3.24 Chemistry of Cannabis

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3.24.1 An Introduction to the Cannabis Plant

Almost no plant has been studied as much as the Cannabis plant (*Cannabis sativa* L.); more than 10 000 papers have been published describing various aspects of Cannabis as a biologically active plant. Nonetheless, it is hard to think of a medical topic that can so strongly divide the research community as the medicinal use of Cannabis. It may even be stated that Cannabis is the most controversial plant in the history of mankind. But imagine if *C. sativa* were to be discovered today, growing in some remote spot of the world, it would be hailed as a wonder of nature; a new miracle plant with the potential to treat anything ranging from headaches to neurological disorders to cancer. Still, the potential of Cannabis was largely ignored until the discovery of the human endocannabinoid system, about a decade ago. Nowadays, it is known that many of our own body functions are controlled by Cannabis-like substances in our brain, immune system, and other organs.

But Cannabis plants are interesting to human society in more ways. As a fiber plant, Cannabis produces some of the best and most durable fibers of natural origin, historically used to produce ropes and sails for sea ships, paper, banknotes, and even the first Levi's jeans. Modern applications include dashboards for exclusive cars and insulation for houses. The oil of the hempseed was found to be well balanced with regard to the ratio of omega-3- to omega-6 fatty acids for human nutrition, and can be used as a sustainable alternative to fish oil. Furthermore, the oil is ideal as an ingredient for body oils and lipid-enriched creams.

The medicinal use of Cannabis has a very long history. However, the availability of alternative treatments, absence of quality control, and sociopolitical pressure led to a decline in the medical use of Cannabis by the beginning of the twentieth century. As a result, in the past decades its medicinal potential continued to be disputed. But despite its illegality, people have continued to obtain Cannabis on the black market for self-medication.

At least one bioactivity of Cannabis is undisputed: the psychoactive effect of delta-9-tetrahydrocannabinol (THC) is one of the best-studied biological activities in the world. As a result, the attention has shifted from the Cannabis plant as a whole, to its main psychoactive component. Interestingly, THC, a terpenophenolic compound, contains no nitrogen atom and therefore is not an alkaloid, which is rare among the psychotropically active compounds. Furthermore, therapeutically used THC is among the most nonpolar compounds used in medicine today. Chemically, THC belongs to a group of closely related compounds known as cannabinoids, and they are considered the main bioactive components of Cannabis. Up to date, already 70 different cannabinoids have been described, several of which were found to be bioactive in one or more ways.

Cannabis has the potential to evolve into useful and much needed new medicines, but this is seriously obstructed by its classification as a dangerous narcotic. But as shown in the case of the poppy plant (*Papaver somniferum*) and the opiates derived from it (e.g., morphine, codeine), the distinction between a dangerous drug of abuse and a medicine can be made by proper, unbiased, and well-conducted research, combined with a rational approach. Relevant biological activities, as shown by thorough research in the laboratory, and finally confirmed through properly conducted clinical trials, are the best guarantee for the future of Cannabis as a medicine. The information presented in this chapter should help researchers of various disciplines to understand the current scientific status of the Cannabis plant and its constituents.

3.24.1.1 The Different Forms of Cannabis

Together with coffee and tobacco, Cannabis is the most commonly used psychoactive drug worldwide, and it is the single most popular illegal drug. Worldwide over 160 million people are using Cannabis regularly and these numbers are still rising.[1] With such high popular demand, it is not surprising that Cannabis and its products are known under a large variety of names. Some of the most widely used ones are defined here.

The commonly used term 'marijuana' or 'marihuana' traditionally describes the Cannabis plant when used as a recreational drug, and is frequently associated with the negative effects or social impact of the drug. 'Weed' is another name for Cannabis when used as a recreational drug. In contrast, when the term 'hemp' is used, it usually refers to the use of Cannabis as a source of fiber, making the term fiber-hemp therefore somewhat superfluous. Because of the inexact and unscientific nature of these terms, they will not be used in this chapter. Instead, the proper scientific name 'Cannabis' will be consistently used to describe the plant *C. sativa* L. in all its varieties.

When discussing about Cannabis for recreational, medicinal, or scientific use, what is usually referred to are the female flowers (also known under the Latin name *flos*), being the most potent part of the plant. The dried resin obtained from these flowers is generally known as 'hash', or 'hashish', although a large variety of names exist throughout the world. This resin is the source of the most important bioactive components of the Cannabis plant, the cannabinoids, which will be the main focus throughout this chapter.

Finally, 'dronabinol' is another name for the naturally occurring (−)-*trans*-isomer of THC, often used in a medical context in the scientific and political literature, and adopted by the World Health Organization.

3.24.1.2 The Botany of *Cannabis sativa*

The basic material of all Cannabis products is the plant *C. sativa* L. (**Figure 1**). It is an annual, usually dioecious, more rarely monoecious, wind-pollinated herb, with male and female flowers developing on separate plants. It propagates from seed, grows vigorously in open sunny environments with well-drained soils, and has an abundant need for nutrients and water. It can reach up to 5 m (16 ft) in height in a 4–6-month growing season. However, in modern breeding and cultivation of recreational Cannabis, the preferred way to propagate the plants is by cloning, using cuttings of the so-called mother plant. As this term indicates, female plants are used for this purpose, as they produce significantly higher amounts of psychoactive compounds than the male plants.

The sexes of Cannabis are anatomically indistinguishable before they start flowering, but after that, the development of male and female plants varies greatly. Shorter days, or more accurately longer nights, induce the plant to start flowering.[2] The female plant then produces several crowded clusters of individual flowers (flower tops); a large one at the top of the stem and several smaller ones on each branch, whereas the male

Figure 1 *Cannabis sativa*. A female plant in full bloom. Photo courtesy by Bedrocan BV, The Netherlands.

flowers hang in loose clusters along a relatively leafless upright branch. The male plants finish shedding their pollen and die before the seeds in the female plants ripen, that is 4–8 weeks after being fertilized. A large female can produce over 1 kg of seed. If the seed survives, it may germinate the next spring.

According to current botanical classification, Cannabis belongs, only with *Humulus* (hops), to the small family of Cannabinaceae (also Cannabaceae or Cannabidaceae).[3–5] Despite this close relationship, cannabinoids (see Section 3.24.2.1) can only be found in *C. sativa*. In the genus *Humulus*, even in grafting experiments between *Cannabis* and *Humulus*, no cannabinoids have been found,[6,7] but instead a variety of the so-called bitter acids, such as humulone, adhumulone, and cohumulone are produced. The close relationship between both plant species is clearly shown by the fact that both compounds (cannabinoids and bitter acids, respectively) are derived from similar biosynthetic pathways (see Section 3.24.2.2). Furthermore, both are excreted as a resinous mixture by glandular hairs, mainly found on female flowers.

The current systematic classification of Cannabis is:[8]

Division Angiosperms
Class Dicotyledon
Subclass Archichlamydeae
Order Urticales
Family Cannabinaceae
Genus *Cannabis*
Species *sativa* L.

Because of centuries of breeding and selection, a large variation of cultivated varieties (or cultivars) has been developed. Already, more than 700 different cultivars have been described[9] and many more are thought to exist. As a result, there has been extensive discussion about further botanical and chemotaxonomic classification. So

far, several classifications of Cannabis have been proposed. Originally, this was a classification into *C. sativa* L., *Cannabis indica* Lam., and *Cannabis ruderalis* Janisch[10–12] or *C. sativa* L. subsp. *sativa* and *C. sativa* subsp. *indica*.[13–15] However, it is becoming commonly accepted that Cannabis is monotypic and consists only of a single species *C. sativa*, as described by Leonard Fuchs in the sixteenth century.[16–19]

To solve the controversy in a biochemical way, a first chemical classification was done by Grlic,[20] who recognized different ripening stages. Fetterman *et al.*[21] described different phenotypes based on quantitative differences in the content of main cannabinoids and he was the first to distinguish the drug and fiber types. Further extension and perfection of this approach was subsequently done by Small and Beckstead,[22] Turner *et al.*,[23] and Brenneisen and Kessler.[24] However, it was found that a single plant could be classified into different phenotypes, depending on its age. Although these chemotaxonomic classifications do not strictly define the contents of the main cannabinoids for each chemotype, it does provide a practical tool for classification. A final validation of Cannabis classification awaits further chemotaxonomic and genetic research.

For forensic and legislative purposes, the most important classification of Cannabis types is that into the fiber type and the drug type. The main difference between these two is found in the content of the psychotropically active component THC, and its acidic precursor tetrahydrocannabinolic acid (THCA, see Section 3.24.2.2): a high content of THC + THCA classifies as drug-type Cannabis, whereas a low THC + THCA content (below 0.2–0.3% of dry weight) is found in fiber-type Cannabis. All Cannabis varieties presently used for medicinal purposes belong to the drug type, because of their high content of the biologically active THC. But although fiber-type Cannabis is currently not used for medicinal or recreational purpose, it does contain components that have been found to be biologically active, most notably the cannabinoid cannabidiol (CBD). This indicates that the distinction between the two types may have limited relevance for medicinal research into Cannabis.

3.24.1.3 A Short History of Cannabis

Cannabis most likely originates from Central Asia, as archeological evidence indicates that it was already cultivated in China for food and fiber 10 000 years ago. Even in ancient Egyptian mummies, clues have been found for the use of Cannabis as food or medicine.[25] In fact, Cannabis is one of the oldest known medicinal plants and is described in almost every ancient handbook on plant medicine, most commonly in the form of a tincture or a tea.[26,27] Some religions were closely related with the properties of the Cannabis plant. For example, in Hindu legend, Cannabis is believed to be the favorite food of the god Shiva, because of its energizing properties. As Cannabis spread from Asia toward the West, almost every culture came into contact with this miracle plant. Nowadays, varieties of Cannabis can be found in all temperate and tropical zones, except in humid, tropical rain forests.[28]

Despite the fact that Cannabis was grown on a large scale in many countries, the abuse as a narcotic remained uncommon in Western countries until relatively recently. People were largely unaware of the psychoactive properties of Cannabis and it is unlikely that early cultivars, selected mainly for their seed or fiber qualities, contained significant amounts of the psychoactive THC. The medicinal use of Cannabis was introduced in Europe only around 1840, by a young Irish doctor, William O'Shaughnessy, who served for the East India Trading Company in India, where the medicinal use of Cannabis was widespread. Unlike the European fiber Cannabis, these Indian varieties did contain a reasonable amount of bioactive cannabinoids. In the following decades, the medicinal use of Cannabis saw a short period of popularity both in Europe and in the United States. At the top of its popularity, more than 28 different medicinal preparations were available with Cannabis as active ingredient, which were recommended for indications as various as menstrual cramps, asthma, cough, insomnia, support of birth labor, migraine, throat infection, and withdrawal from opium use.[27]

However, because no tools existed for quality control, it was impossible to prepare a standardized medicine, so patients often received a dose that was either too low, having no effect, or too high, resulting in serious side effects. Moreover, Cannabis extract was not water-soluble and therefore could not be injected (in contrast to, e.g., the opiates), whereas oral administration was found to be unreliable because of its slow and erratic absorption. Because of such drawbacks, the medicinal use of Cannabis increasingly disappeared in the beginning of the twentieth century, and in 1937 Cannabis was removed from the US pharmacopoeia, a move that was

followed by most other Western countries.[27] Isolation and structure elucidation of the first pure active substances from Cannabis was not achieved until the 1960s.[29]

Only since the flower-power-time of the 1960s, the smoking of Cannabis as a recreational drug has become a widely known phenomenon in the Western world. From then on, import of stronger varieties from the tropics, combined with a growing expertise in breeding and cultivation, led to a steady increase in psychoactive potency. Contemporary recreational Cannabis has increasingly become a high-tech crop, grown indoors under completely artificial conditions.

An extensive review on the history of Cannabis and its uses by humans has recently been published.[30]

3.24.1.4 Chemical Constituents of Cannabis

With currently 538 known constituents, Cannabis is one of the chemically best-studied plants.[31] But because most of these constituents have not yet been properly characterized for biological activity, the Cannabis plant could be called a 'neglected pharmacological treasure trove'.[32] Extensive reviews of Cannabis constituents are available in the literature.[4,27,33] The most important classes are listed in **Table 1**. The most interesting among these constituents are those found in the secretions of the head cells of glandular hairs (trichomes) distributed across the surface of the Cannabis plant. Although trichomes can be found all over the male and female plants, they are particularly concentrated on the bracts that support the female inflorescence. Solitary resin glands, consisting of one or two dozen cells, most often form at the tips of slender trichome stalks that form as extensions of the plant surface, as shown in **Figure 2**. The resin excreted by the glands contains a variety of constituents, any of which might play a role in the biological activities of the Cannabis plant. Among these are terpenoids, flavonoids, and cannabinoids. Resin collects under a thin waxy membrane surrounding the secretory head cells. In these extracellular resin pockets, the secreted compounds are segregated from the secretory cells, protecting it from both oxidative degradation and enzymatic change. A layer of abscission cells at the base of each secretory head allows the gland to be easily removed.[34]

The adaptational significance of the resin glands remains speculative. Although the resin gives a certain defense against insect and fungal attack, Cannabis crops are still vulnerable to attack by a wide variety of pests, particularly under greenhouse conditions. Certainly, the intoxicating effects of Cannabis resin have increased Cannabis predation by humans, as well as encouraged its domestication, thus dramatically widening its distribution. It has been shown that the cannabinoids, cannabigerolic acid (CBGA) and THCA, induce cell

Table 1 An overview of compounds identified in Cannabis

Compound class	*Compounds identified*
Terpenoids	>120
Cannabinoids	>70
Hydrocarbons	50
Sugars and related compounds	34
Nitrogenous compounds	27
Noncannabinoid phenols	25
Flavonoids	23
Fatty acids	22
Simple acids	21
Amino acids	18
Simple ketones	13
Simple esters and lactones	13
Simple aldehydes	12
Proteins, glycoproteins, and enzymes	11
Steroids	11
Elements	9
Simple alcohols	7
Pigments	2
Vitamin	1 (vitamin K)

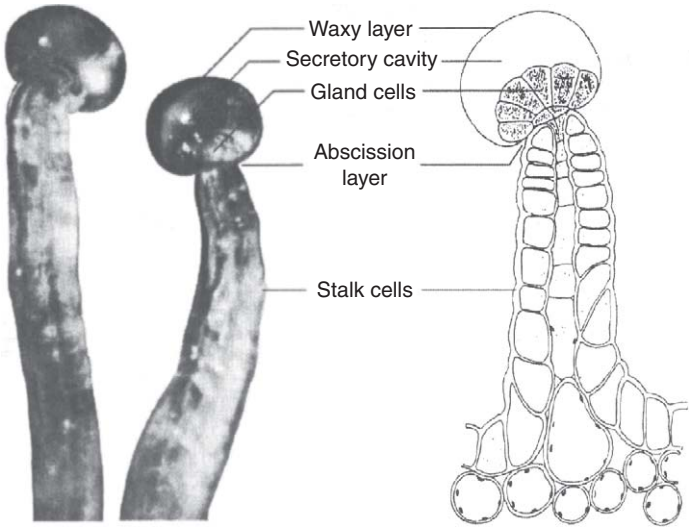


Figure 2 Microscope photograph and drawing of a Cannabis resin gland, with secretory head cells visible underneath the transparent cannabinoid- and terpenoid-rich resin. Photo courtesy by Hashish and R. Clarke, Los Angeles: Red Eye Press, 1998. Reprinted with permission. Drawing from HASHISH!, by R. Clarke.

death through apoptosis in some plant cells as well as insect cells.[35] Furthermore, formation of THCA was found to be linked to hydrogen peroxide formation, which may contribute to the self-defense of the Cannabis plant. These results strongly suggest that cannabinoids act as plant defense compounds, which is a common function of plant secondary metabolites.[36]

The compounds described in **Table 1** have all been identified as a constituent of some preparation of Cannabis: herbal plant material, whole extracts, and chromatographic fractions, or illicit material such as hashish. In many cases, the material used has been obtained from an uncontrolled source (e.g., materials confiscated by authorities) and its quality cannot be guaranteed. It is therefore not certain how many compounds, identified from such materials, should be considered as artifacts, resulting from oxidation, and enzymatic, thermal, or other degradation. In fact, even THC itself is not produced by the metabolism of the Cannabis plant but rather is formed by thermal decarboxylation (loss of CO_2) of THCA. Further degradation of THC results in the formation of cannabinol (CBN) or delta-8-tetrahydrocannabinol (delta-8-THC). Also, many terpenoids are known to be susceptible to degradation upon storage or extraction. As such, the chemical composition of any given Cannabis preparation depends not only on its biosynthetic composition, but also on factors such as age, conditions of storage, and method of extraction. Any biological activity claimed for such preparations should therefore be considered with some care.

Cannabinoids, terpenoids, flavonoids, and fatty acids (hemp oil) comprise the most interesting classes of biologically active compounds from Cannabis. They will be discussed in detail in Sections 3.24.2 and 3.24.5, and constituents in these classes that deserve more scientific attention will be highlighted. Several less significant classes of secondary metabolites found in Cannabis will be discussed shortly in Section 3.24.5.4.

3.24.2 Cannabinoids

Cannabinoids are considered to be the main biologically active constituents of the Cannabis plant, and they can be found nowhere else in nature (for an exception, see Section 3.24.2.2). The majority of biological activities attributed to Cannabis have so far been linked to cannabinoids, and more specifically to THC. The naturally occurring cannabinoids form a complex group of closely related compounds of which currently 70 are known and well described.[4,33,31] New cannabinoids, although present in very minor quantities, have been discovered very recently. These include 11 new cannabinoid esters and a number of other cannabinoid structures.[37,38]

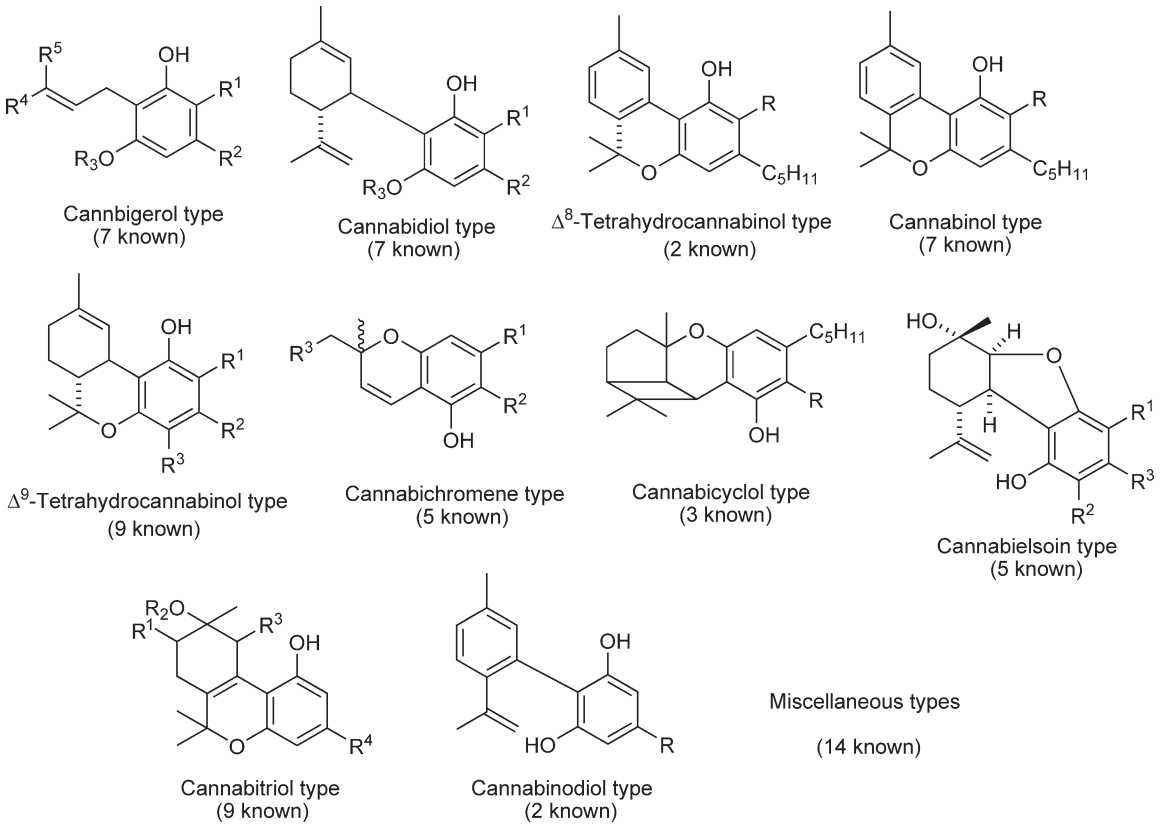


Figure 3 The structural types of cannabinoids found in *Cannabis sativa* L.

There are trace compounds that might have a limited occurrence in certain varieties, but they may add to our increasing understanding of the complexity of cannabinoid biosynthesis.

Cannabinoids can be divided into 10 main structural types (**Figure 3**). All other compounds that do not fit into the main types are grouped as miscellaneous.

3.24.2.1 Cannabinoids Defined

Until the 1980s, the term cannabinoids represented by definition the group of typical terpenophenolic C_{21} compounds present in *C. sativa*, their carboxylic acids, analogues, and transformation products. But from this rather restricted pharmacognostic definition, considerable expansion is now required. A modern definition will put more emphasis on synthetic chemistry and on pharmacology, and would also include related structures or compounds that affect cannabinoid receptors. This, however, creates several chemical subcategories of cannabinoids. The term 'cannabinoids' now represents the whole set of endogenous, natural, and synthetic ligands of the cannabinoid receptors, belonging to a wide variety of chemical families. The plant-derived cannabinoids are now often termed phytocannabinoids. When the word cannabinoids is used in this chapter, the naturally occurring phytocannabinoids are meant, unless indicated otherwise. It should be emphasized that not all phytocannabinoids bind to the cannabinoid receptors.

The first cannabinoid was isolated in 1940.[39] Chemical analysis indicated it to be an alcohol, so it obtained the rather straightforward name CBN ($C_{21}H_{26}O_2$). It was, however, found to be inactive as a psychoactive compound. Chemically, the cannabinoids belong to the terpenophenols, which are common in nature. Cannabinoids are accumulated in the glandular hairs (see Section 3.24.1.4), where they typically make up more than 80% of the subcuticular secretion. In general, all plant parts can contain cannabinoids, except for the seeds. The traces of cannabinoids found in seeds are most likely a result of contamination with Cannabis resin

from the flowers.[17,40] Essentially, there are no qualitative differences in cannabinoid spectrum between plant parts, only quantitative differences.[21,41] The highest cannabinoid concentrations (in percentage of dry weight plant material) can be found in parts of the flowers and fruits. In the foliage leaves the content is lower, and in the stems and, even more so, the roots the content is very low.[42] Cannabis grown outdoors generally has lower levels of cannabinoids when compared to indoor grown plants. When grown under artificial, high-yielding conditions, Cannabis flowering parts can be obtained with a resin content of up to 25–30%, mainly consisting of THCA, the acidic precursor of THC (see Section 3.24.2.2).

3.24.2.2 Biosynthesis of the Cannabinoids

The cannabinoids most commonly detected in herbal Cannabis materials are shown in **Figure 4**. For the chemical numbering of cannabinoids, five different nomenclature systems have been used so far,[43] but the most commonly used system nowadays is the dibenzopyran numbering, which is also adopted by Chemical Abstracts. In Europe, the monoterpenoid system based on *p*-cymene has also been widely used. As a result, the major cannabinoid delta-9-THC is sometimes described as delta-1-THC in older manuscripts. In this chapter, the dibenzopyran numbering is consistently used, therefore THC is fully described as (−)-*trans*-Δ^9-tetrahydrocannabinol (**Figure 5**).

It is commonly thought that cannabinoids are unique compounds only found in Cannabis. However, some exceptions exist in the plant kingdom: In *Helichrysum umbraculigerum* Less., a species from the family Compositae, the presence of CBGA, cannabigerol (CBG), and analogues to CBG was reported.[44] Moreover, in liverworts from *Radula* species the isolation of geranylated bibenzyls analogous to CBG was reported,[45] suggesting the homology of genes from the cannabinoid pathway in at least some other species.

3.24.2.2.1 The acidic cannabinoids

In all biosynthetic pathways for cannabinoids that were postulated until 1964, CBD or cannabidiolic acid (CBDA) was regarded as the key intermediate, which was supposedly built from a monoterpene and olivetol or olivetolic acid (OA), respectively. However, Gaoni and Mechoulam[46] showed that CBG is the common precursor of cannabinoids, biosynthesized through the condensation of geranyldiphosphate and olivetol or OA. Subsequently, they concluded that CBD, THC, and CBN all derive from CBG and differ mainly in the way this precursor is cyclized.[47–50] A further improvement of our understanding of cannabinoid biosynthesis came when Shoyama *et al.*[51,52] concluded that neither the free phenolic (noncarboxylic acid) forms of the cannabinoids nor cannabinolic acid (CBNA) were produced by the living plant. Instead, they postulated a biosynthetic pathway based on geraniol and a polyketoacid, resulting in the production of the acidic cannabinoids. The same conclusion was reached by Turner and Hadley[53] after the study of African Cannabis types.

It is now known that cannabinoids are produced by the metabolism of the plant in the form of carboxylic acids, where the substituent at position 2 is a carboxyl moiety (–COOH).[52] Incorporation studies with ^{13}C-labeled glucose have confirmed that geranyl diphosphate (GPP) and OA are specific intermediates in the biosynthesis of cannabinoids.[54,55] The first specific biosynthetic step is the condensation of GPP with OA into CBGA, catalyzed by the prenylase enzyme geranyldiphosphate:olivetolate-geranyltransferase (GOT).[54] Furthermore, biosynthetic pathways finally became clear by identification and subsequent cloning of the genes responsible for the conversion of CBGA to THCA, CBDA, and cannabichromenic acid (CBCA), respectively.[56–58] Further oxidation of THCA leads to the formation of CBNA, which is still formed after the plant material is harvested and high levels could be due to poor storage conditions (**Figure 6**).

The terpenoid GPP is derived from the deoxyxylulose phosphate/methyl-erythritol phosphate (DOXP/MEP) pathway.[55,59] Not much is known about the biosynthesis of OA yet, but it has been proposed that a polyketide synthase (PKS) could be involved.[59] However, a PKS specifically yielding OA has not been found to date. Interestingly, OA itself has never been isolated from the plant material, possibly indicating it to be a very short-lived intermediate.

Mahlberg and Kim[60] reported that glandular trichomes are exclusively specialized to synthesize high amounts of cannabinoids and that other tissues contain only very low amounts. These authors distinguished three types of glandular trichomes in Cannabis, with different localization, morphology, and cannabinoid content. Cannabinoids are deposited in the noncellular, secretory cavity of glandular trichomes. However, after

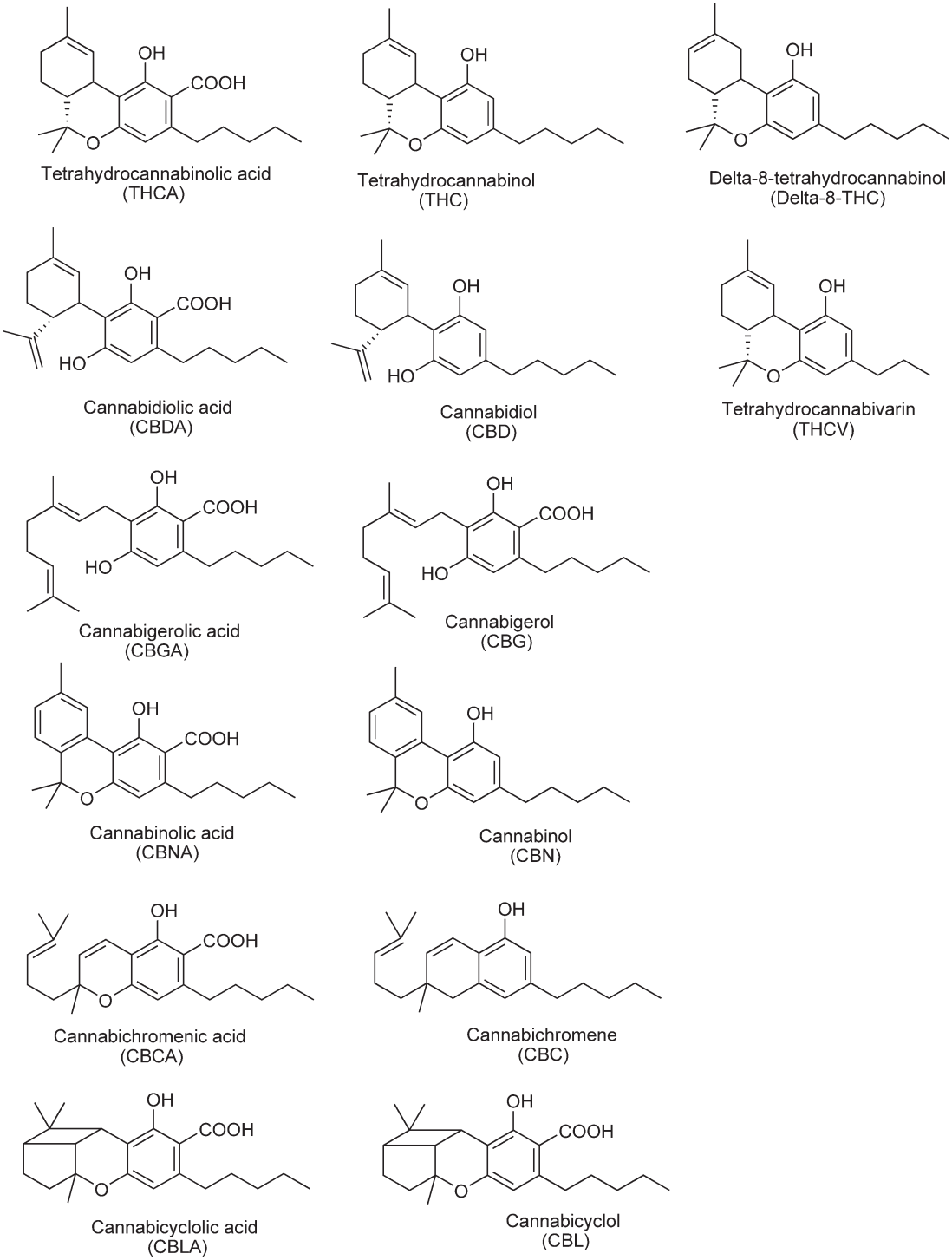
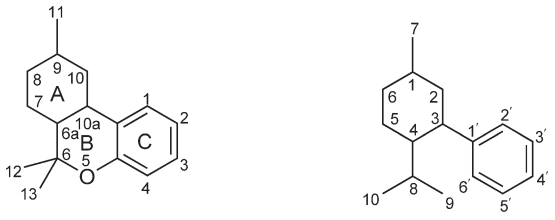


Figure 4 Structures of the cannabinoids most commonly found in Cannabis plant materials. All cannabinoids have the (6a*R*,10a*R*)-orientation, according to the chemical numbering shown in **Figure 5**.

confirming the presence of the central precursor CBGA, as well as THC synthase activity in the secretory cavity, it was suggested that this is not only the site of cannabinoid accumulation, but also the site of cannabinoid biosynthesis.[35]



Dibenzopyran-numbering Monoterpene-numbering based on *p*-cymene

Figure 5 Two most commonly used numbering systems for the cannabinoids. The dibenzopyran system is used in this chapter.

3.24.2.2.2 Occurrence of short-chain cannabinoids and other homologues

Most commonly, the acidic cannabinoids produced by plant metabolism contain a pentyl side chain, derived from the OA moiety. Cannabinoids with propyl side chains result if GPP condenses with divarinic acid instead of OA, into cannabigerovarinic acid (CBGVA). The three known cannabinoid synthase enzymes are not selective for the length of the alkyl side chain, and will convert CBGVA into the propyl homologues of CBDA, THCA, and CBCA.[61] All chain lengths from –methyl to –pentyl have been found in naturally occurring cannabinoids, probably all arising from the incorporation of shorter chain homologues of OA. The side chain is important for the affinity, selectivity, and pharmacological potency for the cannabinoids receptors.

Many other minor acidic cannabinoids have been identified over the years, including monomethyl and other types of esters.[38,62] The biosynthetic pathways explaining this variation have been studied.[59]

3.24.2.3 A Phytochemical Classification of Cannabinoids

Cannabinoids are produced by the metabolism of the plant in the form of carboxylic acids as discussed in Section 3.24.2.2. However, a range of other types of cannabinoids have been detected in Cannabis. For a clear phytochemical discussion of the cannabinoids, they can most conveniently be divided into three groups: acidic cannabinoids; neutral cannabinoids; and 'artifacts'. This practical classification of the cannabinoids is shown in **Figure 7**.

An important distinction that can be made within the group of cannabinoids is between the so-called acidic and neutral cannabinoids. Consequently, in fresh plant material almost no neutral cannabinoids can be found, but theoretically all cannabinoids are present in this acidic form. These can be converted into their decarboxylated analogues under the influence of light, heat, or prolonged storage, by losing the relatively unstable carboxylic group in the form of carbon dioxide.[63] For the purpose of this chapter, the original plant-derived carboxylic acids will be indicated as 'acidic' cannabinoids, whereas their decarboxylated counterparts are indicated as 'neutral' cannabinoids, even though the presence of the phenolic moiety in the neutral cannabinoids may of course classify them as acids as well.

The group of the acidic cannabinoids includes a large number of structures. The most common types of acidic cannabinoids found in a typical drug-type Cannabis plant are THCA, CBDA, CBGA, and CBCA. These acids can be converted to their neutral counterparts by decarboxylation to form THC, CBD, CBG, and cannabichromene (CBC), respectively. An example of this conversion is shown in **Figure 8**.

The group of cannabinoids that occur as a result of degradative conditions deserve some special attention, because their presence is largely the result of variable and unpredictable conditions during all the stages of growing, harvesting, processing, storage, and use. As a result, a well-defined Cannabis preparation may change rapidly into a product with significantly different biological effects. Degradation of THC results in the formation of CBN and delta-8-THC, whereas THCA can further degrade into CBNA.[4] Particularly, in samples that have been stored for an extended period, CBN can be found in relatively large amounts. The isomerization of delta-9-THC to delta-8-THC is well documented and occurs particularly at elevated temperature, where the equilibrium is toward the delta-8 isomer.[49,50,64] The cannabinoids cannabicyclol

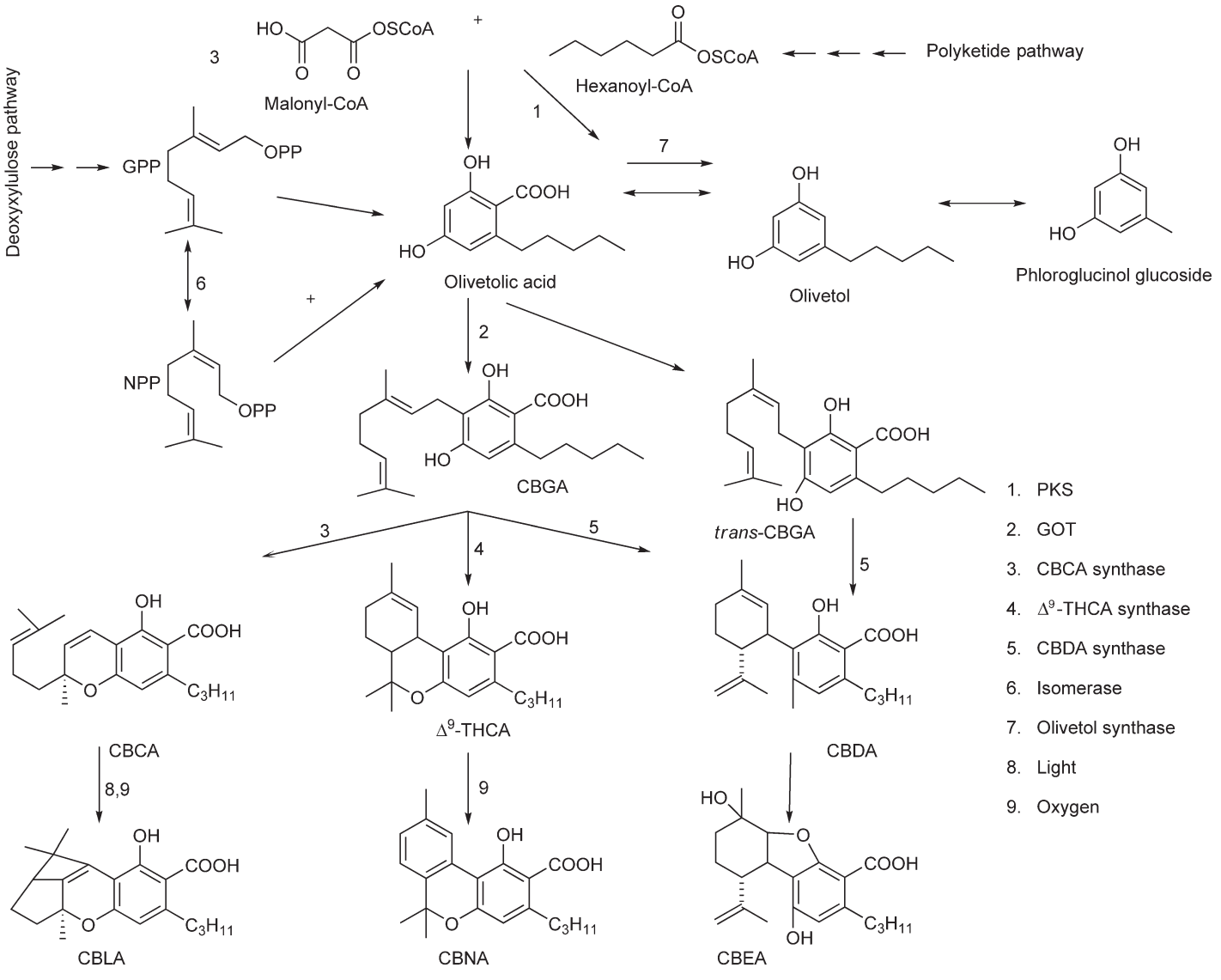


Figure 6 General overview of the biosynthesis of cannabinoids and putative routes. Reproduced with permission from I. J. Flores-Sanchez; R. Verpoorte, *Phytochem. Rev.* **2008**, *7*, 615–639.

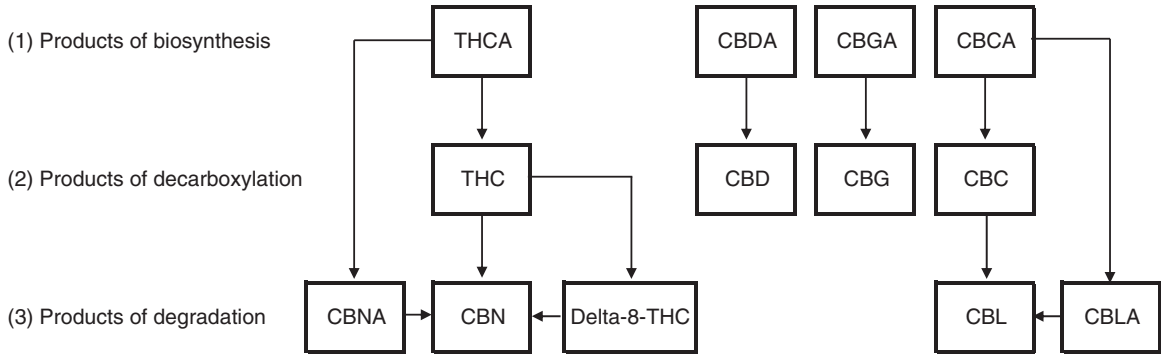


Figure 7 A practical way to categorize the relationships between the major cannabinoids found in herbal Cannabis. Three different groups may be distinguished: (1) cannabinoids produced by biosynthesis of the plant; (2) cannabinoids resulting from natural decarboxylation of acidic cannabinoids; and (3) degradation products resulting from various influences, such as UV-light, oxidation, or isomerization. Arrows indicate the routes of conversion.

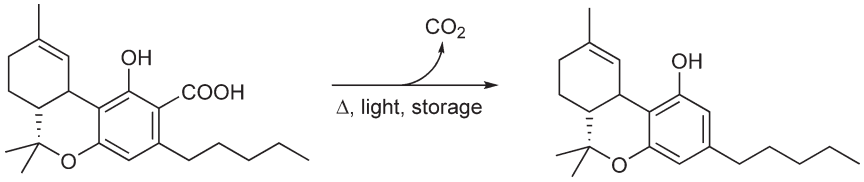


Figure 8 Decarboxylation: the example THCA → THC is shown.

(CBL) and cannabicyclolic acid (CBLA) arise by the exposure of CBC and CBCA, respectively, to UV radiation, leading to cross-linking of two double bonds in the molecule.[65]

Although a large variety of cannabinoids is known, this does not mean that all cannabinoids are detectable in all Cannabis products. They were identified over several decades of Cannabis research, studying many different Cannabis products and different and sometimes rare types of Cannabis plants from a variety of origins and qualities. There can be an enormous variation in their quantitative ratios. The different chemical types of cannabinoids have been well described.[4,66] Understanding how the cannabinoids are (chemically) related to each other is important when studying Cannabis samples, as degradation and changes in the cannabinoid profile might occur as a result of storage or breeding conditions, variations in preparation of medicines, heating, and so on.

3.24.2.4 Chromatographic Analysis of Cannabinoids

For the analysis of highly pure, single cannabinoid preparations, specific analytical procedures can be easily developed. However, most phytochemical and pharmacological studies are far more complex than that. For example, studies on cannabinoid biosynthesis and localization in Cannabis require accurate identification of the whole cannabinoid profile in fresh plant tissues. Also, the identification, classification (e.g., fiber type vs drug type), and individualization (e.g., source tracing) of Cannabis plants and products do not often rely on the analysis of only a single cannabinoid. Furthermore, increasingly *in vitro* and *in vivo* studies are being conducted with whole Cannabis preparations, either as raw plant materials (e.g., powdered flower tops) or as extracts.[67–70] The bioactivities found for such preparations are possibly the result of the acidic cannabinoids,[71] and consequently a method must be available to identify and quantify neutral as well as acidic cannabinoids present in the plant materials used.

For the chemical analysis of cannabinoids, the analytical methods that are available have been extensively reviewed by Raharjo and Verpoorte.[72] Because of the complex chemistry of Cannabis, advanced separation techniques, such as high-performance liquid chromatography (HPLC) and gas chromatography (GC), often coupled with mass spectrometry (MS) detection, are often necessary for the acquisition of the typical chemical profiles of Cannabis constituents. However, especially for screening purposes and on-site field testing, noninstrumental techniques like thin-layer chromatography (TLC) and color reactions may be helpful, too.

3.24.2.4.1 Decarboxylation

Owing to the thermal lability of the acidic cannabinoids, indirect methods have been widely used for their determination. These indirect methods are based on the decarboxylation of the acids and subsequent HPLC determination of the neutral cannabinoids formed. But although decarboxylation occurs naturally over time, during storage of cannabinoids, it is difficult to perform a quantitative decarboxylation under experimental conditions. When performing the thermal decarboxylation of cannabinoid acids in either the presence or absence of organic solvents in an open reactor, an optimum temperature at which the velocity of the decarboxylation would be high enough and simultaneous evaporation of neutral cannabinoids would not occur could not be found.[63] Consequently, it is not possible in this manner to obtain an amount of neutral cannabinoids equivalent to that of the cannabinoid acids from which they were decarboxylated. During the decarboxylation on different sorbent surfaces, the evaporation of cannabinoids was hindered by sorptive effects, but simultaneous side-reactions occurred, causing chemical changes of the neutral cannabinoids.[63]

3.24.2.4.2 Microscopy

Identifying a plant sample as *C. sativa* L. may be done simply by using macroscopic and microscopic evaluation of the intact plant material. The botanical identification of plant specimens consists of physical examination of the intact plant morphology and habit (leaf shape, male and female inflorescences, etc.) followed by the microscopical examination of leaves for the presence of cystolith hairs (as shown in **Figure 2**). The very abundant trichomes, which are present on the surface of the fruiting and flowering tops of Cannabis, are the most characteristic features to be found in the microscopic examination of herbal Cannabis products.[62]

3.24.2.4.3 Color reactions

The most common color spot tests include those developed by Duquenois and its modifications. A study of 270 different plant species and 200 organic compounds has shown that the Duquenois–Levine modification is most specific.[73] The fast blue B salt test is the most common color reaction for the visualization of TLC patterns (see below) but may also be used as spot test on a filter paper.[74]

It must be stressed that positive reactions to color tests are only presumptive indications of the possible presence of Cannabis products or materials containing Cannabis products. A few other materials, often harmless and uncontrolled by national legislation or international treaties, may react with similar colors to the test reagents.[62]

3.24.2.4.4 Thin-layer chromatography

One- and two-dimensional TLC is suited for the acquisition of qualitative cannabinoid profiles from plant material. Both normal-phase and reversed-phase TLC methods have been described.[75] For selective visualization of cannabinoids, the TLC plate can be sprayed with 0.5% fast blue B salt (*o*-dianisidine-bis-(diazotized)-zinc double salt) in water, followed by 0.1 mol l^{-1} NaOH.[74] For quantitation, instrumental TLC coupled to densitometry is necessary. High-pressure TLC and overpressured layer chromatography have been developed for the reproducible and fast determination and isolation of neutral and acidic cannabinoids.[76–78]

3.24.2.4.5 Gas chromatography

The use of GC, commonly coupled to flame ionization detection (FID) or MS detection, permits the analysis of a large variety of cannabinoids with very high resolution. However, derivatization is necessary (e.g., silylation and methylation) when information about cannabinoid acids, the dominating cannabinoids in the plant, is required.[62] Because it is hard to perform a quantitative derivatization for all components in a complex mixture, GC analysis may have only limited value when studying the authentic composition of Cannabis products.

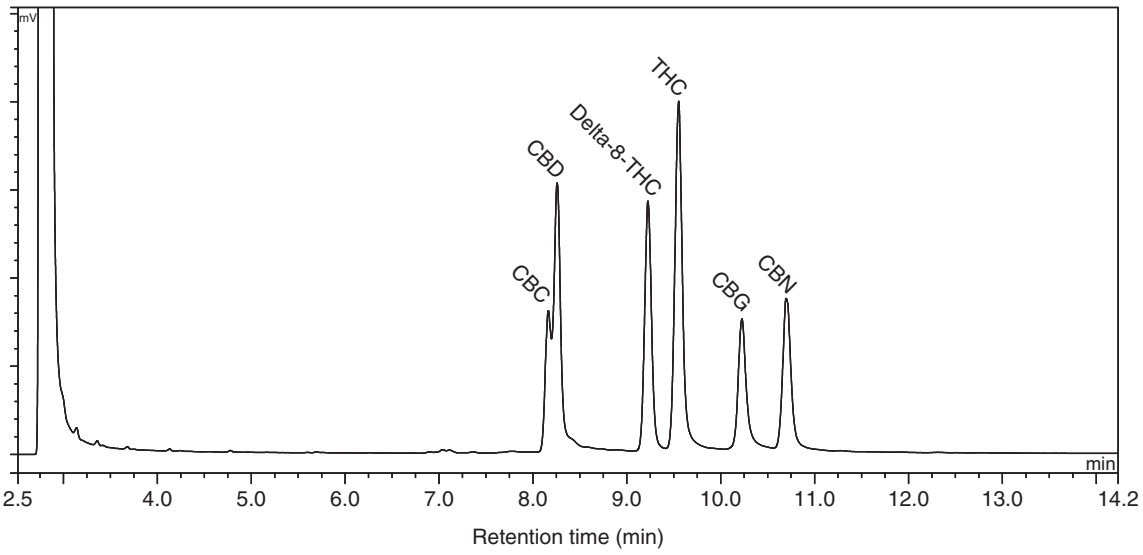


Figure 9a A typical GC chromatogram (FID detection) obtained according to Hazekamp *et al.*[75] Column: Durabond fused silica capillary column (30 m × 0.25 mm inner diameter) coated with DB-1 at a film thickness of 0.1 μm (J&W Scientific Inc., Rancho Cordova, CA). The oven temperature was programmed from 100 to 280 °C at a rate of 10 °C min^{-1}.

GC/MS is the method of choice for creating Cannabis profiles and signatures (chemical fingerprints), a tool for attributing the country of origin, the conditions of cultivation (indoor, outdoor), and so on. A representative GC-FID chromatogram is shown in **Figure 9(a)**.

3.24.2.4.6 High-performance liquid chromatography

HPLC has made it possible to simultaneously detect cannabinoids in both the acid and neutral forms, without the need of derivatization. By making use of an UV- or photodiode array (PDA) detector, cannabinoids can be efficiently analyzed without causing degradation of sample components. Thus, HPLC has become the method of choice for most laboratories. A representative HPLC chromatogram is shown in **Figure 9(b)**. However, the analysis of all major cannabinoids in a typical Cannabis extract is not easily achieved, because of the complex composition resulting in chromatographic overlap of peaks. To overcome this problem, the use of MS detection (LC–MS) to distinguish between overlapping chromatographic peaks is becoming increasingly important.[75,79]

Validated HPLC methods exist for the analysis of cannabinoids according to the American United States Pharmacopoeia (USP) or German the German Drug Codex (DAC) guidelines. However, these were specifically developed for the analysis of impurities in highly pure preparations of THC, derived from either synthetic (USP method) or natural source (DAC method). They were not intended, and hence not validated, for use with whole Cannabis plant materials. More recently, a fully validated pharmacopoeia method was developed for the quality control of Cannabis produced for the Dutch medicinal Cannabis program.[80] This ultra-performance LC (UPLC) method has been validated according to International Conference on Harmonization (ICH) guidelines and is suitable for the analysis of a wide range of authentic cannabinoids in herbal Cannabis. A representative UPLC chromatogram is shown in **Figure 9(c)**.

3.24.2.4.7 Other techniques

Occasionally, new methods are explored for the analysis of cannabinoids. The applicability of capillary electrochromatography with photodiode array UV detection for the analysis of cannabinoids has been demonstrated.[81] Also, supercritical fluid chromatography has been studied,[82] but with limited success. Supercritical fluid chromatography is characterized by shorter analysis times than GC or HPLC and does not require derivatization.

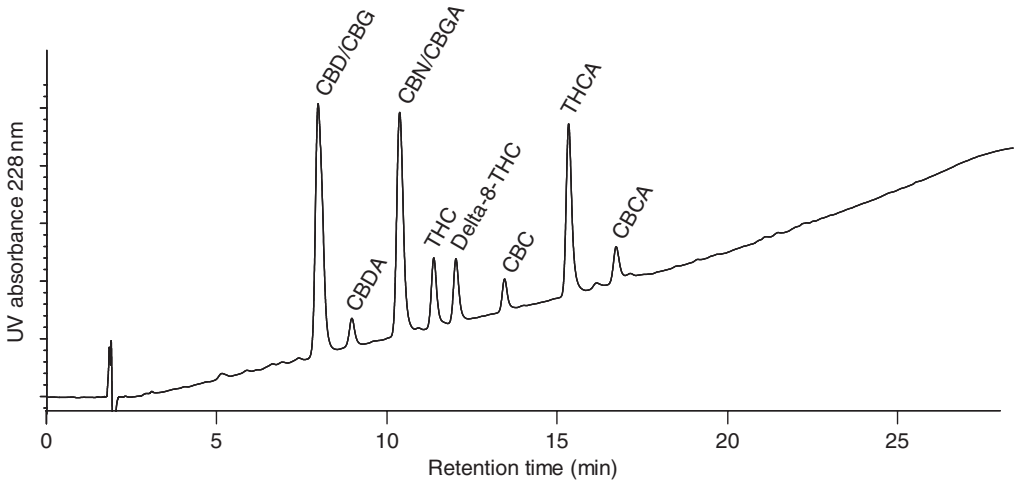


Figure 9b A typical HPLC chromatogram (228 nm) obtained according to Hazekamp *et al*.[115] Column: Waters XTerra MS C_{18} (2.1 × 150 mm, 3.5 μm); eluent: methanol/water gradient with linear increase of methanol from 65 to 100% over 25 min; flow rate: 1.5 ml min^{-1}; detection wavelength: UV at 228 nm. The baseline was not corrected for the influence of the methanol gradient.

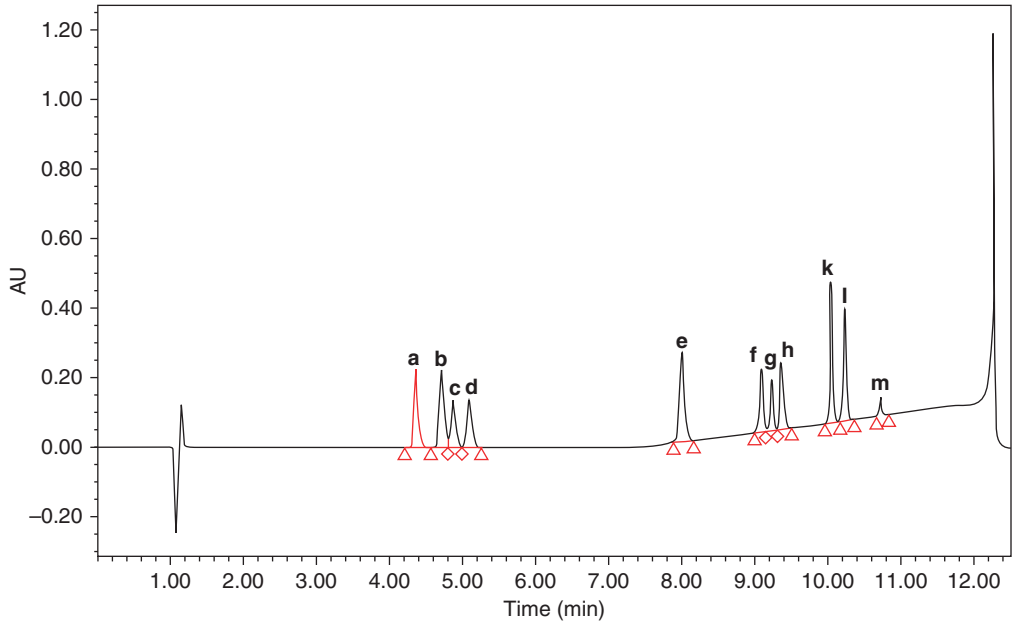


Figure 9c A typical UPLC chromatogram obtained using the following method: Column: Waters C_{18} analytical column (1.7 μm, 2.1 × 150 mm); eluent: acetonitrile/water/0.1% formic acid; 0–6 min isocratic 70% acetonitrile, 6–10.5 min gradient to 100% acetonitrile, 10.5–11 min hold at 100% acetonitrile; flow rate: 0.3 ml min^{-1}; detection wavelength: UV at 228 nm. a, CBDA; b, CBGA; c, CBG; d, CBD; e, CBN; f, THC; g, delta-8-THC; h, CBNA; k, CBC; l, THCA; m, CBCA.

3.24.2.4.8 Spectroscopic and chromatographic data

A final important factor in the effective analysis of the cannabinoids is the availability of reliable spectroscopic and chromatographic data. Although such data have been published for most known cannabinoids during isolation and identification experiments (see Turner *et al*.[4] for an overview), they were scattered over a huge amount of scientific papers. In 2005, Hazekamp *et al*.[75] determined chromatographic and spectroscopic data for 16 different naturally occurring cannabinoids as well as two human metabolites under standardized conditions.

Spectroscopic analyses performed were UV-absorbance, infra-red spectral analysis, (GC–) mass spectrometry, and spectrophotometric analysis. Also, the fluorescent properties of the cannabinoids were presented. Chromatographic data includes relative retention times in HLPC, GC, and TLC. In a similar standardized fashion, the complete ^{1}H- and ^{13}C-NMR assignments of several major cannabinoids have been summarized.[83]

3.24.3 Sites and Mechanisms of Action of Cannabinoids

The majority of studies on the biological effects of Cannabis constituents have been done with cannabinoids. No other class of Cannabis compounds received anything near as much attention. The reason for this is clear: cannabinoids are unique to the Cannabis plant, whereas all the other classes of compounds can also be found elsewhere in nature. Therefore, the majority of this chapter is dedicated particularly to the cannabinoids. And among the cannabinoids, virtually all studies have focused on the effects of THC. So almost by necessity, this section will focus on THC to discuss what is known about the receptor-binding, its metabolism, and other mechanisms involved in understanding the biological effects of cannabinoids. However, other cannabinoid constituents will be discussed where possible, based on the literature available.

In general, biological effects take place through activation of receptors, so it needs no explanation that the psychoactive effects of THC led to the hunt for specific binding sites. Analogues of THC, chemically modified and radiolabeled, served as a tool for the identification of cannabinoid receptor 1 (CB1) in the rat brain, soon followed by the discovery of the CB2 receptor. These findings prompted the search for endogenous ligands, which was guided by the chemical concept that, by homology to the highly lipophilic THC, physiological cannabinoid receptor ligands were to be looked for among endogenous lipids rather than the more polar peptides like the endorphins.

It is now known that cannabinoid receptors can be found in most parts of the brain, as well as in the immune system and a variety of other organs. Their distribution seems to explain many of the observed effects of Cannabis consumption. Such a variety of effects was concisely summarized by Di Marzo *et al.*:[84] endocannabinoids make you 'feel less pain, control your movement, relax, eat, forget (posttraumatic), sleep, and protect your neurons'. The activation of the endogenous cannabinoid system could represent a crucial and important component for each of these functions. The endocannabinoid system that is responsible for our physiological response to Cannabis use is analogous to the morphine–endorphin system. Interestingly, cross talk between the two systems has been shown.[85]

In this section, the sites and mechanisms of action of the cannabinoids are discussed. After describing the discovery of Cannabis receptors, the endocannabinoids will be discussed. The understanding of this endogenous system explains the effects of Cannabis on human physiology, which will be further clarified by looking in more detail at THC. Information on absorption, distribution, metabolism, and elimination will help to explain the possible role of synergy and interaction between Cannabis components. Finally, the effect of administration forms on the observed biological effects of cannabinoids is discussed.

3.24.3.1 The Cannabinoid Receptors: CB1 and CB2

The cannabinoid receptors are a class of receptors under the G-protein-coupled receptor superfamily. Their ligands are known as cannabinoids or endocannabinoids depending on whether they come from external or internal (endogenous) sources. Cannabinoid receptors have a protein structure defined by an array of seven transmembrane-spanning helices with intervening intracellular loops and a C-terminal domain that can associate with G proteins of the $G_{i/o}$ family.[86]

Until the discovery of specific Cannabis receptors, the biochemical mode of action of cannabinoids was much debated. Because of their lipophilic character, cannabinoids can penetrate cellular membranes by simple diffusion. Therefore, possible explanations for cannabinoid activity initially included unspecific membrane binding resulting in fluidity and permeability changes of neural membranes, the inhibition of acetylcholine synthesis, an increase in the synthesis of catecholamines, and an interaction with the synaptosomal uptake of serotonin.[87,88] However, it was established in the mid-1980s that cannabinoid activity is highly stereoselective,[89] indicating the existence of a receptor-mediated mechanism.

The first reliable indications that cannabinoids act through receptors came when it was shown that cannabinoids can act as inhibitors of the adenylate cyclase second messenger pathway in brain tissue and neuroblastoma cell lines. This activity was dose-dependent, stereospecific, and could be modulated by pertussis toxin.[90–94] Finally, a stereospecific G-protein-coupled cannabinoid receptor was found and cloned.[95] It was named 'cannabinoid-binding receptor type 1' or CB1. The CB1 receptor is most clearly localized in the central nervous system (CNS), therefore it is often called the 'central receptor', but it is also found in certain peripheral organs and tissues, such as lungs, liver, and kidneys.[96] CB1 receptors are thought to be the most widely expressed G-protein-coupled receptors in the brain. Activation leads to the inhibition of adenylate cyclase activity.[97] The CB1 receptor also modulates ion channels, inducing, for example, the inhibition of N- and P/Q-type voltage-sensitive Ca^{2+} channels and the activation of G-protein inwardly rectifying K^{+} channels. Besides these well-established cannabinoid receptor-coupled events, cannabinoid receptors have also been shown to modulate several signaling pathways that are more directly involved in the control of cell proliferation and survival, including extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, focal adhesion kinase (FAK), and the ceramide pathway.[98]

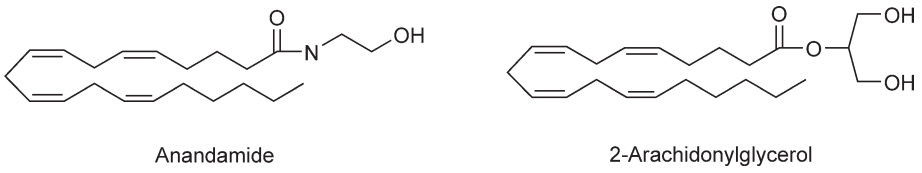
Subsequently, a second cannabinoid receptor (CB2) was found with a possible role in immunological processes.[99] The CB2 receptor was first described as a peripheral G-protein-coupled receptor (GPCR), mainly localized in the immune system, therefore it is often called the 'peripheral receptor'. However, nowadays it appears that the situation is more complex, as CB2 expression was also reported to be present in neurons of the brain.[100] It is primarily expressed by immune tissues such as leukocytes, spleen, and tonsils, and it shows a different selectivity than the centrally acting CB1. So far, the physiological roles of CB2 receptors are proving more difficult to establish, but at least one seems to be the modulation of cytokine release.[101] Recently, it has been recognized that CB2 may play a functionally relevant role in the CNS, mediated through microglial cells.[102]

The cannabinoid signaling system is teleologically millions of years old, as it has been found in mammals, fish, and invertebrates down to very primitive organisms such as the hydra.[103] Surprisingly, the protein sequences of CB1 and CB2 show only about 45% homology. There are indications that CB receptors are evolutionary related to the vanilloid receptors.[104] The transient receptor potential vanilloid receptor 1 (TRVR1) can be activated by the fatty acid amide compound capsaicin. Based on the chemical similarities between capsaicin and endocannabinoids (see Section 3.24.3.2), it was hypothesized that TRVR1 and proteins of the endocannabinoid system share common ligands. This was confirmed when it was demonstrated that the endocannabinoid anandamide activates TRVR1 receptors.[105] Also, it was found in isolated blood vessel preparations that some endocannabinoids can activate vanilloid receptors on sensory neurons,[106] which raises the possibility that endocannabinoids are endogenous agonists for vanilloid receptors.[107] These receptors might therefore be putatively regarded as CB3 receptors.

There is mounting evidence of novel receptors expressed in endothelial cells and in the CNS that have cannabimimetic and therapeutic effects independent of the mechanisms described above.[108] In 2007, the binding of several cannabinoids to a GPCR (GPR55) in the brain was described.[109] These receptors are more likely to be functionally related than structurally, as there is currently no evidence for additional cannabinoid receptors in the human genome. However, not all of the effects of cannabinoids can be explained by receptor-mediated effects, and it is believed that at least some effects are nonspecific and caused through membrane perturbation.[110,111]

3.24.3.2 The Endocannabinoid System

Based on the observation that all natural cannabinoids are highly lipid-soluble, an attempt was made to isolate endogenous ligands for the cannabinoid receptors from fatty tissues of animals. Finally, a single compound could be isolated from the brain tissue of pigs, with a high affinity for the CB1 receptor. It was chemically identified as arachidonic acid ethanolamide, and named anandamide, from the Sanskrit word for 'eternal bliss'.[112] A few years later, a related compound was isolated from canine gut with an affinity for both cannabinoid receptors: 2-arachidonylglycerol (2-AG).[113] Structures of these two compounds are shown in **Figure 10**. In recent years, a variety of compounds with endocannabinoid activity have been isolated or



Anandamide 2-Arachidonylglycerol

Figure 10 Structures of the two major endocannabinoids.

synthesized,[114,115] interestingly, all having an eicosanoid-related structure. Cannabinoid receptors and their endogenous ligands together constitute what is referred to as the endogenous cannabinoid, or endocannabinoid, system.

The endocannabinoid system is now known to be a ubiquitous neuromodulatory system with wide-ranging actions. It consists of cannabinoid receptors, endogenous cannabinoids, and enzymes responsible for their production, transport, and degradation. The endocannabinoid system can be found even in very primitive organisms, indicating it has a fundamental role in basic physiology. There are currently two main families of endocannabinoids that have been extensively characterized. The first are amides of arachidonic acid and ethanolamide; the typical example of this family is anandamide. The second family includes glycerol esters related to 2-AG. The biosynthetic pathways for both families of endocannabinoids are complex, but well reviewed.[116,117]

There are several pathways known for the synthesis and degradation of endocannabinoids, so there appears to be a high redundancy. Basically, endocannabinoids exist intracellularly as precursors in the plasma membrane of neurons as part of certain phospholipids. They are produced on demand by distinct biochemical pathways involving phospholipases C and D, as well as other enzymes. These events are triggered by the enhancement of intracellular calcium concentrations that follow cell depolarization or the mobilization of intracellular calcium stores subsequent to the stimulation of protein-coupled receptors from the G_q/G_{11} family. Accordingly, the enzymes catalyzing anandamide and 2-AG are calcium-sensitive. After formation, endocannabinoids are transported across the cell membrane for interaction with their extracellular binding sites on cannabinoid receptors.[116,117]

Endocannabinoids serve as extracellular retrograde messengers, with characteristics very different from other neurotransmitters such as acetylcholine, γ-aminobutyric acid (GABA), and dopamine. Endocannabinoids are described as retrograde transmitters because they most commonly travel backward against the usual synaptic transmitter flow: they are released from the postsynaptic cell and act on the presynaptic cell, where the target receptors are densely concentrated. Like the endorphins, endocannabinoids exert a homeostatic function. But because of their chemical (nonwater-soluble) nature, they cannot travel unaided for long distances in the aqueous medium surrounding the cells from which they are released. Therefore, endocannabinoids do not typically function like hormones, which can affect cells throughout the body, but instead they act as local (autocrine or paracrine) mediators. Activation of the cannabinoid receptors temporarily reduces the amount of conventional neurotransmitter released, thereby controlling the incoming synaptic signal. The ultimate effect of this process depends on the nature of the transmitter that is controlled, which itself depends on the function of the tissue where the cannabinoid receptors are expressed. Simply said, endocannabinoids produced by a certain neuron are modulators of the flow of other neurotransmitters produced by that same neuron.

Degradation is an important mechanism to regulate endocannabinoid activity, as the duration of endocannabinoid effect is dependent on the localization of the degrading enzymes.[117] The degradation system involves reuptake into the presynaptic cell, followed by rapid hydrolysis of the amide or ester bonds. How endocannabinoids move from the extracellular space to the interior of a cell for degradation remains unclear, but there is indirect evidence for specific proteins facilitating the membrane transport.[118] 2-AG exhibits higher selectivity and efficacy for CB1 and CB2 receptors than anandamide, which also interacts with noncannabinoid receptor targets. Therefore, it is not surprising that the levels of the two compounds are regulated in different ways. However, the main enzyme that inactivates both anandamide and 2-AG (and others) by hydrolysis is fatty acid amide hydrolase (FAAH). It was isolated after the synthesis of inhibitors of endocannabinoid degradation, which were then used for affinity chromatography purification of the degrading enzyme.[119] 2-AG is also inactivated by monoacylglycerol lipase (MAGL).

Interestingly, in *Arabidopsis thaliana*, a functional homologue of the mammalian FAAH has been cloned,[120] and fatty acid ethanolamines with high homology to anandamide have been discovered in several plant species.[121] These findings provide support at the molecular level for a conserved mechanism between plants and animals for the metabolism of *N*-acylethanolamines.

3.24.3.3 Pharmacokinetics of the Cannabinoids (ADME)

Cannabinoid pharmacokinetic research has been especially challenging because of low analyte concentrations in serum, rapid and extensive metabolism, and physicochemical characteristics that hinder the separation of drugs of interest from biological matrices, and from each other. Although many other administration forms have been developed (see Section 3.24.3.5 on administration forms), oral administration, often in the form of synthetic THC (Marinol), has been most extensively studied.

Although the metabolic fate of THC is well known,[27] not much has been reported on the other cannabinoids. After oral administration, THC is almost completely absorbed (90–95%); however, because of the first-pass metabolism by the liver and high lipid solubility, 90% or more of orally administered THC never reaches the sites of activity in the body in its native form.[122,123] THC and its metabolites are extensively protein bound in the blood (~97%) and rapidly distributed to highly vascularized tissues and the brain. Serum concentrations peak at approximately 0.5–4 h after oral dosing, and decline over several days. After oral administration, THC has an onset of action of approximately 1–2 h and duration of psychoactive effects is 4–6 h after administration.

In humans, plasma THC concentration profiles are similar after smoking or intravenous administration, with prompt onset and steady decline. Metabolism occurs mainly in the liver by microsomal hydroxylation, and oxidation catalyzed by enzymes of the P-450 complex. Nearly 100 metabolites have been identified for THC alone. Besides the liver, other tissues like the heart and lungs are also able to metabolize cannabinoids, albeit to a lesser degree. The two major metabolites of THC are 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (11-COOH-THC). 11-OH-THC is the most important psychotropic metabolite, being about twice as psychoactive as THC, and it has a similar kinetic profile as the parent molecule.[124] In contrast, 11-COOH-THC has no psychotropic activity. Most of the 11-COOH-THC is finally converted into its glucuronide form, with a glucuronic acid moiety linked to the carboxylic group. This is the major form of THC excreted into urine. When THC is inhaled through smoking or vaporizing, it avoids first-pass metabolism, and conversion into 11-OH-THC and further metabolites takes place much slower.[124]

Metabolism is the major route for the elimination of THC from the body. The elimination is biphasic; there is a rapid distribution phase (initial half-life about 4 h), believed to be due to the highly lipophilic nature of the drug and redistribution into lipid-rich tissues, and a terminal half-life of around 25–30 h for THC and 11-OH-THC. Plasma half-life for 11-COOH-THC may be even as long as 25–75 h.[125,126]

Only negligible amounts of THC are excreted in unchanged form; less than 5% of an oral dose is recovered unchanged in the feces. Most of the absorbed dose (65–80%) is excreted as metabolites in the feces and a lesser amount in the urine (20–35%). Among the metabolites, 11-COOH-THC is a major one identified in both urine and feces, both in its native form and in the form of its glucuronide. Because of its large volume of distribution ($\sim 10\,l\,kg^{-1}$), THC and its metabolites may be excreted at low levels for prolonged periods of time. Following single-dose administration, low levels of THC metabolites have been detected for more than 5 weeks in the urine and feces.[126]

For the other cannabinoids, only the metabolism of CBD and CBN has been described to some extent.[127] An important aspect of CBD is that it inactivates certain types of cytochromes, which may be important because serious drug–drug interactions may occur in the case that CBD is coadministered with drugs that are metabolized mainly by the enzyme system containing these P-450 isozymes.[128]

So far, virtually nothing was reported on absorption, distribution, metabolism, and excretion (ADME) of the major cannabinoid THCA. In a case study (unpublished data by the author), it was found that the oral consumption of 30 mg pure THCA did not lead to psychotropic effects and no THC metabolites could be detected in urine by a standardized GC–MS detection method for THC. So, despite their structural similarity, the metabolism of THCA seems to be quite distinct from THC.

3.24.3.4 Structure–Activity Relationships of Cannabinoids

There is a central problem in the discussion about making Cannabis or THC medicinally available: the curative properties of Cannabis/THC are mediated mainly by the same receptors that cause its unwanted psychoactive side effects. So, just as for other psychoactive drugs (e.g., morphine, benzodiazepines), the accepted medicinal applications are limited. As a result, a major goal of cannabinoid research nowadays is to separate beneficial from unwanted effects by means of medicinal chemistry, studying structure–activity relationships (SARs). The same approach is also used to develop agonists or antagonists with a high selectivity for only one of the cannabinoid receptors.

The absolute configuration of naturally occurring THC is *trans*-(6a*R*,10a*R*), resulting in a negative specific rotation. The preparation of the *cis*-(+)-enantiomer of THC, and subsequent pharmacological comparison to its natural counterpart, gave a decisive argument for the stereospecificity of the binding, and thereby reinforcing the cannabinoid–receptor interaction hypothesis. Since this discovery, cannabinoids have been extensively studied to understand the relationships between their structure and affinity for the Cannabis receptors. For this purpose, a very large number of synthetic cannabinomimetics have been made and systematically tested for receptor binding on CB1 and CB2.

Large numbers of compounds have been studied for the SAR of both known CB receptors. But in addition to CB1 and CB2 receptors, pharmacological studies have strongly suggested the existence of other cannabinoid receptor subtypes,[129] the most likely candidate being the vanilloid TRVR1 receptor (see Section 3.24.3.1). This essentially means that all available cannabinoids produced for SAR studies could be tested again for binding affinity to this receptor. This may lead to new clues about the mechanism of action for certain bioactivities of cannabinoids.

Classical cannabinoids and endocannabinoids are both agonists of the CB receptors, but because of the different structural features of CB1 and CB2, their SAR is not entirely similar. The CB1 receptor has been proposed to exists in two different conformational states; one where it is bound to the secondary system (by G-proteins) and one in which it is uncoupled.[130] Agonists of the CB1 receptor bind to the precoupled stage activating the receptor, whereas antagonists bind to the receptor without activating it. Because antagonists prevent endogenous cannabinoids from binding, activation is interrupted. Inverse agonists bind to the uncoupled receptor, blocking it and avoiding precoupling of the receptor.

The SAR of classical cannabinoids and endocannabinoids has been studied intensively, and several reviews have been published.[131–136] The major cannabinoids THC[113] and delta-8-THC[137] bind to the CB1 receptor with moderate affinity and do not show specificity for either. CBN, however, does show a slight specificity for the CB2 receptor.[138] It should be noted that values published for CB-receptor affinity may be strongly dependent on the type of tissue and animal species used in the study. Although rat CB1 is 97% homologous with human CB1, critical differences do exist.[139] Also, the type of agonist to be displaced has an influence on the value of K_i reported. As a result, binding efficiencies reported in the literature often show a range of values. An overview is presented by Pertwee,[140] and a summary is presented in **Table 2**.

By introducing a large variety of chemical modifications, four pharmacophores have so far been identified for the classical cannabinoids prototype. They are listed below. For chemical numbering of carbon positions, see **Figure 5**.

Table 2 Range of reported K_i values for the major phytocannabinoids, according to Pertwee[140]

Phytocannabinoid	*CB1 K_i (nmol l^{-1})*	*CB2 K_i (nmol l^{-1})*
THC	5.05–80.3	3.13–75.3
Delta-8-THC	44–47.6	39.3–44
THCV	46.6–75.4	62.8
CBN	120.2–1130	96.3–301
CBD	4350–27 542	2399–4200
CBG	440	337

3.24.3.4.1 An alkyl substituent at C-3

An alkyl group on the C-3 aromatic position seems necessary for binding affinity to the CB receptors, and in the naturally occurring cannabinoids this side chain ranges from the most commonly observed pentyl ($-C_5H_{11}$), down to methyl ($-CH_3$). Changes in the alkyl group of natural cannabinoids lead to wide variations in affinity and selectivity for the cannabinoid receptors. It is now well established that the introduction of a dimethylalkyl side chain greatly increases affinity,[141] suggesting that the introduction of a branched substituent enhances the affinity of CB receptor ligands. The best substituents found so far are 1,1-dimethylheptyl or 1,2-dimethylheptyl.[137,142] One of the strongest CB1 agonists ever made falls into this class: HU210. It has a binding affinity to CB1 of up to 800 times more potent than THC, but without the psychoactive effects.[143]

Sometimes, varying the side chain may also lead to surprising results: the propyl analogue of THC, naturally occurring in the Cannabis plant, is an antagonist of both CB1 and CB2 receptors with fairly high affinity, instead of a weak agonist, as was expected based on its structural similarity to THC.[144]

3.24.3.4.2 A hydroxyl substituent at C-1

The phenolic hydroxyl group at C-1 has to be freely available for significant CB_1 binding. Removal of this hydroxyl group or conversion into a methoxy group leads to selective CB2-binding affinity.[137] A possible explanation for the inactivity of the acidic cannabinoids in receptor binding may be the occurrence of hydrogen bonding between the hydroxyl group and the adjacent carboxyl group.

3.24.3.4.3 The substituent at C-9/C-11

The methyl group at C-9 is not an absolute requirement for binding affinity. Introduction of a hydroxyl group at C-11 was in fact shown to increase affinity of THC as well as delta-8-THC for both CB1 and CB2 receptors.[145] The 11-hydroxy substituent is present in the primary metabolite of THC, 11-OH-THC, which has a more potent psychoactive effect than THC. Further oxidation (to aldehydes or carboxylic acids) eliminates the psychoactive effect but induces analgesic and anti-inflammatory properties. Ajulemic acid (AJA, a carboxylic acid) and nabilone (an aldehyde) are examples of synthetic analogues of THC developed for clinical use (see Section 3.24.6.2).

3.24.3.4.4 An aliphatic hydroxyl at C-6

The hydroxyl group attached to C-6 should be bound to an optimal chain length of three carbon atoms.[146] In the structure of THC, this pharmacophore is a part of ring B (see **Figure 5**, carbon# 6, 12, and 13). In contrast, in the structure of CBD these aliphatic carbons are spatially separated from the hydroxyl, and consequently CBD does not bind to either cannabinoid receptor.

There are several structural similarities between endocannabinoids and the plant-derived cannabinoids that bind to the CB receptors: both classes have a polar head group and a hydrophobic chain with a terminal *n*-pentyl group. More specifically, the pentyl side chain in cannabinoids is present in the endocannabinoids as the last five carbons of the fatty acid chain, and the OH at the C-3 position might correspond to the polar hydroxyl end of the endocannabinoids. Furthermore, the relative distances between these functional groups are comparable because of the ring system in cannabinoids, which can be mimicked by the four double bonds in the endocannabinoids.[147] The fatty acid acyl chain of endocannabinoids should be 20–22 carbons long, with at least three homoallylic double bonds.[148] It is proposed that the acyl chain can assume more than one conformation and that flexibility is necessary to mimic the tricyclic core of the classical cannabinoids.[149] Recently, evidence was also presented for common binding sites to the CB receptors.[150]

The knowledge obtained from these SAR studies has played a crucial role in the development of some of the cannabinoids in clinical use, as described in Section 3.24.6.2. However, the resemblance between phytocannabinoids and endocannabinoids, as described above, does not often apply to the cannabinomimetics, which may structurally be much unrelated to endocannabinoids.

3.24.3.5 Administration Forms

Studies with Cannabis or Cannabis-based preparations have been performed with a large variety of administration forms, ranging from pulmonary (smoking and vaporizing), to sublingual, topical, and oral preparations

(tea, milk decoctions, and baked products). A common factor of all administration forms of (herbal) Cannabis is a heating step, which is essential for the conversion of the acidic cannabinoids into their pharmacologically more active neutral counterparts. Based on the administration form, many changes to the original profile of compounds may occur by, for example, the extent of heating (decarboxylation of acidic cannabinoids), the type of storage (e.g., tea is stored differently from Cannabis cigarettes), degradation, loss by evaporation (e.g., terpenoids), and metabolism. As a result, a different spectrum of compounds is finally entering the bloodstream, and consequently a different type and duration of effects may be observed.

Smoking and oral administration are the two most commonly studied administration forms in clinical trials. The few studies that have directly compared the two forms of THC delivery show smoking to be more effective than oral administration.[151–153] Inhalation of THC avoids the first-pass effect but causes a rapid peak in blood levels accompanied by a spike in psychoactivity. A dose of 2–5 mg of THC consumed through smoking reliably produces blood concentrations above the effective level within a few minutes.[154,155] As a result, Cannabis smoking is a convenient method of administration, allowing self-titration of the desired effects. However, inhalation of toxic compounds during Cannabis smoking poses a serious hazard. This risk is not thought to be due to cannabinoids, but rather due to noxious pyrolytic by-products.[156,157] Consequently, the shortcomings of smoked Cannabis have been widely viewed as a major obstacle for the approval of crude (herbal) Cannabis as a medicine by public health authorities.[158] Nevertheless, inhaling is about equal to intravenous injection in efficiency, while considerably more practical.[159,160] Smoking Cannabis in the presence of tobacco can almost double the release of THC into the smoke, compared to smoking pure Cannabis. The mechanism for this is however still unclear.[161]

Cannabis vaporization or volatilization is a technique aimed at suppressing irritating respiratory toxins by heating Cannabis to a temperature where active cannabinoid vapors are formed, but below the point of combustion where pyrolytic toxic compounds are released. Vaporization offers patients who use medicinal Cannabis the advantages of the pulmonary route of administration, that is, rapid delivery into the bloodstream, ease of self-titration, and concomitant minimizing the risk of over- and underdosing, while avoiding the respiratory disadvantages of smoking. A few studies have been performed in recent years showing that vaporizing can be considered an efficient way of administration of Cannabis as well as pure cannabinoids,[162] resulting in bioavailability of THC comparable to smoking.[163]

In contrast, despite its convenient use, oral THC is notoriously unreliable in its effects.[164] Drawbacks of this administration route include its large variability in bioavailability, and extensive first-pass metabolism. The onset of effects is slow, precluding effective individual titration. In a study performed with orally administered THC, 2 h after oral administration of 10–15 mg, 84% of the subjects had no measurable level of THC in their blood. After 6 h, 57% still had none.[165]

When Cannabis is consumed in the form of a decoction, it is often referred to as 'tea'. Although Cannabis tea is a relatively popular administration form for self-medication among medicinal users, virtually no standardized studies have been performed with it. However, a recent study[166] showed Cannabis tea to be a robust and reproducible administration form for cannabinoids, with relatively high levels of acidic cannabinoids present.

As a result of the factors described above, the choice of administration form can have a major influence on the observed biological effects. For example, early studies indicated that oral doses of THC were no more effective for pain than codeine, and produced a significant amount of dysphoric effects.[167,168] Thus, it was believed that THC could only produce analgesia at doses that were high enough to cause behavioral side effects, and therefore was dropped as potential analgesic.[169] However, when using the parenteral or systemic route, THC and a range of cannabinomimetics have demonstrated potent analgesic effects up to 10 times that of morphine in animal models of acute and neuropathic pain.[170–173]

3.24.4 Biological Effects of the Cannabinoids

Virtually all studies on the biological activities of cannabinoids have been performed on the neutral cannabinoids. However, it should be stressed that most of the neutral cannabinoids discussed below are not biosynthesized by the Cannabis plant as such. Instead, acidic cannabinoids (carboxylic acids) are formed, that will yield neutral cannabinoids upon heating (e.g., recreational use by smoking) or prolonged storage, as

discussed in more detail in Section 3.24.2.4.1. However, it is now becoming increasingly clear that the acidic cannabinoids may have biological activities of their own, and should not be merely considered as 'precursors' of the active, neutral cannabinoids.

It would be impossible here to give a comprehensive overview of all described bioactivities of the cannabinoids, in particular for THC. However, they have been well reviewed in a number of papers and books.[27,174,175] Only the most important or remarkable biological activities will be discussed here, whereas their clinical implications will be described in more detail in Section 3.24.6.1 of this chapter.

3.24.4.1 Delta-9-Tetrahydrocannabinol

THC is the pharmacologically and toxicologically most relevant constituent found in the Cannabis plant, producing a myriad of effects in animals and humans. A frequently used way to review the biological effects of THC is by distinguishing central from peripheral effects, reflecting the classical physiological distribution of the cannabinoid-binding receptors CB1 (the 'central' receptor) and CB2 (the 'peripheral' receptor), as discussed in Section 3.24.3.1. However, the exact mechanism of action of cannabinoids is not exactly clear, as CB1 receptors are increasingly found outside the CNS,[96] whereas CB2 receptors are now known to be present in the nervous system, for example, in rat microglial cells and other brain-associated cells during inflammation.[176]

In a toxicological sense, the CB1-mediated central effects of THC are most important, because they are directly related to the psychological effects of Cannabis use. The most conspicuous psychological effects of THC in humans have been divided into four groups:[177] affective, sensory, somatic, and cognitive. In fact, most documented cannabinoid effects are mediated by the central cannabinoid receptor, and the behavioral effects caused by Cannabis or THC are generally consistent with the anatomical distribution of the cannabinoid receptors, in particular CB1 in the brain. However, neuroprotective properties in ischemia and hypoxia are examples of some known receptor-independent actions of THC and other cannabinoids. Furthermore, both THC (CB1- and CB2-binding) and CBD (nonbinding) potentiate the extinction of conditioned incentive learning, indicating that screening of receptor binding does not necessarily show the potential of cannabinoids.[178]

The best-established palliative effect of THC is the inhibition of chemotherapy-induced nausea and vomiting, mainly in cancer patients. Today, oral capsules containing dronabinol (Marinol) or its synthetic analogue nabilone (Cesamet) are approved for this purpose (see Section 3.24.6.2). Also, herbal Cannabis has been shown to reduce nausea in the majority of users, when ingested or inhaled. The effect of THC on nausea and vomiting has been confirmed in clinical trials.[179,180] It is however unclear how Cannabis or THC compares to the more recently developed, and very efficient serotonin ($5HT_3$) receptor antagonists for the treatment of nausea. Other potential palliative effects of THC in cancer patients include appetite stimulation and pain inhibition.

THC increases the metabolic rate in the brains of animals and humans,[181] and it decreases body temperature, but only at high doses. The increase in heart rate observed after THC administration is clearly dose-dependent and closely associated with THC plasma concentrations. As a result, cardiovascular problems are generally considered a contraindication for the medicinal use of Cannabis or THC. The results of a well-designed clinical trial using inhaled THC suggest that the increase in heart rate is not mediated by brainstem centers but is established by a direct effect of THC on the heart.[124] In the same study a wide array of CNS and non-CNS parameters were monitored after administration. THC had clear dose-dependent effects on postural stability, and body sway was found to be a very reliable indicator of THC blood levels. The high densities of CB1 receptors found in the basal ganglia, cerebellum, amygdala, and forebrain may explain these observations.

In particular in inexperienced users, THC can induce unpleasant effects including anxiety, panic, and paranoia. There are suggestions that in a small number of cases THC is capable of precipitating psychosis, involving delusions and hallucination.[182] If these disorders exist they seem to be rare, because they most likely require very high doses of THC, the prolonged use of highly potent forms of Cannabis, or a preexisting genetic vulnerability.[183] The causal link between Cannabis use and the development of psychosis has not been definitely proven, because of the large amount of parameters to be considered. However, there is enough reason to be precautious and communicate these 'suspicions' in a fair and balanced way. Although the

psychological effects caused by THC or Cannabis are a major drawback in their medical applications, many physical effects are already achieved below the threshold of psychological effects.

The effect that THC decreases intraocular pressure and improves blood circulation in the eye was found serendipitously as part of a study that tried to find easy physiological markers to screen drivers for driving under the influence of drugs. As a result, a variety of studies have targeted THC and other cannabinoids as potential new drugs in the treatment of glaucoma, the leading cause of irreversible blindness. The neuroprotective properties of THC may also be useful in this respect, leading to a dual effect in the protection of the retina and optic nerve.[184]

Anticonvulsant effects have been described for psychotropic as well as nonpsychotropic cannabinoids, including THC, CBD, CBN, 11-OH-THC, and delta-8-THC. THC relaxes muscles and has hypokinetic and anticonvulsant effects. This is one of the major reasons why THC is studied as a treatment for multiple sclerosis, and it may also have significance in epilepsy.

THC exerts an atropine-like effect on salivary secretion resulting in dry mouth. It also causes bronchodilation, comparable to the standard drug salbutamol.[185] This indicates that there is a potential for THC-like substances to treat asthma. However, no recent studies have been performed in this field.

Receptors of the CB2 type are present on white blood cells and affect the immune system, which may be a reason why Cannabis is often used as self-medication by immunocompromised individuals. THC is now considered an immunomodulator, capable of either enhancing or suppressing the function of a range of immune cells. These effects may be modulated by other constituents present in Cannabis.[186] Many described anti-inflammatory effects of THC and other cannabinoids are probably mediated by complex interactions with the immune system.

In early studies, THC was suggested to be mutagenic or carcinogenic. But in fact, an increasing number of studies are showing its anticancer properties.[187] Currently, there is convincing evidence that THC may play a role in the treatment of several types of cancer. In addition to apoptosis and inhibition of proliferation, THC might exert its antitumor effects by inhibiting tumor angiogenesis and metastasis. However, there is some controversy here: although THC has antiproliferative effect in tumors expressing cannabinoid receptors, those with low or no expression suffer increased growth and metastasis due to THC-induced suppression of the antitumor immune response.[188] More research is needed to clearly identify the therapeutic role of THC in cancer treatment.

A promising recent discovery is that THC relaxes the colon and reduces the colonic motility and tone after a meal.[189] This points out the potential for CB receptors to modulate colonic motor function in intestinal disease such as irritable bowel syndrome or Crohn's disease.

An important aspect of the evaluation of THC effects is that it may have a biphasic effect, causing opposite effects at high versus low concentrations. For example, in hefty doses, THC may protect the brain against various types of damage, whereas in tiny doses, potentially adverse effects would come through.[190] The potentially adverse doses would be much lower than those normally obtained from smoking a joint. However, a large dose inevitably becomes a small one as the body slowly clears it out. This may explain the many conflicting results obtained in clinical studies on THC and Cannabis: many studies use low concentrations of THC to prevent possible psychological effects in test subjects. Inadvertently, these low doses may cause exactly those adverse effects the study tries to prevent.[190]

3.24.4.2 Cannabidiol

CBD is, together with CBG, the major nonpsychotropic cannabinoid found in Cannabis. It is the principal cannabinoid present in fiber-type Cannabis (in the form of its carboxylic acid CBDA), a plant that is easily available to researchers, in contrast to the strictly controlled drug-type Cannabis varieties. Second to THC, the pharmacological effects of CBD have been best studied of all cannabinoids. It has powerful antioxidant properties, more potent than ascorbate and α-tocopherol. Also, it has notable anti-inflammatory and immunomodulatory effects.[191] Furthermore, sedating, hypnotic, antiepileptic, and antidystonic effects have been described. Also, CBD is a modulator of some types of opioid receptors,[192] and can modulate sleep in rats.[193]

CBD was found to have antianxiety effects.[194] In a clinical trial, oral administration of 400 mg of CBD resulted in decreased anxiety and increased mental sedation in test subjects.[195] It was concluded that CBD

possesses anxiolytic properties, possibly mediated by an action on limbic and paralimbic brain areas, where it reduced regional cerebral blood flow. These anxiolytic properties might prove useful in psychiatry. Possibly the most significant conclusion of this study is that a dose as high as 400 mg of CBD had no adverse effects. CBD was furthermore found to have antipsychotic benefits.[196]

A prominent effect of CBD was found in a variety of cancer studies. In a mouse model of metastatic breast cancer, CBD reduced the aggressiveness of breast cancer cells, by inhibiting a crucial protein for cancer development.[197] The study concluded that CBD represents the first nontoxic exogenous agent that can significantly inhibit metastatic breast cancer cells leading to the downregulation of tumor aggressiveness. Currently, there is a limited range of options in treating certain aggressive forms of cancer. CBD offers the hope of a nontoxic therapy that could achieve significant results without any of the painful side effects associated with standard therapy. Both *in vitro* and *in vivo* CBD were able to produce a significant antitumor activity on glioma cells. This antiproliferative effect of CBD was shown to be correlated to induction of apoptosis, which suggests a possible application of CBD as an antineoplastic agent. Effects were partially prevented by a (nonpsychoactive) CB2 receptor antagonist, suggesting a role for CB2 in cancer treatment.[198]

In another study performed on a panel of tumor cell lines with a variety of plant-derived cannabinoids, CBD was the most potent inhibitor of cancer cell growth, with significantly lower potency in noncancer cells. A CBD-rich Cannabis extract was equipotent to CBD, whereas CBG and CBC followed in the rank of potency.[199] It was suggested that the observed effect was due to the capability of CBD to induce apoptosis through cannabinoid receptors, or cannabinoid/vanilloid receptor-independent elevation of intracellular Ca^{2+} and reactive oxygen species. These data support the further testing of CBD and CBD-rich extracts for the potential treatment of cancer.

In many Cannabis varieties CBD is present in significant amounts.[200] However, only since a few years there is serious attention for THC–CBD interaction and this is mostly in studies on multiple sclerosis. Earlier studies focusing on the effect of THC alone have generally shown the use of Cannabis to be ineffective in many disease models, and such negative results unfortunately helped to shape the controversy in the discussion on the moral and ethical sides of Cannabis use in multiple sclerosis and other diseases.[201] It is known that CBD inhibits the metabolism of THC, by blocking its conversion to the more psychoactive 11-OH-THC by cytochrome P-450 (CYP) 3A11.[202] Possibly this is the reason why CBD is known to antagonize the psychotropic effects of THC.[203] Even though higher doses of THC are capable of inducing psychotic problems in some users, CBD seems to have an antipsychotic effect, its presence balancing the negative impact of THC consumption.[26] This property of CBD is exploited in the Cannabis-based medicine Sativex (discussed in Section 3.24.6.2).

3.24.4.3 Delta-8-Tetrahydrocannabinol

Delta-8-tetrahydrocannabinol(Delta-8-THC) is a positional isomer of delta-9-THC with a similar pharmacological profile and slightly lower psychoactive potency. Even though delta-8-THC has been very important for SAR studies on the classical cannabinoids, not many bioactivity studies have been done with the pure compound. It is probably not produced by plant metabolism, but rather it is an artifact caused by the degradation of THC (Section 3.24.2.3). In very low concentrations (0.001 mg kg^{-1} in mice, intraperitoneal (i.p.) injection), it increased food consumption, more than THC, whereas performance and activity of the animals were similar.[204] This low dose is equivalent to about 0.1 mg for an average human, an amount that could easily be formed by degradation of THC during the smoking of Cannabis (or be already present in aged plant material). Consequently, it could, at least partially, be responsible for the 'munchies', a popular name for Cannabis-induced increase in appetite.

In a rat study, it was found that behavioral suppression by delta-8-THC was mediated by activation of the arachidonic acid cascade through the CB1 receptor.[205] This may be a useful model to study the amotivational syndrome in humans.

3.24.4.4 Cannabigerol

CBG is one of the major cannabinoids found in most Cannabis varieties. It has shown relevant antibiotic effects,[206] and could decrease intraocular pressure.[207] CBG has been called 'inactive' when compared to THC, but it has slight affinity for CB1 receptors, approximately equal to that of CBD.[90] Like CBD, it has analgesic and

anti-inflammatory properties, indicating that there is scope for developing cannabinoid drugs that do not have the psychoactive properties of THC.[208] In one study,[209] CBG was evaluated for antitumor efficacy against mouse skin melanoma cells and showed a significant *in vivo* activity using an methylthiazoltetrazolium (MTT)-based cell viability assay.

Of several cannabinoids tested, CBG had the strongest potency to inhibit platelet aggregation.[210] However, in recent years no further studies have been reported on the biological activities of CBG.

3.24.4.5 Cannabinol

In 1940, CBN was the first cannabinoid to be isolated and purified from Cannabis. Although CBN is not produced by the metabolism of the plant, it is easily formed from THC by degradation during drying, storing, and consumption (heating) of Cannabis products. As a result, it may play a significant role in several effects attributed to Cannabis consumption. It is a very weakly psychotropic cannabinoid, whose effect is only measurable after intravenous administration. CBN has significant anticonvulsant, sedative, and other pharmacological activities likely to interact with the effects of THC.[211] It was shown to decrease heart rate without affecting coronary blood flow,[212] to decrease intestinal motility,[213] and to inhibit platelet aggregation.[204] Furthermore, CBN is a downregulator of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby counteracting the effects of THC, which increases NF-κB.[214]

3.24.4.6 Cannabichromene

This cannabinoid has hardly been studied at all. However, in most Cannabis varieties analyzed, CBC (in the form of CBCA) can be detected in significant amounts. CBC was shown to have sedative effects. By itself it has only a low analgesic effect in mice, but it increased the analgesic action of THC when administered together.[215] A Cannabis extract rich in CBC altered behavioral despair on the mouse tail suspension test of depression.[216]

3.24.4.7 Tetrahydrocannabivarin

Tetrahydrocannabivarin (THCV) is structurally similar to THC, except for a shortened side chain; it has a propyl ($-C_3$) side chain instead of a pentyl ($-C_5$), and for a long time it was thought to be a slightly less potent little brother of THC, exhibiting similar properties. However, quite unexpectedly, there is evidence that THCV is a CB1 and CB2 receptor antagonist.[144] Although the mechanism of action is not yet fully understood, a very recent study suggest that THCV, alongside standard CB1 receptor antagonists, has therapeutic potential to combat diseases involving cerebellar dysfunction and hyperexcitability, such as epilepsy.[217]

3.24.4.8 The Acidic Cannabinoids

Isolated in 1955, CBDA was the first discovered cannabinoid acid, whereas CBCA was isolated from Cannabis in 1968.[218] Up to date, only sporadic reports have been made on CBCA or CBDA, and rarely have the pure compounds been used for the evaluation of biological activity. As an exception to this rule, pure CBDA was found to display a potent antimicrobial effect.[219] In a study examining the composition of hemp seed oil and its potential as an important source of nutrition, it was observed that extracts containing higher concentrations of CBDA displayed more pronounced antimicrobial activity.[220] Because it is now known that seeds do not produce cannabinoids, the observed levels of CBDA must have resulted from external contamination of seeds by the hemp flowers surrounding it.

Not much is known about the biological effects or human metabolism of the acidic cannabinoids, but older studies at least indicate that the most common acidic cannabinoid, THCA, is not psychoactive in monkeys.[221] CB-receptor-binding assays indicate that the acidic cannabinoids, as well as their esters, are not binding. In the more potent varieties of Cannabis, THCA may be present in levels up to more than 20% of dry weight. However, a quantified, highly pure standard of THCA, as needed for analytical research as well as studies on biological effects, has not been available until recent years.[222] As a result, the potential value of THCA as an immunomodulating agent has only been discovered very recently.[71] Further studies on the biological activities of THCA, and on clinical formulation of this compound, are currently under way.

The acidic cannabinoids are biosynthesized by specialized trichomes, and stored extracellularly, which may indicate that they are cytotoxic. Therefore, the toxicity of CBGA and THCA, in suspension-cultured Cannabis cells and tobacco BY-2 cells, was compared with that of OA, the phenolic moiety in cannabinoids.[35] In 10-day-old suspension-cultured cells of *C. sativa*, 24-h treatment with CBGA and THCA at 50 $\mu mol\,l^{-1}$ caused 100% cell death as demonstrated by trypan blue staining, whereas OA did not have any effect on the cells. The same study also showed that both CBGA and THCA induced apoptosis not only in plant cells but also in insect (*Spodoptera frugiperda*, Sf9) cells, suggesting that cannabinoids may act as plant defense compounds. Since cannabinoid-producing glandular trichomes are distributed in physically fragile young tissues of the Cannabis plant, THCA and CBGA, and possibly other acidic cannabinoids, would protect these tissues from predators such as insects. This was the first report suggesting the physiological importance of THCA and CBGA as apoptosis-inducing defense compounds.

In a later study, it was observed that besides THCA, CBCA also has the ability to induce necrotic cell death through mitochondrial dysfunction in the leaf cells of the Cannabis plant itself.[223]

3.24.5 Noncannabinoid Constituents of Cannabis

Besides some major cannabinoids, no constituents derived from Cannabis plant material have been developed for medical use. Nevertheless, there are several constituents or even whole classes of compounds that may play a significant role in the observed effect of some Cannabis-based preparations. Many of these preparations are a part of self-medication by patients, but they have not been studied in controlled experiments.

Cannabis contains a large number as well as a significant amount of terpenoids. These compounds can be easily evaporated and are consequently inhaled by smoking. Smoke of Cannabis contains a high level of carcinogens, tar, and obnoxious gases (such as CO). However, smoking of Cannabis (without tobacco) does not seem to be associated with lung disease. In contrast, there are even positive effects reported on asthma. It is thought that the positive properties of the terpenoids are at least partially responsible for this. Indeed, several terpenoids identified in Cannabis have known anti-inflammatory, antiapoptosis, or neuroprotective effects (discussed in more detail in Section 3.24.5.1). Furthermore, Cannabis terpenoids and flavonoids may increase cerebral blood flow, enhance cortical activity, and kill respiratory pathogens.[224]

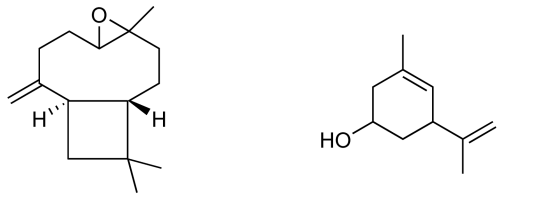
Plants offer a wide range of chemical diversity and have been a growing domain in the search for effective cannabinoid receptor ligands.[225] An increasing number of natural compounds from other species is found to bind to the CB receptors. An exciting discovery was that certain isobutyl analogues of anandamide from *Echinacea* species constitute a new class of cannabinomimetics, which specifically engage and activate CB2.[226,227] More recently, the ubiquitous sesquiterpene β-caryophyllene was found to bind to CB2.[228] Relatively high concentrations of this compound can be found in many plant species, including Cannabis.

The examples mentioned above illustrate the limitations of focusing solely on the cannabinoids. Even though many Cannabis constituents are found ubiquitous in other species, many of them have only been poorly characterized. At the same time, we have strong indications that the biological activities ascribed to Cannabis use cannot be explained by the presence of cannabinoids alone. The following sections will therefore list the most relevant noncannabinoid constituents found in Cannabis. For a complete overview of compounds found in Cannabis materials, the reader is referred to many reviews.[4,33,62]

3.24.5.1 Terpenoids

Terpenoids make up a large percentage of the essential oil of *C. sativa* L. To date, more than 120 terpenoids have been found in Cannabis, including 58 monoterpenoids, 38 sesquiterpenoids, 1 diterpenoid, 2 triterpenoids, and 4 other terpenoids. Two excellent reviews have been published summarizing these compounds and how they were identified.[4,33] Terpenoids display a wide range of biological activities and hence may play a role in some of the pharmacological effects of various Cannabis preparations.

Although cannabinoids are odorless, the volatile monoterpenoids and sesquiterpenoids are the compounds that give Cannabis its distinct smell. The sesquiterpenoid β-caryophyllene-epoxide (**Figure 11**), for example, is the main compound that search dogs are trained to recognize.[229] Only one unusual terpenoid can be found in



β-Caryophyllene-epoxide *m*-Mentha-1,8(9)-dien-5-o1

Figure 11 Two special terpenoids found in Cannabis.

Cannabis: the monoterpenoid *m*-mentha-1,8(9)-dien-5-ol. All others can be found ubiquitously in nature. For this reason the terpenoids of Cannabis did not receive much scientific interest, until it was found that the terpenoid profile of Cannabis products can help in determining the origin of Cannabis in custom seizures.[230]

3.24.5.1.1 Biosynthesis and composition of Cannabis essential oils

The terpenoids in Cannabis are frequently extracted from herbal material by steam distillation or vaporization. Typical yields of the terpene essential oils from fresh plant material range from 0.05 to 0.29% (v/w).[224] The essential oil of Cannabis is mainly composed of monoterpenoids and sesquiterpenoids with monoterpenoids dominating. Since self-administered Cannabis plant material is usually consumed as an (air-)dried product, the change in terpenoid content and concentration in relation to the fresh plant material is important to note. It has been reported that the essential oil content of a Cannabis plant changed from 0.29% essential oil (v/w) in the fresh product to 0.8% (v/w) after 1 week of drying, as a result of water loss. Following storage at room temperature for up to 3 months in a paper bag, the total essential oil was then reduced to 0.57% (v/w). Furthermore, it was observed in the essential oil that the relative percentage of monoterpenoids decreased whereas the relative percentage of sesquiterpenoids increased.[231]

Environmental conditions such as plant density, harvest time, pollination, and climate conditions may all play a role in composition and yield of Cannabis essential oils.[232] The cultivar of the plant also plays a role in the terpenoid composition. A study that analyzed the terpenoids of 157 different strains of Cannabis from various known origins found statistically significant differences in terpenoid composition. Even though these differences were not always indicative of what chemotaxonomic type the Cannabis strains belonged to, it may play a role in differential medicinal effects.[233]

3.24.5.1.2 Biological activities of terpenoids

The observation that whole Cannabis extracts may produce effects greater than expected from THC content alone has led researchers to postulate as to what other components in Cannabis could be responsible for enhancing or modulating the effects of THC. The terpenoids present in Cannabis display a wide range of biological activities that may be involved in regulating the effects of THC as well as producing their own unique pharmacological effects.[224] An overview of some of the known biological activities of terpenoids that have been identified in Cannabis is shown in **Table 3**.

Some undesired side effects of THC may be decreased or modulated in the presence of terpenoid compounds. For example, THC is known to cause acetylcholine deficits in the hippocampus, which may lead to short-term memory loss. This effect can be alleviated in rats by administering tacrine, an alkaloid that inhibits acetylcholine esterase, the primary enzyme involved in the breakdown of acetylcholine in cholinergic receptors.[223] Indeed, tacrine has blocked THC-induced memory loss behavior in rats. Interestingly, many of the terpenoids present in Cannabis display similar acetylcholine esterase inhibition, including pulegone, limonene, limonene oxide, α-terpinene, γ-terpinene, terpinen-4-ol, carvacrol, L- and D-carvone, 1,8-cineole, *p*-cymene, fenchone, and pulegone-1,2-epoxide.[224] For this reason, terpenoids are investigated for the treatment of Alzheimer's disease.

THC has been known to cause negative psychological reactions such as anxiety and depersonalization.[175] Some of these effects may again be alleviated by the terpenoids present in Cannabis, because of their sedative and antidepressive effects.[224] Cannabis terpenoids such as linalool, citronellol, and α-terpinene were shown to

Table 3 Summary of terpenoid biological activity[224,228]

Terpenoid	*Known properties*
β-Myrcene	Analgesic, anti-inflammatory, antibiotic, antimutagenic
β-Caryophyllene	Anti-inflammatory, cytoprotective, antimalarial, CB2 agonist
D-Limonene	Immune potentiator, antidepressant, antimutagenic
Linalool	Sedative, antidepressant, anxiolytic, immune potentiator
Pulegone	Acetylcholinesterase (AChE) inhibitor, sedative, antipyretic
1,8-Cineol	AChE inhibitor, stimulant, antibiotic, antiviral, anti-inflammatory, antinociceptive
α-Pinene	Anti-inflammatory, bronchodilator, stimulant, antibiotic, antineoplastic, AChE inhibitor
α-Terpineol	Sedative, antibiotic, AChE inhibitor, antioxidant, antimalarial
Terpineol-4-ol	AChE inhibitor, antibiotic
p-Cymene	Antibiotic, anticandidal, AChE inhibitor

have significant sedative effects, as indicated by decreased activity in a mice motility model after the inhalation of these compounds.[234] Limonene is a common component of Cannabis essential oil,[231] and it was shown to have a strong antidepressant effect by inhibiting the secretion of hypothalamic–pituitary–adrenal (HPA) stress hormones and normalization of CD4:CD8 ratios.[235] Limonene is also under investigation as an antimutagenic compound because of its multiple anticarcinogensis mechanisms. These effects may reduce some of carcinogenic effects of compounds present in Cannabis smoke.[224]

Cannabis and Cannabis extracts are used in pain relief.[175] Although many of the pain-relieving properties of Cannabis have been attributed to cannabinoids, terpenoids present in Cannabis may also exhibit pain-relieving effects. One of the most abundant terpenoids in Cannabis is β-myrcene,[231] which exhibits a potent analgesic effect as well as anti-inflammatory effect.[236,237] Other terpenoids present in Cannabis, such as carvacrol, exhibit a potent anti-inflammatory effect, even greater than that of THC.[238]

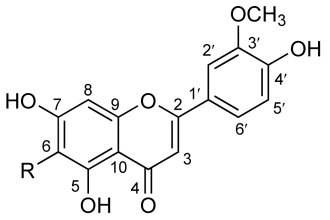
Cannabis extracts are known to effect blood–brain barrier (BBB) permeability,[239] thereby potentially altering the pharmacokinetics of THC and other cannabinoids. Since terpenoids are well known to interact with lipid membranes, they may be responsible for this observed activity. Terpenoids have also been shown to increase cerebral blood flow,[240] which may enhance cognitive brain functions in a way similar to ginkgolides in *Ginkgo biloba*.[224]

Terpenoids known to be present in Cannabis have a variety of effects, including antibacterial, antifungal, antiviral, and antimalarial activity. Besides the general health-promoting effect of these antimicrobial activities, they may also be important in reducing the dangers of recreational smoking of herbal Cannabis contaminated with microbial organisms.[224] A number of studies have investigated the antimicrobial effects of Cannabis essential oil.[241] One conclusion was that terpenoids from hash oil (obtained from drug cultivars of Cannabis, high in THC content) displayed an antimicrobial effect that was greater than essential oil derived from fiber cultivars.[242]

Finally, terpenoids present in Cannabis may play an important role in the chemical ecology of the plant. For example, they have been shown to be involved in the pesticidal properties of the Cannabis plant.[224] Terpenoids have been detected in the pollen of male Cannabis plants, which may play an important role in either attracting organisms involved in pollination or in repelling harmful organisms.[243]

3.24.5.2 Flavonoids

In total, 23 flavonoids have been reported from Cannabis.[31] Some bioactivity studies have been performed on flavonoids from Cannabis, although not nearly as much as on the cannabinoids or terpenoids. Much has been speculated about the role of these compounds in the therapeutic effect of Cannabis. They are often believed to synergistically enhance some beneficial effects, or reduce unwanted side effects of cannabinoids when Cannabis is taken in a crude form. Much remains to be learnt about these flavonoids, related to their effect not only on consumers of Cannabis but also on their role in the plant, and how various factors affect their biosynthesis and distribution in the plant.



R = CH_2-CH=$C(CH_3)_2$, Cannflavin B
R = CH_2-CH=$C(CH_3)CH_2$-CH_2-CH=$C(CH_3)_2$, Cannflavin A

Figure 12 Structures of cannflavins A and B.

Flavonoids have been extracted from the leaves,[244,245] flowers,[83] pollen,[246] and stems[247] of the plant. The aglycones or conjugated *O*-glycosides of kaempferol, quercetin, apigenin, and luteolin have been found, as well as the C-glycosides of vitexin, isovitexin, orientin, and their *O*-glycosides.[4,62] Two flavonoids are so far unique to the Cannabis plant; these are the prenylated flavonoids called cannflavin A and cannflavin B, as shown in Figure 12.[244,248]

In older studies, flavonoid glycosides were hydrolyzed with acid to yield aglycones, before identification. These were characterized with UV spectral properties and behavior in various chromatographic systems.[247,249] The result is that for the glycosides, the exact number and linkage positions of the sugar moieties are mostly not known.[4] In a few later studies, additional spectroscopic techniques were used to determine the structures of these flavonoids more precisely.[246,250]

3.24.5.2.1 Biosynthesis of flavonoids in Cannabis

Although the flavonoid pathway has been extensively studied in several plants, there is no specific data on the biosynthesis of flavonoids in Cannabis. However, the general pathway for flavone and flavonol biosynthesis as it is expected to occur in Cannabis is described by Flores-Sanchez.[251] There is currently no evidence indicating the presence of flavonoids in glandular trichomes.

A few studies have investigated differences in flavonoid content between different strains of Cannabis. Clark and Bohm[252] investigated the flavonoid content of 53 different Cannabis varieties grown from seeds from nine different countries, and found considerable plant to plant variation. The distribution of flavonoids in different varieties followed a pattern based on agronomic use, with clear differences between high-cannabinoid- and low-cannabinoid-producing strains.

Vanhoenacker *et al.*[253] found that cannabinoid-free Cannabis did not produce prenylated flavonoids in leaves or flowers, indicating that the biosynthesis of flavonoids in Cannabis may be linked to that of cannabinoids, and therefore these two polyketide biosynthetic pathways may be competitive. So far, not much is known about how biotic and abiotic factors influence levels and distribution of flavonoids in Cannabis.

3.24.5.2.2 Biological effects of flavonoids

Flavonoids have many roles in the physiology of plants. They provide plant pigmentation and flavor, are involved in plant growth and development, provide resistance to pathogens and predators, as well as protect against harmful effects of UV radiation.[254,255]

Flavonoids also show a large variety of pharmacological and biological activities.[256] As phenolic compounds, they can act as metal chelators and free radical scavengers, and more recently much attention has been given to the role of flavonoids as antioxidants.[257–259] Flavonoids are able to modify the activities of many enzyme systems in the human body, and have been shown to alter the function of various cell types in mammalian cell systems.[260] Many of these properties are related to immune functions, cellular transformation, and tumor growth and metastasis. Other biological activities reported include antibacterial, antifungal, and antiviral effects.[261,262]

Segelman *et al.*[263] isolated orientin and two flavone C-glycosides (orientin-2″-*O*-D-glucopyranoside and vitexin-2″-*O*-*β*-D-glucopyranoside) from a Mexican strain of Cannabis. These compounds were tested in a rat

lens aldose reductase enzyme assay, and found to have inhibitory properties against this enzyme, that is implicated in the pathogenesis of cataracts in humans with diabetes and galactosemia.

In a recent review of the chemistry and pharmacology of marijuana smoke,[264] no mention is made of flavonoids in Cannabis smoke. Nevertheless, it is likely that some flavonoids are present in Cannabis smoke. Most authors cite the work of Sauer *et al.*[265] who assumed that the estrogenic effect of marijuana smoke condensate was due to the presence of apigenin. Lee *et al.*[266] also tested marijuana smoke condensate for estrogenic effects, and went further by fractionating it to find out which components were responsible for the observed effect. They identified phenol and one phenolic derivative in the active fraction, but no flavonoids were found.

3.24.5.2.3 Therapeutic potential

Flavonoids may be important for the overall therapeutic effect of THC and the other cannabinoids by either synergistically enhancing them or reducing their side effects.[224] Flavonoids may counteract some unwanted effects caused by THC, such as the upregulation of tumor necrosis factor-alpha (TNF-α).[267] Flavonoids are particularly adept at inhibiting CYP monooxygenase enzymes, thereby potentially altering the pharmacokinetics of THC, which is converted into 11-OH-THC by the same enzymes. Such CYP-suppressing flavonoids may therefore act as chemoprotective agents by blocking the conversion of procarcinogens such as benzo[α]pyrene and aflatoxin B1, two harmful agents potentially found in Cannabis smoke, as a result of contamination of herbal Cannabis with molds.

Some flavonoids isolated from Cannabis have been tested for pharmacological effects. Cannflavins A and B were found to inhibit prostaglandin E2 in human rheumatoid synovial cells. Cannflavin A did so with 30 times more potency than aspirin.[244] Cannflavin A inhibited cyclooxygenase (COX) and lipoxygenase enzymes and thus had anti-inflammatory properties.[268] It is perhaps not surprising that these compounds show strong biological activities, because substitution with a prenyl group increases lipophilicity of flavonoids and give the molecules strong affinity for biological membranes.[269] Prenylated flavonoids are attracting increasing attention from the scientific community because of their potent antioxidant and anticancer effects, and their potential for treating menopausal problems.[270] Therefore, it is possible that the cannflavins will be shown to possess more biological properties in the future.

Clinical studies on flavonoids have shown that often very little unchanged aglycone is present in human plasma, but that conjugated metabolites such as glucuronic acid conjugates are present at high concentrations.[271] The bioactive forms *in vivo* may thus not be the naturally occurring phytochemical forms but rather their metabolites derived from them after absorption in the body.[272] The uptake of flavonoids and their *in vivo* metabolites are also different for different cell types. Consequently, the concept of oral bioavailability and activity of dietary flavonoids is clearly a complex topic. Nonetheless, it is possible that flavonoids in Cannabis taken orally have potential beneficial effects.

3.24.5.3 Hemp Oil

When Cannabis is cultivated for the production of fiber or seeds, only specially selected varieties with a very low THC content are legally allowed to be used. In that case, it is usual to use the term hemp instead of Cannabis (see Section 3.24.1.1). In recent years, scientific knowledge on the composition and benefits of hemp oil has increased significantly. The oil of Cannabis seeds has been promoted as a good source of the healthy polyunsaturated fatty acids, and may be considered a sustainable alternative to fish oil. It is widely used in body care products, lubricants, paints, and for other industrial uses, while its antimicrobial properties and emollient effect make it a useful ingredient for soaps, shampoos, and detergents.

Hemp oil is obtained from mature hemp seeds, grown outdoors.[273] After harvest, the seed is dried to reduce its moisture content, which also prevents sprouting during storage. Hemp seed contains about 30–35% oil by weight.[273,274] Because hemp oil is considered to be a relatively unstable product, it is not extracted by means of steam or organic solvents, but mainly by cold-pressing methods. Cold-pressed, unrefined hemp oil is light green, with a nutty, grassy flavor, whereas refined hemp oil is clear with little flavor. Chlorophyll and the carotenoid pigments found in mature seeds provide the natural dark green color to the oil.

3.24.5.3.1 Composition of hemp oil

Hemp seed typically contains about 25% high-quality protein and 35% fat in the form of an excellent quality oil. It has a remarkable fatty acid profile, being high in the desirable omega-3 fatty acids and also delivering some γ-linolenic acid (GLA), which is deficient in the average Western diet.[275] Although work by Ross *et al.*[276] showed no significant difference in the fatty acid composition of the oil generated from drug- or fiber-type seeds, the content of such higher fatty acids may vary considerably with variety, climate, and growing conditions.

Hemp oil typically contains 50–70% linoleic acid (LA; C18:2, an omega-6 fatty acid) and 15–25% α-linolenic acid (ALA; C18:3, an omega-3 fatty acid),[273] which is roughly in the 3:1 ratio that matches our nutritional needs (see Section 3.24.5.3.3). Furthermore, hemp oil provides significant amounts of some higher fatty acids such as GLA (C18:3; omega-6) and stearidonic acid (SDA; C18:4; omega-3).[273] Oleic acid (C18:1) and saturated fatty acids (mainly palmitic, stearic acids) both make up about another 10% of the oil.[27] In some hemp varieties, the omega-9 fatty acid eicosenoic acid (EA; C20:1) is present in amounts up to 0.5%;[273,277] however, most varieties typically contain much less.

Because hemp oil contains a high proportion of polyunsaturated fatty acids,[278] the double bonds that provide such unsaturation may be degraded because of oxidation by exposure to air, light, and/or elevated temperatures. At temperatures above 200 °C, undesirable *trans*-fatty acids are gradually formed, which may lead to the formation of aldehydes, causing the oil to become rancid. As a result, it is generally recommended that hemp oil should not be used for frying or baking, but preferably should be consumed cold.[273,275] However, results obtained by Molleken and Theimer,[277] who subjected hemp oil to a series of heat treatments before analyzing the fatty acid composition, showed that the stability of hemp oil is much better than generally assumed: *trans*-fatty acids were not formed under normal cooking conditions, and heated native hemp oils were quite stable under high-temperature conditions (up to 250 °C), presumably because of the presence of significant amounts of the antioxidant γ-tocopherol. In general, extra addition of tocopherols is recommended as preservative for hemp oil.[279]

Besides fatty acids, moderate to high concentrations of the vitamin E are present in hemp oil as well as small amounts of phytosterols, phospholipids, chlorophyll, carotenes, and several minerals.[27]

3.24.5.3.2 Therapeutic potential

Many edible oils (e.g., hemp, sunflower, soybean, pumpkin, and canola) contain significant amounts of the health-promoting omega-6 fatty acid LA. However, only some of these oils simultaneously provide significant amounts of the omega-3 ALA (**Figure 13**). It is important to notice that only hemp oil provides a ratio of LA to ALA close to 3:1, which is suggested as optimal for human nutrition.[273,280,281] Furthermore, hemp oil contains GLA and SDA. No other edible plant oil has these nutritional advantages.

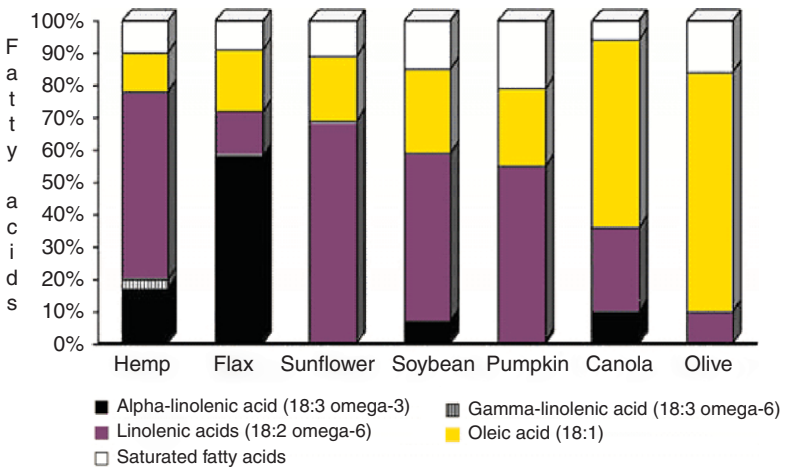


Figure 13 Typical fatty acid composition of vegetable oils. Reproduced with permission from G. Leson; P. Pless; J. Roulac, *Hemp Foods and Oils for Health*; Hemptech: Sebastopol, CA, 1999.

The unbalanced intake of omega-6 and omega-3 fatty acids is associated with many chronic diseases such as cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma, and depression.[282] The average Western diet provides a ratio of omega-6 to omega-3 of about 15:1. An increased intake of omega-3 fatty acids, through their eicosanoid metabolites, has been shown to result in lower blood pressure and blood cholesterol levels, playing an important role in the prevention and treatment of coronary artery disease, cancer, and hypertension. Moreover, it helps normalize fat metabolism and decreases insulin dependence in diabetics. Omega-3 fatty acids also increase overall metabolic rate and membrane fluidity, and exhibit anti-inflammatory properties, specifically with regard to relieving arthritis.[281,283]

Nutritionists suggest that daily requirements should range from 9 to 18 g of LA and 6 to 7 g of ALA, which would be equivalent to the consumption of three to five tablespoons of hemp oil. However, individuals who consume a diet high in saturated fatty acids or *trans*-fatty acids will require more, as well as people who are overweight or under great stress.[220,281]

3.24.5.3.3 Cannabinoid contamination of hemp oil products

Because hemp oil is produced for applications in food, the fear exists that the oil may be contaminated with significant amounts of the psychoactive component THC. Although no cannabinoids are metabolically produced by the hemp seed itself, they may be detected in hemp oil because cannabinoids as well as other components present in the resin may be transferred from the flowers and leaves onto the seeds, and subsequently to the oil during pressing. Thorough cleaning of the seeds, including the removal of the seed coat (dehulling), and the use of varieties with a certified low THC content (or more accurately: THCA content, see Section 3.24.2.3) are ways of preventing such contamination.[27] Certified low-THC hemp seed is currently available from Canada, Europe, and China and is under development in Australia and the United States. Today, hemp is grown throughout the world – except in the United States, where it is illegal to grow the plant but allowed to import, manufacture, and sell products made from it.

In order to ensure the safety of hemp products (oil and other), strict legal limits have been set for the level of THC allowed, ranging from 10 ppm in Canada to 50 ppm in Switzerland.[284] Nowadays, THC quantities observed in hemp oil are usually so small that there is no possibility of intoxication and hence no potential negative effects on human health. Use of cosmetics based on hemp oil typically does not result in positive urine tests for marijuana use. The minimal amounts of THC in hemp oil are probably not absorbed through the skin and/or do not cause any relevant uptake into the bloodstream.

3.24.5.4 Other Components Found in Cannabis

Besides the major classes of compounds described above, some other classes are worth mentioning as well. They will be described shortly in the following sections. An excellent review of the functions, occurrence, and biosynthetic pathways for the production of these minor components of *C. sativa* has been given by Flores-Sanchez and Verpoorte.[60]

3.24.5.4.1 Alkaloids and nitrogenous compounds

In Cannabis, 27 nitrogenous compounds have been detected, of which 10 have been identified as alkaloids.[4,33] Some of the more unusual constituents of Cannabis include an amide formed between *p*-hydroxy-(*trans*)-cinnamic acid and 2-(*p*-hydroxyphenyl)-ethylamine, which was isolated from the roots of Mexican Cannabis, and the spermidine-type alkaloids cannabisativine and anhydrocannabisativine (see **Figure 14**), isolated from the roots and aerial parts of various Cannabis strains.[4]

Other interesting alkaloids include choline, neurine, L-(+)-isoleucine-betaine and muscarine (protoalkaloids), hordenine (a phenethylamine), and trigonelline (a pyridine). The concentration of choline and neurine from dried roots was only 0.01%.[285] The presence of muscarine in Cannabis plants has later been questioned.[286] Methods for the synthesis of cannabisativine[287] as well as the biosynthesis of choline and atropine by hairy root cultures of *C. sativa*[288] have been reported.

Although alkaloids are generally considered to be a most interesting class of compounds for biological activity, there is currently no relevant information on the pharmacological profile of these Cannabis alkaloids.

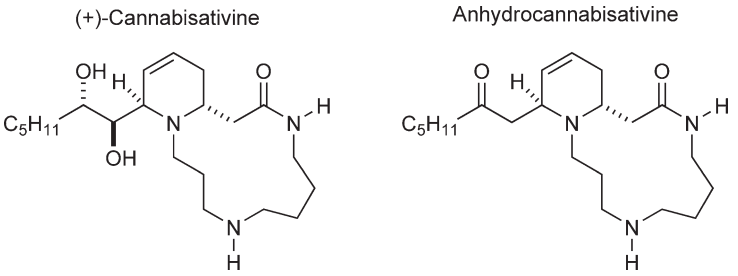


Figure 14 The structures of the Cannabis alkaloids cannabisativine and anhydrocannabisativine.

Some studies suggest pharmacological activities of smoke condensate and aqueous or crude extracts containing Cannabis alkaloids.[289,290]

3.24.5.4.2 Noncannabinoid phenols

Twenty-five noncannabinoid (and nonflavonoid) phenols were identified in Cannabis. These include simple phenols such as eugenol and related phenols, dihydrostilbenes or bibenzyl compounds (e.g., canniprene), and dihydrophenanthrenes and spiro-indans (e.g., Cannabispiran, Cannabispirenone). The dihydrostilbenes and spiroindans are closely related, and they possibly have the same biosynthetic origin. Several spiroindans have only been found in Cannabis.[291]

Only recently, the phenanthraquinone denbinobin (**Figure 15**) was identified in Cannabis extracts. First isolated from *Ephemerantha lonchophylla*, this compound inhibits NF-κB and causes apoptosis in human leukemic cells through reactive oxygen species. Furthermore, in a concentration-dependent manner, it induces apoptosis in human leukemic cells through Akt inactivation, Bad activation, and mitochondrial dysfunction.[292]

3.24.5.4.3 Stilbenoids

Nineteen stilbenoids have been identified in Cannabis.[4,33] They have been isolated from stem, leaves, and resin.[60] Studies have reported antibacterial activity for certain Cannabis stilbenoids.[293] It has been suggested that their biosynthesis could have a common origin, with dihydroresveratrol as a central intermediate.[60] However, no comprehensive reports about the biosynthesis of spirans or about the regulation of the stilbenoid pathway in Cannabis currently exist.

3.24.5.4.4 Lignanamides and phenolic amides

Cannabis fruits and roots have yielded 11 compounds identified as phenolic amides and lignanamides.[60] The phenolic amides include *N-trans*-coumaroyltyramine, *N-trans*-feruloyltyramine, and *N-trans*-caffeoyltyramine; the lignanamide group includes Cannabisins A–G and grossamide. In general, lignanamides belong to the lignan group, and the Cannabis lignanamides are classified as lignans of the arylnaphthalene derivative type.

The phenolic amides have cytotoxic, anti-inflammatory, antineoplastic, cardiovascular, and mild analgesic activity. For the lignanamides grossamide Cannabisin-D and Cannabisin-G, a cytotoxic activity was reported.[60] The presence and accumulation of phenolic amides in response to wounding and UV light suggests a chemical defense against predation in plants.[294] For the lignanamides Cannabisin-B and Cannabisin-D, a potent feeding deterrent activity was reported.[60]

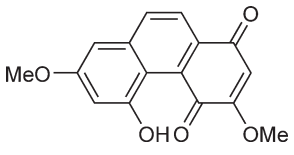


Figure 15 The structures of the Cannabis phenanthraquinone denbinobin.

The structures of the lignanamides and phenolic amides from Cannabis suggest condensation and polymerization reactions in their biosynthesis starting from the precursors tyramine and CoA-esters of coumaric, caffeic, and coniferic acids. However, it has also been suggested that these lignanamides could be isolation artifacts.[295,296] Further biosynthesis studies are necessary to elucidate their origin.

3.24.6 Cannabis as a Medicine

The clinical potential of the cannabinoids is large; some people suggest that Cannabis could be the 'aspirin of the twenty-first century', pointing out the impulse secondary metabolites from Cannabis may give to contemporary medicine.[9] However, much of the evidence for the medicinal use of Cannabis or cannabinoids is anecdotal and it turns out to be very challenging to confirm many of these findings by clinical trials. Also, it is often unknown which constituents are responsible for the effects observed after administration of herbal Cannabis or extracts. The lack of appropriate animal models with the complexity of the human brain hampers the study of the behavioral effects of these compounds. Therefore, experimental studies have concentrated on measurable physiological effects, and, as a result, the understanding of the underlying biology is only slowly improving. But despite these limitations, a number of cannabinoids of natural as well as synthetic origin have been developed for clinical use; most often as agonists or antagonists of CB receptors. These compounds are often the result of extensive studies on SARs performed on the plant-derived cannabinoids, their chemical derivatives, and their metabolites. In contrast, the clinical evaluation or development of the noncannabinoid constituents of Cannabis is minimal.

Although the structure of THC was elucidated by means of NMR spectroscopy already in 1964, relatively little clinical research took place for a long time. Research on the medicinal potential of cannabinoids got a new impulse after the discovery of the cannabinoid receptors in the 1990s. During the extended period between these two events, the cannabinoid character of a large variety of compounds was assessed through a panel of *in vivo* assays, one of the earliest being the dog ataxia test.[297] However, the most widely used set of assays were known as the cannabinoid tetrad,[298] which comprised four different behavioral tests performed mostly in mice: diminution of temperature (hypothermia), immobility in a multiple photoelectric cell chamber (diminution of locomotion), a ring test or bar test (catalepsy), and a hot-plate or tail-flick test (analgesia). A positive response in all four tests was the criterion to consider a compound as a 'classical' cannabinoid. To date, activity in this mouse behavioral battery has been a reliable predictor of psychotomimetic activity in humans. Nowadays, it is understood that the observed effects in the cannabinoid tetrad can in fact be attributed to CB1 activation.

Cannabis preparations have been used in the treatment of numerous diseases, with marked differences in the available supporting data. Clinical studies with single cannabinoids (natural or synthetic) or whole plant preparations (e.g., smoked Cannabis, encapsulated extract) have often been inspired by positive anecdotal experiences of patients using crude Cannabis products for self-treatment. The antiemetic,[299] appetite-enhancing,[300] analgesic,[301] and muscle relaxant effects,[302] and the therapeutic use in Tourette's syndrome,[303] were all discovered or rediscovered in this manner. Incidental observations have also revealed therapeutically useful effects. The discovery of decreased intraocular pressure with THC administration, potentially useful in the treatment of glaucoma, was made serendipitously during a systematic investigation of healthy Cannabis users.[304] However, anecdotes as to the efficacy of Cannabis or THC in indications that have not been confirmed in controlled studies have to be judged with caution. Nevertheless, the therapeutic potential of Cannabis and the cannabinoids is large.

3.24.6.1 Therapeutic Potential of Cannabinoids

The therapeutic potential of cannabinoids can be clarified by pointing out the central physiological importance of the endocannabinoid system, as described in Section 3.24.3. The cannabinoid system is involved in a wide range of physiological functions and might be related to a general stress–recovery system. One yet unproven but intriguing idea is that endocannabinoids may set the 'analgesic tone' of the body, with the level of their production acting as a kind of pain thermostat.[305] It is likely that such a system relies on the combined activities of a range of compounds. Strategies to modulate endocannabinoid activity include inhibition of reuptake into

cells and inhibition of their degradation to increase concentration and duration of action. The effect of cannabinoids or synthetic cannabinomimetics interacting with such an endocannabinoid system could be on multiple levels, other than receptor binding alone. Some of such interactions have already been described.[305]

Cannabinoids make up a significant group of compounds with diverse properties, and even based on the limited data available it may be expected that at least several of them have therapeutic potential. Most known cannabinoids have been tested to describe their relative (psychoactive) potency in comparison to THC, either in receptor-binding assays or in THC-specific assays. However, testing non-THC cannabinoids as serious candidates for new leads can sometimes lead to completely counterintuitive results, as shown in the case of THCV; although its psychoactive potency is roughly similar to THC (about 75%)[4,306], later *in vivo* testing surprisingly showed that THCV should rather be considered an antagonist of THC activity.[144]

An exciting notion is that cannabinoids, and possibly also noncannabinoids present in the plant, may exert their effect independent of cannabinoid receptors altogether. In this respect, most studies are focused on the interaction between the cannabinoid and the opioid system. It is becoming increasingly clear that cross talk between the cannabinoid and the opioid system exists,[85] but our understanding of this field is only just beginning, as discussed in more detail in Section 3.24.6.3.4.

3.24.6.2 Current Status of Cannabinoid Medicines

An increasing number of pharmaceutical companies start to pick up the idea of cannabinoids or their antagonists as therapeutic drugs. At present a number of preparations based on the biological activities of the cannabinoids are available, but not all of these have been fully registered as drugs. Most preparations are pure compounds based on the pharmacological actions of THC. The major ones are shown in **Figure 16**.

The most commonly prescribed cannabinoid-based medicines are Marinol (synthetic THC in sesame oil, Solvay Pharmaceuticals) and Cesamet (nabilone, Valeant Pharmaceuticals International). They are registered for the indication of nausea and vomiting associated with cancer chemotherapy. Marinol is also approved for anorexia and cachexia in HIV/AIDS. The patent on Marinol will expire in 2011, opening the way for the development of generic preparations of synthetic, as well as naturally derived THC.

Although there are clear indications that some pharmacological effects may vary according to the fact if a cannabinoid is taken alone, or in combination with other cannabinoids, not much work has been done on the

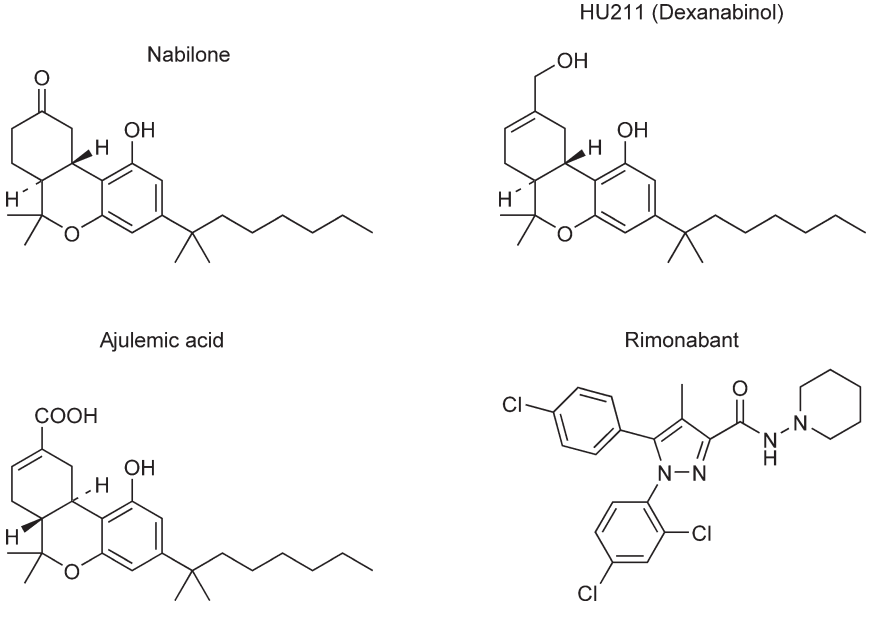


Figure 16 The structures of several cannabinoid receptor agonists currently in clinical use or under development.

activities of combined cannabinoids. However, there is a major exception: Sativex (GW Pharmaceuticals, UK) is a sublingual spray based on a mixture of two distinct standardized Cannabis extracts. The final preparation contains equal amounts of THC and CBD. The presence of CBD is thought to reduce the metabolism of THC by cytochromes in the liver, thereby increasing the half-life of THC in the blood. Because of the use of whole extracts, nonstandardized amounts of ballast components are also present, such as minor cannabinoids and terpenoids.[307] Sativex is currently registered only in Canada, but registration is pending in several European countries.

Cannador (European Institute for Oncological and Immunological Research, Germany) is an oral capsule containing a Cannabis extract, with a ratio of THC:CBD that does not appear to be fully standardized. Although it has been used in several clinical trials, it has not yet been registered as a drug. It has been clinically tested for the reduction of tremor in multiple sclerosis,[308] and postoperative pain management.[70]

Rimonabant (Acomplia, by Sanofi-Aventis) was the first selective CB1 receptor blocker to be approved for use anywhere in the world.[309] It is an inverse agonist for the cannabinoid receptor CB1, intended as a new prescription antiobesity drug. It was released in the form of a tablet under the name Acomplia, but has very recently been pulled off the market out of concern over its depressive side effects. Many other pharmaceutical companies were working on similar CB1 agonists (e.g., Taranabant by Merck & Co, and SR141716 by Eli Lilly), but most have discontinued their work after the withdrawal of Rimonabant.

AJA (also known as CT-3, developed by the University of Massachusetts Medical School, Worcester, Massachusetts, USA)[310] is a synthetic analogue of the human THC metabolite 11-carboxy-THC (see Section 3.24.3.3). Although the mechanism of AJA action remains largely unknown, it has potent analgesic and anti-inflammatory activity,[311] without the psychotropic action of THC. Psychoactive effects may be limited as a result of reduced crossing of the BBB, and greater activity at peripheral rather than central cannabinoid receptors.[312] A major advantage of AJA is that, unlike the nonsteroidal anti-inflammatory drugs (NSAIDs), it is not ulcerogenic at therapeutic doses. It has been studied in clinical trials for chronic neuropathic pain.[313]

Dexanabinol (also known as HU-211, developed at The Hebrew University, School of Pharmacy, Jerusalem, Israel) is a dihydroxylated synthetic cannabinoid resembling THC that is devoid of cannabimimetic effects and does not bind to cannabinoid receptors.[314] The neuroprotective effect of dexanabinol is related to its unique capacity to act as a noncompetitive antagonist of the NMDA receptor, to block COX-2 enzymes, and to prevent inflammation by inhibiting secretion of TNFα and other inflammatory cytokines in the CNS.[315] Dexanabinol may be useful in the treatment of traumatic brain injury.[316]

3.24.6.3 Approaches for Further Development

Extensive information about Cannabis as a medicament goes beyond the possibilities of this chapter and the readers are referred to several comprehensive books[27,317] and recent reviews[69,131,307] on this subject. However, some major developments will be briefly discussed here. The following sections discuss some suggested targets for further development of Cannabis compounds as a drug.

Cannabinoids, as found naturally occurring in the plant, provide the pharmaceutical developer with a variety of challenges, including low water solubility, variable bioavailability, and psychoactive side effects. As a result, current research on synthetic cannabinoids focuses mainly on new chemical entities (NCEs) that can agonize or antagonize one of the CB receptors specifically, or that can affect the endocannabinoid system otherwise (e.g., FAAH inhibitors). Possible fields of application include obesity, anorexia, and neuroprotection. Some of these molecules have proven to be powerful agonists. But although they are extremely useful for nonclinical studies to uncover the functions of the endocannabinoid system, they may be too psychoactive to be used in human subjects.

3.24.6.3.1 Improving the biological availability of cannabinoids

THC is a light yellow resinous oil, sticky at room temperature that hardens upon refrigeration. THC is highly lipophilic, practically insoluble in water,[318] having an octanol:water partition coefficient (at pH 7) of at least 6000:1.[319] As a result, it has been difficult to develop effective formulations for human use.[162] So far, every attempt to make classical cannabinoids more water soluble has yielded only compounds without significant CB

binding. Several examples illustrate the difficulties in handling the high lipophilicity of cannabinoids; the synthetic cannabinoid dexanabinol was evaluated in phase I clinical trial by intravenous (i.v.) infusion in cremophor–ethanol vehicle diluted with saline, whereas Sativex is a sublingual spray containing ethanol and propylene glycol as solubilizers.[320] A possible way to handle this obstacle is the creation of water-soluble prodrugs. Examples are glycinates and salts of amino acid esters containing tertiary and quaternary heterocyclic N-atoms.[321] Also, the use of cyclodextrins as solubilizers seems to be feasible.[322]

3.24.6.3.2 Selective activation of cannabinoid receptors

There is a fundamental problem with using the cannabinoid receptor as a drug target: the main target for most therapeutic activities is CB1 and this is the same receptor that causes most of the adverse effects. Dissociation of the adverse effects from the therapeutic effects of Cannabis may therefore never be truly possible. Furthermore, excessive stimulation of CB1 leads to receptor tolerization and this is a particular problem of strong agonism.[323] Also, there may be risks associated with the long-term use of CB blockers (antagonists): a good example is the increased risk of depression with the prolonged use of Rimonabant.

But although CB1 is generally considered to be centrally active, it is also expressed on nerves outside the CNS, for example, on nerve terminals, dorsal root ganglia, and the vasculature. Therefore, a possible strategy for drug development is to develop compounds that are excluded from the BBB, to selectively activate the peripherally located CB1 receptors. This may limit psychoactivity while producing benefits for disorders such as pain, asthma, and glaucoma.

Cannabinoids inhibit pain in virtually every experimental pain paradigm via either CB1- or CB2-like activity, dependent on the type of nociceptive pathway being studied. This finding is consistent with high concentrations of CB1 receptors on primary afferent nociceptors, particularly in the dorsal spinal cord, whereas peripheral CB2 receptors have been implicated in the control of inflammatory pain.[135] CB2 selective agents, working on the peripherally located CB2 receptors, without activating the CB1 receptors that may induce a psychoactive effect, may have therapeutic value. Guindon and Hohmann[324] reviewed behavioral, neurochemical, and electrophysiological data, which identify cannabinoid CB2 receptors as a therapeutic target for treating pathological pain states with limited, centrally mediated side effects. Cheng and Hitchcock[325] reviewed the present development of cannabinoid agonists with an emphasis on selective CB2 agonists and peripherally restricted CB1 or CB1/CB2 dual agonists for the treatment of inflammatory and neuropathic pain.

3.24.6.3.3 Modulating the endocannabinoid system

Endocannabinoids, through interaction with the CB receptors, have a range of effects on the nervous system. They are weak agonists and these agents naturally stimulate receptors without much potential for inducing psychoactive effects. For this reason, modulation of the endocannabinoid system is an exciting target for cannabinoid therapy. But although endocannabinoids may be interesting as therapeutic agents, their instability and rapid metabolism limit their utility in preclinical and clinical research. To date, no endocannabinoid agents have been administered to humans.

How many and what functions of the endocannabinoids occur tonically under conditions of physiological homeostasis is unclear at present. The fact that CB1 and CB2 receptor knockout, at least in certain genetic backgrounds, does not produce a strong phenotype in unchallenged animals suggests that this system becomes important mostly under pathological conditions. Indeed, endocannabinoid signaling often undergoes dramatic tissue-specific changes in both animal models of disorders and in human diseases.[117]

As discussed in Section 3.24.3.2, endocannabinoids are made on demand, act only locally, and are metabolized immediately after action. As a result, the duration of their action is very limited. Compounds that affect the levels of endocannabinoids, by inhibiting membrane transport or hydrolysis, thereby prolonging their life span, offer promising potential for further research and pharmaceutical development. During a variety of diseases there are changes in endocannabinoid concentrations at the site of pathology. Targeting of endocannabinoid degradation through inhibition of the reuptake mechanism or enzymes that cause degradation could locally target sites of damage while limiting effects in uninvolved areas.

3.24.6.3.4 Interaction with other neurotransmitter pathways

An exciting observation is that THC reduces chronic pain in patients who do not get sufficient pain relief from opioids alone. Therefore, a promising development is the combined use of THC with opioids. Although the brain has more CB1 than opioid receptors, a review by the U.S. Institute of Medicine has commented on how little is known about cannabinoids in comparison with opiates.[326] The obvious analogy between the history of research on opiates and cannabinoids suggests good reason for optimism about the future of cannabinoid drug development.[327,328] Many studies have been performed on Cannabis and pain, but they are hard to compare because of the large variety of pain models used, and the subjective nature of pain. However, a variety of synthetic analogues and derivatives of THC and other cannabinoids have been designed with an improved analgesic effect, but without the psychotropic side effects of THC.

Opioids and cannabinoids share several pharmacological effects, including antinociception, hypothermia, inhibition of locomotor activity, hypotension, and sedation.[169] It is therefore not surprising that cross talk between the two systems has been shown.[85] The coupling of both receptor types, through inhibitory $G_{i/o}$ proteins, to similar intracellular signaling pathways underlies, to some extent, their similarities in actions.[86] Interactions between the pathways probably explain why antagonists of each receptor type sometimes counteract the pharmacological effects induced by the stimulation of the other type.[327] Coadministration of various cannabinoids with morphine produced a greater-than-additive effect with respect to antinociception in mice.[329] Although both cannabinoids and opioids are accompanied by undesirable side effects at high doses, it was found that THC can enhance the potency of opioids such as morphine, thereby dramatically reducing the dose needed for pain control in some clinical indications.[330,331]

Yet another potential target for interaction with (endo)cannabinoids is ceramide, a ubiquitous sphingolipid second messenger that plays an important role in the control of cell fate. Cannabinoid-induced acute ceramide generation might rely on *de novo* synthesis through the induction of sphyngomyelin hydrolysis. As a result of this activity, cannabinoids, like other ceramide-generating agents,[332] might be considered as potential therapeutic drugs for the management of malignant tumors.

3.24.7 Practical Aspects of Cannabis Research

Thanks to the relatively recent discovery of the human endogenous cannabinoid system, it seems that Cannabis-based medicines may have a bright future. But there are a lot of obstacles to be taken first. Some are a direct result of the nature of the plant and the chemical characteristics of its constituents, but others are clearly the result of social, cultural, and as a result, legal bias toward a hazardous plant. Some of the most important aspects are discussed below.

3.24.7.1 Legal Aspects

Starting from 1954, the World Health Organization (WHO) has claimed that Cannabis and its preparations no longer serve any useful medical purpose and are therefore essentially obsolete. Up to that moment, Cannabis legislation had been based on a large number of conventions, causing considerable confusion in the execution of treaties. Under pressure of increasing reports that Cannabis was a drug dangerous to society, it was proposed to combine all in single convention, the draft of which was finally accepted by the United Nations in 1961. In the following years, several complementary treaties were made to strengthen it. Under the 'Single Convention on Narcotic Drugs', Cannabis and its products were defined as dangerous narcotics with a high potential for abuse and no accepted medicinal value. It reflected the belief that Cannabis was a dangerous narcotic with a threat that was equal to the most dangerous opiates, as it was strongly believed that Cannabis use could serve as stepping stone to the use of such drugs. Since the Single Convention, the potential danger of Cannabis abuse by recreational users has been much higher on the political agenda than any of its benefits as a source for fiber, food, or medicines. The distinction between medicinal and recreational use is thereby made only in a few countries.

It can be observed that new scientific insights on Cannabis are only slowly and reluctantly incorporated into new legislation. However, in recent years a large variety of scientific and clinical data has become available,

further showing the physiological effects of cannabinoids and the endocannabinoid system. And in several Western countries important obstacles for a real acceptance of medicinal Cannabis have already been addressed, as serious steps are taken toward decriminalization of Cannabis use or even providing medicinal Cannabis products to patients. These shifts constitute the first steps away from the dominant drug policy paradigm advocated by the United States, which is punishment-based prohibition, and it signals that the Single Convention may start to reach its expiry date. The legislation that follows it will depend for a large part on the quality of the research available. However, good arguments will finally not be enough; what is most needed is a change in mentality; in politics, but also in the way research is conducted.

3.24.7.2 Availability of Plant Materials and Reference Standards

Although a huge number of scientific papers have been published on Cannabis over the past decades, many aspects still remain unclear. The world today is full of Cannabis myth and mystery. A major reason is the unavailability of standardized plant materials. As a result of a prohibition on the breeding of the Cannabis plant, researchers worldwide have virtually no access to fresh plant materials. In fact, most plant material used for Cannabis research comes from customs seizures or governmental agencies. The type of Cannabis (cultivar), the breeding and storage conditions, and age of the plant materials are often unknown to the researcher. Microbiological contamination of such herbal Cannabis has been frequently described. Although it remains a speculation, the production of toxins by these microbes may play a significant role in at least some of the observed adverse effects in medical studies.[80]

For more than half a century, the medicinal research has been driven by the search for the components responsible for the psychoactive effects of Cannabis. As a result, THC has been in the spotlights for decades, but other Cannabis constituents were largely neglected. By doing so, it is often forgotten that more than 700 different varieties of Cannabis have been described. In many research papers, it is assumed that differences between Cannabis cultivars are only defined by the total content of THC (which is defined as THC + THCA). The total fingerprint of other compounds present is usually not reported and very often these compounds were not even analyzed. As a result, such studies can never be repeated, because the (exact) same type of Cannabis cannot be obtained by other researchers trying to duplicate the results. It is obvious that there is a serious need for the availability of standardized herbal Cannabis for research. Such materials are currently only available from a very limited number of sources. The best example is the Netherlands, where pharmaceutical-grade Cannabis is produced as part of the medicinal Cannabis program of the Dutch Health Ministry, and this material can be exported for research worldwide.[80]

Finally, independent of the method used for cannabinoid analysis, reliable standards are needed for the compounds to be studied, to allow high-quality, quantitative research on the pharmacological and medicinal aspects of Cannabis. However, up to very recently, only a few of the major cannabinoids were commercially available (THC, CBD, CBN, and delta-8-THC). Without a doubt, this lack of reference standards has been a great obstacle for the detailed study and understanding of Cannabis. In recent years, however, the number of suppliers of cannabinoid standards has been growing (e.g., Echo Pharmaceuticals, Lipomed, THC Pharm, Sigma-Aldrich).

3.24.7.3 Social Aspects

Although the complex chemical nature of the Cannabis plant and its constituents has complicated its study, a significant role was also played by social and cultural views on Cannabis in recent history. Although Cannabis has a long history, the twentieth century advent of modern purified pharmaceuticals made Cannabis products increasingly less popular with physicians. Only after the biochemical basis of Cannabis activity has been elucidated, which is in fact only in the last 10–15 years, scientific interest has been somewhat revived.

A major argument of health authorities against the medicinal use of herbal Cannabis as currently available has been that it is a highly variable product with respect to composition and (microbiological) contamination. This may be true when comparing different varieties, but the composition of single well-defined varieties of Cannabis can be highly standardized. This fact has been clearly shown by the medicinal Cannabis program currently going on in the Netherlands, where growing under the regime of Good Agricultural Practice (GAP),

in combination with technical and hygienic measures, has shown to produce Cannabis of high, pharmaceutical quality.[80] Furthermore, procedures for standardized prescription botanical products have been formalized by the FDA, providing a further blueprint for regulatory approval of phytochemicals and botanical medicines.[333]

Cannabis studies often have to deal with problems that are largely unknown to other fields of research. These include difficulties to find funding, get results published, or to obtain permission to perform clinical trials. Also, the restrictions on import/export of Cannabis materials and its extracts or pure components can postpone studies for long periods of time. Fortunately, in several Western countries such restrictions are slowly becoming less strict. Still, the continuing fear of potential psychoactive effects of Cannabis frequently interferes with performing, mostly clinical, studies. In fact, the continuous increase in (psychoactive) potency of modern Cannabis varieties is boosting political as well as societal fears of addiction and health problems, and this seems to make the acceptance of Cannabis as a source of potential new medicines even harder.

It is clear that, in time, Cannabis-based medicines should be standardized, efficacious, and safe preparations, as much as any other approved medicine. Therefore, the main challenges for the near future are standardization of Cannabis-based medicines, obtaining clinical proof of its claimed activities, and improving the acceptance among authorities and health professionals. The dominant view is that the proof of the activities of Cannabis must come from statistically significant randomized clinical trials, acceptable to regulatory bodies in various countries and adhering to the modern scientific method. However, times are changing, and other plant-based medicines, such as Chinese traditional medicine, are gaining ground as part of Western medicine. In some cases, traditional use of such herbal medicine by large groups of patients may be accepted by the authorities as sufficient proof of safety and efficacy. It remains to be seen whether Cannabis can benefit from such developments.

3.24.8 Conclusion

Perhaps Cannabis is best known for its use as a psychoactive drug. However, it should also be recognized as provider of the strongest fibers found in the plant kingdom, and source of some of the healthiest and most nutritious edible oils. In fact, the Cannabis plant can probably meet more of human's needs than any other plant can. As a medicinally active plant, Cannabis has been used by cultures all over the world for millennia, making it one of the oldest known medicinal plants.

But despite the great potential of Cannabis, its classification as a narcotic drug and its increasing demonization in most cultures around the world have so far delayed its successful development into modern medicines. Since the United Nations adopted the 'Single Convention on Narcotic Drugs' in 1961, Cannabis and its products have been politically defined as dangerous narcotics with a high potential for abuse and no accepted medicinal value. Following that, a huge number of studies have been published on all aspects of Cannabis use, but there is currently still no scientific consensus on the usefulness of Cannabis as a medicine. New scientific insights on Cannabis are only slowly and reluctantly incorporated into new legislation. The reasons for this are diverse, and they have been discussed in more detail in this chapter. As a result, the world today appears to be full of Cannabis myth and mystery.

At least one bioactivity of Cannabis is undisputed: the psychoactive effect of THC is one of the best-studied biological activities in the world. As a result, scientific attention has largely shifted from the Cannabis plant as a whole, to its main psychoactive component(s). Chemically, THC belongs to a group of closely related compounds known as cannabinoids, and they are commonly considered the main bioactive components of Cannabis. These terpenophenolic compounds are unique to the Cannabis plant and are found nowhere else in nature. Up to date, already 70 different cannabinoids have been described, but only a few of the major ones have been characterized for biological activities, including CBD and CBN. Nevertheless, the activities that have been discovered so far provide enough reasons to find out what else the Cannabis plant has to offer us.

Besides cannabinoids, Cannabis contains over 450 other identified components. Much remains to be learnt about most of these compounds, as the medicinal properties of Cannabis do not seem to be completely understood based on the cannabinoids alone. What we need to learn about these constituents is related not only to their effect on consumers of Cannabis, but also to their role in the plant, and how various factors affect their biosynthesis and distribution in the plant.

Most interesting among the Cannabis constituents are the secretions of the glandular trichomes, found in high density on the female flowers. Besides cannabinoids, terpenoids are present in high amounts, and more that 100 different types have been identified in Cannabis. Although none of them are unique to Cannabis, many of them have well-described biological activities. And because they are easily volatilized, they are present in Cannabis smoke, which is the most commonly used form of Cannabis administration for both recreational and medicinal users. Although it would be very useful to understand the interaction between the cannabinoids and terpenoids in a variety of medical or psychological conditions, such studies are yet to be undertaken.

Hemp oil is obtained from the mature seeds of Cannabis, and may be an upcoming 'superfood'. Hemp seed is very rich in easily digestible protein content, and its oil has one of the healthiest lipid compositions among the edible plant oils. However, the general confusion between hemp and 'marijuana' still stands in the way of accepting hemp as a major new food crop in most countries. Nutritional studies focused on the health benefits of a hemp-oil-enriched diet may help to increase the acceptance of this valuable resource.

Many additional classes of compounds can be found in Cannabis, including flavonoids, alkaloids, and stilbenoids, and all have been covered in this chapter. But because most of these constituents have not yet been properly characterized for biological activity, the Cannabis plant could be called a 'neglected pharmacological treasure trove'.[32] There is still plenty of work to do for the coming generation of plant researchers, to make us truly understand the potential of the Cannabis plant.

The pharmacological effects of the Cannabis plant have intrigued scientists for centuries, and after the elucidation of the structure of THC in 1964, this led to a hunt for specific binding sites. Finally, the discovery of such sites, the CB1 and CB2 receptors, has provided us with an increasingly clear understanding of the effects of Cannabis. It is now known that cannabinoid receptors can be found in most parts of the brain, as well as in the immune system and a variety of other organs. Their distribution seems to explain many of the observed effects of Cannabis consumption.

Cannabinoid receptors are part of the endocannabinoid system, which is now known to be a ubiquitous neuromodulatory system with wide-ranging actions. It comprises cannabinoid receptors, endogenous cannabinoids, and enzymes responsible for their production, transport, and degradation. The endocannabinoid system can be found even in very primitive organisms, indicating that it has a fundamental role in basic physiology. Its activation seems to represent a crucial and important component for the proper functioning of a wide range of physiological functions. The discovery of the endocannabinoid system has opened up a whole new and exciting field of medical and biological research.

The medicinal potential of Cannabis was largely underestimated until the discovery of the human endocannabinoid system. Now that the significance of this system is becoming increasingly clear, Cannabis as a subject for scientific study should have a brighter future. It is now understood that many biological activities of Cannabis are mediated through a real mechanism, involving not only the endocannabinoid system, but potentially also through cross talk with other systems, including the opioid receptors. And there is increasing evidence that the vanilloid receptor may have a double function as a putative CB3 receptor. Obviously, the discovery of new receptors and ligands may only further our interest in this field.

An increasing number of pharmaceutical companies have started to pick up the idea of (synthetic) cannabinoids and their antagonists as therapeutic drugs. At present a number of preparations based on the biological activities of the cannabinoids are already available, as mentioned in more detail in this chapter. A considerable number of cannabinoid-based medicines are expected to enter the market in the coming years, particularly in the field of synthetic cannabinoid receptor agonists and antagonists. A future with Cannabis-based medicines therefore seems very likely, and a further understanding of Cannabis as a medicine through scientific research is warranted.

However, there is a fundamental problem with using the cannabinoid receptor as a drug target: the main target for most therapeutic activities is CB1 and this is the same receptor that causes most of the (psychoactive) adverse effects. Furthermore, their pharmacokinetic properties set the cannabinoids apart from almost any other type of biologically active compounds used in medicine: the cannabinoids are virtually insoluble in water, causing them to bind to adipose and other tissues, and remain in the body for extended periods of time, up to several months. A major goal of SAR studies is therefore to create more water-soluble cannabinoids that are easier to administer and to dose in an effective manner. Other major goals include the development of CB1

agonists that are excluded from the BBB, and specific CB2 agonists that may be used in treating indications related to the immune system and inflammation.

The Cannabis constituents make up a significant group of compounds with diverse properties, and even based on the limited data available it may be expected that at least several of them have therapeutic potential. Unfortunately, much of the evidence for the medicinal use of Cannabis or cannabinoids is anecdotal and it turns out to be very challenging to confirm these findings by clinical trials. Also, it is often unknown which constituents are actually responsible for the effects observed after the administration of herbal Cannabis or extracts. Only a few indications have been more or less confirmed by clinical testing, including multiple sclerosis, cancer- and AIDS-related nausea and vomiting, chronic pain, and Tourette's syndrome. However, many more indications are currently under some form of investigation, one of the most exciting recent findings being that cannabinoids may be very effective in the treatment of some forms of cancer.

But even without considering these pharmaceutical developments, research on the medicinal use of Cannabis is important simply because Cannabis is already used for self-medication by an huge number of chronically ill people worldwide, often risking harsh legal punishments by doing so. They use Cannabis medicinally, with a large array of different administration forms, to ameliorate the symptoms of diseases varying from cancer and multiple sclerosis, to epilepsy, psychological disorders, and irritable bowl syndrome. It is interesting to note that Cannabis in such situations often seems to be used for ailments that cannot sufficiently be treated with conventional medicine, indicating a specific niche for Cannabis medications. The presence of such a large group of experienced users provides the (medical) researcher with an enormous potential reservoir of knowledge, comparable to ethnopharmacological field studies performed in remote places like the Amazon rainforest, or central Africa. It would therefore probably be wise if future studies on the biological activities of Cannabis would consider including these experiences into a more multidisciplinary approach.

Phytochemical analysis of the Cannabis plant, and in particular its cannabinoids, has been complicated in the past, because of overlapping spectroscopic and chromatographic properties, combined with a severe lack of reliable standards. For the analysis of highly pure, single cannabinoid preparations, specific analytical procedures can be easily developed. However, most phytochemical and pharmacological studies are far more complex than that. Because of the complex chemistry of Cannabis, advanced separation techniques are often necessary for the acquisition of the typical chemical profiles of Cannabis constituents.

More recently, the increasing availability of quantified standards has led to new impulses for analytical science to develop reliable, validated methods for the analysis of the many different types of Cannabis preparations that are available today. Unfortunately, most scientific publications still only mention the THC content of the Cannabis material used in the study, refraining from analyzing or publishing the content of other potentially important biologically active ingredients. But it is likely that using only the total THC content to characterize different Cannabis varieties is not sufficient to understand the complex biological effects of this plant. Ideally, a comprehensive overview of the cannabinoid content (i.e., the chemical fingerprint) of Cannabis preparations used in studies should become an integral part of scientific reports on the effects of Cannabis.

It may be concluded that *C. sativa* as a biologically active plant is currently at an exciting crossroads of science, politics, and culture. Advanced modern techniques such as NMR spectroscopy, principal component analysis, high-resolution MS detection, and various chromatographic improvements make it possible to isolate, identify, and study virtually any constituent that is wished. Currently, there seem to be no more major analytical obstacles for a full understanding of the composition, effects, and usefulness of the Cannabis plant. But although numerous laboratories in the world are allowed to work with even the most dangerous and addictive class/schedule I drugs, many of them do not have permission to work with the relatively mild Cannabis plant in any way or form. In this respect, Cannabis as a subject for scientific research is clearly in a league of its own.

So what is needed now is very clear: scientists must be able to take up the challenges that lay ahead, without the restrictions that are currently holding them back. Traditionally, the function of science is to perform unbiased, peer-reviewed, and reproducible research that is open to discussion after the results have been presented. However, in the case of Cannabis-related studies, these basic principles are all too often challenged by public opinion, political barriers, and legal restrictions, even before the studies can take place.

Clearly, this approach does not stimulate a science-based evolution of our perception of Cannabis as a dangerous drug without medicinal value. Access to (research-grade) Cannabis materials, reference standards,

and validated analytical methods are among the basic requirements to set up the types of studies that should tell us if, and when, Cannabis and its biologically active constituents may be useful in modern medicine. Hopefully, this chapter has been able to inform a new generation of Cannabis researchers about the work to be done, so they can help to make the best of the Cannabis plant and its preparations.

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Biographical Sketches

Dr. Arno Hazekamp was born on 15 March, 1976 in the Netherlands. He studied at Leiden University, the Netherlands, where he obtained his Bachelor's diploma in the field of molecular biology from the School of Biology. He continued his studies at the same university, School of Pharmaceutical Science, where he worked for several years in the Department of Pharmacognosy, under Professor Rob Verpoorte. In 2000, Arno finished his M.Sc. research on the ethnopharmacology of a Thai medicinal plant, and graduated with honors. Subsequently, he was employed as a technician and laboratory manager at Leiden University.

In November 2001, Arno started as a Ph.D. student under the supervision of Professor Rob Verpoorte. His research project was focused on the medicinal properties of the Cannabis plant, and on the practical obstacles that stand between this plant and its development into a modern medicine. Arno worked closely with the official grower of medicinal Cannabis in the Netherlands, Bedrocan BV, and was involved in numerous projects regarding quality control, product development, and basic research regarding medicinal Cannabis. Arno was actively involved in setting up the medicinal Cannabis program of the Dutch Health Ministry, and was a strong advocate of a more science-based approach on the medicinal use of Cannabis in the Netherlands and abroad. During his Ph.D., Arno spent several periods at the Institut Universitaire de Médecine Légale (IUML) in Lausanne, Switzerland, focusing on the forensic aspects of Cannabis use.

After finishing his Ph.D. in 2007, Arno started a phytochemical contract laboratory, and was the first to make a wide range of highly pure cannabinoid standards commercially available. He was also involved in the early phase of Echo Pharmaceuticals, a Dutch pharmaceutical company, developing a sublingual administration form of THC and other cannabinoids. Arno is currently working as a phytochemist with a larger consortium of biotech companies that work together under the name Product Isolation from Nature (PRISNA), providing phytochemical services and consultancy for the development of plant-based products.

Arno continues to have a strong interest in the medicinal use of Cannabis, with a specific focus on controlled growing, quality control, and safe access for medical patients. In 2009, Arno became a board member of the International Association for Cannabis as Medicine (IACM). Througout his career, Arno has maintained contact with patients, caretakers, and patient-organizations. These connections and experiences serve as an inspiration for evolving research projects. He is an active traveller and lecturer, and is considered a professionally trained medicinal Cannabis advocate. However, most of his time is still spent in the laboratory.

Andrea Lubbe was born in Belville, South Africa. She completed a B.Sc. degree in Molecular and Cellular Biology at the University of Stellenbosch, South Africa, followed by an honours degree in Plant Physiology at the same university. She obtained an M.Sc. degree in Natural Products at the University of Leiden in The Netherlands, and is currently at the same institution pursuing a Ph.D. degree. The focus of her current work is agronomic factors affecting alkaloid production in *Narcissus* species.

Justin T. Fischedick was born in the United States in 1984. He is currently a Ph.D. student at Leiden University studying various aspects of the Cannabis plant. He is specifically interested in the interactions and synergy among the many biologically active components of Cannabis. Mr. Fischedick earned his Masters degree in Biology at Leiden University in 2008 and his B.Sc. degree from the State University of New York College of Environmental Science and Forestry in 2006.

Renee Ruhaak was born in Leiden, The Netherlands. She completed a B.Sc. degree in BioPharmaceutical Sciences at Leiden University, The Netherlands, where she continued her studies for a Master degree and finished in 2005. Her interests are in methods of application for Cannabis and cannabinoids, which was the topic of her master thesis. Currently, she holds a Ph.D. position at the Leiden University Medical Centre on the topic of glycosylation analysis in the study of healthy ageing.

Mónica Llano Díez was born in Oviedo, Spain, and graduated in Biochemistry at the University of Oviedo, Spain. She did an internship training program in the Department of Molecular Structural Biology, University of Greifswald, Germany, and participated as postgraduate student in the project 'Metabolic profiling of *Catharanthus Roseus* cells elicited with *Pythium* and jasmonic acid using ^{1}H-NMR' at the University of Leiden, The Netherlands. Currently, she is doing her Ph.D. in Neurophysiology at the University of Uppsala, Sweden.