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Chemistry and Biological Activity of Tetrahydrocannabinol and its Derivatives

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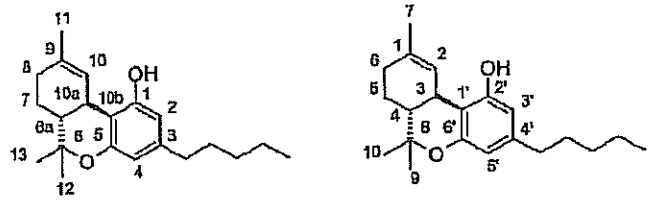
Abstract Cannabinoids and in particular the main psychoactive Δ9-THC are promising substances for the development of new drugs and are of high importance in biomedicine and pharmacy. This review gives an overview of the chemical properties of Δ9-THC, its synthesis on industrial scale, and the synthesis of important metabolites. The biosynthesis of cannabinoids in *Cannabis sativa* is extensively described in addition to strategies for optimization of this plant for cannabinoid employment in medicine. The metabolism of Δ9-THC in humans is shown and, based on this, analytical procedures for cannabinoids and their metabolites in human forensic samples as well as in *C. sativa* will be discussed. Furthermore, some aspects of medicinal indications for Δ9-THC and its ways of administration are described. Finally, some synthetic cannabinoids and their importance in research and medicine are delineated.

Keywords Tetryhydrocannabinol · *Cannabis sativa* · Analytical methods · Medicinal applications

1 Chemistry

1.1 Nomenclature

Natural cannabinoids are terpenophenolic compounds that are only biosynthesized in *Cannabis sativa* L., Cannabaceae. For these compounds five different systems of nomenclature are available, well described by Shulgin [1] and by ElSohly [2]. Two of these systems are mainly employed for the description of tetrahydrocannabinol in publications – the dibenzopyrane numbering system (1.1 in Fig. 1) and the terpene numbering system (1.2), based on *p*-cymene. Because of historical and geographical reasons, the missing standardization is not uniform and is the main reason for ongoing confusion in the literature, leading to discussions regarding the numbering and



(1.1) Dibenzopyrane numbering system

(1.2) Terpene numbering system

Fig. 1 Commonly used numbering systems for cannabinoids

its order. As an example, the use of the terpene numbering system gives the name Δ1-tetrahydrocannabinol; in contrast, using the dibenzopyrane numbering system leads to the name Δ9-tetrahydrocannabinol for the same compound. The dibenzopyrane numbering system, which stands in agreement with IUPAC rules, is commonly used in North America whereas the terpene numbering system, following the biochemical nature of these compounds, was originally developed in Europe [3]. According to IUPAC rules, the dibenzopyrane system is used despite the fact that this system has a general disadvantage because of a complete change in numbering after loss of the terpenoid ring, as found in many cannabinoids.

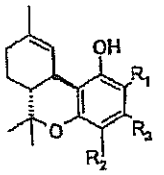
The chemical name of Δ9-THC according to the dibenzopyrane numbering system is 3-pentyl-6,6,9-trimethyl-6*a*,7,8,10*a*-tetrahydro-6*H*-dibenzo-[*b*, *d*]pyran-1-ol as depicted in 1.1 (Fig. 1).

Alternatively, Δ9-tetrahydrocannbinol or simply tetrahydrocannabinol is frequently used in the scientific community. When using the short name tetrahydrocannabinol or just THC it always implies the stereochemistry of the Δ9-isomer.

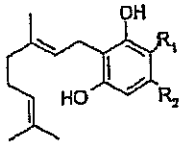
On the market are two drugs under the trade names of Dronabinol, which is the generic name of *trans*-Δ9-THC, and Marinol, which is a medicine containing synthetic dronabinol in sesame oil for oral intake, distributed by Unimed Pharmaceuticals.

1.2 Chemical and Physical Properties of Δ9-THC

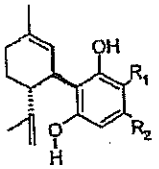
Δ9-THC (2.1 in Fig. 2) is the only major psychoactive constituent of *C. sativa*. It is a pale yellow resinous oil and is sticky at room temperature. Δ9-THC is lipophilic and poorly soluble in water (3 $\mu g\,mL^{-1}$), with a bitter taste but without smell. Furthermore it is sensitive to light and air [4]. Some more physical and chemical data on Δ9-THC are listed in Table 1. Because of its two chiral centers at C-6*a* and C-10*a*, four stereoisomers are known, but only (–)-*trans*-Δ9-THC is found in the *Cannabis* plant [5]. The absolute configuration of the



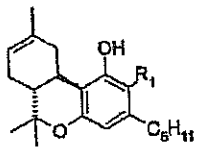
(2.1) R_1=H R_2=H R_3=C_5H_{11}
(2.2) R_1=COOH R_2=H R_3=C_5H_{11}
(2.3) R_1=H R_2=COOH R_3=C_5H_{11}
(2.4) R_1=H R_2=H R_3=C_3H_7
(2.5) R_1=H R_2=H R_3=C_4H_9



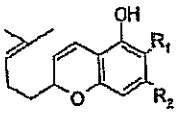
(2.6) R_1=H R_2=C_5H_{11}
(2.7) R_1=COOH R_2=C_5H_{11}
(2.8) R_1=H R_2=C_3H_7



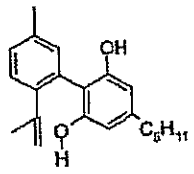
(2.9) R_1=H R_2=C_5H_{11}
(2.10) R_1=COOH R_2=C_5H_{11}
(2.11) R_1=H R_2=C_3H_7
(2.12) R_1=H R_2=C_4H_9



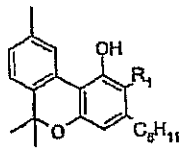
(2.13) R_1=COOH
(2.14) R_1=H



(2.15) R_1=H R_2=C_5H_{11}
(2.16) R_1=COOH R_2=C_5H_{11}
(2.17) R_1=H R_2=C_3H_7



(2.18)



(2.19)

Fig. 2 Chemical structures of some natural cannabinoids

natural product was determined as (6a*R*,10a*R*) [6]. Depending on the position of the double bond in the terpenoid ring six isomers are possible, whereof the Δ9-isomer and the Δ8-isomer are most important. Conformational studies of Δ9-THC using NMR techniques were done by Kriwacki and Makryiannis [7]. The authors found that the arrangement of the terpenoid ring and pyrane ring of this compound is similar to the half-opened wings of a butterfly. An excellent

Table 1 Chemical and physical properties of (–)-*trans*-Δ9-THC [4]

Molecular weight	314.47
Molecular formula	$C_{21}H_{30}O_2$
Boiling point	200 °C (at 0.02 mm Hg)
Rotation of polarized light	$[a]_D^{20} = -150.5°$ ($c = 0.53$ in $CHCl_3$)
UV maxima	275 nm and 282 nm (in ethanol)
Mass fragments (m/z)[a]	314 (M+); 299; 271; 258; 243; 231
pK_a	10.6
Stability	Not stable in acidic solution ($t_{1/2} = 1$ h at pH 1.0 and 55 °C)
Partition coefficient (octanol/water)[b]	12 091
Solubility	Highly insoluble in water (~ 2.8 mg L^{-1} at 23 °C)

[a] These mass fragments were found by our own measurements
[b] In the literature, values between 6000 and 9 440 000 can be found [102]

review by Mechoulam et al. has been published providing more information on this topic and discussing extensively the stereochemistry of cannabinoids and Δ9-THC, with special focus on the structure–activity relationship [8].

It must be noted that Δ9-THC is not present in *C. sativa*, but that the tetrahydrocannbinolic acid (THCA) is almost exclusively found. Two kinds of THCA are known. The first has its carboxylic function at position C-2 and is named 2-carboxy-Δ9-THC or THCA-A (2.2); the second has a carboxylic function at position C-4 and is named 4-carboxy-Δ9-THC or THCA-B (2.3).

THCA shows no psychotropic effects, but heating (e.g., by smoking of *Cannabis*) leads to decarboxylation, which provides the active substance Δ9-THC. Δ9-THC is naturally accompanied by its homologous compounds containing a propyl side chain (e.g., tetrahydrocannabivarin, THCV, THC-C_3, 2.4) or a butyl side chain (THC-C_4, 2.5).

1.3 Further Natural Cannabinoids

Seventy cannabinoids from *C. sativa* have been described up to 2005 [2]. Mostly they appear in low quantities, but some of them shall be mentioned in the following overview – especially because of their functions in the biosynthesis of Δ9-THC and their use in medicinal applications.

1.3.1 Cannabigerol (CBG)

Cannabigerol (CBG, 2.6) was historically the first identified cannabinoid [9]. It can be comprehended as a molecule of olivetol that is enhanced with

2,5-dimethylhepta-2,5-diene. In plants, its acidic form cannabigerolic acid (CBGA, 2.7) and also the acid forms of the other cannabinoids prevail. CBGA is the first cannabinoidic precursor in the biosynthesis of Δ9-THC, as discussed in Sect. 2. Although the *n*-pentyl side chain is predominant in natural cannabinoids, cannabigerols with propyl side chains (cannabigerovarin, CBGV, 2.8) are also present.

1.3.2 Cannabidiol (CBD)

The IUPAC name of cannabidiol is 2-[(1*S*, 6*R*)-3-methyl-6-prop-1-en-2-yl-1-cyclohex-2-enyl]-5-pentyl-benzene-1,3-diol. Cannabidiol (CBD, 2.9) in its acidic form cannabidiolic acid (CBDA, 2.10) is the second major cannabinoid in *C. sativa* besides Δ9-THC. As already mentioned for Δ9-THC, variations in the length of the side chain are also possible for CBD. Important in this context are the propyl side chain-substituted CBD, named cannabidivarin (CBDV, 2.11), and CBD-C_4 (2.12), the homologous compound with a butyl side chain. Related to the synthesis starting from CBD to Δ9-THC as described in Sect. 3.1, it was accepted that CBDA serves as a precursor for THCA in the biosynthesis. Recent publications indicate that CBDA and THCA are formed from the same precursor, cannabigerolic acid (CBGA), and that it is unlikely that the biosynthesis of THCA from CBDA takes place in *C. sativa*.

1.3.3 Δ8-Tetrahydrocannabinol (Δ8-THC)

This compound and its related acidic form, Δ8-tetrahydrocannabinolic acid (Δ8-THCA, 2.13) are structural isomers of Δ9-THC. Although it is the thermodynamically stable form of THC, Δ8-THC (2.14) contributes approximately only 1% to the total content of THC in *C. sativa*. In the synthetic production process, Δ8-THC is formed in significantly higher quantities than in plants.

1.3.4 Cannabichromene (CBC)

Among THCA and CBDA, cannabichromene (CBC, 2.15) and the acidic form cannabichromenic acid (CBCA, 2.16) are formed from their common precursor CBGA. Besides CBC, its homologous compound cannabiverol (CBCV, 2.17) with a propyl side chain is also present in plants.

1.3.5
Cannabinodiol (CBND) and Cannabinol (CBN)

Cannabinidiol (CBND, 2.18) and cannabinol (2.19) are oxidation products of CBD and Δ9-THC formed by aromatization of the terpenoid ring. For the dehydrogenation of THC a radical mechanism including polyhydroxylated intermediates is suggested [10, 11]. CBN is not the sole oxidation product of Δ9-THC. Our own studies at THC-Pharm on the stability of Δ9-THC have shown that only about 15% of lost Δ9-THC is recovered as CBN.

2
Biosynthesis of Cannabinoids

The biosynthesis of cannabinoids can only be found in *C. sativa*. These cannabinoids are praised for their medical and psychoactive properties. In addition, the plant material is used for fiber, oil, and food production [12]. For these applications it is important to gain knowledge of the cannabinoid biosynthetic pathway. As an example, fiber production is not allowed if the plant contains more than 0.2% (dry weight) THC. Higher THC content is illegal in most Western countries and cultivation is strictly regulated by authorities. Interestingly, the content of other cannabinoids is of less importance because no psychoactive activity is claimed for them. Furthermore, for forensic purposes the information may be used to discriminate the plants by genotype, which is correlated to the chemotype (see Sect. 2.2), in the early phase of their development. This may help both the cultivator and legal forces. Here the cultivation of illegal plants may be found and controlled by both of them. For the cultivator, to exclude illegally planted plants and for the police to control illegal activities by the cultivators or criminals. Moreover, the information can be used by pharmaceutical companies and scientists. Here it can be used for the studies on controlled production of specific cannabinoids that are of interest in medicine. For instance, THC has been investigated for its tempering effect on the symptoms of multiple sclerosis [13], but CBG and CBD may also have a role in medicine. Both CBD and CBG are related to analgesic and anti-inflammatory effects [14, 15].

In this section, the latest developments and recent publications on the biosynthesis of Δ9-THC and related cannabinoids as precursors are discussed. Special points of interests are the genetic aspects, enzyme regulation, and the environmental factors that have an influence on the cannabinoid content in the plant. Because of new and innovative developments in biotechnology we will give a short overview of new strategies for cannabinoid production in plant cell cultures and in heterologous organisms.

2.1 Biochemistry and Biosynthesis

The biosynthesis of major cannabinoids in *C. sativa* is located in the glandular trachoma, which are located on leaves and flowers. Three known resin-producing glandular trachoma are known, the bulbous glands, the capitate sessile, and the capitate stalked trichoma. It has been reported that the latter contain most cannabinoids [16]. The capitate stalked trichoma become abundant on the bracts when the plant ages and moves into the flowering period. The capitate sessile trichoma show highest densities during vegetative growth [17, 18].

As depicted in Fig. 3, in glandular trichoma the cannabinoids are produced in the cells but accumulate in the secretory sac of the glandular trichomes, dissolved in the essential oil [17–21]. Here, Δ9-THC was found to accumulate in the cell wall, the fibrillar matrix and the surface feature of vesicles in the secretory cavity, the subcutilar wall, and the cuticula of glandular trichomes [19].

As mentioned before, the cannabinoids represent a unique group of secondary metabolites called terpenophenolics, which means that they are composed of a terpenoid and a phenolic moiety. The pathway of ter-

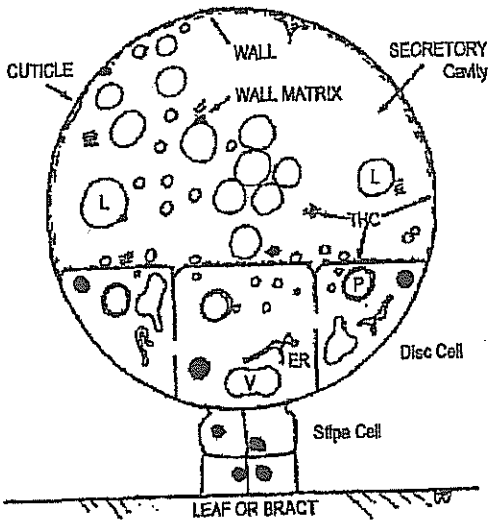


Fig. 3 Representation of mature secretory gland originated from *C. sativa*. The separate compartments of the glandular trichome are clearly shown, and the places where THC accumulates. *Black areas* nuclei, *V* vacuole, *L* vesicle, *P* plastid, *ER* endoplasmic reticulum. Picture obtained from: http://www.hempreport.com/issues/17/malbody17.html

penoid production is already reviewed exhaustively [22–25]. The phenolic unit of cannabinoids is thought to be produced via the polyketide pathway [26–28]. Both the polyketide and terpenoid pathways merge to the cannabinoid pathway and this combination leads to the final biosynthesis of the typical cannabinoid skeleton. Here we will discuss the different aspects of the cannabinoid pathway for most already-found cannabinoids, like cannabigerolic acid (CBGA), tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA). For convenience the abbreviations of the acidic form will be used through this section because

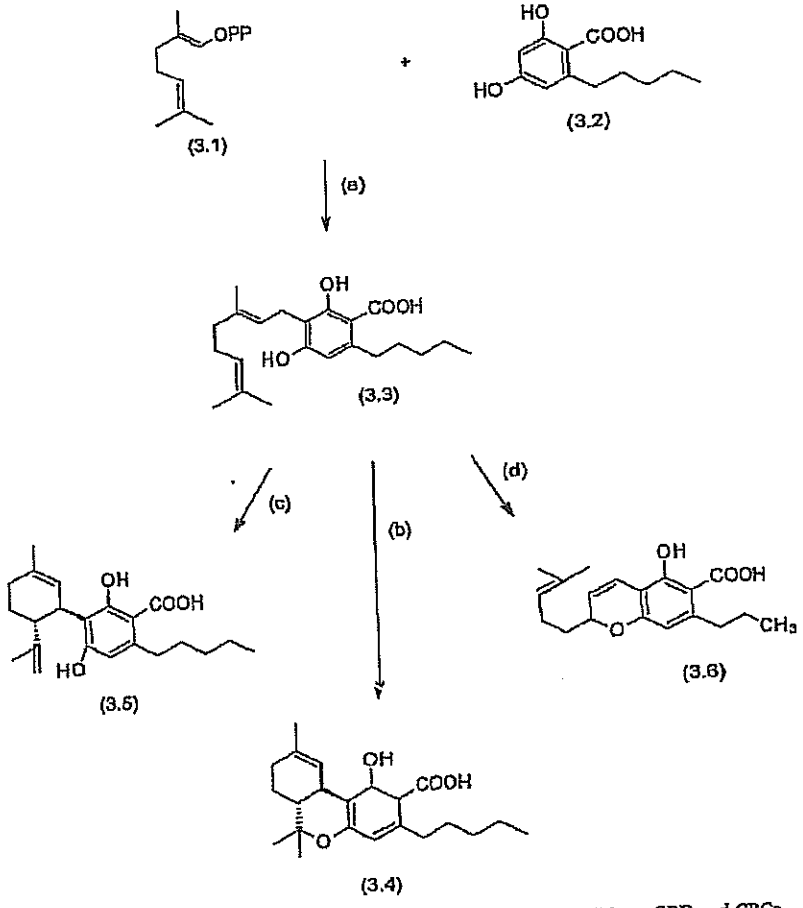


Fig. 4 Biosynthesis of THC and related cannabinoids: *a* GOT, *b* THCs, *c* CBDs, *d* CBCs

they occur as genuine compounds in the biosynthesis. Under plant physiological conditions the decarboxylated products will be absent or be present only in small amounts.

The late cannabinoid pathway starts with the alkylation of olivetolic acid (3.2 in Fig. 4) as polyketide by geranyl diphosphate (3.1) as the terpenoid unit. Terpenoids can be found in all organisms, and in plants two terpenoid pathways are known, the so called mevalonate (MEV) and non-mevalonate (DXP) pathway as described by Eisenrich, Lichtenthaler and Rohdich [23, 24, 29, 30]. The mevalonate pathway is located in the cytoplasm of the plant cells [30], whereas the DXP pathway as major pathway is located in the plastids of the plant cells [29] and delivers geranyl diphosphate as one important precursor in the biosynthesis.

The polyketide pathway for olivetolic acid is not yet fully elucidated. It is assumed that a polyketide III synthase will either couple three malonyl-CoA units with one hexanoyl-CoA unit [26], or catalyze binding of one acetyl-CoA with four malonyl-CoA units [28] to biosynthesize olivetolic acid [26–28, 31, 32]. Olivetolic acid as precursor for Δ9-THC contains a pentyl chain in position C-3 of its phenolic system, but shorter chain lengths have also been observed in cannabinoids [33]. These differences in chain length support the hypothesis of production by a polyketide, as it is a known feature of these enzymes [34]. It was recently described that crude plant cell extracts from *C. sativa* are able to convert polyketide precursors into olivetol [26]; however here no olivetolic acid was detected. On the contrary, Fellermeier et al. [32] showed that only olivetolic acid and not olivetol could serve in the enzymatic prenylation with GPP or NPP. An older article described that both olivetol as olivetolic acid can be incorporated. Here the incorporation of radioactive labeled olivetol has been detected in very low amounts and olivetolic acid in high amounts. These reactions were performed in planta, whereas the previous reactions were performed in vitro [35]. It still remains unclear which structure, olivetol or olivetolic acid, is really preferred. Horper [36] and later Raharjo [26] suggested that the aggregation of the enzymes could prevent the decarboxylation of olivetolic acid. This explanation suggests that the enzymes are either combined or closely located to each other so that the olivetolic acid is placed directly into the site responsible for prenylation. This hypothesis has still to be proven, but supports the fact that olivetolic acid cannot be found in *Cannabis* extracts [35].

Until recently no enzymes able to produce olivetol-like compounds have been isolated. In an article by Funa et al., polyketide III enzymes were responsible for the formation of phenolic lipid compound [34], a natural product group that olivetol belongs to. Although the biosynthesized compounds contained a longer chain, which increased over time, the study supported the hypothesis of olivetolic acid production by a polyketide III synthase. Further studies on the genetic and protein level are essential to elucidate the mode of mechanism by which olivetolic acid is formed in *C. sativa*.

The precursor of the major cannabinoids is proven to be cannabigerolic acid (CBGA, 3.3) [32, 35]. The formation of this compound is catalyzed by an enzyme from the group of geranyltransferases [28, 32]. This enzyme was studied in crude extracts made from young expanding leafs, were it exhibited activity only with olivetolic acid as the substrate. Despite the fact that no sequence has been published yet, the enzyme was designated geranylpyrophosphate: olivetolate geranyltransferase (GOT). Recently [37] the structure and characterization of a geranyltransferase, named orf-2 and originating from *Streptomyces* CL109, was reported. The authors claimed that the enzyme is able to geranylate both olivetol and olivetolic acid and thus it may be highly similar to the CBGA synthase. Although the authors made this firm statement, they based it on the results obtained by thin layer chromatography. For confirmation of this activity more precise analytical techniques, like LC-MS or NMR, must be performed for structure elucidation of the product produced. Although we have more information about GOT than about polyketide synthase (see Table 2), the mechanism of activity remains uncertain. This means more studies must be performed to obtain the gene sequence.

The last enzymatic step of the cannabinoid pathway is the production of THCA (3.5), CBDA (3.4) or CBCA (3.6). The compounds are produced by three different enzymes. The first enzyme produces the major psychoactive compound of cannabis, THCA [21, 38]; the second and third are responsible for the production of CBDA [39] and CBCA [40], respectively. All of these enzymes belong to the enzyme group oxidoreductases [38–41], which means that they are able to use an electron donor for the transfer of an electron to an acceptor. From these enzymes only the THCA and the CBDA synthase gene sequence have been elucidated. Their product also represents the highest constituent in most *C. sativa* strains.

The enzyme responsible for THCA formation is fully characterized and cloned into several heterologous organisms. When cloned in a host organism, the highest activity was mostly seen in the media. Here the only exception was the introduction of the gene into hairy root cultures made from tobacco [42]. Studies performed on the enzyme sequence indicated that it contained a signal sequence upstream of the actual enzyme. This was found to be 28 amino acids (84 bp) long, suggesting that the enzyme, under native conditions, is localized to another place than where it is produced. Later studies proved that the enzyme is localized in the storage cavity of the glandular trichomes [21]. In the first publication it was determined that no cofactor is used by the enzyme [41], but this research was performed with purified protein from the *C. sativa* extract. Later studies indicated that a flavin adenine dinucleotide (FAD) cofactor was covalently bound to the enzyme. This was later confirmed by nucleotide sequence analysis in silico, revealing the binding motive for the FAD cofactor.

CBDA synthase is though to be an allozyme of THCA synthase and shows 87.9% identity on a nucleotide sequence level. Although the sequence of this

Table 2 Properties of enzymes found in cannabinoid biosynthesis

Enzyme	pH optimal /pI	Reaction rate k_{cat} (in vitro) [s^{-1}]	Localization	Metal ion	Cofactors	Mw[a] [kDa]	Comments	Substrates	Refs.
GOT	7/N.R.	N.R.	?	Mg^{2+}				Olivetolic acid with GPP or NPP	[32]
CBDA	5/6.1	0.19[b]	?[d]	None	None[a]	~75		CBGA and CBNRA	[39]
THCA	7.1/6.4	0.2[b]–0.3[c]	Storage cavity of glandular trichome	None	FAD[e]	~74		CBGA and CBNRA	[38, 41]
CBCA	6.5/7.1	0.04[b]	?	None	None[e]	~71	Probably homo-dimeric	CBGA and CBNRA	[40]

[a]Obtained from protein isolation, not heterologously expressed
[b]Determined by purified Cannabis extract
[c]Determined by recombinant proteins isolates
[d]CBDA synthase shown to carry a highly similar N-terminal signal sequence to THCA synthase. It is thus suggested that this enzyme is localized at the same position as THCA synthase. Furthermore, the precursor CBGA has been shown to be toxic for plant cells and is probably localized in the secretory cavity of the glandular trichome. This suggests that CBDA, THCA, CBCA are all localized in the storage cavity
[e]Activity test with crude extract did not show the need for cofactors; however, from analysis performed on THCA synthase, it became clear that FAD is covalently bound to the enzyme. Furthermore, analysis of the enzymes THCA and CBDA showed the motif that is conserved for FAD binding
N.R. not reported

gene is known [43], there are no reports of studies where they produced and characterized it. All information gained about the enzyme was obtained using purified protein from *C. sativa* extracts [39]. Although not tested yet, the deposited sequence shows the same conserved FAD binding motive as found and proven for THCA synthase. Because the CBDA synthase carries the same signal sequence as the THCA synthase it suggests that the CBDA is localized in the same place as the THCA synthase.

For CBCA synthase hardly any information has been published. The enzyme was characterized after it was purified from *C. sativa* extracts and until this moment no sequence has been deposited. After purification of the protein it was found to be a homodimeric enzyme, meaning that enzyme is formed by two identical domains. This was observed after the purification, when the enzyme had a molecular weight of 136 kDa, and after denatured electrophoresis, when it had a molecular weight of ~ 71 kDa. Furthermore, the CBCA synthase has shown to bear higher affinity for CBGA (1717 $M^{-1}S^{-1}$) than THCA synthase and CBDA synthase (respectively 1382 $M^{-1}s^{-1}$ and 1492 $M^{-1}s^{-1}$), which is probably due to its homodimeric nature [40].

From the biosynthetic route a lot of knowledge has been gathered through the years. Up to now only one enzyme has been reasonably characterized, but much information has been gained through crude extract activity studies. This information has already proven to be a solid basis for genetic testing and will be useful for further investigations of the biosynthetic route. Although it must be stated that high polymorphism is detected in the genes [44] and high genetic diversity found within, *C. sativa* can still give unexpected results in other investigations. The information gained from the research reported above is already used frequently in the breeding and detection of certain chemotypes and for the development of new ones, as we will see in the next section.

2.2 Genetics of *Cannabis Sativa*

The majority of *C. sativa* strains exist as a dioeciously (separate sexes) plant species and are wind-pollinated. Under normal conditions it is an annual herb, although longer-living *C. sativa* have been observed [45, 46]. Some *Cannabis* strains appear as monoecious (containing both male and female parts) cultivars, such as the Ukrainian cultivar USO31 [47], or as hermaphrodites. Most of these cultivars are not seen in nature. It is estimated that only 6% of the flowering plants are dioecious and generally they are seen as the most evolved species within the plant kingdom [48, 49]. The *C. sativa* genome is normally a diploid one and contains ten chromosome pairs ($2n = 20$). Here, eighteen are autosomal and two are sex-linked chromosomes. The genome was measured in both female (XX) as well as in male plants (XY). In contrast to animals, the male genome was found to be bigger by 47 Mbp [50, 51]. It must be stated

that dioecious plants are able to change sex during their development. This ability is mostly used as a strategy for survival, however it can be chemically induced. Within the *C. sativa* species lots of phenotypes are known. Generally the *C. sativa* plant are believed to be a monotypic species [47] called *Cannabis sativa* L. with further divisions in subspecies. However, Hillig [46] showed, by allozyme analysis in combination with morphological traits, that a separation may be made between *C. sativa* L. and the *C. indica* Lam. He also suggested a putative third one named *C. ruderalis* Janisch. The polytypic species within *C. sativa* was already suggested several years ago when the plants were determined only by their phenotypic traits or drug potential properties [46]. There is still discussion about whether or not the *C. sativa* species are monotypic or polytypic, but in most literature they are referred to as *C. sativa* with further division into the subspecies *indica* or *ruderalis*.

C. sativa is mostly divided into three major chemotypes. The chemotypes boundaries are set by the ratio CBD : THC and are calculated as percentage of dry weight. These three chemotypes consist of the "fiber"-type (CBD > THC), the "intermediate"-type (CBD≈THC) and the "drug"-type (CBD < THC). The chemotypes have been recently shown to be dependent either on one locus on the chromosome, or two closely linked loci [47], but the former theory is the most likely one [52–54]. The locus is called the B locus and until now it is proven to consist of at least two alleles, namely B_t and B_d. There is also an indication for a third allele. This one was named B_0 and seems to be responsible for a CBGA-dominant chemotype [54]. The alleles, B_t and B_d, show co-dominance and the B_0 allele is recessive or an inactive B_d allele. The B_0 is believed to be an inactive B_d allele because it can be indicated by molecular markers specific for the CBDA gene (B_d allele). The evidence for these alleles was gained by breeding with chemotypes and molecular analysis [47]. In crossings made with fiber-type and drug-type, the intermediate chemotype was obtained as offspring. Intercrosses of these F1 plants gave a representative Mendelian ratio (1 : 2 : 1) of chemotypes. This Mendelian ratio suggests that one locus is responsible for the chemotypes. Furthermore, Pacifico et al. [47] proved, with the help of multiplex PCR, that a 100% identification of specific chemotype (from the three accepted chemotypes) could be made. This multiplex PCR was performed with three primers, one of which was designed to anneal with both the THCA synthase and the CBDA synthase gene, while the other two were specific for one or both. The results showed that the intermediate chemotype was heterozygote and thus contained both the CBDA synthase and the THCA synthase genes. The drug- and the fiber-type were shown to be homogeneous for the THCA synthase and the CBDA synthase genes, respectively. Although the genes are not themselves detected, their products are. For instance, the fiber-type group that is shown to be homogenous for the B_d allele, still produces low amounts of THCA. It is thus still possible that the homogeneous type carries the THCA synthase gene; however, it is not detected due to the polymorphisms within the gene, as shown by Kojoma et al. [44].

Recently it has been suggested that there are two more chemotypes. The first (chemotype 4) has a high content of CBGA (B_D allele) and the second is a strain totally lacking cannabinoids [47]. These strains are of interest because they can serve as good and safe strains for the production of fiber.

2.3 Environmental Factors

Cannabis seems to react to several environmental influences. The most known are hydration, soil nutrients, wounding, competition and UV-B radiation. Proper use of these environmental influences can increase the glandular density and the cannabinoid content. Environmental factors have also been shown to induce sex change in *C. sativa*. Moreover, when some chemotypes are grown in a different environment their cannabinoid content seem to be changed. With genetic analysis it must be possible to determine if a strain is indeed a fiber strain or if it is an intermediate strain that has been suppressed for its cannabinoid content due to the environment of cultivation. Some of the major environmental factors influencing the cannabinoid content are described below. It must be stated that environmental stress also affects the growth of the plant.

2.3.1 Dehydration

In times of less accessibility of water, the plants seem to increase the cannabinoid content. It is suggested that the plant will cover itself with the oily cannabinoids to prevent water evaporation. For instance Sharma (1975) found increased glandular trichome densities in the leaves of Cannabis grown under dry circumstances [55].

2.3.2 Nutrients in Soil

It is clear that the nutrients in soil are important for plant development and that a good nutrient supply within the soil gives healthy plants. However, no profound research results have yet been published on the most optimal soil conditions.

2.3.3 Light

Light has a major influence on plants, and for *Cannabis* plants it is mostly important for growth and flowering. Long daylight induces strong vegetative growth and shorter daylight leads to flowering of the plants. Furthermore, it

has been shown by Lydon et al. that the level of THC increase is linear with the increase in UV-B dose [56–58].

2.4 Growing of *Cannabis Sativa* and Optimization of THC Yield

2.4.1 Cultivation of *Cannabis*

C. sativa is cultivated for several purposes. Actually, the main legal purpose is the production of hemp fibers and pulp. From these materials paper, clothes and ropes are made [12] and several Western countries have already legalized the cultivation of *C. sativa* for these purposes. In research, the drug-type of *C. sativa* is also cultivated, however, only for the investigation and determination of forensic studies for chemotype separation. The growth for medicinal purposes is hardly performed. In the Netherlands *C. sativa* is cultivated for medicinal purposes under strictly controlled regulations by the company Bedrocan. In this chapter we discuss basic aspects of the cultivation of *C. sativa* and the optimization of THC content in the plant.

2.4.2 Optimization of THC Yield

The optimization of THC yield is mostly performed through breeding programs. Because of the illegality of the plant in most countries, it is performed on small scale or by illegal drug cultivators. In the previous section we have already discussed the fact that cannabinoid production is mostly genetically determined. This knowledge could thus be used to increase the production of certain compounds and decrease the others. Furthermore, the total THC yield is dependent on the amount of accessible precursors and the level acceptable for the plant.

Within the *C. sativa* strain the total content of cannabinoids varies. In THCA-dominant plants variations have also been noted. Some have low detectable amounts of CBDA whereas others have none. Furthermore, some plants have been shown to contain detectable amounts of CBGA while others had none. There is still a question as to what extent THCA production can be increased in the plant by breeding programs and genetic modifications. From the genetic point of view it should be noted that the yield of THC is not only dependent on the B_t allele, but also depends on the amount of biomass, the density of trichomes, and the production of precursors indicating a complex spectrum of different possibilities for professional plant breeders. The yield of THCA in THCA-dominant plants can be increased by environmental influences. In cultivations of the drug-type, mostly done by illegal cultivators, the male plant is excluded from the field. The background is that this will induce

more biomass of the female plant because it cannot be inseminated. But, the exclusion of male plants will not give a more constant increase of THC yield.

Genetic modification seems to be an option for increased yield since the THCA production is mainly dependent on genetic factors (see Sect. 2.1). Here we could think of increasing glandular trichoma densities, increasing precursor production, increasing enzyme activity and knocking out enzymes that use the general precursor of THCA (CBGA). Applying some of these techniques have already shown an increase in the amount of secondary metabolites in microbial organisms.

As stated above, breeding programs could increase the total yield of THCA. Within *C. sativa* there are many phenotypes, e.g., while one has the ability to grow over a few meters high, another stays small. Furthermore, variations in glandular trichoma densities have also been observed in THCA content and ratios. By combining the phenotypes of various plants with each other, a plant could be grown that is large in growth, high in glandular trichoma density, or high in THCA content. Through breeding techniques Meijer et al. [54] have already created a high CBGA-producing plant and in the drug culture the same has been reached for THCA [59]. In the latter, preparations were found containing more than 20% THC, while in the literature and exported Cannabis the normal values lie at 6–10%.

2.4.3 *Cannabis* Standardization

Just like all herbal medicinal preparations, *C. sativa* should be standardized if extracts or whole plant material are to be used for medicinal purposes. Basic requirements are that all detectable constituents should be known, but also a sustainable quality control system must be established to achieve the same quality over all batches. For industrial use of cannabis, standardization could also be necessary to equalize the quality of the product. However, it must be stated that cultivation for this purposes is mostly performed outdoors. Outdoor growth makes standardization of the product difficult due to the environmental changes. For this reason the Dutch medicinal *C. sativa* is grown under strictly controllable conditions, and therefore indoors, by the company Bedrocan. At this company clones are used for breeding to maintain high standards for quantity and quality. After a strictly selective breeding procedure a plant line has been established fulfilling all criteria as a herb for medicinal use.

2.5 Alternative Production Systems for Cannabinoids

It is clear that production of cannabinoids should be controllable to obtain a constant quality of certain cannabinoids. With the knowledge of the biosyn-

thetic route towards cannabinoid production it is now possible to develop biological production systems as an alternative to chemical synthesis. The major advantage of biological systems is not only having the right natural product by structure, but also the only isomer in high yield. Here, three alternative production strategies are introduced. Although two of them are still hypothetical, it should be possible to be realize them in the near future.

2.5.1 Cell Cultures

In the literature several reports can be found on the growth of callus and cell suspension cultures [60–62]. Most of them document that no cannabinoids can be found within these cultures. Although one article by Heitrich and Binder [60] mentioned that variations in media can induce cannabinoid secretion, no second report could confirm these results. Callus and cell suspension are induced by standard techniques for plant cell manipulation. The induction of callus seems to vary per *C. sativa* variant [61]. To obtain cell suspensions from the callus, the same media is used as for callus growth, with the exception of agar as solidifier. In the literature, the cell suspensions made from *C. sativa* callus are mostly used for bioconversion studies. There is one report that described the use of cannabinoid precursors to determine if cannabinoid production can be induced by feeding with specific biosynthetic precursors [60]. When production of cannabinoids can be achieved in cell cultures from *C. sativa* material, it must still be considered that cannabinoids are toxic to the plant cell itself. These compounds induce the apoptotic response [21]. Thus, at high levels of cannabinoid content, techniques have to be developed to extract them from the growth media for continuous production.

2.5.2 Transgenic Plants

Although the use of transgenic plants is not generally accepted for medicinal herbal preparations, transgenic plants could be used to express certain preferable traits. The THCA yield could be increased by manipulation of metabolic pathways or by making knock-outs of biosynthetic genes. With the use of these techniques, the plant could be made resistant to certain parasites and diseases. General plant manipulating strategies can be used to obtain transgenic plants. There is no literature available for the production or use of transgenic *C. sativa* plants.

At the moment, strategies for the production of transgenic plants are already used for maize, tobacco, potato, and rice. The main purpose is to increase their resistance toward diseases [63]. Some plants also get newly introduced products, such as vitamins [64]. Another purpose of transgenic plants is their use for production of vaccines; for instance hepatitis B vaccine

in potato plants [65]. The examples shown here are a selection of many to show the possible transgenic plants uses.

2.5.3 Heterologous Expression of Cannabinoid Biosynthetic Genes

Until now there have only been two reports on heterologous expression of *C. sativa* origin genes into host organisms. In these reports the yeast *Pichia pastoris*, hairy root cultures of tobacco, BY-2 tobacco cell cultures, and insect cells were used to produce the THCA synthase enzyme [38, 42]. In the literature, the use of heterologous expression of plant metabolic enzymes have been shown to be useful in the production of several compounds [25]. The same strategy is probably useful for the production of cannabinoids. The production of cannabinoids will probably ask for specific cultivation parameters because some of its constituents may be toxic to the host in certain concentrations. One method could be a constant refreshment of the growth medium. To date, no publications discuss the efforts of using the heterologous production of cannabinoids. This strategy could be, however, of high interest to pharmaceutical companies when some cannabinoids are approved for medicinal use.

3 Chemical Synthesis

3.1 Synthesis Routes for Δ9-THC

After identification of Δ9-THC as the major active compound in *Cannabis* and its structural elucidation by Mechoulam and Gaoni in 1964 [66], a lot of work was invested in chemical synthesis of this substance. Analogous to the biosynthesis of cannabinoids, the central step in most of the Δ9-THC syntheses routes is the reaction of a terpene with a resorcin derivate (e.g., olivetol). Many different compounds were employed as terpenoid compounds, for example citral [67], verbenol [68], or chrysanthenol [69]. The employment of optically pure precursors is inevitable to get the desired (–)-*trans*-Δ9-THC.

A general problem during the syntheses of Δ9-THC is the formation of the thermodynamically more stable Δ8-THC, which reduces the yield of Δ9-THC. It is formed from Δ9-THC by isomerization under acidic conditions. While the usage of strong acids such as *p*-TSA or TFA leads mainly to Δ8-THC, the yield of Δ9-THC can be increased by employment of weak acids, e.g., oxalic acid [70].

Recently the most employed method for the production of Δ9-THC on industrial scale is the condensation of (+)-*p*-mentha-2,8-dien-1-ol (5.1 in

Fig. 5) with olivetol (5.2) in the presence of boron trifluoride etherate, BF_3-$OC(C_2H_5)_2$ with CBD as a key intermediate. This one-step synthesis of Δ9-THC is also used for the production of synthetic dronabinol, which is used in the medicinal application named Marinol. The mechanism of this synthesis is particular described by Razdan et al. [71] and is shown in Fig. 5

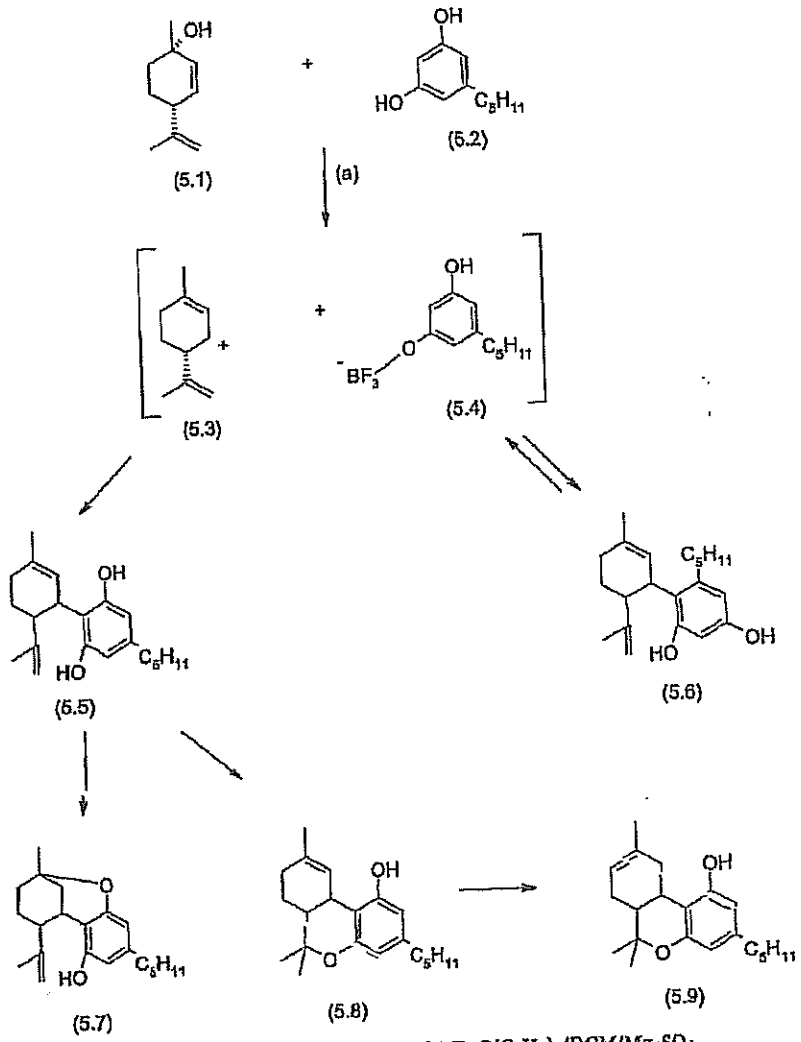


Fig. 5 Commonly used synthesis of Δ9-THC (*a*) $BF_3{\cdot}O(C_2H_5)_2$/DCM/Mg_2SO_4

with the most important side products. There are two possibilities for the condensation of the active terpenoid moiety (5.3) with activated olivetol (5.4). The fusion of these compounds leads to two intermediates, normal CBD (5.5), which has the same structure as natural CBD, and "abnormal" CBD (5.6) with transposed positions of the pentyl side chain and a hydroxy group. Fortunately, the latter compound is less stable than the normal CBD and decompensates more easily. The normal CBD directly undergoes a further cyclization to Δ9-THC (5.7). If the double bond in the terpenoid ring is used for the cyclization, a isomeric compound named *iso*-tetrahydrocannabinol (*iso*-THC, 5.8) will be formed. The reaction has to be stopped here otherwise the stable isomer Δ8-THC (5.9) arises by decreasing the yield of Δ9-THC. Purification of the reaction mixture is implemented as a liquid chromatographic process using a silica-based stationary phase and a weak polar eluent (e.g., heptane with 2% *tert*-butyl methyl ether). Further cleaning up is possible with vacuum distillation procedures.

3.2 Synthesis of Δ9-Tetrahydrocannabinol from Natural Cannabidiol (Semisynthetic Δ9-THC)

As discussed, the cultivation of *C. sativa* with high content of Δ9-THC (drug-type) is not allowed in many countries. Because of this, there is no opportunity to harvest a high amount of the medicinally important substance Δ9-THC directly from plant material. In the synthesis route for semisynthetic Δ9-THC, natural CBD from fiber hemp plants is employed. It can be extracted with non-polar solvents such as petroleum ether and purified by recrystallization in *n*-pentane. This procedure avoids the formation of "abnormal" CBD and gives the opportunity to produce Δ9-THC from fiber hemp. Semisynthetic Δ9-THC is distinguishable from the synthetic compound because it contains, besides the major product, small amounts of Δ9-THC-C3 and Δ9-THC-C4, which are not available in the synthetic product.

3.2.1 Derivates of Δ9-THC

Most relevant for the affinity for Δ9-THC and analogs to CB-receptors are the phenolic hydroxyl group at C-1, the kind of substitution at C-9, and the properties of the side chain at C-3. Relating to the structure–activity relationships (SAR) between cannabinoids and the CB-receptors, many different modified structures of this substance group were developed and tested. The most important variations include variations of the side chain at the olivetolic moiety of the molecules and different substitutions at positions C-11 and C-9. One of the most popular analogous compounds of Δ9-THC is HU-210 or (–)-*trans*-11-OH-Δ8-THC-DMH, a cannabinoid with a 1′,1-dimethylheptyl side

chain (8.1). It was constructed in consideration of SAR and has a potency that is about 100 times higher than that of Δ9-THC itself, while its enantiomer HU-211 (Dexanabinol, 8.2) does not show this property [8]. In the synthesis of HU-210, 5-(1,1-dimethylheptyl)-resorcin is merged with modified [1*S*,5*R*]-myrtenol [72].

Nabilone (8.3) is a 11-nor-9-ketohexahydrocannabinoid with a 1′,1′-dimethylheptyl side chain. It is a synthetic analogous compound of THC and is distributed as Cesamet. Usage of diethyl-α-acetoglutarate as "terpenoid" module in the synthesis of Δ9-THC gives nabilone as an intermediate [73]. In spite of the fact that this synthesis was developed for the forming of Δ9-THC it also could be used for the synthesis of nabilone. A newer synthesis route is described by Archer et al. [74]. The pentyl side chain homologous compound of nabilone, 11-nor-9-ketohexahydrocannabinol, is a useful precursor in the chemical synthesis of the major metabolites of Δ9-THC, e.g., 11-nor-9-carboxy-Δ9-THC (THC-COOH) [75].

Direct oxidation of Δ9-THC at position C-11 involves mainly an isomerization to Δ8-THC; another opportunity in the synthesis of Δ9-THC-metabolites is the pretreatment of terpenoid synthons by introduction of protective groups, e.g., 1,3-dithiane (6.1 in Fig. 6) followed by the condensation with olivetol (6.2) [76]. The formed product is a protected derivate

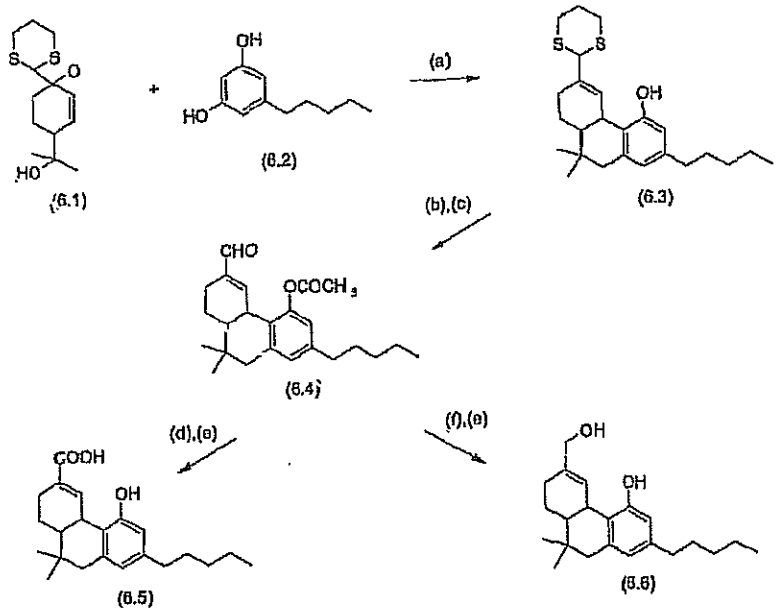
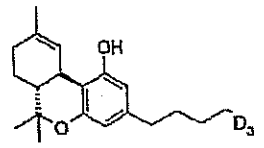


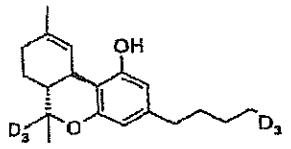
Fig. 6 Synthesis of main metabolites of Δ9-THC: *a* CH_3SO_3H, *b* $(C_2H_5O)_2O$/pyridine, *c* $HgO/BF_3{\cdot}O(C_2H_5)_2$, *d* $NaCN/CH_3COOH/MnO_2$, *e* NaOH/THF, *f* $NaBH_4$/EtOH

of Δ9-THC (6.3), which will be modified further. Protection of the phenolic group by esterification, for example, is necessary before the removing of the 1,3-dithiane masking group with mercury oxide. The corresponding aldehyde (6.4) can be further oxidized. Deprotection of the phenolic group by alkalic hydrolysis gives the 11-nor-9-carboxy-Δ9-THC (THC-COOH, 6.5). Under reductive conditions ($NaBH_4$ or $LiAlH_4$) the corresponding alcohol is formed from the aldehyde. This leads to 11-OH-THC (6.6), which is the first major metabolite from Δ9-THC formed in humans [77].

When [^{2}H]-labeled precursors are employed the resulting compounds can be used as internal standards for analysis, especially by utilization of mass spectrometric methods. Appropriate deuterated standards are shown in Fig. 7. The introduction of deuterium into the Δ9-THC precursors can be done with Grignard reagents such as C[$^2H_3$]MgI or reducing substances such as LiAl[$^2H_4$]. The general procedures for the synthesis with these [^{2}H]-labeled precursors are the same as described above for the unlabeled compounds [76, 78].



(7.1)



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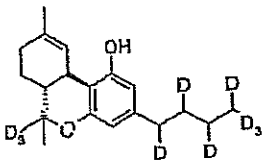

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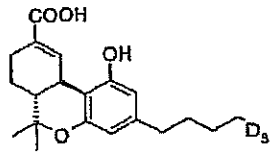

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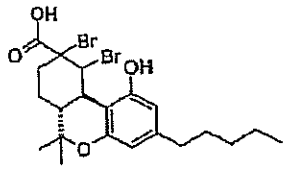

(7.5)

Fig. 7 Deuterated and brominated cannabinoids as internal analytical standards

While the compounds described above contain fundamentally the cannabinoidic structure, there are also compounds with radical changes but which still show high affinity to CB-receptors. Exchange of oxygen with nitrogen in the pyran ring leads to a phenanthridine structure, which can be found in levonantradol (8.4 in Fig. 8). A compound with total loss of the heterocyclic ring is CP-55,940 (8.5). It can be comprehended as a disubstituted cyclohexanole and was synthesized by Pfizer in 1974. This compound was never marketed because of its high psychoactivity, but it is often used for CB-receptor binding studies [79]. Another group of multicore chemical compounds based on the indol structure as a central module in these molecules also shows affinity

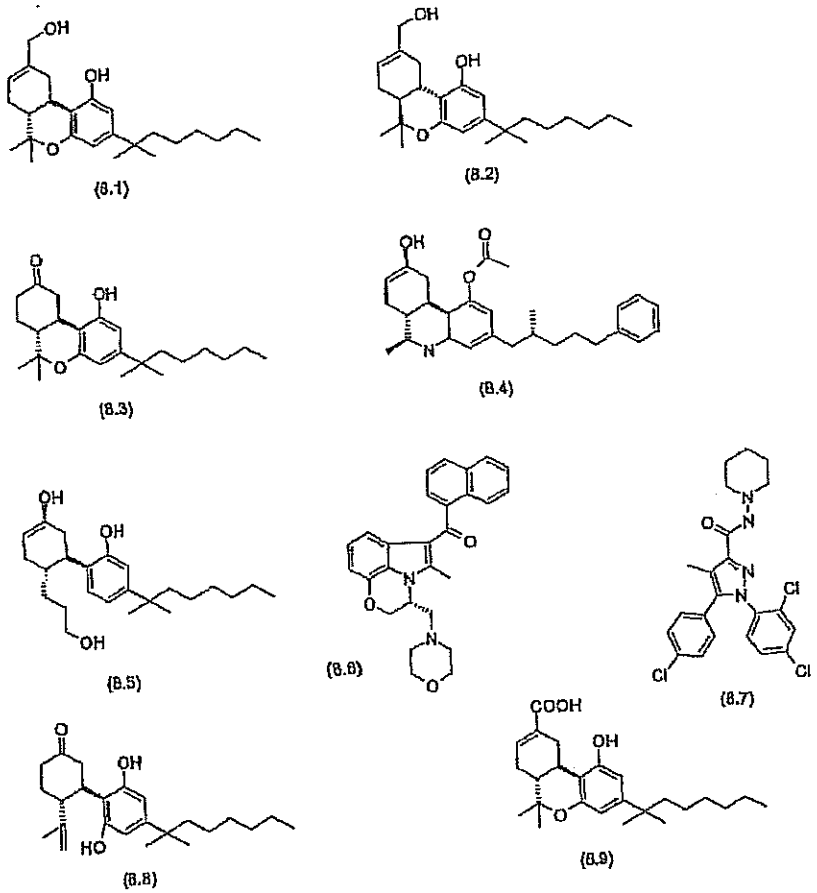

Fig. 8 Synthetic derivatives of Δ9-THC

to CB-receptors. The prototype of this class of aminoalkylindole cannabinoids is the substance named WIN-55,212-2 (8.6), which is quite similar to pravadoline, an anti-inflammatory drug [80].

4 Analytics

4.1 Detection of Cannabinoids in Plant Material

The chemical composition of *C. sativa* is very complex and about 500 compounds in this plant are known. A complete list can be found in [81] with some additional supplementations [2, 82]. The complex mixture of about 120 mono- and sesquiterpenes is responsible for the characteristic smell of *C. sativa*. One of these terpenoic compounds, carophyllene oxide, is used as leading substance for hashish detection dogs to find *C. sativa* material [83]. It is a widespread error that dogs that are addicted to drugs are employed for drug detection. Δ9-THC is an odorless substance and cannot be sniffed by dogs.

The aim of the analysis of cannabinoids in plants is to discriminate between the phenotypes (drug-type/fiber-type). Quantification of cannabinoids in plant material is needed if it will be used in medicinal applications, e.g., in *C. sativa* extracts. The ratio between Δ9-THC and CBN can be used for the determination of the age of stored marijuana samples [84].

4.1.1 Analytical Methods for Detection of Δ9-THC and Other Cannabinoids in Plants

Many methods for determination of cannabinoids in plant material have been developed. Commonly HPLC or GC is used, often in combination with mass spectrometry. Molecular techniques are also available to detect these compounds and will be discussed in this section.

4.1.1.1 Sample Preparation

Usually the first step is an extraction of the desired compounds from plant material. This extraction can be done by different solvents, e.g., methanol [85], *n*-hexane [86], petroleum ether or solvent mixtures such as methanol/chloroform [87]. The use of a second liquid–liquid extraction (LLE) with 0.1 M NaOH after extraction with a non-polar solvent like *n*-hexane makes a separate analysis of acidic cannabinoids possible, which can be found

as their salts in the water phase [86]. These methods are useful for analysis of plant compartments like flowers or leaves, whereas for seeds a solid phase extraction (SPE) is preferred because of their very low content of cannabinoids [88]. The extracts are commonly used directly for analysis. For analysis of acidic cannabinoids, as they normally appear in plant material, using GC-based methods a previous derivatization of the analytes is usually necessary.

4.1.1.2 Gas Chromatographic Methods (GC)

GC is commonly used for the analysis of cannabinoids, mostly in combination with mass spectrometry (GC-MS). Despite the fact that a lot of different cannabinoids are known almost all of them can be separated by using silica-fused non-polar columns. It is not possible to use GC-based methods for profiling of *C. sativa* samples. The high temperatures that are used in GC cause the decarboxylation of acidic cannabinoids. To detect an acidic cannabinoid such as THCA together with its neutral form such as Δ9-THC, a derivatization is required. This procedure increases the stability of the compounds whereas their volutility is maintained. The most often used reagents for derivatization of cannabinoids in herbal samples are compounds that introduce trimethylsilyl groups (TMS) into the analytes, for example *N,O*-bis(trimethylsilyl)trifuoroacetamide (BSTFA), *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA), or *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA). Furthermore, mixtures of these compounds with catalysts, e.g., trimethylchlorosilane (TMCS), are used for a quantitative derivatization [89]. While the employment of established detectors such as the flame ionization detector (FID) or electron capture detector (ECD) can only give information about the quantity of a compound, the usage of mass spectrometry (MS) provides additional information about the structures of detected compounds because of their characteristic fragmentation. For the quantification of cannabinoids three-, six-, or even tenfold deuterated compounds such as shown in 7.1, 7.2 and 7.3 (Fig. 7) are often used as internal standards. The fragmentation of cannabinoids in mass spectrometry is extensively explained by Harvey and the interested reader can find more information about this topic in [90]. A table of about 50 cannabinoids containing free, derivated, and deuterated compounds with their typical mass fragmentations has been published by Raharjo and Verpoorte [89].

4.1.1.3 Liquid Chromatographic Methods (HPLC)

In comparison to GC, an advantage in using HPLC is that there is no decomposition of the acidic forms of cannabinoids. Commonly reversed-phased (RP) materials are used as the stationary phase. Mostly the octadecyl-type

(C-18) is employed. Furthermore, the employment of a guard cartridge containing the same material as used as for the stationary phase is normally recommended. Typical mobile phases are mixtures of methanol and water or acetonitrile and water, acidified with phosphoric acid or formic acid. While for the separation of the main cannabinoids (Δ9-THC, CBD and CBN) an isocratic method is sufficient; the separation of all cannabinoids makes a gradient elution necessary [87]. The use of a photodiode array detector (PDA) is recommended for identification of herbal cannabinoids because of their characteristic UV spectra. If a PDA is used for the detection of cannabinoids Δ8-THC can be employed as an internal standard [91]. According to the law of Lambert–Beer a quantification of cannabinoids based on the strength of the absorption signal is possible. An excellent summary of the most important cannabinoids with their UV spectra and other specific analytical data can be found in [92]. As described in the section on GC-based methods, the employment of mass spectrometry gives the opportunity to identify the structures combined with a better limit of detection (LOD), whereas the use of a UV detector lacks this sensitivity. Another possibility structural identification gives the coupling of HPLC with NMR. The interpretation of [^{1}H]-signals that are specific for different substances can also be used for quantification [93].

4.1.1.4 Immunologically Based Techniques

The enzyme-linked immunosorbent assay (ELISA) technique is often used in laboratories for detection of proteins, but it is also possible to detect small organic molecules by this technique. This assay is based on antibodies that bind with high affinity to certain molecular structures. Testing of cannabinoids by antibodies has been under investigation since the 1970s. The first detections were performed with radiolabeled antibodies made by injection of conjugates from THC, its hemisuccinate, and bovine serum albumin [94]. It was found that the antibody was able to detect cannabinoids and its metabolites from urine and plasma collected from rabbits administered with intravenous cannabinoids. In 1990, Elshoy et al. proved their antibodies to be specific for cannabinoids and related metabolites [95]. Furthermore, they tested against human cannabinoid metabolites excreted via urine and showed that the antibodies against plant cannabinoids were also highly selective and did not bind to any of the non-cannabinoid phenolics. In the early days these studies were all performed with polyclonal antibodies, later monoclonal antibodies were tested and documented the same results [96, 97]. These antibodies may also be used for research. For instance, labeled antibodies have been used against the THC structures to show that THC structures accumulate in the glandular trichoma. Moreover, with this technique it was possible to detect the specific place of accumulation within the trichoma [19]. This indicates that detection by antibodies has an added value over other detection methods such as HPLC

and GC. It is thus possible to use these tests either with enzyme, fluorescent or radioactive labels to detect cannabinoids and their metabolites.

4.1.1.5
Molecular Markers and PCR

These detection mechanisms are not able to detect the small organic structure of the cannabinoids. These techniques are designed to make a selection between plant material on a genetic basis. For instance, by the use of only three polynucleotides (*primers*) and by the use of PCR, discrimination of the major chemotypes (as discussed in Sect. 2.2) was possible. Within the groups selected PCR allowed 100% identification of the chemotypes without any cross reactivity [47]. Furthermore, by a simple PCR technique (two primers used) a separation could be made between drug-type and fiber-type plants [44]. However, it must be stated that a very small number of plants was used and even then polymorphism on the THCA synthase gene was found. The PCR technique cannot be used to detect cannabinoids itself, but maybe it will be of value in plant breeding and cultivation. Furthermore, it may find its place in the detection of illegal *C. sativa* (drug-type) within a population of legal (fiber-type) cultivated plants. However, for this technique, it would be convenient to have the genome of the *C. sativa* plant sequences. This would bring specific information of the differences between male and female plants and could make the design of markers for specific traits easier.

4.2
Detection of Δ9-THC and its Human Metabolites in Forensic Samples

Δ9-THC and its main metabolites are detected and quantified in forensic samples. Determination of these compounds in human beings is needed to make decision on abuse of Δ9-THC-containing drugs by individuals. A careful interpretation of the results is very important to avoid fallacies with regard to the behavior of individuals. The *Cannabis* influence factor (CIF), for example, is an useful tool for distinguishing between acute and chronic intake of Δ9-THC [98].

4.2.1
Metabolism of Δ9-THC by Humane Cytochrome P450 Enzymes

Like other xenobiotics, cannabinoids also undergo extensive metabolism in the human body to increase their hydrophilic properties for a facilitated elimination. The metabolism of Δ9-THC has been very well investigated. More than 100 metabolites of Δ9-THC are known [99] and a good overview of the most important human metabolites is given in [100]. Metabolism takes place mainly in hepatic microsomes, but also in intestines, brain,

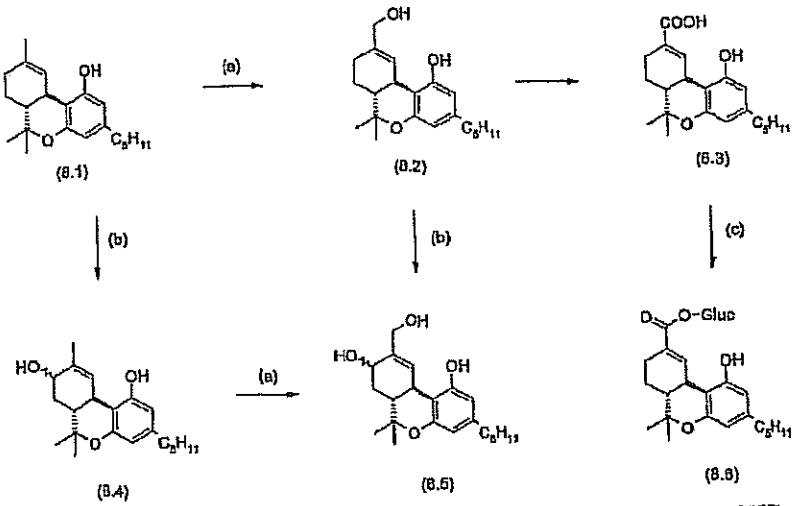

Fig. 9 Main metabolic pathways of Δ9-THC in humans: *a* CYP 2C9, *b* CYP 3A4, *c* UGT

heart, lung, and nearly all tissues of the body. Main metabolites of Δ9-THC are mono-, di- and trihydroxylated compounds, which become carboxylated and glucuronidated further. The metabolism pathway of Δ9-THC and its most important metabolites are shown in Fig. 9. Mostly responsible for metabolism of Δ9-THC in the primary pathway in humans is the cytochrome P450 isoenzyme CYP 2C9 [101]. Hydroxylation of Δ9-THC (9.1) at C-11 leads to 11-hydroxy-Δ9-THC (11-OH-THC, 9.2), which undergoes further oxidation to 11-nor-9-carboxy-Δ9-THC (THC-COOH, 9.3). 11-OH-THC shows similar psychotropic properties to Δ9-THC whereas THC-COOH is a non-psychotropic compound [102]. CYP 3A4 is the second major cytochrome P450 isoenzyme that is involved in metabolism of Δ9-THC – mainly with hydroxylation at C-8 to 8-OH-Δ9-THC, (9.4) [101]. The epoxidation of Δ9-THC at C-9 and C-10 is also described, in addition to oxidation of the alkyl side chain and a following cleavage [8]. Monohydroxylated Δ9-THC can be hydroxylated again, which leads to 8,11-dihydroxy-Δ9-THC, (9.5), for example. Metabolites that are formed by CYP 3A4 represent a minority in comparison to those of CYP 2C9. The glucuronide of THC-COOH, (9.6), which is formed in the secondary pathway is a human metabolite of Δ9-THC.

4.2.2 Analytical Methods for Detection of Δ9-THC and It Metabolites

As described for the analysis of the plant, GC, HPLC, and immunoassays are commonly used for the analysis of body fluids. Although the general proced-

ures are quite similar to those used in the analysis of *C. sativa* (see Sect. 4.1.1) some differences must be pointed out.

4.2.2.1 Sample Preparation

The typical procedure for analysis of cannabinoids from plasma, urine or oral fluids includes preliminary steps such as a SPE for enhancement of the analytes and for minimizing interfering effects of the matrices. Because the metabolites in humans are often conjugated, an anterior hydrolysis of these conjugates either with chemicals like sodium hydroxide or with enzymes [103] is recommended.

Pretreatment of hair samples also includes an extraction, usually with an alkaline sodium hydroxide solution, followed by cleaning up with LLE with *n*-hexane/ethyl acetate. Instead of LLE, the employment of SPE is also possible. Furthermore, the solid phase microextraction (SPME) in combination with head-space analysis is usable [104–106]. In the case of using hair samples, possible external contamination (e.g., by passive smoking of *Cannabis*) has to be considered as false positive result. False positive results can be avoided by washing of the hair samples previous to extraction [107]. Storage of collected samples is another important fact that can cause false results in their content of Δ9-THC and metabolites [108–110].

4.2.2.2 Gas Chromatographic Methods (GC)

The preferred detection method for cannabinoids in forensic samples is GC-MS with or without preceding derivatization. As described before in the analysis of plant materials, the employment of silica-fused columns is recommend in the analysis of human body fluids. While in analysis of *Cannabis* TMS-reagents are mostly employed for derivatization, in the case of human body material fluoric compounds such as pentafluoropropionic anhydride (PFPA) or 2,2,3,3,3-pentafluoro-1-propanol (PFPOH) as derivatization reagents are used [89]. Halogenation of the analytes in these ways allows the use of an electron capture detector (ECD) to find the desired compounds. In comparison with other detectors such as the flame ionization detector (FID), the detection sensitivity of cannabinoids can be increased by using an ECD. This is important because the amount of these compounds is very low in human forensic samples. However, as mentioned above, these detectors are commonly not used in routine analyses of forensic samples. Among PFPA and PFPOH, acylation reagents such as trifluoroacetic anhydride (TFAA) and *N*-methyl-bis(trifluoroacetamide) (MBTFA) are also used for analysis of cannabinoids in human materials [111–114]. Trideuterated THC-COOH (7.4) is the most commonly used internal standard for the analysis of metabolites

with GC-MS. Baptista et al. [103] have shown that the limit of quantification (LOQ) for the important metabolite THC-COOH is much more better if negative chemical ionization (NCI) is used instead of electron ionization (EI).

4.2.2.3
Liquid Chromatographic Methods (HPLC)

Whilst for the analysis of plant material for cannabinoids both GC and HPLC are commonly used, in analytical procedures the employment of GC-based methods prevails for human forensic samples. Nonetheless, the usage of HPLC becomes more and more of interest in this field especially in combination with MS [115–120]. Besides the usage of deuterated samples as internal standards Fisher et al. [121] describe the use of a dibrominated THC-COOH (see 7.5). The usage of Thermospray-MS and electrochemical detection provide good performance and can replace the still-used conventional UV detector. Another advantage in the employment of HPLC rather than GC could be the integration of SPE cartridges, which are needed for sample preparation in the HPLC-system.

4.2.2.4
Immunoassays

Most of the tests that were developed for detection of cannabinoids in plants have shown that antibodies are specific for the cannabinoid structure. Because of this specifity these tests can be extensively applied for the detection of cannabinoids and metabolites in human body fluids such as plasma, urine, and oral fluids. Many different kits based on these methods were developed and they are commercially available, for example Oratect, Branan or Uplink, and OraSure. We must consider, however, that no humans have the same metabolite profile in their blood and that cross-reactivity may always occur [122, 123]. Nevertheless, these tests offer a simple way of excluding most of the suspicious samples, but the results still have to be confirmed with a second method such as GC-MS [124, 125].

5
Medicinal use of *Cannabis* and Cannabinoids

5.1
Historical Aspects

Human use of *C. sativa* goes back over 10000 years and the medicinal use can be definitely found in ancient Chinese writings from 1000 BC [126]. Modern medicinal use was mainly introduced by William B. O'Shaugnessy who

was one of the first physicians who systematically explored its therapeutic potential [127]. Studying the literature of the 19th century it is impressive how efficiently most indications, which are now under intensive research, where already depicted by observation and simple trial and error.

5.2 Modern Use

5.2.1 Natural Cannabinoids

A serious problem in the early Western medicinal use of *C. sativa*, mainly as a tincture, was its highly variable activity and inconsistent results. Medicinal preparations have to handle several particularities due to the structure of the active ingredients of *C. sativa*. The identity of the main active constituent of *C. sativa*, Δ9-tetrahydrocannabinol (INN dronabinol) remained unknown until 1964 [128]; standardized *C. sativa* preparations were not available. The plant itself is found in several different chemotypes, which added to the unpredictable nature of early medicinal preparations.

Cannabinoids are highly lipophilic compounds making bioavailability very dependent on the formulation and the mode of administration. Cannabinoid occurrence in the plant is predominantly in the form of the carboxylic acids, which are pharmacologically totally different and rather unstable, decarboxylating over time to their active neutral form. The carboxylic acids, although not active at the CB receptor, nevertheless add to the overall effect as they possess antibiotic and anti-inflammatory effects.

Last but not least the identification of THC as the main active constituent of *C. sativa* was preceded by an almost total ban on the plant as a narcotic drug, practically ending medicinal research.

So, the 20th century actually led to an almost total disappearance of *C. sativa* for medicinal purposes. The only source for THC, which became the focus of scientific research, was the rather tedious extraction and purification from confiscated hashish or marihuana. In 1972 the first commercially viable total synthesis of Δ9-THC was established and it became the first cannabinoid available as a modern medicine in the form of soft gel capsules (the active ingredient being called dronabinol from tetrahydrocannabinol) under the trade name Marinol for the prevention of nausea and vomiting during cancer chemotherapy.

Interestingly this indication resulted from the observation of marihuana-smoking patients rather than from pharmacological research.

In contrast to the *C. sativa* tincture, Marinol soft gel capsules possess clear advantages. Firstly, they contain a single component in an accurate dosage. Secondly, it uses sesame oil as the carrier, making resorption significantly more reliable and also stabilizing the rather sensitive THC molecule.

The indication "prevention of nausea and vomiting during cancer chemotherapy" came from experiences of marihuana-smoking patients, not from pharmacological research [129].

The second indication, being licensed for THC several years later, came from an observation that had been known for a long time for *C. sativa*, namely its appetite-stimulating effects. This sometimes very impressive effect (popularly known as "munchies") was regarded as a side effect until it became apparent that loss of appetite and weight (the "AIDS wasting syndrome") was one of the determining factors influencing mortality of HIV patients [130].

Pharmacological research and the non-prescriptional use of *C. sativa* by patients gave way to new indications. Now well established are the efficacies for the following indications:

- Nausea and vomiting [129]
- Appetite stimulation [131, 132]
- Spasticity [133, 134]
- Tourette syndrome [135]
- Neuropathic pain [136]
- Multiple sclerosis [137]
- Mood elevation
- Glaucoma [138]
- Pruritus
- Asthma
- Epilepsia
- Migraine

After the discovery of specific endocannabinoid receptors, the amount of scientific literature quickly rose and not only new potential indications were established, but also the mechanisms for the already known effects were clarified. Although the most prominent effect of *C. sativa* is clearly related to THC and its activity at the CB1 receptor, most other natural cannabinoids are not active there. Today two other natural cannabinoids CBD and THCV are the focus of medicinal research.

CBD was first isolated from *C. sativa* in 1940 [139]. Unlike the resinous air-sensitive THC, CBD is a crystalline stable substance. Its plant precursor, the carboxylic acid CBDA can be isolated from fiber hemp by extraction and shows potent antibiotic activity. Upon heating it decarboxylates to CBD.

CBD has no activity at the CB1 or CB2 receptor. It is well known that CBD influences the activity of THC if co-administered [140]. Another effect of CBD is the inhibition of cytochrome oxidase [141], which inversely to its antagonistic activity strongly potentiates THC effects above a certain threshhold. CBD is also active as a mild antipsychotic [142] and was proposed as a treatment for anxiety and panic attacks. The mechanism is not fully understood, but it might be caused by an interference with the endocannabi-

noid system. It is now also under research for the treatment of diabetes and obesity [143].

5.2.2 Synthetic Cannabinoids

Until today only a few synthetic cannabinoids have made their way into clinical use.

5.2.2.1 Nabilone

In contrast to THC (an oxygen-sensitive resin), nabilone (8.3) is a crystalline stable substance. It is about five to ten times more potent than THC [144]. It was developed by Lilly and marketed as Cesamet in several countries, mainly for the prevention of nausea and vomiting during chemotherapy. Recently it was approved in the USA for the treatment of neuropathic pain.

5.2.2.2 Levonantradol

Levonantradol (8.4) was synthesized with the intention to introduce a basic amino function into the heterocycle in the hope of obtaining water-soluble salts. Although the solubility of the hydrochloride is not good it was possible to get stable aqueous micellar solutions with the aid of emulsifiers [145] and the compound made its way as an injectable into clinical trials, but never was approved.

5.2.2.3 CP-55,940

CP-55,940 (8.5) was developed during the search for novel analgesics [146]. Although it is more potent than morphine it was never approved. Nevertheless, in its tritium-labeled form it became a very important tool for research and helped in the first identification of the cannabinoid receptor.

5.2.2.4 WIN-55,212-2

In the search for new anti-inflammatory drugs structurally derived from indomethacine [147], Pravadoline showed psychotropic side effects in clinical trials. It became apparent that these effects are mediated through the cannabinoid receptor. Optimization of the structure finally led to WIN-55,212-2 (8.6), which has a higher affinity to the CB1 receptor than THC [148]

and became an important research tool. The side effects of substances possessing agonistic activity on the CB1 receptor (mainly psychotropic effects similar to those of cannabis) limited its clinical use and changed the focus of research to the development of compounds without this drawback.

5.2.2.5 Rimonabant

Rimonabant or SR-141716A (8.7) is an antagonist at the CB1 receptor [149] and got approval for the treatment of obesity and as an aid in the cessation of cigarette smoking. It is now marketed in Europe under the tradename Acomplia. Interestingly the naturally occurring THCV (the propyl homolog of THC) also acts as an antagonist on the CB1 receptor and might become a competitor for rimonabant.

5.2.2.6 PRS-211,096

PRS-211,096 (8.8) is a CB2-selective agonist, thus avoiding the psychotropic side effects related to CB1. It is currently in clinical trial for the treatment of multiple sclerosis.

5.2.2.7 HU-211

HU-210 is (8.1) among the most potent cannabinoids known. Its enantiomer HU-211 (8.2) does not bind to the cannabinoid receptor and lacks psychotropic side effects (as long as optical purity is guaranteed). In animal models it shows analgesic and antiemetic activity. It also shows neuroprotective effects after brain injury and was tested in humans as anti-trauma agent, where it did not meet the expectations in a clinical phase III trial.

5.2.2.8 Ajulemic Acid

Ajulemic acid (CT3, 8.9) is the dimethylheptyl homolog of the main metabolite of Δ8-THC. It has no psychotropic activity, but has analgesic and anti-inflammatory effects.

5.3 Drug Delivery

The classical way of application of Δ9-THC from *C. sativa* is smoking of dried *Cannabis* flowers or leaves by patients in traditional medicine. Smoking of

Cannabis as an illegal drug is popular, but not only these drug users but also regular patients suffering from various diseases as discussed above use this form of unprescribed self-medication.

Besides the inhalative use, the development of a drug formulation for Δ9-THC has to address other bioavailability questions. A major problem is the lipophilicity and poor solubility in water, limiting oral uptake when given orally. Because of this, other parenteral routes of application are under investigation like pulmonal uptake by vaporization, sublingual or intranasal administration, and application by injection of Δ9-THC incorporated in liposomes.

Marinol and Sativex are given orally to the patient but, as indicated, the poor solubility of Δ9-THC is responsible for its slow onset and release from drug carriers like soft gelatine capsules [150]. Quite frequently a large variety in the bioavailability and a significant first pass effect can be observed in animal tests and patients. One solution to the solubility problem is the development of new Δ9-THC derivatives with improved solubility (e.g., dexanabinol, which is a hemisuccinate prodrug). However, this strategy is mostly not desirable because of the high risk involved in the cost and time-consuming drug approval process to gain all toxicological and clinical data.

The main strategies in pharmaceutical technology to improve solubility are the reduction of particle size and the increase of particle surface according to the Kelvin equitation. These two strategies have been applied for Δ9-THC production by solid dispersion technology and production of nanosuspensions. Van Drooge et al., created a solid dispersion of inulin in which Δ9-THC was incorporated [151]. Applying freeze drying techniques for evaporation of a mixture of water and tertiary butyl alcohol, which acts as dissolving medium for Δ9-THC and inulin, forms amorphous Δ9-THC in a fast-dissolving solid inulin matrix. The main advantage of the technique is to protect Δ9-THC from degeneration and to optimize the dissolution rate from tablets [151]. A second and easy way to increase the solubility can be achieved by reduction of the particle size. In unpublished work by the author's group, nanosuspensions of Δ9-THC have been achieved indicating a first significant improvement on the physical properties. The main drawbacks of the technique is the poor stability of the highly energetic suspensions and the risk of forming cluster and microparticles without sufficient stabilization of the nanosuspension. Perlin et al., applied Δ9-THC incorporated in gelatine capsules and administered these orally to rhesus monkeys at a dose of 2.5-mg/kg doses and compared the plasma levels with parenteral intravenous and intramuscularly injections [152]. The authors concluded that intramuscularly injection is favorable because of a bioavailability of 89% ± 16% (i.m.) versus 26% ± 14% (p.o.). Interestingly Perlin et al. mentioned that rectal administration was not successful and no significant blood levels were detected [152]. More recently Munjal et al., developed a transmucosal system based on polyethylene oxide (PEO) polymers, which are commonly used for

the production of suppositories [150]. In this study the heat-labile Δ9-THC hemisuccinate was used to produce suppositories varying in PEO composition by the hot-melt technique (120 °C). Temperature led to a degradation of between 13.5% and 49.4% depending on the composition, but incorporation of vitamin E succinate reduced processing degradation to 9.2% and gave a shelf half-life of 8 months. No data have been published yet to characterize the bioavailability or pharmacological effect.

To achieve reliable elevated plasma levels and to overcome the first pass effect, alternative parenteral administration systems have been developed. The most obvious route is vaporization of the *Cannabis* plant material or the Δ9-THC directly. Hazekamp et al., conducted an intensive study using the Volcano device [153]. The main principle is evaporation of Δ9-THC from *Cannabis* plant material by a hot air flow. Evaporated compounds are collected in a detectable plastic balloon, which can be removed and fitted with a mouthpiece for inhalation. The main advantage of the Volcano vaporizer is that Δ9-THC is vaporized below the point of combustion, avoiding the production of lung-irritating toxins. Other advantages for the self-medicating patient is the ease of self-titration, fast drug release, and fast reaching of therapeutic blood levels. To compare with alternative smoking procedures, the Δ9-THC recovery was 54% for the Volcano and 39% for the water pipe.

Pulmonal application can be still unpleasant for non-smokers, which is why other administration routes like sublingual or intranasal uptake are also of interest. Valiveti et al., investigated nasal application for Δ9-THC and WIN-55,121-2 mesylate in rats [154]. The latter is a synthetic cannabinoid with a short half life time and a highly variable bioavailability. Both drugs were formulated in ethanol and propylene glycol and were successfully administered. In comparison with i.v. applied reference drugs, a tenfold higher nasal dose (10 mg/kg Δ9-THC) showed similar AUC values with a slightly increased half-life time.

A second alternative is sublingual application, as introduced by Mannila et al., based on cyclodextrin matrices [155]. Cyclodextrins are a group of cyclic oligosaccharides that have been shown to improve aqueous solubility, dissolution rate, and bioavailability of various lipophilic drugs such as testosterone or prostaglandin E, to give two examples. Cyclodextrins have also been successfully studied in a few sublingual and buccal formulations, e.g., hydroxypropyl-β-cyclodextrin (HP-β-CD) led to the effective absorption of sublingual testosterone.

In this study, complexation of Δ9-THC and cannabidiol (prepared by freeze drying) with randomly methylated β-cyclodextrin and hydroxypropyl-β-cyclodextrin (HP-β-CD) was studied by the phase-solubility method. The aqueous solubility of CBD and THC increased as a function of CD concentration, and the dissolution increased for THC and CBD cyclodextrin complexes significantly in contrast to plain THC and CBD. These results demonstrate that cyclodextrins increased both the aqueous solubility and dissolution rate

of these cannabinoids, making the development of novel sublingual formulation possible, which has been shown by in vivo studies in New Zealand rabbits.

References

1. Shulgin AT (1968) J Psychedelic Drugs 2:14
2. Elsohly MA, Slade D (2005) Life Sci 78:539
3. Pate DW (2004) In: Grotenhermen F (ed) Cannabis un Cannabinoide. Hans Huber, Bern, p 33
4. Garrett ER, Hunt CA (1974) J Pharm Sci 63:1056
5. Uliss DB, Handrick GR, Dalzell HC, Razdan RK (1978) Tetrahedron 34:1885
6. Gaoni Y, Mechoulam R (1971) J Am Chem Soc 93:217
7. Kriwacki RW, Makriyannis A (1989) Mol Pharmacol 35:495
8. Mechoulam R, Devane WA, Glaser R (1999) In: Nahas GG, Sutin KM, Harvey DJ, Agurell S (eds) Marihuana and medicine. Humana, New Jersey, p 65
9. Gaoni Y, Mechoulam R (1964) J Am Chem Soc 86:1646
10. Miller IJ, McCallum NK, Kirk CM, Peake BM (1982) Cell Mol Life Sci (CMLS) 38:230
11. Turner CE, Elsohly MA (1979) J Heterocycl Chem 16:1667
12. Ranalli P, Venturi G (2004) Euphytica 140:1
13. Collin C, Davies P, Mutiboko IK, Ratcliffe S (2007) Eur J Neurol 14:290
14. Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L, Laezza C, Portella G, Bifulco M, Di Marzo V (2006) J Pharmacol Exp Ther 318:1375
15. Williamson EM, Evans FJ (2000) Drugs 60:1303
16. Charles T, Hammond PGM (1975) Am J Botany 60:542
17. Turner JC, Hemphill JK, Mahlberg PG (1981) Bull Narc 33:63
18. Turner JC, Hemphill JK, Mahlberg PG (1981) Bull Narc 33:59
19. Eun-Soo Kim PGM (1997) Am J Botany 84:336
20. Jocelyn C, Turner JKH, Mahlberg PG (1978) Am J Botany 65:1103
21. Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S, Shoyama Y (2005) Plant Cell Physiol 46:1578
22. Lange BM, Rujan T, Martin W, Croteau R (2000) Proc Natl Acad Sci USA 97:13172
23. Eisenreich W, Bacher A, Arigoni D, Rohdich F (2004) Cell Mol Life Sci 61:1401
24. Rohdich F, Kis K, Bacher A, Eisenreich W (2001) Curr Opin Chem Biol 5:535
25. Julsing MK, Koulman A, Woerdenbag HJ, Quax WJ, Kayser O (2006) Biomol Eng 23:265
26. Raharjo TJ, Chang W-T, Choi YH, Peltenburg-Looman AMG, Verpoorte R (2004) Plant Sci 166:981
27. Raharjo TJ, Chang WT, Verberne MC, Peltenburg-Looman AM, Linthorst HJ, Verpoorte R (2004) Plant Physiol Biochem 42:291
28. Fellermeier M, Eisenreich W, Bacher A, Zenk MH (2001) Eur J Biochem 268:1596
29. Lichtenthaler HK, Schwender J, Disch A, Rohmer M (1997) FEBS Lett 400:271
30. Lichtenthaler HK (1999) Annu Rev Plant Physiol Plant Mol Biol 50:47
31. Austin MB, Noel JP (2003) Nat Prod Rep 20:79
32. Fellermeier M, Zenk MH (1998) FEBS Lett 427:283
33. Shoyama Y, Hirano H, Nishioka I (1984) Phytochemistry 23:1909
34. Funa N, Ozawa H, Hirata A, Horinouchi S (2006) Proc Natl Acad Sci USA 103:6356
35. Kajima M, Piraux M (1982) Phytochemistry 21:67

36. Horper W, Marner F-J (1996) Phytochemistry 41:451
37. Kuzuyama T, Noel JP, Richard SB (2005) Nature 435:983
38. Sirikantaramas S, Morimoto S, Shoyama Y, Ishikawa Y, Wada Y, Shoyama Y, Taura F (2004) J Biol Chem 279:39767
39. Taura F, Morimoto S, Shoyama Y (1996) J Biol Chem 271:17411
40. Morimoto S, Komatsu K, Taura F, Shoyama Y (1998) Phytochemistry 49:1525
41. Taura F, Morimoto S, Shoyama Y, Mechoulam R (1995) J Am Chem Soc 117:9766
42. Taura U (2004) Research report, Graduate School of Pharmceutical Sciences, Kyushu University. Available at http://www.nisr.or.jp/englishHP/report2004/NISR04taura.pdf, last visited: 29 June 2007
43. *Yoshikai K, Morimoto S, Shoyama Y (2001) Japanese Patent 2000–78979*
44. Kojoma M, Seki H, Yoshida S, Muranaka T (2006) Forensic Sci Int 159:132
45. Cherniak L (1982) The great books of *Cannabis*, vol I, Book II. Damele, Oakland, CA
46. Hillig KW (2005) Gene Res Crop Evolut 52:1573
47. Pacifico D, Miselli F, Micheler M, Carboni A, Ranalli P, Mandolino G (2006) Mole Breed 17:257
48. Renner SS, Ricklefs RE (1995) Am J Bot 82:596
49. Sakamoto K, Abe T, Matsuyama T, Yoshida S, Ohmido N, Fukui K, Satoh S (2005) Genome 48:931
50. Sakamoto K, Akiyama Y, Fukui K, Kamada H, Satoh S (1998) Cytologica 63:459
51. Mandolino GAC, Bagatta MVM, Moliterni C, Ranalli P (2002) Euphytica 126:211
52. Mandolino G, Bagatta M, Carboni A, Ranalli P, Meijer E (2003) J Industrial Hemp 8:51
53. de Meijer EPM, Bagatta M, Carboni A, Crucitti P, Moliterni VMC, Ranalli P, Mandolino G (2003) Genetics 163:335
54. de Meijer EPM, Hammond KM (2005) Euphytica 145:189
55. Sharma GK (1975) Bull Torrey Bot Club 102:199
56. Jansen M, Gaba V, Greenberg BM (1998) Trends Plant Sci 3:131
57. Lydon J (1986) Effects of ultraviolet-B radiation on the growth, physiology and cannabinoid production of *Cannabis sativa* L. Dissertation, Maryland University, USA
58. Lydon J, Teramura AH, Coffman CB (1987) Photochem Photobiol 46:201
59. Pijlman FTA, Rigter SM, Hoek J, Goldschmidt HMJ, Niesink RJM (2005) Addiction Biol 10:171
60. Heitrich A, Binder M (1982) Cell Mol Life Sci 38:898
61. Feeney M, Punja ZK (2003) In Vitro Cell Develop Biol Plant 39:578
62. Braemer R, Paris M (1987) Plant Cell Reports 6:150
63. Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M, Kadwell S, Launis K, Lewis K, Maddox D, McPherson K, Meghji MR, Merlin E, Rhodes R, Warren GW, Wright M, Evola SV (1993) Nat Biotech 11:194
64. Herbers K (2003) J Plant Physiol 160:821
65. Mason HS, Lam DM, Arntzen CJ (1992) Proc Natl Acad Sci USA 89:11745
66. Gaoni Y, Mechoulam R (1964) J Am Chem Soc 86:1646
67. Mechoulam R, Braun P, Gaoni Y (1972) J Am Chem Soc 94:6159
68. Mechoulam R, Braun P, Gaoni Y (1967) J Am Chem Soc 89:4552
69. Razdan RK, Handrick GR, Dalzell HC (1975) Experientia 31:16
70. Petrzilka T, Haefliger W, Sikemeier C (1969) Helv Chim Acta 52:1102
71. Razdan RK, Dalzell HC, Handrick GR (1974) J Am Chem Soc 96:5860
72. Mechoulam R, Lander N, University A, Zahalka J (1990) Tetrahedron: Asymmetry 1:315
73. Fahrenholtz KE, Lurie M, Kierstead RW (1967) J Am Chem Soc 89:5934

74. Archer RA, Blanchard WB, Day WA, Johnson DW, Lavagnino ER, Ryan CW, Baldwin JE (1977) J Org Chem 42:2277
75. Kachensky DF, Hui AHF (1997) J Org Chem 62:7065
76. Szirmai M (1995) Total synthesis and analysis of major human urinary metabolites of dl-tetrahydrocannabinol, the principal psychoactive component of *Cannabis sativa* L. Dissertation, Uppsala University, Sweden
77. Siegel C, Gordon PM, Razdan RK (1989) J Org Chem 54:5428
78. Seltzman HH, Begum KM, Wyrick DC (1991) J Labelled Compds Radiopharm 29:1009
79. Melvin LS, Milne GM, Johnson MR, Subramaniam B, Wilken GH, Howlett AC (1993) Mol Pharmacol 44:1008
80. Rissenstat MA, Bell MR, D'Ambra TE, Alexander EJ, Daum SJ, Ackerman JH, Gruett MD, Kumar V, Estep KG, Olefirowicz EM, et al. (1995) J Med Chem 38:3094
81. Turner CE, Elsohly MA, Boeren EG (1980) J Nat Prod 43:169
82. Ross SA, El-Sohly MA (1995) Zagazig J Pharm Sci 4:1
83. Mediavilla V, Steinemann S (1997) J Inter Hemp Assoc 4:80
84. Ross SA, ElSohly MA (1998) Bull Narcotics
85. Bacigalupo MA, Ius A, Meroni G, Grassi G, Moschella A (1999) J Agric Food Chem 47:2743
86. Lercker G, Bocci F, Frega N, Bortolomeazzi R (1992) Farmaco 47:367
87. Lehmann T, Brenneisen R (1995) J Liquid Chrom 18:689
88. Ross SA, Mehmedic Z, Murphy TP, Elsohly MA (2000) J Anal Toxicol 24:715
89. Raharjo TJ, Verpoorte R (2004) Phytochem Anal 15:79
90. Harvey DJ (1987) Mass Spectrom Rev 6:135
91. Rustichelli C, Ferioli V, Baraldi M, Zanoli P, Gamberini G (1998) Chromatographia 48:315
92. Hazekamp A, Peltenburg A, Verpoorte R, Giroud C (2005) J Liquid Chrom Rel Technol 28:2361
93. Hazekamp A, Choi YH, Verpoorte R (2004) Chem Pharm Bull 52:718
94. Teale JD, Forman EJ, King LJ, Piall EM, Marks V (1975) J Pharm Pharm 27:465
95. ElSohly MA, Jones AB, ElSohly HN (1990) J Anal Toxicol 14:277
96. Tanaka H, Goto Y, Shoyama Y (1996) J Immunoassay 17:321
97. Tanaka H, Shoyama Y (1999) Forensic Sci Int 106:135
98. Musshoff F, Madea B (2006) Ther Drug Monit 28:155
99. Brenneisen R (2004) In: Grotenhermen F (ed) Cannabis und Cannabinoide. Hans Huber, Bern, p 89
100. Agurell S, Halldin M, Lindgren JE, Ohlsson A, Widman M, Gillespie H, Hollister L (1986) Pharmacol Rev 38:21
101. Watanabe K, Yamaori S, Funahashi T, Kimura T, Yamamoto I (2007) Life Sci 80:1415
102. Grotenhermen F (2003) Clin Pharmacokinet 42:327
103. Baptista MJ, Monsanto PV, Pinho Marques EG, Bermejo A, Avila S, Castanheira AM, Margalho C, Barroso M, Vieira DN (2002) Forensic Sci Int 128:66
104. Sporkert F, Pragst F (2000) Forensic Sci Int 107:129
105. Musshoff F, Lachenmeier DW, Kroener L, Madea B (2003) Forensic Sci Int 133:32
106. de Oliveira CDR, Yonamine M, de Moraes Moreau RL (2007) J Sep Sci 30:128
107. Boumba VA, Ziavrou KS, Vougiouklakis T (2006) Int J Toxicol 25:143
108. Skopp G, Potsch L (2004) J Anal Toxicol 28:35
109. Jamerson MH, McCue JJ, Klette KL (2005) J Anal Toxicol 29:627
110. Moore C, Vincent M, Rana S, Coulter C, Agrawal A, Soares J (2006) Forensic Sci Int 164:126

111. Kim JY, Suh SI, In MK, Paeng KJ, Chung BC (2005) Arch Pharm Res 28:1086
112. Nadulski T, Sporkert B, Schnelle M, Stadelmann AM, Roser P, Schefter T, Pragst F (2005) J Anal Toxicol 29:782
113. Jamerson MH, Welton RM, Morris-Kukoski CL, Klette KL (2005) J Anal Toxicol 29:664
114. Moore C, Rana S, Coulter C, Feyerherm B, Prest H (2006) J Anal Toxicol 30:171
115. Franski R, Tezyk A, Wachowiak R, Schroeder G (2004) J Mass Spectrom 39:458
116. Concheiro M, de Castro A, Quintela O, Cruz A, Lopez-Rivadulla M (2004) J Chromatogr B Analyt Technol Biomed Life Sci 810:319
117. Maralikova B, Weinmann W (2004) J Mass Spectrom 39:526
118. Teixeira H, Proenca P, Verstraete A, Corte-Real F, Vieira DN (2005) Forensic Sci Int 150:205
119. Kolmonen M, Leinonen A, Pelander A, Ojanpera I (2007) Anal Chim Acta 585:94
120. Laloup M, Ramirez Fernandez MM, Wood M, De Boeck G, Henquet C, Maes V, Samyn N (2005) J Chromatogr A 1082:15
121. Fisher DH, Broudy MI, Fisher LM (1996) Biomed Chromatogr 10:161
122. Walsh JM, Crouch DJ, Danaceau JP, Cangianelli L, Liddicoat L, Adkins R (2007) J Anal Toxicol 31:44
123. Clarke J, Wilson JF (2005) Forensic Sci Int 150:161
124. Moody DE, Fang WB, Andrenyak DM, Monti KM, Jones C (2006) J Anal Toxicol 30:50
125. Cirimele V, Villain M, Mura P, Bernard M, Kintz P (2006) Forensic Sci Int 161:180
126. Li HL (1974) Econ Bot 28:437
127. O'Shaughnessy WB (1838) Trans Med Phys Soc Bengal, p 71
128. Gaoni Y, Mechoulam R (1964) J Am Chem Soc 86:1646
129. Sallan SE, Zinberg NE, Frei E 3rd (1975) N Engl J Med 293:795
130. Palenicek JP, Graham NM, He YD, Hoover DA, Oishi JS, Kingsley L, Saah AJ (1995) J Acquir Immune Defic Syndr Hum Retrovirol 10:366
131. Beal JE, Olson R, Laubenstein L, Morales JO, Bellman P, Yangco B, Lefkowitz L, Plasse TF, Shepard KV (1995) J Pain Symptom Manage 10:89
132. Beal JE, Olson R, Lefkowitz L, Laubenstein L, Bellman P, Yangco B, Morales JO, Murphy R, Powderly W, Plasse TF, Mosdell KW, Shepard KV (1997) J Pain Symptom Manage 14:7
133. Brenneisen R, Egli A, Elsohly MA, Henn V, Spiess Y (1996) Int J Clin Pharmacol Ther 34:446
134. Maurer M, Henn V, Dittrich A, Hofmann A (1990) Eur Arch Psychiatry Clin Neurosci 240:1
135. Muller-Vahl KR, Schneider U, Kolbe H, Emrich HM (1999) Am J Psychiatry 156:495
136. Finnegan-Ling D, Musty RE (1994) Symposium on the cannabinoids, Burlington, Vermont. International Cannabinoid Research Society, p 53
137. Petro DJ, Ellenberger C Jr (1981) J Clin Pharmacol 21:413S
138. Merritt JC, McKinnon S, Armstrong JR, Hatem G, Reid LA (1980) Ann Ophthalmol 12:947
139. Jacob A, Todd AR (1940) J Chem Soc, p 649
140. Karniol IG, Carlini EA (1973) Psychopharmacologia 33:53
141. Jones G, Pertwee RG (1972) Br J Pharmacol 45:375
142. Zuardi AW, Morais SL, Guimaraes FS, Mechoulam R (1995) J Clin Psychiatry 56:485
143. Weiss L, Zeira M, Reich S, Har-Noy M, Mechoulam R, Slavin S, Gallily R (2006) Autoimmunity 39:143
144. Einhorn LH, Nagy C, Furnas B, Williams SD (1981) J Clin Pharmacol 21:64S

145. Aguiar AJ, Rasadi B (1983) US Patent 4406888. Pfizer, New York
146. Johnson MR, Melvin LS Jr (1983) US Patent 4371720. Pfizer, New York
147. Haubrich DR, Ward SJ, Baizman E, Bell MR, Bradford J, Ferrari R, Miller M, Perrone M, Pierson AK, Saelens JK, et al. (1990) J Pharmacol Exp Ther 255:511
148. D'Ambra TE, Estep KG, Bell MR, Eissenstat MA, Josef KA, Ward SJ, Haycock DA, Baizman ER, Casiano FM, Beblin NC, et al. (1992) J Med Chem 35:124
149. Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere JC, Le Fur GL (1998) J Pharmacol Exp Ther 284:644
150. Munjal M, ElSohly MA, Repka MA (2006) AAPS Pharm Sci Tech 7:71
151. van Drooge DJ, Hinrichs WL, Wegman KA, Visser MR, Eissens AC, Frijlink HW (2004) Eur J Pharm Sci 21:511
152. Perlin E, Smith CG, Nichols AI, Almirez R, Flora KP, Cradock JC, Peck CC (1985) J Pharm Sci 74:171
153. Hazekamp A, Ruhaak R, Zuurman L, Van Gerven J, Verpoorte R (2006) J Pharm Sci 95:1308
154. Valivesti S, Agu RU, Hammell DC, Paudel KS, Earles DC, Wermeling DP, Stinchcomb AL (2007) Eur J Pharm Biopharm 65:247
155. Mannila J, Jarvinen T, Jarvinen K, Tarvainen M, Jarho P (2005) Eur J Pharm Sci 26:71

FORENSIC ANALYSIS OF MARIJUANA AND THE KURZMAN MYSTERY: A CASE STUDY OF FLAWED LOGIC IN DETERMINATION OF GUILT

by Frederic Whitehurst[*]

Recent revelations concerning the number of innocent people our justice system has incarcerated and then found to be innocent by DNA analysis causes concern that our justice system may have an unacceptable error rate.[1] Why do we convict as many innocent people as we do? Aside from outright prosecutorial misconduct, failings of the defense bar to properly represent clients, flaws in eyewitness identifications, biased police lineups, and false confessions, we should also be naturally concerned with the inherent problems within crime laboratories.[2] The national media has exposed problems in crime laboratories all across the United States, from the crime laboratory of the Federal Bureau of Investigation, to local crime labs in Washington, Texas, Florida, and beyond.[3] Determinations of innocence necessarily guide us to this question: If DNA has consistently lead to findings of innocence, then has the rest of forensic science found guilt when in fact innocence exists?

We need a model to ask and answer this question. We need a forensic technique, a protocol, that is easily understood and that has resulted in the conviction of hundreds of thousands of Americans. One such protocol is the forensic analysis of marijuana.[4] We need to determine which protocol is being used to identify marijuana, and if this protocol is logical and valid. If there are flaws in the protocol, then we should determine how long these flaws have

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1. *E.g.*, D. Michael Risinger, *Innocents Convicted: An Empirically Justified Factual Wrongful Conviction Rate*, 97 J. CRIM. L. & CRIMINOLOGY 761, 763 (2007).

2. *See* Fredric Whitehurst, *Forensic Crime Labs: Scrutinizing Results, Audits & Accreditation—Part I*, CHAMPION, Apr. 2004, at 6, 6.

3. *See id.*

4. RYAN S. KING & MARC MAUER, THE WAR ON MARIJUANA: THE TRANSFORMATION OF THE WAR ON DRUGS IN THE 1990S, at 1-4 (2005).

existed, why we have not recognized them, and why our justice system could continue utilizing a flawed protocol without testing its validity. Utilizing this model, we can go beyond simple science and question the justice system's error rate. Can we discover methods to ensure valid forensic techniques, or at the very least, should we simply stop accepting the opinions of individuals in white laboratory coats and seriously review their work product?

I. CANNABIS, THE MODEL

The plant *Cannabis sativa*, also known as marijuana, presents unique issues in our justice system, especially regarding the identification of the substance by law enforcement officers as well as forensic crime laboratories.[5] A review of the *Handbook of Forensic Drug Analysis* notes that "[t]he identification of marijuana or its chemical constituents has long been one of the most often performed analyses in the forensic drug laboratory. This includes analysis of the very common botanical samples, ranging from whole plants to finely chopped vegetation."[6] In 1972, John Thornton and George Nakamura presented an analytical protocol for the identification of marijuana, which requires microscopic analysis of botanical features as well as the Duquenois-Levine test, a chemical spot test,[7] which is being used in most crime labs throughout the U.S. But what are we identifying with this protocol? In a 1969 paper, Nakamura noted that *Cannabis sativa* is classified as follows:

Division:	Spermatophyta (seed plants)
Class:	Angiospermae (flowering plants)
Subclass:	Dicotyledons (dicots); 31,874 species
Order:	Urticales (elms, mulberries, nettles, and hemps); 1,753 species
Family:	Cannabinaceae (hops and marijuana); 3 species
Genus:	*Cannabis*
Species:	*sativa*[8]

Therefore, we are looking for a plant that (1) produces seeds, (2) has flowers, (3) is a dicotyledon, and (4) has some or all of the features of elms, mulberries, nettles, and hemps.[9]

5. *See* discussion *infra* Part I.

6. Charles Tindall et al., *Cannabis: Methods of Forensic Analysis*, *in* HANDBOOK OF FORENSIC DRUG ANALYSIS 43, 43 (Frederick P. Smith ed., 2005).

7. J.I. Thornton & G.R. Nakamura, *The Identification of Marijuana*, 12 J. FORENSIC. SCI. SOC. 461, 461 (1972); *see also* G.R. Nakamura, *Forensic Aspects of Cystolith Hairs of Cannabis and Other Plants, Drug Abuse Control*, 52 J. ASS'N OFFICIAL ANALYTICAL CHEMISTS 5, 5 (1969)

8. Nakamura, *supra* note 7, at 6.

9. *See id.*

We must start marijuana analysis with an understanding of the form in which the alleged marijuana presents itself to the analysts.[10] Most submissions to forensic laboratories are in the form of crushed plant materials that no longer retain gross botanical features.[11] The crushed form of these submissions means law enforcement officers seize plant materials they recognize as marijuana despite the fact that it no longer retains identifying features.[12] This seizure, of course, depends upon the law enforcement officer's ability to determine that the sample is plant material.[13] On the surface, believing that any individual could not determine that a plant is a plant seems ridiculous. We see plants every day and recognize them as trees, grass, ornamental flowers, and the ever present weeds in our gardens. On this level of understanding, we would most appropriately go to a dictionary to determine the definition of a plant not to a treatise on botany. *The American Heritage Dictionary* defines a plant as "[a]n organism of the vegetable kingdom, characteristically having cellulose cell walls, growing by synthesis of inorganic substances, and lacking the power of locomotion."[14] So when a law enforcement officer seizes crushed plant material, we hope that the officer would refer to a standard, like the dictionary definition, when determining if a substance is plant material.

Can we determine from a field examination of crushed material if the seized material is composed of cells with cellulose cell walls? Well, not really. This determination requires at least a microscopic analysis as well as a chemical analysis.[15] History books tell us about the excitement when newly invented microscopes detected the presence of the cellular structure of living matter. If we could not see those cells before microscopes came along, then how can the police officer on the street see those cells with the naked eye? The cellulose making up the walls of those cells requires a chemical analysis; therefore, how can a police officer determine the cell walls are composed of cellulose? And although the officer can determine if the material lacks locomotion (even parts of a dead animal or pieces of newspaper lack locomotion), the proper question is whether the seized material, when in its natural state, existed in an object that lacked locomotion? An officer cannot know that. The material is no longer in its natural state. Finally, can the law enforcement officer know whether the seized material grows by the synthesis of inorganic substances? The answer to this question, of course, is no. So how does the law enforcement officer know whether the seized material is a plant?

But suppose that by some method, the police officer can determine that the seized plant material is a plant. If Nakamura's classification of marijuana is correct, the next level of analysis determines if the seized material is a seed

10. *See* Tindall et al., *supra* note 6, at 45.
11. *See* Nakamura, *supra* note 7, at 5.
12. *See* Tindall et al., *supra* note 6, at 43-44.
13. *See id.*
14. THE AMERICAN HERITAGE DICTIONARY 948 (Margery S. Berube et al. eds., 2d College ed. 1982).
15. *See* Thornton & Nakamura, *supra* note 7, at 461.

plant (i.e., a spermatophyte).[16] My own law enforcement experience in the investigation of marijuana cases has shown that marijuana samples are often accompanied by what appear to be seeds. But are these objects really seeds? How do we determine that? Do we plant the seeds to see if they grow? Do we open them up to see if there are two halves (two cotyledons) and the tiny beginnings of a plant?[17] Even if we can determine whether these seeds are present, how do we know that they are seeds of *Cannabis sativa*? How do we differentiate these seeds from any other seeds?

Assume, however, that we have answered the plant material and seed questions. Next, we must ask if the seized seed plant has flowers (i.e., is an angiosperm).[18] To answer, we must know what a flower is, the different parts of flowers, the kinds of flowers growing in a marijuana standard, and if the flowers seen in marijuana are like flowers in any other kind of plant. Just because marijuana flowers have particular features, one cannot assume that no other plants have flowers with the same features.[19] Because the form of most marijuana samples seized is crushed plant material there may be difficulty in determining whether these crushed flowers are identical to marijuana flowers.[20]

Now suppose that we can determine that we have plant material with seeds and flowers. Do we then know whether they are dicotyledons? Anyone who has ever planted a bean, a watermelon seed, or a peanut knows what a dicotyledon is. Those first little fat leaves from the seed itself are the cotyledons,[21] and plants that have two cotyledons are referred to as dicotyledons.[22] We see these seed leaves and initially wonder what we have planted and why it looks so different from what we expected from our planting project. But soon those cotyledons give way to tiny little leaves and our plants grow up to look like we expected. At one time there were 31,874 known dicotyledons.[23] Because marijuana is one of these dicotyledons, the real question becomes: can the naked eye of a drug analyst or police officer determine that the crushed flower-producing seed is a dicotyledon?[24] Without careful scrutiny, this cannot be determined.[25] Though a trained botanist might

16. *Id.* at 495-96.

17. A cotyledon is "an embryonic leaf in seed-bearing plants, one or more of which are the first leaves to appear from a germinating seed." THE NEW OXFORD AMERICAN DICTIONARY 385 (Erin McKean ed., 2d ed. 2005).

18. *See supra* note 8 and accompanying text.

19. *See generally* Robert F. Thorne, *How Many Species of Seed Plants Are There?*, 51 TAXON 511 (2002) (discussing the numerous types of seed plants).

20. *See* Thornton & Nakamura, *supra* note 7, at 495.

21. *See supra* note 17 (defining cotyledon).

22. *See* THE NEW OXFORD AMERICAN DICTIONARY, *supra* note 17, at 470 (defining dicotyledon).

23. *See* Nakamura, *supra* note 7, at 6.

24. *See* Thornton & Nakamura, *supra* note 7, at 495 (discussing the difficulty of identifying crushed plants).

25. *See* Tindall et al., *supra* note 6, at 48 (discussing the difficulty of identifying marijuana).

be able to discern that a plant is a dicotyledon, "most seized drug analysts are not trained as botanists."[26]

Now suppose that we have plant material that has seeds and flowers and is a dicotyledon. Because there are many dicotyledons, we must discern proper marijuana plants from other dicots.[27] A further subdivision of dicotyledons, an order named urticales, contains 1,753 species of elms, mulberries, nettles, and hemp.[28] Therefore, can the law enforcement officer determine from examining crushed plant material in a baggy if the material originated from a plant in the order urticales?

Now put this paper down and take a break. Go into your back yard and look at the types of plants you see. Look at the myriad of different leaves, shapes, plants, and even weeds. You might be looking at 100 species of plants right now. Take some of those leaves into your office and let them dry for a week or two. Then crush them up. Can you now differentiate those leaves in their crushed form just by looking at them, and can you tell which plant you took them from?

Leaves are classified according to leaf orientation, organization, shape, margin, texture, gland position, petiole, types of venation, and elements of tooth architecture.[29] Looking at the form of leaf margin, we see classification concepts including entire, lobed, toothed, crenate, erose, revolute or enrolled, sinuses, spacing, and series.[30] Without even defining the meaning of any one of these, we can ask ourselves whether the plants being examined fit into the classification scheme. To do so, we need to know how the leaves appeared before they were crushed up and prepared for distribution. Yet this information may be unavailable, so how can we say we are looking at marijuana?

If we have found that the material is a seed-bearing, flower-producing dicotyledon, and has the characteristics of those plants in the order urticales, then we may further ask about the description of the flowers. We know from our experience of simply looking into our gardens that all flowers were not created equally. Day lilies certainly look different than roses. Thus, we can assume that the flowers of marijuana are different from other plants' flowers. Professor Herman E. Hayward's treatise, *The Structure of Economic Plants*, provides a seemingly exhaustive description of the inflorescences of marijuana: "[a]lthough hemp is disecious, it is not uncommon for an individual plant to bear both staminate and caepellate flowers."[31] So, have we seen evidence of either staminate or carpellate flowers in the crushed material that the law enforcement officer has presented as marijuana?

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26. *Id.* at 48.
27. *See* Nakamura, *supra* note 7, at 6 (noting the variety of dicotyledons).
28. *See, e.g., id.*
29. Leo J. Hickey, *A Revised Classification of the Architecture of Dicotyledonous Leaves*, *in* 1 ANATOMY OF THE DICOTYLEDONS 25, 28-30 (C.R. Metcalfe & L. Chalk eds., 2d ed. 1979).
30. *Id.* at 28-29.
31. HERMAN E. HAYWARD, THE STRUCTURE OF ECONOMIC PLANTS 217 (1938).

Additionally, "[t]he staminate flowers develop in small, drooping, branched panicles, which arise in the axils of foliage leaves . . . [t]he flowers of the panicle may occur singly on slender pedicels or in groups, and usually the terminal branches bear three flowers . . . ."[32] Do we see drooping branched panicles, located in the axils of foliage leaves, and if we do not, then can we say with any certainty that we are either looking at or have identified this material as marijuana? And do the flowers of the panicle occur singly on slender pedicels or do they occur in groups?

And further, "[t]he individual flowers are apetalous with a deeply parted calyx having five greenish-yellow or red lobes that are widespread at maturity."[33] Have we seen the apetalous flowers with a deeply parted calyx that have five lobes, either greenish-yellow or red, that are widespread at maturity?

And further, "[t]he oval sepals are acuminate, the outer surface and margins being covered with multicellular glands and slender, pointed unicellular hairs with crystals of calcium oxalate deposited in their swollen bases. The inner epidermis is practically devoid of hairs and stomata which are present in the outer epidermis."[34] Has the law enforcement officer who identified the green leafy material as marijuana (or even the forensic lab examiner) been able to determine if the oval sepals are acuminate with the outer surface and margins covered with multicellular glands in addition to slender pointed unicellular hairs with crystals of calcium oxalate deposited in the swollen bases? Has anyone in the identification process even determined the presence of calcium oxalate?

So far, in order to determine if we have marijuana, we need to know if we have plant material, if that material is from seed-bearing and flower-bearing dicotyledonous plants of the order urticales, and if the botanical features of marijuana described by Hayward are identified. An alternative method involves determining if marijuana has unique characteristics that set it apart from the universe of plants on earth.[35] Obviously *Cannabis sativa* is unique as a species, or we would not have called it a plant species. In the 1970s, the issue of whether the genus *Cannabis* was composed of a number of different species came to the attention of the legal community when debating whether legal statutes properly proscribed the possession and distribution of all species of *Cannabis* or simply *Cannabis sativa*. The arguments were rendered moot by legislators and will not be discussed here. We will merely ask whether one can identify *Cannabis sativa* to the exclusion of all other plants by (1) utilizing the protocol suggested by Nakamura and Thornton and recommended by others,[36]

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32. *Id.* at 218.

33. *Id.*

34. *Id.* at 219.

35. *See id.* at 214-15 (describing the various species of the genus *Cannabis*).

36. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) notes the use of both macroscopic and microscopic examinations of cannabis only as methods of analysis in its February 2006 report. SCIENTIFIC WORKING GROUP FOR THE ANALYSIS OF SEIZED DRUGS (SWGDRUG),

or (2) simply looking at the material and comparing it to one's memory of marijuana seen at the police academy some number of years ago during training, as seems to be the trend in the justice system at this time.

II. THE PROTOCOL

Nakamura's 1969 paper presented a protocol for analysis of seized alleged marijuana samples as follows:

> A leaf specimen (100 mg sample) was macerated in 25 ml petroleum ether, filtered into a beaker, evaporated to dryness without heating, and tested by the Duq. L test as described by Butler.
>
> For morphological examination, leaf specimens were studied under stereoscopic binoculars, 10 to 50X, and a simple compound microscope, 50-100X; 50-100: the subject was illuminated with narrowly directed reflected light of "Flexilight" unit (Iota-Cam Corp., 28 Teal Rd., Wakefield, Mass.) which is capable of producing 3,000-11,000 candle powers.
>
> Photomacrography was conducted through a 16 mm Zeiss Luminar lens mounted on a 35 mm Leica by aid of Visoflex reflex and bellows attachments. Kodak Panatomic film was used. Unless otherwise indicated, all prints were enlarged to a final 60X magnification for all specimens to provide a size comparison.[37]

The analysis is that simple. Nakamura further notes that "[s]ince most marijuana examined in forensic laboratories is crushed and no longer retains gross botanical characteristics, the presence of cystolith hairs on leaf fragments has been used as the principal criterion for morphological identification."[38] What are these cystolith hairs described here? A cross section of a marijuana leaf will reveal the presence of bear-claw-shaped hairs on the top surface of the leaf as well as clothing hairs on the bottom of the leaf.[39] The bear claws should also have large areas containing amorphous (noncrystalline) calcium carbonate in their base.[40] Some of the clothing hairs will also have cystoliths that are smaller than the cystoliths in the bear claws. Though not exactly like the image

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RECOMMENDATIONS 14 (2d ed. 2006), *available at* http://www.swgdrug.org/OLD/SWGDRUG%20Recommendations_080907.pdf

The United Nations' 1987 pamphlet *Recommended Methods for Testing Cannabis, Manual for Use by National Narcotics Laboratories* recommends the examination of macroscopic and microscopic features of suspected marijuana as well as the use of the Duquenois-Levine test. DIVISION OF NARCOTIC DRUGS, UNITED NATIONS, RECOMMENDED METHODS FOR TESTING CANNABIS: MANUAL FOR USE BY NATIONAL NARCOTICS LABORATORIES 19-23, 26 (1987), *available at* http://www.unodc.org/pdf/publications/report_cannabistest_1987-02-01_1.pdf.

37. Nakamura, *supra* note 7, at 6 (footnote omitted).

38. *Id.* at 5.

39. DRUG ANALYSIS BY CHROMATOGRAPHY AND MICROSCOPY 126 (Egon Stahl et al. eds., 1973); *see infra* fig. 1.

40. DRUG ANALYSIS BY CHROMATOGRAPHY AND MICROSCOPY, *supra* note 39.

in Figure 1, Figure 2 shows a bear claw on the upper surface of the leaf and longer hairs on the bottom surface of the leaf.[41]

Figure 1: A photomicrograph of a cross section of a marijuana leaf.[42]

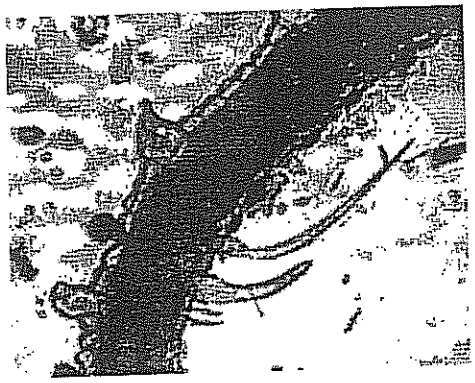
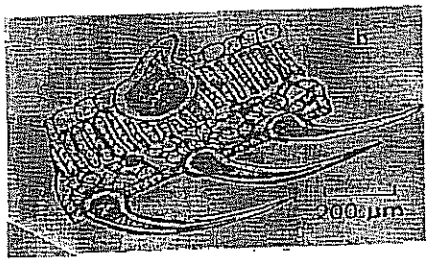


Figure 2: A cross section of a marijuana leaf.[43]



The aim of the forensic analyst is to observe these features through observations, through microscopic analysis of a suspected marijuana leaf, or through simply microscopically observing the top and bottom of the leaf.[44] Nakamura notes the importance of microscopic examination:

> Only after a studied examination, under high magnification, can the cystolith hairs of marihuana be tentatively identified. Microscopic identification of marihuana, therefore, depends not only upon the presence of cystolith hairs but on its association with the longer clothing, or nonglandular hairs, on the other side of the leaf, and if present, the fruits and their hulls, the glandular

41. *Id.*
42. This photomicrograph was taken by the author.
43. *See* DRUG ANALYSIS BY CHROMATOGRAPHY AND MICROSCOPY, *supra* note 39.
44. *See* Nakamura, *supra* note 7, at 15.

> hairs, and the flowering tops as set forth in U.S. Treasury Department Manual. The Duq. L test should be used in final confirmation.[45]

Nakamura's dependence upon not only the cystolithic hairs, but also, if present, the fruits, hulls, glandular hairs, and flowering tops is troubling to the analyst who is left with a choice of a protocol without clear parameters. Who will define a protocol in which the minimum characteristics that need to be detected are clearly described? Do we need to see the fruits, hulls, glandular hairs, and flowering tops, or can we simply stop with the cystolithic hairs?

III. ANALYSIS OF THE PROTOCOL

Critical review of Nakamura's paper leads one to question his numerical data. The number of dicotyledons, 31,874, is a particularly intriguing figure. Nakamura cites the authors Solereder, Metcalfe, Chalk, and Hayward when discussing this figure.[46] A review of Solereder's book immediately reveals that it was written in 1908.[47] If Solereder's information is the basis for the number of dicotyledons noted by Nakamura, then one must wonder if botanists have discovered any new dicotyledons in the past one hundred years. Solereder's book was not the principal source of the number of dicotyledons but rather of discussions concerning the antagonistic relation between the size of cystoliths and the size of the hairs in which they are found.[48] This is useful information when examining bear claws and clothing hairs, which vary in length, but it does not help determine the origin of the number 31,874.

Hayward's book also does not provide a clue as to where Nakamura came up with his figure, but it does give us a very in-depth description of the complete marijuana plant.[49] Hayward wrote the book in 1938[50] during the Great Depression, which begs the question as to how many resources he actually had at his disposal during that time to thoroughly investigate *Cannabis sativa*.

Both editions of Metcalfe and Chalk's *Anatomy of Dicotyledons* provide a list of plant families in which certain diagnostic features occur.[51] The book lists, in particular, the families of plants that contain simple (unbranched) short

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45. *Id.* at 16 (footnote omitted).

46. *See id* (listing references upon which his paper relies).

47. HANS SOLEREDER, SYSTEMATIC ANATOMY OF THE DICOTYLEDONS: A HANDBOOK FOR LABORATORIES OF PURE AND APPLIED BOTANY (D.H. Scott ed., L.A. Boodle trans., 1908).

48. *See id.* at 11-12. The bear claws are shorter than the clothing hairs and have larger cystolithic deposits in them. *Id.* at 11.

49. *See generally* HAYWARD, *supra* note 31, at 214-45 (describing the general morphology of cannabis *sativa*).

50. *See id.*

51. *See* R.C. METCALFE & L. CHALK, ANATOMY OF DICOTYLEDONS: LEAVES, STEM, AND WOOD IN RELATION TO TAXONOMY 1326-59 (1st ed. 1950); *List of Families in which Certain Diagnostic Features Occur*, *in* 1 ANATOMY OF THE DICOTYLEDONS, *supra* note 29, at 190-221.

hairs, as well as those which contain simple long hairs.[52] We can reasonably infer that Metcalfe and Chalk were the original source from which Nakamura derived his number of dicotyledons. Nakamura probably cross referenced those plant families that had both types of hairs, determined the number of species in each family, and added up those numbers. But Metcalfe and Chalk published their first edition in 1950,[53] so one must question the thoroughness of information given that it is over fifty years old. Possibly more dicotyledons have been discovered and classified since then.

So how many dicotyledons are known today? Is it still 31,874, or have scientists discovered more species? Robert F. Thorne reports that there are 199,350 known species of dicotyledons.[54] Thorne also notes the disagreement within the scientific community concerning the size of this number but cites other researchers as proffering numbers of flowering plants between 200,000 and 400,000.[55] Obviously, botanists have been rather busy in the past fifty years, and many more flowering plants have been discovered and classified. What does this mean for identifying marijuana based on its botanical features and for the reaction of a plant to the Duquenois-Levine test? Nakamura responds with the following:

> Representative species that bear cystolith hairs or hairs accompanied by independent calcified growth in the leaf, most of which are similar in structure to those of *Cannabis*, are listed below. (No attempt was made to prepare a comprehensive listing because of the sheer magnitude of the task of examining 31,874 dicotyledons . . . .)[56]

Nakamura microscopically examined 600 of the 31,874 dicotyledons and found that he could not differentiate eighty-two of those using his microscope.[57] He then subjected those eighty-two to the Duquenois-Levine test and found that only one of them gave a positive for marijuana—the marijuana itself.[58] But Nakamura admitted that the "sheer magnitude" of examining all known dicotyledons prohibited him from examining them all.[59] We are then left without really knowing how many plants other than the 600 microscopically examined would have given false positive results for the presence of marijuana. Nakamura's paper is as unclear about this as the 1972 paper by Nakamura and Thornton.[60]

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52. *See* METCALFE & CHALK, *supra* note 51, at 1326-29 (listing types of hair); *List of Families in which Certain Diagnostic Features Occur*, *supra* note 51, at 190-93.
53. METCALFE & CHALK, *supra* note 51.
54. Thorne, *supra* note 19, at 511.
55. *Id.*
56. Nakamura, *supra* note 7, at 15.
57. *See id.* at 5.
58. *See id.* at 5, 15.
59. *Id.* at 15.
60. *See id* at 5, 15; Thornton & Nakamura, *supra* note 7, at 15.

The number of possible alternative plants that may have cystolithic hairs of the same description as marijuana has expanded significantly.[61] If we were to apply the same analytical scheme to the 199,350 plants proposed by Thorne,[62] what would be the result? I have found nothing in the forensic or scientific literature that discusses this issue. Papers that exist assume that the only plant that will give a positive test for marijuana using the Nakamura/Thornton protocol is marijuana.[63] When we identify marijuana we declare that the features seen and the data collected is unique to marijuana to the exclusion of all other plants.[64] Can we say that today? Can law enforcement officers without any training, experience, or education in botany—not to mention the taxonomic features of plants—say that what they are seeing in the seized evidence is marijuana to the exclusion of all other plants? Can forensic lab examiners, after having detected the presence of bear claws and clothing hairs on leaf surfaces, and then subjecting the material to the Duquenois-Levine test, say that those tests uniquely identify marijuana to the exclusion of all other plants? According to Thorne there are 199,349 other plants that share characteristics with marijuana.[65] Have we tested all of them?

Let's consider what that testing would entail. First, we need to acquire the plant specimens themselves, which is not a simple task. This requires travelling to an arboretum, or a number of them, and asking for specimens of plants whose leaves have hairs similar to those found on marijuana leaves. We need to know the names of those specimens so that they are easy to locate. Then we have to microscopically analyze each of those plants that have been determined to have long and short single-celled nonglandular hairs.[66] Although Nakamura microscopically tested 600 of 31,874 dicotyledons based on the classification of Metcalfe and Chalk,[67] that does not mean that we would necessarily test 600, 6,000, or 60,000. We cannot infer that simply because we have expanded our database by a factor roughly of six that we will then have to microscopically analyze six times 600 species, or 3,600 species. We just will not know the number of plants that we must analyze until we find which new species have those hairs.

The analysis also entails the use of the Duquenois-Levine test, which gives rise to another level of complexity.[68] At approximately the same time that Nakamura and Thornton were publishing their study of marijuana analysis, Fochtman and Winek of the Toxicology Department of the Allegheny County (Pa.) Coroner's Office published a note concerning marijuana testing and the

61. *See* Thorne, *supra* note 19, at 511.
62. *Id.*
63. *See* Thornton & Nakamura, *supra* note 7, at 461-65.
64. *See* Nakamura, *supra* note 7.
65. *Cf.* Thorne, *supra* note 19, at 511 (noting the number of dicotyledons).
66. *See* Nakamura, *supra* note 7, at 6.
67. *Id.* at 5.
68. *See infra* text accompanying notes 83-87.

Duquenois-Levine test.[69] Although the Duquenois-Levine test had been used routinely over the past several decades, Fochtman and Winek recommend that identification be made after the use of microscopic and chemical analysis because of the importance of positive identification of marijuana.[70] They recommend the use of a thin layer chromatography or gas chromatography for the positive identification of the cannabinols in marijuana and specifically advised that "[t]he microscopic and Duquenois-Levine chemical test should be used as a screening method only."[71]

C.G. Pitt, working under a grant from the Law Enforcement Association Agency and the State of North Carolina, also agrees with Fochtman and Winek regarding the need for chromatographic testing:

> In conclusion, it is believed that if the criteria for a positive Duquenois test are rigorously adhered to, and botanical evidence is also available, then the Duquenois color test is a reliable screen for cannabinoids. However if botanical evidence is not available, the ubiquitousness of phenols in nature and their diversity of structure makes it mandatory to supplement the colorimetric test with chromatographic evidence. This conclusion is substantiated by [Fochtman's recent report] that certain commercial brands of coffee give a positive Duquenois-Levine color test.[72]

Thornton and Nakamura seem to disagree with these conclusions regarding chromatographic testing.[73] They note that "although a rigorous identification of the marijuana plant may be effected through an examination of its botanical characteristics, it is generally considered advisable to perform a chemical test in most instances, and necessary to perform it in others."[74] While they go on to note that "the Duquenois test, the most widely used chemical test, is a somewhat enigmatic reaction whose mechanism is poorly understood," one is led to believe that the protocol can be used to rigorously identify marijuana.[75] They did not, however, address the issue of possible false positives.[76]

The theme of identity continues through the paper of Hughes and Warner, Drug Enforcement Administration chemists with the Mid-Atlantic Regional Laboratory in Washington, DC.[77] Despite testing a limited number of materials

69. Fredrick W. Fochtman & Charles L. Winek, *A Note on the Duquenois-Levine Test for Marijuana*, 4 CLINICAL TOXICOLOGY 287, 287-89 (1971).

70. *Id.* at 288-89.

71. *Id.* at 289.

72. C.G. Pitt et al., *The Specificity of the Duquenois Color Test for Marijuana and Hashish*, 17 J. FORENSIC SCI. 693, 699 (1972) (footnotes omitted).

73. *See generally* Thornton & Nakamura, *supra* note 7 (discussing the benefits of chemical analysis on cannabis samples).

74. *Id.* at 461.

75. *Id.* at 462.

76. *See id.* at 461-62 (failing to discuss the problem of false positive outcomes with the Duquenois-Levine test).

77. R.B. Hughes & V.J. Warner, *A Study of False Positives in the Chemical Identification of Marihuana*, 23 J. FORENSIC SCI. 304 (1978).

and presenting no data concerning the number of possible chemicals one might find in the plant kingdom, Hughes and Warner, with a flair for the ipse dixit, note that "if glandular, clothing, and unicellular cystolithic hairs are present then either a modified Duquenois-Levine test or TLC when sprayed with Fast Blue B salt are positive evidence that cannabis is present in the sample."[78] They do not say that cannabis is conclusively present, just that the test results are positive evidence that cannabis is present.[79] This is akin to saying that because my car has four tires there is positive evidence that my car was involved in the bank robbery where a car with four tires was used as a get-away vehicle.

The myth involving the infallibility of the Duquenois-Levine test is passed on in such papers as that written by Coutts and Jones. They cite Pitt as stating that "[f]ew, if any, other plant products react identically in the Duquenois-Levine test."[80] Without reading the Pitt paper, we would be left with the impression that we had the solution to this identification issue. We would not know of the pitifully small number of samples Pitt actually tested, nor would we have any idea of the significance of the number of chemicals found in plants.[81]

IV. THE KURZMAN MYSTERY

This critique of marijuana testing follows Marc G. Kurzman and Dwight S. Fullerton's paper, *Winning Strategies for Defense of Marijuana Cases: Chemical and Botanical Issues*.[82] For any scientist, the title of this paper alone is a strong warning that the contents are biased, are meant as winning strategies, and may be suspect. But this long and detailed treatise lays out the fundamental flaws in the classical forensic marijuana analytical scheme so clearly that even lay readers can understand.[83] This paper is not a trick to be played on unprepared prosecutors and triers of fact but instead is actually a thorough study of the problem.[84] Because Kurzman wrote his paper in 1975, it would seem that the use of the hairs on marijuana leaves and the purple alchemy of the Duquenois-Levine test would have long since been successfully challenged and would no longer be useful as evidence in courts of law.[85] At the very least, one would hope that the original experimental design proposed by Nakamura would be revisited, and that a proper analysis would be conducted of

78. *Id.* at 309. Hughes and Warner limited their study to those substances reported to give a positive response for marijuana under various tests, such as the Duquenois-Levine test. *Id.* at 304

79. *See id.*

80. R.T. Coutts & G.R. Jones, *A Comparative Analysis of Cannabis*, 24 J. FORENSIC SCI. 291, 291 (1978) (citing Pitt et al., *supra* note 72, at 693-700).

81. *See* Pitt et al., *supra* note 72, at 694-99.

82. M.G. Kurzman & D.S. Fullerton, *Winning Strategies for Defense of Marijuana Cases: Chemical and Botanical Issue*, 1 NAT'L J. CRIM. DEF. 487, 522-31 (1975).

83. *See generally id.* (giving a comprehensive look at the successful acquittals of marijuana possession cases, the methods which identify cannabis, the inconsistencies in the law, and forensic analyses).

84. *See id.* at 489.

85. *See id.* at 518, 522.

the over 250,000[86] flowering plants known at this time. But this has not happened. In fact, many jurisdictions still only conduct microscopic analysis and chemical tests.[87] In some jurisdictions, identification is even carried out by law enforcement officers with no more than visual analysis, and suspected marijuana is never even sent to a crime lab.[88] The issue that we are left with in this mystery is stated so well by Tobin and Thompson:

> [T]he next step for assessment of forensic significance involves estimation of probabilities for determination of probative value. As noted earlier, there are two crucial questions: (1) how likely are the observed results if the samples had a common source; and (2) how likely are the observed results if the samples did *not* have a common source?[89]

A review of the scientific literature concerning the identification of marijuana by utilizing microscopic analysis of cystolithic hairs on alleged marijuana leaves, as well as the chemical test known as the Duquenois-Levine test, reveals that the validity of the results is unknown if we do not know whether the samples did or did not have a common source.[90] There has not been enough basic research nor was the protocol properly validated as time went on.[91] The Kurzman mystery here is simple: why is this protocol still being utilized to decide whether human beings should be confined to cages and at times, to death chambers?

V. SUMMARY

In 1969 and 1972, George Nakamura and John Thornton published scientific papers that were based on good logic, employed a disciplined approach to a very real problem, and offered a good protocol for the analysis of marijuana.[92] That protocol depended upon the knowledge available to them at the time.[93] Neither Nakamura nor Thornton was a botanist; however, their logic was correct. They were attempting to identify crushed up plant material as marijuana to the exclusion of all other dicotyledon plants.[94] Dr. Nakamura's review of the scientific literature revealed the presence of 31,874

86. *See* Thorne, *supra* note 19, at 511.

87. *See* Paul Giannelli, *Expert Testimony and the Confrontation Clause*, 22 CAP. U. L. REV. 45, 57-58 (1993).

88. M.D. Blanchard & G.J. Chin, *The Enemy in the War on Drugs: A Critique of the Developing Rule Permitting Visual Identification of Indescript White Powder in Narcotics Prosecutions*, 47 AM. U.L. REV. 557, 588-89 (1998).

89. William A. Tobin & William C. Thompson, *Evaluating and Challenging Forensic Identification Evidence*, CHAMPION, July 2006, at 12, 17.

90. *See id.*

91. *See id.*

92. *See* Nakamura, *supra* note 7.

93. *See id.*

94. *See* Thornton & Nakamura, *supra* note 7, at 461.

dicotyledons.[95] After determining that there were 600 dicotyledons that had trichomes on the surfaces of the leaves, he microscopically observed those 600 plants but could not microscopically differentiate eighty-two of them.[96] He then applied a chemical test which resulted in only one plant, *Cannabis sativa*, of the 31,874 considered, passing through the sieve of his protocol.[97]

In 1975, Marc Kurzman and coauthors published a critical review of the Nakamura/Thornton papers that has essentially been ignored by the legal and scientific community.[98] This gave courts the answer to a vexing problem, but courts did not question the validity of the Nakamura/Thornton protocol. Though a number of other authors have noted issues with the original protocol, forensic crime laboratories across the United States continue to use the original Nakamura/Thornton protocol to identify marijuana.[99] Questions about validity were not dealt with in such a manner that the protocol was serviced.[100] Servicing would have required newly discovered plant species to undergo the Nakamura/Thornton protocol to determine if they passed the gauntlet of microscopic and chemical analyses resulting in false positives.[101] But this never happened.

Considering the marijuana identification problem as a model leaves us with another question: Has anyone ever established an effective mechanism within the justice system to determine if scientific protocols used to determine the truth are actually valid? Unless we know if we are getting the right answers from scientific laboratories, how will we know if we are convicting the innocent based on flawed scientific evidence? When our answer to this question is that defendants in courts of law are allowed to review the evidence against them to stop flawed scientific evidence from being admitted in court, then the naiveté of the court system is exposed. Judges and lawyers without scientific credentials have obviously failed to detect serious flaws in crime laboratories as long as crime labs have existed.[102]

The model presented by forensic marijuana analysis in our attempt to understand how innocent citizens can be convicted of crime is clear.[103] We are

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95. *See* Nakamura, *supra* note 7, at 6.

96. *See id.* at 5.

97. *See id.*

98. *See* Kurzman & Fullerton, *supra* note 82.

99. *See generally id.* (discussing methods and inconsistencies in the identification and law of marijuana).

100. *See id.*

101. *See id.* at 488-92 (discussing proper procedures for testing protocols).

102. *See id.* at 488.

103. Ryan King and Marc Mauer report the recent marijuana statistics in their treatise:

> Of the 450,000 increase in drug arrests during the period 1990-2002, 82% of the growth was for marijuana, and 79% for marijuana possession alone;
>
> Marijuana arrests now constitute nearly half (45%) of the 1.5 million drug arrests annually;
>
> Few marijuana arrests are for serious offending: of the 734,000 marijuana arrest in 2000, only 41,000 (6%) resulted in felony conviction . . . .

KING & MAUER, *supra* note 4.

arresting vast numbers of citizens for the possession of a substance that we cannot identify by utilizing the forensic protocol that is presently in use in most crime labs in the United States.[104] We have no idea what the error rate of marijuana analysis is despite professed concerns of our justice system for fairness and a need to determine the probative value of evidence put before it. Do we really care about innocence?

104. *See generally* Kurzman & Fullerton, *supra* note 82 (discussing methods and inconsistencies in the identification and law of marijuana).

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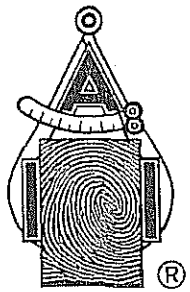

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3. Brennan, J.; Bramble, S.; Crabtree, S.; Wright, G. Fuming of Latent Fingerprints using Dimethylaminocinnamaldehyde. *J. For. Ident.* **1995**, *45* (4), 373-380
4. Katzung, W. Translated version of Neue Reagenzien zur Chemicschen Sichtbarmachung Latenter Papillarleistenspuren auf Papier und Möglichkeiten ihrer Anwendung (New Reagents for the Chemical Development of Latent Fingerprints on Paper and Their Possible Applications). *Krimiminalistik und For. Wissenschaften* **1985**, 58, 82-89.
5. Sasson, Y.; Almog, J. Chemical Reagents for the Development of Latent Fingerprints. 1: Scope and Limitations of the Reagent 4-Dimethylamino-Cinnamaldehyde. *J. For. Sci.* **1978**, *23* (4), 852
6. Brennan, J. S. The Development of Fingerprints by Fuming with Dimethylaminocinnemaldehyde (DMAC). Proceedings of the International Symposium on Fingerprint Detection and Identification, Ne'urim, Israel, June 1995, 85-90.
7. Creer, K. E. Concept into a Successful Aid to Major Crime Scene Management. Presented at 11th Int. ICPO-Interpol Forensic Science Symposium, November 1995.
8. Ramotowski, R. Fluorescence Visualization of Latent Fingerprints on Paper Using p-Dimethylaminocinnamaldehyde (PDMAC). Proceedings of the International Symposium on Fingerprint Detection and Identification, Ne'urim, Israel, June 1995, 91-94.
9. Flynn, J.; Stoilovic, M.; Lennard, C. Detection and Enhancement of Latent Fingerprints on Polymer Banknotes: A Preliminary Study. *J. For. Ident.* **1999**, *49* (6), 594-613.
10. Stimac, J. T. Thermal & Carbonless Papers: A Fundamental Understanding for Latent Friction Ridge Development. *J. For. Ident.* **2003**, *53* (2), 185-197.
11. Rongliang, M.; Qun, W. Chemical Fuming: A Practical Method for Fingerprint Development on Thermal Paper. *J. For. Ident.* **2006**, *56* (3), 364-373.
12. Bowman, V., Ed. *Manual of Fingerprint Development Techniques*, 2nd ed.; Home Office, Police Scientific Development Branch: Sandridge, U.K., revised 2004.
13. Takatsu, M.; Kageyama, H.; Hirata, K.; Akashi, T.; Yoko Ta, T.; Shiitani, M.; Kobayashi, A. Development of a New Method to Detect Latent Fingerprints on Thermal Paper with o-alkyl Derivative of Ninhydrin. Report of the National Research Institute of Police Science, Japan, 1991, 44, 1-6.
14. Lee, J. Evaluation of ThermaNin, Final Year Project Report, University of Lincoln, June 2007.

Article

The Visual Characterization and Identification of *Cannabis sativa* (Marijuana) Seeds

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Abstract: A study was conducted to create a visual standard and basis for the comparison and identification of seeds of *Cannabis sativa* (marijuana) based on their appearance. *Humulus* (hop) seeds were examined, because *Humulus* is phylogenetically related to *Cannabis sativa* and is the only other genus in the Cannabinaceae family. Seeds of other plants whose leaf material had been previously shown to have some similarity to the leaf material of *Cannabis sativa* were examined, and additionally, a survey of approximately one thousand other seeds was conducted to ascertain whether other seeds exist that could reasonably be confused with *Cannabis sativa*. This work is intended to give forensic workers more complete information relative to the visual identification of marijuana seeds.

Introduction

Within the forensic science literature, and for that matter, within the botanical literature as well, there is very little documentation concerning the identification of marijuana seeds. Although certainly the seeds have been described, the descriptions tend to be brief, and in the forensic literature, there has never been a systematic study to address the issue of whether other seeds have a similar appearance to that of marijuana. Under federal law (21 U.S.C. § 502 (16)), possession of *Cannabis sativa* seeds is illegal, and under some state laws (e.g., California

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Uniform Controlled Substances Act § 11018), possession is unequivocally illegal or illegal unless the seeds are sterile and incapable of germination. The identification of seeds as being those of marijuana and not of any other plant may therefore be an element of a criminal prosecution. In a forensic laboratory, using a chemical test to identify the seeds is problematic at the very least, because the seeds rarely test positive for the presence of cannabinoids by means of the classic Butler (Levine)-modified Duquenois-Negm test unless they have come into direct contact with the foliage or buds of the *C. sativa* plant.

The present work sought to create a visual standard and a basis for comparison for the appearance and identification of marijuana seeds and to relate the visual appearance of marijuana seeds to other seeds that could possibly be mistaken for marijuana. It is hoped that this study will help the forensic science and botany communities correctly identify *C. sativa* seeds and give these communities a basis for comparison to the seeds of other plant species, both similar and dissimilar to *C. sativa*. It is also hoped that this will help to eliminate any confusion surrounding the appearance and characterization of marijuana seeds and augment the confidence with which *C. sativa* seed identifications are made.

The classic work on the identification of marijuana is *Marihuana, Its Identification* [1], a 1938 publication of the U.S. Treasury Department Bureau of Narcotics. Forensic workers as well as other personnel in law enforcement rely heavily on this guide. The publication is almost exclusively devoted to detailed descriptions of the foliage and flowering tops of the plant, and very little information and photographic documentation is devoted to the seeds. Curiously, within the forensic science community, there has not been a study of the appearance of marijuana seeds in comparison to other seeds. Given the fact that chemical tests for marijuana are essentially inapplicable to the identification of *C. sativa* seeds, the identification must proceed by means of a microscopic examination, and clearly it is very important to rule out other possible seed identities. At the present, an expert witness testifying in court would have virtually no basis to answer questions such as, What else could this seed have been? What other seeds closely resemble marijuana seeds? It would be especially beneficial to the expert witness to have some knowledge of seeds that have a similar appearance to marijuana seeds, as well as some knowledge as to whether plants that have a known similarity to *C. sativa* based on the microscopic appearance of their leaf material also share a similarity in the appearance of their seeds.

Other works [2, 3] treat the subject of *C. sativa* seeds, but are silent with respect to any comparison to seeds of other species. The number of "other species" is immense. Thorne [4] gives the number as approximately 260,000 species in 13,553 accepted genera.

Humulus (hop) is phylogenetically related to *C. sativa*, being the only other genus in the Cannabinaceae family. The forensic literature has heretofore not provided illustrations of hop seeds.

The 1972 work of Thornton and Nakamura [5] indicated that a number of plants have a similar appearance to marijuana with respect to structures of the leaf material as seen under the microscope. The occurrence of cystolith hairs is an important criterion in the identification of marijuana leaf material. Cystolith hairs were reported to occur among several dicotyledonous families, notably the Moraceae (mulberry), Boraginaceae (forget-me-not), Loasaceae (evening star), and Ulmaceae (elm) families. Other potentially similar plants, with respect to leaf material, are in families Urticaceae (nettle), Verbenaceae (verbena), Cucurbitaceae (gourd), Acanthaceae (acanthus), and Campanulaceae (bellflower). However, no comparison has heretofore been made between *C. sativa* seeds and the seeds of these other plant families.

Method

C. sativa seeds were acquired from the California Department of Food and Agriculture (CDFA) Seed Herbarium in Sacramento, California, and the Napa, California, Sheriff's Department. A Leica EZ4D stereoscopic microscope with integral digital camera was used to photograph the seeds. The seeds were photographed on a background of millimeter square graph paper for purposes of measurement. Seeds of other plants were obtained from the CDFA Seed Herbarium. The CDFA Seed Herbarium houses the second largest seed collection in North America.

The present study addressed three issues:

1. Do hop seeds show any similarity to marijuana seeds, given that *Humulus* is the closest plant to *C. sativa* in the phylogenetic tree?
2. Do any of the plants described by Thornton and Nakamura [5] as having leaf material in some way similar to that of *C. sativa* share a similar appearance with *C. sativa* with respect to seeds?
3. In an accessible reference collection of the seeds of a large number of plants, such as the collection maintained by the CDFA Seed Herbarium, are there other seeds with similar appearance to marijuana?

Results and Discussion

The seeds of approximately one thousand seed plant species were examined. Based on Thorne's approximation of there being approximately 260,000 accepted seed plant species, approximately 0.4% of the seed plant population was examined. However, the CDFA collection includes most of the common agronomically significant seeds.

Cannabis sativa Seeds

For a point of reference, *C. sativa* seeds were examined and photographed. They measure approximately 4 to 5 mm long by 3 to 4 mm wide and are ovoid in shape. However, there is a possibility that *C. sativa* seed dimensions could vary depending on whether or not the seed is wet or on the freshness of the seed. A ridge runs around the circumference on one axis only. They range in color from golden yellow to dark brown, with golden brown to black marbling on the seed shell. The marbling is characteristic and distinctive, but is difficult to subjectively describe in words. The marbling is a lacy network, but a photograph (Figures 1 and 2) will invariably be more appropriate than a verbal description.

Humulus Seeds

Humulus is the only other genus in the Cannabinaceae family besides *C. sativa*. Species examined included *Humulus lupulus* (common name: common hop) and *Humulus japonica* (common name: Japanese hop). The botanical difference between *Humulus* and *C. sativa* has been described by Nakamura [6]. Though both plants have bear-claw shaped cystolith hairs, *Humulus* is differentiated from *C. sativa* by the presence of distinctive two-armed unicellular hairs seated on the epidermis directly or on a multicellular pedestal. A comparison between *C. sativa* and *H. lupulus* shows a slight resemblance between the two seeds. *H. lupulus* seeds are generally a bit smaller, measuring approximately 3 mm long by 3 mm wide, and they are more round in shape without the ridge running around the circumference of the seed on one axis as seen in *C. sativa* seeds. They have dark brown coloring, and dark marbling can sometimes be seen on the seed shell, similar to but distinguishable from the markings of *C. sativa*. The *H. lupulus* seeds (Figure 3) tend to have a lighter brown, rough outer covering (the hull) over the smooth seed shell.

Humulus japonica seeds (Figure 4) are about the same size as *H. lupulus* seeds and are also spherical in shape. They are dark brown to black in color, and there is a consistent overlapping pattern to their seed shell, resembling the scales of a snake. The scaling pattern ranges in colors from gray to dark brown. To the naked eye, there are visual similarities between marijuana and hop seeds (similar sizes, coloring, and the presence of surface features). Under the stereomicroscope, however, there is no reasonable mistaking the difference between the two.

Seeds of Plants with a Similar Leaf Material Appearance to Cannabis sativa Plants

Seeds from plants with a botanical appearance similar (but not identical) to marijuana with respect to leaf material were examined and photographed. These plants were chosen based on the 1969 work of Nakamura [6] and the 1972 work of Thornton and Nakamura [5], where plants with cystolith hairs, leaf hairs, and so-called stinging hairs were microscopically compared to *C. sativa*. Urticaceae (nettles), Moraceae (mulberry), and Ulmaceae (elm) are taxonomically closely related to the Cannabinaceae (hemp) family.

Moraceae Family

The seeds that were examined from plants in the Moraceae (mulberry) family included *Broussonetia papyrifera* (common name: paper mulberry) and *Ficus carica* (common name: edible fig).

B. papyrifera seeds (Figure 5) measure approximately 2 mm by 2 mm and are spherical in shape. The seeds range in color from golden brown to dark brown, and the seed surface is covered in small bumps. Based on appearance, it is unlikely that marijuana seeds would be confused with paper mulberry seeds. *F. carica* seeds (Figure 6) are golden yellow to orange in color. They measure approximately 1.5 mm by 1.5 mm and are also spherical in shape. The seeds have a rough surface with small dimples, reminiscent of an orange peel. Based on the differences in size and seed color, it would be nearly impossible to mistake *F. carica* seeds for marijuana seeds.

Loasaceae Family

The seeds that were examined from plants in the Loasaceae (evening star) family included *Cajophora lateritia* (common name: Chile nettle) and *Petalonyx thurberi* (common name sandpaper plant). *C. lateritia* seeds (Figure 7) are quite small, measuring approximately 0.5 mm wide by 1 mm long. They are irregularly shaped and are dark brown with small, light brown craters covering the entire surface of the seed. The seeds are distinct looking and share no similarities with *C. sativa* seeds. *P. thurberi* seeds (Figure 8) are cream colored, and have a very distinct appearance. They are covered in small, spiny, thistle-like structures and have the shape of a budding flower. They are larger than marijuana seeds, measuring approximately 5 mm by 5 mm. *P. thurberi* and *C. sativa* seeds share no similarities in their appearances.

Boraginaceae Family

The seeds that were examined from plants in the Boraginaceae (borage) family included *Anchusa officinalis* (common name: common bugloss) and *Lithospermum officinalis* (common name: common gromwell). *A. officinalis* seeds (Figure 9) measure approximately 2 mm wide by 2.5 mm long. They are irregularly shaped and are dark brown in color with small, light brown bumps covering the entire seed surface. Some of the seed edges have ridges. The seeds do not possess a similar appearance to *C. sativa* seeds. *L. officinalis* seeds (Figure 10) are white, and the seed surface is very shiny and smooth. The seeds measure approximately 3 mm long by 3 mm wide and are ovoid in shape. Although their size and shape are similar to *C. sativa* seeds, the differences in their color and markings makes it easy to distinguish between the two seeds.

Urticaceae Family

The seeds that were examined from plants in the Urticaceae (nettle) family included *Urtica dioica* with stinging hairs (common name: stinging nettle), *Urtica urens* with stinging hairs (common name: dwarf nettle), and *Boehmeria nivea* (common name: Chinese grass). *U. dioica* (Figure 11) seeds are light brown in color and ovoid in shape. They have small hairs covering the entire seed surface, and the seeds measure approximately 1.5 mm by 1 mm, which is significantly smaller than marijuana seeds. There are small "wings" emerging from each side at the base of the seed. The small hairs also cover the "wings". *U. dioica* and *C. sativa* seeds share no similarities in appearance. *U. urens* seeds (Figure 12) are very similar to *U. dioica* seeds. They are deep green in color, however, and do not appear to have the "wings" coming off each side at the base of the seed. *U. urens* seeds could not reasonably be visually mistaken for *C. sativa* seeds. *B. nivea* seeds (Figure 13) are very small, measuring approximately 0.75 mm by 0.75 mm. They are brown in color and have small hairs covering the seed surface. They are also ovoid in shape, but the extreme differences in size and texture would prevent these seeds from being confused for marijuana seeds. It is possible that the accompanying photographs and the foregoing descriptions above of *U. dioica*, *U. urens*, and *B. nivea* seeds are not describing the naked seeds. It is possible that the naked seed resides inside of a hair-covered hull; however, these seed specimens are so small that a dissection could not be performed to make this determination, and in any event, they will not be reasonably confused with *C. sativa*.

Verbenaceae Family

The seeds that were examined from plants in the Verbenaceae (verbena) family included only *Lantana camara* (common name: lantana). *L. camara* seeds (Figure 14) measure approximately 4.5 mm long by 4 mm wide. They are dark brown to black in color. They are irregularly shaped with small, dark craters covering the entire seed surface. These characteristics would prevent *L. camara* seeds from being mistakenly identified as *C. sativa* seeds.

Acanthaceae Family

The seeds that were examined from plants in the Acanthaceae family included only *Acanthus mollis* (common name: bear's breech). *A. mollis* seeds (Figure 15) are, relatively speaking, very large; they measure approximately 12 mm by 8 mm. They are dark brown in color. They are circular in shape, but flat. They have rippling along the entire seed surface that looks similar to water ripples in a body of water. Based on size alone, there is no credible possibility of *A. mollis* seeds being mistaken for marijuana seeds.

For this study, there were several plant specimens mentioned in the work of Thornton and Nakamura [5] that could not be procured, either at the CDFA Seed Herbarium or the University of California Davis Herbarium. These were the species: *Dorstenia contrajerva* (common names: contra heirba or tusilla - Moraceae family), *Morus nigra* (common name: black mulberry - Moraceae family), *Loasa chelidonifolia* (Loasaceae family), *Eucnide lobata* (Loasaceae family), *Mentzelia albescens* (common name: wavy leaf blazing star - Loasaceae family), *Symphytum officinalis* (common name: comfrey - Boraginaceae family), *Lithospermum purpurocaeruleum* (common name: purple gromwell - Boraginaceae family), *Cordia gerascanthus* (common name: yauco - Boraginaceae family), *Tournefortia scabra* (common name: West Indian soldier brush - Boraginaceae family), *Parietaria officinalis* (common name: upright pellitory - Urticaceae family), *Pilea pubescens* (Urticaceae family), *Lippia citridora* (Verbenaceae family), *Ulmus campestris* (Ulmaceae family), *Celtis occidentalis* (common name: common hackberry - Ulmaceae family), *Melothria gualapensis* (Cucurbitaceae family), and *Campanula americana* (common name: American bellflower - Campanulaceae family). It is disappointing that these seeds are not accessible, but reflects the reality of the situation with respect to seed collections. Jim Effenberger, Senior Seed Botanist of the CDFA Seed Herbarium states [7]:

> During the past 100 years plant herbaria collection resources and related floristic references have dramatically improved in size and diversity. Conversely, during the same period associated seed collection resources and corresponding descriptive references have expanded at a much slower rate and many are limited to seeds primarily from agricultural crops and weeds. This restriction of reference resources has created significant issues contributing to the complexities of identifying and describing unknown seed specimens including: the lack of seed specimens matching existing herbarium specimens and corresponding written morphological descriptions; the sparse geographical distribution of seed collections; and other ensuing problems involved with accessing limited resources, collecting, preserving and photographing appropriate specimen material.
>
> The Federal government's Noxious Weed Seed Project initiated by the United States Department of Agriculture in 2001 to create useable photographs and written descriptions of Federal Noxious Weed Seeds experienced delays and accessibility problems due to the limited collection resources of seed specimens. Approximately 98 species of noxious weed seeds were included on the project list for identification. Over a period of many months the Supervising Botanist was compelled to access numerous herbaria and seed collections in different States in order to obtain accurate comprehensive photographic documentation. Many of the seed collections lacked written descriptions and thus required development by the Project. Completion of the project spanned more than two years as a result of the limited availability of pertinent collection material and references.

Although some of the desired seed specimens could not be examined or described, it is offered that the conclusions of this study are still credible and helpful in the visual characterization and identification of *C. sativa* seeds. Although the *Dorstenia contrajerva* and *Morus nigra* species within the Moraceae family were not examined, the *Broussonetia papyrifera* and *Ficus carica* species within this family were examined. Although the *Parietaria officinalis* and *Pilea pubescens* species within the Urticaceae family were not examined, the *Urtica dioica*, *Urtica urens*, and *Boehmeria nivea* species within this family were examined. Although the seeds of a number of plants within the Boraginaceae family were not available for inspection, the seeds of *Lithospermum officinalis* within this family were examined. In a similar fashion, although a number of the seeds of plants within the Loasaceae family were not available, the seeds of *Cajophora lateritia* and *Petalonyx thurberi* within this family were examined. And although the seeds of *Lippia citridora* within the family Verbenaceae were not examined, the seeds of *Lantana camara* in this family were examined.

Seeds with a Similar Appearance to Cannabis sativa Seeds

For this portion of the study, seeds were examined for similarities to marijuana seeds, independent of plant botany. This was the "shotgun" approach to the question of whether other seeds will show a similarity to those of *C. sativa*. The literature, together with the extended seed collection of the CDFA Seed Herbarium, was surveyed for any seed that might be confused with *C. sativa*. It was found that several plants, unrelated to *C. sativa*, have seeds with a similar appearance to *C. sativa*.

Using the U.S. Department of Agriculture's handbook [8], the photographs of approximately 735 seed species were examined. Using the photographs as a guide, 15 different seed species were selected as potentially being similar in appearance to marijuana seeds based on size, shape, and markings. The 15 seed species were then screened at the CDFA Seed Herbarium and included the following: *Echinochloa colona* (common name: jungle rice), *Digitaria ischaemum* (common name: smooth crabgrass), *Panicum bergii* (common name: Berg's panicgrass), *Panicum miliaceum* (common name: broomcorn millet), *Setaria italica* (common name: foxtail bristlegrass), *Muscari comosum* (common name: tassel grape hyacinth), *Polygonum persicaria* (common name: spotted ladysthumb), *Fumaria officinalis* (common name: drug fumitory), *Cardaria pubescens* (also called *Hymenophysa pubescens* common name: hairy whitetop), *Raphanus sativus* (common name: cultivated radish), *Lathyrus sylvestris* (common name: flat pea), *Lathyrus tuberosus* (common name: tuberous sweetpea), *Pisum sativum* (common name: garden pea), *Vicia sativa* (common name: garden vetch), and *Croton setiger* (common name: dove weed). Based on size, shape, color, and surface markings, none of these seeds have an appearance that could reasonably be confused with *C. sativa*.

Additionally, as part of the "shotgun" approach, the seeds of the CDFA Seed Herbarium were browsed. Several species yielded interesting results. The following seeds were selected while browsing the CDFA seed collection, or at the suggestion of Jim Effenberger, Senior Seed Botanist and AOSA Certified Seed Analyst at the CDFA Seed Herbarium. The first similar looking seed is in the Apiaceae (carrot) family, genus and species *Coriandrum sativum* (common name: coriander or cilantro). *C. sativum* seeds (Figure 16) measure approximately 4.5 mm long by 4 mm wide. They are spherical in shape and have solid, dark brown coloring with random light brown blotches. They have

transverse ridges running from the top to the bottom of the seed. The size, shape, and coloring of coriander seeds are similar to marijuana seeds to the naked eye. However, when viewed under magnification, the ridges and coloring are clearly distinguishable from marijuana seeds. The ridges are a clear feature to distinguish *C. sativum* from *C. sativa*.

Another similar looking seed is in the Chenopodiaceae (goosefoot) family – *Spinacia oleraceae* (common name: spinach). Spinach seeds (Figure 17) measure approximately 4 mm long by 3 mm wide. They are ovoid in shape, but are flatter than marijuana seeds. Their rippled surface gives the appearance of the marbling on a marijuana seed. They are golden brown in color with some randomly placed darker brown spots. Their similarities in size, shape, and perceived color patterns make *S. oleraceae* seeds likely candidates for confusion in unaided visual identification.

Another similar looking seed is in the Fabaceae (legume) family – *Lupinus albifrons* (common name: silver lupine). The silver lupine seeds (Figures 18 and 19) display considerable similarities to marijuana seeds. They measure approximately 5 mm long by 4 mm wide and are ovoid in shape. They are golden brown to dark brown with dark brown marbling on their shell. They do appear to have a glossier surface than most marijuana seeds and, under magnification, it is apparent that the patterns on the seed shell are different from those on marijuana seeds. The marbling appears more mottled than *C. sativa* and lacks the well-defined lacy network characteristic of *C. sativa*.

Additionally, another species (Figures 20 and 21) from the *Lupinus* genus was examined – *Lupinus texensis* (common name: Texas lupine). They, too, are golden brown with dark brown marbling on their shell. However, in comparison with *C. sativa*, they are more round and flat in shape. They measure approximately 4 mm by 4 mm, and they have what looks like a tail at one end of the seed. The seed surface appears to be glossy, as with the silver lupine seeds. To the unaided eye, these seeds could also be misidentified as marijuana seeds.

Another similar looking seed is in the Vitaceae (grape) family – *Vitis girdiana* (common name: desert wild grape). Grape seeds (Figures 22 and 23) may be expected to be within the experience of virtually everyone, unlike many of the other seeds mentioned in this work. Nevertheless, they deserve some

mention. *V. girdiana* seeds measure approximately 4.5 mm long by 4 mm wide. They are dark brown in color and have lighter brown markings and indentations covering the entire seed surface. They have an irregular ovoid shape. To the naked eye, the similarities in color, size, and shape could, under some circumstances, cause confusion with marijuana seeds. However, under the stereomicroscope, the desert wild grape and marijuana seeds could not be credibly confused for one another.

Another similar looking seed is in the Brassicaceae (mustard or cabbage) family – *Crambe abyssinica* (common name: crambe). *C. abyssinica* seeds (Figure 24) have cream to light brown coloring with small, black spots randomly distributed over the seed's surface. They measure approximately 3 mm by 3 mm and are spherical in shape. Their seed surface is textured like an orange peel, and very faint ridges can be seen running from the top to the bottom of the seed. At first glance, the similarities in size and coloring cause them to look similar to marijuana, but the spherical shape allows for differentiation upon a microscopic examination.

Another similar looking seed is in the Euphorbiaceae (spurge) family – *Croton californicus* (common name: California croton). *C. californicus* seeds (Figures 25 and 26) measure approximately 5.5 mm long by 4 mm wide. They are ovoid shaped and have a smooth surface. They are golden brown in color with darker brown marbling covering nearly the entire shell surface. Some of the surface markings appear to be black in color. Like the silver lupine seed, the California croton seed also has one of the most similar appearances to marijuana seeds, although the surface marbling does not have the definition seen on marijuana seeds.

Conclusion

The benefit from this research is the information it will provide to members of the forensic science and botany communities regarding the physical identification and appearance comparison of marijuana seeds. It is hoped that this study will also provide weight and credibility to a potential expert witness testimony regarding the positive identification of marijuana seeds. The expert witness will now have a basis for comparison to other potential similar looking seeds. The present study fills a portion of the gap in *C. sativa* botanical research.

The seeds of *Humulus*, being the closest relative to *C. sativa*, are clearly distinguishable from those of *C. sativa*. To the extent that their seeds were available for the present study, none of the plants whose leaf material is in some way similar to that of *C. sativa* display seeds that cannot be clearly distinguished from *C. sativa*. Seeds that do have a similar appearance to *C. sativa* are unrelated to the *C. sativa* genus and species. Of the approximately 1,000 species surveyed, the Lupine species and the California croton seeds have the most similar appearances to *C. sativa* seeds. Caution should be exercised that these seeds of these species are not confused with *C. sativa*.

It is appropriate here to set forth a caveat, lest the findings of this study be used in support of an offered opinion that the evidence in question is *C. sativa* and no other plant. The identification of *C. sativa*, whether leaf material, buds, or seeds, has always rested on rather fragile and tenuous grounds. The 1972 work by Thornton and Nakamura surveyed 146 genera in 26 families. That work attempted to include common plants and spices, but it cannot be construed as a complete survey of the plant kingdom, and for that reason alone, the botanical identification of leaf material must be approached with a significant measure of diffidence. The same comment must be made with respect to the current work. Although approximately one thousand seeds were examined in the present study, there is no absolute guarantee that a survey of another thousand would not reveal a plant with seeds so similar, based on visually observable characteristics, as to represent a threat to the identification of *C. sativa*. The present study does resolve an important previously unanswered question concerning *Humulus*, and the present study does in part resolve the question concerning plants whose leaf material is known to have some similarity to *C. sativa*, although some species with previously described similarity were not examined in the present study. The fact that some plants within a genus show differences in seed morphology is troubling; the question remains, If different plants within a genus show distinct differences, is it possible that plants in different genera will show distinct similarities? The present study cannot answer this question. It is apparent that the entire display and arrangement of seed features, although diverse, are spread across the plant kingdom and are not confined to certain genera, or even to certain families. And finally, in the "shotgun" phase of the present study, an extended museum of seeds was consulted for seeds having a similar appearance to *C. sativa*, "similar" meaning close accord in size, a ridge running around the circum-

ference on one axis, the presence of lacy "marbling", and color, the latter feature being given less weight given the fact that seeds of a species may vary from specimen to specimen. The seed herbarium consulted is constantly augmenting its collection, and there is no guarantee that the next specimen submitted will not be similar to *C. sativa* in all observable features.

Acknowledgments

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References

1. *Marihuana, Its Identification*. U.S. Treasury Bureau of Narcotics, U.S. Government Printing Office: Washington, DC, 1938.
2. Joyce, C. R.; Curry, S. H., Eds. *The Botany and Chemistry of Cannabis*. J. & A. Churchill: London, 1970; pp 11-38.
3. Stearn, W. T. The Cannabis Plant: Botanical Characteristics. In *The Botany and Chemistry of Cannabis*, Joyce, C. R., Curry, S. H., Eds.; J and A Churchill: London, 1970; pp 1-10.
4. Thorne, R. F. How Many Species of Seed Plants Are There? *Taxonomy* **2002**, *51* (3), 511-512.
5. Thornton, J. I.; Nakamura, G. R. The Identification of Marijuana. *J. For. Sci. Soc.*, **1972**, *12* (3), 461.
6. Nakamura, G. R. Forensic Aspects of Cystolith Hairs of Cannabis and Other Plants. *J. Assoc. Official Anal. Chemists*, **1969**, *52* (1), 5.
7. Effenberger, J. California Department of Food and Agriculture Seed Herbarium, Sacramento, CA. Personal communication, 2008.
8. *Manual for Testing Agricultural and Vegetable Seeds*; Agriculture Handbook No. 30.; U.S. Department of Agriculture, Supt. of Documents: Washington, D.C., 1952.

Appendix A

Cannabinaceae *Cannabis sativa* Seeds

Figure 1

Cannabis sativa (marijuana). Actual seed length 4.5 mm.

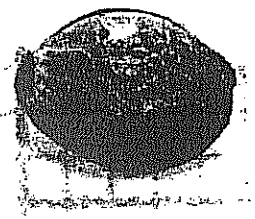

Figure 2

Cannabis sativa (marijuana). Actual seed length 5 mm.