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 C_{21} 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).

From: *Forensic Science and Medicine: Marijuana and the Cannabinoids*
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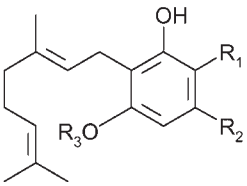
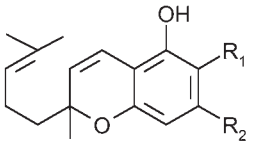
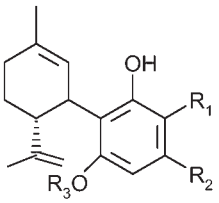
Table 1
Cannabinoids

Compound	Structure	Main pharmacological characteristics
Cannabigerol class		
Cannabigerolic acid (CBGA)	 R_1 = COOH, R_2 = C_5H_{11}, R_3 = H	Antibiotic
Cannabigerolic acid monomethylether (CBGAM)	R_1 = COOH, R_2 = C_5H_{11}, R_3 = CH_3	
Cannabigerol (CBG)	R_1 = H, R_2 = C_5H_{11}, R_3 = H	Antibiotic Antifungal Anti-inflammatory Analgesic
Cannabigerol monomethylether (CBGM)	R_1 = H, R_2 = C_5H_{11}, R_3 = CH_3	
Cannabigerovarinic acid (CBGVA)	R_1 = COOH, R_2 = C_3H_7, R_3 = H	
Cannabigerovarin (CBGV)	R_1 = H, R_2 = C_3H_7, R_3 = 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)***

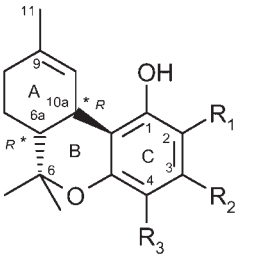
Compound	Structure	Main pharmacological characteristics
Cannabichromene class		
Cannabichromenic acid (CBCA)	 R_1 = COOH, R_2 = C_5H_{11}	
Cannabichromene (CBC)	R_1 = H, R_2 = C_5H_{11}	Anti-inflammatory Antibiotic Antifungal Analgesic
Cannabichromevarinic acid (CBCVA)	R_1 = COOH, R_2 = C_3H_7	
Cannabichromevarin (CBCV)	R_1 = H, R_2 = C_3H_7	
Cannabidiol class		
Cannabidiolic acid (CBDA)	 R_1 = COOH, R_2 = C_5H_{11}, R_3 = H	Antibiotic
Cannabidiol (CBD)	R_1 = H, R_2 = C_5H_{11}, R_3 = 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	
Cannabidiorcol (CBD-C_1)	R_1 = H, R_2 = CH_3, R_3 = H	
Delta-9-tetrahydrocannabinol class		
Delta-9-tetrahydrocannabinolic acid A (THCA-A)	 R_1 = COOH, R_2 = C_5H_{11}, R_3 = H	
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)*.

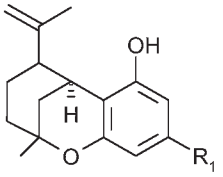
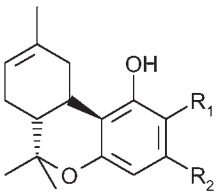
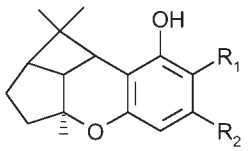
Table 1 ***(continued)***

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.

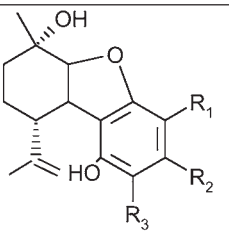
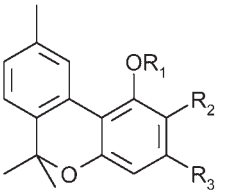
Table 1 ***(continued)***

Compound	Structure	Main pharmacological characteristics
Delta-7-*cis*-iso-tetrahydrocannabivarin	 $R_1 = C_3H_7$	
Delta-8-tetrahydrocannabinol class		
Delta-8-tetrahydrocannabinolic acid (Δ^8-THCA)	 $R_1 = COOH$, $R_2 = C_5H_{11}$	
Delta-8-tetrahydrocannabinol (Δ^8-THC)	$R_1 = H$, $R_2 = C_5H_{11}$	Similar to THC (less potent)
Cannabicyclol class		
Cannabicyclolic acid (CBLA)	 $R_1 = COOH$, $R_2 = C_5H_{11}$	
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.

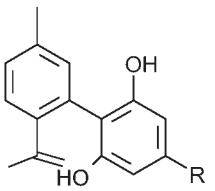
Table 1 *(continued)*

Compound	Structure	Main pharmacological characteristics
Cannabielsoin class		
Cannabielsoic acid A (CBEA-A)	 R_1 = COOH, R_2 = C_5H_{11}, R_3 = H	
Cannabielsoic acid B (CBEA-B)	R_1 = H, R_2 = C_5H_{11}, R_3 = COOH	
Cannabielsoin (CBE)	R_1 = H, R_2 = C_5H_{11}, R_3 = H	
Cannabinol and cannabinodiol class		
Cannabinolic acid (CBNA)	 R_1 = H, R_2 = COOH, R_3 = C_5H_{11}	
Cannabinol (CBN)	R_1 = H, R_2 = H, R_3 = C_5H_{11}	Sedative Antibiotic Anticonvulsant Anti-inflammatory

(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)*.

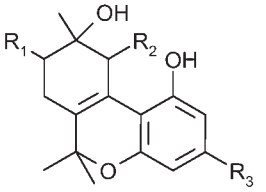
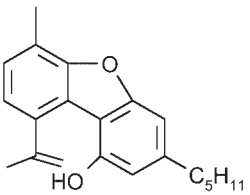
Table 1 ***(continued)***

Compound	Structure	Main pharmacological characteristics
Cannabinol methylether (CBNM)	$R_1 = CH_3$, $R_2 = H$, $R_3 = C_5H_{11}$	
Cannabinol-C_4 (CBN-C_4)	$R_1 = H$, $R_2 = H$, $R_3 = C_4H_9$	
Cannabivarin (CBV)	$R_1 = H$, $R_2 = H$, $R_3 = C_3H_7$	
Cannabinol-C_2 (CBN-C_2)	$R_1 = H$, $R_2 = H$, $R_3 = C_2H_5$	
Cannabiorcol (CBN-C_1)	$R_1 = H$, $R_2 = H$, $R_3 = CH_3$	
Cannabinodiol (CBND)	 $R = C_5H_{11}$	
Cannabinodivarin (CBVD)	$R = C_3H_7$	

(continued)

9. Cannabitriol (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 tetrahydrocannabitriol 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.

Table 1 *(continued)*

Compound	Structure	Main pharmacological characteristics
Cannabitriol class		
Cannabitriol (CBT)	 R_1 = H, R_2 = OH, R_3 = C_5H_{11}	
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}	
Cannabitriolvarin (CBTV)	R_1 = H, R_2 = OH, R_3 = C_3H_7	
Ethoxy-cannabitriolvarin (CBTVE)	R_1 = H, R_2 = OC_2H_5, R_3 = C_3H_7	
Miscellaneous cannabinoids class		
Dehydrocannabifuran (DCBF)		

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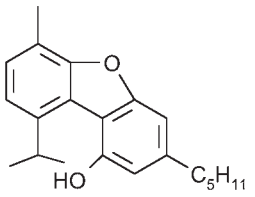
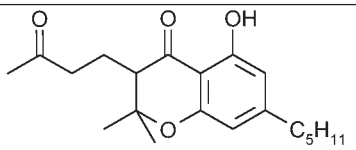
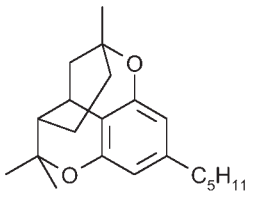
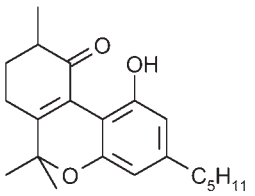
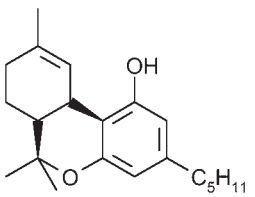
1.1.3. THC Potency Trends

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

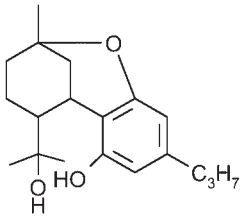
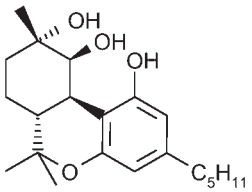
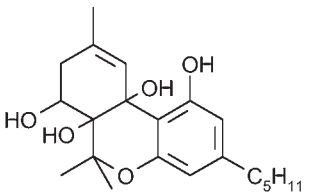
Table 1 ***(continued)***

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

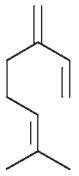
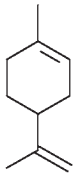
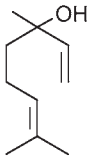
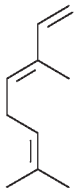
The typical scent of *Cannabis* results from about 140 different terpenoids. Isoprene units (C_5H_8) form monoterpenoids (C_{10} skeleton), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), and triterpenoids (C_{30}; *see* Table 2). Terpenoids may be acyclic, monocyclic, or polycyclic hydrocarbons with 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
trans-Ocimene	M			2.3–5.7

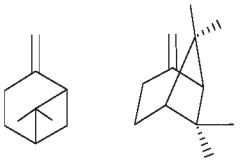
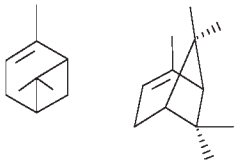
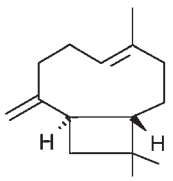
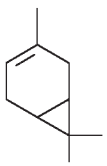
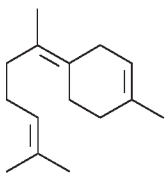
(continued)

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 quartenary 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	

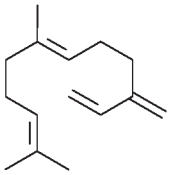
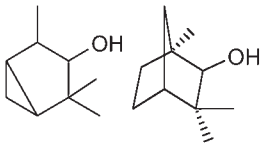
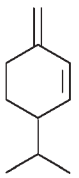
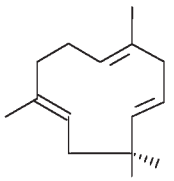
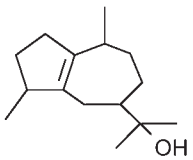
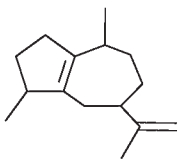
(continued)

zeatinnucleoside; the six enzymes are edestinase, glucosidase, polyphenoloxydase, 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 monosacharides (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*
trans-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	

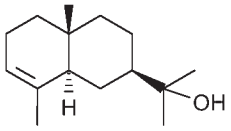
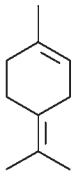
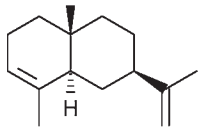
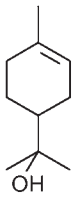
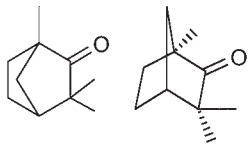
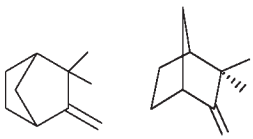
(continued)

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	

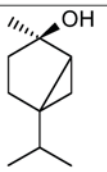
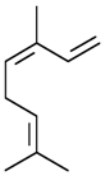
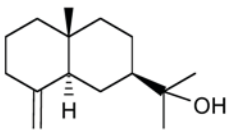
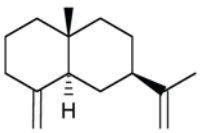
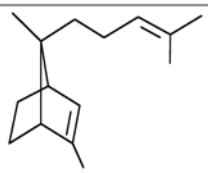
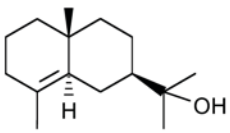
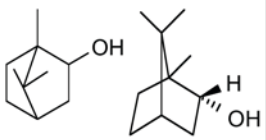
(continued)

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-

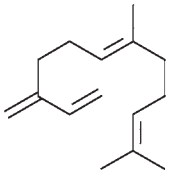
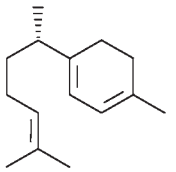
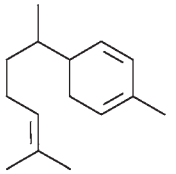
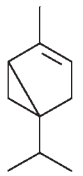
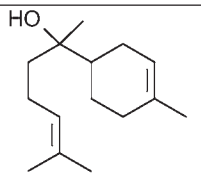
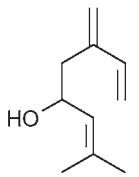
Table 2 *(continued)*

Compound	Class[a]	Structure	Percentage	
			Ref. *32*	Ref. *29*
cis-Sabinene hydrate	M		0.2–0.5	
cis-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-*trans*-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-

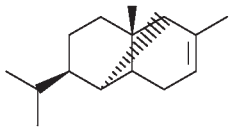
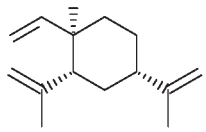
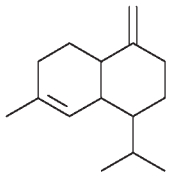
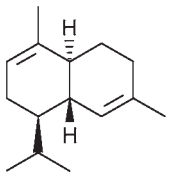
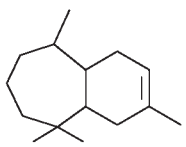
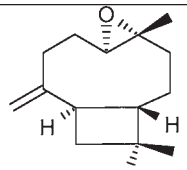
Table 2 *(continued)*

Compound	Class[a]	Structure	Percentage	
			Ref. *32*	Ref. *29*
cis-beta-Farnesene	S		0.1–0.3	0.6–0.9
gamma-Curcumene	S		0.1–0.3	
cis-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-*cis*-Bergamotene	S		traces–0.6	
gamma-Muurolene	S		traces–0.1	
alpha-Cadinene	S		traces–0.1	
alpha-Longipinene	S		traces–0.1	
Caryophyllene oxide	S		traces–0.8	

[a]M, 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

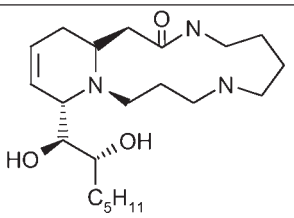
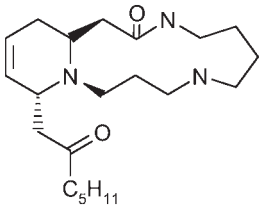
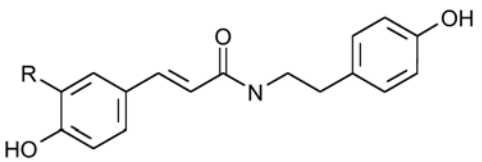
Compound	Structure
Cannabisativine	
Anhydrocannabisativine	

Table 4
Amides

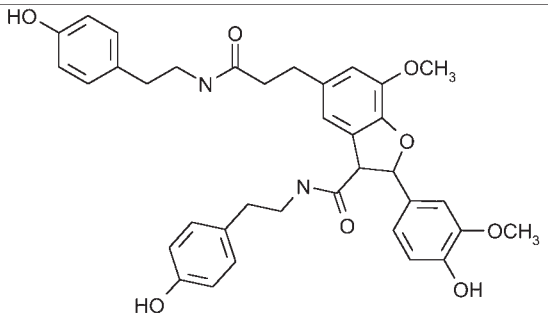
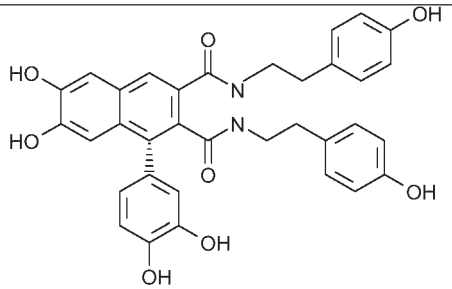
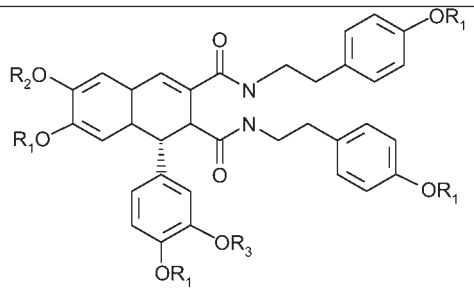
Compound	Structure
N-trans-Feruloyltyramine	 R = OCH_3
N-p-Coumaroyltyramine	R = H
N-trans-Caffeoyltyramine	R = OH

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.

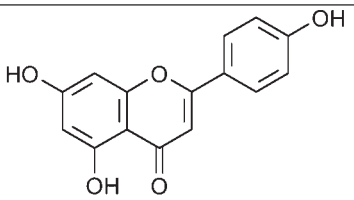
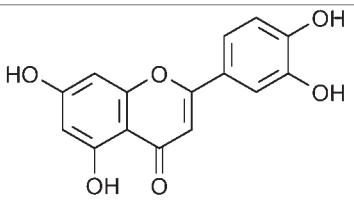
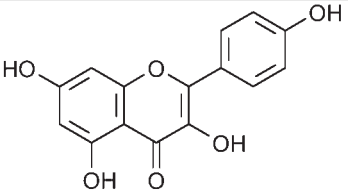
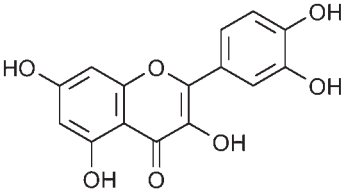
Table 5
Lignanamide Derivatives

Compound	Structure
Grossamide	
Cannabisin-A	
Cannabisin-B	 $R_1 = R_2 = R_3 = H$
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

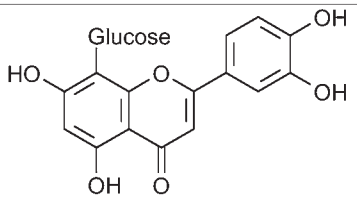
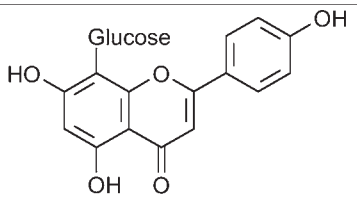
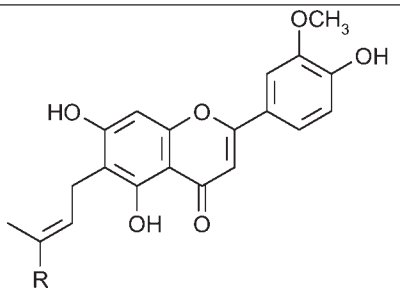
Compound	Structure
Apigenin	
Luteolin	
Kaempferol	
Quercetin	

(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

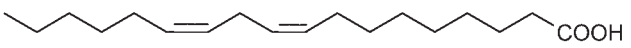
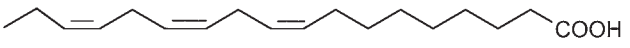
Table 6 *(continued)*

Compound	Structure
Orientin	
Vitexin	
Cannflavin A	 R = H_2C-CH=C-$(CH_3)_2$
Cannflavin B	R = CH_3

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	

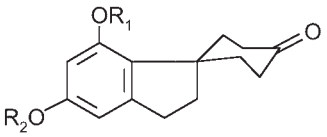
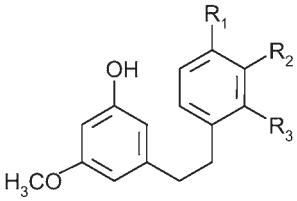
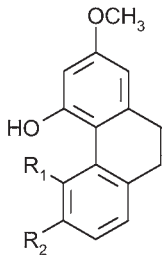
It has been suggested that the terpenoid constituents of *Cannabis* modulate THC activity, for example, by binding to cannabinoid receptors, modulating the THC receptor affinity, or altering its pharmacokinetics (e.g., by changing the blood–brain barrier; ref. *60*). Whereas the anti-inflammatory and 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, excerts 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	 R_1 = H, R_2 = CH_3
Isocannabispiran	R_1 = CH_3, R_2 = H
Cannabistilbene-I	 R_1 = OH, R_2 = isoprenyl, R_3 = H
Cannabistilbene-II	R_1 = OCH_3, R_2 = OH, R_3 = OCH_3 or R_1 = OCH_3, R_2 = OCH_3, R_3 = OH
Cannithrene-1	 R_1 = H, R_2 = OH
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 inflorescenses, 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), an 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*.

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