The Botany of Cannabis sativa L.

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EMERGING ISSUES IN ANALYTICAL CHEMISTRY

BRIAN F. THOMAS, SERIES EDITOR

THE ANALYTICAL CHEMISTRY OF CANNABIS

Quality Assessment, Assurance, and Regulation of Medicinal Marijuana and Cannabinoid Preparations

BRIAN F. THOMAS
MAHMOUD A. ELSOHLY

The Analytical Chemistry of Cannabis

Emerging Issues in Analytical Chemistry

Series Editor
Brian F. Thomas

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DEDICATION

This work is dedicated to my wife Cathy, and my mentors Billy Martin, Ed Cook, Bob Jeffcoat, and Ken Davis.

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FOREWORD

Cannabis has been used for thousands of years for recreational, medicinal, or religious purposes. However, the determination of the chemical structures of its cannabinoids, terpenes, and many other constituents, and of the pharmacological actions and possible therapeutic uses of some of these compounds, began less than 100 years ago. This book begins by describing the cultivation, harvesting, and botanical classification of cannabis plants, and then goes on to specify how these plants produce some of their chemical constituents. Subsequent chapters focus on medical formulations of cannabis and cannabis-derived drugs, on the routes of administration of these formulations, and on analytical methods that are used in the formulation development and for the quality control or stability assessment of cannabis constituents. The penultimate chapter deals with regulatory and additional formulation-related issues for medical cannabis and cannabinoids, while the final chapter identifies ways in which analytical chemistry will most likely contribute to the development of cannabinoid therapeutics in the future. This book provides much needed insights into the important roles that analytical chemistry has already played and is likely to continue to play in the development of cannabis and its constituents as medicines.

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PREFACE

Pharmacology began with natural products and, over some years on either side of 1900, evolved into a rigorous scientific discipline dominated, at least in the West, by well-defined chemical entities, either extracted and processed or synthesized. The two traditions evolved together, each informing the other, the natural strain by long experience pointing the way toward how a drug development program might be structured, the synthetic strain contributing molecular specificity, with analytical chemistry a common element. The resultant contribution to modern medicine, with all its caveats and controversies, must be accounted as one of the great advancements in science.

Natural products pharmacology is very much alive. However, that "natural" is one cause of the popular misconception that herbs are in some way better or safer than pills. Though some herbal remedies do appear to be safe and effective, the opposite is closer to the truth. Cannabis is a good example. The number of parameters on which cannabis products can vary is enormous, from strain, growing conditions, harvesting methods, and handling to storage and processing of the raw material to combination with a wide variety of foods and other excipients in manufacturing to methods of administration (eating, smoking, "vaping," applying to mucous membranes). At every step, from planting through consumption, myriad influences can alter dose, absorption rate, interactions among constituents, exposure to toxins, and a host of other factors that can result in underdosing, overdosing, and various types and levels of acute and chronic poisoning, not excepting an increase in the probability of lung cancer. Even if quality were well controlled, which on the whole is very much not the case, this complexity means that governmental oversight of cannabis products cannot be as close and complete as that for prescription and over-the-counter pharmaceuticals. Caveat emptor.

Governments around the world are coming slowly to the conclusion that, in the absence of draconian enforcement, and to a nontrivial extent in its presence, people are going to use cannabis for medicine and recreation. The Internet spreads knowledge of genetic sequencing,

metabolomics, proteomics, and other disciplines such that people are going to manipulate cannabis, as they have long done by selective breeding, to maximize its mental and physical effects and tailor the quality of those effects. The present legal status in the United States and elsewhere cannot stop these activities by amateurs, but it does inhibit research by professionals to investigate the basic science of cannabis, and to use this information to better understand neurophysiological function, develop new medicines for people and animals, and find ways to deal with cannabis addiction. Tight control of marijuana and inhibition of legal research has arguably led to another paradoxical effect: driving the chemistry underground, which has resulted in the proliferation of new and more dangerous synthetic cannabinoids. There needs to be more involvement by elements of the US Food and Drug Administration rather than the Drug Enforcement Agency.

Clearly, the policy, regulatory, and research challenges that accompany the study and understanding of cannabis are unique. Despite all the issues, research continues, understanding of cannabis and its effects is evolving, policies are in flux, and the literature is ever-changing. The aim of this book is to provide the reader with a detailed understanding of the analytical chemistry of cannabis and cannabinoids as the foundation for quality, safety, and utility of cannabis-derived therapeutics, and offer direction for future advancements.

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We appreciate the opportunity to work with the editorial and production team at Elsevier—Katy Morrissey, Amy Clark, Vijayaraj Purushothaman, and the many who go unmentioned—in bringing this first volume in the series "Emerging Issues in Analytical Chemistry" to fruition.

Chapter 1, "The Botany of *Cannabis sativa* L.," was prepared collectively by Dr Suman Chandra, Dr Hemant Lata, and Dr Mahmoud A. ElSohly at the University of Mississippi, whose work was supported in part with federal funds from the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, USA, under contract No. N01DA-10-7773.

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CHAPTER 1

The Botany of *Cannabis sativa* L.

Cannabis sativa L. is a widespread species in nature. It is found in various habitats ranging from sea level to the temperate and alpine foothills of the Himalayas, from where it was probably spread over the last 10,000 years.[1,2] The age-old cultivation makes its original distribution difficult to pinpoint.[3] Cannabis has a long history of medicinal use in the Middle East and Asia, with references as far back as the 6th century BCE, and it was introduced in Western Europe as a medicine in the early 19th century to treat epilepsy, tetanus, rheumatism, migraine, asthma, trigeminal neuralgia, fatigue, and insomnia.[4,5]

As a plant, it is valued for its hallucinogenic and medicinal properties, more recently being used for pain, glaucoma, nausea, asthma, depression, insomnia, and neuralgia.[6,7] Derivatives are used in HIV/AIDS[8] and multiple sclerosis.[9] The pharmacology and therapeutic efficacy of cannabis preparations and its main active constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) have been extensively reviewed.[10–12] The other important cannabinoid constituent of current interest is cannabidiol (CBD). There has been a significant interest in CBD over the last few years because of its reported activity as an antiepileptic agent, particularly its promise for the treatment of intractable pediatric epilepsy.[13,14] Other than Δ^9-THC and CBD, tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabigerol (CBG), and cannabichromene (CBC) are major isolates. Fig. 1.1 shows chemical structures.

Cannabis is also one of the oldest sources of food and textile fiber.[15–17] Hemp grown for fiber was introduced in Western Asia and Egypt and subsequently in Europe between 1000 and 2000 BCE. Cultivation of hemp in Europe became widespread after 500 CE. The crop was first brought to South America (Chile) in 1545, and to North America (Port Royal, Acadia) in 1606.[18] Now its cultivation is prohibited or highly regulated in the United States.

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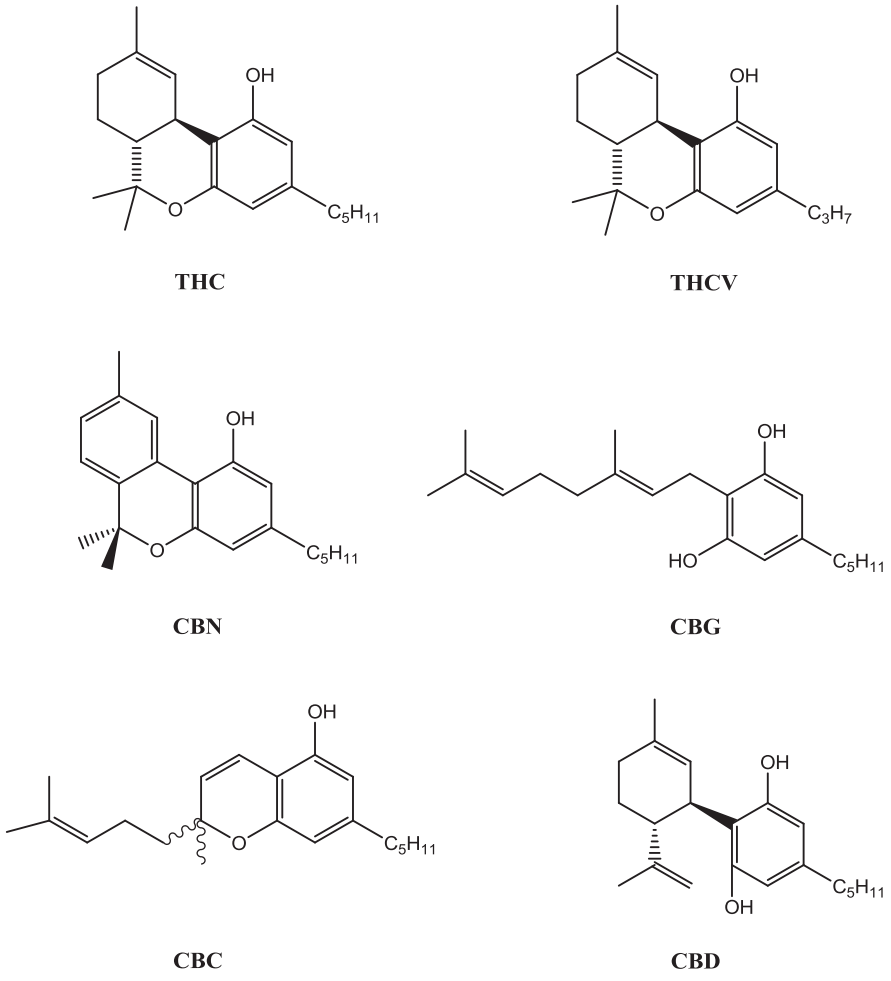

Figure 1.1 Chemical structures of major cannabinoids present in Cannabis sativa. Δ^9-THC, *Δ^9-tetrahydrocannabinol;* THCV, *tetrahydrocannabivarin;* CBN, *cannabinol;* CBG, *cannabigerol;* CBC, *cannabichromene;* CBD, *cannabidiol.*

BOTANICAL DESCRIPTION

Table 1.1 describes the botanical nomenclature of *C. sativa* L. Cannabis is a highly variable species in terms of botany, genetics, and chemical constituents. The number of species in the *Cannabis* genus has long been controversial. Some reports proposed *Cannabis* as a polytypic genus.[19–22] However, based on morphological, anatomical, phytochemical, and genetic studies, it is generally treated as having a single, highly polymorphic species, *C. sativa* L.[23–26] Other reported species

Table 1.1 Botanical Nomenclature of *Cannabis sativa* L.

Category	Botanical Nomenclature
Kingdom	Plantae—Plants
Subkingdom	Tracheobionta—Vascular plants
Superdivision	Spermatophyta—Seed plants
Division	Magnoliophyta—Flowering plants
Class	Magnoliopsida—Dicotyledons
Subclass	Hamamelididae
Order	Urticales
Family	Cannabaceae
Genus	*Cannabis*
Species	*Cannabis sativa* L.

are *Cannabis indica* Lam. and *Cannabis ruderalis* Janisch, but plants considered to have belonged to these species are now recognized as varieties of *C. sativa* L. (var. *indica* and var. *ruderalis*, respectively). *Cannabis sativa* and *indica* are widely cultivated and economically important; *Cannabis ruderalis* is hardier and grows in the northern Himalayas and the southern states of the former Soviet Union but is rarely cultivated for drug content.

The main morphological difference between *indica* and *sativa* is in their leaves. The leaves of *sativa* are much smaller and thinner, whereas those of *indica* have wide fingers and are deep green, often tinged with purple; at maturity, they turn dark purple. *Indica* plants are shorter and bushier, usually under 6 ft tall and rarely over 8 ft *Indica* has short branches laden with thick, dense buds, which mature early, usually at the beginning of September in the Northern Hemisphere. *Indica* buds also vary in color from dark green to purple, with cooler conditions inducing more intense coloration. *Indica* flowers earlier. The natural distribution of *indica* is Afghanistan, Pakistan, India, and surrounding areas. The plants of *sativa* have long branches, with the lower ones spreading 4 ft or more from the central stalk, as on a conical Christmas tree. Height varies from 6 ft to more than 20 ft, with 8–12 ft being the most common. Buds are long and thin and far less densely populated than in *indica*, but longer, sometimes 3 ft or more. Maturation time varies considerably depending on the variety and environmental conditions. Low Δ^9-THC Midwestern *sativa* varieties (ditchweed) mature in August and September, while equatorial

varieties mature from October to December. Buds of *sativa* require intense light to thicken and swell; *indica* does not. *Sativa* tends to be higher in Δ^9-THC and lower in CBD than *indica*. S*ativa* is found all over the world and comprises most of the drug type equatorial varieties such as Colombian, Mexican, Nigerian, and South African, where marijuana plants can be very potent. Cannabis has many local common names, some of which are given in Table 1.2.

Normally, cannabis exhibits a dioecious (male and female flowers develop on separate plants) and occasionally a monoecious (hermaphrodite) phenotype. It flowers in the shorter days (below 12-h photoperiod) and continues growing vegetatively in the longer photoperiod. Sex is determined by heteromorphic chromosomes (males being heterogametic XY, females homogametic XX). Male flowers can be differentiated from female by their different morphological appearance. At the vegetative stage, differentiation is difficult because of morphological similarities. Molecular techniques, however, can differentiate at an early stage.[27–32]

Cannabis is wind pollinated. For the production of cannabinoids (or phytocannabinoids), female plants are preferred for several reasons. First, they produce higher amounts of cannabinoids. Second, once pollinated, female plants produce seeds at maturity, whereas seed-free

Table 1.2 Common Cannabis Names in Different Languages

Language	Common Names
Arabic	Bhang, hashish qinnib, hasheesh kenneb, qinnib, tîl
Chinese	Xian ma, ye ma
Danish	Hemp
Dutch	Hennep
English	Hemp, marihuana
Finnish	Hamppu
French	Chanvre, chanvre d'Inde, chanvre indien, chanvrier
German	Hanf, haschisch, indischer hanf
Hindi	Bhang, charas, ganja
Japanese	Mashinin
Nepalese	Charas, gajiimaa, gaanjaa
Portuguese	Cânhamo, maconha
Russian	Kannabis sativa
Spanish	Cáñamo, grifa, hachís, mariguana, marijuana
Swedish	Porkanchaa

plants (sinsemilla, a Spanish word) are preferred for their higher yield of secondary metabolites. Third, if several cannabis varieties are being grown together, cross-pollination would affect the quality (chemical profile) of the final product. To avoid this, removing male plants as they appear, screening female clones for higher metabolite content, and conservation and multiplication using biotechnological tools ensures the consistency in chemical profile that is desirable for pharmaceuticals.

CHEMICAL CONSTITUENTS AND PHENOTYPES OF *C. SATIVA* L.

CBN was the first cannabinoid to be isolated[33,34] and identified[35–37] from *C. sativa*. The elucidation of CBN led to speculation that the psychotropically active constituents of cannabis could be THCs. The nonpsychotropic compound CBD was subsequently isolated from Mexican marijuana[38] and the structure was determined.[39] Gaoni and Mechoulam, two pioneers of cannabis research, determined the structure of Δ^9-THC after finally succeeding in isolating and purifying this elusive compound (see Mechoulam Close-up: How to Pamper an Idea).[40] Since then, the number of cannabinoids and other compounds isolated from cannabis has increased continually, with 545 now reported. Of these, 104 are phytocannabinoids (Table 1.3). From the isolation and structural elucidation of Δ^9-THC in 1964 until 1980,

Table 1.3 Constituents of *Cannabis sativa* L.

No.	Groups	Number of Known Compounds
1	CBG type	17
2	CBC type	8
3	CBD type	8
4	Δ^9-THC type	18
5	Δ^8-THC type	2
6	CBL type	3
7	CBE type	5
8	CBN type	10
9	CBND type	2
10	CBT type	9
11	Miscellaneous	22
12	Total cannabinoids	104
13	Total noncannabinoids	441
	Total	545

61 phytocannabinoids were isolated and reported.[41] Only nine new ones were characterized between 1981 and 2005,[42] but 31 were reported between 2006 and 2010. The 13 chemical constituent type groups shown in Table 1.3 suggests the chemical complexity of the cannabis plant.[42]

The concentration of Δ^9-THC and CBD, the most abundant cannabinoids, can be characterized qualitatively and quantitatively.[43] Qualitative characterization is based on the Δ^9-THC/CBD ratio and assigning the plant to a discrete chemical phenotype (chemotype). In 1971, cannabis was initially characterized in two phenotypes, drug type and fiber type, by Fetterman et al.[44] A Δ^9-THC/CBD ratio >1 was drug type, a lesser ratio was fiber type. In 1973, Small and Beckstead proposed three categories based on the ratio: drug type if >1, intermediate if close to 1, and fiber if <1.[45,46] In 1987, Fournier et al. added a rare chemotype that was characterized by a very low content of Δ^9-THC and CBD with CBG as the predominant constituent.[47]

Quantitatively, the plant is characterized by potency through measuring the level of its most abundant cannabinoids, Δ^9-THC and CBD, in its tissues (Fig. 1.2). The levels of cannabinoids are controlled by the interaction of several genes and also influenced by the growth environment of the plant.[48–52] Numerous biotic and abiotic factors affect cannabinoid production, including the sex, growth stage, environmental parameters, and fertilization.[23,50,53–56]

CANNABIS BIOSYNTHESIS

Fig. 1.3 is a schematic of cannabinoid biosynthesis. In the plant, Δ^9-THC, CBD, and CBC are in their acid forms.[57–59] Two independent pathways, cytosolic mevalonate and plastidial methylerythritol phosphate (MEP), are responsible for terpenoid biosynthesis. The MEP pathway is reported to be responsible for the biosynthesis of the terpenoid moiety.[12] Olivetolic acid (OLA) and geranyl diphosphate (GPP) are derived from the polyketide and the deoxyxylulose phosphate (DOXP)/MEP pathways, respectively, followed by condensation under the influence of the prenylase olivetolate geranyltransferase, yielding cannabigerolic acid (CBGA). CBGA, in turn, is oxidocyclized

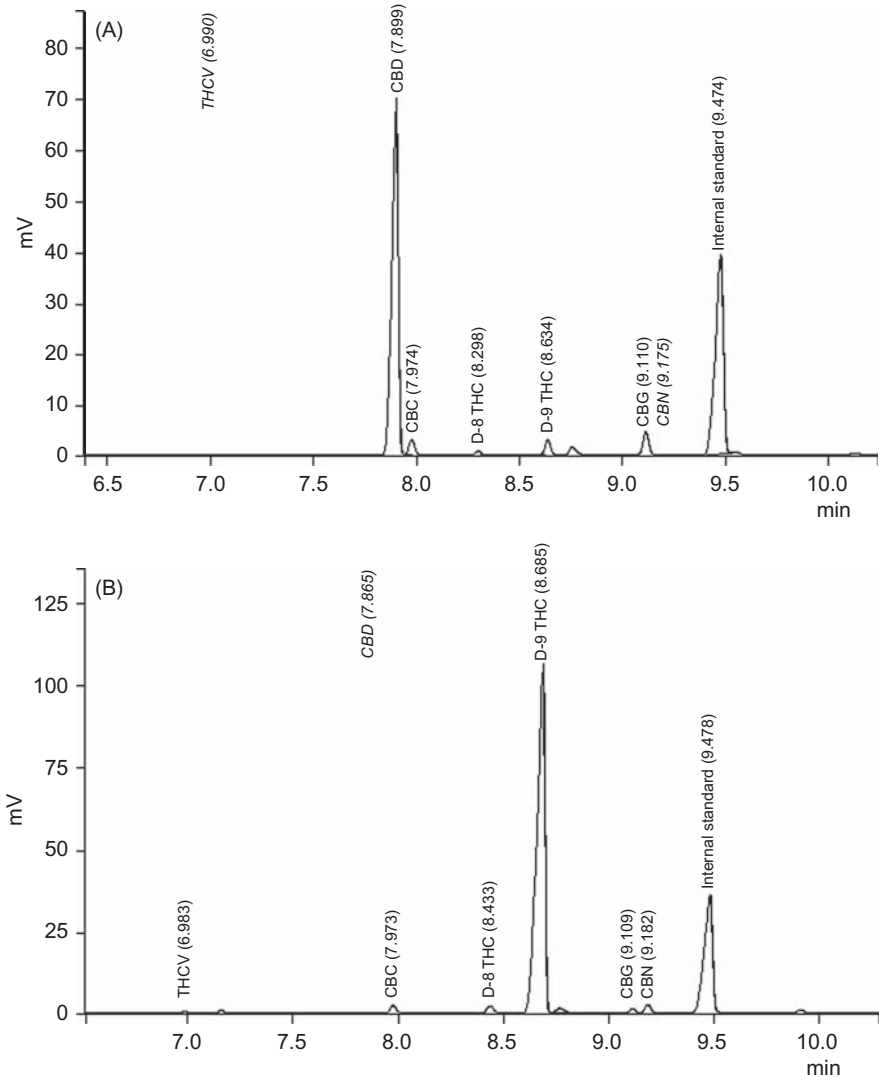

Figure 1.2 Gas chromatography-flame ionization detector (GC-FID) analysis of (A) a high CBD type and (B) a high Δ^9-THC type cannabis plant.

by flavin adenine dinucleotide-dependent oxidases, namely, cannabichromenic acid (CBCA) synthase, cannabidiolic acid (CBDA) synthase, and Δ^9-THCA synthase, producing CBCA, CBDA, and Δ^9-THCA, respectively.[60,61]

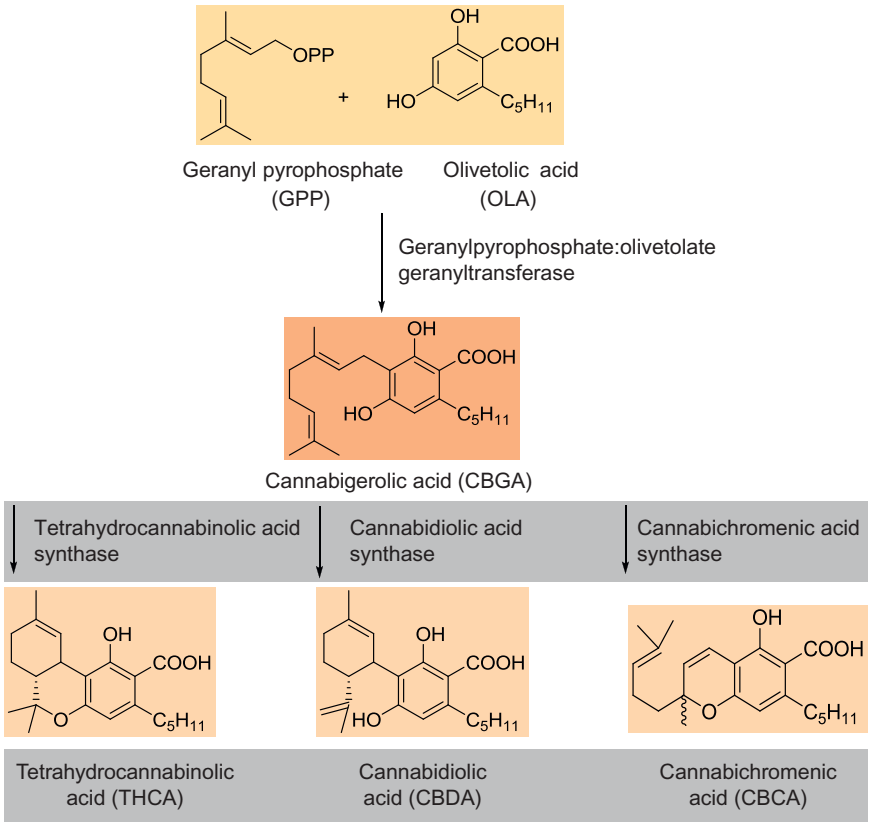

Figure 1.3 Biosynthetic pathway of tetrahydrocannabinolic acid, cannabidiolic acid, and cannabichromenic acid.

SELECTION OF ELITE CLONES FOR PLANT PROPAGATION

The quality, safety, and efficacy of starting material are basic prerequisites in the pharmaceutical industry. Cannabis as a feedstock is more challenging because it is a chemically complex and highly variable plant due to its allogamous nature. The chemical composition of cannabis biomass is affected by a range of factors such as genetics, environment, growth conditions, and harvesting stage. Therefore, selection of elite starting material (female clone) based on chemical composition, conservation, and mass multiplication using advanced biotechnological tools is a suitable way to ensure the consistency in chemical profile of a crop for pharmaceuticals.

In our laboratory, we developed a GC-FID method for screening and selection of elite biomass based on major cannabinoid content.

Briefly, quantitative analysis of seven major cannabinoids (THCV, CBD, CBC, Δ^8-THC, Δ^9-THC, CBG, and CBN) is done by solvent extraction followed by analysis using capillary gas chromatography, a method offering short analysis time and resolution of all cannabinoids on a single column. Three samples (100 mg each) are used for analysis from each manicured biomass sample. A 3-mL internal standard (IS) extraction solvent (100 mg of 4-androstene-3,17-dione + 10 mL chloroform + 90 mL methanol) is added to the sample and allowed to rest at room temperature for 1 h. The extract is then filtered through a cotton plug and the clear filtered material is transferred to an autosampler vial. Samples are placed onto the GC instrument along with vials of ethanol, internal standard/Δ^9-THC mixture (unextracted standard), and controls. The results are calculated by obtaining an average percentage of each cannabinoid from the two chromatograms of each sample. It must be noted that the response factor for the cannabinoids relative to IS is 1. Therefore, the area of each cannabinoid divided by that of the IS multiplied by the amount of IS added (3 mg) gives the percentage of each cannabinoid in the sample, as 100 mg of sample is used for analysis. For example, a cannabinoid with the same peak area as that of the IS represents a 3% concentration in the sample. The method has been validated to meet FDA-GMP requirements.

Once a female cannabis plant is screened and selected based on its cannabinoids profile, it can be used as a mother stalk for future propagation.

PLANT GROWTH AND CULTIVATION

Cannabis is an annual species and can be grown from seed or vegetative cuttings under indoor and outdoor conditions. Indoor cultivation under controlled environmental conditions can generate three or four crops per year, depending upon required per-plant biomass yield; outdoor cultivation is limited to one crop per year. Selection of starting material or variety depends upon the composition of active ingredients required in the end product.

Propagation Through Seed

Fig. 1.4 shows the typical seeds of a high-Δ^9-THC-yielding Mexican variety of *C. sativa* L. Cultivation through seed is an ancient and

Figure 1.4 Cannabis sativa *seeds (high-yielding Mexican variety).*

traditional method. Seeds can be sown in small biodegradable jiffy pots containing soil with good aeration and should be kept moist by watering with a light spray when the upper surface begins to feel dry. During winter, a heat mat can be used below the pots to increase the soil temperature and enhance germination.

Normally seeds start sprouting by the fourth day of plantation, and most of the viable seeds germinate within 15 days. Variation in the rate of seed germination depends on the variety, seed age, storage conditions, and soil and water temperatures. Germinated seedlings can be kept under cool fluorescent light with an 18-h photoperiod until the seedlings are big enough to transplant to bigger pots. Once transplanted, they can be kept under full-spectrum grow light (1000 W high-pressure sodium or metal-halide bulbs) with an 18-h photoperiod for further vegetative growth.

Upon completion of desired vegetative growth, plants may be exposed to a 12-h photoperiod for flowering. (At this stage, cuttings of selected healthy plants can be made and maintained at vegetative stage for screening purposes.) Onset of flowering normally occurs in 2 weeks, depending upon variety. Being a dioceous species, seed raised plants normally turn 50% male and 50% female, depending on the variety. Onset of male flowers normally occurs a week earlier than female flowers. For the production of useful secondary metabolites, female plants are preferred as they produce higher cannabinoid content. At this early flowering stage, male plants can be identified and separated from female

plants. Once all the male plants are removed, female plants can be grown to full maturity for the production of sinsemilla (seedless) buds. Mature buds can be analyzed for cannabinoid content using GC-FID. Based on this analysis, elite high-yielding clones can be identified and their vegetative backup cuttings can be used as mother plants for future propagation.

Vegetative Propagation in Soil

The pharmaceutical industry requires consistency in the active ingredients of source material. Using cannabis as a source raw material remains especially challenging. In spite of being grown from seeds derived from a single cannabis mother plant, a considerable degree of variation in chemical composition of juvenile plants may be observed. Therefore, screening of high-yielding female plants and mass propagation of vegetative clones is the most suitable way to meet the demand for uniformity of the final product.

Once a best candidate female clone with a specific chemical profile is screened and selected, a fresh nodal segment about 6 to 10 cm long containing at least two nodes can be used for vegetative propagation. A soft apical branch is cut at a 45-degree angle just below a node and immediately dipped in distilled water. The base of the cutting is subsequently dipped in rooting hormone and planted in biodegradable jiffy pots (2×2 in) containing coco natural growth medium and a mixture (1:1) of sterile potting mix and fertilome. At least one node is covered by soil for efficient rooting. Plants are regularly irrigated and kept under controlled environmental conditions. Rooting initiates in 2 to 3 weeks, followed by transplantation to bigger pots after 5 to 6 weeks. The cuttings can be maintained at constant vegetative state under 18-h photoperiod (Fig. 1.5).

Vegetative Propagation in Hydroponics

Vegetative cuttings can also be grown in a hydroponics system. A small branch consisting of a growing tip with two or three leaves is cut and immediately dipped in distilled water. The base of the cutting is dipped in rooting hormone and inserted ($\sim$1 in) deep into a rockwool cube or a hydrotone clay ball supporting medium. Plants are supplied with vegetative fertilizer formula and exposed to fluorescent light under 18-h photoperiod. Rooting initiates in 2 to 3 weeks.

(A)

(B)

Figure 1.5 Indoor vegetative propagation of Cannabis sativa. *(A) Vegetative cuttings under fluorescent lights, (B) plant growing under full-spectrum metal-halide lamps.*

In Vitro Plant Regeneration

Tissue culture methods offer an alternative means of vegetative propagation. Clonal propagation through tissue culture, commonly called micropropagation, can be achieved in a short time and a small space. It is possible to produce plants in large numbers starting from a single clone. However, the process involves several stages, from initiation and establishment of aseptic cultures to multiplication, rooting of regenerated shoots, and hardening in soil. Direct organogenesis is the most reliable method for clonal propagation because it upholds genetic uniformity among progenies.[62–64] An efficient micropropagation protocol for mass growing of drug type varieties using apical nodal segments containing axillary buds[65–67] (Fig. 1.6), as well as the micropropagation of a hemp variety using shoot tips,[68] have been reported. Recently, our group developed an improved one-step micropropagation protocol using meta-topolin.[69]

Plant tissue culture is also considered the most efficient technology for crop improvement by the production of somaclonal

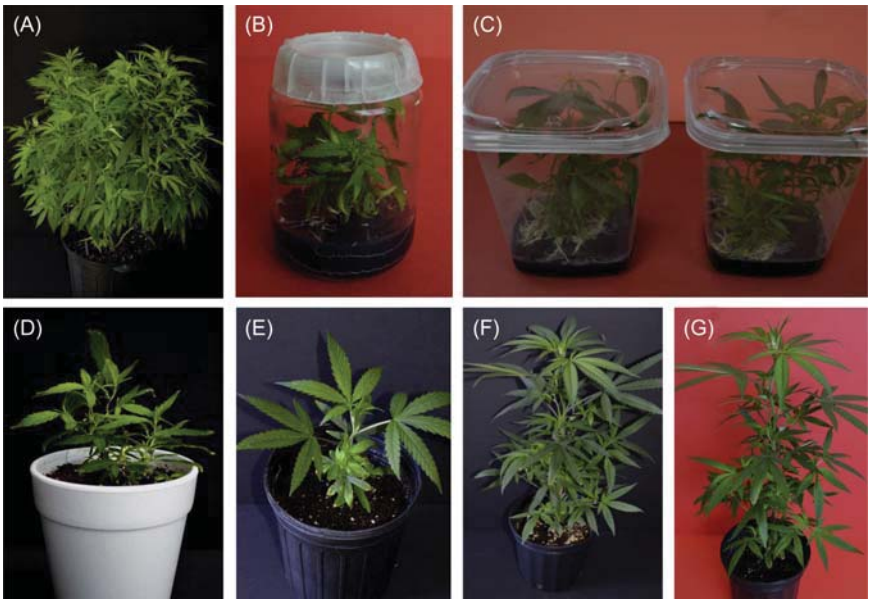

Figure 1.6 Micropropagation of Cannabis sativa. *(A) A representative mother plant, (B and C) fully rooted cannabis plants, (D) micropropagated plants under the acclimatization condition, and (E–G) well-established micropropagated plants in soil.*

and gametoclonal variants. The callus-mediated cultures have inheritable characteristics different from those of parent plants due to the possibility of somaclonal variability,[70] which may lead to the development of commercially important improved varieties. Micropropagation through callus production has been reported, including production of roots through cannabis calli,[71] occasional shoot regeneration,[63] and high-frequency plant regeneration from leaf tissue–derived calli.[72]

Quality Control of In Vitro Regenerated Plants

The sustainability of the regeneration systems depends upon the maintenance of the genetic integrity of micropropagated plants. Despite its potential, in vitro techniques are known to induce somaclonal variations. Further, the frequency of these variations varies with the source of explants and their regeneration pattern, media composition, and cultural conditions. Tissue culture–induced variations can be determined at the morphological, cytological, biochemical, and molecular levels with several techniques. At present, molecular markers are powerful tools used in the analysis of genetic fidelity of in vitro propagated plantlets. These are not influenced by environmental factors and generate reliable and reproducible results.

In our laboratory, DNA-based intersimple sequence repeat (ISSR) markers have been successfully used to monitor the genetic stability of the micropropagated plants of *C. sativa*.[73,74] Fully mature in vitro propagated plants were also analyzed for their chemical profile and cannabinoid content, and compared with mother plants and vegetatively grown plants from the same mother plant using GC-FID for quality assurance.[75]

Our results showed that micropropagated plants were highly comparable to the mother plant and vegetatively grown plants in terms of genetics, chemical profile, and cannabinoid content. These results confirm the clonal fidelity of in vitro propagated plants and suggest that the biochemical mechanism used to produce the micropropagated plants does not affect genetics or metabolic content. So these protocols can be used for mass propagation of true to type plants of *C. sativa* for commercial pharmaceutical use.

Conservation of Elite Germplasm

The conservation of plant genetic resources is vital for the maintenance and improvement of existing gene pool and plant breeding programs.[76] In the last few decades, in situ conservation methods have played an important role in the conservation of elite plant germplasm. We have developed protocols for the conservation of elite C. sativa clones using vegetative propagation, slow growth conservation techniques, and alginate encapsulation.[66,77]

INDOOR CULTIVATION

Indoor cultivation under controlled environmental conditions allows total control of the plant life cycle and the quality and quantity of the biomass as starting material for the production of a desirable cannabinoid profile for pharmaceutical use. Parameters such as light (intensity and photoperiod), temperature, carbon dioxide level, air circulation, irrigation, relative humidity, and plant nutrition are the most important factors.

Light

Light is a vital component for the photosynthesis in plants. Suitable light quality, optimum light intensity, and desirable photoperiod are important in the indoor cultivation of cannabis. Our study shows that cannabis plants can use high photosynthetic photon flux density ($\sim$1500 μmol/m^2/s) for efficient gas and water vapor exchange between leaves and the environment.[50,78] Different light sources can be used: fluorescent (for cuttings), metal-halide bulbs, high-pressure sodium lamps (for well-established plants), conventional bulbs, and light emitting diodes. However, with indoor lighting, it is difficult to match photosynthetically active radiation received in the bright outdoor sunlight. An 18-h photoperiod is optimum for vegetative growth; 12-h is recommended for the initiation of flowering.

Irrigation and Humidity

The amount of water and the frequency of watering vary with the growth stage, size of the plants and pots, growth temperature, humidity, and many other factors. During the early seedling or vegetative stage, keeping the soil moist is recommended. Once the plants are established, the top layer of soil must be allowed to dry out before the plants are watered again. Overall, the soil should not be kept

constantly wet and the plant should not be allowed to wilt. In general, watering should be done based on the requirement of the plant depending on its growth stage and the size of the container. Vegetative cuttings require regular moisture on the leaves to maintain a high humidity in its microclimate until the plants are well rooted. Humidity around 75% is recommended during the juvenile stage and about 55% to 60% during the active vegetative and flowering stages.

Temperature

Depending upon the original growth habitat and the genetic makeup, the temperature response of photosynthesis varies with the cannabis variety. Growth temperature of 25°C to 30°C is ideal for most varieties.[50,51,79]

Level of Carbon Dioxide

Air circulation in the growing room is another important factor and is necessary for indoor cultivation of healthy plants. An elevated CO_2 level enhances photosynthetic carbon assimilation and so may accelerate growth and improve productivity. A doubling of CO_2 concentration has been reported to increase the crop yield by 30% or more in experiments conducted under close environmental conditions such as in greenhouses and growth chambers.[80–84] Doubling of CO_2 concentration ($\sim$750 μmol mol^{-1}) was reported to stimulate the rate of photosynthesis and water use efficiency by 50% and 111%, respectively, as compared to ambient CO_2 concentration.[50,85] Therefore, supplementing CO_2 and proper air circulation in the growing room are recommended and will scale up the rate of photosynthesis and overall growth.

Plant Nutrition

Cannabis requires a minimum amount of nitrogen and a high level of phosphorus to promote early root growth. For vegetative growth, a higher level of nitrogen is required. For flowering, more potassium and phosphorous for the production of buds is needed.

OUTDOOR CULTIVATION

Cannabis is an annual. In the natural environment, it flowers at the end of summer (shortening days) irrespective of planting date or plant age. Seed is set before the arrival of winter and plant dies, if not

harvested. Outdoor planting normally starts during late March or early April, depending on weather conditions, and could last into November or early December for some varieties. Starting from seed, plants may be raised in small jiffy pots and the selected healthy seedlings transplanted to the field (seeds also may be directly planted in the field). Male flowers start appearing within 2 to 3 months, around the middle of July, followed by female flowers. Male plants are generally removed from the fields for reasons stated above. Vegetative propagation of selected elite female clones and their field plantation is generally preferred over seedlings for the consistency in the chemical profile of the end product. Similar to seedlings, propagation of cuttings can be done in small jiffy pots and rooted cuttings then planted in the field by hand or by automated planters. Fig. 1.7 shows a typical outdoor cultivation through vegetative cuttings.

Throughout the growing season, a few randomly selected plants from different plots are periodically analyzed for cannabinoid content. We found that the amount of Δ^9-THC increases with the age of the plant, reaching the highest level at the budding stage and plateauing before the onset of senescence. The maturity of the crop is determined

Figure 1.7 Outdoor cultivation of cannabis crop. (A and B) plants at vegetative stage, (C and D) plants at budding stage.

visually and confirmed using GC-FID based on the Δ^9-THC and other cannabinoid content in samples collected at different growth stages. Since the whole plant does not mature at the same time, mature upper buds are harvested first and other branches are given more time to achieve maturity.

Outdoor cultivation has a few advantages and disadvantages in comparison to indoor. Field-cultivated plants are normally bigger and have higher biomass. Growing in the natural environment does not require the intensive investment in equipment and maintenance that indoor does. The primary disadvantage is less control over growing conditions. The weather may be unfavorable for harvest when the plants have reached maturity, or a thunderstorm may seriously damage plants when they are ready to harvest. Outdoor plants need a longer growing season than indoor.

HARVESTING AND PROCESSING

Fig. 1.8 shows harvesting, drying, and processing of field-grown cannabis biomass.

Figure 1.8 Harvesting, drying, and processing cannabis biomass. (A) Harvesting mature plants, (B) drying biomass, (C) dried cannabis buds, and (D) processed plant material in barrel.

Harvesting

Determining the optimum harvesting stage is a critical step in cannabis cultivation. Too early or too late can significantly affect the yield of Δ^9-THC. Periodical monitoring of Δ^9-THC level allows harvesting material with the desired content. Harvesting should be done in the morning because Δ^9-THC level peaks before noon and then gradually declines. Within the plant, the mature top buds may be harvested first and the others allowed time to mature.

Handling, Drying, Processing, and Storage

During harvest, drying, and processing, gloves are recommended. If the biomass is being used as starting material for pharmaceuticals, contact with the ground should be avoided. Dry and large leaves may be removed from mature buds before drying (Fig. 1.8).

The drying facility is based on the size of cultivation. For large-scale growing, a commercial tobacco drying barn (such as BulkTobac, Gas-Fired Products, Inc., Charlotte, NC) can be used. For small samples, a simple laboratory oven will suffice for overnight drying at 40°C.

When the material is dried, it can be hand manicured. Big leaves should be separated from buds. The buds can be gently rubbed through screens of different sizes to separate small stems and seeds (if any) from the dried biomass. Automated machines designed for plant processing can separate big stems and seeds from the useable biomass.

Storing Biomass

Properly dried and processed biomass can be stored in FDA-approved sealed fiber drums containing polyethylene bags at 18°C to 20°C for the short term. For the long term, −10°C in a freezer is recommended. Stability of Δ^9-THC and other cannabinoids in biomass and products has been reviewed by several authors.[86–88]

CONCLUSION

Cannabis is very special in the plant kingdom in that it belongs to a family (Cannabaceae) with a single genus (*Cannabis*) with only one species (*sativa*) that has many varieties. The plant is very rich in constituents, the most specific of which are the cannabinoids that have not been reported in any

other plant, and it has broad pharmacological properties with tremendous medical potential in the treatment of epilepsy, spasticity, inflammation, irritable bowel syndrome, pain, and other disorders. Methods for growing, harvesting, processing, formulation, and use continue to evolve towards an important position in the pharmacopeia.

Close-Up: How to Pamper an Idea

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A scientific idea has not only to be pursued, but also pampered—in particular, if it is not welcome.

In the early 1960s as a newly appointed junior scientist at the Weizmann Institute in Rehovot, I was supposed to work with the head of my department, but could also explore some ideas of my own. I was interested in the chemistry and biological effects of natural products. I looked for biologically active plants that had not been previously well investigated. To my great surprise, I noted that the chemistry and hence the various activities of *Cannabis sativa*, the hashish plant, were not well known.

Morphine and cocaine had been isolated in the 19th century, and the availability of these alkaloids had made possible biochemical, pharmacological and clinical work with them. Why not cannabis? Actually its chemistry and biology had been investigated. There were numerous publications in the 19th century in mostly obscure journals. More recently, in the late 1930s and early 1940s, Roger Adams, a prominent US chemist, and Lord Alexander Todd, a Nobel Prize winner, had looked at the chemistry of cannabis, but apparently the active constituents had never been isolated in pure form and no definite structures had been put forward. The reasons may have been technical. We know today that the cannabinoids—a term I coined some years later—are present in the plant as a mixture of constituents with closely related chemical structures which presumably could not be separated by the methods available then.

Later, legal obstacles made work on cannabis almost impossible, particularly in North America and Europe. Cannabis—an illicit entity—was not readily available to most scientists, and research with it was next to impossible for academics, as few of them could follow effectively the security regulations required. By the mid 1940s cannabis research had effectively been eliminated.

I was not aware of the legal problems. And neither were the administration of the Weizmann Institute and even the police! In early 1963, through the administrative head of the Institute, I requested hashish for

research from the police and was asked to come over to their store of confiscated smuggled material in Tel Aviv. There I drank a cup of coffee with the elderly person in charge, he told me how the police had caught hashish smugglers from the Lebanon and I told him what we wanted to explore. I received 5 kg of hashish in the form of 10 "hashish soles," signed a receipt and boarded a bus to the Weizmann Institute some 15 miles away. On the bus travelers commented on the pleasant smell of the "vegetables" I was carrying.

We had all actually broken the strict laws on illicit substances. The Ministry of Health was supposed to have approved the research, the police should not have given me such a dangerous substance and I was essentially a criminal. But at the Ministry some of the bureaucrats in charge were my ex-colleagues, and after I was severely scolded for breaking the laws, we drank together some more coffee and I got a properly signed and stamped document. Living and doing research in a small country where people working in related areas generally know each other has at times its positive aspects.

The first thing my colleague Yuval Shvo and I did with the now legal hashish was to reisolate cannabidiol (CBD)—already isolated previously by Adams and Todd—and elucidate its structure. Not surprisingly, for over 40 years after our work on CBD in 1963, very few scientists and clinicians became interested in CBD, although we showed, together with Brazilian colleagues, already in 1980, that it is a good, novel antiepileptic drug and together with Israeli and British colleagues and friends published, in the early years of the 21st century, that it is also a potent drug in autoimmune diseases. Gratifyingly, today CBD is widely acclaimed as a novel antiepileptic drug in children.

In 1964 Yehiel Gaoni, a recent PhD chemist from the Sorbonne, joined the hashish group, and together we isolated for the first time the psychoactive constituent of hashish, which we named Δ^1-tetrahydrocannabinol (Δ^1-THC). The chemical nomenclature rules demanded a change, and today Δ^1-THC is known as Δ^9-THC. However, the academic administration of the Weizmann Institute were not happy with our research. Why could not we work on more respectable scientific topics? With a heavy heart in 1966 I moved to the supposedly more conservative Hebrew University in Jerusalem, where, surprisingly, I had full support for our work and where we continued to pamper cannabinoid ideas for over 50 years.

Over the next decades many other cannabinoids were isolated, their structures were elucidated and compounds were synthesized by our group. We looked at the metabolism of the cannabinoids and we collaborated with numerous biologists in exploring the various cannabinoid activities.

Anandamide and 2-AG were discovered by my group in the 1990s, and, with colleagues in many countries, we found that the newly discovered endocannabinoid system is involved in a large number of biological reactions and clinical conditions. The pampered idea has become a scientific adult.

Looking ahead. Shall we have endocannabinoid drugs soon? By modification of the endocannabinoid system, possibly through epigenetics, can we possibly treat clinical conditions in the future? As we have previously speculated, is the subtle chemical disparity of the many dozens of endocannabinoid-like compounds in the brain somehow involved in the huge variability in personality—an area in psychology that is yet to be fully understood?

What next?

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CHAPTER 2

Biosynthesis and Pharmacology of Phytocannabinoids and Related Chemical Constituents

In addition to nucleic acids, proteins, lipids, and carbohydrates, cannabis produces a large number of additional constituents, or secondary metabolites, including phytocannabinoids, terpenoids, and phenylpropanoids.[1,2] While phytocannabinoids are often referred to as the "active" ingredients in cannabis, these other chemical constituents have a broad spectrum of pharmacological properties and can contribute to the effects seen upon cannabis ingestion or combustion and inhalation, and may also be contained within and contribute to the activity of extracts, tinctures, and other cannabis formulations.[3] This overview of cannabis constituents will focus on the phytocannabinoids, terpenoids, and flavonoids that make up a large percentage of the pharmacologically active ingredients of current or emerging interest.

PHYTOCANNABINOID CONSTITUENTS IN CANNABIS

Phytocannabinoids are a structurally diverse class of naturally occurring chemical constituents in the genus *Cannabis* (Cannabaceae). This chemical classification is broadly based on their derivation from a common C21 precursor (cannabigerolic acid,[4] CBGA), or its C19 analog (cannabigerovaric acid,[5] CBGVA), the predominate phytocannabinoid precursors formed through the reaction of geranyl pyrophosphate with olivetolic and divarinic acid, respectively (Fig. 2.1).

Enzymatic conversion of cannabigerolic and cannabidivaric acid produces a wide variety of C21 terpenophenolics,[6] including (−)-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabidiol (CBD), cannabinodiol (CBND), and cannabinol (CBN), and their C19

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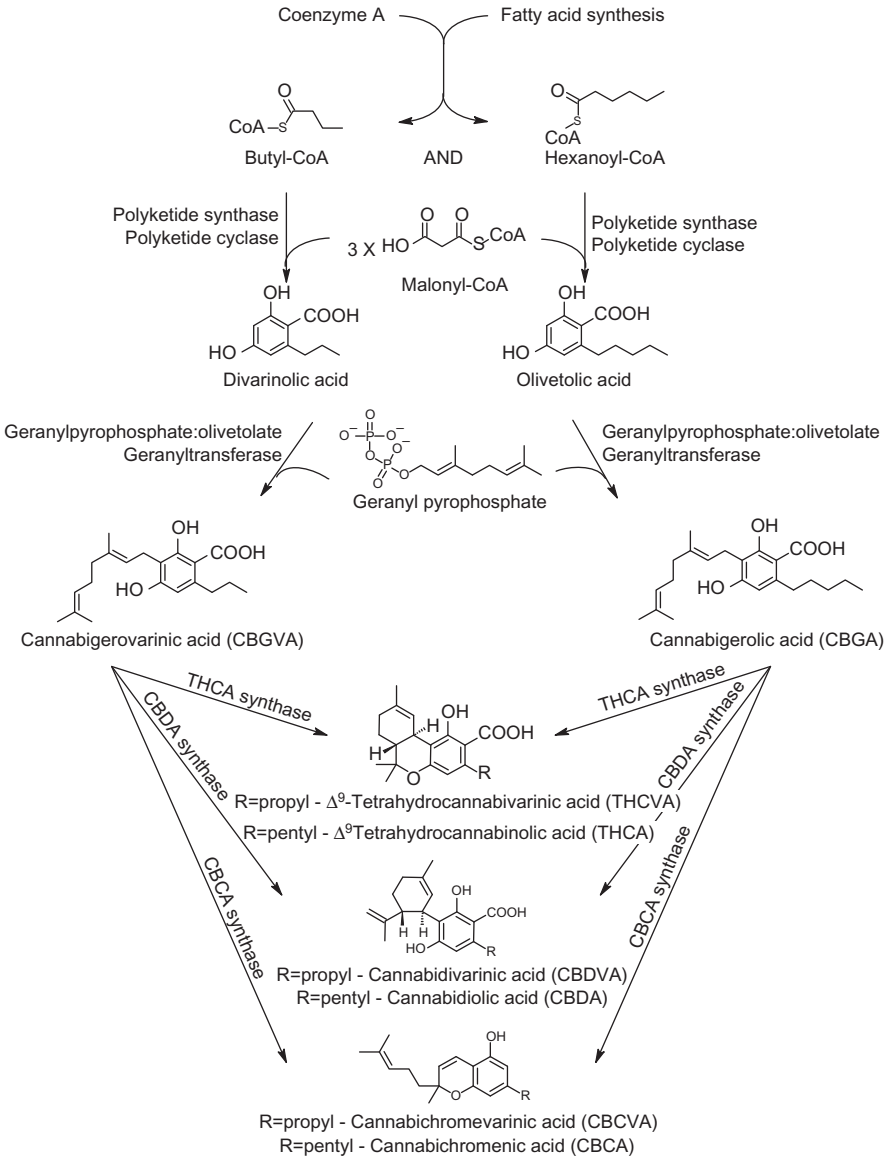

Figure 2.1 Biosynthesis of phytocannabinoids.

homologs Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabivarin (CBV), and cannabidivarin (CBDV). More than 100 phytocannabinoids across 11 chemical classes have been isolated and identified to date.[7] In the growing *Cannabis sativa* plant, most of these cannabinoids are initially formed as carboxylic acids (eg, Δ^9-THCA, CBDA, CBCA, and

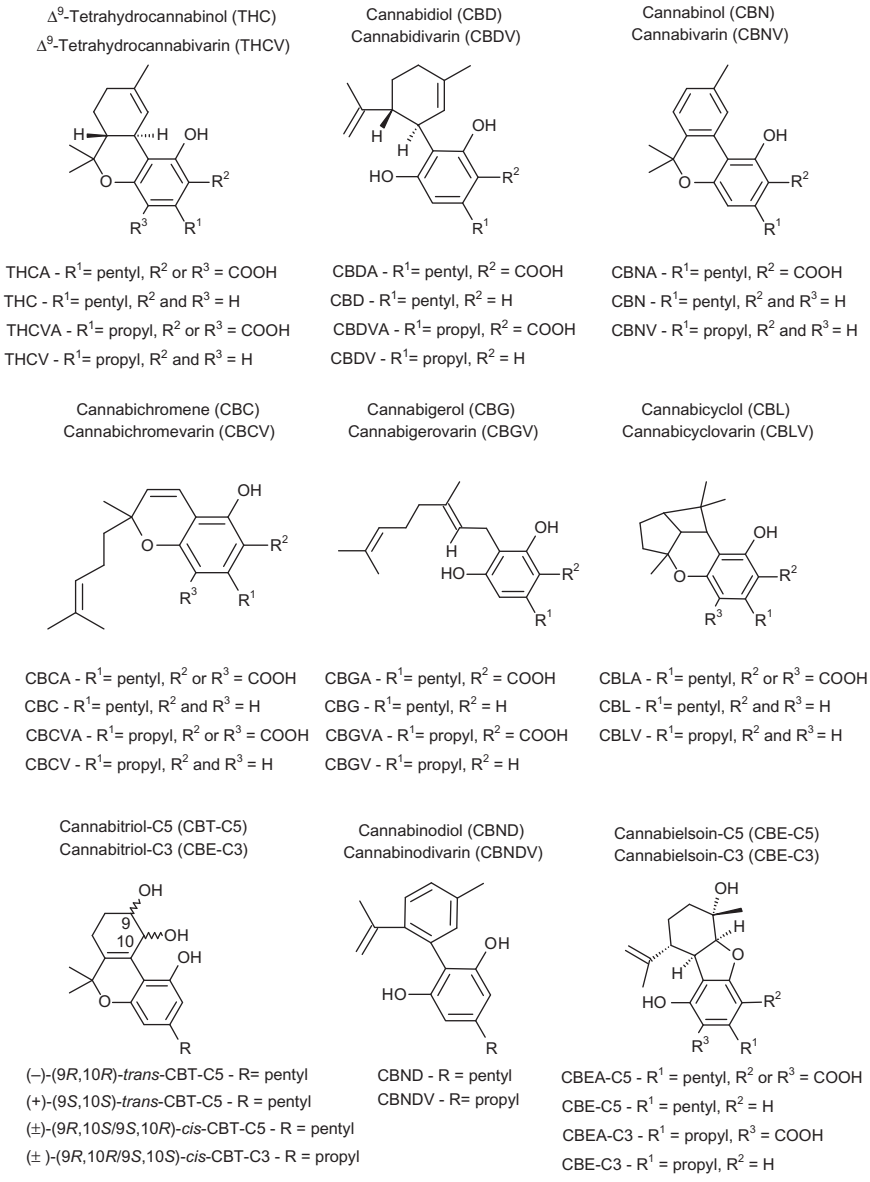

Figure 2.2 Primary phytocannabinoid constituents in cannabis.

Δ^9-THCVA) that are decarboxylated to their corresponding neutral forms as a consequence of drying, heating, combustion, or aging (Fig. 2.2). There are also different isomers of phytocannabinoids resulting from variations or isomerization in the position of the double bond

in the alicyclic carbon ring (eg, (−)-*trans*-Δ^8-THC). It is important to note that CBN is not formed biosynthetically, but is an oxidative degradant of Δ^9-THC.[2]

Regulation of cannabinoid content in each plant phenotype (chemotype) has been proposed to involve genetic control of the expression of a variety of synthetic enzymes by four independent loci.[8] Qualitatively, the cannabinoid chemotype is controlled by the variation in expression of these phytocannabinoid synthetic enzymes, resulting in progenies and populations that have discrete distributions of chemical composition (ie, chemical ratios of phytocannabinoids, such as the Δ^9-THC/CBD ratio). Quantitatively, the phytocannabinoid content is controlled by polygenic mechanisms, and is strongly influenced by environmental factors, such that a Gaussian distribution of total cannabinoid content is typically observed. In addition, the cannabinoid content and profile changes over time as the plant grows, matures, and ages.[9] Wild-type chemotypes can therefore differ between Δ^9-THCA predominance and CBDA predominance in discrete populations, but vary dramatically in total cannabinoid content, with clones of both types reaching total cannabinoid content levels of up to 25−30% (w/w) of the dry and trimmed inflorescences. Spontaneous mutations and selective breeding have produced unique chemotypes that show CBGA-,[10] CBCA-,[11] or Δ^9-THCVA predominance,[8] as well as cannabinoid-free chemotypes.[12] Selective breeding has produced hundreds of strains that differ in appearance and chemical composition, and patients and recreational users often prefer specific strains for their purported ability to produce specific pharmacological effects. De Meijer speculates that future breeding might produce novel terpenophenolic compounds such as those with branched alkyl or aromatic side chains, or chemotypes with increased ratios of currently minor constituents such as methyl, butyl, or farnesyl cannabinoids.[8]

The current variation in phytocannabinoid content across and within chemotypes has important implications in medicinal cannabis and cannabis-based formulations and dosing. This has become increasingly apparent and can be recognized by the plethora of varieties of cannabis being cultivated, manufactured, and marketed as dosing formulations in the medicinal and recreational market. Similarly, the nonphytocannabinoid composition of cannabis is receiving increasing pharmacological attention, particularly terpenoids and flavonoids.[13]

MONOTERPENOID, SESQUITERPENOID, AND DITERPENOID CONSTITUENTS OF CANNABIS

Geranyl pyrophosphate is the precursor in the synthesis of the more ubiquitous terpenoids (Fig. 2.3), leading to the formation of limonene and other monoterpenoids in secretory cell plastids,[14] or coupling with isopentenyl pyrophosphate in the cytoplasm to form farnesyl pyrophosphate, a key intermediate in the biosynthesis of sesquiterpenoids and triterpenoids.[13,15] Addition of another isopentenyl group to farnesyl pyrophosphate leads to geranylgeranyl pyrophosphate, which is the precursor of the diterpenoids.[15] Some of the predominate volatile monoterpenes found in cannabis are β-myrcene, (*E*)-β-ocimene, terpinolene, limonene, and β-pinene.[16,17] β-caryophyllene, α-caryophyllene

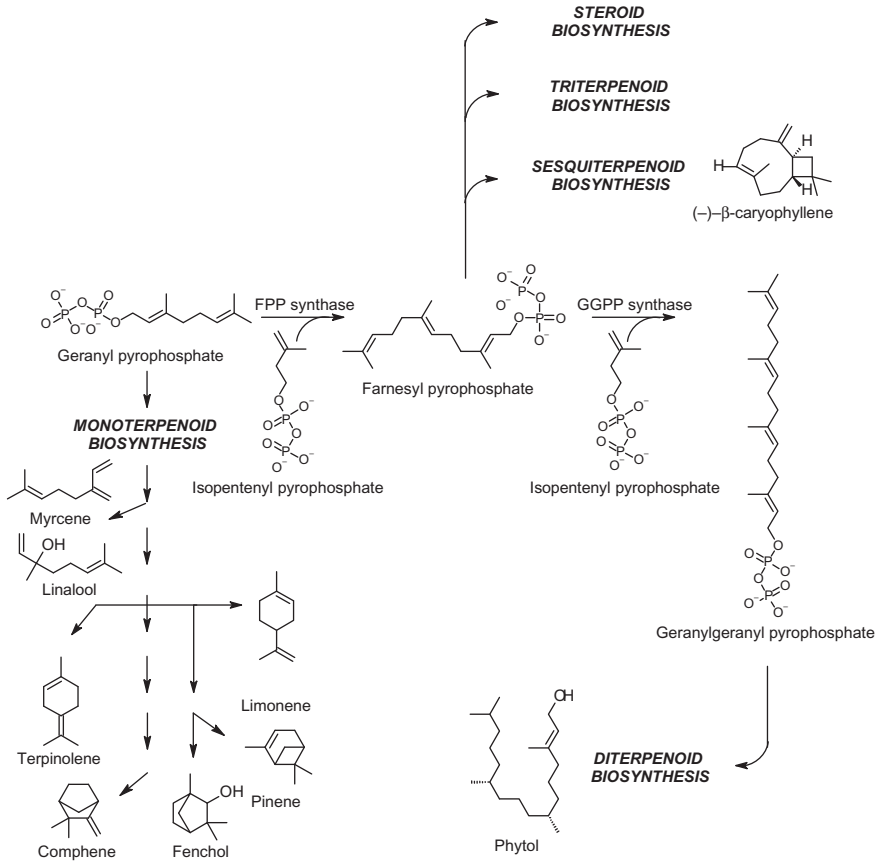

Figure 2.3 Biosynthesis of terpenoids.

(humulene), longifolene, α-zingiberene, and β-cedrene are among the major sesquiterpenes found in prototypical cannabis samples.[16]

PHENYLPROPANOID CONSTITUENTS OF CANNABIS

Phenylpropanoids are of interest for their diverse pharmacological and industrial applications.[18] Their biosynthesis begins with phenylalanine, derived from the shikimate pathway, which is converted by phenylalanine ammonia lyase into cinnamic acid (Fig. 2.4). After hydroxylation of cinnamic acid by cinnamate-4-hydroxylase to form *p*-coumaric acid, it is converted in *p*-coumaroyl CoA by addition of a CoA thioester by a 4-coumarate:CoA ligase enzyme. This common high energy intermediate is used in the biosynthesis of cell wall constituents (lignins), pigments (flavonoids, antocians), and UV protectant and pest resistance compounds (stilbenoids, flavonoids, isoflavonoids, coumarins, and furanocoumarins). A key enzyme in the flavonoid biosynthesis pathway is chalcone synthase (CHS), a protein in the superfamily of polyketide synthase that includes stilbene synthase, phlorisovalerophenone synthase, isobutyrophenone synthase (BUS), and olivetol synthase activities that can be detected during the development and growth of glandular trichomes on bracts of cannabis.[18,19] The activities of polyketide synthases and the resulting biosynthesis of cannabinoids, stilbenoids, and flavonoids in the plant are induced in response to a wide range of stimuli such as UV light, pathogens, hormones, elicitors, growth substances, and wounding. Some of the naturally occurring flavonoid constituents are orientin, vitesin, luteolin-7-*O*-β-D-glucuronide, and apigenin-7-*O*-β-D-glucuronide.[20]

THERAPEUTIC INDICATIONS FOR MEDICINAL CANNABIS AND CANNABIS-DERIVED DOSAGE FORMULATIONS

Cannabis and cannabis-derived dosage formulations such as hashish have a long history of medicinal use. However, the intoxicating effects and associated abuse liability, scheduling, and control efforts have limited the number of methodologically rigorous clinical studies. The most widely supported indications for herbal cannabis and cannabis-derived medicines are nausea and vomiting in cancer chemotherapy, anorexia and cachexia in HIV/AIDS, chronic and neuropathic pain, and spasticity in multiple sclerosis and spinal cord injury.[21] A recent review of the randomized clinical trials investigating cannabis and

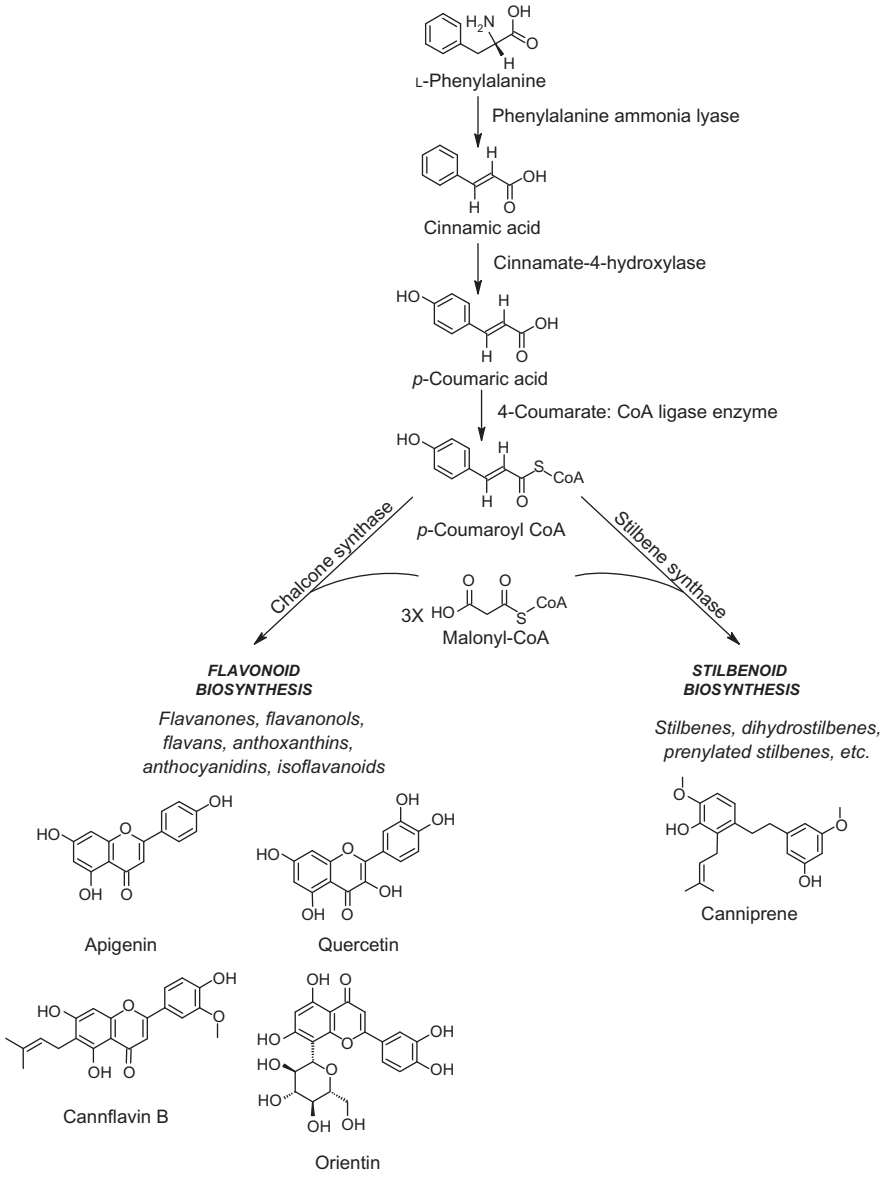

Figure 2.4 Biosynthesis of phenylpropanoids.

cannabinoid therapeutics provides some support for these indications as well as for sleep disorders and Tourette's syndrome.[22] Glaucoma was one of the more frequently cited medical indications in the late twentieth century; however, this indication has received diminished

support from the medical community due to the success of surgical approaches and the availability of drugs with greater efficacy.[21] Cannabis also has a long history of use to control epileptic seizures, the subject of recent reviews.[23] The use of cannabis or cannabinoid-derived drugs for seizures is not fully supported by the Institute of Medicine; however, CBD has gained considerable attention and anecdotal support as a treatment for Dravet Syndrome and other specific seizure disorders.[24] Other indications being investigated are irritable bowel syndrome[25–28] and posttraumatic stress disorder.[29,30]

PHARMACOLOGICAL EFFECTS OF CANNABIS CONSTITUENTS

The contribution of the various chemical constituents in cannabis to its therapeutic and organoleptic effects varies because of several factors, including their differing concentrations (content), chemical properties (eg, stability, volatility), pharmacological actions (eg, receptor affinities, efficacies), physicochemical parameters (eg, lipophilicity, solubility), pharmacokinetics, and pharmacodynamics. The principal acidic forms of phytocannabinoids produced by plant biosynthesis have generally been considered devoid of psychoactivity in man and laboratory animals.[31] Δ^9-THCA, CBNA, and CBGA have previously been reported to lack affinity at the CB1 receptor.[32] More recently, however, Δ^9-THCA showed antinausea and antiemetic effects in animals, with greater potency than Δ^9-THC,[33] and the effect was reversed by rimonabant, the CB1 receptor antagonist. Δ^9-THCA was recently reported to bind to CB1 and CB2 receptors with greater affinity than Δ^9-THC.[34] Δ^9-THCA has shown immunomodulatory actions that are CB1- and CB2 receptor–independent.[35] Thus, further studies of the pharmacological activity and mechanism of action of Δ^9-THCA are needed.

The primary psychoactive phytocannabinoid is often considered to be Δ^9-THC because of its rapid formation from Δ^9-THCA through decarboxylation during combustion of plant material resulting in its high concentration in smoke during inhalation and its potency at cannabinoid receptors at physiologically relevant concentrations. In support of this hypothesis, radioligand binding studies have shown that Δ^9-THC binds to the CB1 receptor with high affinity (K_i values $\sim$50 nM), while CBN has an approximately 10-fold lower affinity, and CBD and CBG have K_i values estimated to be greater than

500 nM.[36,37] This receptor binding affinity correlates to both the inhibition of adenylate cyclase in vitro and the analgesic and psychoactivity of these compounds in vivo.[38] Δ^9-THC also binds to the CB2 receptor with similar high affinity and inhibits adenylate cyclase.[39] However, it is important to note that Δ^9-THC acts as a partial agonist in GTP-γ-S assays[40] as compared to the effects seen with more efficacious synthetic cannabinoid agonists such as CP-55940 and WIN55212-2.[41] Because it acts as a partial agonist at both CB1 and CB2, it elicits a response that is strongly influenced by the tissue-specific expression levels, their extent of constitutive signaling, and the ongoing endogenous cannabinoid release or tone of the receptor system.[42] In addition to its production of psychoactivity in man, Δ^9-THC produces a myriad of additional cannabinoid receptor–mediated pharmacological effects in laboratory animals and man.[43]

Several other phytocannabinoids bind to and modulate cannabinoid receptor function. Δ^8-THC, for example, binds to CB1 and CB2 with affinities approximating those of Δ^9-THC and acts as an agonist.[44–46] Δ^9-THCV also binds to CB1 and CB2 with nanomolar affinities[47] and acts as a cannabimimetic agonist. High doses of Δ^9-THCV have been reported to produce a psychoactive effect characterized as mild intoxication in man[31,48] and catalepsy[49] and analgesia[50] in laboratory animals. However, Δ^9-THCV can also antagonize cannabinoid receptor agonists in CB1-expressing tissues in a manner that is both tissue- and ligand-dependent.[42,50] Moreover, this compound is reported to be capable of behaving either as a CB1 antagonist or, at higher doses, as a CB1 agonist in vivo. CBN can also bind to CB1[51] and CB2, but does so with lower affinity than Δ^9-THC. It also fails to inhibit forskolin-stimulated cAMP increase at doses up to 1 μM in transfected cell lines expressing CB1 or CB2 receptors,[52] and produces only mild intoxication in man at high intravenous doses.[31] Other than Δ^9-THC, Δ^8-THC, CBN, and Δ^9-THCV, no phytocannabinoids have been reported to activate CB1 or CB2 receptors with nanomolar or low micromolar potency.[53] Neither CBD nor CBDA is psychoactive in man,[31] nor does CBD bind to the CB1[51] or CB2[54] receptor with high affinity. However, Pertwee has reported that CBD displays high potency as an antagonist in CB1- and CB2-expressing cells or tissues.[42]

The phytocannabinoids in cannabis can act via a plethora of other noncannabinoid receptor–mediated systems to produce a wide variety

of additional pharmacological effects. As reviewed by Pertwee in 2010, Δ^9-THC and other phytocannabinoids have been shown to modulate the activity of GPR-55[55] and many other receptors and enzyme systems.[56] For instance, CBD, CBN, and Δ^9-THC produce noncompetitive/allosteric interactions with μ and δ opioid receptors,[57,58] and Δ^9-THC allosterically modulates glycine receptor activation at nanomolar concentrations.[59] Δ^9-THC and CBD inhibit Ca(V)3 channels at pharmacologically relevant concentrations, and Δ^9-THC but not CBD may increase the amount of calcium entry following T-type channel activation by stabilizing open states of the channel.[60] These phytocannabinoids also interact with transient receptor potential (TRP) channels and enzymes of the endocannabinoid system.[61] De Petrocellis et al. showed that both CBDV and CBD activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels, and that CBC disrupts transport of the endocannabinoid anandamide in vitro.[62] CBG, CBGV, and Δ^9-THCV stimulate and desensitize human TRPV1, while Δ^9-THC, CBC, CBD, and CBN are potent rat TRPA1 agonists and desensitizers. All of these cannabinoids except CBC and CBN also potently activate and desensitize rat TRPV2.[62] Interestingly, in three models of seizure, cannabis-derived "botanical drug substances" rich in CBDV and CBD exerted significant anticonvulsant effects that were not mediated by the CB1 receptor and were of comparable efficacy with purified CBDV.[63] Whether the anticonvulsant activity produced by certain phytocannabinoids and cannabis-derived drug formulations in vivo is related to these effects on calcium channels remains to be determined; however, these actions are indicative of potential therapeutic utility in the treatment of neuronal hyperexcitability. Both Δ^9-THC and CBN induce a cannabinoid receptor–independent release of calcitonin gene-related peptide (CGRP) from capsaicin-sensitive perivascular sensory nerves.[64] Thus, the antinociceptive actions of phytocannabinoids may rely on the activation of inhibitory cannabinoid receptors (CB1) in the peripheral and central nervous systems, as well as on the activation of excitatory ionotropic TRP channels coexpressed with CB1 in primary nociceptive neurons that contain and release CGRP upon activation.[65] Δ^9-THC can also act as an agonist at the peroxisome proliferator-activated receptor, whereas Δ^9-THCV does not. Thus it appears that these highly lipophilic phytocannabinoids[66] can gain access to and modulate a variety of non-CB1, non-CB2 G protein–coupled receptors, transmitter-gated channels, ion channels, and/or nuclear receptors.[56]

Nonphytocannabinoid terpenoids in cannabis contribute to the organoleptic properties of the plant, but can also modulate the activity of cannabinoid receptors and contribute to a wide variety of noncannabinoid receptor–mediated pharmacological effects.[13] For example, the sesquiterpenoid β-caryophyllene has been shown to bind to CB2 with nanomolar affinity.[67] Upon binding to CB2, it acts like a prototypical CB2 agonist and inhibits adenylate cylcase, produces intracellular calcium transients, and activates the mitogen-activated protein kinase Erk1/2 and p38 pathways in primary human monocytes. β-Caryophyllene reduces the carrageenan-induced inflammatory response in wild-type mice but not in mice lacking CB2 receptors, evidence that this terpenoid exerts cannabimimetic effects in vivo. In addition to modulation of cannabinoid receptors, terpenoids and phenylpropanoids in cannabis have potent antioxidant,[68] anticancer,[69,70] and antiinflammatory activity.[71–75] Several monoterpenic alcohols, including geraniol, nerol, and citronellol, have been reported to be promiscuous TRP modulators.[76–78] It has been hypothesized that the broad spectrum and prolonged sensory inhibition produced by phytocannabinoids and terpenoids may allow them to act synergistically as therapeutics for allodynia, itch, and other types of pain involving superficial sensory nerves and skin.[78–80] Russo has proposed therapeutic synergies and interactions among phytocannabinoids, terpenoids, and phenylpropanoids. If clinically proven, this increases the likelihood of an extensive pipeline of new therapeutic products and cannabis-derived botanical drug products.[13]

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