# Recent Advances in the Chemistry and Biochemistry of Cannabis

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*Received December 6, 1974 (Revised Manuscript Received March 18, 1975)*

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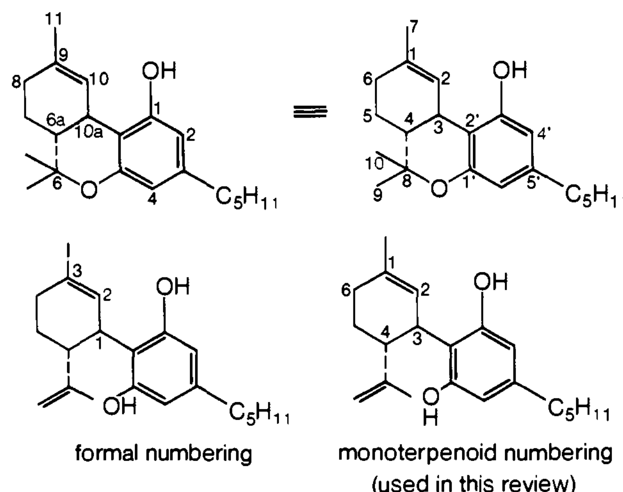
## *I. Introduction*

In the present review we propose to update the chemical and biochemical chapters in the monograph on cannabis published in 1973,[1] in which the literature was reviewed up till about the middle of 1972. In the last years the published data in the cannabis field have increased considerably, partly owing to public interest in the field. All areas of cannabis research have received considerable attention. The most significant chemical advances, in our view, have been the development of methods for the determination of cannabinoids in body fluids and a more detailed understanding of the metabolism of cannabinoids. We are under the impression, however, that there is no direct relationship between the vast number of published papers and the advancement of our knowledge in the field.

### A. Nomenclature

The use of two different numbering systems continues to bedevil the literature. Most American publications use the formal numbering based on the pyran ring, while most European




ones use the numbering based on the monoterpenoid moiety. IUPAC has undertaken to issue definitive rules, which are eagerly awaited. In the present review the monoterpenoid system will be employed in view of the increasing interest in and importance of cannabinoids which do not possess a pyran ring (i.e., cannabidiol).

### B. New Secondary Literature on Cannabis

Two new, important literature tools, which cover the entire area of drug dependence, including cannabis, have appeared in the last few years. The abstract journal *Drug Dependence* is published[2] by the Excerpta Medica group in Amsterdam. It represents the Dutch contribution to the UN Fund for Drug Abuse Control. While articles dealing with the organic chemistry of cannabis are abstracted in less detail than in *Chemical Abstracts*, biochemical and pharmacological papers are presented in considerable length.

DACAS is a new alert-service journal[3] which is published by the National Institute on Drug Dependence. Only titles of articles are published; however, a cross index is available in every issue.

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Several reviews covering specific aspects of cannabinoid research have been published.[4] Overlap of these with the present one is marginal.

## II. Chemistry of Cannabis

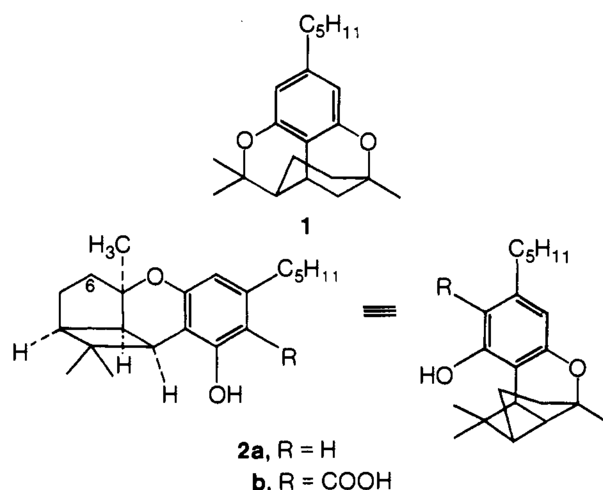
## A. Cannabis Constituents

Analyses of cannabis in its numerous forms (fresh plant, hashish, marijuana, dagga, "green hashish oil"[5]) are reported at an ever-increasing rate. A large number of new minor cannabinoids, as well as non-cannabinoid components, have been isolated. Essentially all the cannabinoids isolated recently represent variations of known constituents. Most of the newly found non-cannabinoids in *C. sativa* are also present in many other plants and seem to be of minor interest.

### 1. Cannabinoids

#### a. Cannabicitran (1)

The tetracyclic cannabinoid 1 ("citrylidene cannabis") is a product obtained in several syntheses and transformations of cannabinoids.[6-8] Closely related natural products (bruceol, rubramine)[9] are known; however, 1, although predicted[10] as a natural product, had not hitherto been isolated from cannabis. Bercht et al.[11] have now reported that 1 (renamed cannabicitran) is indeed present in Lebanese hashish.




2a, R = H
b, R = COOH

Cannabicitran was isolated from an ethanolic extract of *C. sativa* by countercurrent distribution and column chromatography. Its structure, which was suggested by the spectral data, was confirmed by direct comparison with a synthetic sample.

#### b. Cannabicyclol (2a)

The full details of the single-crystal x-ray analysis of cannabicyclol have been published.[12] The cyclobutane ring has been found to be close to planar, and this plane forms angles of 50 and 72° with the mean planes of the aromatic and cyclopentane rings. The angle between these two is 79°. All atoms of the dihydropyran ring (except the atom bearing the oxygen atom) are in the plane of the aromatic ring. The cyclopentane ring adopts an envelope conformation, with C-6 out of plane.

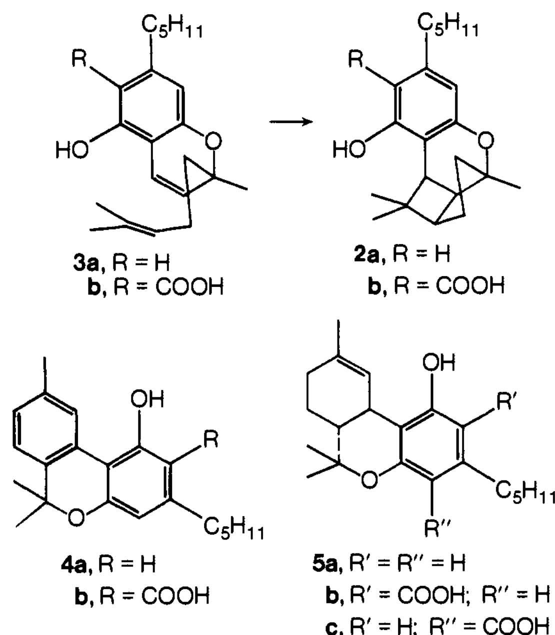
#### c. Cannabinoid Acids

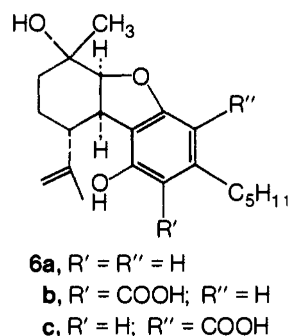
The carboxylic acid counterparts of the major neutral cannabinoids, namely, cannabidiolic acid, $\Delta^1$-THC acids A and B, cannabinolic acid, cannabigerolic acid, and cannabichromenic acid are well known.[13] More recently those of some of the minor cannabinoids were also isolated. The list will undoubtedly grow as additional cannabis samples are looked into.

*Cannabicyclolic acid* (**2b**),[14] mp 152–155°, was isolated from dried *Cannabis sativa* leaves (Kumamoto strain). The amount of **2b** present was small (60 mg of **2b** as isolated from 2.2 kg of raw material as compared to 7.5 g of $\Delta^1$-THC acid). Cannabicyclolic acid (**2b**) on heating gives the known cannabicyclol (**2a**). The position of the carboxyl group was determined by the presence of an internal hydrogen bond (phenolic proton, $\delta$ 11.83).

Cannabicyclolic acid (**2b**) is an optically inactive substance (as is the neutral cannabicyclol, **2a**) despite the presence of four chiral centers. It has been assumed[15] that **2a** (and therefore also **2b**) are artifacts formed from the optically inactive cannabichromene (**3a**) and its acid (**3b**) on irradiation. The experimental conversion of **3a** into **2a** has indeed been previously reported.[15] Shoyama et al.[14] have now found that the acid **3b** is likewise converted into the acid **2b** on irradiation.

The presence of only one cannabicyclolic acid, in which the carboxyl group is ortho to the free phenolic group, follows from the presence in cannabis of only one cannabichromenic acid (**3b**). Neither the isomeric cannabichromenic acid nor the isomeric cannabicyclolic acid has been observed. Cannabinolic acid (**4b**) is also known in one form only; the isomeric acid has not been isolated.[16] This is not, however, a general



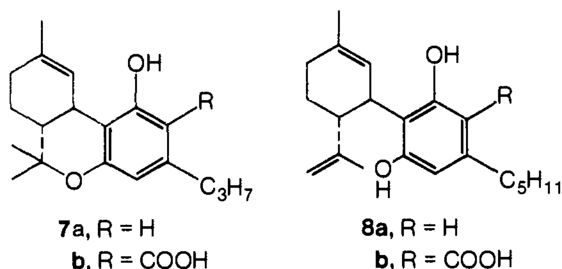




6a, R' = R'' = H
b, R' = COOH; R'' = H
c, R' = H; R'' = COOH

phenomenon. Both THC acid A (**5b**) and B (**5c**),[13] as well as the cannabielsoic acids A (**6b**) and B (**6c**),[17] are known in nature. However, both B acids **5c** and **6c** are present in quantities considerably lower than those of the A series. THC acid B is actually present in some, but not all, hashish samples. It is unknown whether these observations are of biogenetic importance.

*$\Delta^1$-Tetrahydrocannabivarolic acid (propyl-$\Delta^1$-THC acid,*

7b) has been isolated by Paris and coworkers[18] from fresh leaves of *Cannabis sativa* grown from seeds originating from South Africa. The structure was determined by mass spectrometry of a silylated derivative. The presented data do not eliminate the remote possibility that the carboxyl group is ortho to the etheric oxygen, rather than to the free phenolic one.



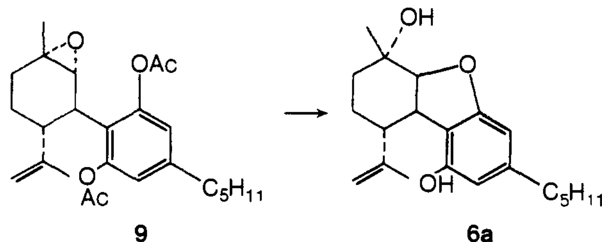

**7a**, R = H
**b**, R = COOH

**8a**, R = H
**b**, R = COOH

The same group has described[19] in detail the isolation of cannabidiolic acid (**8b**) and $\Delta^1$-THC acid A (**5b**) by preparative TLC. Column chromatography was found insufficient for complete separation of **8b** and **5b**. Pure cannabidiolic acid (**8b**) was found to be stable; however, pure $\Delta^1$-THC A (**5b**) was not. It is assumed[19] that in the plant the acids are stabilized by "des stabilisants capables de maintenir l'integritè de ces principes acides . . ."; however, these stabilizers, if present, have not yet been isolated. The instability of $\Delta^1$-THC acid A has also been commented upon by De Zeeuw et al.[20] They were unable to isolate a sample, which could serve as an analytical reference standard, owing to rapid deterioration. However, extracts after storage "for at least ten years on the shelves of the University pharmacy still contained large amounts of $\Delta^1$-THC acid".

The carboxylic acid cannabinoids seem to be present in all *fresh* samples of cannabis. In stored cannabis the ratio of neutral to acid cannabinoids increases;[21] apparently the acids are decarboxylated to the neutral phenols. Several groups[22] have speculated that indeed the noncarboxylic cannabinoids are artifacts formed on heating or on storage. This assumption has yet to be fully substantiated. In view of the notorious variability of the chemical content of cannabis, it is possible that while in some plants only acids are present others contain both acids and neutral cannabinoids.

*The stereochemistry at C-1 of cannabielsoic acid A* (**6b**) has been established by recent work in two laboratories. One group[17b] has synthesized both **6b** and its C-1 isomer and, by comparison of their NMR spectra as well as by chemical modifications, in particular the difference in dehydration patterns of both isomers, has established the structure **6b.** Another group[23] has cyclized the epoxide **9** (whose stereochemistry of C-1, C-2 is based on analogy[24]) to **6a.**

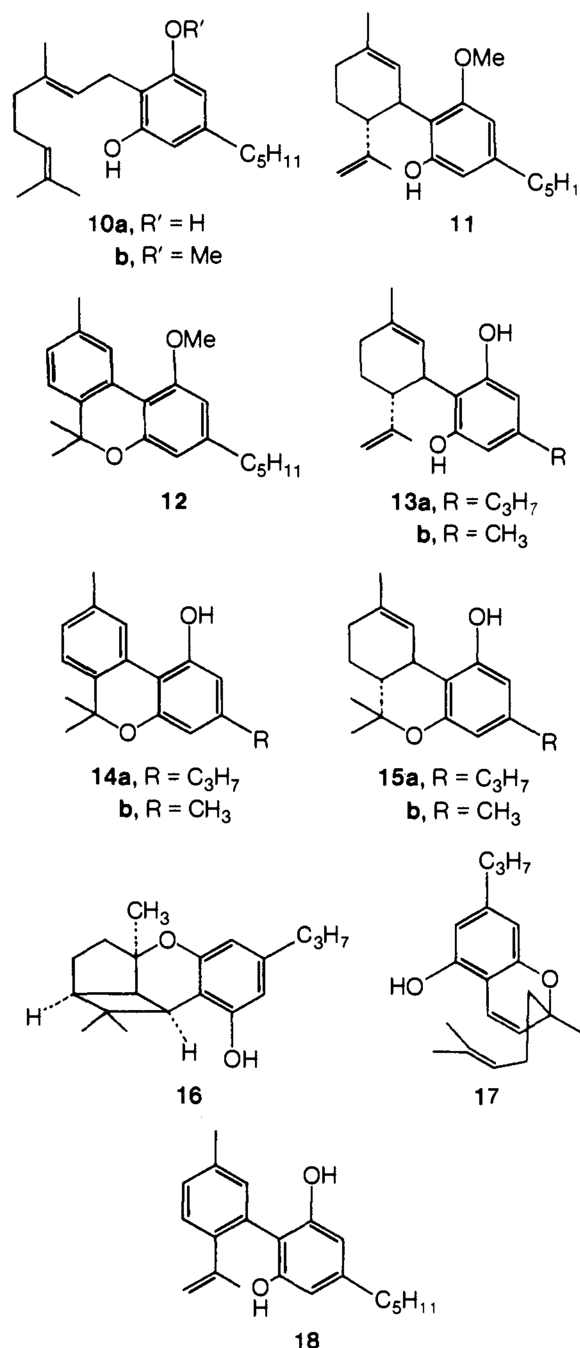



**9** **6a**

d. Natural Monomethyl Ethers of Cannabinoids

A monomethyl ether of cannabigerol (**10b**) was found in Japanese cannabis by Yamauchi et al.[25] in 1968. This particular component has now been reported to be consistently present in plants grown from seeds originating from northeast Africa.[26] Shoyama and coworkers[27] have found a further component of this type, cannabidiol monomethyl ether (**11**). The structure was determined from its spectral characteristics, which are similar to the well-known cannabidiol, and by comparison with a synthetic sample.

Bercht et al.[28] have observed the presence of cannabinol methyl ether (**12**) as well as cannabidiol monomethyl ether (**11**) in hashish. Structure **12** was determined by combined gas chromatography–mass spectrometry (GLC–MS) which precluded the need for isolation. A synthetic sample of **12** showed the same GLC retention time and MS pattern at different electron voltages as cannabinol methyl ether in cannabis. The presence of the methyl ether of $\Delta^1$-THC was also indicated (by comparison with the ms pattern of a synthetic sample); however, the relative retention time was in the region generally obscured by large amounts of cannabidiol.




**10a**, R′ = H
**b**, R′ = Me

**11**

**12**

**13a**, R = $C_3H_7$
**b**, R = $CH_3$

**14a**, R = $C_3H_7$
**b**, R = $CH_3$

**15a**, R = $C_3H_7$
**b**, R = $CH_3$

**16**

**17**

**18**

e. Neutral Cannabinoids with Propyl or Methyl Side Chains

Neutral cannabinoids with a pentyl side chain are generally accompanied by homologs with a propyl side chain. Cannabidivarol (propylcannabidiol, **13a**) was the first member of this class to be isolated.[29] Later cannabivarol (propylcannabinol)

(**14a**) and $\Delta^1$-tetrahydrocannabivarol (propyl-$\Delta^1$-THC) (**15a**) were shown to be present in all cannabis samples, though usually at concentrations considerably lower than those of the pentyl homologs.[30]

Of particular interest in the identification of the above homologs is a new GLC-MS method described by Vree et al.[31] They observed that at an electron energy of 70 eV and ion source temperature of 250°, it was impossible to obtain a mass spectrum that could be used for the identification of cannabinoids with the same molecular weight. The fragmentation pattern was too similar to yield significant information about differences in molecular structure. However, if the mass spectra were obtained at various electron energies (usually 10-20 eV) and the relative intensities of particular mass fragments were plotted vs. eV, characteristic graphs were obtained for each cannabinoid (electron voltage-mass fragment intensity graphs; eV-mf intensity graphs). The eV-mf intensity graphs of the propyl homologs were essentially parallel to those of the pentyl cannabinoids. The crossing point between the eV-mf intensity graphs of two fragments of the same compound are identical for cannabinoid homologs. This method should prove of considerable interest in the identification of homologous natural products available in minute amounts.

By the use of the above method the Dutch group has identified two additional propylcannabinoids in hashish: propylcannabicyclol (**16**)[32] and propylcannabichromene (**17**)[33]. While **16** was only observed as a GLC peak, **17** was also isolated by TLC. Similarly the methyl homologues cannabidiorcol (methylcannabidiol) (**13b**), cannabiorcol (methylcannabinol) (**14b**), and $\Delta^1$-tetrahydrocannabiorcol (methyl-$\Delta^1$-THC) (**15b**) were observed and identified.[34] All three compounds showed eV-mf intensity graphs identical with those of the respective pentyl and propyl homologues except that the fragments were 56 and 28 mass units smaller.

### f. Cannabinodiol

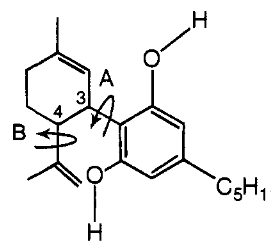
van Ginneken and coworkers[35] have reported the presence of a new cannabinoid, named cannabinodiol (**18**), in Nepalese hashish and Brazilian marijuana. Structure **18** was suggested on the basis of the retention time on GLC and the mass fragmentation pattern on ms. The molecular ion 310, the mass fragments 295 and 354, and the metastable peak at 281 recall the molecular weight and fragmentation of cannabinol (**4a**). In contrast to cannabinol, however, the crossing point of the M and M − 15 eV-mf intensity graphs was at 19.5 eV, about 5 eV higher than for cannabinol. It is suggested that this difference is due to an increase in conjugation of the ring system, leading to more possible resonance structures and thus to a higher stability of the new molecule. The cannabinol-like mass fragmentation suggests that in the mass spectrometer **18** is first cyclized to cannabinol.

Cannabinodiol was only observed as a GLC peak and was not isolated. Hence, NMR, uv, and ir spectral curves, which could have proved the structure unequivocally, are not available.

The presence of propylcannabinodiol was likewise indicated.

### g. Conformation of Cannabidiol

Weiner and Meyer[36] have noticed a temperature dependence of those chemical shifts which are associated with the benzene moiety of cannabidiol. Upon cooling, the aromatic protons which appear as a singlet at 25° move to a lower field and the singlet splits into two. This observation was interpreted as an indication of restricted rotation and a theoretical investigation of the conformation of the molecule was undertaken. It was found, by PCILO calculations, that the cyclohexane ring is a somewhat flattened half-chair. The C-3 and C-4 protons are quasi-axial; the aryl and isopropenyl substituents on C-3 and C-4 are quasi-equatorial with axes $A \sim 30°$ and $B \sim 120°$.




### h. Cannabinoids in Cannabis Smoke. Stability of Cannabinoids

Fehr and Kalant[37] have analyzed cannabis smoke obtained under different combustion conditions. A special smoking machine was constructed which allowed air flow parameters to vary within potential human physiological limits. The temperature of combustion, relevant to marijuana smoking, was found to be about 600°. The maximum recovery after complete combustion was about 60%, with small change in cannabinoid ratios, the most important one being an increase in cannabinol. This increase was particularly evident (from 2.7 to 6.8%) when the material smoked was tobacco injected with $\Delta^1$-THC, rather than marijuana. The percentage recovery in the smoke increased slightly with increasing air flow rate, but was unaffected by continuous vs. intermittent flow. The total dry residue (tar content) of cannabis smoke was comparable to that of tobacco smoke—about 5% on leaving a 30-mm butt.

$\Delta^1$-THC is not formed from cannabidiol on smoking marijuana. However, in tobacco cigarettes injected with cannabidiol this *acid-catalyzed* cyclization does take place. Indeed, water suspensions of the cigarettes used (Pall Mall) had a pH of 5.72; water suspensions of the marijuana (no firm indicated) were alkaline (pH 8.14).[38] The above results may be of practical importance. In the last few years the use of "hashish oil" has increased. Frequently this oil (which contains all cannabinoids including cannabidiol) is used to spike cigarettes.

The above data are generally compatible with previous observations.[39,40]

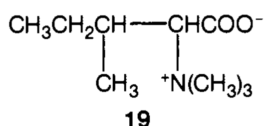
It is considered that cannabinoids are labile materials. This is true under certain conditions only. As indicated (see above) the major cannabinoids are quite stable to heat. In the presence of oxygen, $\Delta^1$-THC is slowly converted into cannabinol, but the rest of the major cannabinoids seem to be more stable than their phenolic nature would lead us to expect. On irradiation $\Delta^1$-THC and $\Delta^6$-THC are slowly converted into unknown decomposition products;[41] cannabidiol gives a number of products, including $\Delta^1$-THC.[41,42] The transformations of cannabinoids under acidic and basic conditions have been reviewed.[43] The decomposition of cannabidiol in chloroform solution has been discussed.[44] It was found that the amount gradually decreased. However, its fate remained unknown.

The solubility, protein binding, and stability of THC have been discussed in detail by Garett and coworkers.[44a]

## 2. *Non-Cannabinoid Components of C. Sativa*

The presence of nitrogen-containing compounds in *C. sativa* has been noted for about a century.[45] Most of the identified constituents of this type are well-known plant components. Recently, however, Bercht et al.[46] have reported the occurrence of L-(+)-isoleucine betaine (**19**) in ground fresh hemp seed (103 mg of **19** from 300 kg of seeds). This is apparently the first report of the natural occurrence of this be-

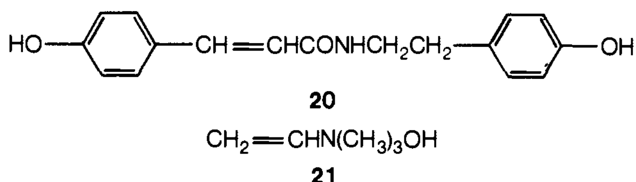
taine, although it has been known[47] as a synthetic product since 1932. The structure was determined by spectroscopic methods and confirmed by synthesis. Preliminary pharmacological assays on analgesic, hypothermal, rotating rod, and toxicity effects of **19** on mice did not reveal any acute symptoms.




The amino acid content of hemp has been determined.[48] A remarkable observation is that the sulfur-containing amino acids methionine, cystine, and cysteine are absent in hemp. This is in accordance with findings on another member of a related plant family, the hop (*Humulus lupulus*, fam. Moraceae), where, likewise, methionine could not be detected. The presence of proline in marijuana has been independently reported by another group.[49]

The free amines in hemp have been analyzed[48] by capillary gas chromatography. At least 36 compounds could be detected, and the following could be identified by comparison with reference samples: ammonia, methylamine, isobutylamine, *sec*-butylamine, dimethylamine, diethylamine, and pyrrolidine. Also tentatively identified were *n*-pentylamine, isoamylamine, β-phenetylamine, cadaverine, ethanolamine (or histamine), and benzylamine (or tyramine). The pharmacological activity of the above mixture, as tested in a syndrome assay, was practically nil, although more refined pharmacological tests will be required in order to conclude that the amine mixture is indeed inactive.

A rather unusual amide, *N*-(*p*-hydroxy-β-phenylethyl)-*p*-hydroxy-*trans*-cinnamamide (**20**) has been isolated from *C. sativa* roots. This amide was characterized by synthesis. The only previous report of this compound was in 1968 when it was isolated from the bark of *Evodia belahe* B. (Rutaceae). Analgesic activity was noted in a mouse behavioral test, but as this compound has not been observed in marijuana or hashish (which are prepared from the leaves and/or the resin of *C. sativa*), it is doubtful whether it is relevant to cannabis activity. The parent acid (*p*-hydroxycinnamic acid) has been previously identified in *C. sativa*.[51]

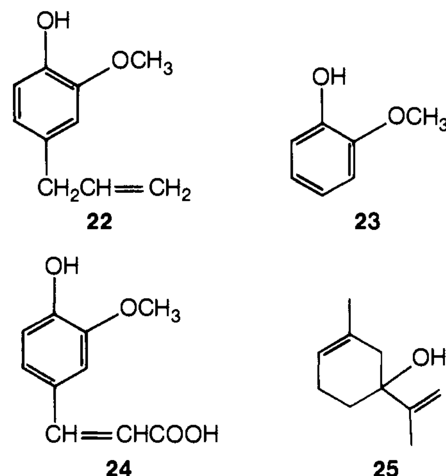



Neurine (**21**) has been reported,[49] for the first time, in the roots of *C. sativa*, at a concentration of 0.01%. It is accompanied by choline. Neurine has some curare-like action; it is very toxic and causes pronounced muscarinic and nicotinic effects. Again, its relevance to marijuana activity is unknown.

*Triterpenes and steroids* have been found, as expected, in *C. sativa*. White crystalline material was found in red oil extract of Yugoslavian cannabis. Fenselau showed[52] by GLC–MS and high-resolution MS that it consisted of the well-known campesterol, stigmasterol, and β-sitosterol. Her results have been confirmed[49] for an Indian variety of *C. sativa*. A comparison of the amounts of these three 3β-hydroxysterols in an American and a Thai variety has been made.[53] In the American sample most of the sterols were found in the form of esters or glycosides with small amounts of free sterols; in the Thai one no free sterols were found. Friedeline and epifriedelanol have been found in *C. sativa* roots.[50]

The *carbohydrate and cyclitol content* of *C. sativa* has been examined.[54,55] The importance of these investigations is that much of the phenols found in the mainstream of cigarette smoke derive from the carbohydrate content of the flue-cured tobacco leaf.[56] It is reasonable to assume that this applies to smoked marijuana as well. In one investigation[54] D-*manno*-heptulose, *altro*-heptulose, D-glucero-D-mannoctulose, myoinositol, quebrachitol, glycerol, erythritol, arabinitol, and xylitol were found. The sugars were isolated from an extract after fermentation with yeast, while the alcohols were isolated from an unfermented extract. In an independent investigation[55] it was found that a U.S. variety of *C. sativa* contained ribitol, fructose, glucose, sucrose, quebrachitol, bornesitol, and myoinositol. A Thai sample contained in addition (+)-inositol, whereas a sample from Viet Nam contained erythritol.

The *non-cannabinoid phenols* found in the fresh plant and in marijuana smoke have been investigated quite thoroughly. In the late fifties and early sixties, eugenol (**22**), guaiacol (**23**),[57] as well as ferulic acid (**24**), cinnamic acid[58] and, as mentioned above, *p*-hydroxycinnamic acid[51] were isolated from the plant. Fentiman and coworkers[59] have now reported on the phenolic content of marijuana smoke condensate. They used GLC–MS [both electron impact (EI) MS and chemical ionization (CI) MS]. CI mass spectrometry has been




shown to complement electron impact (EI) mass spectrometry effectively in the structural analysis of a wide variety of organic compounds.[60] The CI spectra obtained using methane and isobutane as reactant gases characteristically show abundant protonated molecular ions, even when the corresponding EI mass spectra do not show detectable molecular ions. Recently helium has been employed as carrier–reactant gas.[61] The resulting spectra are very similar to standard EI spectra. Using both techniques several aromatic phenols previously not detected in cannabis were identified: *p*-hydroxyacetophenone, catechol, *o*- and *p*-cresol, *p*-ethylphenol, phenol, *p*-vinylphenol, and tentatively, 4-hydroxy-3-methoxystyrene. The last two compounds are probably formed during the smoking process by decarboxylation of *p*-hydroxycinnamic acid and ferulic acid, respectively.[59]

The physiological effects of phenols in *tobacco smoke* have been investigated.[62] Cocarcinogenesis and ciliostasis have been observed. The presence of related phenols in cannabis smoke is an important observation, which obviously may have health implications. It should be pointed out, however, that the amount of tobacco smoked by one is usually much higher than that of cannabis; hence simplistic conclusions should be avoided.

Nine *carboxylic acids* previously unreported in cannabis have been identified in cannabis smoke.[59] They include ben-

zoic acid, furoic acid, as well as long-chain fatty acids. The presence of these acids is not surprising and, from a pharmacological point of view, is probably not particularly significant.

The presence of *alkanes* in cannabis has been mentioned for decades in the chemical and analytical literature.[63] Two systematic studies have been published recently. De Zeeuw et al.[64] have found that leaves of young *C. sativa* plants contain appreciable amounts of *n*-heptacosane and *n*-nonacosane. The whole range of $C_{19}$–$C_{32}$ alkanes were detected as minor components in the leaves and in a variety of stored samples. Adams and Jones[65] have reported closely related results obtained by GLC–MS. They have also compared the composition of the plant extract with that of the smoke condensate. Nonacosane is the major component of both mixtures, although its relative percentage is three times greater in the plant extract. The percentages of almost all hydrocarbons present in the smoke condensate have increased significantly relative to nonacosane. This effect (due to pyrolytic cracking) has previously been observed,[66] though to a lesser extent, in tobacco smoke. Some alkanes have retention times similar to those of the major cannabinoids and thus interfere with GLC analyses. A simple method for the partial elimination of this problem, which has been employed by us, is the selective solution of the cannabinoids in a minimal amount of methanol. The parafins are only slightly soluble in this solvent while the cannabinoids dissolve with ease. Repetition of this process several times eliminates most of the alkanes.

Interest in the *essential oils* of cannabis continues unabated. The very characteristic, slightly sweet, and not unpleasant odor of marijuana, hashish, and fresh hemp is well known. As with most natural odors it is due to the total mixture of volatile components rather than to a specific one. Bercht et al.[67] have subjected freshly harvested plant material to "nitrogen distillation", by passing a continuous flow of nitrogen through the material. The distillate was trapped at −80° and then analyzed by GLC. In this fashion thermolysis of some of the volatile compounds was prevented. The presence of at least 23 volatile components was indicated; 17 were shown to be known monoterpenes. Some of these monoterpenes had not previously been reported in cannabis. All the identified monoterpenes are well-known compounds such as thujene, $\Delta^1$-carene, etc., and are by no means specific for cannabis. A comparison between the head-space components and those of the essential oil has also been reported.[68] The compounds were identified by GLC–MS. Twenty-seven compounds, all of which had previously been found in other plants, accounted for 96% of the essential oil in Mexican marijuana. The major ones were found to be $\beta$-caryophyllene (37.5%) and $\beta$-humulene (13.9%). In the head space, only 17 compounds were found. $\alpha$-Pinene (55.5%) and $\beta$-pinene (16.4%) were the major components. Oxygenated terpenes such as fenchyl alcohol, borneol and $\alpha$-terpineol were all present in the oil, but none were detected in the head space. It is suggested that this is due, in addition to low volatility, to the affinity of the oxygenated species for the plant medium.

Stahl and Kunde[69] have investigated the steam distillate of brown Lebanese hashish. On GLC with a capillary column, 230 components were observed. Longifolene was found to be the major component (33%) in addition to $\beta$-caryopyllene (23%) and humulene (7%). Two humulene epoxides were also identified. The presence of longifolene is unexpected, in view of previous analyses of cannabis material (see above). It should be of interest to determine whether longifolene is a characteristic constituent of Lebanese hashish, while it is absent in cannabis from other sources. A component isolated from cannabis which has not been previously observed as a natural product is *m*-mentha-1,8(9)-dien-5-ol (**25**). The structure of this unusual monoterpene was determined on the basis of its physical data, in particular, its detailed nmr spectrum.

The determination of the volatile components may be of importance in the possible development of a GLC "sniffer" for the detection of cannabis. The results described above, however, make a successful development rather uncertain. The absence of major unique components [except for *m*-mentha-1,8(9)-dien-5-ol, whose status as a typical constituent is yet unknown] may turn to be too high a hurdle. A gas chromatograph could possibly determine numerous components; however, most of these are also present in other plant products, perfumes, or cosmetics. The nose of a police "sniffer" dog is still a tool which chemists cannot copy, as it determines the total small rather than individual components. However, it should be possible to determine the geographical origin of cannabis samples by detailed analysis of their essential oils. This may be achieved after more examples are published and significant quantitative (and hopefully qualitative) differences are determined.

### 3. Chemobotanical Aspects

Although the taxonomic literature on the plant *Cannabis* is complicated by a plethora of names of varieties, the genus has been generally considered to be monotypic, i.e., to consist of a single species, *Cannabis sativa* L. This monotypic concept is reflected in chemical publications as well as in laws governing illicit drugs. Schultes and his coworkers[70], of the Harvard Botanical Museum, on the basis of a superb review of the literature, a reexamination of herbaria specimens preserved since the 18th century, and on a botanical expedition to Afganistan, conclude that ". . . we have little hesitation with the evidence available at this point in accepting [a] polytypic concept." They distinguish at least three *Cannabis* species: *C. sativa, C. indica*, and *C. ruderalis*.[71] This new classification has not, of course, been taken into account in the chemical literature up till now. Hence many reported analyses of *C. sativa* may, in fact, be of these other species. It will be of considerable interest to establish definite chemical differences between the three (or possibly more) species. The lack of solidly based chemical differentiation between the newly established species may cause serious legal problems as many national laws specifically indicate "*Cannabis sativa*". Some *Cannabis* samples (such as *Cannabis* resin) probably cannot be assigned a specific species origin on the basis of a morphological examination. Hence identification for legal purposes may have to be based on chemical differences.

Prior to Schultes' publication, workers in the field, tacitly accepting the existence of a single species, tried to reconcile the considerable differences in chemical constitution of *Cannabis* plants by assuming the existence of two phenotypes—fiber and drug. The first phenotype has been defined[72] as having a ratio of $\Delta^1$-THC/cannabidiol (and their acids) of less than unity. In the drug phenotype, the ratio is more than unity. Cannabinol is taken into account, being produced from $\Delta^1$-THC on storage. Small and Beckstead[26] have examined 350 fresh samples of plants experimentally grown in Canada from seeds originating from various countries. They have found the above classification inadequate. Their results suggest the existence of *three* main *Cannabis* phenotypes. Most strains of type I originated from countries south of latitude 30°N. They possessed large amounts of $\Delta^1$-THC (>0.3%) and low amounts (<0.5%) of cannabidiol. In types II and III cannabidiol was more than 0.5%. In type II the $\Delta^1$-THC content was high (>0.3%); in type III it was low (<0.3%). These varieties usually originated from countries north of latitude 30°N. In both types II and III the female plants possessed substantially higher contents of cannabinoids than did the males. In type I both sexes possessed the same amounts per given weight but the female plants were larger. These observations give experi-

mental support to the agricultural practice of eliminating male plants from cannabis plantations. They are at variance, however, with previous reports that male and female plants on a weight basis produce the same amounts of cannabinoids[72,73]. It is difficult to reconcile these differences, and more work is obviously needed.

In all samples examined by Small and Beckstead $\Delta^6$-THC was present in very limited quantity. Cannabinol was rarely found. In view of the large number of samples analyzed, these results indicate that cannabinol is seldom, if ever, a true natural product, and that $\Delta^6$-THC is, at best, only marginally relevant to cannabis activity.

Less extensive and mostly local analyses of cannabis samples have been reported. $\Delta^1$-THC is present in Argentine marijuana in amounts varying between 0.1 to 8.3%. Cannabidiol is usually a minor component (mostly less than 1%).[74] Large variations have also been observed in analyses of samples in the Lyons area in France.[75]

Some cannabinoids seem to be present mostly in samples from specific geographic locations. Thus, the propylcannabinoids (see above) are most frequently reported in samples of Asian origin. However, this type of cannabinoid cannot serve as "locator" as it occurs sometimes in other samples as well.[76]

The effect of light on cannabinoid content has been examined.[77] Greenhouse plants kept in complete darkness for 15 days produced an amount of THC comparable to that produced in similar plants allowed to grow under normal lighting.

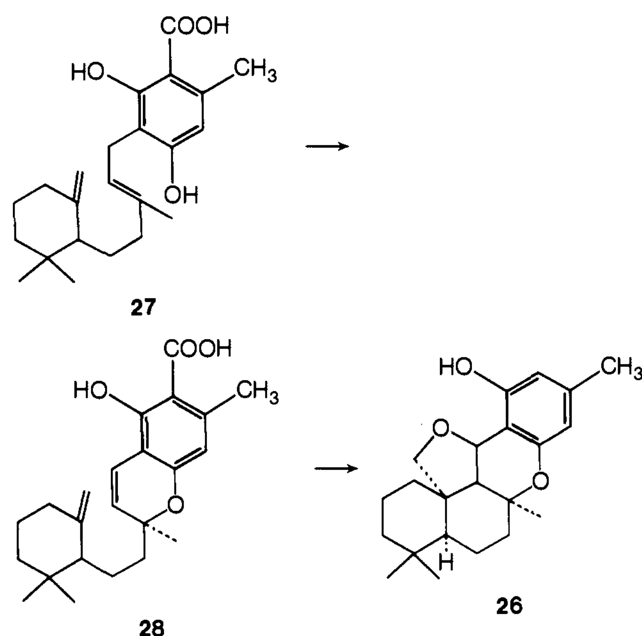
Shoyama et al.[14] have reported that larger amounts of cannabicyclolic acid (**2b**) are present in *Cannabis sativa* plants during the vegetative phase than during the reproductive phase. Amounts of the same material also increased on storage, apparently due to conversion from cannabichromenic acid (**3b**).

Fairbairn and Liebmann[77] have confirmed previous reports that the production of cannabinoids seems to depend less on environmental factors ("hot sunny climate") than on genetic constitution. Whether this is the case after a number of generations and under stressful conditions (cf. ref 78) remains an open question (see, however, Addendum).

On summarizing the work of his own and other groups Fairbairn[77] concludes that ". . . [cannabinoid content] constancy must be tested more widely and especially through several generations, but if confirmed we would be justified in speaking of two chemical races. There is some evidence of existence of an intermediate type with approximately equal proportions of THC and cannabidiol." It will be of considerable chemotaxonomic interest to find whether these "chemical races" are the same as the "botanical species" defined by Schultes.[70]

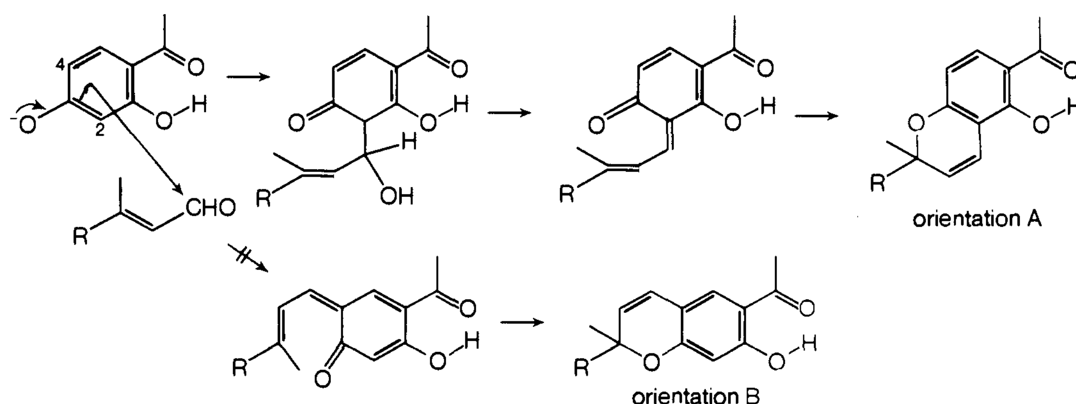
The biogenesis of cannabinoids remains a virgin field of research. However, it has been suggested[79] that in plants in which cannabidiol is absent, cannabinoid biogenesis follows a different path from those plants in which cannabidiol is present. The above suggestion will have to wait substantiation by experiment.

Suzuki and Nozoe[80] have investigated the biosynthesis of the terpenophenolic siccanin (**26**), which is structurally closely related to the cannabinoids. It was found that, as suggested for the cannabinoids,[81] mevalonic acid is a precursor of the terpenoid moiety. The intermediate presiccanochromenic acid (**27**) is oxidatively converted into siccanochromenic acid (**28**). This oxidative step had previously been postulated[81] for the conversion of cannabigerol (**10a**) into cannabichromene (**3a**).




An intriguing point in the biogenesis of natural terpenophenols is the high regiospecificity observed. In all natural cannabinoids the terpene is attached to the phenolic ring at the C-2 rather than the C-4 position. In most laboratory syntheses of cannabinoids, however, the condensation step between olivetol and the terpene leads to mixtures. Clarke et al.[82] have suggested that the regiospecificity of phenol chromenylation in the reaction between citral and appropriate phenols is due to the stability of the transition state leading to the dienone intermediate. Thus 2,4-dihydroxyacetophenone on reaction with $\alpha,\beta$-unsaturated aldehydes leads to compounds of orientation A only. The isomer with orientation B is not formed. Apparently orientation A allows retention of the stabilization energy of the chelate system in the dienone-forming reaction; in orientation B it is lost (see Scheme I).

SCHEME I



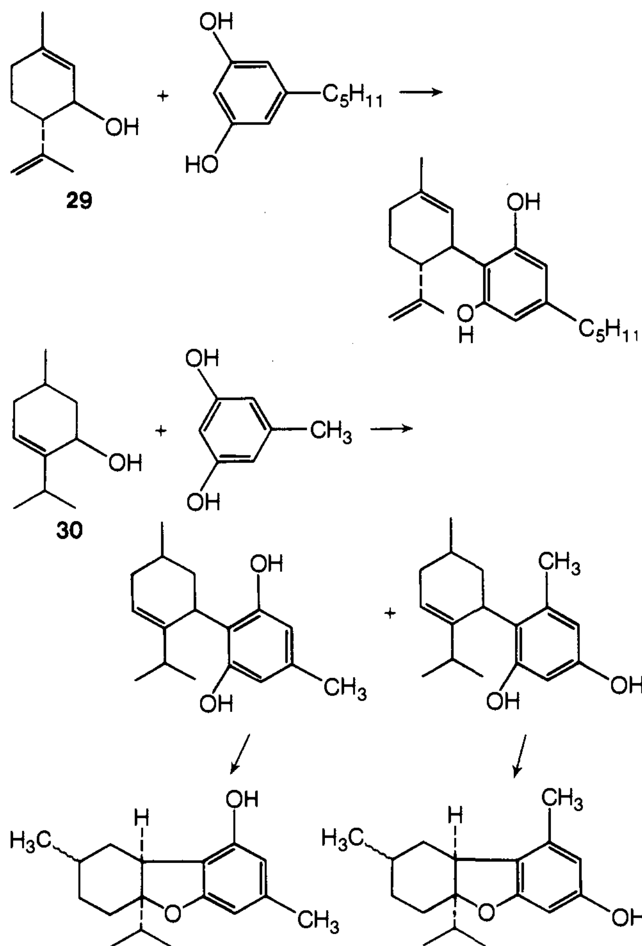
A related effect may be the basis for the biogenetic regiospecificity in the cannabinoids; it is possible that orientation A will, again, be preferred to orientation B (Scheme II). This point should be examined experimentally. Thus, with olivetolic acid as a biogenetic precursor, only cannabinoids with orientation A will probably be obtained. With olivetol, however, presumably both orientations could be present.

## B. Syntheses of Cannabinoids

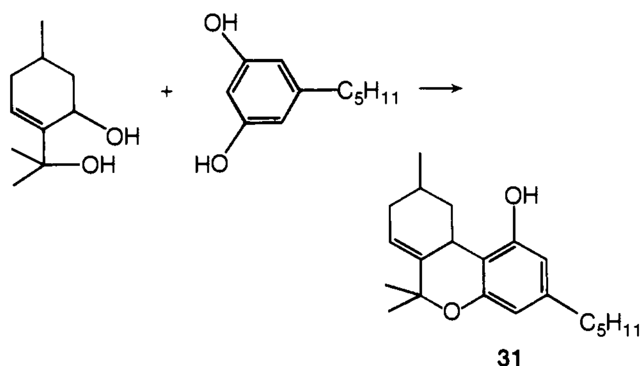
### 1. *Synthesis of Plant Cannabinoids and Closely Related Derivatives*

No radically new approaches toward the syntheses of cannabinoids occurring in *C. sativa* or its preparations have been reported in the last few years.

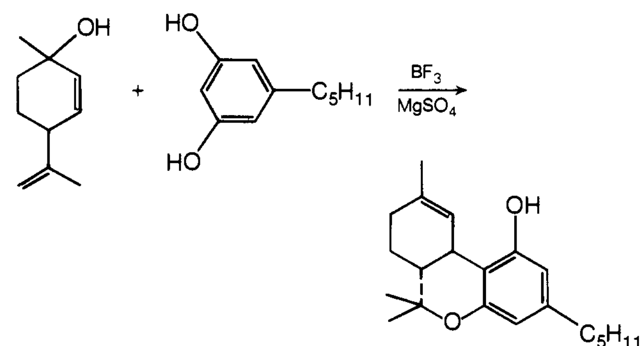
Cardillo et al.[83] have published a detailed paper on their previously announced synthesis of cannabidiol from *p*-menta-1,8-dien-3-ol (**29**) and olivetol in aqueous acid. For this particular purpose the procedure is not attractive as the yields are low and the preparation of the monoterpenoid starting material is laborious. However, an interesting application is the synthesis of "unnatural" cannabinoids, such as the hitherto unknown $\Delta^4$ derivatives. Thus, menth-4-en-3-ol (**30**) with orcinol gives a mixture of uncyclized alkylation products in addition to secondary products obtained by acid-induced cyclization of the former ones. The stereochemistry of the numerous products obtained was established by conformational analysis. It is of interest that the rings A and B of the hexahydrodibenzofurans so formed are invariably with cis junction.




Using the same type of approach, the Italian group[84] has synthesized $\Delta^4$-THC (**31**) by the condensation of *p*-menth-4-ene-3,8-diol with olivetol.




Razdan and coworkers[85] have reported a modification of the Petrzilka[86] cannabinoid synthesis. *p*-Mentha-2,8-dien-1-ol, olivetol, and boron trifluoride gave $\Delta^1$-THC (rather than $\Delta^6$-THC) when a drying agent was added to the reaction mixture. This modification may represent a useful direct route to $\Delta^1$-THC.




Montèro, in a thesis,[87] has reported that 3-carene oxide (**32**) condenses with olivetol, in the presence of *p*-toluenesulfonic acid, to give $\Delta^6$-THC in ca. 20% yield. Ion **33** was put forward as an intermediate in the reaction. While, from a practical point of view, this synthesis does not represent an advance over existing procedures, it does raise a mechanistic question. Razdan and Handrick[88] have suggested that the mechanism of the closely related reaction of 2-carene oxide (**34**) with olivetol proceeds via the enol ether **35**. However, in view of the reactivity of 3-carene oxide (**32**) in the same reaction, it seems that the ion **33** is a more probable intermediate (see Scheme III).

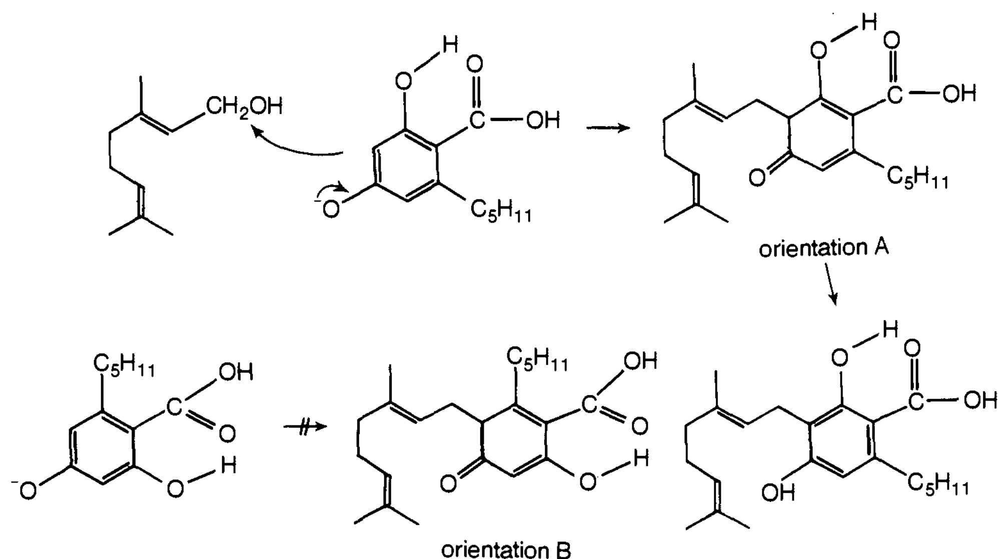
The mechanism and stereochemistry of the citrylidene cyclization has been elucidated. In the cannabinoid series this reaction has been utilized to prepare cannabichromene (**3a**) and cannabicitrane (**1**).[89] The chromene **3a** was obtained on condensation of citral with olivetol in pyridine. On heating, **3a** cyclizes to **1** (see section II.A.3). This cyclization has been represented hitherto as an ionic process which proceeds via ion i (eq 1). The Crombie group in Nottingham[90] has now found that *trans*-2,*trans*-6-farnesal and *trans*-2,*cis*-6-farnesal yield products which differ in stereochemistry at C-8. This observation eliminates ion i as an intermediate. The explanation offered is that the reaction is electrocyclic.

Shani and Mechoulam have described[17b] the synthesis of cannabielsoic acid A, previously reported in a communication.[17a] It was found that cannabidiolic acid can be oxidatively cyclized to cannabielsoic acid A (**6b**) by either irradiation in the presence of air or with manganese dioxide (Scheme IV). The latter oxidation is faster and the yields are generally higher in the presence of oxygen. Both procedures yield complicated mixtures.

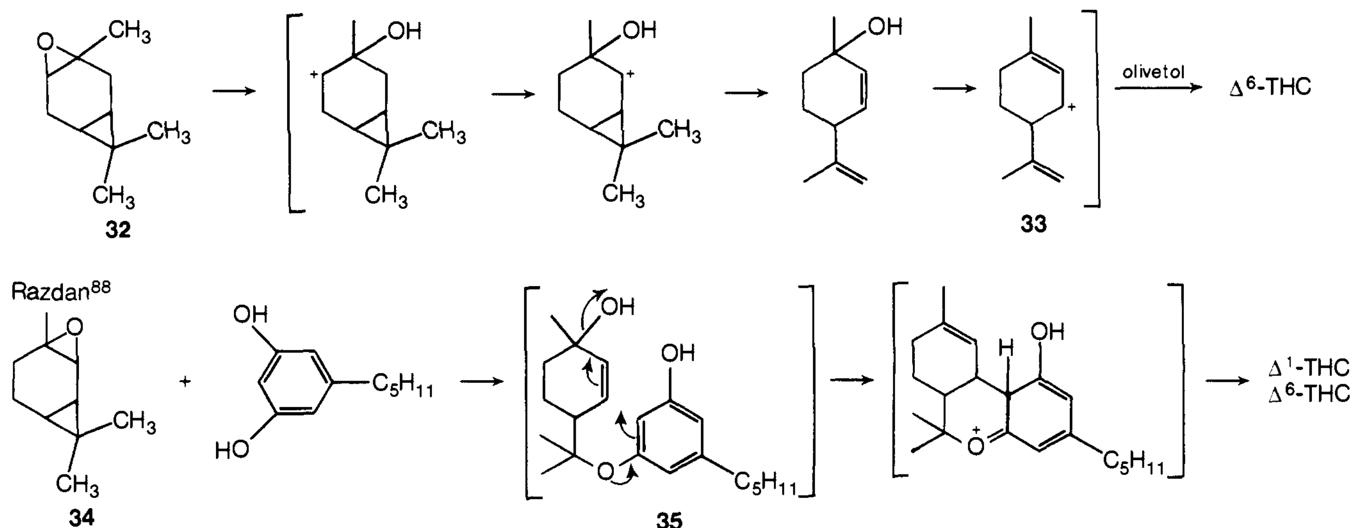
An interesting observation made is that, in the presence of 10% 2-propanol in the solvent (cyclohexane), the reaction rate is increased sevenfold.

The major product obtained[91] by pyrolysis of cannabidiol in

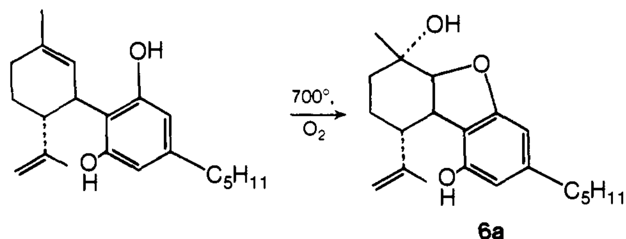
SCHEME II




SCHEME III



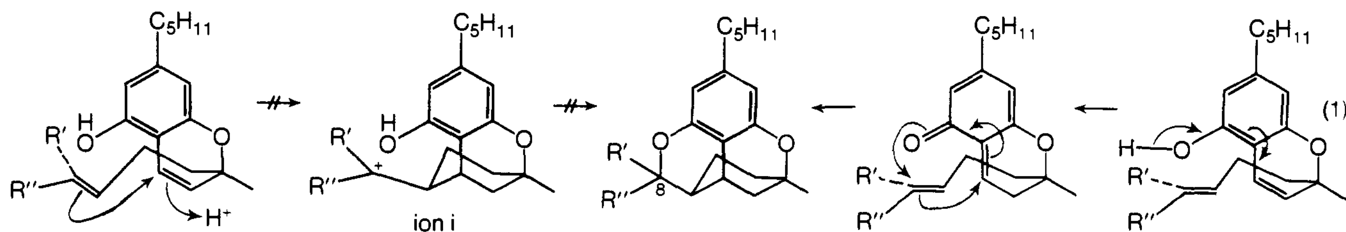

air at 700° is cannabielsoin (6a)—also a decarboxylation-dehydration product of the cannabielsoic acids.[17b]

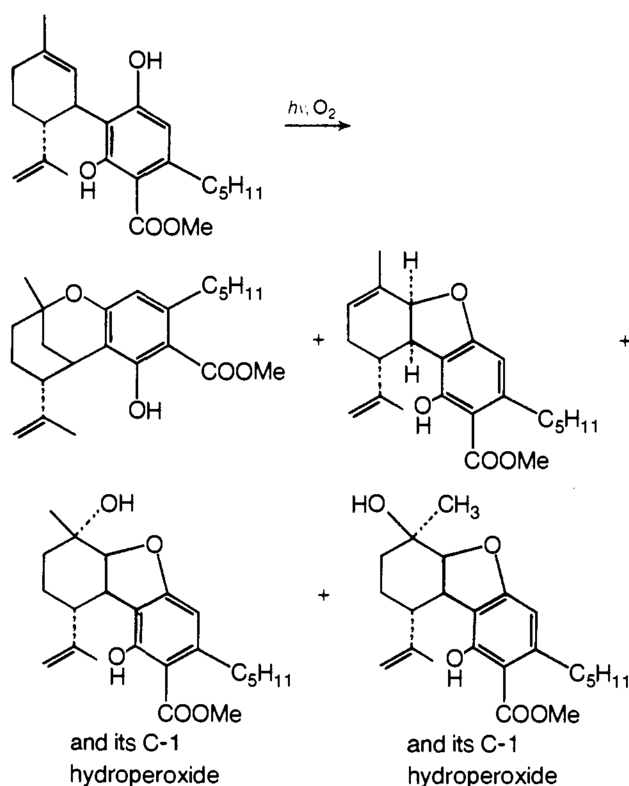



*2. Synthesis of Cannabinoid Metabolites*

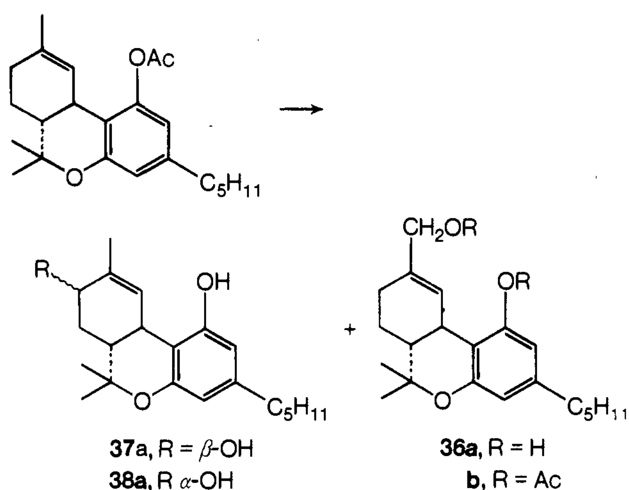
The pronounced biological activities of $\Delta^1$- and $\Delta^6$-THC metabolites[92] has prompted numerous groups to explore synthetic routes to these materials. The earlier synthetic work has been summarized.[93] Several new attempts to synthesize 7-OH-$\Delta^1$-THC (**36a**), a major active metabolite of $\Delta^1$-THC, have been reported. However, the goal of a simple, high-yield synthesis has yet to be achieved (see, however, Addendum). Other metabolites of cannabinoids have, however, been prepared by methods which are practical.

The group at the Research Triangle Institute[94] has reported that allylic halogenation of $\Delta^1$-THC acetate with sulfuryl chloride followed by acetoxylation with silver acetate, gives a mixture of acetoxylated $\Delta^1$-THC derivatives. After saponification, 6$\beta$-OH-$\Delta^1$-THC (**37a**) (14%), 6$\alpha$-OH-$\Delta^1$-THC (**38a**) (1%), and 7-OH-$\Delta^1$-THC (**36a**) (5%) were isolated by chromatography.



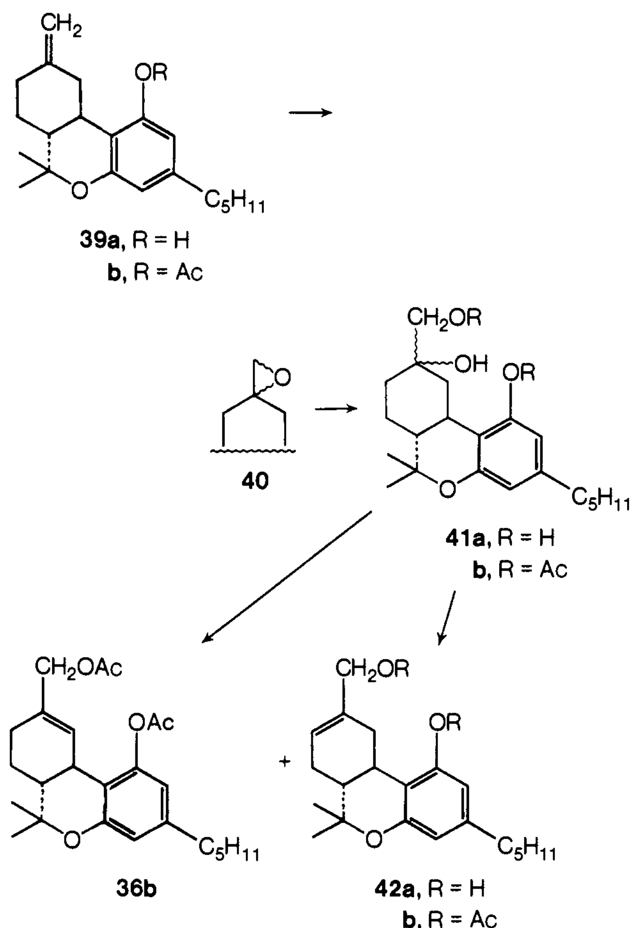



A number of other allylic halogenating and oxygenating reagents were examined with the object of exploiting any variation in selectivity of attack at the primary and secondary allylic sites of $\Delta^1$-THC. All the methods which were tried produced predominantly 6$\beta$-OH-$\Delta^1$-THC with only minor amounts of the 7-hydroxy isomer.




Razdan et al.[95] have treated $\Delta^{1(7)}$-THC acetate (**39b**) with *m*-chloroperbenzoic acid to get the epoxide **40**, which opened under *basic* conditions to the triol **41**a. The diacetate **41b**, on dehydration with thionyl chloride, led to a mixture of the two metabolite acetates **36b** and **42b**, in a ratio of 1:2. These were separated by high-pressure liquid chromatography. This separation proved to be exceptionally difficult as the optimized resolution of the two isomers was only marginal. The $\alpha$ value (a measure of peak-to-peak separation) was only 1.08, and thus several recycles were needed for a good separation.[96] The total yield of the synthesis was mediocre.

The diacetate **41b** can be easily converted on dehydration with *p*-toluenesulfonic acid into 7-OH-$\Delta^6$-THC diacetate (**42b**) in 75% yield from **39a.**




Razdan et al.[95] obtained the starting material **39**a in only ca. 3% yield as a by-product in the Petrzilka synthesis of $\Delta^1$-THC. However, better routes to **39**a are also known.[97]

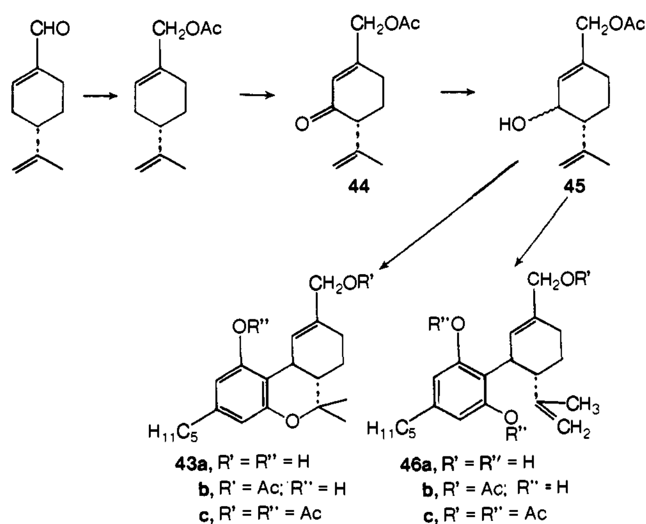
A total synthesis of (+)-7-OH-$\Delta^1$-THC (**43**a) has been completed by Lander et al.,[98] who started from the commercially available (−)-perillyl aldehyde. The latter compound was converted by reduction and acetylation into perillyl alcohol acetate which, on oxidation with freshly prepared dry chromium trioxide–pyridine complex, gave the $\alpha,\beta$-unsaturated ketone **44.** Selective reduction led to the corresponding allylic alcohol **45**, which, depending on the reaction conditions, could be converted into either (+)-7-hydroxycannabidiol acetate (**46b**) or (+)-7-OH-$\Delta^1$-THC acetate (**43b**). This synthesis can be employed to prepare the natural (−) isomers; however, the necessary starting material, (+)-perillyl aldehyde, is not commercially available and has to be synthesized.[99]

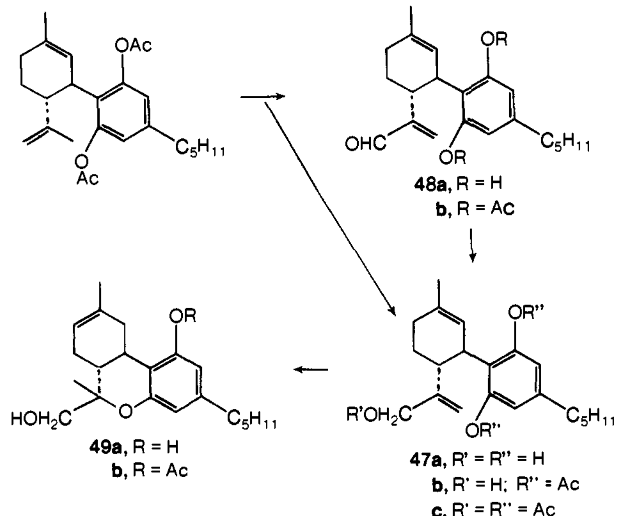
Nilsson et al.[100b] have reported that 10-hydroxycannabidiol (**47a**) is formed on incubation of cannabidiol with rat liver homogenate. The structure of this metabolite awaits confirmation.[101] In order to obtain a synthetic sample of **47a** for direct comparison with the metabolic product, Lander et al.[98] have oxidized cannabidiol diacetate with selenium dioxide to give a mixture of **47b** and **48b.** Reduction of the mixture followed by acetylation gave **47c**. The metabolite and the synthetic material, though apparently closely related, were not identical.

Treatment of **47**a with acid leads to 10-OH-$\Delta^6$-THC (**49**).[98] The same compound has also been prepared via a different route.[23]

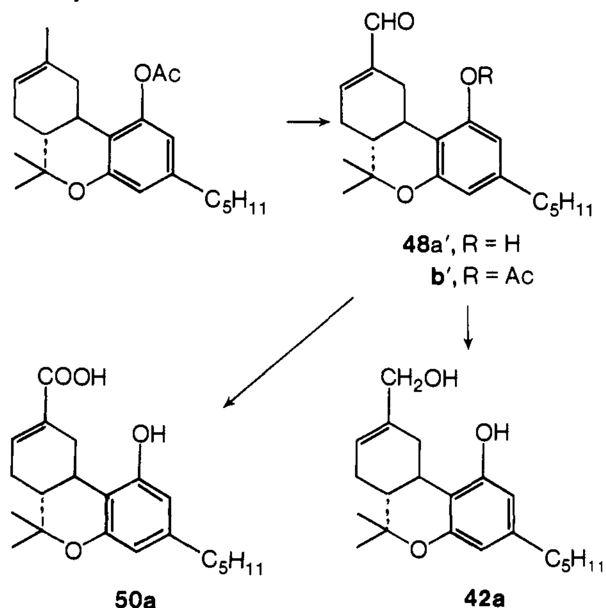
The most direct and facile route to 7-OH-$\Delta^6$-THC (**42a**) is by oxidation of $\Delta^6$-THC acetate with selenium dioxide in ethanol for a prolonged period.[102,103] The aldehyde **48b′** is obtained in 27–33% yields; reduction of **48b′** leads to **42a.**

The same route has been utilized[102] for the synthesis of $\Delta^6$-THC-7-oic acid (**50a**), a metabolite of 7-OH-$\Delta^6$-THC. The aldehyde **48b′** was oxidized in 59–63% yield to the methyl

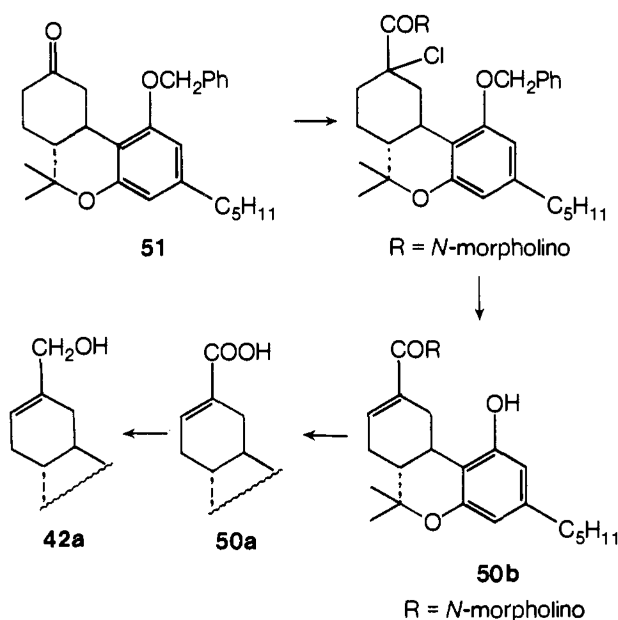



ester acetate of **50a** by reaction with manganese dioxide and sodium cyanide in methanol.

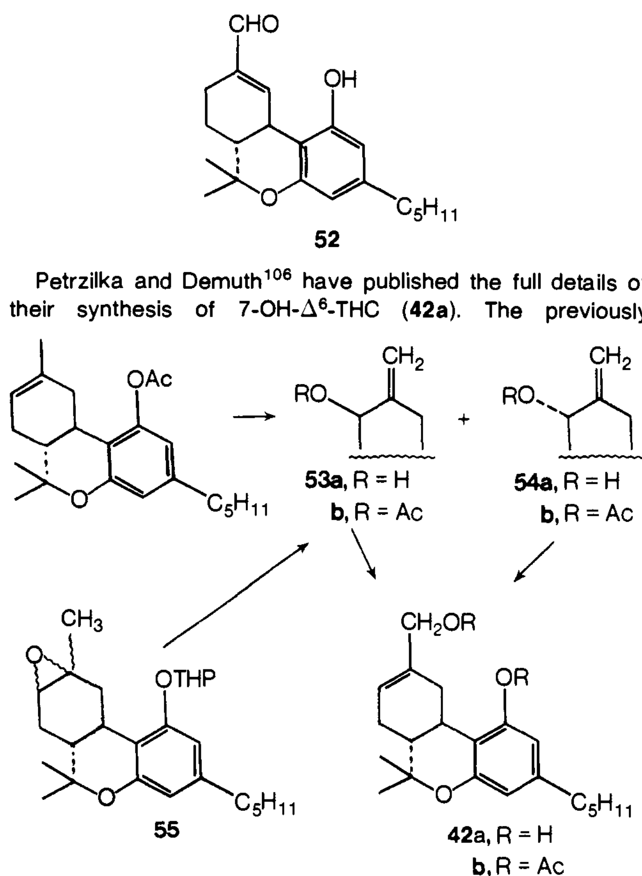



The metabolic acid **50a** has also been synthesized by Pitt et al.[94] by a somewhat longer route, whose main disadvantage is the difficulty of preparation of the starting material, 7-nor-1-oxohexahydrocannabinol *O*-benzyl ether (**51**). Treatment of this compound (as the morpholino enamine) with trichloracetic acid, followed by cleavage of the benzyl protecting group and formation of the phenoxide anion to effect




elimination of hydrochloric acid, gave only the $\Delta^6$-amide (**50b**). Despite precedent[104] no $\Delta^1$-amide, which would be derived from intramolecularly assisted elimination, was formed. Saponification of the $\Delta^6$-amide afforded the carboxylic acid **50a** which could be converted into 7-OH-$\Delta^6$-THC (**42a**).

The free acid **50a**, its methyl ester, and the aldehyde **48a'** have been tested in rhesus monkeys.[103] Neither **50a** nor its methyl ester showed any activity in doses up to 10 mg/kg. These observations contrast sharply with the activity recorded in the same, or other, tests for $\Delta^1$,$\Delta^6$-THC and their 7-hydroxy metabolites. However, the aldehyde **48a'** showed cannabis-like activity at 1 mg/kg. This observation may be of biological relevance as 7-oxo-$\Delta^1$-THC (**52**) has recently been shown[105] to be a metabolite obtained on incubation of $\Delta^1$-THC with rat liver microsomes.




Petrzilka and Demuth[106] have published the full details of their synthesis of 7-OH-$\Delta^6$-THC (**42a**). The previously

known[107] 6β-OH-$\Delta^{1(7)}$-THC (**53**a) and 6α-OH-$\Delta^{1(7)}$-THC (**54a**) were now prepared by a photochemical ene reaction of $\Delta^6$-THC acetate with oxygen and a sensitizer followed by reduction of the mixture of hydroperoxides. The same allylic alcohols were also prepared by treatment of the two epimeric 1,6-epoxides[108,109] **55** (as their tetrahydropyranyl ethers) with base followed by removal of the protecting group. On heating at 290° the diacetates **53b** and **54b** underwent allylic rearrangement to 7-OH-$\Delta^6$-THC diacetate (**42b**). The yields of these procedures are an improvement over those reported previously[107] for the same rearrangements under different experimental conditions. It may be more than a chance observation that in the thermal rearrangement[106] the compound with the equatorial acetoxyl group (**54**, acetate) gives a higher yield than the one with the axial acetoxyl group (**53** acetate). In the acid-catalyzed rearrangements[107] of the free alcohols **54** and **53**, the reverse holds true.

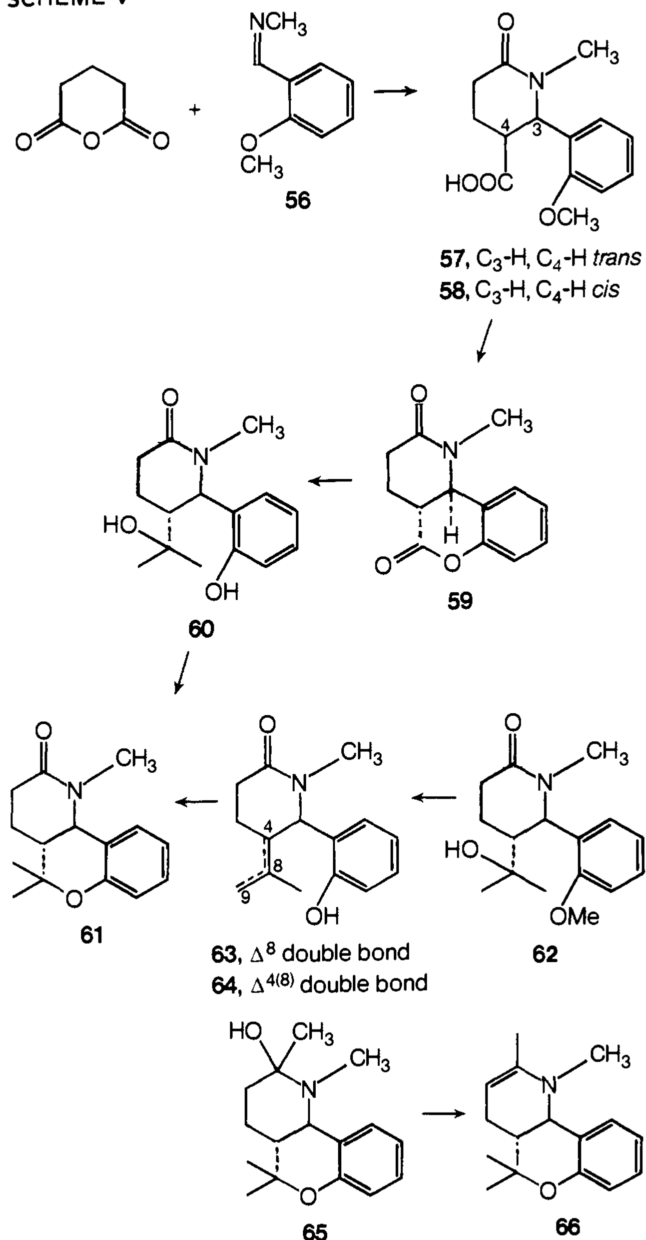
### *3. Synthesis of Tetrahydrocannabinol Analogs. New Cannabinoid Transformations*

Cushman and Castagnoli[110] have published a novel approach to the synthesis of nitrogen-containing analogs of the THC's. In contrast to previously reported[111] methods leading to other nitrogen analogs, the present route preserves the integrity of the trans ring fusion (as in the natural $\Delta^1$-THC and $\Delta^6$-THC).

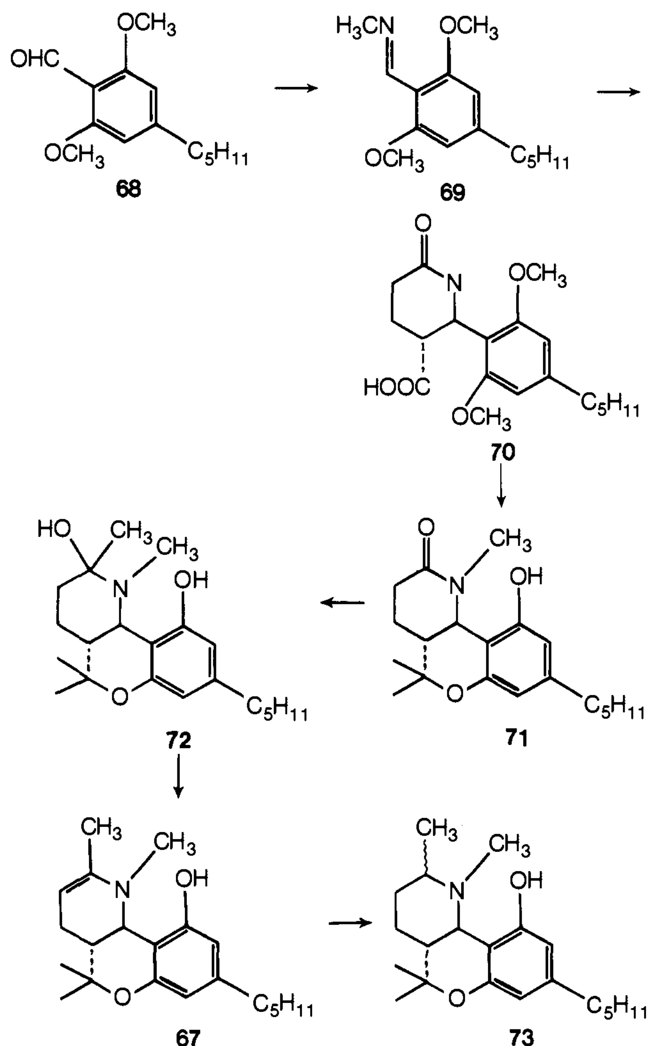
The condensation of *o*-anisylidenemethylamine (**56**) and glutaric anhydride gave the trans and cis piperidones **57** and **58**. Subsequent O-demethylation and cyclodehydration of **57** led to the lactone **59** which was converted into the *gem*-dimethyl alcohol **60**. Cyclodehydration of the latter gave the tricyclic intermediate **61**. The same compound was obtained also via the tertiary alcohol **62** and the olefins **63** and **64**. Stereochemical assignments were made by NMR spectroscopy. The key intermediate **61** was used to make the corresponding carbinolamine (**65**), enamine (**66**), and related compounds (Scheme V).

The above synthesis has been extended[112] to the preparation of the $\Delta^6$-THC analog **67** in which the methylene grouping at 2 of $\Delta^6$-THC has been exchanged with a methylamino group. Structurally, this is the closest nitrogen analog of a natural tetrahydrocannabinol so far prepared. The synthesis of **67** follows that of the model compound **66**. The known aldehyde **68** was converted into the Schiff base **69**, which again, on condensation with glutaric anhydride gave **70**. This acid, by minor modifications of the previously described model synthesis, gave the amide **71** from which, via the unstable tertiary alcohol **72**, the desired enamine **67** was obtained. Reduction led to the diastereoisomeric mixture **73** which was shown to possess both antidepressant and anticonvulsant activity (Scheme VI).

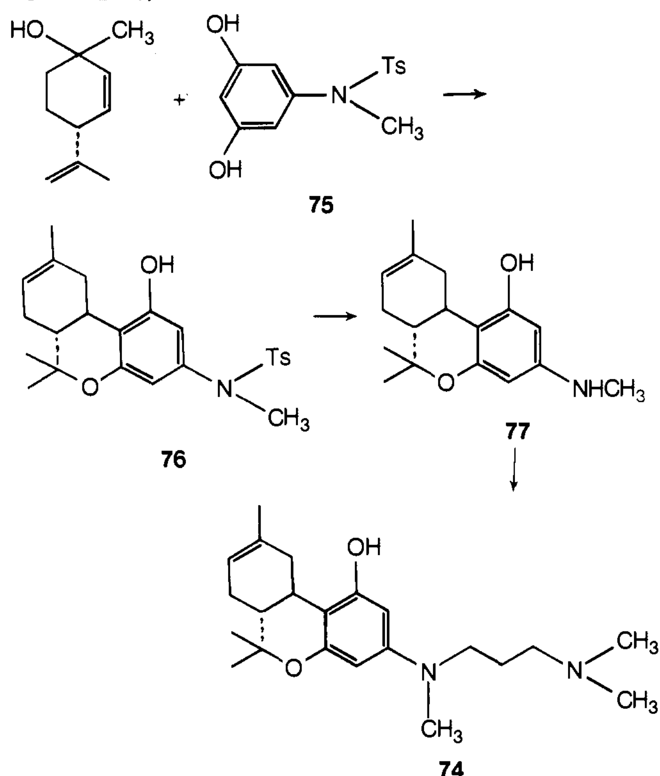
SCHEME V




SCHEME VI

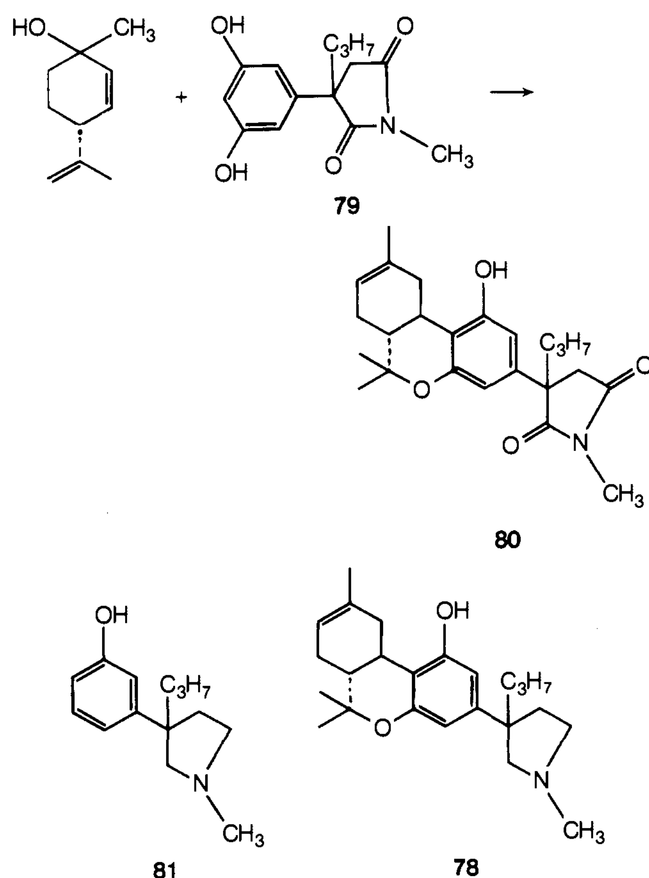


SCHEME VII




Petrzilka and Lusuardi[113] have reported the synthesis of a $\Delta^6$-THC analog (**74**) in which the pentyl side chain has been replaced by a methyl(3-dimethylaminopropyl)amino side chain. 5-Methylaminoresorcinol tosylate (**75**) was condensed with *trans-p*-2,8-menthadien-1-ol to give **76**, which on reduction in sodium in liquid ammonia led to the amine **77**. The lithium salt of the amine reacted with 3-dimethylamino-1-propyl chloride to give the diamine **74** (Scheme VII).

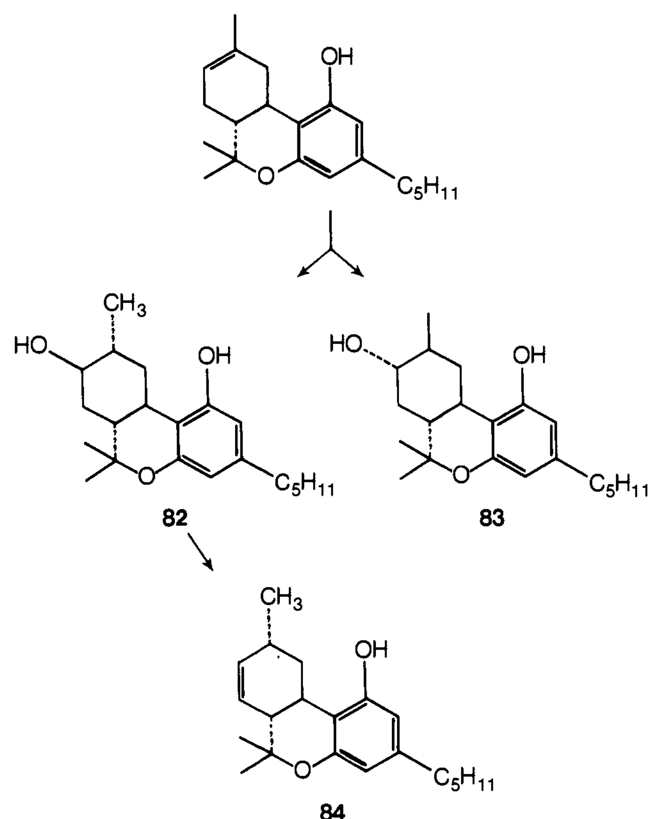
SCHEME VIII




A further $\Delta^6$-THC analog, with a side chain containing a pyrrolidine ring (**78**), was prepared by a condensation of $\alpha$-(3,5-dihydroxyphenyl)-$\alpha$-propyl-*N*-methylsuccinimide (**79**) with *p*-2,8-menthadien-1-ol, followed by reduction of the cannabinoid–succinimide derivative **80** so obtained (Scheme VIII).[114] The resorcinol derivative **79** was prepared by a standard route previously followed[115] in the synthesis of the analgesic profadol **81**. The pharmacological activity of profadol apparently was the raison d'être for the above syntheses. However, no biological data are reported for compounds **74–80.**

The reactions leading from $\Delta^6$-THC to the 6$\beta$- and 6$\alpha$-hydroxy-hexahydrocannabinols (**82** and **83**) and to $\Delta^5$-tetrahydrocannabinol (**84**), previously mentioned,[116] have now been described in detail.[117] Hydroboration of $\Delta^6$-THC leads to **82** and **83**; tosylation of **82** followed by elimination with potassium *tert*-amylate gave $\Delta^5$-THC (**84**) (Scheme IX).

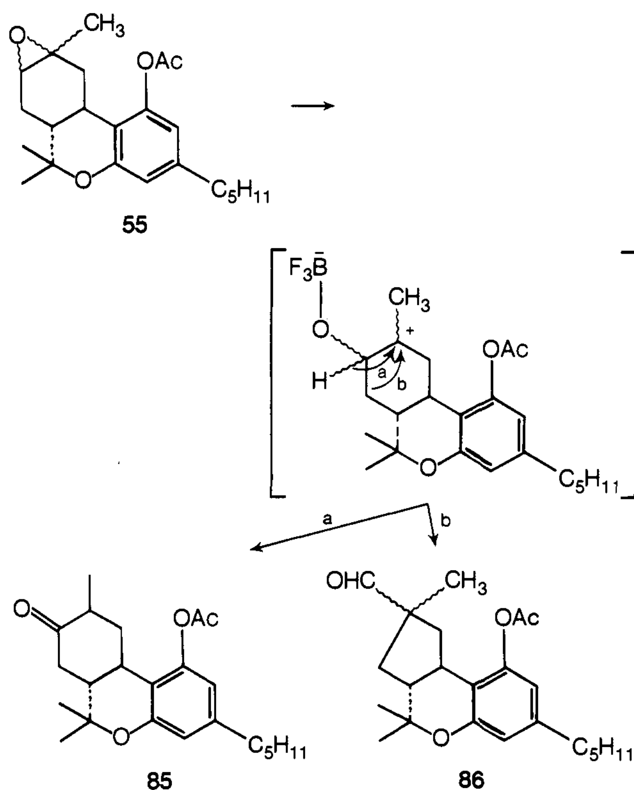
SCHEME IX




A new cannabinoid rearrangement has been described.[117] 1,6-Epoxyhexahydrocannabinol acetate (**55**) on treatment with boron trifluoride in benzene gave 6-oxohexahydrocannabinol acetate **85**, mp 57°, and the aldehyde **86**, mp 63° (Scheme X). The structure of **85** was determined by attempted base equilibration of the ketone. The stability of **85** to these conditions established the equatorial nature of the C-1 methyl group. The formation of the aldehyde **86** takes place by a C-5/C-6 bond break leading to ring contraction. The stereochemistry of **86** at C-1 based on mechanistic consideration in the original paper is not necessarily correct as the starting material, the epoxide **55**, may consist of a mixture of isomers.[108]

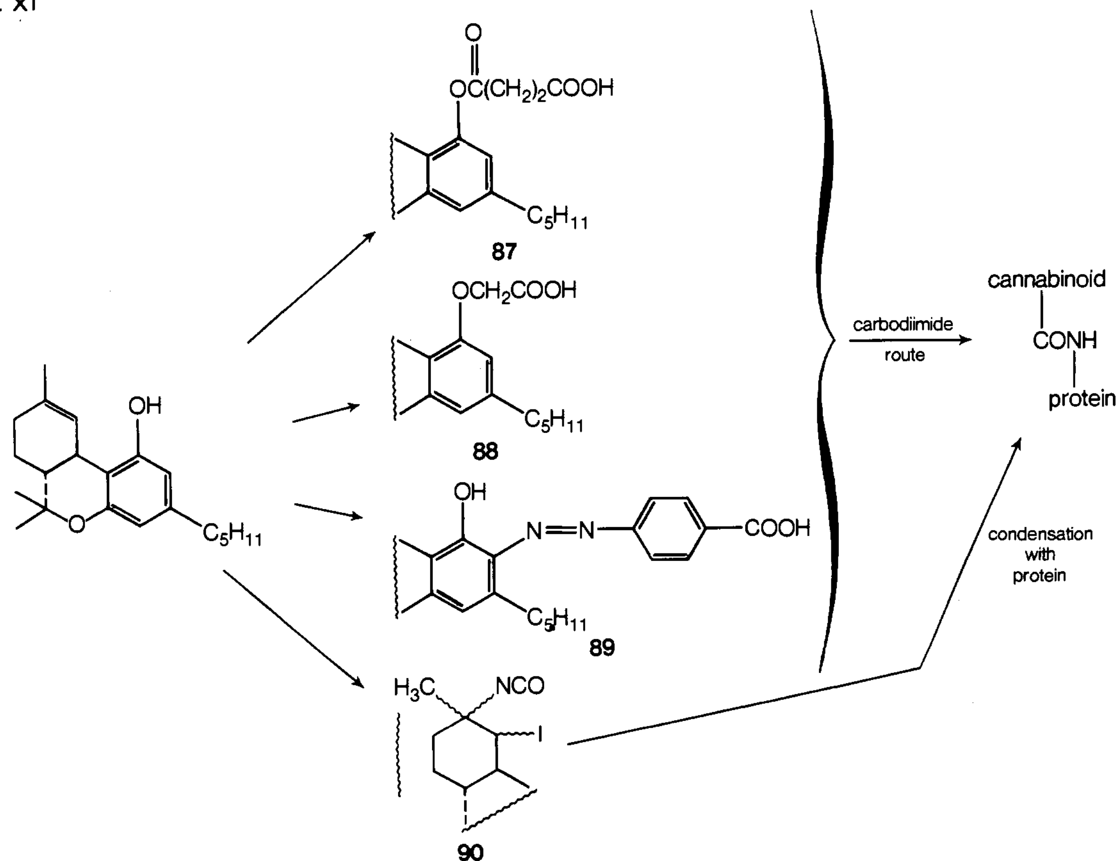
$\Delta^1$-*THC–Protein Conjugates* (see also section III.D.3). Antibodies specific for small molecules (haptens) can be produced by immunization with conjugates consisting of given hapten covalently linked to carrier molecules such as proteins, polypeptides, etc. Tsui et al.[118] have reported several such conjugates of $\Delta^1$-THC. Chemical moieties capable of

SCHEME X




reacting with a protein were attached at several positions of the $\Delta^1$-THC molecule. As antibodies produced by immunization with a conjugate may not be configurationally complementary to the complete hapten (but mainly to those parts of the hapten which are not modified by the addition of the bulky carrier molecule) different parts of the $\Delta^1$-THC molecule were tried for conjugation.

SCHEME XI



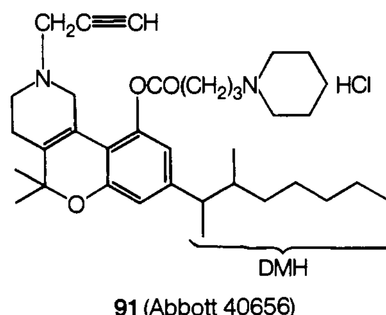

$\Delta^1$-THC was converted into the hemisuccinate ester **87**, the carboxymethyl ether **88**, the 4′-*p*-azobenzoic acid (**89**), and the 2-iodo-1-isocyanate (**90**) by standard methods (Scheme XI). The first three compounds, each containing a free carboxyl group, were coupled directly to proteins with a water-soluble carbodiimide. The THC isocyanate **90** was conjugated directly to proteins. All four haptens were conjugated to three proteins: porcine $\gamma$-globulin, sheep $\gamma$-globulin, and human serum albumin. By analysis it was determined that 9–36 THC residues were coupled to the three proteins.

Active immunization with the $\Delta^1$-THC–*O*-hemisuccinate–porcine $\gamma$-globulin was found to neutralize the depressant effect of $\Delta^1$-THC on the motor activity of rats.

$\Delta^1$-THC-4′-*p*-azobenzoic acid (**89**) has also been prepared by the same method by Grant et al.[119] It was used in the development of an immuno assay for THC.

## C. New Data on Structure–Activity Relationships in the Cannabinoid Series

Although a considerable amount of drug-oriented cannabinoid research has apparently been done in numerous pharmaceutical firms, very few publications have as yet appeared. At least one compound, the water-soluble **91**, is undergoing clinical trails.[120] It has sedative–hypnotic and an-

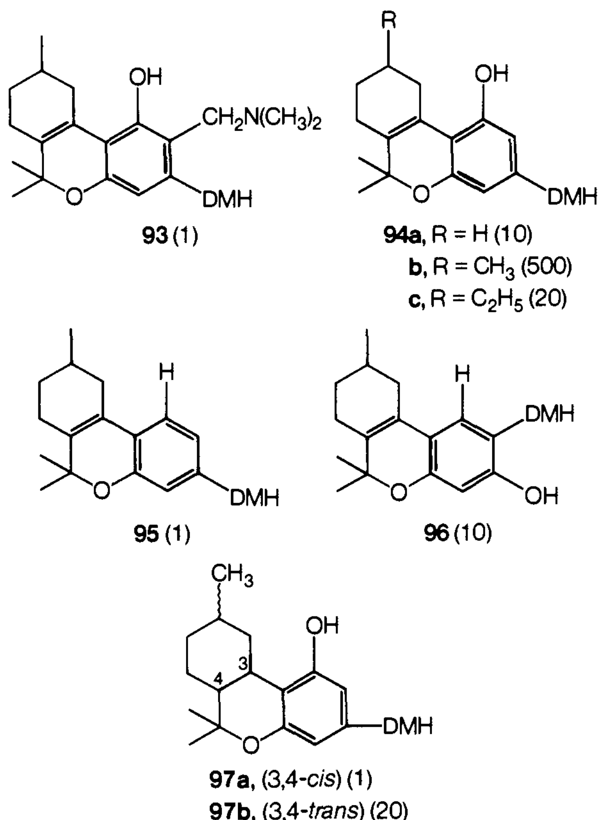



**91** (Abbott 40656)

algesic activity. It is ca. six times more active than codein in the hot-plate test for analgesia; in other tests (acetic acid writhing in mice) it is as active as codein.

Loev and coworkers[121] have reexamined in rats the effects of structural modifications on the central nervous system (CNS) potency of certain cannabinoids, mostly $\Delta^3$-THC derivatives. The observed structure–activity relationship correlations differ significantly from those originally reported[122] by Adams, Loewe, and Todd in the dog and rabbit. The rat was chosen as a test animal since this species, rather than the dog or the rabbit, is most frequently used for studies of CNS activities and for many other pharmacological evaluations. A number of new cannabinoids, mostly with a dimethylheptyl (DMH) side chain prepared by essentially standard methods, were also tested.

Some of the pharmacological results obtained are given in Table I. A few additional results are indicated below formulas **93** to **96**. The activity, relative to that of **92** (see footnote *b* in Table I) is indicated in parentheses. The novel (or contrary to previous[122]) results are as follows:




1. Elongation of the side chain (above *n*-hexyl) enhances, rather than decreases activity.

2. While the 1,2-dimethylheptyl side chain can cause a sharp increase in potency, the most active compound is (again contrary to previous results) the 1,1-dimethylheptyl isomer.

One can possibly rationalize this result on the basis of the known, major metabolic pathway which involves oxidation at the C-1″ position, which in this case is blocked.

3. Substitution of the alkyl side chain by an alkoxy one (in which the alkyl is 1-methylhexyl) results in a compound five times as potent as the natural $\Delta^1$-THC. This was unexpected in view of the insignificant activities for the *n*-alkyl ethers reported previously.[122]

4. The C-1 methyl group is not essential for activity; however, exchange by a hydrogen (**94a**) or an ethyl group (**94c**) reduced the activity ca. 25–50 times (as compared to **94b**).

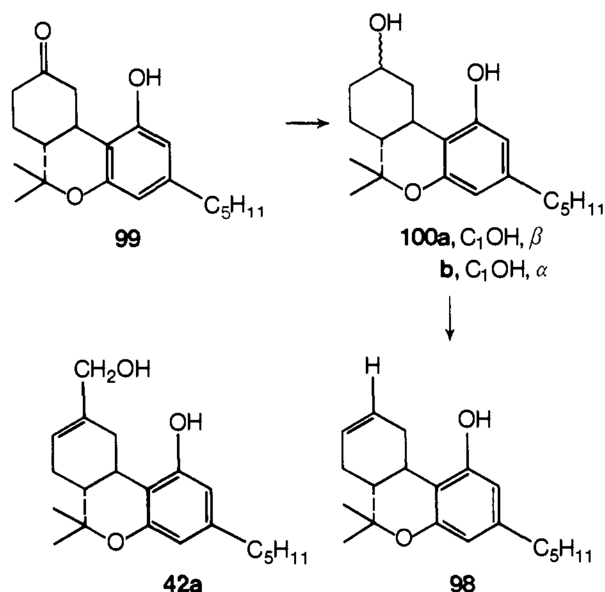
5. Surprisingly, exchange of the free phenolic group with a hydrogen does not completely eliminate activity.

TABLE I.[a] Activity of Various Cannabinoids Tested in Rats[b]

| Compound tested | Relative CNS activity |
|---|---|
| $R = C_5H_{11}$ | 0.5 |
| **92**, $R = C_6H_{13}$ | 1 |
| $R = C_9H_{19}(n)$ | 4 |
| $R = CH(CH_3)C_6H_{13}$ | 100 |
| **94b**, R = DMH | 500 |
| $R = C(CH_3)_2C_6H_{13}$ | 1000 |
| $R = C(CH_3){=}C(CH_3)C_5H_{11}$ | 500 |
| $R = OCH(CH_3)C_5H_{11}$ | 10 |
| $\Delta^1$-THC | 2 |

[a] This table is a abbreviated form of the one published which lists 35 compounds. [b] Quoted as relative values compared with **92** taken as the standard.

The observation that the 7-methyl group is not an absolute requirement for activity has been confirmed by Wilson and May,[123a] who prepared 7-nor-THC (**98**). 7-Nor-1-oxohexahydrocannabinol (**99**), on reduction with sodium borohydride, gave 7-nor-1-hydroxyhexahydrocannabinol (**100a,b**) which on subsequent dehydration gave **98**. This norcannabinoid caused ataxia in dogs at the same minimal effective dose as $\Delta^6$-THC. Compound **100a**, in which the C-1 hydroxyl group is equatorial, was found[123b] to be a potent analgetic, while **100b** in which the C-1 hydroxyl group is axial was found to be inactive. A related observation has already been recorded[124]: hexahydrocannabinol in which the methyl group at C-1 is equatorial is much more potent (in CNS effects) than the axial isomer.

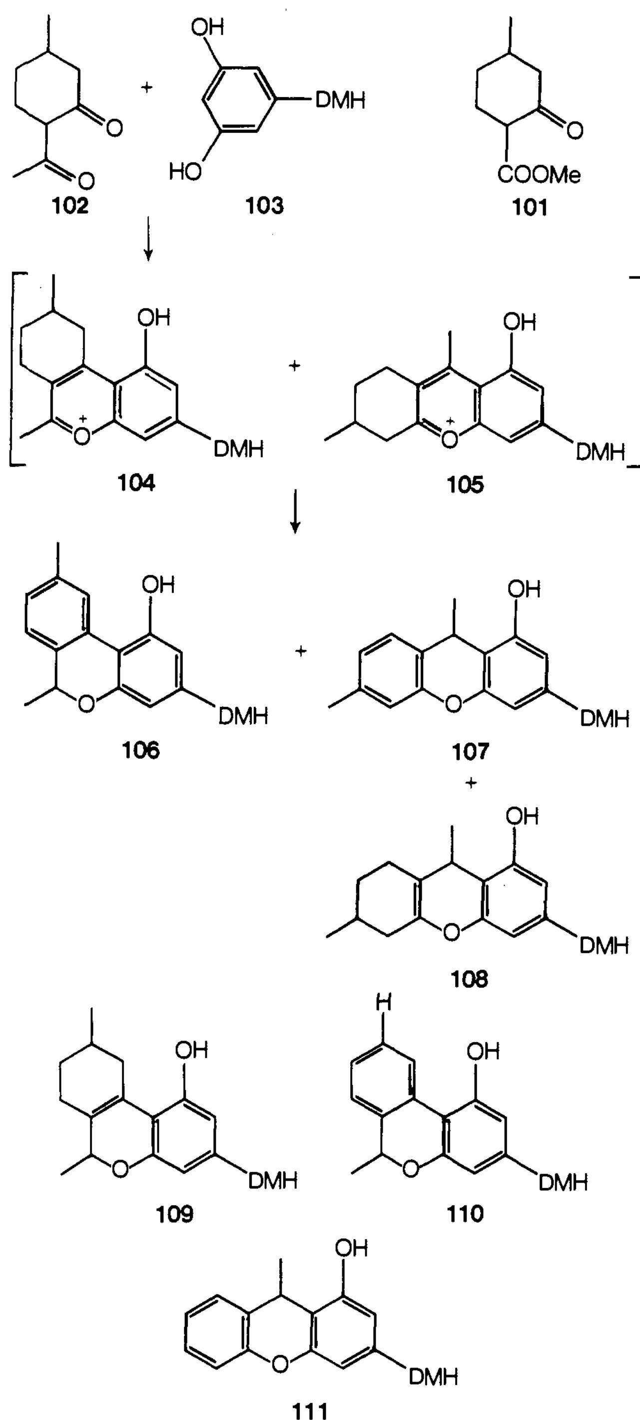



7-Hydroxy-$\Delta^6$-THC (**42a**) has analgesic potency comparable to that of morphine.[123b] Its activity is antagonized by naloxone, a morphine antagonist. Surprisingly **98** has no analgesic activity. This seems to be the first case in which it has been possible to separate the ataxic and analgesic properties in the cannabinoid series.

The norcannabinoid **98** (as the methyl ether) has independently been prepared[125] by decarboxylation of $\Delta^6$-THC-7-oic acid (**50a**) methyl ether by boiling for 15 min in quinoline at 237° in the presence of copper chromite.

Bender et al.[126] have prepared some 9-norcannabinoids

SCHEME XII




and related xanthenes using a modification of th original $\Delta^3$-THC synthesis of Adams and Todd.[1] Instead of the ester **101** they employed the diketone **102** which on acid-catalyzed condensation with 5-DMH-resorcinol (**103**) gave a mixture of what were apparently the pyrylium salts **104** and **105.** Heating the mixture of pyrylium salts caused a disproportionation giving 9-norcannabinol-DMH (**106**), the xanthene (**107**), and the tetrahydroxanthene (**108**) (Scheme XII). The disproportionation is selective: only the tetrahydroxanthene **108** is produced, while the 9-nortetrahydrocannabinol-DMH homolog **109** is not formed. The latter compound can be produced by sodium cyanoborohydride reduction of the mixture of pyrylium salts. Several related compounds were also prepared using the above procedures.

Compounds **107** and **109** as well as the bis-nor analogs **110** and **111** showed CNS effects which were qualitatively and quantitatively similar to those observed with natural $\Delta^1$-THC. As the $\Delta^3$-THC–DMH homolog (**94b**) is ca. 250 times more active than $\Delta^1$-THC, the above results really mean that exchange of a 9-methyl group with a hydrogen atom reduces the activity.

Houry et al.[127] have described a new series of active cannabinoids in which the anomatic moiety is attached to the terpene ring at C-2, rather than at C-3 as in the natural constituents. Michael addition of olivetol to carvone (**112**) gave a mixture of three isomers (**113a**, **114a**, and **115a**) which were converted by standard methods into the respective alcohols (**116a**, **117a** and **118a**), olefins (**119a**, **120a** and **121a**), and saturated analogs (**122a** and **123a**). The stereochemistry at the various chiral centers was determined by conformational analysis and by NMR.

Reaction of olivetol with pinene or limonene leads to **123a** and to **124a**, a previously reported isocannabinoid.[128,129] The latter compound is apparently the result of attack by the aromatic moiety at C-3. One can assume[130,131] that this is made possible by an isomerization of the $\Delta^8$ double bond to the $\Delta^3$ position.

Most of the respective DMH analogs were also prepared.

When tested in rats for decrease in motor activity, hypothermia, and several related effects, compounds **123a**, **123b**, **116a**, and **117b** were found[127] to be 5–50 times more active than $\Delta^1$-THC. Of particular interest is the observation that **123a** and **116a** (which belong to the pentyl side chain series) are more active than $\Delta^1$-THC.

The results summarized above also emphasize that cannabinoid-type activity is not confined to "flat" molecules; the compounds described by Houry et al.[127] cannot take up such a conformation.

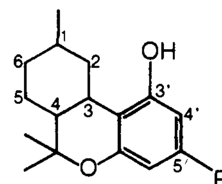
Several additional cannabinoids have been tested for cannabis-type activity in the rhesus monkey.[132] The test method has been described previously.[133] 7-Hydroxycannabinol (**125**), a cannabinol metabolite is ca. 50 times less active than $\Delta^6$-THC. Surprisingly, the tosylate of $\Delta^6$-THC is only very slightly active.

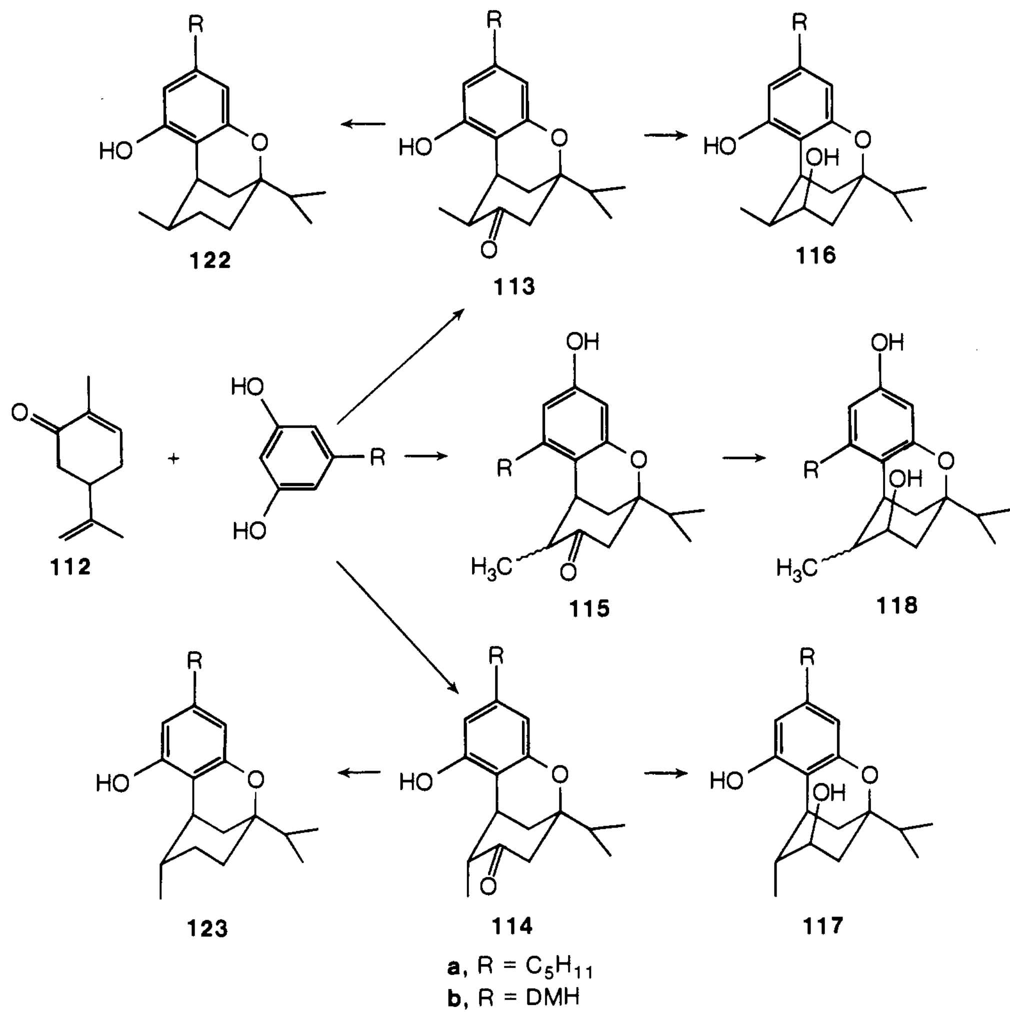
Cannabielsoin (**126**) caused ataxia and increase in body temperature in mice, though at a somewhat high dose (50 mg/kg).[91] No comparison with $\Delta^1$-THC was reported. Assuming that the experimental conditions of the mouse test were close to those reported by Pertwee,[134] cannabielsoin seems to be ca. five times less active than $\Delta^1$-THC. Dehydrocannabielsoin (**127**) has been tested in rhesus monkeys.[132] It was found to be inactive up to 10 mg/kg. It is difficult to reconcile these results.

Leander has synthesized[135] a number of $\Delta^6$-THC derivatives hydroxylated on the side chain (**128**, **129**, **130**, and **131**). The first three are as active in rhesus monkeys[136] as $\Delta^6$-THC; the last one which had been prepared and tested earlier is essentially inactive.[137] From these observations, as well as from the well-known influence of methyl substituents on the C-1″ benzyl position (see above), it is obvious that this position is intimately involved with cannabinoid activity.

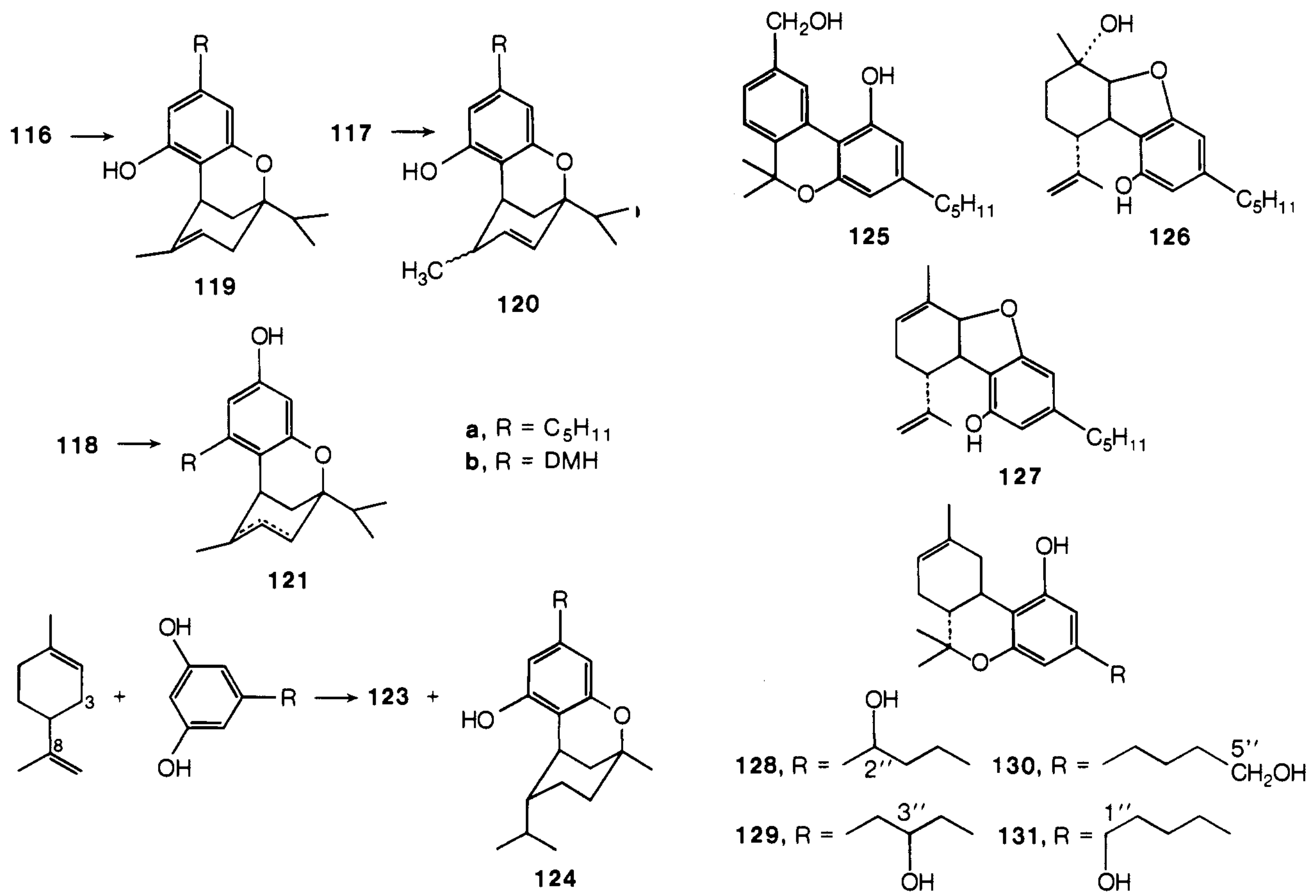
What are then the requirements for cannabinoid ("psychotomimetic") activity? In conjunction with the results summarized[122] previously, one can tentatively suggest the following:

1. A benzopyran (or a xanthene) type of structure with a hydroxyl group at the 3′ aromatic position and an alkyl group on the 5′ aromatic position seems to be requirement. Open-





R
HO
O
OH
H3C
122
113
116
112
115
118
123
114
117
a, R = C5H11
b, R = DMH

116
117
118
119
120
121
a, R = C5H11
b, R = DMH
CH2OH
OH
C5H11
125
126
127
123
124
128, R =
2''
129, R =
3''
130, R =
5''
CH2OH
131, R =
1''

ing of the pyran ring leads to complete loss of activity.

2. The aromatic hydroxyl group has to be free or esterified. Blocking of the hydroxyl group as an ether inactivates the molecule.

3. When alkyl groups are substituted on the phenolic ring at C-4′, activity is retained. Substitution at C-6′ eliminates activity. Electronegative groups such as carboxyl, carbomethoxyl, and acetyl at either C-4′ or C-6′ eliminate activity.

4. A certain length of the aromatic side chain is a requirement for activity. Branching of the side chain may lead to considerable increase in potency. A 1,2-dimethylheptyl or a 1,1-dimethylheptyl side chain seems to be best.

5. Not all the theoretically possible THC's are active. Thus $\Delta^1$- and $\Delta^6$-THC are active in the 3*R*, 4*R* series only; $\Delta^5$-THC and $\Delta^7$-THC are inactive; $\Delta^3$-THC is active; $\Delta^1$-3,4-*cis*-THC is inactive.

6. The terpenoid and pyran rings may be modified considerably. These modifications do not seem to follow a regular pattern, and even tentative rules cannot yet be put forward.

## *III. Analytical Aspects of Cannabis Chemistry*

### A. Introductory Comments

The analysis for cannabinoids has raised problems for scientists of widely differing fields. Well-established chromatographic methods for analysis and isolation of the many cannabinoid and noncannabinoid constituents of *Cannabis sativa* have served the natural product chemist well, but until very recently pharmacologists, toxicologists, and forensic scientists have been severely handicapped by the lack of sufficiently sensitive and specific techniques. Recent developments have changed this situation, and we may expect to see further advances in these fields in the near future. Even the "well-established chromatographic methods" of thin layer chromatography (TLC), gas chromatography (GLC), and column chromatography have undergone considerable refinement, and the introduction of mass spectrometry (MS) has revolutionized recent investigations of the minor constituents of cannabis.

The literature up to the middle of 1972 has already been comprehensively surveyed,[138] and this chapter is mainly intended to cover analytical advances published since. Section III.B will discuss the analytical techniques in detail, and subsequent sections will be confined to a discussion of the relative merits of the techniques as applied to specific problems.

### B. Analytical Techniques

#### *1. Isolation of Cannabinoids*

The isolation of cannabis constituents has been discussed earlier in this review (section II). In addition to the chromatographic methods already mentioned, silica gel–silver nitrate has found application for more difficult cannabinoid separations. Preparative layer chromatography (PLC) separations using silica gel G/silver nitrate (3:1) achieve very good resolutions for smaller quantities of cannabinoids[139] and silica gel/silver nitrate/calcium sulfate (6:2:1) has been used by the same workers for column chromatography of larger quantities.[140] In the latter case the calcium sulfate binder may be omitted and column chromatography on 20% silver nitrate/silica gel has been used for a partial separation of THC isomers.[141] We have used 25% silver nitrate on silica gel for a considerably abbreviated isolation of $\Delta^1$-THC, cannabinol (CBN), and cannabidiol (CBD). In this case careful gradient elution (between light petroleum and 40% ethyl acetate in light petroleum) of the light petroleum extracts of cannabis or hashish yields pure THC, CBN, and CBD although some fractions intermediate between THC and CBN generally need rechromatography.

The use of accelerated microparticulate gel chromatography for the separation of milligram amounts of cannabinoids is described in section III.B.7.

When cannabinoids or their metabolites are being isolated from biological fluids, a different approach is often necessary. Burstein et al.[142] and later Melikian, et al.[143] use ion exchange (XAD-2) for a preliminary separation of the metabolites from inorganic salts and other highly polar material found in urine. In Burstein's method, a chloroform extract of the eluate is then chromatographed on Sephadex G-15 and DEAE Sephadex as a further purification step, and the final separation of the hydroxylated metabolites is achieved by TLC.[142] Agurell et al. employ Sephadex LH 20[144] for a preliminary separation of cannabinoids from both blood[145] and urine[146] extracts prior to GLC–MS.

#### *2. Thin Layer Chromatography*

Good resolution of cannabinoids by TLC is quite difficult because of their structural similarity, and only two types of thin layer systems have been found to give good results. The first of these is based on modification of the absorbent properties of silica gel by the presence of the bases (dimethylformamide or diethylamine) and elution with nonpolar solvents (cyclohexane for the former and toluene for the latter)[138,147] or pyridine with hexane–methanol (18:75:7).[148] Silver nitrate has also been used to modify cannabinoid separations by silica gel to good effect. In this case silver nitrate can complex with molecular double bonds, and a very good resolution can be achieved when the chromatogram is eluted with a solvent such as toluene.[149–151]

Still the most widely used method for detection of cannabinoids on these thin layer chromatograms is spraying with a freshly prepared solution of di-*o*-anisidinetetrazolium chloride (Fast Blue Salt B) which offers both excellent sensitivity of detection (down to approximately 50 ng) and different color reactions for different components.[152] The need for even more sensitive TLC techniques led to the method of Forrest et al.[153] in which the cannabinoids are converted to 1-dimethylaminonaphthalenesulfonates and separated by TLC. The compounds may be detected by virtue of their strong fluorescence under ultraviolet light, down to levels of 0.5 ng.[153–155] Later work by Just, Werner, and Weichmann extended this method to the analysis of blood and saliva.[156,157]

The use of TLC can be extended to quantitative work, the method chosen depending on how the cannabinoids are visualized on the chromatogram. If Fast Blue Salt B is used as a spray, individual cannabinoids may be quantified by scraping off the color zones and measuring them spectrophotometrically, or more simply, by photodensitometric scanning of the plate.[158,19]

For biological investigations in particular, the use of radioactive labeled compounds has become relatively popular since radioactive scanning provides a very sensitive method for quantification of cannabinoids on thin layer chromatograms.[159] Here the mode of scanning also can be a limiting factor in the resolution of cannabinoids and considerable care must be taken in choice and excercise of the chosen method. These methods will be discussed in detail in section III.D.

TLC techniques have also been applied to the problem of isolating cannabinoids. Thus, the silica gel from a particular spot may be removed from a thin layer chromatogram and the compound eluted by a polar solvent and subjected to further characterization by GLC, MS, or uv. However, if the compound is present in only microgram amounts, it can be difficult to remove from the silica gel[160] and may even require elution with solvents as polar as hot MeOH.[161] With very small amounts of compounds, the number of impurities which can be contributed by the silica gel makes this method im-

practicable for a clean-up technique.[162]

Preparative layer chromatography (PLC) employing thicker layers of silica gel (1 mm) and elution systems similar to those used for TLC is a useful technique for larger scale separations.

### 3. Gas Chromatography

Gas chromatography is the method of choice for rapid qualitative and quantitative identifications. A large variety of stationary phases have been found to provide excellent separations of the cannabinoids on packed columns,[163] and the use of capilliary columns has been found to improve separations by an order of magnitude.[164] In the few reports on capilliary column separation of cannabinoids, both glass[160,165] and stainless steel columns[67] have been used, and the separations achieved were little short of spectacular.[165] It is to be expected that the use of capilliary columns[166] will expand very rapidly over the next few years.

The development of more sensitive gas chromatographic techniques has also been the subject of considerable interest. Flame ionization detection, normally used with GLC, is reported to give a maximum sensitivity of approximately 50 ng.[167] Sawa et al. have found that formation of trimethylsilyl derivatives increases the maximum sensitivity of detection to about 10 ng.[168] This derivatization has also been recorded to aid separation of THC isomers,[169] facilitate the quantification of cannabinoidic acids, which would otherwise decarboxylate under GLC conditions[20,170] and allow total resolution of cannabidiol and cannabichromene.[171]

The use of electron capture detection for suitably derivatized cannabinoids has provided big improvements in sensitivity of detection. Nielson et al. report that the use of chloroacetyl derivatives gives maximum sensitivities of approximately 0.04 ng,[172] and later work successfully applied this to the analysis of urines.[173] Garret and Hunt demonstrate a maximum sensitivity of detection of approximately 5 pg for $\Delta^1$-THC pentafluorobenzoate,[174] while 1 pg of $\Delta^1$-THC heptafluorobutyrate can be detected when a capilliary column and low volume coaxial electron capture detector are used.[160] It has been shown, however, that pentafluorobenzoates do give a greater sensitivity of detection than other phenol derivatives under identical conditions,[160,175] and, since these compounds are considerably more stable than polyfluoropropionates and -butyrates, their use as an alternative to heptafluorobutyrates in Fenimore's method[160] should still be considered. Working with these small amounts of compounds in biological extracts presents considerable problems for purification which Fenimore et al. managed to solve using a dual column system. The derivatized extract is first injected onto a packed column, and at the appropriate retention time a small fraction of the eluent gas is trapped and subjected to further GLC on a capilliary column.[160] This appears to be an effective and relatively rapid "clean-up" procedure which should find application in many other toxicological and pharmacological problems.

Quite a different approach to this problem was taken by McCallum, who developed a method of analysis for phenols involving GLC with flame photometric detection of their phosphate esters.[176] Those who have worked with electron capture detection will be aware that it has certain disadvantages. First, sensitivity of detection can fluctuate very rapidly and markedly. Second, in biological samples there are present a large number of electron-capturing compounds which may interfere drastically with analyses of picogram amounts. The flame photometric detection of cannabinoids gives a maximum sensitivity of 0.5 ng/injection, which is not as good as electron capture detection. On the other hand, perfectly stable baseline and sensitivity is obtained, and detection is so specific that preliminary "clean-up" procedures are unnecessary and considerable shortening of analysis time is the result.[176]

The combination of GLC–mass spectrometry (GLC–MS) is the final alternative for sensitive detection, and it has been successfully applied to natural product work and the analysis of biological fluids. The technique is discussed under the heading Mass Spectrometry.

### 4. Mass Spectrometry

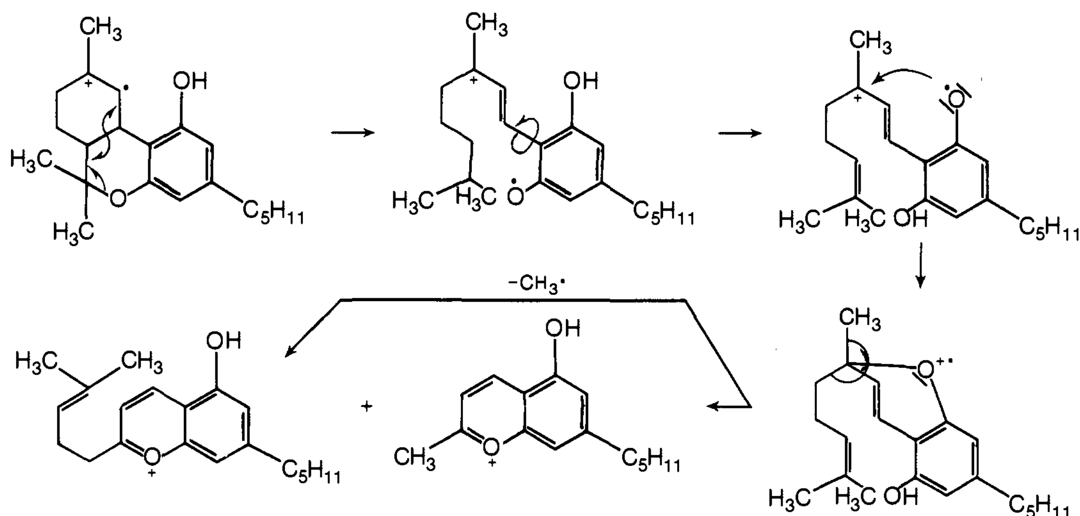
The mass spectra of the common cannabinoids were first studied by Budzikiewicz et al.[177] and Claussen, Fehlhaber, and others.[178,179]

The spectra of these compounds appear to be relatively simple. The most general patterns involve fragments corresponding to retro-Diels–Alder fission of the alkene ring (e.g., with CBD and $\Delta^1$- and $\Delta^6$-THC's), loss of a methyl group from the pyran ring,[177–179] and a smaller contribution from fragmentation of the pentyl side chain.[31] The individual examples are given in each of the three quoted papers.

Although it was clear that $\Delta^6$-THC can decompose via a retro-Diels–Alder reaction, it was necessary for Budzikiewicz to postulate that $\Delta^1$-THC isomerizes to $\Delta^6$-THC to allow it to give the same fragments (246, 231) by this mechanism.[177] However, recent work by Vree and Nibbering found that methylation of the phenolic group in $\Delta^1$-THC greatly reduces mass fragment intensities corresponding to this fragmentation pathway, and they therefore proposed that the 246 and 231 fragments mainly arise from a phenolic proton transfer to the $\Delta^1$ double bond rather than a simple double-bond migration.[180] Still later work by Burgers et al., who examined the spectra of $\Delta^1$-THC with the phenolic proton replaced by deuterium, observed retention of this label in the formation of the *m/e* 231 ion,[181] indicating that the mechanism proposed by Vree and Nibbering[180] is incorrect. As an alternative mechanism it is proposed that the fragmentation can proceed via an iso-THC type structure (see Scheme XIII), and this has been borne out by experiments involving deuterium labeling at C-7. In the case of $\Delta^6$-THC this mechanism is still a minor one compared to the retro-Diels–Alder reaction for the production of the 231 fragment. For $\Delta^1$-THC, the initial $\beta$-hydrogen transfer is unnecessary, and this mechanism becomes the major fragmentation route.[181]

Given these fragmentation patterns, it is not surprising that the naturally occurring homologues, differing only in the side chain, have some spectral peaks in common with the pentyl compounds. However, even the pentyl side-chain cannabinoids have many mass spectral peaks in common with each other,[30b,31] and it is therefore important to exercise caution when interpreting spectra if there is a possibility that the sample is not pure. Similarly, the use of high resolution mass spectrometry for the determination of molecular weight requires pure compound else the wrong molecular ion might be determined. Purification techniques prior to mass spectrometric analysis then must be rigorous. Purification of large amounts of compound is not too difficult since any one or combination of a number of chromatographic and physical techniques may be employed. With submilligram amounts the problem becomes more difficult. In the case of TLC pure compounds may be eluted from the plate and introduced directly into the mass spectrometer, provided a good resolving system is used and sufficient compound is present (see section III.B.2). GLC, however, is still to be preferred for separations, and much recent work has concentrated on the combination of gas chromatography and mass spectrometry in a single apparatus (GLC–MS). This apparatus allows direct mass spectral monitoring of compounds as they leave the column, and it has been used to considerable effect of routine structure elucidation and identification (see also sections III.C

SCHEME XIII. Fragmentation Pattern of $\Delta^1$-THC




and III.D). Vree et al.[31] however, maintain that the operating conditions used by most workers (70 eV, ion source temperature between 100 and 250°) gives cannabinoid fragmentation patterns (especially those of cannabinoids with the same molecular weight) which are too similar to be used for identification or structure elucidation. He points out, however, that the change of intensity of mass fragments under different spectrometric conditions is characteristic of the structure of the compound. By plotting relative mass fragment intensities against eV (usually 10–20 eV with the ion source held at 250°) from the mass spectra of a number of cannabinoids, he obtains a series of curves which are unique for each compound. The rate of change of intensity of each mass fragment with change of eV is a reflection on the ease of production of that fragmentation of the structure, and he suggests that these curves are a much more reliable method for identification of cannabinoids than a mass spectrum taken at only one-electron energy value.[31] This method has, in fact, been utilized in a number of subsequent publications dealing mainly with natural products.

The maximum sensitivity of GLC–MS is dependent on the machine used and the relative abundance of the ions recorded in the spectrogram. Normally most compounds can be detected down to somewhere in the nanogram range, and mass fragmentometry can offer a further improvement in sensitivity of detection. In mass fragmentometry, the spectrometer is set to monitor certain mass numbers of the compounds emerging from the gas chromatograph. The abundance of each mass number is recorded in the same fashion as a signal from a normal GLC detector, and the operator receives the mass fragment intensities as superimposed gas chromatograms. It is possible to choose characteristic mass numbers having high intensity for a given group of compounds and thereby achieve greatly improved sensitivity and selectivity of detection. In the case of chlorpromazine, for example, it has been found possible to detect amounts as low as 1 pg.[182]

Choice of the right mass peaks would not only provide selectivity of detection for complex mixtures but could also be used for pinpointing likely metabolites. Agurell et al.[145] and Skinner[183] have applied this technique to the detection of $\Delta^1$-THC in biological fluids (see section III.D).

Direct mass fragmentometry (DMF) is a recent development which promises to provide a very rapid and sensitive method for the detection of drugs of abuse. DMF is similar to the mass fragmentometry mode of combined GLC–MS but without the GLC inlet system. One or more mass fragments of the substance of interest can be monitored and, in the case of more advanced instruments, this can be accomplished for a large number of compounds simultaneously. The sample is introduced into a molecular separator, which admits the organic compounds for mass fragmentometry but excludes most of the accompanying air gases and water. It is claimed that unique identification of most commonly abused drugs can be achieved by observing five fragment ions without even considering relative intensities. Data processing systems are now available for the simultaneous monitoring of more than 100 selected fragment ions while ascertaining whether they are within the prescribed limits of the proper intensity ratios. Green reports that this technique has been used for the detection of cannabinoids, but no details are published. The method has not as yet been applied to the problem of measuring these compounds in biological fluids.[184]

Chemical ionization MS has proved a useful complement to conventional electron impact MS. The spectra obtained from this technique show abundant protonated molecular ions and only very simple fragmentation patterns. In the case of the cannabinoids, similar molecular weights and structures make it unlikely that this technique will find extensive use for characterizations although it has been useful for identification of noncannabinoid compounds such as *p*-hydroxyacetophenone, catechol, and other compounds found in marijuana smoke.[59]

### 5. Ultraviolet Spectroscopy

In spectrophotometric studies, El-Darawy et al. record spectral values for CBD and cannabidiolic acid (CBDA) (also documented in earlier literature), and from these values calculate p$K_a$ for CBD to be 4.25.[185] This value seems questionable since p$K_a$ of resorcinol is 9.81.[186]

Absorption spectra of iron(III)–CBDA solutions in organic solvents have also been investigated, and it is confirmed that a 1:3 iron–CBDA complex is formed.[187]

Ultraviolet spectrophotometry has been used to study the binding of cannabinoids with the cytochrome of the rat liver microsomal drug metabolizing system. Thus $\Delta^1$- and $\Delta^6$-THC, CBN, and CBD have been found to produce type I spectral changes, indicating the formation of an enzyme–substrate complex, but 7-hydroxy-$\Delta^1$-THC does not. Several studies of this drug metabolizing system have been done using this technique.[188–192]

### 6. Nuclear Magnetic Resonance Spectroscopy

NMR has proved to be a very powerful tool for structure elucidations although its application is confined to the study of pure compounds. Archer et al. give a lot of data on the THC

isomers, hexahydrocannabinol and cannabinol, which they use to confirm detailed conformational analyses of these compounds done from theoretical considerations.[193] Weiner and Meyer use the same technique to study the conformation of cannabidiol.[36]

A review by Mechoulam and Gaoni lists the NMR data of most common naturally occurring cannabinoids.[194]

$^{13}C$ magnetic resonance spectrometry ($^{13}C$ NMR) is becoming a useful adjunct to NMR for structure elucidation.[195] The advantages of $^{13}C$ NMR arise mainly from the simplicity of proton-decoupled spectra, the large spread of chemical shifts, and the sensitivity of these chemical shifts to changes in molecular structure. Carbon-13 is also expected to be a wholly safe tracer to use for metabolic studies in humans, and $^{13}C$ NMR would be an elegant way of locating the positions of tagged atoms in the transformed molecules. An unfortunate disadvantage of the method is that relatively large samples are necessary for analysis.

Chemical shifts have been assigned to all carbons of the THC isomers by off-resonance proton decoupling and other means,[196,197] and these compounds will provide useful models for the analysis of spectra of related compounds.

### 7. Other Methods

Some work is still being done on color tests for cannabinoids.[198–203] Many of these tests are claimed, a posteriori, to be specific for cannabis, but it is hoped that their use is nevertheless confined to field tests and that laboratory work is conducted on a more systematic basis. Cannabinoid color reactions have also been utilized for staining in microscopy work to aid the identification of marijuana glandular hairs (Fast Blue Salt B)[203] and for demonstrating tetrahydrocannabinols in fresh and frozen tissue sections (Fast Garnet GBC).[204]

A very different approach to the problem of separating cannabinoids was first taken by Petcoff et al. In this chromatographic technique, centrifugal force is used to accelerate the migration of compounds through columns of densely packed microparticulate silica gel in an eluent of light petroleum–diethylamine (99:1). The columns are extruded after centrifugation for a given length of time and sprayed with Fast Blue Salt B to reveal how far each component has migrated. Separations appear to be quite good although the sensitivities of detection are inferior to that achieved by TLC.[205] Modifications of this technique have resulted in its application as a good preparative method for milligram quantities of cannabinoids.[206]

It has been shown that THC inhibits the chemotactic response of *Pseudomonas fluorescens* down to a level of 10 $\mu$g/ml and suggested that this may form the basis of a bioassay.[207] It seems unlikely that such a technique would offer advantages over established ones unless a considerable improvement in sensitivity is achieved.

## C. Analysis of Cannabis Constituents

The various analytical techniques for analysis of cannabis constituents have been discussed in detail earlier (section III.B). It was noted that GLC is the method of choice, and it is found that GLC now largely supersedes TLC for routine purposes. However, the use of TLC will persist where analytical requirements are not stringent and where speed and convenience are of greater importance. Relevant literature for this technique is confined largely to earlier publications[138] (see also section III.B) although some recent work also describes TLC and GLC analysis of the more abundant cannabinoids—$\Delta^1$-THC, CBN, CBD—and their propyl homologues which are almost as abundant in Asian hashish.[30b,208,209]

The gas chromatographic behavior of a large number of natural and synthetic cannabinoids has been found to be characteristic of their molecular structures. For example, an increase in the cannabinoid side chain increases GLC retention times by 42% per carbon atom, shifting the side chain from ortho to para on the aromatic ring increases retention times by 130%, silylation reduces them by 53%, and other factors such as branched side chains, saturation of double bonds, and cis-trans isomerization also have quantifiable effects.[210,211] More recently it has been found that the changes resulting from increased length of side chain for $\Delta^6$-THC are not quite linear as was maintained earlier. Bailey proposed that plotting retention times of $\Delta^6$-THC homologues against the corresponding 1-substituted-3,5-dihydroxybenzene retention times graphically gives improved straight-line plots, even when the side chain is branched or functionalized.[212] It is probable that these theoretical techniques can be utilized for preliminary identifications of natural and synthetic cannabinoid homologues.

Normally, however, GLC identifications will be effected by a comparison of retention times between the unknown constitutent and authentic samples. The choice of columns that may be used for these analyses[213] is wide (see section III.B.3), and it has been found that any identification should be at least confirmed on a different column or by other methods such as TLC, MS, etc.

In the absence of authentic samples for GLC comparison, the identity of the compound can be established from physical data reported at the time of isolation and which, for almost all the minor constituents, is furnished by GLC–MS (see section II.A). In fact, ideally, the identity of a constituent would be always best confirmed by MS (see section III.B).

De Zeeuw has reported that a number of long-chain alkanes, with retention times on many columns similar to cannabinoids, can interfere with GLC analyses.[64] Turner maintains that the similarity between cannabichromene and cannabidiol on many columns can also cause erroneous results.[171] In both these cases, confirmation of identity by TLC, MS, or GLC of the silylated extract compared to silylated standards will expose the error in identification.

Quantification of cannabinoids in plant material is usually done by the modified method of Lerner[72,214] which utilizes chloroform for an efficient cannabinoid extraction[215] and which is claimed to give good reproducible results.[216] Other workers report that extraction with light petroleum can also give good, reproducible results when an internal standard is used.[217] The internal standards, methadone, *n*-eicosane, methyl stearate, cholestane, and 4-androstene-3,17-dione have all been used. Of these, Willinsky recommends only cholestane or 4-androstene-3,17-dione since the longer retention times of these compounds make their interference with cannabinoid peaks unlikely.[217] Even the use of an internal standard does not guarantee improved accuracies of determination. Small losses of cannabinoids relative to the standard are possible on low coated columns,[167] and it is advisable to check the linearity of detector response against the amount injected in the same range as the quantifications are to be done.

The difference between quantitative analyses of silylated and unsilylated extracts enables the determination of cannabinoidic acids in samples to be administered by routes other than smoking.[20,170] However, if the sample is to be smoked, there is a case for quoting the apparent cannabinoid amounts determined as the unsilylated extract, since any acids present in the sample will be decarboxylated by the smoking process. In these samples, cannabinoid losses during smoking are commonly 50% or even higher, depending on the method of sample preparation and combustion conditions.[37] Thus, although it is desirable to administer cannabinoids experimen-

tally in a manner closely approximating to "field" conditions, if it is done, a reliable relation between sample analysis and administered dose will be forfeited.

Individual analytical problems such as interference by alkanes and similar cannabinoid retention times (see above) must certainly keep appearing depending on the sample, which compounds are being analyzed for, and the individual columns being used. Rather than devise specific solutions for each instant, it would seem that the most sensible approach would be to convert to the use of the very high-resolution capilliary columns. In the relatively unlikely event of a preliminary purification being necessary, the use of Sephadex LH 20[144,145] is to be recommended over conventional column chromatography[64] to minimize small sample losses.

## D. Cannabinoids in Body Fluids

### 1. *Analytical Requirements*

The detection of cannabinoids in biological fluids has long been a problem for pharmacologists and toxicologists. The quantification of these compounds in human blood in particular has been of the most pressing importance, and because of the very small amounts involved it has posed the biggest challenge.

Until recently it has been assumed that $\Delta^1$-THC is mainly responsible for the biological activity of cannabis.[218] Now, it seems that a full explanation of cannabis intoxication in terms of molecular species present in blood plasma or brain is by no means such a simple matter, and it is probable that techniques will be eventually required for the quantification of at least $\Delta^1$-THC and $\Delta^1$-tetrahydrocannabivarin and possibly also CBN, cannabivarin, the metabolites 6$\beta$-OH-$\Delta^1$-THC and 7-OH-$\Delta^1$-THC,[219] and perhaps even a further unidentified metabolite (see section III.D.2).

From the point of view of sensitivity of the analytical techniques required, blood plasma poses a very difficult problem. For humans, subjective effects from smoking usual amounts of marijuana appear to persist for up to 3 hr and labeled $\Delta^1$-THC studies indicate an analytical method capable of detecting down to about 2 ng/ml is needed for THC analyses after this period.[38,220] Even the development of such a sensitive technique does not ensure a successful method. At these low levels of detection the number of compounds present in biological samples which may interfere with the analysis are legion and, to a large extent, dependent on the method of detection chosen. Thus, for example, the electron capture methods of Garret and Hunt[174] and Fenimore, Freeman, and Loy[160] will detect all strongly electron-capturing compounds plus any others (e.g., having hydroxy or amino groups) extracted from the plasma and capable of reacting with the derivatizing agents used.

Analytical selectivity then is as big a problem as analytical sensitivity. For adequate selectivity, a preliminary purification is generally necessary before the final analysis. Such a process, however, can be costly in terms of time as well as material and equipment, and the advantage of a 30-min procedure over a 3-hr one need not be spelled out.

### 2. *Radioisotope Tracer Methods*

Until recently radioisotope labeling techniques provided the only method sufficiently sensitive to study the metabolism of cannabinoids. Either $^{14}$C or $^{3}$H compounds may be prepared[221] and administered to the animal or, in some cases, man. The cannabinoids and metabolites are recognizable by the active label and quantified down to even subpicogram amounts by a variety of counting procedures. At these levels, however, positive identification of the compounds involved is now often more difficult than the quantification.

Lemberger and coworkers had the simplest solution to this, rather difficult, problem. Human plasma was extracted with heptane–isoamyl alcohol (1.5%) and the extract was assayed by liquid scintillation spectroscopy. The efficiency of extraction of $\Delta^1$-THC by this solvent is known to be 95 $\pm$ 5%, whereas the more polar metabolites must be extracted with ether. It was thus argued that the radioactivity of this extract was a measure of the $\Delta^1$-THC present at different times in the blood, and the identity of the $\Delta^1$-THC was confirmed by the radiohistogram of a silica gel thin layer chromatogram developed with hexane–acetone (3:1).[220,222]

There appear to be two problems in this approach. Firstly, TLC was carried out on pooled samples, i.e., blood samples taken after the first hour, the first day, and the second and third days. If relatively rapid metabolic changes take place within any of these periods, they may not be detectable when samples of the whole period are combined. Secondly, as discussed in section III.B.2, cannabinoids can be very difficult to separate on TLC, and it seems quite probable that the method used would not be adequate to resolve $\Delta^1$-THC from possible metabolites of similar polarity (e.g., $\Delta^6$-THC, CBN, or other "nonpolar metabolites"). More recent work does indicate that CBN occurs in relatively high concentrations in the blood for a short period after smoking[176] and even after injection of $\Delta^1$-THC.[223] It appears that the "$\Delta^1$-THC blood levels" recorded are in reality the sum of the nonpolar cannabinoid concentrations.

Other work by Lemberger et al. with dimethylheptyltetrahydrocannabinol (DMHP) (**94b**) describes the assay of DMHP after TLC analysis of individual blood extracts.[224] This overcomes the first objection against the method used in earlier papers but it is still questionable whether the cited TLC system is capable of resolving possible metabolites of similar polarity.

Gill, Jones, Pertwee, and coworkers employ an ethyl acetate extraction of the biological material and chromatograph the concentrated extract on Whatman SG 81 silica-impregnated paper (solvent 1% v/v methanol in chloroform). Strips corresponding to the $R_f$ of the authentic compound are cut out of the chromatogram, placed in counting vials, eluted directly with scintillator solution, and counted in situ.[225] Again it seems possible that nonpolar metabolites are not resolved from $\Delta^1$-THC in this system. A recent metabolic study of 7-OH-$\Delta^1$-THC according to the same method found "a mobile metabolite which could be $\Delta^1$-THC, cannabinol, or an unidentified metabolite" which accounted for 50% of the radioactivity in the blood of mice.[225d] After subsequent GLC analysis, it was tentatively suggested that this was actually a mixture of CBN and THC, and thus what was thought to be $\Delta^1$-THC in earlier papers "is probably a mixture of compounds of similar chromatographic mobility".[225d,226] Work by Wall et al. on human plasma tentatively suggests that isomerization of $\Delta^1$-THC may also be a metabolic process. These workers also stress that TLC techniques are not adequate in the face of such possibilities.[227]

Similar comments appear to apply to the work of MacMillan et al. who used TLC on silica gel with an eluent of hexane–acetone (3:1)[228] and to that of Fehr and Kalant who used a silica gel TLC eluent of hexane–acetone–ether (4:3:2)—reportedly good for separations of metabolites (although no $R_f$'s are given)[229] but questionable for resolving THC from other nonpolar metabolites. In fact, the only workers who report verifying that the TLC system they used is adequate for separating THC isomers and CBN are Ho et al. (*n*-hexane–acetone, 10:1, followed by *n*-hexane–benzene, 1:1)[230] and Leighty (heptane–benzene–methanol–ethyl acetate 55:15:20:10).[231]

In spite of these methodological criticisms, it is possible that the application of some of these systems by careful

workers could result in adequate resolution. Nevertheless, it should be stressed that TLC alone is not a sufficient confirmation of the identity of $\Delta^1$-THC or individual metabolites, and such work should also incorporate high-resolution GLC or, if possible, mass spectrometry.[232]

### 3. Immunoassay Methods

A number of laboratories are presently developing immunoassay methods (see section III.B.7), but as yet none of the methods are capable of being applied to the qualification of $\Delta^1$-THC in human plasma. A radioimmunoassay method developed by Teale et al. is reported to have a detection limit of 5 ng/ml. It is capable of analyzing for the cannabinoids as a group and could thus provide the basis for a facile screening procedure.[232a] Gross et al.[232b] have described an immunoassay method which can detect 25–50 ng/ml. It can be used to estimate cannabinoids in chronic marijuana users, but probably not in occasional users. Immunoassay methods can have considerable advantages over the existing ones based on gas chromatography. It is to be hoped, therefore, that more specific and more sensitive antisera will be developed in the near future.

### 4. Thin Layer Chromatographic Methods

TLC analysis of pooled biological samples has been used in a number of metabolic studies of animals and men,[233,234] but the analysis of individual samples has presented a much greater problem.

As early as 1967 da Silva claimed to have detected cannabinol in blood, urine, and saliva of people intoxicated with cannabis,[235] but in the case of the blood and urine no subsequent workers have confirmed his results. It is possible that he may have detected CBN shortly after smoking but certainly not after the longer periods stated.

Salaschek, Matte, and Seifert have reviewed the methods used for reported identifications of cannabinoids in urine by TLC. Fifty samples of human urine taken after the intake of hashish were compared with 22 control samples according to these described methods. It is reported that positive identification with possibility of misinterpretation could be achieved in only four cases and that reliable routine detection of hashish ingestion, from urine samples, is not possible by these methods. This fact is in at least part due to the large number of interfering compounds, some of which could, if the analyst were in an optimistic frame of mind, be taken for cannabinoids.[148] Kisser has reported detection of cannabinoids in the urine for 2 out of 40 cases after acid hydrolysis and TLC analysis of the extract.[236] Neither of these groups of workers mention confirmations by alternative analytical means.

Hollister, on the other hand, confirms TLC identification of CBN and CBD by GLC when examining urines of patients administered with hashish. He found that CBN and CBD[237] but not $\Delta^1$-THC[238] are excreted in the urine after ingestion of the pure compounds although in the latter case two possible new metabolites were found.[238] It seems probable that a successful detection of hashish smoking from the urine is dependent on large amounts of these compounds being present in the hashish.

Woodhouse has confirmed the presence of 7-hydroxy-$\Delta^1$-THC in the urine of marijuana smokers with TLC followed by elution of the compound from the silica gel for confirmation by mass spectrometry and GLC.[161]

Although the method of Just et al.[156] offers greatly improved sensitivity, the problem of the large number of compounds in urines (differing from person to person and time to time),[148] which may also form 1-dimethylaminonaphthalenesulfonates and thus interfere with the analysis, still remains. In the blood, it is possible to perceive a pattern of naturally occurring compounds and avoid the above problem. However, the resolution provided by TLC is not great, the cannabinoids and nonpolar metabolites are difficult to separate, and, as we have discussed earlier (section III.B.3), a GLC method would be preferable. Since the appearance of this method for blood analysis, no further publications have appeared reporting its use, so a more thorough evaluation is not, as yet, possible.

It would therefore seem possible to use TLC for the detection of cannabinoids (other than $\Delta^1$-THC) and their metabolites in urine, but the lack of sensitivity and specificity of this method are severe limiting factors. It is essential that any findings with this technique be confirmed by alternative means.

### 5. Gas Chromatographic Methods

GLC and GLC–MS methods have been successful in several metabolic studies of pooled biological samples,[233,234] but as with TLC, the analyses of individual biological samples present a much greater problem.

Recently Repetto and Menendez reported the detection of cannabinoids in both urine[239] and blood[240] by the use of GLC with flame ionization detection. Considering the relative lack of sensitivity of the technique involved (see section III.B.3), the latter result is rather surprising unless the blood sample was taken only very shortly after smoking.[176,220]

Of the available methods of GLC detection (see sections III.B.3 and 4), only mass fragmentometry or electron capture and flame photometric detection of cannabinoid derivatives are capable of providing adequate sensitivity.

The method of Fenimore, Freeman, and Loy utilizing electron capture detection of cannabinoid heptafluorobutyrates has been developed to the point where it may be applied to analysis of human bloods. The bloods examined by these workers were those of rabbits administered 0.1 mg/kg $\Delta^1$-THC, and the limits of detection were claimed to be about 0.1 ng/ml.[160]

Garret and Hunt use pentafluorobenzoates for electron capture detection of $\Delta^1$-THC in the blood of dogs. These workers use dichloromethane with 1% isoamyl alcohol as an extraction solvent and, surprisingly, no clean-up procedures are used and no problems with interference from other compounds extracted are reported. Maximum sensitivities of detection are claimed to be between 40 and 125 pg/ml of plasma.[174] It is difficult to reconcile the problems of interfering compounds experienced by Fenimore et al. using rabbit plasma[160] with the apparent ease of analysis by Garret and Hunt using dog plasma. It is possible that either dog plasma is easier to analyze or that pentafluorobenzoates are a better choice of derivatives to analyze. Whatever the reason, it seems probable that the analysis of human bloods is not simply a question of substituting "human" for "rabbit" or "dog" in the experimental procedure and that the extracts may well form heptafluorobutyrates or pentafluorobenzoates interfering with the analysis of $\Delta^1$-THC. It could need considerable manipulation of column stationary phases and derivatizing agents before a good combination is found.

So far only two methods have been demonstrated to work for human plasma—those of McCallum and Agurell. The method of McCallum, utilizing the flame photometric detection of phosphate ester derivatives, is claimed to have a sensitivity of detection below 2 ng/ml of plasma which is quite adequate for routine $\Delta^1$-THC analyses.[176] The use of capilliary instead of packed columns in this method would further improve sensitivity of detection, allow shorter retention times, and thus facilitate detection of the less volatile dihydric cannabinoid phosphate esters which have not been analyzed for thus far. The possibility of using temperature-programmed GLC with

flame photometric detection provides an additional advantage over electron capture detection.

Skinner has reported the use of GLC–MS for the detection of $\Delta^1$-THC and metabolites in human urine and rat blood and urine. By selection of the 299 mass fragment, excellent maximum sensitivity of detection is recorded, but the need for monitoring other fragments (see section III.B.4) will unfortunately considerably reduce this.[183] $\Delta^1$-THC levels in human urines and rat blood are higher than normally encountered with human blood, and the reason why successful analyses of the latter were not reported is presumably due to the lack of an adequate clean-up procedure. Agurell et al., however, report that a preliminary purification of the extract from human plasma by chromatography on Sephadex LH-20 provides adequate clean-up for subsequent quantification of the $\Delta^1$-THC by mass fragmentometry. Their method[145] has been found suitable for the measurement of $\Delta^1$-THC down to levels of 0.3 ng/ml when fragmentograms of the 299 and 314 mass fragments (at 50 eV) are used.[241] Here mass fragmentometry provides what is in effect a highly specific GLC detector. The more mass fragments monitored, the more certain one can be that a peak at a given retention time is the one of interest. On the other hand, the more of these minor fragments that are used, the less sensitive is the detection. It should be remembered also that many cannabinoids have common mass fragments and thus, when using this method for analyzing blood of cannabis smokers, one still has to be sure that the cannabinoid to be measured has a GLC retention time discrete from the other cannabinoids.

### *6. General Comments*

Presumably, it will be some time before the use and further development of the methods described above allow a realistic evaluation of them. Meanwhile there are aspects of each method which could be applied to advantage in the others.

It will be noticed that Fenimore,[160] Agurell,[145] and Skinner[183] advocate the use of light petroleum or heptane containing 1.5% isoamyl alcohol as the extraction solvent because of the high recoveries of $\Delta^1$-THC it is claimed to give.[222a] Although the use of this solvent does give high extraction efficiencies, the amount of extraneous material also extracted is great. McCallum found that only when a pure light petroleum extraction is used, can the preliminary clean-up procedure necessitated by this extraneous material be dispensed with.[176] With McCallum's method, good selectivity of detection is also a contributing factor, and it remains to be seen whether light petroleum extraction in the other procedures could result in simplification of the purification step. Reported extraction efficiencies using 1.5% isoamyl alcohol are "often in excess of 90%",[160] whereas with pure light petroleum it can be in the order of 70%[176,242] and some workers report even higher yields.[222c] This loss of extraction efficiency can be offset by elimination of losses during clean-up which, with Sephadex LH-20, for example, are about 30%.[145] In addition to the efficient extraction of $\Delta^1$-THC present free in the plasma, McCallum has noted that denaturing the plasma protein also increases the amount of $\Delta^1$-THC extracted.[176]

Recoveries of $\Delta^1$-THC, the extent of derivatization, possible losses on glass, and injection errors are not always constant and necessitate the addition of an internal standard to the plasma. The use of CBN as an internal standard[174] is not satisfactory since it is a major constituent of most of the cannabis smoked and it is also present as a metabolite.[176] The easily prepared $\Delta^6$-THC,[243] only a very minor constituent of cannabis which has not been found to be a metabolite, has provided a convenient internal standard for quantifications.[244] Fenimore et al. have used hexahydrocannabinol as an internal standard.[160] Possibly the best solution would be to use both, for example, one at a level of 2 ng/ml and the other at 40 ng/ml, thus providing standardization for the whole range of cannabinoid concentrations likely to be encountered. Agurell[145] uses a deuterium-labeled $\Delta^1$-THC internal standard for mass fragmentometric detection, an ideal solution in this instant but valueless for other methods.

If clean-up procedures are necessary, that of Fenimore et al.[160] appears to offer advantages of speed and high efficiency of recovery over Sephadex LH-20.

The use of capilliary columns in the methods of Agurell and McCallum would have obvious advantages in terms of improved sensitivity and resolution.

The criteria for an acceptable identification and quantification of cannabinoids are important. Only after it has been demonstrated that a large number of plasma samples contain no compounds which may be misinterpreted as the cannabinoids of interest or the internal standard is it possible to say that the new peaks observed are cannabinoids or their metabolites. Ideally the identity of these compounds should then be verified on a second GLC column with a dissimilar stationary phase.

## E. Forensic Methods

Color tests, briefly discussed earlier (section III.B.7), have found extensive use over the years for the identification of cannabis plant material and resin. By their nature these tests (e.g., Duquenois-Levine test)[245] are not of necessity specific and should therefore be confined in their use to the field work and for screening while more modern techniques should be used for positive identifications.

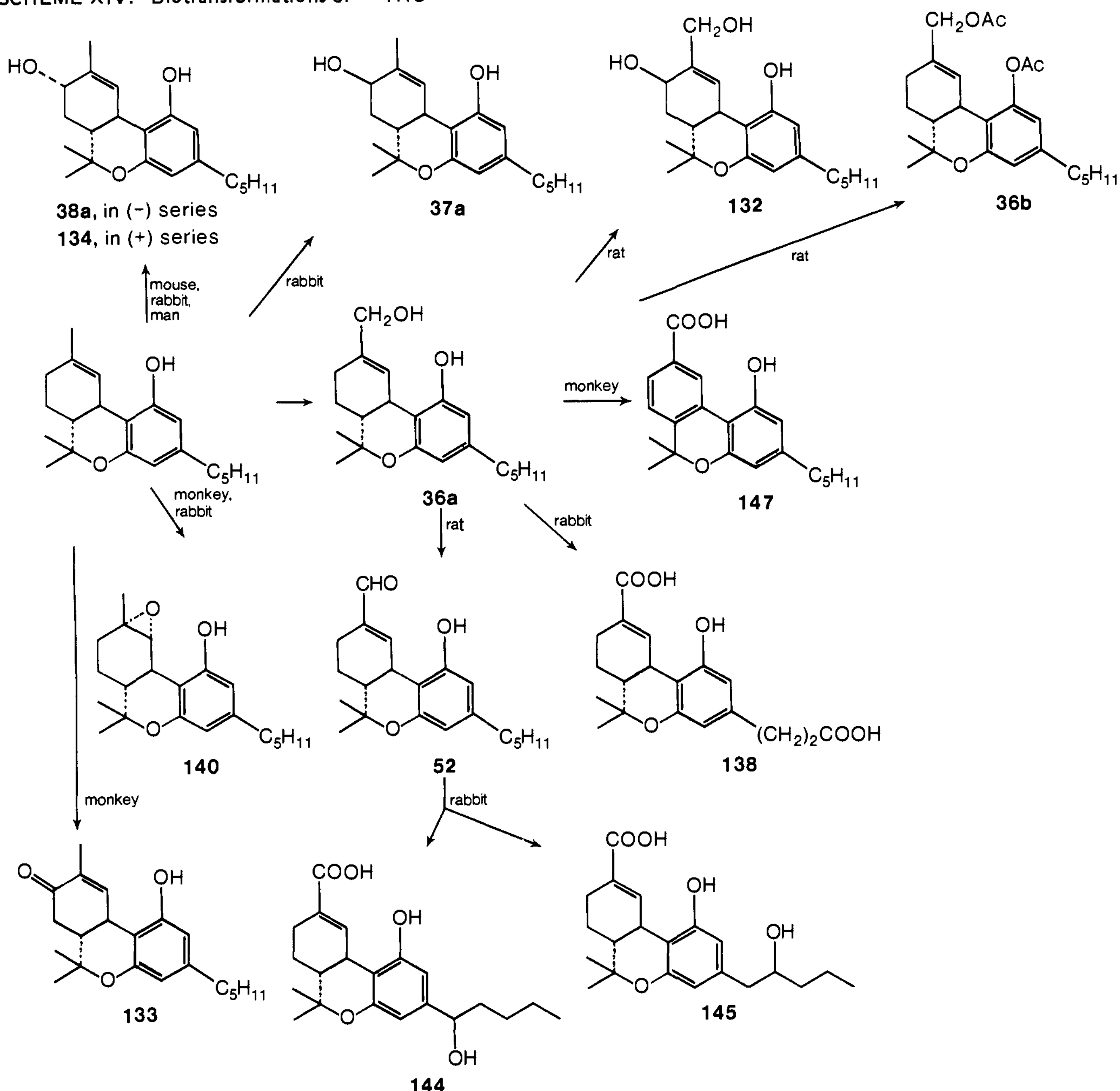
The analysis of small samples of plant material may be effected without prior solvent extraction by volatilization of the cannabinoids onto the walls of a small glass container and then direct application of them onto a TLC plate. Alternatively, direct introduction of the plant sample into the GLC is also possible.[246]

Although it is not possible to assign a sample of cannabis to a definite geographical source, it does seem possible that samples seized in different places might be shown to originate from a common lot. After a detailed study Stromberg could only conclude: "if two or more hashish samples are quantitatively analyzed with regard to some 40 components and the chromatograms are found to be in accordance, it may be assumed that they originated from a common larger lot of homogenous composition" [247]—not a very general conclusion.

Many of the volatile constituents responsible for the characteristic odor of marijuana have now been identified by GLC and MS (see section II.A.2).[67-69] This work may well reveal which marijuana components "sniffer" dogs detect,[68,69] but it seems unlikely that it will result in any further forensic applications. Experiments by Novotny, Lee, and others indicate that it is possible to characterize marijuana smoke in a similar fashion.[59,165] McCarthy and van Zyl have attempted to develop a breath test for cannabis smokers. Subjects were required to breathe onto a paper dampened with aqueous Fast Blue Salt B, and an orange-pink color reaction was found to indicate that cannabis had been smoked up to 2 hr before testing. Unfortunately smoked tobacco gives false positives, and further work is necessary to obtain a reliable method.[248]

In those forensic investigations where simply detection of the use of marijuana is required, analysis of saliva or fingertip washings rather than blood is the easiest approach. Just[156] and later Hackel[249] report detection of cannabinoids in the saliva between 1 and 2 hr after the smoking of marijuana when the TLC technique of Forrest (see section III.D.4) is em-

SCHEME XIV. Biotransformations of $\Delta^1$-THC[a]




[a] The reaction sequences are hypothetical.

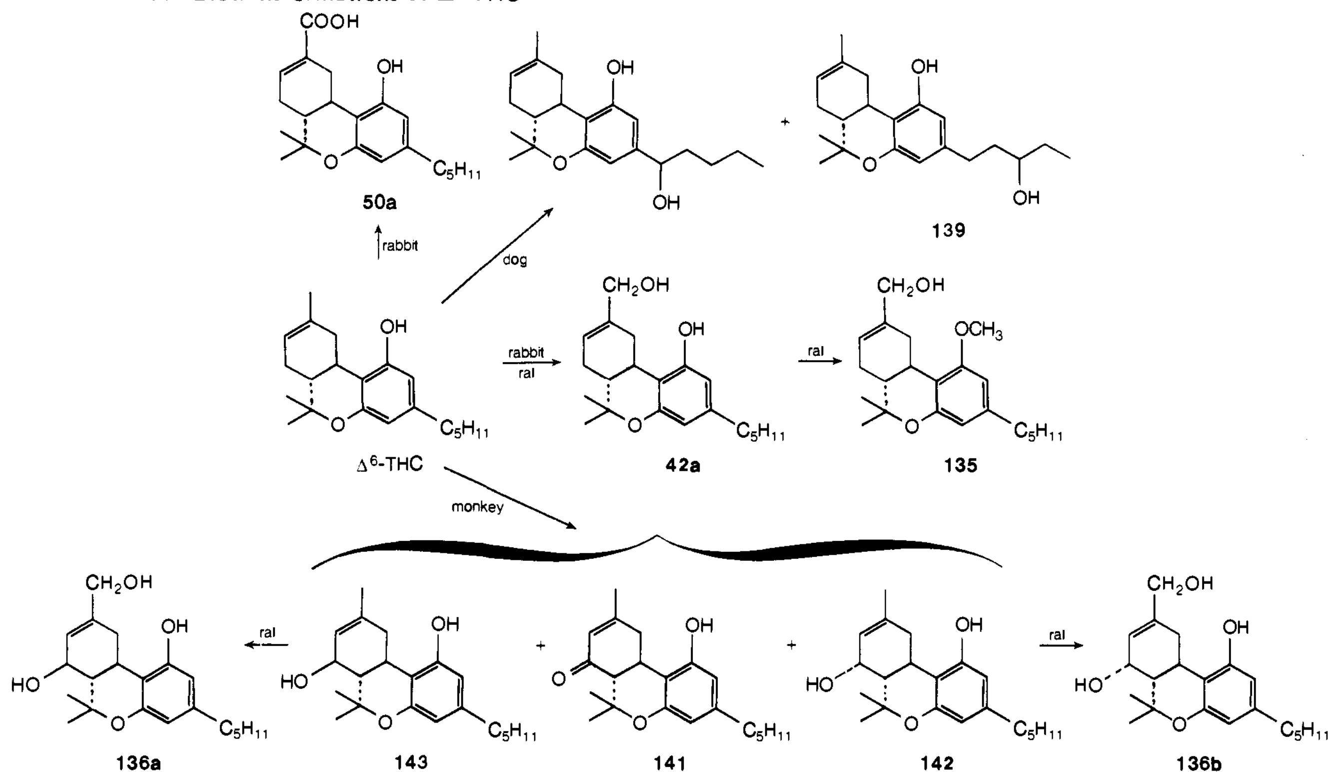
ployed. Stone found that cannabinoids can be detected on fingertips 1 to 3 hr after smoking with even less sensitive techniques.[250] The substitution of sensitive GLC techniques (section III.D.5) in these methods would greatly improve their utility. Quantitative analyses of other biological fluids have been discussed (section III.D). It will probably be some time before it is possible to interpret cannabinoid levels in terms of intoxication as it is now possible with alcohol and other drugs; and the present forensic applications of these methods is therefore limited.

### IV. Biotransformations

The number of publications in this area has nearly doubled since our previous review[251] which covered the prior literature. The major results discussed in that review are summarized in Schemes XIV and XV. The most important transformations of $\Delta^1$-THC and $\Delta^6$-THC discovered were the formation of their 7-hydroxy derivatives (**36a** and **42a**). Interest has been focused on these reactions not only because of their widespread occurrence, but also because of the activity of **36a** and **42a**. The further oxidation products with one exception were found to be inactive in the usual cannabis assays. The general metabolic pathways for the cannabinoids apparently proceed via an initial allylic hydroxylation followed by other reactions. These latter steps probably have a role in the detoxification of the drugs. Recently, a dehydrogenation pathway of metabolism of $\Delta$- and $\Delta^6$-THC has been found. The generality of this route leading to cannabinol (**4a**) and acids derived from cannabinol such as **146** and **147** (see Scheme XIX) is yet to be determined.

The findings during the period reviewed now are probably best characterized as mostly an expansion of what was already known rather than the discovery of major new facts. This is not to minimize the importance of the recent work on cannabinoid metabolism since a number of valuable reports have appeared and the role of metabolism in the pharmacology of cannabis is as debatable a topic as ever.

SCHEME XV. Biotransformations of $\Delta^6$-THC[a]




[a] The reaction sequences are hypothetical.

## A. Metabolism in the Mouse

Although mice have been used in studying the biological activities of the cannabinoids, no metabolic studies have been reported until recently. The first data on metabolism in the mouse were published by Christensen et al.,[252] who showed that liver microsomes rapidly convert $\Delta^1$-THC into several metabolites. Blood and spleen were less active while brain and small intestine were relatively inactive. Tentative identification of two products by TLC indicated the presence of 7-OH-$\Delta^1$-THC (**36a**) and 6,7-di-OH-$\Delta^1$-THC (**132**). The stereochemical nature of the latter was not reported.

Gill and Jones[225a] have found 7-OH-$\Delta^1$-THC in the blood and brains of mice given $\Delta^1$-THC intravenously. They also showed that prior administration of SKF 525A or piperonyl butoxide altered the levels of the metabolite.

Ben-Zvi et al.[253] have provided more definitive evidence for the nature of the mouse metabolites. Mass spectral as well as GLC and TLC comparisons confirmed the presence of 7-OH-$\Delta^1$-THC and established 6$\alpha$-OH-$\Delta^1$-THC (**38a**) as a major metabolite in a hepatic microsomal system. Jones et al.[254a] have also identified the same two monohydroxy metabolites using the 10,000*g* supernate from liver homogenates. In addition they have isolated 6-oxo-$\Delta^1$-THC (**133**), suggesting that this may be produced by a soluble enzyme since microsomes alone did not yield this metabolite.[253]

The mouse hepatic hydroxylase system is also capable of metabolizing the enantiomeric substance (+)-$\Delta^1$-THC. Jones et al.[255] reported the identification of (+)-6$\alpha$-OH-$\Delta^1$-THC (**134**) and (+)-7-OH-$\Delta^1$-THC (**43a**) as the major metabolites in such an experiment. There were significant differences in the proportions of each product, indicating a certain degree of stereospecificity.

The appearance of metabolites less polar (TLC) than $\Delta^1$-THC has been reported by Ryrfeldt et al.[254b] Reaction of the phenolic hydroxyl of $\Delta^1$-THC is the most reasonable possibility for this product which, if correct, would represent a novel metabolic pathway of this drug.

## B. Metabolism in the Rat

Work in this species has continued at a rapid pace and has concentrated mainly on in vitro methods as in the past. In addition to 7-OH-$\Delta^1$-THC, Nakazawa and Costa[256] have reported several other transformation products of $\Delta^1$-THC using both lung and liver microsomes. One of these was intermediate in TLC mobility between $\Delta^1$-THC and its 7-OH metabolite. Ben-Zvi et al.[253] have isolated a similar substance using both mouse and rat hepatic microsomes and have shown that it is 6$\alpha$-OH-$\Delta^1$-THC (**38a**).

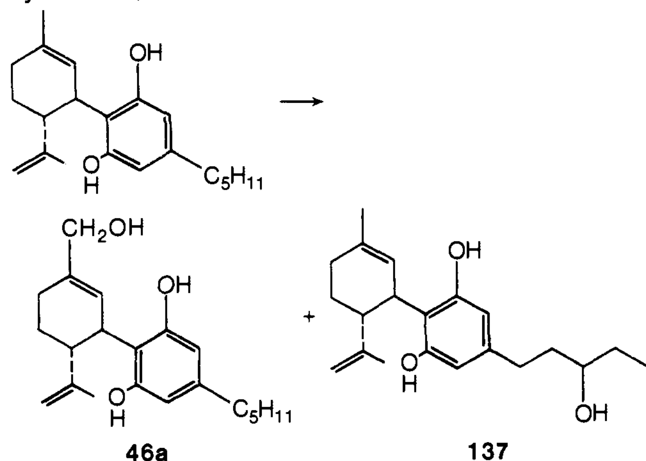
Until recently it was believed that $\Delta^1$-THC was completely transformed by the intact animal. However, Turk et al.[257] have demonstrated that $\Delta^1$-THC is excreted in the feces of rats following oral administration; this was not the case with intravenous injection. Such a result could be explained by incomplete absorption of the drug; however, Ben-Zvi and Burstein (unpublished observations) have shown that in the monkey iv administration also results in excretion of unmetabolized drug.

The metabolite composition of rat bile has been studied by Widman et al.[258] after iv administration of [$^3$H]-$\Delta^1$-THC. Most of the radioactivity rapidly appeared in the bile and consisted mainly of acids and water-soluble conjugates. Some 7-OH-$\Delta^1$-THC and other unidentified monohydroxy THC's were found as well as very small amounts of unmetabolized $\Delta^1$-THC (0.1%) and CBN (0.05%). The authors felt that the CBN in the bile could not originate from CBN in the administered THC.

McCallum[223,259] has identified CBN in the blood of rats within 35 sec after iv administration of $\Delta^1$-THC or $\Delta^6$-THC (both of which contained less than 0.1% cannabinol). The metabolite was identified by GLC and TLC, as well as by isolation and crystallization of $^{14}$C-labeled cannabinol (as its acetate) to constant specific activity. The formation of CBN from $\Delta^6$-THC, as well as $\Delta^1$-THC, provided further confirmation of the contention that the CBN could not be an artifact of the isolation procedure since the dehydrogenation of $\Delta^6$-THC to

SCHEME XVI. Transformations of Cannabidiol (CBD) by Rat Liver in Vitro




CBN requires drastic conditions.

A likely intermediate in the transformation of $\Delta^1$-THC to $\Delta^1$-THC-7-oic acids has been isolated by Ben-Zvi and Burstein.[105] A small yield of 7-oxo-$\Delta^1$-THC (**52**) was obtained by incubation of $\Delta^1$-THC with rat liver microsomes followed by a careful analysis of the products. On chemical grounds it was postulated that this aldehyde is a metabolic intermediate which leads to the acidic products of $\Delta^1$-THC. Its role in the pharmacology of $\Delta^1$-THC may be important since $\alpha,\beta$-unsaturated ketones are highly reactive systems.

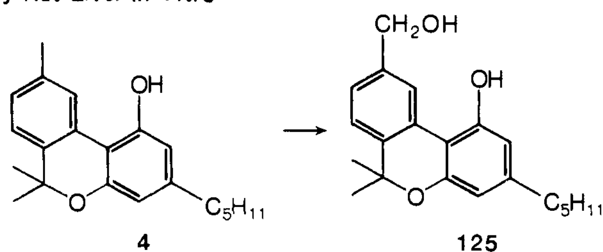
The further transformations of 7-OH-$\Delta^6$-THC in the intact rat were reported by Estevez et al.[230b] The brains and livers were removed from the animals 30 min after iv administration of the cannabinoid. Chromatographic (TLC) analysis of the extracts led to the isolation of 3′-*O*-$CH_3$-7-OH-$\Delta^6$-THC (**135**) and 5,7-di-OH-$\Delta^6$-THC (**136**) as well as two unidentified metabolites. The methyl ether was identified by comparison with a synthetic sample and is the first example reported of this type of transformation. The mixed function oxidase inhibitor, SKF 525A, was found to increase the proportion of methyl ether in both liver and brain.

The metabolism of cannabidiol has been studied in rat liver using the postmitochondrial supernate.[100a] The major metabolite was identified by analysis of its mass and nuclear magnetic resonance spectra. 7-Hydroxycannabidiol (**46a**) was postulated as the most likely structure which is analogous to the major metabolites of the other cannabinoids. A second substance which gave a molecular ion of 330 was also isolated. This again corresponds to a monohydroxy derivative, and a careful analysis of the mass spectrum suggested a side-chain position. Direct comparison with synthetic materials showed this to be 3″-OH-CBD (**137**).

In connection with a study on brain uptake of cannabinoids, Ho et al.[260] have confirmed the earlier finding of Widman et al.[261] on the metabolism of CBN by rat liver. They also isolated 7-OH-CBN (**125**) as the major product.

A metabolite less polar than the parent drug was reported by Leighty.[231] Intraperitoneal injection of both $\Delta^1$- and $\Delta^6$-THC led to the occurrence of the same substance in the livers and spleens of rats after 15 days. The compound was not identified; however, a GLC–MS study showed that it did not correspond to any of the common cannabinoids.

SCHEME XVII. Transformation of Cannabinol (CBN) by Rat Liver in Vitro



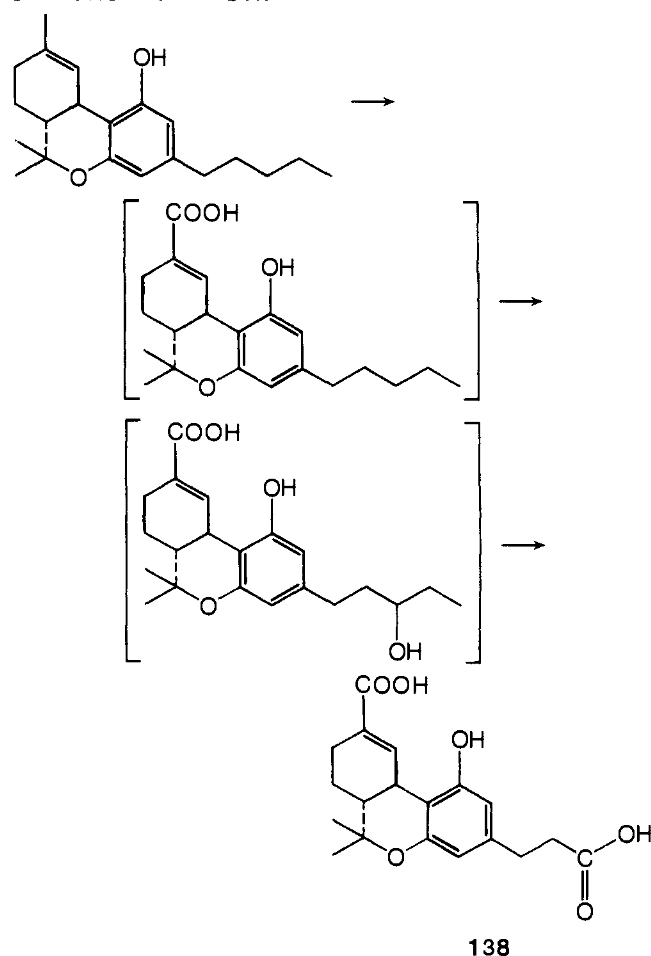

## C. Metabolism in the Rabbit

Evidence that the acidic metabolites of $\Delta^1$-THC are produced via 7-OH-$\Delta^1$-THC has been provided by Nilsson et al.[262] and Mechoulam et al.[103] Injection of [$^3$H]-7-OH-$\Delta^6$-THC to rabbits gave rise to $\Delta^6$-THC-7-oic acid (**49**). The work was done in the $\Delta^6$ series because of the difficulties in preparing standards in the $\Delta^1$ series. Since both series show similar metabolic properties, it is reasonable to assume that the results are applicable to $\Delta^1$-THC.

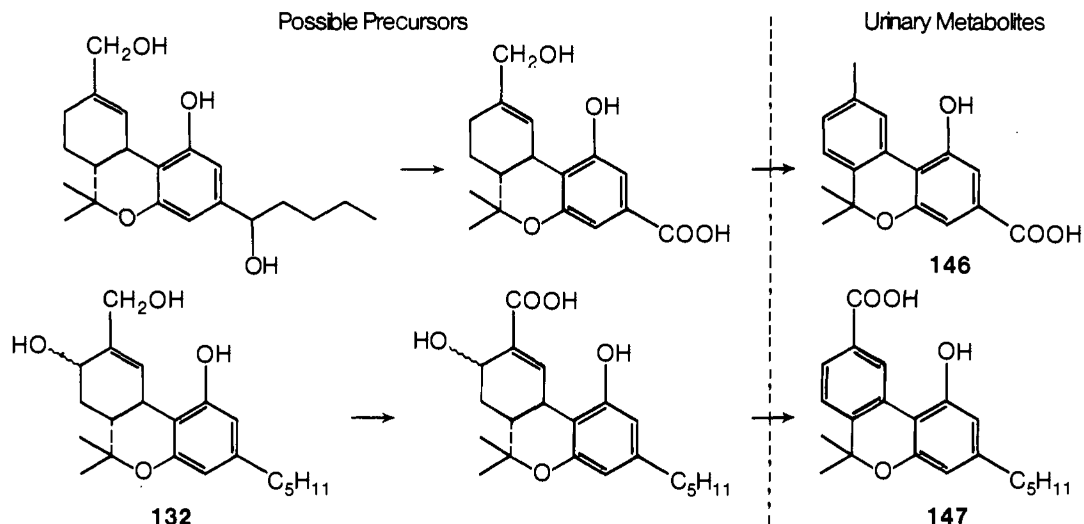
An unusual dicarboxylic acid metabolite, 4″,5″-bisnor-$\Delta^1$-THC-7,3″-dioic acid (**138**) has been isolated from rabbit urine.[263] Scheme XVIII shows a possible pathway for the genesis of this substance from $\Delta^1$-THC. The exact sequence of steps may vary; however, precedents have already been established for all of the intermediates. Metabolic oxidation of the 7-methyl function to a carboxyl group is known (Burstein et al.[142]). Hydroxylation at 3″ has been shown in the $\Delta^6$ series by Maynard et al.[264] in the dog (see **139**). A similar reaction here followed by side-chain cleavage between 3″ and 4″ would lead to the metabolite **138**.

6$\beta$-Hydroxy-$\Delta^1$-THC (**37a**) has previously been obtained by incubation of $\Delta^1$-THC with rabbit liver;[265] however, the 6$\alpha$ isomer (**38a**) has more recently been found by Ben-Zvi et al.[266] under similar conditions. In addition, they reported the presence of 1,2$\alpha$-epoxy-$\Delta^1$-THC (**140**) which had also been found in the monkey by Gurny et al.[267] The possibility, there-

SCHEME XVIII. A Possible Route for the Metabolism of $\Delta^1$-THC in the Rabbit



SCHEME XIX. Some of the Biotransformations of $\Delta^1$-THC in the Monkey




fore, is raised that this epoxide, which is active in monkeys, may also be a human metabolite.

## D. Metabolism in the Monkey

Several unique metabolites of THC have been isolated and characterized using squirrel monkey liver microsomes by Gurny et al.[267] Incubation of $\Delta^1$-THC led to the production of 6-oxo-$\Delta^1$-THC (**133**) and 1,2-epoxyhexahydrocannabinol (**140**). The configuration of the epoxy group was not determined by the authors; however, Mechoulam et al.[164] have shown that it is $\alpha$.

Gurny et al.[267] have also studied the metabolism of $\Delta^6$-THC with the above system. They isolated the analogous 5-oxo-$\Delta^6$-THC (**141**); however, no epoxide was found in this case. Two other metabolites were identified; 5$\alpha$- and 5$\beta$-OH-$\Delta^6$-THC (**142** and **143**) which may be precursors of the ketone (or vice versa). Although **142** and **143** have not been found in the rat, they could be precursors of the allylic diols **136a** and **136b** which were reported by Wall[265] to be products of $\Delta^6$-THC in rat liver. Once again the reaction sequence has not been determined, and 7-hydroxylation may well precede 5-hydroxylation. All five of the squirrel monkey metabolites have been synthesized by Mechoulam et al.[268] and their physical constants compared well with those reported for the metabolites. These syntheses establish the stereochemistry at the C-5 chiral center of **142** and **143.**

Melikian et al.[269] have examined monkey urine after administration of $\Delta^1$-THC. They did not identify any of the metabolites; however, they did observe that a large fraction consisted of carboxylic acids.

The metabolism of $\Delta^1$-THC in the rhesus monkey has been studied in our laboratory.[266] Two substances similar to the rabbit metabolites **144** and **145** were isolated from the monkey urine; however, there was insufficient material for a complete characterization. In addition, two fully aromatic metabolites were isolated (Scheme XIX) representing the first examples of such compounds being formed from THC by a metabolic process. The identity of **146** rests mainly on its mass spectrum, while **147** was further compared with an authentic sample (Syva Corp.).

The genesis of these unusual products allows for some speculation and this has been summarized in Scheme XIX. We have shown some time ago (Burstein et al.[270]) that 7-OH-$\Delta^6$-THC readily aromatizes under laboratory conditions to give CBN. Thus a similar sequence may occur in the monkey which, when coupled with side-chain hydroxylation and cleavage, could give rise to **146.** A 6,7-diol could serve as a precursor for cannabinol-7-oic acid (**147**) or, alternatively, CBN may be formed first (as indicated for the rat[259]) and subsequently oxidized. At the moment, the reaction sequences are completely arbitrary.

## E. Metabolism in Man

The first definitive report on human metabolites of $\Delta^1$-THC has been published by Wall et al.[227] They have identified 7-OH-$\Delta^1$-THC (**36a**) and 6,7-di-OH-$\Delta^1$-THC (**132**) in a pooled plasma sample (1200 ml) from five subjects who took the drug orally. Tentative evidence was found for the presence of 6$\alpha$ (**38**a) and 6$\beta$-OH-$\Delta^1$-THC (**37**a), and an unknown metabolite was separated from the unchanged $\Delta^1$-THC by GLC. The GLC–mass spectrum of this substance indicated an isomeric structure with one-half the retention time. The authors speculated on structures such as $\Delta^2$- or $\Delta^3$-THC based on the fragmentation patterns; however, it seems unlikely that a double bond isomer would have such a reduced GLC retention time.

A series of studies by Lemberger and coworkers on the kinetic aspects of the metabolism in man have been summarized in a single publication.[271] They followed the production of 7-OH-$\Delta^1$-THC in the plasma and concluded that the metabolite is the active species.

Two preliminary reports on urinary metabolites in man have appeared.[238,272] Unfortunately, labeled THC was not used so that the interpretation of the results is made difficult.

The urine of marijuana smokers has been examined[161] and found to contain 7-OH-$\Delta^1$-THC. Evidence was presented for the presence of 7-hydroxycannabinol (**125**) which presumably arose from the CBN in the cigarette.

Relatively large amounts of CBN were found by McCallum in the blood of THC smokers.[176] While part of the CBN observed undoubtedly could be formed from $\Delta^1$-THC, he found that the smoking of pure CBN gave relatively very low blood levels of CBN, and he therefore concluded that the CBN observed in the blood was probably of metabolic origin.[219] This argument, however, does not take into account possible interactions between $\Delta^1$-THC and CBN.[259]

## F. Metabolism of Unnatural Cannabinoids

One paper has appeared (Lemberger et al.[224]) describing the metabolism of DMHP (**94b**). Experiments in the rabbit, mouse, rat, guinea pig, and dog showed that the liver microsomes from these species were capable of hydroxylating DMHP to a mixture of at least three substances. Various comparisons of metabolism were made between $\Delta^1$-THC and DMHP.

# *V. Other Biochemical Effects*

## A. Binding and Distribution

Some time ago it was shown that $\Delta^1$-THC can bind to human plasma proteins.[274] It was reported that greater than 80% of the drug was associated with lipoproteins during electrophoresis. Widman et al.[275] have done similar studies

with 7-OH-$\Delta^1$-THC which again showed extensive (94–99%) binding to plasma proteins. In this case, however, a major portion of the cannabinoid was bound to albumin as well as lipoprotein. This probably reflects the greater lipophyllic character of $\Delta^1$-THC as compared to its hydroxylated metabolites. Since the bound cannabinoids are not as available for action on target cells, it seems that the required amounts for such actions is quite small. Binding constants were not measured in these studies.

Fehr and Kalant[229] found similar results when studying the in vivo distribution of $\Delta^1$-THC in rat plasma. They administered the drug in two different vehicles and as smoke and found in each case that most of the radioactivity was bound to the lipoproteins. At later times, a higher proportion was found in the albumin fraction which agrees with the findings of Widman et al.[275] on binding of 7-OH-$\Delta^1$-THC.

The binding of $\Delta^1$-THC to subcellular fractions has been examined by Dingell et al.[276] Using perfused rat livers, they reported that the cell nuclei and microsomes bound most of the drug in about equal amounts. Interestingly, they also found that the nuclei were able to reduce the metabolism of THC by the microsomal fraction. They suggested that an inhibitor such as NADPH-pyrophosphatase was responsible.

Membrane binding of $\Delta^1$-THC was studied by Seeman et al.[277] in which they used tissues derived from guinea pig brain synaptosomes and human erythrocytes. Their results with the synaptosomes suggested the presence of two distinct binding sites.

Colburn et al.[278] have found that synaptosomes from rat brain also bind $\Delta^1$-THC and its polar metabolites. They felt this was a result of the hydrophobic nature of the cannabinoids rather than the presence of specific binding sites.

The whole-body distribution in the mouse of both $\Delta^1$ and $\Delta^6$-THC has been studied by Ryrfeldt et al.[254b] In a rather thorough report, they showed that the drugs are rapidly cleared from the blood and taken up primarily in the liver, lung, and kidney. Little is absorbed by the brain and radioactivity is retained for long periods by the spleen, bone marrow, and liver. They also examined the appearance of metabolites in various organs at time intervals up to 96 hr. Metabolites less polar than THC were found to accumulate in the spleen (vide supra).

Freudenthal et al.[279] have also reported on the distribution of $\Delta^1$-THC in the mouse in which they found a similar pattern. Both groups observed a high uptake in reproductive tissues suggesting some interaction with the normal functioning of these organs (vide infra).

Willinsky et al.[280] have reinvestigated the distribution of $\Delta^1$-THC in the mouse in a more comprehensive manner. Their findings were in agreement with the previous reports cited above. They also compared distribution after iv and ip administration of the drug and found that in the latter route a large proportion remained in the peritoneal cavity. This finding should be considered when data obtained using various routes of administration are compared.

As expected by its lipophyllic character, $\Delta^1$-THC was found to accumulate in fat after chronic dosage (Kreuz and Axelrod[222c]). In accord with its more polar nature, 7-OH-$\Delta^1$-THC showed less tendency to accumulate; surprisingly, 6,7-di-OH-$\Delta^1$-THC was stored in both fat and liver. The authors felt that this depot of drug may play a role in chronic usage.

The uptake and secretion of $\Delta^1$-THC and its metabolites by the mammary gland has been demonstrated by Jakubovic et al.[281] Injection of [$^{14}$C]-$\Delta^1$-THC to lactating sheep resulted in the appearance of radioactivity in their milk and in the urine and feces of suckling lambs as well.

Gill et al.[225d] have correlated the brain levels of $\Delta^1$- and 7-OH-$\Delta^1$-THC after intravenous injection with a behavioral response (inhibition of spontaneous motor activity). From the results, they concluded that the metabolite was seven times more potent that the drug. Ben-Zvi et al.[253] have since shown that 6$\alpha$-OH-$\Delta^1$-THC (**38**a) is an abundant metabolite in the mouse. Since the 6$\alpha$-hydroxy is active as well, the situation has been complicated somewhat and needs to be reexamined. In the course of their studies, Gill et al.[225d,226] observed the production of a metabolite of 7-OH-$\Delta^1$-THC which was less polar, and they speculated that it could be either CBN or $\Delta^1$-THC. Ben-Zvi and Burstein[105] have recently shown that rat liver can metabolize $\Delta^1$-THC to 7-oxo-$\Delta^1$-THC, presumably through 7-OH-$\Delta^1$-THC. This raises a third possibility for the unknown metabolite. Since alcohol–aldehyde interconversions are facile biotransformations, this last possibility seems quite plausible.

More recently Gill and Lawrence[282] have found that both $\Delta^1$-THC and 7-OH-$\Delta^1$-THC remain in the brain ventricles of the mouse whether injected intravenously or directly into the ventricles. They felt, therefore, that the potencies of the two substances were influenced by the rates at which they diffuse from the ventricles into the brain itself. The slow diffusion they observed was attributed to the very lipophilic nature of these two cannabinoids.

## B. Effects on Enzyme Systems

The recent work in this area has centered mainly around four systems. They are: the "drug metabolizing" enzymes in general and aryl hydrocarbon hydroxylase in particular, adenosine triphosphatase, prostaglandin synthetase, and the adenylcyclase–phosphodiesterase system. While the first two systems may have important toxicological implications, the latter ones are probably involved in the mode of action of cannabis as well.

Some time ago, Cohen et al.[188] reported that $\Delta^1$-THC was a "type I" substrate for rat liver microsomes. The data were obtained by the usual difference spectra that such drugs produce with cytochrome P-450. They showed further that it was a competitive inhibitor for the N-demethylation of ethylmorphine by liver microsomes. This approach has subsequently been extended by Kupfer et al.[189] to include $\Delta^6$-THC, CBN and 7-OH-$\Delta^6$-THC as well. Both $\Delta^6$-THC and CBN gave type I spectral changes while the metabolite 7-OH-$\Delta^6$-THC showed no interaction. This latter result prompted the authors to suggest that the 7-hydroxy metabolites are not further oxidized by cytochrome P-450 to produce the diallylic alcohols (Schemes XIV and XV). One obvious implication of these findings is that the use of cannabis may alter the biotransformation of other drugs in vivo.

Paton and Pertwee[283] have shown that a cannabis extract inhibits microsomal activity to a degree greater than could be accounted for based on THC content. They showed, in fact, by studying pentobarbitone sleeping time and phenazone metabolism that CBD was more potent in this respect than $\Delta^1$-THC and could account for the activity of the extract. The potent biochemical actions of CBD were also demonstrated by Fernandes et al.[191] They further reported type I spectral interactions for $\Delta^1$-THC, $\Delta^6$-THC, CBN, and CBD. Aminopyrine demethylation was inhibited competitively; however, no effect was observed on aniline hydroxylation. CBD was the most active substance studied, and the authors concluded that inhibition of THC metabolism by CBD could account for the somewhat different activity of crude cannabis extracts.

Dingell et al.,[276] using rat liver microsomes, found that both hexobarbital and aminopyrine oxidation were reduced by $\Delta^1$-THC. They also found that the conjugation of estradiol and *p*-nitrophenol to glucuronic acid was inhibited and, by contrast, that the reduction of *p*-nitrobenzoic acid was enhanced. Since the magnitude of the effects of conjugation was lower than those on the redox enzymes, it was felt that $\Delta^1$-THC interacts primarily with the P-450 systems, which agrees with the findings cited above.

Aryl hydrocarbon hydroxylase activity can be stimulated by

both cannabis resin and THC.[284] It was found that the hydroxylation of benzpyrene in rat liver and lung homogenates was increased in a dose related fashion by prior administration of cannabis resin containing about 55% $\Delta^1$-THC. When comparable doses of pure $\Delta^1$- and $\Delta^6$-THC were given, induction was confined mainly to the lung enzymes. Marcotte and Witschi[285] also showed that smoke from marijuana cigarettes produced a similar effect again on lung but not on liver hydroxylase. Interestingly, placebo smoke generated from cannabinoid free plant material was also effective. This suggests a general effect rather than a specific property of THC. Induction was also demonstrated by intratracheal injection of marijuana smoke condensate in hamsters.[286]

The effects of $\Delta^1$-THC on adenosine triphosphatase activity in several systems has been studied. Jain et al.[287] reported that the administration of $\Delta^1$-THC to mice increased ATPase activity in the brain. Since morphine and ethanol also caused increases, but LSD caused a decrease in enzyme activity, they felt that the results correlated best with the sedative actions of THC.

The actions of THC on isolated mitochondria have been studied by two groups,[288,289] who have reported similar findings. Profound changes in the morphology and ATPase activities were observed. The direction of the effect on ATPase was dependent on concentration, high levels causing inhibition. Mahoney and Harris[289] reported that $Mg^{2+}$ enhanced the effects of $\Delta^1$-THC on mitochondrial membranes. They suggested that phospholipids such as cardiolipin were involved and that THC may alter nerve function by such an interaction with membranes.

A further difference in the actions of THC vs. LSD on the molecular level has been reported by Poddar and Ghosh.[290] They found that the rat liver enzymes tyrosine $\alpha$-ketoglutarate transaminase and tryptophan pyrolase were stimulated by pretreatment of the animals with $\Delta^1$-THC but not with LSD. In a later communication[291] it was reported that CBD had little effect by itself but in combination with $\Delta^1$-THC had a potentiating effect. They suggested that this could be explained either by inhibition of metabolism or alteration of cellular permeability by CBD.

The effects of $\Delta^1$-THC and CBD on rat liver lysosomal membranes were studied by Raz et al.[292] They measured the changes in the release of acid phosphatase from the organelles as an indication of membrane alteration. Both cannabinoids were found to cause small but significant decreases at low concentrations ($2 \times 10^{-6}$ *M*); at high concentrations extensive damage occurred releasing large amounts of the enzyme. They concluded that both $\Delta^1$-THC and CBD were capable of permanent damage to cells.

Rat liver lysosomes are lysed by $\Delta^1$-THC at elevated concentrations releasing acid hydrolases.[293] It was pointed out that vitamin A also produces a similar effect and that liver toxicity and cirrhosis accompanying chronic cannabis use could be caused by such an action.

The actions of several drugs such as aspirin have been related by Vane[294] to their abilities to inhibit prostaglandin biosynthesis. The possibility that certain effects of THC could be explained on a similar basis was suggested by Burstein and Raz.[295] $\Delta^1$-THC was shown by them to inhibit the formation of prostaglandin $E_2$ from [$^{14}$C]arachidonic acid by a microsomal preparation from sheep seminal vesicles. By similar methods Burstein et al.[296] found that other cannabinoids were also effective inhibitors of prostaglandin $E_1$ formation, CBN being the most potent. The activity was apparently due to the olivetyl moiety common to the entire series, the terpene portion of the molecule serving to modify the effect.

These findings were confirmed and extended by Crowshaw and Hardman,[297] who reported that 7-OH-$\Delta^1$- and $\Delta^6$-THC as well as $\Delta^1$-THC and DMHP (**94b**) were inhibitors of $PGE_2$ formation. They further observed that with rabbit kidney microsomes the production of $PGF_{2a}$ was stimulated by all of the above cannabinoids. Since the release of prostaglandins has been associated with a wide variety of conditions, it seems reasonable that at least some of the actions of THC and the other cannabinoids may be mediated by this mechanism.

Adenylcyclase activity in isolated cells can be stimulated by PGE and by epinephrine. Kelly and Butcher[298] have tested the action of $\Delta^1$-THC on these stimulations and found that both effects were antagonized in a concentration dependent manner similar to the effects of opiates on cAMP in other systems.[299] Moreover, the action of $\Delta^1$-THC could be removed by washing the cells, indicating that gross toxicity had not occurred. While these findings may not be related to the above reports on prostaglandin synthetase, they do suggest another approach for studying the mode of action of THC.

Low doses of $\Delta^1$-THC (0.1 to 1.0 mg/kg) have been reported by Dolby and Kleinsmith[300] to elevate cAMP levels in mouse brain while higher doses cause a depression. The authors suggested that this biphasic response of $\Delta^1$-THC parallels the changes which occur in biogenic amine levels, temperature regulation and behavior, implicating cAMP as a mediator.

Two groups have found that cannabinoids lower acetylcholine esterase in rats.[301,302] Luthra and Rosenkrantz[302] studied the effects of large doses of cannabis extract chronically administered and also observed decreases in brain protein, RNA, and succinic dehydrogenase and monoamine oxidase activity. Askew et al.[301] reported on an acute study using moderate doses of $\Delta^1$- and $\Delta^6$-THC in which the most pronounced effect was a lowering of brain acetylcholine. The $\Delta^6$-THC was more active, and they suggested this could explain its anticholinergic properties.

## C. Effects of Agents on Cannabinoid Metabolism

The effect of metabolic blocking agents on the in vitro conversion of $\Delta^1$-THC to the 7-hydroxy metabolite was studied by Burstein and Kupfer.[303] They showed that while hexobarbital had little or no effect, $\beta$-diethylaminoethyldiphenylpropyl acetate (SKF-525A) significantly inhibited this reaction. Sofia and Barry[304] have reported a complementary study which was in accord with the findings of Burstein and Kupfer. They measured the prolongation of hexabarbital sleeping time in mice produced by $\Delta^1$-THC. The presence of SKF-525A further extended sleeping time, suggesting a longer half-life for the $\Delta^1$-THC. This prompted the conclusion that, at least for this effect, $\Delta^1$-THC was an active agent and conversion to 7-OH-$\Delta^1$-THC was not a required step for drug action.

The possibility that other cannabinoids may affect the metabolism of THC is of great importance since the commonly used forms of cannabis contain relatively large amounts of substances such as CBN and CBD. Jones and Pertwee[225b] have examined the in vivo effects of CBD on $\Delta^1$-THC metabolism in the mouse. They found that the brain levels of $\Delta^1$-THC and 7-OH-$\Delta^1$-THC were increased by the prior injection of the mice with substantial amounts (50 mg/kg) of CBD. They concluded that CBD was inhibiting the further metabolism of both substances. They also observed no change in behavioral response (immobility index) with CBD pretreatment which they interpret as meaning that 7-OH-$\Delta^1$-THC is not the only active agent. It may be well to examine the role of 6$\alpha$-hydroxylation in this question.

Conflicting reports have appeared concerning the effect of pretreatment of rats with $\Delta^1$-THC on its own metabolism. Ho et al.[305] have found that hydroxylating activity in the liver is increased by long term (4 weeks) daily injections; activity in the lung was unaffected. Kupfer et al.,[190] using a shorter treatment period (9 days), did not find any significant change in

liver microsomal activity. They were, however, able to induce metabolism by pretreating the animals with DDT, a known stimulator of mixed function oxidases. This later finding would imply that exposure to other drugs and environmental contaminants may alter THC metabolism. The discrepancy between the two reports probably can be attributed to differences in experimental design such as vehicle, duration of pretreatment, etc.

## D. Cellular Effects

In an effort to find a biochemical basis for the effects of cannabis on memory and learning, several studies on the influence of THC on macromolecular brain synthesis have been carried out. Roberge and Witschi[306] injected rats with $\Delta^1$-THC and subsequently measured [$^{14}$C]uridine uptake. They found no change in the radioactivity content of DNA isolated from several brain regions.

Using a different approach, Jakubovic and McGreer[307] observed significant inhibitions of brain protein and nucleic acid synthesis. Normal rat brain slices were incubated with the appropriate precursors in the presence of $\Delta^1$-THC, CBD, and cannabigerol. CBD and THC showed comparable levels of inhibitory activity; however, cannabigerol was inactive. While these results are intrinsically of interest they probably represent conditions (drug levels) which would not ordinarily be reached by cannabis users.

The inhibition of uridine incorporation discussed above could be explained by the results of Hodgson et al.[308] They found that chromatin from $\Delta^1$-THC treated rats had a lower capacity to promote RNA synthesis than control rats. They further showed that the effect was not due to an RNA polymerase inhibitor or to increased ribonuclease and suggested that THC depressed brain chromatin template activity.

$\Delta^1$-THC was reported to lower respiration in mouse brain. Nazar et al.[309] found that the effect diminished upon repeated exposure to the drug showing that tolerance had developed. Since this could not be linked to changes in drug distribution, they concluded that it was a cellular effect. Nahas et al.[310] have reported the development of a cellular immune response with $\Delta^1$-THC which they felt was related to tolerance.

The effect of $\Delta^1$-THC on potassium ion influx in rat erythrocytes has been studied by Gibermann et al.[311] They concluded that it was an inhibitor which was pH dependent in a manner similar to certain anesthetics. Schurr et al.[311a] have reported that glucose efflux from erythrocytes is inhibited by cannabinoids.

The influence of cannabinoids on the transformation of cultured rat embryo cells has been studied by Price et al.[312] They observed that $\Delta^1$-THC had moderate activity which was much less than the known carcinogen 3-methylcholanthrene. CBN, CBD, and surprisingly $\Delta^6$-THC showed no activity. This is one of the rare cases where the isomeric THC's show different biological activities.

The production of bone marrow cells in rats can be disrupted by injection of $\Delta^1$-THC.[313] While these results suggest that the use of cannabis may diminish defense against injection, the authors also point out that the drug may be beneficial in combatting certain forms of leukemia.

## E. Endocrine and Hormonal Effects

A clinical study of several biochemical factors was reported by Hollister et al.[314] in which they examined the effects of 30–70 mg of THC and 50–150 mg of synhexyl administered orally. Plasma cortisol levels and platelet serotonin content were not affected; however, the excretion of vanilmandelic acid decreased slightly. The latter finding suggested either a decrease or a shift in catacholamine metabolism.

Kubena et al.[315] reported that corticosterone plasma levels were significantly raised in rats given $\Delta^1$-THC. The response was dose related in the range of 1–4 mg/kg and could not be evoked in hypophysectomized animals. The authors, therefore, concluded that the effect was mediated through the central nervous system.

$\Delta^1$-THC stimulates ACTH secretion and inhibits growth hormone secretion in rats without the development of tolerance even after 20 days (Kokka and Garcia[316]). These effects were similar to those obtained in response to other stressful stimuli showing that $\Delta^1$-THC can alter hypothalamo-pituitary function.

Shahar and Bino[317] have examined the effects of $\Delta^1$-THC on fresh bull sperm. By the use of scanning electron microscopy, they observed a swelling of the mitochondria when exposed to the drug. They also found a decrease in respiration and ATP content as well as changes in sperm motility.

Effects have been reported on both male and female reproductive systems which might be expected from the results of the studies on THC distribution (vide supra). Nir et al.[318] found that the characteristic cyclic luteinizing hormone peak in female rats was completely abolished by two 10-mg injections of $\Delta^1$-THC. They also observed that ovulation had been greatly reduced, an effect which they had also seen with indomethacin. This latter drug is a potent inhibitor of prostaglandin biosynthesis, a property which has also been found for a number of cannabinoids including $\Delta^1$-THC (Burstein et al.[296]). This suggests that the two drugs may be acting on the ovary by similar mechanisms; however, more evidence as needed.

Involution of the thymus gland in rats was reported by Ling et al.[319] with subchronic doses (4–16 mg/kg) of $\Delta^1$-THC. No change was found in the weights of accessory sex organs, testes, kidney, and liver. Adrenal corticosteroid output and ACTH response was also unaffected when determined in vitro following pretreatment with THC. This latter finding does not concur with the above-mentioned report of Kubena et al.[315]

Because of its psychoactive properties, it would be expected that THC should affect at least certain aspects of biogenic amine metabolism. Sofia et al.[320] have reported that serotonin uptake by rat brain synaptosomes is inhibited by $\Delta^1$-THC. Gallager et al.,[321] on the other hand, have found that brain levels of serotonin and 5-hydroxyindoleacetic acid were not altered by treatment of rats with THC. The turnover rates of serotonin were also measured under two sets of conditions and found to be insensitive to the presence of THC. They concluded that the dynamics of the cerebral serotonergic system is unaffected by doses of THC which produce significant behavioral changes. Banerjee et al.[322] have, however, published data which support the report by Sofia et al. $\Delta^1$-THC inhibits the accumulation of norepinephrine and serotonin into hypothalamic preparations and dopamine into the corpus striatum. GABA uptake into cerebral cortical preparations was inhibited less. The affinities of $\Delta^6$-THC, 7-OH-$\Delta^1$-THC, 7-OH-$\Delta^6$-THC, and cannabidiol for the transports of the above neurotransmitters are similar to values for $\Delta^1$-THC, while cannabigerol, cannabinol and $\Delta^6$-THC-7-oic acid have substantially less affinity. Thus hydroxylation of C-7 in $\Delta^6$-THC does not alter inhibitory potency, but its oxidation to an acid and aromatization of ring A greatly reduce affinity. The phenolic hydroxyl is critical for inhibition of uptake, since its acetylation or methylation abolishes activity. Inhibition of neurotransmitter uptake by all cannabinoids examined is noncompetitive. Onl, about 1% of $\Delta^1$-THC and $\Delta^6$-THC and 5% of cannabidiol are fully soluble under the experimental conditions. If uptake inhibition involves only the soluble moiety, then cannabinoids may be considerably more potent inhibitors of the synaptosomal uptake of neurotransmitters than is apparent from the effects of suspensions.

Catecholamine synthesis in rat brain and adrenals can be

increased by the chronic administration of $\Delta^1$-THC (Mazurkiewicz-Kwilecki and Filczewski[323]). However, the levels of norepinephrine, dopamine, and epinephrine in these tissue remained unchanged. This could be explained by the stimulation of tyrosine hydroxylase which has been reported by Ho et al.[324] following administration of $\Delta^1$-THC to rats. This is reasonable since tyrosine hydroxylase is believed to be a rate-controlling enzyme in norepinephrine synthesis.

Immobilization of rats has been found to produce an increase in adrenal tyrosine hydroxylase which may be responsive to certain drugs. Lamprecht et al.[325] using this model have found that $\Delta^1$-THC enhanced enzyme activity which was the opposite of the effects produced by other psychoactive drugs such as chlorpromazine. The authors point out that this may relate to the clinical observation that nonpharmacological factors can affect THC response.

The influence of cannabinoids on biogenic amine concentrations in various brain regions has also been reported by Yagiela et al.[326] and Waters et al.[327] While some effects were observed, detailed mechanisms have not been proposed as yet.

In a series of papers Sabelli and coworkers[328] have studied the effect of $\Delta^1$-THC on the levels of the neuroamine phenylethylamine. They have shown, for example, that a low dose of $\Delta^1$-THC causes a dramatic increase in phenylethylamine levels in the rabbit brain. Since phenylethylamine is believed to "modulate affective behavior", the authors suggest that some of the actions of cannabis could be explained on this basis.

## VI. Addendum

***I. Introduction.*** During the past 10 months the literature on cannabis has continued to grow at the estimated rate of a paper per day. Paton[329] has published a review which updates the pharmacological chapters of the 1973 book on cannabis.[1] The present review supplements Paton's with regard to chemistry and biochemistry in the same monograph. The fourth annual report to the U.S. Congress on marijuana and health has been published.[330] These reports represent excellent surveys of the biological and social aspects of cannabis research. The proceedings of two Cannabis symposia are in press.[331,332] Two short critical overviews on Cannabis chemistry are available.[333] The report of an U.N. Working Group on the chemistry of cannabis smoke has been published.[334]

***II. Chemistry of Cannabis.*** **A. Cannabis Constituents.** Four new minor cannabinoids have been isolated and identified.[335] This brings the grand total of plant natural cannabinoids to 37. Two of the new constituents belong to the cannabielsoin group;[17] another is 2-oxo $\Delta^3$-THC and one is a chromanone.

The first crystallographic report on a THC has been published.[336] The cyclohexane and the pyran rings of $\Delta^1$-THC acid B[13] are in half-chair conformation. The benzene ring is considerably strained. The carboxylic and phenolic groups are significantly out of the plane of the aromatic system. The angle between the aromatic plane and that through the cyclohexene ring is 37.7°. The pentyl side chain takes up an extended gauche conformation. The absolute configuration is confirmed as 3*R*,4*R*.

In the 1973 book on marijuana[337] natural (−)-cannabidiol is wrongly assigned the 3*R*,4*R* configuration (rather than 3*S*,4*R*), due to a technical error, although the formula is correctly drawn and the reaction sequences leading to (−)-(3*R*,4*R*)-$\Delta^1$-THC and (−)-(3*R*,4*R*)-$\Delta^6$-THC leave no doubt as to the configuration.

A new spermidine alkaloid, cannabisativine, as well as the known alkaloid hordenine, have been isolated from *C. sativa*.[338] Zeatine,[339] some common steroids,[340] and flavanoids,[341] as well as 29 known mono- and sesquiterpenes[342] have also been identified.

The fate of the cannabinoid components in marijuana during smoking has been investigated.[343] Approximately 50% of the $\Delta^1$-THC was destroyed on smoking. Contrary to previous findings, some of the cannabinoid acids survived the smoking process. A hitherto unknown material with a GLC retention time equal to that of $\Delta^1$-THC was found. It is probably cannabielsoin, a product of the oxidative cyclization of cannabidiol[91] (or with decarboxylation, of cannabidiolic acid[17]). Carbazole, indole, and skatole (in amounts of 1–15$\mu$/cigarette) were found in marijuana smoke condensate obtained from smoking 30,000 cigarettes in a smoking machine.[344]

*Chemobotanical Aspects.* Further evidence has been presented that the genus Cannabis is polytypic, being comprised of at least three species.[345] This may be a reason for the considerable variations seen in pattern and content of resin and fiber, in particular of $\Delta^1$-THC, cannabidiol, and cannabichromene.[346] However, evidence from the phytotron in Gif-sur-Ivette suggests that genetics alone cannot account for the variability. After several generations of growth the cannabinoid profile tends to drift from the resin type (more THC) to the fiber type (less THC) in a temperate climate and from the fiber type to the resin type in a hot climate.[347] (For a partially conflicting report see ref 77.)

The effect of drying time and temperature on the cannabinoid profile of stored leaf tissue has been investigated.[348]

Interracial grafts between high and low $\Delta^1$-THC strains of *C. sativa,* as well as cross grafts with two Humulus (hop) species has been effected.[349] *C. sativa* strains continue to produce essentially their own characteristic mixtures of cannabinoids. No cannabinoids were found in Humulus stock below the Cannabis graft. Apparently, no cannabinoid beer is forthcoming.

**B. Syntheses of Cannabinoids.** Several new olivetol syntheses have been reported.[350] A one-step synthesis of $\Delta^1$-THC from chrysanthenol has been described.[351] As this monoterpene is not readily available, this synthesis is not of particular advantage over previously described ones. Crombie has reported conditions for the facile syntheses of cannabinoids on a miniature scale.[352] While the routes described are not novel, they are improved to such an extent that they make the various natural cannabinoids rather easy to prepare.

New syntheses of the metabolites 7-OH-$\Delta^1$-THC (**36a**) and 6$\alpha$- and 6$\beta$-$\Delta^1$-THC (**38**a and **37**a) and the first syntheses of the metabolites 6$\alpha$,7-di-OH-$\Delta^1$-THC, 6$\beta$,7-di-OH-$\Delta^1$-THC (**132**), and $\Delta^1$-THC-7-oic acid have been described.[353] The straightforward synthesis of the important metabolite **36a** appears to be the first practical one reported so far (20% yield from $\Delta^1$-THC). It is based on allylic rearrangement of 2-OH-$\Delta^{1(7)}$-THC with hydrobromic acid to give 7-Br-$\Delta^1$-THC which in turn is converted into **36**a. This route follows, in general lines, a synthesis (via a parallel allylic rearrangement) of 7-OH-$\Delta^6$-THC (**42a**) reported earlier.[107]

The full paper on the syntheses of cannabinoid analogs from phloroacetophenone has appeared.[354]

*New Cannabinoid Transformations.* For obvious reasons the products formed on pyrolysis of cannabinoids are of considerable interest. Salemink and his collaborators[355] have now identified in the pyrolysate of cannabidiol several aromatic products formed on cracking. These are olivetol, 2-methylolivetol, 2-ethylolivetol, two benzopyrans, and a benzofuran. Cannabidiol also produces $\Delta^1$-THC, cannabinol, the cannabidiol isomer $\Delta^4$-dihydrocannabidiol, and a new bicyclic cannabinoid, in addition to the previously described cannabielsoin.[91] The effect of heat on hashish has again been discussed.[356a]

Solutions of cannabinol when irradiated at 285 nm, in ethanol, undergo a photochemical transformation yielding a stable

highly fluorescent photoproduct,[356b] which has now been shown to be 4-hydroxy-6,9-dimethyl-2-pentylphenanthrene.[357] The reaction takes place in two sequential stages. The first involves ring opening of cannabinol to form cannabinodiol,[358] which then undergoes photoinduced dehydration, and ring closure to the phenanthrene. Similar irradiation of other cannabinoids yielded the same phenanthrene as the end product.

The lithium-ammonia reduction of a $\Delta^3$-THC homolog and several nitrogen analogs has been described.[359] Opening of the pyran ring was the main reaction in all cases.

The structure and stereochemistry of one of the dihydrobenzofurans (**30**) prepared by condensation of menth-4-en-3-ol with orcinol has been shown (by crystallography) to be 8,5a-*trans*-5a,9a-*cis*-1,8-dimethyl-5a-isopropyl-5a,6,7,8,9,9a-hexahydrodibenzofuran-3-ol.[360]

**C. Structure-Activity Relationships.** One of the main technical problems of testing cannabinoids, especially in vitro or in isolated biological systems, is their insolubility in water. Numerous methods have now been reported aimed at overcoming this difficulty.[361]

The various biological effects of the cannabinoids are not subject to the same structure-activity relationships. Some of the more striking examples are the inactivity of $\Delta^1$-THC, vs. the potent activity of 7-OH-$\Delta^1$-THC, in blocking the impulse conduction in the giant axon of the squid;[362] the development of (and later disappearance of) tolerance to locomotor activity, intestinal motility, and lowering of body temperature in mice which follow different time schedules;[363] the previously noted dichotomy between analgesic activity and locomotor activity;[123] the antiepileptic potency of the psychotomimetically inactive cannabidiol and related compounds[364,365] which parallels that of THC; etc.

The synthesis of polymers of $\Delta^6$-THC and cannabidiol has been reported.[366] The *O*-methacryloyl derivatives of $\Delta^6$-THC and of cannabidiol were synthesized and subjected to free radical polymerization. The parent compounds were also linked to the terminal carbons of polyethylene oxide (PEO) of different molecular weights by a carbonate bond, after transforming the terminal hydroxyl of PEO into their chlorocarbonate derivatives. The hydroxyl groups of $\Delta^6$-THC and cannabidiol were converted to the corresponding chlorocarbonate derivatives which were utilized for the synthesis of a carbonate dimer of $\Delta^6$-THC and of a polycarbonate of cannabidiol, respectively, as well as for the synthesis of carbamate derivatives with amines and amino acids. The psychotropic activity in monkeys of the THC compounds was tested, and some of them showed similar activity to the original $\Delta^6$-THC.

The synthesis of the dimethylheptyl homolog of 9,10-dinor-$\Delta^3$-THC has been reported.[367] This is the first cannabinoid which has no alkyl substitution on the pyran ring $\alpha$ to the oxygen atom. It is only 0.01 times as potent as the corresponding dimethyl compound **94b** in a CNS depression test in rats.

The importance of the phenolic group for the CNS activity of cannabinoids has been pointed out again.[368]

The structure of $\Delta^1$-THC and that of thujone (the active principle in absinthe) have been compared.[369] A similarity of both the molecular geometry and of the biological activity is claimed. Hence a common mechanism of action is suggested. Numerous critical criteria have to be satisfied before the acceptance of this hypothesis.

(+)-$\Delta^1$-THC has been shown[225e] to be at least 13 times less active than natural (−)-$\Delta^1$-THC. As the optical purity of the starting material (verbenol) was not absolute, it is possible that the activity was even lower. This observation confirms previous reports.[1]

Several cannabidiol metabolites and derivatives have been tested for antiepileptic activity. None was more active than cannabidiol itself;[365] 6-oxocannabidiol diacetate at 100–200 mg/kg, however, did not cause decrease of spontaneous motor activity, which cannabidiol does at these high doses.

At least one new cannabinoid has reached the clinical research stage. It is the 1,1-dimethyl homologue of 7-nor-1-oxohexahydro-THC (cf. **99**).[370]

***III. Analytical Aspects of Cannabis Chemistry.* B. Analytical Techniques.** Recently, a number of amines have been tested as impregnating agents for silica gel TLC. Only triethylamine was found to provide a long-term storage capability combined with good resolving power for cannabinoids.[371] Tewari et al., on the other hand, report excellent separations of cannabinoids using TLC on alumina.[372,373]

The use of high-resolution capillary columns for GLC work has been hampered by the need for using either very small sample volumes (0.1–1.0 $\mu$l) or depending on inlet splitters. Both make analysis of low concentrations difficult, and the latter is known to prejudice quantitative results.[374] McCallum has developed a simple device which allows the analysis of large sample volumes (over 10 $\mu$l) without stream splitting and makes the advantages of using capillary columns, e.g., high resolution and its attendant increased sensitivity of detection, available for all types of quantitative analysis for cannabinoids.[375]

Plasma chromatography has been advocated as an analytical method for the determination of cannabinoids in the microgram to picogram range. $\Delta^1$-THC is identifiable by its characteristic positive ion mobility spectra, and it has been suggested that this could become a useful detector for GLC analysis[376] although the technique has yet to be successfully applied in the manner suggested. High-pressure liquid chromatography (HPLC) has been compared favorably to GLC and TLC for analyzing cannabinoid mixtures,[377] and the technique has been extended to separation of the fluorescent 1-dimethylaminonaphthalenesulfonate cannabinoid derivatives, achieving excellent subnanogram sensitivity of detection.[378] Cannabinoid fluorescence is also reported to be produced by a simple thermal treatment allowing down to ca. 100 ng to be detected.[379]

**C. Analysis of Cannabis Constituents.** Wheals et al. claim that chromatograms from HPLC facilitate the classification of cannabis samples into geographical source groups, apparently by recording constituents not revealed by GLC and TLC.[377] No attempt was made to identify the various chromatographic peaks. Gas chromatographic data for a number of cannabinoids (including several, such as cannabidivarol, cannabicyclol, and unnatural isomers of CBD, with retention times shorter than CBD) have been reported for three stationary phases by Stromberg.[380] Turner has published a considerable amount of data on silyl derivatives of cannabinoids and advocates the silyl procedure as a routine method for the analysis of cannabinoids which are difficult to separate in the underivatized form.[381] High-resolution glass capillary columns for GLC offer a simple alternative to silylation for the resolution of "difficult" cannabinoids—as illustrated by the work of Friedrich-Fiechtl and Spiteller.[335] Such quantitative analyses could be conveniently performed using McCallum's inlet device.[375] It is recommended that standard $\Delta^1$-THC solutions are prepared using uv spectroscopy rather than gravimetric methods on account of the notorious difficulty of removing traces of solvent from oils. Dilute secondary standards (in the order of micrograms per milliliters) should be freshly prepared and, if possible, silanized glassware used to minimize absorption.[44a]

**D. Cannabinoids in Body Fluids.** Details and improvements have been published[382] on a radioimmunoassay previously reported.[232a] The detection limit has been reduced to 1 ng/ml, and this assay can be used directly for urine analysis of tricyclic cannabinoids.

Cais et al.[383] have developed a free radical immunoassay

(comparable to the one available for morphine). Preliminary experiments with extracts from human urines collected from both casual and habitual hashish smokers indicate a positive, significant increase in the ESR signal. The sensitivity of this method does not seem to be high enough to allow direct screening of urine samples rather than extracts.

Two-dimensional TLC has found application as a preliminary clean-up procedure for blood and saliva extracts prior to mass spectrometry. Detection of $\Delta^1$-THC in saliva was often successful up to 2 hr after smoking but was unsuccessful in blood except for two instances after an unspecified time interval.[384]

The first investigation on cannabis from the USSR to appear in many years is an analytical patent from Tbilisi.[385] It describes the identification of hashish on extraction of acidified urine of hashish smokers with ethyl acetate. Successful procedures involving TLC separations have been reported for the isolation of $\Delta^1$-THC and metabolites from human urine.[386–388]

An alternative mass fragmentographic method to that of Agurell[145] has been described by Rosenfeld et al.[389] This analytical technique is based on the analysis of $\Delta^1$-THC as its *O*-methyl ether, using the perdeuterio-*O*-methyl ether of $\Delta^1$-THC as an internal standard. The clean-up procedure is based on the selective extractibility of lipid soluble phenols from hexane by Claisen's alkali, and it should be more convenient than Agurell's method which employs chromatography on Sephadex LH 20.[145] Binder et al. have presented MS and NMR data on a number of side-chain hydroxylated derivatives of CBD, CBN and $\Delta^6$-THC. As the mass spectra of the trimethylsilyl ethers of these compounds showed fragmentations specific to the site of hydroxylation, these data are useful as a general method for the identification of side-chain hydroxylated cannabinoid metabolites.[390]

***IV. Biotransformations.*** New interest in the metabolism of CBN has been generated by the observations of several groups that CBN may be an important intermediate in some of the transformations of $\Delta^1$-THC.[258,259,269a] In vitro studies by Widman et al.[391] using rat and rabbit liver homogenates have shown that a series of monohydroxylated derivatives is readily formed with 7-OH-CBN being the major product in both species. Interestingly, 4″-OH-CBN was also an important product in the rabbit indicating that side-chain hydroxylation might be more prevalent in this species. Other side-chain monohydroxy derivatives were also isolated and identified although they occurred in minor amounts; the synthesis of the 2″-, 3″-, and 5″-hydroxy products were also reported.

In vivo studies on the transformations of CBN have also been described by Burstein and Varanelli.[392] Administration of [$^{14}$C]-CBN to the mouse gave rise to **146** and **147** (Scheme XIX) which had been previously reported by the same laboratory to be metabolites of $\Delta^1$-THC in the monkey.[269a] The occurrence of CBN derivatives as common metabolites of $\Delta^1$-THC and CBN lends support to the possibility that CBN is indeed an intermediate in the metabolism of $\Delta^1$-THC.

Lung and liver metabolism of $\Delta^1$-THC were compared in a study using perfused dog lung.[393] In addition to the usual in vitro products, 3″- and 4″-OH-$\Delta^1$-THC were both identified in each tissue. It seems that almost every nonaromatic position in the THC structure can be oxygenated; the major exceptions thus far are the 9 and 10 (*gem*-dimethyl) positions.

Siemens and Kalant[394] have studied the metabolism of $\Delta^1$-THC in rats tolerant to cannabis extract. No differences were found either in the extent or pattern of metabolism when compared with vehicle treated controls. Chronic treatment with phenobarbital, on the other hand, did increase the proportion of polar metabolites. Several of the quantitative aspects of THC metabolism in the rat were investigated by the same authors.[395] They reported values for the apparent $K_m$ ($1.35 \times 10^{-4}$ *M*) and $V_{max}$ (0.18 $\mu$g of THC/mg of protein/min) and studied the effects of dose and time on the course of metabolism. The effects of certain psychoactive drugs on THC metabolism in rats were also examined by Siemens et al.[396] Morphine and mescaline showed no effects while amphetamine and barbiturates decreased metabolism in vitro; none of these drugs had any effect in vivo.

The distribution of $\Delta^1$- and $\Delta^6$-THC in the monkey has been studied by Just et al.[397] They also examined the ratio 7-OH-THC to unchanged drug in several tissues and concluded that the $\Delta^1$ isomer is more rapidly transformed in vivo than the $\Delta^6$ isomer. Irvin and Mellors[398] have determined the subcellular distribution of [$^{14}$C]-$\Delta^1$-THC in rat liver cells. They reported that the radioactivity was concentrated in the lysosomes and suggested that this might cause certain toxic manifestations associated with chronic marijuana use.

Both the acute and chronic administration of $\Delta^1$-THC to rats causes an increase in monoamineoxidase activity in certain tissues.[399] One of the largest increases was in the hypothalamus, suggesting this as an important site of action for THC. Yoshimura et al.,[400] on the other hand, have shown a correlation between rat behavior and brain acetylcholine metabolism. A possible underlying mechanism which might accommodate these and other observations would be the modulation by THC of the prostaglandin–cyclic nucleotide regulatory system (vide supra). In this connection, Burstein et al.[401] have reported finding additional inhibitors of prostaglandin biosynthesis in cannabis. Investigation of the volatile oil fraction revealed the presence of at least two highly active substances, one of which was identified as eugenol.

Gaul and Mellors[402] report that $\Delta^1$-THC depresses the activity of macrophage migration inhibition factor. This effect was related to clinical observations in the literature.

The effects of cannabinoids on endocrine secretions such as prolactin,[403,404] plasma testosterone,[405,406] plasma corticosterone,[407] growth hormone, luteinizing hormone, and follicle stimulating hormone[408] have been reported. Several of these are of a conflicting nature (prolactin and testosterone) indicating, perhaps, that more subtle factors may be involved.

Finally, Gill and Lawrence[409] have reexamined their previous experiments on the metabolism of [$^3$H]-7-OH-$\Delta^1$-THC (see ref 226). The earlier report of a less polar metabolite was shown to be due to the presence of $\Delta^1$-THC in the injection mixture. They also found that both blood and brain levels of $\Delta^1$-THC were raised in the presence of 7-OH-$\Delta^1$-THC and suggested that this might be caused by competition for tissue binding sites.

*Acknowledgment.* The preparation of this study was supported through the Special Foreign Currency Program of the National Library of Medicine, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Maryland, under an agreement with the *Israel Journal of Medical Sciences*, Jerusalem, Israel.

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