, with its sub-species of Grey poppy and Blue poppy, is a cultivated plant, which presumably originates from the Poppy of Troy (or Dwarf Breadseed Poppy, *Papaver setigerum*) and has been grown for ages as a source of oil, spice, drugs and smoking materials, and also as an ornamental plant in the Euro-Asiatic region.

5.48 *The petals and seed-capsules of the opium poppy and a field of corn poppy. The bright red colour of the petals of corn poppy stems from the anthocyanin dyestuff, mecocyanin, which were used in former times for the manufacturing of red ink. – The aglycones of anthocyanin dyes are called anthocyanidins, the basic structure of which (red) is a 2-phenylbenzopyrylium salt. Many other blue and red petal colours are also due to anthocyanidins, e.g. those of scarlet pelargoniums and orange dahlias (pelargonidin), red roses, red dahlias, black cherries and plums (cyanidin), as well as violet pansies and black grapes (delphinidin).*

The opium poppy is an annual plant, containing a milky juice, which grows to a height of *ca.* 1.5 metres, with serrated, grey-green leaves and individual white to red-violet blossoms. These form a hat-shaped fruit capsule (pod) with scars, which upon ripening develop holes at their endings to release the numerous seeds, which are mostly blue-black, rich in oil and as fine as grains of sand.

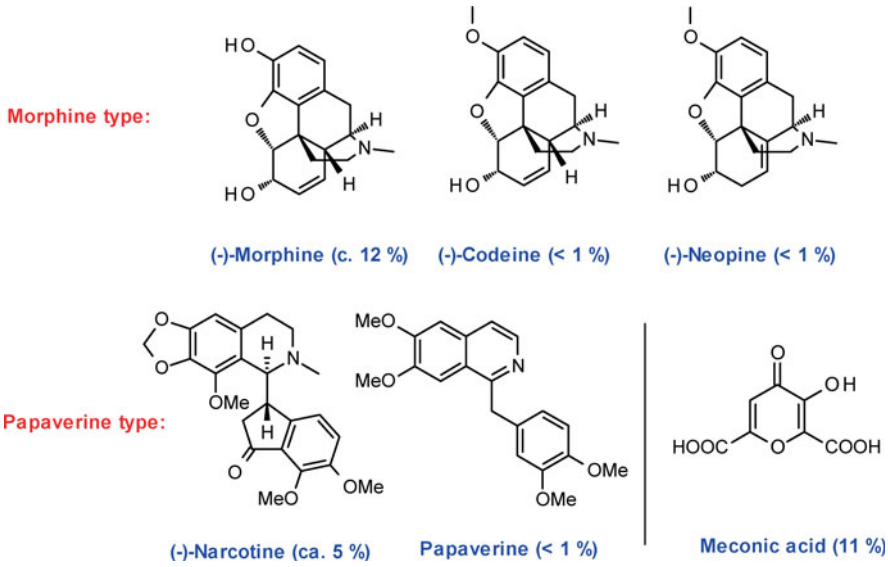
Important areas for growing poppy legally are found in India and Australia, followed by the USA, Turkey, Spain and France. In terms of output of opium, the most important producers are Afghanistan, Pakistan and the countries of the "Golden Triangle", namely Myanmar (Burma), Laos and Thailand.

In 2011, more than 5,800 tonnes of opium were produced illegally in Afghanistan. Due to adverse weather conditions, the opium production in 2012 was estimated at only 3,700 tonnes. [80]

5.3.4 Ingredients of Poppy

Morphine alkaloids are a characteristic of the plant genus *Papaver*. However, for medical use, in only two of the species, *Papaver somniferum* and *Papaver bracteatum*, opiates are found in quantities sufficient for industrial exploitation.

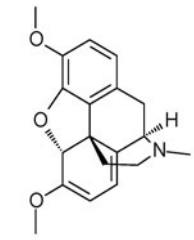
Indian opium (from *Papaver somniferum*) contains more than 30 different isoquinoline alkaloids (22 %), which can be divided into those of a morphine type and those of a papaverine type (Fig. 5.49). The main alkaloids are morphine (Greek *Morpheus*: God of dreams), around 12 %, and narcotine, around 5 %. The remainder comprises meconic acid (11 %), water (14 %), sulfuric and lactic acid (8 %), fats, proteins, sugars and waxes (*ca.* 44 %). [72, 81]



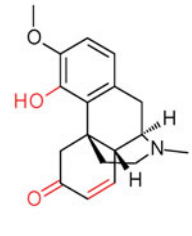
5.49 *The alkaloids of Papaver somniferum.*

The main alkaloid in the juice of *Papaver bracteatum* is thebaine (up to 26 %) (Fig. 5.50). [82]

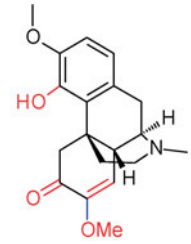
Poppy seeds contain up to 50 % fatty oil, along with protein and lecithin, but only traces of alkaloids.



(-)-Thebaine (< 26 %)



(-)-Thebainone



(+)-Methoxy thebainone

5.50 *In 1835, Pierre-Joseph Pelletier (1788–1842) discovered thebeine, the main alkaloid of Papaver bracteatum.*

Alcoholics and morphine-takers use, directly or indirectly, the Pictet-Spengler reaction for the synthesis of isoquinoline alkaloids. [84] In the liver, with the production of the corresponding redox equivalents of NADH, ethanol is oxidised *via* acetaldehyde to acetic acid. The kinetics of the reactions is such that for an extended period during the alcohol degradation, there exists a quasi-stationary concentration of 1 μg/ml acetaldehyde in the blood. Acetaldehyde reacts with the catecholamines to produce tetrahydroisoquinolines. These interact competitively at the catecholamine receptors, and thereby disturb the equilibrium of the vegetative nervous system, which in the course of time leads to addiction.

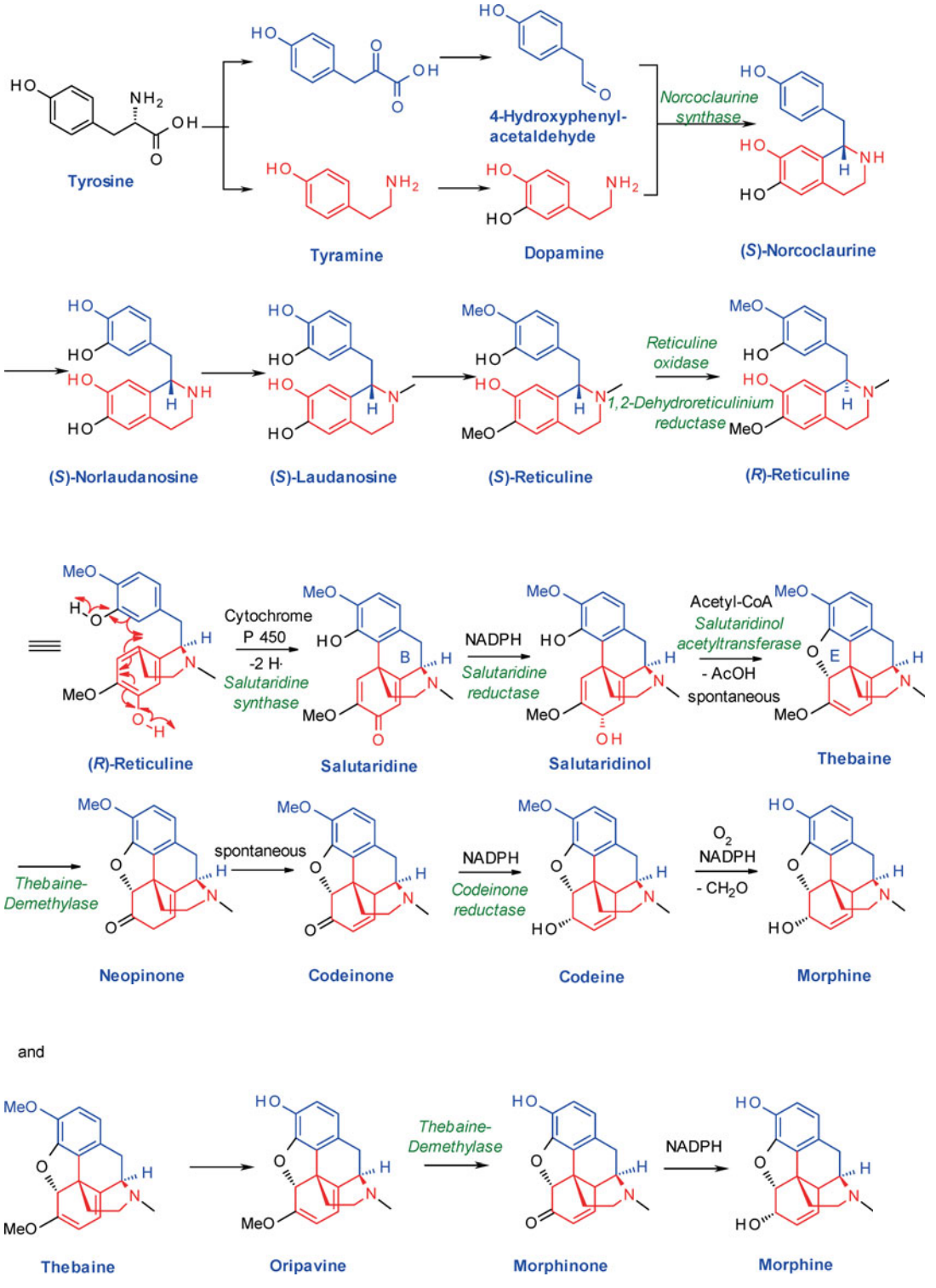
Reticuline is an important alkaloid, which is remarkably found in Nature in both enantiomeric forms: (*S*)-(+)-reticuline in *Annona reticulata* (the fruit is called custard-apple, also bullock's heart or bull's heart), in *Phylica rogersii*, and in *Papaver somniferum*, while (*R*)-(–)-reticuline occurs in Coulter's Matilija poppy (*Romneya coulteri var. trichocalyx*). [83]

5.3.5 Biosynthesis

The biosynthetic pathway to morphine in *Papaver somniferum* comprises 15 distinct stages, which could be elucidated by labelling experiments and enzyme studies. [81, 83] The starting material for benzylisoquinoline alkaloids is tyrosine; this is either degraded by deamination and decarboxylation into 4-hydroxyphenylacetaldehyde, or else by decarboxylation and aromatic hydroxylation to dopamine. These two compounds then condense in an enzyme-catalysed Pictet-Spengler reaction (an intramolecular Mannich reaction) to (*S*)-norcoclaurine, which is hydroxylated, and then stepwise methylated, first at the nitrogen and then at two of the hydroxy-groups, to give (*S*)-reticuline.

In the opium poppy then follows the dehydrogenation to an iminium salt, and an enantioselective hydrogenation with NADPH. Thereby, the stereogenic centre is inverted to (*R*)-reticuline, as proven by tritium labelling. Cytochrome P450 catalyses the oxidative, radical phenol coupling to salutaridine. This reaction is highly regio- and stereoselective and similar to the synthesis of Pummerer's ketone [81], which results from oxidation of *p*-cresol. Subsequently, the carbonyl group of the quinonoid system is reduced with NADPH to give salutaridinol. Acetyl-Coenzyme A acetylates the hydroxy-function at C-7, whereupon a spontaneous cyclisation to thebaine ensues. Demethylation at C-6 is accompanied by a double bond migration, and the isomerisation of neopinone to codeinone follows spontaneously. (This is one of the rare instances in the biosynthesis of natural products, where a reaction is not catalysed by enzymes.) Afterwards, codeinone is reduced enantioselectively with NADPH to codeine, and finally demethylated to produce morphine.

In addition, another biosynthetic pathway from thebaine to morphine can be demonstrated in the opium poppy. The stepwise demethylation of thebaine leads *via* oripavine to morphinone, and finally by reduction to morphine.


Tyrosine
4-Hydroxyphenyl-acetaldehyde
Tyramine
Dopamine
Norcoclaurine synthase
(S)-Norcoclaurine
(S)-Norlaudanosine
(S)-Laudanosine
(S)-Reticuline
Reticuline oxidase
1,2-Dehydroreticulinium reductase
(R)-Reticuline
(R)-Reticuline
Cytochrome P 450
-2 H·
Salutaridine synthase
Salutaridine
NADPH
Salutaridine reductase
Salutaridinol
Acetyl-CoA
Salutaridinol acetyltransferase
- AcOH
spontaneous
Thebaine
Thebaine-Demethylase
Neopinone
spontaneous
Codeinone
NADPH
Codeinone reductase
Codeine
O2
NADPH
- CH2O
Morphine
and
Thebaine
Oripavine
Thebaine-Demethylase
Morphinone
NADPH
Morphine

5.51 *Also in amphibians like the cane toad, Bufo marinus, considerable amounts of morphine can be detected in the skin.*

By targeted mutation of *Papaver somniferum*, it was possible to knock-out the enzyme thebeine-demethylase, which is responsible for the 6-*O*-demethylation of thebeine, and as well oripavine. Correspondingly, these mutants produce only thebeine and oripavine, and are thus free of morphine and codeine. [85]

Morphine can be detected in the skin of toads (Fig. 5.51), [86] rats and rabbits, as well as in the hypothalamus and kidneys of cattle and the cerebrospinal fluid of humans. Also breast milk and cow's milk contain 200–500 ng/litre of morphine. It is therefore assumed, that all species employ the same biosynthetic route as the opium poppy. [87] Meinhart H. Zenk (1933–2011) was able to prove that human neuroblastoma and pancreas-carcinoma cells can produce [^{18}O]-labelled morphine in the presence of $^{18}O_2$. [88]

5.3.6 Preparation of Opium and Opium Alkaloids

5.52 *Scratched poppy capsule.*

GlaxoSmithKline, Johnson and Johnson, Johnson Matthey and Mayne produce opiates in Tasmania and Australia, Sanofi Aventis in France, Shionogi in Japan and Macfarlan Smith (now part of Johnson Matthey) in Britain. Mallinckrodt, Noramco, Abbott Laboratories and Purdue Pharma import these from Turkey and India to the USA.

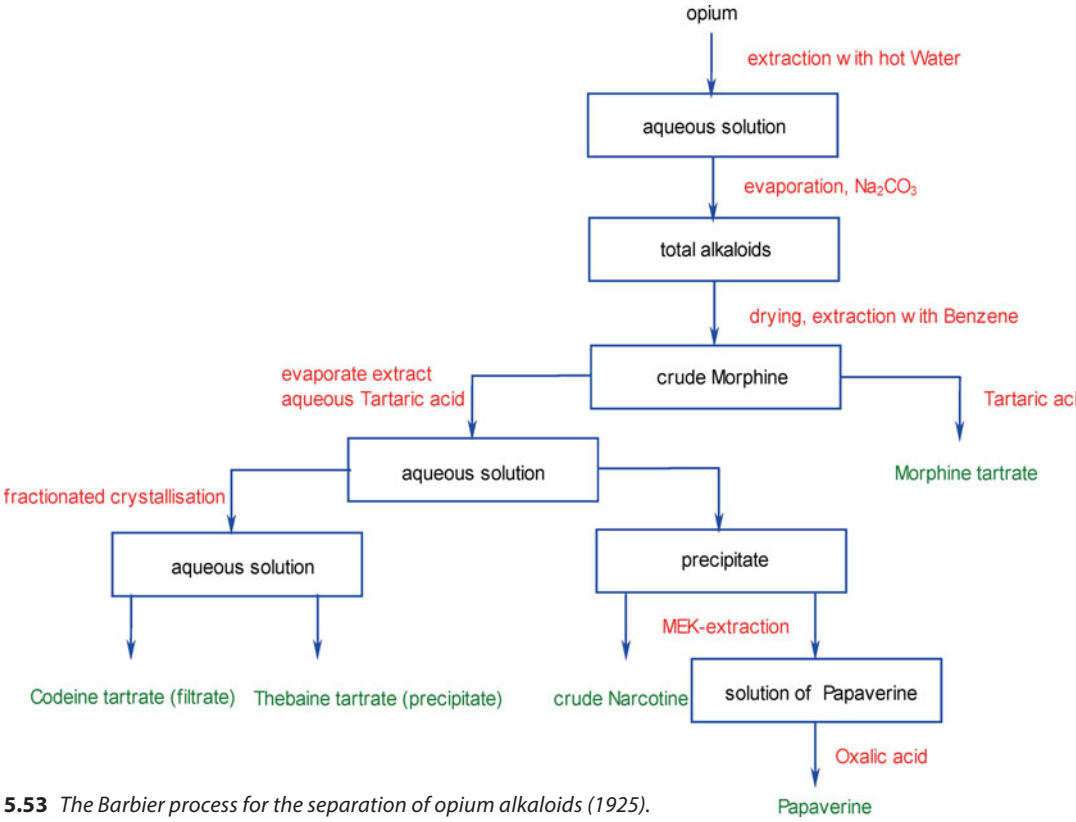
Between blooming and ripening, the opium poppy produces in its tubular system a milky juice, which can be harvested 15 days after the petals fall by scratching the unripe fruit capsules. The exuded rose-coloured juice hardens in the air to resinous brown crude opium, which is then wiped off the capsules. By repeated scratching, three or four additional crops of crude opium can be harvested (Fig. 5.52).

The further work-up of the crude opium comprises several steps. The crude opium is heated in copper pots, and then the brew is squeezed, formed into a cake and reheated. The paste is allowed to oxidise in the air, whereby it obtains its aroma, and afterwards it is ripened for four to five months in stone jugs. The opium consumer prepares his smoking material for him(her)self: For this, the opium is boiled three times with distilled water and filtered off. The product thus obtained is syrup-like and almost completely water-soluble. [82]

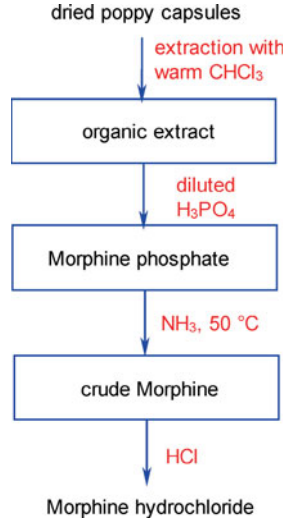
The commercial preparation of morphine takes place from opium and from poppy straw at approximately equal proportions. [72] Since 1960, the production of thebeine has gained growing significance, due to an increased demand for semi-synthetic opiates. This is generated from opium like morphine, codeine and papaverine. The key step is removal of the crude morphine with tartaric acid. The various alkaloids are subsequently separated by fractional crystallisation and extraction (Fig. 5.53).

The key advantage in obtaining morphine from poppy straw lies in the high degree of mechanisation of the agricultural cultivation methods. "Poppy straw" refers to the dried capsules, free of seeds, which are crushed. Since poppy seeds for the food industry are as profitable as morphine for the pharmaceutical industry, the capsules are allowed to ripen – although green capsules produce more morphine, and the drying process entails additional costs. The yield of morphine from poppy straw amounts to around 10 % (Fig. 5.54). Thebeine is accessible from *Papaver bracteatum* by similar procedures.

While in 2011, approximately 7,000 tonnes of opium were illegally produced from the cultivation of 207,000 hectares of poppy plantations, the production figures vary considerably; thus, in the preceding year 4,736 tonnes were reported.



5.53 *The Barbier process for the separation of opium alkaloids (1925).*



5.54 *The Merck process for the isolation of morphine (1945).*

Heroin is still the most frequently used opiate. The United Nations estimate that in 2011 between 12 and14 million heroin addicts worldwide have consumed 467 tonnes of this drug. [80] According to a United Nations report, the current annual demand for morphine and thebeine as raw materials for medical purposes ranges around 450 and 150 tonnes respectively. [82]

5.3.7 Discovery of Morphine

5.55 *Friedrich Wilhelm Sertürner (1783–1841)*

The history of the discovery of the poppy alkaloids begins with Charles Derosne (1780–1846), a pharmacist from Paris, who in 1803 isolated narcotine and named it "Sel de Derosne". The French chemist Antoine Baumé (1728–1804) had also isolated this compound. In the same year, Friedrich Wilhelm Sertürner (Fig. 5.55), a pharmacist in Paderborn, Germany, isolated the *principium somniferum* (Latin: "the sleep-making principle") of opium, which he later (1817) named "morphine". [89] Sertürner also recognised its alkaline character. As a result, the pharmacist Meißner introduced the term "alkaloids" in 1818. Sertürner tried out morphine in animal experiments, and on himself – something, which he barely survived. Purified morphine elicited a greater biological response than opium, and Sertürner became addicted for the rest of his life.

The world's oldest pharmaceutical companies, such as MacFarlan Smith Ltd. in Edinburgh or Merck at Darmstadt, were founded on the alkaloid business.

MacFarlan's history as an apothecary supplier can be traced back to 1780. In 1815, John Fletcher MacFarlan, licentiate of the Royal College of Surgeons, became owner of the family business and began to manufacture laudanum. Since 1832, the company was able to produce chemically pure morphine as its acetate and hydrochloride. [90]

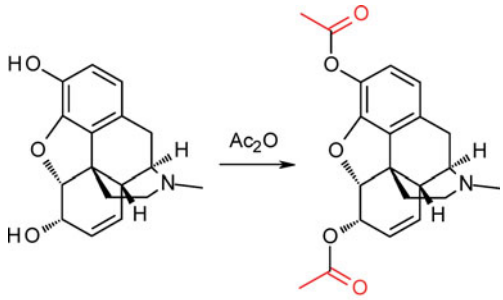
Almost at the same time, in 1827, the Prussian Government allowed physicians and pharmacists in Germany to acquire drugs from factories, which would otherwise require laborious procedures. Heinrich Emanuel Merck (1794–1855) took advantage of this act and started his business with alkaloids, which he had isolated or prepared in purified form (Fig. 5.56 and Fig. 5.57). The example of Merck in Darmstadt was followed later by Schering and Riedel in Berlin. [90]

During the American Civil War (1861–1865) opium was still used for the treatment of wounded soldiers. However, the dosage of pure morphine proved more reliable, and the invention of the hypodermic syringe by the French physician Charles-Gabriel Pravaz (1791–1853) enabled a superior mode of administration. Morphine became the preferred drug for pain treatment.

5.56 *Since 1668, the Engel pharmacy in Darmstadt has been under the ownership of the Merck family.*

5.57 *Heinrich Emanuel Merck (1794–1855) produced morphine in the Engel pharmacy, and sold it to other pharmacists, chemists and physicians.*

In 1834, codeine was isolated from opium by Pierre Jean Robiquet (1780–1840). One year later, Pierre-Joseph Pelletier (1788–1842) and M. Thiboumery discovered thebaine (named after the ancient Egyptian city of Thebes). In 1848, at the University of Giessen, papaverine was extracted from poppy waste by Georg Merck (1825–1873), the son of Heinrich Emanuel Merck. In 1870, Augustus Matthiessen (1831–1870) and Charles R. A. Wright (1844–1894) recognised that codeine is the monomethyl ether of morphine. After the selective methylation of morphine for the preparation of codeine had failed, Felix Hoffmann (1868–1946) tried out in 1897 a selective acetylation, in analogy to the synthesis of aspirin from salicylic acid. [91]



Morphine Heroin

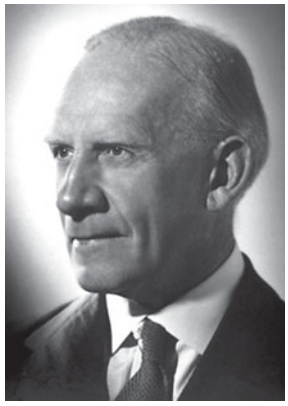
Heroin was first synthesised in 1874 by Charles Romley Alder Wright (1844–1894), an English chemist, working at St. Mary's Hospital Medical School in London. However, his invention did not lead to any further developments, until it was more than two decades later independently re-synthesised by Felix Hoffmann.

Just one year after Hoffmann's synthesis, the Bayer company started the production of heroin (the word is related to *heroic*, on account of its anxiolytic effect). The drug, which is also known as diamorphine, was used for the treatment of severe coughs, labour pains and to premedicate narcoses (Fig. 5.58). It was the first semi-synthetic opiate on the drug market, and found use for severely wounded soldiers during the First World War, and for certain neurotic illnesses. As a consequence of the associated potential for addiction, which had been completely underestimated in the beginning, the drug had disappeared by 1931 again from almost all pharmacopoeias.

Since the seminal work of Sertürner, there were substantial efforts to elucidate the chemical structure of the *principium somniferum*. Auguste Laurent (1807–1853) and Justus von Liebig (1803–1873), a close friend of Heinrich Emanuel Merck, correctly deduced in 1847 the empirical formula of morphine as $C_{17}H_{19}NO_3$. Liebig also realised that the basic character of morphine originates from an amine. In 1903, Freund and Becker suggested the correct structure for narcotine, which was confirmed by synthesis in 1911 by Perkin and Sir Robert Robinson (1886–1975). Degradation experiments performed by Eduard Vongerichten, Ludwig Knorr, Robert Pschorr, Heinrich Otto Wieland and also Robinson himself, finally

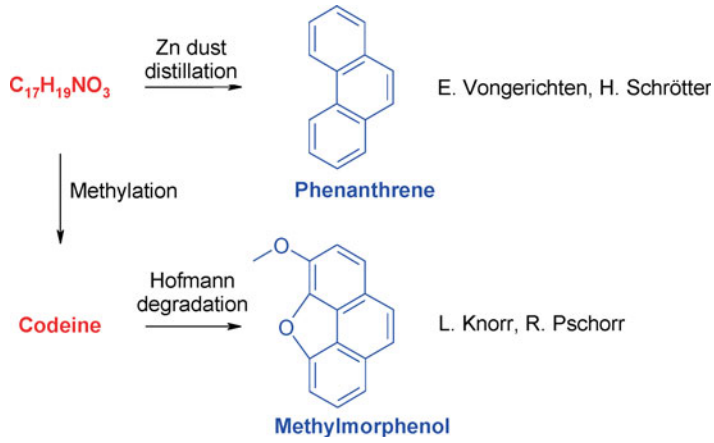
5.58 *Bottle with heroin in powder form.*

However, diamorphine continues to be widely used in palliative care in the United Kingdom. From 1995 to 2002, global manufacture of heroin varied between 200 kg and 500 kg. While in 2003, a sharp increase to 1,163 kg was noted, production declined afterwards to 66 kg in 2006. This fluctuation reflects primarily manufacturing problems in the United Kingdom. In 2011, the production reached a quantity of 900 kg. [82]



5.59 *Sir Robert Robinson (1886–1975) was an English organic chemist and Nobel laureate, recognised in 1947 for his research on anthocyanins and alkaloids.*

enabled the latter to propose the actual chemical structures of morphine, codeine and thebeine in 1925 (Fig. 5.59). [92]



Tab. 5.6 *Total syntheses of morphine.*

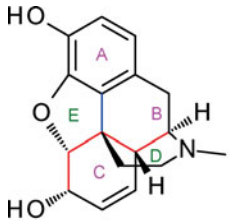
Principle Author	Date	Product	Steps	Yield (%)
Gates	1952	morphine	23	0.01
Ginsberg	1954	*rac*-dihydrothebainone	21	8.89
Grewe	1967	*rac*-dihydrothebainone	9	0.81
Rice	1980	*rac*-dihydrocodeinone (morphine)	10 (17)	29.0 (13.0)
Evans	1982	*rac*-O-methylthebainone	12	16.7
Rapaport	1983	*rac*-codeine	26	1.15
Fuchs	1988	*rac*-codeine	22	1.53
Tius	1992	*rac*-thebainone-A	28	0.97
Parker	1992	*rac*-dihydrocodeinone	12	9.42
Overman	1993	(–)-dihydrocodeinone	14	4.43
Mulzer	1996	(–)-dihydrocodeinone ((–)-morphine)	15 (22)	11.5 (5.16)
Parsons	1996	morphine		0.88*
White	1997	(+)-morphine	28	3.00
Hudlicky	1998	10-hydroxy-*ent*-*epi*-dihydrocodeinone	14	2.70
Cheng	2000	*rac*-desoxicodeine-D	15	13.3
Ogasawara	2000	*rac*-3,4-dimethoxy-6-morphinanone	29	0.25
Ogasawara	2001	(–)-dihydrocodeinone ethylene ketal	24	0.37
Taber	2002	(–)-morphine	27	0.51
Trost	2002	***(–)*-morphine**	15	5.68
Fukuyama	2009	(+/–)-morphine	25	< 9.2

**: last 5 steps.*

In 1952, Marshall D. Gates and Gilg Tschudi reported the first total synthesis of morphine. [93, 94] Since then, about 20 syntheses of racemic morphine have been published. [95] Only in 1993, Larry E. Overman could obtain (–)-morphine by an enantioselective synthesis. [96] Further syntheses followed: in 1996 by Johann Mulzer [97], in 1997 by James White [98], in 2002 by Douglas Taber [99] and Barry Trost [100], and by many others [101] as well (Tab. 5.6). Measured by the number of stages and the overall yield, the total syntheses by Rice in 1980 and Trost in 2002 are particularly noteworthy.

5.3.8 The First Enantioselective Total Synthesis of Morphine

The most demanding tasks in the total synthesis of a natural product lie in building up the target molecule by starting from simple materials, as far as possible under avoidance of protecting groups, by a short, convergent route with high stereo-and regio-control, and with high overall yield. The total synthesis of morphine poses from its very beginning one of the formidable challenges in natural product synthesis. At the centre of the problem is, as so often, the complexity of the structure (Fig. 5.60).



(-)-Morphine

3 condensed carbocycles

2 heterocycles

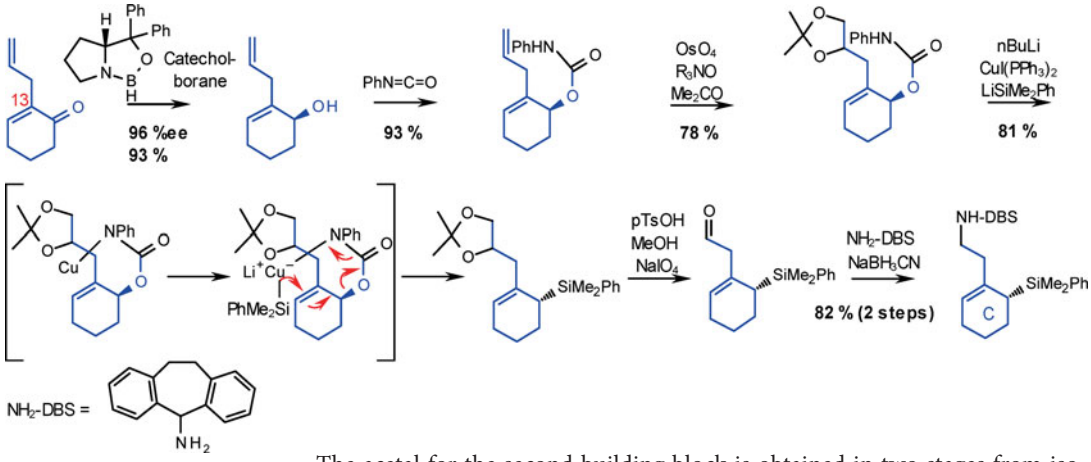
5 consecutive stereo centres

1 quaternary benzylic carbon atom

5.60 *The challenges in the total synthesis of morphine.*

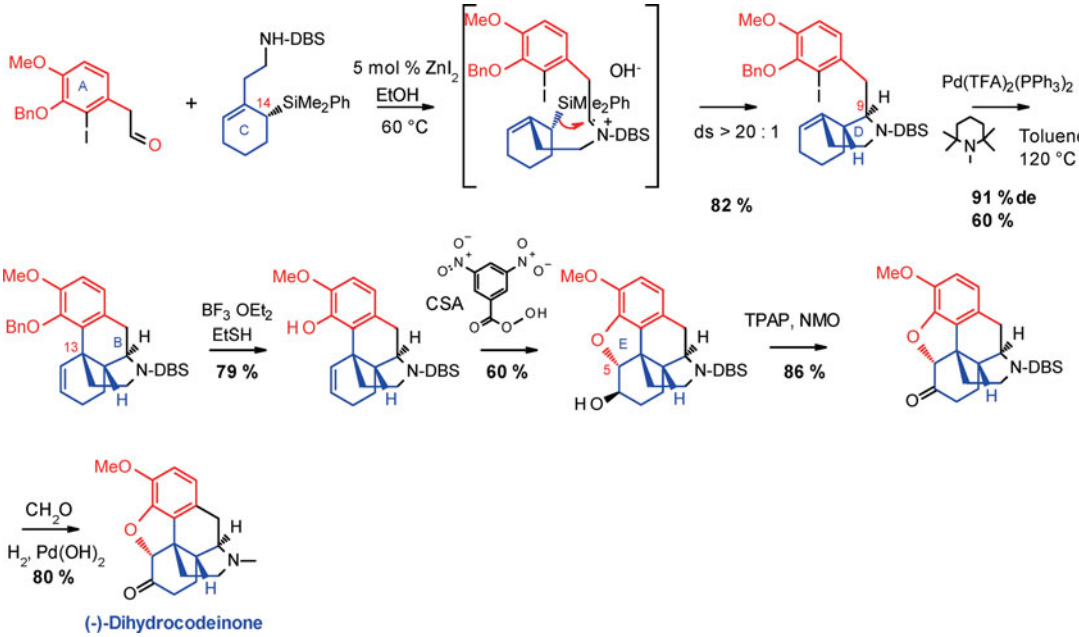
The starting point of Overman's synthesis is 2-allylcyclohexenone, which is reduced enantioselectively with a proline-derived borane. Thereby the first stereogenic centre is generated (in the eventual C-ring) with a selectivity of >96 %. From here, all of the remaining centres are constructed by diastereoselective reactions. (+)-Morphine is in consequence obtained at the end by use of the opposite enantiomerically pure reducing agent.

The reaction of the hydroxy-function with phenyl isocyanate generates a protecting group, which also serves as a leaving group for the next but one step. The catalytic dihydroxylation occurs selectively at the terminal double bond; the carbamate can then be displaced in a *syn*-facial S_N2' reaction [102] by a silyl residue, without significant loss of enantiomeric purity. Oxidative cleavage of the diol and reductive amination with dibenzosuberylamine (DBS-NH_2) gives the first central building block in this convergent synthesis, and appears ultimately as ring C of the morphine skeleton.

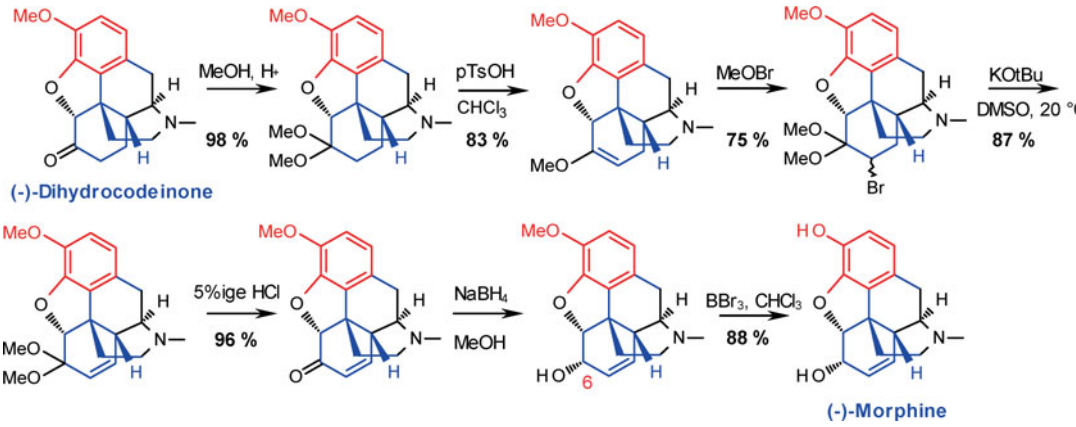


The acetal for the second building block is obtained in two stages from isovanillin in 96 % yield. After the introduction of iodine, the hydroxy-group is reprotected and the aldehyde converted into its homologue by reaction with a sulfur ylide, and then by Lewis acid-catalysed opening of the epoxide. In this way, there results an appropriate building block for ring A of the morphine skeleton.

Ring D of the morphine skeleton is constructed by a zinc iodide-mediated condensation of the two previously synthesised building blocks. The ring closure takes place from the less sterically hindered side with high diastereoselectivity. Intramolecular arylation using the Heck reaction serves to construct the quaternary centre. By migration of the double bond, ring B is closed and the third stereogenic centre generated. After the benzyl protecting group is cleaved using boron trifluoride/ethanethiol, ring E is closed by diastereoselective epoxidation and acidic *trans*-diaxial cleavage of the oxiran. Reductive cleavage of the protecting group is not allowed, because the DBS residue must still be retained. Under the reaction conditions, oxidation to the tetracyclic *o*-quinone is largely avoided. The fourth stereogenic centre is established by the epoxidation. Enantiomerically pure (–)-dihydrocodeinone is finally obtained by oxidation with tetra-*n*-propylammonium perruthenate, then reductive cleavage of the DBS group, reductive amination with formaldehyde, and a single recrystallisation.



The closing sequence comes from Kenner C. Rice and comprises seven stages. [103] Acetalisation of the keto-function and elimination of methanol with *p*-toluenesulfonic acid in chloroform is followed by addition of methyl hypobromite. Elimination of HBr and hydrolysis of the ketal leads to the unsaturated ketone, which is reduced stereoselectively with sodium borohydride to produce (–)-codeine. This generates the fifth stereogenic centre. The methyl ether is finally cleaved with boron tribromide.



Due to the complexity of the total synthesis, the relatively low overall yield, and the comparatively easy accessibility of morphine from natural sources by extraction, this synthesis is not of industrial interest.

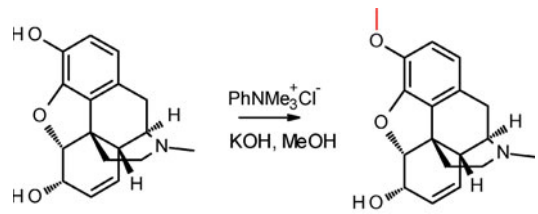
5.3.9 Partial Synthesis of Opioids with a Morphine Skeleton

The chemistry of semi-synthetic opiates, which are mostly derived from morphine or thebeine, is however rather versatile. [104] There are also several totally synthetic analgesics with the morphine skeleton, though most of the opioids generated by total synthesis show only a distant similarity to morphine. The greatest structural alterations to the morphine skeleton are found in ring C, and in the oxygen functions. Thereby, an augmented pharmacological activity can be obtained in many cases. Further variations may be made in ring D. Modification of the *N*-methyl group may result in partial or full opioid antagonists, while the receptor affinity is retained. Nowadays, a continuous spectrum of opioid analgesics is known, reaching from pure agonists to pure antagonists. For example, a substance can act as an agonist at the κ-receptor and as an antagonist at the μ-receptor. Pure antagonists (*e.g.* naloxone) offset the activity of hypnoanalgesics and serve as an antidote of opioid poisoning. Dualistically acting compounds have been developed to reduce the addictive potential of strong analgesics. By now, this objective has however not been achieved yet.

Codeine

Codeine is the monomethyl ether of morphine. It is metabolised to morphine, and possesses accordingly a morphine-like activity spectrum, although its potency is five to ten times weaker. It shows good oral bioavailability and is frequently used for the treatment of low to medium levels of pain, and also to suppress the urge to cough. Its potential for addiction at therapeutic doses is low.

Even today, codeine is occasionally obtained by extraction from opium, although mostly by methylation of (–)-morphine. This methylation can be carried out selectively in a phase- transfer reaction with trimethylphenylammonium chloride. [105]

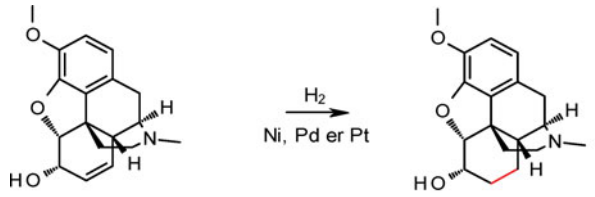


(-)-Morphine (-)-Codeine

Codeine is by far the most widely used legal opiate in the world. The manufactured tonnage increased to 381 tonnes in 2011, the highest level ever reported. [82]

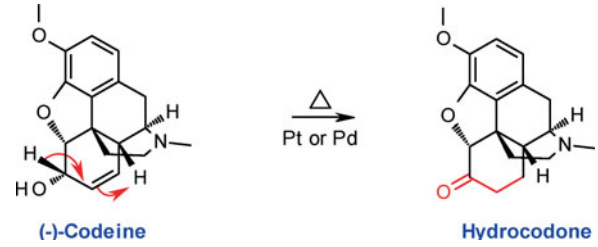
Dihydrocodeine

Dihydrocodeine possesses a pharmacological profile related to that of codeine. It is generally administered orally. During chronic medication it may lead to dependence. The annual output fluctuates between 27 and 32 tonnes. [82]



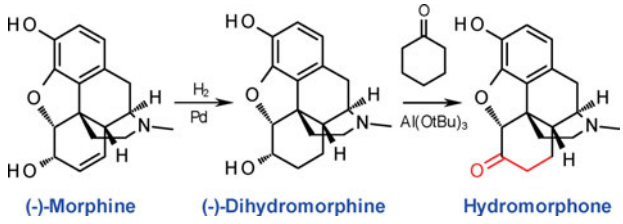
Hydrocodone

Hydrocodone (trade name in Germany: *Dicodid*®, though marketing approval has been withdrawn due to addiction risk) is an opioid as well, with a pharmacology that is comparable to codeine. Particularly pronounced is its antitussive activity. Hydrocodone has higher potency than codeine, and is therefore used for moderate to severe pain. It is formed by palladium- or platinum-catalysed double bond isomerisation of codeine. Hydrocodone production has seen an almost constant upward trend over the past 20 years, reaching 56 tonnes in 2011. It is exclusively manufactured in the United States, where it is used in combination products, *e.g.* with acetaminophen in *Vicodin*®. [82]



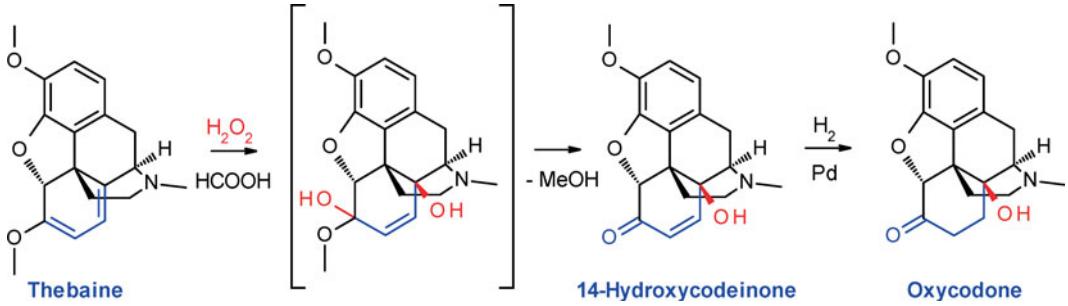
Hydromorphone

Hydromorphone (launched by Knoll in 1926 under the brand name *Dilaudid*®; with intended connotation to '*laudanum*') is more active than morphine, and is indicated for medium to severe pain. Hydromorphone can cause severe dependence. The starting material for its synthesis is morphine, which is first hydrogenated, and its alcohol function is then submitted to an Oppenauer oxidation with cyclohexanone.



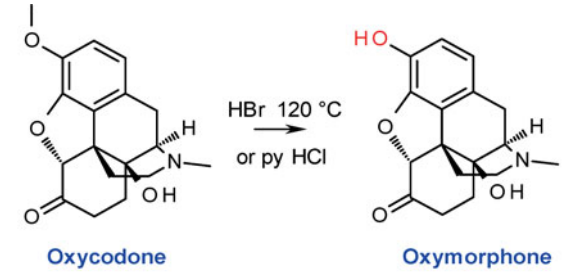
Oxycodone

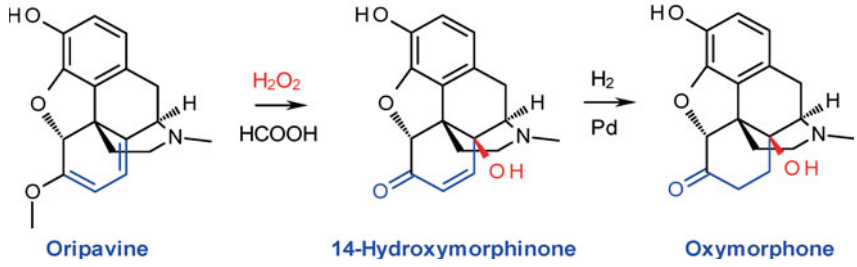
Oxycodone (trade names in Germany: *Oxygesic®*, *Eukodal®*, in the USA: *OxiContin®*) is also used in combination with paracetamol or aspirin for the control of severe pain. It is obtained from thebeine by oxidation with hydrogen peroxide and hydrogenation of the vinylogous double bond.



Oxymorphone

Oxymorphone (trade name: *Numorphan®*, *Opana®*) is a five- to ten-times more potent morphine derivative, which is marketed in the USA and Canada as parenteral and rectal application forms to treat more severe pain. It is prepared by cleavage of the methyl ester of oxycodone, or from oripavine by oxidation and hydrogenation.



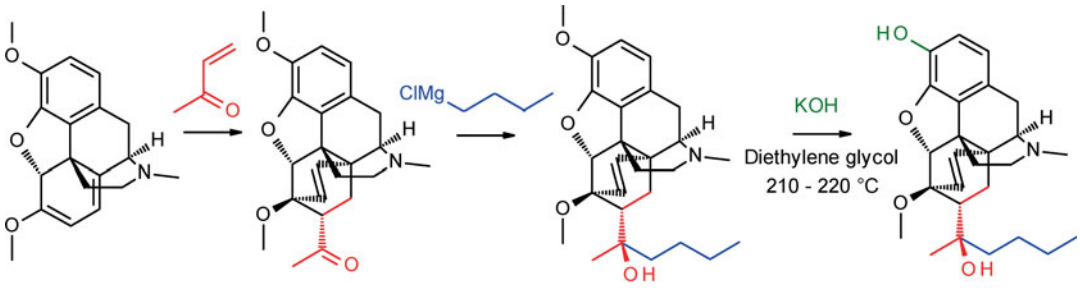


Etorphine

In the early 1960s, Kenneth Bentley and his group at MacFarlan Smith in Edinburgh discovered extremely strong and rapid-acting μ-opioids. Among these compounds was etorphine, 400–1,000 times stronger than morphine. The substance is very rapidly absorbed through the skin or mucous membrane, so that

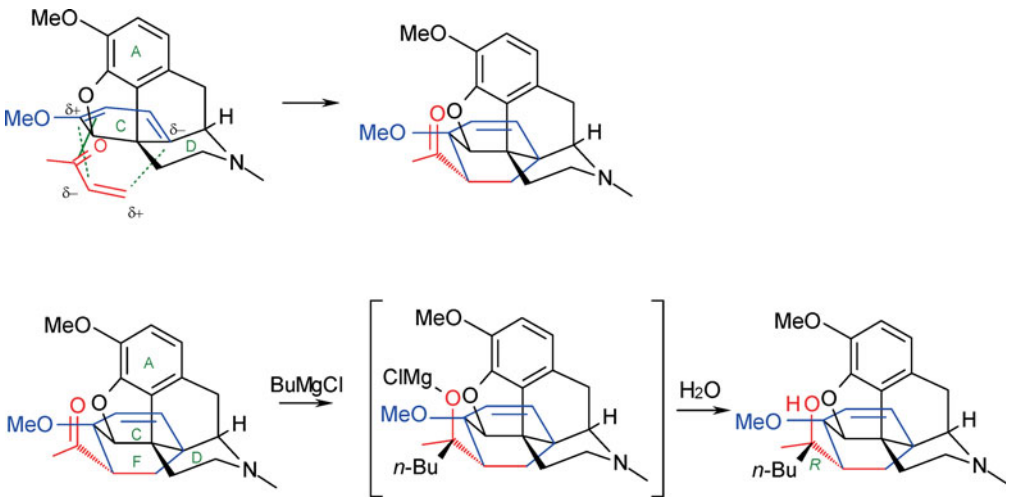
extraordinary care must be exercised to avoid contamination. Naloxone or diprenorphine is effective as an antagonist. Etorphine is used mostly in veterinary medicine for the immobilisation of large animals (*e.g.* rhinoceros or elephants). Correspondingly, its trade name in Britain is *Immobilon*®.

The synthesis starts from thebeine, which is subjected to a Diels-Alder reaction with methyl vinyl ketone and then reacted with *n*-butylmagnesium chloride. Finally, in a nucleophilic substitution, the methoxy-group is replaced by hydroxy.



Thebaine **Etorphine**

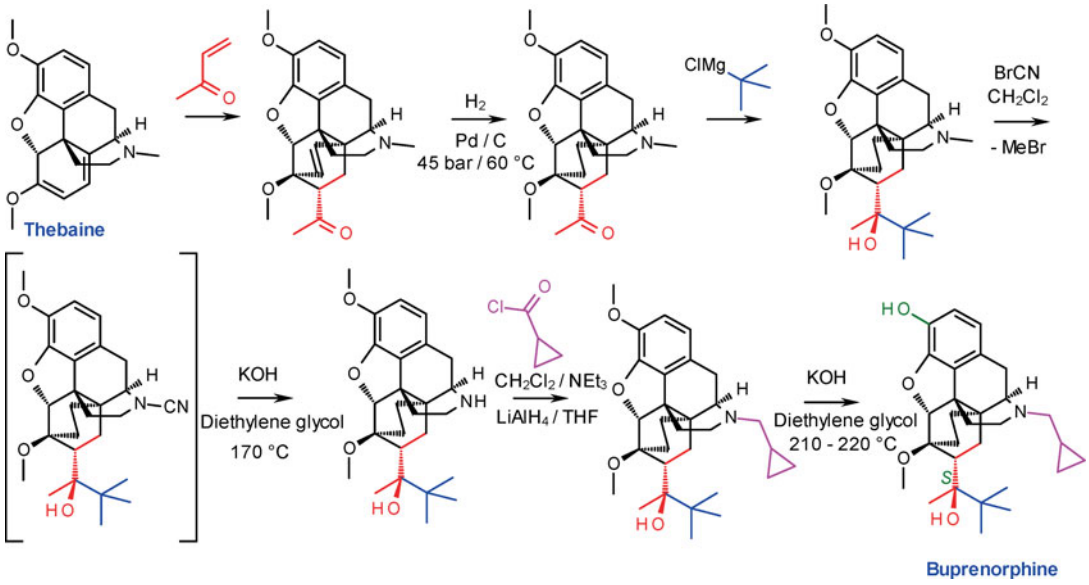
The Diels-Alder reaction is facilitated by electron-donating groups on the diene system and electron acceptors on the dienophile. The diene system of ring C lies almost in a plane with the piperidine ring (Ring D, chair conformation), whereby the aromatic ring A of thebaine very effectively shields the "upper half". The partial polarisation and secondary orbital interaction determine the high regio-and *endo*-selectivity. Also the Grignard reaction proceeds with strict control of diastereoselectivity. The methoxy-function on ring C serves as a stereo-differentiating group through chelation control.



Buprenorphine

Buprenorphine (trade name in Germany: *Temgesic*®, in the USA: *e.g. Suboxone*®) is a mixed opioid μ-agonist-δ, κ-antagonist [106] with long duration of action, a property which is explained by its slow dissociation from the opioid receptor. This compound is 20–30 times more potent than morphine. As to its effects and side-effects, the profile of a μ-opioid agonist predominates. Despite its partial antagonistic character, buprenorphine possesses a considerable potential for addiction. However, due to its long-lasting activity, it is also used for addiction therapy.

The synthesis of buprenorphine has a certain similarity to that of etorphine. After the Diels-Alder reaction of thebaine with methyl vinyl ketone, the double bond is hydrogenated. Then follows a Grignard reaction with *t*-butylmagnesium chloride. For modification of the tertiary amine, this is demethylated with cyanogen bromide (von Braun reaction) and treated with cyclopropanecarbonyl chloride. Following reduction with lithium aluminium hydride, the aromatic methoxy-group is finally replaced by nucleophilic substitution under forcing conditions.



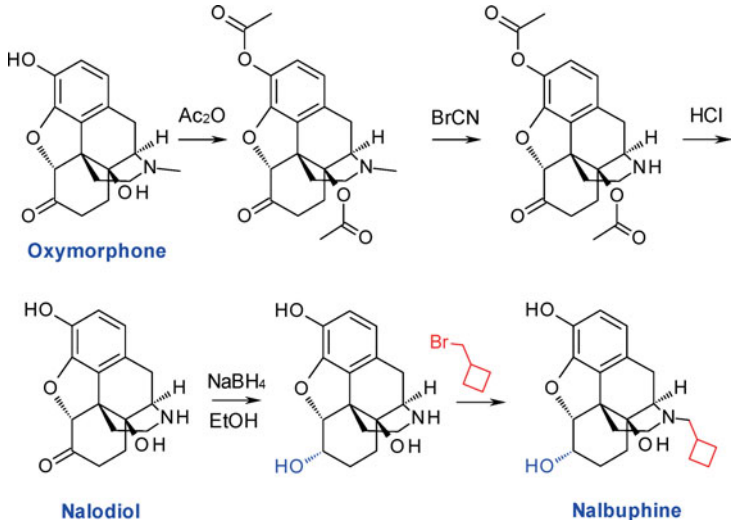
Apart from the transformation with cyanogen bromide, the *N*-demethylation of the morphinan and tropane skeletons may also be carried out with chloroformates, dialkyl azodicarboxylates, photochemically, fermentatively or by means of Polonowski-like reactions with iron salts. [107]

The growing consumption of buprenorphine in recent years is mainly the result of its extended use in detoxification and substitution programmes to treat opioid dependence. In 2009, global production reached a peak level of 6.5 tonnes. [82]

Nalbuphine

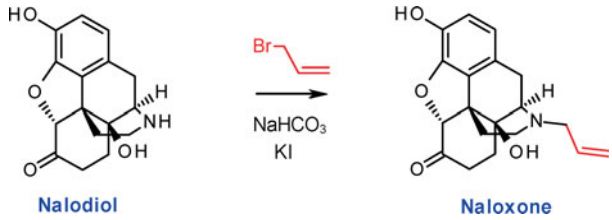
Nalbuphine (trade name in Germany and elsewhere: *Nubain*®) is a mixed agonist-antagonist. The danger of hallucinations and psychotic effects is lower than for other dualistic opioids. The addiction potential is likewise low, so that nalbuphine is not subject to regulatory control as a narcotic.

The starting material for the synthesis is oxymorphone, the hydroxy-groups of which are first esterified with acetic anhydride, before the *N*-methylgroup is cleaved off with cyanogen bromide. After acid hydrolysis of the esters, the keto-group is reduced wth sodium borohydride, and the nitrogen finally alkylated with cyclobutylmethyl bromide. [108]



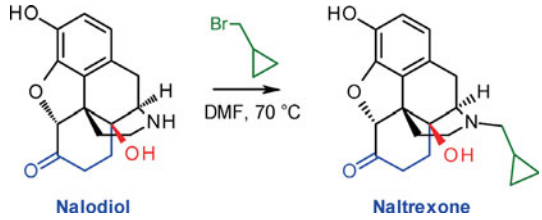
Naloxone

Naloxone (trade names: *Nalone*®, *Narcanti*®) is the prototype of pure antagonists. Naloxone binds to the receptor, however does not activate it. After parenteral application, the onset of actvity occurs very rapidly, though its duration is relatively short. Due to a considerable *first-pass*-metabolism in the intestine and liver, the oral bioavailability is poor. With a low side-effect rate, naloxone is used for therapy of opioid intoxication. For its synthesis, Nalodiol (one of the intermediate stages of the route to nalbuphine) is selectively allylated at the nitrogen atom.



Naltrexone

Naltrexone (trade names: *Revia*®, *Vivitrol*®, *Nemexin*®) is a pure opioid antagonist like naloxone, however with a more pronounced and longer-lasting effect. It has sufficient oral bioavailability to be used for the treatment of drug addicts. Its synthesis proceeds in an analogous manner to that of naloxone.



Nalodiol Naltrexone

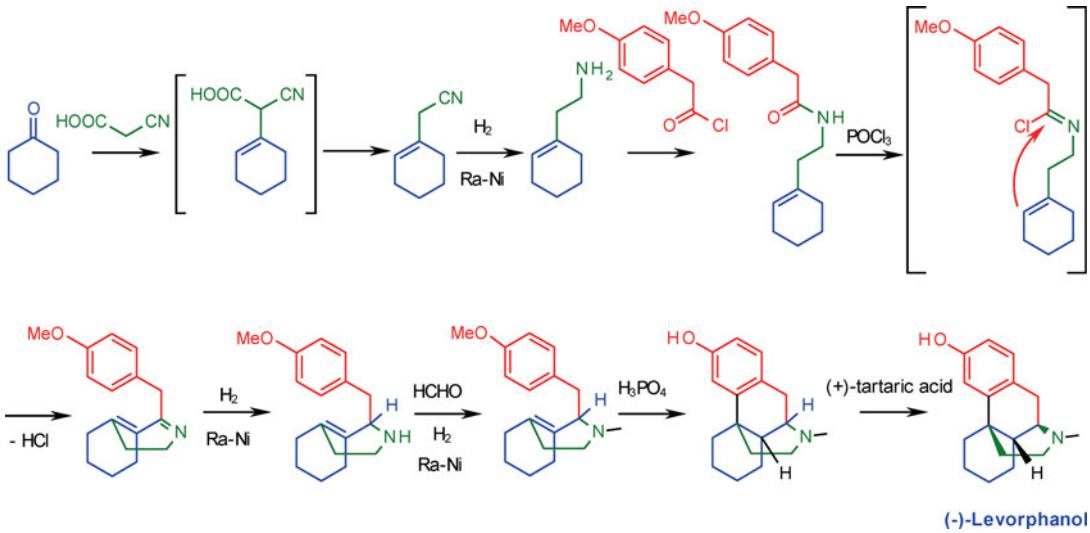
5.3.10 Total Synthesis of Opioids with a Morphinan-like Skeleton

Levorphanol

The third important isoquinoline synthesis is the Pomeranz-Fritsch reaction. Starting from benzaldehydes and an acetal of amino-acetaldehyde, one can obtain isoquinolines, often in moderate yield, by warming and treatment with sulfuric acid.

Levorphanol (trade name in the USA: *Levo-Dromoran*®) is a totally synthetic opioid agonist for parenteral and oral administration. Its potency by parenteral route is four to five times higher than for morphine. Similarly advantageous is its long activity duration (up to eight hours) after oral dosing.

Starting from cyclohexanone, the condensation with cyanoacetic acid leads remarkably to cyclohexenyl acetonitrile (and not as might be expected to 2-cyclohexylideneacetonitrile). [109] The key step is a Bischler-Napieralski reaction (an intramolecular Vilsmeier reaction), by which the reduced isoquinoline ring is closed. After reductive methylation with formaldehyde, heating with phosphoric acid cleaves the methyl ether and also alkylates the aromatic ring, whereby the quaternary benzylic centre is constructed. The active enantiomer is, as expected, the (–)-isomer, which can be obtained in a final stage by crystallisation with (+)-tartaric acid. Levorphanol is a tetracyclic compound. For its pharmacological effect, ring D is obviously only of secondary importance.

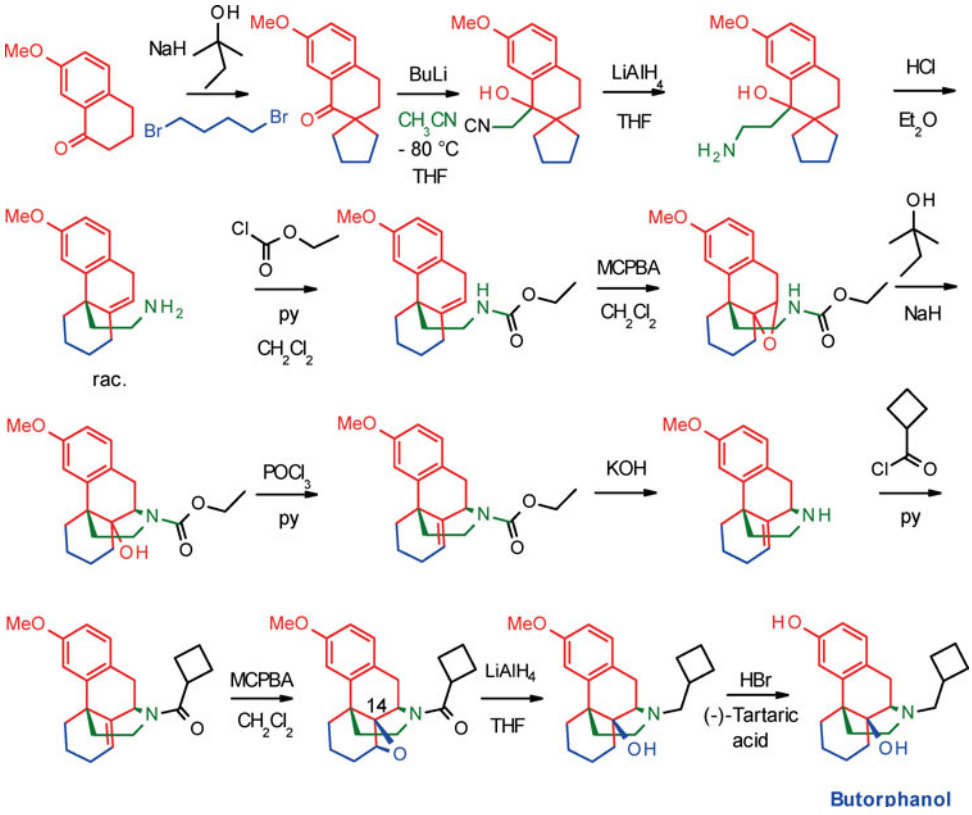


(-)-Levorphanol

Butorphanol

Butorphanol (trade name in the USA: *Stadol*®) is a fully synthetic analgesic for the treatment of moderate to severe pain, and is a component of various anaesthetics. It behaves as a partial agonist at the μ-receptor and a full antagonist at the κ-receptor.

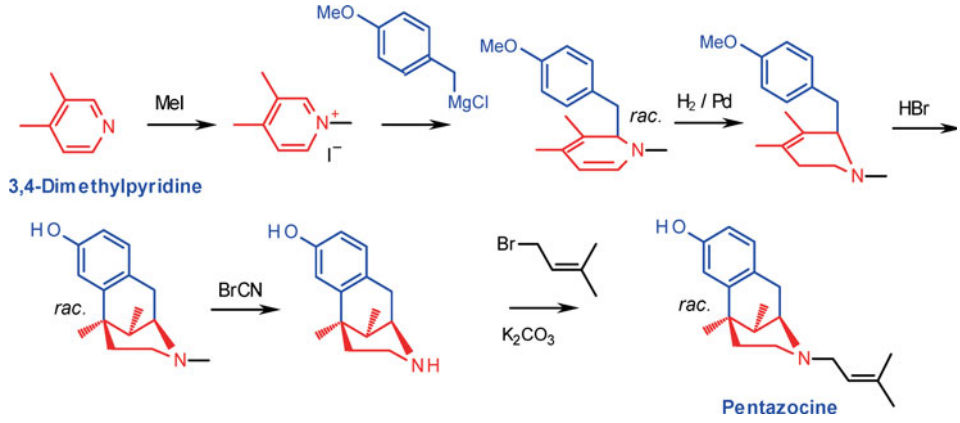
The synthetic route is relatively lengthy. Noteworthy is the construction of the quaternary centre by a Wagner-Meerwein rearrangement, which leads to the racemate. Closure of the piperidine ring is achieved *via* an epoxide ring opening. By re-epoxidation, an oxygen function with the desired absolute configuration can be generated at C-14. In course of the reduction of the amide to the tertiary amine, the epoxide is also reduced to the alcohol. On account of the high diastereoselectivity of the individual reaction steps, at the end of the sequence, a racemate is obtained, which only has to be separated by crystallisation with (–)-tartaric acid.



Pentazocine

Pentazocine (trade names: *Talwin*®, *Fortwin*®, *Fortral*®) is likewise a mixed opioid agonist-antagonist, although with an almost complementary activity profile compared to butorphanol. It behaves as a partial μ-antagonist and a κ-agonist. The structure of pentazocine is still further simplified, relative to morphine. Apart from ring D being missing, ring C is just mimicked by two methyl groups.

The synthesis of pentazocine is comparatively short. The Grignard reaction with a pyridinium salt is followed by partial hydrogenation. Cleavage of the methyl ether is accompanied by acid-catalysed alkylation, which leads to the buildup of the quaternary centre. Finally, the amino-function is demethylated with cyanogen bromide and alkylated with isopentenyl bromide.



5.3.11 Totally Synthetic Opioids without a Morphinan Structure

Whereas, in the above-described fully synthetic opiates, the morphinan core is still recognisable, in spite of attempts to alter and simplify the structures even further, from the 1930s on, a series of active compounds has been discovered, which lack the morphinan scaffold, but bind selectively at the μ-subtype of opioid receptors, like morphine. [110]

Pethidine

The synthesis of pethidine (trade names: *Dolantin*® in Germany, *Meperidine*® in the USA) in 1937 is regarded as an important milestone in opioid research. In search for spasmolytics (cramp-easing medicaments) Otto Schaumann and Otto Eisleb discovered at Hoechst, that mice showed morphine-like symptoms after dosing with structural variants of atropine: *e.g.* the Straub-tail phenomenon (S-shaped erection of the tail, discovered by the German pharmacologist Walther Straub (1874–1944)). Extensive and careful pharmacological experiments then led to pethidine, the first totally synthetic opioid. Its oral bioavailability is around 50 %. The *first-pass* metabolism in the liver leads *via* demethylation to the pharmacologically active metabolite norpethidine.

The synthetic concept is simple. Benzyl cyanide is alkylated twice with *β*, *β*'-bis-(chloroethyl)methylamine, and the nitrile function transformed into the ethyl ester in a Pinner reaction with ethanol.

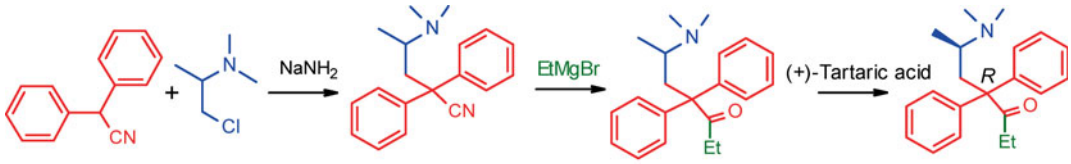
The annual consumption of pethidine has shown a declining trend, and levelled off in 2011 at around 9 tonnes. [82]

Pethidine

Levomethadone

In the 1940s, it was discovered that derivatives of 3,3-diphenylpropyl-*N*,*N*-dimethylamine possessed analgesic properties as well. A structural comparison with pethidine reveals the relationship. The piperidine ring is basically cut open and the structure stiffened again by another phenyl ring. The first compound in this class, which was clinically tested in 1946, was methadone. The (*R*)-enantiomer is the pharmacologically active isomer, which resembles morphine in its activity and side-effects. The oral bioavailability (*ca.* 80 %) is extremely high for an opioid. It shows high plasma-protein binding and a long elimination half-life (24 hours). The withdrawal syndrome is actually longer-lasting, but milder than with morphine. On account of severe pain at the injection site after parenteral application, the potential for abuse is relatively low. The pure (–)-enantiomer (levomethadone) is on the market in Germany.

The synthesis is likewise simple. Alkylation of diphenylacetonitrile is followed by a Grignard reaction, and separation of the enantiomers is achieved with (+)-tartaric acid. Global production of racemic methadone has increased steadily over the last two decades, and reaches an annual amount of 35–45 tonnes. [82]



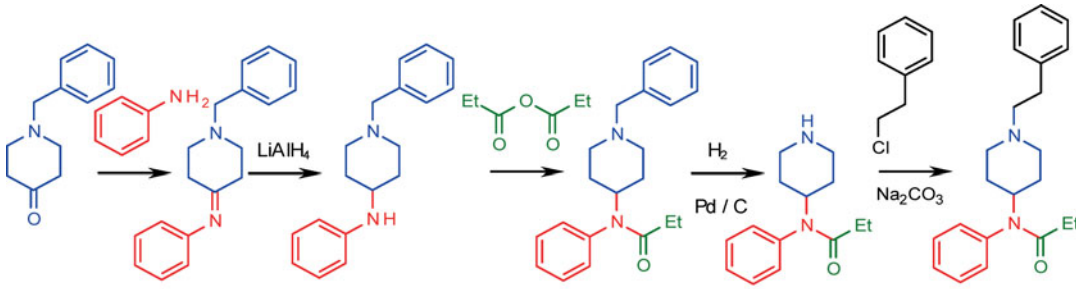
Levomethadone

Fentanyl

A relationship to pethidine can be recognised in fentanyl as well, which was discovered in 1959 by Paul Janssen (1926–2003). The pethidine nitrogen atom is linked through a short carbon chain to an aromatic ring. C-4 of the piperidine ring carries a bulky substituent. Fentanyl is a very lipophilic opioid with a 100 times stronger potency than morphine. It finds use in anaesthesia and in the treatment of severe chronic pain. Fentanyl can be administered by a transdermal patch, in order to provide constant exposure of the opioid over a period of 48 to 72 hours, or as a flavoured lollipop of fentanyl citrate (*Actiq*®) for rapid onset (Fig. 5.61).

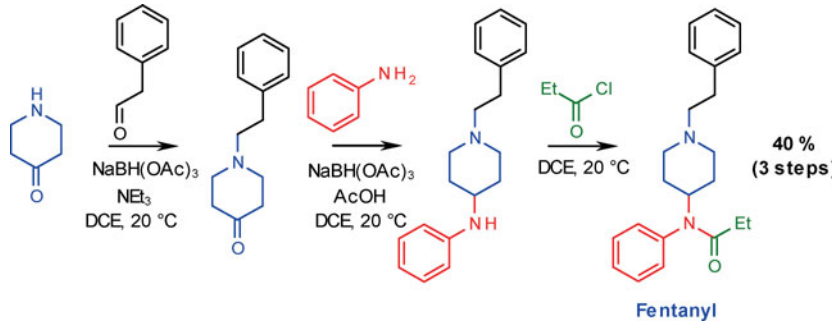
5.61 *Fentanyl is frequently used as a painkiller by the Danish Army and the United States Air Force Pararescue during combat operations.*

Compared to previous examples, its synthesis is somewhat more elaborate. One advantage is, however, that the achiral nature of the compound does not require a separation of enantiomers.



Fentanyl

In 2005, Ramesh Chandra Molhotra published a remarkable three-step-one-pot procedure, based on two consecutive reductive amination reactions with sodium triacetoxyborohydride. The overall-yield is 40 %. [111]



Fentanyl

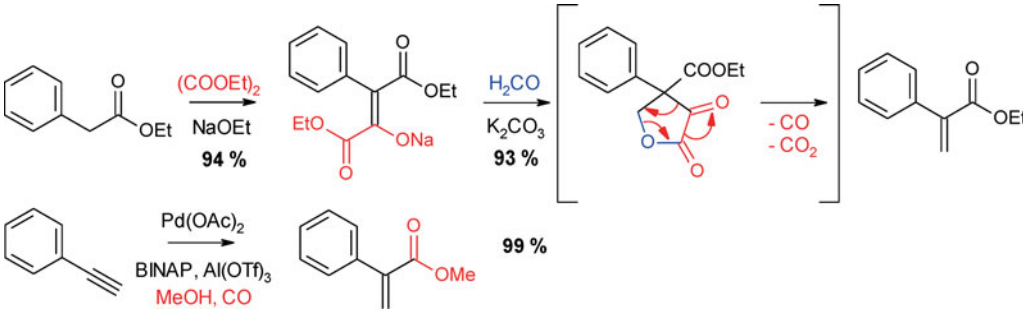
The fentanyl production grew rapidly during the last two decades, and has reached a record level of more than four tonnes in 2010. [82]

Tilidine

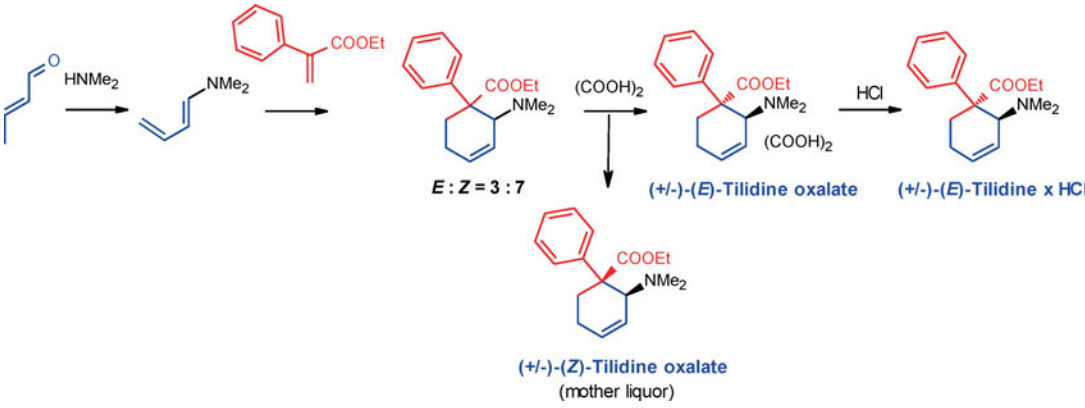
Also in the 1960s, there were discovered various dimethylaminocyclohexanes, like tilidine from Goedecke AG, which showed a strong analgesic effect. As with pethidine, it is not only tilidine itself which works as a painkiller, but also its metabolites, nortilidine and bisnortilidine.The risk of tilidine abuse is high; therefore, enteral dosage forms contain in many countries the μ-antagonist naloxone in addition. This serves as tilidine antidote to prevent harm also after inappropriate parenteral application. After regular oral administration as prescribed, naloxone is metabolised sufficiently fast, so that the analgesic effect of tilidine remains unaffected. The absorption rate is too slow for a "kick" to materialise, which renders the drug uninteresting for abuse.

Tilidine synthesis is impressively simple. [112] The cyclohexene system is constructed by a Diels-Alder reaction of an enamine, derived from crotonaldehyde, and ethyl atropate.

The atropic ester typically is produced by condensation of ethyl phenylacetate and formaldehyde. The condensation reaction can be performed in the presence of ethyl oxalate to obtain the product in higher yield. A more modern approach is the Pd-catalysed alkoxylcarbonylation of phenylacetylene.

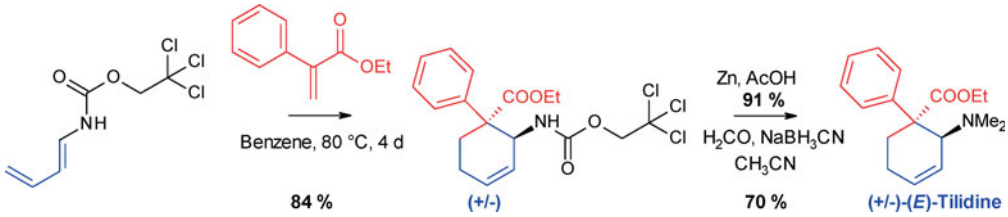


The Diels-Alder reaction generates an *E*/*Z* mixture of 3 : 7, which can be separated *via* crystallisation of the (*E*)-tilidine oxalate salt. Tilidine is marketed as the racemate.



A highly stereoselective tilidine synthesis was published by Larry Overman. [113] The Diels-Alder reaction of a related carbamate with ethyl atropate

affords exclusively the (*E*)-diastereomers. Cleavage of the carbamate and reductive methylation leads selectively to (+/–)-(*E*)-tilidine.



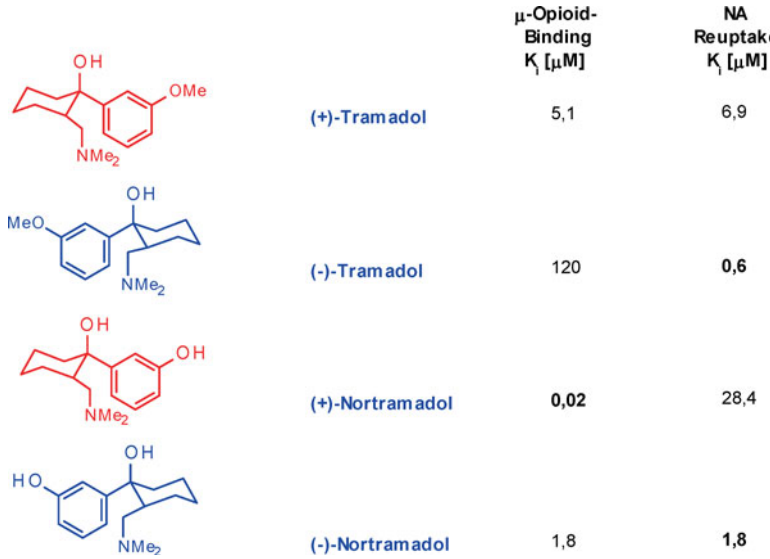
Global output of tilidine reached a record level of 77 tonnes in 2008, but decreased three years later to some 30 tonnes. Most tilidine is consumed in Germany, which accounted for 92 % of the world production in 2011. [82]

Tramadol

Tramadol is used to treat moderate to moderately severe pain, and most types of neuralgia. It was developed by the German pharmaceutical company Grünenthal GmbH in the late 1970s, and is marketed under the trade name *Tramal*®.

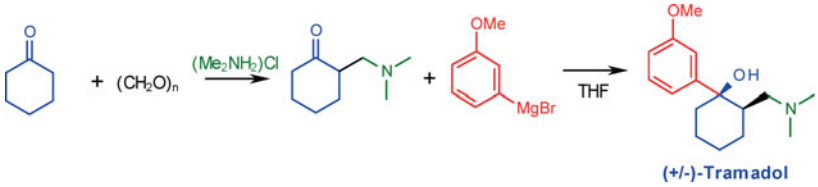
Tramadol is an analgesic with an atypical activity profile. The original objective of its development was, to find a new antitussive agent derived from codeine. With tramadol, however, a substance was discovered, which possessed only moderate opioid properties, but a clear monaminergic activity, *i.e.* the inhibition of noradrenaline- and serotonin-reuptake, leading to the inhibition of spinal pain transmission. With its moderate opioid-receptor affinity, the risk of tramadol abuse is low, and the corresponding drugs are available on prescription worldwide, without further limitations. The global turnover in 2012 was estimated at more than 1.4 billion Euro.

Tramadol is sold as the racemate, though both enantiomers, as well as their metabolites – in particular the *O*-demethylated derivatives – show different pharmacological activity (Fig. 5.62).



5.62 *Whereas the main metabolite of (+)-tramadol is primarily responsible for the opioid effect, (–)-tramadol and equally (–)-nortramadol cause inhibition of the re-uptake of neurotransmitters.*

The synthesis is in this case also simple. It consists of the reaction between a Grignard reagent, derived from *m*-bromoanisole, and the Mannich base produced from dimethylamine, formaldehyde and cyclohexanone.



The diastereomers can be separated by crystallisation. While the marketed products contain the racemic mixture of (*R*,*R*)-and (*S*,*S*)-enantiomers, their separation may be possible using tartaric acid, its *O*,*O*'-dibenzoyl or *O*,*O*'-di-*p*-toluoyl derivatives, or mandelic acid. [114, 115]

5.63 *Nauclea latifolia is an evergreen multi-stemmed shrub or tree, which is native to the humid tropical rainforests and savannah woodlands of West and Central Africa.*

Tramadol – a Pseudo-Natural Product?
In 2013, researchers at the Université Joseph Fourier in Grenoble discovered unexpectedly racemic tramadol in the root bark of the sub-Saharan African peach (pincushion tree, *Nauclea latifolia*) (Fig. 5.63). While in Cameroon the decoction of this root bark is well known in traditional medicine for the treatment of pain, malaria, fever, epilepsy, and infantile convulsions, the association with tramadol is questionable. A most recent investigation explains the unusual occurrence of tramadol by "anthropogenic" contamination, an alarming example of unintended drug distribution into the biosphere. [116]

5.3.12 Closing Remarks

In Germany alone, there are more than a million patients, who suffer from pain over a prolonged period or even permanently. [117] Of all drug classes, analgesics are the most frequently prescribed today. Together with antirheumatics, these two indications account for 80 % of all prescriptions. [118]

For the treatment of severe acute, as well as chronic pain, especially resulting from accidents, post-surgery conditions and tumours, opioids are still the drugs

of choice, despite their numerous undesirable side-effects. Because of their tranquillising effect, hypnoanalgesics are also indicated in cases of heart attacks or acute pulmonary oedema. It remains the responsibility of both, the physician and the patient, to use painkillers sensibly.

Also for patients with chronic pain, it is not about completely eliminating any and all pain, but to reduce pain to a tolerable level. If this guidance is observed, the risk of developing an addiction remains low.

In this context, the treatment of tumour patients is of particular importance. Its primary goal is no longer to extend the lifespan of a terminally ill patient under all circumstances, but to secure his or her quality of life, and that for the remaining time the disease impact is as bearable as possible.

Summary in Bullet Points

- Opiates play still a significant role in the control of severe pain.
- The morphine-derived analgesic core structure can be found in a multitude of painkillers.
- Despite a series of newer enantioselective total syntheses of morphine and thebaine, these compounds will continue to be isolated from "renewable raw materials" and then converted by chemical methods into the corresponding drug substances.
- Moreover, there is a series of "structurally simplified opiates", which are accessible by chemical synthesis *via* a comparatively short route.

5.4 Tetrahydrocannabinol

5.64 *Hemp (Cannabis sativa L.)*

Hemp (*Cannabis sativa* L.) belongs among the oldest cultivated plants (Fig. 5.64). Originally native to India, Afghanistan and Iran, hemp is now found almost everywhere in the world, at times even in our allotments and in balcony cultivation.

The word "cannabis" is presumably derived from the Hebrew "kaneh bosm" and is therefore found in various passages of the Old Testament in its original version (*e.g.* Exodus 30: 23, Ezekiel 27: 19 and Song of Songs 4: 14). Interestingly, in the first translation of the Hebrew Bible into Greek, the Septuaginta, in the third century BC, "kaneh bosm" was confused with Calamus (*Acorus calamus*) and translated erroneously. The error survived many subsequent translations unchanged, including the one of Martin Luther (1534) and in the famous King James Bible (1611). Over the course of time the two words "kaneh bosm" were contracted into "kanabos", the original form of the Greek word. [119]

The utilisation of hemp as a source of fibres was known in China for at least 4,800 years. Also the Egyptians had used it, as documented by texts dating back to the end of the Fifth Dynasty (*ca.* 2350 BC), carved in pyramid stones, and hemp fibres were found in the tomb of Amenhotep IV (alias Akhenaten or Echnaton, *ca.*1334 BC). [119] The Greeks encountered hemp only in the 5th century BC

and referred to it as "Kannabis". Its psychotropic effect was recognised early on; already Herodotus (490–430 BC) described an ancient cannabis orgy. He recounts that the Scythians, inhabitants of the territories to the north of the Black Sea, throw hemp seeds onto stones, which they have heated to glow, releasing smoke and steam... and the Scythians happily breathe in this vapour and utter screams ... [119]

Around the same time Germanic tribes were also cultivating hemp. The Celts had known hemp since the Hallstatt period (700–450 BC). In the 3rd century BC, the Gauls in the Rhône valley used hemp to manufacture ropes and clothing. Hiero II (308–215 BC), the tyrant of Syracuse, Sicily, imported hemp from there for the production of marine ropes. In Roman literature, hemp is first mentioned by Gaius Lucilius (180–102 BC).

At the time of Charlemagne (742–814), hemp was cultivated extensively in Europe. In the monasteries, hemp oil lamps were used. The monks did their transcripts on hemp paper. At a later date, Gutenberg (1454) printed the Bible on this material, and also the American Declaration of Independence is written on hemp paper. Christopher Columbus (1492) and later the crew of the Mayflower (1620) took hemp to America. In the 19th century, hemp possessed military and strategic importance for Britain. Ropes and sails consisted principally of hemp, which was imported from Italy and Russia. During the Napoleonic Continental Blockade, King George III promoted hemp cultivation in the south of England.

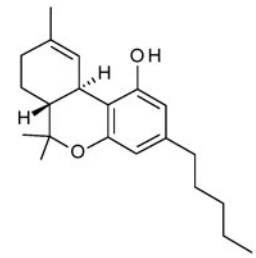
One of the oldest descriptions of cannabis as a medicament comes from the pharmacopoeia of the Chinese Emperor Shen Nung, dating back to the 28th century BC. In Europe, the narcotic constituents of hemp were first recognised through the influence of Arabian medicine. Hildegard of Bingen (1098–1179) described hemp as a medicinal plant. Since 1660, cannabis from the Dutch Cape Colony in South Africa reached Amsterdam, and there it was smoked in "coffee shops". In the 19th century, the drug attained undreamt-of popularity. At the Hôtel de Lauzun (also called Hôtel de Pimodan) in Paris resided the "Club des Hashischins", to which belonged such as Charles Pierre Baudelaire, Eugène Delacroix, Honoré de Balzac, Alexandre Dumas, and Gustave Flaubert. Artists and authors smoked the hookah (also known as the water-pipe or hubble-bubble) and tasted the "confection" of the French psychiatrist Dr. Jacques-Joseph Moreau (1804–1884). Sir John Russell Reynolds, the physician of Queen Victoria (1837–1901), was one of the early advocates of cannabis as a drug. Around 1900, the German Empire was one of the greatest hemp importers – not only for the production of ropes. Indian immigrants brought cannabis to Mexico. From there the drug also reached the southern USA. [120] The Prohibition during the 1920s promoted the consumption of marijuana (Spanish: Maria-Johanna (code name)) and hashish (Arabic: dried grass) as much as the hippie culture in the second half of the 1960s. In 1981, against the backdrop of drug problems, Germany prohibited the cultivation of hemp. Since 1996, varieties with low levels of tetrahydrocannabinol (THC), which instead possess an increased content of cannabidiol, are again being cultivated under licence.

On a worldwide basis, cannabis is nowadays the most frequently consumed drug. The United Nations Organisation estimates that between 2.8 and 4.5 % of

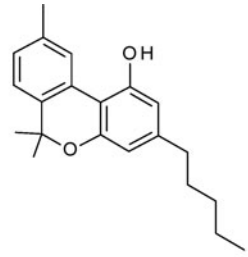
the world population have used this drug at least once in the preceding year. Hemp for drugs is cultivated over an area of 200,000–641,800 ha. This corresponds to the production of cannabis plants ranging from 13,300 to 66,100 tonnes, and of cannabis resin from 2,200 to 9,900 tonnes.

Global licit production of cannabis grew steadily in the last decade and stabilized at a level of about 6 tonnes. After an increase in 2007 (10 tonnes), the production of cannabis declined to 4–5 tonnes in 2010. In 2011, a sharp rise to almost 25 tonnes were registered. [121]

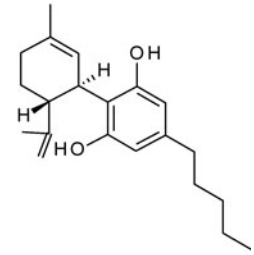
5.4.1 Botany and Use



Δ^9-Tetrahydrocannabinol (THC)



Cannabinol



Cannabidiol

Hemp (*Cannabis sativa* L.) is a plant of the genus Cannabis (family *Cannabaceae*) and is classified with regard to its drug content. In view of the fact, that tetrahydrocannabinol (THC) can be oxidised to cannabinol, it is categorised by the quotient of both ingredients ([THC] + [cannabinol])/[cannabidiol]. If this quotient amounts to less than 1, the phenotype is labelled as fibre hemp; if it is greater than 1, it counts as drug hemp. [122] The annual plant grows up to five metres high. The leaves are lobed with palmate shape. Hemp is dioecious (*i.e.* the male and female flowers are on separate plants). The female plants are more extensively branched and more richly leaved than the male counterparts. Hemp is harvested after the male flowers have withered.

From the bark of the hemp stems, bast is obtained. The bast fibres are separated mechanically from the woody parts: modern separation methods employ enzymes or steam pressure, surfactant or ultrasound processes. The best hemp fibres are obtained from the male plants: being very durable. They are used for the manufacture of ropes, cords, nets, strings, yarn, carpets, textiles and sailcloth. The wood is used for the manufacture of insulating material, but it is also well suited for the production of paper, cardboard and cartons, since its lignin content amounts to only 10 % (compared to 20–25 % from trees).

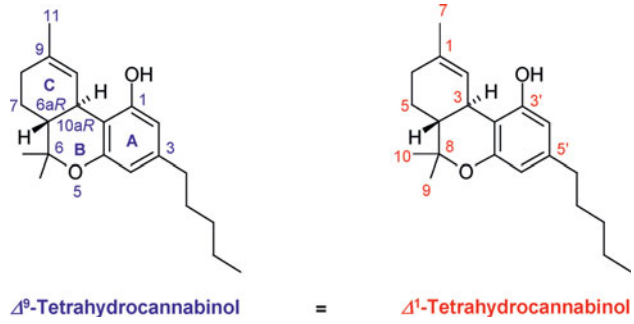
The oval fruited bodies, small smooth grey nuts, several millimetres in size and with a fat content of 30–35 %, are crushed and pressed for their oil. The greenish, medium dried hemp oil contains in its glycerides 46–70 % linoleic and 14–28 % linolenic acids. It finds use as edible oil, and serves as a substitute for linseed oil in paint. Apart from that, it is also used for the manufacture of margarine and soft green soap.

Especially the female hemp plant secretes from their blossoms, leaves and stems a resin, which is however produced in considerable amount only in warm regions. In order to obtain drug material, the female blossoms are harvested, dried and sold as marijuana or sinsemilla (Spanish: *sin semilla*, without seeds), or they are pulverised on rugs, whereby the sticky resin remains adhering to the rug fibres. After separation of the blossom components, the resin is kneaded into small "loaves". Another harvesting method consists of collectors wearing leather aprons walking through a hemp field during the flowering season (July–August), and later scraping off the sticky resin from the leather. Morocco and Afghanistan are particularly known as the hashish suppliers for Europe. [119] Marijuana and hashish are smoked along with tobacco ("joints"). Apart from that, hashish is smoked in the water-pipe or inhaled through a mouthpiece. Other forms of consumption comprise brewing up like tea, or the processing in pastry.

5.4.2 The Drug

Marijuana/sinsemilla contains as the psychotropic substance 2–20 % Δ^9-tetrahydrocannabinol (THC); in hashish its proportion amounts to 8–40 %. The concentrations of THC vary widely, according to the country of origin, the cultivation and the preparation; but in general, during the last decade a significant rise in the THC concentration has been observed. [121]

By smoking marijuana, 20 % of the THC is absorbed; after oral application of hashish, 6 % of the tetrahydrocannabinol reaches the bloodstream. Due to its lipophilic character, Δ^9-tetrahydrocannabinol is stored in tissue in metabolised form. After a week only 30 % of the substance is eliminated through faeces and urine. Complete clearance extends over a month. For the psychotropic effects, it is the (6a*R*,10a*R*)-enantiomer which is responsible (Fig. 5.65).



5.65 *Along the terpene nomenclature, the synonymous name Δ^1-tetrahydrocannabinol is found in the literature.*

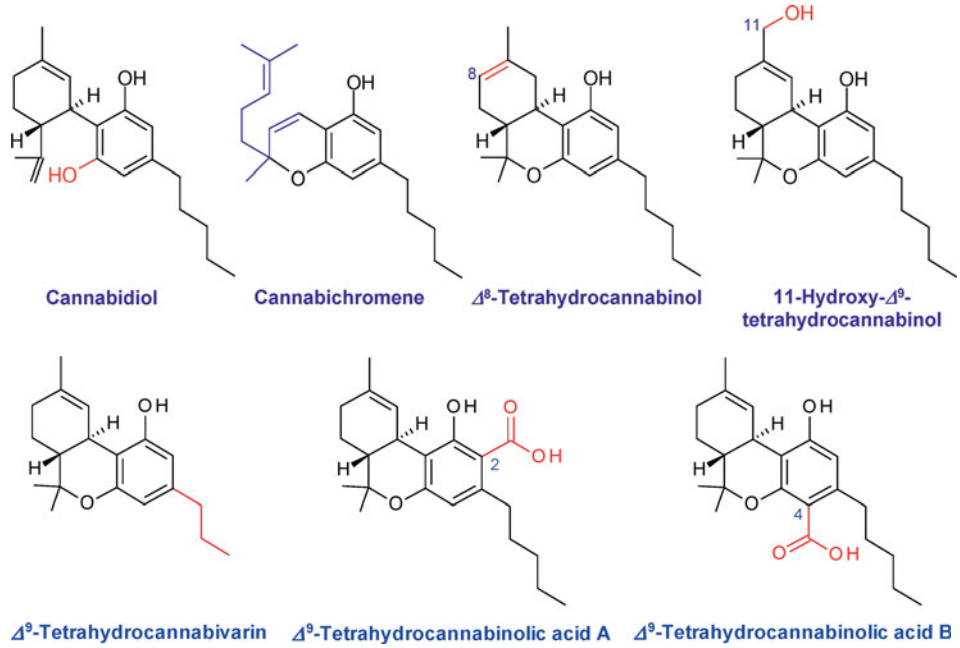
Another important constituent is cannabidiol. This excerts bacteriostatic activity, acts as a pain-reliever and is sedative, although not mind-altering. The analgesic and anti-inflammatory potency is several hundred times higher than that of aspirin, and is based on a dual cyclooxygenase- and lipoxygenase-inhibition. [123] This provides cannabidiol as an ideal lead structure in the search for new non-steroidal anti-inflammatories. Moreover, its neuroprotective proper-

ties are also of interest in case of acute or chronic neuro-degeneration like Parkinson's disease. Cannabichromene possesses also anti-inflammatory as well as fungicidal and antibacterial properties.

Of similar activity as Δ^9- is Δ^8-tetrahydrocannabinol, although it occurs in hemp only in small amounts. Oral application of hashish generates a metabolite, hydroxylated at C-11, which shows as well psychotropic effects, equivalent to those of THC.

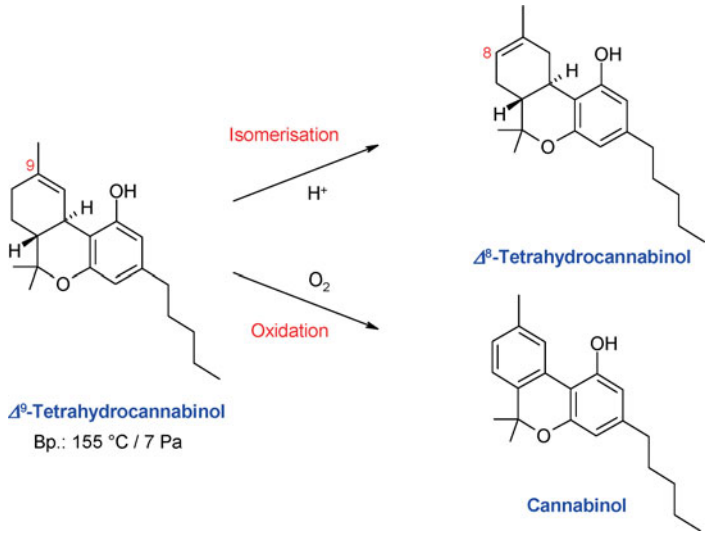
In special hemp varieties, there are found increased amounts of Δ^9-tetrahydrocannabivarin, the side-chain of which is shortened by two carbon atoms. This compound is a CB1- and CB2-receptor antagonist (see below).

Further cannabinoids are Δ^9-tetrahydrocannabinolic acids A and B, which contain an additional carboxylic acid function at C-2 or C-4. [124] By smoking of cannabis these undergo decarboxylation and therefore raise the THC level (Fig. 5.66).



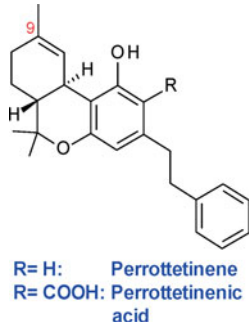
5.66 *Phytocannabinoids: further active substances and metabolites of cannabis.*

Around 1940, cannabinol was isolated independently by Sir Alexander R. Todd (1907–1997) at the University of Manchester and Roger Adams at the University of Illinois. However, it was determined that this compound was not responsible for the psychotropic effects of cannabis, but actually an artefact, which originated by oxidation of THC during the isolation process. [125] An additional difficulty in determining the structure of THC was, that the double bond isomerises readily in presence of acid into the thermodynamically favoured 8-position. The problem of isolation was simply technical in nature: in contrast to the alkaloids, which are readily purifyable by salt formation as crystalline compounds, this is not possible with Δ^9-tetrahydrocannabinol.



By improved chromatographic methods, and thanks to the new techniques of NMR- and mass spectroscopy, Raphael Mechoulam at the Weizmann Institute of Science in Israel, succeeded in the 1960s in the isolation, structure elucidation and total synthesis of Δ^9-tetrahydrocannabinol, the pharmacological principle of hemp. [126, 127]

It was believed for a long time that cannabinoids occurred only in hemp, until perrottetinene was discovered in 1994 in the small leafy-stem liverwort (*Radula perrottetii*). Later, this compound was also found in *Radula laxiramea,* and along with its carboxylic acid in the New Zealand liverwort (*Radula marginata*) as well. [119] At the present time, around 70 phytocannabinoids (*i.e.* plant-derived cannabinoids) are known.

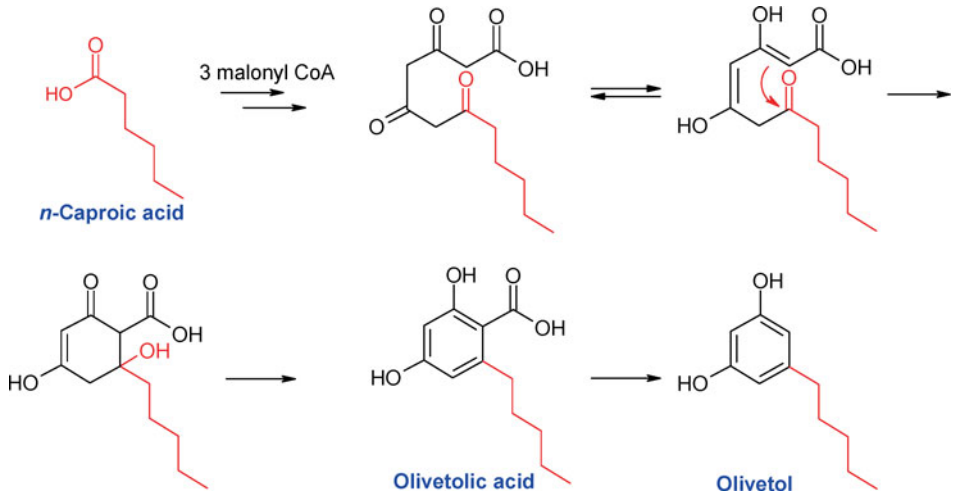


5.4.3 Biosynthesis

Studies to elucidate the biosynthetic pathway to phytocannabinoids by feeding plants with radioactively labelled nutrients proved initially difficult, because these were not incorporated to a sufficient extent. From the mid-1990s, several research groups began to identify the key enzymes, one prenyltransferase and

various oxidoreductases, which participate in the biogenesis of cannabinoids. This endeavour has largely deepened our understanding of this process.

The starting point of the biosynthesis is *n*-caproic acid, from which a triketocarboxylic acid is constructed in successive polyketide steps. Olivetolic acid is formed by an intramolecular aldol reaction, its decarboxylation generates olivetol.

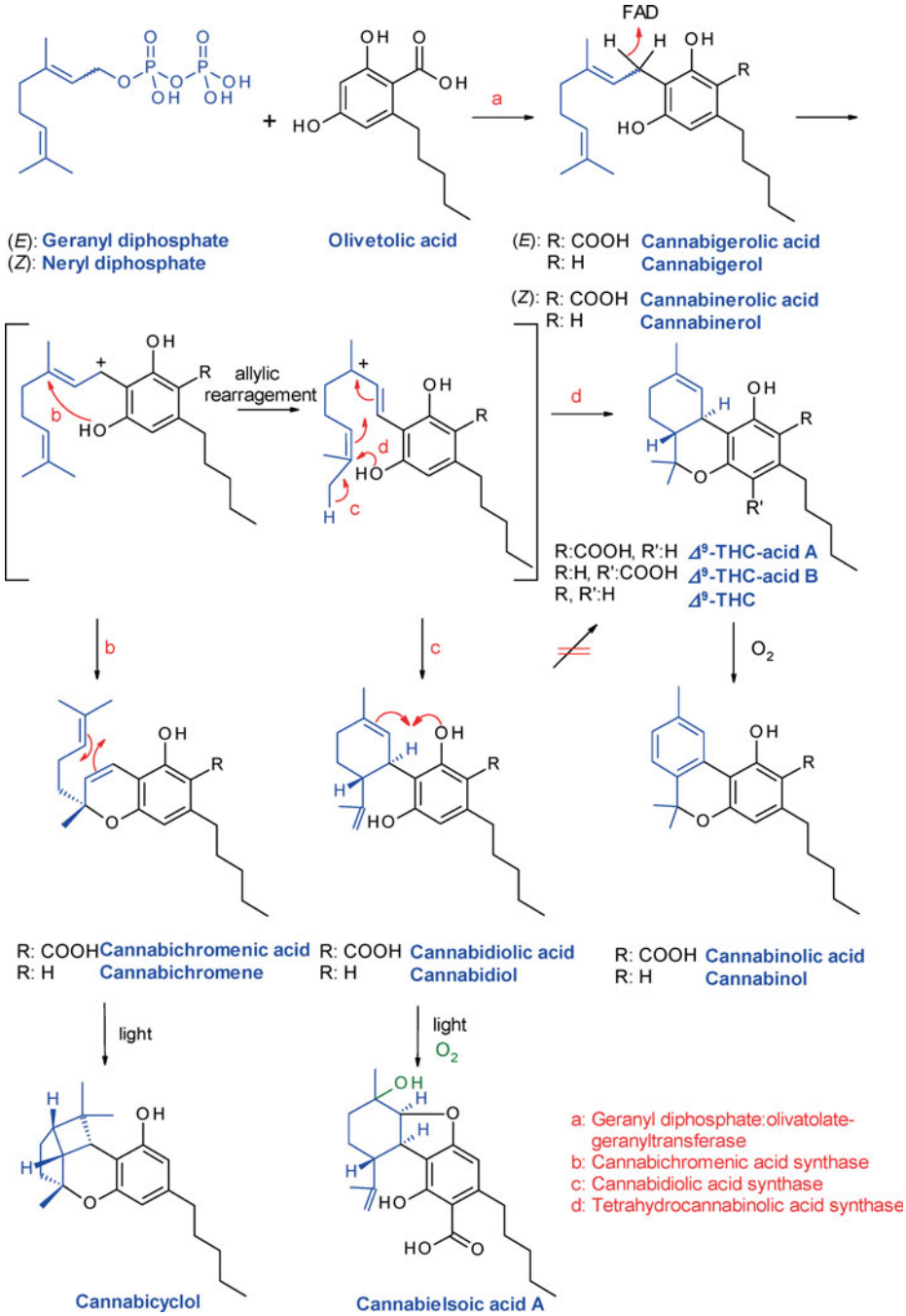


In the next step, olivetolic acid is condensed with geranyl diphosphate in the presence of a particular prenyltransferase. Olivetol itself is not active as a prenyl-acceptor. [128] Tracer experiments with ^{13}C-labelled glucose show that in hemp the terpene part of cannabinoids is constructed according to the newly-discovered deoxyxylulose phosphate pathway (the Rohmer route). Since cannabinerolic acid is found as a second component, it appears likely that also neryl diphosphate is transformed by the same enzyme.

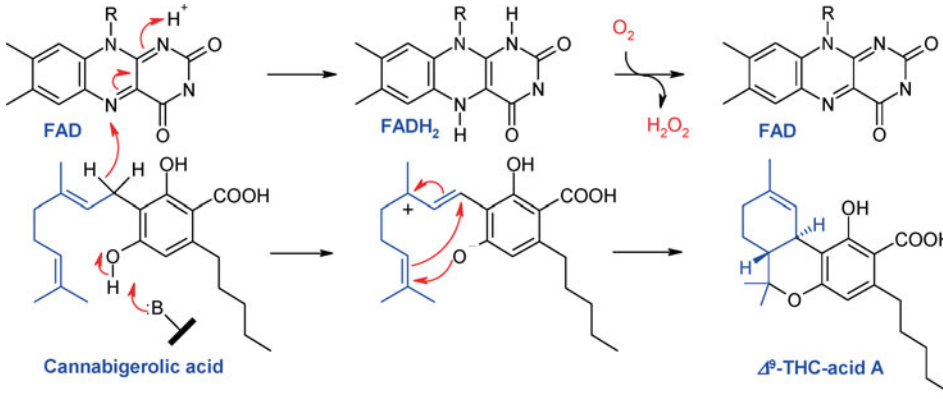
Cyclisation to the cannabinoidcarboxylic acids takes place on various oxidoreductases. Here, neither of the neutral cannabinoids, cannabigerol and cannabinerol, are substrates for the enzymes. Tetrahydrocannabinolic acid synthase transforms cannabigerolic acid and cannabinerolic acid to Δ^9-tetrahydrocannabinolic acid (Δ^9-THC-acid). The enzyme contains as a cofactor flavin adenine dinucleotide (FAD), covalently bonded to His-114. For the cyclisation of cannabigerolic acid, oxygen is required, and hydrogen peroxide is formed as a by-product. Remarkably, cannabidiolic acid, a plausible intermediate in the cyclisation, is not a substrate for the tetrahydrocannabinolic acid synthase. [129]

The formation of cannabidiolic acid and cannabichromenic acid takes place independently on other oxidoreductases. Cannabidiolic acid is the main cannabinoid in fibre hemp. Cannabichromenic acid is also produced in drug hemp. In contrast to other cannabinoids, it is produced as a 5 : 1 mixture of enantiomers. Therefore, seen from the genetic standpoint, the expression of the cannabidiolic acid synthase or of the tetrahydrocannabinolic acid synthase determines if the phenotype correlates with drug or fibre hemp.

Other cannabinoids may be considered as potential artefacts, generated by light, heat and/or oxygen, *e.g.* during drying of the branches or collection of the resin. It is also conceivable that at temperatures of 50–55 °C, partial decarboxylation may already have taken place in the foliage of the plants.

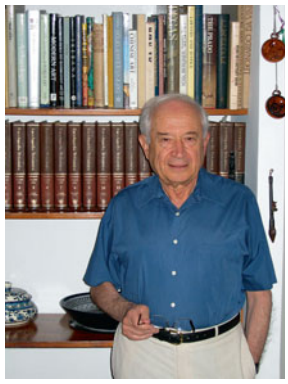


Tetrahydrocannabinolic acid is highly toxic for insect and plant cells, including those of hemp, and it is therefore produced extracellularly in the storage cavity of the glandular trichomes. Along with hydrogen peroxide, this ensures that hemp is effectively protected against predators and pests.



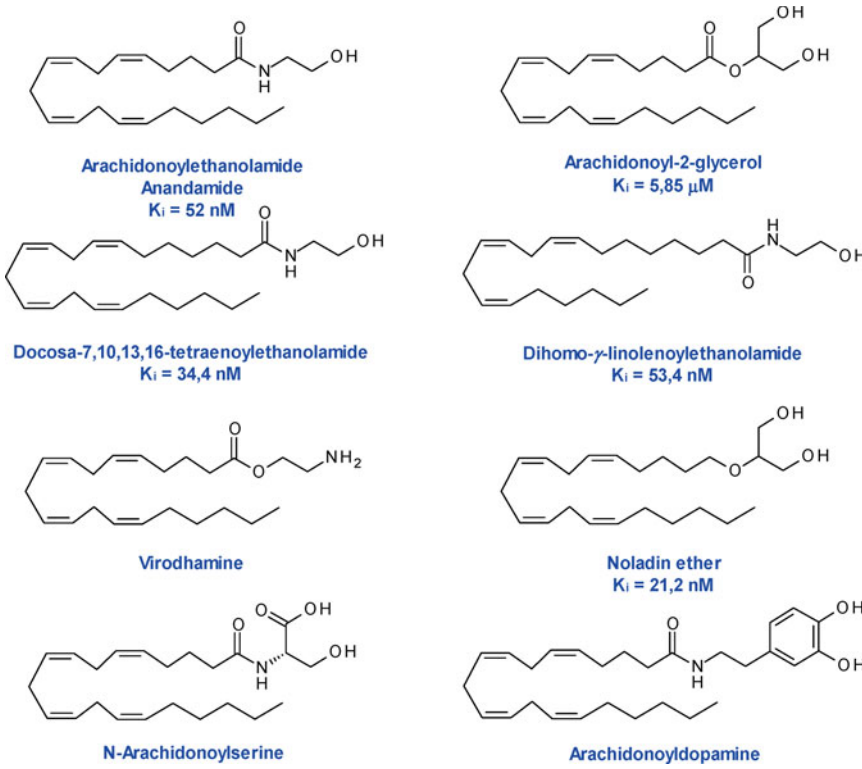
5.4.4 Endocannabinoids

Although cannabis, like opium, has been used as a drug for thousands of years, our pharmacological and toxicological understanding of cannabinoids, as opposed to opioids, was first emerging in the 1990s. [130] Their mode of action was explained categorically as a disturbance of the membrane potential of nerve cells caused by high lipophilicity, accumulation and persistence of the tetrahydrocannabinoids. However, detailed investigation of structure-activity relationships casted doubt on this explanation. Thus, in part even minor structural alterations led to a complete loss of activity. In addition, the stereospecificity pointed to the involvement of a particular receptor. In 1988, the first receptor of this kind, the cannabinoid receptor type 1 (CB1), could eventually be characterised, and also cloned in 1990. [125] As is now known, this is one of the receptors most frequently expressed in the brain. In 1993, the first non-cerebral cannabinoid receptor, type 2 (CB2), was identified in the spleen.



5.67 *Raphael Mechoulam (*1930).*

Starting from the thought that a "lock" had been discovered, there came now the question of the endogenous "key". Altough Δ^9-tetrahydrocannabinol from hemp fits nicely into this "lock", it could be mere serendipity; the more so as THC, unlike morphine, had not been detected in the human body as an endogenous compound. In 1992, Raphael Mechoulam (Fig. 5.67), at the Hebrew University in Jerusalem, discovered the first endocannabinoid, which he extracted from a pig's brain; he demonstrated its affinity to the CB1-receptor by classical displacement studies. Mechoulam named it "anandamide" (Sanskrit, *ananda*: bliss). In chemical terms it is arachidonoylethanolamide (Fig. 5.68). Anandamide (K_i: 52 nM) has practically the same affinity as Δ^9-tetrahydrocannabinol (K_i: 41 nM) at the CB1-receptor, but in contrast to THC, the former is rapidly deactivated hydrolytically by the enzyme anandamide amidohydrolase.



5.68
Endocannabinoids

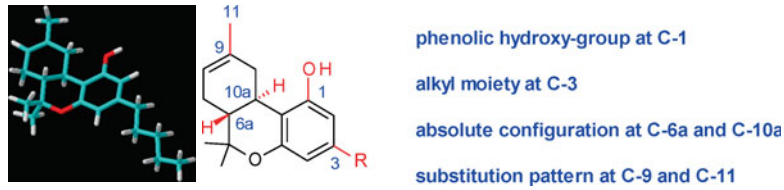
In veterinary medicinal studies it could be shown that anandamide sedates pigs. Thereby the body temperature drops slightly, respiration is slower, and the animals lie down more often. It is hypothesised that the significance of anandamide as a neurotransmitter for cognitive and emotional processes in the brain is similar to dopamine and serotonin, the functions of which are now much better understood. Anandamide appears to play an important role as well in the regulation of mood, memory, appetite and the sensation of pain. Outside the brain, the highest concentration of anandamide is found in the uterus shortly before nidation of the embryo. It is therefore understandable that tetrahydrocannabinol endangers a pregnancy during the first weeks.

Two more fatty acid amides of aminoethanol were later discovered in extracts of the pig brain. These bind to the CB1 receptor with comparable K_i-values. [119] Further endocannabinoids were discovered in the gastro-intestinal tract of the dog, and in the spleen of the mouse. These are monoglyceride esters. 2-Arachidonoylglycerol could be detected in the brain at a 170 times higher concentration than anandamide. This compound has the same effect as anandamide, both, *in vivo* and *in vitro*.

In 1996, Daniele Piomelli at the Neurosciences Institute in San Diego found anandamide in chocolate and cocoa powder. [131] It is thus possible to speculate whether or not consumption of chocolate prompts the cannabinoid receptors to induce a pleasant mood.

5.4.5 Structure-Activity Relationships

On the basis of empirical structure-activity relationships, it is possible to develop a three-dimensional model of the CB1-receptor. Important for a high binding affinity are: the phenolic hydroxy group at C-1, the alkyl residue at C-3, the (*R*)-configuration at both stereogenic centres, as well as the substitution pattern at C-9 and C-11 (Fig. 5.69 and Tab. 5.7).



5.69 *Structure-activity relationships.*

Tab. 5.7 *Binding affinities of Δ^8-tetrahydrocannabinoids at the CB1-receptor*

Substituent R	Binding affinity K_i (nM)
n-butyl	65
n-pentyl	44
n-hexyl	41
n-heptyl	22
n-octyl	8.5
(1′*S*,2′*R*)-dimethylheptyl	0.46
1′,1′-dimethylethyl	14
1′,1′-dimethylpentyl	3.9
1′,1′-dimethylhexyl	2.7
1′,1′-dimethylheptyl	0.77
1′,1′-dimethyloctyl	0.09
1′,1′-dimethylnonyl	1.6
1′,1′-dimethyldodecyl	126

The binding affinity of Δ^8-tetrahydrocannabinol (K_i: 44 nM) and Δ^9-tetrahydrocannabinol (K_i: 41 nM) are equal within experimental error. On the other hand, significant effects are observed by alteration of the length and branching of the side-chain. Shortening of the side-chain of Δ^8-tetrahydrocannabinol by one carbon atom raises the K_i-value to 65 nM. The lengthening from pentyl to octyl leads to a stepwise reduction of the K_i-value to 8.5 nM. A hundred-fold higher affinity relative to Δ^8-tetrahydrocannabinol is found for a cannabinoid with a (1'*S*,2'*R*)-dimethylheptyl side-chain (K_i: 0,46 nM). The 1',1'-dimethylheptyl-substituted isomer shows a somewhat lower affinity (K_i: 0,77 nM). In the case of the 1',1'-dimethyloctyl-substituted compound, the binding to the receptor (K_i: 0,09 nM) is further enhanced by a factor of 8. However, if the chain is

extended further, the K_i-value rises again. In the case of the 1',1'-dimethyldodecyl substituent, a K_i-value of 126 nM was determined. [132]

5.4.6 Pharmacology

At the beginning of the 1970s, there was increasing anecdotal evidence from young cancer patients, that smoking of marijuana eased their nausea and vomiting during chemotherapy. Several government- and industry-sponsored studies subsequently confirmed the anti-emetic potential of cannabinoids. [133]

Clinically important are today:

- the suppression of nausea and vomiting of cancer patients in connection with their chemotherapy;
- the stimulation of appetite in AIDS-associated complications (cachexia, wasting syndrome);
- the reduction of muscular cramp and spasms in multiple sclerosis and paraplegia patients; and
- chronic pain therapy (*e.g.* phantom pain, migraine).

The onset of pharmacological effects starts at a dose of 0.1 mg/kg body weight of Δ^9-tetrahydrocannabinol. The drug causes a feeling of hunger and accelerates the heartbeat. The conjunctiva is reddened because of an increased blood supply.

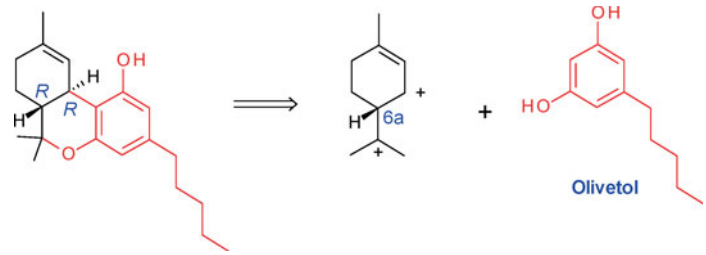
The psychic and hallucinogenic effects are not uniform. At small doses (5–7 mg), Δ^9-tetrahydrocannabinol acts sedative and at high doses (beyond 15 mg) as a stimulant. This stimulation may intensify to a psychotic state. Frequently it amounts to a feeling of relaxation, to the forgetting of everyday problems and to a mild euphoria. But sometimes THC consumption leads to a restless anxiety and to aggressive irritability. Visual and audible perceptions are distorted, thought processes are slowed, and the ability to articulate is partially disturbed. Hashish consumers, in contrast to morphine- or alcohol-dependents, do not require ever increasing doses in order to attain the desired effect, even after prolonged drug consumption. There is no documented physical drug dependence, but a psychic one.

As a rule, cannabis-dependent patients prefer the natural product to pure synthetic Δ^9-tetrahydrocannabinol. In comparative human studies it was shown, especially for women and older people, that the cannabidiol contained in cannabis calms those states of anxiety, as they are caused by pure THC.

However, the problem with hashish and marijuana as a medication is their route of application and their standardisation. Resorption kinetics and bioavailability are highly dependent on the administration form. In addition, the content of active ingredients in cannabis fluctuates considerably, what makes it hard to compare data from different clinical studies, apart from any potential planning errors that may also have occurred. Microbial pollutants, which lead to allergies and breathing illnesses, pose another problem. All this favours the chemical total synthesis of the pure active material. Thereby, a targeted co-administration of several cannabinoids may become feasible in the future.

5.4.7 Chemical Syntheses

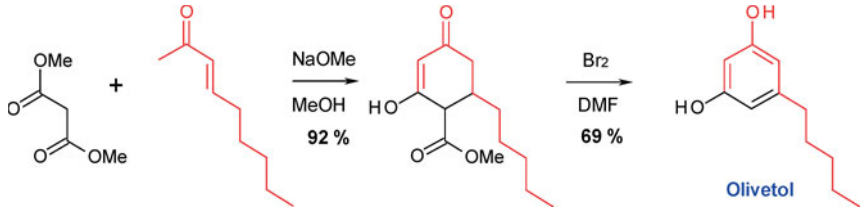
In terms of the chosen strategy, practically all total syntheses follow the biosynthetic route. Olivetol is built up from appropriate precursors, and then alkylated with an enantiomerically pure terpenoid building block. The eventual stereogenic C-6a thereby retains its configuration in the final product. [134]



Through the choice of the terpenoid building block and the reaction conditions, it is possible to influence the position of the double bond, at C-8 or C-9.

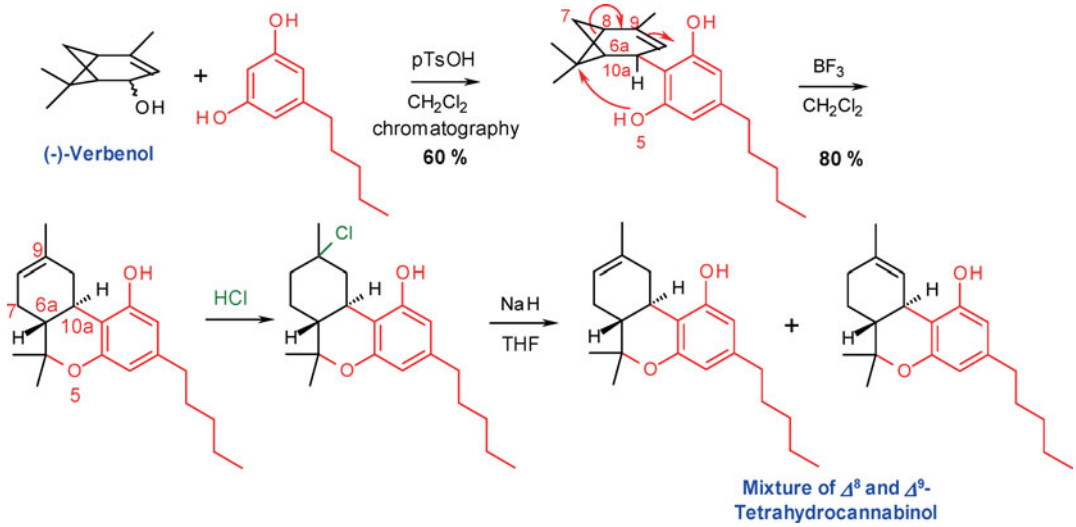
Olivetol

The indeed simplest synthetic path to olivetol was described by Hoffmann-La Roche. [135] In this, dimethyl malonate is condensed with non-3-en-2-one. After hydrolysis and decarboxylation, aromatisation is achieved, *e.g.* with bromine in DMF.

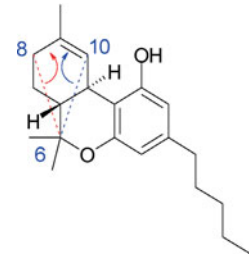


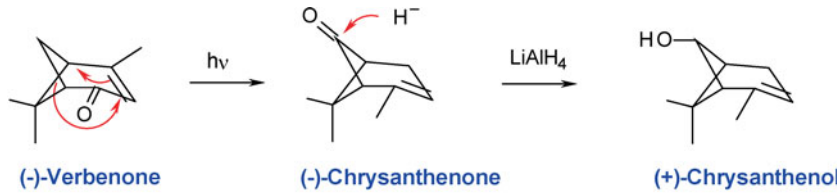
Δ^9-Tetrahydrocannabinol

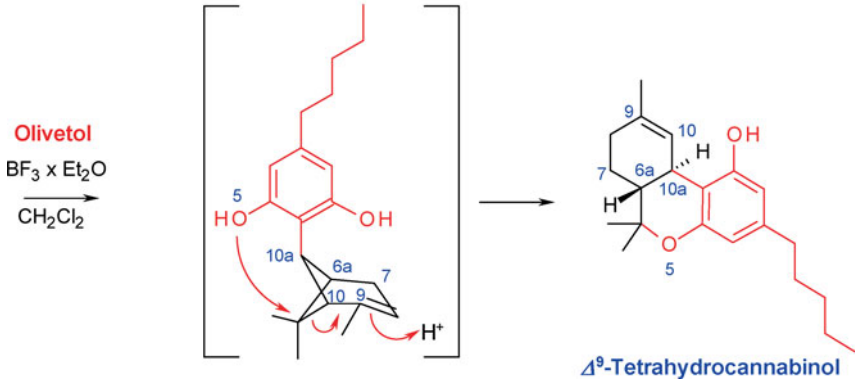
The first synthesis of Δ^9-tetrahydrocannabinol originates from Mechoulam in 1967. [136] Olivetol is condensed with (–)-verbenol (*cf.* section 8.5, pheromones) in presence of *p*-toluenesulfonic acid. The attack proceeds selectively *anti*- to the sterically very demanding isopropylidene group. After chromatographic purification, reaction with boron trifluoride renders Δ^8-tetrahydrocannabinol. BF_3 increases the Broensted-acidity of the phenol, which leads to the protonation of the double bond and finally to the rearrangement of the ring system. Of preparative interest are the addition of HCl and the elimination, leading to a mixture of Δ^8- and Δ^9-tetrahydrocannabinol, which can be separated by chromatography.



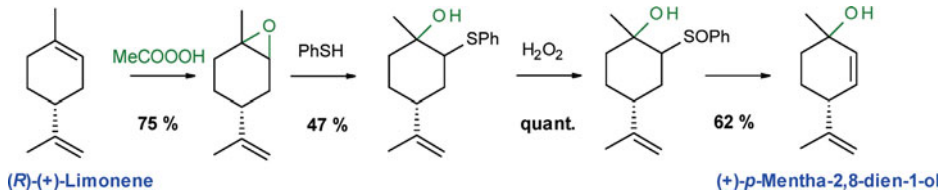
The regiochemical analysis of the initial synthesis showed that (–)-verbenol is the ideal educt for Δ^8-tetrahydrocannabinol. For Δ^9-tetrahydrocannabinol, the hydroxy-group of the terpenoid component is in the wrong position. THC should be obtained directly if (–)-verbenol is replaced by (+)-chrysanthenol. [137] The latter is obtained from (–)-verbenone by photochemical rearrangement and stereoselective reduction with lithium aluminium hydride. Ring closure leads, regio- and enantioselectively, to the Δ^9-isomer, although the product is contaminated with other by-products and must likewise be purified by chromatography.



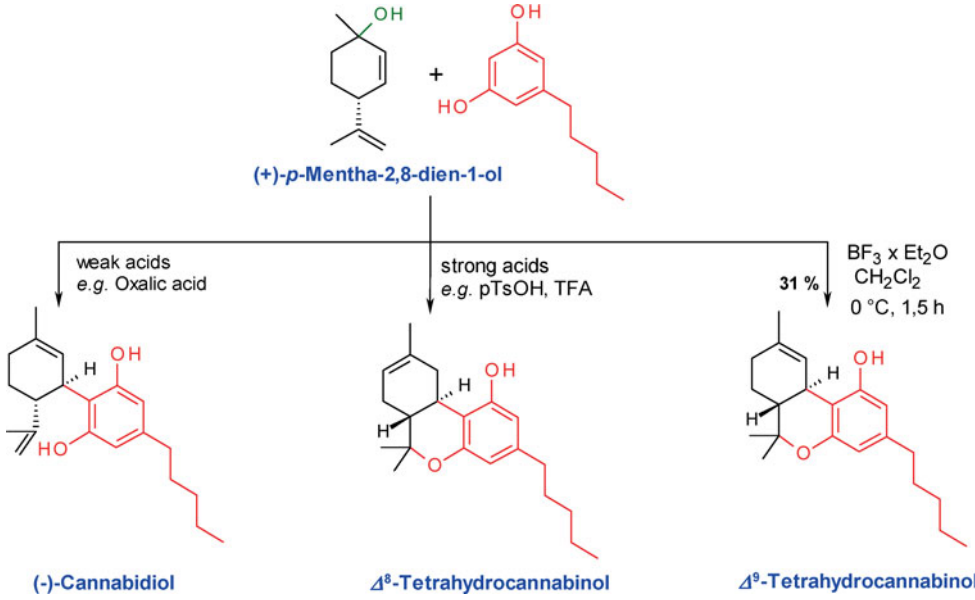




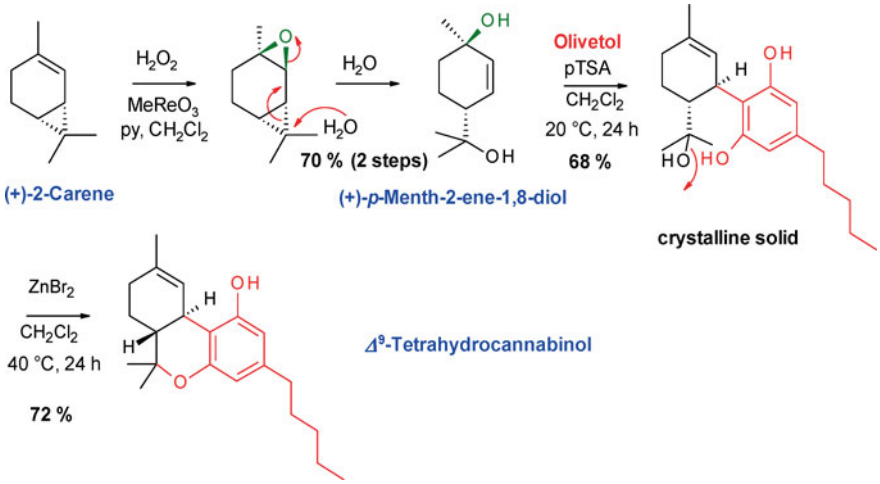
The starting material for the first industrial synthesis was (*R*)-(+)-limonene. In four stages a *cis*/*trans*-mixture of (+)-*p*-mentha-2,8-dien-1-ol is accessible. [138]



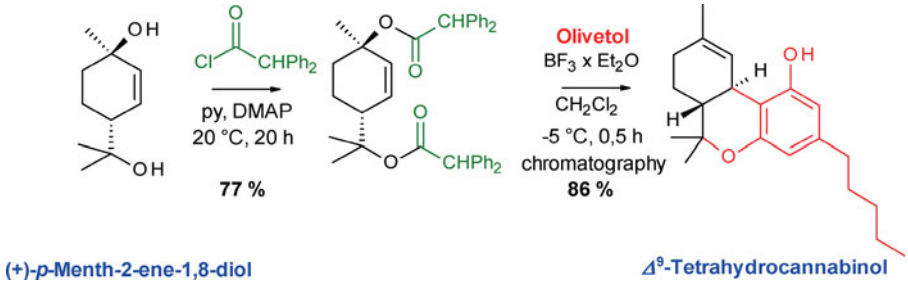
Raj K. Razdan investigated the spectrum of products obtained in the diastereoselective allylic substitution, depending to the cyclisation conditions. With weak acids, cannabidiol predominates. In case of strong acids, Δ^8-tetrahydrocannabinol results as the main product. Only under optimised conditions, using boron trifluoride, a 31 % yield of THC can be achieved.



Probably one of the best syntheses, developed at Johnson Matthey, starts with (+)-2-carene. In two steps (+)-*p*-menth-2-ene-1,8-diol is obtainable. The latter reacts with olivetol in presence of *p*-toluenesulfonic acid to form a crystalline solid, which is easily purified by recrystallisation. Ring closure is achieved with zinc bromide in surprisingly good yields. [139–141]

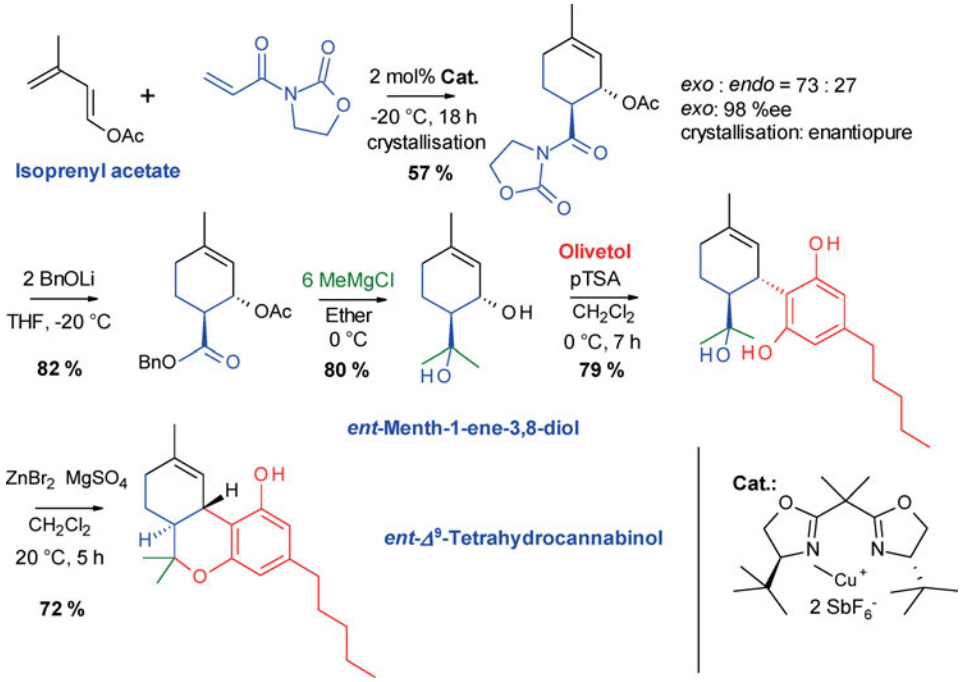


The yield may be further increased, if (+)-*p*-menth-2-ene-1,8-diol is esterified with diphenylacetic acid, although this requires a chromatographic step at the final stage. [142]



One approach to *ent*-Δ^9-tetrahydrocannabinol, which is independent of the chiral pool, was developed by David Evans (reaction scheme see next page). [143] *Via* enantioselective Diels-Alder reaction on a copper-bis-(oxazoline) complex, there is obtained a cyclohexenecarboxamide, which after conversion into its benzyl ester and an exhaustive Grignard reaction gives *ent*-menth-1-ene-3,8-diol. Also this isomer can be converted in an analogous manner stepwise into tetrahydrocannabinol. Δ^9-Tetrahydrocannabinol would be correspondingly accessible by use of the antipode of the catalyst.

Dronabinol (2.5 mg THC per capsule, *Marinol*®) was approved in the United States in 1985 by the FDA (Food and Drug Administration) for the treatment of nausea and vomiting during cancer chemotherapy. In 1992, additional approval was granted for the treatment of anorexia associated with weight loss in AIDS patients. In Germany, the drug is available since 1998 in pharmacies upon presentation of a narcotics prescription. Patients, who had legal problems before, when they used cannabis preparations for medicinal purposes, can since then be supplied with this active ingredient. The legislature has thus created a medicinally acceptable operational framework for the physicians in charge.

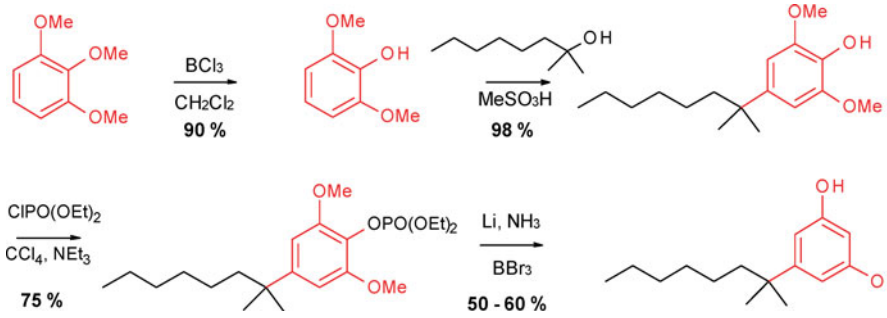


Nabilone

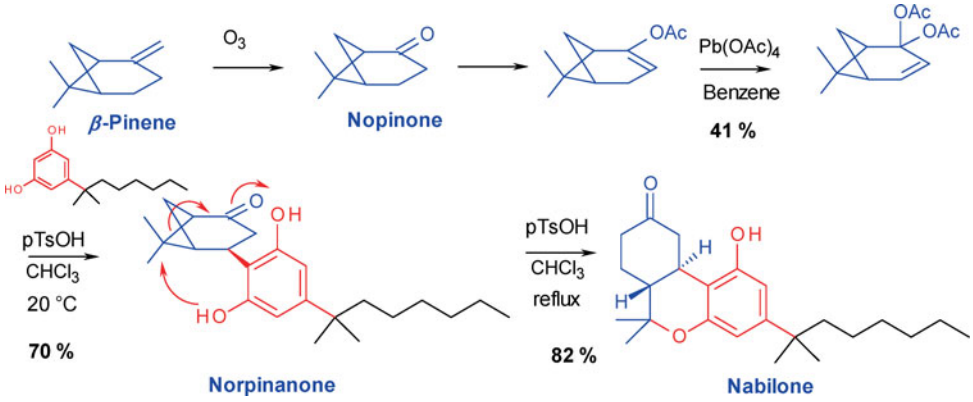
Nabilone is a synthetic cannabinoid with a pharmacological profile comparable to dronabinol. It was developed by Eli Lilly to treat vomiting during cancer treatment, and is marketed as the racemate. [144] However, Eli Lilly has also developed syntheses of the enantiomerically pure product, starting from *β*-pinene. [145, 146]

The alkylated resorcinol is obtained from tri-*O*-methyl pyrogallol by regioselective demethylation with borontrichloride, followed by a Friedel-Crafts alkylation with the appropriate tertiary alcohol. The demethylation is highly regioselective, because the central methoxy group sticks out of the plane of the benzene ring so that the oxygen is more basic than its neighbours. [147]

After phosphorylation, Birch reduction converts the aromatic system to a cyclohexadiene; re-aromatisation occurs then under elmination of the phosphate moiety. The resorcinol is subsequently liberated with boron tribromide.



For the second building block, the enol acetate derived from nopinone is oxidised with lead tetraacetate. A Michael reaction and subsequent Wagner-Meerwein rearrangement finally yield the active compound.



Summary in Bullet Points

- In contrast to opiates, cannabinoids were first approved as pharmaceuticals in the 1980s and 1990s. Their main areas of use are in the treatment of cancer and AIDS patients.
- Investigation into structure-activity relationships led to the discovery of endocannabinoids.
- Over the years, elegant syntheses of Δ^9-tetrahydrocannabinol, starting from the chiral pool or by enantioselective synthesis, have been developed.

5.5 Nonsteroidal Anti-Inflammatory Drugs

The history of non-steroidal anti-inflammatory drugs (NSAIDs) dates right back to ancient times – beginning with Hippocrates of Kos (460–377 BC) and the collection of medical writings known as the *Corpus Hippocraticum*. It is therefore as old as medical science itself. Hippocrates already knew of the pain-relieving (analgesic) and fever-reducing (antipyretic) effects of willow bark (Fig. 5.70).

He advised expectant mothers to chew willow bark in order to deaden the pain experienced during labour. Greek and Roman surgeons recommended aqueous extracts from willow, poplar and evergreens to suppress the pain caused by wounds and other injuries. In the Middle Ages, women herbalists such as Hildegard von Bingen (1098–1179) made use of a decoction from willow bark to combat pain. In addition, from Renaissance times onward, pure meadowsweet [148] (*Filipendula ulmaria* [149]; formerly *Spiraea ulmaria*) was used to treat colds, rheumatism and gout [150] (Fig. 5.71 and Fig. 5.72). [151]

5.70 *The white willow (Salix alba).*

5.71 *Meadowsweet (Filipendula ulmaria). For its essential oils, the blossoms of this plant were formerly used to produce mead, a fermented brew similar to beer.*

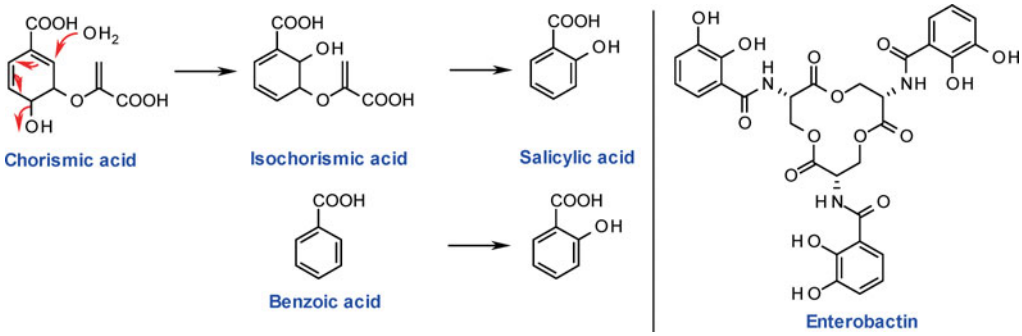
5.72 *Like Frederick the Great (left), Charles V, Henry VIII (right) and Albrecht Wenzel Eusebius von Waldstein (Wallenstein, a Bohemian military leader and politician) also suffered from rheumatism and gout. It is fair to assume that they made use of willow bark and meadowsweet, in order to treat their "minor ailments" or "rheumatic pain", as gout was popularly called.*

As a result of his observations of Nature, Edward Stone (1702–1768), a curate from Chipping Norton in Oxfordshire, used willow bark in 1763 to treat more than 50 patients with malaria. In 1792, the physician Samuel James (1763–1831) used the bark of goat willow (or "pussy-willow", *Salix caprea*) to reduce fever. Stones' work first found general recognition in Nicholas Culpeper's *Complete Herbal,* in the edition of 1802, and willow bark was acknowledged thereafter as

the most important natural remedy for fever. It rapidly replaced the widely-used cinchona bark, which was imported mainly from Peru, especially when the supply of cinchona was stopped by Napoleon's Continental Blockade in 1806. After the Napoleonic Wars, several pharmacists and chemists (among them Pierre-Joseph Pelletier, Joseph Bienaimé Caventou, Johann Samuel Friedrich Pagenstecher and Johann Andreas Buchner) set about the isolation of the active ingredient in willow bark and the determination of its structure. Eventually Henri Leroux, a pharmacist from Vitry le François, succeeded in preparing salicin from meadowsweet, and in 1838, Raffaele Piria (1814–1865) at the Sorbonne oxidised the aglycone of salicin to a carboxylic acid, which he named salicylic acid. Later, Jean Baptiste Dumas (1800–1884) was able to establish that this acid was identical with the one isolated directly from meadowsweet. [152] Finally in 1899, the Bayer company brought acetylsalicylic acid to the market as a pain-suppressing and fever-reducing remedy under the name *Aspirin*® ("A"= acetyl, and "spirin" from *Spiraea*, the genus name of meadowsweet).

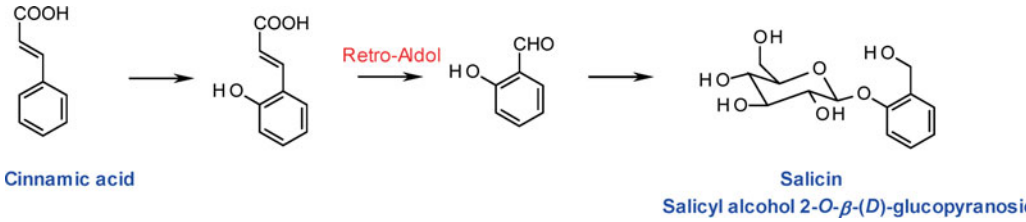
5.5.1 Biosynthesis of Salicylates

There are many diverse biosynthetic routes to salicylates. Microorganisms produce salicylic acid starting from chorismic acid, and also by hydroxylation of benzoic acid.



This is presumably a side-path in the biosynthesis of the much more widely important 2,3-dihydroxybenzoic acid. The latter serves, for example, as a synthetic building block for enterobactin (a siderophore), a critical iron transporter (Fe^{3+} affinity: $K = 10^{52}\ M^{-1}$) in bacteria.

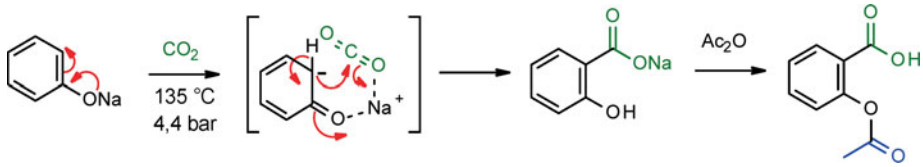
Plants produce salicin as a secondary metabolite from cinnamic acid: this involves *ortho*-hydroxylation followed by a retro-aldol condensation.



The functions of salicylates in plants are manifold. It is known, for example, that they are produced in larger amounts as a result of tissue damage or viral disease, and that they play an important role in thermogenesis during the flowering season. [149]

5.5.2 Chemical Synthesis of Salicylic acid

The structure elucidation of salicylic acid we owe to Hermann Kolbe (1818–1884) and Victor Meyer (1848–1897). Also its industrial synthesis, from phenol and carbon dioxide, can be traced back to Kolbe, who developed this process in 1874. Previously, salicylic acid was produced by hydrolysis of its methyl ester, which in turn was obtained from wintergreen oil, a product of the steam distillation of the leaves of the American wintergreen or teaberry (*Gaultheria procumbens*) and the spice birch (*Betula lenta*).

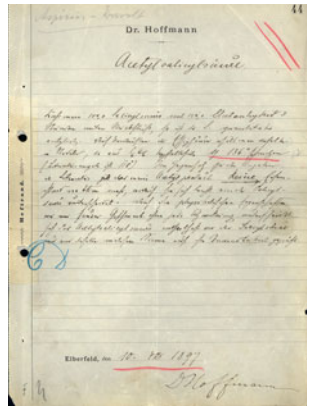


Friedrich von Heyden, a student of Hermann Kolbe (1818–1884), founded a small factory in Dresden for the production of salicylic acid, from which later emerged the Arzneimittelwerke Dresden (Dresden Pharmaceutical Works).

Acetylsalicylic acid was obtained in impure form for the first time in 1853 by the French chemist, Charles Frédéric Gerhardt (1816–1856), and was later isolated in crystalline form by Karl Johann Kraut (1829–1912).

5.5.3 Discovery of *Aspirin*®

5.73 *Felix Hoffmann (1868–1946).*



5.74 *Hoffmann's laboratory notebook of August 10, 1897. Only a few days later, on August 21 1897, Felix Hoffmann prepared heroin by the double acetylation of morphine.*

There are several legends about the discovery of *Aspirin*®, which are connected to German history in a tragic way. At the centre of the commonly known version is a chemist at Bayer, Felix Hoffmann (Fig. 5.73). His father suffered from arthritis and was being treated with the bitter-tasting sodium salicylate, which was known for causing stomach irritation, and also vomiting in patients. He therefore asked his son to go after a better tolerated alternative. Thereupon, Felix Hoffmann

studied the relevant literature, and being aware of Gerhardt's work, he acetylated salicylic acid as described in his laboratory notebook of August 10, 1897 (Fig. 5.74).

Less well known is the following story, on which no further light was shed in Bayer's centenary anthology, dedicated to its Corporate Research. [153, 154] It originates from Arthur Eichengrün (Fig. 5.75), who wrote it down a few months before the end of the Second World War at the concentration camp in Theresienstadt, and published it shortly before his death in 1949 in the journal *Die Pharmazie*. [155, 156]

In this paper, Eichengrün claimed that the idea of acetylating known drugs, in order to improve their solubility, bioavailability and tolerability, came from him. Heinrich Dreser (Fig. 5.76), the Head of the Pharmacological Institute at Bayer, did not want to pursue the studies on acetylsalicylic acid any further, due to its putative cardiotoxicity, which was investigated in those days on frogs' hearts. Without authorisation, Eichengrün conducted self-experiments, by which he took a daily dose of 5 grams of acetylsalicylic acid over a 14-day period. Moreover, without consulting Dreser, he had initiated clinical studies in Berlin, during the course of which a dentist accidentally discovered that acetylsalicylic acid possessed not only antipyretic but also analgesic properties. Eventually, the alleged heart-damaging side-effects could be disproved. Heinrich Dreser could no longer defy such positive results, and upon further pressure from Bayer's Head of Research, Carl Duisberg (1861–1935), further clinical studies were conducted. *Aspirin*® was brought to the market in 1899, initially as a powder and later as the first pharmaceutical to be available in tablet form.

The irony of this story, however, culminated, that Heinrich Dreser gained glory and wealth, for "the discovery of the most successful drug of all time". In contrast to Hoffmann and Eichengrün, Dreser received a share in the sales of all of Bayer's pharmaceuticals. Finally, when *Aspirin*® was put on display at the "Deutsches Museum" (Munich) in 1941, only the names of Hoffmann and Dreser appeared on the exhibit, while at the entrance the sign was posted: "No admittance to non-Arians". Arthur Eichengrün was Jewish.

Even before the First World War, *Aspirin* was economically such a great success that in 1918 the trademark was reckoned by the Allied Powers to be especially valuable, and thus, together with relevant patents and the manufacturing plant, it was confiscated as reparation claim. Only in 1994, Bayer was allowed to repurchase these rights, along with the Bayer Cross, for 1 billion dollars. The annual world production of acetylsalicylic acid amounts today to approximately 50,000 tonnes (Fig. 5.77). [152]]

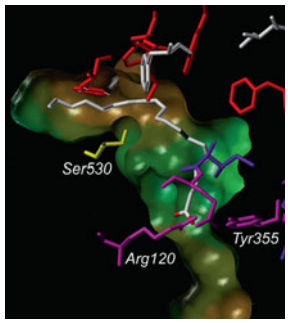
5.75 *Arthur Eichengrün (1867–1949).*

5.76 *Heinrich Dreser (1860–1924).*

5.77 *On the occasion of the hundredth birthday of Aspirin® in March 1999, Bayer's high-rise at its Leverkusen headquarters was transformed into the largest Aspirin® carton in the world.*

5.5.4 Mode of Action of *Aspirin*®

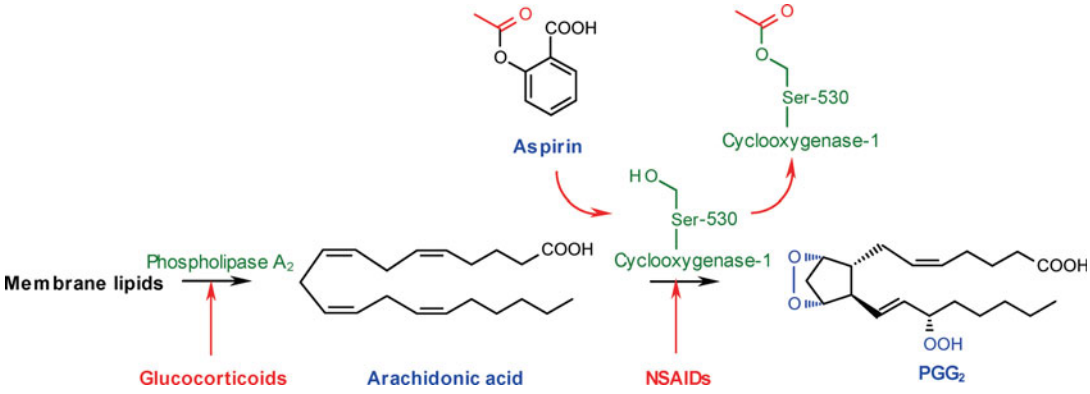
Notwithstanding the medicinal successes of *Aspirin*®, its pharmacological mode of action remained obscure for a long time. In the 1940s, the Californian physician Lawrence Craven (1883–1957) observed, that the use of *Aspirin*® as a painkiller following tonsil surgery (tonsillectomy), led to massive bleeding. He tested *Aspirin*® as an anticoagulant on several thousand patients suffering from car-



5.78 *A view of the COX channel with arachidonic acid (in white).*

diac infarction, and was able to show unequivocally that it is a highly effective thrombocyte-aggregation inhibitor. It is an ironic twist of fate that Craven, despite his self-medication, died of a heart attack prior to completion of his studies.

The dominant mode of action of acetylsalicylic acid (ASA) was clarified in 1971 by the British pharmacologist Sir John R. Vane (1927–2004), an achievement, for which he was awarded the Nobel Prize for Medicine in 1982. Through acetylation of serine-530, acetylsalicylic acid irreversibly inhibits cyclooxygenase-1 (COX-1). For this, ASA first diffuses through a narrow channel of the enzyme into its active pocket, and binds, like arachidonic acid, to arginine-120 (*cf.* section 5.6, prostaglandins) *via* an ionic interaction. Thereby, acetysalicylic acid is lined up in a favourable direction for transacetylation – only around 5Å beneath serine-530 – so that the transfer of the acetyl group can finally take place easily (Fig. 5.78). [157, 158]

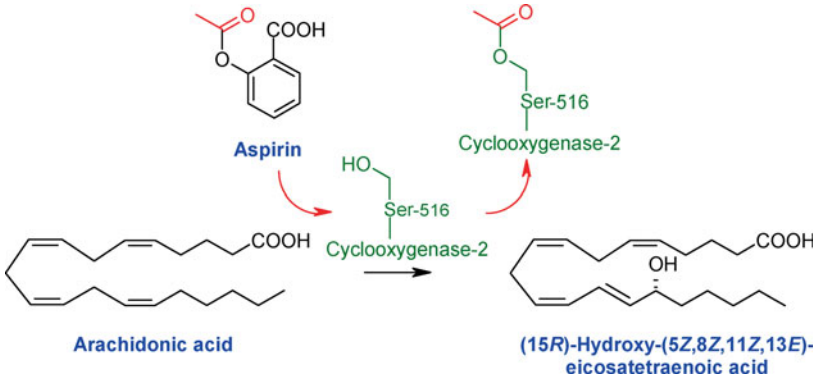


Vane's work also unveiled the relationship of acetylsalicylic acid to the glucocorticoids, the steroidal inflammation inhibitors, especially to cortisol and its derivatives. These block the enzyme phospholipase A_2 and therefore the liberation of arachidonic acid from membrane phospholipids. Due to the very diverse effects of glucocorticoids, these are of restricted value as anti-inflammatory and analgesic drugs, so that the search for non-steroidal anti-inflammatory drugs (NSAID) took on great importance. [159]

Cyclooxygenase-1 is a membrane-bound, ubiquitously occurring enzyme. It is located upstream of the extensively branched biosynthetic pathways to prostaglandins, which play a significant part in the origin of pain and inflammatory processes. [152]

Interestingly, it was shown that the analgesic and anti-inflammatory effect of acetylsalicylic acid could be further enhanced by increased dosage, even if the cyclooxygenase activity was already inhibited to the 100 per cent level. The discovery by Daniel L. Simmons at Brigham Young University in Provo, Utah in 1991 of an isoform of cyclooxygenase, COX-2 provides the explanation for this phenomenon. [160, 161] This enzyme promotes preferably, although not exclusively, the synthesis, of those prostaglandins, which participate in analgesic and inflammatory processes.

Due to a less precise alignment in the catalytic centre of this enzyme, acetylsalicylic acid inhibits COX-2 only at elevated concentrations. Interestingly, acetylation occurs here at position 516, whereupon only (15*R*)-hydroxyeicosatetraenoic acid, a metabolite of 15-lipoxygenase, can be produced. [162, 163]



However, it is also of significance that inflammation mediators upregulate the expression of COX-2. Kenneth K. Wu could demonstrate that acetylsalicylic acid and, remarkably, salicylic acid as well inhibit the binding of transcription factors in the promoter region of the COX-2-gene, and thereby inhibit the synthesis of COX-2. Only this second mechanism explains satisfactorily why salicylic acid itself is able to relieve pain and reduce inflammation. [164]

5.5.5 Other Natural Cyclooxygenase Inhibitors

The deepened understanding of the mode of action of *Aspirin*® was an important prerequisite that in the following years a number of other natural products were discovered, which, like salicylic acid from willow bark (*Cortex salix alba*), exhibit analgesic and anti-inflammatory activity.

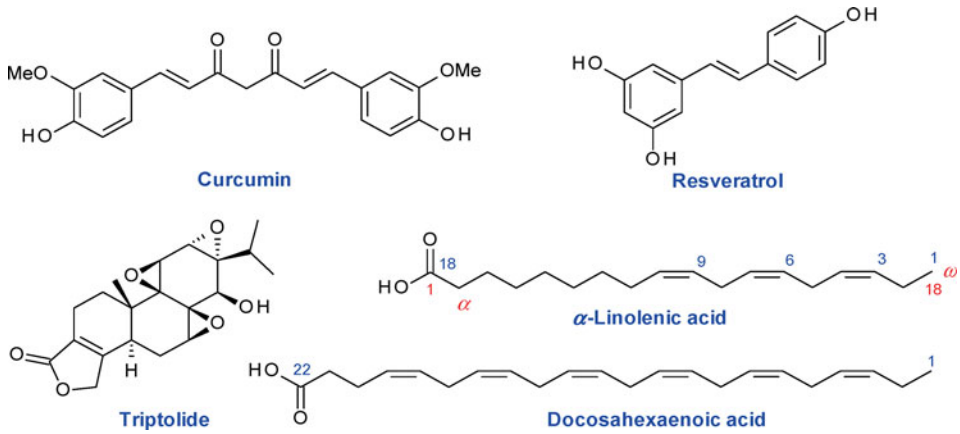
Fan Zhang at Cornell University in New York and Ajay Goel at the University of California in San Diego found that curcumin, the yellow dye in turmeric (*Curcuma longa*), inhibits in a concentration- and time-dependent manner the expression of cyclooxygenase-2 and the formation of prostaglandin E_2 (PGE_2) (Fig. 5.79 and Fig. 5.80). [165, 166]

A similar effect is now as well ascribed to resveratrol, a phytoalexin from the skin of black grapes, and to the Japanese knotweed (*Polygonum cuspidatum*). The active compound, widely used in traditional Chinese herbal medicine, prevents the increased expression of human cyclooxygenase-2 and hence a higher COX-2 activity.

Extracts from Wilford's three-wing fruit (Thunder God Vine, *Tripterygium wilfordii*) have likewise been used in traditional Chinese medicine since ancient times for the treatment of rheumatoid arthritis. The pharmacologically important component of the extract proves to be triptolide, which inhibits the lipopolysaccharide-induced COX-2 expression and the release of PGE_2, and has thereby an anti-inflammatory effect. [167, 168] It is remarkable that the extract does not affect the COX-1 activity.

Lastly omega-3 fatty acids, which are physiologically valuable nutritional components of various vegetable and fish oils, are attributed to the selective inhibition of COX-2 mRNA, without impairing COX-1 expression. [169, 170]

5.79 *Turmeric (Curcuma longa) and black grapes (Vitis vinifera).*



5.80 *Natural products with anti-inflammatory activity.*

While none of these substances come anywhere close to the activity of acetylsalicylic acid, their structures offer potentially interesting starting points for new drug developments. [171]

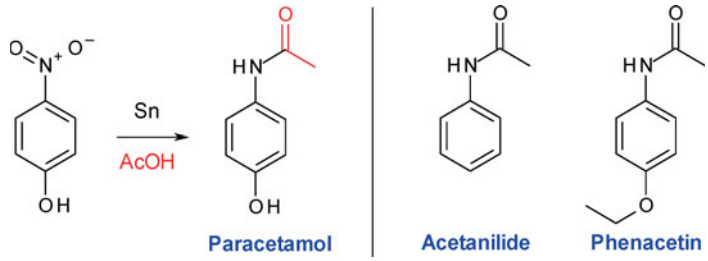
5.5.6 Paracetamol

Whereas the mode of action of acetylsalicylic acid is now well understood, that of paracetamol (also known as "acetaminophen", especially in the USA, Canada, Japan, Hong Kong, Iran and Colombia), still remains a mystery, in spite of being equally powerful in terms of both, its breadth of use and its market presence.

Paracetamol was first prepared in 1878 by Harmon Northrop Morse (1848–1920) at the Johns Hopkins University by reduction of *p*-nitrophenol with tin in acetic acid. [172, 173] In 1887, Josef von Mehring (1849–1908) used the compound for the first time as a drug, which did however not achieve any great therapeutic significance.

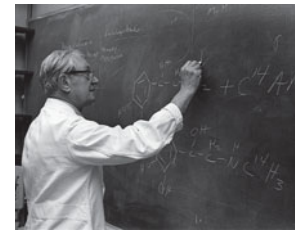
A much wider use was found for two molecules closely related to paracetamol, namely acetanilide and phenacetin. The serendipitous discovery of acetanilide

(and thereby the basic structure of the compound class) is due to confusion of two substances by a pharmacist; this compound was marketed in 1886 by Kalle & Co. under the trade name *Antifebrin*®. Phenacetin was discovered by Oscar Hinsberg (1857–1939), who worked during his University holidays in the laboratory of Carl Duisberg (1861–1935), at the erstwhile dyestuff-manufacturing Farbenwerke Friedrich Bayer & Co. in Wuppertal-Elberfeld. The substance was the first pharmaceutical, which Bayer brought to the market in 1888, and thereby laid the foundation for a worldwide operating pharmaceutical business. [174]



In 1893, paracetamol was discovered for the first time in human urine from a man who had been treated with phenacetin. Shortly before the turn of the century, it became clear that paracetamol is a metabolite of acetanilide. This information however attracted no attention for almost 50 years.

It was only after the Second World War that David Lester (1916–1990) and Leon Greenberg, and also shortly afterwards, Bernard Brodie (1907–1989) and Julius Axelrod (1912–2004) at the New York City Department of Health, came across this discovery for a second time. Brodie and Axelrod were seeking new pain-killing drugs on behalf of the American Government (Fig. 5.81). They were able to show that the pharmacological effect of acetanilide and phenacetin is almost wholly attributable to their common metabolite, paracetamol; and they suggested development of this compound as a drug.

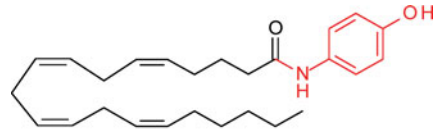


5.81 *Julius Axelrod in 1973.*

In 1955, paracetamol was launched in the USA as *Tylenol*® by the firm McNeil Laboratories, and in the following year in Britain by the Sterling Drug Co. under the trade name *Panadol*®. Introduction to the German market followed in 1959 by the Munich-based company, *bene*-Arzneimittel, under the trade name *Ben-u-ron*®. Since 1977, paracetamol is listed among the "indispensable" pharmaceuticals by the World Health Organisation. It was quickly recognised that by comparison with *Aspirin*®, this compound presented a lesser risk of stomach damage, which was used specifically as an argument in advertising to promote the sales of paracetamol.

Traditionally, the drug counts as a non-steroidal inflammation inhibitor, although its anti-inflammatory effect is comparatively low, while its antipyretic and analgesic action predominate. There has been a series of studies so far to determine the molecular mode of action. [174]

Apart from modulating the serotonin system, another interaction with the endocannabinoid system could be detected *in vivo* (*cf.* section 5.4 – tetrahydrocannabinol). The amide from arachidonic acid and *p*-aminophenol bind like the endocannabinoids at the vanilloid receptor TRPV1 and thus impact the nociception and the body temperature.



(5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(4-hydroxyphenyl)-5,8,11,14-icosatetraenamide

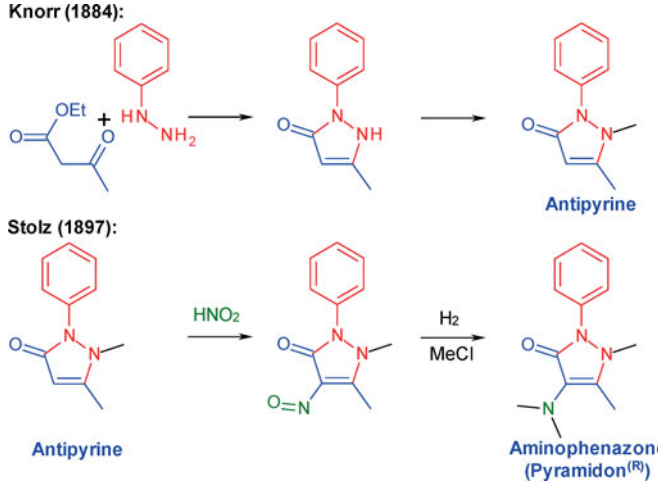
Based on the work of John Vane, an interaction of cyclooxygenase was also taken into further consideration, whereby it was possible to explain the low anti-inflammatory activity by virtue of the missing carboxylic acid function, which suggests a different systemic distribution compared to *Aspirin*®.

Roderick J. Flower and John Vane recognised already in 1972 that prostaglandin biosynthesis in the brain is ten times more sensitive to inhibition by paracetamol than, for example, in the spleen. [175] But only in 2005, Graham and Scott proved that paracetamol has at therapeutic doses pharmacological effects similar to selective COX-2 inhibitors in intact cells of the central nervous system. [176] Paracetamol inhibits both, the basal and the lipopolysaccharide-induced PGE_2 production in cerebral endothelial cells. [177] One factor is still unexplained: how this happens at the molecular level.

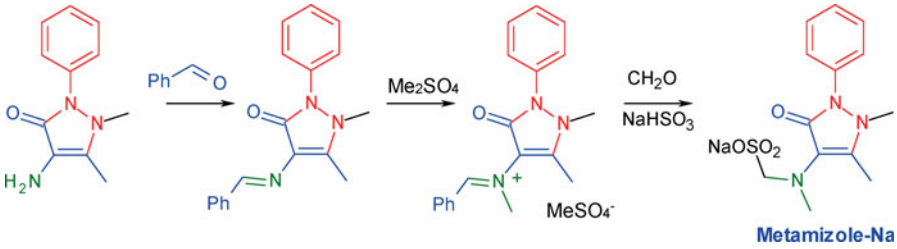
Paracetamol is extremely toxic for cats, because they lack the glucuronyltransferase important for detoxification. Also for snakes the compound possesses high toxicity, so that it has been considered as a means to exterminate especially the brown tree snake (*Boiga irregularis*) in Guam. [173]

5.5.7 Pyrazolones

A different non-steroidal inflammation inhibitor, which was discovered almost at the same time as paracetamol and its prodrugs acetanilide and phenacetin, was first synthesised in 1884 by Ludwig Knorr (1859–1921) in Erlangen: this was *Antipyrine*®, which he obtained in the course of the pyrazolone synthesis, which is named after him, followed by methylation. The active compound was produced at a small dyework on the outskirts of Frankfurt, out of which the Hoechst company (now part of Sanofi), emerged. [178] Later, Friedrich Stolz (1860–1936) at Hoechst also synthesised *Pyramidon*® from antipyrin by nitrosation, hydrogenation and methylation. [179] This drug, like many other members of its structural class, has in the meantime disappeared from the market.



Nowadays, only metamizole (*Novalgin*®) is still of limited relevance, as a water-soluble intravenously-applied formulation of its sodium salt for the relief of severe colic-like pain. In newer studies it could be shown that this compound also inhibits COX-2.



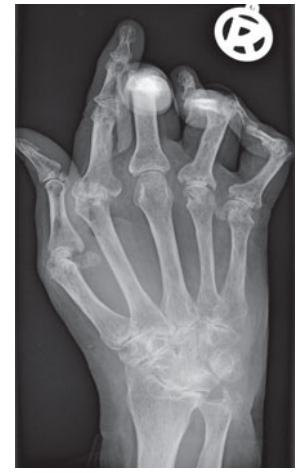
5.5.8 Discovery of the Classical Non-steroidal Anti-inflammatory Drugs

The standard therapy for rheumatoid arthritis relyed in the 1950s on corticosteroids, in spite of their massive side-effects (Fig. 5.82). The single non-steroidal substance, which was known in the 1950s and could help in relief of rheumatoid arthritis, was *Aspirin*®. But due to the associated serious risk of damaging the gastric mucosa at elevated doses, it could not be used for long-term application.

Stewart Adams and John Nicholson at the Boots Company in Nottingham took acetylsalicylic acid as a lead template and looked for related anti-inflammatory structures, which would be devoid of the ASA-related side-effect. In 1955, they discovered that their anti-inflammatory compounds reduced skin-reddening (erythema) in guinea-pigs, caused by ultraviolet irradiation. Thereby they had found a simple and feasible assay system to test a whole host of compounds. In 1961, out of a group of several hundred substances they were able to select the one, which was introduced to the British market in 1969 under the name of ibuprofen (Fig. 5.83). [180]

Also other members of the profen-family, among them naproxen, flurbiprofen, ketoprofen and tiaprofenic acid, are of great importance today. In general, these are 2-aryl- or 2-heteroaryl-propionic acids, of which *in vitro* only the (*S*)-(+)-enantiomer inhibits cyclooxygenase-1 (Fig. 5.84). *In vivo,* depending on the particular animal species, and also in humans, the (*R*)-(−)-enantiomer is converted to various degrees into its (*S*)-enantiomer with the aid of α-methylacyl-CoA-racemase, *via* the Coenzyme A-thioester. In addition, more recent investigations could show that (*R*)-ibuprofen and (*R*)-flurbiprofen themselves have also anti-inflammatory properties. Many of the profens, with the exception of naproxen, are therefore marketed as racemates.

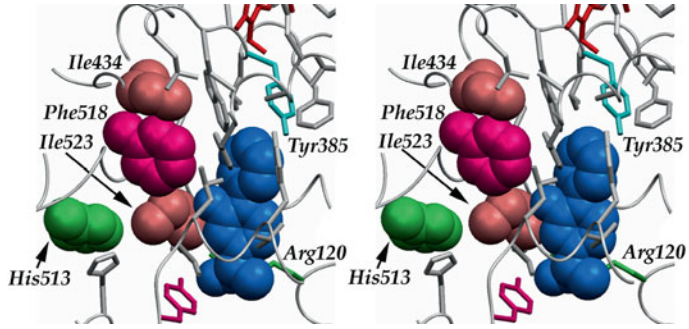
Several drug companies have developed, apart from profens, additional classes of NSAIDs (Fig. 5.85). Many of these compounds, like the profens and acetylsalicylic acid, as well as the natural prototype, arachidonic acid, possess as



5.82 *Typical X-ray picture of rheumatoid arthritis.*

It was reported that Dr. Adams had also employed the drug for the treatment of his hangover. [181]

5.83 *A bottle of generic Ibuprofen.*



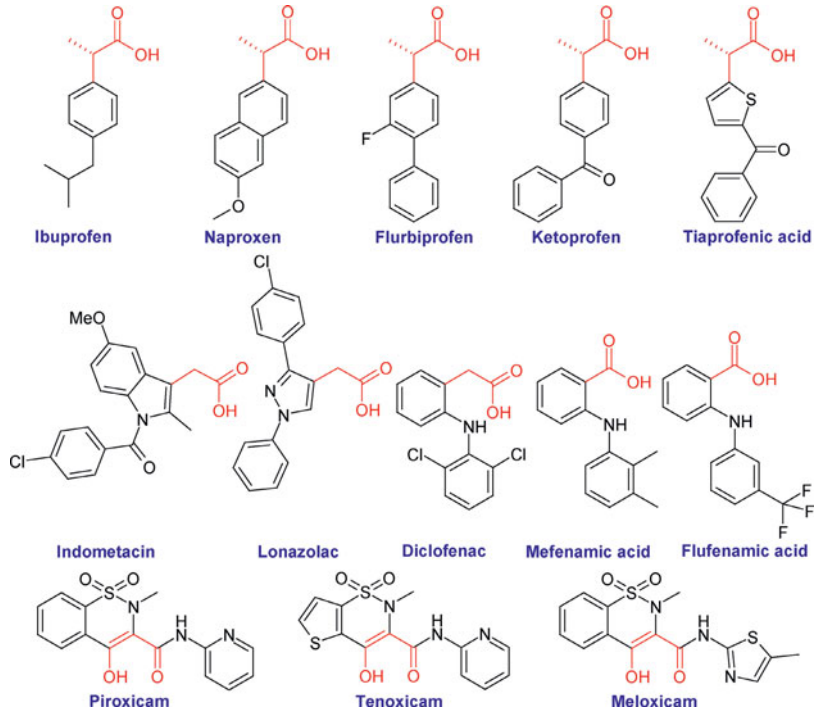
5.84 *A stereo diagram of Flurbiprofen (blue) binding to the COX active site. [182]*

their common structural characteristic an acidic or optionally vinylogous carboxylic function. [183]

Indometacin (*Amuno*®) provides a representative example of the fenac class. The search for better-tolerated drugs led in1974 to diclofenac (*Voltaren*®). [184] This is well-tolerated and nowadays available in a variety of application forms. For the prevention of gastrointestinal ulceration, a combination product with the prostaglandin misoprostol (*Cytotec*®) (*cf.* section 5.6, prostaglandins) has been developed.

The oxicams comprise a class of vinylogous carboxylic acids, which were first launched in the 1990s. [183] The basic structure is a 4-hydroxy-2-methyl-1,1-dioxo-5,6-dihydrothiazine-3-carboxamide. Most of these substances show a long duration of action and are used in rheumatism therapy.

More recently, diclofenac has met with criticism due to its toxicological and ecotoxicological properties. [184] It leads to acute kidney failure in vultures (*Aegypiinae*), if these feed on dead animals (and humans, as for example those of the Pakistani and Indian Zoroastrian Parsi religion in the "towers of silence") who have been treated with diclofenac shortly before their death. The decline in vulture population led to growing numbers of wild dogs (*Cuon alpinus*) and leopards (*Panthera pardus*), which for their part endanger humans either directly or through rabies. Moreover, as a consequence of its high persistence, the drug has shown to be harmful to freshwater fish through excreted urine.

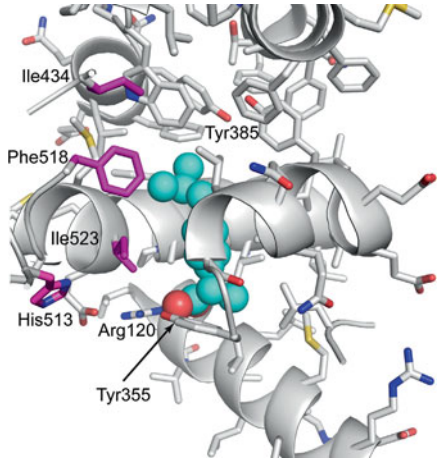


5.85 *Classical Non-Steroidal Anti-Inflammatory Drugs.*

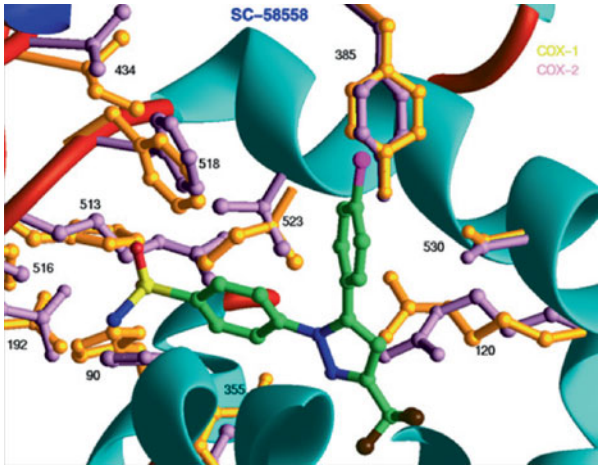
5.5.9 Mode of Action of Competitive COX-Inhibitors

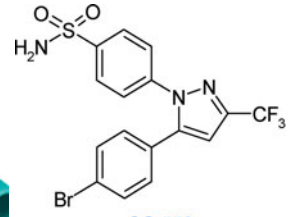
The classical non-steroidal anti-inflammatory drugs, except acetylsalicylic acid, bind competitively to cyclooxygenases (Fig. 5.86). Usually, a very strong ionic bond to Arg-120 is formed. Additional lipophilic interactions at the site of the channel narrowing or in the active pocket enhance the binding further, so that arachidonic acid can no longer access this pocket. [158]

The rational basis for the development of cyclooxygenase-2-selective inhibitors was the discovery that there are structural differences between both isoforms in the region of the binding pocket (Fig. 5.87). The end of the hydrophobic access channel is bordered by a side-pocket, which is in the case of cy-



5.86 *Ibuprofen (turquoise) is shown in the active site of COX-1. Residues Ile434, His513, Phe518, and Ile523 (colored in magenta) create a side-pocket, but the bulkiness of Ile434 and Ile523 ensures that Phe518 blocks the entry into this pocket.*





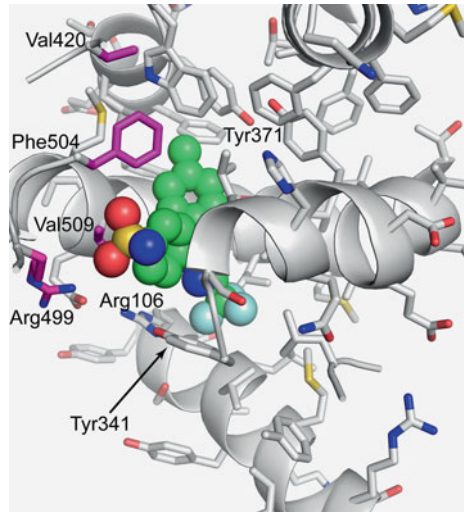
5.87 *Superposition of the active site of COX-1 (yellow) with COX-2 (purple) and the COX-2-selective inhibitor SC-558. The larger NSAID binding pocket is clearly visible. The access to this side-pocket is more restricted in COX-1 because of the larger isoleucine instead of valine-523.*

clooxygenase-1 blocked by a bulky isoleucine residue. In cyclooxygenase-2, the smaller valine-523 occupies the corresponding position, which eventually enlarges the pocket by around 17 %. [185] R. G. Kurumbail was able to show that the COX-2-selectivity of 1,2-diaryl-substituted heterocycles with sulfonamide side-chains is based upon hydrogen bonding with polar amino acid residues in the side-pocket, such as histidine, glutamine and arginine. [186] Eventually, this amounts to an inhibitor-induced conformational change and thereby the formation of a stable complex.

5.5.10 Development of Selective COX-2 Inhibitors

The development of essential structural motifs for modern COX-2 inhibitors dates back to the 1970s, when an attempt was made to improve the tolerability of arylacetic acid derivatives by replacing the carboxylic acid function with a methanesulfonamido-group. Additional strongly electron-withdrawing substituents like nitro-, cyano- or acetyl-, increase the acidity of the sulfonamide to an extent, which is sufficient for the pharmacological effect at the receptor. The typical representative of this series is nimesulide. However, nimesulide gained its actual significance only, once it was recognised in the 1990s that the cyclohexane analogue NS-398 preferentially inhibits COX-2. In spite of extensive clinical research, NS-398 never reached the market, though for a long time it assumed great experimental value as reference material. An early development candidate with the corresponding structural motif was flosulide. Despite a high COX-2 selectivity *in vitro*, its development was terminated, after no advantage in stomach tolerability could be found *in vivo*.

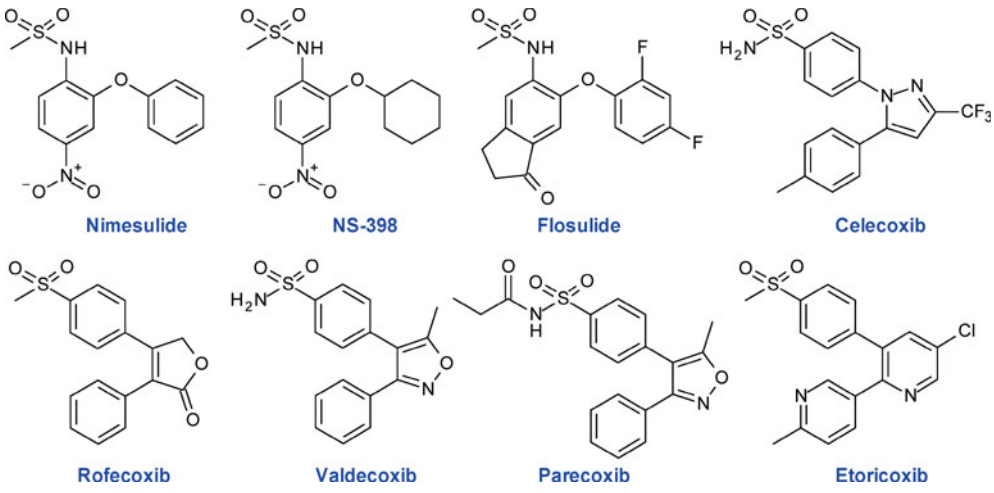
Celecoxib was the first modern COX-2 inhibitor, which was introduced to the market in 1998. Developed by Monsanto/Searle, this drug is 375 times more selective for COX-2 over COX-1, based on assays with recombinant human enzymes (Fig. 5.88). [187]



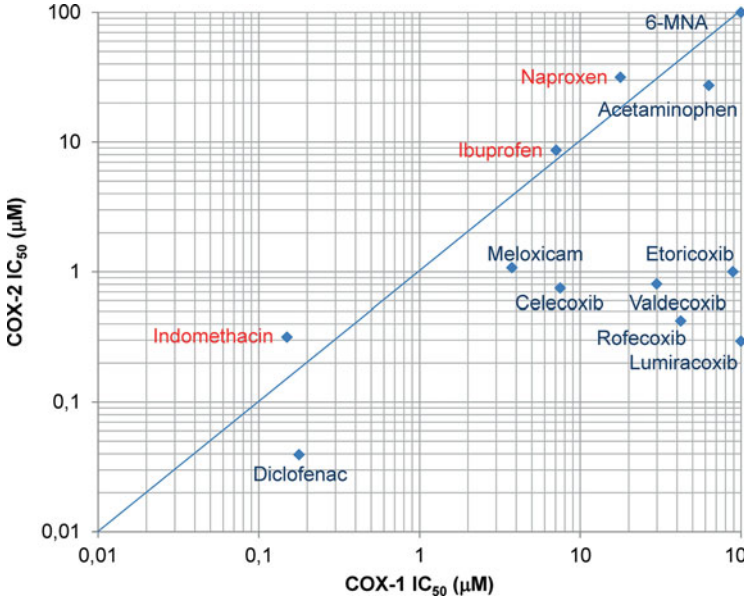
5.88 *Celecoxib is shown in the COX-2 active site. The COX-2 residues equivalent to Ile434, His513, Phe518, and Ile523 in ovine COX-1 are Val420, Arg499, Phe504, and Val509, respectively, and are colored in magenta. Because of the isoleucine to valine substitutions for the residues that surround Phe504 in COX-2, the phenylalanine can move out of the way, which opens up the side-pocket. Now, polar substituents, such as the benzenesulfonamide of celecoxib, can extend into the side-pocket of COX-2, in contrast to inhibitors binding to COX-1.*

The second drug, which was marketed almost at the same time by Merck, was rofecoxib (*Vioxx*®). Within a few years, *Vioxx*® became the company's product with the highest sales revenue, amounting to 2.6 billion US dollars. However, Merck had to withdraw the drug from the market unexpectedly in 2004, because of serious side-effects, like increased risk of heart attacks, unstable Angina pectoris and strokes.

A similar fate befell a series of other coxibs as well: These, like Valdecoxib (*Bextra*®) had to be withdrawn from the market by Pfizer, or, like parecoxib (Pfizer) or etoricoxib (Merck), did not obtain regulatory approval in major markets at all (Fig. 5.89).



5.89 *Selective COX-2 inhibitors.*

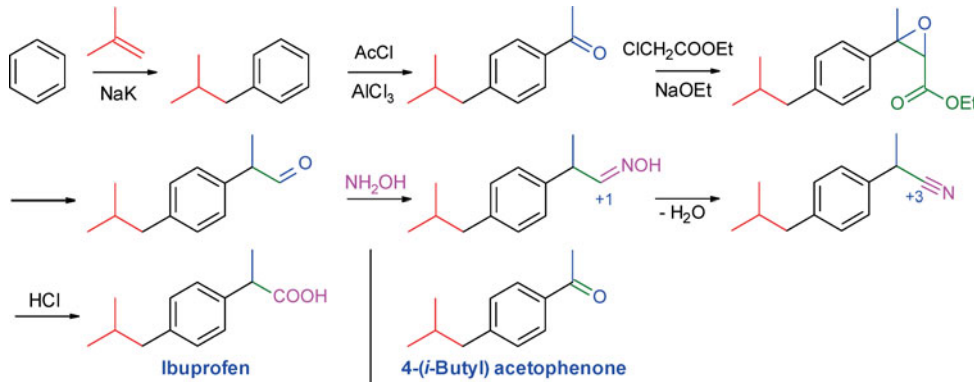


5.90 *IC_{50} values of COX inhibitors at COX-1 and COX-2.*

The selectivity of drugs is generally assessed with the aid of *in vitro* or *ex-vivo* assay systems, and also in human whole blood as appropriate. Occasionally, however, the data show considerable variability. This appears to apply in particular to meloxicam, where, depending on the experimental set-up, almost any selectivity factor may be obtained. [188] COX- inhibitors show in general affinity to both isozymes, cyclooxygenase-1 and -2. In many cases the selectivity factor ranges from 1 to 100 (Fig. 5.90). [189]

5.5.11 Syntheses of Modern Non-steroidal Anti-inflammatory Drugs

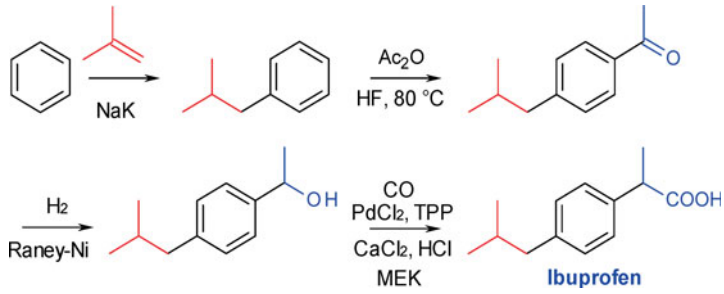
The industrial synthesis of ibuprofen is a well-known case study for "green chemistry". [190] The original Boots route, starting from benzene, comprised seven steps. *p*-Isobutylacetophenone is accessible by alkylation of benzene with isobutene, followed by a Friedel-Crafts acylation with acetic chloride. Homologation to the corresponding aryl-propionaldehyde is achieved by a Darzens reaction. Transformations *via* the oxime and nitrile, and hydrolysis of the latter, finally give ibuprofen.



In this context, the oxidation method of the aldehyde to the carboxylic acid is noteworthy: this is eventually accomplished by dehydration of the corresponding oxime. The redox potentials would suggest more direct methods. However, readily enolisable aldehydes tend to undergo C-C bond cleavage and hence result in poor yields. [191] Conventional oxidations, *e.g.* with permanganate, chromate, or ruthenium trichloride/periodate or electrochemical methods lead to the formation of larger amounts of the corresponding acetophenone. Oxidations with silver oxide or silver salts actually produce ibuprofen in better yields, but are too expensive on an industrial scale. [192] Only the development of special oxidation methods, like that of the bisulfite adduct with DMSO/acetic anhydride [191], or with sodium chlorite in aqueous acetonitrile [193] allowed for the conversion of the aldehyde into ibuprofen in good yields (81 and 95 % respectively).

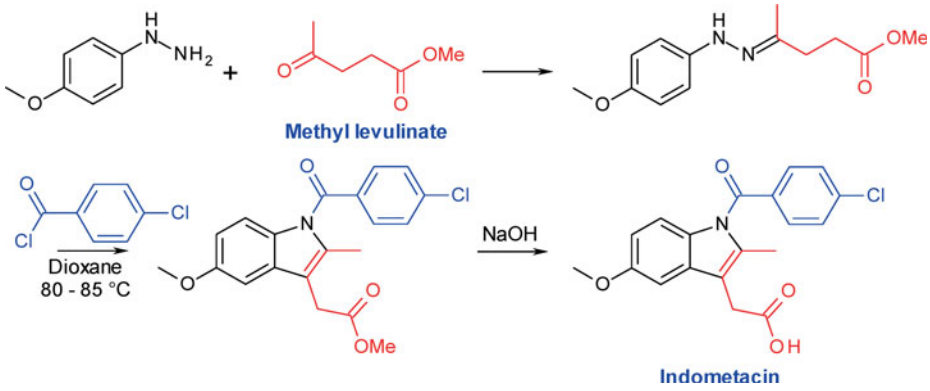
The optimised synthesis of ibuprofen by Boots-Hoechst-Celanese however comprises only four reaction steps: *p*-Isobutylacetophenone is prepared by a

process, which is formally similar to the original synthesis. However, stoichiometric quantities of aluminium chloride are replaced by catalytic amounts of hydrogen fluoride, which are recovered and reused. Then follow a hydrogenation over Raney nickel and finally a palladium-mediated carbonylation. In 1997, this route achieved recognition by the Greener Synthetic Pathways Award.



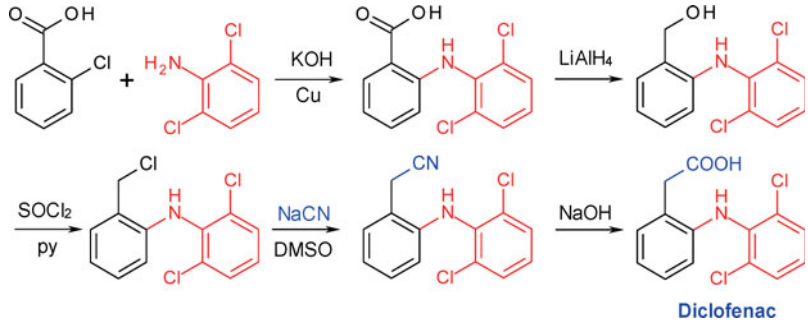
Indometacin was discovered at the beginning of the 1960s by the Merck Sharp & Dohme company, and was approved by the FDA in 1965 for the United States market. The industrial synthesis involves a Fischer indole synthesis of 4-methoxyphenylhydrazine with methyl levulinate. The charme of this route is that the indole synthesis is carried out stepwise, and that 4-chlorobenzoyl chloride is added at the hydrazone stage. In this way, the methyl ester of indometacin is produced, which requires only hydrolysis to generate the active material.

An article by J.-P. Rieu, A. Boucherle, H. Cousse and G. Mouzin provides an excellent overview of the early synthetic methods for the preparation of 2-arylpropionic acids. [194]



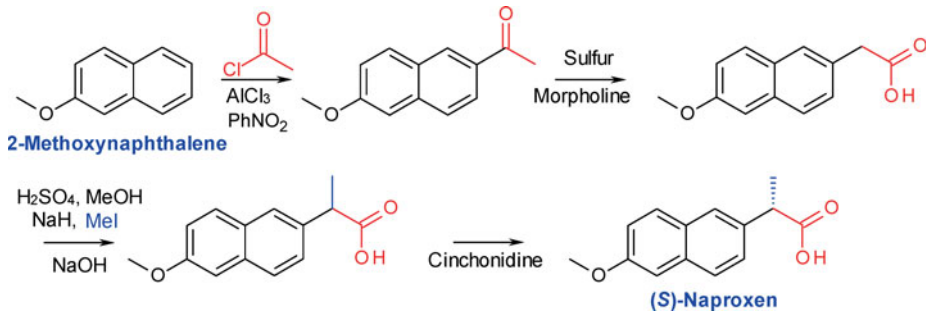
Diclofenac dates from the beginning of the 1970s, and was developed by Ciba-Geigy (now part of Novartis). It is marketed as its sodium or potassium salt in various formulations and dosage forms (injectable solutions, suppositories and capsules).

The synthesis is comparatively simple. An Ullmann reaction is followed by reduction with lithium aluminium hydride and a one-carbon elongation of the side-chain with cyanide.



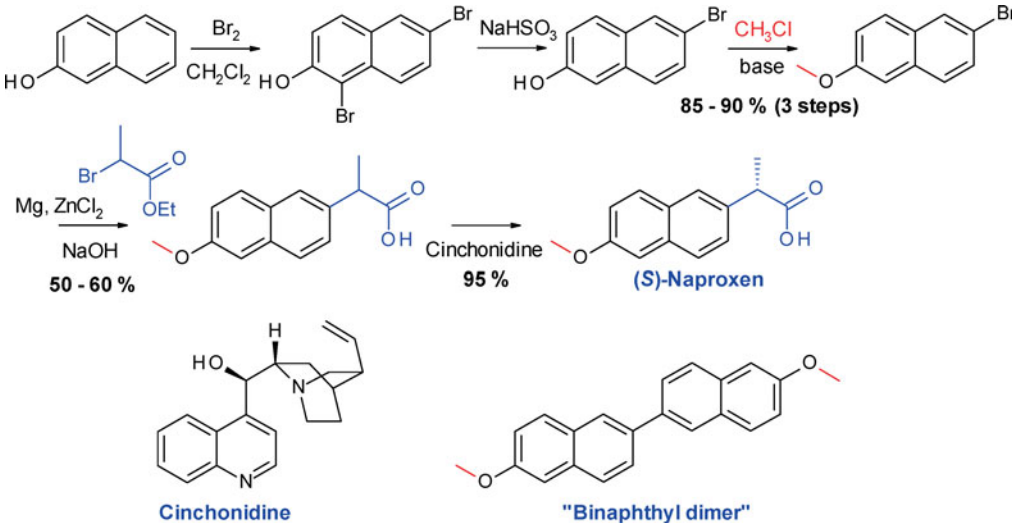
(*S*)-Naproxen was the first enantiomerically pure non-steroidal inflammation inhibitor, which was developed almost at the same time as diclofenac, and marketed by Syntex in 1976. [195] At the beginning of the 1990s (*S*)-naproxen was reckoned among the drugs with the highest turnover: in 1995, shortly after the expiry of the patent protection, this amounted to 1.05 billion dollars. The initial 500 kg of the drug were produced in 1970 according to the following synthesis:

Friedel-Crafts acylation of 2-methoxynaphthalene (nerolin) is followed by a Willgerodt-Kindler oxidation of the methyl ketone. After esterification, the methyl group is introduced by alkylation, and the enantiomers are finally separated by co-crystallisation with cinchonidine.



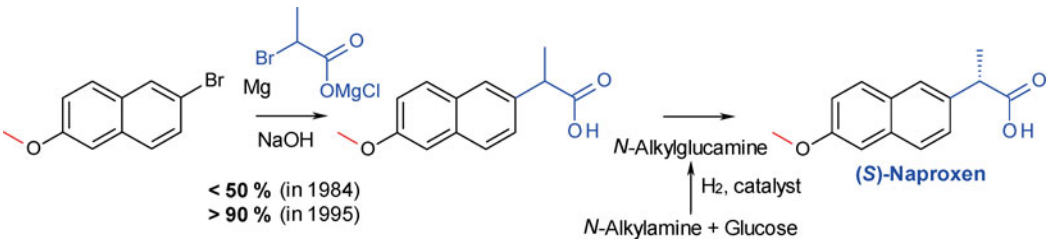
The synthesis had a series of disadvantages, like a poor regioselectivity in the Friedel-Crafts acylation, in which the 1-isomer is also produced, accompanied by educts and by-products with safety-critical properties (nitrobenzene, sodium hydride, methyl iodide, sulfide salts) and an undesirable waste disposal problem, demanding a different synthetic path for the larger production scale.

From 1972 to 1975, Syntex synthesised the drug by bromination of 2-naphthol. The labile bromine atom at C-1 was removed with sodium bisulfite. After methylation of the naphthol came a zinc-mediated coupling reaction. The enantiomers were separated as before with cinchonidine.



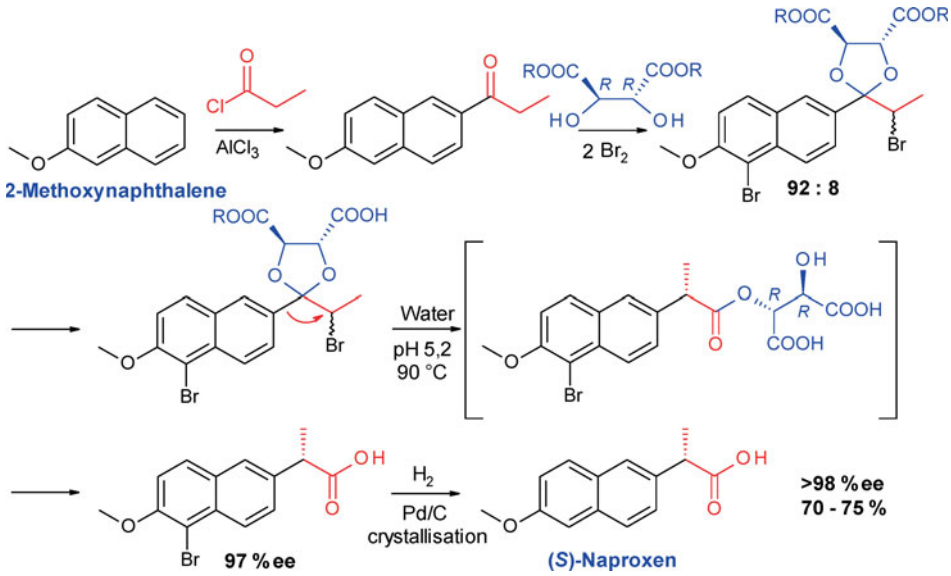
This synthesis had some disadvantages as well, which related basically to the organometallic coupling step. The yield is comparatively poor. Zinc chloride is used in stoichiometric quantities. During the reaction considerable amounts of a sparingly soluble binaphthyl dimer are produced by homo-coupling. By reduction, the volatile 2-methoxynaphthalene occurs as well. The dimer is filtered off along with the zinc paste and dumped. The 2-methoxynaphthalene, because of its "sweet grape" smell, repeatedly led to complaints from the communities neighbouring the production plant.

The fundamental improvement of the process encompassed replacing the naphthylzinc-coupling by a direct reaction of the Grignard reagent with the salt of bromopropionic acid. Hereby, it was first of all possible to reduce drastically the formation of both by-products, and after additional process optimisations, also to increase the yield to above 90 %. Furthermore, cinchonidine was replaced by *N*-alkylglucamine, which was easily and inexpensively accessible by reductive amination of (*D*)-glucose. The (*R*)-enantiomer of naproxen was recovered by what is effectively a Pope-Peachy resolution. In this, the racemic acid is reacted with 0.5 equivalents of an achiral amine and 0.5 equivalents of an enantiomerically pure amine (the *N*-alkylglucamine). The salt of the chiral amine with (*S*)-naproxen precipitates and is flitered off. The salt of (*R*)-naproxen with the achiral amine remains in the mother-liquor, and is racemised under heating. The racemic mixture is then resubmitted to the resolution cycle. Thereby the yield in the resolution exceeds 95 %.



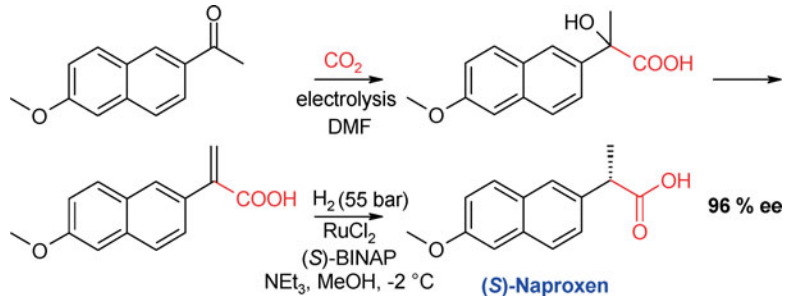
However, there remained the basic problem of a stereochemically unselective synthesis, an issue, which in consequence had been addressed in many papers published over the years. [196]

An interesting solution was provided by a process developed by the Zambon company. [197] Friedel-Crafts acylation of 2-methoxynaphthalene is followed by a ketalisation with diethyl (*R*,*R*)-tartrate and a double bromination, at C-1 and in the side-chain, yielding a 92 : 8 mixture of diastereomers. After hydrolysis of the tartrate, the reaction mixture is heated to 90 °C in water. Thereby, under kinetic resolution and by a [1,2]-aryl migration with complete inversion at the stereogenic centre, bromonaproxen is formed, which is finally converted by reductive dehalogenation into enantiomerically pure (*S*)-naproxen.

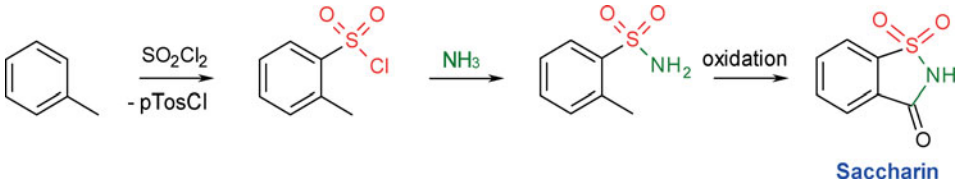


An essentially more elegant and shorter synthesis is the asymmetric hydrogenation of naphthacrylic acid, which is accessible by electrocarboxylation of 2-acetyl-6-methoxynaphthalene in an undivided cell with a sacrificial aluminium anode. [198]

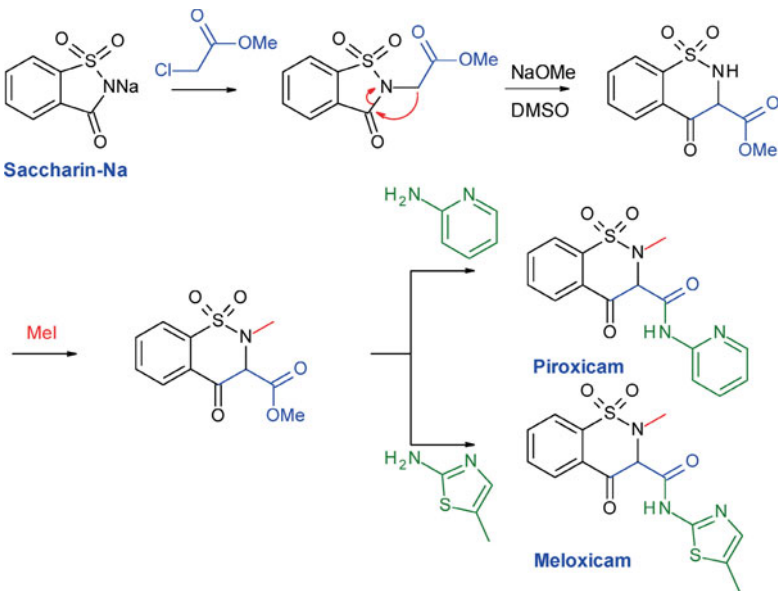
Enantioselective hydrogenation can be carried out with a ruthenium-BINAP catalyst. [199] The enantiomeric excess increases by lowering the temperature and rising the hydrogen pressure. [200]

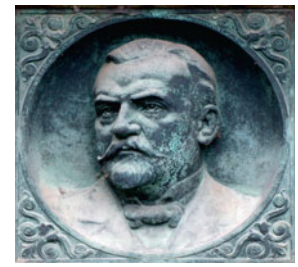


The starting material for meloxicam [201] and piroxicam [202], two oxicams from Thomae GmbH, respectively Pfizer, is the sweetener saccharin, which is accessible in a few steps from toluene according to the Remsen-Fahlberg procedure (Fig. 5.91). [203, 204]



The sodium salt of saccharin is first alkylated with methyl chloroacetate, and then rearranged with sodium methoxide to a benzothiazinone. Methylation at the nitrogen and then reaction with 2-amino-5-methylthiazole or with 2-aminopyridine gives meloxicam and piroxicam respectively.

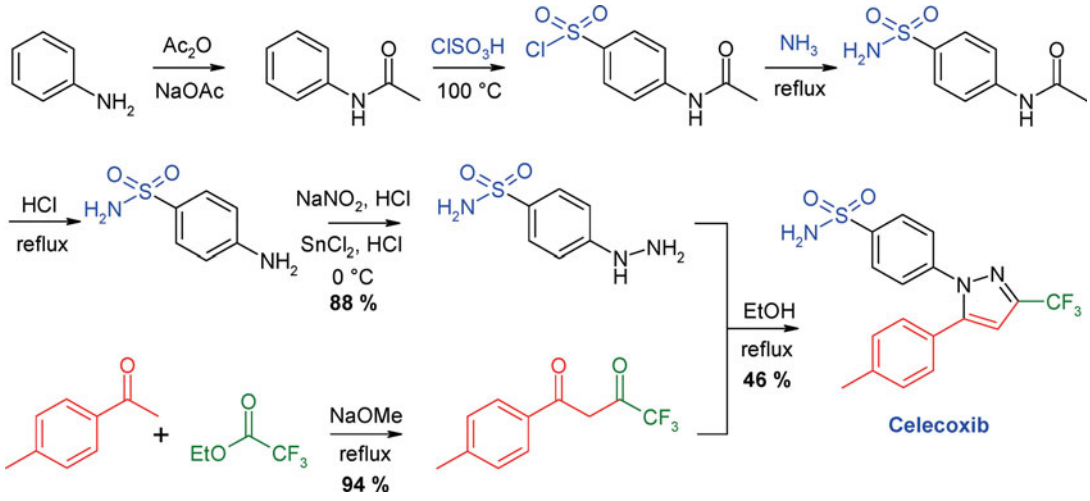




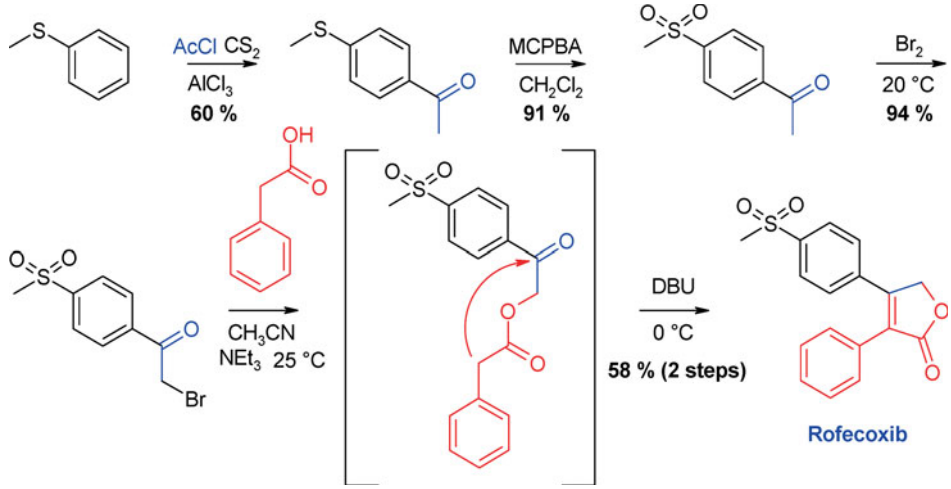
5.91 *Saccharin, the first synthetic sweetener, was discovered accidentally in 1878 by Constantin Fahlberg (1850–1910) in Ira Remsen's laboratory at the Johns Hopkins University. In the course of his work on coal tar, an experiment was allowed to boil over; afterwards at dinner, Fahlberg noted a strikingly sweet taste on his hands.*

5.5.12 Syntheses of COX-2 Inhibitors

Celecoxib is prepared in a convergent synthesis by a classical condensation reaction, which gives a pyrazole from the corresponding 1,3-diketone and a hydrazine. [205] The hydrazine is obtained from aniline by sulfonation, diazotisation and reduction. The 1,3-diketone is accessible in one step from 4-methylacetophenone and ethyl trifluoroacetate.



Rofecoxib is also accessible by a comparatively short synthesis. [206] The starting material, methyl phenyl sulfide, is converted by Friedel-Crafts acylation and oxidation into the required sulfonylacetophenone. This is brominated and finally condensed with phenylacetic acid to result in the active compound.



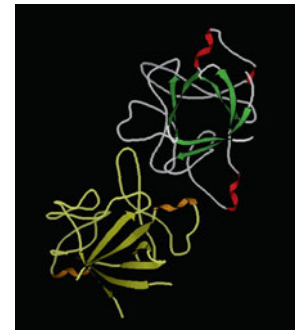
5.5.13 Outlook

The dream of Stewart Adams, to find not only a palliative treatment but also a cure for rheumatoid diseases, did unfortunately not come true. [180] Pro-inflammatory cytokines like interleukin-1 and -6, or the tumour-necrosis factor-α (TNF-α) play key roles in diseases of the rheumatoid spectrum, and impact a multitude of signalling cascades within the immune system. Accumulating insight into the pathophysiology, biochemistry, cellular and molecular biology of these illnesses has clearly improved the opportunities for their treatment at the

beginning of the 21st century. The new therapeutics are antibodies, soluble receptors or antagonists of such cytokines. [207]

Based on the work of Tadamitsu Kishimoto on the properties of interleukin-6, the Japanese pharmaceutical company Chugai developed the interleukin-6-receptor-blocker tocilizumab (*RoActemra*®) for the treatment of rheumatoid arthritis, Castleman's disease, systemic juvenile idiopathic arthritis and other chronic inflammatory indications. [208]

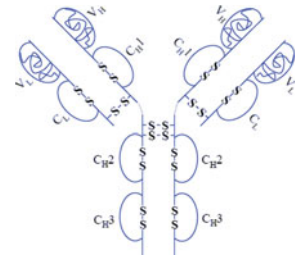
The interleukin-1-receptor-antagonist Anakinra (*Kineret*®) with its sequence of 153 amino acids was patented in 1989 by the company Synergen, and later developed to the market by Amgen (Fig. 5.92). It is produced using recombinant *Escherichia coli* strains, and is applied as a combination product for the treatment of severe rheumatoid arthritis. [209]



5.92 *Anakinra.*

The pioneering work on monoclonal antibodies against TNF-α originate from Junming Le and Jan Vilcek at the New York University School of Medicine. Infliximab (*Remicade*®) is a chimeric, monoclonal anti-TNF-α-antibody (human/mouse), which is approved for the treatment of rheumatoid arthritis, Crohn's disease (a chronic inflammatory disease of the intestine) and psoriasis. [210]

The first fully human monoclonal anti-TNF-α-antibody, adalimumab (*Humira*®) was developed by BASF and its affiliate Knoll Pharma (now part of Abbott Laboratories/AbbVie) in collaboration with the British biotechnology firm Cambridge Antibody Technology (acquired by AstraZeneca). The product was launched in 2003, and by 2013 its projected annual sales figure surpasses the $10 billion mark, becoming one of the best selling drugs of all time. (Fig. 5.93). [211] In 2009, another human monoclonal anti-TNF-α-antibody, golimumab (*Simponi*®), from Centocor Inc., won approval in Europe. [212]



5.93 *Adalimumab structure.*

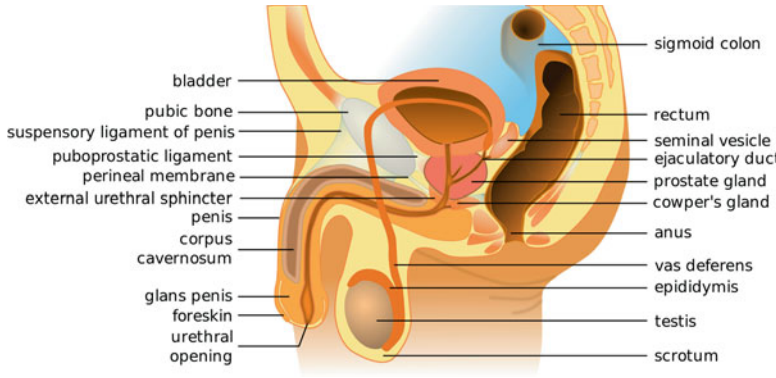
Summary in Bullet Points

- Non-steroidal anti-inflammatory drugs inhibit cyclooxygenase, an enzyme critical for the biosynthesis of prostaglandins. This is the basis for their analgesic, anti-inflammatory and antipyretic effect.
- The elucidation of the mode of action of acetylsalicylic acid paved the road for many other drug classes within this spectrum of indications.
- Whereas the development of selective COX-2 inhibitors proved problematic, modern "biological drugs" spur new hope for the effective treatment of rheumatoid diseases.

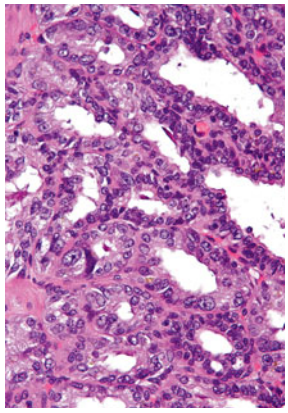
5.6 Prostaglandins

In 1935, the Swedish physiologist Ulf Svante von Euler-Chelpin (1905–1983; Nobel Prize in Physiology or Medicine, 1970; his father had been a Nobel laureate as well) (Fig. 5.94) made an unusual discovery: he observed that human seminal fluid acts to lower the blood pressure and causes contraction of the uterine tissue. Guided by the assumption that the pharmacological principle was produced in the prostate gland, he called this prostaglandin. [213] Later, it was

5.94 *Ulf Svante von Euler-Chelpin.*



5.95 *Section through the male pelvis.*



found that prostaglandins are not synthesised in the prostate, but in the seminal vesicles (*Vesicula seminalis*) (Fig. 5.95 and Fig. 5.96); by that time however, the name had already been adopted into common use. The prostaglandin concentration in human seminal fluid is, at 300 µg/ml, especially high. Prostaglandins and related compounds (eicosanoids) are also found in nearly all other tissues, at concentrations of 0.01–1 µg/g of wet weight.

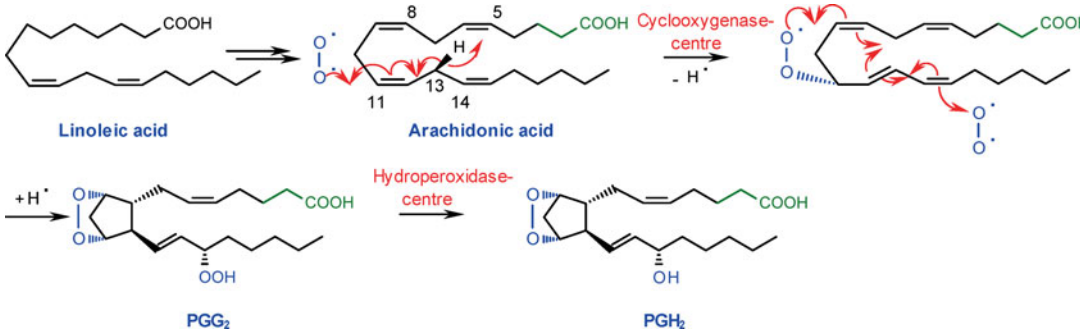
5.96 *Microscopic view of a human seminal vesicle gland cross section (haematoxylin-eosin stain): strong muscular wall (below in the picture) and typical "net-like" arrangement of the mucous membrane. The dark red-coloured areas are remains of the gland secretion.*

5.6.1 Biosynthesis of Eicosanoids

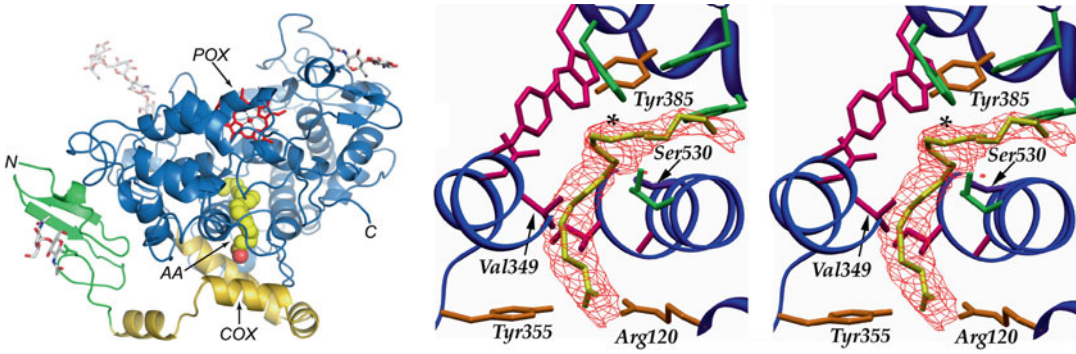
The saturated C_{20} fatty acid arachidic acid is a minor constituent of peanut oil (peanut: *Arachis hypogaea*).

The most important precursor of eicosanoids (Greek, *eikosi*: twenty) in humans is arachidonic acid (5,8,11,14-eicosatetraenoic acid), a non-conjugated, tetraunsaturated C_{20}-carboxylic acid, which is formed from linoleic acid ((9*Z*,12*Z*)-octadecadienoic acid) by successive desaturation and elongation. Starting from arachidonic acid, the metabolic pathways are branching off. One leads to cyclic products, the prostaglandins, prostacyclin and thromboxanes; another one to acyclic products, the leukotrienes (see page 338).

Prostaglandin-H_2-synthase (PGH_2-synthase, cyclooxygenase) produces cyclic metabolites. In presence of this iron-porphyrin-containing synthase [214], the cyclopentane skeleton is constructed by addition of oxygen, and a hydroxy-group is introduced into the side-chain. Reduction of the hydroperoxide in PGG_2 is glutathione-dependent. Glutathione (γ-Glu-Cys-Gly) is thereby oxidised to the disulfide. In this way PGH_2 is formed. [215]



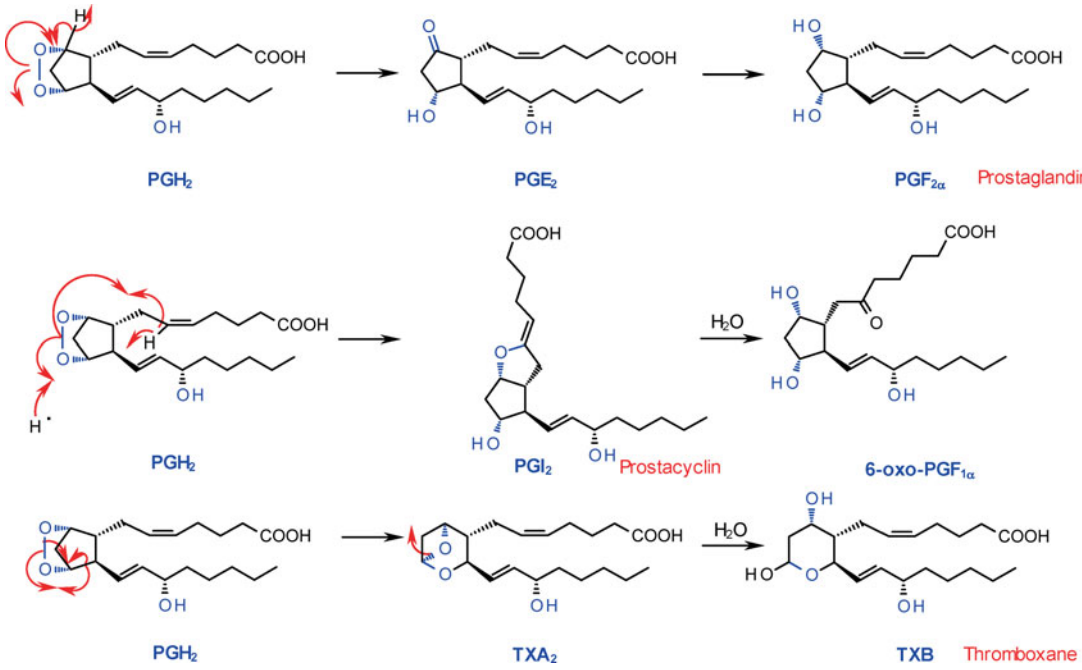
The essential individual steps are the following: First, arachidonic acid bonds ionically to arginine-120 of prostaglandin-H_2-synthase. The oxoiron(IV)-porphyrin complex abstracts a hydrogen radical from tyrosine-385. This then cleaves off, regio- and stereo-selectively, the allylic (13-*pro*-*S*)-hydrogen atom of arachidonic acid, and thereby initiates the cycloaddition with oxygen. In the reaction centre of the hydroperoxidase, the hydroperoxide is then reduced to the alcohol in a second step (Fig. 5.97). [216]



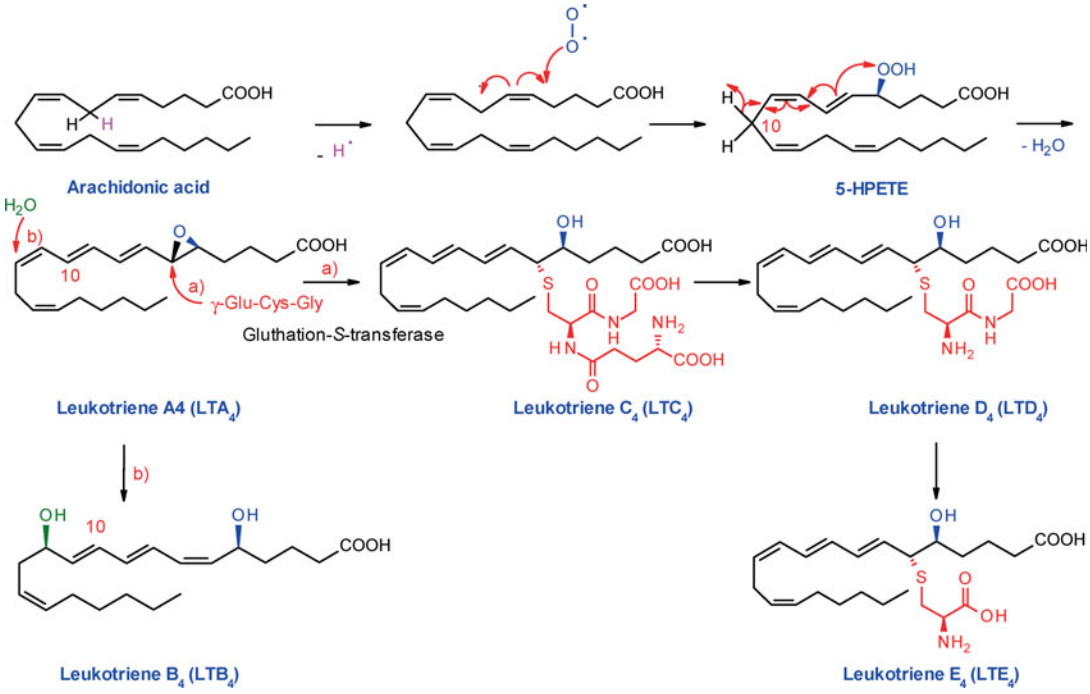
5.97 *Prostaglandin-H_2-synthase. A ribbon drawing of the cyclooxygenase-1 monomer with arachidonic acid (in yellow) in the COX active site. – The stereo diagram is based on crystallographic data and chemical evidence for an appropriate structure for oxygenation and cyclisation of PGG_2, positioning of Tyr-385 (copper) aligned with the (13-proS)-hydrogen (*) of arachidonate, and interaction between the carboxylate of arachidonate and the guanidino group of Arg-120.*

PGH_2 is the key compound, from which the type-2 prostaglandins, prostacyclin and the thromboxanes are eventually generated by ring-opening of the endoperoxide.

Which of the downstream products are formed, depends on the corresponding enzymes, and their expression level in the different tissue types. The blood platelets produce almost exclusively thromboxane TXA_2 (a vasoconstrictor and inhibitor of platelet aggregation); foremost the vascular endothelium and the inner lining of blood vessels release prostacyclin PGI_2 (a vasodilator and inhibitor of platelet activation/aggregation), while heart muscle cells synthesise PGI_2, PGE_2 and $PGF_{2\alpha}$.

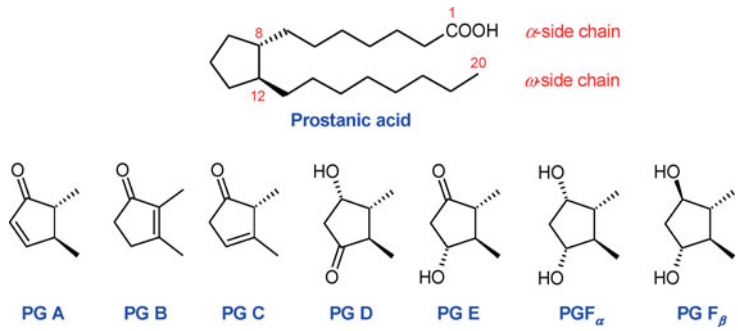


A linear biosynthetic route, which is not influenced by acetylsalicylic acid, leads by means of lipoxygenase *via* a simple addition of oxygen to an epoxide, which is transformed by addition of water or glutathione into the various leukotrienes.



5.6.2 Nomenclature

Prostaglandins, prostacyclins and thromboxanes are all derived formally from prostanic acid, which does not occur in Nature (Fig. 5.98).



5.98 *Nomenclature of prostanoids.*

The letters PG stand for prostaglandins and prostacyclins, TX for thromboxanes. The various substitution patterns on the ring system are denoted by a further capital letter. For prostacyclins this is an I. The different substitution patterns (and the number of double bonds) in the side-chains are denoted by indices. The suffix α or β indicates the stereochemical position of the hydroxy-group at C-9. In thromboxanes, the cyclopentane fragment is replaced by tetrahydropyran.

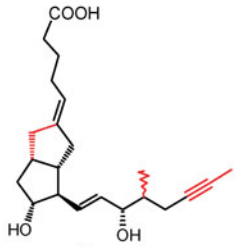
5.6.3 Pharmacology of Eicosanoids

The action of eicosanoids is both, intracellular (autocrine) and intercellular on the cells immediately adjacent to their site of origin (paracrine). However, they are not transported like the endocrine hormones through the bloodstream to a distant target. In particular, the eicosanoids participate in:

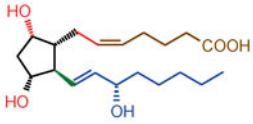
- inflammatory reactions like rheumatoid arthritis or psoriasis;
- the origin of pain and fever;
- regulation of blood pressure;
- blood coagulation;
- growth and reproductive functions, like setting off labour; and also
- regulation of the sleep-wake rhythm.

Corresponding to their omnipresence, they possess also broad therapeutic interest. However, as a rule, these therapeutic agents are derivatives of the natural products, since the latter are metabolised very rapidly. In addition, through structural modification it is possible, to tune the selectivity of the intended activity.

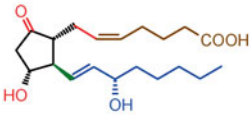
Misoprostol



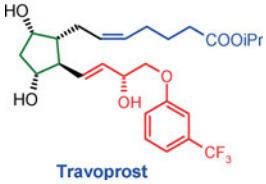
Iloprost



$PGF_{2\alpha}$
Dinoprost



PGE_2
Dinoprostone



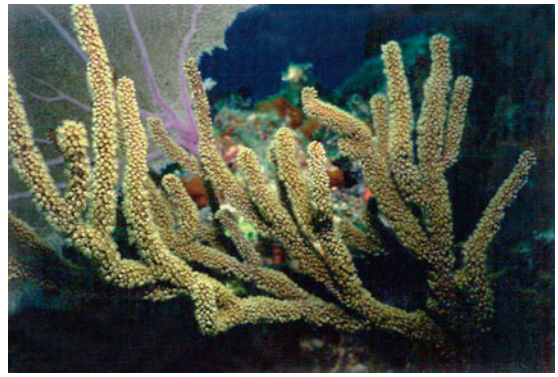
Travoprost

- Prostanoids play a significant role in diseases of the gastrointestinal tract. Their cytoprotective and accelerated healing effects make them the remedy of choice for the treatment of stomach ulcers. The prostaglandin-E_1 derivative misoprostol (*Cytotec*®, Searle) serves, for example, as an ulcer drug.
- Prostaglandins of the A- and E-series lower the blood pressure. Thromboxanes and carbacyclins are used for the treatment of cardiovascular diseases. Corresponding drugs are *e.g.* alprostadil (*Prostavasin*®, Schwarz Pharma, Ono Pharmaceutical) or iloprost (*Ilomedin*®, Bayer/Schering).
- Prostaglandins of the E- and F-series, like dinoprostone (*Minprostin* E_2®, Pharmacia and Upjohn/Pfizer) and dinoprost (*Minprostin* $F_{2\alpha}$®, Pharmacia and Upjohn/Pfizer) lead to contractions of the uterine smooth muscle, and are used for termination of pregnancy and in obstetrics.
- Prostaglandins of the F- and D-series reduce intraocular pressure, and thus offer attractive therapeutic options for the treatment of glaucoma. Marketed drugs are, for example, latanoprost (*Xalatan*®, Pfizer) and travoprost (Alcon).
- Prostaglandins of the F-series also find application in veterinary medicine. They are used for synchronisation of the ovulation in cows, pigs, sheep and horses. This allows for a scheduled insemination.

5.6.4 Partial Synthesis of Natural Products

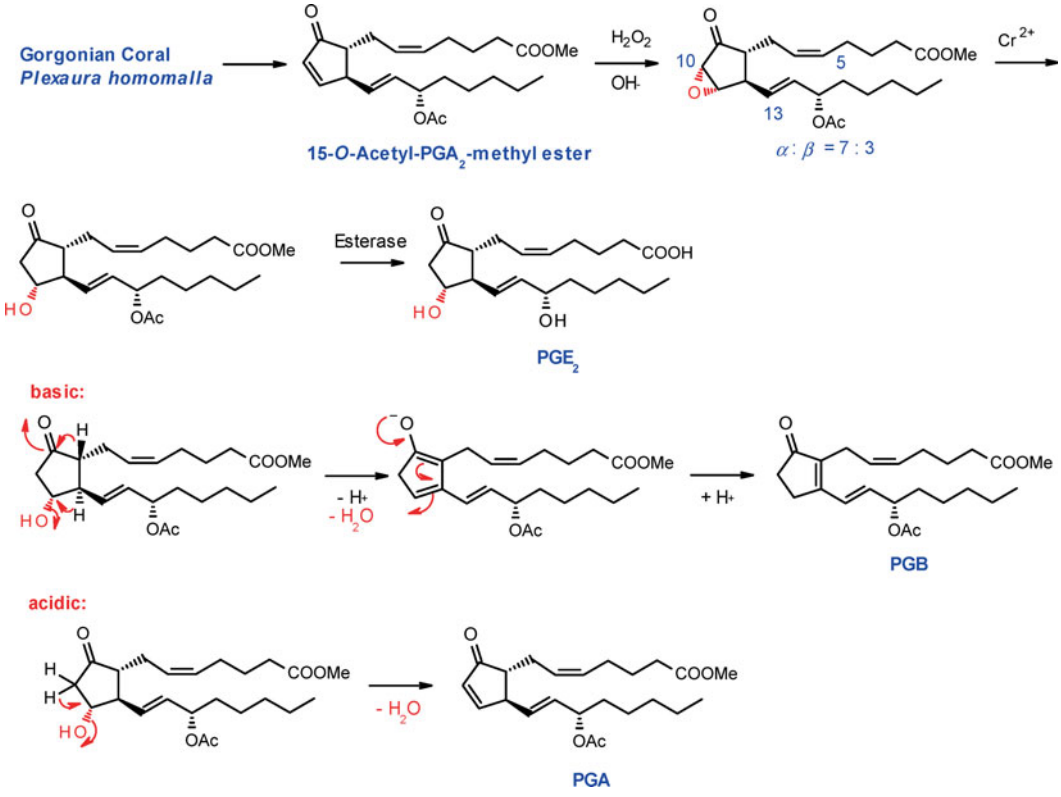
With the recognition of the pharmacological potential of prostaglandins, there arose interest in efficient syntheses. Just as in the era of the steroids and the β-lactam antibiotics, prostaglandin research also enriched us with an abundance of new synthetic strategies and synthetic methods, which are of general importance.

Because of the complexity of the structures, the initial objectives were to synthesise the prostaglandins PGE_2 and $PGF_{2\alpha}$ from natural starting materials. The dry mass of the Gorgonia coral *Plexaura homomalla*, which is native to the Caribbean, contains, for example, 1.5 % 15-*O*-acetyl-PGA_2 methyl ester (Fig. 5.99). [217] In basic media the vinylogous double bond is epoxidised with

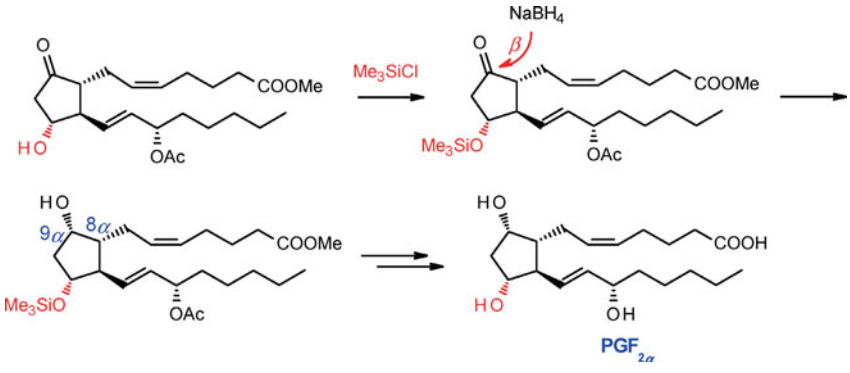


5.99 *The Black sea rod or Caribbean sea whip (Plexaura homomalla) is widely distributed in the Caribbean from the Florida Keys to the northern coast of Venezuela.*

hydrogen peroxide, without the double bonds at positions 5 and 13 being affected; however, the stereoselectivity (7 : 3) is not overly good. The reductive ring-opening with chromium(II) salts produces the hydroxy-group at C-11. Every attempt to hydrolyse the ester, in acid or base, fails at the sensitive β-hydroxyketone stage: this compound very readily loses water. Only enzymatic hydrolysis under neutral conditions yields PGE_2.



$PGF_{2\alpha}$ is obtained by protecting the hydroxy-group of the hydroxyketone, and reducing stereoselectively the keto-group with sodium borohydride. Since the hydride is introduced β-facially, the 9α-alcohol is formed with good selectivity.



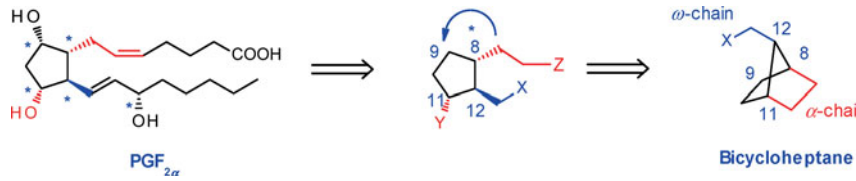
5.6.5 Total Synthesis of Natural Products

Bicycloheptane Route

5.100 *Elias James Corey Jr. (*1928)*

Talking about the syntheses of prostaglandins, one cannot but mention the first total synthesis of PGE_2 and $PGF_{2\alpha}$ by E. J. Corey in 1969 (Fig. 5.100). It represents a pivotal milestone in the development of modern synthetic chemistry. Corey's original design was steadily improved in the course of decades, and was eventually transferred to a larger scale of around 50 kg. [218]

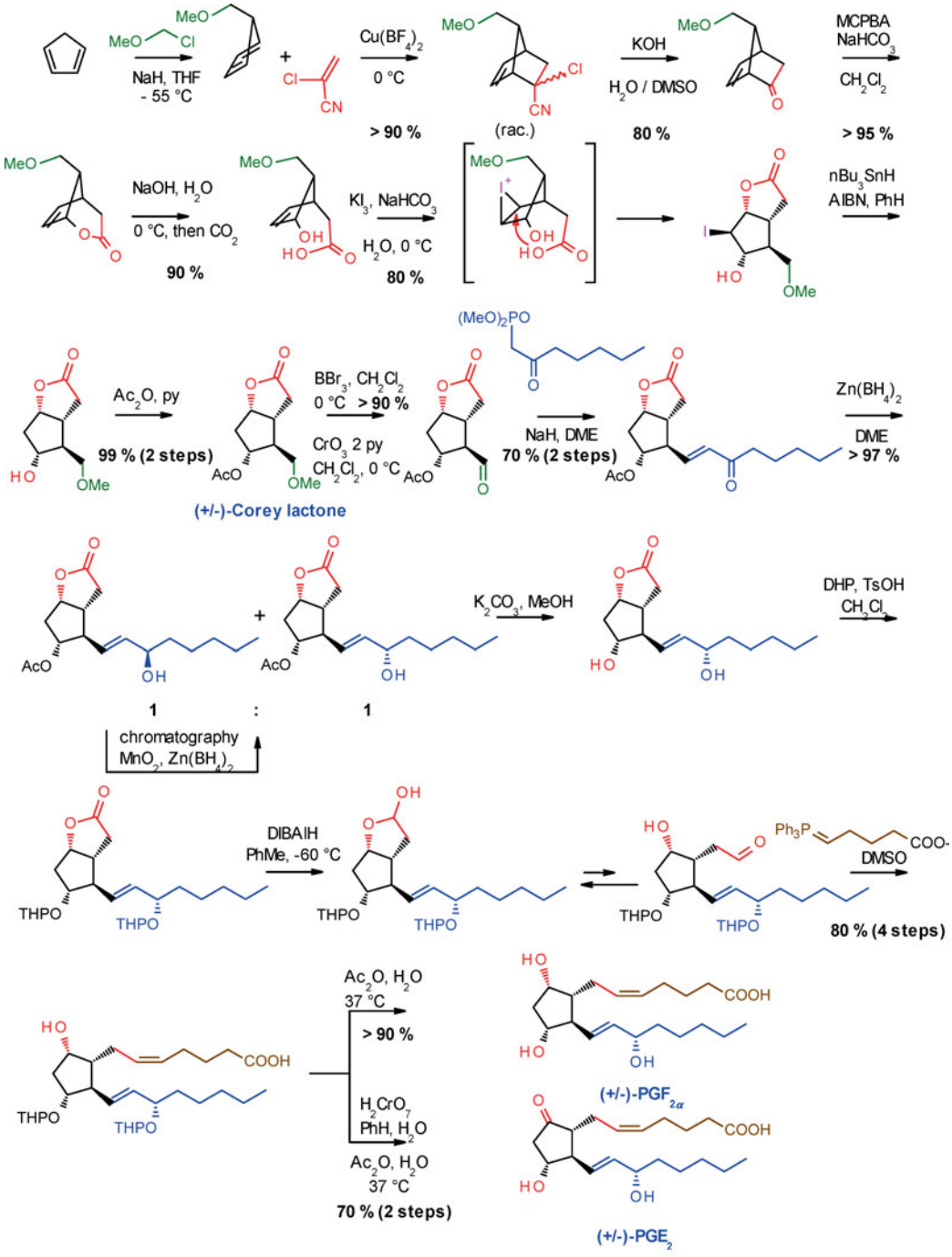
The central idea in Corey's original synthetic strategy was to fix the relative stereochemistry of three out of the total of five stereocentres within a bicycloheptane skeleton. In this, the Z-group has to be positioned such, that it is suitable for attachment to a *cis*-double bond and formation of the α-chain. Moreover, the configuration at C-8 ought to serve in the generation of a new stereogenic centre at C-9. Ring-opening of the bicycle ought to take place in a way, that Y preferably comprises already an oxygen function, since the correct relative stereochemistry is already defined by the bicyclic system. For the ring-opening, carbocationic and radical processes come into consideration, since it is expected that thereby secondary C-atoms migrate more easily than primary. The stereochemistry of the ω-chain on the five-membered ring has to be set when forming the bicycloheptane. X is therefore chosen so that the chain can be extended by attachment of a *trans*-double bond. The stereocentre in the side-chain must be generated separately.



Corey's synthesis starts with cyclopentadiene, which is alkylated with monochlorodimethyl ether at -55 °C and then reacted at 0 °C with 2-chloroacrylonitrile in presence of a copper catalyst. Acids, bases or even slightly raised temperatures lead, *via* a 1,5-hydrogen shift, to isomeric double-bonds, which are of no use for the synthesis. For steric reasons, the methoxymethyl group is positioned *anti* to the chloroacrylonitrile. 2-Chloroacrylonitrile serves as the synthetic equivalent for the introduction of a keto-function, which can be liberated using KOH. In course of the Baeyer-Villiger oxidation, the double bond is not epoxidised. The bridgehead carbon migrates very selectively. After hydrolysis of the lactone, iodolactonisation serves to generate a new stereogenic centre at C-9. The so-called Corey-lactone is obtained by deiodination with tributylstannane. A few steps later, oxidation with Collins' reagent produces an aldehyde, which lacks stability, and therefore has to be submitted immediately to the subsequent Horner-Wadsworth-Emmons reaction.

The enone moiety in the ω-side-chain is chemoselectively reduced with zinc borohydride. The 1 : 1 mixture of diastereomers is separated by chromatography, and the 15β-epimer recycled by oxidation with activated manganese dioxide. Reduction of the lactone with DIBAlH produces a lactol, the equilibrium of

which is shifted far enough to the open-ring structure, so that it can directly undergo a Wittig reaction. $PGF_{2\alpha}$ and PGE_2 are accessible in racemic form by cleavage of the THP-protecting groups and, respectively, by mild oxidation with subsequent cleavage of the THP-groups.

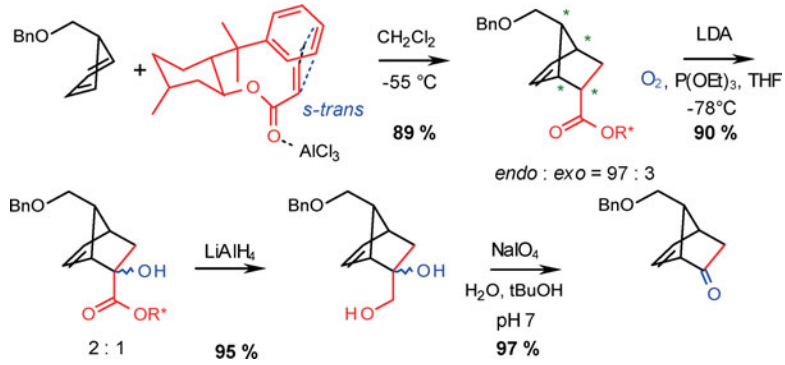


During the 1980s and 1990s, Corey published results from his efforts to overcome the fundamentally weak point of the synthesis, the lack of stereocontrol. This explicitly concerns attempts to find stereoselective conditions for the Diels-Alder reaction and the reduction of the keto-function. Both areas of the work subsequently gained general importance.

For the initial preparation of enantiomerically pure prostaglandins PGE_2 and $PGF_{2\alpha}$, Corey separated the enantiomers after the Baeyer-Villiger oxidation of the bicycloheptenone and hydrolysis of the resulting lactone by salt formation and crystallisation with ephedrine.

It appeared more attractive to carry out a Diels-Alder reaction with control of both, its regio- and stereo-chemistry. In 1975, Corey published a diastereoselective Diels-Alder reaction with the use of 8-phenylmenthol as a chiral auxiliary. The Lewis acid fulfils two tasks: on the one hand, it increases the reactivity by lowering the LUMO of the dienophile [219]; on the other hand, it stabilises the s-*trans* conformation for steric reasons. The attack of the diene on the s-*cis*-dienophile would lead to the opposite absolute stereochemistry in the product. The Diels-Alder reaction occurs diastereoselectively, because the *Si*-facial half of the prochiral double bond is effectively shielded by π-stacking with the phenyl ring of the auxiliary. In this reaction, four new stereogenic centres are generated all at once. The yield of the desired diastereomer amounts to 89 %.

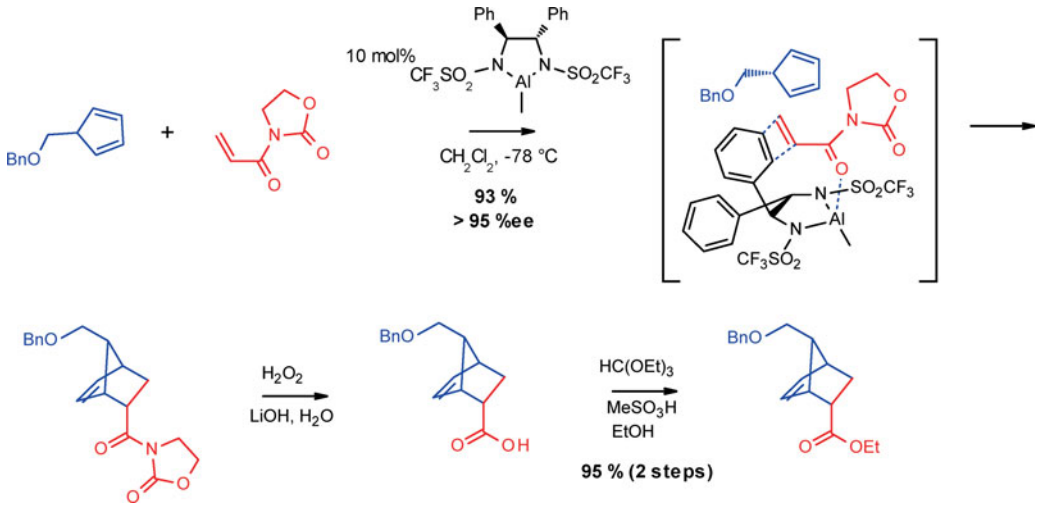
By treatment with LDA and oxygen, a hydroxy-group can be introduced at the α-position of the ester. The initially formed hydroperoxide is then reduced with triethyl phosphite. Reduction with lithium aluminium hydride leads to elimination of the 8-phenylmenthol, which can be recovered and used again, and the formation of a diol. The latter can finally be converted by periodate cleavage into nearly enantiomerically pure bicycloheptenone.



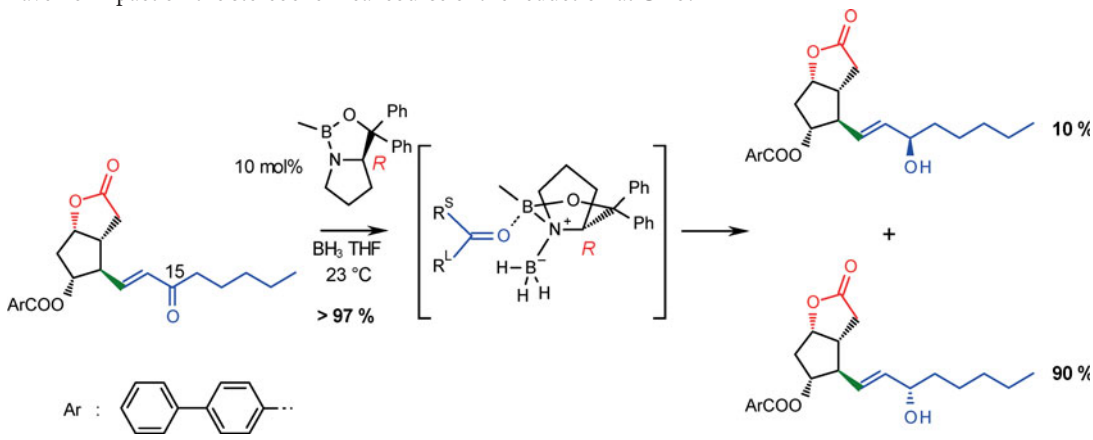
The era of diastereoselective reaction methods was replaced by that of enantioselective methods, resulting in an augmented efficiency of the syntheses. In the case of enantioselective catalyses, there are savings not only from skipping the attachment and cleavage procedures of chiral auxiliaries to/from the actual substrate, but also in terms that an enantiomerically pure catalyst is only required in sub-stoichiometric amounts.

In a specific case, Corey developed in 1991 an enantioselective Diels-Alder reaction with a C_2-symmetric aluminium complex. For the stereo-differentia-

tion of the prochiral centre, the same principles apply as for the diastereoselective reaction. However, 10 mole % of the catalyst was sufficient to obtain the desired amide in 93 % yield and enantiomeric purity of > 95 %. Mild cleavage of the amide with lithium hydroperoxide and Fischer esterification with triethyl orthoformate gave the bicycloheptenecarboxylic ester in a yield of 95 %.



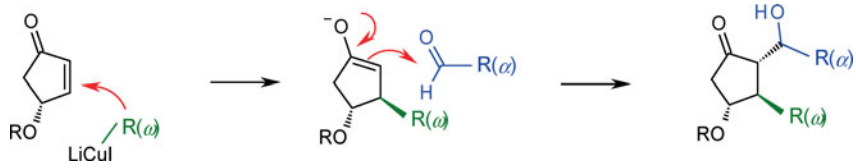
The second stereochemical problem, enantioselective reduction of the keto-group in the ω-side-chain, was solved by Corey already in 1987. The reduction was successful with borane-THF in presence of 10 mole % of the (*R*)-proline-derived (*R*)-oxazaborolidine (Corey-Bakshi-Shibata reduction). If the enone was reduced in presence of the corresponding (*S*)-oxazaborolidine, the inverse product distribution resulted. Obviously, the other stereocentres in the educt have no impact on the stereochemical course of the reduction at C-15.



Three-component Route

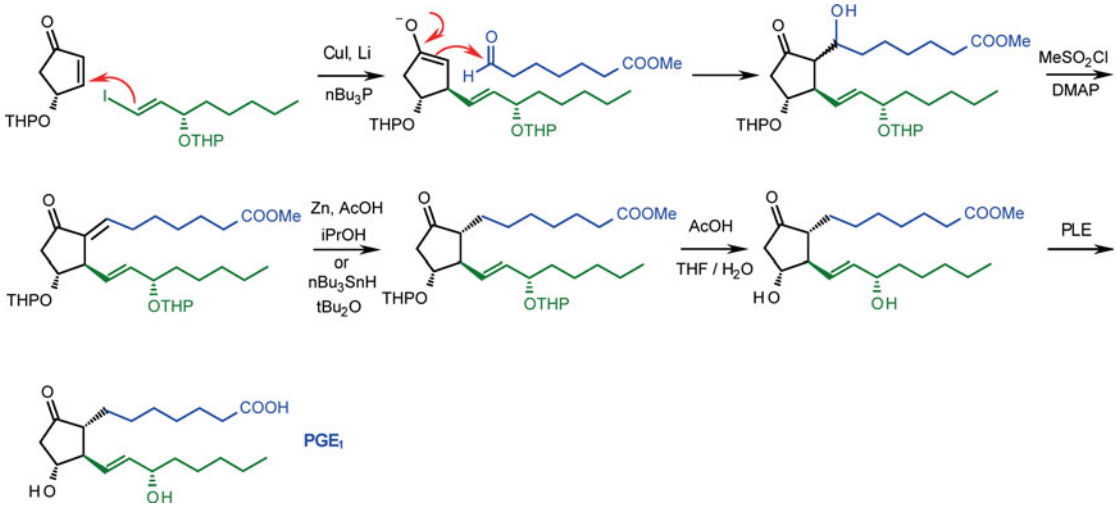
One of the most elegant routes to the formation of prostaglandins is the three-component coupling according to Ryōji Noyori [220], which starts from an

enantiomerically pure cyclopentenone with α- and ω-chains. [217] The synthetic concept consists of a diastereoselective Michael addition of the ω-chain to the cyclopentenone using an organocopper reagent, whereafter the resulting enolate launches a nucleophilic attack on the aldehyde function of the α-chain.

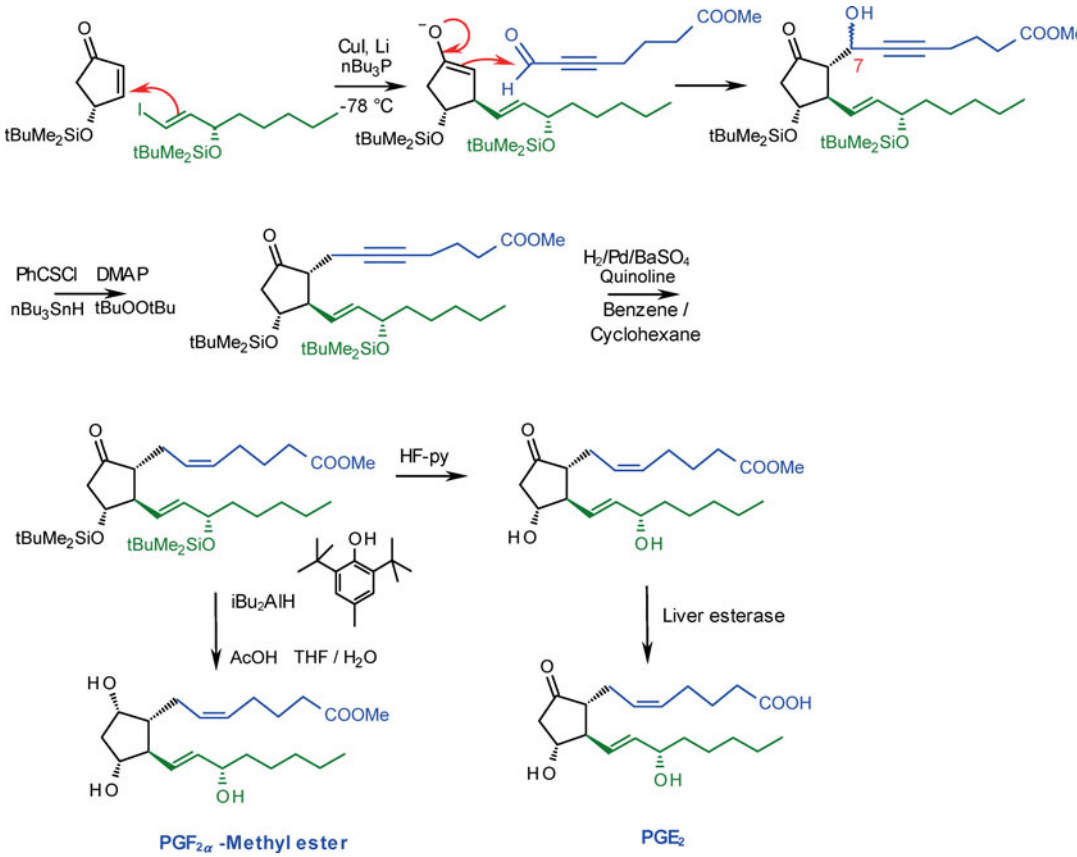


Enantiomerically pure cyclopentenones are accessible *via* enzyme-catalysed processes. By photochemical oxidation and acetylation, *cis*-1,4-diacetoxycyclopent-2-ene is obtained. Taking advantage of the *meso-trick* (*cf.* section 5.1.3) with an enzyme from *Pseudomonas fluorescens*, the latter can be converted upon recrystallisation and oxidation into (*R*)-acetoxycyclopentenone. (The (*S*)-enantiomer is obtainable by hydrolysis with pig liver esterase.) [221, 222]

In a two-step, "one-pot" process, the building blocks of both side-chains undergo a diastereoselective reaction with the enantiomerically pure cyclopentenone. [217] Here, the stereochemistry is determined by the chirality of the cyclopentenone. After elimination of the hydroxy-group in the α-chain, the desired absolute configuration is set by regioselective reduction of the vinylogous double bond with tributylstannane or zinc/acetic acid. In consequence of the sensitivity of prostaglandin-PGE$_1$, the protecting groups are removed with aqueous acetic acid and pig liver esterase respectively.



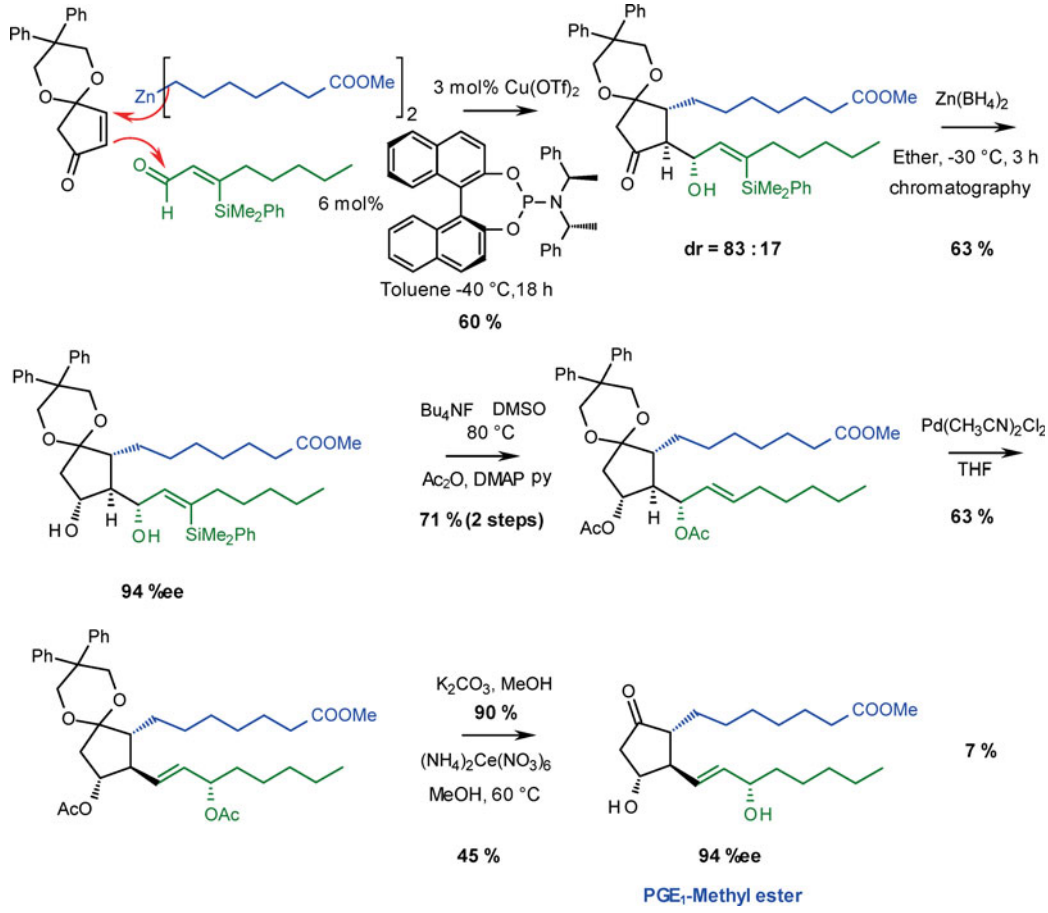
For a series of prostaglandins, the *cis*-configured double bond in the α-chain can be installed with corresponding alkynal precursors, where the resulting alkynes are amenable to a Lindlar reduction. The hydroxy-group at C-7 has then to be removed *via* the thiobenzoate; its reduction is carried out under mild conditions with tributylstannane under addition of di-*t*-butyl peroxide. The alkyne thus stabilises the radical, which appears as an intermediate. The cyclopentanone can be reduced stereoselectively with diisobutylaluminium hydride. Cleavage of the protecting groups is achieved by conventional methods.



Catalytic, enantioselective Michael reactions of cyclopentenones have attracted particular attention in recent years. Ben L. Feringa, Albert S. C. Chan, Andreas Pfaltz and Amir H. Hoveyda described various ligand systems for stereoselective addition of organo-zinc reagents to cyclopentenones. With the aid of a phosphoramidite derived from BINOL, Feringa developed an enantioselective, catalytic domino-Michael/aldol reaction for the preparation of (+)-PGE_1 methyl ester. [223]

Since unsaturated organo-zinc reagents do not give the desired products, it was necessary to introduce the side-chains in reverse order, starting with the monoketal of the cyclopentenedione. In Feringa's synthesis, the α-chain was introduced with the organo-zinc reagent, and the ω-chain *via* the aldehyde. The

cyclopentanone can be reduced very selectively with zinc borohydride (β-facial hydride addition). The transposition of the allylic acetate group is achieved by means of palladium chloride with complete retention of the absolute configuration. Using catalytic amounts of ceric ammonium nitrate, the cleavage of the ketal works successfully under almost neutral conditions. The overall yield amounts to 7 %.

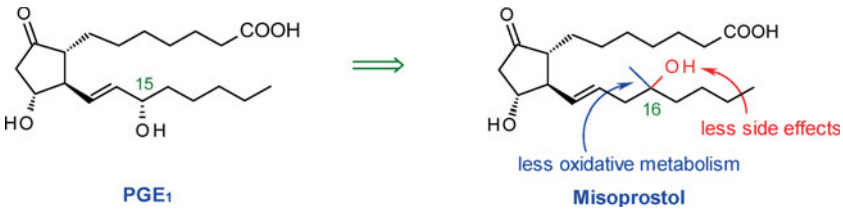


5.6.6 Prostaglandin Derivatives

Misoprostol and Enisoprost

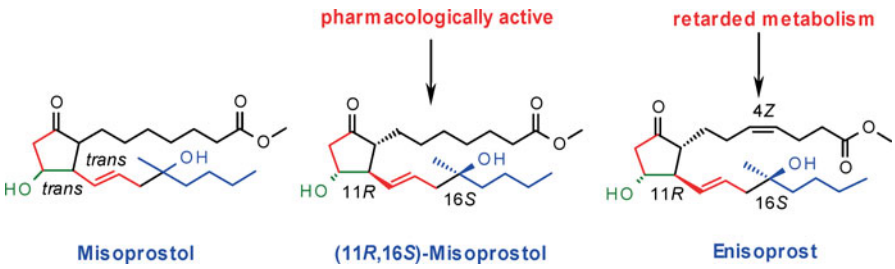
One of the most important prostaglandins on the drug market is misoprostol from Searle. It is marketed worldwide under the name *Cytotec*® as a medicament for stomach ulcers, which can be induced by non-steroidal antiinflammatory drugs. Formally, it is a PGE_1 derivative. However, underlying the drug design is the observation that moving the hydroxy-group from C-15 to C-16 does not affect the antisecretory activity in the stomach, but undesirable side-effects are clearly reduced. In addition, through the introduction of a methyl group at C-16, the oxidative metabolism is impaired.

Humans produce daily 1.2–1.5 litres of gastric juice. Owing to its HCl content it is strongly acidic, and it also contains a series of proteases. The gastric mucosa protects the underlying stomach tissue from self-digestion: it produces mucus, which is very viscous and prevents the penetration of acid. Additional protection comes from bicarbonate, secreted as well by surface epithelial cells in order to buffer the hydrochloric acid. Prostaglandins like PGE_2 very effectively inhibit the release of gastric acid. The protection afforded by the gastric mucosa against high temperatures, alcohol, aspirin, bile acids or high concentrations of salt rests upon prostaglandins, which regulate gastric secretion, and initiate repair mechanisms.



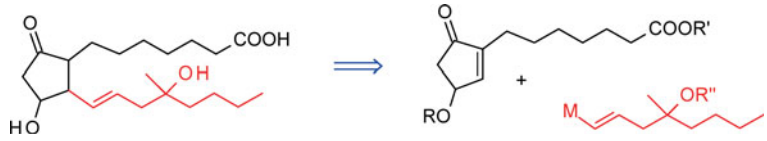
Misoprostol is marketed as a mixture of four stereoisomers, although it is only the (11*R*,16*S*) isomer that carries the pharmacological activity. The product consists of a mixture of the (*R*/*S*)-stereoisomers of the enone and of the ω-side-chain. The high stereoselectivity of the cuprate reaction prevents the formation of further stereoisomers.

Enisoprost is the Δ^4-(*Z*)-derivative, which was developed by Searle as a second-generation back-up candidate for misoprostol to enter the antiulcer market. Associated with the incorporation of the (4*Z*)-double bond (at an "unnatural" position) was the hope to slow down the metabolic degradation of the α-chain and thus to prolong the pharmacological activity. While this effect had indeed been observed in animal studies, clinical trials failed to demonstrate such benefit in humans. Further development of enisoprost for this indication was therefore discontinued. However, enisoprost attracted attention as an immunosuppressant for organ transplantations (Fig. 5.101).



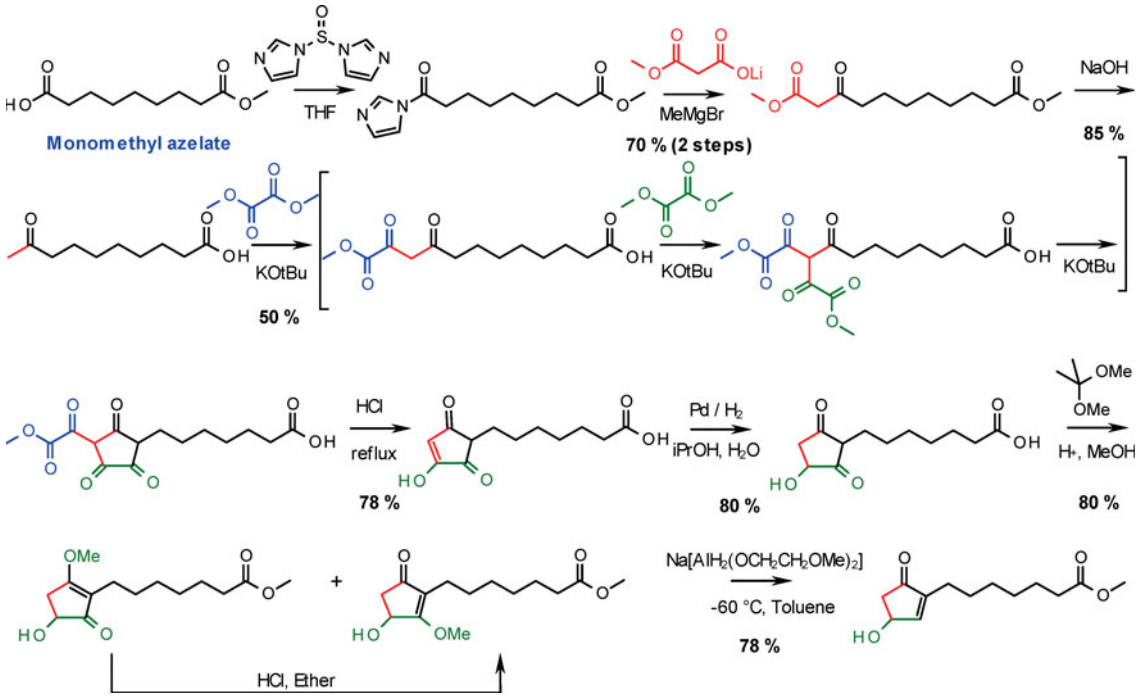
5.101 *From misoprostol to enisoprost.*

The Michael addition of the ω-chain to a cyclopentenone with an α-chain already in place, a synthetic concept developed at Searle, served as the model for a whole series of industrial production processes for synthetic prostaglandins.

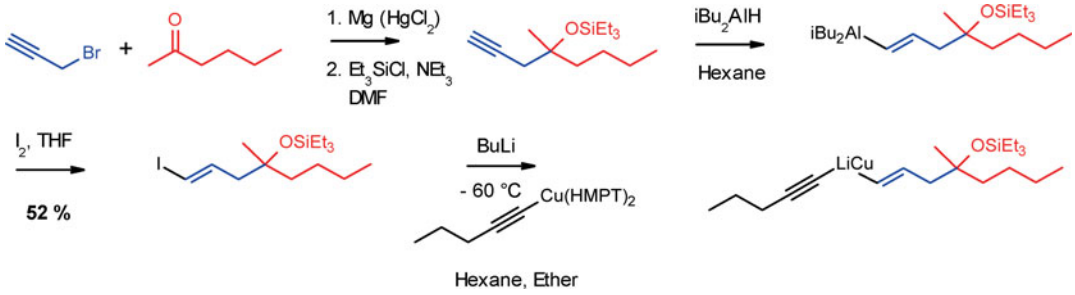


The challenge in this is the selective *trans*-addition of the organometallic reagent. For the optimisation of this reaction, Searle developed an elaborate concept of transmetallation.

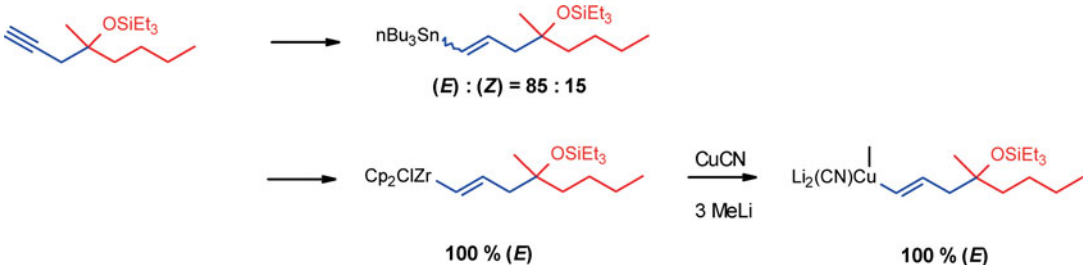
Monomethyl azelate is the starting material for the construction of the cyclopentenone with the α-chain. The carboxylic acid is activated with thionyldiimidazole, freshly prepared from thionyl chloride and imidazole, and is then reacted with the magnesium salt of the malonate half-ester, which is generated *in situ* from its lithium precursor. Use of the lithium salt offers the advantage that monomethyl malonate can be better isolated and purified by crystallisation than by distillation of the free acid. Condensation with dimethyl oxalate in presence of an excess of potassium *t*-butoxide, followed by an acidic retro-aldol reaction, gives the enolised triketone. After regioselective hydrogenation of one keto-group and methylation with acetone dimethyl ketal, two isomeric enol ethers are obtained; of these, the undesired isomer is the main product. Fortunately, the minor component crystallises out and can be isolated in this way. The equilibrium of the two enol ethers can even be shifted completely towards the desired product, if the concentrated mother-liquor is recrystallised from ethereal hydrochloric acid. The cyclopentenone is finally obtained by reduction of the enol ether with Red-Al at low temperature. [224]



The original access to the fully functionalised ω-side-chain involved addition of DIBAlH to silyl-protected 4-methyloct-1-yn-4-ol, which was prepared by a mercury(II)chloride-activated Grignard reaction. Further reaction with iodine and then with butyllithium and pent-1-ynyl-copper formed a copper complex, which selectively transfers the ω-side-chain to the cyclopentenone.

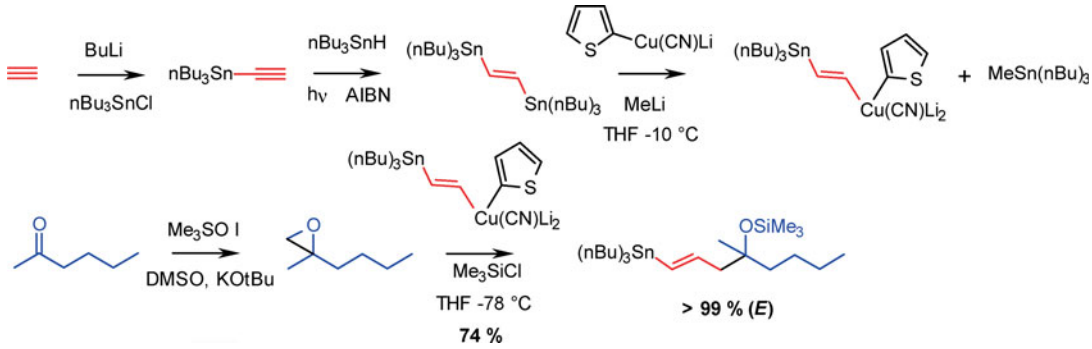


Direct hydrostannylation of the alkyne, on the other hand, gave an 85 : 15 (*E*/*Z*)-mixture, which was not suitable for an industrial synthesis. However, hydrozirconation with $Cp_2Zr(H)Cl$ proceeded under *cis*-addition and resulted in 100 % of the (*E*)-vinylzirconate. Transmetallation with complex lithium cuprates proceeded analogously to the vinylstannanes. Along this route, misoprostol is obtainable in 73 % yield.

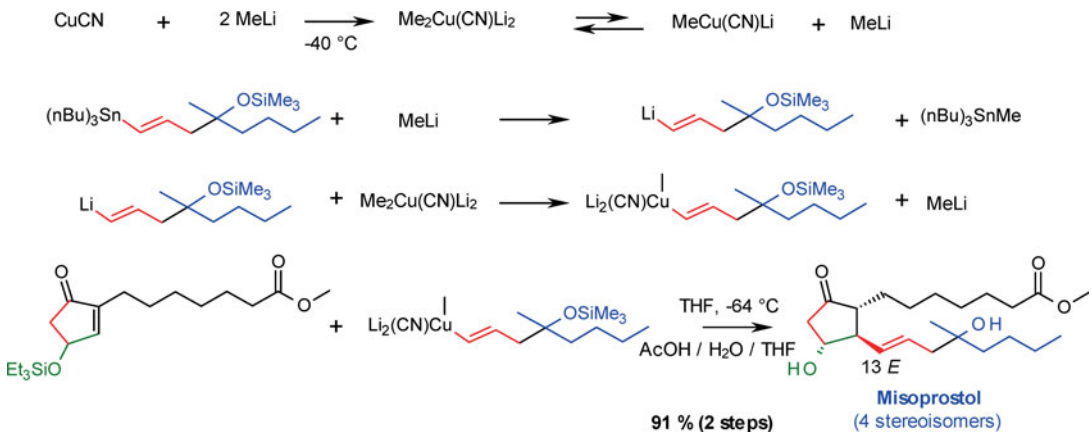


However, these paths were not followed for larger scale production, in particular because of the explosive nature of copper acetylides. Nonetheless, prompted by the work of Corey, it was found that the corresponding stannanes offered a better and more reliable way for the stereoselective synthesis of the side-chain. [225]

Key intermediates for the industrial synthesis are firstly, the (*E*)-bis-(tributylstannyl)-ethylene, which is obtained from *trans*-selective hydrostannylation of the corresponding alkyne under irradiation and in presence of a radical initiator, and secondly, the geminally disubstituted epoxide, which is accessible from the corresponding ketone by a Corey reaction. The (*E*)-stannyl-ω-side-chain is then formed by a regiospecific epoxide ring-opening with a thienyl cyanocuprate.



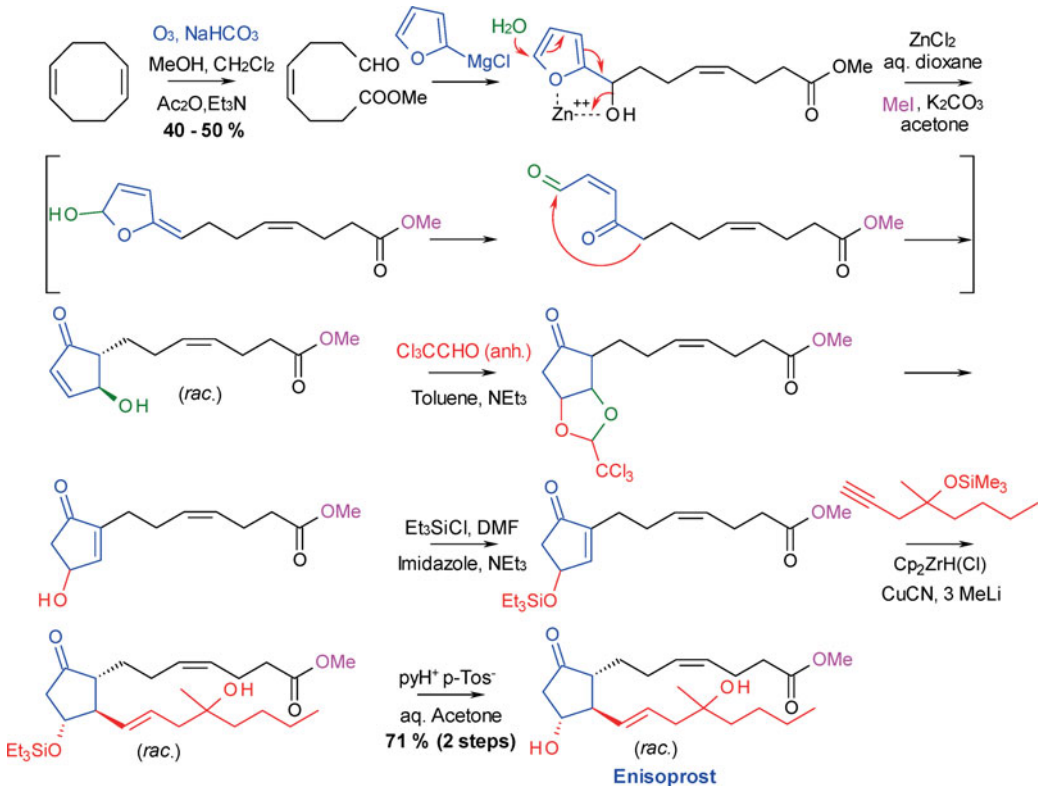
Based on the work of Bruce Lipshutz [226, 227], it was found that vinylstannanes undergo transmetallation with dilithiodimethyl cyanocuprate, the driving force being the irreversible formation of tributylmethylstannane. The involvement of free methyllithium was postulated, but this could not be confirmed in a corresponding NMR experiment. Thus, during the course of the reaction this species is only present in very low concentrations – if at all. The transmetallation proceeds with retention of the stereochemistry, which is not lost in the subsequent Michael reaction either.



For the preparation of enisoprost, Searle developed a decidedly appealing and elegant synthesis. [228] The starting material is (*Z*,*Z*)-cycloocta-1,5-diene, from which the corresponding aldehydo-ester may be obtained in one step by ozonolysis. After the Grignard reaction with 2-furylmagnesium chloride follows an interesting zinc chloride-catalysed rearrangement, brought about by heating in aqueous dioxane. The ester, which gets hydrolysed under these conditions, is then regenerated. Finally, the cyclopentenol undergoes a rearrangement with the aid of catalytic amounts of water-free chloral, and the hydroxy-group is silylated.

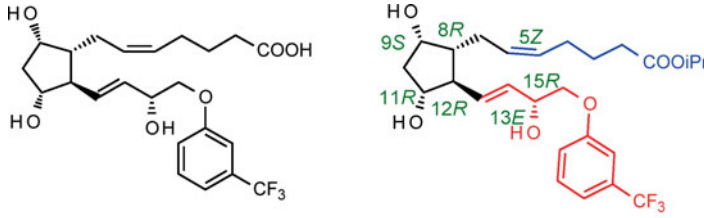
The introduction of the ω-side-chain can be carried out in one step from the corresponding alkyne by hydrozirconation, *in situ*-transformation to the dilithio-cyanocuprate and coupling with the cyclopentenol. Enisoprost is ultimate-

ly obtained by deprotection with pyridinium *p*-toluenesulfonate in aqueous acetone.



Travoprost

Fluprostenol was marketed in form of its racemate by ICI as a contraceptive in the veterinary sector. The synthetic route developed for this product proceeds *via* the Corey lactone, whereby the side-chains are built up using Horner-Wadsworth-Emmons or Wittig reactions. Travoprost, the enantiomerically pure isopropyl ester of fluprostenol, is a potent anti-glaucoma drug, for which Chirotech Technology Ltd. has published an interesting synthesis (Fig. 5.102). [229]



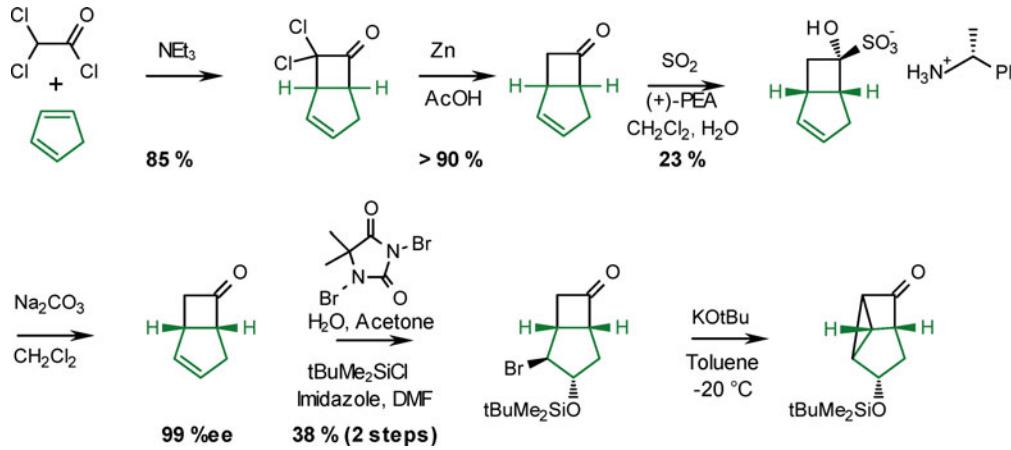
Fluprostenol (racemic) **Travoprost**

5.102 *From fluprostenol to travoprost.*

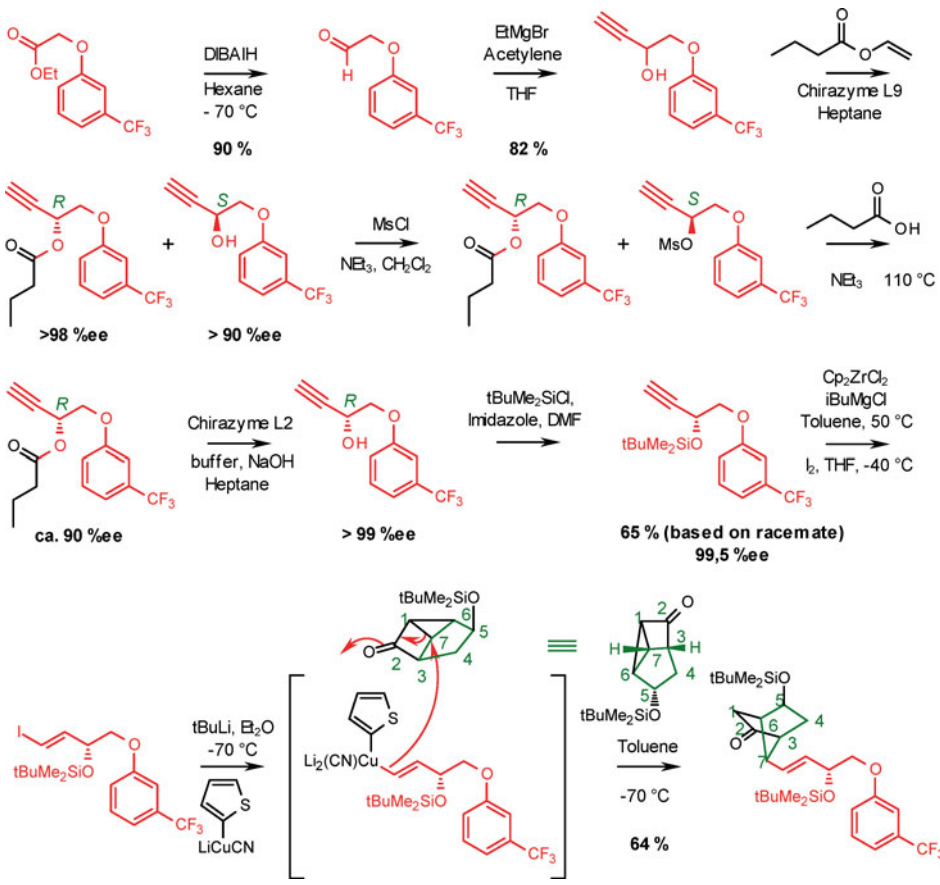
The challenge encompasses not only to make travoprost accessible by a route as convergent as possible without elaborate protecting-group chemistry, and in good yields; but also to generate five stereocentres correctly and with high stereoselectivity. In addition, the configuration of both double bonds must be given due consideration.

As the starting material for travoprost, Chirotech chose racemic bicyclo[3.2.0]hept-2-en-6-one, which is accessible in good yields by [2 + 2]-cycloaddition of cyclopentadiene with dichloroketene, followed by a zinc reduction. [230]

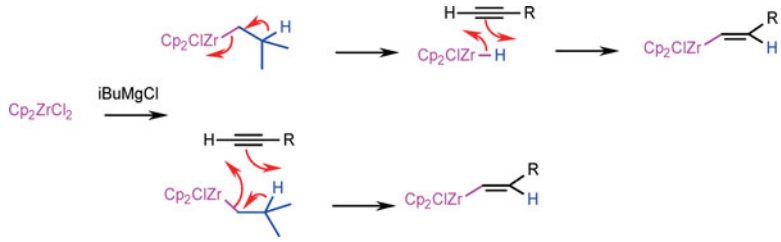
It is remarkable that the two diastereomeric salts of the bisulfite adduct can be separated with phenylethylamine. After being liberated, the less stable bicycle is converted directly with excellent regio- and diastereoselectivity *via* an *exo*-bromonium ion [231] into the silyl-protected bromohydrin. The tricyclic ketone is then generated with potassium *t*-butoxide; the lability of this intermediate requires that it is used for the next reaction step as crude material.



The enantiomerically pure ω-side-chain is obtained by enzymatic kinetic resolution of the corresponding ethynylcarbinol. [232] The unreacted (*S*)-enantiomer is likewise converted by mesylation, followed by an S_N2 reaction with butyric acid, into the valuable product. Enzymatic cleavage of the butyrate ester increases the enantiomeric purity from around 90 % to more than 99 %.



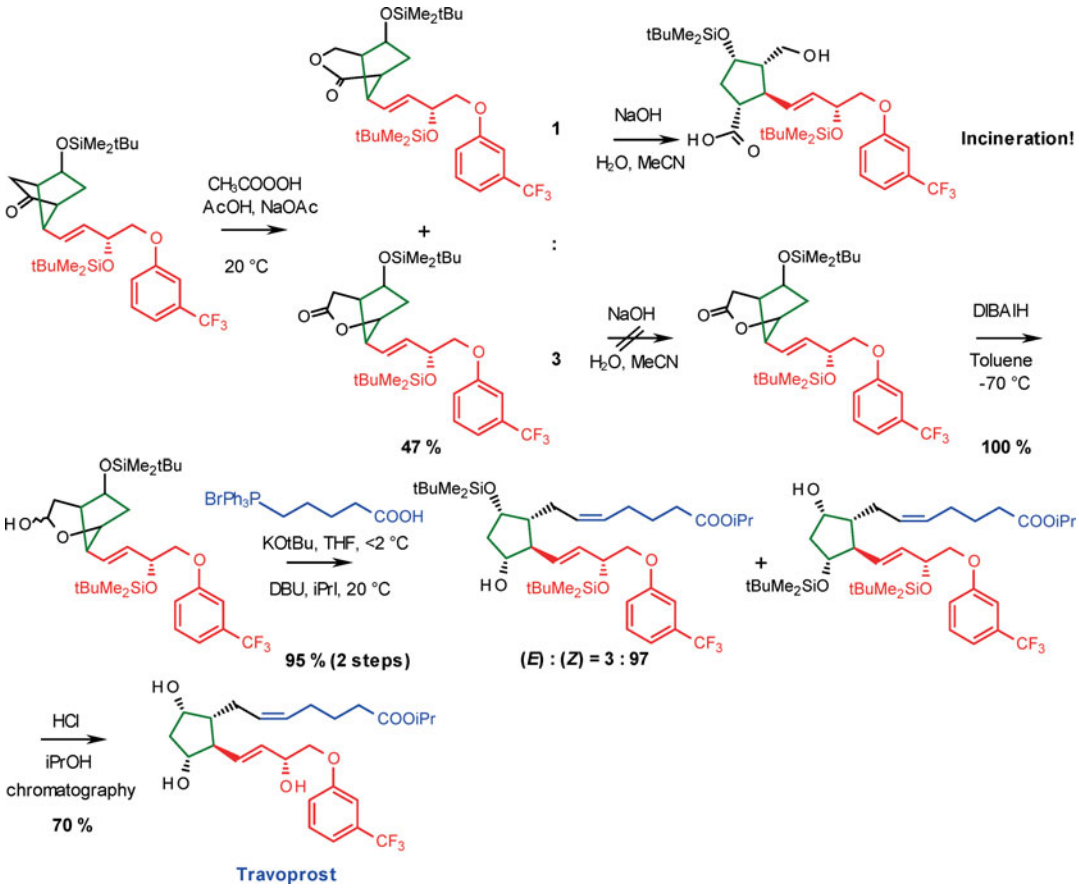
The *trans*-vinyl iodide is obtained by hydrozirconation, in which zirconocene dichloride is converted *in situ* into the analogously reactive *i*-butylzirconocene chloride. [233] Zirconocene dichloride is readily available, and less expensive than the Schwartz reagent, zirconocene hydridochloride.



By zirconium-iodine and then iodine-lithium exchange, the *trans*-alkenyllithium is obtained, which is reacted first with lithium 2-thienylcyanocuprate and then with the tricycle. Attack at the "homo vinylogous" 7-position produces a bicyclic compound, in which the five-membered ring has the *ω*-chain already with the correct stereochemistry.

This amounts formally to a homo-Michael reaction, for which there is abundant precedent in the literature concerning simple cyclopropylcarbonyl compounds. [234]

The most difficult step in the overall synthesis is the following Baeyer-Villiger oxidation. The reaction with peracetic acid in presence of sodium acetate gives a 1 : 3 mixture of isomers. This ratio is nearly independent of the type of peracid, and is in accordance with similar examples from the literature. Astonishingly, the undesired isomer can be selectively hydrolysed, and separated by crystallisation of the lactone. After reduction of the remaining lactone to the lactol, the α-chain is established in a Wittig reaction. The olefination proceeds with high stereoselectivity. A mixture of disilylated products is obtained, which is attributed to silyl-group migration. However, this is not of relevance at this point, since the protecting groups are cleaved off anyway in the next step. Travoprost is finally purified by chromatography to drug quality.



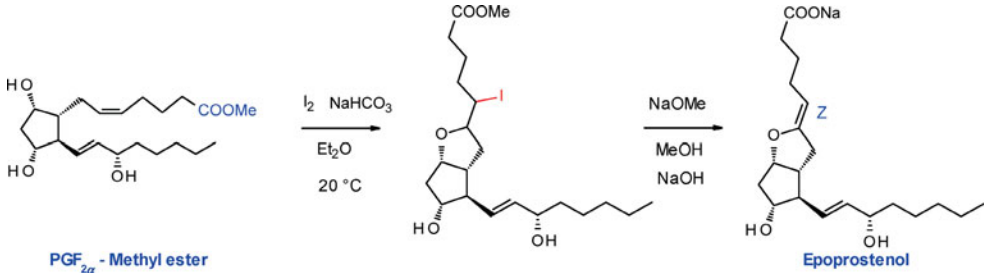
The highlight of this synthesis is certainly that, after separation of the enantiomers of the tricyclic precursor *via* its bisulfite adducts, four stereogenic centres are correctly formed. By attachment of the properly functionalised ω-side-chain, a key building block results, which after a regioselective Baeyer-Villiger oxidation and a stereoselective Wittig reaction, provides the target product. By this route, 450 grams of the active material were prepared. In Chirotech's view, this procedure is also suitable for syntheses at the kilogram scale.

5.6.7 Prostacyclin PGI_2

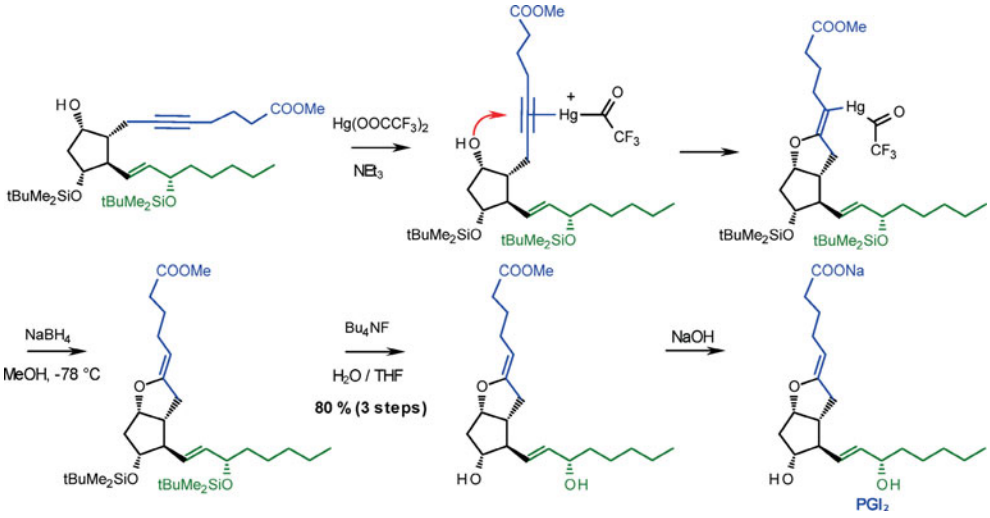
Prostacyclin PGI_2 is an extraordinarily potent vasodilator and inhibitor of blood platelet aggregation. These properties make PGI_2 a versatile agent for treating thromboembolic and cardiovascular diseases. A problem with this compound is its inherent instability, exhibiting a relative chemical stability only in strongly alkaline solutions (pH > 10.5), which are incompatible with parenteral (*e.g.* intravenous) applications. This severely limits the clinical utility.

Epoprostenol is the sodium salt of natural prostacyclin PGI_2. As an injectable solution it was introduced to the market in Britain by Wellcome under the name *Flolan*®, and it is used as an anticoagulant and platelet aggregation-inhibitor for the treatment of dialysis patients and during bypass surgery.

The iodocyclisation gives a pair of diastereomers, which yield from the subsequent elimination exclusively the desired (*Z*)-alkene. After the hydrolysis, carried out as a "one-pot" reaction, its sodium salt, which is stable in solid form at -30 °C for at least two months, is isolated by freeze-drying. [235]

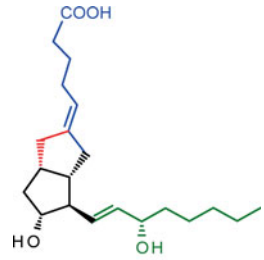


A more modern way is the mercury trifluoroacetate-induced cyclisation according to Noyori. The starting material is obtainable from a three-component coupling reaction. The stereospecific cyclisation proceeds *via* a mercurinium ion in what amounts to a 5-*exo*-*dig*-cyclisation. Critical for the (*E*/*Z*)-isomer ratio is the subsequent mercury cleavage. Normally, this reaction proceeds radically with loss of stereochemical integrity. However, if the mercury residue is removed reductively in protic solvents, then the stereochemistry is preserved.



5.6.8 Carbacyclins

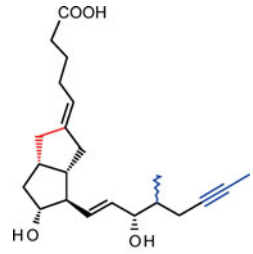
Considering the inherent instability of PGI_2, very great efforts were made early on to prepare more stable prostacyclins with the same pharmacological effect. Thus, the obvious question was, whether it might be possible to replace the enol ether oxygen, the source of the instability, with a carbon, while retaining the pharmacological profile. In this way, carbacyclins were discovered, which are pharmacologically similar to prostacyclin. The lead structure was developed by Upjohn in the 1970s as a drug for the treatment of thromboembolic diseases.



Carbacycline

However, after oral application within the therapeutic range, marked side-effects occured: such as headaches, facial reddening, high pulse-rate (tachycardia) and fluctuating blood pressure. Never-the-less, this structural motif served as a guidance and further analogues were developed.

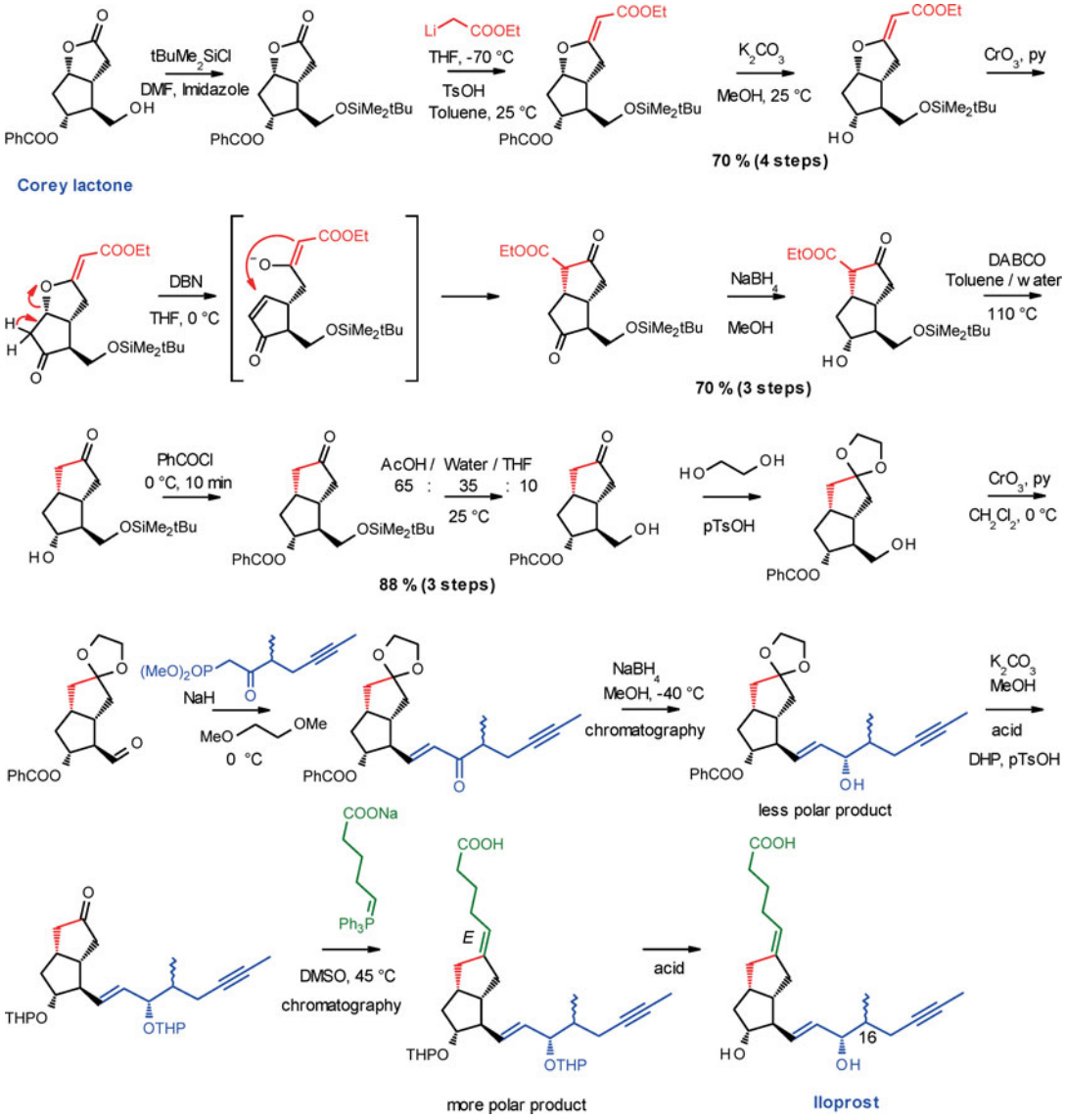
Iloprost is a carbacyclin from Schering for the treatment of cardiovascular diseases. This compound differs from the basic carbacyclin structure by an altered ω-side-chain. Hereby, the metabolic rate is reduced. [235]



Iloprost

The starting material for iloprost is the enantiomerically pure Corey lactone, which is treated with the lithium salt of ethyl acetate. After oxidation, treatment with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) leads to an exceptionally interesting rearrangement. Presumed cleavage of the cyclic ether generates an enolate, which adds to the cyclopentenone in a Michael reaction. Decarbethoxylation is achieved with 1,5-diazabicyclo[2.2.2]octane (DABCO), and the ω-chain is constructed under Horner-Wadsworth-Emmons conditions. The subsequent reduction leads to a mixture of allyl alcohols, which can be purified by chromatography, and the alcohol from the less polar product then be released by transesterification. Both alcohol functions are then protected as THP-ethers. The

synthesis of the carbon skeleton of iloprost is completed by a Wittig reaction. Here again, purification by chromatography is required. The more polar fraction finally yields the target compound after the THP-protecting group is cleaved off. [236]



Iloprost is a 1 : 1 mixture of the 16α- and 16β-diastereomers, which can be separated by crystallisation with (-)-cinchonidine or (+)-3-(aminomethyl) pinane. *In-vitro*, the (16*S*)-diastereomer is five times more potent than the (16*R*)-isomer with regard to inhibiting the ADP-induced blood platelet aggregation.

Summary in Bullet Points

- The first prostaglandins were prepared by partial synthesis.
- Auxiliary-controlled diastereoselective and enantioselective Diels-Alder reactions (Corey) and diastereoselective three-component reactions (Noyori), examples of which were tested for the first time in the total synthesis of prostaglandins, enriched the arsenal of methods in modern preparative organic chemistry.
- Prostaglandins are highly active and possess an extraordinarily broad spectrum of activities.
- The drug demand ranges in most cases clearly below 100 kg per annum. This allows the application of otherwise at industrial production scale unusual methods and reagents.

5.7 Tetrahydrolipstatin

In the Museo del Prado in Madrid, there is a wooden table-top with a painting by Hieronymus Bosch (*ca.* 1450–1516): *The Seven Deadly Sins and the Four Last Things*. [237] This table would once have been found in that suite of rooms in the Escorial, where Philip II (1527–1598) lived (Fig. 5.103).

The Deadly Sins are mentioned for the first time by the Greek theologian and mystic, Evagrius of Pontus (346–399). In the 6th century, Pope Gregory I cut the original eight deadly sins down to seven: Pride, Envy, Wrath, Sorrow,

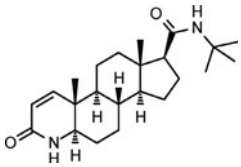
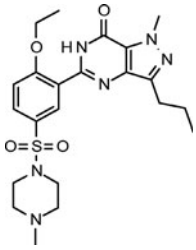
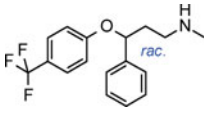
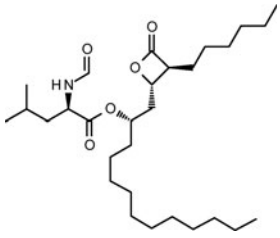
5.103 *Hieronymus Bosch: "The Seven Deadly Sins and the Four Last Things".*

Covetousness, Gluttony and Lust. In the 7th century, "Sorrow" and "Covetousness" were redefined as "Sloth" and "Greed".

Bosch labelled the individual pictures with the seven sins: Pride (Lat. *superbia*: haughtiness, arrogance, as well as pride), Lust (Lat. *luxuria*: indulgence, licentiousness), Sloth (Lat. *segnitia*: slowness, inertia, listlessness), Gluttony (Lat. *gula*: gullet, feasting), Greed (Lat. *avaritia*: avarice), Envy (Lat. *invidia*: covetousness, resentment, jealousy) and Wrath (Lat. *ira*: anger, bitterness, rage).

For or against these individual vices there have recently been developed so-called "lifestyle-drugs", which are intended to turn life better and more pleasant. Since many people long for a better life, unlimited markets beckon. Tab. 5.8 gives an overview of important lifestyle-drugs. [238, 239]

Tab. 5.8 *Modern lifestyle-drugs*

Structure	Name/ producer	Action	Indication
	*Propecia*®/ Merck	5α-reductase inhibitor, prevents hormone-driven male baldness	Superbia
	*Viagra*®/ Pfizer	phosphodiesterase inhibitor, slows down the degradation of cGMP and thereby increases local blood supply	Luxuria
	*Prozac*®/ Eli Lilly	serotonin-reuptake inhibitor, mood elevator, anti-depressant	Segnitia
	*Xenical*®/ Hoffmann-La Roche	lipase inhibitor, treats obesity when combined with a suitable diet	Gula

Sadly, for Greed, Envy and Wrath there is as yet no adequate remedy!

5.7.1 Adiposity

This chapter is concerned with the intemperance of food and drink. Apposite to the painting by Hieronymus Bosch, we read in *Le livre des bonnes moeurs* ("The Book of Good Manners") by the Augustinian Friar Jacques Legrand (Jacobus Magnus, *ca.* 1365–1425):

"And indeed: By gluttony man takes leave of sense and reason, and often uncovers hidden folly. Gluttony allows man to age prematurely and become ugly; and in drunkenness man utters multifarious sounds and is like cattle."

Obesity is obviously not an illness of recent times. Evidence of overweight humans is already found in the Stone Age, in Egyptian mummies and Greek sculptures. Adiposity – this is the scientific name for obesity or corpulence – occurs widely in affluent regions of the world. In view of the 800 million people, who suffer from hunger and malnutrition, there are an estimated 800 million others, who are overly well-nourished. [240] The spread of adiposity is increasingly significant, in men and women, young adults and children. This development contrasts the trend of a low-fat and low-cholesterol diet and of the desire for more physical exercise. Adiposity becomes one of the main causes for diabetes, high blood pressure and cardiovascular diseases.

There are several causes of adiposity. In animal experiments and in humans, genetic defects have recently been demonstrated to influence appetite control and adipogenesis. Such inherited dysregulations are however rather rare. Statistical investigations show that psychological, socio-economic and cultural factors contribute to adipogenesis. American studies document that individuals with poor education and low income are more prone to become overweight.

How people plan and conduct their life and what attitude to life they adopt, are important indicators as to whether someone will ultimately develop adiposity. Hence, parents function as role models as well, and can have a decisive influence on the eating habits of their children (Fig. 5.104).

The Body-Mass-Index (BMI) is calculated as the quotient of body weight (in kilograms) and height (in metres) squared. Insurance companies have determined that men and women with a BMI of 22–25 have the least disease risk and the lowest mortality rate. At a BMI beyond 27, the mortality rate begins to rise significantly. These assumptions apply to people with a lower or with an only average exercise level. Due to their distribution of fat and muscle, for highly trained, muscular athletes, these BMI values have to be revised upwards.

5.104 *"Gluttony" by Hieronymus Bosch: from "The Seven Deadly Sins and the Four Last Things".*

5.7.2 Pharmacology

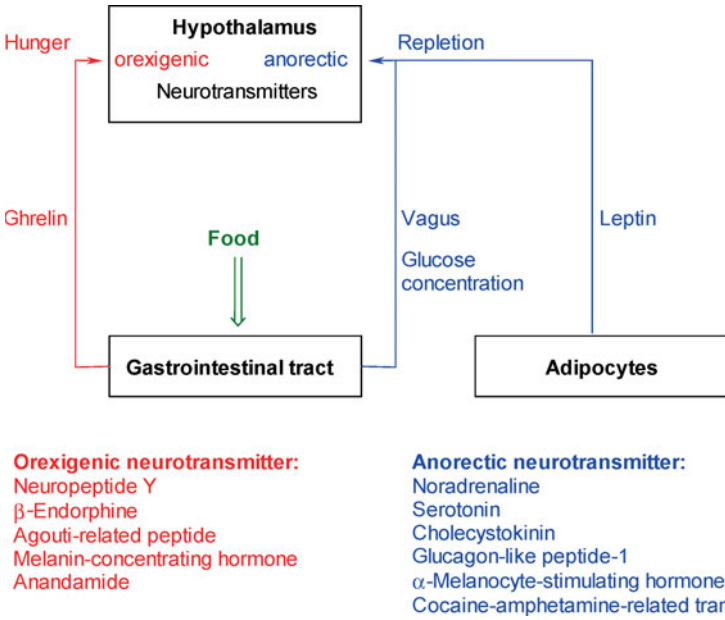
Over the years a plethora of natural product based medications, mainly of herbal origin, has been tried to treat adiposity. [241] Many of them are sold and used globally with very little proof of efficacy or quality. The continued search for new therapies has revealed multiple targets to combat obesity.

Nowadays, there are in principle two therapeutic strategies for the treatment of adiposity. The first aims at creating a feeling of satiety and increasing metabolism; the second aims at reducing the resorption of fat.

Regulation of Food Intake

The interplay of hunger and repletion results from a fairly complex interaction of hormonal regulators, where predominantly the gastro-intestinal tract, the adipose tissue and the hypothalamus are involved (Fig. 5.105). [242] By the intake of food, the stomach is being filled, whereby the stretching stimulus is transmitted to the hypothalamus *via* the afferent vagus nerve. Additionally, chemoreceptors in the intestines and the liver register the energy content of the food. The releases of insulin, controlled by the glucose level in the blood, and cholecystokinin and leptin, excreted by fat cells, eventually trigger the release of anorectic neurotransmitters, and this leads to the sensation of repletion.

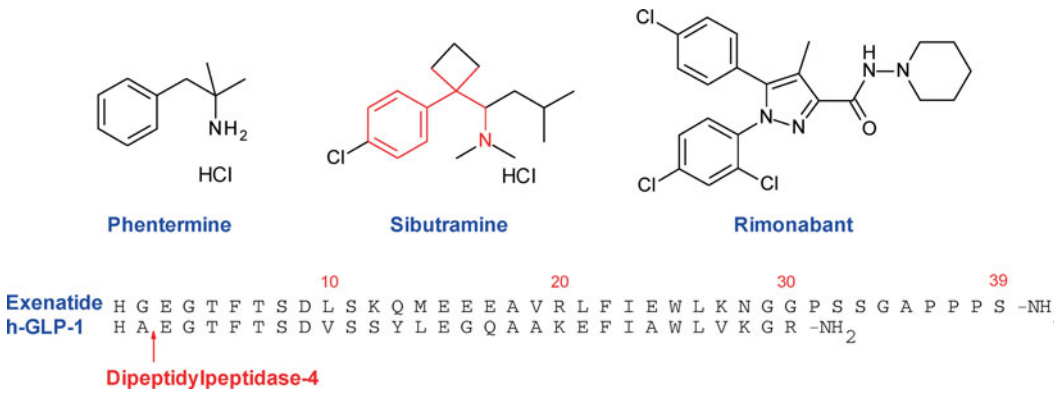
While the food is digested, these signals become weaker until finally, under the influence of ghrelin, which is produced in the stomach as well, orexigenic neurotransmitters are increasingly released, conveying again the sensation of hunger.



5.105 *Regulation of the sensations of hunger and repletion.*

Remarkably, amongst the orexigenic and anorectic neurotransmitters, there is not a single one that stands out as the most important. Selective blockage of one or the other messenger is automatically compensated by a different neurotransmitter. In addition, along with the enhancement of the anorectic activity goes also an increased orexigenic tone. This is a principal problem, which inevitably restricts the therapeutic efficiency of drug intervention.

Nevertheless, phentermine, from SmithKlineBeecham, became the first appetite suppressant (anorectic), which was approved by the FDA in 1959 (Fig. 5.106). [243] Phentermine leads to increased release of catecholamines like dopamine, adrenaline (epinephrine) and noradrenaline (norepinephrine), which reduce the sensation of hunger. However, the effect declines in the course of several weeks. Due to grave side-effects (sleeplessness, nervousness, nausea, obstipation, Angina pectoris problems and acute psychoses) the drug has meanwhile been withdrawn from a number of markets.



5.106 *Cerebrally-active anorectics.*

In the 1980s the Boots company developed the serotonin- and norepinephrine-reuptake inhibitor sibutramine for reduction of severe overweight. [242, 244] Sibutramine has a dual function: to increase the feeling of satiety and to enhance metabolism. In 1997, the drug was launched under the trade name *Meridia*®, however after an unfavourable risk/benefit assessment and the consequent ban by the European Medicines Agency (EMA) and the FDA, it was ultimately suspended by Abbott in 2010.

Rimonabant is an anorectic, which was originally developed by Sanofi-Aventis for smoking cessation. [242, 245] In contrast to the amphetamines and sibutramine, it acts not as an agonist of the anorectic, but as an inhibitor of the orexigenic system. Rimonabant is an inverse agonist of the cannabinoid-1 receptor (CB1). The drug was launched in Europe in 2006 under the trade name *Acomplia*®. Due to the risk of severe depression and suicidal thoughts, Sanofi-Aventis withdrew the product in 2008 upon a warning issued by the EMA.

One of the latest anorectics was discovered in 1992 by John Eng at Veterans' Affairs Medical Center, Bronx, New York, in the saliva of the Gila lizard (Fig. 5.107). [246] The toxin is produced in the lizard's salivary glands in the lower jaw, and it contains serotonin together with a multitude of peptides

5.107 *The Gila lizard (Heloderma suspectum, or Gila monster), lives in desert regions of south-western North America. It is reckoned among the few toxic lizards: though rather painful, its bite has not been reported fatal to humans.*

and proteins, like hyaluronidase, phospholipase A_2 and several kallikrein-like glycoproteins.

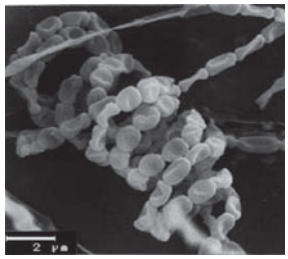
Another pharmacologically active component is exendin-4, a 39 amino acid peptide; its corresponding C-terminal amide is called exenatide. [247] Interestingly, exendin-4 and exenatide share a 50 % sequence homology with the 30 amino acid human glucagon-like-peptide-1 (h-GLP-1), but, in contrast to the latter, are resistant to degradation by dipeptidylpeptidase-4 (DPP-IV). Exenatide binds to GLP-1 receptors expressed in pancreatic beta cells and acts there as an agonist, thereby stimulating indirectly synthesis and excretion of insulin. Therefore, this compound became very attractive as an antidiabetic treatment option.

During the clinical development by Amylin Pharmaceuticals and Eli Lilly, it was discovered that exenatide is also acting on GLP-1 receptors in the brain: gastric emptying is retarded, and appetite control increases the sensation of repletion [247], which leads to a significant weight loss.

Exenatide is manufactured by a convergent solid-/liquid-phase synthesis [248] and was launched in the USA for the treatment of *Diabetes mellitus* type 2 in 2005 under the trade name *Byetta*®. The European approval followed in 2006.

Regulation of Fat Resorption

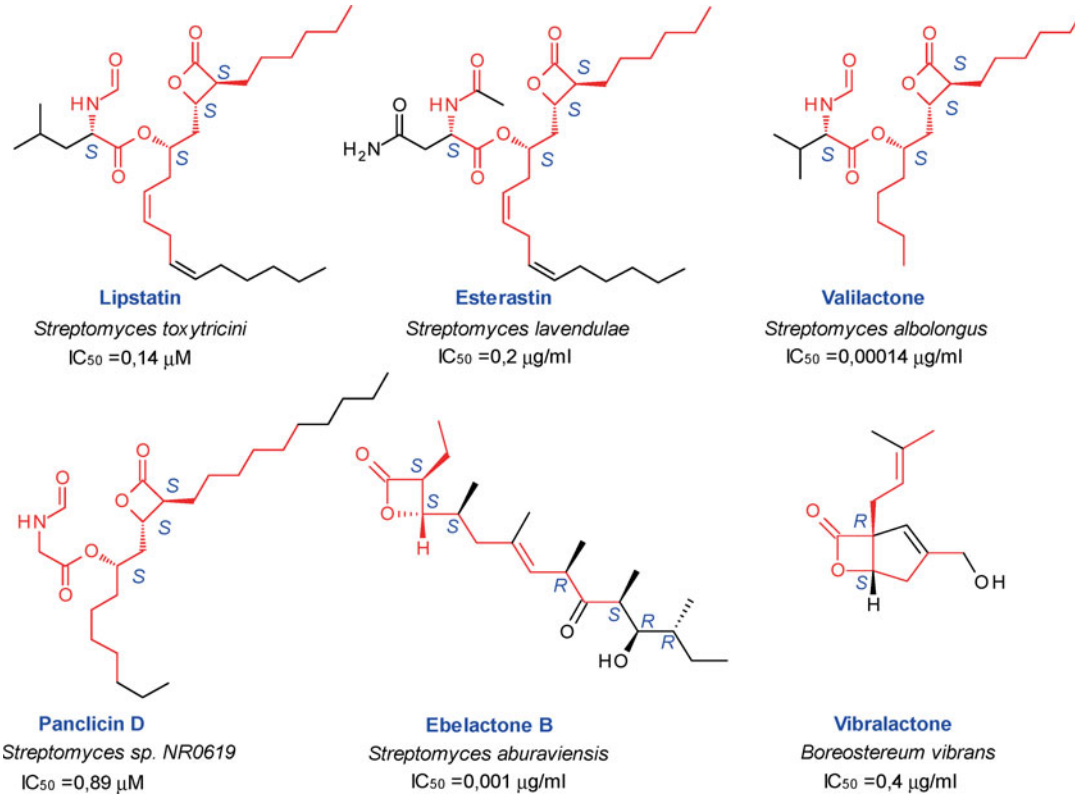
A fundamentally different approach to treat adiposity is to lower the intake of high-calory food. Since humans possess only a very limited capability for *de novo* biosynthesis of fat, adiposity can be prevented by reducing or blocking the resorption of dietary fat. This can in principle be achieved as well through an appropriate nutritional regimen. However, low-fat dishes often appear less tasty (fat is an important flavour carrier), so that the compliance of the patients to



5.108 *Scanning electron micrograph (×10,000) of a spore chain of Streptomyces toxytricini.*

adhere to a strict diet is rather poor. The key enzyme for the digestion of fat is a pancreas-lipase, which hydrolyses triglycerides. Subsequently, the free fatty acids and monoglycerides are stored in micelles and finally resorbed in the small intestine. If the pancreas-lipase is blocked, the fat passes through the intestine without being resorbed.

In 1987, scientists at Hoffmann-La Roche discovered in two soil samples from Mallorca and also from Gstaad (Switzerland) a grey and white variant of *Streptomyces toxytricini*, which produce lipstatin. This compound inhibits very selectively and irreversibly pancreas-lipase (Fig. 5.108). [249] In the following years, a number of structurally closely related lipase inhibitors were discovered, like valilactone, panclicin D and others. [250, 251] Esterastin was already known since 1978 (Fig. 5.109). [252]



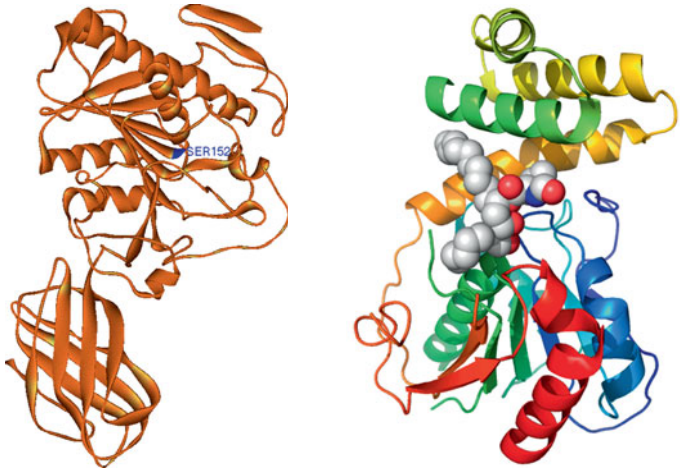
5.109 *Inhibitors of the pancreas-lipase.*

Other pancreatic enzymes, like phospholipase A2, amylase, trypsin, chymotrypsin and liver-esterase, are not blocked by lipstatin. Though, it is known that tetrahydrolipstatin also inhibits other serine-hydrolases such as stomach-lipase and pancreas-carboxylic-ester lipase. [253]

All these inhibitors have as their central pharmacophore a β-lactone structure, which is formed by a branched long-chain carboxylic acid, carrying hydroxy-groups at the 3- and 5-positions, and mostly linked to an amino acid. All

stereogenic centres of the lactone moiety have the (*S*)-configuration. [254] Lipstatin and esterastin differ only in the amino acid side-chain. In both cases, hydrolysis results in the same long-chain doubly-unsaturated C_{22}-dihydroxy-carboxylic acid. [255]

Nature produces obviously not only interesting β-lactams as in the antibiotics, but also structurally corresponding β-lactones. The high reactivity of the oxetanone system is crucial to the irreversible enzyme inhibition: The oxetanone acylates a serine residue (Ser-152) in the active centre of the pancreas-lipase and thereby blocks its function (Fig. 5.110). [256] Some derivatives, *e.g.* tetrahydrolipstatin, also inhibit the thioesterase domain of the human fatty acid synthase. [257]



5.110 *Pancreas lipase (left) and human Fatty acid synthase (right) inhibited by tetrahydrolipstatin.*

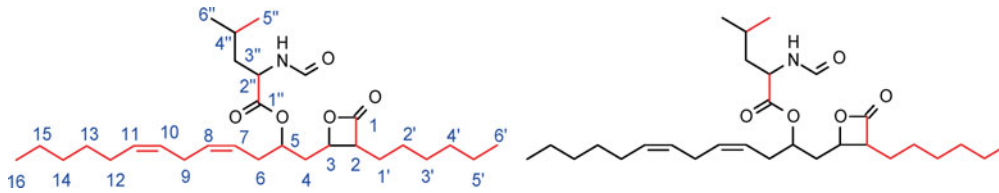
5.7.3 Biosynthesis

The elucidation of biosynthetic pathways uses nowadays isotopically labelled precursors. Labelling experiments are frequently carried out with the radioactive ^{14}C-isotope. Once the radioactive metabolites are identified, the precise position of the label can be determined by degradation reactions. For ^{13}C-labelled compounds, there is also the possibility, not only to identify the metabolites with the aid of modern NMR spectroscopy, but also to directly resolve their structure and labelling pattern.

The labelling with ^{13}C intensifies the corresponding NMR signal and ^{18}O leads to a high-field shift of the neighboring C atom. The use of doubly labelled compounds, such as 1,2-^{13}C-acetate, is of additional value. The multiplicity and coupling constants reveal the incorporation of the entire labelled fragment or of its rearrangement products.

Initial investigations of the biogenesis of lipstatin using ^{14}C-labelled acetate were inconclusive. However, the use of fully ^{13}C-labelled lipids from algae, which had been fed with $^{13}CO_2$, was successful. Corresponding NMR studies showed that the lactone was formed directly from fatty acids by a Claisen reaction. [258]

Since labelled building blocks are incorporated together with unlabelled material in a *de novo* synthesis, no universal label can be expected. Correspondingly, the missing coupling between C-2 and C-3 reveals the linking position of the Claisen reaction. If a mixture of fully hydrogenated, labelled fatty acids (shown in red) and unhydrogenated, unlabelled fatty acids (shown in black) is utilised, experiments demonstrate that the unsaturated side-chain is exclusively formed from the unlabelled fatty acids. On the other hand, the saturated side-chain is fully labelled (Fig. 5.111).

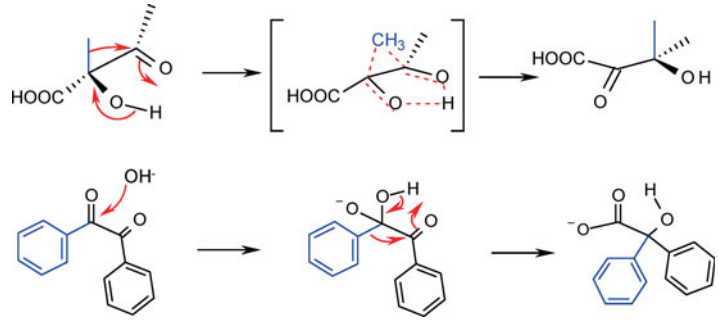


5.111 *^{13}C-Labelling experiments for elucidation of the biosynthesis.*

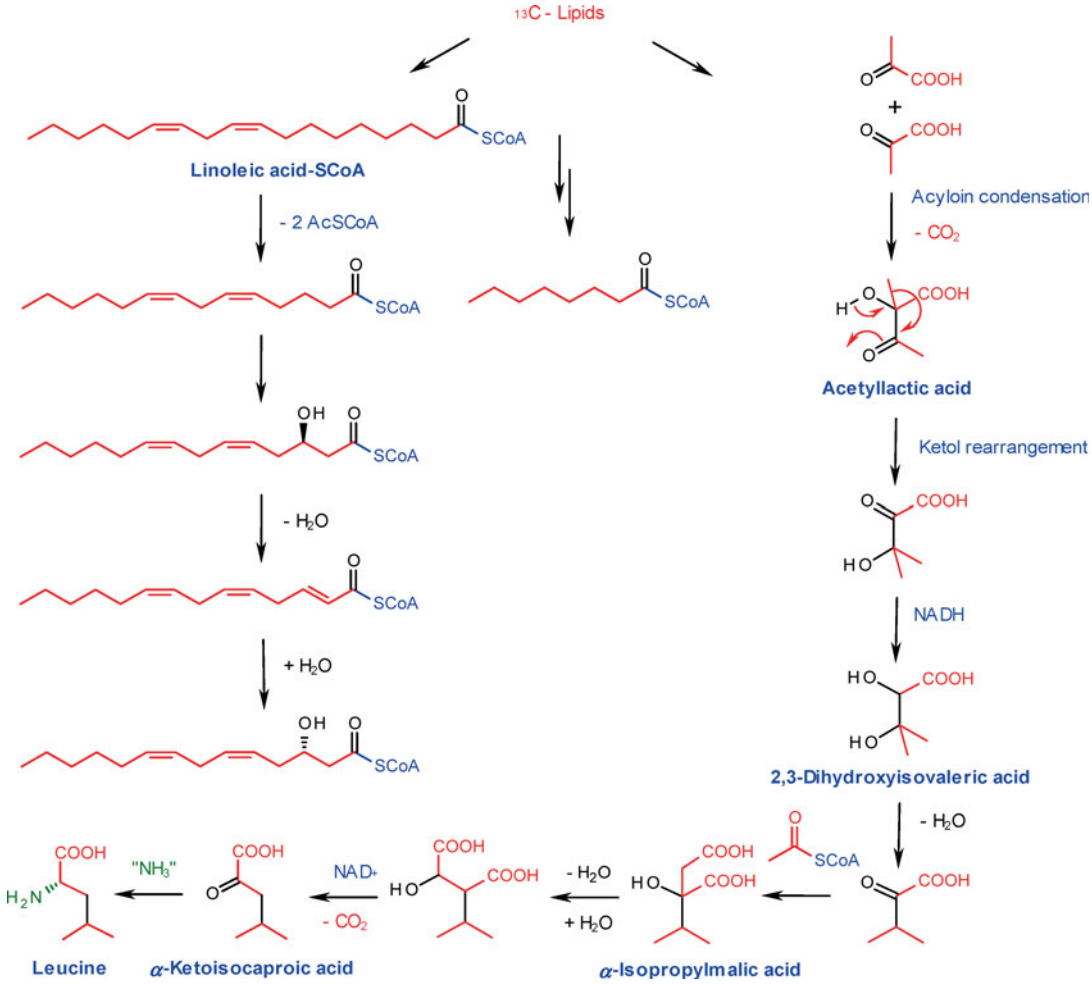
The longer side-chain originates from linoleic acid, which is shortened by four carbon atoms *via* β-oxidation and cleavage of two acetate units. The shorter side-chain is generated similarly. By dehydration and then addition of water, the required hydroxy-acid is formed. [259]

The labelling pattern in leucine corresponds, as expected, to the known biosynthetic pathway. Although this was not explicitly investigated, one can assume that pyruvic acid is produced from glycerol during the fermentation process.

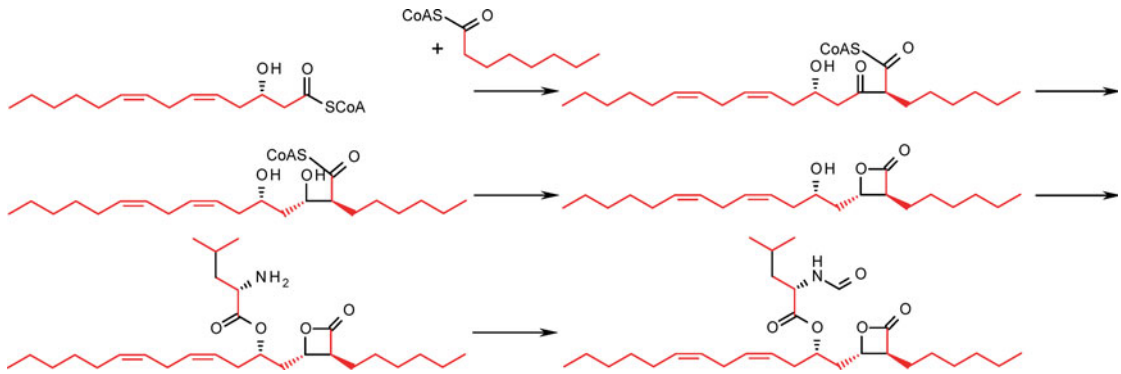
Acetyllactic acid results from an acyloin condensation and loss of carbon dioxide, followed by a very interesting ketol rearrangement, which proceeds in a stereochemically uniform manner. Herein, the hydroxy- and keto-groups are oriented *syn*-periplanar, so that the methyl group is transferred suprafacially on the (*Re*)-side, a mechanism, which is supported by data from NMR spectroscopy on model compounds [260] and by preparative examples. [261, 262] The ketol rearrangement is to some extent related to the benzil-benzilic acid rearrangement.



The keto-group is subsequently reduced and water is eliminated. By aldol addition, α-isopropylmalic acid is obtained. This is first dehydrated, followed by another addition of water; in this way, β-isopropylmalic acid is formed. Oxidative decarboxylation yields α-ketoisocaproic acid, which is finally converted into leucine by reductive amination.



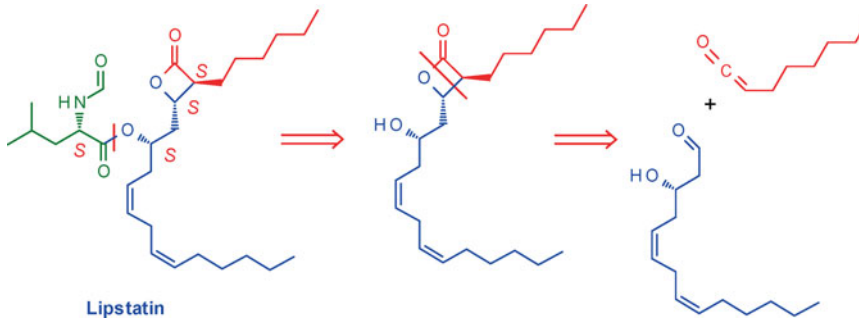
After the Claisen reaction of the CoA-activated carboxylic acids, the β-keto-group is reduced and the lactone ring closed. Finally, esterification with the amino acid and formylation occur. [263]



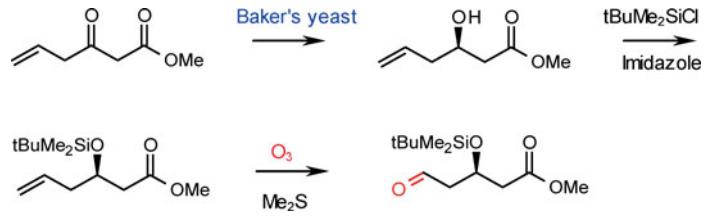
In the initial fermentation experiments, lipstatin was obtained in low time-volume yield of a few milligrams per litre. With an understanding of the biosynthesis, it became possible to increase the product concentration to 150 mg/l per 138 hours by specificly dosed supplementation of the fermentation broth with fatty acids and leucine. In order to avoid inhibition, it appears important to maintain a low *steady-state* concentration of the substrates by slow addition. [264, 265] For industrial production, however, these time-volume yields are still too low, at least by a factor of 500.

5.7.4 Synthesis of the Natural Product

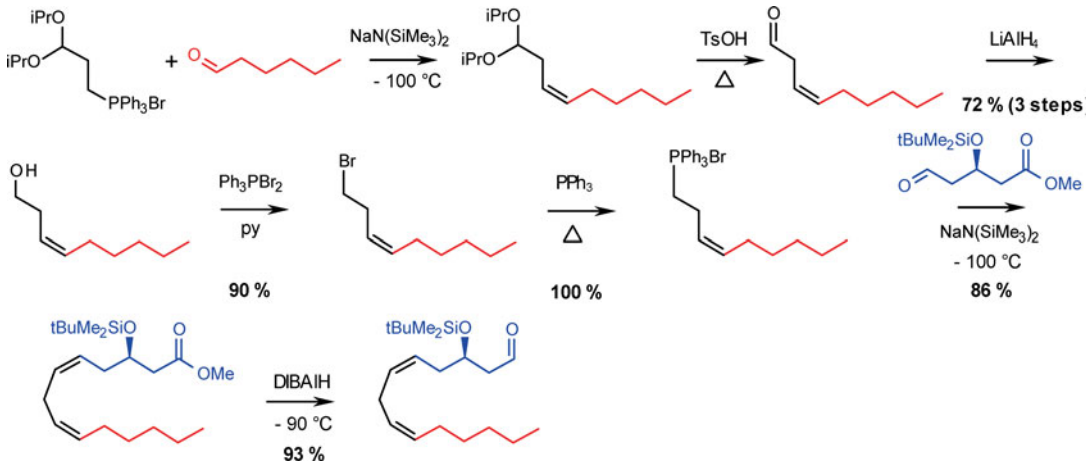
The first synthesis of (-)-lipstatin came from Jean-Marc Pons and Philip Kocienski. [266] The key step is a Lewis acid-catalysed, diastereoselective [2 + 2]-cycloaddition of an aldehyde with a ketene. The unsaturated side-chain is formed by two Wittig reactions.



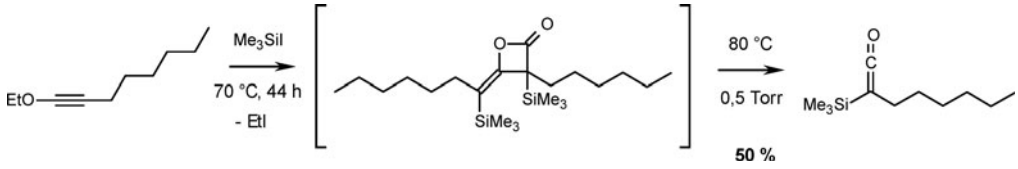
The synthesis starts with the enantioselective reduction of methyl 3-oxohex-5-enoate with baker's yeast. The enantiomeric excess amounts to 78 %, at a yield of around 60–70 %. Subsequently, the hydroxy-group is protected and the aldehyde function is introduced by ozonolysis. [267, 268]



For the second part of the side-chain, hexanal is first extended by three carbon atoms *via* a Wittig reaction. The acetal is hydrolysed and then, in a sequence of several reaction steps converted into a phosphonium salt, which is used in another, so-called "Li-salt-free", Wittig reaction, leading to the expected high (*Z*)-selectivity. Finally, the ester is reduced to the corresponding aldehyde with diisobutylaluminium hydride; the overall yield of the (*Z*,*Z*)-dienaldehyde, based on hexanal, amounts to 50 %.

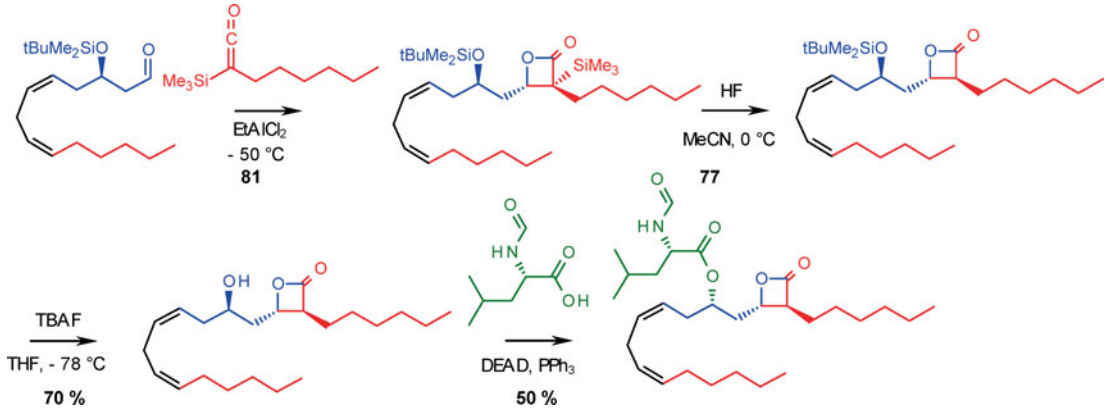


The ketene for the [2 + 2]-cycloaddition is obtained by a method of Hedeki Sakurai, from 1-ethoxyoct-1-yne and trimethylsilyl iodide. [269] The initially formed diketene is then thermolysed and can be stored as the monomeric ketene for a certain time.



The ethylaluminium dichloride-catalysed [2 + 2]-cycloaddition gives a 75:15:10 mixture of diastereomers. Without further purification, the silyl protecting groups are then cleaved off, and *N*-formylleucine introduced in a Mitsunobu reaction.

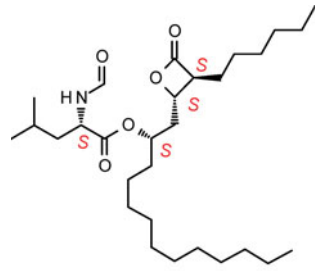
The silyl-protected hydroxy-group provides in the diastereoselective [2 + 2]-cycloaddition the asymmetric induction of the *β*-lactone, and is in the Mitsunobu reaction by inversion of the stereogenic centre converted into the desired absolute configuration. The end-product, a slightly yellowish oil, still contains around 10 % of unwanted diastereomers, which cannot be separated off. However, enantiomerically pure tetrahydrolipstatin can be obtained by hydrogenation and crystallisation.



5.7.5 Syntheses of Tetrahydrolipstatin

The saturated analogue of lipstatin, namely tetrahydrolipstatin, has pharmacological properties comparable with those of the natural compound, but in chemical terms it is substantially more stable, and possesses advantageous physical properties. It is a solid, which may be purified by crystallisation. [250] This was the main reason for Hoffmann-La Roche to develop the saturated compound rather than the natural product as a drug for adiposity (Fig. 5.112). [270]

In the past 15 years, tetrahydrolipstatin served many research groups as a showcase example, in order to test new and old methods for the stereoselective synthesis of β-lactones. In what follows, some of these especially interesting syntheses are compared with one another.



Tetrahydrolipstatin

5.112 *Tetrahydrolipstatin was developed by Hoffmann-La Roche as a drug for the treatment of adiposity.*

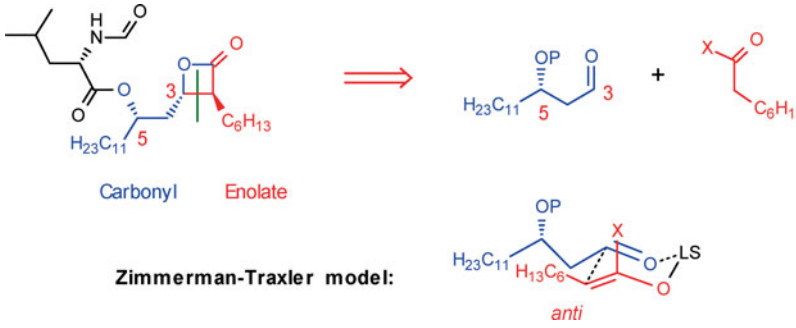
Several years prior to the market introduction of a drug, it is necessary to define exactly the ultimate synthetic methods of the production process for the active ingredient. The material manufactured precisely in that same manner has to be used to generate all the biological, pharmacological and toxicological information relevant to submit for regulatory review and possible approval. More elegant syntheses are often found later on, which however rarely reach industrial-scale realisation due to the potential cost involved seeking approval for a product manufactured differently.

Diastereoselective Mukaiyama Aldol Reaction

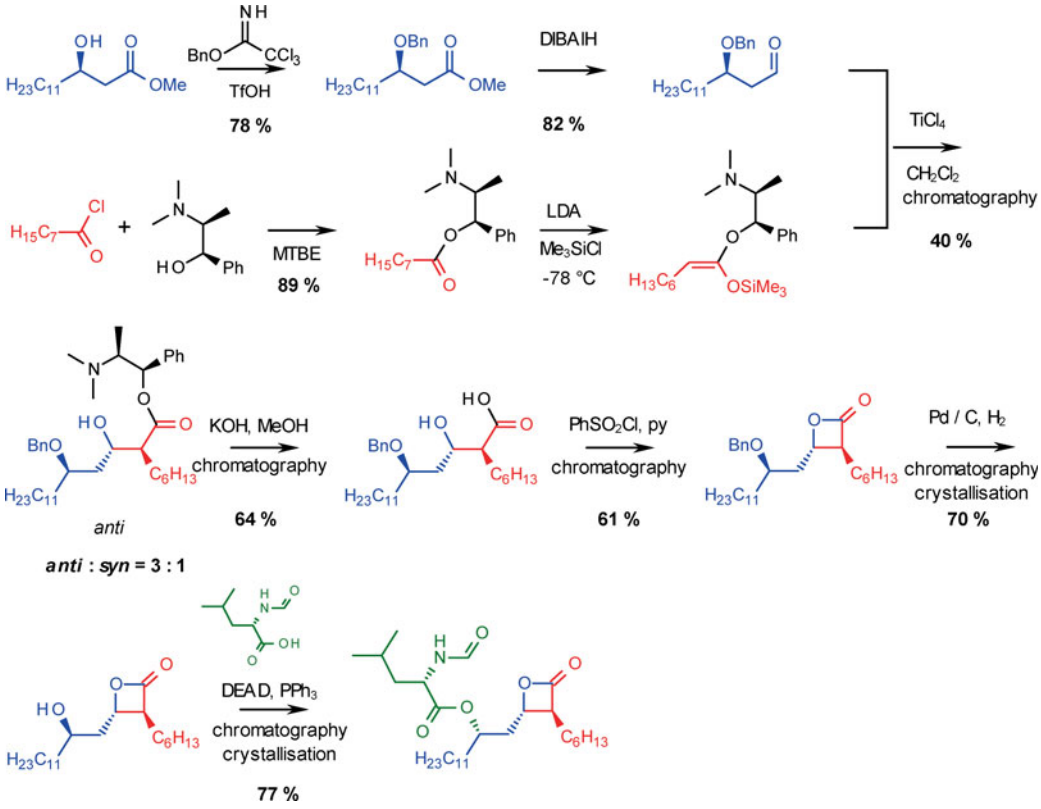
The reactions of aldehydes with carboxylate enolates are stereochemically related to the Ivanov reaction (dianions of arylacetic acids + carbonyl compounds), named after the Bulgarian chemist Dimitar Ivanov Popov (1894–1975). In this reaction, the *anti*-product is preferentially formed. The Ivanov reaction is known to proceed through the Zimmerman-Traxler transition state model.

In order to construct the basic skeleton of tetrahydrolipstatin, an aldol addition is virtually predestined. The structure of tetrahydrolipstatin may be regarded formally as an α-branched carboxylic acid with oxygen functions at positions 3 and 5. These ought to be established easily by an *anti*-selective aldol reaction from a β-hydroxyaldehyde and an enolate component at the oxidation level of a carboxylic acid. The hydroxy-function on the stereogenic centre at position 5 can be used moreover as a stereodifferentiating structural fragment.

Hoffmann-La Roche used for their initial syntheses the Mukaiyama variant. [271] The aldehyde component is obtained by reduction of the corresponding enantiomerically pure β-hydroxy-ester with diisobutylaluminium hydride. The silylketene acetal is accessible from the ester of (–)-*N*-methylephedrine by silylation. The titanium tetrachloride-catalysed Mukaiyama aldol-addition gives the desired *anti*-product in a 3:1 ratio. After saponification of the ester, the



lactone ring is closed with benzenesulfonyl chloride. The benzyl protecting group is removed by hydrogenation and the side-chain introduced in a Mitsunobu reaction with inversion of the absolute configuration.

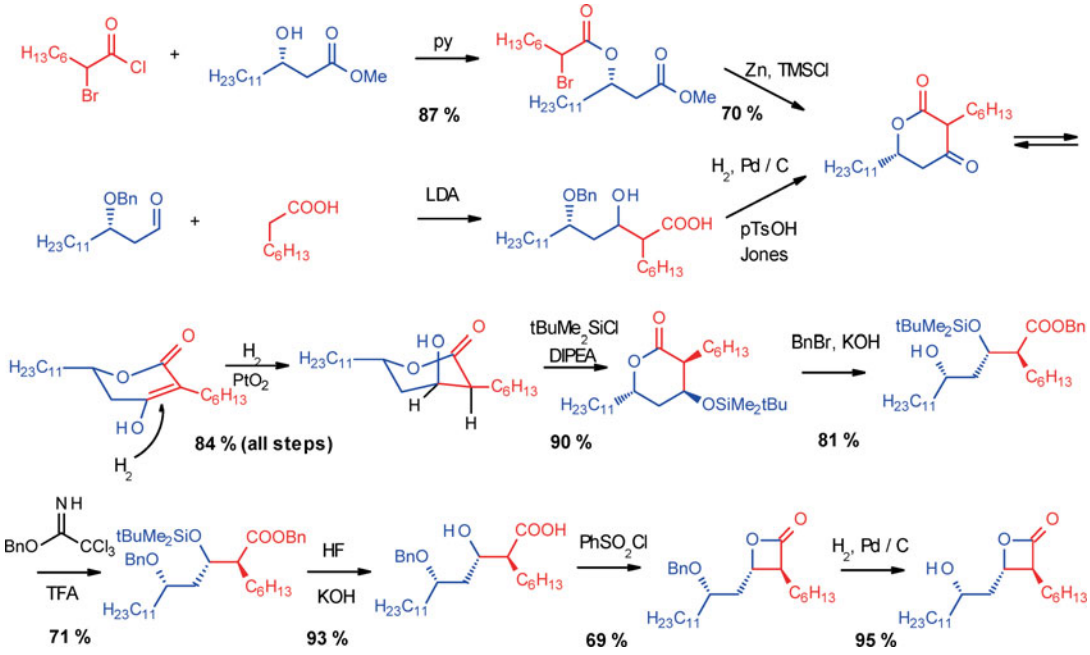


Disadvantages of this synthesis are the many chromatography steps and the poor *anti*-/*syn*-selectivity.

Diastereoselective Hydrogenation

In a further synthesis, Hoffmann-La Roche took the diastereoselective hydrogenation of a δ-lactone into consideration. [272–274] The reaction of the enantiomerically pure hydroxy-ester with 2-bromooctanoyl chloride leads after an in-

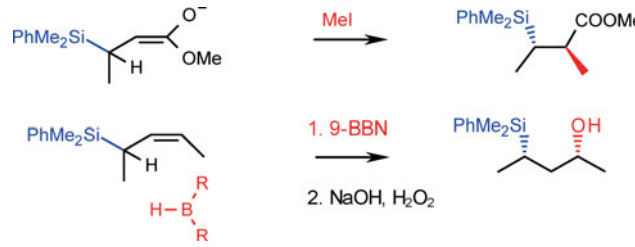
tramolecular Reformatzky reaction to the δ-lactone. Another approach involves the Ivanov reaction of the benzyl-protected hydroxy-aldehyde with octanoic acid. Reductive removal of the protecting group, cyclisation with acid and Jones oxidation gives the same δ-lactone. The diastereoselective hydrogenation then yields a tetrahydropyrone.



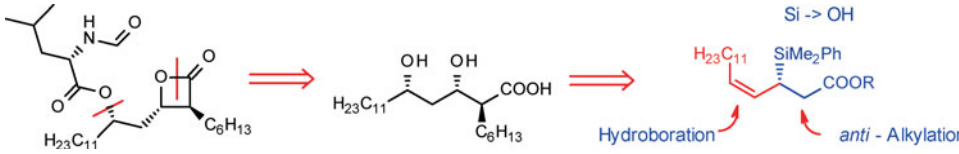
A series of protecting group manipulations follows, until the β-lactone is finally obtained. Perhaps apart from the diastereoselective hydrogenation of the δ-lactone, this reaction sequence conveys the overall impression of being devoid of any elegance as an incentive for its implementation. To be technically viable, the synthetic strategy, and above all the concept of protecting groups, has to be drastically simplified.

Diastereoselective Michael Reaction

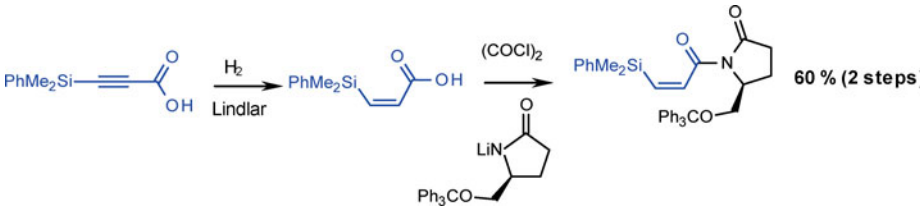
The basic idea of the following tetrahydrolipstatin synthesis is the combination of two methods with high stereoselectivity for the generation of two new stereogenic centres in the 1,2- and 1,3-positions relative to the silyl substituent: (1) alkylation of β-silyl-enolates and (2) hydroboration of allylsilanes. [275, 276]



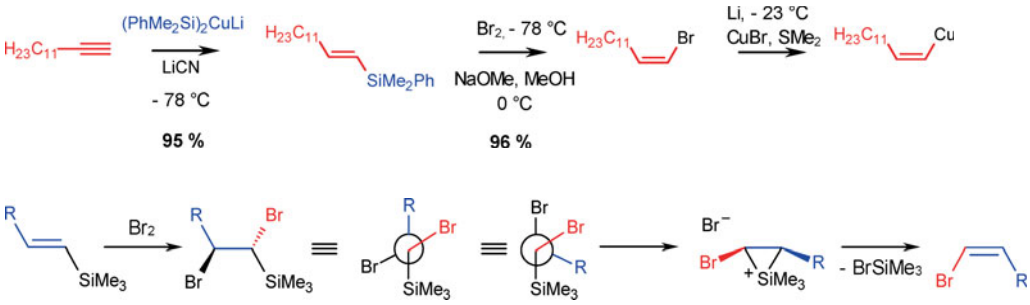
The corresponding retrosynthesis of tetrahydrolipstatin indicates the task to construct an unsaturated β-silylcarboxylic ester, which is then alkylated in the α-position and hydroborated in the allyl position.



At the beginning of the synthesis, a *cis*-silyl-substituted acrylic acid is prepared from the corresponding alkynoic acid by reduction with a Lindlar catalyst, then activated with oxalyl chloride and reacted with Koga's auxiliary.



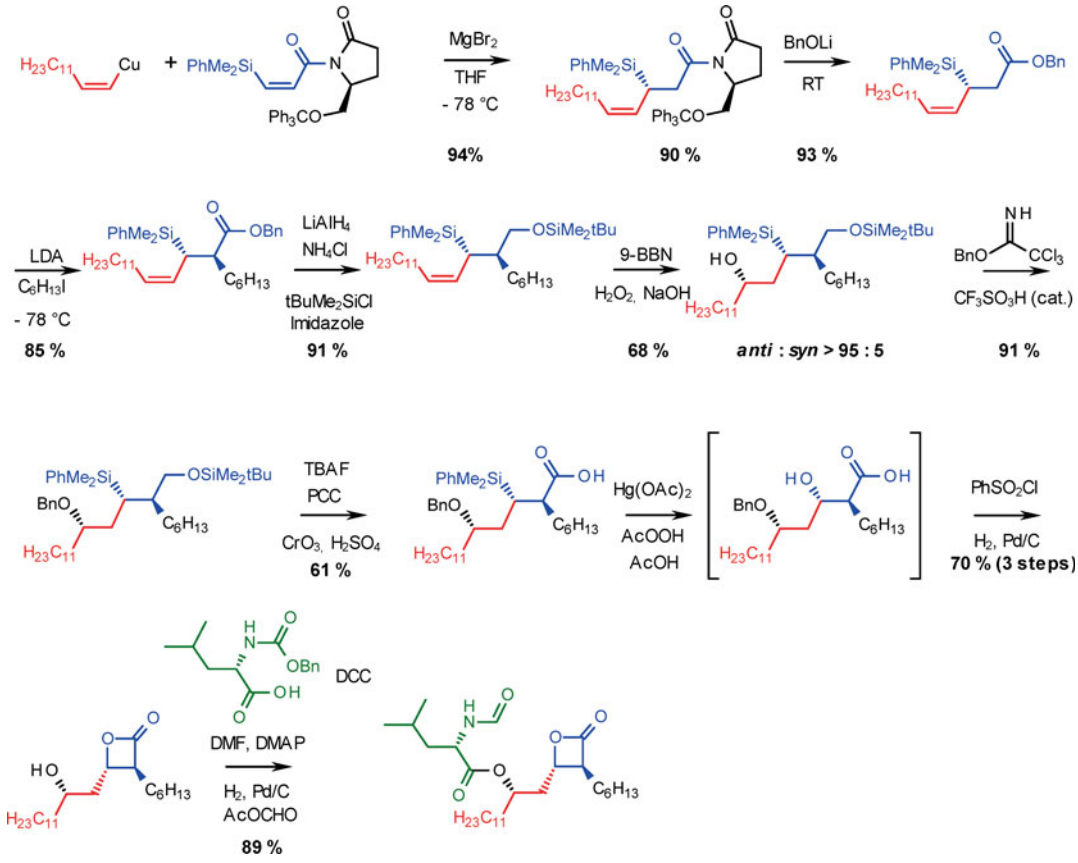
For the hydroboration, a *cis*-allylsilane is required, resulting from a *cis*-vinylcuprate. This precursor is obtained by reaction of the terminal alkyne with the lithium cuprate/lithium cyanide system, by which the terminal *trans*-vinylsilane is stereospecifically generated. Bromodesilylation then gives a *cis*-vinyl bromide, which is finally converted into the corresponding cuprate with retention of the stereochemistry. [277]



The subsequent reaction of the cuprate with the enantiomerically pure acrylamide gives, with good diastereoselectivity, the key building block for the carbon skeleton, from which tetrahydrolipstatin can be formed.

The chiral auxiliary is cleaved off first and replaced by a benzyloxy-group. Alkylation at the α-position proceeds stereospecifically. However, the projected next step with 9-BBN leads also to side-products involving the ester function. This is a conceptual disadvantage of the overall synthesis, since the ester must first be reduced and then protected (total of four additional steps). The introduction of the hydroxy-group proceeds with high stereoselectivity. The new hydroxy-group is protected as its benzyl ether under acid catalysis, since the basic

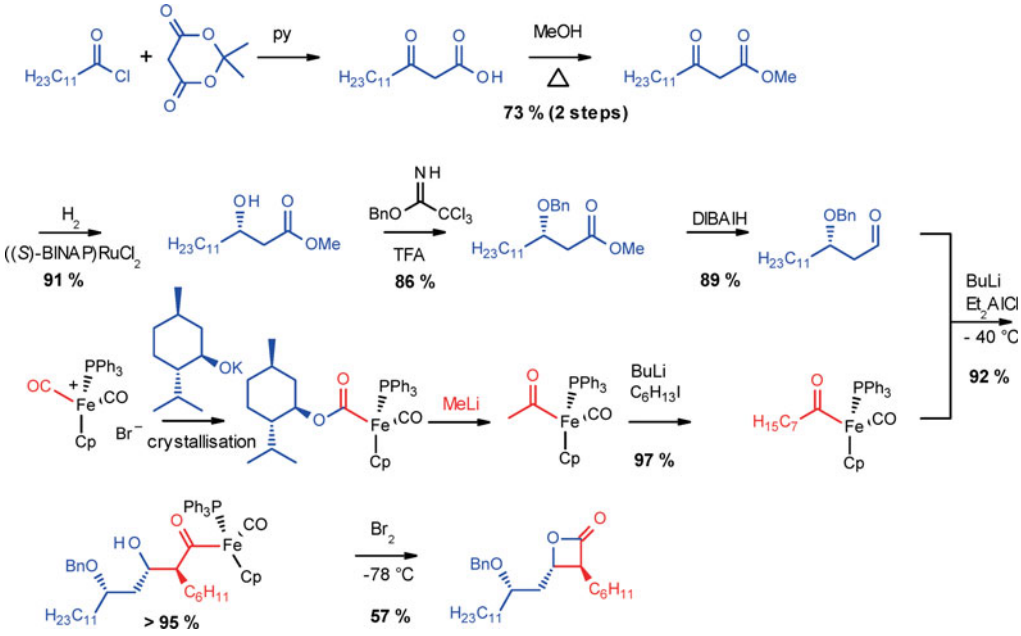
method (BnBr, NaH) leads *via* intramolecular substitution of the phenyl substituent by the alkoxy-function to a cyclic silyl ether. In the next stage, the carboxylic acid moiety is re-constructed stepwise with pyridinium chlorochromate and Jones oxidation. Finally, the silyl function is replaced by a hydroxy-group with mercury(II) acetate and peracetic acid, the β-lactone ring is closed and the side-chain introduced. By a single recrystallisation of the tetrahydrolipstatin, an enantiomerically pure product is obtained.



Diastereoselective Aldol Reactions with Acyl-Iron Complexes

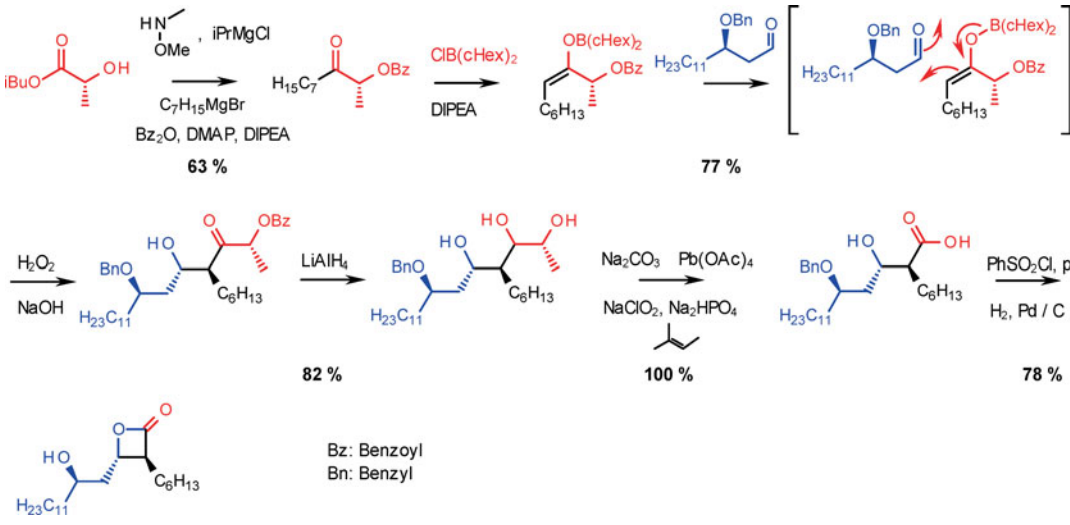
Another original synthesis of tetrahydrolipstatin comes from Stephen Davies. [278] The two central building blocks are on the one hand an enantiomerically pure β-hydroxyaldehyde, which is accessible by acylation of Meldrum's acid with dodecanoyl chloride, followed by an enantioselective hydrogenation of the β-keto-ester along Noyori's method, and on the other hand an enantiomerically pure acyl-iron complex [279], which is obtained by alkylation of the acetyl complex. [280] It is noteworthy that from the beginning the aldehyde component possesses the correct absolute configuration. The side-specific course of the *anti*-aldol addition is exclusively determined by the iron complex. First, the iron complex is deprotonated with butyl-lithium, then transmetallated with diethylaluminium chloride, and finally reacted with the aldehyde. The re-

action product contains less than 5 % of the other diastereomers. The lactone results directly from decomplexation with bromine, and it is finally converted into tetrahydrolipstatin by means of DCC coupling.



Diastereoselective Borinate-mediated Aldol Reaction

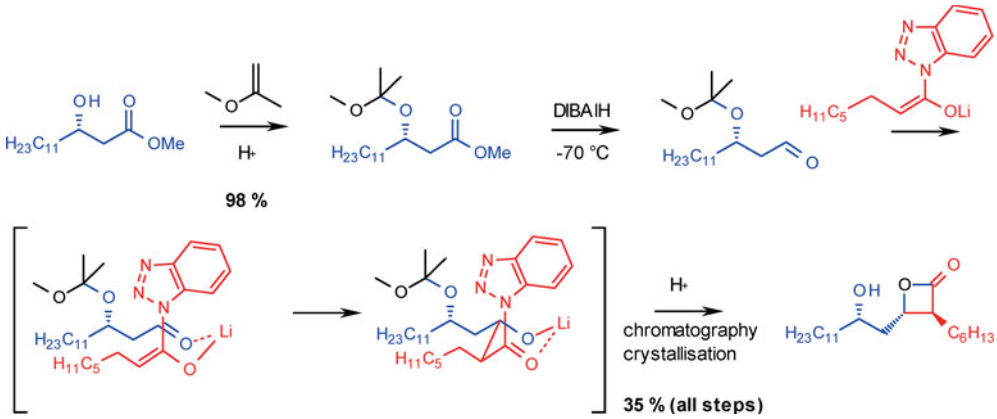
Impressively short is a total synthesis by Ian Paterson [281], who uses a lactate ester as chiral auxiliary. This is subsequently converted into a ketone *via* a Grignard reaction with its Weinreb amide. The boron-mediated aldol reaction, after an oxidative work-up, gives the aldol with a diastereoselectivity of >98 %. Since also the reaction with propionaldehyde shows the same diastereoselectiv-



ity, the steric differentiation of the sides by the benzyloxy-group in the aldehyde is of secondary importance. Finally, the lactate-derived side-chain is degraded by reduction with lithium aluminium hydride, oxidation with lead tetraacetate (to the aldehyde) [282] and with sodium chlorite ($NaClO_2$) to the carboxylic acid. The remainder of the synthesis resembles what has been described above.

Industrial Synthesis of WITEGA

Of equally striking simplicity is the synthesis, which originates from the WITEGA company. [250] The protected hydroxy-aldehyde is reacted with the amide enolate from 1-octanoylbenzotriazole and lithium hexamethyldisilazide. The preferential formation of the *anti*-aldol product can be explained by the Zimmerman-Traxler model. The (*Re*)-side of the aldehyde is attacked by the (*E*)-enolate forming a transition state in a chair-conformation. Both side-chains are in the equatorial position. The (*Si*)-side is very efficiently shielded by the 2-methoxyisopropoxy residue. The benzotriazolyl group provides not only for a high *anti*-selectivity (by comparison, for example, with the corresponding phenyl ester), but it also allows the ring-closure of the lactone in a domino-reaction upon acidic work-up.

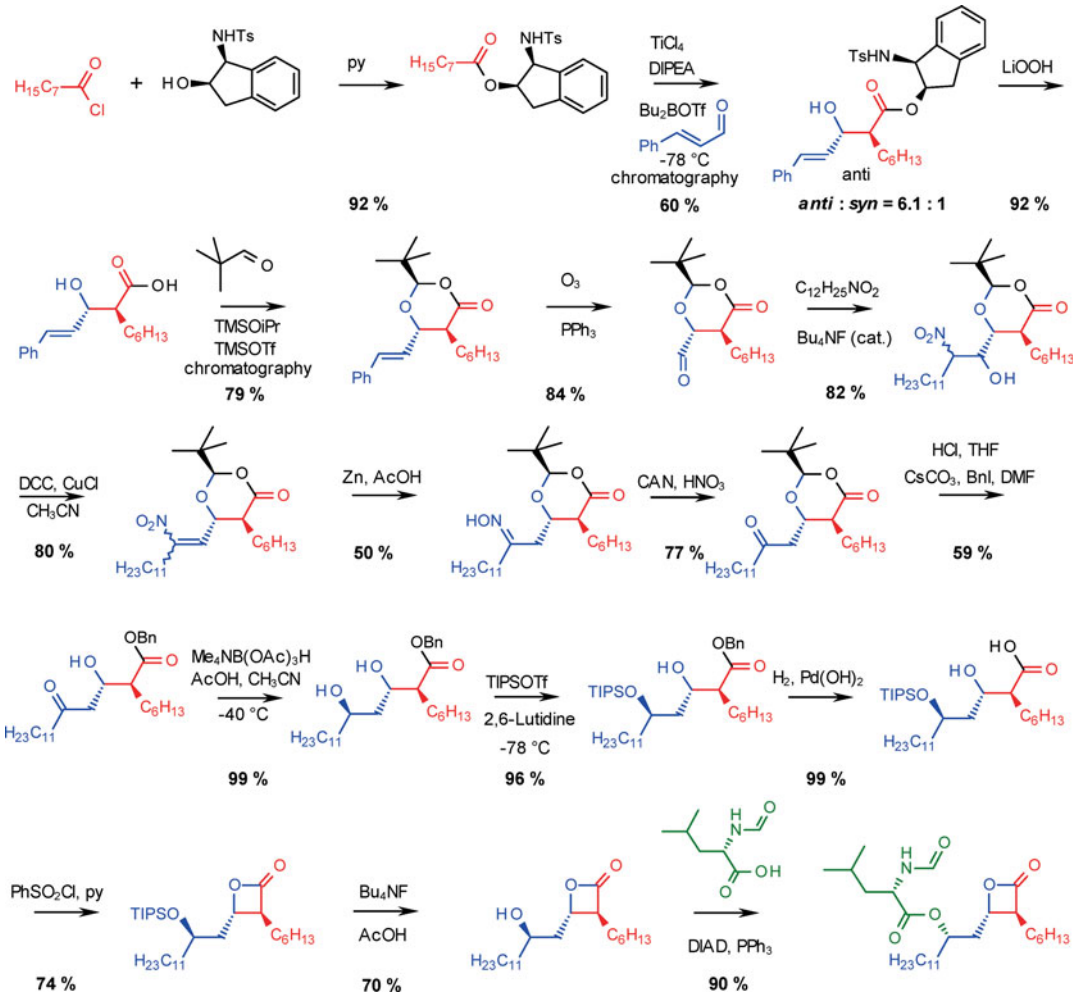


Diastereoselective Titanium Enolate *anti*-Aldol Reaction

The following synthesis contains almost everything that makes academic research nice and expensive. [283] Key steps are a titanium enolate *anti*-aldol reaction, a nitroaldol reaction and the diastereoselective reduction of a β-hydroxy-ketone.

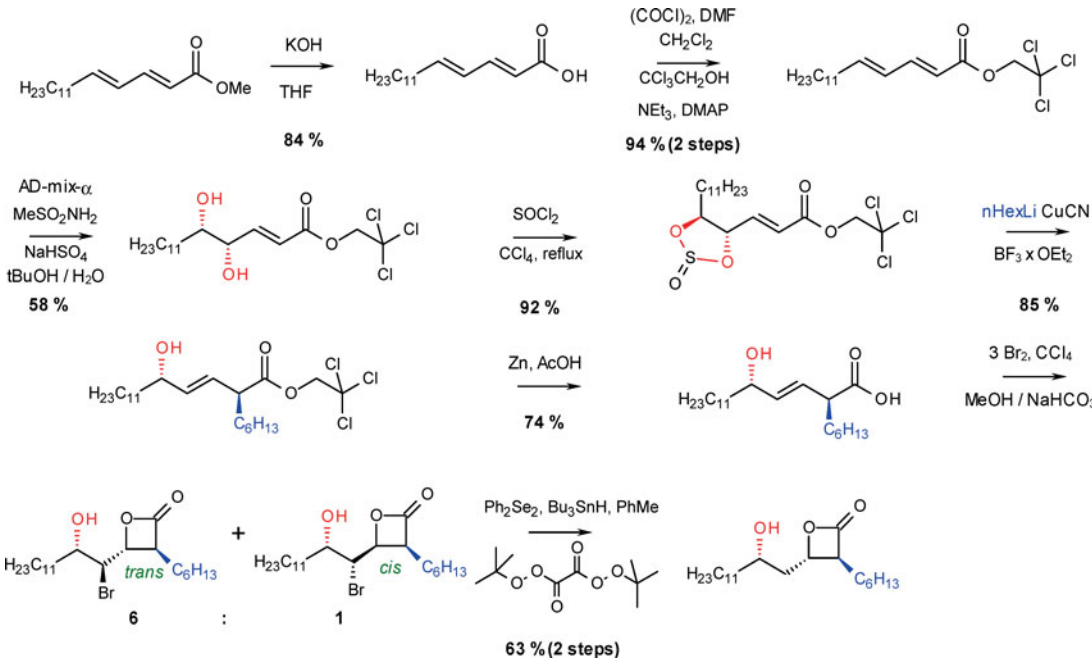
Arun Ghosh used aminoindanol as the chiral auxiliary. The titanium enolate was formed by reaction of the ester with titanium tetrachloride and diisopropylethylamine. This was reacted at –78 °C with cinnamaldehyde, pre-complexed with Bu_2BOTf. The desired *anti*-enantiomer was obtained in a 6.1 : 1 excess. The diastereomers were separated by column chromatography. The chiral auxiliary was subsequently cleaved off in a very mild procedure with lithium hydroperoxide. Attempts to protect the hydroxy-carboxylic acid in the usual way, with pivalaldehyde in presence of camphor-10-sulfonic acid or *p*-toluenesulfonic acid, failed. Eventually, isopropoxytrimethylsilane, TMSOTf and molecular

sieve were successful. [284–286] The dioxolane was obtained as an 11:1 mixture of diastereomers. Ozonolysis and reductive work-up with triphenylphosphane gave the corresponding aldehyde, which was reacted with nitrododecane in a Henry reaction. After dehydration with DCC and copper(I) chloride, a 1 : 1.7 mixture of (*E*)- and (*Z*)-isomers was obtained. The nitro-alkene was reduced with zinc to the oxime, and the latter oxidatively hydrolysed to the ketone with ceric ammonium nitrate. Hydrolysis of the acetal was followed by esterification using benzyl iodide; then the keto-group was stereoselectively reduced by Evans' method, with tetramethylammonium triacetoxyborohydride. The reduction proceeded *via* a chair-like transition state, and gave the *anti*-product. [287] Prior to ring closure, the hydroxy-group at C-5 was selectively protected. The remainder of the synthesis is the same as described above.



Diastereoselective Bromolactonisation

Diastereoselective bromolactonisation offers an attractive approach to tetrahydrolipstatin. [288, 289] The doubly unsaturated ester as starting material is obtained in a Horner-Wadsworth-Emmons reaction from dodecanal and methyl diethoxyphosphinylbutenoate. The methyl ester has to undergo transesterification first, because its hydrolysis prior to lactonisation produces only very poor yields; the trichloroethyl ester however, can be cleaved off reductively at a later stage. By means of a Sharpless dihydroxylation with AD-mix-α, two stereogenic centres are created, of which the one at C-5 possesses already the final absolute configuration. Reaction with thionyl chloride gives a cyclic sulfite. The following allylic substitution with lithium *n*-hexylcuprate is highly *anti*-stereoselective. Bromolactonisation leads under optimised conditions to a *cis*/*trans*-mixture in a 1 : 6 ratio of the bromohydrins, which are not amenable to purification by chromatography. Thus, for the subsequent radical debromination the crude mixture is used. Surprisingly, the *trans*-β-lactone is obtained exclusively: the *cis*-compound probably decomposes.

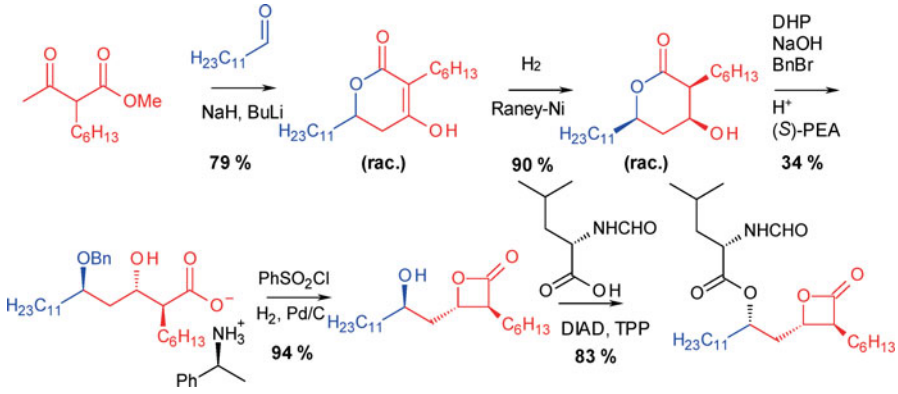


The remaining three reaction steps, namely the introduction of Z-Leu with DCC, cleavage of the protecting group and formylation with acetic formic anhydride (AcOCHO), are more or less the same as in the previously described syntheses. The overall yield in the twelve-stage synthesis lies at around 11 %.

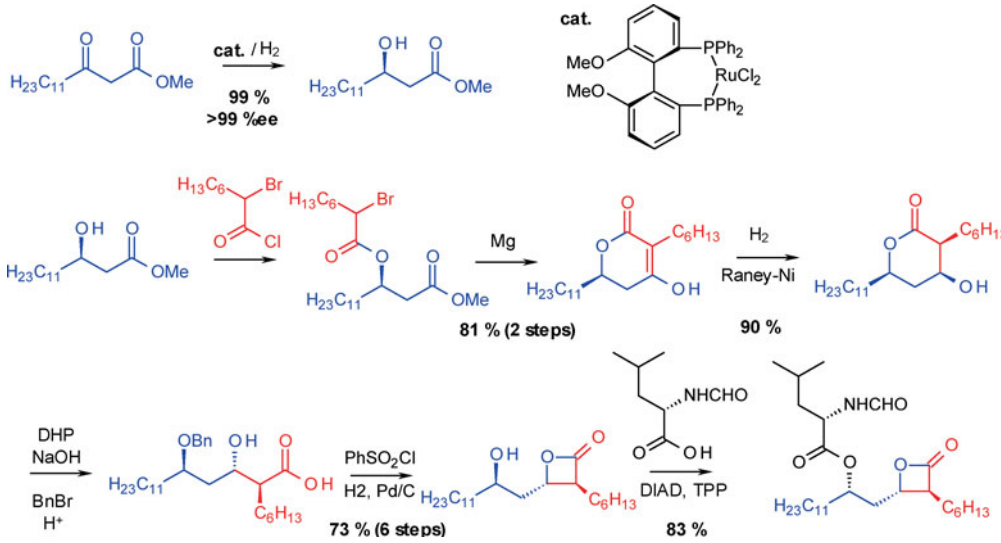
Hoffmann-La Roche's Industrial Synthesis

Concerning the current industrial synthesis evolving for tetrahydrolipstatin, Hoffmann-La Roche initially decided to follow conceptually its laboratory synthesis described above. Starting materials are methyl hexylacetoacetate and do-

decanal. The aldol reaction is followed by spontaneous cyclisation to a dihydropyrone, which is subjected to hydrogenation over Raney nickel. This leads with high selectivity to the racemic *all-syn*-tetrahydropyrone. As the next key step, the isomers are separated *via* classical resolution with (*S*)-phenylethylamine. The β-lactone ring is closed by activation with benzenesulfonyl chloride, and the benzyl group is cleaved off reductively. Tetrahydrolipstatin results from inversion of the stereogenic centre at C-5 by means of a Mitsunobu reaction. The yield over all steps lies at around 19 %. [290]



A serious disadvantage of this synthesis, however, is the loss of 50 % of the material at the racemate-resolution stage. For this reason, Hoffmann-La Roche developed a succeeding process, reflecting the experience gained from earlier syntheses. Key step is now an enantioselective hydrogenation for preparation of the β-hydroxy-tetradecanoate. Instead of a Reformatzky reaction, the intramolecular cyclisation can also be carried out using magnesium. The remainder of the synthesis is very similar to the existing industrial process, apart from the fact that a crystallisation to separate the enantiomers is no longer necessary.



As expected, compared to the previous procedure, the overall yield is doubled. Moreover, the new process can be carried out with significantly increased time-volume yield in the existing plant, requiring only slight modifications. [291]

5.7.6 Concluding Remarks

5.113 *In 2010, the production of tetrahydrolipstatin had climbed to 83.9 tons, and Xenical® reached a sales volume of 587 million Swiss francs.*

Tetrahydrolipstatin was marketed in 1998 under the name *Xenical®* (Fig. 5.113). Since then, the product has been approved in more than 20 countries. As of 2007, tetrahydrolipstatin is available "over-the-counter" (prescription-free).

Summary in Bullet Points

- Tetrahydrolipstatin is an important lifestyle-drug for the treatment of adiposity. Its action is based on the irreversible acylation of the pancreas-lipase, which is important for the resorption of fat.
- The preparation of lipstatin by fermentation with an economically acceptable time-volume yield has not been successful.
- For the commercial drug, tetrahydrolipstatin is chosen over the natural product, due to stability and ease of purification reasons.
- The sequence of listed drug syntheses reflects the virtual evolution of a manufacturing process. In industrial practice, it is a matter of selecting the most viable of the syntheses available at a specified point in time, although even better syntheses are often found at a later date!

5.8 *Taxol®*

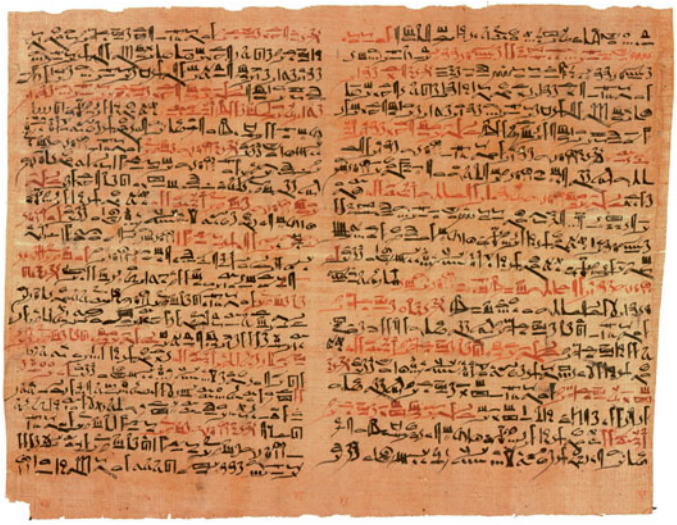
Cancerous diseases are not a novel phenomenon of our days, but have accompanied humankind since prehistoric times. [292] Thus, pathological bone lesions, caused by a Burkitt lymphoma, have been found in the jawbone of an *Australopithecus* from Kenya, and, due to an osteosarcoma, in the thigh-bone of a *Homo erectus* from Java. The deformed upper arm bone (humerus) from the tomb of a warrior at Münsingen in Switzerland, dating back to the late Iron Age (500 BC) is regarded as one of the earliest fossil evidence for malignant bone tumours in *Homo sapiens*. [293] In mummies from Egypt, China and Peru, not only bone cancers have been detected, but also mummified tissue malignancies.

5.8.1 Historical Facts about Cancer

The first written indication of malignant diseases can be found in the old Egyptian "Kahun Papyrus" as well as the "Ebers Papyrus", where cancers of the uterus, breast, bladder, and also Kaposi-sarcoma are mentioned. In the "Papyrus of Edwin Smith", even surgical techniques for cancer are described (Fig. 5.114). Already at that time, it had been recognised that surgical intervention has to

remove the surrounding healthy tissue as well. The first references to chemotherapy are found in old Indian as well as old Chinese medicine. Pastes based on arsenic and mercury were used to spread on the tumour.

Kaposi sarcoma is a form of skin cancer, which nowadays is frequently associated with AIDS. It was described for the first time in 1872 by Moritz Kaposi (1837–1902), a dermatologist from Vienna.



5.114 *The Edwin Smith papyrus is an ancient Egyptian medical text, which belongs among the earliest written documents on medical healing procedures. It was discovered in Thebes and purchased by Smith in Luxor from an Egyptian dealer named Mustafa Agha in 1862.*

During Greek and Roman Antiquity, physicians also devoted attention to the origin and treatment of cancer. The first descriptions of facial and stomach cancer are found in the "Aphorisms" and "On Ancient Medicine" by Hippocrates of Cos (460–370 BC). In other parts of the *Corpus Hippocraticum* he writes about the blood supply *via* large vessels surrounding breast cancer tissue, which were reminiscent of the legs and pincers of crabs. Such malignant, non-ulcer-forming tumours he called *"karkínos"* (Greek: crab). Hippocrates was also in the position to differentiate between benign and malignant tumours.

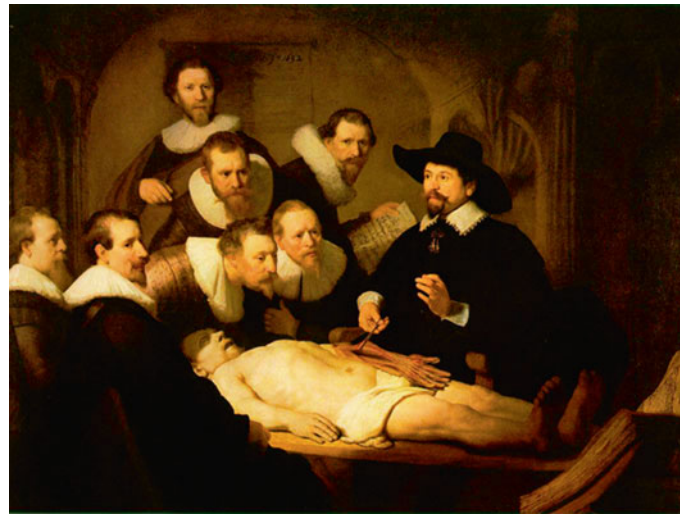
Claudius Galenus (also known as Galen of Pergamon, 129–216 AD) already propagated the surgical removal of breast carcinomas. He described 60 various types of cancer, termed a tumour lump "oncos" (Greek word for swelling), and thus became the founder of oncology.

Cancer research made significant progress with the Arab (Moorish) physicians from Seville and Córdoba, Ibn Zuhr (Avenzoar, 1091–1161) and his student Ibn Rushd (Averroës, 1126–1198), who described cancer not only of the stomach but also of the oesophagus (Fig. 5.115). With the aid of a feeding tube, he succeeded to provide his patients food.

The basic understanding of cancerous lesions goes back to the French physician and founder of histology, Marie-Francois-Xavier Bichat (1771–1802), who, without having a microscope at his disposal, recognised cancer as a pathological overgrowth of tissue. The German physiologist Johannes Peter Müller (1801–1858) established, with the aid of a microscope, that cancerous tissue, like healthy tissue, consists of cells; and Rudolf Virchow (1821–1902) at the Charité in Berlin formulated with "omnis cellula e cellula" [294] the concept that all cancerous growth originates from a body cell.

5.115 *Ibn Rushd (Averroës, born 1126 in Córdoba; died 10th December 1198 in Marrakesh) was a Spanish-Arabian philosopher, physician and mystic. He wrote a medical encyclopaedia (Kulliyat) and commented extensively on the work of Aristotle.*

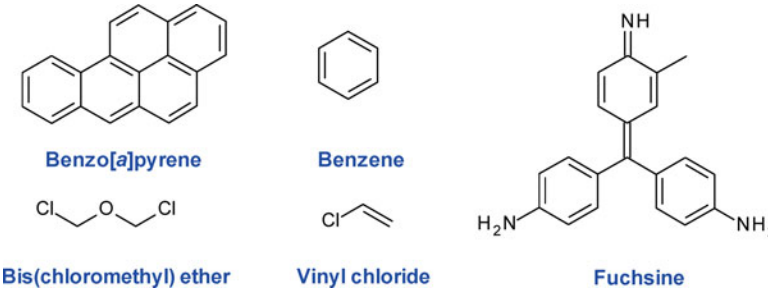
While the Dutch surgeon Nicolaes Tulp (1593–1674) (Fig. 5.116) believed that cancer was a contagious disease, the London physician Sir Persivall Pott (1714–1788) established for the first time in 1775 a link between the soot-blackened clothing of young chimney sweeps and the later appearance of cancer of the scrotum, penis and groin. [295] It became clear only much later that soot and coal dust contain benzo[*a*]pyrene (benzo[*def*]chrysene), which is responsible also for lung cancer among miners and smokers (Fig. 5.117).



5.116 *Nicolaes Tulp (1593–1674) in the painting "The Anatomy of Dr. Tulp" by the Dutch artist Rembrandt van Rijn (1606–1669).*

It was realised in 1894, that electromagnetic radiation can trigger the onset of cancer, as for example the skin cancers of seafarers by UV light, and then later those of hospital staff, who were exposed to the radiation from newly developed X-ray machines.

With the emergence of the dyestuffs industry, it was observed that fuchsine caused bladder cancer among the workers. In the first half of the last century, an increased incidence of lung cancer was noted, amongst those, who were exposed over an extended period to chromate, nickel and asbestos (a collective term for various naturally occurring fibre-shaped silicates). In the 1970s, halogenated ethers and vinyl chloride became conspicuous as potent carcinogenic substances (Fig. 5.117).



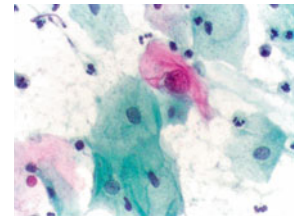
5.117 *Carcinogenic substances from various areas of application, like solvents, intermediate products, monomers, dye stuffs and combustion products.*

The induction of cancers by viruses was first described in 1908 by the Danish pathologists Vilhelm Ellermann and Oluf Bang, who succeeded in transmitting chicken leukosis to other chickens *via* cell-free filtrates. Thereby the first proof was established of an infectious type of cancer.

In 1910, the American pathologist Francis Peyton Rous (1879–1970), at the Rockefeller Institute for Medical Research in New York, succeeded in the transmission of the virus later named after him (the Rous sarcoma virus) from one diseased chicken to other chickens. For this he was awarded the Nobel Prize in Physiology or Medicine in 1966.

Nowadays, it is estimated that approximately 20 % of all cancers worldwide are associated with infections. First and foremost, a number of different viruses are of importance: like the Epstein-Barr virus (Burkitt lymphoma, naso-pharyngeal cancer), the human herpes virus type 8, the human papilloma virus, the hepatitis-B- and -C-viruses, and above all, the human immunodeficiency virus (Kaposi sarcoma). The bacterium *Helicobacter pylori* is the leading contributor to stomach cancer. Parasites, like the flatworm *Schistosoma haematobium* (the cause of bilharzia or snail fever) lead in Egypt to an increased incidence of bladder cancer, and types of liver flukes (*Opisthorchis viverrini*, *Clonorchis sinensis*) are important risk factors, particularly in south-east Thailand and in southern China, for the appearance of Cholangio carcinoma and hepatocellular carcinoma. [296]

The Pap-test [297], developed by the Greek physician George Nicolas Papanicolaou (1883–1962) for the early screening of cervical cancer, constituted an important milestone in cancer research. This test assesses under a microscope the stained cells of a smear sample taken from the surface of the cervical canal (Fig. 5.118).



5.118 *The Pap-test, Papanicolaou staining, 400x. Among normal cells, an atypical one can also be seen.*

Methods of Treatment

With the discovery in 1934 of artificial radionuclides by Frédéric Joliot (1900–1958) and Iréne Curie (1897–1956) at the *Institut du Radium* in Paris, the path to radiotherapy was paved as a second effective treatment option for cancer, other than surgical procedures. A prominent example is the irradiation of thyroid carcinoma with iodine-131.

Already during the First World War physicians had determined that the poison gas, bis-(2-chloroethyl)-sulfide (also called "sulfur mustard") has antiproliferative (growth-inhibiting) properties. After the war, there was developed for therapeutic use in lymphoma patients the less toxic "nitrogen mustard", *N,N*-bis-(2-chloroethyl)methylamine, which had undergone experimental studies at Yale University as classified trials in 1942. Together with the accidental discovery of the *Vinca* alkaloids in Madagascar periwinkle (especially *Vinca rosea*) [298] in 1952, which were known from folk medicine in Jamaica as a remedy for diabetes, and the discovery of platinum complexes [299] (*e.g.cis*-$Pt(NH_3)_2Cl_2$), which were found in 1965 through experiments looking at the effect of electrical current on cell cultures and using a platinum electrode, the foundation for chemotherapy was laid as the third important variant of cancer treatment.

Another critical advancement in the battle of cancer resulted from the work of the Heidelberg physician Harald zur Hausen (laureate of the 2008 Nobel Prize

5.119 *Harald zur Hausen (Nobel Prize for Physiology or Medicine in 2008).*

for Physiology or Medicine) on cervical cancer induced by Papilloma virus, and the development of a vaccine against this type of cancer (Fig. 5.119).

5.8.2 Biological Fundamentals

Out of a fertilised egg cell (zygote), complex organisms, comprising many billions of cells, are developed through cell division and cell differentiation. In the case of humans, these cells number 10^{13}. Important for the maintenance of life is cellular proliferation by cell division, a process that is highly regulated at the different stages of the cell cycle. This is where the balance is kept between cellular growth and cell death; cells that are no longer needed can be disposed *via* apoptosis (programmed cell death). Dysregulation of the cell cycle leads to hyper- or hypoproliferative diseases.

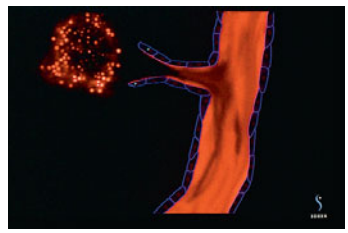
Cancer cells evade these regulatory mechanisms through genetic mutations. Functional loss of a tumour-suppressing gene (antioncogene), leads *e.g.* to an inefficient DNA-repair mechanism, or to a blockade of driving cells into apoptosis. The best-investigated human tumour suppressor is p53 (gene product of TP53, located on chromosome 17), which is mutated in 50 % or more of all colon, breast and lung cancers. In the course of cancer formation (oncogenesis), proto-oncogens, which are normal genes regulating cell growth and differentiation, become transformed by mutation into oncogenes (cancer genes). These encode atypical growth factors, receptors for growth factors or intracellular molecules for signal transduction. The consequence is continuous growth stimulation and uncontrolled cell proliferation. Cancer cells gain virtually immortal growth through an activated enzyme system (telomerase), which counteracts the normal shortening with each cell division of a genetic sequence (telomere) on the chromosome ends. By extending the telomeres again, the normal ageing process, steering cells ultimately into senescence and death, is abolished.

In the course of tissue augmentation (hyperplasia), the tumour outgrows into the surrounding tissue. To grow further and satisfy the increased demand for nutrients, the tumour requires the formation of new blood vessels (angiogenesis) (Fig. 5.120). [300] Eventually, cancer cells are released into the blood or lymph system and disseminated into other organs to establish secondary tumours (metastases).

The growth of a single cancer cell into a fully-developed tumour can be disrupted by cytostatics at a number of development stages. Important intervention points, each targeted by one or several classes of cytostatics, are the replication of DNA (alkylating agents, platinum complexes), cell division (antitubulin drugs) and angiogenesis (signal transduction inhibitors). [301]

While during the interphase of the cell cycle, DNA is replicated, in the mitotic phase (M-phase) the nuclear chromosomes are separated into two identical sets. The latter step lasts in humans for around one hour.

On 1st February 1951 Henrietta Lacks, who was suffering from cervical cancer, had – without her consent – samples of her cervix removed at the Johns Hopkins Hospital in Baltimore. She deceased on 4th October of the same year. A subset of her malignant cells was characterised and found to divide beyond the normal limit. From a single of these cells, researchers were able to establish the first immortal human cell line, which they labelled HeLa-cells, in memory of the donor. From 1952 right up to the present time, this cell line is used worldwide for research purposes, as *e.g.* for the development of the polio vaccine by Jonas Salk at the University of Pittsburgh School of Medicine, which underwent clinical trials already in 1954.



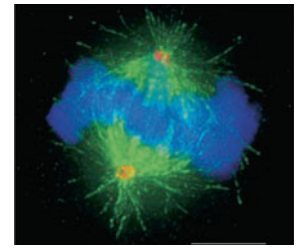
5.120 *A newly forming blood vessel is "attracted" by a tumour (left).*

Essential for cell division is the formation of the spindle apparatus (Fig. 5.121). [302] This consists of axonemes (Fig. 5.122), which are part of the cytoskeleton, and which are, along with some other types of filaments, responsible for the mechanical stabilisation of the cell and its outer shape, for active movement of the cell as a whole, as well as for movements and transport within the cell.

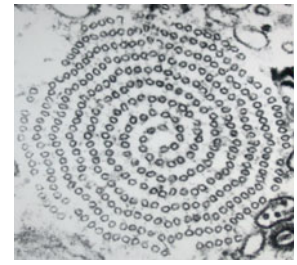
The building blocks of the axoneme are microtubules, which are tube-shaped protein filaments (Fig. 5.123 and Fig. 5.124). They consist of helically arranged, spherical proteins, α- and β-tubulin, each of *ca.* 450 amino acids (*ca.* 50 kDa) in size. Both form a heterodimer, to which two molecules of guanosine triphosphate (GTP) are bonded (Fig. 5.125). One GTP is tightly bonded and can be removed only by denaturation of the heterodimer; the second GTP can easily be replaced, and is involved in the "dynamic instability" of the microtubule, which is explained further down.

In the presence of additional GTP, the heterodimers form long protein fibres by head-to-tail coupling. After an induction phase, typically of a few minutes, 12 or 13 protofilaments line up side by side and form a C-shaped sheet. This finally closes to a tube, the microtubule, with an outer diameter of 24 nm and an inner diameter of 15 nm. Microtubule-associated proteins (MAPs) protect the microtubule from depolymerisation.

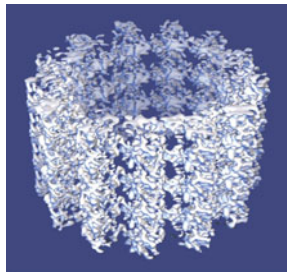
Microtubules are not static constructs. They exist in an equilibrium, by which heterodimers add permanently to one end (the "plus-end") and are shed from the other (the "minus-end"). In the heterodimer, the α-tubulin bonded GTP at the interface of the dimer is enclosed by a loop of β-tubulin and thus protected from hydrolysis. The GTP attached to β-tubulin is however hydrolysed to GDP shortly after the addition of another heterodimer. This destabilises the microtubules, and causes a more facile depolymerisation of the microtubules from the minus-end in the direction of the plus-end. Both processes (polymerisation and depolymerisation) occur in the cell simultaneously, and



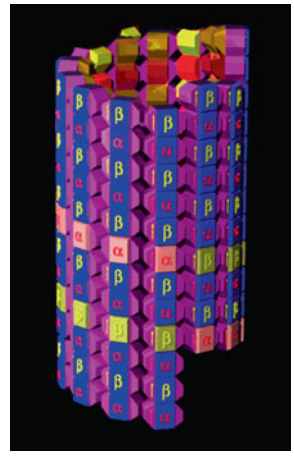
5.121 *Immunofluorescence-microscope photograph of a dividing human cell. The chromosomes are coloured blue and the spindle apparatus is in green.*



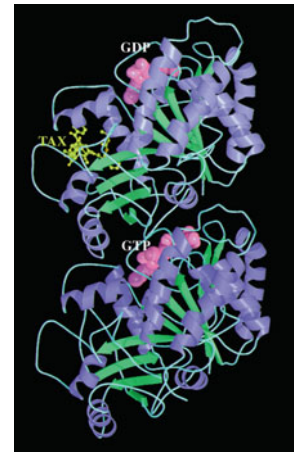
5.122 *Cross-section through an axoneme (axial filament). As substructures, two spiral-shaped rows of microtubules are visible. Transmission electron microscope photograph, 65,000 times enlarged.*



5.123 *Cryo-electron microscopic 3D-reconstruction of an intact microtubule (Resolution: ca. 0.8 nm).*



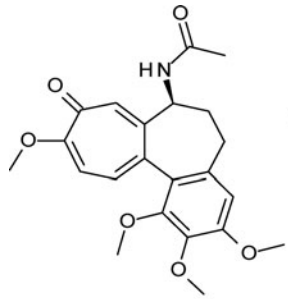
5.124 *Model of the structure of a microtubule built from α, β-tubulin dimers.*



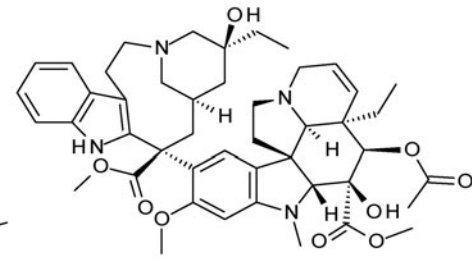
5.125 *Taxane-binding sites of β-tubulin (view from the interior of the microtubule).*

thus affect the dynamic instability of the microtubule network. The fine balance between growth and shrinking, and therefore the control of the length of the microtubules, is a fundamental prerequisite for the vital processes in the cell. In case the dynamic equilibrium of the microtubules is imbalanced, cancer cells are prevented from mitotic division. This makes microtubules a favourable target for cancer therapy.

At the end of the 1930s, with the alkaloid colchicine [303] from the autumn crocus (*Colchicum autumnale*, also known as the meadow saffron or naked lady) the first natural product was discovered, which attacks the spindle apparatus. Whereas colchicine and the *Vinca* alkaloids interfere with the polymerisation of tubulins, taxanes and epothilone inhibit their depolymerisation.



Colchicine



Vinblastine

In this context, a problem is, that healthy cells, which divide at a relatively high rate (show a high mitotic index), like those in skin, hair, intestines, as well as cells of the immune and reproductive systems, are likewise harmed, leading to serious side-effects, like hair loss, intestinal bleeding and increased susceptibility to infections.

5.8.3 Discovery of Paclitaxel

Excursus

Yews are very slow-growing evergreen bushes or small- to medium-sized trees. [304] They can live for more than 1,000 years and have among needle-bearing trees a very rare property, namely that they can start to bud from the trunk. The flaky bark is reddish-brown. The needles are arranged in double rows relative to the branches. The red fruit wraps around the seed in the form of a cup. Yews are mostly dioecious (the male and female flowers are carried on separate plants). Their wood was already used by our ancestors and relatives of *Homo sapiens* for making hunting weapons. Thus, in Lehringen (Lower Saxony, Germany) there was found the skeleton of a straight-tusked elephant (*Palaeoloxodon antiquus*) with a lance in his thorax, 2.38 m in length and made of yew; this was attributed to Middle Palaeolithic Neanderthal Man. Homer describes in the Iliad the use of bows made from yew. It was reckoned in the Middle Ages as the best wood for that purpose, because of its hardness and elasticity. By using

yew bows, the forces of William the Conqueror had a decisive advantage at the battle of Hastings in 1066. During the late Middle Ages, every merchant ship, which wanted to carry on commerce with England, had to bring along a certain number of workpieces from yew wood for the construction of longbows. By the late 16th century, yew wood was in such high demand, that in northern Europe the European Yew was almost extinct. Between 1531 and 1590 alone, around 500,000 yew bows were exported from Nuremberg and Bamberg, *via* Cologne, to the West. In addition, there came the local demand for wood-turning, wood-carving, and construction wood for boats and carts. Also for manufacturing lutes, the highly elastic yew was the wood of choice for its teardrop-shaped sound board. Nowadays, this tree is a protected species in Germany, as well in parts of the UK, and its wood is relatively expensive.

5.126 *Memorial to King Ambiorix in Tongeren (a town in what is now Belgium). The Eburones lived 54 BC in a dual kingship under the rule (sub imperio) of Ambiorix and Catuvolcus. The Eburones were a Celtic tribe, whose territory lay between the rivers Rhine and Maas, the Rhineland, the northern Ardennes and the Eifel.*

The history of taxol begins with a murder in ancient times. According to Greek mythology, the goddess of hunting, Artemis, used poisoned arrows made from yew to murder the seven daughters of Niobe, who had boasted of her 14 children. [305] The Celts poisoned their arrow tips with a decoction of yew needles. Julius Caesar reported in his "Gallic Wars" that Catuvolcus, King of the Eburones, committed suicide in 53 BC with taxus, after his rebellion against the Romans had been quelled (Fig. 5.126 and Fig. 5.127). [306]

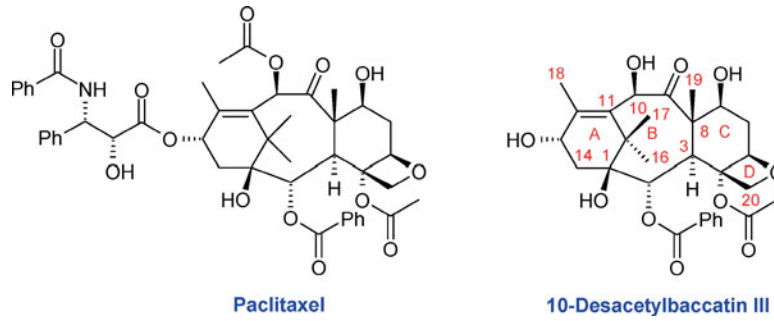
Dioscurides writes in his pharmacology teachings (*De Materia Medica*) that yews in Narbonia (the present-day *arrondissement* of Narbonne) are so poisonous that people even resting in their shade come to harm and may often die. In the Middle Ages, yew extracts were used for the treatment of epilepsy,

5.127 *The European yew (Taxus baccata) (left) and the Pacific yew (Taxus brevifolia). The Pacific yew is a slow-growing conifer, rarely more than 12 m high, which grows in the coastal regions of the Pacific, from Annette Island in Alaska to Montecino County in California, as well as in the Cascade mountain range in Washington and Oregon, on the western slopes of the Rockies and in the Lewis mountain range in Montana. Before the discovery of paclitaxel, the tree had no commercial use.*

The timber, bark, needles and seeds of the yew contain toxic compounds, which are called taxanes. In the past, farmers in particular had been eager to clear the land from yews, since their toxic ingredients had poisoned livestock again and again. And the chronicles of the Père Lachaise cemetery in Paris report that the hearse horses fell victim to the yews, which grew in the cemetery, because during the burials they repeatedly fed on the branches of these plants.
The red and sweetish aril is however non-poisonous. It may be preserved as a jam, so long as the poisonous seeds are removed carefully.

diphtheria, rheumatism, skin rashes and scabies. The decoction from yew needles was also used as an abortifacient.

At the beginning of the 1960s, the National Cancer Institute in the USA began an extensive programme to identify natural products with antineoplastic properties. [307, 308] As part of this initiative, Arthur Barclay, a botanist at the United States Forest Service, collected bark of a Pacific yew (*Taxus brevifolia* Nutt) (Fig. 5.127) in the Gifford Pinchot National Forest, on a hillside 7 miles north of Packwood, Washington. Monroe Wall and Mansukh C. Wani, chemists at the Research Triangle Institute in North Carolina, discovered that extracts from the bark showed cytotoxic properties against leukaemia cells and a range of solid tumours. They succeeded in isolating the active principle and, together with Andrew McPhail at Duke University in Durham, North Carolina, in elucidating its structure by X-ray crystallography. They named the compound paclitaxel (Fig. 5.128). Out of more than 110,000 compounds from 35,000 plant species, which were tested within the scope of this programme between 1960 and 1981, paclitaxel appeared as the most interesting. This was based largely on the observation Susan Horwitz of the Albert Einstein College of Medicine in New York made in 1979, that paclitaxel inhibits the depolymerisation of microtubules.



5.128 *The highly functionalised, tetracyclic 10-desacetylbaccatin III was likewise found in the Pacific yew, and proved later to be the key intermediate for partial syntheses.*

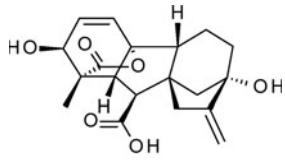
Phase I clinical studies began in 1983. Problematic from the beginning, however, was the supply of paclitaxel. A 100-year old yew has around 3 kg of bark, out of which 300 mg of paclitaxel can be isolated. Bristol-Myers-Squibb (BMS) patented a process for the extraction of paclitaxel from the yew bark. In 1992, there were harvested just 1,400 tonnes of bark, from which 139 kg of paclitaxel could be isolated. A year later already, paclitaxel came on to the market under the trade name *Taxol*®.

It was foreseeable that with growing demand, the total stock of Pacific yews in the USA would be wiped out in fewer than ten years. This is why a desperate search for alternative drug sources was initiated.

5.8.4 Paclitaxel from Microorganisms

A theoretical possibility for meeting the supply bottleneck existed in producing paclitaxel by fermentation. However, up to the beginning of the 1990s, no bac-

teria or fungi had been known, which produced paclitaxel. In their search for such microorganisms, Andrea and Donald Stierle together with Gary Strobel from the University of Montana were guided by a discovery the Japanese plant pathologist Teijiro Yabuta (1888–1977) had made: He found already in 1935 that the phytopathogenic fungus *Gibberella fujikuroi* (*Fusarium moniliforme*), which is responsible for a disease of the rice seedlings, produces gibberellins (Fig. 5.129). [309] This is nothing unusual in itself, if gibberellic acid – a highly functionalised diterpene like paclitaxel – were not also a ubiquitously occurring phytohormone in higher plants, where it is produced along the same biosynthetic pathway as in *Gibberella fujikuroi,* starting from geranylgeranyl diphosphate.



Gibberellic acid

5.129 *Gibberellins are phytohormones, which regulate plant growth.*

It is really surprising that two taxa, phylogenetically so remote from each other like fungi and higher plants, should produce secondary metabolites by the same biosynthetic route. A plausible explanation for this is horizontal gene transfer. The microorganism, which developed the biosynthetic pathway, has the corresponding genes generally located in one gene cluster, so that it can be transferred in a single transition to the plant. This is in contrast with higher organisms, like plants, where such genetic information is scattered throughout the entire genome. The reverse direction of transmission is therefore very unlikely, because many horizontal gene transfers would be necessary for the complete transmission of all required genes. In any case, such gene transfers are possible only between organisms, which live in close association.

If the search for paclitaxel-producing microorganisms were successful, then there was a high probability of finding these in the yew itself. In 1993, Stierle and Strobel actually discovered in the inner bark, the phloem, a fungus of a Pacific yew from Montana, which had not been described previously and which synthesised paclitaxel [310, 311], this fungus they named *Taxomyces andreanae*. By ^{14}C-labelling experiments it could be confirmed that the isolated plant cells, like those of the fungus, produce paclitaxel independently of one another, and that cross-contamination could be excluded.

In 2000 and 2001, other paclitaxel producing fungi were discovered in the phloem of the European yew and the Himalayan yew (*Taxus wallichiana*), such as *Sporormia minima*, *Trichothecium sp.* and *Pestalotiopsis microspora*. Korean scientists showed, that a fungal isolate from the forest floor under a stand of yews likewise produces paclitaxel. Finally, Italian researchers found with a *Kitasatospora* strain the first bacterium, which had the capability of synthesising paclitaxel.

Unfortunately, the concentration of paclitaxel in every case lies at best in the range of a few micrograms per litre of fermentation broth, and could not be

significantly improved until now by optimisation of the fermentation conditions – contrary to penicillin, as an example. Thus, the production of paclitaxel from microorganisms still appears not attractive from a commercial viewpoint.

More recent research in the USA and Singapore with genetically modified microorganisms suggests so far, that only early-stage precursors of paclitaxel can be biosynthetically produced. [312]

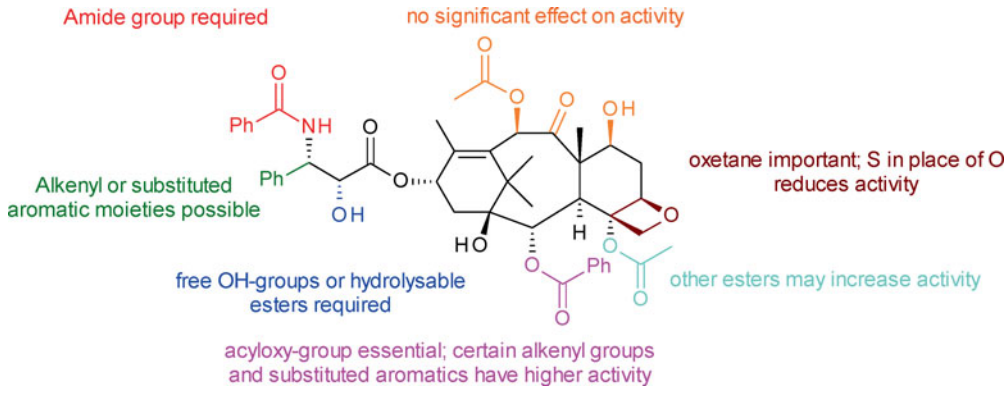
5.8.5 Total Synthesis

In general, this is a favourable opportunity for total synthesis. In case of paclitaxel, many research groups around the globe were engaged in this topic. The first successful synthesis originates from Robert Holton. [313, 314] Almost simultaneously, Kyriacos Costa ("K. C.") Nicolaou [315, 316] published a total synthesis, and a little later Samuel Danishefsky as well. [317, 318] Additional total syntheses were published by Wender, Kuwajima and Mukaiyama. [319]

However, it became clear very quickly that none of these syntheses would have a chance of being used at an industrial scale for reasons of technical feasibility and overall yield. Holton's total synthesis of paclitaxel, for example, comprised around 40 preparative stages, and the overall yield was clearly below 2 %.

5.8.6 The Paclitaxel Pharmacophore

Nevertheless, the work on the total synthesis of paclitaxel made extraordinarily valuable contributions to the understanding of the structure-activity relationships. The synthesis of many hundred analogues and the associated activity studies gave a comprehensive picture of the paclitaxel pharmacophore (Fig. 5.130). [319]



5.130 *Structure-activity relationships of paclitaxel.*

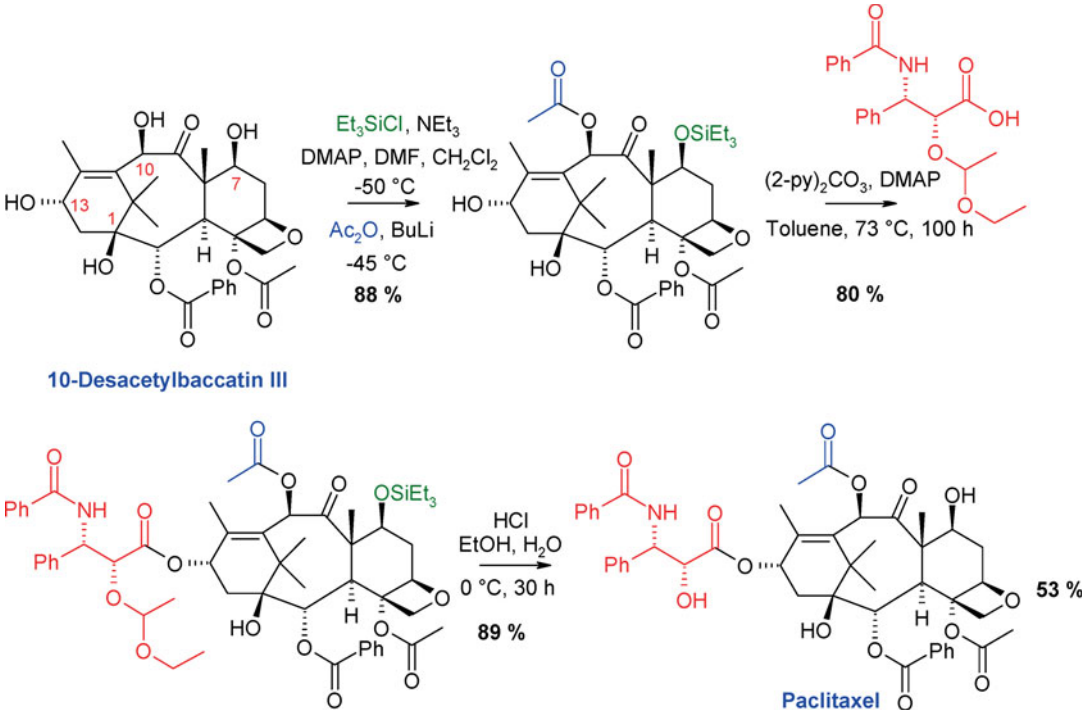
5.8.7 Manufacturing of Paclitaxel

Partial Synthesis

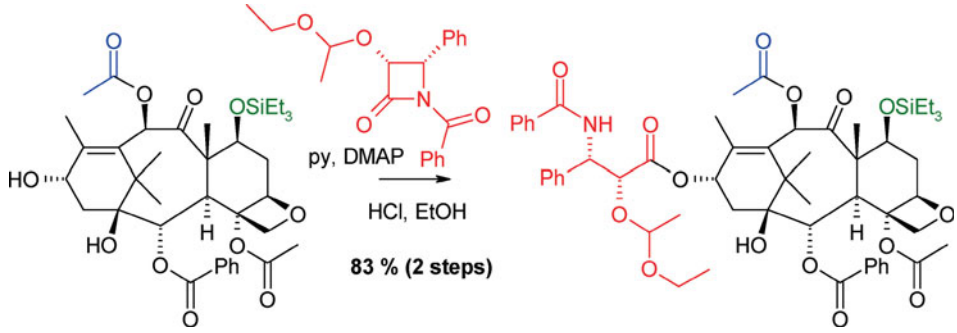
A first way out of the supply bottleneck was offered by partial synthesis, after it was discovered, that approximately 1 kg of 10-desacetylbaccatin III was extractable with ethanol from three tonnes of needles from the European yew (*Taxus baccata*), and can be purified by chromatography. [320, 321] In 1988, starting from the needles of numerous European yews growing on the university campus at Gif-sur-Yvette, near Paris, Pierre Potier and Andrew Greene succeeded in carrying out the first partial synthesis of paclitaxel. [322, 323]

The basic problem in the synthesis lies in differentiating between the alcohol functions at C-7, C-10, C-13 and C-1, the reactivity of which decreases in that same order. For the introduction of the side-chain at C-13, the hydroxy-groups at C-7 and C-10 have to be protected. Further, it is necessary to consider that under weakly basic conditions C-7 has a tendency to undergo a retro-aldol reaction.

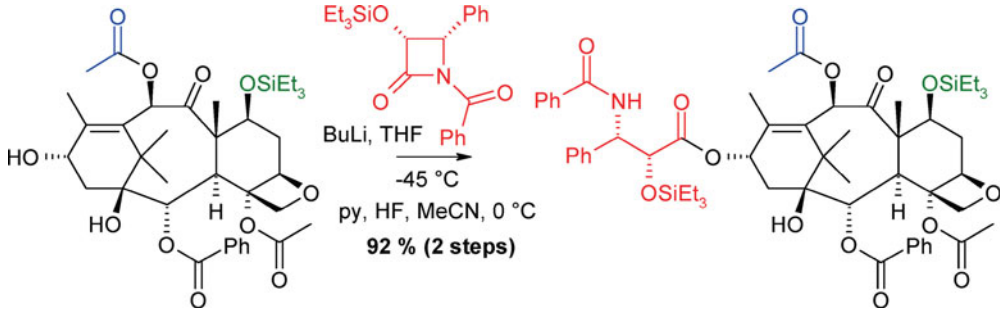
Following silylation of the alcohol group at C-7 under optimised conditions, it is possible to acetylate [324] regioselectively the alcohol at C-10, and to introduce the side-chain required for paclitaxel at C-13. The drastic reaction conditions and long reaction times are due to the steric hindrance of the hydroxy-group at C-13.



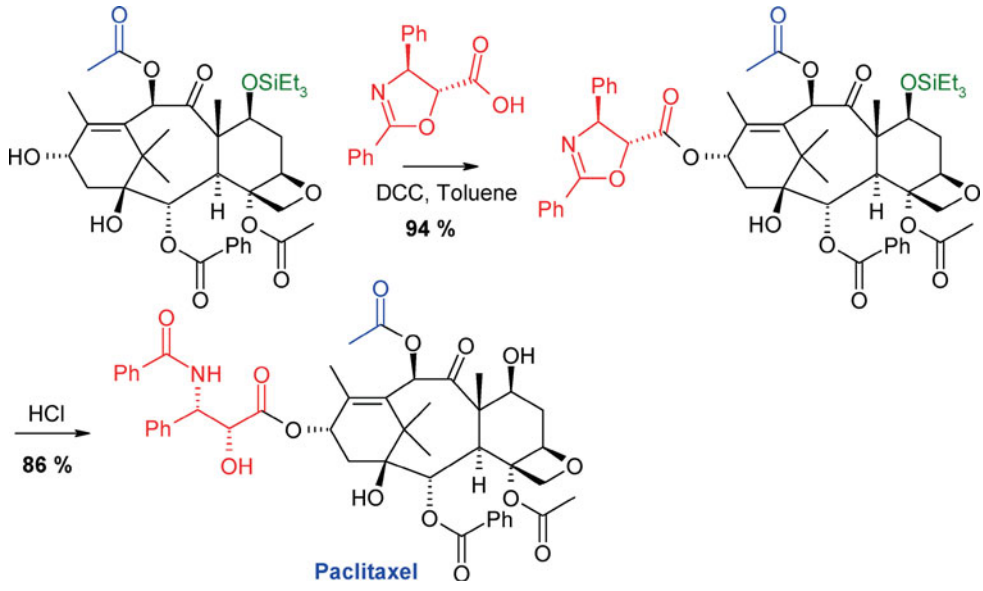
Robert Holton at Florida State University was able to react the baccatin III derivative in a similar way with an enantiomerically pure β-lactam. The overall yield across the comparable stages was around 12 % better. [325]



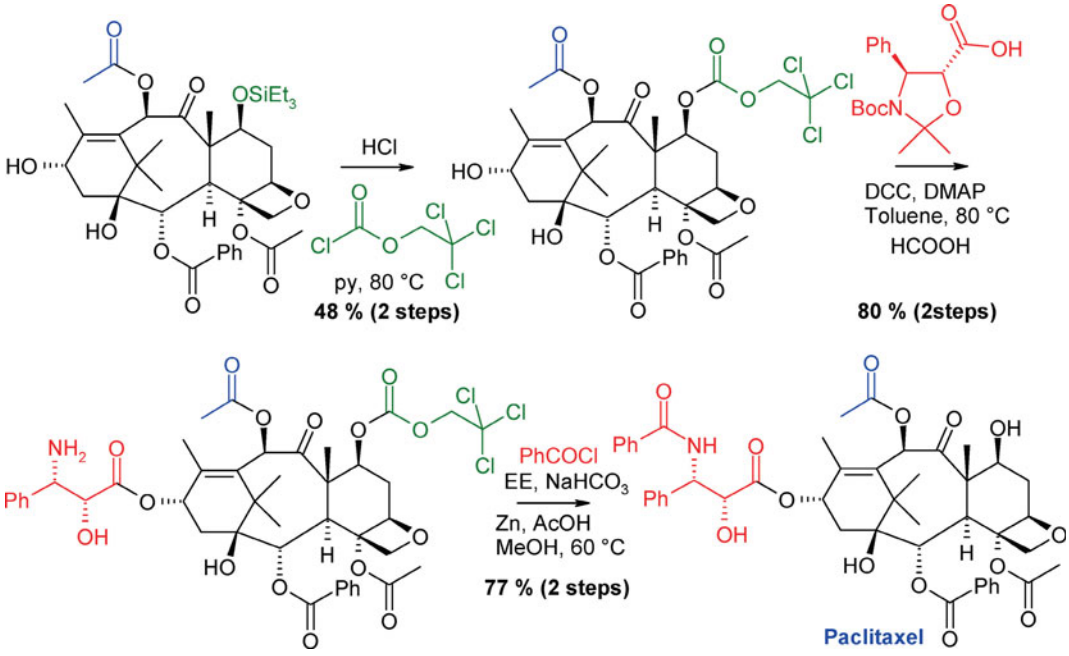
By the so-called metal alkoxide paclitaxel synthesis, the yield *via* these stages was increased to 92 %. [326] BMS obtained a licence and started producing paclitaxel along this route at their plant in Swords, Ireland.



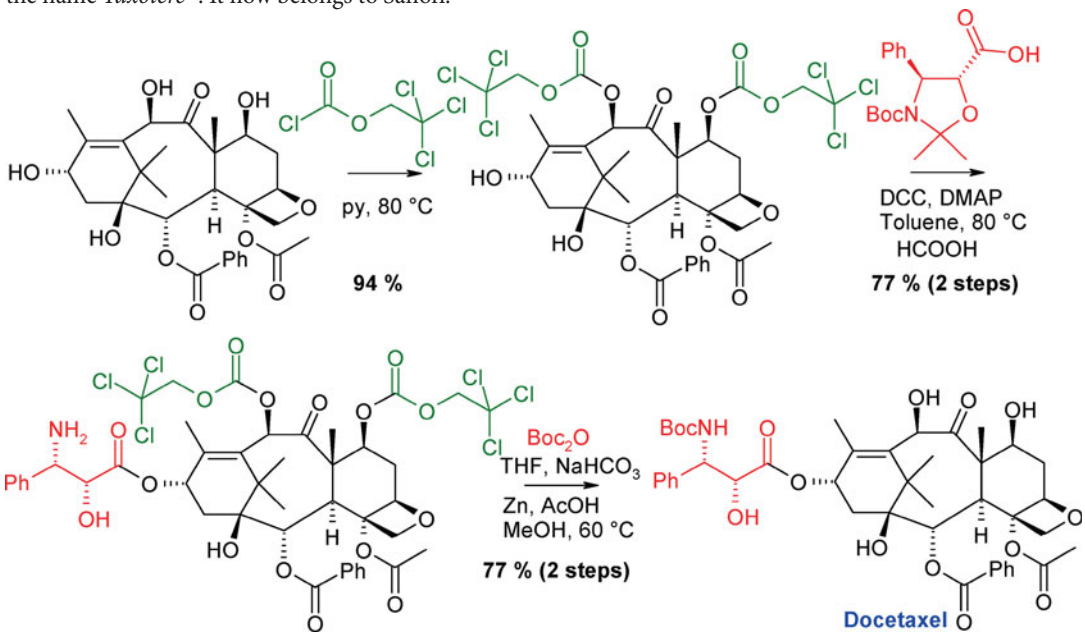
The use of an appropriately substituted oxazoline as a building block for the side-chain is particularly attractive, because the protecting group for the amine and alcohol functions is subsequently an integral part of the drug structure. [324]



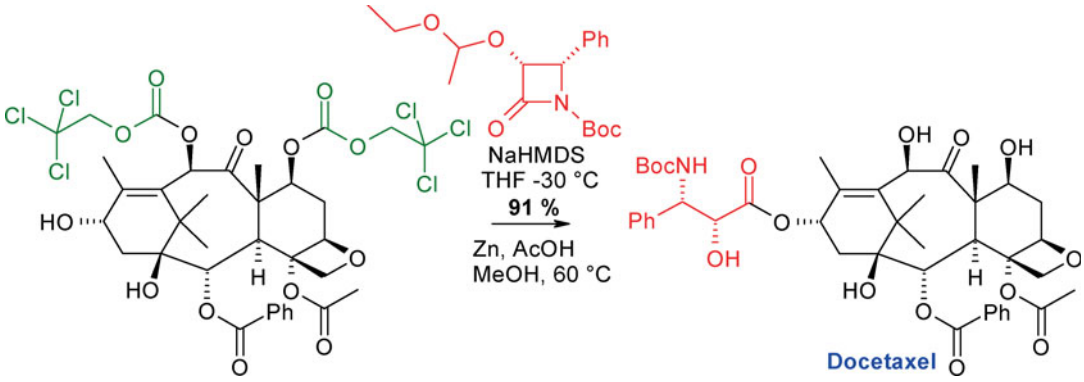
Alain Commerçon of the Rhône-Poulenc-Rorer company used (4*S*,5*R*)-2,2-dimethyl-4-phenyl-1,3-oxazolidine-5-carboxylic acid as a building block for the side-chain, and he likewise obtained paclitaxel, although in lower yield. [327]



Docetaxel, a paclitaxel analogue, is accessible in a very similar way. [320, 328] The drug was developed by Rhône-Poulenc-Rorer and marketed in 1995 under the name *Taxotere*®. It now belongs to Sanofi.



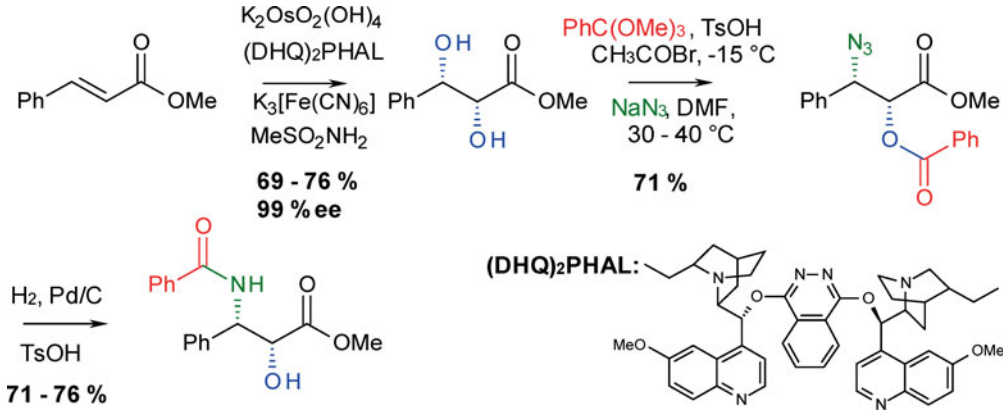
Ojima developed a second route to docetaxel, in which the side-chain was introduced by deprotonation with sodium hexamethyldisilazide and reaction with a β-lactam. [329]



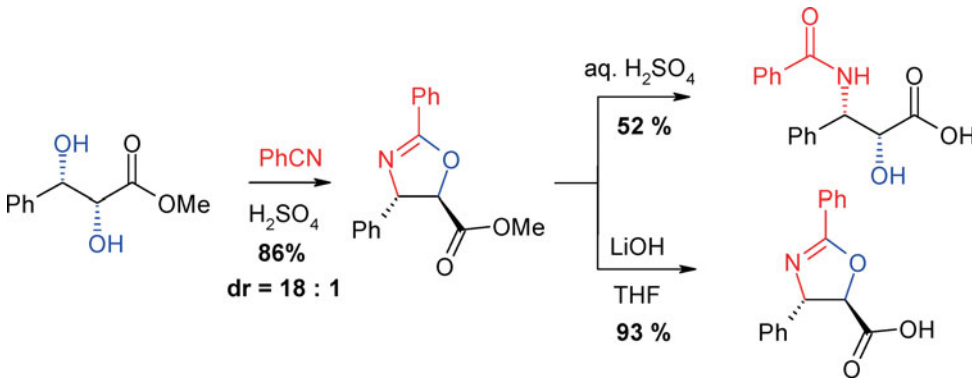
Side-Chain Syntheses

For the synthesis of (2*R*,3*S*)-3-phenylisoserine, a whole series of efficient approaches were developed, of which only selected examples are featured below. [307]

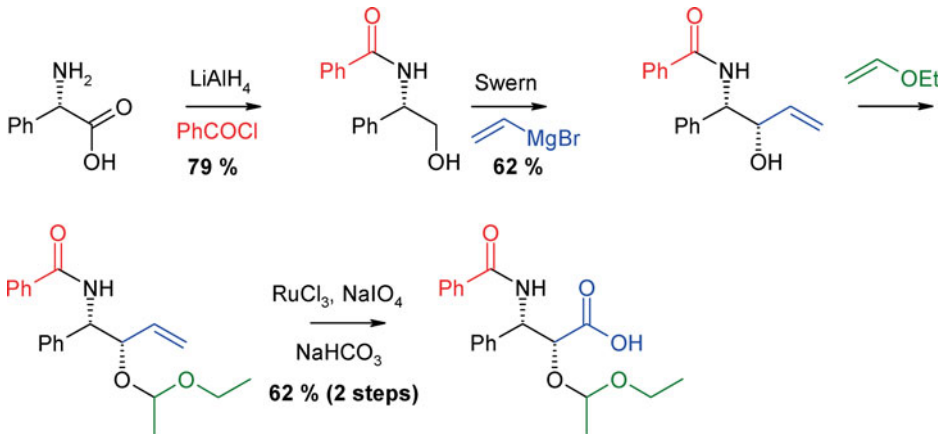
An attractive route, which employs the Sharpless oxidation method, dihydoxylates methyl cinnamate with high enantioselectivity. After regioselective esterification with trimethyl orthobenzoate, the amino-function is introduced (*via* the azide). The desired product is obtained from benzoyl group migration.



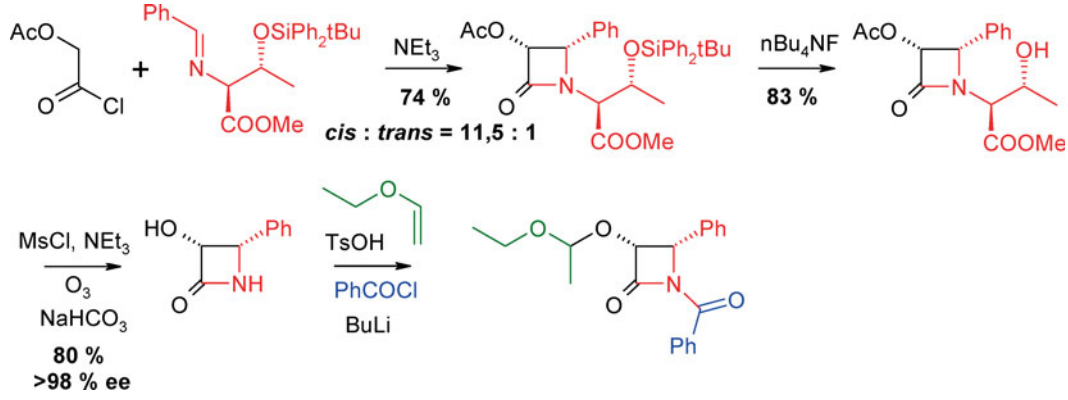
An appealing alternative to the substitution with azide is provided by the Ritter reaction with benzonitrile, from which the oxazolines can be isolated as well. [324, 330] It is interesting that the Ritter reaction produces a high (18:1) diastereomeric ratio. It may be assumed that the configuration of the benzyl cation is stabilised by the neighbouring hydroxy-group in the α-position.



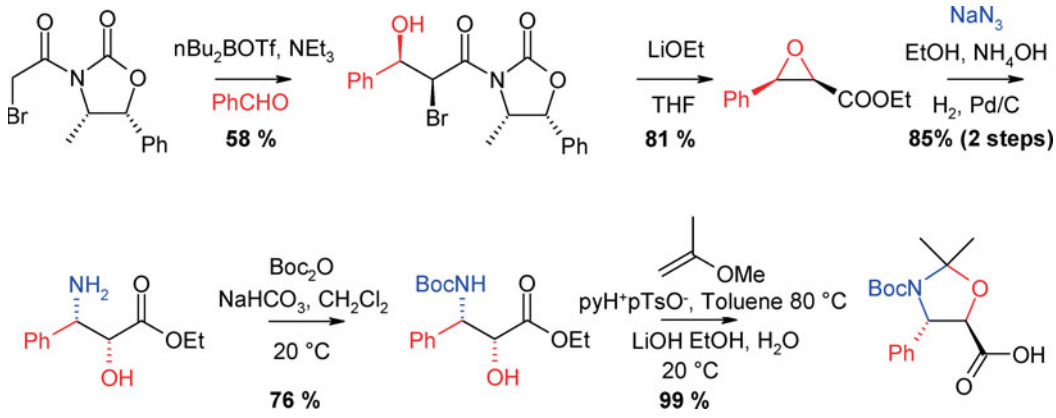
Greene's synthesis starts from (*S*)-phenylglycine. [331] The corresponding amino-alcohol is reacted with benzoyl chloride. After Swern oxidation and a Grignard reaction, an allyl alcohol is obtained. After protection of the alcohol function, the desired product results from periodate cleavage.



The β-lactam building block is obtainable from the condensation of acetoxyacetyl chloride with the imine of a threonine ester. Following oxidative degradation of the amino acid, the alcohol function is protected as the acetal, and the lactam treated with benzoyl chloride.



Alain Commerçon synthesised the protected 3-phenylisoserine *via* a diastereoselective, dibutylboron triflate-mediated aldol condensation. By a sequence of standard methods, the intended, configurationally stable carboxylic acid was obtained.



Biosynthesis and Plant Cell Fermentation

The elucidation of the biosynthetic pathway to natural products is frequently of purely academic interest, provided, that the substance is readily accessible by organic synthesis. [312] In case of paclitaxel this is different. Apart from partial synthesis, fermentation from cell cultures provides the most promising method for the larger-scale production of this drug. Importantly, the latter requires a deepened understanding of the biosynthetic routes, the participating genes and enzymes, as well as of the regulation mechanisms.

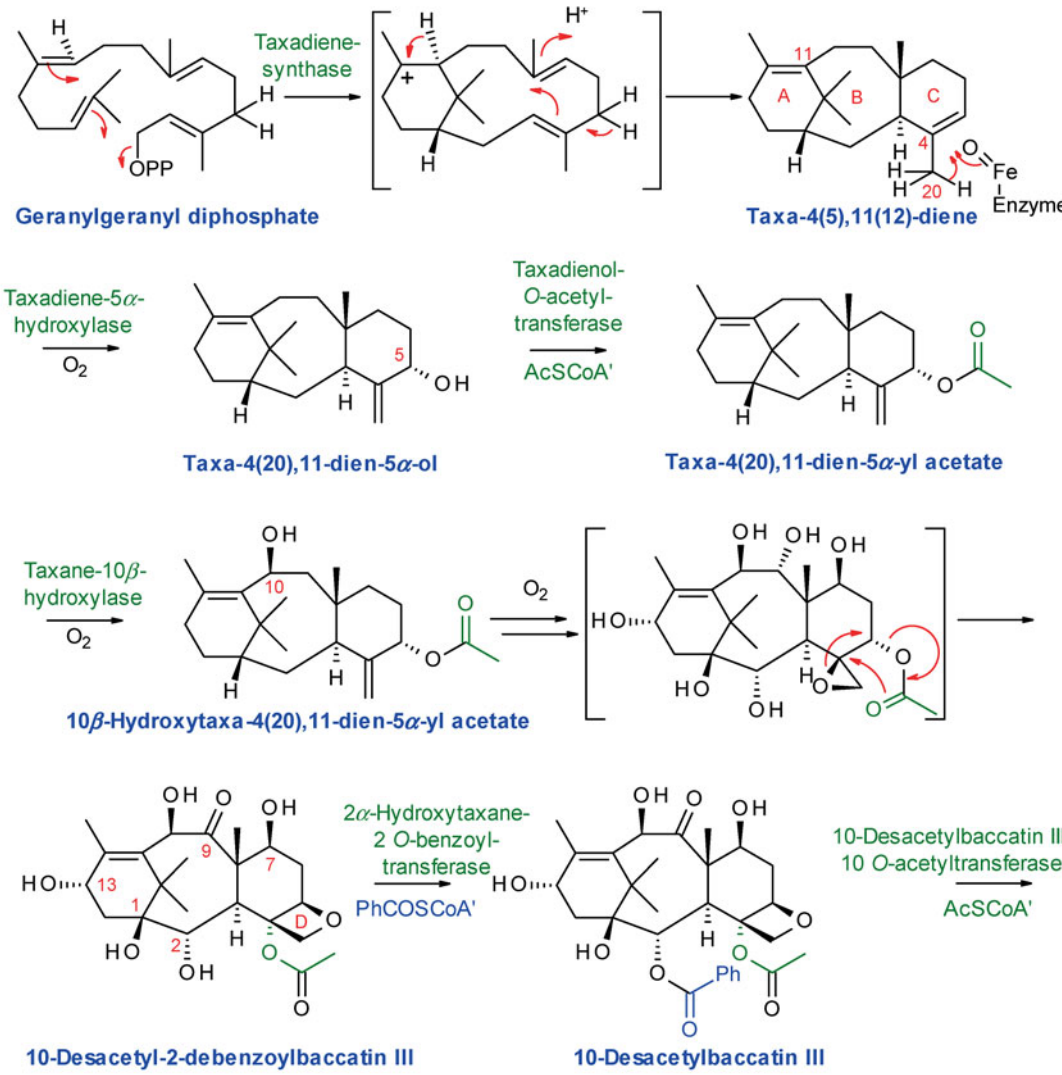
Studying the structure of paclitaxel, reveals that its basic tricyclic framework, taxa-4(5),11(12)-diene, is a diterpene. This can be traced back to geranylgeranyl diphosphate (GGPP), the product of the enzyme GGPP synthase, which is involved in primary metabolism. In case of the yew, GGPP is synthesised through the pyruvate-glyceraldehyde-phosphate pathway (the Rohmer pathway). [332, 333] In the first of the 19-step biosynthesis of paclitaxel, geranylgeranyl diphosphate is cyclised by the taxadiene synthase. Meanwhile, there is a fairly detailed understanding of this cyclisation process. [334] The main product of the cyclisation (about 94 %) is taxa-4(5),11(12)-diene. The by-product (*ca.* 6 %) is taxa-4(20),11(12)-diene.

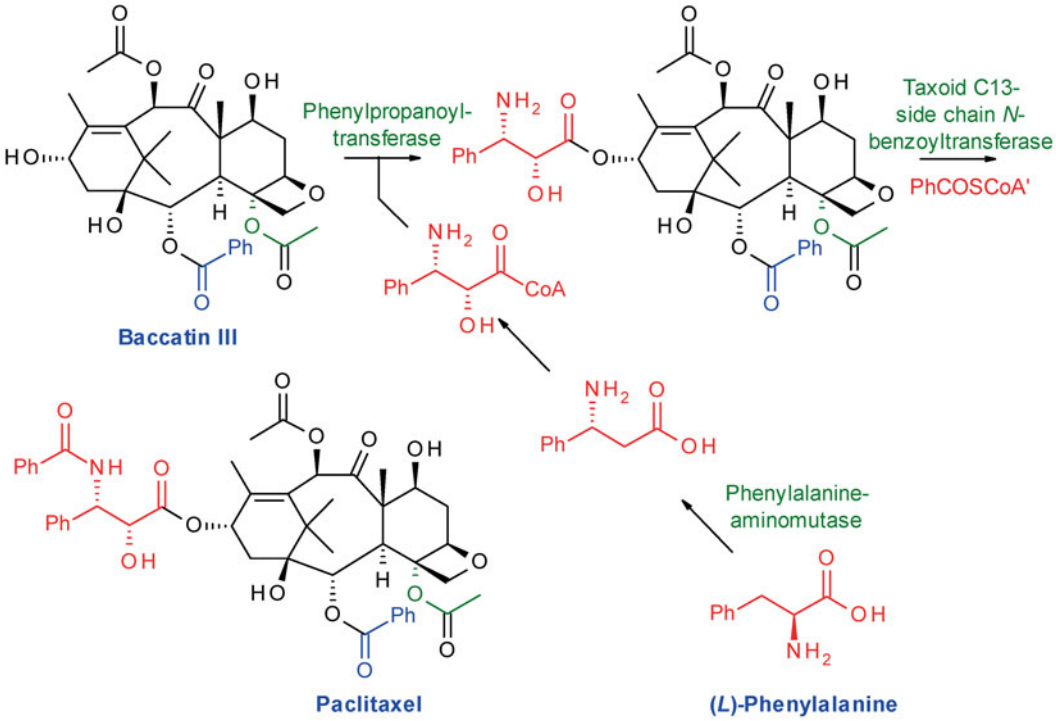
Subsequently, taxa-4,11-diene is oxidised stepwise at eight positions with atmospheric oxygen in presence of cytochrome-P450-monooxygenases, and is esterified at three of the hydroxy-groups by Coenzyme A-dependent acyltransferases. The first hydroxylation takes place at C-5, where by cytochrome P450 abstracts a hydrogen atom, and the resulting allyl radical is then hydroxylated. Interestingly, taxa-4(20),11(12)-diene is likewise a substrate for the enzyme. Subsequently occur hydroxylations at C-10, C-2, C-9 and C-13, and finally at C-7 and C-1.

Hitherto, there are still relatively few clear experimental data supporting the indicated sequence of the following steps. A plausible mechanism for the forma-

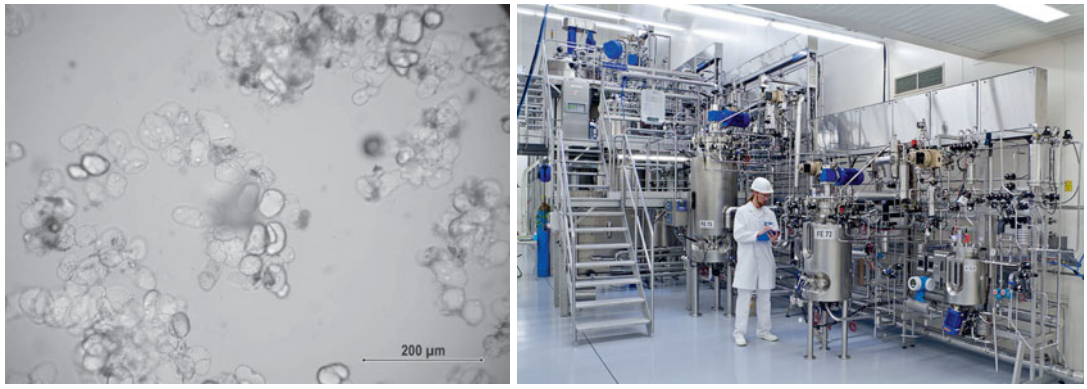
tion of the D-ring is a 4 β-facial epoxidation, followed by an acid-catalysed rearrangement. Afterwards, the hydroxy-group at C-9 may be oxidised.

The studies on the synthesis and installation of the side chain held a particular surprise. The phenylpropanoyl residue stems from phenylalanine, not from cinnamic acid. (*L*)-Phenylalanine is converted into phenyl-β-alanine by means of phenylalanine-aminomutase, and then hydroxylated, followed by esterification with baccatin III. In the final step of the paclitaxel biosynthesis, this ester is converted into the amide. Interestingly, the benzamide is essential for the pharmacological activity of paclitaxel.





With knowledge of the biosynthetic pathway, paclitaxel can be produced nowadays by cultivation of *Taxus* cells in fermenter plants (Fig. 5.131). The largest plants for this purpose, with a nominal capacity of 75,000 litres, are sited at the Phyton company in Ahrensburg, Germany. Phyton Biotech was founded in 1990 by two committed postdoctoral fellows from Cornell University in Ithaca, New York, with the aim of using plant-cell culture technology for the production of plant constituents, difficult to access otherwise, like paclitaxel, by a reliable,

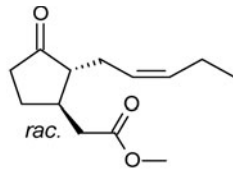


5.131 *Microscope picture of cultivated cells of Taxus chinensis, and the fermenter plant for the cell culture technology based drug production at the Phyton company in Ahrensburg, Germany.*

standardised and environment-friendly route. Safeguarded by long-term contracts, Phyton manufactures paclitaxel in Germany for BMS. [335] In the meantime, a second producer of paclitaxel from plant cell cultures, especially for the Asian market, is Samyang Genex in Korea.

The yew cells, necessary for the inoculation of culture media, are first submitted to a selection process, before being stored under frozen conditions, and reactivated upon demand to be grown in a cascade of fermenters of increasing volume. Paclitaxel is finally isolated from production-scale fermenters. Since the yew cells excrete paclitaxel rather selectively into the culture medium, its isolation by extraction turns out comparatively easily. Such a production cycle lasts for around eight months.

A discovery made by Yukihito Yukimune of Mitsui Petrochemical Industries in 1996 became crucial for achieving higher time-volume yields. [336, 337] He had found that cultivated cells of *Taxus media* and *Taxus baccata* produced significantly more paclitaxel and baccatin III, if traces of (+/–)-methyl jasmonate were added to the culture medium (Fig. 5.132).

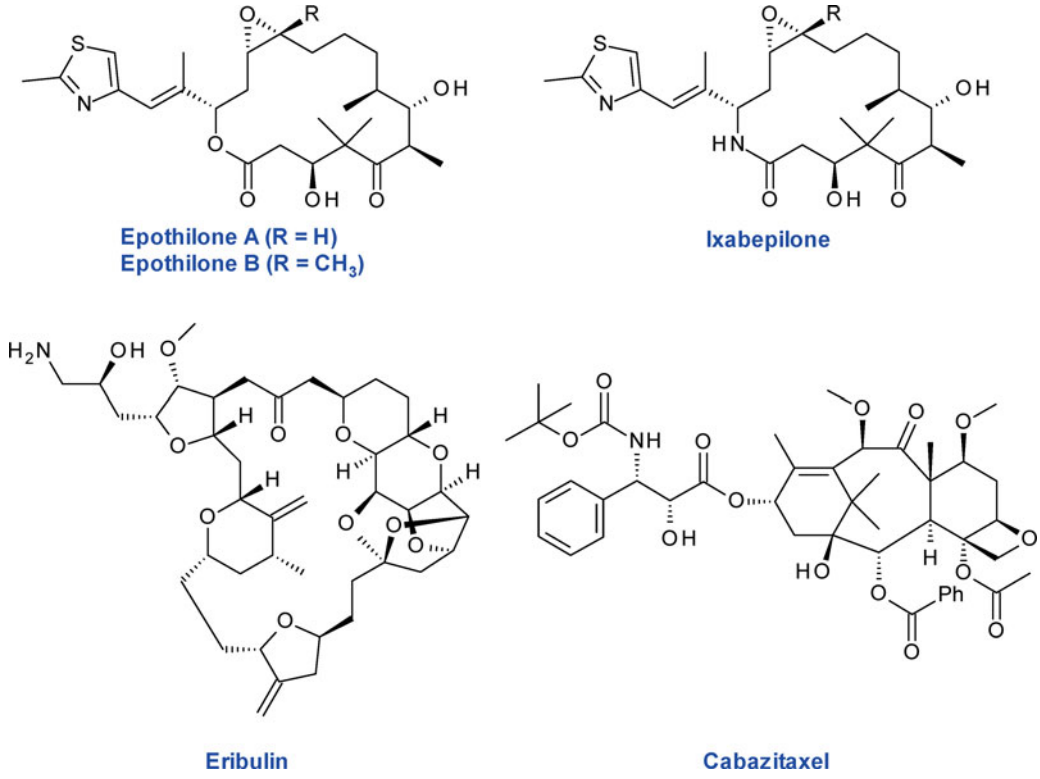


Methyl (+/-)-jasmonate

5.132 *For many plants, methyl jasmonate is a key elicitor, which initiates a series of response mechanisms to herbivores, like the synthesis of protease inhibitors and the increased formation of secondary metabolites (cf. section 3.3 – Jasmonoids). The effects were already well-known for tomato and tobacco plants, but also for Catharanthus and Cinchona seedlings and for soya beans. In the case of yew cells, this additive enabled to increase the paclitaxel production from 3 to 117 mg, and later to 295 mg per litre of fermentation broth.*

5.8.8 Outlook

From today's perspective, paclitaxel represents one of the most important milestones in natural product synthesis as well as in cancer research. Meanwhile, a series of other natural products with the same mode of action has been discovered. The most promising amongst them are the epothilones (Fig. 5.133), which were originally identified as metabolites from a myxobacterium species. With several epothilone derivatives and analogues undergoing advanced preclinical and clinical studies, ixabepilone (*IXEMPRA*®), developed by BMS, became the first of this novel class of microtubule inhibitors to win marketing approval in the USA in 2007. Another recent example is provided by the halichondrin B derivative eribulin. Since late 2010, this product is marketed as well in the USA by Eisai under the trade name *Halavan*®. [338]



5.133 *Novel microtubule inhibitors.*

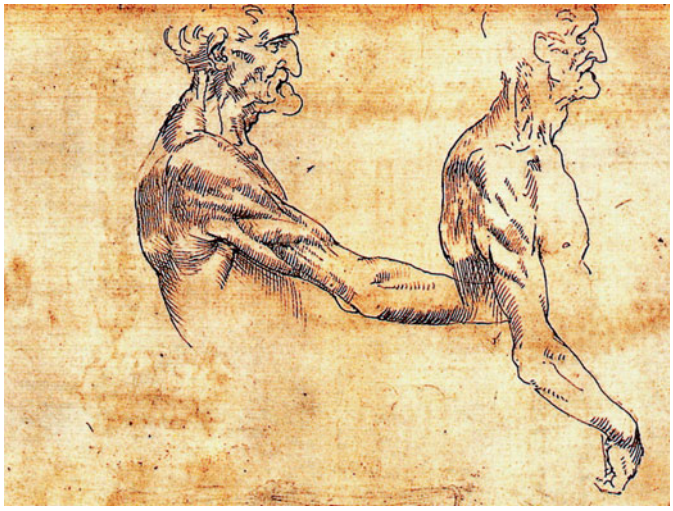
Apart from nab-paclitaxel (*n*anoparticle *a*lbumin *b*ound paclitaxel) and cabazitaxel, which have enjoyed clinical success and are FDA-approved, Sanofi, BMS and also other companies have various paclitaxel analogues in advanced clinical development. In 2010, paclitaxel exceeded worldwide sales of 1.4 billion dollars, and docetaxel's revenues were in excess of 2.8 billion dollars. Since these drugs can be produced by fermentation and partial synthesis, there is no longer the risk of supply restriction for the foreseeable future.

Summary in Bullet Points

- Cancer is a disease, which has been known since antiquity. The discovery of paclitaxel and the development of *Taxol*® are milestones in natural product synthesis and also in cancer research.
- Over the years, numerous total syntheses from prestigious research groups have appeared in the literature; though, initially the clinical demand was covered by extraction of yew bark and later on by partial synthesis.
- Since the 1990s, paclitaxel has also been produced by plant cell fermentation.

5.9 Statins

In the winter of 1507, there died at the Hospital Santa Maria Nuova in Florence an old man (*il vecchio*) (Fig. 5.134) who, shortly before his death, stated that in spite of his age, he felt no illness apart from a certain weakness. His age was one hundred years, more than double the statistical life expectancy at that time.



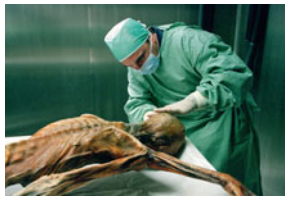
5.134 *"An old man", drawing by Leonardo da Vinci.*

The autopsy (see below) on his body was carried out by Leonardo da Vinci. [339] At the time, such a procedure was quite unusual, if not forbidden. Leonardo was interested in the question as to why old people sometimes died without the fever indicative of an infectious disease. He recognised that the walls of various blood vessels of the old man were thicker than those of young individuals. Leonardo da Vinci then logically describes in his *Dell'Anatomia* the pathogenesis of arteriosclerosis. [340] Arteries harden in the course of one's life, and gradually occlude – in the case of atherosclerosis through plaque-forming lipid deposition. This leads to an inadequate blood supply to the affected tissue and the internal organs. The old man had eventually died as a consequence of the occlusion of a coronary artery.

5.9.1 Discovery of Arteriosclerosis/Atherosclerosis

Arteriosclerosis is one of the few internistic disease forms, the appearance of which can be demonstrated over many thousands of years. We owe this insight mainly to two circumstances: favourable climatic conditions, and also the fact, that a number of ancient peoples wished their bodies not to be cremated or buried, but mummified and in this way preserved. [342] The disease is detectable by radiological investigations and dissection of mummies from Egypt, China

A patient with arteriosclerosis (hardened arteries) may not have atherosclerosis (plaque), but a patient with atherosclerosis does have arteriosclerosis. Patients often have both conditions, which can cause a decrease in the blood flow to the heart muscle. [341]



5.135 *The autopsy of the body of the Ice-Man was not only the much-belated investigation of a criminal case, but it also led to valuable information concerning the living conditions of men in the Neolithic period.*

and the Canary Islands as well as, for example, from Arctic regions. Prominent examples are the atherosclerosis of a 45-year-old man from the Neolithic Age (around 3340 BC) (Fig. 5.135), whose mummified body was discovered in 1991 by two German alpinists on the Hauslabjoch in the Ötztal Alps, and the calcified arteries of Pharaoh Ramesses II (*ca.*1303–1213 BC) and his son Merenptah (*ca.* 1273–1203 BC). [343]

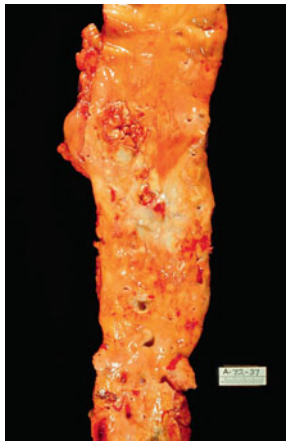
Descriptions of some of the main secondary diseases following from arteriosclerosis have already been handed down to us from ancient times. Thus, one can find in the Hippocratic writings (*Corpus Hippocraticum*) indications of gangrene, stroke (*apoplexia*) and cardiac failure. But the explanation of the cause of such diseases first began in early modern times, when autopsies were carried out in increasing numbers to clarify the cause of death.

The rebellion of the Israelites and the beginning of their journey into Palestine under the leadership of Moses happened during the reign of Merenptah (*ca.* 1213 – 1203 BC). [344]

5.136 *English country doctor Caleb Hillier Parry (1755–1822).*

Autopsy

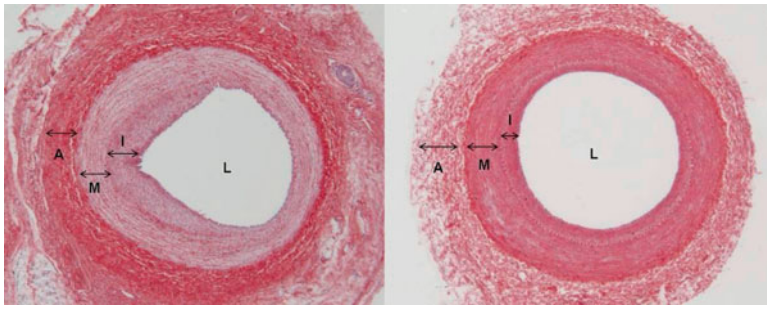
Autopsy, which is synonymous with dissection, post-mortem examination and necroscopy, served originally the studies of anatomy and to establish the causes of disease and death. The first autopsies in this sense were carried out by the Greek physician Herophilus (335–280 BC), and later almost notably by Galen of Pergamon (129–216 AD). Emperor Frederick II (1196–1250) ordered that every year the body of an executed person had to be delivered to the medical schools (among others, in Salerno) to train physicians in anatomy. In 1302, the municipal council of Bologna authorised for the first time an autopsy to investigate the cause of a death. While Leonardo da Vinci and Michelangelo carried out autopsies for anatomical studies, the Flemish personal physician to Charles V and Philip II, Andreas Vesalius (1514–1564), is reckoned as the founder of modern anatomy. He authored a most influential and richly illustrated book: De humani corporis fabrica (On the Fabric of the Human Body). Vesalius also donated a skeleton, named after him, to the town of Basel, where it is on display as part of the University's Anatomical Museum. The Vesalius skeleton originates from a public dissection of the criminal Jakob Karrer from Gebweiler, who was beheaded in 1543. It was not unusual for Vesalius' to cut the corpses for his studies down from the gallows. Pioneering for modern pathological anatomy were also the book De sedibus et causis morborum per anatomen indagatis (The Seats and Causes of Disease Investigated by Anatomy) by Giovanni Battista Margagni (1682–1771) from Padua, and also the contributions of the Viennese physician Carl von Rokitansky (1804–1878) and the Berlin pathologist Rudolf Virchow (1821–1902).



5.137 *Pathological changes in the aorta.*

In July 1788, the English country doctor Caleb Hillier Parry (Fig. 5.136) reported at a meeting of the Gloucestershire Medical Society at the Fleece Inn in Rodborough on the connection between Angina pectoris (chest pain) and pathological changes in the coronary arteries and aorta (Fig. 5.137). [345] Together with Edward Jenner, he had performed autopsies like Leonardo da Vinci, and thereby had detected something hard and gritty in the coronary arteries. According to the legend, at first he conceived that some plaster had fallen down from the old and crumbling ceiling of his laboratory (see also 'Hardening of the Arteries', below).

Contrary to the perception of Leonardo da Vinci, who believed atherosclerosis to be a degenerative manifestation of the ageing process, in 1815, Joseph Hodgson (1788–1869) [346], a surgeon at the Birmingham General Hospital, was the first to describe atherosclerosis as an inflammatory process, which takes place in the intima, the innermost cell layer of the blood vessels (Fig. 5.138 and Fig. 5.139). In 1856, Rudolf Virchow, a physician at the Charité in Berlin, confirmed these findings and provided an extensive description of the disease. The essential features of their concept regarding the induction of arterio- and atherosclerosis still stands nowadays.



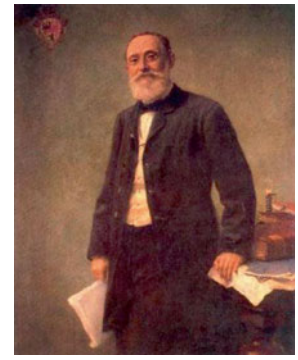
5.139 *Section through a blood-vessel narrowed by disease (left) and a normal blood-vessel. The inside space of a blood-vessel (lumen (L)) is surrounded by a monocellular layer of endothelial cells (intima (I)). This is encased by several layers of smooth muscle cells (media (M)). Between intima and media there is an elastic thin sheet of fibers, the basement membrane. The connective tissue layer enclosing the blood-vessel (adventitia (A)) anchors the vessel to nearby organs.*

The endothelial cells of the intima fulfil a host of tasks: the endothelial layer is responsible for the transport of materials from the blood; through the production of nitric oxide and the associated vasodilation it regulates blood pressure; in addition, it has antithrombotic properties by preventing excessive adhesion of thrombocytes and leukocytes.

Smoking, hypercholesterolaemia, diabetes mellitus, arterial hypertension and various infections damage the endothelial layer and therefore constitute increased risk factors for atherosclerosis.

At the beginning of the last century, Russian physicians developed the hypothesis that atherosclerosis could be associated with eating habits. The Russian bacteriologist Ilya Ilyitsch Mechnikov (1845–1916; Director of the Pasteur Institute in Paris, and laureate of the Nobel Prize together with Paul Ehrlich in 1908), postulated in 1903 that a very protein-rich diet was harmful to humans, and accelerated the ageing process. [347] Alexander Ignatowski, a young pathologist in the Military Academy in Saint Petersburg, tried to prove this hypothesis by feeding rabbits with large quantities of meat, eggs and milk. In young rabbits, this actually led to adrenal and liver damage. In adult animals he observed in the arteries lesions, which resembled atherosclerosis in humans. Since such lesions were regarded as a typical characteristic of the ageing process, Ignatowski was convinced, he had proven Mechnikov's hypothesis. [348]

Edward Jenner (1749–1823) is known for the development of the modern vaccination against smallpox.



5.138 *Rudolf Ludwig Karl Virchow (1821–1902), around 1856.*

5.140 *Adolf Windaus (1876–1959) (a) and Ludwig Aschoff (1866–1942) (b).*

From 1901 on, Adolf Windaus in Freiburg was engaged in the chemistry of cholesterol. Karl Albert Ludwig Aschoff (discoverer of the atrioventricular node, a specialised segment of the heart muscle), who was considered to be one of the most influential pathologists at that time, taught likewise in Freiburg. Aschoff sent Windaus autopsy samples from normal and atherosclerotic aortae, requesting to determine their cholesterol content (Fig. 5.140). Subsequently, Windaus described in 1910 that the level of cholesterol ester in atheromatous aortas was over twenty-fold higher than in normal arteries. [349]

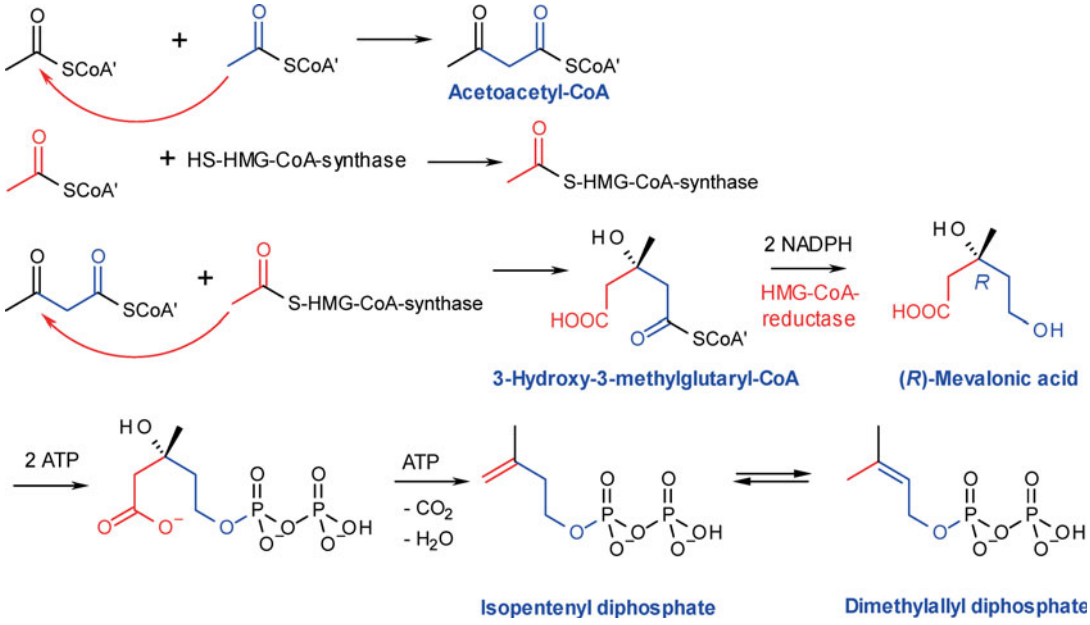
Cholesterol

Cholesterol (Greek, chole: bile and stereos: solid) is a central building block in the biosynthesis of steroid hormones, of bile acids, and of glycolipids, which are the most important constituents of the cytoplasmic membrane in eukaryotes, apart from phospholipids. Steroids were originally considered as fats, because they form lustrous flakes, which feel fatty. Around 1770, cholesterol was first isolated by various researchers from gallstones. Michel Eugène Chevreul (1786–1889), who also named the material, recognised in 1812 that this compound, unlike the triglycerides, could not be hydrolysed. The structure of cholesterol was fully elucidated by Adolf Windaus in 1932, based in part on an X-ray structure analysis by John Desmond Bernal (1901–1971) in Cambridge. The first total synthesis was achieved by Robert B. Woodward in 1951. Clarification of the biosynthetic pathway came in the 1950's from research of the German biochemists Feodor Lynen and Konrad Emil Bloch (Fig. 5.141).

For this pioneering research, both, Lynen and Bloch were awarded the 1964 Nobel Prize for Physiology or Medicine.

5.141 *Feodor Lynen (1911–1979) (a) and Konrad Emil Bloch (1912–2000) (b).*

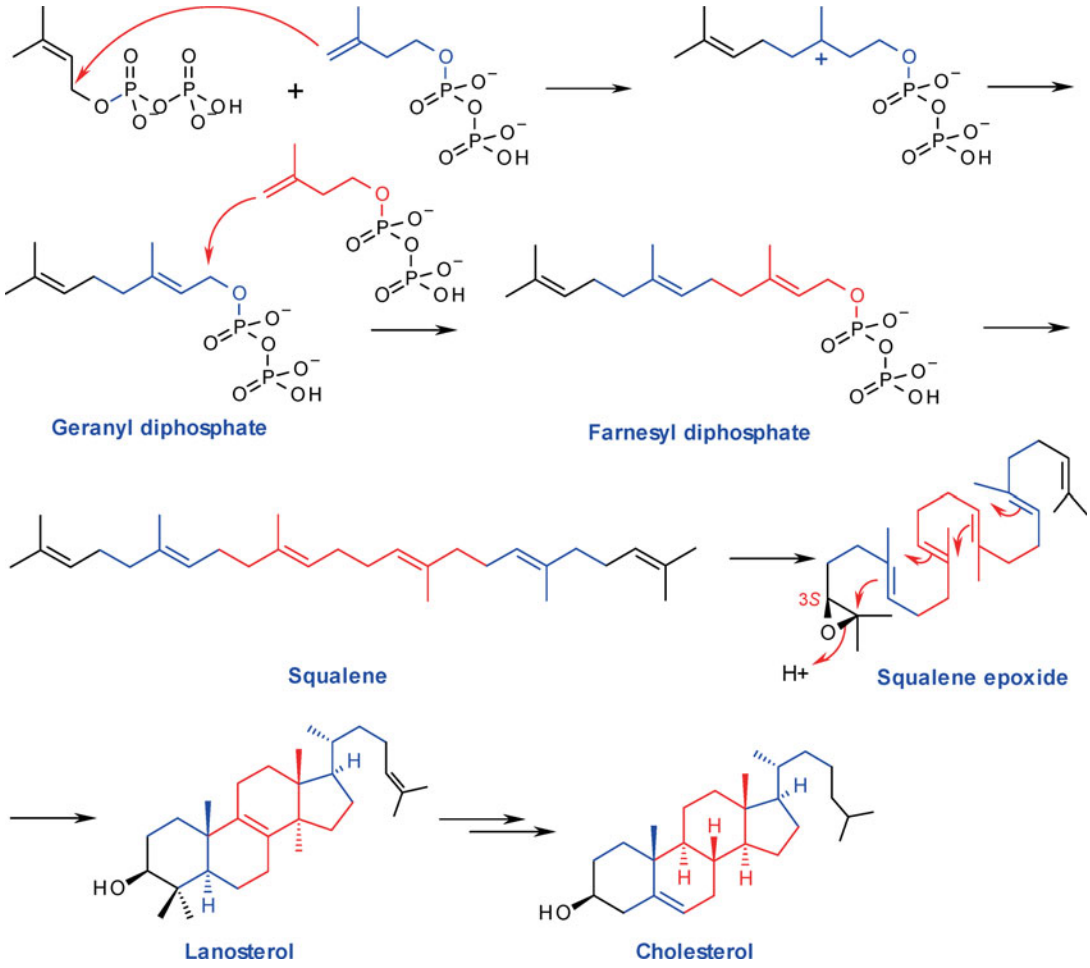
The German-American biochemist Fritz Albert Lipmann (1899–1986) had discovered Coenzyme A in 1948, while working at the Massachusetts General Hospital in Boston. In 1951, Lynen succeeded at the University of Munich in the isolation of acetyl- Coenzyme A, (acetyl-CoA, the activated form of acetic acid) from yeast cells. Karl Folkers at Merck Sharp & Dohme recognised in 1956 mevalonic acid as a critical unit in the terpene biosynthesis, while Lynen documented its formation from three acetyl-CoA moieties and the further route to fatty acids and terpenoids. Recently, another non-mevalonate pathway to terpenes (Rohmer pathway) has been discovered, which is not present in humans.



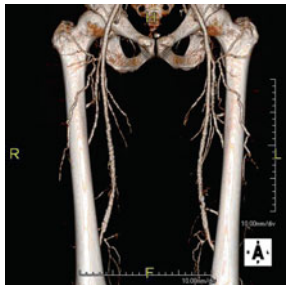
Bloch had to flee as a Jew from the Nazi régime in 1936 *via* Switzerland to the USA. At Columbia University in New York, he was able to show together with David Rittenberg in 1942, using isotopically labelled acetate, that this was a precursor for the cholesterol synthesis in animals. The polyene cyclisation of the triterpene squalene produces lanosterol, which is degraded to cholesterol, the central intermediate of all human steroids. [350]

At the beginning of the last century, Nikolay Nikolaevich Anichkov, a colleague of Alexander Ignatowski at the Military Academy in Saint Petersburg, took up as well on atherosclerosis as a research topic (Fig. 5.142). In animal experiments he was able to prove that the blood-vessel-damaging can be attributed to cholesterol and not to protein-rich foodstuffs. He fed rabbits with cholesterol dissolved in sunflower oil and observed vascular lesions closely resembling those of human atherosclerosis, while the vehicle, sunflower oil itself, did not produce such lesions. [351] These findings marked the inception of modern atherosclerosis research. [352]

5.142 *Nikolay Nikolaevich Anichkov (1885–1964).*



However, this landmark discovery remained largely unappreciated for the next thirty years, because it was not in accordance with the spirit of the times. Atherosclerosis was held to be an age-related degeneration of the arteries, which developed in the course of the decades. By 1915 however, the Düsseldorf pathologist Johann Georg Mönckeberg (1877–1925) had already discovered considerable sclerotic changes in the aortae and coronary arteries from autopsies of 140 young soldiers from the First World War. [353, 354]



5.143 *Medial calcinosis, shown by computer tomographic angiography.*

Arterial Calcification

Morbus Mönckeberg (medial arterial calcification) or medial calcinosis is the deposition of hydroxyapatite ($Ca_5(PO_4)_3OH$) in the media. Most frequently affected are the small to medium sized arteries of the lower extremities, the pelvic arteries and the abdominal aorta. An occurrence in the coronary arteries is rare. As a rule, Morbus Mönckeberg frequently coincides with atherosclerosis. At an advanced stage, the media is almost entirely replaced by a homogeneous calciferous layer, which displays itself in the computer tomogram almost like a second skeleton (Fig. 5.143). In contrast to atherosclerosis, lipids do not contribute to the deposition. [355]

Anitchkov's findings were received in professional circles with great scepticism. The cardiovascular effects on rabbits, strict herbivores, were held as artefacts, which were not transferable to the human omnivore. A few research groups repeated Anitschkow's experiments with rats and dogs, but could not confirm his results. As carnivores, dogs are adapted to a cholesterol-rich diet. By its very efficient conversion into bile acid and subsequent excretion, a largely constant cholesterol level is maintained in dog's blood.

The first indication of a connection between a diet-related elevated cholesterol exposure and heart disease in humans came from the Dutch physician Cornelis D. de Langen. In 1916, he reported from the Dutch East Indies (nowadays Indonesia), that the natives had a considerably lower cholesterol level than the Dutch colonists. He attributed this to their different dietary habits. Whereas the diet of the Dutch was very rich in meat, the natives lived mainly on vegetables and rice. Within the framework of an epidemiological study, he could prove that the cholesterol level of the latter rose by around 27 %, if they changed their diet to that of the Dutch. Regrettably, de Langen published his results in Dutch, in a journal, which received little attention from the experts.

Similar results were collected in 1953 by the American physician, Major William F. Enos, from 300 autopsies of young American soldiers, who had lost their lives in the Korean War (1950–1953). The coronary arteries of many soldiers showed considerable atherosclerotic lesions. Remarkably, these pathological changes were not found in the Korean soldiers, a fact, which Enos, like de Langen, attributed to differences in their diet. [356]

A deepened understanding of the pathogenesis of atherosclerosis came from research addressing the question of how lipophilic compounds were transported in blood. Along with food, humans take up various lipids (fats, cholesterol and its esters), which are important sources of energy, essential precursors for the biogenesis of steroid hormones, and indispensable components of many cell membranes. For transport in a blood vessel, the lipids form non-covalent aggregates with various proteins, known as lipoproteins.

In 1929, Michel A. Macheboeuf (1900–1953), working at the Pasteur Institute in Paris, was the first to isolate and characterise the α-lipoprotein from the serum of horse blood. [357] The American biochemist Edwin Josef Cohn (1892–1953) succeeded in separating human blood plasma into different fractions. During the Second World War he developed at the Harvard Medical School for the American forces industrial processes for the production of serum albumin, γ-globulin, fibrinogen and fibrin. These were urgently needed for the treatment of the wounded. In the course of these studies, he also isolated and characterised two different lipid fractions from human blood serum.

A few years after the Second World War the physicist John William Gofman (1918–2007) finally succeeded in separating, and comprehensively characterising, the lipoproteins of human blood; this was accomplished with the aid of an ultracentrifuge, developed in Sweden by Theodor H. E. Svedberg (1884–1971) (Fig. 5.144).

The work of Anitschkow was known to Gofman, and he was firmly convinced that an elevated cholesterol level leads to atherosclerosis. Gofman used, like Kai O. Pedersen (1901–1991) at the University of Uppsala, Sweden, one of

In 1943, Gofman had obtained his doctorate on uranium isotopes under the supervision of Glenn T. Seaborg (1912–1999, discoverer of ten transuranic elements). Later, at the University of California in Berkeley, and by the order of Julius Robert Oppenheimer (1904–1967) he prepared plutonium in sufficient quantity for experiments within the framework of the Manhattan Project to build the first atomic bomb in Los Alamos, New Mexico.

5.144 *John Gofman (1918–2007) at the ultracentrifuge around 1950. In modern ultracentrifuges, rotating velocities from 60,000 to over 100,000 rpm can be achieved and gravitational fields of more than 500,000 times the gravitational acceleration. In operation, the centrifuge is evacuated in order to reduce atmospheric resistance and to prevent frictional heating.*

the first ultracentrifuges to separate out the constituents of the blood serum (blood plasma without fibrinogen). Whereas Pedersen tried to separate out a lipoprotein by sedimentation, which was unsuccessful, because the sedimentation rate was too low, Gofman added sodium chloride before centrifugation and could isolate the lipoprotein from the supernatant. With the aid of the ultracentrifuge, he was even able to characterise further sub-classes of lipoproteins (Tab. 5.9).

Lipoproteins are globular, micelle-like particles, with a non-polar nucleus of triglycerides and cholesterol esters, and an amphiphilic 2 nm thick casing of proteins, phospholipids and cholesterol. Being subject to constant metabolic change, their compostition and properties vary. The density of the particles increases with decreasing diameter, since the density of the shell is higher than of the nucleus. The protein portion of the lipoprotein is called the apolipoprotein or apoprotein for short (Fig. 5.145).

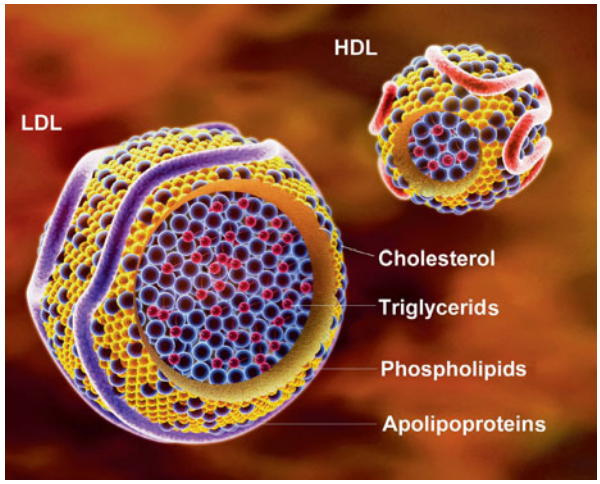
An enormous advance in atherosclerosis research was made at the beginning of the 1960s by the observation of Donald S. Frederickson (1925–2002) and Robert S. Lees that lipoproteins could also be separated by paper electrophoresis. This was much simpler and cheaper than ultracentrifugation and could be carried out in numerous ways in every hospital. In the end, for the first time large-scale epidemiological studies on atherosclerosis became feasible with this new technique.

The separation of lipoproteins in gravitational or electric fields enabled their characterisation, although their function remained unclear as well as their contribution to the pathogenesis of atherosclerosis. [358]

In 1974, Russell Ross (1929–1999) and John Glomset at the University of Washington in Seattle discovered that during coagulation, a growth factor was

Tab. 5.9 *Lipoproteins are subdivided nowadays into five classes according to their density and functional properties.*

Lipoprotein class	Apoprotein	Density (g/cm³)	Diameter (nm)	Function
Chylomicron	A-I, A-II, B-48, C-I, C-II, C-III, E	< 0.95	80–500	Largest lipoproteins. Formed in the intestine, serve to transport lipids out of food, are rapidly dissolved
Very-low-density Lipoprotein (VLDL)	B-100, C-I, C-II, C-III, E	0.95–1.006	30–80	Structurally similar to the chylomicrons, but smaller, produced in the liver, transport mainly endogenously- formed triglycerides
Intermediate-density Liproprotein (IDL)	B-100, C-III, E	1.006–1.019	25–35	Like VLDL, however smaller
Low-density Lipoprotein (LDL)	B-100	1.019–1.063	18–28	Formed in the cardiovascular system from VLDL, transport 60–70 % of the plasma cholesterol
High-density Lipoprotein (HDL)	A-I, A-II, C-I, C-II, C-III, D, E	1.063–1.210	5–12	Smallest, most widespread lipoprotein, transport cholesterol from peripheral tissue back to the liver, contain *ca*. 20–30 % of the plasma cholesterol



5.145 *Lipids are transported in the blood by lipoproteins. The lipid droplets are wrapped up in a monomolecular layer of cholesterol, phospholipids and specific, amphiphilic apolipoproteins.*

released from the blood platelets. This stimulates the proliferation (cell growth) of smooth muscle cells in the blood vessel. Ross and Glomset picked up Rudolf Virchow's idea, and postulated their "response to the injury hypothesis", assuming that the endothelial layer suffers a mechanical injury, which leads to blood coagulation and results in a thickening of the vessel wall, into which lipids are also deposited. Apart from mechanical damage, chronic hypercholesterolemia was also recognised as a possible source of injury. [359]

5.146 *Michael S. Brown (left) and Joseph L. Goldstein (right).*

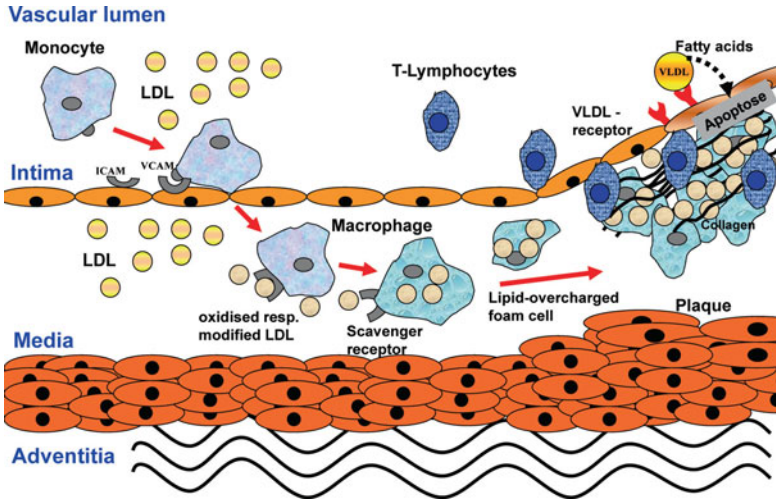
Decisive contributions to the understanding of the origin of atherosclerosis came in the following years from Joseph L. Goldstein and Michael S. Brown, for which they were awarded the 1985 Nobel Prize for Physiology or Medicine (Fig. 5.146). The starting point for their research work were patients with familial hypercholesterolemia, the link of which to coronary heart disease was first described in 1937 by the Norwegian physician Carl Arnoldus Müller (1886–1983). The disease is caused by a defect of a single gene and is inherited as an autosomal dominant disorder, according to the principles of genetics established by the Augustinian friar Gregor Mendel (1822–1884). Homozygous carriers of this congenital disease, with cholesterol values of 650–1000 mg/100 ml blood, show a three- to five-fold increase in their LDL-level, and run the risk of suffering a heart attack, even as children or adolescents. For heterozygous carriers, with cholesterol values of 270–550 mg/100 ml blood, cardiovascular diseases frequently manifest themselves after the age of 30. Goldstein and Brown recognised that the defective gene in familial hypercholesterolemia encodes a receptor for LDL, which is required for its cellular uptake.

The concept of receptor-mediated endocytosis was new, and pointed the way ahead for cell biology of the 20th century. This general mechanism enables cells to incorporate large molecules, bound to specific cell-surface receptors, *via* a membrane invagination process. LDL receptors are found on almost all cells of the body. Those in the liver are however crucial for homoeostasis, since this is where excess of cholesterol is metabolised to bile acids and excreted.

The work of Brown and Goldstein got serendipitous support from the discovery, a Japanese veterinarian had made at Kobe University. In 1973, Yoshio Watanabe (1927–2008) noticed incidentally that a rabbit from his colony showed a tenfold increased blood cholesterol level. By appropriate breeding, he managed to establish a strain of rabbits with this characteristic dysregulation. All of them developed a coronary heart disease, closely resembling its human variant. Thus, suddenly there was an animal model available to investigate familial hypercholesterolemia much better than with the elaborate method of Brown and Goldstein, who had used tissue cultures of skin cells, in lack of sufficient access to human hepatocytes (liver cells). [360]

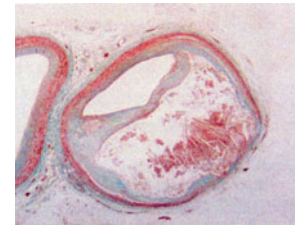
5.9.2 Pathogenesis of Atherosclerosis

As to our current understanding, atherosclerosis has its roots in an endothelial dysfunction, which makes it possible for LDL to increasingly infiltrate the intima (Fig. 5.147). At the beginning of the 1980s, James R. Hessler in Cleveland and Daniel Steinberg in La Jolla were able to show, independently of each other, that LDL is oxidised in the endothelial layer by superoxide (O_2^-). The monocytes circulating in the blood-vessels are penetrating the endothelial layer and are transformed into tissue macrophages in the subendothelial matrix. Brown and Goldstein observed, however, that these do not accumulate LDL. The macrophages rather have a large number of particular receptors (scavenger-receptors) to bind oxidised LDL (OxLDL), which is then internalised *via* endocytosis and transforms macrophages into foam cells.



5.147 *Damage of the endothelium by oxidised LDL.*

The foam cells then form deposits of fat in the intima and media of the blood vessel wall, which are called "fatty streaks", the early form of plaques. This initiates an inflammatory process, as a result of which even more monocytes are recruited into the sub-intimal space. The proliferation of the smooth muscle cells leads to the thickening of the blood-vessel wall, accumulation of more lipids, and development of a lipid-necrotic nucleus, which is covered with fibrous tissue, forming the fibrous cap of a plaque. The narrowing of the vessel can remain unnoticed by the patient for many years. However, in the course of time, a stable plaque can also become unstable, when activated cells within the plaque secrete matrix proteases, which degrade the fibrous cap (Fig. 5.148). This leads within minutes to rupture and thrombus formation, causing occlusion of blood-vessels and resulting in a heart attack, unstable angina pectoris or a stroke.



5.148 *Histological preparation of a lipid-containing plaque with a thin fibrous cap in a coronary artery.*

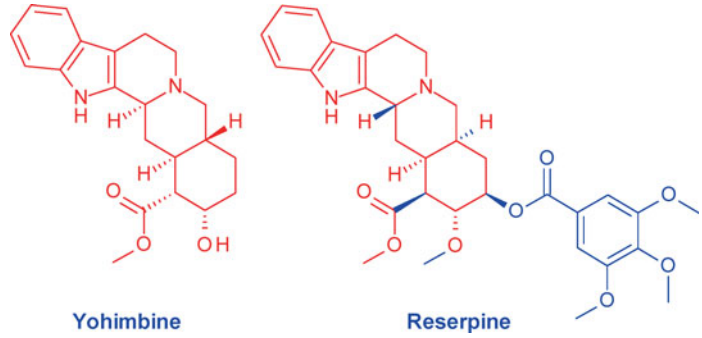
Interestingly, atherosclerosis-triggered heart attacks are substantially more frequent than strokes. The assumption that this is due to high mechanical stress on the coronary arteries is not likely, because infarctions of the plantar arteries, which have a similar mechanical load, do not arise. More plausible is the so-called "Marburg hypothesis", by which the heart becomes the victim of its energy demand, up to 70 % thereof – like in no other organ – is covered by supply of fatty acids. [361] In contrast, the brain uses mainly glucose as its energy source. The heart of an adult needs around 40 g of fatty acids per day, being delivered through the blood stream in the form of VLDL and LDL, from which the lipoprotein lipase of the endothelial layer releases the fatty acids. An elevated level of unsaturated fatty acids from VLDL can activate a proteinphosphatase 2C (PP2C), which triggers endothelial cell apoptosis, and thereby plaque formation. The proposed anti-apoptotic role of HDL is still under debate. [362]

5.9.3 First Treatment Methods for Atherosclerosis

5.149 *The Indian snakeroot (Rauwolfia serpentina) was named after the physician and botanist Leonhart Rauwolf (ca. 1540–1596). The South-Asian evergreen bush, which grows to a height of around 1 metre, belongs to the dogbane family (Apocynaceae), the roots of which are used especially in Indian folk medicine. The pulverised roots serve for the treatment of high blood pressure and snakebite, and as a sedative for various mental illnesses.*

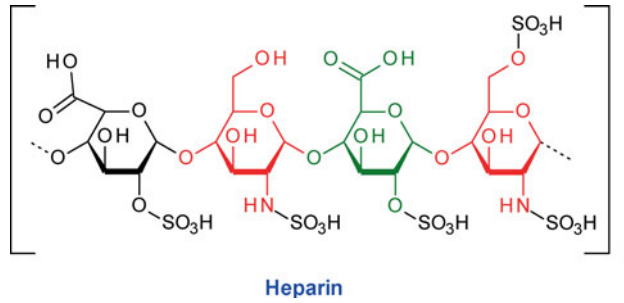
Rauwolfia serpentina

Up to the 1960s, the treatment of atherosclerosis was only symptomatic. [363] Thus, for example for lowering blood pressure, there was used an extract from the snake-shaped roots of *Rauwolfia serpentina*, which was already known to the ancient Hindus (Fig. 5.149). [364] In 1952, Emil Schlittler (1906–1979) of the Ciba company isolated reserpine as its hypotensive principle, and determined its constitution. In particular, he also recognised the relationship to the large class of yohimbine alkaloids. The first constitutional total synthesis was achieved in 1956 by R. B. Woodward. [365–367] It counts as one of his most notable contributions to modern synthetic chemistry and formed the basis of industrial processes for the synthesis of this drug. In 1989, Gilbert Stork published a stereospecific total synthesis. [368]



Heparin

Another drug, which was assumed to intervene in the metabiolism of lipoprotein or cholesterol, was heparin. Nowadays, we know that heparin indeed releases a lipoprotein-lipase from the surface of vascular endothelial cells, which then dissolves triglycerides in the chylomicrons and VLDL.

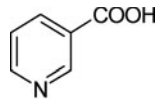


Heparin consists of alternately (*D*)-glucuronic (green) and (*L*)-iduronic (black) acid moieties and a molecule of glucosamine (red), which are irregularly sulfated. The molecular mass ranges between 6,000–30,000 g/mole. The coagulation-inhibiting polysaccharide ranks among the strongest acids occurring in the human body. It was discovered in 1922 by the American physiologist William

Henry Howell (1860–1945) and is extracted nowadays from the mucous membranes in the small intestine of pigs, or from the lungs of cattle. The drug inhibits the transformation of fibrinogen into fibrin, and is now used for the treatment of thromboses, embolisms and for the prophylaxis of cardiac infarction and thrombosis. A disadvantage is, that heparin is not resorbed from the gastrointestinal tract, and has to be administered *via* an intravenous, subcutaneous or percutaneous route.

Nicotinic Acid

In 1955, nicotinic acid (vitamin B3, niacin) moved into the focus of research as a drug to lower cholesterol levels. The Canadian psychiatrist Abram Hoffer (1917–2009) had achieved good results with nicotinic acid in the treatment of schizophrenic patients. [369]



Nicotinic acid

However, as a side-effect of the therapy, his patients suffered from a massive "flush syndrome" (harmless but mostly unpleasant reddening of the skin on the face, neck, chest and upper arm). For a better assessment, Hoffer himself took gram quantities of niacin over several months, and discovered to his surprise that this treatment had healed his serious inflammatory gum disease. Inspired by Rudolf Virchow's hypothesis, that atherosclerosis was an inflammatory process, which was associated with serum cholesterol, Rudolf Altschul (1901–1963), Hoffer's former histology teacher, demonstrated in his hypercholesterolemic rabbit model, that within a few days niacin brought cholesterol levels back to normal. This prompted corresponding studies by Hoffer in the psychiatric patients.

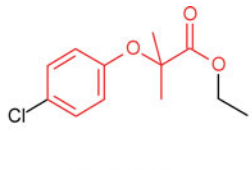
With the discovery of the nicotinic acid receptor in 2003 by a team at GSK, and, among others, by Stefan Offermanns of the Pharmacological Institute at the University of Heidelberg, the unique effects of nicotinic acid on lipid metabolism are substantially better understood nowadays. In binding to its receptor (HM74A) in spleen cell membranes and on the surface of adipocytes (fat cells), nicotinic acid inhibits adenylylcyclase, which converts ATP into cAMP, a necessary mediator for the hydrolysis of triglycerides to free fatty acids (lipolysis). With lower blood levels of fatty acids, the liver reduces the synthesis of triglycerides and the formation of VLDL, which leads again to decrease of LDL and facilitates through the cholesterol ester transfer protein (CETP) in humans a massive rise in HDL. [370]

Because of the flush syndrome, nicotinic acid was initially marketed in the USA only in 1997, after the development of an extended-release form. In Germany, Merck KGaA launched the drug as *Niaspan*® for the first time in 2004.

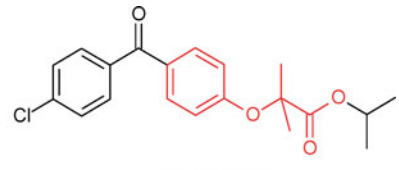
Now, Merck reported in the wake of results from a combination study, that it had withdrawn all its niacin containing drugs.

Fibrates

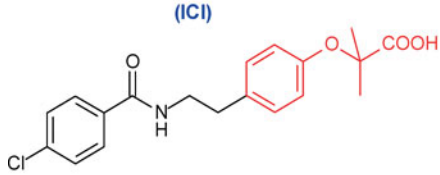
In awareness of the lipid-lowering effect of oestrogen observed in the 1960s, ICI conducted a targeted search for substances, which possess no oestrogenic but lipid-lowering properties. They struck it lucky with compounds related to 2-phenoxyisobutyric acid (red). In 1968, clofibrate came to the market. There followed a series of other drugs with similar structures and the same mode of action.



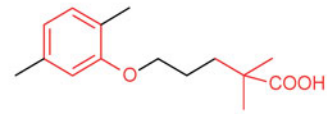
Clofibrate
(ICI)



Fenofibrate
(Orchimed)



Bezafibrate
(Boehringer Mannheim)

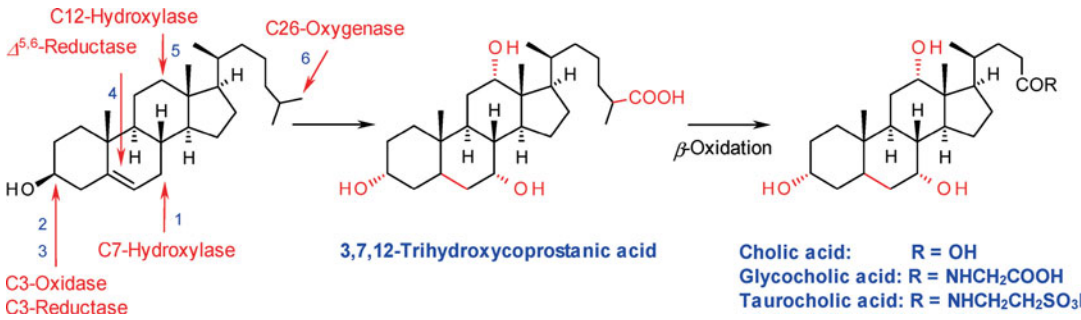


Gemfibrozil
(Parke Davies)

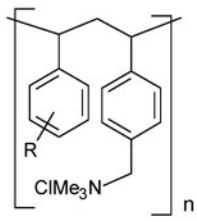
However, this mechanism remained unknown until the 1990s. [371] With the discovery of the peroxisome-proliferator-activating receptors (PPARα-γ), the effects on lipid metabolism could be understood. The endogenous ligands are fatty acids and eicosanoids, whereof polyunsaturated fatty acids turned out to be particularly strong receptor activators. The fibrates are mimetics of fatty acids, which act selectively on the PPARα, the central regulator of hepatic lipid metabolism. Its activation leads to an increased HDL level and fatty acid degradation, a drop in triglyceride synthesis and reduced VLDL secretion.

Colestyramine and Ezetimibe

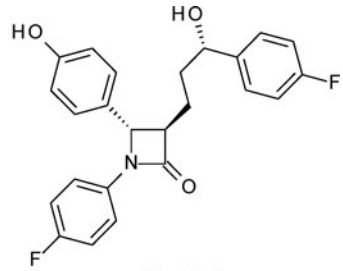
In the 1950s and 1960s anion-exchange resins, such as colestyramine, were developed with the aim of binding bile acids in the intestine, which originate from cholesterol elimination in the liver, and thus disrupt their enterohepatic circulation. The sequestered bile acids are then excreted with the faecal stools. Since the transport of the bile acids back to the liver can no longer occur, the serum cholesterol level drops.



The cholesterol resorption inhibitor ezetimibe, introduced to the market in 2003 as *Zetia*® by the Merck company, works as well as a sequestrant. The compound localises at the brush border of the small intestine and selectively blocks the transport of cholesterol into the circulation and its liver storage. The reduction of the exogenous cholesterol metabolism leads eventually to a drop in LDL cholesterol and a rise in HDL levels. [372]



Colestyramine
(Bristol-Myers, Merck)



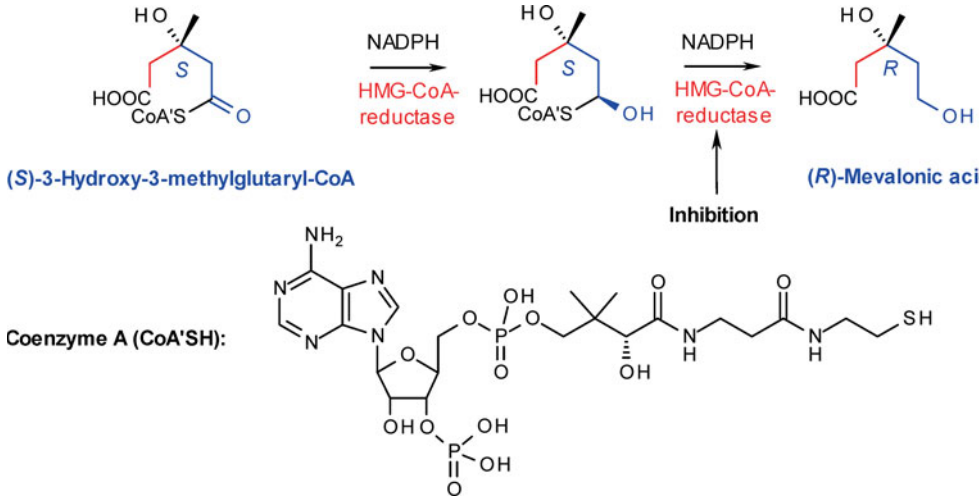
Ezetimibe
(Schering-Plough, Merck)

5.9.4 Discovery of HMG-CoA-Reductase Inhibitors (Statins)

The first drugs to inhibit HMG-CoA-reductase emerged from the quest for new antibiotics. [373] Inspired by the epochal discoveries of penicillin by Alexander Fleming in 1928 and of streptomycin by Selman Abraham Waksman (1888–1973) in 1943, Akira Endo (Fig. 5.150) and Masao Kuroda at the Sankyo company in Tokyo began in 1971 to search fermentation broths for secondary metabolites, which interfere with the biosynthesis of steroids and isoprenoids. After two years of futile screening efforts and more than 6,000 tests, Endo and Kuroda discovered in the fermentation broth of *Pythium ultimum* citrinin, an active compound that turned out to be too toxic, and afterwards from a *Penicillium citrinum* culture a remarkably potent inhibitor of cholesterol biosynthesis, which was initially given the name ML-236B, compactin, and later mevastatin.

5.150 *Akira Endo.*

The further evolution of this project and the approach as a whole was favoured by tremendous fortune. The compounds were tested initially in rats. For lack of the material, these in-vivo assays were generally run only once. By repeated administration they noticed that the results were not reproducible. At this point, they might have abandoned their approach, and the whole research would have been in vain. Fortunately, however, they then also tested their substances in dogs, and later in laying hens, rabbits and monkeys. In all these cases they obtained results with good reproducibility. In hindsight it was clear why the results with rats were so inconclusive. Endo and Kuroda had chosen an unsuitable test model. Rats naturally have an extraordinarily low LDL level, and most cholesterol is bound in HDL, so that even small measuring errors translate into a large effect, when expressed as a percentage.

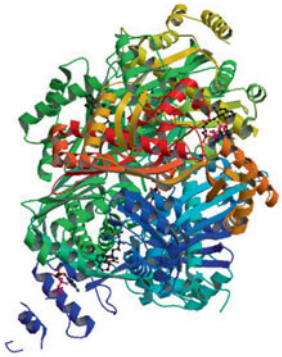


Cholesterol in a Breakfast Egg

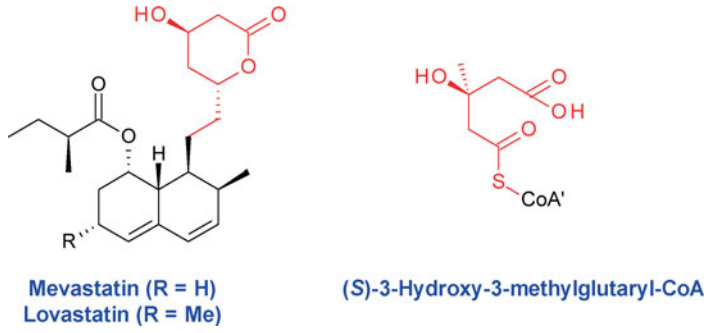
One egg contains 300 mg of cholesterol, of which around 100 mg is produced daily by *de novo* synthesisin a hen. The "supplementation" of hen feed with 0.1 % of mevastatin reduces the egg's cholesterol content by around 10–15 %. Laying hens make therefore a well-suited test model. [373]

Out of 600 litres of fermentation broth, after repeated chromatography on silicagel and crystallisation, they isolated 23 milligrams of the active compound. Endo could show that mevastatin inhibits the incorporation of acetate, but not of mevalonate, into cholesterol. He eventually discovered that mevastatin competitively inhibits HMG-CoA-reductase (Fig. 5.151).

With the structure determination of mevastatin in 1973, by a combination of degradation reactions, X-ray analysis and spectroscopic methods, the structural similarities to HMG-CoA were also recognised (Fig. 5.152).



5.151 *Human HMG-CoA-reductase with mevastatin.*



5.152 *Mevastatin and lovastatin differ from each other only by one methyl group, which is of negligible importance for the pharmacological activity.*

Like Sankyo, scientists at Beecham Pharmaceuticals in Britain were almost at the same time in search for new antibiotics and discovered mevastatin in the strain *Penicillium brevicompactum*; being unaware of the earlier Japanese work, they named the compound compactin. [374] However, their efforts were narrowly focussed on antibiotics. While they used exclusively rats in their experimental animal models, the enormous potential of the compound, which they held in their hands, went unrecognised.

Prompted by Endo's initial publications, Merck Sharp & Dohme signed an agreement with Sankyo to receive a sample of compactin. Once Merck confirmed Endo's results, they started their own search for HMG-CoA-reductase inhibitors in October 1978. In contrast to Endo, the group led by Alfred W. Alberts identified a compound with the desired activity after only 18 fermentation samples and two weeks of screening. Out of the fermentation broth of a mould, *Aspergillus terreus,* they isolated mevinolin, which they later named lovastatin. This compound has pharmacological properties very similar to those of mevastatin, and merely differs from the latter, just by a single methyl group on the ring system (Fig. 5.152).

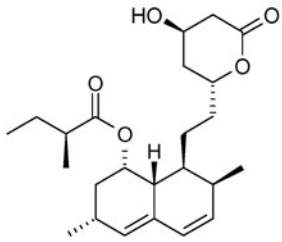
Endo left Sankyo at the beginning of 1979, and became an Associate Professor at the Noko University in Tokyo, where he continued his research on HMG-CoA reductase inhibitors. In August 1979, he isolated from the fungus *Monascus ruber* a compound, which he named monacolin K, but which soon proved to be identical with lovastatin.

In 1980, Sankyo surprisingly stopped the development of mevastatin, upon reports from chronic tox-studies of carcinogenic effects in dogs, observed at a 200-fold excess of the therapeutic dose. Worried about these findings, Merck Sharp & Dohme stopped their clinical development immediately. Several years later, and encouraged by additional experimental results, Merck resumed the development of lovastatin. In retrospect it emerged that this compound is carcinogenic neither in humans nor in the experimental animals. In 1984, large-scale clinical trials were initiated with more than 1,200 patients. In 1987, lovastatin won approval by the Food and Drug Administration (FDA) as the first statin for the treatment of hypercholesterolemia, and the product was launched in the USA as *Mevacor*®.

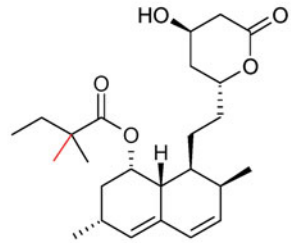
Prompted by the success with lovastatin, Merck developed the semi-synthetic statin simvastatin, which differs from the former by an additional methyl group in its side-chain. Pravastatin was the first statin to be marketed by Sankyo in 1989. It is obtained from mevastatin by microbial hydroxylation. The efficacy of these statins differs in patients very little from that of lovastatin.

All other statins, which entered the market, are produced by total synthesis (Fig. 5.153). Fluvastatin is marketed as the racemate. After the *Lipobay*® scandal, cerivastatin was withdrawn from the market worldwide in 2001, due to reports of fatal rhabdomyolysis associated with renal failure. The reasons behind these toxicities were a too narrow therapeutic window (effective dose compared to toxic dose) and the drug interaction with fibrates, especially with gemfibrozil.

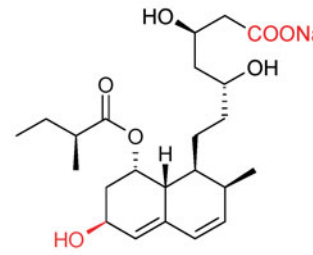
Natural and semisynthetic Statins



Lovastatin, IC_{50} = 3-11 nM
(Merck, 1987)

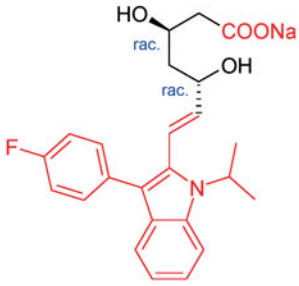


Simvastatin, IC_{50} = 2,7-11 nM
(Merck, 1988)

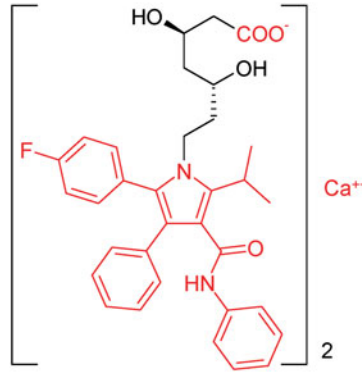


Pravastatin, IC_{50} = 6,9 nM
(Sankyo, BMS, 1989)

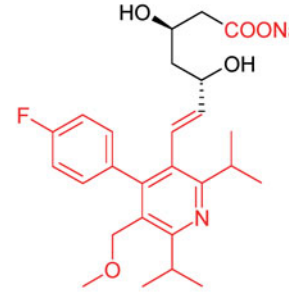
Synthetic Statins



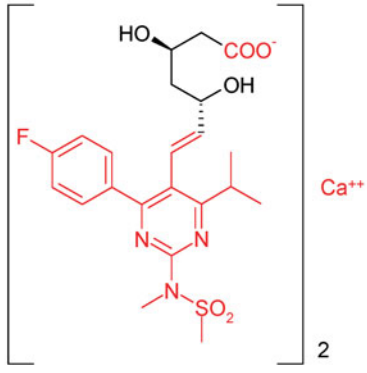
Fluvastatin, IC_{50} = 3,8-28 nM
(Sandoz, Novartis, 1994)



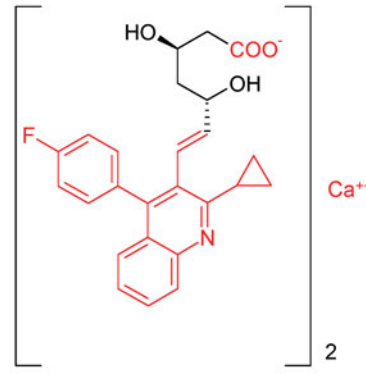
Atorvastatin, IC_{50} = 1,2-8 nM
(Warner-Lambert, Pfizer, 1997)



Cerivastatin, IC_{50} = 3,5-10 nM
(Bayer, 1997)



Rosuvastatin, IC_{50} = 0,2-5 nM
(Shionogi, AstraZeneca, 2002)

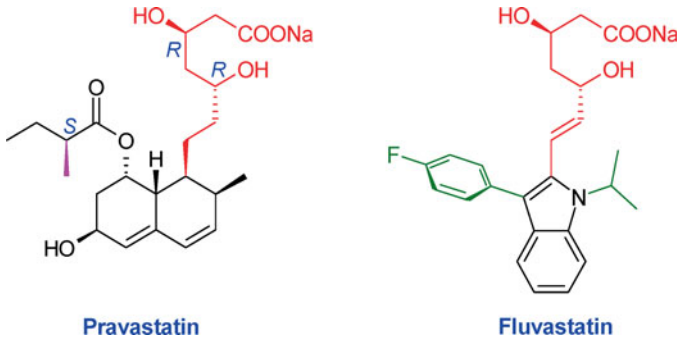


Pitavastatin, IC_{50} = 6,8 nM
(Nissan, 2003)

5.153 *Natural, semi-synthetic and totally synthetic statins for the treatment of hypercholesterolemia.*

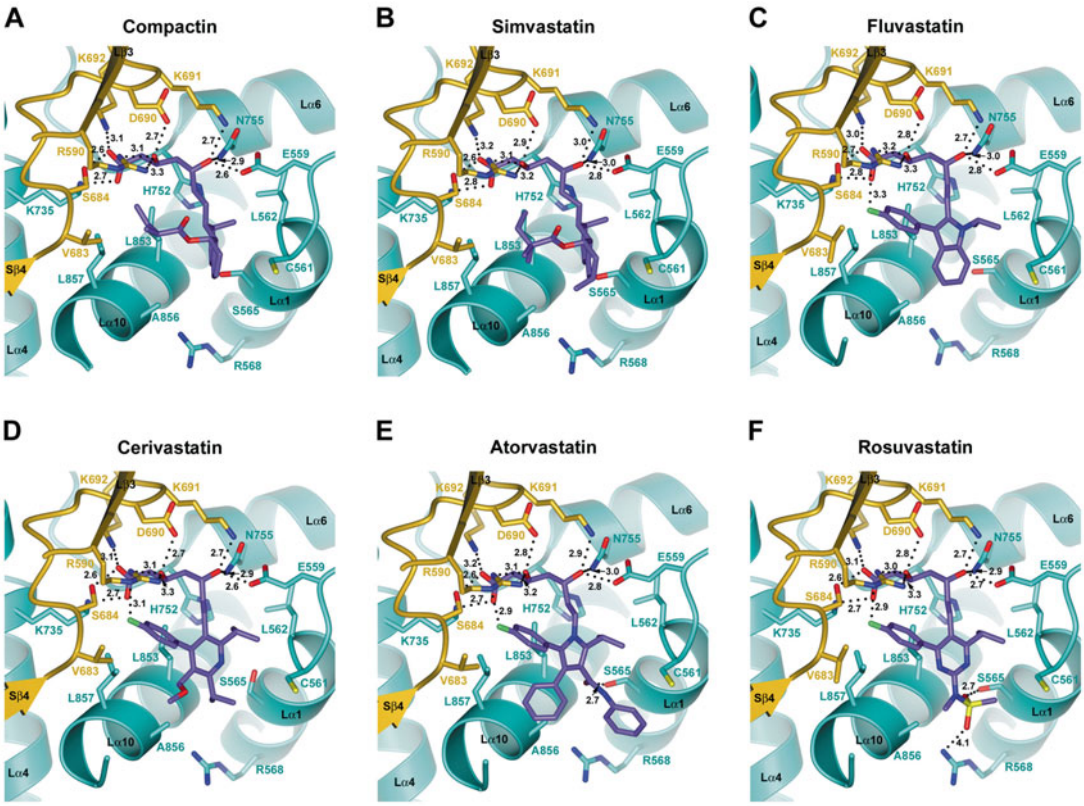
5.9.5 Mode of Action and Structure-Activity Relationships

All statins block competitively and reversibly the HMG-CoA reductase. By comparison with the natural substrate, (*S*)-3-hydroxy-3-methylglutaryl-CoA ($K_m = 4$ µM), they possess a more than 1,000-fold higher enzyme affinity. [375] The 3,5-dihydroxyheptanoyl moiety (shown in red) (Fig. 5.154) is indispensable for the activity of the statins. The lactone moieties of mevastatin, lovastatin and simvastatin have prodrug character, and are rapidly hydrolysed in the intestinal mucosa and the liver. The stereochemistry of both hydroxy-groups is critical for the drugs' activity, indicating that the statins act as *transition state analogue* inhibitors to block the reduction of the hemithioacetal intermediate after the first reduction step. [376] On the other hand, the absolute configuration of the 2-methylbutanoyl residue has hardly an impact. The hexahydronaphthalene moiety can only be replaced by a cyclohexane ring under considerable loss of activity. In the case of heterocyclic statins, these should be substituted in the vicinity of the pharmacophore (red) by a non-coplanar *p*-fluorophenyl group and on the opposite side by a branched aliphatic substituent (shown in green).



5.154 *Structure-activity relationships.*

The crystal structure of human HMG-CoA reductase with various statins shows that these in fact occupy the binding pockets for the (*S*)-3-hydroxy-3-methylglutaryl residue and also in part for the Coenzyme A site (Fig. 5.155). All of the statins investigated show a very similar binding mode. Surprisingly, none of the statins reaches the NADPH binding pocket, which would promise an even greater binding affinity and stronger efficacy. [375, 377]

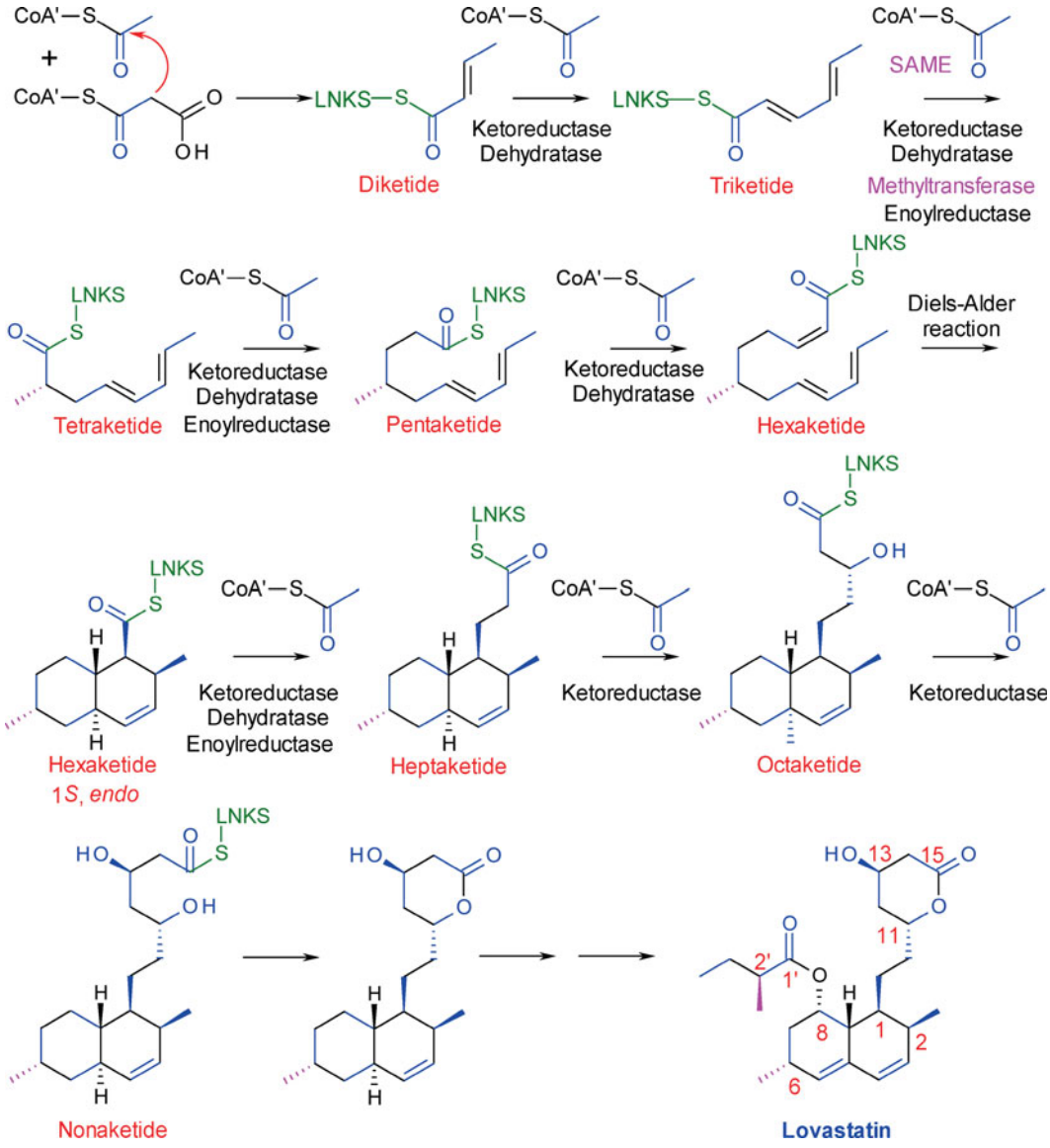


5.155 *Structural analysis of human HMG-CoA reductase with various statins.*

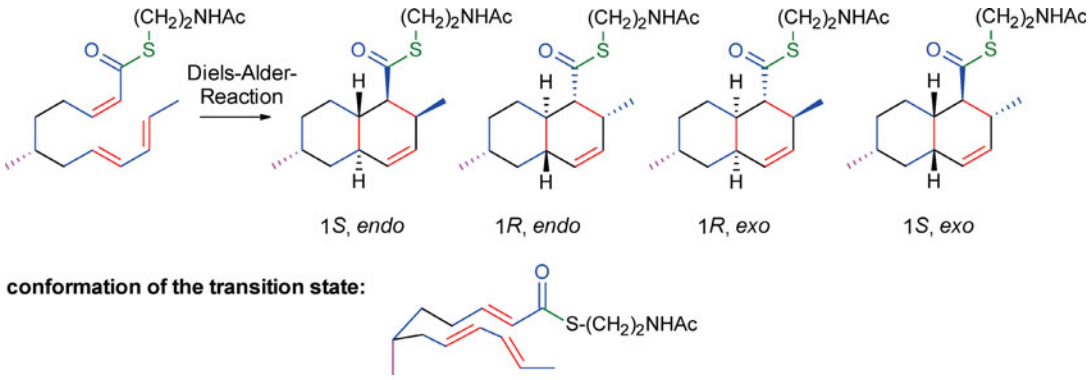
5.9.6 Biosynthesis

The biosynthesis of lovastatin was elucidated with the aid of feeding studies on *Aspergillus terreus* with ^{13}C-, ^{18}O- and ^{2}H-labelled acetate by John C. Vederas at the University of Alberta. [376, 378, 379] Lovastatin is a polyketide, which consists of a C_{18}- and a C_4-chain. These are constructed by head-to-tail coupling of acetate units by lovastatin-nonaketide synthase (LNKS). The biosynthesis resembles that of fatty acids, although some of the reduction steps are by-passed, whereby the carbon skeleton is functionalised with hydroxy-groups and double bonds. The nonaketide is cleaved off from the lovastatin-nonaketide synthase and then further functionalised by other enzymes. The oxygen atoms at C-11, C-13, C-15 and C-1' originate from acetate, whereas the one at C-8 is incorporated from the atmospheric oxygen. Both of the methyl groups, at C-6 and C-2', are transferred to the ketide by *S*-adenosylmethionine (SAME). The decalin skeleton is the product of an *endo*-selective Diels-Alder reaction.

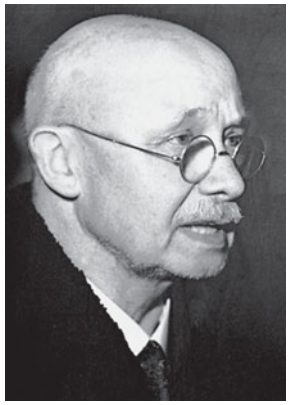
Until recently, it was unclear whether Nature's repertoire also comprises enzyme-catalysed Diels-Alder reactions. [380, 381] Thus, the elucidation of the



biosynthetic route to lovastatin was a sensation. It provided, apart from a few other examples, proof that Diels-Alderases do exist. The Diels-Alder reaction takes place at the hexaketide stage, because only this possesses an acceptor-substituted dienophile. A hydrogen bond to the carbonyl oxygen in the active centre of the enzyme thereby lowers the electron density of the dienophile. In model reactions with *N*-cystamine acetate-substituted hexaketide, out of the four conceivable stereoisomers only the (1*S*)-*endo*-isomer is obtained in presence of intact lovastatin-nonaketide synthase (Fig. 5.156). The enzyme appears to bind the substrate in a conformation, which resembles that of the (1*S*)-*endo*-producing transition state. [382]



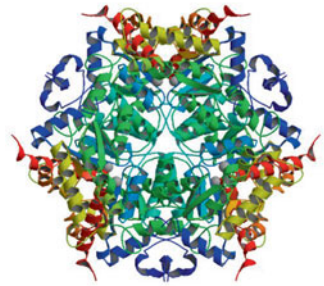
5.156 *Model Diels-Alder reaction at the hexaketide stage.*



5.157 *Otto Paul Hermann Diels (1876–1954). He and his student, Kurt Alder (1902–1958), discovered the [4 + 2] cycloaddition reaction at the University of Kiel, Germany in 1928, for which they were awarded the Nobel Prize in Chemistry in 1950. [384]*

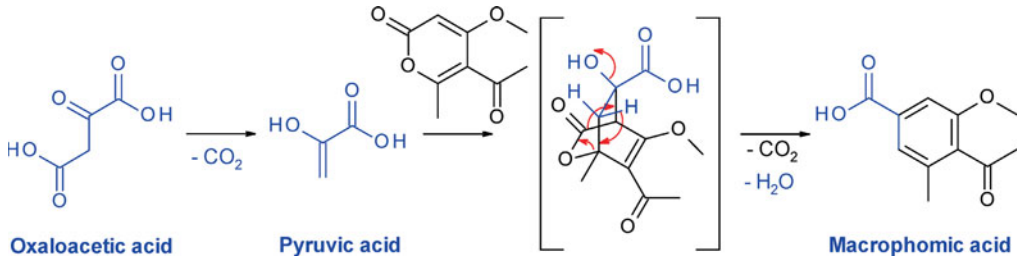
Enzyme-catalysed Diels-Alder Reactions

The participation of enzymes in Diels-Alder reactions (Fig. 5.157) was postulated for more than 100 natural products. [381, 383] However, the structure of Diels-Alderases, and therefore the mechanism of the biocatalysed [4 + 2]-cycloaddition, remained undetermined for a long time. Other enzyme-catalysed electrocyclic reactions, like the Cope rearrangement of chorismic acid into prephenic acid by chorismate-mutase, were already known for a while and are well investigated. The first determined structure of a Diels-Alderase was that of the macrophomate-synthase of the phytopathogenic fungus *Macrophoma commelinae* (Fig. 5.158).



5.158 *By complexation of diene and dienophile on the macrophomate-synthase, the [4 + 2]-cycloaddition is both electronically and sterically favoured.*

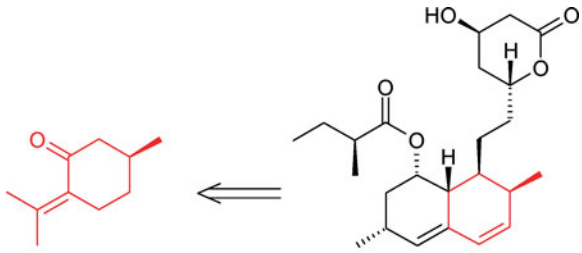
Macrophomic acid, a derivative of benzoic acid, is surprisingly synthesised neither *via* the shikimate nor the polyketide pathway, but in an intermolecular Diels-Alder reaction starting from oxaloacetic acid and a 2-pyrone.



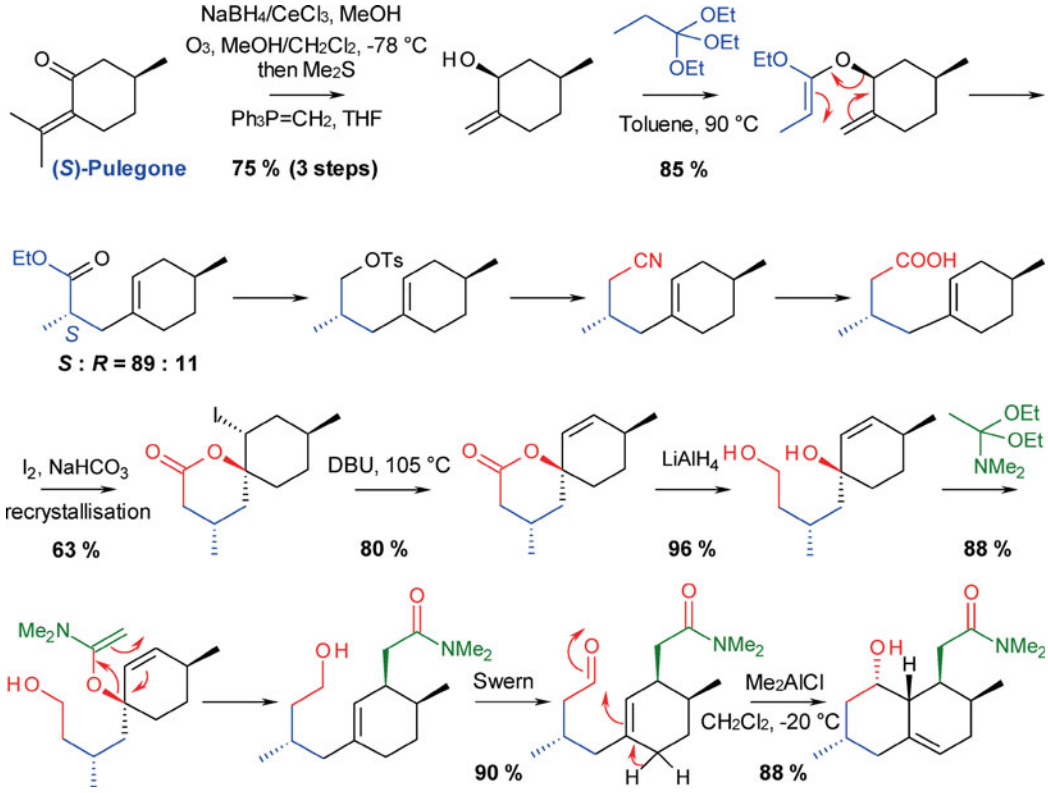
In the first step, the oxaloacetic acid is decarboxylated. Thereby, the enolate form of the pyruvic acid is stabilised with a magnesium ion in the macrophomate-synthase. This then undergoes the Diels-Alder reaction with the pyrone favourably positioned for the cycloaddition, while the enzyme is activated by hydrogen bonding. The *anti*-elimination of water and carbon dioxide leads ultimately to macrophomic acid.
However, the possibility of a Michael-aldol reaction should be mentioned as an alternative route.

5.9.7 Total Synthesis

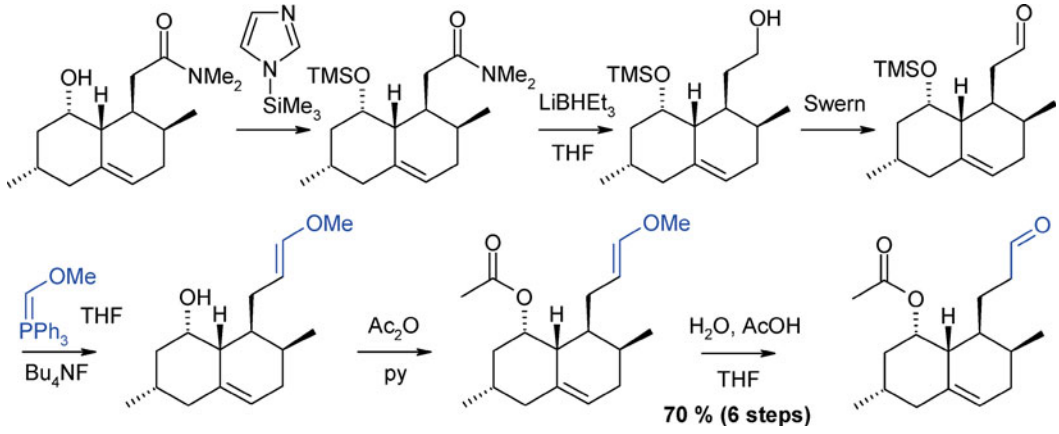
It is presumably unnecessary mentioning that molecules like mevastatin and lovastatin attracted even shortly after their discovery the undivided interest of many natural product chemists. Consequently, in the course of a few years a series of total syntheses was published. [385–388] Only one of these, which are without exception highly interesting and attractive syntheses, will be presented here. [389] The starting material for Hoffmann-La Roche's synthesis is (*S*)-pulegone, the stereogenic centre of which then determines in highly diastereoselective reactions all other stereocentres in the hexahydronaphthalene and lactone fragments.



In the first segment of the synthesis, (*S*)-pulegone is converted by stereoselective reduction of the keto-group, ozonolysis and a Wittig reaction into an allylic alcohol. By means of an orthoester-Claisen rearrangement, the second stereocentre is generated in the hexahydronaphthalene-ring system. Subsequent to the homologation [390] and formation of the iodo-lactone, the main enantiomer is purified by crystallisation. After the elimination of hydrogen iodide and reduction of the ester, the Eschenmoser variant of the Claisen rearrangement is used to introduce the side-chain in a stereospecific manner. The hexahydronaphthalene ring is finally obtained by Swern oxidation and a stereospecific ene-reaction with an excess of dimethylaluminium chloride. The strong stereodirecting effect of both substituents in the cyclohexenyl ring is responsible for the 1,3-diaxial orientation of the hydroxy- and methyl groups in the product.



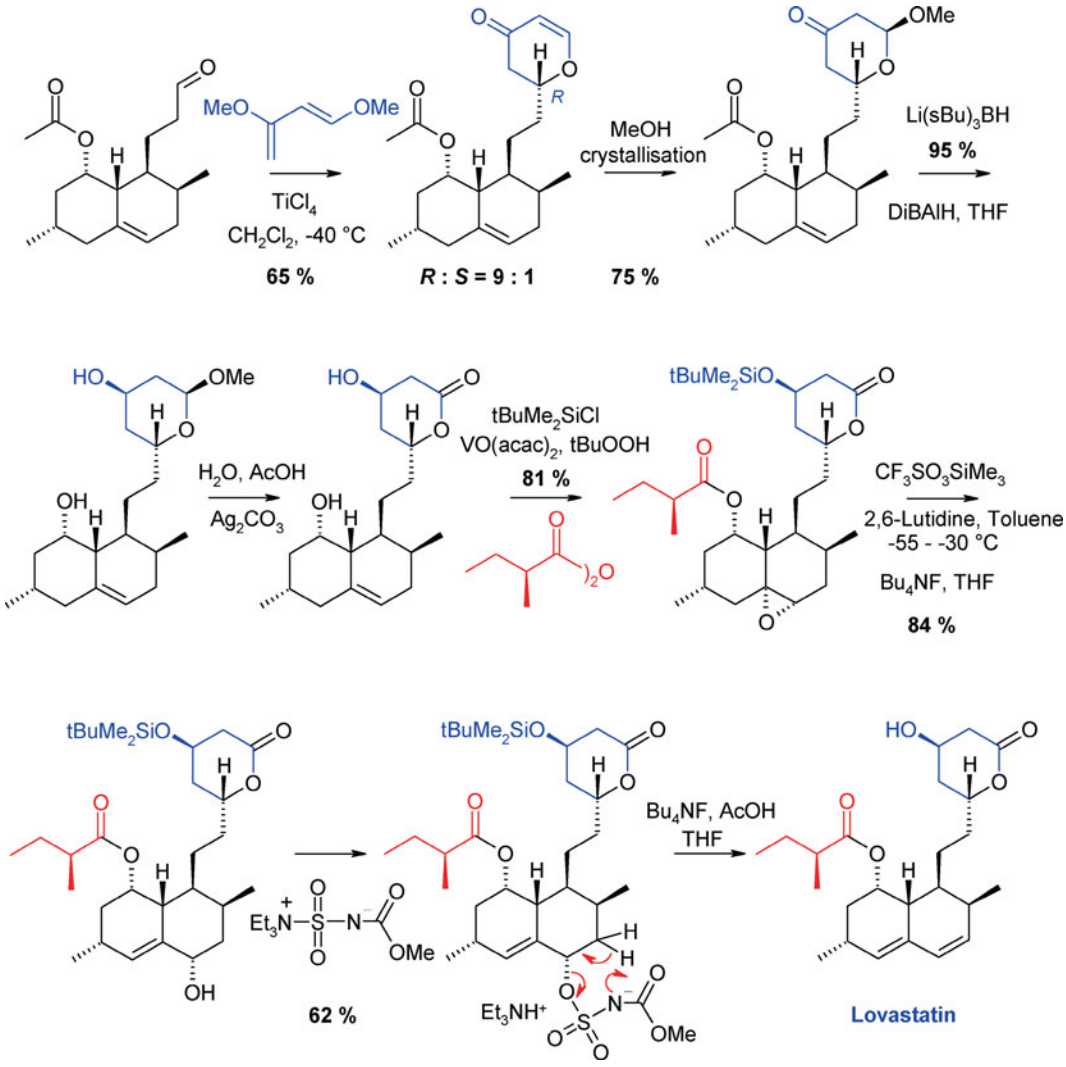
In the second segment of the synthesis, the buildup of the side-chain is initiated. After silylation of the alcohol, the amide function is converted into the corresponding aldehyde and then homologated by a Wittig reaction.



The most remarkable step in the third segment is the diastereoselective Diels-Alder reaction of the aldehyde with the Danishefsky diene to form the pyranone. It appears that the macrocyclic surroundings, which arise from complexation of

the aldehyde and the acetate to the Lewis acid, shield very effectively the desired diastereotopic side of the aldehyde. The purification is successful by crystallisation at the methanol-adduct stage. After stereoselective reduction of the pyranone and reductive cleavage of the acetate, the acetal is hydrolysed and directly oxidised to the lactone with silver carbonate.

Following regioselective monosilylation, the double bond is stereospecifically epoxidised. The transformation with trimethylsilyl triflate and desilylation generates the allylic alcohol, which is converted into the diene using the Burgess reagent. In the last step, the *t*-butyldimethylsilyl group is cleaved off.



After around 30 stages, lovastatin is obtained in an overall yield of under 3 %. Fascinating key steps in the total synthesis are the diastereoselective Claisen rearrangements, the ene-reaction and the Diels-Alder reaction.

5.9.8 Industrial Syntheses

In spite of a multitude of total syntheses of statins, those which occur naturally are obtained on an industrial scale by fermentation, and in the case of simvastatin, by partial synthesis. Only those statins containing a nitrogen heterocycle are obtained by total syntheses. [391] Hereafter, selected representative examples are presented.

Lovastatin

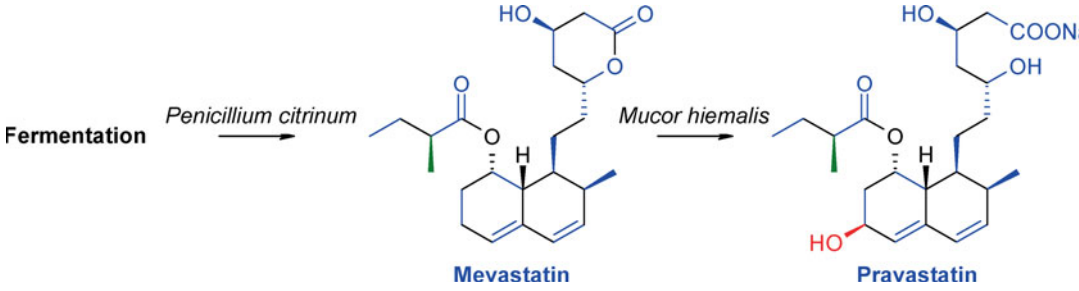
Lovastatin (*Mevacor*®, Merck) is produced by fermentation of *Aspergillus terreus* or *Monascus ruber* strains. The worldwide demand is estimated at around 300 tonnes per year. Production at Merck began in 1980. In the course of process development, important fermentation parameters, like culture homogeneity, the influence of various carbon sources, the pH-value, the aeration and the stirrer geometry, were optimised. By precise pH control and slow dosing of the carbon source, mostly glycerol, the productivity could be increased five-fold. When scaling-up from 800 to 19,000 litres, it was noticed that the oxygen supply limited the productivity due to the high viscosity of the fermentation broth. This problem was solved by an improved stirrer design. Eventually, the Finnish company Metkinen Oy, using the *Aspergillus terreus* ATCC 20542 strain, achieved a productivity of 7–8 g/l. [392]

With the expiry of the Merck patent in 2001, today the largest production capacity is located in China and India. Biocon in Bangalore was the first Indian company to be granted a licence from the FDA for the production of a pharmaceutical by fermentation.

Lovastatin, being a marketed product itself, serves also as the starting material for the preparation of simvastatin.

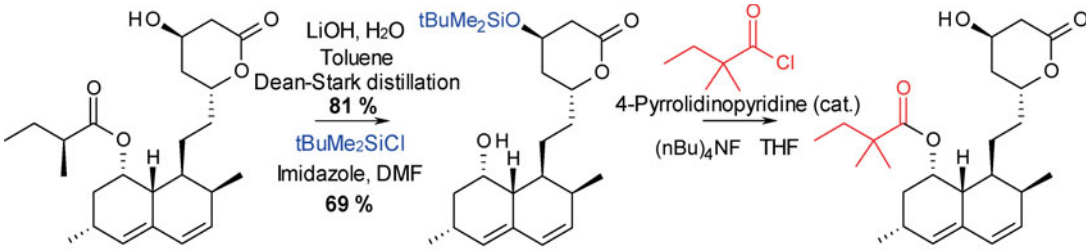
Pravastatin

For the preparation of pravastatin (*Mevalotin*®, Sankyo), at first mevastatin is obtained from fermentation of *Penicillium citrinum*; its regio- and stereo-selective enzymatic hydroxylation can be achieved with a number of different microbial strains: *Mucor hiemalis*, *Penicillium coccineus*, *Rhizoctonia solani* or *Nocardia*. *Streptomyces carbophilus* is used today to produce mevastatin; under optimised conditions a productivity of around 5 g/l or even higher can be obtained. [392]

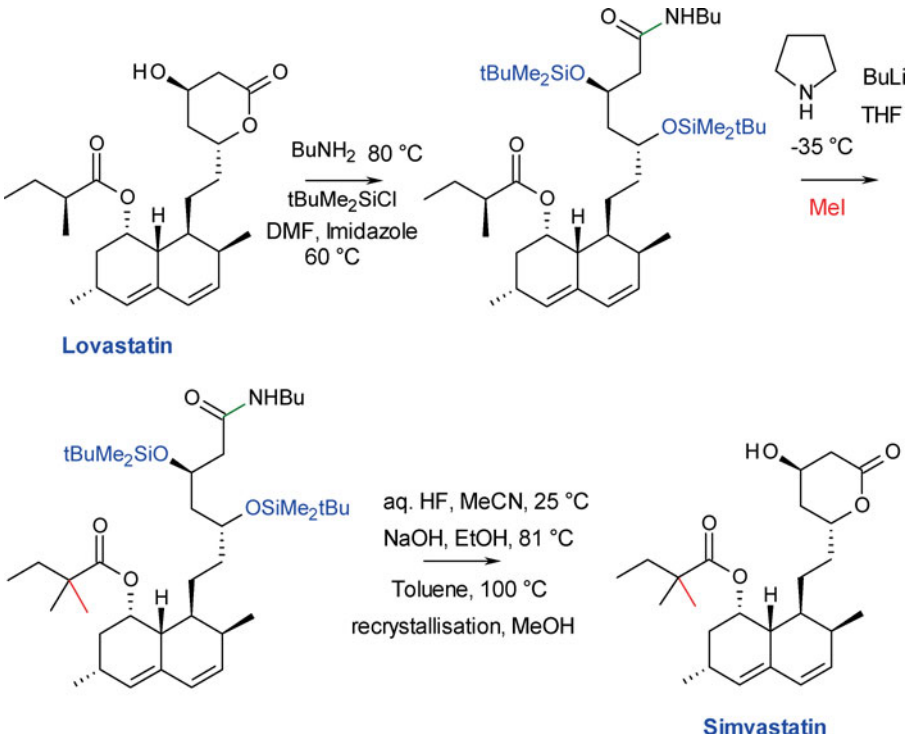


Simvastatin

Simvastatin (*Zocor*®, Merck) is prepared from lovastatin. The methylbutyroyl ester is first hydrolysed with lithium hydroxide, whereby the lactone is opened as well. The latter is cyclised again by azeotropic distillation with toluene. After regioselective protection of the hydroxy-group in the lactone ring, the intermediate is esterified with dimethylbutyryl chloride, and the silyl protecting group is finally cleaved off.



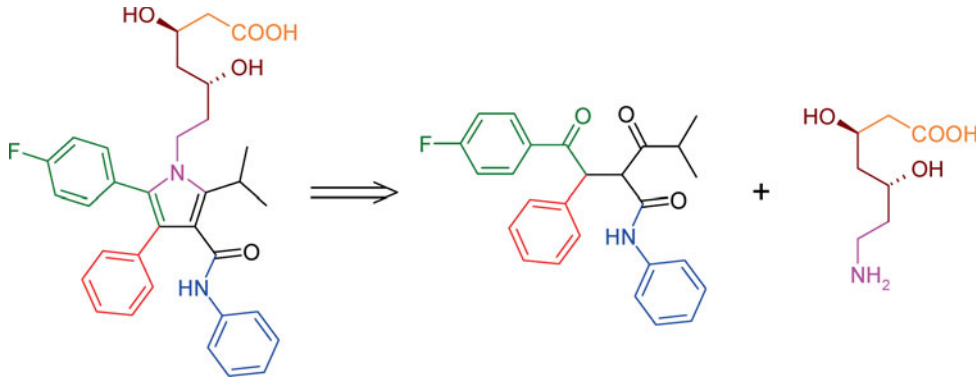
Alternatively, the α-position of the side-chain of lovastatin can be directly methylated, if this is first converted into the butylamide and the alcohol functions are silylated or protected as their THP ether. Simvastatin is then obtained by removal of the protecting groups, lactonisation and purification by recrystallisation from methanol. [393, 394]



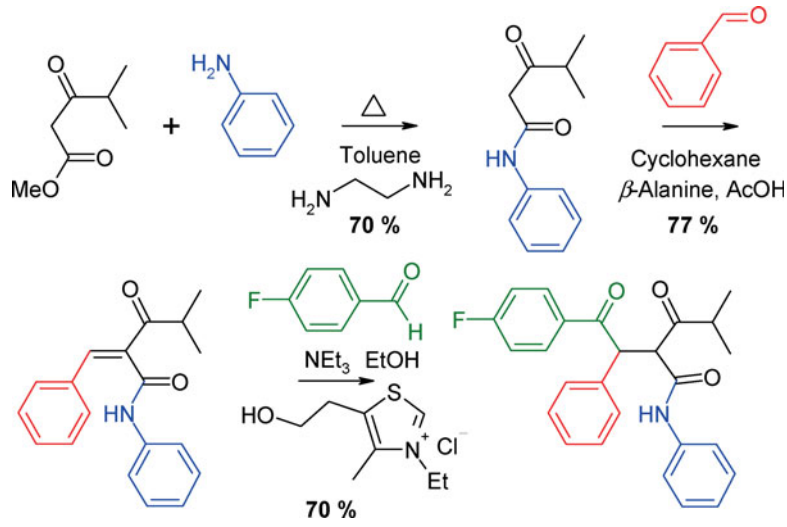
Atorvastatin

Since 2001, atorvastatin had been the best selling drug worldwide (peak sales were reached in 2008 with 15.2 billion dollars). The annual demand has in the meantime reached more than 200 tonnes. The drug was originally developed by Warner Lambert and co-marketed with Pfizer in 1997 under the trade name *Lipitor*®, until the strategically driven company acquisition by Pfizer in 2000. With the patent expiration in November 2011, atorvastatin became generic in 2012.

The compound's core comprises a pentasubstituted pyrrole, which results from a convergent synthesis with a Paal-Knorr reaction as its crucial step. This succeeds only under specially designed conditions; the reaction faced initially a seemingly insurmountable obstacle, and therefore a range of linear syntheses had been developed as well. Although they are scientifically very interesting, they will not be discussed here in any greater detail. [395, 396]



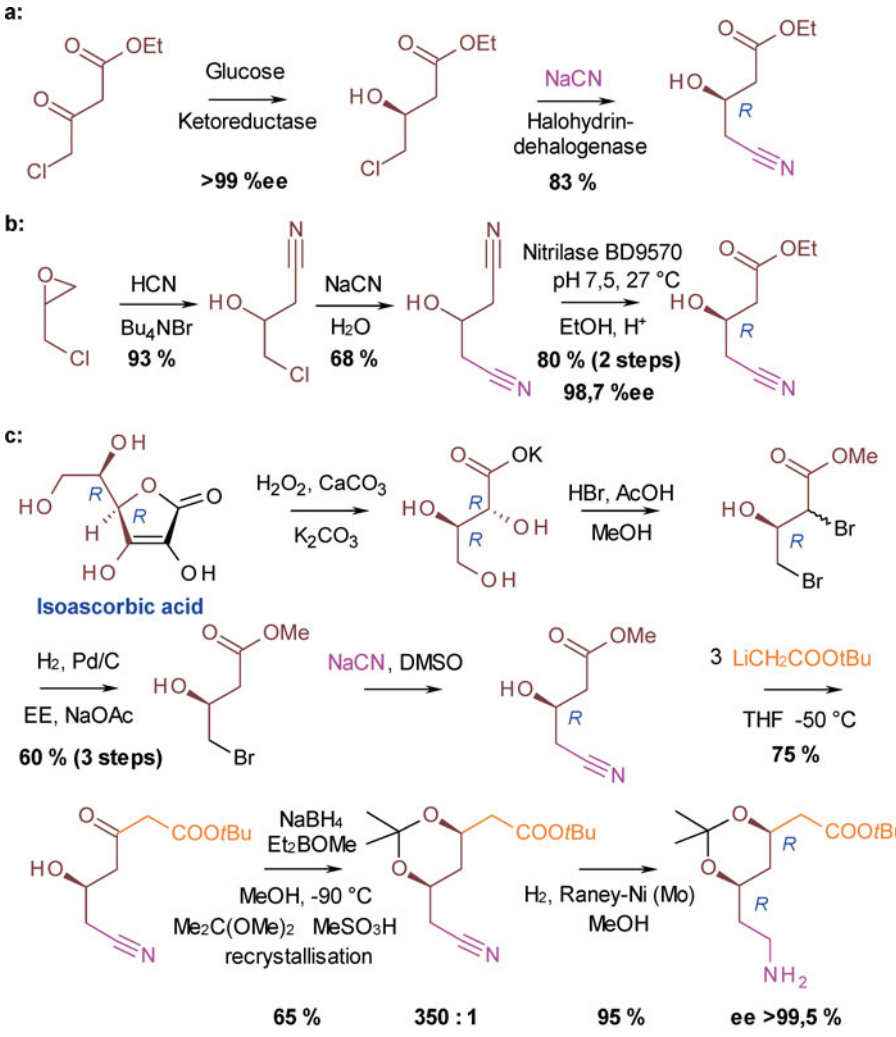
The key step for building the 1,4-diketone, subsequently used in the Paal-Knorr pyrrole synthesis, is a Stetter reaction, whereby *p*-fluorobenzaldehyde is added to a β-ketoamide in presence of a thiazolium salt.



Essential for the economics of the overall process is a simple way to make the amino-component available. A pivotal intermediate is therefore the (*R*)-4-cyano-3-hydroxybutyrate ester, which is accessible by various routes. [397] In variant **a**) ethyl 4-chloroacetoacetate is reduced enantioselectively with a ketoreductase, and the chlorine is replaced enzymatically by cyanide (Codexis' technology). [398]

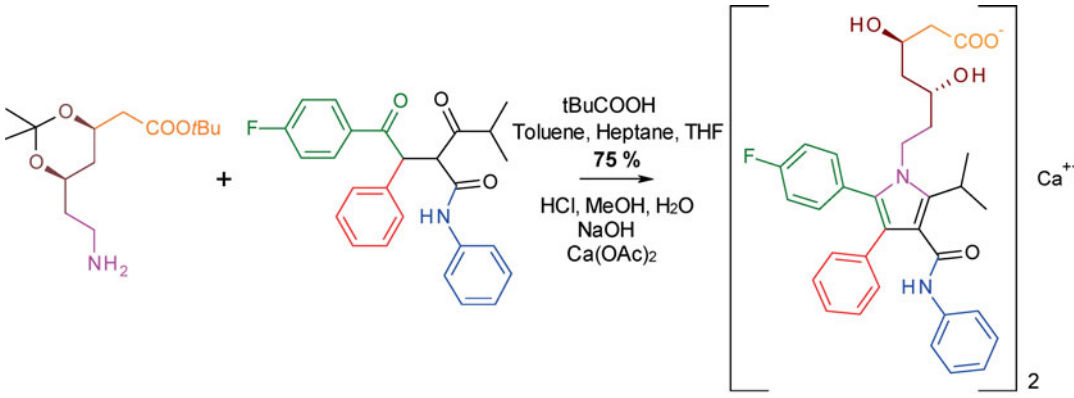
Another attractive route (variant **b**) is the double substitution of epichlorohydrin by cyanide and the subsequent enzymatic hydrolysis of one nitrile function (Dowpharma's process). [399, 400]

Alternatively (variant **c**), it is also possible to start from isoascorbic acid, which already possesses the desired substitution pattern and the required stereochemistry. The oxidative degradation of isoascorbic acid provides the same synthetic building block after a few steps. [401, 402]



A very efficient chain extension consists of the reaction with the lithium salt of *t*-butyl acetate. The *δ*-hydroxy-*β*-keto-ester is reduced with high selectivity, using sodium borohydride/diethylmethoxyborane, to the *syn*-1,3-diol with a diastereomeric ratio of 100:1. The acetonide is a well-crystallisable substance; its purification in a single recrystallisation step results in a diastereomeric ratio of 350:1. Reduction of the nitrile function with molybdenum-doped Raney nickel produces the side-chain.

The Paal-Knorr synthesis succeeds especially well in presence of one equivalent of pivalic acid, in a solvent mixture of toluene/heptane/THF at a ratio of 1:4:1. Deprotection and salt formation with calcium acetate finally yield atorvastatin.



Atorvastatin

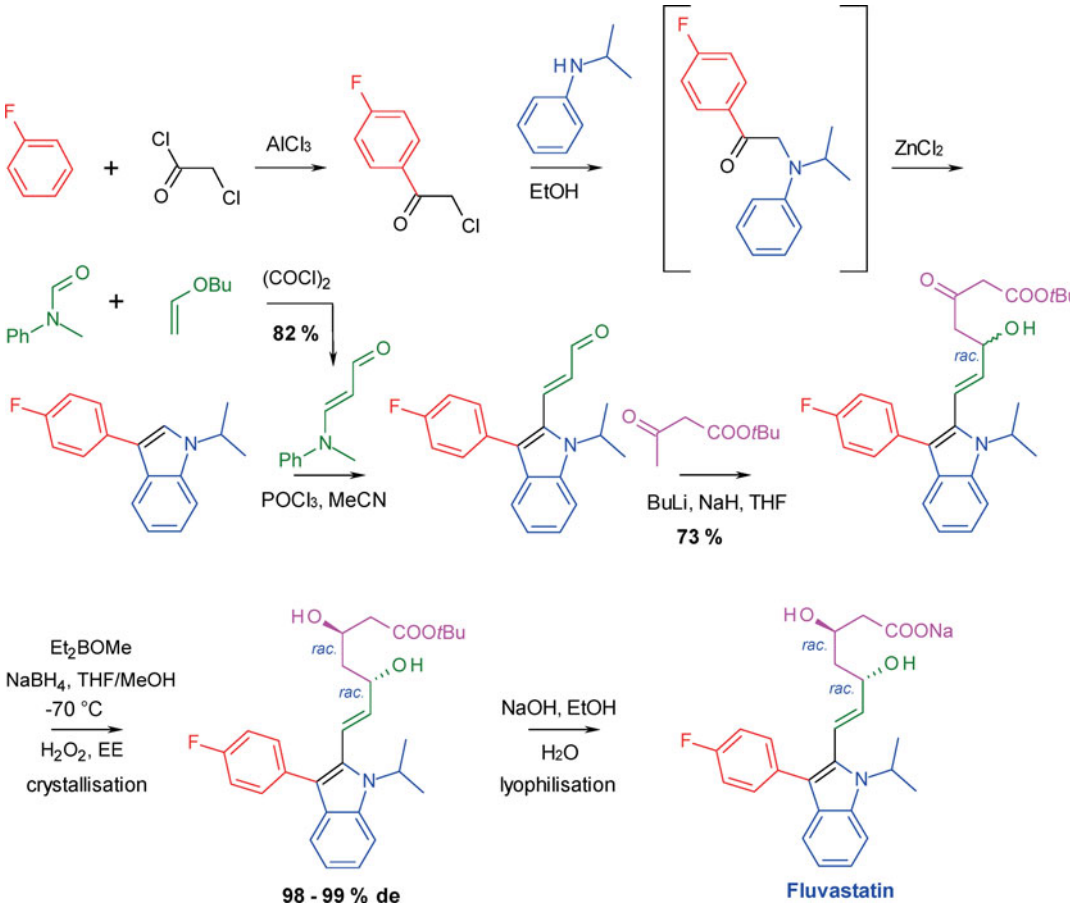
All the other structures of marketed statins contain a double bond in their side-chain. This indicates, compared with atorvastatin, additional challenges for a synthetic strategy.

Fluvastatin

Fluvastatin (*Lescol*®, Novartis) is synthesised in a linear sequence of only six stages starting from *α*-chloro-4-fluoroacetophenone, which in turn is prepared by a Friedel-Crafts acylation of fluorobenzene with chloroacetyl chloride. [403] After reaction with *N*-isopropylaniline, the indole ring is closed in a "one-pot" reaction with zinc chloride (a Bischler-Möhlau-type indole synthesis with stoichiometric amounts of the aniline). An important step in the synthesis is the following Vilsmeier reaction with the vinylogous aldehyde, *N*-methyl-*N*-phenylaminoacrolein, which is likewise obtained in good yield from the Vilsmeier reaction of *N*-methyl-*N*-phenylformamide and butyl vinyl ether. The preparation and the reactions of *N*-methyl-*N*-phenylaminoacrolein proceed considerably faster, more uniformly, and in higher yields than the corresponding synthesis with DMF as starting material.

The condensation with *t*-buylacetoacetate delivers the starting material for the diastereoselective reduction to the racemic *syn*-1,3-diol. The reason for using the *t*-butyl ester is, that this, unlike a methyl or ethyl ester, does not cyclise subsequently to a lactone, which moreover would epimerize readily at the allyl

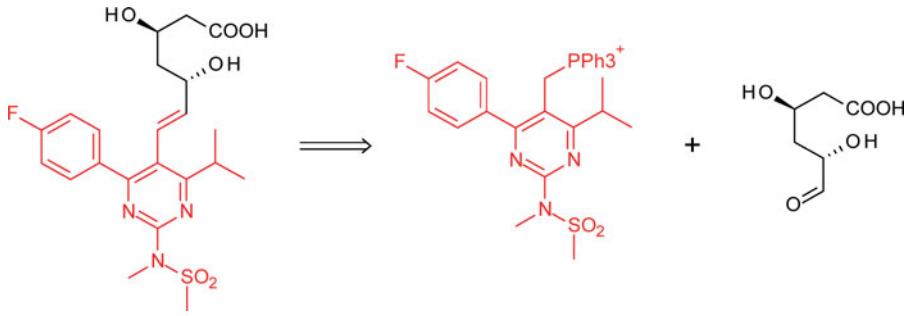
position to give the *anti*-diol. Furthermore, the yield of the addition and the stereoselectivity of the reduction are also increased by using the *t*-butyl ester. The following addition of hydrogen peroxide serves to simplify the separation of the excess reducing agent in the form of borate. Since the *t*-butyl ester of fluvastatin is a solid, it can easily be purified by crystallisation. The active compound is finally obtained by hydrolysis with sodium hydroxide and freeze-drying.



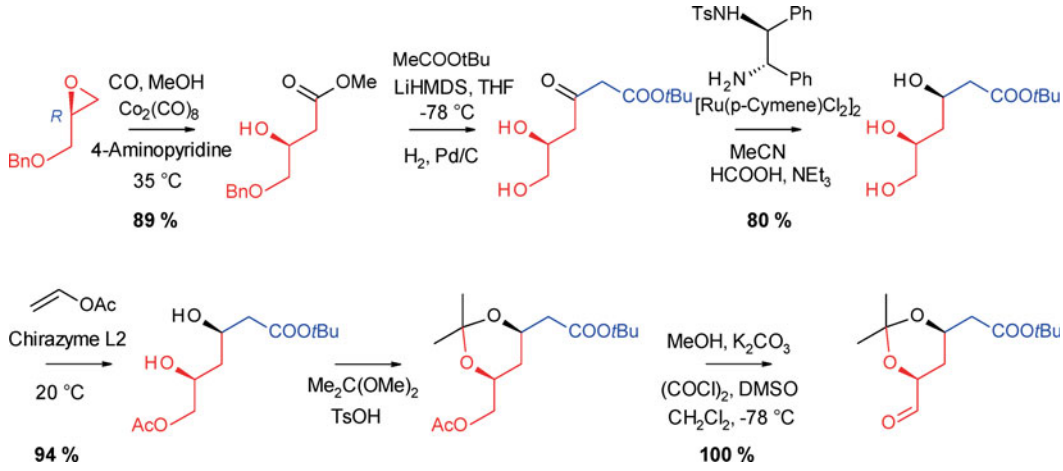
Rosuvastatin

Rosuvastatin was discovered by Shionogi and developed together with AstraZeneca. It was marketed in 2002 under the trade name *Crestor*®.

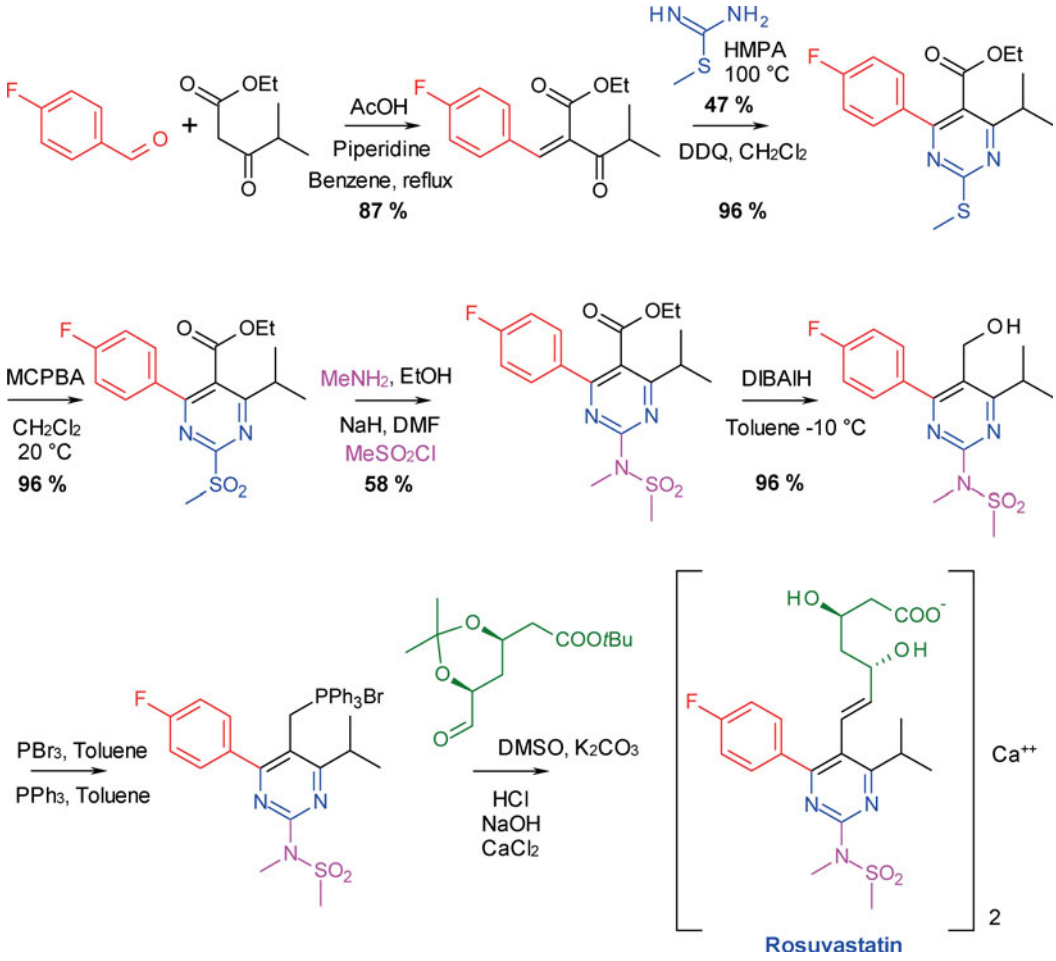
The enantiomerically pure side-chain of the drug is attached by a Wittig reaction. While in the Shionogi synthesis the side-chain bears the phosphonium salt, AstraZeneca developed a process, in which the side-chain contains the aldehyde function.



The starting material for the side-chain is (*R*)-glycidyl benzyl ether, which is first transformed by methoxycarbonylation into the corresponding *β*-hydroxyester, followed by a condensation with *t*-butyl acetate. The enantioselective transfer hydrogenation with a ruthenium catalyst produces *t*-butyl 3,5,6-trihydroxyhexanoate. Its primary alcohol function is regioselectively esterified with a lipase. After protection of the two other alcohol functions and methanolysis of the acetate, the desired building block is obtained by Swern oxidation. [404]



The syntheses of the pyrimidine with *S*-methylisothiourea and of the methanesulfonamide by oxidation and substitution proceed along standard methods. After reduction of the ester with DIBAlH to the alcohol, the latter is converted into the phosphonium salt. The Wittig reaction leads first to the protected rosuvastatin *t*-butyl ester, from which the protecting groups are cleaved off in acid, and the active compound is finally precipitated as its calcium salt.



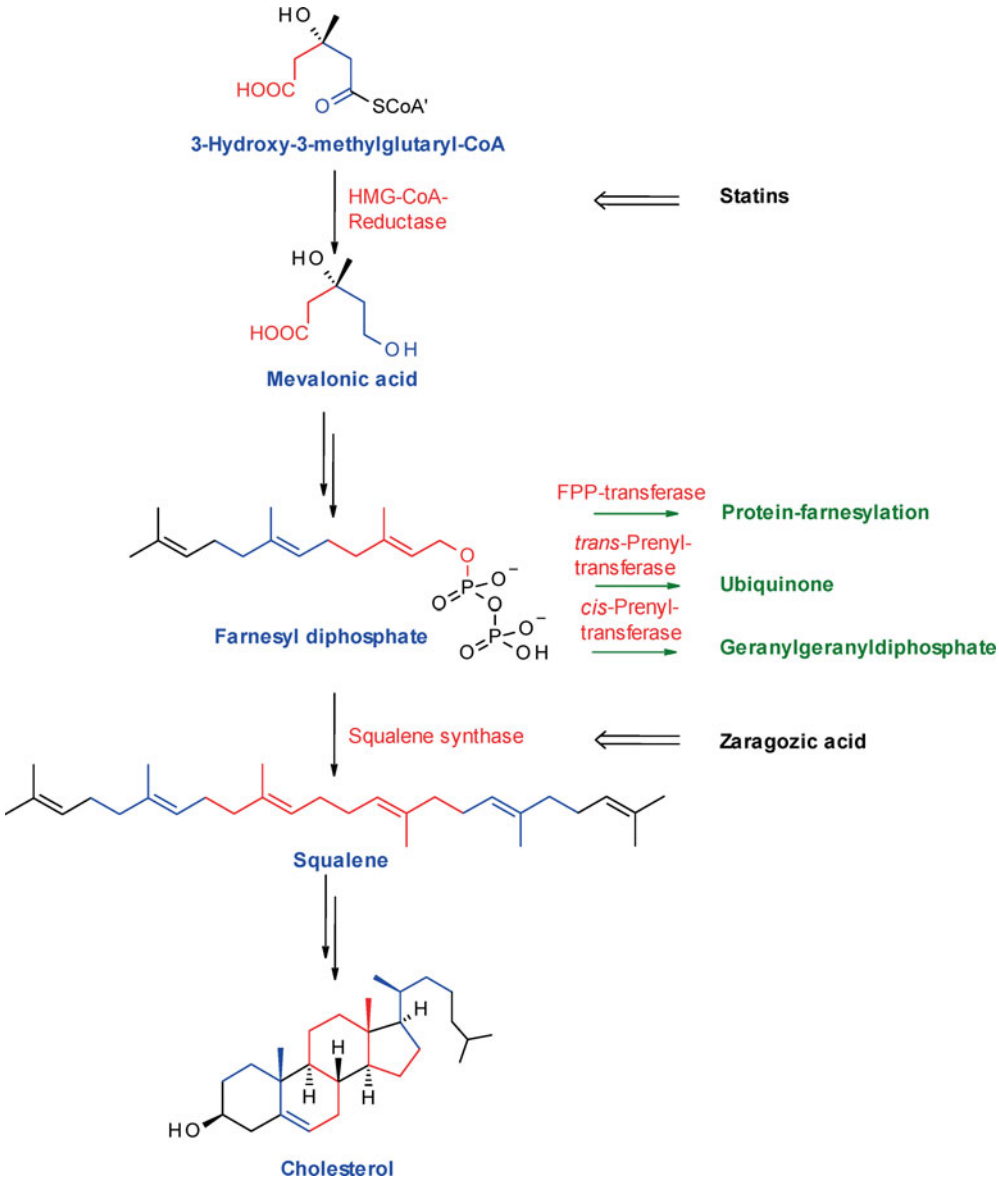
5.9.9 Outlook

With regard to the biosynthesis of cholesterol, it is really astonishing that its inhibition at such an early stage, like HMG-CoA reductase, does not produce diverse and serious side-effects. [405] Thus, farnesyl diphosphate is for example a critical downstream intermediate in the cholesterol pathway, needed for the farnesylation of numerous proteins. It constitutes an essential building block for the biosynthesis of ubiquinone and geranylgeranyl diphosphate along with their secondary products, *e.g.* the biologically important dolichols (polyprenyldihydroprenols with 16–21 isoprene units). Therefore, the availability of farnesyl diphosphate is essential for cell growth and for metabolism.

The concept of HMG-CoA reductase inhibitors as suitably safe drugs is only viable, as the research of Goldstein, Brown and Faust revealed at the beginning of the 1980s, because squalene synthase has a comparatively low affinity towards farnesyl diphosphate. [406] This facilitates a diversion of mevalonate metabolites

into the ubiquinone pathway. Nevertheless, in patients who are being treated with high doses of lovastatin, reduced ubiquinone levels are observed. Therefore, it appeared attractive to intervene further downstream in the biosynthetic pathway to cholesterol (Fig. 5.159).

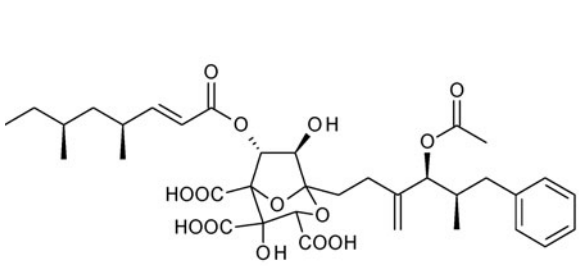
There is another incentive to search for cholesterol lowering agents, which can overcome limitations, apparently intrinsic to statins. These drugs achieve a reduction of LDL levels between *ca.* 18 and 55 %. This pharmacological efficacy



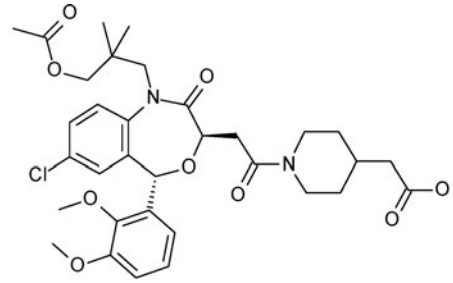
5.159 *The inhibition of squalene synthase is an attractive target for the inhibition of the biosynthesis of cholesterol.*

is unsurpassed by any other class of drugs on the market. Nevertheless, under statin treatment the majority of patients with a moderate to high risk of cardiovascular disease do not get below the WHO-recommended LDL threshold of 77 mg/dl. Since higher doses of statins are not advised for toxicological reasons, the search will have to continue for drugs with a novel mode of action. [407]

In 1991 and 1992, scientists at Merck, Glaxo and Mitsubishi Kasei isolated a compound from three different fungi, which inhibits a downstream target, the squalene synthase, and correspondingly the formation of squalene from farnesyl diphosphate. Merck discovered this compound, which they named zaragozic acid A (Fig. 5.160), from the uncharacterised sterile fungus ATCC 20986, in the filtrates of the river Jalon in the Spanish province of Zaragoza. Glaxo isolated the same compound from the fungus *Phoma sp.* C2932 in a soil sample from Armacao de Pera in Portugal, while finally Mitsubishi Kasei identified it from the fungus *Setosphaeria khartoumensis*. Glaxo and Mitsubishi Kasei named the compound, related to its activity, squalestatin S1.



Zaragozic acid A / Squalestatin S1



Lapaquistat

5.160 *A natural and a synthetic inhibitor of squalene synthase.*

More recently, Merck and Glaxo discovered a whole series of other zaragozic acids, which differ in their substitution pattern of the side-chain. By cultivating the fungi in 5 to 11m^3 fermenters, it was possible to produce larger amounts of the zaragozic acids, and to purify these, thanks to the three carboxylic functions, rather easily by ion-exchange chromatography.

In studies with rats, dogs and Rhesus monkeys, the zaragozic acids showed the much hoped-for effect on the cholesterol level, but unfortunately also serious side-effects and toxic symptoms (acidosis). These are considered mechanism-based and likely result from an accumulation of farnesol-derived dicarboxylic acids. A risk assessment therefore precluded the further deveopment of this target class.

A whole series of other development compounds have shared the same fate. For example, lapaquistat; this first squalene synthase inhibitor in Phase III clinical trials was abandoned by Takeda in 2008 due to liver toxicity, which occurred even at low doses (Fig. 5.160). [408]

Thus, it remains open, when LDL-lowering drugs with a new mode of action will emerge and make their way through the clinic to the market. High medical need and substantial scientific interest are in any case certain.

Summary in Bullet Points

- 3-Hydroxy-3-methylglutaryl-coenzyme A-reductase (HMG-CoA-reductase) catalyses the formation of mevalonic acid, an early intermediate in the biosynthesis of cholesterol.
- The statins are HMG-CoA-reductase inhibitors, which efficiently lower serum cholesterol levels and are widely prescribed in the treatment of hypercholesterolemia, a condition that causes atherosclerosis.
- This section provided insight into the pharmacological background of this disease and described the discovery of the statins, their biosynthesis and methods of industrial production.
- Atorvastatin became the best selling active pharmaceutical ingredient worldwide.

5.161 *Fortunately, Friedrich Schiller survived his illness due to "eating cinchona bark like bread", and only therefore we owe to him plenty of masterful plays and poems, not at least the Ode to Joy (Ode an die Freude), written in the summer of 1785. The lyric inspired Ludwig van Beethoven after Schiller's early death to the choral movement in his Ninth Symphony. In 1972, this tune was adopted as the Anthem of Europe.*

5.10 Artemisinin

During the night hours of 23rd September of 1782, the young military physician Friedrich Schiller (1759–1805), who would become a leading German dramatist and poet, succeeded in a risky and adventurous escape from his regiment in Stuttgart. Schiller's first play, "*Die Räuber*" (The Robbers), a protest against injustice and the abuse of power, had brought him into serious trouble with his employer, Karl Eugen, the 12th Duke of Württemberg (1728–1793), who wanted to detain him. Thus, he deserted, criss-crossing the country *via* Mannheim, Frankfurt am Main, the Palatinate and Thuringia, to finally arrive back in Mannheim, where he found refuge. He carried with him manuscripts of the drama "*Die Verschwörung des Fiesko zu Genua*" (Fiesco; or, the Genoese Conspiracy), the tragedy "*Kabale und Liebe*" (Cabal and Love), and an initial draft of "*Don Carlos*", all of which would later contribute to his fame. It was in Mannheim in September of 1783, that he fell sick and suffered from chills, which he described in a letter to a friend as vicious "cold fever" (Fig. 5.161). [409] The actual cause of his illness was malaria, and associated with the swampy flatlands along the river Rhine. These did not dry up until the river was straightened during a period from 1817 to 1876, according to plans designed by Johann Gottfried Tulla (1770–1828), an engineer from the Baden region, who died from a Malaria infection. The correlation between the disease and these swamps was only recognised much later.

The World Malaria Report of 2013 lists around 100 countries, where Malaria is endemic (Fig. 5.162). For 2012, the report estimates an incidence rate of 207 million malaria cases (80 % thereof in just 17 countries), and a mortality rate of around 627,000 deaths worldwide. Most affected are African countries like the Democratic Republic of the Congo, Nigeria, Tanzania, Uganda and Mozambique, but also Asian countries like India, Indonesia and Myanmar face a grave situation. There is a strong association between malaria mortality and poverty: The mortality rate is increased in countries with populations, where a larger fraction has an available income of less than $1.25 per capita per day. [410]

In 1783, a malaria epidemic in Mannheim claimed roughly 2000 lives. [409]

5.10.1 History of the Disease

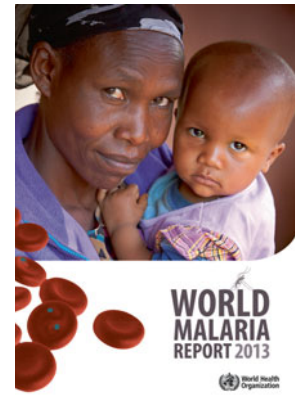
The earliest indicator of a malaria parasite (*Plasmodium dominicana*, precursor of the prevalent *Plasmodium juxtanucleare* species, which infects birds) was discovered in the body-cavity of a female *Culex*-mosquito from Tertiary amber in the Dominican Republic; a fossil, which is estimated to date back 25 to 45 million years (Fig. 5.163). [411]

Since the first human-like primates (hominids) evolved during the Tertiary Period, it is reasonable to assume from a phylogenetic viewpoint that malaria parasites have accompanied mankind all along its development path. *Plasmodium falciparum* is thought to have been transmitted to humans initially from gorillas. [412] But presumably, malaria epidemics might have occurred only some 10,000 years ago, when humans settled down and started farming.

The first experimental confirmation of a malaria epidemic was found at the river Tiber. About 100 km upstream north of Rome, a team of archaeologists from the University of Arizona, led by David Soren, researched from 1988 to 1992 a Late Roman infant cemetery at the site of Lugnano in Teverina, which dates to around the year 450. [413] The excavations revealed that more than 50 young children had died within one year, and that the burials took place during the summer time, as concluded from plant remains found as grave goods. The large portion of prenatal deaths indicated a fatal disease, which also gives rise to a high rate of miscarriages in pregnant women, and suggested a malaria epidemic as plausible cause. The fact that malaria pathogens are not only affecting erythrocytes, but also their precursor cells produced in the bone marrow, increased the chances to find evidence for this hypothesis by analysing bone material from the site. In 2001, Robert Sallares from the University of Manchester was able to extract 89 base-pair long fragments from two separate DNA samples he had retrieved from the skeleton at burial no. 36 (a 2–3 year old female). Amplification of these fragments by polymerase chain reaction (PCR) and comparison with DNA sequences [414] from parasites ultimately confirmed *Plasmodium falciparum* as the cause for this deadly infection (Fig. 5.164).

It is interesting to note, that archaeological and microbiological findings match nicely with the assumption, that Attila the Hun had abandoned his plan to conquer Rome in the year 452 for reasons other than being personally coaxed out of this by Pope Leo I, or being offered a large amount of gold. A more likely explanation would be that his army faced supply issues, and the outbreak of the "Roman fever" in his camp.

The microbiological evidence of a far earlier *Plasmodium falciparum* prevalence came from the Munich pathologist Andreas Nerlich. In 2008, he discovered in biopsies from two mummified Egyptian skeletons, by the DNA extraction and PCR-amplification technology, gene fragments of this malaria pathogen. The samples were retrieved in Thebes-West, and dated from the New Kingdom to the Late Period (1500–500 BC). [415] They originated from two adults with a chronic anaemic disorder, as concluded from osteopathology. Around this period (ca. 1550 BC), the first written reports on malaria infections in Egypt are recorded, as *e.g.* in the Edwin Smith Papyrus and the Ebers Papyrus.

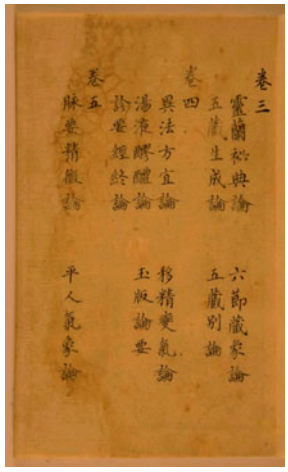


5.162 *The most common malaria victims are children, and thus featured on the front page of the World Malaria Report 2013, as they did in previous editions.*

5.163 *Mosquito in amber from a mine near the northern Cordillera Septentrional mountain range of the Dominican Republic.*

AJ426488
agaaataaca atacaatatc gaaaaatgat tttgtaattg gaatgatagg aatttacaag gttcctagag aaaccattgg agggcaagt
AJ426487
agaaataaca atacaatatc gaaaaatgat tttgtaattg gaatgatagg aattgacaag gttcctagag aaacaattgg agggcaagt

5.164 *The skeleton of the girl in grave no. 36 at Lugnano's infant cemetery and DNA fragments of Plasmodium falciparum. – Since malaria was also in Late Antiquity often associated with demons, according to pagan rites, the hands and feet of a dead infant were weighted down with heavy stones in order to prevent these evil spirits from escaping and inflicting further harm. The Roman physician and savant Quintus Sammonicus Serenus (around 200 AD) advised in his medical poem "De medicina praecepta saluberrima" wearing an amulet with the magic spell ABRACADABRA (Aramaic: "Create as I say") as a shield against malaria.*



5.165 *TheYellow Emperor's Inner Classic is even nowadays a pivotal element of training in Chinese Medicine.*

However, the earliest written documentation on malaria can be found in the legendary "*Canon of Medicine*", *TheYellow Emperor's Inner Classic* (Huangdi Neijing), dating from 2698 to 2598 BC. This book describes epidemics, characterised by febrile paroxysms (fever attacks occurring in flushes) in conjunction with enlarged spleen, which can be interpreted as *Plasmodium vivax*- or *Plasmodium malariae*-infections (Fig. 5.165). [416, 417]

Evidence of diseases marked by periodic (tertian or quartan) fever [418] attacks is also provided by Indian traditional medicines (Ayurveda). Already the oldest reference texts, like the Charaka Samhita, distunguish between fever episodes recurring every third or fourth day (caused by *Plasmodium vivax* or *Plasmodium ovale* (malaria tertiana), and respectively by *Plasmodium malariae* (malaria quartana)). The Susruta Samhita suggests as well that such illnesses are related to insect bites or stings.

In addition, there is sufficient indication of malaria being widespread in the European Mediterranean area centuries before Christ. [419] Thus, Hippocrates (around 460–370 BC) illustrates clearly in that portion of the Hippocratic Collection (Latin: *Corpus Hippocraticum*), which refers to Epidemics, that the intermittent fever is a disease, which goes along with spleen enlargement, and emerges usually in the vicinity of swamps, notably during the fall harvest, and coinciding with the appearance of Sirius in the night sky. (See also Homer, Iliad XXII (ca. 850 BC): "Sirius, harbinger of fevers, the evil star"). [420]

The Roman scholar and historian Marcus Terentius Varro (116–27 BC) authored at the age of 80 three books on agriculture (*Rerum rusticarum libris tres*), where he warns of minute creatures, which are bred in swamps (Fig. 5.166)

5.166 *Marcus Terentius Varro (medieval illustration) was appointed by Julius Caesar (100–44 BC) to plan a grand public library in Rome. After Caesar's assassination, this project remained unfinished. Having been outlawed and prosecuted by Mark Antony, Varro's life was saved by Octavian (the later Augustus), and he spent the rest of his life in seclusion at his estate in the Sabini Mountains engaged in his literary pursuits.*

and are responsible for dreaded febrile illnesses. He also mentions nets or screens, with which to cover windows, so that no animal may carry evil into the home (*fenestrae reticuletae nequod animal maleficium introire queat*). [421] Another Roman writer, Lucius Iunius Moderatus Columella († around 70 AD), cautioned in his twelve-volume companion "*Rei rusticae libri duodecim*" as well

Tab. 5.10 *Celebrities, who died from malaria*

Name	Occupation	Year	Remarks
Alaric I.	King of the Visigoths	ca. 370–410	Died in Consenza, Calabria
Augustine	Archbishop of Canterbury	† 604	Founder of the English Church
Otto II	Holy Roman Emperor	955–983	Died in Rome
Gregory V	Pope	972–999	Descended from Salian dynasty
Damasus II	Pope	† 1048	Died in Palestrina, after a short pontificate of only 24 days
Frederick IV	Duke of Swabia	1144–1167	Died on a campaign to Rome with Emperor Barbarossa in August
Henry VI	Holy Roman Emperor	1165–1197	Died in Messina in September
Henry VII	Holy Roman Emperor	1278–1313	Died near Siena in August
Dante Alighieri	Italien poet	1265–1321	Died in Ravenna in September
Leo X	Pope	1475–1521	Died in Rome on 1 December
Albrecht Dürer	German painter	1471–1528	Died in Nuremberg in April, likely from recurrent malaria attacks
Charles V	Holy Roman Emperor	1500–1558	Died in September in San Jerónimo de Yuste from malaria tropica
Francesco I de' Medici	Grand Duke of Tuscany	1531–1587	Died in October at his villa in Poggio a Caiano in Tuscany
Sixtus V	Pope	1521–1590	Died in Rome from "marsh fever" (malaria) in August
Urban VII	Pope	1521–1590	Died in Rome in September
Álvaro de Mendaña de Neyra	Spanish navigator and explorer	1541–1591	Died on Santa-Cruz-Islands in October
Oliver Cromwell	Lord Protector of the Commonwealth of England, Scotland and Ireland	1599–1658	Contracted malaria on a trip to Ireland. He suffered as well from kidney and urinary tract infection and might actually have died due to septicaemia.
George Gordon Noel Lord Byron	British poet	1788–1824	Died in Missolonghi, Greece in April
Josef Ludwig Franz Ressel	Inventor of ship propellers and the steam ship	1793–1857	Died in Ljubljana in October
Rebka Chenashu	Ethiopian sprint medalist	1986–2003	Died from cerebral malaria in April

5.167 *Not only the Grand Duke of Tuscany, Francesco I de' Medici and his second wife, Bianca Cappello (1548–1587), died from malaria. 15 Years earlier already, three other family members, Eleonora di Toledo (1522–1562) and her sons Giovanni (1543–1562) and Don Garzia (1547–1562), had fallen victim to this disease within two months. [424]*

5.168 *The Puritan leader Oliver Cromwell became infected with malaria in 1649/50, while traveling in Ireland. He refused to take the recently discovered remedy, a tree bark powder, called "Jesuits' powder" (see below), and died in 1658 from a malaria-relapse in combination with a kidney disease. [419]*

5.169 *Self-portrait by Albrecht Dürer: "There, where the yellow spot is located, and where I point my finger at, there I feel pain". The enlarged spleen (splenomegaly) is a common concomitant feature of malaria.*

to locate houses near swamps, since from there emerge animals, which bite and transfer hidden diseases that are difficult to identify, even by physicians. [422] The term malaria is based on the perception that fumes of bad air (Italian: mala aria or mal'aria) emanate from swamps and cause the health problems.

Malaria has presumably been disseminated across Northern Europe as far as England and the shores of the Baltic Sea by legionnaires and mercenaries of the Roman Empire. Throughout the centuries, many millions of people have fallen victim to the disease, among them numerous celebrities (Tab. 5.10, Fig. 5.167 and Fig. 5.168). [423]

Others survived the infection, but faced the risk of relapses for the rest of their life. Among them were the American presidents George Washington, Abraham Lincoln, Theodore Roosevelt and John F. Kennedy, and Royalties like King Charles II of England, Scotland and Ireland, King Philip II of Spain, and Cesare Borgia, the Duke of Valentinois and Romagna. Thereto belong as well politicians like Ho Chi Minh, Leon Trotzky and Mahatma Gandhi, also military leaders like Hernán Cortés, Lord Horatio Nelson and General John J. Pershing, the explorers Christopher Columbus, Henry Morton Stanley, and David Livingstone, also the authors Sir Arthur Conan Doyle, and Ernest Hemingway, actors like Errol Flynn, Michael Caine, Christopher Lee and George Clooney, but also infamous robbers and gang leaders as Jesse James, and benefactors like Mother Teresa.

William Shakespeare (1564–1616) mentions the fever attack or ague in eight of his plays, and Albrecht Dürer (1471–1528) conveys in a letter to his doctor with an attached sketch: Do der gelb fleck ist und mit dem finger drawff dewt do ist mir we. (Fig. 5.169).

It is historically accepted, that around 1557/1558 a malaria epidemic swept across large parts of Europe. [425] The French physician Julien Le Paulmier (1520–1588) published in 1578 one of his monographs on contagious diseases (*De morbis contagiosis libri septem*), where he noted in one of the chapters (*De Febre Pestilenti*, page 398): Tota tamen Europa febribus variis maxima ex parte intermittentibus iisque diuturnis iactata est, sed iis minime lethalibus. (… all of Europe has been haunted by diverse and protracted fevers, which were worrisome, though in most cases of little lethality).

Up to the 19th century, more epidemics of the "marsh fever" were to follow, affecting in particular coastal areas with their fen- and marshlands. In East Frisia, a German region at the North Sea, close to the Netherlands, one in two children were purportedly infected by malaria tertiana. The physician Aemil Storm (1833–1897), younger brother of the German poet and novelist Theodor

Storm, wrote in 1857 a paper on this marsh fever: *De febre sic dicta marchica* (also called Dithmarsch disease). Only towards the end of the 19th century, river regulations (as mentioned above in case of the river Rhine), draining of swamps and canalisation measures gradually dried up the mosquito breeding grounds and led to the disappearance of malaria in Northern Germany. It is remarkable that all this was achieved prior to identifying the actual pathogen or elucidating the transmission path of the disease.

Nevertheless, Giovanni Maria Lancisi (1654–1720) an Italian physician and epidemiologist, had already postulated in 1717 in his monograph, titled "Noxious Emanations of Swamps and Their Cure", mosquitoes as potential carriers of the disease.

5.10.2 The Pathogenic Agent and its Vector

Since the discovery of bacteria in the late 17th century, the view had developed over time that most likely germs were to blame for all deadly epidemics. Accordingly, the concept of a microbial infection (called *Bacillus malariae*) had won a lot of support in the medical community. In 1880, while working at a military hospital in Constantine, Algeria, the French physician Charles Louis Alphonse Laveran (1845–1922) examined blood of a soldier, who had come down with malaria tropica. [426] For the first time, he discovered on 6 November of that year a parasite in red blood cells of his patient (Fig. 5.170) and suspected that this could cause the pathogenesis of malaria. Subsequent studies led him to the conclusion that the discovered pathogen was a parasitic protozoan, which he named *Oscillaria malariae*, but would later become known as *Plasmodium falciparum*. The initial reports Laveran published on his findings were met with great skepticism. But his further investigations, conducted at the San Spirito Hospital in Rome with blood from patients who had become infected with malaria in the surrounding Roman Campagna, confirmed his earlier hypothesis – as did research from scientists elsewhere.

In those days, different staining techniques and the development of the oil-immersion microscope objective by the Carl Zeiss Company had largely facilitated studies on the malaria parasite. The Italian pathologist Camillo Golgi (1843–1926) was able to distinguish those protozoans, which cause two forms of tertian malaria (a benign infection by *Plasmodium vivax*, occurring in springtime, and a deadly variety by *Plasmodium falciparum*, occurring in summer and autumn), froma species (*Plasmodium malariae*) responsible for the quartan malaria (Fig. 5.171). [427] He also documented their life cycles and recognised the link to their different periodicities (48 and 72 hours respectively).

While it was still obscure and unexplainable how the disease is transmitted, an initial answer to this crucial question came from Sir Ronald Ross (1857–1932), a British-Indian physician (Fig. 5.172). Serving at the Presidency General Hospital in Calcutta, and subsequently as the Acting Garrison Surgeon in Bangalore, he was engaged between 1881 and 1899 in malaria research, collecting and studying mosquitoes (Spanish: *mosquito* is a "small fly"; from Latin: *musca*, "fly"). Being afterwards transferred to the Osmania University in Secunderabad, Ross made a break-through discovery: He found pigmented bodies on the stomach wall of a dappled-winged mosquito (later classified as an *anopheline*), which he had let feed on an infected patient (Fig. 5.173). [428] Ross detected, that these pigments then ruptured, releasing rod-like structures, which

5.170 *Charles Louis Alphonse Laveran and his drawings of the malaria pathogen. Dark-brown pigments (haemozoin), iron-containing degradation products of haemoglobin, and also crescentic bodies, flagella-like structures in blood samples of his patients were indicative of the infection. In 1907, Laveran was awarded the Nobel Prize in Physiology or Medicine.*

5.171 *Camillo Golgi made numerous discoveries, many of them named after him, as e.g.: Golgi's method of staining nerve tissue with silver nitrate, or the Golgi apparatus; one of the main tasks of this organelle, critical for most eukaryotic cells, is to provide a kind of "postal service" of grouping, packaging, and shipping material to intra- and extra-cellular destinations. Golgi became in 1906 a Nobel Laureate in Physiology or Medicine.*

5.172 *Ronald Ross himself contracted the disease in 1897 when he was posted in Ooty, India. In 1902, he was awarded the Nobel Prize in Physiology or Medicine "for his work on malaria, by which he has shown how it enters the organism and thereby has laid the foundation for successful research on this disease and methods of combating it".*

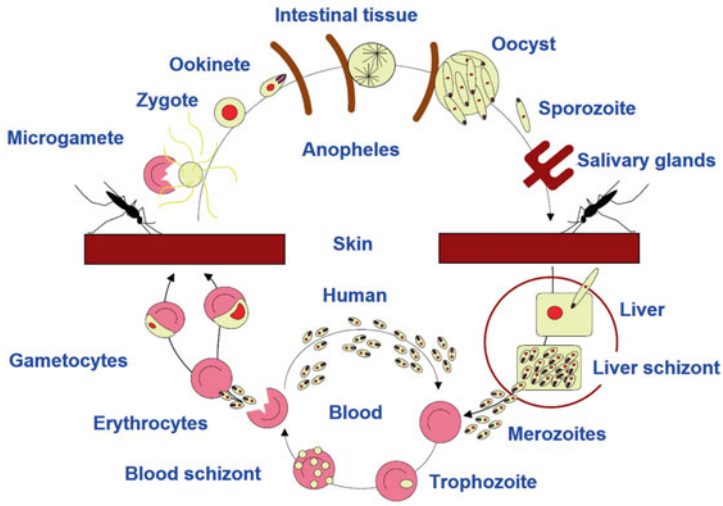
invaded the mosquito's salivary glands. Over the years, many other scientists had joined the research effort and contributed to the elucidation of the different development stages of the parasite; among them were Patrick Manson, Amico Bignami, Giuseppe Bastianelli, Félix Mesnil, Émile Roubaud, Henry Shortt, Cyril Garnham and Miles B. Markus. [429]

The life cycle of the malaria pathogen encompasses humans as intermediate and *anopheline* mosquitoes as definitive hosts (Fig. 5.174). In humans, they undergo asexual reproduction, in mosquitoes, they multiply sexually. Once an infected *anopheline* insect bites a human, it transmits with its anticoagulant-containing salivary juice several hundred sporozoites of the parasite *via* the skin wound into the blood stream (Fig. 5.175). [430] Reaching the host's liver, the sporozoites invade hepatocytes, multiply asexually in a phase called exoerythrocytic schizogony, and mature to uninucleate merozoites, which are released again into the blood circulation. In the case of *Plasmodium vivax* and *Plasmodium ovale*, a small fraction of the merozoites outlasts in an exoerythrocytic dormant stage as hypnozoites, which can be re-activated after months or years and cause the characteristic relapse pattern of malaria tertiana.

The initially cone-shaped merozoites target preferentially and invade red blood cells to start their replication cycle (erythrocytic schizogony) by adopting a ring structure. They then re-organise the biological armamentarium of the host cell to take full control of its enzyme machinery. Developing into trophozoites (Greek: *trophes* = nourishment), they digest the haemoglobin protein of the erythrocytes as an energy source, leaving behind the iron complex of haemozoin (called malaria pigment). Growing trophozoites morph into the schizont stage, where within 3–4 days numerous new merozoites are formed and released into the blood stream, ready to infect other erythrocytes. This recurrent cycle leads to the symptoms typical of malaria: fever, chills, and anaemia. If not treated properly, the pathological consequences of this acute and systemic disease are ischaemia, damage to the central nervous system (cerebral malaria), coma, and death.

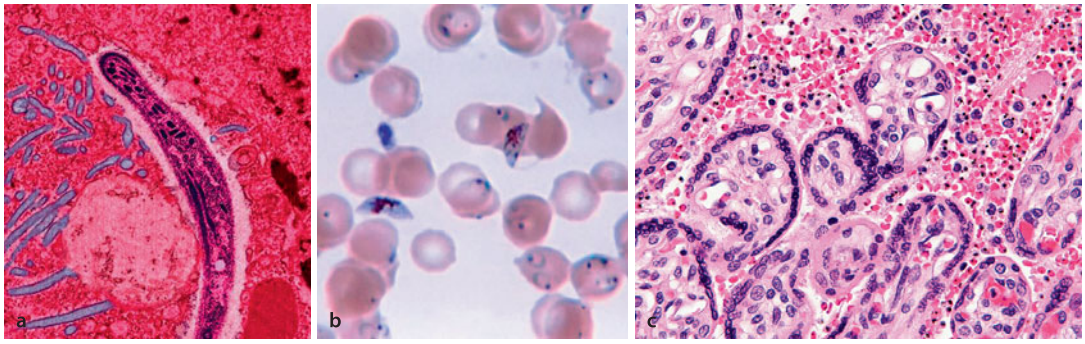
5.173 *Female Anopheles gambiae at a blood meal (their male counterparts feed on plant nectar). Besides Anopheles funestus, this malaria vector belongs to the most prominent in Afrika. There are around 460 anopheline species. Many of them feed on blood from animals (zoophilic), e.g. birds, mice and cattle, some 30–40 of them (anthropophilic) prefer however human blood and thereby transfer malaria.*

With progressing infection, not only merozoites, but gametocytes are formed and released into the blood circulation, from where female *anopheline* mosquitoes take them up when they feed. After maturation into male and female gametocytes and fertilisation within the insect, motile zygotes (ookinetes) develop, which penetrate into the lumen of the insect's gut and form oocysts. Here another replication cycle (sporogony) begins with the production of sporozoites. Bursting of the oocyst enables these sporozoites to migrate *via* the haemolymph to the salivary glands of the mosquito, from where a blood meal on humans closes the infection loop.



5.174 *Life cycle of malaria parasites. The Russian physician Nikolai A. Sakharov reported in 1890 that he had injected himself with the intestinal contents of a leech, which had fed on a malaria patient a couple of days earlier, and that 12 days later he had come down with malaria. This heroic self-experiment confirmed the infection path of the pathogen in principle. [431]*

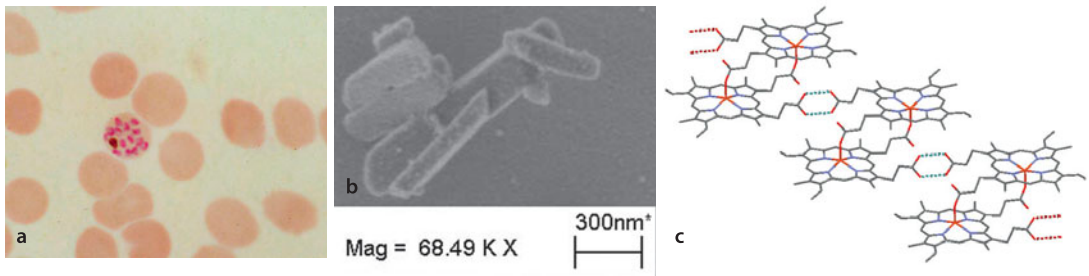
Sickle cell anemia is an inherited illness, marked by abnormal, crescent-shaped red blood cells. Linus Pauling (1901–1994) called it a 'molecular disease', when he discovered in 1949 the association with an altered protein structure, based upon a genetic disorder. Individuals with two mutated hemoglobin gene copies develop sickle cell anemia, while they are not affected by *Plasmodium* infection. Carriers of one faulty copy don't become anemic and are highly protected against malaria. Miguel P. Soares at the Instituto Gulbenkian de Ciência in Oeiras, Portugal, unraveled in 2011 the molecular mechanism of this infection tolerance. [430]



5.175 *Sporozoite of a plasmodium (a), ring-forms and gametocytes of Plasmodium falciparum in human blood (b), and a micrograph of a placenta from a stillbirth due to maternal malaria. (Haematoxylin and eosin stain) (c). Red blood cells are anuclear; blue/black staining in bright red structures (red blood cells) indicate foreign nuclei from the parasites.*

While the degradation of haemoglobin is essential for the survival of blood-feeding parasites, the simultaneously inevitable formation of dark pigment involves serious risks and turns out to be the proverbial Achilles heel – in this case of malaria parasites. Rudolf Virchow (1821–1902) recognised, while working at the University of Würzburg in 1849, a connection between the appearance of such pigments and malaria. [432] Haematophagous organisms like *Plasmodium* species, but also *Schistosoma* species (causing the flatworm disease bilharziasis), have only a very limited capability to synthesise amino acids *de-novo*. Thus, they metabolise up to 80 % of a host's erythrocyte haemoglobin in their digestive food vacuole under acidic conditions (at a pH of 4.5–5.2). In cases of severe malaria infection, as much as 100 g of the on average 750 g haemoglobin in the circulation of a healthy human can be catabolised. [433]

In the end, there remains only the free haeme (ferrous protoporphyrin IX (Fe(II)PPIX)) left in the food vacuole of the parasite (Fig. 5.176). This monomeric complex is highly toxic for trophozoites due to catalysing very efficiently the formation of reactive oxygen species. In 1891, Tito Carbone (1863–1904) at the University of Turin, and subsequently Wade H. Brown (1878–1942) at the University of Wisconsin, could demonstrate that the malaria parasite oxidises the complexed ferrous to ferric iron. The resulting crystalline haemozoin (ferriprotoporphyrin IX (Fe(III)PPIX)) is an aggregate of dimers, which is now insoluble and can cause therefore no longer harm to its environment. [434] This process, called biocrystallisation, provides an excellent intervention point for developing anti-malaria drugs.



5.176 *Giemsa-stained human erythrocyte, infected by Plasmodium falciparum; haemozoin is visible as a brown particle (a). Electron micrograph, 68,490-fold magnification, showing distinct rod-shaped haemozoin crystals (b). Crystalline ferriprotoporphyrin IX (Fe(III)PPIX) forms coordinate bonds (red) between iron atoms and the oxygen of propionate residues to yield centrosymmetric dimers; hydrogen bonding (dotted lines) leads to aggregation (c). [435]*

5.10.3 Malaria Treatment and its History

Quinine

Prior to Renaissance, it was only possible to address the symptoms of malaria. The first effective natural product, which became available in Europe for treatment of the disease, originated from Peru, where malaria had not been endemic prior to colonial times. Living in Lima, the Jesuit missionary and apothecary Agostino Salumbrino (1561–1642) observed that the Quechua-Indians, who

worked in cold and flooded silver mines of the Spanish conquistadors, drank an extract from bark of the yellow Cinchona tree (*Cinchona officinalis*) to treat chivering. [436] His intention to use this remedy for malaria as well was scientifically completely unfounded, and can be counted among the fortunate discoveries, where a valuable result came out of a misconception. The Jesuit priest Barnabé de Cobo (1582–1657) is thought to have been the first to bring in 1632 Cinchona bark (also called Jesuits' or Peruvian bark) to Spain and later to Rome. The Swedish "Father of Taxonomy", Carl von Linné (1707–1778), classified this tree genus (with some 38 species like *C. officinalis*, *C. pubescens*, *C. ledgeriana* and *C. calisaya*) as Cinchona. The name refers to the Countess of Chinchón, Señora Ana de Osorio, wife of Don Luis Gerónimo Fernández de Cabrera de Bobadilla Cerda y Mendoza, whom King Philip IV had appointed as ruler of the Spanish South American colonies. Legend has it that on a journey to Lima in 1629, the Countess contracted malaria (tertian fever) and was cured by her physician, Juan de Vega, who applied a brew prepared from bark of the Cinchona tree. [437] The fact that the English apothecary Sir Robert Talbor (1642–1681) successfully treated King Charles II of England, Scotland and Ireland (1630–1685) for his malaria with a slurry of powdered Cinchona bark in white wine, significantly augmented the popularity of "Jesuits' Powder", and made it soon into an export hit of the Spanish colonies (Fig. 5.177).

5.177 *Robert Talbor travelled also to France, where he cured the son of Louis XIV. During his lifetime, he nevertheless kept the ingredients of his remedy under wraps. Soon after his death, Talbor's secret was however first disclosed in a French book, and one year later in an English translation under the title: The English Remedy: Talbor's Wonderful Secret for Curing of Agues and Fevers. (a) – Also the famous English physician Thomas Sydenham (1624–1689) (b) recommended Cinchona bark (Cinchona officinalis) (c) for treatment of intermittent fever.*

London native Charles Ledger (1818–1905), an adventurer and explorer, had spent four years in the Bolivian Andes and, with support from his local guide and taxonomy expert Manuel Incra Manami, he managed to identify Cinchona trees of superior quality, and to collect their seeds. In 1865, he smuggled a box

5.178 *Prior to further processing, labourers, including women and children, sorted out cinchona bark on the Cinchona estate Tjinjiroean, West-Java.*

5.179 *Quinine is a highly fluorescent compound, which makes it in beverages easily detectable under UV light. Today's tonic water is not intended for medical use and has significantly lower quinine content than in Colonial times. In the United States, FDA regulations have set the current limit at 83 ppm.*

filled with these seeds, which were later named after him *Cinchona ledgeriana*, to London. Some were also sold to the Dutch government, whereupon the Dutch East Indies plantations in Java (Fig. 5.178) grew more than 20,000 trees and thus established a dominant role on the world market for Cinchona bark, while the economies in Bolivia and Peru suffered. Ledger himself did not gain a lot of financial benefit from this endeavour, being ultimately buried in a pauper's grave. [438]

Already in 1820, the chemist Pierre-Joseph Pelletier (1788–1842) and the pharmacist Joseph Bienaimé Caventou (1795–1877) were able to isolate in pure form quinine as the active principle of Cinchona bark by extraction with alcohol. [439] Three years later, the pharmacist Friedrich Koch (1786–1865) established in the city of Oppenheim (upper Rhine region) a viable industrial production process for this alkaloid.

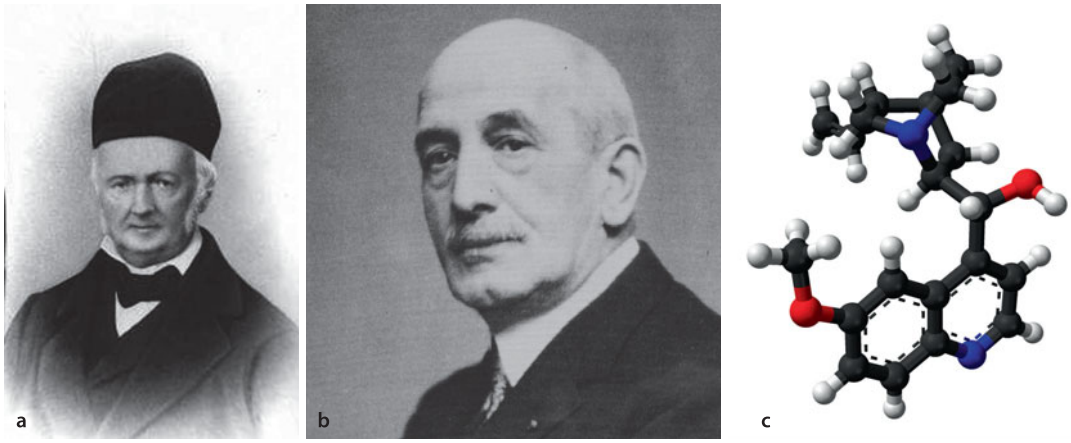
Quinine was regarded soon the mainstay of malaria treatment and prophylaxis for countries in the tropics, in particular in South Asia and Africa. From the mid 1800s on, carbonated (tonic = energising) water with large amounts of quinine became increasingly a commodity for British military to protect from ague while in India or other malaria ridden places (Fig. 5.179). Since sweetening the water appeared not sufficient to offset the bitter taste of the drink, the British colonials in India mixed it with gin and thereby created the gin-tonic cocktail, still popular around the world.

Among the first attempts to synthesise quinine, though unsuccessful, is the one by William Henry Perkin (1838–1907), an 18-year-old student at the Royal College of Chemistry in London. While the constitution of quinine was still unknown, Perkin thought it would be possible to produce this alkaloid by oxidising N-allyl toluidine. Instead, he prepared accidentally aniline purple, which became better known as mauveine, the first industrial dyestuff and a cornerstone for the development of the chemical industry.

Only in 1912, the Swiss chemist Amé Jules Pictet (1857–1937) elucidated at the University of Geneva the structure of quinine (Fig. 5.180). [440]

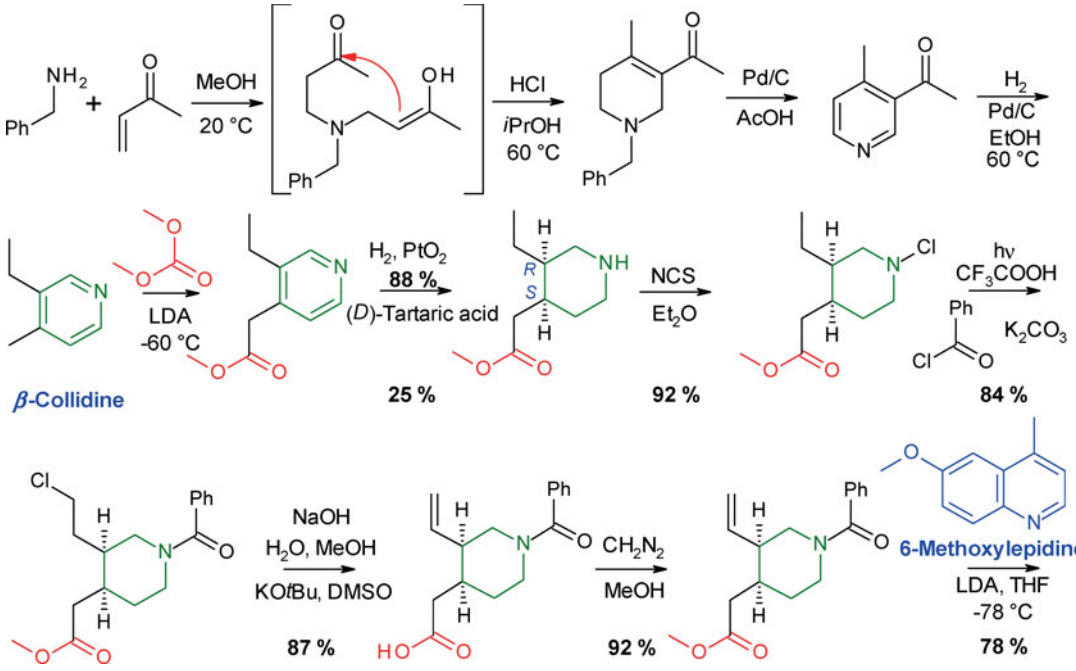
Whilst the Germans had occupied the Netherlands and Japan controlled Java, supply shortages of quinine during World War II took a huge toll on Allied troops, who lost thousands of soldiers in Africa and Asia dying from malaria. Since this had a serious impact on military operations, there was accordingly a tremendous strategic interest in accessing other drug sources. This meant to explore as well synthesis options for quinine. In 1944, Robert Burns Woodward (1917–1979) at Harvard University and William von Eggers Doering (1917–2011) at Columbia University published the (controversially discussed [441]) non-stereoselective total synthesis of a precursor alkaloid. [442] The first complete total synthesis of enantiomerically pure quinine was only published in 1978 by Milan R. Uskokovic at the Chemical Research Department of Hoffmann-La Roche in Nutley, New Jersey. [443]

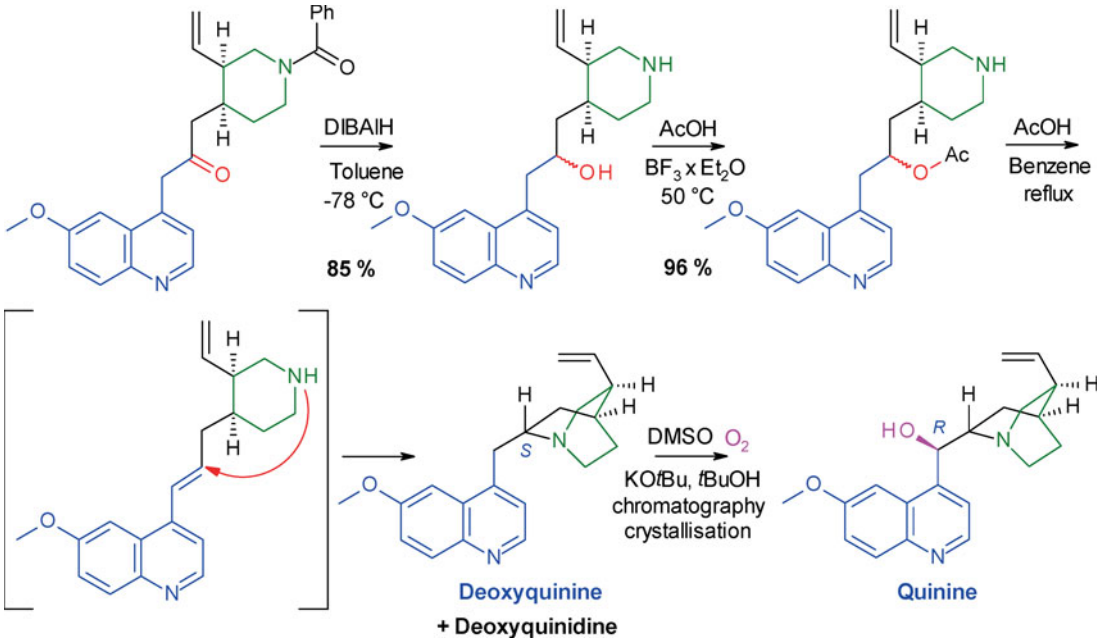
As starting material serves 3-ethyl-4-methylpyridine (β-collidine), which is accessible by condensation of benzylamine with methyl vinyl ketone, followed by dehydration and reduction. After introduction of the ester moiety, hydrogenation and separation of the enantiomers, a Hofmann-Löffler-Freytag reaction leads to the stepwise build-up of the vinyl side chain. Reaction with

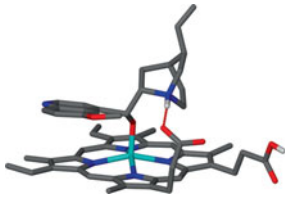


5.180 *German apothecary Friedrich Koch (1786–1865) (a); Swiss chemist Amé Jules Pictet (1857–1937) (b); 3D-model of quinine structure (c).*

6-methoxylepidine generates the carbon scaffold of quinine. The resulting ketone is reduced with diisobutylaluminium hydride, and after esterification, the quinuclidine ring is formed under acidic conditions at elevated temperature. In a final step, the deoxyquinine/deoxyquinidine mixture is oxidised, and the resulting quinine and quinidine are separated *via* chromatography and crystallisation.





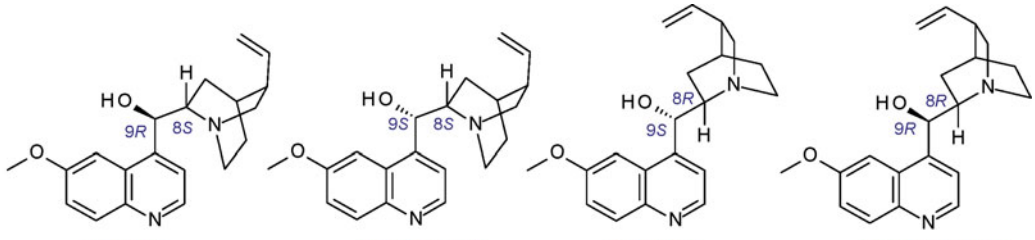


5.181 *Complex of ferriprotoporphyrin IX and quinine.*

Katherine A. de Villiers at Stellenbosch University in South Africa was able to demonstrate in 2012 by single crystal X-ray diffraction how quinine interacts with ferriprotoporphyrin IX and thereby inhibits the formation of haemozoin (Fig. 5.181). [444] Iron possesses the coordination number five in this ferriprotoporphyrin IX-quinine complex, where the benzylic alkoxide group of the alkaloid attaches to the fifth binding site. The complex gains further stability from a salt bridge between the protonated quinuclidine nitrogen and a propionate residue, as well as from a π-stacking between the almost coplanar oriented quinoline and the pyrrole of the porphyrin system. Thus, ferriprotoporphyrin IX has lost its capability of forming haemozoin dimers and undergoing further biocrystallisation, while its toxicity for *plasmodium* species is retained.

The crystal structure allows rationalising the differences in activity between the diastereomers in position 8 and 9 of the molecule as well. An *in-vitro* assay with *Plasmodium falciparum* showed that quinidine is roughly twice as potent as quinine. Switching to the corresponding 9-epimers is more dramatic and results in virtually inactive compounds (Fig. 5.182). [445]

5.182 *IC_{50}-Data from an in vitro-assay with Plasmodium falciparum.*

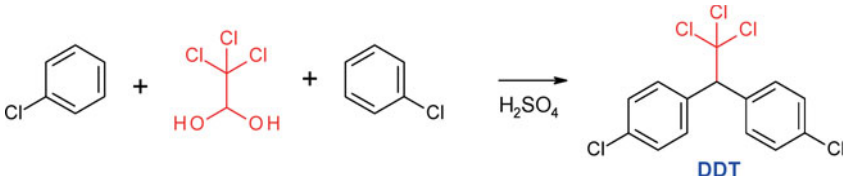


Insecticides to Combat Malaria

5.183 *Paul Hermann Müller was the first non-physician to be awarded the Nobel Prize for Physiology or Medicine in 1948 "for his discovery of the high efficiency of DDT as a contact poison against several arthropods".*

A completely different approach in the fight against malaria is to target its vector, the *anopheline* mosquito. The first agent to be used for this purpose in considerable amounts was dichloro-diphenyl-trichloroethane (DDT). [446] The compound was initially synthesised in 1874 by the Austrian chemist Othmar Zeidler (1859–1911) in the course of his dissertation under the guidance of Adolf von Baeyer in Strasbourg. More than 60 years later however, it was the Swiss chemist Paul Hermann Müller (1899–1965), who discovered in 1939 its insecticidal properties, while he was working at Geigy AG in Basel (Fig. 5.183). DDT, which is easily accessible from chloral hydrate and chlorobenzene in an acid-resistant vessel, was marketed only three years later as an agricultural insecticide (*e.g.* against the Colorado potato beetle), as an agent to control stock pests, and in the hygiene sector (*e.g.* as pediculicide = killing lice).

Towards the end of World War II, DDT was employed in the South Pacific to contain the spread of malaria. The US Air Force sprayed aerially many islands extensively with 220–280g of DDT per hectare. In Italy, the inner walls of entire houses were treated with up to 2g of DDT per square metre in order to ensure that the contact poison was lethal to *anopheline* mosquitoes, when they rested after a blood meal on such walls. The procedure had to be repeated twice a year for three to four years (Fig. 5.184).



5.184 *As shown here in case of Orosei in Sardinia, the dated sign next to the entrance marked homes, where the interior had been sprayed with DDT. Under a trial programme to eradicate malaria, sponsored by the Rockefeller Foundation, the number of infections in Sardinia could be dramatically reduced from 75,000 in 1946 to just 9 cases in 1951. [447]*

Based on these impressive results, the physician Fred Lowe Soper (1893–1977), Director of the Pan American Sanitary Bureau, persuaded the WHO to launch in the mid 1950s a worldwide campaign to eliminate the disease. Its initial success turned out to be remarkable, foremost in India, Pakistan, Sri Lanka, Paraguay, Venezuela, and Middle America. – In 1949, the USA was declared free of malaria, thereby resolving a significant public health problem; by the 1960s the disease had also been eradicated in Europe. Both of these achievements are probably by and large owed to the use of DDT.

After a few years of DDT application however, resistant anopheles mosquitoes started to emerge in El Salvador, Mexico, India and elsewhere. That would not remain the only concern this once versatile pesticide caused. The marine biologist Rachel Carson (1907–1964) wrote in 1962 three articles in the New Yorker magazine, which were then also published in book form, entitled "Silent Spring", where she warned of the dangerous ecotoxicological impact DDT and some other agrochemicals have when they are used uncontrolled and

unexamined. The environmental persistence, accumulation in the food chain, carcinogenicity and effects on the reproductive system led internationally to the nowadays very restricted use (and production) of DDT. In 1996, a risk assessment of 12 chemicals, including DDT, reflects this change of thought and of policies and promoted the Stockholm Convention on persistent organic pollutants (POPs), which entered into force in 2004.

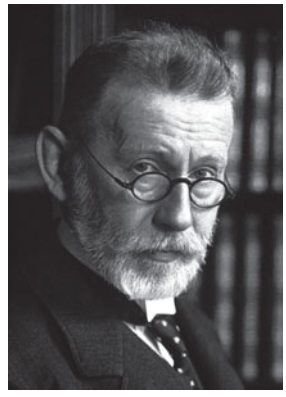
5.185 *Insecticide-treated bed nets are used to prevent malaria effectively.*

The expectation had been, that with the development of novel insecticides (*e.g.* pyrethroids, phosphoric acid esters) and new antimalarial drugs it might be possible to eradicate the disease and its vectors. In addition to the technique of indoor-residual spraying, in particular insecticide-treated nets (Fig. 5.185) proved to be effective in highly endemic regions. Nevertheless, the overall success has remained fairly limited, foremost due to a continuous problem of increasing resistance at the drug- and insecticide-level.

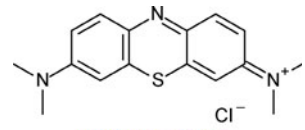
To complement existing strategies, the WHO has outlined a roadmap for the ongoing development of a malaria vaccine, which is in phase III clinical trials and could potentially become available in 2015.

Synthetic Antimalarial Drugs

The first synthetic drugs to treat malaria can be traced back to Paul Ehrlich's experiments to stain microorganisms. [448] Around 1889, Ehrlich succeeded in preferentially staining *plasmodium* species in red blood cells with methylene blue, leading him to the idea that this phenothiazine dye could also be useful for the therapy of malaria (Fig. 5.186). Only two years later, a butler and a sailor were cured from malaria, when they were administered methylene blue at the Moabit hospital in Berlin. [449] The drug remained in use throughout World War II, though it was not very much liked among the Allied forces in the South Pacific. As General Douglas MacArthur (1880–1964) recalled in his memoirs, they complained about two obvious, though reversible side effects of the medication: their blue urine ("Even at the loo we see, we pee, navy blue") and a blue colouration of the sclera (whites of the eye). More recently, the drug (under the label *Proveblue*®) experiences a notable renaissance, taking advantage of its selective inhibition of glutathione reductase from *Plasmodium falciparum*, and being administered in combination with other antimalarials. [450]

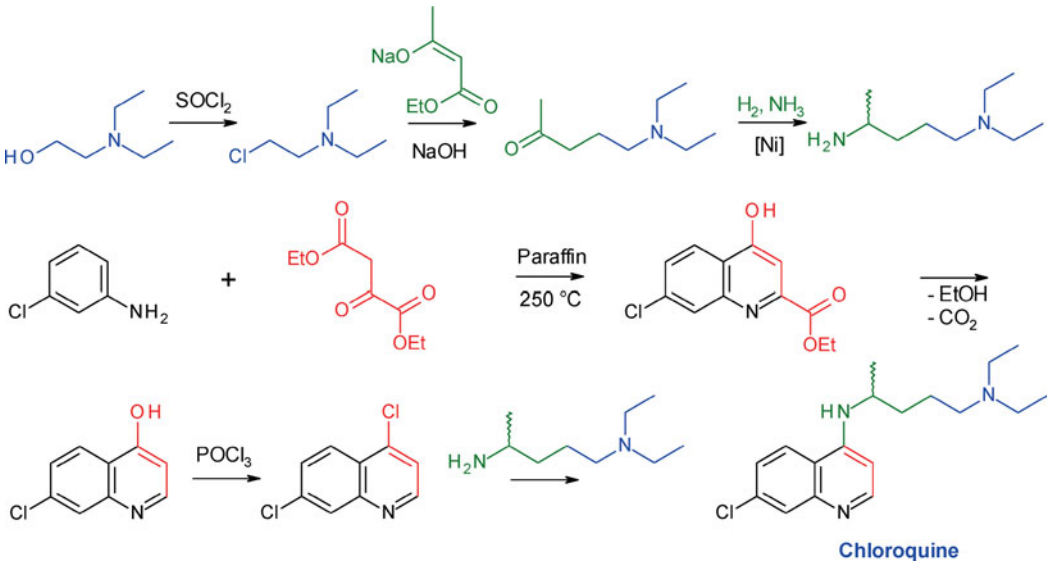


5.186 *Paul Ehrlich (1854–1915), Nobel Laureate in Physiology or Medicine of 1908, and methylene blue. The compound was first synthesised in 1876 by Heinrich Caro (1834–1910) at BASF, and a year later, the company was hereby awarded the first German patent on a coal tar dye.*



Methylene blue

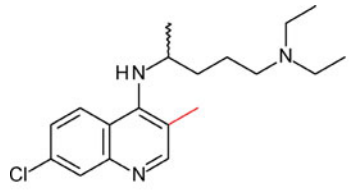
Immediately after World War I, Ehrlich's former coworker, Wilhelm Röhl (1881–1929), initiated at Bayer AG a series of biological assays with synthetic antimalarial test compounds. The driving force was twofold: uncertainties of both, the supply of quinine, in particular during war times, and also the drug's spectrum of side effects under chronic and sometimes even acute dosing, summarised as cinchonism (symptoms include blurred vision, tinnitus, headache, dizziness, confusion, tremor, diarrhea, nausea, vomiting and anorexia). Over the years, some 12,000 molecules were synthesised and tested at Bayer, and finally in 1934, Hans Andersag (1902–1955) discovered within this series chloroquine (*Resochin*®). [451] Chloroquine can be obtained in a few steps *via* a Conrad-Limpach reaction to form 4-hydroxyquinoline with subsequent substitution of the hydroxyl function by a corresponding amine.



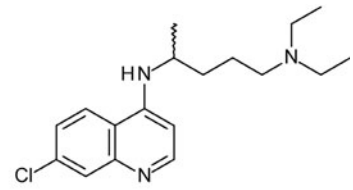
Early toxicological evaluation led however to the perception, that the compound would not be suited for application in humans. Andersag found two years later with sontochin (3-methyl chloroquine) a significantly safer alternative, which was moved through clinical trials and reached the market. Sontochin was also used during World War II by the German army as malaria prophylaxis, in view of its numerous casualties due to the high infection rate in occupied territories like Greece, the Ukraine, southern Russia and northern Africa.

After Allied troops had captured Tunis in May of 1943, the drug fell together with pharmacological reports into the hands of Americans, who confirmed back home its superior efficacy compared to quinine and atabrine. The latter, an acridine dye, also known as mepacrine, originated as well from the discovery programme at Bayer and was first synthesised by Fritz Mietzsch and Hans Mauss and tested by Walter Kikuth around 1932. Bayer's collaboration and distribution partner in the US, Winthrop Chemical Corporation, had increased its production of atabrine by 1944 to annually 3,500 million tablets, with an additional 2 million tablets from the British ICI, in support of Allied forces. The

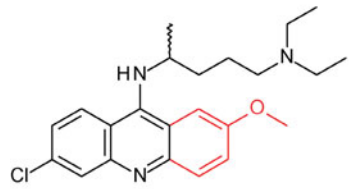
soldiers in East Asia were suspicious of the drug. The pills tasted bitter, turned the skin yellow and rumours spread that atabrine "causes loss of virility" (Fig. 5.187). Structural analysis of sontochin rekindled within the American malaria programme the research effort to pursue the 4-aminoquinoline motif with highest priority, which led to a rediscovery of chloroquine, ultimately replacing sontochin in the market for toxicology and pharmacology reasons. [452]



Sontochin



Chloroquine



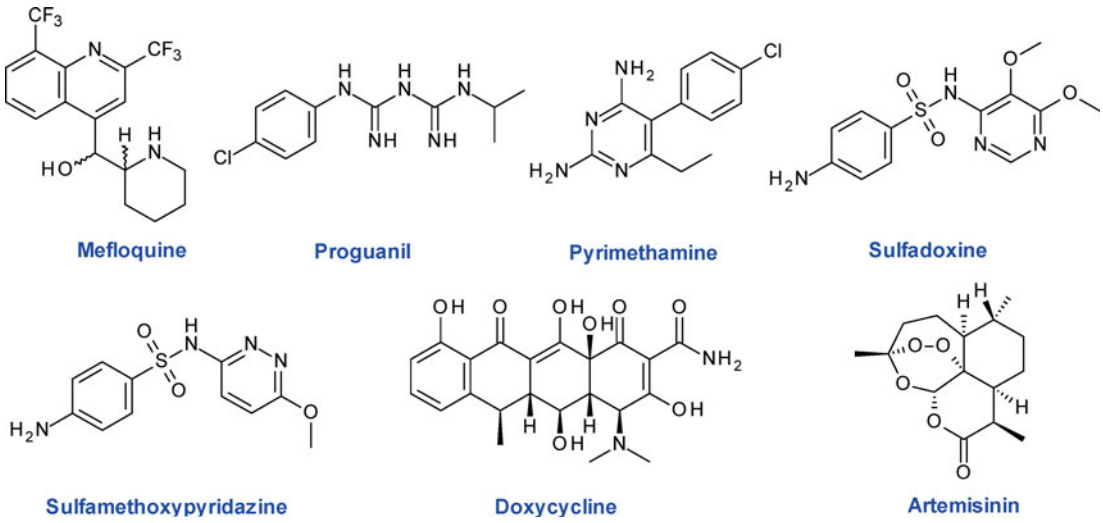
Atabrine

5.187 *The first synthetic malaria drugs were marketed as racemic mixtures. The call for patient's compliance appears perhaps a bit blunt.*

The mechanism of action for chloroquine and quinine is similar. The basic drug accumulates in the acidic digestive vacuole of the erythrocytic schizont and inhibits the formation of haemozoin. For more than 20 years, choroquine had been the standard of care for malaria patients. While the long-term experience with the drug, its reasonable cost of goods and facile availability was certainly advantageous, reports on cases of resistance in Thailand, Cambodia and Columbia occurred as early as in the 1950s. There was increasing evidence of disseminating resistant malaria-strains, which gave rise to a higher mortality rate worldwide, markedly in Africa since the 1990s. [429] The exact mechanism of chloroquine resistance is still under debate, though a mutated membrane protein (Pf-CRT) of the digestive vacuole has been identified, which prevents the drug from reaching a toxic concentration, either by inhibiting its influx, or by facilitating its expulsion.

To battle resistant malaria pathogens, a diverse range of new drugs have been developed, some of them possessing also a novel mechanism of action. [453] Mefloquine is a potent schizonticide, which was discovered from a screening programme of over 250,000 compounds at the Walter Reed Army Institute of Research, shortly after the Vietnam War had ended. In the mid-1970s, the US Department of Defense had sponsored the human phase I and phase II trials with mefloquine in prisoners at the Joliet Correctional Center, Illinois, and at the Maryland House of Correction. The drug received expedited approval by the FDA in 1986, and was licensed to Hoffmann-La Roche for production and mar-

keting under the brand name Lariam, which is the mefloquine hydrochloride salt. The obviously neglected comprehensive safety evaluation and omission of further clinical trials caused already months after the product launch major concern among prescribing physicians and their patients, when serious side effects, such as harm to the central nervous system and even fatalities were reported. Today, Lariam is marketed in the US by generics companies; it has a 'black box warning' from the FDA, emphasising its potentially permanent neurological and psychiatric side effects (Fig. 5.188). [454]



5.188 *New drugs have been developed to overcome resistance of malaria pathogens, also featuring novel mechanisms of action.*

Aside from the schizonticidal agents, a number of compounds have been developed after Word War II, which interferes with the disease process at a different level. Dihydrofolate reductase inhibitors, like proguanil and pyrimethamine, block the biosynthesis of purine and pyrimidine, and therefore also the parasite's DNA replication. Different sulfonamide drugs, *e.g.* sulfadoxine and sulfamethoxypyridazine, exert a similar mechanism of action.

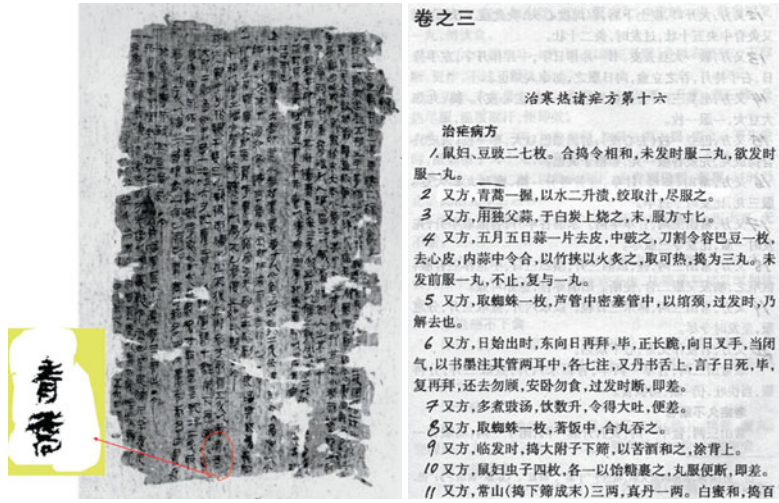
Tetracyclines, like doxycycline, are broad-spectrum antibiotics with extended use as cheap and effective antibacterials. They act at the level of protein synthesis, and impair, in case of *Plasmodium falciparum*, the formation of merozoites. Due to a slow onset of activity, doxycycline is used in combination with faster acting drugs for malaria therapy; as a single compound it is today applied in prophylaxis, frequently in regions with known chloroquine resistance.

However, the most modern drugs in our armoury to fight malaria infections are derivatives of artemisinin. This natural product originates from the sweet wormwood, (*Artemisia annua*), a traditional Chinese herbal medicine, which had already been in use for malaria treatment some 1,300 years prior to quinine, albeit its active principle was only discovered in the 1970s.

5.10.4 The Discovery of Artemisinin

During archaeological excavations in the Chinese province of Hunan between 1972 and 1974, three tombs dating back to the early Western Han-Dynasty (207 BC to 9 AD) were unearthed. One of them could be assigned to a nobleman, the "Marquis of Dai", whereas the other two had presumably been dedicated to his wife and his son. In the third tomb was found a male in his 30s, who is thought to have died in 168 BC. There was as well a silk scroll with 283 known prescriptions discovered, titled "Medical Treatments for 52 Diseases" (Wu Shi Er Bing Fang), mentioning for the first time sweet wormwood (qinghao, 青蒿, see yellow box in Fig. 5.189) as a remedy to treat haemorrhoids.

The earliest indication of *qinghao* as an antimalarial medication stems from a famous healer and alchemist, Ge Hong (284–346), during the Jin Dynasty (265–420 AD), who documented instructions to prepare extracts in his medical text "Emergency Prescriptions Kept up one's Sleeve" (*Zhou hou bei ji fang*) (Fig. 5.189). [455]


卷之三

治寒热诸症方第十六

治疟病方

1 鼠妇、豆豉二七枚。合捣令相和，未发时服二丸，欲发时服一丸。

2 又方，青蒿一握，以水二升渍，绞取汁，尽服之。

3 又方，用独父蒜，于白炭上烧之，末，服方寸匕。

4 又方，五月五日蒜一片去皮，中破之，刀割令容巴豆一枚，去心皮，内蒜中令合，以竹挟以火炙之，取可热，捣为三丸。未发前服一丸，不止，复与一丸。

5 又方，取蜘蛛一枚，芦管中密塞管中，以绪颈，过发时，乃解去也。

6 又方，日始出时，东向日再拜，毕，正长跪，向日叉手，当闭气，以书墨注其管两耳中，各七注，又丹书舌上，言子日死，毕，复再拜，还去勿顾，安卧勿食，过发时断，即差。

7 又方，多煮豉汤，饮数升，令得大吐，便差。

8 又方，取蜘蛛一枚，著饭中，合丸吞之。

9 又方，临发时，捣大附子下筛，以苦酒和之，涂背上。

10 又方，鼠妇虫子四枚，各一以饴糖裹之，丸服便断，即差。

11 又方，常山（捣下筛成末）三两，真丹一两。白蜜和，捣百

5.189 *The "Medical Treatments for 52 Diseases" (on the left) were written on silk fabric and in part also on small bamboo strips. With its 9,950 characters, this is the most extensive medical text found in ancient Chinese tombs. – Ge Hong's 16th recipe in part Nr. 3 of his four-volume corpus Zhou hou bei ji fang (on the right) describes the preparation of extracts from qinghao to treat "heat vexation" and (intermittent) "bone-steaming" fevers (nüe, Malaria).*

As ordered by Mao Zedong (1893–1976) in the 1960s, the China Academy of Traditional Chinese Medicine received support from the People's Liberation Army Research Institute in the covert task, code-named "Project 523" (meaning: May 23rd 1967, when the project launch was decided), of identifying new antimalarial compounds. The reason was an urgent request from North Vietnam, who saw more of its soldiers dying from malaria than from warfare. In October 1971, the Chinese phytochemist and pharmacologist Youyou Tu and her team at the Academy succeeded to obtain an extract from sweet wormwood, which proved very efficacious in animal models of malaria and well tolerated, when courageously tested in a self-experiment. [456] After many failures and inconsistent data, the researchers fortunately followed more strictly the procedure Ge Hong had outlined: 'Another recipe: *qinghao*, one bunch, take two *sheng* [2 × 0.2 l] of water for soaking it, wring it out, take the juice, ingest it in its

entirety'. [455, 457] It turned out that treatment with cold water yielded extracts of higher potency than the normally employed method, which used hot solvents.

The actual active ingredient *Qinghaosu* ((+)-artemisinin) was as well isolated by Y. Tu in 1972. When in 1975 the structure of (+)-artemisinin was elucidated at the Chinese Academy of Sciences (CAS), Institute of Biophysics, by degradation reactions and crystal X-ray diffraction techniques, as published [458] in 1977, it became clear why the compound had a stability problem at elevated temperature. The peroxide ketal and the acetal moiety of this oxygen-rich sesquiterpene are easily cleaved under such conditions in aqueous media.

For several years, the Chinese government prohibited any publication of the scientific results – in particular outside of China –, and equally the export of precious plant material. Only towards the end of the 1970s and during the 1980s, the first English language papers on this research topic appeared, submitted in part by anonymous authors. [459] Once the WHO finally supported the new drug after the turn of the century, artemisinin, and subsequently artemisinin-based combination therapy (ACT) would become the treatment of choice for malaria of all degrees of severity. In 2011, Youyou Tu received the prestigious Lasker-DeBakey Clinical Medical Research Award for "the discovery of artemisinin, a drug therapy for malaria that has saved millions of lives across the globe, especially in the developing world".

5.10.5 Sweet Wormwood (*Artemisia annua*)

Artemisia annua is also known as sweet wormwood, sweet annie, sweet sagewort or annual wormwood. This annual herb belongs to the *Compositae*/*Asteraceae*-family (as *e.g.* daisies and sunflowers) with fern-like leaves and a camphor-like scent. Its native geographic distribution across regions in Eurasia with warm and humid summers extends from the Balkans to India, Vietnam and China. Being naturalised in many other countries, *e.g.* in Germany along the river Elbe (Fig. 5.190), the plant could even be cultivated in Finland.

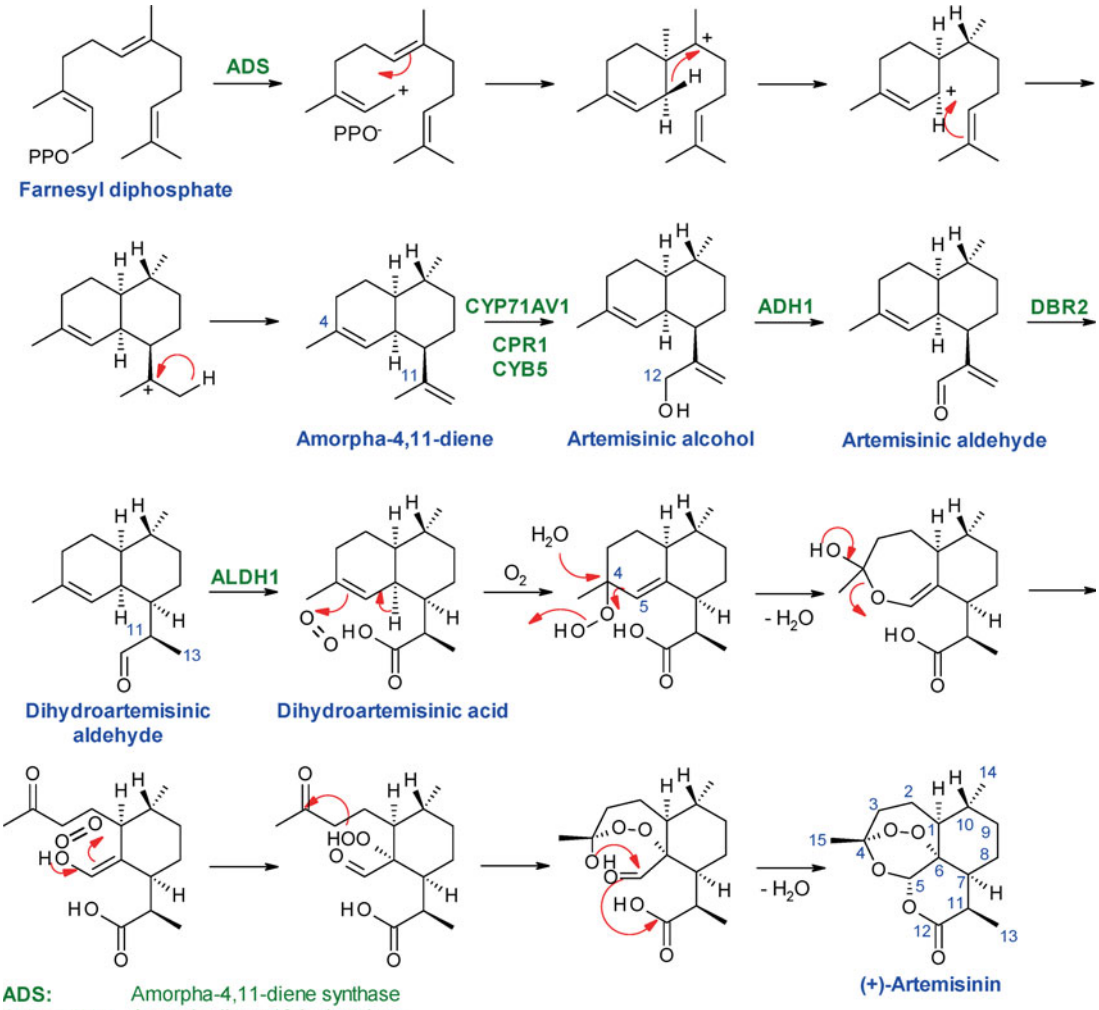
5.190 *Sweet wormwood (Artemisia annua) was first described in 1753 by the Swedish botanist and physician Carl von Linné (1707–1778) in Species Plantarum. [460]*

5.10.6 Biosynthesis

Primarily the glandular secretory trichomes in all aerial parts of *Artemisia annua* synthesise and sequester artemisinin, with leaves and flower buds showing the highest level. [461] Starting material of the biosynthesis is farnesyl diphosphate, produced in the plant cell's cytosol along the mevalonic acid pathway. Interestingly, ^{13}C-labelling experiments however revealed that plastidial geranyl diphosphate originates from the methylerythritol phosphate pathway (Rohmer-path), and is partially exported across the plastid's membrane into the cytosol compartment for further transformation into farnesyl diphosphate. [462]

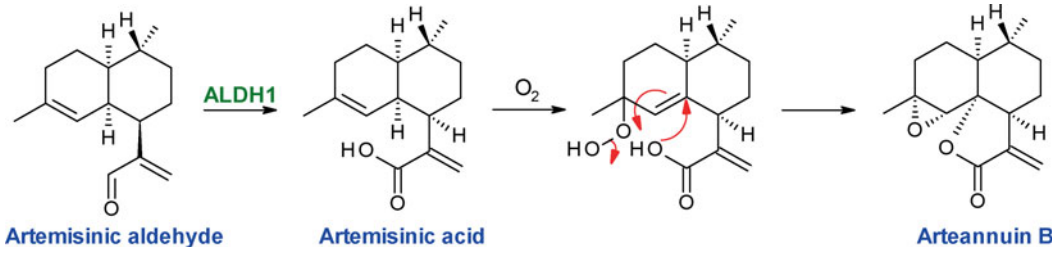
In the initial biosynthetic step, amorpha-4,11-diene synthase facilitates the cyclisation of farnesyl diphosphate to amorpha-4,11-diene, which is then sequentially converted to artemisinic alcohol by a set of three cytochrome P450 enzymes, and further oxidised to dihydroartemisinic aldehyde under

ADH1-catalysis. [463] The double bond at C-11/13 is reduced by DBR2, a member of the enoate reductase family, and the aldehyde dehydrogenase ALDH1 leads to the oxidation product dihydroartemisinic acid. [464] The further oxidation cascade is non-enzymatic, however stereoselective, and yields (+)-artemisinin *via* relatively unstable intermediates. Geoffrey D. Brown at the University of Reading was able to demonstrate, that the Hock rearrangement of a hydroperoxide, followed by oxidation of the resulting enol with triplet oxygen, are the key steps of this sequence. [465]



ADS: Amorpha-4,11-diene synthase
CYP71AV1: Amorphadiene-12-hydroxylase
CPR1: Cytochrome P450 reductase
CYB5: Cytochrome B5 reductase
ADH1: Alcohol dehydrogenase
DBR2: Artemisinic aldehyde-Δ11(13)-reductase
ALDH1: Aldehyde dehydrogenase 1

Experiments with ^{13}C- and ^{2}H-labelled precursors provided unambiguous evidence, that there are several branch points in the biosynthesis of artemisinin. The most prominent sesquiterpene metabolites in sweet wormwood are, aside from artemisinin, artemisinic acid and arteannuin B, which branch off at the artemisinic aldehyde stage. [465]

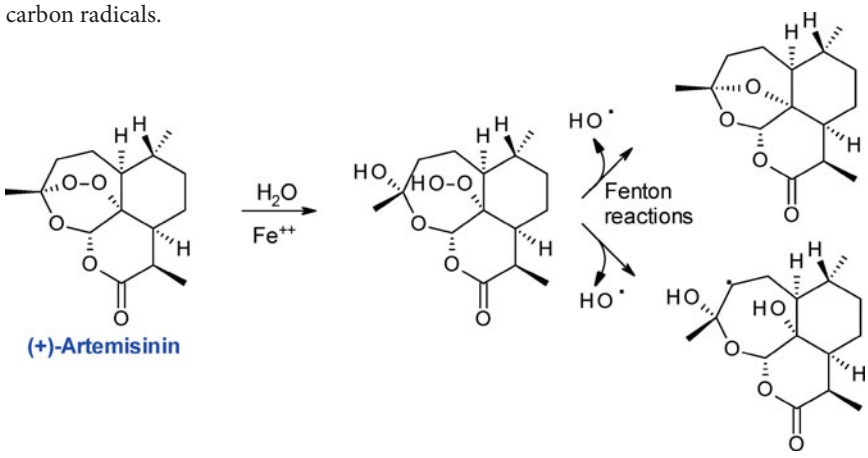


Notable is as well the impact of methyl jasmonate on the biosynthesis of artemisinin, which regulates as a phytohormone the response of a plant to abiotic or biotic stress (*e.g.* drought, wind, or attacks from herbivores). In case of sweet wormwood, methyl jasmonate stimulates the expression of amorpha-4,11-diene synthase (ADS) and the cytochrome P450 monooxygenase (CYP71AV1), leading ultimately to an enhanced formation of artemisinin. [464]

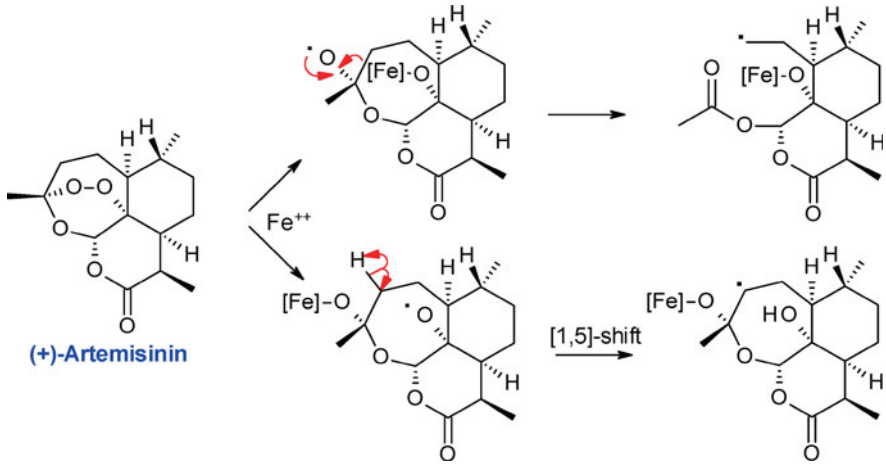
5.10.7 Pharmacology of Artemisinin

The striking activity of artemisinin is characterised by its potency, also against a number of multidrug-resistant parasites, its rapid onset, and the ability to clear the asexual and early sexual forms of *Plasmodium*. However, the mechanism of action of artemisinin is not yet fully understood, and its initial molecular target is under controversial debate. [466] The 1,2,4-trioxane structural motif of the sesquiterpene seems to be the generally accepted critical pharmacophore. While one school of thought favours the endoperoxide activation and cleavage by ferrous ions (Fe^{2+} from haeme) as initial step, other groups have *e.g.* generated data, which support the direct interaction of artemisinin and a calcium ion pump. [467]

In case the trioxane moiety is cleaved unsymmetrically, the resulting hydroperoxide fragments in a Fenton-like reaction, into energy-rich OH radicals and carbon radicals.



The symmetric cleavage of the endoperoxide generates high-energy carbon radicals as well, which can alkylate proteins of the parasite and damage the membrane of the food vacuole.

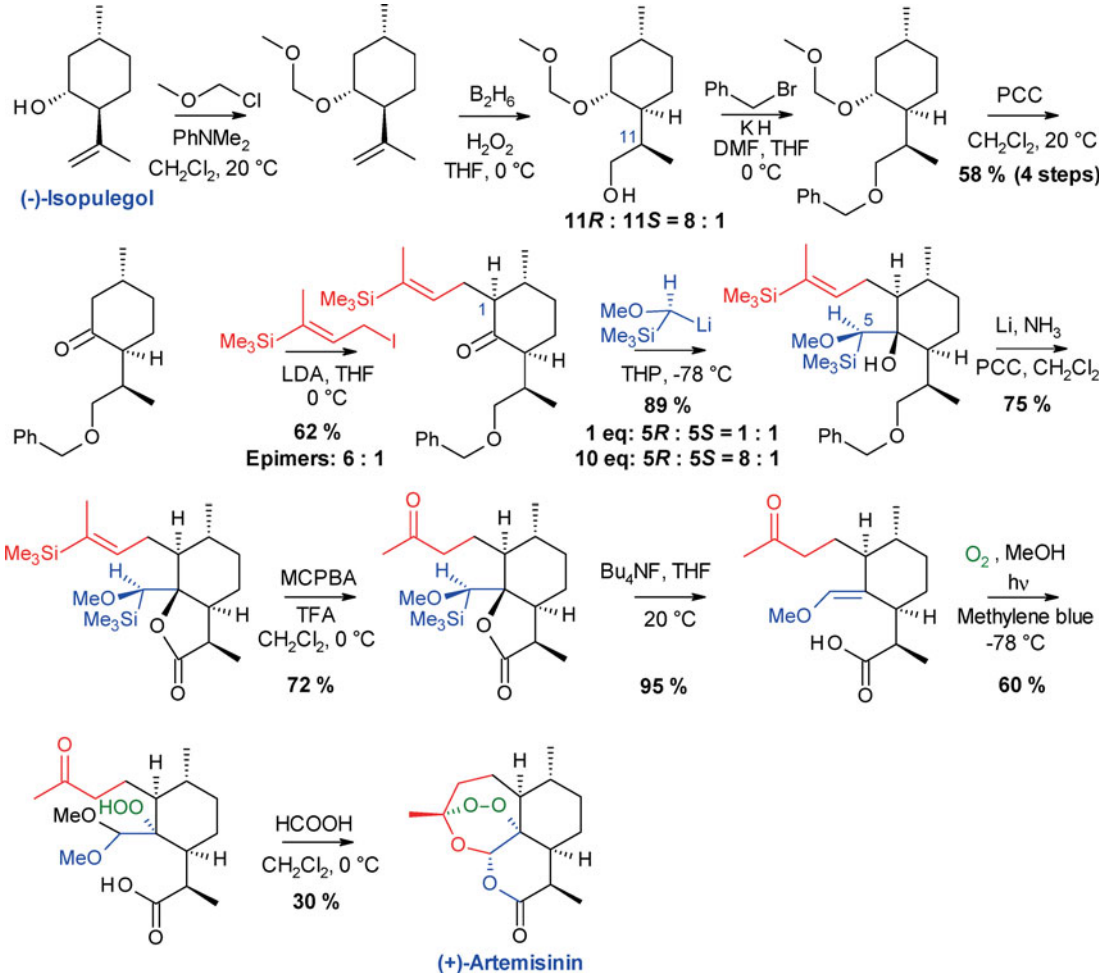


It seems clear, that interference with the pathophysiological degradation of haemoglobin is an essential part of the pharmacological effect of artemisinin. Current research focuses on the possibility that artemisinin can harm *Plasmodium* species in other ways, as *e.g.* through interaction with flavoenzyme disulfide reductases, and thereby perturbing the parasite's redox homeostasis [468], and/or through inhibition of PfATP6, which is a calcium ion pump of the sarco/endoplasmatic reticulum (SERCA). [469] Attempts are underway to rationalise this concept by computational means.

5.10.8 Total Syntheses

Soon after the structure of the natural product and its properties became known, Hoffmann-La Roche initiated a synthesis programme. [470] In 1983, Gerard Schmid and Werner Hofheinz published a total synthesis, which gained attention worldwide.

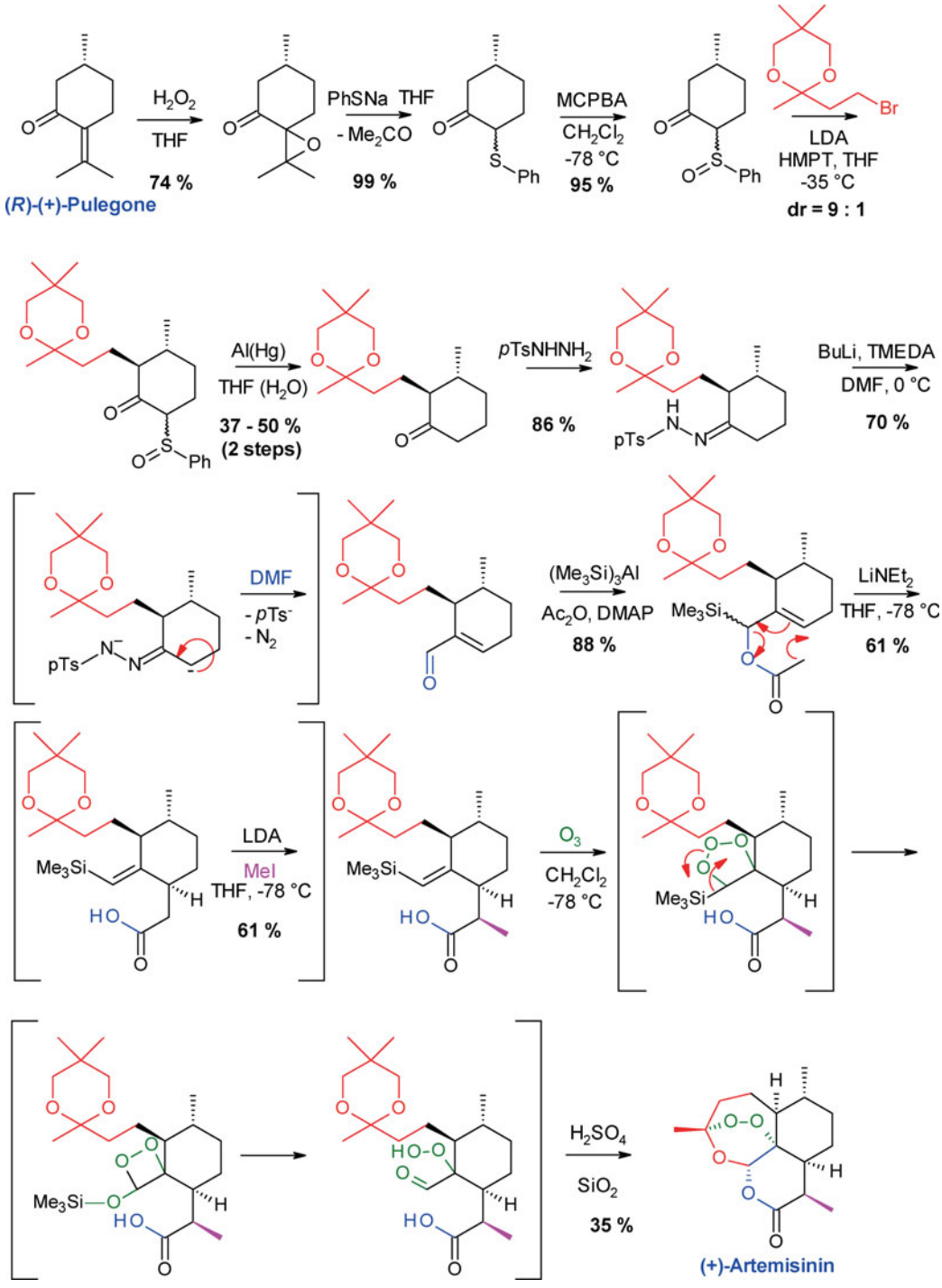
The starting material is (–)-isopulegol, which is protected in a first step as an MOM-ether. Hydroboration and oxidative workup yield the corresponding alcohol as a diastereomeric mixture with an 8 : 1 ratio. Benzylation, acetal cleavage and oxidation gives benzyloxymenthone with an overall yield of 58 % related to (–)-isopulegol. Kinetic deprotonation and alkylation of the corresponding enolate produces an epimeric mixture of 6 : 1. The selectivity of the subsequent keton transformation with lithium methoxy(trimethylsilyl)methylide can be greatly increased by using the alkylating reagent in large excess. Debenzylation and oxidation generates a bicyclic lactone, the vinyl silane function of which is afterwards converted with *m*-chloroperoxybenzoic acid into the corresponding ketone. The desilylation is a synchronous, antiperiplanar process. Photooxygenation in methanol at –78 °C leads to a complex reaction mixture, which is treated with formic acid at 0 °C to give artemisinin in 30 % isolated yield.



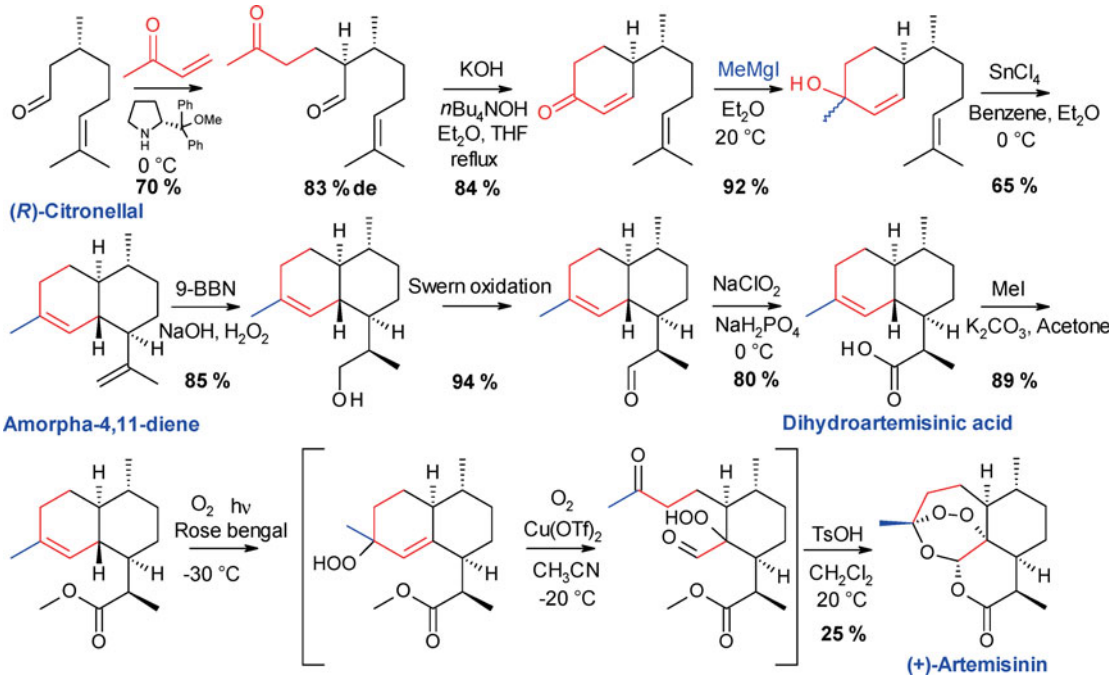
Over the following years, there were a number of additional total syntheses published. [471] The one reported by Mitchell Avery at the University of North Dakota in 1987 is particularly worth highlighting. [472]

Starting with (*R*)-(+)-pulegone, the isopropylidene residue is first degraded to a sulfoxide by epoxidation, retro-aldol fragmentation and oxidation. The following alkylation occurs with a diastereoselective ratio of 9 : 1. Removal of the sulfoxide with aluminium amalgam and reaction with *p*-toluenesulfonyl hydrazide provides a hydrazone, which is submitted to a Bamford-Stevens-type reaction. The resulting vinyl anion thereby reacts with DMF to form the α,β-unsaturated aldehyde. [473] At this stage, all impurities are removed by chromatography. Whereas the trimethylsilyl salts of lithium, sodium and potassium show unsatisfying results in the subsequent 1,2-addition reaction, the aluminium salt leads in surprisingly high yield to an alcoholate, which can be scavenged by acetic anhydride. The next step forms the vinylsilane residue and the desired carboxylic acid intermediate from an Ireland-Claisen rearrangement, which is directly methylated after the formation of a dianion with LDA.

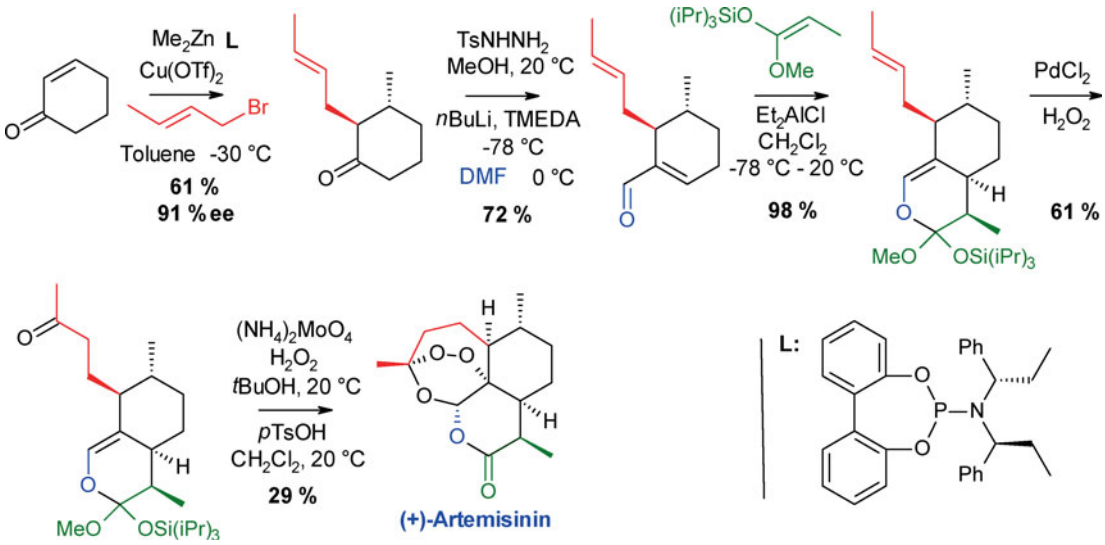
The final step generates artemisinin in 35 % yield from ozonolysis of the vinylsilane *via* an interesting fragmentation of the primary ozonide. [474]



Particularly remarkable is a nine-step total synthesis by Jhillu Singh Yadav from 2010, which does not require protecting groups and follows a biomimetic path for the oxidtion steps. [475] Starting material is (*R*)-(+)-citronellal, which is reacted with methyl vinyl ketone *via* an enamine mediated organocatalysis. After an intramolecular aldol condensation follows a Grignard reaction with unselective stereochemistry, which is however not relevant for the further synthesis. Subsequent cyclisation under tin tetrachloride catalysis leads after chromatographic purification to the desired amorpha-4,11-diene in 65 % yield. By stereo- and as well regioselective hydroboration and stepwise oxidation reactions, dihydroartemisinic acid is obtained, which is then further oxidised to artemisinin, following a procedure developed by Richard K. Hanyes at the University of Sydney. [476] The course of the reaction follows most likely the biosynthetic path.



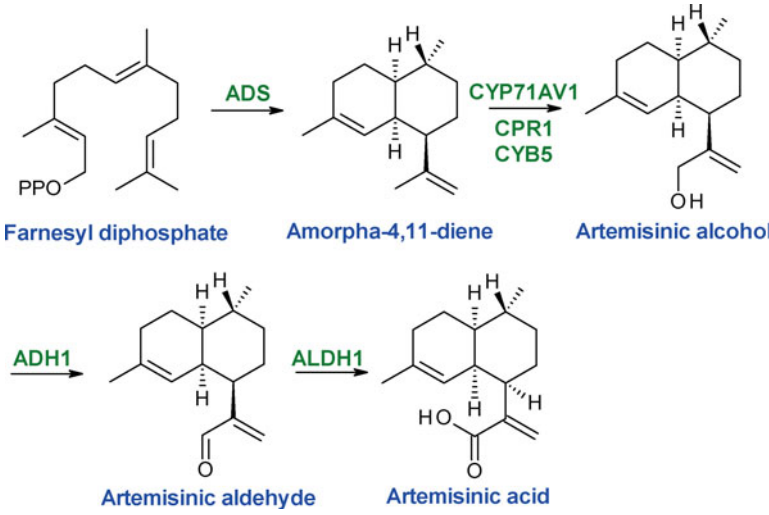
The by far most elegant total synthesis of artemisinin was published in 2012 by Silas Cook at Indiana University in Bloomington. [477] The target molecule is obtained in only six steps. The first one produces in an enantioselective domino Michael-alkylation sequence two stereocentres, which direct all subsequent diastereoselective transformations. Formation of the α,β-unsaturated aldehyde is followed by an unusual Diels-Alder reaction, leading to the desired cyclic orthoester. The ketone function is introduced into the side chain through a palladium-catalysed oxidation with hydrogen peroxide. In the last step, the enolic orthoester is reacted with singlet oxygen, generated by an ammonium molybdate/peroxide system (according to a protocol from Jean-Marie Aubry). Removal of the protecting groups with *p*-toluene sulfonic acid gives finally (+)-artemisinin in 7.6 % yield over all stages.



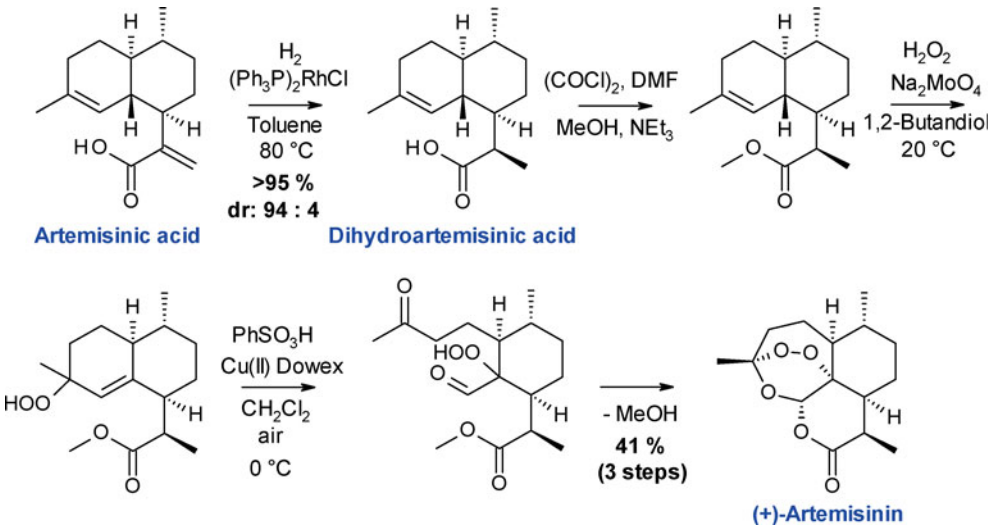
5.10.9 Production

From the examples given above, it is easy to recognise the impressive progress that was achieved as to concepts and methods towards the total synthesis of artemisinin. Nevertheless, sweet wormwood plants have remained until quite recently the only relevant industrial source. [455, 478]. Major producers are China, Vietnam, Madagascar, and several east African countries. The active ingredient is extracted from dried plant leaves with hexane, and after evaporation of the solvent purified by crystallisation. The average artemisinin content in dried material from wild-type plants ranges between 0.1 and 0.4 percent by weight. Specifically bred varieties have reportedly achieved up to 1.4 percent by weight. In recent years, the production volume has fluctuated significantly, leading to supply shortages and causing price swings between 120 and 1,200 US Dollar/kg.

To address low inventory situations better, it appeared attractive accessing artemisinin *via* partial synthesis, and to have its artemisinic acid precursor produced by fermentation. Funded generously by the Bill & Melinda Gates Foundation, the pivotal basis for this endeavour came from the American biochemist Jay D. Keasling at the University of California in Berkley, who succeeded in 2006 to establish a genetically engineered baker's yeast strain, which contained all the enzymes required for the production of artemisinic acid. He was able to achieve secretion levels of the acid reaching up to 100 mg/l medium. [479] Further optimisation work in collaboration with Amyris Biotechnologies has meanwhile augmented the productivity to 25 g/l. [463]. The fermenation is carried out in presence of isoamyl myristate to enhance the solubility of artemisinic acid in a biocompatible way. The product is re-extracted from the separated organic phase under moderately basic conditions (pH 10.7) and crystallises upon acidification (pH 5.0).



The subsequent diastereoselective reduction of artemisinic acid to dihydroartemisinic acid works on a rhodium catalyst in excellent yield and with high selectivity. There follows an esterification, since the esters show better selectivity during the ensuing oxidation steps. The latter can be performed without purification of the intermediate in a quasi one-pot reaction. Artemisinin is obtained after chromatography in 41 % yield.



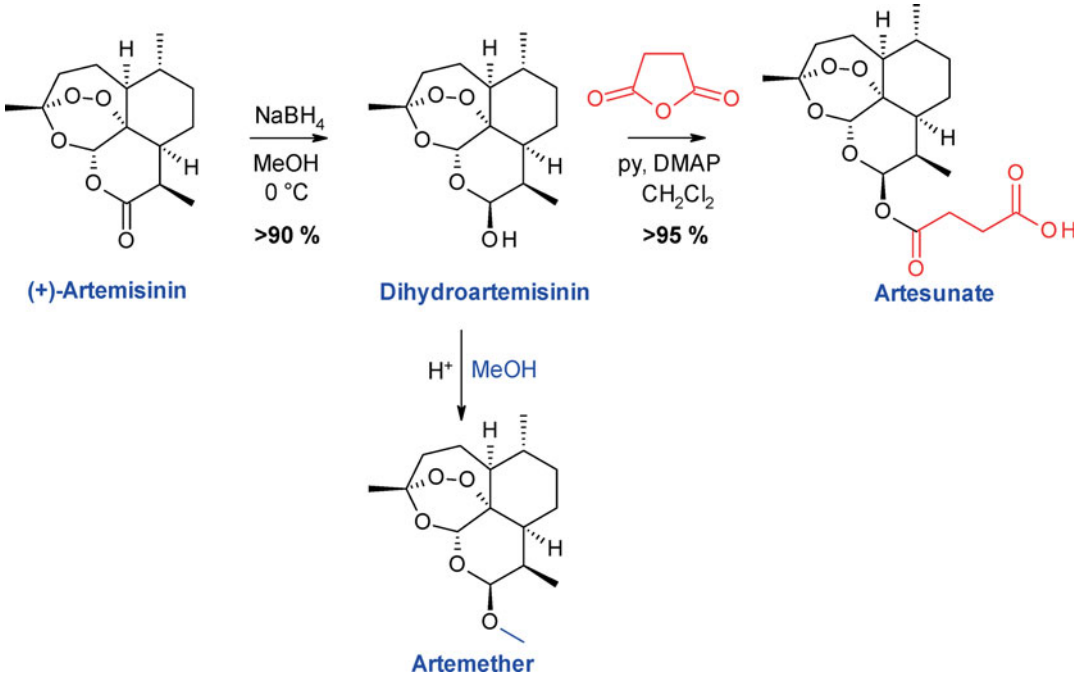
Peter Seeberger at the Max-Planck-Institute of Colloids and Interfaces in Potsdam accomplished in 2012 a comparable synthesis, where dihydroartemisinic acid is converted to artemisinin in a flow apparatus with photochemically produced singlet oxygen. [480]

The industrial process uses this photochemical step as well: In April 2013, Sanofi announced the launch of a semi-synthetic production facility for artemis-

inin at their Garessio site in Italy. [481] The fermentation part of the process corresponds to the one developed by Sanofi's partner Amyris. However, the hydrogenation is performed on a Ru-segphos-catalyst and the singlet oxygen for the Schenck-ene reaction is generated in three large-scale continuous-flow photoreactors. The projected annual capacity of this plant is around 50 to 60 tonnes, covering one third of the artemisinin demand worldwide per year. The anticipated production cost of 350–400 US Dollar/kg is in line with that from botanical sources. In case this price declines significantly, cultivation of *Artemisia annua* and the extraction route would become unprofitable, push the business of farming sweet wormwood into ruin, and lead again to supply shortages. [482]

5.10.10 Derivatives

Since poor bioavailability and a short half-life limit the pharmacological activity of artemisinin, several derivatives have been developed to address such disadvantages. A key compound among them is dihydroartemisinin, which serves not only as an intermediate, but is used as a drug by itself. Reduction of the lactone is achieved under very mild conditions with sodium boranate in methanol at 0 °C. Esterification with succinic acid anhydride leads to artesunate, a water soluble compound, which is suitable for different routes of application (oral, rectal, intramuscular, and intravenous). Acetalisation of dihydroartemisinin with methanol produces artemether, which is soluble in lipids and can therefore be formulated for oral, rectal and intramuscular dosing.



5.10.11 Final Remarks

While experimental resistance to artemisinin had been induced earlier, blood samples from Cambodia, Senegal and French Guiana provided in 2005 the first hints on drug-resistant *Plasmodium falciparum* isolates in the field. [483] The first clinical cases were reported in 2008 from Pailin and other provinces in western Cambodia [484], and in 2012, resistant strains were also found in neighbouring Thailand. [485] In these countries, artemisinin and its derivatives had been frequently used as mono-therapy and in an uncontrolled fashion. While there is an enormous effort under way to elucidate the mechanisms of resistance development, the WHO urges to only apply artemisinin-based combination therapy (ACT) for the treatment of malaria in order to secure and maintain the efficacy of our most recent – and at the same time oldest – weapon in the combat of this devastating disease for as long as possible. [486]

Summary in Bullet Points

- Artemisinin is the most modern drug against malaria, an infectious disease that has been our evil companion throughout the history of mankind.
- The elucidation of the parasite's reproductive cycle has opened up a rational access to efficient therapies, *e.g.* with schizonticidal agents like quinine and mefloquine, or novel drugs like artemisinin.
- While the mode of action of artemisinin is not yet completely understood, the drug was isolated in the 1970s from sweet wormwood (*Artemisia annua*). The plant known in Traditional Chinese Medicine to treat malaria for at least 1,600 years.
- During the last three decades, a number of interesting total syntheses of this complex natural product have been published, illustrating in an impressive way the advancements of modern organic chemistry.
- On an industrial scale, artemisinin is obtained from plant extracts of sweet wormwood, and by fermentation combined with partial synthesis.
- Complete eradication of malaria might not be possible, and the fight against the disease will remain a constant battle in search for new drugs and in promoting practical strategies to delay the development of resistance.

5.11 Caffeine

Today, coffee is along with crude oil, natural gas, copper, silver, gold, sugar, cotton, corn, and wheat one of the world's largest trading commodities – worth more than 12 billion dollars in trade every year – and the 2nd most consumed beverage worldwide after water. Most of the world's coffee crop is produced in Southern and Central America, Asia and Africa. The United States is the biggest importer, while Finland is the nation that consumes the most per capita.

The popularity of coffee is certainly attributable to its caffeine content. The alkaloid caffeine acts mildly euphorigenic, stimulating or relaxing, devoid of leading to any physical dependency, not even to a compensatory depressive phase or state of exhaustion. [487]

5.11.1 History

Originally indigenous to the highlands of Abyssinia (the present-day Ethiopia), the coffee tree was brought very early to Arabia. The oldest written traditions relating to coffee come from the Persian physicians Muhammad ibn Zakariyā Rāzī (Rhazes, 865–925) and Abū Alī al-Husain ibn Abdullāh ibn Sīnā (Avicenna, 980–1037). They describe *kahwa* as a tonic, which supposedly originates from Yemen. The coffee beans were exported together with other merchandise *via* the sea port of Al Mukha (Mocha) to Jeddah, and from there with ships and galleys on to Suez, which was connected to the central markets in Cairo and Damascus through major caravan routes.

5.191 *A coffee-house in Vienna.*

In 1554, the first coffee-house was opened in Constantinople (the present-day Istanbul). In the second half of the 17th century, in the wake of the expansion and fall of the Ottoman Empire, coffee houses spread all across Europe, to Vienna, Venice, Paris, Marseille, London, Hamburg and to other places (Fig. 5.191).

At around the same time, the Dutch brought the first coffee plants to Java. From the first half of the 18th century, cultivation of coffee is also traceable in the West Indies, in Ecuador, Venezuela and Brazil. [488]

In 1669, the Turkish Ambassador Soliman Aga gave coffee beans as a gift to Ludwig XIV. In 1734, Johann Sebastian Bach composed the "Coffee Cantata" (*Kaffeekantate,* BWV 211). Ludwig van Beethoven (1770–1827) composed "Rage over the Lost Penny" (*Die Wut über den verlorenen Groschen,* op. 129), while counting 60 beans for one cup of coffee. Voltaire (1694–1778) and Balzac (1799–1850) were said to have drunk up to 30 cups of coffee per day. Frederick the Great (1712–1786) however took the view that soldiers who drank coffee, as opposed to beer, could never win any battles. [489]

5.11.2 Botany and Occurrence

Around 80 coffee plant varieties are known. For the production of coffee, however, only two varieties are cultivated in practice: *Coffea arabica* (Arabian coffee) and *Coffea canephora* (robusta coffee), in Java and Sumatra, in India, in the Arabian Peninsula and in a few African and South American countries. Arabica coffee grows in regions at an elevation of 600–1,800 metres above sea level, from where some coffee variants are ranked among the best in the world. The share of arabica coffee in the world market amounts to 70–75 %. Robusta coffee grows already at an elevation of 300–600 metres. It is more resistant to lower temperatures and diseases.

Coffee plants are trees or bushes with white blossoms (Fig. 5.192) and cherry-like fruits (Fig. 5.193). Each fruit contains one or two coffee beans. The

5.192 *Coffee blossom of Coffea arabica at a plantation in Brazil.*

5.193 *Coffee cherries ripening on a Coffea arabica tree.*

caffeine content of *Coffea arabica* beans amounts to 1.2 % and that of *Coffea canephora* around 2 %. [490, 491]

Caffeine has an antimycotic activity, and thus protects the raw coffee from mould and the production of mycotoxins. Under the conditions of roasting (220–250 °C), it is stable. The most important reactions the coffee ingredients undergo during the roasting process are the Strecker degradation and Maillard reactions. Only a small fraction of the caffeine sublimes [487] during roasting. The majority of the weight loss (around 20 %) is due to evaporation of water and release of carbon monoxide and carbon dioxide. Thus, the roasted coffee contains more caffeine than raw coffee.

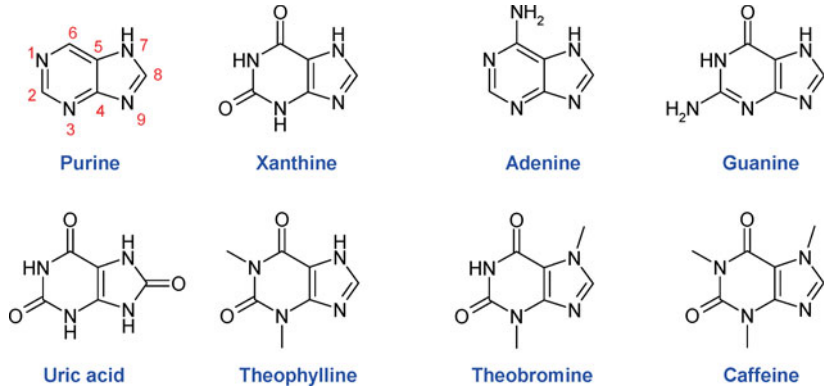
Caffeine is also found in tea leaves from *Camellia sinensis* (up to 5 %) and in the seeds of cola nuts (*Cola nitida*, up to 2 %). Maté (Paraguay tea, *Ilex paraguariensis*, with 0.3–1.5 %) and the seeds of the cocoa plant (*Theobroma cacao*, with 0.05 %) show a significantly lower content. The main alkaloid in cocoa beans is theobromine.

The Boston Tea Party (1773) could be considered as a gigantic experiment to prepare tea with cold salt water. This obviously failed, but fueled instead America's zeal for independence and may have subsequently also promoted Americans' preference for coffee, thereby further contrasting their former colonial masters.

5.194 *Friedlieb Ferdinand Runge (1784–1867) discovered caffeine.*

5.11.3 Structure Determination

Purine derivatives, which include caffeine, belong to the first natural products, for which structure determination was attempted. In 1776, Carl Wilhelm Scheele (1742–1786) isolated a compound from bladder stones, which Antoine François Comte de Fourcroy (1755–1809) in 1793 named *acide ourique* (uric acid). In 1819, Johann Wolfgang von Goethe presented a box of coffee beans to the chemist Friedlieb Ferdinand Runge (Fig. 5.194). Runge noted down about that encounter in his *Hauswirthschaftliche Briefe* ("housekeeping papers"): "After Goethe had expressed his great satisfaction to me ... he handed over to me a box of coffee beans, which a Greek had sent him as something exquisite. 'You can use these for your experiments as well!' said Goethe. – He was right about that; soon after, I discovered therein 'caffeine', what had become so famous for its high nitrogen content." [492]



In 1834, Justus von Liebig (1803–1873) and Eilhard Mitscherlich (1794-1863) determined the exact molecular formula of uric acid, and in 1875, Ludwig Medicus (1847–1915) proposed a bicyclic structure. In 1841, the Russian chemist Alexander Woskresensky (1809–1880) isolated theobromine from cocoa beans, and in 1844 Julius Bodo Unger (1819-1885) obtained guanine from guano. In 1885 Albrecht Kossel (1853–1927) recognised that adenine is a constituent of nucleic acids. Three years later, he isolated theophylline from tea leaves. In 1889, the Knoll company marketed theobromine as a diuretic. Emil Fischer (1852–1919) eventually recognised the structural relationship between uric acid, theophylline, theobromine and caffeine. [493, 494]

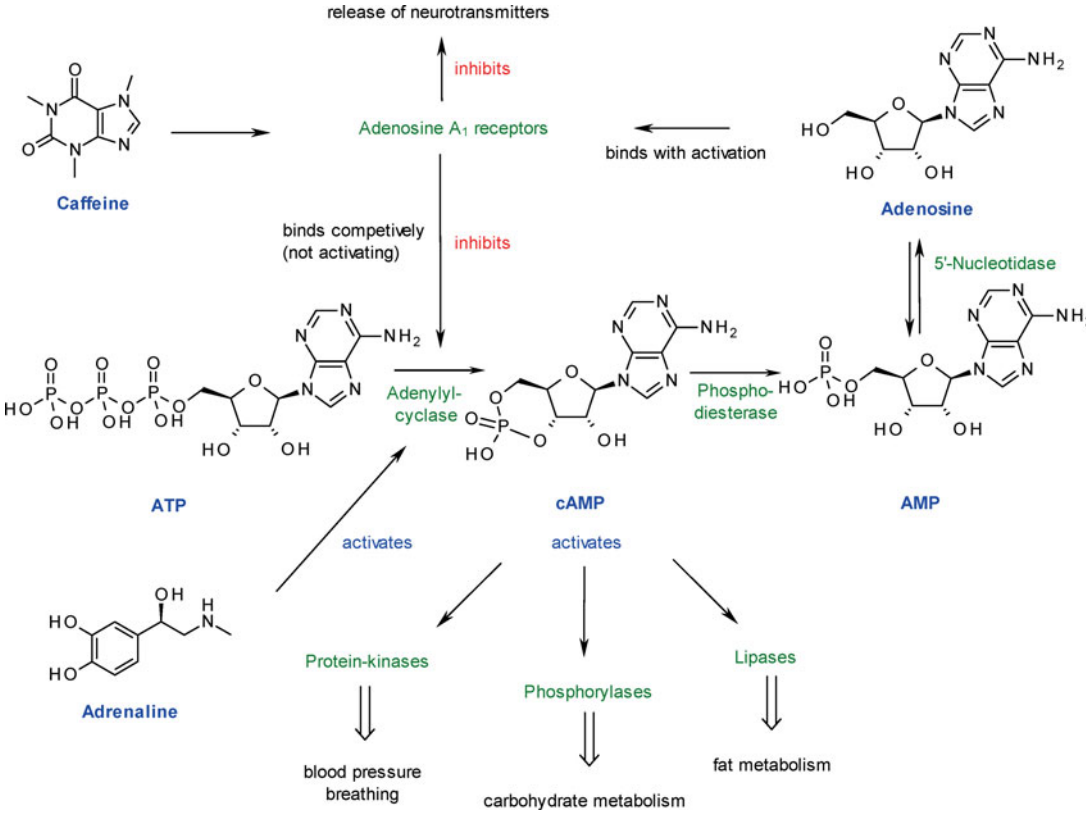
5.11.4 Pharmacology

In 1685, the French apothecary Philippe Sylvestre Dufour (1622–1687) published a famous book: "Traités nouveaux & curieux du café, du thé et du chocolate", wherein he ascribed, in contrast to popular belief, a number of beneficial pharmacological effects to coffee. He argued that the ingredients of the drink counteracted drunkenness and nausea as well as menstrual disorders, and

additionally: 'Coffee banishes languor and anxiety, gives to those who drink it a pleasing sensation of their own well-being and diffuses through their whole frame, a vivifying and delightful warmth'.

Caffeine leads to constriction of the blood vessels, whereby the blood pressure rises. The bronchial vessels are dilated, which eases breathing. Moreover, fat and carbohydrate metabolism is stimulated.

Many of these effects can be explained by an interference with a central signal cascade (Fig. 5.195). Adrenaline activates adenylylcyclase, which converts adenosine triphosphate (ATP) into cyclic 3',5'-adenosine monophosphate (cAMP). This is a secondary messenger, which is deactivated by a phosphodiesterase to adenosine monophosphate (AMP). Dephosphorylation with a 5'-nucleotidase releases the neuromodulator adenosine, which is enriched extracellularly in the waking state and is degraded during sleep. When adenosine binds in the presynaptic cleft to adenosine-A_1-receptors of the nerve cells, the release of most neurotransmitters, like glutamate, γ-aminobutyric acid, norephedrine, serotonin and acetylcholine is inhibited. In addition, adenosine inhibits adenylylcyclase.



5.195 *Caffeine interferes with a central signal cascade.*

At a "therapeutic dose", caffeine is an unselective adenosine-receptor antagonist. It replaces adenosine from the receptors without triggering any effect; overall, the neuronal activity is thereby increased, and the formation of cAMP promoted. [495, 496]

The activity of formamidine insecticides is based on the excessive synthesis of cAMP. Since caffeine causes the same effect, it acts synergistically. In addition, molluscs (snails) are very sensitive towards caffeine, which becomes rapidly absorbed through their foot slime. Unfortunately, caffeine is of limited value as a repellent in the open field due to its high water-solubility. [496–498]

cAMP now activates protein kinases in muscle tissues, which leads to a narrowing of the blood-vessels and a dilation of the bronchial vessels. By the activation of phosphorylases, glycogen is degraded to glucose-1-phosphate in the skeletal muscles and the liver. Furthermore, cAMP activates a lipase in the fatty tissue, whereby the blood fat level is raised.

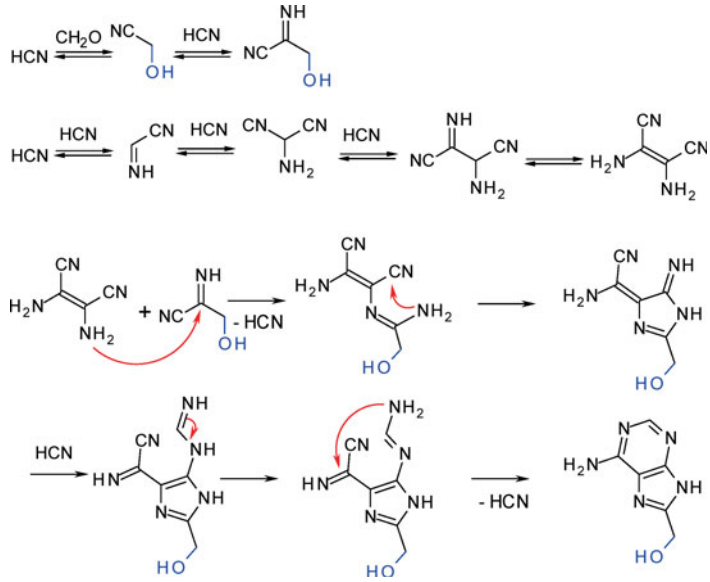
Caffeine is usually absorbed rapidly, especially in the small intestine, and overcomes the blood/brain barrier without problems. The bioavailability is greater than 90 %. The drug is deactivated in the liver by cytochrome P 450 enzymes. The methyl groups are an essential part of the pharmacophore. After their oxidative removal, caffeine loses its activity. The primary metabolites are theophylline, theobromine and 1,7-dimethylxanthine.

5.11.5 Prebiotic Synthesis

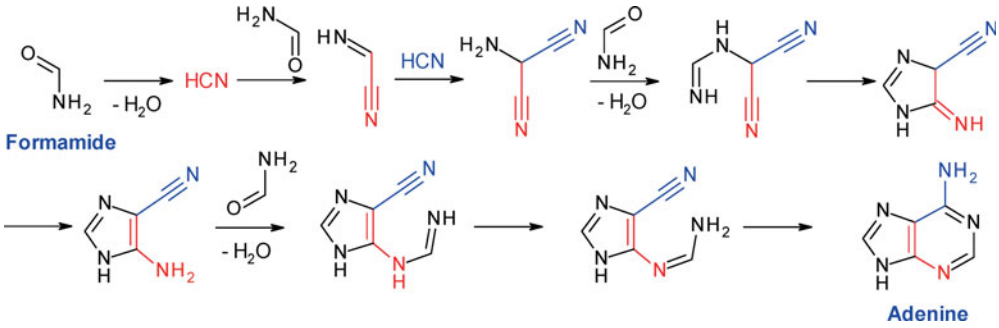
The spontaneous, prebiotic formation of purines from hydrogen cyanide or formamide is a fascinating subject. Adenine is formally the pentamer of hydrogen cyanide.

There is good reason to assume that formaldehyde, being formed photochemically from carbon dioxide and water vapour, or by electrical discharge from methane and water vapour, was ubiquitous on the newly formed Earth. Hydrogen cyanide results likewise from electrical discharge of nitrogen/methane mixtures.

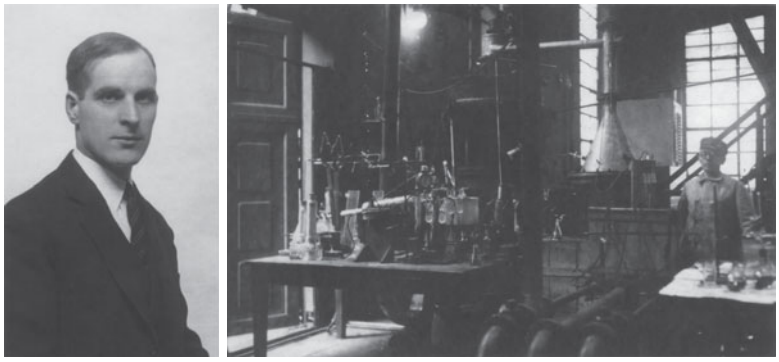
In their experiments, Joan Oró and later Leslie E. Orgel and Alan W. Schwartz [499] could detect purines as oligomerisation products of hydrogen cyanide or hydrogen cyanide/formaldehyde mixtures. Furthermore, formaldehyde accelerates the oligomerisation of hydrogen cyanide.



Most recently, Greg Springsteen proposed a pathway for the formation of adenine from formamide. [500]



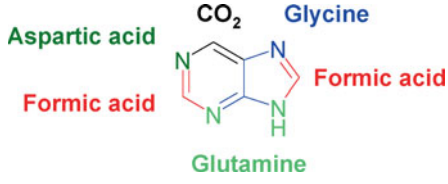
Hydrogen cyanide is produced industrially by thermolysis of formamide, or from methane and ammonia on platinum-rhodium catalysts (following a process developed by Leonid Andrussow (1896–1988) in 1927 at BASF in Ludwigshafen) (Fig. 5.196).



5.196 *Leonid Andrussow and the pilot plant for the Andrussow process.*

5.11.6 Biosynthesis

The biosynthesis of purines was essentially elucidated by John M. Buchanan (1917–2007) at the Massachusetts Institute of Technology in Cambridge and G. Robert Greenberg (1918–2005) at Case Western Reserve University, Cleveland. [501] In 1948, Buchanan fed pigeons with isotopically-labelled compounds and afterwards isolated the excreted uric acid. [502] His investigations gave information about the origin of the atoms in the purine skeleton, and thereby the first indications of the biosynthetic pathway (Fig. 5.197). [503]



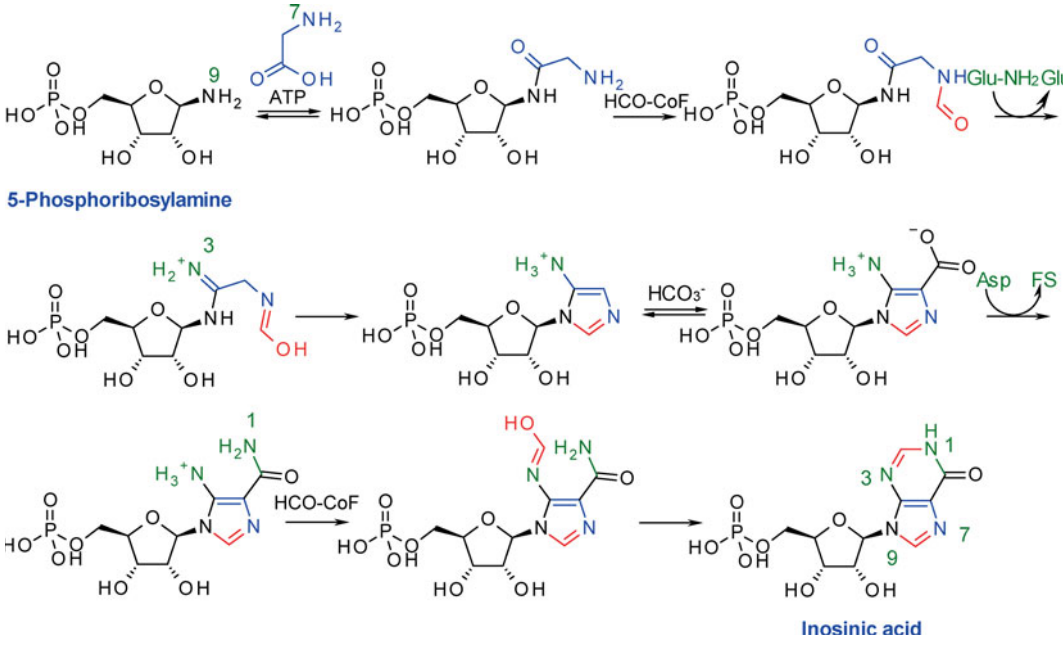
5.197 *The biosynthesis of purines requires various amino acids, formic acid and carbon dioxide.*

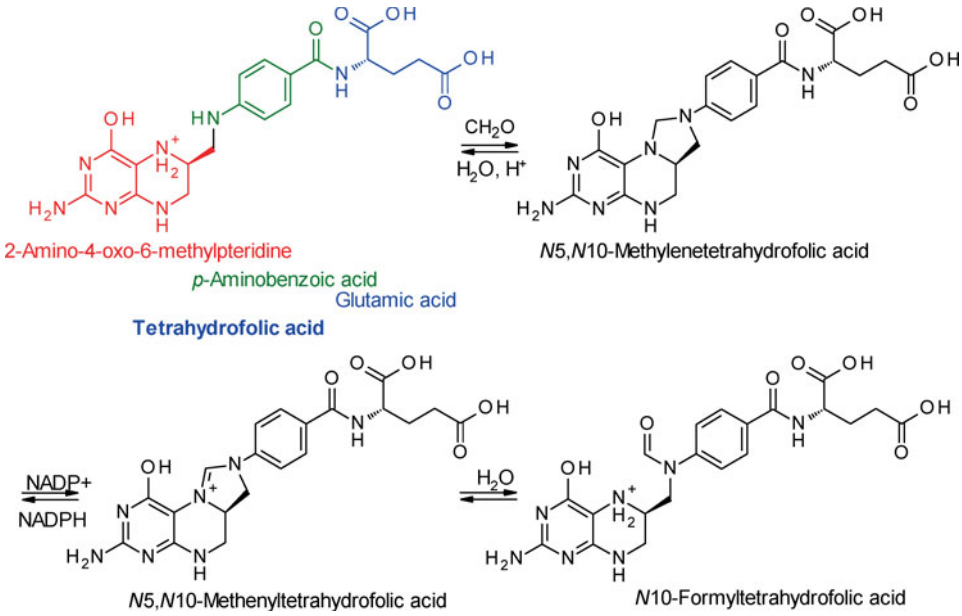
Uric acid is the end-product of amino acid metabolism in birds, land-based reptiles and many insects. In contrast to urea, it has low water solubility and is excreted as a solid. This enables the animals to save on water and therefore weight. In humans, the purine metabolism generates urea, which is excreted *via* the kidneys at a rate of approximately 1 gram per day.

Further research revealed that the bioneogenesis of purines is the same in *Escherichia coli*, yeast, pigeons and humans. Although, the conversion of preformed purines into caffeine cannot be excluded, it was also possible to demonstrate the *de novo* synthesis of caffeine in young tea plants. [504] By radioactive labelling experiments and targeted blocking of individual enzymes of the biosynthetic pathway, the origin of particular plant constituents can now be traced back very precisely.

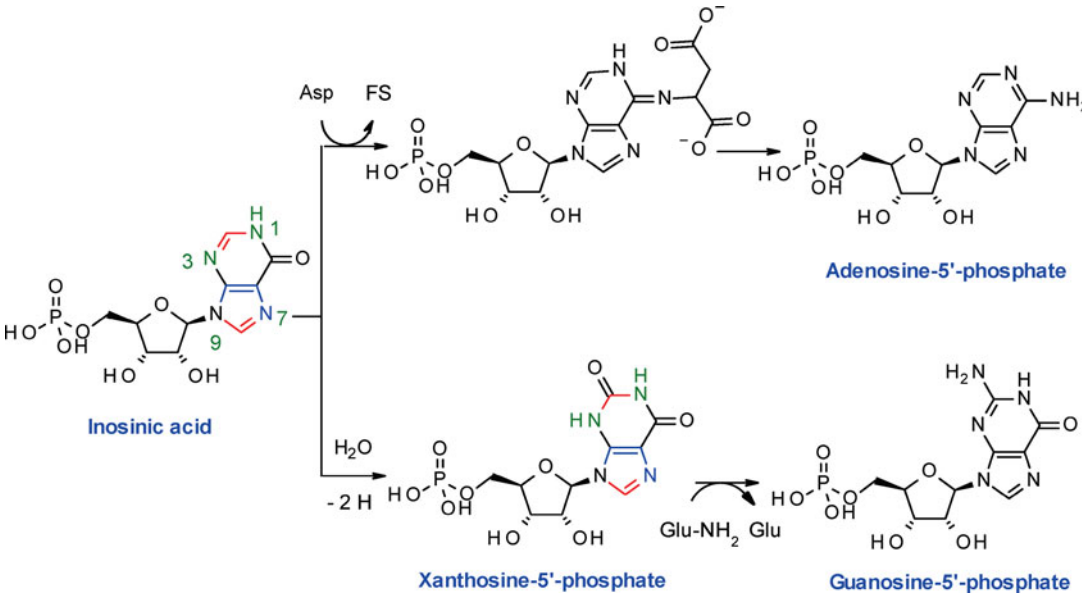
The biosynthesis of purine derivatives is remarkable in several respects. [505] It starts with 5-phosphoribosylamine, on the amino-group of which the bicyclic system is built up in a stepwise manner. This amino-group nitrogen ends up as *N*-9 of the purine skeleton. The ring formation is characterised by a sequence of partially repeated synthetic steps.

After activation of glycine with ATP, this is condensed with 5-phosphoribosylamine, and subsequently formylated by *N*10-formyl-tetrahydrofolate (HCO-CoF). After glutamine serves as the source again for the later nitrogen (*N*-3), ring closure and carboxylation of the imidazole ring occur directly with bicarbonate, requiring no activation by ATP. The last nitrogen (*N*-1) comes from aspartic acid, which is correspondingly deaminated to fumaric acid. Another transfer of formic acid with *N*10-formyl-tetrahydrofolate is followed by ring closure to yield inosinic acid.

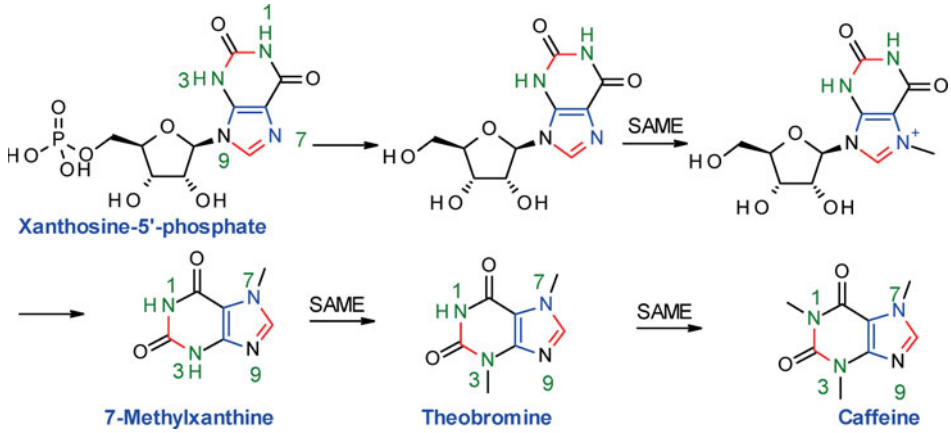




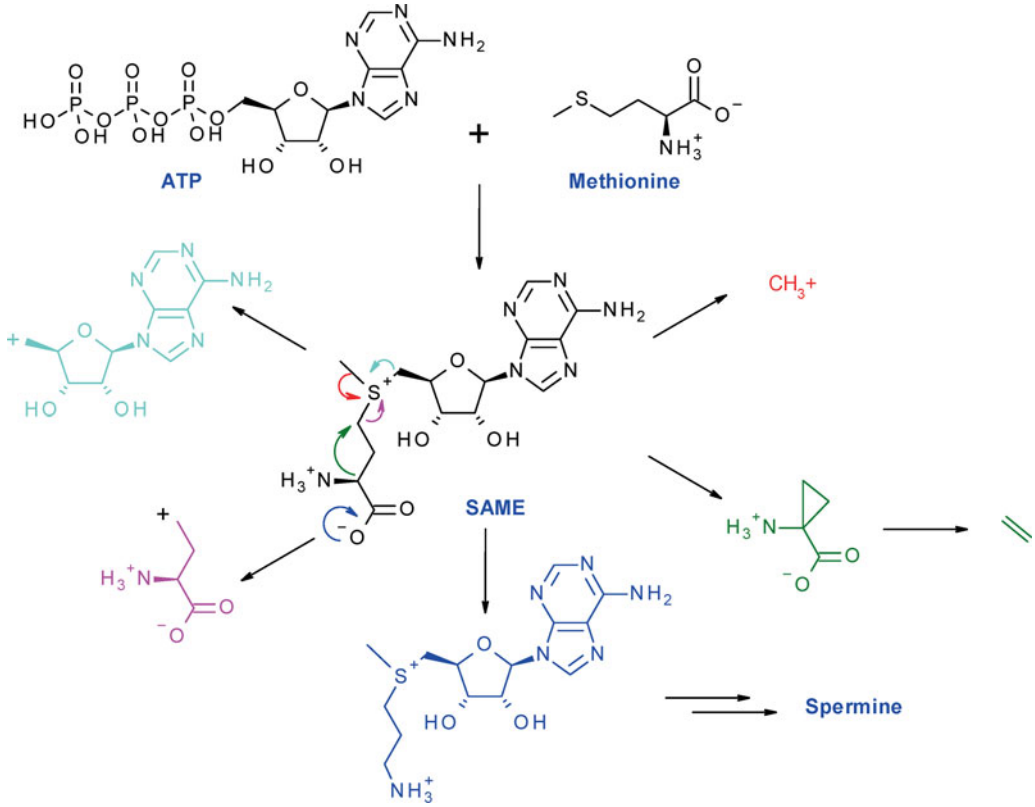
After that, aspartic acid transfers another amino moiety, leading to the formation of adenosine-5'-phosphate. Guanosine-5'-phosphate results from addition of water, oxidation and amination, this time by glutamine.



The biosynthesis of caffeine takes the same path up to xanthosine-5'-phosphate, which is here dephosphorylated and methylated at *N*-7. However, there are also reports that xanthosine-5'-phosphate can be methylated directly. [506] After the sugar residue is cleaved off, two additional methylation steps take place *via* the intermediates 7-methylxanthine and theobromine. [507]



From a chemical point of view, the successive methylations of the xanthine skeleton are particularly interesting.[507, 508] They are accomplished with *N*-methyltransferases [509, 510] and *S*-adenosylmethionine (SAME) as methyl source, which is formed from ATP and methionine. Moreover, SAME is used by Nature in a large number of other biosyntheses. The compound is available as a dietary supplement (neutraceutical) over-the-counter, and on prescription in Europe to treat osteoarthritis. It is also promoted to alleviate joint pain, depression and some other conditions.



Most recently, genetic engineering opened the opportunity to knock-out caffeine-synthase and eliminate both of the last steps in the biosynthesis, methylation of 7-methylxanthine and theobromine respectively. The goal is to cultivate thereby caffeine-free tea and coffee, without affecting their high polyphenol content as current decaffeination processes do. [511, 512]

5.11.7 Caffeine from Natural Sources

Since the beginning of the 20th century, coffee has been decaffeinated on a commercial scale. [513] The raw coffee is first swelled with superheated steam and then extracted with an organic solvent. Residual solvent is then vaporised (deodorisation), and the beans are dried and roasted. Initially, ethyl acetate was used as a solvent. Since this caused repeatedly explosions, it was replaced by dichloromethane. Although, the final solvent content in roasted coffee amounts to only a few parts per million, given the toxicological risk of chlorinated hydrocarbons, renowned coffee roasters changed their process to a "destraction" (a coinage of "*dest*illation" and ext*raction*) of raw coffee with supercritical carbon dioxide (Fig. 5.198). This is a very elegant method, since the solubilising properties of carbon dioxide can be controlled through water content, pressure and temperature. In the roasted product, there remains no xenobiotic material at all. Approximately 5,000 tonnes of caffeine are obtained worldwide by destraction per year.

5.198 *Decaffeination of raw coffee at the pilot plant of the Max-Planck-Institut für Kohlenforschung in Mülheim/Ruhr.*

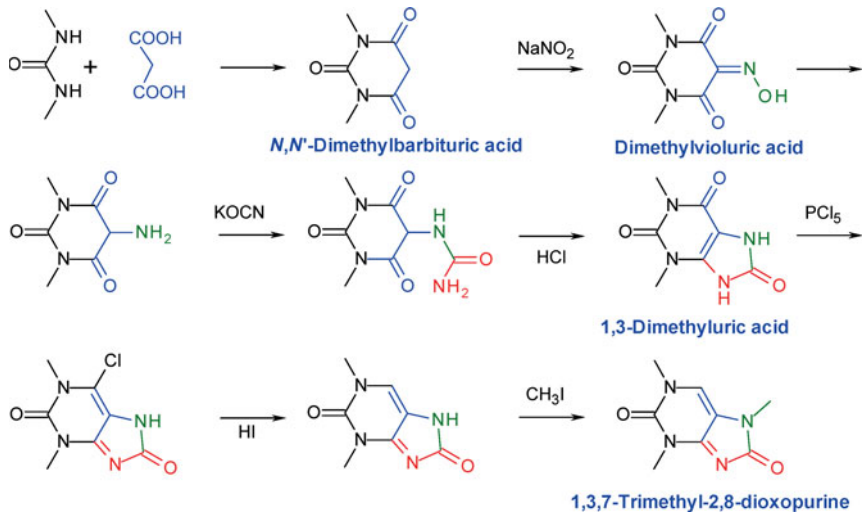
The process of destraction dates back to a discovery by Kurt Zosel (1913–1989) at the Max-Planck-Institut für Kohlenforschung in Mülheim, who was engaged in normal-pressure polymerisation of ethylene. He had incidentally noticed, that residues from the distillation of oil, but also waste oil, vegetable fats and oils from natural products, could be extracted very well with supercritical gases. [514] This methodology offered correspondingly a number of other applications, as to extract hop aroma from hops, unsaturated fatty acids from fish oils, vitamin E from vegetable oils, and paraffins or phenols from bituminous tar.

5.11.8 Syntheses

In 1895, Emil Fischer (1852–1919) published what was supposedly the first synthesis of caffeine. [515, 516] However, this was not the case, because the assumed structures of xanthine, theophylline [517] and caffeine were in fact wrong. He had believed, that they were 2,8-dioxopurines.

The starting point for his synthesis was *N*,*N*'-dimethylurea, the reaction of which with malonic acid gave *N*,*N*'-dimethylbarbituric acid. With "nitrous acid" (sodium nitrite and a mineral acid) he produced dimethylvioluric acid, which gave dimethylpseudouric acid upon reduction and further reaction with potassium cyanate. Ring closure by heating with hydrochloric acid then led to 1,3-dimethyluric acid. [518] Its reaction with phosphorus pentachloride and reduction with hydrogen iodide then produced the supposed theophylline; final

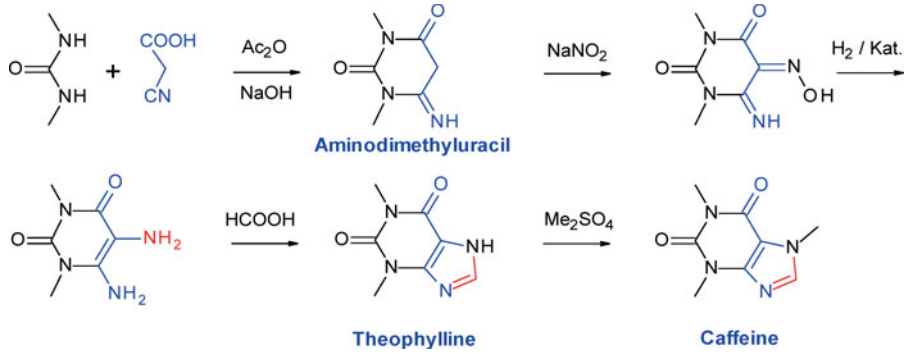
methylation ought to have produced caffeine. In fact, he prepared 1,3,7-trimethyl-2,8-dioxopurine by this route.



Theophylline is like the β-sympatomimetics a powerful bronchospasmolytic and finds use in the therapy of acute asthmatic attacks and in asthma prophylaxis. [520]

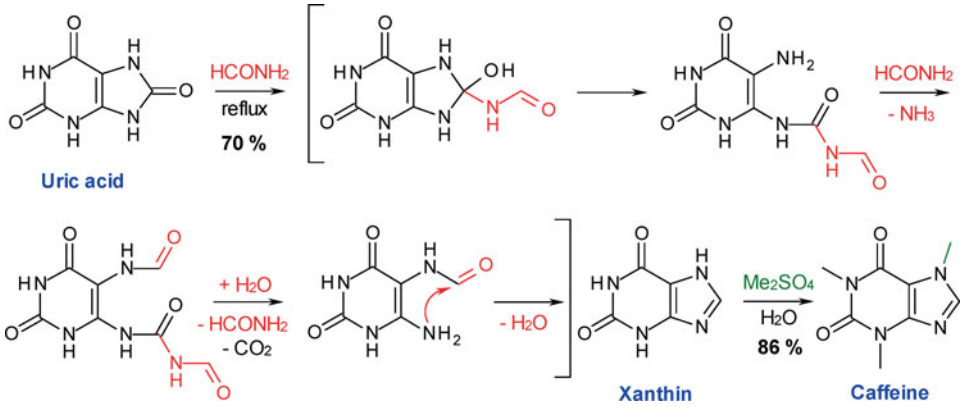
The first correct synthesis of caffeine was achieved by Wilhelm Traube (1866–1942) in 1900. [519] In spite of some modifications that were introduced over the years, Traube's procedure still serves today as the basis for the industrial synthesis of caffeine, theobromine and theophylline.

Starting material for the industrial synthesis of caffeine is dimethylurea as well. Reaction with cyanoacetic acid leads to dimethylaminouracil, which upon reaction with nitrous acid, reduction, and condensation with formic acid gives theophylline. Methylation with methyl chloride or dimethyl sulfate finally leads to caffeine.



Still noteworthy is the elegant two-stage synthesis of caffeine, which Hellmut Bredereck (1904–1981) published in 1950. [521] Thereby, uric acid – as a matter of choice, the excrement of snakes may be used as well – is heated to reflux in formamide. The precipitated xanthine is dissolved in aqueous sodium hydroxide, purified with activated charcoal, and finally methylated with dimethyl sulfate. With pure uric acid, the overall yield of caffeine is 60 %, and in the case of

snake excrement, remarkable 47 % can be achieved (under the certainly incorrect assumption, the faeces would consist of pure uric acid).



5.11.9 Uses

The worldwide demand for caffeine reaches almost 20,000 tonnes per annum. Approximately a quarter of this originates from natural sources; the rest is obtained by chemical synthesis. The largest producer of synthetic caffeine is BASF, followed currently by the Chinese firms Shandong Xinhua Pharmaceutical, Jilin Shulan Synthetic, Shijiazhuang Pharmaceutical and Tianjin Zhongan.

A small proportion of caffeine finds application in pharmaceuticals for the treatment of migraine, pain and fever. It is occasionally administered in combination with analgesics, since caffeine augments their activity. The cosmetics industry uses caffeine as a blood-circulation-enhancing agent for skin preparations. Caffeine is needed for the production of copier paper ("Diazopaper"). The lion's share however accounts for sodas and energy drinks (*Coca-Cola®*, *Pepsi-Cola®*, *Red Bull®*), but also a host of other caffeinated food products, which has already attracted the FDA's attention.

Worldwide, caffeine is the most-consumed psychostimulant. If the amount of caffeine in one cup of coffee, around 100 mg, were filled into a gelatin capsule, it would be obtainable only on prescription. Thus, a hundred million people drink every day one billion cups of coffee, without even noticing that they are taking a drug in quantities, which could have potentially mandated an 'on prescription only' label.

Summary in Bullet Points

- Caffeine is an adenosine-receptor antagonist. Thereby, caffeine promotes neuronal activity, carbohydrate and fat metabolism, and raises blood pressure.
- Purines, the class of compounds to which caffeine belongs, originate from the simplest building blocks in a prebiotic fashion.

- The biosynthetic route to caffeine follows that of the purines. The nitrogen atoms are methylated by *S*-adenosylmethionine.
- For the industrial production of caffeine, the Traube synthesis is still used. Around a quarter of pure caffeine results from extraction of natural sources (*e.g.* by destraction of coffee with supercritical carbon dioxide).

5.12 Nicotine

Smoking damages one's health. At present, the consumption of tobacco products is widespread around the world. In principle, there are still no serious legal regulations, in spite of the fact, that smoking continues to play the dominant role in fatalities, morbidity and the associated economic damage, ahead of adiposity and alcohol abuse. The tremendous costs for medical care of those smoking-related illnesses, combined with loss of earnings and other expenses, exceed the tax revenues from tobacco by far. For those reasons alone, not even considering all the personal sorrow, smoking cessation programmes and addiction counselling of young people have become imperative.

5.12.1 Historical

The Indians described smoking-pipes as "Tabaco". [522]

The Spanish sailor Rodrigo de Jerez, after his return with the *Niña,* was sentenced by the inquisition to seven years' imprisonment: when he gave off clouds of smoke from both, his mouth and nose, this was held to be the work of the Devil. Thereby, he was only showing his compatriots in Ayamonte (Andalusia) what he had observed with the Arawak Indians in San Salvador (an island of the Bahamas) and Hispaniola (a major Caribbean island located between Cuba and Puerto Rico) during the expedition with Christopher Columbus in 1492. [522, 523] The hermitic monk and geographer, Ramón Pané, and a supervisor of the West Indian gold smelters, Gonzola Fernandez de Oviedo y Valdés, described likewise in 1497, how the Indians smoked tobacco through wooden pipes. [524] Bernal Díaz del Castillo, the war correspondent under Hernán Cortés, reported that in Tenochtitlán (the present-day Mexico City) there was a trade in smoking-pipes and tobacco leaves. Illustrations of such pipes are to be found in the famous Mayan "Troano Codex" in Madrid (Fig. 5.199). [525]

5.199 *From the Mayan Codex in Madrid. On the orders of the bishop of Yucatán, Diego de Landa Calderón, in 1562, all but three of the Mayan Codices were burnt. These are now located in Madrid, Paris and Dresden. [526]*

Apart from smoking tobacco, the Indians of North and South America were also familiar with snuff from powdered tobacco leaves, chewing tobacco, and drinking tobacco water. They used tobacco as a gift to the gods, for

medicinal purposes in the treatment of pain, tiredness, syphilis, and for wound healing, during peace treaty ceremonies, at ritual dances and for the creation of delirious visions.

In 1511, the first tobacco plants reached Spain. In the middle of the 16th century, Francisco Hernández de Toledo, personal physician to Philip II, and André Thevet, a Franciscan monk who converted to Calvinism, cultivated Mapacho (*Nicotiana rustica*) as an ornamental plant. In 1560, the French Envoy to Portugal, Jean Nicot de Villemain (Fig. 5.200) sent tobacco plants (*Nicotiana tabacum*) to Catherine de Medici in Paris.

5.200 *Jean Nicot de Villemain (1530–1600).*

At the French Court, tobacco became enormously popular for smoking as well as for pharmaceutical purposes. Around 1570, smoking was common among Dutch seamen, and during the Thirty Years' War, the soldiers of Tilly, Gustav Adolf and Wallenstein eventually extended the consumption of tobacco across the whole of Europe. The Swedish botanist Carl von Linné (1707–1778) named the plant genus after Jean Nicot, where also the name of its main alkaloid is derived from.

Bishop Bartholomé de las Casas (1474–1566) already reported that Spanish soldiers and priests who smoked were no longer able to rid themselves of this. In 1590, the Dutch Medical Faculty warned about smoking, "because the brains thereby become black". In Britain, James I (1566–1625) argued that tobacco is unhealthy (Fig. 5.201). His paper "The Counterblast to Tobacco" of 1604 is reckoned nowadays to be one of the first publications against smoking. In 1634, the Russian Tsar Mikhail Fyodorovich Romanov (1596–1645) banned the trade of tobacco and threatened its consumption as an offence carrying capital punishment. From 1660 on, smoking became liable to severe punishment also in the canton of Bern. [527] However, with the introduction of a tobacco tax (in England in 1652) as an additional source to generate revenue for the treasury, these bans were relaxed again.

> Sometimes they booze on tobacco, at other times they feed on it, and quite often they sniff it; it therefore makes me wonder why I have still not found anyone who has it stuck in his ears. (Hans Jacob Christoffel von Grimmelshausen (1621–1676). [522]

In 1612, John Rolfe (1585–1622, husband of Pocahontas (1595–1617)) of Jamestown in Virginia succeeded in the first tobacco cultivation as an export crop. In the following years, the American settlers spread tobacco production across the whole of Virginia and Maryland. Tobacco achieved great importance for the British Merchant Navy as a material for barter with the American colonies. At the time of the American Declaration of Independence, in 1776, tobacco cultivation had been extended to North Carolina, Kentucky, Tennessee, Ohio and Missouri.

5.201 *James became king of Scotland in 1567 (as James VI) on the abdication of his mother Mary; he also inherited the English throne (as James I) on the death of Elizabeth I in 1603.*

Whereas in the 16th and 17th centuries pipe-smoking was fashionable, in the 18th century, snuff-taking became more popular. While in the 19th century cigars were preferred, these were finally displaced by cigarettes (French: *cigarette*, a small cigar). King Frederick I of Prussia founded the *Tabakskollegium* (Tobacco Council), an evening club open for discussion

5.202 *The "Tabakskollegium" of Frederick I of Prussia, around 1710 and a snuff-box of Frederick the Great.*

and amusement to those privileged, who enjoyed the pleasure of pipe-smoking. His son, Frederick the Great preferred the more elegant snuff-taking to smoking, and he was a passionate collector of precious snuff-boxes (*Tabatière*) (Fig. 5.202).

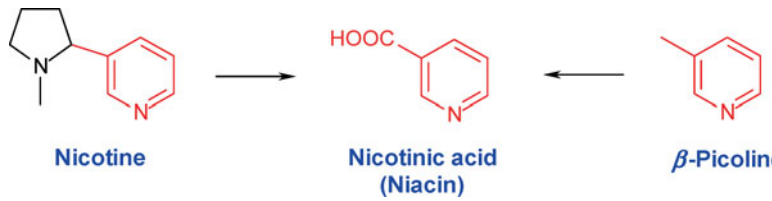
5.12.2 Isolation and Structure Determination

Louis-Nicolas Vauquelin (1763–18 29) was a multifaceted scientist: He also discovered chromium in a lead ore from Siberia, and beryllium in beryl. In 1806, along with Pierre-Jean Robiquet (1780–1840), he isolated the first amino acid, asparagine, from asparagus.

In 1807, Gaspare Cerioli, Professor at the Lyceum in Cremona, reported on an "Olio essentiale" (an essential oil), which he had obtained by distillation of aqueous tobacco extracts, and two years later, the well-known French chemist Louis-Nicolas Vauquelin (1763–1829) described the preparation of the "Essence de Tabac". In 1828, the chemist Karl Ludwig Reimann (1804–1872) and the physician Wilhelm Heinrich Posselt (1806–1877) delivered a paper "About tobacco, its chemistry and physiology" at the University of Heidelberg, in which they described the isolation of the alkaloid nicotine and its toxic effect on dogs and rabbits.

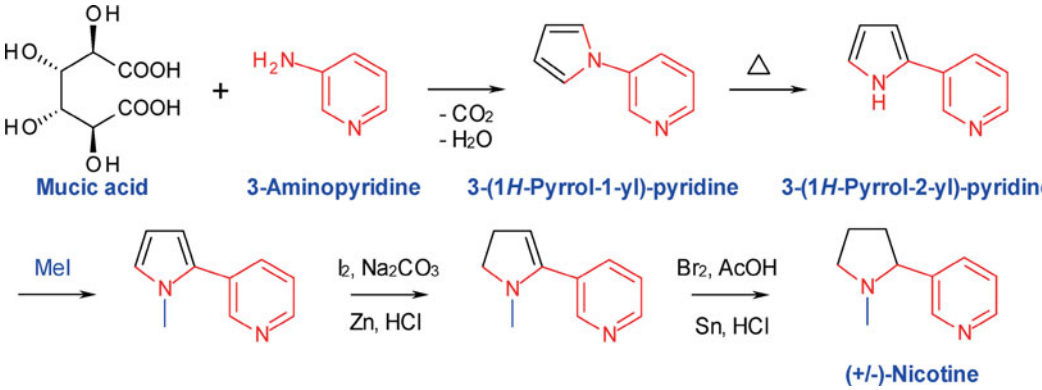
For the structure determination, Carl Huber, Hugo Weidel (1849–1899) and Richard Laiblin oxidised nicotine *inter alia* with chromic acid and nitric acid, and they obtained pyridine-3-carboxylic acid (nicotinic acid), which is also accessible by oxidation of β-picoline.

In 1937, it was discovered that a nicotinic acid (niacin) deficiency leads to pellagra (a form of dermatitis, which occurs endemically where unprocessed millet and maize (*Amer.*: corn) are the staple food), and can also cause diarrhoea and dementia, or be even fatal. A daily dose of ten to twenty milligrams of nicotinic acid can prevent these deficiency signs. In the body, niacin is converted into the corresponding amide, an integral part of the nicotinamide nucloeotides (NAD, NADP). Niacin is produced industrially for animal feed by nitric acid oxidation of 5-ethyl-2-methylpyridine.

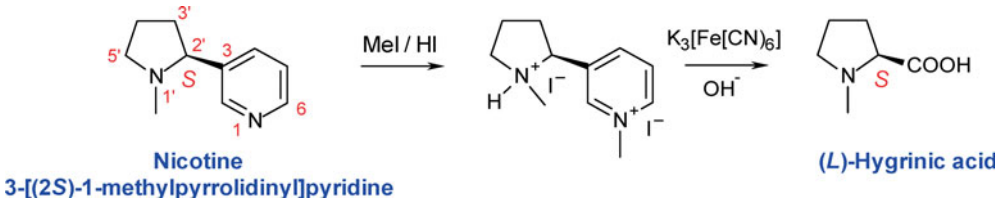


Adolf Pinner (1842–1909), Professor of Chemistry at the Veterinary Institute in Berlin, elucidated the structure of nicotine in 1893 by oxidative degradation with bromine. [528] In addition to nicotinic acid, he also found methylamine and malonic acid. With the knowledge of the overall chemical formula, and the fact that nicotine had to contain two tertiary amine functions, he postulated it as a 3-substituted pyridine linked to a 2-substituted *N*-methylpyrrolidine.

In 1895 Amé Pictet (1857–1937) published the first nicotine synthesis. [529, 530] Key steps are the formation of 3-(1*H*-pyrrol-1-yl)-pyridine from 3-aminopyridine and mucic acid and its rearrangement to 3-(1*H*-pyrrol-2-yl)-pyridine at high temperatures. *N*-Methylation and sequential reduction give eventually racemic nicotine, which can be separated with tartaric acid into its enantiomers.



In 1925, Paul Karrer succeeded in determining the absolute configuration of nicotine, by deconstructing it to (*L*)-hygrinic acid, and further to proline (Fig. 5.203). [531]



Nicotine is one of the few volatile alkaloids. It is a colourless and odourless oily liquid (b.p. 246 °C). In air and under the influence of light, nicotine becomes brown and develops an intensive smell of tobacco. Nicotine counts as one of the most potent plant toxins (fatal respiratory paralysis is reported in humans at an oral dose below 1 mg/kg of body weight).

5.203 *Paul Karrer (1889–1971) in his laboratory.*

5.12.3 Occurrence and Botany

Nicotine belongs to the *Solanaceae* (nightshade family) alkaloids. It is the principal alkaloid of tobacco, but occurs also as a trace component in *Acacia*, *Sedum*, *Erythroxylum*, *Equisetum* and *Lycopodium* species. The two economically most important tobacco species are *Nicotiana tabacum* (Virginia tobacco), which grows up to 3 metres in height, has reddish flowers and lancet-shaped, pointed leaves, and the 1.2-metre high *Nicotiana rustica* species (known in South America as Mapacho and in Vietnam as Thuoc Lao), with greenish-yellow flowers and egg-shaped leaves (Fig. 5.204).

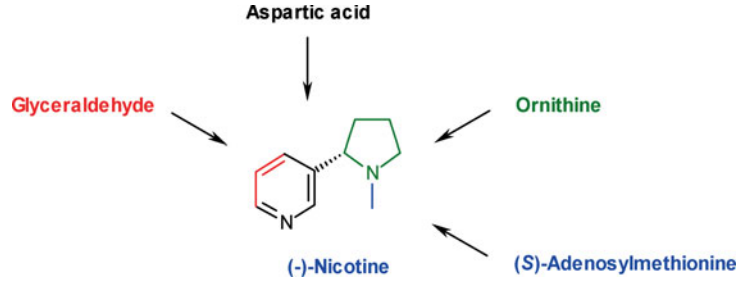
5.204 *Nicotiana tabacum (a) and Nicotiana rustica (b).*

Nicotine is formed mainly in the roots and transported to the leaves to be stored there. The nicotine content in the different tobacco varieties ranges from 0.3 to 7 or even 9 % (*Nicotiana rustica*) of dry weight. In so-called nicotine-free tobaccos, the alkaloid is enzymatically demethylated, which reduces the nicotine content to below 0.1 %. [532]

For smoking, snuff, and chewing, *Nicotiana tabacum* is cultivated from the tropics to the temperate regions, corresponding to climate and soil requirements of a particular variety (*e.g.* Virginia tobacco, Oriental tobacco, Burley tobacco, Kentucky tobacco, Havana tobacco, Sumatran tobacco, Brazilian tobacco). The biggest producer by far is China, with Brazil, India and the USA as distant followers. *Nicotiana rustica* is produced in Poland, in Belarus, Ukraine, Kazakhstan and Russia, and also in the USA.

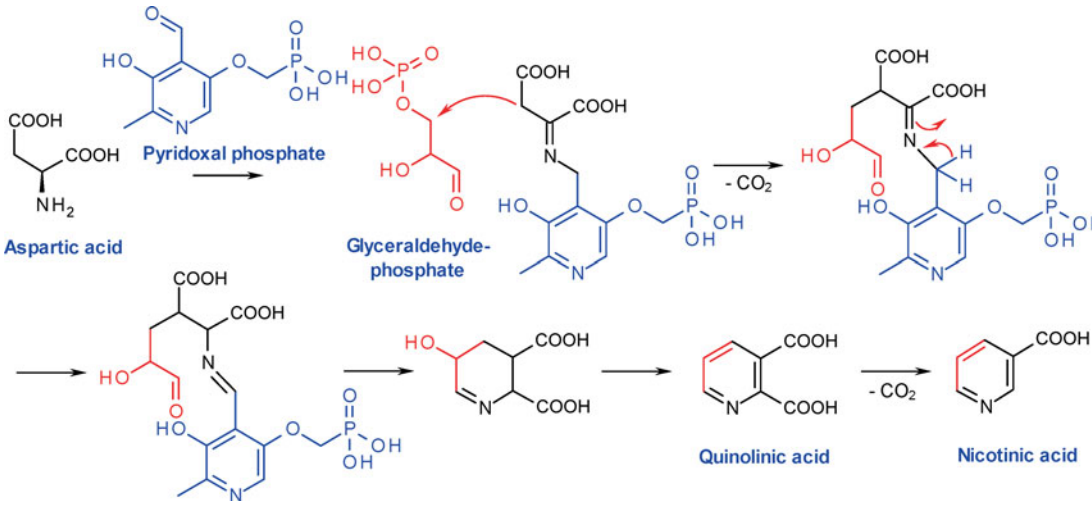
5.12.4 Biosynthesis

The tobacco plant generates nicotine from various essential amino acids (Fig. 5.205).

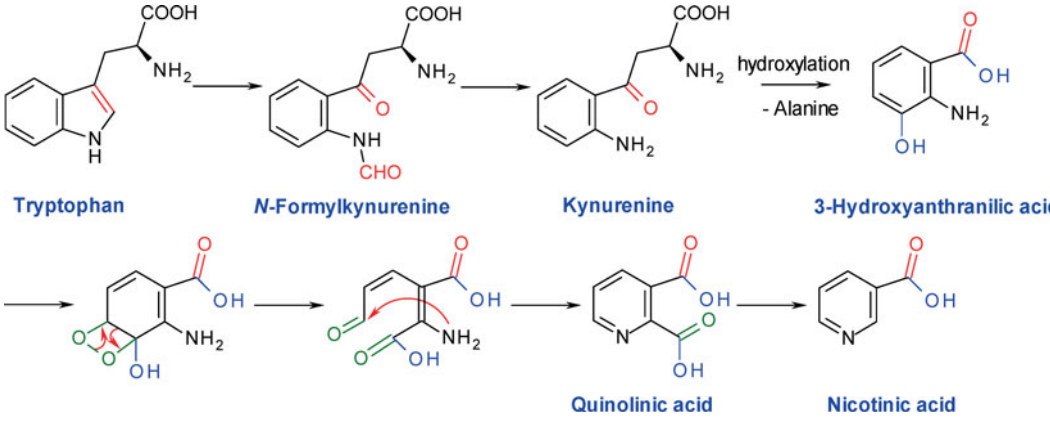


5.205 *Biogenesis of nicotine.*

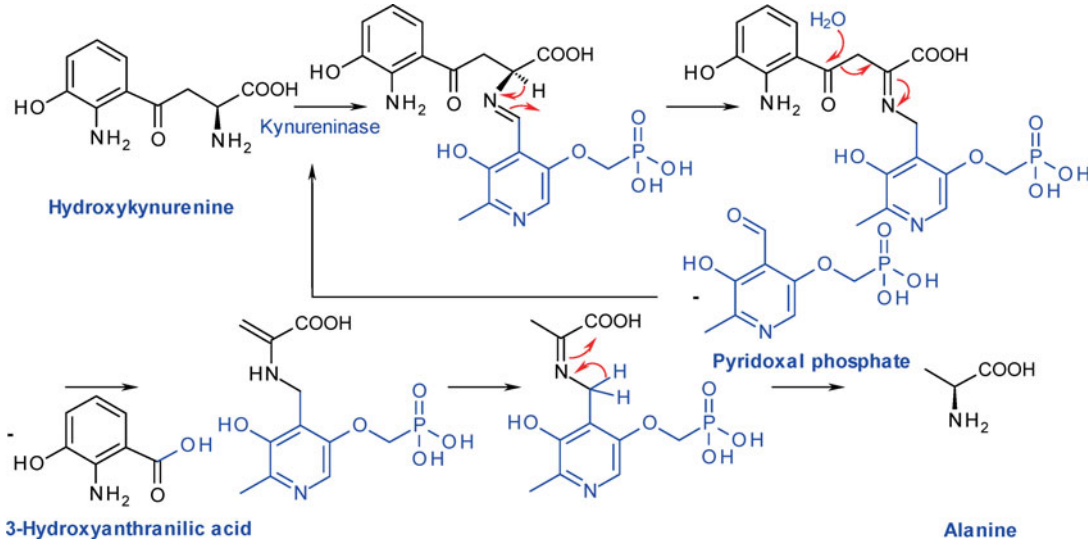
The pyridine moiety of (-)-nicotine is derived biogenetically from nicotinic acid, which is formed in prokaryotes and plants starting from aspartic acid and glyceraldehyde-3-phosphate. [533] In this, the aspartic acid is activated by pyridoxal phosphate. After cyclisation and aromatisation, quinolinic acid is formed first, and then decarboxylation leads to nicotinic acid.



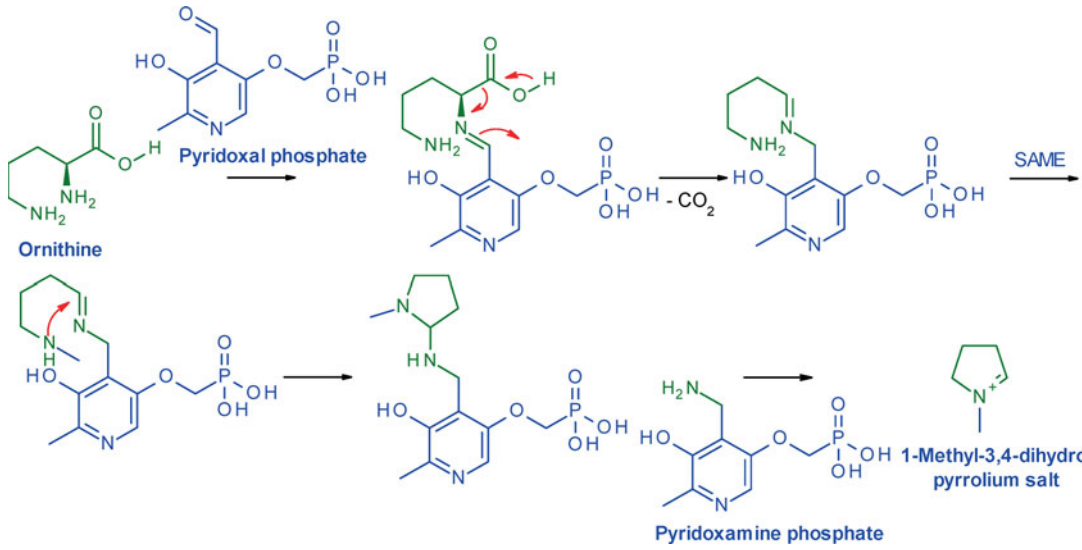
Interestingly, animals and various fungi synthesise nicotinic acid by a different route, the degradation of tryptophan. Oxidative ring-opening and deformylation gives kynurenine, which is subsequently hydroxylated. After cleavage of the alanine residue, the aromatic system is broken down oxidatively by a dioxygenase. Reclosure gives quinolinic acid, which is decarboxylated to give nicotinic acid.



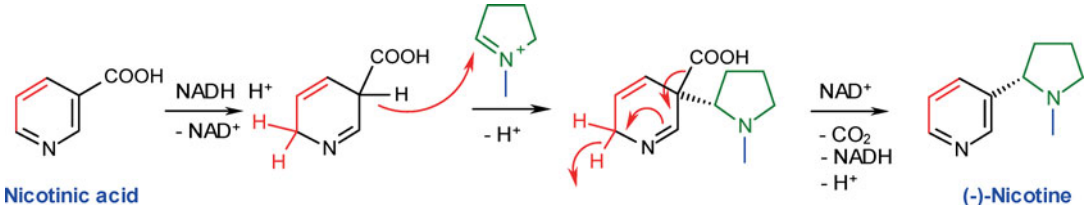
Really intriguing is the pyridoxal phosphate-dependent cleavage of the alanine residue. [534, 535]



The pyrrolidine moiety in nicotine originates from ornithine, which is first decarboxylated in a pyridoxal phosphate-mediated step. After methylation with *S*-adenosylmethionine (SAME) and ring-closure, cleavage of pyridoxamine phosphate yields the 1-methyl-3,4-dihydropyrrolium salt.

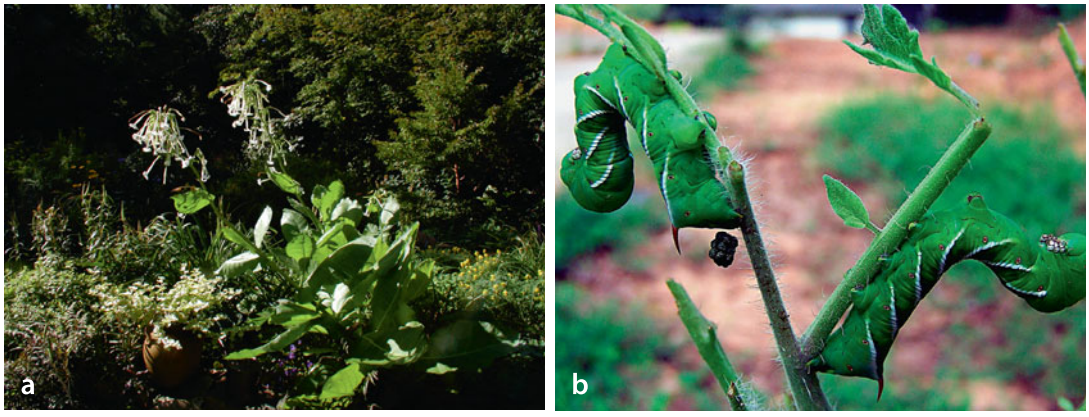


In the tobacco plant, the nicotinic acid is reduced first, and a nucleophilic attack of deprotonated dihydronicotinic acid on the 1-methyl-3,4-dihydropyrrolium salt gives (–)-nicotine. [536]



Nicotinic acid (-)-Nicotine

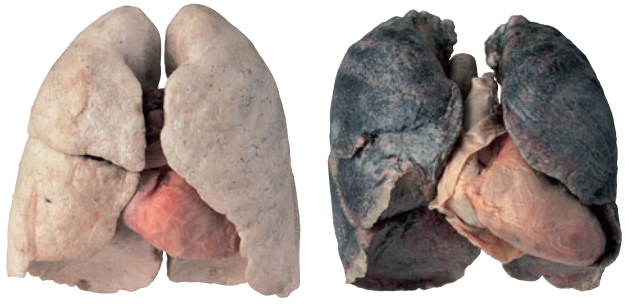
The wild-growing tobacco variety *Nicotiana sylvestris* reacts to mechanical damage, for example by herbivores like the caterpillar of the American tobacco hornworm *Manduca sexta*, by increasing its nicotine production up to four-fold (Fig. 5.206). The tobacco farmer pursues the same objective by pinching out the shoots of young plants. Their response is mediated by jasmonic acid. Unharmed tobacco plants show higher nicotine levels as well, if the phytohormone is supplied *e.g.* with irrigation water to the roots. [537, 538]



5.206 *Nicotiana sylvestris (a) and the caterpillar of the American tobacco hornworm (Manduca sexta) (b).*

5.12.5 Pharmacology

Tobacco smoke has considerable adverse effects on the cardiovascular and digestive systems, and is the cause of many cancers (Fig. 5.207). This holds for both, smokers and passive smokers. It is estimated that smoking shortens the lifespan on average by around 10 years. Around half of the 1.25 billion smokers worldwide die from diseases, which are associated with this addiction.



5.207 *The plastinated lungs of a non-smoker (left) and a smoker (right).*

After inhalation and absorption, (*S*)-nicotine rapidly crosses the blood/brain barrier, and binds to and activates $\alpha_4\beta_2$-nicotinic acetylcholine-receptors (*cf.* section 8.4 – Neonicotinoids) in the A*rea tegmentalis ventralis* of the midbrain. The *nucleus accumbens* and the prefrontal cortex in the forebrain are stimulated *via* dopaminergic axons. As for many of the drugs in abuse, this induces an increased release of dopamine in the *nucleus accumbens.* This region of the *striatum* is thought to play a crucial role in processing reward, mediating pleasure, aggression and fear, but also in harbouring the root of addiction.

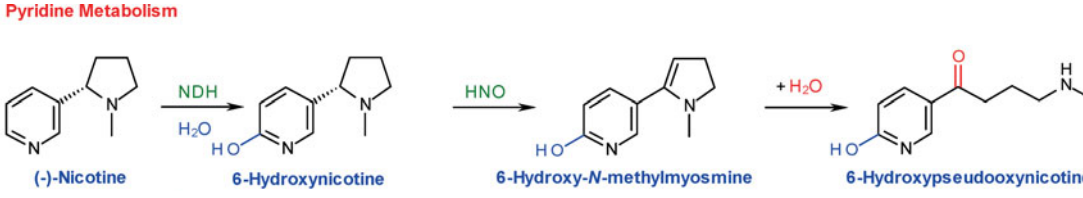
On the other hand, nicotine inhibits the monoaminoxidases, which degrade dopamine in the brain. Compared to non-smokers, smokers have an up to 40 % reduced monoaminoxidase activity. This may explain the epidemiological observation that smokers contract Parkinson's disease significantly less frequently. The disease can be traced back to a lack of dopamine-producing neurones. Nicotine therefore represents an interesting lead structure for the development of medicaments to treat Parkinson's disease. Nicotine might serve as an interesting starting point also for the treatment of other neurodegenerative diseases like Alzheimer's disease. Psychological disturbances like schizophrenia, certain forms of epilepsy and Tourette's syndrome, or the management of pain might be potential target indications. [539]

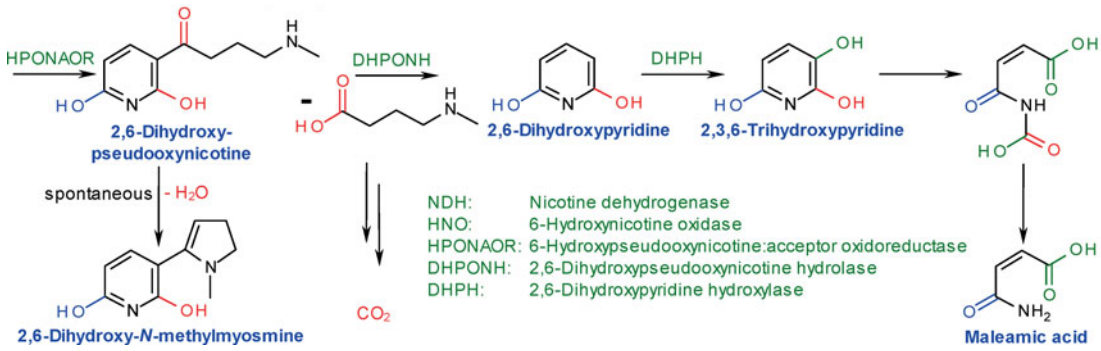
5.208 *Despite its high toxicity, nicotine was used as an insecticide in agriculture, since there were hardly any compounds with higher selectivity. Around 1950, the world's annual demand for nicotine came to around 2,800 tonnes.*

For centuries, nicotine has found application to treat cattle, sheep and poultry against mange, ticks and lice. In veterinary medicine, nicotine is used as an anthelmintic (a worming agent). Over the last hundred years, nicotine sulfate has been used in agriculture to control aphids, and since 1885 as an insecticide in viniculture (Fig. 5.208). [540, 541]

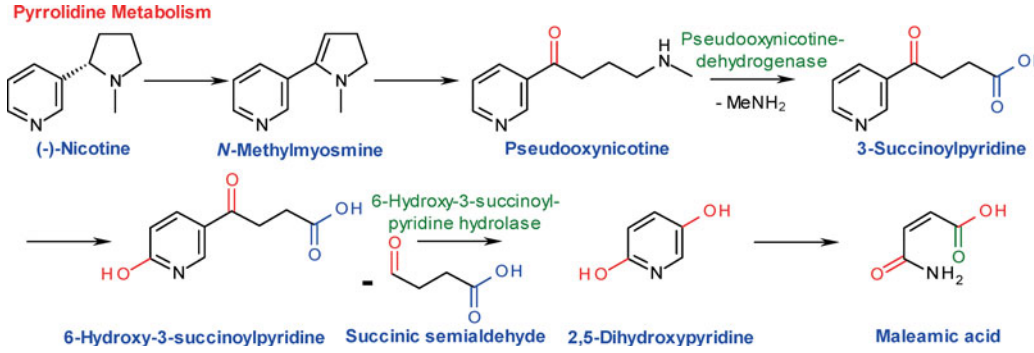
5.12.6 Detoxification

Bacteria and some fungi catabolise nicotine with the aim of producing energy-rich carboxylic acids for their primary metabolism. [542, 543] The bacterium *Arthrobacter nicotinovorans* degrades both enantiomers of nicotine: the pyridine ring is first hydroxylated by a molybdenum-containing nicotine dehydrogenase. Both enantiomers of 6-hydroxynicotine are converted into 6-hydroxypseudooxynicotine, which is further transformed to 2,6-dihydroxypyridine and *via* 2,3,6-trihydroxypyridine finally broken down to maleamic acid. 2,6-Dihydroxy-*N*-methylmyosmine is the metabolic end-product in the soil bacterium *Arthrobacter*, and formed in a spontaneous condensation from 2,6-dihydroxypseudooxynicotine.

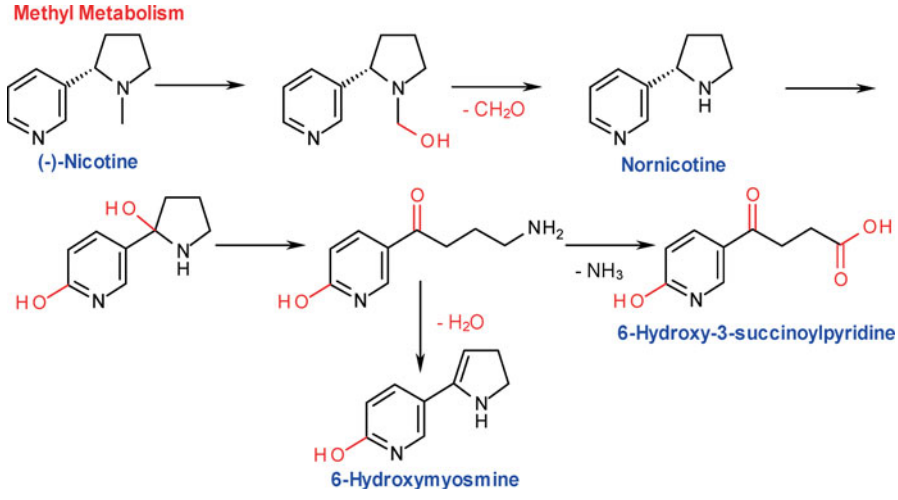




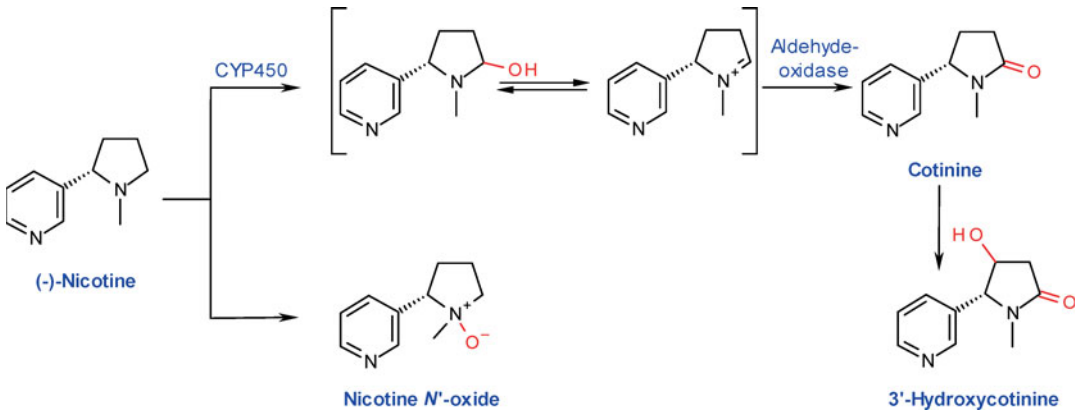
Pseudomonas species degrade nicotine first to 6-hydroxypseudooxynicotine, or in the case of *Pseudomonas putida* to pseudooxynicotine. After oxidative deamination and oxidation of the aldehyde to the acid, the side-chain is cleaved off and yields succinic semialdehyde and 2,5-dihydroxypyridine, which is transformed into maleamic acid.



Finally, tobacco plants and several fungi, *e,g. Pellicularia filamentosa*, are able to degrade nicotine to nornicotine. *Arthrobacter nicotinovorans* and *Pseudomonas* strains then metabolise this further to 6-hydroxy-3-succinoylpyridine.



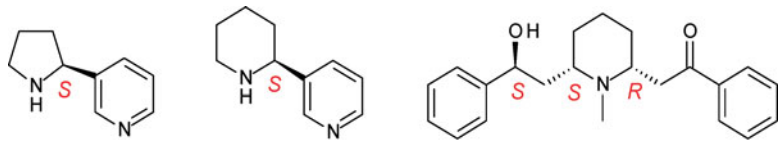
The detoxification of nicotine in humans takes place mainly in the liver. The elimination half-life of the unaltered compound in blood plasma lies at around 2 hours, though its terminal excretion half-life in urine averages 11 hours. Nicotine is oxidised mainly (70–80 %) to cotinine, but also about 4 % to (1'*S*,2'*S*)- and (1'*R*,2'*S*)-nicotine *N*'-oxide. Cotinine, with an elimination half-life of up to 20 hours, is further hydroxylated in the 3'-position. Nicotine, cotinine and 3'-hydroxycotinine are excreted in the urine largely as glucuronic acid conjugates. [544]



5.12.7 Nicotinoids – Nicotine-related Natural Products

Nornicotine and anabasine count among the minor alkaloids of tobacco. Whereas *Nicotiana* species contain the (*S*)-nornicotine, *Duboisia hopwoodii* produces the (*R*)-enantiomer. Anabasine is with up to 2.6 % the principal alkaloid of *Anabasis aphylla*. The anabasine content in *Nicotiana glauca* is comparatively high and reaches 1 %.

Indian tobacco (*Lobelia inflata*) belongs to the genus of bell-flowers (*Campanulaceae*) and is native to the open North American woodlands. The principal alkaloid of *Lobelia inflata* is lobeline, which acts as a partial agonist at the nicotinic acetylcholine $\alpha_4\beta_2$ receptor and thereby desensitises the receptor through alteration of its molecular architecture and inhibits dopamine uptake into synaptic vesicles. Thus, lobeline provides an interesting lead structure for new smoking cessation drugs (Fig. 5.209 and Fig. 5.210).



5.209 *Nicotine-related alkaloids from plants.*

5.210 *Duboisia hopwoodii (a), Nicotiana glauca (b) and Lobelia inflata (c).*

There is a whole range of natural products, which are not just structurally related to nicotine, but also with regard to their pharmacological activity. Of these, only lobeline, (–)-epibatidine, (–)-cytisine and (+)-anatoxin A will be discussed here (Fig. 5.211).

5.211 *Nicotine-related alkaloids from dart-poison frogs, laburnum and cyanobacteria.*

Lobeline

Indian tobacco (*Lobelia inflata*), also known as "pukeweed", has been used for centuries by the Penobscots people in the Northeastern United States and the Canadian Maritimes, as an entheogenic and emetic drug, before Samuel Thompson (1769–1843), an herbalist from New Hampshire, claimed it as a valuable remedy. The roots were used for the treatment of sexually transmitted diseases, *e.g.* syphilis, and skin diseases were medicated with poultices from smashed leaves. By parenteral application, Indian tobacco is effective against oedema and asthma. Thus, *Lobelia inflata* was found since 1820 in many pharmacopoeias.

At the beginning of the last century, Heinrich Otto Wieland (1877–1957) explored the alkaloids of Indian tobacco and thereby discovered lobeline, which was brought to the market as an active substance for the treatment of agonal respiration by Boehringer Ingelheim in 1921. Initially, the drug was extracted from the plant. "Lobelin Ingelheim" became a big commercial success and initiated considerable efforts to develop a total synthesis. However, it was not before 1937, when Boehringer succeeded in launching "Lobeton", the fully synthetic API. [545]

Over many decades, Lobeline has also been widely used in commercial smoking cessation remedies. Unfortunately, there are up to now no adequate long-term trials, which could provide evidence that Lobeline can indeed help people stop smoking.

Epibatidine

Alexander von Humboldt (1769–1859) was the first to report that *Strychnos toxifera* is a source of curare. Information about a South American vine, *Chondrodendron tomentosum*, as another source had come from Hippolyto Ruiz and Joseph Pavonin 1794.

For centuries, the native Indians in Ecuador and Colombia used besides curare [546] from *Strychnos toxifera* and *Chondrodendron tomentosum* also the skin secretion of several dart-poison frogs of the genus *Phyllobates* and *Dendrobates* to tip the darts of their blowpipes for hunting (Fig. 5.212).

During a research trip to south-west Ecuador, John W. Daly (1933–2008) of the National Institutes of Health in Bethesda, Maryland, discovered that the toxin of a dart-poison frog, *Epipedobates tricolor*, only a few centimetres in size, possesses interesting pharmacological properties. From the skin of 750 frogs of this species, he isolated in 1976 on a microgram scale a component of the poison, epibatidine, which triggers the Straub tail phenomenon in mice. [547] Thereby, the mice bend their tails over their backs – a reaction, which proved to be a typical effect for opiates, although it is not specific.

5.212 *Phantasmal poison frog (Epipedobates tricolor): The adventurer Captain Charles Stuart Cochrane reported for the first time in 1823 that the natives ("Indians") in the rain-forests of Colombia obtained dart poisons from small brightly coloured frogs.*

However, Daly was only able to elucidate the structure of epibatidine in 1992 by meanwhile considerably upgraded NMR spectroscopy. [548] The structure was a surprise: it contained a 7-azabicyclo[2.2.1]heptane skeleton, something without precedent at that time in Nature, and a 2-chloropyridyl moiety, likewise rare in natural products. Interestingly, *Epipedobates tricolor* does not synthesise epibatidine by itself, but picks up the alkaloid with food from an unknown source. Frogs, which are bred in captivity, do not contain epibatidine.

The observation that the analgesic action, despite the Straub tail phenomenon, cannot be inhibited by the opiate antagonist Naloxone, but by the non-competitive nAChR antagonist mecamylamine, indicates that epibatidine binds not at opiate receptors, but instead at nicotinic acetylcholine receptors. [547, 549, 550]

Depending on the test system used, epibatidine is around 200 times more potent than morphine and nicotine. Native Americans have known its analgesic property for many centuries, and meanwhile it has been verified experimentally in numerous pharmacological studies. [551]

Cytisine

Cytisine is the principal alkaloid of the laburnum or "Golden Chain" (*Laburnum anagyroides* or *Cytisus laburnum*), indigenous to southern Europe and south-west Asia; however, since the 16th century it has also been cultivated in northern Europe as an ornamental plant due to its beautiful blooms (Fig. 5.213). Cytisine was isolated for the first time in 1865 by August Husemann (1833–1877) and Wilhelm Marmé (1832–1897) in Göttingen from ripe laburnum seeds. [552] This alkaloid is also found in a number of other leguminous plants (*Fabaceae*), like the Common or Scots broom (*Cytisus scoparius*), the German broom (*Genista germanica*), the Spanish gorse or Dyer's whin (*Genista tinctoria*), in the seeds of the Buttercup family (*Ranunculacea*), Fetid bugbane (*Cimicifuga euro-*

paea) and in the English holly (*Ilex aquifolium*) from the Holly (*Aquifoliaceae*) family of several hundred species.

Whereas laburnum is well tolerated by goats, sheep and hares, its seeds are highly toxic especially for humans (cytisism). Cytisine, like nicotine, leads at low concentrations to the excitation of the peripheral sympathetic nerves. At higher doses, it paralyses the stimulus transmission to the vegetative innervated organs and the motor endplates of the neuromuscular junction in the somatic nerve system, especially that of the respiratory centre.

Cytisine finds nowadays application in homeopathy and as a drug for smoking cessation (*Tabex*®, so far approved in Eastern Europe only). Its analogue, varenicline (*Chantix*®, or *Champix*®), is marketed globally (see below).

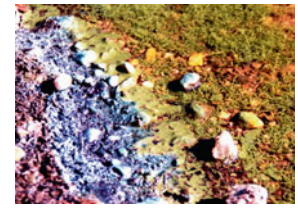
5.213 *The "Golden Chain" (Laburnum anagyroides) was already known to the Greeks and Romans.*

Anatoxin A

Anatoxin A is the fast-acting and highly effective poison of the cyanobacterium *Anabaena flos-aquae*, which is ubiquitous in freshwater. Anatoxin A, also known as "Very Fast Death Factor", was isolated from *Anabaena flos-aquae* in 1977 by Paul Gorham at the National Research Council in Ottawa. [553, 554] The structure had already been determined in 1972 by X-ray analysis of its *N*-acetyl derivative. [555] Later, the presence of anatoxin A was detected in a range of other toxic strains of *Oscillatoria*, *Anabaena circinalis*, *Aphanizomenonflos-aquae*, *Cylindorsperum pp.* and *Raphidiopsis mediterranea*.

Cyanobacteria

Cyanobacteria, which were previously described as blue-green algae, are amongst the oldest living organisms on Earth, and responsible for the development of an oxygen-containing atmosphere. Reference to algal blooms, the exponential growth of these bacteria, can be found in the Old Testament (Exodus, chapter 7, verses 20–21). The first of the ten Biblical plagues may be interpreted as a massive appearance of planktonic cyanobacteria *Planktothrix rubescens* (Burgundy-blood algae). [556] Historically, such algal blooms have carpeted several Swiss lakes – the Murtensee (1825), the Baldegger See (1884), the Lake Zurich (1898) and the Rotsee (1910). In 1878, George Francis described for the first time acute poisonings of cattle, sheep, dogs and pigs through an algal bloom ("Conferva") in the lakes close to the mouth of the Murray River in Australia. [557] Humans came to harm by intoxication from the drinking water and bathwater. Recently, in many parts of Germany algal blooms were observed: on the Bleiloch dam (in Thuringia), the Wahnbach dam (near Cologne), in the Schlachtensee (near Berlin), Tegeler See (near Berlin), Ammersee (in Bavaria) and on the Bergknappweiher (near Munich) (Fig. 5.214). Indeed, Algal blooms are becoming a global problem, increasingly affecting people's water supply on all continents. Recent data from Lake Zurich show that global warming contributes to aggravate the situation.



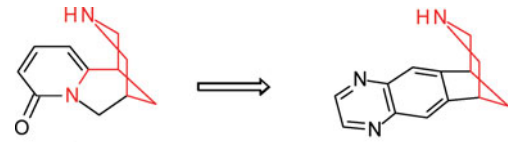
5.214 *Algal blooms in 1998 on the Bergknappweiher near Munich.*

The toxins of the cyanobacteria can be classified into categories corresponding to the organs they affect and the type of toxicity they exert. One group encompasses small cyclic peptides (microcystines), which, in relation to their specific transport features, are highly liver-toxic and lead within a few hours to a haemorrhagic shock. Another group consists of neurotoxins, which can be fatal within a few minutes. Anatoxin A binds irreversibly to the nicotinic acetylcholine

receptor with a higher affinity than nicotine and causes pre- and postsynaptic depolarisation. The exact lethal dose for humans is not known, but is estimated as less than 5 milligrams.

5.12.8 Varenicline – an Anti-smoking Drug

In 2006, Pfizer brought varenicline (*Champix*® in the EU, *Chantix*® in the USA) to the market as a drug for smoking cessation. This compound is derived structurally from cytisine.

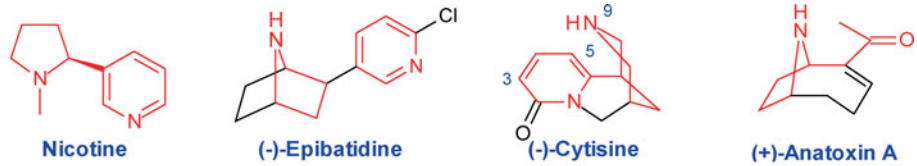


(-)-Cytisine Varenicline

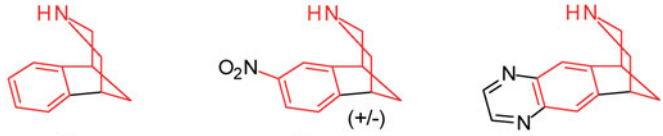
Wartime experience guided the selection of the lead structure: Due to shortage of tobacco, heavy smokers found a surrogate in laburnum leaves, or they chewed laburnum seeds. [558] In this way, they tried to cope with their addiction. Abstinence from nicotine results in chronically low mesolimbic dopamine levels, which leads to withdrawal symptoms and eventually to a high rate of relapse. For this reason, Pfizer's research aimed at a partial agonist at the $\alpha_4\beta_2$-nicotinic acetylcholine receptor, which sustainably upregulates dopamine levels. It should also possess a higher affinity than nicotine at the $\alpha_4\beta_2$-receptor, so that in the case of a relapse, a sharp rise of dopamine in the mesolimbic system is prevented. [559] Furthermore, the drug should be better resorbed than cytisine and cross the blood/brain barrier as easily as possible.

Structure-activity investigations of numerous natural products with high activity and as the profile of a partial agonist at the nicotinic $\alpha_4\beta_2$-receptor provided a suitable pharmacophore model. This suggested a core structure with a basic nitrogen, which is protonated under physiological conditions, and in most cases an electron-deficient aromatic system, which is separated by two or three C–C bonds.

Thus, the cytisine skeleton should be altered within the boundaries of the model, but without abandoning the molecule's rigidity. Initial structural variations at positions 9 and 5 were accompanied by a dramatic loss of activity. Conversely, substituents at position 3 appeared to have less of an impact. The tricyclic benzazepine **A** shows a lower affinity than cytisine, which however, may be significantly increased again by nitration (**B**). The doubly nitrated benzazepine was an important intermediate, from where upon hydrogenation a series of heterocyclic compounds became accessible. These efforts finally resulted in varenicline with a 15-fold higher affinity at the $\alpha_4\beta_2$-receptor than nicotine. The binding profile demonstrates good selectivity with at least three orders of magnitude lower affinity at other nicotinic receptors, like the $\alpha_3\beta_4$-, the $\alpha_1\beta\gamma\delta$- and the α_7-receptor (Fig. 5.215). Nevertheless, also this drug is not



	Nicotine	(-)-Epibatidine	(-)-Cytisine	(+)-Anatoxin A
K_i ($\alpha_4\beta_2$):	0,95 nM	0,07 nM	0,17 nM	4,4 nM
Agonist (10 µM):	100 %		56 %	
Antagonist (10 µM/ 10µM Nicotine):	/		30 %	



	A	B	Varenicline
K_i ($\alpha_4\beta_2$):	20 nM	0,75 nM	0,06 nM
Agonist (10 µM):	0 %	65 %	68 %
Antagonist (10 µM/ 10µM Nicotine):	35 %	20 %	34 %

5.215 *Structure-activity relationships for various nicotinoids.*

free of side effects, and exacerbation of neuropsychiatric conditions has been reported.

The partial agonistic activity was determined *via* a functional electrophysiological assay in *Xenopus oocytes* at 10 µM of the test compound relative to 10 µM of nicotine, or upon co-administration of both. These competition experiments demonstrated that varenicline possesses only 68 % of the efficacy of nicotine, while the efficacy of nicotine itself is reduced to 34 %. Extensive *in vivo* studies showed that oral administration of varenicline raises the release of dopamine in the *Nucleus accumbens,* but on the other hand, it attenuates the effect of nicotine.

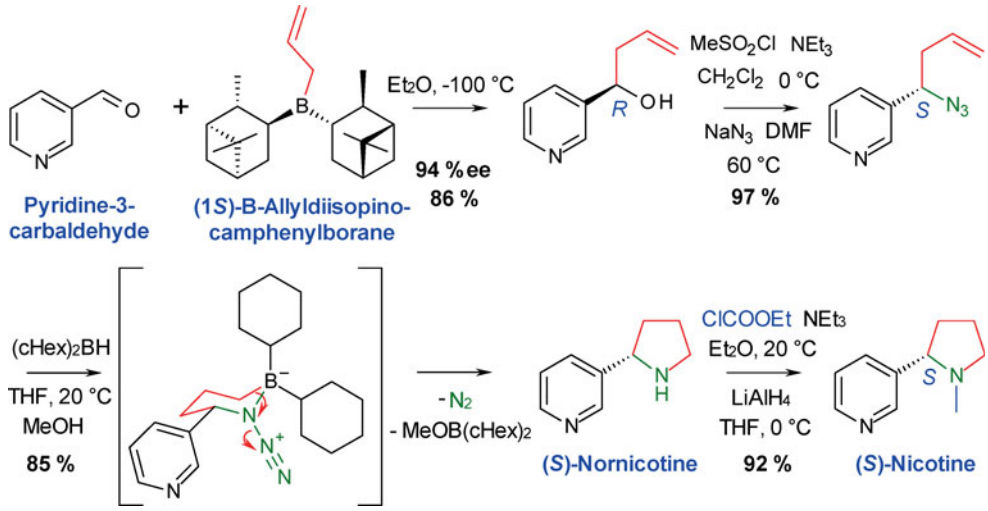
5.12.9 Chemical Syntheses

Nicotine

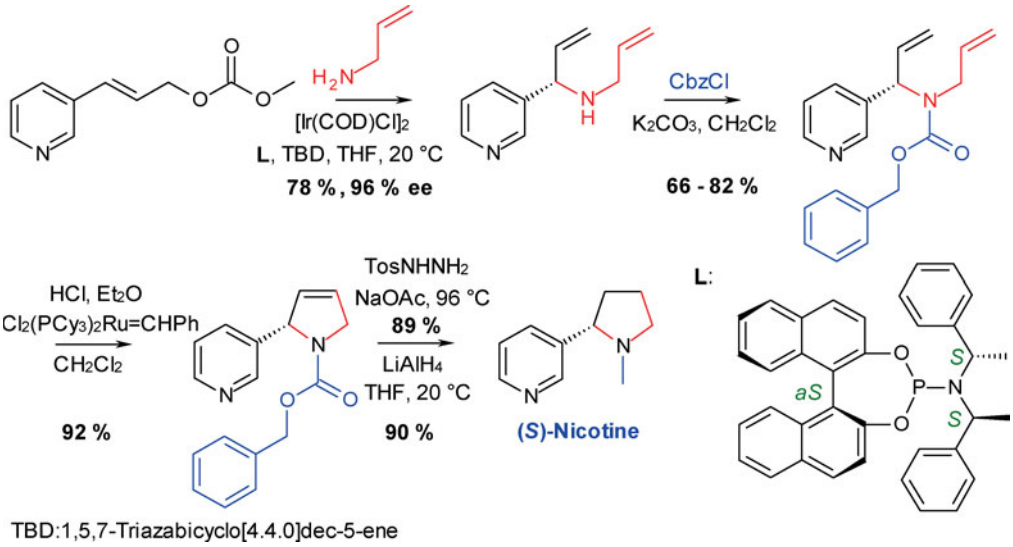
Since the first nicotine synthesis by Amé Pictet (1857–1937) in 1895, a whole host of other syntheses – mostly of racemic nicotine – has been recorded. [522, 541] The enantiomers can be separated with tartaric acid. The (*R*)-enantiomer is also obtainable by microbial degradation of the (*S*)-nicotine in the racemate. [560] Surprisingly, only a few enantioselective total syntheses of nicotine have been published up to the present time. [561] Two of them, which look particularly elegant, are discussed below.

The first comes from Jacques Lebreton, and comprises as a key step an enantioselective allylation with (1*S*)-*B*-allyldiisopinocamphenylborane, which is readily accessible from allylmagnesium chloride and (1*S*)-*B*-chlorodiisopinocamphenylborane. [561] Since the pyridine nitrogen complexes to the borane, at least two equivalents are necessary to obtain good yields and high enantioselectivity. After mesylation, the azide moiety is introduced with highly selective

inversion at the stereogenic centre. Equally elegant is the intramolecular hydroboration/cycloalkylation with concurrent cleavage of nitrogen, which leads directly to (*S*)-nornicotine. While the pyrrolidine ring may be methylated using methyl iodide (78 % yield), higher yields are however obtained by reaction with ethyl chloroformate and reduction with lithium aluminium hydride. The enantiomeric excess of the so prepared nicotine lies around 92–93 %.



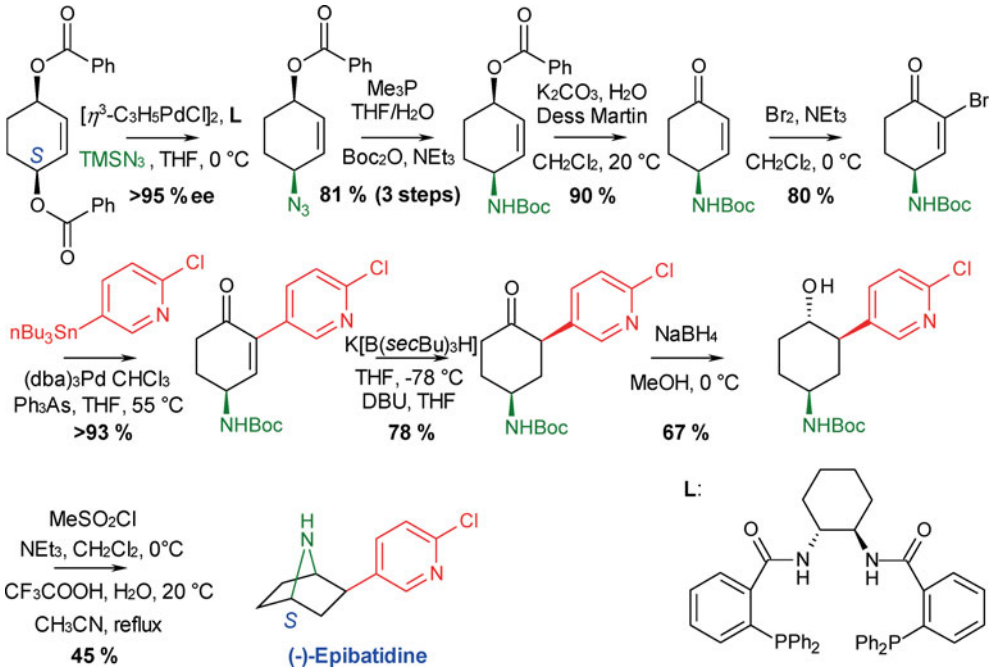
The second nicotine synthesis to be presented here comes from Günter Helmchen and comprises as a key step an enantioselective iridium-catalysed allylic aminaton in presence of an enantiomerically pure phosphoramidite ligand. [562] Also is the case, the ring-closure metathesis is successful only with the pyridinium salt. In a diimine reduction with tosylhydrazine, the double bond can be reduced while preserving the Cbz-protecting group. The reduction with lithium aluminium hydride gives exclusively (*S*)-nicotine with unaffected enantiomeric purity.



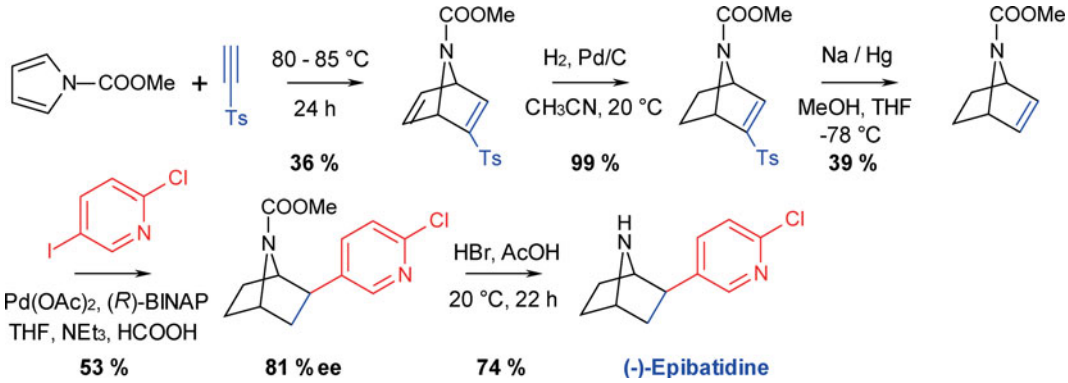
Epibatidine

Once the attractive structure of epibatidine and its interesting pharmacological properties became known, there appeared a plethora of publications on the synthesis of the racemic natural product. [563] Over the years, also a few enantioselective total syntheses have been developed, two of which are discussed here.

Barry Trost used in 1996 *cis*-1,4-dibenzoylcyclohexene as starting material, in which the (*S*)-configured benzoyl group is substituted by azide in presence of an enantiomerically pure palladium catalyst. [564] Without work-up, the azide group is then reduced with trimethylphosphine and the resulting amine protected. Hydrolysis, oxidation with the Dess-Martin periodate reagent, bromine addition and dehydrobromination produce the educt for the introduction of the chloropyridyl moiety, a core reaction in this sequence. The organo-tin compound is obtained from 5-bromo-2-chloropyridine by selective sequential halogen-metal exchange, first with *n*-butyllithium and then with tri-*n*-butyltin chloride. The palladium-catalysed cross-coupling succeeds in producing excellent yields with triphenylarsine as a ligand. The double bond is reduced chemoselectively with K-Selectride. From the mixture of *cis*- and *trans*-isomers in a 4:1 ratio, the *cis*-isomer can be separated by crystallisation, and the *trans*-isomer is isomerised with DBU in THF. Reduction of the keto-group with sodium borohydride gives the *trans*-amino-alcohol in 67 % yield and its *cis*-isomer in 29 % yield. The Boc-protecting group directs the selective mesylation of the *trans*-amino-alcohol. After cleavage of the Boc-group, heating under reflux in acetonitrile produces epibatidine, which forms colourless needles.



Another elegant enantioselective total synthesis comes from Dieter Kaufmann, and draws on an enantio-unselective synthesis by Andrew Regan, published in 1993. [565, 566] The central compound, a 7-azabicyclo[2.2.1]heptene, is obtained by the Diels-Alder reaction of 1-methoxycarbonylpyrrole and *p*-toluenesulfonylacetylene. In the next step, the unsubstituted double bond is chemoselectively reduced, and the tosyl group cleaved off with sodium amalgam. The key step is an enantioselective, reductive Heck hetarylation with an (*R*)-BINAP-palladium catalyst in THF and formic acid as the reducing agent. This produces only the *exo*-enantiomer. Under optimised conditions, the enantiomeric excess amounts to 81 %. Eventually, epibatidine is obtained by cleavage of the carbamate with HBr in acetic acid.



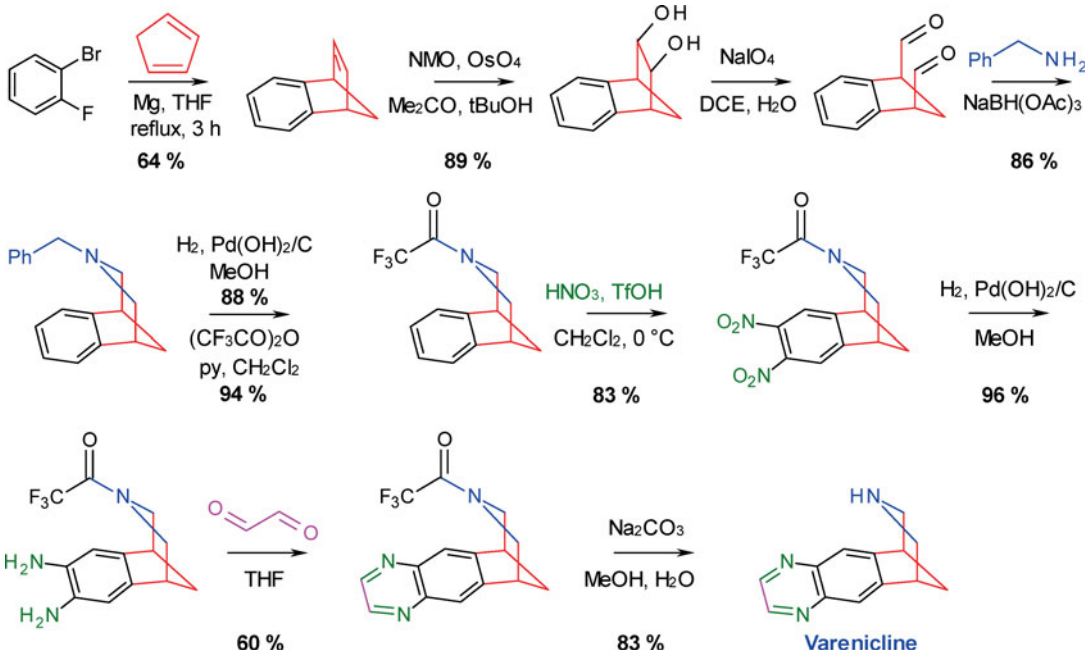
5.12.10 Production of Nicotine and Varenicline

Nicotine

Nicotine is obtained from tobacco plants by extraction or steam distillation. Particularly suitable for this, just as for caffeine, is destraction with supercritical carbon dioxide. [567] Nicotine-rich Mapacho is cultivated for this purpose in the USA; but also the waste from tobacco processing is a suitable source. The annual worldwide production for pharmaceutical purposes reaches a total of around 35–40 tonnes, whereof most is attributed to nicotine replacement therapy (nicotine containing patches, nasal spray, chewing gum, lollipops) for smoking cessation.

Varenicline

Starting material for the synthesis of varenicline is *o*-bromofluorobenzene, which reacts (*via* benzyne) with cyclopentadiene in a Diels-Alder reaction. Oxidative ring-opening and reductive amination provides a *N*-benzylbenzazepine derivative. After *N*-protection with trifluoroacetic anhydride, nitration with a mixture of nitric and trifluoromethanesulfonic acids, reduction, and condensation with glyoxal, hydrolysis of the trifluoroacetamide as a final step provides the active compound in good overall yield. [568, 569]



Summary in Bullet Points

- Tobacco and its principal alkaloid, nicotine, are of great cultural-historical significance. They served primarily people's hedonism and to indulge themselves in risk-taking pleasures.
- Besides nicotine, there is a number of other related natural products, like anabasine, epibatidine, cytisine and anatoxine A, which possess a comparable pharmacology.
- The precursors in the biogenesis of nicotine are amino acids.
- Nicotine was one of the first insecticides, which has been successfully applied in agriculture since the end of the 19th century.
- The primary use of nicotine is nowadays for products in nicotine replacement therapy to support smoking cessation.

References

References to Introduction

1. J. Davis, *Scrip Magazine*, Jan. 2000, 37
2. H. J. Quadbeck-Seger, R. Faust, G. Knaus, A. Maelicke, U. Simeling, Chemie-rekorde, Wiley-VCH, Weinheim, 2nd Edn., 1999, 1.

References to 5.1 ACE Inhibitors

3. S. H. Ferreira, D. C. Bartelt, L. J. Greene, *Biochemistry* **9** (1970) 2583; S. Müller, I. Paegelow, S. Reismann, *Signal Transduction* **6** (2006) 5.
4. H. Remane, Y. Remane, *Pharm. Unserer Zeit* **39** (2010) 340; C. Roegler, J. Lehmann, *Pharm. Unserer Zeit* **39** (2010) 351.
5. P. Dominiak, *Pharm. Unserer Zeit* **32** (2003) 24.
6. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1992, 138.
7. M. A. Ondetti, N. J. Wiliams, E. F. Sabo, J. Pluscec, E. R. Weaver, O. Kocy, *Biochemistry* **19** (1971) 4033; D. W. Cushman, M. A. Ondetti, *Hypertension* **17** (1991) 589.
8. M. L. Nunes-Mamede, F. G. De Mello, A. R. Martins, *J. Neuroscience Methods*, **31** (1990) 7.
9. www.laskerfoundation.org/awards/library/1999remarksgsclin2.shtml.
10. L. D. Byers, R. Wolfenden, *Biochemistry* **12** (1973) 2070.
11. H. Kubinyi, Wirkstoffdesign, Graduiertenkolleg University Heidelberg, Feb. 1999.
12. R. R. Chirumamilla, R. Marchant, P. Nigam, *J. Chem. Technol. Biotechnol.* **76** (2001) 123.
13. A. Kleemann, J. Engel, Pharmaceutical Substances, 3rd Edn., Thieme, Stuttgart, 1999, 310.
14. M. J. Wyvatt, A. A. Patchett, *Med. Res. Rev*. **5** (1985) 483.
15. C. Chevillard, S. Jouquey, F. Bree, M.-N. Mathieu, J. P. Stepniewski, J. P. Tillement, G. Hamon, P. Corvol, *J. Cardiovascular Pharm. 23rd Suppl.* **4** (1994) S11.
16. F. Brion, C. Marie, P. Mackiewicz, J. M. Roul, J. Buendia, *Tetrahedron Lett.* **33** (1992) 4889.
17. H. G. Eckert, M. J. Badian, D. Gantz, H.-M. Kellner, M. Volz, *Arzneim.-Forsch./ Drug Res*. **34** (1984) 1435.
18. R. Natesh, S. L. U. Schwager, E. D. Sturrock, K. R. Acharya, *Nature* **421** (2003) 551.
19. R. Natesh, S. L. U. Schwager, H. R. Evans, E. D. Sturrock, K. R. Acharya, *Biochemistry* **43** (2004) 8718.
20. http://en.wikipedia.org/wiki/Discovery_and_development_of_angiotensin_receptor_Blockers.
21. http://en.wikipedia.org/wiki/Renin_inhibitor.
22. http://en.wikipedia.org/wiki/Pepstatin.
23. G. Klebe, Wirkstoffdesign, 2nd Edn., Spektrum Verlag, Heidelberg, 2009, 384.
24. http://en.wikipedia.org/wiki/Aliskiren.

References to 5.2 β-Lactam Antibiotics

25. G. Greene, *The Third Man*, Heinemann, London, 1950; reprinted Vintage Books, London, 2001; extracts from Chapter 10, pp. 63–64.
26. J. Nosek, R. Radzio, U. Kück, *Chemie in unserer Zeit* **31**(1997) 172.
27. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 6.
28. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 258.
29. E. Mutschler, Arzneimittelwirkungen, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1991, 577.

30. C. Friedrich, *Pharmazie in unserer Zeit*, **35** (2009) 392; Kirk-Othmer, Encyclopedia of Chemical Technology, Wiley, New York, 4th Edn., Vol. 2 (Antibiotics), 893; http://en.wikipedia.org/wiki/Ernest_Duchesne.
31. H. Remane, K. Begitt, *Nachrichten aus der Chemie* **51** (2003) 1180.
32. A. Fleming, *Brit. J. Exp. Pathol.* **10** (1929) 226.
33. D. Crawfoot Hodgkin, C. Bunn, B. Rogers-Low, A. Turner-Jones, *Chemistry of Penicillin*, Princeton University Press, Princeton, NJ, 1949; K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 41.
34. http://veprints.unica.it/148/1/orru-poster.pdf.
35. D. Crawfoot Hodgkin, E. N. Maslen, *Biochem. J.* **79** (1961) 393.
36. R. B. Woodward, *Science* **153** (1966) 487; R. B. Woodward, *Angew. Chem*. **78** (1966) 557. R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, H. Vorbrüggen, *J. Am. Chem. Soc*. **88** (1966) 852.
37. J. Weil, J. Miramonti, M. R. Ladisch, *Enzyme Microbial Techn.***17** (1995) 85.
38. P. L. Roach, I. J. Clifton, C. M. H. Hensgens, N. Shibata, C. J. Schofield, J. Hajdu, J. E. Baldwin, *Nature* **387** (1997) 827.
39. W. A. Schenk, *Angew. Chem. Internat. Edn.* **39** (2000) 3409.
40. N. I. Burzlaff, P. J. Rutledge, I. J. Clifton, C. M. H. Hensgens, M. Pickford, R. M. Adlington, P. L. Roach, J. E. Baldwin, *Nature* **401** (1999) 721.
41. J. E. Baldwin, K.-C. Goh, M. E. Wood, C. J. Schofield, R. D. G. Cooper, G. W. Huffman, *Bioorg. Med. Chem. Lett.* **1** (1991) 421.
42. J. E. Baldwin, E. Abraham, *Nat. Prod. Rep*. **5** (1988) 129.
43. T. Fekner, J. E. Baldwin, R. M. Adlington, T. W. Jones, C. K. Prout, C. J. Schofield, *Tetrahedron* **56** (2000) 6053.
44. S. Kukolja, *J. Am. Chem. Soc*. **93** (1971) 6267.
45. S. Kukolja, *J. Am. Chem. Soc*. **93** (1971) 6269.
46. R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, S. L. Andrews, *J. Am. Chem. Soc*. **85** (1963) 1896.
47. R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, S. L. Andrews, *J. Am. Chem. Soc*. **91**(1969) 1401.
48. M. A. Wegman, M. H. A. Janssen, F. van Rantwijk, R. A. Sheldon, *Adv. Synth. Catal*. **343** (2001) 559.
49. Ullmann's Encyclopedia of Industrial Chemistry, 5thEdn., Vol. B8, 302.
50. L. Cao, F. van Rantwijk, R. A. Sheldon, *Org.Lett*. **2** (2000) 1361; R. A. Sheldon, *Adv. Synth. Catal.* **349** (2007) 1289.
51. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 249.
52. M. Sunagawa, A. Sasaki, *Heterocycles* **54** (2001) 497.
53. I. Kawamoto, *Drugs Fut.* **23** (1998) 181.
54. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 343.
55. H. Nozaki, S. Moriuta, H. Takaya, R. Noyori, *Tetrahedron Lett.* **1966**, 5239; H. Nozaki,H. Takaya, S. Moriuta, R. Noyori, *Tetrahedron* **24** (1968) 3655.
56. G. Sedelmeier in H. Waldmann, Organic Synthesis Highlights II, VCH, Weinheim, 1995, 277; and references cited therein.
57. P. J. Reider, E. J. J. Grabowski, *Tetrahedron Lett.* **23** (1982) 2293.
58. T. N. Salzmann, R. W. Ratcliffe, B. G. Christensen, F. A. Bouffard, *J. Am. Chem. Soc*. **102** (1980) 6161.
59. F. A. Bouffard, T. N. Salzmann, *Tetrahedron Lett.* **26** (1985) 6285; T. Kametani, S.-C. Chu, T. Honda, *J. Chem. Soc. Perkin Trans.* 1 (1988) 1593.
60. J. Prous, J. Castaner, *Drugs Fut.* **13** (1988) 534.
61. X. Lu, Z. Xu, G. Yang, R. Fan, *Org. Proc. Res. Dev*. **5** (2001) 186.
62. M. Sunagawa, H. Matsumura, T. Inoue, M. Fukasawa, M. Kato, *J. Antibiot.* **43** (1990) 519.

63. U. Holzgrabe, *Pharmazie in unserer Zeit* **35** (2006) 410; M. S. Butler, M. A. Cooper, *J. Antibiotics* **64** (2011) 413.
64. L. P. Kotra, D. Golemi, S. Vakulenko, S. Mobashery, *Chem. Ind. (London),* **2000**, 341.
65. C. Walsh, *Nature* **406** (2000) 775.
66. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 256.
67. W. Dürckheimer, J. Blumbach, R. Lattrell, K. H. Scheunemann, *Angew. Chem. Internat. Edn.* **24** (1985) 180.

References to 5.3 Opiates

68. S. Shaik, *Angew. Chem. Internat. Edn*. **42** (2003) 3208 (Endorphins and love).
69. M. Seefelder, Opium – Eine Kulturgeschichte, Athenäum, Frankfurt, 1987.
70. E. Pallenbach, *Pharmazie in unserer Zeit* **31** (2002) 90;http://www.drogenbeauftragte.de/fileadmin/dateiendba/Presse/Downloads/Rauschgifttote_5 2_ Geschlecht_110324_Drogenbeauftragte.pdf; http://en.wikipedia.org/wiki/ Papaver_somniferum.
71. F. I. Carroll, L. L. Howell, M. J. Kuhar, *J. Med. Chem*. **42** (1999) 2721; http://www.drugabuse.gov/publications/drugfacts/nationwide-trends.
72. R. J. Bryant, *Chem. Ind.(London)* (1988) 146.
73. E. Mutschler, Arzneimittelwirkung, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1991, 161.
74. F.-J. Kuhlen, *Pharmazie in unserer Zeit* **31** (2002) 13 (Historical, on the theme of pain).
75. E. Friedrichs, W. Straßburger, *Pharmazie in unserer Zeit* **31** (2002) 32.
76. M. Eguchi, *Med. Res. Rev*. **24** (2004) 182.
77. W. Straßburger, E. Friedrichs, *Pharmazie in unserer Zeit* **31** (2002) 52.
78. W. G. Donner, *Pharmazie in unserer Zeit* **15** (1986) 33.
79. M. Hesse, Alkaloide–Fluch oder Segen der Natur?, Wiley-VCH, Weinheim, 2000, 344.
80. 2011 International Narcotics Control Strategy Report: UN World Drug Report; http://www.incb.org/pdf/technical-reports/narcotic-drugs/2011/Nar_Repor_2011_English/Part_FOUR_Comments_NAR-Report-2011_English.pdf; 2012 International Narcotics Control Strategy Report: UN World Drug Report; http://www.unodc.org/documents/data-and-analysis/WDR2012/WDR_2012_ web_small.pdf; http://www.unodc.org/unodc/secured/wdr/wdr2013/World_ Drug_Report_2013.pdf.
81. J. Frackenpohl, *Chemie in unserer Zeit* **34** (2000) 99; D. H. R. Barton, A. M. Deflorin, O. E. Edwards, *Chem. Ind.* (1955) 1039.
82. International Narcotics Control Board, 2012;http://www.nacada.go.ke/drugs/ heroin/; CD Römpp Chemie Lexikon, Thieme, Stuttgart, 1995.
83. M. Hesse, Alkaloide–Fluch oder Segen der Natur?, Wiley-VCH, Weinheim, 2000, 275; J. M. Finefield, D. H. Sherman, M. Kreitman, R. M. Williams, *Angew. Chem.* **124** (2012) 4886.
84. G. Bringmann, *Naturwissenschaften* **66** (1979) 22.
85. A. G. Millgate, B. J. Pogson, I. W. Wilson, T. M. Kutchan, M. H. Zenk, W. L. Gerlach, A. J. Fist, P. J. Larkin, *Nature* **431** (2004) 413.
86. K. Oka, J. D. Kantrowitz, S. Spector, *Proc. Natl. Acad. Sci. USA* **82** (1985) 1852.
87. T. Amann, M. H. Zenk, *Deut. Apotheker Zeit.* **136** (1996) 519.
88. C. Poeaknapo, J. Schmidt, M. Brandsch, B. Dräger, M. H. Zenk, *PNAS* **1 01** (2004) 14091.
89. M. Booth, Opium, A History, St. Martin's Griffin, New York, 1996.
90. http://en.wikipedia.org/wiki/MacFarlan_Smith; C. Schuster, *Die BASF* **21** (1971) 47.

91. 100 Jahre Chemisch-Wissenschaftliches Laboratorium der Bayer AG, Firmenschrift, 1996, 28.
92. J. M. Gulland, R. Robinson, *Mem. Proc. Manch. Lit. Soc*. **69** (1925) 79.
93. M. Gates, G. Tschudi, *J. Am. Chem. Soc*. **74** (1952) 1109.
94. M. Gates, G. Tschudi, *J. Am. Chem. Soc*. **78** (1956) 1380.
95. M. Maier, H. Waldmann (Ed.), Organic Synthesis Highlights II, VCH, Weinheim, 1995, 357 (Review of non-enantioselective morphine syntheses).
96. C. Y. Hong, N. Kado, L. E. Overman, *J. Am. Chem. Soc*. **115** (1993) 11028.
97. J. Mulzer, G. Dürner, D. Trauner, *Angew. Chem. Internat. Edn.* **35** (1996) 2832; D. Trauner, S. Porth, T. Opatz, J. W. Bats, G. Giester, J. Mulzer, *Synthesis* (1998) 653.
98. J. D. White, P. Hrnciar, F. Stappenbeck, *J. Org. Chem*. **62** (1997) 5250.
99. D. F. Taber, T. D. Neubert, A. L. Rheingold, *J. Am. Chem. Soc.* **124** (2002) 12416.
100. B. M. Trost, W. Tang, *J. Am. Chem. Soc*. **124** (2002) 14542.
101. Review: J. Zezula, T. Hudlicky, *Synlett* (2005) 388; K. Uchida, S. Yokoshima, T. Kan, T. Fukuyama, *Heterocycles* **77** (2009) 1219, and references cited therein.
102. H. L. Goering, S. S. Kantner, C. C. Tseng, *J. Org. Chem*. **48** (1983) 715.
103. I. Iijima, J. Minamikawa, A. E. Jacobson, A. Brossi, K. C. Rice, *J. Org. Chem*. **43** (1978) 1462.
104. Ullmann's Encyclopedia of Industrial Chemistry, 6thEdn., Wiley-VCH, Weinheim, 2000, Electronic Release.
105. C. H. Boehringer, DE 247180 (1909).
106. G. Geißlinger, *Deut. Apotheker Zeit*. **143** (2003) 62.
107. Review of *N*-demethylation: S. Thavaneswaran, K. McCamley, P. J. Scammells, *Nat. Prod. Commun.* **1** (2006) 885; G. B. Kok, P. J. Scammells, *Bioorg. Med. Chem. Lett.* **20** (2010) 4499.
108. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 1294.
109. O. Schnider, J. Hellerbach, *Helv. Chim. Acta* **33** (1950) 1437; O. Schnider, A. Grüssner, *Helv. Chim. Acta* **34** (1951) 2211; V. J. Harding, W. N. Haworth, W. H. Perkin jun., *J. Chem. Soc.* **93** (1908) 1944.
110. H. Buschmann, B. Sundermann, C. Maul, *Pharmazie in unserer Zeit* **31**(2002) 44.
111. P. K. Gupta, K. Ganesan, A. Pande, R. C. Malhotra, *J. Chem. Res.* (2005) 452.
112. G. Satzinger, W. Herrmann, DE 1923619 (1969); G. Satzinger, W. Herrmann, DE 1923620 (1969); K.-O. Vollmer, F. W. Koss, B. Liedtke, G. Heinzel, *Arzneimittelforsch.* **20** (1970) 990; GB 1226318 (1969); H. Schinz, M. Hinder, *Helv. Chim. Acta* **39** (1947) 1349; C. W. Jefford, A. Zaslona, *Tetrahedron Lett.* **26** (1985) 6035; G. M. Ksander, J. E. McMurry, M. Johnson, *J. Org. Chem.* **42** (1977) 1180; G. Fogliato, G. Fronza, C. Fuganti, P. Grasselli, S. Servi, *J. Org. Chem.* **60** (1995) 5693; D. B. G. Williams, M. L. Shaw, T. Hughes, *Organometallics* **30** (2011) 4968.
113. L. E. Overman, C. B. Petty, R. J. Doedens, *J. Org. Chem.* **44** (1979) 4183.
114. G. R. Evans, P. D. Fernández, J. A. Henshilwood, S. Lloyd, C. Nicklin, *Org. Proc. Res. Dev.* **6** (2002) 729.
115. RWTH, Arbeits- und Ergebnisbericht, Transferbereich 11, Stereoselektive Synthese (2001).
116. A. Boumendjel, G. Sotoing Taiwe, E. Ngo Bum, T. Chabrol, C.l Beney, V. Sinniger, R. Haudecoeur, L. Marcourt, S. Challal, E. Ferreira Queiroz, F. Souard, M. Le Borgne, T. Lomberget, A. Depaulis, C. Lavaud, R. Robins, J.-L. Wolfender, B. Bonaz, M. De Waard, *Angew. Chem.* **125** (2013) 11996; S. Kusari, S. J. N. Tatsimo, S. Zühlke, F. M. Talontsi, S. F. Kouam, M. Spiteller, *Angew. Chem.* **126** (2014) 12269.
117. R. F. Schmidt, *Pharmazie in unserer Zeit* **31** (2002) 23.
118. G. Seitz, *Pharm. Z.* **137** (1992) 87.

References to 5.4 Tetrahydrocannabinol

119. Bibliographisches Institut & F. A. Brockhaus AG, 2001, Electronic Release, Hanf; E. B. Russo, *Chem. Biodivers*. **4** (2007) 1614; L. O. Hanus, *Med. Res. Rev*. **29** (2009) 213.
120. http://en.wikipedia.org/wiki/Club_des_Hashischins;www.hanf-cannabis-sativa.org/geschichte-kultur.htm.
121. http://www.unodc.org/documents/data-and-analysis/WDR2012/WDR_2012_web_small.pdf; http://www.incb.org/documents/Narcotic-Drugs/Technical-Publications/2012/Narcotic_Drugs_Report_2012.pdf.
122. C. Giroud, *Chimia* **56** (2002) 80.
123. E. M. Williamson, F. J. Evans, *Drugs* **60** (2000) 1303.
124. Römpp, Electronic Release, Thieme, 2004, Tetrahydrocannabinols.
125. P. Nuhn, Naturstoffchemie, S. Hirzel Verlag, Stuttgart, Leipzig, 1997, 616.
126. Y. Gaoni, R. Mechoulam, *J. Am. Chem. Soc.* **86** (1964) 1646.
127. R. Mechoulam, S. Ben-Shabat, *Nat. Prod. Rep*. **16** (1999) 131.
128. M. Fellermeier, M. H. Zenk, *FEBS Letters* **427** (1998) 283.
129. F. Taura, S. Morimoto, Y. Shoyama, *J. Am. Chem. Soc*. **117** (1995) 9766, F. Taura, S. Sirikantaramas, Y. Shoyama, Y. Shoyama, S. Morimoto, *Chem. Biodivers*. **4** (2007) 1649.
130. D. M. Lambert, C. J. Fowler, *J. Med. Chem*. **48** (2005) 5059.
131. E. di Tomaso, M. Beltramo, D. Piomelli, *Nature*, **382** (1996) 677.
132. J. W. Huffman, J. R. A. Miller, J. Liddle, S. Yu, B. F. Thomas, J. L. Wiley, B. R. Martin, *Bioorg. Med. Chem*. **11** (2003) 1397; further structure-activity relationships: E. Stern, D. M. Lambert, *Chem. Biodivers*. **4** (2007) 1707.
133. S. Kreutz, *Pharmazie in unserer Zeit* **36** (2007) 389; B. Costa, *Chem. Biodivers*. **4** (2007) 1664.
134. R. K. Razdan, The Total Synthesis of Natural Products, Vol. 4, J. ApSimon, Ed., Wiley, New York, 1981, 185 (Review).
135. A. Focella, S. Teitel, A. Brossi, *J. Org. Chem*. **42** (1977) 3456.
136. R. Mechoulam, P. Braun, Y. Gaoni, *J. Am. Chem. Soc*. **89** (1967) 4552.
137. R. K. Razdan, G. R. Handrick, H. C. Dalzell, *Experientia* **31** (1975) 16.
138. Dronabinol, BIC 3000, Becker Associates 1999–2004, 24/11/2004.
139. L. Crombie, W. M. L. Crombie, S. V. Jamieson, C. J. Palmer, *J. Chem. Soc., Perkin. Trans. 1* **1988**, 1243.
140. P. Stoss, P. Merrath, *Synlett* **1991**, 553.
141. Johnson Matthey: WO02/096846 (2001).
142. Johnson Matthey: WO02/096899 (2001).
143. D. A. Evans, E. A. Shaughnessy, D. M. Barnes, *Tetrahedron Lett.* **38** (1997) 3193.
144. R. A. Archer, DE 2451936 (1973).
145. S. J. Dominianni, C. W. Ryan, C. W. DeArmitt, *J. Org. Chem*. **42** (1977) 344.
146. R. A. Archer, W. B. Blanchard, W. A. Day, D. W. Johnson, E. R. Lavagnino, C. W. Ryan, J. E. Baldwin, *J. Org. Chem*. **42** (1977) 2277.
147. C. F. Carvalho, M. V. Sargent, *J. Chem. Soc., Chem. Commun*.(1984) 227.

References to 5.5 Nonsteroidal Anti-Inflammatory Drugs

148. W. Eisenreich, A. Handel, U. E. Zimmer, Tier- und Pflanzenführer, BLV, München, 14th Edn., 1997, 104.
149. K. Dombrowski, A. W. Alfermann, *Pharmazie in unserer Zeit* **22** (1993) 275.
150. http://www.zm-online.de.
151. Bibliographisches Institut & F. A. Brockhaus AG, Electronic Release, 2001, Aspirin.
152. N. Kuhnert, *Chemie in unserer Zeit*, **33** (1999) 213.
153. Bayer AG, 100 Jahre Chemisch-Wissenschaftliches Laboratorium der Bayer AG in Wuppertal-Elberfeld, 1996, 28.

154. H. Henderson, *Science* **286** (1999) 1090.
155. A. Eichengrün, *Die Pharmazie* **4** (1949) 582.
156. W. Sneader, *Brit. Med. J.* **321** (2000) 1591.
157. J. R. Vane, *Nat. New. Biol.* **25** (1971) 232; P. J. Loll, D. Picot, M. Garavito, *Nat. Struct. Biol.* **2** (1995) 637; R. S. Sidhu, J. Y. Lee, C. Yuan, W. L. Smith, *Biochem.* **49** (2010) 7069.
158. D. L. Simmons, R. M. Botting, T. Hla, *Pharm. Rev.* **56** (2004) 387; R. M. Garavito, A. M. Mulichak, *Annu. Rev. Biophys. Biomol. Struct.* **32** (2003) 183.
159. E. Mutschler, Arzneimittelwirkungen, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 6th Edn., 1991, 177.
160. D. L. Simmons, W. Xie, J. G. Chipman, G. E. Evett, Plenum Press, New York, 1991, 67.
161. W. Xie, *Proc. Nat. Acad. Sci. USA* **88** (1991) 2692.
162. E. A. Meade, W. L. Smith, D. L. DeWitt, *J. Biol. Chem.* **268** (1993) 6610.
163. K. N. Allen, *Nature Medicine* **1** (1995) 882.
164. K. Schrör, *Pharmazie in unserer Zeit* **38** (2009) 306.
165. F. Zhang, N. K. Altorki, J. R. Mestre, K. Subbaramaiah, A. J. Dannenberg, *Carcinogenesis* **20** (1999) 445.
166. A. Goel, C. R. Boland, D. P. Chauhan, *Cancer Lett.* **172** (2001) 111; T. Esatbeyoglu, P. Huebbe, I. M. A. Ernst, D. Chin, A. E. Wagner, G. Rimbach, *Angew. Chem. Int. Ed.* **51** (2012) 5308.
167. http://en.wikipedia.org/wiki/Tripterygium_wilfordii.
168. Y. Gong, B. Xue, J. Jiao, L. Jing, X. Wang, *J. Neurochem.* **107** (2008) 779.
169. R. Ariza-Ariza, M. Mestanza-Peralta, M. H. Cardiel, *Semin. Arthritis Rheum.* **27** (1998) 366.
170. C. L. Curtis, C. E. Hughes, C. R. Flannery, C. B. Little, J. L. Harwood, B. Caterson, *J. Biol. Chem.* **275** (2000) 721.
171. A. Almada, "Natural COX-2 Inhibitors: The Future of Pain Relief" in http://www.chiro.org/nutrition/FULL/Natural_COX-2_Inhibitors.shtml.
172. H. N. Morse, *Ber. Dtsch. Chem. Ges.* **11** (1878) 232.
173. Wikipedia: Paracetamol.
174. G. Benz, R. Hahn, C. Reinhardt, 100 Jahre Chemisch Wissenschaftliches Laboratorium der Bayer AG in Wuppertal-Elberfeld, Bayer, 1997, p. 22; G. G. Graham, M. J. Davies, R. O. Day, A. Mohamudally, K. F. Scott, *Inflammopharmacology* **21** (2013) 201.
175. R. J, Flower, J. R. Vane, *Nature* **240** (1972) 410.
176. G. G. Graham, K. F. Scott, *Am. J. Ther.* **12** (2005) 46.
177. B. Kis, J. A. Snipes, S. A. Simandle, D. W. Busija, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288** (2005) R897.
178. K. Brune, T. Egger, *Pharmazie in unserer Zeit* **31** (2002) 133.
179. W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 21st Edn., 1969, 187.
180. Wikipedia: Ibuprofen; S. S. Adams, *J. Clin. Pharmacol.* **32** (1992) 317; http://www.telegraph.co.uk/health/3351540/Dr-Stewart-Adams-I-tested-ibuprofen-on-my-hangover.html.
181. V. Lambert, Dr. Stewart Adams: "I tested ibuprofen on my hangover", The Telegraph Magazine, 8.10.2007.
182. R. M. Garavito, in A. Messerschmidt, R. Huber, T. Poulos, K. Wieghardt, Wiley, Chichester, 2001, 245.
183. H. Ulbrich, G. Dannhardt, *Pharmazie in unserer Zeit* **31** (2002) 146.
184. Wikipedia: Diclofenac.
185. G. Dannhardt, L. Hahn, *Phamazie in unserer Zeit* **29** (2000) 100.
186. R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, R. A. Stegeman, J. Y. Pak, D. Gildehaus, J. M. Miyashiro, T. D. Penning, K. Seibert, P. C. Isakson, W. C. Stallings, *Nature* **384** (1996) 644.

187. K. Antoniou, M. Malamas, A. A. Drosos, *Expert Opin. Pharmacother*. **8** (2007) 1719.
188. S. Laufer, *Pharmazie in unserer Zeit* **31** (2002) 164.
189. S. Chaiamnuay, J. J. Allison, J. R. Curtis, Am. *J. Health Syst. Pharm*. **63** (2006) 1837; D. H. Solomon, *Arthritis & Rheumatism* **52** (2005) 1968; G. A. FitzGerald, C. Patrono, *N. Engl. J. Med.* **345** (2001) 433.
190. RSC: "Ibuprofen – A Case Study in Green Chemistry" in http://www.rsc.org/Education/Teachers/Resources/green/ibuprofen/ibuprofen.pdf.
191. P. G. M. Wuts, C. L. Bergh, *Tetrahedron Lett*. **27** (1986) 3995.
192. K. Kogure, K. Nakagawa, US 3965161 (1976).
193. B. R. Babu, K. K. Balasubramaniam, *Org. Prep. Proc. Int*. **26** (1994) 123.
194. J.-P. Rieu, A. Boucherle, H. Cousse, G. Mouzin, *Tetrahedron* **42** (1986) 4095.
195. P. J. Harrington, E. Lodewijk, *Org. Proc. Res. Dev.* **1** (1997) 72.
196. H. R. Sonawane, N. S. Bellur, J. R. Ahuja, D. G. Kulkarni, *Tetrahedron: Asymmetry* **3** (1992) 163.
197. C. Giordano, G. Castaldi, S. Cavicchioli, M. Villa, *Tetrahedron* **45** (1989) 4243.
198. A. S. C. Chan, T. T. Huang, J. H. Wagenknecht, R. E. Miller, *J. Org. Chem.* **60** (1995) 742.
199. T. Ohta, H. Takaya, M. Kitamura, K. Nagai, R. Noyori, *J. Org. Chem.* **52** (1987) 3174.
200. A. S. C. Chan, US 4994607 (1991).
201. A. Kleemann, J. Engel, Pharmaceutical Substances, 3rd Edn., Thieme, Stuttgart, 1999, 1156.
202. A. Kleemann, J. Engel, Pharmaceutical Substances, 3. Edn., Thieme, Stuttgart, 1999, 1549.
203. Wikipedia: Saccharin.
204. Wikipedia: Constantin Fahlberg.
205. Celecoxib, BIC 3000, Becker Associates 1999–2010, 08/12/2010; S. Dadiboyena, A. T. Hamme II, *Curr. Org. Chem.* **16** (2012) 1390.
206. Rofecoxib, BIC 3000, Becker Associates 1999–2010, 08/12/2010.
207. http://en.wikipedia.org/wiki/Rheumatoid_arthritis
208. http://en.wikipedia.org/wiki/Tocilizumab
209. http://en.wikipedia.org/wiki/Anakinra
210. http://en.wikipedia.org/wiki/Infliximab
211. http://en.wikipedia.org/wiki/Adalimumab
212. http://en.wikipedia.org/wiki/Golimumab

References to 5.6 Prostaglandins

213. Encyclopedia Britannica, Electronic Release, 2001, Prostaglandins.
214. E. Matsui, Y. Naruta, F. Tani, Y. Shimazaki, *Angew. Chem. Interrnat. Edn.* **42** (2003) 2744.
215. P. Karlson, Kurzes Lehrbuch der Biochemie, Thieme, Stuttgart, 1970, 32; S. Peng, N. M. Okeley, A.-L. Tsai, G. Wu, R. J. Kulmacz, W. A. van der Donk, *J. Am. Chem. Soc.* **124** (2002) 10785.
216. R. M. Garavito, A. M. Mulichak, *Annu. Rev. Biophys. Biomol. Struct.* **32** (2003) 183; R. M. Garavito, in A. Messerschmidt, R. Huber, T. Poulos, K. Wieghardt, Wiley, Chichester, 2001, 245.
217. G. Habermehl, P. E. Hammann, H. C. Krebs, Naturstoffchemie, 2nd Edn., Springer, Berlin, 2002, 525.
218. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 65.
219. R. Brückner, Reaktionsmechanismen, Spektrum Akademischer, Heidelberg, 1996, 443.
220. M. Suzuki, T. Kawagishi, T. Suzuki, R. Noyori, *Tetrahedron Lett*. **23** (1982) 4057.

221. K. Laumen, M. P. Schneider, *J. Chem. Soc. Chem. Commun*. (1986) 1298.
222. K. Laumen, M. P. Schneider, *Tetrahedron Lett*. **25** (1984) 5875.
223. L. A. Arnold, R. Naasz, A. J. Minnaard, B. L. Feringa, *J. Am. Chem. Soc*. **123** (2001) 5841.
224. P. W. Collins, E. Z. Dajani, D. R. Driskill, M. S. Bruhn, C. J. Jung, R. Pappo, *J. Med. Chem.* **20** (1977) 1152.
225. J. R. Beehling, P. W. Collins, J. S. Ng, *Adv. Metal-Org. Chem*. **4** (1995) 65.
226. B. H. Lipshutz, R. S. Wilhelm, D. M. Floyd, *J. Am. Chem. Soc*. **103** (1981) 7672.
227. B. H. Lipshutz, J. A. Kozlowski, R. S. Wilhelm, *J. Org. Chem*. **49** (1984) 3943.
228. J. H. Dygos, J. P. Adamek, K. A. Babiak, J. R. Behling, J. R. Medich, J. S. Ng, J. J. Wieczorek, *J. Org. Chem.* **56** (1991) 2549.
229. L. T. Boulton, D. Brick, M. E. Fox, M. Jackson, I. C. Lennon, R. McCague, N. Parkin, D. Rhodes, G. Ruecroft, *Org. Proc. Res. Dev*. **6** (2002) 138.
230. P. A. Grieco, *J. Org. Chem*. **37** (1972) 2363.
231. Z. Grudzinski, S. M. Roberts, *J. Chem. Soc. Perkin Trans. 1* (1975) 1767.
232. G. A. Tolstikov, M. S. Miftakhov, N. A. Danilova, F. Z. Galin, *J. Org. Chem. USSR (Engl. Trans.)* **19** (1983) 1624.
233. D. R. Swanson, T. Nguyen, Y. Noda, E. Negishi, *J. Org. Chem*. **56** (1991) 2590.
234. C. Mioskowski, S. Manna, J. R. Falck, *Tetrahedron Lett*. **24** (1983) 5521 (with primary, secondary and tertiary lithium dialkylcuprates); I. Prowotorow, J. Wicha, K. Mikami, *Synthesis* (2001) 145 (with magnesium cuprates); T. Hiyama, Y. Morizawa, H. Yamamoto, H. Nozaki, *Bull. Soc. Chem. Jpn*. **54** (1981) 2151 (with organoaluminium compounds); E. J. Enholm, Z. J. Jia, *J. Org. Chem.* **62** (1997) 9159 (with organotin compounds); P. Herrington, M. A. Kerr, *Tetrahedron Lett*. **38** (1997) 5949 (lanthanoid-catalysed homo-Michael reactions).
235. P. W. Collins, S. W. Djuric, *Chem. Rev.* **93** (1993) 1533.
236. W. Skuballa, H. Vorbrüggen, *Angew. Chem. Internat. Edn.* **20** (1981) 1046.

References to 5.7 Tetrahydrolipstatin

237. R. H. Marijnissen, P. Ruyffelaere, Bosch, VCH, 1988, 329; http://www.lucifuge.de/themen/religio/suenden.htm.
238. H. Wolff, C. Kunte, *Pharmazie in unserer Zeit* **29** (2000) 153.
239. M. Henningsen, *Chemie in unserer Zeit* **34** (2000) 179.
240. C. Leitzmann, *Biologie in unserer Zeit* **31** (2001) 408.
241. I. Vermaak, A. M. Viljoen, J. H. Hamman, *Nat. Prod. Rep* **28** (2011) 1493.
242. V. Schusdziarra, J. Erdmann, *Pharmazie in unserer Zeit* **35** (2006) 500.
243. http://en.wikipedia.org/wiki/Phentermine.
244. http://en.wikipedia.org/wiki/Sibutramine.
245. http://en.wikipedia.org/wiki/Rimonabant.
246. http://en.wikipedia.org/wiki/Gila_monster; J. Eng, W. A. Kleinman, L. Singh, G. Singh, J.-P. Raufman, *J. Biol. Chem.* **267** (1992) 7402.
247. http://en.wikipedia.org/wiki/Exenatide; A. Hamann, Diabetes Klinik Bad Nauheim, http://www.dgem.de/material/pdfs/Ernaehrung08_PDFs/Hamann%20J%20med%20BehAdi%20120608.pdf.
248. http://www.medknowledge.de/neu/2003/II-2003-21-exenatide-p.htm; M. Giraud, A.-S. Droz, S. Varray, E. D. Rekai, M.-H. Brichard, D. Latassa, C. Devijver, P. Gilles, J.-M.Cauvin, F. Albericio, M. P. Bas, US 20110046349 (2011).
249. E. K. Weibel, P. Hadvary, E. Hochuli, E. Kupfer, H. Lengsfeld, *J. Antibiot*. **40** (1987) 1081; R.-Y. Wu, M.-H. Chen, *Bot. Bull. Acad. Sin.* **36** (1995) 201.
250. C. Wedler, B. Costisella, H. Schick, *J. Org. Chem.* **64** (1999) 5301.
251. A. Pommier, J.-M.Pons, *Synthesis (*1995) 729 (review on natural 2-oxetanones); J. W. Yun, *Phytochem.* **71** (2010) 1625.
252. H. Umezawa, T. Aoyagi, T. Hazato, K. Uotani, F. Kojima, M. Hamada, T. Takeuchi, *J. Antibiot.* **31** (1978) 639.

253. H. Stalder, G. Oesterhelt, B. Borgström, *Helv. Chim. Acta* **75** (1992) 1593.
254. P. Barbier, F. Schneider, *Helv. Chim. Acta* **70** (1987) 196.
255. E. Hochuli, E. Kupfer, R. Maurer, W. Meister, Y. Mercadal, K. Schmidt, *J. Antibiot*. **40** (1987) 1086.
256. A. Pommier, J.-M. Pons, P. J. Kocienski, L. Wong, *Synthesis* (1994) 1294; P. Hadvary, W. Sidler, W. Meister, W. Vetter, H. Wolfer, *J. Biol. Chem*. **266** (1991) 2021.
257. C. W. Pemble, L. C. Johnson, S. J. Kridel, W. T. Lowther, *Nat. Struct. Mol. Biology* **14** (2007) 704.
258. W. Eisenreich, E. Kupfer, W. Weber, A. Bacher, *J. Biol. Chem*. **272** (1997) 867.
259. U. Kaulmann, C. Hertweck, *Angew. Chem. Internat. Edn*. **41** (2002) 1866.
260. D. H. G. Crout, C. R. McIntyre, N. W. Alcock, *J. Chem. Soc., Perkin Trans.* **2** (1991) 53.
261. D. H. G. Crout, D. L. Rathbone, *J. Chem. Soc., Chem. Commun*. (1987) 290.
262. Daicel Chemical Industries Ltd., DE 3731290 (1987).
263. C. A. Schuhr, W. Eisenreich, M. Goese, P. Stohler, W. Weber, E. Kupfer, A. Bacher, *J. Org. Chem*. **67** (2002) 2257.
264. H. G. W. Leuenberger, P. K. Matzinger, B. Wirz, *Chimia* **53** (1999) 536.
265. A. Bacher, P. Stohler, W. Weber, EP 0803576 (1997).
266. J.-M. Pons, A. Pommier, J. Lerpiniere, P. Kocienski, *J. Chem. Soc., Perkin Trans. 1* (1993) 1549.
267. F. Bennett, D. W. Knight, G. Fenton, *J. Chem. Soc., Perkin Trans. 1* (1991) 519.
268. F. Bennett, D. W. Knight, G. Fenton, *Tetrahedron Lett*. **29** (1988) 4865.
269. J.-M. Pons, P. Kocienski, *Tetrahedron Lett*. **30** (1989) 1833.
270. J. Prous, N. Mealy, J. Castaner, *Drugs of the Future* **19** (1994) 1003.
271. P. Barbier, F. Schneider, U. Widmer, *Helv. Chim. Acta* **70** (1987) 1412.
272. N. K. Chadha, A. D. Batcho, P. C. Tang, L. F. Courtney, C. M. Cook, P. M. Wovkulich, M. R. Uskokovic, *J. Org. Chem*. **56** (1991) 4714.
273. J. J. Landi Jr., L. M. Garofalo, K. Ramig, *Tetrahedron Lett*. **34** (1993) 277.
274. P. Barbier, F. Schneider, *J. Org. Chem*. **53** (1988) 1218.
275. I. Fleming, N. J. Lawrence, *Tetrahedron Lett*. **31** (1990) 3645.
276. I. Fleming, N. J. Lawrence, *J. Chem. Soc., Perkin Trans.* **1** (1998) 2679.
277. A. W. P. Jarvie, A. Holt, J. Thompson, *J. Chem. Soc. (B)* (1969) 852.
278. S. C. Case-Green, S. G. Davies, C. J. R. Hedgecock, *Synlett* (1991) 781.
279. H. Brunner, E. Schmidt, *J. Organometal. Chem*. **36** (1972) C18; S. J. Cook, J. F. Costello, S. G. Davies, H. T. Kruk, *J. Chem. Soc. Perkin Trans. 1* (1994) 2369.
280. N. Aktogu, H. Felkin, S. G. Davies, *J. Chem. Soc., Chem. Commun*. (1982) 1303.
281. I. Paterson, V. A. Doughty, *Tetrahedron Lett*. **40** (1999) 393.,
282. J. March, Advanced Organic Chemistry, 4th Edn., Wiley, New York, 1992, 1175 (mechanism).
283. A. K. Ghosh, S. Fidanze, *Org. Lett*. **2** (2000) 2405; meantime a further and similarly lengthy synthesis has appeared: A. K. Ghosh, K. Shurrush, S. Kulkarni, *J. Org. Chem*. **74** (2009) 4508.
284. D. Crich, X.-Y. Jiao, M. Brunko, *Tetrahedron Lett*. **53** (1997) 7127.
285. M. Kurihara, N. Miyata, *Chem. Lett*. (1995) 263.
286. T. Tsunoda, M. Suzuki, R. Noyori, *Tetrahedron Lett*. **21** (1980) 1357.
287. D. A. Evans, K. T. Chapman, E. M. Carreira, *J. Org. Chem*. **53** (1988) 3560.
288. J. A. Bodkin, E. J. Humphries, M. D. McLeod, *Tetrahedron Lett*. **44** (2003) 2869.
289. J. A. Bodkin, E. J. Humphries, M. D. McLeod, *Aust. J. Chem*. **56** (2003) 795.
290. U. Zutter, M. Karpf, EP 443449 (1991).
291. K. Püntener, "With Asymmetric Hydrogenation Towards a Stereoselective Synthesis of *Xenical*®", Proceedings of the 11th International Conference & Exhibition, Organic Process Research and Development, 25–28 April 2005, Barcelona, Spain.

References to 5.8 *Taxol*®

292. M. Reitz, Krebs als ständiger Begleiter der Menschheit, *Neue Züricher Zeitung*, April 2004, 7.
293. http://www.geschichte-muensingen.ch/fileadmin/user_upload/ortsgeschichte_muensingen/dokumente/1.2_Von_keltischem_Schmuck.pdf, page 46.
294. "Every cell comes from another cell"
295. http://flexikon.doccheck.com/Krebs
296. http://www.zeit.de/1966/43/Wie-koennen-Viren-Krebs-ausloesen; H. zur Hausen, *Angew. Chem. Internat. Edn*. **48** (2009) 5798; C. de Martel, J. Ferlay, S. Franceschi, J. Vignat, F. Bray, D. Forman, M. Plummer, *Lancet Oncology* **13** (2012) 607.
297. http://en.wikipedia.org/wiki/Pap_smear.
298. http://de.wikipedia.org/wiki/Rosafarbene_Catharanthe; J. R. Wright, *Can. Med. Ass.J.* **167** (2002) 1391.
299. http://zptech.net/media/Platinum%20Salts.pdf.
300. H. Gießen, *Pharmazie in unserer Zeit* **32** (2003) 90.
301. B. Kutscher, G. A. Curt, C. J. Allegra, R. L. Fine, H. Mujagic, G. C. Yeh, B. A. Chabner, Ullmann's Encyclopedia of Industrial Chemistry, Weinheim, 2005, Electronic Release, Cancer Chemotherapy.
302. A. Jordan, J. A. Hatfield, N. J. Laurence, A. T. McGowan, *Med. Res. Rev*. **18** (1998) 259; R. A. Stanton, K. M. Gernert, J. H. Nettles, R. Aneja, *Med. Res. Rev.* **31** (2011) 443.
303. T. Graening, H.-G. Schmalz, *Angew. Chem. Internat. Edn.* **43** (2004) 3230.
304. http://de.wikipedia.org/wiki/Eiben; K. C. Nicolaou, R. K. Guy, *Angew. Chem. Internat. Edn.* **34** (1995) 2079.
305. http://de.wikipedia.org/wiki/Europ%C3%A4ische_Eibe.
306. G. Julius Caesar, *Commentarii de Bello Gallico*: English translations by, among others, S. A. Handford ("The Conquest of Gaul") and K. Gilliver ("Caesar's Gallic Wars, 58–50 BC"); see also online synopses.
307. K. C. Nicolaou, W.-M. Dai, R. K. Guy, *Angew. Chem. Internat. Edn*. **33** (1994) 15.
308. W. C. Shortly, R. Minorca, in S. P. Raychaudhuri, K. Maramorosch, Biotechnology and Plant Protection in Forestry Science, Science Publishers, (place of publication unknown), 1999, 1.
309. T. Yabuta, *Agriculture and Horticulture* **10** (1935) 17.
310. A. Stierle, G. Strobel, D. Stierle, *Science* **260** (1993) 214; A. Stierle, G. Strobel, D. Stierle, P. Grothaus, G. Bignami, *J. Nat. Prod*. **58** (1995) 1315.
311. E. Leistner, *Pharmazie in unserer Zeit* **34** (2005) 98.
312. P. K. Ajikumar, W.-H. Xiao, K. E. J. Tyo, Y. Wang, F. Simeon, E. Leonard, O. Mucha, T. H. Phon, B. Pfeifer, G. Stephanopoulos, *Science* **330** (2010) 70.
313. R. A. Holton, H.-B. Kim, C. Somoza, F. Liang, R. J. Biediger, P. D. Boatman, M. Shindo, C. C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K. K. Murthi, L. N. Gentile, J. H. Liu, *J. Am. Chem. Soc*. **116** (1994) 1597.
314. R. A. Holton, H.-B. Kim, C. Somoza, F. Liang, R. J. Biediger, P. D. Boatman, M. Shindo, C. C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K. K. Murthi, L. N. Gentile, J. H. Liu, *J. Am. Chem. Soc*. **116** (1994) 1599.
315. K. C. Nicolaou, Z. Yang, J. J. Liu, H. Ueno, P. G. Nantermet, R. K. Guy, C. F. Claiborne, J. Renaud, E. A. Couladouros, K. Paulvannan, E. J. Sorensen, *Nature* **367** (1994) 630.
316. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis: Targets, Strategies, Methods, VCH, Weinheim, 1996, 655.
317. J. J. Masters, J. T. Link, L. B. Snyder, W. B. Young, S. J. Danishefsky, *Angew. Chem. Internat. Edn*. **34** (1995) 1723.

318. S. J. Danishefsky, J. J. Masters, W. B. Young, J. T. Link, L. B. Snyder, T. V. Magee, D. K. Jung, R. C. A. Isaacs, W. G. Bornmann, C. A. Alaimo, C. A. Coburn, M. J. Di Grandi, *J. Am. Chem. Soc*. **118** (1996) 2843.
319. Summary: D. G. I. Kingston, P. G. Jagtop, H. Yuan, L. Samala, *Prog. Chem. Org. Nat. Prod*. **84** (2002) 53; L. Sun, C. Simmerling, I. Ojima, *Chem Med Chem* **4** (2009) 719.
320. V. Sénilh, F. Guéritte, D. Guénard, M. Colin, P. Potier, *C. R. Acad. Sci. (Paris)*, *Série 2*, **299** (1984) 1039.
321. P. G. M. Wuts, *Curr. Opin. Drug Discov. Dev*. **1** (1998) 329.
322. J. N. Denis, A. E. Greene, D. Guénard, F. Gueritte-Voegelein, L. Mangatal, M. T. Adeline, P. Potier, *J. Am. Chem. Soc.* **110** (1988) 5917.
323. http://www.rinr.fsu.edu/fall2002/taxol.html.
324. M. A. Poss, J. L. Moniot, I. D. Trifunovich, D. J. Kucera, J. K. Thottathil, S.-H. Chen, J. Wei, WO 94/14787 (1994).
325. R. A. Holton, EP 400971 (1990).
326. R. A. Holton, US 5274124 (1993); R. A. Holton, US 6683196 (2004).
327. A. Commerçon, D. Bézard, F. Bernard, J. D. Bourzat, *Tetrahedron Lett*. **33** (1992) 5185.
328. F. Gueritte-Voegelein, V. Sénilh, B. David, D. Guénard, P. Potier, *Tetrahedron* **42** (1986) 4451; alternative synthesis and x ray structure: N. M. Sekhar, P. Vishweshwar, P. V. R. Acharyulu, Y. Anjaneyulu, *Synth. Commun.* **42** (2012) 3482.
329. I. Ojima, M. Zucco, O. Duclos, S. D. Kuduk, C. M. Sun, Y. H. Park, *Bioorg. Med. Chem. Lett.* **3** (1993) 2479.
330. M. V. Vononkov, A. V. Gontcharov, Z.-M. Wang, *Tetrahedron Lett*. **44** (2003) 407.
331. J.-N. Denis, A. Correa, A. E. Greene, *J. Org. Chem.* **56** (1991) 6939.
332. R. Croteau, R. E. B. Ketchum, R. M. Long, R. Kaspera, M. R. Wildung, *Phytochem. Rev*. **5** (2006) 75.
333. S. C. Roberts, *Nature Chemical Biology* **3** (2007) 387.
334. Y. J. Hong, D. J. Tantillo, *J. Am. Chem. Soc.* **133** (2011) 18249.
335. http://www.phytonbiotech.com/about_history.htm.
336. Y. Yukimune, H. Tabata, Y. Higashi, Y. Hara, *Nature Biotechnology* **14** (1996) 1129.
337. H. Tabata, *Adv. Biochem. Engin./Biotechnol*. **7** (2004) 1.
338. J. A. Yared, K. H. R. Tkaczuk, Drug Design, Development and Therapy 6 (2012) 371.

References to 5.9 Statins

339. E. Crispino (Ed.), Leonardo, art and science, Giunti Gruppa Editionale, Firenze, 2001, 112.
340. K. D. Keele, *Med. Hist*. **17** (1973) 304.
341. http://www.mayoclinic.com/health/arteriosclerosis-atherosclerosis/DS00525; http://surgery.about.com/od/beforesurgery/f/ArteriosclerosisAtherosclerosis.htm.
342. E. H. Ackerknecht, *Gesnerus* **32** (1975) 229.
343. K. Michel, *Zeit online* **37** (2007) 42; O. Gabel, K. H. Künzel, *Exp. Gerontol.* **33** (1998) 655; A. R. David, A. Kershaw, A. Heagerty, *The Lancet* **375** (2010) 718, A. G. Nerlich, I. Wiest, J. Tübel, *J. Paleopathology* **9** (1997) 83.
344. Encyclopedia Britannica, Electronic Release, 2001.
345. Published in 1799 in the paper "Inquiry into the Symptoms and Causes of Syncope Anginosa, commonly called Angina pectoris"
346. Joseph Hodgson, "On Wounds and Diseases of Arteries and Veins", printed by J. Moyes, Greville Street, Hatton Garden for T. Black, Edinburgh; J. Smith and Son, Glasgow; and J. Cumming, Dublin, 1815.

347. I. Metschnikow, Études sur la Nature Humaine: Essai de Philosophie Optimiste, Paris, Masson, 1903.
348. A. Ignatowski, *Virchows Arch. Pathol. Anat*. **198** (1909) 248.
349. A. Windaus, *Hoppe-Seyler's Z. Physiol. Chem*. **67** (1910) 174.
350. http://www.jci.org/articles/view/102881/files/pdf; http://www.nobelprize.org/nobel_prizes/medicine/laureates/1964/bloch-lecture.pdf.
351. N. Anitschkow, *Beitr. Pathol. Anat*. **56** (1913) 379; N. N. Anitschkow, S. Chalatov, *Zentralbl. Allg. Pathol*. **24** (1913) 1; I. E. Konstantinov, N. Mejevoi, N. M. Anichkov, *Tex. Heart Inst. J.* **33** (2006) 417.
352. D. Steinberg, *J. Lipid Res*. **45** (2004) 1583.
353. J. G. Mönckeberg, *Zentralbl. Herz Gefäßkrankheiten* **7** (1915) 7.
354. J. G. Mönckeberg, *Zentralbl. Herz Gefäßkrankheiten* **7** (1915) 336.
355. P. Lanzer, C. Shanahan, *Deutsches Ärzteblatt* **97** (2000) A-1746.
356. W. F. Enos, R. H. Holmes, J. Beyer, *JAMA* **152** (1953) 1090.
357. M. A. Macheboeuf, *Bull. Soc. Chim. Biol.* **11** (1929) 268.
358. D. Steinberg, *J. Lipid Res*. **46** (2005) 2037.
359. R. Ross, J. Glomset, L. Harker, *Am. J. Pathol*. **86** (1977) 675.
360. R. M. Roberts, Serendipity, Wiley, New York, 1989, 137.
361. K. Arnheim, *Deutsches Ärzteblatt* **103** (2006) A-796; J. R. Schaefer, B. Hufnagel, S. Klumpp, B. Malsch, J. Krieglstein, *Diabetologie* **2006**, 187.
362. M. Reinbold, B. Hufnagel, T. Kewitz, S. Klumpp, J. Krieglstein, *Mol. Nutr. Food. Res.* **52** (2008) 581; J. W. Heinecke, *Circulation* **127** (2013) 868.
363. S. Bernschneider-Reif, *Pharmazie in unserer Zeit* **36** (2007) 88.
364. G. Hahn, *Erfahrungsheilkunde* **51** (2002) 384.
365. R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey, R. W. Kierstead, *J. Am. Chem. Soc.* **78** (1956) 2023; R. B. Woodward, F. E. Bader, H. Bickel, A.. J. Frey, R. W. Kierstead, *J. Am. Chem. Soc.* **78** (1956) 2657.
366. R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey, R. W. Kierstead, *Tetrahedron* **2** (1958) 1.
367. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 55.
368. G. Stork, *Pure Appl. Chem*. **61** (1989) 439.
369. http://www.whale.to/a/hoffer5.html.
370. Alan Wise, S. M. Foord, N. J. Fraser, A. A. Barnes, N. Elshourbagy, M. Eilert, D. M. Ignar, P. R. Murdock, K. Steplewski, A. Green, A. J. Brown, S. J. Dowell, P. G. Szekeres, D. G. Hassall, F. H. Marshall, S. Wilson, N. B. Pike, *J. Biol. Chem.* **278** (2003) 9869; K. Ahmed, S. Offermanns, *Pharmazie in unserer Zeit* **36** (2007) 120.
371. V. Guetta, R. O. Cannon III, *Circulation* **93** (1996) 1928; D. Steinhilber, M. Schubert-Zsilavecz, *Pharmazie in unserer Zeit* **36** (2007) 108.
372. W. Schunack, *Pharmazie in unserer Zeit* **32** (2003) 498.
373. A. Endo, in "Statins, The HMG-CoA Reductase Inhibitors in Perspective", 2nd Edn., Martin Dunitz Ltd., London, 1994, 32; Takuji Hara, Innovation in the Pharmaceutical Industry: The Process of Drug Discovery and Development, Edward Elgar Publishing Ltd., Cheltenham, 2003, 132; D. Steinberg, *J. Lipid Res*. **47** (2006) 1339.
374. A. G. Brown, T. C. Smale, T. J. King, R. Hasenkamp, R. H. Thompson, *J. Chem. Soc. Perkin I.* (1976) 1165.
375. H. Stark, *Pharmazie in unserer Zeit* **32** (2003) 464.
376. A. Sutherland, K. Auclair, J. C. Vederas, *Curr. Opin. Drug Dev*. **4** (2001) 229.
377. E. S. Istvan, E. S. Deisenhofer, *Science* **292** (2001) 1160.
378. D. J. Witter, J. C. Vederas, *J. Org. Chem*. **61** (1996) 2613.
379. K. Wagschal, Y. Yoshizawa, D. J. Witter, Y. Liu, J. C. Vederas, *J. Chem. Soc. Perkin Trans. 1*, (1996) 2357.

380. E. M. Stocking, R. M. Williams, *Angew. Chem. Internat. Edn.* **42** (2003) 3078.
381. H. Oikawa, *Bull. Chem. Soc. Jpn*. **78** (2005) 537; A. Minami, H. Oikawa in Biomimetic Org. Synth.Wiley, 2011, 751.
382. K. Auclair, A. Sutherland, J. Kennedy, D. J. Winter, J. P. Van den Hoever, C. R. Hutchinson, J. C. Vederas, *J. Am. Chem. Soc*. **122** (2000) 11519.
383. Not-concerted reaction mechanism: see also G. Pohnert, *ChemBioChem* **4** (2003) 713.
384. O. Diels, K. Alder, *Liebigs Annalen der Chemie* **460** (1928) 98.
385. H. Hagiwara, T. Nakano, M. Kon-no, H. Uda, *J. Chem. Soc. Perkin Trans. 1* (1995) 777.
386. C. M. Blackwell, A. H. Davidson, S. B. Launchbury, C. N. Lewis, E. M. Morrice, M. M. Reeve, J. A. R. Roffey, A. S. Tipping, R. S. Todd, *J. Org. Chem*. **57** (1992) 5596.
387. D. L. J. Clive, K. S. K. Murthy, A. G. H. Wee, J. S. Prasad, G. V. J. Da Silva, M. Majewski, P. C. Anderson, *Stud. Nat. Prod. Chem.* **11** (1992) 335.
388. M. Hirama, M. Iwashita, *Tetrahedron Lett*. **24** (1983) 1811.
389. P. M. Wovkulich, P. C. Tang, N. K. Chadha, A. D. Batcho, J. C. Barrish, M. R. Uskokovic, *J. Am. Chem. Soc*. **111** (1989) 2596.
390. Unfortunately homologation is not further described in the original publication.
391. A. Kleemann, Cardiovascular Drugs, Ullmann Encyclopaedia, Electronic Release, Wiley-VCH, Weinheim, 2008.
392. M. Manzoni, M. Rollini, *Appl. Microbiol. Biotechnol*. **58** (2002) 555; M. Bizjak, S. Kranjc, R. Mencigar, P. Mrak, D. Smodis, G. Stampar, WO2008092950 A1 (2008).
393. T. R. Verhoeven, D. Askin, US 4820850 (1989).
394. R. K. Singamsetty, S. K. Vujjini, N. Manne, B. R. M. V. Naga, V. Himabindu, A. Battacharya, M. R. Ghanta, R. Bandichhor, *Synth. Commun*. **38** (2008) 4452.
395. B. D. Roth, *Prog. Med. Chem*. **40** (2002) 1.
396. R. R. Sagyam, H. Vurimidi, P. R. Padi, M. R. Ghanta, *J. Heterocycl. Chem*. **44** (2007) 923.
397. M. Müller, *Angew. Chem. Internat. Edn.* **44** (2005) 362.
398. S. C. Davis, J. H. Grate, D. R. Gray, J. M. Gruber, G. W. Huisman, S. K. Ma, L. M. Newman, R. Sheldon, L. A. Wang, WO 2005/018579.
399. S. Bergeron, D. A. Chaplin, J. H. Edwards, B. S. W. Ellis, C. L. Hill, K. Holt-Tiffin, J. R. Knight, T. Mahoney, A. P. Osborne, G. Ruecroft, *Org. Proc. Res. Dev*. **10** (2006) 661.
400. M. Hajkova, B. Kratochvil, S. Radl, *Chem. Listy* **102** (2008) 3.
401. M. Sletzinger, T. R. Verhoeven, R. P. Volante, J. M. McNamara, E. G. Corley, T. M. H. Liu, *Tetrahedron Lett*. **26** (1985) 2951.
402. K. Bock, I. Lundt, C. Pedersen, *Acta Chim. Scand. B* **37** (1983) 341.
403. O. Repic, K. Prasad, G. T. Lee, *Org. Proc. Res. Dev*. **5** (2001) 519.
404. Becker Associates, BIC 3000, Rosuvastatin, 2008.
405. A. Nadin, K. C. Nicolaou, *Angew. Chem. Internat. Edn*. **35** (1996) 1622.
406. J. R. Faust, J. L. Goldstein, M. S. Brown, *Arch. Biochem. Biophys.* **192** (1979) 86; M. S. Brown, J. L. Goldstein, *J. Lipid Res.* **21** (1980) *505*.
407. M. H. Davidson, *Curr. Athereoscl. Rep*. **9** (2007) 78.
408. R. K. Elsayed, J. D. Evans, *Expert Opinion Emerging Drugs*, **13** (2008) 309.

References to 5.10 Artemisinin

409. Friedrich Schiller, letters to Henriette von Wolzogen, Mannheim, Donnerstag 11. September 1783, and Mannheim, Donnerstag, 13. November 1783; http://www.wissen-im-netz.info/literatur/schiller/briefe/1783/178309111.htm; R. Klimm, *Dtsch. Ärztebl.* **99** (2002) A-420.

410. http://www.who.int/malaria/publications/world_malaria_report_2013/en/; http://www.ncbi.nlm.nih.gov/books/NBK2624/.
411. G. Poinar, Systematic *Parasitology* **61** (2005) 47.
412. W. Liu, Y. Li, G. H. Learn, R. S. Rudicell, J. D. Robertson, B. F. Keele, J.-B. N. Ndjango, C. M. Sanz, D. B. Morgan, S. Locatelli, M. K. Gonder, P. J. Kranzusch, P. D. Walsh, E. Delaporte, E. Mpoudi-Ngole, A. V. Georgiev, M. N. Muller, G. M. Shaw, M. Peeters, P. M. Sharp, J. C. Rayner, B. H. Hahn, *Nature* **467** (2010) 420.
413. R. Sallares, S. Gomzi, Ancient *Biomolecules* **3** (2001) 196; R. Sallares, A. Bouwman, C. Anderung, *Med Hist.* **48** (2004) 311.
414. M. J. Gardner, N. Hall, E. Fung, O. White, M. Berriman, R. W. Hyman, J. M. Carlton, A. Pain, K. E. Nelson, S. Bowman, I. T. Paulsen, K. James, J. A. Eisen, K. Rutherford, S. L. Salzberg, A. Craig, S. Kyes, M.-S. Chan, V. Nene, S. J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M. W. Mather, A. B. Vaidya, D. M. A. Martin, A. H. Fairlamb, M. J. Fraunholz, D. S. Roos, S. A. Ralph, G. I. McFadden, L. M. Cummings, G. M. Subramanian, C. Mungall, J. C. Venter, D. J. Carucci, S. L. Hoffman, C. Newbold, R. W. Davis, C. M. Fraser, B. Barrell, *Nature* **419** (2002) 498.
415. http://de.wikipedia.org/wiki/Malaria#Geschichte; A. G. Nerlich, B. Schraut, S. Dittrich, T. Jelinek, A. R. Zink, Plasmodium falciparum in ancient Egypt, http://wwwnc.cdc.gov/eid/article/14/8/08-0235_article.htm
416. F. E. G. Cox, Clin. *Microbiol. Rev.* **15** (2002) 595;
417. http://www.malariasite.com/malaria/history_literature.htm.
418. C. R. Garcia, R. P. Markus, L. Madeira, *J. Biol. Rhythms.* **16** (2001) 436.
419. http://www.gigers.com/matthias/malaria/history.htm.
420. C. B. Cunha, B. A. Cunha, *J. Vector Borne Dis.* **45** (2008) 194.
421. http://de.wikipedia.org/wiki/Hygiene_im_R%C3%B6mischen_Reich.
422. S. Winkle, Sonderdruck aus Hamburger Ärzteblatt, issue 6 and 8 (1984).
423. http://www.malariasite.com/malaria/history_victims.htm.
424. G. Fornaciari, V. Giuffra, E. Ferroglio, S. Gino, R. Bianucci, *Trans. R. Soc. Trop. Med. Hyg.* **104** (2010) 583.
425. M. K. Dalitz, Martin-Luther-Universität Halle-Wittenberg, Dissertation, 2005.
426. L. F. Haas, *J. Neurol. Neurosurg. Psychiatry* **67** (1999) 520; http://de.wikipedia.org/wiki/Alphonse_Laveran; http://www.nobelprize.org/nobel_prizes/medicine/laureates/1907/laveran-bio.html.
427. http://de.wikipedia.org/wiki/Camillo_Golgi.
428. http://en.wikipedia.org/wiki/Ronald_Ross.
429. F. E. G. Cox, *Parasites Vectors* **3** (2010) 5; http://en.wikipedia.org/wiki/History_of_malaria.
430. http://www.gigers.com/matthias/malaria/circle.htm; http://www.niaid.nih.gov/topics/malaria/pages/lifecycle.aspx; A. Ferreira, I. Marguti, I. Bechmann, V. Jeney, Â. Chora, N. R. Palha, S. Rebelo, A. Henri, Y. Beuzard, M. P. Soares, *Cell* **145** (2011) 398.
431. A. P. C. Fiks, Self-Experimenters: Sources for Study, Praeger Publishers, Westport CT, 2003, 196.
432. R. Virchow, *Arch. Pathol. Anatomie Physiol. Klin. Med.* **2** (1849) 587.
433. J. Ziegler, R. Linck, D. W. Wright, *Current Medicinal Chemistry* **8** (2001) 171.
434. T. Carbone, *Giornale della R Accademia di Medicina di Torino* **39** (1891) 901; W. H. Brown, *J. Exp. Med.* **13** (1911) 290; .
435. S. Pagola, P. W. Stephens, D. S. Bohle, A. D. Kosar, S. K. Madsen, *Nature* **404** (2000) 307; D. S. Bohle, R. E Dinnebier, S. K. Madsen, P. W. Stephens, *J. Biol. Chem.* **272** (1997) 713.
436. http://en.wikipedia.org/wiki/Cinchona; I. W. Sherman (Hrsg.), Malaria: Parasite Biology, Pathogenesis, and Protection, ASM Press, Washington, 1998, 341.
437. http://de.wikipedia.org/wiki/Chinin.

438. G. Gramiccia, *Parassitologia* **29** (1987) 207.
439. S. Streller, K. Roth, *Chemie in unserer Zeit* **46** (2012) 228; http://www.chemistryviews.org/details/ezine/4701281/From_Pharmacy_to_the_Pub_A_Bark_Conquers_the_World_Part_1.html.
440. E. Schwab, Römpp Online – Version, Georg Thieme Verlag, Stuttgart, 2013.
441. G. Stork, D. Niu, A. Fujimoto, E. R. Koft, J. M. Balkovec, J. R. Tata, G. R. Dake, *J. Am. Chem. Soc.* **123** (2001) 3239.
442. R. Woodward, W. Doering, *J. Am. Chem. Soc.* **66** (1944) 849.
443. Total synthesis: M. R. Uskokovic, J. Gutzwiller, T. Henderson, *J. Am. Chem. Soc.* **92** (1970) 203; Enantiopure Quinine: J. A. W. Gutzwiller, M. R. Uskokovic, US 3663554 (1972); M. R. Uskokovic, T. Henderson, C. Reese, H. L. Lee, G. Grethe, J. Gutzwiller, *J. Am. Chem. Soc.* **100** (1978) 571; J. Gutzwiller, M. R. Uskokovic, *J. Am. Chem. Soc.* **100** (1978) 576.
444. K. A. de Villiers, J. Gildenhuys, T. le Roex, *ACS Chem. Biol.* **7** (2012) 666.
445. J. M. Karle, I. L. Karle, L. Gerena, W. K. Milhous, *Antimicrob. Agents Chemother.* **36** (1992) 1538.
446. http://de.wikipedia.org/wiki/Dichlordiphenyltrichlorethan.
447. E. Tognotti, *Emerg. Infect. Dis.* **15** (2009) 1460.
448. R. H. Schirmer, H. Adler, M. Pickhardt, E. Mandelkow, *Neurobiol. Aging* **32** (2011) 2325.
449. P. Guttman, P. Ehrlich, *Berliner Klinische Wochenschrift* **39** (1891) 953.
450. http://en.wikipedia.org/wiki/Paul_Ehrlich#Methylene_blue; H. Schirmer, B. Coulibaly, A. Stich M. Scheiwein, H. Merkle, J. Eubel, K. Becker, H. Becher, O. Müller, T. Zich, W. Schiek, B. Kouyaté, *Redox Rep.* **8** (2003) 272; http://www.malariajournal.com/content/pdf/1475-2875-12-127.pdf.
451. K. Krafts, E. Hempelmann, A. Skórska-Stania, *Parastol. Res.* **111** (2012) 1.
452. S. Pou, R. W. Winter, A. Nilsen, J. X. Kelly, Y. Li, J. S. Doggett, E. W. Riscoe, K. W. Wegmann, D. J. Hinrichs, M. K. Riscoe, *Antimicrobial Agents and Chemotherapy* **56** (2012) 3475.
453. M. A. Biamonte, J. Wanner, K. G. Le Roch, *Bioorg. Med. Chem. Lett.* **23** (2013) 2829; H. M. Staines, l. Sanjeev Krishna, Treatment and Prevention of Malaria, Springer, Basel, 2012; M. Schlitzer, *Arch. Pharm. Chem. Life Sci.* **341** (2008) 149.
454. A. M Croft, *J. Royal Soc. Med.* **100** (2007) 170; http://en.wikipedia.org/wiki/Mefloquine.
455. E. Hsu, *Br. J. Clin. Pharmacol.* **61** (2006) 666.
456. U. S. Neill, *J. Clin. Invest.* **121** (2011) 3768.
457. Y. Tu, *Nature Medicine* **17** (2011) 1217.
458. Anonymus, *Kexue Tongbao (Chin.)* **3** (1977) 142; J.-M. Liu, M.-Y. Ni, J.-F. Fan, Y.-Y. Tu, Z.-H. Wu, Y.-L. Wu, W.-S. Chou, *Huaxue Xuebao* **37** (1979) 129.
459. Anonymus, *Chin. Med. J. (Engl.)* **92** (1979) 811; D. L. Klayman, *Science* **228** (1985) 1049.
460. Carl von Linné, Species Plantarum 2, Stockholm, 1753, 847.
461. L. Olofsson, A. Engström, A. Lundgren, P. E. Brodelius, *BMC Plant Biology* **11** (2011) 45.
462. N. Srivastava, A. Akhila, *J. Plant Interactions* **6** (2011) 265.
463. C. J. Paddon, P. J. Westfall, D. J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, D. R. Polichuk, K. H. Teoh, D. W. Reed, T. Treynor, J. Lenihan, M. Fleck, S. Bajad, G. Dang, D. Dengrove, D. Diola, G. Dorin, K. W. Ellens, S. Fickes, J. Galazzo, S. P. Gaucher, T. Geistlinger, R. Henry, M. Hepp, T. Horning, T. Iqbal, H. Jiang, L. Kizer, B. Lieu, D. Melis, N. Moss, R. Regentin, S. Secrest, H. Tsuruta, R. Vazquez, L. F. Westblade, L. Xu, M. Yu, Y. Zhang, L. Zhao, J. Lievense, P. S. Covello, J. D. Keasling, K. K. Reiling, N. S. Renninger, J. D. Newman, *Nature* **496** (2013) 528.

464. Z.-X. Yu, J.-X. Li, C.-Q. Yang, W.-L. Hu, L.-J. Wang, X.-Y. Chen, *Mol. Plant* **5** (2012) 353.
465. L.-K. Sy, G. D. Brown, *Tetrahedron* **58** (2002) 897; G. D. Brown, L.-K. Sy, *Tetrahedron* **63** (2007) 9548; G. D. Brown, *Molecules* **15** (2010) 7603; J. Kong, Y. Yang, W. Wang, K. Chenga, P. Zhu, *RSC Advances* **3** (2013) 7622.
466. http://www.nature.com/srep/2013/130829/srep02513/full/srep02513.html#affil-auth.
467. P. M. O'Neill, V. E. Barton, S. A. Ward, *Molecules* **15** (2010) 1705.
468. R. K. Haynes, K.-W. Cheu, K.-Y. Li, M. M.-K. Tang, H.-N. Wong, M.-J. Chen, Z.-F. Guo, Z.-H. Guo, P. Coghi, D. Monti, *Chem Med Chem* **6** (2011) 1603.
469. S. Pulcini, H. M. Staines, J. K. Pittman, K. Slavic, C. Doerig, J. Halbert, R. Tewari, F. Shah, M. A. Avery, R. K. Haynes, S. Krishna, *J. Infect. Dis.* **208** (2013) 468.
470. G. Schmid, W. Hofheinz, *J. Am. Chem. Soc.* **105** (1983) 624.
471. X. X. Xu, J. Zhu, D. Z. Huang, W. S. Zhou, *Tetrahedron* **42** (1986) 819; T. Ravindranathan, M. A. Kumar, R. B. Menon, S. V. Hiremath, *Tetrahedron Lett.* **31** (1990) 755; H. J. Liu, W. L. Yeh, S. Y. Chew, *Tetrahedron Lett.* **34** (1993) 4435; M. G. Constantino, M. Beltrame, G. V. J. daSilva, *Synth. Commun.* **26** (1996) 321; H. J. Liu, W. L. Yeh, *Heterocycles* **42** (1996) 493.
472. M. A. Avery, C. Jennings-White, W. K. M. Chong, *Tetrahedron Lett.* **28** (1987) 4629; M. A. Avery, W. K. M. Chong, C. Jennings-White, *J. Am. Chem. Soc.* **114** (1992) 974.
473. P. C. Traas, H. Boelens, H. J. Takken, *Tetrahedron Lett.* (1976) 2287.
474. G. Büchi, H. Wuest, *J. Am. Chem. Soc.* **100** (1978) 294.
475. J. S. Yadav, B. Thirupathaiah, P. Srihari, *Tetrahedron* **66** (2010) 2005.
476. S. C. Vonwiller, J. A. Warner, S. T. Mann, R. K. Haynes, *J. Am. Chem. Soc.* **117** (1995) 11098.
477. C. Zhu, S. P. Cook, *J. Am. Chem. Soc.* **134** (2012) 13577; P. Alsters, W. Jary, V. Nardello-Rataj, J.-M.Aubry, *Org. Proc. Res. Dev.* **14** (2010) 259.
478. http://de.wikipedia.org/wiki/Artemisinin.
479. D. K. Ro, E. M. Paradise, M. Ouellet, K. J. Fisher, K. L. Newman, J. M. Ndungu, K. A. Ho, R. A. Eachus, T. S. Ham, J. Kirby, M. C. Y. Chang, S. T. Withers, Y. Shiba, R. Sarpong, J. D. Keasling, *Nature* **440** (2006) 940.
480. F. Lévesque, P. H. Seeberger, *Angew. Chem. Int. Ed.* **51** (2012) 1706.
481. http://www.rsc.org/chemistryworld/2013/04/sanofi-launches-malaria-drug-production; J. Turconi, F. Griolet, R. Guevel, G. Oddon, R. Villa, A. Geatti, M. Hvala, K. Rossen, R. Göller, A. Burgard, *Org. Proc. Res. Dev.* **18** (2014) 417.
482. M. Peplow, *Nature* **494** (2013) 160.
483. R. Jambou, E. Legrand, M. Niang, N. Khim, P. Lim, B. Volney, M. T. Ekala, C. Bouchier, P. Esterre, T. Fandeur, O. Mercereau-Puijalon, *The Lancet* **366** (2005) 1960; http://www.who.int/malaria/publications/atoz/arupdate042012.pdf.
484. H. Noedl, Y. Se, K. Schaecher, B. L. Smith, D. Socheat, M. M. Fukuda, *N. Engl. J. Med.* **359** (2008) 2619; N. J. White, *Science* **320** (2008) 330.
485. A. P. Phyo, S. Nkhoma, K. Stepniewska, E. A. Ashley, S. Nair, R. McGready, C. ler Moo, S. Al-Saai, A. M. Dondorp, K. M. Lwin, P. Singhasivanon, N. P. J. Day, N. J. White, T. J. C. Anderson, F. Nosten, *The Lancet* **379** (2012) 1960.
486. http://www.who.int/malaria/publications/atoz/9789241500838/en/.

References to 5.11 Caffeine

487. http://www.biggone.com/2013/02/08/top-10-most-traded-commodities-in-the-world-2013/; H. G. Maier, *Chemie in unserer Zeit* **18** (1984) 17.
488. http://coopcoffees.com/resources/coffee/coffee-timeline; F. Ferré, Kaffee: Eine Kulturgeschichte, Wasmuth, Tübingen, 1991.
489. B. Sicard, *Spektrum der Wissenschaft*, June 2003, 65.

490. H. Steinhart, *Chem. Ind. (London)* (2002) 26.
491. E. A. Starbird, *National Geographic* **159** (1981) 388.
492. F. F. Runge, Hauswirthschaftliche Briefe, VCH Verlagsgesellschaft, Weinheim, 1988, Thirty-sixth letter: Mein Besuch bei Goethe im Jahre 1819 (My visit to Goethe in the year 1819), 165.
493. H. H. Lenz, Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, 1998, Electronic Release.
494. S. Rau, *Pharmazie in unserer Zeit* **35** (2006) 286.
495. B. Best, www.benbest.com/health/caffeine.html.
496. B. B. Friedholm, K. Bättig, J. Holmén, A. Nehlig, E. E. Zvartau, *Pharm. Rev*. **51** (1999) 83; P. Araque, H. Casanova, C. Ortiz, B. Henao, C. Reláez, *J. Agric. Food Chem.* **55** (2007) 6918.
497. S. R. Waldvogel, *Angew. Chem. Internat. Edn.* **42** (2003) 604.
498. P. Bützer, www.educeth.ch/chemie/diverses/coffein.
499. J. Oró, *Nature* **191** (1961) 1193; J. Oró, A. P. Kimball, *Arch. Biochem. Biophys*. **94** (1961) 217; R. A. Sanchez, J. P. Ferris, L. E. Orgel, *J. Mol. Biol.* **30** (1967) 223; A. W. Schwartz, C. G. Bakker, *Science* **245** (1989) 1102.
500. J. S. Hudson, J. F. Eberle, R. H. Vachhani, L. C. Rogers, J. H. Wade, R. Krishnamurthy, G. Springsteen, *Angew. Chem.* **124** (2012) 5224.
501. P. Karlson, Kurzes Lehrbuch der Biochemie, Thieme, Stuttgart, 1970, 103.
502. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1992, 764.
503. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1992, 745.
504. E. Ito, H. Ashihara, *J. Plant Physiol*. **154** (1999) 145.
505. H. Rosemeyer, *Chem. Biodivers.* **1** (2004) 361 (Review on natural purine derivatives).
506. A. Crozier, T. W. Baumann, H. Ashihara, T. Suzuki, G. R. Waller, *Coll. Sc. Int. sur le Café* **17** (1997) 106.
507. T. Suzuki, E. Takahashi, *Phytochem*. **15** (1976) 1235.
508. J. E. Prenosil, M. Hegglin, T. W. Baumann, P. M. Frischknecht, A. W. Kappeler, P. Brodelius, D. Haldimann, *Enzyme Microb. Technol*. **9** (1987) 450.
509. S. S. Mösli Waldhauser, J. A. Kretschmar, T. W. Baumann, *Phytochem*. **44** (1997) 853.
510. M. Kato, T. Kanehara, H. Shimizu, T. Suzuki, F. M. Gillies, A. Crozier, H. Ashihara, *Physiol. Plant*. **98** (1996) 629.
511. Anonymous, *Chem. Ind. (London)* (2006) 556.
512. M. Kato, K. Mizuno, A. Crozier, T. Fujimura, H. Ashihara, *Nature* **406** (2000) 956.
513. S. N. Katz, *Spektrum der Wissenschaft*, Mai 1999, 157.
514. K. Zosel, *Angew. Chem*. **90** (1978) 748.
515. E. Fischer, L. Ach, *Ber. Dtsch. Chem. Ges*. **28** (1895) 2473.
516. E. Fischer, L. Ach, *Ber. Dtsch. Chem. Ges*. **28** (1895) 3135.
517. A. Kossel, *Z. physiol. Chem*. **13** (1889) 298.
518. R. Behrend, O. Roosen, *Liebigs Ann. Chem*. **251** (1888) 235 and 248 (the first uric acid synthesis starting from urea).
519. W. Traube, *Ber. Dtsch. Chem. Ges*. **33** (1900) 3035.
520. E. Mutschler, Arzneimittelwirkungen, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1991, 239.
521. H. Bredereck, H.-G. von Schuh, A. Martini, *Chem. Ber.* **83** (1950) 201; H. Bredereck, R. Gompper, H.-G. von Schuh, G. Theilig, *Angew. Chem.* **71** (1959) 753.

References to 5.12 Nicotine

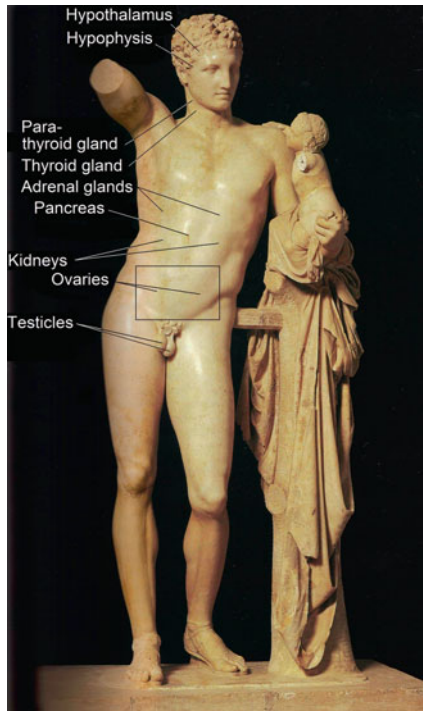
522. F. Eiden, *Pharmazie in unserer Zeit* **5** (1976) 1.
523. http://en.wikipedia.org/wiki/Rodrigo_de_Jerez.
524. E. Ritz, *Dtsch. Med. Wochenschr*. **130** (2005) 2947.
525. http://www.fmsi.org/mayawriting/codices/madrid.html.
526. R. Feser, R. Hilschmann, Weltreiche, Band 3, Zweiburgen Verlag, Weinheim, 193.
527. M. Hesse, Alkaloide, Wiley-VCH, Weinheim, 2000, 365.
528. A. Pinner, *Arch. Pharm.* **231** (1893) 378.
529. A. Pictet, *Ber. Dtsch. Chem. Ges*. **28** (1895) 1904.
530. A. Pictet, *Ber. Dtsch. Chem. Ges*. **37** (1904) 1225.
531. P. Karrer, R. Widmer, *Helvetica Chimica Acta* **8** (1925) 364; P. Nuhn, L. Wessjohann, Naturstoffchemie, Hitzel, Stuttgart, 2006, 587.
532. Nicotin, Roempp Chemie-Lexikon, Thieme, Stuttgart, electronic release.
533. G. Habermehl, P. E. Hammann, H. C. Krebs, Naturstoffchemie, Springer, Berlin, 2002, 160.
534. S. V. Koushik, J. A. Moore III, B. Sundararaju, R. S. Phillips, *Biochemistry* **37** (1998) 1376.
535. R. S. Phillips, B. Sundararaju, S. V. Koushik, *Biochemistry* **37** (1998) 8783.
536. D. R. Dalton, The Alkaloids, Marcel Dekker, New York, 1979, 145; K.-M. Oksman-Caldentey, *Phytochemistry* **68** (2007) 2773.
537. I. T. Baldwin, *J. Chem. Ecol*. **15** (1989) 1661.
538. I. T. Baldwin, E. A. Schmelz, T. E. Ohnmeiss, *J. Chem. Ecol*. **20** (1994) 2139.
539. M. N. Romanelli, P. Gratteri, L. Guandalini, E. Martini, C. Bonaccini, F. Gualtieri, *Chem Med Chem* (2007) 746.
540. T. S. Tso, Tobacco, Ullmann Encyclopaedia, Wiley-VCH, Weinheim, 2005, Electronic Release.
541. F. F. Wagner, D. L. Comins, *Tetrahedron* **63** (2007) 8065.
542. H.-J. Eberhardt, *Beiträge Tabakforschung Int*. **16** (1995) 119.
543. B. Vickers, M. Fitzgerald, S. Anderson, M. Turnbull, University of Minnesota, 2008, http://umbbd.msi.umn.edu/nic/nic_map.html; R. Brandsch, *Appl. Microbiol. Biotechnol.* **69** (2006) 493; C.-B. Chiribau, M. Mihasan, P. Ganas, G. L. Igloi, V. Artenie, R. Brandsch, *FEBS* **273** (2006) 1528.
544. R. B. Silverman, Medizinische Chemie, VCH, Weinheim, 1995, 314; N. L. Benowitz, *Ann. Rev. Pharmacol. Toxicol.* **36** (1996) 597; J. Hukkanen, P. Jacob III, N. L. Benowitz, *Pharm. Rev.* **57** (2005) 79.
545. L. F. Stead, J. R. Hughes, Cochrane Database Syst. Rev. 2012;2:CD000124; http://de.wikipedia.org/wiki/Lobelin.
546. M. R. Lee, *J. R. Coll. Physicians Edinburgh* **35** (2005) 83.
547. J. W. Daly, H. M. Garraffo, T. F. Spande, M. W. Decker, J. P. Sullivan, M. Williams, *Nat. Prod. Rep.* **17** (2000) 131; H. M. Garraffo, T. F. Spande, M. Williams, *Heterocycles* **79** (2009) 207; D. L. J. Bilbey, H. Salem, M. H. Grossman, *Brit. J. Pharmacol.* **15** (1960) 540.
548. T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell, J. W. Daly, *J. Am. Chem. Soc.* **114** (1992) 3475.
549. B. Badio, J. W. Daly, *Mol. Pharmacol*. **45** (1994) 563.
550. G. Bejeuhr, *Pharmazie in unserer Zeit* **23** (1994) 105.
551. C. E. Müller, *Pharmazie in unserer Zeit* **25** (1996) 85.
552. A. Husemann, W. Marmé, *Z. Chem*. (1865) 161.
553. J. P. Devlin, O. E. Edwards, P. R. Gorham, N. R. Hunter, R. K. Pike, B. Stavric, *Can. J. Chem*. **55** (1977) 1367.
554. S. Wonnacott, T. Gallapher, *Marine Drugs* (2006) 228.
555. C. S. Huber, *Acta Cryst*. **B28** (1972) 2577.
556. M. G. Weller, *Nachrichten aus der Chemie* **50** (2002) 700.

557. G. Francis, *Nature* (1878) 11; http://www.epa.gov/cyano_habs_symposium/monograph/Ch28.pdf.
558. W. Schunack, *Pharmazie in unserer Zeit* **37** (2008) 494.
559. J. W. Coe, P. R. Brooks, M. G. Vetelino, M. C. Wirtz, E. P. Arnold, J. Huang, S. B. Sands, T. I. Davis, L. A. Lebel, C. B. Fox, A. Shrikhande, J. H. Heym, E. Schaeffer, H. Rollema, Y. Lu, R. S. Mansbach, L. K. Chambers, C. C. Rovetti, D. W. Schulz, F. D. Tingley, B. T. O'Neill, *J. Med. Chem.* **48** (2005) 3474; P. Rucktooa, C. A. Haseler, R. van Elk, A. B. Smit, T. Gallagher, T. K. Sixma, *J. Biol. Chem.* **287** (2012) 23283; B. Billen, R. Spurny, M. Brams, R. van Elk, S. Valera-Kummer, J. L. Yakel, T. Voets, D. Bertrand, A. B. Smit, C. Ulens, *PNAS* **109** (2012) 9173; X. Da Silva Tavares, A. P. Blum, D. T. Nakamura, N. L. Puskar, J. A. P. Shanata, H. A. Lester, D. A. Dougherty, *J. Am. Chem. Soc.* **134** (2012) 11474; J. B. McClure, G. E. Swan, L. Jack, S. L. Catz, S. M. Zbikowski, T. A. McAfee, M. Deprey, J. Richards, H. Javitz, J. Gen. Intern Med. **24** (2009) 563; R. L. Papke, C. Trocmé-Thibierge, D. Guendisch, S. A. A. Al Rubaiy, S. A. Bloom, *J. Pharm. Exp. Ther.* **337** (2011) 367.
560. W. B. Edwards III, R. McCuen, *J. Org. Chem.* **48** (1983) 2484.
561. S. Girard, R. J. Robins, J. Villiéras, J. Lebreton, *Tetrahedron Lett.* **41** (2000) 9245.
562. C. Welter, R. M. Moreno, S. Streiff, G. Helmchen, *Org. Biomol. Chem*. **3** (2005) 3266.
563. E. V. Dehmlow, *J. prakt. Chem*. **337** (1995) 167.
564. B. M. Trost, G. R. Cook, *Tetrahedron Lett.* **37** (1996) 7485.
565. S. C. Clayton, A. C. Regan, *Tetrahedron Lett*. **34** (1993) 7493.
566. J. C. Namyslo, D. E. Kaufmann, *Synlett* (1999) 804.
567. R. Prasad, H. J. Grubbs, US 5497792 (1996).
568. J. Wadsworth Coe, P. R. Palmer Brooks, US 7144882 (2006).
569. J. Wadsworth Coe, P. R. Palmer Brooks, US 6410550 (2002).

6 | Hormones

Hormones are endogenous substances, which are produced by internal secretory cells [1, 2]. Through the interstitial fluid and the bloodstream they reach in small amounts their target cells/organs, where the interaction with specific receptors triggers their physiological effects. The effective concentrations range between 10^{-10} and 10^{-12} mole/l. Hormones are differentiated into three classes, corresponding to the distance they travel to their target:

1. Autocrine hormones affect the function of the cell they are secreted from.
2. Paracrine hormones act on neighbouring cells.
3. Endocrine hormones are produced by endocrine glands and specialised neurosecretory cells of the hypothalamus. They are released into the bloodstream and transported to remote sites in the body to exert their activity (Fig. 6.1).



6.1 *The endocrine glands in humans (Hermes and the Infant Dionysos, traditionally attributed to Praxiteles, 4th century BC, Archaeological Museum of Olympia).*

B. Schaefer, *Natural Products in the Chemical Industry*,
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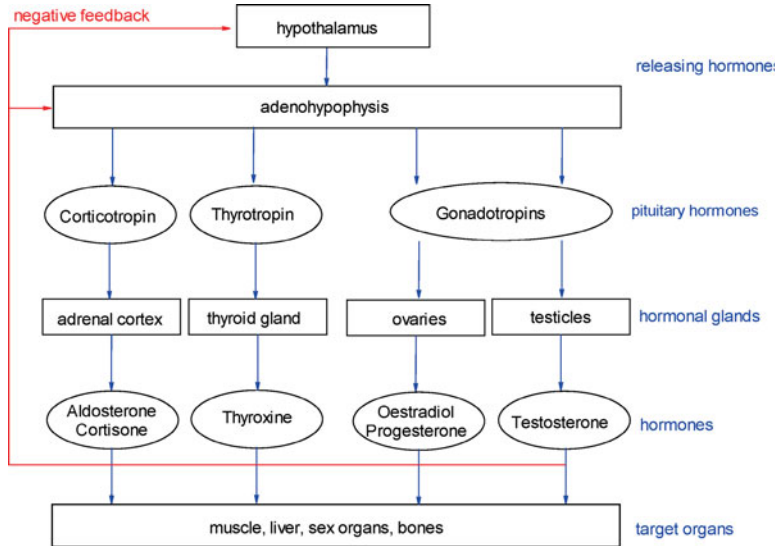
The word "hormone" is derived from Greek, where it means "to set in motion" or "to rush". Ernest Henry Starling (1866–1927) introduced the term in 1905. From a chemical viewpoint, there are four structurally distinct classes of hormones known:

- Amines
- Prostaglandins
- Steroids
- Peptides, proteins

The hormonal system is closely related to the nervous system. Adrenaline, for example, is not only a hormone but also a neurotransmitter in the synaptic cleft of nerve cells. Both systems serve the coordination of bodily functions and are centrally connected *via* the hypothalamus (Fig. 6.2).



6.2 *Hormonal regulation of bodily functions.*

The hypothalamus produces the releasing-hormones, which lead to the release of pituitary hormones in the anterior lobe of the pituitary gland (adenohypophysis). For their part, these stimulate the further secretion of hormones by particular hormonal glands, which in turn trigger the effects on the organs they target. This system is regulated by a negative feedback mechanism, in which the increased hormone level act on the adenohypophysis and the hypothalamus by inhibiting further release of the pituitary hormones.

The endocrine system in humans secretes a broad spectrum of hormones, with the objective of homoeostasis, the response to external stimuli and the modulation of regulatory cycles and development programmes. Insulin and glucagon, for example, ensure that the blood sugar level remains roughly constant after a sumptuous meal or during a period of fasting. Adrenaline and

noradrenaline put us in the position to either flee or fight in case of danger; and the sex hormones control the anlage and maturing of the sex organs, the menstrual cycle and pregnancy.

Summary in Bullet Points

- Hormones are endogenous substances with important physiological functions.
- They control, comparable to the nervous system, the coordination of bodily functions.
- They act at very low concentrations in the range of 10^{-10} to 10^{-12} mole/l.

6.1 Steroids and Hormonal Contraceptives

In 1790, the Venetian monk Gianmaria Ortis came to the conclusion that population growth cannot continue indefinitely. Eight years later, Thomas Robert Malthus (1766–1834) wrote an essay on the *Principle of Population*. There he raised the issue that along with geometric population growth the consumption of resources increases only arithmetically. Being an Anglican cleric, he recommended premarital chastity, late marriage and sexual abstinence. Malthus's work prompted many questions, which eventually inspired Charles Darwin (1809–1882) to develop the theory of biological evolution. Despite associated concerns, the awareness of population growth remained right up to 1960 merely of theoretical interest. Nowadays however, seeing the numbers grow by leaps and bounds is perceived as an existential threat. Thus, efficient control of population-growth poses one of the toughest challenges for future well-being of mankind.

The technical means of contraception, which are now at our disposal, are ample and well-developed:

- Periodic abstinence method (Knaus-Ogino)
- Prolonged lactation
- Physical barriers
- Chemical vaginal contraceptives
- Intra-uterine devices
- Hormonal contraceptives
- Sterilisation
- Post-coital contraception

However, they differ strongly in their acceptance and reliability. Moreover, there are moral and religious concerns, which impact the social discussion. The following chapter is focused on the "chemical aspects" of contraception. [3]

6.1.1 Historic Methods of Contraception

The first hint on vaginal contraception can be found in 3,550-year-old Egyptian papyri (collected in *Ebers Papyrus* about Egyptian medical texts of 1550 BC [4]). Tampons from acacia leaves were soaked with honey and inserted into the vagina. Thereby contact of sperm with the cervical os was effectively prevented.

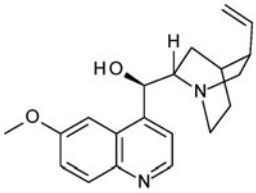
Pliny the Elder, Pedanius Dioskurides (*De materia medica*, 77 AD) and a little later, the most important gynaecologist of ancient times, Soranus of Ephesus (*On Midwifery and the Diseases of Women*, 100 AD), already wrote about contraception and abortion. Reference thereof can also be found in the thriving Arabic medicine of the 10th century, as, for example, in works by Muhammad ibn Zakariyā Rāzī (Rhazes, *Quintessence of Experience*), Ali ibn al-'Abbas al-Majusi (Haly Abbas, *Royal Book*) and Abū Alī al-Husain ibn Abdullāh ibn Sīnā (Avicenna, *Canon of Medicine*).

According to Augustine (354–430), the sexual act serves solely the conception of children and is not meant to be enjoyed for its own sake. In the Middle Ages, *Coitus interruptus* was practised, though birth control supposedly did not play a major role, given a high infant mortality and low life expectancy (Fig. 6.3). Since around the 15th century, the use of condoms is known, the first tractable description of which appeared in *De Morbo Gallico* (The French Disease). It originates from the Italian anatomist Gabriel Fallopius (1523–1562) and was published posthumous in 1564. Condoms, fabricated from fish bladders, animal

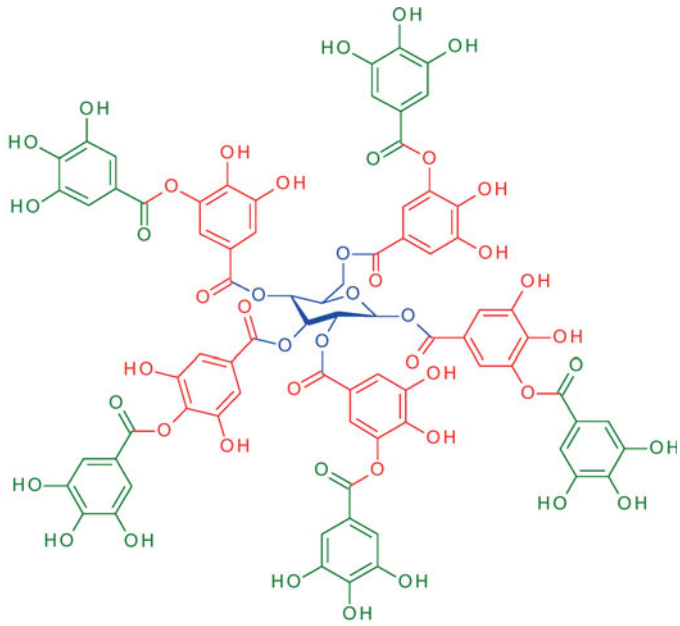
6.3 *In the Protestant Cyriakus church in Bönnigheim, Germany, a late Gothic painted wooden panel from 1508 commemorates Barbara Schmotzerin (ca. 1448–1503), whose 35-year marriage to Adam Stratzmann produced 53 children: 38 sons are grouped below along with their father on the left, and 15 daughters are portrayed along with their mother on the right. In 29 pregnancies she gave birth to 18 single children, also five pairs of twins, four times triplets and even once sextuplets and septuplets each. Contrary to the representation in the painting, all of the children died before their parents, while 19 had been stillborn.*

intestines, linen or silk, were not so much applied to prevent a pregnancy but rather to protect from sexually transmitted diseases (*e.g.* syphilis). A while after Charles Goodyear (1800–1860) had discovered the vulcanisation process for rubber in 1839, a number of new applications – beyond tyres – emerged, amongst them also reusable condoms.

The first writings on systematic investigations of contraception are to be found at the beginning of the 19th century. Already in 1855, Albert von Kölliker (1817–1905), a Swiss physiologist, explored the influence of chemicals on the mobility of sperm. The first commercial vaginal contraceptive, a suppository of cocoa butter containing quinine sulfate as a spermicide, was developed and manufactured by the London pharmacist Walter Rendell in the 1880s. Other preparations with boric acid, tannic acid or mercuric chloride followed. [4]



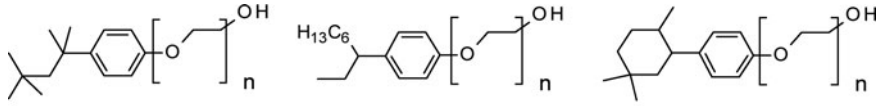
Quinine



Tannic acid
(Decagalloylglucose)

Adam Raciborski (1809–1871), a Polish physician in Paris, was the first to publish a statistical analysis on the periodicity of the menstrual cycle. Hermann Knaus (1929) and Kyusaku Ogino (1930) proved, independently of each other, that ovulation occurs 14 days prior to the next menstruation, on which the Knaus-Ogino calendar method of contraception is based.

In the 1940s, at the Ortho research laboratories, the first spermicidal surfactants (octoxynol (*Triton X-100*®) and nonoxynol-9 (*Triton N*®)) were discovered and developed. Both of these substances became very quickly the leading spermicidal agents worldwide. Another product, a foaming tablet containing menfegol from the Eisai company, followed in the late 1960s, but was introduced only in Europe.



Triton X-100 Triton N Menfegol

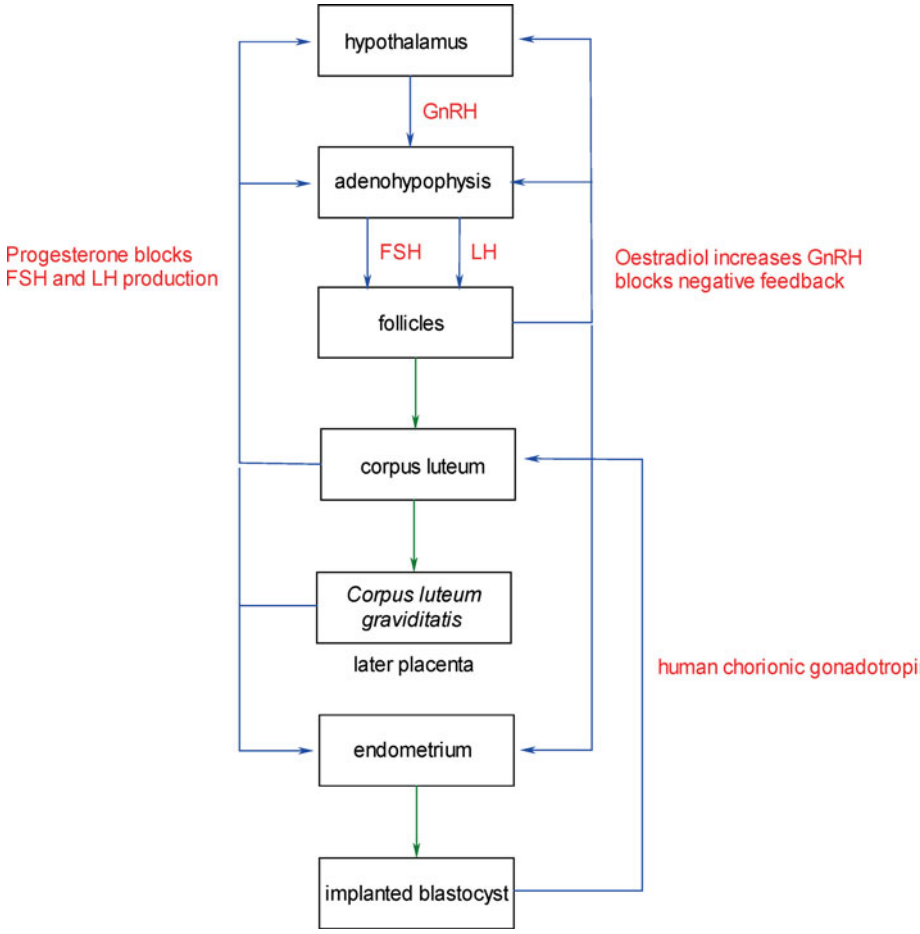
The most fundamental societal change remained however reserved to the discovery of the "pill". In times of economic awakening, student upheaval, women's emancipation movement, hippie culture, liberalisation in the Roman Catholic Church (in the wake of the 2nd Vatican Council, 1962–1965) and sexual laxity, the first hormonal oral contraceptives came on the market. In 1964, 2 % of the women of child-bearing age took ovulation inhibitors; by 1968, this number had already increased to 12 %, and by 1986, to more than 35 %. Nowadays, the "pill" is ranked as one of the safest contraceptives. Not only the active ingredients were improved, but also their dosages and dosing schedules. Through the experience over the last 30 years with many millions of women on the "pill", the associated risks and side-effects are now well-known.

6.1.2 Biological Essentials

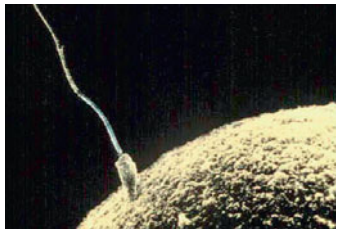
The hypothalamus [5], the adenohypophysis, the ovaries and the uterus control the monthly cycle of a sexually mature woman (Fig. 6.4). The hypothalamus produces the gonadotropin-releasing hormone (GnRH, pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2). This leads to the situation that the adenohypophysis (anterior pituitary gland) releases the gonadotropins FSH (follicle-stimulating hormone, follitropin-alpha) and LH (luteinising hormone, lutropin). The gonadotropins are glycoproteins with a molecular weight of 20,000–50,000 g/mole. Under the influence of FSH, a cohort of follicles matures: these increasingly produce oestrogens, especially oestradiol, which inhibit the negative feedback corresponding to their concentration. This means that with oestrogen levels rising, the hypothalamus secretes also more GnRH, which leads in turn to an increased release of LH and FSH. Under the influence of oestrogen, the frequency of the pulsating release of GnRH during the first half of the cycle is around 90 minutes, and during the second half of the cycle, under the influence of gestagens, between 3–4 hours. This rhythm is of crucial importance for a woman's fertility. Continuous supply of GnRH or alteration of the frequency leads to a reduced release of FSH and LH.

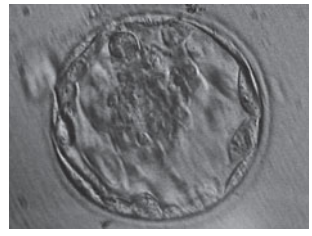
In the male, follitropin stimulates spermatogenesis in the seminiferous tubules. Lutropin triggers testosterone synthesis and release in the testicular tissue. The term *gestagens* (gestational hormones) refers to progesterone and a series of synthetic drugs, which possess similar properties.

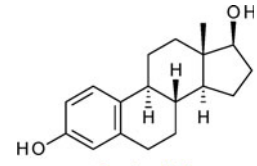
During the so-called proliferation phase, oestrogen induces the formation of the uterine mucous membrane and the liquefaction of the mucous membrane at the neck of the uterus. At the middle of the cycle, the LH-concentration surges and leads to ovulation: *i.e.* the rupture of a mature follicle and the discharge of an ovum (oocyte). The ruptured follicle transforms into a new tissue, called the *Corpus luteum* (Latin for "yellow body"), which synthesises and releases progesterone. In the secretory phase, progesterone causes the conversion of the uterine mucous membrane into a pregravid stage and prepares for the implantation of the fertilised egg. Under the influence of progesterone, the cervical mucus once again becomes more viscous.



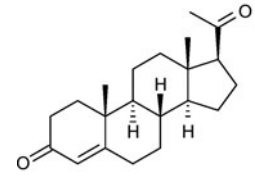
6.4 *Hormonal regulation of female sexual functions and two images showing a sperm cell fertilizing an egg cell and a human blastocyst five days after conception.*







Oestradiol



Progesterone

The decreasing oestradiol level enhances a negative feedback. The FSH- and LH-production is inhibited by progesterone and their concentrations return to the initial levels. This ensures that during the second half of the cycle and likewise during pregnancy another ovulation and conception does not occur.

If the egg is fertilised and has been implanted, the *Corpus luteum* – stimulated by the chorionic-gonadotropin (CG), a hormone of the uterine mucous membrane – develops into the *Corpus luteum graviditatis* and raises the production of progesterone, which is subsequently taken over by the placenta. For pregnancy tests, nowadays immunoassays are used, which allow the detection of CG in the blood stream or urine already within a few days after fertilisation.

In case the egg is not fertilised, the *Corpus luteum* stops progesterone production, causing its degeneration to the *Corpus albicans* and the retraction of the coiled arteries in the uterine mucous membrane. Falling levels of progesterone trigger menstrual bleeding and the beginning of the next cycle.

Most hormonal contraceptives contain progesterone derivatives, which prevent mid-cycle release of the maximal amount of FSH and LH, and thereby ovulation, but consequently simulate a mock pregnancy. [6] Birth control pills can be devided into several groups:

- **Monophasic drugs:** This single-phase method combines a constant dose of oestrogen/gestagen combination to be administered over 21 days. Three to four days after discontinuation, a hormone-withdrawal bleeding starts, which is comparable to menstrual bleeding. Due to the anti-gonadotropic effect of the steroid hormone, ovulation is suppressed. Even if ovulation takes place, the egg cannot be implanted on account of an incomplete transformation of the uterine mucous membrane. In addition, the hormones increase the viscosity of the cervical mucus, and thereby inhibit sperm penetration.
- **Two- or three-phase medications:** This method is more in line with the natural menstrual cycle. Starting initially with a low combined dosage of oestrogen and gestagen, during the middle and towards the end of the cycle, the gestagen dose is increased to achieve likewise an inhibition of the ovulation. The success rate is comparable with the monophasic procedure.
- **Mini-pills:** These medications contain a low dose of gestagens. These drugs are recommended to women at late reproductive age or when combined medications are contraindicated. They merely raise the viscosity of the cervical mucus, and their reliability is lower than for the aforementioned methods.
- **Post-coital contraception:** These emergency hormonal contraception (EHC) drugs contain high doses of oestrogens and/or gestagens as previously discussed. They are used after unprotected sexual intercourse or contraceptive failure and may be accompanied by higher rates of undesirable side effects. They should not be confused with the "abortion-pill" mifepristone (RU486, *Mifeprex*®).

6.1.3 Natural Steroids and Steroid Hormones

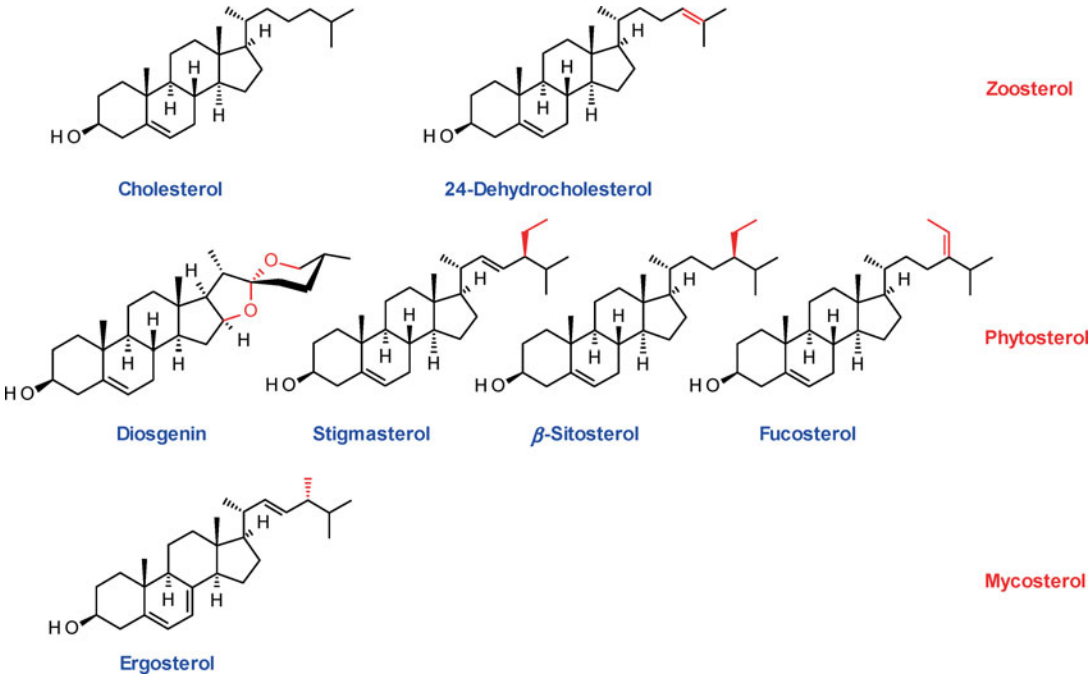
Natural Steroids

Cholesterol (*Greek*: solid bile) is the most important steroid of vertebrate animals (the "zoosterols"). It is a central intermediate in the biosynthesis of steroids.

Isolated for the first time in 1775 from gallstones by the physician Benjamin Gottlob Friedrich Conradi (Jena, Germany), cholesterol occurs everywhere in the human body. With high levels especially in the brain, the spinal cord and the suprarenal glands, the total amount of cholesterol in an adult adds up to some 240 grams. Invertebrates, on the other hand, synthesise in addition 24-dehydrocholesterol in considerable quantity.

Plants produce cholesterol derivatives (phytosterols), the side-chains of which form a spiroketal, as in diosgenin, or else bear an additional ethyl group, as in the stigmastane series. Stigmasterol was obtained for the first time in pure form by Adolf Otto Reinhold Windaus (1876–1959) from the oil of Calabar beans (*Physostigma venenosum*). Widely distributed are the sitosterols, which were originally isolated from grain (*sitos*). The main representative is β-sitosterol. Fucosterol was isolated from algae.

In most fungi, lichens and algae, ergosterol (mycosterol) occurs. This was isolated from ergot fungus (most prominent member: *Claviceps purpurea*) in 1889 by the Parisian apothecary Charles Tanret (1847–1917). It is the main sterol in baker's yeast (*Saccharomyces cerevisiae*), and acts as provitamin D (Fig. 6.5). [7]



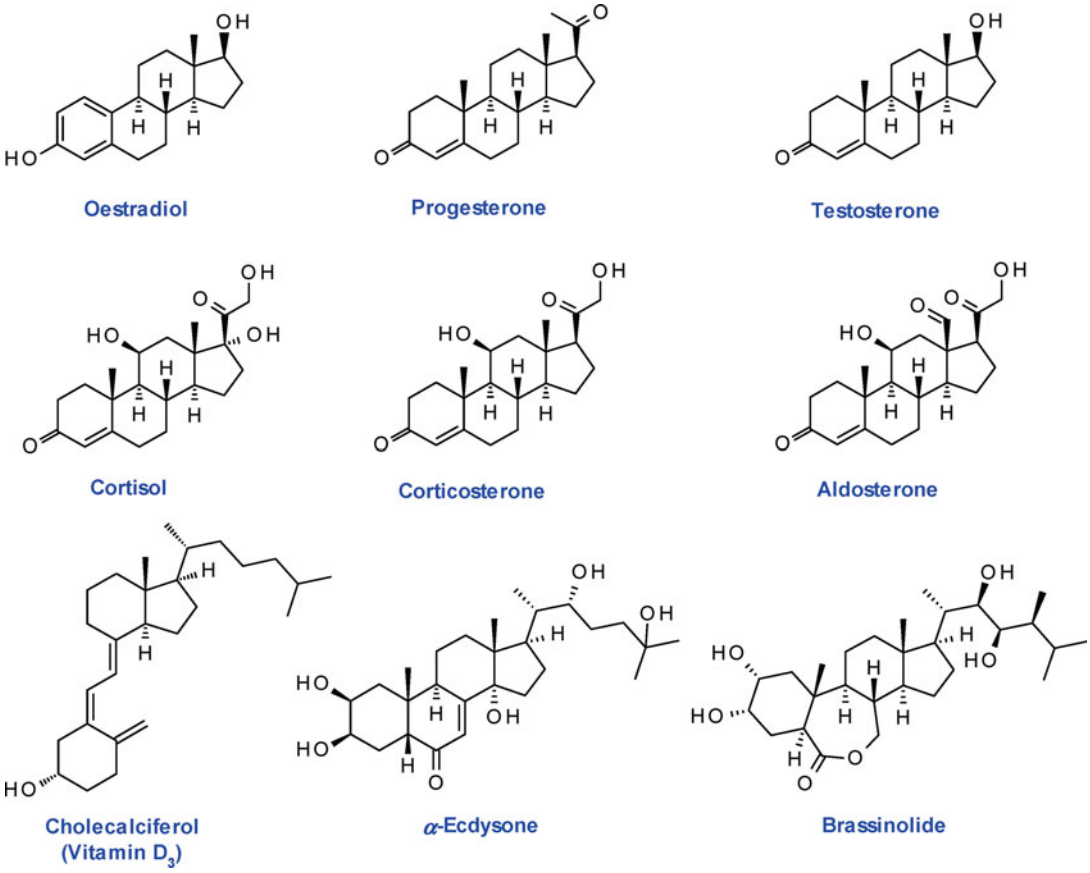
6.5 *Examples of steroids of animals, plants and fungi.*

An important raw material for the partial synthesis of steroid hormones (and Vitamin D) is cholesterol, which (prior to the BSE crisis) was isolated from the spinal cord of cattle. Another important source is the fat in sheep's wool (lanolin), which contains around 15 % of cholesterol. Among the plant sterols, stigmasterol is of great economic significance as the starting material for the partial synthesis of steroids. It is contained from 12 to 25 % in the non-hydrolysable

fraction of soybean oil, and is also found in sugar-cane wax. Sitosterol, likewise another important raw material, occurs, besides in soybean oil, also in tall oil, a byproduct of the paper industry. Diosgenin, found in various *Liliaceae* and *Dioscoreaceae* species, is relevant for partial syntheses as well.

Steroid Hormones

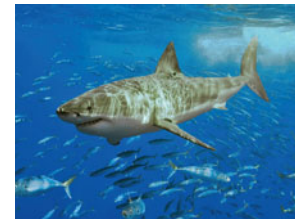
Steroid hormones play a critical role in a whole range of vital processes like sexuality, reproduction and stress (Fig. 6.6). They can be differentiated into four physiological categories:



6.6 *Steroid hormones.*

- **Sex hormones** are, according to their physiological activity, subdivided into oestrogens, gestagens and androgens. While oestrogens and gestagens control a woman's menstrual cycle, the androgen testosterone promotes the development of secondary sexual characteristics in men, raises the libido, reduces the release of gonadotropins, impacts the mental behaviour of a man, and increase *e.g.* protein synthesis in skeletal muscles (anabolic action). A range of other species shares the same sex hormones as humans.

- **Adrenal steroids** are produced in the adrenal cortex, and they can be divided into two subsets: glucocorticoids and mineralocorticoids. The glucocorticoids (cortisol, corticosterone) promote the formation of glycogen in the liver and the gluconeogenesis from proteins. Both raise blood sugar levels. Mineralocorticoids (aldosterone) control the electrolyte and water balance.
- **Calcium-regulating sterols** control the resorption of calcium ions into the gastro-intestinal tract, and influence bone metabolism. Vitamin D_3 (cholecalciferol), generated by photolysis of 7-dehydrocholesterol, is its most prominent member.
- **Insect skin-shedding hormones** regulate the metamorphosis from the larva via the pupa into the mature insect. In 1954, the first moulting hormone, α-ecdysone, was isolated from the silkworm *Bombyx mori* and its X-ray structure solved. In the course of time, a large number of other moulting steroid hormones was discovered, among them those also occurring in plants (phytoecdysone), like brassinolide from rapeseed (*Brassica napus*) pollen.

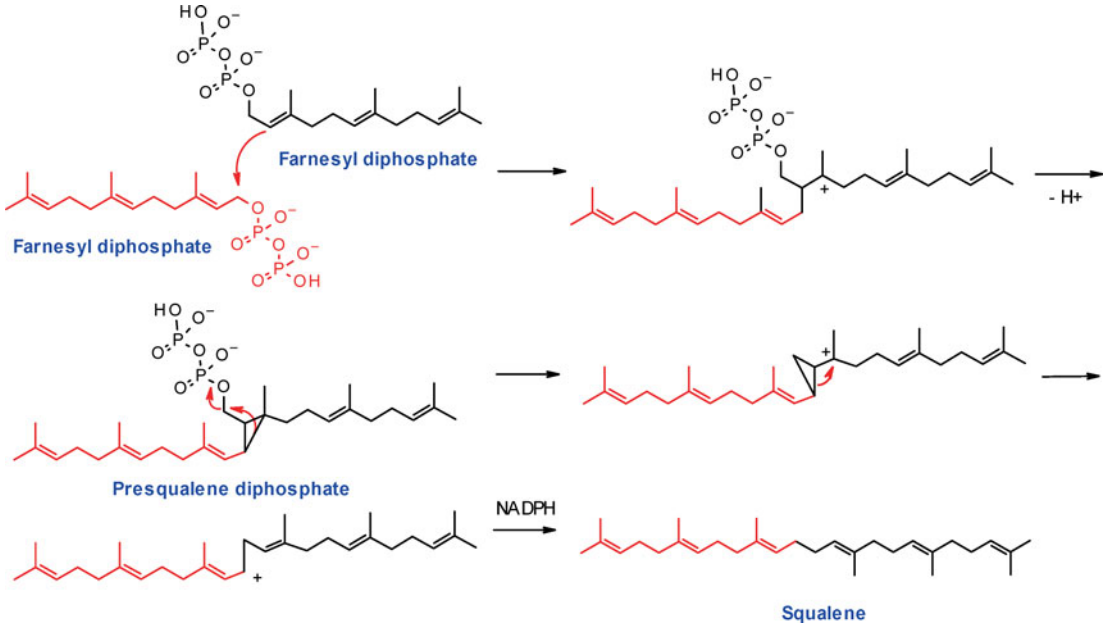


6.7 *Squalene is a hydrocarbon, which was first isolated in 1906 by the Japanese chemist Mitsumaru Tsujimoto from shark liver oil (Latin: squalus, shark). Later, small amounts of squalene were discovered in mammals, in ergot and in olive oil as well.*

6.1.4 Biosynthesis

The biosynthesis of steroids proceeds *via* farnesyl diphosphate and squalene (Fig. 6.7). It is fascinating that Nature applies here the same principles as they are also found in the biosynthesis of carotenoids and pyrethroids.

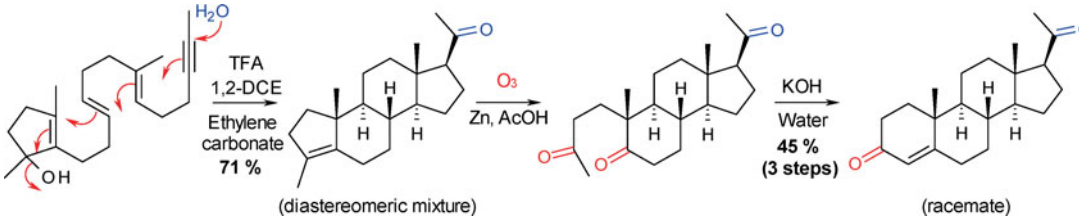
The tail-to-tail coupling of two farnesyl diphosphate molecules leads *via* a cyclopropane intermediate to squalene. The primary cyclopropane derivative, presqualene diphosphate, cleaves off the diphosphate residue, and the resulting cyclopropylmethyl carbocation opens the ring again. The allyl cation is reduced by NADPH to squalene.



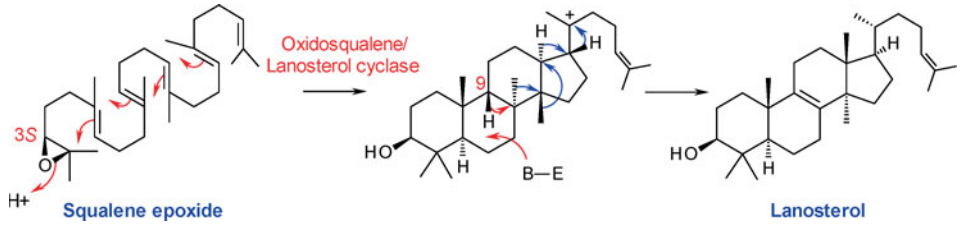
In the middle of the 20th century, Konrad Bloch (1912–2000) at Harvard and Leopold Ružička at the ETH in Zurich were able to show that the enzymatic epoxidation of a terminal double bond precedes the cyclisation of squalene.

Surprisingly, Eugene Earle van Tamelen (1925–2009) could demonstrate that the terminal functionalisation with hypochlorous acid in a water/THF solvent mixture succeeds also without an enzyme being involved. The reason lies probably in the folding of the molecule. The highly lipophilic chain coils itself up so that only the double bond on the surface is exposed to epoxidation.

A pioneering step in synthetic polyene cyclisation for the preparation of steroids was the synthesis of racemic progesterone in a few stages and with excellent yields by William S. Johnson (1913–1995) in 1971. [8] The first diastereoselective polyene cyclisation, starting from an enantiomerically pure epoxide, originates from Elias J. Corey (*cf.* section 3.7.5 – Polyene cyclisations). [9]

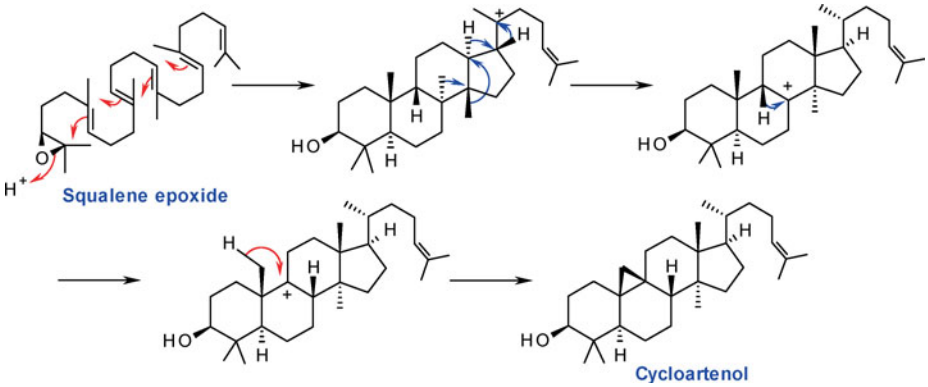


The epoxidation is followed by a proton-induced cyclisation reaction and Wagner-Meerwein rearrangements, which are among the most fascinating in biochemistry. The proteolytic epoxide ring-opening by oxidosqualene/lanosterol cyclase, which occurs only with the (3*S*)-enantiomer, produces under stereocontrol first a tetracycle. Subsequently, induced by deprotonation at C-9 and after a cascade of Wagner-Meerwein rearrangements, lanosterol, the principal component of the non-hydrolysable portion of wool fat, is formed. [10] This is the key building block to all animal steroids.



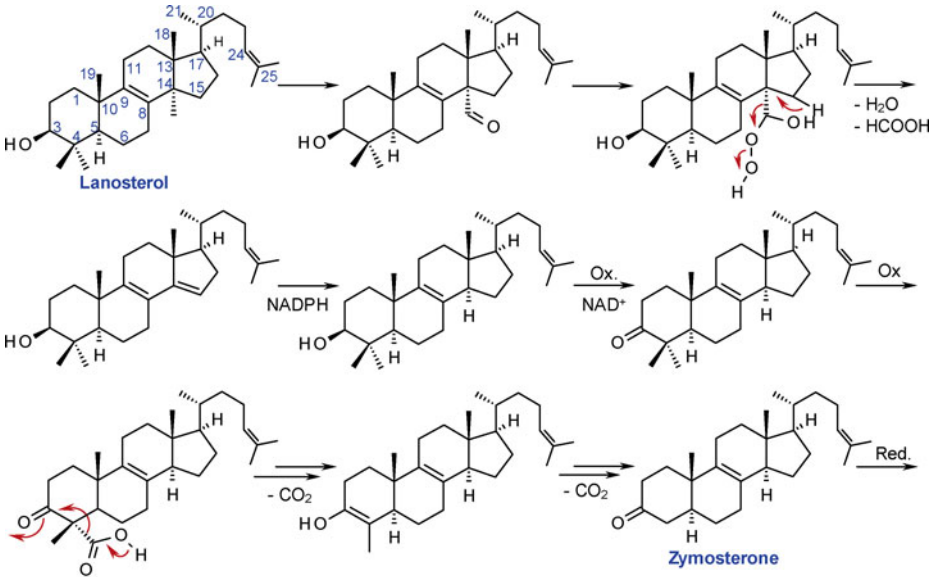
In plants, the rearrangements take a somewhat different course and lead to cycloartenol. It is plausible that the Wagner-Meerwein rearrangements then proceed stepwise, *via* the corresponding alkenes or alcohols, since these are also found. [11]

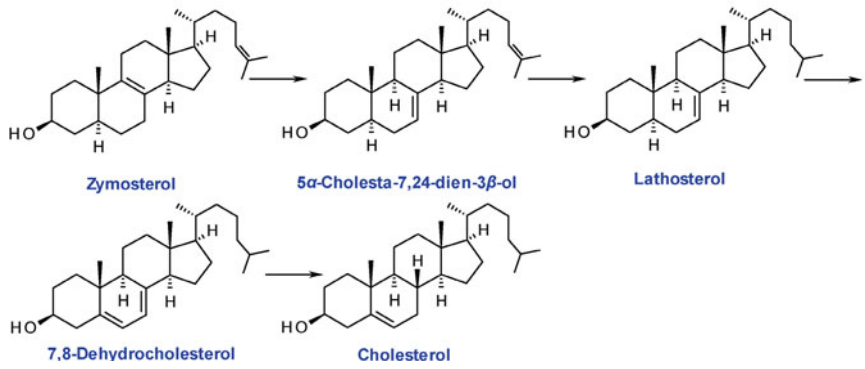
Cholesterol represents the next "milestone" in the biosynthesis of the sex steroids. En route from lanosterol to cholesterol, the methyl groups at C-4 and C-14 have to be removed, the double bond is to migrate from C-8 to C-5, and the $\Delta^{24,25}$-double bond must be reduced.

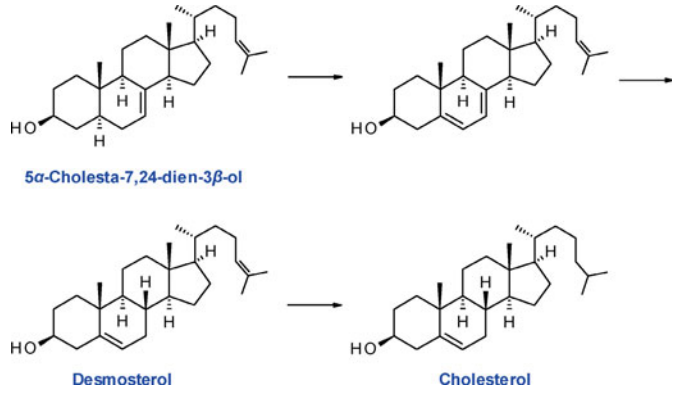


The methyl group at C-14 is removed first, as can be observed in ^{13}C-NMR studies. Formally, hydrogen peroxide is added at the aldehyde stage, and formic acid is eliminated. Afterwards, the resulting diene is partially reduced with NADPH/H$^+$. The removal of both methyl groups at C-4 is more laborious. First of all, the hydroxy-group at C-3 is oxidised: the chirality is thereby lost. Then, the α-methyl group (pointing below the drawing plane) is oxidised to a carboxylic acid, and carbon dioxide is cleaved off. The responsible enzyme system operates in a highly selective mode. As model reactions show, no residues other than methyl are tolerated. Since the required stereochemistry is strictly defined, prior to repeating this cycle, the newly created stereocentre must be epimerised. After the second methyl group is oxidised and removed, the keto-group at C-3 is reduced again under asymmetric induction to the previous configuration.

The formal shift of the C-8-double bond to C-5 proceeds stepwise by means of an isomerase and the 5α,6α-dehydrogenase. The double bonds at C-7 and C-24 are reduced in no specific order. Important intermediates are lathosterol, 7,8-dehydrocholesterol and desmosterol.



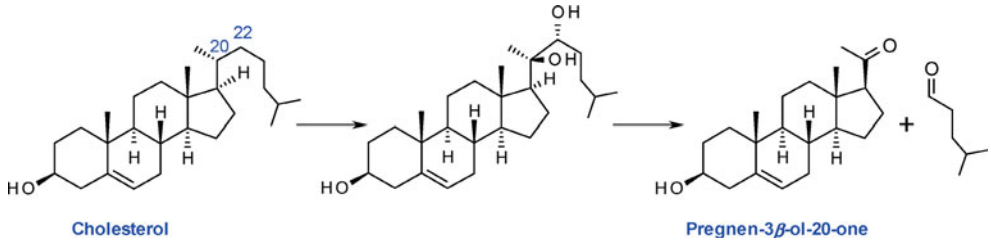




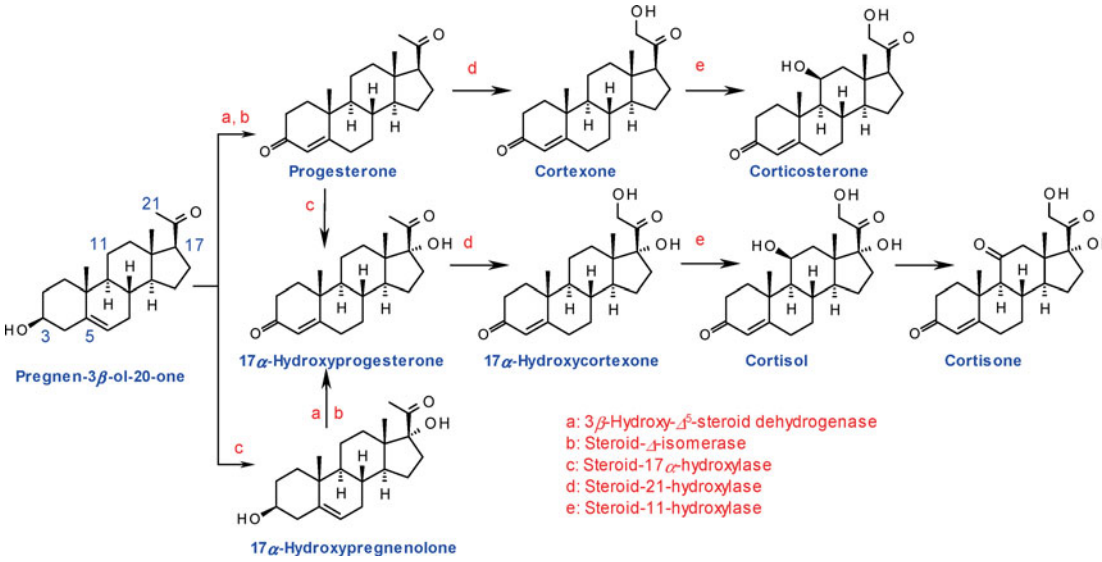
While starting from squalene, the ring-closure and the following Wagner-Meerwein rearrangements appear elegant, but the rest of the synthesis to its final product cholesterol is long-winded and cumbersome. In that respect, biosynthesis does not look essentially different from many natural product syntheses in our laboratories. Although, individual reaction steps are hard to outperform in terms of selectivity, the impression prevails that Nature aims at its objective without a lot of ingenuity, but tends to rely mostly on its repertoire of proven methods and reliable intermediates. The same reaction types are employed again and again – albeit in a virtuoso manner.

Cholesterol is the biochemical precursor for both, the adrenocorticosteroid hormones cortexone and cortisol, and the sex hormones testosterone and oestradiol. Cholesterol is first hydroxylated at C-20 and C-22. The next tangible intermediate is pregnen-3β-ol-20-one (Latin: *praegnatio*: pregnancy). After oxidation and a retro-acyloin reaction, the rest of the molecule is formally cleaved off as isocaproic aldehyde (4-methylpentanal).

In the next reaction steps, the enzymes 3β-hydroxy-Δ^5-steroid dehydrogenase, steroid Δ-isomerase and steroid 17α-hydroxylase are involved. Oxidation at C-3 and double bond migration leads to progesterone, the hormone of the *corpus luteum*. Afterwards, the C-17 position is hydroxylated to give 17α-hydroxyprogesterone. The reverse reaction sequence is also known and



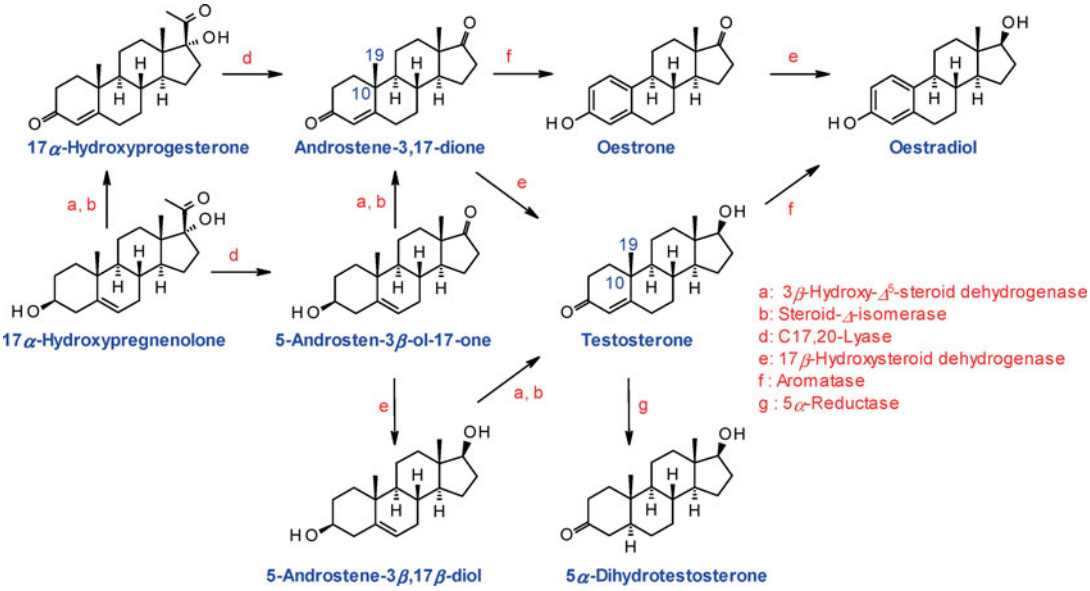
proceeds by way of 17α-hydroxypregnenolone. Starting from progesterone, hydroxylation at C-21 and C-11 gives rise to corticosterone, while starting from 17α-hydroxyprogesterone, cortisol is generated, which can be oxidised at C-11 to cortisone. The hydroxylation occurs with O_2 and NADPH + H^+. All the three enzymes, steroid-11-hydroxylase, steroid-17α-hydroxylase and steroid-21-hydroxylase act highly regio- and stereo-selective, which is in part accompanied by very precise substrate recognition. Therefore, steroids hydroxylated at C-21 are not further hydroxylated by the 17α-hydroxylase. That is the reason why, for example, cortisol is not accessible from corticosterone.



17α-Hydroxyprogesterone and 17α-hydroxypregnenolone are degraded by C17,20-lyase to C_{19}-steroids (retro-acyloin reaction). 17β-Hydroxysteroid dehydrogenase reduces C-17 in 5-androsten-3β-ol-17-one and androstene-3,17-dione to 5-androstene-3β,17β-diol and testosterone respectively. Demethylation of testosterone at C-10 leads, *via* aromatisation of the A-ring, to oestradiol, which is also accessible from androstene-3,17-dione in the reverse sequence *via* oestrone.

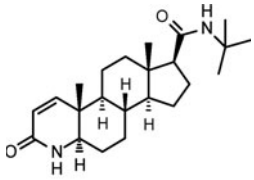
6.8 *The Court dwarf Don Sebastian de Morra, by Diego Rodriguez de Silva y Velazquez (1599–1660), ca. 1645, National Museum El Prado, Madrid.*

In case of a hereditary occurring steroid-21-hydroxylase deficiency, the synthesis of all the corticoid hormones is down regulated, and in consequence of the normal feedback mechanism, adrenocorticotropic hormone levels are boosted; this leads to an adrenal hyperplasia with an overproduction of pregnen-3β-ol-20-one and androgens. The most striking clinical effect of this congenital disorder in girls is the virilisation immediately after birth. In boys, precocious puberty is initiated a few months after birth. The hereby accelerated bone maturation results as a consequence in dwarfism (Fig. 6.8). [12]



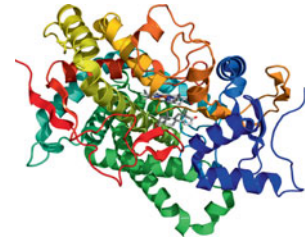
Excursus: Hair loss

Every second man observes during his life the thinning of his hair. Key factors for androgenetic alopecia (hair loss) are genetic predisposition, androgen level, and age. Over the years, the hair follicles become sensitive towards androgens. Already Aristotle reported that eunuchs do not become bald. However, it is not testosterone itself which causes hair loss. This was at least the conclusion of a discovery in 1974 with pseudohermaphrodites ("Guevedoces") from remote villages in the Dominican Republic, who suffered from a genetically-caused 5α-reductase deficiency. Thus, the culprit is instead 5α-dihydrotestosterone, which equally causes a benign enlargement of the prostate at an older age. Men with androgenic hair loss have significantly higher 5α-reductase activity in the affected region of the skin. Inhibition of the 5α-reductase type 2 is therefore conceptually a promising approach to both disorders. Merck Sharp & Dohme originally developed finasteride (*Proscar*®, 5 mg tablet) as a 5α-reductase inhibitor for the indication of benign prostate hyperplasia; the drug was later approved as well for the indication of androgenetic alopecia (*Propecia*®, 1 mg dosages), which has now become economically even more important, as reflected by the annual sales figures. [13]



Finasteride (Propecia (R))

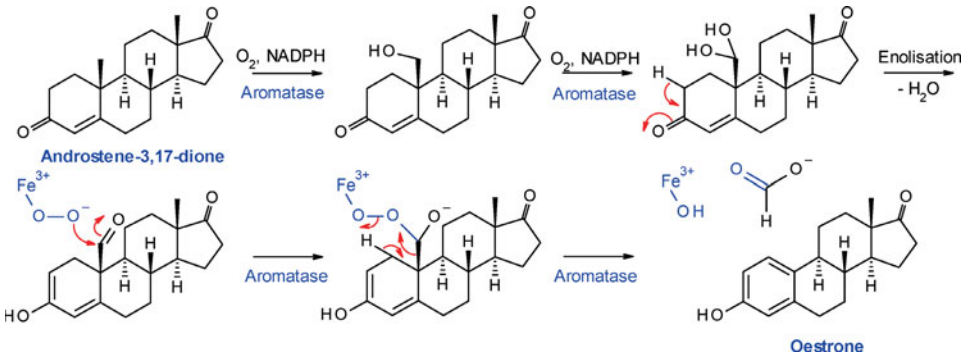
Only a few years ago, the crystallisation and structure elucidation of human placental aromatase has been achieved (Fig. 6.9). [14] Now, it became possible to gain a deeper understanding of how the enzyme operates and why it has such a high substrate selectivity – in contrast to many other cytochrome P450 reductases.



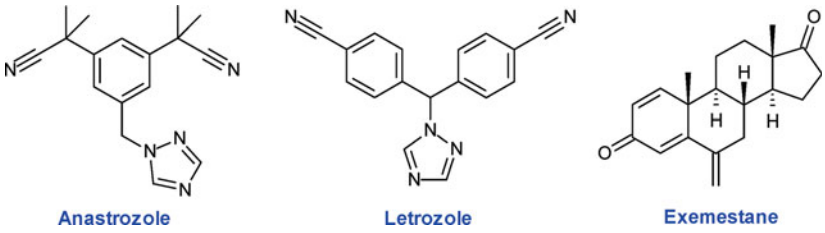
6.9 *Crystallographic structure of the human placental aromatase cytochrome P450 (N-terminus: blue, C-terminus: red) in a complex with the cofactor protoporphyrin IX and the substrate androstene-3,17-dione (carbon = white, oxygen = red, nitrogen = blue, iron = orange).*

The aromatisation is in principal a three-stage process; in the first two steps, the β-oriented angular methyl group at C-19, positioned exactly opposite to the iron-porphyrin, is dihydroxylated by consuming one equivalent of each, oxygen and NADPH. After dehydration and enolisation, there follows another oxidation step with loss of formic acid, and accompanying aromatisation.

The human aromatase gene CYP19A1 is located on chromosome 15.



In vertebrates, the aromatase is the sole enzyme, which catalyses the formation of oestrogens from androgens. This makes it a favoured target for the treatment of oestrogen-dependent cancers, such as breast cancer. Correspondingly, a range of aromatase inhibitors has already been developed (Fig. 6.10).



6.10 *Aromatase inhibitors for the treatment of oestrogen-dependent cancers.*

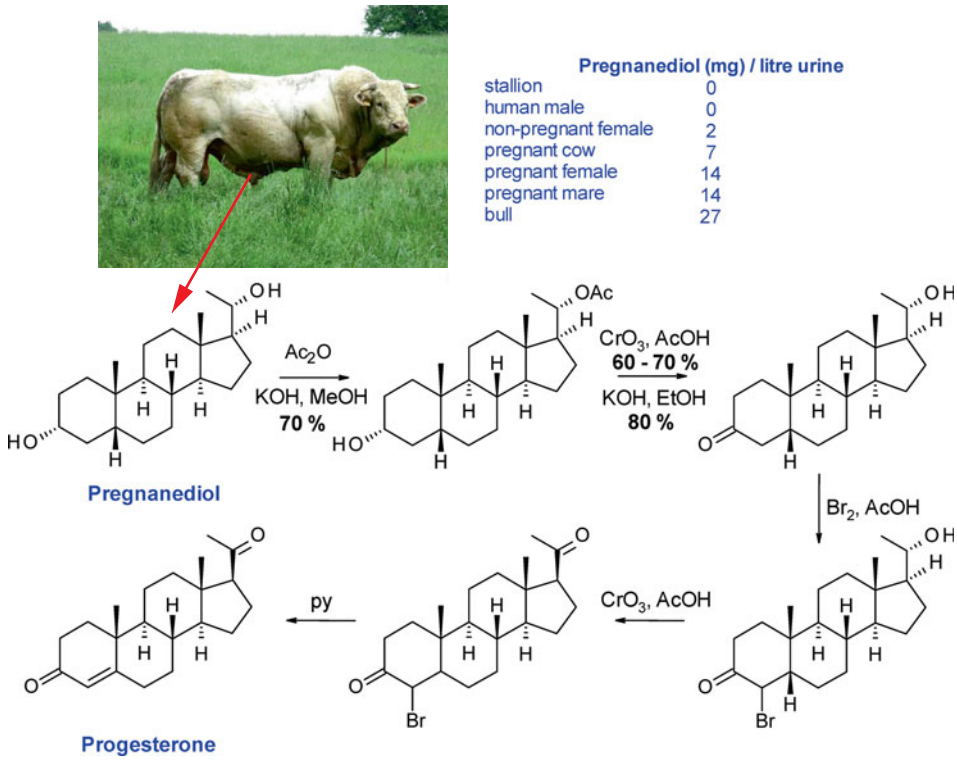
6.1.5 Discovery of Steroid Hormones

The beginnings of steroid chemistry go back to Adolf Windaus (1876–1959), who laid the foundations of this captivating field in 1905. Important contributions came from Adolf Butenandt (1903–1995) and Heinrich Otto Wieland (1877–1957). [15] Oestrone was the first sex hormone to be isolated from the urine of pregnant women in 1929 by Butenandt in Göttingen. [16] Later, he used

the urine from pregnant mares. For the isolation of the hormone, a test was needed in order to identify the oestrone-containing fractions. In this, spayed female mice were injected with samples of the various fractions, and the hypertrophic changes of the vaginal epithelium observed (the Allen-Doisy test). Out of 625 kilograms of ovaries from 50,000 pigs, Butenandt obtained 20 milligrams of pure progesterone. Ernst Laqueur (1880–1947) in Amsterdam had isolated approximately 10 milligrams of testosterone from 100 kilograms of bovine testicles. In 1934, progesterone was synthesised by four research groups. [17–20] In 1935, artificial testosterone was produced for the first time. [21] By 1943, already 28 steroids had been identified, including cortisone, cortisol and corticosterone. In the 1950s, Merck developed the first process for the preparation of cortisone acetate. [22]

6.1.6 Partial Syntheses

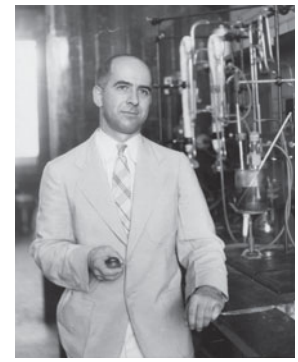
The story of hormonal contraceptives began in 1921 with Ludwig Haberlandt (1885–1932) in Innsbruck. [23, 24] He showed that mice and rabbits can be temporarily sterilised with an extract of *corpus luteum*. [25] Progesterone served to manage menstrual pain (Dysmenorrhea) and to prevent miscarriages. It was manufactured in the laboratories of various European pharmaceutical firms, and a very expensive product.



6.11 *Pregnanediol was isolated from bull's urine and converted into progesterone.*

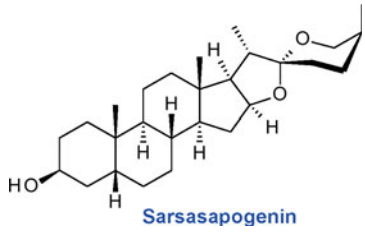
Russell E. Marker (1902–1995), a chemist at Pennsylvania State University, while sponsored by a research grant from Parke-Davis & Co., isolated pregnanediol in the 1930s from the urine of pregnant women. He transformed this into progesterone following a protocol from Butenandt. [26] In his search for a more productive source of pregnanediol, Marker discovered that bull's urine is very rich in this steroid [27], what made Parke-Davis & Co. consider to set up a large stable of bulls for the production of progesterone (Fig. 6.11).

It was perfectly clear to Russell Marker, that the future demand for steroid hormones could not be satisfied on the basis of bull's urine. By the end of the 1930s, he had already found a way of preparing progesterone also from various sapogenins (Fig. 6.12).



6.12 *In November 1941, Russell E. Marker found the starting material for the partial synthesis of progesterone in yam roots.*

Excursus: Saponin
The saponin to be investigated first came from the red foxglove (*Digitalis purpurea*). Saponins are widespread plant glycosides, which produce in water a soapy froth. Their name is derived from the Common Soapwort (*Saponaria officinalis*), a vespertine flower, the root of which was used for several thousand years as a soap. The aglycones are referred to as sapogenins. Steroids are mostly glycosylated through their 3β-hydroxy-group.



Sarsasapogenin

Marker's synthesis was initially based on sarsasapogenin as starting material from root extracts of the Mexican sarsaparilla plant (*Smilax aristolochiaefolia*), and later on diosgenin. Diosgenin is found as the 3-glycoside (dioscin) in numerous *Liliaceae* and *Dioscoreaceae* species, and may be extracted with ethanol from the air-dried rhizomes of *Dioscorea tokoro*, *Dioscorea macrostachya*, *Dioscorea mexicana*, *Dioscorea floribunda* and *Dioscorea composita* (Barbasco). The extract is evaporated and heated with dilute hydrochloric or sulfuric acid to cleave the glycosidic bond. Subsequently, diosgenin is filtered off and used as such for the synthesis of steroid hormones.

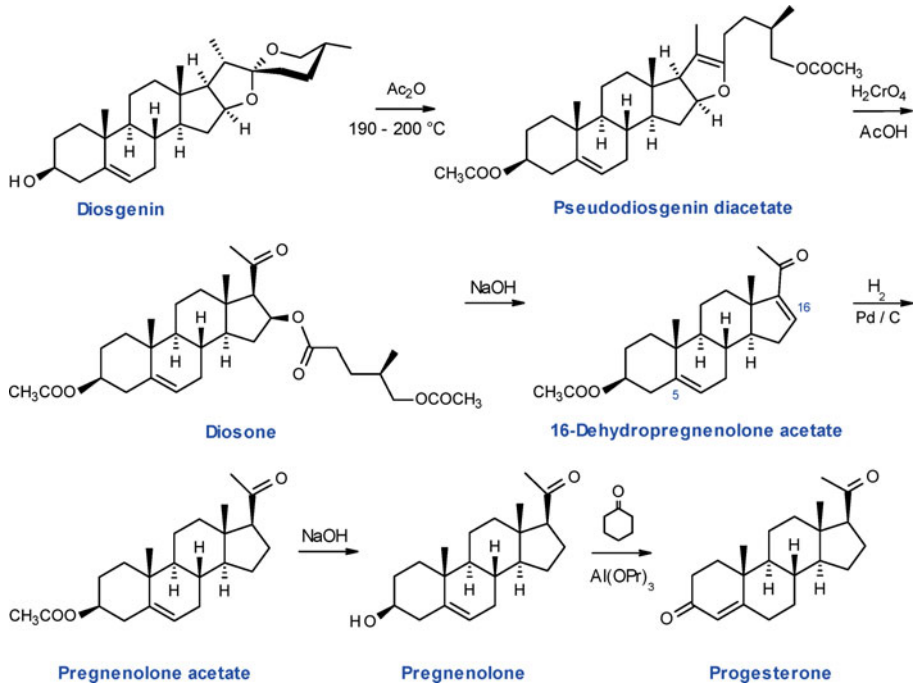
Initially, Marker wanted to obtain diosgenin from the roots of the inedible yam *Dioscorea mexicana*, which grows wild in Mexico (Fig. 6.13). [27] Parke-Davis & Co. and other pharmaceutical companies did not want to engage in supply of plant material from Mexico for political and reliability reasons. Thus, Marker quit his position at the Pennsylvania State University in 1943, travelled to Mexico, rented a small house and explored the jungle in the mountains of Veracruz with a mule.

Healers of the native Chinantec people in northern Oaxaca, Mexico, already used the tuber of *Dioscorea composita* as an abortifacient, long before it gained any industrial significance. [27]

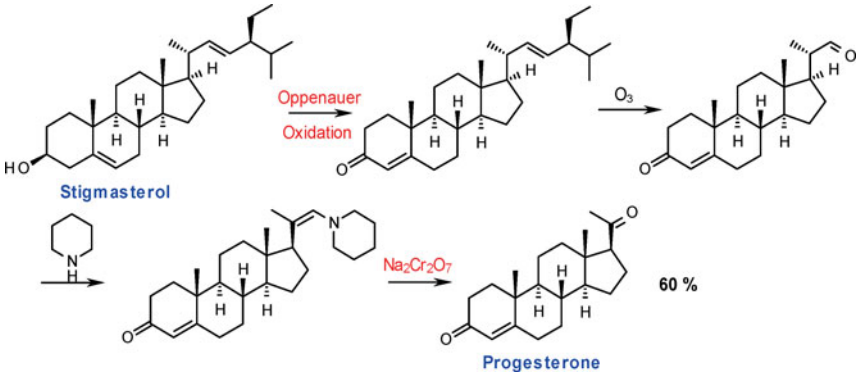
6.13 *Dioscorea mexicana (Mexican yam or cabeza de negro) is a species of yam in the genus (Dioscorea). It has either a partly to completely above-ground dome-shaped caudex with a thick, woody outer layer, which resembles the shell of a tortoise. It is divided into polygonal plates that are scored by deep furrows.*

With the support of Mexican campesinos, Marker eventually harvested some 10 tonnes of yam roots, from which he isolated crude diosgenin as a syrup in a rented laboratory in Mexico City. Once back in the USA, he made an arrangement to generate the product in a friend's laboratory for granting a share in the profits. The ultimate yield exceeded 3,000 grams of progesterone, which had a value of some 240,000 US dollars.

To transform the diosgenin (Marker degradation), he heated batches of the raw material in acetic anhydride under pressure to almost 200 °C. Thereby, pseudodiosgenin diacetate is formed, which can undergo mild oxidation to diosone with chromic acid in acetic acid. [28] Hydrolysis with dilute sodium hydroxide leads to dehydropregnenolone acetate in an overall yield of *ca*. 45 %. Hydrogenation over palladium/carbon allows selective reduction of the conjugated double bond at C-16 with preservation of the Δ^5-double bond. Hydrolysis and Oppenauer oxidation finally give progesterone in yields of 85 %.



The Upjohn company developed later an elegant procedure for the production of progesterone from stigmasterol. In this, the hydroxy-group of the stigmasterol is first oxidised to the corresponding ketone by an Oppenauer oxidation. Ozonolysis, carried out under special conditions, cleaves only the side-chain. Azeotropic distillation with piperidine gives initially the enamine, which is then converted oxidatively into progesterone. [28] The overall yield amounts to 60 %.

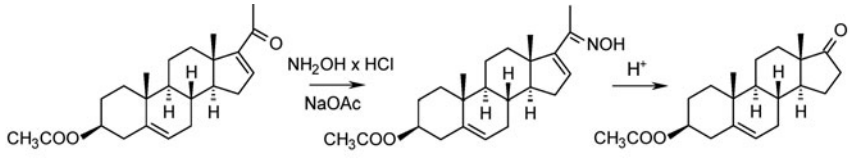


In 1944, Marker founded together with two Mexican partners a pharmaceutical company, which he called Laboratorios Syntex SA (*Synt*hesis in M*ex*ico). However, the collaboration was not successful, and Marker left the firm almost a year later to form another company (Botanica-Mex S.A.). When his second attempt to build up a steroid business failed as well, Marker brought in 1949 an abrupt end to his previous scientific career and moved into manufacturing and merchandising Mexican replicas of French silver artworks of the 18th century.

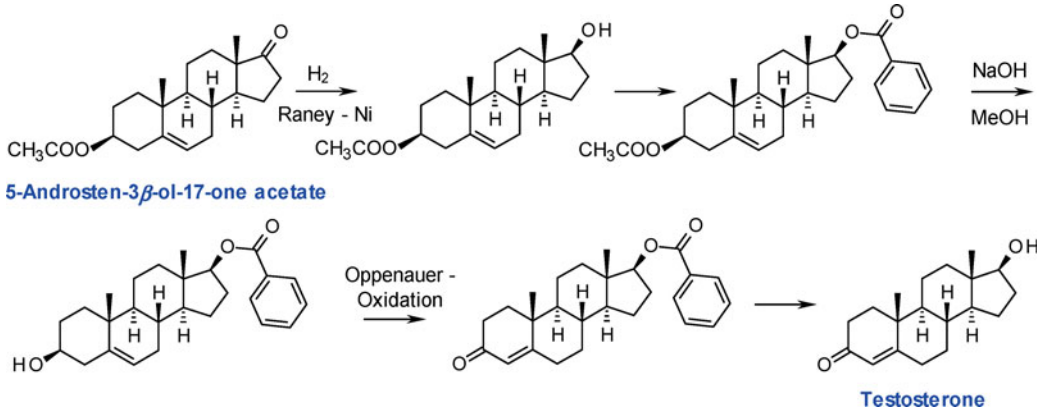
In the 1950s to the 1970s, a flourishing Barbasco trade developed in Mexico. As it turned out, Barbasco (*Dioscorea composita*) has a higher diosgenin content compared to *Dioscorea mexicana* and became therefore the preferred source for the Mexican steroid industry. In its heyday, by the mid of the 1970s, 125,000 Mexican peasants (*barbasqueros*) earned their living from the Barbasco business, and each week about 10 tonnes of yam roots were harvested. [27]

The Mexican owners of Syntex recruited after Marker's departure George Rosenkranz. Within two years, Rosenkranz succeeded not only in the large-scale production of progesterone, but he also established the synthesis of the male sex hormone, testosterone, from yam roots. The synthesis starts from 16-dehydropregnenolone acetate, which is converted into its oxime; Beckmann rearrangement of the latter is followed by hydrolysis and yields 5-androsten-3β-ol-17-one acetate.

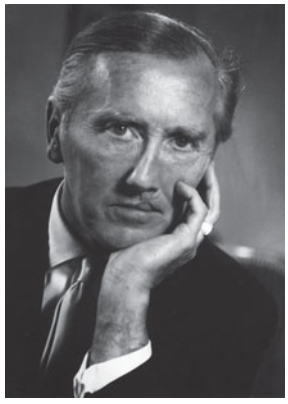
At the present time, important customary intermediates for the partial synthesis of sex hormones are pregnenolone, 16-dehydropregnenolone and 5-androsten-3β-ol-17-one, which can be obtained from diosgenin and stigmasterol. [7]



5-Androsten-3β-ol-17-one acetate is reduced over Raney nickel to the 17β-alcohol, which is protected as a benzoate. This allows the selective hydrolysis of the acetate with methanolic sodium hydroxide solution. A final Oppenauer oxidation leads to testosterone benzoate, hydrolysis of which gives the target compound.



In 1949, when Carl Djerassi took over the leadership of a research group at Syntex in Mexico City, nobody thought of hormonal contraceptives yet. In fact, meanwhile the biological function of progesterone had become known, but the hormone was not orally available and could be only administered by injection. Djerassi's aim was the synthesis of cortisone and possibly oestradiol. Cortisone had been discovered a few years earlier by Edward Calvin Kendall (1886–1972), and was at that time regarded as a miracle drug. Oestradiol was used for the treatment of problems during puberty and menopause.

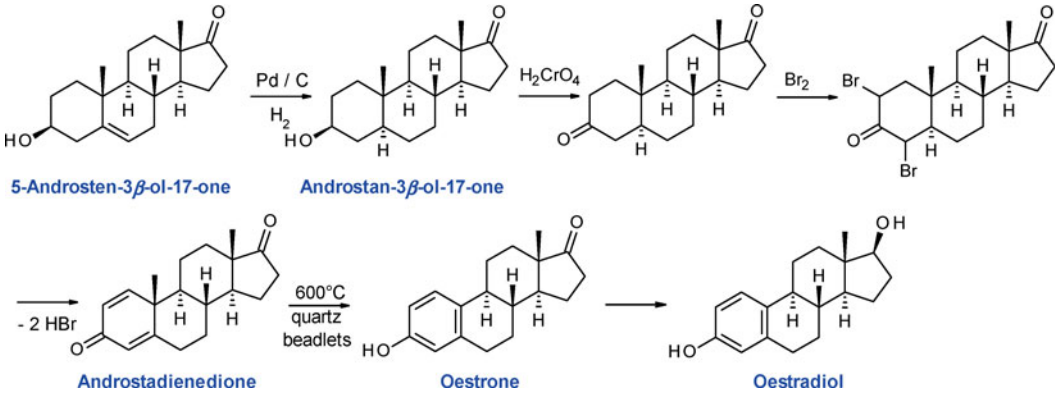


6.14 *Hans Herloff Inhoffen.*

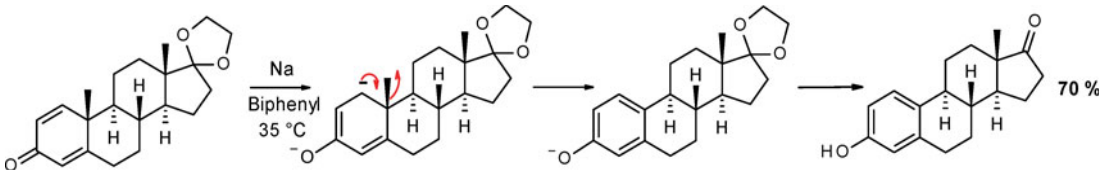
Oestradiol

The chemistry concerning the preparation of oestradiol was already basically known. Hans Herloff Inhoffen (1906–1992) (Fig. 6.14), from Schering AG in Berlin, published in the late 1930s the following route, which had been at least inspired by his earlier work assignment at Edward Charles Dodds' laboratory at the Courtauld Institute of Biochemistry in London:

The double bond in 5-androsten-3β-ol-17-one is first hydrogenated over palladium/carbon. Androstan-3β-ol-17-one is next oxidised with chromic acid, and the diketone selectively dibrominated in the A-ring. By a double elimination of HBr, androstadienedione is produced. Flash pyrolysis on quartz beadlets at 600 °C in tetralin or mineral oil gives then oestrone, which requires only hydrogenation to yield oestradiol. [29, 30] This remarkable achievement was a significant milestone on the long road to synthetic sex hormones.

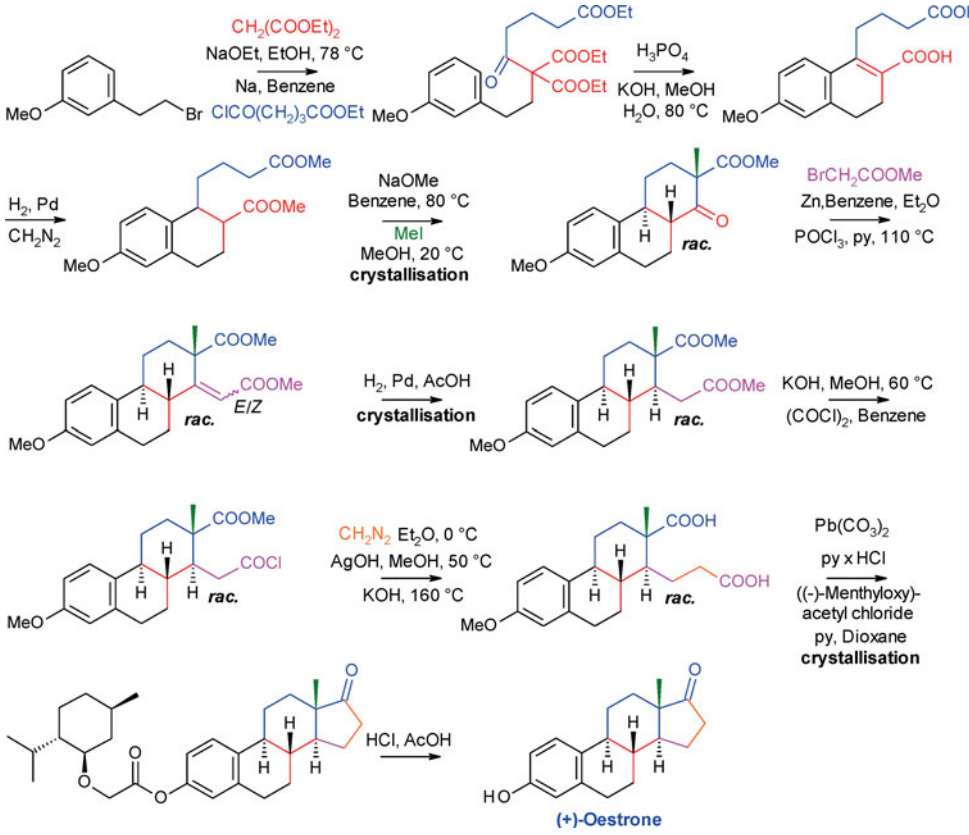


For elimination of the methyl group, further methods were later developed, *e.g.* by Hugh L. Dryden Jr. (1923–2001) in the early 1960s at the Searle company. [31] Androstadienedione 17-ethyleneketal is reacted with sodium biphenylide in diglyme. Thereby two electrons are transferred to the A-ring. Along with its aromatisation, the methyl group is eliminated as a methyl anion.



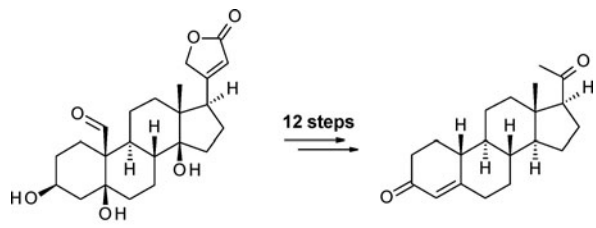
Almost 10 years after and independent of Inhoffen's pioneering research, Karl Miescher (1892–1974) and George Anner from Ciba AG in Basel published in 1948 the first total synthesis of enantiopure (+)-oestrone. [32] Their approach was based on earlier work from Robert Robinson (1886–1975) in Oxford and Werner Emmanuel Bachmann (1901–1951) in Ann Arbor, Michigan. As another highlight in steroid chemistry, their synthesis stands out as one of the earliest attempts to circumvent the challenging demethylation step. Key reaction sequences are a malonic ester synthesis, a Dieckmann cyclisation, a Reformatzky reaction and the application of the Arndt-Eistert reaction. Remarkable is here as well the high art of crystallisation: In the first fractional crystallisation, three racemic diastereomers were separated and in the second one two. Finally, enantiopure (+)-oestrone is obtained by stereoselective crystallisation of the desired diastereomer of the ((–)-menthyloxy) acetate.

The Arndt-Eistert reaction [33] was discovered in 1927 at the University of Breslau (Wrocław, Poland) by Bernd Eistert (1902–1978), while he was working on his PhD thesis with Fritz Arndt (1885–1969). In 1933, Arndt was forced by the Nazi regime to abandon his position at Breslau University. After a short stay at Oxford University, he accepted a professorship at the University of Istanbul. – Eistert worked at BASF from 1929 to 1957; from 1943 on, he taught at the University of Heidelberg, and after the war he became professor in Darmstadt and later in Saarbrücken, Germany.



Norsteroids

Another milestone was achieved in 1944 by Maximilian Ehrenstein (1899–1968) at the University of Pennsylvania. He prepared in a twelve-step sequence a minute amount of 19-norprogesterone, starting from the cardiac glycoside strophanthidin, which is present in lily of the valley (*Convallariae herba*), *inter alia*. [34]

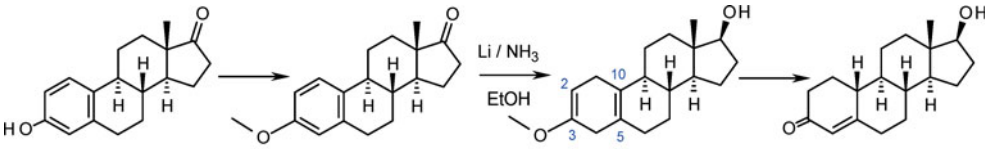


Strophanthidin — 19-Norprogesterone

When Arthur Birch, an Australian chemist, was working in 1940 under the guidance of Robert Robinson on his PhD thesis at Oxford University, his steroid chemistry caught the attention of the Royal Air Force. The RAF was concerned about rumours that German fighter pilots were administered corticoid hormones to tolerate high altitude. Therefore, they were interested in having the same available for British pilots. [35]

Roy Hertz (1909–2002) an endocrinologist at the National Cancer Institute in Bethesda, Maryland, discovered that local injections of 19-norprogesterone were surprisingly four to eight times more efficient than the natural hormone in the treatment of cervical cancer. This result contradicted the school of thought at that time, and directed a special scientific interest towards 19-norsteroids.

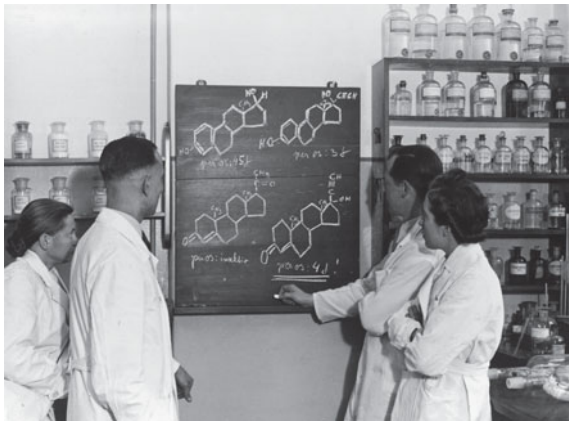
19-Nortestosterone and 19-norprogesterone were considerably easier to obtain as soon as Arthur John Birch (1915–1995) had discovered the reduction of aromatics. [36] While the reactivity of deprotonated oestrone is too low for a direct Birch reduction, the methyl ethers were used in particular, because electron donors lower the reaction rate. The donor substituent is always attached at the remaining double bond, and the product is, on account of the *principle of least motion*, a 1,4-cyclohexadiene, in this case the 2,$\Delta^{5,10}$-doubly unsaturated steroid skeleton. The second conceivable isomer, with double bonds at the 3,6-positions, is for steric reasons thermodynamically less favoured (Bredt's Rule). [37]

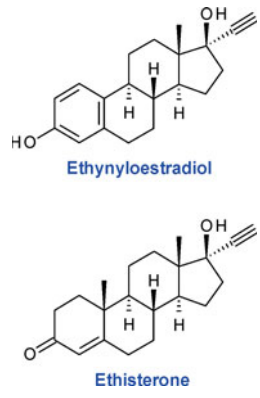


17*α*-Ethynyl-steroids

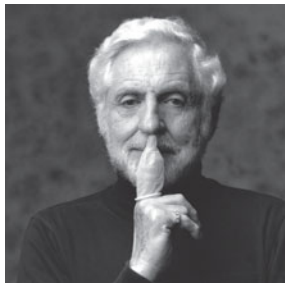
However, the remaining problem was the administration route of the drug. Shortly before the Second World War, Inhoffen had prepared 17*α*-ethynyl-oestradiol, and had noticed that this derivative is surprisingly stable in the stomach. The initial objective of Inhoffen's synthesis was actually oestradiol-17-carboxylic acid, which ought to have been produced by ethynylation and ozonolysis. Fortunately, the intermediate was also checked for its oral bioavailability. In fact, the carboxylic acid was only synthesised 50 years later and proved to be completely inactive. [24]

Encouraged by the bioavailability data, Inhoffen reacted androstene-3,17-dione with acetylene, hoping to obtain an orally active androgen, for which there was likewise great clinical demand. However, he obtained a compound with completely different properties. Ethisterone (17*α*-ethynyltestosterone) is practically inactive as an androgen; however, it possesses progestagenic properties and showed indeed some oral availability (Fig. 6.15).



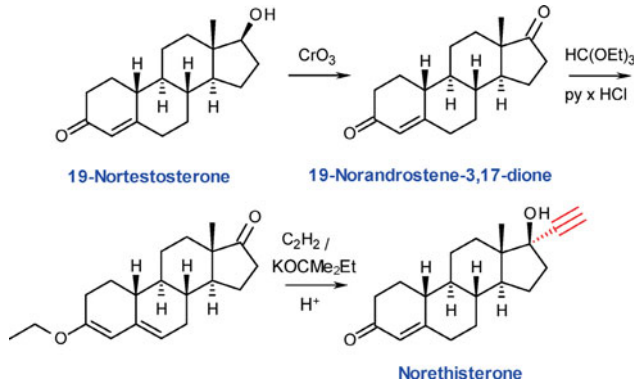


6.15 *Ethynyloestradiol (top right) and Ethisterone (bottom right) at Schering.*

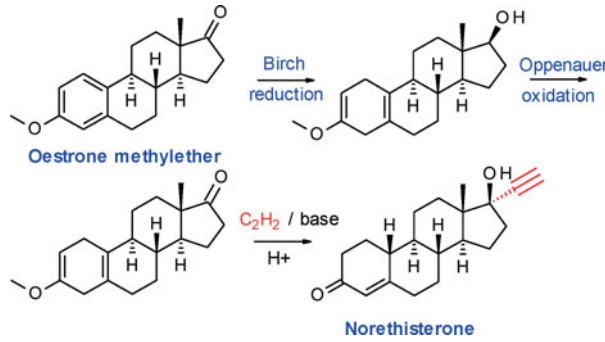


6.16 *Carl Djerassi has also achieved popularity in recent years as a novelist ("Science-in-Fiction") and playwright.*

Although ethisterone never attained greater medicinal importance, this compound provided the crucial hint, which led to the synthesis of norethisterone. Carl Djerassi (Fig. 6.16) at Syntex oxidised 19-nortestosterone with chromium trioxide to 19-norandrostene-3,17-dione. Treatment with triethyl orthoformate in presence of pyridinium chloride gave an enol ether, which was reacted with acetylene and potassium *t*-amyloxide, and could subsequently undergo acid hydrolysis to the desired product. [38]



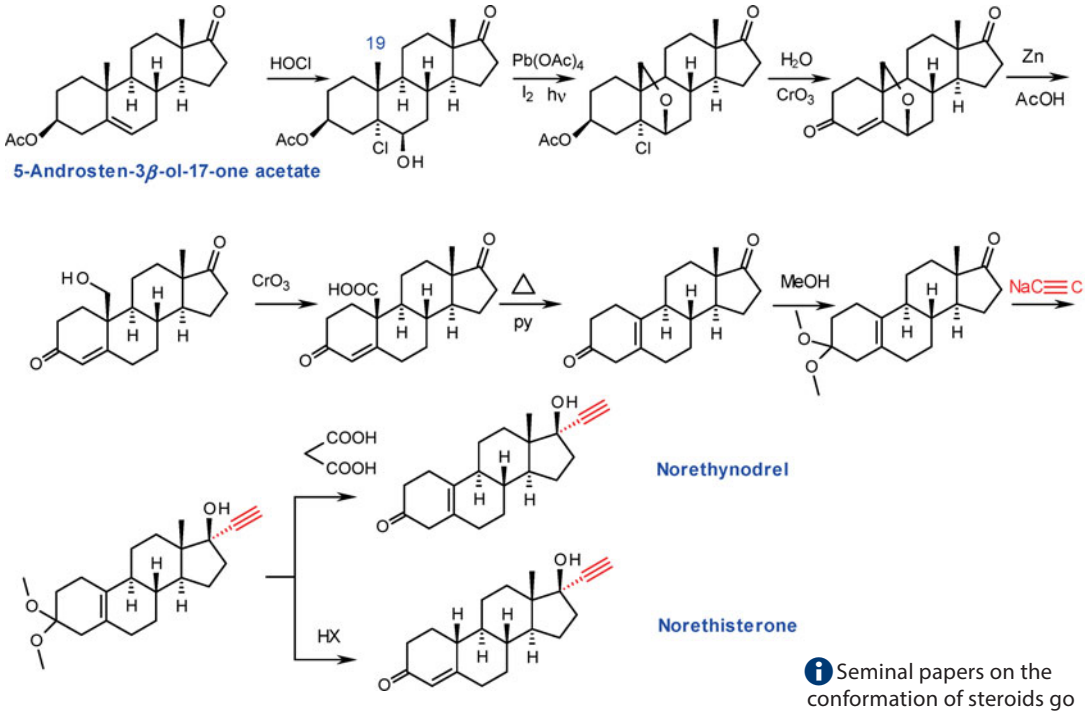
Norethisterone is more elegantly prepared from oestrone methyl ether by a sequence of Birch reduction, Oppenauer oxidation and ethynylation. [39, 40]



At the beginning of the 1950s, norethisterone was the best orally available progestagenic steroid and became the active constituent of almost half of all oral contraceptives.

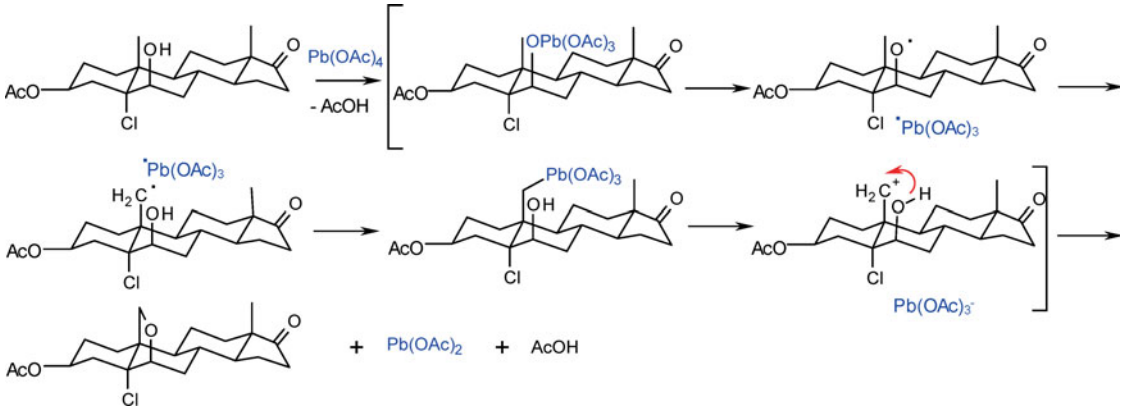
In 1952, Frank B. Colton (1923–2003) at Searle synthesised and later patented norethynodrel, an isomer of norethisterone. An improved synthesis emerged, in which both compounds were obtained, while the aromatisation and Birch reduction could be avoided. [41, 42] As starting point serves 5-androsten-3β-ol-17-one acetate, to which hypochlorous acid is added. Notable is the thermal or photochemical functionalisation of C-19 with lead tetraacetate/iodine, [43] under formation of a cyclic ether. Hydrolysis of the ester and oxidation with chromium trioxide leads to a Δ^4-enedione, while HCl is eliminated. Reductive opening of the ether gives the 19-hydroxy-compound, which can be oxidised with chromium trioxide to the 10β-carboxylic acid and then decarboxylated in

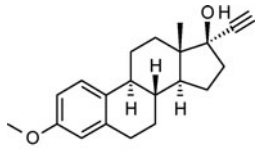
pyridine. Partial ketalisation at the 3-position enables the selective ethynylation of the unprotected keto-function. After hydrolysis with weak acids, like malonic acid, norethynodrel is obtained, whereas with strong mineral acids norethisterone is formed.



Albert Bowers (1930–1990) [46, 47] at Syntex observed for secondary steroidal alcohols, that these are amenable to cyclisation with lead tetraacetate, supposed the stereochemistry in the δ-position is adequate. The key step is a radical 1,5-H-abstraction, which is intrinsically more favourable than a 1,4- or 1,6-abstraction. This reaction is mechanistically related to the Hofmann-Löffler-Freytag reaction and the Barton reaction. [48]

Seminal papers on the conformation of steroids go back to Sir Derek Barton (1918–1998), and are the basis of our understanding of selective reactions at the steroid skeleton. [44, 45] Barton was also the first to discover specific functionalisations of non-activated carbon atoms in steroids.



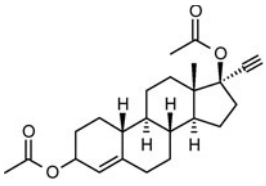


Mestranol

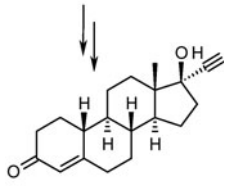
In 1953/54, Gregory Goodwin Pincus (1903–1967), director of the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, had collected from various companies some 200 synthetic steroids, which structurally resembled progesterone. When tested for their activity as ovulation inhibitors in rabbits, his colleague, the biologist Min-Chueh Chang, found Syntex's norethisterone and Searle's norethynodrel as particularly effective. Dr. John Rock (1890–1984), a gynaecologist and member of the Harvard Medical School, operated a private clinic in Brookline, Massachusetts, where he treated infertile patients. He would become the third important contributor to the development of oral contraceptives, besides Djerassi and Pincus. [24, 49] Since use of birth control devices was a felony in Massachusetts at that time, Rock conducted the first human studies in Puerto Rico to show that both compounds could be used not only for the treatment of menstrual problems, but also as contraceptives.

Pincus found out by chance that the simultaneous administration of small amounts of oestrogens reduced the side-effect of the new contraceptives. Whereas the first studies proceeded with extraordinary success, breakthrough bleeding occurred during subsequent series of trials. Careful analysis revealed that the norethynodrel used in the studies originated from different batches. The initial one contained a larger percentage of a hidden impurity, the oestrogen mestranol, than the following batches. By intentionally adding mestranol and thus creating a combination drug product, not only the number of intermenstrual bleedings was reduced, but also the reliability and efficacy of the new contraceptives were increased.

It is interesting to recall that prior to 1972 unmarried women in the USA did not have legal access to oral contraceptives.



Ethynodiol diacetate



Norethisterone

In 1957, this combination was introduced to the market to treat menstrual disorders (*Enovid® 10 mg*), for legal reasons with the clear warning of a possible contraceptive side-effect. Due to the opposition from the Catholic Church, which spoke out very strongly against birth control, Searle feared that this might reflect negatively on other Searle products as well and hurt sales. Nevertheless, Pincus succeeded later in convincing Searle to file an application for approval of the product by the FDA (Food and Drug Administration) as a hormonal contraceptive. The pharmaceutical preparation was called *Enovid® 5 mg* and contained 5 mg of norethynodrel and 0.075 mg of mestranol. In 1966, Searle substituted norethynodrel by the more potent ethynodiol diacetate. It is noteworthy, that this compound has to be metabolised *in vivo* to norethisterone in order to unfold its activity.

While Syntex was lacking resources to commercialise its hormones efficiently, they had granted a marketing license to Parke-Davis. Thus, by the end of the 1950's, norethisterone had been introduced in the USA as a menstrual regulator. Parke-Davis pursued a conservative strategy and was as reluctant as Searle to file an application for norethisterone as an oral contraceptive.

Therefore, Syntex felt forced to choose the Ortho Division of Johnson and Johnson as their new partner, who finally won FDA approval in 1963 for norethisterone as *Ortho-Novin®* in the indication contraception.

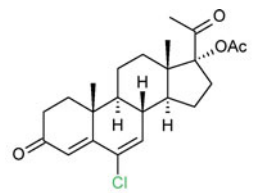
The first "pill" from Schering AG, *Anovlar®*, was launched in 1961 and contained 4 mg of norethisterone acetate and 0.05 mg of ethynyloestradiol (Fig. 6.17).

6.17 *Anovlar®*

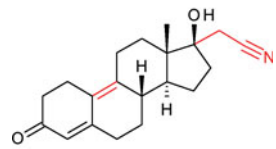
Dienogest

Right from its foundation in 1950, the VEB Jenapharm (Volkseigener Betrieb = nationally-owned enterprise) was engaged in research and development of sex hormones. In the GDR (East Germany), the first oral contraceptive, *Ovosiston*®, with chlormadinone acetate and mestranol as active ingredients, came to the market in 1965 (under a licence from Merck Darmstadt). During the following years, Jenapharm grew to become the leading manufacturer of the "pill" in Central and Eastern Europe. [50]

In the 1970s, Kurt Ponsold (1926–2003) at the Friedrich-Schiller-University in Jena collaborated with VEB Jenapharm to synthesise and develop a new gestagen under the code name STS 557. Compared to known drugs, it featured two uncommon structural elements: an additional double bond in the Δ9,10-position and a cyanomethyl group, which replaced the usual ethynyl residue. [51] In spite of its compared to levonorgestrel (see below) approximately ten-fold higher activity, STS 557 entered the market only in 1995 under the generic name Dienogest, six years after the fall of the Berlin Wall and the end of the GDR. Over the years, its combination with ethinyloestradiol became under the brand name *Valette*® one of the most prescribed oral contraceptives in Germany today.



Chlormadinone acetate

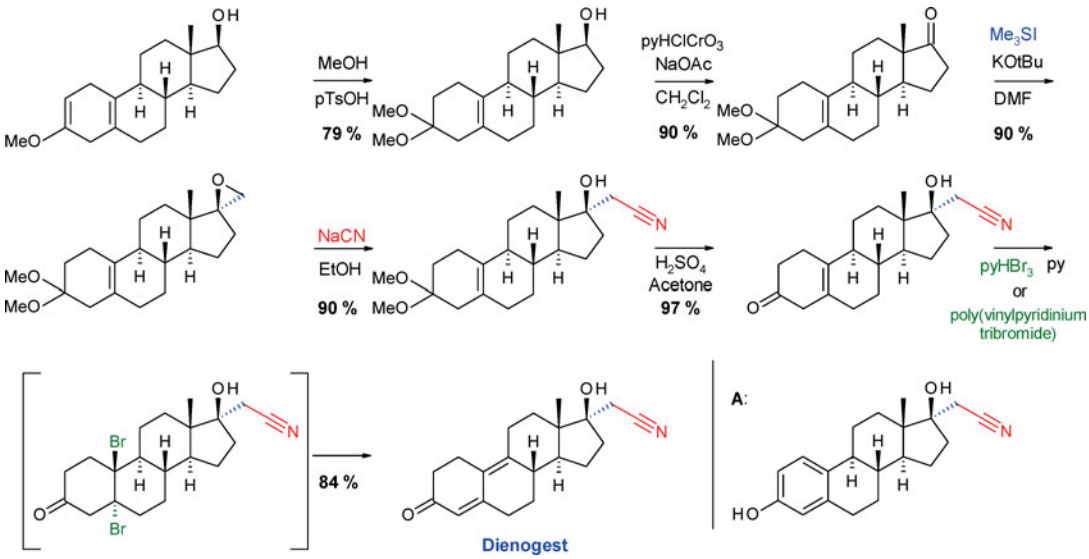


Dienogest (STS 557)

In one of the first production processes, after a Birch reduction of oestrone methyl ether, methanol was added to the A-ring; the hydroxy-group at C-17 was then oxidised with pyridinium chlorochromate in presence of sodium acetate, serving as a buffer. Reaction with dimethylsulfo-

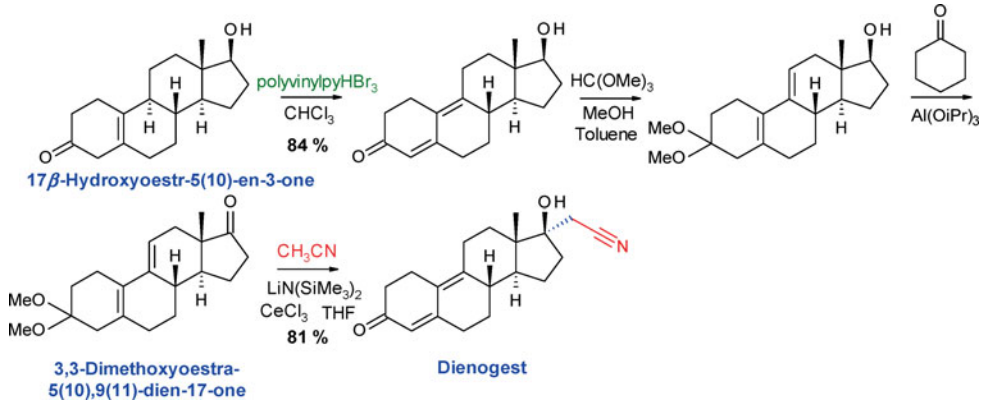
During the second half of the last century, the political world was divided into an Eastern and a Western Bloc. This led behind the "Iron Curtain" to a series of interesting technical and scientific developments, which were achieved independently. The nuclear and space technology arenas remained not the only examples. Also the pharmaceutical sector contributed its share: Besides the synthesis of (*L*)-DOPA, as mentioned above (*cf.* section 4: Amino acids), steroid research is another example.

Chlormadinone acetate was developed almost at the same time at Syntex and at E. Merck (Darmstadt). It was licensed by Syntex to Grünenthal and by Merck to VEB Jenapharm. [50]



nium methylide gave the corresponding oxirane, which underwent nucleophilic ring-opening with sodium cyanide. Hydrolysis of the ketal with dilute sulfuric acid occurred without isomerisation of the double bond. A bromination-dehydrobromination procedure introduced the double bond into the B-ring. This is an especially tricky step, which succeeds only with pyridine reagents. The elimination with strong bases or under acid catalysis favours in general aromatisation to byproduct A. In case pyridinium tribromide is replaced by poly(vinylpyridinium tribromide), the yield of Dienogest can even be increased to 84 %. [52]

A more recent synthesis uses 3,3-dimethoxyoestra-5(10),9(11)-dien-17-one as a key intermediate, which is in a few steps accessible from 17β-hydroxyoestr-5(10)-en-3-one. The reagent, (dichlorocerio)acetonitrile, is formed here *in situ* by reaction of acetonitrile with lithium hexamethyldisilazide and transmetallation with cerium(III) chloride, and is superbly suitable for the transformation of ketones. [52, 53]



6.1.7 Total Syntheses

Another important drug within the group of highly active oral contraceptives is levonorgestrel, which was introduced into the market by Wyeth Laboratories in 1968. Levonorgestrel is a wholly synthetic drug, which brought in the wake of the rapidly growing market for hormonal contraceptives relief to an increasing shortage of raw material. Moreover, the total synthesis allowed incorporating structural elements, which were otherwise difficult to access from diosgenin. The synthesis is also remarkable for a completely different reason: Already in 1967, a microbial reduction had been used for the stereoselective synthesis of a β-hydroxy-ketone. This enabled now the preparation of the enantiomerically pure drug.

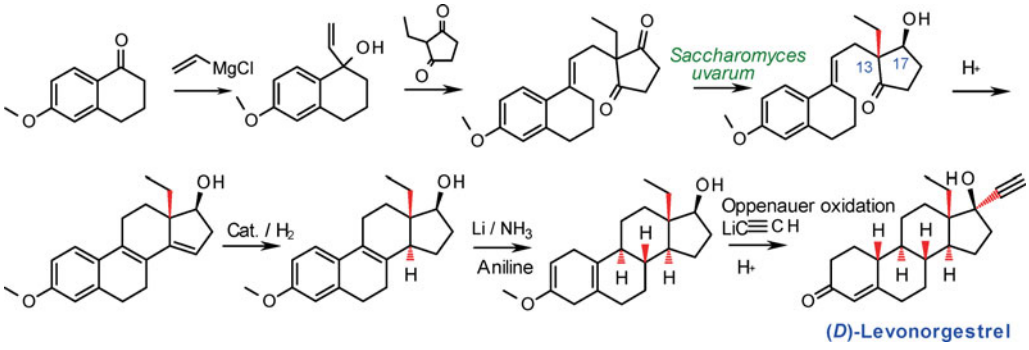
The prefix "*seco*" denotes the fission of a ring system, in the present case of the steroid skeleton, as *e.g.* in vitamin D.

Starting material of the synthesis is 6-methoxy-1-tetralone, which is first reacted with vinylmagnesium chloride to give the corresponding vinyl alcohol. Condensation with 2-ethylcyclopentane-1,3-dione under basic or acidic catalysis (*p*-TsOH, benzene, 82 %) [54] leads, according to Torgov [55], to a *seco*-dione, which is then reduced with *Saccharomyces uvarum* to the β-hydroxy-ketone. [56]

By exploiting the *meso-trick* (*cf.* section 5.1.3) two stereogenic centres are thereby generated: at C-13, a quaternary carbon atom, and at C-17. These absolute configurations correspond to those present in natural steroids. All of the subsequent reactions are stereospecific, so that the microbial reduction eventually predetermined the route to enantiomerically pure norgestrel (levonorgestrel). The cyclisation in acid, in the manner of a carbonyl-ene reaction, leads to an arylbutadiene system, which is subsequently reduced by catalytic hydrogenation and a Birch reduction. The rest of the synthesis is well established. Oppenauer oxidation produces the keto-function at C-17; ethynylation and hydrolysis of the methyl ether finally give (*D*)-levonorgestrel. [57]

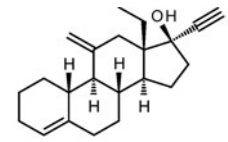
Due to the lack of a good understanding of dose/response relations, the "first-generation" oral contraceptives contained relatively high-dosed oestrogen-gestagen combinations. Epidemiological studies in the 1960s and 1970s pointed to an increased risk of thrombo-embolic complications, especially for female smokers, which correlated with the oestrogen dosage.

Sofya Nikolaevna Ananchenko and Igor Vladimirovich Torgov (1912–2007) focused in the late 1960's on the synthesis of anabolic steroids, like (*D*)-homooestrone 3-methyl ether or 19-nor-(*D*)-homotestosterone. [58] This effort paved the way for steroid research to gain "olympic fame" with the help of athletes in particular from former Eastern Bloc countries, but also from elsewhere.



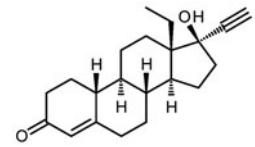
(*D*)-Levonorgestrel

In the course of more liberal legislation in many countries concerning birth control, larger clinical studies could be conducted, which resulted in recommendations to reduce the daily oestrogen dose from 100–150 µg to 30–35 µg without loss of efficacy, but with better tolerability. Since the early 1990s, these low-dose "second-generation" contraceptives have taken over the bulk of the market share.



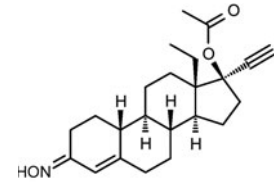
Desogestrel

Considerable progress was made in developing selective gestagens. Careful studies revealed that the original gestagens led to weight gain, affected lipid metabolism, and possessed androgen activity. Consequently, Ortho, Organon and Schering developed drugs with an improved activity/side-effect profile. Desogestrel, levonorgestrel and norgestimate are examples of such "second-generation" drugs.



Levonorgestrel

As oestrogens, exclusively mestranol and ethynyloestradiol are used. In the 1990s, desogestrel was worldwide the most prescribed contraceptive with several hundred million dollars in sales. Although the daily dose of 0.15 mg desogestrel + 0.03 mg ethynyloestradiol is low, the production cost (cost of goods) plays a significant role, in particular considering the limited purchasing power of markets in developing countries. The current partial synthesis, as practised in industry, starts from diosgenin, and includes a cost-intensive microbial oxidation at C-11. [59] While the ethyl group is on the one hand responsible for



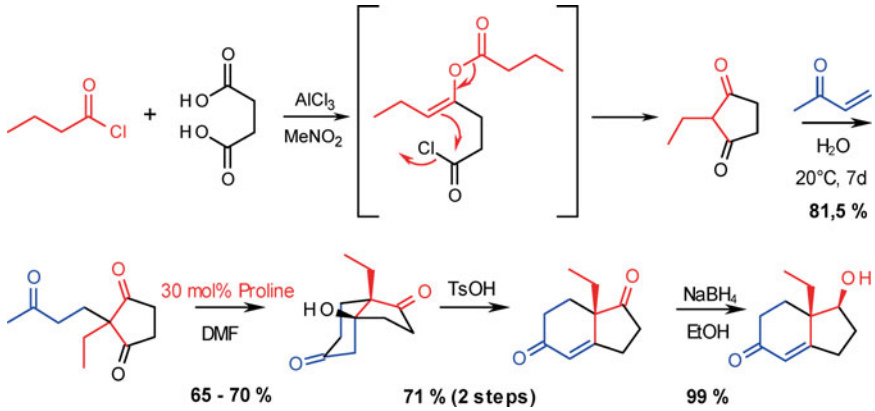
Norgestimate

the 50-fold higher activity of desogestrel and constitutes therefore an indispensable structural element, it causes on the other hand unanticipated challenges for the partial syntheses, which makes it laborious and costly.

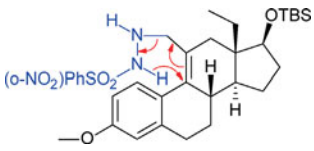
Corey has recently proposed a total synthesis, which gives in 14 stages enantiomerically pure desogestrel in remarkably high yields. [60] This laboratory synthesis is still a long way from an industrial process (see the red markings), but it includes some synthetically interesting steps.

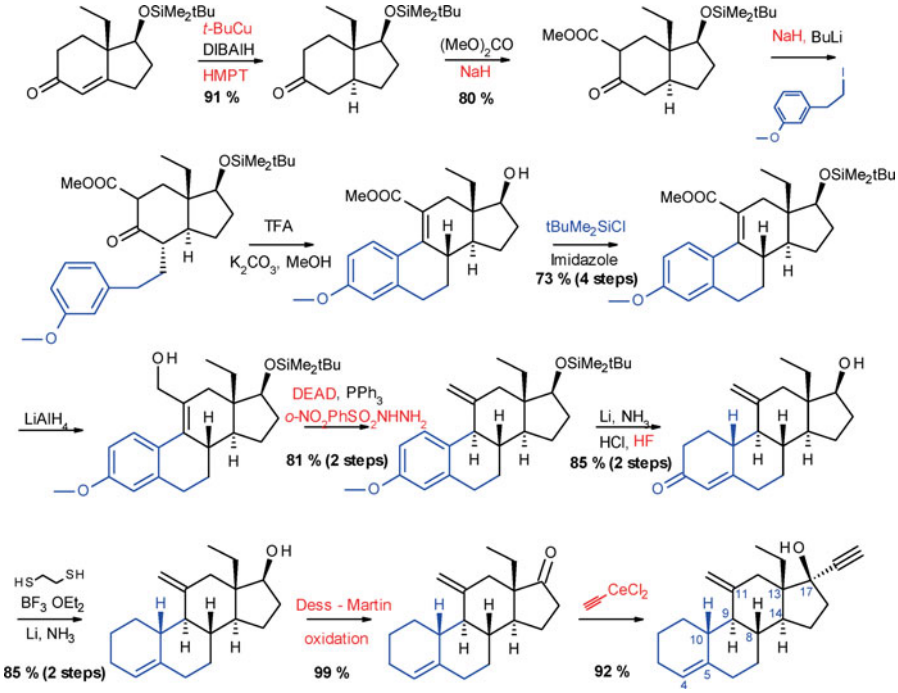
Around 1970, chemists at Schering (Ulrich Eder, Gerhard Sauer, Rudolf Wiechert) and concurrently at Hoffmann-La Roche (Zoltan Hajos, David Parrish) had found an improved Michael addition of 2-ethylcyclopentane-1,3-dione [61] to methyl vinyl ketone. If water is used in place of methanol, and catalytic amounts of potassium hydroxide are present, then the yield is increased from 54 to 81 %. [62, 63] The higher homologues can be synthesised in an analogous manner as well. [64] Robinson annulation, in presence of 30 mole % proline, leads in good yield to a bicyclic hydroxy-ketone. After dehydration, crystallisation and reduction with sodium borohydride, the enantiomerically pure bicyclic ketone is obtained, which is required for Corey's synthesis.

This is the first example of an enantioselective, organocatalytic aldol addition, which went unnoticed for 30 years. Initiated, among others, by the work of Carlos Barbas III, David MacMillan and Benjamin List, only in recent years the thriving research area of organocatalysis evolved from that original report. [65–67]



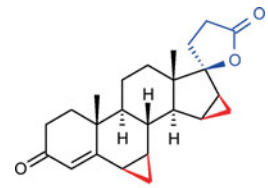
In the first step, the double bond is stereoselectively hydrogenated with the DIBAlH/HMPT complex in presence of *t*-butylcopper. The reduction product possesses a *trans*-fused ring system. Introduction of the ester group with sodium hydride and dimethyl carbonate in a refluxing THF/hexane mixture leads with high regioselectivity to methoxycarbonylation at the future C-11. After a double deprotonation of the enol of the *β*-keto-ester, stereoselective alkylation takes place at C-8. The cationic cyclisation with trifluoroacetic acid leads unfortunately to cleavage of the TBS-protecting group, so that the hydroxy-group has to be reprotected (blue marking). Reduction with lithium aluminium hydride gives the corresponding alcohol, which is reacted with *o*-nitrobenzenesulfonylhydrazine. A [3,3]-sigmatropic rearrangement of the allylic diazine generates the exomethylene group and establishes the absolute configuration at C-9. The A-ring is hydrogenated in the known way through a Birch reduction, followed by removal of the TBS-group. Deoxygenation and a Dess-Martin oxidation are routine steps. Finally, the ethynyl group is stereoselectively introduced with lithium acetylide in presence of cerium (III) chloride. The overall yield amounts to 28 %. [68]





6.1.8 The Third-Generation "Pill"

In the 21st century, the "pill" has to meet many more requirements than merely preventing conception. [69] Drospirenone is a suitable example thereof. This gestagen exhibits a pharmacological profile, which is to some extent comparable to that of the natural hormone progesterone. It has in addition some other beneficial features like its antimineralocorticoid and antiandrogenic activity. Clinical studies demonstrated that the combination of drospirenone with ethynyloestradiol offers very reliably effective contraception and good cycle control. After three cycles, 90 % of the women are free from mid-cycle bleedings. The antiandrogenic component lowers the sebaceous gland activity and alleviates acne and seborrhoea problems. The antimineralocorticoid properties reduce the retention of sodium and water and thereby lower body weight. Obvious advantages over second-generation drugs, like desogestrel, result in better compliance [70] and an impressive commercial success for Bayer and other companies. However, serious concerns remain. A significantly higher incidence rate of drospirenone-related blood clot formation (venous thromboembolisms) and associated fatalities has been reported. [71]

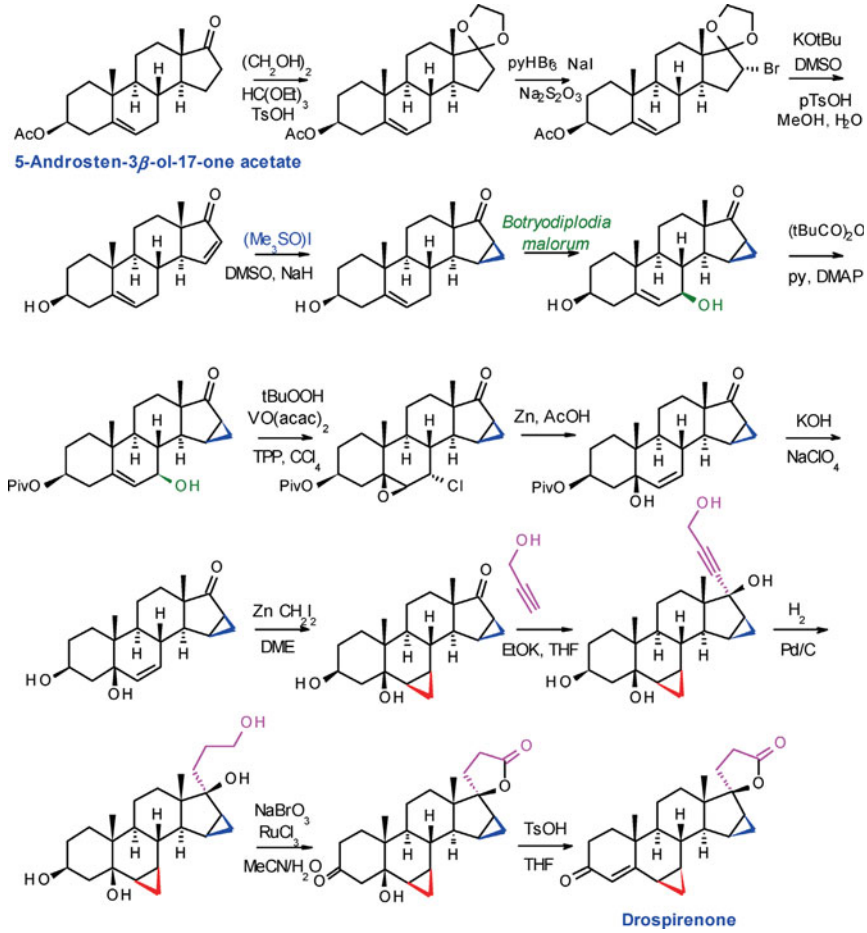

Drospirenone

It is noteworthy that Rudolf Wiechert (Fig. 6.18) at Schering had already synthesised drospirenone back in the 1970s. [72] However, it took around 25 years before the pharmacological potential of this compound was fully recognised and the drug received market approval. From a chemical viewpoint, drospirenone is an attractive molecule. Unusual structural characteristics are the two cyclopropane rings and the spirolactone moiety. In contrast to the previous

6.18 *Rudolf Wiechert (1928–2013).*

paradigm, replacement of the ethynyl substituent, which is a crucial substituent in almost all important gestagenes, by a lactone is feasible without compromising the oral bioavailability.

The synthesis starts with 5-androsten-3β-ol-17-one acetate, into which a double bond can be inserted at the 15-position, *via* a few steps of regio- and stereo-selective bromination and elimination. [73] After a Corey-cyclopropanation [74], the 7β-position is hydroxylated with the aid of the fungus *Botryodiplodia malorum*. [75] The hydroxy-group at C-3 is then selectively esterified with pivalic anhydride. Epoxidation with *t*-butyl hydroperoxide/$VO(acac)_2$ leads stereospecifically to the 5β,6β-epoxide. Subsequently, reaction with triphenylphosphine and carbon tetrachloride (Appel reaction, Rolf Appel (1921–2012), Bonn University in Bonn, Germany), followed by reduction with zinc and acetic acid, produce an allyl alcohol. After cleavage of the pivalate ester, the Simmons-Smith reaction occurs stereospecifically synfacial. [76] The spirolactone ring is constructed stereoselectively by reaction with propargyl alcohol, catalytic hydrogenation of the alkyne, and ruthenium-catalysed oxidative lactonisation with sodium bromate. In the course of the latter step, the hydroxy-group at C-3 is also oxidised. The final product is obtained by elimination of water. [77, 78]



In the year 2000, Schering introduced the contraceptive *Yasmin*®, which contains 3 milligrams of drospirenone and 30 micrograms of ethynyloestradiol. In 2004 followed *Angeliq*®, a combination product as hormone replacement for women during menopause, and for prevention of post-menopausal osteoporosis, with 0,5 milligrams of drospirenone and 1 milligram of oestradiol.

6.1.9 Further Developments

To further improve tolerability, convenience of dosing and therefore compliance, recent development efforts have been focused on alternative delivery systems for contraceptives. These include transdermal, as well as vaginal, and implantable, or injectable systems. [79]

The subcutaneous (SC) or intramuscular (IM) injection of a progestin exhibits a depot effect and blocks ovulation for up to 10 or 12 months. SC-implantation of small polymer rods, filled *e.g.* with levonorgestrel, has proven particularly efficacious and lasts for up to 5 years. Transdermal patches represent another meanwhile established delivery route. Contraceptive vaginal rings (CVRs) have the advantage of circumventing the "first-pass-effect" (initial hepatic metabolism), and achieving better absorption rates. These devices can also be placed and removed by the users themselves. Particular treatment regimen have been developed as emergency contraceptives (ECs), which inhibit ovulation, fertilisation or implantation up to 72 hours after unprotected intercourse; they do not cause an abortion. Contraceptive vaccines hold additional promise to tackle birth control on a global scale. A number of companies are pursuing this approach in close collaboration with the World Health Organisation (WHO). Most advanced are vaccines targeting human chorionic gonadotropin (CG), which demonstrated encouraging results in Phase II clinical trials. [80]

Finally, control of male fertility represents also an important option. The ideal reversible contraceptive would induce azoospermia (absence of sperm cells in semen), without influencing a man's libido or sexual potency. The current best prospect for success is the combination of testosterone and a progestin with or without a GnRH-antagonist. This regimen has shown efficient suppression of spermatogenesis and no serious adverse effects.

6.1.10 Final Remarks

There still exists an unmet need for inexpensive contraceptives with long duration of action, which are reliable and can be easily applied. Since this field does not appear to promise another "blockbuster", large research-oriented pharmaceutical companies direct their resources instead to disease indications, which are prevalent in the growing geriatric communities of Japan, North America and Europe. The problems of poor paediatric countries in South America, Africa and Asia attract only modest attention. [81]

With the "pill" being available now for more than half a century, one has to realise in hindsight that this discovery brought sexual liberation – uncoupling

of sex and reproduction – to mostly economically prosperous regions of the world, while it sadly had very little impact on slowing the population boom in less developed countries.

Summary in Bullet Points

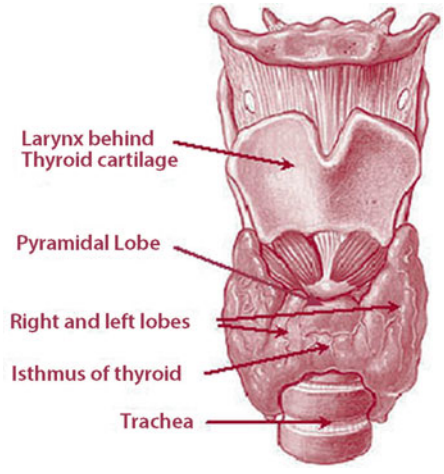
- Hormonal contraceptives rank among the safest methods of birth control. They simulate a pregnancy and prevent ovulation.
- Steroids are secondary products of the terpene metabolism. The key intermediate is cholesterol.
- The first hormonal contraceptives were prepared by partial synthesis, starting from diosgenin isolated from yam roots.
- Structure-activity analyses paved quickly the way to fully synthetic drugs.

6.2 Thyroxine

The goitre-preventing effects of seaweed were already known to the legendary Chinese emperor Shen-Nung as early as around 3000 BC, and also the Greek physicians in the ancient world had this knowledge available. Nevertheless the pharmacological principle remained undiscovered until the early 19th century. [82]

In 1802, the French coroner François-Emmanuel Fodéré (1764–1835) published a paper, which concerned the combined occurrence of goitre (struma) and cretinism in the Aosta valley (Italy) and in Valais (Switzerland). He systematically collected information about the customs and traditions of the region, its vegetation and minerals, and its folk medicine. Fodéré believed that cretinism was a hereditary disease.

The Scottish physician Allan Burns (1781–1813) was the first to differentiate thyroid cancer from goitre. In 1830, a connection was found between diseases of the thyroid gland (Fig. 6.19), and certain heart and eye diseases. In 1835,



6.19 *The thyroid gland (Latin: Glandula thyreoidea) is an organ weighing 20–60 grams, which is located in the neck below the larynx, and surrounds the trachea with two butterfly-shaped lobes.*

Robert J. Graves (1796–1853), an eminent Irish surgeon, recognised that an accelerated heart-beat can be the consequence of an enlargement (hypertrophy) of the thyroid gland. Five years later, the Merseburg physician Carl von Basedow (1799–1854) reported on four cases of an overactive thyroid (hyperthyroidism) (Fig. 6.20). The characteristic symptoms are rapid heart-beat (tachycardia), bulging eyes (exophthalmos) and goitre. Despite many attempted explanations, the cause of these symptoms remained in the dark until the end of the 19th century.

The understanding of cretinism was facilitated through enhancements in anaesthesia for surgical procedures, and towards the end of the 1870s, by improved insight into anatomical pathology from autopsies. In children, just as in adults, there was the assumption of a link between an atrophied or absent thyroid gland and the occurrence of cretinism. Physicians removed the goitre from patients with thyroid hyperactivity, in spite of the associated high risk of infection, and noticed that these suffered afterwards from symptoms of cretinism. Over time, it became evident that an underactive thyroid (hypothyroidism) led to cretinism in children. Their physical and intellectual development is thereby seriously disturbed. William Ord (1834–1902) introduced the term myxoedema for hypothyroidism, which can be considered the adult counterpart of cretinism. Those affected are lethargic and tired, their heart-beat is slow, they feel cold, their face becomes thick and swollen (oedematous), the eyelids are narrowed and the eye movements are just as slow as the overall facial expression.

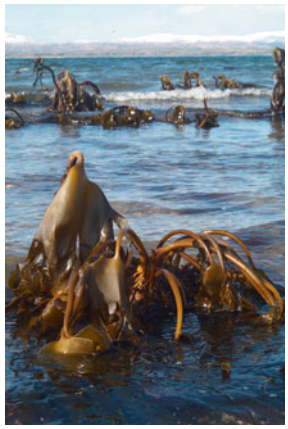
In 1896, Eugen Baumann (1846–1896, German chemist, best known for the Schotten-Baumann reaction) demonstrated in the thyroid gland the presence of iodine, associated with organic material at levels of 0.05 to 0.45 %. Gaspard Adolphe Chatin (1813–1901), a French physician, recognised the connection between the iodine content in drinking water and the morbidity (frequency of occurrence) of cretinism. The human body contains around 10–30 mg of iodine, of which 70–80 % is located in the thyroid gland. The iodide from diet, *e.g.* seafood, bread, or salt (recommended daily amount: 0.15–0.2 mg), serves as a source for the iodination of tyrosine. According to an estimate by the World Health Organisation (WHO), more than 2 billion individuals suffer from inadequate iodine intake, 266 million of which are school-aged children.

6.20 *Carl Adolph von Basedow (1799–1854), a renown physician, engaged also in preventive health and hygiene, showed in 1844, that volatile organoarsenic compounds are released from Paris Green by Penicillium brevicaule. That explained the cases of intoxication from mouldy wallpapers printed with this pigment.*

Hibernating mammals reduce their basal metabolism by lowering their thyroxine level. Correspondingly, injection of thyroid hormones interrupts the hibernation cycle.

6.2.1 Iodine Supply

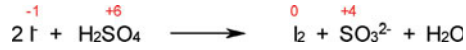
The supply of iodine is dependent on a unique accumulation process, performed by inconspicuous marine organisms, namely algae. The average concentration of total dissolved iodine in sea-water is about 450 nM. Brown algae (*Phaeophyceae*), such as *Laminaria digitata* (a species of kelp, oarweed) and *Fucus vesiculosus*, are believed to be the most potent iodine accumulators, with an average iodine content of 1.0 % of dry weight (Fig. 6.21). They use iodide as an antioxidant. At low tide, high iodide concentration at the algal surface reacts with atmospheric oxidants, thereby forming molecular iodine. Further oxidation by ozone in the marine boundary layer results in ultrafine particles of hygroscopic iodine oxides, which are transported as aerosols in the coastal atmosphere.



6.21 *Brown algae on the shore at Dunstaffnage, Scotland, at low tide.*

A recent study in Ireland supports the hypothesis that human iodine intake in coastal regions depends significantly on the abundance of seaweed. [83]

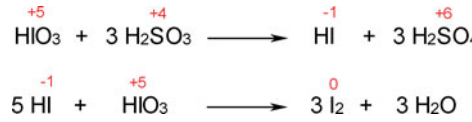
The technical exploitation of iodine may also be traced back to brown algae. Iodine was first discovered during the Napoleonic wars (1803–1815) in 1811 by the French chemist Bernard Courtois (1777–1838) in the course of isolating sodium carbonate from seaweed ash for the production of gunpowder. The addition of concentrated sulfuric acid to this ash not only resulted in serious corrosion of his copper vessels, but also led to the emission of a previously unobserved violet vapour. [83]

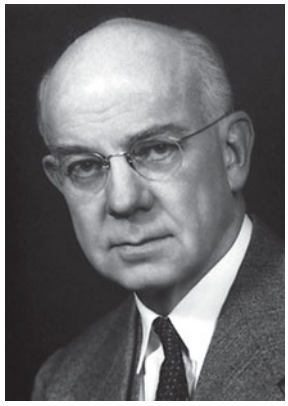


In the 19th century, iodine production from seaweeds became a major economic activity in the coastal regions of Europe, in particular in parts of Brittany, Normandy, Ireland, and Scotland.

Today, iodine production is conducted in areas where brines from natural gas and oil fields contain high iodine concentrations. One of these sources is the brine from the Southern Kanto gas field in Japan, which contains approximately 100 ppm of iodine.

However, worldwide the largest production source of iodine is the Atacama Desert in Chile. In addition to nitrate, the mineral *caliche* contains considerable amounts of iodate, which by reduction with sulfur dioxide and an intermolecular redox reaction (comproportionation) is converted into elementary iodine. The iodine produced is supplied to the market as a flaked, granulated, or prilled solid, with a purplish-black metallic lustre. [83]



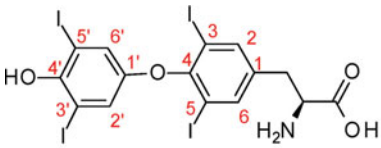


6.22 *In 1950, along with Tadeusz Reichstein and Philip Showalter Hench, Edward Calvin Kendall (1886–1972) was awarded the Nobel Prize in Physiology or Medicine for their discoveries relating to the hormones of the adrenal cortex.*

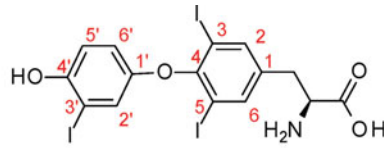
Nowadays, iodine is widely used for the manufacturing of X-ray contrast media, antimicrobial products, as tinctures of polyvinylpyrrolidone-iodine (Povidone-iodine), catalysts in chemical processes (*e.g.* for the production of acetic acid by carbonylation of methanol in the presence of a rhodium iodide-catalyst (Monsanto process) or an iridium iodide-catalyst (Cativa process)), and also on a smaller scale for the production of pharmaceuticals like thyroid hormones. [83]

6.2.2 Discovery of Thyroid Hormones

In 1915, Edward Kendall (1886–1972) was the first to isolate thyroxine from the thyroid glands of pigs (Fig. 6.22). [82] From 3,000 kilograms of thyroid glands he obtained by alkaline hydrolysis 33 grams of pure thyroxine. In 1926/27, Sir Charles R. Harington (1897–1972) elucidated the structure of thyroxine, and in 1950, Rosalind Pitt-Rivers (1907–1990) and Jean Roche (1901–1992) simultaneously discovered that by comparison with thyroxine, 3,3',5-triiodothyronine was five times more active (Fig. 6.23).



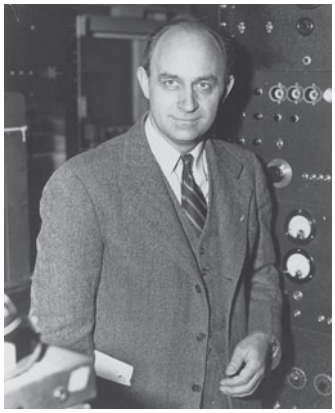
Thyroxine



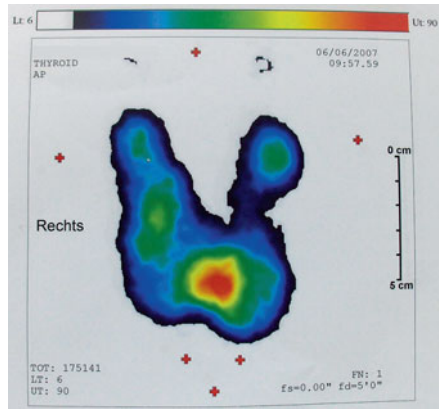
3,3',5-Triiodothyronine

6.23 *The thyroid hormones belong to a group of approximately 110 known iodine-containing natural products, most of which originate from marine organisms.*

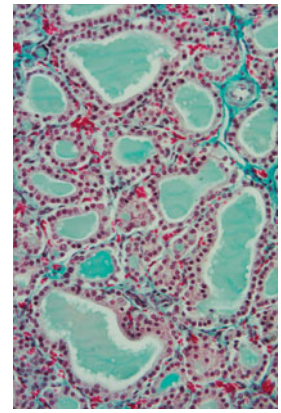
After Enrico Fermi (Fig. 6.24) had described in 1934 the first radioactive isotope of iodine, ^{128}I, it became possible to explore the biosynthesis of thyroxine. For the first time, radioiodine enabled as a "theranostic" agent the diagnosis of thyroid cancer by autoradiography and also its radiologic treatment (Fig. 6.25).



6.24 *On 2 December 1942, Enrico Fermi (1901–1954) succeeded in carrying out the first nuclear chain reaction with equipment, which had been built in a makeshift laboratory under the grandstands of Chicago's Stagg Field Stadium.*



6.25 *Scintigram of the thyroid gland.*

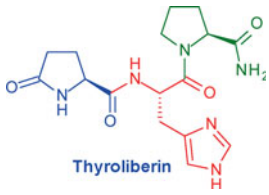


6.26 *Microscopy of the human thyroid gland. The tissue consists of spheric vesicles (follicular cells), which are surrounded by epithelial cells and store in their colloid-filled lumen thyroid hormones, bound to thyroglobulin.*

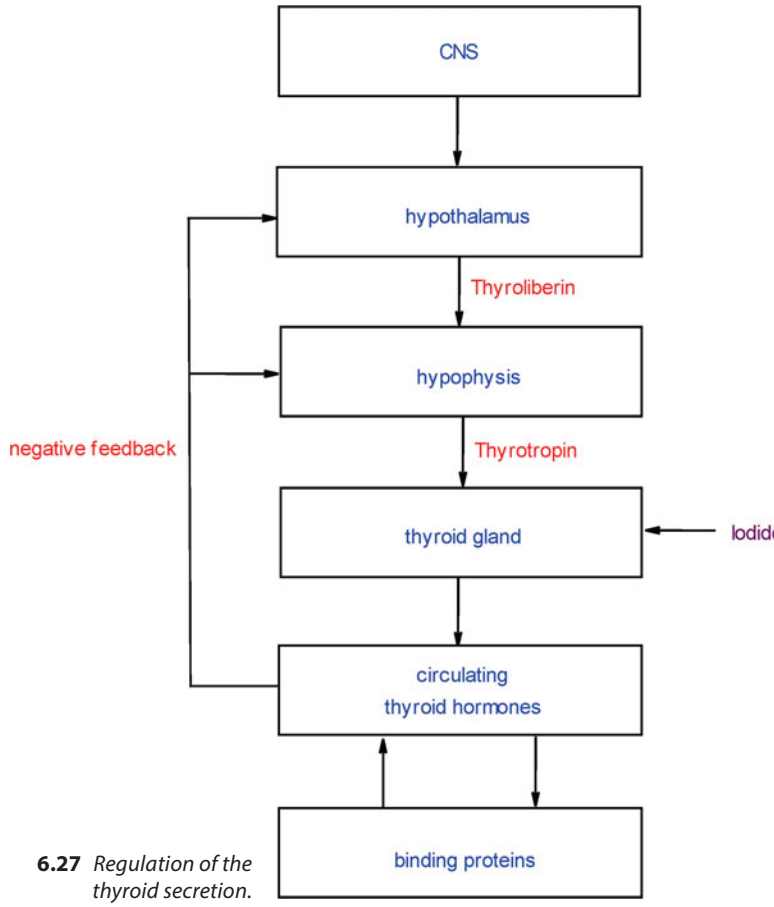
Radioactive iodine gained notoriety through the nuclear disaster at the Chernobyl power plant in 1986, which resulted in an increase of thyroid carcinomas among small children by a factor of around 10–30. It is now presumed that many of these cancer cases might have been prevented by prophylactic administration of iodide. The longer term consequences of the nuclear fallout from the Fukushima Daiichi accident in 2011, where also a number of different radionuclides were released, are still being evaluated.

6.2.3 Physiology

The iodine is stored predominantly as thyroxine, and to a lesser extent as 3,3',5-triiodothyronine, in colloid-filled vesicles (follicular cells), which are surrounded by connective tissue within the thyroid gland (Fig. 6.26). [84] The hormone is bound to a 660 kD protein, thyroglobulin. Stimulation by the tripep-



tide thyroliberin (thyrotropin-releasing hormone (TRH)) and the 26–30 kD glycoprotein thyrotropin (thyroid-stimulating hormone (TSH)), initiates enzymatic proteolysis and releases the hormone into the blood stream (Fig. 6.27). In the blood, thyroxine binds to three carrier proteins, thyroxine-binding globulin (TBG), transthyretin (TTR, prealbumin) and serum albumin.



6.27 *Regulation of the thyroid secretion.*

The thyroid hormones control metabolism. They increase cardiac output and the excitability of the nervous system. In children, the maturing of the cerebral cortex, of the skeleton, the musculature and the genitalia, is stimulated. Both hormones are active only in their protein-unbound state. Free thyroxine and 3,3',5-triiodothyronine bind to receptors in the cell nucleus and in the mitochondria, where they activate protein synthesis and the production of adenosine triphosphate.

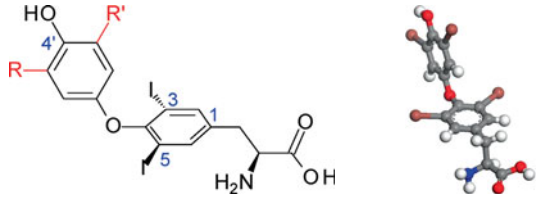
The endogenous secretion is estimated at a daily rate of around 90 µg of thyroxine and 10 µg of 3,3',5-triiodothyronine. The liver and kidneys deiodinate thyroxine to 3,3',5-triiodothyronine, the hormone, which binds with higher affinity at the nuclear receptor.

Clinical studies have demonstrated that a daily dosage of 1.5 to 1.3 µg thyroxine/kg is adequate for replacement therapy after thyroidectomy. Corresponding to its high potency, strict health and safety precautions are mandatory for the handling of this hormone. By comparison, the acute toxicity of a few well-known poisonous compounds is in a similar range. The LD_{50}-value of tetrodotoxin (from the puffer fish) lies at 10 µg/kg, of dioxin at 22 µg/kg, and of soman (nerve agent: *O*-pinacolyl methylphosphonofluoridate) at 80 µg/kg. The LD_{50}-value of thyroxine lies with 20,000 µg/kg twofold higher than of KCN.

Further metabolism of the thyroid hormones takes place in the liver and kidneys by deamination, decarboxylation, sulfatation or conjugation, but especially by deiodination, whereby around 20 % of the iodide, which is set free, is reused for the synthesis of thyroxine. The biological half-life of thyroxine is around 190 hours; that of 3,3',5-triiodothyronine is 19 hours. About 15 % of the thyroid hormones are excreted *via* the faeces, and only minor amounts into the urine.

6.2.4 Structure-Activity Relationships

In search for mimetics of the thyroid hormones, more than a hundred analogous compounds were prepared and the structural requirements for thyroxine-like potency investigated in binding studies, *e.g.* at the rat liver nuclear receptor (Fig. 6.28 and Tab. 6.1). [85]



6.28 *Structure-activity relationships of thyroxine mimetics.*

Tab. 6.1 *Relative binding affinity of thyroxine mimetics at the rat liver nuclear receptor.*

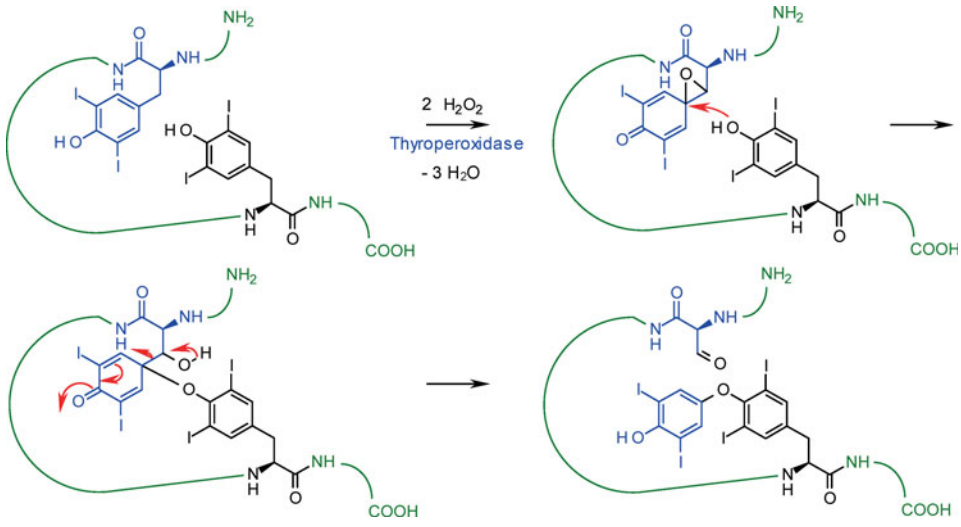
R	R'	Relative binding affinity
I	H	1
i-Pr	H	0.89
s-Bu	H	0.78
i-Pr	Cl	0.53
n-Pr	H	0.24
i-Pr	Br	0.22
Br	H	0.16
I	I	0.14
i-Pr	I	0.12
t-Bu	H	0.08
Br	Br	0.05
Cl	H	0.04
Cl	Cl	0.04
Me	H	0.03
F	H	0.02
i-Pr	i-Pr	0.01

Important for the hormonal activity of thyroid mimetics are:

- Two aromatic rings, which are electronically isolated from each other. Oxygen, sulfur or carbon may serve as the linking atoms, with a preferred bond angle close to 120 °.
- There may be alkyl or halogen substituents at the 3- and 5-positions. They should however be sterically demanding, in order to align the two aromatic systems orthogonally to each other.
- Position 1 should carry an acidic function, which is separated from the ring system by two or three carbon atoms. While an (*L*)-alanyl moiety does reduce the binding affinity at the receptor level, it prolongs however the *in vivo* activity by lowering metabolic degradation and decreasing the excretion rate.
- Isosteric residues in the 4'-position, like NH_2, lower the activity. Substituents, which can not be metabolised to OH turn the compounds pharmacologically inactive.
- At least one lipophilic substituent, for example an isopropyl group, should be *ortho* to the 4'-position. Both atropisomers possess different biological activity. As a rule, the receptor affinity of the isomer with distal (remote) conformation is higher than of the proximal one.

6.2.5 Biosynthesis

Although the biosynthesis of thyroid hormones has been investigated for more than 50 years, our understanding of some important steps is still incomplete. There is agreement that iodide is oxidised by peroxidases to a species, which iodinates tyrosine twice; further, that thyroxine is formed in a peroxide mediated inter- or intra-molecular coupling reaction [86, 87] of two 3,5-diiodotyrosine units by a radical [88] or an ionic mechanism. Supported by mass spectrometry (ESI-MS) data generated with a nonapeptide, Charles Sih was able to prove that aminomalonic semialdehyde occurs as C_3 fragment, and he postulated the following mechanism:

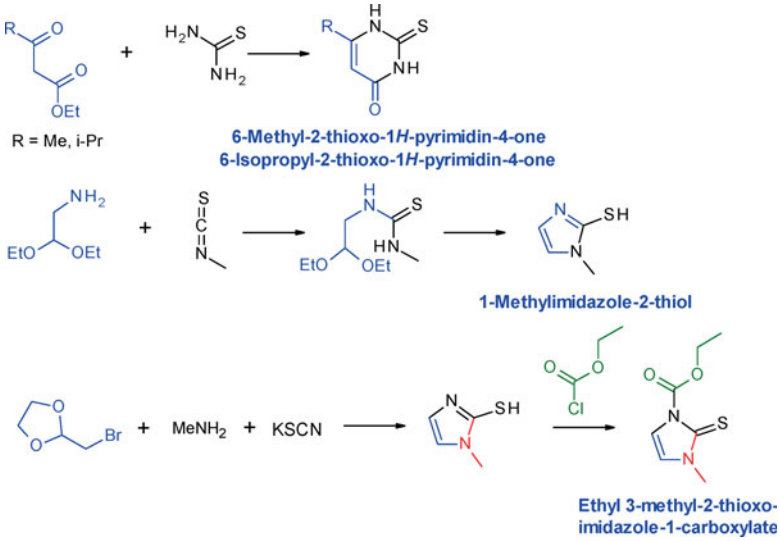


6.2.6 Hyperthyroidism

For the treatment of thyroid hyperactivity, there is a range of options:

- Surgical removal of parts of the thyroid gland ("hot" nodules or tumour tissue)
- Destruction of thyroid tissue by radioactive iodine (^{131}I, β-radiation)
- Blocking of the biosynthesis of thyroxine with thionamides
- Inhibition of thyroxine release with lithium preparations
- Inhibition of peripheral deiodination of thyroxine to the active 3,3',5-triiodothyronine with thiouracil derivatives
- Increased excretion of thyroid hormones by sequestration with bile acids
- Application of competitive antagonists at the thyroid receptors level to disrupt signal transduction

Apart from surgery, blocking of the biosynthesis of the thyroid hormones is of particular interest. There are several hundred thionamides known, which exert their activity by inhibiting thyroperoxidase, an enzyme required for iodination and coupling of the tyrosine residues. However, only a few of these drugs are approved and in clinical use. Their synthesis employs conventional heterocyclic chemistry.

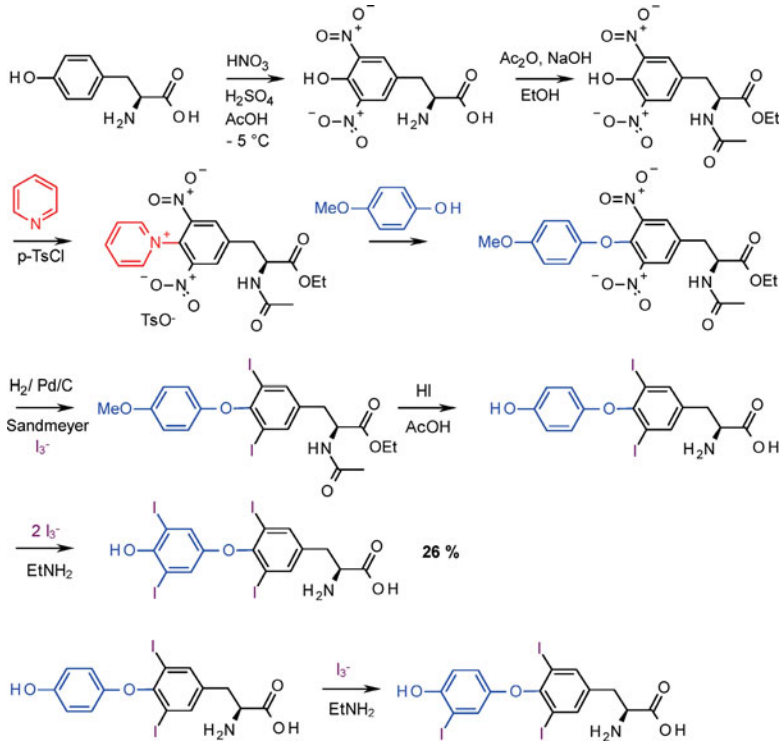


A disadvantage for 6-isopropyl-2-thioxo-1*H*-pyrimidin-4-one is its short plasma half-life. The corresponding glucuronide is excreted very rapidly *via* the urine. To overcome the bitter taste of 1-methylimidazole-2-thiol, the corresponding ethylcarbamate has been developed as a suitable prodrug.

6.2.7 Industrial Synthesis

Prior to the synthetic preparation of thyroxine, hypothyroidism was treated with extracts of dried thyroid glands from animals. Standardisation was however not easy, and the use of these nutritional supplements caused occasionally serious side-effects. Industrial manufacturing of the pure hormone began around 60 years ago. Its synthetic challenges consist in the formation of the diphenyl ether linkage and the iodination of the aromatic rings. [89]

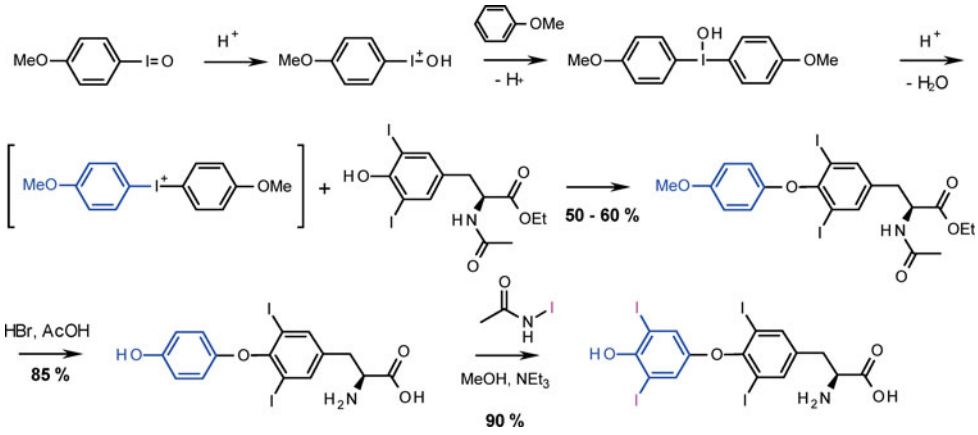
The first of the methods described here for the production of thyroxine is the so-called Glaxo synthesis. [90] (*L*)-Tyrosine serves as the starting material. Dinitration and *N*-protection of the amino acid function lead to an important intermediate. Conversion of the phenolic hydroxy-group with tosyl chloride and pyridine gives, supported by the activating nitro-groups, the pyridinium salt, which undergoes nucleophilic substitution by *p*-methoxyphenol. Tosyl chloride can be advantageously replaced by methanesulfonyl chloride. The nitro-groups are then reduced and the resulting amino-functions replaced by iodine in a Sandmeyer reaction. After removal of the protecting groups, iodination takes place at 3'- and optionally also at 5'. The overall yield amounts to 26 %.



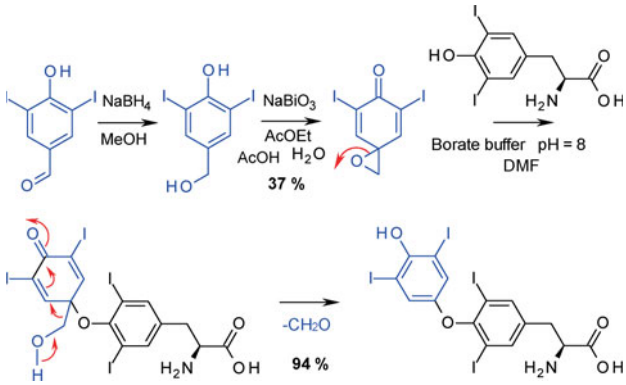
Iodonium salts are excellent arylating reagents, which usually undergo nucleophilic substitution reactions. Their reactivity is comparable to that of a diazonium group. Carboxylates lead to aryl esters, alkoxides and phenoxides to ethers. Thiols react analogously. Sulfite gives sulfonic acids, and sulfinates are converted into sulfones. The reaction with nitrite produces nitro-compounds. Amines give arylamines. From the reaction with cyanide ion, aryl nitriles are obtained. In addition, the reaction with organo-lithium or Grignard reagents leads to the formation of C-C-bonds. The reaction with lithium aluminium hydride removes the functionality. [91]

The advantage of this synthesis is that it provides both hormones in pure form. The modular construction of the diphenyl ether allows also introducing any substituent at the 3'- and 5'-positions, which is extraordinarily useful for exploring structure-activity relationships.

A considerable simplification of the synthesis is owed to the work of Günther Hillmann (1919–1975) and his wife Anneliese. [92] 3,5-Diiodotyrosine can be etherified directly with a 4,4'-dimethoxyphenyliodonium salt. [93, 94] After acidic cleavage of the protecting groups, iodination occurs with *N*-iodoacetamide.

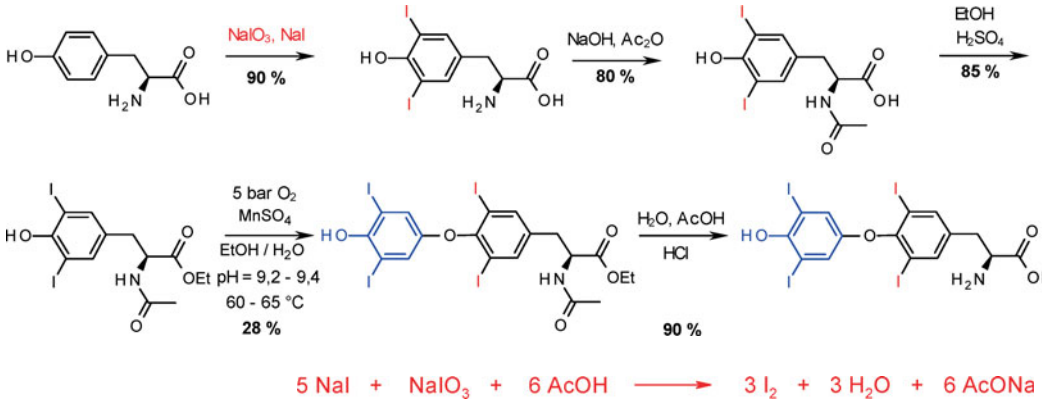


In 1997, Charles J. Sih published an interesting coupling reaction of the two aromatic systems. [95] 4-Hydroxy-3,5-diiodobenzaldehyde is reduced with sodium borohydride to the corresponding benzyl alcohol. Oxidation with sodium bismuthate produces a quinonoid oxirane (unfortunately only in 37 % yield), which can be converted in a smooth reaction with diiodotyrosine (under loss of formaldehyde) into thyroxine.



One of the most impressive and unusual natural product syntheses in the chemical industry is the biomimetic oxidation of 3,5-diiodotyrosine. [96] In view of the extremely high potency of the hormone, the added value is considerable, and therefore the yield and associated manufacturing cost play only a tangential role.

In the actual production process, *N*-acetyl-3,5-diiodotyrosine ethyl ester is oxidised in aqueous ethanol at 60–65 °C with oxygen, in the presence of catalytic amounts of manganese(II) sulfate, at pH 9.2–9.4 over a period of 20 hours.

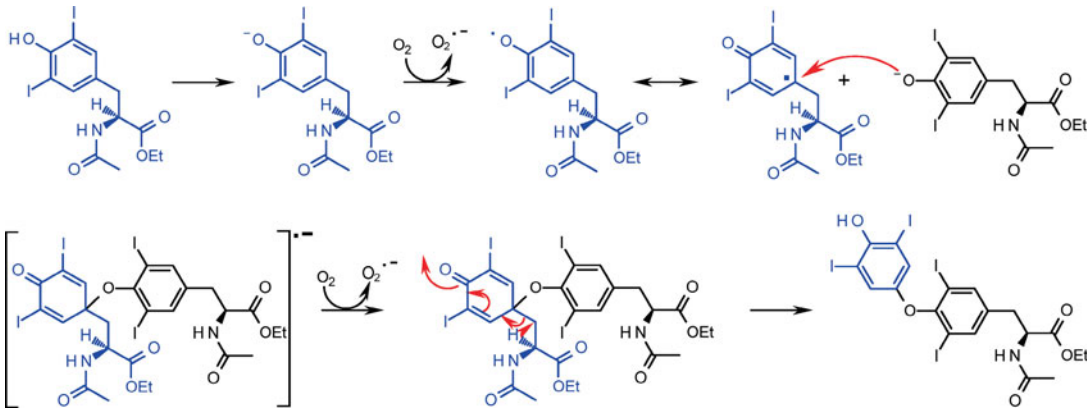


A slightly elevated temperature is necessary for the reaction, which is accelerated by excess oxygen pressure. Control experiments without manganese sulfate give the correct product, although in poor yield. No reaction occurs in the absence of oxygen, neither as a redox reaction of diiodotyrosine nor through oxidation with stoichiometric amounts of tri- or tetra-valent manganese salts (Tab. 6.2).

Tab. 6.2 *Optimisation experiments of oxidative coupling*

Catalyst	O_2 or N_2	Pressure	Temperature	Reaction Time	Product Yield
$MnSO_4$	O_2	5 bar	25 °C	48 h	1 %
$MnSO_4$	O_2	atmospheric	reflux	48 h	22 %
$MnSO_4$	O_2	5 bar	60–70 °C	20 h	28 %
–	O_2	5 bar	reflux	48 h	16 %
$MnSO_4$	N_2	5 bar	reflux	48 h	0 %
1 eq. MnO_2	N_2	atmospheric	reflux	48 h	0 %
1 eq. $Mn(OAc)_3$	N_2	atmospheric	reflux	48 h	0 %

Following a mechanism also discussed for the biosynthesis, oxygen oxidises the phenoxide to a phenoxy radical, which is very rapidly converted by another phenoxide into a radical anion. The initially formed radical anion originally contains the unpaired electron in an antibonding C-O σ*-orbital. Through rapid reorganisation of the orbitals, the radical anion is stabilised, so that the unpaired electron is then located in a π*-orbital. In the second oxidation step, the electron is transferred to oxygen. The aromatisation provides the driving force for the subsequent fragmentation. Since the C_3-fragment was not unambiguously characterised, the mechanism remains up to now incompletely understood. [97]



After hydrolysis of the protecting groups, the free amino acid is converted with sodium carbonate into its sodium salt, which has better bioavailability. In the commercial product, the drug is present as its pentahydrate (Fig. 6.29).

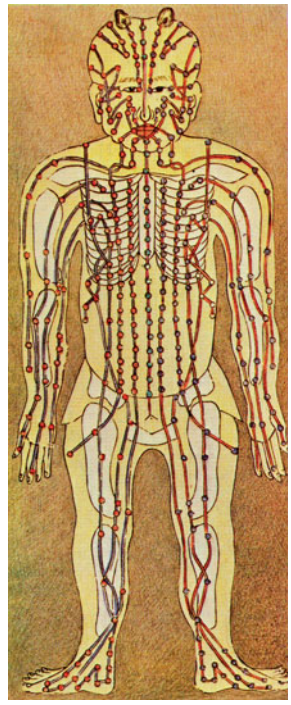
6.29 *Synthroid® contains synthetic thyroxine as the active ingredient.*

Summary in Bullet Points

- An overactive or underactive thyroid gland can lead to a range of serious illnesses.
- The hormones produced by the thyroid gland are thyroxine and the five times more potent 3,3',5-triiodothyronine.
- The biosynthesis starts from tyrosine, which is iodinated and oxidatively coupled.
- The annual demand worldwide amounts to only a few tonnes of thyroxine; since the market price of the active ingredient is high, and an enormous value is added by a smart synthesis, the production process can even afford partially capricious methods.

6.3 Adrenaline

Over the last few decades, a deeper medical understanding of the hormones, produced by the medulla of the adrenal gland (Latin: *glandula suprarenalis*), has been developed. For both hormones, adrenaline and noradrenaline (also known as "epinephrine" and "norepinephrine"), the empirical pharmacology dates back to more than five thousand years. The Chinese Emperor Huang-ti wrote according to legend in the third millennium BC the *Canon of Internal Medicine*, known as *Huangdi Neijing* (Fig. 6.30) [98], which has been treated as the fundamental doctrinal source for traditional Chinese medicine for more than two millennia. Even nowadays, significant importance is ascribed to this famous classic. The *Golden Mirror* compiles recovered medical writings from the Han Dynasty (202 BC–220 AD). Unsurpassed, however, is the *Materia Medica* of Li Shih-chen (1518–1593). His *Pen-ts'ao kang-mu* from 1578 summarises in 52 volumes the



6.30 *Chinese medicine is based on the philosophy of a dualistic cosmic theory, Yin and Yang. These complimentary forces are interacting through a network of channels in the human body. The objective of the "art of healing" is to restore the equilibrium of Yin and Yang.*

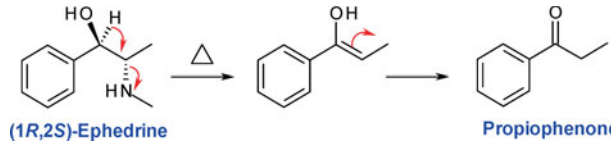
6.31 *Ephedra sinica belongs to the Ephedraceae family, which comprises around 45 species. These perennial plants with a strong pine-like smell and an astringent taste grow as large bushes up to over 1 metre high.*

complete available medical knowledge towards the end of the Ming Dynasty (1368–1644). It contains 142 illustrations and descriptions of more than 1,000 plants, 400 animals and 200 minerals. Li Shih-chen also assembled in his compendium more than 11,000 recipes for drug formulations, of which 8,000 new ones he had collected himself.

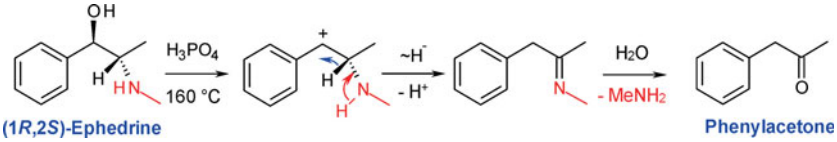
Among these preparations are a few, which have found their way into Western pharmacology. These include extracts from the camphor tree (*Cinnamomum camphora*), from hemp (*Cannabis sativa*), from various *Rauwolfia* species (*e.g.* reserpine), from ginseng (*Panax quinquefolium* and *Panax schinseng*) and from Ma-huang (*Ephedra sinica*).

In 1887, Nagajosi Nagai (1844–1929) discovered that the active substance in Ma-huang is ephedrine (Fig. 6.31). [99] The drug was extracted in basic solution with benzene. Washing the organic phase with dilute hydrochloric acid and concentrating the aqueous solution led to the crystallisation of ephedrine hydrochloride.

Upon dry heating, ephedrine decomposes into propiophenone (along with small amounts of phenylacetone) and methylamine. [100]



In elucidating its structure further, it turned out, that the drug undergoes hydramine cleavage when heated with acids. At 160 °C, predominantly phenylacetone and methylamine are formed by elimination of water, hydride migration and hydrolysis. [101]



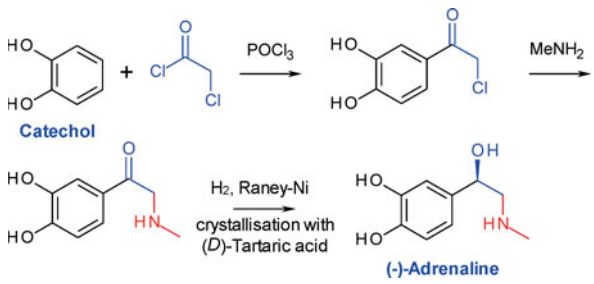
Ephedrine was also found later in the aconite or monkshood (*Aconitum napellus*), in the European yew (*Taxus baccata*) and in khat (or qat; *Catha edulis*). [102]

Ma-huang served for almost 2000 years in traditional Chinese medicine as a remedy to treat asthma and hay fever. The introduction of ephedrine into Western medicine in 1924 represented an important advance in the management of asthma and various allergic reactions.

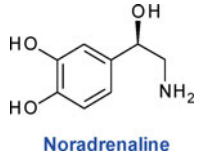
6.3.1 Discovery of the Hormones of the Adrenal Medulla

In 1856, the French physician Edmé Félix Alfred Vulpian (1826–1887) recognised the chromaffin tissue in the inner part of the adrenal gland (= adrenaline containing cells) by the green colour from staining with ferric chloride. Shortly before the turn of the century, in 1895, Edward Albert Sharpey-Schäfer (1850–1935) and George Oliver (1841–1915) observed that injection of the extract from the adrenal gland resulted in strong blood pressure increase in various animal species. Finally, in November 1900 Keizo Uenaka, an assistant to Jokichi Takamine isolated for the first time the pure crystalline hormone, which Parke-Davis licensed and marketed soon after under the trade name "*Adrenalin*" (Fig. 6.32). [103]

The constitution of adrenaline was independently elucidated and published in 1901 by Thomas Bell Aldrich (1861–1938) at Parke-Davis. Friedrich Stolz (1860–1936), a chemist at Hoechst, was able to confirm the structure through synthesis in 1904. The enantiomers can be separated by crystallisation with tartaric acid. The natural (–)-adrenaline is 15 times more potent than the (+)-enantiomer.



In 1946, the Swedish physiologist Ulf Svante von Euler-Chelpin (1905–1983) (see also *cf.* section 5.6 – Prostaglandins; Fig. 5.94) was the first to discover noradrenaline. This hormone has the same structure as adrenaline, except that it lacks the *N*-methyl group.



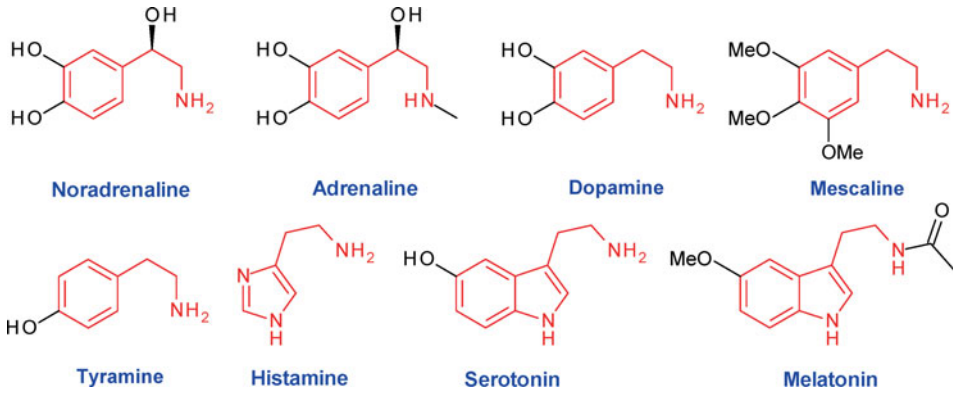
The blood pressure-raising effect of adrenaline is caused by a constriction of the blood-vessels. If, for example, mucous membranes are brushed with a highly diluted adrenaline solution (1 : 10,000), these become completely bloodless. To take advantage of this effect for small surgical procedures, adrenaline is added to local anaesthetics.

The utility of adrenaline in the treatment of asthma, as mentioned before, is due to its bronchodilation and antispasmodic effect. However, its poor oral bioavailability of around 3 %, and the short half-life of around 2 minutes, are

6.32 *Jokichi Takamine (1854–1922). It was his assistant Keizo Uenaka who got the crystals, but Takamine filed the patent application and claimed the fame.*

Takamine, who became a rather wealthy entrepreneur, might be remembered as well for funding more than 3,000 cherry trees, the mayor of Tokyo donated to the city of Washington, D.C. in 1912. Planted along the Potomac River and the Tidal Basin, these trees later inspired the National Cherry Blossom Festival, a highly treasured event to herald the beginning of spring in the US capital.

major disadvantages and limit its application range; therefore, adrenaline can be administered most effectively by intravenous infusion. Apart from other medications, oral inhalation of adrenaline can be used to alleviate acute asthma attacks. On the other hand, it was shown that oral administration of ephedrine, though less potent, had a considerably longer antispasmodic effect compared with adrenaline. Since the 1920s, synthetic ephedrine has therefore been used for the treatment of bronchial asthma, in order to relieve symptoms of cold by vasoconstriction of nasal capillaries and thus decreasing swelling of the mucous membrane, but also to treat allergic reactions and urinary incontinence.



6.33 *Pharmacologically active arylethylamines.*

Excursus

The catecholamines noradrenaline, adrenaline and dopamine belong to a larger group of pharmacologically highly active arylethylamines, which are likewise derived from amino acids (tyrosine, histidine and tryptophan) (Fig. 6.33). Tyramine raises the blood pressure and acts as a stimulant on smooth muscle (*e.g.* the uterus). Histamine leads to a dilation of the blood-vessels, increases secretion of gastric juice, and is a mediator of allergic reactions. Serotonin (5-HT) is a neurotransmitter found in many tissues, including blood platelets, intestinal mucosa, and the central nervous system. The latter molecule is the natural ligand of a large family of 5-HT receptors, including many subtypes, which translate into its rather complex pharmacology. Already in 1917, it was discovered that an extract of the pineal gland (epiphysis) of cows caused skin-lightening in tadpoles. The underlying effect is due to melatonin, a hormone which is involved in a broad spectrum of physiological and pathophysiological conditions. Its level controls and responds to the circadian and seasonal rhythmicity of physiological functions in humans.

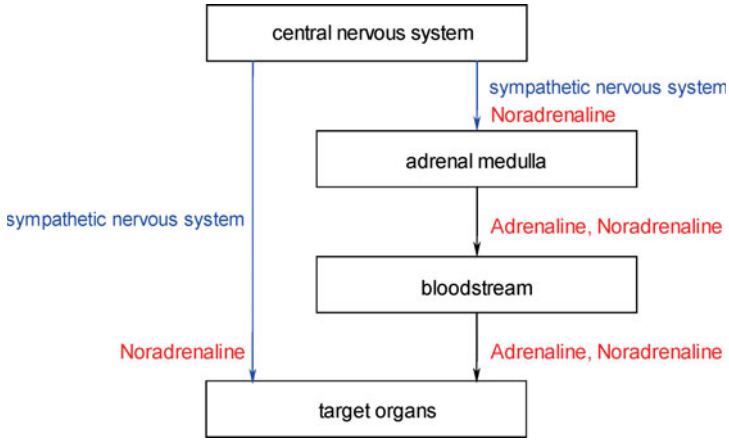
In contrast, mescaline [104] is an exogenous arylethylamine from the Mexican cactus species Peyote (Aztec: "root that excites"; *Lophophora williamsii*), which causes visual hallucinations, and which had already been used by the indigenous inhabitants of Mexico in the pre-Columbian era as a narcotic for their ritual festivals (Fig. 6.34). In 1896, mescaline was isolated for the first time from *Lophophora williamsii* by Arthur Heffter (1859–1925). In 1919, Ernst Späth (1886–1846) recognised its structural relationship to the catecholamines.

6.34 *Lophophora williamsii is native to southern North America and Mexico.*

6.3.2 Physiology

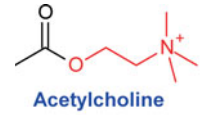
The autonomic nervous system [105], as part of the peripheral nervous system, acts largely independent of conscious control (involuntarily); it serves to maintain the organism's internal balance (circulation, breathing, peristalsis, muscle tone and secretion), and controls also visceral functions. According to Walter Holbrook Gaskell (1847–1914), the autonomic nervous system is sub-divided into two branches, which he named sympathetic and parasympathetic nervous system, in relation to their morphology and function. Stimulation of the sympathetic system mobilises the body's fight-or-flight response. Activation of the parasympathetic nerves is associated with protection, conservation, and restoration of body resources.

Sympathetic nerve fibres connect the brain stem to the target organs, such as eyes, heart, lungs, bronchi, stomach, kidneys, intestines, bladder and genitals. In case of stimulation, noradrenaline is released at the nerve endings into the synaptic cleft, from where it binds to postsynaptic receptors on the cell membranes of the target organ and initiates the actual effect. To avoid over-stimulation, excess noradrenaline binds to presynaptic receptors as well, whereby its further release is inhibited. If the entire organism is involved, *e.g.* in situations of stress, the sympathetic nervous system initiates not only the release of noradrenaline, but predominantly of adrenaline from the adrenal medulla, which travel to the target organs *via* the blood circulation (Fig. 6.35). [106]

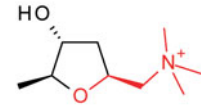


6.35 *Schematic description of the sympathetic nervous system.*

Upon stimulation, acetylcholine (Ach) is released at the parasympathetic nerve endings and displays activity on a number of different cell types, mediated by two major classes of receptors. While muscarine, an alkaloid of the fly agaric (*Amanita muscaria*) and other mushrooms, binds more selectively than acetylcholine at certain ACh-receptors, these are called muscarinic receptors or mAChRs. On the other hand, nicotinic-type AChRs function as ligand-gated ion channels and show selectivity for nicotine over ACh.



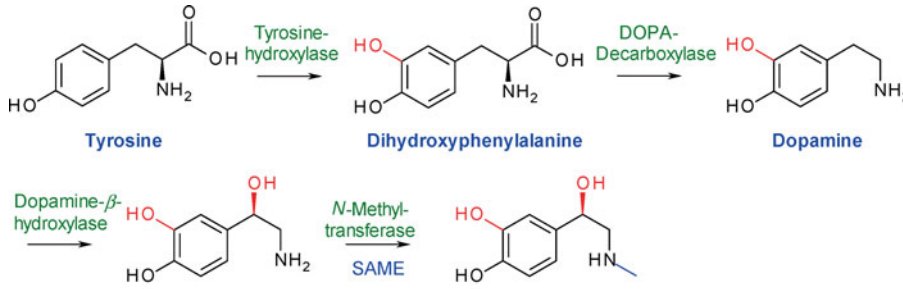
Acetylcholine



Muscarine

6.3.3 Biosynthesis

Like the thyroid hormone thyroxine, noradrenaline and adrenaline originate as well from the amino acid tyrosine, which is formed in the liver by hydroxylation of phenylalanine. Tyrosine is hydroxylated a second time in the aromatic ring and, subsequent to decarboxylation, again in the side-chain. The *N*-methylation of noradrenaline with *S*-adenosylmethionine in the chromaffin cells of the adrenal medulla leads finally to adrenaline. [107]

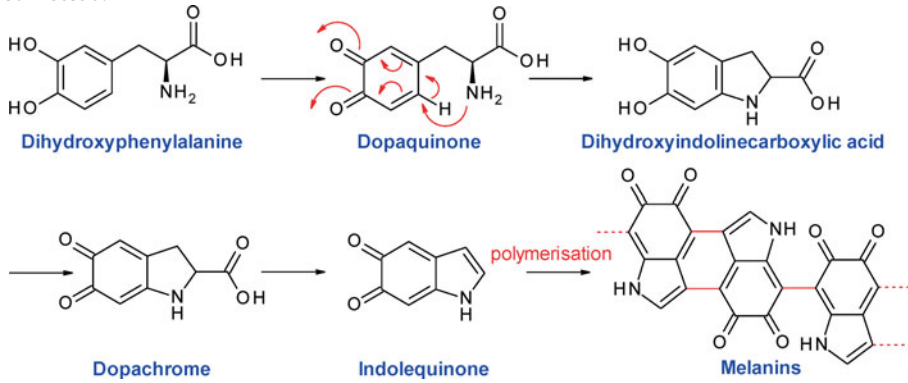


In other areas of the brain, especially in the *substantia nigra*, phenylalanine metabolism ends at the stage of the neurotransmitter dopamine. Current therapeutic intervention of Parkinson's disease, a degeneration of the *substantia nigra*, aims primarily at substitution of the reduced dopamine levels. Since dopamine itself does not cross the blood-brain barrier, dihydroxyphenylalanine (DOPA) is administered instead.

Which of the catecholamines is formed in a cell, depends on its armamentarium of available enzymes. The adrenal medulla produces, besides noradrenaline, mainly adrenaline; the former is as well generated in particular sections of the brain stem.

Melanin

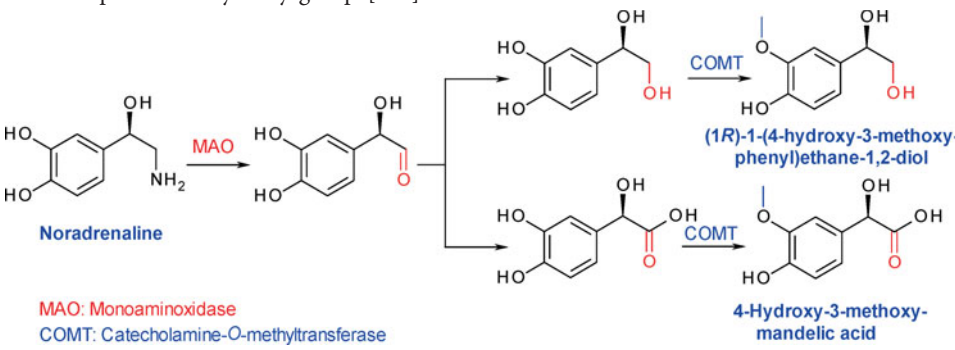
Dihydroxyphenylalanine is also an intermediate for the generation of the brown-black pigments, melanins, which are responsible for skin pigmentation. Following oxidation of DOPA with a copper-containing phenoloxidase to the corresponding quinone, spontaneous ring-closure to the dihydroxyindolinecarboxylic acid occurs. Further oxidation steps on the same enzyme and decarboxylation lead to the indolequinone, which finally polymerises to melanins of variable molecular masses. [108]



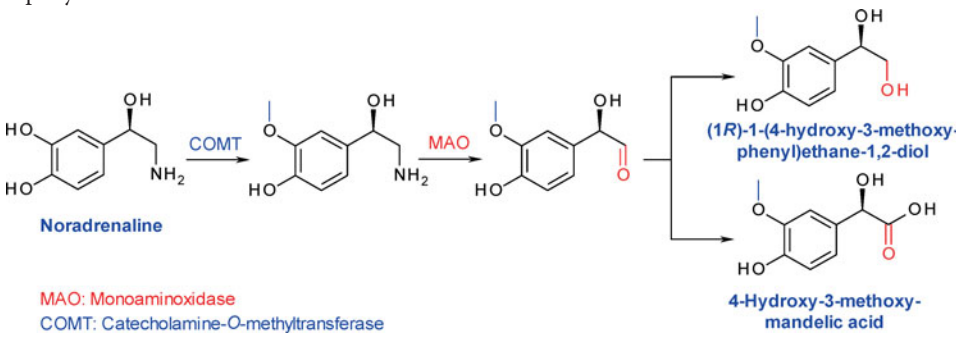
6.3.4 Deactivation

A precise control of the noradrenaline concentration in the synaptic cleft is crucial for an efficient signal transduction. For deactivation, there are several mechanisms available. Prior to reaching the post-synaptic receptors, around 90 % of the noradrenaline is reabsorbed into the pre-synaptic axoplasm. A certain amount is bound by extraneuronal cells, while another portion is deactivated by methylation and oxidative deamination. The oxidative deamination occurs *via* an imine in the mitochondria of nerve endings, in cells of the target organ, and in the liver. After oxidation or reduction of the aldehyde function,

catecholamine-*O*-methyltransferase in the target organ or in the liver methylates the *meta*-positioned hydroxy-group. [109]



The reaction may also occur in an inverse sequence. This route is more relevant for the degradation, because the methylation of noradrenaline proceeds more rapidly than the oxidative deamination.



The metabolites are released into the blood and renally excreted. Thus, urine analysis provides insight into the activity of the sympathetic nervous system, and possibly reveals as well indicators of diseases. Over the last decade, diagnostic capabilities have been expanded by the rapidly growing arena of "metabolomics", where advanced NMR and mass spectrometry techniques enable the identification of biomarkers and disease signatures from different body fluids.

6.3.5 Pharmacology

In 1948, the American pharmacologist Raymond Perry Ahlquist (1914–1983) published a paper [110] that initially drew little attention, but should ultimately greatly enhance our understanding of the pharmacology underlying noradrenaline and adrenaline. His concept that adrenergic receptors fall into two basic classes, namely α- and β-receptors, was able to explain the activity of several existing drugs, and also paved the way to develop new drugs, in particular for the nowadays widely prescribed beta blockers.

Ahlquist's research resulted in the conclusion that various organs express different receptor subtypes. For example, the effects of the sympathetic nervous system on the heart are predominantly mediated through β_1-receptors, but those

Already in 1910, George Barker (1878–1939) and Sir Henry Hallett Dale (1875–1968) had coined the term "sympathomimetic" for adrenergically active amines. [103, 111]

on the bronchi through β_2-receptors. At present, α_1-(with α_{1A}, α_{1B}, α_{1D}), α_2-(with α_{2A}, α_{2B}, α_{2C}), β_1-, β_2- and β_3-receptors are known. All these receptors are located in the cell membrane and belong to the large group of G-protein-coupled receptors (GPCRs) with seven membrane-spanning domains (TMD). This classification of adrenergic receptors into sub-types, and the insight into their organ-related expression profile, proved very helpful to advance pharmacotherapy, and to develop subtype-selective drugs with improved side-effects properties (Tab. 6.3).

Tab. 6.3 *Effects of selective stimulation of adrenergic receptor sub-types*

Receptor	Effect/Location
α_1	Activation of phospholipase C (PLC), which ultimately triggers release of calcium ions from the sarcoplasmatic reticulum and their cellular efflux, leading to smooth muscle contraction Located in smooth muscle, heart and liver
α_2	Inhibition of adenylate cyclase, which in a cascade of signalling events leads to the opening of cellular calcium channels and thereby increased calcium influx into the cytosol. This causes, besides numerous other effects, *e.g.* vasoconstriction of certain arteries Located in platelets, vascular smooth muscle, nerve termini and pancreatic islets
β	Activation of adenylate-cyclase, thereby increased formation of cAMP and stimulation of cAMP dependent kinase activity; leads to smooth muscle relaxation, but *e.g.* to increased contraction force and rate of the cardiac tissue
β_1	Located mainly in the heart
β_2	Located mainly in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle
β_3	Located in brown adipose tissue (fat cells), intestinal smooth muscle, and blood vessels

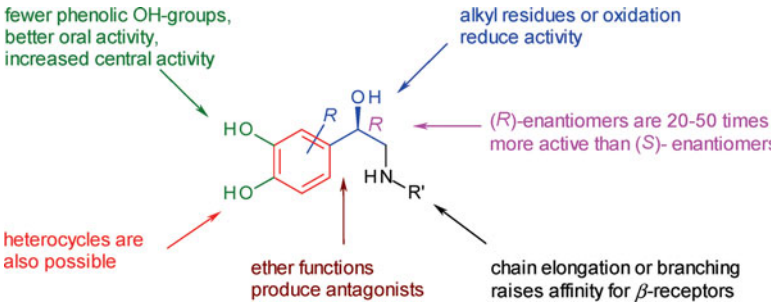
Noradrenaline and adrenaline, the endogenous neurotransmitters, display a fairly unselective affinity profile towards the adrenergic receptors. Whereas adrenaline binds in general somewhat tighter than noradrenaline to α_1-, α_2- and β_1 -receptors, at β_2-receptors, adrenaline has significantly stronger affinity. Therefore, its effect on the smooth muscle of the intestines and the bronchi is most pronounced. In addition, human lung tissue possesses, with 30,000–40,000 β_2-receptors per cell, the highest β_2-receptor density.

Adrenaline shows correspondingly a relaxing effect on intestinal and bronchial musculature, with decreased peristalsis and ease of oxygen uptake, whereas noradrenaline has rather low potency at these sites.

Noradrenaline acts predominantly as an α-mimetic.

6.3.6 Structure-Activity Relationships

The pharmacological difference between adrenaline and noradrenaline can be attributed to the *N*-methyl group. Replacing this methyl group in a synthetic sympathomimetic molecule by larger alkyl substituents, increases the affinity towards β-receptors, while α-receptors become less affine (Fig. 6.36).



6.36 *Structure-activity relationships of sympathomimetics.*

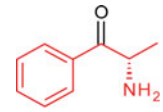
Alkylation of the phenylethane skeleton, or oxidation of the alcohol function (as in (*S*)-cathinone) weakens the activity. Of greater importance for pharmacological efficacy is the absolute configuration of the alcohol. The (*R*)-enantiomers are clearly more potent than the (*S*)-enantiomers. If the number of phenolic hydroxy-groups is reduced, the oral availability is improved, and central effects exceed the peripheral ones. The benzene ring is itself not essential and can be replaced by various heterocycles. If an ether function is introduced between the aromatic moiety and the side-chain of a sympathomimetic, the typical structural pattern of first and second generation antagonists is obtained.

The metabolic stability of the drugs can be enhanced by replacing the hydroxyl-functions of the catechol with other substituents, so that they are no longer substrates for catecholamine-*O*-methyltransferase and undergo deactivation by methylation. Substituents at the nitrogen or its neighbouring carbon can minimise oxidative degradation by the monoaminoxidase. [113]

In 1986, the β_2-adrenergic receptor became the first GPCR cloned. [114] Since then, a plethora of molecular- and cellular-biology publications have largely advanced our understanding of drug-receptor interactions (Fig. 6.37). [115] Like many G-protein-coupled receptors, the β_2-receptor has also two agonist binding sites with different affinity, which result from two different receptor conformations. Recently, the crystal structure of these low- and high-affinity conformers has been successfully determined. [116]

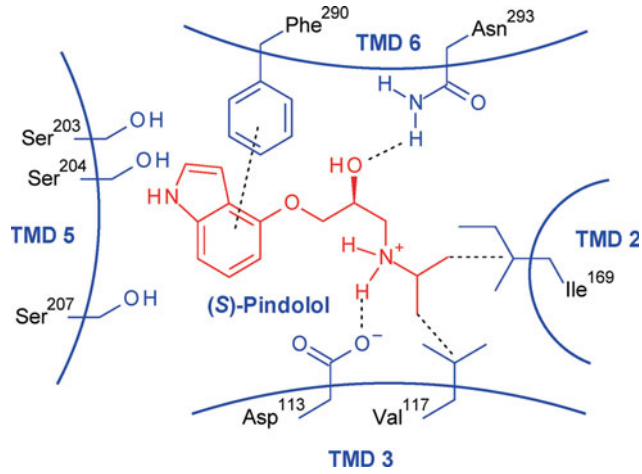
The adrenergic ligands form an ionic interaction (salt bridge) with aspartic acid-113 (Asp-113), which is highly conserved in the third transmembrane domain (TMD 3) of all aminergic receptors. Other important sites that contribute to the ligand-receptor affinity are a hydrogen bridge to asparagine-293 (Asn-293) and the π/π-interactions with phenylalanine-290 (Phe-290) in the sixth transmembrane domain (TMD 6). Lipophilic interactions occur between the

Khat (*Catha edulis*), indigenous to the higher elevations of Yemen and Somalia, has been mentioned for the first time around 1300. Its principal alkaloid is (–)-(*S*)-cathinone. The freshly-cut leaves of the tree, which grows up to a height of 25 metres, are chewed and induce a mild state of intoxication. Tiredness fades away, physical work becomes easier and the sensation of hunger is suppressed. Side-effects upon long-term use are gastritis, loss of appetite, impotence and constipation. [112]



(-)-(S)-Cathinone

second and third transmembrane domains and the *N*-alkyl substituent. If a drug is in the position to form hydrogen bonds to the serine residues in the fifth transmembrane domain (TMD 5), it acts as a full or partial agonist.



6.37 *Drug-receptor interactions as exemplified by an adrenoceptor-antagonist at the β_2-receptor.*

6.3.7 Adrenergic Drugs

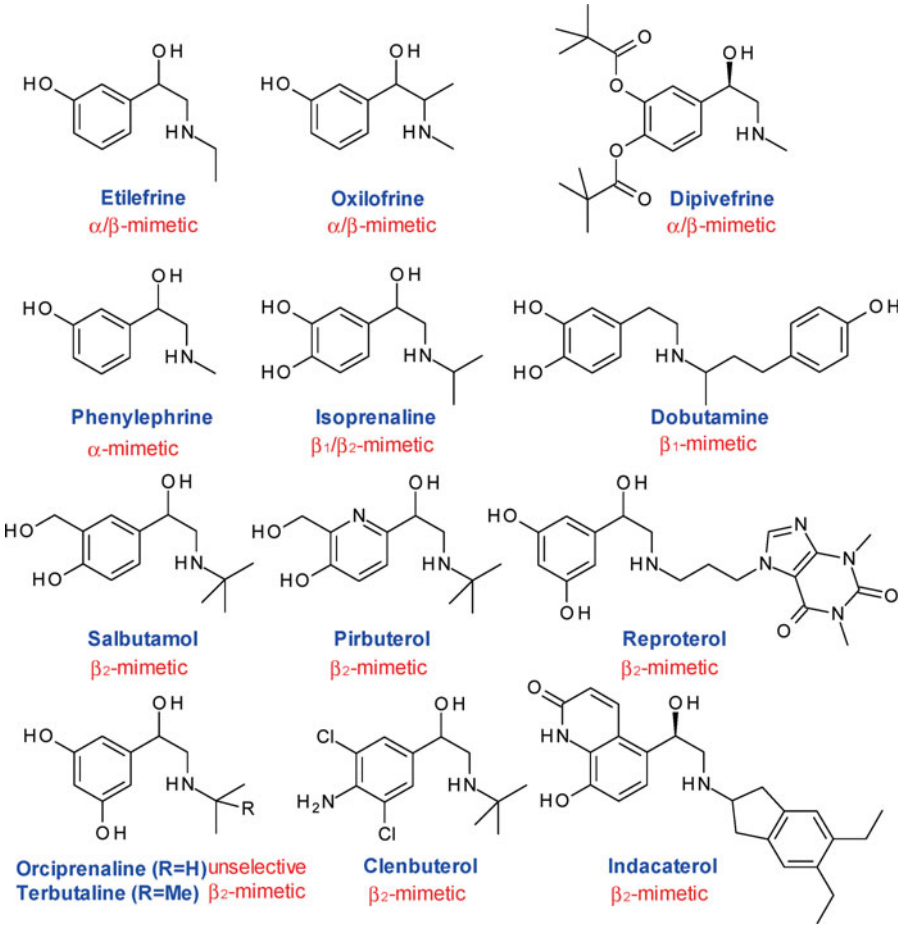
Oxilofrine had been popular amongst cyclists and sprinters, as doping tests uncovered.

Directly acting α- and β-sympathomimetics (α- and β-agonists) stimulate the adrenergic receptors by binding to these like noradrenaline or adrenaline. Nowadays, a broad variety of both, non-selective and selective sympathomimetics is at our disposal (Fig. 6.38). Etilefrine and oxilofrine are non-selective agonists, which elevate the blood pressure. This can be ascribed to an α-adrenergical vasoconstriction and a β_1-adrenergic effect on the heart, which increases the heart rate and the cardiac contractile force. Dipivefrin lowers the intraocular pressure by decreasing the production and increasing the outflow of vitreous humour; it is therefore used in the treatment of glaucoma. This bis-pivalate prodrug of adrenaline penetrates the retina unaltered and takes advantage of particular metabolic capabilities in the eye, where the drug is hydrolysed around 20 times more rapidly than in other tissues. Phenylephrine, an *α*-selective sympathomimetic, shrinks swollen mucous membranes.

The Austrian pharmacologist Heribert Konzett (1912–2004) had discovered in 1940 isoprenaline, which should become the prototype of β-selective sympathomimetics. Due to its bronchodilatory properties, but without causing hypertention, it was considered the drug of choice to treat asthmatic attacks. With almost equally strong effect on β_1- and β_2-receptors, it was of concern, that isoprenaline acts as a very potent cardiac stimulant. Further structural optimisation then led to more selective β_1-sympathomimetics, like dobutamine, for the treatment of heart failure and cardiogenic shock, while selective β_2-sympathomimetics were aimed at safer therapies of bronchial asthma.

β_2-Sympathomimetics can be grouped into different structural clusters, which all take into account, that modification of the catechol moiety affects

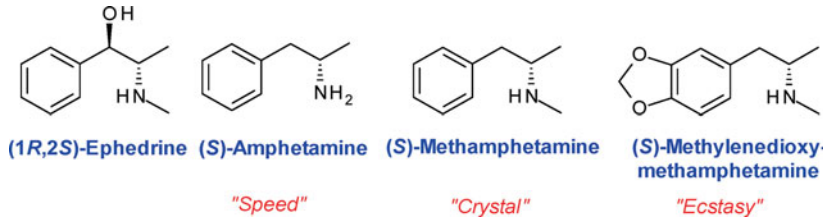
catechol-*O*-methyltransferase metabolism. Accordingly, in the compound classes originally developed by Allen & Hanburys (now part of GlaxoSmith-Kline), the catechol motif was replaced by saligenin (salicyl alcohol), as for example in salbutamol or in its pyridyl analogue in pirbuterol. The variants from Boehringer Ingelheim and Astra used however resorcinol as structural alternative, exemplified by orciprenaline (Boehringer, 1961), terbutaline (Astra, 1966), and reproterol. [103] In a third cluster, the hydroxyl-residues are replaced by other functionalities, as in clenbuterol and in the newly-approved (2010), enantiomerically pure indacaterol, which possesses a long duration of action.



6.38 *Direct α- and β-sympathomimetics.*

Some β_2-sympathomimetics, like clenbuterol, show anabolic side-effects, when over-dosed. As light and shadow are not infrequently in close proximity, these drugs have earned a dubious popularity for their appearance as performance enhancing substances. There are reports of misuse in the cattle-rearing industry, as well as cases of abuse in professional cycling and other competitive sports. [117]

Indirectly acting sympathomimetics release noradrenaline from the nerve endings and/or inhibit its re-uptake. Thereby, the noradrenaline concentration in the synaptic cleft is raised, and the sympathetic tone therefore increased. After repeated administration of higher doses, this effect is however restricted by the limited supply of newly synthesised noradrenaline. Typical representatives are ephedrine and the amphetamines (Fig. 6.39). The high polarity of catecholamines, associated with the two aromatic hydroxyl-groups, necessitates intravenous administration, and confines their activity to the periphery. Ephedrine, amphetamine, and as well methamphetamine exhibit in addition to their peripheral sympathomimetic effect also a central stimulating activity.



6.39 *Indirect sympathomimetics with central stimulating effect.*

6.40 *Methamphetamine forms coarse bright crystals.*

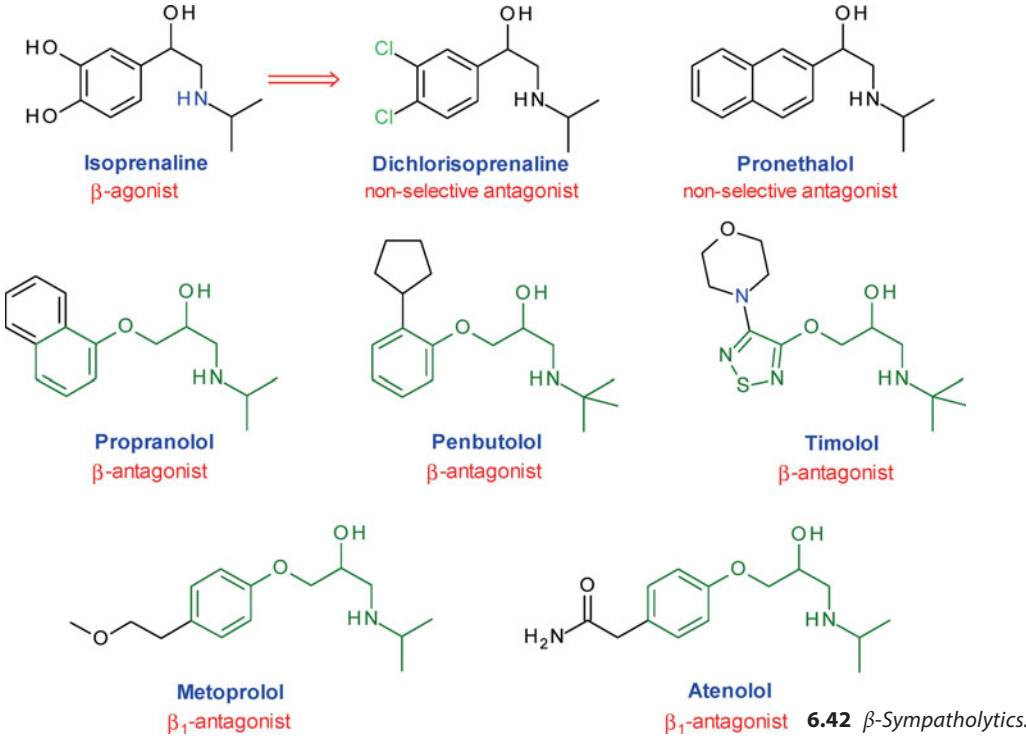
Ephedrine is mostly combined with other compounds in preparations for the treatment of bronchitis, of asthma and as an ingredient in nose drops for local vasoconstriction. Since amphetamines possess a clear potential for addiction, they are prescribed only reluctantly.

Excursus: Amphetamines

Similar to corticosteroids, as mentioned before, also the amphetamines gained some early military attention. [118] The Japanese pharmacologist Akira Ogata (1887–1978) was in 1919 the first to obtain methamphetamine in its crystalline form. The drug became widely used during World War II, when the German and Japanese as well as the Allied forces distributed many millions of tablets as "Pilot's chocolate" or "Pilot's salt" to enhance performance and increase concentration among their tank crews and aircraft personnel. Later, the "crystals" found widespread usage during the Vietnam War (1955–1975) as well as in the Gulf War (1990–1991) (Fig. 6.40). Nowadays, the focus is on their misuse as "party drugs" and as drugs for doping purposes in competitive sports (Fig. 6.41).

6.41 *Galina Kulakova, cross country skier from the former USSR and multiple Olympic gold medalist at the Winter Games in 1972, was stripped of her bronze medal at the 1976 Winter Olympics in Innsbruck, when it was discovered that her nasal spray contained ephedrine. [98]*

Among the sympatholytic agents, which block the adrenergic receptors, the β-selective antagonists (beta-blockers) assume an especially important role as to frequency and scope of their application. The target indications encompass coronary heart disease, functional cardiovascular disturbances, cardiac arrhythmia and hypertension (Fig. 6.42). By blocking β_1-receptors of the heart in a competitive manner, they inhibit the increase of the heart rate and cardiac contractile force, which is elicited by endogenous catecholamines. This is in most cases the desired therapeutic effect. Inhibition of β_2-receptors by non-selective beta-blockers or by higher dosing regimen leads generally to unwanted side-effects.



6.42 *β-Sympatholytics.*

Dichloroisoprenaline, a drug derived from the β-agonist isoprenaline by replacing both hydroxyl-groups with chlorine, was the first, though still non-selective β-receptor antagonist. This compound was an extremely valuable tool in experimental pharmacology, but it never advanced into therapeutic practice due to its serious side-effects. Pronethalol was another non-selective drug, which never attained therapeutic relevance, because it caused dizziness, nausea and vomiting in humans. In 1965, ICI brought propranolol to the market, the first beta-blocker for treatment of high blood pressure and Angina pectoris (Fig. 6.43). [119] A novel feature in this structure was the ether bridge, which became a guiding motif for this class of drugs. The nitrogen bears usually an isopropyl or a *t*-butyl group, and the naphthyl moiety can be exchanged by a heteroaromatic unit, as in timolol, or by other aromatic systems. Beta-blockers with distinct β_1-receptor selectivity resulted from *para*-alkyl-substituted phenyl residues, as *e.g.* in the case of atenolol or metoprolol. [115]

6.43 *The development of the first beta-blockers by ICI was essentially based on the scientific work of the Scottish pharmacologist Sir James Black (1924–2010). For his "discoveries of important principles for drug treatment", i.e. propranolol and cimetidine, he was awarded the 1988 Nobel Prize for Physiology or Medicine.*

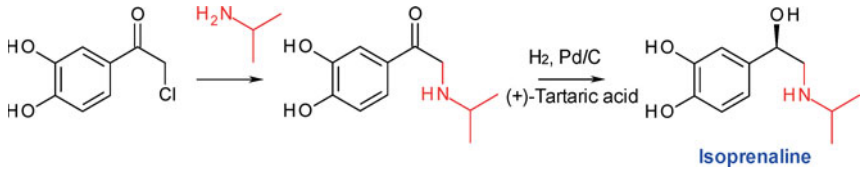
6.3.8 Chemical Syntheses

Most of the sympathomimetics and beta-blockers presently on the market are racemates, irrespective of the fact that the individual enantiomers have often a strikingly different pharmacological profile. The use of enantiomerically pure drugs would in many cases be advantageous to patients, thereby avoiding chal-

lenges to their organism by the other enantiomer, an unnecessary or even side-effect-prone material.

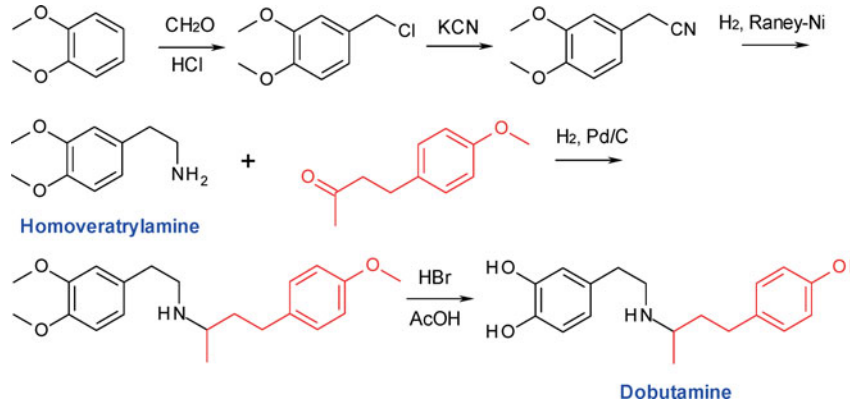
Isoprenaline

Isoprenaline was developed by Boehringer Ingelheim, and a patent was applied for in 1939. [120] The synthesis starts from 2-chloro-3',4'-dihydroxyacetophenone, which is reacted with isopropylamine. Then the keto-group is hydrogenated in presence of a palladium catalyst. Resolution of the racemate into its enantiomers is achievable with (+)-tartaric acid. Irrespectively, the commercial product is the racemate.



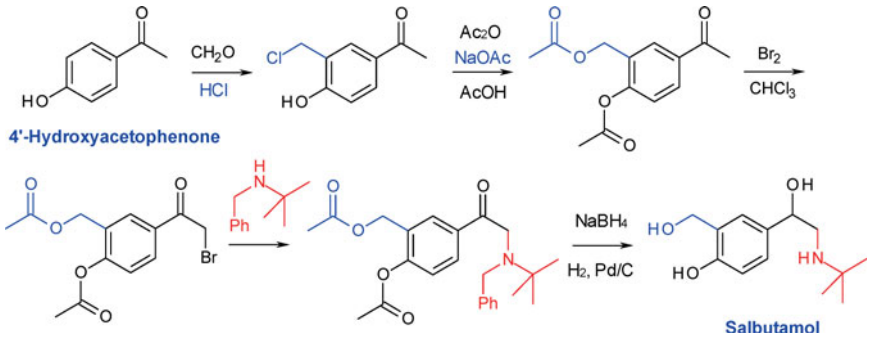
Dobutamine

Dobutamine is a development compound from Eli Lilly in the 1970s. [121] Homoveratrylamine is obtained by chloromethylation of 1,2-dimethoxybenzene, nucleophilic substitution with cyanide, and hydrogenation. The carbon skeleton is extended by reductive amination, and the methyl groups are cleaved off with HBr.

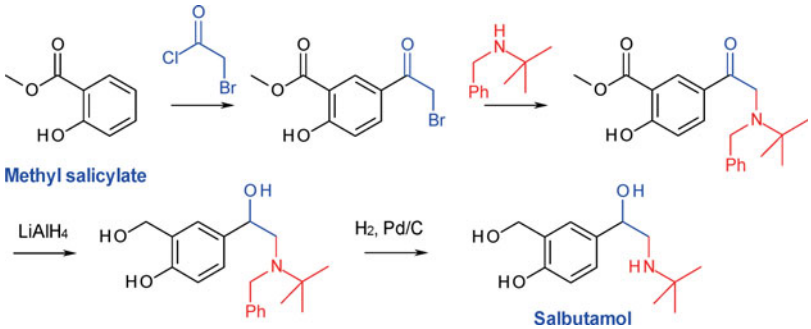


Salbutamol

For salbutamol, Allen & Hanburys developed two different synthetic routes in the 1960s. [122] The first one starts from 4'-hydroxyacetophenone, which, after chloromethylation and protection of both oxygen functions, is brominated and then reacted with *t*-butylbenzylamine. Treatment with sodium borohydride cleaves off both of the acetyl protecting groups and reduces the keto-group. The active compound is finally obtained by catalytic, reductive cleavage of the benzyl residue.

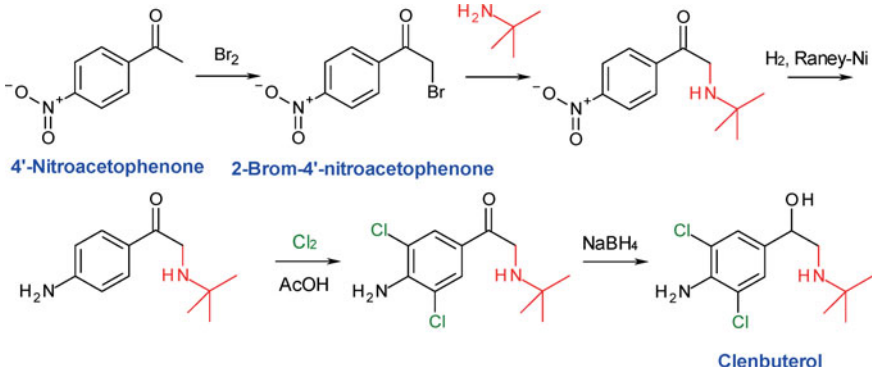


The starting material for the second route is methyl salicylate, which is treated with bromoacetyl chloride in a Friedel-Crafts acylation. The amine is then introduced as in the first synthesis, the ester and keto functions are reduced with lithium aluminium hydride, and the benzyl residue is finally cleaved off.

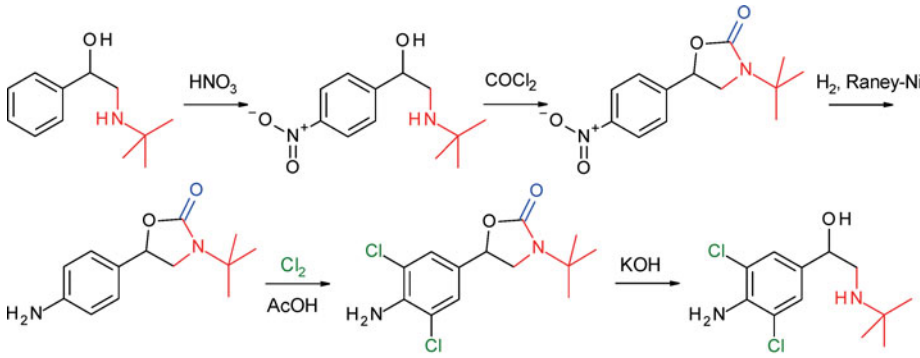


Clenbuterol

Clenbuterol is a product from Boehringer Ingelheim. [123] There are several syntheses reported for this product, which were in part developed by Thomae as well. The starting material for the first synthesis presented here is 4'-nitroacetophenone. The amine is again introduced in analogy to the previous syntheses. The target compound is obtained by chlorination of the aromatic ring and reduction of the ketone with sodium borohydride.



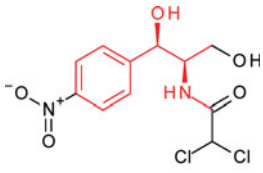
An alternative synthesis starts with an amino-alcohol, which is initially nitrated. Prior to reduction and chlorination, the alcohol function is protected with phosgene. The active compound is obtained by alkaline cleavage of the oxazolidinone.



Clenbuterol

Ephedrine

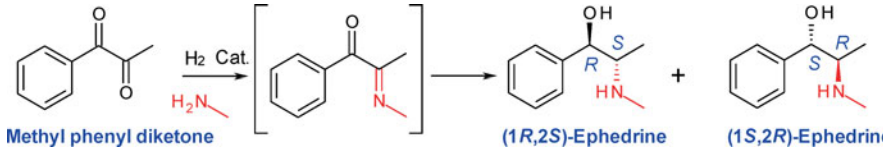
Structurally related to (*D*)-(–)-pseudoephedrine (shown in red) is the antibiotic chloramphenicol, isolated from the filamentous Gram-positive bacterium *Streptomyces venezuelae*, and used to treat bacterial conjunctivitis topically and in certain cases also meningitis systemically.



Chloramphenicol

One of the older syntheses of ephedrine originates from Aladar Skita (1876–1953) in 1929 (Fig. 6.44). Methyl phenyl diketone is catalytically hydrogenated in presence of methylamine. Thus, ephedrine is produced in a single step, devoid of the other diastereomers of pseudoephedrine (the *R*,*R*- and *S*,*S*- enantiomers). [100]

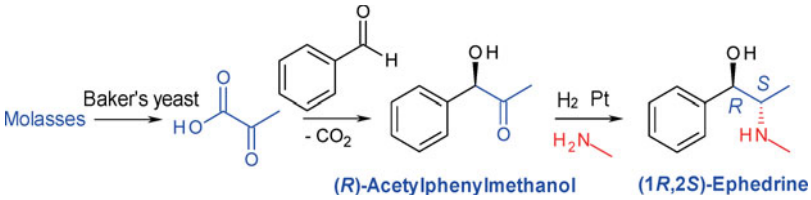
6.44 *Drugs containing Ephedrine.*



Methyl phenyl diketone (1*R*,2*S*)-Ephedrine (1*S*,2*R*)-Ephedrine

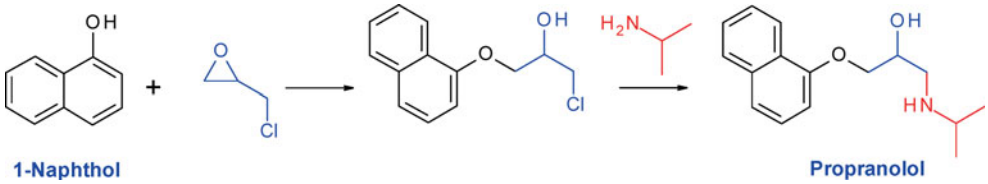
The enantioselective production of ephedrine belongs to the earliest industrial examples of biocatalysis. Around 1920, Carl Neuberg (1877–1956) had discovered at the Kaiser-Wilhelm-Institut in Berlin, that pyruvate-decarboxylase in baker's yeast (*Saccharomyces cerevisiae*) converted benzaldehyde with acetalde-

hyde, the yeast's endogenous metabolite, enantioselectively into (*R*)-acetylphenylmethanol. In the 1930s, Gustav Hildebrandt and Wilfried Klavehn at Knoll AG in Ludwigshafen developed an industrial process for the enantioselective production of ephedrine, which is still operated today. [124]



Propranolol

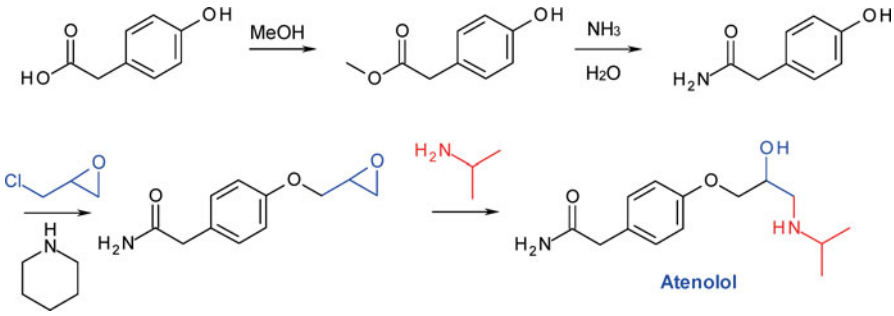
ICI's first patents for propranolol date back to the year 1962. [125] 1-Naphthol is reacted with epichlorohydrin. Propranolol is then obtained in a second step by reaction with isopropylamine.



These are the key steps, which are applied as well to many syntheses of this structural type.

Atenolol

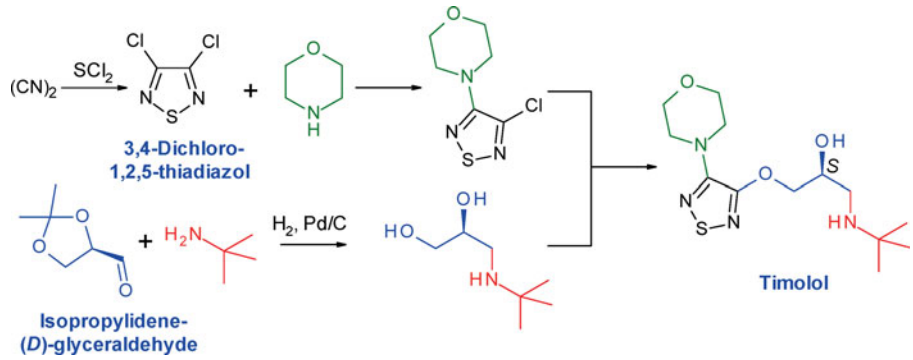
For the synthesis of atenolol, ICI used actually the same strategy [126]:



Timolol

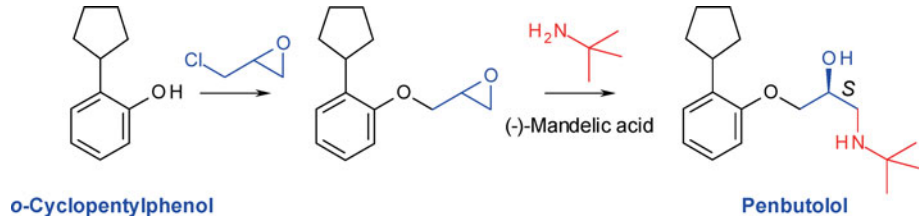
The first papers on timolol came from C. E. Frosst & Co. In the 1970s, Merck Sharp & Dohme filed patent applications as well for their synthesis. [127] Timolol is one of the few beta-blockers, which are marketed as pure enantiomers. Whereas in the first syntheses the racemic product was separated into its enantiomers with (+)-tartaric acid, later efforts were directed towards direct formation of the enantiomerically pure drug from isopropylidene-(*D*)-glyceraldehyde.

This resulted in a convergent synthesis. The side chain is reacted with an appropriately substituted 1,2,5-thiadiazole, which is easily accessible from cyanogen and sulfur dichloride. [128]

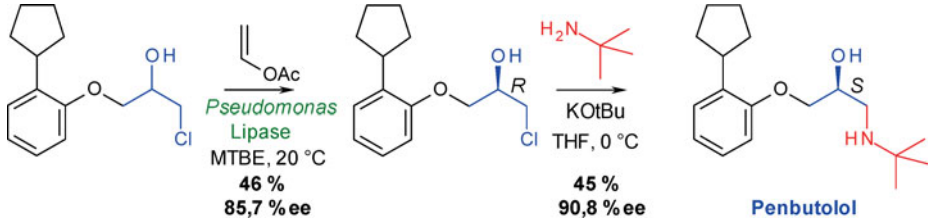


Penbutolol

Penbutolol is a highly potent β-antagonist with long duration of action, which was developed by Hoechst. The synthesis process follows the common scheme, where the enantiomers are ultimately separated with (–)-mandelic acid. [129]



In addition, several research groups have also developed enantioselective syntheses [130] and enzymatic processes [131] for this class of drugs. A 1-aryloxy-3-chloropropan-2-ol can for example be esterified enantioselectively with vinyl acetate in presence of a *Pseudomonas*-lipase. The unreacted, enantiomerically-enriched (*R*)-chlorohydrins are then transformed with potassium *t*-butoxide to the corresponding oxiranes, which are without isolation reacted further to the target compound. In case of penbutolol, the enantiomerically pure (*S*)-beta-blocker is obtained by recrystallisation of its hydrochloride salt.



Summary in Bullet Points

- Adrenaline is the hormone of the adrenal medulla. In response to excitation of the sympathetic nervous system, adrenaline is released into the bloodstream, and enables us to perform and to interact with our environment.
- The biosynthesis starts from the amino acid tyrosine. Intermediates are the neurotransmitters dopamine and noradrenaline.
- The separation of adrenergic receptors into subtypes and the understanding of their different expression levels in target organs proved essential for developing suitable pharmacotherapies with subtype-selective drugs to minimise undesired side-effects.
- β_2-Sympathomimetics are indicated for the therapy of bronchial asthma.
- Beta-blockers with selectivity for β_1-receptors are used for the treatment of cardiovascular diseases.

References

References to Introduction

1. http://en.wikipedia.org/wiki/Hormone.
2. M. Rashid, D. Singla, A. Sharma, M. Kumar, G. P.S. Raghava, *BMC Genomics* **10** (2009) 307.

References to 6.1 Steroids and Hormonal Contraceptives

3. Ullmann's Encyclopedia of Industrial Chemistry, Sixth Edition, 1999, Electronic Release, Contraception.
4. Encyclopaedia Britannica: Birth control.
5. E. Mutschler, Arzneimittelwirkungen, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 6th Edn., 1991, 281; http://en.wikipedia.org/wiki/Menstrual_cycle.
6. D. Voet, J. G. Voet, Biochemie, VCH Weinheim, 1994, 1170.
7. P. Nuhn, Naturstoffchemie, S. Hirzel Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1990, 498.
8. W. S. Johnson, M. B. Gravestock, B. E. McCarry, *J. Am. Chem. Soc.* **93** (1971) 4332.
9. E. J. Corey, G. Luo, L. S. Lin, *J. Am. Chem. Soc.* **119** (1997) 9927; E. J. Corey, G. Luo, L. S. Lin, *Angew. Chem. Internat. Edn.* **37** (1998) 1126.
10. Mechanistical details of polyene cyclisation: B. A. Hess, L. Smentek, *Angew. Chem.* **125** (2013) 11235.
11. I. Abe, *Chem. Rev.* **93** (1993) 2189 (Review: Squalene cyclisation); M. M. Meyer, M. J. R. Segura, W. K. Wilson, S. P. T. Matsuda, *Angew. Chem. Internat. Edn.* **39** (2000) 4090.
12. A. Gossauer, Struktur und Reaktivität der Biomoleküle, Verlag Helvetica Chimica Acta AG, Zürich, 2006, 270.
13. H. Wolff, C. Kunte, *Pharmazie in unserer Zeit* **29** (2000) 153; G.-J. Fan, W. Mar, M. K. Park, E. W. Choi, K. Kim, S. Kim, *Bioorg. Med. Chem. Lett.* **11** (2001) 2361; L. S. Marks, *Rev. Urology* **6,** Suppl. 9 (2004) S11; U. Holzgrabe, E. Bennack, *Pharmazie in unserer Zeit* **37** (2008) 310; J. M. Williams, in N. Yasuda, The Art of Process Chemistry, Wiley-VCH, Weinheim, 2011, 77.
14. D. Ghosh, J. Grisawold, M. Erman, W. Pangborn, *Nature* **457** (2009) 219.
15. U, Meyer, *Pharmazie in unserer Zeit* **33** (2004) 352.
16. A. Butenandt, *Hoppe-Seyler's Z. physiol. Chem.* **191** (1929) 727.

17. A. Butenandt, U. Westphal, W. Holweg, *Hoppe-Seyler's Z. physiol. Chem*. **227** (1934) 84; A. Butenandt, *Wiener Klein. Wochenschr*. **30** (1934) 934.
18. K. H. Slotta, H. Ruschig, E. Fels, *Ber. Dtsch. Chem. Ges*. **67** (1934) 1270.
19. W. M. Allen, O. Wintersteiner, *Science* **80** (1934) 190; O. Wintersteiner, W. M. Allen, *J. Biol. Chem*. **107** (1934) 321.
20. M. Hartmann, A. Wettstein, *Helv. Chim. Acta* **17** (1934) 1365.
21. K. David, E. Dingemanse, J. Freud, E. Laqueur, *Hoppe-Seyler's Z. physiol. Chem.* **233** (1935) 281.
22. S. H. Pines, *Org. Proc. Res. Dev*. **8** (2004) 708.
23. C. Djerassi, *Science* **84** (1984) 127; R. M. Roberts, Serendipity, Wiley, New York, 1989, 128.
24. S. Streller, K. Roth, *Chemie in unserer Zeit* **45** (2011) 270.
25. L. Haberlandt, *Münch. Med. Wochenschr*. **68** (1921) 1577.
26. A. Butenandt, J. Schmidt, *Ber. Dtsch. Chem. Ges.* **67** (1934) 1901.
27. R. E. Marker, E. L. Wittle, E. J. Lawson, *J. Am. Chem. Soc.* **60** (1938) 2931; http://en.wikipedia.org/wiki/Mexican_barbasco_trade; http://en.wikipedia.org/wiki/Dioscorea_composita.
28. A. Goswami, R. Kotoky, R. C. Rastogi, A. C. Ghosh, *Org. Proc. Res. Dev*. **7** (2003) 306 (Cleavage with potassium permanganate); R. P. Holysz jr., DE932799 (1955) (Cleavage of the enamine).
29. H. H. Inhoffen, *Naturwissenschaften* **25** (1937) 125.
30. H. H. Inhoffen, Schering AG US 2 361 847 (1941).
31. L.J. Chinn, H. L. Dryden, *J. Org. Chem.* **26** (1961) 3904; H. L. Dryden, G. M. Webber, J. Weiczorek, *J. Am. Chem. Soc*. **86** (1964) 742.
32. G. Anner, K. Miescher, *Helv. Chim. Acta* **31** (1948) 2173; W. E. Bachmann, S. Kuschner, A. C. Stevenson, *J. Am. Chem. Soc.* **64** (1942) 974; F. Litvan, R. Robinson, *J. Chem. Soc.* (1938) 1997; K. Heusler, J. Kalvoda, *Steroids* **61** (1996) 492 (review on Ciba's steroid chemistry).
33. F. Arndt, B. Eistert, *Chem. Ber.* **68** (1935) 200.
34. W. M. Allen, M. Ehrenstein, *Science* **100** (1944) 251; M. Ehrenstein, *J. Org. Chem.* **9** (1944) 435.
35. A. J. Birch, *J. Chem. Education* **52** (1975) 458.
36. A. J. Birch, *J. Chem. Soc.* (1944) 430; A. J. Birch, *J. Chem. Soc.* **1950**, 367; H. Pellissier, M. Santelli, *Org. Prep. Proc. Int.* **34** (2002) 609.
37. J. March, Advanced Organic Chemistry, Wiley, New York, 1992, 781; H. R. Christen, Organische Chemie, Sauerländer, Diesterweg, Salle, Frankfurt, 1970, 419.
38. C. Djerassi, L. Miramontes, G. Rosenkranz, F. Sondheimer, *J. Am. Chem. Soc*. **76** (1954) 4092.
39. F. B. Colton, L. N. Nysted, B. Riegel, A. L. Raymond, *J. Am. Chem. Soc*. **79** (1957) 1123.
40. F. B. Colton, Searle, US 2 655 518 (1952).
41. H. Ueberwasser, K. Heusler, J. Kalvoda, Ch. Meystre, P. Wieland, G. Anner, A. Wettstein, *Helv. Chim. Acta* **46** (1963) 344.
42. T. B. Windholz, M. Windholz, *Angew. Chem. Internat. Edn*. **3** (1964) 353.
43. C. Meystre, K. Heusler, J. Kalvoda, P. Wieland, G. Anner, A. Wettstein, *Experientia* **17** (1961) 475.
44. D. H. R. Barton, *J. Chem. Soc*. **1953**, 1027.
45. D. H. R. Barton, *Experientia* **6** (1950) 316.
46. A. Bowers, L. C. Ibanez, M. E. Cabezas, H. J. Ringold, *Chem. Ind*. (*London*) (1960) 1299.
47. A. Bowers, E. Denot, L. C. Ibanez, M. E. Cabezas, H. J. Ringold, *J. Org. Chem*. **27** (1962) 1862.
48. J. March, Advanced Organic Chemistry, Wiley, New York, 4th Edn., 1992, 704, 1153.

49. K. S. Davis, "The Story Of The Pill", American Heritage Magazine **29** (1978) (no page number). http://www.americanheritage.com/content/story-pill.
50. K. Brückner, DE 1075114 (1960) (application date: 29.4.1958); K. Brückner, B. Hampel, U. Johnsen, *Chem. Ber.* **94** (1960) 1225; H. J. Ringold, E. Batres, A. Bowers, J. Edwards, J. Zderic, *J. Chem. Soc.* **81** (1959) 3485; K. Roth, S. Steller, *Chem. Unserer Zeit* **45** (2011) 270; C. Djerassi, *Chem. Unserer Zeit* **45** (2011) 424;http://de.wikipedia.org/wiki/Chlormadinone; http://www.jenapharm.de/unternehmen/geschichte/.
51. http://de.wikipedia.org/wiki/Dienogest; M. Oettel, A. Kurischko, *Contraception* **21** (1980) 61.
52. K. Ponsold, B. Menzenbach, M. Huebner, M. Oettel, K. Barnikol-Oettler, G. Teichmueller, DD 160418 (1979); B. Menzenbach, M. Huebner, K. Ponsold, *J. prakt. Chem.* **326** (1984) 893; B. Menzenbach, M. Huebner, *Z. Chem.* **26** (1986) 371.
53. D. Grawe, S. Gliesing, H. Gerecke, P. Hoesel, U. Mueller, T. Michel, R. Eilers, U. Knabe, B. Erhart, M. Mosebach, D. Voigtlaender, U. Tilstam, J. Jacke, K. Bahl, U. Bohlmann, D. Wehmeier, M. Sander, WO 2007/118717 (2007); M. Alpegiani, W. Capri, T. Cristiano, R. Strigaro, T. I. Danielli, EP 1935898 (2007); H.-J. Liu, N. H. Al-said, *Tetrahedron Lett.* **32** (1991) 5473.
54. S. N. Ananchenko, T. Jeng-o, I.V. Torgov, *Bull. Acad. Sci. USSR*, *Div. Chem. Sci.* (1962) 275.
55. S. N. Ananchenko, I. V. Torgov, *Doklady Akad. Nauk SSSR* **127** (1959) 553; Engl. transl.: *Doklady*, *Chemistry Section* **127** (1959) 567.
56. K. Fuhshuku, N. Funa, T. Akeboshi, H. Ohta, H. Hosomi, S. Ohba, T. Sugai, *J. Org. Chem.* **65** (2000)129 (kinetic resolution of the Wieland-Miescher ketone).
57. C. Rufer, H. Kosmol, E. Schröder, K. Kieslich, H. Gibian, *Liebigs Ann. Chem*. **702** (1967) 141.
58. S. N. Ananchenko, V. Y. Limanov, V. N. Leonov, V. N. Rzheznikov, V. N. Torgov, *Tetrahedron* **18** (1962) 1355; V. M. Rzheznikov, S. N. Ananchenko, I. T. Torgov, *Bull. Acad. Sci. USSR, Div. Chem. Sci.* (1962) 431.
59. M. J. van den Heuvel, C. W. Bokhoven, H. P. de Jongh, F. J. Zeelen, *Recl. Trav. Chim. Pays-Bas* **107** (1988) 331.
60. E. J. Corey, A. X. Huang, *J. Am. Chem. Soc*. **121** (1999) 710.
61. H. Schick, G. Lehmann, G. Hilgetag, *Chem. Ber*. **102** (1969) 3238.
62. U. Eder, G. Sauer, R. Wiechert, *Angew. Chem. Internat. Edn*. **10** (1971) 496.
63. Z. G. Hajos, D. R. Parrish, *J. Org. Chem*. **39** (1974) 1612.
64. Z. G. Hajos, D. R. Parrish, *J. Org. Chem.* **39** (1974) 1615; R. A. Micheli, Z. G. Hajos, N. Cohen, D. R. Parrish, L. A. Portland, W. Sciamanna, M. A. Scott, P. A. Wehrli, *J. Org. Chem.* **40** (1975) 675.
65. B. List, *Angew. Chem. Internat. Edn*. **49** (2010) 1730.
66. P. I. Dalko, L. Moisan, *Angew. Chem. Internat. Edn.* **116** (2004) 5138; W. Notz, F. Tanaka, C. F. Barbas III, *Acc. Chem. Res.* **37** (2004) 580; B. M. Trost, C. S. Brindle, *Chem. Soc. Rev*. **39** (2010) 1600.
67. A. Berkessel, H. Gröger, Asymmetric Organocatalysis, Wiley-VCH, Weinheim, 2005; S. Mukherjee, J. W. Yang, S. Hoffmann, B. List, *Chem. Rev.* **107** (2007) 5471.
68. F. Damkaci, B. B. Jarvis, *Chemtracts – Org. Chem.* **12** (1999) 1013.
69. E. Boschitsch, Speculum – *Z. Gynäkol. Geburtsh.* **20** (2002) 34.
70. Press information from Schering, *Pharmazie in unserer Zeit* **33** (2004) 416; I. H. Thorneycroft, *Eur. J. Contracept. Reproduct. Health Care* **7** (2002) 13.
71. http://en.wikipedia.org/wiki/Drospirenone.
72. R. Wiechert, D. Bittler, U. Kerb. J. Casals-Stenzel, W. Losert, DE 2652761 (1976).
73. D. Liu, L. M. Stuhmiller, T. C. McMorris, *J. Chem. Soc., Perkin Trans. 1* (1988) 2161.
74. O. Schmidt, K. Prezewowsky, R. Wiechert, DE 1593500 (1966).
75. K. Petzoldt, R. Wiechert, H. Laurent, K. Nickisch, D. Bittler, DE 3042136 (1980).

76. D. Bittler, H. Hofmeister, H. Laurent, K. Nickisch, R. Nickolson, K. Petzoldt, R. Wiechert, *Angew. Chem. Internat. Edn*. **21** (1982) 696.
77. J.-T. Mohr, K. Nickisch, DE 19633685 (1996).
78. P. Norman, J. Castaner, R. M. Castaner, *Drugs Future* **25** (2000) 1247.
79. Practice Committee of the American Society for Reproductive Medicine, *Fertility and Sterility, Suppl. 3*, **90** (2008) S103.
80. A. R. Lemons, R. K. Naz, *Am. J. Reprod. Immunol.* **66** (2011) 13.
81. C. Djerassi, *Science* **272** (1996) 1857.

References to 6.2 Thyroxine

82. P. Fragu, *Ann. Endocrinol.* **60** (1999) 10; V. Leoutsakos, *Hormones* **3** (2004) 268.
83. F. C. Küpper, L. J. Carpenter, G. B. McFiggans, C. J. Palmer, T. J. Waite, E.-M. Boneberg, S. Woitsch, M. Weiller, R. Abela, D. Grolimund, P. Potin, A. Butler, G. W. Luther III, P. M. H. Kroneck, W. Meyer-Klaucke, M. C. Feiters, *PNAS* **105** (2008) 6954; F. C. Küpper, M. C. Feiters, B. Olofsson, T. Kaiho, S. Yanagida, M. B. Zimmermann, L. J. Carpenter, G. W. Luther III, Z. Lu, M. Jonsson, L. Kloo, *Angew. Chem. Int*. Ed. **50** (2011) 11598; http://www.iccidd.org/newsletter/idd_nl_feb08_iodine_production.pdf; E.F. Verhaeghe, A.Fraysse, J. L.Guerquin-Kern, T. D. Wu, G.Devès, C.Mioskowski, C.Leblanc, R.Ortega, Y.Ambroise, P. Potin . *J. Biol. Inorg. Chem.* **13** (2008) 257.
84. CD Römpp Chemie Lexikon-Version 1.0, Stuttgart/New York, Georg Thieme Verlag, 1995, Thyroid-Hormone.
85. M. B. Bolger, E. C. Jorgensen, *J. Bio. Chem*. **255** (1980) 10271.
86. Y.-A. Ma, C. J. Sih, A. Harms, *J. Am. Chem. Soc.* **121** (1999) 8967.
87. Y.-A. Ma, C. J. Sih, *Tetrahedron Lett*. **40** (1999) 9211.
88. J. M. Gavaret, H. J. Cahnmann, J. Nunez, *J. Biol. Chem*. **256** (1981) 9167; J. M. Gavaret, J. Nunez, H. J. Cahnmann, *J. Biol. Chem*. **255** (1980) 5281.
89. S. R. Chemburkar, K. C. Deming, R. E. Reddy, *Tetrahedron* **66** (2010) 1955.
90. J. R. Chalmers, S. T. Dickson, J. Elks, B. A. Hems, *J. Chem. Soc.* (1949) 1448.
91. M. Beringer, A. Brierley, M. Drexler, E. M. Gindler, C. C. Lumpkin, *J. Am. Chem. Soc*. **75** (1953) 2708.
92. G. Hillmann, Z. *Naturforsch.* **11B** (1956) 419; G. Hillmann, A. Hillmann-Elies, Hoppe-Seyler's *Zeitschrift für physiologische Chemie* **305** (1956) 177.
93. M. Beringer, M. Drexler, E. M. Gindler, C. C. Lumpkin, *J. Am. Chem. Soc*. **75** (1953) 2705.
94. M. J. Peacock, D. Pletcher, *Tetrahedron Lett*. **41** (2000) 8995 (electrochemical synthesis of iodonium salts).
95. G. M. Salamonczyk, V. B. Oza, C. J. Sih, *Tetrahedron Lett*. **40** (1997) 6965.
96. A. J. Boyle, A. Zlatkis, US 2835700 (1958); L. G. Ginger, P. Z. Anthony, US.2889363 (1959); P. Z. Anthony, L. G. Ginger, US 2889364 (1959).
97. N. V. Bell, W. R. Bowman, P. F. Coe, A. T. Turner, D. Whybrow, *Can. J. Chem*. **75** (1997) 873.

References to 6.3 Adrenaline

98. Encyclopedia Britannica, Electronic Release, 2001.
99. N. Nagai, *Pharm. Ztg.* **32** (1887) 700; N. Nagai, *Yakugaku Zasshi* **120** (1892) 109; E. A. Abourashed, A. T. El-Alfy, I. A. Khan, L. Walker, *Phytother. Res*. **17** (2003) 703.
100. W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 1969, 413.
101. H.-H. Otto, *Pharmazie in unserer Zeit* **9** (1980) 26.
102. T. A. Smith, *Phytochemistry* **16** (1977) 9.
103. http://pubs.acs.org/subscribe/journals/mdd/v04/i12/html/12timeline.html; A. Helmstädter, *Pharmazie in unserer Zeit* **40** (2011) 378; F. Stolz, *Ber. Dtsch. Chem. Ges.* **37** (1904) 4149.

104. H. Becker, *Pharmazie in unserer Zeit* **14** (1985) 129; Römpp Chemielexikon, Thieme, Stuttgart, 2006: Electronic Release, Mescaline.
105. A.-H. Maehle, *Med. Hist.* **48** (2004) 153.
106. E. Mutschler, Arzneimittelwirkungen, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1991, 239.
107. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 713.
108. P. Karlson, Biochemie, Thieme, Stuttgart, 1970, 155.
109. J. Vidgren, L. A. Svensson, A. Liljas, *Nature* **368** (1994) 354 (X-ray structure).
110. G. Barger, H. H. Dale, *J. Phys.* **41** (1910) 19.
111. R. P. Ahlquist, *Am. J. Physiol*. **153** (1948) 586; M. R. Bristow, *Circ. Res.* **109** (2011) 1176.
112. M. Hesse, Alkaloide, Wiley-VCH, Weinheim, 2000, 79.
113. Ullmann's Encyclopedia of Industrial Chemistry, 6th Edition, 2000: Electronic Release, Antiasthmatic Agents.
114. R. A. Dixon, B. K. Kobilka, D. J. Strader, J. L. Benovic, H. G. Dohlman, T. Frielle, M. A. Bolanowski, C. D. Bennett, E. Rands, R. E. Diehl, *Nature* **321** (1986) 75.
115. P. P. Griffin, M. Schubert-Zsilavecz, H. Stark, *Pharmazie in unserer Zeit* **33** (2004) 442; L. Hein, *Pharmazie in unserer Zeit* **33** (2004) 438; D. M. Perez, S. S. Karnik, *Pharm. Rev.* **57** (2005) 147.
116. C. Lamers, M. Schubert-Zsilavecz, H. Stark, *Pharmazie in unserer Zeit* **40** (2011) 423; D. M. Rosenbaum, C. Zhang, J. A. Lyons, R. Holl, D. Aragao, D. H. Arlow, S. G. F. Rasmussen, H.-J. Choi, B. T. DeVree, R. K. Sunahara, P. S. Chae, S. H. Gellman, R. O. Dror, D. E. Shaw, W. I. Weis, M. Caffrey, P. Gmeiner, B. K. Kobilka, *Nature* **469** (2011) 236; S. G. F. Rasmussen, H.-J. Choi, J. J. Fung, E. Pardon, P. Casarosa, P. S. Chae, B. T. DeVree, D. M. Rosenbaum, F. S. Thian, T. S. Kobilka, A. Schnapp, I. Kronetzki, R. K. Sunahara, S. H. Gellman, A. Pautsch, J. Steyaert, W. I. Weis, B. K. Kobilka, *Nature* **469** (2011) 175.
117. W. Schänzer, *Chemie in unserer Zeit* **31** (1997) 218.
118. http://en.wikipedia.org/wiki/Methamphetamine.
119. R. P. Rubin, *Pharm. Rev.* **59** (2007) 289**;** L. Hein, *Pharmazie in unserer Zeit* **33** (2004) 434.
120. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 1035.
121. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 652.
122. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 1701.
123. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 465.
124. A. Gottschalk, *Nature* **178** (1956) 722; R. Stürmer, M. Breuer, *Chemie in unserer Zeit* **40** (2006) 104.
125. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 1613.
126. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 132.
127. A. Kleemann, J. Engel, Pharmaceutical Substances, Thieme, Stuttgart, 1999, 1883.
128. L. M. Weinstock, P. Davis, B. Handelsman, R. Tull, *J. Org. Chem*. **32** (1967) 2823.
129. G. Härtfelder, H. Lessenich, K. Schmitt, *Arzneim.-Forsch*. **22** (1972) 930.
130. J. M. Klunder, T. Onami, K. B. Sharpless, *J. Org. Chem*. **54** (1989) 1295.
131. U. Ader, M. P. Schneider, *Tetrahedron: Asymmetry* **3** (1992) 521.

7 | Vitamins

Seafarers in the Middle Ages feared not only sea monsters, adverse winds and hobgoblins, but also scurvy (Fig. 7.1). On voyages lasting for months, many suffered from this deficiency disease. Antonio Pigafetta (1480–1534), the Venetian chronicler of Ferdinand Magellan (1480–1521), reported in March 1521:

"We travelled for three months and 20 days, without taking on fresh provisions. The rusks had disintegrated into dust, full of maggots and rat droppings. The drinking water was cloudy and foul-smelling. We ourselves ate the hard leather of the square rig, which had been consistently at the mercy of the weather. It had first to be soaked for days in sea-water, and then roasted in glowing ashes, before it became edible. Rats constituted a delicacy and were bought for a half-crown per piece. To make matters worse, we then had scurvy, from which 19 men died. If God and his Holy Mother had not sent us good weather on this long journey, we should surely all have perished in this open sea. I believe that no one will undertake such a journey ever again." [1]

Scurvy also occurs in animals, especially dogs, pigs and guinea-pigs: this was also called "mouth-rot" or "bristle-rot".

7.1 *Replicas of the sailing ships Niña, Pinta and Santa Maria of Christopher Columbus, with which he sailed off in 1492 in order to find the sea-route to India, and instead discovered America.*

This disease occurred also among prisoners in jail. It was characterised by weight loss, bleeding gums, tooth loss, bleeding in the gastrointestinal tract, the musculature and the skin, tiredness and a weak heart. Since the Renaissance, the curative effect of the Lesser celandine *(Ranunculus ficaria)* had been known. In 1720, the Austrian Army physician Johann Georg Heinrich Kramer (1684–1744) pointed out that scurvy originated through an inappropriate diet. He was able to show that the disease may be rapidly cured by the consumption of fresh vegetables and fruit. The British Naval physician James Lind (1716–1794) also came to a similar conclusion in 1747 in a "clinical study" with twelve sailors on

B. Schaefer, *Natural Products in the Chemical Industry*,
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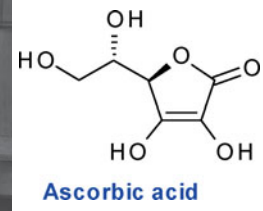
7.2 *James Lind (1716–1794) and his famous publication "A Treatise of the Scurvy in Three Parts" (1753) and 'Ambersweet' oranges (Citrus sinensis).*

HMS Salisbury, in which he was able to cure scurvy with lemons and oranges (Fig. 7.2). [2]

Since 1760, there has existed in the British Royal Navy a regulation that lemons are to be carried on board its ships. In 1907 Axel Holst (1860–1931) and Theodor Frølich (1870–1947) published a paper which is now considered the most important single contribution to elucidating the aetiology of scurvy. [3] For proof and quantification, guinea-pigs served as the appropriate test system. In 1928, Albert von Szent-Györgyi isolated a crystalline material with strong reducing properties, first from the adrenal cortex and later from lemons, cabbage and sweet pepper (Fig. 7.3). At the time, he did not know that this was pure Vitamin C. Josef K. Tillmans (1876–1935) was able to show that the reductive capacity of fruit and vegetable juices accompanies their antiscorbutic effect. In 1932, Szent-Györgyi proved that his reducing substance was the long-sought Vitamin C (ascorbic acid). Fritz Micheel (1900–1982) at the Universität Münster, Sir Norman Haworth (1883–1950) at St Andrews and then Birmingham Universities, and his erstwhile assistant Sir Edmund Hirst (1898–1975) at Bristol and Edinburgh Universities, elucidated the structure and prepared synthetic ascorbic acid for the first time. [4] Less than a milligram daily is enough to prevent scurvy in guinea-pigs.



Ascorbic acid

7.3 *Albert von Szent-Györgyi (1893–1986) and Vitamin C.*

Tadeusz Reichstein (1897–1996) eventually succeeded in developing an industrial synthesis starting from (*D*)-glucose. Merck in Darmstadt and Hoffmann-La Roche in Basel have produced ascorbic acid by this method since the 1930s.

Another disease resulting from a deficiency is beriberi, which has been known in China for more than 1,000 years. It occurs above all in southern and eastern Asia, but also in Alaska, Brazil, South Africa, the Congo and Senegal. The ailment is caused by a slow degeneration of the peripheral nerves (manifesting as polyneuritis in birds). The first symptom is the beginning of impaired sensory perception in the skin of the lower limbs, followed by muscle weakness, especially in the legs, but in severe cases also in hips and hands, and finally dropsy and heart weakness. Untreated, the illness leads to death. On account of

the demographic observation that beriberi occurs especially in those countries, where rice is the traditional foodstuff, the connection was made between the illness and the diet. The first comprehensive description came from Jacob de Bondt (1599–1631), a physician in the Dutch East India Company (Fig. 7.4).

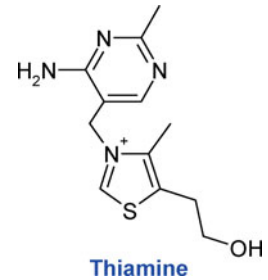
7.4 *The book De Medicina Indorum by Jacob de Bondt, published (posthumously) in 1642, is the first important treatise on the diseases of the East Indies, in which he describes beriberi as "the sheep's walk" on account of the characteristic wobbling, unsightly gait of patients, using the words of the local people in Batavia (the present-day Jakarta).*

In 1882, the Japanese military physician Kanehiro Takaki (1849–1920) recommended a change to the diet of the Japanese navy. Instead of rice, more meat, bread, fruit and vegetables should be consumed. This led to the almost complete disappearance of beriberi among the crews.

The actual origin of beriberi was however discovered by the Dutchman Christiaan Eijkman (1858–1930) at a military hospital of the Dutch colony of Java in 1896, and he thereby provided the basis of vitaminology (Fig. 7.5). [5] He recognised that rice, as such, did not make the patients and staff ill, but "polished rice", which had been mechanically freed from the rice bran. Eijkman suspected an indispensable nutrient in the rice bran, since it emerged that the bran, or extracts from it, cured beriberi. The chance observation, that chickens (*Gallus gallus domesticus*) at the hospital (which were fed with "polished" rice, because their usual feed had run out) developed characteristic symptoms of a beriberi-like illness, led Eijkman to the conclusion, that poultry could also be taken ill with beriberi. Thus, for the first time, experimental researching into beriberi was possible.

7.5 *Christiaan Eijkman (1858–1930) recognised in beriberi an "avitaminosis", and thereby laid the foundation stone of vitamin research.*

In 1911, Kazimierz (Casimir) Funk (1884–1967) isolated from rice bran a water-soluble, crystalline substance, which he called "beriberi-vitamin". He chose the name "vitamin", a technical term derived from the Latin *vita* (life) and *amine*, on account of the basic character of the substance. Although, in the period that followed it was recognised that most of the essential nutritional factors are not amines, the term "vitamin" stuck. In 1916, Elmer Verner McCollum (1879–1967), biochemist at the University of Wisconsin, and later, in 1920, Jack Drummond (1891–1952), the first Professor of Biochemistry at University College London, suggested that vitamins should be classified by letters of the alphabet, according to the order of their discovery (Fig. 7.6). Those vitamins, which were discovered later, were integrated into this system. The beriberi-vitamin received the name Vitamin B_1. In recognition of its chemical constitution, the elucidation of which involved a larger number of researchers, including Barend Coenraad Petrus Jansen (1884–1962), Adolf Otto Reinhold Windaus (1876–1959), Rudolf Grewe (1910–1968) and Robert Runnels Williams (1886–1965), Vitamin B_1 also received the name thiamine. In 1936, Vitamin B_1 was successfully synthesised for the first time by R. R. Williams. [6, 7]



7.6 *Sir Jack Cecil Drummond (1891–1952).*

An Unsolved Crime Mystery

Sir Jack Cecil Drummond was a distinguished British biochemist, noted for his scientific work on nutrition during the Second World War. In 1944, he was elected Fellow of the Royal Society and knighted in the same year. After the war, he became Director of Research at the Boots Company in Nottingham.

Early in August 1952, he spent his holydays together with his wife Lady Anne and their 10-year-old daughter Elizabeth in south-eastern France. In the night of 4 August 1952, the Drummond family has been murdered and a young peasant farmer, Gustave Dominici, found their corpses next morning close to their picturesque farm house, called La Grande Terre, 6 km south of Peyruis. Over the years, several legends appeared, in France referred to as *l'affaire Dominici*, from an assassination by a Soviet agent to industrial or military espionage, but up to now the background for these homicides remains a mystery. [8]

Nowadays, the term "vitamin" is understood to mean an essential organic compound, which is not synthesised in sufficient quantities or at all in the human body (or in most animals), and has therefore to be supplied through the diet or other sources. Vitamins are defined by their biological function, but not as a chemically uniform group of substances. In contrast to energy-providing foodstuffs, they are needed in only very small amounts. By this definition, there are 13 compounds or compound families known, which constitute vitamins for humans (Tab. 7.1). [9] However, there are additional substances of value as dietary supplements and with vitamin-like properties: fatty acids (Vitamin F), lipoic acid, ubiquinone, choline, myoinositol and *S*-adenosylmethionine.

Vitamins are classified as water- or fat-soluble. While fat-soluble vitamins are stored in the liver and fatty tissues, water-soluble vitamins are more rapidly excreted and require regular replacement. An exception is the water-soluble Vitamin B_{12}, which can be stored in the body for 3–5 years. The fat-soluble Vitamin K, on the other hand, reveals its deficiency immediately after interruption of its nutritional supply.

The individual vitamin demand varies widely and is also dependent on the personal constitution. Stressful situations and *e.g.* pregnancy require a higher vitamin intake. For men, the recommended daily dose of Vitamin A, B_1, B_2, B_6, K and niacin is usually higher than for women. There are series of studies, which confirm the breadth of application and the tolerability of vitamins and other micronutrients. The pharmacological effects with high doses of antioxidant provitamins and vitamins (especially β-carotene and Vitamins C and E) in the treatment of cardiovascular and other diseases have been encouraging, albeit further controlled clinical studies will be needed to substantiate these observations.

All of the vitamins, except Vitamin B_{12}, are produced nowadays by chemical syntheses. Many of them are also obtained *via* biotechnological processes (Vitamins B_1, B_6, B_{12}) or isolated from natural products (Vitamins A, D, E, K). For Vitamin C, the exclusively chemical process (the Reichstein-Grüssner synthesis) has been replaced by a mixed chemical/fermentation procedure.

The volume of the global vitamin market amounts currently to 2.5–3.0 billion Euro per annum; 50 % thereof accounts for animal feed, 40 % for nutritional supplements and food and 10 % for cosmetics.

Tab. 7.1 *Table Vitamins – their discovery and function*

	Vitamin	Discovery/ Isolation	Isolated from	Active form	Function
1.	Vitamin A	1909/1931	Fish liver oil	11-(*Z*)-Retinal	Visual process
2.	Vitamin B_1	1897/1911	Rice	Thiamine diphosphate Thiamine triphosphate	Decarboxylation of α-oxo-acids, aldehyde group transfer, PO_4^{3-}-donor
3.	Vitamin B_2	1920/1933	Eggs	Flavin mononucleotide Flavin adenine dinucleotide	Hydride/electron transfer
4.	Vitamin B_6	1934/1938	Rice	Pyridoxal-5-phosphate	Amino-group transfer
5.	Vitamin B_{12}	1926/1948	Liver	Coenzyme B_{12}	1,2-Hydrogen shift
6.	Vitamin C	1912/1928	Citrus fruits		Hydroxylation, subcellular antioxidant
7.	Vitamin D	1918/1932	Fish liver oil		Regulation of calcium and phosphate homeostasis
8.	Vitamin E	1922/1936	Wheat germ oil	α-Tocopherol	Intracellular antioxidant
9.	Vitamin K	1929/1939	Alfalfa		Biosynthesis of prothrombin
10.	Biotin	1931/1935	Liver	Biocytin	Carboxyl group transfer
11.	Folic acid	1941/1941	Liver	Tetrahydrofolic acid	Formyl group transfer
12.	Niacin	1936/1935	Liver	Nicotinamide adenine dinucleotide	Hydride/electron transfer
13.	Panthothenic acid	1931/1938	Liver	Coenzyme A	Acetyl group transfer

Summary in Bullet Points

- "Vitamins" are essential organic compounds, which are not synthesised in sufficient quantities or at all in the human body (or in most animals), and have therefore to be supplied through the diet or other sources.
- There are 13 compounds or compound families known, which constitute vitamins for humans.
- The annual sales volume for vitamins is currently estimated at 2.5–3.0 billion Euro globally.

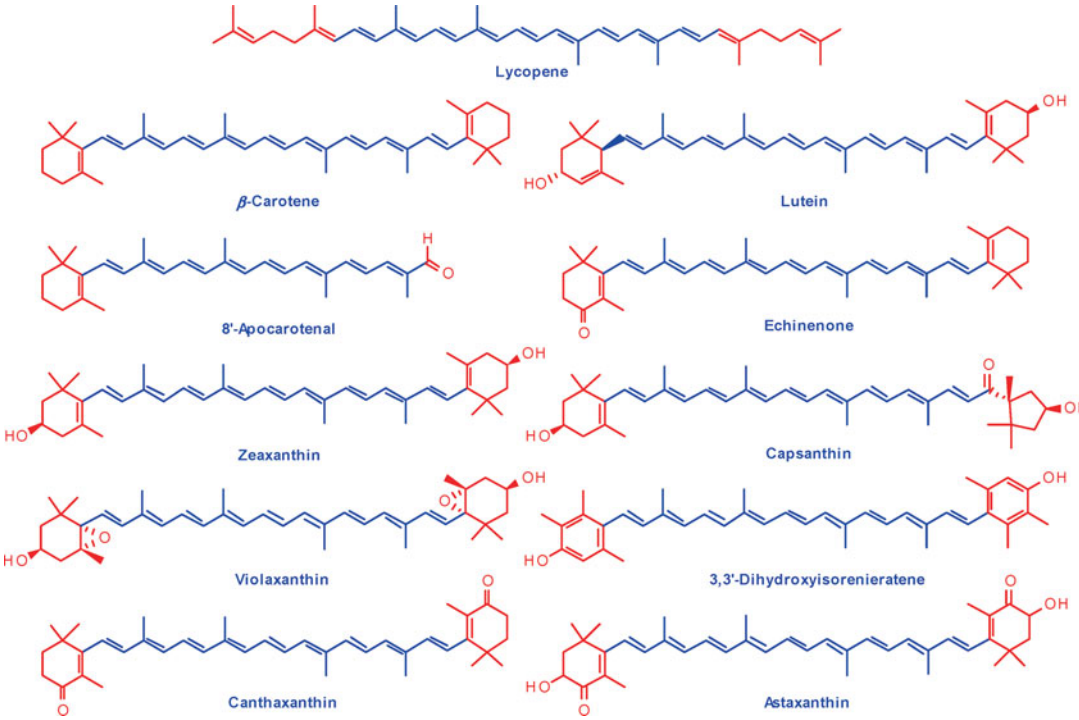
7.1 Vitamin A and Carotenoids

The colourants in carrots (β-carotene), tomatoes, rose-hips (lycopene), peppers (capsanthin), maize (zeaxanthin), pumpkins (violaxanthin), oranges (apocarotenal), chanterelles (canthaxanthin) and red smear cheese (3,3'-dihydroxyisorenieratene) are naturally-occurring carotenoids. [10] They give rise to the various colours of fall foliage (lutein), or the rose-pink plumage of the flamingo and ibis,

7.7 *Carotenoids are widespread in Nature and important pigments in living organisms.*

and the red of starfish and sea-urchins (echinenone), goldfish, crabs, krill (*Euphausiacea*, animal plankton) and lobsters (astaxanthin) (Fig. 7.7, Fig. 7.8 and Fig. 7.9).

In order to colourise margarine, vegetable oils, cheese, soups, lemonade and custard, carotenoids are added to these foodstuffs. Carotenoids also play an important role as additives to animal feed. Worldwide, 2,400,000 tonnes of salmon are farmed annually in net cages. In order to ensure that salmon fillet from aquaculture has the same colour as that from wild salmon, their feed is



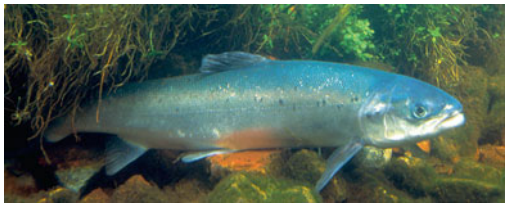
7.8 *A selection of natural carotenoids. From a chemical viewpoint, these food colourants are all closely related to one another. They differ only in their terminal groups.*

supplemented with astaxanthin as amorphous nanoparticles for better absorption (Fig. 7.10).

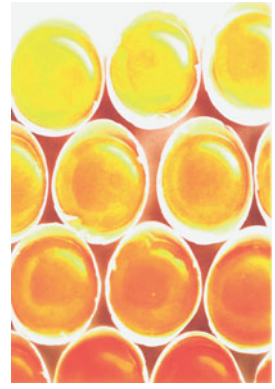
Pigmentation is also a priority aspect for using carotenoids in poultry feed, in order to meet regional colouring preferences: pale, almost colourless egg yolks in Norway, a strong yellow in Germany, an orange-red yolk in duck eggs from Thailand, and in Spanish-speaking countries the yellow-pigmented skin of broiler-chickens, in order to match the appearance of maize-fed chickens (Fig. 7.11).

On account of their highly unsaturated structure, carotenoids are ideal antioxidants. They quench singlet oxygen and scavenge radicals. The significance of carotenoids in the prevention of chronic diseases of the immune and cardiovascular system, or of cancer, has been investigated in numerous medical studies. [11]

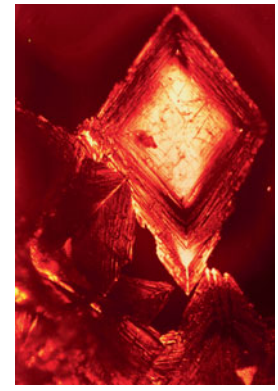
7.9 *The carotenoids absorb light in the blue-green region, and pass on this energy to the photosynthesis system. Hereby, an extension of the absorption spectrum is achieved, along with an increased efficiency of carbohydrate photosynthesis in the plant. While in autumn the colour of the leaves fades through degradation of the dominant chlorophyll, the carotenoids become visible.*



7.10 *Of economic importance is the European salmon (Salmo salar), a migratory fish, 1–1.5 metres long and up to 36 kilograms in weight, which has been farmed, for example in Norway and Scotland, since the 1980s in coastal aquacultures.*

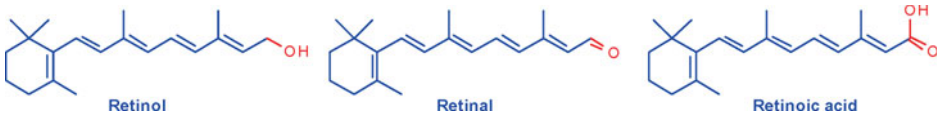


7.11 *Depending on the carotenoid content of the poultry feed, the colour intensity of egg yolks varies.*



7.12 *The term "Vitamin A" is here collectively used to encompass (all E)-retinol and (all E)-retinal, also their 3-dehydro-derivatives and double bond isomers, and retinol esters.*

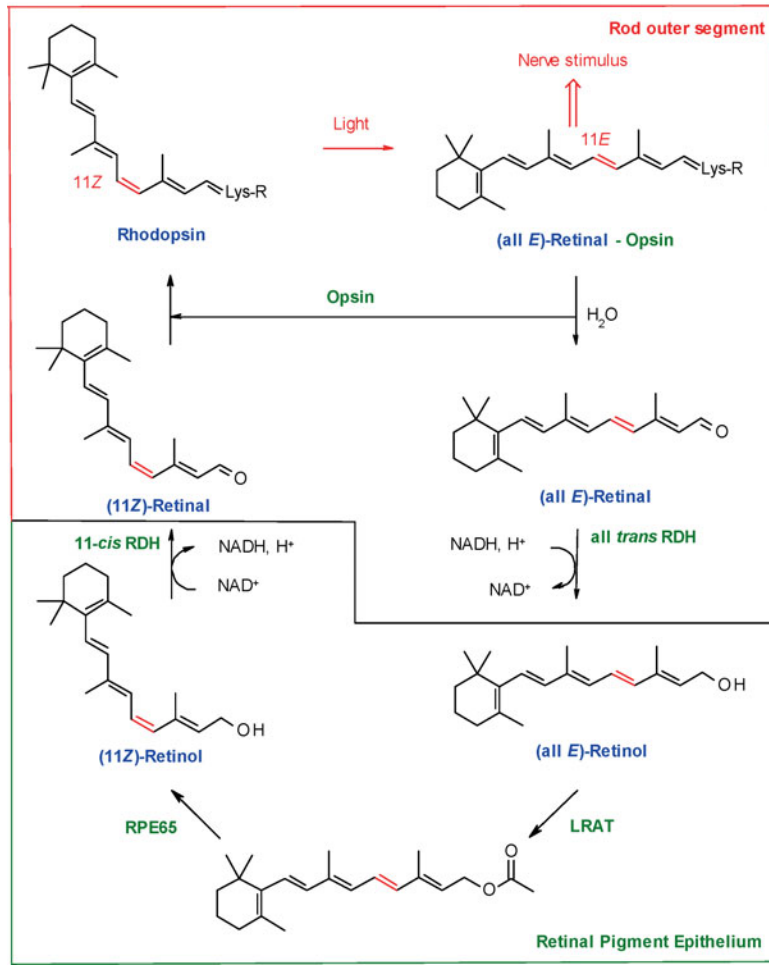
Vitamin A (retinol) (Fig. 7.12) and its metabolic oxidation products assume a critical physiological role in growth, development and differentiation of epithelial tissue, the maintenance of vision, and as well in spermatogenesis and the normal development of the placenta and the foetus. (all*E*)-Retinoic acid and (13*Z*)-retinoic acid are important medicaments for the treatment of acne and psoriasis; the former has also demonstrated complete remission in most cases of acute promyelocytic leukemia (APL).



Vitamin A is used as a feed additive in farming to enhance the regeneration and the protection of the skin, and to increase the fertility of animals. In addition, Vitamin A supports growth and protects against infectious diseases.

Apart from its protective and health-promoting properties, Vitamin A is essential for the perception of light. Our current understanding of the visual cycle is based on the seminal work of Ragnar Arthur Granit (1900–1991), Haldan Keffer Hartline (1903–1983) and George Wald (1906–1997), who shared the 1967 Nobel Prize in Physiology or Medicine, "for their discoveries concerning the primary physiological and chemical visual processes in the eye". [12]

In mammals, (11*Z*)-Retinal, generated from retinol in the retina, is the photo-reactive chromophore, which forms a Schiff-base to a lysine residue of opsin, a G-protein-coupled receptor (GPCR) protein, to give rhodopsin. This "visual purple" is concentrated in the outer parts of the rod and cone photoreceptors. Upon light absorption, the chromophore converts photons into a chemical signal by isomerisation to (all *E*)-retinal, which causes a conformational change of



the GPCR moiety of rhodopsin to accommodate the cytosolic binding of G proteins and to initiate the downstream signal transduction.

To regenerate the rhodopsin pigment, the (all *E*)-retinal is cleaved off from its bleached rhodopsin complex and recycled by reduction to (all *E*)-retinol (RDH: retinol dehydrogenase) in the rod outer segment, followed by Lecithin retinol acyltransferase (LRAT) mediated esterification in the retinal pigment epithelium. These (all *E*)-retinyl esters are stored in retinosomes and / or reutilised *via* hydrolysis and isomerisation into the (11*Z*)-retinol, which is catalysed by a retinal pigment epithelium-specific 65 kDa protein (RPE65). Reoxidation and transport to the rod outer segment starts the cycle again.

Carotenoids such as lutein and zeaxanthin are found in the macular pigment, primarily in the central retina. They function like internal "sun glasses" and help to protect the retina and the embedded photoreceptors from the damage, caused by high-energy light. Lutein and zeaxanthin are found in green leafy vegatables (like spinach and kale), but also in corn, eggs and other foods. Due to the potential benefit of preventing an early onset of macular degeneration, which poses an increasing problem in an aging population, dietary intake of lutein is now recommended by ophthalmologists, and the industry has responded by including this component in its multivitamin preparations.

7.1.1 Discovery of Carotenoids

Crude *β*-carotene was isolated in 1831 from carrots (*Daucus carota*) by Heinrich Wilhelm Ferdinand Wackenroder (1798–1854) as the first representative of the carotenoids. However, when better isolation techniques became available, it was possible to obtain chemically pure material. In 1907, Richard Willstätter (1872–1942) was the first to correctly identify the molecular composition of *β*-carotene. [13] In 1928, a more intensive research effort started in this area: László Zechmeister (1889–1972) and Paul Karrer (1889–1971) recognised, that the chromophoric group of *β*-carotene and its isomer lycopene, the colourant in tomatoes (*Solanum lycopersicum*), arises from conjugated double bonds within a $C_{40}H_{56}$ hydrocarbon system. Hydrogenation experiments indicated that *β*-carotene contains 11 double bonds, although lycopene contains 13. [14, 15]

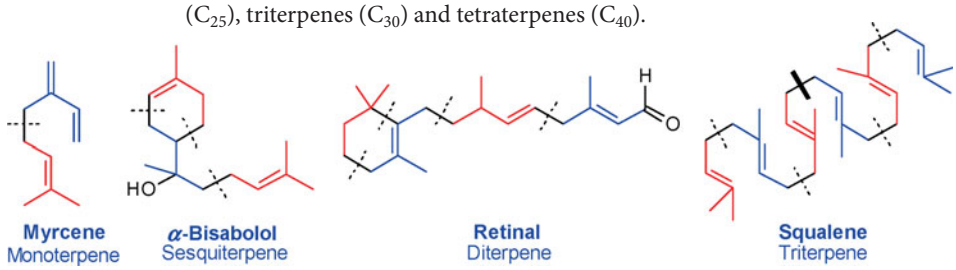
In 1929, Thomas Moore (1900–1999) at The Nutritional Laboratory in Cambridge was able to prove clearly, on the basis of animal experiments, that *β*-carotene is provitamin A, and thus distinct from all the other carotenoids known at the time. [16] It seemed however plausible, that their structures were all closely related. In 1931, Richard Kuhn (1900–1967) and Paul Karrer (1889–1971) used, besides fractional crystallisation, for the first time chromatography as a novel separation technique for the purification of carotenoids. [17, 18] In the same year, Karrer isolated almost pure retinol from shark liver oil. [19] The structural elucidation of retinol was particularly challenging due to its initially unrecognised sensitivity towards oxidation. However, further light was shed on the structure of *β*-carotene through oxidative degradation experiments using ozone, potassium permanganate and chromic acid. Karrer was thereby able to determine the structure of *β*-carotene and to suggest the first correct structure

Retinal was first synthesised in 1946 by the Dutch chemists Josef Ferdinand Arens (1914–2001) and David Adriaan van Dorp (1915–1995). [20]

of retinol. In 1937, Kuhn synthesised retinol for the first time. [20] In 1946, James G. Baxter and Charles D. Robeson (1916–2010) isolated (13*Z*)-retinol in crystalline form. [21] Starting from β-ionone, Karrer and Conrad Eugster (1921–2012) succeeded in 1950 to synthesise β-carotene [22], and finally in 1952, the structure of retinol could be elucidated and confirmed with the synthesis of 3-dehydroretinol. [23]

7.1.2 Biosynthesis

Otto Wallach (1847–1931) (laureate of the 1910 Nobel Prize for Chemistry) and Konrad Julius Bredt (1855–1937) recognised at the beginning of the last century that terpenes are formed from C_5-building blocks (Fig. 7.13): semiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes (C_{25}), triterpenes (C_{30}) and tetraterpenes (C_{40}).

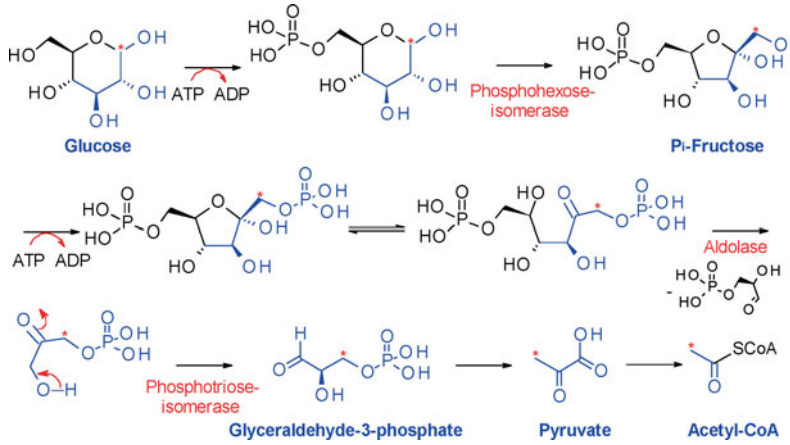


7.13 *The concepts of Bredt, Wallach and Ružička would nowadays be labelled as "retrosynthesis" or "retrosynthetic analysis".*

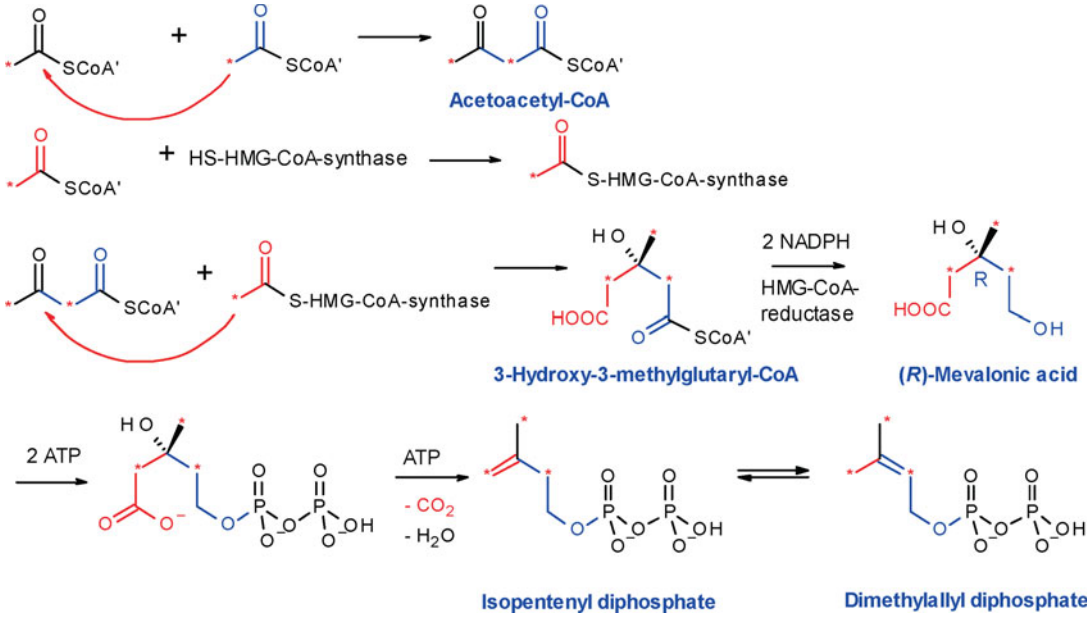
In 1921, Leopold Ružička (1887–1976) established the isoprene rule for the biosynthesis of terpenes through a formal head-to-tail coupling of isoprene units. Deviations from the rule (as in the case of chrysanthemic acid), as well as Wagner-Meerwein rearrangements, occasionally complicate the analysis. Only in the mid-1950s it could be shown that terpenes are indeed formed by "active isoprene units". [24, 25]

Mevalonate Pathway

If the degradation of 1-^{13}C-labelled glucose, *e.g.* in *Escherichia coli* or *Alicyclobacillus acidoterrestris*, is investigated, the label is found at the terminal carbon atom (C-3) of glyceraldehyde-3-phosphate, pyruvate and acetyl-CoA.



When correspondingly the biosynthetic pathway to terpenes is tracked, three carbons in dimethylallyl diphosphate carry the label. First, two molecules of acetyl-CoA condense to give acetoacetyl-CoA. Then an enzyme-bound acetyl residue is transferred in the manner of an aldol addition, and reduction with NADPH finally produces (*R*)-mevalonic acid. Phosphorylation by ATP, dehydrative decarboxylation and isomerisation of the isopentenyl diphosphate lead to dimethylallyl diphosphate.



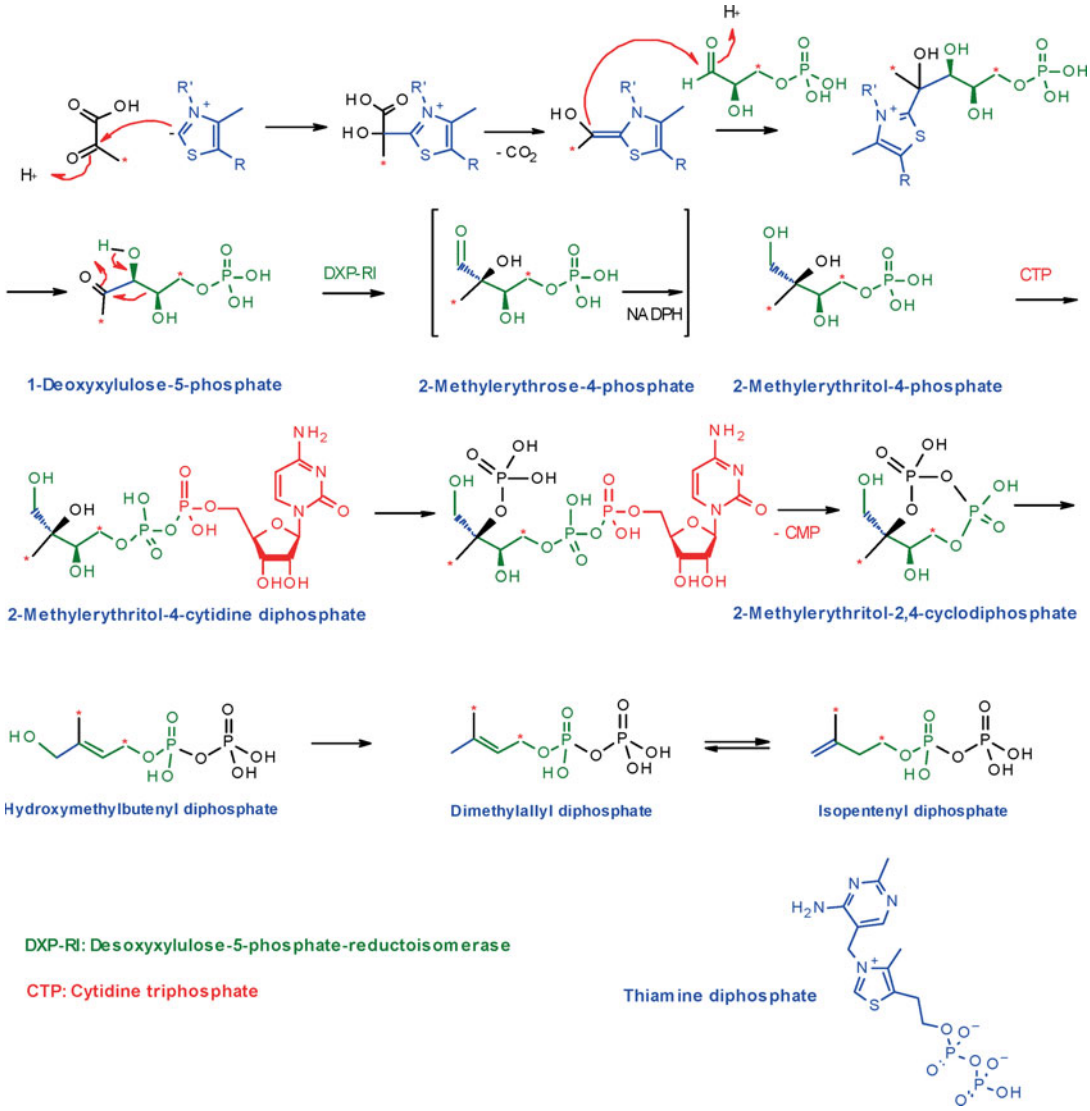
Triose-pyruvate Pathway

Since the elucidation of the biosynthetic route to sterols, especially cholesterol in the liver and ergosterol in yeast, it was generally accepted that their basic building blocks were uniformly synthesised in all living organisms *via* mevalonic acid. Only in the past few years, however, an alternative biogenesis of isoprenoid building blocks in plants and bacteria has become recognised. This biosynthetic route is called, after its discoverer, the Rohmer route or triose-pyruvate pathway (Fig. 7.14). [26]

7.14 *Michel Rohmer.*

This route had been simply overlooked for several decades, because all experimental data were interpreted to match the previous dogma, in spite of the contradictory nature of some results. As an example, in plants radioactively labelled mevalonic acid is frequently incorporated only incompletely into terpenes. Furthermore, in chloroplasts of many plants, the existence of some essential enzymes of the mevalonate pathway, *e.g.* 3-hydroxy-3-methylglutaryl-CoA-reductase (HMG-CoA-reductase), could not be demonstrated. In the meantime, the triose-pyruvate pathway was found in a whole range of higher plants. [27]

Noteworthy is the bacterium *Streptomyces aeriouvifer*, because it uses the mevalonate route along with the triose-pyruvate route. In higher plants, this is



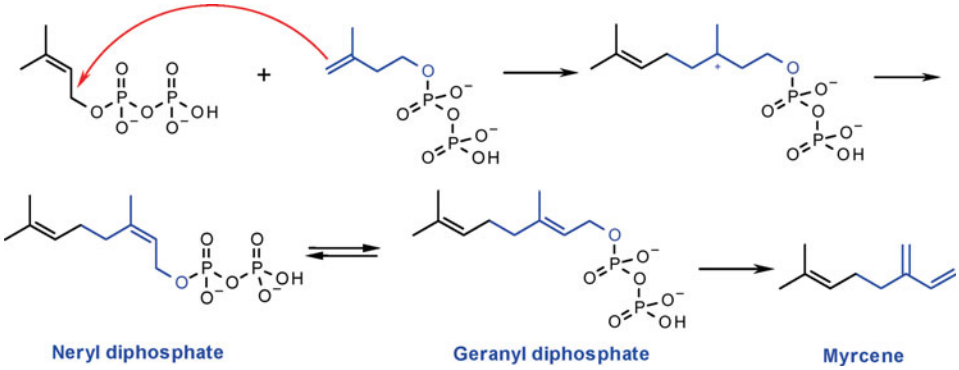
the rule. By contrast, there is so far no indication yet of the triose-pyruvate pathway in fungi and animals.

The mechanistic details were confirmed by a whole series of labelling experiments. [28, 29] By starting again from 1-^{13}C-glucose, and following the triose-pyruvate route, ^{13}C-labelling is found in only two carbon atoms of the dimethylallyl diphosphate. The reaction of pyruvate with glyceraldehyde-3-phosphate (triose-3-phosphate) is thiamine diphosphate-dependent and leads to 1-deoxyxylulose-5-phosphate. Remarkable is its rearrangement into 2-methylerythrose 4-phosphate. The stereochemical course of this step is comparable to the ketol rearrangement in the biosynthesis of valine and isoleucine. The hydroxy- and keto-groups are arranged in a *syn*-periplanar orientation, so that the glycol residue is transferred suprafacially to the *Re*-side. In the presence of

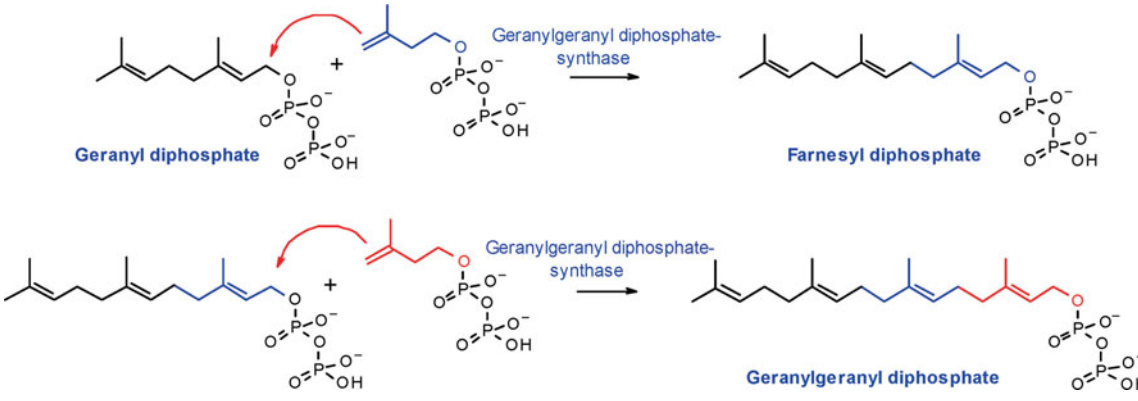
cytidine triphosphate (CTP), a cytidine phosphate moiety is transferred by the corresponding transferase. [30, 31] Subsequently, a kinase phosphorylates the hydroxy-group at the 2-position. By cleavage of cytidine monophosphate, 2-methylerythritol-2,4-cyclodiphosphate is formed. [32] The cyclic diphosphate undergoes reductive ring-opening. The hydroxymethylbutenyl diphosphate-synthase is a [4Fe-4S]-protein, on which, in presence of NADPH, the reduction proceeds. [33] An analogous process is discussed for the final step in the biosynthesis of isopentenyl diphosphate and dimethylallyl diphosphate. [34]

Biosynthesis of Higher Terpenes

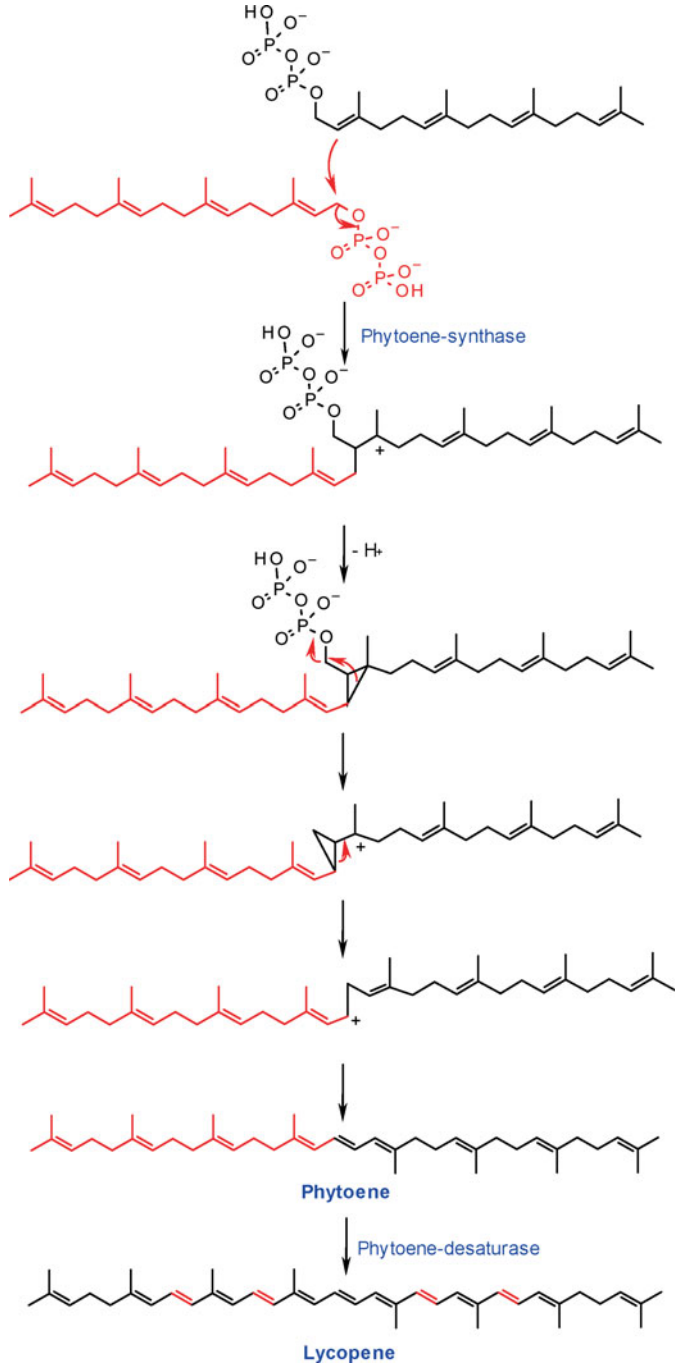
The enzymatic cleavage of diphosphate from dimethylallyl diphosphate leads to a resonance-stabilised prenyl cation, which reacts *in statu nascendi* further with nucleophiles. Monoterpenes are formed by reaction with isopentenyl diphosphate. The cleavage of the diphosphate residue from geranyl diphosphate produces myrcene.



The reaction of geranyl diphosphate with isopentenyl diphosphate gives farnesyl diphosphate, and repetition of this sequence leads to geranylgeranyl diphosphate.



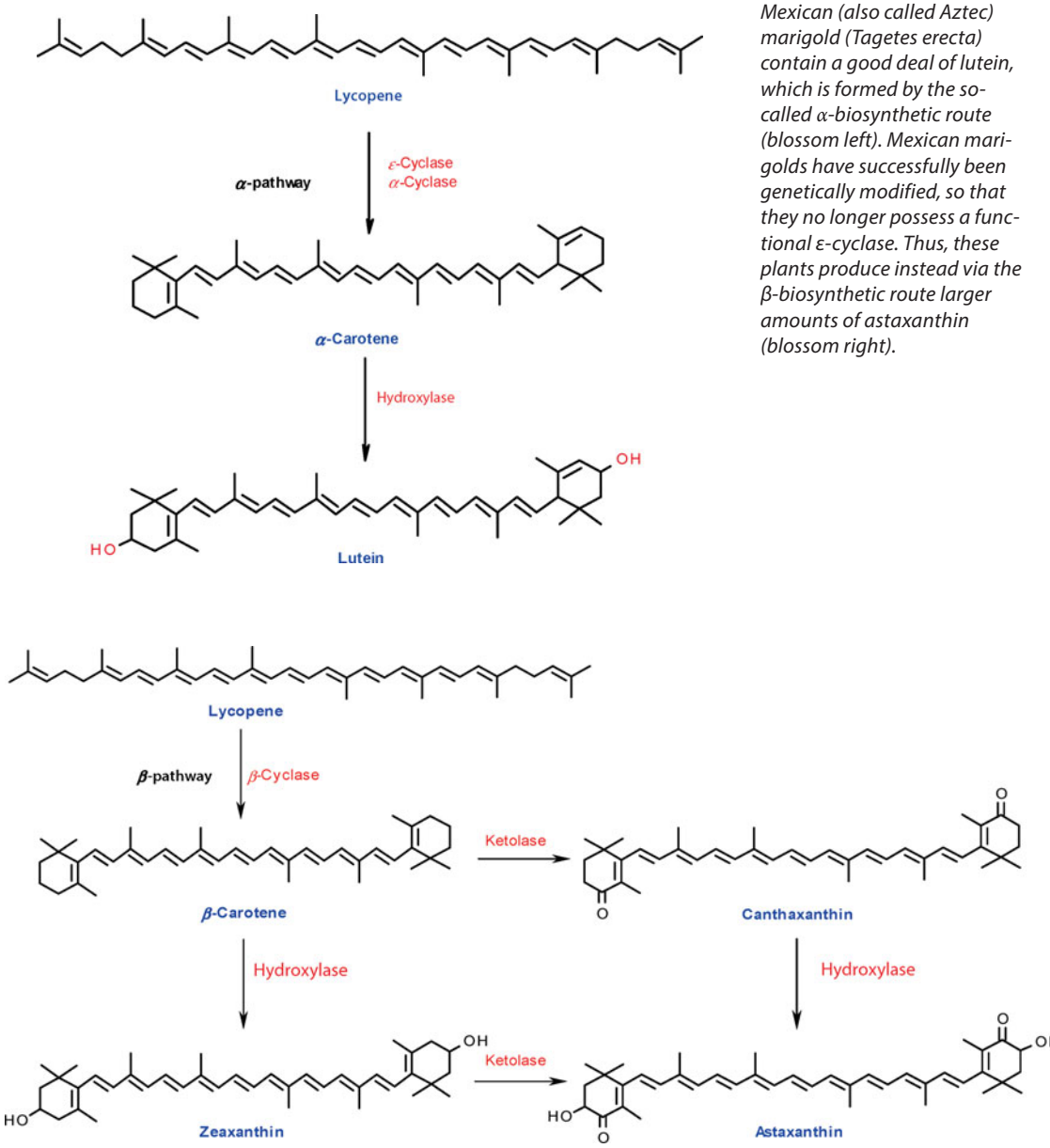
The tail-to-tail coupling of two geranylgeranyl diphosphate units proceeds *via* a cyclopropane intermediate. Cleavage of the diphosphate from the primary cyclopropane derivative, ring-opening of the resulting cyclopropylmethyl carbo-


Phytoene-synthase
- H+
Phytoene
Phytoene-desaturase
Lycopene

cation, and deprotonation then give phytoene. Finally, dehydrogenation produces lycopene, which imparts the red colour to tomatoes and rose hips.

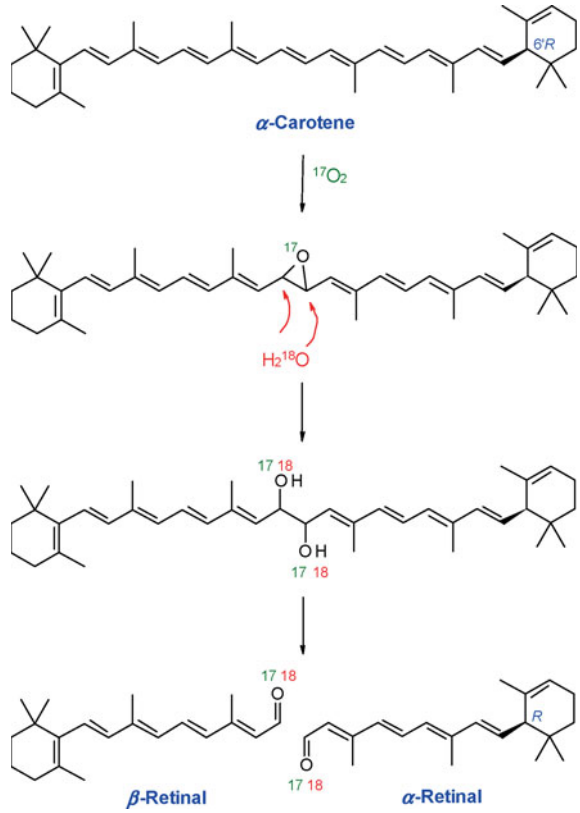
Lycopene is the central intermediate for all the more highly functionalised carotenoids. The ends are cyclised under acid catalysis to α- or β-carotene. Hydroxylation of the former yields lutein. The xanthophylls, (3*R*,3'*R*)-zeaxanthin, canthaxanthin and astaxanthin, result, as in the example shown here, from oxidations of β-carotene (Fig. 7.15).

7.15 *The petals of the Mexican (also called Aztec) marigold (Tagetes erecta) contain a good deal of lutein, which is formed by the so-called α-biosynthetic route (blossom left). Mexican marigolds have successfully been genetically modified, so that they no longer possess a functional ε-cyclase. Thus, these plants produce instead via the β-biosynthetic route larger amounts of astaxanthin (blossom right).*



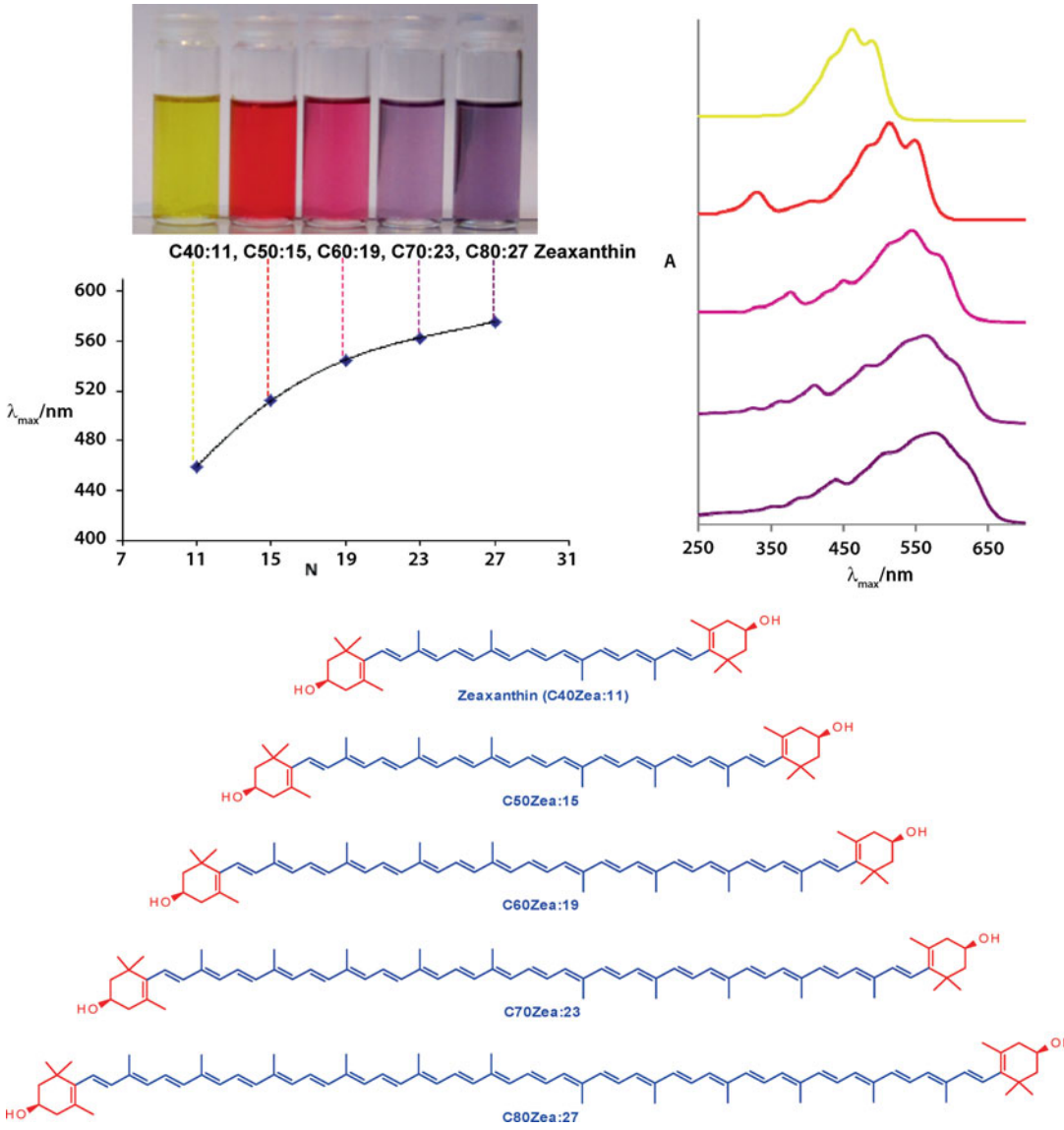
Biosynthesis of Retinoids

While the previous school of thought was, that the degradation of β-carotene to retinal takes place by an iron dioxygenase mediated [2 + 2]-cycloaddition of oxygen, followed by fragmentation of the dioxetane; however, new investigations with a purified enzyme revealed, that a monooxygenase is the enzyme behind. [35] Labelling experiments to monitor the degradation of α-carotene *via* epoxidation with $^{17}O_2$ and hydrolysis with $H_2{}^{18}O$ produced α- and β-retinal with its carbonyl group carrying equal labels of ^{17}O and ^{18}O. The degradation of β-carotene follows presumably the same mechanism.



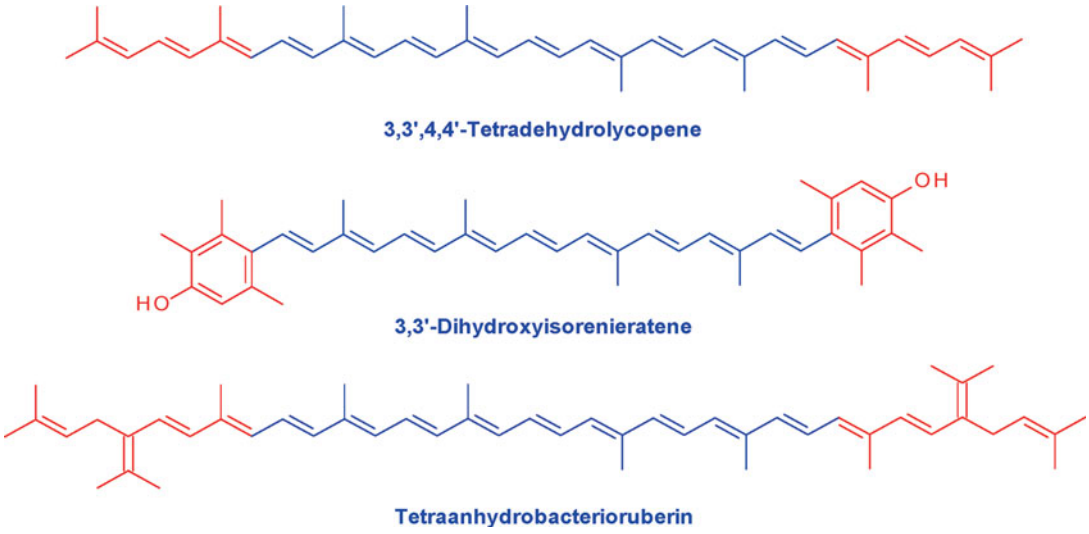
7.1.3 The Colours of Carotenoids

Natural carotenoids are typically tetraterpenes, which absorb blue light in the range of 400–500 nm. Hereby, the absorption maximum (λ_{max}) is primarily related to the number of conjugated double bonds. Whereas, for example, natural zeaxanthin (C40Zea:11) has its absorption maximum at 461 nm, λ_{max} of the as yet longest artificial ca rotenoid C80Zea:27 is at 576 nm. With the increasing number of double bonds, the hue turns in solution correspondingly from yellow to red and blue (Fig. 7.16). [36, 37]



7.16 *Based on zeaxanthin, the bathochromic shift is related to the number of double bonds (N) and it gradually decreases with increasing N (λ_{max} of C40Zea:11 = 461 nm, C50Zea:15 = 514 nm, C60Zea:19 = 547 nm, C70Zea:23 = 563 nm, C80Zea:27 = 576 nm).*

Nature did however not develop blue carotenoids by increasing the number of double bonds. The longest conjugated systems in natural carotenoids, as known so far, extends to 15 double bonds and corresponds to a red hue (Fig. 7.17). Examples are tetraterpenes such as 3,3',4,4'-tetrahydrolycopene (in unripe tomatoes) and 3,3'-dihydroxyisorenieratene (in *Brevibacterium linens*

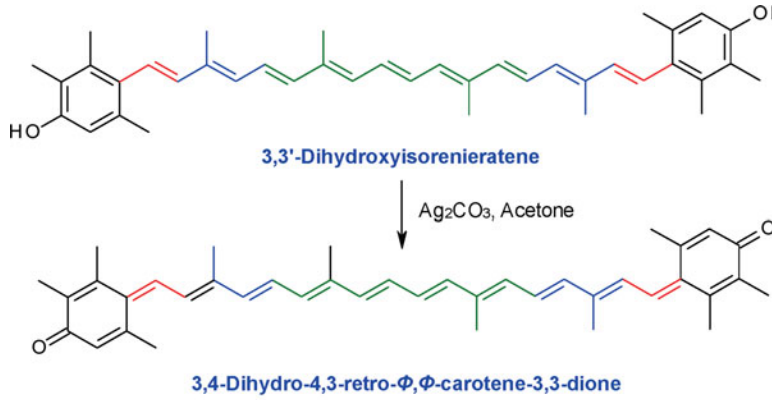


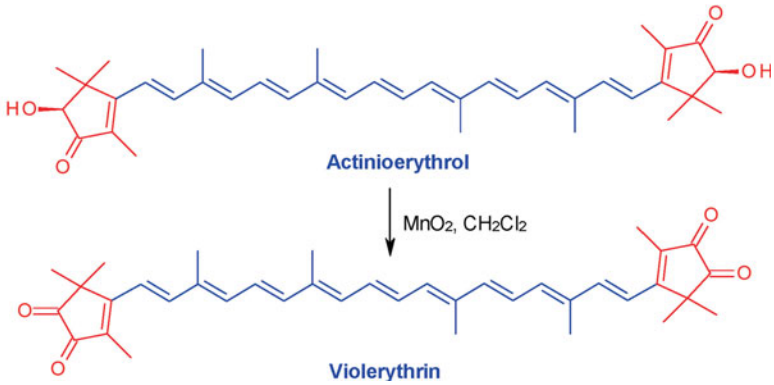
7.17 *The longest conjugated systems in natural carotenoids.*

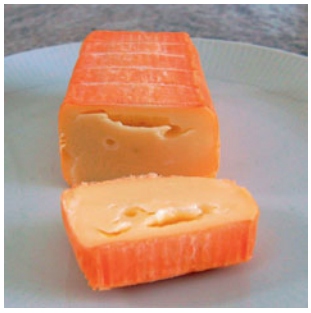
In the 1940s, Robert B. Woodward and Louis F. Fieser developed empirical rules for the incremental estimation of λ_{max}. Nowadays, for this purpose we have elaborated quantum mechanical methods at our disposal; however, in the case of vast conjugated systems, we still encounter considerable problems to calculate the UV/Vis spectra.

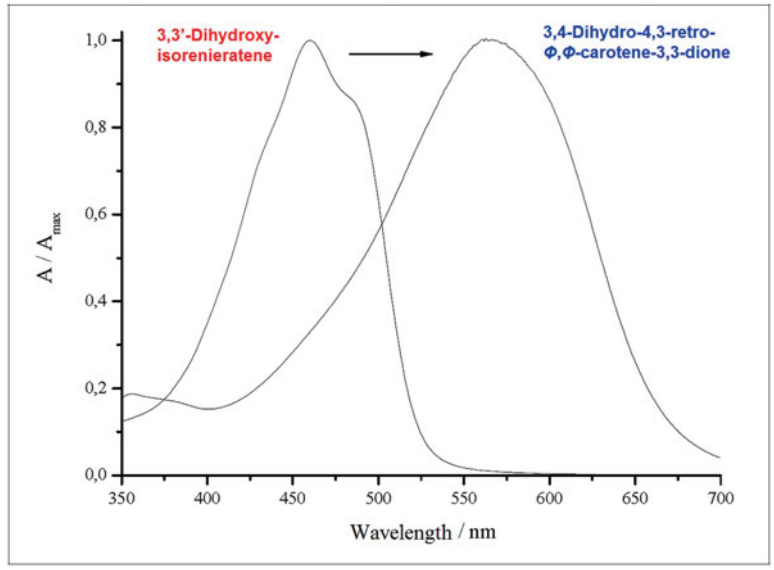
(Fig. 7.18)) and pentaterpenes such as tetraanhydrobacterioruberin (from *Halobacterium salinarum*). [38]

Nevertheless, based on relatively simple carotenoids, virtually a true kaleidoscope of colours, up to blue shades, can be found in Nature. A few oxidation products of natural carotenoids, such as violerythrin (λ_{max} = 549 nm in acetone) from actinioerythrol (isolated from sea anemones, λ_{max} = 500 nm in acetone) and 3,4-dihydro-4,3-retro-β-carotene-3,3-dione (λ_{max} = 563 nm in acetone) from 3,3'-dihydroxyisorenieratene (λ_{max} = 460 nm in acetone) have a distinguished blue colour (Fig. 7.18 and Fig. 7.19). [39] While it takes just one reaction step, oxidation is not a preferred route how Nature affords blue hues.

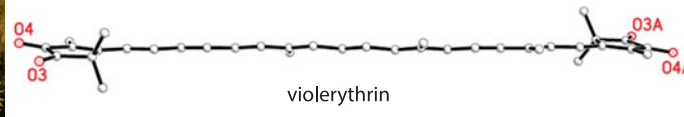






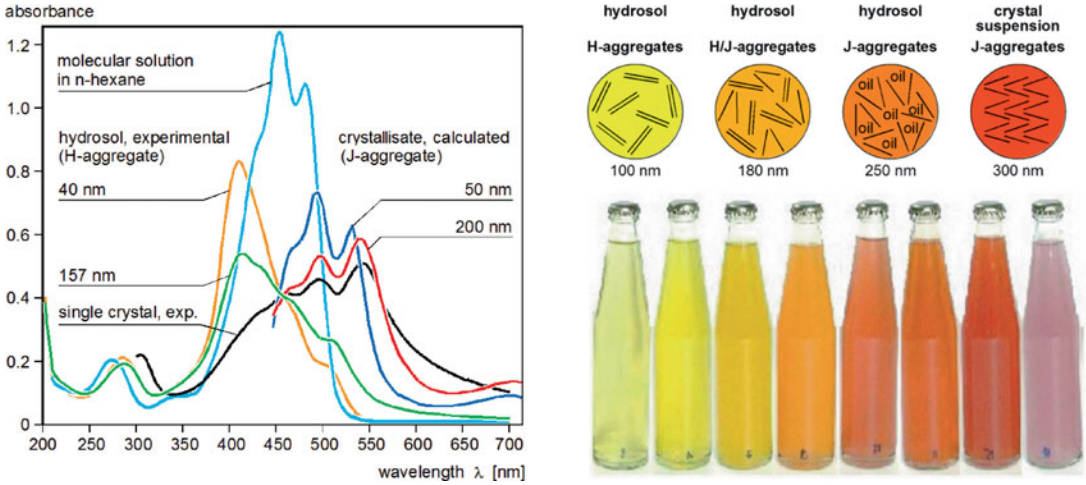


7.18 *Brevibacterium linens is ubiquitously present, and populates also the human skin, where it causes e.g. foot odour. It is contained in the reddish smear coat of Remoudou and other Belgian cheeses. The oxidation of its red pigment leads to a tremendous bathochromic shift (about 100 nm).*



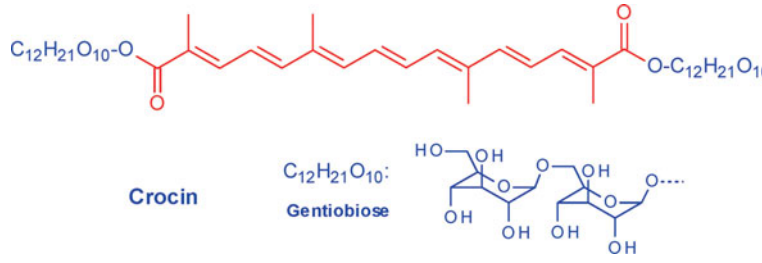
7.19 *Sea anemones are a group of water-dwelling, predatory animals of the order Actiniaria. Actinioerythrol was isolated, for example, from the sea anemone Actinia equina L., which is also known as beadlet anemone or pomodoro di mare, and which can be seen on the bottom left side of the painting. Its oxidation product, violerythrin, bears an almost planar, cross-conjugated chromophore, containing 30 π-electrons.*

Remarkably nice plays of colours can be observed when carotenoids are forming aggregates by supramolecular self-organisation. Depending on the type of aggregation, hypsochromic (H-aggregates) as well as bathochomic (J-aggregates, referring to Edwin E. Jelley, who discovered this phenomenon in 1936) shifts compared to the mono-molecular solution can be observed, as it has been demonstrated *inter alia* for the lipophilic β-carotene (Fig. 7.20). [40]

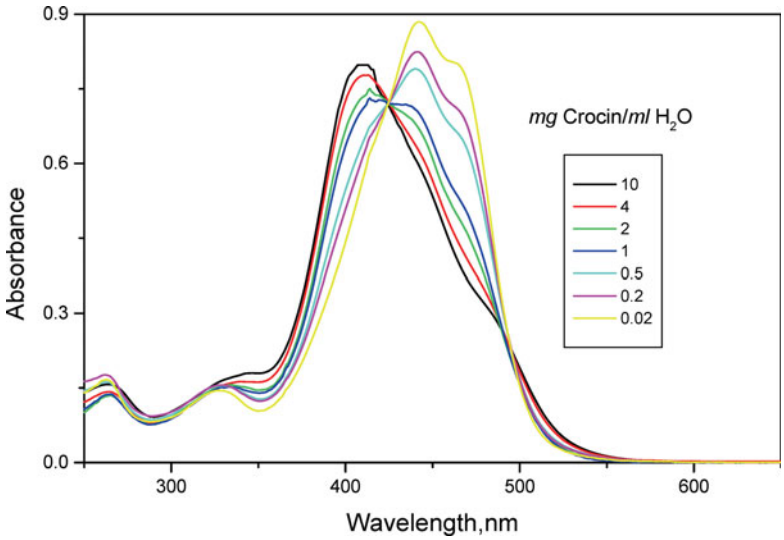


7.20 *Influence of aggregation structure and particle size on the colour shade of nanodispersed β-carotene hydrosols. – Thereby, and by using blends of carotenoids, such as carotene, lycopene, 8′-apo-β-carotenal and canthaxanthin, the colour of soft drinks can be adjusted from light yellow to red violet.*

A nice hypsochromic shift was also observed with one of the rare water-soluble carotenoids: crocin, the ingredient, which is primarily responsible for the colour of saffron (Fig. 7.21). The critical aggregation concentration in water was found to be 0.8 mg/ml. Upon increasing the concentration, a hypsochromic shift from λ_{max} = 445 to 410 nm was observed due to a change in the equilibrium between monomeric and aggregated forms (Fig. 7.22). The UV/Vis spectra of crocin in water at concentrations below 0.8 mg/ml resemble those in organic solvents and represent the monomeric form (λ_{max} = 445 nm). [41]



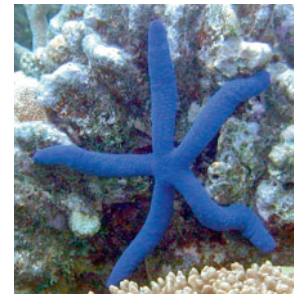
7.21 *Saffron (Crocus sativus) blossom with crimson stigmas. Crocin is a potent antioxidant. It has anticarcinogenic, antidepressant and aphrodisiac properties (at least according to an Iranian study in normal male rats). [42]*



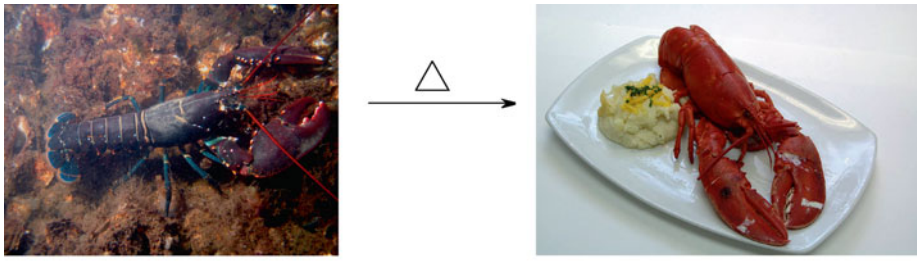
7.22 *UV/Vis Spectra of crocin in water as a function of concentration.*

However, the most prominent and unsurpassed way, how Nature generates huge bathochromic shifts and ultimately affords blue colours, is by incorporating carotenoids into proteins. [39] Examples for these carotenoproteins are allophorin (λ_{max} = 545 nm) in the soft coral *Allopora californica*, linckiacyanin (λ_{max} = 612 nm) in the blue star fish *Linckia laevigata* (Fig. 7.23) and α-crustacyanin (λ_{max} = 632 nm) (which is an octamer of β-crustacyanin (λ_{max} = 587 nm)) in the lobster (*Homarus gammarus*) (Fig. 7.24).

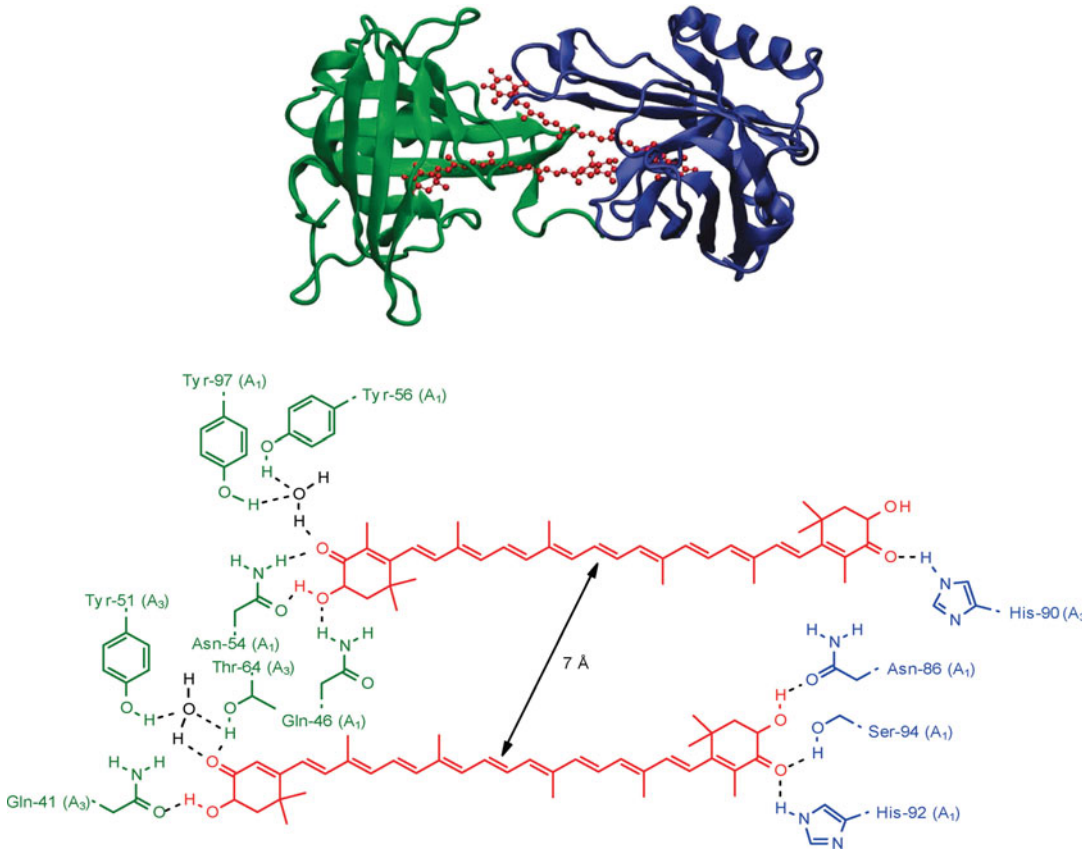
In β-crustacyanin, several amino acids of the apoproteins form hydrogen bonds to two molecules of astaxanthin (Fig. 7.25). [39, 43] The two astaxanthin molecules are at a distance of 7 Å and constitute an exciton coupling system. Further, and perhaps more significant contributions to the enormous bathochromic shift are due to the elongation of the π-system, caused by the coplanarity of the cyclic end groups and by partial polarisation of the keto-groups.



7.23 *Linckia laevigata lives in shallow waters of the tropical Indian and Pacific Ocean. The blue carotenoprotein linckiacyanin has been isolated from the skin of starfish.*



7.24 *Astaxanthin (λ_{max} = 472 nm) is the typical colourant of lobster. In live animals, astaxanthin is complexed by proteins. The absorption maximum of these complexes lies at 587 and 632 nm, so that the animals appear blue. When lobster is cooked, the protein complexes are destroyed and the red colour of free astaxanthin becomes evident.*



7.25 *β-Crustacyanin is a 1 : 1 astaxanthin-protein dimer.*

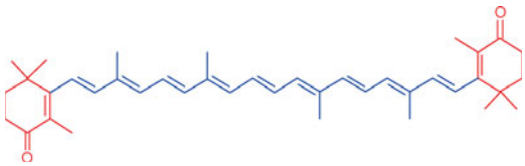
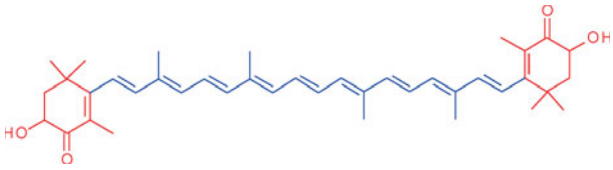
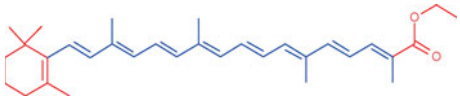
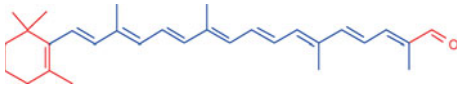
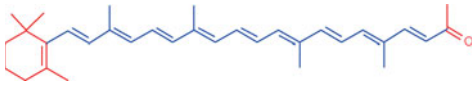
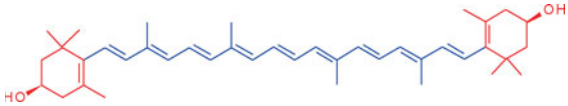
7.1.4 Industrial Manufacturing Processes

The companies' names used in the following are those, by which these entities were known at the time of the development of the processes. Meanwhile, some of the companies still exist, but have divested their vitamin business, or they have been absorbed into other businesses by mergers and acquisitions.

The industrial synthesis of nature-identical carotenoids began in 1954 at Hoffmann-La Roche (the Vitamin division of which belongs now to DSM) and in 1960 at BASF. In the course of time, a number of other firms followed, such as Rhône-Poulenc (now Solvay/Rhodia and Adisseo, a member of the Chinese BlueStar group), Sumitomo, Kuraray, Glaxo and Philips. In 1995, the annual global sales of carotenoids reached 500 million US dollars, equalling that of Vitamin A. The carotenoid market was estimated in 2011 at almost 1.2 billion, and is expected to increase further to 1.4 billion US dollars by 2018. [44] Out of the approximately 700 known natural carotenoids, only a few have gained greater industrial and commercial significance. Since 1990, the business, in particular for β-carotene and astaxanthin, has grown steadily (Tab. 7.2).

Universal methods for coupling of the synthetic building blocks are the Wittig reaction, the Horner-Wadsworth-Emmons reaction, the sulfone coupling by Julia's procedure, the enol ether condensation (Müller-Cunradi-Pieroh reaction), and the Saucy-Marbet rearrangement. Since in very many cases mixtures

Tab. 7.2 *Commercially important carotenoids*

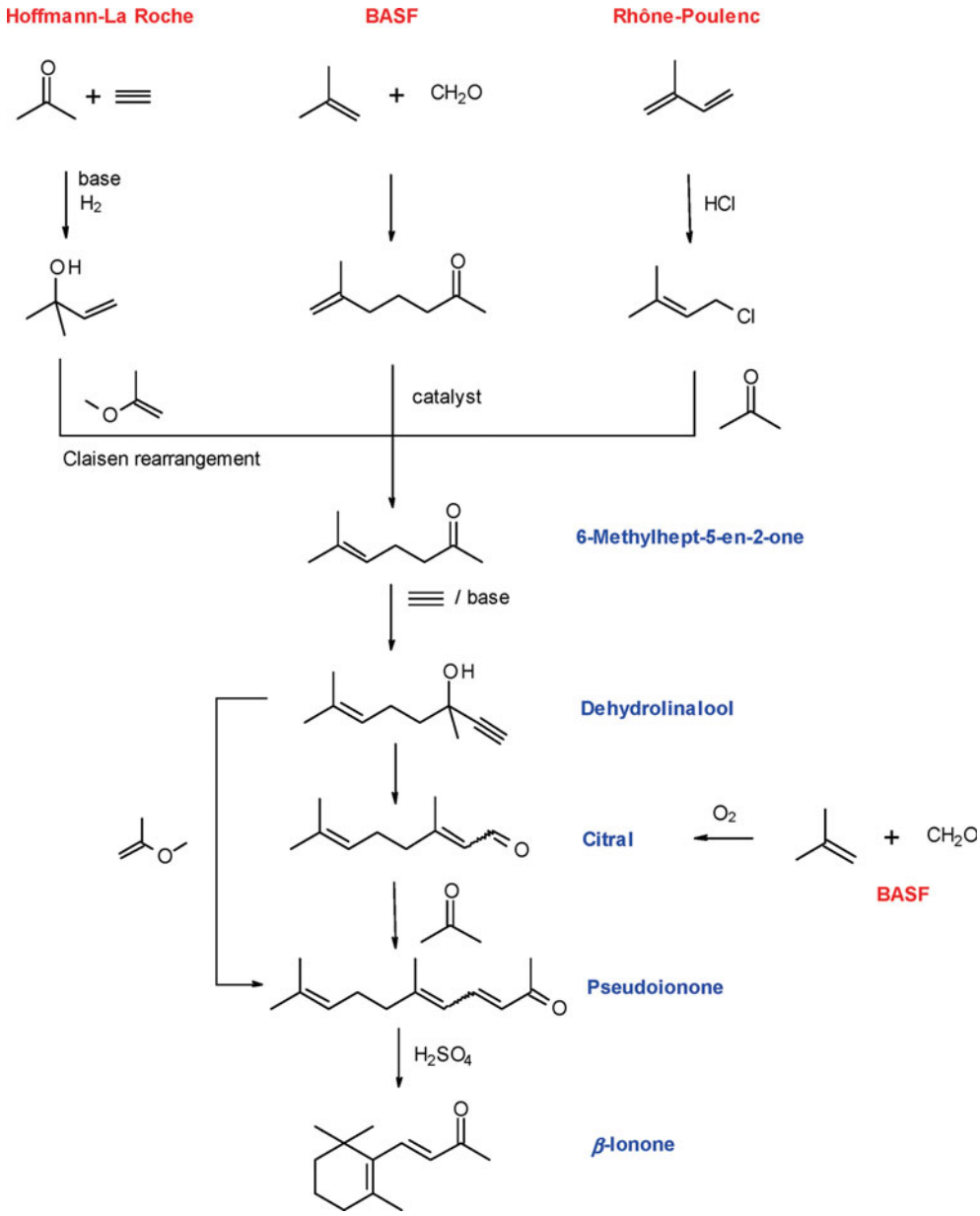
Structure	Name	Use
	*β*-carotene	margarine fruit juices nutritional supplement fertility enhancer of livestock
	canthaxanthin	egg yolk colourant broiler skin-pigmentation fish farming
	astaxanthin	salmon farming lobster farming
	ethyl 8′-*apo*-*β*-carotenoate	egg yolk colourant broiler skin-pigmentation
	8′-*apo*-*β*-carotenal	processed cheese salad dressing
	citranaxanthin	egg yolk colourant
	lycopene	nutritional supplement multivitamin preparations
	zeaxanthin	nutritional supplement

of (*E*)- and (*Z*)-isomers are produced, a subsequent isomerisation, *e.g.* by heating, is often necessary.

The central intermediate for all manufacturing methods of Vitamin A and *β*-carotene is *β*-ionone, for which the production process has already been discussed in connection with the fragrances of violets (*cf.* section 3.2.3). To briefly recapitulate the essentials of the different strategies: The starting material used by Hoffmann-La Roche was acetylene, while BASF used isobutylene, and

It is still under debate, whether citranaxanthin is a genuine natural carotenoid, or an artefact of its isolation process from treatment of natural extracts with acetone. [45]

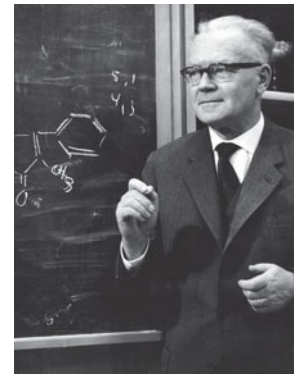
Rhône-Poulenc isoprene. 6-Methylhept-5-en-2-one is an important intermediate, which is transformed *via* citral into pseudoionone and finally cyclised to β-ionone.



Wittig Olefination

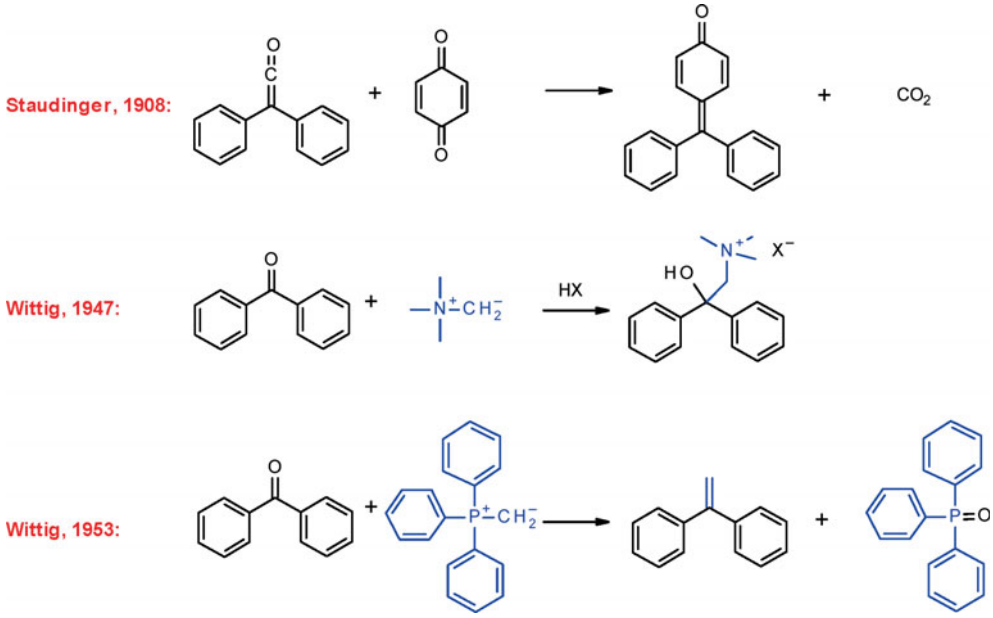
In the course of working on pentavalent phosphorus compounds, Georg Wittig (1897–1987) [46] discovered in 1953 the olefination reaction later named after him (Fig. 7.26). [47] As in many truly great discoveries, serendipity plays a ma-

jor role, and they may be not at all that new, if examined more thoroughly. In case of the Wittig olefination, this reaction of methylenetriphenylphosphorane with benzophenone was unintended; and there were also parallels to a reaction, which Hermann Staudinger (1881–1965) [48] had carried out 45 years earlier [49], but what Wittig initially did not notice. In 1947, Wittig had reacted nitrogen ylides with benzophenone to give (2-hydroxy-2,2-diphenylethyl)-trimethylammonium salts. [50] Correspondingly, phosphorus ylides were expected to give phosphonium salts. Wittig instead obtained 1,1-diphenylethene in a yield of approximately 84 %. [51]



7.26 *Georg Wittig.*

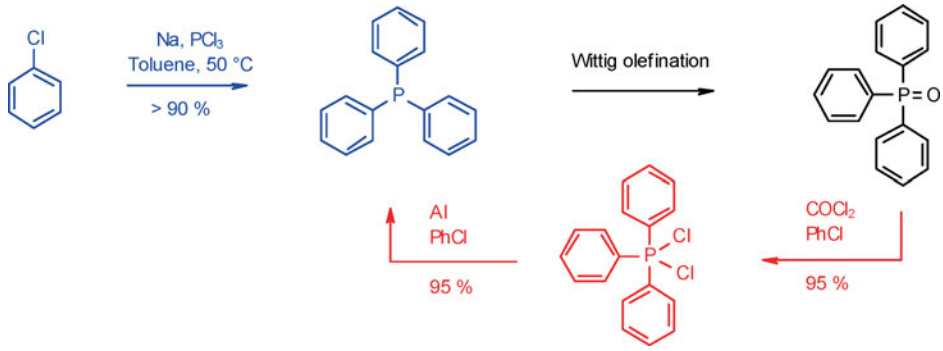
Already in 1956, the first patent was published, in which the retinoic acid ester synthesis by a Wittig reaction was claimed. [52–54] Special about Wittig's discovery was, that this unanticipated reaction enabled for the first time the generation of carbon-carbon double bonds under very mild conditions. Moreover, the method was widely applicable. Wittig recognised both of these advantages very quickly. Nevertheless, it was not simple to answer the question, whether this new synthesis method could prove utility on an industrial scale.



Challenges of Wittig Reactions

Triphenylphosphane oxide

One of the serious drawbacks of the Wittig reaction is the unavoidable production of triphenylphosphane oxide in stoichiometric quantities. Whilst its direct reduction with boron, aluminium or silicon hydrides would be possible, these reagents are too expensive for a viable process. In fact, distilled triphenylphosphane oxide is reacted with phosgene, generated *in situ*, to give the corresponding dichloride, which is then reduced with metals, like aluminium. [55]

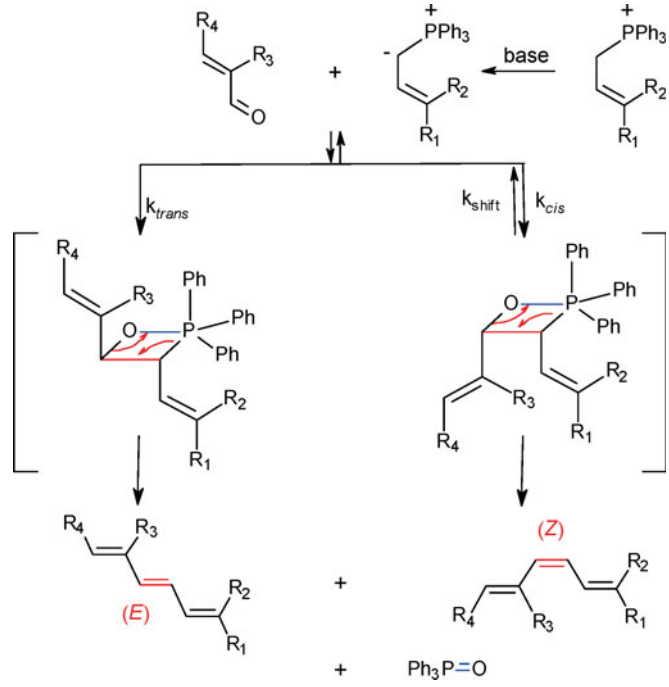


At present, more than 5,000 tonnes of triphenylphosphane are manufactured annually worldwide. The most important producers are BASF, Atochem and Hoko. Chlorobenzene is reacted with a suspension of sodium in toluene and phosphorus trichloride. On an industrial scale, this happens in a two-stage cascade reactor. After an aqueous work-up, isolation of the commercial product is achieved by distillation.

Around half of the triphenylphosphane finds application in polyene chemistry, the remainder for other Wittig reactions in the pharmaceutical industry, and a small proportion as a ligand for metal catalysts.

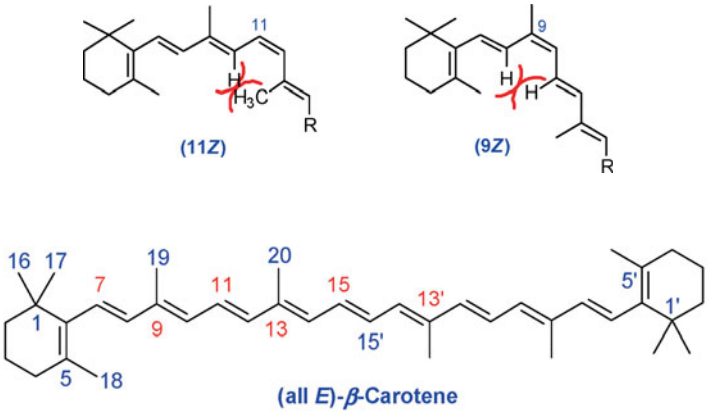
(*E*/*Z*)-Isomers

Another general problem of Wittig reactions with unsaturated and therefore semi-stabilised phosphoranes is the low (*E*/*Z*)-selectivity.



The (all *E*)-isomer is the usually desired product. The mixtures of (*E*)- and (*Z*)-isomers are mostly isomerised immediately after the Wittig reaction by different methods. Simple heating is often sufficient, and the addition of catalytic amounts of iodine, acids, or bases may accelerate the establishment of the (*E*/*Z*)-equilibrium. Noble metal catalysts are as well of importance for facilitating the isomerisation as are photochemical methods.

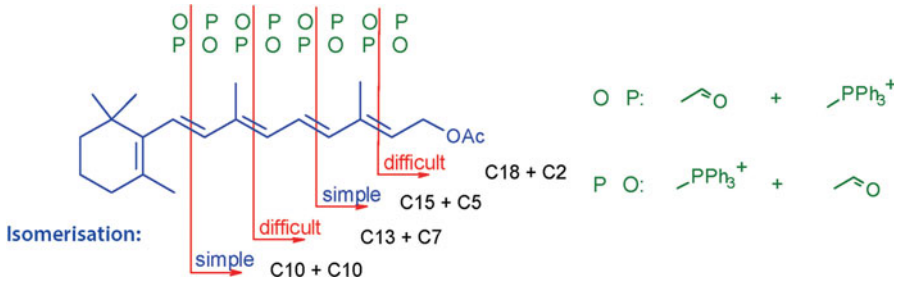
For choosing the appropriate isomerisation conditions, it must however be taken into consideration, that double bonds isomerise for stereochemical reasons at various rates, and that the state of equilibrium does not necessarily lie at 100 % in favour of the (all *E*)-isomers (Fig. 7.27). In case of (all *E*)-*β*-carotene, the (13*Z*)-isomer is the fastest to form, while this isomer accounts for 25 % at the final equilibrium. The (9*Z*)- and (13*Z*,13'*Z*)-isomers are formed at a distinctly slower rate. The proportion of (15*Z*)-*β*-carotene is at equilibrium very small, and in the case of the double bonds at C-7 and C-11, the equilibrium lies exclusively on the side of the (*E*)-configuration. This has direct implications for the synthetic strategy.



7.27 *Isomerisation of the carotenoid skeleton is not aimed at the thermodynamic equilibrium, but at a selective isomerisation of the doubly-substituted double bonds.*

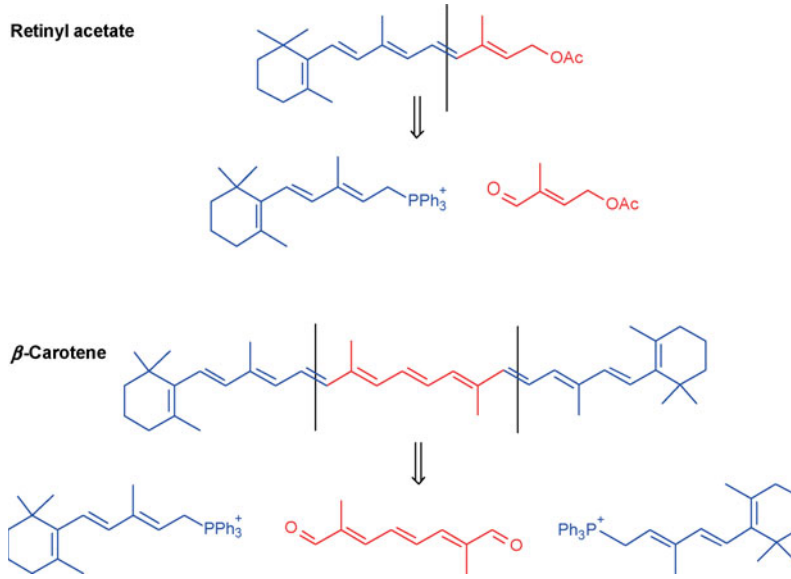
Retrosynthesis

For the side-chain of retinol acetate, there are four options to cut the double bond system retrosynthetically (Fig. 7.28). [55] Four double bonds give theoretically rise to 16 stereoisomers. Knowing that disubstituted (*Z*)-double bonds, as in *β*-carotene, are isomerised under milder conditions than trisubstituted ones, limits the retrosynthetic choices.



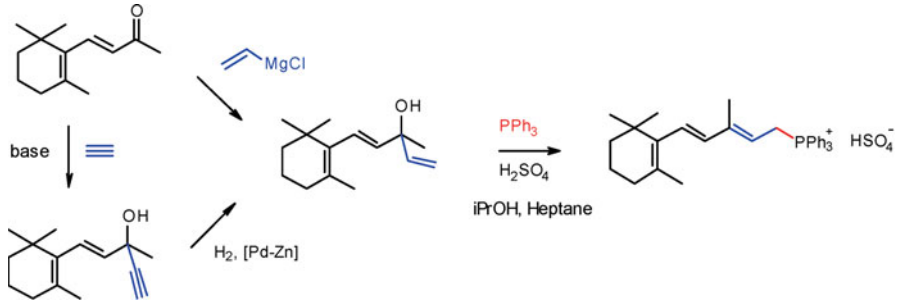
7.28 *The retrosynthetic analysis of retinol acetate reveals eight options for the carbonyl building block and its matching phosphorane.*

Additional restrictions result from the availability of the different synthetic building blocks. Considering the choice between cyclocitral (C_{10}) and ionone (C_{13}) as precursors for Vitamin A, it turns out that the most suitable building blocks are a C_{15}-phosphonium salt from β-ionone ($C_{13} + C_2$) and a C_5-aldehyde, since pseudoionone is cyclised to β-ionone more easily than citral to cyclocitral. Consequently, the retrosynthesis of β-carotene suggests a C_{10}-dialdehyde as building block.



Vitamin A Acetate (BASF)

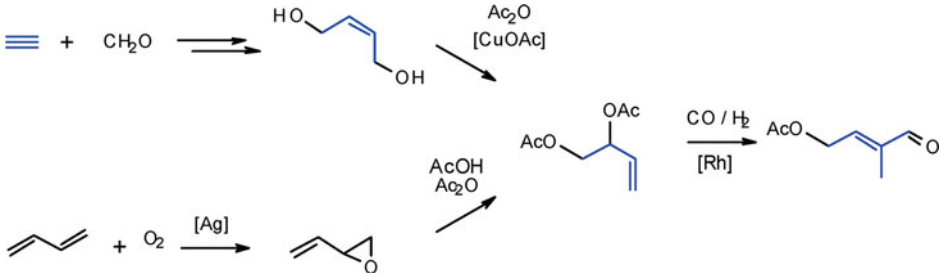
The reaction of β-ionone with vinylmagnesium chloride gives vinyl-β-ionol directly, which could also be accessed *via* ethynylation and partial hydrogenation. The allyl alcohol is converted immediately with triphenylphosphane under acidic conditions to the corresponding β-ionylidenethyltriphenylphosphonium salt. [55]



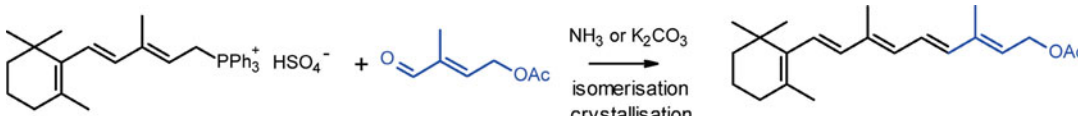
Whether β-ionone is reacted with acetylene, depends primarily on the availability of the latter, whereas the use of vinylmagnesium chloride depends largely on the capability to handle this reagent safely, which always contains a few per

cent of the hazardous vinyl chloride. Direct formation of the phosphonium salt from the allyl alcohol has the advantage that the corresponding labile β-ionylidenethyl halide can be avoided. The crystalline phosphonium salt can be isolated in excellent yields.

For the C_5-aldehyde moiety, there are several access routes available. A comparatively simple option is the hydroformylation of 1,2-diacetoxybut-3-ene, which is obtainable from but-2-ene-1,4-diol by esterification with acetic anhydride and a copper-catalysed rearrangement. Critical for this synthetic route is to control the regioselectivity of the hydroformylation. A recently developed process to prepare vinyloxirane from butadiene over a silver catalyst opens up a second attractive approach. [56, 57]

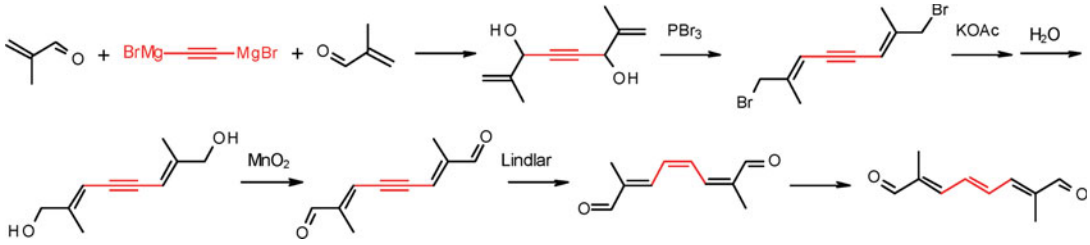


The Wittig reaction of the β-ionylidenethyltriphenylphosphonium salt with (*E*)-4-acetoxy-2-methylbut-2-enal leads in high yields directly to (*E*/*Z*)-retinol acetate, which subsequently has to be isomerised and crystallised. [55]



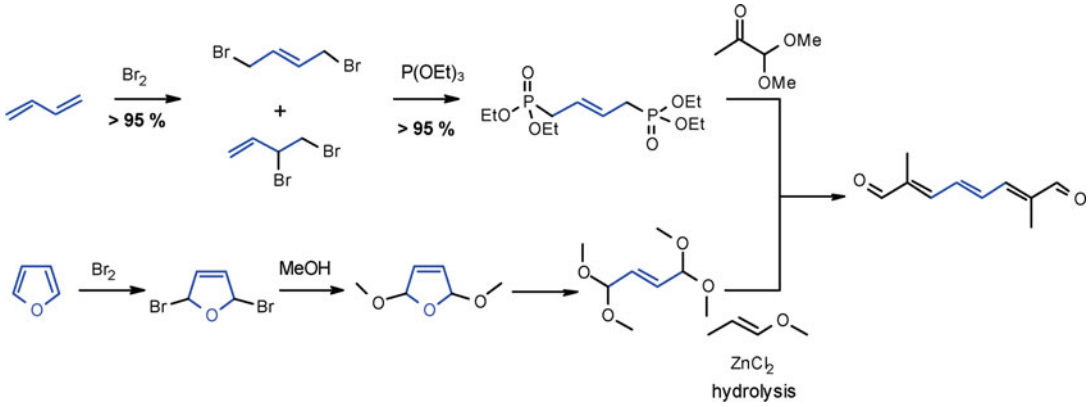
β-Carotene (BASF)

Analogously, the C_{15}-phosphonium salt is reacted with 2,7-dimethylocta-2,4,6-trienedial to give β-carotene. [55] The C_{10}-dialdehyde is accessible from methacrolein and acetylene di-(magnesium bromide). The diol is converted with phosphorus tribromide into the terminal dibromide *via* an S_N2' reaction. Substitution of both halogens with potassium acetate, followed by hydrolysis, gives the corresponding alcohol, which is oxidised with MnO_2; hydrogenation in presence of a Lindlar catalyst and isomerisation leads finally to the C_{10}-dialdehyde.



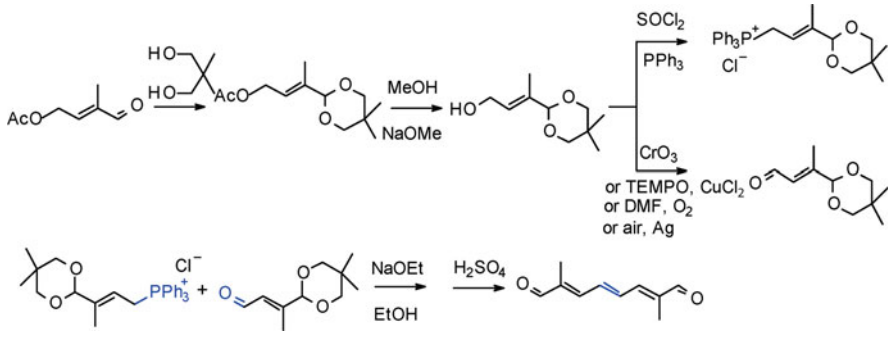
Several industrial processes have been described to generate 2,7-dimethylocta-2,4,6-trienedial. Of great importance for the large-scale synthesis is a gas-phase bromination of butadiene, which gives a mixture of 1,2- and 1,4-dibromobut-2-ene. The diphosphonate is obtained by an Arbusov reaction in almost quantitative yield, and undergoes a double Horner-Wadsworth-Emmons reaction with pyruvaldehyde dimethyl acetal, followed by hydrolysis, to yield the desired 2,7-dimethylocta-2,4,6-trienedial as a mixture of its (*E*/*Z*)-isomers. It is interesting that the analogous Wittig reaction shows yields of less than 10 %, which is caused by the considerably higher steric demand of the triphenylphosphane residue.

Alternatively, furan may also be brominated and then subjected to an exhaustive methanolysis. A zinc chloride-catalysed double enol ether condensation with 1-propenyl methyl ether (Müller-Cunradi-Pieroh reaction) gives finally the crystalline (all *E*)-C_{10}-dialdehyde in an overall yield of >50 %.



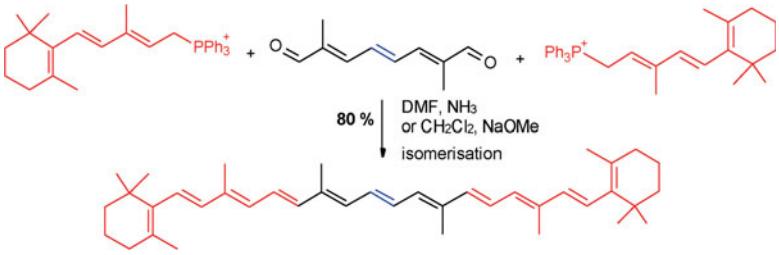
A third synthetic route follows the $C_5 + C_5$ strategy. In principal, (*E*)-4-acetoxy-2-methylbut-2-enal is on the one hand oxidised to a dialdehyde, and on the other hand, the same precursor is converted into the corresponding phosphonium salt. A Wittig reaction of both of these intermediates gives then the desired C_{10}-building block.

Thus, the aldehyde function of (*E*)-4-acetoxy-2-methylbut-2-enal is protected as an acetal by reaction with 2,2-dimethylpropane-1,3-diol, and then the acetoxy-group undergoes alkaline methanolysis. While the phosphonium salt is accessible by reaction with thionyl chloride, and subsequently with triphe-



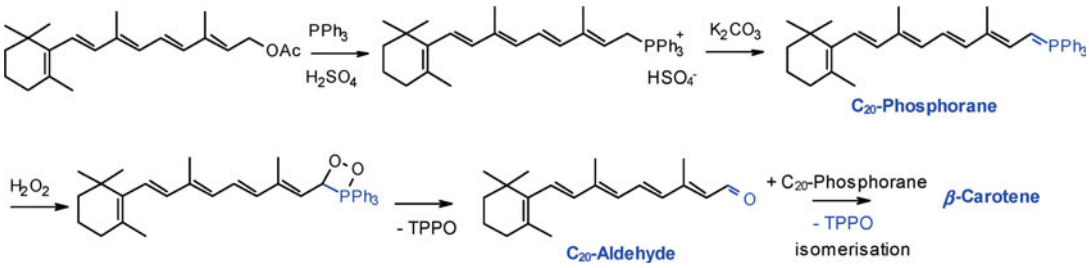
nylphosphane, the aldehyde is obtained by Jones oxidation, TEMPO oxidation or other suitable oxidation methods. After the Wittig olefination, the cyclic diacetal is cleaved with sulfuric acid.

The crude product from the Wittig reaction of the C_{10}-dialdehyde with the C_{15}-phosphonium salt contains up to approximately 35 % of the (11*Z*)-isomer, which is isomerised in boiling heptane. Triphenylphosphane oxide is separated off by extraction with a mixture of either DMF and water or alcohol and water. Analytically pure *β*-Carotene is then isolated in a yield of 80 %.



An alternative *β*-carotene process, currently in use at BASF, starts from retinol acetate [55, 58], which is reacted with triphenylphosphane in the presence of an acid to give the C_{20}-phosphonium salt. Partial alkaline oxidation with hydrogen peroxide leads directly to (*E*/*Z*)-*β*-carotene, which is then isomerised to its (all *E*)-form in an overall yield of around 70 %.

In many oxidation reactions, particular attention must be paid to safety precautions. Basically, the work is carried out below the lower explosion limit, which means, by lowering the reaction temperature, the vapour pressure of the volatile compounds is reduced to a sufficient level. In case the temperature cannot be decreased sufficiently, the protective effect may also be achieved by passing nitrogen into the reaction vessel. Any source of ignition like, for example, a fast-running stirrer, has to be removed. Electrostatic discharges may be excluded by choosing conductive reaction media and appropriate vessel design. An additional degree of safety is provided by reaction vessels, capable of withstanding pressures 40 times higher than expected during normal operation.

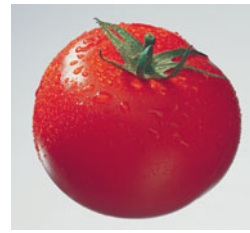


The mechanism is still not fully understood. Hydrogen peroxide formally transfers oxygen to the nucleophilic phosphorane, which then cleaves off triphenylphosphane oxide to give the aldehyde; this finally reacts in a normal Wittig reaction to produce *β*-carotene.

In more recent reports on the oxidative coupling of phosphonium salts, oxygen is used in presence of 1 mole % VO (acac)$_2$. [59]

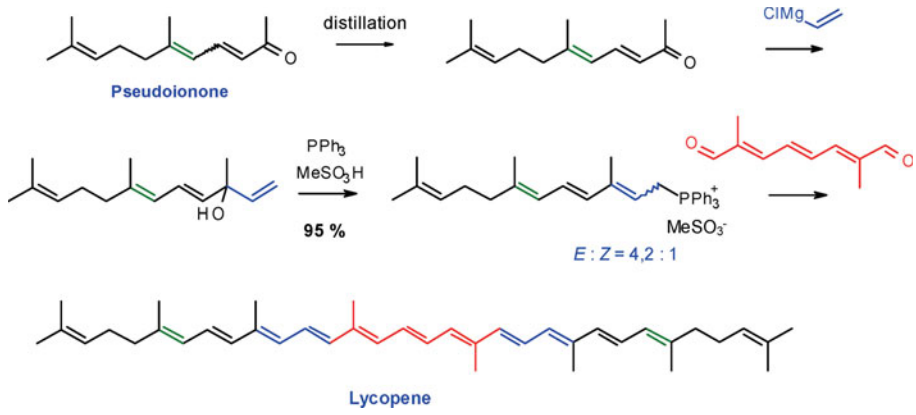
Lycopene (BASF)

The starting material for lycopene is pseudoionone, which is available as a mixture of (*E*)-and (*Z*)-isomers from the citral production (Fig. 7.29). Since isomerisation of triply-substituted double bonds produces equilibrium mixtures, the isomers are separated by fractional distillation. At BASF, pseudoionone is reacted with vinylmagnesium chloride



7.29 *Tomatoes (Solanum lycopersicum) contain large amounts of lycopene.*

and then with triphenylphosphane and methanesulfonic acid to give the C_{15}-phosphonium salt. By the appropriate choice of acid and temperature management, an (*E*/*Z*)-ratio of 4.2 : 1 can be attained. Lycopene is finally obtained by a double Wittig reaction with the C_{10}-dialdehyde. [60]

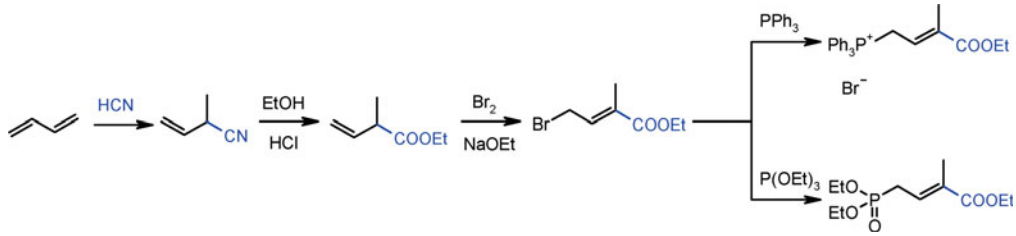


Apocarotenoids (BASF)

The unsymmetrical structure of apocarotenoids poses particular technical and economic challenges. It is remarkable that these compounds can be constructed economically in linear syntheses from the appropriate building blocks. The viability of the process has therefore to be attributed to the fact that nearly all of the reaction steps are shared with and can be integrated into other production processes. However, for the apocarotenoid syntheses, additional C_5-phosphonium salts and C_5-phosphonate esters are required. A multitude of different synthetic routes have been described. [61] Only the more recent developments are discussed here.

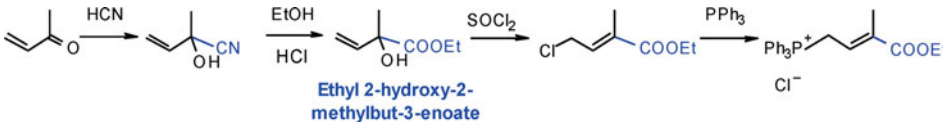
The Greek prefix "*apo-*" means: from, off, away from.

At BASF, the following path was developed for the synthesis of the C_5-building block: the Pinner reaction with 2-methylbut-3-enonitrile produces ethyl 2-methylbut-3-enoate, which is converted by bromination-dehydrobromination into the corresponding bromo-derivative. This can be further reacted to both, the corresponding phosphonium salt and as well, by means of an Arbusov reaction, the phosphonate ester. [62]

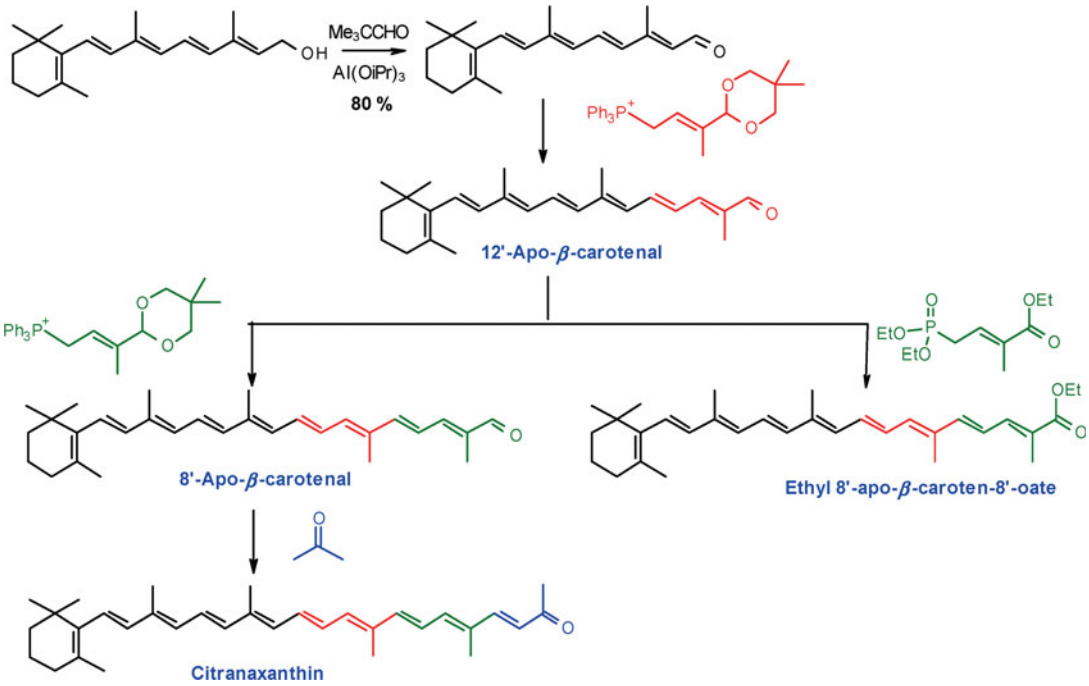


Recently, Hoffmann-La Roche also established a synthetic route to the C_5-phosphonium salt. The cyanhydrin, generated from methyl vinyl ketone is subjected to ethanolysis, which produces ethyl 2-hydroxy-2-methylbut-3-enoate. Further

reaction with thionyl chloride and triphenylphosphane gives also the desired intermediate.

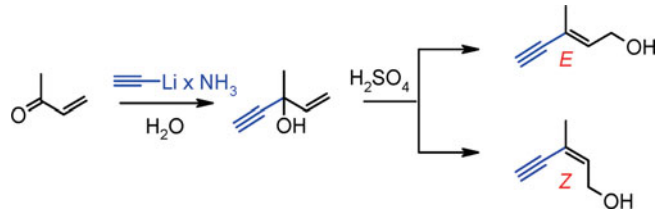


8'-*Apo*-*β*-carotenal, ethyl 8'-*apo*-*β*-carotenoate and citranaxanthin are readily accessible by this modular synthesis. The central building block is 12'-*apo*-*β*-carotenal, which itself may be synthesised in diverse ways by Wittig reactions from the intermediates mentioned above. Of particular appeal is a synthesis, which starts from retinol. First, the retinol is oxidised to retinal by an Oppenauer or TEMPO oxidation. Successive reactions of retinal with C_5-phosphonium salts or phosphonate esters, followed by isomerisation, lead to 8'-*apo*-*β*-carotenal and ethyl 8'-*apo*-*β*-carotenoate. Citranaxanthin is obtained by an aldol condensation of 8'-*apo*-*β*-carotenal with acetone. [55]



Vitamin A Acetate (Hoffmann-La Roche)

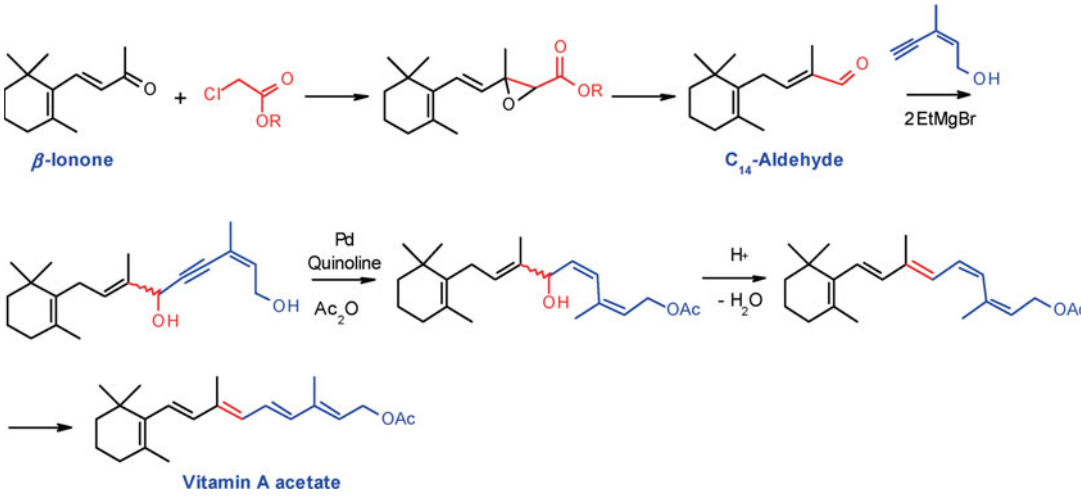
Starting from their experience in manufacturing *β*-ionone, Hoffmann-La Roche initially favoured acetylene as the universal building block for further syntheses. The reaction of methyl vinyl ketone with lithium acetylide in ammonia gives a tertiary alcohol, which is isomerised with sulfuric acid into a mixture of the (*E*)- and (*Z*)-isomers of 3-methylpent-2-en-4-ynol. The isomers can be separated by distillation. Whereas the main component, the (*Z*)-isomer, is used for the production of Vitamin A, the (*E*)-isomer finds application in carotenoid synthesis.



Acetylene and ammonia, the gases, which are used in excess in this process, are almost completely recycled, the lithium salts are returned to the lithium metal manufacturer and there converted into the marketable lithium hydroxide. In the 1980s, the whole process was optimised to reduce the waste streams, and it was regarded as a classic example of an environment-friendly and economic production process.

The Lindlar catalysts (palladium + quinoline) were developed for this example by Herbert Lindlar-Wilson (*1909) at Hoffmann-La Roche. [45]

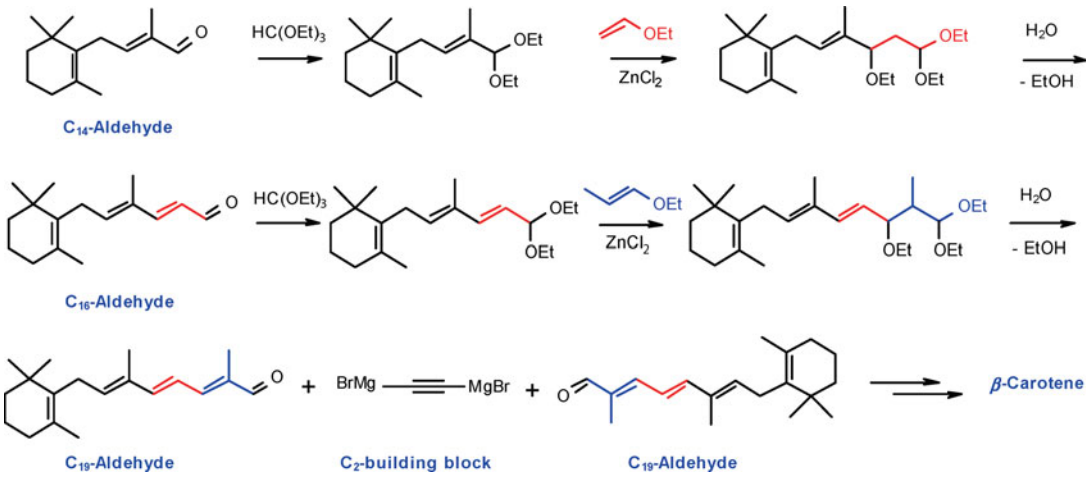
β-Ionone is first converted by a Darzens reaction into the homologous aldehyde, which is then reacted in a Grignard coupling with 3-methylpent-2-en-4-ynol. Partial hydrogenation of the triple bond on a Lindlar catalyst, acetylation, elimination and isomerisation lead eventually to Vitamin A acetate, which is isolated by crystallisation. [63]



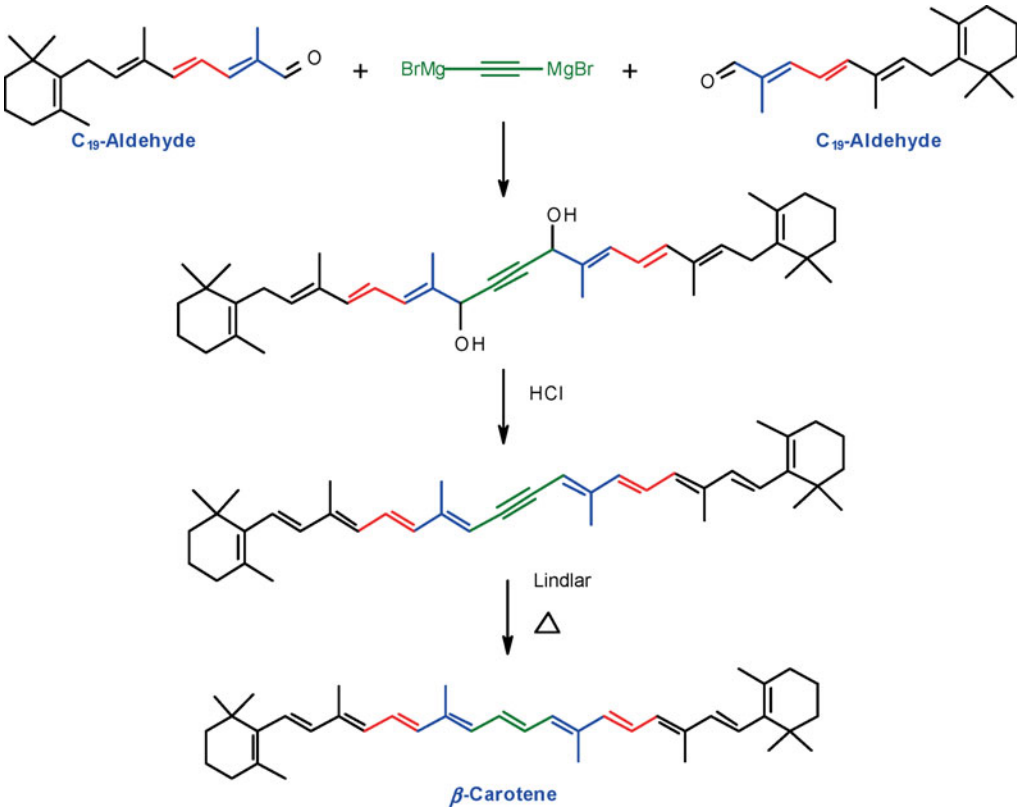
The Lewis acid-mediated coupling of alkyl enol ethers with acetals was discovered in 1937 by Martin Müller-Cunradi (1902–1945) and Kurt Pieroh at BASF, and is one of the lesser-known key reactions in the chemistry of carotenoids; it can be employed in miscellaneous ways and repeatedly for the construction of complex structures. [64]

β-Carotene (Hoffmann-La Roche)

The first industrial synthesis of *β*-carotene by Hoffmann-La Roche followed the $C_{19} + C_2 + C_{19}$ principle. With the C_{14}-aldehyde from the Vitamin A synthesis as the starting point, the sequence of acetal formation, Lewis acid-catalysed insertion of an enol ether, hydrolysis and elimination of ethanol, produces initially a C_{16}-aldehyde. Repetition of this sequence with ethyl 1-propenyl ether gives the C_{19}-aldehyde.



The C_{19}-aldehyde is first reacted with acetylene di-(magnesium bromide), and water is then eliminated with acid. Partial hydrogenation with a lead salt-Lindlar catalyst gives the (*Z*)-isomer of *β*-carotene. The less soluble (all *E*)-*β*-carotene is finally obtained by thermal isomerisation and crystallisation. The yield, based on the C_{19}-aldehyde, amounts to 60 %.

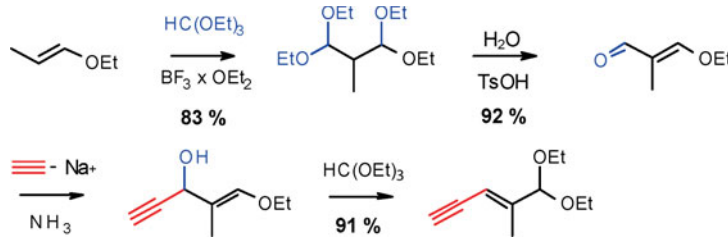


This process was carried out for around 40 years, and produced annually around 200 tonnes of β-carotene. In recent years, Hoffmann-La Roche adopted the procedure originally developed by BASF for the production of β-carotene from the C_{15}-phosphonium salt and the C_{10}-dialdehyde.

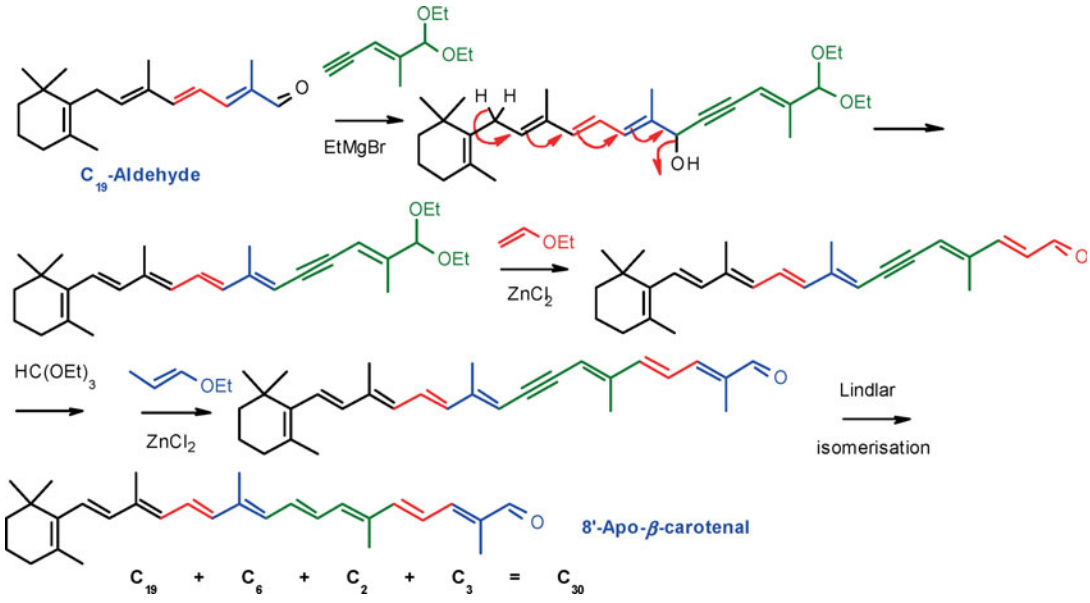
Apocarotenoids (Hoffmann-La Roche)

The C_{19}-aldehyde is also an ideal starting material for various apocarotenoids. The charm of these procedures is based on the fact, that many of the synthetic steps, and often even the reagents, may be retained across the different target syntheses. Only the sequence or single building blocks have to be varied.

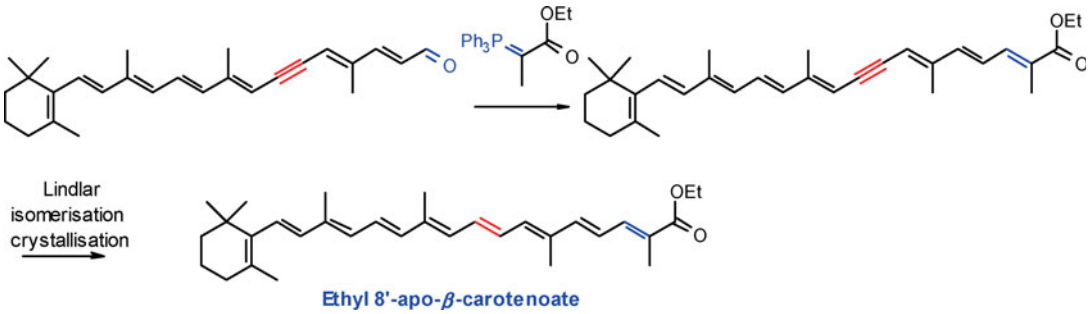
For the production of 8'-*apo*-β-carotenal, a C_6-aldehyde, in form of its diethyl acetal, was required as the middle section of the carotene skeleton. This was obtained by a boron trifluoride-catalysed condensation of ethyl 1-propenyl ether with triethyl orthoformate. After partial hydrolysis and reaction with sodium acetylide, the reaction with triethyl orthoformate was repeated. The yield over all steps was around 50 %.



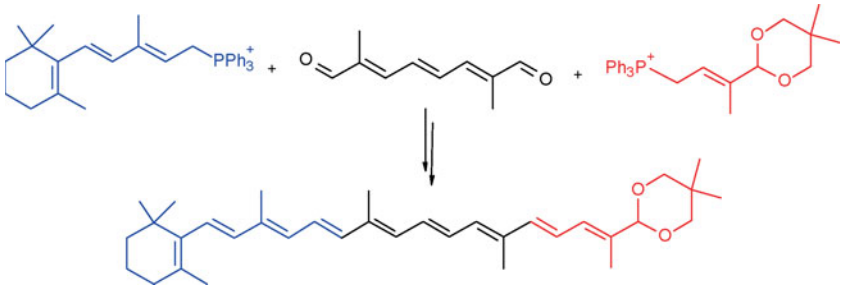
Starting again from the C_{19}-aldehyde, a coupling with the C_6-alkyne and hydrolysis now produced a C_{25}-aldehyde, the carbon chain of which was extended according to the same sequence as for the C_{19}-aldehyde, with ethyl vinyl ether and ethyl 1-propenyl ether.



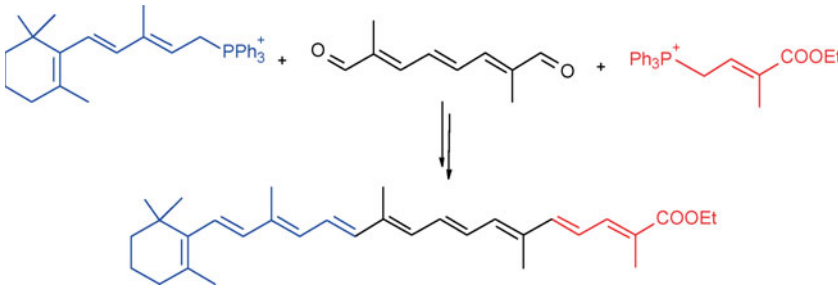
The second apocarotenoid, ethyl 8'-*apo*-β-carotenoate, is accessible by means of a Wittig reaction.



Later, however, for the synthesis of 8'-*apo*-β-carotenal Hoffmann-La Roche preferred a Wittig olefination, starting from the C_{15}-phosphonium salt, the C_{10}-dialdehyde and a C_5-phosphonium salt. [63]



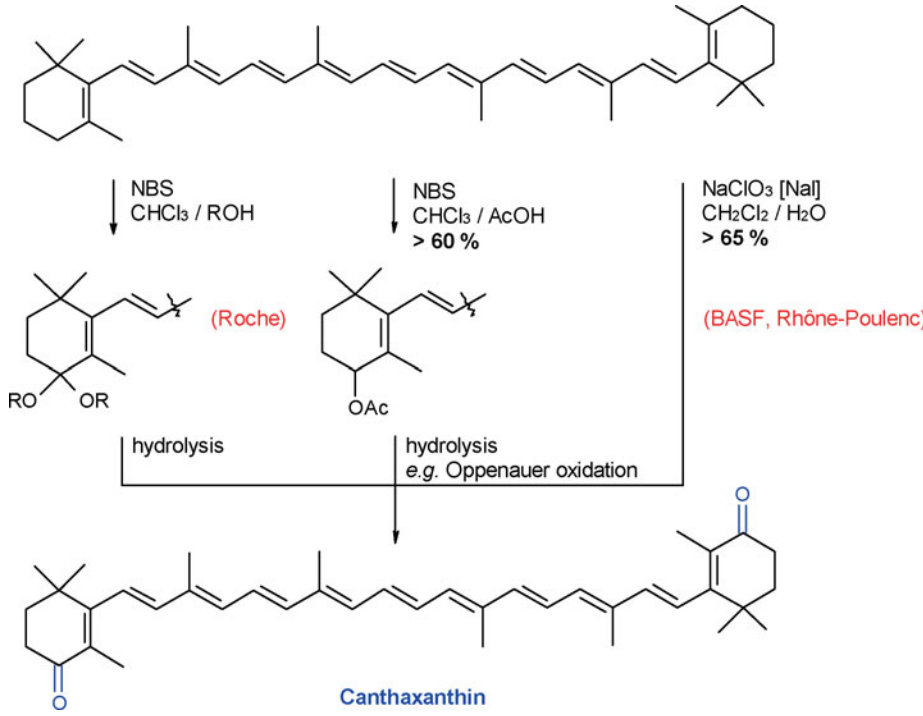
For standardisation of the synthesis strategy, it was obvious to construct as well the ethyl 8'-*apo*-β-carotenoate from C_{15} + C_{10} + C_5-building blocks.



Canthaxanthin (Hoffmann-La Roche)

Already in the 1960s, Hoffmann-La Roche developed a process for the production of canthaxanthin. Selective oxidation of β-carotene may be carried out with various reagents. Hoffmann-La Roche used NBS in chloroform/alcohol or chloroform/acetic acid. [65] BASF [66] and Rhône-Poulenc [67], on the other hand, employed halogenates as oxidants. The BASF procedure uses a biphasic system

of dichloromethane-water. β-Carotene is oxidised by sodium chlorate in presence of catalytic amounts of sodium iodide.

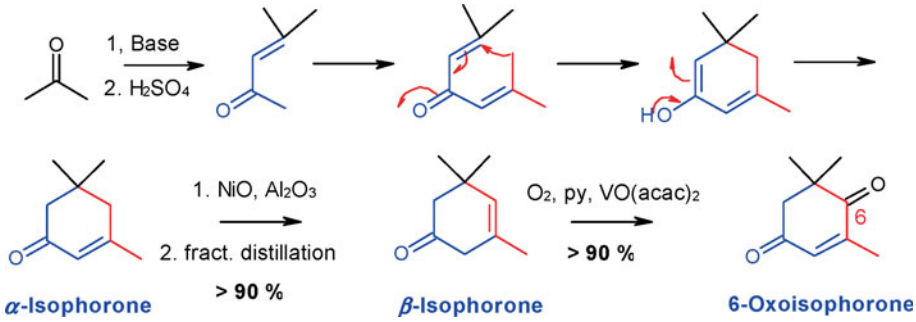


Astaxanthin (Hoffmann-La Roche)

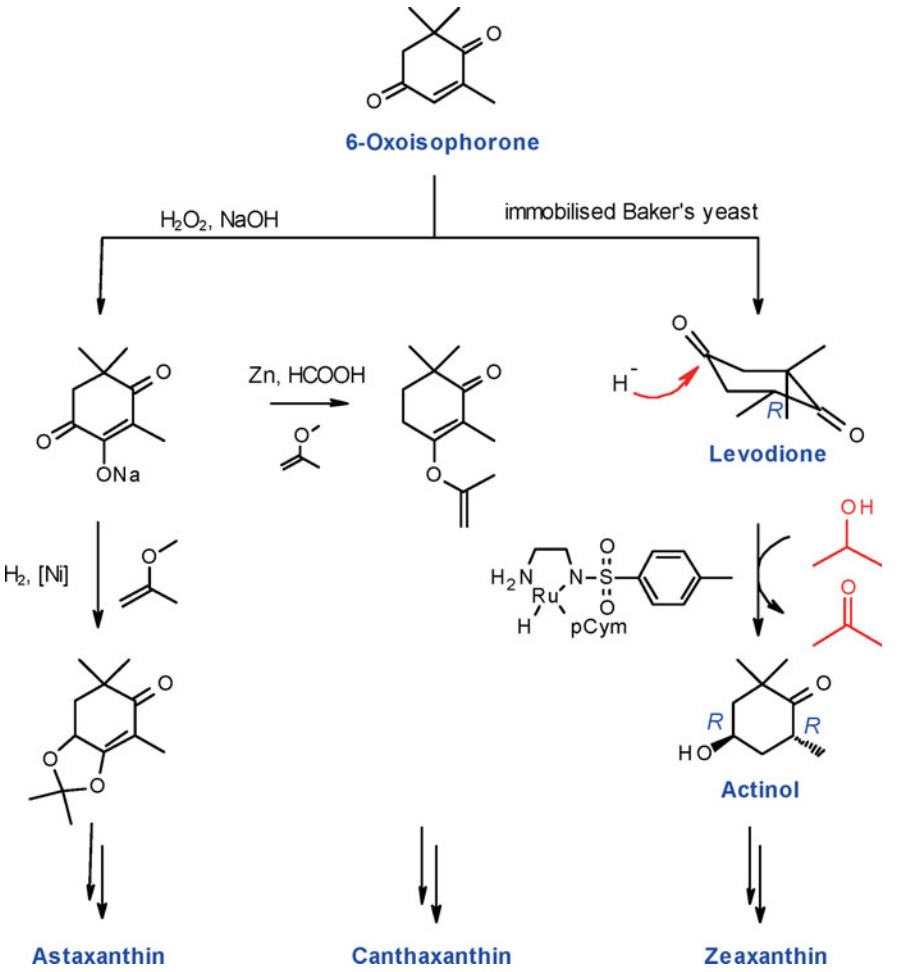
Although, as was shown above, it is possible to introduce an oxygen function into the rings of β-carotene without oxidising the polyene system unselectively, Hoffmann-La Roche developed in the 1970s a new strategy for the synthesis of xanthophylls; this has been used in the case of astaxanthin at production scale since 1984.

Starting from the inexpensive α-isophorone, which is obtainable from acetone *via* an aldol condensation and an intramolecular Michael reaction [68], 6-oxoisophorone is produced in two steps. Gas-phase isomerisation of the double bond in presence of nickel oxide catalysts leads to the lower-boiling β-isophorone, which is separated off by distillation. The oxidation proceeds with manganese salts in DMF (Rhône-Poulenc) or with vanadyl acetylacetonate in pyridine (Hoffmann-La Roche). The yield over both stages exceeds 80 %. 6-Oxoisophorone is the perfect building block for the synthesis of a whole range of xanthophylls.

For manufacturing of astaxanthin, 6-oxoisophorone is first oxidised with alkaline hydrogen peroxide. Catalytic hydrogenation and reaction with isopropenyl methyl ether lead to the required building block. Reduction of 4,6-dioxoisophorone with zinc in formic acid and subsequent etherification give the starting material for canthaxanthin. Finally, enantioselective reduction of 6-oxoisophorone succeeds with immobilised baker's yeast. A diastereoselective

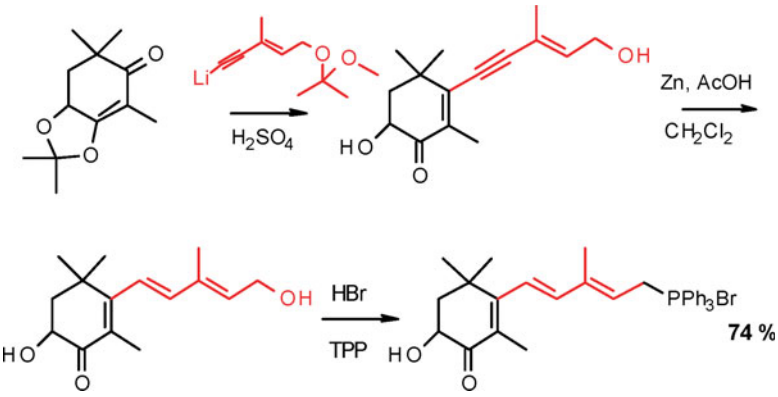


transfer hydrogenation with a ruthenium catalyst produces enantiomerically pure actinol, the precursor of zeaxanthin. It is remarkable that the baker's yeast generates a stereogenic centre, which is levelled out later on in the zeaxanthin synthesis; whereas however, in course of the hydrogenation the methyl group is used as an efficient stereodifferentiating substituent.

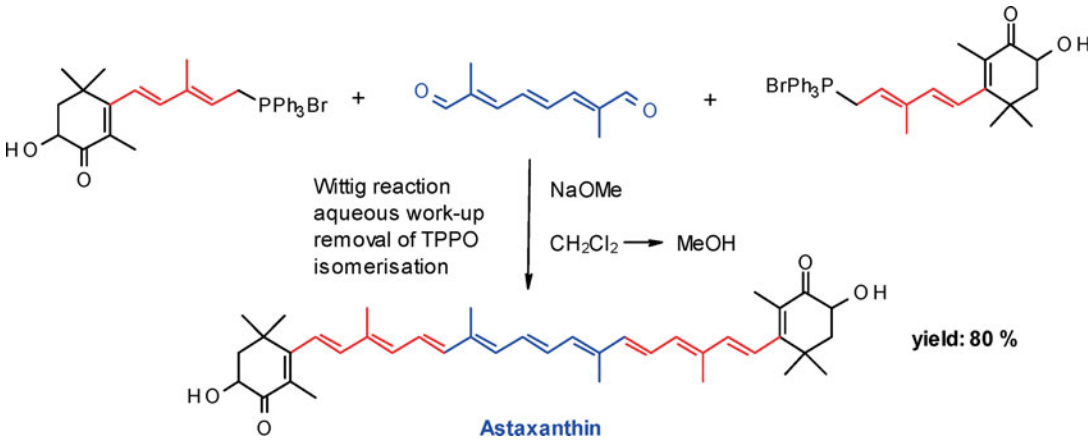


The construction of the xanthophylls succeeds according to the pattern $C_9 + C_6 = C_{15}$ and $C_{15} + C_{10} + C_{15} = C_{40}$. For this, the C_6-building block originates from the Vitamin A synthesis. The final stage is a double Wittig reaction, analogous to the β-carotene synthesis with the C_{10}-dialdehyde.

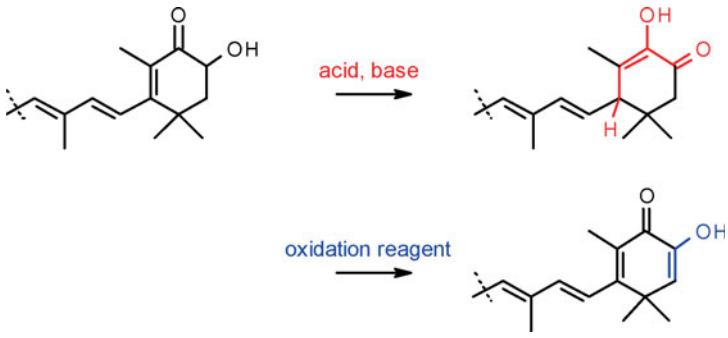
The C_{15}-phosphonium salt for astaxanthin is obtained by addition of a lithium acetylide (with *n*-butyllithium [63]) to the ketal-protected ketone, followed by the cleavage of the alcohol protecting groups, and crystallisation from diisopropyl ether. The triple bond is reduced with zinc and acetic acid in dichloromethane or with hydrogen on a Lindlar catalyst. The alcohol is converted into the corresponding bromide in a biphasic system of HBr in dichloromethane, and finally reacted with triphenylphosphane to give the phosphonium salt.



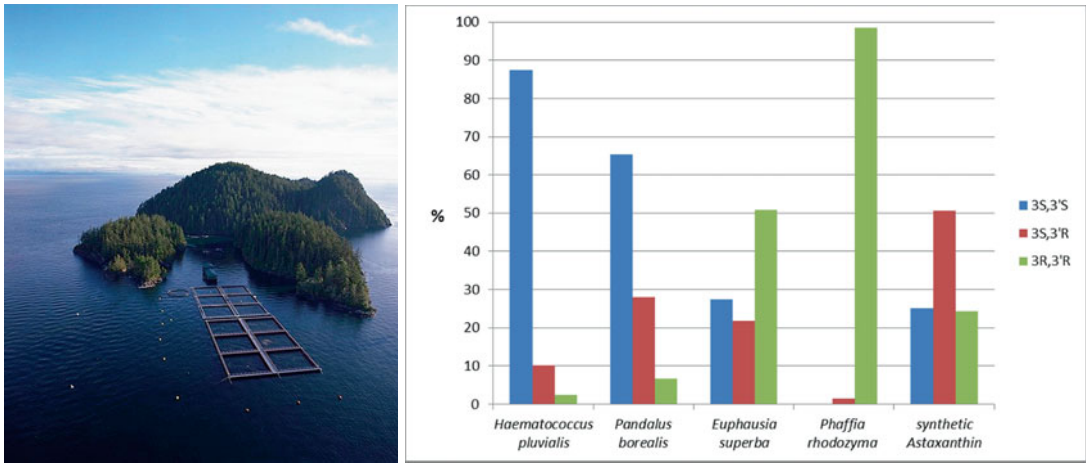
For the concluding Wittig reaction, sodium methoxide in dichloromethane/methanol is used as the base. After an aqueous work-up, the product is recrystallised by a simultaneous solvent exchange from dichloromethane to methanol. Triphenylphosphane oxide remains thereby in solution. Thermal isomerisation finally gives (all *E*)-astaxanthin in a yield of around 80 % and purity higher than 98 %.



The synthesis is strongly impacted by the following decomposition reactions: On the one hand, under the influence of strong acids or bases, astaxanthin gives products with a diosphenol structure. On the other hand, astaxanthin is sensitive to oxidation and thereby α,β-unsaturated 1,2-diketones (*i.e.* semiastacene and astacene) are readily formed.



Synthetic astaxanthin is a diastereomeric mixture. When fed to salmon, these isomers, (3*S*,3'*S*) : (3*S*,3'*R*) : (3*R*,3'*R*), are found in the expected ratio of 1 : 2 : 1. The enantiomers and the *meso*-form of astaxanthin can be separated by reverse-phase HPLC (Fig. 7.30).

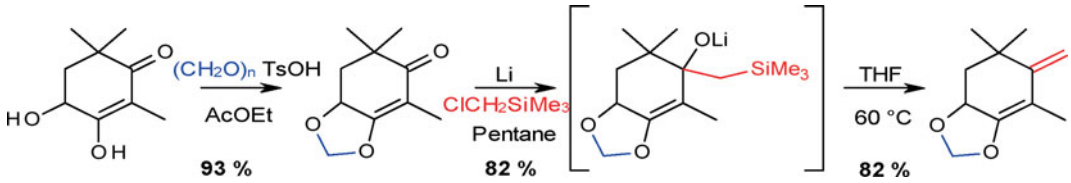


7.30 *Salmon farm: Wild salmon contains predominantly the (3S,3'S)-enantiomer. In farmed salmon, fed with shrimp bran, which is mainly produced from northern prawn (Pandalus borealis) or with an extract from the green alga Haematococcus pluvialis, the (3S,3'S)-enantiomer is also the main enantiomer. However, if the astaxanthin comes from the basidiomycetous yeast Phaffia rhodozyma or its sexual form Xanthophyllomyces dendrorhous, or from the Antarctic krill (Euphausia superba), the (3R,3'R)-enantiomer prevails. [69]*

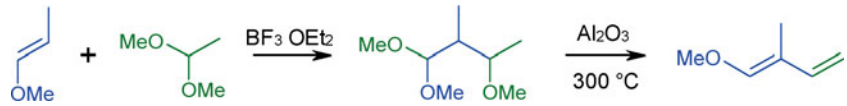
Astaxanthin without Wittig Reactions (DSM/Hoffmann-La Roche)

Interestingly, there are new studies by DSM/Hoffmann-La Roche on the laboratory-scale synthesis of various carotenoids, like astaxanthin and also canthaxanthin, entirely without a Wittig reaction. [70] They follow the synthetic concept

$C_{10} + C_{20} + C_{10}$. The C_{10}-building block for astaxanthin is obtained from 6-oxoisophorone, which, after oxidation and hydrogenation, is protected not with acetone but with formaldehyde. A Peterson olefination gives the required dienol ether.

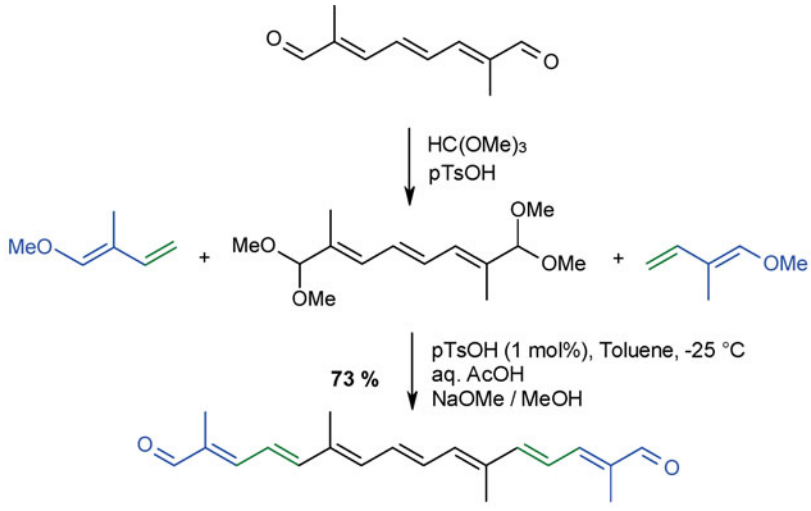


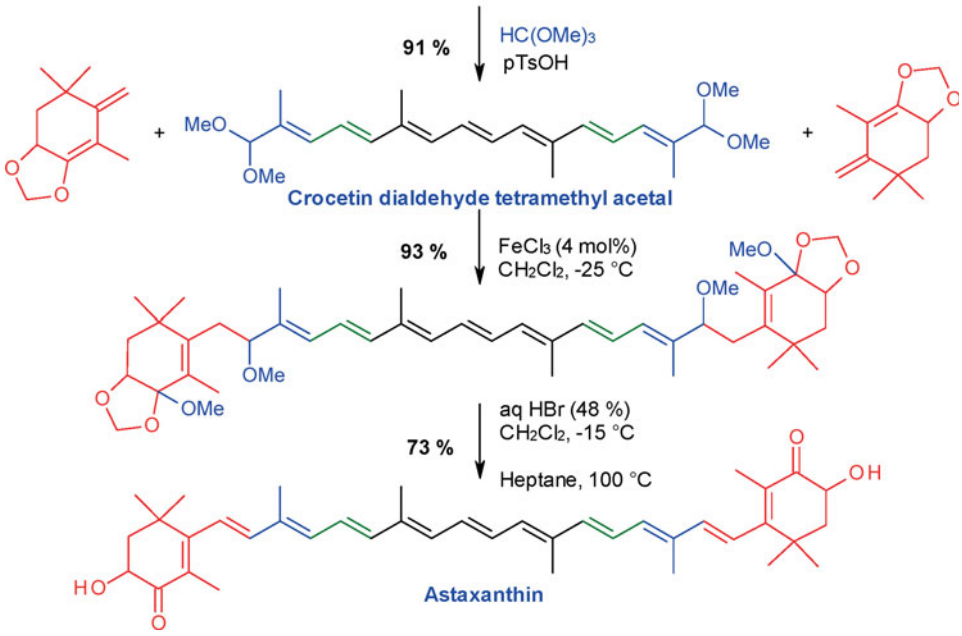
The synthesis of crocetindialdehyde tetramethyl acetal, as the C_{20}-building block, follows the $C_5 + C_{10} + C_5$ strategy. The required methoxyisoprene is accessible from acetaldehyde dimethyl acetal and methyl propenyl ether in a Lewis acid-mediated reaction.



The catalytic dienol ether condensation, an important extension of the Müller-Cunradi reaction, was discovered in 1958 by Ivan N. Nazarov (1906–1957) and S. M. Makin. Thereby it became feasible to extend acetals, not merely by two or three, but by five carbon atoms at once. A general issue with these reactions relates to the formation of telomers, which is influenced considerably by the choice of catalyst and solvent.

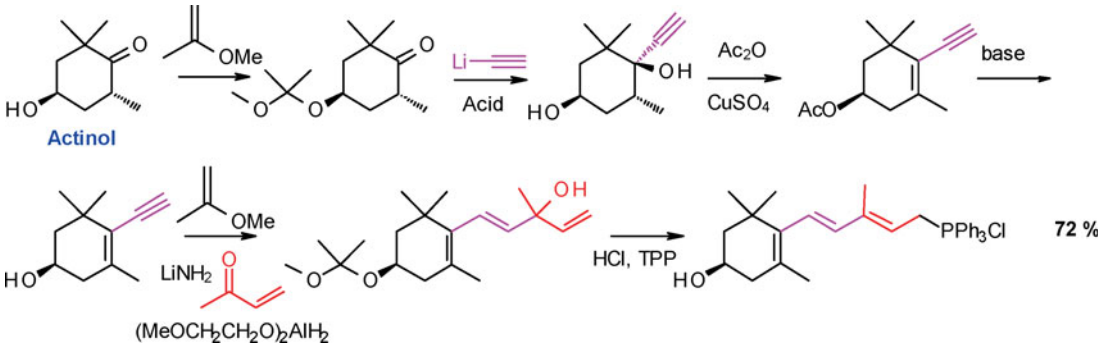
For the synthesis of crocetindialdehyde from the C_{10}-dialdehyde tetramethylacetal, the use of Brønsted acids, like *p*-toluenesulfonic acid, proves advantageous. After re-acetalisation with trimethyl orthoformate, a second, iron(III) chloride-mediated dienol condensation follows with subsequent cleavage of methanol in presence of 48 % aqueous HBr. The crude product consists of several stereoisomers, which are isomerised by heating in heptane. Recrystallisation from dichloromethane/acetone leads to a product, which comprises around 95 % of (all *E*)-astaxanthin.





Zeaxanthin (Hoffmann-La Roche)

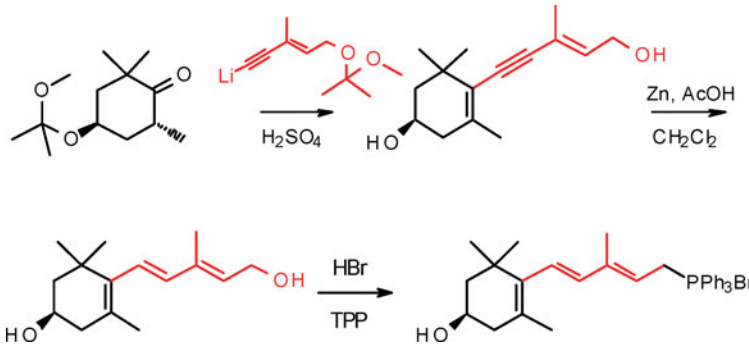
For the industrial production of zeaxanthin, Hoffmann-La Roche developed the following process:



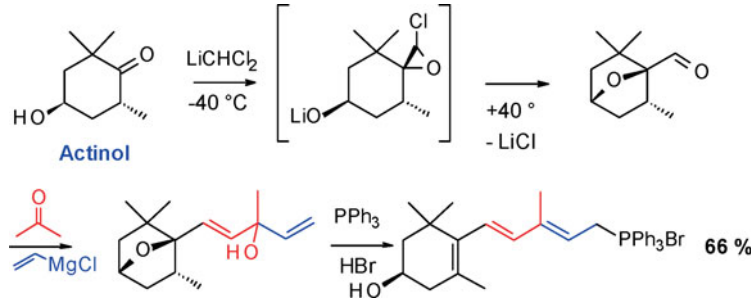
The synthesis of the phosphonium salt follows the pattern $C_9 + C_2 + C_4 = C_{15}$. Notably, the yield reaches around 72 %, because several of the reaction stages can be carried out as a "one-pot" reaction.

Alternatively, the C_{15}-phosphonium salt for zeaxanthin may be prepared along the $C_9 + C_6 = C_{15}$ route, analogously to the astaxanthin building block, starting from the protected actinol.

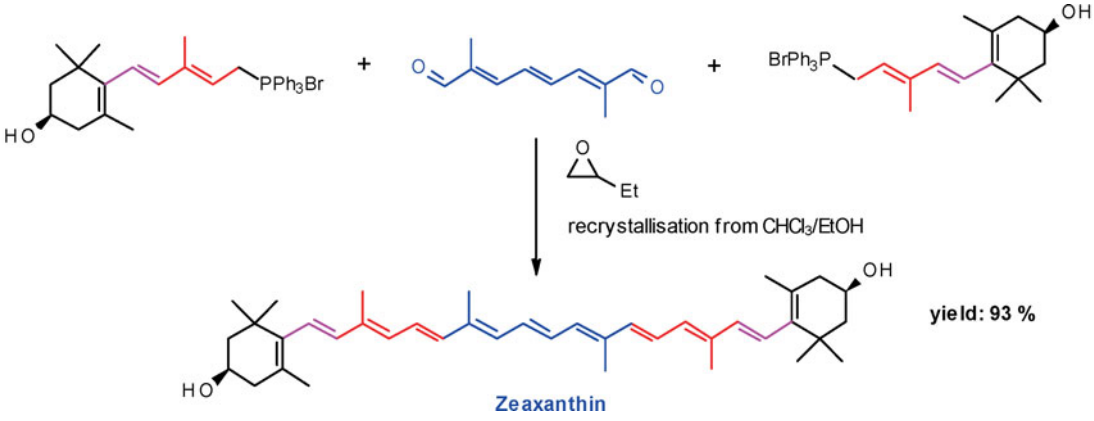
BASF worked out a procedure (second scheme on next page), which does not require any protecting group chemistry. Actinol is reacted at low temperature with dichloromethyllithium to form initially a chloro-oxirane, which undergoes upon warming a rearrangement to a bicyclic aldehyde. The side-chain is



assembled stepwise by an aldol condensation and a Grignard reaction. Under the conditions of the phosphonium salt formation, the cyclic ether is ring-opened regioselectively under retention of the absolute configuration. The yield over all four stages lies around 66 %. [60]



Joachim Buddrus from the Technical University of Berlin developed a variant of the Wittig reaction, by using an epoxide as HBr scavenger; this method produces carotenoids of high purity. [71] Thermal (*Z*/*E*)-isomerisation and crystallisation follow simultaneously under solvent-exchange (chloroform/ethanol). The yield of 93 % is the best that has been achieved in Wittig reactions of this type.

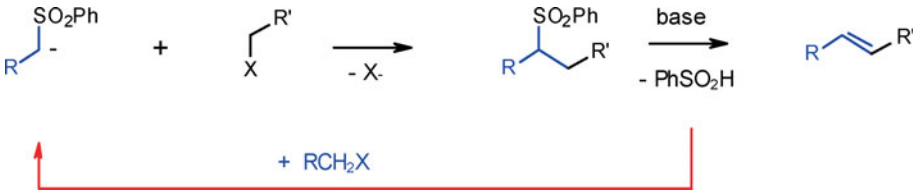


Julia Olefination (Rhône-Poulenc)

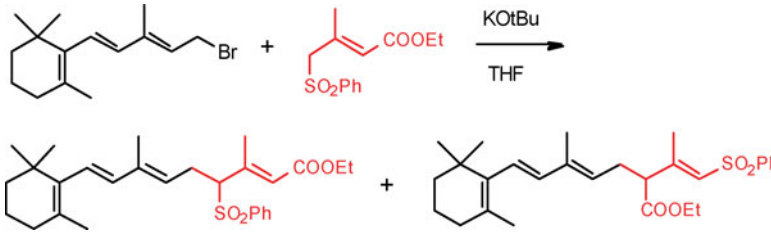
In view of all the technical challenges, initially associated with the Wittig reaction, Rhône-Poulenc *e.g.* opted for the olefination reaction developed by Marc Julia (1922–2010) at the beginning of the 1970s (Fig. 7.31). [72]

7.31 *Marc Julia (1922–2010).*

The Julia olefination allows the generation of a double bond by alkylation of a sulfone, followed by a base-induced elimination of benzenesulfinic acid. The water-soluble sulfinate can be separated off, and recovered as the free sulfinic acid. The sulfone is then obtained by reaction of the sulfinic acid with the appropriate alkyl halide. [73]

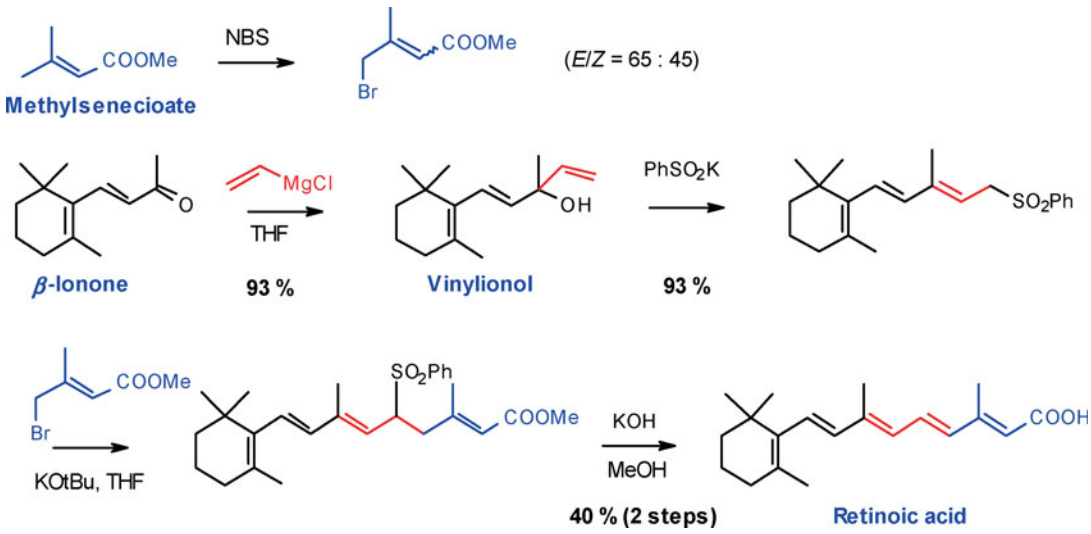


The Julia olefination found its first industrial application for the production of retinoic acid. [74, 75] The original design of the synthesis, however, concealed a difficulty: The allyl anion, substituted on both sides with electron-accepting substituents, does not react regioselectively.

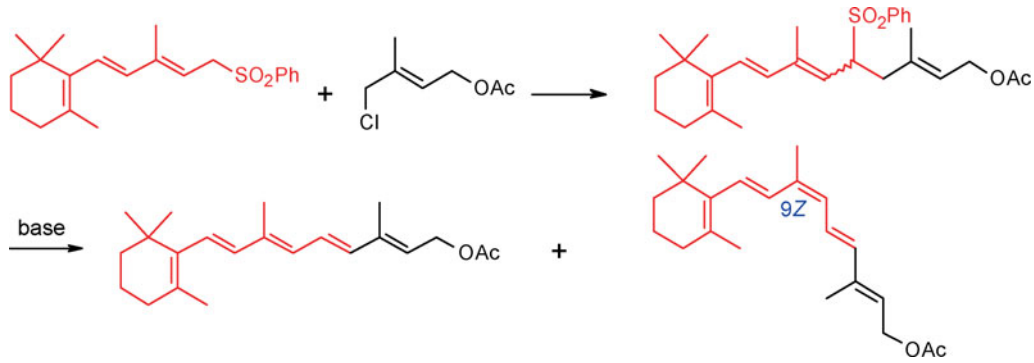


More successful was the inverse concept, by which retinoic acid is obtainable in good yields. The C_5-building block is accessible by allylic bromination of methyl senecioate (3-methylbut-2-enoate) with NBS. The C_{15}-sulfone is obtained, similar to the C_{15}-phosphonium salt of the BASF process, from vinylionol and potassium benzenesulfinate.

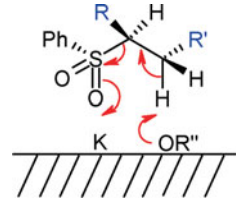
Rhône-Poulenc followed a corresponding strategy for Vitamin A acetate as well. [76] (*E*)-4-Chloro-3-methyl-2-butenyl acetate is obtainable from isoprene in acetic acid and *t*-butyl hypochlorite. The corresponding bromoacetate is obtained using NBS. A disadvantage is the unsatisfactory yield, which stays usually below 50 %. [77] The elimination of benzenesulfinic acid in a homogeneous phase, with potassium alkoxides in methanol or pyridine, leads to a mixture of (all *E*)- and (9*Z*)-isomers. This is remarkable, since besides the (all *E*)-actually



the (11*Z*)-isomer might be expected. In fact, however, partial formation of the (9*Z*)-isomer can be interpreted by loss of the sulfinate residue prior to deprotonation. Thus, the stereochemistry of the heptatrienyl cation depends on the leaving group and the elimination conditions.



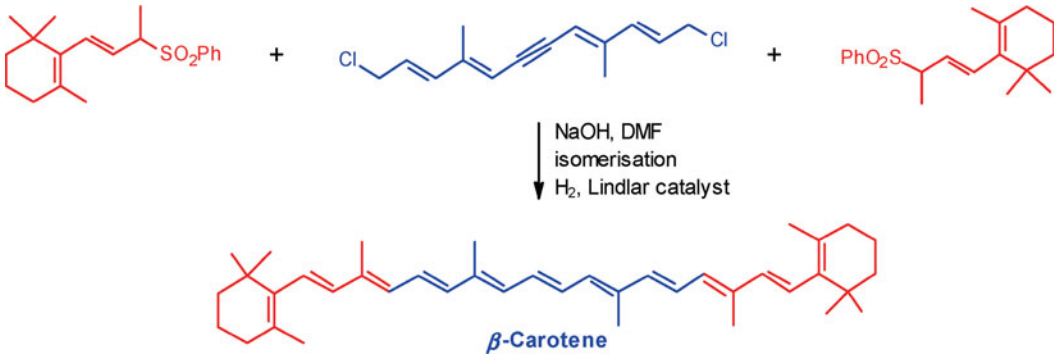
Heterogeneous systems, *e.g.* potassium alkoxides in petroleum ether, yield better stereoselectivity, which can be explained by a concerted *syn*-elimination on the surface of the solid base.



Also Hoffmann-La Roche investigated this reaction in depth, hoping to find milder and more selective conditions by variation of the sulfinate moiety. In one of the first series of experiments, the use of benzenesulfinic acid gave the (all

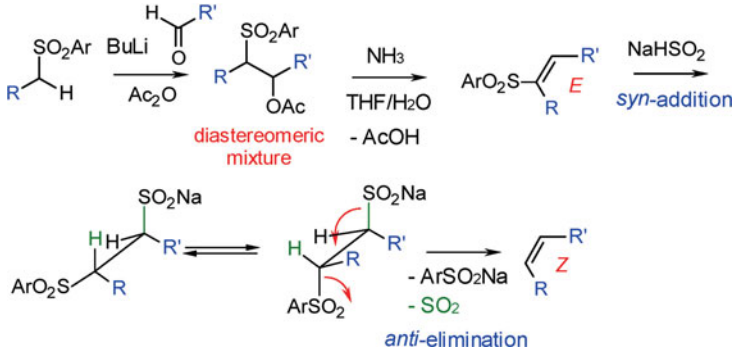
E)-isomer with 83 % selectivity. Later, this result was significantly improved further by the use of *p*-chlorobenzenesulfinic acid. [45]

The Julia olefination was also adapted to the synthesis of *β*-carotene, where the C_{13}-sulfone was reacted with a C_{14}-dichloride, isomerised with iodine, and the triple bond reduced with a Lindlar catalyst. [78]



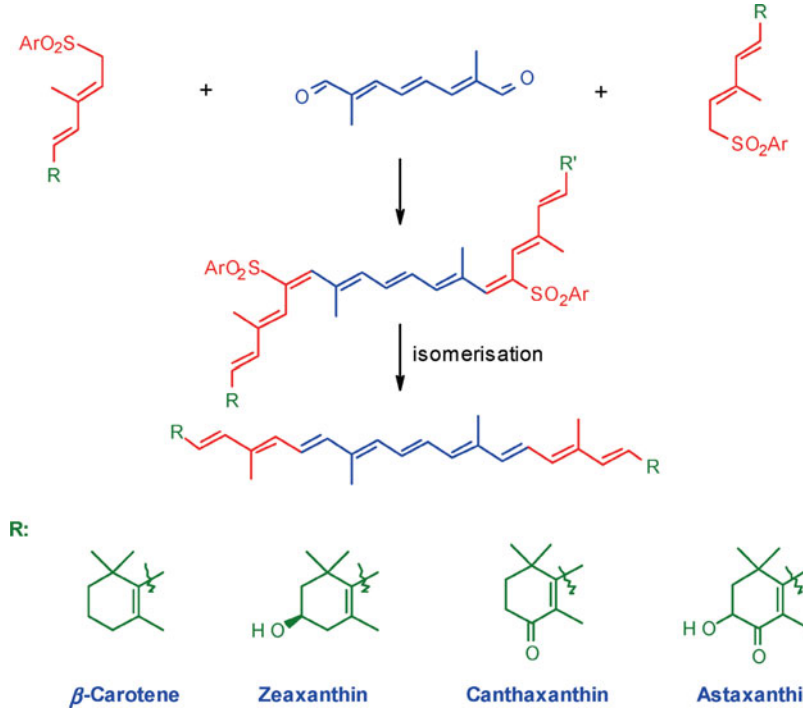
While it became evident that almost all of these carotenoid syntheses led primarily to (*E*/*Z*)-mixtures, and that the isomerisation step could not be avoided, this triggered increased efforts to find at least a more general methodology, and to employ the less expensive aldehydes building blocks. The primarily formed *α*-hydroxysulfones were directly esterified by the addition of acetic anhydride. The stereoconvergent step is the elimination of acetic acid in aqueous THF or 1,2-dimethoxyethane in presence of ammonia or an amine. The reaction gives, with a high degree of selectivity, the (*E*)-vinyl sulfone. The arylsulfinyl group is reductively cleaved with sodium dithionite. Thereby hydrogen sulfoxylate is added *syn*-facially to the double bond. The *anti*-elimination gives, with high stereoselectivity, the (*Z*)-alkene in a yield of up to 90 %. [79, 80]

The reduction of the diastereomeric acetoxysulfone with sodium amalgam gives selectively the (*E*)-alkene.

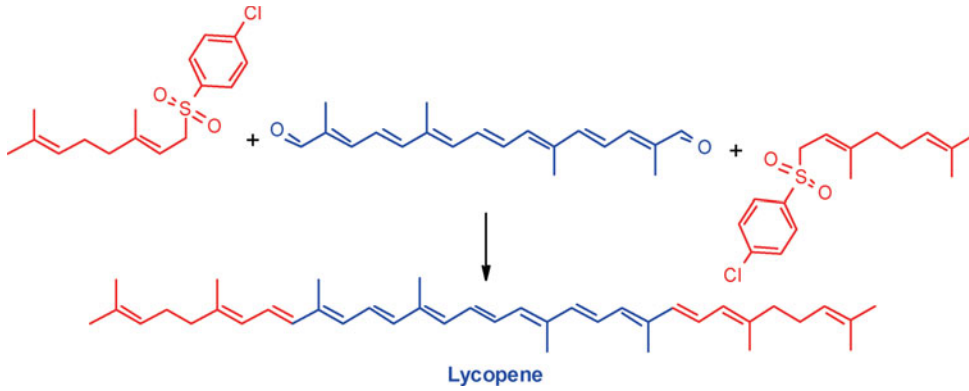


The concept of using two C_{15}-sulfone moieties and one C_{10}-dialdehyde may not only be applied to *β*-carotene, but also to zeaxanthin, canthaxanthin and astaxanthin. For canthaxanthin and astaxanthin, it is necessary to protect the func-

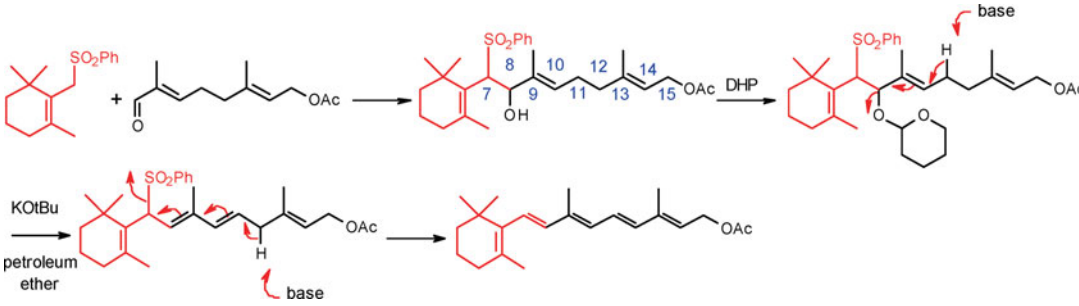
tional groups as ketals or ethers, respectively, due to their incompatibility with the reaction conditions of the Julia-olefination and dithionite reduction. After the isomerisation, the (all *E*)-portion amounts to around 90 %.



Lycopene is also obtained by the same methodology.



In the 1980s, the Japanese firm Kuraray developed a stereoselective Vitamin A synthesis, which follows the $C_{10} + C_{10}$ concept. The synthetic building blocks are cyclogeranyl phenyl sulfone and a C_{10}-aldehyde, which is obtainable by allylic oxidation of geranyl acetate with *t*-butyl hydroperoxide. [81] The alcohol function is protected as the THP-ether, and the sulfinic acid and hydroxytetrahydropyran are eliminated with potassium *t*-butoxide in petroleum ether. By means of this double elimination, the reduction step is avoided.



The elimination is initiated by a deprotonation at C-11. After the cleavage of the ether function, a 1,6-elimination of the sulfinic acid then occurs. It is remarkable, that in this case the (all *E*)-portion reaches around 95 %.

Carotenoids by Extraction

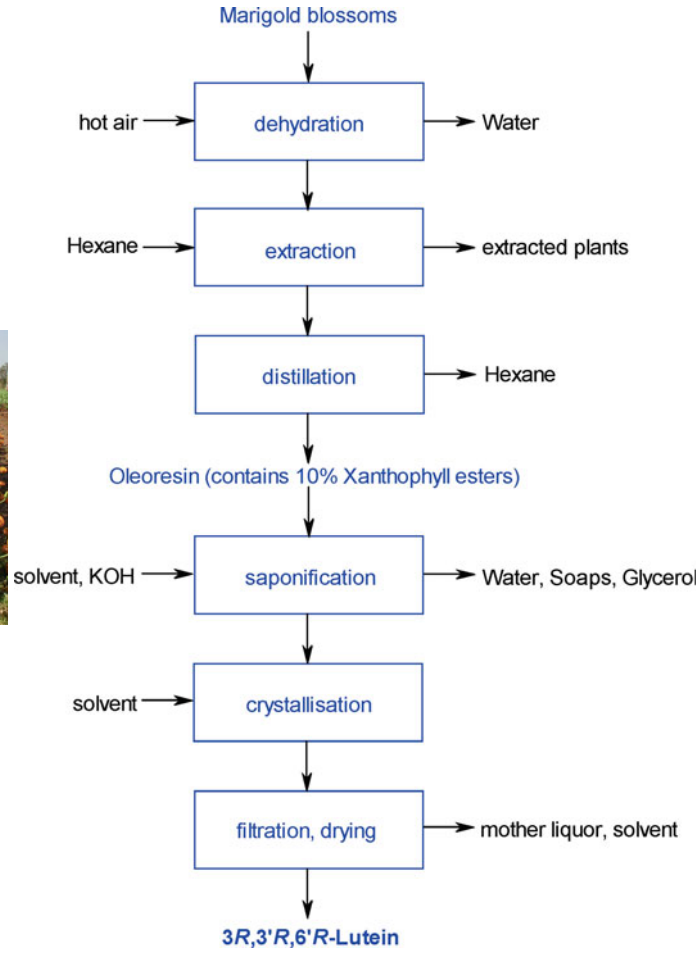
Driven by modern consumer behaviour, organic carotenoids have meanwhile achieved a relevant market share, in spite of their frequently higher manufacturing costs and consequently a higher retail price, compared to synthetic carotenoids (on the basis of pure substance). It is often the case, that the label "organic" by itself guarantees a market presence in the food sector. The utility claims extend thereby from nutritional supplements (*β*-carotene), over tanning pills (canthaxanthin), and preparations to improve fertility (astaxanthin) to applications in ophthalmology (lutein and zeaxanthin) and as antioxidants for cancer prophylaxis (lycopene).

A diverse array of sources and preparative procedures are available to obtain organic carotenoids. – Lutein may be extracted from the petals of the Mexican marigold (*Tagetes erecta*) (Fig. 7.32). [69] Since xanthophylls occur in Nature mostly as esters, and while the free alcohol form is advantageous for many applications, the extraction process with a hydrocarbon is often followed by a hydrolytic step. [63]

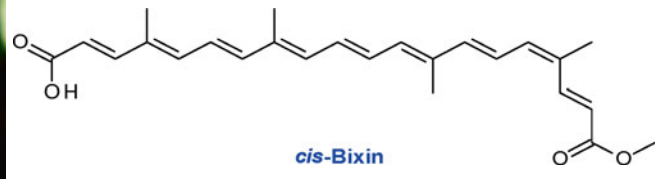
In terms of quantity, annatto is a very important carotenoid colourant for foodstuffs, which is isolated from the seeds of the achiote (also known as "aplop-pas", indigenous to the tropics: *Bixa orellana*) (Fig. 7.33). The principal component is *cis*-bixin, an apocarotenoid with two carboxylic acid functions, one of which is esterified. Annatto is marketed either as a solution in various edible oils or as a spray-dried powder, and serves as colourant of margarine, pasta, cheese and yoghurt. [63]

LycoRed, an Israeli company, obtains lycopene from Tangerine tomatoes, which are especially cultivated for this purpose. Another source of lycopene is the biomass from fermentation of the fungus *Blakeslea trispora*, which is extracted with isobutyl acetate and crystallised. [82] DuPont and Microbia Inc. (now DSM) developed processes for the production of canthaxanthin with the aid of the recombinant oleaginous yeast *Yarrowia lipolytica*. [83]

Algae are also superbly suited for the isolation of carotenoids. Thus, the freshwater green alga *Haematococcus pluvialis* is used to obtain astaxanthin. When a pond starts to dry up, or nutrients become limited, the algae form a protective cell wall, encyst and enter a dormant phase. Massive amounts of astaxanthin are



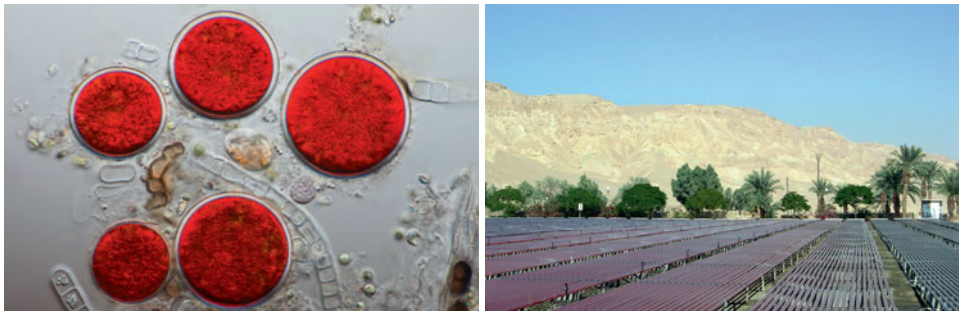
7.32 *Extraction process of lutein from the marigold, and a marigold field in India. For centuries, this flower has also been cultivated there for ornamental purposes and for the decoration of temples.*



7.33 *The achiote is cultivated mainly in Latin America and Asia. The isolated pigments were used already by the Mayas and Aztecs, not only as a food colourant but also as red ink, as a dye for body-painting, as an insect repellent, and to ward off evil.*

produced as protection against UV-light and oxidative stress (Fig. 7.34). [84] Relative to the dry weight, the algae contain up to 4 % of astaxanthin. In the course of the work-up, the algae are decanted off, the cells are ruptured by a high-pressure homogeniser and dried to a residual moisture content of under 5 %.

Alternatively, astaxanthin may be obtained by extraction of a *Haematococcus* oleoresin with supercritical carbon dioxide, which results in a dark red powder with standardised astaxanthin content.



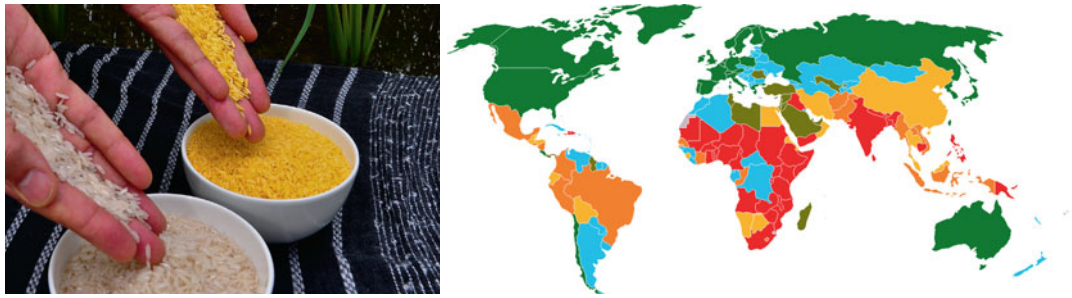
7.34 *The cysts of the microalga Haematococcus pluvialis occasionally cause a spectacular blood-red appearance to dried-out lakes, ponds, river beds or even the stoups in churches, and can be associated with the natural phenomenon described as "blood rain" (left). – Algae production plant in the kibbutz Ketura in the Negev desert in Israel (right).*

The algal species *Dunaliella salina* [85], a halophilic, unicellular green microalga, has been used for a long time to produce β-carotene. The first pilot plants had already been built by 1966 in the USSR. The production procedures range from low-tech extensive cultivation in lagoons to intensive cultivation at high cell densities under carefully controlled conditions. In general, after separation of the aqueous phase, the algae are extracted with hot oil, from which β-carotene crystallises out upon cooling, and is then separated by centrifugation. [61] Cognis (now part of BASF) operates large ponds in Australia for the manufacturing of natural β-carotene (*Betatene*®), which is offered on the market in diverse formulations (Fig. 7.35).

7.35 *The Hutt Lagoon in Western Australia, where Cognis/BASF grows Dunaliella salina.*

7.1.5 Modern Trends

In recent years, we have witnessed the advent of entirely new carotenoid production technologies. The most prominent example might be the 'golden rice project'. In the year 2000, Ingo Potrykus at the ETH Zurich and Peter Beyer at the University of Freiburg developed by genetic engineering a new variety of rice (*Oryza sativa*), which produces β-carotene in the edible part of the rice grains (the endosperm) and gives them a distinctive yellow colour (Fig. 7.36). [86]



7.36 *Golden rice and the prevalence of Vitamin A deficiency (red: clinical → green: Vitamin A deficiency under control; blue: no data available).*

The golden rice project was conducted in order to combat Vitamin A deficiency, which is still prevalent in many parts of the world, but most frequently found in nearly all countries in Africa and in India. According to a WHO study, it is estimated that Vitamin A deficiency affects at least 190 million young children and 19 million pregnant women per year. This malnutrition leads to an annual rate of 1–2 million deaths, 500,000 cases of irreversible blindness and millions of cases of xerophthalmia. [87]

Summary in Bullet Points

- Vitamin A and the carotenoids are products of the terpene metabolism.
- Industrial manufacturing processes are based on modular building blocks.
- Universal methods to couple these building blocks are the Wittig reaction, the Horner-Wadsworth-Emmons reaction, the sulfone coupling according to Julia, the Müller-Cunradi enol ether condensation and the Saucy-Marbet rearrangement.
- The carotenoids are important colourants for the foodstuff industry.

7.2 Vitamin D

7.2.1 Discovery

The first reports on rickets in infants originate from Soranus of Ephesus, a Greek physician, who practiced in Alexandria and subsequently in Rome at about 100 AD. As we know nowadays, rickets predominant cause is a deficiency or impaired metabolism of Vitamin D. Particularly in malnourished children, this results in the disruption of growth and softening and deformation of the bones, as a consequence of insufficient mineralisation of the collagen matrix (Fig. 7.37).

As first comprehensively described [88] in 1645 by Daniel Whistler (1619–1684) in his doctoral thesis "De morbo puerilli anglorum", rickets had grown in the wake of industrialisation to a major health problem across all of northern Europe and North America. The urbanisation of a society, previously shaped by agriculture, led to circumstances, which were conducive to the so-called "English Disease" (Fig. 7.38).

Already at the beginning of the 19th century, the Polish physician and chemist Jędrzej Śniadecki (1768–1838) had established a connection between exposure to sunlight and rickets. In 1890, a similar observation was made by Theobald Adrian Palm (1848−1928), a Scottish medical missionary in Niigata in Japan. He compared the prevalence of rickets in northern European urban areas with similar areas in Japan and concluded that sunlight deficiency was implicated in the aetiology of rickets.

In 1919, Sir Edward Mellanby (1884–1955) succeeded inducing rickets experimentally in dogs and curing the illness with fish liver oil. A short time later, the Berlin physician Kurt Huldschinsky (1883–1941) observed that children with rickets, who play outside in the sun, rapidly become healthy again. The same effect was achieved artificially by exposing children to UV light of a wavelength between 230 and 315 nm (Fig. 7.39).

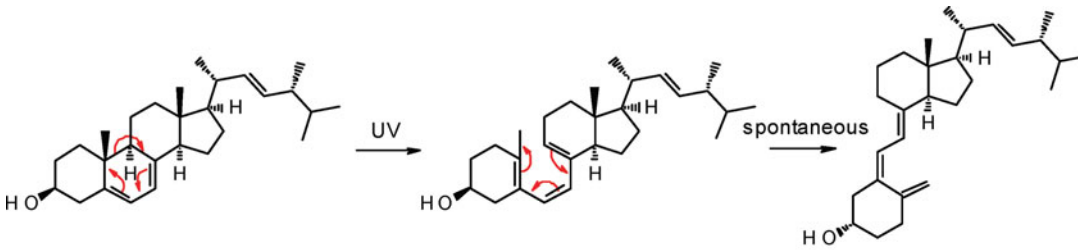
7.37 *Typical symptoms of rickets, like softening of the skull bones (craniotabes), the bulging of cartilage-bone interfaces in the growth plate regions, and the flattening of the back of the head, leading to a squared shape (caput quadratum), were already so frequent in small children, that Albrecht Dürer (1478–1521) captured such a case in 1512 in his painting "Virgin and child with a pear".*

7.38 *A working class family in the courtyard of Langer Jammer in Brauerknechtsgraben (working class quarter) in Hamburg around 1900. These were dark dwellings with narrow back yards, which were, along with an inadequate and unbalanced diet, leading to rickets.*

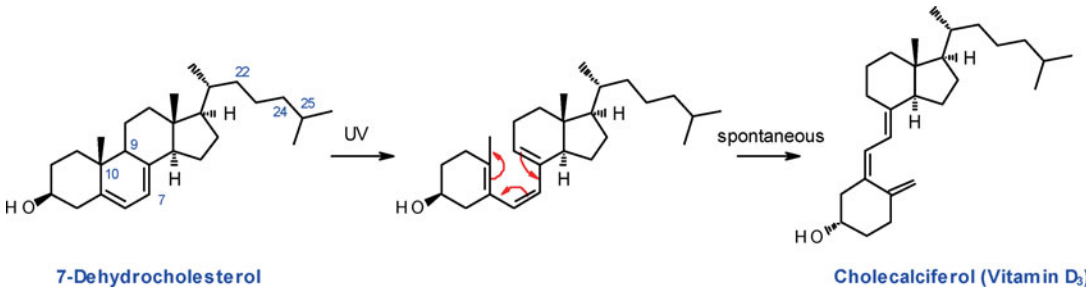
7.39 *In the 1930s BASF employed a "radiation facility" for the children of their employees.*

While not many natural foods contain significant levels of Vitamin D, fatty fish (tuna, salmon, mackerel) are regarded the best source. Nowadays, in particular in the United States, most milk is fortified with Vitamin D, which is also a component in many breakfast cereals, some beverages and margarine, to prevent rickets in babies and small children, and also osteomalacia (bone softening) in adults. The Vitamin was originally tracked by the observation, that certain foodstuffs and vegetable oils, which had been irradiated with UV light, showed a similar therapeutic effect as codliver oil. UV spectroscopy enabled to attribute this effect to trace quantities of an ingredient in the non-hydrolysable portion of vegetable oil. It was not possible, however, to separate off this component by crystallisation.

This material was also detectable in crude cholesterol from animals. Later it was found, that irradiation of ergosterol, a steroid from baker's yeast, led to a mixture of products with extraordinarily high anti-rachitic activity. At the end of the 1920s, Otto Rosenheim, Thomas Arthur Webster and Adolf Windaus succeeded, independently of each other, in isolating first the unpurified Vitamin D (Vitamin D_1, a mixture of ergocalciferol and lumisterol$_2$) and later the pure crystalline Vitamin D (Vitamin D_2, ergocalciferol). The structure determination followed by classical chemical degradation.



Hans Brockmann (1903–1988) isolated Vitamin D_3 from tuna liver oil. The synthesis was achieved by irradiation of 7-dehydrocholesterol, a minor component of crude cholesterol. Adolf Windaus (1876–1959) and Sir Ian Morris Heilbron (1886–1959) determined the structure and Hans Herloff Inhoffen (1906–1992) confirmed it by total synthesis.



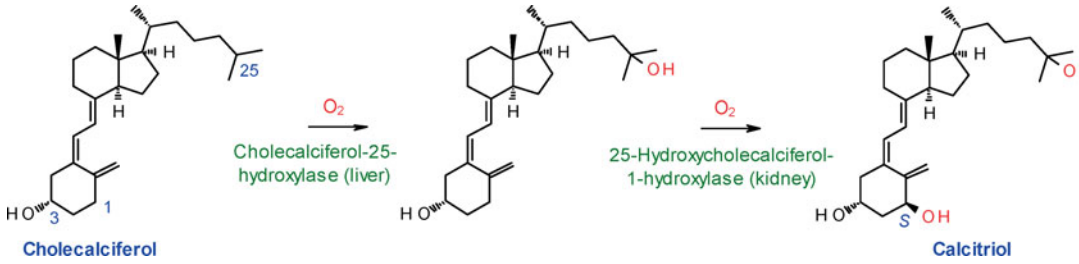
Vitamins D_2 and D_3 are the two economically important forms of the D-vitamins. A few years ago, however, it was shown that in the breeding of cattle and pigs, and the keeping of horses, ergocalciferol possesses a lower efficacy

than cholecalciferol (Vitamin D_3), and in poultry farming it is actually almost inactive. In contrast, humans can utilise both of these forms. [89]

7.2.2 Physiology

The synthesis of Vitamin D_3 *in vivo* takes place in the skin. UV-B radiation (β = 290–315 nm) cleaves 7-dehydrocholesterol, an intermediate product of cholesterol metabolism, in the epidermis, which isomerises spontaneously at body temperature to cholecalciferol. While the photolysis is very efficient, it is subject to seasonal and climatic fluctuations. Strong pigmentation and aged skin lead to a considerably reduced capacity to produce Vitamin D.

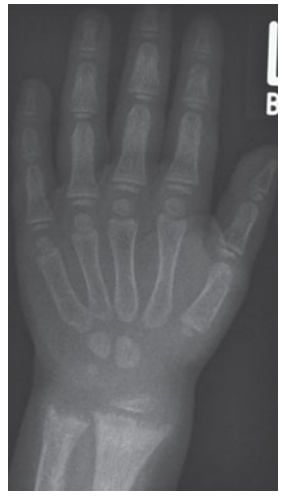
Over the years 1966–1971, Anthony Norman, Egon Kodicek and Hector De Luca found out, with the aid of labelled cholecalciferol, that this is hydroxylated stepwise in the liver and kidneys of warm-blooded animals, and that in reality the resulting metabolite, calcitriol, is the active principle. In the liver, the side-chain is first hydroxylated at C-25 by the cytochrome P450-enzymes CYP27A1 and CYP2R1. 25-Hydroxycholecalciferol is a depot form of Vitamin D with a biological half-life of 1–2 months. The second hydroxylation takes place in the kidneys at C-1, whereby these function as endocrine hormonal glands. The half-life of calcitriol in humans ranges between 5–8 hours. [90]



Intracellular calcitriol receptors are widespread in the body. They are found, for example, in the small intestine, the kidneys, the bones, and the parathyroid gland. Calcitriol binds to these receptors and induces at the DNA level the transcription of hormone-sensitive genes. It influences cell differentiation and proliferation, stimulates an increased uptake of calcium ions from the intestine through enhanced formation of calcium-binding proteins, and also controls the release of calcium from the bones. [91] Since the counter-ion of the calcium is mostly phosphate, calcitriol raises consequently the phosphate level in blood as well. While the release of calcium from the bones appears counterproductive, this effect is however over-compensated by the increased intestinal calcium resorption, resulting in an elevated serum concentration.

In humans, the recommended average daily intake of Vitamin D_3 lies for adults at 15–20 micrograms, for infants up to 12 months at 10 micrograms, for children up to 8 years, and pregnant or breast feeding women at 15 micrograms. Exact thresholds are difficult to define, but the safe upper limits per day range from 25 micrograms in infants to 100 micrograms in adults. Considering these

7.40 *In grazing livestock, which consume glycosylated calcitriol-containing plants with their fodder (e.g. golden oat grass (Trisetum flavescens)), calcinosis has repeatedly caused considerable damage.*



7.41 *The hand of a five-year-old boy with chronic kidney disease, and thereby dysregulation of Vitamin D metabolism, which leads to rickets if it remains untreated. The typical cup-shaped distension is visible at the ulna and hints thereof also at the radius, which indicates abnormal mineralisation in the region of the growth plates.*

small daily doses, Vitamin D is one of the most potent vitamins, and excess intake, in particular of 25-hydroxycholecalciferol, can cause health problems by leading to hypercalcaemia, possibly resulting in irreversible calcification of soft tissue like kidneys, lungs, heart, pancreas and the aorta. Especially vulnerable are the kidneys, where kidney stones are formed, leading potentially to organ failure (Fig. 7.40).

Under normal physiological conditions, humans can synthesise sufficient amounts of Vitamin D_3 in their skin upon exposure to sunlight (*ca*. 0.25 micrograms of Vitamin D per hour and per square centimetre of skin surface). In contrast, Vitamin D_2 has to be absorbed from food. In this respect, Vitamin D_3, like calcitriol, acts more like a hormone than a vitamin.

The most important medicinal applications of Vitamin D preparations target the prevention of rickets and osteomalacia (bone-weakening). Inadequate kidney function (Fig. 7.41), as in dialysis patients, indicates the supplementation of Vitamin D, just as post-menopausal or age-related osteoporosis (bone atrophy) do. Apart from this, Vitamin D and its analogues are as well used for the treatment of proliferative diseases like psoriasis and cancer.

Examples of Vitamin D supplementation in the farming sector include poultry feed to increase egg production and the stability of egg-shells. Vitamin D serves also to prevent milk fever in cows and leg weakness in turkeys.

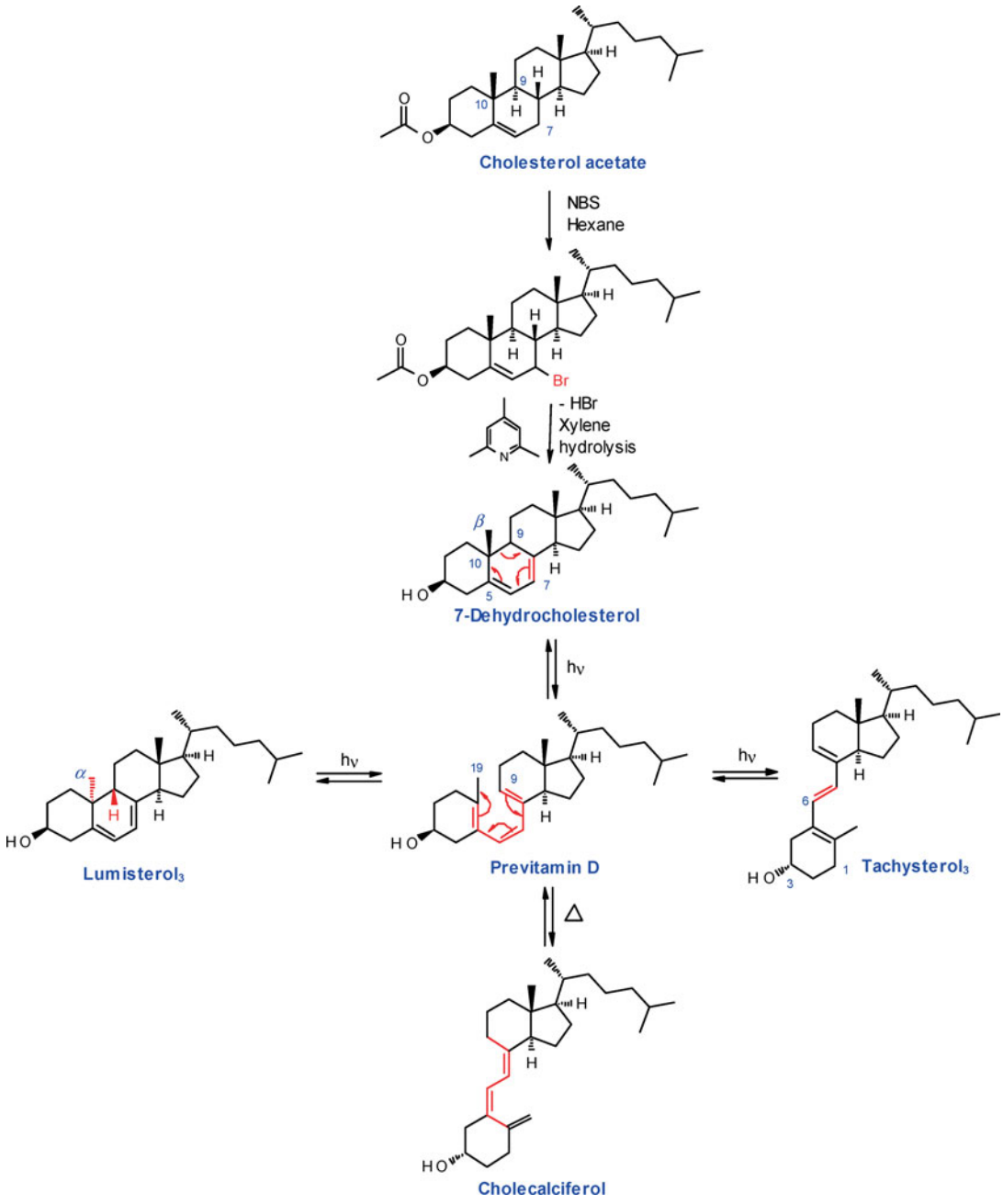
7.2.3 Industrial Syntheses

Since 1978, Hoffmann-La Roche/DSM produces Vitamin D by a semi-synthetic route on industrial scale. Fish oil, with its relatively high levels of Vitamin D, serves merely as a source to generate Vitamin D concentrates.

Cholecalciferol

Cholecalciferol (Vitamin D_3) is obtained from cholesterol, accessible by extraction from wool-fat (which contains around 15 % cholesterol) and the spinal marrow from animals. In an analogous fashion, ergocalciferol (Vitamin D_2) may be obtained from ergosterol, which in turn is extracted from the non-hydrolysable portion of vegetable oil, and from baker's yeast. The use of ergosterol has however declined over the years, since chickens, pigs, cattle and horses utilise Vitamin D_3 better than ergocalciferol, which in addition offers no advantage in manufacturing costs.

The hydroxy-group of cholesterol is protected either as the acetate or benzoate. Bromination in the allylic position by Karl Ziegler's method using *N*-bromosuccinimide or 1,3-dibromo-5,5-dimethylhydantoin, then dehydrobromination with 2,4,6-collidine and hydrolysis gives 7-dehydrocholesterol. Photolysis of 7-dehydrocholesterol and ergosterol, respectively, with a mercury vapour lamp in an inert solvent (*e.g.* peroxide-free diethyl ether, methanol, cyclohexane

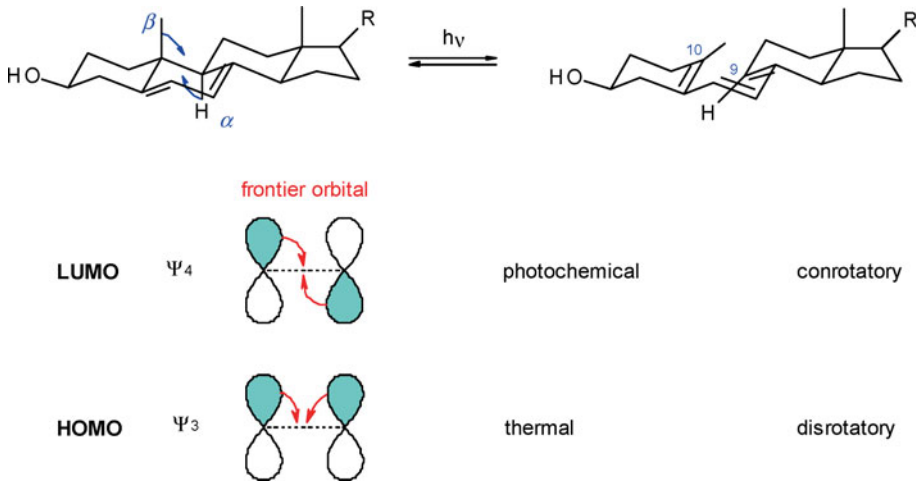


or dioxane) leads to a reversible cleavage of the B-ring between C-9 and C-10 ($\pi \rightarrow \pi^*$excitation, λ_{max} = 291 nm, ε = 12,000). The reaction solution is pumped through a water-cooled quartz reactor. Addition of salts (*e.g.* lead acetate) to the cooling water serves as a chemical filter to screen out unwanted lower frequencies. Glass filters may also be used. Addition of sensitisers (*e.g.* eosin) to the reaction solution enhances the emission of certain UV-frequencies and has an influence on the product ratio.

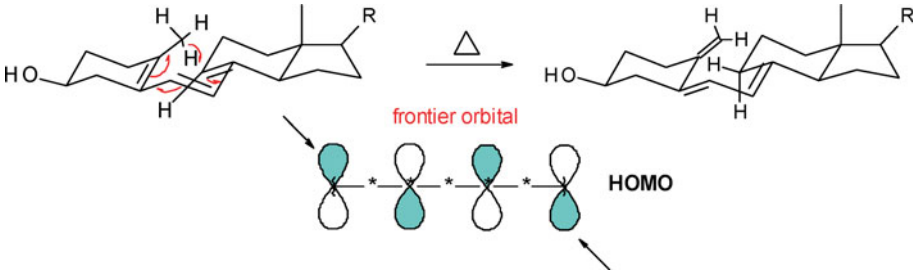
The Previtamin D exists in equilibrium with the starting material, its 9,10-epimer, lumisterol$_3$ and the (6*E*)-isomer tachysterol$_3$. The position of the equilibrium, and therefore the product ratio, is dependent on the wavelength of the UV light. In order to avoid photolytic decomposition reactions, radiation is terminated after a conversion rate of about 40 %. For protection against oxidation, the reaction mixture is stabilised with < 1 % by weight of butylated hydroxyanisole or butylated hydroxytoluene. Subsequently, the photolysis product is warmed to 80 °C. This induces a reversible thermal double bond migration in the manner of a 1,7-hydrogen shift from C-19 to C-9.

The reactivity and stereochemistry of 7-dehydrocholesterol and the downstream products may be described by the Woodward-Hoffmann rules. [92] In 7-dehydrocholesterol, the hydrogen atom at C-9 is α-configured, and the methyl group at C-10 in a β-orientation. A thermally-induced disrotatory ring-opening would turn one of these substituents into the fused ring system; energetically, this is highly unfavourable. On the other hand, the photochemically-induced conrotatory ring-opening proceeds smoothly. Correspondingly, the substituents at C-9 and C-10 in lumosterol$_3$, which results from the photochemical ring-closure reaction, are also *anti*-orientated (Fig. 7.42 and Fig. 7.43).

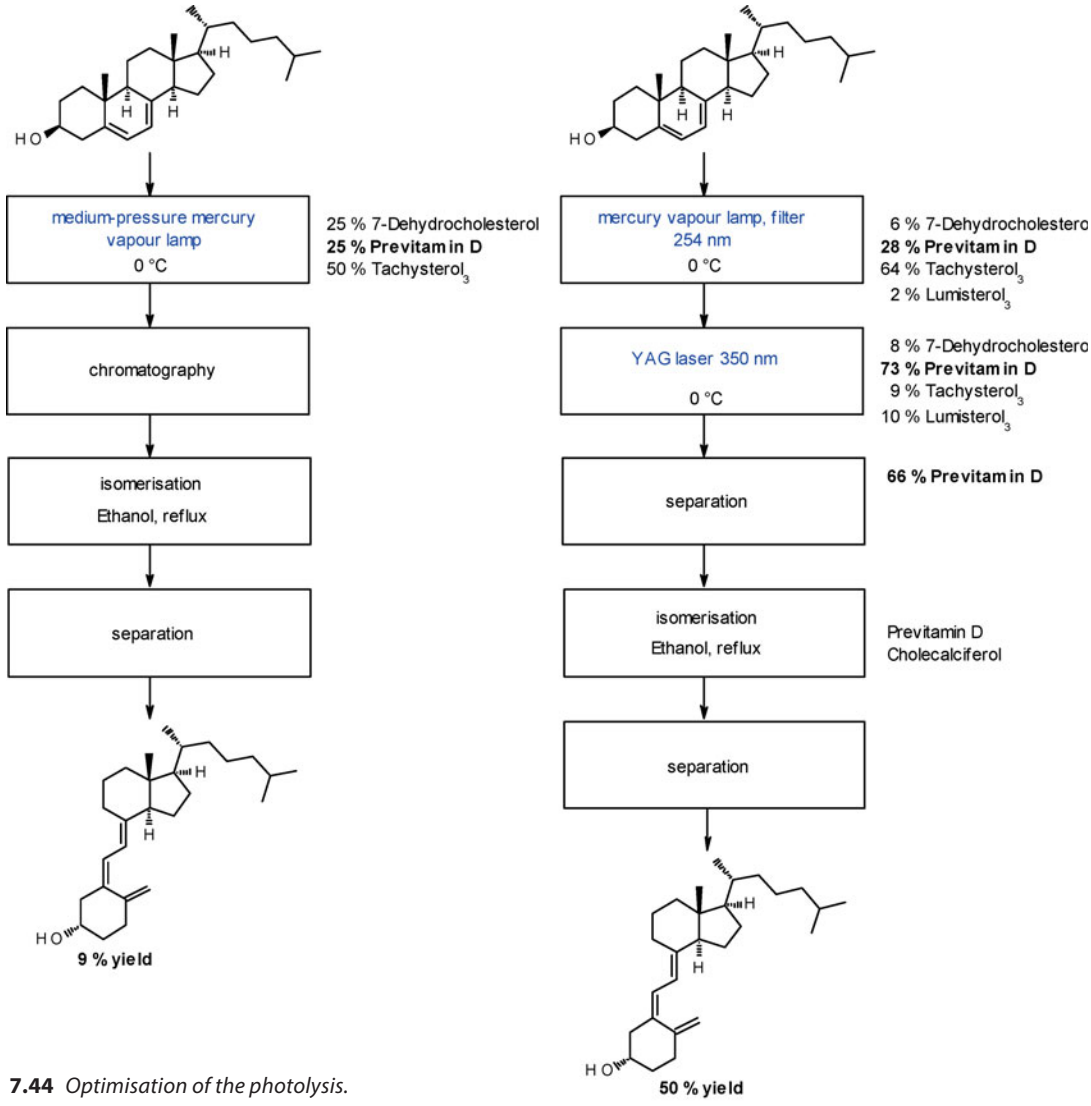
The simple broad-band irradiation with a medium-pressure mercury lamp delivers, after chromatography, thermal isomerisation and purification, cholecalciferol in a yield of only 9 %, based on 7-dehydrocholesterol. The greatest losses result from the formation of tachysterol$_3$ (Fig. 7.44). [93]



7.42 *Rules for electrocyclic ring-closure reactions of a 6 π-electron system.*



7.43 *The thermal [1,7]-hydrogen shift proceeds antarafacially from the non-bonding allyl orbital. On account of the size of the system and the preconformation, such rearrangements are possible.*



7.44 *Optimisation of the photolysis.*

The yield of cholecalciferol may be significantly increased, if the photolysis conditions are improved. A wavelength-controlled, two-stage irradiation, by which the unwanted isomers are re-isomerised, largely simplifies the workup procedure and leads to a yield of 50 %.

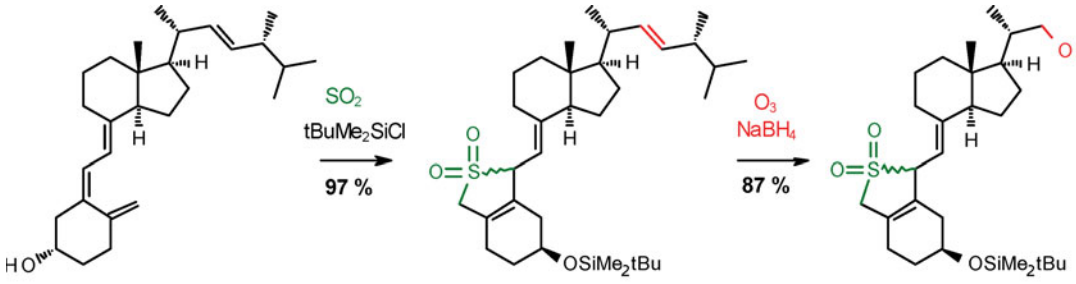
UV light with a wavelength of 254 nm can be generated with a mercury vapour lamp and the appropriate filters. UV radiation with a wavelength of 350 nm is obtained with an yttrium-aluminium-garnet (YAG) laser. Optional is as well the irradiation with a KrF laser (248 nm), or a nitrogen laser (337 nm).

In the workup sequence, following concentration of the reaction solution and the addition of methanol, the sparingly-soluble $\Delta^{5,7}$-diene components (lumisterol$_3$, lumisterol$_2$, 7-dehydrocholesterol, and ergosterol, respectively) can be removed by filteration. Tachysterol$_3$ is separated off in the form of its Diels-Alder adduct with maleic anhydride. The mother liquor is evaporated and may be used directly as an additive for animal feed (Vitamin D$_3$-resin). Vitamin D for the food and pharmaceutical sector is first purified as its butanoate or 3,5-dinitrobenzoate by recrystallisation, and then hydrolysed and again recrystallised.

Calcitriol

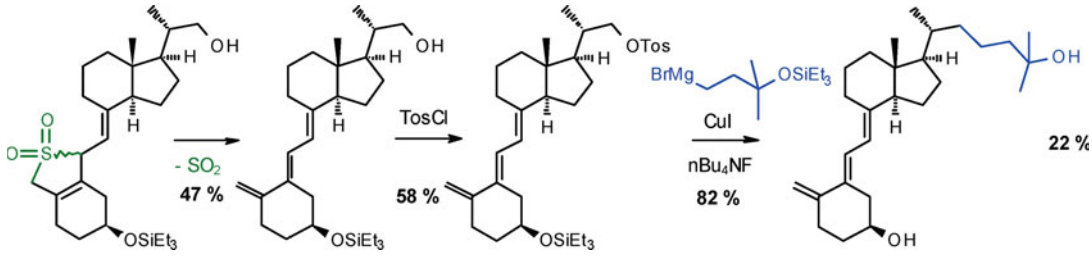
The discovery that calcitriol is actually the active vitamin / hormone, has reinvigorated Vitamin D research, and has initiated numerous efforts to prepare this compound. [93, 94] Hoffmann-La Roche's process for manufacturing calcitriol in commercial quantities [95] follows conceptually a synthesis method of Sir Derek H. R. Barton (1918–1998) and Robert H. Hesse. [96]

The starting material is ergocalciferol, the triene system and alcohol group of which are protected as a sulfur dioxide adduct and a silyl ether, respectively. The side-chain is degraded by ozonolysis at –10 °C in dichloromethane. Direct reduction with sodium borohydride destroys the ozonide and reduces the aldehyde to the alcohol. The yield amounts to 87 %.

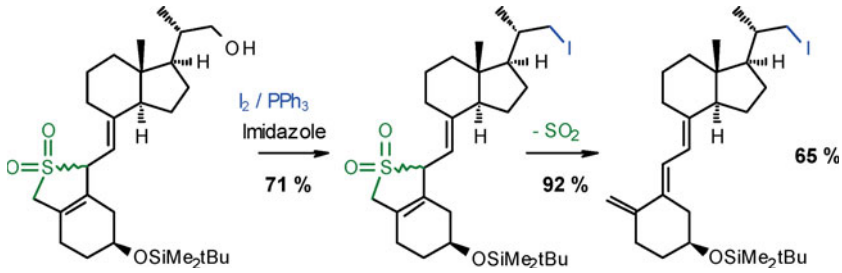


Ergocalciferol

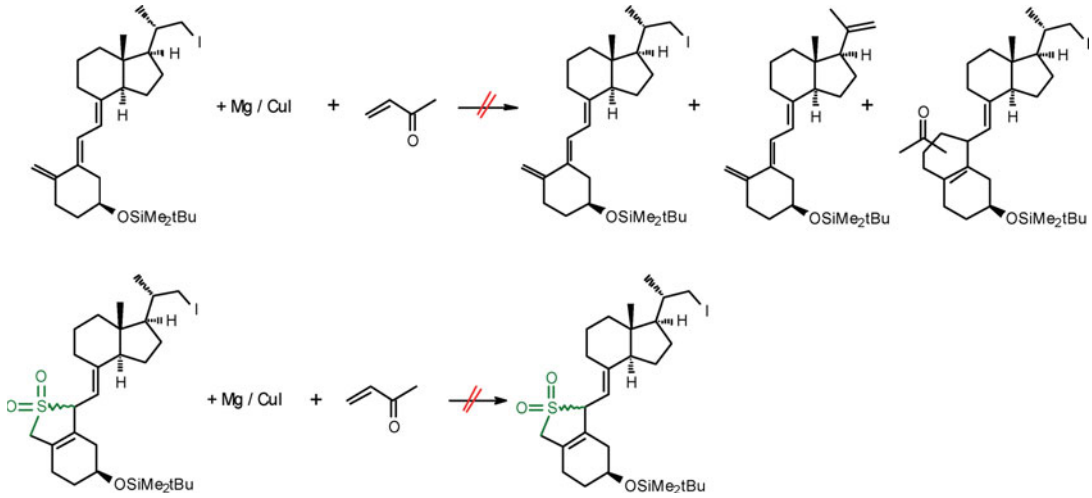
In the Barton-Hesse synthesis, following extrusion of sulfur dioxide, the alcohol is tosylated, and reacted with the cuprate, formed from copper(I) iodide and 3-methyl-3-(triethylsiloxy) butylmagnesium bromide. The coupled product is obtained after chromatography in a yield of 82 %.



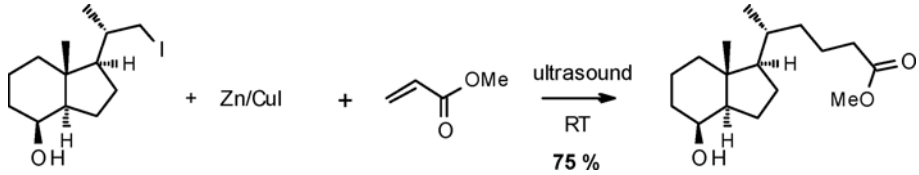
In the Roche synthesis, the alcohol is converted in the next step into the iodide with iodine and triphenylphosphane, followed by extrusion of sulfur dioxide.



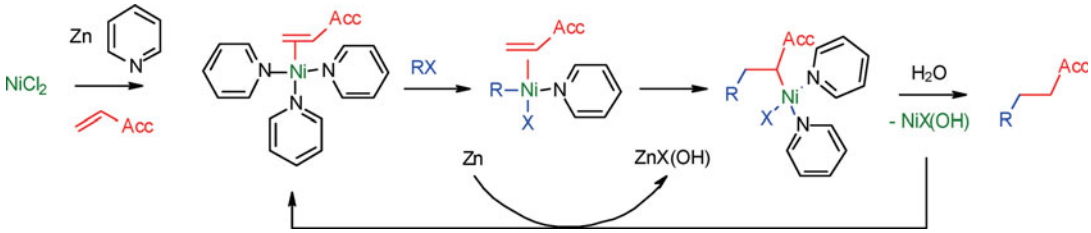
The objective was, to add the corresponding cuprate [97] to methyl vinyl ketone. However, the experiments failed, and in most cases the educt was recovered unchanged, and contaminated with the desired product. In other cases, the corresponding alkene or the Diels-Alder adduct of the iodide, and methyl vinyl ketone were obtained. The attempted coupling at the sulfone-stage was not successful either.



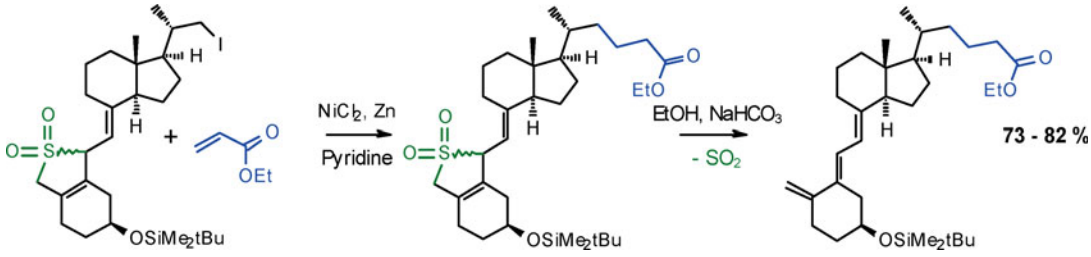
This came as a surprise, since a closely related system underwent the reaction easily under Luche-conditions (with ultrasound). [98–100]



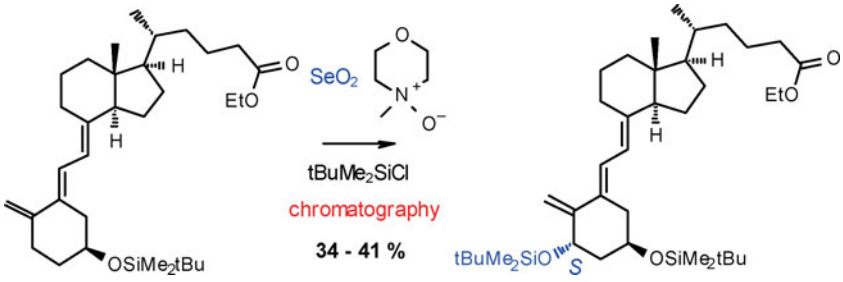
The solution to this synthesis problem goes back to the work of Reiner Sustmann, who successfully carried out the formal, nickel(0)-mediated addition of alkyl or aryl halides to electron-deficient alkenes, like acrylate esters or acrylonitrile. [101] Sub-stoichiometric amounts of nickel(II) chloride hexahydrate (15–20 mole %) are reduced with zinc in presence of pyridine to nickel(0), which forms together with pyridine a complex with the alkene. Oxidative addition of the alkyl halide leads to an alkyl-nickel species, the carbon-metal bond of which undergoes an alkene insertion. Hydrolysis gives ultimately the product. Heck reaction products are not observed.



The Ni(0)-mediated coupling reaction could be successfully applied to Vitamin D derivatives. Nickel chloride is reduced with zinc powder in pyridine to nickel(0), and this forms a brick-red complex with the acrylate ester. This, in turn, is smoothly alkylated by the alkyl iodide. The reaction is widely applicable; it can also be carried out successfully with the sulfur dioxide adducts, and ethyl acrylate may be replaced by methyl vinyl ketone as well.

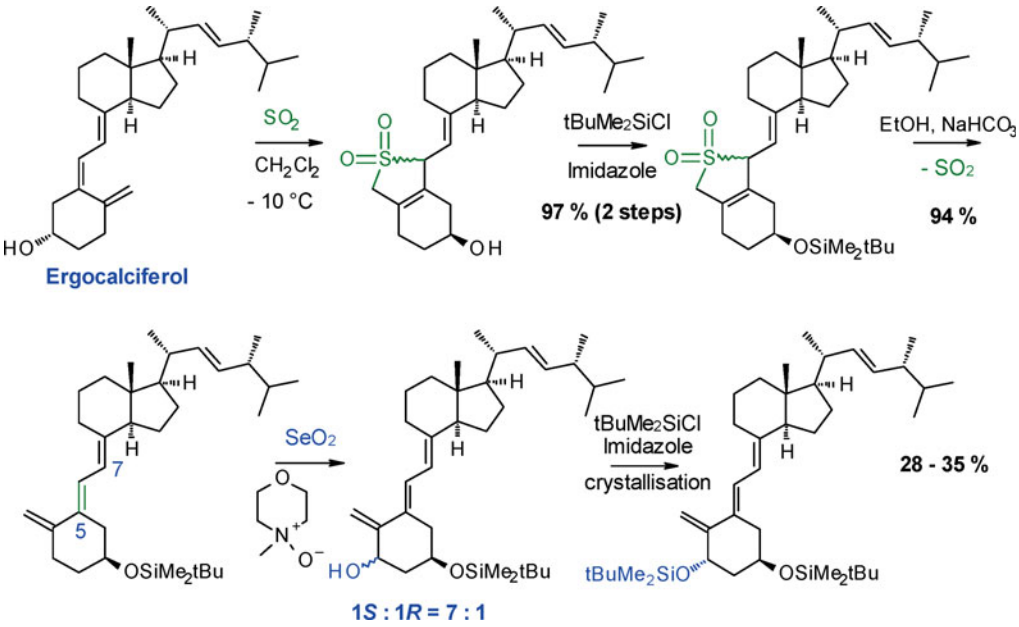


Extrusion of sulfur dioxide, by warming in ethanol in the presence of sodium hydrogen carbonate, is followed by hydroxylation at C-1 with sub-stoichiometric amounts of selenium dioxide and *N*-methylmorpholine *N*-oxide as the oxidant. The stereoselectivity of the oxidation lies at a ratio of 7 : 1 in favour of the (*S*)-diastereomer. After silylation with *t*-butyldimethylsilyl chloride and chromatographic purification, the desired diastereomer is obtained in a yield of 41 %.



Problematic are the low yields at this rather advanced synthesis stage, which calls the whole strategy into question. The yield over all the reaction steps amounts to around 18 %. Whether perhaps an inverse reaction order, that is introduction of the hydroxy-group at the 1-position prior to functionalising the side-chain, would produce better overall yields, remained an option worth addressing.

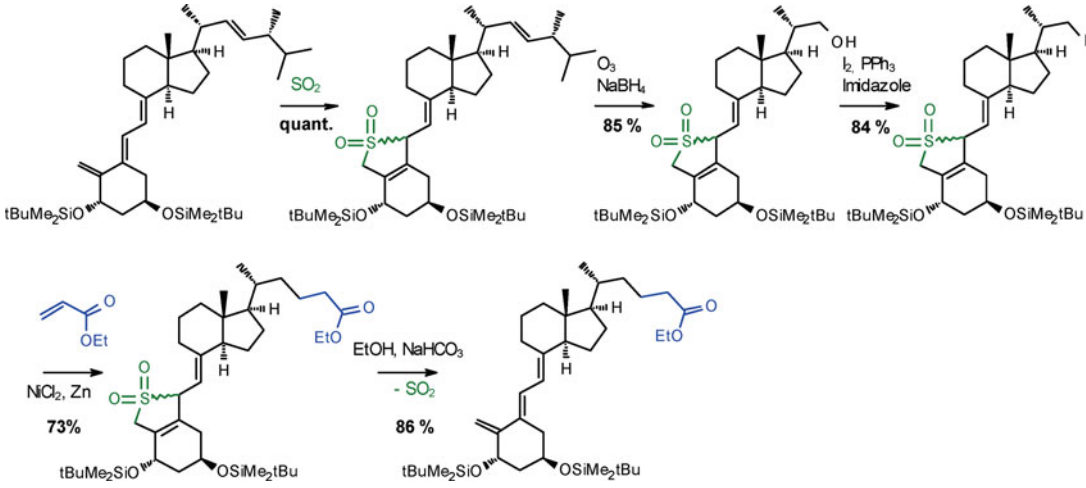
Thus, ergocalciferol is reacted with sulfur dioxide at –10 °C, and then the hydroxy-group is silylated. The chelotropic extrusion of sulfur dioxide produces the (5*E*,7*E*)-isomer. Allylic hydroxylation by Barton's method with selenium dioxide and *N*-methylmorpholine *N*-oxide gives a mixture of (1*S*)- and (1*R*)-isomers in the ratio of 7 : 1. Silylation of the hydroxy-group at the 1-position and crystallisation lead finally to the (1*S*,5*E*)-hydroxy-derivative in pure form. The yield over all the reaction stages is around 28–35 %. [102, 103]



Regrettably, the experiments on direct hydroxylation of the silyl-protected (5*Z*)-ergocalciferol were unsuccessful.

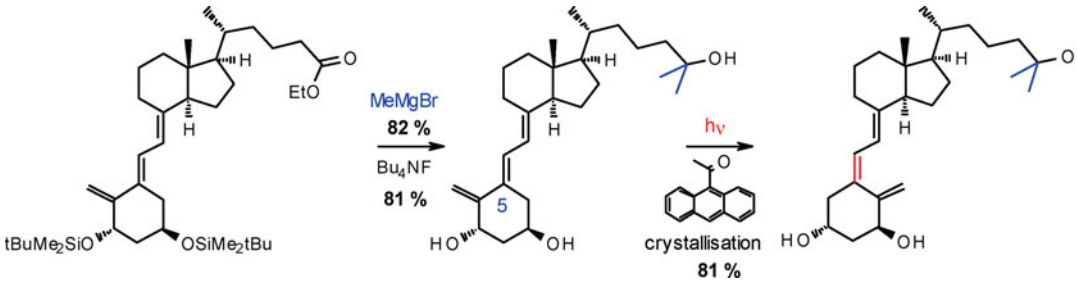
The sequence of functionalising the side-chain corresponds to the already described reaction pathway. Renewed sulfur dioxide addition is followed by

ozonolysis. The conversion of the alcohol into the corresponding iodide is then followed by the nickel(0)-mediated addition to ethyl acrylate and extrusion of sulfur dioxide. The order of the last two stages can also be reversed.



The combined yield over the whole reaction sequence, up to the ethyl ester, reaches only around 15 %. Nevertheless, this strategy offers the advantage, that those transformations with poor yields have been moved to the beginning of the overall synthesis.

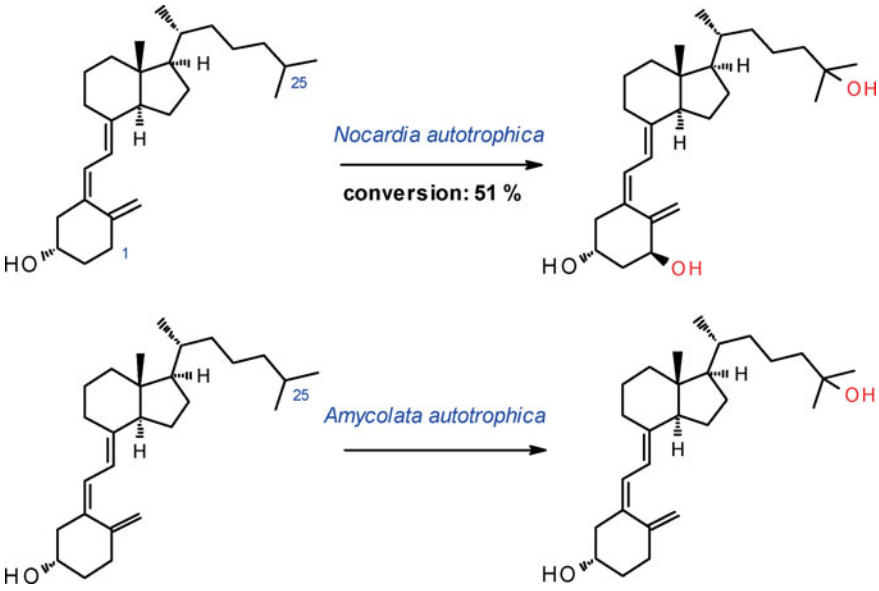
To generate the final product, the ethyl ester is reacted with an excess of methylmagnesium chloride, the silyl protecting groups are cleaved off with tetrabutylammonium fluoride, and the double bond in the 5-position is lastly isomerised in a triplet-sensitised photoisomerisation.



The yield of calcitriol, based on ergocalciferol, lies at 8 %. If the industrially cost-efficient crystallisations, following the hydroxylation at C-1 and the photoisomerisation, are replaced by considerably more expensive chromatographic purification steps, the yield can be increased to more than 10 %. [104]

7.2.4 New Approaches

Patent filings from Taisho Pharmaceutical Co. had claimed already by 1990 a new and promising approach towards calcitriol *via* microbial (1*S*,25)-dihydroxylation of cholecalciferol. [105, 106] *Nocardia autotrophica* hydroxylates both positions, whereas *Amycolata autotrophica*, in contrast, shows a preference for mono-hydroxylation in the side-chain.



This fermentation procedure has meanwhile developed into a novel opportunity to provide calcitriol at much lower cost. [107]

7.2.5 Economic Aspects

The pharmaceutical industry produces Vitamin D_3 and, on a smaller scale, Vitamin D_2 in pure crystalline form, or as a solution in vegetable oil (1 million IU/g) for manufacturing drugs, and as nutritional supplement (Multivitamin specialities). The lion's share of Vitamin D_3 is sold as the crude product, Vitamin D_3 resin (around 500,000 IU/g), for the production of cattle feed. The bulk manufacturers of Vitamin D are DSM (Switzerland, D_3), Dishman (India, Netherlands, D_2, D_3), and Synthesia (Czech Republic, D_2). The annual global production comes to 1.5×10^{15} IU (1 IU is the biological equivalent of 0.025 µg), which translates into 37.5 tonnes of Vitamin D.

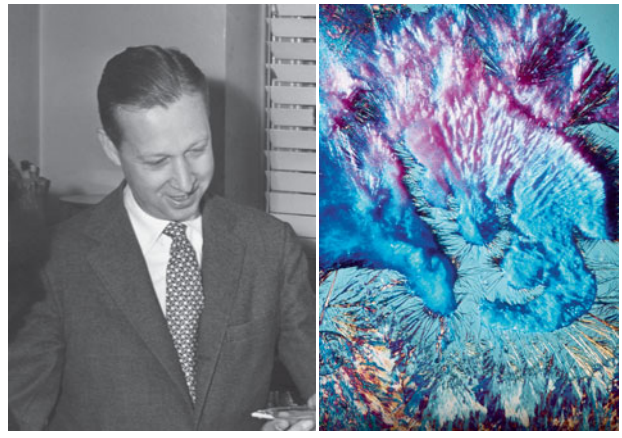
The annual global production of calcitriol amounts to several hundred grams; key producers are Hoffmann-La Roche and Abbott. Tritium-labelled Vitamin D-derivatives for experimental purposes, like biological assays, are produced by Amersham (Great Britain) and by PerkinElmer/New England Nuclear Corp. (USA).

Summary in Bullet Points

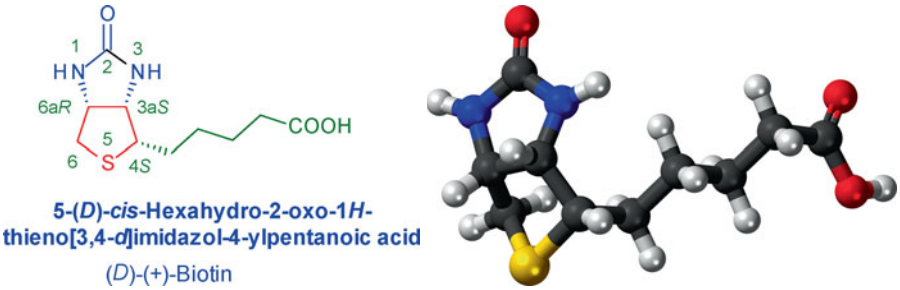
- The successful industrial synthesis starts from photolysis of 7-dehydrocholesterol.
- Vitamin D serves to prevent rickets, and has great importance in animal feed.
- Prior to unfolding its physiological activity, cholecalciferol has to be hydroxylated stepwise to calcitriol, first in the liver, and then in the kidneys.
- Until now, calcitriol has been produced for commercial purposes chemically in 100-gram quantities. Biotechnological processes are very promising.

7.3 Biotin

Biotin is one of the most potent pharmacological compounds, essential for cellular metabolism and cell proliferation. The critical dependence of baker's yeast (*Saccharomyces cerevisiae*) on biotin as a nutrient marked the beginning of its discovery. An accelerated growth rate of yeast can even be detected down to the minute concentration of one milligram of biotin in 400,000 litres of culture medium. In 1901, Eugene Wildiers (1878–1908) in Antwerp discovered this, and biotin was recognised as a yeast growth factor, called "bios". It turned out to be identical with Vitamin H, isolated in 1936 from egg yolk by Fritz Kögl (1897–1959) at the Utrecht University, Netherlands and from liver by Albert von Szent-Györgyi (1893–1986) at the University of Szeged, Hungary. Between 1940 and 1942, Vincent du Vigneaud (1901–1978) at the Cornell University, Ithaca, New York elucidated the structure of biotin by degradation reactions. In 1943, Stanton Avery Harris (1902–1992) and Karl August Folkers (1906–1997) at Merck's Research Laboratory in Rahway, New Jersey, confirmed the structure by the first total synthesis (Fig. 7.45). In 1949, in Nutley, New Jersey, the chemists Moses Wolf Goldberg (1905–1964) and Leo Henryk Sternbach



7.45 *Karl August Folkers and biotin crystals in polarised light.*



7.46 *From the chemical viewpoint, biotin is a quite appealing enantiomerically pure heterocyclic compound with a basic thienoimidazole structure and three adjacent stereogenic centres, which are relative to the thiophane ring all-cis-configured.*

(1908–2005) from Hoffmann-La Roche successfully synthesised enantiomerically pure (*D*)-(+)-biotin. The absolute configuration was first confirmed in 1966 by James Trotter and Jean A. Hamilton *via* X-ray analysis. [108] Biotin possesses three stereogenic centres, so that eight diastereomers are conceivable. However, only the (3a*S*,4*S*,6a*R*)-enantiomer shows full biological activity (Fig. 7.46).

7.3.1 Demand and Occurrence

The normal recommended intake for biotin is age-dependent: Infants require 10–20 µg/day, children 25–30 µg/day and adolescents and adults 30–100 µg/day. Biotin deficiency is rare, but can lead to seborrhoea (excessive secretion of sebum from the sebaceous glands in the skin), dermatitis, loss of appetite, muscular pain, lethargy, and nervous disturbances (Tab. 7.3). [109]

A deficiency of biotin in humans may result from an unbalanced diet, alcohol abuse, side-effects of medication (*e.g.* with sulfonamides, antiepileptics), or genetic defects. In babies, this deficiency may appear after prolonged breastfeeding (> 4 months), as the biotin content of the mother's milk decreases. There has

Tab. 7.3 *Biotin content of selected foods*

Foods	Biotin Content (µg/g)
Chicken liver, cooked	1872
Beef liver, cooked	416
Egg, yolk, cooked	272
Yeast	202
Peanuts, roasted, salted	175
Salmon, pink, canned in water	59
Pork chop, cooked	45
American cheese	31

also been discussed a connection between the sudden infant death syndrome (SIDS, sometimes also referred to as "cot death" or "crib death") and biotin deficiency.

In general, the amount of biotin consumed with food is sufficient to meet the recommended daily intake (RDI). While unchanged biotin is excreted in humans primarily through the renal system, biliary excretion plays only a minor role. Surprisingly large amounts are found however in the faeces, which originate from colonic bacteria. Thereby, sewage sludge contains 50–70 µg of biotin per 100 g of dry matter – a source of biotin, which of course can not be readily used.

The fact that biotin deficiency is a rare phenomenon is owed to a genuine symbiosis between humans and their intestinal flora. These microorganisms – like a variety of bacteria, most yeast, lower fungi and a few phytoplankton species – excrete biotin into the surrounding medium, from where reabsorption can occur by a specific transport mechanism, and also by a normal diffusion process. Although plants can take up biotin through their roots, many are as well able to synthesise biotin, employing pathways and transport mechanisms, which are still under active investigation (Fig. 7.47).

7.47 *Among a variety of plant species studied, cultured green cells of lavender (Lavandula angustifolia, syn. Lavandula officinalis, Lavandula vera) were found to contain the greatest amount of free biotin.*

For animal feed, biotin is also of high importance. [110] Whereas ruminants have normally a sufficient supply of biotin provided by their fodder and by the amounts of biotin synthesised in the gastrointestinal tract, deficiencies occur more often in pigs, especially in piglets. Poultry tends to underutilise the biotin in their feed, and their enteral biotin synthesis is poor. Turkeys have an especially high demand for biotin. The use of sulfonamides and other antibiotics in animal husbandry affects the intestinal flora and may necessitate biotin-fortified feed (Tab. 7.4).

Tab. 7.4 *Symptoms associated with biotin-deficiency*

Animals	Symptoms
poultry	• poor feather formation • dermatitis (toes, legs, beak) • slow growth • swelling of the eyelids • perosis (deformed ankle joint) • lower rates of hatching • leg weakness (turkeys)
pigs	• inflammation of the hooves • skin ulceration • diarrhoea • eye inflammation • changes in the oral mucosa • poor fertility
furry animals	• grey hair • hair loss • tail-biting
fish	• poor growth • blue-slime disease
cats, dogs	• coat lacking gloss • hair loss • eczema • hyperkeratosis
horses	• damaged horn in the hooves

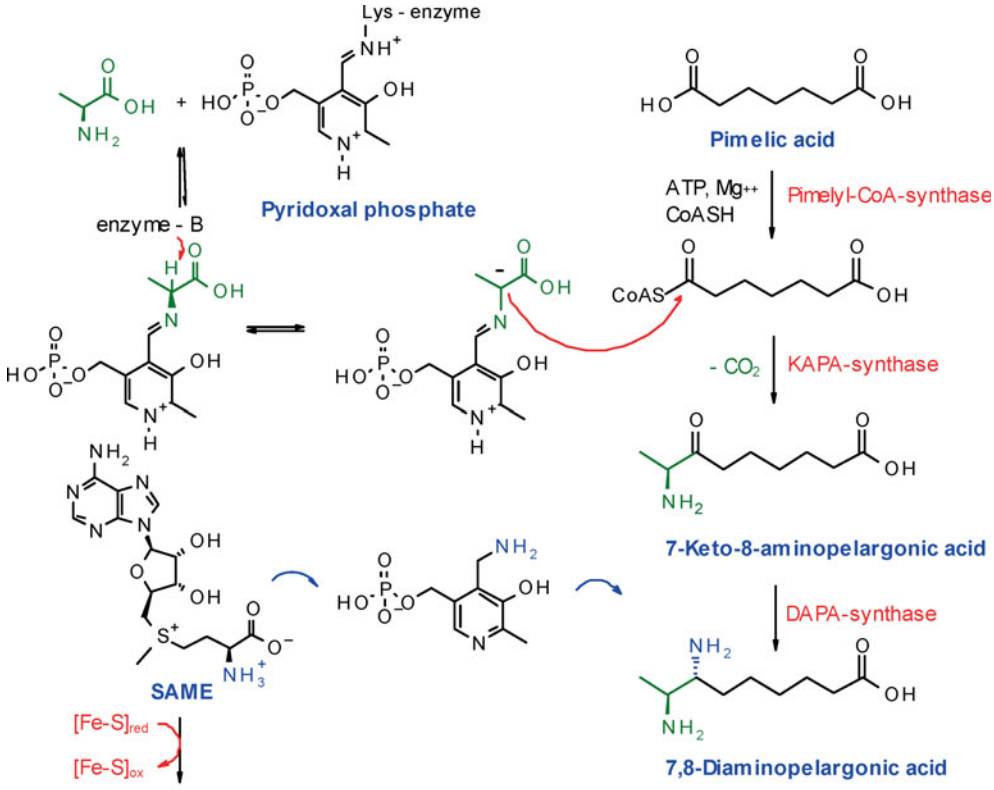
The growth of many microorganisms used in biotechnology, such as *Corynebacterium glutamicum*, is biotin-dependent, so that supplementation of the fermentation broth with biotin is advantageous.

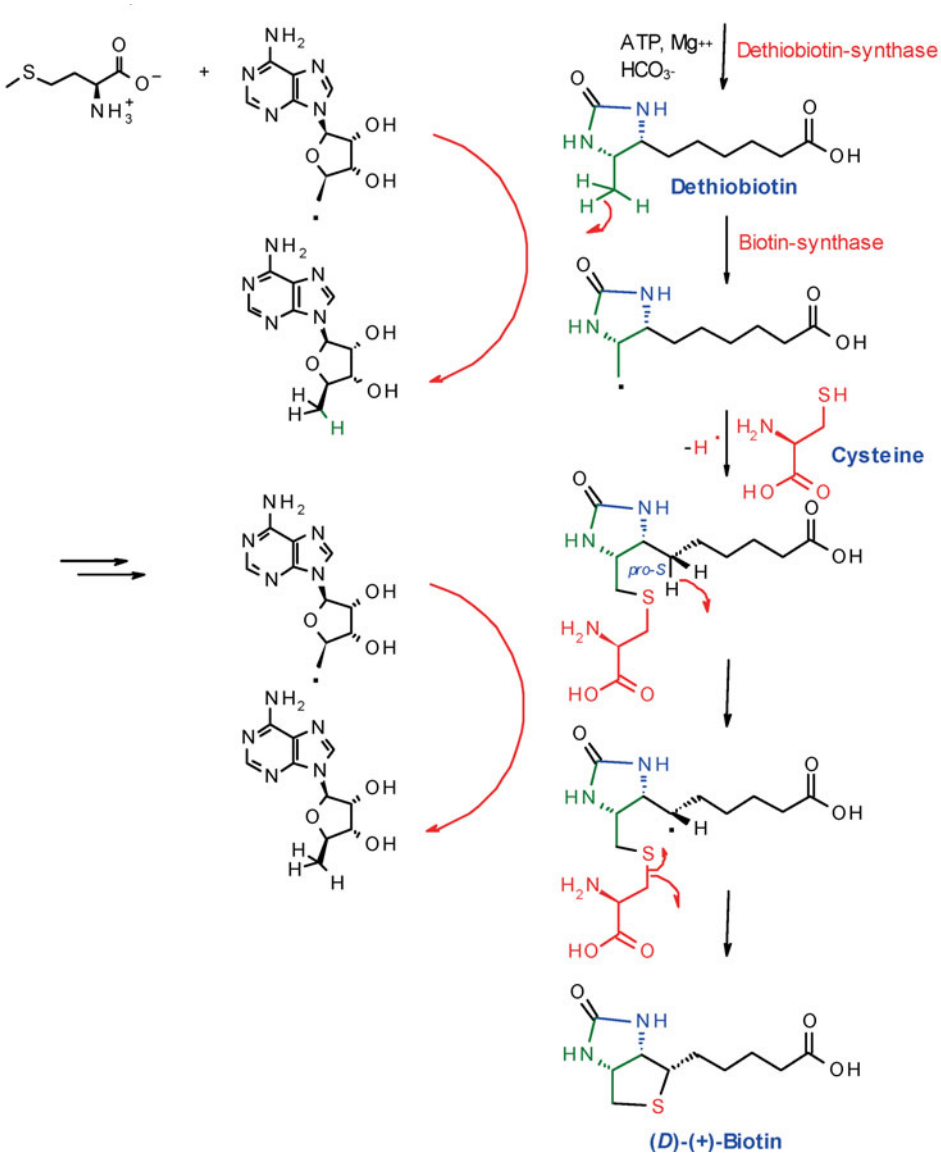
7.3.2 Antagonists

Many foodstuffs contain a metabolic intermediate of biotin, biocytin (*ε-N*-biotinyllysine), which is cleaved in the intestinal tract by the enzyme biotinidase. Only free biotin can be resorbed in the proximal small intestine, a process, which can be blocked by avidin, a glycoprotein with a molar mass of *ca.* 70,000. Avidin occurs in greater amounts in egg-white, and forms with biotin an extraordinarily stable molecular complex (dissociation constant at 25 °C: $K = 10^{-15}$ M), which can be cleaved neither by acids nor by peptidases. Only irradiation or longer exposure to heat leads to denaturation of avidin and thereby the release of biotin. This is another reason why a breakfast egg ought to be cooked for at least 4½ minutes. In this way avidin is denatured and loses its harmful effect. Similarly stable complexes are formed by biotin with neutravidin (de-glycosylated avidin), streptavidin and stravidin from certain *Streptomyces* and *Saccharomyces* species respectively.

7.3.3 Biosynthesis

The biosynthesis of biotin was investigated in a range of microorganisms, including also *Escherichia coli* and *Aspergillus niger*. The starting material is pimelic acid, which is in an initial step activated by conversion into its Coenzyme A thioester. *E. coli* synthesises pimelic acid by a modified fatty acid synthetic pathway. In a pyridoxal phosphate-dependent reaction, which formally resembles the Dakin-West reaction, alanine is condensed with pimelic acid, with loss of carbon dioxide. [111] The ammonia for the reductive amination comes from *S*-adenosylmethionine, and is transferred with the aid of pyridoxal phosphate. The imidazolidinone is formed with bicarbonate in presence of ATP and magnesium salts. Introduction of the sulfur into the dethiobiotin succeeds by a most unusual and fascinating reaction, in which *S*-adenosylmethionine also participates. This is reductively cleaved by an iron-sulfur cluster, which is coupled to NADPH and flavodoxin, resulting in the formation of methionine. The adenosyl radical abstracts a hydrogen atom from dethiobiotin, so that stoichiometric amounts of deoxyadenosine are found. The source of the sulfur for biotin is probably cysteine. The tetrahydrothiophene ring is closed by the enantioselective abstraction of the (4-pro-*S*)-hydrogen atom, with retention of the absolute configuration. For the ring-closure, two equivalents of *S*-adenosylmethionine are needed overall. [108, 112–114]





Up to the present time, fermentation is not an option for industrial manufacturing, because apart from the formation of CoA-pimelic acid, biotin very efficiently inhibits every step of the biosynthesis through a negative feedback. Hitherto only low biotin titres can be attained in the fermentation broths, so that the procedures remain uneconomic on a preparative scale (Tab. 7.5). [108, 112] To rival the established chemical synthesis, significantly higher concentrations than 1,000 mg/l would be required.

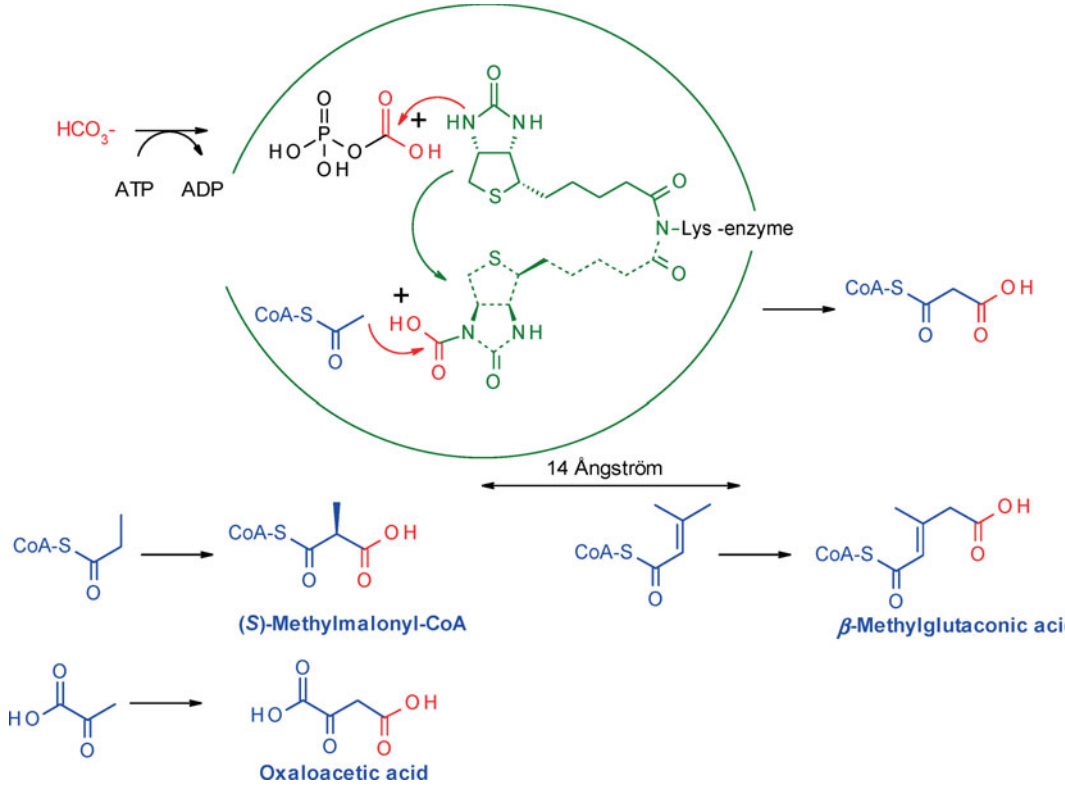
Tab. 7.5 *Biotin titres in the fermentation broth of various microorganisms*

Microorganism	Titre (mg/l)
Serratia marcescens	600
Bacillus sphaericus	365
Rhizopus delemar	0.6
Brevibacterium flavum	0.5

7.3.4 Biological Function

The biological function of biotin is that of an essential prosthetic group, sometimes referred to as a cofactor, which is required to enable the enzymatic activity, in this case for carboxylases. Biotin plays a critical role in the biosynthesis of sugars and fatty acids.

The first biotin-dependent enzyme to be discovered was acetyl-CoA-carboxylase, which forms malonic acid and is necessary for the synthesis of fatty acids (*cf.* section 8.5.3). It is assumed that biotin is bonded to a 14-Ångström-long lysine linker, so that it can swing back and forth between two active centres in the enzyme complex. In the first centre, biotin is carboxylated under the consumption of ATP; in the second, the carboxylic acid function is transferred. [115, 116]



In an analogous manner, a carboxyl group may also be transferred to propionyl-CoA (for the biosynthesis of branched or odd-numbered fatty acids, isoleucine synthesis, or cholesterol metabolism) or to 3-methylcrotonyl-CoA (a degradation product of leucine; after addition of water, there results hydroxymethylglutaryl-CoA, the precursor of mevalonic acid; *cf.* section 7.1.2). [117] Oxaloacetic acid is derived from pyruvate, which is of central importance for gluconeogenesis. [108] In addition, biotin participates also in the transfer of carboxylic acid functions. In prokaryotes, biotin functions as a cofactor for decarboxylases (Tab. 7.6).

Tab. 7.6 *Biotin-dependent enzymes*

Enzyme classes	Enzyme
Carboxylases	Acetyl-CoA-carboxylase
	Propionyl-CoA-carboxylase
	β-Methylcrotonyl-CoA-carboxylase
	Pyruvate-carboxylase
	Geranyl-CoA-carboxylase
	Aminocarboxylase
Transcarboxylases	Methylmalonyl-CoA: pyruvate-carboxyltransferase
Decarboxylases	Methylmalonyl-CoA-decarboxylase
	Oxaloacetate decarboxylase

7.3.5 Chemical Syntheses

Since its first synthesis, 70 years ago, biotin has repeatedly attracted the interest of research groups as a target molecule, and this led to a fairly large number of total syntheses. [118–120] These have been summarised in excellent reviews by Pierre J. De Clercq and Masahiko Seki (Tab. 7.7). [121]

Apart from the first synthesis, the following sections discuss selected examples of syntheses conducted on an industrial scale, of synthesis optimisation, and the probably most elegant synthesis of (+)-biotin, developed by Eike Poetsch and Michael Casutt.

First Chemical Synthesis

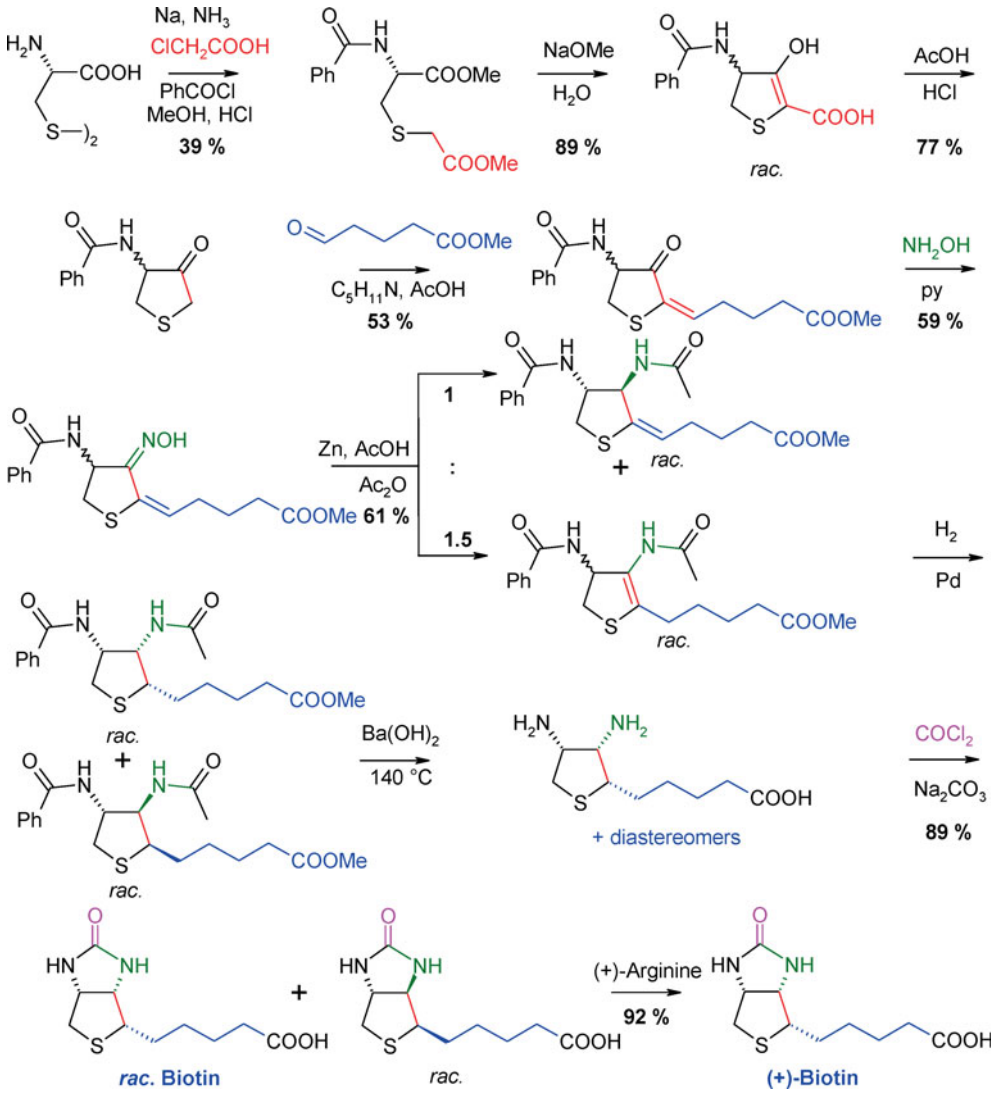
In 1943, Stanton A. Harris (1902–1992) of the Merck Research Laboratory in Rahway, New Jersey, reported the first stereo-unselective synthesis of biotin. He separated the enantiomers with (–)-mandelic acid, and determined the optical rotation and melting point. [122] In the following years, the full scope of the synthesis was outlined in a total of six publications. The starting material was cystine, which was first reduced and suitably derivatised. The conditions of the Dieckmann cyclisation, which created the thiophene ring, led to racemisation of the product. After decarboxylation, the side-chain was introduced and the oxime formed. A two-stage reduction led to a mixture of diastereomers, which

Tab. 7.7 *Biotin syntheses*

	Author/company	Starting material	Number of stages	Product	Comments
1943–45	Harris/Merck USA	(*L*)-Cystine	11	*rac*-Biotin	first synthesis
1945	Grüssner/HLR	5-Methoxyhexanoic acid	13	*rac*-Biotin	
1945–50	Cheney/Parke-Davis	Pimelic acid	14	*rac*-Biotin	
1947	Baker/Lederle	Pimelic acid	17	*rac*-Biotin	
1949	Sternbach/HLR	Fumaric acid	15	(+)-Biotin	industrial synthesis
1962	Nishimura	Thiophene	12	*rac*-Biotin	
1965	Fabrichnyi	Thiophene	12	*rac*-Biotin	
1968–73	Isaka/Zavyalov	Methylimidazolone	7	*rac*-Biotin	
1970	Gerecke/HLR	Fumaric acid	11	(+)-Biotin	industrial synthesis
1975	Marquet	Fumaric acid	12	*rac*-Biotin	
1975	Ohrui	(*D*)-Mannose	16	(+)-Biotin	
1975	Confalone/HLR	(*L*)-Cystine	19	(+)-Biotin	
1976	Confalone/HLR	Pimelic acid	11	*rac*-Biotin	
1977	Ogawa	(*D*)-Glucose	23	(+)-Biotin	longest synthesis (!)
1977	Confalone/HLR	Pimelic acid	13	*rac*-Biotin	
1977	Marx/Syntex	Methyl hexanoate	8	*rac*-Biotin	
1978	Sueda	(*D*)-Glucosamine	14	(+)-Biotin	
1978	Field/HLR	Methyl hexanoate	12	(+)-Biotin	
1980	Vogel	(*D*)-Arabinose	15	(+)-Biotin	
1980	Confalone/HLR	Cycloheptene	11	*rac*-Biotin	
1980	Hohenlohe-Oehringen	2*H*-Chromene	11	*rac*-Biotin	
1981	Rossy/BASF	Methyl chloroacetate	10	*rac*-Biotin	
1982	Schmidt	(*D*)-Arabinose	11	(+)-Biotin	
1982	Baggiolini/HLR	(*L*)-Cystine	11	(+)-Biotin	
1983	Whitney	Dimethylimidazolinone	11	*rac*-Biotin	
1983	Volkmann/Pfizer	Ethyl 7-oxoheptanoate	7	(+)-Biotin	shortest synthesis
1984	Ravindranathan	(*D*)-Glucose	19	(+)-Biotin	
1987	Poetsch/E. Merck	(*L*)-Cysteine	9	(+)-Biotin	industrial synthesis
1988	McGarrity/Lonza	Tetronic acid	12	(+)-Biotin	
1988	Corey	(*L*)-Cystine	13	(+)-Biotin	
1990	Moran	*N*-Phenylglutarimide	11	*rac*-Biotin	
1991	Poetsch/Merck	(*L*)-Cystine	13	(+)-Biotin	
1993	Ravindranathan	(*L*)-Cysteine	12	(+)-Biotin	
1993	De Clercq	(*L*)-Cysteine	12	(+)-Biotin	
1994	De Clercq	(*L*)-Cysteine	14	(+)-Biotin	
2000	Chen	Ethyl acetacetate	10	(+)-Biotin	
2001	Ravindranathan	(*L*)-Cystine	11	(+)-Biotin	
2003	Seki/Tanabe	(*L*)-Cysteine	10	(+)-Biotin	

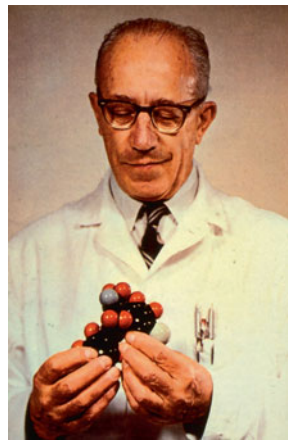
(HLR = Hoffmann-La Roche)

was partly separable. After deprotection with barium hydroxide, the imidazolidinone ring was closed using phosgene, and separation of the enantiomers with (+)-arginine gave optically pure (+)-biotin. [123]



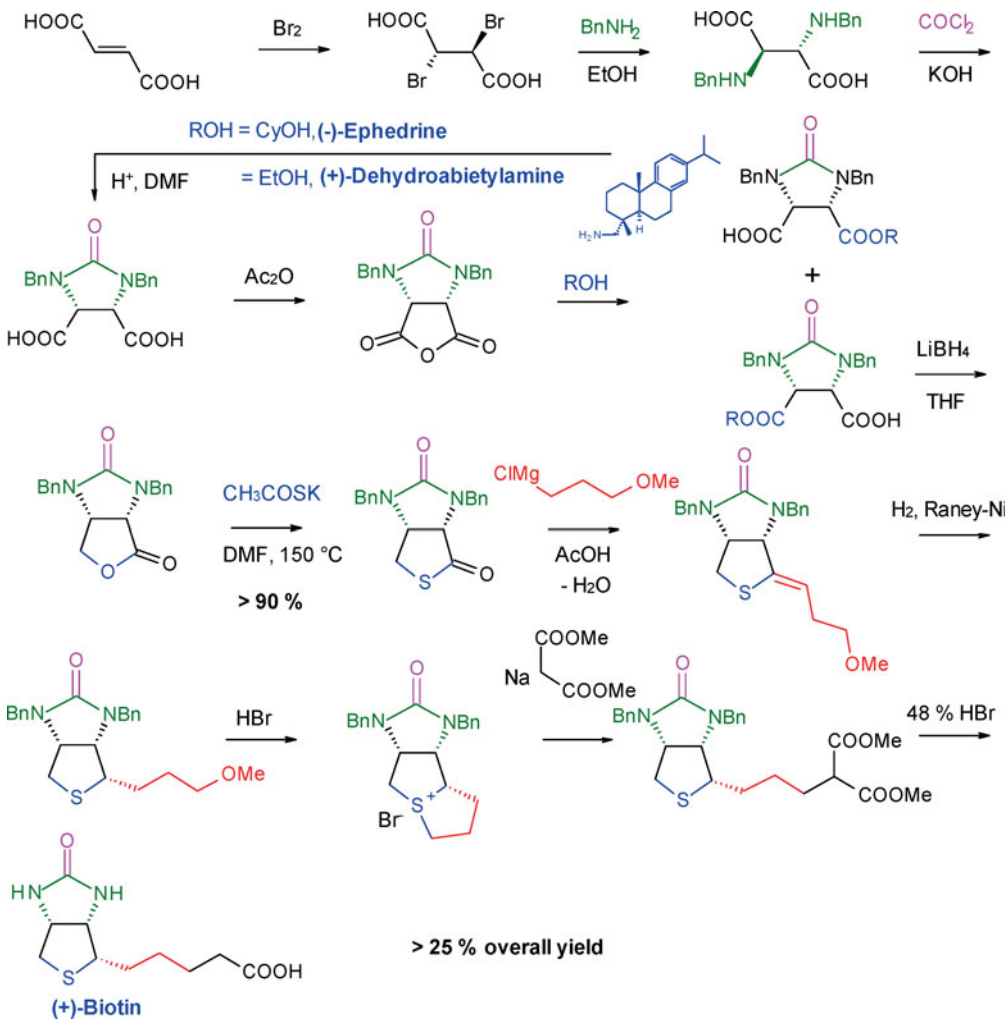
First Industrial Synthesis (Hoffmann-La Roche)

In 1946, Moses W. Goldberg (1905–1964) and Leo H. Sternbach from Hoffmann-La Roche filed the first of several patent applications relating to the synthesis of biotin (Fig. 7.48). The starting material was fumaric acid, which was *trans*-dibrominated. Nucleophilic substitution with benzylamine, cyclisation with phosgene and treatment with acetic anhydride gave the *cis*-substituted *meso*-imidazolidinone, which was converted with cyclohexanol into the half-ester. The enantiomers were then separated with (–)-ephedrine. Alternatively,



7.48 *Leo Henryk Sternbach (1908–2005) was a highly gifted chemist, who did not only develop the first technical process of biotin, but is also credited with the discovery of several well-known drugs (e.g. chlordiazepoxide (Librium®), diazepam (Valium®), flurazepam (Dalmane®), nitrazepam (Mogadon®), flunitrazepam (Rohypnol®), clonazepam (Klonopin®), and trimethaphan (Arfonad®)).*

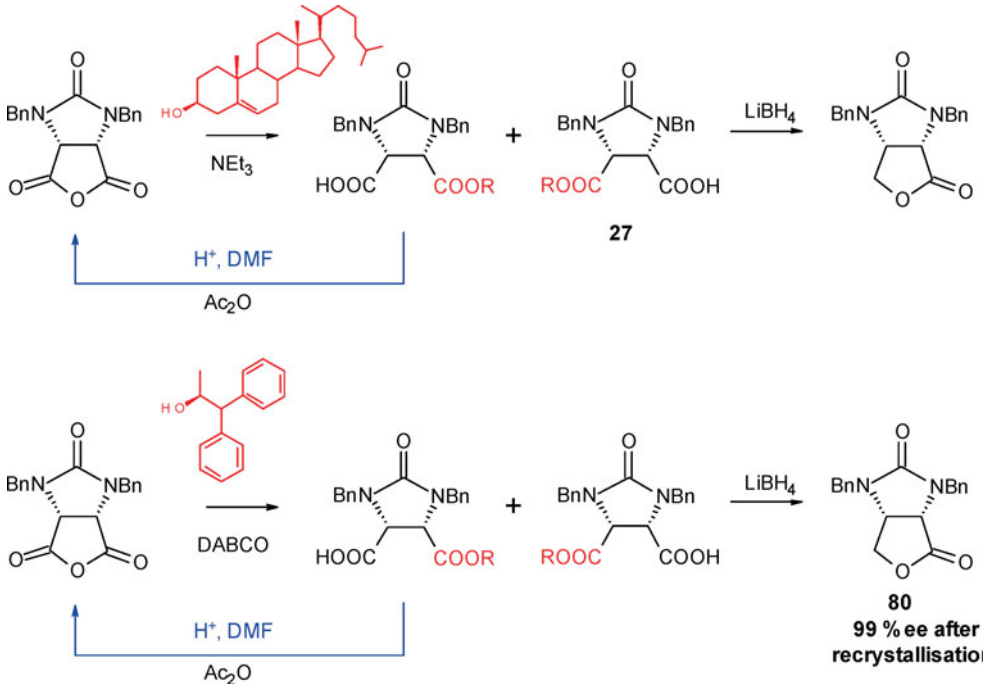
the corresponding ethyl half-ester may be separated with (+)-dehydroabietylamine. The undesired isomer was hydrolysed and the *meso*-compound recycled. The target isomer, however, was reduced with lithium borohydride and cyclised to the lactone. A crucial step on the route to (+)-biotin was the conversion of the lactone into the thiolactone with potassium thioacetate in DMF at 150 °C, which was published by Max Gerecke in 1970. The major portion of the side-chain was introduced by a Grignard reaction, followed by dehydration, to give the exocyclic double bond with (*Z*)-configuration. [124] A stereospecific hydrogenation



lead to the all-*cis* product, which was then treated with HBr to give a tetrahydrothiophenium salt, and the latter was reacted further with sodium dimethyl malonate. Alternatively, a masked carboxylic acid function could be introduced by the action of cyanide on the homotetrahydrothiophenium salt. Hydrolysis of the esters, and decarboxylation, as well as cleavage of the benzyl protecting groups was achieved by heating with 48 % aqueous HBr. The combined yield over all steps was > 25 %. [108, 113, 121]

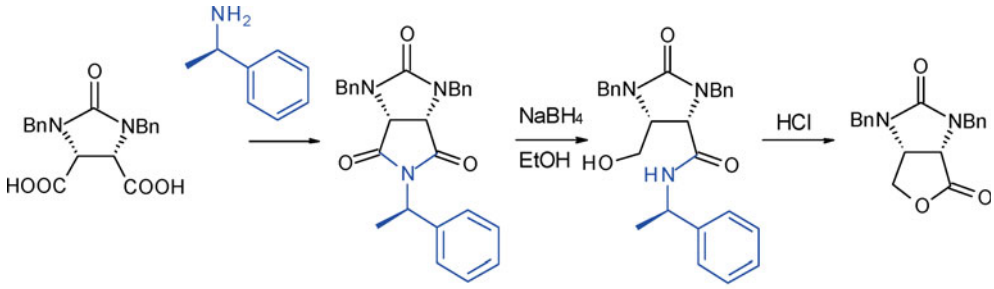
The most significant disadvantages of the Sternbach-Goldberg synthesis lie in the separation and partial recycling requirement of the enantiomers at the half-ester stage, and in a series of less than efficient reaction steps. First, a lactone is synthesised, only to convert it then into a thiolactone. The side-chain is built up in two segments, requiring a subsequent degradation cascade. Thus, the ether has to be cleaved, the diester hydrolysed and the diacid then decarboxylated. All of these procedures render the overall synthesis labourious and affect the space-time yield.

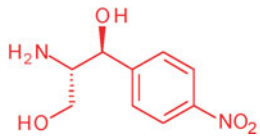
Over the years, different firms and academic research groups worked out considerable improvements or suitable variations based on the Sternbach synthesis of (+)-biotin. Thus, Max Gerecke found that the *meso*-anhydride could be converted into its cholesteryl half-ester and the enantiomers separated as a triethylammonium salt. In case cholesterol was replaced by (*S*)-1,1-diphenylisopropanol, the lactone was obtained after a single recrystallisation with an enantiomeric excess of 99 %.



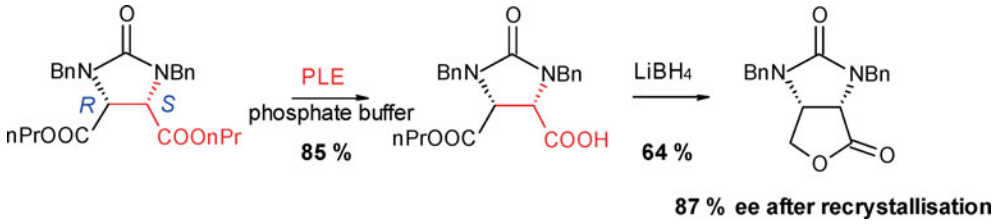
In 1975, chemists from Sumitomo described the formation of the imide from phenylethylamine and its enantioselective reduction with sodium borohydride.

If phenylethylamine is replaced by an analogous precursor of chloramphenicol, the yield of diastereomerically and enantiomerically pure product can be increased to 60–65 %.

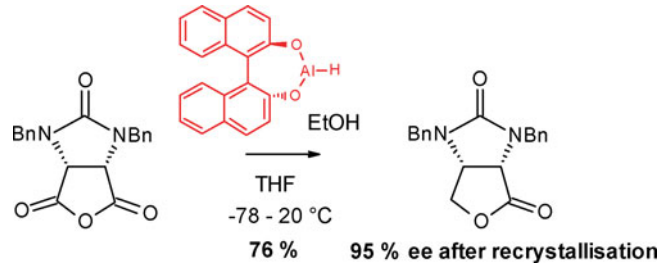




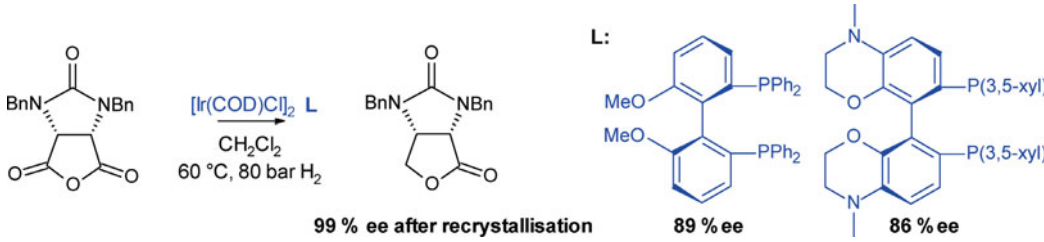
The *meso*-dipropyl ester may be hydrolysed enantioselectively with pig liver esterase, which selectively cleaves the (*S*)-configured ester.



In 1993, Kenji Matsuki reported an enantioselective reduction of the anhydride with Noyori's binol-aluminium hydride-ethanol complex. [125]

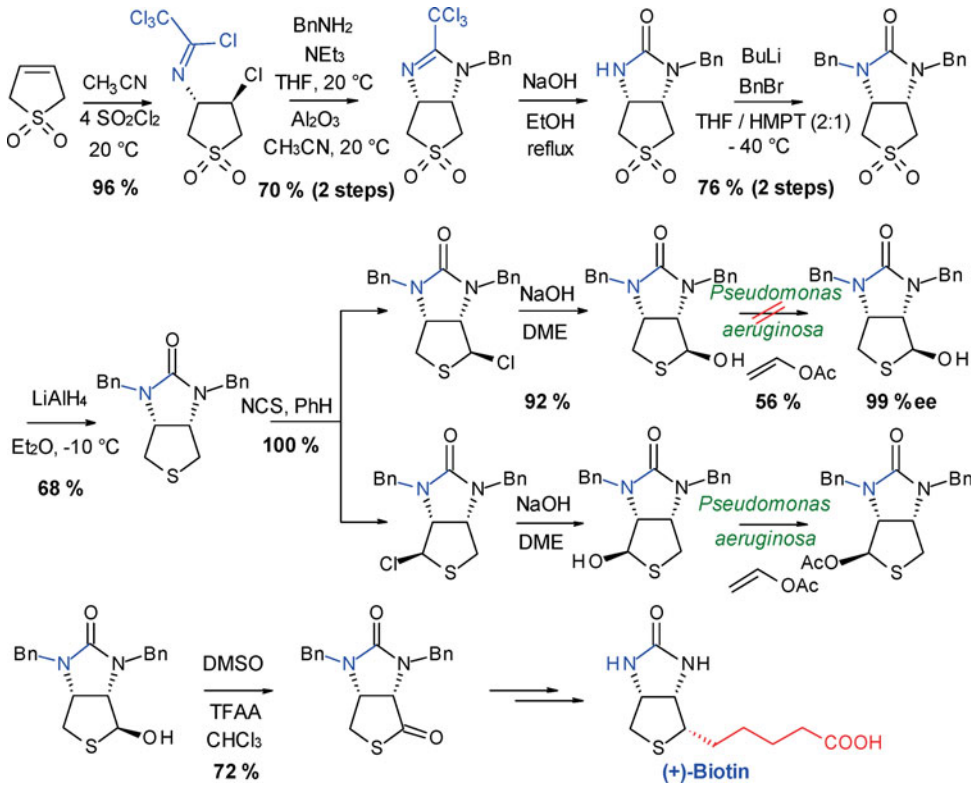


Finally, Werner Bonrath, Reinhard Karge and Felix Roessler from DSM succeeded in reducing the anhydride by enantioselective catalytic hydrogenation. An initial catalyst screening boosted the enantioselectivity up to 90 % ee; further optimisation of the reaction conditions and the substrate / catalyst ratio to 5,000 : 1 lead to more than 95 % ee, and recrystallisation enhanced the optical purity even further to more than 99 % ee. [126]



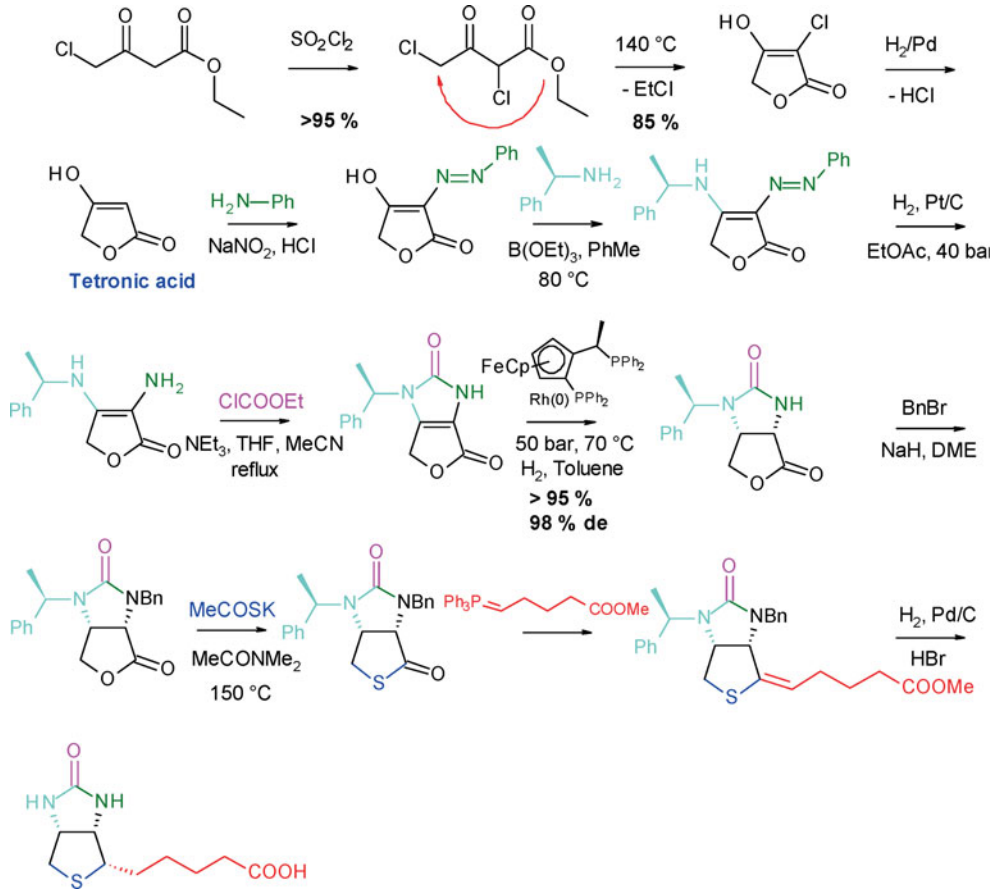
Marquet's Optimised Synthesis

A route conceptually independent of the Roche synthesis was developed by Andrée Marquet and Hideki Kinoshita. [127] The synthetic strategy is attractive, because it starts from inexpensive materials and the target compound is reached by a relatively linear synthesis. The starting material is sulfolene, which is treated with four equivalents of sulfuryl chloride in acetonitrile. This gives an imidoyl chloride, which is cyclised with benzylamine and heated with aqueous sodium hydroxide to obtain the urea. After protection of the second nitrogen, the sulfone group is reduced and the tetrahydrothiophene functionalised with a hydroxy-group *via* a Pummerer chlorination. Chemists from Takeda succeeded in separating the alcohol isomers by an enzymatic esterification. There is a disadvantage that the undesired diastereomer cannot be easily recycled. Oxidation with DMSO/trifluoroacetic anhydride finally provides a building block for the Sternbach synthesis.



Industrial Synthesis (Lonza)

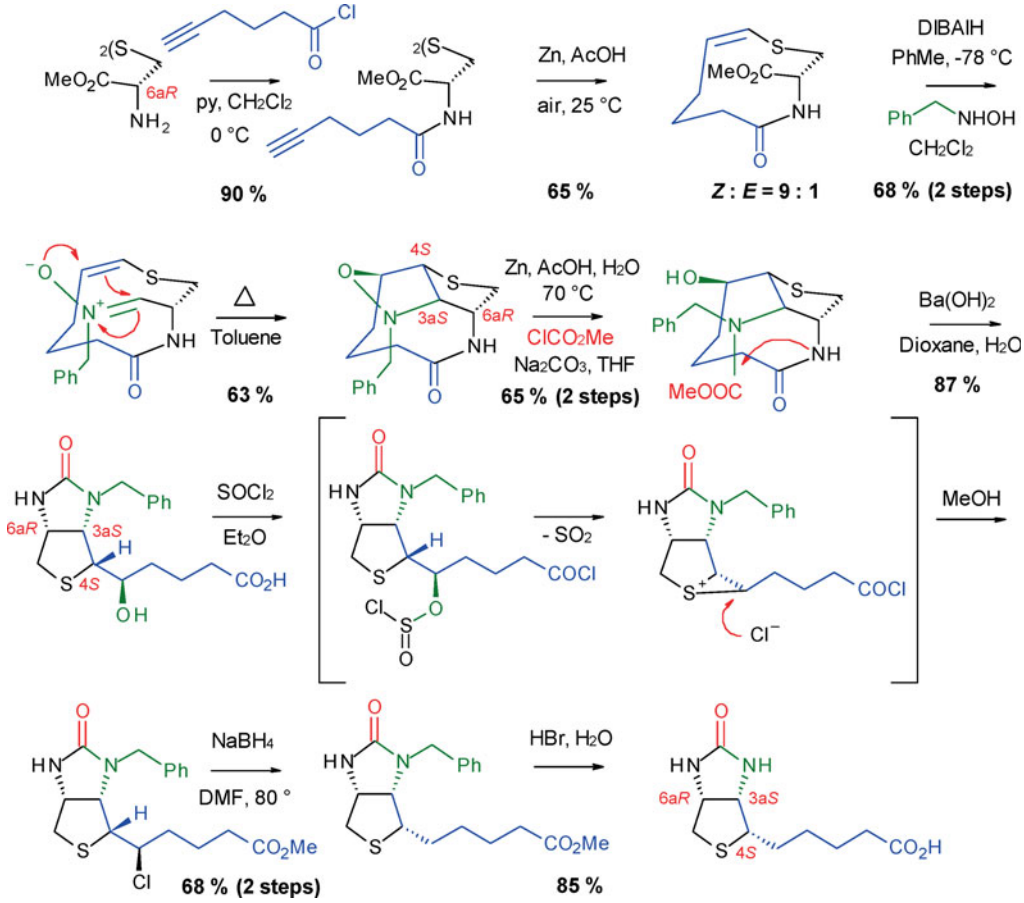
The Lonza company developed a synthesis route, which starts from tetronic acid. [128] For the introduction of the first nitrogen, this compound is reacted with the diazonium salt from aniline. The keto-group is converted into the enamine with (*R*)-phenylethylamine. After reductive cleavage of the diazo-compound, the furoimidazolinone ring is closed with ethyl chloroformate. The enantioselective hydrogenation with a ferrocenylphosphane-rhodium complex is remarkable. The unprotected nitrogen is benzylated and the sulfur introduced by Gerecke's method. The Wittig reaction shortens the construction of the side-chain considerably. Hydrogenation of the exocyclic double bond may be carried out with a palladium catalyst. Finally, as in the Roche process, the protecting groups are cleaved with HBr.



Diastereoselective Synthesis (Hoffmann-La Roche)

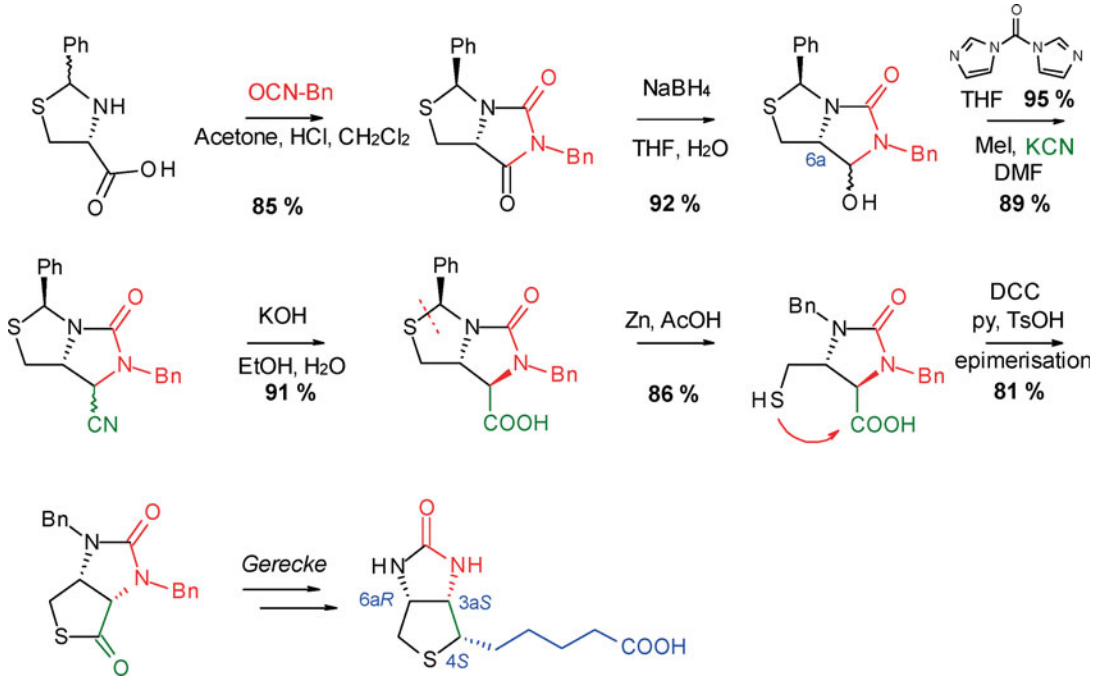
In 1982, chemists from Hoffmann-La Roche published an elegant synthesis of biotin, in which, starting from cystine, the tetrahydrothiophene ring is built up by an intramolecular [3 + 2]-cycloaddition of a nitrone. [129] The cyclisation occurs *via* a ten-membered ring system and generates two new stereogenic centres.

The actual starting material is cystine dimethyl ester, which provides an elegant protection for the sulfur function. After reaction with hex-5-ynoyl chloride, reduction of the disulfide with zinc generates a thiol, which in the presence of air adds spontaneously to the alkyne. The (*E*/*Z*)-selectivity, critical for the subsequent cycloaddition, is 1 : 9. After removal of the (*E*)-isomer, the ester is reduced to an aldehyde, and converted into the nitrone with benzylhydroxylamine. By heating in toluene under reflux, the stereospecific cycloaddition takes place. The isoxazolidine ring is easily opened by zinc dust reduction. The cyclic urea is then formed using methyl chloroformate. X-ray analysis showed that the "superfluous" hydroxy-group in the side-chain is, as a first step, amenable to conversion into the corresponding chloride with net retention of configuration. The reaction is probably facilitated by anchimeric assistance from the adjacent sulfur. The chloride can then be removed by reduction with sodium borohydride. Biotin is finally obtained after cleavage of the protecting groups with aqueous HBr. [121, 130]

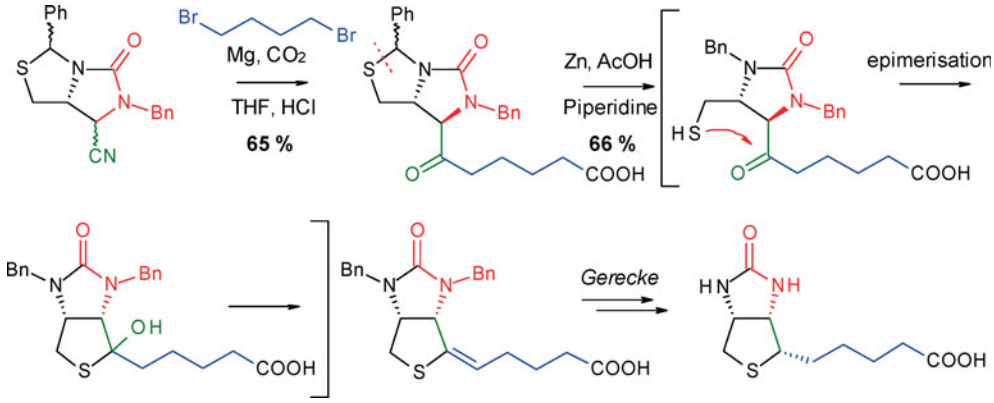


Diastereoselective Synthesis (E. Merck)

One of the shortest synthetic routes to enantiomerically pure (+)-biotin came from Eike Poetsch and Michael Casutt at the E. Merck company in Darmstadt. [131] Reaction of the benzaldehyde-protected cysteine with benzyl isocyanate leads to a hydantoin, which is a surprisingly uniform compound, according to its ^{1}H NMR spectrum. Reduction with sodium borohydride gives a hemiaminal, which is configurationally stable at the 6a-carbon atom. D_2O-exchange experiments prove that the ring-opened aminoaldehyde, which might epimerise by keto-enol tautomerisation, is also not present at equilibrium. For the introduction of cyanide, the reaction with carbonyldiimidazole produces the alkoxycarbonylimidazole, which, after *N*-methylation at the imidazole system, can be readily substituted with cyanide. Hydrolysis leads to the enantiomerically pure carboxylic acid. After reductive ring-opening of the thiazolidine, the carboxylic acid is epimerised upon activation with dicyclohexylcarbodiimide, and cyclises to the thiolactone. From this stage onward, enantiomerically pure (+)-biotin is obtained along the Hoffmann-La Roche process.



Even more attractive is however to react the nitrile with 1,4-dibromobutane and carbon dioxide in a double Grignard reaction. The thiazolidine ring is in this case as well opened reductively to a ketone intermediate, which is epimerised and spontaneously forms the benzyl-protected dehydrobiotin. Hydrogenation and deprotection have already been discussed for Gerecke's route.



7.3.6 Economic Aspects

Around 100 tonnes of biotin are manufactured annually. [126] Animal feed accounts for the largest proportion of the market, followed by food-supplements (multivitamin tablets, dragees, capsules or syrup), and pharmaceutical preparations (5–10 %). A small fraction is used for biochemical and analytical purposes (immunoassays), and for cosmetic applications.

Important manufacturers, apart from an increasing number of Chinese companies, are DSM, Tanabe and Sumitomo. On the world market, the price for pure (+)-biotin ranges around 500 to 600 US-dollars per kilogram.

Summary in Bullet Points

- Biotin assumes a prominent role as supplemental ingredient in food and in animal feed.
- The biological function of biotin is to regulate important metabolic pathways and enable critical enzyme functions.
- Production of biotin *via* fermentation has not reached industrial maturity, because almost every step of the biosynthesis is hampered by product inhibition.
- The industrial synthesis of Goldberg and Sternbach (Hoffmann-La Roche) was a landmark achievement, which allowed for the first time to produce enantiomerically pure biotin in large quantities.
- Particularly elegant syntheses were developed by Lonza and E. Merck.

References

References to Introduction

1. http://www.oppisworld.de/poesie/philo/entdeck/magel.htm
2. W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 21st Edn. (1969) 268; C. Staiger, *Pharmazie in unserer Zeit* **38** (2009) 112; M. Bartholomew, *Postgrad. Med. J.* **78** (2002) 695.
3. A. Holst, T. Frølich, *J. Hygiene* **7** (1907) 634; K. R. Norum, H. J. Grav, *Tidsskr. Nor. Laegeforen* **122** (2002) 1686.
4. F. Micheel, *Hoppe-Seyler's Z. physiol. Chem*. **215** (1933) 215 and **216** (1933) 233.
5. W. Langenbeck, W. Pritzkow, Lehrbuch der Organischen Chemie, Verlag Theodor Steinkopff, Dresden, 21st Edn. (1969) 419.
6. R. Grewe, *Naturwissenschaften* **24** (1936) 657.
7. R. R. Williams, J. K. Cline. *J. Am. Chem. Soc.* **58** (1936) 1504; H. Andersag, K. Westphal, *Ber. Dtsch. Chem. Ges.* **70** (1937) 2035.
8. J. Henley, Spy theory revives French murder mystery, *The Guardian*, Monday 29 July, 2002; Boots chemist Sir Jack Drummond's death still a mystery, *BBC Nottingham*, 2011; http://en.wikipedia.org/wiki/Jack_Drummond.
9. M. Eggersdorfer, D. Laudert, U. Létinois, T. McClymont, J. Medlock, T. Netscher, W. Bonrath, *Angew. Chem. Int. Ed. Engl.* **51** (2012) 12960.

References to 7.1 Vitamin A and Carotenoids

10. E. Guggolz, *Nachr. Chem. Tech. Lab*. **47** (1999) 1322; http://en.wikipedia.org/wiki/Salmon.
11. H. Nishino, H. Tokuda, Y. Satomi, M. Masuda, P. Bu, M. Onozuka, S. Yamaguchi, Y. Okuda, J. Takayasu, J. Tsuruta, M. Okuda, E. Ichiishi, M. Murakoshi, T. Kato, N. Misawa, T. Narisawa, N. Takasuka, M. Yano, *Pure Appl. Chem.* **71** (1999) 2273; A. V. Rao, L. G. Rao, *Pharm. Res.* **55** (2007) 207.
12. X. E. Zhou, K. Melcher, H. E. Xu, *Acta Pharmacol. Sin*. **33** (2012) 291; M. Lidén and U. Eriksson, *J. Biol. Chem.* **281** (2006) 13001; G. Moiseyev, Y. Chen, Y. Takahashi, B. X. Wu, J.-X. Ma, *Proc. Nat. Acad. Sci.* **102** (2005) 12413; M. Jin, Q. Yuan, S. Li, G. H. Travis, *J. Biol. Chem.* **282** (2007) 20915; D. Kernell, *J. Hist. Neurosci.* **9** (2000) 280.
13. R. Willstätter, W. Mieg, *Liebigs Ann. Chem*. **355** (1907) 1.
14. L. Zechmeister, L. v. Cholnoky, V. Vrabely, *Ber. Dtsch. Chem. Ges.* **61** (1928) 566.
15. P. Karrer, R. Widmer, *Helv. Chim. Acta* **11** (1928) 751.
16. T. Moore, *Biochem. J.* **23** (1929) 803.
17. R. Kuhn, E. Lederer, *Ber. Dtsch. Chem. Ges.* **64** (1931) 1349.
18. P. Karrer, R. Morf, *Helv. Chim. Acta* **14** (1931) 833.
19. P. Karrer, R. Morf, K. Schöpp, *Helv. Chim. Acta* **14** (1931) 1040 and 1431.
20. R. Kuhn, C. J. O. R. Morris, *Ber. Deutsch. Chem. Ges.* **70** (1937) 853; J. F. Arens, D. A. van Dorp, *Nature* **157** (1946) 190.
21. C. D. Robeson, J. G. Baxter *J. Am. Chem. Soc.* **69** (1947) 136.
22. H. H. Inhoffen, F. Buhlmann, K. Bartram, G. Rummert, H. Pommer, *Liebigs Ann. Chem*. **570** (1950) 54.
23. K. R. Farrar, J. C. Hamlet, H. B. Henbest, E. R. H. Jones, *J. Chem. Soc.* **1952**, 2657.
24. P. M. Dewick, *Nat. Prod. Rep*. **16** (1999) 97 (Review).
25. K. Ogura, *Chem. Rev.* **98** (1998) 1263 (Review).
26. M. Rohmer, M. Knani, P. Simonin, B. Sutter, H. Sahm, *Biochem. J*. **295** (1993) 517.
27. W. Knöss, *Pharmazie in unserer Zeit* **28** (1999) 247; V. A. Paseshnichenko, *Biochemistry (Mosc)*. **63** (1998) 139.
28. M. Rohmer, *Nat. Prod. Rep*. **16** (1999) 565.
29. A. Jux, G. Gleixner, W. Boland, *Angew. Chem. Internat. Edn.* **40** (2001) 2091.

30. T. Kuzuyama, M. Takagi, K. Kaneda, T. Dairi, H. Seto, *Tetrahedron Lett.* **41** (2000) 703.
31. T. Kuzuyama, M. Takagi, K. Kaneda, H. Watanabe, T. Dairi, H. Seto, *Tetrahedron Lett.* **41** (2000) 2925.
32. M. Takagi, T. Kuzuyama, K. Kaneda, H. Watanabe, T. Dairi, H. Seto, *Tetrahedron Lett.* **41** (2000) 3395.
33. M. Seemann, B. Tse Sum Bui, M. Wolff, D. Tritsch, N. Campos, A. Boronat, A. Marquet, M. Rohmer, *Angew. Chem. Internat. Edn.* **41** (2002) 4337.
34. W. Brandt, M. A. Dessoy, M. Fulhorst, W. Gao, M. H. Zenk, L. A. Wessjohann, *ChemBioChem* **5** (2004) 311.
35. M. G. Leuenberger, C. Engeloch-Jarret, W.-D. Woggon, *Angew. Chem. Internat. Edn.* **40** (2001) 2613.
36. M. Zeeshan, PhD Thesis, Norwegian University of Science and Technology, Trondheim, 2012.
37. M. Zeeshan, H.-R. Sliwka, V. Partali, A. Martinez, *Org. Lett.* **14** (2012) 5496.
38. F. P. Zscheile, *Arch. Biochem. Biophys.* **113** (1966) 554; H.-D. Martin, S. Kock, R. Scherrers, K. Lutter, T. Wagener, C. Hundsdörfer, S. Frixel, K. Schaper, H. Ernst, W. Schrader, H. Görner, W. Stahl, *Angew. Chem. Int. Ed.* **48** (2009) 237; M. Kelly, S. Norgard, S. Liaaen-Jensen, *Acta Chem. Scand.* **24** (1970) 2169; N. J. C. Fong, M. L. Burgess, K. D. Barrow, D. R. Glenn, *Appl. Microbiol. Biotech.* **56** (2001) 750.
39. M. Korger, PhD Thesis, Heinrich-Heine-Universität Düsseldorf, 2005; C. Kock, PhD Thesis, Heinrich-Heine-Universität Düsseldorf, 2007; F. Kienzle, R. E. Minder, *Helv. Chim. Acta* **61** (1978) 242, S. Liaaen-Jensen, G. Kildahl-Andersen, *ARKIVOC* (2008) 5.
40. Dieter Horn and Jens Rieger, *Angew. Chem. Int. Ed.* **40** (2001) 4330.
41. S. N. Naess, A. Elgsaeter, B. J. Foss, B. Li, H.-R. Sliwka, V. Partali, T. B. Melø, and K. R. Naqvia, *Helv. Chim. Acta* **89** (2006) 45.
42. H. Hosseinzadeh, T. Ziaee, A. Sadeghi, *Phytomedicine* **15** (2008) 491.
43. J. Neugebauer, J. Veldstra, F. Buda, *J. Phys. Chem. B* **115** (2011) 3216; W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graphics* **14.1** (1996) 33; M. Cianci, P. Rizkallah, A. Olczak, J. Raftery, N. Chayen, P. Zagalsky, J. Helliwell, *Proc. Natl. Acad. Sci. USA* **99** (2002) 9795; N. Christensson , K. Zídek, N. C. Magdaong, A. M. Lafountain, H. A. Frank, D. Zigmantas, *J. Phys. Chem. B* (2013) 11209.
44. http://www.bccresearch.com/report/carotenoids-global-market-fod025d.html.
45. H. Lindlar, *Helv. Chim. Acta* **35** (1952) 446; K. Meyer, personal communication, 2005.
46. R. W. Hoffmann, *Angew. Chem. Internat. Edn.* **40** (2001) 1411.
47. G. Wittig, U. Schöllkopf, *Chem. Ber.* **87** (1954) 1318.
48. H. Staudinger, *Ber. Dtsch. Chem. Ges.* **41** (1908) 1355.
49. R. M. Roberts, Serendipity, Wiley, New York, 1989, 213.
50. G. Wittig, M.-H. Wetterling, *Liebigs Ann. Chem.* **557** (1947) 193.
51. G. Wittig, G. Geissler, *Liebigs Ann. Chem.* **580** (1953) 44.
52. H. Pommer, *Angew. Chem. Internat. Edn.* **16** (1977) 423.
53. G. Wittig, H. Pommer, DE 950552 (1956).
54. H. Pommer, G. Wittig, W. Sarnecki, DE 1026745 (1958).
55. J. Paust, in G. Britton, S. Liaanen-Jensen, H. Pfander, Carotenoids, Birkhäuser, 1996, Vol. 2, 258–292; T. Mori, T. Onishi, K. Yamamoto, US 5087762, 1992; H. Ernst, *Pure Appl. Chem.* **74** (2002) 2213.
56. S. Boeck, K. Herzog, R. Fischer, H. Vogel, M. Fischer, DE 4241942 (1992).
57. R. K. Müller, in G. Britton, S. Liaanen-Jensen, H. Pfander, Carotenoids, Birkhäuser, 1996, Vol. 2, 123 (further methods).
58. H. J. Bestmann, O. Kratzer, R. Armsen, *Liebigs Ann. Chem.* **1973**, 760.
59. M. Shi, B. Xu, *J. Org. Chem.* **67** (2002) 294.
60. H. Ernst, *Pure Appl. Chem.* **74** (2002) 2213.

61. R. K. Müller, in G. Britton, S. Liaanen-Jensen, H. Pfander, Carotenoids, Birkhäuser, 1996, Vol. 2, 126.
62. G. H. Knaus, H. Ernst, M. Thyes, J. Paust, US 4937308 (1990).
63. K. Meyer, *Chemie in unserer Zeit* **36** (2002) 178; W. C. Evans, *Biologist (London)* **47** (2000) 181; C. Ulbricht, R. C. Windsor, A. Brigham, J. K. Bryan, J. Conquer, D. Costa, N. Giese, J. Guilford, E. R. Higdon, K. Holmes, R. Isaac, S. Jingst, J. Kats, L. Peery, E. Rusie, A. Savinainen, T. Schoen, T. Stock, S. Tanguay-Colucci, W. Weissner, *J. Diet. Suppl.* **9** (2012) 57.
64. M. Müller-Cunradi, K. Pieroh, US 2165962 (1939) (filed 22.10.1937).
65. R. Entschell, P. Karrer, *Helv. Chim. Acta* **41** (1958) 402; H. Ernst, personal communication, 2011.
66. J. Paust, J. Schneider, H. Jaedicke, DE 2534805 (1975); mechanistic investigations with the example of ionone: L. Changhui, L. Yiming, T. Ruiren, Y. Huawu, *Huaxue Fanying Gongcheng Yu Gongyi* **21** (2005) 437.
67. J. Morel, US 3646149 (1967); J. Morel, US 3790635 (1972).
68. K. Weissermel, H.-J. Arpe, Industrielle Organische Chemie, VCH, Weinheim, 4th Edn., 1994, 303.
69. U. Ostermeyer, T. Schmidt, *Deutsche Lebensmittel-Rundschau* **100** (2004) 437; V. M. Moretti, T. Mentasti, F. Bellagamba, U. Luzzana, F. Caprino, G. M. Turchini, I. Giani, F. Valfrè, *Food Additives and Contaminants* **23** (2006) 1056.
70. B. Wüstenberg, R. T. Stemmler, U. Létinois, W. Bonrath, M. Hugentobler, T. Netscher, *Chimia* **65** (2011) 420; A. Rüttimann, *Pure Appl. Chem.* **71** (1999) 2285; I. N. Nazarov, Zh. A. Krasnaia, *J. Gen. Chem. USSR* **28** (1958) 2477; S. M. Makin, I. N. Rozhov, *J. Gen. Chem. USSR* **31** (1961) 3096.
71. J. Buddrus, *Angew. Chem. Internat. Edn.* **7** (1968) 536; J. Buddrus, *Chem. Ber.* **107** (1974) 2050.
72. M. Julia, J.-M. Paris, *Tetrahedron Lett.* **49** (1973) 4833; J. Otera, in G. Britton, S. Liaanen-Jensen, H. Pfander, Carotenoids, Birkhäuser, 1996, Vol. 2, 103.
73. J. March, Advanced Organic Chemistry, Wiley, New York, 4th Edn., 1992, 410.
74. M. Julia, D. Arnould, *Bull. Soc. Chim. Fr.* (1973) 746.
75. D. Arnould, P. Chabardes, G. Farge, M. Julia, *Bull. Soc. Chim. Fr.* (1985) 130.
76. P. Chabardes, J. P. Decor, J. Varagnat, *Tetrahedron* **33** (1977) 2799.
77. J. H. Babler, W. J. Buttner, *Tetrahedron Lett.* **4** (1976) 239.
78. A. Fischli, H. Mayer, *Helv. Chim. Acta* **58** (1975) 1584.
79. M. Julia, H. Lauron, J.-P. Stacino, J.-N.Verpeaux, *Tetrahedron* **42** (1986) 2475.
80. K. Bernhard, H. Mayer, *Pure Appl. Chem.* **63** (1991) 35.
81. J. Otera, H. Misawa, T. Onishi, S. Suzuki, Y. Fujita, *J. Org. Chem.* **51** (1986) 3834.
82. http://www.vitatene.com/Pdf/BLAKESLEA%20TRISPORA.pdf.
83. P. L. Sharpe, R. W. Ye, Q. Q. Zhu,WO/2008/073367 (2008); R. Bailey, K. T. Madden, J. Trueheart, US Patent 2011/0021843 (2011).
84. http://en.wikipedia.org/wiki/Haematococcus_pluvialis; M. Olaizola, *J. Appl. Phycology* **12** (2000) 499; Alga Technologies: http://www.algatech.com/; http://www.cyanotech.com/pdfs/bioastin/axbul60.pdf.
85. http://en.wikipedia.org/wiki/Dunaliella_salina; A. Oren, Saline Systems (published online in 2005).
86. X. Ye, S. Al-Babili, A. Klöti , J. Zhang, P. Lucca, P. Beyer, I. Potrykus, *Science* **287** (2000) 5451.
87. WHO. Global prevalence of Vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. Geneva, World Health Organisation, 2009.

References to 7.2 Vitamin D

88. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1st Edn., 1994, 1163; C. Staiger, *Pharmazie in unserer Zeit* **38** (2009) 112; R. W. Chesney, *Nutrients* **4** (2012) 42; J. Ekpe, *J. Med. Biogr.* **17** (2009) 155.
89. Kirk-Othmer, Encyclopedia of Chemical Technology, 4th Edn., Vol. 25, Wiley, New York, 1998, 217.
90. Ullmann's Encyclopedia of Industrial Chemistry, Electronic Release, 6th Edn. (1999) 469; P. Nuhn, *Pharmazie in unserer Zeit* **38** (2009) 136.
91. S. Klumpp, J. E. Schultz, *Pharmazie in unserer Zeit* **14** (1985) 19.
92. R. B. Woodward, R. Hoffmann, *Angew. Chem. Internat. Edn*. **8** (1969) 781; http://www.chem.ox.ac.uk/vrchemistry/Pericyclics/page06.htm.
93. G.-D. Zhu, W. H. Okamura, *Chem. Rev*. **95** (1995) 1877.
94. H. Dai, G. H. Posner, *Synthesis* **1994**, 1383.
95. P. S. Manchand, G. P. Yiannikouros, P. S. Belica, P. Madan, *J. Org. Chem*. **60** (1995) 6574.
96. D. R. Andrews, D. H. R. Barton, R. H. Hesse, M. M. Pechet, *J. Org. Chem*. **51** (1986) 4819.
97. B. H. Lipshutz, S. Sengupta, *Org. React*. **41** (1992) 135.
98. J. L. Luche, C. Allavena, *Tetrahedron Lett.* **29** (1988) 5369.
99. J. L. Luche, C. Allavena, C. Petrier, C. Dupuy, *Tetrahedron Lett*. **29** (1988) 5373.
100. J. L. Mascarenas, J. Pérez-Sastelo, L. Castedo, A. Mourino, *Tetrahedron Lett*. **32** (1991) 2813.
101. R. Sustmann, P. Hopp, P. Holl, *Tetrahedron Lett.* **30** (1989) 689.
102. M. J. Calverley, *Tetrahedron* **43** (1987) 4609.
103. S. C. Choudhry, P. S. Belica, D. L. Coffen, A. Focella, H. Maehr, P. S. Manchand, L. Serico, R. T. Yang, *J. Org. Chem.* **58** (1993) 1496.
104. M. Okabe in K. G. Gadamasetti, Process Chemistry in the Pharmaceutical Industry, Marcel Dekker, New York, 1999, 73.
105. K. Takeda, K. Kimura, K. Okamura, R. Okamoto, J. Sasaki, T. Adachi, S. Omura, EP 0461921 (1990).
106. S. Omura, A. Mikami, J. Sasaki, K. Mizoue, EP 0298757 (1987).
107. US8530205 (2008); CN 201110095568 (2011).

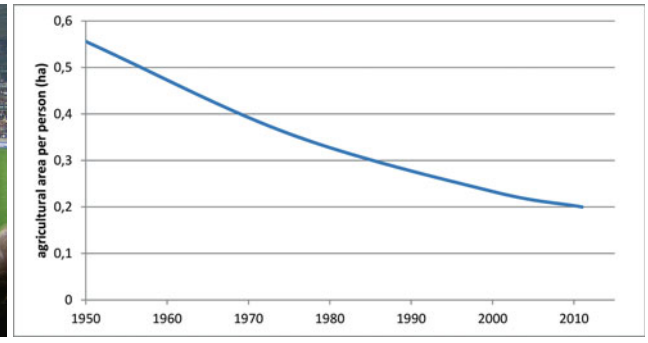
References to 7.3 Biotin

108. Ullmann's Encyclopedia of Industrial Chemistry, VCH, Weinheim, 5th Edn., (1996) 566; Römpp Chemielexikon, Thieme, Stuttgart, Electronic Release, 2003.
109. C. G. Staggs, W. M. Sealey, B. J. McCabe, A. M. Teague, D. M. Mock, *J. Food Compost. Anal.* **17** (2004) 767.
110. *Lutavit*® H 2, BASF Produktinformation.
111. P. Karlson, Biochemie, Thieme, Stuttgart, 1970, 135.
112. Kirk-Othmer, Encyclopedia of Chemical Technology, Wiley, New York, 4th Edn., 1998, Vol. 25, 48.
113. Kirk-Othmer, Encyclopedia of Chemical Technology, Wiley, New York, 3rd Edn., 1984, Vol. 24, 41.
114. F. Escalettes, D. Florentin, B. Tse Sum Bui, D. Lesage, A. Marquet, *J. Am. Chem. Soc.* **121** (1999) 3571.
115. S. A. Henderson, J. O'Connor, A. R. Rendina, G. P. Savage, G. W. Simpson, *Aust. J. Chem*. **48** (1995) 1907.
116. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1992, 628.
117. P. Karlson, Biochemie, Thieme, Stuttgart, 1970, 142 and 196.
118. F.-E. Chen, Y.-D. Huang, H. Fu, Y. Cheng, D.-M. Zhang, Y.-Y. Li, Z.-Z. Peng, *Synthesis* (2000) 2004.
119. S. P. Chavan, R. B. Tejwani, T. Ravindranathan, *J. Org. Chem*. **66** (2001) 6197.

120. M. Seki, M. Kimura, M. Hatsuda, S. Yoshida, T. Shimizu, *Tetrahedron Lett.* **44** (2003) 8905.
121. P. J. De Clercq, *Chem. Rev*. **97** (1997) 1755; M. Seki, *Med. Res. Rev.* **26** (2006) 434 (reviews; see references cited therein).
122. S. A. Harris, D. E. Wolf, R. Mozingo, K. Folkers, *Science* **97** (1943) 447.
123. D. E. Wolf, R. Mozingo, S. A. Harris, R. C. Anderson, K. Folkers, *J. Am. Chem. Soc*. **67** (1945) 2100.
124. K. Meyer, personal communication, 2006.
125. K. Matsuki, H. Inoue, A. Ishida, M. Takeda, M. Nakagawa, T. Hino, *Chem. Pharm. Bull.* **42** (1994) 9.
126. W. Bonrath, R. Karge, F. Roessler, WO2006108562 (2006); W. Bonrath, R. Karge, T. Netscher, F. Roessler, F. Spindler, *Chimia* **63** (2009) 265.
127. S. Bory, M. J. Luche, B. Moreau,S. Lavielle, A. Marquet, *Tetrahedron Lett.* (1975) 827; S. Lavielle, S. Bory, B. Moreau, A. Marquet, *J. Am. Chem. Soc.* **100** (1978) 1558; H. Kinoshita, M. Futagami, K. Inomata, H. Kotake, *Chem. Lett.* (1983) 1275.
128. T. Meul, R. Miller, L. Tenud, *Chimia* **41** (1987) 73.
129. E. G. Baggiolini, H. L. Lee, G. Pizzolato, M. R. Uskokovic, *J. Am. Chem. Soc*. **104** (1982) 6460.
130. K. C. Nicolaou, E. J. Sorensen, Classics in Total Synthesis, VCH, Weinheim, 1996, 285.
131. E. Poetsch, M. Casutt, *Chimia* **41** (1987) 148.

8 | Agrochemicals

Plant protection is more necessary today than ever before. It is of prime importance for the safeguarding of our food supply. Whereas over the last 50 years the usable agricultural area has remained more or less constant (1.4 billion hectares worldwide) [1], the human population has grown during the same period from 2.5 to more than 7 billion, which translates statistically into only around 0.2 hectares (~ 0.5 acres) per capita nowadays (Fig. 8.1).



8.1 *A plot roughly the size of a quarter of a football field must grow all the necessities of life for each of us.*

Since the Neolithic Age, around 10,000 years ago, Man has cultivated the land. The crops, as we know them today, were bred from wild plant species. A sophisticated irrigation technique in Mesopotamia and Egypt allowed more people to be fed. Hence, the first advanced civilisations emerged, especially in North Africa and the Middle East, some 6000 years ago. As we learned from Sumerian texts and from Egyptian decriptions, locusts, beetles, rodents and fungal diseases threatened the harvests already in those days (Fig. 8.2). [2]

Also Chinese documents from the Shang Dynasty (1523–1027 BC) reveal the threats posed by insects. Therefore, attempts were made to battle, for example, the migratory locust *Locusta migratoria manilensis* with fire. In Greek and Roman writings, there are reports of plant damage, preventive measures, and pest control possibilities. For instance, Pliny the Elder describes the use of ashes, crushed cypress leaves and diluted urine as insect repellents.

8.2 *Locusts on the gravestones at Sakkara, Upper Egypt, 2400 BC.*

In the years 1845–1850, a fungal infection of potatoes (Solanum tuberosum L.) with Phytophtora infestans was rife in Ireland: this led to a starvation, known as the "Great Irish Famine", as a result of which, more than a million people died and two million emigrated.

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In 1765, Otto von Münchhausen (1716–1774) had reported that ergot was a fungus with sponge-like structures, but this theory was only proved in 1815 by the Swiss botanist Augustin-Pyrame de Candolle (1778–1841). [2]

8.3 *In damp summers, the fungus Claviceps purpurea (ergot) infests mainly rye, and less frequently wheat, so that in the Middle Ages, especially the poorer members of the population, whose crop was affected, suffered foremost from ergot poisoning.*

8.4 *Wing-panel of the Isenheim altar-piece by Matthias Grünewald (ca. 1480–1528). "St Anthony's fire" causes severe bodily disfigurement. The swollen and miscoloured body is covered all over with festering sores.*

The people were rendered somewhat helpless, however, in dealing with fungal diseases, since their causes were unknown. The so-called "St Anthony's fire" (ergotism) has been known since the Middle Ages. The disease is caused by the fungus ergot (*Claviceps purpurea*), which grows on ears of grain (Fig. 8.3). On a side-wing of Matthias Grünewald's altar-piece in Isenheim, a sufferer from ergotism is depicted (Fig. 8.4).

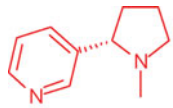
Only once the cultivation of potatoes as an important, starch-containing food source had spread, fungicides were applied, and decontamination of grains was ultimately established, ergotism disappeared.

Around the middle of the 17th century, the German pharmacist Johann Rudolf Glauber (1604–1670) was the first, who tried to control by chemical means smut and rust, both being fungal diseases in cereals. In 1720, copper sulfate, and, since 1784, an arsenic-containing preparation were sold in Europe as disinfectants for coating of seeds. Around the same time, Mathieu Tillet (1714–1791) had shown in his dissertation, that common wheat bunt (*Tilletia tritici*), a fungus later named after him, was responsible for a grain infection that turns its seeds black.

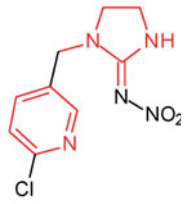
From 1841 on, powdered sulfur was applied to control powdery mildew (*Erysiphaceae*) in orchards, and since 1885 nicotine was used as an insecticide in vineyards. Ten years later, mercuric chloride ($HgCl_2$, corrosive sublimate) was introduced for coating of seeds. As weed-killers, corrosive metal salts, like copper(II) and iron(II) sulfate, dilute sulfuric and nitric acids, and sodium chlorate were employed.

The first synthetic insecticides were salts of arsenic. Paris Green (copper(II) acetoarsenite, $Cu(OAc)_2 \cdot Cu_3(As_3O_6)_2$), also known as Schweinfurt Green [4], is a bright green pigment, which was used originally in paints. In France, winegrowers sprayed their vines in autumn with this colourant as a defence against

The neonicotinoids, a new type of insecticide, are formally derived from the natural product nicotine (*c.f.* section 5.12). At present, the most well-known neonicotinoid is *imidacloprid*®. [3]

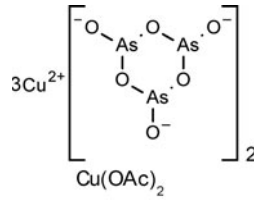


Nicotine



Imidacloprid

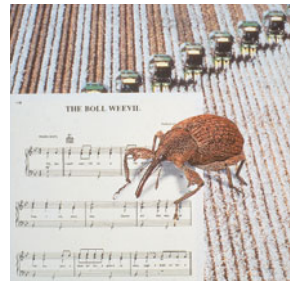
grape thieves. Around 1865 it was discovered that Paris Green is also effective in viticulture against herbivorous insects. [5]



Paris Green

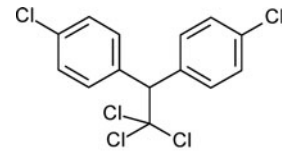
Paris Green may also be used as a blue colourant for fireworks.

Natural fibres are, beyond foodstuffs, another group of crucial agricultural products. An important pest in the cotton industry is the boll weevil, a beetle, which feeds on buds and flowers of the plant; it migrated in 1892 from Mexico into the United States. The *Boll Weevil Song* describes the unsuccessful struggle of farmers against this new pest, which turned out to be not controllable by Paris Green (Fig. 8.5). [6]



8.5 *Nowadays, the Cotton boll weevil population has been largely eradicated thanks to the use of pheromone traps as part of an integrated pest management programme.*

Insects not only cause great damage to agriculture, but they also transmit harmful diseases like malaria (> 40 species of *Anopheles* mosquitoes), sleeping sickness (Tsetse flies), leishmaniasis (sandflies), Chagas' disease (assassin bugs, *Triatoma megista* and *Rhodnius prolixus*), Dengue and yellow fever (gnats: *Aedes aegypti*), trachoma (houseflies), typhus (fleas and lice), and Lyme disease (ticks). In 1943, shortly after the liberation of Naples, the outbreak of a typhus epidemic posed a serious threat to the Allied troops and civilians likewise. By mixing DDT with an inert powder and dusting it on soldiers and refugees, the American military successfully stopped the disease from spreading (Fig. 8.6). In 1939, Paul Müller (1899–1965) at the Swiss company Geigy had discovered the insecticidal activity of this already long-known substance. [7] In 1942, DDT came on to the market and has proved as an invaluable remedy in the fight against malaria (Tab. 8.1). [8] However in 2010, an estimated 219 million people were infected with this disease, and some 660,000 have died.



DDT

8.6 *A U.S. soldier is demonstrating the dusting procedure for administering DDT to eliminate body lice.*

Even in Germany malaria was not a rare phenomenon at all. In 1783, Friedrich von Schiller (1759–1805), a famed German poet and playwright, contracted this serious infection in Mannheim, which he called "cold fever". Also today, supposedly well guarded celebreties, like the Hollywood actor George Clooney, are not immune to falling prey to the disease.

For decades, up to 100,000 tonnes of DDT were produced every year. The American marine biologist and author Rachel Carson's book *Silent Spring*, which appeared in 1962, described the threat posed by DDT to birdlife, and contributed to a fundamental rethinking of plant protection agents. The careless and often thoughtless use of these was followed by a process of mind-change,

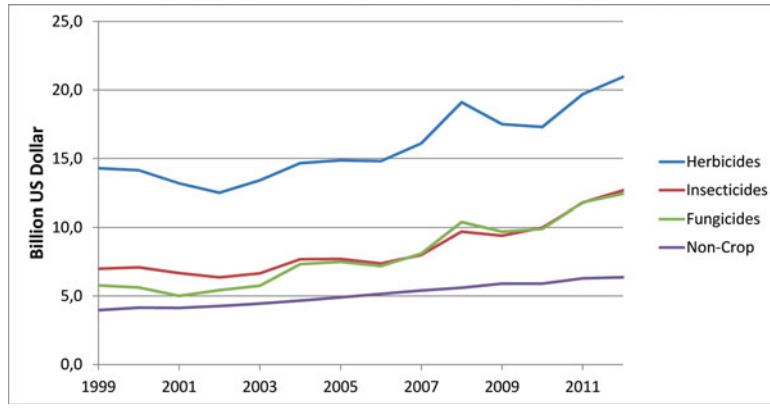
Tab. 8.1 *Decline in cases of malaria after the introduction of DDT.*

Country	Malaria occurrences per annum prior to DDT use	Malaria occurrences per annum following DDT use
Italy	411,602 (1946)	37 (1969)
Turkey	1,188,969 (1950)	2,173 (1969)
India	*ca.* 75 million	*ca*. 750,000 (1969)
Sri Lanka	2.8 million (1946)	110 (1961) 31 (1962) 17 (1963) 2.5 million (1968/69) [a]
Taiwan	> 1 million (1950)	9 (1969)
Venezuela	817,115 (1943)	800 (1958)

[a:] after discontinuation of DDT use (1963).

coupled with the intensive search for environmentally acceptable products. Nowadays, a considerable portion of the development work on a new agrochemical is dedicated to *non-target* organisms, to which not only humans and birds belong, but also fish and a multitude of useful insects, like honey- and bumble-bees.

In spite of scientific hurdles and societal requirements [9], all segments of the plant protection business have recorded significant growth. [10] In 1970, the total market volume amounted to almost 3 billion US dollars, by 2000 this number had grown to 32 billion and by 2012 to almost 53 billion (Fig. 8.7).



8.7 *Development of the plant protection market from 1999 to 2012.*

Herbicides possess now the largest market share worldwide, followed by insecticides and fungicides, which in recent years effectively accounted for the same market volume. The herbicide segment is dominated by amino acid derivatives (*Roundup*®). The fungicides with the highest turnover are strobilurins and the triazoles, and the top-selling insecticides are neonicotinoids, followed by pyrethroids and organophosphates.

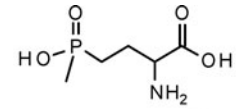
Summary in Bullet Points

- Plant protection is more necessary today than ever before. It is of prime importance for safeguarding our food supply.
- Worldwide, herbicides have the largest market share, followed by insecticides and fungicides, which in recent years virtually accounted for the same market volume.
- In 2012, the total market for plant protection products amounted to 53 billion US dollars.

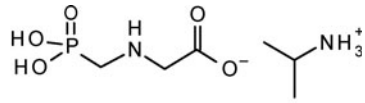
8.1 Amino Acid Herbicides

Maize (corn) and soya are crops, which require especially intensive cultivation during the spring, until the plants cover the whole field. Until a few years ago, much manual cultivation was required in order to keep the young plants free from faster-growing weeds. In recent decades, total (broad-spectrum) herbicides gained increasingly importance in agriculture, because for the first time it became possible to control the weeds without great expenditure of labour, either before germination of the crops (pre-emergence) or in the crops (post-emergence), as long as these are resistant to the total herbicides.

In the following sections, the historical development of two herbicides is discussed, which were developed independently of each other, though their structural similarity may suggest some connection. Both herbicides intervene in the amino acid metabolism of plants, however at different points. One of the two compounds is derived from a natural product, the other one is a substance out of a chemical catalogue (Fig. 8.8).



Glufosinate (*Basta*(R))



Glyphosate (*Roundup*(R))

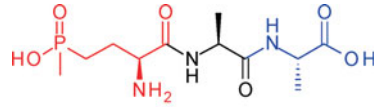
8.8 *Amino acid herbicides of both, natural and artificial origin.*

The one compound results from a systematic development programme, the other is a serendipitous discovery without any structure optimisation. The spectrum of modern plant protection research cannot be broader.

8.1.1 *Basta*®

In 1972, Ernst Bayer (1927–2002) and Hanspaul Hagenmaier (1934–2013) [11] at the University of Tübingen observed that the *Streptomycetes* strain Tü 494 of the species *Streptomyces viridochromogenes* produced several closely related antibiotics. The main component is a peptide, which shows good activity against Gram-positive and Gram-negative bacteria and which inhibits the growth of fungi like *Botrytis cinerea*. The structure elucidation revealed, that it was a

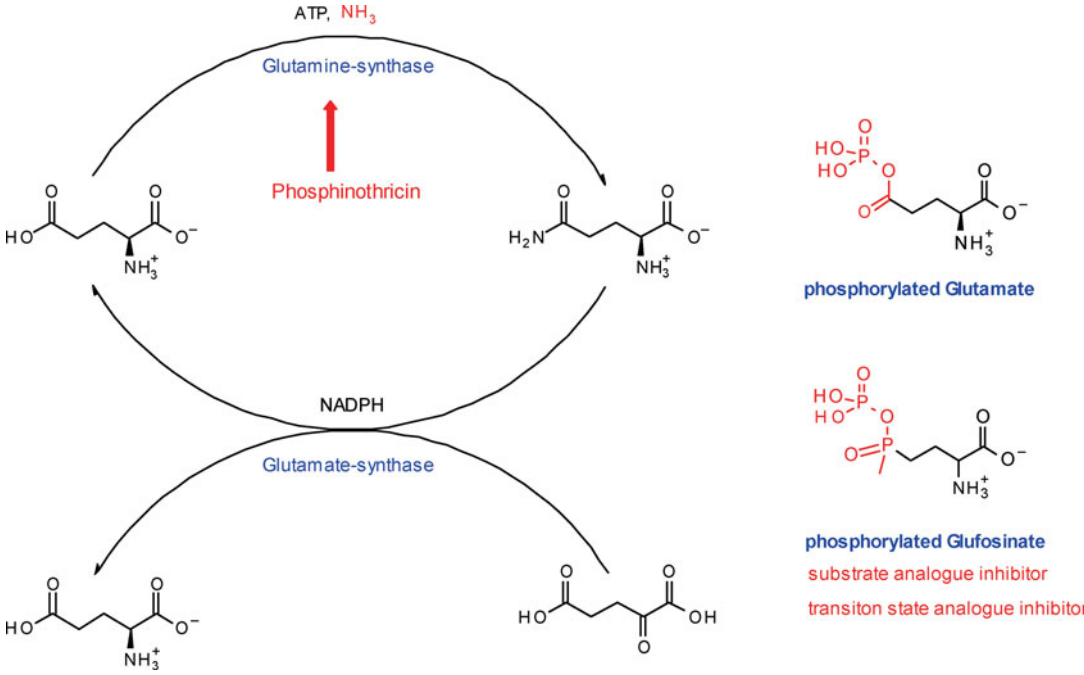
tripeptide, in which two of the amino acids are (*L*)-alanine. The third amino acid was new and proved to be (2*L*)-amino-4-(methylphosphino)-butyric acid (phosphinothricin) (Fig. 8.9).



8.9 *Sequence analysis by means of the Edman degradation led to the structure of the peptide.*

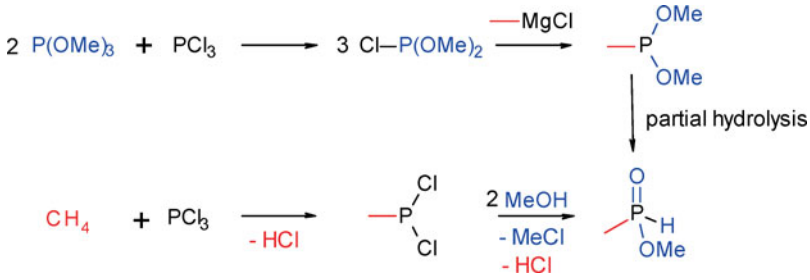
The antibiotic activity may be offset by the addition of (*L*)-glutamine. All the other natural amino acids show no such effect. If *Bacillus subtilis* is cultivated with glutamine as the only carbon source, even at a 250-times higher than the normally active concentration of phosphinothricylalanylalanine, no inhibition is detectable. This leads to the conclusion that glutamine synthase is affected. To exhibit its herbicidal activity, the tripeptide has to be hydrolysed *in vivo* [12], and thereby releases phosphinothricin as the actual blocking agent of glutamine synthase.

For the biosynthetic transfer of ammonia, the carboxylic acid function of glutamic acid is activated by ATP. In case phosphinothricin is present, this undergoes the same activation. The resulting phosphorylated phosphinothricin inhibits glutamine synthase in a competitive manner and functions as a *substrate analogue*- and *transition state analogue*-inhibitor. [13]

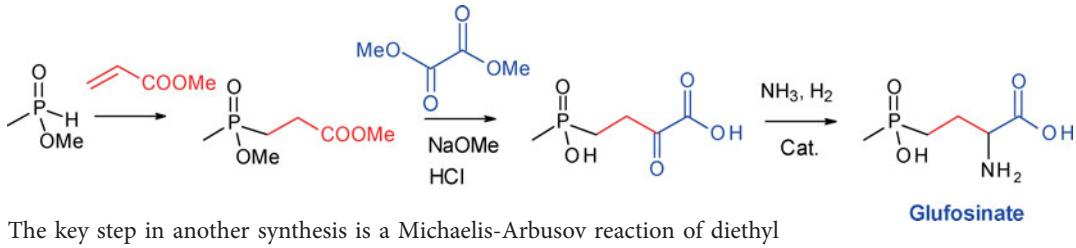


The accumulation of toxic concentrations of ammonia and depletion of amino acids leads to chlorosis, desiccation and necrosis, and eventually to the death of the plant.

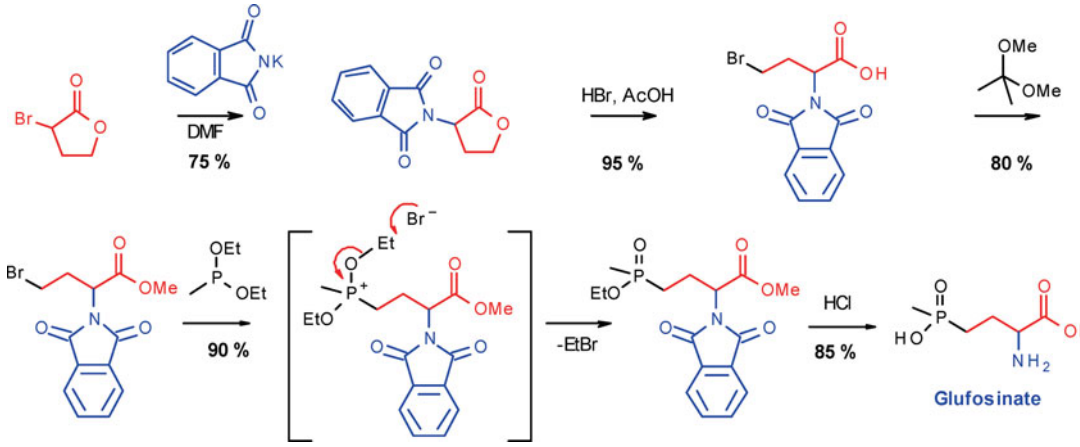
Meiji Seika obtains the tripeptide (Bialaphos) by fermentation and sells it under the trade name *Herbiace*®. Hoechst developed a chemical synthesis for glufosinate (the diastereomeric mixture of phosphinothricin). The methylphosphonite monoester serves as the key building, and is readily accessible by two routes:



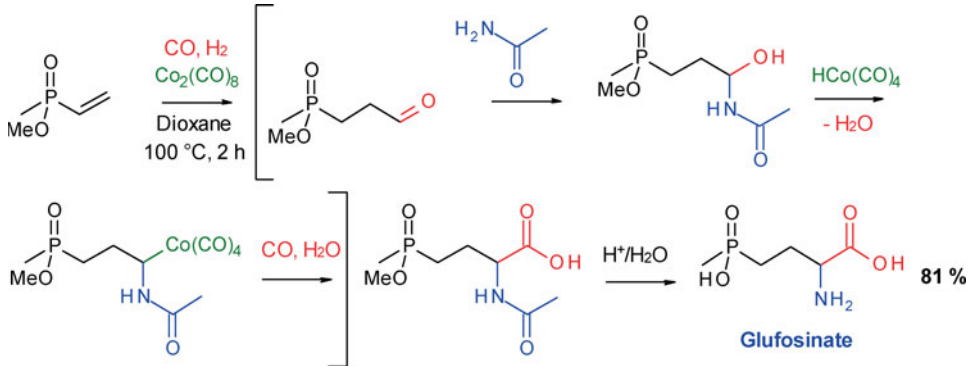
After addition of monomethyl methylphosphonite to methyl acrylate, the product is converted in a Claisen reaction with dimethyl oxalate into the corresponding α-keto-acid, which is then subjected to a reductive amination. [14]



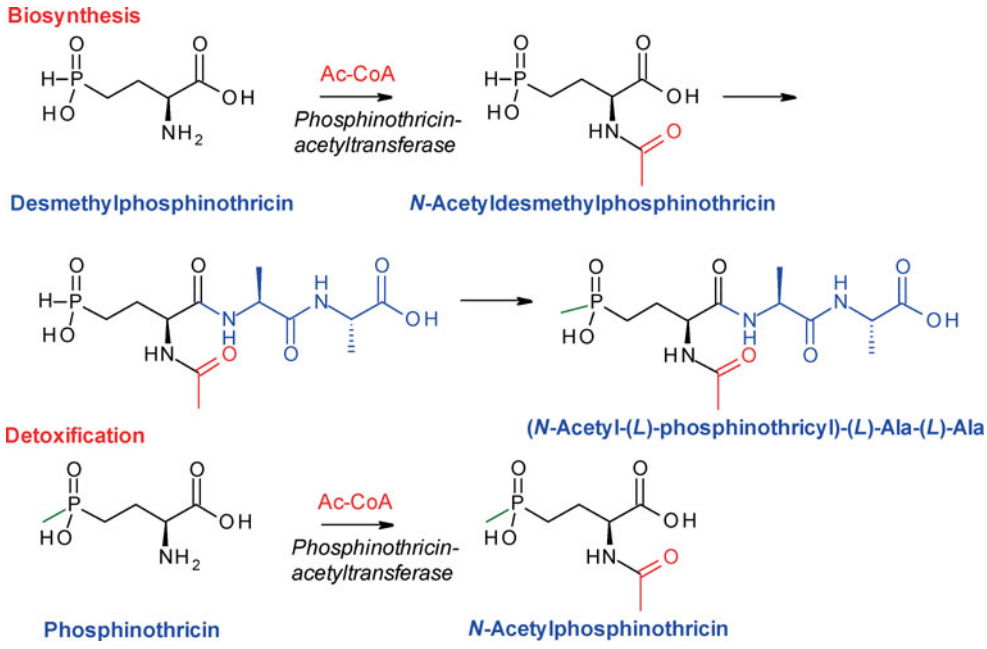
The key step in another synthesis is a Michaelis-Arbusov reaction of diethyl methylphosphonite with the *N*-protected 2-amino-4-bromobutanoic acid. According to a paper from the Monsanto company [15], the latter may be obtained from bromobutyrolactone, which is first treated with phthalimide, and after ring-opening, esterified. [16]



The probably most elegant synthesis of glufosinate is based on the discovery of amidocarbonylation of aldehydes by Hachiro Wakamatsu at Ajinomoto in 1971. [17] Erhard Jägers from Hoechst found, that 3-oxopropylphosphinate esters could also be used. [18] Chemists from Nissan succeeded in manufacturing phosphinothricin esters by starting from easily accessible methylvinylphosphinates *via* a domino-hydroformylation/amidocarbonylation. In spite of a regioselectivity challenge at the hydroformylation step, glufosinate is obtained following hydrolysis in an overall yield of 81 %. [19]



The marketed product is a racemate, although only the (*L*)-form inhibits glutamine synthase. [11] The broad-spectrum herbicide *Basta*® finds application against annual and perennial broad-leaved weeds and grasses on railway tracks, in fruit plantations, vineyards, oil and rubber plantations and for ornamental plant cultivation. It is also applied pre-emergent in vegetables. The major use of



Basta® is nowadays however in genetically modified, glufosinate-resistant crops. Researchers not only succeeded in isolating of phosphinothricin from the soil bacterium *Streptomyces viridochromagenes*, they also discovered its correspondingly detoxifying resistance gene, which encodes the enzyme phosphinothricin acetyltransferase, and is involved in the biosynthesis of bialaphos.

First, desmethylphosphinothricin is *N*-acetylated, then coupled with two alanine residues, and finally the phosphorus is methylated. [20] In this way, the formation of free phosphinothricin is avoided. In addition, the phosphinothricin acetyltransferase transfers an acetyl group to phosphinothricin itself, whereby this is inactivated and can no longer bind to glutamine synthase (see scheme on page 684).

Although phosphinothricin is a glutamic acid mimetic, glutamic acid itself, like all other proteinogenic amino acids, is not acetylated. This is a critical feature for the efficiency of the detoxification mechanism.

It was possible to transfer the phosphinothricin-acetyltransferase gene to various crops, such as rape, maize, lucerne, barley, soya beans, tomato, rice and sugar-beet. These transgenic crops now synthesise phosphinothricin-acetyltransferase and are therefore able to escape the toxic effects of glufosinate.

Epilogue

In recent years, growing concern over reproductive hazards associated with glufosinate led to a biocide ban proposed by the Swedish Chemicals Agency. The ban was approved by the European Parliament in 2009. [21] While further restrictions are to be executed in the EU by end of 2013, growing demand for this herbicide in the US market encouraged Bayer to announce at the same time an expansion of their production facility in Mobile, Alabama.

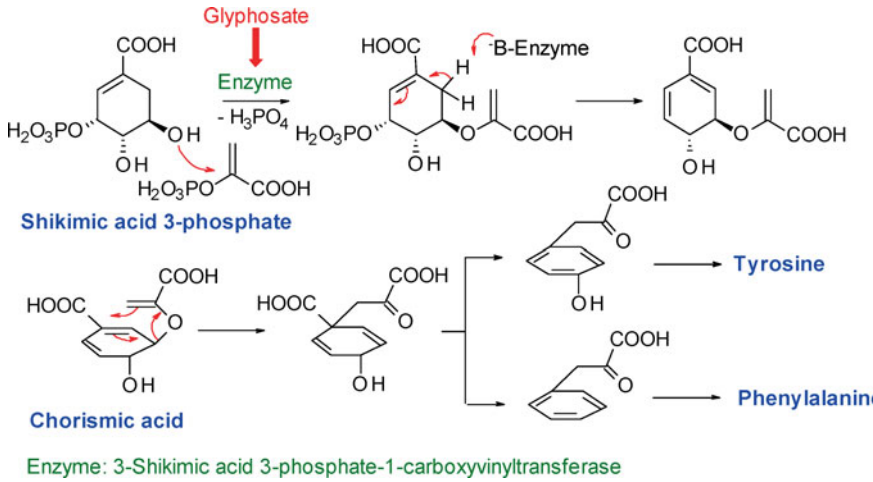
8.1.2 *Roundup*®

Roundup® is a broad-spectrum systemic herbicide, effective against annual and perennial mono- and dicotyledonous root spreading weeds and grasses. It is employed in plantations, on arable fields and pastures, in forests, vineyards, orchards and for grassland renewal (Fig. 8.10). [22] Nowadays, Monsanto's *Roundup*® is the total herbicide with the largest turnover (in 2001: 230,000–250,000 tonnes were sold [14], generating revenues of around 3 billion US dollars; in 2010, its market share had grown to 4.08 billion US dollars [13]). The active ingredient (glyphosate) is absorbed through the leaves and works systemically, which is essential, for example, for effectively controlling wheatgrasses (*Elymus* species).

8.10 *Roundup*®*-use as an alternative to mowing in apple orchards.*

The herbcidal effect is caused by the interference of glyphosate with the biosynthesis of aromatic amino acids. By competing with the natural substrate, glyphosate blocks an enzyme, which catalyses the reaction of phosphoenol pyruvate with shikimic acid. As a consequence, shikimic acid accumulates, and the plant is starved of phenylalanine. There are indications that glyphosate interacts at the phosphate binding site of phosphoenol pyruvate. [23] The fatal effect on the plant is towfold: first, due to the phenylalanine-deficiency, the starting material for the synthesis of lignin and other phenylpropanoid downstream metabo-

lites is lost; and second, there is not sufficient phenylpyruvic acid available to sequester the toxic ammonia from glutamate. [24]

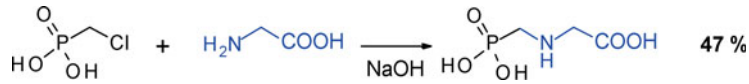


Glyphosate is an unselective herbicide. It damages crops just the same way as weeds and grasses.

Glyphosate-resistant plants are engineered by point mutations of the gene for 3-shikimic acid-3-phosphate 1-carboxyvinyltransferase (EPSP synthase), whereby two amino acids in the enzyme protein are exchanged (Thr^{97} → Ile and Pro^{101} → Ser). Such resistant gene mutants were originally isolated from bacteria (*Agrobacterium* sp.). A different path to resistance is programming plant cells to overexpress the EPSP enzyme and thereby to overcompensate the impact of glyphosate. In this way, the so called 'Green Biotechnology' has provided another example of conferring specific herbicide resistance, this time to soya and maize plants. [25, 26] For a number of years, glyphosate-resistant weeds had no significant economic impact, but recent surveys among farmers in the US show that such weeds emerge as a rapidly growing and spreading problem.

Discovery

In 1950, Henri Martin of Cilag in Schaffhausen prepared glyphosate for the first time. The synthesis was conceivably simple. Martin treated glycine with chloromethylphosphonic acid in sodium hydroxide solution, and obtained *N*-aminomethylphosphonylacetic acid in a yield of 47 %.

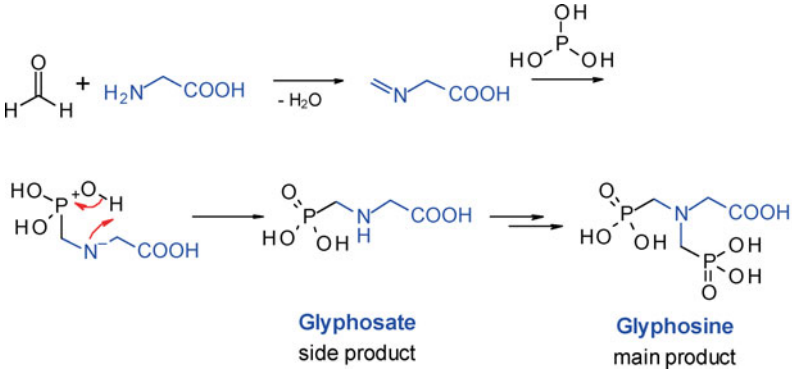


The substance however was not submitted to biological assays, but gathered dust on the shelves. Later, Martin came to believe that his *N*-aminomethylphosphonylacetic acid could be a good chelating agent. In 1959, Johnson & Johnson acquired Cilag, and many of Cilag's research compounds were then bought up

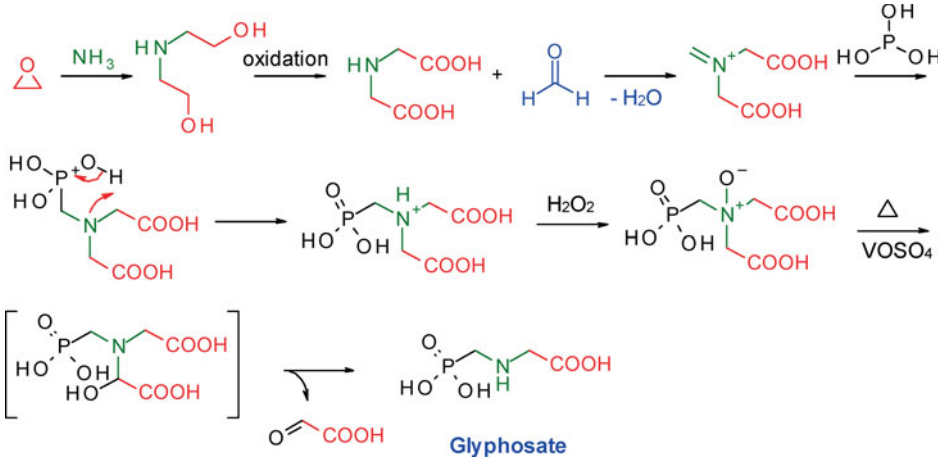
by the chemicals supplier Aldrich. *N*-Aminomethylphosphonylacetic acid was listed in their 1966 catalogue *Library of Rare Chemicals* under the code S39,860-8. Several companies purchased small samples of the material, mostly for screening purposes, without recognising its biological properties.

Only in 1970, John E. Franz at Monsanto observed its outstanding herbicidal activity (Fig. 8.11). Monsanto registered the use of substance CP67573 worldwide as a herbicide, without however being able to claim patent protection of the compound itself ('composition of matter'). It is remarkable that Henri Martin's substance was developed as a commercial product without any structural modification.

In the industrial synthesis, phosphoric acid and glycine are heated and treated with formaldehyde. The phosphoric acid is aminomethylated in the manner of a Mannich reaction. However, the target product, glyphosate, is amenable to a second Mannich reaction, so that a doubly phosphonomethylated glycine results as the main product.



It is therefore necessary to use a protected glycine. This is provided by iminodiacetic acid, which can be generated from the inexpensive precursor diethanolamine. After the same addition reaction, the protecting group is cleaved off oxidatively in a vanadyl sulfate-catalysed Polonovsky-like reaction. [14, 28, 29]



8.11 *In 1970 John E. Franz discovered the most successful herbicide of all time.*

Subsequent research at Novartis confirmed, that the structure is so unique in that any modification leads to a loss of its herbicidal activity. [27]

Over the years, *Roundup*® has developed into the commercially most successful herbicide ever, superior by far to all other plant protection agents. [30] A major contributor to this 'blockbuster' story was the timely development of genetically modified (GM), *Roundup*®-resistant crops, especially soya and maize. Thereby, the loss in profit from the patent expiration of glyphosate in 2000 was not only compensated, Monsanto even managed to extend the life cycle of *Roundup*® by tying it to its new and prosperously growing seed business.

There is a major divide between North America, Europe and Japan concerning the acceptance of GM-crops and GM-foods, as well as their labelling requirements. This impacts significantly the advancement of agrobiotechnology in the different regions of the world.

Summary in Bullet Points

- *Roundup*® from Monsanto is regarded as the commercially most successful plant protection agent of all time.
- *Basta*® from Hoechst is derived from a natural product.
- Both of these agents act by interfering with the amino acid biosynthesis in plants.

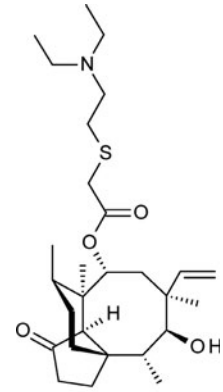
8.2 Strobilurins

Fungi have accompanied humankind through their entire cultural history. [31] They are indispensable aids in the production of foodstuffs. For 5000 years *Aspergillus oryzae* has been used for the production of koji from rice, and for 4000 years *Penicillium roqueforti* has been used in the manufacture of cheese. In Asia, soya sauce has been prepared for at least 3000 years with the aid of fungi, and in Egypt, yeast has been used for just as long to leaven the bread dough. Various American and Asiatic cultures have been drawing on fungi as producers of hallucinogens for certain ritual ceremonies. Since ancient times, the sporocarp (also known as fruiting body or fruit body) of fungi has been valued as welcome enrichment of our menu. The appreciation of truffles, for example, is still hardly surpassed. When Alexander Fleming discovered in 1928 the broad-spectrum antibiotic, penicillin, its source was a fungus, which has saved innumerable human lives up to this day. Since that time, fungi have been employed extensively for the manufacture of pharmaceuticals.

Fungi (New Latin: *Mycetes*) are divided into four phyla: Archemycota, Microsporidia (parasites of animals), Ascomycota ("Sac fungi") and Basidiomycota ("higher fungi").

The basidiomycota, with penny bun (*Boletus edulis*) and fly agaric (*Amanita muscaria*) as examples, were initially not in the focus of research, probably because they grow mycelia only slowly and they are not so easy to cultivate. The first chemical studies on basidium fungi originated from Sir Ewart R. H. Jones (1911–2002), best known for the invention of the Jones oxidation, and Viktor Thaller at Oxford, who recognised that this fungal class, like a few higher plants, especially *Apiaceae* and *Asteraceae*, was capable of forming polyalkynes. [32] In the 1940s and 1950s Marjorie Anchel, Annette Hervey and Frederick Kavanagh extracted hundreds of fruit bodies in the search for new antibiotics. [33, 34]

The outstanding result of these studies was tiamulin, a pleuromutilin antibiotic, which was developed by Sandoz and is still deployed in veterinary medicine. [35, 36]

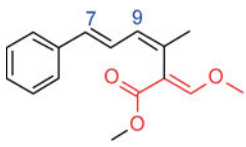


Tiamulin

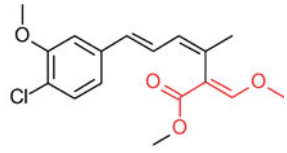
In 1969, Vladimir Musilek isolated a substance from Porcelain fungus (*Oudemansiella mucida*), which he called mucidin. [37] In the now Czech Republic, formerly Czechoslovakia, this compound was used as the active ingredient of an antimycotic ointment (*Mucidermin*®), manufactured by Spofa a.s. for the treatment of skin infections. Musilek was however never able to elucidate the structure unequivocally.

In 1977, Timm Anke in Tübingen isolated two substances, which he called strobilurin A and B, from the mycelium of the Pinecone cap (*Strobilurus tenacellus*) (Fig. 8.12). These showed a strong fungicidal effect on a range of fungi, and a cytotoxic effect on Ehrlich-Ascites tumour cells (Fig. 8.13). *In vivo* tests in cancer models at the National Cancer Institute (NCI, USA) however did not result in convincing data, though no acute toxicity was observed either. Wolfgang Steglich succeeded ultimately in elucidating the structure of strobilurine, and was surprised by its simplicity.

8.12 *The Pinecone cap (Strobilurus tenacellus) (left) is a small, brown coloured mushroom commonly found throughout Europe, except the Iberian Peninsula and Ireland. – The porcelain fungus (Oudemansiella mucida) (right) belongs to the genus Oudemansiella, which is widely distributed in tropical and temperate regions. It is named after the Dutch physician, botanist and mycologist Cornelis Antoon Jan Abraham Oudemans (1825–1906).*



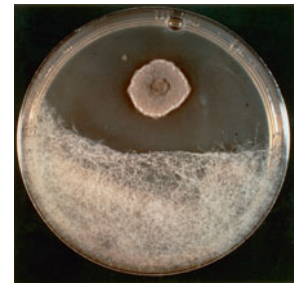
Strobilurin A



Strobilurin B

In the course of their attempt to establish the identity of mucidin, Anke and Steglich isolated from the Porcelain fungus, apart from strobilurin A, also (–)-oudemansin A, which possesses likewise potent fungicidal activity.

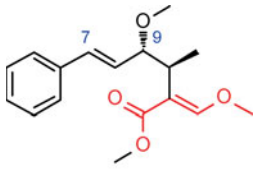
Only in 1986, it could finally be demonstrated that mucidin is identical to strobilurin A. In the meantime, more than 15 strobilurins and oudemansins became known, some with more complex structures. Strobilurin and oude-



8.13 *In a Petri-dish assay, Strobilurus tenacellus forms a halo.*

Later, Strobilurin A was also found in a culture of *Cyphellopsis* and in two *Mycena* species.

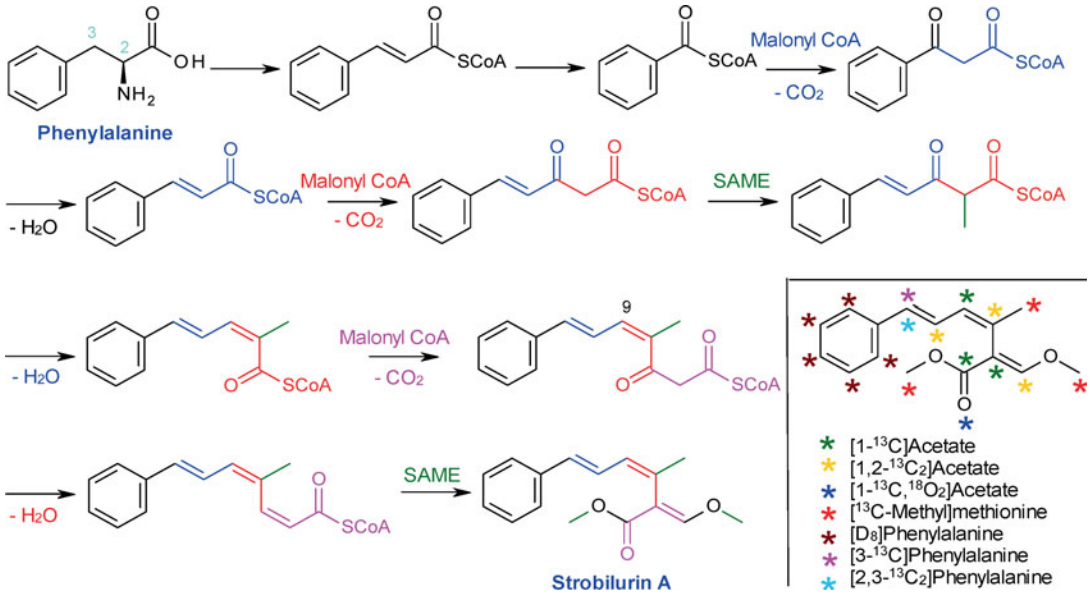
mansin producing fungi occur in all climate zones and belong, with a very few exceptions, like the sac fungi *Bolinea lutea*, to the basidiomycete class.



(-)-Oudemansin A

8.2.1 Biosynthesis

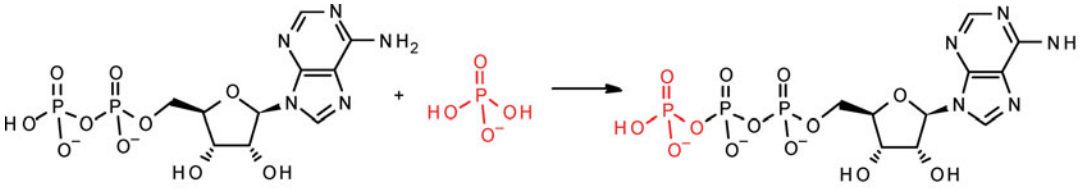
The biosynthesis of strobilurin and oudemansin had already been studied in 1981 by Vladimir Musilek in Prague, and in 1997 and 2004, had also been subject of the doctoral theses by Anna Mampe Soares-Sello in Tom J. Simpson's group in Bristol and by Gerald Thormann from Axel Zeeck's group in Göttingen [38] respectively. Through feeding experiments with labelled precursors, it could be shown that phenylalanine, from the shikimate/chorismate pathway, is degraded to benzoic acid. This serves as a starter unit for the polyketide biosynthesis, which is less common and had previously been observed in only a few other cases (*e.g.* aureothin, isolated from *Streptomyces thioluteus*). After the side-chain is extended by two acetate units, the methyl group is introduced by *S*-adenosylmethionine (SAME); reduction of the keto-group, and elimination of water produces the (*Z*)-configured double bond in position 9. Then the chain is extended by a third acetate unit; an intramolecular rearrangement, which is still not completely understood, and a further methylation with *S*-adenosylmethionine then give strobilurin A.



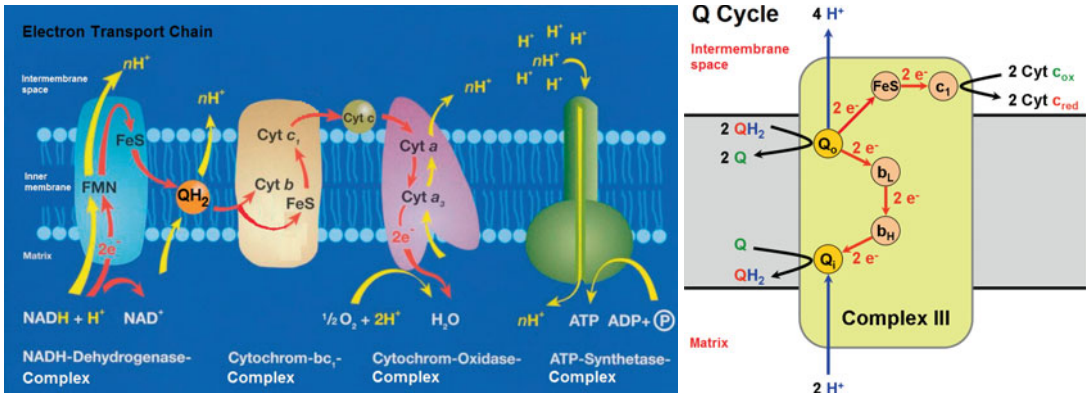
8.2.2 Biochemical Activity of Strobilurins and Oudemansins

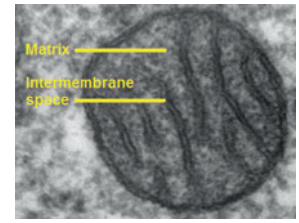
Experiments with Ehrlich-Ascites carcinoma cells led to the conclusion that inhibition of mitochondrial respiration is the mode of action of strobilurin and

oudemansin; the electron transport chain is the actual site, where these agents exert their activity. [39] From degradation of glucose to carbon dioxide and water arise ten equivalents of NADH and two of $FADH_2$. This energy source is utilised in the mitochondria for the oxidative phosphorylation of adenosine diphosphate to adenosine triphosphate. The synthesis of ATP takes place within and at the inner membrane of the mitochondria.



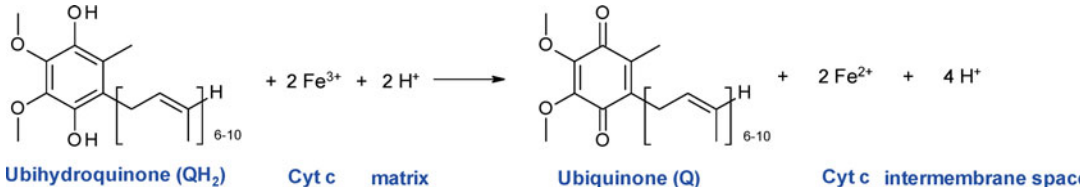
Four protein complexes, three of them function as proton pumps, are embedded in the inner mitochondrial membrane and constitute essential components of the electron transport chain. Every complex consists of a different set of proteins with a variety of redox-active prosthetic groups. All in all, through oxidation of NADH, a proton gradient between the matrix and the intermembrane space is created, which eventually drives the ATP synthase-complex. The correspondingly released electrons are consumed in the reduction of oxygen to water. Both, the NADH oxidation and the oxygen reduction, as well as the ATP synthesis, take place in the matrix of the mitochondrion (Fig. 8.14).

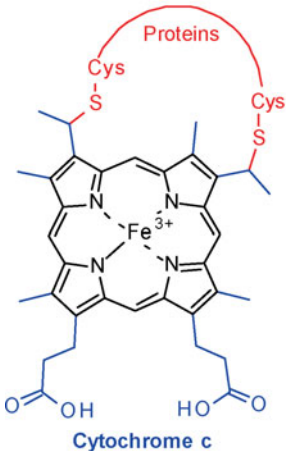




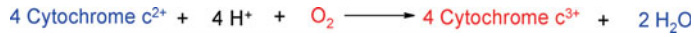
8.14 *Strobilurins halt the production of ATP by blocking the electron transport at the level of the bc_1-complex, located within the mitochondrial inner membrane, which separates the matrix from the intermembrane space (transmission electron microscope image). In a process, the so-called Q cycle, which was first proposed by the British biochemist Peter Dennis Mitchell (1920–1992), ubihydroquinone (also known as ubiquinol) is oxidized to ubiquinone, thereby transfering an electron to each, the Rieske iron-sulfur complex and the b_L heme. While this cycle operates twice, four protons in total are pumped into the intermembrane space, and generate a proton gradient.*

The cytochrome-bc_1-complex (also called coenzyme Q or "Complex III") is composed of multiple subunits, containing two cytochromes and a [2Fe-2S] cluster (Rieske cluster). This complex passes on the electrons from ubihydroquinone (QH_2) to cytochrome c. Cytochromes are families of redox-active proteins with a haem (heme) moiety as prosthetic group, that is linked *via* cystein residues; sequence and length of the polypeptidic chain are species dependent.





Cytochrome c is only loosely attached to the inner mitochondrial membrane, and shuttles the electrons within the intermembrane space in a rotating mode from the cytochrome-bc_1 complex to the cytochrome-oxidase complex. Repeated electron transfer cycles within the cytochrome-oxidase complex ultimately reduce oxygen to water.



It could be shown that strobilurins and oudemansins as well as myxothiazol A disrupt the electron transport at the bc_1-complex (Fig. 8.15). [40] The (*E*)-*β*-methoxyacrylate moiety is the essential toxicophore and interacts reversibly with cytochrome b_L at the outer ubihydroquinone oxidation centre (Qo). Detailed biochemical and structural biology data have been generated, which provide good experimental evidence of how this so-called "QoI" group of inhibitors operates at the molecular level.

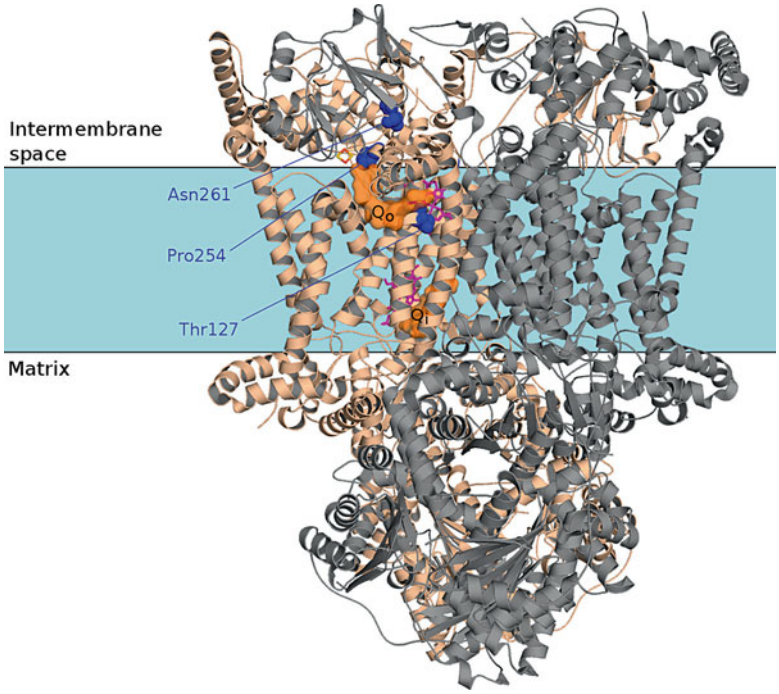
8.15 *Myxothiazol A was isolated originally from the bacterium Myxococcus fulvus, which was found in a soil sample from Java.*

Depriviaton of ATP, caused by disruption of the electron transport chain, affects rapidly the energy-dependent cellular proliferation and overall survival. This applies equally to the growth inhibition of tumour cells and to the prevention of spore germination and mycelial growth in fungi.

At the first glance, it seems surprising that the toxicity of strobilurins against *non-target* organisms, except fish and amphibians like tadpoles, is generally low. [41] Almost all eukaryotes use the same bc_1-complex in their mitochondrial electron transport chain, and the amino acid sequence in the region of the strobilurin binding pocket is highly conserved across many species (*e.g.* fungi, houseflies, rats, maize (corn) and humans). This would imply a lack of any sig-

nificant species-selectivity at the target site. Thus, the reason for the actually observed selectivity may be based on the pharmacodynamics and pharmacokinetics of these compounds. Metabolic effects in non-fungal species lead supposedly to a rapid detoxification prior to reaching the target enzyme. The acute oral toxicity in mice (LD_{50}) for strobilurin A is 500 mg/kg, however for myxothiazol the oral LD_{50} is 2 mg/kg.

The strobilurin and oudemansin producing fungi feature an intrinsic resistance to their cytotoxic products, though these are generated in considerable amounts. This self-protection is achieved by specific mutations of the amino acid sequence that forms the binding pocket for these fungicides in the target protein complex. Comparing a sensitive and a non-sensitive fungus, the example below illustrates the three mutation loci around the Qo region. *Saccharomyces cerevisiae* → *Strobilurus tenacellus*: Thr127 → Ile127, Pro254 → Gln254 and Asn261 → Asp261. Especially the switch to the bulkier isoleucine at position 127 hinders strobilurins and oudemansins from binding, while the affinity of ubihydroquinone remains unaffected (Fig. 8.16).



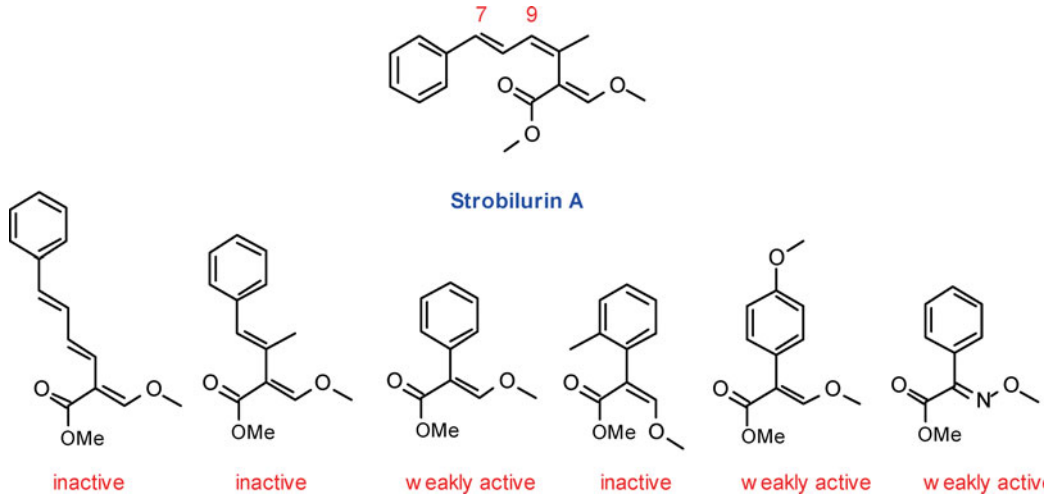
8.16 *The cytochrome bc_1 complex of Saccharomyces cerevisiae. A few amino acids (shown in blue) in Strobilurus tenacellus are responsible for its natural resistance to strobilurins and oudemansins (binding pockets Qo and Qi: ochre, b_L and b_H haem: pink, FeS cluster: yellow and brown).*

Already in the late 1990s it was reported, that a single point mutation (Codon: GGT → GCT) at the gene level of cytochrome b in cucurbit powdery mildew, which translates into a switch from glycine to alanine at position 143, had caused wide-spread strobilurin resistance in the field. [42] Nowadays, the development of resistance can be retarded and managed by applying fixed combinations of agents that interfere at different stages of the fungal life cycle.

8.2.3 Structure Optimisation

In collaboration with Hoechst, Anke and Steglich had initially in mind to develop a strobilurin derivative as an antimycotic for application in humans. Since the activity observed in assay systems was however far below standard antimycotics in the market, the project was dropped, and the corresponding patents were later abandoned. While operating a crop protection business, Hoechst had not noticed the fungicidal potential of this novel structural class. In 1982 however, Zeneca (at that time still part of ICI, now Syngenta) started to work on oudemansin.

In 1977, Steglich had already begun with structural variations to improve the fungicidal activity of strobilurin A, though the resulting series of compounds turned out to be even inferior when tested against *Penicillium notatum* (Fig. 8.17).



8.17 *The first structure variations of strobilurin A.*

Nevertheless, some interesting structure-activity relationship could be derived from this series: The first compound came from an incorrect structure elucidation of the natural product, which was initially believed to possess the (*E*)-configuration at the 9-position. It seemed therefore logical to simplify the structure and prepare (9*E*)-norstrobilurin A. For the second and third compound, the double bonds were then stepwise cut back. Compound four provided information on the impact of the stereochemistry of the double bond in the acrylate system. The fifth one showed that substituents on the aromatic ring are permitted, and the sixth that carbon atoms in the acrylate moiety may be replaced by heteroatoms.

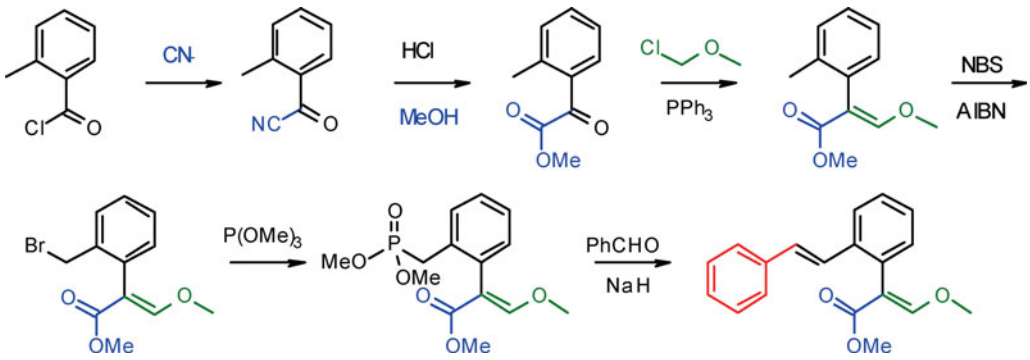
While searching for new active compounds, it is necessary to achieve a reasonable affinity for the target enzyme, but this is by no means a sufficient requirement. Of crucial importance are also resorption and transport within the plant, metabolism, and elimination of the parent compound and its metabolites, as well as the environmental impact of the plant protection agent. Such a data

package requires, in part very labourious and expensive investigations and cannot be compiled for every single molecule that goes through the initial test. Therefore, a stepwise screening and evaluation cascade is employed to select those compounds that meet certain criteria to be submitted to more in-depth assays. In the course of a discovery programme for plant protection agents, a rather large body of analytical, physicochemical and biological data is accumulated for the most promising set of compounds, from which a development candidate is chosen. Those data form ultimately the basis to apply for regularatory approval with the different national authorities to bring a new product to the market. Besides pharmaceuticals, plant protection agents are nowadays the best-characterised compounds.

The analysis of structure-activity relationships needs to take into account the following properties in particular:

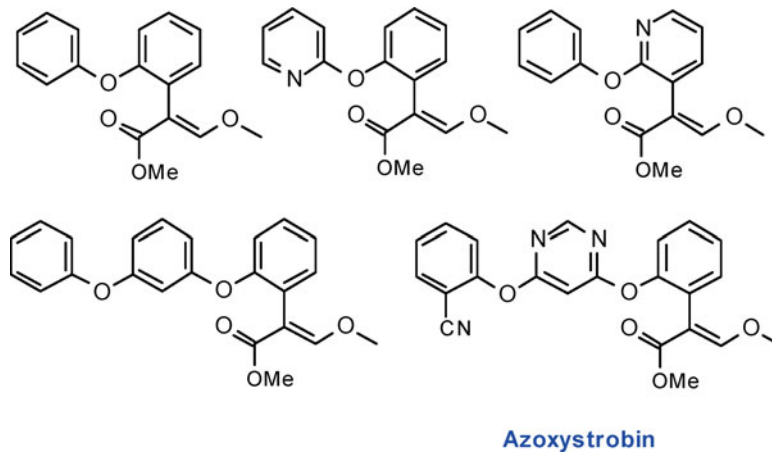
- Melting point: The compounds are often applied in suspension, so that crystallisation phenomena may critically influence the rate of uptake and the bioavailability (depot effect).
- Vapour pressure: Even comparatively low vapour pressures are sufficient for a quasi-systemic mobility of the active ingredient.
- Lipophilicity: Aqueous solubility and lipophilicity are important indicators for resorption and transport processes.
- Photostability: Active compounds with insufficient photostability suffer degradation when applied in the field, which usually affects their potency.

In 1983, BASF started their structure optimisation programme of strobilurin A. The initial *in vivo* tests of the natural product, using fungally infected plants in the greenhouse, were disappointing. However, the photolytic and/or oxidative degradation of the diene system was soon recognized as the culprit. The idea of stabilising the double-bond system by means of an aromatic ring led to stilbene derivatives, which are easily accessible by a Horner-Wadsworth-Emmons reaction:



The activities found in the greenhouse were encouraging and paved the way to field trials. A new lead structure had been discovered, the substituents of which were now easily amenable to a systematic variation.

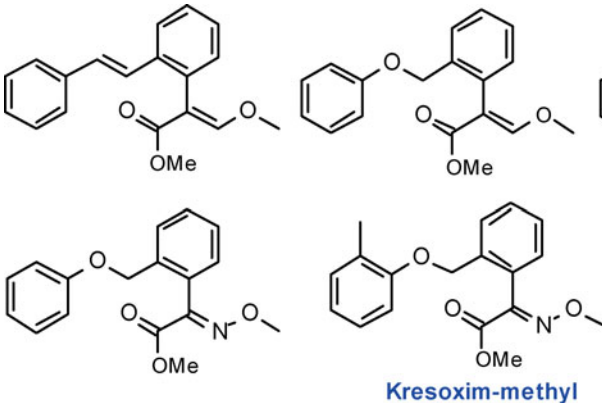
At the same time, starting from oudemansin, Zeneca had drawn the same conclusions, and within the scope of their development programme, they had synthesised a host of analogues (Fig. 8.18). Compared to the stilbene derivatives, bis-phenyl ethers proved even more photostable and entailed another advantage, for which Zeneca had deliberately optimised their structures: systemic mobility in the sap of the crop. Consequently, the active agent was able to be transported also within the plant to those parts, which the spray mist did not reach. However, a slight phytotoxic damage was noticeable, which prompted Zeneca's endeavour to minimise the lipophilicity of the lead series. The introduction of nitrogen atoms increased the selectivity, albeit derivatives with a phenoxydiphenyl ether substructure showed superior activity. On the other hand, the lipophilicity of the latter was so high that these compounds lost their systemic availability. At the end of the optimisation process, Zeneca found a compromise between these diverging property features and selected azoxystrobin (oral LD_{50} (rats): > 5 g/kg) to became its commercial product *Amistar*®.



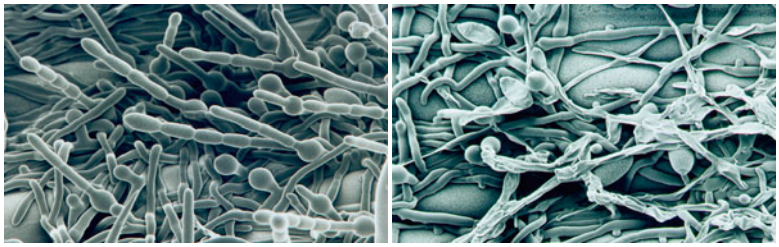
8.18 *Structure optimisation along the way to azoxystrobin.*

The research groups at BASF focused on modifying the pharmacophore, in spite of the paradigm that the methoxyacrylate fragment was crucial and any variation in this part of the molecule might substantially impair the activity (Fig. 8.19). Since Steglich had already prepared oxime ethers at a very early stage of his investigation, and had found that these were not totally inactive, BASF saw a chance therein and picked it up. Considering earlier results, the stilbene fragment was replaced by a benzyl phenyl ether moiety, and an oxime ether was introduced.

This optimisation effort led to kresoxim-methyl (oral LD_{50} (rats): > 5 g/kg), which was introduced to the market under the trade name *Diskus*® (in Japan *Stroby*®) at the same time as azoxystrobin was launched (Fig. 8.20).



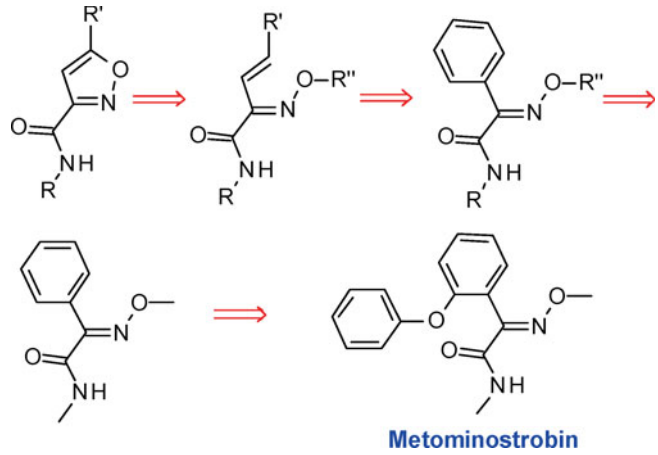
8.19 *Structure optimisation along the way to kresoxim-methyl.*



8.20 *Untreated fungus Erysiphe graminis tritici on wheat (left). Kresoxim-methyl damages both, the mycelium and the exospores (right).*

In this neck-and-neck race, Zeneca had varied the pharmacophore as well, and optimised structures with the oxime ether motif. Their patent application was however only filed two days after the corresponding one from BASF.

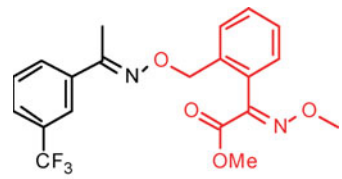
Shionogi pursued a quite different approach to strobilurins. Their starting point for optimisation was a carbamoylisoxazole screening hit with antifungal activity against rice pathogens. Variations of the isoxazole system led to a development compound in close structural proximity to the strobilurin scaffold (Fig. 8.21).



8.21 *The development path to metominostrobin.*

To understand a structure-activity relationship better and evaluate options for optimisation, it is common practice to open or close (hetero)cyclic ring systems and probe the impact of rigidity. This exercise led to structures, which Steglich had already varied. Corresponding to the development route BASF took, the double bond was replaced by an aromatic ring, and a phenoxy-residue was introduced along the pattern from Zeneca. Metominostrobin is registered by Shionogi as *Oribright*® in Vietnam and other Asian countries to prevent and cure rice blast.

The roots for another sub-class of strobilurins originate from the Swiss company Dr. Rudolf Maag AG, where the research was quite distinct from BASF's approach, and focused on modifications at the side-chain. Through acquisition, their results were passed on *via* Hoffmann-La Roche to Ciba-Geigy and then, after merging with Sandoz in 1990, to the agro division of Novartis (meanwhile spun off and combined with Zeneca's agro business as Syngenta). Within the course of their fermentation research in the 1980s, Ciba-Geigy had independently isolated strobilurins from their fermentation trials and recognised their fungicidal activity, but did not follow up on these results further. The patent applications from Novartis were primarily directed towards protecting the intellectual property around strobilurins with an oxime ether side chain. The development compound trifloxystrobin (Fig. 8.22) was ultimately marketed in the so-called *FLINT*® product line, which was divested to Bayer in 2000, in the wake of creating Syngenta.



Trifloxystrobin
(Kresoxim-methyl motif)

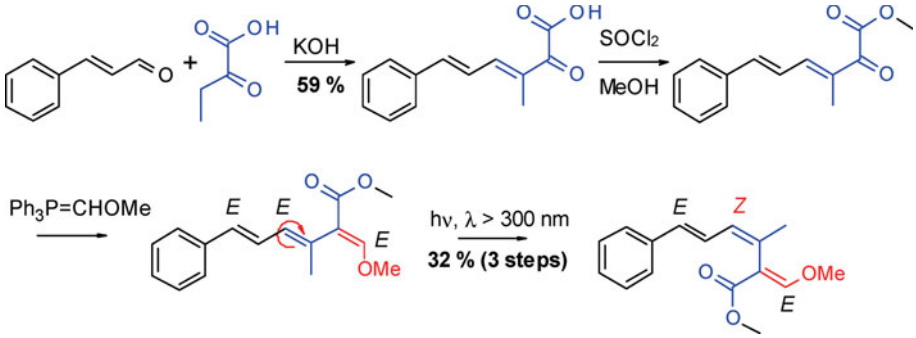
8.22 *Trifloxystrobin is a double oxime ether with a clearly recognisable kresoxim-methyl motif.*

The strobilurins are reckoned among the most important fungicide innovations of the 1990s. Altogether, more than 20 firms and research institutes were engaged in this area, and filed more than 500 related patent applications. Up to the present, an estimated number of more than 30,000 strobilurin derivatives have been synthesised. The global market volume of this fungicide class aggregated in 2010 to 2.76 billion US dollars. [43]

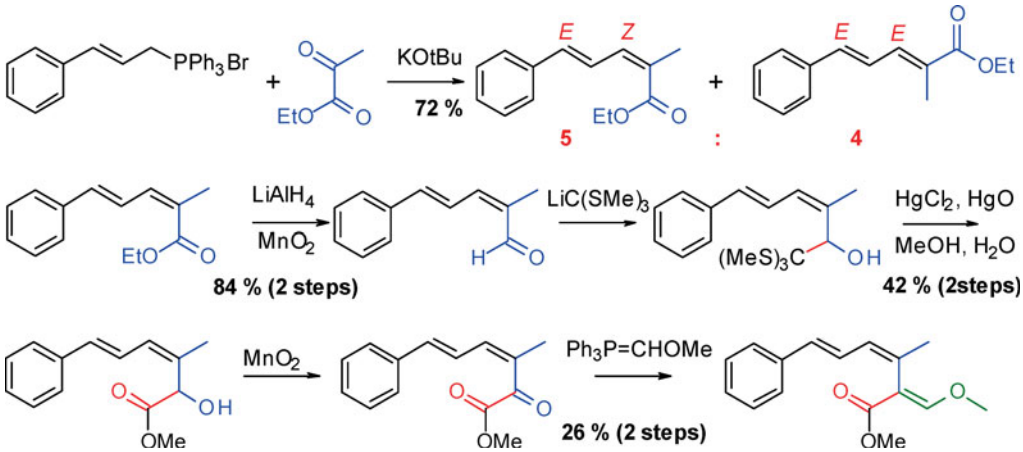
8.2.4 Natural Product Syntheses

The first synthesis of strobilurin A held an uncertainty regarding the configuration of the double bond at position 9. [44] The key steps of Steglich's synthesis are an aldol condensation of cinnamaldehyde and 2-oxobutanoic acid and a Wittig reaction. The natural product is obtained by photochemical isomerisa-

tion of the double bond at C-9, a step, which had presumably been overlooked initially. [45]

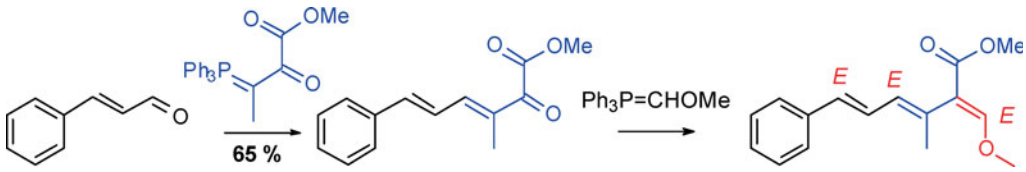


Due to its strong interest in strobilurins, Zeneca developed independently stereoselective syntheses for both the (*E*)- and (*Z*)-isomers. [46] A Wittig reaction, with cinnamylphosphonium bromide and ethyl pyruvate as the starting materials, gives a 5:4 mixture of the corresponding (*E*,*Z*)- and (*E*,*E*)-dienoic esters. These are separated by chromatography and then converted into strobilurin A and its (all *E*)-isomer respectively. The key steps are here the introduction of the carboxylic acid function with $LiC(SMe)_3$ and the final Wittig reaction. The last two stages of the strobilurin A synthesis are carried out in the dark, in order to avoid partial photoisomerisation, because the photochemical half-life of strobilurin A amounts to only a few seconds. [47] The concluding Wittig reaction has to follow immediately after the oxidation, since the α-keto-ester is isomerised exceptionally readily. Chromatographic purification yields spectroscopically pure strobilurin A, which is indistinguishable from the natural product, also in terms of its fungicidal properties. The (all *E*)-isomer is obtained by the same route, starting from the (all *E*)-dienoic ester.

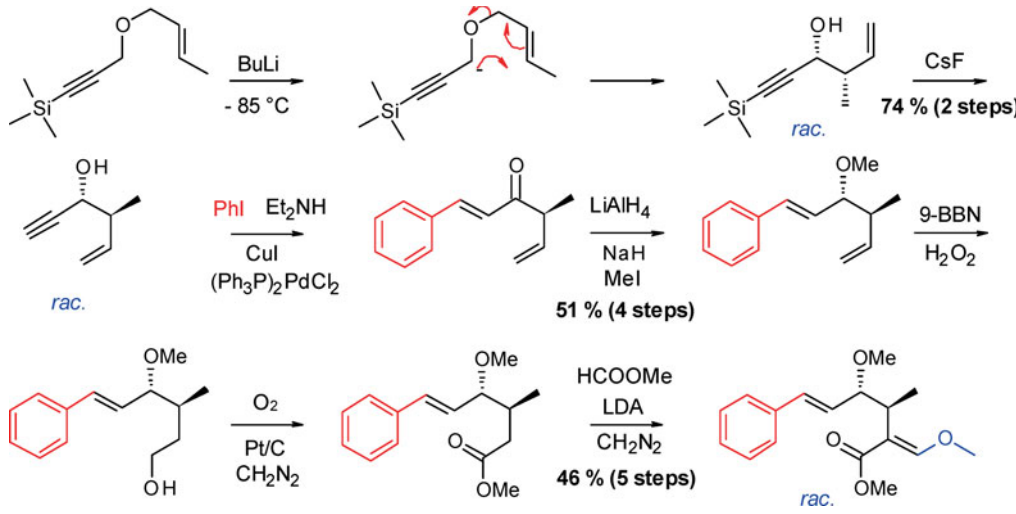


The (all *E*)-isomer of strobilurin A possesses no fungicidal properties.

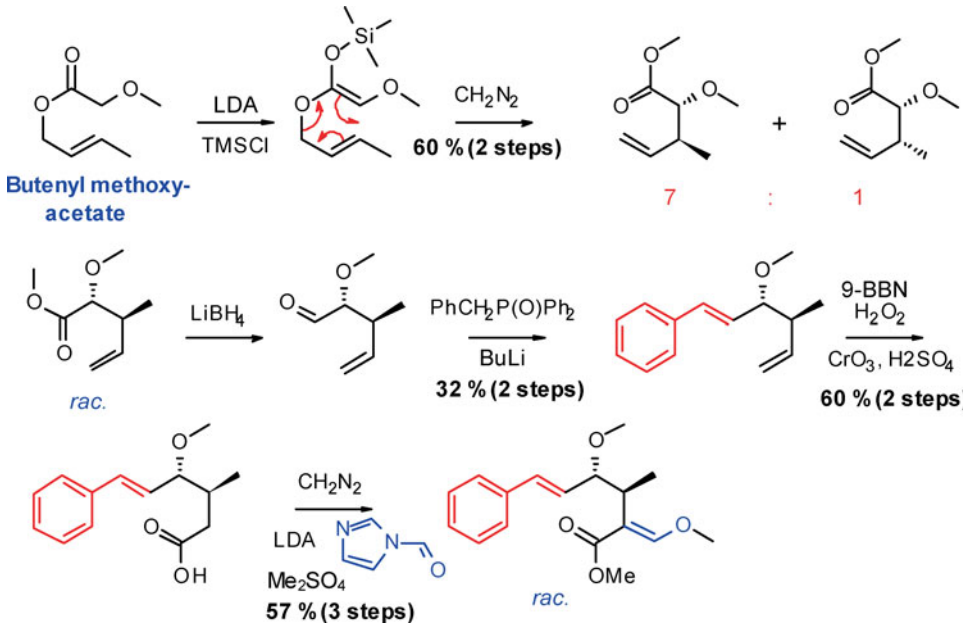
A sequence of two Wittig reactions provides a very elegant access to the (all *E*)-isomer.



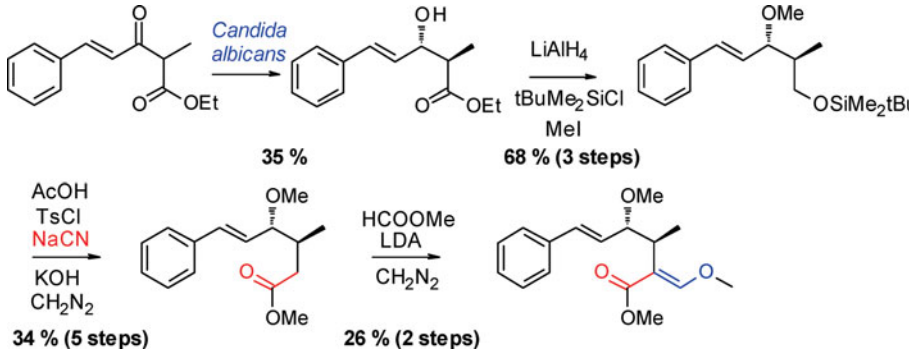
Several syntheses of racemic oudemansin A have been described. The basic skeleton is obtained in a highly diastereoselective [2,3]-Wittig rearrangement, starting from a silyl-protected butenyl propargyl ether. The alkyne is arylated in a Stephens-Castro coupling. Through a palladium-catalysed isomerisation of the triple bond, a vinylogous ketone is obtained; this undergoes a stereoselective reduction with lithium aluminium hydride. Hydroboration with 9-BBN and oxidation introduce the carboxylic acid function. The Claisen condensation with methyl formate and methylation with diazomethane lead finally to racemic oudemansin A. [48–50]



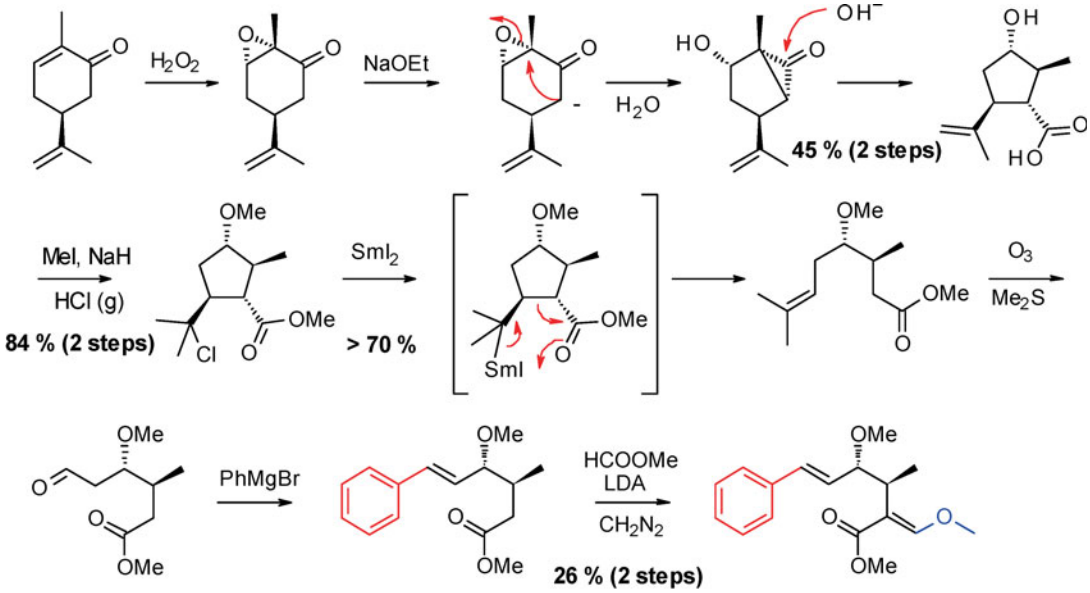
Alternatively, the central synthetic building block can also be generated *via* a diastereoselective Ireland-Claisen rearrangement of butenyl methoxyacetate. Introduction of the styryl fragment results from a Horner-Wadsworth-Emmons reaction. The remainder of the synthesis is closely related to the sequence described above. [51, 52]



In the 1980s, Hiroyuki Akita and Takeshi Oishi published an enantioselective synthesis of oudemansin A, where a β-keto-ester is reduced stereoselectively to the β-hydroxy-ester with *Candida albicans*. After chromatographic purification from small amounts of diastereomers, the ester is then homologised in a classical reduction and cyanide substitution sequence. The remainder of the synthesis for establishing the acrylate system follows the previously described methodology. [53–55]

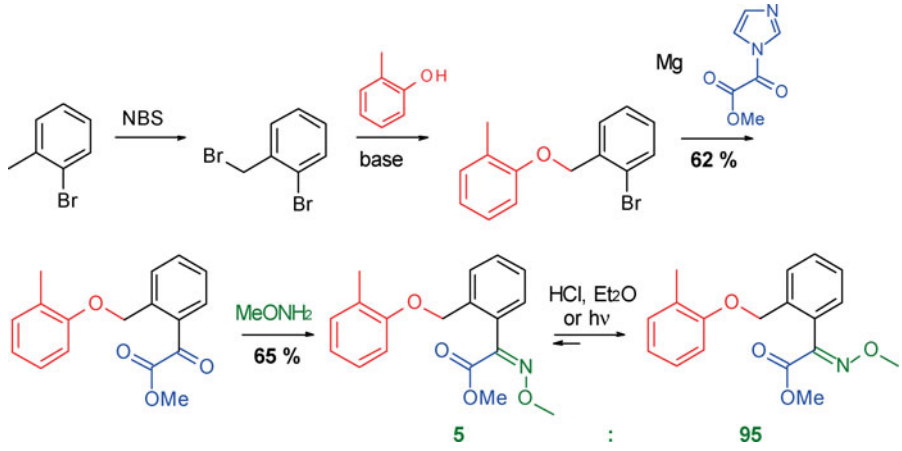


Yukio Suzuki took advantage of the *chiral pool* and synthesised oudemansin A starting from (+)-carvone. The diastereoselective epoxidation is followed by a Favorskii rearrangement to give a highly substituted cyclopentanol. After exhaustive methylation and addition of hydrogen chloride, the five-membered ring was reductively cleaved with samarium iodide. Ozonolysis and a Grignard reaction produce the key building block, which was then reacted analogously to the previous syntheses to give oudemansin A. [56]



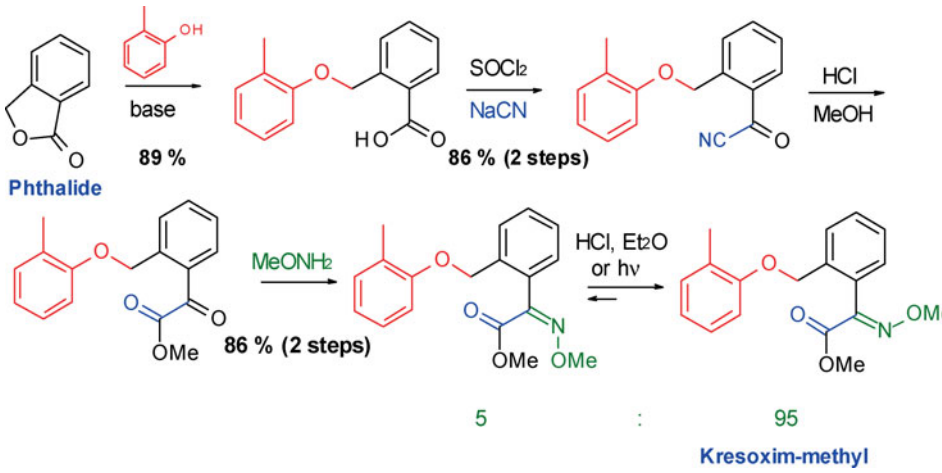
8.2.5 Industrial Syntheses of Active Compounds

The initial laboratory-scale syntheses of kresoxim-methyl started from *o*-bromotoluene, which was first brominated in the side-chain with NBS. Substitution with *o*-cresol leads to the benzyl phenyl ether. The carbon skeleton of the final product resulted from a Grignard reaction with methyl 1-imidazolyloxalate. Reaction with methoxyamine gave a mixture of (*E*)- and (*Z*)-isomers, which can be isomerised, either with the aid of acids or photochemically. Fortunately, the biologically active isomer is also the thermodynamically more stable one. [57, 58]

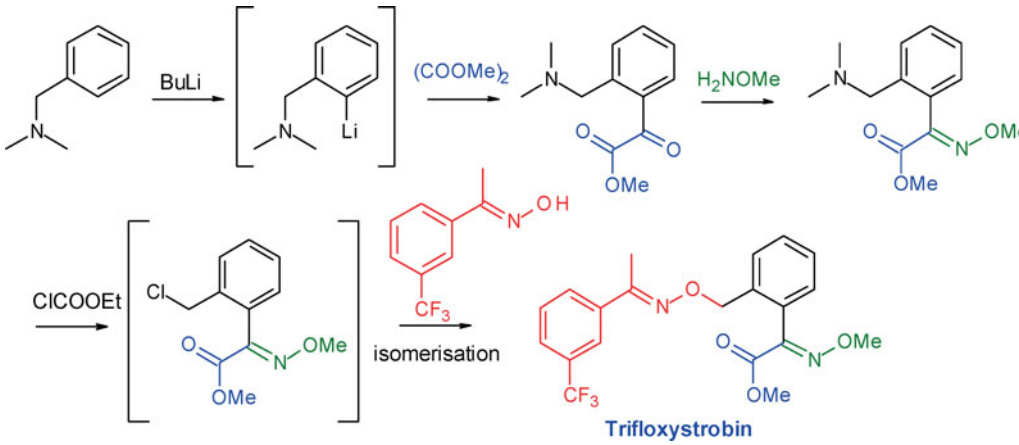


For large-scale production, this synthesis is not suitable on account of the cost involved in the side-chain bromination and the Grignard reaction. In addition, the yields are unsatisfactory.

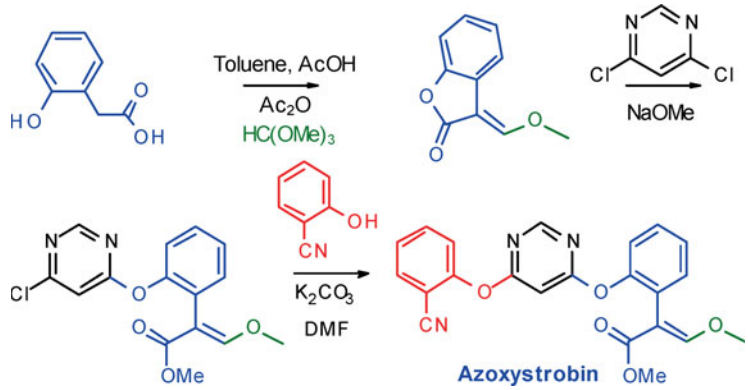
Starting material for the industrial synthesis is phthalide, which may be obtained by hydrogenation of phthalic anhydride. Reaction with *o*-cresol leads to the corresponding carboxylic acid, which is converted *via* its acid chloride into the acyl cyanide. A Pinner reaction and treatment with methoxyamine finally give kresoxim-methyl. [59]



Despite its high similarity to kresoxim-methyl, the analogous synthetic access to trifloxystrobin is prohibited for patent reasons. Starting material is here benzyldimethylamine, which is *ortho*-lithiated and reacted with dimethyl oxalate. After formation of the oxime ether, the dimethylamino-group is exchanged for chlorine using ethyl chloroformate, and the latter substituted by the side-chain fragment. The active compound is isolated following isomerisation and separation of the unwanted (*Z*)-isomer. [60]



Azoxystrobin is built up in an elegant and convergent synthesis from three building blocks: 3-(methoxymethylene)-benzo-2(3*H*)-furanone, 4,6-dichloropyrimidine and 2-hydroxybenzonitrile. [61, 62]



Summary in Bullet Points

- The strobilurin class of fungicides is derived from a secondary metabolite of *Strobilurus tenacellus*.
- Strobilurins interrupt the mitochondrial electron transport at the bc_1-complex.
- The essential pharmacophore of strobilurins is an (*E*)-*β*-methoxyacrylate unit.
- The strobilurins belong to the most important innovations among plant protection agents during the 1990s.

8.3 Pyrethroids

The first records of pyrethrum as an insecticide date back to China's late Zhou Dynasty (*ca.* 1046–256 BC). Since medieval times, the flowers were traded along the old Silk route through Tashkent to Persia and eventually to the Dalmatian coast. Later, during the Napoleonic Wars (1804–1815) French soldiers appreciated pyrethrum as an agent to control fleas and body lice. [63]

The Dalmatian insect powder, as its name implies, was prepared from the dried, crushed blooms of the Dalmatian insect-flower (*Tanacetum cinerariifolium*, syn.: *Chrysanthemum cinerariifolium*, *Pyrethrum cinerariifolium*) (Fig. 8.23).

On the other hand the Armenian, Persian and Caucasian insect powder was obtained from the Caucasian insect-flower (*Tanacetum coccineum*, syn.: *Chrysanthemum coccineum*, *Pyrethrum roseum*, *Pyrethrum carneum*), native to the Caucasus.

In former times, either species was a genus of its own, but both are placed now in the *Tanacetum* genus of about 160 species of flowering plants in the family *Asteraceae*.

The active material is located in the mature, fully-opened flower-head. The average content of toxic components in chrysanthemums from Kenya is 1.3 %,

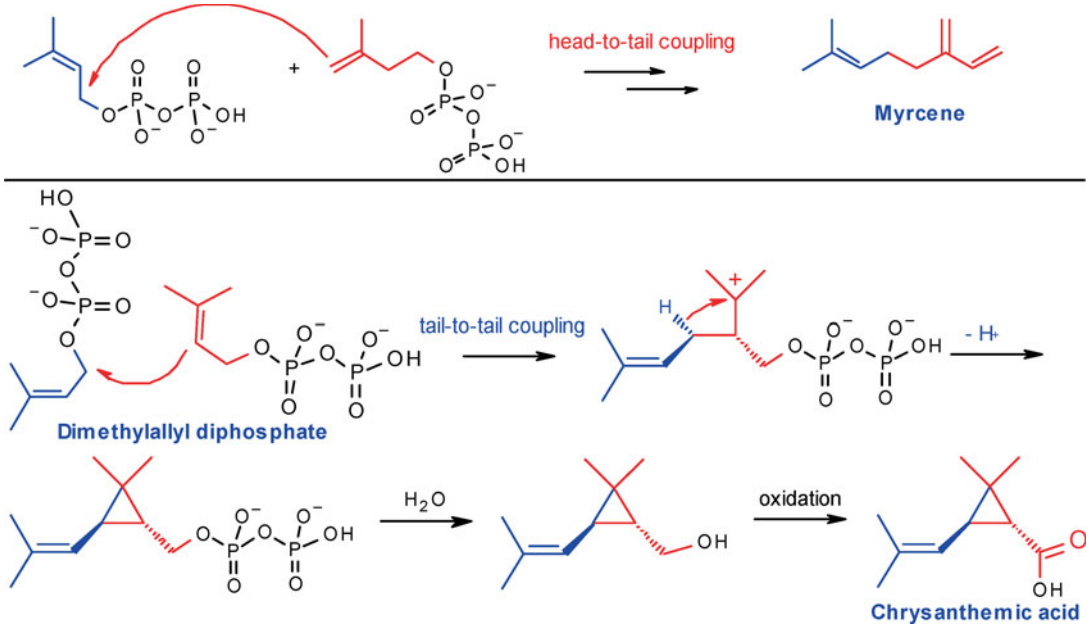
from Japan 1.0 % and from Dalmatia 0.7 %. Apart from grinding the dried blooms into a powder, an active concentrate was prepared by extraction of the blooms with petroleum ether, dichloroethane, methanol, acetone or acetic acid. The extract was purified by adsorption on wax and re-extracted with nitromethane or methanol. Adsorption on activated charcoal led finally to a 25 % concentrate. The dusty powders produced were described as pyrethrum, and were employed in particular against fleas, lice and flies, mosquitoes in households, and also for the protection of livestock.

The production of flea and louse powders began around 1828 (human flea: *Pulex irritans;* head louse: *Pediculus humanus capitis*). After 1851, once this utility was published, a rapid world-wide use was recorded. Up to the First World War, Dalmatia was the most important source of pyrethrum. Later on, Japan became the largest exporter, on account of the higher content of active material in the local blooms. In Kenya, cultivation of chrysanthemums began in 1932, and eight years later it was already the biggest producer of pyrethrum. In 1975, the World production of dried chrysanthemum blooms amounted to 23,000 tonnes annually, more than half of which originated from Kenya. Since then, synthetic pyrethroids have surpassed the natural products in the market. Altogether, the market volume of pyrethroids totaled 1.64 billion US dollars in 2000, and 2.23 billion in 2010. [63]

8.23 *Chrysanthemums (Chrysanthemum cinerariifolium) belong to the aster family (Compositae, Asteraceae). These perennials grow up to one metre in height with feathery and hairy leaves; they bloom in June and July.*

8.3.1 Biosynthesis

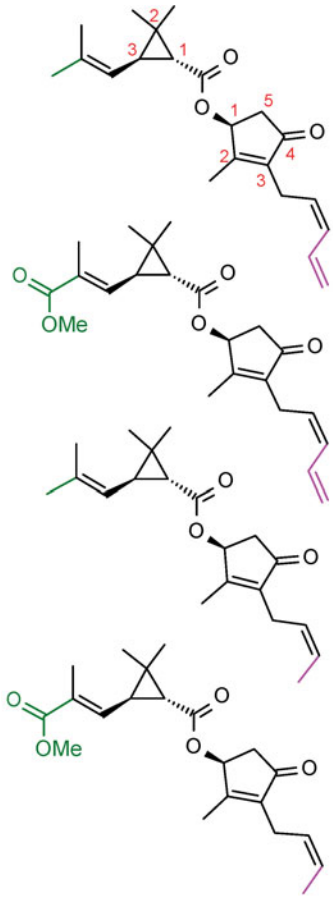
Terpenes, like myrcene, are formed according to the terpene rule by a head-to-tail coupling of two isoprene units. This is for example contrasted by the tail-to-tail coupling of two geranylgeranyl diphosphate molecules to phytoene, which collides with this rule; the same is true for the mechanistically related biosynthesis of chrysanthemic acid from two molecules of dimethylallyl diphosphate.



The initial reaction corresponds to the linkage between two molecules of geranylgeranyl diphosphate. One dimethylallyl diphosphate molecule is subject to a nucleophilic attack by a second one. Deprotonation in the allyl position by a basic centre in the enzyme leads to the formation of the three-membered ring. Whereas in the phytoene synthesis a rearrangement of the three-membered ring occurs under cleavage of the diphosphate moiety, in the biosynthesis of chrysanthemic acid a simple hydrolysis of the diphosphate takes place, followed by an oxidation to give the carboxylic acid (*cf.* section 7.1.2).

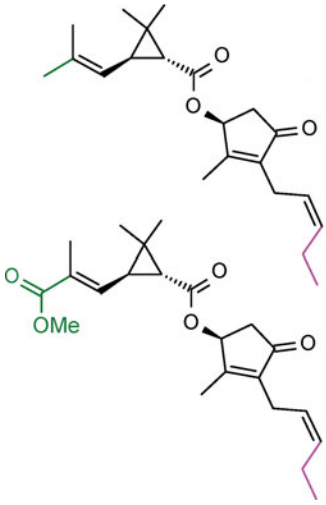
8.3.2 Structure Determination

The fundamental work on the structure elucidation goes back to Hermann Staudinger (1881–1965) and Leopold Ružička (1887–1976); in the 1920s, they isolated pyrethrins from Dalmatian insect-powder, and in particular (+)-*trans*-chrysanthemic acid [64], and recognised their key structural features (Fig. 8.24). [65, 66]



8.24 *The most important constituents of pyrethrum.*

8.24 *(continued)*



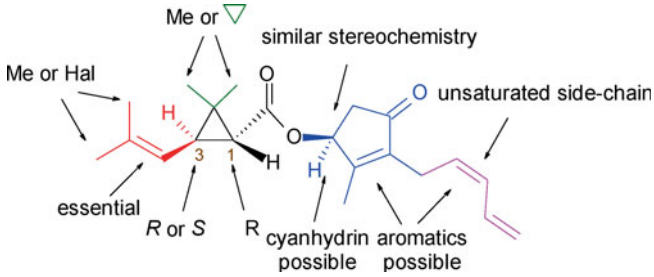
Jasmolin I 1 %
(+)-*trans*-Chrysanthemic acid
(+)-*Jasmolone*

Jasmolin II 1.1 %
(+)-*trans*-Pyrethric acid
(+)-*Jasmolone*

Substantial advances were later provided by the work of Frederick B. La Forge (1882–1958) and William F. Barthel at the US Department of Agriculture. [67] The active compounds consist of two different acids, (+)-*trans*-chrysanthemic acid and (+)-*trans*-pyrethric acid, along with three different alcohols, (+)-pyrethrolone, (+)-cinerolone and (+)-jasmolone. The esters are all optically active. The absolute configuration in the acid portion is (1*R*,3*R*); the side-chain in the pyrethric acid has the *trans*-configuration. In the alcohol moiety, the absolute configuration is (*S*), and the side-chain has the *cis*-orientation.

8.3.3 Structure-Activity Relationships

Once extensive structural variations of the natural pyrethrin I had been carried out, these disclosed insight into the structure-activity relationships of pyrethroids (Fig. 8.25). [68]

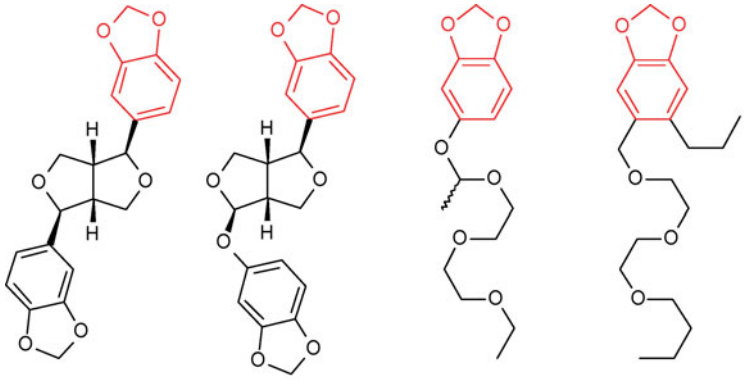


8.25 *The distinctive dependence of the biological activity on the stereochemistry points to a three-dimensional interaction of the active compounds with their neuronal molecular targets, where the isobutenyl group, the two methyl groups at the cyclopropane, and the cyclopentenone moiety play a critical role.*

- The isobutenyl moiety (red) may be replaced by a 2,2-dihalogenovinyl group.
- The two methyl groups (green) at the cyclopropane ring are crucial for the insecticidal activity. Compounds lacking these substituents are inactive.
- The cyclopentenone residue (blue) may be replaced by structures with similar stereochemistry.
- The alcohol components ought to bear a side-chain (magenta). This must be unsaturated, but may be varied from 1-alkenyl or 1-cycloalkenyl even to an aromatic system.
- The absolute configuration (brown) at C-1 and C-3 has to be (1*R*,3*S*) or (1*R*,3*R*).

The natural pyrethroids are contact poisons. They penetrate the exoskeletal cuticula of an insect very fast and exert their toxic activity by altering the function of voltage-gated sodium channels in the neuronal membranes, which leads to a disruption of electrical signaling in the nervous system. Natural and synthetic pyrethroids without a cyano-group, classified as Type I compounds, show a rapidly-decaying sodium tail current in cellular patch-clamp experiments, whereas nitrile-substituted (Type II) pyrethroids cause a prolonged opening of the sodium channels with long-lived sodium tail current. More recent studies suggest that also voltage-gated calcium and chloride channels appear to be involved in the insecticidal effect of pyrethroids. Cell and molecular biology techniques have helped to shed light on a plausible target site, where pyrethroids interact at the α-subunit of sodium channels. The resulting data explain the altered sensitivity observed for different species and as well the resistance phenomena.

Upon exposure to pyrethroids, insects initially undergo with rapid onset a phase of excitation, followed by failure of movement coordination, paralysis and finally death. The paralysis, also termed *knock-down effect*, which is attained with only a few other insecticide classes, provides an important practical parameter for pest control agents, though it is not a sufficient descriptor of efficacy. The *knock-down* dose is in many cases not necessarily lethal, since pyrethrin and cinerin undergo rapid metabolism and are thereby detoxified by microsomal oxidases. Cytochrome P450 oxidises the double bonds of the side-chains to epoxides. Terminal methyl groups are oxidised to carboxylic acids. The ester linkage is however fairly stable: no hydrolysis products are detected. Already in the late 1930s, synergists have been searched for to enhance the insecticidal activity of pyrethrum and to reduce the cost of this expensive natural product. The synergistic action of sesame oil was discovered in 1940, which contained the lignans sesamin and sesamolin, both characterised by a methylenedioxyphenyl (MDP) moiety in the molecule. Subsequent screening for more potent synergists with this particular structural feature led to the identification of a number of suitable combination partners. Piperonyl butoxide proved to be quite effective to be used together with natural pyrethroids. Its role is not only to retard their detoxication by competing as substrate for the oxidising enzymes, but also to increase their resorption into the insects.

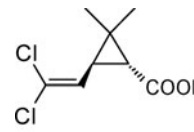


Contributing reasons for the favorable selective toxicity of pyrethroids towards warm-blooded creatures are the species dependent genetic polymorphism of the target ion channels, and also the limited metabolic stability of the active compounds. However, some synthetic derivatives are highly toxic for mammals as well. The lack of a specific antidote augments the danger of acute intoxication. A disadvantage is the toxicity of pyrethroids towards bees and fish (LC_{50}-range: around 0.001 ppm), which may partially be compensated by their insect-repellent properties.

Pyrethrum is commercially available in aerosol pressure-bottles. The formulation contains 0.04–0.25 % of the active ingredient and around five- to ten-fold the amount of piperonyl butoxide, or another synergist, and in some cases also phosphate esters or carbamates. The majority of these products find application in the hygiene sector. For plant protection, pyrethrum is only of limited use due to its high air- and light-sensitivity. The stability may be improved by addition of antioxidants like hydroquinone or resorcinol.

8.3.4 Synthetic Pyrethroids

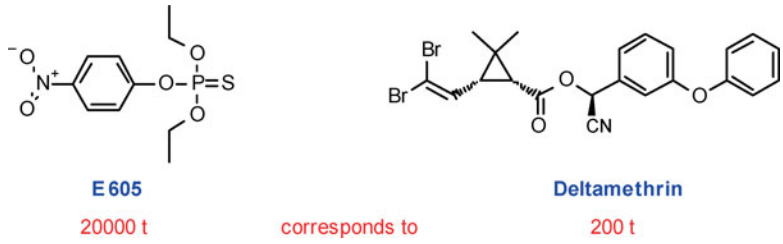
The high production costs of pyrethrum and its lack of stability have triggered an intensive search for new synthetic molecules with improved properties, which started in the 1940s. Through structure variations, broad-spectrum insecticides have been discovered with a 10 to 20 times higher potency than many agrochemicals from other classes. Aside from neonicotinoids, the pyrethroids are still reckoned at present among the commercially most important insecticides on the plant protection market, with a global production amounting to several thousand tonnes per annum.



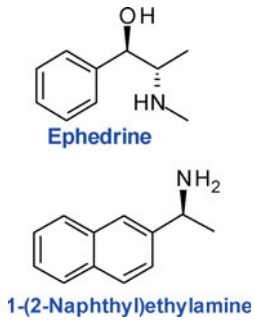
Permethric acid

Given the disadvantages mentioned before, the manufacture of chrysanthemic acid is economically less important than that of permethric acid. Scientifically, the syntheses of both compounds are of interest. The aim of (mostly industrial) research was to identify the simplest and cheapest way to access this structurally demanding class of substances. Nowadays, pyrethroid research is however largely a matter of the past, the insecticide market is dominated by

neonicotinoids, newer classes of compounds with alternative mechanisms of action are emerging, and even totally different concepts of insect control, *e.g.* pheromones, have gained ground. Nevertheless, pyrethroids mark the beginning of a rethinking process for the manufacture of agrochemicals. For the first time the question arose, whether it is economically feasible to use enantiomerically pure compounds in agriculture, against the backdrop of the fact that only the (1*R*)-diastereomers of pyrethroids are active as insecticides (Fig. 8.26). [69]



8.26 *The 20,000 tonnes per annum of Parathion (E 605®), easily available in few reaction steps, are in terms of activity equivalent to the 200 tonnes of an enantiomerically pure pyrethroid (deltamethrin), which has to be manufactured in a multi-step synthesis.*

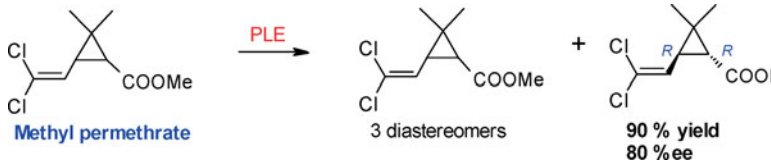


Enantiomerically Pure Active Compounds

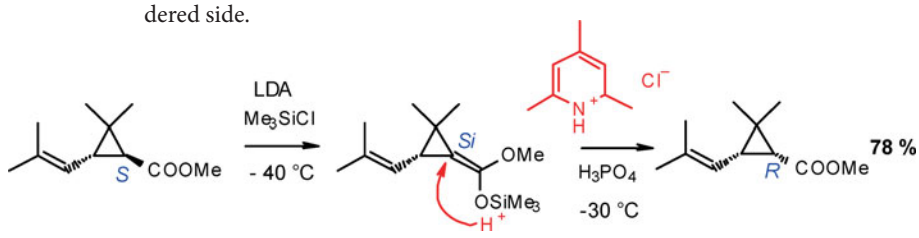
In spite of their different activities, a mixture of pyrethroid (1*R*)- and (1*S*)-diastereomers was marketed initially. However, in view of the rising standards for agrochemicals on the one hand, and advances in stereoselective synthesis on the other, enantiomerically pure plant protection agents are nowadays no longer overly exotic entities. [70]

Classical resolution of racemates by means of enantiomerically pure amines is an established methodology, which is also used at the industrial scale. (1*R*)-*trans*-Chrysanthemic acid may be separated out of the *trans*-racemate by using for instance 1-(2-naphthyl)ethylamine or ephedrine.

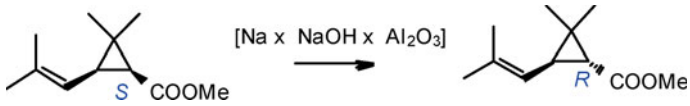
Enzymatic kinetic resolution has also been successfully achieved with cell-free liver esterases [71] or by whole-cell microorganisms. [72]



The disadvantage is in both cases that the undesired diastereomers have to be racemised or discarded. Racemisation of compounds with two stereogenic centres is not a simple task. The (1*S*)-*trans*-isomer can be converted under gentle conditions into the (1*R*)-*cis*-isomer by diastereoselective protonation of the silyl enol ester: the protonation takes place preferentially on the sterically less hindered side.



The isomerisation of the (1*S*)-*cis*-isomers is thermodynamically favoured and can be successfully carried out with a superbase on solid support. [73]

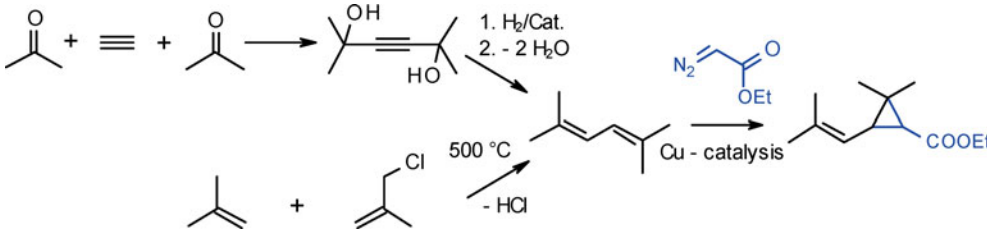


Though in all these cases it remains obvious that the resolution of enantiomers and racemisation are still only stepping stones towards increased efficiency and improved economy. Retrospectively, and according to today's standards, the objective of developing a synthesis procedure is clear: a simple and stereoselective route.

Syntheses of Chrysanthemic acid

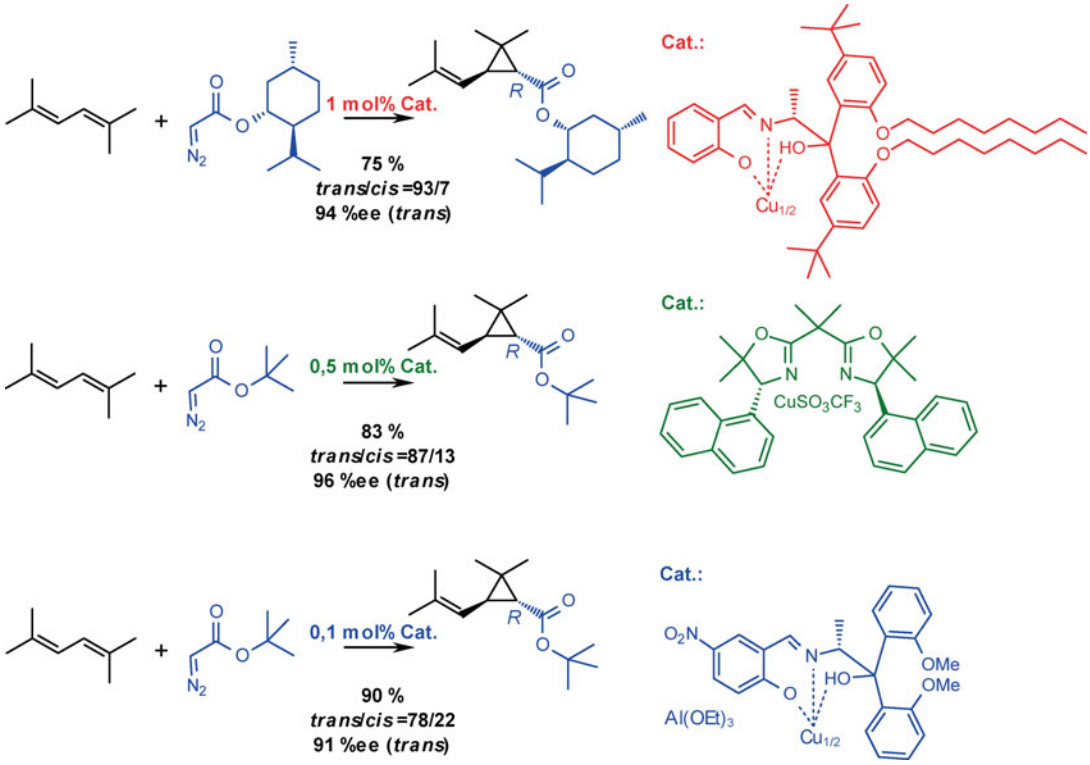
[2 + 1]-Cycloaddition

The first synthesis of chrysanthemic acid, as a *cis*/*trans*-isomeric mixture, goes back to Staudinger, who reacted ethyl diazoacetate with 2,5-dimethylhexa-2,4-diene. From this approach, the first industrial process emerged in 1950 at the Carbide and Carbon Chemical Corporation (later known as Union Carbide, and now part of Dow Chemical Corporation, USA) and at Sumitomo (Japan), which is still in practice these days. The diene is accessible from acetone and acetylene, or from isobutene and methallyl chloride at 500 °C. The decomposition of the diazo-ester in presence of copper at elevated temperature may be improved through use of palladium acetate or rhodium acetate at 20 °C. Sterically demanding diazoacetates give a higher proportion of the desired *trans*-chrysanthemate ester.



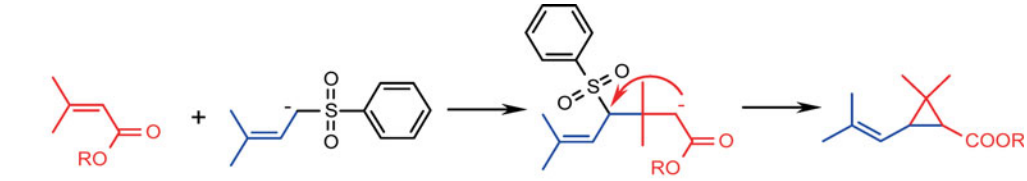
Based on Hitosi Nozaki's and Ryōji Noyori's seminal work, Tadatoshi Aratani developed in 1975 at Sumitomo in Osaka a copper-salicylaldimine complex, with which it was possible for the first time to prepare menthyl (1*R*)-*trans*-chrysanthemate with high (*E*/*Z*)-selectivity and optical purity. [74, 75] This synthesis provided the foundation for the industrial manufacturing process at Sumitomo.

Since then, a whole host of catalysts have been published, *e.g.* by Masamune, Kanemasa, Scott and Itagaki, for the enantioselective [2+1]-cycloadddition, but only a few give good yields for the reaction of 2,5-dimethylhexa-2,4-diene and the readily available *t*-butyl diazoacetate. [76] In the meantime, systematic optimisation work at Sumitomo has made highly active catalysts available, not only of the copper-salicylaldimine type, but also with bisoxazoline ligands, which enable excellent yields and selectivities.



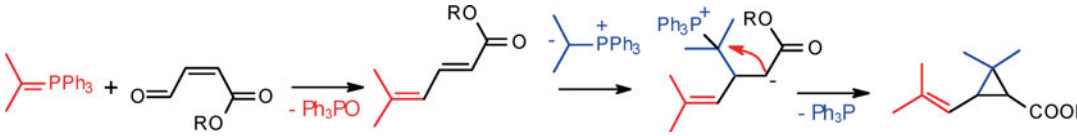
1,3-Cycloelimination

The 1,3-cycloelimination is particularly interesting, because it follows in principle the biosynthetic route. The thermodynamically more stable *trans*-isomer is favoured. Essential contributions came from work at Rhône-Poulenc (in collaboration with Marc Julia at the Pasteur Institute) and at Roussel-Uclaf (Jacques J. Martel), who reacted the senecioate (3-methylbut-2-enoate) ester with an allyl phenyl sulfone. [77–81]

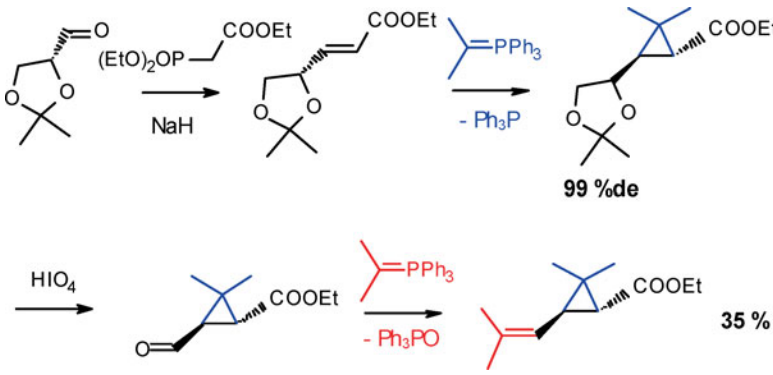


Alkyl senecioate

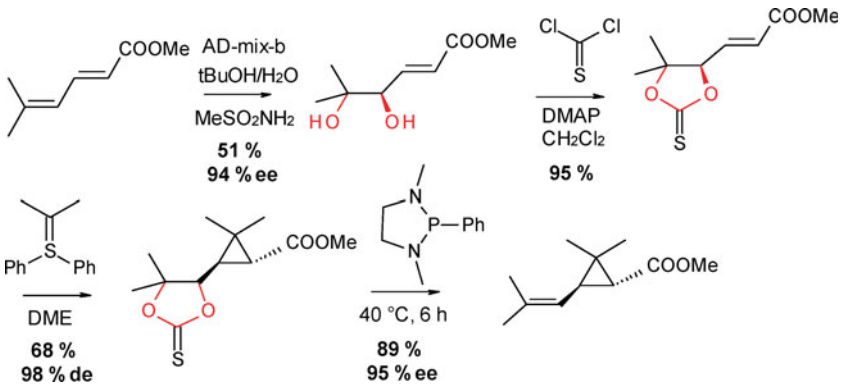
A "one-pot" synthesis, which had been developed by Alain Krief and is almost unrivalled for elegance, converts a malealdehydic ester with two equivalents of isopropylidenetriphenylphosphorane to chrysanthemic ester. [82, 83] The first equivalent gives in a Wittig reaction the 5-methylsorbate ester, while the second equivalent performs a nucleophilic addition to this intermediate at the β-position, and a cycloelimination leads then with the loss of triphenylphosphine to the product.



In case optically pure (*R*)-glyceraldehyde is used as starting material, a similar reaction sequence yields enantiomerically pure ethyl (1*R*)-*trans*-chrysanthemate. [84, 85] Comparable diastereoselectivity is also achieved, if the cyclopropanation (according to Elias J. Corey) is carried out with sulfur ylides.

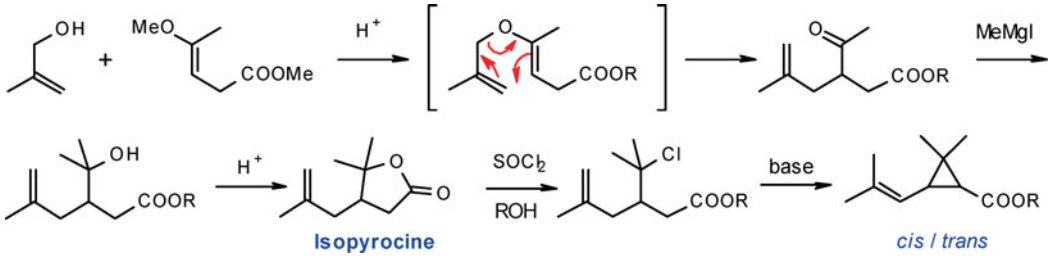


Methyl 5-methylsorbate can be dihydroxylated, regio- and stereoselectively, by Barry Sharpless's method. After protection of the two hydroxy-groups with thiophosgene, formation of the three-membered ring is effected with diphenylisopropylidenesulfurane. Methyl (1*R*)-*trans*-chrysanthemate is finally obtained by removal of the protecting group according to a method of Corey and Winter. [86]

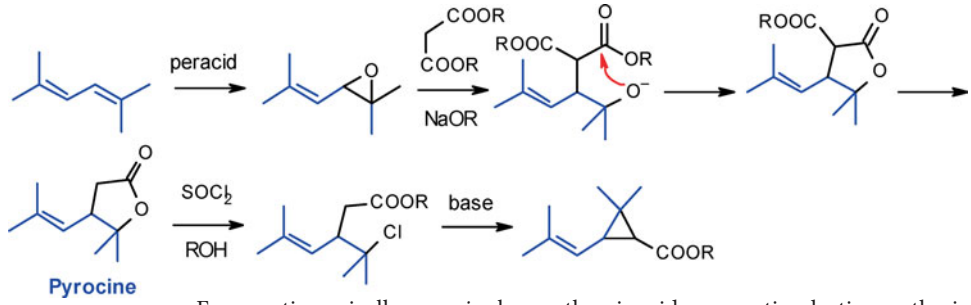


The synthesis sequence of a Claisen rearrangement and a 1,3-cycloelimination was developed by Marc Julia, and turned out highly relevant to pyrethroid chemistry. [87] Methallyl alcohol is etherified with the methyl enol ether of methyl laevulinate. After the subsequent Claisen rearrangement, the keto-group is selectively reacted with methylmagnesium iodide. Acid hydrolysis leads to isopyrocine, a central building block, which is also accessible by other routes. Con-

version with thionyl chloride is followed by base treatment and results in a *cis*/*trans*-mixture of chrysanthemic acid isomers.

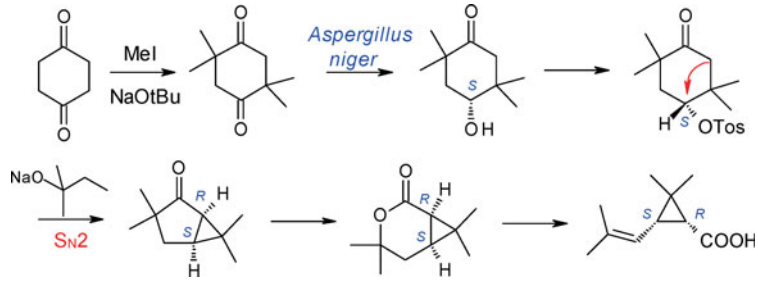


In a short and elegant synthesis, pyrocine is accessible *via* the Lehmann-Traube method, in which the monoepoxide of 2,5-dimethylhexa-2,4-diene is reacted with a malonic ester. After hydrolysis and decarboxylation, pyrocine is obtained, which, like isopyrocine, can be converted into chrysanthemic acid by thionyl chloride and base-induced 1,3-cycloelimination. [88]



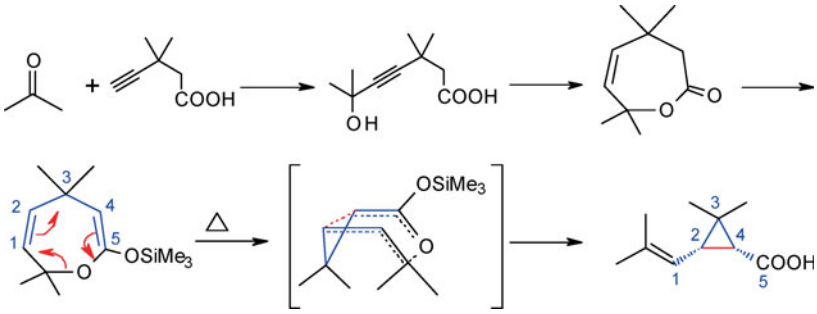
For enantiomerically pure *cis*-chrysanthemic acid, an enantioselective synthesis is used, which takes advantage of the ability of microorganisms to differentiate between two enantiotopic, but chemically equivalent groups (the so called *meso-trick*: *cf.* section 5.1.3). In contrast to the resolution of two enantiomers, the turnover amounts here to 100 % with an enantiomeric excess of > 98 %. The fact that the sequence starts from a cyclic compound, ensures that the *cis*-configuration is obtained.

Cyclohexane-1,4-dione is tetramethylated with methyl iodide and sodium *t*-butoxide. The enantioselective enzymatic reduction is successful with *Aspergillus niger*, *Aspergillus ochraceus* or *Curvularia lunata*. 1,3-Cycloelimination of the corresponding tosylate and a Baeyer-Villiger oxidation give a bicyclic *cis*-configured lactone, which undergoes ring-opening with magnesium bromide in pyridine to produce (1*R*)-*cis*-chrysanthemic acid. [89]



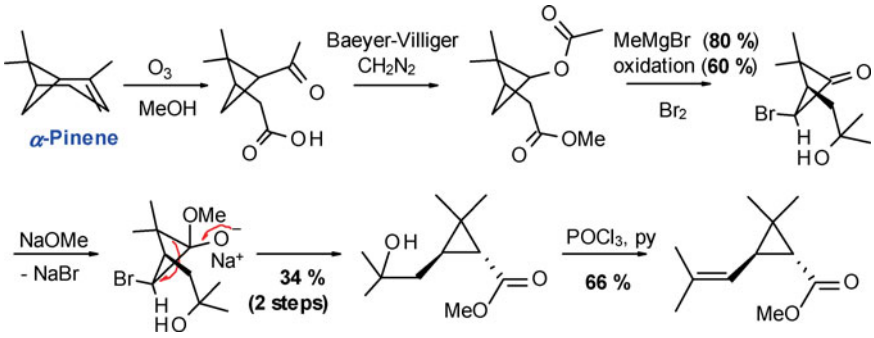
Claisen Rearrangement

The same stereochemical trick may be used in the Claisen rearrangement of cyclic dienes for the targeted preparation of *cis*-chrysanthemic acid. The conversion of an *ω*-alkynylcarboxylic acid with acetone and following ring closure gives a lactone, the silyl enol ether of which is rearranged on warming to *cis*-chrysanthemic acid. [90, 91]



Diastereoselective Synthesis

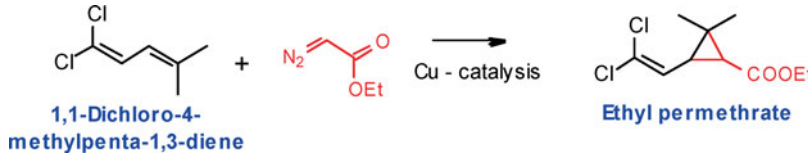
α-Pinene is the ideal starting material for the preparation of (1*R*)-*trans*-chrysanthemic acid. Ozonolysis gives a cyclobutyl methyl ketone, which is degraded to cyclobutanone. After a Grignard reaction with methylmagnesium bromide, there follows a stereoselective bromination. The Favorskii rearrangement finally leads to (1*R*)-*trans*-chrysanthemic acid. [92]



Syntheses of Permethric Acid

A breakthrough in the development of pyrethroid insecticides for agriculture came with the discovery that the esters of permethric acid are both highly active and – most of all – stable upon exposure to light. They became the basic building blocks for many pyrethroids, which were developed in the 1970s. First and foremost, the patent literature contains numerous ingenious contributions as to how these compounds could be prepared by the simplest and cheapest possible route. Hereinafter, the attention is focused on those stereoselective syntheses, which are in particular chemically interesting.

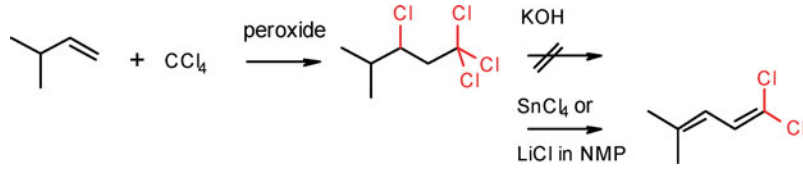
The initial synthesis originated from J. Farkaš. [93] By analogy with the synthesis of chysanthemic acid, Farkaš used as the key step the [2 + 1]-cycloaddition, starting from ethyl diazoacetate and 1,1-dichloro-4-methylpenta-1,3-diene.



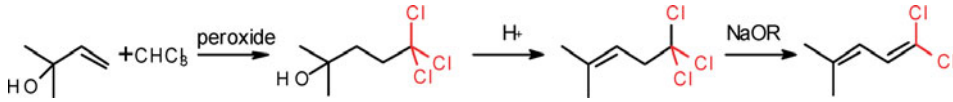
1,1-Dichloro-4-methylpenta-1,3-diene

Ethyl permethrate

To employ this laboratory method at an industrial scale, the simplest possible access to the diene had to be found. Tetrachloromethane may indeed be added radically to 3-methylbut-1-ene, however the direct elimination of hydrogen chloride with potassium hydroxide does not occur. This elimination is on the other hand successful with catalytic amounts of tin(IV) chloride, lithium chloride in NMP, or lithium bromide in DMF.

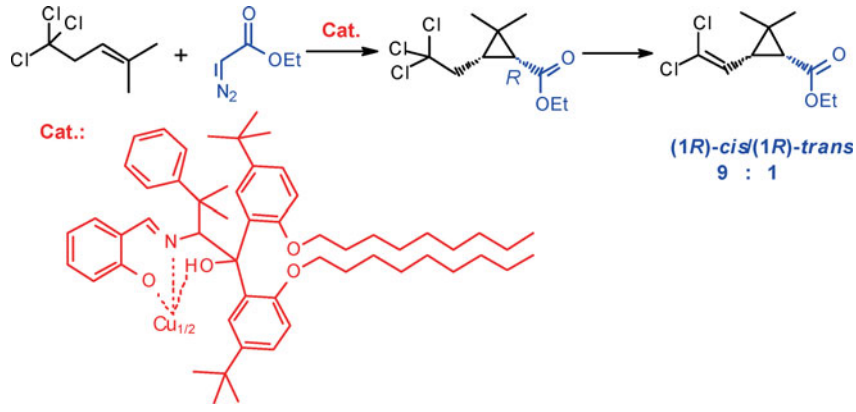


In a similar way, chloroform may be added to isoprenol (which derives from acetone and acetylene). Acid-induced dehydration leads to 5,5,5-trichloro-2-methylpent-2-ene, which can be used directly for the reaction with the diazoacetate ester, or it may optionally be first converted with base to the desired diene.



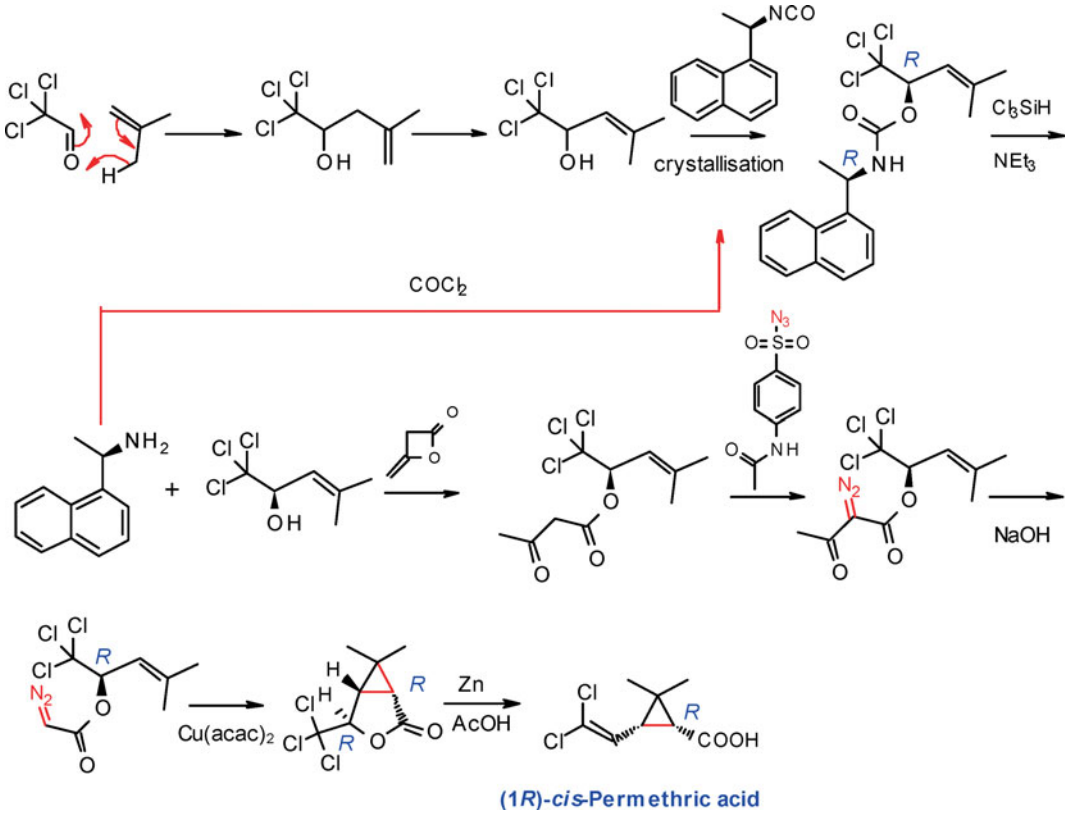
[2 + 1]-Cycloaddition

In general, for the cycloaddition with the diazo-ester, the use of rhodium benzoate is advised, since thereby the reaction temperature may be lowered to 20 °C. The *cis*/*trans*-isomer ratio lies at 2 : 3. On account of the reduced reactivity of the diene, compared to its chlorine-free analogue, it has to be used in a large excess to avoid a range of side-reactions. 5,5,5-Trichloro-2-methylpent-2-ene possesses in contrast higher reactivity and is the preferred substrate for the enantioselective [2 + 1]-cycloaddition, though, at the end an additional dehydrohalogenation step is required.



(1*R*)-*cis*/(1*R*)-*trans*
9 : 1

The process from the FMC company involves as the pivotal step an intramolecular stereoselective [2 + 1]-cycloaddition. In a Prins reaction [94] of chloral and isobutene, followed by an isomerisation, a racemic, trichloromethyl-substituted allyl alcohol is obtained. Reaction with the isocyanate from (*R*)-naphthylethylamine enables separation of the diastereomers by crystallisation. The carbamate is cleaved by trichlorosilane/triethylamine, thus permitting the recycling of the chiral auxiliary. The optically pure (*R*)-allyl alcohol is reacted with diketene, to produce the *β*-keto-ester. After diazo transfer and basic cleavage, the diazoacetate is obtained; catalysed by a copper salt, this is converted in a [2 + 1]-cycloaddition into a bicyclic lactone. The Boord reaction (discovered by Cecil E. Boord in 1930) [95] finally gives (1*R*)-*cis*-permethric acid. [96]



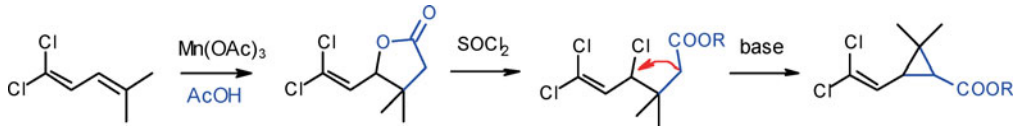
(1*R*)-*cis*-Permethric acid

A few years ago, a research group in Israel published a fundamentally revised version of the procedure. [97] Significant improvements were found in the synthesis of the optically pure allyl alcohol, which was prepared by an enzymatic kinetic resolution, and the diazo transfer is replaced by diazotisation of the corresponding glycine ester.

1,3-Cycloelimination

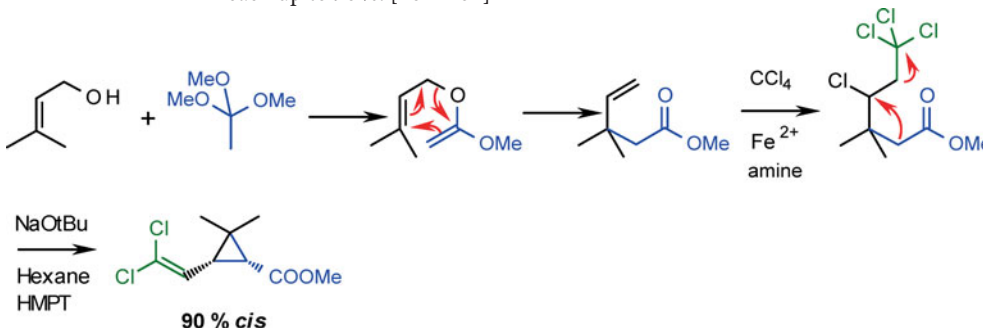
Of striking brevity is the direct addition of acetic acid to 1,1-dichloro-4-methyl-penta-1,3-diene by means of one-electron oxidising agents like manganese (III) acetate [98], cerium(IV) [99] or vanadium(V) salts. [100, 101] The further

transformations by already well-established methods correspond to those for the preparation of chrysanthemic acid.



Immediately after the discovery of the advantageous properties of permethric acid, many research laboratories initiated enhanced efforts to find the most convenient synthesis of this compound. The Japanese Sagami Research Institute developed a synthetic route based on a Claisen rearrangement and a 1,3-cycloelimination, for which FMC took out a licence. A few weeks later, two other firms, Sankyo and Kuraray, filed patent applications for similar syntheses.

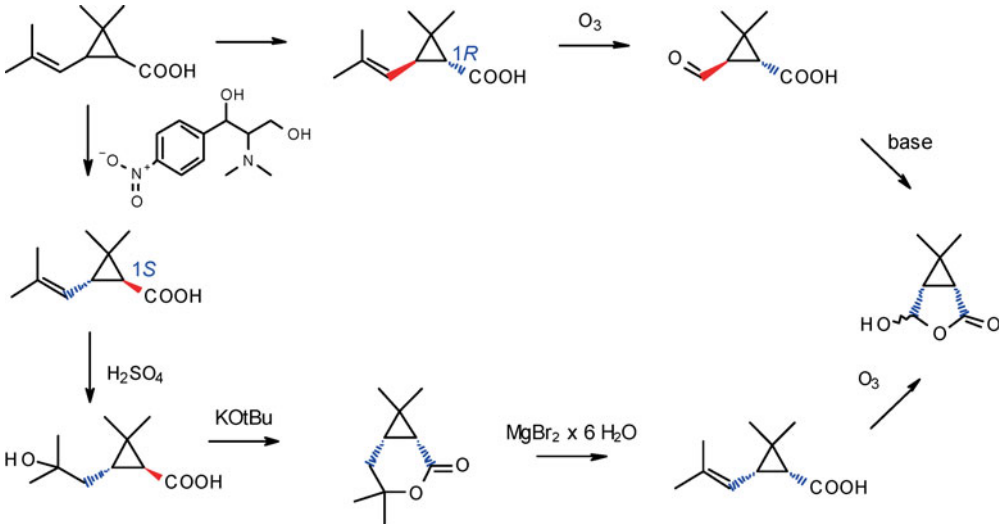
According to the Sagami synthesis, prenol is reacted with methyl orthoacetate. A Claisen rearrangement yields methyl 3,3-dimethylpent-4-enoate, which undergoes a radical addition to tetrachloromethane. The double elimination of hydrogen chloride gives finally permethric acid. In this transformation, the base and the solvent have a critical impact on the *cis*/*trans*-ratio. Under the most favourable conditions (*t*-BuONa, hexane, HMPT) the *cis*-proportion can reach up to 90 %. [102–104]



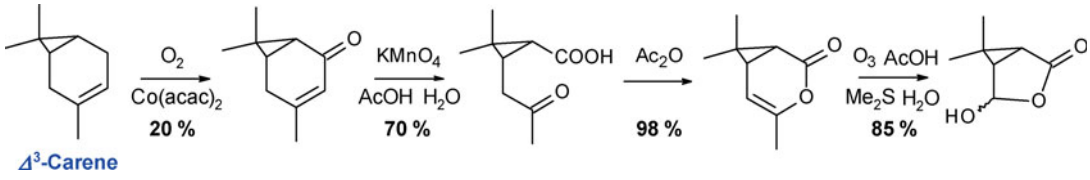
The comparatively expensive orthoester may be replaced by the enol ether of the *β*-keto-ester.

(1*R*)-*cis*-Caronaldehyde Hemiacetal as the Central Synthetic Building Block

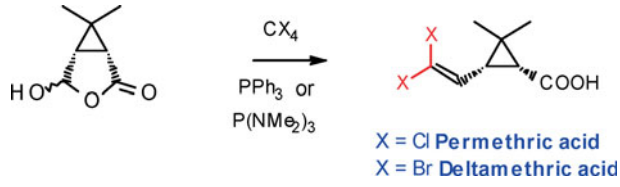
The process for the industrial production of permethric acid by Roussel-Uclaf starts from racemic *trans*-chrysanthemic acid, which is in turn accessed by the Martel synthesis (*cf.* 1,3-cycloelimination). After separation of the enantiomers with an amino-alcohol, the (1*R*)-enantiomer is subjected to ozonolysis. Basic epimerisation gives (1*R*)-*cis*-caronaldehyde hemiacetal. Water is added to the (1*S*)-enantiomer in the presence of a catalytic amount of sulfuric acid; then the carboxylic acid function is epimerised with the formation of a lactone. Magnesium bromide-catalysed ring-opening leads to (1*R*)-*cis*-chrysanthemic acid, which is converted into (1*R*)-*cis*-caronaldehyde hemiacetal by an analogous route. [105]



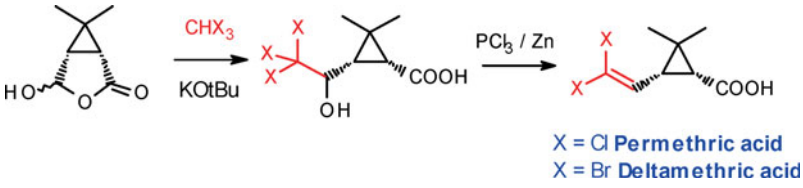
Caronaldehyde hemiacetal is also obtainable in a few stages and in enantiomerically pure form from Δ^3-carene, which occurs in certain varieties of turpentine oil. [106]



Caronaldehyde hemiacetal can be transformed in a simple manner by the Corey-Fuchs reaction [107] into permethric acid, and as well its bromo-analogue deltamethric acid. Instead of triphenylphosphane, tris-(dimethylamino) phosphane may also be used.

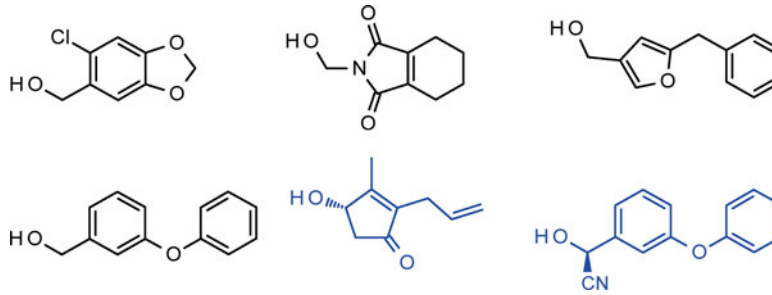


It is however less expensive to introduce the dihalogenomethyl moiety by means of haloform. The dehalogenation is achieved with zinc.



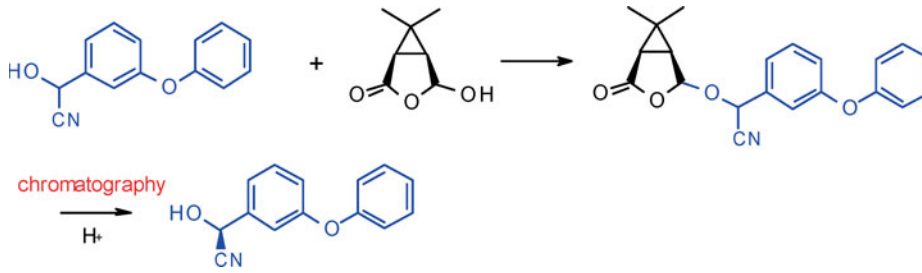
8.3.5 The Alcohol Moiety of Synthetic Pyrethroids

The alcohol residue of the commercial pyrethroids shows a considerable diversity. Just a few of the alcohols are listed here, the discussion of which focuses only on the chiral compounds.

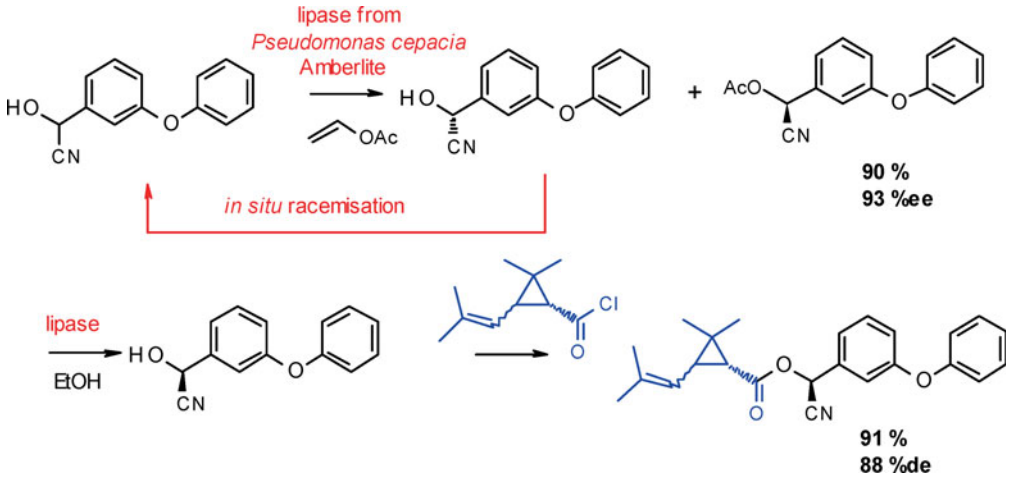


Enantiomerically Pure Cyanohydrins

The insecticidal effect of esters of *meta*-phenoxymandelonitrile is associated with the (*S*)-configuration of the latter. The synthetic challenge is given by the fact that cyanohydrins are readily racemised, especially in basic media. Phenoxymandelonitrile is obtained by Ullmann coupling of potassium phenoxide and *m*-bromobenzaldehyde, followed by reaction with hydrogen cyanide. In 1980, Jacques J. Martel was the first to succeeded in reacting the cyanohydrin as its racemic mixture with (1*R*)-*cis*-caronaldehyde hemiacetal and separating the (*S*)-lactol ether by chromatography. [108]

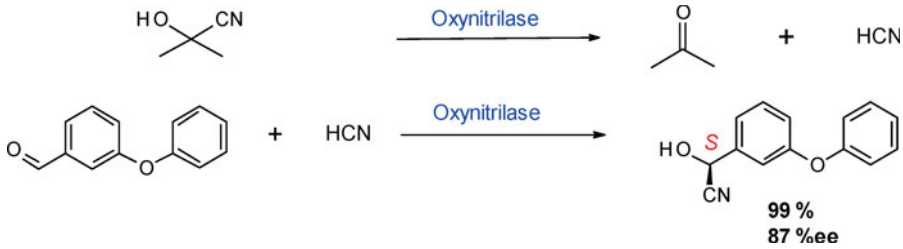


Markedly elegant are the enzymatic kinetic resolution and *in situ* racemisation of the cyanohydrin (dynamic kinetic resolution). [109, 110] The propensity of cyanohydrins for racemisation allows to esterify stereoselectively just the (*S*)-enantiomer with lipases. In a subsequent step, the ester is then enzymatically hydrolysed.



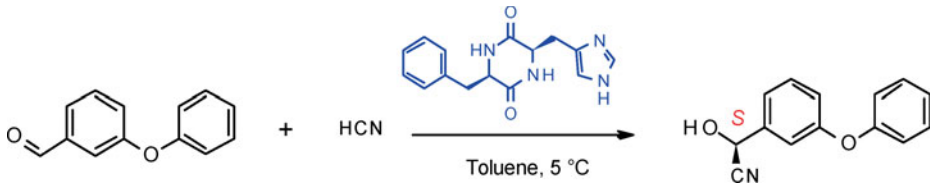
Addition of hydrogen cyanide to aldehydes by means of oxynitrilases gives cyanohydrins in high yields and high optical purity. It is often unnecessary to work with free hydrogen cyanide; instead, the cyanohydrin of acetone can be used to form hydrogen cyanide *in situ*, which is then reacted with the aldehyde. The oxynitrilases are compatible with organic solvents or multiphase solvent systems. [111, 112]

Oxynitrilases are enzymes, which are part of the defence systems of certain plants, like the bitter almond (*Prunus amygdalus*), rubber tree (*Hevea brasiliensis*), cassava (also called manioc or yucca, *Manihot esculenta*) and sorghum (*Sorghum*) against herbivores. In a "defence situation", they release hydrogen cyanide from the corresponding glycosides, as *e.g.* amygdalin.

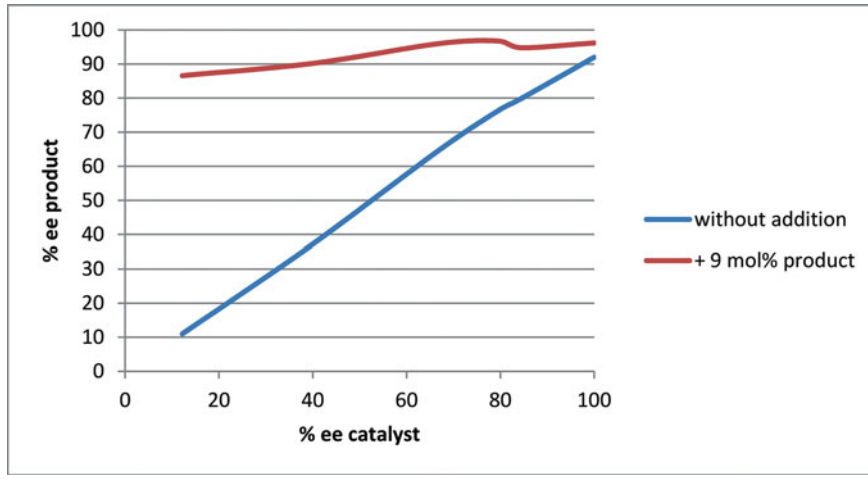


DSM developed a recombinant oxynitrilase from the rubber tree (*Hevea brasiliensis*), with which it is possible to produce the cyanohydrin of (*S*)-3-phenoxybenzaldehyde with an enantiomeric excess of 98.5 % and a space/time-yield of 1,000 g/l/day. [113]

One of the most interesting developments in recent years is the non-enzymatic, enantioselective, organocatalytic addition of hydrogen cyanide to aldehydes. This reaction is, for example, very efficiently catalysed by optically pure diketopiperazines, such as *cyclo*-(*R*)-Phe-(*R*)-His), which are readily accessible from the corresponding amino acids. [114–116]



Enantioselective autoinduction was also investigated (Fig. 8.27). [117] The reaction of an aldehyde with hydrogen cyanide in presence of the chiral diketopiperazine, the enantiomeric purity of which varies from 12 to 100 %, gives the cyanohydrin in the same optical purity as that of the catalyst (blue curve). However, if to the mixture of the aldehyde and the diketopiperazine with varying optical purity, 9 mole % of (*S*)-cyanohydrin in an optical purity of 92 % ee is added prior to the addition of hydrogen cyanide, the enantiomeric excess in the product is almost independent of the optical purity of the catalyst itself (red curve).

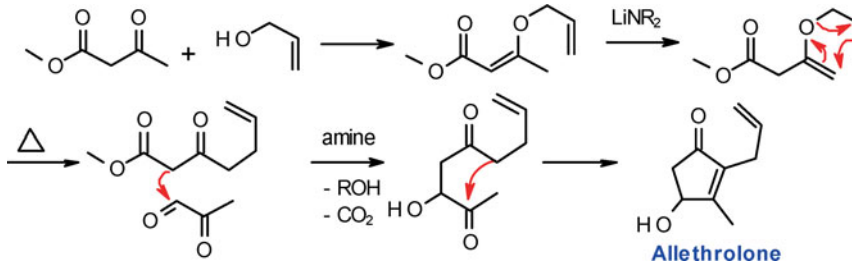


8.27 *Autocatalytic formation of an enantiomerically pure cyanohydrin.*

If almost racemic diketopiperazine is used under these conditions, after all, an enantiomeric excess of 81 % can still be achieved. However, in case the reaction is carried out in the absence of the diketopiperazine, no transformation is observed. The active catalytic species is probably an adduct of the diketopiperazine and the cyanohydrin, being a more powerful catalyst than the diketopiperazine alone.

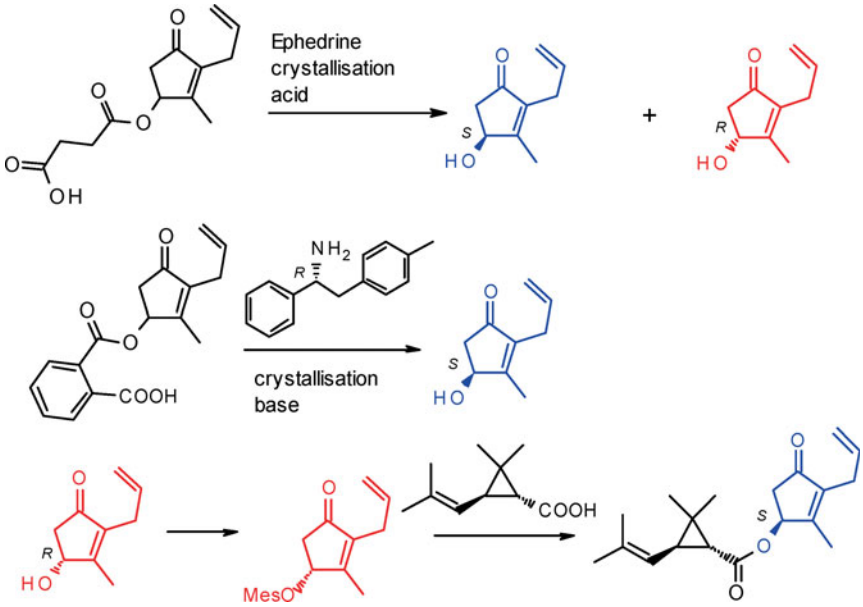
Cyclopentenolones

The industrial synthesis of allethrolone follows a route developed by Frederick B. La Forge. [118, 119] Methyl acetoacetate is etherified with allyl alcohol. Deconjugation of the double bond enables a Claisen rearrangement. The resulting β-keto-ester is reacted with pyruvaldehyde in presence of an amine, and an intramolecular aldol condensation leads then to racemic allethrolone.

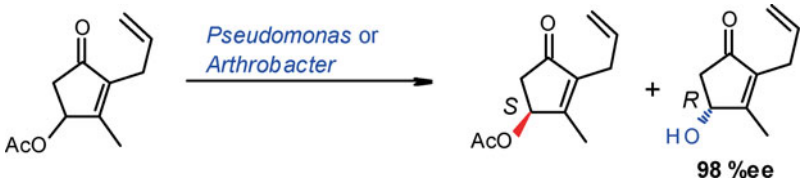


If the final aldol condensation is carried out in presence of cinchonine, one enantiomer is formed preferentially. [120] Higher homologues and the corresponding alkyne may be prepared in an analogous fashion.

Optically pure allethrolone can be accessed by the classical route. The half-ester of succinic acid is separated with ephedrine and the half-ester of phthalic acid with (–)-1-phenyl-2-*p*-tolylethylamine. The (*R*)-enantiomer of the corresponding mesylate is subsequently converted with chrysanthemic acid into the desired product.



Enzymatic kinetic resolution provides another way to enantiomerically pure allethrolone. [121]



Summary in Bullet Points

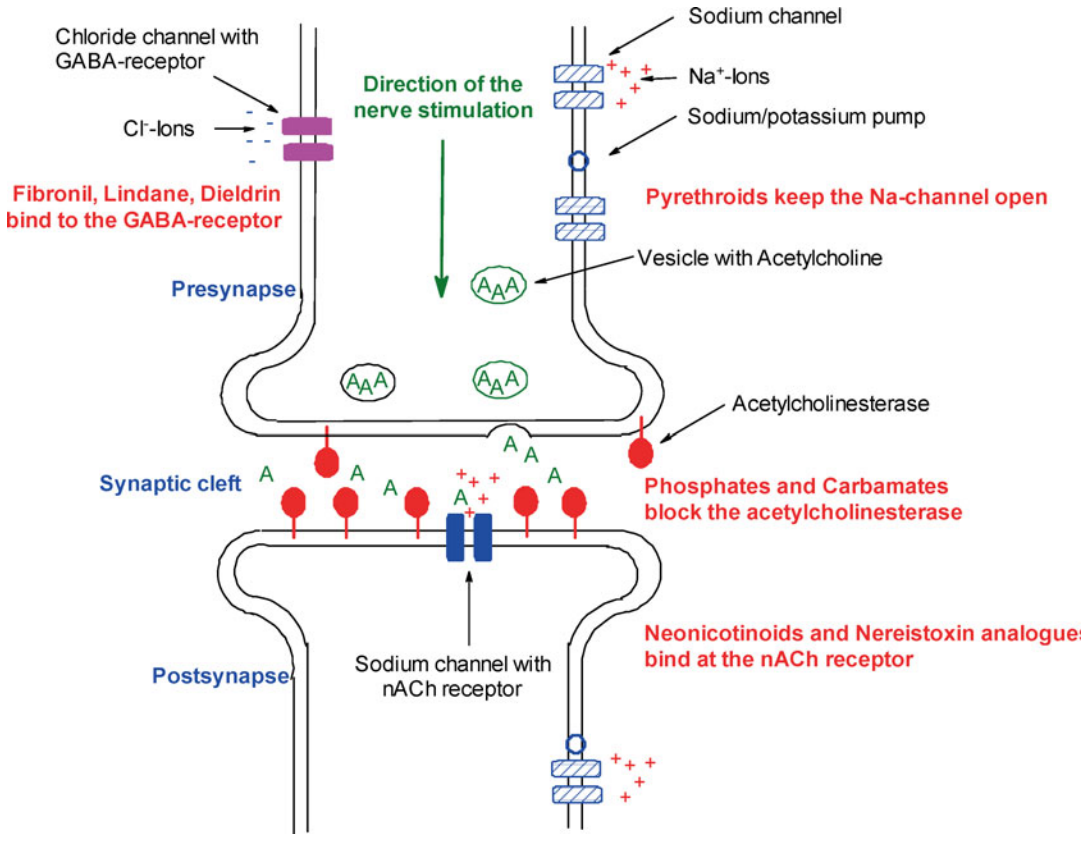
- Pyrethrum has been obtained for a long time from the blooms of chrysanthemums.
- Contrary to the terpene rule, the biosynthesis involves tail-to-tail coupling of two molecules of dimethylallyl diphosphate.
- The toxic effect is based on a disruption of electrical signaling in the nervous system. The pyrethroids alter primarily the proper function of neuronal sodium channels.

- Only the (1*R*)-diastereomers possess insecticidal activity.
- A breakthrough in the use of pyrethroids as agricultural insecticides came with the discovery that esters of permethric acid are both highly potent and exhibit remarkable photostability.
- Important synthetic concepts are [2 + 1]-cycloaddition and 1,3-cycloelimination.

8.4 Neonicotinoids

8.4.1 Insecticides Acting at the Cholinergic Synapse

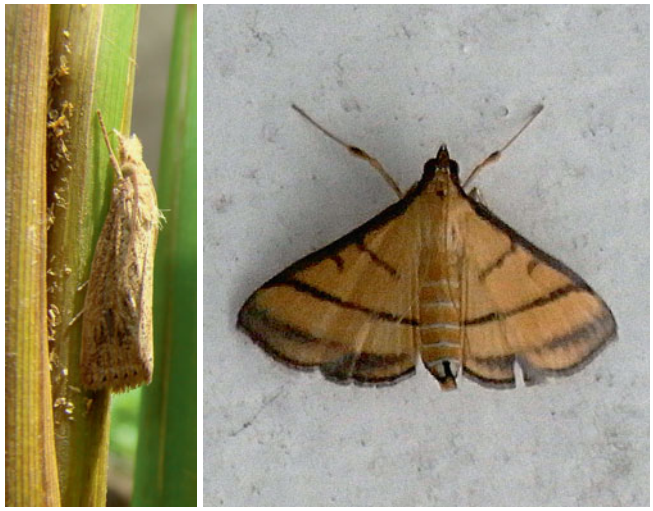
The insect nervous system provides the preferred taget pool for all major insecticide classes (Fig. 8.28). Whereas the pyrethroids deregulate signal transduction by keeping the presynaptic sodium channel open for a prolonged period, the carbamates inhibit reversibly and the phosphate esters irreversibly acetylcholinesterase, leading to an overload of the neurotransmitter acetylcholine in the synaptic cleft. Fipronil blocks the ion-flow in GABA-regulated chloride chan-



8.28 *The target of many insecticides is the cholinergic synapse of the insects.*

nels, which causes excessive neural excitation. A range of chlorinated hydrocarbons, like lindane, interact at the same target.

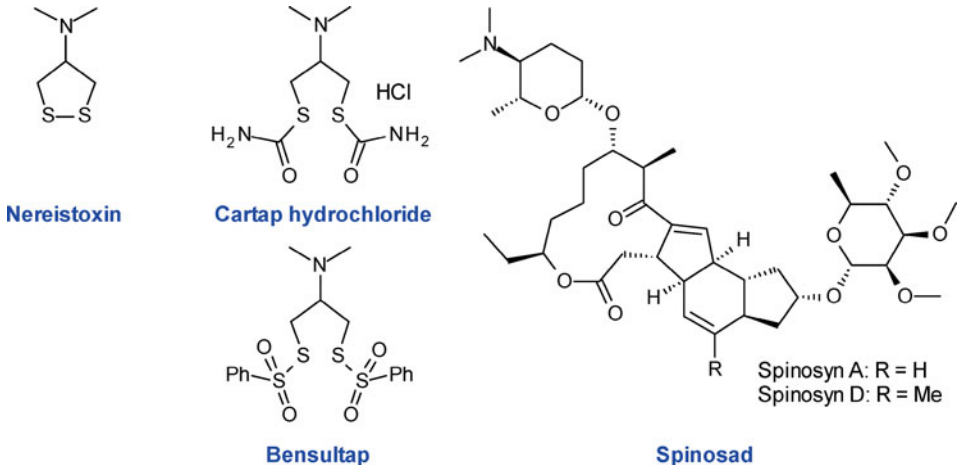
Nicotine was the first insecticide to be identified as targeting the cholinergic postsynapse. The second class of compounds aiming at this same target comprises nereistoxin and its analogues. In the 1960s, Takeda marketed two products to control important rice pests like the striped rice stem borer (*Chilo suppressalis*) and the rice leafroller (*Cnaphalocrocis medinalis*); these agents were derived from nereistoxin, the poison of the marine annelid *Lumbrineris heteropoda* (Fig. 8.29).



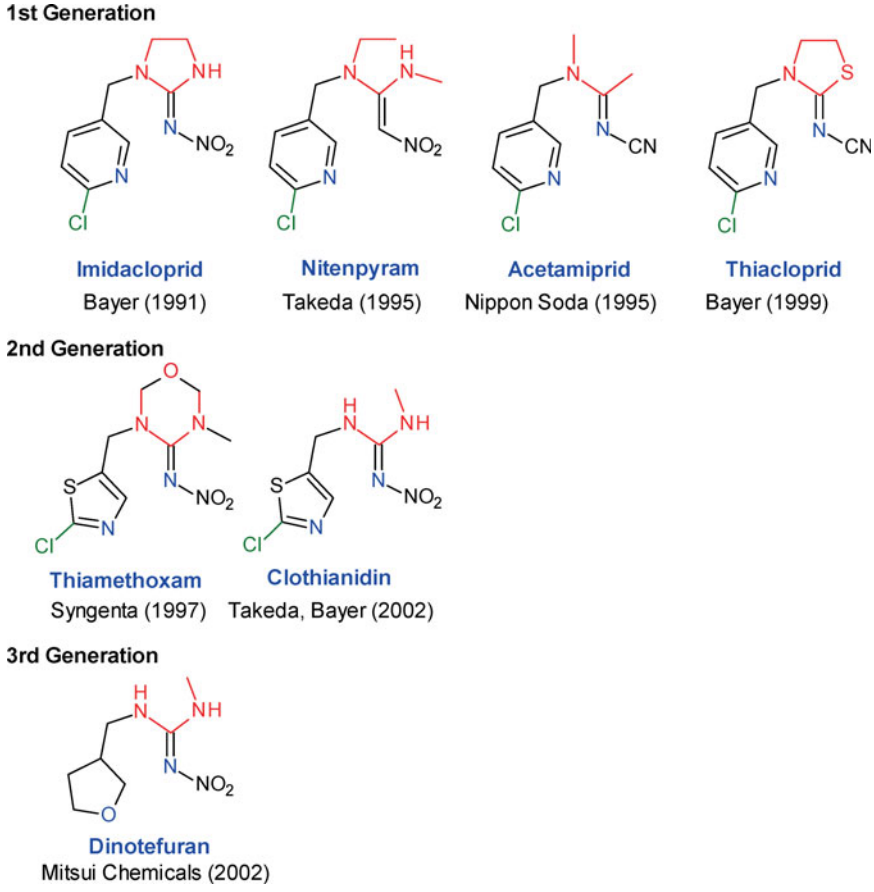
8.29 *Striped Rice Stem Borer (Chilo suppressalis) (left) and Rice Leafroller (Cnaphalocrocis medinalis) (right).*

The spinosyns act in a similar, though rather unique way on the cholinergic postsynapse, where they activate the nicotinic acetylcholine- (nACh), but antagonise the γ-aminobutyric acid (GABA)-receptors, without however interacting at any of the known binding-sites. [122] Spinosad is a defined combination of two polyketide-derived macrocyclic lactones, spinosyns A and D, in the ratio of around 85 : 15; these were isolated from fermentation broths of the soil bacterium *Saccharopolyspora spinosa*, which belongs to the *Actinomycetales*. The species was originally isolated by researchers at Eli Lilly from soil samples collected at a rum still in the Virgin Islands and was characterised by its pale yellowish pink aerial hyphae that bear long chains of spores encased in distinctive spiny spore sheaths. [122] In 1997, Dow AgroSciences brought Spinosad on to the market as an insecticide for the control of moths (*Lepidoptera*) and beetles (*Coleoptera*) (Fig. 8.30).

Up to now, a total of seven neonicotinoids have been commercially launched (Fig. 8.31). [122] These can be divided into generational sub-classes with distinct structural features. The active compounds of the first generation contain a 6-chloro-3-pyridyl substituent, whereas the second generation agents share a 2-chloro-5-thiazolyl moiety. Dinotefuran is the first representative of the third generation, with a tetrahydrofuran-3-yl substituent.



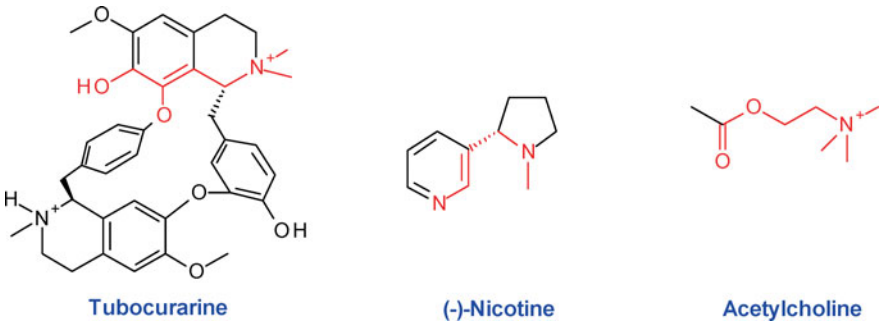
8.30 *Insecticidal agents, which target at the cholinergic postsynapse.*



8.31 *The structures of neonicotinoids.*

8.4.2 Physiology and Pharmacology

In 1905, John Newport Langley (1852–1925) reported on his experiments, carried out at Cambridge University, with nicotine and curare on the skeletal muscles of frogs and chickens, where he formulated the concept of cellular "receptive substances" (we would say 'structures'), which mediate the activity of these agents. [123] This represents the actual foundation of a neuronal receptor theory (Fig. 8.32), and led Paul Ehrlich two years later to propose more generally the existence of "chemoreceptors" for drugs.



8.32 *Tubocurarine and nicotine act like acetylcholine at its receptor on the postjunctional motor endplate of the neuromuscular junction. Both alkaloids were of crucial importance for the development of the neuronal receptor theory at the beginning of the last century.*

In 1914, Sir Henry Hallett Dale postulated acetylcholine as a possible natural neurotransmitter (Fig. 8.33). [124] In the course of time it became apparent that acetylcholine is widely distributed, and plays an important role in both, the central and the peripheral nervous system.

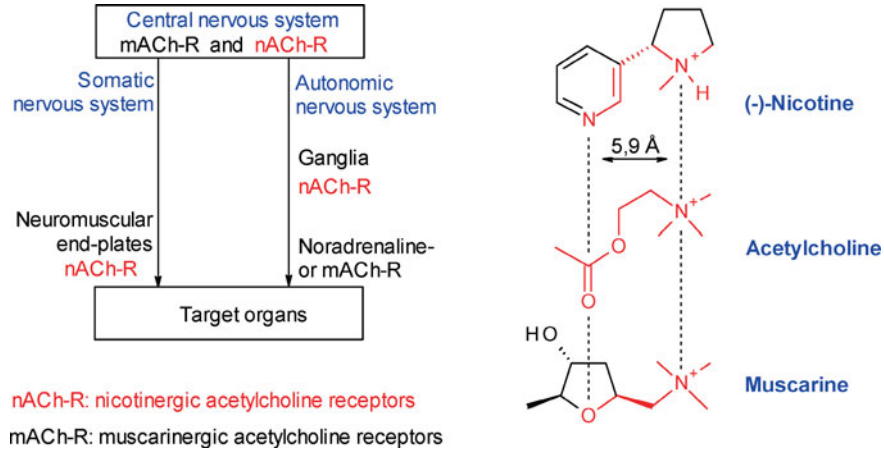
8.33 *Sir Henry Hallett Dale (1875–1968).*

Nicotinic Acetylcholine Receptors

According to H. H. Dale, the acetylcholine receptors are divided into a muscarinic (mAChR) and a nicotinic (nAChR) subtype, depending on which ligand, muscarine or nicotine, preferentially binds to these receptors (Fig. 8.34 and Fig. 8.35).

A pharmacophore model of the three ligand structures suggests, that for recognition at the acetylcholine receptor sites, the pyridine nitrogen, the tetrahydrofuran oxygen and the acetyl group should be in an approximately equidistant position to the ammonium group, and that each of these moieties serves as a hydrogen bond acceptor. [125]

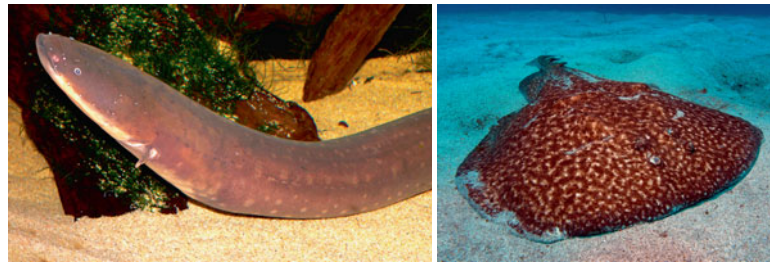
8.34 *In 1869, Oswald Schmiedeberg (1838–1921) and Richard Koppe at the University of Dorpat (Tartu, Estonia) discovered muscarine in the fungus fly agaric (Amanita muscaria).*



8.35 *Distribution of nicotinic and muscarinic acetylcholine receptors in the central and peripheral nervous system of vertebrate animals (Vertebrata).*

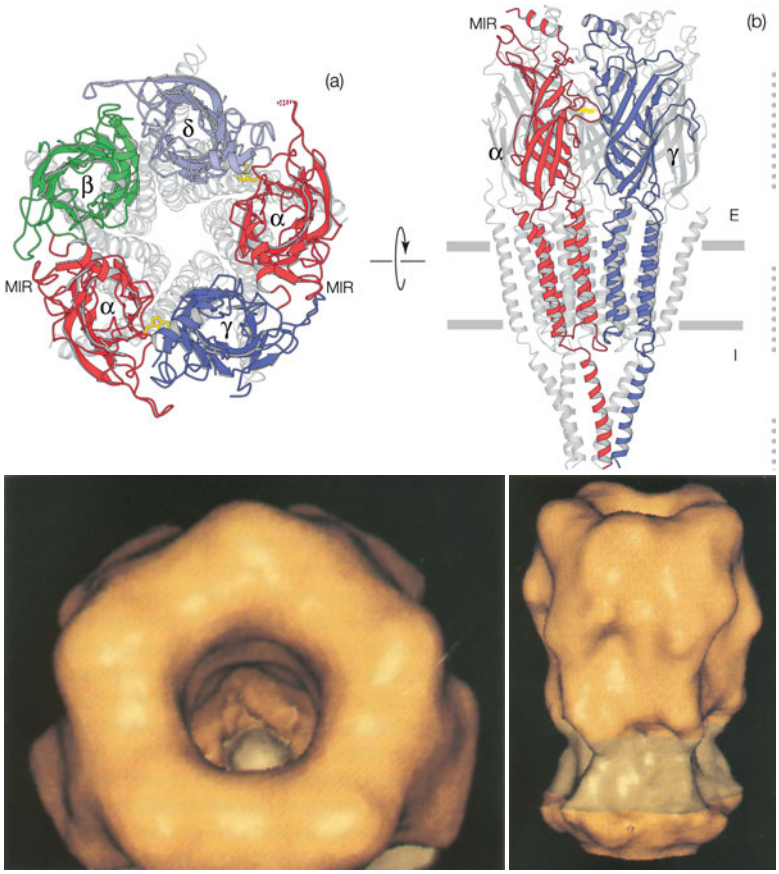
Up to the present, the most thoroughly investigated are the vertebrate nicotinic acetylcholine receptors. They are not only found in the brain, on the neuromuscular endplates of the somatic nervous system, and in the ganglia of the autonomic nervous system, but also on nonexcitable cells like keratinocytes. This more recent paradigm shift indicates that even more scientific revelations are to be expected in clarifying the underlying signaling mechanisms within this sophisticated system. [126]

The richest source of nicotinic acetylcholine receptors are the electric organs of the South American electric eel (*Electrophorus electricus*), the African electric catfish (*Malapterurus electricus*) and the electric ray (*e.g.* the marbled electric ray of the eastern Atlantic Ocean (*Torpedo marmorata*) and the Californian electric ray in the northeastern Pacific (*Torpedo californica*)) (Fig. 8.36).



8.36 *Electric eel (Electrophorus electricus) (left) and marbled electric ray (Torpedo marmorata) (right).*

The nicotinic acetylcholine receptor was initially isolated, as the first ligand-gated receptor, from the electric organ of the electric ray, which contains more than 100 mg of receptor protein per kg of wet tissue. The purification of this 290 kDa protein involved density gradient centrifugation and affinity chromatography on columns with immobilised cobratoxin. Meanwhile, the receptor morphology has been studied by high-resolution electron microscopy to yield refined structures at 4 Å resolution. Cloning of this extensive super-family of receptors has provided valuable insight into its complex pharmacology (Fig. 8.37). [127]



8.37 *The nicotinic acetylcholine receptor is a ligand-gated ion channel. The five rod-shaped protein subunits are aligned with five-fold symmetry over almost their entire length. The binding site of acetylcholine (gold) is located between the α-γ- and α-δ-subunits (a). The receptor has a length of 16 nm and a diameter of 8 nm (b). It extends around 6 nm into the extra-cellular space and only 1.5 nm into the cytoplasm. The channel runs perpendicularly to the plane of the phospholipid bilayer. [125, 128]*

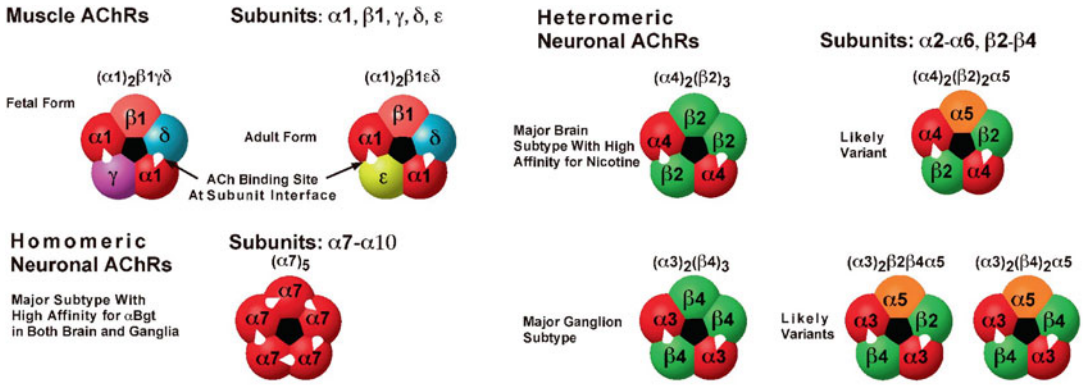
In the electric organs there are stacks of up to 5,000 flat disk-like cells (electroplaques), which can generate at any one time a voltage of 130 mV. When suddenly discharged in series, voltages of up to 650 V and currents of up to 30 A can be generated, with which the electric fish defend themselves and kill their prey. [129]

Evolution has spawn over time a large variety of nicotinic acetylcholine receptor subtypes. The receptor of the electric ray consists of two identical α_1-subunits and another three different, β_1, γ and δ subunits. The structure of the nicotinic acetylcholine receptor at the neuromuscular endplate is similarly configured.

In contrast, the nicotinic acetylcholine receptors in the ganglia of the autonomic nervous systems are formed, for example, by $\alpha_{2\text{-}4}$ and $\beta_{2\text{-}4}$-units, and those of the central nervous system are assembled from α_7- and α_8-units. Up to the present time, at least 17 sub-types of nicotinic acetylcholine receptors ($\alpha_{1\text{-}10}$, $\beta_{1\text{-}4}$, γ, ε, δ) are known (Fig. 8.38).

In foetal muscular acetylcholine receptors, the binding pockets for acetylcholine are located between the α-γ- and α-δ-subunits, whereby the two show different binding affinities to acetylcholine, being higher for the α-δ- than for

Interestingly, the Roman physicians Claudius Galenus (129–199 AD) and Scribonius Largus (court physician to the Roman emperor Claudius) had already described in their collections of medical recipes (around 47 AD) the use of electric shocks with the Atlantic electric ray (*Torpedo nobiliana*) for the treatment of gout.



8.38 *Subtypes of the acetylcholine receptor.*

the α-γ-subunit. The region of these binding pockets is in all receptors highly conserved. The *α*-subunit contains a set of aromatic amino acids, which contribute to binding acetylcholine by cation-π-interactions. These features are found in the adult form of the muscular receptors, as well as in both, the homomeric and heteromeric forms of the neuronal acetylcholine receptor.

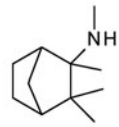
Ligands of the Nicotinic Acetylcholine Receptors

There are different functional effects of how ligands can modulate receptors. Also the neurotoxins, which interact with the nicotinic acetylcholine receptor, are classified accordingly:

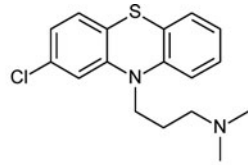
1) Agonists: Three kinds of agonistic ligands (either full, or partial, or inverse agonists) are normally distinguished, when they compete with acetylcholine at the nAChR binding sites and produce the respective functional response of the ion channel. However, agonistic and antagonistic properties can be concentration dependent. In the case of nicotine, both enantiomers function as full or partial agonists, depending on the individual subtype of nicotinic acetylcholine receptors; the (*S*)-enantiomer is however one to two orders of magnitude more potent. [130] The neonicotinoids cause also an agonistic response at the corresponding insect receptor.
2) Antagonists: Reverse the effects of receptor agonists; they can act either in a competitive or non-competitive manner.
   a. Competitive antagonists: These displace acetylcholine from the receptor binding sites without triggering the opening of the ion channel. Examples are α-bungarotoxin, the poison of the Taiwanese many-banded krait (*Bungarus multicinctus*), the α-conotoxins of the cone snails (*Conidae*), nereistoxin from *Lumbrineris heteropoda* and tubocurarine from the seeds of *Strychnos toxifera* and *Chondrodendron tomentosum*.
   b. Non-competitive antagonists: These block the ion channel, although without displacing acetylcholine from its binding sites. They either clog the ion channel, or bind in an allosteric mode, *i.e.* altering the protein conformation from a remote site and thereby preventing the interaction with acetylcholine. Examples are mecamylamine and chlorpromazine.

Excursus

Mecamylamine was developed by Merck in the 1950s as an antihypertensive agent. Nowadays, it finds more frequent use to manage smoking cessation. Its (*S*)-enantiomer has been recently evaluated in clinical trials for antidepressant properties. – Chlorpromazine, the prototype of phenothiazine drugs, has been hailed as "the single biggest advance in psychiatric treatment", which dramatically improved the prognosis of patients with illnesses like schizophrenia. In his search for a new antihistamine, Paul Charpentier first synthesised this molecule (code name: 4560 RP) at Rhône-Poulenc; in November of 1952 (!), the drug became already available on prescription in France. The interaction of Chlorpromazine with multiple molecular targets, and the associated 'polypharmacology' required the subsequent development of more specific drugs.



Mecamylamine



Chlorpromazine

Not surprisingly, different ligands exhibit a characteristic individual affinity profile at the subtypes of the nicotinic acetylcholine receptors (Tab. 8.2). [131] These compounds served as suitable tools to investigate the tissue distribution of such receptors in the central and peripheral nervous systems.

Whereas acetylcholine has a relatively high affinity for the $(\alpha_1)_2\beta_1\delta$- and the $(\alpha_4)_2(\beta_2)_3$-receptor, and binds in addition to the muscarinic receptor at the nanomolar level, (*S*)-nicotine shows the same preference, but does not bind to the latter. Cytisine binds more selectively to the $(\alpha_4)_2(\beta_2)_3$-receptor, while epiba-

Tab. 8.2 *Ligand binding profiles at various nicotinic acetylcholine receptor subtypes*

Ligand	K_i $((\alpha_1)_2\,\beta_1\,\delta)$ [nM]	K_i $((\alpha_3)_2\,(\beta_4)_3)$ [nM]	K_i $((\alpha_4)_2\,(\beta_2)_3)$ [nM]	K_i $(\alpha_7)_5$ [nM]
Acetylcholine	15	520	29	2200
Choline		/	7000	180,000
(*S*)-Nicotine	1	73	1.0	1600
(*R*)-Nicotine		810	38	16,000
(*S*)-Nornicotine		610	23	>10,000
Anabasine		38	65	58
Epibatidine	2*	0.051	0.058	21
Cytisine		54	0.12	260
Anatoxin A		2.5	0.34	91
α-Bungarotoxin	0.35–3.5*	very low affinity	very low affinity	0.35–3.5
Mecamylamine	37**	0.86	2.4	1.1

**: muscle type*
***: foetal receptor*

8.39 *α-Bungarotoxin is a peptide of 74 amino acids from the toxin of the many-banded krait (also known as Taiwanese krait or Chinese krait, Bungarus multicinctus), a member of the poison viper family, which occurs mainly in Taiwan.*

8.40 *A hunting cone snail (Conus textile). Currently, more than 2,000 species are known. They occur in every ocean in the World. From the phylogenetic viewpoint, cone snails have a strikingly broad spectrum of prey. This stretches from other snails via worms to fish. Each species has its own toxins, consisting of diverse cocktails of up to 200 (from a pool of ca. 50,000) different neuroactive peptides, some of which can also be dangerous to humans.*

tidine and anatoxin A possess in addition equal or similar affinity for the $(\alpha_3)_2(\beta_4)_3$-subtype.

Apart from these alkaloids, also small peptides, like α-bungarotoxin (Fig. 8.39) and various α-conotoxins, proved to be very valuable biochemical tools to investigate these receptor species. α-Bungarotoxin binds with high affinity and irreversibly to the heteropentameric receptors $(\alpha_1)2\beta_1\gamma\delta$, $(\alpha_1)_2\beta_1\varepsilon\delta$, and the homomeric receptors α_7, α_8 and α_9, whereas no affinity was detected at heteropentameric receptors of the neuronal type, which are built up from α_2–α_6 and β_2–β_4-subunits.

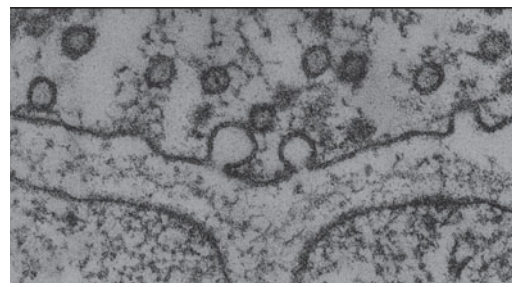
The α-conotoxins of cone snails, twelve to sixteen amino acids in length, are among the smallest known peptidic, nAChR antagonists (Fig. 8.40). [132, 133] These bind with remarkable selectivity at the different subtypes of neuronal nicotinic receptors and at those of the neuromuscular endplate. The α-conotoxin MII (GlyCysCysSerAsnProValCysHisLeuGluHisSerAsnLeuCysNH_2 [134]) from *Conus magnus,* for instance, inhibits the $(\alpha_3)_2(\beta_2)_3$-receptor in the low to sub-nanomolar region, with a > 200-fold selectivity over related receptors.

Conversely, α-conotoxin ImI (GlyCysCysSerAspProArgCysAlaTryArgCysNH_2 [134]) from the cone snail *Conus imperialis* preferentially inhibits the homomeric α_7- and α_9-receptors, but not, like α-bungarotoxin, receptor combinations composed of α_1-subunits. Selectivities across species have been documented as well: Whereas the poison from the many-banded krait acts primarily on mammalian nerve cells, α-conotoxin ImI blocks for example also the nicotinic α_7-receptors of locusts (*Locusta migratoria*).

Mode of Action of the Acetylcholine Receptor

The acetylcholine receptors control ion channels and thus facilitate the propagation of an arriving action potential and the depolarisation of a postsynaptic target cell. Whereas the muscarinic receptor-type (mAChR) is coupled to a G-protein (guanosine nucleotide-binding membrane protein), which mediates the intracellular signal transduction to open an ion channel, the nicotinic acetylcholine receptor (nAChR) itself is an ion channel, operated by the ligand interaction directly. [126]

In the case of a stimulus, acetylcholine is released from presynaptic vesicles (exocytosis) into the synaptic cleft and diffuses in fewer than 100 µs to the membrane of a postsynaptic target cell, where it interacts with its receptor (Fig. 8.41).



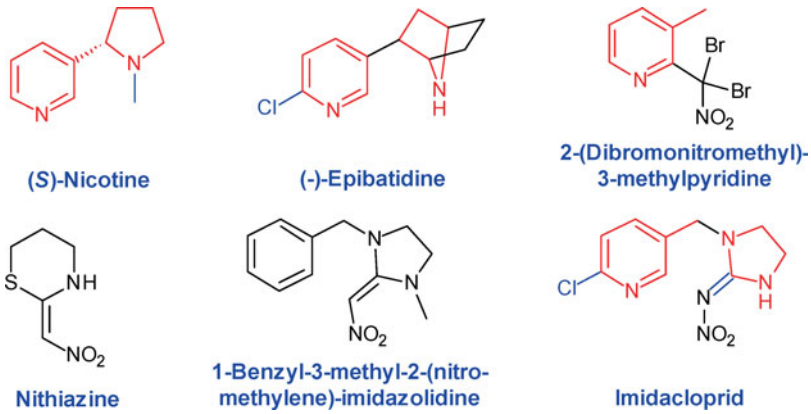
8.41 *Electron-microscopic photograph of a frog motor nerve terminal, capturing the presynaptic vesicle docking, fusion and exocytosis at the neuromuscular junction.*

Upon cooperative interaction with an agonistic ligand, like acetylcholine or nicotine, at both α-subunits of the receptor, the cation-selective channel undergoes a conformational change that widens its pore to a diameter of 0.7 to 2.5–3 nm. This enables the cross-membrane flow of extracellular sodium or calcium ions into the cytosol of the cell. While homopentameric receptors have five binding sites, it is already sufficent to open the ion gate, as soon as two of them are occupied.

This leads to depolarisation of the postsynaptic membrane and thus triggers a new action potential. After one to two milliseconds, acetylcholine diffuses off the receptor, whereupon the channel closes again. Acetylcholine is hydrolysed in the synaptic cleft by acetylcholinesterase prior to arrival of the next nerve signal. The generated choline is reabsorbed through the presynaptic membrane and re-esterified by choline acetyltransferase. At low concentration, nicotine depolarises the postsynaptic membrane just like acetylcholine, which leads to comparable stimulation of the ganglia. At high concentration, however, the continuous depolarisation of the cell membrane causes a functional antagonism (depolarisation block), muscular paralysis and death due to respiratory failure.

8.4.3 Discovery of the Neonicotinoids

Comparing the structures of nicotine and epibatidine to the first neonicotinoid, imidacloprid, one may suspect the example of a consequent further development of a highly potent lead compound from the natural products pool (Fig. 8.42). This is indeed not the case. Nicotine has been used as an insecticide for more than a hundred years. However, Izuru Yamamoto's structure variations in the 1960s did not result in any promising active substances. [135] Epibatidine, the nAChR agonist from a poisonous frog skin, which could have served as a pharmacophore template, had still not been discovered at the time when neonicotinoids were being developed.



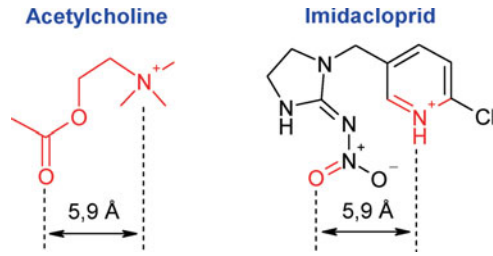
8.42 *Natural and synthetic compounds related to imidacloprid.*

Nithiazine

In 1978, at a IUPAC conference in Zurich, Samuel Barney Soloway from the Shell organisation reported on the insecticidal activity of nitro-substituted ketene aminals. These compounds resulted from syntheses around 2-(dibromonitromethyl)-3-methylpyridine, a molecule, which Henry Feuer at Purdue University had submitted for screening at Shell in 1970. Nithiazine turned out to be the most active representative of this series, and was selected as a development candidate. However, due to its photolability, the compound never reached the market, except as the active ingredient in a flytrap. Additional structure variations at Shell led, among others, also to 1-benzyl-3-methyl-2-(nitromethylene) imidazolidine, a compound almost devoid of activity.

Imidacloprid

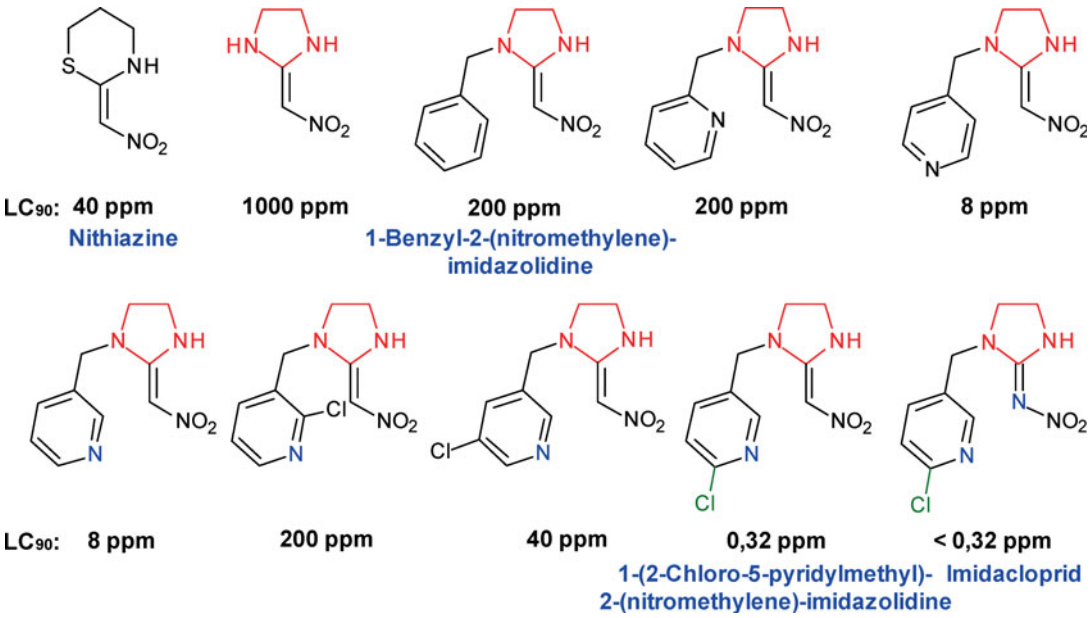
While the plant protection division of Shell was sold to Dupont in 1986, the neonicotinoid research came to a halt. Independently, chemists at Nihon Tokushu Noyaku Seizo K. K. (now part of Bayer CropScience) in Japan had begun to modify the nitromethylene lead structure already at the beginning of the 1980s. Among other changes, they replaced the phenyl substituent in 1-benzyl-2-(nitromethylene)-imidazolidine by a 2-pyridyl unit, and found that this derivative had comparatively low insecticidal activity. With a 3- or 4-pyridyl substituent, the potency increased however by a factor of around 25. Retrospectively, it became clear why the introduction of a 3-pyridyl residue was so beneficial (Fig. 8.43). [136, 137]



8.43 *Structural resemblances between acetylcholine and imidacloprid.*

An additional chloro-substituent at the carbon atom next to nitrogen provided a further gain in activity by a factor of around 625. The combination of the apparently xenobiotic 2-chloro-5-pyridyl residue with the novel nitroguanidine moiety led after some 2,000 structure variations eventually to the identification of imidacloprid, a very potent, light-stable insecticide with systemic activity and low toxicity for vertebrates (Fig. 8.44).

LC_{90}: the lethal concentration, at which 90 % of the insects die within four days.



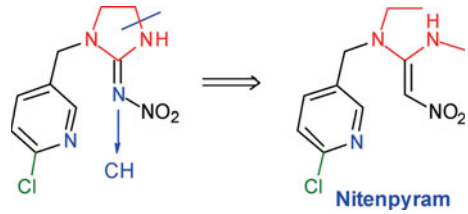
8.44 *Selected structure-activity relationship along the development path from nithiazine to imidacloprid.*

Nitenpyram

Whereas S. B. Soloway had originally assumed that the for insecticidal activity critical nitromethylene unit would have to be attached to a heterocycle, scientists at Takeda Chemicals were able to show that acyclic compounds possessed good insecticidal properties as well. They developed a product to control two important pests in rice, both are cicada species: the brown planthopper (*Nilaparvata lugens*) and the green rice leafhopper (*Nephotettix cincticeps*) (Fig. 8.45).

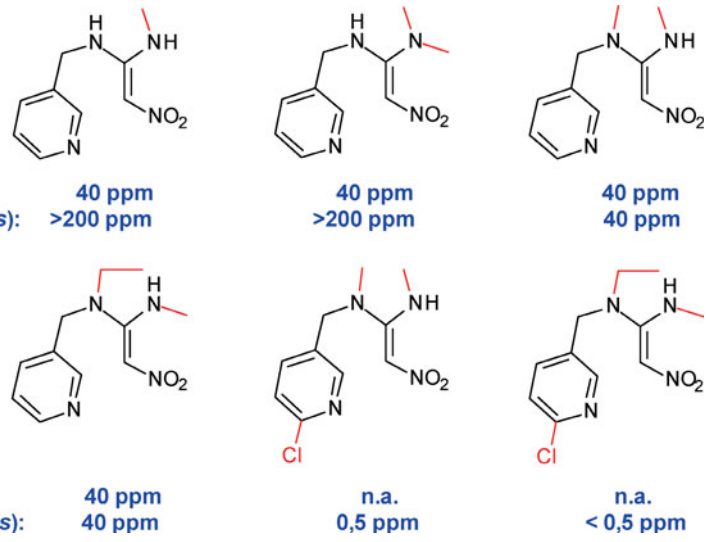
8.45 *The brown planthopper (Nilaparvata lugens) (left) and green rice leafhopper (Nephotettix cincticeps) (right).*

A ring-open derivative from Nihon's development programme, nitenpyram, became in the end the commercial product (Fig. 8.46).



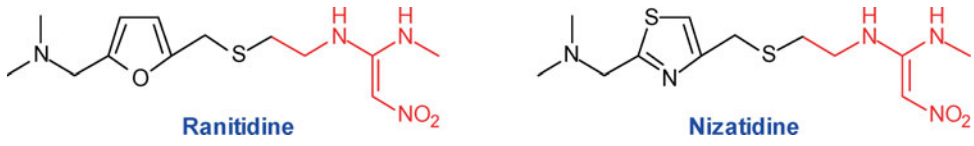
8.46 *Minor structural variations led to nitenpyram, the second neonicotinoid on the insecticide market.*

Even such seemingly marginal modifications are usually backed by extensive investigations of the structure-activity relationship. A small selection of the respective structural variations and their biological data is detailed below (Fig. 8.47).



8.47 *Selected structure-activity relationship within the nitenpyram series of compounds.*

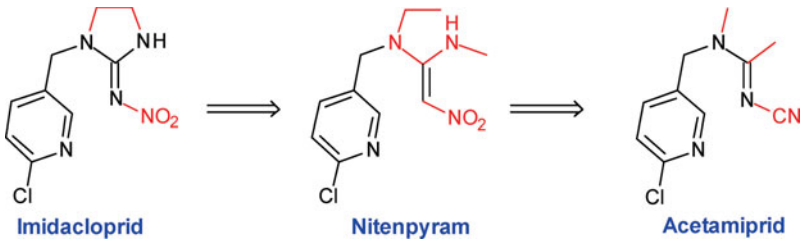
Interestingly, some histamine H_2-receptor antagonists, like ranitidine or nizatidine, in spite of their different pharmacological profile, show a certain structural relationship to nitenpyram (Fig. 8.48). Prior to the discovery of proton-pump inhibitors, these were the most important drugs for the treatment of stomach ulcers.



8.48 *Ranitidine and nizatidine reduce the secretion of stomach acid as competitive and reversible inhibitors of the histamine-H_2-receptor.*

Acetamiprid

The results which Shell had achieved with nithiazine also stimulated research work at Nippon Soda. From the publication of patents by Nihon, the excellent activity of imidacloprid against phytophagous (feeding on plant sap, or plant-sucking) insects like *Homopterans* (*e.g.* aphids and cicadas) and *Heteropterans* (*e.g.* true bugs) had become obvious. A more intensive analysis of the biological data revealed the weakness of imidacloprid against *Lepidopterans* (*e.g.* moth and butterfly caterpillars), but also that compounds in the structural vicinity, which carry a cyanoimino-group, showed impressive effects, *e.g.* against *Blattella germanica* (German cockroach) (Fig. 8.49 and Fig. 8.50). Takeda had already demonstrated that open-chain neonicotinoids may have high insecticidal activity. Eventually, it appears, that acetamiprid emerged from an optimisation programme within remaining holes of the patent landscape that was in the public domain at that time.



8.49 *Structural variations lead from imidacloprid via nitenpyram to acetamiprid.*

8.50 *The German cockroach (Blattella germanica).*

Thiamethoxam

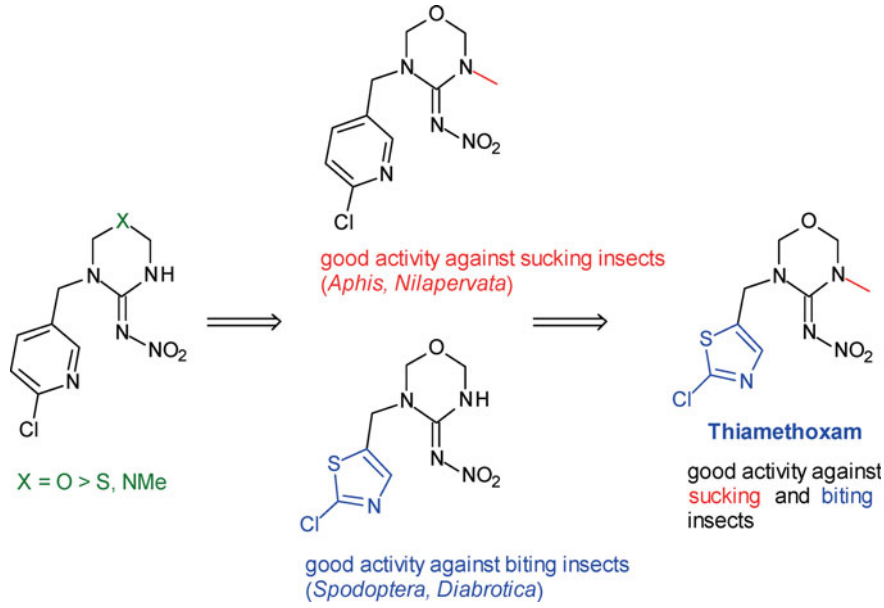
Ciba-Geigy (later Novartis, then Syngenta) launched their research programme on neonicotinoids in 1985. [138] Among the early compounds were 6-chloro-3-(pyridylmethyl)-substituted 1,3,5-oxadiazinanes, 1,3,5-thiadiazinanes and hexahydro-1,3,5-triazines, the first of which proved to be the most active one. Replacing the 6-chloro-3-pyridyl substituent by a 2-chloro-5-thiazolyl group clearly enhanced the activity against biting insects. The activity spectrum included also sucking insects when the oxadiazinane carried a methyl group. Combining both of these modifications, lead ultimately to thiamethoxam (Fig. 8.51).

The active ingredient has been profiled in comparison to analogous derivatives in a battery of assay systems (*inter alia*, against *Aphis craccivora*, *Myzus persicae*, *Nilaparvata lugens*, *Spodoptera littoralis* and *Diabrotica balteata*), and proved to be superior (Fig. 8.52 and Fig. 8.53).

From this, some general structure-activity relationships could be concluded (Fig. 8.54):

- The nitroimino-group is an essential constituent of the pharmacophore. Any alteration lowered the activity.
- The 2-chloro-5-thiazolyl residue was associated with the highest potency, followed by the 6-chloro-3-pyridyl moiety. All other heterocycles assayed were clearly less active.

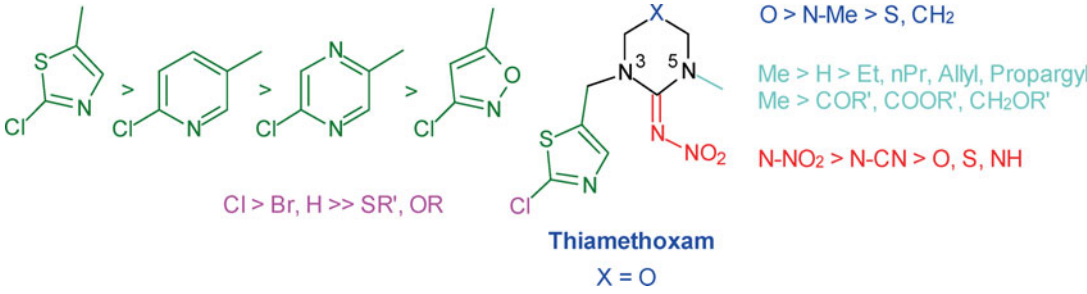
- Also 1,3,5-oxadiazinanes proved more active than 1,3,5-thiadiazinanes and hexahydro-1,3,5-triazines.
- The introduction of a methyl group at N-5 increased the insecticidal activity. All other substituents at this position however impaired the efficacy.



8.51 *Species-directed structure optimisation in the development of thiamethoxam.*

8.52 *The cowpea aphid (Aphis craccivora) (left) and green peach aphid (Myzus persicae) (right).*

8.53 *The Egyptian cotton worm (Spodoptera littoralis larva) (left) and banded cucumber beetle (Diabrotica balteata) (right).*



8.54 *Structure-activity relationships in the development of Thiamethoxam.*

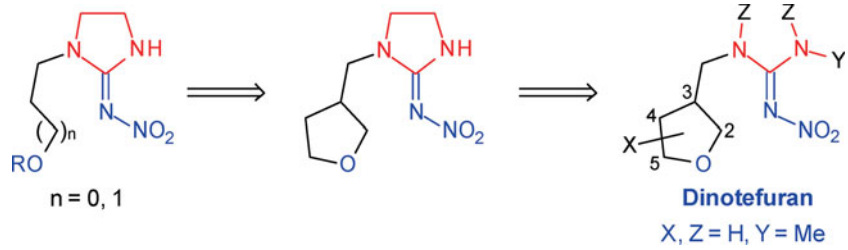
Under physiological conditions, and contrary to 1,3,5-thiadiazinanes and hexahydro-1,3,5-triazines, the 1,3,5-oxadiazinane ring is not liable to hydrolysis, suggesting that thiamethoxam is not a prodrug. [137]

Biochemical experiments with several neonicotinoids on insect membranes showed that both, thiamethoxam and imidacloprid bind to the nicotinic acetylcholine receptor. Imidacloprid however inhibits the binding of thiamethoxam, while not competing for the same binding site. Thiamethoxam and other equally non-competitive neonicotinoids, which only served as research tools, share as a common structural element the *N*-methyl group at position 5 of the 1,3,5-oxadiazinane ring.

Dinotefuran

Mitsui Chemicals initiated their research efforts on neonicotinoids in 1993. [139] The basic idea was to start from imidacloprid and replace the chloropyridyl substituent with an oxygen-containing residue, which resembled the natural neurotransmitter acetylcholine more closely than nicotine. As in acetylcholine, the distance between the hydrogen acceptor and the cationic centre was supposed to be around 5.9 Ångströms, which corresponds to a carbon chain of two or three atoms. Out of a range of esters, free alcohols and methyl ethers, the latter gave the best results, when tested against *Nephotettix cincticeps*. Cyclic ethers demonstrated even better efficacy, and the (*R*/*S*)-3-methyltetrahydrofuranyl substituent constituted a considerable improvement. In the further course of development, it was shown that substituents at this ring lowered the insecticidal activity.

Optimisation at the "cationic centre" led to open-chain nitroguanidines, among which the monomethylated derivatives revealed outstanding activity, while other substitution patterns of the nitroguanidine possessed a species-dependent insecticidal profile. Whereas the sensitivity of *Nephotettix cincticeps* towards analogues with an additional acetyl-, benzoyl- or methylcarbamate-substituent, was approximately the same in comparison to the parent compound, *Laodelphax striatellus* (the small brown planthopper) proved clearly less sensitive. Careful analysis of the data led eventually to the decision, which structure to select and develop further (Fig. 8.55 and Tab. 8.3).



8.55 *Structure variations in the development of dinotefuran.*

Tab. 8.3 *Species-dependent structure-activity relationships in the vicinity of dinotefuran.*

Test model	Substituents	Activity profile
Nephotettix cincticeps and Laodelphax striatellus	Z = H, Y = Me	X: H > 5-Me > 4-Me >> 3-Me, 2-Me, 4-Et (mixture of diastereomers)
Nephotettix cincticeps and Laodelphax striatellus	X, Z = H	Y: Me > Et > H, higher alkyl
Nephotettix cincticeps	X = H, Y = Me	Z: H, Ac, Bz, COOMe > allyl > benzyl, Me
Laodelphax striatellus	X = H, Y = Me	Z: H > Ac > Bz, COOMe, allyl > benzyl, Me

Dinotefuran is marketed as a racemate, although the (*S*)-(+)-enantiomer is clearly more active against some species. [137]

8.4.4 Selectivity: Insects versus Vertebrates

In 1984, Mark E. Schroeder and Roger F. Flattum at Shell explored for nithiazine as the example, at which site these nitromethylene insecticides interact to effect their pharmacological activity. In cockroaches, the nicotinic acetylcholine receptor turned out to be the target. [140] In insects, the expression of these receptors is limited to the nervous system. There they reach a density, which is comparable only to the electric organ of the South American electric eel (*Electrophorus electricus*). [141] From ligand displacement studies with nerve cell receptors of the American cockroach (*Periplaneta americana*), the honey-bee (*Apis mellifera*) and the common housefly (*Musca domestica*), the α-subunit of the receptor became evident as the prime locus of interaction for neonicotinoids.

Most notable is however the observed safety margin of nithiazin; in contrast to nicotine, it is highly toxic for insects, but much less so for mammals. The same holds true for imidacloprid and related neonicotinoids. [142] While the protein structure of nAChRs is fairly similar across species, from vertebrates to insects and nematodes, recent studies attribute the selective activity/toxicity of neonicotinoides, at least in part, to different binding geometries of these ligands. Their nitromethylene or nitroimino motif is not only responsible for a higher affinity to the acetylcholine receptors of insects, its electronegativity appears to direct these ligands to a distinct cationic receptor subsite. Basic amino acids of the insect proteins accommodate here such an interaction much better than the corresponding protein sequence of vertebrates. [137]

These structural insights may also enable the future development of novel neonicotinoids with confirmed safety for bees and other pollinators.

8.4.5 Industrial Syntheses

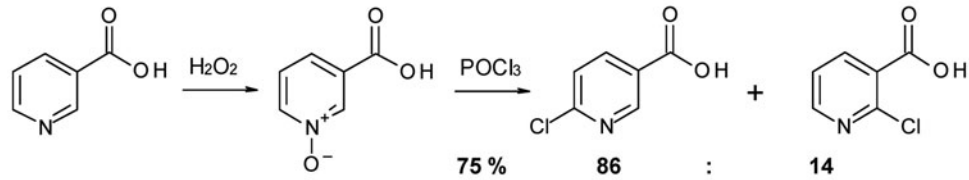
A simple retrosynthetic analysis reveals that the neonicotinoids consist of a chloromethyl-substituted heterocycle and in most cases a nitroguanidine part. For discovery research as for industrial synthesis, the accessibility to these building blocks is of considerable importance. Prior to the emergence of neonicotinoids, heterocycles of this structural type had not drawn particular attention, what has clearly changed ever since. [143, 144]

Synthesis of the Building Blocks

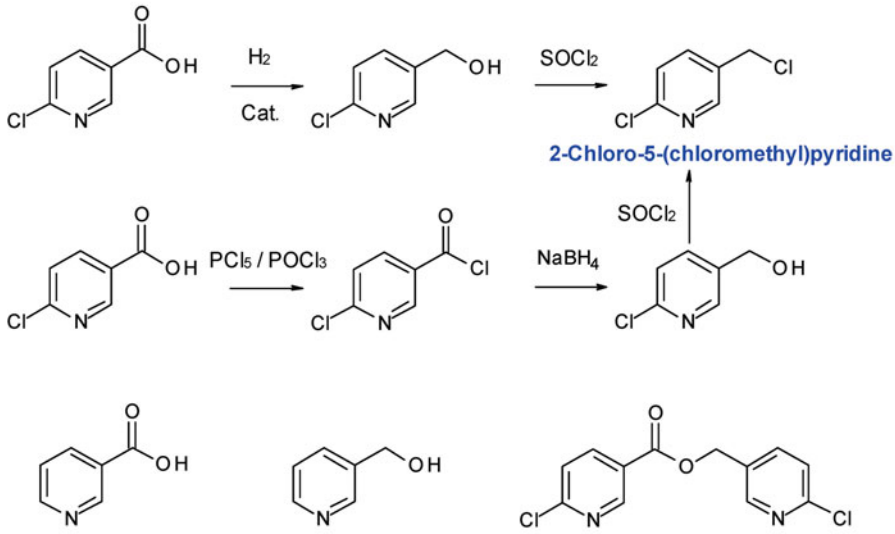
2-Chloro-5-(chloromethyl)pyridine

2-Chloro-5-(chloromethyl)pyridine is an essential synthetic building block for a number of neonicotinoids. The starting material for the first laboratory synthesis of imidacloprid was 6-chloronicotinic acid, which is accessible *via* the *N*-oxide (**a**). Despite substantial development work, some fundamental problems at the subsequent stages could not be solved. For example, the catalytic reduction of 6-chloronicotinic acid led also to partial dehalogenation. Transformation of the acid chloride with stoichiometric quantities of sodium borohydride gave always significant amount of the ester. While using borohydride in large excess provided much better yields, the high reagent costs turned the synthesis uneconomic (**b**). Starting from 6-chloronicotinic acid, the most favourable route is *via* esterification with triethyl orthoformate, sodium borohydride reduction, and reaction with thionyl chloride (**c**). 2-Chloro-5-methylpyridine can also be elegantly obtained from acyclic compounds. The reaction of propionaldehyde with morpholine produces a mixture of the corresponding enamine and aminal, which may be cyclised without separation with 2-chloroacrylonitrile to a cyclobutane derivative. Hydrolysis with sulfuric acid in acetonitrile gives 2-chloro-4-methyl-5-oxo-pentanenitrile, which affords the desired compound by elimination of hydrogen chloride and water (**d**). Propionaldehyde is also the starting material in the following straight forward synthesis. Reaction with benzylamine gives the corresponding imine, which is then acetylated with acetic anhydride. Reaction with the Vilsmeier salt from DMF and phosphoryl chloride results in the desired product under concurring cleavage of benzyl chloride and aromatisation (**e**). *β*-Picoline (3-methylpyridine) is likewise an appealing starting point, even though direct chlorination of the ring and the side-chain fails due to the low reactivity of the ring system. Under harsher conditions, the methyl group becomes fully chlorinated. The conversion of *β*-picoline into 2-amino-5-methylpyridine with sodium amide examplifies a Tschitschibabin reaction as practised in industry. Diazotisation and thermal decomposition of the diazonium salt intermediate, in the manner of a Sandmeyer reaction, gives 2-chloro-5-methylpyridine. In the simplest case, the latter may then be converted with chlorine at 60 °C in presence of sodium carbonate (to sequester the hydrogen chloride) into the target compound **(f).** By-products are chloropicolines with di- or tri-chlorinated side-chains.

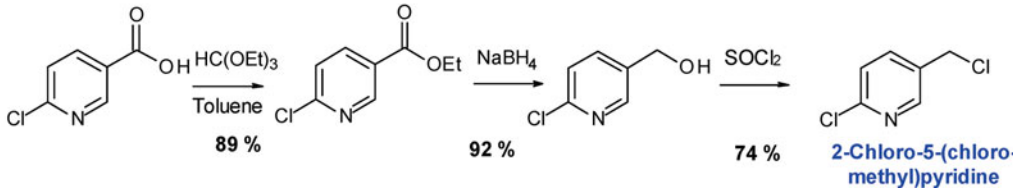
a)


H2O2
POCl3
75 %
86 : 14

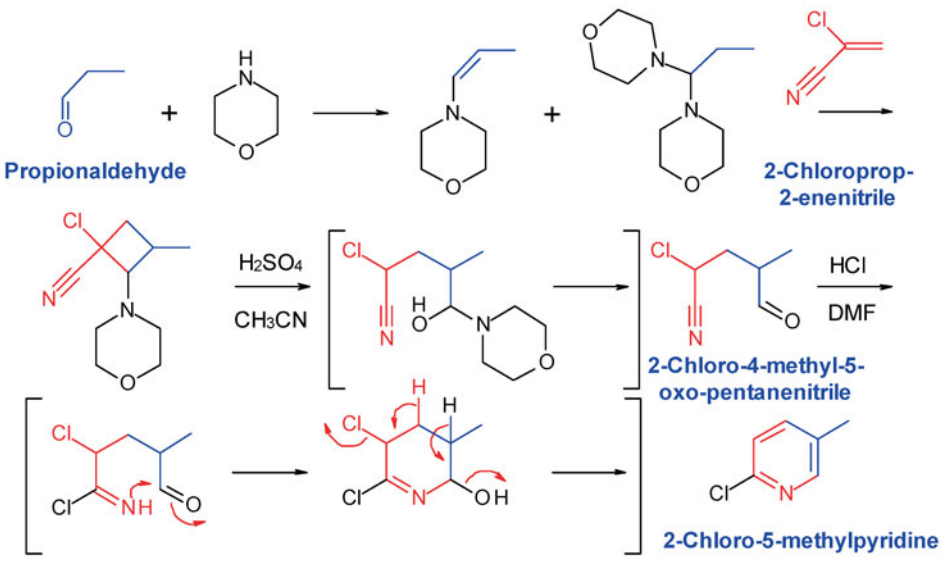
b)


H2
Cat.
SOCl2
2-Chloro-5-(chloromethyl)pyridine
PCl5 / POCl3
NaBH4
SOCl2

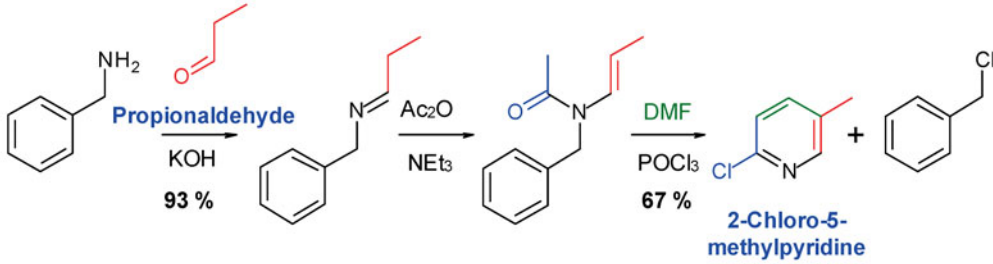
c)


HC(OEt)3
Toluene
89 %
NaBH4
92 %
SOCl2
74 %
2-Chloro-5-(chloro-methyl)pyridine

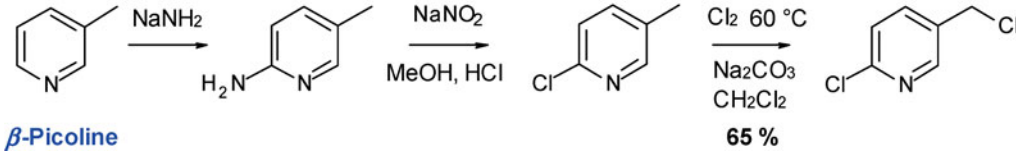
d)



e)



f)



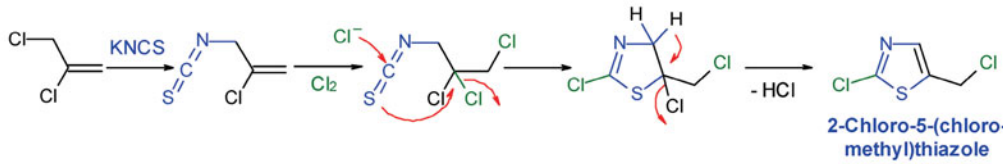
2-Chloro-5-(chloromethyl)thiazole

For a number of neonicotinoids, 2-chloro-5-(chloromethyl)thiazole is a sought-after building block, which replaces the chloropyridyl substituent.

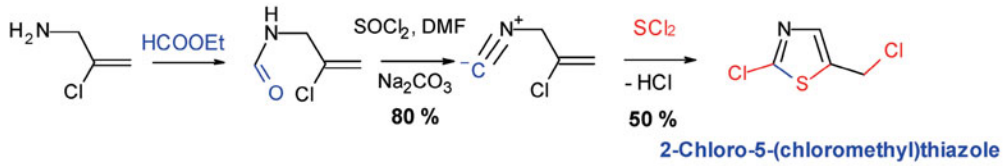
The reaction of 2,3-dichloropropene with potassium thiocyanate and chlorination with chlorine or sulfuryl chloride leads directly to 2-chloro-5-(chloromethyl)thiazole (**a**). The reaction of 3-amino-2-chloropropene with ethyl for-

mate, followed by dehydration, gives an isonitrile, which may be converted directly into the desired building block with sulfur dichloride (**b**). A surprisingly short synthesis with excellent yields starts from propargylamine, to which carbon disulfide is added. Chlorination gives 2-chloro-5-(chloromethyl)thiazole directly (**c**).

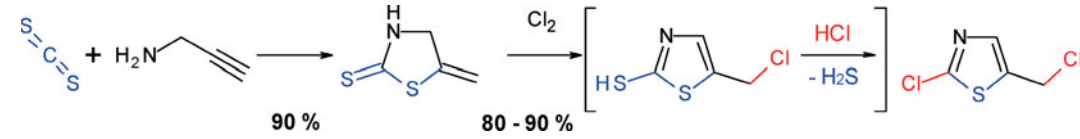
a)



b)

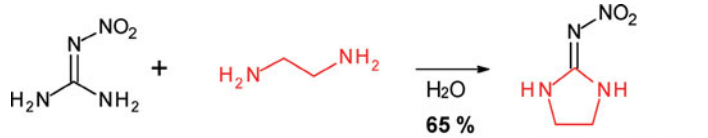


c)



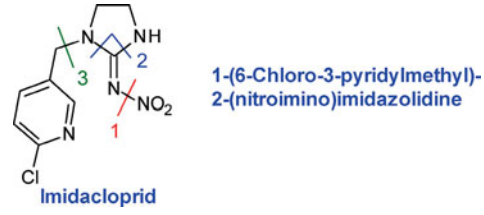
2-(Nitroimino)imidazolidine

The simplest method for the preparation of 2-(nitroimino)imidazolidine is the heating of nitroguanidine with ethylenediamine in water.



Syntheses of Active Compounds

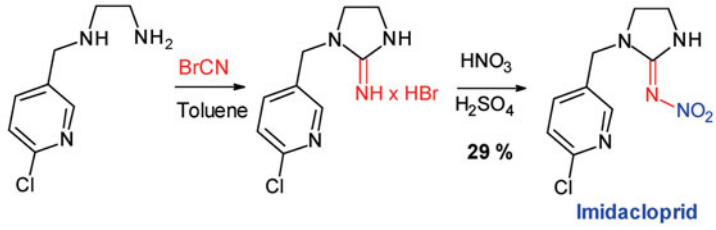
For the synthesis of imidacloprid, three principal routes were developed. The active agent can be obtained either by modification of the basic carbon scaffold, or by building up the imidazolidine or by coupling of the two ring systems (Fig. 8.56).



8.56 *Retrosynthetic analyses for imidacloprid.*

The first syntheses were developed in the 1980s by Kozo Shiokawa at Nihon Bayer Agrochem. The iminoimidazolidine was formed by reaction of the diamine with cyanogen bromide; however, this product could be nitrated on the nitrogen in only poor yields (**a**). [145] Shigeru Kojima prepared imidacloprid by reaction of the diamine with dimethyl nitrocarboimidodithioate in dichloromethane (**b**). [146] The best synthesis is probably the reaction of 2-chloro-5-(chloromethyl)pyridine and 2-nitroiminoimidazolidine with potassium carbonate in acetonitrile. [147] The reaction may be catalysed by 3–5 mole percent of caesium chloride (**c**). [148]

a)



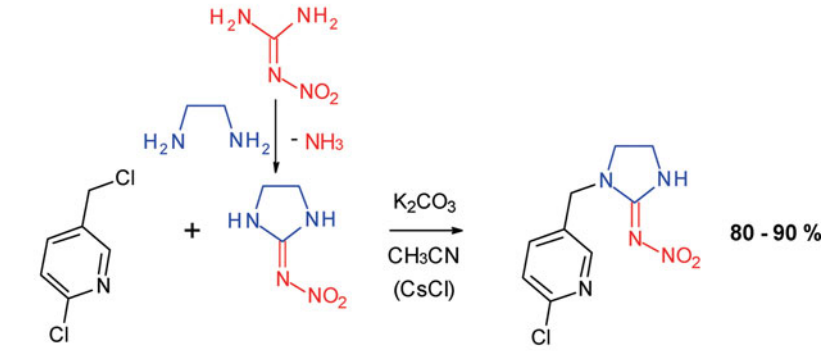
Imidacloprid

b)

Dimethylnitro-carboimidodithioate

Imidacloprid

c)

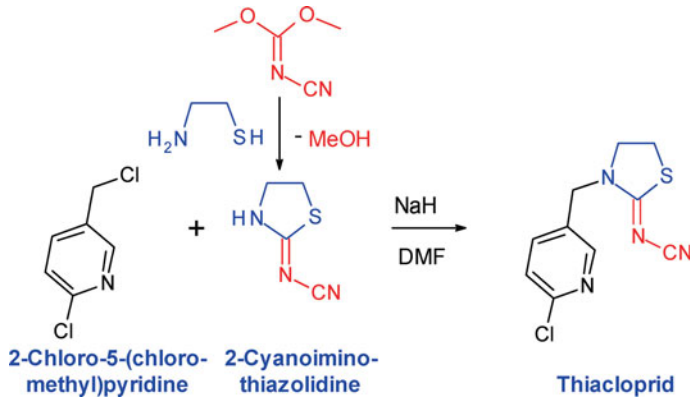


2-Chloro-5-(chloromethyl)pyridine

2-Nitroimino-imidazolidine

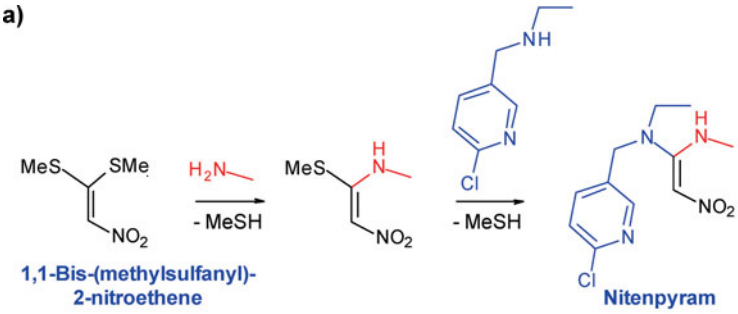
Imidacloprid

Along a similar methodology, thiacloprid is produced by Nihon Bayer Agrochem. 2-Cyanoiminothiazolidine is obtained by reaction of cysteamine with dimethyl *N*-cyanoiminocarbonate. For the subsequent alkylation, *e.g.* sodium hydride in DMF can be used as the base. [137, 149] According to quantum mechanical calculations, the C=N-CN double bond in thiacloprid has the (*Z*)-configuration.

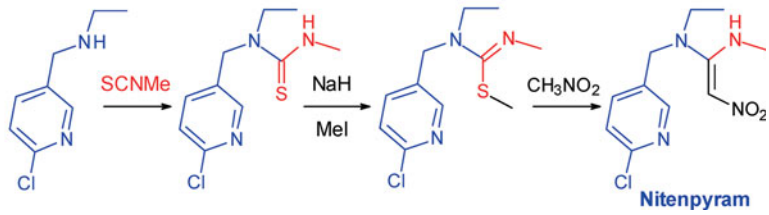


Nitenpyram, from Takeda Chemical Industries, is accessible by starting from 1,1-bis-(methylsulfanyl)-2-nitroethene *via* sequential aminolysis with methylamine and 2-chloro-5-(ethylaminomethyl)pyridine, in either order (**a**). [150, 151] Alternatively, 2-chloro-5-(ethylaminomethyl)pyridine may also be reacted with methyl isothiocyanate, to give the corresponding thiourea; this then gives the active agent by *S*-methylation and reaction with nitromethane (**b**).

a)

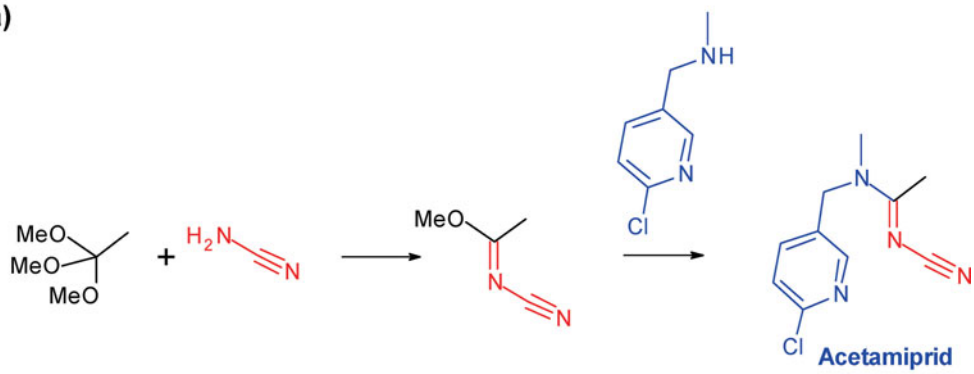


b)

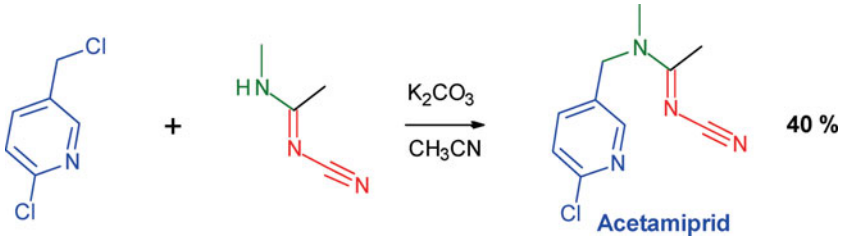


Acetamiprid, from Nippon Soda, is obtained in good yields, from trimethyl orthoformate, by sequential reaction with cyanamide and 2-chloro-5-(methylaminomethyl)pyridine (**a**). [152] Michael Y. Liu and John E. Casida synthesised acetamiprid by alkylation of *N*-cyano-*N*'-methylacetamidine with 2-chloro-5-(chloromethyl)pyridine in a yield of 40 % (**b**). [153]

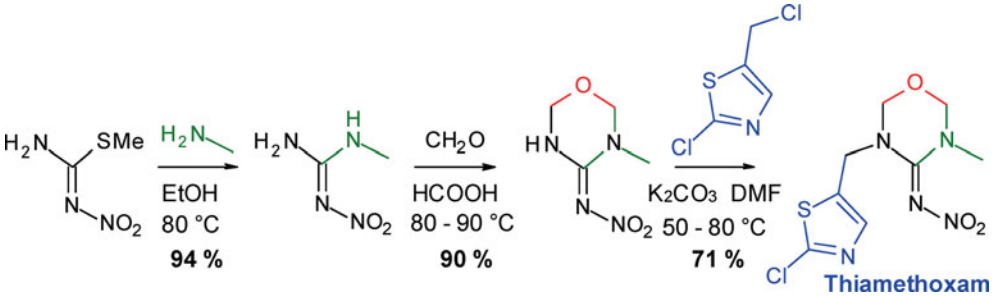
a)



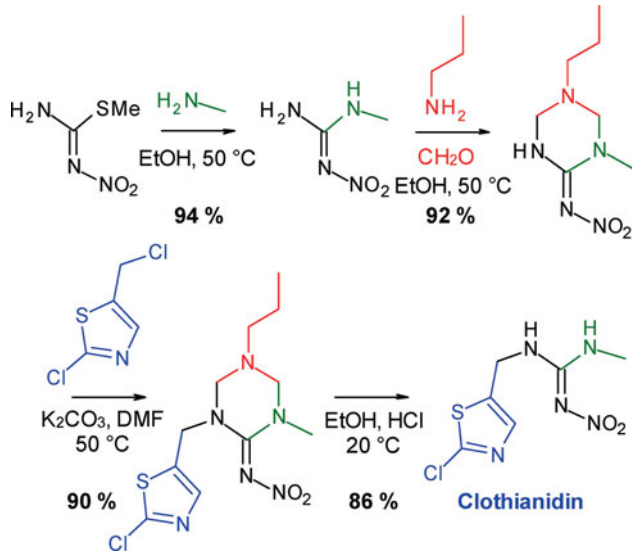
b)



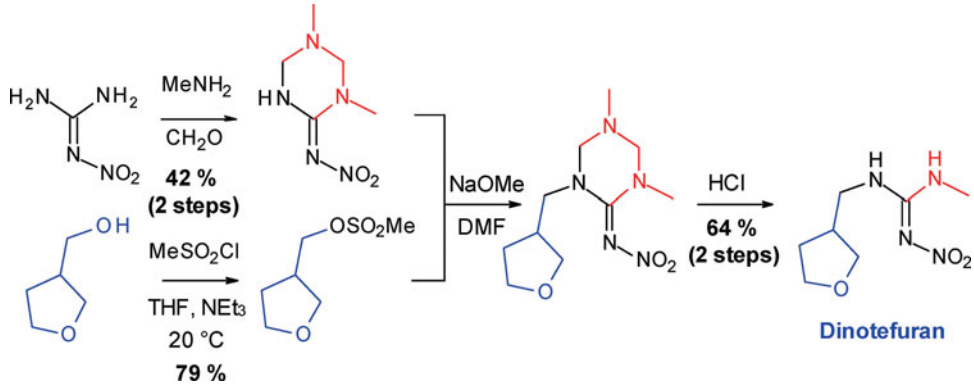
The first syntheses of thiamethoxam originate from 1991, when scientists at Ciba-Geigy (now part of Syngenta) discovered that compounds with a 1,3,5-oxadiazinane motif possess pronounced insecticidal activity. [154] Starting material for the industrial synthesis is *S*-methyl-*N*-nitroisothiourea, which reacts with methylamine to give *N*-methyl-*N*'-nitroguanidine. Its reaction with formaldehyde under optimised conditions in presence of formic acid produces the 1,3,5-oxadiazinane skeleton. The active compound is finally obtained by alkylation with 2-chloro-5-(chloromethyl)thiazole. [155, 156]



One of the best syntheses of clothianidin [157], another Takeda product, starts as well from *S*-methyl-*N*-nitroisothiourea. Just as for thiamethoxam, this compound is reacted with methylamine, and with formaldehyde and propylamine, a hexahydrotriazine is built up as a protecting group to alkylate the appropriate nitrogen with 2-chloro-5-(chloromethyl)thiazole. Hydrolysis with hydrochloric acid finally gives the active agent. [158] Quantum mechanical calculations, NMR spectroscopic studies and X-ray analysis confirm the (*E*)-configuration of the C=N-NO_2 group. [137]



The convergent synthesis of dinotefuran, the third-generation neonicotinoid from Mitsui Chemicals, applies a strategy analogous to clothianidin, but starts from nitroguanidine. Sequential transformations, first with methylamine and then with a mixture of methylamine and formaldehyde in water, form a hexahydrotriazine. This is alkylated with activated racemic 3-(hydroxymethyl)tetrahydrofuran; acid hydrolysis then gives the active compound. [139]



8.4.6 Economic Importance

In today's pest management market, the neonicotinoids are regarded as the most important insecticides. At a sales volume in 2010 of 2.65 billion US dollars, the growth rate of this product segment has been above average. Contributing factors for this success are the successive withdrawal of older insecticides from the market, like phosphate esters, but also that these newer agents demonstrate efficacy against insects, which had become resistant. Ranked by their turnover in 2010, the leading neonicotinoids are imidacloprid (*Admire®*, *Confidor®*, *Gaucho®*) at 980 million US dollars and thiamethoxam (*Actara®*, *Cruiser®*) at 905 million US dollars. [159] Imidacloprid represents the most successful insecticide worldwide, licensed in more than 120 countries, and applied in more than 140 agricultural crop commodities, as well as in the veterinary area and in control of termites. [137]

The neonicotinoids possess a wide spectrum of activity against numerous sucking insects, like lice, whiteflies, cicadas and thrips. Affected are also phytophagous (biting) beetles, like Colorado beetles (*Leptinotarsa decemlineata*), the Asiatic rice stem borer (*Chilo suppressalis*), rice leafroller (*Cnaphalocrocis medinalis*), pigimy mangold beetle (*Atomaria linearis*), rice weevil (*Calandra oryzae*), wireworms (beetle larvae), as well as beet leafminers (*Pegomya hyoscyami*), onion flies (*Delia antiqua*), and caterpillars of various butterfly species. Several neonicotinoids, *e.g.* imidacloprid or nitenpyram, are fairly water-soluble and therefore systemically active. Their potency is high enough that a single seed treatment is sufficient to provide full and long-lasting protection of sugar beets, maize, winter- and spring-cereals, rice and cotton. Furthermore, they are suitable for vegetable, fruit and citrus cultivation. Particular importance is also attached to the protection of buildings and to animal health. Their high activity against termites, fleas and lice extends the application as well to the protection of wooden construction material and of pets or companion animals likewise. [160]

Summary in Bullet Points

- The cholinergic synapse is the key target area for all major insecticides.
- Nicotine and the neonicotinoids bind to the postsynaptic nicotinic acetylcholine receptor.
- The neonicotinoids were not generated through lead-optimisation of natural products, *e.g.* nicotine or epibatidine, but of nitro-substituted ketene aminals, a serendipitous discovery.
- Today, the neonicotinoids are reckoned as the most important class of insecticides in the crop protection market. Many smart syntheses have been published.
- Emerging resistance to insecticides is an ongoing challenge and requires a tailored and integrated pest management.
- Growing concern, especially over toxic effects on pollinators, will require additional field studies with neonicotinoids, and safety strategies. [122]

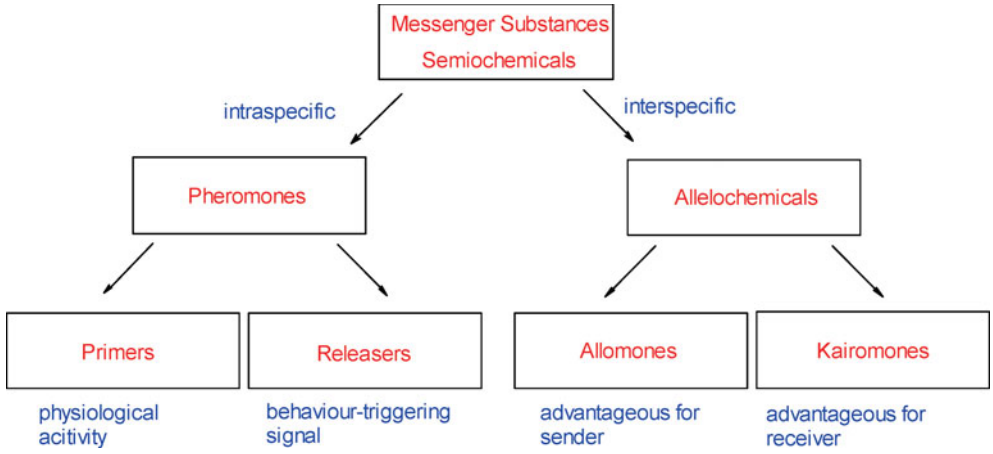
8.5 Pheromones

The village of Vosne Romanée is located in the Côte de Nuit district, where one can find some of the most reputed Grand-Cru wine estates of the region: Richebourg, La Romanée, Romanée-Conti, Romanée-Saint-Vivant, and La Tâche.

In Vosne Romanée, a small village just 20 km south of Dijon, the capital of the Côte-d'Or département, one of the best red wines of the Bourgogne (Burgundy) region is produced. The generation of this high-priced product faces a number of challenges, not the least of which involves the sex-life of butterflies in the vineyards. Like many other insects, these use chemical compounds for their communication.

Insects communicate with one another concerning gender identification, sexual maturity, mate selection and reproduction, the tracking down of prey, marking of territory and routes, feeding and nesting places, as well defence and alarm behaviour, or the regulation of social and caste systems. [161] (In these terms their behaviour hardly differs from that what humans do.)

Chemical messengers (semiochemicals; Greek, *semeion*: sign or signal) represent, from the phylogenetic viewpoint, a long-established communication system (Fig. 8.57). Messenger compounds, which act on individuals of the same species, are called pheromones. These are sub-divided, according to their biological effect, into primers and releasers. Primers cause a particular physiological reaction, whereas releasers trigger a particular behaviour. [162] Interspecific messenger substances (allelochemicals), on the other hand, act between organisms of different species. The term *allomone* is used if the messenger molecule is advantageous to the sender, and *kairomone* indicates an advantage for the receiver.



8.57 *Chemical Communication Systems.*

The "queen substance" of honey-bees can exhibit physiological effects and modulate behaviour. [164] It operates as a primer pheromone in suppressing the development of ovaries in female workers. Though, some of its components function as releasers; they trigger a retinue response by attracting worker bees to the queen, and are crucial for the coordination of swarming. By the use of different pheromone bouquets, the insect may send out different messages. The

range of the message may also be influenced by the vapour pressure. Although releaser-pheromones are most widely distributed in insects (*Hexapoda*), they also play an important role in protozoa (unicellular eukatyotic organisms), arthropods (invertebrate animals, *e.g.* crustaceans), and in higher animals like fish, reptiles and mammals (*e.g.* pigs, cattle, sheep, goats, dogs, house-mice, antelopes, elephants and gorillas). Also in humans their activity is discussed (*cf.* Chapter 3 – Flavours and fragrances).

South American female Bolas spiders use an allomone blend, which contains the sex attractant (9*Z*)-tetradecenyl acetate of moths, to entice their prey "under false pretences", and then catch it with a sticky woven globule at the end of a silk thread, known as a "bolas". By swinging the bolas with a foreleg at flying male moths nearby, the spider may angle its target rather like a fisherman snagging a fish on a hook.

For the colonisation of trees, the Western pine beetle (*Dendroctonus brevicomis*) secretes different pheromones, which also act as kairomones. *Exo*-brevicomin attracts not only the males of the pine beetle, but also the bark-gnawing beetle (*Temnochila virescens*). In order to attract females, male pine beetles emit frontalin, which at the same time also draws snakeflies (*Raphidioptera*). The black-bellied clerid (*Enoclerus lecontei*) is attracted by *trans*-verbenol of the female pine beetle and the ipsdienol of the male European spruce bark beetle (*Ips typographus*). All these predators deposit their eggs in the egg galleries (tiny tunnels in the live inner bark) of the pine beetle, to have their larvae feed on those of the beetle.

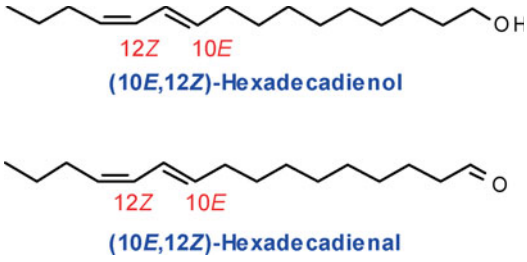
Interestingly, bitches in heat (*Canis lupus familiaris*) use the same sex pheromone as some swallowtail butterflies: methyl *p*-hydroxybenzoate. (*Z*)- 7-Dodecenyl acetate is not only a pheromone of more than 100 butterfly and moth species, but also of the female Asian elephant (*Elephas maximus*). This indicates that pheromones are among to the oldest tools in the evolution of communication. [163]

8.5.1 Discovery

While the importance of semiochemicals has been known for a very long time, intensive research activity in this area only started in 1959 with the work of Adolf Butenandt (Fig. 8.58) on bombykol, the sex attractant of the female silkworm moth (*Bombyx mori*). [165] It required the collection of some 500,000 female moths, to obtain enough material for the structure elucidation of the messenger compound, which in the end proved to be a mixture of (10*E*,12*Z*)-hexadecadienol and (10*E*,12*Z*)-hexadecadienal (Fig. 8.59).

8.58 *Adolf Butenandt (1903–1995).*

The substances are released into the air from the abdominal scent glands (Sacculi lateralis) of the female insects. When these reach the antennae of their



8.59 *The sex attractant of the silkworm moth (Bombyx mori).*

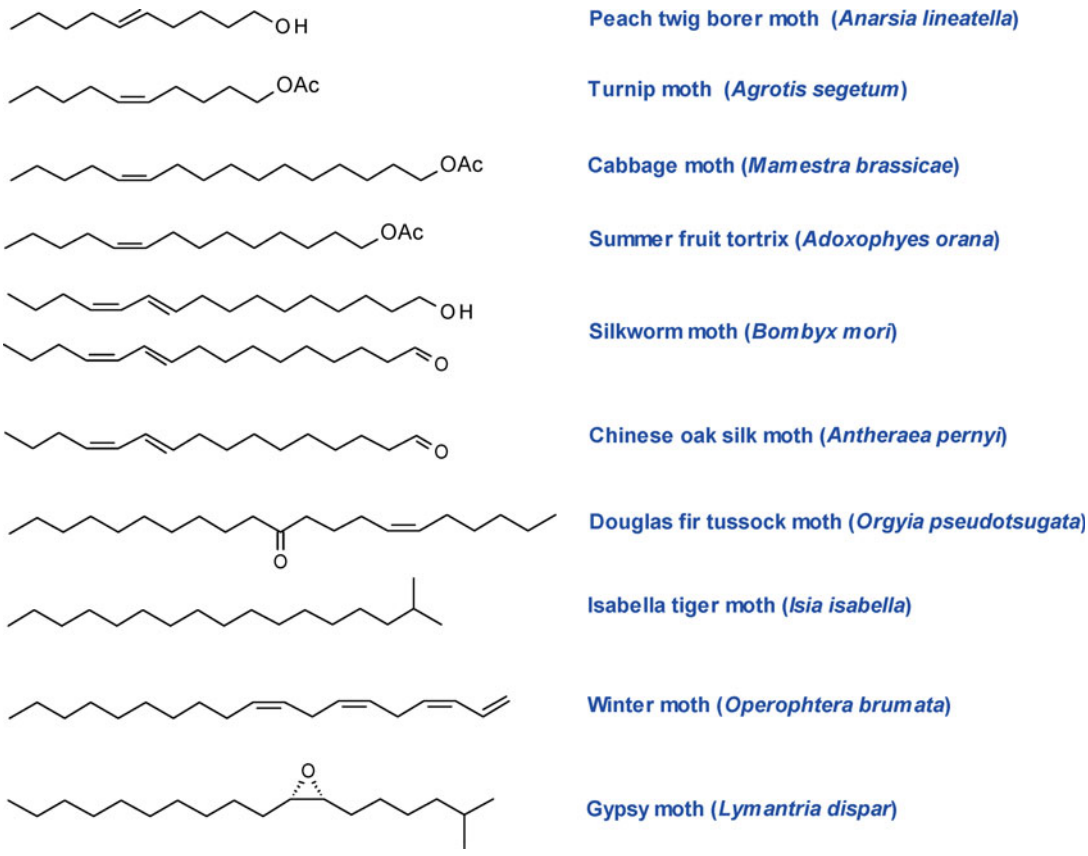
To a human, bombykol is odourless.

mates, a specific behavioural sequence is triggered in their central nervous system, and they move against the wind direction along the gradient of the odour. The pheromone is effective even at concentrations as low as 10^{-13} to 10^{-15} g/l of air.

55 years ago, Peter Karlson (1918–2001) and Martin Lüscher (1917–1979) coined the term *pheromone* (Greek; *pherein*: to carry or bring; *hormon*: to stimulate), for a substance, which is emitted by an animal, in order to produce an alteration in behaviour or a physiological response in another animal of the same species. [166]

8.5.2 Examples

Among the sex attractants of butterflies and moths (*Lepidoptera*), most are long-chain unsaturated alcohols and their acetates or aldehydes, which are emitted by the females to attract males. In a few species (*Geometridae*, *Arctiidae*, *Noctuidae*), saturated hydrocarbons, polyenes and epoxides have been discovered (Fig. 8.60).

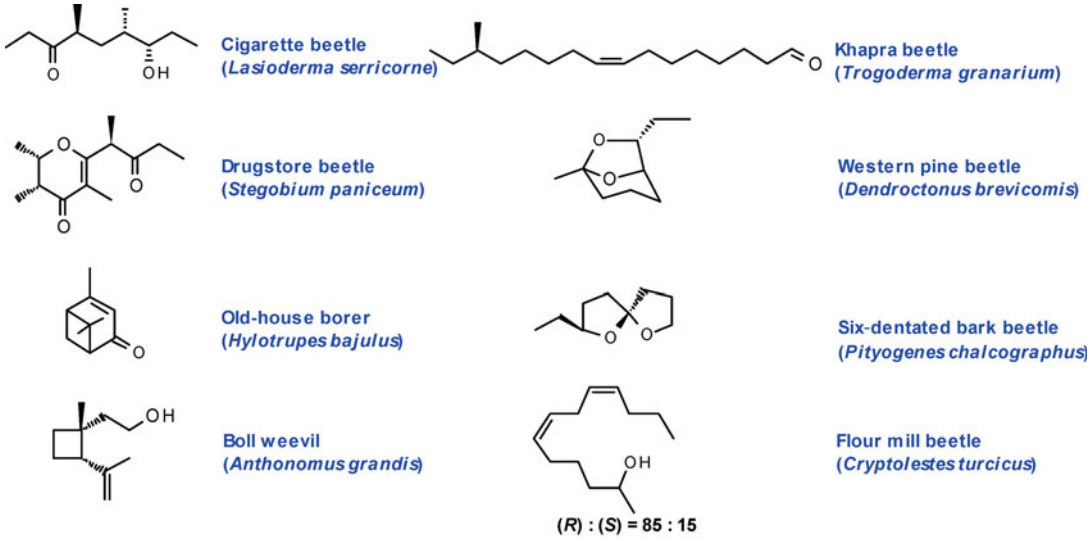


8.60 *Sex pheromone components of female moths (Lepidoptera).*

On account of its multitude of species (worldwide around 150,000), butterflies often use a mixture of compounds with a defined composition (pheromone complexes) to secure species-specificity and exclude cross-attraction. Remarkable in this connection is the effect of (11*Z*)-hexadecenol on males of the cabbage moth. Whereas the corresponding acetate is the sex attractant released by the female, the corresponding free alcohol acts as an antagonist. The latter is not produced by a female moth, which is not just in the mood for mating, but by an evolutionarily related butterfly species, which in this way warns of "infidelity", *i.e.* prevents cross-copulation. Such types of species separation are ubiquitous in the insect world.

Also in beetles (*Coleoptera*), the largest insect class (around 350,000 species), specific pheromones for males and females are wide-spread. As an example, aggregation pheromones lead bark beetles (*Scolytidae*) to colonise suitable host trees (Fig. 8.61).

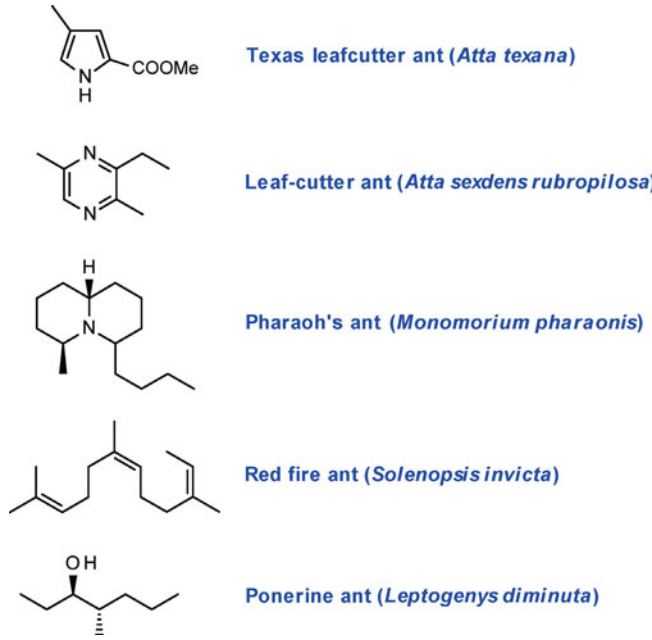
Structurally, beetle pheromones are difficult to classify. They are often fatty acid or oxygen-containing terpene derivatives, occasionally with a complex polycyclic structure.



8.61 *Aggregation and sex pheromones of beetles (Coleoptera).*

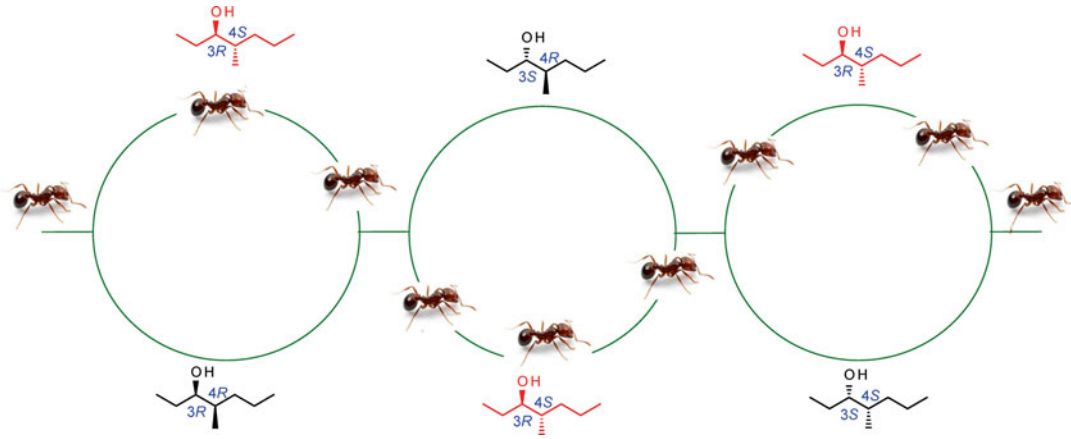
The hymenopterous insects (*Hymenoptera*), to which the species-rich families of ants, honey-bees, wasps and bumble-bees belong, often live in large colonies and use pheromones to maintain their highly structured social order.

Some ant species (*Formicidae*) use nitrogen heterocycles as trail pheromones; others however use hydrocarbons or alcohols (Fig. 8.62). In the rectal glands of a few *Lasius* and *Formica* species, 3,4-dihydroisocoumarins have been identified. The scents are emitted by exocrine glands like the poison apparatus or the sternal gland. The trail pheromone of the leafcutter ant (*Atta texana*) is effective at extremely low concentrations of 80 fg/cm (1f = 10^{-15}). One milligram of methyl 3-methylpyrrole-2-carboxylate is sufficient to lay a trail three times around the Earth.



8.62 *Trail pheromones of ants (Formicidae).*

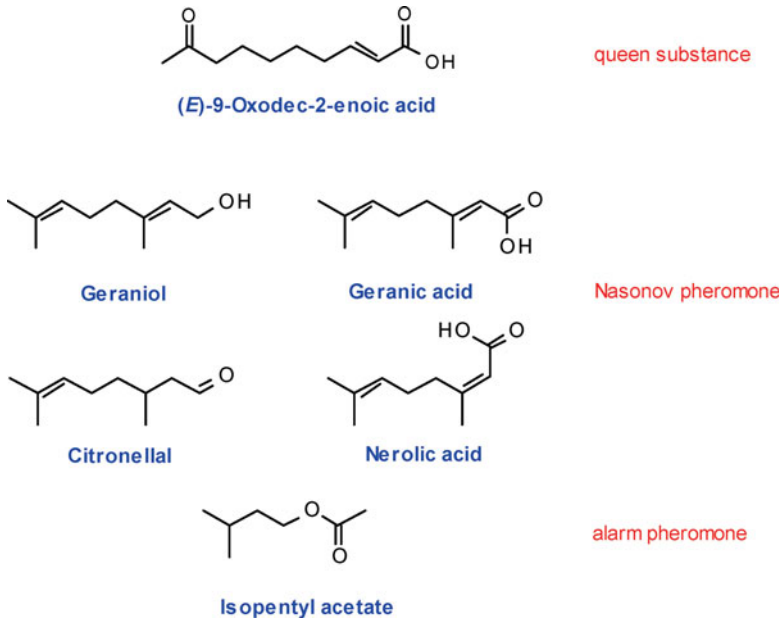
An example illustrating the "enantioselective" recognition capabilities of ants is provided by trail-following experiments with Ponerine ants (*Leptogenys diminuta*). If the four diastereomers of 4-methylheptan-3-ol (5 ng/cm) are drawn out in circular arcs, the ants tenaciously follow only the trail of the (3*R*,4*S*)-diastereomer (Fig. 8.63). The concentration limit for (3*R*,4*S*)-4-methylheptan-3-ol lies at 50 fg/cm.



8.63 *Trail-following experiment with Leptogenys diminuta.*

The pheromones of honey-bees (*Apis mellifera*), which they produce in their mandibular glands, the Nasonov-glands, and in the sting apparatus, are well known (Fig. 8.64). The "queen substance", also referred to as *queen rentinue pheromone* (QRP), is a mixture of eight components, attracting swarming bees

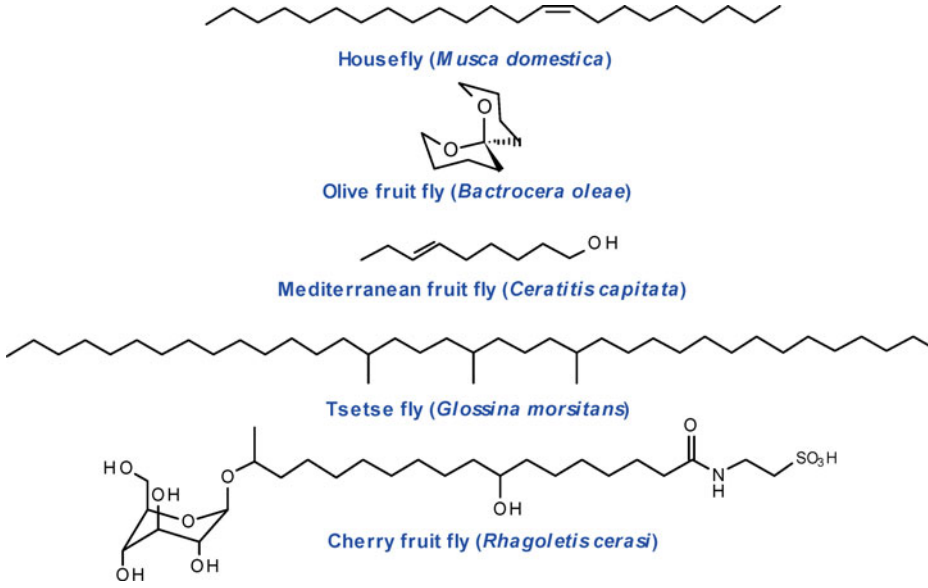
from far away, and organising them into a stable cluster. The main component, 9-oxodec-2-enoic acid (9-ODA), acts as a releaser pheromone to attract worker bees, and at the same time as a primer pheromone that suppresses the development of the worker's ovaries. Above a minimum altitude, it acts also as a long-distance sex pheromone, attracting mature drones on their mating flight. The worker bee produces in its Nasonov-gland the colony-specific Nasonov pheromone cocktail, which serves for feeding and nest entrance marking as well as for stabilisation of the swarm. From the sting chamber, the honey-bee secretes the alarm pheromone isopentyl acetate. [167]



8.64 *Pheromones of the honey-bee (Apis mellifera).*

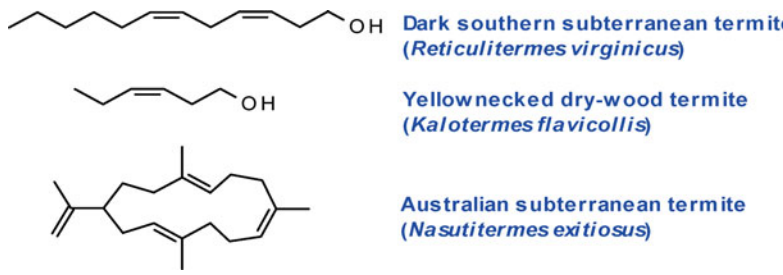
South American colony-forming robber bees (*Lestrimelitta limao*) steal nectar and pollen from the nests of related bee species. During their attack, they secrete large amounts of citral as an allomone, which raises their own aggressiveness but confuses the victim, rendering it defenceless. [168]

Although flies (*Brachycera*) are visual creatures, they also employ pheromones. The sex pheromone of the Tsetse fly (*Glossina morsitans*) consists notably of a saturated hydrocarbon set (Fig. 8.65) with a chain length of 37 carbon atoms, which is produced in the waxy cuticle of the insects' wings. The female of the European cherry fruit fly (*Rhagoletis cerasi*) marks the cherries, on which she has laid her eggs, with a glucoside, in order to deter other flies from doing the same.



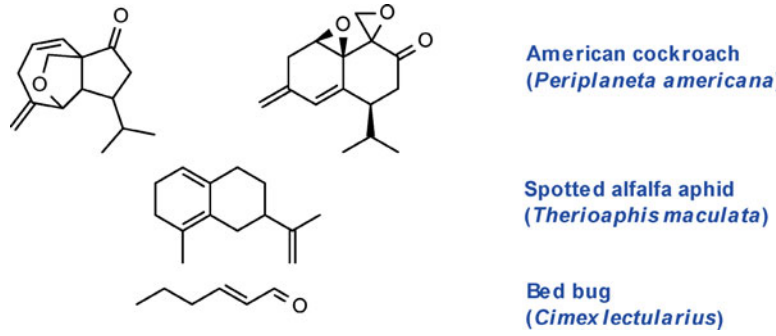
8.65 *Sex pheromones of flies (Brachycera).*

Termites (*Isoptera*) are related to cockroaches and mantids (however not to ants). Occurring mostly in the tropics, they are an order of social, often wingless and blind insects, which mark their paths with trail-following pheromones. These are usually unsaturated alcohols or macrocyclic hydrocarbons (Fig. 8.66).



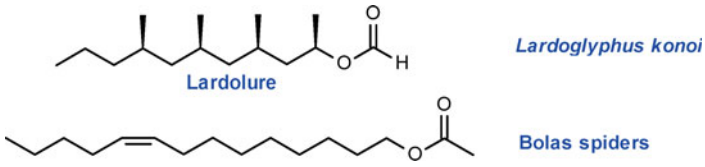
8.66 *Trail pheromones of termites (Isoptera).*

The sex pheromone of the American cockroach is a mixture of two sesquiterpenes. Some aphids use hydrocarbons as alarm pheromones, but irritated or injured bed bugs use hex-2-enal as their alarm messenger (Fig. 8.67).



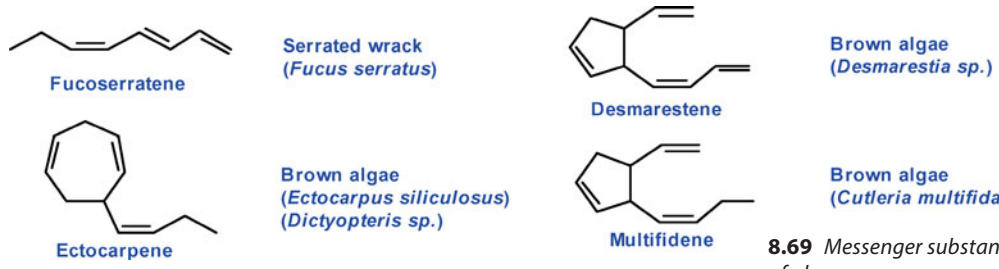
8.67 *Messenger substances of cockroaches (Blattodea), aphids (Aphidoidea) and bugs (Heteroptera).*

Also spiders (*Arachnida*) use pheromones for chemical communication (Fig. 8.68). Lardolure is the aggregation pheromone of the fish mite *Lardoglyphus konoi* [169], and bolas spiders use a moth pheromone as an allomone. [162]



8.68 *Messenger substances of spiders (Arachnida).*

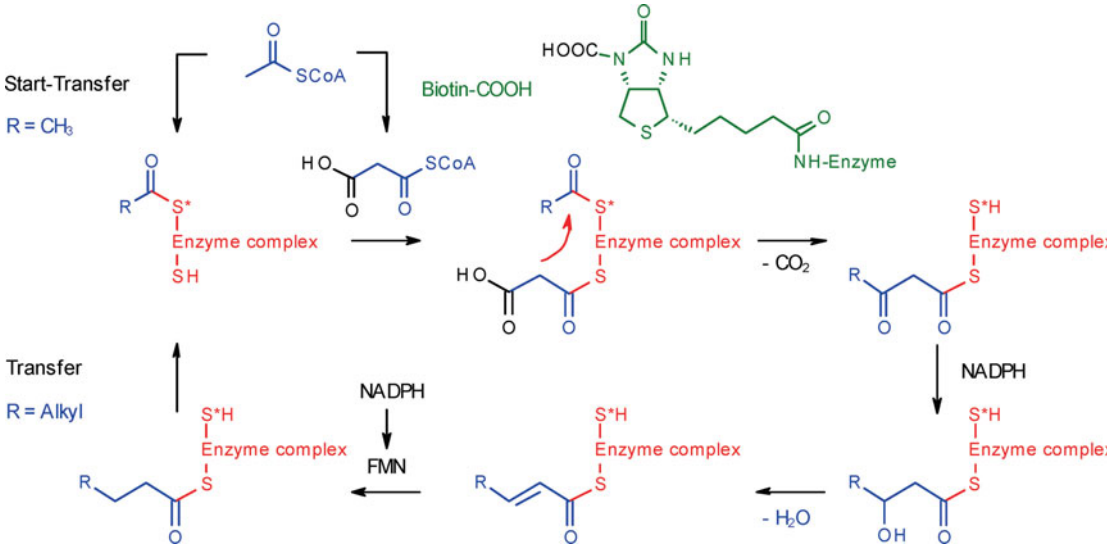
It might be surprising that algae draw on pheromones as well (Fig. 8.69). Female gametes (sex cells) of numerous algae emit semiochemicals into the water, in order to attract spermatozoids. These attractants are normally polyunsaturated hydrocarbons, which are effective even at extreme dilution. (+)-Multifidene has shown a threshold concentration of 6×10^{-12} M. [162]



8.69 *Messenger substances of algae.*

8.5.3 Biosynthesis

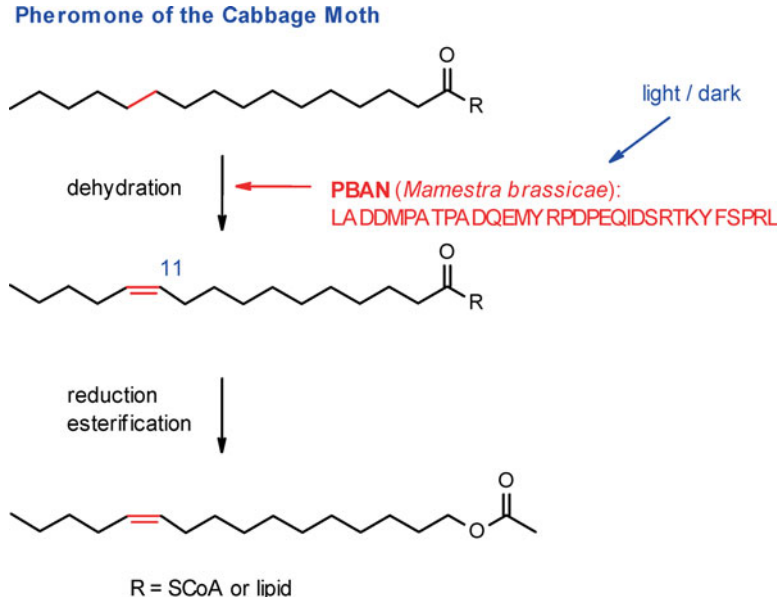
The pheromone-biosynthesis of most female butterflies starts *de novo* from acetate, which originates from the carbohydrate metabolism, on a multi-enzyme complex along the general pathway for the biosynthesis of fatty acids.



Mammals are also able to synthesise fatty acids *de novo*, as proven for pigs, which become fat, even when fed only with potatoes and bran.

Fatty acids are dehydrated by special enzyme systems, then reduced to aldehydes or alcohols and esterified if necessary. These steps are not substrate-specific. If other fatty acids are applied topically to the pheromonal gland of a butterfly, these compounds are converted into the acetates as well, which is scientifically interesting, though the message from the female is ruined, since it is no longer understood.

In a few *Lepidoptera*, like the silkworm moth (*Bombyx mori*), heliothide species as the corn earworm (*Helicoverpa zea*) and the cabbage moth (*Mamestra brassicae*) it has been demonstrated that a neuronal hormone (PBAN, *pheromone biosynthesis activating neuropeptide*) is synthesised by a cephalic organ in their brain, dependent on the day/night rhythm. This mediator controls the sex pheromone production in these moths. [161, 170]



Arctiidae
A family of butterflies with around 11,000 species. These are mostly small to medium in size, with coloured rear wings, marked yellow or red with black. The caterpillars are often very hairy and cause considerable damage by feeding on more than 50 plant species.

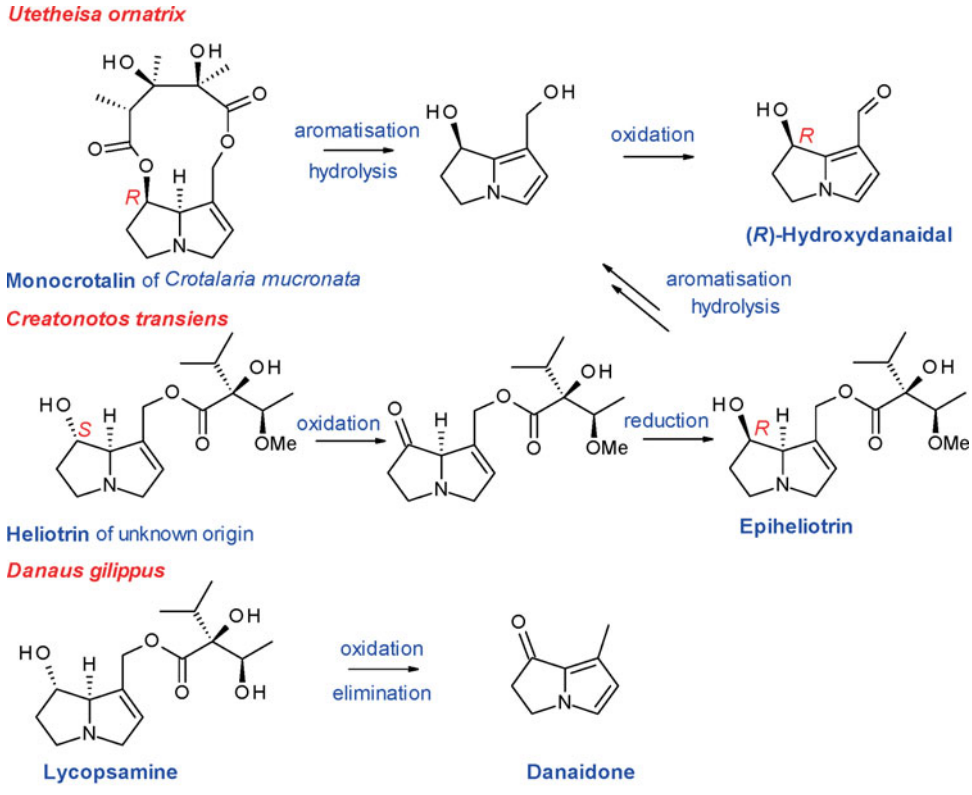
Danaidae
A small family of tropical or subtropical butterflies with only a few members like the milkweed or monarch species. Their wingspan extends to 7–10 cm, and their annual mass migration is spectacular; the North American Monarch butterflies (*Danaus plexippus*) fly in autumn from southern Canada as far south as Mexico and Florida.

Other insect species convert plant constituents into pheromones. The caterpillar of certain *Arctiidae*- and *Danaidae*-species [171] utilise pyrrolizidine alkaloids, *e.g.* from food plants, which remain even in the fully-developed insect (imag-

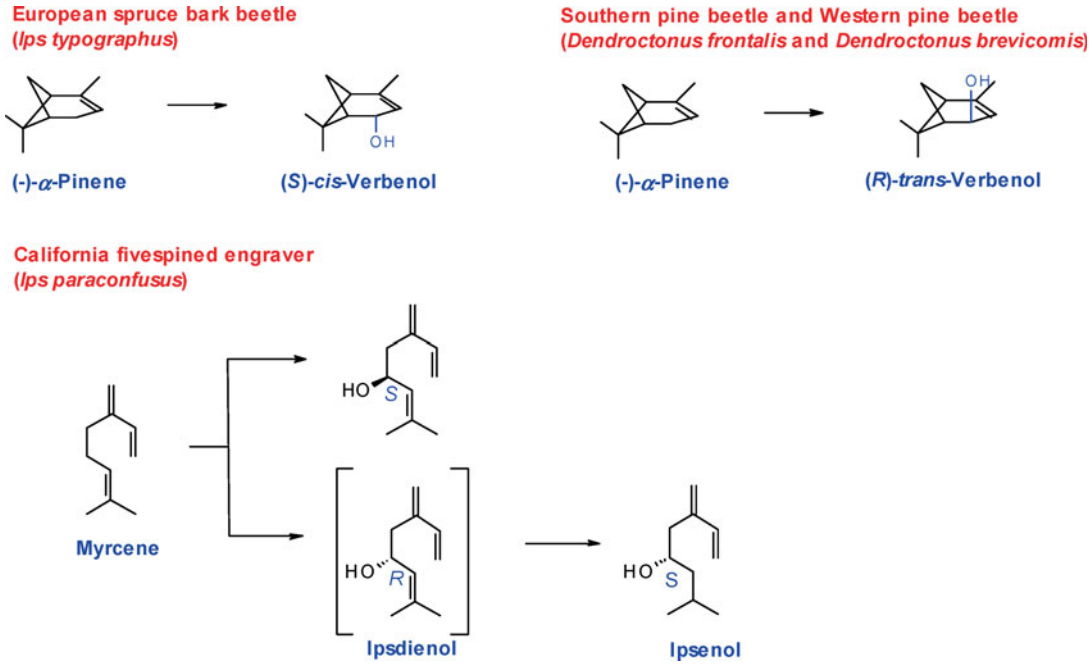
ines) to some extent intact. The male (on the right of the picture) metabolises these compounds partially to pheromones, which are transferred during copulation to the female and the eggs, where they deter predators (Fig. 8.70). [172]

8.70 *Ornate moths (Utetheisa ornatrix).*

The caterpillar of the ornate moth *Utetheisa ornatrix* feeds on the leaves of *Crotalaria* species (rattlepods) and thereby absorbs monocrotalin, which gets metabolised to (*R*)-hydroxydanaidal. [173] Along with its food, *Creatonotos transiens*, an Asiatic species of ornate moths, takes up heliotrin, which exhibits a 7-hydroxy-function in the (*S*)-configuration. By means of a redox sequence, this "mistake" is corrected, and eventually (*R*)-hydroxydanaidal is produced. [174] Adult *Danaidae* species, like *Danaus gilippus*, take up pyrrolizidine alkaloids from their food plants and metabolise them to danaidone, a structurally related pheromone.



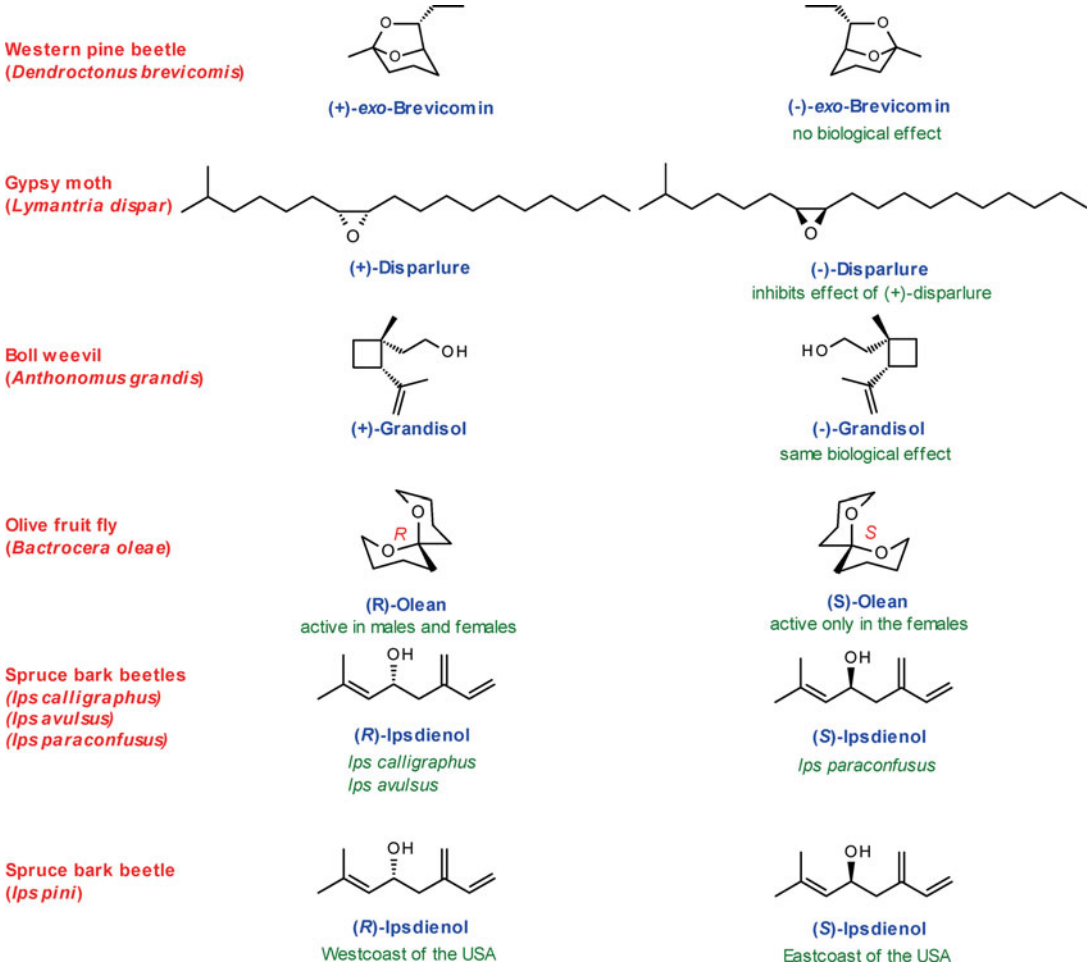
Bark beetles oxidise toxic monoterpenoid hydrocarbons in their respiratory air, which originate from the resin of the tree they colonise, and thereby circumvent this defense barrier of the tree. Converting these compounds into less toxic secondary metabolites serves another purpose, by providing pheromones to attract insects of the same species, of both sexes. Therefore, an oxygen function is often introduced species-specifically and stereoselectively. European spruce bark beetles oxidise (–)-α-pinene to (*S*)-*cis*-verbenol, whereas pine beetles produce (*R*)-*trans*-verbenol. In the California fivespined engraver (*Ips paraconfusus*), myrcene is converted into (*S*)-ipsdienol and (*S*)-ipsenol. But *Ips pini* and *Ips paraconfusus* are also able to synthesise their pheromones *de novo via* the classical mevalonate biosynthetic pathway.



8.5.4 Stereochemistry and Biological Action

It is generally expected that biological activity depends strongly on the stereochemistry of a compound. This often applies to pheromones as well. However, there is a range of exceptions, where this relationship is considerably more complex (Fig. 8.71). [175]

1. In the simplest case, as for *exo*-brevicomin, only one enantiomer is biologically active. The antipode does not interfere with the biological activity.
2. While also only one enantiomer of disparlure is active, the antipode however functions as an inhibitor.
3. Grandisol serves as an example, in which all the stereoisomers possess comparable biological activity.



8.71 *Stereochemical structure-activity relationships.*

4. An interesting correlation is observed in the case of olean, the pheromone of the Olive fruit fly. Whereas in males only the (*R*)-enantiomer carries activity, the female, which produces the racemate, responds to both antipodes – also as a self-stimulant.
5. It happens, that some species within a genus utilise the same enantiomer, whereas other species employ the opposite enantiomer. For example, ipsdienol is an important pheromone of spruce bark beetles. *Ips calligraphus* and *Ips avulsus* use (*R*)-ipsdienol as their attractant; *Ips paraconfusus*, on the other hand, uses the (*S*)-enantiomer; its (*R*)-enantiomer acts even as an inhibitor. [176]
6. One of the most uncommon cases relates to geographical differences. The *Ips pini* at the East Coast of the USA uses (*S*)-ipsdienol as the pheromone; in contrast, the *Ips pini* at the West Coast uses the (*R*)-enantiomer. This shows that the very same species has developed two different pheromone receptors for communication.

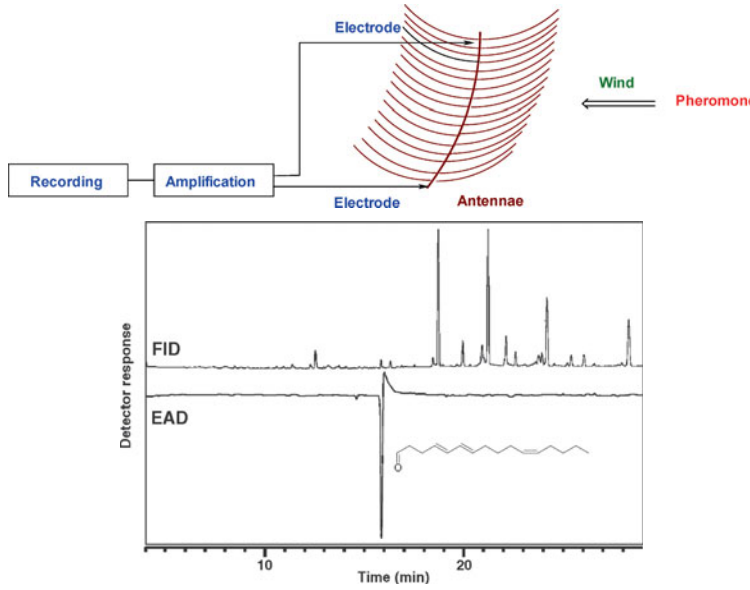
8.5.5 Communication

8.72 *The Atlas moth (Attacus atlas) from Southeast Asia has a wingspan of up to 30 centimetres and is considered among the largest butterflies in the world.*

The pheromonal scent from butterflies often covers a range of several hundred metres. Location of the female is facilitated by a light wind, against which the male then approaches. Insects pick up the pheromones with special olfactory cells, which are mostly found in their antennae (Fig. 8.72).

The scent is adsorbed on to the scent hairs (sensilla), and moves through pores of the cuticle into the so-called pore kettle filled with sensillar lymph. At this air-liquid interface, the pheromones are thought to be shuttled by small protein carriers (*e.g.* pheromone-binding proteins, PBPs) to the olfactory receptors, located in the dendrite membrane of sensory neurons. [177] Interaction with these ion-channel receptors causes their opening and leads to depolarisation of the nerve cells. This change in potential may be recorded as an electroantennogram. For this, the severed antenna, which remains still "alive" for a certain time, is connected to microelectrodes, from where the amplified signal is displayed. This provides the only method, which allows measuring the impact of pheromones at their natural concentrations (Fig. 8.73).

When combined with gas chromatographic separation of the pheromone bouquet, the electroantennographic detector (EAD) system offers a unique possibility of identifying physiologically active components in trace quantities. [178]



8.73 *An electroantennographic detector system (EAD) and an electroantennogram.*

In the insect, the scent bouquet causes a specific stimulation pattern at the sensory neurons, which is then processed in the central nervous system into an unambiguous reaction and eventually leads to a characteristic response by the insect.

Since the use of pheromones in pest control interferes with the natural communication system of insects, these agents are not expected to suffer the development of resistance, as is the case with conventional insecticides. In addition to the high environmental compatibility, this provides another distinct advantage in agriculture and forestry.

8.5.6 Applications in Plant Protection

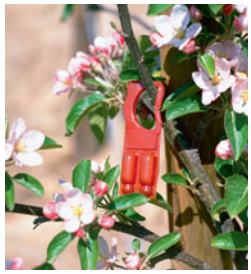
The idea of using pheromones in plant protection goes back to Adolf Butenandt. Due to the difficulty of working with natural products at the nanogram scale, beginning from the isolation and structure elucidation challenges up to the determination of their biological function, it took two decades of hard work before pheromones found use as plant protection agents to a larger extent.

In forestry it was known for centuries, how to attract spruce bark beetles by fallen spruces, and intervene at the onset of the reproductive cycle. Once larval galleries had developed at such "trap trees", the infested bark was peeled off and burned to destroy as many beetles and larvae as possible. Subsequently, this method has been improved by priming of the "trap trees" with an insecticide. In Norway, the first large-scale field trial was conducted in 1979 to control the spruce bark beetle with artificial attractants, consisting of 600,000 pheromone traps. Hoechst developed monitoring-traps (*Biotrap*®) for forestry, and Cela Merck/Shell, as well as Borregaard produced funnel traps (*Pheroprax*®) to cut back on the peak populations of forest pests. At the end of the 1980s, BASF developed the first industrial syntheses of pheromones for their application in agriculture. The mating disruption method enabled for the first time to control the reproduction of vine- and apple-moths by natural means. [179] Nowadays, all three procedures are adopted in a variety of ways:

- **Monitoring:** In orchards, vineyards and in woodland, glue-traps are used for monitoring purposes (Fig. 8.74). In this way, it is possible to trap, species-specifically, the male butterflies, the caterpillars of which are the actual pests. The appearance of insects in these traps allows to determine the exact timing of the insecticide application, and thus to avoid unnecessary spraying.
- **Mass trapping:** Mass trapping with sex pheromone baits aims at the timely capturing of as many males of a pest species as possible, to prevent these from copulating, and to keep the subsequent generations small. By using aggregation pheromones, the bark beetle is effectively controlled. Cylinder traps are perforated plastic tubes with a rough outside. The beetles land on the tube, crawl inside through the beetle-sized openings, then slide along the smooth inner surface and fall into a collecting container. Undesired captives of indifferent or beneficial species are rare. Multi-funnel traps are designed as flight interception traps (Fig. 8.75). Many insects, while hitting a solid object in flight, tend to drop to the ground. Here, they slide through a cascade of funnels and land in a collector attached to the bottom funnel. Besides the pheromone bait, the trunk-shaped form of this trap contributes to its efficiency. Mis-trapping can be minimised if the trap is dark coloured, so that flower-seeking insects are not attracted. [180]
- **Mating disruption method:** This pest management technique interferes directly with the communication system between the male and female, and has demonstrated successes for more than 25 years. By means of exposure to an extensive cloud of pheromone, the male insects are confused. They can no longer locate their mate, which reduces the population of the next

8.74 *Monitoring traps.*

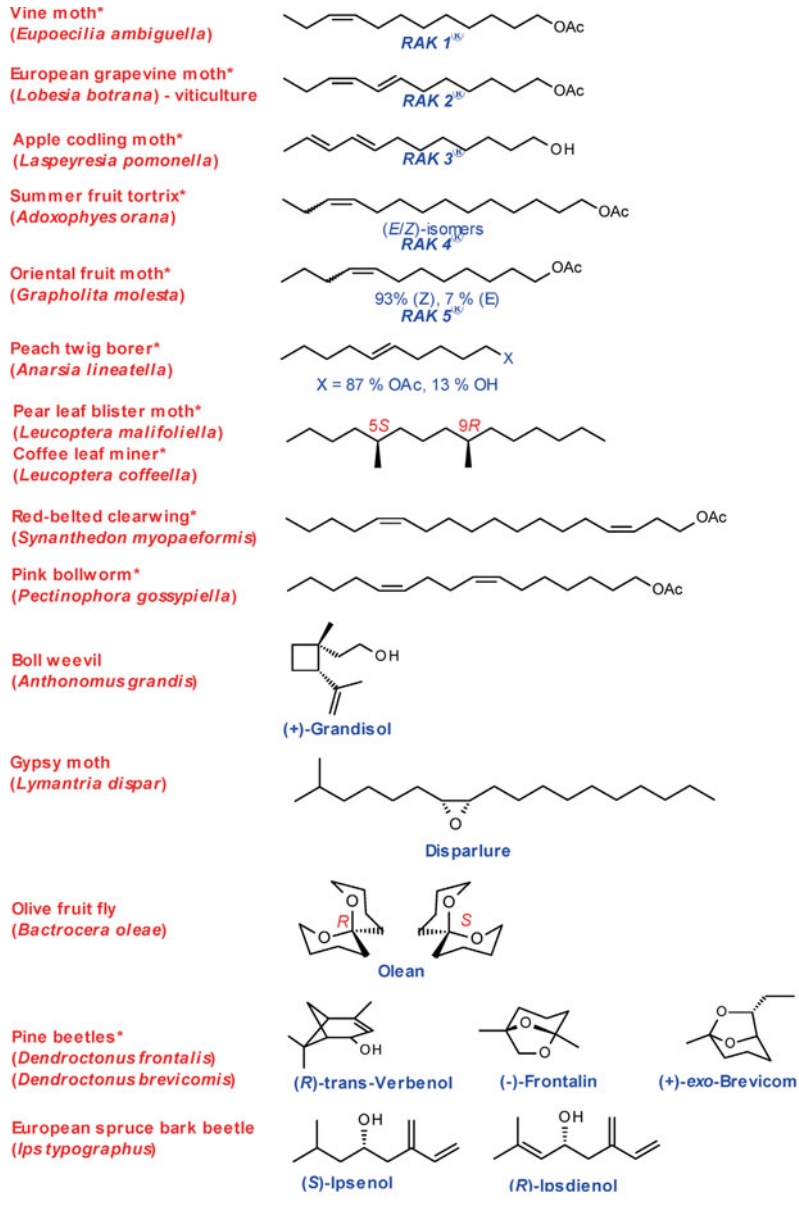
8.75 *Lindgren funnel trap.*



8.76 *A pheromone dispenser.*

generation. For this, permeable plastic dispensers, containing the sex attractant, are placed in the target crop area along a tailored pattern, from where the scent diffuses slowly, but evenly to generate a disorientating pheromone plume. Multi-chambered dispensers allow various insect pests to be controlled at the same time (Fig. 8.76). [181]

Some of the pests, which are relevant in agriculture and forestry, and the corresponding pheromones, applied in mass trapping or for the mating disruption, are listed in Fig. 8.77. [182]



8.77 *Pests and their pheromones relevant to agriculture and forestry. (* used via mating disruption method).*

8.5.7 Chemical Synthesis

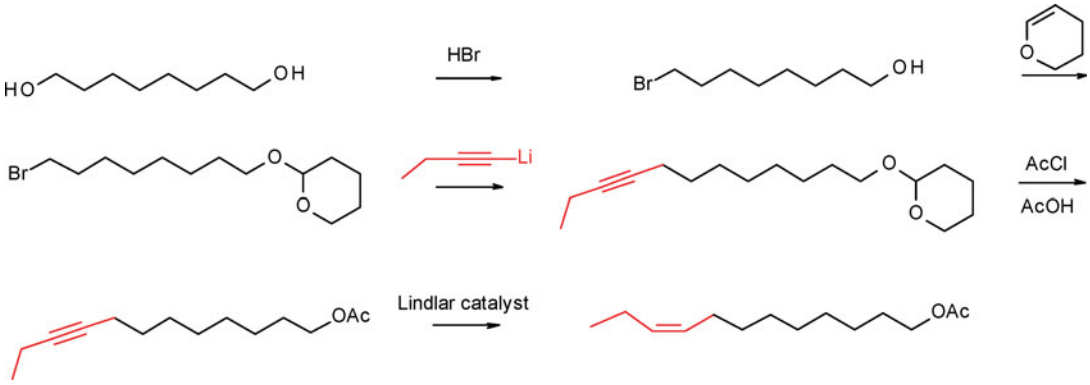
Since Butenandt's time, pheromone research has enjoyed a brisk and growing interest. Not least the unusual and diverse structures have kept this area of research alive. [183] Up to the present time, more than 800 pheromones are known. The following sections concentrate on the synthesis of attractants pertinent to agriculture and forestry.

8.78 *The European grape-vine moth (Lobesia botrana).*

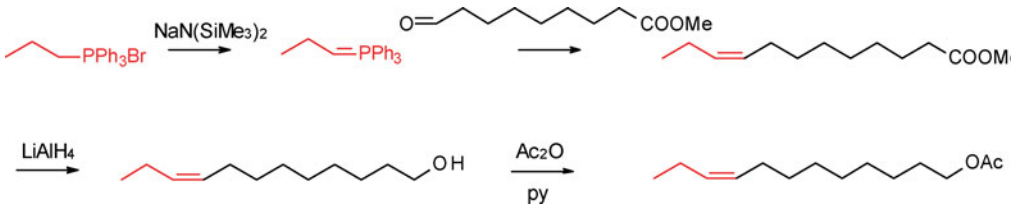
Vine moths (*RAK 1*®)

The pheromones of the vine moths (*Eupoecilia ambiguella* and *Lobesia botrana*) are typical for the sex attractants used by the large families of tortrix moths (Tortricidae) and owlet moths (Noctuidae) (Fig. 8.78).

Key reactions for the commercial production of these attractants with (*Z*)-configured double bonds are alkyne syntheses in combination with Lindlar hydrogenations, and Wittig reactions. The α,ω functionalised starting materials are obtained in a few steps from cyclooctadiene or from unsaturated fatty acids.



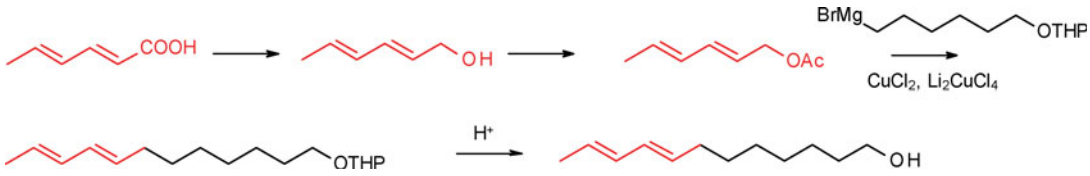
The (*Z*)-selective Wittig reaction with non-stabilised ylides, with exclusion of lithium salts, offers the possibility of avoiding alkynes, which are often thermally labile.



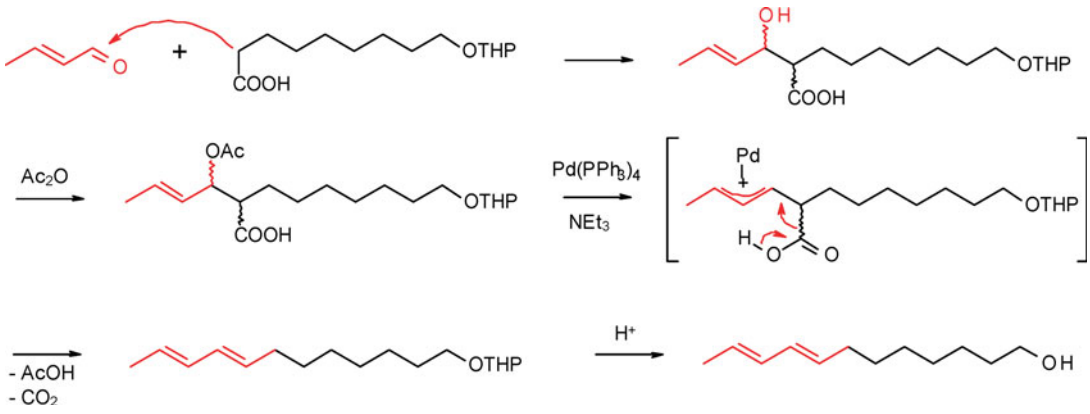
Apple codling moth (Codlemon, *RAK 3*®)

The Apple codling moth (*Laspeyresia pomonella*) is a pest of worldwide distribution (Fig. 8.79). Its pheromone can be produced in a few stages starting from sorbic acid, *via* a cuprate-mediated Grignard coupling (a Schlosser-Fouquet reaction) as the essential step. [162]

8.79 *The Apple codling moth (Laspeyresia pomonella).*



Codlemon is also accessible from crotonaldehyde, by means of an elegant palladium-catalysed elimination of carbon dioxide to generate the diene system. [184]



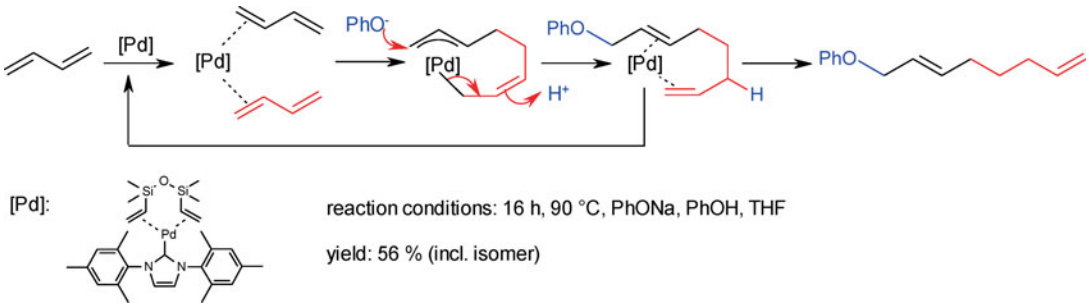
8.80 *The Peach twig borer (Anarsia lineatella).*

Peach twig borer (*CheckMate*®)

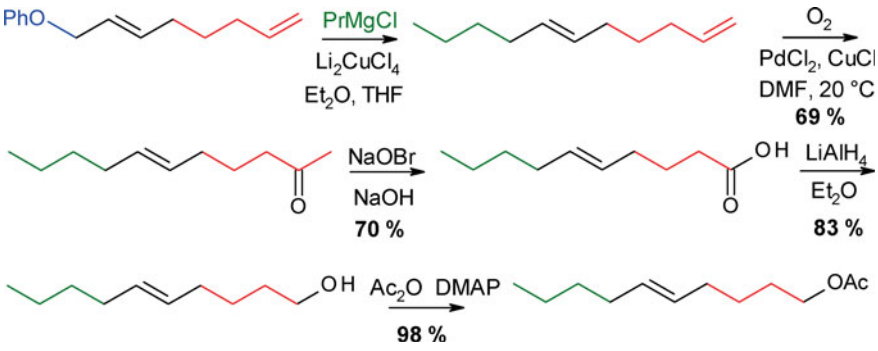
The Peach twig borer (*Anarsia lineatella*) is a moth commonly found in Europe, but was introduced to California in the 1880s (Fig. 8.80). It is a significant pest of peach, nectarine and apricot orchards and also of local almond plantations. [185]

Starting material for the preparation of the major component of its pheromone bouquet is 1-phenoxy-(2*E*,7)-octadiene, which is accessible by telomerisation of butadiene on a noble metal catalyst. Butadiene telomers find nowadays extensive use as plasiticiser alcohols, solvents, corrosion inhibitors and monomers for polymers.

In 2002, Matthias Beller described a palladium-carbene catalyst with excellent catalytic efficiency (turnover numbers of up to 1,500,000 and turnover frequencies of up to 100,000 h^{-1}). [186]



The cuprate-mediated reaction of 1-phenoxy-(2*E*,7)-octadiene with propylmagnesium chloride leads to undeca-1,6-diene, which may be selectively oxidised at the terminal double bond in a Wacker oxidation. Further oxidation with sodium hypobromite (haloform reaction) and reduction with lithium aluminium hydride then lead to (5*E*)-decenol, which can be esterified with acetic anhydride. The alcohol and the acetate are then mixed corresponding to their ratio in the natural pheromone composition. [187]



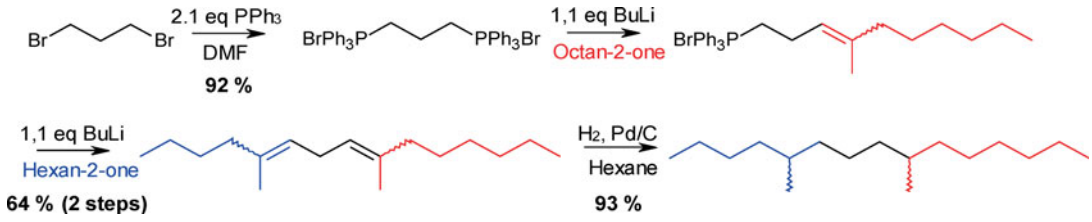
Coffee leaf miner

One of the most troublesome pests in Brazilian coffee plantations is the Coffee leaf miner moth (*Leucoptera coffeella*) (Fig. 8.81). In 1988, Wittko Francke recognised that the main component of the sex attractant of females is 5,9-dimethylpentadecane. [188]

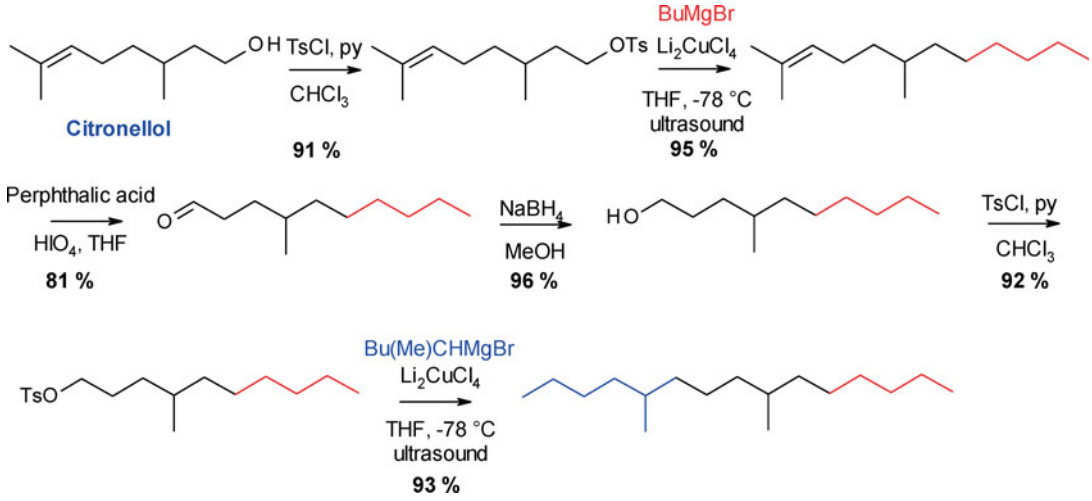
Field studies with monitoring traps, which contained the different stereoisomers, revealed the (5*S*,9*R*)-isomer as the most active component. [189]

For practical applications, the mixture of diastereomers can be used none the less. One of the shortest syntheses comes from Paulo Zarbin, and comprises an unsymmetrical double Wittig olefination. [190]

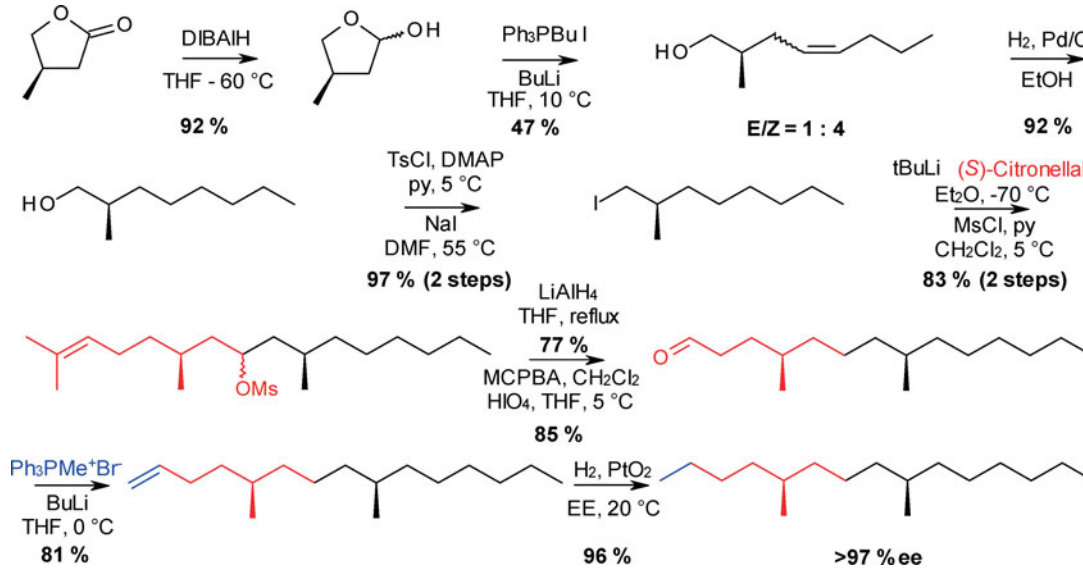
8.81 *The larva of the Coffee leaf miner (Leucoptera coffeella).*



Likewise in 2007, Fritz Duus published an elegant synthesis, the core steps in which are two consecutive ultrasound-assisted Schlosser-Fouquet cross-couplings, starting from the corresponding tosylates and Grignard reagents. The combined yield over all stages amounts to 57 %. [191]



One of the shortest syntheses of an enantiomerically pure pheromone comes from Kenji Mori. [192] The key building blocks of the synthesis are (3*R*)-methyl-4-butanolide, available from Mitsubishi Rayon Co., and (*S*)-citronellal, which are coupled by way of a corresponding organolithium reaction. The overall yield comes to 16 %.

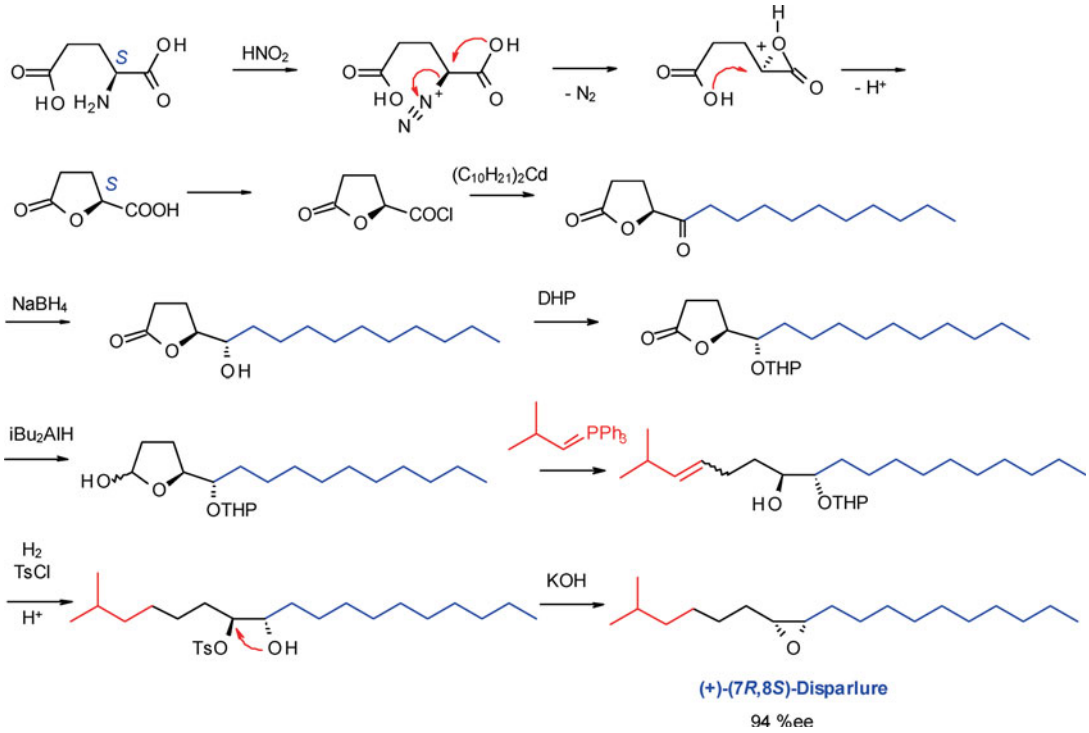


Gypsy moth (Disparlure)

The Gypsy moth (*Lymantria dispar*) is a dangerous forest pest (Fig. 8.82). Its caterpillars have reportedly defoliated and killed several hundred of tree and shrub species. They destroyed in the United States alone over 75 million acres of woodland since 1970. As noted above, of the sex attractant disparlure, only the (+)-enantiomer carries the bioactivity.

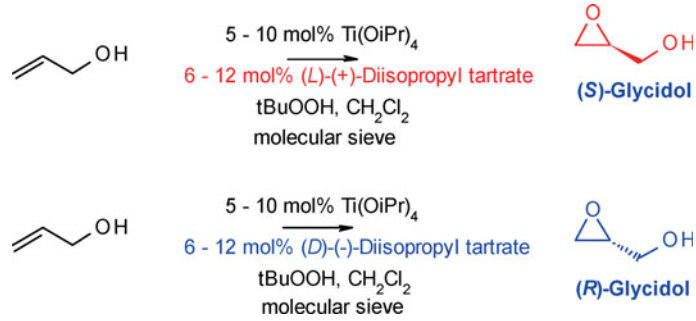
An interesting stereoselective laboratory synthesis draws on the *chiral pool*. Starting material is the inexpensive (*S*)-glutamic acid. By reaction with "nitrous acid" (prepared *in situ* from nitrite salt and mineral acid) a *γ*-lactone is obtained. The reaction proceeds with retention of the absolute configuration, since the neighbouring carboxylic acid moiety effects a stereochemical differentiation, and the (*Si*)-facial attack of the *γ*-situated carboxylic acid function is directed by the carbenium ion. Essential in this synthesis sequence are as well the subsequent steps of establishing the side-chain with cadmium didecanoate and the Wittig reaction of the lactol. The epoxide ring is finally closed by elimination of tosylate. An optical purity of 94 % was achieved.

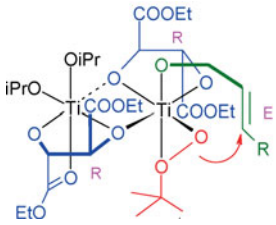
8.82 *The Gypsy moth (Lymantria dispar) – male (above) and female (below).*



The industrial synthesis is considerably more elegant. It involves one of the few non-enzymatic, enantioselective synthetic methods, which can be widely transferred to the industrial scale: the Sharpless epoxidation. Through the use of molecular sieves, K. B. Sharpless succeeded in carrying out the reaction with catalytic amounts of the enantiomerically pure titanium complex. Only this discovery rendered the reaction suitable for industrial dimensions.

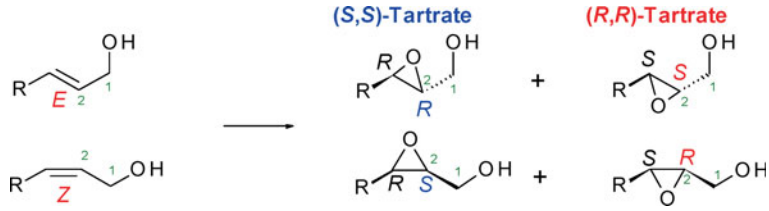
In case of the enantioselective epoxidation of allyl alcohol to glycidol, the Sharpless procedure, as conducted by ARCO, has to compete with an enzymatic resolution process from DAISO; however, for the preparation of disparlure the Sharpless epoxidation proves unsurpassed.



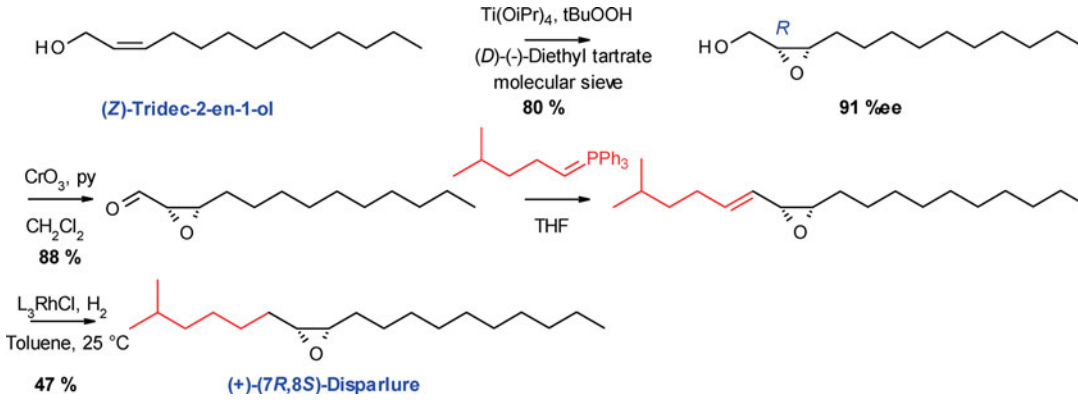


The reaction probably proceeds *via* the two-centred titanium complex, proposed by Elias J. Corey, in which the alcohol functions of the tartrate esters occupy the equatorial positions of the octahedrally coordinated titanium. The *t*-butyl hydroperoxide spans across an axial-equatorial edge; the allyl alcohol is axially complexed. The C_2-equivalent positions on the neighbouring titanium are occupied by alkoxides and one carboxylate function. In the epoxidation, the oxygen is transferred "from below" to the allylic double bond. [193]

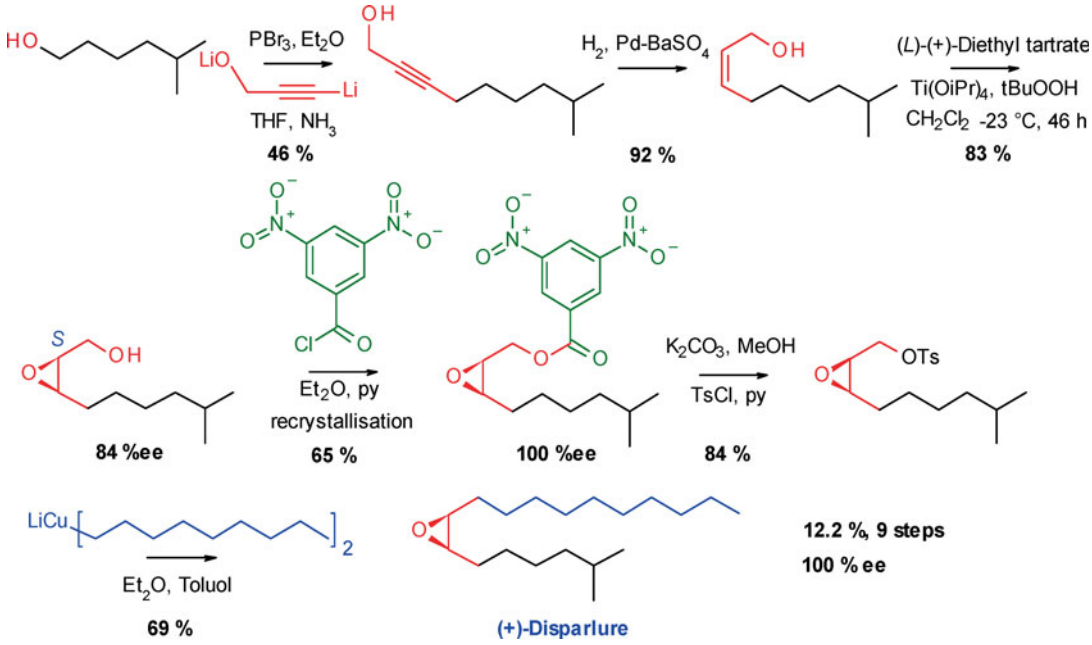
The use of (*E*)-allyl alcohols gives (*E*)-epoxides, and the use of (*Z*)-allyl alcohols gives (*Z*)-epoxides. According to the choice of tartrate ester, one diastereomer is obtained in excess.



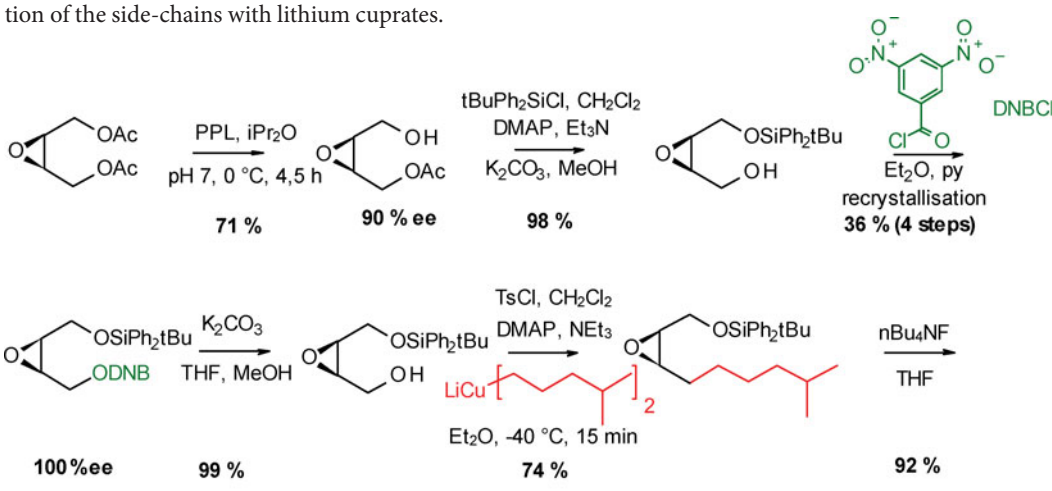
Whereas primary allyl alcohols may be converted to the extent of 100 %, for secondary alcohols, kinetic resolution is used to obtain one diastereomer in excess. The J. T. Baker Company harked back to the original work of Sharpless [194] and developed from it an industrial process for the production of (+)-disparlure.

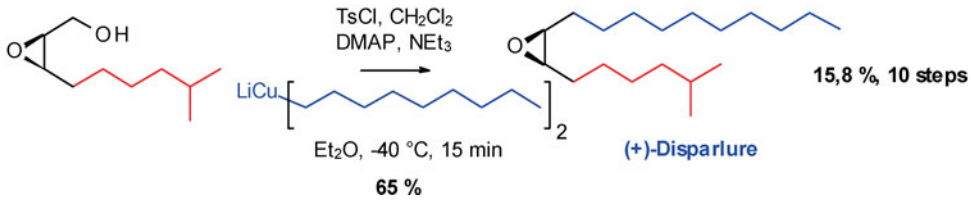


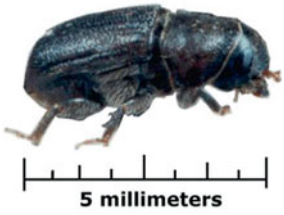
At the same time, Kenji Mori published a similar route to (+)-disparlure. [195] Since disparlure may virtually be built up from the other end, for the Sharpless epoxidation diethyl (*L*)-tartrate is used instead of the (*D*)-enantiomer. By recrystallisation of the 3,5-dinitrobenzoate, the desired enantiomer of the intermediate can be enriched. Enantiomerically pure (+)-disparlure is finally obtained by chain extension with lithium dinonylcuprate.



Also from Mori stems an enzymatic method for the preparation of (+)-disparlure. [196] With butyne-1,4-diol as the starting material, the *meso-trick* (*cf.* section 5.1.3) is used in order to generate the mono-protected diol in good yield and enantiomeric purity. The attractant is then obtained by stepwise introduction of the side-chains with lithium cuprates.





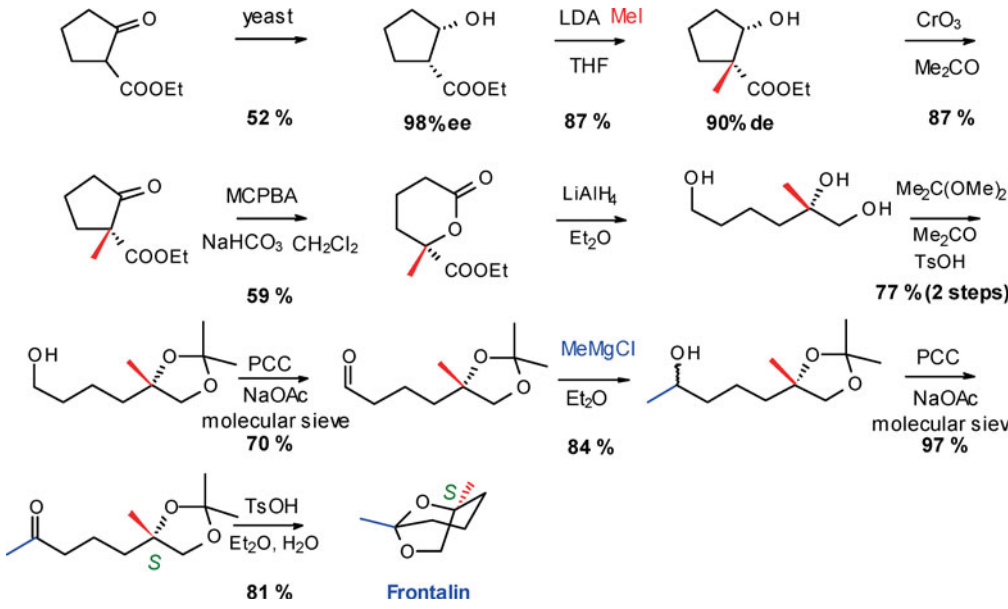


8.83 *The Mountain pine beetle (Dendroctonus ponderosae).*

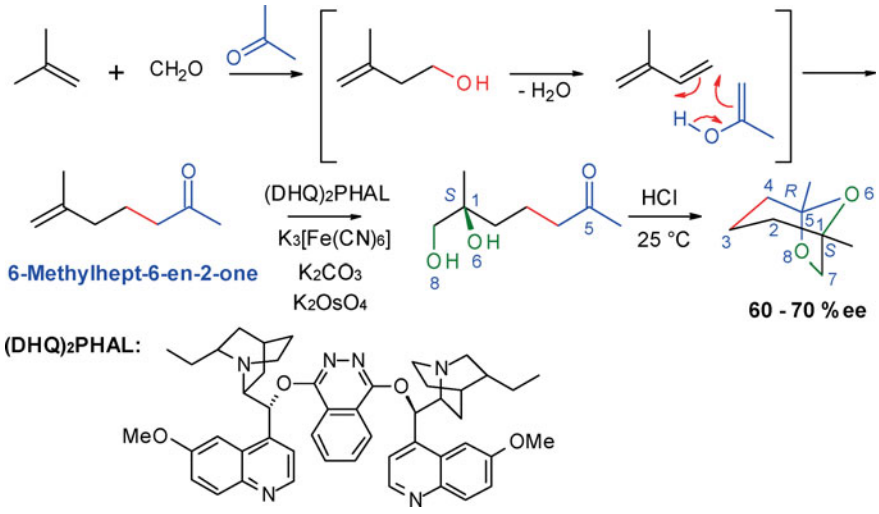
Male Southern pine beetle (Frontalin)

One of the most destructive pests in pinewoods in the south-east of the USA is the Southern pine beetle (*Dendroctonus frontalis* Zimmermann), and in western North America the Mountain pine beetle (*Dendroctonus ponderosae*) (Fig. 8.83).

In 1969, Glenn W. Kinzer [197] isolated frontalin, an important component of the aggregation pheromone of these beetle species. [198] In 1998, Mori published an enantioselective synthesis of this pheromone. [199] Key steps are the enantioselective reduction of the β-keto-ester, the diastereoselective methylation and the Baeyer-Villiger oxidation. The methylation proceeds under chelation control, and gives the desired methylation product with excellent diastereoselectivity.



One of the most elegant syntheses comes from Joseph A. Turpin and Leland O. Weigel. Starting material is 6-methylhept-6-en-2-one, which is as well an intermediate in the ionone synthesis, and can be accessed from isobutene, formaldehyde and acetone. By means of a Sharpless dihydroxylation and acid ring-closure, frontalin is obtained with an enantiomeric excess of 60–70 %. [200]

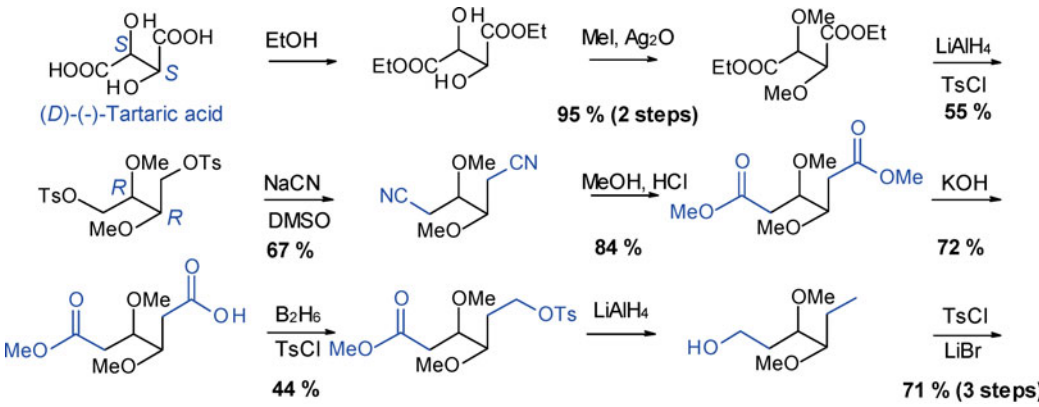


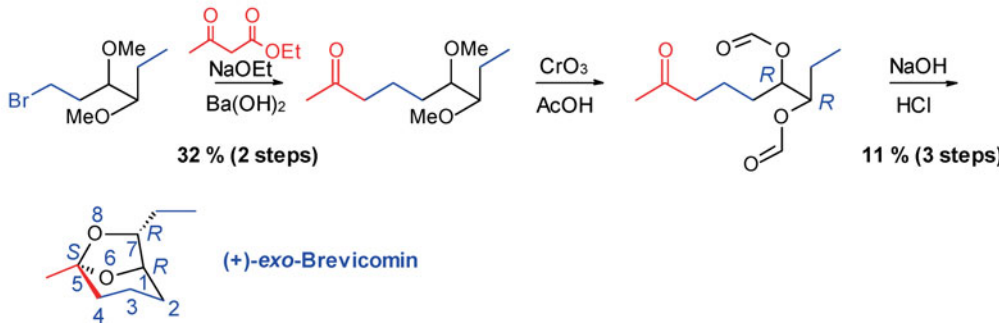
The male Southern pine beetle produces an 85 : 15 mixture of the (1*S*,5*R*)- and (1*R*,5*S*)-enantiomers [201] of frontalin, which corresponds to a "formal" enantioselectivity of only 70 %; therefore, the Sharpless dihydroxylation yields exactly the correct mixture.

Female Western pine beetle ((+)-*exo*-Brevicomin)

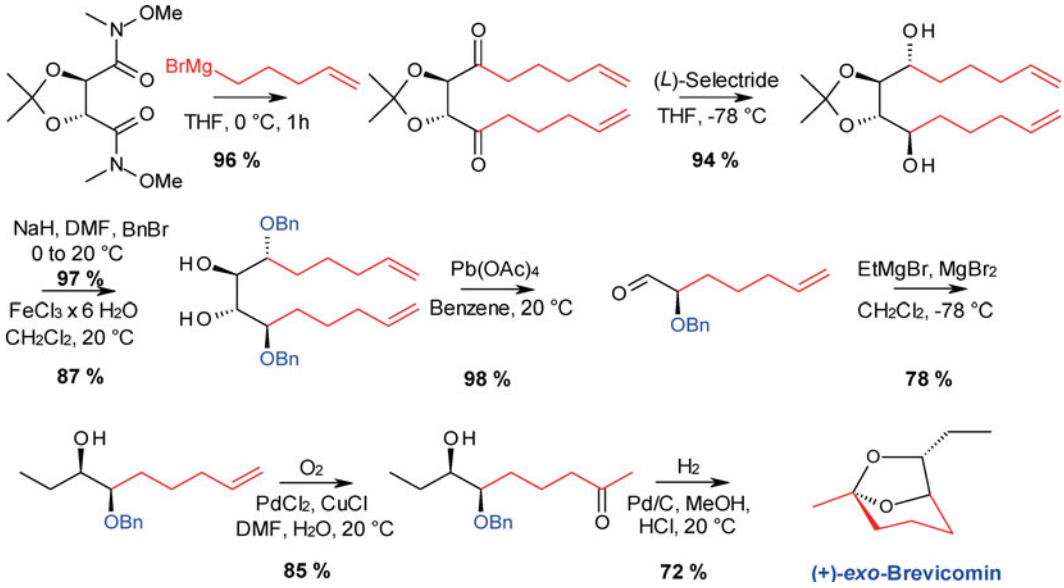
The sex attractant of the female Western pine beetle (*Dendroctonus brevicomis*) is the enantiomerically pure (1*R*,5*S*,7*R*)-(+)-*exo*-brevicomin. The (–)-enantiomer is not biologically active. In contrast, the pheromone component (1*R*,5*S*,7*S*)-(+)-*endo*-Brevicomin caused variable responses in the Southern pine beetle (*Dendroctonus frontalis* Zimmermann). [202]

One of the first syntheses of enantiomerically pure brevicomin is likewise due to Mori. [203] The starting material is (*D*)-(–)-tartaric acid. After elongation of the carbon chain, one ester is selectively hydrolysed, and in three steps reduced to an ethyl group. The keto-function is introduced with ethyl acetoacetate. The methyl ethers are oxidatively degraded and the rings closed with acid catalysis to yield brevicomin.

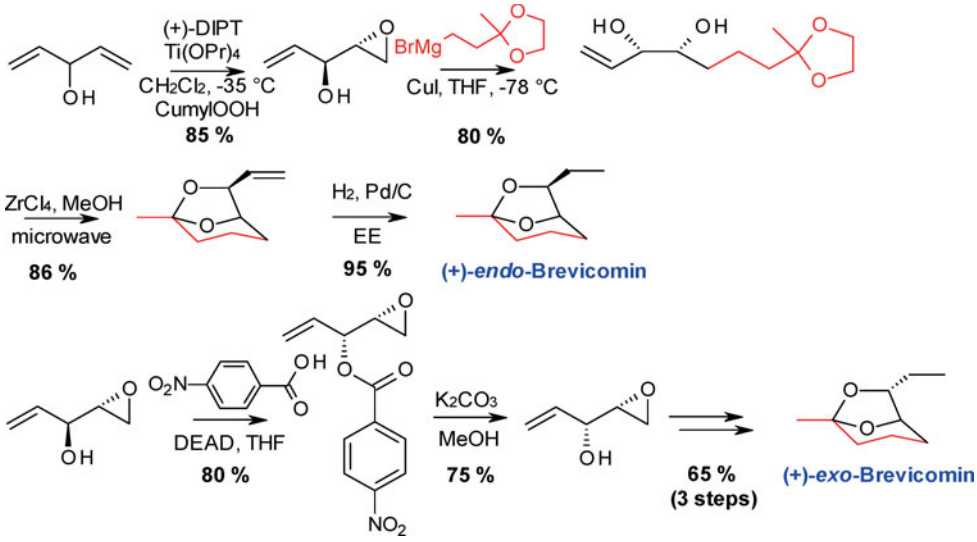




Kavirayani R. Prassad's elegant brevicomin synthesis starts also from tartaric acid, although actually from its Weinreb amide, which is converted into the desired intermediate by a double Grignard reaction. Other key steps in the synthesis are the lead tetraacetate-mediated cleavage of the diol and the Wacker oxidation of the terminal alkene. [204]



The essential steps of the intriguing route to (+)-*endo*-brevicomin by Patrick Guiry are a Sharpless epoxidation, ring-opening of the epoxide with a Grignard reagent in presence of copper iodide, and the microwave-assisted zirconium tetrachloride-catalysed *trans*-ketalisation. After inversion of the stereogenic centre of the allyl alcohol in a Mitsunobu reaction, (+)-*exo*-brevicomin is accessible using the same synthetic strategy. [205]

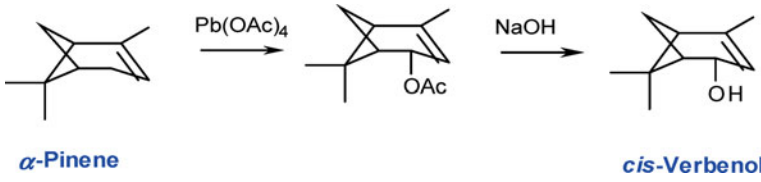


Male European spruce bark beetle (cis-Verbenol, (S)-Ipsenol, (R)-Ipsdienol)

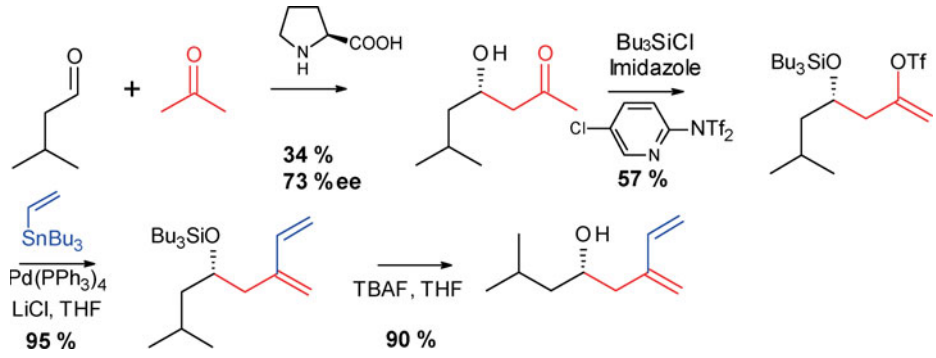
One of the most serious pest insects in old stands of spruce is the European spruce bark beetle (*Ips typographus*) (Fig. 8.84). While colonising a tree, 2-methylbut-3-en-2-ol and (*S*)-*cis*-verbenol are secreted first. After establishing a mating chamber, the polygamous male attracts females with ipsenol and ipsdienol. [180]

8.84 *The European spruce bark beetle (Ips typographus).*

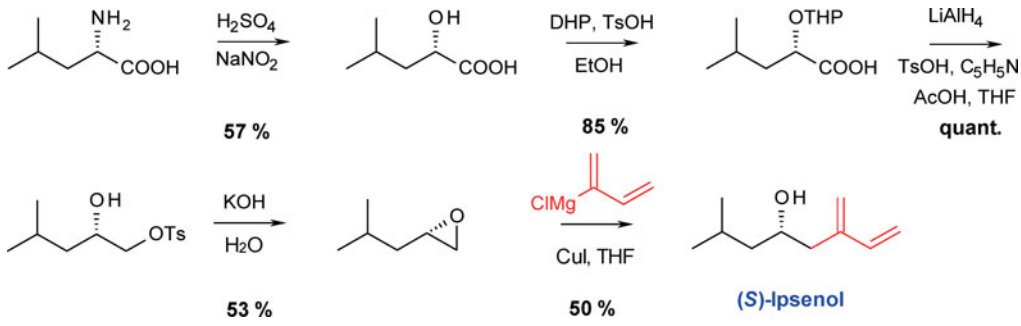
cis-Verbenol is obtained from α-pinene by oxidation with lead tetraacetate and subsequent hydrolysis.



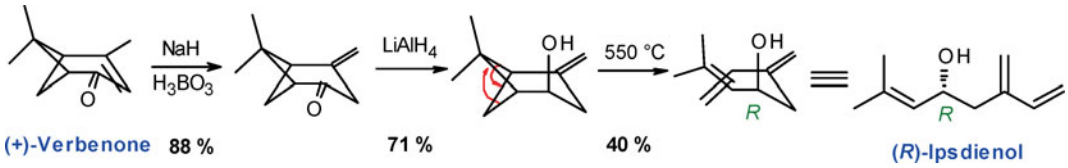
For ipsenol, Benjamin List published in 2001 a synthesis starting from 3-methylbutanal and acetone. [206] The enantioselective aldol reaction is catalysed by proline to yield the hydroxy-ketone with an enantiomeric excess of 73 %. After protection of the alcohol function, the enol triflate is formed regioselectively. The vinyl substituent is introduced by means of a Stille coupling, and followed by deprotection.



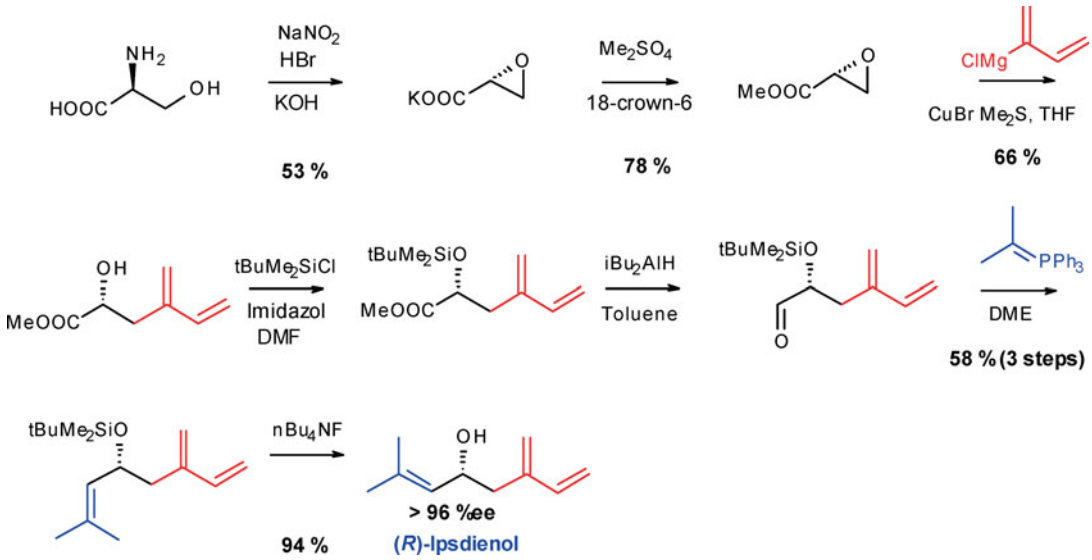
In 1979, Mori synthesised enantiomerically pure (*S*)-ipsenol based on (*S*)-leucine. [207] This is converted in a few steps into an enantiomerically pure epoxide, which gives (*S*)-ipsenol with a Grignard reagent derived from chloroprene.



(*R*)-Ipsdienol is obtained from (+)-verbenone in a short reaction sequence, which comprises as the critical step a thermolysis at 550 °C. [208]



(*R*)-Ipsdienol, like ipsenol, is also accessible from the amino acid (*S*)-serine. After generation of the enantiomerically pure epoxypropanoate ester, the carbon skeleton is built up by a Grignard reaction and a Wittig reaction. [176]

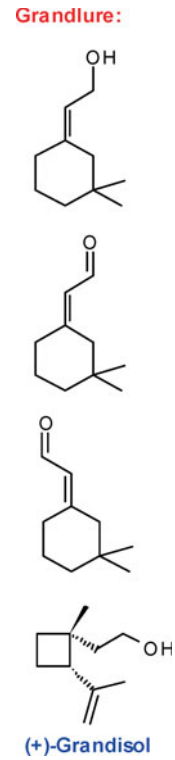


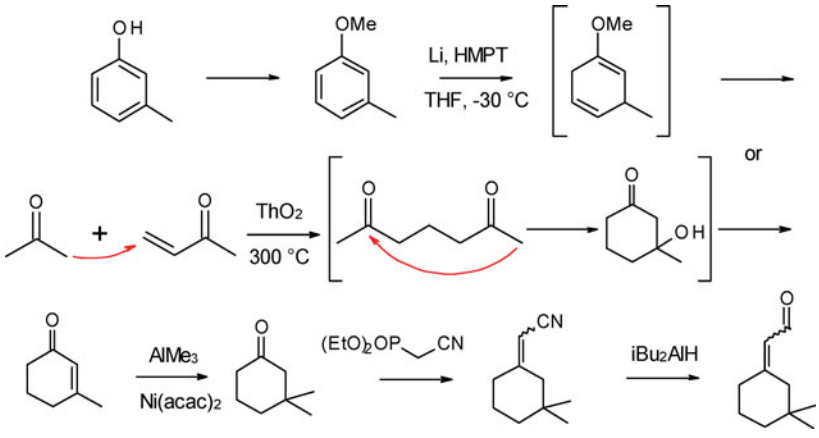
Male Boll weevil (Grandlure)

The aggregation pheromone grandlure of the male Boll weevil (*Anthonomus grandis*) (Fig. 8.85) consists of four components. In field trials, the attractive effect of grandlure on both sexes was recognised. [209]

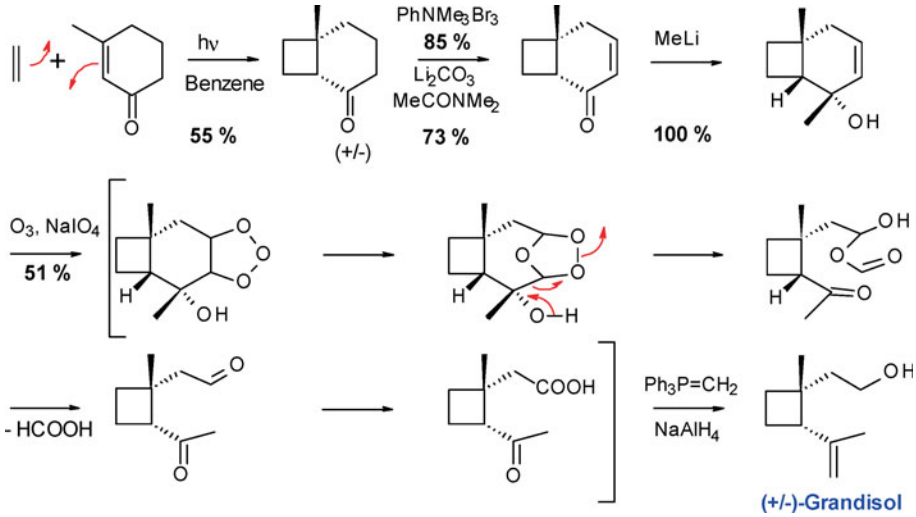
The dimethylcyclohexane compounds are obtained in a Horner-Wadsworth-Emmons reaction from dimethylcyclohexanone with subsequent reduction. As possible starting materials, *m*-cresol [210], just like acetone and methyl vinyl ketone [211], can be considered. The second methyl group may be inserted advantageously using trimethylaluminium. [212]

8.85 *The Boll weevil (Anthonomus grandis).*

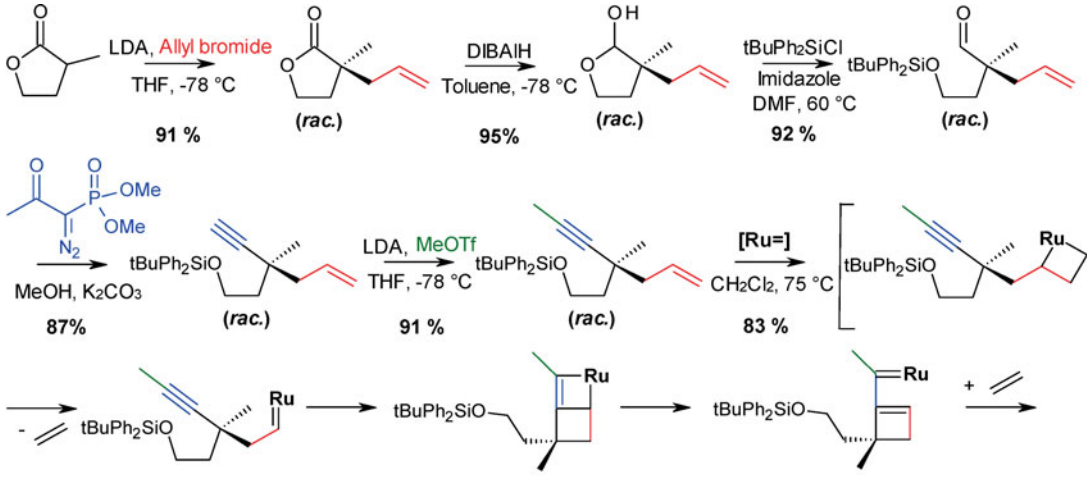


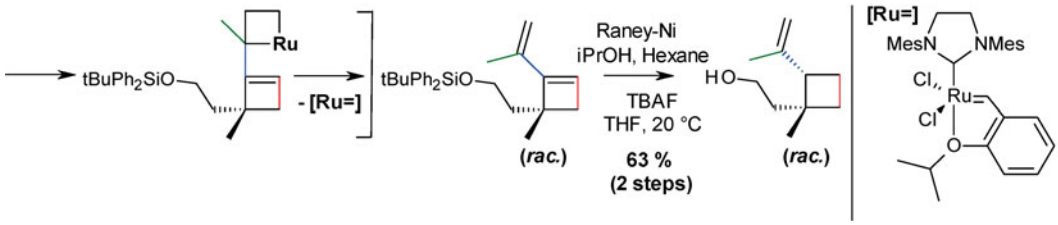


Racemic grandisol is likewise accessible from 3-methylcyclohexenone by a photochemical [2 + 2]-cycloaddition with ethylene under formation of the *cis*-fused bicycle. A double bond is introduced into the six-membered ring by a bromination-dehydrobromination sequence. Attack by methyllithium is controlled by the four-membered ring system, which is however insignificant for the further transformations, because during the following ozonolysis in presence of periodate, the configuration at the stereocentre is lost again. A final Wittig reaction and reduction provide racemic grandisol. [209]



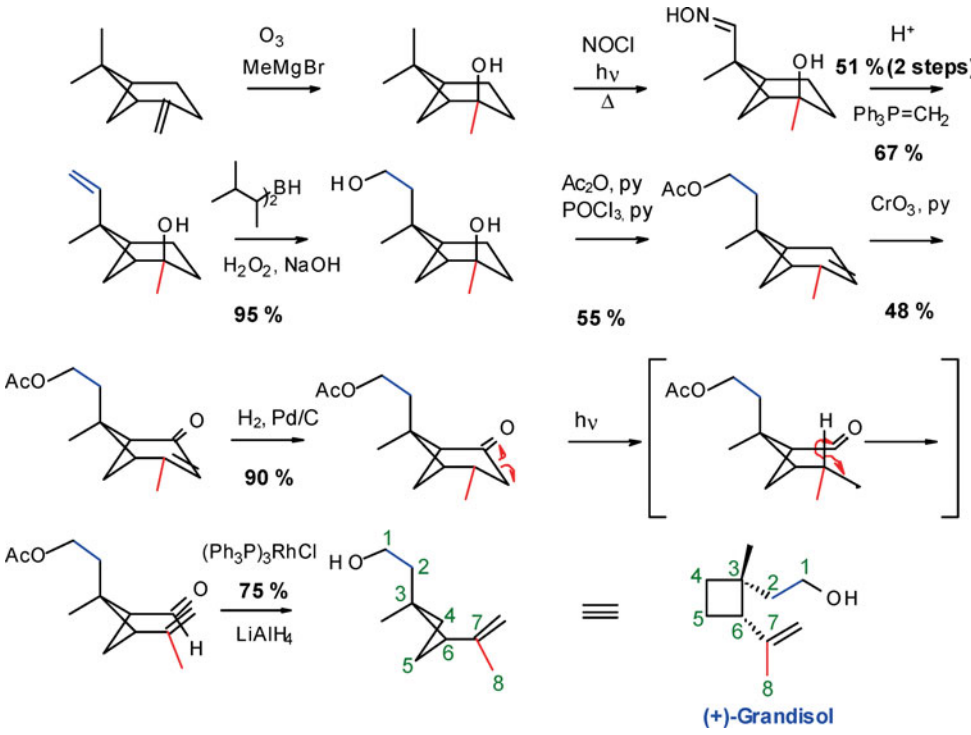
A short synthesis of racemic grandisol, starting from readily available α-methyl-γ-butyrolactone, was published by Brian C. Goess in 2010. Key steps are the Seyferth-Gilbert homologation (conversion of an aldehyde into an alkyne by the use of the Bestmann-Ohira reagent) and an enyne metathesis reaction. Remarkable is also the hydrogenation, which uses hydrogen-saturated Raney-Ni to avoid a hydrogen atmosphere, and to prevent complete reduction of the diene. The overall-yield of this eight-step-synthesis is 33 %. [213]





Enantiomerically pure (+)-grandisol is obtainable from (–)-β-pinene. [214] In this case, one can take advantage of the fact that pinene contains a four-membered ring system with the proper stereochemical substitution pattern. After ozonolysis, the resulting ketone is reacted with methylmagnesium chloride. For steric reasons, the addition is highly diastereoselective. By means of nitrite ester photolysis (the Barton reaction), an unactivated C-H bond of the *endo*-methyl group is attacked, and transformed to the aldoxime. Hydrolysis, *via* an intramolecular hemiacetal, and a Wittig reaction lead to an alkene. The remaining steps are a hydroboration with oxidative work-up, an allylic oxidation and a hydrogenation. Key is the Norrish-Type-2 reaction, which generates the isopropenyl group. The formyl group is removed with stoichiometric amounts of the Wilkinson complex, and the acetate cleaved off by reduction with lithium aluminium hydride. Basic hydrolysis is not possible, due to isomerisation at C-6 to the more stable *trans*-grandisol.

The term "Norrish-Type-2 cleavage" is understood as the photolysis of aldehydes or ketones at 230–330 nm, generating alkyl and acyl radicals, which stabilise themselves, for example, by intramolecular hydrogen abstraction or fragmentation. [215]



8.86 *The Olive fruit fly (Bactrocera oleae).*

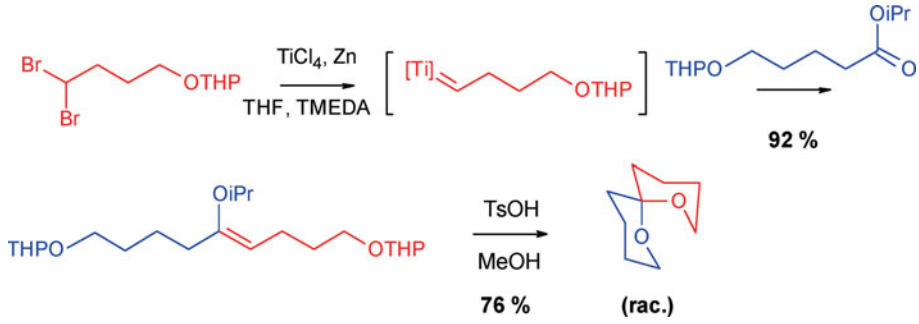
Olive fruit fly (Olean)

The Olive fruit fly (*Bactrocera oleae*) originates most likely from the Mediterranean countries, where infestations have been reported even in BC times. The pest spread further to South Africa, across the Middle East to Pakistan and northern India. Since 1998, also olive plantations in California are threatened (Fig. 8.86). The pheromone of the Olive fruit fly is olean, a spiroketal (1,7-Dioxaspiro[5.5]undecane).

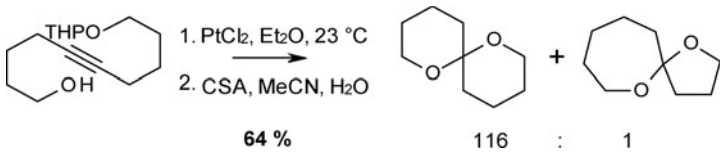
Absolute configuration of olean

The stereogenic centre of olean has only two different substituents. The C2 symmetry of the molecule is based on the *spiro*-linkage of the two identical rings. For assigning the absolute configuration, a less common rule for stereogenic centres, similar to the axial chirality for atropisomeric compounds is used: "front before rear". First, the molecule is oriented so that there is a front and a rear ring. The substituent with the lowest priority is the carbon in the rear ring. The three remaining substituents can then be determined along the usual Cahn-Ingold-Prelog priority rules. The oxygen in the rear ring has a lower priority, because it is linked in the ring with the lowest-priority substituent. Accordingly, the displayed structure possesses the (*R*)-configuration. [216]

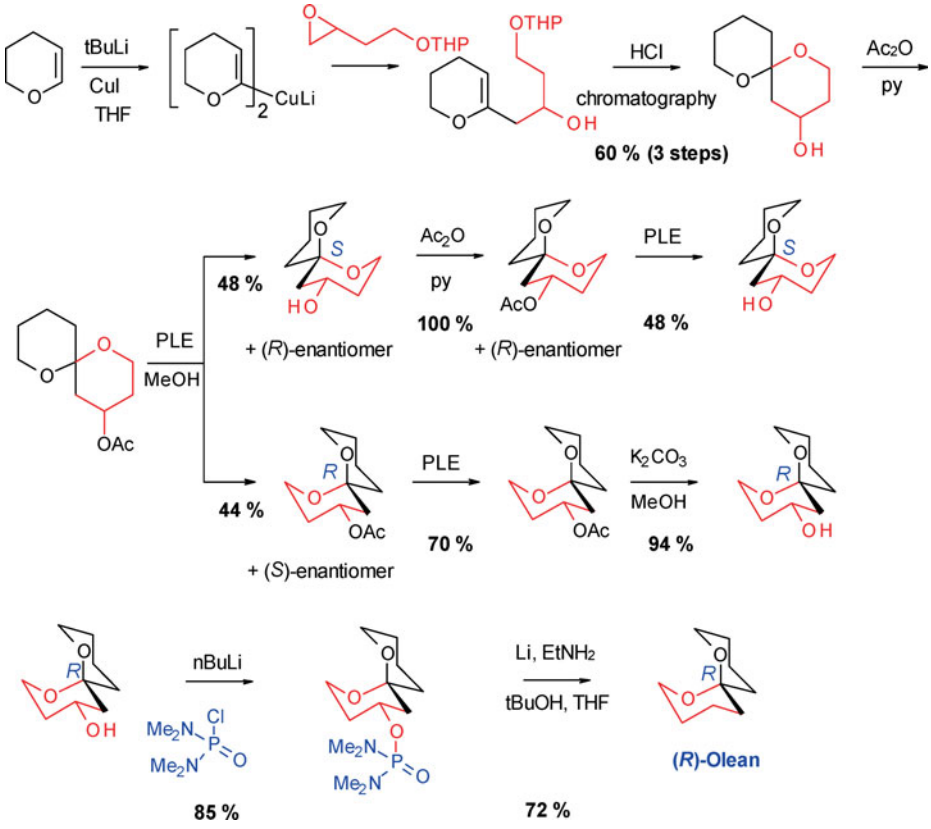
Philip Kocienski published an elegant synthesis of racemic olean. The starting material is the THP ether of 4,4-dibromobutanol. In spite of the acid-sensitivity of the acetal, the formation of a carbene complex with titanium tetrachloride and zinc can be achieved. Its reaction with a corresponding ester leads to an enol ether, which cyclises to olean under acidic conditions. [217]



Another very short route to racemic olean comes from Jef De Brabander, and is based on the platinum-catalysed intramolecular hydroalkoxylation of internal alkynes. Under optimised conditions, the 6-*exo-dig*/7-*endo-dig* ratio is more than 100 : 1. [218]



The crucial step in an enantioselective synthesis by Mori [219] is the pig liver esterase-catalysed hydrolysis of the corresponding acetoxy-derivative. Regrettably, the selectivity is not sufficiently high, so that the resolution has to be carried out twice to obtain enantiomerically pure material. In the final step, the alcohol function is reductively removed.



Summary in Bullet Points

- From a phylogenetic point of view, chemical messengers belong to a very old communication system.
- Although pheromones are most prominent and wide-spread in the insect world, they also play an important role in protozoa, arthropods, and even in higher animals like fish, reptiles and mammals.

- Nowadays, there are three application methods used for pest control in agriculture and forestry: monitoring, mass trapping and mating disruption.
- The active ingredients are produced, formulated and marketed by established chemical companies and, while their quantities are comparatively small, in part also by research institutes.

References

References to Introduction

1. B. Fugmann, F. Lieb, H. Moeschler, K. Naumann, U. Wachendorff, *Chemie in unserer Zeit* **25** (1991) 317.
2. W. Schwab, *Pharmazie in unserer Zeit*, **29** (2000) 107; A. Sinz, *Pharmazie in unserer Zeit* **37** (2008) 306; M. R. Lee, *J. R. Coll. Physicians Edinb.* **39** (2009) 179.
3. Ullmann's Encyclopedia of Industrial Chemistry, 6th Edn., 1999, Electronic Release, Insect Control; http://www.theguardian.com/environment/2013/mar/22/us-government-sued-pesticides-bee-harm.
4. The Columbia Encyclopedia, 6th Edn., 2001–05, Electronic Release, Paris Green.
5. F. Müller in Winnacker, Küchler, Chemische Technik, Vol. 8, Wiley-VCH, Weinheim, 2005, 213.
6. http://www.nationalhistoryday.com/03_educators/teacher/doc7.htm.
7. A. v. Baeyer, O. Zeidler, J. Weiler, O. Fischer, E. Jäger, E. Ter Mer, W. Hemilian, E. Fischer, *Ber. dtsch. chem. Ges*. **7** (1874) 1181.
8. T. H. Jukes, *Naturwiss.* **61** (1974) 6; http://www.who.int/malaria/publications/world_malaria_report_2012/wmr2012_factsheet.pdf.
9. J. Stetter, F. Lieb, *Angew. Chem. Internat. Edn*. **39** (2000) 1724.
10. A. Hübenthal, *Nachr. Chem*. **53** (2005) 735; Phillips McDougall – AgriService, Products Section – 2012 Market.

References to 8.1 Amino Acid Herbicides

11. E. Bayer, K. H. Gugel, K. Hägele, H. Hagenmaier, S. Jessipow, W. A. König, H. Zähner, *Helv. Chim. Acta* **55** (1972) 224.
12. J. R. Corbett, K. Knight, A. C. Baillie, The Biochemical Mode of Action of Pesticides, Academic Press, 2nd Edn., 1984, 283; G. Hoerlein, *Rev. Environ. Contam. Toxicol.* **138** (1994) 73.
13. T. Seitz, M. G. Hoffmann, H. Krähmer, *Chemie in unserer Zeit* **37** (2003) 112; Phillips McDougall – AgriService, Products Section – 2010 Market.
14. K.-J. Haack, *Chemie in unserer Zeit* **37** (2003) 128.
15. E. W. Logusch, *Tetrahedron Lett.* **27** (1986) 5935.
16. L. Maier, P. Lea, *Phosphorus Sulfur* **17** (1983) 1 (further syntheses).
17. H. Wakamatsu, J. Uda, N. Yamakami, *Chem. Commun*. (1971) 1540.
18. E. Jägers, H. Erpenbach, F. Bylsma (Hoechst) DE 3823886 (1988).
19. S. Takigawa, S. Shinke, M. Tanaka, *Chem. Lett*. (1990) 1415.
20. G. Donn in R. De Prado, J. Jorrin, L. Garcia-Torres, Weed and Crop Resistance to Herbicides, Kluwer Academic, Dordrecht, 1997, 221.
21. http://en.wikipedia.org/wiki/Glufosinate; http://news.agropages.com/News/NewsDetail---9598.htm;
22. K.-H. König, *Chemie in unserer Zeit* **24** (1990) 217.
23. B. Hock, C. Fedtke, R. R. Schmidt, Herbizide, Thieme, Stuttgart, New York, 1995, 190 ff.
24. J. R. Corbett, K. Knight, A. C. Baillie, The Biochemical Mode of Action of Pesticides, Academic Press, New York, 2nd Edn., 1984, 276.

25. W. Schwab, *Pharmazie in unserer Zeit* **29** (2000) 107; T. Funke, Y. Yang, H. Han, M. Healy-Fried, S. Olesen, A. Becker, E. Schönbrunn, *J. Biol. Chem.* **284** (2009) 9854.
26. B. Hock, C. Fedtke, R. R. Schmidt, Herbizide, Thieme, Stuttgart, 1995, 194.
27. L. Maier, *Phosphorus, Sulfur and Silicon* **144** (1999) 429.
28. D. L. Field Jr. (Monsanto), US 5095140 (1992).
29. N. C. Aust, T. Butz, M. Fischer (BASF), WO 2001/12639 (2001), catalysis with sulfur dioxide); N. C. Aust, T. Butz, M. Fischer (BASF), WO 2001/12640 (2001), catalysis with KSCN.
30. A. Bader, *Aldrichimica Acta* **21** (1988) 15.

References to 8.2 Strobilurins

31. H. Sauter, W. Steglich, T. Anke, *Angew. Chem. Internat. Edn.* **38** (1999) 1328; U. Hölke, M. Höfer, J. Lenz, *Appl. Microbiol. Biotechnol.* **64** (2004) 175.
32. E. R. H. Jones, V. Thaller, Handbook of Microbiology, CRC Press, Boca Raton, Vol. 5, 2nd Edn., 1984, 83; R. E. Minto, B. J. Blacklock, *Prog. Lipid Res.* **47** (2008) 233.
33. M. Anchel, A. Hervey, F. Kavanagh, J. Polatnick, W. J. Robbins, *Proc. Natl. Acad. Sci. USA* **34** (1948) 498.
34. H. Anchel, A. Hervey, W. J. Robbins, *Proc. Natl. Acad. Sci. USA* **36** (1950) 300; F. Kavanagh, A. Hervey, W. J. Robbins, *Proc. Natl. Acad. Sci. USA* **37** (1951) 570.
35. H. Egger, J. Reinshagen, *J. Antibiot.* **29** (1976) 915.
36. H. Egger, J. Reinshagen, *J. Antibiot.* **29** (1976) 923.
37. V. Musilek, J. Cerna, V. Sasek, M. Semerdzieva, M. Vondracek, *Folia Microbiol. (Prague)* **14** (1969) 377.
38. F. Nerud, P. Sedmaera, Z. Zouchova, V. Musilek, V. Miloslav, *Coll. Czech. Chem. Comm.* **47** (1981) 1020; A. M. Soares-Sello, PhD Thesis, Bristol, 1997; G. Thormann, A. Zeeck, unpublished results; G. Thormann, PhD Thesis, Göttingen, 2004.
39. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 540; Z. Zhang, L. Huang, V. M. Shulmeister, Y.-I. Chi, K. K. Kim, L.-W. Hung, A. R. Crofts, E. A. Berry, S.-H. Kim, *Nature* **392** (1998) 677; J. M. Clough, *Nat. Prod. Reports* **10** (1993) 565; P. Kraiczy, U. Haase, S. Gencic, S. Flindt, T. Anke, U. Brandt, G. von Jagow, *Eur. J. Biochem.* **235** (1996) 54; http://en.wikipedia.org/wiki/Electron_transport_chain.
40. J. M. Clough, *Nat. Prod. Rep.* **10** (1993) 565; A. R. Crofts, *Annual Review of Physiology* **66** (2004) 689; U. Brandt, U. Haase, H. Schägger, G. von Jagow, *J. Biol. Chem.* **266** (1991) 19958.
41. http://edis.ifas.ufl.edu/pi104**.**
42. H. Ishii, B. A. Fraaije, T. Sugiyama, K. Noguchi, K. Nishimura, T. Takeda, T. Amano, D. W. Hollomon, *Phytopathology* **91** (2001) 1166; P. Leroux, M. Gredt, M. Leroch, A.-S. Walker, *Appl. Environ. Microbiol.* **76** (2010) 6615.
43. Phillips McDougall – AgriService, Products Section – 2010 Market.
44. J. M. Clough, *Nat. Prod. Rep.* **10** (1993) 565 (Review).
45. T. Anke, G. Schramm, B. Schwalge, B. Steffan, W. Steglich, *Liebigs Ann. Chem.* (1984) 1616.
46. K. Beautement, J. M. Clough, *Tetrahedron Lett.* **28** (1987) 475.
47. K. Beautement, J. M. Clough, P. de Fraine, C. R. A. Godfrey, *Pestic. Sci.* **31** (1991) 499.
48. C. J. Kowalski, M. S. Haque, K. W. Fields, *J. Am. Chem. Soc.* **107** (1985) 1429.
49. K. Mikami, K. Azuma, T. Nakai, *Chem. Lett.* (1983) 1379.
50. K. Mikami, K. Azuma, T. Nakai, *Tetrahedron* **40** (1984) 2303.
51. J. Kallmerten, M. D. Wittman, *Tetrahedron Lett.* **27** (1986) 2443.
52. J. Kallmerten, M. D. Wittman, *J. Org. Chem.* **52** (1987) 4303.

53. H. Akita, H. Koshiji, A. Furuichi, K. Horikoshi, T. Oishi, *Tetrahedron Lett*. **24** (1983) 2009.
54. T. Oishi, H. Akita, *J. Synth. Org. Chem. Jpn*. **41** (1983) 1031.
55. H. Akita, A. Furuichi, H. Koshiji, K. Horikoshi, T. Oishi, *Chem. Pharm. Bull*. **31** (1983) 4376.
56. T. Honda, K. Naito, S. Yamane, Y. Suzuki, *J. Chem. Soc., Chem. Commun*. (1992) 1218.
57. H. Sauter, E. Ammermann, F. Roehl, Crop Protection Agents from Nature, Royal Society of Chemistry, Cambridge, 1996, 50.
58. B. Wenderoth, C. Rentzea, E. Ammermann, E.-H. Pommer, W. Steglich, T. Anke, BASF, EP 0 253 213 (1987).
59. H. Wingert, B. Wolf, R. Benoit, H. Sauter, M. Hepp, W. Grammenos, T. Kuekenhöhner, BASF, EP 0 493 711 (1991).
60. K.-J. Haack, *Chemie in unserer Zeit* **37** (2003) 128.
61. Zeneca, WO 9208703 (1992); US 5760250 (1998).
62. V. V. Zakharychev, L. V. Kovalenko, *Russ. Chem. Rev*. **67** (1998) 535.

References to 8.3 Pyrethroids

63. John E. Casida, *Environmental Health Perspectives* **34** (1980) 189; http://www.pyrethrum.com/About_Pyrethrum/History.aspx; F. Müller in Winnacker, Küchler, Chemische Technik, Vol. 8, Wiley-VCH, Weinheim, 2005, 213; Phillips McDougall – AgriService, Products Section – 2010 Market.
64. H. Staudinger, L. Ruzicka, *Helv. Chim. Acta* **7** (1924) 177.
65. H. Staudinger, L. Ruzicka, *Helv. Chim. Acta* **7** (1924) 201, 212, 236, 245, 448.
66. H. Staudinger, O. Muntwyler, L. Ruzicka, S. Seibt, *Helv. Chim. Acta* **7** (1924) 390.
67. F. B. La Forge, W. F. Barthel, *J. Org. Chem*. **12** (1947) 199.
68. M. Elliot, N. F. Janes, in J. E. Casida, Pyrethrum, the Natural Insecticide, Academic Press, New York, 1973, 90; D. M. Soderlund, *Arch. Toxicol*. **86** (2012) 165; D. M. Soderlund, J. M. Clark, L. P. Sheets, L. S. Mullin, V. J Piccirillo, D. Sargent, J. T. Stevens, M. L. Weiner, *Toxicology* **171** (2002) 3.
69. K. Naumann, Chemistry of Plant Protection, Vol. 5, Springer, Berlin, 1990.
70. N. Kurihara, J. Miyamoto, Chirality in Agrochemicals, Wiley, New York, 1998.
71. M. Schneider, *Angew. Chem. Internat. Edn.* **23** (1984) 64.
72. Bayer, DE 3418374 (1984).
73. G. Suzukama, M. Fukao, M. Tamura, *Tetrahedron Lett*. (1984), 1595.
74. H. Nozaki, H. Takaya, S. Moriuti, R. Noyori, *Tetrahedron* **24** (1968) 3655.
75. T. Aratani, Y. Yoneyoshi, T. Nagase, *Tetrahedron Lett.* (1975) 1707; T. Aratani, Y. Yoneyoshi, T. Nagase, *Tetrahedron Lett.* (1977) 2599.
76. M. Itagaki, K. Masumoto, Y. Yamamoto, *J. Org. Chem.* **70** (2005) 3292; M. Itagaki, K. Masumoto, K. Suenobu, Y. Yamamoto, *Org. Proc. Res. Dev.* **10** (2006) 245; M. Itagaki, K. Suenobu, *Org. Proc. Res. Dev.* **11** (2007) 509.
77. M. Julia, FR 1506425 (1966).
78. R. V. M. Campbell, L. Crombie, D. A. R. Findley, R. W. King, G. Pattenden, D. A. Whiting, *J. Chem. Soc., Perkin. Trans.* 1 (1975) 897.
79. J. J. Martel, G. Nomine, *C. R. Acad. Sci.* (*Paris*) (1969) 2199.
80. J. J. Martel, C. Huynh, *Bull. Soc. Chim. Fr.* (1967) 985.
81. M. Julia, A. Guy-Rouault, *Bull. Soc. Chim. Fr.* (1967) 1411.
82. Roussel-Uclaf, BE 827651 (1975).
83. A. Krief, M. J. Devos, *Tetrahedron Lett*. (1979) 1511.
84. BASF, DOS 3229537 (1984).
85. J. Mulzer, M. Kappert, *Angew. Chem. Internat. Edn.* **22** (1983) 63.
86. A. Krief, L. Provins, A. Froidbise, *Tetrahedron Lett*. **43** (2002) 7881.
87. Rhône-Poulenc, FR 1356954 (1962).
88. M. J. Devos, A. Krief, *Tetrahedron Lett*. (1978) 1845.

89. J. d'Angelo, G. Revial, R. Azerad, D. Buisson, *J. Org. Chem.* **51** (1986) 40.
90. R. L. Funk, J. D. Munger, *J. Org. Chem*. **50** (1985) 707.
91. A. G. Cameron, D. W. Knight, *Tetrahedron Lett*. (1985) 3503.
92. R. B. Mitra, A. S. Khan, *Synth. Commun*. **7** (1977) 245.
93. J. Farkaš , F. Sorm, P. Kourim, *Coll. Czech. Chem. Commun*. **24** (1959) 2230.
94. J. March, Advanced Organic Chemistry, Wiley, New York, 1992, 968.
95. J. March, Advanced Organic Chemistry, Wiley, New York, 1992, 1034.
96. K. Naumann, Chemistry of Plant Protection, Vol. 5, Springer, Berlin, 1990, 64.
97. A. Fishman, D. Kellner, D. Ioffe, E. Shapiro, *Org. Proc. Res. Dev*. **4** (2000) 77.
98. Mobil Oil, US 4328363 (1968).
99. Mobil Oil, US 4285868 (1968).
100. Kuraray, JA 5257163 (1975).
101. W. Costain, B. W. H. Terry, M. Blackley, N. Punja, P. J. Cleare, A. Vernon, DE 2630981 (1975); S. O. Badanyan, A. N. Stepanyan, A. R. Mikaelyan, E. M. Ovivyan, G. A. Panosyan, *Russ. J. Org. Chem.* **33** (1997) 34.
102. N. R. Norton, R. D. Dillard, *J. Org. Chem*. **27** (1962) 3602.
103. Sagami, DOS 2538895 (1974).
104. Sagami, FMC, EP 3683 (1978).
105. K. Naumann, Chemistry of Plant Protection, Vol. 5, Springer, Berlin, 1990, 73.
106. K.-J. Haack, *Chemie in unserer Zeit* **37** (2003) 128; A. K. Mandal, D. P. Borude, R. Armugasamy, N. R. Soni, D. G. Jawalkar, S. W. Mahajan, K. R. Ratnam, A. D. Goghara, *Tetrahedron* **42** (1986) 5715; R. B. Mitra, G. H. Kulkarni, P. N. Khanna, *Synth. Commun.* **17** (1987) 1089.
107. E. J. Corey, P. L. Fuchs, *Tetrahedron Lett.* **36** (1972) 3769.
108. J. J. Martel, J. P. Demoute, A. P. Teche, J. R. Tessier, *Pestic. Sci.* **11** (1980) 188.
109. M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Org. Chem.* **57** (1992) 5643.
110. M. Inagaki, J. Hiratake, T. Nishioka, J. Oda, *J. Am. Chem. Soc*. **113** (1991) 9360.
111. H. Griengl, *Tetrahedron* **54** (1998) 14477.
112. H. Griengl, H. Schwab, M. Fechter, *Trends in Biotechnol*. **18** (2000) 252.
113. P. Poechlauer, W. Skranc, M. Wubbolts in H. U. Blaser, E. Schmidt, Asymmetric Catalysis on Industrial Scale, Wiley-VCH, Weinheim, 2004, 151.
114. D. W. Stoutamire, C. H. Tieman, W. Dong, EP 109681 (1984).
115. A. Mori, Y. Ikeda, K. Kinoshita, S. Inoue, *Chem. Lett.* (1989) 2119.
116. K. Tanaka, A. Mori, S. Inoue, *J. Org. Chem*. **55** (1990) 181.
117. H. Danda, H. Nishikawa, K. Ohtaka, *J. Org. Chem*. **56** (1991) 6740.
118. H. J. Sanders, A. W. Taff, *Ind. Eng. Chem*. **46** (1954) 414.
119. M. S. Schechter, F. B. La Forge, US 2574500 (1950).
120. Sumitomo, JA 601148 (1983).
121. S. Mitsuda, S. Nabeshima, H. Hirohara, *Appl. Microbiol. Biotechnol*. **31** (1989) 334.

References to 8.4 Neonicotinoids

122. W. Krämer and U. Schirmer, Modern Crop Protection Compounds, Wiley-VCH, Weinheim, 2007, 941; P. Jeschke, R. Nauen, M. E. Beck, *Angew. Chem.* **125** (2013) 9640; F. P. Mertz, C. Y. Raymond, *Int. J. Syst. Bact.* **40** (1990) 34; H. A. Kirst, *J. Antibiotics* **63** (2010) 101; http://en.wikipedia.org/wiki/Spinosad#cite_ref-one_0-0; B. W. Reynard, PhD thesis, Penn State University, 2012; http://repository.upenn.edu/cgi/viewcontent.cgi?article=1050&context=mes_capstones.
123. J. N. Langley, *Proc. R. Soc. London,* Ser. B **33** (1905) 347; W. C. Bowman, *Brit. J. Pharm.* **147** (2006) S277.
124. H. H. Dale, *J. Pharmacol. Exp. Ther*. **6** (1914) 147; H. H. Dale, W. Feldberg, M. Vogt, *J. Physiol.* **86** (1936) 353.
125. C. E. Müller, *Dtsch. Apothek. Z.* **135** (1995) 3253.

126. N. S. Millar, P. C. Harkness, *Molecular Membrane Biology* **25** (2008) 279; E. X. Albuquerque, E. F. R. Pereira, M. Alkondon, S. W. Rogers, *Physiol. Rev.* **89** (2009) 73.
127. N. Unwin, *Cell* **72** (1993) 31; N. Unwin, *J. Mol. Biol.* **346** (2005) 967; J. L. Popot, J. P. Changeux. *Physiol Rev.* **64** (1984) 1162.
128. M. Haass, W. Kübler, *Card. Drugs Ther.* **10** (1996) 657.
129. D. Voet, J. G. Voet, Biochemie, VCH, Weinheim, 1994, 1185.
130. A. Nordberg, *Med. Chem. Res.* **2** (1993) 522.
131. J. W. Daly, *Cell. Mol. Neurobiol.* **25** (2005) 513.
132. J. M. McIntosh, A. D. Santos, B. M. Oliveira, *Ann. Rev. Biochem.* **68** (1999) 59.
133. R. M. Jones, G. Bulaj, *Curr. Pharm. Design* **6** (2000) 1249.
134. The cysteine units marked in the same colour are in each case linked with each other by disulfide bridges. These are critical in determining the spherical structure of the toxins; see also R. Halai, D. J. Craik, *Nat. Prod. Rep.* **26** (2009) 526.
135. I. Yamamoto and J. E. Casida, Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor, Springer, Tokyo, 1999, 30.
136. W. Leicht, *Pflanzenschutz-Nachrichten Bayer* **49** (1996) 71.
137. M. Tomizawa, J. E. Casida, *Acc. Chem. Res.* **42** (2009) 260; W. Krämer and U. Schirmer, Modern Crop Protection Compounds, Wiley-VCH, Weinheim, 2007, 927; P. Jeschke, R. Nauen, M. Schindler, A. Elbert *J. Agric. Food Chem.* **59** (2011) 2897.
138. P. Maienfisch, M. Angst, F. Brandl, W. Fischer, D. Hofer, H. Kayser, W. Kobel, A. Rindlisbacher, R. Senn, A. Steinemann and H. Widmer, *Pest Manag. Sci.* **57** (2001) 906.
139. T. Wakita, K. Kinoshita, E. Yamada, N. Yasui, N. Kawahara, A. Naoi, M. Nakaya, K. Ebihara, H. Matsuno and K. Kodaka, *Pest. Manag. Sci.* **59** (2003) 1016.
140. M. E. Schroeder, R. F. Flattum, *Pestic. Biochem. Physiol.* **22** (1984) 148; W. D. Kollmeyer, R. F. Flattum, J. P. Foster, J. E. Powell, M. E. Schroeder, S. B. Soloway, Discovery of the Nitromethylene Heterocycle Insecticides, in Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor, Springer, Tokyo, 1999, 71.
141. K. Matsuda, S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso and D. B. Satelle, *Trends Pharmacol. Sci.* **22** (2001) 573.
142. I. Yamamoto and J. E. Casida, Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor, Springer, Tokyo, 1999, 91; Kazuhiko Matsuda, Satoshi Kanaoka, Miki Akamatsu, and David B. Sattelle, *Mol. Pharmacol.* **76** (2009) 1; M.Tomizawa, J. E. Casida, *J. Agric. Food Chem.* **59** (2011) 2825; M. Tomizawa, J. E. Casida, *Entomology* **48** (2003) 339.
143. I. Yamamoto and J. E. Casida, Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor, Springer, Tokyo, 1999, 109.
144. N. V. Kovganko, Z. N. Kashkan, *Russ. J. Org. Chem.* **40** (2004) 1709; J. Legocki, I. Polec, *Pestycydy.* (2008) 143.
145. K. Shiokawa, S. Tsuboi, S. Kagabu, K. Moriya, EP 192060 (1986).
146. S. Kojima, M. Funabora, N. Kawahara, Y. Iiyoshi, US 5453529 (1995).
147. C.-L. Yeh, C.-H. Chen, US 6307053 (2001).
148. H.-J. Diehr, DE 3830238 (1988).
149. K. Shiokawa, S. Tsuboi, S. Kagabu, S. Sasaki, K. Moriya, Y. Hattori, EP 235725 (1987).
150. I. Minamida, K. Iwanaga, T. Okauchi, EP 302389 (1988).
151. I. Minamida, K. Iwanaga, T. Tabuchi, H. Uneme, H. Dantsuji, T. Okauchi, *J. Pestic. Sci.* **18** (1993) 31.
152. K. Ishimitsu, J. Suzuki, H. Ohishi, T. Yamada, R. Hatano, N. Takakusa, J. Mitsui, EP 456826 (1990).

153. M. Y. Liu, B. Latli, J. E. Casida, *Pestic. Biochem. Physiol*. **52** (1995) 170.
154. P. Maienfisch, L. Gsell, A. Rindlisbacher, *Pestic. Sci*. **55** (1999) 351.
155. P. Maienfisch, H. Huerlimann, A. Rindlisbacher, L. Gsell, H. Dettwiler, J. Haettenschwiler, E. Sieger, M. Walti, *Pest Manag. Sci*. **57** (2001) 165.
156. P. Maienfisch, *Z. Naturforsch*. **61b** (2006) 353.
157. P. Maienfisch, O. Kristiansen, L. Gsell, EP 483062 (1991).
158. P. Maienfisch, H. Huerlimann, J. Haettenschwiler, *Tetrahedron Lett*. **41** (2000) 7187.
159. Phillips McDougall – AgriService, Products Section – 2010 Market.
160. K. Naumann, *Nachr. Chem. Tech. Lab*. **42** (1994) 255; Alfred Elbert, M. Haas, B. Springer, W. Thielert, R. Nauen, *Pest. Manag. Sci*. **64** (2008) 1099.

References to 8.5 Pheromones

161. H. J. Bestmann, O. Vostrowsky, *Chemie in unserer Zeit* **27** (1993) 123; D. G. Velázquez, J. J. Marrero Tellado, A. G. Ravelo, *Studies Nat. Prod. Chem*. **34** (2008) 393.
162. C. A. Olsen, A. S. Kristensen; K. Stromgaard, *Angew. Chem. Internat. Edn*. **50** (2011) 11296; O. Vostrowsky, www.organik.uni-erlangen.de/vostrowsky/natstoff/naturstoffe.html.
163. D. R. Kelly, *Chem. Biol*. **3** (1996) 595.
164. S. D. Kocher, C. M. Grozinger, *J. Chem. Ecol*. **37** (2011) 1263.
165. A. Butenandt, R. Beckmann, D. Stamm, *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **324** (1961) 84.
166. P. Karlson, M. Lüscher, *Nature* **183** (1959) 55.
167. K. W. Wanner, A. S. Nichols, K. K. O. Walden, A. Brockmann, C. W. Luetje, H. M. Robertson, *PNAS* **104** (2007) 14383; E. Urlacher, B. Francès, M. Giurfa, J.-M. Devaud, *Frontiers Behav. Neurosci*. **4** (2010) 157.
168. J. Javier, G. Quezada-Euán, J. Ramirez, T. Eltz, T. Pokorny, R. Medina, R. Monsreal, *Ani. Behav*. **85** (2013) 817.
169. K. Mori, S. Kuwahara, *Tetrahedron* **42** (1986) 5545.
170. P. Karlson, Biochemie, Thieme, Stuttgart, 1970, 197; E. Jacquin-Joly, M. Burnet, M.-C. Francois, D. Ammar, P. Nagnan-Meillour, C. Descoins, *Insect Biochem. Mol. Biol*. **28** (1998) 251; S. Matsumoto, A. Ohnishi, J. M. Lee, J. J. Hull, *Vitam. Horm*. **83** (2010) 435.
171. Bibliographisches Institut and F. A. Brockhaus AG, 2001.
172. M. Hesse, Alkaloide, Wiley-VCH, Weinheim, 2000, 63 and 295.
173. K. Roth, *Chemie in unserer Zeit* **39** (2005) 72.
174. J. A. Tillman, S. J. Seybold, R. A. Jurenka, G. J Blomquist, *Insect Biochem. Mol. Bio*. **29** (1999) 481.
175. K. Mori, *Mitteilungsblatt Chem. Ges. DDR* **34** (1987) 194.
176. K. Mori, H. Takikawa, *Tetrahedron* **47** (1991) 2163.
177. M. Tegoni, V. Campanacci, C. Cambillau, *Trends Biochem. Sci*. **29** (2004) 257.
178. J. P. Vité, W. Franke, *Chemie in unserer Zeit* **19** (1985) 11.
179. E. M. Hansen. J. C. Vandygriff, R. J. Cain, D. Wakarchuk, *J. Econ. Entomol*. **99** (2006) 373.
180. D. Schneider, *Z. Vergl. Physiol*. **40** (1957) 8; J. G. Millar, J. S. McElfresh, C. Romero, M. Vila, N. Marí-Mena, Carlos Lopez-Vaamonde, *J. Chem. Ecol*. **36** (2010) 923.
181. RAK1 Plus, BASF-Firmenschrift, Limburgerhof.
182. K.-H. König, *Chemie in unserer Zeit* **24** (1990) 292.
183. K. Mori, *Topics Curr. Chem*. **239** (2004) 1.
184. G. Habermehl, P. E. Hammann, H. C. Krebs, Naturstoffchemie, 2nd Edn., Springer, Berlin, 2002, 607.
185. http://en.wikipedia.org/wiki/Anarsia_lineatella.

186. R. Jackstell, M. Gómez Andreu, A. Frisch, K. Selvakumar, A. Zapf, H. Klein, A. Spannenberg, D. Röttger, O. Briel, R. Karch, M. Beller, *Angew. Chem. Internat. Edn.* **41** (2002) 986; R. Jackstell, S. Harkal, H. Jiao, A. Spannenberg, C. Borgmann, D. Röttger, F. Nierlich, M. Elliot, S. Niven, K. Cavell, O. Navarro, M. S. Viciu, S. P. Nolan, M. Beller, *Chem. Eur. J.* **10** (2004) 3891.
187. L. I. Zakharkin, E. A. Petrushkina, *J. Org. Chem. USSR* **18** (1982) 1419; L. I. Zakharkin, E. A. Petrushkina, *Bull. Acad. Sci. USSR, Div. Chem. Sci.* (1985) 193.
188. W. Francke, M. Tóth, G. Szöcs, W. Krieg, H. Ernst, E. Buschmann, *Z. Naturforsch.* **43c** (1988) 787.
189. E. A. Malo, J. C. Rojas, G. Lopez-Guillen, J. F. Barrera, *Florida Entomologist* **92** (2009) 548.
190. P. H. G. Zarbin, J. L. Princival, E. R. de Lima, A. A. dos Santos, B. G. Ambrogio, A. R. M. de Oliveira, *Tetrahedron Lett.* **45** (2004) 239.
191. N. N. Doan, T. N. Le, H. C. Nguyen, P. E. Hansen, F. Duus, *Molecules* **12** (2007) 2080.
192. K. Mori, *Tetrahedron: Asymmetry* **19** (2008) 861.
193. T. Katsuki, V. S. Martin, *Org. React*. **48** (1996) 1.
194. B. E. Rossiter, T. Katsuki, K. B. Sharpless, *J. Am. Chem. Soc*. **103** (1981) 464.
195. T. Ebata, K. Mori, *Tetrahedron Lett*. **22** (1981) 4281; T. Ebata, K. Mori, *Tetrahedron* **42** (1986) 3471.
196. J.-L. Brevet, K. Mori, *Synthesis* (1992) 1007.
197. G. W. Kinzer, A. F. Fentiman, T. F. Page, R. L. Foltz, J. P. Vite, G. B. Pitman, *Nature* **221** (1969) 447.
198. http://www.faidherbe.org/site/cours/dupuis/fronta2.htm
199. Y. Nishimura, K. Mori, *Eur. J. Org. Chem*. (1998) 233.
200. J. A. Turpin, L. O. Weigel, *Tetrahedron Lett*. **33** (1992) 6563.
201. K. Mori in N. Kurihara, J. Miyamoto, Chirality in Agrochemicals, Wiley, New York, 1998, 208.
202. B. T. Sullivan, M. J. Dalusky, K. Mori, C. Brownie, *J. Chem. Ecol.* **37** (2011) 403.
203. K. Mori, *Tetrahedron* **30** (1974) 4223.
204. K. R. Prasad, P. Anbarasan, *Tetrahedron: Asymmetry* **16** (2005) 3951.
205. S. Singh, P. J. Guiry, *J. Org. Chem*. **74** (2009) 5758.
206. B. List, P. Pojarliev, C. Castello, *Org. Lett*. **3** (2001) 573.
207. K. Mori, T. Takigawa, T. Matsuo, *Tetrahedron* **35** (1979) 933.
208. G. Ohloff, W. Giersch, *Helv. Chim. Acta* **60** (1977) 1496.
209. G. Habermehl, P. E. Hammann, H. C. Krebs, Naturstoffchemie, Springer, Berlin, 2002, 613.
210. W. Kotlarek, *Pol. J. Chem*. **57** (1983) 809.
211. IG Farbenindustrie, DE 714314 (1938).
212. L. Bagnell, *Aust. J. Chem*. **28** (1975) 801.
213. T. J. A. Graham, E. E. Gray, J. M. Burgess, B. C. Goess, *J. Org. Chem*. **75** (2010) 226.
214. P. D. Hobbs, P. D. Magnus, *J. Chem. Soc., Chem. Commun*. (1974) 856; P. D. Hobbs, P. D. Magnus, *J. Am. Chem. Soc*. **98** (1976) 4594.
215. J. March, Advanced Organic Chemistry, Wiley, New York, 1992, 243.
216. Personal communication from Prof. H. Kunz, Mainz, 2004.
217. M. Mortimore, P. Kocienski, *Tetrahedron Lett.* **29** (1988) 3357.
218. B. Liu, J. K. De Brabander, *Org. Lett.* **8** (2006) 4907.
219. Y. Yokoyama, H. Takikawa, K. Mori, *Bioorg. Med. Chem*. **4** (1996) 409.

List of Abbreviations

Ac	acetyl
acac	acetylacetonate
AcOH	acetic acid
AIBN	2,2'-azobis(2-methylpropionitrile)
AMP	adenosine monophosphate
cAMP	cyclic adenosine monophosphate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
9-BBN	9-borabicyclo[3.3.1]nonane
BINAP	2,2'-bis(diphenylphosphano)-1,1'-binaphthyl
BINOL	2,2'-dihydroxy-1,1'-binaphthyl
Bn	benzyl
Boc	*N*-*t*-butoxycarbonyl
Boc_2O	di-*t*-butyl dicarbonate
Bu	*n*-butyl
sBu	*sec.*-butyl
tBu	*t*-butyl
Bu_2BOTf	di-*n*-butylboryl trifluoromethanesulfonate
BuOH	*n*-butanol
tBuOH	*t*-butanol
Bz	benzoyl
CAN	ceric ammonium nitrate
CBS reduction	Corey-Bakshi-Shibata reduction
Cbz	carbobenzyloxy protecting group
CoA	coenzyme A
CSA	campher-10-sulfonic acid
DABCO	1,4-diazabicyclo[2.2.2]octane
dba	dibenzylideneacetone
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DBS	dibenzosuberyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DHP	dihydropyrane
DIAD	diisopropyl azodicarboxylate

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DIBAlH	diisobutylaluminium hydride
DIC	diisopropylcarbodiimide
DIGLYME	bis(2-methoxyethyl) ether
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	ethyl
EtOH	ethanol
FAD	flavin adenine dinucleotide
Hex	*n*-hexyl
cHex	cyclohexyl
HMG	3-hydroxy-3-methylglutaryl
HMPT, HMPA	hexamethylphosphoric triamide
HOMO	highest occupied molecular orbital
IC_{50}	concentration of a substance that is required for 50% inhibition
ICI	Imperial Chemical Industries
LD_{50}	lethal dose, where 50% of test subjects exposed would die
LDA	lithium diisopropylamide
LUMO	lowest unoccupied molecular orbital
MCPBA	*m*-chloroperbenzoic acid
Me	methyl
MeOH	methanol
Mes, mesylate	methanesulfonate
MTBE	*t*-butylmethylether
NAD	nicotinamide adenine dinucleotide
NaHMDS	sodium bis(trimethylsilyl)amide
NBS	*N*-bromosuccinimide
NCS	*N*-chlorosuccinimide
NMO	*N*-methylmorpholine-N-oxide
NMP	*N*-methyl-2-pyrrolidone
PCC	pyridinium chlorochromate
PEA	1-phenylethylamine
PEG	polyethylene glycol
Ph	phenyl
PLE	pig liver esterase
PPA	1-propanephosphonic acid cyclic anhydride*
Pr	*n*-propyl
iPr	*iso*-propyl
PrOH	*n*-propanol
iPrOH	isopropanol
PTC	phase-transfer catalysis
py	pyridine

Ra-Ni	Raney nickel
Red-Al	sodium bis(2-methoxyethoxy)aluminium hydride
SAME	S-adenosylmethionine
TBAF	tetra-*n*-butylammonium fluoride
TBS	*t*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofurane
THP	tetrahydropyrane
TIPSOTf	triisopropylsilyl trifluoromethanesulfonate
TMEDA	tetramethylethylenediamine
TMSOiPr	isopropoxy trimethylsilane
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tos, tosylate	4-toluenesulfonate
TPAP	tetra-*n*-propylammonium perruthenate
TPP	triphenylphosphane
TPPO	triphenylphosphane oxide
Triflate	trifluoromethanesulfonate
Trityl	triphenylmethyl
TsOH, pTSA	*p*-toluenesulfonic acid

*: unless otherwise indicated

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2. Colourants

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