

Chapter 2

Chemistry and Analysis of Phytocannabinoids and Other Cannabis Constituents

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1. THE CHEMISTRY OF PHYTOCANNABINOIDS AND NONCANNABINOID-TYPE CONSTITUENTS

1.1. Phytocannabinoids

1.1.1. Introduction

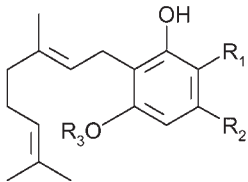
The *Cannabis* plant and its products consist of an enormous variety of chemicals. Some of the 483 compounds identified are unique to *Cannabis*, for example, the more than 60 cannabinoids, whereas the terpenes, with about 140 members forming the most abundant class, are widespread in the plant kingdom. The term “cannabinoids” represents a group of C₂₁ terpenophenolic compounds found until now uniquely in *Cannabis sativa* L. (1). As a consequence of the development of synthetic cannabinoids (e.g., nabilone [2], HU-211 [dexanabinol; ref. {3}], or ajulemic acid [CT-3; ref. 4]) and the discovery of the chemically different endogenous cannabinoid receptor ligands (“endocannabinoids,” e.g., anandamide, 2-arachidonoylglycerol) (5,6), the term “phytocannabinoids” was proposed for these particular *Cannabis* constituents (7).

1.1.2. Chemistry and Classification

So far, 66 cannabinoids have been identified. They are divided into 10 subclasses (8–10) (see Table 1).

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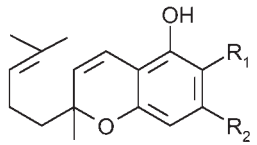
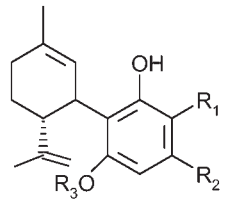
Table 1
Cannabinoids

Compound	Structure	Main pharmacological characteristics
Cannabigerol class		
Cannabigerolic acid (CBGA)	 <p>$R_1 = \text{COOH}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{H}$</p>	Antibiotic
Cannabigerolic acid monomethylether (CBGAM)	$R_1 = \text{COOH}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{CH}_3$	
Cannabigerol (CBG)	$R_1 = \text{H}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{H}$	Antibiotic Antifungal Anti-inflammatory Analgesic
Cannabigerol monomethylether (CBGM)	$R_1 = \text{H}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{CH}_3$	
Cannabigerovarinic acid (CBGVA)	$R_1 = \text{COOH}, R_2 = \text{C}_3\text{H}_7, R_3 = \text{H}$	
Cannabigerovarin (CBGV)	$R_1 = \text{H}, R_2 = \text{C}_3\text{H}_7, R_3 = \text{H}$	

(continued)

1. Cannabigerol (CBG) type: CBG was the first cannabinoid identified (11), and its precursor cannabigerolic acid (CBGA) was shown to be the first biogenic cannabinoid formed in the plant (12). Propyl side-chain analogs and a monomethyl ether derivative are other cannabinoids of this group.
2. Cannabichromene (CBC) type: Five CBC-type cannabinoids, mainly present as C5-analogs, have been identified.
3. Cannabidiol (CBD) type: CBD was isolated in 1940 (13), but its correct structure was first elucidated in 1963 by Mechoulam and Shvo (14). Seven CBD-type cannabinoids with C1 to C5 side chains have been described. CBD and its corresponding acid CBDA

Table 1 (continued)

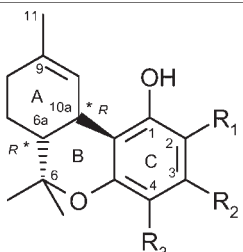
Compound	Structure	Main pharmacological characteristics
Cannabichromene class		
Cannabichromenic acid (CBCA)	 <p>$R_1 = \text{COOH}, R_2 = \text{C}_5\text{H}_{11}$</p>	
Cannabichromene (CBC)	$R_1 = \text{H}, R_2 = \text{C}_5\text{H}_{11}$	Anti-inflammatory Antibiotic Antifungal Analgesic
Cannabichromevarinic acid (CBCVA)	$R_1 = \text{COOH}, R_2 = \text{C}_3\text{H}_7$	
Cannabichromevarin (CBCV)	$R_1 = \text{H}, R_2 = \text{C}_3\text{H}_7$	
Cannabidiol class		
Cannabidiolic acid (CBDA)	 <p>$R_1 = \text{COOH}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{H}$</p>	Antibiotic
Cannabidiol (CBD)	$R_1 = \text{H}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{H}$	Anxiolytic Antipsychotic Analgesic Anti-inflammatory Antioxydant Antispasmodic

(continued)

are the most abundant cannabinoids in fiber-type *Cannabis* (industrial hemp). Isolated in 1955, CBDA was the first discovered cannabinoid acid.

4. Δ^9 -Tetrahydrocannabinol (THC) type: Nine THC-type cannabinoids with C1 to C5 side chains are known. The major biogenic precursor is the THC acid A, whereas

Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
Cannabidiol monomethylether (CBDM)	$R_1 = H, R_2 = C_5H_{11}, R_3 = CH_3$	
Cannabidiol- C_4 (CBD- C_4)	$R_1 = H, R_2 = C_4H_9, R_3 = H$	
Cannabidivarinic acid (CBDVA)	$R_1 = COOH, R_2 = C_3H_7, R_3 = H$	
Cannabidivarin (CBDV)	$R_1 = H, R_2 = C_3H_7, R_3 = H$	
Cannabiorcol (CBD- C_1)	$R_1 = H, R_2 = CH_3, R_3 = H$	
Delta-9-tetrahydrocannabinol class		
Delta-9-tetrahydrocannabinolic acid A (THCA-A)	 <p>$R_1 = COOH, R_2 = C_5H_{11}, R_3 = H$</p>	
Delta-9-tetrahydrocannabinolic acid B (THCA-B)	$R_1 = H, R_2 = C_5H_{11}, R_3 = COOH$	

(continued)

THC acid B is present to a much lesser extent. THC is the main psychotropic principle; the acids are not psychoactive. THC (6a,10a-*trans*-6a,7,8,10a-tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[*b,d*]pyran-1-ol) was first isolated in 1942 (15), but the correct structure assignment by Gaoni and Mechoulam took place in 1964 (16).

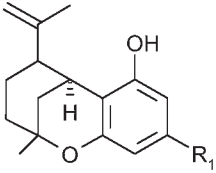
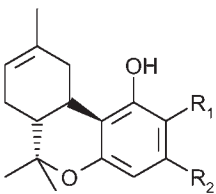
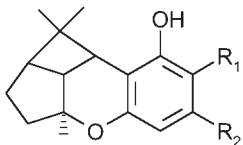
Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
Delta-9-tetrahydrocannabinol (THC)	$R_1 = H, R_2 = C_5H_{11}, R_3 = H$	Euphoriant Analgesic Anti-inflammatory Antioxidant Antiemetic
Delta-9-tetrahydrocannabinolic acid-C ₄ (THCA-C ₄)	$R_1 = COOH, R_2 = C_4H_9, R_3 = H$ or $R_1 = H, R_2 = C_4H_9, R_3 = COOH$	
Delta-9-tetrahydrocannabinol-C ₄ (THC-C ₄)	$R_1 = H, R_2 = C_4H_9, R_3 = H$	
Delta-9-tetrahydrocannabivarinic acid (THCVA)	$R_1 = COOH, R_2 = C_3H_7, R_3 = H$	
Delta-9-tetrahydrocannabivarin (THCV)	$R_1 = H, R_2 = C_3H_7, R_3 = H$	Analgesic Euphoriant
Delta-9-tetrahydrocannabiorcolic acid (THCA-C ₁)	$R_1 = COOH, R_2 = CH_3, R_3 = H$ or $R_1 = H, R_2 = CH_3, R_3 = COOH$	
Delta-9-tetrahydrocannabiorcol (THC-C ₁)	$R_1 = H, R_2 = CH_3, R_3 = H$	

(continued)

5. Δ^8 -THC type: Δ^8 -THC and its acid precursor are considered as THC and THC acid artifacts, respectively. The 8,9 double-bond position is thermodynamically more stable than the 9,10 position. Δ^8 -THC is approx 20% less active than THC.

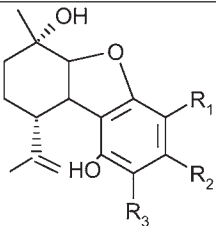
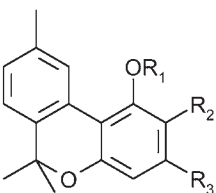
Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
Delta-7- <i>cis</i> -isotetrahydrocannabivarin	 <p style="text-align: center;">$R_1 = C_3H_7$</p>	
Delta-8-tetrahydrocannabinol class		
Delta-8-tetrahydrocannabinolic acid (Δ^8 -THCA)	 <p style="text-align: center;">$R_1 = COOH, R_2 = C_5H_{11}$</p>	
Delta-8-tetrahydrocannabinol (Δ^8 -THC)	$R_1 = H, R_2 = C_5H_{11}$	Similar to THC (less potent)
Cannabicyclol class		
Cannabicyclolic acid (CBLA)	 <p style="text-align: center;">$R_1 = COOH, R_2 = C_5H_{11}$</p>	
Cannabicyclol (CBL)	$R_1 = H, R_2 = C_5H_{11}$	
Cannabicyclovarin (CBLV)	$R_1 = H, R_2 = C_3H_7$	

(continued)

6. Cannabicyclol (CBL) type: Three cannabinoids characterized by a five-atom ring and C_1 -bridge instead of the typical ring A are known: CBL, its acid precursor, and the C_3 side-chain analog. CBL is known to be a heat-generated artifact from CBC.
7. Cannabielsoin (CBE) type: Among the five CBE-type cannabinoids, which are artifacts formed from CBD, are CBE and its acid precursors A and B.

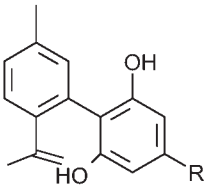
Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
Cannabielsoin class		
Cannabielsoic acid A (CBEA-A)	 <p>$R_1 = \text{COOH}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{H}$</p>	
Cannabielsoic acid B (CBEA-B)	<p>$R_1 = \text{H}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{COOH}$</p>	
Cannabielsoin (CBE)	<p>$R_1 = \text{H}, R_2 = \text{C}_5\text{H}_{11}, R_3 = \text{H}$</p>	
Cannabinol and cannabinodiol class		
Cannabinolic acid (CBNA)	 <p>$R_1 = \text{H}, R_2 = \text{COOH}, R_3 = \text{C}_5\text{H}_{11}$</p>	
Cannabinol (CBN)	<p>$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{C}_5\text{H}_{11}$</p>	<p>Sedative Antibiotic Anticonvulsant Anti-inflammatory</p>

(continued)

8. Cannabinol (CBN) and Cannabinodiol (CBND) types: Six CBN- and two CBND-type cannabinoids are known. With ring A aromatized, they are oxidation artifacts of THC and CBD, respectively. Their concentration in *Cannabis* products depends on age and storage conditions. CBN was first named in 1896 by Wood et al. (17) and its structure elucidated in 1940 (18).

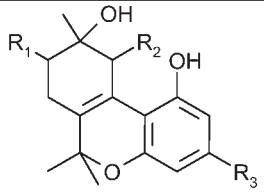
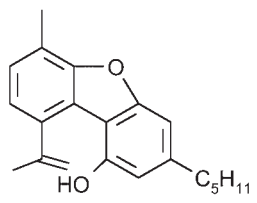
Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
Cannabinol methylether (CBNM)	$R_1 = \text{CH}_3, R_2 = \text{H}, R_3 = \text{C}_5\text{H}_{11}$	
Cannabinol-C ₄ (CBN-C ₄)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{C}_4\text{H}_9$	
Cannabivarin (CBV)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{C}_3\text{H}_7$	
Cannabinol-C ₂ (CBN-C ₂)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{C}_2\text{H}_5$	
Cannabiorcol (CBN-C ₁)	$R_1 = \text{H}, R_2 = \text{H}, R_3 = \text{CH}_3$	
Cannabinodiol (CBND)	 $R = \text{C}_5\text{H}_{11}$	
Cannabinodivarin (CBVD)	$R = \text{C}_3\text{H}_7$	

(continued)

9. Cannabitrilol (CBT) type: Nine CBT-type cannabinoids have been identified, which are characterized by additional OH substitution. CBT itself exists in the form of both isomers and the racemate, whereas two isomers (9-a- and 9-b-hydroxy) of CBTV were identified. CBDA tetrahydrocannabitrilol ester (ester at 9-hydroxy group) is the only reported ester of any naturally occurring cannabinoids.
10. Miscellaneous types: Eleven cannabinoids of various unusual structure, e.g., with a furano ring (dehydrocannabifuran, cannabifuran), carbonyl function (cannabichromanon, 10-oxo- δ -6a-tetrahydrocannabinol), or tetrahydroxy substitution (cannabiripsol), are known.

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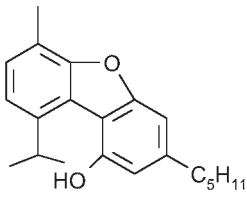
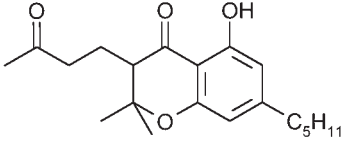
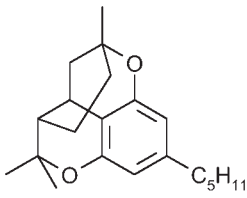
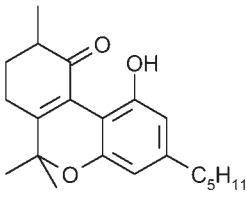
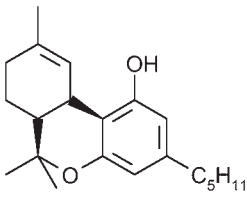
Compound	Structure	Main pharmacological characteristics
Cannabinol class		
Cannabinol (CBT)	 <p style="text-align: center;">$R_1 = H, R_2 = OH, R_3 = C_5H_{11}$</p>	
10-Ethoxy-9-hydroxy-delta-6a-tetrahydrocannabinol	$R_1 = H, R_2 = OC_2H_5, R_3 = C_5H_{11}$	
8,9-Dihydroxy-delta-6a-tetrahydrocannabinol	$R_1 = OH, R_2 = H, R_3 = C_5H_{11}$	
Cannabinolvarin (CBTV)	$R_1 = H, R_2 = OH, R_3 = C_3H_7$	
Ethoxy-cannabinolvarin (CBTVE)	$R_1 = H, R_2 = OC_2H_5, R_3 = C_3H_7$	
Miscellaneous cannabinoids class		
Dehydrocannabifuran (DCBF)		

(continued)

1.1.3. THC Potency Trends

From 1980 to 1997, a total of 35,213 samples of confiscated *Cannabis* products (*Cannabis*, hashish, hashish oil) representing more than 7717 tons seized in the United States were analyzed by gas chromatography (GC) (19). The mean THC concentration increased from less than 1.5% in 1980 to 4.2% in 1997. The maximum levels found were 29.9 and 33.1% in marijuana and sinsemilla *Cannabis*, respectively. Hashish

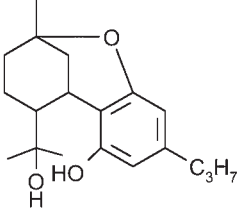
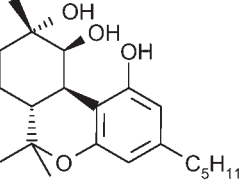
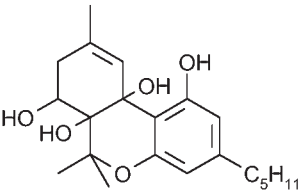
Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
Cannabifuran (CBF)		
Cannabichromanon (CBCN)		
Cannabicitran (CBT)		
10-Oxo-delta-6a-tetrahydrocannabinol (OTHC)		
Delta-9- <i>cis</i> -tetrahydrocannabinol (<i>cis</i> -THC)		

(continued)

and hashish oil showed no particular potency trend. The highest THC concentrations measured were 52.9 and 47.0%, respectively. Two studies performed in Switzerland from 1981 to 1985 (20) and 2002 to 2003 (21) found mean THC concentrations in marijuana samples of 1.4 and 12.9%, respectively. Maximum levels were 4.8 and 28.4%, respectively. Reasons for this enormous increase in potency include progress in breed-

Table 1 (continued)

Compound	Structure	Main pharmacological characteristics
3,4,5,6-Tetrahydro-7-hydroxy-alpha-alpha-2-trimethyl-9-n-propyl-2,6-methano-2H-1-benzoxocin-5-methanol (OH-iso-HHCV)		
Cannabiripsol (CBR)		
Trihydroxy-delta-9-tetrahydrocannabinol (triOH-THC)		

ing, the tendency to cultivate under indoor conditions, and the worldwide access to and exchange of seeds originating from high-THC cultivars via the Internet (22).

1.1.4. THC in Hemp Seed Products

The presence of THC in hemp seed products is predominantly the result of external contact of the seed hull with cannabinoid-containing resins in bracts and leaves during maturation, harvesting, and processing (23–25). The seed kernel is not entirely free of THC but contains, depending on the hemp variety, less than 0.5 µg/g. Studies on hemp oil conducted in the United States, Germany, and Switzerland have shown THC levels from 11 to 117, 4 to 214, and up to 3568 µg/g, respectively (24,26–28). These high levels were attributed to seeds from THC-rich, “drug-type” varieties, and the lack of adequate cleaning procedures. In recent years, more careful seed drying and cleaning have considerably lowered the THC content of seeds and oil available in the United States (23,24). However, oils and hulled seeds containing 10–20 and 2–3 µg/g THC, respectively, are still found on the US market.

1.2. Noncannabinoid-Type Constituents

1.2.1. Terpenoids

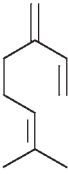
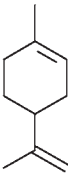
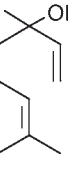
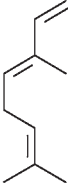
The typical scent of *Cannabis* results from about 140 different terpenoids. Isoprene units (C_5H_8) form monoterpenoids (C_{10} skeleton), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), and triterpenoids (C_{30} ; see Table 2). Terpenoids may be acyclic, monocyclic, or polycyclic hydrocarbons with substitution patterns including alcohols, ethers, aldehydes, ketones, and esters. The essential oil (volatile oil) can easily be obtained by steam distillation or vaporization. The yield depends on the *Cannabis* type (drug, fiber) and pollination; sex, age, and part of the plant; cultivation (indoor, outdoor etc.); harvest time and conditions; drying; and storage (29–31). For example, fresh buds from an Afghani variety yielded 0.29% essential oil (32). Drying and storage reduced the content from 0.29 after 1 week and 3 months to 0.20 and 0.13%, respectively (32). Monoterpenes showed a significantly greater loss than sesquiterpenes, but none of the major components completely disappeared in the drying process. About 1.3 L of essential oil per ton resulted from freshly harvested outdoor-grown *Cannabis*, corresponding to about 10 L/ha (29). The yield of nonpollinated (“sinsemilla”) *Cannabis* at 18 L/ha was more than twofold compared with pollinated *Cannabis* (8 L/ha) (30). Sixty-eight components were detected by GC and GC/mass spectrometry (MS) in fresh bud oil distilled from high-potency, indoor-grown *Cannabis* (32). The 57 identified constituents were 92% monoterpenes, 7% sesquiterpenes, and approx 1% other compounds (ketones, esters; refs. 9 and 32). The dominating monoterpenes were myrcene (67%) and limonene (16%). In the essential oil from outdoor-grown *Cannabis*, the monoterpene concentration varied between 47.9 and 92.1% of the total terpenoid content (29). The sesquiterpenes ranged from 5.2 to 48.6%. The most abundant monoterpene was β -myrcene, followed by *trans*-caryophyllene, α -pinene, *trans*-ocimene, and α -terpinolene. “Drug-type” *Cannabis* generally contained less caryophyllene oxide than “fiber-type” *Cannabis*. Even in “drug-type” *Cannabis*, the THC content of the essential oil was not more than 0.08% (29). In the essential oil of five different European *Cannabis* cultivars, the dominating terpenes were myrcene (21.1–35.0%), α -pinene (7.2–14.6%), α -terpinolene (7.0–16.6%), *trans*-caryophyllene (12.2–18.9%), and α -humulene (6.1–8.7%; ref. 33). The main differences between the cultivars were found in the contents of α -terpinolene and α -pinene.

Other terpenoids present only in traces are sabinene, α -terpinene, 1,8-cineole (eucalyptol), pulegone, γ -terpinene, terpineol-4-ol, bornyl acetate, α -copaene, alloaromadendrene, viridiflorene, β -bisabolene, γ -cadinene, *trans*- β -farnesene, *trans*-nerolidol, and β -bisabolol (29,32,34).

1.2.2. Hydrocarbons

The 50 known hydrocarbons detected in *Cannabis* consist of *n*-alkanes ranging from C_9 to C_{39} , 2-methyl-, 3-methyl-, and some dimethyl alkanes (10,35). The major alkane present in an essential oil obtained by extraction and steam distillation was the *n*- C_{29} alkane nonacosane (55.8 and 10.7%, respectively). Other abundant alkanes were heptacosane, 2,6-dimethyltetradecane, pentacosane, hexacosane, and hentriacontane.

Table 2
Terpenoids of the Essential Oil From *Cannabis*

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
Myrcene	M		32.9–67.1	29.4–65.8
Limonene	M		16.3–17.7	0.9–1.5
Linalool	M		2.8–5.1	0.002
<i>trans</i> -Ocimene	M			2.3–5.7

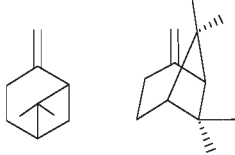
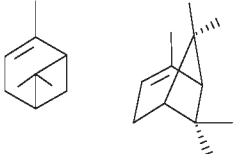
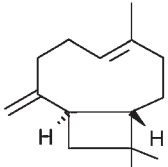
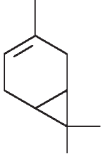
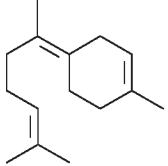
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1.2.3. Nitrogen-Containing Compounds

Cannabis sativa L. is one of the rare psychotropic plants in which the central nervous system activity is not linked to particular alkaloids. However, two spermidine-type alkaloids (see Table 3) have been identified among the more than 70 nitrogen-containing constituents. Other nitrogenous compounds found are the quaternary bases choline, trigonelline, muscarine, isoleucine betaine, and neurine. Among the 8 amides are, for example, *N-trans*-feruloyltyramine, *N-p*-coumaroyltyramine, and *N-trans*-caffeoyltyramine (see Table 4). Five lignanamide derivatives have been isolated, including cannabisin A, B, C, and D (see Table 5).

Twelve simple amines, including piperidine, hordenine, methylamine, ethylamine, and pyrrolidine, are known. The three proteins detected are edestin, zeatin, and

Table 2 (continued)

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
beta-Pinene	M		2.2–2.5	1.3–1.6
alpha-Pinene	M		1.1–1.6	6.0–8.4
beta-Caryophyllene	S		1.3–5.5	19.5–31.4
delta-3-Carene	M			0.8–1.0
trans-gamma-Bisabolene	S		0.7–3.9	

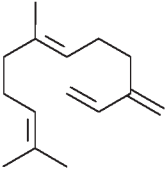
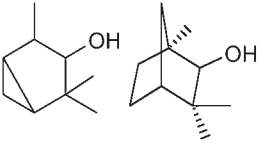
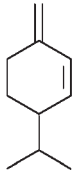
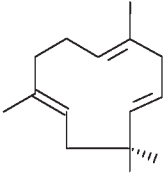
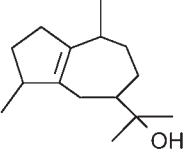
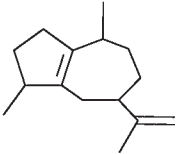
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zeatin nucleoside; the six enzymes are edestinase, glucosidase, polyphenol oxidase, peptidase, peroxidase, and adenosine-5-phosphatase. The 18 amino acids are of a structure common for plants.

1.2.4. Carbohydrates

Common sugars are the predominant constituents of this class. Thirteen monosaccharides (fructose, galactose, arabinose, glucose, mannose, rhamnose, etc.), two disaccharides (sucrose, maltose), and five polysaccharides (raffinose, cellulose, hemicellulose, pectin, xylan) have been identified so far. In addition, 12 sugar alcohols

Table 2 (continued)

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
<i>trans</i> -alpha-Farnesene	S		0.6–2.7	
beta-Fenchol	M		0.4–1.0	
beta-Phellandrene	M			0.4
alpha-Humulene (alpha-Caryophyllene)	S		0.3–2.1	3.3–3.4
Guajol	S		0.3–1.8	
alpha-Guaiene	S		0.3–1.2	

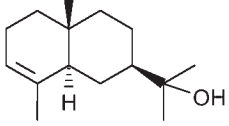
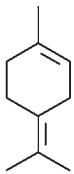
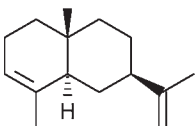
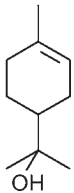
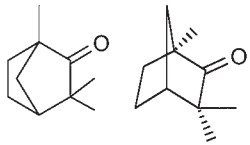
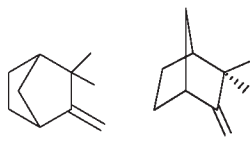
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and cyclitols (mannitol, sorbitol, glycerol, inositol, quebrachitol, etc.) and two amino sugars (galactosamine, glucosamine) were found.

1.2.5. Flavonoids

Twenty-three commonly occurring flavonoids have been identified in *Cannabis*, existing mainly as *C*-/*O*- and *O*-glycosides of the flavon- and flavonol-type aglycones

Table 2 (continued)

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
alpha-Eudesmol	S		0.2–1.4	
Terpinolene	M		0.2–1.1	3.4–5.6
alpha-Selinene	S		0.2–0.7	
alpha-Terpineol	M		0.2–0.5	
Fenchone	M		0.2–0.4	
Camphene	M		0.2–0.4	


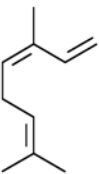
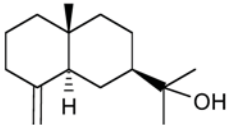
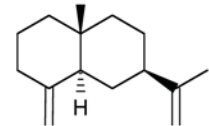
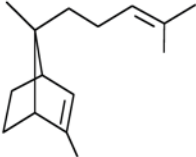
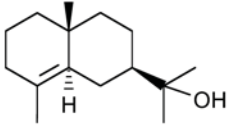
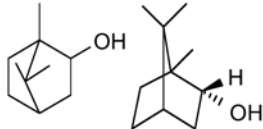
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apigenin, luteolin, quercetin, and kaempferol (see Table 6; ref. 36). Orientin, vitexin, luteolin-7-*O*-glucoside, and apigenin-7-*O*-glucoside were the major flavonoid glycosides present in low-THC *Cannabis* cultivars (37). The cannflavins A and B are unique to *Cannabis* (38,39).

1.2.6. Fatty Acids

A total of 33 different fatty acids, mainly unsaturated fatty acids, have been identified in the oil of *Cannabis* seeds. Linoleic acid (53–60% of total fatty acids), α -

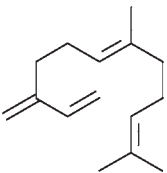
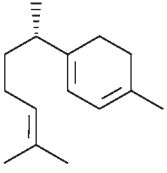
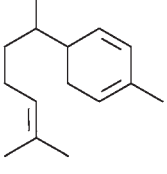
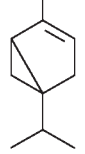
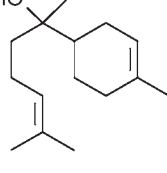
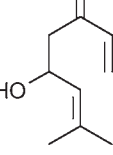
Table 2 (continued)

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
<i>cis</i> -Sabinene hydrate	M		0.2–0.5	
<i>cis</i> -Ocimene	M		traces–0.2	0.2–0.3
beta-Eudesmol	S		0.1–1.1	
beta-Selinene	S		0.1–0.6	0.2–0.4
alpha- <i>trans</i> -Bergamotene	S		0.1–0.5	0.4–0.6
gamma-Eudesmol	S		0.1–0.5	
Borneol	M		0.1–0.3	0.008

(continued)

linolenic acid (15–25%), and oleic acid (8.5–16%) are most common (see Table 7) (40). Other unsaturated fatty acids are γ -linolenic acid (1–4%), stearidonic acid (0.4–2%), eicosanoic acid (<0.5%), *cis*-vaccenic acid, and isolinolenic acid. The saturated fatty acids are palmitic acid (6–9%), stearic acid (2–3.5%), arachidic acid (1–3%), behenic acid (<0.3%), myristic acid, lignoceric acid, caproic acid, heptanoic acid, ca-

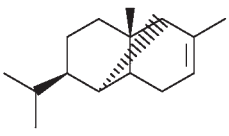
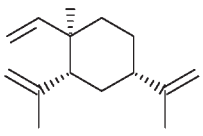

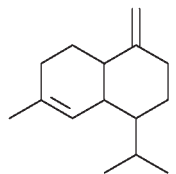
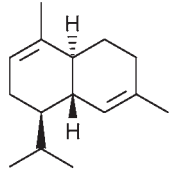
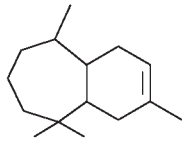
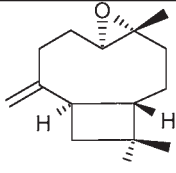
Table 2 (continued)

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
<i>cis</i> -beta-Farnesene	S		0.1–0.3	0.6–0.9
gamma-Curcumene	S		0.1–0.3	
<i>cis</i> -gamma-Bisabolene	S		0.1–0.3	
alpha-Thujene	M		0.1–0.2	
epi-alpha-Bisabolol	S		0.1–1.2	
Ipsdienol	M		traces–0.1	

(continued)

prylic acid, pelargonic acid, capric acid, lauric acid, margaric acid, and isoarachidic acid. The fatty acid spectrum of *Cannabis* seeds does not significantly vary in oil produced from drug (THC) or low-THC (hemp, fiber) type *Cannabis* (41). For the THC content of *Cannabis* seeds and seed oil, see Section 1.1.4.

Table 2 (continued)

Compound	Class ^a	Structure	Percentage	
			Ref. 32	Ref. 29
alpha-Ylangene	S		traces–0.1	
beta-Elemene	S		traces–0.2	
alpha- <i>cis</i> -Bergamotene	S		traces–0.6	
gamma-Murolene	S		traces–0.1	
alpha-Cadinene	S		traces–0.1	
alpha-Longipinene	S		traces–0.1	
Caryophyllene oxide	S		traces–0.8	

^aM, monoterpene; S, sesquiterpene.

1.2.7. Noncannabinoid Phenols

Thirty-four noncannabinoid phenols are known: nine with spiro-indan-type structure (e.g., cannabispiran, isocannabispiran), nine dihydrostilbenes (e.g., cannabistilbene-

Table 3
Spermidine Alkaloids

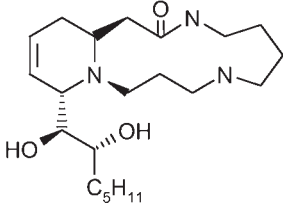
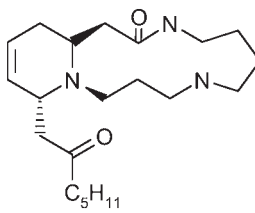
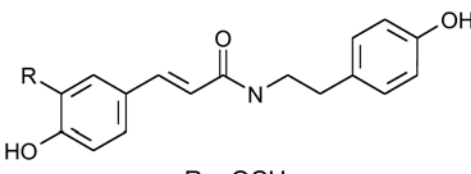
Compound	Structure
Cannabisativine	
Anhydrocannabisativine	

Table 4
Amides

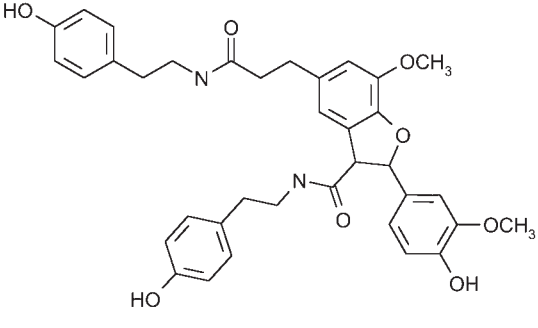
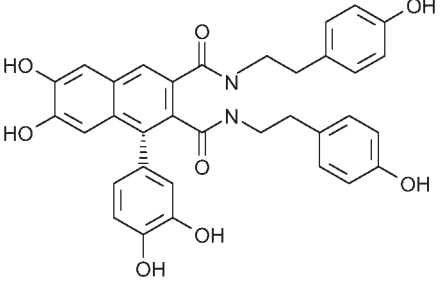
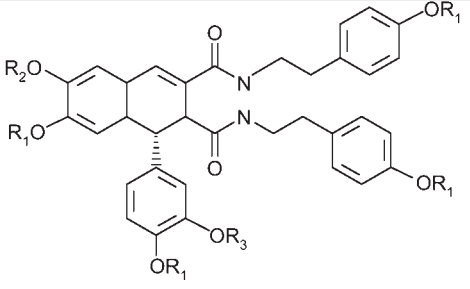
Compound	Structure
<i>N-trans</i> -Feruloyltyramine	 R = OCH ₃
<i>N-p</i> -Coumaroyltyramine	R = H
<i>N-trans</i> -Caffeoyltyramine	R = OH

I, -II), three dihydrophenanthrenes (e.g., cannithrene-1, -2), and six phenols, phenol methylethers, and phenolic glycosides (phloroglucinol glucoside; *see* Table 8).

1.2.8. Simple Alcohols, Aldehydes, Ketones, Acids, Esters, and Lactones

Seven alcohols (e.g., methanol, ethanol, 1-octene-3-ol), 12 aldehydes (e.g., acetaldehyde, isobutyraldehyde, pentanal), 13 ketones (e.g., acetone, heptanone-2, 2-methyl-2-heptene-6-one), and 21 acids (e.g., arabinic acid, azealic acid, gluconic acid) have been identified.

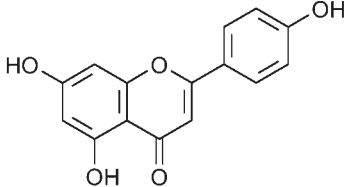
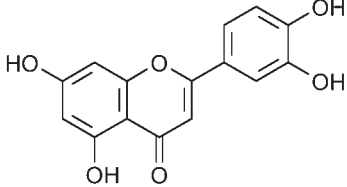
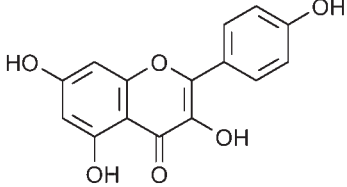
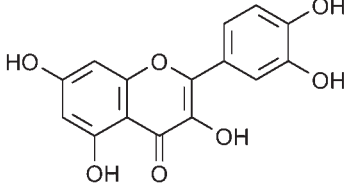
Table 5
Lignanamide Derivatives

Compound	Structure
Grossamide	
Cannabisin-A	
Cannabisin-B	 <p style="text-align: center;">$R_1 = R_2 = R_3 = H$</p>
Cannabisin-C	$R_1 = R_3 = H, R_2 = CH_3$
Cannabisin-D	$R_1 = H, R_2 = R_3 = CH_3$

1.2.9. Other

Among the 11 phytosterols known are campesterol, ergosterol, β -sitosterol, and stigmasterol. Vitamin K is the only vitamin found in *Cannabis*, whereas carotene and xanthophylls are reported pigments. Eighteen elements were detected (e.g., Na, K, Ca, Mg, Fe, Cu, Mn, Zn, Hg).

Table 6
C- and O-Glycosides Forming Flavonoid Aglycones and C-Glycosides

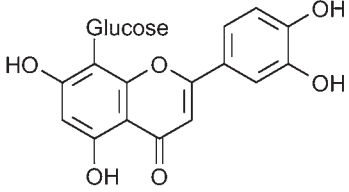
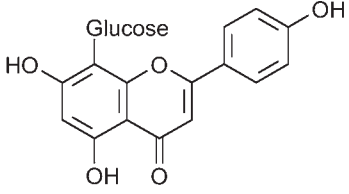
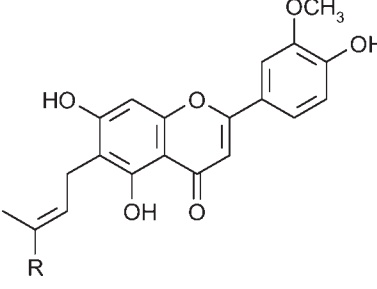
Compound	Structure
Apigenin	
Luteolin	
Kaempferol	
Quercetin	

(continued)

1.3. Pharmacological Characteristics of Cannabinoids and Other Cannabis Constituents

THC is the pharmacologically and toxicologically most relevant and best studied constituent of the *Cannabis* plant, responsible for most of the effects of natural *Cannabis* preparations (42). (A MEDLINE search covering the period 1993–2003 and using the keywords “tetrahydrocannabinol” and “pharmacology” produced about 1000 citations.) THC mainly acts through binding to the CB-1 receptor (see Chapter 6). The natural (-)-*trans* isomer of THC is 6- to 100-fold more potent than the (+)-*trans* isomer. A review of the pharmacology, toxicology, and therapeutic potential of *Cannabis*, cannabinoids, and other *Cannabis* constituents is given in refs. 43–53. It is claimed that *Cannabis* as a polypharmaceutical herb may provide two advantages over




Table 6 (continued)

Compound	Structure
Orientin	
Vitexin	
Cannflavin A	 <p data-bbox="779 1081 1048 1120">$R = H_2C-CH=C-(CH_3)_2$</p>
Cannflavin B	<p data-bbox="860 1149 967 1178">$R = CH_3$</p>

single-ingredient synthetic drugs: (1) the therapeutic effects of the primary active *Cannabis* constituents may be synergized by other compounds, and (2) the side effects of the primary constituents may be mitigated by other compounds (34). Thus, *Cannabis* has been characterized as a “synergistic shotgun,” in contrast, for example, to dronabinol (synthetic THC, Marinol®), a single-ingredient “silver bullet” (54). A recent study compared the subjective effects of orally administered and smoked THC alone and THC within *Cannabis* preparations (brownies, cigarettes; refs. 55 and 56). THC and *Cannabis* in both application forms produced similar, dose-dependent subjective effects, and there were few reliable differences between the THC-only and whole-plant conditions.

CBD is the next-best phytocannabinoid after THC. An overview of the pharmacology and clinical relevance of CBD can be found in refs. 34, 57, and 58. Of clinical relevance could be its reported ability to reduce anxiety and the other unpleasant psychological side effects of THC. Among the underlying mechanisms is the potent inhibition of the cytochrome P450 3A11, which biotransforms THC to the fourfold more psychoactive 11-hydroxy-THC (59).

Table 7
Unsaturated Fatty Acids From *Cannabis* Seed Oil

Compound	Structure
Linoleic acid	
alpha-Linolenic acid	
Oleic acid	

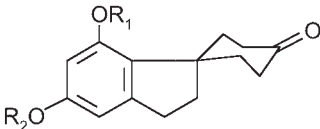
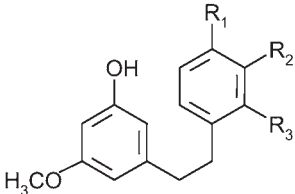
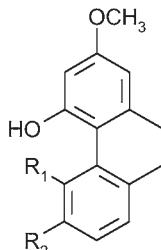
It has been suggested that the terpenoid constituents of *Cannabis* modulate THC activity, for example, by binding to cannabinoid receptors, modulating the THC receptor affinity, or altering its pharmacokinetics (e.g., by changing the blood–brain barrier; ref. 60). Whereas the anti-inflammatory and antibiotic activity of *Cannabis* terpenoids is known and has been used therapeutically for a long time, the serotonergic effect at 5-HT_{1A} and 5-HT_{2A} receptors of the essential oil, which could explain *Cannabis*-mediated analgesia and mood alteration, has only recently been demonstrated (61). β -Myrcene, the most abundant monoterpene in *Cannabis*, has analgesic, anti-inflammatory, antibiotic, and antimutagenic properties (34). β -Caryophyllene, the most common sesquiterpene, exhibits anti-inflammatory, cytoprotective (gastric mucosa), and antimalarial activity. The pharmacological effects of other *Cannabis* terpenes are discussed by McPartland and Russo (34).

Apigenin, a flavonoid found in nearly all vascular plants, exerts a wide range of biological effects, including many properties shared by terpenoids and cannabinoids. It selectively binds with high affinity to benzodiazepine receptors, thus explaining its anxiolytic activity (62). The pharmacology of other *Cannabis* flavonoids is reviewed in ref. 34.

2. ANALYSIS OF PHYTOCANNABINOIDS

Instrumental methods are most often used for the identification, classification (e.g., fiber type, drug type), and individualization (e.g., source tracing) of *Cannabis* plants and products. Because of the complex chemistry of *Cannabis*, separation techniques, such as GC or liquid chromatography, often coupled with MS, are necessary for the acquisition of the typical chemical profiles and the sensitive, specific, qualitative, and/or quantitative (e.g., THC potency) determination of *Cannabis* constituents. However, especially for screening purposes and on-site field testing, noninstrumental techniques like thin-layer chromatography (TLC) and color reactions are helpful, too.

Table 8
Noncannabinoid Phenols

Compound	Structure
Cannabispiran	 <p>$R_1 = H, R_2 = CH_3$</p>
Isocannabispiran	$R_1 = CH_3, R_2 = H$
Cannabistilbene-I	 <p>$R_1 = OH, R_2 = \text{isoprenyl}, R_3 = H$</p>
Cannabistilbene-II	<p>$R_1 = OCH_3, R_2 = OH, R_3 = OCH_3$</p> <p>or</p> <p>$R_1 = OCH_3, R_2 = OCH_3, R_3 = OH$</p>
Cannithrene-1	 <p>$R_1 = H, R_2 = OH$</p>
Cannithrene-2	$R_1 = OH, R_2 = OCH_3$

2.1. Microscopy

Identifying a plant sample as *Cannabis sativa* L. is the first step. 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 (22,63–69). 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 *Cannabis* products (not liquid *Cannabis*, hashish oil). Sometimes microscopic evidence is still available in smoked *Cannabis* residues.

2.2. Color Reactions

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. It is mandatory for the laboratory to confirm such results by the use of an alternative technique, which should be based on MS (70). The most common color spot tests include those developed by Duquenois and its modifications (70–74). A study of 270 different plant species and 200 organic compounds has shown that the Duquenois–Levine modification is most specific (71). The fast blue B salt test is the most common color reaction for the visualization of TLC patterns but may also be used as spot test on a filter paper (70).

2.3. Chromatographic Techniques

2.3.1. Thin-Layer Chromatography

One- and two-dimensional TLC is suited for the acquisition of qualitative cannabinoid profiles from plant material (70,73,75,76). Fast blue salt B or BB are used for visualization and result in characteristically colored spot patterns (68). 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 (77–79).

2.3.2. Gas Chromatography, Gas Chromatography/Mass Spectrometry

GC with flame ionization or MS detection is now the best established method for the analysis of *Cannabis* and its products (25,32,70,77,80–92). Derivatization is necessary (e.g., silylation or methylation) when information about cannabinoid acids, the dominating cannabinoids in the plant (see Section 1.1.), is required. The total cannabinoid content, i.e., the amount of neutral cannabinoids plus the neutral cannabinoids formed by decarboxylation of the acidic cannabinoids, is determined when the GC analysis is performed without derivatization (89). 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 (see Chapter 3; refs. 21 and 87).

2.3.3. High-Performance Liquid Chromatography

High-performance liquid chromatography makes possible the simultaneous determination of neutral and acidic phytocannabinoids without derivatization. Reversed-phase columns and preferably solvent programmed gradient systems are required for the separation of major and minor cannabinoids and their corresponding acids, e.g.,

for chemotyping (CBD-, THC, CBD/THC-type etc.), estimating the age (ratio acidic/neutral cannabinoids) of *Cannabis*, studying the effect of manufacturing processes and storage conditions, batch comparison, or direct quantification of THC in aqueous herbal preparations (e.g., *Cannabis* tea) (81,82,93–98). Detection is usually performed by UV (70,80,87,98–101) and diode array photometers (93), as well as by fluorescence, electrochemically (102), and, recently, MS (103).

2.3.4. Other Techniques

The applicability of capillary electrochromatography with photodiode array UV detection for the analysis of phytocannabinoids has been demonstrated (104). Supercritical fluid chromatography coupled to atmospheric pressure chemical ionization/MS is characterized by shorter analysis times than GC or high-performance liquid chromatography and does not require derivatization (105).

2.4. DNA Testing

After a *Cannabis* sample has been identified and classified, it may become important to individualize the specimen for forensic and intelligence purposes (22). Tracing the source of origin can be performed on a chemical, e.g., by using chromatographic–spectroscopic profiles (see also Chapter 3) or a genetic base. For DNA profiling (22,106–110), the following techniques are used: randomly amplified polymorphic DNA (111), amplified fragment length polymorphism (112), short tandem repeats (113,114), inter-simple sequence repeats (115), internal transcribed spacer II (116), and microsatellite markers (117). An overview and description of the different DNA testing methods is given in ref. 22.

REFERENCES

1. Mechoulam, R. and Gaoni, Y. (1967) Recent advances in the chemistry of hashish. *Fortschr. Chem. Org. Naturst.* **25**, 175–213.
2. Ward, A. and Holmes, B. (1985) Nabilone. A preliminary review of its pharmacological properties and therapeutic use. *Drugs* **30**, 127–144.
3. Mechoulam, R., Lander, N., Breuer, A., and Zahalka, J. (1990) Synthesis of the individual, pharmacologically distinct, enantiomers of a tetrahydrocannabinol derivative. *Tetrahedron Asymmetry* **1**, 315–318.
4. Burstein, S. H., Audette, C. A., Breuer, A., et al. (1992) Synthetic nonpsychotropic cannabinoids with potent antiinflammatory, analgesic, and leukocyte antiadhesion activities. *J. Med. Chem.* **35**, 3135–3141.
5. Di Marzo, V. and Fontana, A. (1995) Anandamide, an endogenous cannabinomimetic eicosanoid: ‘killing two birds with one stone’. *Prostaglandins Leukot. Essent. Fatty Acids* **53**, 1–11.
6. Devane, W. A., Hanus, L., Breuer, A., et al. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.
7. Pate, D. (1999) Anandamide structure-activity relationships and mechanisms of action on intraocular pressure in the normotensive rabbit model, PhD thesis, University of Kuopio, Kuopio, Finland.
8. Turner, C. E., Elsohly, M. A., and Boeren, E. G. (1980) Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J. Nat. Prod.* **43**, 169–234.
9. Ross, S. A. and ElSohly, M. A. (1995) Constituents of *Cannabis sativa* L. XXVIII—A review of the natural constituents: 1980–1994. *Zagazig J. Pharm. Sci.* **4**, 1–10.

10. ElSohly, M. (2002) Chemical constituents of Cannabis, in *Cannabis and cannabinoids—Pharmacology, Toxicology and Therapeutic Potential* (Grotenhermen, F. and Russo, E., eds.), Haworth Press, New York, pp. 27–36.
11. Gaoni, Y. and Mechoulam, R. (1964) The structure and synthesis of cannabigerol, a new hashish constituent, in *Proc. Chem. Soc.*, London, p. 82.
12. Shoyama, Y., Yagi, M., Nishioka, I., and Yamauchi, T. (1975) Biosynthesis of cannabinoid acids. *Phytochemistry* **14**, 2189–2192.
13. Adams, R., Hunt, M., and Clark, J. (1940) Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp. I. *J. Am. Chem. Soc.* **62**, 196–199.
14. Mechoulam, R. and Shvo, Y. (1963) Hashish—I. The structure of cannabidiol. *Tetrahedron* **19**, 2073–2078.
15. Wollner, H., Matchett, J., Levine, J., and Loewe, S. (1942) Isolation of a physiologically active tetrahydrocannabinol from Cannabis sativa resin. *J. Am. Chem. Soc.* **64**, 26–29.
16. Gaoni, Y. and Mechoulam, R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **86**, 1646–1647.
17. Wood, T., Spivey, W., and Easterfield, T. (1896) XL. Charas. The resin of Indian hemp. *J. Chem. Soc.* **69**, 539.
18. Adams, R., Baker, B., and Wearn, R. (1940) Structure of cannabinol III. Synthesis of cannabinol, 1-hydroxy-3-n-amy-6,6,9-trimethyl-6-dibenzopyran. *J. Am. Chem. Soc.* **62**, 2204–2207.
19. ElSohly, M., Ross, S., Mehmedic, Z., Ararat, R., Yi, B., and Banahan, B.F., 3rd (2000) Potency trends of delta-9-THC and other cannabinoids in confiscated marijuana from 1980–1997. *J. Forens. Sci.* **45**, 24–30.
20. Brenneisen, R. (1986) The cannabinoid content in Cannabis products confiscated in Switzerland. *Arch. Kriminol.* **177**, 95–104.
21. Brenneisen, R. and Meyer, P. *Swiss Cannabis Profiling Project*, University of Bern and Swiss Federal Office of Public Health (unpublished data).
22. Miller Coyle, H., Palmbach, T., Juliano, N., Ladd, C., and Lee, H. C. (2003) An overview of DNA methods for the identification and individualization of marijuana. *Croat. Med. J.* **44**, 315–321.
23. ElSohly, M. (2003) Practical challenges to positive drug tests for marijuana. *Clin. Chem.* **49**, 1037–1038.
24. Leson, G., Pless, P., Grotenhermen, F., Kalant, H., and ElSohly, M. A. (2001) Evaluating the impact of hemp food consumption on workplace drug tests. *J. Anal. Toxicol.* **25**, 691–698.
25. Ross, S. A., Mehmedic, Z., Murphy, T. P., and Elsohly, M. A. (2000) GC-MS analysis of the total delta-9-THC content of both drug- and fiber-type cannabis seeds. *J. Anal. Toxicol.* **24**, 715–717.
26. Bosy, T. Z. and Cole, K. A. (2000) Consumption and quantitation of delta-9-tetrahydrocannabinol in commercially available hemp seed oil products. *J. Anal. Toxicol.* **24**, 562–6.
27. Mediavilla, V., Derungs, R., Känzig, A., and Mägert, A. (1997) Qualität von Hanfsamenöl aus der Schweiz. *Agrarforschung* **4**, 449–451.
28. Lehmann, T., Sager, F., and Brenneisen, R. (1997) Excretion of cannabinoids in urine after ingestion of cannabis seed oil. *J. Anal. Toxicol.* **21**, 373–375.
29. Mediavilla, V. and Steinemann, S. (1997) Essential oil of Cannabis sativa L. strains. *J. Int. Hemp Assoc.* **4**, 80–82.
30. Meier, C. and Mediavilla, V. (1998) Factors influencing the yield and the quality of hemp (Cannabis sativa L.) essential oil. *J. Int. Hemp Assoc.* **5**, 16–20.
31. Lehmann, T. (1995) Chemical profiling of Cannabis sativa L., PhD thesis, University of Bern, Dep. of Pharmaceutical Sciences, Bern, Switzerland.
32. Ross, S.A. and ElSohly, M. A. (1996) The volatile oil composition of fresh and air-dried buds of Cannabis sativa. *J. Nat. Prod.* **59**, 49–51.

33. Novak, J., Zitterl-Eglseer, K., Deans, S. G., and Franz, C. M. (2001) Essential oils of different cultivars of *Cannabis sativa* L. and their antimicrobial activity. *Flavour Fragr. J.* **16**, 259–262.
34. McPartland, J. M. and Russo, E. B. (2001) Cannabis and cannabis extracts: greater than the sum of their parts? *J. Cann. Therap.* **1**, 103–132.
35. Hendriks, H., Malingré, T. M., Batterman, S., and Bos, R. (1977) Alkanes of the essential oil of *Cannabis sativa*. *Phytochemistry* **16**, 719–721.
36. McPartland, J. and Mediavilla, V. (2002) Noncannabinoid components, in *Cannabis and Cannabinoids—Pharmacology, Toxicology, and Therapeutic Potential* (Grotenhermen, F., and Russo, E., eds.), Haworth Press, New York, pp. 401–409.
37. Vanhoenacker, G., Van Rompaey, P., De Keukeleire, D., and Sandra, P. (2002) Chemotaxonomic features associated with flavonoids of cannabinoid-free cannabis (*Cannabis sativa* subsp. *sativa* L.) in relation to hops (*Humulus lupulus* L.). *Nat. Prod. Lett.* **16**, 57–63.
38. Barrett, M. L., Scutt, A. M., and Evans, F. J. (1986) Cannflavin A and B, prenylated flavones from *Cannabis sativa* L. *Experientia* **42**, 452–453.
39. Barrett, M. L., Gordon, D., and Evans, F. J. (1985) Isolation from *Cannabis sativa* L. of cannflavin—a novel inhibitor of prostaglandin production. *Biochem. Pharmacol.* **34**, 2019–2024.
40. Leson, G., Pless, P., and Roulac, J. (1999) *Hemp Foods and Oils for Health*, Hemptech, Sebastopol, CA.
41. Ross, S., ElSohly, H., ElKashoury, E., and ElSohly, M. (1996) Fatty acids of cannabis seeds. *Phytochem. Anal.* **7**, 279–283.
42. Grotenhermen, F. (2002) Effects of Cannabis and the cannabinoids, in *Cannabis and Cannabinoids—Pharmacology, Toxicology, and Therapeutic Potential* (Grotenhermen, F. and Russo, E., eds.), Haworth Press, New York, pp. 55–65.
43. Grotenhermen, F. and Russo, E. (eds.) (2002) *Cannabis and Cannabinoids—Pharmacology, Toxicology, and Therapeutic Potential*, Haworth Press, New York, p. 439.
44. Iversen, L. (2003) Cannabis and the brain. *Brain* **126**, 1252–1270.
45. Croxford, J. L. (2003) Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs* **17**, 179–202.
46. Kumar, R., Chambers, W., and Pertwee, R. G. (2001) Pharmacological actions and therapeutic uses of cannabis and cannabinoids. *Anaesthesia* **56**, 1059–68.
47. Hirst, R. A., Lambert, D. G., and Notcutt, W. G. (1998) Pharmacology and potential therapeutic uses of cannabis. *Br. J. Anaesth.* **81**, 77–84.
48. Ashton, C. H. (1999) Adverse effects of cannabis and cannabinoids. *Br. J. Anaesth.* **83**, 637–49.
49. Williamson, E. M. and Evans, F. J. (2000) Cannabinoids in clinical practice. *Drugs* **60**, 1303–1314.
50. Campbell, F. A., Tramèr, M. R., Carroll, D., Reynolds, D. J., Moore, R. A., and McQuay, H. J. (2001) Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. *Br. Med. J.* **323**, 13–16.
51. Tramèr, M. R., Carroll, D., Campbell, F. A., Reynolds, D. J., Moore, R. A., and McQuay, H. J. (2001) Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. *Br. Med. J.* **323**, 16–21.
52. Walker, J. M. and Huang, S. M. (2002) Cannabinoid analgesia. *Pharmacol. Ther.* **95**, 127–135.
53. Voth, E. A. and Schwartz, R. H. (1997) Medicinal applications of delta-9-tetrahydrocannabinol and marijuana. *Ann. Int. Med.* **126**, 791–798.
54. McPartland, J. M. and Pruitt, P. L. (1999) Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. *Altern. Ther.* **5**, 57–62.

55. Wachtel, S. R., ElSohly, M. A., Ross, S. A., Ambre, J., and de Wit, H. (2002) Comparison of the subjective effects of delta-9-tetrahydrocannabinol and marijuana in humans. *Psychopharmacology* **161**, 331–339.
56. Hart, C. L., Ward, A. S., Haney, M., Comer, S. D., Foltin, R. W., and Fischman, M. W. (2002) Comparison of smoked marijuana and oral Delta(9)-tetrahydrocannabinol in humans. *Psychopharmacology* **164**, 407–415.
57. Mechoulam, R., Parker, L. A., and Gallily, R. (2002) Cannabidiol: an overview of some pharmacological aspects. *J. Clin. Pharmacol.* **42**, 11S–19S.
58. Consroe, P. (1998) Brain cannabinoid systems as targets for the therapy of neurological disorders. *Neurobiol. Dis.* **5**, 534–551.
59. Bornheim, L. M., Kim, K. Y., Li, J., Perotti, B. Y., and Benet, L. Z. (1995) Effect of cannabidiol pretreatment on the kinetics of tetrahydrocannabinol metabolites in mouse brain. *Drug Metab. Dispos.* **23**, 825–831.
60. Meschler, J. P. and Howlett, A. C. (1999) Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. *Pharmacol. Biochem. Behav.* **62**, 473–480.
61. Russo, E. (2001) Hemp for headache: an in-depth historical and scientific review of cannabis in migraine treatment. *J. Cann. Ther.* **1**, 21–92.
62. Salgueiro, J. B., Ardenghi, P., Dias, M., Ferreira, M. B., Izquierdo, I., and Medina, J. H. (1997) Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats. *Pharmacol. Biochem. Behav.* **58**, 887–891.
63. Mitosinka, G. T., Thornton, J. I., and Hayes, T. L. (1972) The examination of cystolithic hairs of Cannabis and other plants by means of the scanning electron microscope. *J. Forens. Sci. Soc.* **12**, 521–529.
64. Thornton, J. I. and Nakamura, G. R. (1972) The identification of marijuana. *J. Forens. Sci. Soc.* **12**, 461–519.
65. Gigliano, G. (2001) Cannabis sativa L.—botanical problems and molecular approaches in forensic investigations. *Forens. Sci. Rev.* **13**, 2–17.
66. Stearn, W. T. (1970) The Cannabis plant: botanical characteristics, in *The Botany & Chemistry of Cannabis* (Joyce, C. and Curry, S., eds.), J. & A. Churchill, London, p. 1.
67. Nordal, A. (1970) Microscopic detection of Cannabis in the pure state and in semi-combusted residues, in *The Botany & Chemistry of Cannabis* (Joyce, C. and Curry, S., eds.), J. & A. Churchill, London, pp. 61–68.
68. Petri, G., Oroszlan, P., and Fridvalszky, L. (1988) Histochemical detection of hemp trichomes and their correlation with the THC content. *Acta Biol. Hung.* **39**, 59–73.
69. Bruni, A., Barni Comparini, I., and Menziani Andreoli, E. (1983) A histofluorescent procedure for identifying marijuana cannabinoids. *Experientia* **39**, 886–888.
70. United Nations (1987) *Recommended Methods for Testing Cannabis*, ST/NAR/8, Division of Narcotic Drugs, United Nations, New York.
71. Bailey, K. (1979) The value of the Duquenois test for cannabis—a survey. *J. Forens. Sci.* **24**, 817–841.
72. Butler, W. (1962) Duquenois-Levine test for marijuana. *J. Assoc. Off. Anal. Chem.* **45**, 597–600.
73. Tewari, S. N. and Sharma, J. D. (1982) Spot tests for cannabis materials. *Bull. Narc.* **34**, 109–112.
74. Pitt, C. G., Hendron, R. W., and Hsia, R. S. (1972) The specificity of the Duquenois color test for marihuana and hashish. *J. Forens. Sci.* **17**, 693–700.
75. Mali, B. D. and Parulekar, P. P. (1988) Diazotized dapsone as a reagent for the detection of cannabinoids on thin-layer chromatographic plates. *J. Chromatogr.* **457**, 383–386.

76. Baker, P. B., Gough, T. A., and Taylor, B. J. (1980) Illicitly imported Cannabis products: some physical and chemical features indicative of their origin. *Bull. Narc.* **32**, 31–40.
77. Debruyne, D., Albessard, F., Bigot, M. C., and Moulin, M. (1994) Comparison of three advanced chromatographic techniques for cannabis identification. *Bull. Narc.* **46**, 109–121.
78. Pothier, J., Galand, N., and Viel, C. (1992) Rapid characterization of stupeficient and toxic substances by pressurized thin-layer chromatography. *J. Toxicol. Clin. Exp.* **12**, 495–501.
79. Oroszlan, P., Verzar-Petri, G., Mincsovcics, E., and Szekeley, T. (1987) Separation, quantitation and isolation of cannabinoids from Cannabis sativa L. by overpressured layer chromatography. *J. Chromatogr.* **388**, 217–224.
80. Ferioli, V., Rustichelli, C., Pavesi, G., and Gamberini, G. (2000) Analytical characterisation of hashish samples. *Chromatographia* **52**, 39–44.
81. Debruyne, D., Moulin, M., Bigot, M. C., and Camsonne, R. (1981) Identification and differentiation of resinous cannabis and textile cannabis: combined use of HPLC and high-resolution GLC. *Bull. Narc.* **33**, 49–58.
82. Tsatsakis, A. M., Tutudaki, M., Stiakakis, I., Dimopoulou, M., Tzatzarakis, M., and Michalodimitrakis, M. (2000) Characterisation of cannabis plants phenotypes from illegal cultivations in Crete. *Boll. Chim. Farm.* **139**, 140–145.
83. Ross, S. A. and ElSohly, M. A. (1996) The volatile oil composition of fresh and air-dried buds of Cannabis sativa. *J. Nat. Prod.* **59**, 49–51.
84. Barni Comparini, I. and Centini, F. (1983) Packed column chromatography, high-resolution gas-chromatography and high pressure liquid chromatography in comparison for the analysis of cannabis constituents. *Forens. Sci. Int.* **21**, 129–137.
85. Harvey, D. J. (1990) Stability of cannabinoids in dried samples of cannabis dating from around 1896–1905. *J. Ethnopharmacol.* **28**, 117–128.
86. Turner, C. E., Bouwsma, O. J., Billets, S., and Elsohly, M. A. (1980) Constituents of Cannabis sativa L. XVIII—Electron voltage selected ion monitoring study of cannabinoids. *Biomed. Mass Spectrom.* **7**, 247–256.
87. Brenneisen, R. and ElSohly, M. A. (1988) Chromatographic and spectroscopic profiles of Cannabis of different origins: Part I. *J. Forens. Sci.* **33**, 1385–1404.
88. Bosy, T. Z. and Cole, K. A. (2000) Consumption and quantitation of delta-9-tetrahydrocannabinol in commercially available hemp seed oil products. *J. Anal. Toxicol.* **24**, 562–566.
89. Lercker, G., Bocci, F., Frega, N., and Bortolomeazzi, R. (1992) Cannabinoid acids analysis. *Farmaco* **47**, 367–378.
90. Vree, T. B. (1977) Mass spectrometry of cannabinoids. *J. Pharm. Sci.* **66**, 1444–1450.
91. Novotny, M., Lee, M. L., Low, C. E., and Raymond, A. (1976) Analysis of marijuana samples from different origins by high-resolution gas-liquid chromatography for forensic application. *Anal. Chem.* **48**, 24–29.
92. Raharjo, T. J. and Verpoorte, R. (2004) Methods for the analysis of cannabinoids in biological materials: a review. *Phytochem. Anal.* **15**, 79–94.
93. Lehmann, T. and Brenneisen, R. (1995) High performance liquid chromatographic profiling of Cannabis products. *J. Liq. Chromatogr.* **18**, 689–700.
94. Barni Comparini, I. and Centini, F. (1983) Packed column chromatography, high-resolution gas-chromatography and high pressure liquid chromatography in comparison for the analysis of cannabis constituents. *Forens. Sci. Int.* **21**, 129–37.
95. Zoller, O., Rhyh, P., and Zimmerli, B. (2000) High-performance liquid chromatographic determination of delta9-tetrahydrocannabinol and the corresponding acid in hemp containing foods with special regard to the fluorescence properties of delta9-tetrahydrocannabinol. *J. Chromatogr. A* **872**, 101–110.

96. Baker, P. B., Gough, T. A., and Wagstaffe, P. J. (1983) Determination of the distribution of cannabinoids in cannabis resin from Morocco using high-performance liquid chromatography. Part II. *J. Anal. Toxicol.* **7**, 7–10.
97. Baker, P. B., Taylor, B. J., and Gough, T. A. (1981) The tetrahydrocannabinol and tetrahydrocannabinolic acid content of cannabis products. *J. Pharm. Pharmacol.* **33**, 369–372.
98. McDonald, P. A. and Gough, T. A. (1984) Determination of the distribution of cannabinoids in cannabis resin from the Lebanon using HPLC. Part III. *J. Chromatogr. Sci.* **22**, 282–284.
99. Brenneisen, R. (1984) Psychotropic drugs. II. Determination of cannabinoids in Cannabis sativa L. and in cannabis products with high pressure liquid chromatography (HPLC). *Pharm. Acta Helv.* **59**, 247–259.
100. Rustichelli, C., Ferioli, V., Baraldi, M., Zanolì, P., and Gamberini, G. (1998) Analysis of cannabinoids in fiber hemp plant varieties (Cannabis sativa) by high-performance liquid chromatography. *Chromatographia* **48**, 215–222.
101. Baker, P. B., Fowler, R., Bagon, K. R., and Gough, T. A. (1980) Determination of the distribution of cannabinoids in cannabis resin using high performance liquid chromatography. *J. Anal. Toxicol.* **4**, 145–152.
102. Nakahara, Y. and Tanaka, K. (1988) Studies on discrimination of confiscated cannabis products by high performance liquid chromatography with electrochemical detector. *Eisei Shikenjo Hokoku*, Bulletin of National Institute of Hygienic Sciences, pp.11–18.
103. Rustichelli, C., Ferioli, V., Vezzalini, F., Rossi, M. C., and Gamberini, G. (1996) Simultaneous separation and identification of hashish constituents by coupled liquid chromatography-mass spectrometry (HPLC-MS). *Chromatographia* **43**, 129–134.
104. Lurie, I. S., Meyers, R. P., and Conver, T. S. (1998) Capillary electrochromatography of cannabinoids. *Anal. Chem.* **70**, 3255–3260.
105. Backstrom, B., Cole, M. D., Carrott, M. J., Jones, D. C., Davidson, G., and Coleman, K. (1997) A preliminary study of the analysis of Cannabis by supercritical fluid chromatography with atmospheric pressure chemical ionisation mass spectroscopic detection. *Sci. Justice* **37**, 91–97.
106. Miller Coyle, H., Ladd, C., Palmbach, T., and Lee, H. C. (2001) The green revolution: botanical contributions to forensics and drug enforcement. *Croat. Med. J.* **42**, 340–345.
107. Miller Coyle, H., Palmbach, T., Juliano, N., Ladd, C., and Lee, H. C. (2003) An overview of DNA methods for the identification and individualization of marijuana. *Croat. Med. J.* **44**, 315–321.
108. Cole, M. D. and Linacre, A. M. T. (2002) The identification of controlled plant drugs using phytochemistry and DNA. *Curr. Topics Phytochem.* **5**, 129–140.
109. Linacre, A. and Thorpe, J. (1998) Detection and identification of cannabis by DNA. *Forens. Sci. Int.* **91**, 71–76.
110. Siniscalco Gigliano, G., Caputo, P., and Cozzolino, S. (1997) Ribosomal DNA analysis as a tool for the identification of Cannabis sativa L. specimens of forensic interest. *Sci. Justice* **37**, 171–174.
111. Gillan, R., Cole, M., Linacre, A., Thorpe, J. W., and Watson, N. D. (1995) Comparison of Cannabis sativa by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoids: a preliminary study. *Sci. Justice* **35**, 169–177.
112. Miller Coyle, H., Sutler, G., Abrams, S., et al. (2003) A simple DNA extraction method for Marijuana samples used in amplified fragment length polymorphism (AFLP) analysis. *J. Forens. Sci.* **48**, 343–347.
113. Hsieh, H. M., Hou, R. J., Tsai, L. C., et al. (2003) A highly polymorphic STR locus in Cannabis sativa. *Forens. Sci. Int.* **131**, 53–58.

114. Gilmore, S., Peakall, R., and Robertson, J. (2003) Short tandem repeat (STR) DNA markers are hypervariable and informative in *Cannabis sativa*: implications for forensic investigations. *Forens. Sci. Int.* **131**, 65–74.
115. Kojoma, M., Iida, O., Makino, Y., Sekita, S., and Satake, M. (2002) DNA fingerprinting of *Cannabis sativa* using inter-simple sequence repeat (ISSR) amplification. *Planta Med.* **68**, 60–63.
116. Gigliano, G. (1998) Identification of *Cannabis sativa* L. (Cannabaceae) using restriction profiles of the Internal Transcribed Spacer II (ITS2). *Sci. Justice* **38**, 225–230.
117. Alghanim, H. J. and Almirall, J. R. (2003) Development of microsatellite markers in *Cannabis sativa* for DNA typing and genetic relatedness analyses. *Anal. Bioanal. Chem.* **376**, 1225–1233.