Progress in the Chemistry of Organic Natural Products

A. Douglas Kinghorn
Heinz Falk
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Jun'ichi Kobayashi *Editors*

103

Phytocannabinoids

Unraveling the Complex Chemistry and Pharmacology of *Cannabis sativa*

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Volume 103

With contributions by

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Preface

In his 1974 paper on the "Structure–Activity Relationships in Man of Cannabis Constituents, and Homologs and Metabolites of Δ^9-Tetrahydrocannabinol," the late great Professor Leo E. Hollister stated "The more one studies cannabis chemically, the more complicated it becomes." Never have truer words been spoken about the science of cannabis generally, and it is likely that Professor Hollister knew precisely how complex, diverse, and far reaching the study of this intriguing plant would become. Cannabis science has exploded in recent years with new natural product chemistry, which may not have been predicted, new synthetic analogues as drugs of abuse, new uses and medicinal targets, and from the socioeconomic perspective, as an approved medicine in its purified state and as a decriminalized regulated material in its crude form, generating tax dollars for the US economy.

Indeed at the time of writing of this volume, more than half of the US states have decriminalized possession of cannabis for medicinal use, with some going further and allowing non-medicinal usage. This has presumably occurred due to the fact that many US citizens were already self-medicating illegally for pain, to reduce spasticity, and for joint inflammation for some considerable time, and the opportunity to generate taxable revenue was appealing to state governments and not distasteful to the electorate. However, there is a considerable body of evidence to suggest that both short- and long-time use of cannabis can lead to psychosis and schizophrenia, and the long-term mental health issues in the states that have allowed easier access to this material remain to be evaluated.

Despite potential harms associated with crude cannabis drug materials, the great medicinal potential of its components singly and in mixtures is profound. Sativex©, a product of the UK-based company GW Pharmaceuticals, has been approved for spasticity associated with multiple sclerosis in nearly 30 countries including the UK, Canada, Germany, Italy, and Spain, and its use in treating cancer-associated pain is under development. Excitingly, in April 2016, the US Food and Drug Administration (FDA) granted the GW Pharmaceuticals experimental cannabis-derived antiepilepsy drug Epidiolex© "orphan" status for patients affected by

Dravet and Lennox-Gastaut Syndrome, which are rare forms of epilepsy affecting children and adults, respectively. Epidiolex© is pure cannabis-derived cannabidiol, and this non-psychoactive compound has potential in a number of areas, particularly in inflammation and even as an anti-infective.

Cannabis science has also generated many synthetic cannabinoids that were initially lead molecules for pharmaceutical companies seeking analgesic products; unfortunately, some of these compounds are highly potent at the CB_1 receptor, and they consequently have undesirable psychoactivity. These compounds have been seized upon by chemists wishing to manufacture and market Novel Psychoactive Substances (NPS), and they are very common as additions to smoking mixtures, e.g., as "Spice" or even as single pure NPS. Unfortunately, they are highly potent, in some cases being 1000 times the strength of THC, and at the time of writing of this editorial, five deaths and four acute intoxications associated with the synthetic cannabinoid 5F-MDMB-PINACA (5F-ADB) have been reported by the EU Early Warning System.

Despite the toxicity associated with cannabinoids acting at the CB_1 receptor, there are a plethora of druggable targets that phytocannabinoids interact with, notably the glycine receptors, and this target, which is covered in this volume, has great potential in the treatment of neuropathic pain and inflammation. Indeed, it may be that some of the work surrounding cannabis and its constituents interacting with CB_1 and CB_2 may have been a pharmacological "red herring" to pain management and anti-inflammation, and many of the targets discussed in this volume are possibly responsible for these effects.

The endocannabinoid system is highly complex and is affected by cannabis natural products in many direct and indirect ways, and as a consequence, we have sought in this volume to focus on natural product cannabinoids: the phytocannabinoids, which have been demonstrated to act at specific receptor targets in the CNS. With this focus in mind, we have elicited contributions from experts in the fields of phytocannabinoid chemistry (ElSohly), synthesis of phytocannabinoids (Carreira), phytocannabinoid molecular pharmacology (Iversen and Whalley), and finally phytocannabinoid molecular targets (Reggio). We believe that this volume represents the current "state of the art" on phytocannabinoid chemistry and pharmacology and will be of much use to those wishing to understand the current landscape of the exciting and intriguing phytocannabinoid science.

Columbus, OH — Douglas Kinghorn
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London, UK — Simon Gibbons
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Phytochemistry of *Cannabis sativa* L.

Mahmoud A. ElSohly, Mohamed M. Radwan, Waseem Gul, Suman Chandra, and Ahmed Galal

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1 Introduction

Cannabis sativa L. (Cannabaceae) is found in a variety of habitats and altitudes, ranging from sea level to the alpine foothills of the Himalayas from which it possibly originated [1]. *Cannabis* cultivation and use are 5000 to 6000 years old, making it difficult to pinpoint the origin of this species [2]. Furthermore, it is one of the oldest plant sources of food and textile fiber [3]. Cultivation of *C. sativa* for textile fiber originated from Egypt and western Asia and was introduced subsequently to Europe between 1000 and 2000 B.C. and to South America (Chile) in 1545. Over 60 years later (1606), hemp cultivation was introduced to North America (Port Royal, Canada) [4]. Current federal laws in the United States prohibit the cultivation of *C. sativa*, including hemp.

Cannabis sativa has a rich history of medicinal use dating back to ancient times. The first account of its medicinal use came from the Middle East and Asia during the sixth century B.C. Its introduction into western medicine occurred much later, during the early nineteenth century [5, 6]. This species has been indicated in the treatment of pain, glaucoma, nausea, depression, and neuralgia [7–11]. The therapeutic value of phytocannabinoids has also been employed for HIV/AIDS symptom management and multiple sclerosis treatment [12, 13].

2 Nomenclature

2.1 Botanical Nomenclature

The taxonomic classification of *Cannabis sativa* is as shown below:

Kingdom:	Plantae (plants)
Subkingdom:	Tracheobionta (vascular plants)
Superdivision:	Spermatophyta (seed plants)
Division:	Magnoliophyta (flowering plants)
Class:	Magnoliopsida (dicotyledons)
Subclass:	Hamamelididae
Order:	Urticales
Family:	Cannabaceae
Genus:	*Cannabis*
Species:	*sativa*
Taxonomic authority abbreviation:	L.

The number of species in the genus *Cannabis* has been the subject of a long debate. Taxonomists have variously characterized the genus "*Cannabis*" based on its polytypic nature [14–16]. Schultes et al. divided this genus into three separate species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis* [17]. In contrast, several other researchers considered the genus to have two major species, *C. sativa* and *C. indica* [18, 19]. However, in spite of these different taxonomic interpretations, *Cannabis* is commonly treated as constituting only a single, highly diverse species, *C. sativa* L. [20–23]. *C. sativa*, *C. indica*, and *C. ruderalis* are now recognized as varieties of *C. sativa* L. (var. *sativa*, var. *indica*, and var. *ruderalis*, respectively). The *sativa* and *indica* varieties are more economically important and widespread, whereas *ruderalis* is considered a hardier variety grown in the northern Himalayas and southern states of the former Soviet Union that is characterized by a sparse, "weedy" growth, and is rarely cultivated for its drug content. Compared to *sativa* for which the average plant height ranges from 2.5 to 3.5 m, plants of the *indica* variety are generally shorter (average height ca. 1.8 m) and bushier with broader and darker green leaves that mature early when cultivated outdoors.

Field-grown *Cannabis sativa* L.: male plants (*left*), female plants (*right*)

2.2 Common Names

Around the world, local populations refer to *Cannabis sativa* by many common names, of which some are summarized below.

Arabic:	Al-Bhango; Al-Hashish; Al-Qanaap
Chinese:	Xian ma; ye ma
Danish:	Hemp
Dutch:	Hennep

English:	hemp; marihuana
Finnish:	Hamppu
French:	Chanvre; chanvre d'Inde; chanvre indien; chanvrier; chanvrier
German:	Hanf; Haschisch; indischer Hanf
Indian:	Bhang; charas; ganja
Japanese:	Mashinin
Nepalese:	Cares; gajiimaa; gaanjaa
Portuguese:	Cânhamo; maconha
Russian:	*Kannabis sativa*
Spanish:	Cáñamo; grifa; hachís; mariguana; marijuana
Swedish:	Porkanchaa

Cannabis sativa is normally a dioecious species, i.e. male and female flowers develop on separate plants. The sex is determined from the heteromorphic chromosomes, with males being heterogametic (XY) and females homogametic (XX). Morphologically, it is difficult to identify male and female plants at the vegetative stage. With sexual dimorphism occurring late in plant development, male plants can be differentiated from female plants after the onset of flowering. Molecular techniques can now be employed to differentiate male from female plants at an early stage [24–29]. The sexual phenotype of *Cannabis* occasionally shows flexibility leading to the formation of hermaphrodite flowers or bisexual inflorescences, i.e. a monecious phenotype.

2.3 Definition of Cannabis

According to the United States Code (USC), the term "marihuana/cannabis" is defined as "all parts of the plant *C. sativa* L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resin. Such term does not include the mature stalks of the plant, fiber produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt, derivative, mixture, or preparation of such mature stalks (except the resin extracted therefrom), fiber, oil, or cake, or the sterilized seed of such plant which is incapable of germination" [30].

"Cannabis" is the drug prepared from the dicotyledonous (flowering plant with two leaves at germination), herbaceous (non-woody plant of which the aerial parts die after fruiting), diecious (the male plants are distinct from the female plants), apetalous (the flower has no corolla), annual herb, *C. sativa* L., and its variants (family: Cannabaceae), which biosynthesizes uniquely the terpenophenolic cannabinoids or phytocannabinoids, accumulating mainly in the glandular trichomes of the plant.

3 Cultivation of *Cannabis sativa*

Cannabis sativa is an annual crop that can be grown successfully under indoor and outdoor conditions, with each having its advantages and disadvantages. While it is possible with indoor cultivation to regulate the photoperiod to trigger the flowering and maturation (three to four crops per year are possible), the cultivation under outdoor conditions is normally limited to one crop per year (requiring five to seven months depending on the variety).

3.1 Outdoor Cultivation

Cultivation of a new crop from seeds usually starts in late March to early April. The crop usually lasts until November to early December in the northern hemisphere but this depends on the variety. Seeds are sown in small biodegradable pots, and the selected healthy seedlings are then transplanted in the field. Alternatively, seeds may be planted directly in the field. Male flowers and subsequently female flowers appear within two to three months (the middle of July) of seeding/transplanting. Culling of male plants is generally done, since male plants contain less Δ^9-tetrahydrocannabinol (Δ^9-THC) (**1**) (see the figure on page 14 of Sect. 6.1.1) compared to female plants, and this also avoids cross-pollination both among different varieties and within the same variety.

Although outdoor cultivated plants have more biomass compared to indoor-grown plants, depending on the weather and the space provided, it is difficult to maintain a constant chemical profile under outdoor conditions if grown from seeds, due to the allogamous (cross fertilization) nature of *C. sativa*. Therefore, vegetative propagation through cuttings is an appropriate method to maintain uniformity in the chemical profiles of the crops produced.

3.2 Indoor Cultivation

Indoor cultivation of *Cannabis sativa* under controlled environmental conditions allows for a complete control of the plant life cycle, resulting in management of the quality and quantity of biomass. This is important when producing *C. sativa* with a specific chemical profile for pharmaceutical use. The environmental parameters such as light level, photoperiod, humidity, temperature, CO_2 concentration, and circulation of air play a critical role in the indoor production of this plant.

Cannabis sativa requires high photosynthetic photon flux density (PPFD) for photosynthesis and growth [31]. In-house studies by the present authors have shown that *C. sativa* can utilize a fairly high level of PPFD and temperature for gas and water vapor exchange processes and can perform much better if grown at ca. 1500 μmol m^{-2} s^{-1} PPFD (comparable to a sunny summer day in northern

Mississippi) and around 25–30°C in temperature. Different light sources can be used for indoor propagation, namely, fluorescent light bulbs for juvenile cuttings and metal halide (MH) and/or high-pressure sodium (HPS) bulbs for established plants. Separate ballasts are required to regulate MH and HPS bulbs. MH and HPS bulbs should be 0.9–1.2 m from the plants to avoid overexposure. However, when using indoor grow bulbs, it is difficult to achieve photosynthetically active radiation (PAR) levels up to 1500 μmol m^{-2} s^{-1} PPFD. Furthermore, photoperiods play an important role in regulating vegetative and flowering growth of *C. sativa*. These plants maintain a vegetative growth stage provided a photoperiod cycle consisting of >18-h light and ≤6-h dark is maintained. A 12-h photoperiod induces *C. sativa* to bloom [31].

In the indoor growing room, the level of humidity is crucial for all stages of plant growth, starting from seed germination or vegetative propagation to budding and harvesting. Juvenile plants require high humidity as compared to well-developed plants. Vegetative cuttings require a regular water spray on the leaves to maintain high humidity (ca. 70–75%) in its microclimate until the plants are well-rooted, while the active vegetative and flowering stages require 55–60% humidity. The amount and frequency of watering for *C. sativa* is dependent on numerous factors, such as growth stage, size of the plants and pots, temperature, humidity, and other parameters. During the early seedling or vegetative stage, it is recommended to keep the soil moist; however, the top layer of soil should be dry before established plants are watered.

Circulation of air is another important aspect of the indoor growing environment. Regulation of gas and water vapor exchange between the leaves and the microenvironment is achieved by having an airflow around the surfaces of the leaves. This affects the leaf boundary layer thermal conductance, energy budget, and ultimately, the physiology and growth of the plant. Electric fans can be employed to manage air circulation. Doubling the ambient CO_2 concentration in the environment is reported to stimulate the rate of photosynthesis and water use efficiency (WUE) in *C. sativa* by 50 and 111%, respectively, resulting in increased overall growth [31].

3.2.1 Propagation Through Seeds

Propagation starting from seeds is the most popular and conventional method of cultivating *C. sativa*. The seeds of this species need a moist environment, in addition to heat and air circulation for optimum germination. Several methods may be used for the germination of seeds. The most popular method is to plant the seeds in small biodegradable jiffy pots containing moist, aerated soil. Seedling heat mats may be placed underneath the pots to increase soil temperature and to enhance germination during the winter.

Seed germination starts after four to five days, with most of the viable seeds germinating within 15 days, depending on the seed variety, age, and storage conditions, as well as the soil and water temperatures. Seedlings should be exposed to cool

fluorescent light (18-h photoperiod) for the initial vegetative growth, followed by transplantation to bigger pots (30 × 30 cm), and exposed to full spectrum growth light conditions (18-h photoperiod) for the desired vegetative growth. Plants can be exposed to a 12-h photoperiod when appropriate vegetative growth has been achieved, which induces flowering in about two weeks. Male flowers appear before female flowers, allowing for separation or culling of the male plants if "sinsemilla" buds are required. Cuttings from the branches of female plants can be used for vegetative propagation. If a specific chemical profile is required, chemical analysis should be performed to aid in the selection of the appropriate chemotype or clone.

3.2.2 Propagation Through Vegetative Cuttings

There are several ways of accomplishing the vegetative propagation of *C. sativa* plants. The three main types are grafting, air-layering and the use of cuttings. These three types are referred to as macropropagation, as opposed to micropropagation or tissue culture. Propagation by cuttings is the most convenient, effective and generally preferred method for *C. sativa*. Once a clone with a specific chemical profile is screened and selected, a fresh nodal segment of about 6–10 cm in length containing at least two nodes from the mother plant can be used for vegetative/conventional propagation. Vegetative propagation of *C. sativa* can be effectively done using either solid (soil) or liquid medium (hydroponics).

For soil propagation, an apical branch is cut at a 45° angle immediately below a node and immediately dipped in distilled water. The base of the cutting (2 cm) is dipped subsequently in rooting hormone (Green Light, USA) and planted in pots (5 × 5 cm) containing coco natural growth medium and a mixture (1:1) of sterile potting mix and Fertilome® (Canna Continental, USA). At least one node must be covered by soil for efficient rooting. Plants are regularly irrigated and kept under controlled environmental conditions. Rooting initiates in two to three weeks, and this is then followed by transplantation to bigger pots (e.g. 30 × 30 cm) after six weeks.

For propagation in hydroponics, the cuttings are inserted 2.5 cm deep into a rockwool cube or a hydrotone clay ball supporting medium. Plants are supplied with a vegetative fertilizer formula (Advance Nutrient, Canada) for vegetative growth. Rooting and transplantation are similar to those of soil propagation. For vegetative growth, plants are kept under an 18-h photoperiod until they reach the desired size, and the light cycle is changed to a 12-h photoperiod for flowering and maturation.

3.2.3 Micropropagation

The tissue culture technique is a powerful tool, which can be employed as an alternative to the conventional method of vegetative propagation with the objective of enhancing the rate of multiplication of the desired genotypes. Furthermore, plant tissue culture is an important frontier area in plant biotechnology and has not only

facilitated mass propagation but has also yielded benefits in terms of crop improvement and germplasm conservation, in particular for vegetatively propagated crops. Although vegetative propagation is standard in *C. sativa*, it is limited by its slow multiplication rate, the poor survivability of some clones, and the need for copious initial planting. Therefore, in vitro-propagation methods offer powerful tools for mass multiplication and germplasm conservation of this important medicinal plant species. In-house methodologies created by the authors have led to optimized simple and effective micropropagation protocols for *C. sativa*, using apical nodal segment (direct organogenesis) and leaf explant (indirect organogenesis) techniques [32, 33].

In the above-mentioned in-house protocol for direct organogenesis, apical nodal segments containing axillary buds (ca. 1 cm in length) were used as the explant for initiation of shoot cultures. Explants were obtained from healthy branches of a screened and selected high-yielding *C. sativa* clone grown in an indoor cultivation facility. Explants were surface-disinfected using 0.5% NaOCl (15% *v/v* bleach) and 0.1% Tween 20 for 20 min. The explants were washed in sterile, distilled water three times for 5 min prior to inoculation on the culture medium. Micropropagation and hardening of micropropagated plants were done following the protocol described by Lata et al. [36]. The best response for shoot induction of this species was observed with Murashige and Skoog (MS) medium containing 0.5 μ*M* thidiazuron (TDZ). Well-developed shoots were then transferred to half-strength MS medium with activated charcoal supplemented by different concentrations of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) for rooting. The highest percentage of rooting in micropropagated plants was achieved in half-strength Murashige and Skoog (1/2 MS) salts with 500 mg/dm^3 activated charcoal supplemented with 2.5 μ*M* IBA.

For indirect organogenesis, callus-mediated plant regeneration is an easy way to obtain somaclonal variants as has been emphasized for several plants [34, 35]. The phenomenon of somaclonal variation through indirect organogenesis offers an opportunity to uncover the natural variability in plants and to use this genetic variability for new product development and crop improvement [36]. Many factors influence the frequency of somaclonal variation, such as plant species, the genotype and the type of explants involved, the culture protocol applied during the in vitro process, and particularly the hormone composition of the medium as well as the number of subcultures [37, 38]. In the laboratory of the present authors, an in vitro system was developed to produce callus culture and regenerated *Cannabis* plants from leaf tissue through indirect organogenesis of a high-yielding variety of *C. sativa* [33]. Calli were introduced from leaf explants on Murashige and Skoog medium supplemented with different concentrations (0.5, 1.0, 1.5, and 2.0 μ*M*) of IAA, IBA, NAA, and 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with 1.0 μ*M* thidiazuron (TDZ). The optimum callus growth and maintenance was with 0.5 μ*M* of these auxins along with 1.0 μ*M* TDZ. The 2-month-old calli were sub-cultured in MS medium containing different concentrations of cytokinins [6-benzoaminopurine (BAP), kinetin (Kn), and TDZ]. The rate of shoot induction and proliferation was highest in 0.5 μ*M* TDZ. Regenerated shoots rooted best on

half-strength MS medium supplemented with 2.5 μ*M* IBA of the various auxins (IAA, IBA, and NAA) utilized.

Application of micropropagation for large-scale production of elite clones is an effective and superior alternative to seed and conventional cuttings. Despite its potential, this technique has not lived up to expectations and is still plagued with many problems. One of its principal limitations is the poor survival of plantlets once transferred from in vitro conditions to the natural uncontrolled environment [39]. These problems have restricted the application of micropropagation technology to the mass production of many high-value medicinal plant species. Furthermore, the clonal fidelity in terms of genetic stability, stable chemical profile, and a comparable chemical content is one of the most important prerequisites of micropropagation of plant species of pharmaceutical interest. Therefore, for commercial utilization, a comparison of chemical profile, useful secondary metabolite content, and genetic stability of in vitro-grown plants with mother plants is of utmost importance. In our laboratory, tissue culture-raised plants of *C. sativa* are compared with mother plants and conventionally grown plants for their genetic stability and cannabinoid profiles and content.

DNA-based inter-simple sequence repeat (ISSR) markers have been used successfully in the laboratory of the authors to monitor the genetic stability of micropropagated plants (using apical nodal segments) of *C. sativa* of up to 30 passages in culture, which are hardened in the soil for eight months [32]. Fifteen ISSR primers generated a total of 115 distinct and reproducible bands. The banding pattern for each primer was uniform and comparable to that of the mother plant from which the cultures had been established. Based on the results obtained, the micropropagation protocol standardized for *C. sativa* can be used commercially with a minimum possibility of any in vitro-induced variability.

To evaluate the stability of the chemical profile in the micropropagated plants, gas chromatography-flame ionization detection (GC-FID) was used to assess the chemical profiles and quantify cannabinoids to identify differences, if any, in the chemical constituents of in vitro-propagated plants versus vegetatively grown plants and the mother plant [32, 40]. In general, the Δ^9-THC (**1**) content in all groups increased with plant age up to its highest level during the budding stage, where the content of this compound plateaued before the onset of senescence. In all plant groups, the pattern of changes observed in the concentration of other cannabinoids with respect to plant age has followed a similar trend. Qualitatively, the cannabinoid profiles obtained using GC-FID in mother plants, vegetatively grown plants, and in vitro-propagated plants were found to be similar to each other and to those of the field-grown mother plants of *C. sativa*. Minor differences observed in cannabinoid concentration within and among the groups were not statistically significant. These results have confirmed the clonal fidelity of our in vitro-propagated plants of *C. sativa* and suggest that the protocols used in these studies to produce micropropagated plants do not affect the resultant metabolic profile. Furthermore, this approach could be used for mass propagation of true-to-type plants of this species for commercial pharmaceutical use.

3.3 Harvesting, Processing, and Storage of *Cannabis sativa* Biomass

Identifying the optimum harvesting stage is a critical step in *C. sativa* cultivation to ensure the required chemical profile, e.g. the optimum Δ^9-THC (**1**) content. This can be achieved through daily analysis of the cannabinoid content in different parts of the plant. The content of **1** increases with plant age up to the highest level during the budding stage, whereupon it reaches a plateau for approximately one to two weeks, followed by a decrease with the onset of senescence. Also, the content of **1** is usually higher before noon. The upper mature buds may be harvested first, allowing time for the lower immature buds to mature. A visual indication of maturity is the color of the stigmas, which shrivel and turn brown as the flowers ripen.

A commercial tobacco drying barn, such as BulkTobac (Gas-Fired Products, Inc., USA), can be used for drying biomass from large-scale cultivation (12–15 h, 40°C); a laboratory oven may be used for small samples (overnight, 40°C). Dead leaves and stems should be removed from mature buds before drying. The whole buds can be dried, or the buds can be cut into smaller pieces. The dried plant material can be hand manicured. Any remaining leaves should be separated from the buds. The buds are carefully rubbed through differently sized screens to separate small stems and seeds. Automated plant processing machines can also be used to separate big stems and seeds from the useable biomass.

Adequately dried and processed *Cannabis* biomass can be stored in FDA-approved polyethylene bags placed in sealable fiber drums (short term: 18–20°C; long term: −10°C). A major concern when storing *C. sativa* is the stability of many of the cannabinoids. Degradation of **1** is negligible during processing, especially when the material is well-dried and sealed; however, it is still extremely sensitive to oxygen and UV light, and slow degradation occurs during room temperature storage through oxidation to cannabidiol (CBD) (**35**). Also, **1** readily converts to (−)-Δ^8-*trans*-tetrahydrocannabinol (Δ^8-THC) (**16**) under thermodynamic control. Therefore, the preferred conditions for long-term storage are low temperature and absence of light.

4 Chemotaxonomy of *Cannabis sativa*

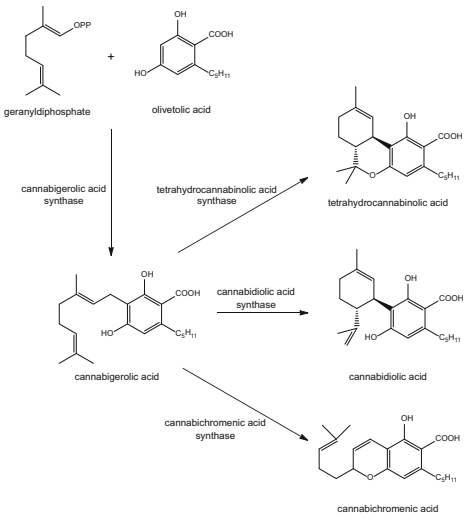
Cannabis sativa is a chemically complex species based on its numerous reported natural secondary metabolite constituents. The concentration of Δ^9-THC (**1**) in the dried inflorescence (leaves and buds) is used to determine its psychoactivity. Quantitative and qualitative analysis of *Cannabis* can be employed to characterize its phenotype and phytocannabinoid profile [25].

Cannabis sativa can be divided into three phenotypes: phenotype I (drug-type), with Δ^9-THC (**1**) (>0.5% and cannabidiol (CBD) (**35**) <0.5% (Δ^9-THC/CBD $\gg$ 1)); an intermediate phenotype II (intermediate-type), with CBD as the major cannabinoid but with Δ^9-THC (**1**) also present at various concentrations (Δ^9-THC/CBD ~ 1); and

phenotype III (fiber-type or hemp), with especially low Δ^9-THC content (Δ^9-THC/CBD $\ll$ 1). Hemp usually contains non-psychoactive cannabinoids as major constituents, e.g. CBD or cannabigerol (CBG) (**21**) [41, 42]. Although environmental factors play a role in determining the amount of cannabinoids present in different parts of the plant at different growth stages [43], the CBD:Δ^9-THC ratios evident in most populations are under genetic control [44].

5 Biosynthesis of Phytocannabinoids

Phytocannabinoids are terpenophenolic compounds chemically related to the terpenes with their ring structure derived from a geranyl pyrophosphate (C_{10} monoterpene subunit). The cytosolic mevalonate and the plastidial methylerythritol phosphate (MEP) are two independent pathways reported to be responsible for plant terpenoid biosynthesis. Plastidial methylerythritol phosphate is reported to be responsible for the biosynthesis of the cannabinoid terpenoid moiety [45]. The first step in the cannabinoid biosynthesis pathway (Scheme 1) is the formation of olivetolic acid, the biosynthesis pathway of which has not been fully elucidated. However, it has been proposed that a type III polyketide synthase, olivetol synthase, could be involved [46]. Olivetolic acid and geranyl diphosphate are coupled under the influence of the prenylase, geranyl diphosphate:olivetolate geranyltransferase, yielding cannabigerolic acid (CBGA). This, in turn, is oxido-cyclized by flavin adenine dinucleotide (FAD)-dependent oxidases, namely, Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) synthase, cannabidiolic acid (CBDA) synthase and cannabichromenic acid (CBCA) synthase, producing Δ^9-tetrahydrocannabinolic acid, cannabidiolic acid, and cannabichromenic acid [47, 48].



Scheme 1 Biosynthesis of tetrahydrocannabinolic acid (THCA) and related cannabinoids

6 Constituents of *Cannabis sativa*

The total number of natural compounds identified or isolated from *C. sativa* has continued to increase over the last few decades. In 1980 Turner et al. reported 423 compounds from this plant [49], and this number increased in 1995 to 483 [50]. Between 1995 and 2005 seven compounds were added [51]. The main focus of this chapter is to provide a chemical account on the recently reported 49 cannabinoids as well as the 26 non-cannabinoid constituents (Table 1), totaling 565 compounds identified to date.

6.1 Cannabinoids

The natural products isolated from *C. sativa* exhibiting the typical C_{21} terpenophenolic skeleton are termed "cannabinoids". This class of compounds has derivatives and transformation products that are also considered as cannabinoids. Since the initiation of the chemical investigations on *C. sativa,* altogether 120 cannabinoids have been isolated to date (Table 1), which can be classified into 11 general types: (−)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC), (−)-Δ^8-*trans*-tetrahydrocannabinol (Δ^8-THC), cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabitriol (CBT), and miscellaneous types.

Table 1 Constituents of *C. sativa* L. by chemical class

Chemical class	2005	2015
Δ^9-THC type	9	23
Δ^8-THC type	2	5
CBG type	8	16
CBC type	6	9
CBD type	7	7
CBND type	2	2
CBE type	5	5
CBL type	3	3
CBN type	7	11
CBT type	9	9
Miscellaneous types	14	30
Total cannabinoids	72	120
Total non-cannabinoids	419	445
Total	491	565

6.1.1 (−)-Δ^9-*trans*-Tetrahydrocannabinol (Δ^9-THC) Type

Gaoni and Mechoulam first reported the structure of Δ^9-THC (**1**) and determined its absolute configuration as *trans*-(6a*R*,10a*R*) [52]. Recently, X-ray crystal analysis of the tosylate ester of **1** has confirmed its previously assigned configuration (Fig. 1). The tosylate ester was used for the X-ray crystallography since the free compound is non-crystallizable [53]. Along with its chemistry, the psychotropic properties of **1** were also disclosed.

The growing interest in *C. sativa* and its constituents has led to intensification of research in this area and, consequently, the appearance of a number of reviews on this subject [49–51]. Recently, Ahmed et al. isolated eight new tetrahydrocannabinol-type compounds [54], as shown in Fig. 2. These new cannabinoids were identified as β-fenchyl Δ^9-tetrahydrocannabinolate (**2**), α-fenchyl Δ^9-tetrahydrocannabinolate (**3**), *epi*-bornyl Δ^9-tetrahydrocannabinolate (**4**), bornyl Δ^9-tetrahydrocannabinolate (**5**), α-terpenyl Δ^9-tetrahydrocannabinolate (**6**), 4-terpenyl Δ^9-tetrahydrocannabinolate (**7**), α-cadinyl Δ^9-tetrahydrocannabinolate (**8**), and γ-eudesmyl Δ^9-tetrahydrocannabinolate (**9**). Their structures (Fig. 2) were established on the basis of NMR and GC-MS spectroscopic analysis, as mono- or sesquiterpenoid esters of Δ^9-tetrahydrocannabinolic acid A, the precursor of Δ^9-THC. Under the high temperature conditions of the GC-MS analysis used, these compounds fragment into their two components to yield Δ^9-THC and the mono- or sesquiterpene. These cannabinoid esters were isolated from a high-potency *C. sativa* variety using multiple chromatographic techniques, including vacuum-liquid chromatography (VLC), C_{18} semi-preparative HPLC, and semi-preparative enantioselective chiral HPLC [54]. In a recent publication, Zulfiqar et al. isolated a dimeric cannabinoid named cannabisol (**10**) (Fig. 2), from illicit samples with high cannabigerol (**21**) content received under a *C. sativa* potency monitoring

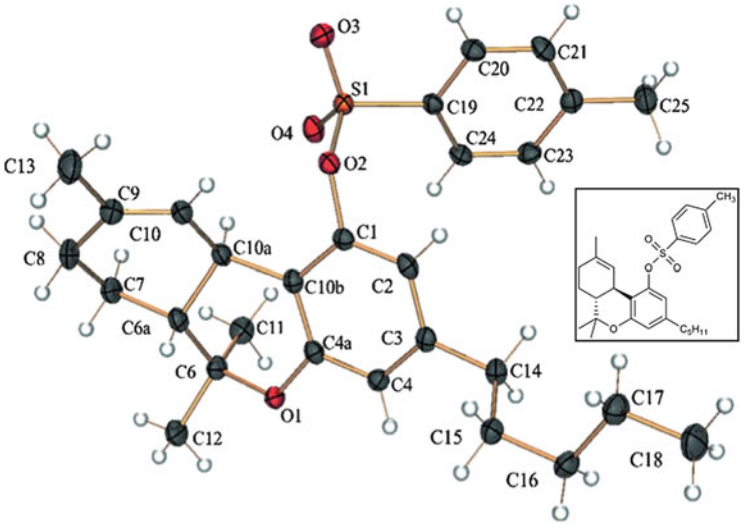


Fig. 1 X-ray crystallographic crystal structure of the tosylate ester of **1**

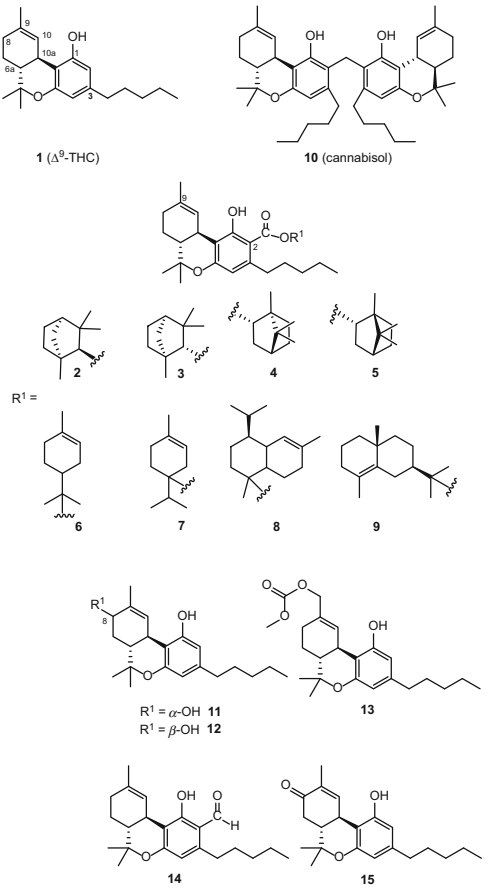


Fig. 2 Structures of (−)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) type cannabinoids

program, employing flash silica gel column chromatography. The GC-MS analysis of cannabisol (**10**) displayed two diagnostic ion peaks at *m*/*z* 314 and *m*/*z* 328, corresponding to Δ^9-THC and 2-methyl-Δ^9-THC [55]. Radwan et al. isolated and identified 8α-hydroxy-Δ^9-tetrahydrocannabinol (**11**), 8β-hydroxy-Δ^9-tetrahydrocannabinol (**12**), and 11-acetoxy-Δ^9-tetrahydrocannabinolic acid A (**13**) from a high-potency sample of *C*. *sativa* [56]. A Δ^9-THC aldehyde (**14**) and 8-oxo-Δ^9-THC (**15**) were also isolated from the same variety [57] (Fig. 2).

6.1.2 (−)-Δ^8-*trans*-Tetrahydrocannabinol (Δ^8-THC) Type

For many years, only two Δ^8-THC–type cannabinoids, namely, Δ^8-*trans*-tetrahydrocannabinol (**16**, Δ^8-THC) and Δ^8-*trans*-tetrahydrocannabinolic acid A (**17**, Δ^8-THCA) (Fig. 3), had been reported since the beginning of chemical investigations of

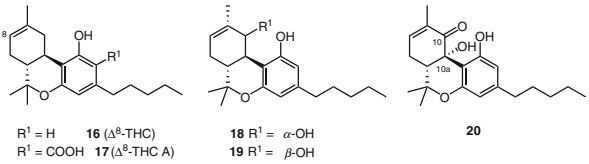


Fig. 3 Structures of (−)-Δ^8-*trans*-tetrahydrocannabinol (Δ^8-THC) type cannabinoids

C. sativa [58, 59]. Recently our group isolated three more compounds of this type [55, 56], which were identified as 10α-hydroxy-Δ^8-tetra-hydrocannabinol (**18**), 10β-hydroxy-Δ^8-tetrahydrocannabinol (**19**), and 10aα-hydroxy-10-oxo-Δ^8-tetrahydrocannabinol (**20**) (Fig. 3).

6.1.3 Cannabigerol (CBG) Type

Cannabigerol (**21**) (CBG) was the first compound to be isolated in a pure form from the resin of *Cannabis sativa* [60]. Of the cannabigerol-type cannabinoids, eight compounds were published through 2005 [51] while an additional nine new cannabinoids of this type (Fig. 4) have been reported recently. Of these, seven compounds (**22**–**28**) were isolated

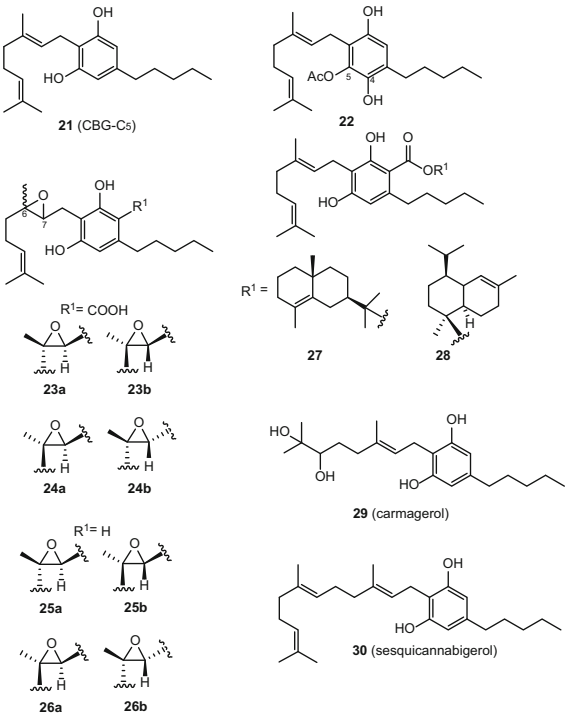


Fig. 4 Structures of cannabigerol (CBG) type cannabinoids

form the buds of the mature female plant of a high-potency variety of *C. sativa*. Radwan et al. isolated 5-acetyl-4-hydroxy-cannabigerol (**22**), (±)-6,7-*trans*-epoxycannabigerolic acid (**23**), (±)-6,7-*cis*-epoxycannabigerolic acid (**24**), (±)-6,7-*cis*-epoxycannabigerol (**25**), and (±)-6,7-*trans*-epoxycannabigerol (**26**) from high-potency *C. sativa* [61, 62]. Two cannabigerolic acid esters, γ-eudesmyl-cannabigerolate (**27**) and α-cadinyl-cannabigerolate (**28**), were isolated using enantioselective HPLC from the same high-potency variety [54]. In 2008, Appendino et al. purified a novel, polar dihydroxy-cannabigerol derivative (carmagerol) (**29**) from the acetone extract of the aerial parts of *C. sativa* variety "carma". In their procedure, a *C. sativa* extract was fractionated on a RP C_{18} silica gel column followed by silica gel column chromatography and subsequent use of normal-phase HPLC to isolate compound **29** [63]. Sesquicannabigerol (**30**), a lipophilic analogue of cannabigerol was isolated by Pollastro et al. from the waxy fraction of the "carma" variety of fiber hemp after hydrolysis of the wax with methanolic KOH and purification by gravity-column silica gel column chromatography, followed by flash chromatography over neutral alumina [64].

6.1.4 Cannabichromene (CBC) Type

The research groups of Claussen et al. and Gaoni and Mechoulam independently disclosed the structure of cannabichromene (**31**) (CBC) in 1966 [65, 66]. ElSohly and Slade subsequently reported six cannabichromene-type cannabinoids [51]. A recent publication has described the isolation from high-potency *C. sativa* of three new cannabichromene-type cannabinoids (Fig. 5), namely, (±)-4-acetoxycannabichromene (**32**), (±)-3″-hydroxy-$\Delta^{4''}$-cannabichromene (**33**), and (−)-7-hydroxycannabichromane (**34**), by applying silica gel vacuum-liquid chromatography (VLC) as well as silica gel and C_{18} HPLC [62].

6.1.5 Cannabidiol (CBD) Type

Cannabidiol (**35**) (CBD) and cannabidiolic acid (**36**) (CBDA) are the major metabolites of the non-psychotropic (fiber-type) varieties of *C. sativa*. Adams et al. isolated cannabidiol (CBD) in 1940 [67], while Petrzilka et al. reported its synthesis and

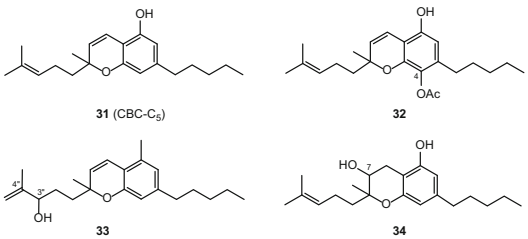


Fig. 5 Structures of cannabichromene (CBC) type cannabinoids

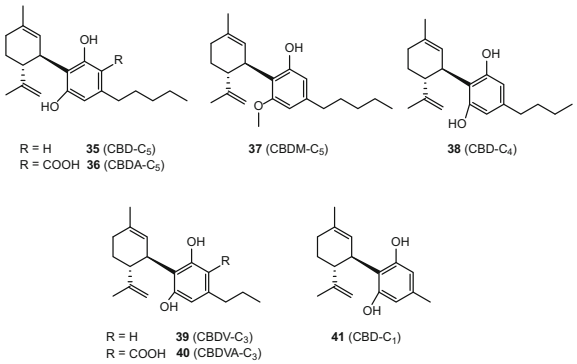


Fig. 6 Structures of cannabidiol (CBD) type cannabinoids

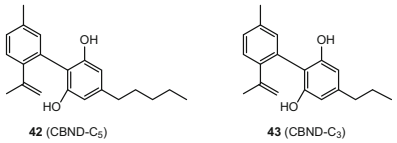


Fig. 7 Structures of cannabinodiol (CBND) type cannabinoids

absolute configuration as (−)-*trans*-(1*R*,6*R*) [68]. Seven cannabidiol-type cannabinoids [(−)-cannabidiol (CBD) (**35**), cannabidiolic acid (CBDA) (**36**), cannabidiol monomethyl ether (CBDM) (**37**), cannabidiol-C_4 (CBD-C_4) (**38**), (−)-cannabidivarin (CBDV) (**39**), cannabidivarinic acid (CBDVA) (**40**), and cannabidiocol (CBD-C_1) (**41**)] were included in a 2005 review by ElSohly and Slade (Fig. 6) [51]. No new CBD-type cannabinoids were reported since 2005.

6.1.6 Cannabinodiol (CBND) Type

Cannabinodiol-type cannabinoids are the aromatized derivatives of cannabidiol (CBD). Cannabinodiol (CBND-C_5) (**42**) and CBND-C_3 (cannabinodivarin) (**43**) (Fig. 7) are the only two compounds from this subclass that have been characterized in *C. sativa* [49, 51].

6.1.7 Cannabielsoin (CBE) Type

Five cannabielsoin (CBE)-type cannabinoids with an identical absolute configuration (5a*S*,6*S*,9*R*,9a*R*) have been described [49], and named cannabielsoic acid A (CBEA-C_5 A) (**44**), cannabielsoin (CBE) (**45**), cannabielsoic acid B (CBEA-C_5 B) (46), cannabielsoic acid B-C_3 (CBEA-C_3 B) (47), and C_3-cannabeilsoin (CB3-C_3) (**48**). These CBE-type cannabinoid isolates were characterized from *C. sativa* [69]. Furthermore, they were identified as mammalian metabolites of CBD [70] (Fig. 8).

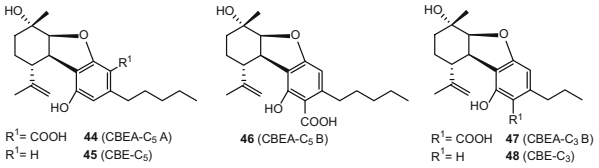


Fig. 8 Structures of cannabielsoin (CBE) type cannabinoids

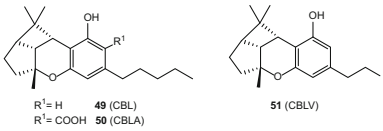


Fig. 9 Structures of cannabicyclol (CBL) type cannabinoids

6.1.8 Cannabicyclol (CBL) Type

Cannabicyclol (CBL) (**49**), cannabicyclolic acid (CBLA) (**50**), and cannabicyclovarin (CBLV) (**51**) (Fig. 9) are the only representatives of this subclass [71–75].

6.1.9 Cannabinol (CBN) Type

Seven cannabinol derivatives were included in a 2005 review [51]. In addition, Ahmed et al. isolated 4-terpenyl cannabinolate (52) (Fig. 10) from a high-potency variety of *C. sativa* [54]. On GC-MS analysis, compound **52** fragmented to CBN and a monoterpenol. This cannabinoid was isolated through a semi-preparative enantioselective HPLC method. Furthermore, 8-hydroxycannabinolic acid A (**53**) and 8-hydroxycannabinol (**54**) (Fig. 10) were isolated from the same variety of *C. sativa* [62]. Compound **54**, which was obtained initially as a synthesized product [76], was isolated for the first time from a natural source using a C_{18} solid-phase extraction (SPE) procedure. Ahmed et al. recently reported the isolation of (1′*S*)-hydroxycannabinol (**55**) (Fig. 10) from a high-potency *C. sativa* specimen [57].

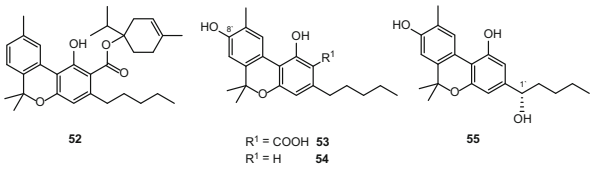


Fig. 10 Structures of cannabinol (CBN) type cannabinoids

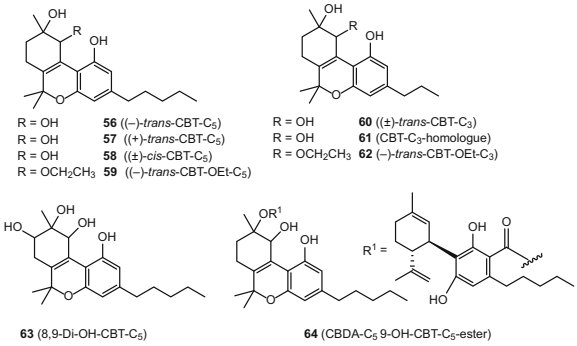


Fig. 11 Structures of cannabitriol (CBT) type cannabinoids

6.1.10 Cannabitriol (CBT) Type

Cannabitriol was originally reported by Obata and Ishikawa in 1966 [77], but its chemical structure was elucidated a decade later in 1976 [78]. The configuration of this compound was later determined by X-ray analysis [79]. In addition, nine CBT-type cannabinoids including (−)-*trans*-cannabitriol ((−)-*trans*-BT-C_5) (**56**), (+)-*trans*-cannabitriol ((+)-*trans*-CBT-C_5) (**57**), (±)-*cis*-cannabitriol ((±)-*cis*-CBT-C_5) (**58**), (−)-*trans*-10-ethoxy-9-hydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol ((−)-*trans*-CBT-OEt-C_5) (**59**), (±)-*trans*-cannabitriol-C_3 ((±)-*trans*-CBT-C_3) (**60**), CBT-C_3 homolog (unknown stereochemistry) (CBT-C_3-homologue) (**61**), (−)-*trans*-10-ethoxy-9-hydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabivarin-C_3 ((−)-*trans*-CBT-OEt-C_3) (**62**), 8,9-dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol (8,9-Di-OH-CBT-C_5) (**63**), and cannabidiolic acid tetrahydrocannabitriol ester (CBDA-C_5 9-OH-CBT-C_5 ester) (**64**) (Fig. 11), were mentioned in a previous review [50]. Nevertheless, the two ethoxy cannabitriols, (−)-*trans*-CBT-OEt-C_5 (**59**) and (−)-*trans*-CBT-OEt-C_3 (**62**), are likely artifacts, since ethanol was used in their isolation from *C. sativa* [80, 81].

6.1.11 Miscellaneous Types

Miscellaneous types of cannabinoids have been summarized in a review by ElSohly and Slade [51]. These compounds included dehydrocannabifuran (DCBF-C_5) (**65**), cannabifuran (CBF-C_5) (6**6**), 8-hydroxy-isohexahydrocannabivirin (OH-iso-HHCV-C_3) (**67**), cannabichromanone-C_5 (CBCN-C_5) (**68**), cannabichromanone-C_3 (CBCN-C_3) (**69**) (Fig. 12), 10-oxo-$\Delta^{6a(10a)}$-tetrahydrocannabinol (OTHC) (**70**), cannabicitran (**71**), (−)-Δ^9-*cis*-(6a*S*,10a*R*)-tetrahydrocannabinol (*cis*-Δ^9-THC) (**72**) cannabicoumaronone (CBCON-C_5) (**73**) (Fig. 13), cannabiripsol (CBR) (**74**), cannabitetrol (CBTT) (**75**), (±)-Δ^7-*cis*-isotetrahydrocannabivarin-C_3 (*cis*-iso-Δ^7-THCV) (**76**), (−)-Δ^7-*trans*-(1*R*,3*R*,6*R*)-isotetrahydrocannabivarin-C_3 (*trans*-iso-Δ^7-THCV) (**77**),

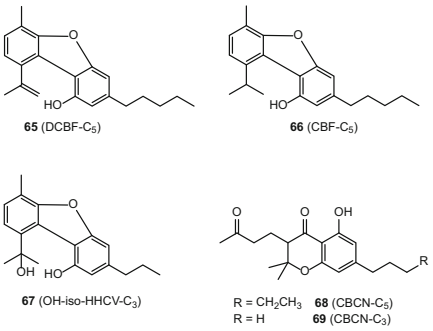


Fig 12 Structures of cannabinoids **65–69**

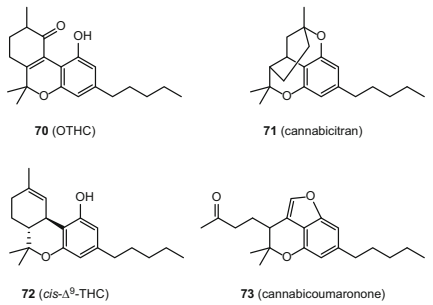


Fig 13 Structures of cannabinoids **70–73**

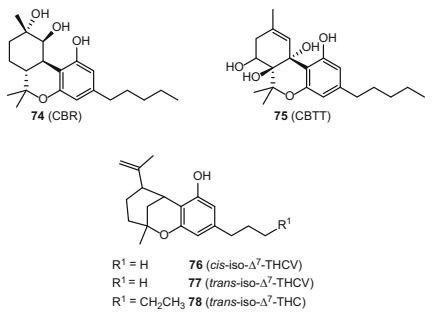


Fig 14 Structures of cannabinoids **74–78**

and (−)-Δ^7-*trans*-(1*R*,3*R*,6*R*)-isotetrahydrocannabinol-C_5 (*trans*-iso-Δ^7-THC) (**78**) (Fig. 14).

However, in a more recent review, cannabichromanones and cannabicoumaronones were placed in separate groups as a result of a refined classification [82]. From a high-potency variety of *C. sativa,* Ahmed et al. isolated three cannabichromanones (Fig. 15) that were named cannabichromanones B (**79**), C (**80**), and D (**81**). The absolute

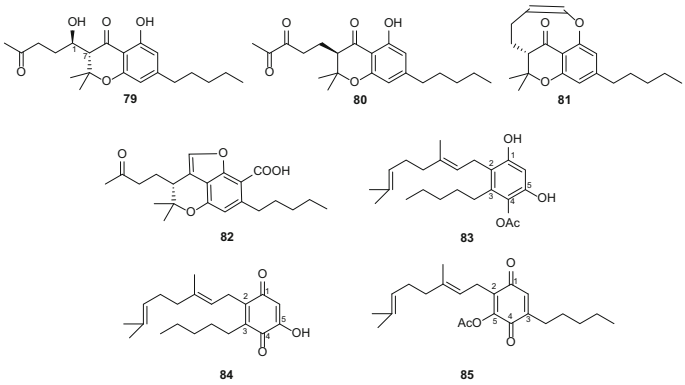


Fig 15 Structures of cannabinoids **79–85**

configuration of the three compounds was assigned on the basis of Mosher ester derivatization and inspection of their circular dichroism spectra [83]. The isolation of these compounds was performed using semi-preparative C_{18} HPLC. (−)-(7*R*)-Cannabicoumarononic acid (**82**), 4-acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol (**83**), and 2-geranyl-5-hydroxy-3-*n*-pentyl-1,4-benzoquinone (**84**) (Fig. 15) were isolated from buds and leaves of the same variety of *Cannabis* by application of several chromatographic techniques, including vacuum-liquid chromatography (VLC) over silica gel, solid-phase extraction column separation (C_{18} SPE), and normal-phase HPLC [62]. The CD spectrum of **82** showed a positive Cotton effect at 246 nm and negative one at 295 nm, indicating the (7*R*) configuration [61]. In addition, 5-acetoxy-6-geranyl-3-*n*-pentyl-1,4-benzoquinone (**85**) (Fig. 15) was isolated by employing silica gel column chromatography followed by normal-phase HPLC [84]. Radwan et al. classified compound **85** erroneously as a non-cannabinoid [83]; however, since it is an acetoxy derivative of a C_{21} terpenophenol, its classification should be revised to be included in the cannabinoid class of constituents.

In 2010, Taglialatela-Scafati et al. isolated a new cannabinoid named cannabimovone (CBM) (**86**) (Fig. 16) from a non-psychotropic variety of *C. sativa* [85]. This unusual metabolite presumably is formed from cannabidiol, and was isolated from the polar fraction of hemp using flash chromatography over reversed-phase C_{18} silica gel followed by normal-phase HPLC [85]. Pagani et al. isolated the tetracyclic cannabinoid cannabioxepane (CBX) (**87**) (Fig. 16) from the "caramagnola" variety of *C. sativa* [86]. Recently, Radwan et al. [55] and Ahmed et al. [57] isolated six cannabinoids from a high-potency variety of *C. sativa* that were identified as 10α-hydroxy-$\Delta^{9,11}$-hexahydrocannabinol (**88**), 9β,10β-epoxyhexahydrocannabinol (**89**), 9α-hydroxyhexahydrocannabinol (**90**), 7-oxo-9α-hydroxyhexahydrocannabinol (**91**) 10α-hydroxyhexahydrocannabinol (**92**), 10a*R*-hydroxyhexahydrocannabinol (**93**), and 9α-hydroxy-10-oxo-$\Delta^{6a,10a}$-tetrahydrocannabinol (**94**) (Fig. 16).

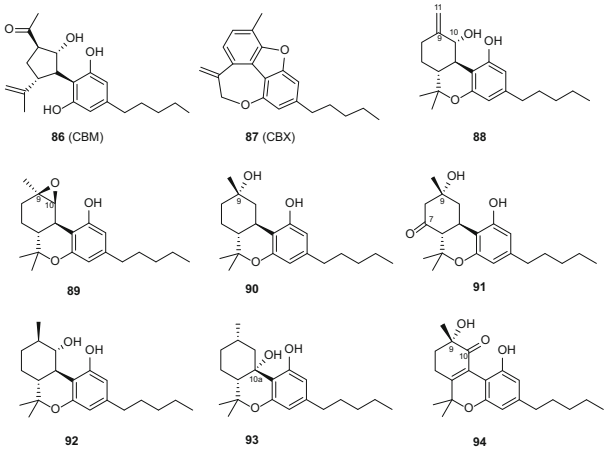


Fig 16 Structures of cannabinoids **86**–**94**

6.2 Non-Cannabinoid Constituents

In addition to the cannabinoids, hundreds of constituents belonging to a broad range of chemical classes have been obtained from *C. sativa* previously [49–51]. In addition to the 419 prior known non-cannabinoid secondary metabolites of *C. sativa*, 26 non-cannabinoids have been isolated from this plant since 2005 (Figs. 17–23). Seven compounds, namely, cannflavin C (**95**), chrysoeriol (**96**), 6-prenylapigenin (**97**), 4,5-dihydroxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene (**98**), 4-hydroxy-2,3,6,7-tetramethoxy-9,10-dihydrophenanthrene (**99**), 4,7-dimethoxy-1,2,5-trihydroxyphenanthrene (**100**) (Fig. 17), and *β*-sitosteryl-3-*O*-*β*-D-glucopyranoside-2′-*O*-palmitate (**101**) were isolated from a high-potency variety of *C. sativa* [84]. Compound **101** was isolated by silica gel column chromatography followed by normal-phase solid-phase extraction, while compound **96** was purified by silica gel column chromatography with subsequent reversed-phase chromatography. Compounds **95**, **99**, and **100** were purified by silica gel column chromatography, normal-phase solid-phase extraction, and C_{18} HPLC. The new compound, 9,10-dihydro-2,3,5,6-tetramethoxyphenanthrene-1,4-dione (**102**) was isolated from the leaves and branches of *C. sativa* by silica gel chromatography and passage over Sephadex LH-20, followed by semi-preparative liquid chromatography [87]. Docosanoic acid methyl ester (**103**) (Fig. 18), acetyl stigmasterol (**104**), *α*-spinasterol (**105**), 1,3,6,7-tetrahydroxy-2-*C*-*β*-D-glucopyranosyl-xanthone (**106**) (Fig. 19), apigenin-6,8-di-*C*-*β*-D-glucopyranoside (**107**), uracil (**108**) and quebrachitol (**109**) were isolated from the petroleum ether and *n*-butanol fractions of the methanol extract of hemp leaves and branches [88]. The authors indicated that quebrachitol (**109**) was isolated for the first time from *C. sativa* [88]; however, this particular compound has long been reported from this species

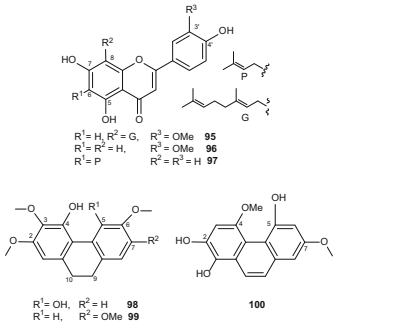


Fig. 17 Structures of non-cannabinoids **95–100**

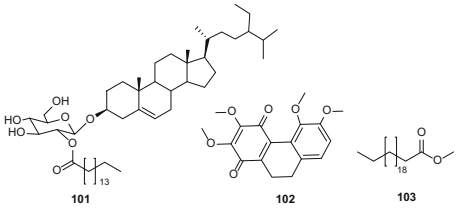


Fig. 18 Structures of non-cannabinoids **101–103**

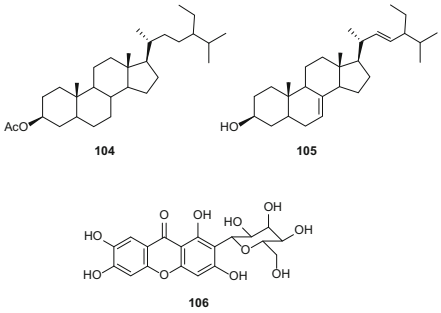


Fig. 19 Structures of non-cannabinoids **104–106**

[49]. Qian et al. isolated cannabsin A (**110**) (Fig. 20), isoselachoceric acid (**111**), *β*-sitosterol (**112**) and mannitol (**113**) from the fruits of *C. sativa* [89]. These compounds were purified by sequential silica gel column and Sephadex LH-20 column chromatography [89]. Although the authors claimed the isolation of these five compounds for the first time from *C. sativa* [89], *β*-sitosterol (**112**) and mannitol (**113**) have previously been described from this species [49]. 5′-Methyl-4-pentylbiphenyl-2,6,2′-triol (**114**) (Fig. 21) and 7-methoxycannabispirone (**115**) were isolated from a high-potency variety of *C. sativa* utilizing normal-phase chromatography with subsequent C_{18} HPLC [61]. In

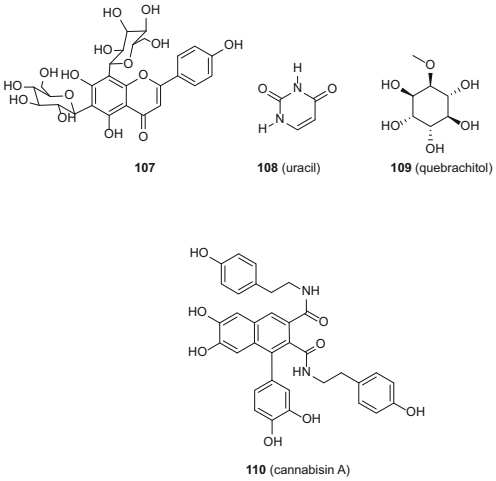


Fig. 20 Structures of non-cannabinoids **107–110**

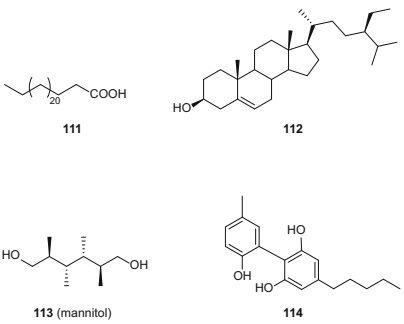


Fig. 21 Structures of non-cannabinoids **111–114**

2011, Pagani et al. reported the isolation of isocannabispiradienone (**116**) and two polyunsaturated hydroxy fatty acids, **117** and **118** (Fig. 22), from a fiber cultivar of *C. sativa* variety "carmagnole" using RP-C_{18} flash chromatography and normal-phase HPLC [86]. In 2015, Yan et al. isolated four new lignanamides, cannabisin M (**119**), cannabisin N (**120**), cannabisin O (**121**), and 3,3′-demethyl-heliotropamide (**122**) from the ethyl acetate extract of hemp seed (Fig. 23), using a combination of medium-pressure reversed-phase chromatography, passage over Sephadex LH-20, and RP-HPLC [90].

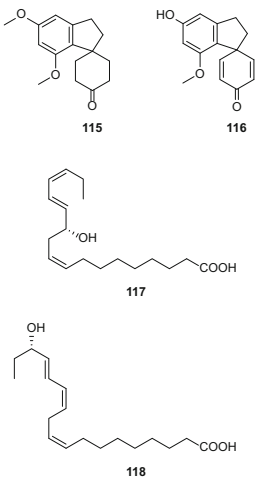


Fig. 22 Structures of chemical structures of non-cannabinoids **115–118**

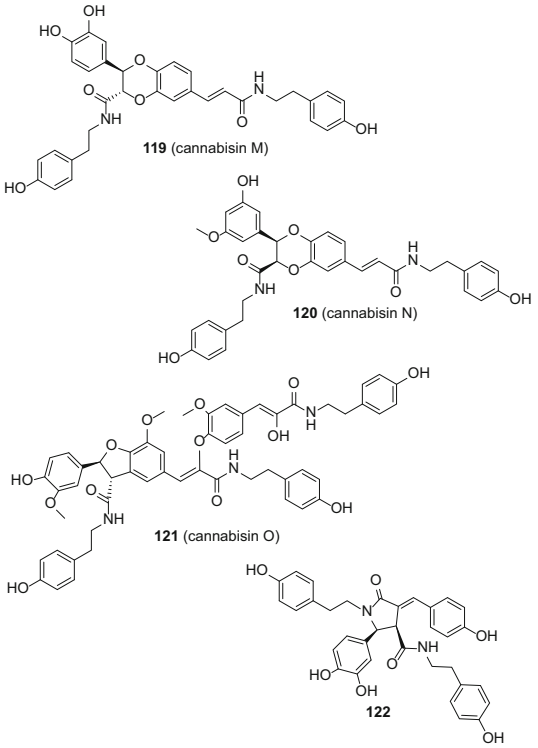


Fig. 23 Structures of non-cannabinoids **119–122**

7 Analysis of Cannabinoids in *Cannabis sativa* and Its Preparations

Several methods for the analysis of *Cannabis sativa* and its products (marijuana, hashish, and hash oil) have been described in the literature. These methods have been focused mainly on the identification, fingerprinting, and quantification of cannabinoids, including both the acidic and neutral substances of this type. Particular emphasis has been directed to these with possible medicinal properties such as (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) (**1**), cannabigerol (CBG) (**21**), cannabichromen (CBC) (**31**), cannabidiol (CBD) (**35**), cannabinol (CBN) (**123**) (Fig. 24), and tetrahydrocannabivarin (THCV) (**124**) (Fig. 24), and also for forensic purposes [91]. Diversified analytical approaches, techniques, and instrumentation with different levels of sensitivity and specificity were utilized to achieve the aforementioned goals. The most substantially employed techniques for analysis of *Cannabis* were GC/MS and HPLC [92–97]. Gas chromatography/mass spectrometry has been much more commonly used for this purpose despite its inherent disadvantage of causing thermal decarboxylation of the acidic cannabinoids to their neutral form, unless the plant extract is derivatized chemically. In contrast, HPLC does not affect the structure of the cannabinoids, which permits analysis of the original compounds present in a plant extract. However, it has the disadvantage of insufficient resolution of the whole array of cannabinoids. The following is an overview of the methods described in the literature for the analysis of *C. sativa* preparations.

Morita and Ando described a GC-MS method for the analysis of several cannabinoids in hash oil. Using this method, eleven compounds, including Δ^9-THC (**1**), CBC (**31**), CBD (**35**), and CBN (**123**) (Fig. 24), along with some C_3 homologs, were resolved and identified. In this report, the authors proposed structures for the Δ^9-THC peak fragments based on their masses observed [98].

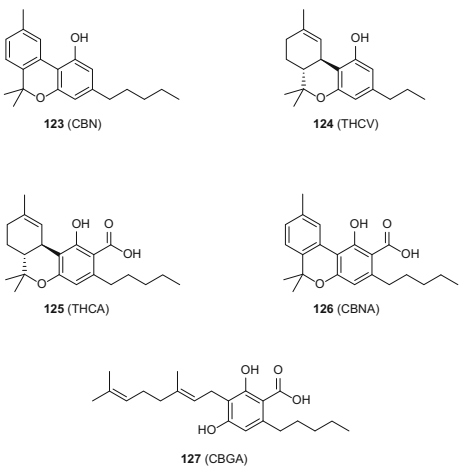


Fig. 24 Structures of cannabinoids **123–127**

Brenneisen and ElSohly described a method that combined high-resolution capillary gas chromatography-flame ionization detection (GC-FID) and GC-MS for identification of the compounds in *C. sativa* extracts in order to establish a correlation between their chemical profiles and the geographical origin of the plant materials. The constituents analyzed comprised terpenes, alkanes, cannabinoids, and non-cannabinoid phenols. Over 100 different compounds were identified, and the procedure proved to be robust and applicable in forensic inspections to trace the geographical origin of *C. sativa* samples through their chemical profiles [99].

In another study, HPLC-UV was employed for the resolution of mixtures of acidic and neutral cannabinoids, using a Beckman Ultrasphere 3 μm ODS column. More than 40 components were detected in the HPLC trace produced [91].

Hida et al. published a procedure for the classification of varieties of hashish on the basis of inspecting their pyrolysis-gas chromatography profiles in the presence of powdered chromium, and the data subsequently were subjected to cluster analysis of the normalized pyrograms. In this work, the peaks in each pyrogram were normalized against the highest peak present. The results of the cluster analysis were presented in clearly interpreted dendrogram visual representations. The dendograms could be exploited to compare unknown hashish samples with those of samples from a diversity of sources for classification purposes [100].

A GC-FID method was developed by Ross et al. for the routine analysis of confiscated marijuana samples and the quantification of cannabinoids including Δ^9-THC (**1**), CBG (**21**), CBC (**31**), CBN (**35**), CBN (**123**) (Fig. 24), and THCV (**124**) (Fig. 24). The procedure used involved extraction of a small amount of sample (100 mg) with a methanol-chloroform mixture (99:1) containing an internal standard (4-androstene-3,17-dione), followed by direct analysis of the extract on a DB-1 column [101].

Veress et al. reported an early method for the comparative analysis of neutral cannabinoids by HPLC, utilizing two different types of bonded-phase columns. In this study, an amino-bonded-phase column was used for the separation of the constituents of the plant material with non-polar solvents, using direct injection of the extract without prior clean-up. The results using this column were compared to those obtained employing a reversed-phase column, where the latter required sample clean up, using a C_{18}-Sep-Pak cartridge prior to the HPLC analysis. The use of an amino-bonded column was shown to be superior to the reversed-phase one for the quantitation of neutral cannabinoids [102].

Several analytical techniques, including TLC, GC-FID with both packed and capillary columns, and HPLC have been described in detail for the analysis of neutral and acidic cannabinoids in *C. sativa* products (marijuana, hashish, and hashish oil) in a manual prepared in 1987 by the United Nations, Division of Narcotic Drugs [103]. This manual included a compilation of methods for sampling and analysis of *C. sativa* products, as recommended for use by the National Narcotics Laboratories. The amount of THC (**1**) in hemp seed oil was determined by a GC-MS analytical procedure published by Bosy and Cole [104]. The content of Δ^9-THC (**1**) and tetrahydrocannabinolic acid (THCA) (**125**) (Fig. 24) in hemp-containing foods were determined by HPLC [105]. Also, the total Δ^9-THC (**1**) concentration levels of both drug- and fiber-type *C. sativa* seeds was determined by

Ross et al. following a GC-MS procedure [106]. Pellegrini et al. published a method combining liquid-liquid extraction with GC-MS for quantification of Δ^9-THC (**1**), CBD (**35**), and CBN (**123**) (Fig. 24) in different hemp-containing foods and beverages. The dietary items included in this study were beer, pastilles, liqueur, seeds, scented grass, and oil. In this procedure, in which Δ^8-THC (**16**) was used an as internal standard, solid and liquid samples were extracted with hexanes/isopropanol (9:1) and analysis was performed on a fused silica capillary column. Silylated samples were analyzed in the selected-ion-monitoring (SIM) mode. Quantification levels were found to be 1 ng/g for Δ^9-THC and CBN and 2 ng/g for CBD. Levels of Δ^9-THC in the different hemp foods varied by a factor of 50-fold. The data obtained showed that the majority of the samples analyzed were derived from drug-type *Cannabis* [107].

Quantification of individual cannabinoids necessitates the use of internal standards which have varied depending on the method employed. These have included the use of long-chain hydrocarbons (e.g. *n*-tetradecane or *n*-docosane), steroids (androst-4-ene-3,17-dione and cholestane), and phthalates (dibenzylphthalate or di-*n*-octylphthalate) [91].

In another published study, HPLC, using a reversed-phase column (7 μm particle size) and a mixture of methanol and 0.01 *M* sulfuric acid (80:20), as the mobile phase, was employed in the analysis of Δ^9-THC (**1**), CBD (**35**), and CBN (**123**), along with their respective carboxylic acid forms, tetrahydrocannabinolic acid (THCA) (**125**) (Fig. 24), cannabidiolic acid (CBDA) (**36**) and cannabinolic acid (CBNA) (**126**) (Fig. 24). The authors applied standard storage conditions for hashish samples along with pure cannabinoids, and concluded that the total values of CBD/CBDA-C_5, CBN/CBNA, and Δ^9-THC/THCA were useful for the evaluation of hashish samples [108].

A ^{1}H-NMR spectroscopic method for the quantitative analysis of cannabinoids in *C. sativa* was developed by Hazekamp and associates. Distinctive signals for the cannabinoids were found to be in the range δ_H 4.0–7.0 ppm of the ^{1}H-NMR spectrum, with anthracene used as an internal standard. The quantitation of the target compounds was achieved by calculating the relative ratio of the peak areas of selected proton signals of the target compound to the known amount of the internal standard. This method permits simple and rapid quantification of cannabinoids without prior chromatographic purification, involving only a 5-min analysis time [109].

Elias and Lawerence, in a book on the analysis of drugs of abuse published in 1991, provided a concise overview of the instrumental methods used in illicit drug interdiction. Methods employed for detecting concealed drugs were classified into two main techniques based on bulk detection and air sampling. The bulk detection techniques included X-ray imaging, gamma backscattering, thermal neutron activation, and other systems, while the air sampling techniques comprised acetone vapor detection, mass spectrometry, gas spectrometry, and ion-mobility spectrometry. The authors reached the conclusion all that these methods have limitations and drawbacks and suggested the need for more efficient and selective methodologies [110].

A recent report described a method for the simultaneous quantification of Δ^9-THC (**1**), cannabidiol (CBD) (**35**), cannabinol (CBN) (**123**) (Fig. 24) when present in *C. sativa*, adopting sequential silica gel column chromatography and reversed-

phase HPLC. The column chromatography stage was used to purify a methanol-soluble extract of *C. sativa* [111].

A reversed-phase HPLC-UV (detection at 220 nm) method for the concurrent determination of Δ^9-THC (1), cannabidiol (CBD) (**35**), and cannabinol (CBN) (**123**) (Fig. 24) in the seed oil of *C. sativa* has been published [112]. The range of detection was established at 3.75–37.5 ng cm^{-3}, 0.125–1.25 ng cm^{-3} and 0.735–7.35 ng cm^{-3} for Δ^9-THC, CBD, and CBN, respectively. These average recoveries were 96.1, 97.2, and 98.0% for Δ^9-THC, CBD, and CBN [112].

De Backer et al. developed a method based on HPLC/DAD for both the identification as well as the quantitative determination of Δ^9-THC (**1**), Δ^8-THC (**16**), CBG (**21**), CBD (**35**), CBDA (**36**), CBN (**123**) (Fig. 24), THCA (**125**) (Fig. 24), and cannabigerolic acid (CBGA) (**127**) (Fig. 24) in samples of drug-type, non-psychotropic, and fiber-type *C. sativa*. Predictive multilinear models were used to establish optimal analytical conditions. The validation of this method was accomplished by utilization of accuracy profiles based on β-expectation tolerance intervals for the total error measurement, and evaluating the measurement uncertainties. The authors claimed this method is useful in the identification of phenotypes, assessment of psychoactivity potency, and quality control of *C. sativa* [41].

In a recently published study, Fischedick et al. developed a normal-phase high-performance TLC-densitometric method for the identification and fingerprinting of the major neutral cannabinoids [Δ^9-THC (**1**), CBG (**21**), CBC (**31**), CBD (**35**), and THCV (**124**) (Fig. 24)] as well as the simultaneous quantification of Δ^9-THC and cannabinol (**123**) (Fig. 24) in two medicinal *C. sativa* cultivars. Decarboxylation of Δ^9-THCA (**125**) (Fig. 24) in the plant material was attained through heating at 100°C for 2 h, prior to analysis. CAMAG instrumentation was used for the different steps of chromatography. The range of quantification was determined to be 50–500 ng, using UV light at 206 nm for detection. The results of this method showed it to be comparable to validated HPLC methods, making it potentially useful for forensic analysis and the quality control of hemp and medicinal *C. sativa* [113].

Hazekamp et al. carried out a study on the chromatographic and spectroscopic analysis of 16 major cannabinoids. Their analytical study utilized UV absorbance, IR, GC-MS, and HPLC profiling of the cannabinoids, spectrophotometric analysis, and identification of the fluorescence characters of these cannabinoids. The GC-MS analysis was conducted without prior derivatization, and led to determination of retention times, molecular weights, and mass spectrometric fragmentation spectra of the cannabinoids [93].

Chemiluminescence has been used recently for the detection of cannabinoids. This process is based on reacting cannabinoids with potassium permanganate under acidic conditions to produce a chemiluminescence effect. This method, in conjunction with HPLC, was used to determine cannabidiol (**35**). The authors concluded this technique has the potential to extend to the analysis of Δ^9-THC (**1**) and other cannabinoids in drug-grade *C. sativa* samples [114].

The most current method described the simultaneous detection and quantification of 11 cannabinoids, both in biomass and in extracts of different varieties of *C. sativa*, using C_{18} HPLC [115]. The cannabinoids that were involved in the analysis included Δ^9-THC (**1**), Δ^8-THC (**16**), CBG (**21**), CBC (**31**), CBD (**35**), CBDA (**36**),

cannabicyclol (CBL) (**49**), CBN (**123**), THCV (**124**), THCA (**125**), and CBGA (**127**). Separation of the cannabinoid mixture was achieved in 22.2 min. The concentration-response patterns of the process exhibited linear relationships between the concentrations and the peak areas [115].

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Synthesis of Phytocannabinoids

Michael A. Schafroth and Erick M. Carreira

Contents

1 Introduction

Descriptions of rites and uses of cannabis, both as a medicinal and recreational drug, can be found across a wide range of cultures [1]. One of the first historical accounts of *Cannabis sativa* originates from the Atharvaveda, a Hindu ancient text composed dating from 1500–1000 BCE, where it is considered a sacred plant, to be employed in various rituals and ceremonies [2]. In "Histories" (400 BCE), Herodotus describes the ceremonial burning of *Cannabis* seeds during a Scythian funeral near the Black Sea [3]. At the beginning of the Common Era, the Greek physicians Pedanius Dioscorides (40–90 AD) and Claudius Galen (129–201/215 AD) described the physiological effects of *Cannabis* seeds and recommended their application in the treatment of earache [4, 5]. Finally, in modern times in the West, medicinal uses of cannabis were introduced by O'Shaughnessy in 1839, who suggested its application in the treatment of tetanus and as an analgesic [2, 4]. In the following two centuries extensive research activities have focused

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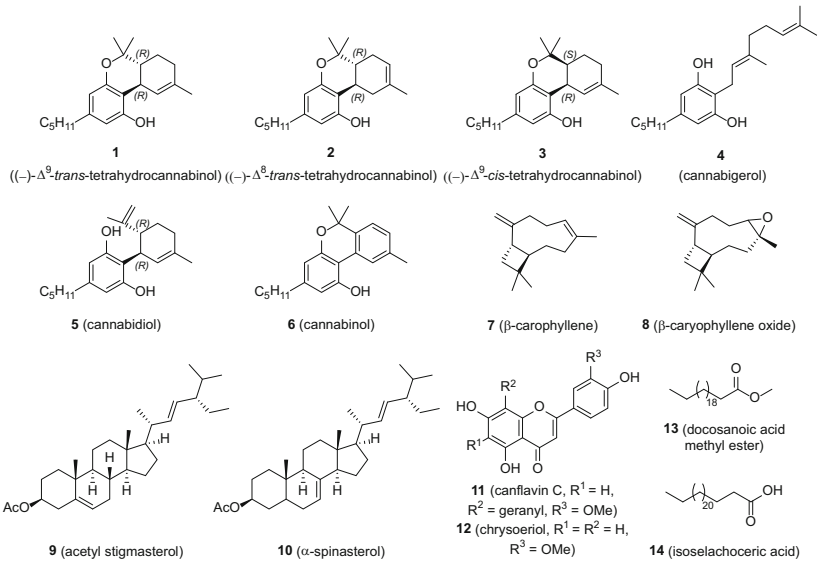
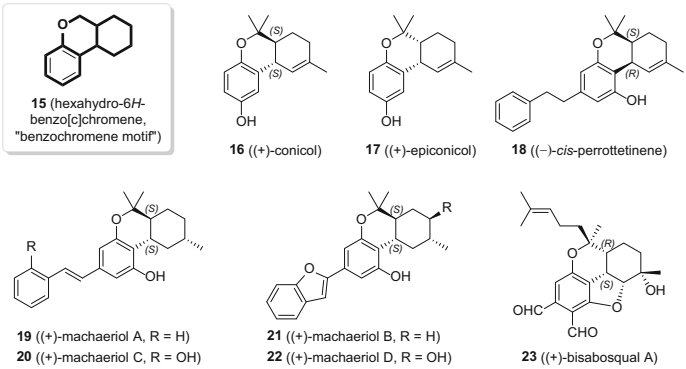
on the identification of the active principle and other constituents of *Cannabis sativa* [6]. These research efforts resulted in seminal work by the research groups of Cahn, Adams, and Todd, which provided the basis for the ground-breaking elucidation of the structure of (−)-*trans*-Δ^9-tetrahydrocannabinol (**1**) by Gaoni and Mechoulam in 1964 [7–10]. To date, more than 500 different natural products have been identified in *Cannabis sativa*, including the thermodynamically more stable (−)-*trans*-Δ^8-tetrahydrocannabinol (**2**), which often occurs as a side product during the synthesis of **1** by isomerization, under acidic conditions or by heating [11–14]. Other constituents include the diastereoisomeric (−)-Δ^9-*cis*-tetrahydrocannabinol (**3**) [15], the THC precursor cannabigerol (**4**) [16], cannabidiol (**5**) [17], and an oxidation product, cannabinol (**6**) [18]. Compounds from other structural classes of secondary metabolites include mono- and sesquiterpenes (**7**–**8**), steroids (**9**–**10**), flavonoids (**11**–**12**), fatty acids (**13**–**14**), and others (Scheme 1) [19].

Various definitions for phytocannabinoids have emerged: in some cases only the C_{21} terpenophenolic substances isolated from *Cannabis sativa* are included [19], while in other cases phytocannabinoids are defined as plant-derived natural products that either interact with the cannabinoid receptors or share a chemical similarity to cannabinoids [20]. In this chapter, we define "phytocannabinoids" as any natural product that shares the central hexa-/tetrahydro-6*H*-benzo[*c*]chromene core (**15**) with tetrahydrocannabinol. Derivative compounds possessing all four stereoisomeric permutations at this core motif have been reported as biologically active natural products (Scheme 2). Compound **1** possesses the (*R*,*R*)-configuration, while the enantiomeric (*S*,*S*)-configuration is found in conicol (**16**), a meroterpenoid isolated from the marine invertebrate *Aplidium conicum* [21] and



Scheme 1 Naturally occurring cannabinoids and other constituents isolated from *Cannabis sativa*



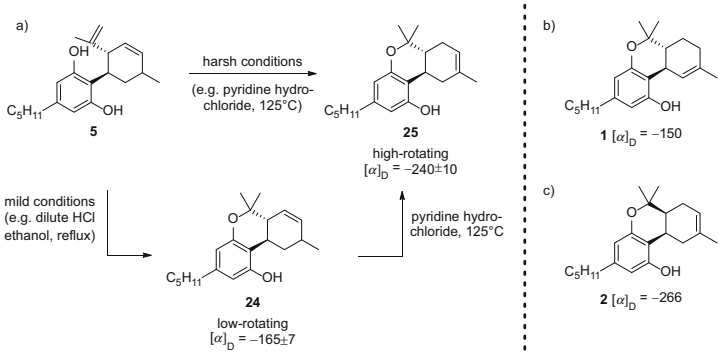
Scheme 2 Other natural products that contain the "benzochromene motif" **15**

machaeriols A–D (**19**–**22**), which show antibacterial, antifungal, or antimalarial activity and were are isolated from the stem bark of *Macherium multiflorum* Spruce, a native Amazonian liana from Peru [22, 23]. The (*S*,*R*)-diastereomer occurs in natural *cis*-tetrahydrocannabinol (**3**) and in perrottetinene (**18**), a bibenzyl cannabinoid isolated from *Radula perrottetti* and related liverworts [24–26]. The (*R*,*S*)-diastereomer is present in bisabosqual A (**23**), a prenylated derivative with squalene synthase inhibition activity found in the *Stachybotrys* genus [27, 28]. Epiconicol (**17**), isolated from *Aplidium conicum*, *Synoicum castellatum*, and *Aplidium* aff. *densum* shows activity against different cancer cell lines and acts as an antibacterial against the Gram-positive bacterium *Micrococcus luteus* [21, 29, 30]. The absolute configuration of the *cis*-isomer has yet to be determined.

The changing legal landscape involving the sale and consumption of *Cannabis* has led to renewed interest in this class of natural products [31]. Accordingly, there is a need for a contribution that summarizes current approaches to cannabinoid-derived natural products. This compilation will highlight several asymmetric approaches that have been implemented in the synthesis of tetrahydrocannabinol and related natural products. Earlier approaches towards the synthesis of cannabinoids have been extensively reviewed by Mechoulam [32, 33], Razdan [34], and Tius [35], and selected examples from these will only be briefly discussed.

2 Early Approaches

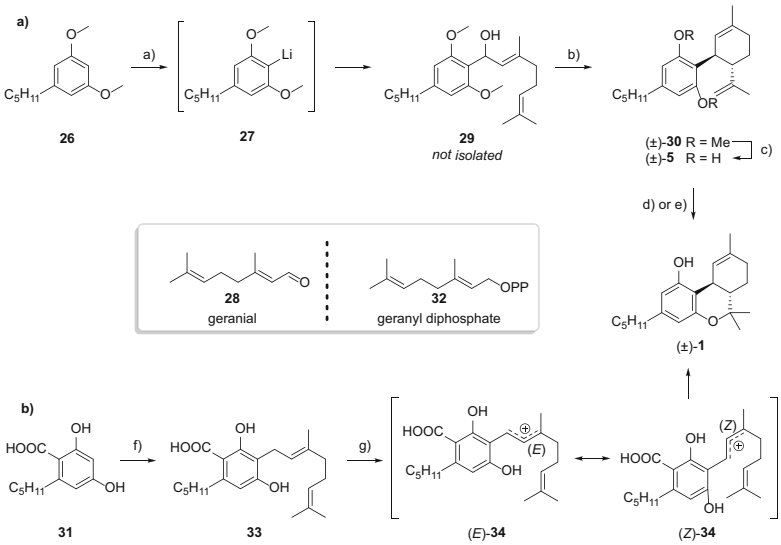
In the 1940s, Roger Adams published a series of structure elucidation studies, along with attempts towards understanding the structure-activity relationship of cannabinoids [8]. In the course of these studies, Adams found that the non-psychoactive cannabidiol (**5**) isomerized upon treatment with acids to a viscous oil with high physiological activity [36–38]. After careful analysis it was reported that "mild conditions" convert **5** into a tetrahydrocannabinol **24** with low optical



Scheme 3 Conversion of cannabidiol (**5**) under "mild" and "harsh" conditions to tetrahydrocannabinols **24** and **25** with low- and high-optical rotation as reported by Roger Adams and co-workers. Note: the double bond is shown as assigned by Adams and co-workers (*left*). Structures and reported optical activities of (−)-Δ^9-*trans*-tetrahydrocannabinol (**1**) [10] and of (−)-Δ^8-*trans*-tetrahydrocannabinol (**2**) (*right*) [11]

rotation, while "harsher more vigorous conditions" lead to a tetrahydrocannabinol **25** with high optical rotation (Scheme 3a). The formation of two different products was explained by invoking the isomerization of the alicyclic double bond under harsher conditions. However, with the limited analytical techniques available at the time for structure elucidation, the position of the double bond remained uncertain and was subsequently misassigned by the authors. Even though the exact nature of **24** and **25** remains unknown, Mechoulam and co-workers re-evaluated the conversion of cannabidiol (**5**) under these conditions and found that (−)-*trans*-Δ^9-tetrahydrocannabinol (**1**) and (−)-*trans*-Δ^8-tetrahydrocannabinol (**2**) are indeed formed, along with other double-bond isomers [10, 11]. These studies demonstrate some of the difficulties that were encountered during the early investigations of cannabinoids, namely, the instability of the major active constituent, and, consequently, the difficulties associated with obtaining pure material.

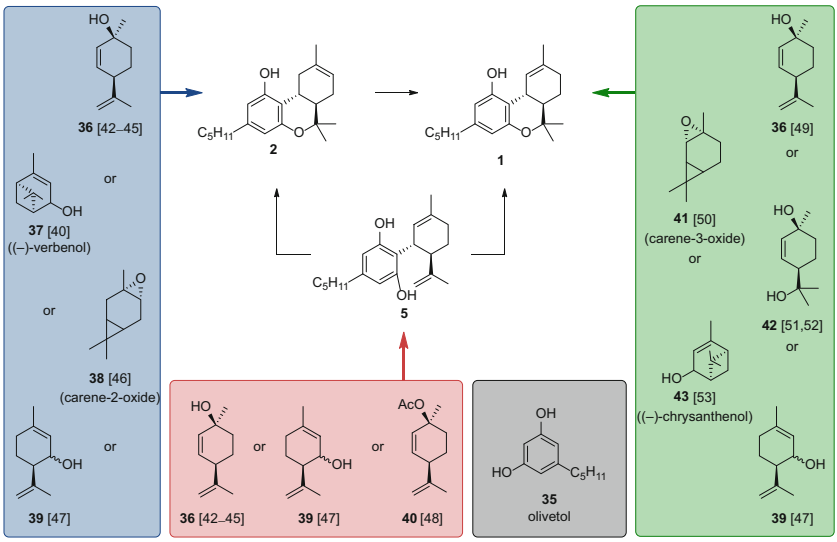
The first synthesis of (±)-Δ^9-*trans*-THC, published by Mechoulam and co-workers, is not only of historical interest, but can be considered the first biomimetic total synthesis of tetrahydrocannabinol (Scheme 4) [40, 41]. *ortho*-Lithiation of olivetol dimethyl ether **26**, followed by addition of the lithiated species **27** to geranial **28** afforded a complex mixture containing allylic alcohol intermediate **29**. As isolation and purification of this material was not successful, the unpurified product was directly treated with tosyl chloride in pyridine to afford cannabidiol dimethyl ether **30** in 7% overall yield. Demethylation of **30** with neat MeMgI at 160°C afforded (±)-cannabidiol (**5**), which was converted under acidic conditions to give (±)-*trans*-THC (**1**) in up to 70% yield.



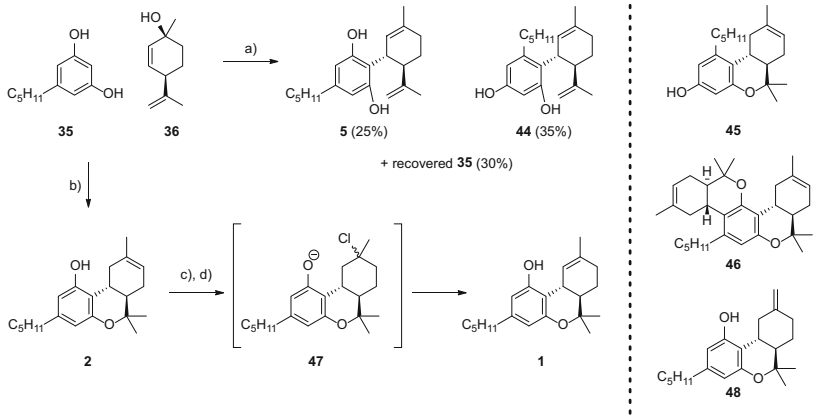
Scheme 4 First synthesis of racemic *trans*-Δ^9-tetrahydrocannabinol by Mechoulam and co-workers (*top*). Reagents and conditions: (a) 1.0 equiv *n*-BuLi, diethyl ether, rt, 2 h; then 1.0 equiv **28**, diethyl ether, rt; (b) 3.5 equiv *p*-TsCl, pyridine, rt, 3 d, 7% over two steps; (c) excess MeMgI (neat), 155–165°C, 15 min, 80%; (d) 0.05% (*v*/*v*) HCl in ethanol, reflux, 2 h, 2%; (e) 0.5 equiv $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 30 min, 70%; (f) biosynthesis of (−)-*trans*-Δ^9-tetrahydrocannabinol (*bottom*). Reagents and conditions: (g) geranyl diphosphate **32**, olivetol geranyltransferase; (h) FAD, tetrahydrocannabinolisc acid synthase (THCA) [39]

3 Stereospecific Approaches

The most common strategy for the synthesis of enantiomerically enriched cannabinoids is based on the condensation of olivetol (**35**) with an optically pure monoterpene [32–35]. A compilation of different monoterpenes that have been employed is shown in Scheme 5. The coupling strategy has been applied in one of the first stereospecific syntheses of (−)-Δ^9-cannabidiol (**5**) and (−)-Δ^9-*trans*-tetrahydrocannabinol (**1**) by Petrzilka and Eschenmoser (Scheme 6) [42–45]. Condensation of olivetol (**35**) with readily available (+)-*cis*/*trans*-*p*-mentha-2,8-dien-1-ol (**36**) was achieved by addition of catalytic amounts of a formamide acetal to give (−)-cannabidiol (**5**) in 25% yield, along with a regioisomeric side product called abnormal cannabidiol (**44**). When strong acids (e.g. *p*-TsOH, HCl, TFA) were used instead of the acetal no cannabidiol was isolated, and instead (−)-Δ^8-*trans*-tetrahydrocannabinol (**2**) was obtained in 53% yield along with abnormal tetrahydrocannabinol (**45**) (13% yield), the bis-adduct **46** (5% yield), and re-isolated starting material. Zinc(II) chloride-catalyzed addition of gaseous HCl to the olefin in **2**, followed by elimination using potassium *tert*-amylate and resulted in formation of **1** as a single product in excellent yield [54]. The excellent regioselectivity observed in the elimination reaction was explained by a mechanism, which involves generation



Scheme 5 Overview of different monoterpenes **36**–**43** that have been used for the synthesis of cannabinoids by condensation or coupling with olivetol (**35**) and olivetol derivatives

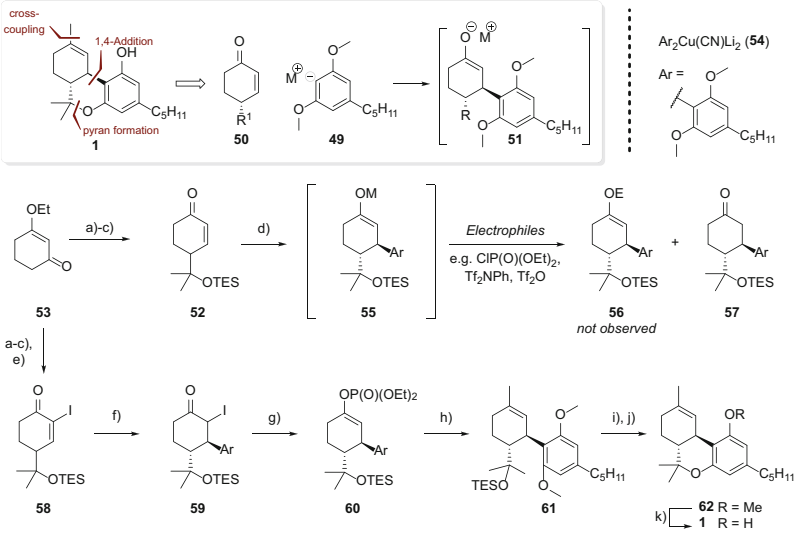


Scheme 6 Total synthesis of cannabidiol and (−)-Δ^9-*trans*-tetrahydrocannabinol (**1**) by Petrzilka and co-workers. Reagents and conditions: (a) 1.3 equiv *N*,*N*-dimethyl formamide-dineopentylacetal, CH_2Cl_2, rt, 63 h, **5** (25%), **34** (35%), and **32** (30%); (b) 0.15 equiv. *p*-TsOH, C_6H_6, reflux, 2 h, **2** (53%), **36** (13%), **37** (5%); (c) 0.67 equiv $ZnCl_2$, HCl, CH_2Cl_2, 0°C to rt, 15 h, quantitative; (d) 3.2 equiv. K-*t*-amylate, C_6H_6, 5–65°C, 15 min, quantitative

of the phenolate anion **47** that serves as an internal base. This route was studied in great detail by Razdan in course of a research program of the National Institutes of Health, who optimized the synthesis for preparation of kilogram-quantities of **1** and **2** [34]. They found that the final elimination step was extremely sensitive to

reaction conditions and was always accompanied by formation of about 5% of an isomeric product **48**, which could be isolated by chromatography on silver impregnated-silica gel [55]. Use of a different promoter ($BF_3{\cdot}OEt_2$) under water-free conditions (CH_2Cl_2, $MgSO_4$), prevented the formation of the double bond isomer and accordingly **1** was directly obtained from the condensation of **36** with olivetol (**35**) in 31–50% yield.

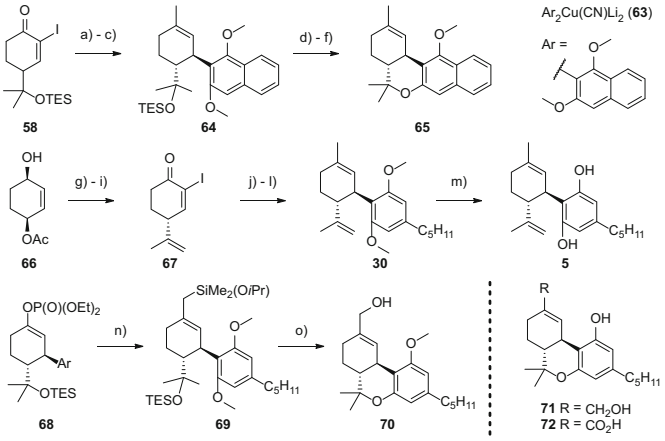
A useful strategy to prevent the formation of abnormal tetrahydrocannabinol and other side products has been developed by Kobayashi and co-workers based on 1,4-addition of olivetol derivative **49** to the cyclic enone **50** (Scheme 7) [56, 57]. The resulting enolate **51** was trapped with ($ClP(O)(OEt)_2$), and a subsequent cross coupling/methylation and cyclization resulted in the formation of **1**. Initial investigations were carried out on the racemic enone **52**, which was obtained from **53** in three steps. 1,4-Addition of the bulky cuprate **54** required addition of $BF_3{\cdot}OEt_2$ as no 1,4-addition product was isolated in the absence of a Lewis acid. Unfortunately, intermediate **55** proved to be unreactive towards reaction with various electrophiles leading to isolation of ketone **57**. Attempts to enhance the reactivity of boron enolate **55** by transmetallation with *n*-BuLi were ineffective, as was the TMSCl-assisted 1,4-addition of the cuprate to the enone. To circumvent these problems, an



Scheme 7 Total synthesis of (−)-Δ^9-*trans*-tetrahydrocannabinol (**1**) by Kobayashi and co-workers. Reagents and conditions: (a) 1.5 equiv LDA, THF, −78°C, 2 h; then 2.0 equiv acetone, −78°C, 30 min, 99%; (b) 1.3 equiv TESCl, 1.6 equiv imidazole, DMF, rt, overnight, 99%; (c) 2.0 equiv DIBAL, THF, −78°C, 1 h, then 2 *N* HCl, 45 min, rt, 72%; (d) 1.5 equiv **54**, 1.0 equiv $BF_3{\cdot}OEt_2$, Et_2O, −78°C, 2 h, 48 (90%); (e) 3.0 equiv I_2, CCl_4, pyridine, 0°C to rt, overnight, 89%; (f) 1.5 equiv **54**, 1.0 equiv $BF_3{\cdot}OEt_2$, Et_2O, −78°C, 2 h, 67%; (g) 1.5 equiv EtMgBr, THF, 0°C, 10 min; then 2.5 equiv $ClP(O)(OEt)_2$, 0°C, 2 h, 70%; (h) 10 mol% $Ni(acac)_2$, 2.6 equiv MeMgCl, THF, 0°C to rt, overnight, 90%; (i) 12.0 equiv NaSEt, DMF, 120°C, 12 h, 73%; (j) 2.0 equiv $ZnBr_2$, $MgSO_4$, CH_2Cl_2, rt, 12 h, 84%; (k) 8.0 equiv NaSEt, DMF, 120°C, 12 h, 45%

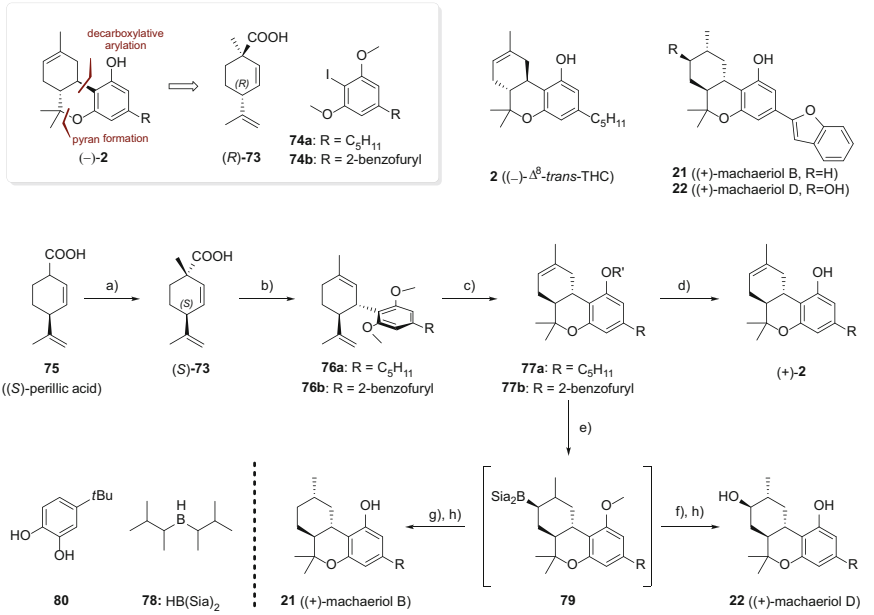
indirect approach was envisioned using the α-iodocyclohexenone **58**. Following successful 1,4-addition, the resulting α-iodoketone **59** was treated with EtMgBr to generate a reactive enolate, which was trapped by addition of $ClP(O)(OEt)_2$ to give enol phosphate **60** in 70% yield. Nickel-catalyzed methylation afforded **61**, cleavage of one methyl ether and the silane protecting group was achieved upon exposure to excess NaSEt in refluxing DMF. The resulting intermediate was then cyclized by the procedure published by Evans ($ZnBr_2$, $MgSO_4$). Final deprotection (NaSEt, DMF, reflux) afforded **1** in 45% yield. Synthesis of optically active Δ^9-tetrahydrocannabinol was achieved by employing enantiomerically enriched enone (*R*)-**52** (which was synthesized from (+)-β-pinene in seven steps) following the same route. It is noteworthy that the strategy allows the introduction of modifications at several positions (Scheme 8). Employing a different cuprate (**63**) in the 1,4-addition, naphthalene analogue **65** was synthesized, and changing the substituent at the γ-position of cyclic enone **66**, enabled the preparation of cannabidiol (**5**) [58], and Ni-catalyzed cross coupling with $ClMgCH_2SiMe_2(OiPr)$ as a CH_2OH-equivalent afforded **70**, a possible precursor for the synthesis of THC metabolites **71** and **72**.

Recently, Studer reported an elegant, divergent synthesis of (+)-Δ^8-*trans*-tetrahydrocannabinol ((+)-**2**) and the structurally related natural products (+)-macheriols B and D (**21** and **22**) using a stereospecific Pd-catalyzed decarboxylative arylation



Scheme 8 Synthesis of cannabidiol (**5**) and derivatives **71** and **72**. Reagents and conditions: (a) 1.5 equiv **63**, 1.0 equiv $BF_3{\cdot}OEt_2$, Et_2O −78°C, 2 h, 74%; (b) 1.5 equiv EtMgBr, THF, 0°C, 10 min; then 2.5 equiv $ClP(O)(OEt)_2$, 0°C, 2 h, 60%; (c) 10 mol% $Ni(acac)_2$, 2.6 equiv MeMgCl, THF, 0°C to rt, overnight, 99%; (d) 8.0 equiv NaSEt, DMF, 120°C, 12 h, 62%; (e) 1.2 equiv TBAF, THF, 0°C to rt, 12 h, 78%; (f) 1.5 equiv $ZnBr_2$, $MgSO_4$, CH_2Cl_2, rt, 12 h, 91%; (g) 3.5 equiv isopropenylzinc chloride, 4.2 equiv TMEDA, 10 mol% $NiCl_2(tpp)_2$, THF, 0°C to rt, 20 min, then 1.0 equiv **66**, rt, overnight, 80%; (h) 1.1 equiv Jones reagent, acetone, 0°C, 15 min; (i) 3.0 equiv I_2, 3 mol% 2,5-di-*tert*-butyl-hydroquinone, pyridine, rt, 2 h, 76% over two steps; (j) 1.5 equiv **54**, 1.0 equiv $BF_3{\cdot}OEt_2$, Et_2O, −78°C, 2 h; (k) 1.26 equiv EtMgBr, THF, 0°C, 10 min; then 2.1 equiv $ClP(O)(OEt)_2$, 0°C, 2 h, 51% over two steps; (l) 20 mol% $Ni(acac)_2$, 2.4 equiv MeMgCl, THF, 0°C to rt, overnight, 84%; (m) excess MeMgI, neat, 155–165°C, 15 min, 80%

of cyclohexenyl carboxylic acid **73** and aryl iodide **74a** or **74b** as the key step [59, 60] (Scheme 9). α-Selective alkylation of (*S*)-perillic acid (**75**) with excess LDA in THF/DMPU, followed by addition of dimethyl sulfate afforded carboxylic acid (*S*)-**73** as a mixture of *anti*/*syn* products in 90% yield. Separation of the isomers was not required, as only the *anti*-isomer reacted during the Pd-catalyzed decarboxylative arylation and resulted in the formation of **76a** in 73% yield as the sole isomer. The synthesis continued with construction of the pyran ring, by employing in situ-generated TMSI as a reagent for the selective mono-demethylation and the subsequent oxocyclization. Over the course of this transformation, the alicyclic double bond isomerized to the thermodynamically more stable Δ^8-position. Finally, deprotection of **77a** under standard conditions resulted in the formation of Δ^8- tetrahydrocannabinol ((+)–**2**). Synthesis of (+)-machaeriols B and D was possible, employing the same steps, using the benzofuryl derivative **74b** in the Pd-catalzyed arylation reaction. Stereoselective hydroboration of **77b** with disiamylborane **78** from the sterically less hindered face, followed by oxidative work-up of the boron intermediate **79** and subsequent deprotection resulted in

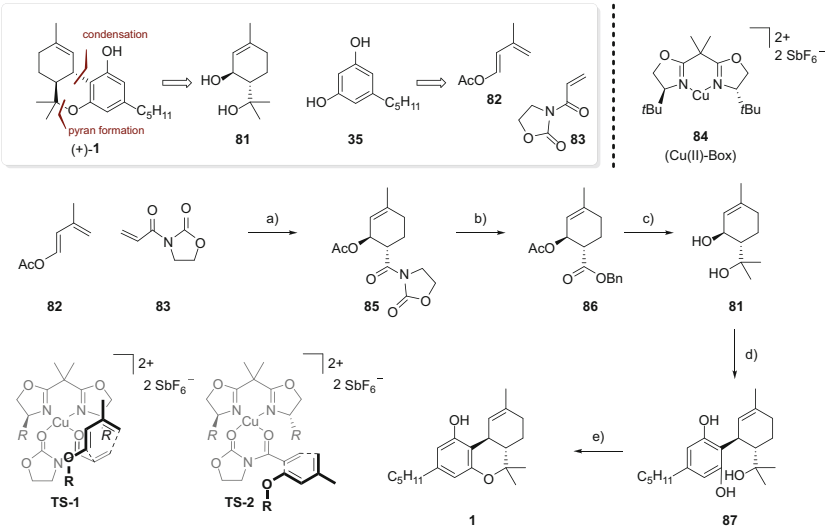


Scheme 9 Total synthesis of (+)-Δ^8-*trans*-tetrahydrocannabinol ((+)-**2**) and machaeriols B and D (**21** and **22**) by Studer and co-workers. Reagents and conditions: (a) 2.5 equiv LDA, THF, −78°C to rt, 2 h; then 10 equiv DMPU, rt, 15 min; then 1.3 equiv dimethyl sulfate, −78°C to rt, 1 h, 90% (*anti*:*syn* 1.7:1); (b) 1.0 equiv **74a** or **74b**, 1.1 equiv Cs_2CO_3, 10 mol% $Pd(dba)_2$ toluene, reflux, 26 h, 74% for **76a**, 81%, for **76b**; (c) 2.0 equiv NaI, 1.5 equiv TMSCl, acetonitrile, reflux, 24 h; then 0.2 equiv NaI, reflux, 24 h; (d) 10 equiv NaSEt, DMF, 140°C, 12 h, 64% over two steps; (e) 4.0 equiv **78**, DCE, 60°C, 2d; then (f) 4.9 equiv H_2O, 6.6 equiv NaOH, 22 equiv H_2O_2, 0°C to rt, 2 h, 51% over two steps or (g) 10 equiv **80**, reflux, 4 h; (h) 10 equiv NaSEt, DMF, 140°C, 12 h, for **21** 39% over 3 steps, for **22** 82%

formation of (+)-machaeriol D (**21**). The same boron intermediate, **79**, was converted to (+)-machaeriol B (**22**) by radical reduction of the carbon-boron bond with 4-*tert*-butylcatechol (**80**) and deprotection of the methoxy group. This reduction procedure gave improved diastereoselectivity when compared to hydrogenation with heterogeneous catalysts (e.g. Pd/C, 3:1 *dr*) or the use of non-chiral and chiral homogeneous catalysts, which favored formation of the undesired diastereoisomer.

4 Enantioselective Approaches

Evans and co-workers completed the first asymmetric synthesis of (+)-Δ^9-*trans*-tetrahydrocannabinol ((+)-**1**) from achiral starting materials based on a strategy that involved the formation of the optically active key intermediate **81** through an enantioselective Diels-Alder reaction (Scheme 10) [61, 62]. Thus, Cu(II)-BOX **84** catalyzed reaction of diene **82** with imide **83** at low temperature and afforded cycloadduct **85** in 57% yield after recrystallization, with a good *trans*/*cis* ratio and excellent enantioselectivity. The preference for the exo-product was suggested to involve **TS-2**, which avoids an unfavorable steric interaction between the methyl and the ligand as illustrated for **TS-1**. The product formed by transesterification of



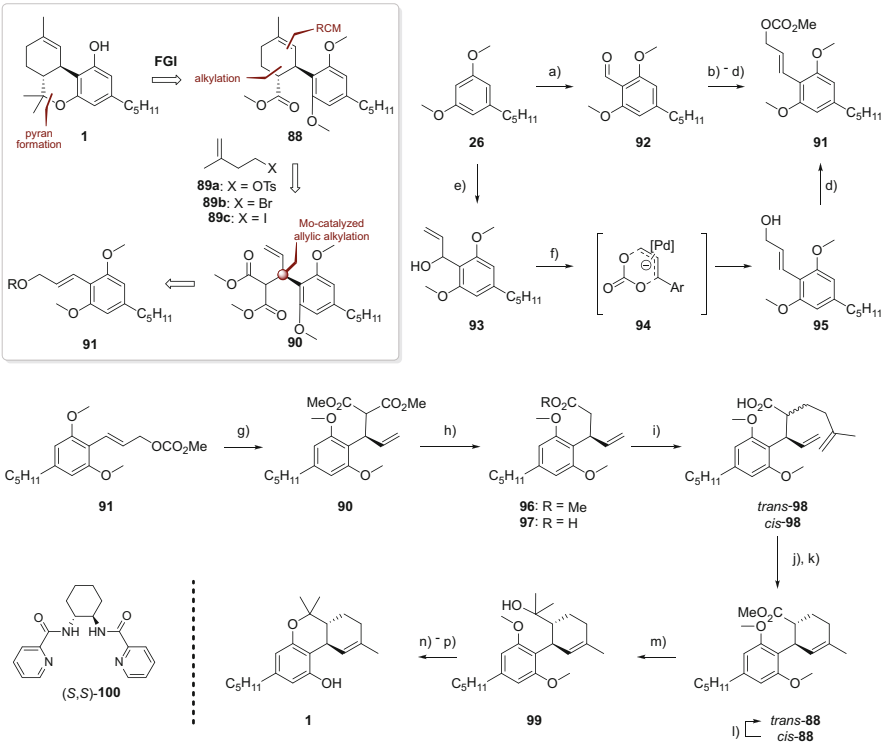
Scheme 10 First enantioselective synthesis of the (+)-Δ^9-*trans*-tetrahydrocannabinol ((+)-**1**) as reported by Evans and co-workers. Reagents and conditions: (a) 3.0 equiv **83**, 2 mol% Cu(II)-BOX, CH_2Cl_2, −20°C, 18 h, 57% (*trans*:*cis* 73:27, 98% *ee*); (b) 2.5 equiv benzyl alcohol, 2.0 equiv *n*-BuLi, THF, −78°C, 10 min, then: 1.0 equiv **85**, −20°C, 3.5 h, 82%; (c) 6.0 equiv MeMgBr, diethyl ether, 0°C, 2.5 h, 80%; (d) 1.0 equiv olivetol **35**, 0.5 equiv *p*-TsOH, CH_2H_2, 0°C, 7 h, 79%; (e) 1.0 equiv $ZnBr_2$, $MgSO_4$, CH_2Cl_2, rt, 5 h, 72%

the imide and acetate groups was difficult to separate from excess benzyl alcohol, and, accordingly, the selective transesterification of the imide under milder conditions afforded the ester **86**. Concurrent deprotection and formation of the tertiary alcohol was achieved by subjecting this compound to excess methyl magnesium bromide. The acid-catalyzed condensation of the diol **81** with olivetol (**35**) was achieved at high dilution to afford the cyclization precursor **87** in good yield. Interestingly enough, formation of abnormal derivatives or bis-adducts was not observed during this reaction (cf. **45** or **46** in Scheme 6), which was explained as arising from increased steric interaction between the amyl group and the tertiary alcohol. The final transformation was achieved by a previously reported $ZnBr_2$-promoted cyclization [52], which was modified by addition of $MgSO_4$ to prevent the formation of isomeric cannabinol derivatives.

In contrast to the previously presented approaches, which rely on the condensation (or addition) of olivetol (or olivetol derivatives) to chiral monoterpenes, Trost and co-workers planned for the construction of the cyclohexene ring in the cyclization precursor **88** by implementation of a ring closing metathesis reaction [63]. This approach would place the double bond in the desired Δ^9-position and hence provide a definitive solution to the often observed complications resulting from isomerization to the thermodynamically more favored isomer (Scheme 11).

The synthesis commenced with the preparation of the allylic carbonate **91** by a four-step sequence: starting with formylation of olivetol dimethyl ether (**26**), followed by Horner-Wadsworth-Emmond reaction, DIBAL reduction and carbonate formation. Alternatively, the branched allylic alcohol **93** was prepared and isomerized in the presence of a palladium catalyst to the more stable linear allylic alcohol **95**, after treatment with *n*-BuLi and dry CO_2 gas.

With allylic carbonate **91** in hand, the crucial allylic substitution was examined. Reaction of the sterically demanding carbonate **91** with sodium dimethyl malonate in the presence of 5 mol% $[Mo(CO)_3C_7H_8]$ and 7.5 mol% of chiral ligand (*S*,*S*)-**100** resulted in formation of the branched product **90** in high yield and enantioselectivity (95% yield, 94% *ee*). Alkylation of the malonate **90** with **89** (X = Br, I, OTs) was unsuccessful due to steric congestion around the malonate carbon and the reaction was accompanied by slow consumption of **89** probably due to 1,2-elimination to give isoprene. Hence, to reduce the steric demand, the monoester **96** was prepared under Krapcho decarboxylation conditions (83% yield). However, alkylation of this ester at low temperatures with **89a** (X = OTf) was sluggish and the alkylated product was isolated in 50% yield. The authors envisioned that a more stable enolate was required to perform the alkylation at room temperature or higher. Hence, the enolate derived from acid **97** was alkylated with iodide **89c** and the adduct **98** was isolated in 84% yield, as a 2.4:1 mixture of *anti*- and *syn*-isomers. Each isomer was converted to the corresponding methyl ester and subjected to ring closing metathesis reaction conditions to result in *anti*- and *syn*-cyclohexene **88** (*anti*-**88**: 94% yield, *syn*-**88**: 81% yield). The *syn*-product **88** was equilibrated in 91% yield to the thermodynamically more stable *trans*-**88** by treatment with excess sodium methoxide at elevated temperature for 3 days. Addition of methyllithium resulted in formation of the tertiary alcohol **99** (92% yield).

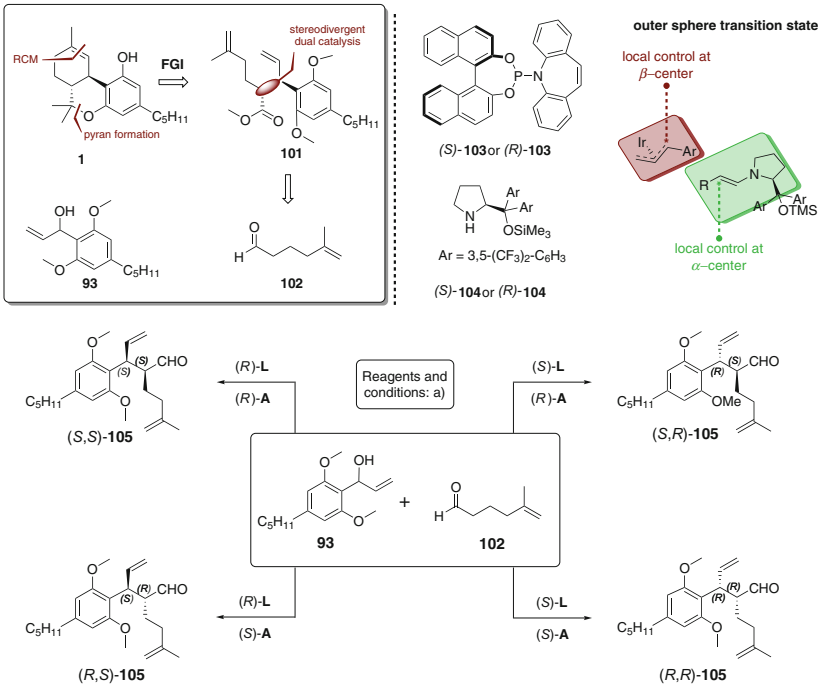


Scheme 11 Asymmetric synthesis of **1** as reported by Trost and co-workers. Reagents and conditions: (a) 1.1 equiv *n*-BuLi, THF, reflux, 2.5 h; then 1.1 equiv dimethyl formamide, rt to reflux, 3 h, 83%; (b) 1.1 equiv NaH, 1.1 equiv triethylphosphonoacetate, THF, −35°C to rt, 1 h; (c) 2.5 equiv DIBAL, diethyl ether, −10°C to 0°C, 30 min, 97%; (d) 1.1 equiv *n*-BuLi, 2.0 equiv methyl chloroformate, diethyl ether, −78°C, 10 min, quantitative; (e) 1.1 equiv *n*-BuLi, THF, −78°C to rt, 2 h, 91%; (f) *n*-BuLi, CO_2, cat. $PdCl_2(CH_3CN)_2$; (g) 1.2 equiv dimethyl malonate, 1.2 equiv NaH, 10 mol% $Mo(CO)_3(C_7H_8)$, 15 mol% (*S*,*S*)-**100**, THF, 75°C, 54 h, 84% (97% *ee*); (h) 1 N aq. NaOH, MeOH, reflux, 6 h; then 160°C, 1 h, 98%; (i) 2.4 equiv LDA, THF, −20°C to 40°C, 30 min; then 1.5 equiv **89c**, −10°C to rt, 3 h, 84% (*trans*/*cis* 2.4:1); (j) 3.0 equiv K_2CO_3, 3.0 equiv dimethyl sulfate, acetone, reflux, 3 h; (k) 1 mol% Grubbs second generation catalyst, CH_2Cl_2, rt, overnight; then 0.5 mol% Grubbs second generation catalyst, CH_2Cl_2, rt, 16 h, 93%; (l) 1 *M* NaOMe, MeOH, reflux, 3 d, 92% (*trans*/*cis* 1:0.03); (m) 3.0 equiv MeLi, diethyl ether, −78°C to rt, 92%; (n) 3.0 equiv NaSEt, DMF, 140°C, 3 h, 97%; (o) 2.0 equiv $ZnBr_2$, $MgSO_4$, CH_2Cl_2, rt, overnight; (p) 10.0 equiv NaSEt, DMF, 140°C, 10 h, 61% over two steps

Concurrent demethylation and pyran ring formation with BBr_3 and other acidic reagents resulted in the formation of complex mixtures of different products. Hence, a stepwise approach was applied. Mono-demethylation with NaSEt in refluxing DMF (97% yield), followed by pyran ring formation under the known conditions of Evans ($ZnBr_2$, $MgSO_4$) and final deprotection of the crude product with sodium thioethanolate in refluxing DMF gave (−)-Δ^9-*trans*-tetrahydrocannabinol (**1**) (61% yield over two steps).

Recently, Carreira and co-workers reported a synthesis of all four stereoisomers of Δ^9-tetrahydrocannabinol relying on a fully stereodivergent dual catalysis strategy [64–66]. They used two chiral catalysts, each of which independently controls one of the two stereocenters, as a general approach to selectively access all diastereoisomers of the chiral benzochromene motif **15** (Scheme 12). Thereby, this synthesis approach is one of the few syntheses that provide access to enantiomerically pure Δ^9-*cis*-tetrahydrocannabinol, which in contrast to the *trans*-isomers is not as well studied.

Stereoselective dual catalytic α-allylation of aldehyde **102** with branched allylic alcohol **93**, using 3 mol% [{Ir(cod)Cl_2}], 12 mol% phosphoramidite ligand (*S*)-**103** or (*R*)-**103**, 15 mol% Hayashi–Jørgensen amine (*S*)-**104** or (*R*)-**104** and 5 mol% $Zn(OTf)_2$ as a promoter, provided all stereoisomers of the adduct **105** in good yield and with excellent selectivities. The use of zinc triflate as a promoter proved to be crucial to prevent degradation of the electron-rich allylic alcohol and epimerization at the C-α center of the product aldehyde. With the key intermediates in hand, the syntheses of the four stereoisomers were finished using a uniform synthetic

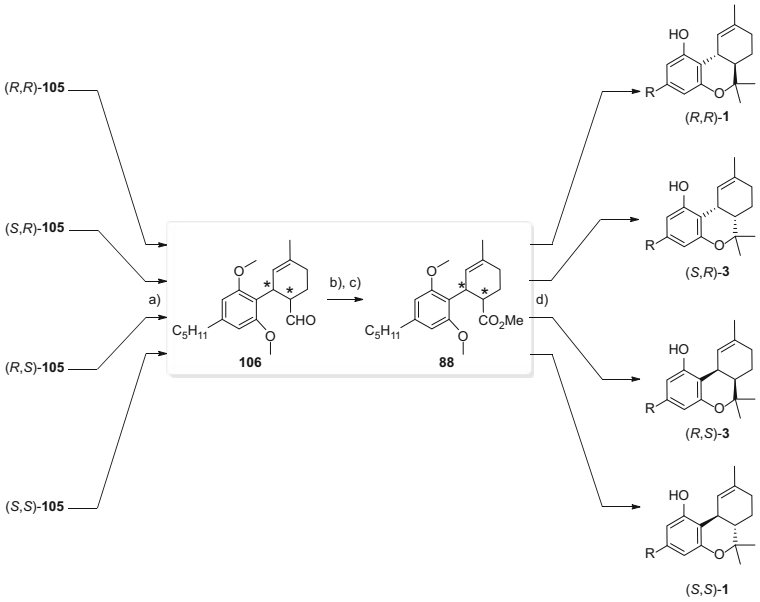


Scheme 12 Stereodivergent total synthesis of Δ^9-*trans*-tetrahydrocannabinol ((+)-**1**) and ((−)-**1**) and Δ^9-*cis*-tetrahydrocannabinol ((+)-**3**) and ((−)-**3**) as reported by Carreira and co-workers. Reagents and conditions: (a) 1.0 equiv **93**, 3.0 equiv **102**, 3 mol% [{Ir(cod)Cl}$_2$], 12 mol% (*S*)-**103** or (*R*)-**103**, 15 mol% (*S*)-**104** or (*R*)-**104**, 5 mol% $Zn(OTf)_2$, DCE, rt, 20 h, (*S*,*S*)-**105** 60% (15:1 *dr*, >98% *ee*), (*R*,*S*)-**105** 55% (20:1 *dr*, >98% *ee*), (*S*,*R*)-**105** 54% (20:1 *dr*, >98% *ee*), (*R*,*R*)-**105** 62% (15:1 *dr*, >98% *ee*)

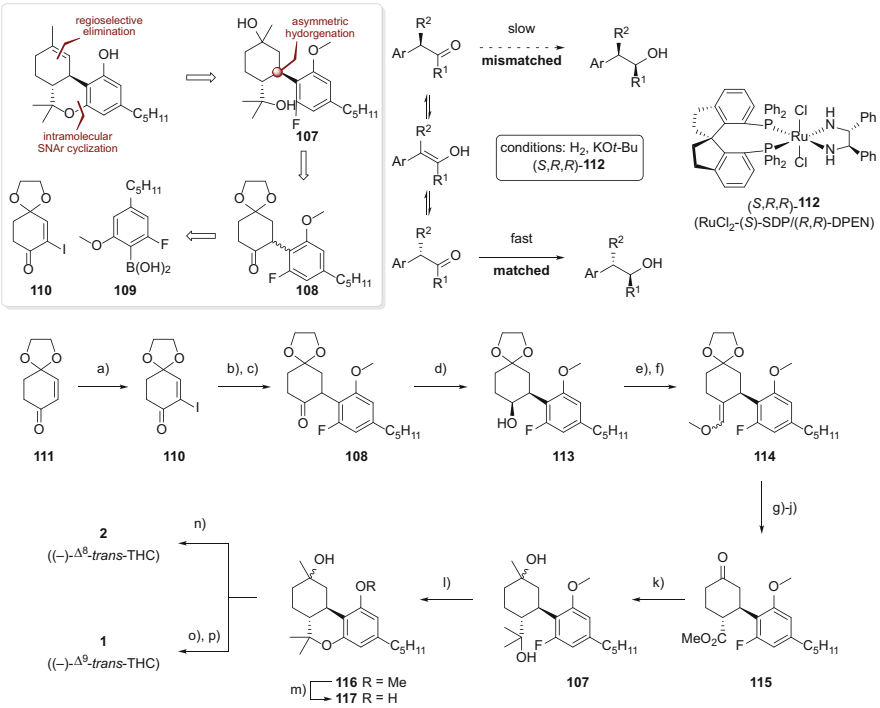
sequence (Scheme 13). Accordingly, ring closing metathesis in the presence of Grubbs second generation catalyst, followed by oxidation of the aldehydes under Pinnick conditions and subsequent addition of trimethylsilyl diazomethane resulted in formation of the corresponding methyl esters **88**. From these intermediates, the desired products were obtained by a three step one-pot sequence: addition of excess MeMgI (0–160°C under reduced pressure) led to the formation of a tertiary alcohol, with subsequent demethylation of both methoxy ethers upon heating, then addition of $ZnBr_2$ and $MgSO_4$ to the organic phase (CH_2Cl_2) after aqueous work up furnished the desired Δ^9-tetrahydrocannabinol stereoisomers (41–65% yield).

Zhou and co-workers reported an enantioselective synthesis of Δ^9- and Δ^8-tetrahydrocannabinol using an asymmetric hydrogenation of ketone **108** via dynamic kinetic resolution and an intermolecular S_NAr etherification as key steps (Scheme 14) [67, 68].

The synthesis commenced with preparation of the racemic ketone **108** in three steps from commercially available **111**, which was then subjected to hydrogenation conditions (50 atm H_2, 0.05 M KO^{t}Bu in ^{i}PrOH) in the presence of the ruthenium catalyst (*S*,*R*,*R*)-**112** to obtain the chiral alcohol **113** in excellent yield and selectivity. The alcohol was then oxidized under Swern conditions to give the



Scheme 13 Adapted from Ref. [42]. Reagents and conditions: (a) 3 mol% Grubbs second generation catalyst, CH_2Cl_2, rt, 18 h, 92% for (*S*,*S*)-**106**, 87% for (*R*,*S*)-**106**, 90% for (*S*,*R*)-**106**, 85% for (*R*,*R*)-**106**; (b) 2.3 equiv $NaClO_2$, 2.0 equiv NaH_2PO_4, 30 equiv 2-methyl-2-butene, *t*-BuOH/H_2O, rt, 3 h; (c) 1.1 equiv Me_3SiCHN_2, C_6H_6/MeOH, 0°C, 45 min, 66% for (*S*,*S*)-**88**, 60% for (*R*,*S*)-**88**, 61% for (*S*,*R*)-**88**, 65% for (*R*,*R*)-**88** over two steps; (d) 10 equiv MeMgI, diethyl ether, 0°C to 160°C, ambient pressure to 150 mmHg; then addition of $ZnBr_2$ and $MgSO_4$ upon workup in CH_2Cl_2, rt, 4 h, 57% for (*S*,*S*)-**1**, 41% for (*R*,*S*)-**3**, 45% for (*S*,*R*)-**3**, 65% for (*R*,*R*)-**1**



Scheme 14 Total synthesis of **1** and **2** by Zhou and co-workers. Reagents and conditions: (a) 1.2 equiv K_2CO_3, 1.5 equiv. I_2, 0.2 equiv DMAP, THF/H_2O, rt, 2 h, 84%; (b) 1.5 equiv **110**, 3.0 equiv LiCl, 2.8 equiv Na_2CO_3, 1.25 mol% 1,2-dimethoxyethane, 80°C, 24 h, 93%; (c) 1 atm H_2, 0.1 equiv 5% Pd/C, ethanol, 12 h, 90%; (d) 50 atm H_2, 0.17 equiv KOtBu, 0.1 mol% (*S*,*R*,*R*)-**112**, iPrOH, rt, 12 h, 98% (96% *ee*, *cis*/*trans* >99:1); (e) 1.2 equiv oxalyl chloride, 2.4 equiv DMSO, CH_2Cl_2, −78°C, 15 min; then 2.4 equiv trimethylamine, −78°C to −10°C over 2 h, 95%; (f) 2.0 equiv (methoxymethyl)triphenylphosphonium chloride, 2.0 equiv *n*BuLi, THF, 0°C to rt, 2 h; 90%; (g) AcOH, H_2O, reflux, 2 h; (h) 1.2 equiv CrO_3, H_2SO_4, H_2O, acetone, 5°C to rt, 2 h; (i) 2.0 equiv K_2CO_3, 2.0 equiv MeI, DMF, rt, 12 h; 76% (*cis*/*trans* 1.4:1) over 3 steps; (j) 4.2 equiv NaOMe, MeOH, rt, 24 h, 100% (*cis*/*trans* 1:31); (k) 10 equiv MeMgBr, THF/diethyl ether, 0°C to reflux, 3 h, 61% (*trans*-**107**) and 30% (*cis*-**107**); (l) 5.0 equiv NaH, DMF, reflux, 1 h, 94% (*trans*- and *cis*-diol); (m) 10 equiv NaH, 10 equiv $Et_2NCH_2CH_2SH$, DMF, 130–140°C, 6 h, 95% (*trans*-diol) and 97% (*cis*-diol); or one-pot (l) and (m) 10 equiv NaH, 5 equiv $Et_2NCH_2CH_2SH$, DMF, 130–140°C, 6 h, 90% (*trans*- and *cis*-diol); (n) 20 mol% *p*-TsOH, C_6H_6, reflux, 4 h, 96%; (o) 25 equiv $ZnCl_2$, aq. HCl, AcOH, rt, 3 h; (p) 5.3 equiv KOt-Bu, C_6H_6, −5°C to 65°C, 15 min, 80%

enantiomerically enriched ketone (*S*)-**108**. Olefination with Wittig reagent furnished the vinyl ether **114**, which was converted to the ester **115** in a four-step sequence involving: acid-promoted rearrangement/isomerization of the vinyl ether to the corresponding aldehyde with concurrent deprotection of the ketal, oxidation of the aldehyde to the carboxylic acid under Jones conditions, esterification with MeI/K_2CO_3, and base-catalyzed epimerization to the thermodynamically more stable *trans*-isomer of **115**. The keto ester was then treated with excess MeMgBr in refluxing THF to provide the diol **107**. Subjecting this diol to basic conditions

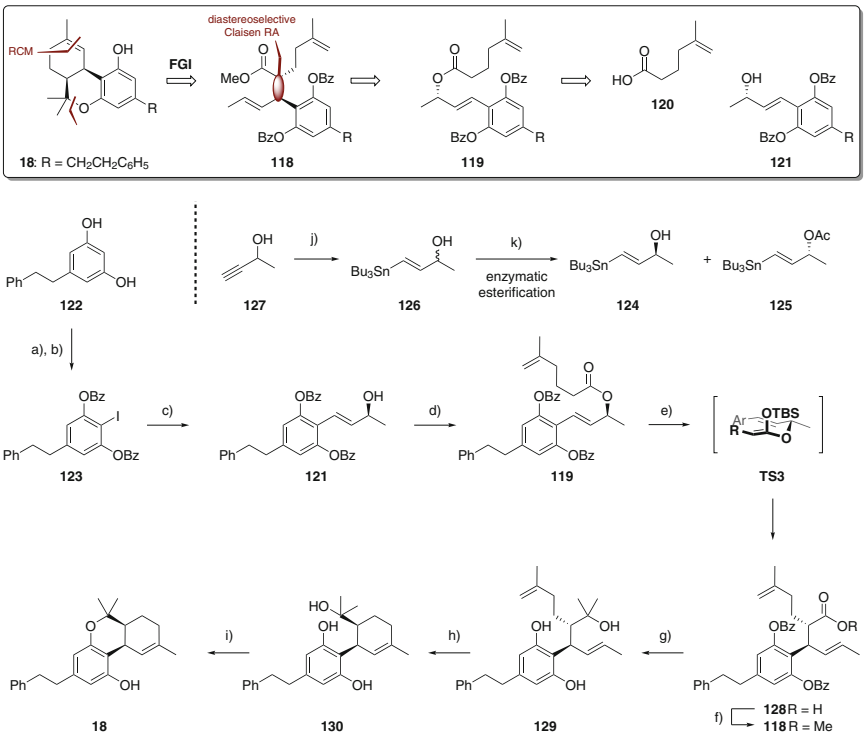
(NaH, DMF, 150°C) promoted the intermolecular S_NAr reaction to form **116**, which was subsequently deprotected with in situ-generated $NaS(CH_2)_2NEt_2$. As the S_NAr cyclization and the demethylation reaction both required similar reaction conditions (strong base, high temperatures) the authors attempted a two-step one pot reaction sequence. Accordingly, when diol **107** was subjected to 10.0 equiv. NaH and 5.0 equiv $HS(CH_2)_2NEt_2$ in refluxing DMF, phenol **117** was isolated in 90% yield. From this common intermediate, (−)-Δ^8-*trans*-tetrahydrocannabinol (**2**) was obtained in 96% yield by acid-promoted dehydration (20 mol% TsOH, benzene, reflux, 4 h) and (−)-Δ^9-*trans*-tetrahydrocannabinol (**1**) in 80% yield by the known two-step sequence reported by Petrzilka (1. $ZnCl_2$/HCl, 2. K-*t*-pentoxide) [45].

The natural occurrence of the bibenzyl cannabinoid *trans*-perrottetinene (*trans*-(**18**)) was predicted by Crombie and co-workers in the late 1980s based on the isolation of common intermediates with cannabinoid natural products [69, 70]. In 1994, this hypothesis was confirmed by Asakawa and co-workers, who isolated **18**, interestingly as the *cis* stereoisomer [24, 26].

The first synthesis and assignment of the absolute configuration of (−)-perrottetinene (**18**) has been published by Kim and co-workers (Scheme 15) [71]. They applied a diastereoselective Ireland-Claisen rearrangement via **TS3** for installation of the *cis*-stereochemistry, followed by ring closing metathesis and acid catalyzed pyran ring formation (Scheme 15). The synthesis started with iodination of known catechol derivative **122**, followed by protection of the two phenol groups as benzoyl esters to give **123**. The chiral stannane building block **124**, which is available from enzymatic esterification of **126** [72], was used in the Stille coupling with aryl iodide **123** to afford the enantiomerically enriched allylic alcohol **121** (78% yield). Subsequent Steglich esterification with 5-methylhex-5-enoic acid (**120**) resulted in formation of **119**. Formation of the required (*Z*)-silyl ketene acetal afforded the desired Claisen rearrangement product **128** (60% yield, 20:1 *dr*), which was subsequently converted to methyl ester **118**. Treatment of this intermediate with excess methyl magnesium bromide in refluxing THF resulted in the formation of the tertiary alcohol with concurrent deprotection of the two benzoyl protecting groups and bisphenol **129** was isolated. The synthesis was completed through the use of ring closing metathesis using Grubbs' catalyst to give **130**, followed by tosic acid promoted pyran ring formation in refluxing benzene to give (−)-perrottetinene (**18**) (55%).

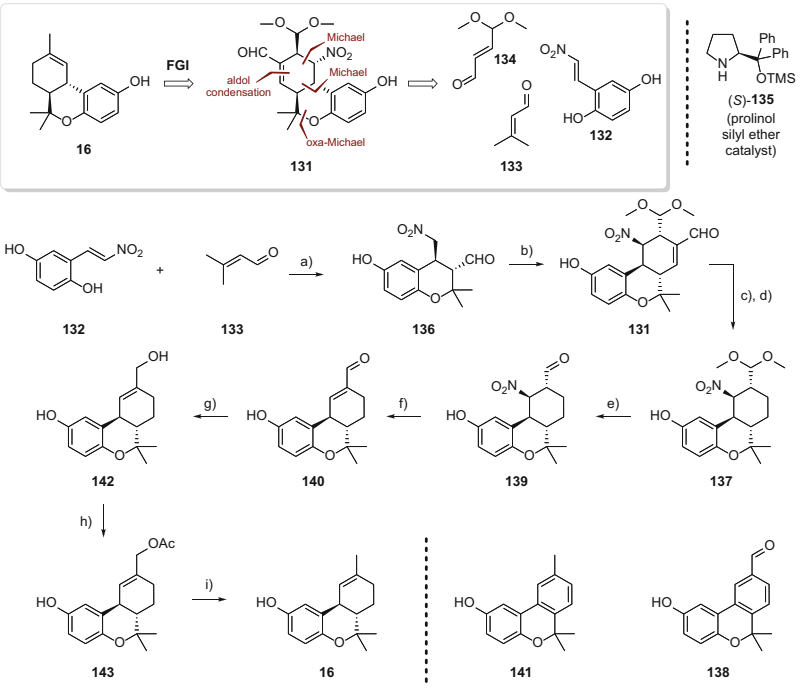
A conceptually different approach for the synthesis of the central benzochromene motif involves the application of two consecutive organocatalytic domino reactions, involving a cascade of oxa-Michael/Michael/Michael/aldol-condensation, and has been applied by Hong and co-workers in the synthesis of the marine metabolite (+)-conicol (**16**) [73, 74] (Scheme 16).

The synthesis commenced with prolinol silyl ether **135** catalyzed tandem oxa-Michael/Michael reaction of the nitro olefin **132** and the α,β-unsaturated aldehyde **133** to give **136** (76% yield, >99% *ee*). Subsequent prolinol silyl ether **135**-catalyzed domino Michael/aldol condensation with aldehyde **134** afforded the benzo-chromene intermediate **131**, which contains the full carbon skeleton of (+)-conicol (**16**). The two organocatalytic cascade reactions were successfully



Scheme 15 Total synthesis of (−)-perrottetinene (**18**) by Kim and co-workers. Reagents and conditions: (a) 1.1 equiv I_2, 1.1 equiv $NaHCO_3$, THF/H_2O, 0°C to rt, 10 min, 85%; (b) 2.2 equiv NaH, 2.2 equiv benzoyl chloride, 0°C to rt, 3 h, 98%; (c) 1.2 equiv **124**, 5 mol% $Pd_2(dba)_3$, 10 mol % P(*t*-Bu)$_3$, 10 mol% CuI, 2.2 equiv CsF, toluene, 80°C, 5 h, 78%; (d) 1.5 equiv **120**, 1.5 equiv DCC, 30 mol% DMAP, CH_2Cl_2, 0°C to rt, 16 h, 98%; (e) 2.0 equiv TBSCl, 2.0 equiv LDA, THF/HMPA (4:1), −78°C to rt, 15 h, 60%; (f) 1.5 equiv DCC, 30 mol% DMAP, CH_2Cl_2/MeOH, rt, 16 h, 91%; (g) 29 equiv MeMgBr, THF, reflux, 2 h, 90%; (h) 5 mol% Grubbs second generation catalyst, CH_2Cl_2, reflux, 16 h, 90%; (i) 2.3 mol% *p*-TsOH, C_6H_6, reflux, 30 min, 55%; (j) 1.3 equiv Bu_3SnH, 1 mol% $Pd(PPh_3)_2Cl_2$, THF, −78°C, 2 h, 81%; (k) lipozyme, diisopropyl ether/vinyl acetate (10:1), 35°C, 12 h, 47% (**124**, >99% *ee*) and 48% (**125**, >99% *ee*)

combined in a one-pot two-step sequence, resulting in the formation of key intermediate **131** directly from **132** and **133**, without isolating **136**. Decarbonylation of **131** with Wilkinson catalyst in refluxing toluene and subsequent hydrogenation in the presence of Pd/C afforded **137**. Hydrolysis of the acetal under mild conditions resulted in the formation of aldehyde **139** along with trace amounts of the aromatic side product **138**. Treating β-nitro aldehyde **139** with DABCO resulted in denitration elimination and afforded the α,β-unsaturated aldehyde **140**. Various attempts to reduce aldehyde **140** under Wolf-Kishner conditions were unsuccessful and resulted in isolation of the aromatic side product **141** or in decomposition. Accordingly, the aldehyde was first reduced to give the alcohol **142**, followed by



Scheme 16 Total synthesis of (+)-conicol (**16**) as reported by Hong and co-workers. Reagents and conditions: (a) 1.5 equiv **133**, 1.0 equiv **132**, 20 mol% AcOH, 20 mol% (*S*)-**145**, $CHCl_3$, rt, 1 h, 76% (>99% *ee*); (b) 2.0 equiv **134**, 20 mol% (*S*)-**145**, $CHCl_3$, rt, 35 h, 69%; or one-pot (a) and (b): 1.5 equiv **133**, 1.0 equiv **132**, 15 mol% AcOH, 15 mol% (*S*)-**135**, $CHCl_3$, rt, 1 h; then 2.0 equiv **134**, rt, 35 h, 55% (>99% *ee*); (c) 1.0 equiv $[Rh(PPh_3)_3Cl]$, toluene, reflux, 4 h, 54%; (d) 1 atm H_2, Pd/C, MeOH, rt, 1 h, 72%; (e) Amberlyst 15, CH_3CN/H_2O (1:1), 80°C, 5 h, 69%; (f) 1.5 equiv DABCO, CH_3CN, 0°C to rt, 2 h, 79%; (g) 3.0 equiv DIBAL, THF, −78°C, 1 h, 73%; (h) 4.0 equiv DMAP, 2.9 equiv trimethylamine, 2.0 equiv acetyl chloride, CH_2Cl_2, 0°C to rt, 1 h, 76%; (i) Li/NH_3, THF, −78°C, 30 min, 73%; (j) hydrazine hydrate, KOH, diethylene glycol, rt to 130°C, 8 h, 63%

acetylation with acetic anhydride and reduction under Birch conditions to afford (+)-conicol (**16**).

5 Conclusion

The chemistry discussed in this contribution highlights various successful approaches to cannabinoid total synthesis. The reexamination of these time-honored targets with an eye to implementing modern methods and tactics provides efficient routes, which can be adapted to new structures from this important family of natural products that may be isolated in the future. Additionally, the routes

enable the investigation of the exciting biology of members of this class of natural products.

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Molecular Pharmacology of Phytocannabinoids

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1 Introduction

Cannabis sativa contains about 120 phytocannabinoids, which are the C_{21} terpenophenolic constituents making up approximately 24% of the total natural products of the plant [1]. To date, eleven different chemical classes of phytocannabinoids have been identified (Table 1). The Δ^9-tetrahydrocannabinol (**1**) type class represents the largest proportion, comprising 17.3% of the total phytocannabinoid content, closely followed by the cannabigerol (**6**) type (see [1] for a detailed review of these different classes). The proportion of each chemical class in the cannabis plant is, however, dependent on the growing conditions, geographical location, plant processing methods, and plant variety or chemotype. Thus, these factors influencing the relative proportions of each phytocannabinoid type will additionally influence the pharmacological effects of whole cannabis extracts, either through a polypharmacological effect of the phytocannabinoids themselves, or through modulation of phytocannabinoid effects by the non-cannabinoid content of the plant [2]. These variances are therefore important to take into account when assessing the effects of whole cannabis plant extracts. In this chapter, focus will be made on the seven individual phytocannabinoids that have been the most thoroughly studied.

Table 1 Constituents of *Cannabis sativa* L. represented as a percentage of the total phytocannabinoid content. Adapted from [1]

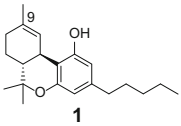
Chemical class type	Percent of total phytocannabinoid content (%)[a]
1 type	17.3
Δ^8-THC type	1.9
3 type	9.6
4 type	7.7
7 type	7.7
6 type	16.3
CBND type	1.9
CBE type	4.8
CBL type	2.9
CBT type	8.7
Miscellaneous type	21.2

[a]Total phytocannabinoid content = ca. 120

Phytocannabinoids have been of recreational, therapeutic, and other interest for thousands of years [3, 4]. Elucidation of the structure of the main phytocannabinoid obtained from cannabis, **1** [5], was reported in 1971. This discovery paved the way for further research that ultimately led to the discovery of the cannabinoid receptors, CB_1 [6], which predominates in the central nervous system, and the principally peripheral cannabinoid receptor, CB_2 [5]. The mammalian endocannabinoid system was then discovered [6], including the endogenous cannabinoid receptor ligands arachidonylethanolamide (AEA) and 2-arachidonylglycerol (2-AG) [7–9]. The psychotropic effect of **1**, mediated by its partial agonist activity at CB_1 receptors, has limited the extent of its use medicinally and it was removed from the *British Pharmacopeia* in 1971, and was declared of no medical benefit and placed under control in the Misuse of Drugs Act 1971 of the United Kingdom [10]. Despite this, patient-led self-medication campaigns claimed various therapeutic benefits, such as control of pain and emesis [11–15], control of seizures [16–21], and anti-inflammatory properties [17, 22], among others. This drove further investigation, leading to some licensed medications containing **1** being now available, such as Sativex®, which is used for the treatment of spasticity associated with multiple sclerosis. Although **1** also exerts some effects through non-CB receptor targets, the absence of psychotropic effects associated with the other phytocannabinoids present in cannabis has driven research into their discrete pharmacology and molecular targets that lie outside of the endocannabinoid system.

Over the years, a variety of molecular targets for plant cannabinoids outside the endocannabinoid system have been identified, such as ion channels, non-CB_1 or CB_2 G-protein coupled receptors, enzymes, and transporters. In this chapter, an overview of the molecular pharmacology of phytocannabinoids is presented, describing both targets within the endocannabinoid system and a wide range of other molecular targets. Since ca. 120 phytocannabinoids have now been identified and many have, as yet, poorly defined or unknown pharmacological profiles, particular focus is paid to phytocannabinoids that: (a) are reported to exert a behavioral effect in animal models or clinical reports, and (b) exert effects via specific molecular targets at sub-micromolar to low micromolar concentrations, which can realistically be achieved in vivo due to the lipophilic nature of these compounds [23].

2 Δ^9-*trans*-Tetrahydrocannabinol



1

2.1 Activity at Cannabinoid Receptors

In 1986, Howlett and colleagues developed a biochemical model system that allowed the indirect identification of cannabimimetic drugs, i.e. those exhibiting properties like **1** (cAMP assay) [24]. This system provided an indication of cannabinoid receptor activation by monitoring the ability of a compound to inhibit forskolin-induced stimulation of cyclic adenosine monophosphate (cAMP) production. Along with the many synthetic CB_1 receptor agonists now developed [6, 25, 26], **1** can inhibit the activity of adenylate cyclase that synthesizes cyclic AMP. However, in this assay, **1** does not inhibit adenylate cyclase to the same extent as several other synthetic CB_1 receptor agonists, which led to its classification as a partial agonist at this receptor [27].

Two years later, in 1988, Devane and co-workers developed a radioligand displacement binding assay using the highly potent, synthetic CB_1 receptor agonist, CP-55940 [28]. In this assay, **1** effectively displaced radiolabeled CP-55940 and showed low micromolar affinity at the CB_1 receptor (Table 2). The properties of **1** as a CB_1 receptor partial agonist were further exemplified in binding assays assessing ligand-induced changes in GTPγS binding in cell membranes [27, 29, 30]. Here, the synthetic CB_1 receptor agonist, JWH-018, increased GTPγS binding in mouse brain membranes to a much greater extent than **1** [29].

Importantly, **1** not only activates CB_1 receptors in vitro but also in vivo as well. In vivo activity of **1** at CB_1 receptors was tested in a battery of animal behavior tasks known to produce outcomes associated with CB_1 receptor activation [31, 32]. The four simple behavioral tests in mice known as the "Billy Martin Tetrad" were reported, and these are: inhibition of locomotor activity; reduced sensitivity to pain; reduced body temperature; and immobility (catalepsy) [31]. At doses of 0.03–20 mg kg^{-1} (i.v.), **1** was active in all of these tests, and the effects were blocked by the CB_1 receptor antagonist, rimonabant (10 mg kg^{-1}) [31–33]. However, it should be noted that rimonabant is not a specific ligand for the CB_1 receptor when employed at concentrations of >1 μ*M* [34, 35] and, therefore, at the concentrations reached in vivo.

With this dose, functional antagonism of these effects could also have been mediated by other targets of rimonabant such as agonism or antagonism of GPR55 receptors [36], antagonism of A1 adenosine receptors [37], and antagonism of TRPV1 channels [38].

In a feeding study in rats, **1** (0.5–4.0 mg kg^{-1}) stimulated hyperphagia. However, while rimonabant predictably inhibited hyperphagia at doses of >0.67 mg kg^{-1}, it also stimulated hyperphagia at lower doses. There was no significant difference in food intake between these two groups and this may be due to the differences in the feeding pattern being masked by effects on non-specific behavioral effects such as reduced motor co-ordination induced by **1** treatment [39].

There is also in vitro and in vivo evidence that **1** binds to, and activates the CB_2 receptor. The binding affinity of **1** at CB_2 receptors is, however, lower than that at CB_1 receptors, as shown in Table 2 [29, 40]. Evidence of a partial agonist effect of

Table 2 Examples of K_i values of Δ^9-*trans*-tetrahydrocannibinol (**1**), Δ^9-*trans*-tetrahydrocannabivarin (**2**), cannabinol (**3**), cannabidiol (**4**), cannabidivarin (**5**), cannabigerol (**6**), and cannabichromene (**7**) and half maximal responses where described

	K_i/μ*M*	EC_{50}/ IC_{50}	Assay	Cell type	Ref.
Δ^9-Tetrahydrocannabinol (**1**)					
CB_1	0.0061	ND	[^{3}H] CP55-940 binding assay	Whole brain/Rat	[28]
	0.005	ND	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Human	[158]
	0.008	ND	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Mouse	
	0.013	ND	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Rat	
	0.021	ND	Filtration assay	Brain membranes/Rat	[86]
	0.035	ND	[^{3}H] CP55-940 binding assay	Brain synaptosomal membrane/Rat	[35]
	0.0395	0.013	[^{3}H] HU-243 binding assay	COS-7 cells/Rat	[40]
	0.0477	ND	[^{3}H] CP55-940 binding assay	Whole brain/Mouse	[90]
	0.053	0.0165	[^{3}H] CP55-940 binding assay	Fibroblast L cells/Rat	[87]
	0.065	ND	[^{3}H] HU-243 binding assay	Synaptosomal brain membrane/Rat	[85]
	0.08	ND	[^{3}H] HU-243 binding assay	COS-7 cells/Rat	
	0.0356	0.087	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
CB_2	0.003	ND	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Human	[158]
	0.0017	ND	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Mouse	
	0.0068	ND	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Rat	
	0.036	ND	Filtration assay	Spleen membrane/Rat	[86]
	0.0039	ND	[^{3}H] CP55-940 binding assay	Spleen membrane/Rat	[35]
	0.040	ND	[^{3}H] HU-243 binding assay	CHO cell/Rat	[40]
	0.075	0.0418	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Rat	[87]
	0.032	ND	[^{3}H] HU-243 binding assay	COS-7 cells/Rat	[85]
	0.0084	0.061	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
Δ^9-Tetrahydrocannabivarin (**2**)					
CB_1	0.075	ND	[^{3}H] CP55-940 binding assay	Whole brain membranes/Mouse	[73]
	0.047	ND	[^{3}H] CP55-940 binding assay	Cortical brain membranes/Rat	[72]
	0.286	ND	[^{3}H] rimonabant binding assay	Cortical brain membranes/Rat	[160]
	0.046	ND	[^{3}H] CPP-940 binding assay	Whole brain/Mouse	[90]

(continued)

Table 2 (continued)

	K_i/μM	EC_{50}/ IC_{50}	Assay	Cell type	Ref.
CB_2	0.225	0.038	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Human	[70]
	0.145	0.143	[^{3}H] CP55-940 binding assay	CHO cell membrane/ Human	[41]
Cannabinol (**3**)					
CB_1	0.326	ND	Filtration assay	Brain/Rat	[86]
	0.129	ND	[^{3}H] CPP-940 binding assay	Whole brain/Mouse	[90]
	1.13	>1	[^{3}H] CPP-940 binding assay	Fibroblast L cells/Rat	[87]
	0.392	ND	[^{3}H] HU-243 binding assay	Synaptosomal brain membrane/Rat	[85]
	0.211	ND	[^{3}H] HU-243 binding assay	COS-7 cells/Rat	
	3.2	ND	[^{3}H] CP55-940 binding assay	Sectioned brain/Rat	[25]
	0.25	ND	[^{3}H] CP55-940 binding assay	Whole brain/Rat	[161]
	0.74	ND	[^{3}H] rimonabant binding assay	Whole brain/Rat	
	0.012	0.017	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
	0.069	ND	[^{3}H] CP55-940 binding assay	CHO cell/Human	[88]
CB_2	0.096	ND	Filtration assay	Spleen/Rat	[86]
	0.301	>1	[^{3}H] CPP-940 binding assay	CHO cell membrane/ Rat	[87]
	0.126	ND	[^{3}H] HU-243 binding assay	COS-7 cells/Rat	[85]
	0.016	0.055	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
	0.07	0.062	[^{3}H] CP55-940 binding assay	CHO cell/Human	[88]
Cannabidiol (**4**)					
CB_1	>10	ND	[^{3}H] CP55-940 binding assay	Whole brain/Mouse	[90]
	0.073	ND	[^{3}H] 5′-trimethylammonium-Δ^9-THC binding assay	Whole brain/Rat	[162]
	>0.5	ND	[^{3}H] CP55-940 binding assay	Whole brain/Rat	[28]
	53	ND	[^{3}H] CPP-940 binding assay	Sectioned brain/Rat	[25]
	4.3	ND	[^{3}H] CP55-940 binding assay	Cortical brain membranes/Rat	[163]
	2.3	ND	[^{3}H] CP55-940 binding assay	Whole brain/Rat	[161]
	1.3	ND	[^{3}H] rimonabant binding assay	Whole brain/Rat	
	>10	ND	[^{3}H] HU-243 binding assay	Whole brain/Rat	[136]
	4.9	ND	[^{3}H] HU-243 binding assay	Whole brain/Mouse	[104]
	1.8	ND/ NE	[^{3}H] rimonabant binding assay	Brain cortical membranes/Rat	[164]
	4.7	ND	[^{3}H] CP55-940 binding assay	Whole brain membranes/Mouse	[118]
	1.45	3.86	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
CB_2	>10	ND	[^{3}H] HU-243 binding assay	COS-7 cells/Rat	[136]
	2.86	ND	[^{3}H] CP55-940 binding assay	CHO cell/Human	[86]
	4.2	0.503	[^{3}H] CP55-940 binding assay	CHO cell/Human	[104]

(continued)

Table 2 (continued)

	K_i/μM	EC_{50}/ IC_{50}	Assay	Cell type	Ref.
	2.86	ND	[^{3}H] CP55-940 binding assay	*E. coli* cell membranes/ Human	[118]
	0.37	2.27	[^{3}H] CP55-940 binding assay	Sf9 cells/ Human	[159]
Cannabichromene (**7**)					
CB_1	>10	ND	[^{3}H] CP55-940 binding assay	Whole brain/Mouse	[90]
	0.71	1.68	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
CB_2	0.256	1.30	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
Cannabigerol (**6**)					
CB_1	275	ND	[^{3}H] CP55-940 binding assay	Sectioned brain/Rat	[25]
	0.896	1.12	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
CB_2	0.153	0.85	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
Cannabidivarin (**5**)					
CB_1	14.7	13.80	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]
	0.127*	ND	[^{3}H] CP55-940 binding assay	MF1 brain membranes/ Mouse	[2]
CB_2	0.57	3.45	[^{3}H] CP55-940 binding assay	Sf9 cells/Human	[159]

ND, not described; CHO, Chinese hamster ovary; COS, CV1 in origin with SV40 genes; Sf, *Spodoptera frugiperda*

1 at CB_2 receptors came from a study where **1** antagonized the inhibition of adenylate cyclase in CHO cells transfected with human CB_2 receptors induced by the agonists HU-293a and HU-210 (Table 2) [40].

As is typical of a partial agonist, **1** has a mixed agonist–antagonist effect. This is likely dependent on the proportion of cannabinoid receptors that are in the "active" state in tissues, coupled to their effector mechanisms, or in the "inactive" state, uncoupled to their effector mechanisms [41]. Moreover, it would also depend on the presence of other synthetic or endogenous cannabinoid receptor agonists, and possibly species differences between studies. As a partial agonist, **1** can be expected to antagonize the actions of full agonists. In a mouse model of hypothermia, **1** alone acted as a partial agonist with less efficacy than the cannabinoid receptor full agonist, AM2389, but when co-administered with this compound, **1** antagonized AM2389's hypothermic effects [42].

In an in vitro study using the GTPγS binding assay in rat brain membranes from rats chronically treated with 10 mg kg^{-1} **1** for 21 days, the stimulation of GTPγS binding by WIN 55212–2 was reduced by up to 70%, suggesting that chronic exposure to **1** led to a desensitization of cannabinoid-activated signal transduction.

In healthy human subjects, the intravenous administration of **1** caused acute psychotic reactions and a temporary decline in cognitive functioning [43].

2.2 Cannabinoid Receptor Independent Activity

The well-known psychotropic effect of **1** is mediated by its partial agonist activity at CB_1 receptors. However, **1** also exerts effects at molecular targets outside of the endocannabinoid system. Some of the physiological effects of **1** may be mediated by more than one target, as detailed below.

In this regard, **1** has been proposed to act in an allosteric manner on specific receptors outside of the endocannabinoid system. In vitro, **1** potently inhibited $5HT_{3A}$-induced currents in HEK293 cells transfected with $5HT_{3A}$ receptor cDNA [44], similar to the reported effect of the synthetic cannabinoid receptor agonist, WIN 55212–2,and also in cultured rat trigeminal ganglion neurons (Table 3) [44, 45]. Together with **1**, other cannabinoids such as WIN 55212–2, anandamide, JWH-015, and CP-55940, have been shown to stereoselectively inhibit currents at this receptor [44].

Cannabinoid receptors and $5HT_3$ receptors are both involved in control of pain and emesis [11–14]. The results above show that the activity of cannabinoid receptor agonists on the control of pain and emesis may be shared by their antagonistic effect on $5HT_3$ receptors [46–50]. This highlights the possibility of a ligand having a physiological effect that can be mediated by multiple targets. Therefore, an effect proven to be mediated through one target does not mean that other targets of the ligand mediating the same physiological effect can be ruled out.

At glycine receptors, low concentrations of **1** also acted through a possible allosteric mechanism by potentiating the amplitude of glycine-activated currents in rat isolated ventral tegmental area neurons via a cannabinoid receptor-independent mechanism (Table 3) [51]. Glycine receptor function was potentiated by **1** at physiologicallyrelevant concentrations. Glycine receptors are involved in pain transmission [52, 53] and dopamine release from ventral tegmental area neurons [54, 55], thus **1** may be important for analgesia and drug addiction. Analgesia is also produced through **1** activity at cannabinoid receptors [11, 12] but some of this analgesic effect may be mediated through glycine receptors as well. This again shows a physiological effect being mediated by more than one target of the same ligand.

Compound **1** (0.1–10 μ*M*) is a peroxisome proliferator-activated receptor gamma (PPARγ) agonist. The studies below outline the relevance of the agonist effect at this nuclear receptor in the cardiovascular system and potentially in cancer treatment. Through agonism of the PPARγ receptor, **1** has time-dependent effects on vasorelaxation of the aorta and superior mesenteric arteries in a dose-dependent manner [56]. This relaxation effect of **1** was similar to the vascular relaxation effect of the PPARγ ligand rosiglitazone (46.7% and 69.7% respectively). Another study by the same group showed differences in the time-dependent effect of **1** on vasorelaxation in different vessel types; in resistance mesenteric arteries no time-dependent effect of **1** on PPARγ mediated vasorelaxation was noted [57]. These studies show that the effect of **1** on endothelium-dependent vasorelaxation is dependent on the predominant relaxing factor in a given artery. Agonism of

Table 3 A comparison of select in vitro studies showing cannabinoid receptor independent activity of Δ^9-*trans*-tetrahydrocannabinol (**1**) according to concentrations, assay types, and cell types used

Target	Concentration/ μ*M*	EC_{50}/ μ*M*	IC_{50}/ μ*M*	Assay	Cell line	Ref.
GPR55	<1		0.008	GTPγS binding assay	HEK293/Human	[67]
	1–10		5	$[Ca^{2+}]$ mobilization assay	HEK293/Human	[165]
	<1		0.64	ERK1/2 MAPK phosphorylation	HEK293/Human	[69]
	1		ND	ERK1/2 MAPK phosphorylation	HEK293/Human	
			ND	B-arrestin assay	HEK293/ND	[66]
GPR18	<1	0.96		MAPK activation assay	HEK293/ Human	[62]
$5HT_{3A}$	<1		0.038	Voltage clamp	HEK293/Human	[44]
Glycine ligand gated ion channels						
α1	<1	0.086		Whole cell patch clamp	*Xenopus laevis* oocytes/ Human	[51]
α1β1	<1	0.073		Whole cell patch clamp		
		0.115			Ventral tegmental area neurons/ Rat	
PPARγ nuclear receptor	<1	ND		Contraction	HEK293/ND	[166]
TRP cation channels						
TRPA1	<1	0.23		Ca^{2+} Fluorescence assay	HEK293/Rat	[80]
TRPV2		0.65			HEK293/Rat	
TRPM8			0.16		HEK293/Rat	
			0.15		HEK293/Rat	[79]
TRPV3	1–10	9.5			HEK293/Rat	[81]
TRPV4		8.5			HEK293/Rat	
CYP1A1	1–10		0.53	Fluorescence assay-FLUOSTAR OPTIMA	Recombinant/ Human	[167]
CYP1A2			4.59			
CYP1B1			1.39			
CYP2C9	1–10		2.84	HPLC	Recombinant/ Human	[168]
Adenosine uptake	<1		0.27	Scintillation counting [^{3}H]adenosine	EOC-20 microglia	[116]
			0.334	Scintillation counting [^{3}H]adenosine	RAW264.7 macrophages	

GPR, G-protein-coupled receptor; 5HT, 5-hydroxytryptamine; PPAR, peroxisome proliferator-activated receptor; TRP, transient receptor potential; CYP, cytochrome P450; HEK, human embryonic kidney; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); Ca^{2+}, Calcium; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase; also see footnote for Table 1

PPARγ by **1** leads to an increase in superoxide dismutase activity, thus leading to an increase in hydrogen peroxide (H_2O_2). In superior mesenteric arteries, H_2O_2 is the predominant relaxing factor and therefore **1** enhances endothelium-dependent vasorelaxation. In resistance mesenteric arteries, however, where endothelium-derived hyperpolarizing factor (EDHF) is the predominant relaxing factor, **1** inhibits EDHF production and therefore inhibits vasorelaxation in these arteries [57].

In vivo, **1** acts via the PPARγ mechanism to reduce tumor growth rate. In mice with induced tumour xenografts, **1** (15 mg kg^{-1}) showed antitumor properties by reducing tumor growth rate, which was prevented by co-administration with the PPARγ antagonist, GW9662 [58]. However, an antagonist-only treatment group was not included in this study and therefore the effect of **1** on tumor growth has not been validated as being mediated by PPARγ and so, as yet, can be considered a functional, rather than molecular antagonism.

Moreover, PPARγ is not only involved in the physiological roles outlined above. It is also involved in adipogenesis, where it is highly expressed, and in the treatment of type 2 diabetes [59, 60] and gastro-inflammatory disorders [61]. Compound **1** may therefore have as yet unproven effects on these disorders. There are other $G_{i/o}$ coupled receptors (GPCR) that are thought to be novel cannabinoid receptors. These are GPR18 and GPR55 [29, 30, 62, 63]. These receptors belong to the same class as CB_1 and CB_2 receptors but do not share many structural similarities [64], which would likely result in differing ligands and physiological effects at these receptors compared to CB_1 and CB_2 receptors.

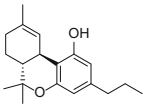
In HEK293 cells transfected with the novel $G_{i/0}$ coupled GPCR cannabinoid receptor, GPR18, **1** acts as a potent agonist (Table 3) [62]. Interestingly, the phytocannabinoid cannabidiol (**4**) can antagonize the effect of the agonists such as **1** at this receptor [62, 63].

There are conflicting reports on the activity of **1** at the GPR55 receptor in vitro. This receptor has been claimed, by many authors, to be a third cannabinoid receptor [29–31, 65]. Using two different assays in the same cell line (HEK293) transfected with human GPR55, **1** weakly activated GPR55 in a β-arrestin assay [66], but potently activated it in a GTPγS binding assay with a submicromolar half maximal response (Table 3) [67]. However, using the same cell line transfected with human GPR55 and the β-arrestin assay, Kapur and co-workers found no detectable activity of **1** at this receptor [68]. Moreover, again in the same cell line also transfected with human GPR55, **1** has been reported to exhibit differential effects in a concentration-dependent fashion. It was reported in the same study that **1** is an inhibitor of the proposed endogenous agonist of GPR55, lysophosphatidylinositol (LPI), at concentrations of 1 μ*M*, by inducing a rightward shift in the log concentration-response

curve of LPI as well as activating this receptor at micromolar concentrations [69]. These findings of agonism and inhibition suggest that there could be two distinct binding sites on GPR55 receptors. By itself, **1** may bind to either an orthosteric binding site or an allosteric binding site producing agonism of the receptor or, by binding to an allosteric site, produces a conformational change in the orthosteric binding site, thus reducing the effect of LPI [69]. The binding of **1** to a particular binding site may be dependent on the concentrations used.

Even though **1** has undesirable psychotropic effects, mediated by CB_1 receptors, it is important to remember that this phytocannabinoid has a range of important therapeutic benefits. These effects may be mediated both by cannabinoid receptors, either CB_1 and CB_2 receptors or novel GPCRs, and non-cannabinoid targets.

3 Δ^9-Tetrahydrocannabivarin



2

Δ^9-Tetrahydrocannabivarin (**2**) is included in the Δ^9-THC chemical class which, as mentioned earlier, constitutes the majority of the phytocannabinoid content [1] of *C. sativa*. This phytocannabinoid is the *n*-propyl analog of **1**, with the slight structural change resulting in some different molecular targets and physiological effects when compared to **1**.

3.1 Activity at Cannabinoid Receptors

In vitro, **2** is a CB_2 receptor partial agonist, as shown by its lower efficacy at CB_2 receptors than the agonist CP-55940 in both CHO cells transfected with human CB_2 receptors and in the GTPγS binding assay in membranes from these cells, as shown in Table 2 [70].

Importantly, there is also in vivo evidence of **2** as a CB_2 receptor partial agonist. Garcia and co-workers showed that **2** (2 mg kg^{-1}) can show signs of neuroprotection in a model of Parkinson's disease in mice that have received

intrastriatal injections of lipopolysaccharide (LPS), similar to the effects shown by the CB_2 selective agonist HU-308 [71]. CB_2 receptor-deficient mice were more vulnerable to LPS-induced lesions, which supports the effects of **2** being mediated, at least in part, by agonism at CB_2 receptors.

At low concentrations (0.1–5 μ*M*), **2** blocks CB_1 receptors both in vitro and in vivo, but interestingly at high doses acts as a CB_1 agonist in vivo but not in vitro. Two studies reported that **2** blocks the agonist effects of CP-55940- and (+)-(*R*)-WIN55212-induced stimulation of GTPγS binding to mouse whole membranes at a low concentration of 1 μ*M* (Table 2) [72, 73], while Dennis and co-workers showed, using the same assay, this antagonistic effect of **2** from the lower concentration of 0.1 μ*M* up to 5 μ*M* on (+)-(*R*)-WIN55212 in the mouse cerebellum and piriform cortex membranes [74]. The antagonist effect of **2** is the same as two established CB_1 receptor-selective antagonists, rimonabant and AM251 [72, 73, 75, 76]. Thus, antagonism of CB_1 receptors by **2** modulates inhibitory neurotransmission in the cerebellum [76].

In vivo, **2** acts as both an antagonist and agonist at low doses and high doses, respectively. This antagonist and agonist phenomenon results in opposing effects on antinociception and on locomotor activity depending on the concentration used. This disparity in pharmacological effect of **2**, dependent upon the concentration used, highlights the importance of knowing the concentration of each phytocannabinoid in whole cannabis plant material and extracts when this is being used for therapeutic use.

At low doses of 0.3 and 3 mg kg^{-1}, **2** blocks the antinociceptive effect of **1** in a mouse model of acute pain and hypothermia [72]. Using the same model, **2** also partially antagonized the CB_1 agonist effects of CP-55940 at a dose of 2 mg kg^{-1} and also partially antagonized CP-55940-induced inhibition of rat locomotor activity in a model of Parkinson's disease [71]. There was no effect of **2** treatment alone on either of these parameters and therefore these studies support the molecular antagonism of **2** at CB_1 receptors.

At higher doses of 3, 10, 30, and 56 mg kg^{-1}, **2** acts as an agonist by producing antinociception in an acute model of pain and causes immobility in the ring test (a quantitative test for measuring catalepsy [65]) [72]. In this study, the CB_1 receptor antagonist rimonabant blocked the agonist effect of **2** on antinociception but not on immobility in the ring test. A rimonabant-only treatment group was not included in this study to rule out whether this antagonist worsens nociceptive pain. It is therefore not clear from this study whether the effect found is functional or molecular.

In other in vivo experiments, **2** (3, 10, 30 mg kg^{-1}) suppressed food consumption in non-fasted mice, similar to the CB_1-selective antagonist AM251 [77]. Signs of motor inhibition, induced by 6-hydroxydopamine, were reduced by **2** (2 mg kg^{-1}), similar to the effect of the CB_1 antagonist, rimonabant [71]. It is unclear without further investigation whether this effect of **2** is via inverse agonism of the CB_1 receptor, competitive inhibition with endogenous cannabinoids at CB_1 receptors or by activity at another target, since comparisons made were based on functional effects of the compounds without confirmation of the molecular targets [78].

3.2 Cannabinoid Receptor Independent Activity

There is little available evidence to suggest that **2** acts at CB_1 or CB_2 receptor-independent targets but it may have other targets within the cannabinoid system, such as the novel cannabinoid receptor GPR55 [69]. There is, however, only one study to date describing agonism of GPR55 receptors by **2** [69]. In this investigation, **2** was an agonist of GPR55 in HEK293 cells expressing human GPR55 with a similar potency to **1** (Table 4) and 1 μ*M* **2** was shown to inhibit LPI induced stimulation of GPR55 with 50% efficacy, higher than that of **1** [69].

The evidence of **2** acting at targets outside the cannabinoid system comes from the proven interaction between **2** and transient receptor potential (TRP) cation channels at higher concentrations than at which it acts at CB_1 or CB_2 receptors [79–81].

Despite there being limited known pharmacological targets for **2**, its activity at TRP channels may have wide-reaching physiological effects. These TRP channels are present in the plasma membrane of a broad range of cell types in many tissues and act as ligand-gated, non-selective cation channels permeable to sodium, calcium and magnesium ions, thereby being powerful regulators of many cell functions [82].

De Petrocellis and co-workers studied the efficacy and potency of numerous phytocannabinoids at various TRP channels [79–81]. At TRPA1 and TRPV1 cation channels, **2** is an agonist with the same high potency and at TRPV2 with a slightly

Table 4 A comparison of selected in vitro studies showing cannabinoid receptor independent activity of Δ^9-*trans*-tetrahydrocannabivarin (**2**) according to concentrations, assay types, and cell types used

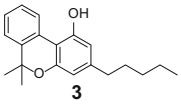
Target	Concentration/ μ*M*	EC_{50}/ μ*M*	IC_{50}/ μ*M*	Assay	Cell type	Ref.
GPR55	>1	0.88		ERK1/2 MAPK phosphorylation	HEK293/Human	[69]
	1		ND	ERK1/2 MAPK phosphorylation	HEK293/ Human	
$5HT_{1A}$	<1	5.4		GTPγS binding assay 8-OH-DPAT	Brainstem membranes/Rat	[169]
		28.3		GTPγS binding assay 8-OH-DPAT	CHO cells/ Human	
TRP cation channels						
TRPA1	1–10	1.5		Ca^{2+} fluorescence assay	HEK293/Rat	[80]
TRPM8	<1		0.87		HEK293/Rat	
TRPV1	1–10	1.5			HEK293/ Human	
TRPV2		4.1			HEK293/Rat	
TRPV3		3.8			HEK293/Rat	[81]
TRPV4		6.4			HEK293/Rat	

See Tables 1 and 2

lower potency. The TRPM8 cation channels are blocked by **2** with relatively high potency (Table 4) [80].

In summary, **2** is known to be an antagonist at CB_1 receptors at low concentrations both in vitro and in vivo but at high concentrations it shows agonistic effects at CB_1 receptors only in vivo. This antagonistic effect at CB_1 receptors has been shown to have adverse effects in the clinic, with removal of the CB_1 receptor antagonist, rimonabant, from the market due to adverse psychological effects [83]. In vivo and in vitro evidence supports partial agonism activity at CB_2 receptors and at higher concentrations than at which it activates cannabinoid receptors it has activity at TRP cation channels, which may have benefits for regulating a variety of cell functions.

4 Cannabinol



3

Cannabinol (**3**) is an oxidation product of **1** and is found in large quantities in dried and aged cannabis material [84]. The acid form of **3** is also found in large quantities in the cannabis plant but upon heating this acid is decarboxylated to **3** [84]. This is important to take into account when considering how cannabis that is being used for medicinal or recreational purposes is processed, and stored, and how it is administered.

4.1 Activity at Cannabinoid Receptors

Cannabinol (**3**) like **1**, acts at both CB_1 and CB_2 receptors but with higher affinity for CB_2 than CB_1 receptors, as shown in Table 2 [85–87]. It is an agonist at CB_1 receptors [29], but there are conflicting reports about its activity at CB_2 receptors. In COS-7 cells transfected with rat CB_2 receptors, **3** acted as a CB_2 receptor agonist in the cyclic AMP assay at 1 μM [85] but in another study performed in CHO cells transfected with human CB_2 receptors, **3** acted as an inverse agonist in the GTPγS binding assay at submicromolar concentrations [88]. These discrepancies may be due to the differences in concentrations of **3** used between the studies and could also depend on the conformational state of the receptors in the tissues. Receptors can either be in the active conformational state, where G-proteins are activated and

elicit a physiological response, or the inactive conformational state, where there is no activation of G-proteins. The amount of receptors in either state can differ in different tissues and under different conditions. If a ligand has a greater affinity for a specific conformational state (active or inactive), then the presence of the ligand will cause a redistribution of the concentrations of each conformational state. Thus, the concentration of ligand present will dictate the distribution of the receptor conformational state and either induce or inhibit a physiological response [89]. Further investigations are warranted to determine the activity of **3** at CB_2 receptors.

In vivo, **3** (50 mg kg^{-1}) has been shown to be a CB_1 receptor agonist by suppressing acetic acid-induced abdominal stretching behavior in mice, which was blocked by the CB_1 antagonist, rimonabant. The administration of rimonabant alone did not significantly affect abdominal stretching, indicating that this effect of **3** is likely to be a molecular one [90]. Moreover, in this study, the effect of **3** on locomotor suppression was also investigated. This was performed to determine whether the effect of **3** on hypomotility could be excluded from the observed effect of **3** on abdominal stretching behavior. The dose of **3** used (50 mg kg^{-1}) did not elicit locomotor suppression thereby indicating the suppression of abdominal stretching was not due to motor dysfunction [90].

Additionally, **3** (0.26–26.0 mg kg^{-1} p.o.) exerts CB_1 receptor-dependent effects on rat feeding behavior by decreasing latency to feed and increasing food consumption over the whole test period with these effects being abolished in the presence of rimonabant [91]. However, a rimonabant-only treatment group was not included in this study and therefore it is not clear whether this effect of **3** is via functional mechanisms or molecular mechanisms. In numerous other feeding studies rimonabant decreases food consumption [92–94], but there is speculation as to whether this is due to suppressive effects of rimonabant on spontaneous locomotion [95, 96] and stimulation of emesis and nausea [97–99]. Together with these studies it is unclear whether the effects of **3** and rimonabant on feeding are mediated via molecular mechanisms.

For further information on binding affinities of **3** at CB_1 and CB_2 receptors, see Table 2.

4.2 Cannabinoid Receptor Independent Activity

Cannabinol also acts at targets outside of the endocannabinoid system. It is a potent agonist of TRPA1 cation channels, potently blocks TRPM8 cation channels, and also desensitizes TRPA1 cation channels to activation by the agonist allyl isothiocyanate (Table 5) [80].

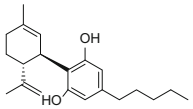
There is little recent literature on the pharmacology of **3** and thus further investigations need to be conducted to determine whether this compound has other therapeutic or recreational effects and how it modulates or enhances the physiological effects of whole cannabis-derived preparations.

Table 5 A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabinol (**3**) according to concentrations, assay types, and cell types used

Target	Concentration/ μ*M*	EC_{50}/ μ*M*	IC_{50}/ μ*M*	Assay	Cell type	Ref.
TRP cation channels						
TRPA1	<1	0.18	0.4	Ca^{2+} fluorescence assay	HEK293/Rat	[80]
TRPM8			0.21		HEK293/Rat	
TRPV1	1–10	6.2			HEK293/ Human	
TRPV2	>10	19.0				
TRPV3	1–10	5.3			HEK293/Rat	[81]
TRPV4	>10	16.1			HEK293/Rat	
CYP1A1	1–10		0.685	fluorescence assay—FLUOSTAR OPTIMA	Recombinant/ Human	[167]
CYP1A2			3.92			
CYP1B1			1.50			
CYP2C9	1–10		2.86	HPLC	Recombinant/ Human	[168]

See Tables 1 and 2

5 Cannabidiol



4

Cannabidiol (**4**) is a non-psychotropic phytocannabinoid and the **4** chemical class type of phytocannabinoids is currently the third most abundant chemical class type in cannabis, after **1** and **6** [1]. Another phytocannabinoid in this class, cannabimovone, was isolated in 2010 [100], thereby increasing the number of phytocannabinoids of this type from seven in 2005 [101] to eight [1]. This class now makes up 7.7% of phytocannabinoid content (Table 1).

5.1 Activity at Cannabinoid Receptors

Cannabidiol (**4**) has been investigated in a number of studies to determine its activity at cannabinoid receptors and shows very low affinity at these receptors (Table 2) [102, 103]. There has been a single report where **4** was shown to act as an antagonist of both CB_1 and CB_2 receptors at submicromolar concentrations [104]. However, a meta-analysis examining interspecies differences in ligand-binding affinity and receptor distribution identified eight methodological covariates

that could explain the discrepancies between results from various studies on cannabinoid receptor affinity for **4** [105]. A more recent meta-analysis from the same group concluded that **4** has very low affinity as an orthosteric ligand for CB_1 receptors (Table 2), but may affect CB_1 receptor activity in vivo via an indirect mechanism [78]. However, a study recently published showed that **4** can act as a negative allosteric modulator of CB_1 receptors [106]. Allosteric modulators alter the potency and efficacy of the orthosteric ligands but do not activate the receptor themselves. The allosteric effects of **4** were studied using an operational model of allosterism [107] and the effects of **4** treatment compared to the well-characterized negative allosteric modulators ORG2759 and PSNGBAM-1 [108–111]. The efficacy of both of the orthosteric ligands, **1** and 2-AG, was reduced by **4** (<1 μ*M*) and **4** displayed negative co-operativity for binding of these ligands. Moreover, **4**-treatment reduced G-protein dependent signaling and arrestin 2 recruitment, similar to the effects of the negative allosteric modulators ORG2759 and PSNCBAM-1 [109, 112]. This allosteric modulation of CB_1 receptors needs to be validated by further studies, but the results from this study could explain the reported ability of **4** to functionally antagonize some effects of **1** in animal studies and clinical studies in humans (for a review see [113]).

Compound **4** has an effect in vitro of inhibiting anandamide uptake and therefore affecting endocannabinoid tone by increasing availability of anandamide. The concentration at which **4** exerts its half maximal response, however, is higher than what would be relevant for a physiological effect in vivo [80].

5.2 Cannabinoid Receptor Independent Activity

Despite **4** showing very little affinity for CB_1 and CB_2 receptors, as described above, there is evidence of an antagonist effect of **4** at the novel cannabinoid receptor GPR55 both in vitro and in vivo. At a concentration of 1 μ*M*, **4** suppressed the activation of GPR55 in rat hippocampal slices, thus suppressing excitatory output from pyramidal cells [114]. In a GTPγS-binding assay, **4** had potent antagonist effects at GPR55 with a submicromolar half maximum response (Table 6) [67]. Whyte and co-workers have shown a role for GPR55 in bone physiology, regulating osteoclast formation and function and bone mass [115]. This group reported that administration of **4** (10 mg kg^{-1}) to mice three times daily for 8 weeks significantly reduced bone resorption in these mice.

Outside of the endocannabinoid system, **4** has numerous targets and its activity at these targets results in a variety of physiological effects. Some of these physiological effects may be mediated by more than one target, such as the anti-inflammatory and immunosuppressive effect of **4**. These effects are mediated by both adenosine mechanisms and via strychnine-sensitive glycine receptors, as detailed in the following paragraphs.

Table 6 A comparison of select in vitro studies showing cannabinoid receptor independent activity of cannabidiol (**4**) according to concentrations, assay types, and cell types used

Target	Concentration/ μ*M*	EC_{50}/μ*M*	IC_{50}/μ*M*	Assay	Cell line	Ref.
GPR55	<1		ND	Two photon Ca^{2+} imaging	Hippocampal slices/Rats	[114]
	<1		0.45	GTPγS binding assay	HEK293/Human	[67]
	<1		0.45	Rho/ERK1/2 activation	Human osteoclasts	[115]
Glycine receptors						
α1 subunit	1–300	12.3 (allosteric)		Patch clamp	HEK293/ND	[117]
		132.4 (direct)				
α1β subunit	1–300	18.1 (allosteric)		Patch clamp	HEK293/ND	
		144.3 (direct)				
α3 subunit	0.01–50	3 (direct)		Patch clamp	HEK293/ND	[118]
$5HT_{1A}$	<1	0.007		GTPγS binding assay	Rat brainstem	[123]
	>10	ND	ND	[3H]-8-OH-DPAT ligand binding	CHO/Human	[122]
				[35S]-GTPγS assay		
				Forskolin		
$5HT_{2A}$	>10	ND	ND	[^{3}H]-Ketanserin	NIH 3 T3 membrane/rat	
$5HT_{3A}$	<1		0.6	Patch clamp	*Xenopus laevis* oocytes	[135]
PPARγ	1–10	5		Contraction	Aorta/rat	[166]

Mitochondrial Complex I Complex II Complex IV	1–10, >10			High resolution respirometry	Brain cortex/Pig	[170]
			8.2			
			19.1			
			18.8			
CYP2C19 CYP3A5 CYP2C9 CYP1A1	1–10		0.5–2.7	HPLC, Fluorescence FLUOSTAR OPTIMA	Recombinant/Human liver microsomes	[167, 168, 171, 172]
Cav3.1–3.3 T-type	1–10		0.78–3.7	Whole cell patch clamp	HEK293/Human, Sensory neurons/Mouse	[173]
TRPA1	<1	0.096		Ca^{2+} Fluorescence assay	HEK293/Rat	[79]
		0.11	0.06		HEK293/Rat	[80]
TRPM8	<1		0.14	Ca^{2+} Fluorescence	HEK293/Rat	[79]
			0.06		HEK293/Rat	[80]
TRPV1	1–10	3.5		Ca^{2+} Fluorescence assay	HEK293/Human	[136]
	1–10	1			HEK293/Human	[80]
TRPV2	1–10	1.25		Ca^{2+}Fluorescence	HEK293/Rat	
	>10	22.2		Ca^{2+} mobilization	U87MG/Human	[174]
TRPV3	1–10	3.7		Ca^{2+} Fluorescence	HEK293/Rat	[81]
TRPV4	1–10	0.8			HEK293/Rat	
Adenosine uptake	<1		0.12	Scintillation counting [^{3}H] Adenosine	EOC-20 microglia	[116]
			0.19	Scintillation counting [^{3}H] Adenosine	RAW264.7 macrophages	
	1–10		3.5	Dual label counting [^{3}H] Adenosine	Striatal tissue synaptosome/ Mouse, Rat	[175]

8-OH-DPAT, 8-hydroxy-2-(dipropylamino)-tetralinhydrobromide; HPLC, high-performance liquid chromatography; also see Tables 1 and 2

Activity of **4** at one target may also elicit various physiological effects. This is shown by **4** having anti-inflammatory effects and antiarrhythmic effects both mediated by adenosine mechanisms. Another example refers to the 5HT serotonin receptors of a target where **4** acts to mediate multiple physiological effects such as acute autonomic responses to stress, nausea and vomiting, cerebral infarction and anxiolytic, panicolytic, and antidepressant effects. The sections below will describe in more detail the studies that support evidence for the numerous and varied physiological targets of **4**.

It is known that **4** has anti-inflammatory and immunosuppressive effects, but these effects have been shown to be mediated by multiple pharmacological targets, as detailed below. The mechanisms by which **4** possibly mediate anti-inflammatory and immunosuppressive effects include: activity at A_{1A} and A_{2A} adenosine receptors and the inhibition of the equilibrative nucleoside transporter [116] and the activation of strychnine-sensitive α_1 and $\alpha_1\beta$ glycine receptors [117, 118].

The effects of **4** mediated via adenosine have been shown in both in vitro and in vivo studies. Uptake of [^{3}H] adenosine was inhibited by **4** in murine microglia and RAW264.7 macrophages by a mechanism of binding to the equilibrative nucleoside transporter 1 (ENT1) and competitively inhibiting this nucleoside transporter with a K_i value of less than 0.25 μM and a submicromolar half maximal response [116] (Table 6). In addition to inhibition of ENT1 uptake of adenosine, the authors also documented in vivo that **4** could bind and activate the A_{2A} receptor, since the effects of **4** on tumor necrosis factor α (TNF α) were abolished by an A_{2A} receptor antagonist and by genetic deletion of this receptor [116]. An in vivo effect of **4** on anti-inflammatory effects mediated by the A_{2A} receptor was shown with lipopolysaccharide-induced inflammation in the rat retina [119] and in the mouse lung [120], both using the A_{2A} receptor antagonist ZM241385. The study by Liou and co-workers that indicated inhibition of adenosine uptake by ENT1 is important in the anti-inflammatory effects of **4** both in vitro and in vivo in the rat retina [119].

These studies clearly indicated that **4** has immunosuppressive effects that are mediated via adenosine mechanisms. This immune-suppressive effect is important in limiting cellular stress and inflammation and perhaps explains the effect of **4** on improving arthritis and multiple sclerosis symptoms. Its immunosuppressive effects in microglia would have considerable benefits for a number of neurodegenerative conditions.

The anti-inflammatory effects of **4** mediated through strychnine-sensitive α_1 and $\alpha_1\beta$ glycine receptors have also been shown in in vitro and in vivo studies but the in vitro study detailed below would be physiologically irrelevant due to the high concentrations used to elicit an effect that would not be achieved in vivo. The study used a whole cell patch clamp technique to show that **4**, at a mid-micromolar range, had positive allosteric modulating effects at these glycine receptor subunits and at higher concentrations showed direct activation of these receptor subunits (Table 6) [117].

It has also been reported that **4** has anti-inflammatory actions and suppresses neuropathic pain in vivo, mediated by glycine receptors. In α3 glycine channel knockout mice injected with Freund's adjuvant into the hind paw, the anti-

inflammatory effects of **4** (50 mg kg^{-1} i.p.) in this model of inflammatory pain were abolished [118].

Another physiological effect of **4** mediated via adenosine mechanisms is its antiarrythmic effect, shown by inhibiting ventricular tachycardia in rats [121]. This effect was shown using a low dose of **4** of 50 μg kg^{-1} and agonism of the A_{1A} receptor by **4** was validated by administration of the selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), at 100 μg kg^{-1}. In the presence of this selective antagonist, these effects of **4** were abolished [121]. Importantly, this effect was also determined to be a molecular effect rather than a functional one since a DCPX-only treatment group showed no effect on the incidence or duration of arrhythmias.

Significant evidence supports **4** producing its effects via serotonin (5-HT) receptors, predominantly the $5HT_{1A}$ receptor subtype but also the $5HT_{3A}$ receptor and less so at the $5HT_{2A}$ receptor. As described above, activity at these receptors mediates a variety of physiological responses.

In two in vitro studies, first in Chinese hamster ovary (CHO) cells [122] and more recently using rat brainstem membranes [123], **4** was found to enhance the ability of a $5HT_{1A}$ agonist, 8-OH-DPAT, to stimulate GTPγS binding at submicromolar concentrations (Table 6).

In vivo, **4** induces various $5HT_{1A}$-mediated physiological responses. These include attenuation of: acute autonomic responses to stress, nausea and vomiting, and cerebral infarction, and induction of anxiolytic, panicolytic, and antidepressant effects [123–133]. Studies with evidence supporting these effects are detailed below.

At doses of 1, 10, or 20 mg kg^{-1} (i.p.) of **4** in male Wistar rats, this compound dose-dependently reduced the acute autonomic response to restraint stress and reduced the anxiety behavior caused by previous exposure to restraint [124]. These effects of **4** were blocked by the $5HT_{1A}$ receptor antagonist, WAY100635 (0.1 mg kg^{-1}), while by itself WAY100635 did not have an effect on cardiovascular or anxiogenic responses, indicating this to be a molecular effect [124]. Another study reported that **4** administration directly into the dorsal periaqueductal gray via an implanted cannula in rats elicits panicolytic effects by inhibiting escape responses in the elevated T maze via $5HT_{1A}$ mediated responses. These responses were blocked by treatment with WAY100635 [125]. In both of these studies, a WAY100635-only treatment group was not used and therefore these results are not indicative of a molecular effect.

Activation of $5HT_{1A}$ receptors has been regularly related to the therapeutic effect of antidepressant treatments [130] and a reduced number/affinity of postsynaptic $5HT_{1A}$ receptors in the brains of depressed individuals has been reported by a number of studies [131, 132]. The first study to investigate whether there is a link between these receptors and the antidepressant effects of **4** was conducted quite recently by Zanelati and co-workers [126]. Mice received i.p. injections of 3, 10, 30, and 100 mg kg^{-1} **4** and were then subjected to the forced swimming test. This test is predictive of antidepressant-like activity [133]. Immobility time was reduced by **4** and showed a bell-shaped response, since **4** was only effective at 30 mg kg^{-1}

and not at the lower or higher doses [126]. The $5HT_{1A}$ receptor antagonist WAY100635 blocked the effects of **4** on antidepressant-like activity but mediation of this effect by $5HT_{1A}$ receptors was not validated by use of a WAY100635-only treatment group.

Various studies have reported **4** to have antiemetic- and antinausea-like effects and this is thought to be mediated by $5HT_{1A}$ receptors. Unlike **1**, for which the antiemetic effects are mediated by both CB_1 receptors and 5HT receptors (as described in Sect. 2.2), it appears that **4** exerts its antiemetic effects primarily through 5HT receptors. Three studies showed that a low dose of **4** (5 mg kg^{-1} i.p.) suppressed nicotine, cisplatin, and lithium chloride-induced vomiting in house musk shrews (*Suncus murinus*) [123, 127, 128] and lithium chloride-conditioned gaping in rats [123]. This suppression of vomiting and conditioned gaping was abolished by pre-treatment with the $5HT_{1A}$ receptor antagonists, WAY100135 and WAY100635 [123], but since an antagonist-only treatment group was not included in this study, an effect of **4** being mediated by $5HT_{1A}$ receptors has not been validated and it only showed a functional effect.

Neuroprotective effects of **4** have been shown through increasing cerebral blood flow and reducing infarct volume in a mouse model of middle cerebral artery occlusion [129]. This effect has been claimed to be CB_1 receptor independent [134] and in this study the effects of **4** were opposed by WAY100135 but not by the CB_1 receptor antagonist, rimonabant [129]. The neuroprotective effects of **4** have been claimed here to be mediated by $5HT_{1A}$ receptors but since a WAY100135-only treatment group was not included, this effect may be functional rather than molecular.

The only study to date investigating **4** activity at $5HT_{3A}$ receptors in vitro was conducted using *Xenopus laevis* oocytes expressing mouse $5HT_{3A}$ receptors using two electrode voltage clamp techniques [135]. In this study, **4** reversibly inhibited 5HT evoked currents in a concentration-dependent manner, which indicated that **4** is a non-competitive antagonist of $5HT_{3A}$ receptors (Table 6) [135]. This antagonist activity of **4** at $5HT_{3A}$ receptors may also be involved in the control of pain and emesis as described for **1** [46–50].

Activity of **4** at $5HT_{2A}$ receptors seems to be minimal and studies to date are not physiologically relevant, as shown with the high concentrations used in the following study. Using NIH/3 T3 cells expressing rat $5HT_{2A}$ receptors, **4** showed activity as a partial agonist but only at a concentration of 32 μ*M* and furthermore it only had 50% efficacy at displacing [^{3}H]-ketanserin [122]. The concentration used here would not be reached in vivo.

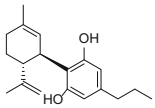
Unlike most of the other phytocannabinoids, **4** has been reported to act at TRP cation channels not just in vitro but in vivo as well. In vitro, **4** has been reported by numerous studies to activate TRPV1, TRPV2, and TRPA1 channels [80, 136–139] in HEK293 cells expressing these channels (Table 6). The TRPA1 channels are potently activated by **4**, with this compound being the second most potent agonist at this channel of all the phytocannabinoids tested in this study (Table 6) [80]. Indeed, all phytocannabinoids mentioned in this chapter, except cannabichromene (**7**), antagonize the Ca^{2+} elevation response induced by the agonist icilin [79, 80], but

4 is the most potent antagonist at this channel (Table 6) [80]. Moreover, in a recent study using whole cell patch clamp techniques on HEK293 transfected cells, it was shown that **4** (3, 10, 30 μ*M*) dose-dependently activated and rapidly desensitized TRPV1, TRPV2, and TRPA1 channels [139]. The TRPV3 channels are activated by **4** with high efficacy (50% higher than that of ionomycin) and high potency (Table 6) [81].

In vivo, **4** shows possible activity at TRPV1 channels in mice and rats [102–104] and TRPA1 channels in rats [140]. The effects of **4** in one of these studies was blocked by the TRPA1 selective antagonist, AP18, and by the TRPV1 selective antagonist, 5-iodo-resiniferatoxin, indicating involvement of these channels in the tail-flick related antinociception effects of **4** in anesthetized rats [140]. However, these antinociceptive effects were also blocked by the CB_1 receptor-selective antagonist, AM251, the $5HT_{1A}$ receptor antagonist, WAY100635, and also the adenosine A_1-selective antagonist [140]. This indicated that the descending pathway of antinociception in rats is possibly mediated by various mechanisms and the mechanism by which **4** mediates antinociception needs to be explored further.

The non-psychotropic quality of **4** provides promise for its use in the clinic and its "taming" of the effects of **1** have also proven beneficial in a licensed cannabis extract medication currently on the market in several countries, Sativex®. This medication is for the treatment of spasticity in multiple sclerosis patients and contains equal ratios of **4** and **1**. Here **4** functionally, not molecularly, antagonizes the undesirable effects of **1**, thus increasing its therapeutic index [113]. This reported "antagonism" may be explained by the negative allosteric modulation of CB_1 receptors as described in Sect. 5.1. Under the names Epidiolex® [141] and Cannabidiol Oral solution [142], **4** has been granted Orphan Drug designation by the U.S. FDA for treatment of Dravet syndrome and Lennox-Gastaut syndrome, both of which being forms of childhood-onset epilepsy. Epidiolex is in Phase 3 trials for Dravet syndrome and Lennox-Gastaut syndrome and Cannabidiol Oral Solution is in Phase 1 clinical trials for both these syndromes [141, 142]. Epidiolex is also nearing the end of Phase 2 trials for tuberous sclerosis, a genetic disease that results in benign tumor growth in the brain and other vital organs. Novagant Corp. has released GoldenCBD™ in capsule and liquid form. This is cannabidiol-rich hemp oil that is being marketed as medical marijuana for people living outside the states of Washington and Colorado in the USA [143].

6 Cannabidivarin



5

Cannabidivarin (**5**) is the *n*-propyl analog of **4**, therefore being part of the **4** chemical class type (Table 1) and like **4** it is non-psychotropic. Little is known about the pharmacological properties of **5** [103] and how it exerts its therapeutic benefits. It was first isolated in 1969 by Vollner and co-workers [103], but, since its classification, relatively few studies have been conducted to determine its pharmacological profile.

6.1 Activity at Cannabinoid Receptors

Pure **5** and **5**-enriched cannabis extracts are known to be CB_1 independent due to the lack of effect on motor function in a battery of motor tasks [2, 144]. Additionally, in a CP-55940 radioligand-binding assay using MF1 whole mouse brain and in CHO cells expressing human CB_1 receptors, pure **5** only displaced CP-55940 at the highest concentration tested (10 μ*M*) and a **5**-enriched extract showed very weak affinity for CB_1 receptors, displacing CP-55940 only weakly [2]. For a summary of **5** binding affinities to CB_1 and CB_2 receptors, see Table 2.

6.2 Cannabinoid Receptor Independent Activity

De Petrocellis and co-workers showed **5** to have agonist and antagonist effects at (TRP) cation channels. At human TRPA1 channels, **5** is a potent agonist and a less potent agonist at human TRPV1 and TRPV2 channels [80]. In this study, when **5** was given to TRPM8 transfected HEK293 cells, it antagonized the Ca^{2+} elevation response elicited by the agonist icilin. With the same potency, **5** induced intracellular Ca^{2+} elevation at the TRPV4 channel and is also an agonist at TRPV3 channels (Table 7) [81].

A recent study using whole cell patch clamp techniques on HEK293 transfected cells reported that **5** (3, 10, 30 μ*M*) dose-dependently activated, and rapidly desensitized, TRPV1, TRPV2, and TRPA1 channels [139]. Previous work has shown **5** to have antiepileptiform activity in rat hippocampal slices [144] and Iannotti and co-workers showed there to be significant TRPV1 transcript expression in rat hippocampal slices [139]. This group therefore conducted multi-electrode array (MEA) experiments, which showed that **5** and the TRPV1 agonist, capsaicin,

Table 7 A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabidivarin (**5**) according to concentrations, assay types, and cell types used

Target	Concentration/ μ*M*	EC_{50}/ μ*M*	IC_{50}/ μ*M*	Assay	Cell type	Ref.
GPR55	<1	0.4		ERK1/2 MAPK phosphorylation	HEK293/ Human	[69]
TRP cation channels						
TRPA1	1–10	ND	ND	Whole cell patch clamp	Hipocampal slices/Rat	[139]
	<1	0.42		Ca^{2+} Fluorescence assay	HEK293/Rat	[80]
TRPM8			0.9		HEK293/Rat	
TRPV1	1–10	3.6			HEK293/ Human	
TRPV2	1–10	ND	ND	Whole cell patch clamp	Hippocampal slices/Rat	[139]
	1–10	7.3		Ca^{2+} Fluorescence assay	HEK293/Rat	[80]
TRPV3		1.7			HEK293/Rat	[81]
TRPV4	<1	0.9			HEK293/Rat	

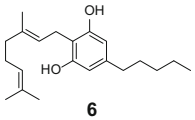
See Tables 1 and 2

produced similar effects on epileptiform activity induced in rat hippocampal slices. The effects of capsaicin on burst amplitude were reversed by the selective TRPV1 antagonist, IRTX, but the effects of **5** were not. This indicated that the anti-epileptiform effects of **5** are not mediated by activity at TRPV1 channels [139].

In vivo, **5** has been shown to display anticonvulsant properties in various acute animal models of seizure [144] and is currently in Phase 2 clinical trials as an antiepileptic drug [141]. The mechanism of action underlying these effects, however, is yet to be determined. In the pentylenetetrazole (PTZ) model of acute seizure, **5** (400 mg kg^{-1} p.o.) exhibited anticonvulsant effects by significantly reducing PTZ-induced seizure activity, in male Wistar rats, which was correlated with changes in gene expression of various epilepsy-related genes [145]. Of note is the clinical relevance of the route of administration used in this study (per os) compared to other in vivo studies where administration is via non-clinically relevant routes. The mechanism by which **5** induces changes in these epilepsy-related genes requires investigation.

Apart from currently being in clinical trials for epilepsy, **5** is also in clinical trials for glioma, type-2 diabetes and schizophrenia and has received U.S. FDA Orphan Drug Designation for neonatal hypoxic-ischemic encephalopathy [141]. This phytocannabinoid, like **4**, is therefore proving to be a promising therapeutic constituent of cannabis.

7 Cannabigerol



6

Cannabigerol (**6**) is another non-psychotropic phytocannabinoid and its chemical class type is the second most abundant in the cannabis plant, making up 16.3% of the phytocannabinoid content [1]. The carboxylic acid form of this phytocannabinoid, cannabigerolic acid (CBGA), is very important for the synthesis of other phytocannabinoids. In fresh cannabis plant material, phytocannabinoids are present in their carboxylic acid forms [146]. Cannabigerolic acid is the precursor to the acid forms of three phytocannabinoids: Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA) [147–149]. Cannabigerovaric acid (CBGVA) is the precursor of the *n*-propyl analogues of the carboxylic acid derivatives Δ^9-THCVA, CBDVA, and CBCVA [146]. Upon heating and storage of cannabis plant material these acid forms undergo decarboxylation to produce the non-acid forms, such as **1** and **2** [150]. Furthermore, under prolonged storage and drying some of these non-acid forms undergo oxidative catabolism to other phytocannabinoids. An example of this is oxidative catabolism of **1** to **3**, as described in Sect. 4 [84]. This phytocannabinoid was first isolated by Gaoni and Mechoulam in 1964 [151] and since then only a few studies have been conducted to investigate its pharmacological actions.

7.1 Activity at Cannabinoid Receptors

The non-psychotropic effect of **6** is explained by its low affinity for CB_1 receptors (Table 2) [103] and it has been shown in vivo to not produce psychotropic effects like **1** [152]. It does however affect endocannabinoid tone indirectly by inhibiting anandamide uptake, thereby increasing levels of anandamide, as shown in Table 8.

Table 8 A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabigerol (**6**) according to concentrations, assay types, and cell types used

Target	Concentration/ μ*M*	EC_{50}/ μ*M*	IC_{50}/ μ*M*	Assay	Cell type	Ref.
GPR55	1–10	2.16		ERK1/2 MAPK phosphorylation	HEK293/ Human	[69]
α2 adrenoceptor	<1	0.0002		GTPγS binding assay	Brain membranes/Mouse	[153]
		0.072		Electrically invoked contractions	Vas deferens/ Mouse	
TRP cation channels						
TRPA1	1–10	3.4		Ca^{2+} Fluorescence assay	HEK293/Rat	[79]
	<1	0.7			HEK293/Rat	[80]
TRPM8			0.16		HEK293/Rat	[79]
			0.16		HEK293/Rat	[80]
TRPV1	1–10	1.3			HEK293/ Human	
TRPV2		1.72			HEK293/Rat	
TRPV3		1.0			HEK293/Rat	[81]
TRPV4		5.1			HEK293/Rat	
Anandamide uptake			11.3	[^{14}C]-AEA uptake	RBL-2H3 cells/Rat	[80]

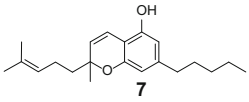
AEA, anandamide (arachidonoylethanolamine); also see Tables 1 and 2

7.2 Cannabinoid Receptor Independent Activity

Despite the relatively few investigational studies conducted, there is evidence of pharmacological actions at a number of targets. In a study using mouse brain membranes, **6** acted as a potent α2 adrenoceptor agonist [153]. The same study found **6** to moderately block $5HT_{1A}$ receptors with a K_B value of 0.0519 μ*M*. This effect is opposite to that of **4** on $5HT_{1A}$ receptors and explains the ability of **6** to antagonize the antinausea and antiemetic effect of **4** [154].

Like many phytocannabinoids, **6** interacts with numerous TRP cation channels. It is a potent TRPA1 agonist, a weak agonist at TRPV1 and TRPV2 and a potent TRPM8 antagonist (Table 8) [80].

8 Cannabichromene



7

Cannabichromene (**7**) is one of the most abundant phytocannabinoids naturally occurring in the cannabis plant, with its chemical class type making up the same percentage as that of the **4** chemical class type (Table 1) [1, 151, 155]. It was discovered independently by Claussen and co-workers and Gaoni and Mechoulam in 1966 [103].

8.1 Activity at Cannabinoid Receptors

Cannabichromene has not been found to have significant affinity for CB_1 or CB_2 receptors as shown in Table 2 but it does, however, affect endocannabinoid tone indirectly by inhibiting cellular uptake of anandamide (Table 9) [80].

8.2 Cannabinoid Receptor Independent Activity

The most notable pharmacological action of **7** to date is most likely its effect at TRP cation channels. At TRPA1 channels, **7** was found to be the most potent agonist of all the phytocannabinoids tested and also desensitized the TRPA1 channel to activation by the agonist allyl isothiocyanate [80]. At a lower potency, but still within the lower micromolar range, **7** was able to activate TRPV3 and TRPV4 channels and also desensitize TRPV4 channels to an agonist (9.9 μM) [81]. At the TRPV2 channel, **7** was only found to desensitize the channel and although **7** was found to block TRPM8 channel activation, this was at a very low potency [80] and would not be deemed physiologically relevant in vivo (Table 9) [23].

At a concentration of 1 μM, **7** has also been reported to act via ATP upregulation and adenosine signaling to raise the viability of adult mouse neural stem/progenitor cells (NSPCs) during differentiation [156]. The adenosine A_{1A} receptor selective antagonist, DPCPX, countered the stimulation of ERK1/2 phosphorylation by **7** and the upregulation of the astrocyte marker nestin by **7**.

Table 9 A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabichromene (**7**) according to concentrations, assay types, and cell types used

Target	Concentration/μ*M*	EC_{50}/μmol	IC_{50}/μ*M*	Assay	Cell type	Ref.
TRP cation channels						
TRPA1	<1	0.06		Ca^{2+} Fluorescence assay	HEK293/Rat	[79]
		0.09			HEK293/Rat	[80]
TRPM8	>10		40.7		HEK293/Rat	
TRPV1		24.2			HEK293/Human	
TRPV3	1–10	1.9			HEK293/Rat	[81]
TRPV4	<1	0.6			HEK293/Rat	
Anandamide uptake	>10		12.3	[^{14}C]-AEA uptake	RBL-2H3 cells/Rat	[80]
Adenosine A_{1A} receptor		ND		MTT (viability) assay	Neuroprogenitor cells (NSPCs)/Mouse	[156]

See Tables 1 and 2

9 Conclusions

This chapter has reviewed the molecular pharmacology of the seven most thoroughly studied phytocannabinoids and demonstrated that each has a diverse set of pharmacological targets with varying therapeutic, recreational and toxicological effects. Even slight structural differences between the phytocannabinoids can produce very diverse and competing physiological effects. Investigations into some of the phytocannabinoids have produced conflicting results, as mentioned in this chapter. Thus, it is critical to take into account the differences in assays used, the species from which the target is taken and the concentrations used in in vitro studies in order to predict the pharmacology of the phytocannabinoids at the system level. It is important that the concentrations used to elicit a response in vitro are indicative of the levels that will be reached after administration in animal models or in the clinic, otherwise no predictions can be made on the physiological relevance of results from in vitro studies. As highlighted by McPartland and co-workers, it is imperative that when analyzing the results of various studies one takes into account interspecies differences in receptor distribution and differences among different tissues and cell types [105]. Moreover, it is also important when designing experiments to look at the therapeutic benefits of a phytocannabinoid that the species used, route of administration of the compound, and concentrations used are clinically relevant, i.e. applicable to the end target species. It is therefore important to assess species differences in receptor orthologues and distribution, remembering that there are molecular divergences between human and rodent orthologues such as, for example, within the endocannabinoid system [157].

This chapter has also highlighted the importance of each individual phytocannabinoid in mediating the therapeutic and recreational effects of cannabis. Two phytocannabinoids, **4** and **5**, may prove to be clinically useful constituents of cannabis. Both phytocannabinoids have been granted Orphan Drug designation by the U.S. FDA for a number of seizure-related disorders and, as a result, Phase II and III clinical trials are underway [141–143]. The conduct of formal clinical trials using these non-Δ^9-tetrahydrocannabinol phytocannabinoids could stimulate new research of cannabis and its constituents and see additional phytocannabinoids objectively assessed for therapeutic potential. Even though research on individual phytocannabinoids has been conducted for many years, still much more research is warranted. The cannabis plant contains about 120 phytocannabinoids, which shows, in reality, how little research has been conducted on these compounds. Further research on the "known" phytocannabinoids as well as the "unknown" phytocannabinoids would greatly advance our understanding of these substances alone as well as in conjunction with each other or as part of a whole in cannabis.

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Molecular Targets of the Phytocannabinoids: A Complex Picture

Paula Morales, Dow P. Hurst, and Patricia H. Reggio

Contents

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1 Introduction

For centuries, hashish and marihuana, both derived from the Indian hemp *Cannabis sativa* L. (Cannabaceae), have been used for their medicinal as well as their psychotropic effects. Phytocannabinoids are oxygen-containing C_{21} aromatic hydrocarbons found in *C. sativa*. To date, about 120 phytocannabinoids have been isolated from *Cannabis*, including two compounds, (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC) (**1**) and (−)-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC) (**4**) (Fig. 1) that have been shown to bind to cannabinoid receptors and elicit the characteristic psychotropic effect associated with *Cannabis* [1]. These compounds also have beneficial effects, such as appetite stimulation [2], analgesia [3], antiglaucoma [4], and antiemetic effects [5]. Non-psychotropic phytocannabinoids are currently emerging as key constituents of *Cannabis* as well. For example, the non-psychotropic phytocannabinoid, cannabidiol (CBD) (**3**), is of great interest because of its anti-inflammatory, analgesic, antianxiety, and antitumor properties [6]. For many years, it was assumed that the beneficial effects of the cannabinoids were mediated by the cannabinoid receptors, CB_1 and CB_2. However, today we know that the picture is much more complex, with the same phytocannabinoid acting at multiple targets. This chapter focuses on the molecular pharmacology of the phytocannabinoids, including Δ^9-THC (**1**) and CBD (**3**), from the perspective of the targets at which these important compounds act.

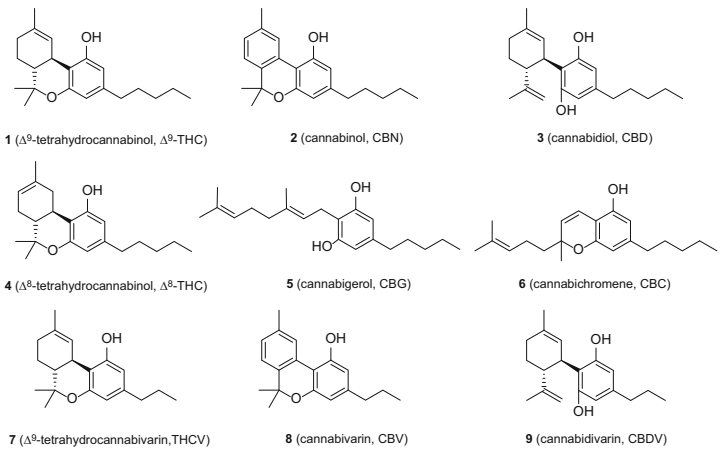

Fig. 1 Structures of the most abundant phytocannabinoids in *Cannabis sativa* L.

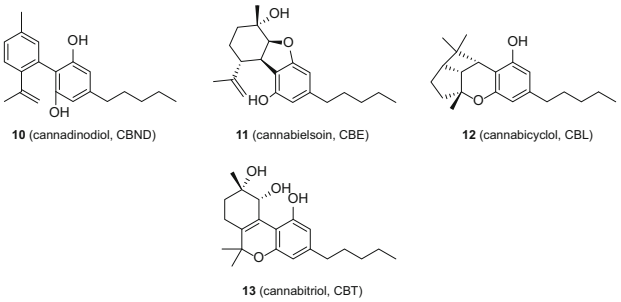

Fig. 2 Structures of phytocannabinoids at lower abundance in *Cannabis sativa* L.

2 Pharmacology of Selected Phytocannabinoids

As mentioned above, ca. 120 cannabinoids, or the so-called phytocannabinoids (pCB), have been isolated to date from the cannabis plant. Contrary to other naturally occurring drugs, such as opioids, nicotine, cocaine, or caffeine, cannabinoids do not contain nitrogen, and hence are not alkaloids. Most phytocannabinoids share common structural features that include a dibenzopyran ring and a hydrophobic alkyl chain. The most abundant cannabinoids in the plant are Δ^9-tetrahydrocannabinol (Δ^9-THC) (**1**), cannabinol (CBN) (**2**), cannabidiol (CBD) (**3**), Δ^8-tetrahydrocannabinol (Δ^8-THC) (**4**), cannabigerol (CBG) (**5**), cannabichromene (CBC) (**6**), Δ^9-tetrahydrocannabivarin (THCV) (**7**), cannabivarin (CBV) (**8**), and cannabidivarin (CBDV) (**9**) (Fig. 1). Despite their lower concentration levels in the plant, other phytocannabinoids such as cannabinodiol (CBND) (**10**), cannabielsion (CBE) (**11**), cannabicyclol (CBL) (**12**), and cannabitriol (CBT) (**13**) have also been the subjects of pharmacological study over the last few decades (Fig. 2) [7].

Phytocannabinoids show different affinities for CB_1 and CB_2 receptors. In addition, over the last years, molecular targets outside the endocannabinoid system have been identified for certain plant cannabinoids. These compounds have been shown to interact with other G-protein coupled receptors such as the putative cannabinoid receptors GPR55 or GPR18, and other well-known GPCRs such as the opioid or the serotonin receptors. In addition, several papers have reported the ability of certain phytocannabinoids to modulate nuclear receptors, ligand-gated ion channels or transient receptor potential (TRP) channels, among others.

2.1 Abundant Cannabinoid Constituents of Cannabis sativa

The table at the end of Sect. 2.2.4 provides a pharmacology summary for each of the abundant constituents of *Cannabis*.

2.1.1 Δ^9-Tetrahydrocannabinol (Δ^9-THC)

Δ^9-Tetrahydrocannabinol (Δ^9-THC) (**1**) is the principal component of the cannabis plant. As demonstrated by numerous in vitro and in vivo assays, **1** is a moderate partial agonist of the CB_1 and CB_2 receptors [8, 52, 53]. As a partial agonist, it presents a mixed agonist–antagonist profile depending on the cell type, the expression of receptors, and the presence of endocannabinoids or other full agonists [9]. This compound is largely responsible for the pharmacological properties as well as the psychoactive effects associated with marijuana use. Δ^9-Tetrahydrocannabinol (**1**) is also a multitarget ligand, with the non-CB_1, non-CB_2 activity of this compound being responsible for some of the physiological effects reported for this phytocannabinoid in vitro and in vivo.

Conflicting reports about the ability of this phytocannabinoid to modulate the putative cannabinoid receptor GPR55 have been published. Δ^9-Tetrahydrocannabinol (**1**) exhibits activation of GPR55 in [^{35}S]GTPγS binding, RhoA assays, and intracellular calcium mobilization in transiently transfected *h*GPR55-HEK293 cells [10–12]. However, this phytocannabinoid was found to be unable to stimulate ERK1/2 phosphorylation or β-arrestin recruitment [13, 14, 16]. It remains to be determined whether this is a consequence of experimental variability, differences in functional readouts or GPR55 intrinsic properties. In addition, studies from Anavi-Goffer and coworkers [17] have shown that **1** is able to inhibit the response generated by lysophosphatidylinositol (LPI), the proposed GPR55 endogenous ligand. For the putative cannabinoid receptor GPR18, studies in different cell models demonstrate that **1** acts as a potent agonist of this receptor [19, 54].

Δ^9-Tetrahydrocannabinol (**1**) has also been proposed to be a serotonin $5HT_{3A}$ receptor antagonist [18, 20] and an allosteric modulator of the opioid receptors [21].

Certain non-GPCRs have also been suggested as targets of **1**. This compound is a peroxisome proliferator-activated receptor gamma (PPARγ) agonist. The vascular relaxation and antitumor effects of **1** have been linked to its agonism at PPARγ [22, 23]. Low concentrations of **1** have been shown to significantly potentiate the amplitudes of glycine-activated currents [55, 56]. The activity of **1** at the glycine receptors seems to contribute to the cannabis-induced analgesia in behavioral mice models [55].

Δ^9-Tetrahydrocannabinol (**1**) did not show a response at the vanilloid type 1 receptor (TRPV1, also known as the capsaicin receptor), whereas several reports describe its agonistic effects at the TRPV2, TRPV3, and TRPV4 channels [24, 25, 57]. As further detailed in this chapter, **1** is also an agonist of the ankyrin channel TRPA1 and an antagonist of the melastatin receptor TRPM8 [24, 58].

2.1.2 Δ^8-Tetrahydrocannabinol (Δ^8-THC)

Δ^8-Tetrahydrocannabinol (**4**) is an isobaric isomer of **1** that differs in the position of the double bond (Fig. 1). Δ^8-Tetrahydrocannabinol (**4**) also displays psychoactivity

and is chemically more stable than **1** [26, 59]. Δ^8-Tetrahydrocannabinol (**4**) shows moderate partial agonistic effects on CB_1 and CB_2 receptors [27, 60]. Likewise, it exhibits similar in vitro and in vivo properties in different studies [28, 59, 61]. There is not much literature reported on the activity of **4** at other targets such as GPR55, GPR18, TRP channels, or PPAR nuclear receptors. However, this compound will presumably present a similar pharmacological profile to **1**.

2.1.3 Cannabinol (CBN)

Cannabinol (**2**) is an oxidized metabolite of **1** [29]. Its carboxylic acid derivative is present also in the plant and CBN is formed upon heating of this acid. CBN is a weak psychoactive compound that binds to the cannabinoid receptors showing higher affinity towards CB_2. Cannabinol (**2**) consistently has been reported to be a weak CB_1 agonist, although different results have been found regarding its CB_2 modulation. Mechoulam and coworkers [31] performed cAMP assays that revealed its agonist capacity, whereas in GTPγS experiments, this compound behaved as a CB_2 inverse agonist [62]. These divergences may be due to the experimental outcome or the dose utilized in each case.

Cannabinol (**2**) has also shown CB_1 and CB_2 independent activity. This compound is a potent agonist of TRPA1 and an antagonist of TPRM8 channels [25]. Besides the TRP channels, its activity at other receptors outside the endocannabinoid system has not been determined.

2.1.4 Cannabidiol (CBD)

Due to its promising therapeutic effects, cannabidiol (**3**) is one of the most studied cannabinoids today. This non-psychoactive compound has demonstrated anti-inflammatory, analgesic, antianxiety, and antitumor properties, among others [6].

Diverse research groups have reported the lack of affinity of **3** for the cannabinoid CB_1 and CB_2 receptors [63]. However, in vitro studies revealed that CBD displays weak CB_1 and CB_2 antagonistic effects [30, 32]. Recent results from Laprairie and colleagues show that CBD behaves as an negative allosteric modulator of Δ^9-THC- and 2-AG [33, 34]. These results may explain some of the in vivo effects of CBD. In addition, **3** is able to inhibit cellular uptake of the endogenous CB_1 ligand, anandamide (*N*-arachidonylethanolamine; AEA), directly affecting endocannabinoid tone. At the GPR55 receptor, this non-psychoactive phytocannabinoid acts as an antagonist preventing [^{35}S]GTPγS binding and Rho activation [12, 35, 36]. However, **3** was inactive in Ca^{2+} mobilization assays [10] and β-arrestin recruitment [13]. As demonstarted by McHugh and coworkers [19, 54], **3** is an antagonist of the putative cannabinoid receptor GPR18.

Cannabidiol (**3**) is further involved in the modulation of different receptors outside the endocannabinoid system (ECS). The serotonin receptors have been implicated in the therapeutic effects of **3**. Different studies revealed that this

phytocannabinoid acts as a full $5HT_{1A}$ agonist, a $5HT_{2A}$ weak partial agonist, and a non-competitive antagonist of $5HT_{3A}$ [37–39]. The ability of **3** to activate the A_{1A} adenosine receptors has also been proposed [40]. Its activity at these receptors could mediate the anti-inflammatory and immunosuppressive effects of **3**. The activity of **3** at the nuclear receptors PPARγ [41–43], the glycine ligand-gated ion channels [44, 45], and $GABA_A$ receptors [46], or at the transient receptor potential channels [25, 57] is summarized in the table at the end of Sect. 2.2.4.

Despite all of these pharmacological data, the mechanistic bases of the effects of **3** remain unclear. Therefore, great efforts are currently being made to fully elucidate the molecular pharmacology of **3**.

2.1.5 Cannabigerol (CBG)

Cannabigerol (**5**) is a non-psychoactive phytocannabinoid found in high concentration in the plant; its carboxylic acid derivative (CBDA, cannabigerolic acid) is the precursor of other important phytocannabinoids. Compound **5** has low affinity for the cannabinoid CB_1 and CB_2 receptors [47, 48, 64], but it affects the endocannabinoid system because of its ability to inhibit anandamide (AEA) uptake [25]. Cannabigerol (**5**) has also been shown to weakly inhibit the LPI response in GPR55 assays [17]. The non-cannabinoid activity reported for CBG involves its ability to potently activate the α2 adrenergic receptor and moderately block the serotonin $5HT_{1A}$ receptor [50].

As with many other phytocannabinoids, **5** interacts with different TRP channels acting as a weak TRPV1 and TRPV2 agonist, a potent TRPM8 antagonist, and a potent TRPA1 agonist [25, 58].

2.1.6 Cannabichromene (CBC)

Cannabichromene (CBC) (**6**) is one of the most abundant phytocannabinoids in the plant; it was discovered in 1966 by Gaoni and Mechoulam [48]. This phytocannabinoid does not display significant affinity for the cannabinoid CB_1 and CB_2 receptors [47]. Nonetheless, it directly influences the endocannabinoid system by inhibiting anandamide (AEA) uptake [25]. The more relevant pharmacological activity of **6** explored so far, is at TRP channels. Among the phytocannabinoids tested by De Petrocellis and coworkers [25], **6** is the most potent agonist of the TRPA1 channels. Although at a lower potency, CBC is also able to activate TRPV3 and TRPV4, and block TPRM8 receptors in the same cellular and functional outcome [25, 57].

2.1.7 Δ^9-Tetrahydrocannabivarin (Δ^9-THCV)

Δ^9-Tetrahydrocannabivarin (Δ^9-THCV) (**7**) is a *n*-propyl analog of **1**. Even though it only varies from **1** by the length of its lipophilic alkyl chain, it possesses a different pharmacological profile at certain molecular targets.

Discrepancies have been found regarding its activity at CB_1 receptors. Although the in vitro evaluation of this compound consistently displays antagonistic/inverse agonistic effects [49, 65, 66], at higher doses, the in vivo effects indicate agonism in an antinociception model [67]. Δ^9-Tetrahydrocannabivarin (**7**) is a CB_2 partial agonist as demonstrated in different in vitro and in vivo assays [51]. Recent studies suggest that this phytocannabinoid is a partial agonist of GPR55 in being also able to inhibit the activity of the full agonist LPI [17]. Beyond the endocannabinoid system, **7** has been reported to activate $5HT_{1A}$ receptors [68], as well as different TRP channel subtypes [25] (See table at the end of Sect. 2.2.4).

2.1.8 Cannabivarin (CBV)

Cannabivarin (CBV) (**8**) is a non-psychoactive phytocannabinoid found in the plant in low concentrations. It is a *n*-propyl derivative of cannabinol (**2**) and can be obtained as an oxidation product of tetrahydrocannabivarin (**7**) [69–71]. Its pharmacology has not been explored so far.

2.1.9 Cannabidivarin (CBDV)

Cannabidivarin (CBDV) (**9**) is a *n*-propyl analogue of cannabidiol (**3**) that lacks psychoactive properties. This compound displays very weak affinity for CB_1 and CB_2 receptors [47, 72]. Its ability to inhibit the activity of the putative endogenous ligand LPI has been reported in *h*GPR55-HEK293 cells [17].

Molecular targets outside the ECS have also been found for CBDV. The TRP channels are tightly involved in the therapeutic potential of this phytocannabinoid. CBDV potently activates human TRPA1 channel, being a weak agonist of the TRPV1, TRPV2, and TRPV3 cation channels [25, 57].

2.2 Less Abundant Cannabinoid Constituents of Cannabis sativa

Other cannabinoid compounds from the cannabis plant have been identified and structurally characterized. Total synthesis approaches have been intended for some of them, but the pharmacology of these phytocannabinoids has not been properly studied. Indeed, to the best of our knowledge, their activity at the well-known

cannabinoid CB_1 and CB_2 receptors, or other molecular targets has not been reported so far.

2.2.1 Cannabinodiol (CBND)

Cannabinodiol (CBND) (**10**) (Fig. 2) is a fully aromatized cannabidiol (**3**) analog that was first characterized in 1977 [73]. This phytocannabinoid can be obtained as a product of CBD photochemical conversion. Although its concentration in the plant is quite low, **10** is one of the psychoactive compounds found in the plant's flowers [74]. There are no available experimental data at present related to its pharmacological action on specific targets.

2.2.2 Cannabielsoin (CBE)

Cannabielsoin (CBE) (**11**) is a phytocannabinoid metabolite that can be produced by photo-oxidation from CBD and CBDA [75, 76], or by biotransformation using tissue cultures under normal growth conditions [77, 78]. The ability of this compound to modulate the cannabinoid CB_1 and CB_2 receptors has not been described thus far.

2.2.3 Cannabicyclol (CBL)

Cannabicyclol (CBL) (**12**) is a photochemical product that originates from the phytocannabinoid cannabichromene under heating conditions [15, 79]. This is important to take into account when considering that cannabis is frequently smoked for both medicinal and recreational purposes. No pharmacological evaluation of this phytocannabinoid has been reported.

2.2.4 Cannabitriol (CBT)

Cannabitriol (CBT) (**13**) was first isolated by Obata and Ishikawa in 1966 [80], but the structures of its *cis* and *trans* isomers were not fully determined until years later [74, 81]. Cannabitriol (**13**) has been synthesized by antibody-catalyzed oxidation of Δ^9-THC (**1**) [82]. No pharmacological evaluation of this phytocannabinoid has been reported.

Cannabinoid and non-cannabinoid molecular targets of selected phytocannabinoids

	CB activity			Non-CB_1/Non-CB_2 activity			
pCB	Target	Functionality	Ref.	Target		Functionality	Ref.
Δ^9-THC (**1**)	CB_1	Partial agonist	[2–4]	GPR55		Agonist	[8]
						NR	[9]
						LPI inhibitor	[10]
				GPR18		Agonist	[11, 12]
				$5HT_{3A}$		Antagonist	[13, 14]
	CB_2	Partial agonist	[3, 4, 15]	μ- and δ-OPR		Allosteric modulator	[16]
				PPARγ		Agonist	[17]
				GlyR	α_1	Positive allosteric modulator	[18]
					α_2	NR	[19]
					α_3	Positive allosteric modulator	[19]
				TRP channels		TRPV1 NR	[20]
						TRPV2, 3, 4 agonist	[20–22]
						TRPM8 antagonist	[23]
						TRPA1 agonist	[20, 23]
Δ^8-THC (**4**)	CB_1	Partial agonist	[24, 25]	–		–	–
	CB_2	Partial agonist	[24, 25]				
CBN (**2**)	CB_1	Agonist	[4]	TRP channels		TRPA1 agonist	[21]
	CB_2	Agonist	[26]			TRPM8 antagonist	[21]
		Inverse agonist	[27]				
CBD (**3**)	CB_1	Antagonist*	[28, 29]	GPR55		Antagonist	[8, 30]
				GPR18		Antagonist	[11, 12]
		Negative allosteric modulator	[31]	$5\text{-}HT_{1A}$		Agonist	[32, 33]
				$5\text{-}HT_{2A}$		Partial agonist*	[32]
				$5\text{-}HT_{3A}$		Antagonist	[34]
				A_{1A}		Agonist	[35]
	CB_2	Antagonist*	[29]	μ- and δ-OPR		Allosteric modulator	[16]
				PPARγ		Agonist	[36–38]
	AEA uptake	Inhibitor	[21]	GlyR	α_1	Positive allosteric modulator	[39]
					α_2	ND	–
					α_3	Positive allosteric modulator	[40]
				$GABA_A$		Positive allosteric modulator	[41]
				TRP channels		TRPV1, 2, 3 agonist	[21, 22]
						TRPA1 agonist	[21]

(continued)

pCB	CB activity			Non-CB_1/Non-CB_2 activity		
	Target	Functionality	Ref.	Target	Functionality	Ref.
CBG (**5**)	CB_1	Partial agonist*	[42–44]	GPR55	LPI inhibitor*	[10]
				$5\text{-}HT_{1A}$	Antagonist	[45]
				α_2-AR	Agonist	[45]
	CB_2	Partial agonist*	[42–44]	TRP channels	TRPV1, 2 agonist	[21]
					TRPM8 antagonist	[23]
	AEA uptake	Inhibitor	[21]		TRPA1 agonist	[21]
CBC (**6**)	CB_1	Agonist*	[42]	TRP channels	TRPV3, 4 agonist	[22]
	CB_2	Agonist*	[42]		TRPM8 antagonist	[21]
	AEA uptake	Inhibitor	[21]		TRPA1 agonist	[21]
Δ^9-THCV (**7**)	CB_1	Antagonist	[46–48]	GPR55	Partial agonist/ LPI inhibitor	[10]
				$5HT_{1A}$	Agonist	[49]
	CB_2	Partial agonist	[50]	TRP channels	TRPV2 agonist	[21]
					TRPM8 antagonist	[21]
					TRPA1 agonist	[21]
CBV (**8**)	CB_1	ND	–	–	–	–
	CB_2	ND	–			
CBDV (**9**)	CB_1	NR	[51]	GPR55	LPI inhibitor	[10]
	CB_2	NR	[42]	TRP channels	TRPV1, 2, 3 agonist	[21, 22]
					TRPA1 agonist	[21]
CBND (**10**)	CB_1	ND	–	–	–	–
	CB_2	ND	–			
CBE (**11**)	CB_1	ND	–	–	–	–
	CB_2	ND	–			
CBL (**12**)	CB_1	ND	–	–	–	–
	CB_2	ND	–			
CBT (**13**)	CB_1	ND	–	–	–	–
	CB_2	ND	–			

*Weak effect
NR no response; *ND* not determined

3 Molecular Targets of Phytocannabinoids

3.1 G-Protein Coupled Receptors

Many of the phytocannabinoids interact with the cannabinoid CB_1 and CB_2 receptors. The cannabinoid CB_1 [83] and CB_2 [84] receptors belong to the Class A (rhodopsin (Rho) family) of G-protein coupled receptors (GPCRs). Figure 3a illustrates that the general topology of a Class A GPCR includes: (1) an extracellular (EC) N terminus; (2) seven transmembrane alpha helices (TMHs) arranged to form a closed bundle; (3) loops connecting TMHs that extend intra- and extracellularly; and (4) an intracellular (IC) C terminus that begins with a short helical segment (Helix 8) oriented parallel to the membrane surface. Ligands for Class A GPCRs are generally thought to enter the receptor via the extracellular space. Figure 3b illustrates an extracellular view of the 2.8 Å resolution μ opioid receptor structure (PDB entry 4DKL). Here one can see the opening that allows the ligand, beta-funaltrexamine, to descend into the receptor binding pocket.

The docking of a GPCR agonist ligand triggers a conformational change in the receptor on its intracellular (IC) side most commonly by altering the proline kink angle in the TMH6 CWXP motif, allowing TMH6 to straighten. This change in angle breaks the IC salt bridge between R3.50 and D/E6.30 that maintains the GPCR inactive state. The overall conformational change creates an IC opening that allows the G-protein alpha-5 helix (which is located intracellularly) to insert into the receptor opening and form a receptor/G-protein complex. This, then, is the beginning of signal transduction.

In many ways, the CB_1 and CB_2 receptors are atypical within the Class A GPCRs [85]. The endogenous ligands for these receptors, *sn*-2-arachidonoylglycerol

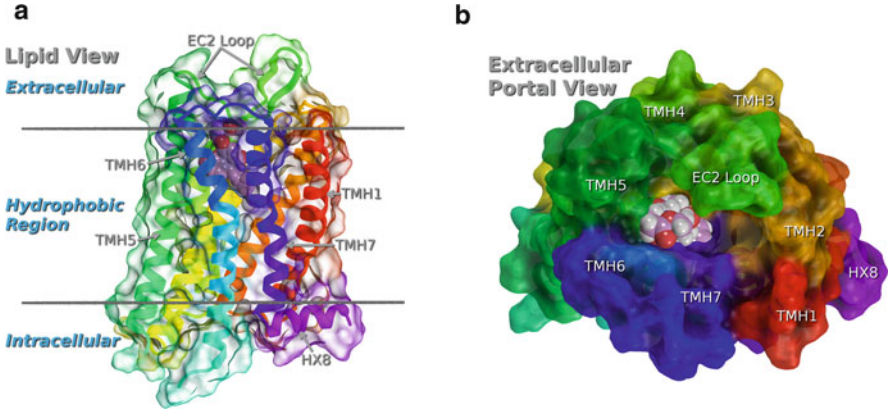

Fig. 3 (**a**) The typical Class A G-protein coupled receptor structure is illustrated by the 2.8 Å structure of the μ opioid receptor (MOR; PDB entry 4DKL). (**b**) An extracellular view of the MOR structure is illustrated. In the MOR, the extracellular loops of the receptor are splayed open, making ligand access from the extracellular milieu possible. Here, the covalent ligand, beta-funaltrexamine is bound

(2-AG) (CB_1 and CB_2) [86, 87] and anandamide (CB_1) [88] are lipid-derived agonists that are made on demand from the lipid bilayer and degraded by membrane associated enzymes [89–91], negating the need for vesicle storage. The CB_1 receptor and its endogenous ligand, 2-AG, have been shown to mediate depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE), at GABAergic and glutamatergic synapses [92]. To accomplish this regulation of neurotransmission, CB_1 has a presynaptic location, an atypical location for neuronal GPCRs. Although neither cannabinoid receptor has yet been crystallized, two Class A GPCRs that recognize lipid-derived ligands have been crystallized. This includes the $S1P_1$ receptor, which has over 60% homology with hCB_1 in the transmembrane helix (TMH) regions for which the endogenous ligand is also lipid-derived, sphingosine-1-phosphate [93]. The second GPCR is GPR40, which binds long-chain free fatty acids [94]. Two very striking features are evident in these crystal structures: (1) the extracellular domain of the receptor is completely covered by either the N-terminus [84] or the EC-2 loop [94], precluding ligand access from the EC milieu; (2) portals between TMHs through which ligands can be shuttled have been identified for each of these receptors and the location of the TMH portal varies between receptors and is dependent on the sequence of each receptor [85]. For the S1P1 receptor, the N-terminus occludes the binding site. Instead, a portal between TMH1 and TMH7 allows ligand access from the lipid

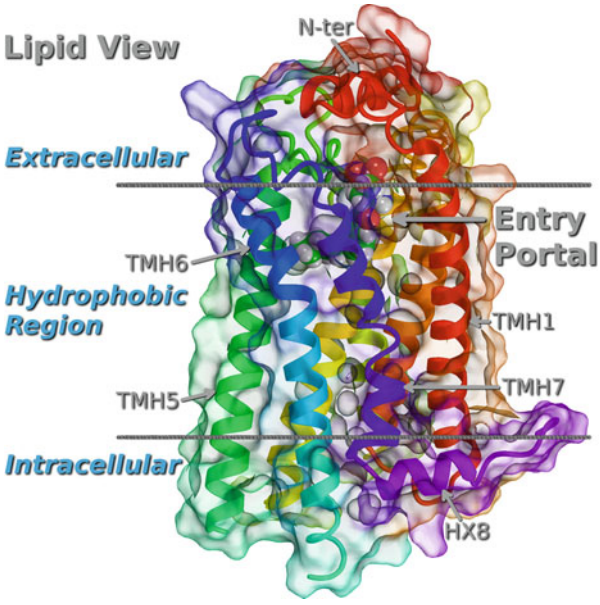

Fig. 4 The 2.8 Å structure of the S1P1 receptor is illustrated (PDB 3V2Y with antagonist, ML056). In this receptor, the N-terminus covers the EC side of the receptor, permitting no ligand access from the EC milieu. Instead, there is a portal between THH1 and TMH7 that allows ligand access from the lipid bilayer

bilayer. This is illustrated in Figure 4 (PDB 3V2Y; antagonist, ML056 bound; 2.8 Å resolution).

How Do Phytocannabinoids Reach the CB_1/CB_2 Binding Domain?
Molecular dynamics (MD) simulations reported by the Reggio laboratory have suggested that for CB_1 and CB_2, there is a ligand portal between TMH6 and TMH7 [95]. Figure 5 illustrates the CB_1/CB_2 ligand, 2-AG entering the CB_2 receptor via the lipid bilayer. This result is supported experimentally by covalent labeling studies from the Makriyannis laboratory, which pinpoint C6.47 (a lipid facing TMH6 residue) in CB_1 and CB_2 as the residue covalently labeled by the classical cannabinoid, AM841 which is isothiocyanate derivatized. This labeling of a *lipid facing residue* occurs despite the fact that other Cys residues face into the ligand binding pocket and are extracellular to C6.47 [96, 97].

Thus, for the cannabinoids, it is likely that high ligand lipophilicity is required for ligand access to the entry portal into CB_1 or CB_2. Table 1 provides calculated Qlog*P* values for the phytocannabinoids. Here it is clear that the phytocannabinoids do possess high lipophilicities.

The table at the end of Sect. 2.2.4 lists additional Class A GPCRs that have been implicated in various phytocannabinoid actions. These include the putative cannabinoid receptors GPR55 and GPR18, the serotonin-1A, -2A, -3A receptors ($5HT_{1A}$, $5HT_{2A}$, $5HT_{3A}$), the μ- and δ-opioid (MOR and DOR) receptors, the adenosine A_{1A}, receptor, and the α_2-adrenergic receptor (α_2-AR).

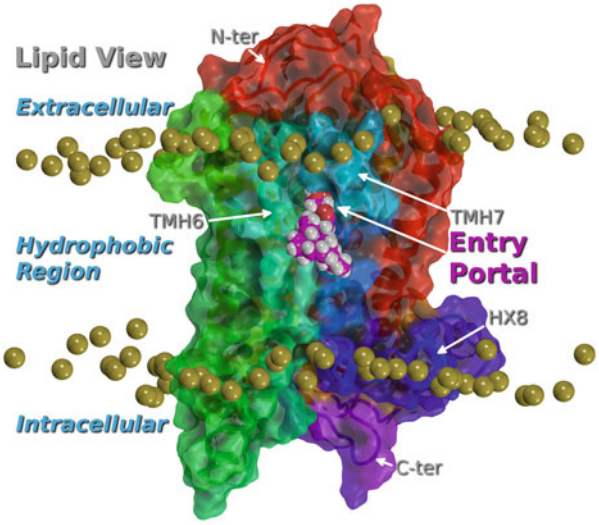

Fig. 5 Results from molecular dynamics simulations in which the CB endogenous ligand, 2-AG, enters the CB2 receptor from the lipid bilayer via a TMH6-TMH7 portal

Table 1 Physicochemical Qlog*P* descriptor of phytocannabinoids **1–13**; **12** and **13** were calculated with QikProp 3.5 integrated in Maestro (Schrödinger LLC, New York)

Compound	Qlog*P*[a]
Δ^9-THC (**1**)	5.627
Δ^8-THC (**4**)	5.630
CBN (**2**)	5.576
CBD (**3**)	5.414
CBG (**5**)	5.790
CBC (**6**)	5.954
Δ^9-THCV (**7**)	4.901
CBV (**8**)	4.855
CBDV (**9**)	4.648
CBDN (**10**)	5.299
CBE (**11**)	4.859
CBL (**12**)	5.575
CBT (**13**)	3.997

[a]Predicted octanol/water partition coefficient –(2.0/6.5) (range of 95% of drugs)

3.2 Beyond GPCRs: PPARs, GlyR, and TRP Channels

3.2.1 Peroxisome Proliferator-Activated Receptors (PPARs)

Increasingly over the past decade, research has shown that cannabinoids can modulate peroxisome proliferator-activated receptors (PPARs) [98–101]. Some of the physiological responses triggered by phytocannabinoids are partially mediated by these nuclear hormone receptors that control the transcription of target genes. Activation of PPARα and PPARγ isoforms is associated with some of the neuroprotective, antinociceptive, antiproliferative, anti-inflammatory and metabolic properties of cannabinoids. Therefore, the activity of phytocannabinoids at these receptors is tighly related with its therapeutic potential for the treatment of pathologies such as cancer, diabetes, obesity, as well as cardiovascular or neurodegenerative disorders.

How Do Phytocannabinoids Reach the PPAR Binding Domain?
Several reports have revealed that certain phytocannabinoids, especially Δ^9-THC (**1**) and CBD (**3**), can activate the transcriptional activity of PPARs and these effects can be blocked by PPAR antagonists. However, the mechanisms facilitating this activity are still under investigation [98, 100]. Based on different studies, direct binding of cannabinoids to the PPAR isoforms has been proposed [102, 103]. The PPAR ligand binding domain has an extensive secondary structure consisting of 13 alpha helices and a beta sheet. Many PPAR crystal structures, including a PPARγ complex with the THC acid synthetic analogue, ajulemic acid (AJA), have been already solved [103]. This crystallographic study revealed a low occupancy of the binding pocket explaining the structural basis for the weak PPAR activation produced by cannabinoids. On the other hand, metabolism of cannabinoids to active PPAR binders has also been suggested as a potential mechanism of interaction with these transcription factors [104]. Another

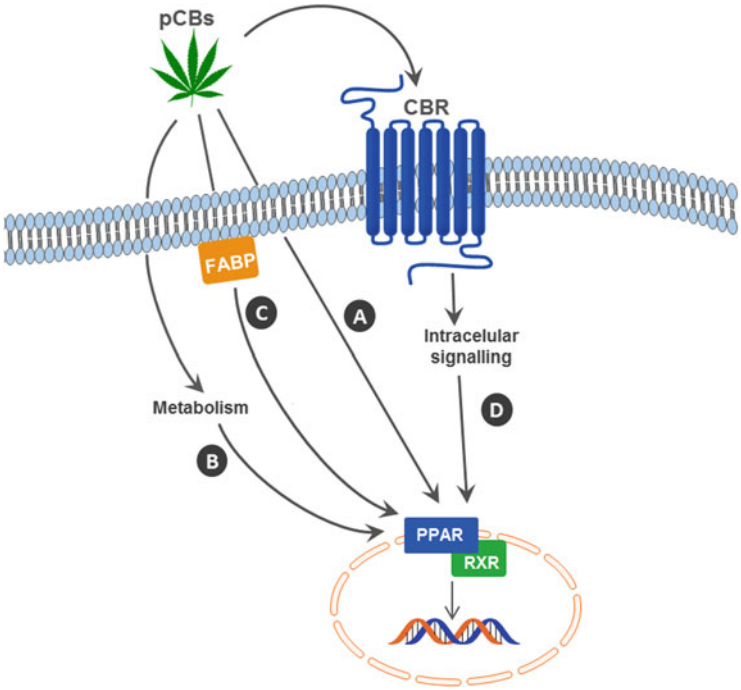

Fig. 6 Potential mechanisms of PPAR-phytocannabinoid interactions: (A) Direct binding of phytocannabinoids to these nuclear receptors; (B) Possible conversion of phytocannabinoids into metabolites that may activate PPARs; (C) Phytocannabinoid transported to the nucleus by FABPs; (D) Another possibility is that phytocannabinoids modulate CBR triggering intracellular signalling pathways that may lead to the activation of PPARs

possible mechanism triggering cannabinoid-PPAR interaction is the active transport of cannabinoid to the nucleus by fatty acid-binding proteins (FABPs). Recent findings have shown that **1** and **3** can be transported to the interior of the cell by these proteins and, therefore, they could be delivered for PPAR activation [105]. Finally, an indirect PPAR activation has been proposed that is triggered by the signaling cascades elicited by CB_1 and/or CB_2 receptors and a direct activation has also been proposed [106]. These four potential mechanisms have been summarized in Fig. 6. The effects of phytocannabinoids at these receptors may be result of a combination of these pathways depending on the cell type, expression of receptors and experimental readout. Whether this activation is different depending on the PPAR isotype or why phytocannabinoids activate them differentially is a question to be explored further.

PPARα Activation by Phytocannabinoids

The alpha PPAR isoform is mainly expressed in liver, kidney, heart, muscle, and adipose tissue. Thus, PPARα activation by cannabinoids is involved in some of their central effects including memory, reward processing, food intake, and lipid metabolism. There is little published data on the activity of phytocannabinoids at

these nuclear receptor isoforms. In 2007, Sun and coworkers [107] reported that Δ^9-THC (**1**) lacks PPARα binding, whereas a recent study demonstrates that this phytocannabinoid is able to increase PPARα transcriptional activity in triple-negative breast cancer cells [108].

PPARγ Activation by Phytocannabinoids

The gamma isoform of these nuclear receptors is predominantly expressed in the heart, muscles, colon, kidney, pancreas, and spleen. These transcription factors are implicated in the regulation of fatty acid storage, glucose metabolism, cell growth and cell differentiation. Activation of PPARγ plays a role in the apoptotic effects of cannabinoids [23, 101].

The phytocannabinoids Δ^9-THC (**1**) and CBD (**3**) extensively have been shown to bind PPARγ, enhancing their transcriptional activity. In addition, their effects have been inhibited selectively by PPARγ antagonists in different experimental in vitro and in vivo models [23, 109–111]. Other phytocannabinoids such as CBG and CBC are also PPARγ agonists [110], whereas THCV (**7**) was not able to increase the transcriptional activity of PPARγ [22]. It is interesting to note that in spite of their ability to activate these nuclear receptors, phytocannabinoids do not modulate PPARs to the same extent as other reported PPAR ligands, and therefore are considered weak agonists. Table 2 provides a summary of the PPAR isotypes that are activated by individual phytocannabinoids.

Synthetic cannabinoids such as abnormal CBD, cannabigerol quinone, and ajulemic acid (AJA), also modulate PPARγ increasing transcriptional activity [103, 110]. Fig. 7 illustrates the 2.8 Å structure of PPARγ with ajulemic acid bound (PDB 2OM9).

Despite all of these data, PPAR-activation has not been reproduced in certain experimental models where **1** and **3** failed to activate either PPARα or PPARγ on an intestinal permeability study [112, 113].

To the best of our knowledge, the PPAR activity of many of the phytocannabinoids discussed in this chapter has not been explored yet. In fact, to date there is little direct evidence of the effects of phytocannabinoids at PPARα, and the potential involvement of the PPARβ/δ isotype on cannabinoid properties remains unknown.

Table 2 Activation of PPAR isotypes by phytocannabinoids (no data for PPARβ/δ are available)

Compound	PPARα	PPARγ
Δ^9-THC (**1**)	Transcriptional activity [103]	Binding assays [105] Transcriptional activity [104] Inhibition by PPARγ antagonists [54, 104]
CBD (**3**)	–	Binding assays [105] Transcriptional activity [36] Inhibition by PPARγ antagonists [36–38]
CBG (**5**)	–	Binding assays [105]
CBC (**6**)	–	Binding assays [105]
THCV (**7**)	–	No response [17]

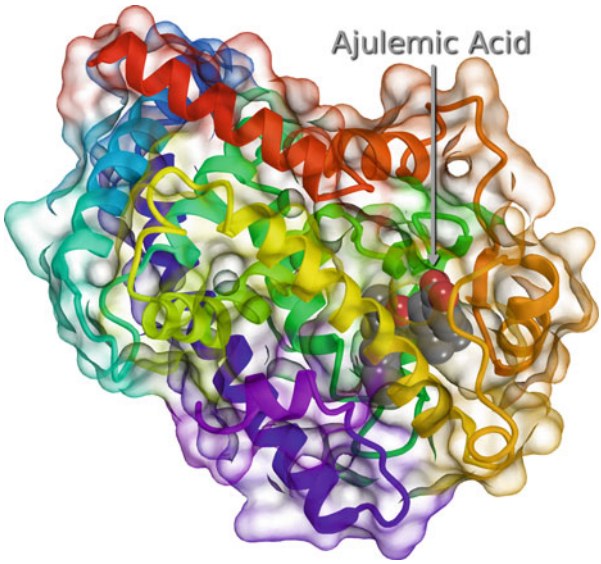

Fig. 7 The 2.8 Å structure of PPARγ with ajulemic acid (PDB 2OM9)

3.2.2 Glycine Receptors (GlyR)

Over the last years, consistent evidence has shown that glycine receptors (GlyR) are relevant targets for CNS cannabinoid action [44, 55, 114, 115]. Glycine receptors mediate synaptic inhibitory neurotransmission involved in crucial physiological and pathological processes [116]. These ionotropic receptors consist of five subunits, each of them composed of a four transmembrane helical segment, surrounding a central chloride-selective ion channel opened by the inhibitory neurotransmitter glycine [117] (Fig. 8). Direct interaction of phytocannabinoids with GlyR has been proposed in the literature [45, 55, 118]. Using mutagenesis and NMR studies, Xiong and coworkers have demonstrated that certain phytocannabinoids can hydrogen bond with the polar residue S296 in the third transmembrane domain of purified α1 and α3 GlyR subunits [45, 55, 119].

The anti-inflammatory and antinociceptive properties of phytocannabinoids are in part mediated by their ability to target glycine receptors. Different cannabinoids, including **1** and **3**, can potentiate glycine currents in native neurons in the hippocampus, amygdala, or spinal cord [44, 56]. In vivo studies in a rodent model have also demonstrated that the analgesic effects **3** and **1** are significantly decreased in mice lacking α3-GlyR, but not in mice lacking CB_1 and CB_2 receptors [45, 55]. Therefore, these receptors likely contribute to the therapeutic effects of phytocannabinoids in the treatment of inflammatory and neuropathic pain.

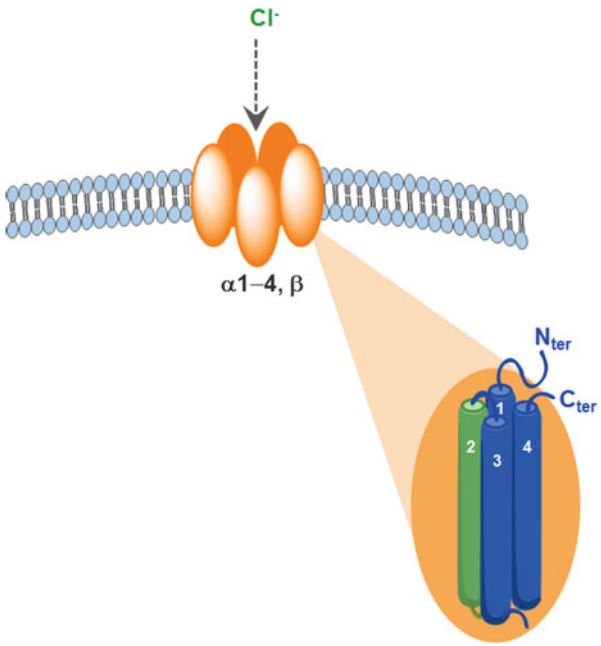

Fig. 8 Structure of glycine receptors: pentamers formed by ∝ and β subunits in a ratio of 2α:3β [115], each subunit consists of four transmembrane segments, the second transmembrane helix of each subunit forms the lining of the ion pore of these ligand-gated ion channels

3.2.3 Transient Receptor Potential Channels

Transient receptor potential (TRP) channels are a group of membrane proteins involved in the transduction of a significant range of chemical and physical stimuli. These channels modulate ion entry mediating a variety of neural signaling processes. They are involved in numerous physiological functions such as temperature sensation, smell, taste, vision, pressure or pain perception, among others [120, 121].

Phytocannabinoids have shown activity at TRP channels from three different subfamilies: TRPV (Vanilloid), TRPA (Ankyrin) and TRPM (Melastatin). These receptors are formed by six transmembrane helixes, a cation-permeable pore (between helix 5 and 6), and intracellular C- and N-termini. The general topology of TRP channels is depicted in Fig. 9. The most striking structural divergence among these three subfamilies is the number of ankyrin repeat domains present in the N-terminus of the receptor. Ankyrin-type channels (TRPA) bear a high number of repeats, whereas the TRPM subfamily lacks ankyrin domains. The vanilloid subfamily present a variable number of ankyrin repeats, depending on the TRPV type.

To date, six types of TRP channels of the aforementioned three subfamilies have been reported to affect phytocannabinoid activity: TRPV1, TRPV2, TRPV3, TRPV4, TRPM8, and TRPA1 [25, 57, 58]. The increasing data regarding cannabinoid interactions with these receptors has prompted some research groups to consider certain TRP

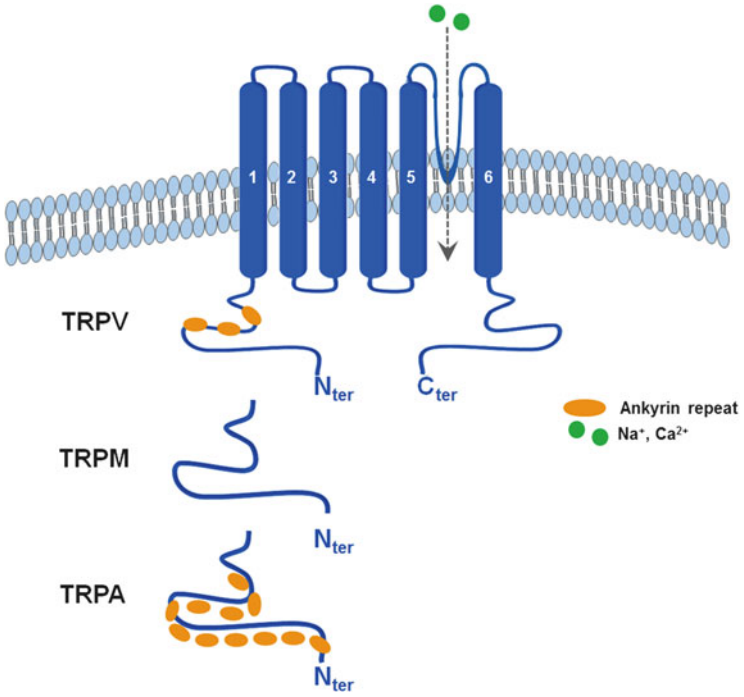

Fig. 9 General structure of the TRP channels modulated by phytocannabinoids: TRPV, TRPM, and TRPA

channels as the "ionotropic cannabinoid receptors" [122–124]. Therefore, these receptors represent potentially attractive targets for the therapeutic use of phytocannabinoids in the treatment of sensory, inflammatory or dermatological pathologies [125].

TRPV1 Channel Activation by Phytocannabinoids

The transient receptor potential channel TRPV1 was first cloned in 1997 as a receptor for the natural product capsaicin. Its structure has been determined in a recent study by a combination of electron cryomicroscopy and lipid nanodisc technology (Fig. 10) [126]. This receptor is widely expressed in brain and sensory neurons (mainly in the dorsal root and trigeminal ganglia), being involved in pain, nociception, and temperature sensing among other physiological and pathological conditions [127]. The transient receptor potential channel TRPV1 colocalizes with CB_1 receptors and CB_2 receptors in sensory and brain neurons respectively [128, 129]. Endocannabinoids and synthetic derivatives have been considered putative endovanilloids based on their high potency towards TRPV1. In fact, anandamide and *N*-arachidonoyl dopamine have been proposed to interact at the same binding site as capsaicin (TMH3-4 region) [130]. Although with less potency and efficacy, many phytocannabinoids are able to activate TRPV1 [25, 122, 131]. As summarized in the table at the end of Sect. 2.2.4, CBD (**3**), CBN (**2**), CBG (**5**), CBC (**6**), Δ^9-THCV (**7**), and CBDV (**9**) are agonists of this ion channel.

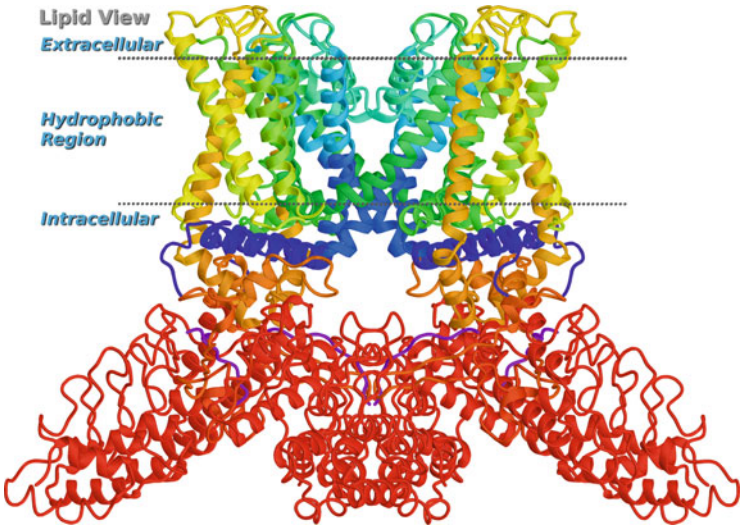

Fig. 10 The 3.27 Å structure of the TRPV1 channel

TRPV2, TRPV3 and TRPV4 Channel Activation by Phytocannabinoids

Phytocannabinoids can also modulate other non-capsaicin-sensitive TRPV channels such as TRPV2, TRPV3, and TRPV4. These receptors are directly involved in the modulation of nociception and temperature perception. As demonstrated through diverse functional outcomes, the phytocannabinoids Δ^9-THC (**1**), CBD (**3**), CBG (**5**), Δ^9-THCV (**7**), and CBDV (**9**) are agonists of TRPV2 [25, 122]. In addition, strong data suggest that some of the analgesic and antiproliferative properties of CBD may be mediated by TRPV2 activation [24, 132].

The activity of phytocannabinoids has also been evaluated in TRPV3- and TRPV4-expressing HEK-293 cells [57]. In this study, phytocannabinoids were not only able to modulate, but also alter, the expression of these TRP channels. These results highlight the therapeutic potential of phytocannabinoids for the treatment of diseases such as gastrointestinal inflammation.

Other TRP Channels Affecting Phytocannabinoid Activity: TRPA1 and TRPM8

The channels TRPA1 and TRPM8 belong to the ankyrin and melastatin subfamilies of TRP channels, respectively. These receptors are also involved in thermosensation, but they are activated by cold temperatures, as well as by different molecules such as menthol. The TRPA1 and TRPM8 channels play a role in cold hypersensitivity associated with inflammatory and neuropathic pain [133]. Therefore, these ion channels may be a potential targets for the treatment of pathophysiological cold pain.

In HEK293 cells expresssing TRPA1, diverse plant-derived cannabinoids were able to efficaciously activate this ion channel. Among others, **1**, **5**, and **6** can induce TRPA1-mediated Ca^{2+} elevation in these cells [25, 58]. Although with lower

potency the activation effect of CBC was also confirmed in DGR neurons. In addition, **3** and **6** were further observed to potently desensitize TRPA1 [58], thus supporting the hypothesis that phytocannabinoids may exert analgesic effects via TRPA1 activation/desensitization.

De Petrocelli and coworkers have characterized phytocannabinoid effects on TRPM8 channels (see table at the end of Sect. 2.2.4). Studies on intracellular Ca^{2+} increase in HEK293 cells transfected with rat recombinant TRPM8, as well as in DRG neurons, have demonstrated that certain phyocannabinoids can efficaciously antagonize the effect of TRPM8 agonists [58, 122]. Interestingly, this activity was shown to be cannabinoid receptor-independent. Even though more studies, especially in vivo, need to be done to fully determine the role of TRP channels in the activity triggered by phytocannabinoids, there is definitely evidence that these molecules are highly involved in the modulation of these receptors.

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