CONSTITUENTS OF *CANNABIS SATIVA* L. XVII. A REVIEW OF THE NATURAL CONSTITUENTS

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Cannabis preparations have been used by man for over 5000 years (1). Early use was associated with medical applications. However, as newer and better medicinal agents were discovered, the use of crude drugs from *Cannabis* lost favor in clinics throughout the world. This trend continued, and today there are no crude *Cannabis* drugs used in modern medical clinics.

The same *Cannabis* preparations once accepted as therapeutically useful drugs have gained acceptance as innocuous drugs of leisure in most countries of the world. The rapid and broad increase in the use and abuse of these drugs resulted in *Cannabis* being controlled by international treaties (2). Local, national, and international agencies are charged with the responsibility of enforcing these treaties. Crude *Cannabis* preparations once of medical use are now illegal. Thus, marihuana, hashish, dagga, bhang, ganja, hash oil, sinsemilla, etc., comprise the world's most common and widely used group of illicit drugs. Worldwide, approximately 300 million people use one or more of these crude drugs. It is estimated that in the United States of America 36 million people have used some form of *Cannabis* (3).

Current scientific opinions about *Cannabis* are not always based on rational and reproducible scientific facts, but on emotion. Although the *Cannabis* and health issue is of interest to all health-related scientific disciplines and agencies of the world, due to the emotional issue, it is possible that scientific experiments may never clarify the pharmacology of *Cannabis*. The general opinion held by much of the broadly diversified scientific community is that *Cannabis* preparations can be evaluated solely on (−)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC) content, thereby, neglecting other cannabinoids and chemicals in the crude drug. It is easy to understand how this concept came to be accepted, since Δ^9-THC is always referenced as the "active compound" in *Cannabis*, and data from "synthetic marihuana" (Δ^9-THC) is taken as being synonymous with data from marihuana. This has fostered the impression that marihuana and other crude drugs from *Cannabis* are singular in composition and uniform in potency with hashish being "X" times as strong as marihuana, etc. Therefore, it is critical to the scientific community that a document fully elucidating the current state of the knowledge of naturally occurring constituents in *Cannabis* be published. Although over 7000 scientific papers (4, 5) have been published on *Cannabis* and its constituents and many reviews have been written on *Cannabis* constituents and cannabinoid chemistry (6–17), all are of limited value in the natural profile of *Cannabis* and are of even less value for elucidating chemically why confusion exists and may continue to exist in the biological evaluation of the crude drugs obtained from *Cannabis*.

The present review is structured around all known natural constituents of *Cannabis sativa* L. Emphasis will be placed on those compounds actually isolated. Some constituents considered to be artifacts within the plant, such as cannabinol, will be discussed; and commonly occurring plant constituents never isolated from

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Cannabis, but shown to be present by spectral data, will be considered. No synthetic chemistry, nor chemistry of the pyrolysis process which occurs when *Cannabis* preparations are smoked, will be considered. These subjects have been adequately covered by Razdan (8) and others (6, 9) and by a United Nations Narcotics Laboratory document (18), respectively.[1] However, the paper will include a brief review of the botany and chemical classification systems used for *Cannabis* with emphasis on the chemical aspects rather than the botanical aspects.

CLASSIFICATION OF *CANNABIS*

Today, in classifying this plant, botanists generally agree on the following list of taxa (19).

Division—Tracheophyta
Subdivision—Pteropsida
Class—Angiospermae
Subclass—Dicotyledoneae
Order—Urticales
Family—Cannabaceae
Genus—*Cannabis*
Species—*sativa* Linné

Previously *Cannabis* was classified as belonging to the Urticaceae (20). Also *Cannabis* was thought to be monotypic, but recently several botanists have proposed a polytypic genus (21). See table 1 for taxa proposed in the literature. Of those species listed in table 1, Schultes *et al.* (21) prefer *sativa*, *indica*, and *ruderalis;* Anderson (22) tentatively favors *sativa* and additional species. Small and Beckstead (23) and Quimby and associates (19, 24, 25) prefer *sativa*. Recently Small and Cronquist (26) and Small *et al.* (27) proposed subspecies of the species *sativa;* whereas, a group of experts consisting of a botanist, a taxonomist (classical, chemo, and wood), a chemist, and a pharmacognosist, agreed it was impossible to subdivide the genus with current published data (28).

TABLE 1. *Cannabis* names used in the scientific literature (21).

1. *Cannabis sativa* Bauhin and Linnaeus.
2. *Cannabis folliisdigitatis* Linnaeus.
3. *Cannabis erratica* Bauhin and Siev.
4. *Cannabis mas* D'AleChamps.
5. *Cannabis femina* D'AleChamps.
6. *Cannabis chinensis* Delile.
7. *Cannabis foetens* Gilib.
8. *Cannabis indica* Lam.
9. *Cannabis rupulus* Scop.
10. *Cannabis macrosperma* Stokes.
11. *Cannabis americana* Pharm. ex Wehmer.
12. *Cannabis generalis* Krause.
13. *Cannabis gigantea* Crevost.
14. *Cannabis ruderalis* Janischevskii.
15. *Cannabis interstitia* Sojak. A hybrid.

[1]Additional information on *Cannabis* can be obtained from the bibliography compiled by Eddy (4) and an annotated bibliography compiled by Waller, *et al.* (5). See pp. 4–7 of ref. 16 for some very early investigations not covered in this review. Only those constituents in the literature as of 6-30-78 or known to be included in accepted manuscripts are included in this review.

Cannabis and the genus *Humulus* make up the family Cannabaceae, which is also written Cannabinaceae and Cannabidaceae (20). Crombie and Crombie (29) have cross-grafted *C. sativa* with *H. lupulus* and *H. japonicus*. The grafting of *Humulus* onto *Cannabis* root stock or vice versa did not change the chemistry of the scions. The success of the grafting experiments between *Cannabis* and two species of *Humulus* is consistent with botanical classifications. Failure was reported in attempts to graft *C. sativa* on *Urtica dioica* (the common nettle, family Uriticaceae).

CHEMICAL CLASSIFICATION BASED ON CANNABINOIDS

Numerous attempts have been made chemically to classify *Cannabis* as a function of its intoxicant and fiber qualities. Grlić first proposed a system based on ripening stages of *Cannabis* plants (30). Grlić's system is based on the chemical composition of the resin from different samples of *Cannabis*. This system is based on the data obtained originally by nine different methods: 1) Beam test; 2) peroxide-sulphuric acid test; 3) ferric chloride reagent; 4) indolphenol reaction; 5) determination of the acid fraction; 6) ultraviolet; 7) infrared; 8) microbiological assay on antibiotic potency; and 9) corneal reflexia test in rabbits. By using two or more of the methods described, Grlić was able to place *Cannabis* samples into one of five different categories associated with the phytochemical process ("ripening" of the resin) by which cannabidiolic acid (CBDA) is gradually converted to cannabidiol (CBD), Δ^9-THC, and finally to cannabinol (CBN). Subsequently, the samples can be classified as 1) "unripe"-predominance of CBDA; 2) "intermediate"-predominance of CBD; 3) "ripe"-predominance of Δ^9-THC; 4) "overripe"-predominance of CBN; and 5) "altered" type consisting of *Cannabis* which has been altered or has decomposed.

Grlić pointed out that *Cannabis* "ripening" is more advanced in samples from tropical areas and that this system only gives a general picture of the chemical composition of the drug and may not be satisfactory when more data are required.

The complexity of Grlić's system, the advancement of analytical techniques, and the need for a system based more on chemical analysis led Waller to propose a phenotype system. This system is based on the combined Δ^9-THC and CBN content divided by the CBD content. Any sample with a value greater than 1 is classified as a drug type and a sample with a value less than 1 is a fiber type (25, 31).

$$\text{phenotype} = \frac{\Delta^9\text{-THC} + \text{CBN}}{\text{CBD}}$$

Small and Beckstead (23) extended the phenotype system and divided *Cannabis* plants into four phenotype classes. Phenotype I exhibits relatively high amounts of THC[2] (more than 0.3%) and low amounts of CBN (less than 0.5%) in both sexes. Phenotype II exhibits high CBD (generally more than 0.5%) and a THC mean content of at least 0.3% in the female. Phenotype III exhibits high CBD (generally more than 0.5%) and relatively little THC present in the female (less than 0.3%). Phenotype IV is discernible with respect to plants consistently showing trace amounts (about 0.05%) of a material having the same retention time as cannabigerol mono-methylether (CBGM).

Both the Waller and Small systems are useful; however, recent advances in

[2]THC is used when authors referred to THC or the exact structure of Δ^9-THC had not been determined.

the quantitation of cannabinoids have shown the gas chromatographic peak normally labelled cannabidiol (CBN) may be a mixture of CBD, cannabichromene (CBC), and cannabivarin (CBV) or any combination of the three (32, 33). Drug types such as Mexican, Columbian, and South African contain primarily CBC and rarely, if ever, contain CBD. Fiber types such as Turkish, Polish, Iranian, French, Russian, etc., normally contain CBD and very little CBC (34). Cannabivarin is found primarily in hashish samples (35). Thus both classification systems are useful for classifying each sample in a very general way but are of limited use in classifying variants. Turner, *et al.* (36) have shown plants from *Cannabis* variants may be classified as drug or fiber and may be placed in different phenotypes depending on the age of the plant when analyzed. They also proposed a classification system based on a ratio of the following cannabinoids which takes into account quantifiable homologs and separates the cannabinoids according to their ring systems.

$$\text{Phenotype} = \frac{\Delta^9\text{-THC} + \Delta^9\text{-THCV} + \text{CBN} + \Delta^8\text{-THC}}{\text{CBDV} + \text{CBD} + \text{CBC} + \text{CBG} + \text{CBGM}}$$

Although this classification system extends the original Waller system, it is (as the others) only good for each sample unless analyses are performed at regular intervals during the entire growing season—an impossibility with crude drugs. No totally reliable chemical classification system based on a single chemical analysis exists. The plasticity of the genus has to date prevented the development of such a system. The system based on isomers, homologs, and ring systems appears to most accurately describe the chemical makeup of a single *Cannabis* sample or variant.

CHEMICAL CONSTITUENTS

Cannabis sativa L. contains many classes of chemical constituents. These constituents can be broken down as follows:

1. Cannabinoids: 61 known
   a. Cannabigerol (CBG) type: 6 known
   b. Cannabichromene (CBC) type: 4 known
   c. Cannabidiol (CBD) type: 7 known
   d. Δ^9-Tetrahydrocannabinol (Δ^9-THC) type: 9 known
   e. Δ^8-Tetrahydrocannabinol (Δ^8-THC) type: 2 known
   f. Cannabicyclol (CBL) type: 3 known.
   g. Cannabielsoin (CBE) type: 3 known
   h. Cannabinol (CBN) type: 6 known
   i. Cannabinodiol (CBND) type: 2 known
   j. Cannabitriol (CBT) type: 6 known
   k. Miscellaneous types: 9 known
   l. Other cannabinoids. See addendum: 4 known
2. Nitrogenous compounds: 20 known
   a. Quaternary bases: 5 known
   b. Amides: 1 known
   c. Amines: 12 known
   d. Spermidine alkaloids: 2 known
3. Amino acids: 18 known
4. Proteins, glycoproteins, and enzymes: 9 known
5. Sugars and related compounds: 34 known
   a. Monosaccharides: 13 known
   b. Disaccharides: 2 known
   c. Polysaccharides: 5 known
   d. Cyclitols: 12 known
   e. Aminosugars: 2 known

6. Hydrocarbons: 50 known
7. Simple Alcohols: 7 known
8. Simple Aldehydes: 12 known
9. Simple Ketones: 13 known
10. Simple acids: 20 known
11. Fatty acids: 12 known
12. Simple esters and lactones: 13 known
13. Steroids: 11 known
14. Terpenes: 103 known
    a. Monoterpenes: 58 known
    b. Sesquiterpenes: 38 known
    c. Diterpenes: 1 known
    d. Triterpenes: 2 known
    e. Miscellaneous compounds of terpenoid origin: 4 known
15. Non-cannabinoid phenols: 16 known
16. Flavanoidglycosides: 19 known
17. Vitamins: 1 known
18. Pigments: 2 known

The total number of compounds known to occur in *Cannabis* is 421 with new compounds constantly being discovered and reported.

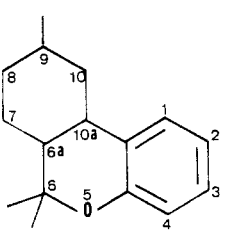
Cannabinoids were defined by Mechoulam and Gaoni (6) "as the group of C_{21} compounds typical of and present in *Cannabis sativa*, their carboxylic acids, analogs, and transformation products." Cannabinoids belong to the chemical class of terpenophenolics, which are widely distributed in nature. Since many terpenophenols are structurally very similar to the cannabinoids, one would expect the cannabinoids to be relatively abundant in nature. From botanical and chemical considerations, cannabinoids would most likely be found in the genus *Humulus*, which with *Cannabis* makes up the family Cannabaceae. However, after an in-depth investigation of *Humulus* by Fenseleau, *et al.* (37) and broad grafting experiments with *Cannabis* and *Humulus* by Crombie and Crombie (29), no cannabinoids were found. These findings do not exclude the possibility of cannabinoids existing in other plants and animals but do indicate the natural uniqueness of cannabinoids.

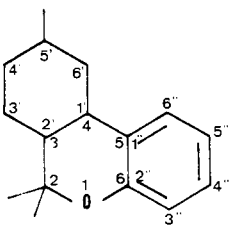
NUMBERING SYSTEMS FOR CANNABINOIDS

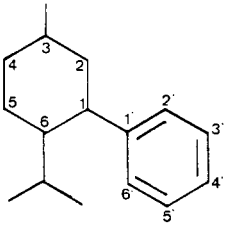
Five numbering systems have been used for the cannabinoids (4) (see figure 1). Currently two numbering systems are in use for these compounds. This review will use the dibenzopyran numbering system, which is mainly used in North America and was adopted by *Chemical Abstracts*. Mechoulam and others (6, 8) and most researchers in Europe use the monoterpene numbering system based on p-cymene; this system accomodates the cannabinoids which do not possess a pyran-ring.

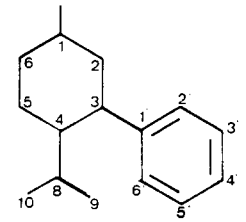
BIOSYNTHESIS OF CANNABINOIDS

Biosynthetic pathways for the cannabinoids have been proposed and studied by several groups. Simonsen and Todd, in 1942, suggested that cannabinoids were produced naturally from a condensation of menthatriene with olivetol. This would result in a cannabidiol structure type molecule which could then cyclize to THC followed by loss of hydrogen to CBN (38, 39). Previously, Adams *et al.* (40) had published data on the conversion of CBD to CBN. Building on the work by Todd's group (38, 39), Farmilo (41) and Farmilo *et al.* (42) presented a detailed biogenesis of cannabinoids in 1961 and 1962, respectively, using the acetic acid route hypothesized for phenol synthesis proposed by Birch (43). The first step in cannabinoid biosynthesis, according to Farmilo *et al.*, would be the condensation of a hexanoic acid with three molecules of acetic acid. This would yield a cyclo-









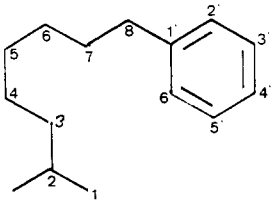


FIG. 1. Numbering of cannabinoids (5).

hexanedione acid as an intermediate which would enolize to dihydroxyphenolic acid (olivetolic acid). Limonene, a menthadiene, instead of the menthatriene as proposed by Todd (38, 39), would condense with olivetolic acid to form cannabidiolic acid which would decarboxylate to CBD or cyclize to form tetrahydrocannabinolic acid. This acid would then decarboxylate to THC and subsequently form CBN by dehydrogenation. This proposed biogenesis was the first to account for the cannabinoids and assume the cannabinoids existed in nature as their carboxylic acid derivatives. Farmilo's (41) proposal depicted a THC acid B years before it was shown to exist. The double bonds in CBD and THC were placed in the Δ^8-position. Others visualized the double bond in the Δ^7-position. Thus, Farmilo's assignment of the double bond was the most nearly correct of the incorrect structures. Farmilo did, indeed, provide the assignment for THC which corresponds to Δ^8-THC. (See figure 2, for Farmilo's pathway).

Ni (44), also in 1962, postulated CBD was derived from a terpene and an acetogenin. The terpenoid, *p*-mentha-3,8-diene, was postulated as being derived from mevalonic acid through mevalonic acid pyrophosphate which lost H_2O and CO_2 to form isopentyl pyrophosphate. Subsequently, two isopentyl pyrophosphate molecules formed the terpene skeleton which, through isomerization and oxidation, provided *p*-mentha-3,8-diene-5-one. The acetogenin was postulated from six acetic acid molecules to form olivetol or olivetolic acid. Condensation of *p*-mentha-3,8-diene-5-one and olivetolic acid via a Michael condensation provided a product at equillibrium between the keto and enol tautomers. Reduction and loss of H_2O led to cannabidiolic acid. Loss of CO_2 and cyclization led to tetrahydrocannabinol and finally to cannabinol. In 1962, the double bond in CBD and Δ^9-THC was believed to be in the Δ^7 position.

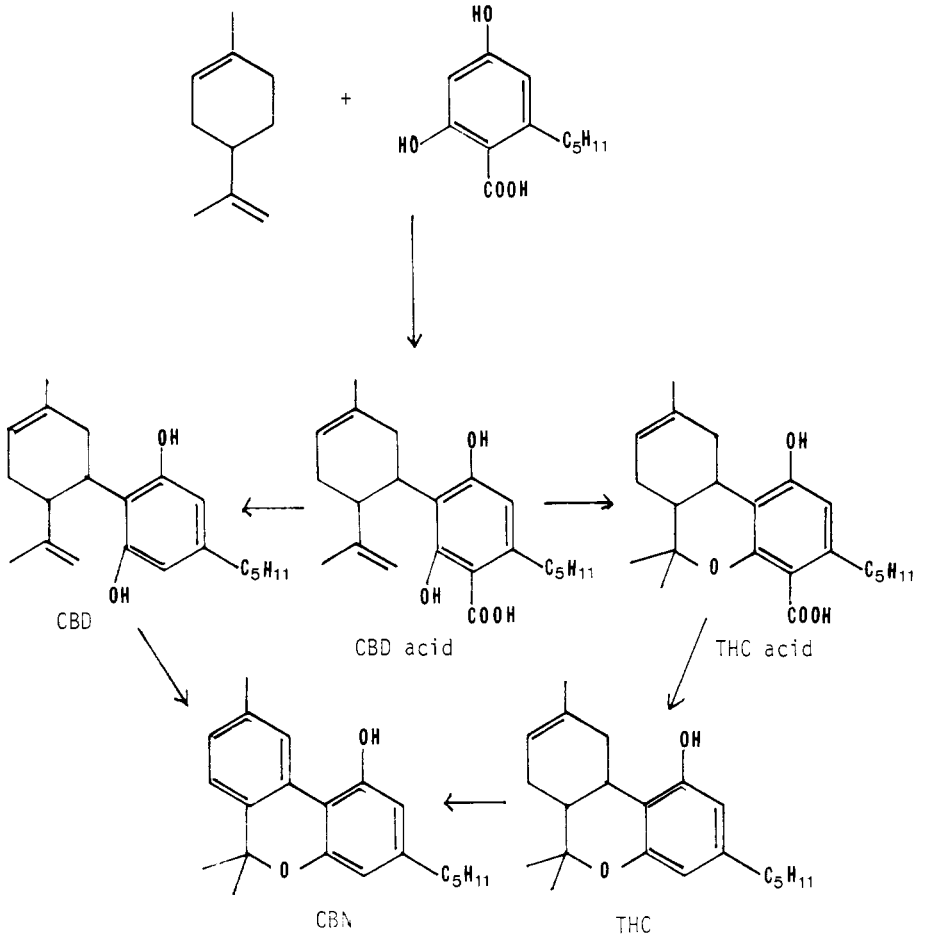


FIG. 2. Biosynthesis of cannabinoids-1961 (41) according to Farmilo (first to assign double bond in 8-position).

Ni's proposal was based on the fact that *p*-cymene and olivetol were obtained from the hydrolysis of CBD. Subsequently, Ni carried out feeding experiments with sodium acetate-1-C^{14}. This way, both *p*-mentha-3,8-diene-5-one and olivetol should be tagged. From feeding experiments, radioactive CBD was isolated as its bis-3,5-dinitrobenzoate, which then provided *p*-cymene and olivetol. *p*-Cymene was oxidized to terephtalic acid. Both the acid and olivetol were radioactive. However, no radioactivity was found in CBN, and radioactive impurities were found with CBD. Thus, this first proposed biosynthetic pathway based on experiments with labelled compounds accounted for neutral cannabinoids and cannabinoid acids. But, as was Farmilo, *et al.* (41, 42), Ni was unaware of the existence of cannabigerol (CBG).

In 1964, Gaoni and Mechoulam suggested CBG was probably formed by condensation of geranyl pyrophosphate and olivetol (45). These authors viewed CBG as being the missing link in the formation of *Cannabis* constitutents. In 1965 these authors visualized CBG as being converted into CBD, Δ^9-THC, and

CBN via two successive enzymatic cyclizations followed by dehydrogenation (46). This pathway would not account for cannabinoid acids; therefore, they proposed that the condensation of geranyl pyrophosphate and olivetolic acid led to cannabinoidic acids. Two parallel biogenesis pathways possessed problems, and in 1967 Mechoulam and Gaoni (6) proposed a cannabinoid biogenesis which accounted for neutral cannabinoids and cannabinoid acids. In 1970 Mechoulam proposed a biogenesis of cannabinoids showing cannabielsoic acid A (CBE acid A), cannabinolic acid (CBNA), and cannabicyclol (CBL) (7). Mechoulam discussed the proposed biogenesis with some additional data in his book (16) (see figure 3, for Mechoulam's pathway).

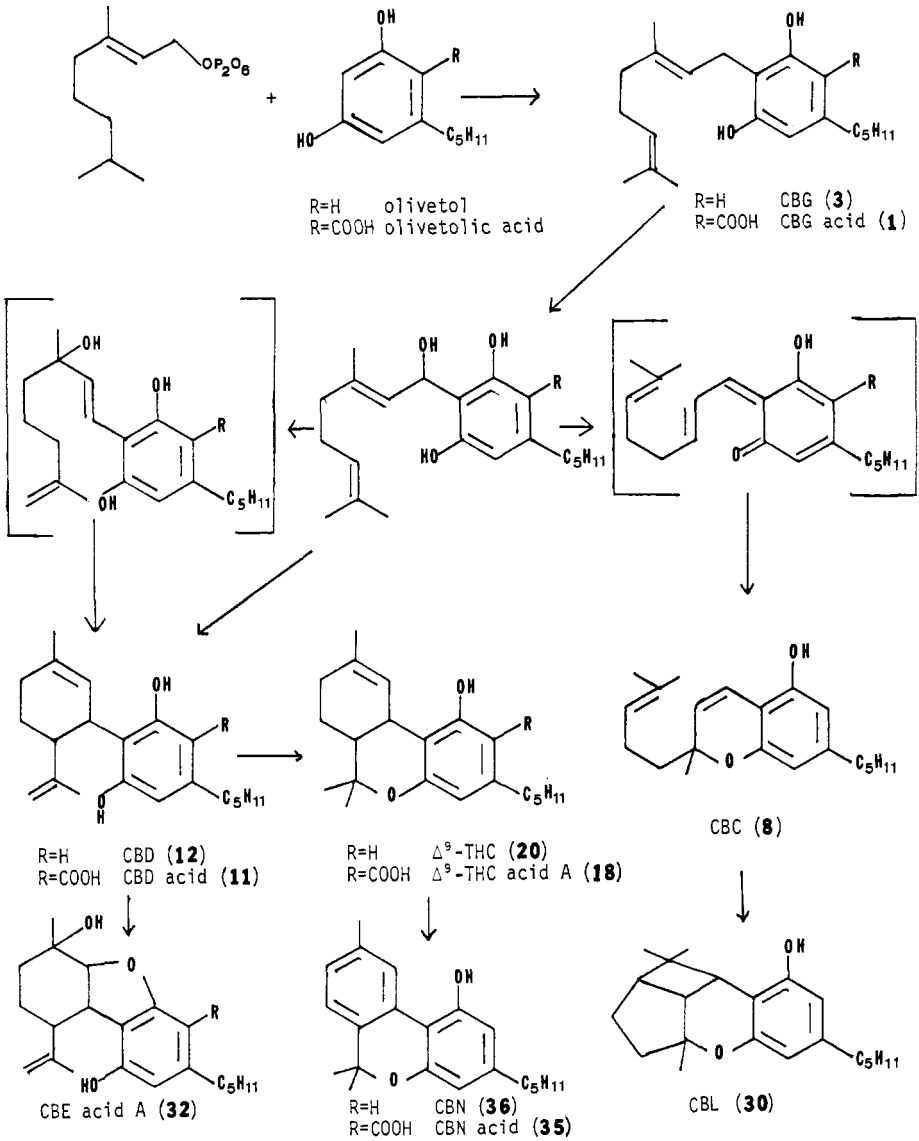


FIG. 3. Biosynthetic pathway of cannabinoids-1970 (7) proposed by Mechoulam.

Nishioka's group (47), in a paper received in 1969 and published in 1970, excluded cannabinolic acid and the other free phenol from original cannabinoids in the living plants. Nishioka's group proposed a biosynthetic pathway which accounted for previous known cannabinoid acids and also for cannabichromenic acid and cannabigerolic acid monomethyl ether, which was found to be a natural cannabinoid.

In 1975, Nishioka's group published biosynthesis data based on ^{14}C-labelled malonate and mevalonate, ^{3}H-labelled geraniol and nerol, and labelled cannabigerolic and cannabidiolic acids (48). Cannabigerolic acid was found to be formed by condensation of geraniol and a C_{12}-polyketide, a precursor for olivetolic acid.

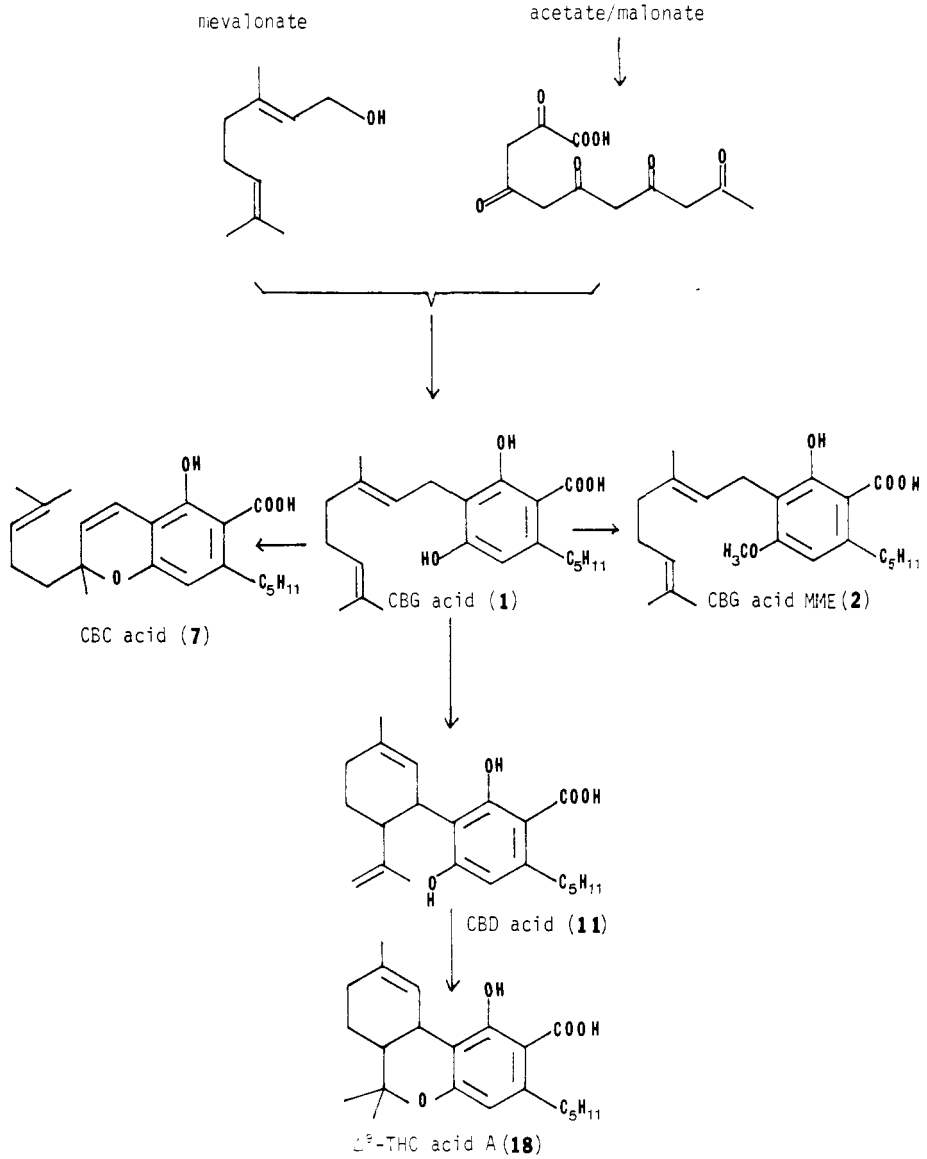


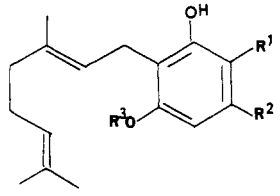
FIG. 4. Biosynthetic pathway of cannabinoids-1970 (47) proposed by Nishioka, *et al.*

When labelled cannabigerolic acid was fed, cannabidiolic acid was the major labelled acid when a domestic variety of *Cannabis* was used. A Mexican and two other variants provided labelled cannabichromenic acid and Δ^9-tetrahydrocannabinolic acid, the latter being the most abundant cannabinoid. This data supports the hypothesis of de Faubert Maunder (49) that *Cannabis* plants from different geographical locations may have different biogenesis. Turner and Hadley (32) had also proposed the possibility of an enzymatic system operative in certain variants which facilitates concomitant allylic rearrangement of hydroxycannabigerol and cyclization of the rearranged intermediate to Δ^9-THC bypassing or preventing the detection of CBD. Thus, Nishioka's biosynthetic pathway proposed in 1970 (47) and validated experimentally through the use of different genetic types of *Cannabis* and labelled compounds in 1975 (48) explains why different ratios of cannabinoid acids and neutral phenols are found in *Cannabis* preparations from different geographical locations. Moreover, the pathway adequately provides for homologs and derivatives found in *Cannabis*. It also substantiates reports that cannabinol, (−)Δ^8-*trans*-tetrahydrocannabinol, cannabicyclol, and their acids are not, in general, naturally occurring cannabinoids but artifacts (47, 50) (See figure 4, for Nishioka's biosynthetic-pathway). For a proposed pathway using a 9,10 epoxide to produce polyhydroxylated cannabinoids, see reference 8.

CANNABIGEROL-TYPE CANNABINOIDS (table 2)

Cannabigerolic acid (CBGA, **1**), cannabigerolic acid monomethyl ether

TABLE 2.



	Cannabigerol-type	R_1	R_2	R_3	Ref
1	Cannabigerolic acid	COOH	C_5H_{11}	H	46
2	Cannabigerolic acid monomethylether	COOH	C_5H_{11}	CH_3	47
3	Cannabigerol	H	C_5H_{11}	H	45
4	Cannabigerol monomethylether	H	C_5H_{11}	CH_3	51
5	Cannabigerovarinic acid	COOH	C_3H_7	H	53
6	Cannabigerovarin	H	C_3H_7	H	52

(CBGAM, **2**), cannabigerol (CBG, **3**), cannabigerol monomethyl ether (CBGM, **4**), cannabigerovarinic acid (CBGA-C_3, **5**), and cannabigerovarin[3] (CBG-C_3, **6**) make up the cannabigerol-type subclass of cannabinoids. Cannabigerol was the first to be isolated. In 1964, Gaoni and Mechoulam (45) obtained CBG when they chromatographed a hexane extract of hashish on Florisil. These authors confirmed the structure and stereochemistry of CBG by physical data and by synthesis. Geraniol and olivetol boiled in decalin for 36 hours provided CBG. Cannabigerol was viewed as being formed in nature by the condensation of geranyl

[3]Cannabigero*varol* has been used to designate the C_3 homologs. In this review we used Cannabigero*varin* because most literature sources use varin.

pyrophosphate with olivetol. Nishioka's group (48) subsequently proved CBG in its acid form was the first cannabinoid formed in the biosynthesis of Δ^9-THC acid A (**18**). Mechoulam and Gaoni (46) in 1965 obtained cannabigerolic acid as its methyl ester from the acidic fraction of a hashish sole. Cannabigerolic acid was the most polar acid compounds found in the hashish sole. Regeneration of the original acid from the ester was unsuccessful.

Nishioka's group (51) isolated cannabigerol monomethyl ether (**4**) from domestic hemp in 1968. They decarboxylated an acid mixture by heating it in toluene for 7 hours and chromatographed the neutral cannabinoids on silica gel with benzene as eluant. Two years later, this same group reported the isolation of cannabigerolic acid monomethyl ether (**2**) (47). The separation procedure involved passing the percolate through a polyamide column to remove chlorophyll and then through a silica gel column to obtain a mixture of cannabigerolic acid monomethyl ether and Δ^9-THC acid A. This mixture was separated on a silver nitrate-silica gel column. Cannabigerolic acid monomethyl ether and cannabigerolic acid were also isolated by Nishioka's group in biosynthesis studies (48).

Nishioka's research group continued their excellent phytochemical investigation of *Cannabis* by isolating the propyl homologue of CBG, cannabigerovarin (CBG-C_3, **6**) in 1975 (52). Cannabigerovarin was obtained from a "Meao variant" of Thailand *Cannabis*. Leaves in the vegetative phase of growth were percolated with benzene and subsequently decarboxylated and chromatographed over silica gel. The most polar compound obtained was CBG-C_3.

Cannabigerovarin acid (CBGA-C_3, **5**) was reported in 1977 by Nishioka's group (53). The "Meao Variant" of Thailand *Cannabis* was used. Dry leaves were extracted with benzene, and the extracts were treated with acetone and chromatographed on a polyamide column to obtain the cannabinoid acid fraction.

To date, no methyl homologs of cannabigerol have been detected in any variant or preparation of *Cannabis*. However, it seems reasonable that the methyl homolog of CBG should be in *Cannabis*.

Cannabigerol type cannabinoids have been referred to as inactive when compared to Δ^9-THC (54, 55). However, CBG-type cannabinoids show considerable antibacterial activity against gram positive bacteria (46). Cannabigerol has been shown to decrease the rate of absorption and excretion of pentobarbital (56) and had a moderate reductive effect in nuclear membrane bound ribosomes of infant rat brain cells (57). Cannabigerol inhibited incorporation of leucine and uridine into protein and nucleic acid of rat brain cortex slices (58). The unavailability of a generous supply for research may account for the limited biological data on CBG.

The relative abundance of CBG-type compounds in *Cannabis* is nominal.

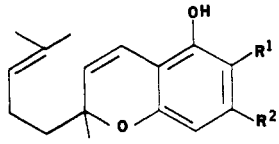
CANNABICHROMENE-TYPE CANNABINOIDS (table 3)

Cannabichromenic acid (CBCA, **7**), cannabichromene (CBC, **8**), cannabichromevarinic acid (CBCA-C_3, **9**), and cannabichromevarin (CBC-C_3, **10**) make up the subclass of cannabinoids referred to as cannabichromene-type. The discovery of CBC was independently reported by Claussen, *et al.* (59) and Gaoni and Mechoulam (60) in 1966. Submission dates were 15 November 1965 and 23 November 1965, respectively. Gaoni and Mechoulam's paper was in English and received more attention than did Claussen's *et al.*, which was in German. Claussen *et al.* obtained CBC as a complex with dimethylformamide, whereas, Gaoni and Mechoulam used a hexane extract of hashish and florisil column chromatography.

Physical data compared satisfactorily. Gaoni and Mechoulam did not use mass spectral data, whereas, Claussen *et al.* used the fragmentation pattern to aid in structure determination. Both groups proposed the name cannabichromene. Mass spectral data published on CBC (61) isolated by Gaoni and Mechoulam did not agree with the data obtained by Claussen *et al.*

In 1968 cannabichromenic acid (CBCA) was isolated from a benzene percolate of hemp by Nishioka's research group (62). A silica gel column was used in the purification procedure.

TABLE 3.



	Cannabichromene-type	R_1	R_2	Ref
7	Cannabichromenic acid	COOH	C_5H_{11}	62
8	Cannabichromene	H	C_5H_{11}	59, 60
9	Cannabichromevarinic acid	COOH	C_3H_7	53
10	Cannabichromevarin	H	C_3H_7	52

Cannabichromevarin (CBC-C_3) was reported in 1973 by de Zeeuw *et al.* (63). This propyl homolog of CBC was found to be present in hashish and marihuana. An Asian hashish sample was extracted with chloroform and subjected to preparative chromatography. Structure determination was based on tlc data and mass-spectra analysis with the variable electron voltage-mass fragment intensity graph technique first used on cannabinoids by Vree *et al.* (64, 65). Recent correspondence with Vree and comparison of the mass spectral data for the C_3 homolog of cannabicyclol (CBC) (66) confirm that de Zeeuw *et al.* (63) misidentified CBC-C_3. Thus, the actual discovery of CBC-C_3 belongs to Nishioka's group (52). This research group actually isolated CBC-C_3 from a benzene percolate of leaves from a "Meao Variant" of Thailand *Cannabis*. Purification was by column chromatography with silica gel. Mass spectral data were supported by other physical data.

Nishioka's research group isolated cannabichromevarinic acid in 1977 (53). This homolog was isolated from a benzene extract of young vegetative parts of the "Meao Variant" of Thailand *Cannabis*. Previously it had been reported that CBCA was exclusively observed in *Cannabis* seedlings prior to the appearance of THCA (62). The amount of CBCA-C_3 was minor when compared to other cannabinoid acids.

No methyl homologs of cannabichromene have been detected in any variant or preparation of *Cannabis*. Since CBC has now been shown to be one of the four major cannabinoids in *Cannabis* (CBD, Δ^9-THC, CBN and CBC) (67), the methyl homolog should be isolated or detected, as has been the case with the C_3 homologs of CBD, Δ^9-THC and CBN.

Biologically cannabichromene was originally reported as being capable of causing sedation and ataxia in the dog (60). Later CBC was found to be "inactive" in human smoking experiments (68). Mechoulam *et al.* (55) found no "Cannabis like" activity in the Rhesus monkey. Razdan and Pars (69) found

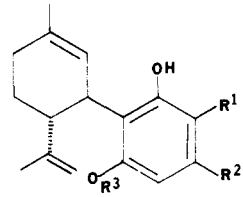
CBC to cause slight loss of muscular coordination and an increase of cyanosis and urination in rats.

Recently CBC has been determined to be present in drug-type *Cannabis* (32, 67). Previously CBC had been misidentified as CBD in most crude drugs from *Cannabis*. Some interaction between Δ^9-THC and CBC has been proposed (70), and the National Institute on Drug Abuse has funded one large study to evaluate CBD and CBC on various biological functions.

CANNABIDIOL-TYPE CANNABINOIDS (table 4)

Cannabidiolic acid (CBDA, **11**), cannabidiol (CBD, **12**), cannabidiol monomethylether (CBDM, **13**), cannabidiol-C_4 (CBD-C_4, **14**), cannabidivarinic acid (CBDV-A **15**), cannabidivarin (CBDV, **16**), and cannabidiorcol (CBD-C_1, **17**) make up the subclass of cannabinoids known as cannabidiol-type.

TABLE 4.

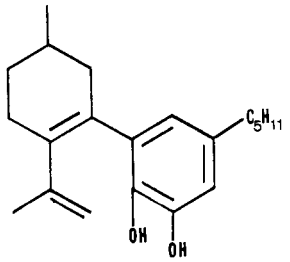


	Cannabidiol-type	R_1	R_2	R_3	Ref
11	Cannabidiolic acid	COOH	C_5H_{11}	H	79, 80
12	Cannabidiol	H	C_5H_{11}	H	71, 72, 76, 77
13	Cannabidiol monomethylether	H	C_5H_{11}	CH_3	84
14	Cannabidiol-C_4	H	C_4H_9	H	85
15	Cannabidivarinic acid	COOH	C_3H_7	H	53
16	Cannabidivarin	H	C_3H_7	H	87, 90
17	Cannabidiorcol	H	CH_3	H	89, 91

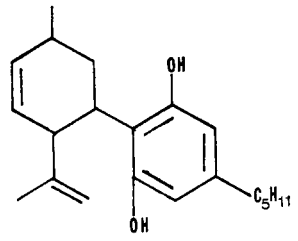
Cannabidiol was first isolated and reported by Adams *et al.* (71) in 1940. Minnesota wild hemp was extracted with ethanol, and the "red oil" obtained was investigated. A pure compound having the formula $C_{21}H_{32}O_2$ was found. This compound was purified as the bis-3,5-dinitrobenzoate and given the name cannabidiol. For the various structures assigned to CBD, see fig. 5. Later in 1940, Jacob and Todd (72) isolated CBD from hashish of Egyptian origin by extracting the "hard kahaki-coloured, flat slabs" with light petroleum. The extract was distilled, and the resin was *p*-nitrobenzoylated and separated into two fractions according to solubility in petroleum ether. Hydrolysis of a low melting impure ester and subsequent acylation with 3,5-dinitrobenzoyl chloride provided a product identical to that reported by Adams *et al.* (71) (see figure 5).

In 1940, Jacob and Todd (73) also isolated cannabidiol through its bis-3,5-dinitrobenzoate from charas. Adams *et al.* (74) in 1940 also crystallized CBD by allowing the oily CBD to stand for several weeks.

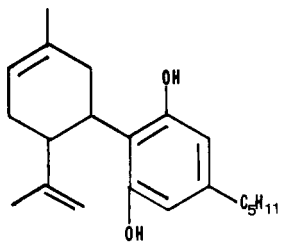
Adams and co-workers published several papers on the structure of CBD in 1940 and 41. After a thorough investigation and using the dibenzopyran numbering system they proposed a structure which had the double bond in the 7 position of the cyclohexene ring (75) (see figure 5).



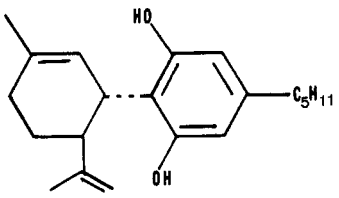
CBD-1940 (71) by Adams et al.



CBD-1941 (75) by Adams et al.



CBD-1961 (41) by Farmilo



CBD-1963 (76) by Mechoulam and Shvo

FIG. 5. Proposed structures of CBD by various researchers.

In 1963, Mechoulam and Shvo (76) determined the structure of CBD as we know it today. Stereochemical assignments were made on the basis of nmr data. It is interesting to note that Šantavý communicated with Mechoulam that he had reached the same conclusions on the structures of CBD (76). Šantavý subsequently published in English his findings on the stereochemical assignments for CBD in 1964 (77). Later Gaoni and Mechoulam (78) presented excellent data on the absolute configuration of CBD and other cannabinoids.

Cannabidiolic acid (CBDA, **11**), the first cannabinoid acid discovered, was isolated in 1955 by Krejčí and Šantavý (79). Fresh tops and leaves of *Cannabis sativa* were rapidly dried and extracted cold with benzene or petrol ether. Partial removal of the solvent under reduced pressure provided a residue, which was extracted with 4% sodium hydroxide. After neutralization, the acidic compounds were extracted with chloroform. Acids from the oily residue were obtained by extraction under a nitrogen atmosphere with aqueous $NaHCO_3$. The acids were in a slightly yellow product. A major acid was subsequently separated as its diacetyl derivative $C_{26}H_{36}O_6$. The uv extinction curve closely resembled that of cannabidiol derivatives and was, therefore, named cannabidiolic acid.

In 1958 and 1959 Krejčí *et al.* (80, 81) assigned the name 3-methyl-6-isopropenyl-4'-n-pentyl-2',6'-dihydroxy-1,2,3,6-tetrahydrodiphenyl-3'-carbonsaüre. Numbering was based on diphenyl. The structure of cannabidiolic acid was correct as to the placement of the carboxy group since only one acid is possible with the CBD series. Placement of the double bond in the cyclohexene ring was in keeping with the accepted 4,5 position based on diphenyl. The position of the double bond was later changed by Šantavý (77).

In fresh plant material 95% of CBD exists as its acid (25).

The crystal and molecular structure of CBD was determined independently in

1977 by research groups from England and Israel, Jones *et al.* (82) and Norway and U.S.A., Ottersen *et al.* (83).

Shoyama *et al.* (84) isolated cannabidiol monomethylether (CBDM, **13**) from domestic hemp. Leaves were percolated with ethanol, and the ethanol extract was then treated with acetone. Decarboxylation was by heating in toluene. Neutral cannabinoids were chromatographed on Florisil with benzene and rechromatographed on silica gel. Cannabidiol monomethyl ether was obtained as a brownish syrup. All physical data were compared with an authentic sample of CBDM. The "Minamioshihara No. 1" domestic hemp which gave CBDM is a CBD type and has been under investigation phytochemically and biosynthetically by the Nishioka group for some years.

Cannabidiol-C_4 (CBD-C_4, **14**) was reported in 1975 by Harvey (85). *Cannabis* resin (hashish) and leaves obtained from police seizures were crushed in a mortar and percolated with ethyl acetate for 1 hour. Filtration and concentration provided a residue which was derivatized and subjected to gas chromatography-mass spectrometry analysis. Butyl cannabidiol was *identified* by its methylene unit and mass spectrum. Harvey stated the C_4 series are naturally occurring cannabinoids. However, hashish and other "commercial" preparations of *Cannabis* are often allowed to mold. Thus it is possible for hydroxylation to occur at carbon C_5 of the side chain (86). Oxidation to the aldehyde, carboxylic acid and decarboxylation would result in a butyl chain. Until the butyl side chains are found in *Cannabis sativa*, the C_4 cannabinoids are suspect, at best, as to their natural occurrence. The occurrence of butyl homologs in fresh plant material is also very unlikely from the biosynthetic standpoint.

Cannabidivarinic acid (CBDVA, **15**) was isolated from a benzene extract of young vegetative parts of the "Meao Variant" of Thailand *Cannabis* (53). In the CBD type, CBDVA was a major component.

Cannabidivarin (CBDV, **16**) was isolated from hashish by Vollner *et al.* (87) in 1969. Hashish was extracted with ligroin and chromatographed on silica gel. The CBDV obtained was compared to a sample synthesized by an unambiguous route. The absolute configuration acccording to ord was identical to CBD. This was the first compound found in hashish which had the divarinyl-group instead of the usual olivetyl-group.

Vree, *et al.* (88, 89) in 1971 reported a new method for identification of unknown compounds in hashish by a combination gc-ms system. Electron energy was varied and plotted against the percent of relative abundance of major ions. When this method is used, several line intensities of certain fragments are very characteristic for certain compounds. By comparison of graphs of the results produced by this method, it was possible to identify CBDV in hashish.

Fetterman and Turner (90) showed that CBDV was present in chloroform extract of vegetative Indian *Cannabis* grown in Mississippi.

Cannabidiorcol (CBD-C_1, **17**) was detected by Vree *et al.* (89, 91) in a *n*-hexane extract of Lebanese hashish. No CBD-C_1 was found in a similar extract of Brazilian marihuana. Lebanese *Cannabis* usually is considered to be of the fiber type, whereas Brazilian *Cannabis* is usually of the drug type (34).

The anticonvulsant action of cannabidiol has been studied by Carlini *et al.* (92), Isquierdo's group (93, 94), and Karler *et al.* (95). Anti-inflammatory activity of CBD was studied by Sofia, *et al.* (96) and behavioral effects where studied by Izquierdo and Nasello (97). Cannabidiol was shown to inhibit incorporation of radio carbon-labelled leucine and uridine, respectively, into protein and nucleic

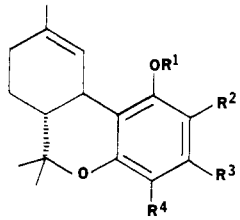
acid of rat brain cortex slices (58). Cannabidiol has been studied extensively; for more details on the pharmacology of CBD consult reference 5.

Cannabidiolic acid has some interesting bactericide effects (81), particularly on *Staphylococcus aureus*, and gave satisfactory antibacterial effects upon several additional microbes (98). Other compounds in the cannabidiol subclass have not been evaluated for pharmacological activity; however, their activity would be expected to mimic CBD and CBDA.

(−)-Δ^9-*TRANS*-TETRAHYDROCANNABINOL-TYPE CANNABINOIDS (table 5)

Δ^9-*Trans*-tetrahydrocannabinolic acid A (Δ^9-THC acid A, **18**), Δ^9-*trans*-tetrahydrocannabinolic acid B (Δ^9-THC acid B, **19**), Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC, **20**), Δ^9-*trans*-tetrahydrocannabinolic acid-C_4 (Δ^9-THC acid-C_4, **21**), Δ^9-*trans*-tetrahydrocannabinol-C_4 (Δ^9-THC-C_4, **22**), Δ^9-*trans*-tetrahydrocannabivarinic acid (Δ^9-THCV acid, **23**), Δ^9-*trans*-tetrahydrocannabivarin (Δ^9-THCV, **24**), Δ^9-*trans*-tetrahydrocannabiorcolic acid (Δ^9-THC acid C_1, **25**), and Δ^9-*trans*-tetrahydrocannabiorcol (Δ^9-THC-C_1, **26**) make up the Δ^9-THC type cannabinoids.

TABLE 5.



	Δ^9-(*trans*)-Tetrahydrocannabinol-type	R_1	R_2	R_3	R_4	Ref
18	Δ^9-(*trans*)-Tetrahydrocannabinolic acid A	H	COOH	C_5H_{11}	H	99, 100
19	Δ^9-(*trans*)-Tetrahydrocannabinolic acid B	H	H	C_5H_{11}	COOH	101, 102
20	Δ^9-(*trans*)-Tetrahydrocannabinol	H	H	C_5H_{11}	H	106
21	Δ^9-(*trans*)-Tetrahydrocannabinolic acid-C_4	H	COOH or H	C_4H_9	H or COOH	85
22	Δ^9-(*trans*)-Tetrahydrocannabinol-C_4	H	H	C_4H_9	H	85
23	Δ^9-(*trans*)-Tetrahydrocannabivarinic acid	H	COOH	C_3H_7	H	33, 53
24	Δ^9-(*trans*)-Tetrahydrocannabivarin	H	H	C_3H_7	H	109, 110
25	Δ^9-(*trans*)-Tetrahydrocannabiorcolic acid	H	COOH or H	CH_3	H or COOH	85
26	Δ^9-(*trans*)-Tetrahydrocannabiorcol	H	H	CH_3	H	91

Δ^9-Tetrahydrocannabinolic acid A was first extracted with petroleum ether from pulverized and homogenized hashish and was reported by Korte *et al.* (99). The acid was isolated as a complex with dimethylformamide by counter current methods. Isolation of pure Δ^9-THC acid A was accomplished by Nishioka's group (100) in 1967. Isolation was from Mexican hemp cultivated in Japan. The acid was obtained as the main component with the aid of chromatography on cellulose powder impregnated with dimethylformamide and *n*-hexane as an eluant

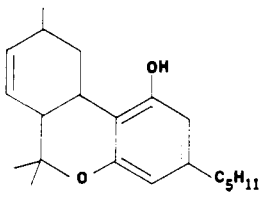
followed by preparative thin-layer chromatography with *n*-hexane-ethylacetate.

Mechoulam *et al.* (101) in 1969 reported the isolation of Δ^9-tetrahydrocannabinolic acid B. Acid B was obtained from the careful chromatography of cannabinoid acidic material from a sole of hashish on silicic acid.

Δ^9-Tetrahydrocannabinolic acid B was isolated from the small leaves and stems of an Indian variant of *Cannabis sativa* L. grown in Mississippi (102). The leaves and stems were percolated twice with hexane. Heating of the extract at 90° decarboxylated the acids except for acid B, the most stable cannabinoid acid, which was thus separated and purified on silica gel (103). A glc method was described for quantitating Δ^9-THC acid A and B by silylation: Δ^9-THC acid B was found to be present in *Cannabis*, but Δ^9-THC acid A was found to be the most abundant (102). Previously it was reported that at least 95% of Δ^9-THC existed in the plant material as Δ^9-THC acids A and B (25).

The crystal and molecular structure of Δ^9-THC acid B was determined in 1975 (104). Acid B crystallizes fairly easy but acid A has never been crystallized.

Δ^9-*Trans*-tetrahydrocannabinol, although only known as natural tetrahydrocannabinol and believed to have the structure shown in figure 6, was first isolated



THC-1942 [105] by Wollner, et al.

FIG. 6.

in 1942 as its acetate by Wollner *et al.* (105) from "red oil" derived from Indian charas. This product was optically active and did possess "marihuana activity," and it was generally accepted that isomeric THC's of this type were the "active components" of marihuana. However, the first isolation of a naturally occurring THC in its pure form with correct structural assignment eluded researchers until 1964 when Gaoni and Mechoulam (106) isolated Δ^9-THC. Δ^9-*Trans*-tetrahydrocannabinol was isolated by column chromatography from a hexane extract of hashish on Florisil. Nuclear magnetic resonance was used to accurately assign the double bond and trans configuration. It should be noted that Wollner *et al.* (105) reported $[\alpha]^{21}D - 120°$ in alcohol for their "crude distillate" from which they obtained the acetate of a THC; Gaoni and Mechoulam reported $[\alpha]D - 140°$ ($CHCl_3$) for pure Δ^9-THC.

Archer *et al.* in 1970 reported detailed conformation of Δ^9-THC using x-ray and proton magnetic resonance analysis (107).

After the breakthrough by Gaoni and Mechoulam on the structure of Δ^9-THC, chemical and pharmacological studies of *Cannabis* constituents flourished. Today Δ^9-THC is the most widely known of the cannabinoids and is often "incorrectly" referred to as the "active principle" of *Cannabis*. For more details on Δ^9-*trans*-THC see the following references: 5–10, 12, 15–16, 18.

Δ^9-*Trans*-tetrahydrocannabinolic acid C_4, presumably the A acid, was detected by combined gas chromatography-mass spectrometry from samples of *Cannabis* of unknown geographical origin and age (85). This group also detected Δ^9-*trans*-

tetrahydrocannabinol-C_4 by utilizing a data system to remove "contamination" from ions of other compounds and column "bleed".

Spectral evidence for Δ^9-*trans*-tetrahydrocannabivarinic acid in fresh *Cannabis* was first presented by Fetterman and Turner (90) in 1971. Subsequently, in 1973, Turner's group (33) reported mass spectral data for Δ^9-THCV acid and stated that Δ^9-THCV existed in fresh plant material as its acid. Fifty-one samples from different geographical locations were evaluated in this report on C_3 homologs of cannabinoids.

Paris *et al.* (108) reported the isolation of Δ^9-THCV acid from fresh leaves of South African *Cannabis sativa* L. However, Δ^9-THCV acid was fully characterized by Nishioka's group (53) in 1977. They obtained pure Δ^9-THCV acid from the benzene extract of dried leaves from a "Meao Variant" of *Cannabis* grown in Japan. Seeds which produced this variant were collected in a Meao Village in Thailand.

Δ^9-*Trans*-tetrahydrocannabivarin was first isolated in 1971 by Gill (109). The starting material was Tincture of *Cannabis* BPC, a commercial product in this case prepared from the flowering tops of Pakistan *Cannabis*. This preparation was extracted with light petroleum and separated by counter-current distribution. Δ^9-*Trans*-tetrahydrocannabivarin was detected in an Indian variant of *Cannabis* (90) and later quantitated in many variants (33). Mole and Turner (110) isolated Δ^9-THCV from small stems, leaves, and flowering tops of an Indian variant grown in Mississippi.

The presence of Δ^9-*trans*-tetrahydrocannabiorcolic acid was detected in low concentrations in *Cannabis* by Harvey (85). Vree *et al.* (91) identified Δ^9-*trans*-tetrahydrocannabiorcol using electron voltage-mass fragment intensity graphs and comparing the graph with those for Δ^9-THCV and Δ^9-THC. Light petroleum or hexane extracts of Brazilian marihuana contained Δ^9-THC-C_1, whereas, Lebanese hashish did not. To date, no Δ^9-THC-C_1 has been isolated or definitely shown to be present in fresh *Cannabis sativa*. However, its presence has been tentatively identified (33).

Δ^9-*Trans*-tetrahydrocannabinol is thought of as the "active principle" in *Cannabis* preparations. The pharmacology of "marihuana activity" has been studied extensively. Over 2500 papers have been written on the subject. Synthetic Δ^9-THC and Δ^9-THC in crude drugs from *Cannabis* continue to be divided and subdivided according to types of activity, drug interactions, etc. To attempt to briefly discuss this area would be folly. There are over 40 review articles listed in reference 5. These, in combination with the work by Braude and Szara (111), Nahas (112), Mechoulam (16), Cohen and Stillman (113), and Kettenes, *et al.* (114), are good sources for the pharmacology of Δ^9-THC.

The acids of Δ^9-THC have been studied only superficially. Tampier *et al.* (115) found Δ^9-THC acid (unknown as to A or B) affected the isolated rabbit intestine and exhibited a non-superable antagonism on contraction of isolated rat ileum induced by acetylcholine, barium chloride or histamine. Unpublished results on Δ^9-THC acid B show it to be a neuro-toxin in frogs (116) and to exhibit no Δ^9-THC-like activity in man (117).

None of the other THC acids have been obtained in large enough quantities for broad pharmacological testing.

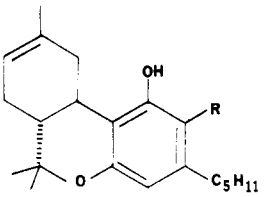
Gill (109) reported biological activity for Δ^9-THCV but was unable to define the type of action. Hollister (118) found Δ^9-THCV to be approximately one-fourth as "active" as Δ^9-THC in humans.

Biological data on other homologs are not available at this time.

(−)Δ^8-*TRANS*-TETRAHYDROCANNABINOL-TYPE CANNABINOIDS (table 6)

There are only two known cannabinoids in this subclass: Δ^8-*trans*-tetrahydrocannabinolic acid A (Δ^8-THCA, **27**) and Δ^8-*trans*-tetrahydrocannabinol (Δ^8-THC, **28**).

TABLE 6.



	Δ_8-(*trans*)-Tetrahydrocannabinol-type	R	Ref
27	Δ_8-(*trans*)-Tetrahydrocannabinolic acid	COOH	119
28	Δ_8-(*trans*)-Tetrahydrocannabinol	H	120

Hanuš and Krejčí (119) isolated Δ^8-*trans*-tetrahydrocannabinolic acid A as its methyl ester from *Cannabis sativa* of Czechoslovakian origin.

Hively *et al.* (120) reported the isolation of Δ^8-THC from a petroleum ether extract of the flowering tops and leaves of marihuana grown in Maryland. Chromatography on silicic acid with benzene followed by chromatography on silica gel-silver nitrate with benzene provided Δ^8-THC. Gas liquid chromatography of freshly grown *Cannabis* nearly always indicated Δ^8-THC; however, the peak normally referred to as free or silylated Δ^8-THC has been recently identified as cannabispiran (121). Generally Δ^8-THC is regarded as an artifact (50). The chemistry in which Δ^9-THC isomerizes to Δ^8-THC is well documented (7, 8, 16). Archer *et al.* (107) have published detailed X-ray and nuclear magnetic resonance data on Δ^8-THC. No biological data are available on Δ^8-THC acid A.

The pharmacology of Δ^8-THC is similar to that of Δ^9-THC, although Δ^8-THC is less "active." In the early years of research on marihuana, synthetic marihuana was actually synthetic Δ^8-THC and, thus, many literature sources on the "activity" of Δ^8-THC are available (5, 9, 16, 111).

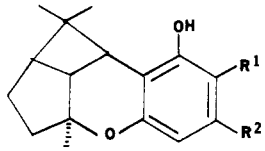
CANNABICYCLOL-TYPE CANNABINOIDS (table 7)

Cannabicyclolic acid (CBLA, **29**), cannabicyclol (CBL, **30**), and cannabicyclovarin (CBLV, **31**) make up the cannabicyclol-type cannabinoids.

Cannabicyclolic acid was isolated in 1972 by Nishioka's group (122). The dried leaves of *Cannabis* from the Kumamato strain were harvested early in the vegetative phase, stored for four months, powdered and percolated with benzene. The benzene extract was treated with acetone at 0° and filtered; the filtrate was evaporated *in vacuo* and chromatographed on a polyamide column using methanol-water. Cannabicyclolic acid was methylated and thus isolated. This acid was determined to be an artifact formed when CBCA is naturally irradiated during storage.

Cannabicyclol was first detected and isolated by Korte and Sieper (123), Korte *et al.* (124) in 1964 and 1965, respectively. Thin layer chromatography of various

TABLE 7.



	Cannabicyclol-type	R_1	R_2	Ref
29	Cannabicyclolic acid	COOH	C_5H_{11}	122
30	Cannabicyclol	H	C_5H_{11}	123–126
31	Cannabicyclovarin	H	C_3H_7	66

hashish and *Cannabis* samples provided "THC I, II and III: THC I proved to be Δ^9-THC, THC II is CBC, and THC III turned out to be CBL. However, Korte's group (125) published a paper in 1968 in which THC–III was named cannabipinol, and the following structure was proposed for cannabipinol based on spectral data (figure 7).

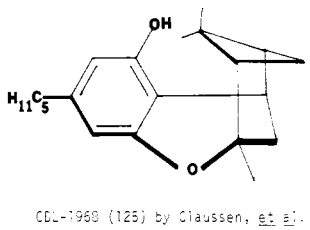
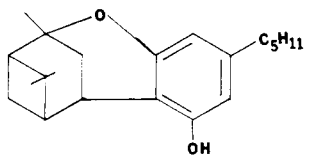


FIG. 7. Cannabipinol.

Mechoulam and Gaoni (6) in 1967 stated that the structure of THC–III has not yet been established and proposed the following structure based on spectral data (figure 8). Mechoulam and Gaoni proposed the name cannabicyclol.



CBL–1967 (6) by Mechoulam and Gaoni.

FIG. 8. Cannabicyclol.

Crombie and Ponsford (126) in 1968 isolated a material from a synthetic mixture which was shown to be identical with cannabicyclol. These authors also assigned a structure to CBL. Subsequently, Crombie's group obtained CBL when CBC was irradiated by uv (127). The structure assigned to CBL by Crombie and Ponsford (126) was determined to be the correct one via X-ray study of dibromocannabicyclol (128).

Cannabicyclovarin, CBL-C_3 was shown to be present in an ether extract of

Congo marihuana (66). Structural identity was based on the comparison of the electron voltage *vs* mass fragment graph for CBL and CBL-C_3.

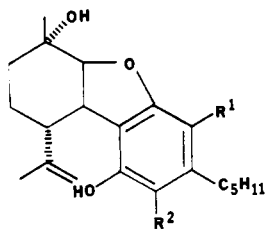
There is no biological data available for CBL acid and CBL-C_3. The activity of CBL is also vague. No activity was reported in rhesus monkeys (129); in mice, irritability when touched was noted along with piloerection and increased respiration at 10 mg/kg (130). Cannabicyclol was found to have a negative mitotropic effect in studies carried out by Nahas *et al.* (131).

Since CBL-type cannabinoids are artifacts obtained from CBC, the recent improvement in synthesis for CBC by Turner's group (132) may lead to renewed interest in the pharmacology of CBC and CBL.

CANNABIELSOIN-TYPE CANNABINOIDS (table 8)

Cannabielsoinic acid A (CBE acid A, **32**), cannabielsoinic acid B (CBE acid B, **33**), and cannabielsoin (CBE, **34**) make up the cannabielsoin-type cannabinoids found in *Cannabis*.

TABLE 8.



	Cannabielsoin-type	R_1	R_2	Ref
32	Cannabielsoic acid A	COOH	H	133
33	Cannabielsoic acid B	H	COOH	133
34	Cannabielsoin	H	H	134

Both cannabielsoinic acid A and B were isolated from a boiling benzene extract of Lebanese hashish (133) which had previously been extracted with light petroleum. A silica gel column was used to accomplish separation.

Both acids were synthesized from CBD and doubt was raised as to the occurrence of cannabielsoin-type cannabinoids in nature.

Cannabielsoin was detected by Bercht *et al.* (134) in 1973 in an ethanolic extract of Lebanese hashish subjected to a 130-step counter-current distribution. A mass fragmentogram as first described by Vree *et al.* (88, 89) was used to confirm the structure.

At this time, no pharmacological data are available on any cannabielsoin type cannabinoids.

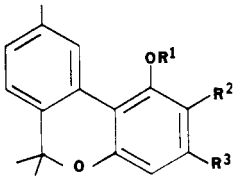
Cannabielsoin type cannabinoids are artifacts formed from CBD.

CANNABINOL-TYPE CANNABINOIDS (table 9)

Cannabinolic acid A (CBN acid A, **35**), cannabinol (CBN, **36**), cannabinol methylether (CBNM, **37**), cannabinol-C_4 (CBN-C_4, **38**), cannabivarin (CBV, **39**), and cannabiorcol (CBN-C_1, **40**) make up the cannabinol type cannabinoids.

Cannabinolic acid A was obtained from the crude acidic constituents found in a

TABLE 9.



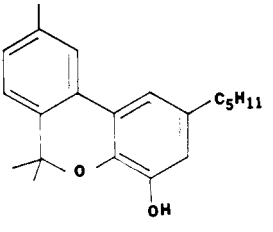
	Cannabinol-type	R_1	R_2	R_3	Ref
35	Cannabinolic acid	H	COOH	C_5H_{11}	46
36	Cannabinol	H	H	C_5H_{11}	135–138
37	Cannabinol methylether	CH_3	H	C_5H_{11}	134
38	Cannabinol-C_4	H	H	C_4H_9	85
39	Cannabivarin	H	H	C_3H_7	139, 140
40	Cannabiorcol	H	H	CH_3	91

hashish sole. The crude acidic fraction was esterified with diazomethane and chromatographed on acid-washed alumina to yield CBN acid A as its methyl ester (46).

Cannabinol was first named by Wood *et al.* (135) in 1896. They extracted two kilos of charas (the exuded resin of Indian hemp) with ether, concentrated the extract to a syrup, distilled it at room pressure to 300° and then re-distilled it under reduced pressure of 15 to 60 mm/Hg. The fraction which was worked with boiled at 270–290° under the above pressures. Paraffins from this fraction were removed by steam distillation and crystallization. The resultant syrup was an oil, amber colored when seen in thin layers, but ruby red when seen in mass, thus, the name "Red Oil" of *Cannabis*. The red oil was evaluated for physiological action and was found to be "extremely active and, taken in doses of 0.05 g, induces decided intoxication followed by sleep. The symptoms produced by it are peculiar to *Cannabis indica* . . ." This substance must be regarded as the active constituent of the plant. This red oil fraction was named "Cannabinol as the compound is undoubtedly a hydroxyl derivative."

Wood *et al.* (136) in 1899 acetylated the crude red oil called "Crude Cannabinol" and obtained pure cannabinol as its acetate. One or more compounds were thought to be present in the resulting oily residue. Formation of the acetate proved cannabinol contained a hydroxy group. The formula $C_{21}H_{25}O_2 \cdot C_2H_3O$ was proposed for the acetate of CBN.

Cahn worked on the molecular structure of CBN for three years from 1930 to 1933. Based on his work, Cahn proposed the structure shown in figure 9 (137).



CBN-1932 (137) by Cahn.

FIG. 9.

The correct structure for CBN was determined by Adams *et al.* (138) in 1940 after a series of brilliant syntheses.

Cannabinol methyl ether was detected by Bercht *et al.* (134) in 1973 from an ethanolic extract of Lebanese hashish which had been subjected to a 130-step counter-current distribution. Detection was by comparison of the mass fragmentogram of synthetic CBNM with the same type spectral data obtained on counter-current fractions.

Cannabinol-C_4 was detected in an ethyl acetate extract of *Cannabis* obtained from police seizures (85). Spectral data were obtained by combined gas chromatography and mass spectrometry.

Cannabivarin was reported in 1971 (139, 140) by Merkus. Merkus observed an intense spot in a thin-layer chromatogram of a sample of hashish from Nepal. Mass spectral data on the isolated material showed the compound to be the C_3 homolog of CBV. Merkus proposed the name cannabivarin in accordance with the naming of CBDV by Vollner *et al.* (87).

Cannabiorcol was identified in the *n*-hexane extract of Brazilian marihuana by Vree *et al.* (91) in 1972. Electron voltage-mass fragment intensity graphs were used to confirm structural integrity. Harvey (85) also detected cannabiorcol by mass spectral data on *Cannabis* of unknown origin.

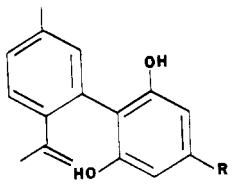
The pharmacology of CBN, a crystalline material when pure, has been extensively investigated. Cannabinol is known to have anti-convulsant activity (95), anti-inflammatory activity (96) and immunological properties (141); CBN has been reported to potentiate the effects of Δ^9-THC (142, 143). Psychomimetically, CBN is about 1/10 as active in man as Δ^9-THC. For other pharmacological properties, see references 5, 9, 11 and 112.

Other cannabinoids of the cannabinol type have not been evaluated for biological activity.

CANNABINODIOL-TYPE CANNABINOIDS (table 10)

Cannabinodiol (CBND, **41**) and cannabinodivarin (CBVD, **42**) make up the subclass of cannabinoids called cannabinodiols.

TABLE 10.



	Cannabinodiol-type	R	Ref
41	Cannabinodiol	C_5H_{11}	144-146
42	Cannabinodivarin	C_3H_7	144

Cannabinodiol was first reported in the literature by van Ginneken *et al.* (144) in 1972. It was detected in hashish by combined gc-ms. However, synthesis work on cannabinodiol by Lousberg, *et al.* (145) in 1977 confirmed that cannabinodiol as reported in the literature was not, in fact, cannabinodiol.

Lousberg *et al.* (145) did isolate a compound from a hexane-ether extract of

"Red" Lebanese hashish with properties similar to those reported for cannabinodiol. This compound did, indeed, turn out to be cannabinodiol. Lousberg *et al.* suggested the van Ginneken *et al.* compound was cannabifuran (CBF, **50**), which was previously reported by Friedrich-Fiechtl and Spiteller (146). These authors noted that the compound they isolated (cannabifuran) was identical to the cannabinodiol reported by van Ginneken *et al.* (144) except the relative retention times were different.

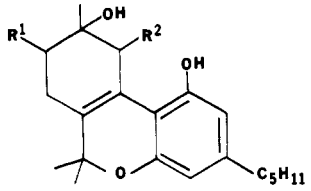
The propyl homolog of cannabinodiol, cannabinodivarin, was also reported to be present in either a hexane extract of Nepalese hashish or Brazilian marihuana, or both. No data were given except cannabinodivarin was detected by gc/ms. Whether or not the propyl side chain was actually cannabinodivarin or the propyl side chain of cannabifuran is unknown; however, since cannabinodiol as reported by van Ginneken *et al.* (144) was actually cannabifuran, it is reasonable to assume the propyl homolog was also misidentified.

No pharmacological data are available on these cannabinoids. They are, indeed, present in trace amounts.

CANNABITRIOL-TYPE CANNABINOIDS (table 11)

(−)-Cannabitriol [(−)-CBO, **43**], (+)-cannabitriol [(+)-CBO, **44**], (±)-9,10-dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol (**45**), (−)-10-ethoxy-9-hydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol (**46**), (±)-8,9-dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol (**47**) and cannabidiolic acid tetrahydrocannabitriol ester (CBD-THO, **48**) make up the subclass of cannabinoids called cannabitriols.

TABLE 11.



	Cannabitriol-type	R_1	R_2	Ref
43	(−)-Cannabitriol	H	OH	147, 148
44	(+)-Cannabitriol	H	OH	149
45	(±)-9,10-dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol	H	OH	150
46	(−)-10-ethoxy-9-hydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol	H	OEt	149
47	(±)-8,9-dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol	OH	H	150
48	Cannabidiolic acid tetrahydrocannabitriol ester (CBD-THO) (CBDA ester at 9-OH group)	H	H	151

The name cannabitriol was first proposed by Obata and Ishikawa in 1966 (147) for a compound they isolated from Japanese hemp. This compound had a mp of 170–172° and gave a positive test with Gibbs' reagent. Structural parameters were not such that Obata and Ishikawa proposed a structure. In 1976 Chan *et al.* (148) obtained cannabitriol from the benzene extract of the dried leaves, twigs, and flowering tops of Jamaican ganja. The benzene extractables were partitioned between light petroleum and 10% aqueous methanol. The neutral

portion of the aqueous layer was chromatographed on alumina with 20% ethyl acetate in benzene. Yield of cannabitriol was 0.025% by dry weight.

(+)-Cannabitriol was reported in 1977 by Elsohly *et al.* (149). This compound was obtained from an ethanolic extract of *Cannabis* furnished by the National Institute on Drug Abuse (NIDA). The residue obtained upon evaporation of 75 ml of the ethanolic extract was dissolved in methanol and adsorbed on silica gel, and the solvent was evaporated. The granular residue was passed through a 60–80 mesh sieve and then applied on a silica gel 60 column and chromatographed. (+)-Cannabitriol was purified by rechromatography on a tlc grade silica gel column.

The rotation reported for (−)-cannabitriol by Chan *et al.* (148) was −107°; whereas, (+)-cannabitriol had a rotation of +7°. This indicates that the isolated (+)-cannabitriol was a partially racemized mixture.

(±)-9,10-Dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol and (±)-8,9-dihydroxy-$\Delta^{6a-(10a)}$-tetrahydrocannabinol were reported by Elsohly *et al.* (150) in 1978. These compounds were obtained from a hexane extract of an Indian variant. Chromatography was performed on silica gel.

(−)-10-Ethoxy-9-hydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol was isolated by Elsohly *et al.* (149) in 1977 from an ethanolic extract of *Cannabis*. For details on the isolation procedure, look at (+)-cannabitriol. The presence of the ethoxy group could suggest that this compound might be an artifact, especially since it was isolated from an ethanolic extract of *Cannabis*. It also suggests the possibility of an epoxide (*m/e* 328) which was detected in Costa Rican marihuana (149). Nucleophilic attack on the epoxide ring by water or ethanol would result in the formation of (+)-cannabitriol and (−)-10-ethoxy-9-hydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol respectively (fig. 10).

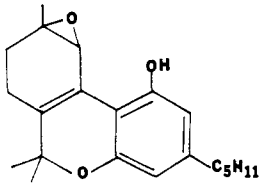


FIG. 10.

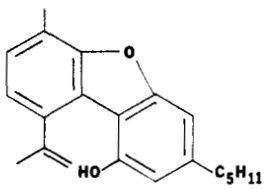
Cannabidiolic acid tetrahydrocannabitriol ester was reported by Von Spulak *et al.* (151) in 1968. The ester was obtained from a "pet ether" extract of hashish chromatographed on silica gel. This is the only reported ester of any cannabinoid occurring naturally.

No pharmacological data are available on cannabitriols.

MISCELLANEOUS CANNABINOIDS (table 12)

Dehydrocannabifuran (DCBF, **49**), cannabifuran (CBF, **50**), cannabichromanon (CBCN, **51**), cannabicitran (CBT, **52**), 10-oxo-$\Delta^{6a(10a)}$-tetrahydrocannabinol (OTHC, **53**), Δ^9-(6a,10a-*cis*)-tetrahydrocannabinol (*cis*-Δ^9-THC, **54**), 3,4,5,6-tetrahydro-7-hydroxy-α,α,2-trimethyl-9-n-propyl-2,6-methano-2H-1-benzoxocin-5-methanol (OH-*iso*-HHCV, **55**), (−)-(6aR,9S,10S,10aR)-9,10-dihydroxyhexahydrocannabinol [cannabiripsol (CBR, **56**)], and 6a,7,10a-trihydroxy-Δ^9-tetrahydrocannabinol (6a,7,10a-triOH-Δ^9-THC, **57**) make up the miscellaneous cannabinoids.

TABLE 12.



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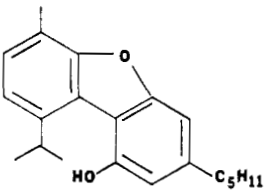

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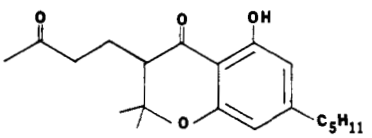
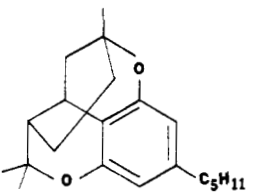
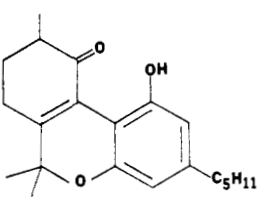


51



52

	Miscellaneous	Ref
49	Dehydrocannabifuran	146
50	Cannabifuran	146
51	Cannabichromanon	146
52	Cannabicitran	152



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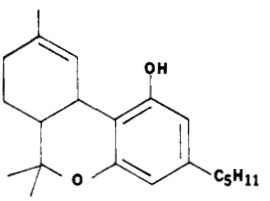

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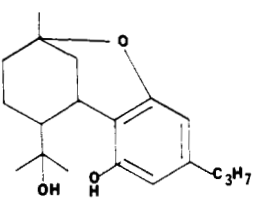

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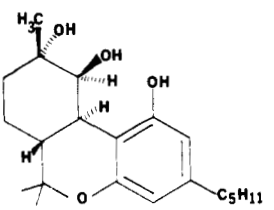
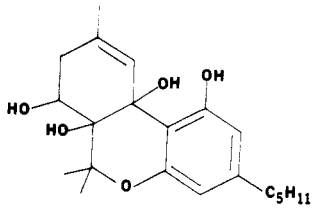


56

53	10-Oxo-$\Delta^{6a(10a)}$-Tetrahydrocannabinol	146
54	Δ^9-(6a,10a,*cis*)-Tetrahydrocannabinol	153
55	3,4,5,6-Tetrahydro-7-hydroxy-α,α-2-trimethyl-9-n-propyl-2,6-methano-2H-1-benzoxocin-5-methanol	154
56	(−)-(6aR,9S,10S,10aR)-9,10-Dihydroxyhexahydrocannabinol [Cannabiripsol]	155

TABLE 12. Continued.



57

	Miscellaneous	Ref
57	6a,7,10a-Trihydroxy-Δ^9-tetrahydrocannabinol	155

Dehydrocannabifuran, cannabifuran, and cannabichromanon were isolated by Friedrich-Fiechtl and Spiteller (146) in 1975. These authors used cyclohexane methanol extracts of green Afghan hashish and subjected the extracts to glass capillary chromatography. Subsequently, micropreparative gas chromatography followed by thin layer chromatography gave pure dehydrocannabifuran, cannabifuran, and cannabichromanon. Structure assignments were made with the usual physical data.

Cannabicitran was isolated by Bercht *et al.* (152) in 1974 from an ethanolic extract of Lebanese hashish. Purification was by counter-current distribution followed by repeated chromatography on silica gel. Previously cannabicitran was called citrylidene-cannabis by Crombie and Ponsford (126). This research team prepared cannabicitran in the course of their synthetic work on cannabinoids and predicted cannabicitran would be found in nature.

10-Oxo-$\Delta^{6a(10a)}$-tetrahydrocannabinol was isolated by Friedrich-Fiechtl and Spiteller (146) using the same materials and methods as reported for dehydrocannabifuran, cannabifuran, and cannabichromenon.

Δ^9-(6a,10a-*Cis*)-tetrahydrocannabinol was isolated from petrol extracts of contraband marihuana in 1977 by Smith and Kempfert (153). Purification was by a Florisil chromatography followed by repeated preparative thin layer chromatography.

3,4,5,6-Tetrahydro-7-hydroxy-α,α,2-trimethyl-9-*n*-propyl-2,6-methano-2H-1-benzoxocin-5-methanol (OH-*iso*-HHCV) was isolated by Turner's group (154) from an ethanolic extract of an Indian variant. The ethanol extract was concentrated *in vacuo* and triturated with hexane. The OH-*iso*-HHCV was obtained from hexane solubles. Structure proof was by synthesis.

(−)-(6aR,9S,10S,10aR)-9,10-dihydroxyhexahydrocannabinol (cannabiripsol) was isolated from a hexane extract of South African *Cannabis* by Turner's group (155). Purification was accomplished by chromatography with silica gel and polyamide. Stereochemical assignment was based on synthesis.

6a,7,10a-Trihydroxy-Δ^9-tetrahydrocannabinol was isolated from a hexane extract of small leaves, stems, and flowering tops of Mexican marihuana produced in Mississippi. The hexane solubles were partitioned with NaOH, and the aqueous layer was neutralized and extracted with petroleum ether. Purification was by column chromatography with silica gel.

No pharmacological data are available on these miscellaneous cannabinoids. However, recent trends in *Cannabis* research may generate interest in these compounds.

NITROGENOUS COMPOUNDS

Nitrogenous compounds in *Cannabis* can be traced to the earliest chemical investigations and publications on *Cannabis*. Preobraschensky (156) in 1876 reported nicotine to be present in Chinese hashish from Taschkent, China. The hashish was extracted with water and evaporated. The residue was macerated with ethanol and treated with sulfuric acid and water and then distilled. The acidic residue provided material which was saturated with sodium bicarbonate and extracted with amyl alcohol. Subsequent work-up provided the alkaloid nicotine.

The findings by Preobraschensky were immediately challenged by several chemists, including Professor Dragendorff and Dr. Marquiss, based on the striking difference between the physiological action of *Cannabis* and tobacco and the fact that crude drugs from *Cannabis* often contain other herbs (157).

In 1881 Siebold and Bradbury (158) reported they could not detect the presence of nicotine in extracts from ten pounds of Indian *Cannabis*. They soaked the plant material in a strong base followed by heating with steam until one-half of the water was removed. Neutralization with oxalic acid followed by slow evaporation was carried out at 70° and below. The dry residue was pulverized and extracted four times with ether. The powder was dried and extracted with alcohol and dissolved in water. Subsequent work-up of the water layer (neutral and basic) with ether did not provide nicotine but, rather, 2 grams of a compound believed to be an unknown alkaloid which was named "cannabinine."

Hay (159) reported the presence of "several alkaloids" in *Cannabis* in 1883. These alkaloids were precipitated by alkaloid precipitants. One alkaloid was isolated by Hay from a water infusion of powdered *Cannabis* by treating it with subacetate of lead and filtering. Ammonia was added to the filtrate and the precipitate removed by filtration. The filtrate was acidified with sulfuric acid, and the alkaloids were precipitated with phosphowolframic acid. Subsequent work-up provided colorless needle-like crystals which caused tetanus in frogs in exactly the same manner of strychnia; thus, this unknown alkaloid was given the name of "tetano-cannabine." Hays did not find nicotine in any of his alkaloid fractions.

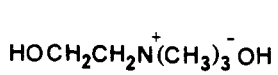
Kennedy (160) in 1886 conducted experiments on tobacco and *Cannabis* to ascertain if nicotine was present in *Cannabis*. The exact same weights, conditions, etc., were used to extract *Cannabis* and tobacco: both were percolated with ethanol for 24 hours. Extracts were divided and worked-up under identical conditions. No nicotine was found in *Cannabis*, but there were indications of alkaloids in *Cannabis*.

No basic structure work was done by these early researchers, but today nitrogenous compounds in *Cannabis* can be classified into four groups: quaternary bases, amides, amines, and spermidine alkaloids. To date 20 nitrogenous compounds have been found in various *Cannabis* plant parts, and 6 were tentatively identified according to R_f.

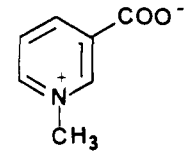
QUATERNARY BASES

Choline (**58**), trigonelline (**59**), muscarine (**60**), L-(+)-isoleucine betaine (**61**), and neurine (**62**) make up the quaternary bases found in *Cannabis*.

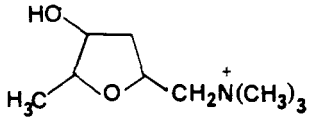
Jahns (161), in 1887, reported the first isolation and identification of a nitrogenous substance from a water extract of Indian *Cannabis* which was evaporated and the residue was redissolved in ethanol. The alkaloid was precipitated from ethanol as its platinum salt and recrystallized from water. It was determined that the unknown alkaloid was choline. Jahns expressed the opinion that choline might be identical to Siebold and Bradbury's (158) "cannabinine" and Hay's (159) "tetano-cannabine."



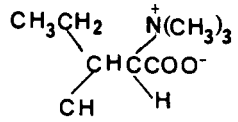
58 Choline (161).



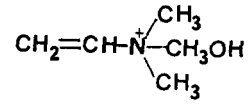
59 Trigonelline (162).



60 Muscarine (166).



61 L-(+)-isoleucine betaine (174).



62 Neurine (175, 176).

FIG. 11. Quaternary bases.

Trigonelline was reported to have been isolated from *Cannabis* by Schulze and Frankfurt (162) as its platinum salt. The structure was proven by synthesis and comparison. No data was given on the isolation procedure. Trigonelline represents the first heterocyclic compound of a known structure isolated from *Cannabis*. The paper was published in 1894, although the paper indicated the work was carried out earlier.

The following year, Marino-Zuco and Vignolo (163) published the first review on the progress of separating nitrogenous compounds from *Cannabis*. Then seven years elapsed before another paper was published on the alkaloids in *Cannabis*, a very brief article by Humphrey (164) in 1902. Thirty-eight years passed before another work on alkaloids in *Cannabis* was published. Merz and Bergner (165) used three *Cannabis* samples grown in Germany. Samples were extracted with ether, petroleum ether, ethanol and water. Alkaloids were obtained from the water fraction, which was acidified with sulfuric acid, extracted with ether, and treated with several precipitating reagents. Choline and trigonelline were obtained. This confirmed the earlier work by Schulze and Frankfurt (162).

Salemink *et al.* (167), in 1965, published an article stating that at least six quaternary bases were present in *Cannabis sativa*. Material used was a mixture of *Cannabis* grown in the Netherlands from seeds obtained from Chile, France, Italy, Yugoslavia, the Netherlands, Poland, Russia, Turkey and Hungary. One base was identified as choline, but the presence of trigonelline was suspected but not absolutely confirmed in Dutch *Cannabis*. Results also showed no muscarine present, although the authors were very interested in muscarine type compounds. *Cannabis* seeds were found to contain choline, trigonelline, and three additional bases. Bercht and Salemink (168), in 1969, investigated the bases in French *Cannabis* seed (Fibrimon-a fiber type). Seeds were ground and extracted with

96% ethanol. Subsequent work-up of the pulp and the ethanol extract, precipitation of the nitrogen bases with Kraut's reagent, provided nitrogen bases which were chromatographed on cellulose powder. Choline chloride and trigonelline hydrochloride were confirmed by R_f-values, mp's, and ir spectra.

Samrah (169) reported the screening of *Cannabis* samples from Madagascar, South Africa, and Spain. Nine precipitating reagents and six color reactions for alkaloids were used. No definite results were reported, but quaternary bases were present.

Gill, *et al.* (170), obtained trigonelline from the watery extract of a tincture of *Cannabis* B.P.C. This commercial product was prepared from *Cannabis* plant material grown in Pakistan. Two muscarinic and one atropinic substances were found. These findings were based on pharmacological data. No additional data have been published by these authors in this regard. Thus, it is highly unlikely muscarinic and atropinic compounds are present in *Cannabis;* rather, it is likely compounds that pharmacologically mimic these types of compounds are present.

Aguar (171) screened *Cannabis* grown in Spain and South Africa, Switzerland and Czechoslovakia and two samples of unknown seed origin for alkaloids. Qualitative results showed all samples contained alkaloids. No attempt was made to separate the alkaloids into classes. Reagents used would detect quaternary bases having a structure similar to the ergot alkaloids.

Paris, *et al.* (172), screened the pollen of *Cannabis* grown in France from seeds of South African and Turkish variants. "Alkaloid type" substances were found to be more abundant in the South African variant.

In 1973, Lousberg and Salemink (173) proposed the structure (see figure 12)

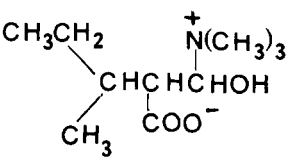


FIG. 12. Betaine base-1973 (172) proposed by Lousberg and Salemink.

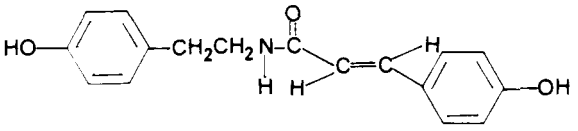
for a betaine base which they obtained from the ethanol extract of *Cannabis* seed of the Fibrimon variant. Purification was on a cellulose column. However, the physical data of this compound are identical with the data of a compound isolated by Bercht (174) (structure **61**), which might indicate that both compounds are the same. Synthesis by Bercht, *et al.* (174) in 1973 proved the structure of the compound proposed by Lousberg and Salemink (173) was actually L-(+)-isoleucine.

After the first new quaternary alkaloid from *Cannabis* since 1894 was proven by Salemink's group (174), Turner's group (175, 176) reported neurine. Neurine was obtained from roots of Mexican *Cannabis* grown in Mississippi which were extracted consecutively with hexane, chloroform, ethanol, water and 5% HCl. The water layer contained a brown syrup which was chromatographed on a strong anion exchange column; purification of the compound on alumina provided neurine and choline. To show neurine to be a naturally occurring compound in *Cannabis* roots, it was demonstrated that the extract was never subjected to forcing conditions required to dehydrate choline.

The pharmacology of quaternary alkaloids from *Cannabis* in the form of crude extracts was reported by Gill, *et al.* (170). Data on the pure alkaloids can be obtained in the literature since they are not indigenous to *Cannabis* and are all well known.

AMIDES

N-(*p*-hydroxy-β-phenylethyl)-*p*-hydroxy-(*trans*)-cinnamide (**63**) is the only amide known to exist in *Cannabis*.



63 N-(p-hydroxy-β-phenylethyl)-p-hydroxy-(trans)-cinnamide (177).

FIG. 13. Amides.

Slatkin *et al.* (177) extracted ground roots of Mexican *Cannabis sativa* grown in Mississippi with ethanol. The ethanol was removed and the residue was dissolved in ether and shaken with 1% HCl. The acid extract was rendered alkaline with concentrated ammonia solution, extracted with chloroform, and purified on silica gel to give the amide. Proof of the structure was by synthesis. The only other report of this amide was in 1968; it was isolated from *Evodia belahe*.

Mild analgesic activity for this amide was observed in the mouse tailflick test.

AMINES (table 13)

Piperidine (**64**), hordenine (**65**), ammonia (**66**), methylamine (**67**), ethylamine (**68**), *n*-propylamine (**69**), *n*-butylamine (**70**), iso-butylamine (**71**), secbutylamine (**72**), dimethylamine (**73**), diethylamine (74), and pyrrolidine (**75**) make up the nitrogenous compounds in this class.

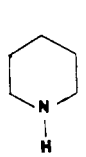
Piperidine was identified in *Cannabis* by Obata, *et al.* (178) in 1960. Leaves and tops of wild *Cannabis* growing in Yubari County in Hokkaido, Japan, were extracted four times with ether. The ether was evaporated and the residue was extracted with 90% alcohol and concentrated *in vacuo*. Piperidine odor was detected in the ethanol distillate and hydrochloric acid was used to adjust the pH to 3. Concentration *in vacuo* followed by neutralization with potassium hydroxide followed by extraction with ether provided a product identical to piperidine. Subsequently, Salemink's group (167, 168) have identified piperidine in *Cannabis*.

Hordenine, the first and only β-arylethylamine isolated from *Cannabis* was reported in 1975 by El-Feraly and Turner (179). Dried leaves of *Cannabis* grown in Mississippi from an unknown drug type were percolated with 95% ethanol at ambient temperature. The residue obtained on evaporation of the solvent was partitioned between 2% citric acid and chloroform. The aqueous phase was rendered alkaline with concentrated ammonia and extracted with chloroform. After further partitioning, the crude alkaloid fraction was chromatographed on silica gel G. A light yellow crystalline material was obtained, recrystallized from acetone-hexane to give needles which were identical to hordenine. Subsequently, Elsohly and Turner (180) isolated hordenine from the leaves and small stems of a Mexican variant of *Cannabis* grown in Mississippi. Elsohly and Turner (181)

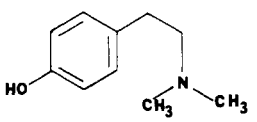
later found hordenine in fifteen variants of *Cannabis* produced in Mississippi from the following countries: Afghanistan, Australia, Czechoslovakia, Hungary, India, Jamaica, Lebanon, Mexico, South Africa, Spain, Thailand, Turkey and Russia.

Previous to the work by Turner's group (179, 180) Klein and Rapoport (182) reported the presence of four alkaloid compounds in confiscated commercial *Cannabis* of Mexican origin. These compounds reported in 1971 were called: cannabamines A, B, C and D. Cannabamine C was thought to be a β-arylethylamine. All conclusions were based on mass spectral information. Data were presented on the pharmacology of compounds A, B, C and D. No further work has been published by this group.

TABLE 13. "Simple" amines.



64 Piperidine (178).



65 Hordenine (179, 181).

No.	Compound	Ref.
66	Ammonia	173
67	Methylamine	173
68	Ethylamine	173
69	n-Propylamine	173
70	n-Butylamine	173
71	iso-butylamine	173
72	sec-butylamine	173
73	Dimethylamine	173
74	Diethylamine	173
75	Pyrrolidine	173

Tentatively Identified:

1 iso-Amylamine Reference 173
2 β-Phenethylamine Reference 173
3 n-Pentylamine Reference 173
4 Cadaverine Reference 173
5 Ethanolamine or histamine Reference 173
6 Benzylamine or tyramine Reference 173

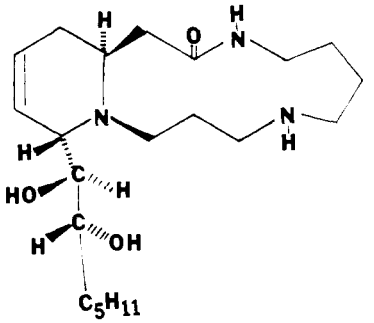
Ammonia, methylamine, ethylamine, *n*-propylamine, *n*-butylamine, iso-butylamine, sec-butylamine, dimethylamine, diethylamine and pyrrolidine were identified in Dutch *Cannabis* by Lousberg and Salemink (173) in 1973. The amine fraction was isolated by steam distillation of the plant material. Capillary gas chromatography was used for identification. Tentatively identified were *n*-pentylamine, isoamylamine, β-phenethylamine, cadaverine, ethanolamine or histamine, and benzylamine or tyramine.

Little pharmacological data is available on crude amine extracts of *Cannabis* (182); however, most are common amines with known biological profiles.

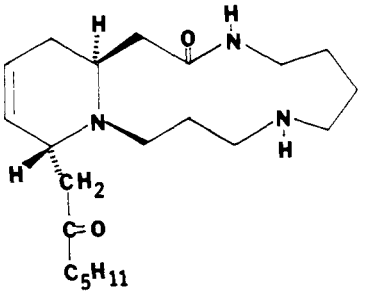
SPERMIDINE ALKALOIDS

Cannabisativine (**76**) and anhydrocannabisativine (**77**) make up the nitrogenous compounds in this class.

Cannabisativine was first reported by Lotter *et al.* (183) in 1975. This report was mainly on X-ray data. Isolation was from a methanol extract of roots of a Mexican variant grown in Mississippi. The methanol extract was partitioned between water and chloroform. The chloroform fraction was then partitioned between petroleum ether and methanol-water. The aqueous-methanol fraction was chromatographed on silicic acid. Elution with 8% methanol-chloroform provided a residue which was subsequently isolated and purified by crystallization from acetone (184).



76 Cannabisativine (183, 184).



77 Anhydrocannabisativine (180, 187).

FIG. 14. Spermidine alkaloids.

Cannabisativine represents the first "new" alkaloid isolated from *Cannabis*. Compounds of this nucleus (palustridine, palustine) have been isolated from the genus *Equisetum* but this nucleus was previously unknown in higher plants (183).

El-Feraly and Turner (185) later isolated cannabisativine from an ethanol percolation of dry leaves and small stems of a Thailand variant of *Cannabis* grown in Mississippi. The extraction procedure was more complex than the isolation from root material. The 95% ethanol percolates were combined and evaporated *in vacuo*. The residue was stirred with chloroform and 2% citric acid in water. The aqueous layer was then separated and the chloroform layer extracted three times with aqueous citric acid. Combined aqueous extracts were basified with concentrated ammonia solution to pH 9 and extracted four times with chloroform. Chloroform layers were combined and dried over anhydrous sodium sulfate, then evaporated. The residue was stirred with 1N HCl and chloroform.

Additional acid-base work-ups provided crude cannabisativine which was purified by thin layer and column chromatography followed by recrystallization from acetone.

Elsohly and Turner (181) screened fifteen variants and found cannabisativine present in all fifteen. However, with tlc and the solvent system of 4% ammonia in methanol, cannabisativine and hordenine were not separated, but were cleanly separated by chloroform-acetone-ammonia (1:1:1).

Waller, *et al.* (186) also reported cannabisativine in a progress report of the separation and isolation of nitrogenous compounds from *Cannabis* by the Mississippi group.

Elsohly and Turner reported anhydrocannabisativine in 1976 (180). They isolated the compound from the dry leaves and small stems of a Mexican variant

grown in Mississippi. Plant material was defatted by extraction with hexane and then extracted with 95% ethanol. The concentrated ethanol extract was partitioned between chloroform and 2% citric acid. The acidic fraction was again washed with chloroform, and the chloroform extract was washed with 2% citric acid. The last acidic washing was combined with the original acidic fraction and rendered alkaline with ammonium hydroxide to pH 9 and extracted with chloroform. Work-up of the chloroform layer provided the crude alkaloidal fraction which was chromatographed on silica gel G. The alkaloid was very difficult to crystallize as was the picrate salt. Structure proof was by physical data and conversion of cannabisativine to anhydrocannabisativine. Elsohly *et al.* (187) reported the isolation of anhydrocannabisativine from the roots and leaves of Mexican *Cannabis*. The procedure for isolation from the root was similar to the procedure used in the isolation from the plant material as reported by Elsohly and Turner (180). Elsohly and Turner (181) reported anhydrocannabisativine to be present in fifteen samples of *Cannabis* from different geographical locations. With tlc and the solvent system of 4% ammonia in methanol, anhydrocannabisativine has a lower R_f than combined hordenine-cannabisativine; whereas, with the solvent system of acetone-chloroform-ammonia (1:1:1), anhydrocannabisativine has a higher R_f than hordenine and cannabisativine which are well separated.

No pharmacological data have been published for cannabisativine or anhydrocannabisativine. The small yields and difficulty in purification have severely limited the amount of material available for further work. It should be noted that cannabamine D reported by Klein and Rapoport (182) has the same molecular formula as anhydrocannabisativine ($C_{17}H_{22}N_3O_2$); therefore, it is possible that both compounds are the same.

The possibility of the presence of indole alkaloids in *Cannabis* has been discussed by Samrah, *et al.* (188). To date, no data exist that indicate indole alkaloids are present in *Cannabis*.

Several review articles are available on the nitrogenous compounds in *Cannabis*. Elsohly and Turner (11) provided physical constants and details on the extraction procedure. Hanuš (14) reviewed the nitrogenous substances prior to the work published by the Mississippi group. A report from the U.N. (28) also reviewed the nitrogenous substances as well as other components in *Cannabis*. Vree, *et al.* (189) have also reviewed *Cannabis'* nitrogenous compounds and selected other topics.

AMINO ACIDS (table 14)

Alanine (**78**), asparatic acid (**79**), cystine (**80**), glutamic acid (**81**), glycine (**82**), serine (**83**), arginine (**84**), histidine (**85**), isoleucine (**86**), leucine (**87**), lysine (**88**), methionine (**89**), phenylalanine (**90**), proline (**91**), threonine (**92**), tryptophane (**93**), tyrosine (**94**), and valine (**95**) make up the amino acids known to occur in *Cannabis*. Obata *et al.* (178) in 1960 extracted the tops of wild hemp with petroleum ether four times, concentrated the extractables and extracted them with 90% ethanol. Subsequent work-up afforded leucine, methionine, threonine, valine, histidine, and lysine.

In 1972 Lousberg and Salemink (173), using an amino acid analyzer, determined alanine, asparatic acid, glutamic acid, glycine, serine, arginine, isoleucine, phenylalanine, proline, and tyrosine were present in *Cannabis* plant material. This group also supported the previous amino acid reported by Obata *et al.* (178) with the exception of methionine. Turner and Mole in 1973 reported the isolation

of proline from an ethanol extract of the stems, leaves, and flowering tops of an Indian variant of *Cannabis* (110, 175). Following the report by Turner's group, Wallace *et al.* (190) reported the amino acid sequence of hemp cytochrome-C. This report showed cystine, methionine, and tryptophane, as well as the other amino acids, to be present in *Cannabis*. The amino acid sequence of Haem peptide was as follows: Lys-Thr-Lys-Cys-Ala-Glu-Cys-His-Thr-Val-Gly-Arg-Gly-Ala-Gly-His. Data are also presented on the non-haem petides.

TABLE 14. Amino acids.

No.	Compound	Ref.
78	Alanine	172
79	Asparatic acid	172
80	Cystine	190
81	Glutamic acid	172
82	Glycine	172
83	Serine	172
84	Arginine	172
85	Histidine	178
86	Isoleucine	172
87	Leucine	178
88	Lysine	178
89	Methionine	178, 190
90	Phenylalanine	172
91	Proline	110, 172
92	Threonine	178
93	Tryptophane	190
94	Tyrosine	172
95	Valine	178

Amino acids of the types found in *Cannabis* occur widely in nature, and no elaborate discussion is required.

PROTEINS, GLYCOPROTEINS, AND ENZYMES (table 15)

Edestin (**96**), zeatin (**97**), zeatin nucleoside (**98**), edestinase (**99**), glucosidase (**100**), polyphenol oxidase (**101**), peptidase (**102**), peroxidase (**103**), and adenosine-5-phosphatase (**104**), and two glycoproteins of unknown structure make up the known compounds in this class.

In 1964 Stockwell *et al.* (191) isolated edestin from *Cannabis* fruits and determined the amino acid composition. Edestin and edestinase were subsequently isolated from viable *Cannabis* fruits by Angello *et al.* in 1968 (192) and 1969 (193), respectively.

TABLE 15. Proteins, glycoproteins, enzymes.

No.	Compound	Ref.
96	Edestin	191
97	Zeatin	193
98	Zeatin nucleoside	193
99	Edestinase	192
100	Glucosidase (emulsin)	197
101	Polyphenol oxidase	198
102	Peptidase (ereptase)	200
103	Peroxidase	198
104	Adenosine-5-phosphatase	199

Zeatin and Zeatin nucleoside were isolated in 1971 by Rybicka and Engelbercht from a water extract of fruits (194). Hillestad and co-workers (195, 196) obtained a water-soluble glycoprotein from the leaves of South African and Thailand *Cannabis*, respectively. The glycoprotein from South African *Cannabis* leaves contained the amino acid hydroxproline, whereas, Thailand *Cannabis* leaves contained a glycoprotein without hydroxyproline.

Glucosidase (emulsin) was detected in *Cannabis* (hemp) fruits by Leoncini (197) in 1931. Polyphenol oxidase and peroxidase were studied in male and female plants by Ostapenko in 1960 (198); the activity was more pronounced in the male plants.

Adenosine-5-phosphatase was detected in dormant cotyledons of *Cannabis* by Bargoni and Luzzati in 1956 (199), and peptidase was detected by Vines in 1903 (200).

SUGARS AND RELATED COMPOUNDS

Sugars occur widely in nature. Fructose, xylose, and glucose, (monosaccharides), were the first sugars reported to be present in *Cannabis* (201). This work was done in 1932 on stalks of *Cannabis*. (−) Quebrachitol, a cyclitol, was the first sugar actually isolated from *Cannabis*. This work was reported by Adam's group (74) in 1940. Work on sugars and related compounds was not in abeyance but received very little attention from 1940 until the early 1970's. During this time, the cannabinoids were investigated with little emphasis on other chemical constituents.

TABLE 16. Monosaccharides.

No.	Compound	Ref.
105	Arabinose	202
106	Fructose	201, 202
107	Galactose	205
108	Galacturonic acid	206
109–110	α and β-D-glucose	201, 203
111	*altro*-Heptulose (*sedo*-heptulose)	202
112	D-*manno*-heptulose	202
113	Mannose	206
114	D-*glycerol*-D-*manno*-Octulose	202
115	Rhamnose	205
116	Ribose	205
117	Xylose	201

Today, the sugars and related compounds in *Cannabis* account for 34 compounds and can be classified into five groups: monosaccharides, disaccharides, polysaccharides, sugar alcohols-cyclitols, and amino sugars.

MONOSACCHARIDES (table 16).—Arabinose (**105**), fructose (**106**), galactose (**107**), galacturonic acid (**108**), α-D-glucose (**109**), β-D-glucose (**110**), alto-heptulose (**111**), D-*manno*-heptulose (**112**), mannose (**113**), D-*glycerol*-D-*manno*-octulose (**114**), rhamnose (**115**), ribose (**116**), and xylose (**117**) make up the monosaccharide compounds found in *Cannabis*.

Although Parisi (201) was the first to report sugars in *Cannabis* with the detection of fructose, xylose, and glucose, Haustveit and Wold (202) carried out the first comprehensive study on the carbohydrates in *Cannabis* in 1973. They isolated sugars from the leaves and stems of *Cannabis* grown in Oslo from seed of Swiss origin. Extraction was by boiling the plant material with 65% ethanol for

20 minutes. After filtration and evaporation, the extractables were treated with lead acetate, deionized, and concentrated to a thick syrup. This syrup was fermentated with baker's yeast. Centrifugation and removal of residual protein with lead acetate, followed by deionization and subsequent work-up on a carbon-celite column, followed by preparative paper chromatography, provided D-*manno*-heptulose, *altro*-heptulose, D-*glycerol*-D-*manno*-octulose and arabinose; glucose and fructose were also isolated. Also, in 1973, Groce and Jones (203) used ethanol and water to extract sugars from Mexican *Cannabis* plants grown in Mississippi. The sugars found in Mexican male *Cannabis* were compared to those found in Thailand and Vietnam *Cannabis* variants obtained from police seizures. Thus, the true country of origin is not absolute. These authors worked up the extracts and analyzed them on a Beckman GO-45 equipped with a 2% OV-17 column; silyl derivatives were formed. Identification was by relative retention times of known compounds. With this procedure, α and β-D-glucose were found in the ethanol and water extracts of all three variants. Of the five fructose isomer peaks, it was observed that in both extracts D-fructose$_3$ was present in the Mexican and Thailand variants but not the Vietnam.

Galactose was found to be present in Indian *Cannabis* by Krishnamurty and Kaushal (204). Dried flowers and leaves were extracted with 50% ethanol. Identification was by paper chromatography.

Bózner, in 1960, (205) analyzed the sugar contents of *Cannabis* by paper chromatography to show it was possible to distinguish between several fiber variants: Rastislavice, Fleischman, and Hungarian. Bózner reported the first detection of galactose, rhamnose and ribose.

Hillestad *et al.* (206) investigated the water-soluble, non-dialysable, carbohydrate-protein material from South African *Cannabis* grown in Oslo. Chromatography on DEAE-cellulose afforded, for the first time, mannose and galacturonic acid. Other simple sugars previously reported were also isolated.

DISACCHARIDES (table 17).—Sucrose (**118**) and maltose (**119**) are the only disaccharides known to be present in *Cannabis*. They were first reported by Bózner (205) in 1960 in several fiber variants of *Cannabis*. Later Groce and Jones (203) reported sucrose in three drug variants: Thailand, Vietnam and Mexican.

TABLE 17. Disaccharides.

No.	Compound	Ref.
118	Sucrose (saccharose)	203, 205
119	Maltose	205

POLYSACCHARIDES (table 18).—Raffinose (**120**) an oligosaccharide, cellulose (**121**), hemicellulose (**122**), pectin (**123**), and xylan (**124**) make up the compounds in the polysaccharide class.

Parisi (201) discussed the yield of xylan from hemp stalks in 1932. The xylan, a white powder, gave xylose when hydrolyzed. Parisi also reported cellulose and hemicellulose.

Pectin was reported by Negro (207) in 1951, and Kostrubin *et al.* (208) added to Parisi's (201) work on hemicelluloses and Negro's work on pectin in 1953. Yarosh (209) did a very thorough investigation into cellulose and hemicellulose in 1963. He found there was a maximum accumulation of carbohydrates in hemp

during budding and flowering. In the mature phase, hemp plants contained 43.0–43.5, 12.0–12.3, and 1.14–4.53%, respectively, of cellulose, hemicellulose and sugars.

TABLE 18. Polysaccharides.

No.	Compound	Ref.
120	Raffinose (oligosaccharide)	205
121	Cellulose	201
122	Hemicellulose	201
123	Pectin	207
124	Xylan	201

SUGAR ALCOHOLS AND CYCLITOLS (table 19).—Arabitol (arabinitol) (**125**), erythritol (**126**), galactitol (**127**), glycerol (**128**), mannitol (**129**), ribitol (**130**), sorbitol (**131**), and xylitol (**132**), make up the sugar alcohols and D(−)-bornesitol (**133**), (+)-inositol (**134**), *myo*-inositol (**135**) and (+)-quebrachitol, (**136**) make up the cyclitols found in *Cannabis*.

TABLE 19. Sugar alcohols and cyclitols.

No.	Compound	Ref.
125	Arabitol (arabinitol)	206
126	Erythritol	202, 203
127	Galactitol	204
128	Glycerol	202
129	Mannitol	204
130	Ribitol	203
131	Sorbitol	204
132	Xylitol	202
133	D(−)-Bornesitol	203
134	(+)-Inositol	203
135	*myo*-Inositol	202, 203
136	(+)-Quebrachitol (1L-2-O-methyl-*chiro*-inositol)	74, 210

The first compound cyclitol, quebrachitol, was isolated from the red oil of Minnesota wild hemp by Adam's group (74) in 1940. No further work was reported on cyclitols until 1953 when Plouvier (210) reported quebrachitol in *Cannabis sativa*.

Groce and Jones (203) first reported, in a paper submitted July 26, 1972, and published in 1973, the cyclitols (+)-inositol, *myo*-inositol, and (−)-bornesitol. Detection was by silylation followed by gc analysis. Thailand, Vietnam and Mexican variants grown in Mississippi were investigated. Only the Thailand sample contained (+)-inositol, whereas, the other cyclitols were found in all variants. The carbohydrate-cyclitol content was Mexican > Thailand > Vietnam.

Groce and Jones (203) also first reported the sugar alcohols erythritol and ribitol. Erythritol was only found in the Vietnam sample, whereas, ribitol was found in all three variants.

In a paper submitted December 1, 1972, and published in 1973, Haustveit and Wold (202) reported *myo*-inositol in *Cannabis sativa* leaves grown in Oslo from Swiss seed. They reported xylitol and glycerol in *Cannabis* for the first time and confirmed erythritol's presence. As in most studies on carbohydrates, Groce and

Jones (203) and Haustveit and Wold (202) reported quebrachitol in all variants investigated.

Krishnamurty and Kaushal (204) reported in 1976, galactitol, mannitol, and sorbitol in *Cannabis sativa* (Indian marihuana). "Dried flowers and top leaves" were used. Detection was by paper chromatography. As did other researchers, this group also found quebrachitol.

Arabinose was first reported by Hillestad *et al.* (206) in 1977. Arabinose was isolated from South African *Cannabis* grown in Oslo. Gel filtration and gc methods were used.

No previously unknown sugar alcohols or cyclitols have been, to date, isolated from *Cannabis*.

AMINO SUGARS (table 20).—Galactosamine (**137**) and glucosamine (**138**) make up the amino sugars known to occur in *Cannabis*.

TABLE 20. Amino sugars.

No.	Compound	Ref.
137	Galactosamine	211
138	Glucosamine	211

Wold and Hillestad (211) reported the isolation of galactosamine and glucosamine in 1976. They used a light petrol extract of milled dried leaves and stems of South African *Cannabis* grown in Oslo. Separation was by ion exchange chromatography on DEAE-cellulose and subsequent gel filtration on Sepharose 4B. Both amino sugars were analyzed as their trimethylsilyl ethers by cg, and both are thought to be part of a carbohydrate protein polymer. Glucosamine was known to exist in higher plants, but the presence of galactosamine was not previously established conclusively.

HYDROCARBONS (table 21)

The following compounds make up the hydrocarbons in *Cannabis: n*-nonane (**139**), *n*-decane (**140**), *n*-undecane (**141**), *n*-dodecane (**142**), *n*-tridecane (**143**), *n*-tetradecane (**144**), 3,6-dimethyl-tridecane (**145**), *n*-pentadecane (**146**), 2,6-dimethyl-tetradecane (**147**), *n*-hexadecane (**148**), *n*-heptadecane (**149**), 2,6-dimethyl-hexadecane (**150**), *n*-octadecane (**151**), 3,6-dimethyl-heptadecane (**152**), 3,7-dimethyl-heptadecane (**153**), *n*-nonadecane (**154**), 3,6-dimethyl-octadecane (**155**), 3,7-dimethyl-octadecane (**156**), *n*-eicosane (**157**), *n*-heneicosane (**158**), 3-methyl-heneicosane (**159**), *n*-docosane (**160**), *n*-tricosane (**161**), 3-methyl-tricosane (**162**), *n*-tetracosane (**163**), 2-methyl-tetracosane (**164**), *n*-pentacosane (**165**), *n*-hexacosane (**166**), 3-methyl-pentacosane (**167**), 2-methyl-hexacosane (**168**), *n*-heptacosane (**169**), 3-methyl-heptacosane (**170**), *n*-octacosane (**171**), 2-methyl-octacosane (**172**), 9-methyl-octacosane (**173**), *n*-nonacosane (**174**), 3-methyl-triacontane (**175**), *n*-triacotane (**176**), 2-methyl-hentriacontane (**177**), *n*-hentriacontane (**178**), 3-methyl-hentriacontane (**179**), *n*-dotriacontane (**180**), 2-methyl-dotriacontane (**181**), *n*-tritriacontane (**182**), tetra-triacontane (183), pentatriacontane (**184**), hexatriacontane (**185**), heptatriacontane (**186**), octatriacontane (**187**), and nonatriacontane (**188**).

Using Indian hemp, Valente (212, 213) carried out the original work on hydrocarbons in 1880 and 1881, respectively. Wood *et al.* (173), in 1896, isolated *n*-

TABLE 21. Hydrocarbons.

No.	Compound	Ref.
139	C_9H_{20} *n*-Nonane	217
140	$C_{10}H_{22}$ *n*-Decane	217
141	$C_{11}H_{24}$ *n*-Undecane	217
142	$C_{12}H_{26}$ *n*-Dodecane	217
143	$C_{13}H_{28}$ *n*-Tridecane	217
144	$C_{14}H_{30}$ *n*-Tetradecane	217
145	$C_{15}H_{32}$ 3,6-Dimethyl-tridecane	217
146	$C_{15}H_{32}$ *n*-Pentadecane	217
147	$C_{16}H_{34}$ 2,6-Dimethyl-tetradecane	217
148	$C_{16}H_{34}$ *n*-hexadecane	217
149	$C_{17}H_{36}$ *n*-heptadecane	217
150	$C_{18}H_{38}$ 2,6-Dimethyl-hexadecane	217
151	$C_{18}H_{38}$ *n*-Octadecane	217
152	$C_{19}H_{40}$ 3,6-Dimethyl-heptadecane	217
153	$C_{19}H_{40}$ 3,7-Dimethyl-heptadecane	217
154	$C_{19}H_{40}$ *n*-Nonadecane	214, 217
155	$C_{20}H_{42}$ 3,6-Dimethyl-octadecane	217
156	$C_{20}H_{42}$ 3,7-Dimethyl-octadecane	217
157	$C_{20}H_{42}$ *n*-Eicosane	214, 217
158	$C_{21}H_{44}$ *n*-Heneicosane	214, 217
159	$C_{22}H_{46}$ 3-Methyl-heneicosane	217
160	$C_{22}H_{46}$ *n*-Docosane	214, 217
161	$C_{23}H_{48}$ *n*-Tricosane	214, 217
162	$C_{24}H_{50}$ 3-Methyl-tricosane	217
163	$C_{24}H_{50}$ *n*-Tetracosane	214, 217
164	$C_{25}H_{52}$ 2-Methyl-tetracosane	215, 217
165	$C_{25}H_{52}$ *n*-Pentacosane	214, 217
166	$C_{26}H_{54}$ *n*-Hexacosane	214, 217
167	$C_{26}H_{54}$ 3-Methyl-pentacosane	215, 217
168	$C_{27}H_{56}$ 2-Methyl-hexacosane	215, 217
169	$C_{27}H_{56}$ *n*-Heptacosane	214, 215, 217
170	$C_{28}H_{58}$ 3-Methyl-heptacosane	215, 217
171	$C_{28}H_{58}$ *n*-Octacosane	214, 215, 217
172	$C_{29}H_{60}$ 2-Methyl-octacosane	218
173	$C_{29}H_{60}$ 9-Methyl-octacosane	215
174	$C_{29}H_{60}$ *n*-Nonacosane	214, 217
175	$C_{31}H_{64}$ 3-Methyl-triacontane	215, 218
176	$C_{30}H_{62}$ *n*-Triacotane	214, 215, 217
177	$C_{32}H_{66}$ 2-Methyl-hentriacontane	218
178	$C_{31}H_{64}$ *n*-Hentriacontane	214, 215
179	$C_{32}H_{66}$ 3-Methyl-hentriacontane	217
180	$C_{32}H_{66}$ *n*-Dotriacontane	214, 217
181	$C_{33}H_{68}$ 2-Methyl-dotriacontane	217
182	$C_{33}H_{68}$ *n*-Tritriacontane	217
183	$C_{34}H_{70}$ Tetra-triacontane	217
184	$C_{35}H_{72}$ Pentatriacontane	217
185	$C_{36}H_{74}$ Hexatriacontane	217
186	$C_{37}H_{76}$ Heptatriacontane	217
187	$C_{38}H_{78}$ Octatriacontane	217
188	$C_{39}H_{80}$ Nonatriacontane	217

nonacosane by extracting *Cannabis* leaves with methanol. Later Krejčí and Šantavý (79) also isolated *n*-nonacosane from the leaves of *Cannabis sativa*.

A major article on alkanes was published by DeZeeuw *et al.* (214) in 1973. Adams and Jones (215) also published a paper on hydrocarbons in 1973. However, the submission date on the paper by DeZeeuw *et al.* was 1972, whereas Adams and Jones submitted their paper in 1973. DeZeeuw *et al.* reported the presence of *n*-alkanes C_{19}–C_{32} in marihuana and hashish from police seizures. This paper was not really a hydrocarbon study but, rather, called attention to

the interference of hydrocarbons in certain gc analyses of *Cannabis* products. Adams and Jones (215) detected hydrocarbons with C_{22} through C_{31}. Methyl substitution was identified at the C_2 and C_3 carbons in six hydrocarbons. See table 21. Nonacosane was the major hydrocarbon in Mexican female *Cannabis* produced in Mississippi.

Novotny *et al.* (216), in 1976, published the detection of six hydrocarbons in the extracts of Turkish and Mexican *Cannabis* produced in Mississippi and Mexican *Cannabis* produced in Indiana. The only branched chain compound was 9-methyl-octacosane.

Hendriks *et al.* (217) published, in 1977, the first comprehensive study on alkanes. They reported forty-five alkanes. Hendriks, *et al.* (218) continued their work and reported two branched chain hydrocarbons, 2-methyl-octacosane, and 2-methyl-hentriacontane in 1978. These two compounds had not previously been found in *Cannabis*.

No unusual hydrocarbons were found in *Cannabis*.

SIMPLE ALCOHOLS (table 22)

Methanol (**189**), ethanol (**190**), 1-octene-3-01 (**191**), octanol-1 (**192**), octanol-3 (**193**), nononol-1 (**194**), and hexadecanol-1 (**195**) make up the compounds in the simple alcohol class.

TABLE 22. Simple alcohols.

No.	Compound	Ref.
189	Methanol	219
190	Ethanol	219
191	1-Octene-3-01	218
192	Octanol-1	218
193	Octanol-3	218
194	Nonanol-1	218
195	Hexadecanol-1	218

Methanol and ethanol were detected by Hood *et al.* (219) in the low boiling fraction of oxygenated compounds (MW < 100) of the headspace volatiles of Mexican and confiscated *Cannabis*.

Hendriks *et al.* (218) used dry "*Cannabis* herb" grown from "bird seed" in an experimental garden in Buitenpost to detect the other simple alcohols. This same "variant" was used in their excellent work on the hydrocarbons. The "herb" was extracted twice for 30 seconds with 500 ml of petroleum ether. Clean-up was by column chromatography. 1-Octen-3-01 and octanol-3 were also found in *Humulus* (218). Identification was by gc/ms.

SIMPLE ALDEHYDES (table 23)

Acetaldehyde (**196**), isobutyraldehyde (**197**), pentanal (198), hexanal (**199**), heptanal (200), octanal (201), nonanal (202), decanal (**203**), undecanal (**204**), dodecanal (**205**), tridecanal (**206**) and *p*-ethylbenzaldehyde (**207**) make up the compounds in the simple aldehyde class.

Hood *et al.* (219) detected acetaldehyde and isobutyraldehyde in the same fraction of the headspace volatile where methanol and ethanol were detected. With the exception of these two aldehydes, all compounds in this class were de-

TABLE 23. Simple aldehydes.

No.	Compound	Ref.
196	Acetaldehyde	219
197	Isobutyraldehyde	219
198	Pentanal	218
199	Hexanal	218
200	Heptanal	218
201	Octanal	218
202	Nonanal	218
203	Decanal	218
204	Undecanal	218
205	Dodecanal	218
206	Tridecanal	218
207	*p*-Ethylbenzaldehyde	218

tected in *Cannabis* "herb" by Hendriks *et al.* (218). The procedure was described under the simple alcohols section. Of these aldehydes, octanal, nonanal, decanal, undecanal, and dodecanal were found in *Humulus*.

SIMPLE KETONES (table 24)

Acetone (**208**), heptanone-2 (**209**), 2-methyl-2-heptene-6-one (**210**), decanone-2 (**211**), undecanone-2 (**212**), dodecanone-2 (**213**), pentadecanone-2 (**214**), octanone-3 (**215**), 2,2,6-trimethylcyclohexanone (**216**), 2,2,6-trimethyl-5-cyclohexenone (**217**), 3-decene-5-one (**218**), 6,10-dimethylundecanone-2 (**219**) and 6,10-14-trimethyl-pentadecanone-2 (**220**) make up the compounds in the simple ketone class.

TABLE 24. Simple ketones.

No.	Compound	Ref.
208	Acetone	219
209	Heptanone-2	218
210	2-Methyl-2-heptene-6-one	219
211	Decanone-2	218
212	Undecanone-2	218
213	Dodecanone-2	218
214	Pentadecanone-2	218
215	Octanone-3	218
216	2,2,6-Trimethyl cyclohexanone	218
217	2,2,6-Trimethyl-5-cyclohexenone	218
218	3-Decene-5-one	218
219	6,10-Dimethyl undecanone-2	218
220	6,10,14-Trimethyl pentadecanone-2	218

Hood *et al.* (219) reported the presence of acetone and 2-methyl-2-heptene-6-one, as constituents of *Cannabis* in 1973. Headspace analyses were obtained on *Cannabis* seized by U.S. Customs and a Mexican variant grown in Mississippi. A gc/ms system was used for identification purposes.

Hendriks *et al.* (218) reported the other compounds in this class in 1978. Only heptanone-2, decanone-2, undecanone-2, and pentadecanone-2 were found in *Humulus* (218).

SIMPLE ACIDS (table 25)

Arabinic (**221**), azelaic (**222**), *trans*-cinnamic (**223**), citric (**224**), glucaric (**225**), gluconic (**226**), glyceric (**227**), p-hydroxybenzoic (**228**), p-hydroxycinnamic (**229**), isocitric (**230**), malic (**231**), malonic (**232**), 2-C-methylaldotetronic (**233**), 3-

methoxy-4-hydroxycinnamic (**234**), phosphoric (**235**), pyroglutamic (**236**), quinic (**237**), succinic (**238**), threonic (**239**), and vanillic acid (**240**) make up the compounds in the simple acid class.

Azelaic acid was the first simple acid to be reported in *Cannabis*. This report was by Bauer and Hazura (220) in 1886; hemp seed oil was used. Citric acid was the second acid to be reported in 1894 by Frankfurt according to Nordal's

TABLE 25. Simple acids.

No.	Compound	Ref.
221	Arabinic	221
222	Azelaic	220
223	*trans*-Cinnamic	80
224	Citric	221, 222
225	Glucaric	221
226	Gluconic	221
227	Glyceric	221
228	*p*-Hydroxybenzoic	224
229	*p*-Hydroxycinnamic (*p*-Coumaric)	224
230	Isocitric	221, 222
231	Malic	221, 222
232	Malonic	221, 222
233	2-C-Methylaldotetronic	221
234	3-Methoxy-4-hydroxycinnamic (Ferulic)	223
235	Phosphoric	221
236	Pyroglutamic	221
237	Quinic	221
238	Succinic	221, 222
239	Threonic	221
240	Vanillic	224

group in Oslo (221)[4]. We have been unable to confirm Frankfurt's report and, thus, prefer to give credit to Nordal's group who have studied the acids in *Cannabis* extensively. They determined citric acid to be present in Mexican, French, Russian, South African (UNC-335), and two Czechoslovakian variants of *Cannabis* grown in Oslo. Basic extraction was with water at 70–80° with stirring x 3. This was followed by dialyses. The dialysates were subjected to column chromatography with Dowex 50 W x 8 (H+), 20–50 mesh and then through Dowex 1 x 8 (HCOO–), 20–50 mesh. Two normal formic acid was used to elute from the last column. Eluates were evaporated to dryness under reduced pressure at 40°. All formic acid was removed by repeated addition and evaporation with toluene. This general procedure was used in all acid work by Nordal's group (221, 222).

The third acid from *Cannabis* was reported by Krejči, *et al.* in 1958 (80). Bate-Smith (223) reported the presence of ferulic acid in *Cannabis* in 1962. Paris and Paris (224), in 1973, reported *p*-hydroxybenzoic, vanillic, and *p*-hydroxycinnamic acid. Lutnes (222) reported, along with citric acid, isocitric, malic, malonic, and succinic acid to be present in several variants of *Cannabis* grown in Oslo. The comprehensive investigations of the acids by Nordal's group (221) led to the discovery of arabinic, glyceric, gluconic, glucaric, 2-C-methylaldoteronic, *p*-coumaric, phosphoric, pyroglutamic, threonic and quinic acid. Table 25–A gives more data on the presence of acids in six variants of *Cannabis* grown in Oslo. Identification was by separation and isolation and/or tlc, gc/ms. None of the simple acids present in *Cannabis* are unusual in nature.

[4]Frankfurt, *Landw. Versuchst.*, **43**, 143 and 307 (1894) was the citation.

TABLE 25–A. Distribution of simple acids in six variants of *Cannabis* grown in Oslo.

ACID	COUNTRY OF ORIGIN					
	Mexico	French	Russian	S. African	CZ–1*	CZ–2*
Arabinic	−	−	−	+	+	+
Citric	+	+	+	+	+	+
p-Cumaric	−	+	−	−	−	−
Glucaric	−	−	+	−	+	−
Gluconic	+	+	+	+	+	+
Glyceric	+	+	+	+	+	+
Isocitric	+	−	−	−	+	+
Malic	+	+	+	+	+	+
Malonic	+	+	−	+	−	+
2-C-Methyl-aldotetronic	+	+	+	+	+	+
Phosphoric	−	+	+	+	+	+
Pyroglutamic	+	+	+	−	+	−
Quinic	−	+	+	−	−	−
Succinic	+	+	−	+	+	+
Threonic	+	+	+	+	+	+

*Czechoslovakian 1 and Czechoslovakian 2.

FATTY ACIDS (table 26)

Arachidic (**241**), behenic (**242**), eicosadienic (**243**), eicosemic (**244**), linoleic (**245**), linolenic (**246**), myristic (**247**), oleic (**248**), palmitic (**249**), palmitoleic (**250**), sativic (**251**), and stearic (**252**) make up the compounds in the fatty acid class.

Bauer and Hazura (220), in 1886, reported the first discovery of fatty acids in hempseed oil. They reported the presence of myristic, palmitoleic, and an acid called sativic which had the emperical formula of $C_{32}H_{62}O_{11}$. Sativic acid has not been reported since 1886. In fact, only one other paper of significance has been published on fatty acids from *Cannabis*. Prior *et al.* (225) in 1968 reported that hempseed oil contained arachidic, behenic, eicosadienic, eicosemic, linoleic, linolenic, oleic, palmitic, and stearic acids. Also the presence of palmitoleic first reported by Bauer and Hazora (220) was confirmed.

Sativic is the only unusual fatty acid to be found in *Cannabis*.

TABLE 26. Fatty acids.

No.	Compound	Ref.
241	Arachidic	225
242	Behenic	225
243	Eicosadienic	225
244	Eicosemic	225
245	Linoleic	225
246	Linolenic	225
247	Myristic	220
248	Oleic	225
249	Palmitic	225
250	Palmitoleic	220, 225
251	Sativic	220
252	Stearic	225

SIMPLE ESTERS AND LACTONES (table 27)

Benzyl acetate (**253**), p-ethyl benzyl acetate (**254**), *cis*-3-hexenyl caproate (**255**), hexyl acetate (**256**), hexyl butyrate (**257**), hexyl caproate (**258**), hexyl isobutyrate (**259**), methyl acetate (**260**), 2-C-methyl-aldotetronolactone (**261**), methyl linoleate (**262**), methyl palmitate (**263**), methyl salicylate (**264**), and octyl caproate (**265**) represent the simple esters and lactones found in *Cannabis*. The only lactone detected was 2-C-methylerythronolactone.

TABLE 27. Simple esters and lactones.

No.	Compound	Ref.
253	Benzyl acetate	218
254	p-Ethyl benzyl acetate	218
255	*cis*-3-Hexenyl caproate	218
256	Hexyl acetate	218
257	Hexyl butyrate	218
258	Hexyl caproate	218
259	Hexyl isobutyrate	218
260	Methyl acetate	219
261	2-C-Methyl-aldotetronolactone	226
262	Methyl linoleate	218
263	Methyl palmitate	218
264	Methyl salicylate	218
265	Octyl caproate	218

Methyl acetate was detected by Hood *et al.* (219) in the low boiling fraction (ms 100) of the headspace volatiles of Mexican *Cannabis*. All other esters, with the exception of methyl palmitate and methyl linolaeate, were reported by Hendricks *et al.* (218) to be present in the essential oil of *Cannabis* leaves. The air-dried leaf material was subjected to steam distillation, and the essential oil was then chromatographed on a silica gel column. Elution with hexane was followed by mixtures of hexane and ether. The hexane ether fractions contained the oxygen- and carbonyl-containing compounds which were identified by gc/ms analysis. Hexyl acetate, hexyl isobutyrate, and p-ethylbenzyl acetate are the only esters detected in the essential oil of *Humulus* (218).

Recently, Nordal's group (226) reported the presence of the lactone-forming acid, 2-C-methylaldotetronic acid (2-C-methyl-aldotetronolactone), in the leaves of six different variants of *Cannabis*. Dried leaves were milled and extracted with water; the aqueous extract was dialyzed 3 times against water. The dialyzable material was then treated with Dowex 50 W x 8 [H^+], 20–50 mesh, followed by Dowex 1 x 8 [$HCOO^-$], 20–50 mesh. The acid fraction was eluted from the latter column with 2 M solution of formic or hydrochloric acid. The identification of the new acid and its lactone form was carried out by gc/ms of TMS derivatives and comparison of the data on the acid with authentic 2-C-methylaldotetronic acid.

STEROIDS (table 28)

Campesterol (**266**), campest-5-en-3β-ol-7-one (**267**), campest-4-en-3-one (**268**), ergosterol (**269**), β-sitosterol (**270**), 5α-stigmasta-7,24(28)-dien-3β-ol (**271**), stigmasta-5,22-dien-3β-ol-7-one (**272**), stigmasta-4,22-dien-3-one (**273**), stigmast-5-en-3-β-ol-7-one (**274**), stigmast-4-en-3-one (**275**), and stigmasterol (**276**) represent the steroids found in *Cannabis*.

TABLE 28. Steroids.

No.	Compound	Ref.
266	Campesterol	227, 228, 229
267	Campest-5-en-3β-ol-7-one	228
268	Campest-4-en-3-one	228
269	Ergosterol	229
270	β-Sitosterol	227, 228, 229
271	5α-Stigmasta-7,24(28)-dien-3β-ol	229
272	Stigmasta-5,22-dien-3β-ol-7-one	228
273	Stigmasta-4,22-dien-3-one	228
274	Stigmast-5-en-3β-ol-7-one (7-Keto-β-sitosterol)	228
275	Stigmast-4-en-3-one	228
276	Stigmasterol	227, 228, 229

The first report of steroids in *Cannabis* was made by Fenselau and Hermann in 1972 (227). Campesterol, stigmasterol and β-sitosterol were identified in the red oil extract of Yugoslavian *Cannabis* by gc and gc/ms (227).

With the exception of 5α-stigmasta-7,24(28)-dien-3β-ol and ergosterol, all compounds in this class have been isolated and identified by Slatkin *et al.* (228) from Mexican *Cannabis* roots. The methanol extract of the roots was partitioned between chloroform and water. The chloroform fraction was then partitioned between light petroleum and 10% aq. methanol. Chromatography of the light petroleum fraction on silicic acid resulted in the isolation of nine sterols in different fractions. The identification was carried out by gc/ms and comparison with authentic samples.

In a comparison of the steroid composition of tobacco and marihuana, Novotny *et al.* (229) detected five phytosterols in the leaves of *Cannabis*. In addition to stigmasterol, campesterol and β-sitosterol, which have been previously identified in the roots, two more phytosterols were detected in the plant material: ergosterol and 5α-stigmasta-7,24(28)-dien-3β-ol. Novotny *et al.* (229) extracted the plant material with acetone in a Soxhlet. The dried extract was then hydrolyzed with ethanolic H_2SO_4 followed by ethanolic KOH. The free sterols were extracted from the reaction mixture by the addition of hexane and enough water to form two layers. The hexane layer was separated and evaporated. To further purify the residue, it was dissolved in boiling ethanol and precipitated with digitonin. The digitonide was then decomposed with pyridine. The purified sterols were identified by gc analysis of the TMS derivatives on capillary columns and by gc/ms. Since the extraction procedure involved hydrolysis of the extract, both free and conjugated steroids (esters and glycosides) were identified. It must be mentioned that in the paper by Novotny *et al.* (229) a chromatogram of the steroids fraction of *Cannabis* was shown. In this chromatogram, a peak was shown for another steroid, namely, fulosterol which was not mentioned in the body of the paper. Also, the steroid 5α-stigmasta-7,24(28)-dien-3β-ol was mentioned in the paper as a constituent of *Cannabis* but was not shown on the chromatogram.

TERPENES

The first reported examination of the so-called "terpene fraction," which is devoid of "hashish activity" and boils at much lower temperatures than the active "crude cannabinol," was carried out by Wood *et al.* (135) in 1869. They reported the separation of a terpene, bp 165–175° ($C_{10}H_{10}$) and a sesquiterpene ($C_{15}H_{24}$),

bp 258–259°. In 1942 Simonsen and Todd (38) re-examined the low- and high-boiling "terpene" fraction of the essential oil of Egyptian hashish. The low boiling terpene fraction was found to consist mainly of *p*-cymene and small amounts of 1-methyl-4-iso-propenylbenzene. From the high boiling terpene fraction, humulene (α-caryophyllene) was obtained by fractional distillation at reduced pressure. The terpenes in *Cannabis* were neglected until recently when investigators directed their attention to the composition of the volatile oil of *Cannabis* with the hope of finding a relationship between the chemical composition of the oil and the geographical origin of the plant material. Today, a total of 103 terpenes have been identified in *Cannabis* which would be classified into 5 subclasses, namely, monoterpenes, sesquiterpenes, diterpenes, triterpenes and miscellaneous compounds of terpenoid origin.

MONOTERPENES (table 29).—Borneol (**277**), borynyl acetate (**278**), camphene (**279**), camphenehydrate (**280**), camphor (**281**), Δ^3-carene (**282**), Δ^4-carene (**283**), carvacrol (**284**), carvone (**285**), β-cyclocitral (**286**), 1,4-cineol (**287**), 1,8-cineol (**288**), citral B (**289**), citronellol (**290**), p-cymene (**291**), p-cymene-8-ol (**292**), dihydrocarveyl acetate (**293**), dihydrocarvone (**294**), frenchyl alcohol (**295**), fenchone (**296**), geraniol (**297**), geranyl acetone (**298**), Limonene (**299**), linalool (**300**), *cis*-linalool oxide (**301**), trans-linalool oxide (**302**), m-mentha-1,8(9)-dien-5-ol (**303**), 1-methyl-4-*iso*-propenylbenzene (**304**), myrcene (**305**), nerol (**306**), nerolidol (**307**), *cis*-β-ocimene (**308**), *trans*-β-ocimene (**309**), perillene (**310**), α-phellandrene (**311**), β-phellandrene (**312**), 3-phenyl-2-methyl-prop-1-ene (**313**), α-pinene (**314**), β-pinene (**315**), α-pinene oxide (**316**), pinocarveol (**317**), pinocarvone (**318**), piperitenone (**319**), piperitone oxide (**320**), piperitenone oxide (**321**), pulegone (**322**), sabinene (**323**), *trans*-sabinene hydrate (**324**), sabinol (**325**), safranal (**326**), α-thujene (**327**), α-terpinene (**328**), γ-terpinene (**329**), α-terpinene-4-ol (**330**), α-terpinolene (**331**), α-terpineol (**332**), β-terpineol (**333**), and thujyl alcohol (**334**) make up the monoterpenes found in *Cannabis*.

TABLE 29. Monoterpenes.

No.	Compound	Ref.
277	Borneol	219, 235
278	Bornyl acetate	218
279	Camphene	231, 173
280	Camphenehydrate	218
281	Camphor	234, 218
282	Δ^3-Carene	173, 232
283	Δ^4-Carene	173, 232
284	Carvacrol	234, 218
285	Carvone	218
286	β-Cyclocitral	218
287	1,4-Cineol	234, 218
288	1,8-Cineol	234, 218
289	Citral B	218
290	Citronellol	218
291	*p*-Cymene	38, 173
292	*p*-Cymene-8-ol	218
293	Dihydrocarveyl acetate	218
294	Dihydrocarvone	218
295	Fenchyl alcohol	219, 234
296	Fenchone	218
297	Geraniol	234, 218
298	Geranyl acetone	218
299	Limonene	230, 173
300	Linalool	231, 219
301	*cis*-Linalool oxide	218

TABLE 29. Continued.

No.	Compound	Ref.
302	*trans*-Linalool oxide	231
303	*m*-Mentha-1,8(9)-dien-5-ol	233, 218
304	1-Methyl-4-*iso*-propenylbenzene	38
305	Myrcene	230, 173
306	Nerol	234, 218
307	Nerolidol	234, 218
308	*cis*-β-Ocimene	173, 232
309	trans-β-Ocimene	173, 232
310	Perillene	218
311	α-Phellandrene	173, 232
312	β-Phellandrene	231, 173
313	3-Phenyl-2-methyl-prop-1-ene	218
314	α-Pinene	231, 173
315	β-Pinene	231, 173
316	α-Pinene oxide	218
317	Pinocarveol	218
318	Pinocarvone	218
319	Piperitenone	235, 218
320	Piperitone oxide	218
321	Piperitenone oxide	218
322	Pulegone	218
323	Sabinene	173, 232
324	*trans*-Sabinene hydrate	231, 218
325	Sabinol	218
326	Safranal	218
327	α-Thujene	173, 232
328	α-Terpinene	231, 173
329	γ-Terpinene	231, 173
330	α-Terpinene-4-ol	231, 234
331	α-Terpinolene	173, 232
332	α-Terpineol	231, 219
333	β-Terpineol	218
334	Thujyl alcohol	218

The first monoterpene indicated to be present in the "terpene fraction" of the Indian *Cannabis* preparation (charas) was reported by Wood *et al.* (135). In 1942, Simonsen and Todd (38) reported the low boiling terpene fraction of Egyptian hashish consisted mainly of *p*-cymene and small amounts of 1-methyl-4-isopropenylbenzene but failed to detect the monoterpene reported by Wood *et al.* (135), which they assumed was myrcene. In 1961, Martin *et al.* (239) were able to identify myrcene along with limonene and two sesquiterpenes in the volatile oil of fresh *Cannabis*. Thirteen samples of fresh male and female plants growing wild in the Canadian provinces of Ontario and Quebec were used. The identification of the components was carried out by gas chromatographic comparison of relative retention times with components in the volatile oils of hops and caryophyllene. Fourteen peaks were found to be characteristic for *Cannabis* oil. In addition, infrared and ultraviolet spectra were recorded on components collected from the gc. Dried *Cannabis*, charas and hashish, were also studied, and similar results were obtained. Thus, the components of the volatile oil may be useful as a means of characterization.

A more detailed examination of the volatile oil of *Cannabis* was carried out by Nigam *et al.* (231)[5]. The essential oils of both male and female *Cannabis sativa* L.

[5]The authors reported in their introduction that S. Dutt, *Indian Soap, J.*, **22**, 242(1957) had established the presence of *p*-cymene, myrcene, limonene and caryophyllene along with unidentified sesquiterpenes and sesquiterpene alcohols. This paper was located while the manuscript was in press.

growing wild in India were prepared by steam distillation. Gas chromatographic and physical data on both oils were similar; thus, only the volatile oil of male plants was examined in detail. The study resulted in the identification of 11 monoterpenes which have not been reported before: camphene, linalool, *trans*-linalool oxide, β-phellandrene, α-pinene, *trans*-sabinene hydrate, α-terpinene, α-terpinolene, α-terpinene-4-ol, α-terpineol, and β-pinene. The volatile oil was fractionally distilled in a Tower's column to provide five fractions. Identification of the constituents was by gas chromatography, comparison of the infra-red spectra of the gc eluates (preparative) with authentic samples, and with published data. The volatile oils of Dutch and Turkish *Cannabis*, prepared by hydrodistillation or by "nitrogen extraction," were compared by capillary gas chromatography (173, 232). In addition to ten previously identified monoterpenes, eight more compounds were identified in this group: Δ^3 and Δ^4-carene, *cis*- and *trans*-β-ocimene, α-phellandrene, sabinene, α-thujene and α-terpinolene.

From the essential oil of *Cannabis*, Stahl and Kunde (233) separated a rather unusual monoterpene, namely, *m*-mentha-1,8-(9)-dien-5-ol. The identification was carried out by ir, nmr and ms. This compound was again detected in the volatile oil of *Cannabis* grown from bird seed by Hendricks *et al.* (218).

Hood *et al.* (219) first detected fenchyl alcohol and borneol along with 15 other monoterpenes during the analysis of headspace volatiles of marihuana. Standard Mexican marihuana samples supplied by the Mississippi group as well as samples from Customs' seizures were used in the analysis. The plant material (1 g) was placed in a microvial and equilibrated for 1 hr at 65°; then, a gas-tight syringe was used to inject a 5 ml sample from the headspace air containing the volatiles into a gas chromatograph. Three fractions were identified based on ascending boiling points: fraction I = low molecular weight (<100) oxygenated compounds; fraction II = monoterpene hydrocarbons and oxygenated compounds (MW>100); and fraction III = sesquiterpene hydrocarbons (MW>200). The identification of components was carried out by comparison of RRT with authentic standards and by ms analysis. Two monoterpene alcohols, borneol and fenchyl alcohol, were later detected in the volatile oil of *Cannabis* (218, 234, and 235). However, a major qualitative difference between headspace volatiles and the volatile oil of *Cannabis* was that monoterpenes α-pinene, β-pinene, myrcene, and limonene represented 85% of the headspace and only 10% of the essential oil and more monoterpenes were identified in the volatile oil of *Cannabis*.

Camphor, carvacrol, 1,4- and 1,8 cineol, geraniol, nerol, and nerolidol were first identified by Bercht and Paris (234) in the volatile oil of Mexican *Cannabis* along with ten previously identified monoterpenes. Separation of hydrocarbon and oxygenated compounds was on a silica column impregnated with silver nitrate. The identification was by gc/ms. Piperitenone was first detected and identified as a minor component in the volatile oil of *Cannabis* by Strömberg (235). Gc/ms was used in the identification.

The most comprehensive study on the essential oil of *Cannabis* was carried out by Hendricks *et al.* (218, 236). Along with other constituents, 55 monoterpenes were reported; twenty-three were reported for the first time. See table 29 where reference 218 is listed first. The volatile oil was prepared by steam distillation of air-dried leaf material. The oil was then chromatographed on a silica column and eluted with hexane to give the terpene fraction. Fractional distillation afforded monoterpene fractions, which were examined by gc, tlc, gc/ms, ir, and nmr.

The only unusual monoterpene found in *Cannabis* is *m*-mentha-1,8(9)-dien-5-ol (218, 233).

SESQUITERPENES (table 30).—Allo-aromadendrene (**335**), α-*trans*-bergamotene (**336**), β-bisabolene (**337**), α-bisabolol (**338**), calamenene (**339**), caryophyllene (**340**), α-caryophyllene (Humulene) (**341**), β-caryophyllene (**342**), α-caryophyllene alcohol (caryophyllenol) (**343**), iso-caryophyllene (**344**), caryophyllene oxide (caryophyllene epoxide) (**345**), α-cedrene (**346**), γ-cadinene (**347**), δ-cadinene (**348**), α-copaene (**349**), α-cubebene (**350**), α-curcumene (**351**), β-curcumene (**352**), γ-elemene (**353**), γ-eudesmol (**354**), β-farnesene (**355**), (Z)-β-farnesene (**356**), *trans-trans*-α-farnesene (**357**), farnesol (**358**), farnesyl acetone (**359**), α-gurjunene (**360**), guaiol (**361**), β-humulene (**362**), humulene epoxide I (**363**), humulene epoxide II (**364**), ledol (**365**), longifolene (**366**), nerolidol (**367**), epi-β-santalene (**368**), α-selinene (**369**), β-selinene (**370**), selina-3,17(11)-dien (**371**) and selina-4(14),7(11)-dien (**372**) make up the compounds in the sesquiterpene class.

TABLE 30. Sesquiterpenes.

No.	Compound	Ref.
335	Allo-aromadendrene	234, 235
336	α-*trans*-Bergamotene	231, 219
337	β-Bisabolene	219
338	α-Bisabolol	218
339	Calamenene	234
340	Caryophyllene	231, 234
341	α-Caryophyllene (Humulene)	38, 230
342	β-Caryophyllene	230, 219
343	α-Caryophyllene alcohol (Caryophyllenol)	233, 218
344	*iso*-Caryophyllene	236, 218
345	Caryophyllene oxide (Caryophyllene epoxide)	231, 219
346	α-Cedrene	218
347	γ-Cadinene	218
348	δ-Cadinene	218
349	α-Copaene	234, 218
350	α-Cubebene	218
351	α-Curcumene	234
352	β-Curcumene	231, 219
353	γ-Elemene	218
354	γ-Eudesmol	218
355	β-Farnesene	231, 219
356	(*Z*)-β-Farnesene	218
357	*trans-trans*-α-Farnesene	218
358	Farnesol	218
359	Farnesyl Acetone	218
360	α-Gurjunene	235, 218
361	Guaiol	218
362	β-Humulene	231, 219
363	Humulene epoxide I	233
364	Humulene epoxide II	233, 218
365	Ledol	218
366	Longifolene	233, 236
367	Nerolidol	234
368	epi-β-Santalene	218
369	α-Selinene	231, 219
370	β-Selinene	236, 218
371	Selina-3,7(11)-diene	236, 218
372	Selina-4(14),7(11)-diene	236, 218

Humulene (α-caryophyllene) is the first sesquiterpene found in *Cannabis*. It was isolated by Simonsen and Todd (38) from the high-boiling terpene fraction of Egyptian hashish by fractional distillation. Caryophyllenes were then identified by Martin *et al.* (230) in the volatile oil of fresh *Cannabis* by gc comparison with hops and caryophyllene oils. The sesquiterpenes α-bergamotene, caryophyllene,

β-farnesene, β-humulene, α-selinene, curcumene, and carophyllene oxide were first reported by Nigam *et al.* (231) in the volatile oil of fresh Indian *Cannabis*. Of these compounds, caryophyllene was reported to have been present in Indian *Cannabis* by Dutt; the reference to this work is cited in footnote 5. The identification was carried out by fractional distillation of the steam distilled oil followed by column and gas chromatography of the fractions.

Investigation of the volatile oil of *Cannabis sativa* L. by Stahl and Kunde (233) resulted in the separation and identification of longifolene, humulene epoxide I, humulene epoxide II and caryophyllene alcohol for the first time.

In a study of headspace volatiles of Mexican *Cannabis* and samples of marihuana obtained from Customs' seizures, Hood *et al.* (219) identified β-bisabolene along with seven other sesquiterpenes previously found in the essential oil. This is the only report of β-bisabolene in *Cannabis* to date.

The sesquiterpenes allo-aromadendrene, calamenene, α-copaene, and α-curcumene were first reported in the essential oil of Mexican *Cannabis* by Bercht and Paris (234). Column chromatography, gc and ms were used in the identification. α-Gurjunene was detected for the first time as a minor component in *Cannabis* resin by gc and gc/ms (235). Its presence in the volatile oil of *Cannabis* was then reported by Hendricks *et al.* (218). In their publication (218), 33 sesquiterpenes were identified. Of these 13 were identified for the first time: α-bisabolol, α-cedrene, γ-cadinene, δ-cadinene, α-cubebene, γ-elemene, γ-eudesmol, (*Z*)-β-farnesene, *trans-trans*-α-farnesene, farnesol, guaiol, ledol and epi-β-santalene.

In a previous report, Hendricks *et al.* (236) had identified iso-caryophyllene, β-selinene, selina-3,17(11)-dien and selina-4(14),7(11)-diene for the first time in the volatile oil of *Cannabis*. They re-identified these compounds in their later report (218).

No unusual sesquiterpenes were found in *Cannabis*.

DITERPENES (table 31).—Phytol (**373**) is the only diterpene reported to exist in the essential oil of *Cannabis* (218). It was detected in the oxygenated compounds fraction obtained by column chromatography of the oil and was identified by gc/ms.

TABLE 31. Diterpenes.

No.	Compound	Ref.
373	Phytol	218

TRITERPENES (table 32).—The only report on triterpenes in *Cannabis* was carried out by the Mississippi group (177) where friedelin (**374**) and epifriedelanol (**375**) were isolated from an ethanolic extract of the roots. The identification of the two triterpenes was carried out by comparison of the spectral data and by comparison with authentic samples.

TABLE 32. Triterpenes.

No.	Compound	Ref.
374	Friedelin (Friedelan-3-one)	177
375	Epifriedelanol	177

MISCELLANEOUS COMPOUNDS OF TERPENOID ORIGIN (table 33).—Four compounds were identified in this group. Bercht *et al.* (237) reported the isophorone type compounds vomifoliol (**376**) and dihydrovomifoliol (**377**). The compounds were isolated from the leaves and stems of Dutch-grown hemp, and their structures determined by spectral data and by synthesis from (+)-α-ionone. Recently, Hendricks *et al.* (218) reported the presence of β-ionone (**378**) and the C_{11} dihydroactinidiolide (**379**) as constituents of the essential oil of *Cannabis*.

TABLE 33. Miscellaneous compounds of terpenoid origin.

No.	Compound	Ref.
376	Vomifoliol (blumenol A)	237
377	Dihydrovomifoliol (blumenol B)	237
378	β-Ionone	218
379	Dihydroactinidiolide	218

NON-CANNABINOID PHENOLS

A total of thirteen non-cannabinoid phenols have been found in *Cannabis*. These can be conveniently divided into six spiro-indan type structures, four dihydrostilbenes, one dihydrophenanthrene, and two simple phenols. In addition, three phenolmethylethers have been detected in the volatile oil.

SPIRO-INDAN COMPOUNDS (table 34).—Acetyl-cannabispirol (**380**), cannabispiradienone (**381**), β-cannabispiranol (cannabispirol) (**382**), cannabispirenone (dehydrocannabispiran) (**383**), cannabispirenone-isomer (**384**), and cannabispirone (cannabispiran) (**385**) are the compounds found in this class. The first reports of the presence of spiro-indans in *Cannabis* were published almost simultaneously by two independent groups (121, 238) in 1976. Cannabispiran and cannabispirone were the names given to the same compound by the Mississippi group (121) and

TABLE 34. Non-cannabinoid phenols.

No.	Compound	Ref.
380	Acetylcannabispirol	242
381	Cannabispiradienone	243
382	β-Cannabispiranol (cannabispirol)	241, 242, 243
383	Cannabispirenone (Dehydrocannabispiran)	238, 239, 242
384	Cannabispirenone-isomer	240
385	Cannabispirone (Cannabispiran)	238, 121, 239
386	3-[2-(4-hydroxyphenyl)ethyl]-5-methoxyphenol	240, 244
387	3-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-5-methoxyphenol	240, 244
388	3-[2-(3-isoprenyl-4-hydroxy-5-methoxy-phenyl)ethyl]-5-methoxyphenol	240
389	3[2-(2-isoprenyl-3-hydroxy-4-methoxyphenyl)ethyl]-5-methoxyphenol (canniprene)	244
390	Cannabidihydrophenanthrene	243
391	Eugenol	218
392	iso-Eugenol	218
	Ethers of Phenols	
393	*cis*-Anethol	218
394	*trans*-Anethol	218
395	Methyleugenol	218

by Bercht *et al.* (238), respectively. Turner's group isolated cannabispiran from the dried leaves of an Indian variant using a silica gel packed column. Structure determination was by X-ray crystallography. Whereas, Bercht *et al.* (238) isolated the same compound from the dried leaves of a South African variant grown in France. Structure assignment was based on extensive mass spectral, ^{1}H nmr and ^{13}C nmr studies.

The dried leaves were extracted with methanol, and the extract was chromatographed on a silica gel column using hexane diethylether. Fractions containing the polar components were partitioned between ether and 5% NaOH. The aqueous layer was acidified and re-extracted with ether; the ethereal extract, when chromatographed repeatedly on silica gel with 12% dioxane in hexane, yielded pure cannabispiran.

Bercht *et al.* (238) also isolated cannabispirenone from the South African variant grown in France. Turner's group (239) isolated the same compound from the South African variant grown in Mississippi. Turner's group called their compound dehydrocannabispiran.

An isomeric compound of dehydrocannabispiran with the hydroxy and methoxy groups interchanged was detected by Bosch and Salemink (240) in Mexican *Cannabis* grown in Mississippi and supplied by Turner's group. It must be mentioned here that the Mississippi group reported the relative retention times of free and silylated cannabispiran as being identical to the relative retention times of (−)-Δ^{8}-THC indicating that the peak normally labelled Δ^{8}-THC in extracts from fresh *Cannabis* might actually be cannabispiran.

In 1977 the Mississippi group, using the South African variant grown in Mississippi, was able to isolate another related compound, namely, β-cannabispiranol (241). The β-configuration was assigned based on the multiplicity of the proton on the carbon carrying the secondary hydroxy group in the ^{1}H nmr spectrum of the acetate derivative. Comparisons were made with the derivatives obtained by the $NaBH_4$ reduction of cannabispiran. Shoyama and Nishioka (242) in 1978 reported the isolation of β-cannabispiranol, which they named cannabispirol, and its monoacetyl derivative from different strains of Japanese domestic *Cannabis*. The acetyl derivative was isolated from the dried leaves by a benzene extraction. The dried benzene extract was washed with acetone, and the acetone solubles were chromatographed on polyamide column. Elution with methanol-water (1:1) afforded a fraction which, upon chromatography on a silica gel column using hexane-ethyl acetate, gave cannabispirol and its monoacetate as well as cannabispiran and dehydrocannabispiran. The structure determination was carried out by spectral means. Although Turner's group (241) had isolated β-cannabispiranol one year earlier, no reference was given to their work. Shoyama and Nishioka (242) also proposed a biosynthetic pathway for all spiro compounds as being totally acetate derived. Recently, Crombie *et al.* (243) reported the isolation of another spiro-compound, cannabispiradienone, from Thailand *Cannabis*. The structure was determined by spectral data and hydrogenation, which yielded cannabispiran. They also indicated that the dienone is a possible intermediate in the biosynthesis of spiro compounds and could be formed through phenol oxidative coupling of 3-[2-(4-hydroxyphenyl)ethyl]-5-methoxyphenol. The latter is thought to be of mixed shikimate acetate origin and has also been found in the plant (240, 244).

All previously mentioned spiro-compounds have only been detected in *Cannabis*. See figure 15 for proposed biosynthesis based on isolation of intermediate compounds.

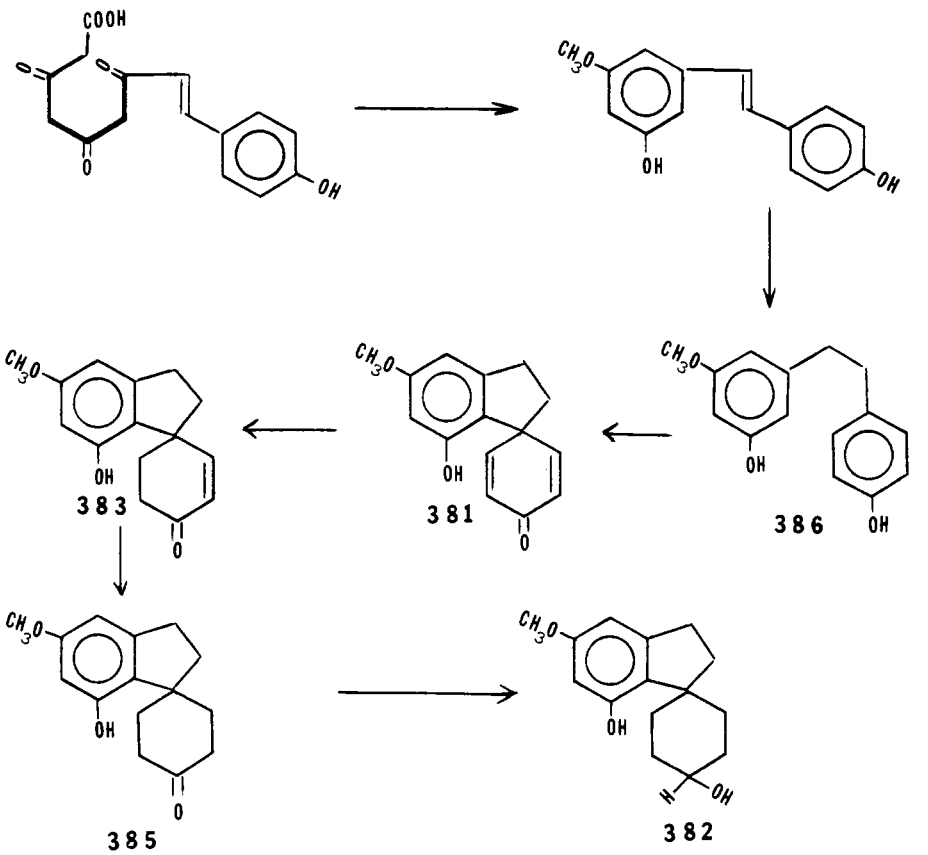


FIG. 15. Proposed Bio-Synthetic Pathway For Spiro-Indan Compounds. (The pathway was originally proposed by F. S. El-Feraly of the Mississippi group in his grant proposal to NIDA (1977) and then appeared in E. G. Boeren's dissertation in 1978.) Subsequently, an acetate derived pathway was proposed in 1978. See reference 242.

DIHYDROSTILBENE TYPE (table 34).—The four dihydrostilbenes reported in *Cannabis* are: 3-[2-(4-hydroxyphenyl)ethyl]-5-methoxyphenol (**386**), 3-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-5-methoxyphenol (**387**), 3-[2-(3-isoprenyl-4-hydroxy-5-methoxyphenyl)ethyl]-5-methoxyphenol (**388**) and canniprene (**389**). Compounds **386**, **387**, and **388** were first reported by Bosch and Salemink (240) to be present in the methylene chloride extract of Mexican marihuana supplied by Turner's group. The structure of the first compound **386** was determined by spectral evidence and confirmed by synthesis. Only spectral data were given for compounds **387** and **388**. Cannabispirenone and its isomer were proposed to be formed from compound **386** through an intramolecular coupling process. Compounds **387** and **388** were also isolated from Thailand *Cannabis* (244) along with another dihydrostilbene called canniprene. The structures were determined by spectral and chemical means; canniprene was later proved by the synthesis of its acid catalyzed cyclization product (243).

Although these dihydrosilbenes are new as such, other similar compounds are found in nature.

CANNABIDIHYDROPHENANTHRENE DERIVATIVE (table 34).—Cannabidihydrophenanthrene (**390**) is the only compound of this type. It was isolated from Thailand *Cannabis* by Crombie *et al.* (243), who assigned the structure based on spectral evidence. They proposed that cannabidihydrophenanthrene could be biogenetically derived from the spirocompound, cannabispiradienone, through a dienone-phenol rearrangment.

SIMPLE PHENOLS (table 34).—The two simple phenols eugenol (**391**) and isoeugenol (**392**) were detected in the essential oil of *Cannabis* cultivated from bird seed (218). The steam distilled oil was chromatographed on a silica gel column, and fractions collected from petroleum ether-ether eluates containing oxygenated compounds were analyzed by gc and gc/ms. Three methyl ethers of phenols were also detected in the same study (218), namely, cis-anethol (**393**), *trans*-anethol (**394**) and methyleugenol (**395**).

FLAVONOID GLYCOSIDES (table 35)

Apigenin-7-O-*p*-coumarylglucoside (**396**), apigenin-7-O-glucoside (cosmosioside) (**397**), apigenin-O-glycoside (**398**), isovitexin-7-O-glucoarabinoside (**399**), isovitexin-O-glucoside (**400**), isovitexin-7-O-rhamnoglucoside (**401**), kaempferol-O-glycoside(s) (**402**), luteolin-O-glycoside (**403**), orientin (**404**), orientin-7-O-glucoside (**405**), orientin-O-glucoside (in sugar moiety) (**406**), orientin-O-glucoside (**407**), orientin-7-O-rhamnoglucoside (**408**), quercetin-O-glucoside(s) (**409**), vitexin-7-O-(6"-glucoside) (**410**), vitexin-O-glucoside (**411**), vitexin-O-rhamnoglucoside (**412**), 2"-O-glucopyranosylorientin (**413**), and 2"-O-glulopyranosylvitexin (**414**) represent the compounds found in *Cannabis* in this class.

TABLE 35. Flavonoid glycosides.

No.	Compound	Ref.
396	Apigenin-7-O-*p*-coumarylglucoside	246, 247
397	Apigenin-7-O-glucoside (Cosmosioside)	224
398	Apigenin-O-glycoside	224, 172
399	Isovitexin-7-O-glucoarabinoside	246, 247
400	Isovitexin-O-glucoside	246, 247
401	Isovitexin-7-O-rhamnoglucoside	246, 247
402	Kaempferol-O-glycoside(s)	245
403	Luteolin-O-glycoside	224, 172
404	Orientin	246, 247
405	Orientin-7-O-glucoside	246, 247
406	Orientin-O-glucoside (in sugar moiety)	246, 247
407	Orientin-O-glucoside	224
408	Orientin-7-O-rhamnoglucoside	246, 247
409	Quercetin-O-glucoside(s)	245
410	Vitexin-7-O-(6"-glucoside)	246, 247
411	Vitexin-O-glucoside	224
412	Vitexin-O-rhamnoglucoside	246, 247
413	2"-O-Glucopyranosylorientin	248
414	2"-O-glucopyranosylvitexin	248

To date, no free flavonoids have been reported in *Cannabis*. However, nineteen flavonoid glycosides have been shown to be present in *Cannabis*. The exact structures of most of these glycosides are not known, and data available is limited to chromatographic analysis on different adsorbents and to characterization of acid hydrolytic products. Most of the compounds in this class are O-glycosides of the C-glycosides orientin, vitexin, and iso-vitexin. A few are the O-glycosides

of the flavonoids apigenin, luteolin, quercitin, and kaempferol. Because of the uncertainty of the exact linkage and/or the number of sugar moieties, the correct number of compounds in this class could be more or less than seventeen.

The first report on flavonoid glycosides was done by Paris and Paris (224) in 1973 using the leaves of *Cannabis* plants grown in Paris. The plant material was extracted with boiling methanol, and the concentrated extract was then partitioned with ether, ethylacetate, and butanol. No free flavonoids were detected in the ether fraction. Two-dimensional paper chromatography of the butanol fraction showed eight spots of flavonoid glycosides, of which six were major. Acid hydrolysis of the butanol fraction followed by ether extraction afforded small amounts of two genins which were characterized as apigenin and luteolin indicating the presence of apigenin-O-glycoside(s) and luteolin-O-glycoside(s). Ethylacetate extraction of the hydrolytic product showed the presence of large amounts of C-flavonoid glycosides not hydrolyzable under acidic conditions. The butanol fraction was then chromatographed on paper followed by cellulose tlc to give a flavonoid glycoside "A_2". Acid hydrolysis of "A_2" afforded orientin and glucose indicating that it was orientin-O-glucoside. Polyamide column chromatography of the butanol fraction gave a flavonoid which yielded vitexin and glucose upon acid hydrolysis indicating a vitexin-O-glucoside structure. In addition a glucoside isolated from the same column was characterized as apigenin-7-O-glucoside by hydrolysis to apigenin and glucose. Only uv and chromatographic procedures were used in the identification.

A contradictory report was then published by Gellert *et al.* (245) in 1974. Qualitative examination of the leaves, flowers, stems, and roots of Thai, South African, Turkish, Nepalese and Hungarian *Cannabis* indicated the presence of flavonoid glycosides in the leaves, flowers, and stems. No flavonoids were detected in the roots. Dried flowering tops were extracted first with benzene and then with methanol. The methanol extract was diluted with water and partitioned with benzene; the concentrated aqueous methanol fraction was then applied on a polyamide column. Elution with 20% methanol in water afforded two flavonoids. Acid hydrolysis of these compounds gave a mixture of quercetin and kaempferol as the aglycones. No data were given on the glycosides or the genins.

Paris *et al.* (172) then examined the extracts obtained from pollen grains of male plants grown under artificial conditions in a phytotron. They detected two flavonoid spots on two-dimensional paper chromatography. Acid hydrolysis provided two genins identified as apigenin and luteolin, indicating the presence of the respective O-glycosides in the pollens.

In a more comprehensive study, Paris and Paris (246, 247) re-examined the flavonoids in *Cannabis* using the same plant material they used before (224). Paper and column chromatography were used in the isolation of the flavonoid glycosides. In this study, ten glycosides were detected and then isolated. These were denoted glycosides A_1, A_2, B_1, B_2, B_3, C_1, C_2, D, E, and F. Most of them were shown to be O-glycosides of C-glycosides. The uv spectrum of each compound was studied in ethanol, ethanol-aluminum chloride, ethanol-sodium acetate and ethanol-sodium acetate-boric acid. Hydrolysis of each glycoside was carried out under acidic conditions. The aglycones were characterized by uv and chromatographic techniques and by comparison with authentic samples. The sugar moieties were also characterized. Their studies indicated the following identities: A_1 = orientin; A_2 = orientin-O-glucoside (with the glucose moeity attached to the sugar part on the C–8 of luteolin); B_1 = orientin-7-O-glucoside; B_2 = orientin-7-O-rhamnoglucoside; B_3 = vitexin-7-O-glucoside (with the link at the 6 position of the

sugar); C_1 = isovitexin-O-glucoside (with glucose attached either to 4 position of the flavonoid or on the sugar moiety); C_2 = vitexin-O-rhamnoglucoside (with rhamnose and glucose either on 4 position of flavonoid or on the sugar moiety); D = apigenin-7-O-*P*-coumarylglucoside; E = isovitexin-7-O-glucoarabinoside; and F = isovitexin-7-O-rhamnoglucoside.

Recently, Segelman *et al.* (248) reported the isolation of orientin and two new glycosides from Mexican *Cannabis* obtained from the Mississippi group. The two glycosides were shown to be 2"-O-glucopyranosylorientin and 2"-O-glucopyranosylvitexin. The structures were based on acid hydrolysis and spectral data (uv, ms and nmr) of the acetate and methylether derivatives.

No unusual flavonoid glycosides were found in *Cannabis*.

VITAMINS (table 36)

Vitamin K (**415**) is the only vitamin reported in *Cannabis*. Dam (249, 250) reported that hemp seeds contain an antihemorrhagic factor, for which he gave the name vitamin K, which was fat soluble and different from vitamins A, D, and C. The seeds were used in the treatment of chick disease characterized by a tendency to large hemorrhages.

TABLE 36. Vitamins.

No.	Compound	Ref.
415	Vitamin K	249, 250

PIGMENTS (table 37)

The only report on pigments in *Cannabis* was done by Manys and Kasprzak (251) in 1969. They determined the carotene (**416**) and zanthophylls (**417**) content in different hemp leaves. Fibrinon 24, Kompolti, Yugoslavian LKCSD, and Polish varieties of hemp leaves were used in this study.

TABLE 37. Pigments.

No.	Compound	Ref.
416	Carotene	251
417	Zanthophylls	251

ADDENDUM

CANNABINOIDS (table 38).—While this manuscript was being prepared, four new cannabinoids were reported. These are cannabichromanone-C_3 (**418**), cannabielsoin-C_3 (**419**), cannabielsoic acid B-C_3 (**420**) and cannabicumaronone (**421**). This brings the total number of cannabinoids known to date to be 61 compounds.

TABLE 38. New cannabinoids.

No.	Compound	Ref.
418	Cannabichromanone-C_3	252
419	Cannabielsoin-C_3	252
420	Cannabielsoin acid B-C_3	252
421	Cannabicumaronone	253

The propyl homologs of cannabichromanone, cannabielsoin, and cannabielsoic acid B were detected in *Cannabis* extracts by Grote and Spiteller (251) using gc/ms.

Cannabicumaronone was isolated by the same group (253) from hashish extracts, and the structure was proved by ozonolysis to give cannabichromanone.

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