Mass spectrometry of the cannabinoids and their metabolites

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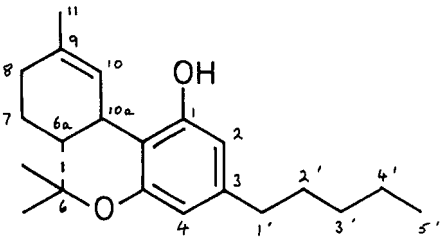
I. INTRODUCTION

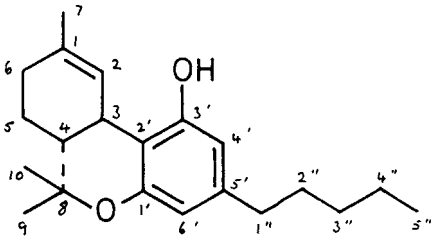
The cannabinoids are a group of over 60 terpene-related compounds (1), unique to the plant *Cannabis sativa* L., and the active constituents of marijuana, one of the earliest known psychoactive drugs. The drug has a recorded history that can be traced back to the Chinese literature of around 3000 BC, and it has been in constant use in many cultures to the present day under a wide range of preparations and names such as *bhang, charas, pot, grass, dagga, hashish, marijuana,* and *Cannabis.* (For general reviews on the chemistry and pharmacology of cannabis preparations see, for example, Refs. 2–4.) In contemporary Western society, marijuana is generally regarded as the most widely used of the popular drugs of abuse, after alcohol and tobacco, and most countries have regulations which attempt to restrict or prevent its consumption. The drug is extremely potent, and only about 10 mg of the major active constituent, delta-9-tetrahydrocannabinol (delta-9-THC, **1**) is sufficient to produce euphoria, hallucinations, and adverse changes in the cardiovascular system. Although acute toxicity is low, the highly lipophilic



1a

Delta-9-tetrahydrocannabinol

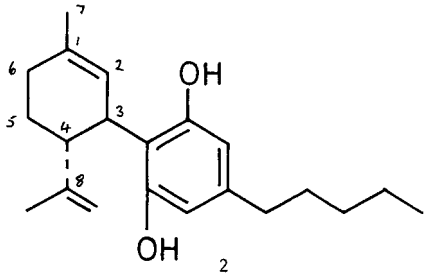
(benzpyran numbering)



1b

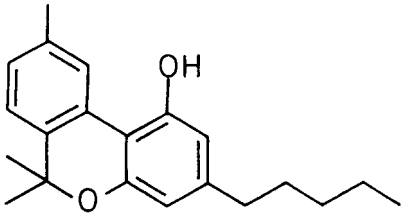
Delta-1-tetrahydrocannabinol

(monoterpene numbering)



2

Cannabidiol (CBD)



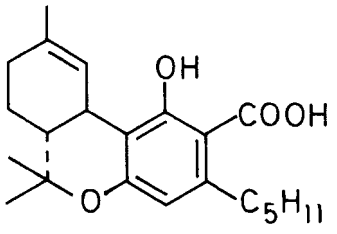
3

Cannabinol (CBN)

nature of the cannabinoids causes considerable accumulation in tissues during chronic use, and this can lead to psychoses, pronounced adverse effects on reproductive function, immunological response and learning, and can induce a state resembling that of partial anaesthesia. Smoking is the preferred route of administration for this drug, but oral consumption is by no means uncommon. Both lead to somewhat different effects; smoking causes some decomposition of the drug with the production of smoke more carcinogenic than tobacco smoke, and oral administration results in higher concentrations of active metabolites in the blood as the result of extensive first-pass metabolism.

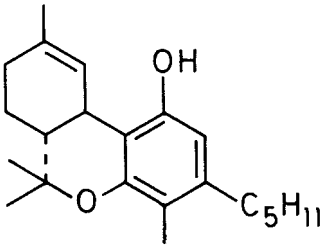
Although the cannabinoids are found in many plant tissues, they are concentrated in the resin associated with the flowering tops of the female plants (5). Unfertilized female plants yield a particularly potent form of the drug known as sinsemilla (6), which is now being extensively cultivated in the United States. The most important members of the cannabinoid group are delta-9-THC, (**1**), cannabidiol (CBD, **2**), and cannabinol (CBN, **3**) of which delta-9-THC is the major psychoactive constituent. Its structure was elucidated as recently as 1964 (7). Unfortunately, two numbering systems, the benzpyran system (**1a**) and the monoterpene system (**1b**), have persisted in the literature for this compound; the benzpyran system is preferred by workers in the United States and will be used in this review. CBD, the biochemical precursor of delta-9-THC and CBN, a chemical decomposition product, are usually also present in relatively high abundance, although the relative proportion of these and other cannabinoids varies greatly depending on the geographical origin, strain, and age of the sample. Cannabis grown in cool climates generally has low concentrations of delta-9-THC, whereas that cultivated in hotter regions can produce a resin containing delta-9-THC concentrations approaching 10% by weight with very little other cannabinoid material present. Cannabinoids are present in the plant as their acid derivatives (e.g., **4** and **5**), but these are relatively unstable, decarboxylating slowly at room temperature but rapidly when the drug is smoked. In addition, most, if not all, cannabinoids are members of homologous series. Homologues having a C_5 side-chain are the most common but also present, in varying amounts depending on strain, and listed in order of decreasing abundance are propyl, methyl, butyl, and possibly ethyl homologues.

After administration to man or animals, the cannabinoids are rapidly taken



4

Delta-9-THC acid-A



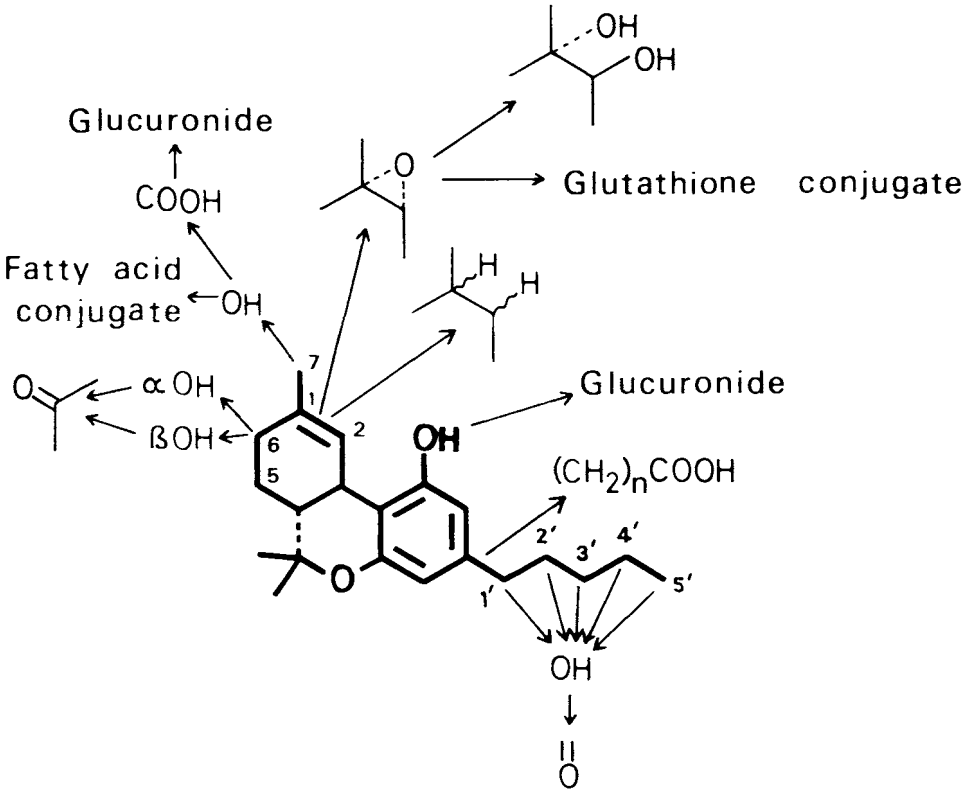
5

Delta-9-THC acid-B

up into adipose tissue (8). This causes their concentration in blood to fall quickly to levels that cannot be measured by even the most sensitive of existing analytical techniques (see refs. 4 and 9–11). Typical concentrations during intoxication are in the mid ng/mL range but these drop to the pg/mL range within a few hours (12). The drop in concentration shows polyphasic kinetics with up to five phases having been reported for several species (13–15). However, the half-life of the final phase describing the distribution of delta-9-THC in man is not yet known with certainty (16), as existing analytical techniques are not sufficiently sensitive to measure the low concentrations of the drug present under these conditions. In rabbits, terminal half-lives of 30 to 123 h have been reported after a single dose (14) and in dogs, a value of 8 days has been found (13) under similar conditions.

Metabolism of the cannabinoids is complex (12) as illustrated by reports of over 100 metabolites from delta-9-THC alone. Although metabolism of only three natural cannabinoids, delta-9-THC, CBD, and CBN and two synthetic isomers of THC (delta-8- and delta-9(11)-THC) have so far been studied, it is apparent that the compounds are extensively oxidized by the cytochrome P-450 system to mono-, di-, and tri-hydroxy metabolites and that most of the metabolically introduced hydroxy groups can be further oxidized to ketones and carboxylic acids. The major metabolic route shown by delta-9-THC is allylic hydroxylation at C-11 followed by oxidation to delta-9-THC-11-oic acid. This acid is excreted in urine predominantly as the acid glucuronide conjugate (17). Other routes of metabolism are outlined in Scheme 1 and the extent to which each route is seen depends on the species in which metabolism is studied. Delta-8- and delta-9(11)-THC, CBD, and CBN are metabolized by similar mechanisms, although the absence of allylic hydroxylation in CBN results in fewer metabolites being produced.

The extent to which Cannabis is now used in Western society has raised the question of whether or not it is harmful. It is generally not regarded to be as dangerous as drugs such as the opiates where dependence and severe withdrawal symptoms are easily demonstrable and there is strong pressure in many quarters for the drug to be legalized. This has led to a considerable research effort to define the drug's toxic effects and to ascertain whether or



Scheme 1 Metabolic routes for delta-9-THC

not such claims for legalization are justified. This has proved not to be easy in the case of Cannabis. Adverse effects such as those already described are now well recognized by the scientific community as the result of Cannabis use. However, defining the extent to which they occur in the general public is complicated by factors such as polydrug use, particularly from alcohol, and by the absence of easily recognizable addictive signs such as withdrawal symptoms.

The reason for this apparent lack of withdrawal is now thought to be related to the drug's high lipid solubility which causes it to be retained in tissues. During chronic use, considerable concentrations of the drug can accumulate in fat and other lipids such as cell membranes and can adversely affect the functions of many body functions. Concentrations of the drug in the brain can be high enough for the drug to have a permanent residual depressive action. Because of the slow onset of this accumulation, this effect is not noticed by users unless they suddenly stop taking the drug. Then a "clarification" of thought processes occurs over a period of months. This slow withdrawal as the drug leaves the system is undoubtedly responsible for the lack of classical withdrawal symptoms as the body has time to adapt to the slowly falling drug concentration.

Quantification of this process has been difficult on account of the low level

of drug in the blood, and the pharmacology is further complicated by the large number of metabolites, several of which are pharmacologically active and are eliminated at different rates. In addition, the great variety of samples of the drug containing cannabinoids in very different relative proportions necessitates careful analysis of the individual components and the determination of any interactions between them. Thus, from a chemical point of view, there is considerable scope for mixture analysis and measurements of drugs and their metabolites at very low concentrations. The large number and low concentration of metabolites has made GC/MS the major analytical method used for their identification. The sensitivity of the technique has been exploited in many laboratories for quantitative measurements and has led to the development of the most sensitive assays available, more sensitive even than radioimmunoassay. On the forensic side, there is scope for metabolite monitoring in the determination of the time of drug abuse and for measurements of cannabinoid ratios in confiscated samples in attempts to determine the nature and origin of suspect material. It must be said, however, that the cost of GC/MS equipment usually restricts these applications to confirmation of data obtained by cheaper but less specific techniques. This review covers all aspects of the application of mass spectrometry to work on the cannabinoids, both qualitative and quantitative, and shows how the technique has been applied in metabolic, pharmacokinetic, and forensic studies of these compounds. Previous reviews on various aspects of the mass spectrometry of cannabinoids are given in Refs. 18–24.

II. FRAGMENTATION OF NATURAL CANNABINOIDS

Knowledge of the fragmentation mechanisms induced by electron ionization has been a key feature in the identification of natural cannabinoids, and many detailed studies have been reported. Much of the structural identification has been conducted with derivatives such as trimethylsilyl (TMS) ethers, and so the fragmentation of these derivatives is discussed together with that of the parent compound in this section. Deuterium labelling has played a major part in these studies. Initially, deuterated cannabinoids were synthesised specifically for mass spectrometric work. However, later work on cannabinoid quantification necessitated internal standards labelled with stable isotopes, and this made available several other deuterated compounds for fragmentation studies.

A. Tetrahydrocannabinols

The mass spectra of isomeric tetrahydrocannabinols contain ions at common masses, but their relative abundance is frequently very different, thus affording ready identification. This can be seen from Fig. 1 (25), which shows the spectra of delta-9- (**1**), delta-8- (**6**), delta-6a(10a)- (**7**), and delta-10-THC (**8**). The spectrum of delta-6a(10a)-THC is the simplest and is dominated by the $[M - CH_3]^+$ ion produced by loss of one of the gem methyl groups

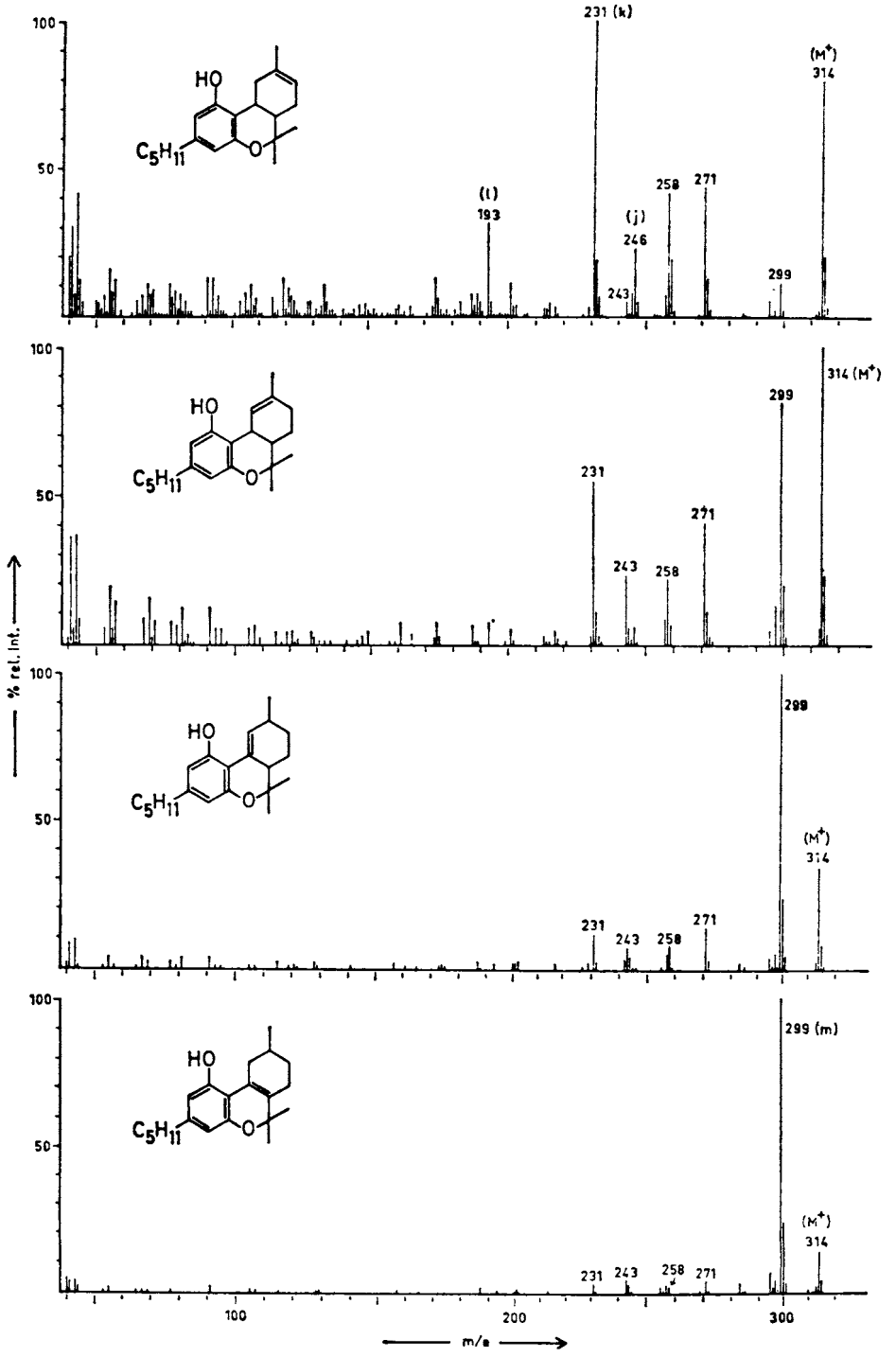
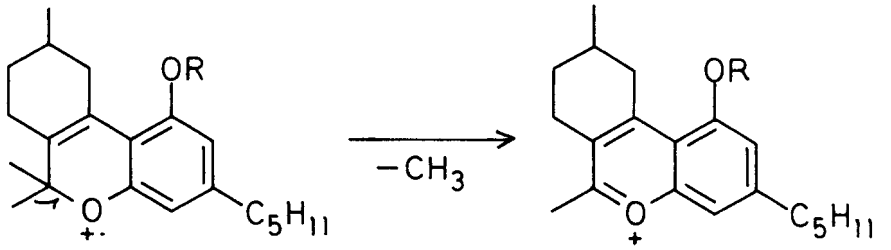


Figure 1. Mass spectra of delta-8-THC, delta-9-THC, delta-10-THC and delta-6a(10a)-THC. (Reproduced with permission from Pergamon Press Ltd.)



a, R = H, m/z 299

R = TMS m/z 371

Scheme 2

(Scheme 2). The corresponding ion in the mass spectrum of the delta-10-isomer is nearly as abundant, but the origin of the eliminated methyl group has not been confirmed. Ions produced by competing fragmentations are much more abundant in the mass spectra of delta-8- and delta-9-THC as can be seen from Fig. 1, and the molecular ions are abundant, reflecting the general stability of these molecules. In general, corresponding ions are also seen in the mass spectra of most derivatives of these compounds (26) as the

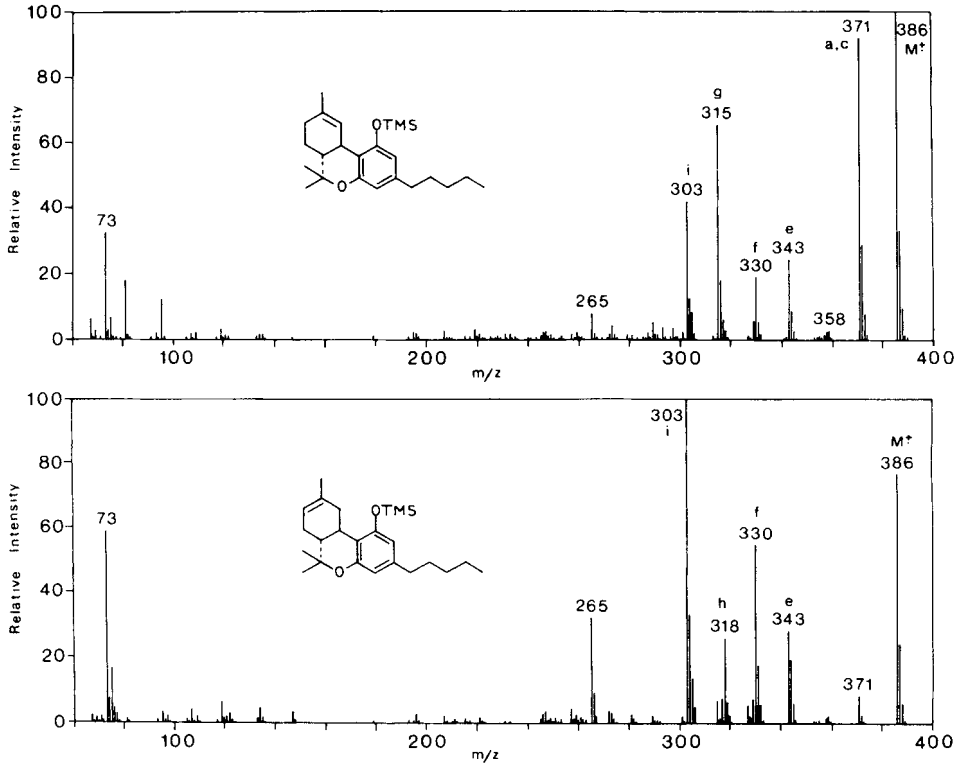
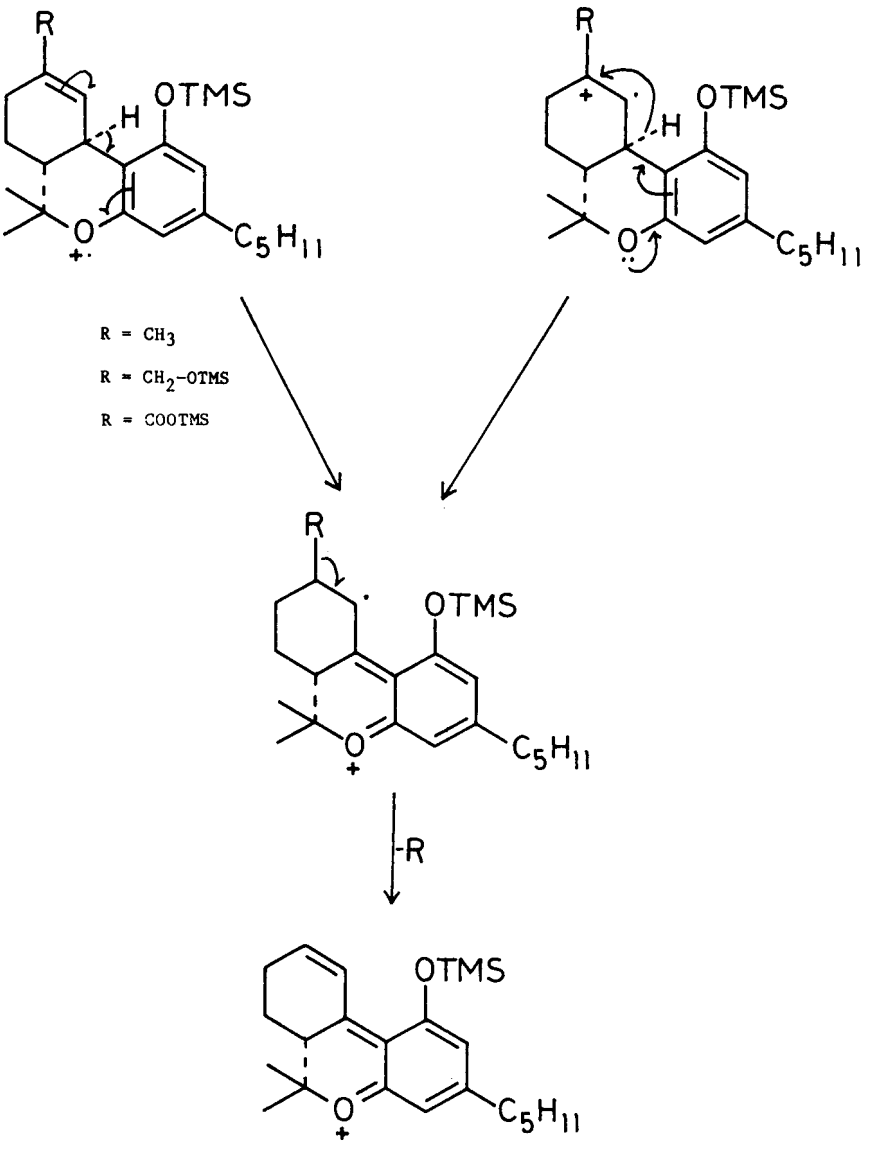


Figure 2. Mass spectra (25 eV) of the TMS derivatives of delta-9-THC and delta-8-THC.

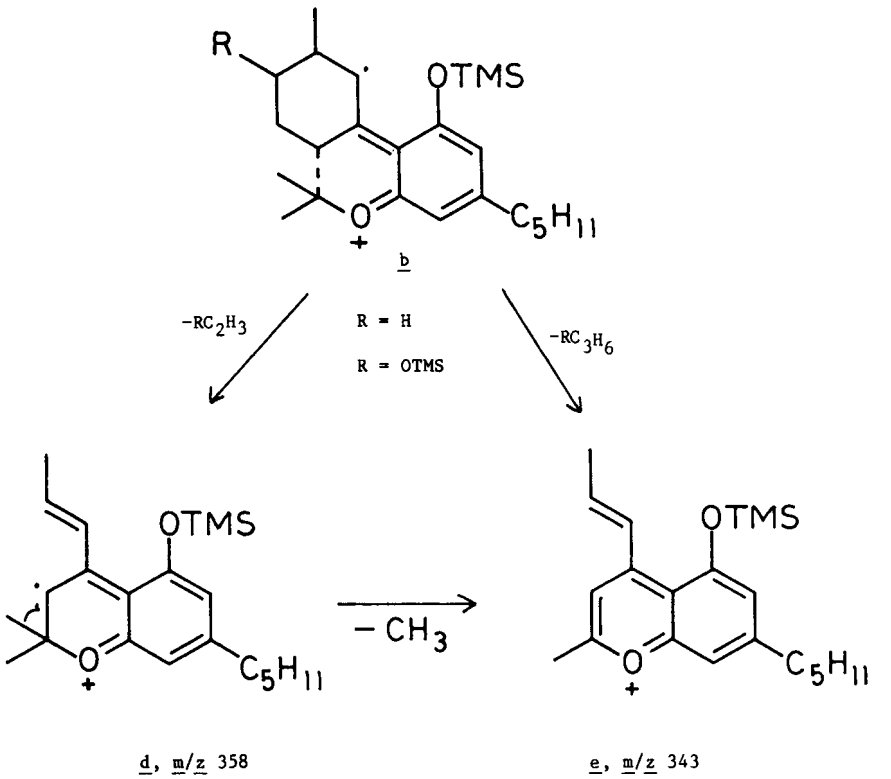


Scheme 3

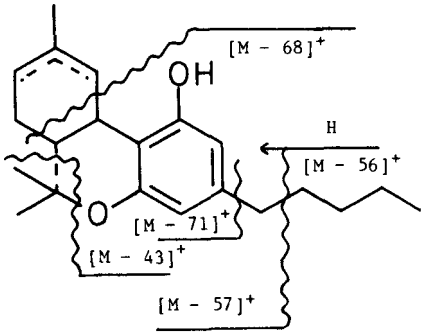
fragmentation is initiated at sites remote from the derivatized function. This can be seen from Fig. 2, which shows the spectra of the TMS derivatives of delta-8- and delta-9-THC. The $[M - CH_3]^+$ (*m/z* 299) ions in the spectra of these two nonderivatized compounds appear to arise by at least two mechanisms, elimination of one of the gem methyl groups as in Scheme 2 or loss of the C-11 carbon atom. The mass spectrum of the TMS derivative of [11,11,11-

$^2H_3$]delta-9-THC shows that 68% of the methyl loss involves C-11 (27) and the fragmentation pathway shown in Scheme 3 can be drawn (27). The low abundance $[M - CH_3]^+$ ion in the spectrum of the TMS derivative of the delta-8-isomer can be rationalized by the absence of this pathway as hydrogen migration would not lead to the conjugated species *c*. Additionally, in the spectrum of the TMS derivative of delta-9-THC, 2% of the methyl loss is from the TMS group as shown by the spectrum of the [$^2H_9$]TMS derivative (27). A higher proportion (27%) of the methyl loss in the spectrum of the TMS derivative of delta-8-THC arises from the TMS group, again reflecting the absence of the mechanism shown in Scheme 3.

Loss of 28 mass units from the molecular ion gives the low abundance ion at *m/z* 286 in the mass spectra of nonderivatized THC's and at *m/z* 358 in the spectra of the TMS derivatives. In the latter spectra, this ion subsequently appears to lose a methyl radical to give the ion at *m/z* 343 ($[M - C_3H_7]^+$, Scheme 4). However, in the case of nonderivatized delta-8-THC, the fragmentation appears to be elimination of both gem-methyl groups together with C-6 (Scheme 5), as shown by deuterium labelling (28). Budzikiewicz et al. (29) proposed a mechanism involving transfer of the hydrogen atom from the C-10a position to C-6, but later work with deuterium-labelled compounds



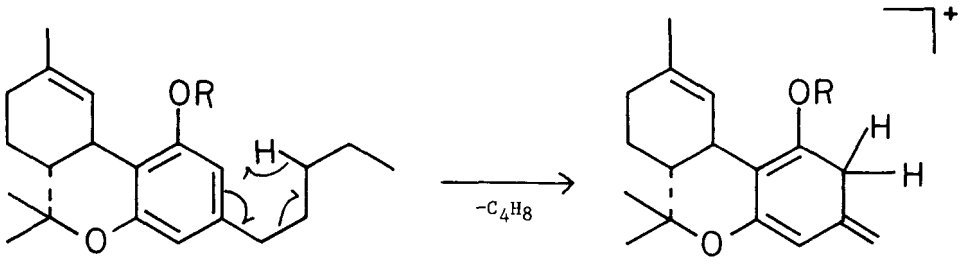
Scheme 4



Scheme 5

(27,28) showed that this mechanism is incorrect as the deuterium at C-10a is retained by both isomers. The fragmentation producing the ion at $[M - 43]^+$ in this spectrum has not yet been elucidated.

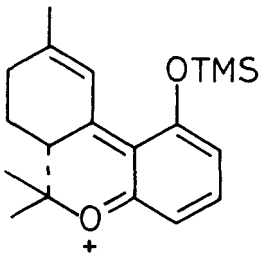
The ion at *m/z* 258 (330 in the spectrum of the TMS derivatives), corresponding to $[M - C_4H_8]^{+\cdot}$, is formed by loss of C_4H_8 from the side-chain according to Scheme 6 (27,29). Formation of the ion one mass unit lower



f, R = H, m/z 258 ([M - 56]+)

R = TMS, m/z 330

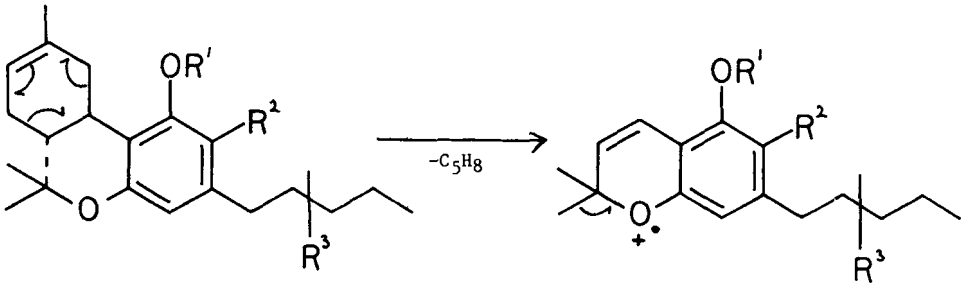
Scheme 6



g, m/z 315

evidently involves cleavage of the same carbon-carbon bond but without hydrogen transfer as shown in Scheme 5 (27). Loss of the entire side-chain gives the ion at $[M - C_5H_{11}]^+$ for which the structure *g* may be drawn (27). A metastable ion indicates direct formation of *g* from the molecular ion, and the higher abundance of ion *g* in the spectrum of delta-9-THC than in that of the delta-8-isomer implicates involvement of the delta-9-double bond in the mechanism for its formation. A mechanism proposed earlier for the formation of this ion (18,29) involving loss of CH_3 from ion *f* is largely inconsistent with the data from deuterium labelling studies and has been found to contribute only 6% to the formation of this ion.

A prominent retro-Diels-Alder ion (*h*, $[M - 68]^{+\cdot}$) is present in the mass spectrum of delta-8-THC and its derivatives (Scheme 7) but not in that of

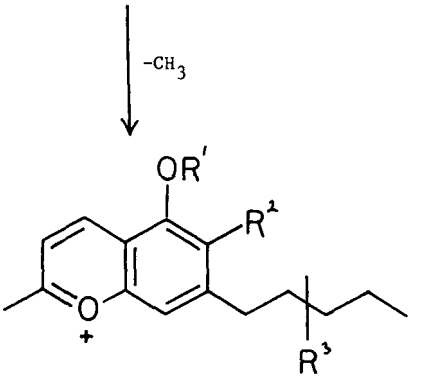


h, $R^1 = R^2 = R^3 = H$, m/z 246

R^1 = TMS, $R^2 = R^3 = H$, m/z 318

$R^1 = R^3$ = TMS, $R^2 = H$, m/z 406

R^1 = TMS, R^2 = COOTMS, $R^3 = H$, m/z 430



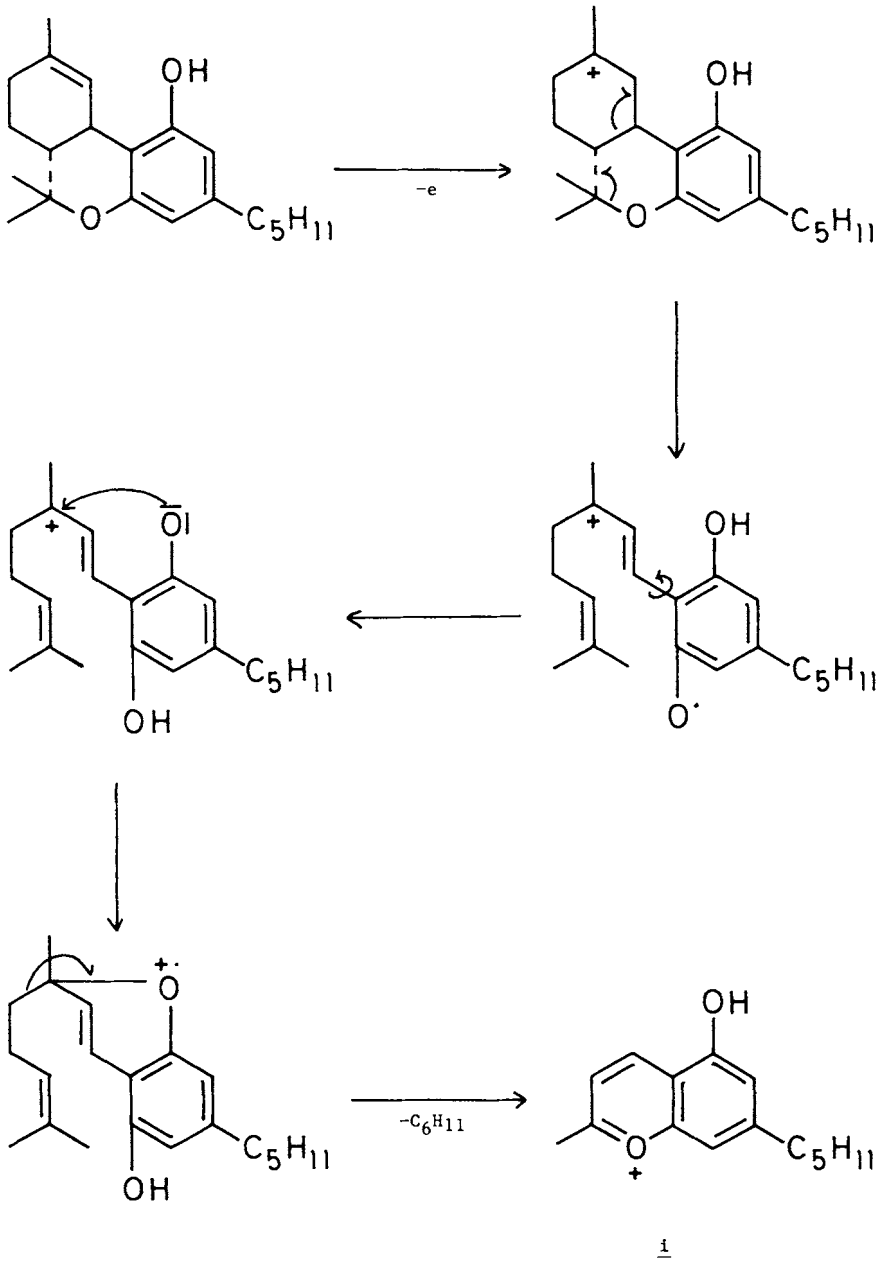
i, $R^1 = R^2 = R^3 = H$, m/z 231

R^1 = TMS, $R^2 = R^3 = H$, m/z 303

$R^1 = R^3$ = TMS, $R^2 = H$, m/z 391

R^1 = TMS, R^2 = COOTMS, $R^3 = H$, m/z 415

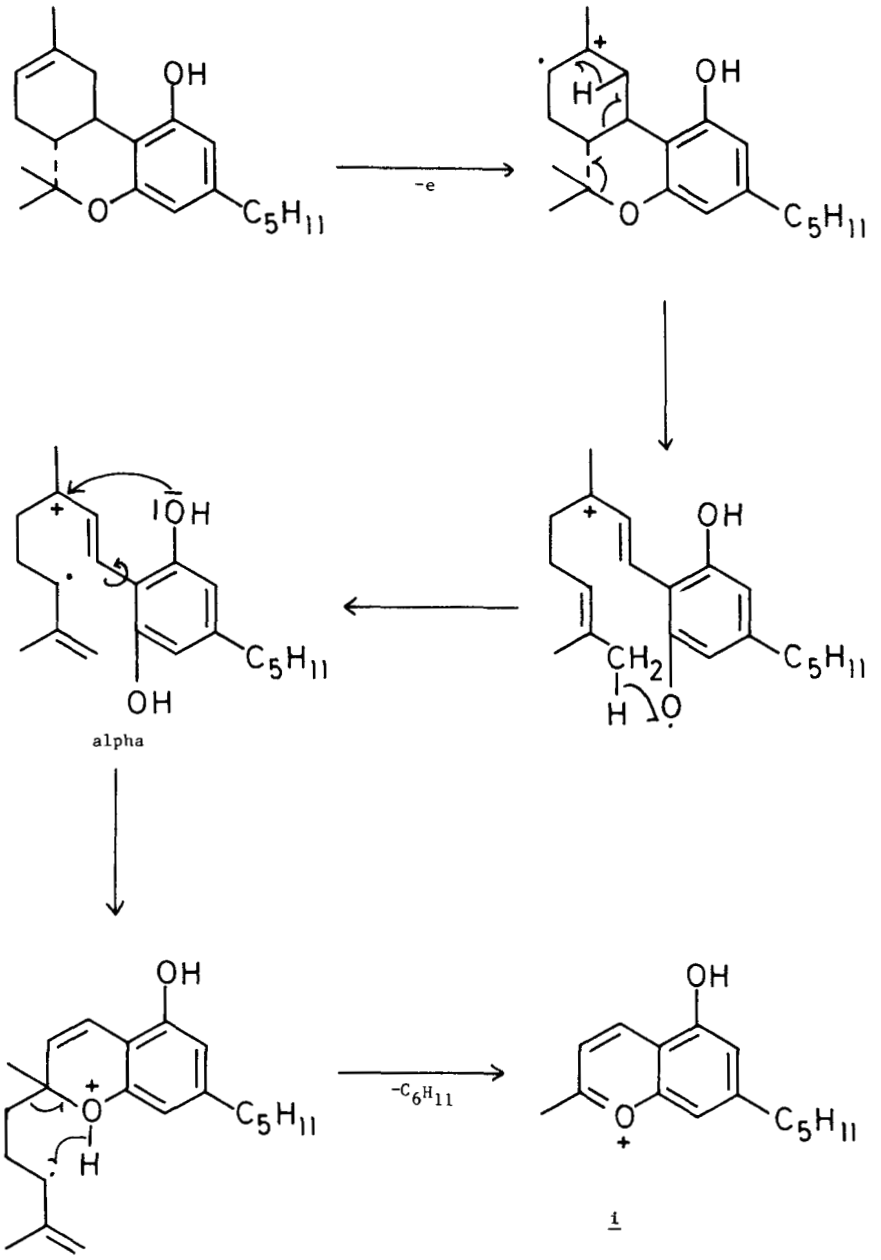
Scheme 7


OH
O
C_5H_{11}
-e
OH
O
C_5H_{11}
OH
O·
C_5H_{11}
OH
OH
C_5H_{11}
O
C_5H_{11}
OH
$-C_6H_{11}$
OH
O
C_5H_{11}

i

Scheme 8

delta-9-THC. Loss of a methyl radical from ion *h* gives ion *i*, which is responsible for the base peak in the spectrum of the delta-8-isomer. This ion has proved of great use in metabolite identification as its presence at the same mass as in the parent compound indicates metabolic transformation in the terpene-derived part of the molecule rather than in the side-chain. Although the retro-Diels-Alder reaction does not lead to fragmentation in



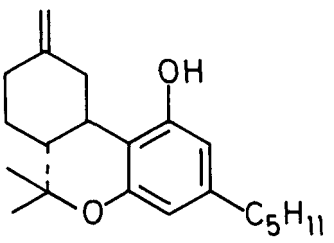
i

Scheme 9

the case of the delta-9-isomer to give ion *h*, ion *i* is formed in reasonable abundance and is, therefore, probably formed via an alternative mechanism. Isomerization to the delta-8-isomer was originally proposed (29) but was later shown to be a minor process (30). In a study of the fragmentation of the delta-6a(10a)-*cis*- and both the *trans-para* and *trans-ortho* isomers, in which the phenol group and pentyl side-chains are reversed, it was concluded that in the nonderivatized compound, transfer of the phenol hydrogen to the terpene ring occurs (30). This mechanism is now thought to be incorrect. Later, metastable evidence (28) showed three pathways to this ion, two dual step processes involving the loss, in either order, of C_5H_{10} as in the retro-Diels Alder mechanism, and of a methyl radical, and a direct pathway from the molecular ion for which the mechanism shown in Scheme 8 was proposed. In later work with the delta-8-isomer (31), labelling of the gem-methyl groups and the C-11 hydrogens with deuterium showed that the fragmentation involved retention of the C-11 carbon atom, and the mechanism shown in Scheme 9 was proposed. Rotation of the 10a–10b bond of intermediate *alpha* in a percentage of the ions was proposed in order to account for the observed hydrogen isotope ratio in the analogue deuterated at the gem-methyl positions.

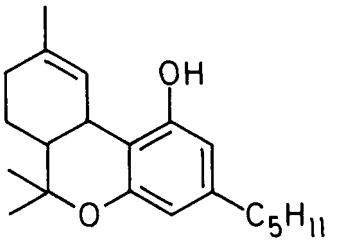
The mass spectrum of the TMS derivative of the synthetic exocyclic isomer, delta-9(11)-THC (**9**) (32) again shows similar ions with a prominent molecular ion and a base peak at *m/z* 371 ($[M - CH_3]^+$). The mass spectrum of the nonderivatized molecule has been reported to contain a prominent ion at $[M + 2]^{+\cdot}$ (33) caused by reduction inside the mass spectrometer.

6a(10a)-*cis*-delta-9-THC (**10**) has been identified as a natural Cannabis constituent in Cannabis samples with a high CBD to THC ratio (34). Its mass spectrum is very similar to that of 6a(10a)-*trans*-delta-9-THC (the major isomer) except that the relative abundance of the ion at *m/z* 231 (*i*) is higher (100%). (±)-*cis-abn*-delta-9-THC (**11**) is a synthetic cannabinoid (35), and its mass spectrum is again similar to that of 6a(10a)-*trans*-delta-9-THC. (±)-*cis*-delta-8-THC has a mass spectrum resembling that of its trans isomer (36).



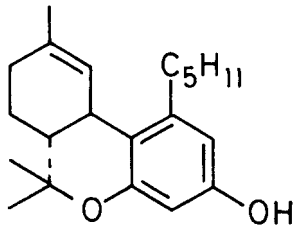
9

Delta-9(11)-THC



10

6a(10a)-cis-delta-9-THC



11

abn-delta-9-THC

B. Hexahydrocannabinol (HHC)

The mass spectrum of the two isomers of this compound, epimeric around C-9 are indistinguishable and similar to that of delta-9-THC. The molecular ion and five fragment ions are abundant with losses of C_3H_7 (to give an ion at *m/z* 273) and C_4H_8 (to give an ion at *m/z* 260) parallelling those in THC (29). It has been proposed that the formation of the *m/z* 231 ion, *i*, involves loss of the terpene ring together with a methyl group (29). The only other major ions are the abundant tropylium ion at *m/z* 193 and an ion at *m/z* 233 whose structure has not been determined but which would appear to be a dihydro analogue of ion *i* (*m/z* 231).

The two isomers of HHC also give similar spectra as their acetate or TMS derivatives. The mass spectrum of the acetate has ion *i* (*m/z* 273) as the most abundant and contains prominent ions produced by loss of ketene and the acetyl radical, whereas the mass spectrum of the TMS derivative parallels that of the nonderivatized compound with the molecular ion as the most abundant. The mass spectrum of the compound labelled with deuterium at the 10a-position shows multiple pathways for the formation of the tropylium ion (*m/z* 265), ion *i* (*m/z* 303), and the ion at *m/z* 305; the mechanisms have not been elucidated.

C. Cannabinol (3)

This was the first cannabinoid to be isolated from *Cannabis sativa* and its structure has been known for some 50 years. Its mass spectrum and those of its derivatives (see Fig. 3) are very simple and consist mainly of the molecular ion (approximately 10% relative intensity) and the most abundant ion which is produced by loss of a gem-methyl group in a similar mechanism to that shown in Scheme 2. Loss of butylene, as in Scheme 6 (29) is observed from the $[M - 15]^+$ ion to give a low abundance ion at *m/z* 239 (6%), and loss of 72 mass units from the molecular ion gives the only other prominent ion at *m/z* 238. This latter ion is at the same mass in the mass spectra of CBN homologues and must, therefore, involve elimination of the carbon atoms from the side-chain. Elimination of C_4H_9 and a methyl group is a possibility although these losses would give an odd-electron ion. Cannabinol mono-

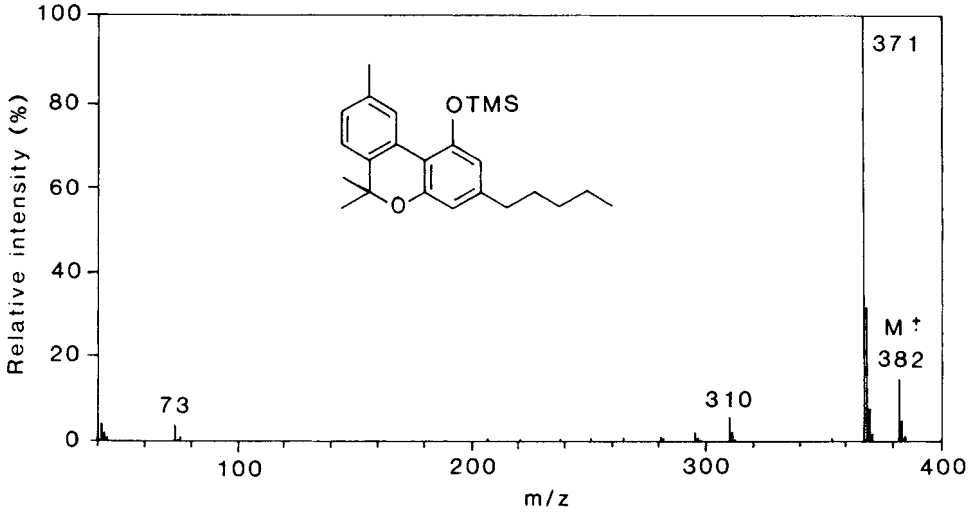


Figure 3. Mass spectrum (25 eV) of the TMS derivative of CBN.

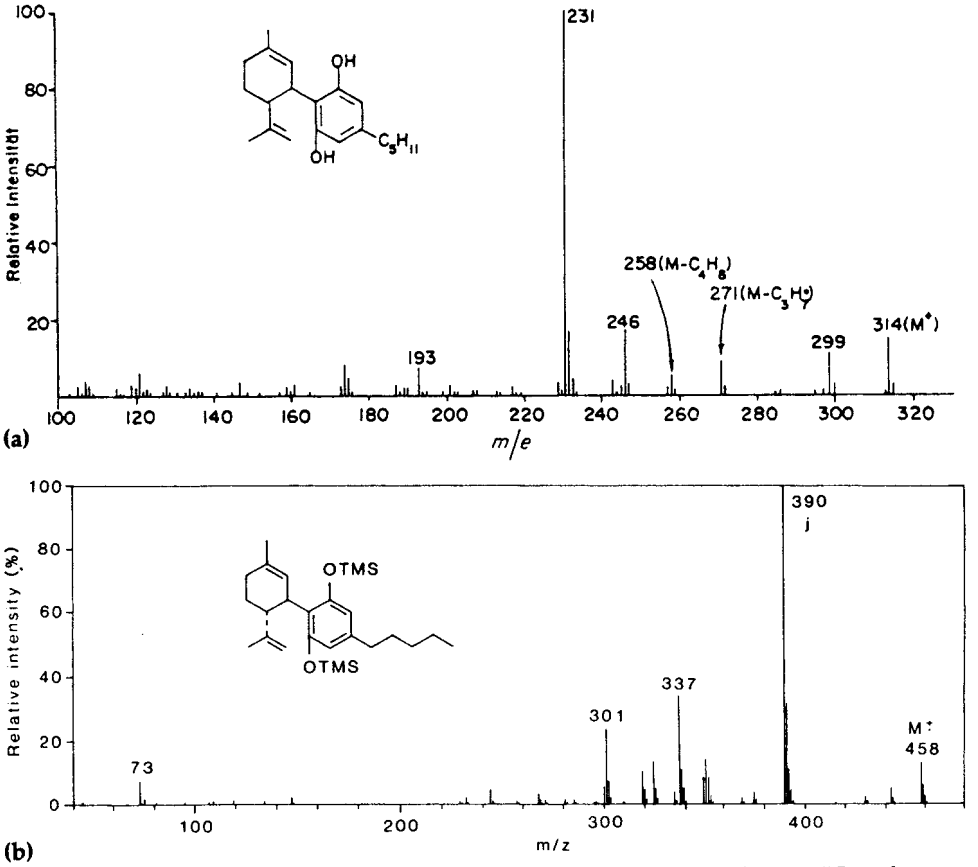
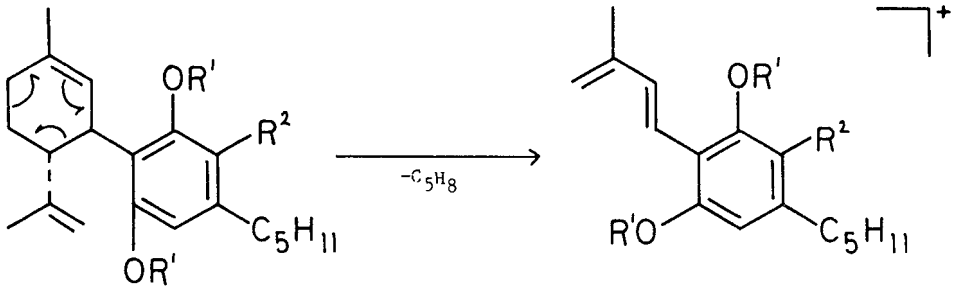


Figure 2. Mass spectra (25 eV) of the TMS derivatives of delta-9-THC and delta-8-THC.

methyl either gives a similar mass spectrum to that of CBN, but all ions show a mass increment of 14 u (37).

D. Cannabidiol (2)

Ions in the mass spectrum of cannabidiol (Fig. 4a) have the same masses as in the spectra of the THCs, and several ions, such as that produced by loss of butylene from the molecular ion, are formed by related mechanisms. Retro-Diels-Alder cleavage of the terpene ring (29) gives the ion at *m/z* 246, *j*, (Scheme 10) in about 20% relative abundance and loss of a methyl radical from this ion gives the most abundant ion at *m/z* 231 (ion *k*). However, in the mass spectrum of the dimethyl ether (25), the retro-Diels-Alder ion (*j*) at *m/z* 274 is the most abundant as confirmed by the spectrum of the deuteromethyl analogue. The same is also true for the TMS ether which gives an ion at *m/z* 390 (Fig. 4b). Ions at the same mass appear in the spectra of the delta-8-isomers as the result of retro-Diels-Alder cleavage, but the eliminated fragment is different as shown in Scheme 11. Loss of a methoxy radical from ion *l* in the mass spectrum of the dimethyl derivative of CBD gives ion

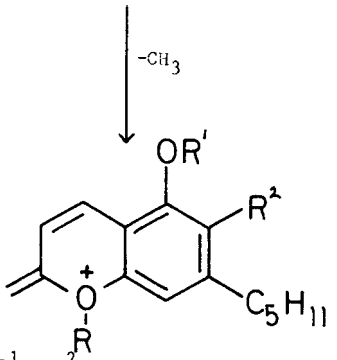


j, $R^1 = R^2 = H$, m/z 246

$R^1 = CH_3$, $R^2 = H$, m/z 274

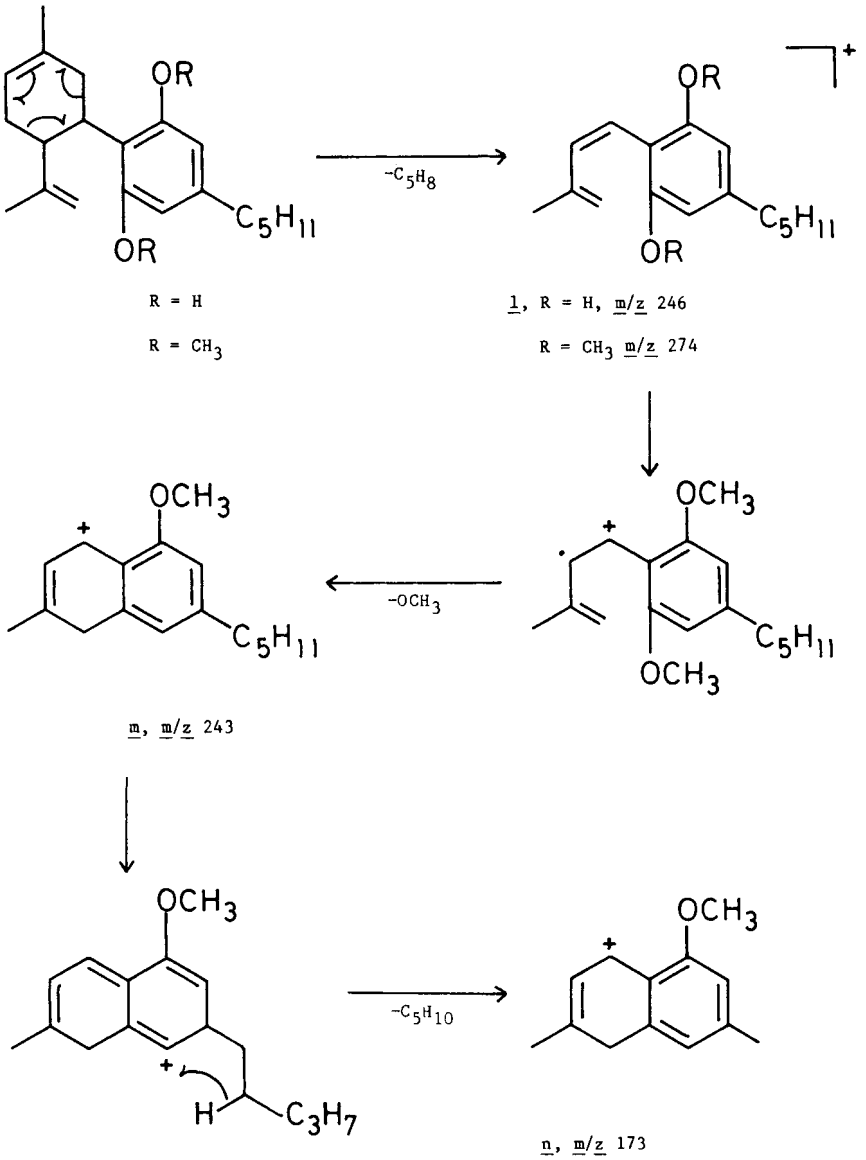
R^1 = TMS, $R^2 = H$, m/z 390

R^1 = TMS, R^2 = COOTMS, m/z 506



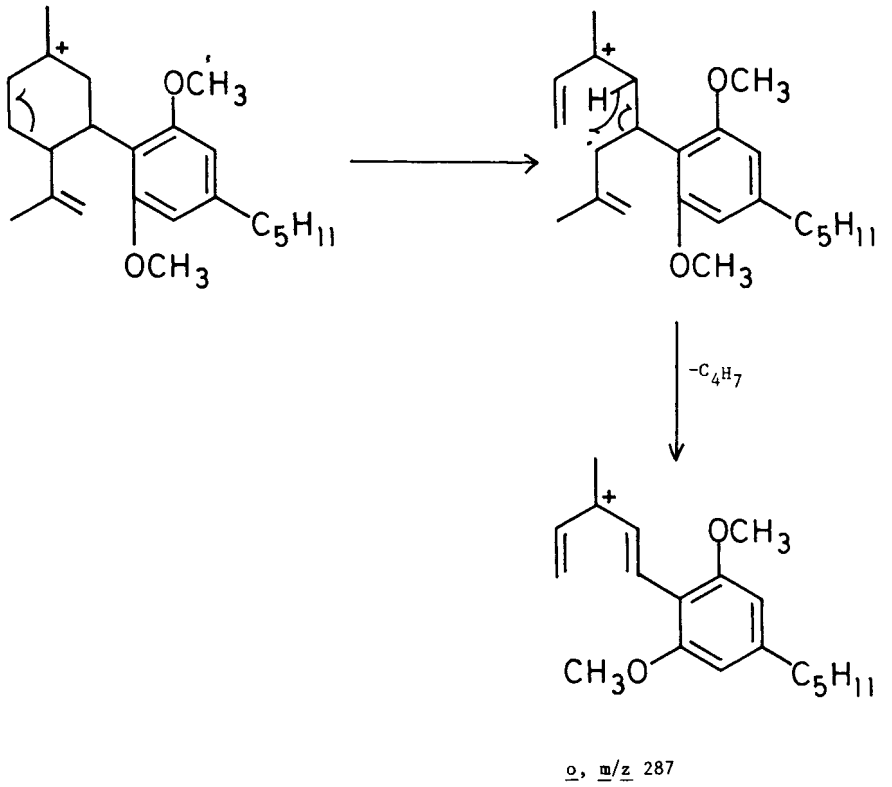
k, $R^1 = R^2 = H$, m/z 231

Scheme 10



Scheme 11

m (Scheme 11) and further elimination of the side-chain from this gives ion *n*. Further studies on the fragmentation of CBD and its methyl ethers were reported by Calussen and Korte (38), who used deuterated analogues and the methyl homologue. Spectra of the dimethyl ether of delta-6-CBD show both qualitative and quantitative differences from that of the delta-1-isomer with delta-6-CBD giving a prominent ion at *m/z* 287 (*o*). The mechanism shown in Scheme 12 has been proposed for its formation, based on evidence from deuterium labelling studies (38). Another less prominent ion in this spectrum is ion *p* (Scheme 13). The tropylium ion (*q*) is prominent in the spectra of both isomers and is the most abundant ion in that of delta-6-CBD (Scheme 14).

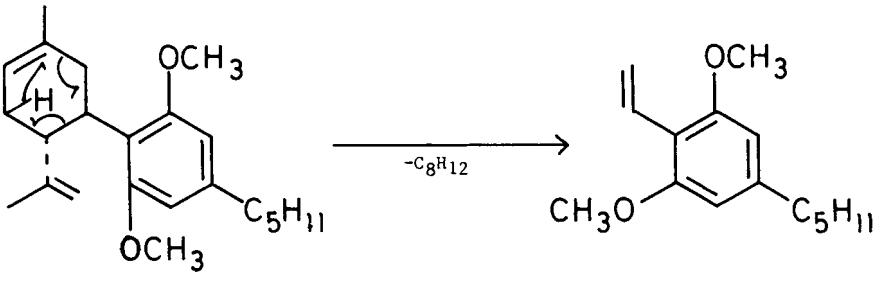


o, m/z 287

Scheme 12

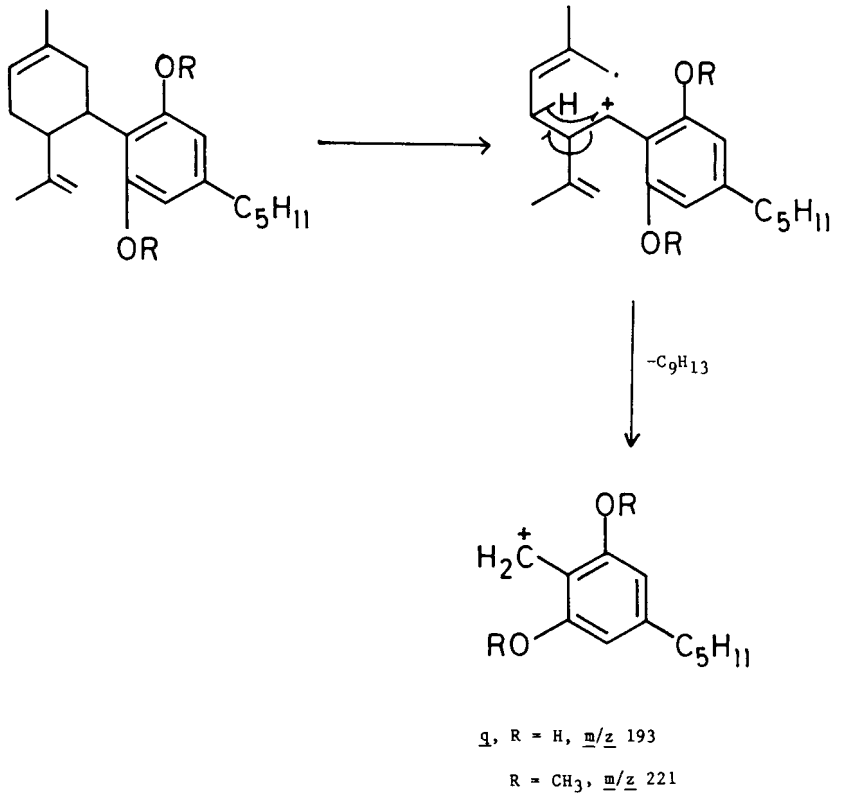
Cannabidiol monomethyl ether was identified in Cannabis samples using mass spectrometry (39). Its mass spectrum is similar to that of CBD itself with a 14 u shift in the mass of the major ions. Mass spectrometric confirmation of the presence of this cannabinoid was provided in the following year by Bercht et al. (37).

In addition to identification of natural cannabidiols, GC/MS was also used to show the absence of CBD from Cannabis originating from Africa (40). A peak in the gas chromatogram in the expected position for CBD was shown by GC/high resolution MS to be cannabichromene. It was later shown that



p, m/z 234

Scheme 13



q, R = H, m/z 193

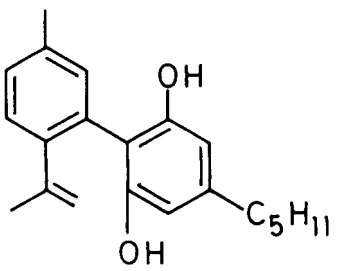
R = CH_3, m/z 221

Scheme 14

the two compounds could be successfully resolved as their TMS ethers (41), and it was pointed out that in earlier Cannabis analyses using nonderivatized samples, that CBC was assumed to be CBD.

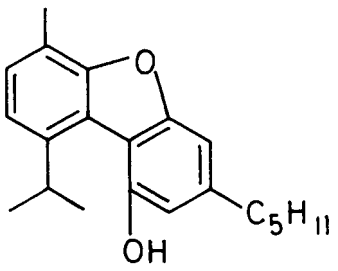
E. Cannabinodiol (12)

This compound, the aromatic analogue of CBD, has been confirmed as a constituent of Cannabis by Lousberg et al. (42). A suspected earlier identi-



12

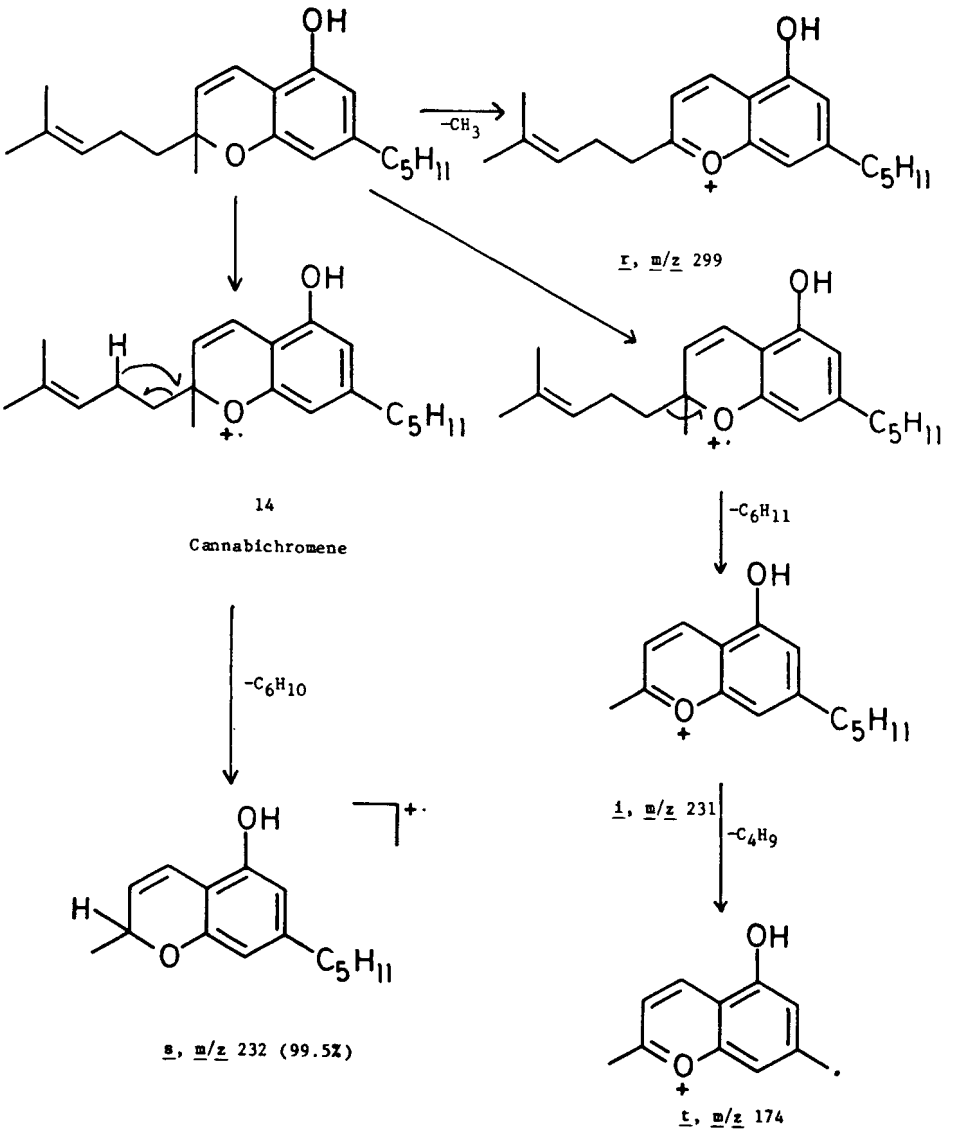
Cannabinodiol



13

Cannabifuran

fication in 1972 from Nepalese and Brazilian hashish (43) was shown to be incorrect; the mass spectra did not agree with each other, and the compound identified in 1977 was shown to form a bis-TMS derivative, whereas the cannabinoid isolated earlier formed only a mono-TMS ether. It appears that the compound isolated in 1972 was cannabifuran (**13**). The true cannabinodiol, whose structure has been confirmed by synthesis, gives a most abundant ion at *m/z* 295 ($[M - CH_3]^+$) and a molecular ion at *m/z* 310 (13.5%). All other fragment ions are of less than 15% relative intensity. Cannabifuran produces a molecular ion that is the most abundant.



Scheme 15

F. Tetrahydrocannabidiol

The mass spectrum of this cannabinoid, the reduced form of CBD, contains four significant ions, $[M]^{+\cdot}$, $[M - C_4H_8]^{+\cdot}$ (from the side-chain), the ion *q* (*m/z* 193), and *m/z* 233 which is probably a dihydro-analogue of the chromenyl ion, *i* (29).

G. Cannabichromene (CBC, 14)

Cannabichromene was identified in 1966 by Claussen and co-workers (44). The major ions in its mass spectrum are summarized in Scheme 15. Loss of the methylpentenyl side-chain produces the most abundant ion at *m/z* 231, *i*, and competitive, though less facile, loss of the methyl group gives rise to the $[M - 15]^+$ ion at *m/z* 299 (*r*, 16.5%). Loss of the methylpentenyl side-chain as C_6H_{10} with transfer of the allylic hydrogen to the chromenyl nucleus gives the very abundant ion at *m/z* 232 (*s*, 99.5%). The only other major ion arises from cleavage of the side-chain from ion *i* to give an ion at *m/z* 174 (*t*, 50%). Somewhat different relative abundances were reported for these ions by the same workers (45) two years later using an Atlas CH_5 with a source temperature of 200°C (Table I); ion *i* was still the most abundant, but all ions were reduced in relative abundance such that the second most abundant ion, *m/z* 232 was only 19.2%.

CBC has been shown to decompose to cannabicyclol in the injection port of gas chromatographs during GC/MS analysis (41). This can be overcome by using the TMS derivative (46). The 25-eV mass spectrum of this derivative shows a very abundant ion of type *i* (*m/z* 303), and all other ions are of less than 5% relative abundance.

H. Cannabicyclol (CBCy, 15)

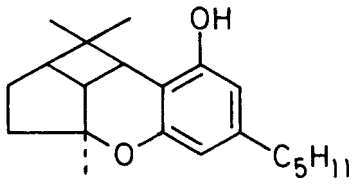
This compound, originally isolated by Claussen and co-workers (45) and assigned the name cannabipinol, is isomeric with THC and has a mass spectrum very similar to that of cannabichromene. As in the spectrum of cannabichromene, the relative abundance of the ions varies with the recording conditions as outlined in Table I.

I. Cannabigerol (CBG, 16)

No work has been reported on the detailed fragmentation of this compound, although the fragmentation was discussed briefly without deuterium labelling. Only four fragment ions are of significant abundance, the *m/z* 231 ion (*i*, 70%), the *m/z* 233 ion, the *m/z* 193 ion (*q*, 100%), and the *m/z* 247 ion

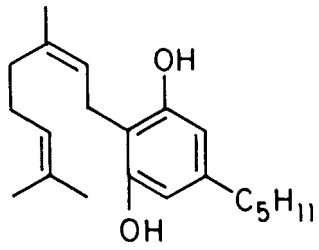
Table I. Variation of ion abundance in the mass spectra shown by cannabichromene and cannabicyclol recorded under different conditions.

Cannabinoid	Mass Spectrometer	Source Temp.	Ions (*m/z*)				
			314	299	232	231	174
Cannabichromene	CH4	200	9.0	5.5	19.2	100.0	7.9
Cannabicyclol	CH4	70	14.9	5.8	18.2	100.0	10.5
Cannabicyclol	CEC-21	92	28.3	16.8	94.5	100.0	50.0



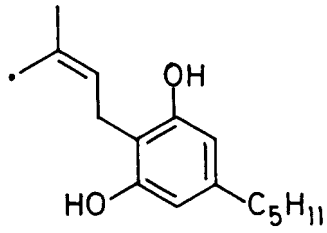
15

Cannabicyclol



16

Cannabigerol

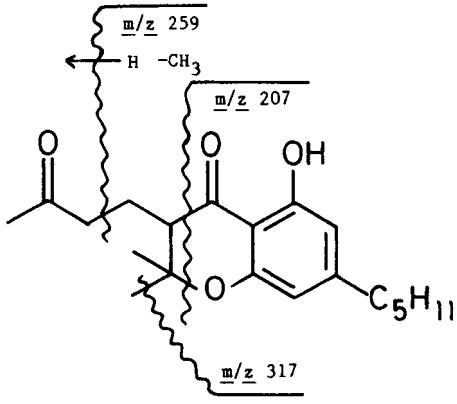


u, m/z 247

(*u*, 25%). Its monomethyl ether was identified by using mass spectrometry as a minor component of Cannabis; its spectrum is similar to that of cannabigerol with all ions shifted by 14 mass units to higher mass (47).

J. Cannabichromanone (17)

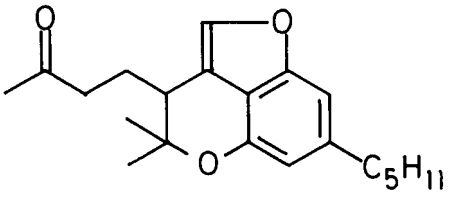
This minor cannabinoid was isolated in 1975 (48), and its structure was determined with the aid of mass spectrometry. Its mass spectrum contains



17

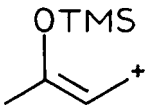
Cannabichromanone

Scheme 16



18

Cannabicumarone



v, m/z 143

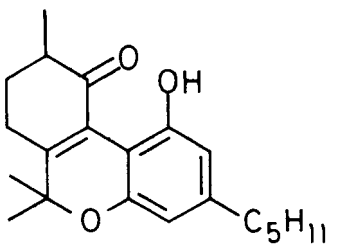
a fairly abundant molecular ion (*m/z* 332, 34%) and the most abundant ion is $[M - CH_3]^+$. Loss of butylene from the side-chain as in Scheme 6 gives an ion at *m/z* 276 (16%), and a McLafferty rearrangement of the butanone chain of the $[M - CH_3]^+$ ion results in loss of C_3H_6O and the formation of the ion at *m/z* 259 (15%). The mechanism for the formation of the ion at *m/z* 207 is shown in Scheme 16.

K. Cannabicumarone (18)

Only one report of this minor cannabinoid has appeared, and mass spectrometry played a major part in its identification (49). Alpha-deuterium exchange of the hydrogen atoms adjacent to the carbonyl group and the observation of a 5 mass unit increase in the molecular mass were used to determine the position of this group. The compound formed a TMS derivative by enolization of the carbonyl group and this derivative produced an abundant ion of *m/z* 143, which shifted four mass units for the analogue which had undergone alpha-exchange of the hydrogens. The structure of this ion was formulated as *v*.

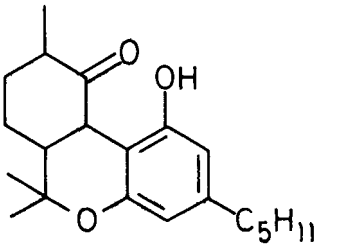
10-Oxo-Delta-6a(10a)-THC (19) and 10-Oxo-HHC (20)

10-Oxo-delta-6a(10a)-THC is another minor cannabinoid (48) identified by employing mass spectrometry. Its mass spectrum contains only four ions over 10% relative abundance. As for other delta-6a(10a)-cannabinoids, loss of an allylic gem methyl group produces the most abundant *m/z* 313 ion. In the spectrum of 10-oxo-HHC, where the delta-10a-double bond is missing, the $[M - CH_3]^+$ ion is only 16% relative abundance and the molecular ion is the most abundant. Loss of C_4H_8 from the side-chain of the delta-6a(10a)-compound is undoubtedly responsible for the ion at *m/z* 272 (18%). Other major ions in the spectrum of 10-oxo-HHC are the molecular ion (*m/z* 330,



19

10-Oxo-delta-6a(10a)-THC



20

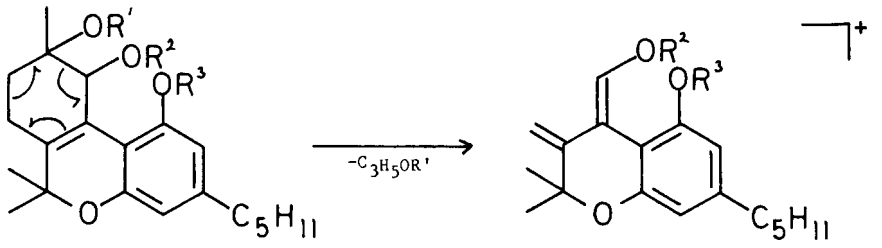
10-Oxo-hexahydrocannabinol

100%) and ions at *m/z* 287 (20%), 274 ([M − C_4H_8]$^{+\cdot}$, 36%), 246 (*h*, 44%), 231 (*i*, 17%), and 193 (*q*, 22%).

M. Polyhydroxy cannabinoids of natural origin

Cannabitriol, 9,10-dihydroxy-delta-6a(10a)-THC was characterized in 1976 by spectroscopic methods (50), but the stereochemistry of the hydroxy groups was not determined. In the following year, (+)-cannabitriol and a related compound, (−)-10-ethoxy-9-hydroxy-delta-6a(10a)-tetrahydrocannabinol, were identified in an ethanolic extract of Cannabis (51), and their mass spectrometric fragmentation was reported. It would appear that these cannabitriols are not true natural cannabinoids but are derived from delta-9-THC by chemical oxidation and hydrolysis (52). Thus, hydroxylation at the allylic/benzylic position, C-10a, followed by elimination of water would introduce the delta-6a(10a) double bond, and epoxidation and hydrolysis of the delta-9-double bond would then account for the two hydroxy groups. Further elimination of water leads to the ultimate decomposition product, CBN (**3**). Support for this scheme is provided by the presence of the ethoxy derivative in an ethanolic solution where opening of the epoxide ring would involve ethanolysis. Cannabitriol and its 10-*O*-ethyl derivative (**21d**) were found in high concentrations in an old ethanolic extract of Indian hemp dating from about 1840 (53), adding further support to this theory. Further work with this old sample revealed the presence of a second, isomeric cannabitriol, and mass spectrometry, together with the preparation of various derivatives, played a prominent role in determining the stereochemistry of these compounds.

The two cannabitriols in the Indian hemp extract showed different reactivity towards trimethylsilylating reagents. Under mild conditions, the major isomer formed only a bis-TMS ether, indicating the presence of a hindered hydroxyl group. That this hydroxyl group was the tertiary hydroxyl at C-9 was confirmed by a prominent retro-Diels-Alder ion at *m/z* 432 (ion *w*, Scheme 17) in its spectrum. Persilylation under more vigorous conditions produced the tris-TMS ether, and a mixed TMS:[$^2H_9$]TMS derivative was prepared using mild and strong conditions sequentially. The spectra of these com-



21a, $R^1 = R^2 = R^3$, Cannabitriol

21b, R^1 = OH, $R^2 = R^3$ = TMS, m/z 490

21c, $R^1 = R^2 = R^3$ = TMS, m/z 562

21d, $R^1 = R^3$ = TMS, $R^2 = C_2H_5$, m/z 518

w, $R^2 = R^3$ = TMS, m/z 432

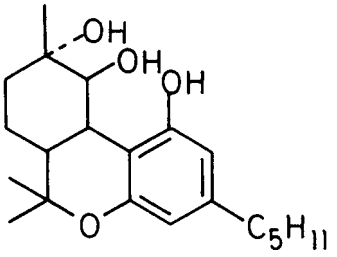
$R^2 = C_2H_5$, R^3 = TMS, m/z 388

Scheme 17

pounds have molecular ions at *m/z* 562 and major fragment ions at $[M - CH_3]^+$, $[M - TMSOH]^{+\cdot}$, $[M - TMSOH - CH_3]^+$, and at *m/z* 432 (retro-Diels-Alder ion). Because of its hindered nature and because its loss accounted for all of the $[M - TMSOH]^{+\cdot}$ ion in the spectrum of the per-TMS derivative (as shown by the spectrum of the mixed TMS: $[^2H_9]$ derivative), the 9-hydroxy group clearly has the axial (alpha) stereochemistry. The unhindered position of the 10a-hydroxy group indicates that it is 10-beta thus giving a *trans*-configuration for the glycol. The major ethoxy analogue also appeared to have this configuration, and its formation in the ethanolic cannabis sample can be rationalized by attack of ethanol on C-10 of the putative epoxide. As the 9-hydroxy group in the other isomer could be silylated under mild conditions, it must have the beta stereochemistry (53). It was not, however, possible to deduce the stereochemistry of the other hydroxy group from the spectra of the TMS derivatives. The loss of a methyl radical from both the molecular ion and $[M - TMSOH]^{+\cdot}$ involves elimination of either the C-11 methyl group or a gem-methyl group and not the TMS moiety.

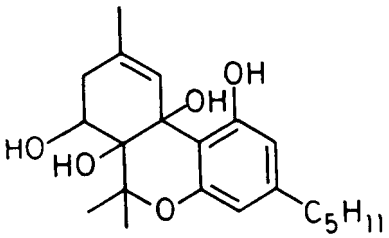
ElSohly and co-workers (54) isolated (±)-9,10-dihydroxy-delta-6a(10a)-from Cannabis in 1978 and showed that it formed a bis- rather than a tris-acetate. The hydroxy group at C-9 must, therefore, be in the 9-alpha (axial) position. As the compound formed an acetonide, it must have a cis-glycol structure, indicating the presence of a 10-alpha hydroxy group. This compound is not the same as the cannabitriol isolated by Chan and co-workers (50), which does not form an acetonide. The mass spectrum of the *bis*-acetate shows typical fragment ions for this type of compound and is dominated by ions produced by elimination of methyl radicals, acetic acid, water, and ketene. By analogy with the mass spectra of other delta-6a(10a)-cannabinoids, the most likely site for the eliminated methyl group is from one of the gem-methyl positions.

Lower homologues of these cannabitriols also exist, as shown by selected ion monitoring of TMS derivatives from the Indian hemp. Isomers of propyl-



22

Cannabiripsol



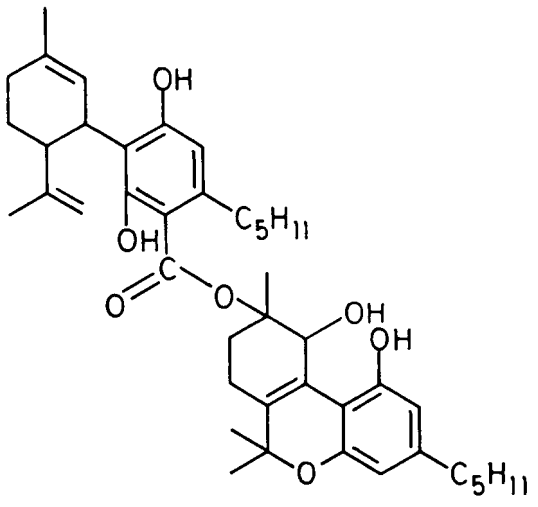
23

Cannabitetrol

cannabitriols and their ethoxy analogues, having similar fragmentation patterns and thus similar structures to the pentyl cannabinoids, were found (53), but no further mass spectral properties have been reported.

Cannabiripsol (−)-(6aR,9S,10S,10aR)9,10-dihydroxy-HHC (**22**) from South African Cannabis is the saturated analogue of the *trans*-cannabitriol isomer (**21**) and was characterized by using GC/MS and other methods (55). The mass spectrum of the nonderivatized compound contains the chromenyl ion at *m/z* 231 (*i*) and prominent ions produced by loss of water and a methyl radical. The mass spectrum of its TMS derivative is discussed later as the compound is also formed as a metabolite of delta-9-THC.

The structure **23** was proposed for a naturally occurring trihydroxylated cannabinoid, cannabitetrol, in 1984 on the basis of spectral data (56). Acetyl and methyl derivatives were prepared for mass spectral analysis, but no details of the fragmentation were reported.



24

CBD acid-B ester of cannabitriol

N. Ester of cannabidiolic acid-B and Cannabitriol (24)

This is the only dimeric cannabinoid yet isolated and is interesting in that the alcohol, cannabitriol, esterified to CBD acid-B had not been identified at the time that the ester was reported. Mass spectrometry played a major part in the characterization of this cannabinoid and spectra were recorded of both the free compound and its TMS derivative (57). The spectrum of the free ester contains a base peak at *m/z* 231 corresponding to ion *i*. Cleavage of the molecule at the ester link gives the cannabitriol ion at *m/z* 346 (8%), and this fragments by loss of two molecules of water and a methyl radical to give the ion of *m/z* 295 (80%). In the spectrum of the TMS derivative, the most abundant ion arises by loss of trimethylsilanol and a methyl radical from the cannabitriol-containing fragment ion.

O. Cannabinoid acids

The existence of the cannabinoids in plant material as their carboxylic acid derivatives of type **4** (for delta-9-THC), the so-called A-acids, appears to have been first shown in 1955 by Krejci and Santavy (58) with the isolation of CBD-acid-A. Subsequently, other cannabinoids were shown to exist mainly in their acid forms (59), but mass spectra were not reported. The isomeric delta-9-THC acid-B (5) was isolated in 1969 (60), and its mass spectrum was reported to be similar to that of delta-9-THC with the addition of the ions at *m/z* 358 ($M^{+\cdot}$) and *m/z* 343 ($[M - CH_3]^+$). Examination of these compounds by using GC/MS requires derivatization, because the free acids undergo thermal decarboxylation (46). The mass spectra of the TMS derivatives of the isomeric acids of delta-9-THC are considerably different, affording ready differentiation (61) as shown in Fig. 5. The spectrum of delta-9-THC acid-A contains a low-abundance molecular ion at *m/z* 502 (5%), and the most abundant ion is at *m/z* 487, corresponding to $[M - CH_3]^+$. $[^2H_9]$TMS labelling (61,62) was used to show that this ion is formed exclusively by elimination of a methyl radical from a TMS moiety and not by elimination of one of the gem- methyl groups as proposed earlier (63). Elimination of one of the methyl groups from the acid-TMS moiety would give the cyclic ion *x* (Scheme 18), accounting for the high abundance of the $[M - CH_3]^+$ ion in the spectrum of this isomer. For the TMS derivative of delta-9-THC acid-B, where this cyclization cannot occur, the $[M - CH_3]^+$ ion is of greatly reduced abundance and the molecular ion gives rise to the base peak. In accord with deuterium labelling of this isomer, the $[M - CH_3]^+$ ion is formed both by elimination of a TMS methyl group and a methyl group from another position, probably one of the gem-methyl groups. Other ions in the spectrum of the A-acid are of low abundance. The ion at *m/z* 413 (4%) arises from loss of $OSi(CH_3)_3$ from the acid group of the molecular ion to give the stable acylium ion as confirmed by the observation of a similar ion at the same mass in the spectrum of the TMS derivative of the bis-deutero-methyl ester (61). An earlier proposal that this ion represented loss of the phenol moiety

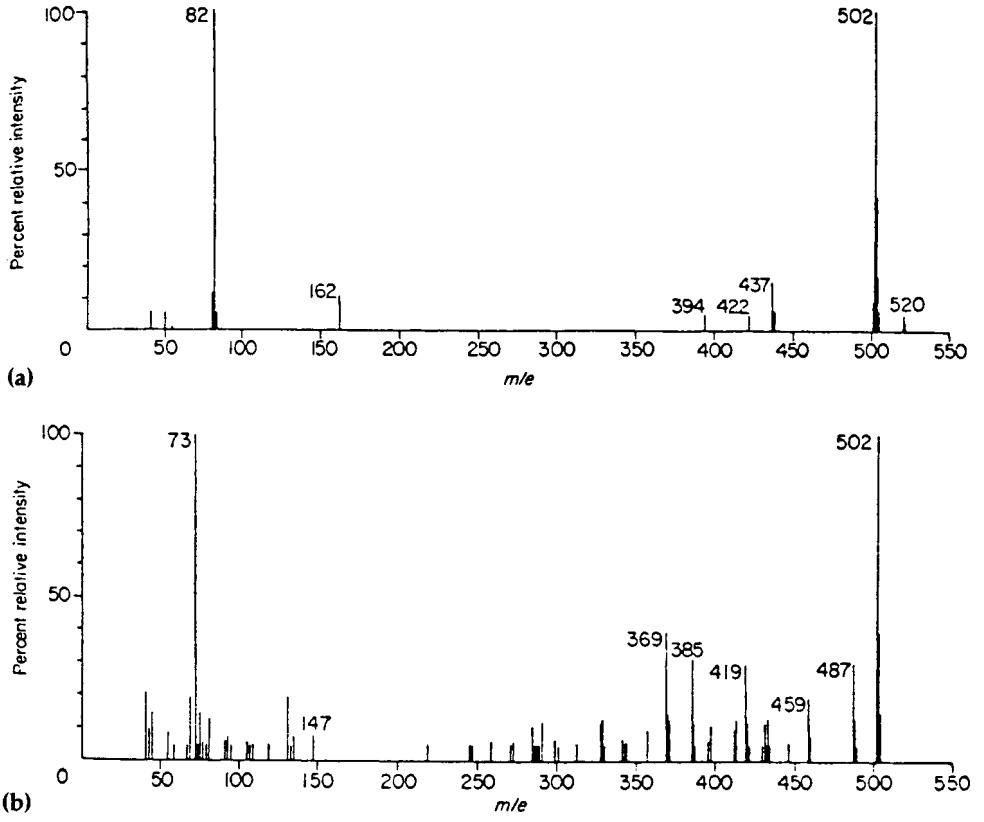
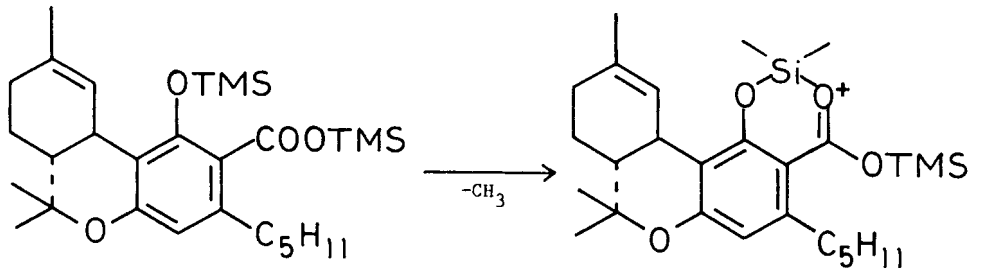


Figure 5. Mass spectra of the TMS derivatives of (a) delta-9-THC acid-A and (b) delta-9-THC acid-B. (Reproduced with permission from Wiley-Heyden Ltd.)



x, m/z 487

Scheme 18

(63) is thus erroneous. Further loss of carbon monoxide from the ion at *m/z* 413 gives the *m/z* 385 ion (5%). The ion at *m/z* 415 is the chromenyl ion *i* (Scheme 7). Results from deuterium labelling experiments (61) are consistent with a mechanism for its formation analogous to that proposed by Terlouw et al. (28, Scheme 8) for the formation of the related ion in the mass spectrum of delta-9-THC. These fragment ions are also present in the spectrum of the TMS derivative of the B-acid but at greater abundance. Also present in the spectrum of this isomer are ions at *m/z* 369 (40%) and 459 (20%). Mechanisms involving elimination of carbon-6 together with the two gem-methyl groups from the $[M - TMSOH]^+$ and molecular ions, respectively, have been proposed for their production (61), but the necessary deuterium labeled isomers have not been examined to substantiate these mechanisms.

The undiagnostic mass spectrum produced by the TMS derivative of delta-9-THC acid-A is the result of the formation of the stable cyclic $[M - CH_3]^+$ ion *x* and localization of the charge at the derivatized functions. A derivative not producing such charge localization should allow fragmentation to be initiated from the terpene moiety as in delta-9-THC itself and thus produce a much more diagnostic spectrum. This was shown to be the case for the cyclic alkane boronates (62) as shown in Fig. 6 where the cyclic butane boronate of delta-9-THC acid-A can be seen to fragment in a very similar manner to delta-9-THC and its TMS derivative. Methyl derivatives behave in a similar manner (64), and the only ion in the spectrum of the permethylated derivative of delta-9-THC acid-A not having an exact counterpart in the mass spectrum of delta-9-THC itself is the ion at $[M - 31]^+$ (loss of OCH_3).

Fragmentation of the TMS derivative of CBD acid-A is a little more diagnostic than that of delta-9-THC acid-A (61). The molecular ion is of low abundance and, as in the spectrum of the delta-9-THC acid-A derivative, the $[M - 15]^+$ ion (56%) involves exclusive loss of a methyl radical from a TMS group. This ion, however, does not give rise to the base peak, but fragments further by the retro-Diels-Alder mechanism typical for CBD and

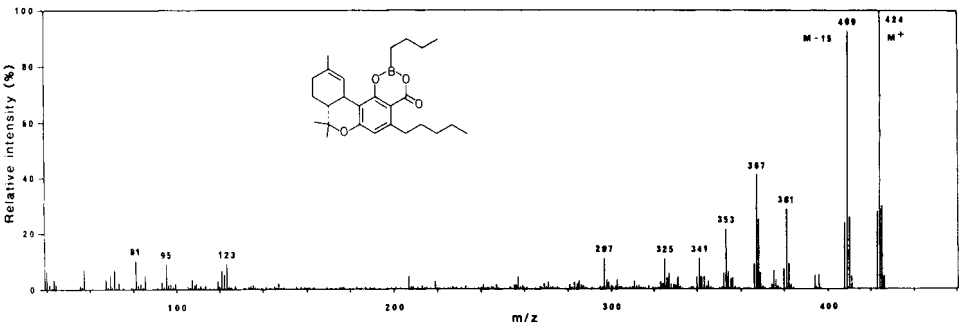


Figure 6. Mass spectrum of the *n*-butane boronate derivative of delta-9-THC acid-A. (Reproduced with permission from Wiley-Heyden Ltd.)

shown in Scheme 10 to give the ion at *m/z* 491 (100%). The only other abundant fragment ion is at *m/z* 453 (15%), and is formed by cleavage of the terpene ring. Propyl THC acid-A fragments in a strictly analogous manner.

The alkane boronate derivatives of CBD acid-A produce spectra similar to those of the parent neutral cannabinoid (62). Thus, the molecular ions, although of low abundance, are more abundant than the $[M - CH_3]^+$ ions, and the base peak is produced by the retro-Diels-Alder cleavage.

The spectrum of the TMS derivative of CBN acid-A is similar to that of the TMS derivative of CBN itself (62) and contains essentially only the molecular and $[M - 15]^+$ ions, the latter giving rise to the base peak. The cyclic alkane boronate derivatives (62) fragment similarly although the abundance of the molecular ions is higher.

Mass spectrometry has also assisted in the identification of a number of other cannabinoid acids, mainly by providing molecular weight information. These include cannabicyclolic acid (65), cannabielsoic acid (66–68) and its propyl homologue (68), the methyl homologue of cannabichromenic acid (69), and the propyl homologues of delta-9-THC, CBD, CBC, and CBG acids (70). The propyl homologues were identified as their permethyl derivatives, and their mass spectra are analogous to those of the permethyl derivatives of the pentyl cannabinoids, but ion masses are shifted by 28 units to lower mass.

Unlike the neutral cannabinoids, none of these acidic cannabinoids appear to fragment by mechanisms involving cleavage of the side-chain.

P. Cannabinoid homologues

The large number of cannabinoids present in *Cannabis sativa* is partly because of the existence of several homologues of the basic structures containing side-chains of different length. All known natural homologues have chains shorter than the C_5 chain found in the major cannabinoids, and evidence for their existence was first reported in the years around 1970 in papers from several laboratories. Propyl homologues were found to be particularly abundant in samples of Cannabis originating from India and Pakistan. Cannabidivarin, the propyl homologue of CBD, was isolated in 1969 (71), and its structure was confirmed by synthesis. Its mass spectrum, which is illustrated in Refs. 72 and 73, is similar to that of CBD itself with the most abundant ion of *m/z* 203 due to loss of a methyl radical from the retro-Diels-Alder fragment ion of type *j* (29%) shown in Scheme 10.

Gill (74) isolated the propyl homologue of delta-9-THC from Pakistani cannabis in 1971 and confirmed its structure by synthesis. Its mass spectrum was similar to that of the pentyl homologue with all ions, except that at *m/z* 258 ($[M - C_2H_4]^+$), which involved loss of the side-chain by the mechanism depicted in Scheme 6, appearing 28 mass units lower. Evidence for the delta-9-position of the bond was obtained from the ratio of the molecular and $[M - CH_3]^+$ ion which was 0.68. A much higher ratio would be expected for the other possible isomer, the pentyl homologue of delta-8-THC. Mercus

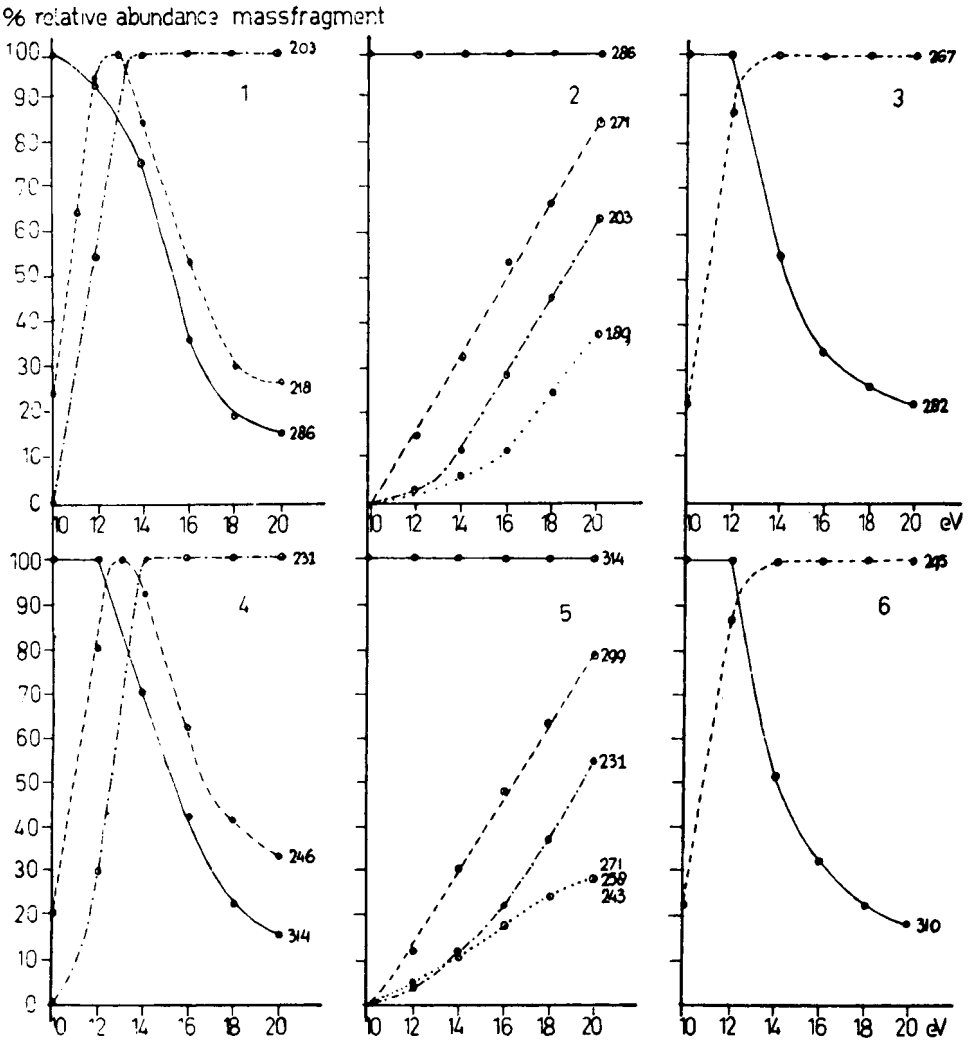


Figure 7. Plots of ion abundance as a function of electron energy for some major cannabinoids and their homologues. 1 = propyl-CBD, 2 = propyl-delta-9-THC, 3 = propyl-CBN, 4 = CBD, 5 = delta-9-THC, 6 = CBN. (Reproduced with permission from Elsevier Scientific Publishing Co. Ltd.)

(75) also isolated this homologue in 1971 together with the propyl homologue of CBN. Ions in the spectrum of the latter compound are comparable to those in that of CBN itself. In another report, Fetterman and Turner used GC/MS to identify the propyl homologues of delta-9-THC and CBD in an Indian variant of Cannabis (76).

Propyl homologues of the three major cannabinoids were also identified by Vree et al. (77) who used GC/MS and plots of electron energy against ion abundance to show similarities in the fragmentation between the two homologues. The relative abundance of the major fragment ions varies with energy in a characteristic fashion for each cannabinoid, and this pattern is reflected in the fragmentation of the lower homologue as shown in Fig. 7.

Vree et al. (78) also identified the propyl homologue of cannabicyclol by using this technique in 1972; electron energy: abundance plots of the molecular ions (*m/z* 286 and 314) and the most abundant ions (*m/z* 203 and 231) were used to compare the propyl and pentyl homologues. Plots of the same pairs of ions also allowed the propyl homologue of cannabichromene to be identified in the following year (79). Shoyama et al. (80) reported mass spectral details of propyl cannabichromene in 1975 and extended the range of known propyl homologues to include propyl cannabigerol. Identification in these cases was by conventional GC/MS and comparison of the spectra of the nonderivatized compounds with those of the pentyl homologues. Propyl-CBC has a spectrum similar to that of CBC with a base peak at *m/z* 203 (corresponding to ion *i*).

In addition to the propyl homologues, methyl homologues of delta-9-THC, CBD and CBN were identified in 1972 in Cannabis by Vree et al. (81) who used GC/MS. As in the authors' earlier work on electron energy, abundance plots were used to characterize the compounds. A further comprehensive study of the effects on ion abundance of varying the electron energy was reported in 1980 by Turner et al. (82). A large number of cannabinoids and their TMS derivatives were examined at electron energies between 5.5 and 21 eV.

All of the above homologues contain odd numbers of carbon atoms in the side-chain and fit a biosynthetic pathway in which different numbers of acetyl groups are incorporated into the half of the molecule containing the side-chain. However, incorporation of small amounts of propionyl groups is often encountered during biosynthetic chain elaboration and this, in the case of the cannabinoids, would be expected to give small concentrations of cannabinoids containing even numbers of carbon atoms in the chain. Evidence that this occurred was first provided by Harvey in 1976 (83), who observed an ion at *m/z* 473 corresponding to the $[M - CH_3]^+$ ion from butyl delta-9-THC acid-A, whose abundance maximized midway in time between the elution of the propyl and pentyl homologues in gas chromatograms of the TMS derivatives of several Cannabis samples. Further work confirmed the presence of this acid, together with the butyl homologues of delta-9-THC, CBD, and CBN. Butyl CBN was particularly abundant in an old sample of tincture of Indian hemp in which most of the cannabinoids had decomposed to cannabinols. Mass spectra of the TMS, $[^2H_9]$TMS, triethylsilyl and tri-*n*-propylsilyl derivatives of all four butyl cannabinoids were recorded, and the nature of the derivative was shown to have minimal effect on fragmentation. Similarly, the side-chain length had little effect except for those ions involving cleavage of the chain. Figure 8 shows a comparison of the mass spectra of the triethylsilyl derivatives of the methyl, propyl, butyl and pentyl homologues of delta-9-THC. The presence of methyl homologues in all examined Cannabis samples was confirmed in this work, although the concentrations were low.

Of even lower concentration are ethyl homologues, of which only ethyl CBN has so far been reported (53). The presence of ethyl CBN as the TMS

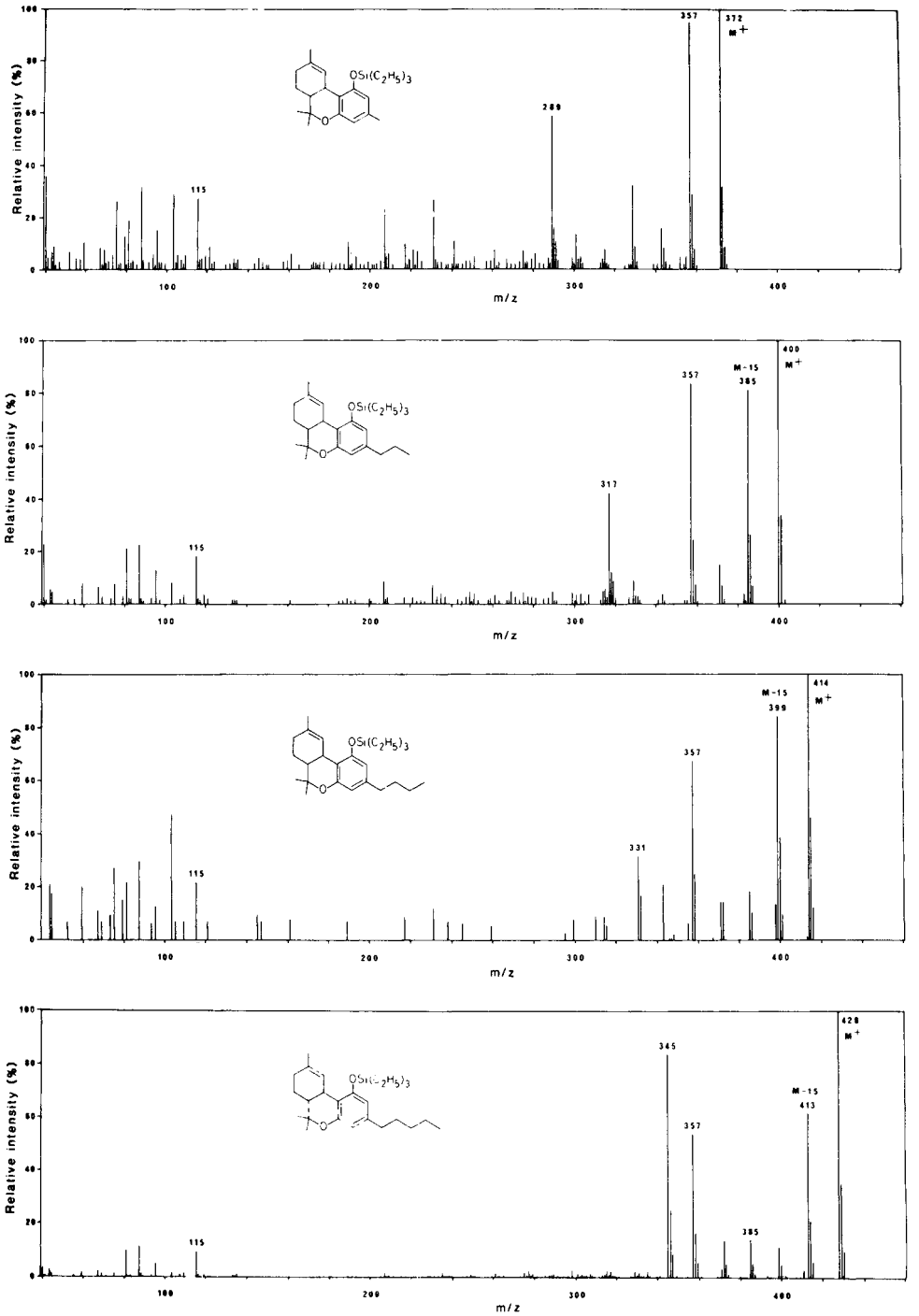


Figure 8. Mass spectra (25 eV) of the triethylsilyl derivatives of homologous delta-9-THC's.

derivative was demonstrated in the sample of tincture of Indian hemp mentioned earlier by using GC/MS and selected ion monitoring of the $[M - CH_3]^+$ ions from each homologous cannabinoid, together with a common ion of *m/z* 310. The sample was first chromatographed on Sephadex LH-20 to remove fatty acids having ions at the same nominal masses.

A number of other homologues of the natural and synthetic cannabinoids have been synthesized, mainly for pharmacological investigation, and their mass spectra have been recorded (84,85). In general, changes in the hydrocarbon side-chain have little effect on fragmentation as illustrated in Fig. 8.

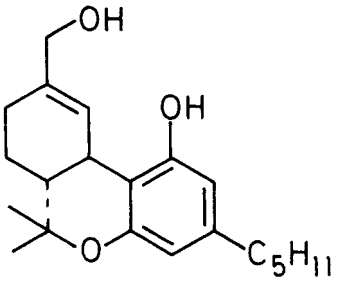
III. CANNABINOID METABOLITES

Mass spectrometry and, in particular GC/MS, has been the most widely used technique for the identification of cannbinoid metabolites. This section of the review outlines the methods adopted and the results obtained, and later sections are devoted to details of the fragmentation pathways.

A. Tetrahydrocannabinols

1. Hydroxy, oxo and acid metabolites

a. **Use of Mass Spectrometry for Metabolite Identification** The first reports of the identification of cannabinoid metabolites appeared in 1970. Burstein et al. (86) reported a compound tentatively identified as 11-hydroxy-delta-8-THC by the use of several chemical techniques including mass spectrometry of the *bis*-acetyl derivative. The spectrum of this derivative is not very diagnostic, as it is dominated by the $[M - 42]^{+\cdot}$ ion (ketene loss from the phenol acetate group), $[M - 60]^{+\cdot}$ (loss of acetic acid), and $[M - 42 - 60]^{+\cdot}$. The structure was later confirmed by synthesis and comparison of the mass spectra of the acetyl derivatives (87). Foltz et al. (88) also identified the same compound in 1970 in rat liver homogenates and obtained high resolution spectra of the free metabolite and of its TMS ether.



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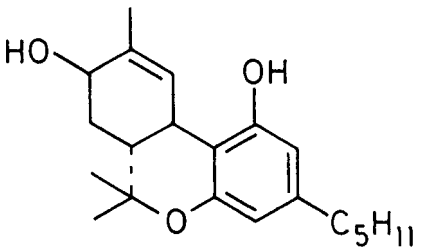
11-Hydroxy-delta-9-THC



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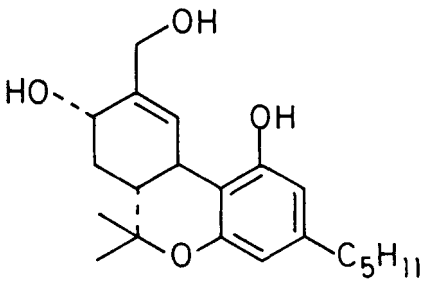
8-hydroxy-delta-9-THC

The use of ion *i* (*m/z* 303, TMS ether) for identifying metabolites substituted in the terpene ring was emphasized.

Delta-9-THC was also shown in 1970 (89,90) to be metabolized by hydroxylation in the 11-position (**25**) and, in addition, another allylic metabolite, 8-hydroxy-delta-9-THC (**26**) was identified in rat liver microsomes (89). Both metabolites in the nonderivatized state were identified by using NMR and mass spectrometry, and their mass spectra show the same type of fragmentation as was later found for the TMS derivatives and which is discussed later in this paper. The mass spectrum of the 11-hydroxy metabolite (**25**) has a base peak which corresponds to an ion at *m/z* 299 formed by elimination of the 11-CH_2OH group. The dihydroxy metabolite, 8,11-dihydroxy-delta-9-THC was also reported (89); and its spectrum contained a base peak corresponding to an ion at *m/z* 328 $[M - 18]^+$ as the result of elimination of water and an ion at *m/z* 297 from further elimination of the 11-CH_2OH group. The presence of ion *i* (*m/z* 231) localized both hydroxyl groups in the terpene ring. Metabolism of both delta-8- and delta-9-THC by liver homogenates from several species, including man, was studied by Wall in 1971 (91), and considerable use was made of mass spectrometry of the nonderivatized compounds in structural elucidation. 11-, 8-alpha-, 8-beta-hydroxy and 8-alpha,11-dihydroxy-delta-9-THC (**27**) were characterized as metabolites of delta-9-THC and 11-hydroxy and both 7-alpha,11- (**28**) and 7-beta,11-dihydroxy-delta-8-THC were formed from the delta-8-isomer. Synthesis of the 8-beta-hydroxy-metabolite was achieved in 1971 by Ben-Zvi et al. (92).

GC/MS made an early appearance in studies of this drug and was used by Mikes, Hofmann, and Waser (93) to identify the same 8,11-dihydroxy metabolite of delta-9-THC in rats as that found by Wall et al. (89). A further compound identified on the basis of its mass spectrum was the bis-acetate of 11-hydroxy-delta-9-THC. However, this is possibly an artefact (91) as its existence has not been confirmed. Wall et al. (94) have also used the technique to identify 11-, 8-alpha- and 8-beta-hydroxy-delta-9-THC and the diol, 8-alpha,11-dihydroxy-delta-9-THC in human plasma after using Sephadex LH-20 to extract the metabolites and Ben-Zvi and co-workers (95) confirmed



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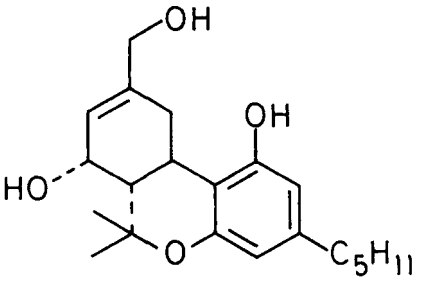
8-Alpha,11-dihydroxy-delta-9-THC



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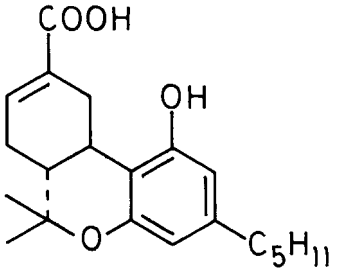
7-Alpha,11-dihydroxy-delta-8-THC



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Delta-8-THC-11-oic acid

the formation of the 8-alpha- and 11-hydroxy-metabolites of delta-9-THC in the mouse by use of the acetyl derivatives.

This work established the major hydroxylation pathways as involving allylic hydroxylation, but further experiments by Wall (91) with CBN, and the use of TMS derivatives resulted in the identification of 2'-hydroxy-CBN, thus suggesting that side-chain aliphatic hydroxylation could also occur with the tetrahydrocannabinols. Hydroxylation of delta-8-THC in the side-chain was reported in 1971 by Maynard et al. (96) using dog liver microsomes. 1'- and 3'-hydroxy-delta-8-THC were identified by synthesis (97) and NMR and their acetyl derivatives by GLC and MS. The high resolution mass spectrum of these derivatives are again dominated by ions produced by losses of ketene and acetic acid. Two side-chain hydroxylated derivatives of delta-9-THC-11-oic acid were reported in the following year (98,99) in rabbit urine and identified as 1'- and 2'-hydroxy-delta-9-THC by using GC/MS of the bis-methyl derivatives (carboxylic acid group and phenol).

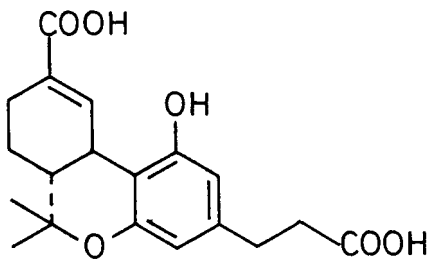
Further oxidation of these hydroxylated metabolites, particularly at C-11, gives major urinary metabolite in most species. Thus, Mechoulam et al., (100) found delta-8-THC-11-oic acid (**29**) in rabbit urine in 1973 and identified it as its methyl ester by comparison with a synthetic standard by using selected ion monitoring of ions at *m/z* 358 ($M^{+\cdot}$), 326, and 302. Watanabe et al. (101) also identified this acid, together with its 7-hydroxy analogue by using the mass spectra of their methyl esters. The intermediacy of an aldehyde in the oxidation of 11-hydroxy-THC's to the 11-oic acids has been demonstrated for both delta-8- (102) and delta-9-THC (103). The acetate derivatives of the two aldehydes have similar mass spectra with abundant ions corresponding to loss of ketene (*m/z* 328) and a further loss of 56 mass units, possibly C_4H_8 from the side-chain. Ion *i* (*m/z* 231) is abundant (103).

The possible formation of cannabinol metabolites from THC's has been controversial for many years as CBN itself and its metabolites have been identified in several laboratories from animals and humans treated with THC. For example, Ben-Zvi et al. (104,105) identified cannabinol-7-oic acid by using GC/MS of its methyl ester: methyl ether derivative as a metabolite of delta-9-THC in the rhesus monkey. It is probable, however, that these CBN's are

not enzymatic products as THC has been shown to oxidize to CBN and thus its metabolites are probably also oxidized to the corresponding CBN metabolites.

The complexity of cannabinoid metabolism became evident in the mid 1970's following work by Agurell's group in Sweden and Harvey's group in England. GC/MS was the main analytical technique, but the sample treatment methods differed between the two groups. Agurell's approach involved considerable pre-GC/MS separation using a variety of column and thin-layer chromatographic systems and, in many cases, enabled metabolites to be isolated in sufficient purity for the recording of NMR and other spectral properties in addition to mass spectra. Thus, in 1974, Widman et al. (106) identified 11-hydroxy-delta-9-THC by using selected ion monitoring and detected the presence of two other conjugated hydroxy-THC's in the bile of rats treated with the drug. One of these metabolites appeared to be hydroxylated in the side-chain but full identification could not be made. Jones et al. (107) separated metabolites of delta-9-THC from mouse brain by using Florisil column chromatography and purified them by Sephadex LH-20 chromatography; TLC, NMR, and GC/MS of the nonderivatized metabolites were used for identification of 8-alpha-hydroxy-, 11-hydroxy-, and 8-oxo-delta-9-THC by employing selected ion monitoring of major ions to compare the metabolites with authentic standards. Studies on the further metabolism of the major metabolite, 11-hydroxy-delta-9-THC (108), revealed a complex pattern of 10 metabolites, but only delta-9-THC-11-oic acid was identified by mass spectrometry, again using selected ion monitoring. Isolation from urine was by chromatography on Amberlite XAD-2 resin, solvent extraction at various pHs, and chromatography on Sephadex LH-20.

By using a similar extraction method, Nordqvist et al. (109), in 1974, isolated a di-acid metabolite 4',5'-bis-nor-delta-9-THC-3',11-dioic acid (**30**) from rabbit urine and identified it as its methyl ester by using NMR and mass spectrometry. This was the first indication that delta-9-THC could be oxidized



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4',5'-bis,nor-delta-9-THC-3',11-dioic acid

by the beta-oxidation pathway. However, further studies revealed that this pathway is used extensively by the rabbit, and in two papers published in 1979 (110,111), Nordqvist et al. reported 18 additional metabolites in rabbit urine, most of which are disubstituted and have undergone beta-oxidation. Isolation was again by XAD-2, LH-20, and Sephadex G-25 column chromatography and identification employed GC/MS of the methyl ester: TMS derivatives. Several of the mono-carboxylic acid metabolites of delta-8-THC were subsequently synthesized (112) and identified in varying proportions by using selected ion monitoring of their methyl ester; TMS ethers, as metabolites of the drug in several species.

Analysis of human urine by Halldin et al. (113) has shown that a similar profile of metabolites is produced. Of the thirteen metabolites identified by GC/MS, eight have degraded side-chains. The other metabolites were delta-9-THC-11-oic acid and four side-chain hydroxylated derivatives. Five di-acids, all with one acid group at C-11 and the other in the side-chain were also reported in subsequent papers (114,115). In addition to the major human urinary metabolite of delta-9-THC, the acid glucuronide of delta-9-THC-11-oic acid (17), Halldin and Widman (116) identified the phenol glucuronide of delta-9-THC as a minor metabolite. Microsomal metabolites of delta-9-THC in humans have also been studied by Agurell's group and shown to consist of 8 (alpha and beta) and 11-hydroxy-delta-9-THC and the 8- and side-chain hydroxylated derivatives of 11-hydroxy-delta-9-THC (117). An epoxide derivative of 11-hydroxy-delta-9-THC was also reported.

Analysis of metabolites of delta-9-THC formed in the lungs of dogs (118), rats (119), and guinea pigs (119) have revealed a different profile from that formed by the liver. In particular, side-chain monohydroxy metabolites are of relatively high abundance, presumably reflecting a different range of cytochrome P-450 mixed function oxidases. In both of the earlier reports, identification of the metabolites involved GC/MS of the TMS derivatives, and the metabolites from the dog represent the first example of the isolation of metabolites of this type from delta-9-THC. In another report from this laboratory, four side-chain monohydroxy metabolites of delta-8-THC together with the three allylic hydroxylation products and six dihydroxy metabolites were identified in the rhesus monkey, mainly by using GLC and GC/MS (120). Again metabolites were identified as their TMS derivatives.

The approach adopted by Harvey et al. (121–125) for the structural determination of metabolites present in tissues involved the minimum of chemical manipulation with the aim of minimizing losses, particularly of minor and possible unstable compounds. Metabolites were extracted from tissue homogenates with the fairly polar solvent, ethyl acetate, and separated from the coextracted lipids by chromatography on Sephadex LH-20 in chloroform. Many of the resulting fractions were complex when examined by GC/MS as shown in Fig. 9, but structural elucidation was achieved with the aid of group-specific derivatives and the use of deuterium labelling (126). An electron energy of 25 eV was used to reduce unspecific fragmentation in the lower mass range and to enhance the abundance of diagnostic fragmenta-

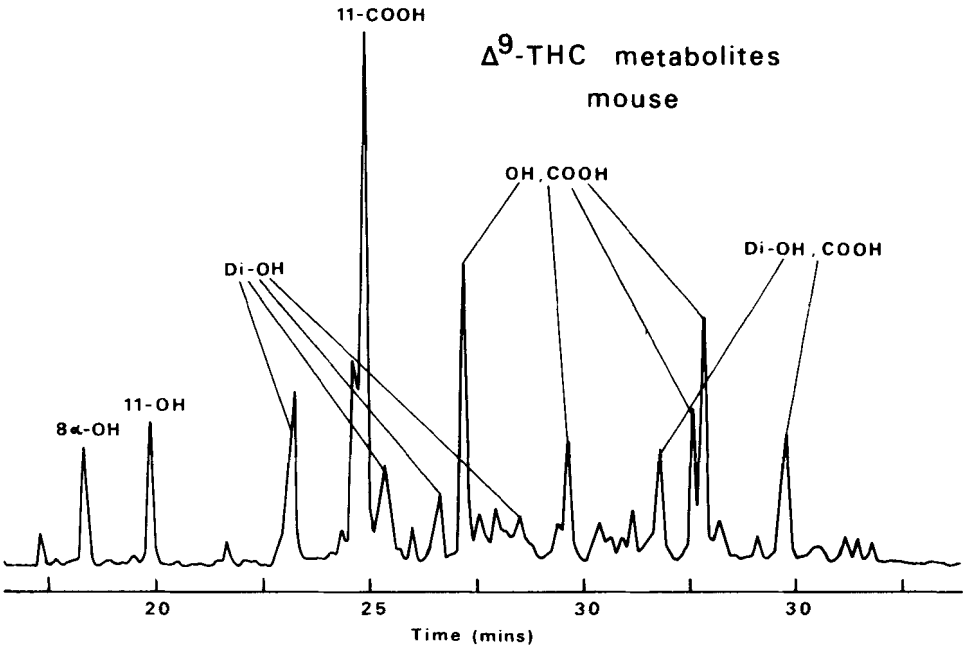


Figure 9. Reconstructed total ion current profile of metabolites of delta-9-THC extracted from mouse liver.

tions. Computer reprocessing of spectra by the MassMax technique (127) was used to enhance the apparent GLC resolution (Fig. 9). Initial screening was performed using TMS derivatives, and the number of TMS groups introduced into each metabolite, reflecting the degree of metabolism, was determined by the preparation of [$^2H_9$]TMS derivatives. Carboxylic acids were identified by preparation of methyl esters by using diazomethane, and carbonyl-containing metabolites, mainly ketones, were identified as methyl oximes.

One feature of the metabolic fractions is that they contain several metabolites having common positions substituted, but with the substituents in different oxidation states, mainly as acids and ketones. Structural correlation between these metabolites was achieved by reduction of the ketones and acids in the oxidized metabolites with lithium aluminium deuteride to give the hydroxy metabolites already present in the mixture. The source of the hydroxy compound in the resulting mixture was determined by the extent of deuterium incorporation. Thus, acids incorporated two atoms of deuterium, and ketones incorporated one atom. An example is shown in Fig. 10, which shows the mass spectrum of 7-beta,11-dihydroxy-delta-8-THC produced by reduction of the metabolic fraction from the liver of a mouse treated with delta-8-THC. The ion at *m/z* 562 is that from the metabolic alcohol and that at *m/z* 563, containing one deuterium atom, is produced by reduction of a carbonyl metabolite, most probably, 7-oxo-11-hydroxy-delta-8-THC. The major ion in the group at *m/z* 564, containing two deuterium atoms, is the product of reduction of 7-hydroxy-delta-8-THC-11-oic acid, and the ion at

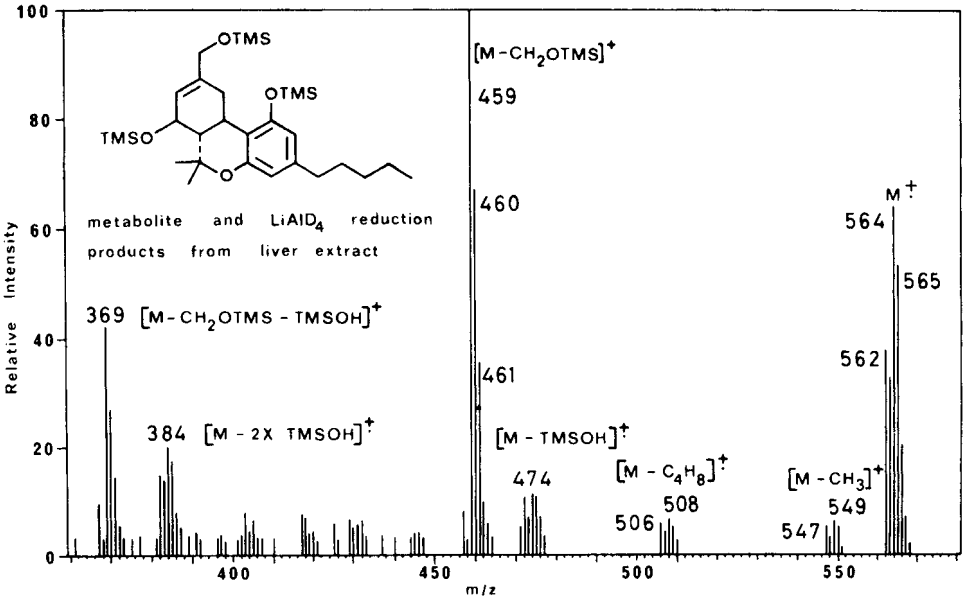


Figure 10. Mass spectrum (25 eV, upper mass range) of the TMS derivative of 7-beta,11-dihydroxy-delta-8-THC obtained by lithium aluminium deuteride reduction of metabolites of delta-8-THC produced by the mouse.

m/z 565, containing three deuterium atoms, derives from 7-oxo-delta-8-THC-11-oic acid (126).

One of the problems encountered with this general approach is the similarity between the spectra of some THC metabolites and endogenous lipids. This is well illustrated in Fig. 11, which shows spectra of the TMS derivatives of 11-hydroxy-delta-9-THC and 1-monopalmitin. Both compounds contain two TMS groups, and their spectra contain similar ions. Retention times on SE-30 are also similar. Identification of the spectra produced by metabolites is best accomplished by using the isotope doublet technique, which involves a study of the metabolism of an equimolar mixture of the drug and an isotopically labelled varient. The spectrum shown at the bottom of Fig. 11 was produced by metabolism of such a mixture of delta-9-THC and [1',1',2',2'-$^2H_4$]delta-9-THC. The doublet peaks, four mass units apart, clearly identify this spectrum as arising from a metabolite (126). Caution must, however, be exercised in the use of this technique in cases where the deuterium atom is at a site of metabolic attack. Replacement of a deuterium atom will cause a reduction in the spacing between the doublets, but removal of a deuterium atom can be used to advantage to identify the site of metabolic substitution. If metabolic replacement of deuterium involves breaking of the carbon-deuterium bond in the rate-determining step of a metabolic reaction, as in the case of cytochrome P-450-mediated aliphatic hydroxylation, the primary kinetic isotope effect causes a reduction in the abundance of the resulting metabolite as has been observed with delta-9-THC in the formation of 2'-hydroxy-metabolites from the [$^2H_4$]analogue (128). In some cases, where this

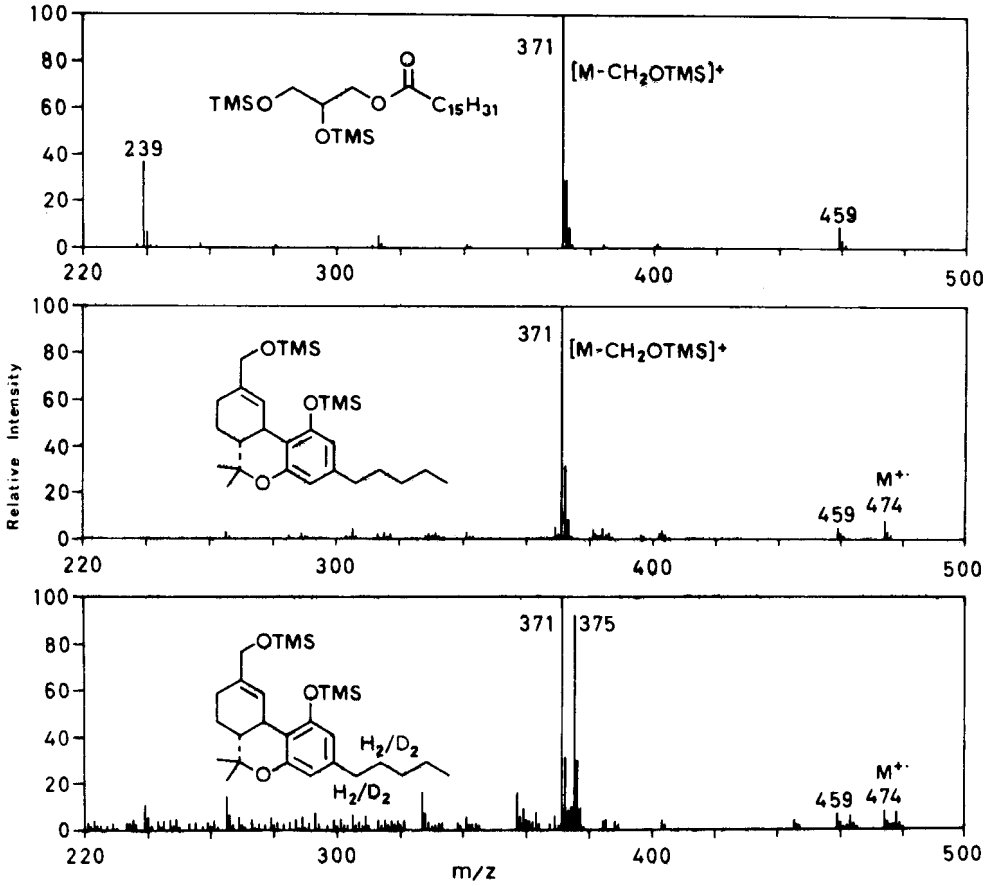


Figure 11. Mass spectra (25 eV) of the TMS derivatives of (a) 1-monopalmitin, (b) 11-hydroxy-delta-9-THC and (c) a 1:1 mixture of 11-hydroxy-delta-9-THC and its [1′,1′,2′,2′ $-{}^{2}H_4$]analogue. (Reproduced with permission from Wiley-Heyden Ltd.)

affects a major metabolic route, another route may be used in preference to give a different metabolic profile, a phenomenon known as "metabolic switching." It has not, however, been observed with the cannabinoids.

Another major problem with mass spectrometric identification of metabolites is the determination of isomers. This occurs frequently in studies of the cannabinoids, as isomeric alcohols can be produced from hydroxylation at several positions of the terpene ring. Many of these metabolites, as their TMS derivatives, fragment to give abundant losses of trimethylsilanol which, in itself, is not stereospecifically informative. However, it has been shown that elimination of water and trimethylsilanol under electron ionization conditions involves specific removal of a hydrogen by the trimethylsilyloxy function in a 1:3 or 1:4 diaxial interaction (129) and that the two interacting groups should be able to approach to within 3.2 Å (130). Reactivity of the abstracted hydrogen is also significant in determining which of several alternative hydrogen atoms is abstracted (131). Thus, if the abstracted hydro-

gen can be determined in elimination reactions involving loss of trimethylsilanol, it should be possible to deduce the stereochemistry of the eliminated trimethylsilyloxy function. This has been examined in tetrahydrocannabinol metabolism (132,133) by using deuterium labelling. The 10a-hydrogen atom is an obvious candidate for abstraction by trimethylsilyloxy groups projecting below the plane of the molecule as it is both axial and chemically active. It was found to be quantitatively abstracted during fragmentation of both 8-alpha-hydroxy-delta-9-THC and 7-alpha-hydroxy-delta-8-THC in which the hydroxy groups project below the plane of the terpene ring but not by the isomeric beta-isomers were the groups project away from the 10a-hydrogen. Similar results were obtained for 9,10-dihydroxy-HHC's where it was found that the 10a-hydrogen is nearly quantitatively abstracted by the 9-alpha-trimethylsilyloxy group (axial) whereas only some 50–60% of the hydrogen is lost when the 9-trimethylsilyloxy group is beta (equatorial). Confirmation that it was the 9-trimethylsilyloxy group that is involved in the first loss of trimethylsilanol was provided by the mass spectra of the mixed-labelled TMS: [$^2H_9$]TMS derivatives. The hydroxy group in the 9-position could be specif-

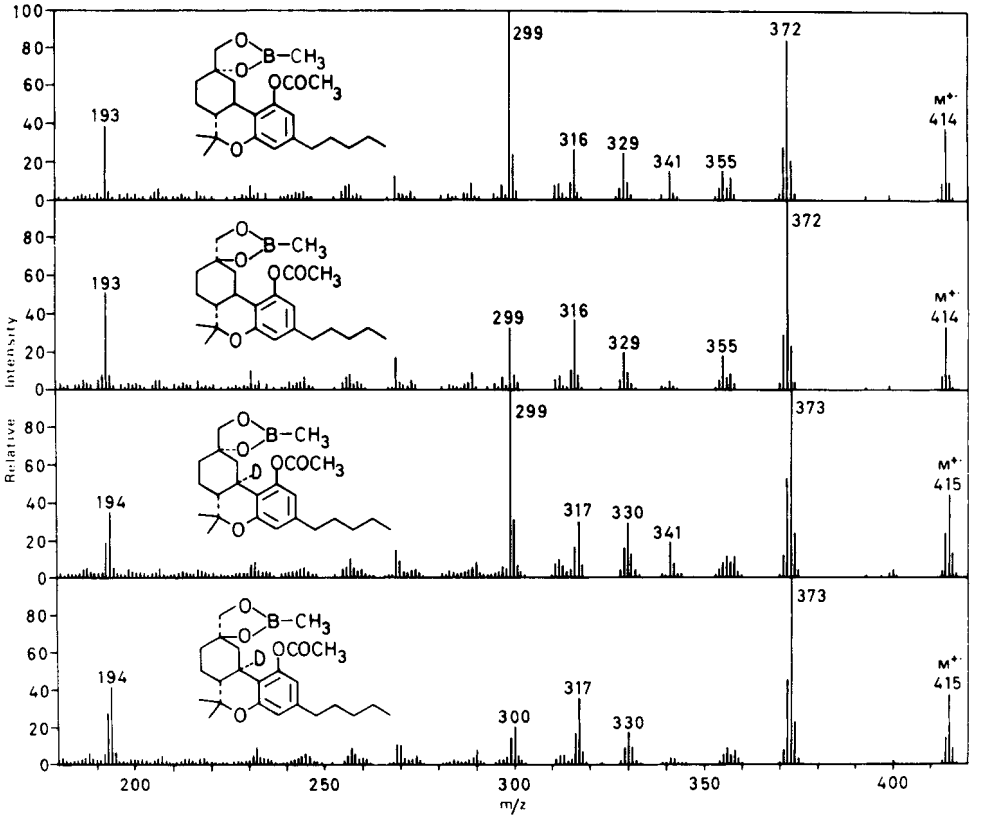
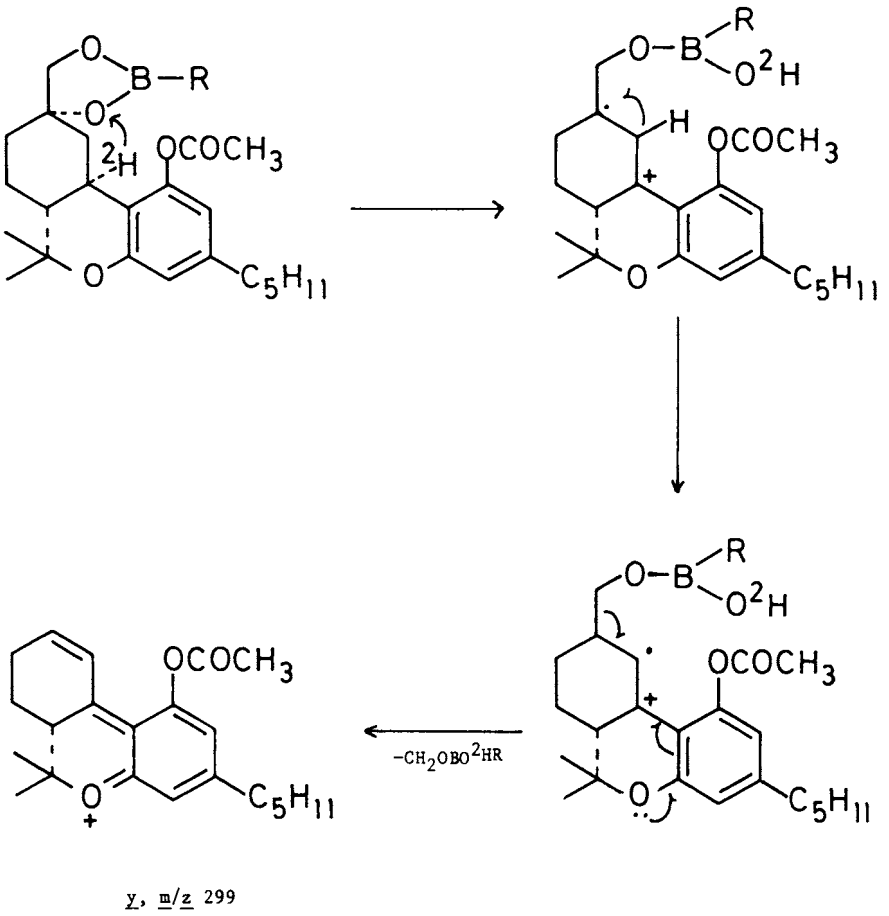


Figure 12. Mass spectra (25 eV) of the methane boronate : acetate derivatives of epimeric 9-alpha and 9-beta,11-dihydroxy-HHC's and their [10a-^{2}H]-analogues. (Reproduced with permission from Wiley-Heyden Ltd.)



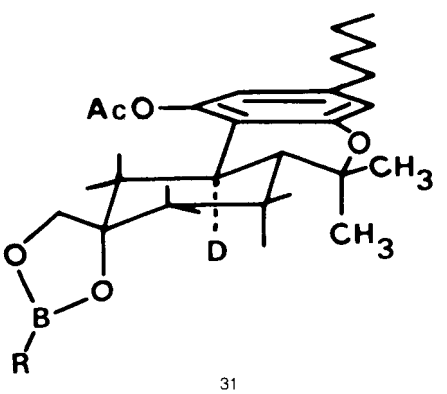
y, m/z 299

Scheme 19

ically labelled as it is less reactive than those in the other positions. This method, however, did not work for the 8,9-dihydroxy derivatives; in all cases the first loss of trimethysilanol involved abstraction of the 10a-hydrogen atom.

A similar non-specific result was obtained from 9,11-dihydroxy-metabolites. However, in this case it is possible to prepare cyclic alkane boronate derivatives of both possible isomers and to show abstraction of the 10a-hydrogen only from the 9-alpha isomer. This occurs during a fragmentation involving loss of the alkane boronate ring together with a hydrogen to give the abundant ion at *m/z* 299 (ion *y*, Fig. 12). The mechanism shown in Scheme 19 can only occur for the 9-alpha-isomer with loss of the deuterium; in the 9-beta-isomer, the ion at *m/z* 299 is of lower abundance and is formed with retention of most of the deuterium. Structures **31** and **32** show the interaction.

Another example of the use of this method is illustrated in Fig. 13. The mass spectra of the TMS derivatives of the two isomeric HHC-11-oic acids are very similar except for an ion at *m/z* 372 of higher relative abundance in



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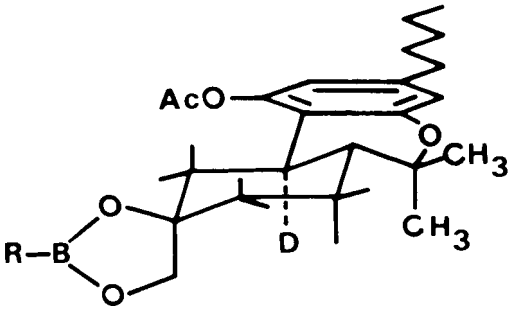


32

the spectrum of the axial-isomer than in that of the other compound. This ion is produced by loss of the carboxy-TMS group together with a hydrogen atom, and deuterium labelling has shown that in the spectrum of the axial-isomer, but not in that of the equatorial isomer, that it is the 10a-hydrogen that is eliminated. Loss of the deuterium label thus indicates the presence of an axial carboxy-TMS group. The main advantage of this approach is that the stereochemistry of several metabolites can be determined in one experiment by treating the experimental animal with the deuterated drug. The presence of deuterium also enables the isotope doublet technique to be used to confirm derivation from the drug of the peak under examination.

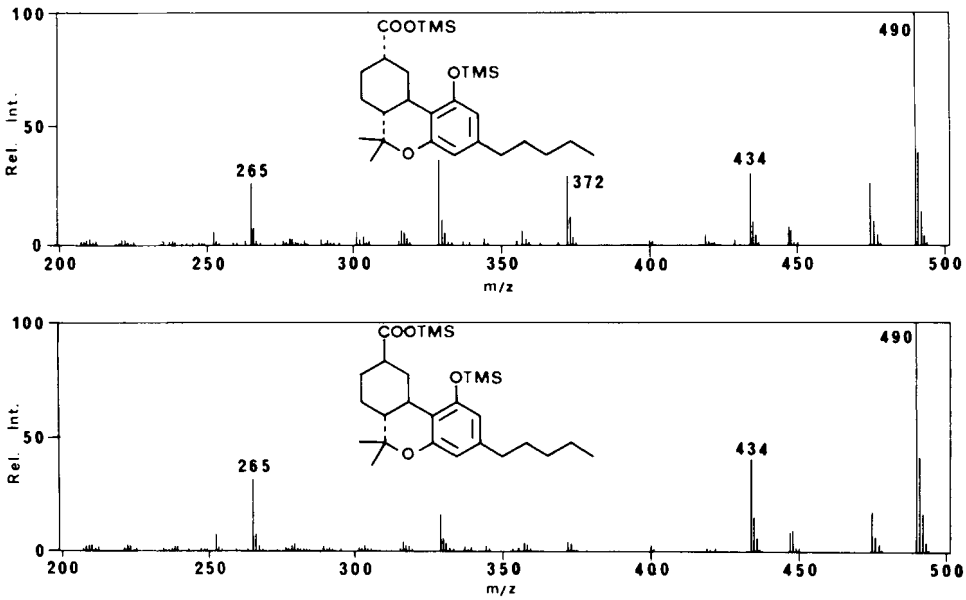


Figure 13. Mass spectra (25 eV) of the TMS derivatives of epimeric HHC-11-oic acids. (Reproduced with permission from Wiley-Heyden Ltd.)

By use of these methods with packed column GC/MS, Harvey et al. identified a large number of acid, hydroxy-acid, dihydroxy-acid, dihydroxy, and trihydroxy metabolites of delta-9-THC in mice (121–125). All of the polysubstituted metabolites contained either a hydroxy or an acid group at C-11 with further hydroxylation at the 8-alpha, 8-beta, 2'-, 3'-, or 4'-positions. Also identified in low concentration were dihydro-derivatives of delta-9-THC-11-oic acid and 11-hydroxy-delta-9-THC. The mass spectra of their TMS derivatives contain abundant molecular ions; those of the acids are shown in Fig. 13 (134). A very different metabolic profile was observed for the guinea pig under the same conditions (123,135,136). 8-Beta- rather than 8-alpha-hydroxylation dominates substitution at C-8, but the major difference is the occurence of several acidic metabolites produced by beta-oxidation of the side-chain. Of these, 4',5'-*bis,nor*-delta-9-THC-3'-oic acid is the most abundant. Yamamoto et al. (137) have also reported 8-beta-hydroxy-delta-9-THC to be the major monohydroxy metabolite of delta-9-THC in a guinea pig microsomal preparation; analysis was by GC/MS using TMS derivatives.

The metabolic routes leading to the dihydroxy metabolites in the mouse were examined by Burstein and Shoupe (138) by studying the metabolism of several monohydroxy metabolites with GC/MS detection and were shown to be specific. Thus, whereas 8-alpha-delta-9-THC is hydroxylated at C-11, the 8-beta-isomer is not. Instead, this metabolite shows an alternative pathway of metabolism involving beta-oxidation of the side-chain to give metabolites similar to those observed from delta-9-THC in the guinea pig (139).

In vivo metabolism of delta-8-THC in the mouse (140) and guinea pig (141) has been shown to be very similar to that of delta-9-THC with a wide range of polysubstituted metabolites being produced.

b. Fragmentation:

i. Delta-9-THC. *Monohydroxy metabolites*—The most abundant ion in the mass spectrum of 11-hydroxy-delta-9-THC is at *m/z* 299 and is produced by loss of the 11-CH_2OH group by the mechanism shown in Scheme 3 (142,143). 8-Alpha- and 8-beta-hydroxy-delta-9-THC give similar spectra with prominent ions resulting from loss of water and of butylene from the side-chain. The elimination of water is more prominent for the 8-alpha-hydroxy isomer (82%) than for the other compound, presumably because of the greater availability of extractable hydrogen atoms. The most abundant ions in both spectra are reported differently in various publications. These are at *m/z* 297 corresponding to loss of water and a methyl radical (107,118) and at *m/z* 271 corresponding to loss of an isopropyl radical (142). The base peak in the mass spectrum of 3'-hydroxy-delta-9-THC is at *m/z* 258 (144) and is the result of elimination of C_4H_8O from the side-chain according to the mechanism related to that shown in Scheme 6. Other major ions are the molecular (*m/z* 330, 39%) and $[M - CH_3]^+$ ions (*m/z* 315, 12%) and *m/z* 247 (25%, ion *i*) (118). Ion *i* is the base peak in the spectrum of the 4'-hydroxy metabolite.

The mass spectra of the *bis*-acetate derivatives of 8-alpha-, 8-beta-, and 11-

hydroxy-delta-9-THC are very similar (142) and not suitable for structural characterization . This can be rationalized by charge localization on the acetyl moiety of the hydroxy group, which results in the formation of the most abundant ion as $[M - CH_3COOH]^{+\cdot}$ in each case. Loss of ketene from the phenolic acetyl group of this ion gives *m/z* 312. All other fragment ions are typical of those from THC itself.

TMS groups have a strong directing influence on the fragmentation of hydroxy metabolites of most of the cannabinoids and, in particular, of delta-9-THC (145) and are, therefore, of much greater value than acetyl derivatives for structural determination. In many cases, the spectra are dominated by a single diagnostic ion unique to a particular metabolite. As these diagnostic ions are also present in the spectra of polysubstituted metabolites, the identification of these compounds by mass spectrometry is reasonably straightforward. In many cases, the mechanisms producing these ions can be identified in the fragmentation of delta-9-THC itself, but the presence of the TMS group greatly enhances their relative abundance.

The mass spectrum of the TMS derivative of 11-hydroxy-delta-9-THC (Fig. 11) is characterized by a very abundant ion at *m/z* 371 produced by elimination of the 11-CH_2OTMS group as shown in Scheme 3 (145). This is similar to the major mechanism producing the $[M - 15]^+$ ion from the parent compound. The spectrum of the TMS derivative of 8-alpha-hydroxy-delta-9-THC (Fig. 14a), on the other hand, has no significant ion at this mass, but is characterized by a most abundant ion at *m/z* 384, corresponding to loss of trimethylsilanol (145). Deuterium labelling indicates the 10a-position as the source of the abstracted hydrogen atom (126,132,133). The high abundance of this ion can be explained by the ease of abstraction of the hydrogen atom as it is both allylic to the double bond and benzylic to the benzene ring, and

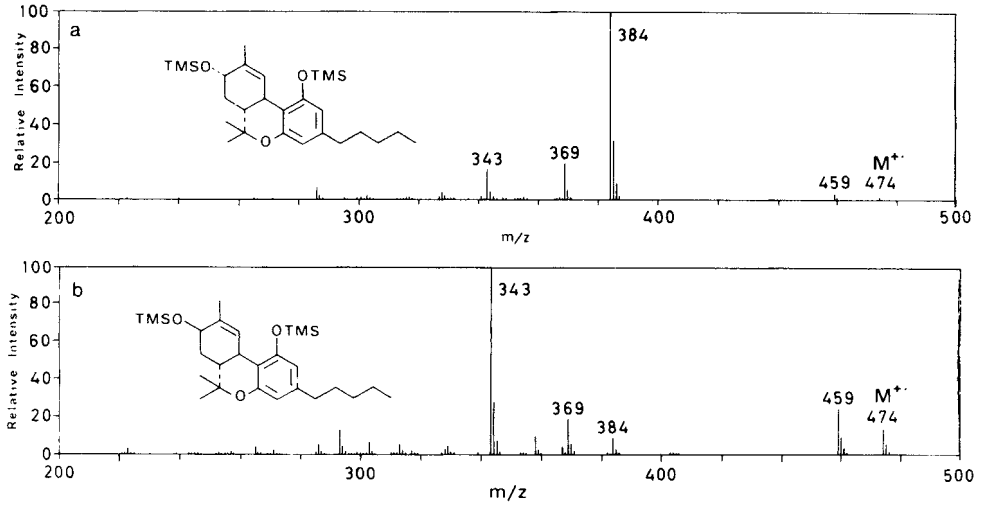


Figure 14. Mass spectra (25 eV) of the TMS derivatives of 8-alpha- (a) and 8-beta- (b) hydroxy-delta-9-THC.

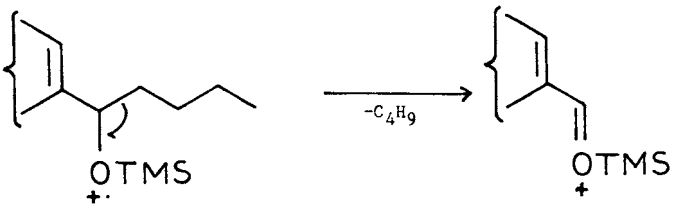
to the fact that the pseudo-axial 8-alpha-OTMS group can approach to within bonding distance. The spectrum of 8-beta-hydroxy-delta-9-THC TMS ether (Fig. 14b), on the other hand, has only a low abundance ion at this mass, as the pseudo-equatorial OTMS group cannot approach a suitable hydrogen atom. In the mass spectrum of the [10a-$^2H_1$]analogue, abstraction of a small amount of the deuterium indicates that ring cleavage is involved to some extent in the formation of the $[M - TMSOH]^{+\cdot}$ ion. The base peak in the spectrum of this compound corresponds to an ion at *m/z* 343 and is equivalent to the $[M - 43]^+$ ion in the spectrum of delta-9-THC TMS ether. Its formation can be rationalized by the mechanism shown in Scheme 4 (145). Loss of a methyl radical from the $[M - TMSOH]^{+\cdot}$ ion of these 8-hydroxy metabolites gives *m/z* 369; the source of the methyl group has not been determined, although the spectra of the [$^2H_9$]TMS derivatives shows that it does not arise from the phenolic TMS group.

Most TMS ethers of side-chain hydroxy metabolites produce diagnostic mass spectra (145–148). 1′-Hydroxy metabolites are characterized by an abundant ion at *m/z* 417 ($[M - 57]^+$, *z*, Scheme 20). 2′-hydroxy metabolites produce most abundant ions at *m/z* 145 (*aa*, Scheme 21). An additional fragmentation of the 2′-hydroxy metabolite involves transfer of the TMS group to the benzene ring with loss of 72 mass units (C_4H_8O, *ab*, Scheme 22). 3′-hydroxy metabolites fragment by a rearrangement reaction analogous to that producing the $[M - C_4H_8]^{+\cdot}$ ($[M - 56]^{+\cdot}$) ion from the parent molecule, to give a base peak at $[M - 144]^{+\cdot}$ (*ac*, Scheme 23). Although an alpha-cleavage ion is produced at *m/z* 117 in the spectra of 4′-hydroxy metabolites (Scheme 24), this is usually of low abundance and of little diagnostic importance. Relative GLC retention times are of more use for structural identification of this isomer; on SE-30 and OV-17 the side-chain hydroxy metabolites elute in numerical order from 1′-hydroxy to 5′-hydroxy although on SE-30 the separation between 3′- and 4′-hydroxy metabolites is marginal (125). The dominant ion in the spectra of the 5′-hydroxy metabolites is at *m/z* 315 (ion *g*). Table II summarizes the diagnostic ions found for the various monohydroxy metabolites.

The mass spectrum of 6-beta-hydroxymethyl-delta-9-THC (**33**) contains a base peak at *m/z* 299 representing loss of the 6-beta-hydroxymethyl group (149). The spectrum of the acetate (149) is less diagnostic with losses of OCH_3,

Table II. Diagnostic ions in the mass spectra of the TMS derivatives of monohydroxy metabolites of delta-9-THC

Hydroxy group	Ion	Structure
1′	$[M - 57]^+$	*z*
2′	*m/z* 145	*aa*
	$[M - 72]^+$	*ab*
3′	$[M - 144]^+$	*ac*
4′	*m/z* 117	*ad*
5′	*m/z* 315	*g*
8-alpha	$[M - 90]^+$	
8-beta	$[M - 131]^+$	*e*
11	$[M - 103]^+$	*c*

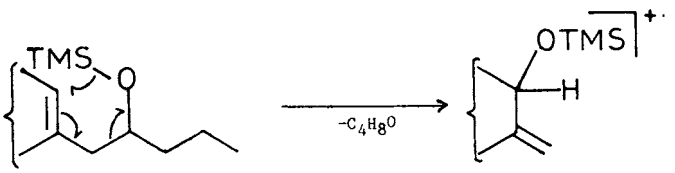


z, m/z 417

Scheme 20

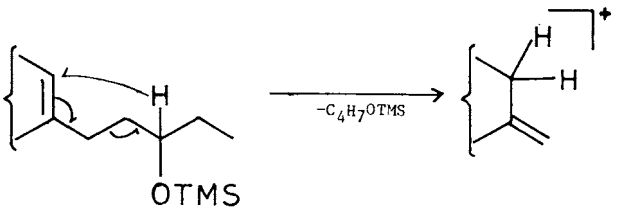
aa, m/z 145

Scheme 21



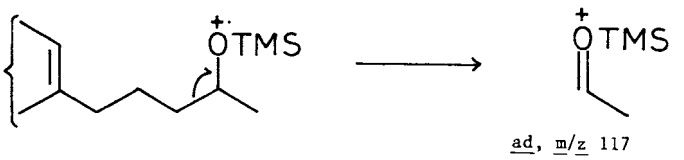
ab, $[M - 72]^+$

Scheme 22



ac, $[M - 144]^+$

Scheme 23



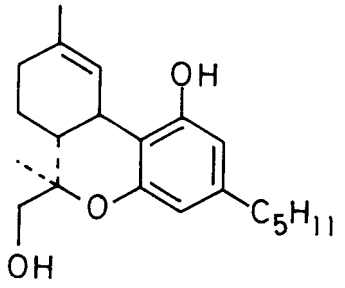
ad, m/z 117

Scheme 24

$COCH_3$, and CH_3COOH dominating the fragmentation. On the other hand, the spectrum of the TMS derivative (Fig. 15) shows a prominent molecular ion and a most abundant ion at *m/z* 384 due to loss of a trimethylsilyloxy group. The source of the eliminated hydrogen has not been determined. Further loss of a methyl radical gives *m/z* 369. Elimination of the trimethylsilyloxymethyl group gives the abundant ion at *m/z* 371.

Dihydroxy metabolites—The positions substituted in the dihydroxy metabolites of delta-9-THC are the same as those found in the monohydroxy metabolites. The mass spectra of the TMS derivatives of these metabolites usually contain the ions diagnostic of both substituted positions with one of them dominating the spectrum. The order of domination is usually 2′ = 3′ > 8-alpha > 11 > 8-beta (145). Thus, the spectrum of the TMS derivative of 8-alpha,11-dihydroxy-delta-9-THC is dominated by the $[M - TMSOH]^+$ ion (*m/z* 472, 100%) formed by elimination of the trimethylsilyloxy group from the 8-alpha position. As loss of trimethylsilanol leaves an odd electron ion, further diagnostic fragment ions may be formed as a fragment of this ion rather than from the molecular ion. The fragmentation of the TMS derivative of 8-beta,11-dihydroxy-delta-9-THC does not show the predicted fragmentation. Although the first stage in the fragmentation is the expected loss of CH_2OTMS from the 11-position to give an ion at *m/z* 459, this fragments further by loss of TMSOH rather than by the route typical of 8-beta-hydroxy-delta-9-THC to give the ion corresponding to the base peak at *m/z* 369. The ion diagnostic of 8-beta-hydroxy substituents ($[M - 131]^+$, *e*) is formed to the extent of only 13%. However, this ion is prominent in the spectra of the dihydroxy metabolites containing a 8-beta-hydroxy substituent and in which the second hydroxy group is in a position other than C-11. Molecular ions in the mass spectra of the TMS derivatives of metabolites containing a 8-alpha-hydroxy group are usually of low abundance or absent, whereas those of the 8-beta-hydroxy metabolites are usually quite prominent. This is a reflection of the very favorable elimination of the 6-alpha-OTMS group and the more stable nature of the derivative of the 8-beta-hydroxy metabolite. Major fragment ions in the mass spectra of the TMS derivatives of identified dihydroxy metabolites of delta-9-THC are given in Ref. 145.

(±)-2′,11-Dihydroxy-delta-9-THC was synthesized (150) and its spectrum



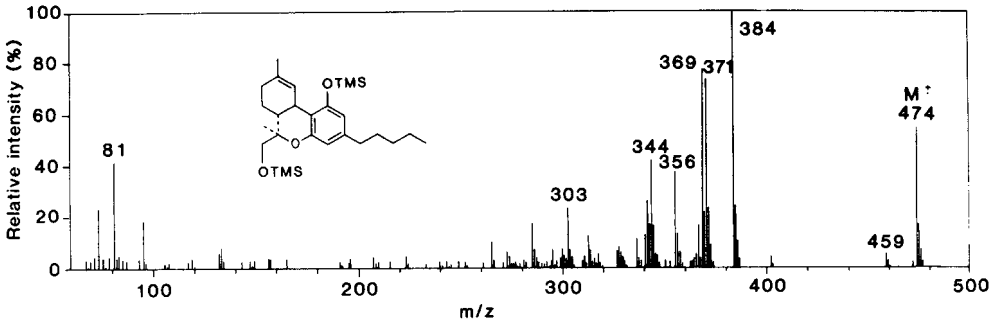


Figure 15. Mass spectrum of the TMS derivative of 6-beta-hydroxymethyl-delta-9-THC.

recorded. Unlike that of the TMS derivative where loss of $TMSOC_4H_8$ from the side-chain gives the ion representing the base peak, the most abundant ion in the spectrum of the nonderivatized compound is produced by loss of the 11-CH_2OH group.

Trihydroxy metabolites—Fragmentation of the TMS ethers of these compounds follows the same pattern as that shown by the dihydroxy metabolites (145). All of the compounds identified to date contain one hydroxy group at C-11, a second at C-8, and the third in the side-chain. The most abundant ion for most metabolites containing an 8-alpha-hydroxy group occurs at $[M - TMSOH]^+$, the exception being the 2',8-alpha,11-trihydroxy metabolite whose mass spectrum has a base peak at *m/z* 145 (ion *aa*). Molecular ion abundance with respect to the stereochemistry of the hydroxy group at C-8 parallels that of the dihydroxy metabolites.

Monocarboxylic acid metabolites—Delta-9-THC-11-oic acid (34) is the major metabolite of delta-9-THC in man, and the mass spectrum of its per-TMS derivative is shown in Fig. 16. The carboxylic acid group has little directing influence over the fragmentation with the normal delta-9-THC fragment ions present in its spectrum. The molecular and $[M - CH_3]^+$ ions are very prominent and the base peak, at *m/z* 371, corresponds to $[M - 117]^+$; this ion is produced by the same type of fragmentation as that giving rise to the most abundant ion in the spectrum of the 11-hydroxy metabolite (ion *c*, Scheme 3). The spectrum of the acid metabolite also contains an $[M - TMSOH]^+$ ion at *m/z* 398 whose formation involves abstraction of about 40% of the 10a-

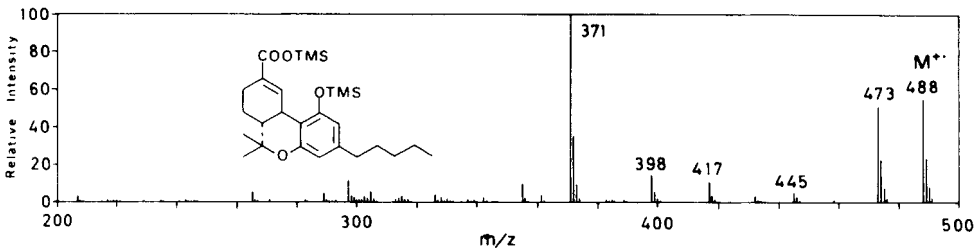


Figure 16. Mass spectrum (25 eV) of the TMS derivative of delta-9-THC-11-oic acid.

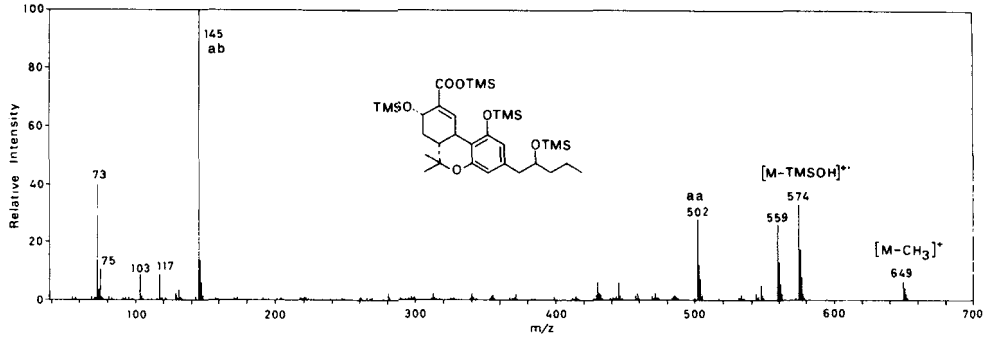


Figure 17. Mass spectrum (25 eV) of the TMS derivative of 2',8-alpha-dihydroxy-delta-9-THC-11-oic acid.

hydrogen. Carboxylic acid metabolites are more usually examined as their methyl ester: TMS ether derivatives. The methyl ester group makes little difference to the fragmentation except to reduce the mass of all ions containing the ester group by 58 mass units.

The weak directing influence of the carboxylic ester group is also seen in the fragmentation of the side-chain mono-carboxylic acid metabolites (135). Thus, the spectra of the derivatives of delta-9-THC-5'-oic acid are very similar to that of the 5'-hydroxy metabolite.

Mono- and di-hydroxy-derivatives of delta-9-THC-11-oic acid—Again the carboxylic ester group has little directing influence on the fragmentation of hydroxy derivatives of delta-9-THC-11-oic acid, and thus the fragmentation of these compounds parallels that of the corresponding hydroxylated metabolites (145). A typical spectrum is shown in Fig. 17, and major ions in identified hydroxy and dihydroxy-acid metabolites are listed in Ref. 145. The TMS derivatives of the 11-hydroxy analogues of side-chain acids are dominated by the ion produced by loss of the 11-hydroxy-substituent (151). Ions from other hydroxylated acids are listed in Refs. 110 and 111.

ii. (+)-cis-delta-9-THC. 11-Hydroxy-(±)-*cis*-delta-9-THC was synthesized (152) and its TMS derivative has a spectrum almost identical to that of the (−)-*trans*-isomer. Thus, the molecular ion is of low abundance (5%) and the most abundant ion (*m/z* 371, *c*) is due to loss of the 11-CH_2-OTMS group.

iii. delta-8-THC. *Monohydroxy metabolites*—The mass spectrum of the TMS derivative of 11-hydroxy-delta-8-THC is similar to that of the TMS derivative of the parent drug (142) in that ion *c* is of low abundance, unlike the spectrum of the delta-9-isomer where formation of this ion is a major fragmentation route. The molecular ion is more abundant than that of the delta-9-isomer and the base peak corresponds to an ion at *m/z* 231 that is the result of retro-Diels-Alder cleavage and loss of a methyl radical (ion *i*, Scheme 7).

The acetate derivative (142) gives a rather uninformative spectrum with a base peak corresponding to an ion formed by loss of acetic acid and ketene and less abundant ions produced by loss of these molecules individually from the molecular ion.

The spectrum of the TMS derivative again appears to be more informative (Fig. 18) and is very different from that of 11-hydroxy-delta-9-THC (145). Ion

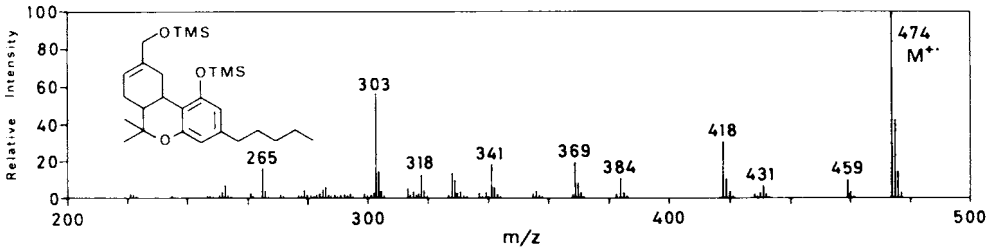
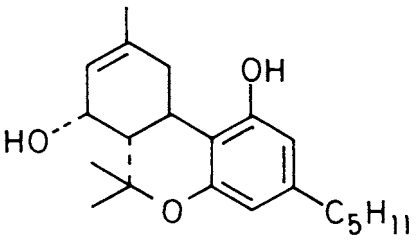


Figure 18. Mass spectrum (25 eV) of the TMS derivative of 11-hydroxy-delta-8-THC.

c (*m/z* 371) is of very low abundance because the mechanism depicted in Scheme 3 cannot occur and the resulting increased stability of the molecule is reflected in the abundant molecular ion (base peak). The major fragmentation is the formation of ion *i*, due presumably to the facile occurrence of the retro-Diels-Alder reaction (as Scheme 7). This ion is diagnostically useful as it enables side-chain hydroxy metabolites to be readily identified because of its mass shift to *m/z* 391. Loss of trimethylsilanol from the molecular ion gives the ion at *m/z* 418, but the source of most of the abstracted hydrogen is unknown. A small amount is abstracted from C-10a. Other fragment ions are conventional THC fragment ions formed either from the molecular ion or from the $[M - TMSOH]^{+\cdot}$ ion. Thus, the $[M - CH_3]^+$ and $[M - TMSOH - CH_3]^+$ ions are seen at *m/z* 459 and 369, respectively. In the case of the $[M - CH_3]^+$ ion approximately 40% arises by elimination of a TMS methyl group whereas the $[M - TMSOH - CH_3]^+$ ion appears to arise by elimination almost exclusively of one of the gem methyl groups.

Related ions are formed from 7-alpha- (**34**) and 7-beta-hydroxy-delta-8-THC (**35**, Fig. 19), but their relative abundance is sufficiently different to afford ready differentiation (145). The facile elimination of the axial trimethylsilyloxy group from the 7-alpha-hydroxy isomer, involving abstraction of the 10a-hydrogen, accounts for the low abundance of the molecular ion (9%) and the abundant $[M - TMSOH]^{+\cdot}$ ion (42%). The high abundance of the ion at *m/z* 369 ($[M - TMSOH - CH_3]^+$, 100%) can be rationalized by the mechanism shown in Scheme 25 (145). In the case of the TMS derivative of the 7-beta-isomer, loss of TMSOH is not as favorable as reflected in the more abundant molecular ion. The retro-Diels-Alder fragmentation thus dominates the spectrum to give *m/z* 303 as the most abundant ion.



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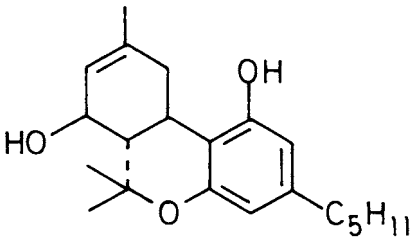

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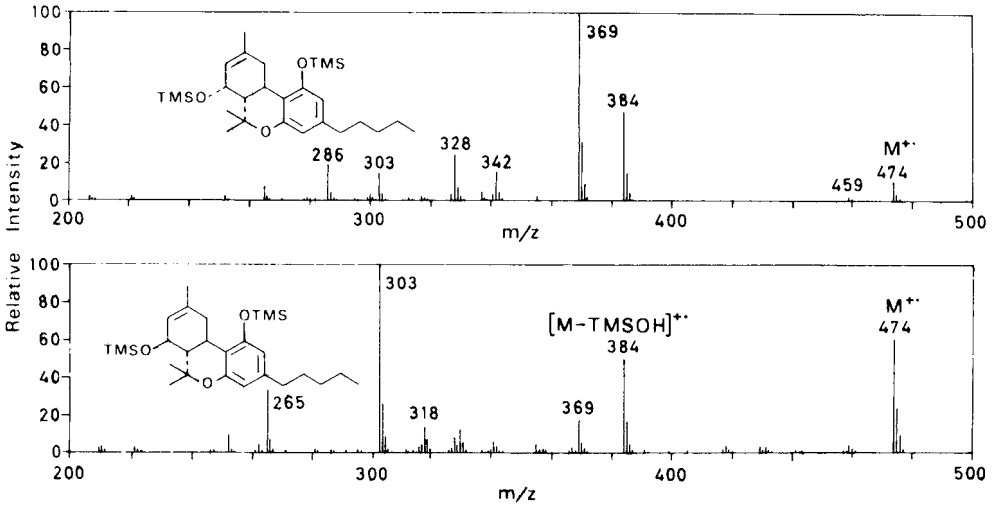
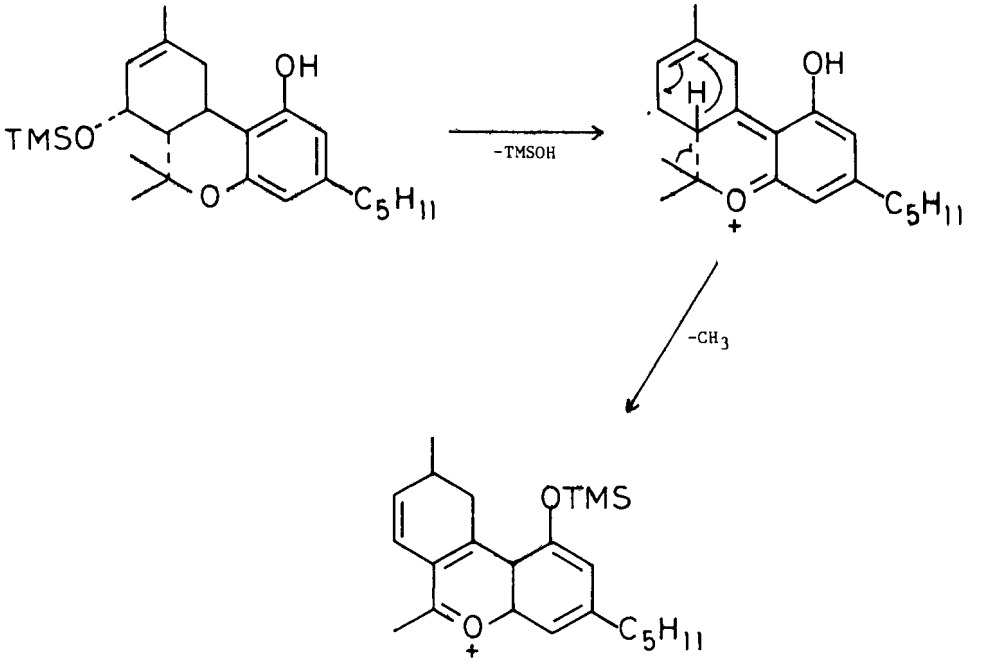
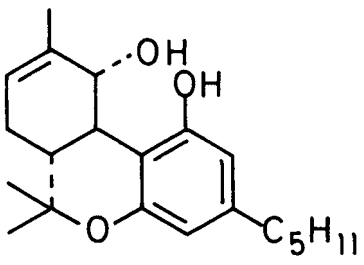


Figure 19. Mass spectra (25 eV) of the TMS derivatives of 7-alpha- and 7-beta-hydroxy-delta-8-THC.



ae, m/z 369

Scheme 25



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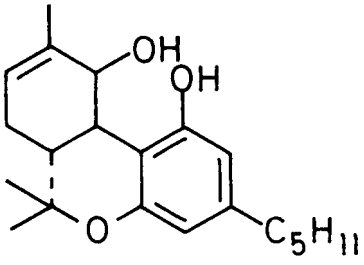

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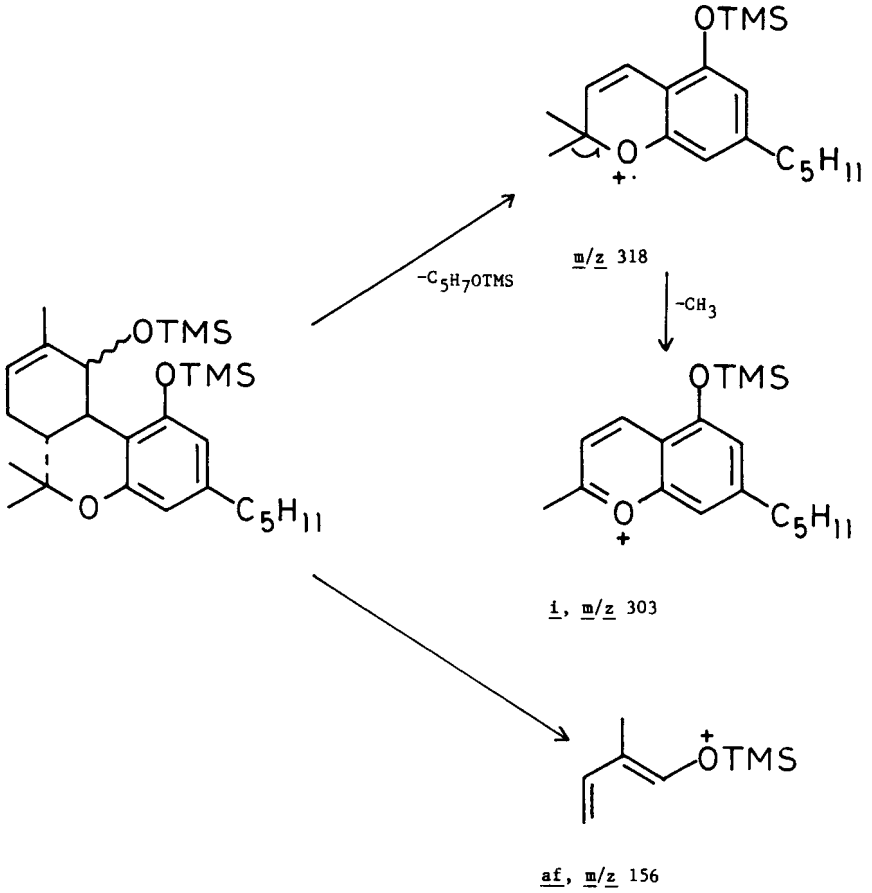
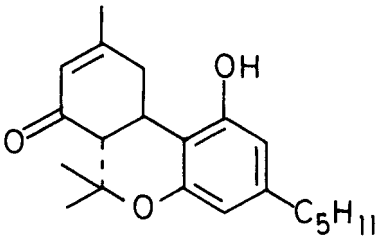
The allylic hydroxy derivatives, 10-alpha- (**36**) and 10-beta-hydroxy-delta-8-THC (**37**) have not yet been identified as metabolites of delta-8-THC, but the spectra of their TMS derivatives demonstrate an important additional fragmentation (145). The spectra of both compounds are almost identical and this can be rationalized by charge localization on the 10-OTMS group resulting in cleavage of the C-10:C-10a bond (Scheme 26). This then leads



Scheme 26



38

to radical (C-10)-induced cleavage to give *m/z* 318, which loses CH_3 to give an ion represented by the base peak at *m/z* 303, or to an ion of *m/z* 156 (8%) if the charge is retained by the OTMS group. These three fragment ions are the only ones having a relative abundance greater than 3%.

Side-chain hydroxylation produces compounds that give the same diagnostic ions as in the spectra of the delta-9-THC metabolites except in the case of the 5'-hydroxy metabolite whose mass spectrum has a base peak at *m/z* 391 (ion *i*) and a very abundant ion at $[M - 144]^+$ formed by a similar mechanism to that in Scheme 6.

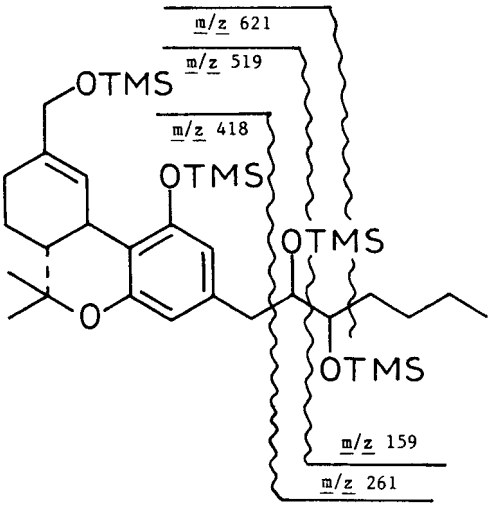
Oxo metabolites—The mass spectrum of 7-oxo-delta-8-THC (**38**) (142) contains an abundant molecular ion and three major fragment ions, all of which are produced by conventional cannabinoid fragmentations; these are the loss of C_4H_8 from the side-chain and the formation of ions *i* (*m/z* 231) and *q* (*m/z* 193). Its acetate derivative displays a similar spectrum with one additional ion produced by loss of ketene from the molecular ion.

Acid metabolites—The mass spectrometric fragmentation of these compounds has not been studied in detail, but several of the compounds were synthesized and the spectra of their methyl ester:TMS and per-TMS derivatives were recorded (112,145,153,154).

Polysubstituted metabolites—Again, no detailed fragmentation studies have been reported for these compounds. Spectra of the methyl and TMS derivatives can be found in Refs. 145 and 153.

iv. Delta-11-THC (8). Only two reports have appeared on the metabolism of this compound (155,156), but the reports between them include mass spectral data on 39 metabolites. In addition, a number of hydroxylated derivatives were synthesized (157) and their mass spectra recorded. Metabolism is similar to that of the other THC's in that allylic and aliphatic hydroxylations are common, but also different because the major route in the mouse, at least, involves epoxidation of the exocyclic double bond and hydrolysis to epimeric dihydroxy-HHC's. Rearrangement of the epoxides gives the 11-aldehyde, which is either oxidized to the corresponding carboxylic acid or reduced to the alcohol. The mass spectra of derivatives of these compounds are discussed under HHC metabolites.

The mass spectra of the nonderivatized 8-alpha- and 8-beta-hydroxy metabolites are complex (157) but show the same general trends to those shown by the other hydroxy-THC's. Thus, the molecular ion is more abundant



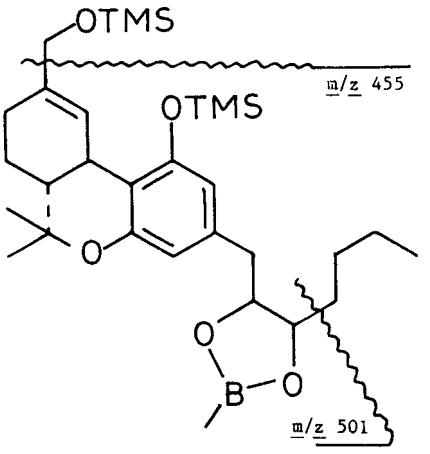
Scheme 27

(100%) in the spectrum of the 8-beta- (equatorial) isomer than in that of the 8-alpha-isomer whereas, in the latter compound, loss of water followed by a methyl radical leads to the ion represented by the base peak (*m/z* 297). In the spectra of the acetates, loss of acetic acid gives the most abundant ion for the alpha-isomer but, although the molecular ion is more abundant in the spectrum of the beta-isomer than in that of the alpha-isomer, it is not responsible for the base peak. Loss of acetic acid is very prominent (*m/z* 312, 86%) and the resulting ion loses a methyl radical to give *m/z* 297 (100%).

v. *n*-heptyl-delta-9-THC (39). The metabolism of this cannabinoid differs from that of the pentyl homologue in that few acid metabolites are produced but, on the other hand, there is an increased tendency for hydroxylation to occur on the side-chain with the result that tri- and tetra-hydroxylated metabolites are formed with two of the hydroxy groups in the side-chain (158). The positions of the other hydroxy groups are, as for the pentyl homologue, C-8 and C-11. Fragmentation of the TMS derivatives of these metabolites follows conventional pathways. Cleavage of the side-chain of metabolites containing two side-chain hydroxy groups produces several abundant fragment ions as outlined in Scheme 27. Confirmation of the 1,2-glycol structure in the side-chain of 2′,3′,11-trihydroxy-*n*-heptyl-delta-9-THC was obtained by the preparation of a cyclic methane boronate. This derivatization considerably simplifies the fragmentation pattern from that shown in Scheme 27 and gives the most abundant ion at $[M - CH_2OTMS]^+$ (Scheme 28), a fragmentation similar to that producing the base peak in the spectrum of the TMS derivative of 11-hydroxy-delta-9-THC (Scheme 2). Another diagnostic ion is loss of C_4H_9 from the side-chain (Scheme 28).

2. Metabolites produced by the epoxide-diol pathway

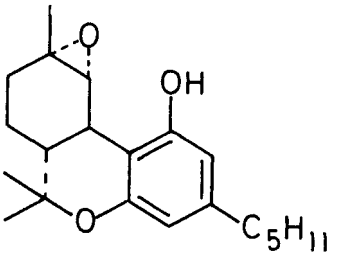
This metabolic route is seen in the metabolism of all THC's, and its importance depends on the position of the double bond. Gurny et al. (159)



Scheme 28

found an epoxide in metabolite fractions obtained from monkey liver homogenates, and its structure, 9-alpha,10-alpha-epoxy-HHC (**40**), was determined by synthesis (160). Other metabolites reported in these publications are 8-oxo-delta-9-THC, 7-oxo-delta-8-THC, and both 7-alpha- and 7-beta-hydroxy-delta-8-THC. Characterization was based on NMR spectra and high resolution mass spectra of the acetates. The latter were used to determine the molecular weights and elemental compositions. The same epoxide, together with 8-alpha- and 11-monohydroxy- and 8-alpha,11-dihydroxy-delta-9-THC acetates, were identified as metabolites in rabbit liver microsomes by using comparisons with synthetic standards and by using GC/MS (161,162). Mice were also shown to be capable of producing 9-alpha,10-alpha-epoxy-HHC (163) with identification, in this case being by GC/MS of the TMS derivative.

Proof of metabolic glycol formation from this epoxide was obtained by Ohlsson and Emanuelson (164) who used GC/MS to examine the metabolism of the oxide itself in rats. In addition to glycol formation, the epoxide is metabolized by hydroxylation at the 8-alpha-, 11-, 2′-, 3′-, and 4′-positions. Metabolites were examined as TMS derivatives, and structural determination



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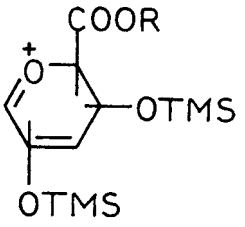


ag, R = CH_3, m/z 317

R = TMS, m/z 375

was based on comparison of GC/MS properties with those of synthetic standards or by the presence of diagnostic fragment ions (Table II) discussed earlier. Two glycol metabolites of 9-alpha,10-alpha-epoxy-HHC were found by Ben-Zvi (165) in the rat and characterized as the trans isomers, 9-alpha,10-beta, and 9-beta,10-alpha-glycols. Other metabolites identified as their acetate derivatives from delta-9-THC are the epoxide itself, the 8-alpha- and 11-monohydroxy metabolites, and 8-alpha-11-dihydroxy-delta-9-THC. In a study of delta-9-THC metabolism by guinea pig microsomes (137), Yamamoto et al. reported the formation of 9-alpha,10-alpha-epoxy-HHC. Further metabolism of this epoxide is by hydroxylation in the terpene ring and side-chain as determined by using GC/MS.

Delta-8-THC epoxide was detected in mice (166–168) by using GC/MS and its structure was determined as 8-alpha,9-alpha-epoxy-HHC. The 22 eV spectrum of its TMS derivative was recorded, and metabolism was reported to lead to the glycol, 8-beta,9-alpha-dihydroxy-HHC (169). This metabolic route appears to be of greater significance for this isomer for which the double bond is less hindered than that in delta-9-THC (140). It is even more prominent for the exocyclic isomer, delta-9(11)-THC, for which this route is the major biotransformation pathway. Fragmentation patterns are discussed later in the paper under HHC metabolites.

3. *Glucuronide conjugates*

In addition to glucuronide conjugates of delta-9-THC-11-oic acid discussed earlier, several other glucuronides of the tetrahydrocannabinols and their metabolites have been characterized by mass spectrometry. A novel *C*-glucuronide of delta-8-THC was synthesized and shown by Yagen et al. (170) to be produced enzymatically. It was later demonstrated as an *in vivo* metabolite of delta-8-THC in the mouse (171). Characterization involved a variety of spectroscopic techniques, mass spectrometry, and GC/MS of acetyl derivatives. Mass spectra of the *tris*- and *tetrakis*-acetyl derivatives of the two possible C-glucuronide derivatives (substitution at positions 2 or 4) were reported, together with that of the *tris*-acetyl derivative of the *O*-glucuronide. The spectra are dominated by ions resulting from elimination of acetic acid and acetyl radicals and, as such, are not particularly diagnostic. More useful spectra are exhibited by the TMS derivatives.

Detailed EI and CI studies of the TMS and methyl ester: TMS derivatives of glucuronide conjugates of delta-9-THC, delta-8-THC, CBD, and CBN were reported by Lyle et al. (172). All of the EI spectra of the Me: TMS derivatives are dominated by the ion at *m/z* 317 (*ag*) formed from the glucuronide moiety, and the corresponding ion at *m/z* 375 produces the base peak in the per-TMS spectra (see Fig. 20). Low abundance ions are present at $M^{+\cdot}$, $[M - CH_3]^+$, and $[M - 58]^{+\cdot}$ and are also found in the mass spectra of the Me:TMS derivatives; the latter ion, which is probably derived from elimination of CH_2CO_2 from the derivatized carboxylic acid function, is thought by Lyle et al. to be thermally enhanced as its relative abundance was lower in spectra recorded from the direct probe than in those from GLC introduction.

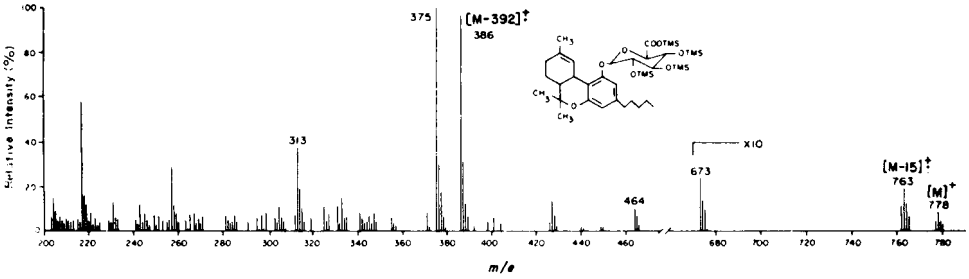
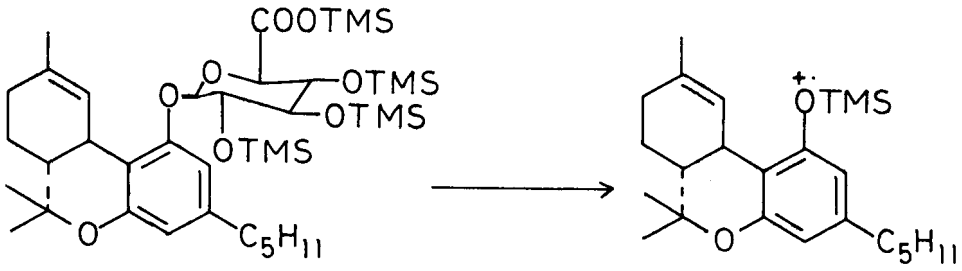


Figure 20. Mass spectrum of the per-TMS derivative of delta-9-THC glucuronide. (Reproduced with permission from Wiley-Heyden Ltd.)

The structure of these glucuronides as phenol-linked metabolites was confirmed by the presence in high abundance of the aglycone ion *ah* (for delta-9-THC) Scheme 29 ($[M - 334]^+$ in the Me:TMS spectra) produced by TMS transfer from the glucuronide moiety to the aglycone. This ion is of higher abundance than that at $[M - 423]^+$ which is produced by direct cleavage. This is again reflected in a later study (173) where the glucuronide conjugates of 5′- and 11-hydroxy-delta-9-THC were studied. Here, two glucuronides are possible for each metabolite depending on the point of attachment of the glucuronic acid residue. In the spectrum of the phenol-linked glucuronide, the aglycone ion produced with TMS transfer is more abundant than the ion produced without transfer by a factor of more than 10:1. In the spectrum of the other glucuronide, the abundance ratio of the two ions is reversed. Two other ions reflecting the site of attachment of the glucuronide residue are *ai* and *aj* of which *aj* is of higher abundance than *ai* in the spectra of all the phenol-linked glucuronides.

Fragment ions typical of the aglycone are also present in the spectra; for example, the spectra of derivatives of 11-hydroxy-THC glucuronide contain prominent ions at *m/z* 371 (*c*), thus aiding identification. Most other major ions in the spectra are typical of those from carbohydrate TMS derivatives.

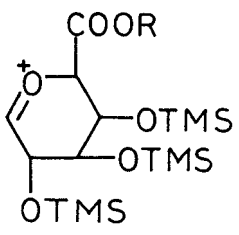
Ammonia CI produces more abundant ions in the molecular ion region ($[M + 1]^+$ and $[M + 18]^+$) than do the EI spectra, but neither of these ions is the base peak in any of the spectra yet examined. The most abundant ion



41

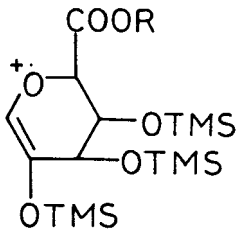
ah, m/z 386

Scheme 29



ai, R = CH_3, m/z 407

R = TMS, m/z 465



aj, R = CH_3, m/z 406

R = TMS, m/z 464

is either the aglycone ion produced with TMS transfer or the glucuronide ion *ag*. In general, these are the only abundant ions in the spectra.

The mass spectrum of the major human urinary glucuronide of delta-9-THC, delta-9-THC-11-oic acid, is shown in Fig. 21. This compound was assigned the structure of the acid glucuronide mainly on the basis of the most abundant ion at *m/z* 371, which, as was argued, could only have been

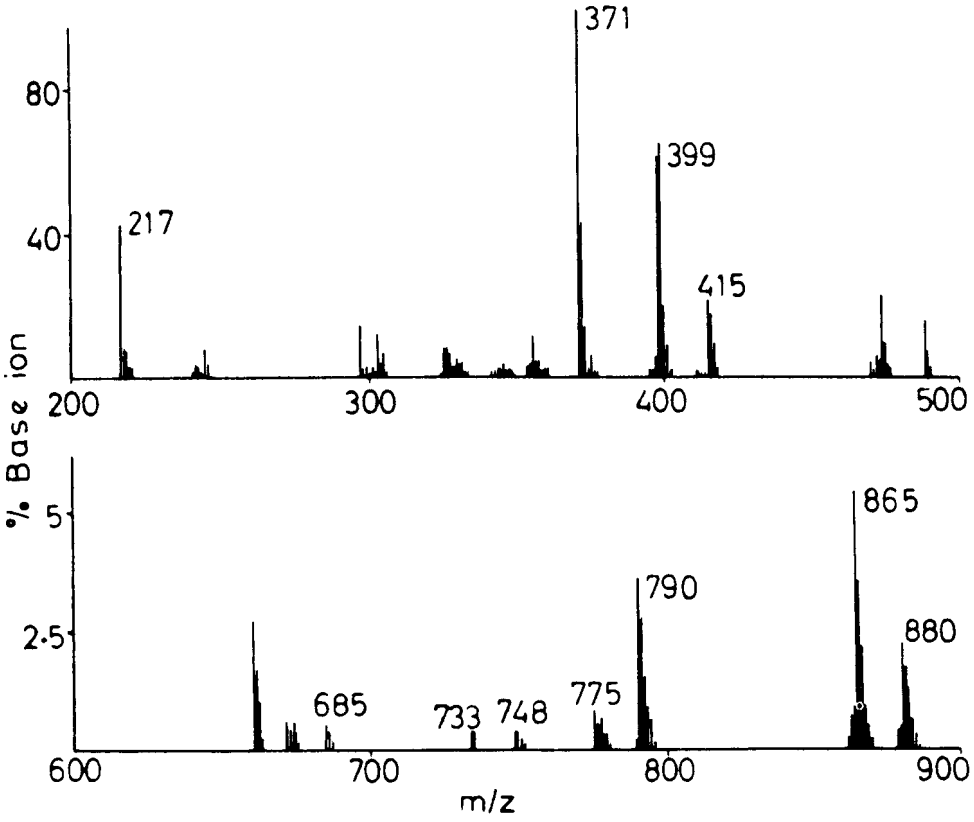


Figure 21. Mass spectrum of the TMS derivative of the acid glucuronide of delta-9-THC-11-oic acid. (Reproduced with permission from the Pharmaceutical Society of Great Britain.)

produced by cleavage of the esterified 11-carboxylic ester function. However, the presence of the ions just below *m/z* 500, which appear to be at *m/z* 488 and 473, would be typical of the aglycone ion formed by TMS transfer (*m/z* 488) and its analogue produced by loss of a methyl radical expected from a phenol-linked glucuronide. The ion at *m/z* 371 could then be produced from *m/z* 488 by loss of 103 mass units as in Scheme 3. The presence of this ion, therefore, cannot be used to define the point of attachment of the glucuronide in this case. However, a major difference in this spectrum and those of the phenol-linked glucuronides discussed earlier, is the absence of the prominent glucuronide-derived ion *ag* which, in this spectrum, would appear at *m/z* 375. Instead, prominent ions are present at *m/z* 398, 399, and 415. These were interpreted as involving loss of HO-(TMS)$_4$-glucuronide, -O-(TMS)$_4$-

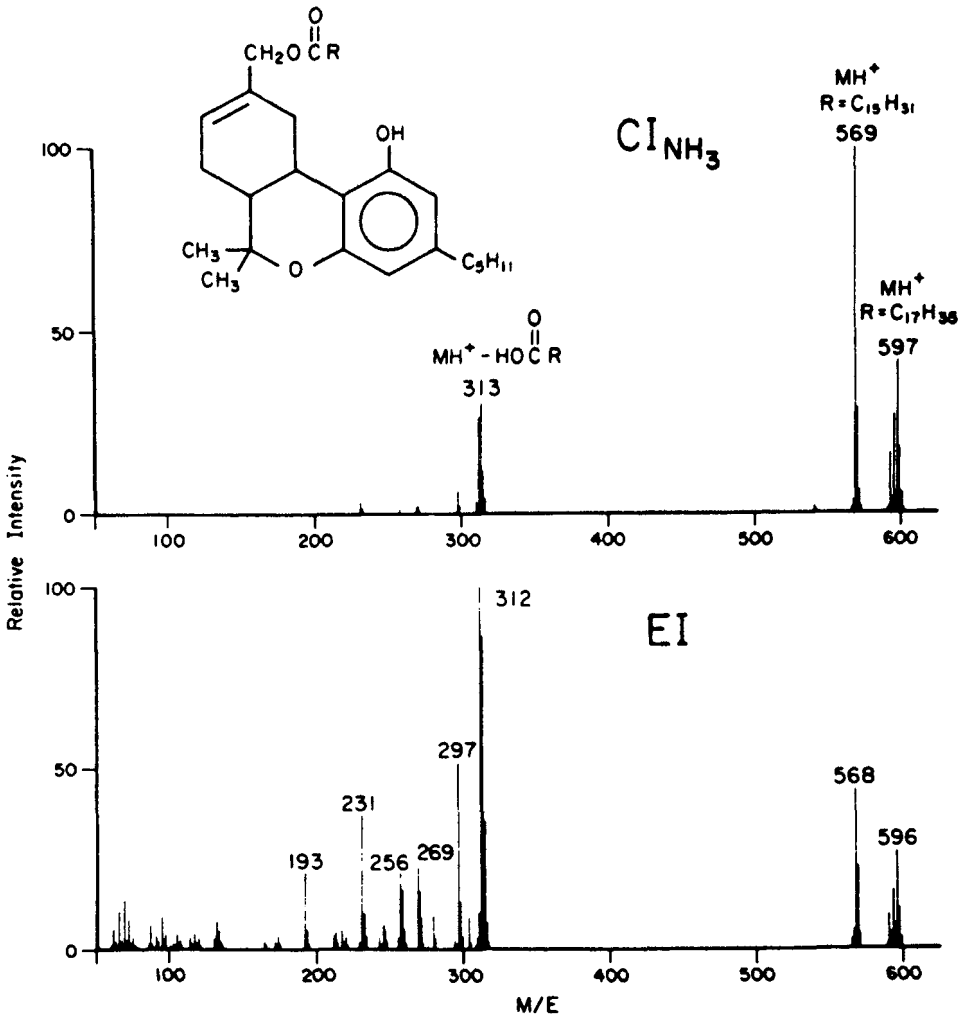


Figure 22. Electron ionization and ammonia chemical ionization mass spectra of the C_{16} and C_{18} fatty acid conjugates of delta-8-THC. (Reproduced with permission from PJD Publications Ltd.)

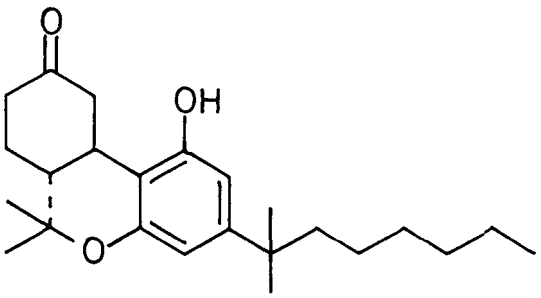
glucuronide, and -$(TMS)_4$-glucuronide, respectively, and would be consistent with the production of an acid glucuronide. The mass spectrum of the only other acidic cannabinoid metabolite to have been published, that of the Me:TMS derivative of the phenol-linked glucuronide of CBN-11-oic acid reported by Harvey (174), has a base peak corresponding to ion *ag*. Other ions in the upper mass range of the glucuronide of delta-9-THC-11-oic acid (Fig. 21) at $M^{+\cdot}$, $[M - CH_3]^+$, $[M - TMSOH]^{+\cdot}$, and $[M - TMSOH - CH_3]^+$ are typical of the spectra of TMS derivatives of sugars.

4. Fatty acid conjugates

Conjugates of 11-hydroxy-delta-9- and -delta-8-THC with long-chain fatty acids such as stearic, oleic, and palmitic acids were identified in rat tissues in 1976 by Leighty and co-workers (175) using MS and GC/MS techniques. Electron-ionization mass spectra of the nonderivatized compounds (e.g., Fig. 22) show prominent molecular ions and a most abundant ion produced by elimination of RCOOH from the esterified portion of the molecule. The ion produced by loss of the acyl radical is nearly as abundant. The conjugates' TMS derivatives give molecular ions of very low abundance, but the main initial fragmentation is again loss of RCOOH to give an ion at *m/z* 384. Acyl radical loss is insignificant. Further fragmentation of this ion by loss of 15, 43, and 56 mass units, and the formation of ions *i* and *q* (*m/z* 303 and 265, respectively) parallels that from the tetrahydrocannabinols. The ammonia CI mass spectra of both the derivatized and nonderivatized compounds gives the MH^+ ions as the most abundant with the only significant fragmentation being loss of RCOOH.

5. Metabolites produced by microorganisms

Several papers have described the characterization of metabolites of THC and other cannabinoids produced by a wide range of microorganisms. Thus, Binder (176) characterized 3'-, 4'-, and 8-alpha-hydroxy-delta-9-THC, 4'-hydroxy-8-oxo-delta-9-THC, 4',11-dihydroxy-, and 4',8-alpha-dihydroxy-delta-9-THC as metabolites of delta-9-THC produced by *Cunninghamalla blackesleeana* Lender, by using their TMS derivatives and utilizing the presence of the diagnostic ions listed in Table II for structural identification. 4'-Hydroxy metabolites of delta-8-, and delta-9-THC, CBD, and CBN were characterized as their TMS derivatives by Robertson and co-workers (177); molecular ions of the hydroxy-THC's are the most abundant and the diagnostic ion at *m/z* 117 is less prominent at 15–20%. TMS derivatives were also used by Christie, Rickards, and Watson (178) to identify 3'-hydroxy-, 3',11-dihydroxy, and 3'-oxo-delta-9-THC from *Chaetomium globosum* and by Robinson et al. (179,180) to identify several hydroxy metabolites and acid intermediates in the beta-oxidation of the side-chain of several cannabinoids. High resolution mass spectrometry and NMR were used by Abbott and co-workers (181) to identify the product of beta-oxidation of the side-chain of the synthetic cannabinoid,



42

Nabilone

nabilone (**42**), and in the same publication, hydroxy and keto metabolites of delta-6a(10)-THC (**7**) were reported. In addition to dihydroxy metabolites, Vidic et al. (182) identified glycols produced from epoxide hydrolysis, thus demonstrating yet another mammalian metabolic route also seen in microorganisms.

B. Cannabidiol (2)

The first metabolites of CBD to be identified were 3″- and 7-hydroxy-CBD (183), formed in rat liver (see Structure 2 for the numbering system). The nonderivatized 7-hydroxy metabolite produces an ion at *m/z* 299 (51%) representing cleavage of the 7-CH_2OH group and a base peak corresponding to *m/z* 244 of unknown origin (possibly [M − C_4H_8 from the side-chain − CH_2O]). The molecular ion (*m/z* 330) is of low abundance (7%), and the presence of ion *i* at *m/z* 231 confirms that hydroxylation has not occurred in the side-chain. In the spectrum of the other metabolite, this ion shift to *m/z* 247 (100%), indicating hydroxylation in the side-chain. The next most abundant ion is at *m/z* 262 (26%), the retro-Diels-Alder ion (*l*), containing the side-chain hydroxy-group. No major ion is present to indicate the position of the hydroxy group in the chain.

Much more informative mass spectra have been obtained from TMS derivatives of these and other monohydroxylated metabolites (184). Molecular ions, particularly from the 6-hydroxy metabolites, are of low abundance; base peaks, in most cases, correspond to the retro-Diels-Alder ion (*l*, *m/z* 478). The ions observed in the spectra of THC, diagnostic of the position of the side-chain hydroxy-group (Table II) are prominent but are, in the case of the 1″- and 3″-hydroxy metabolites, formed from fragmentation of the retro-Diels-Alder ion, *l* (ions, of *m/z* 421 and 334, respectively). 7-Hydroxy-CBD TMS ether fragments with an abundant loss of the 7-CH_2OTMS group to give an ion of *m/z* 443.

The retro-Diels-Alder ions (*m/z* 566) are also prominent in the mass spectra of the TMS derivatives of dihydroxy metabolites (185). For all side-chain hydroxylated derivatives of 7-hydroxy-CBD, loss of the 7-OTMS group dom-

inates the spectrum to give the ion at *m/z* 531. Similar spectra are produced from TMS derivatives of the corresponding hydroxylated metabolites of CBD-7-oic acid (186,187). The 2'-OTMS analogue, however, produces a most abundant ion at *m/z* 145 (ion *aa*). CBD-7-oic acid TMS ether gives a most facile loss of the 7-COOTMS group, a parallel fragmentation to that observed with the 7-alcohol. In the spectra of acids containing the carboxy-TMS group in the side-chain (1', 3', and 5' reasonably stable molecular ions are observed, and the most abundant ions correspond to $[M - 68]^{+\cdot}$ (ion *l*). The carboxylic acid groups have little directing effect on the fragmentation, and thus, the spectra are similar to that of CBD TMS ether itself. Benzyl ions (produced by loss of C-2" to C-5") are prominent and confirm the position of the carboxylic acid group in the side-chain. As would be predicted, the 7-OTMS derivatives of these acids fragment to give the most prominent ion at $[M - 103]^+$ (186). The 6-oxo-derivatives, on the other hand, give the tropylium ion as the most abundant.

Glucuronide conjugates of CBD and its major hydroxy metabolites are the first cannabinoid glucuronides to be identified by using GC/MS (188). Glucuronides have since been identified using GC/MS for many other cannabinoids (189). CBD glucuronide has been synthesized, and its mass spectrum studied in detail (172). In common with the spectra of the TMS derivatives of other phenol-linked glucuronides, migration of a TMS group to the aglycone moiety gives the diagnostic aglycone "molecular type" ion. This ion fragments by the characteristic retro-Diels-Alder reaction to give the *m/z* 390 ion (*l*) as the second most abundant ion in the spectrum. The ammonia CI spectrum of this compound is dominated by ion *ag* from the glucuronide moiety with the aglycone-derived ion (*m/z* 458) being nearly as abundant (172). In the molecular ion region, the $[M + 18]^{+\cdot}$ ion is the most abundant at nearly 20%.

C. Cannabinol (3)

The major biotransformation pathway undergone by this cannabinoid is again hydroxylation at C-11 to give 11-hydroxy-CBN, identified in rat liver preparations in 1971 by Widman et al. (190). Its mass spectrum is similar to that of CBN itself with the most abundant ion at $[M - 15]^+$, produced by loss of a gem-methyl group, and with all ions shifted by 16 mass units from their positions in the mass spectrum of CBN. The spectrum of the TMS ether is similar (174,191) and illustrates the general simplicity of the spectra of all metabolites of CBN. Hydroxylation also occurs in all positions of the side chain with fragmentation of the TMS ethers giving the same diagnostic fragment ions as are found for THC (Table II) (91,174,191,192). The TMS derivatives of the dihydroxy-metabolites, 1'-, 2'-, 3'-, and 4',11-dihydroxy-CBN, also give simple spectra with $M^{+\cdot}$, $[M - 15]^+$, and the diagnostic side-chain fragment ion being the only abundant ions in the spectra (174, 193). The ion, corresponding to $[M - CH_3]^+$, is the most abundant for 1'- and 2',11-dihydroxy metabolites with the side-chain fragment ions being most abundant in the spectra of the other two compounds.

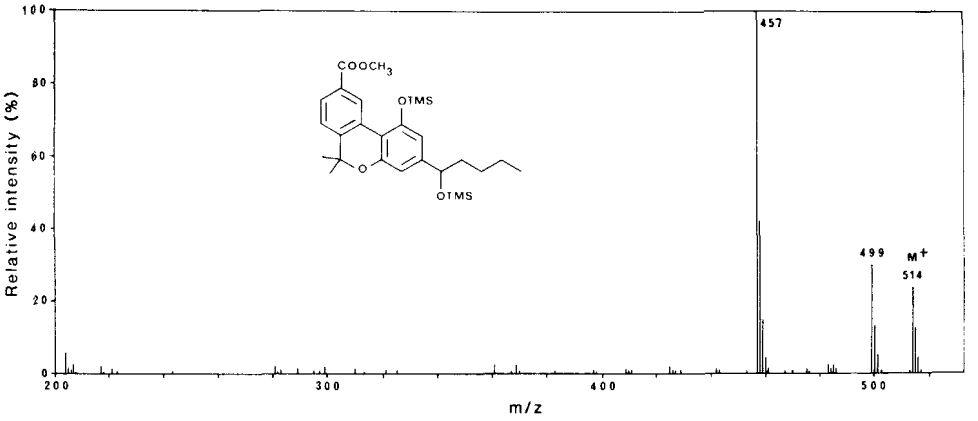


Figure 23. Mass spectrum (25 eV) of the methyl ester: TMS derivative of 1'-hydroxy-CBN-11-oic acid. (Reproduced with permission from Wiley-Heyden Ltd.)

Very similar fragmentation is observed for side-chain hydroxy derivatives of CBN-11-oic acid, both as TMS (174) and methyl ester; TMS ether derivatives (174,194). In common with the mass spectra of the di-hydroxy metabolites, only three ions are prominent in the mass spectra of the 1'-, and 2'-, and 3'-OTMS derivatives of this acid as shown by the example in Fig. 23.

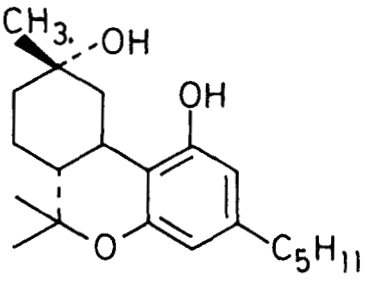
The mass spectra of the Me-TMS and TMS derivatives of the glucuronide conjugates are similarly diagnostic with abundant molecular and $[M - CH_3]^+$ ions (172,174). The base peak in the spectra of conjugates of CBN and its 11-hydroxy and -11-oic acid metabolites corresponds to the glucuronide-derived ion at *m/z* 317 (Me:TMS derivative) and at *m/z* 375 (TMS derivative, ion *ag*) (172). TMS transfer from the glucuronide moiety to the aglycone again gives prominent ions at the mass of the aglycone molecular ion. Abundant ions correspond to loss of a methyl radical from this ion. In the ammonia CI spectrum of the TMS derivative, the aglycone ion at *m/z* 382 is the most abundant with $[M + NH_4]^+$, at nearly 20%, being the most abundant ion in the molecular ion region.

Other reported conjugates of CBN are long-chain fatty acid (palmitic and oleic) esters of 4'-, 5'-, and 11-hydroxy-CBN (195). The mass spectra of their TMS derivatives are characterized by abundant molecular ions (33–50%) and base peaks corresponding to $[M - CH_3]^+$. Abundant metastable ions occur for this fragmentation. The two other major ions in each spectrum correspond to loss of the acyloxy moiety to give an ion of *m/z* 381 and an apparent loss from this of CH_3 to give an ion of *m/z* 366. This is interesting as it appears to represent the loss of two radicals from the molecular ion to give an odd electron ion.

D. Hexahydrocannabinol (HHC)

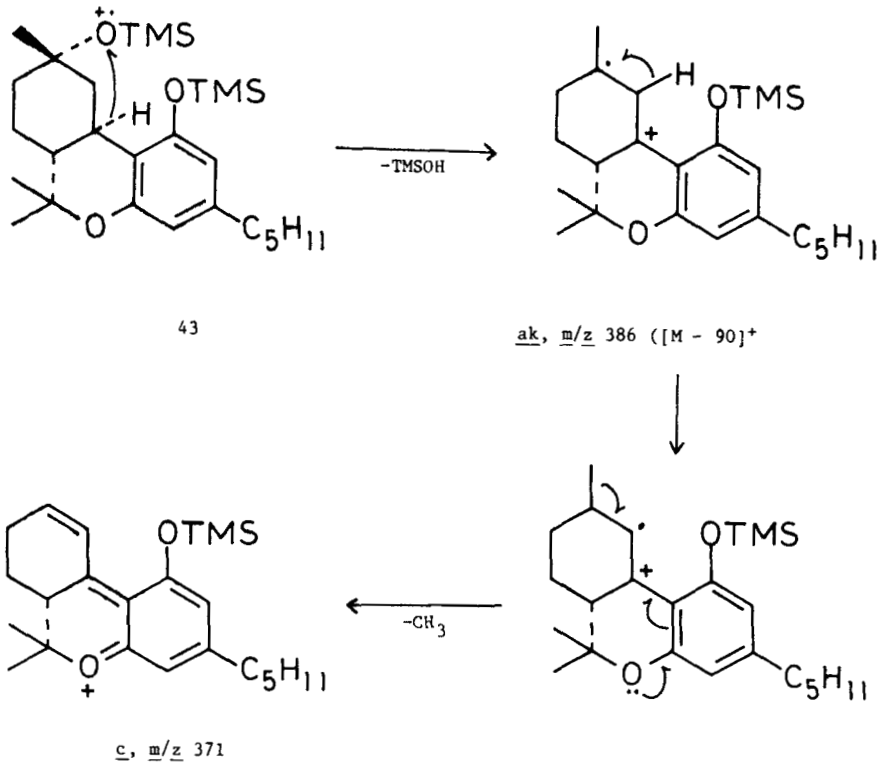
1. Monohydroxy derivatives

a. 9-Alpha-hydroxy-HHC (43) The major fragmentation shown by the TMS derivative of 9-alpha-hydroxy-HHC is loss of the 9-alpha-trimethylsilyloxy



43

group to give the ion at *m/z* 386 (100%) and is due to the favorable abstraction of the 10a-hydrogen by the axial OTMS group (196). Loss of a methyl radical, 74% of which comes from the 11-position, as shown by deuterium labeling (Scheme 30), gives the ion at *m/z* 371 (ion *c*). Other ions in the spectrum, namely those from the losses of 28, 43, 56, 71, and 83 mass units from the $[M - TMSOH]^{+\cdot}$ ion (*ak*) are those typically present in the spectra of THC's. Formation of the ion at $[M - 90 - 83]^{+}$ (*m/z* 303, *i*) involves retention of 60% of the 11-methyl group, a process which can be rationalized by the mechanism similar to that shown in Scheme 9.

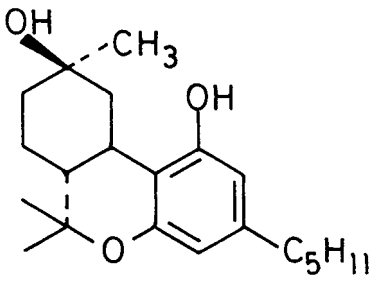


43

ak, m/z 386 ([M - 90]+

c, m/z 371

Scheme 30

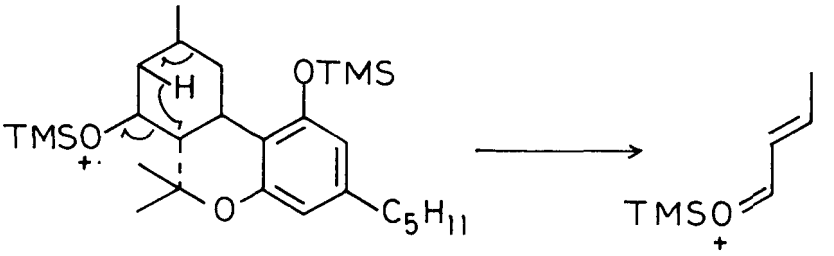


44

b. 9-Beta-hydroxy-HHC (44) In the spectrum of the nonderivatized compound (158), the $[M - H_2O]^+$ ion (*m/z* 314) is prominent (92%) but the most abundant ion is produced by further loss of a methyl radical to give an ion of *m/z* 299. Ions present in the mass spectrum of 9-beta-hydroxy-HHC TMS ether (196) are at the same mass as those from the alpha-isomer but are produced in different relative abundance. As the OTMS group is equatorial, TMSOH loss by direct abstraction of the 10a-hydrogen is precluded, and the $[M - TMSOH]^+$ ion is not as abundant (49%) as in the spectrum of the alpha-isomer. The molecular ion, on the other hand, is correspondingly more abundant (30%). Loss of CH_3 from the $[M - TMSOH]^+$ ion, to give the most abundant ion, involves mainly a gem-methyl group rather than the 11-methyl group, but again, a substantial proportion (49%) of the 11-methyl group is retained during formation of the ion of *m/z* 303 (ion *i*).

c. 8-Beta-hydroxy-HHC The TMS ether of this compound fragments in a similar way to other hydroxy-HHC's by elimination of TMSOH and formation of ions typical of THC's. However, as the OTMS group is equatorial, it is unsuitably placed to abstract a hydrogen atom, and thus, ring cleavage appears to occur first. This is supported by the observation that most of the hydrogen is abstracted from the 10a-position to give the $[M - TMSOH]^+$ ion at *m/z* 386 (22%). Further loss of a methyl radical from this ion gives the fragment at *m/z* 371 (48%). Because this process is relatively unfavorable, a stable molecular ion (100%) results. In the mass spectrum of the nonderivatized compound (158), the molecular ion is again abundant (81%), and the unfavorable loss of water results in the ion at *m/z* 193 (*q*) as the most abundant.

d. 7-Beta-hydroxy-HHC The spectra of both the 9R and 9S isomers of the TMS ethers of these two compounds are similar with base peaks corresponding to the molecular ions. Loss of TMSOH gives the *m/z* 386 ion (45%) and this fragments to give the ions typical of THC (at *m/z* 371, 40%; 343, 41%; 330, 65% and 265, 29%) and the ion at *m/z* 305. Because of the position of the OTMS group adjacent to the tertiary carbon atom at C-6a, cleavage of the C-6a – C-7 bond is initiated and gives rise to the two prominent ions at *m/z* 143 (44%, *al*) and 157 (48%, *am*). Their formation can be rationalized as in Schemes 31 and 32.



al, m/z 143

Scheme 31

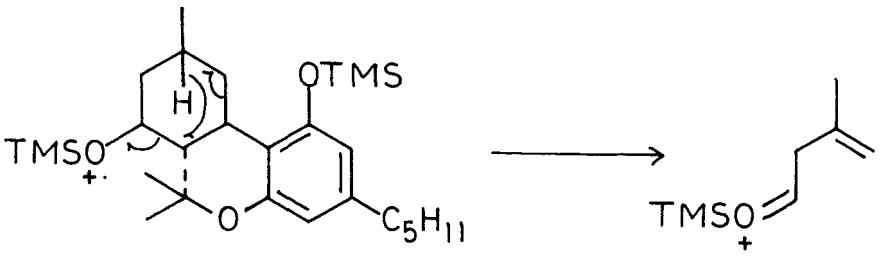
e. 10-Beta-hydroxy-HHC The presence of an equatorial hydroxy group results in a prominent molecular ion (61%). The major fragmentation is loss of water to give the *m/z* 314 ion followed by loss of a methyl radical to form the *m/z* 299 ion (100%). Ion *i* (*m/z* 231) is prominent at 46% (197). The spectrum of the acetate is similar with the base peak corresponding to a fragment formed by acetic acid and ketene elimination (197). No report has been published on the mass spectrum of the TMS derivative.

f. 11-Hydroxy-HHC The 70-eV mass spectrum of the nonderivatized compound was briefly described by Razdan and co-workers (198), but no fragmentation details were given. Major ions were listed as occurring at *m/z* 332 ($M^{+\cdot}$), 289, 276, 231, and 193. The TMS derivatives of both 9R and 9S isomers show similar ions although at somewhat different relative abundance. The molecular ion at *m/z* 476 corresponds to the base peak in the spectrum of both isomers, and fragment ions are formed by similar mechanisms to those for THC.

2. *Dihydroxy derivatives*

These compounds arise metabolically or as chemical artefacts by hydroxylation of metabolic epoxides. Different isomers are produced depending on which carbon-oxygen bond is broken.

a. 8,9-Dihydroxy-HHC The mass spectra of the TMS derivative of 8-alpha,9-alpha-dihydroxy-HHC and of its 9-beta-isomer are shown in Fig. 24



am, m/z 157

Scheme 32

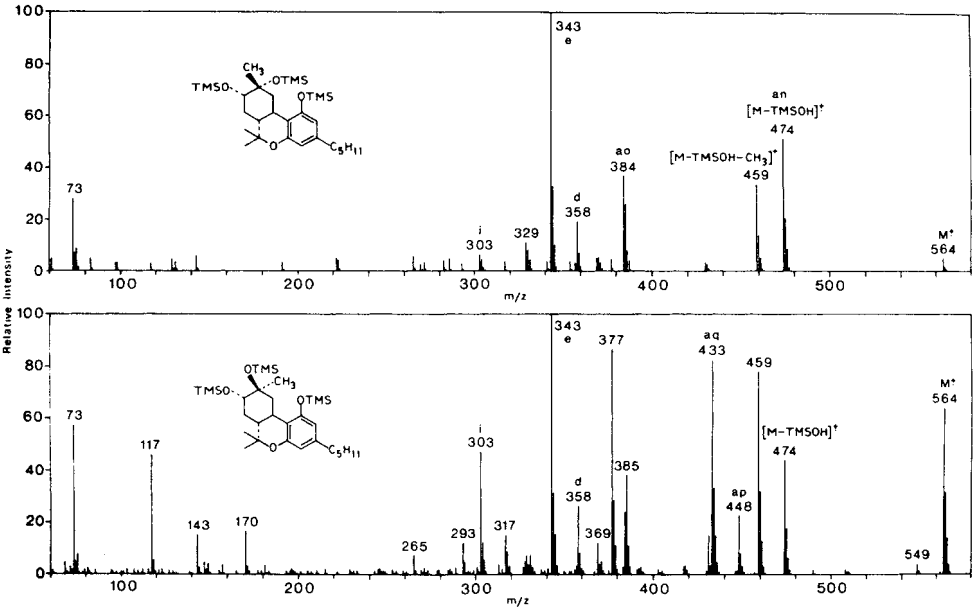


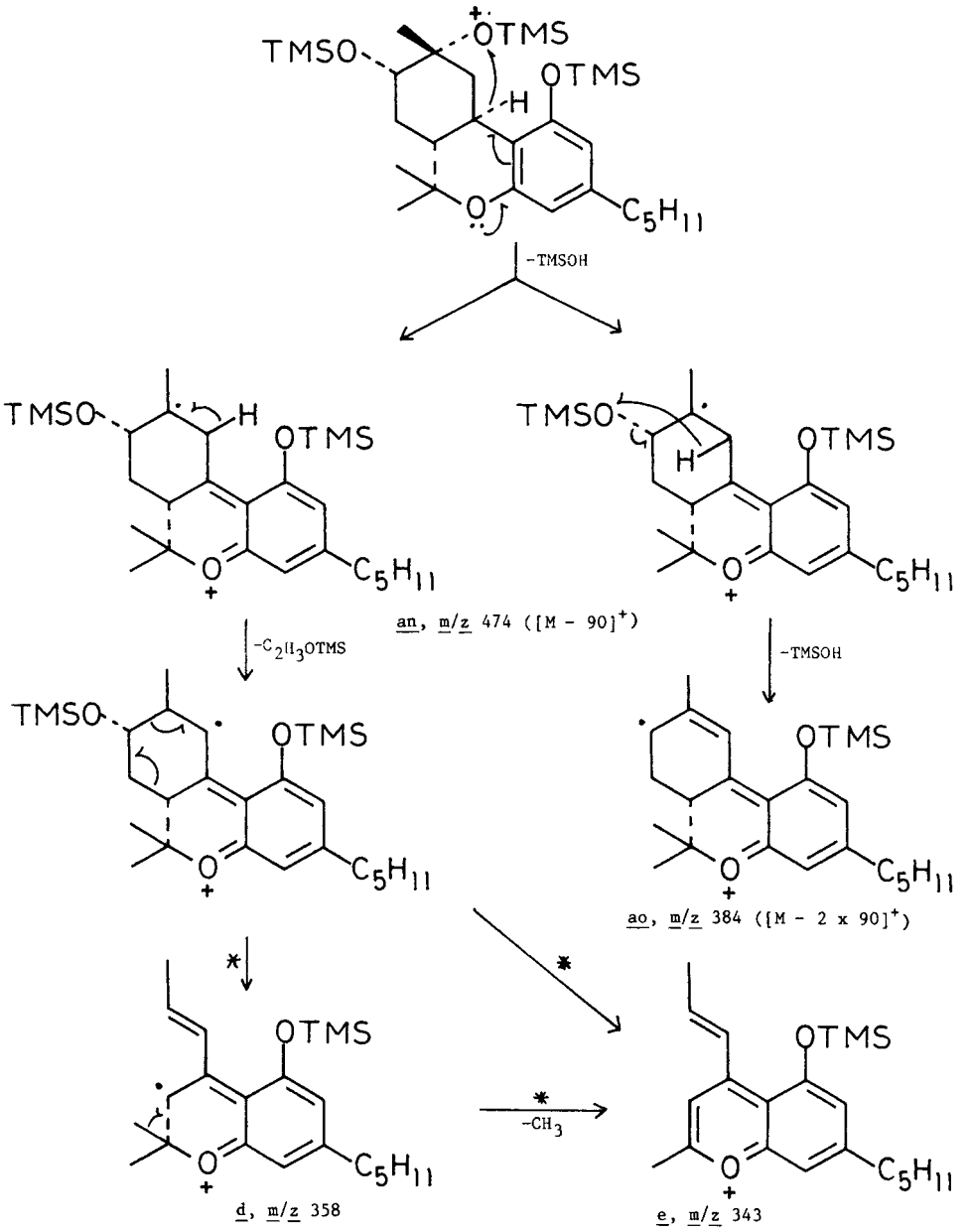
Figure 24. Mass spectra (25 eV) of the TMS derivatives of 8-alpha, 9-alpha-, and 8-alpha,9-beta-HHC.

(196). The influence of the axial 9-alpha-OTMS group is immediately apparent by the low abundance molecular ion and the prominent losses of trimethylsilanol and a methyl radical from the 9-alpha isomer. Selective TMS:[$^2H_9$]TMS labelling, which is possible as the result of the hindered nature of the 9-alpha-OTMS group, indicates initial TMSOH loss from this position by abstraction of the 10a-hydrogen (196). Further deuterium labelling experiments support the fragmentation pathways shown in Scheme 33.

The abundant molecular ion for the per-TMS derivative of 8-alpha,9-beta-dihydroxy-HHC (Fig. 24) reflects the equatorial positions of the OTMS groups, resulting in less favorable elimination of trimethylsilanol. Formation of the ions at *m/z* 448 and 433 can be rationalized as in Scheme 34, but the mechanism producing *m/z* 377 has not been determined.

Fragmentation of the 8-beta,9-alpha-isomer is very similar to that of the 8-alpha,9-alpha-isomer because of the initial favorable elimination of the 9-alpha-OTMS group.

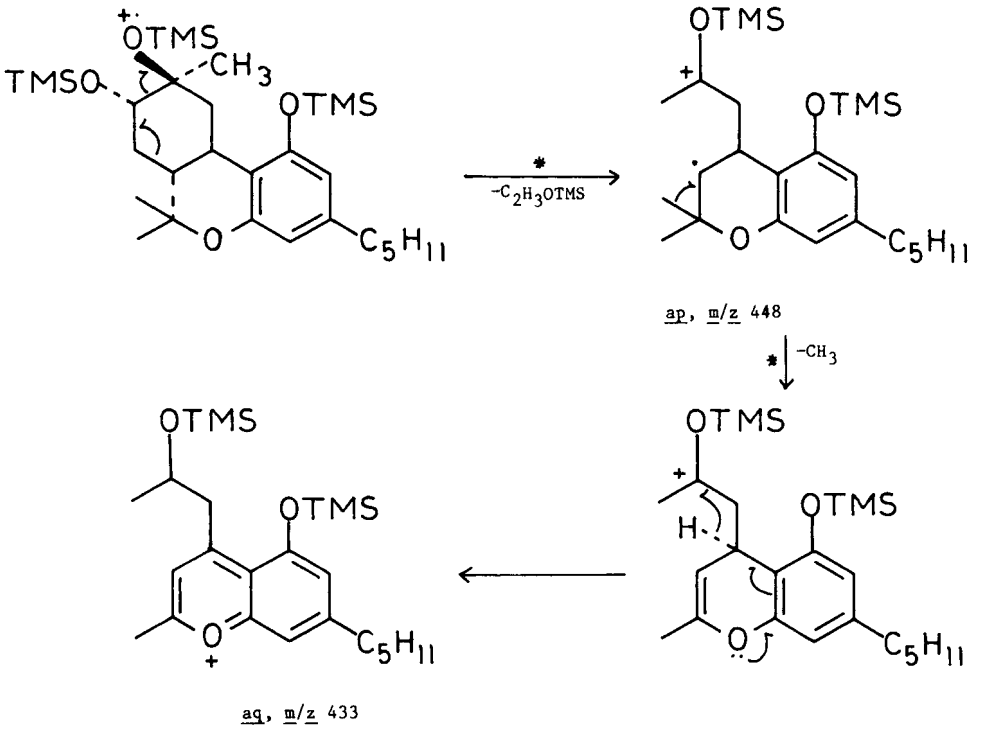
b. 9,10-Dihydroxy-HHC's The spectra of all isomers of these compounds are similar with ions formed by the loss of trimethylsilanol followed by a methyl radical being the most abundant in all cases (Fig. 25). Formation of the most abundant ion involves elimination of the 9-OTMS group from the 9-alpha-isomers accompanied by the 10a-hydrogen atom. This hydrogen atom is largely retained by the isomers containing a 9-beta-(equatorial) OTMS group, an observation that has been used diagnostically to differentiate these compounds (132). Further loss of a methyl radical from the 9-beta-OTMS isomers largely (80%) involves the gem-methyl groups, whereas that from



Scheme 33

the 9-alpha-OTMS isomer is predominantly from C-9. The ion at *m/z* 143 (*ar*) is abundant, and its formation can be rationalized as in Scheme 35.

c. 9,11-Dihydroxy-HHCs These two compounds, epimeric around C-9, are produced as metabolites of the epoxide from delta-9(11)-THC. The mass spectra of their TMS derivatives are almost identical, suggesting that fragmentation involves a common intermediate. This fragmentation is depicted



Scheme 34

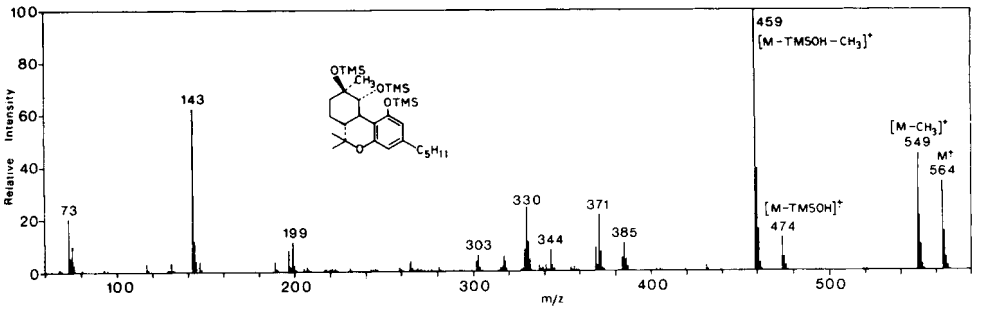
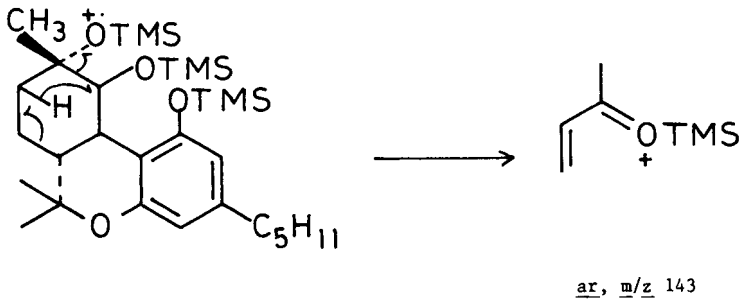
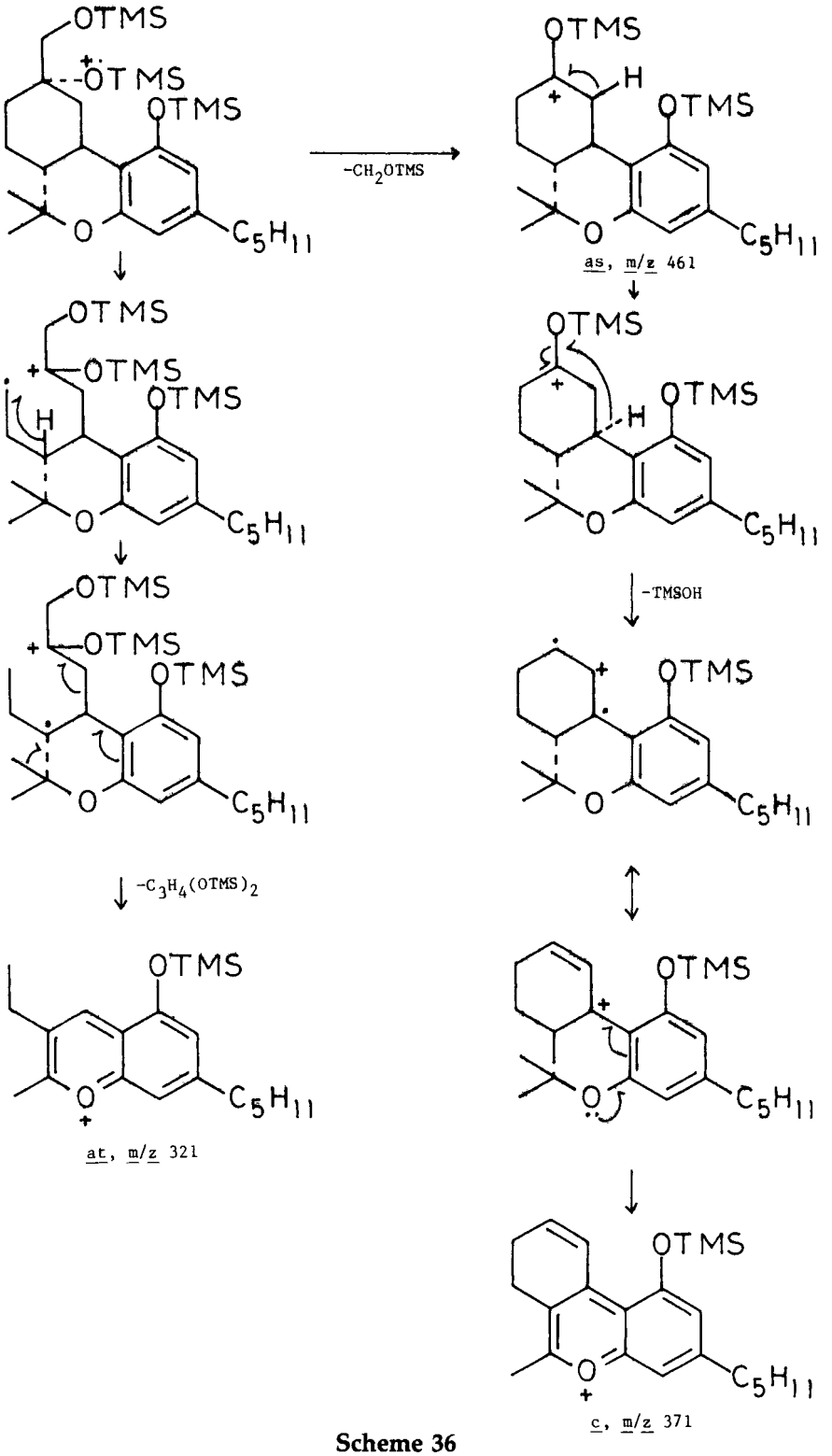


Figure 25. Mass spectrum (25 eV) of the TMS derivative of 9-beta,10-alpha-HHC.



ar, m/z 143

Scheme 35


OTMS
OTMS
OTMS
O
C_5H_{11}
$-CH_2OTMS$
H
$-TMSOH$
$-C_3H_4(OTMS)_2$
as, m/z 461
at, m/z 321
c, m/z 371

Scheme 36

in Scheme 36 (196). Initial cleavage of the C-9 – C-11 bond gives a common ion (*m/z* 461, *as*) from both compounds, thus accounting for the abstraction of the 10a-hydrogen during subsequent loss of TMSOH in both cases.

IV. MISCELLANEOUS DERIVATIVES FOR CANNABINOID ANALYSIS

In addition to the conventional acetyl (A, Table III), TMS (B) and alkyl (e.g., Me, C, Table III) derivatives, many other derivatives have been investigated for mass spectrometric and GC/MS analysis of the cannabinoids. Trialkylsilyl derivatives with alkyl groups larger than methyl (Et, Pr, Bu, Hex) D – G, Table III) were investigated as GLC shift reagents in order to separate monophenolic from diphenolic cannabinoids. A greater retention increment shift is introduced into the diphenolic cannabinoids by virtue of the larger alkyl groups, and differential separation was obtained (199). Fragmentation under EI conditions (200) gives spectra very similar to those observed with the TMS derivatives, even though for other compounds such as steroids and alkanols, elimination of alkyl radicals and alkenes from the trialkylsilyl group dominates the fragmentation. These eliminations are attributed to the fragmentation directed from sites remote from the derivatized function. *t*-Butyl-dimethylsilyl derivatives (H), often prepared to provide an abundant $[M - t\text{-Bu}]^+$ ion for selected ion monitoring purposes, fragment to give the reasonably abundant $[M - t\text{-Bu}]^+$ ions, but of several cannabinoids examined, in only delta-9-THC is this the base peak (32,201). Considerable additional fragmentation occurs from the rest of the molecule, thus limiting the use of these derivatives for high sensitivity work. Allyldimethylsilyl derivatives (I) fragment in a similar way, but additional abundant

Table III. Some derivatives used for mass spectrometric and GC/MS studies on the cannabinoids.

No.	Name	Acronym	Structure
A	Acetyl	Ac	$COCH_3$
B	Trimethylsilyl	TMS	$Si(CH_3)_3$
C	Methyl	Me	CH_3
D	Triethylsilyl	TES	$Si(C_2H_5)_3$
E	Tri-*n*-propylsilyl	TPS	$Si(C_3H_7)_3$
F	Tri-*n*-butylsilyl	TBS	$Si(C_4H_9)_3$
G	Tri-*n*-hexylsilyl	THS	$Si(C_6H_{13})_3$
H	*tert*-Butyldimethylsilyl	TBDMS	$Si(CH_3)_2$-*t*-C_4H_9
I	Allyldimethylsilyl	ADMS	$Si(CH_3)_2CH_2CH=CH_2$
J	Vinyldimethylsilyl	VDMS	$Si(CH_3)_2CH=CH_2$
K	Alkoxyldimethylsilyl	—	$Si(CH_3)_2OR$
L	Trimethylsilylacetyl	—	$COCH_2Si(CH_3)_3$
M	Diethylphosphate	—	$PO_2(OC_2H_5)_2$
N	Trifluoroacetyl	TFA	$COCF_3$
O	Flophemesyl	—	$Si(CH_3)_2C_6F_5$
P	Pentafluoropropionyl	PFP	COC_2F_5
Q	Deuteromethyl	—	C^2H_3
R	Ethyl	Et	C_2H_5
S	Pentafluorobenzyl	—	$CH_2C_6F_5$

ions resulting from loss of C_3H_4 are seen (32). Both *t*-butyl- and allyldimethylsilyl derivatives have been reported to enhance the relative abundance of the molecular ions of a number of cannabinoids.

Other alkyldimethylsilyl derivatives have little effect on the fragmentation of cannabinoids (26). Vinyl- (J) (202,203) and alkoxy-dialkylsilyl derivatives (K) (203,204) of the tetrahydrocannabinols, although producing many of the fragmentations shown by the TMS derivatives, induce an additional fragmentation resulting in loss of the entire cannabinoid side-chain. This ion corresponds to the base peak in the spectrum of the delta-9-THC derivatives.

TMS-acetates (TMS-CH_2-CO-) (L) are of less use for identification purposes as their spectra are devoid of molecular ions (201) on account of a very ready loss of the TMS-acyl radical to give the phenoxy cation. Differentiation between delta-8- and delta-9-THC can be made from the further loss of a methyl radical from this ion. The loss of a methyl radical leaves an odd-electron ion and its unusually high abundance in the spectrum of the delta-9-isomer presumably indicates involvement of the double bond to give a cyclic structure. Diethyl phosphate derivatives (M) of the THC's (201) show spectra having molecular ions of low abundance and abundant ions at *m/z* 297 and 296 produced by elimination of the diethylphosphate radical and diethylphosphoric acid, respectively.

Negative methane CI of trifluoroacetate derivatives (N) of delta-9-THC and its two major metabolites were published (205); the spectra of THC and delta-9-THC-11-oic acid has M^- corresponding to the base peak and a low abundance ion at *m/z* 113 (CF_3COO^-), whereas the 11-hydroxy metabolite fragments to give $[M - CF_3COO]^-$ as the ion of highest mass.

V. MEASUREMENTS OF CANNABINOIDS IN PHYSIOLOGICAL MEDIA

The low concentrations of cannabinoids in blood after cannabis consumption, typically in the low nanogram to picogram range, demand sensitive analytical techniques for their measurement, and mass spectrometry has played a major role in studies requiring measurement of the drug at these concentrations.

In common with most chromatographic methods for cannabinoid quantification, the sensitivity achieved with mass spectrometry for measurements of delta-9-THC and its metabolites in body fluids and tissues is limited by the signal : noise ratio achieved at the detector. Although detection limits in the low picogram to upper femtogram (10^{-15}) range are readily attainable by mass spectrometry when pure compounds are measured, contaminating ions arising from fragmentation of co-extracted endogenous material severely reduce the sensitivity in the "real" situation. Reductions in the order of 1000-fold are not uncommon. Consequently, most assays employing GC/MS contain at least one cleanup stage between the extraction of the cannabinoids from the biological matrix and their ultimate measurement. This, of course, considerably lengthens the analysis time and cost compared with a method employing simple extraction and measurement and, in some cases, tends to

reduce the usefulness of these assays. On the other hand, the high specificity and sensitivity that can be obtained with GC/MS more than justifies its use if the necessary apparatus is available. Nevertheless, the high cost of the equipment has resulted in cheaper techniques such as radioimmunoassay being used as the methods of first choice for forensic analysis, with mass spectrometry-based methods being used for confirmation of results. Most of the reported assays for delta-9-THC and its metabolites have concentrated on the development of highly sensitive techniques in order to measure the drugs at the very low concentrations encountered in the body.

With such low concentrations of drug, it is important to take the usual precautions regarding cleanliness of glassware and solvents that are commonly used in assays of this type. Solvents should be redistilled, preferably twice and not stored in plastic containers. Cannabinoids bind strongly to glass, but this can be prevented by silanizing all glassware with dichlorodimethylsilane (206,207). Some cannabinoids are acid-labile (208–210), decomposing by isomerization, ring opening, and disproportionation; thus low pH's should be avoided during extraction.

The first GC/MS method for the measurement of delta-9-THC in plasma incorporated selected-ion-monitoring and was published by Agurell et al. in 1973 (211). The drug and its internal standard, [1′,1′-$^2H_2$]delta-9-THC were extracted with 1.5% isoamyl alcohol in light petroleum, and cleanup was by Sephadex LH-20 column chromatography. No derivatization was used, but even so, delta-9-THC could be measured to 1 ng/mL. A similar method for the measurement of delta-8-THC to 0.3 ng/mL was reported in the following year (212) with [1′,1′,2′,2′-$^2H_4$]delta-8-THC as the internal standard.

As it is advantageous to use a deuterated standard with a greater mass separation than 2 mass units, the use of other deuterated standards was investigated (213,214) for this assay. Cannabinoids labelled with three ([5′,5′,5′-$^2H_3$]) and seven ([1′,2′,3′,4′,5′,5′,5′-$^2H_7$]) deuterium atoms were synthesized, and the $^2H_7$-analogue was shown to give the least isotopic contamination at the masses (molecular and $[M - CH_3]^+$ ions) used to monitor the drug. It was emphasized in this work that deuterated standards prepared by a non-exchange method were superior to standards produced by exchange techniques as greater incorporation of deuterium could be achieved. It was also demonstrated in this work that CBD and CBN can be measured simultaneously by using [$^2H_2$]CBD and [$^2H_3$]CBN, respectively, as internal standards. In later assays, the available [$^2H_3$]delta-9-THC has been used as the internal standard for measurements of delta-9-THC (215). The method was improved in 1979 by the use of TMS derivatives; these increased the sensitivity to 0.1 ng/mL for delta-9-THC (216) and have been used in later assays.

An example of the usefulness of derivatization in improving sensitivity is shown in Fig. 26. The response of nonderivatized delta-9-THC and that of four derivatives is plotted against concentration (217), and considerable improvements in sensitivity are obtained with all four derivatives investigated (A, B, H, and O, Table III). Molecular ions were monitored in each case, and the similarity in response given by each derivative is accounted for by

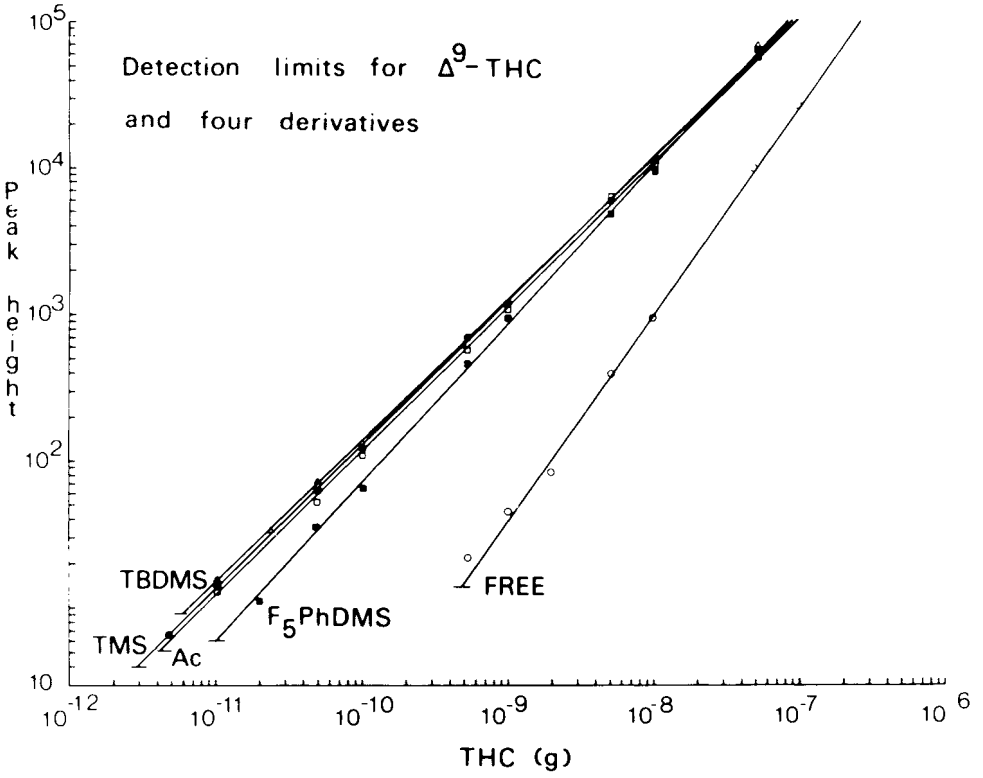


Figure 26. Plots of ion abundance against concentration for delta-9-THC and four of its derivatives. (Reproduced with permission from Pergamon Press Ltd.)

charge localization on the heterocyclic oxygen atom which is remote from the derivatized site.

An interesting modification of the technique developed by Agurell's group for determining the pharmacokinetics of delta-9-THC in cases where the drug is already present in the body as the result of earlier use was recently reported (218). Deuterium-labelled [5′,5′,5′-$^2H_3$]delta-9-THC was given to volunteers as a "pulse" bolus injection and was monitored using 1′,2′,3′,4′,5′,5′,5′-$^2H_7$]THC as the internal standard. In another recent study, delta-9-THC labelled with two deuterium atoms was given to a heavy smoker for 4 days prior to discontinuation of smoking. The decline in the concentration of the deuterated analogue was then followed, by using GC/MS, for 8 days, and a half-life of about 20 h was found (16). By monitoring the deuterated analogue, any interference from natural cannabinoids was avoided.

CBN and CBD were also measured against deuterated internal standards by Agurell's group using a similar method (219). Tri-deuterated internal standards were used in each case, and the base peaks of the TMS derivatives were monitored. Detection limits were 0.1 ng/mL for CBN and 0.03 ng/mL for CBD. The method was used to show little difference in pharmacokinetics between these cannabinoids and those of delta-9-THC following oral admin-

istration in man. More accurate pharmacokinetic studies have since been performed using [$^2H_2$]-labelled analogues of CBD (220) and CBN (221) as the administered drugs with [$^2H_7$]-labelled analogues as the internal standards. *t*-Butyldimethylsilyl derivatives were used in the assay for CBN as they were reported to reduce the amount of interfering ions that were experienced when TMS derivatization was used.

Several GC/MS approaches for simultaneous quantification of delta-9-THC, 11-hydroxy-delta-9-THC and CBN in plasma were investigated by Wall et al. (222–224) at the Research Triangle Institute. In a method similar to that used by Agurell's group, the cannabinoids were extracted with 1.5% isoamyl alcohol in petroleum ether and chromatographed on Sephadex LH-20 in a mixture of petroleum ether, chloroform, and ethanol (10:10:1) to give fractions containing the individual cannabinoids. Trideuterated analogues were used as the internal standards, the deuterium being at C-11 for delta-9-THC and at C-5′ for the other two compounds. TMS derivatives were then prepared from the 11-hydroxy-delta-9-THC fraction only and the compounds were monitored by EI GC/MS by using a 1% SE-30 column. Derivatization of THC and CBN was not adopted in order to "simplify procedure and eliminate potential sources of error" (223). Detection limits were approximately 0.5 ng/mL for THC, but later Agurell's group (225,226) claimed that this method, which is similar to their own, can detect delta-9-THC to as low as 0.06 ng/mL. Hexane extraction was later used in this assay (223) to give cleaner extracts than those obtained with the isoamyl alcohol: petroleum ether mixture. This solvent change did not improve sensitivity but did enable removal of the LH-20 chromatographic step.

In a parallel assay with comparable sensitivity (222), hexane extracts of THC and CBN were purified by using acid : base partition and analyzed as their pentafluoropropionate derivatives (P) by using isobutane CI mass spectrometry with hexahydrocannabinol as the internal standard. The $[M + H]^+$ ions were monitored, and it was stated that endogenous compounds did not interfere in the mass range used (*m/z* 455–465).

Wall's group also developed a method for the measurement of delta-9-THC-11-oic acid in urine and plasma (224). Protein was precipitated with acetone, and the acid metabolite was extracted with ether. Purification was by HPLC, and the acid was measured by using EI mass spectrometry of the di-methyl derivative with [5′,5′,5′-$^2H_3$]delta-9-THC-11-oic acid as the internal standard. The detection limit was 0.5 ng/mL. Human pharmacokinetic studies using these techniques were reported in these and several other recent papers (227,228).

The problem of ions from endogenous sources interfering at the masses commonly used to monitor the TMS derivatives of delta-9-THC (*m/z* 386 and 371) and its 11-hydroxy and acid metabolites (*m/z* 371, base peaks) was also encountered by Bergman and co-workers (229). To overcome this, delta-9-THC was extracted from blood with pentane, the pentane extract was washed with sodium hydroxide, and the compound was converted into its pentafluoropropionate ester. HHC was used as the internal standard; the molec-

ular ions at *m/z* 460 and 462, respectively, were monitored under EI conditions; and a detection limit of 1 ng/mL was achieved.

The poor availability of standards and reference material has been one of the limiting factors in the development of assays of this type. One method for partially overcoming this (230) is to use the [$^2H_3$]methyl ether (Q) as the internal standard as described by Rosenfeld et al. (229). Delta-9-THC was extracted from the plasma using Brodie's solvent, purified by extraction into Claisen's alkali (KOH in aqueous methanol), and methylated by using an on-column technique. The molecular ions from the labelled and unlabelled forms of the drug (*m/z* 331 and 328 respectively) were monitored using EI, MS, and a 0.3 ng/mL detection limit was reported. One potential limitation of this technique, where the internal standard contains the label as part of the derivative rather than as part of the basic molecule, is loss of label through hydrolysis or exchange reactions. However, no loss seems to arise in this assay. Another difficulty arises from the fact that the internal standard was not added until after the extraction stage, and thus no compensation was made for extraction variations. In a modification to the method (231–233), both delta-9-THC and its 7-hydroxy metabolite were measured. Ethylation (derivative R, Table III) rather than methylation was used to overcome possible interference by metabolically produced THC methyl ether. As the on-column alkylation method caused some decomposition of the metabolite, a milder method based on phase-transfer catalysis was developed. The metabolite was extracted into toluene as an ion-pair with tetrahexylammonium hydroxide, which then reacted with ethyl iodide present in the solvent. The 11-hydroxy group was then converted into its TMS derivative and the compound, together with delta-9-THC were measured by monitoring the base peak (*m/z* 327). The corresponding [$^2H_4$]ethyl derivative was used as the internal standard, and the detection limits were 1 ng/mL for the metabolite and 5 ng/mL for delta-9-THC.

Several methods have been used by Foltz et al. to minimize interference by ions from endogenous sources. Hexane was used as the extraction solvent in the GC/MS method described by Detrik and Foltz (234) for the measurement of the drug in plasma, and the noise level was reduced further by the use of chemical ionization using methane and ammonia as the reagent gas (Fig. 27). Some cleanup was necessary, however; this was achieved by washing the extract with dilute alkali followed by acid. [$^2H_3$]THC was used as the internal standard, and delta-9-THC was measured by monitoring the $[M + H]^+$ ion from the TMS derivative. A detection limit of 0.5 ng/mL was achieved.

A development of the method for the analysis of 11-hydroxy-delta-9-THC and delta-9-THC-11-oic acid in plasma was published in 1979 (235). The cannabinoids were extracted by direct injection of the body fluid onto a reversed-phase HPLC column which was eluted with a water : methanol gradient. The quantification step used a 25-m SE-30 capillary column for chromatography of the TMS derivatives and again the [$^2H_3$] analogues of the compounds were used as internal standards. The detection limit for delta-9-THC was in the region of 0.5 ng/mL.

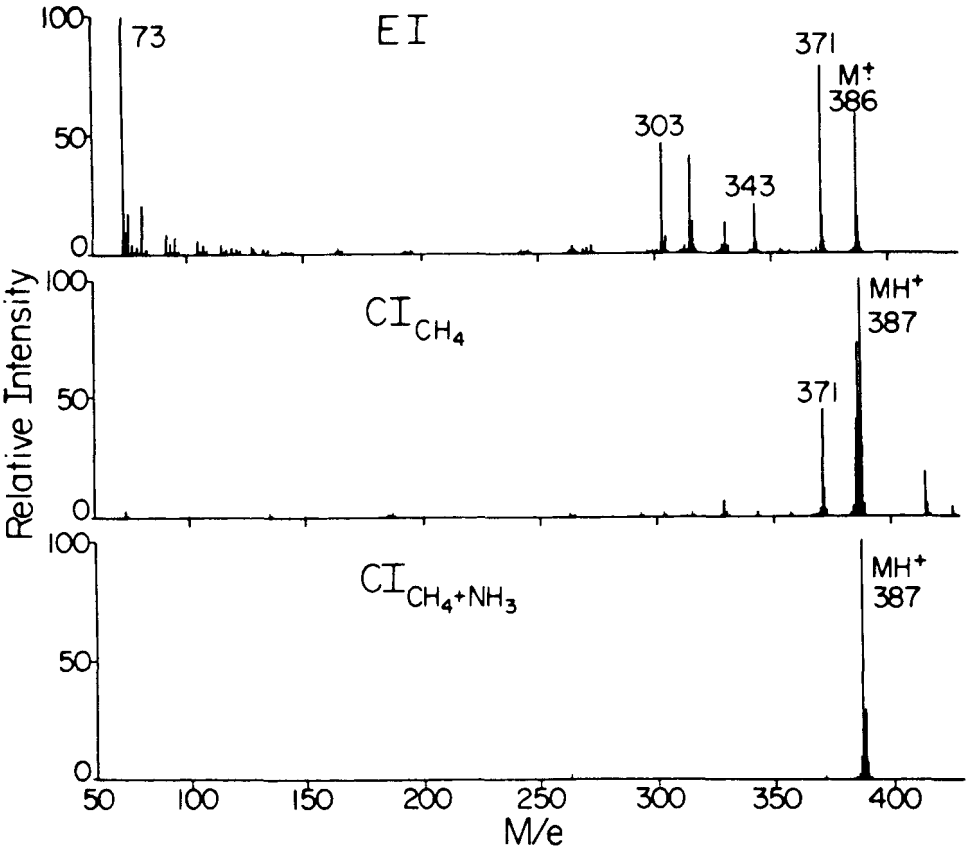


Figure 27. Electron impact and chemical ionization mass spectra (methane and methane : ammonia) of delta-9-THC TMS ether. (Reproduced with permission from DHEW.)

In 1982, Foltz and Hidy (236) reported that a related assay in which delta-9-THC could be measured to 0.2 ng/mL of plasma against [$^2H_3$]delta-9-THC by using positive ammonia CI mass spectrometry had been in use for 4 years at Batelle Columbus Laboratories, and that over 2000 samples had been processed. One person could complete the analysis of 36 samples in two 8-h days. The cannabinoids were extracted with a mixture of hexane and ethyl acetate (7:1), washed with dilute acid, and converted into TMS derivatives for separation by capillary GLC. Even with this selective technique, however, it was reported that interference from endogenous compounds was still a problem.

Foltz and co-workers (205) recently published another GC/MS method based on negative CI mass spectrometry for the measurement of delta-9-THC, 11-hydroxy-delta-9-THC, and delta-9-THC-11-oic acid in blood, plasma, and urine samples with detection limits of 0.2, 0.5, and 0.1 ng/mL for the three cannabinoids, respectively. Samples were deproteinized with acetonitrile; the THC and its 11-hydroxy metabolite were extracted at high pH with a 9 : 1 mixture of hexane:ethyl acetate; and the acid metabolite was subse-

quently extracted with the same solvent after acidification. The neutral cannabinoids were converted into their TFA derivatives, and the acid was methylated with BF_3: methanol before formation of TFA derivatives. GC/MS measurement involved a 15-m fused silica capillary column with hydrogen as the carrier gas and methane as the chemical ionization reagent gas. As examples of the utility of the method, four legal cases were described in which THC was implicated as the presumptive intoxicant in various fatal accidents.

Negative ion CI has also been used by Karlsson et al. (237) in a recent assay for delta-9-THC-11-oic acid in urine. The acid, together with the internal standard, [5′,5′,5′-$^2H_3$]delta-9-THC-11-oic acid, were extracted from the hydrolysed urine with a 1:1 mixture of hexane and diethyl ether and converted directly into their pentafluoropropyl-pentafluoropropionyl derivatives in a single-step process. A comparison of the EI spectrum with both the positive and negative ion CI spectra showed that the sensitivity in the negative ion mode was some 200 times better than in the other modes. The molecular or $[M + H]^+$ ions were the most abundant in the EI and positive CI spectra, whereas the ion produced by loss of a pentafluoropropyl group corresponded to the base peak under methane negative CI conditions. A detection limit better than 1 ng/mL was claimed for the assay with a GC/MS run time of less than 4 min.

Negative ion electron-capture mass spectrometry has great potential for detecting low concentrations of delta-9-THC as demonstrated by Hunt and Crow (238), who had detected 10 fg (0.00001 ng) of the drug as its pentafluorobenzoyl derivative (S). A total of 90.1% of the total ion current was carried by the M^- ion. Unfortunately, the method was not developed into an assay technique for the drug in body fluids.

The most sensitive assay reported to date for the measurement of delta-9-THC in plasma and tissues is that developed by Harvey and co-workers (239) with a detection limit of 0.005 ng/mL. Extraction was again with hexane to minimize the amount of co-extracted lipid, and measurement of delta-9-THC as its TMS derivative was achieved with a double-focussing mass spectrometer tuned to a metastable ion rather than to the molecular or fragment ion. This gave greatly increased selectivity and hence sensitivity, and additional cleanup was not necessary. Focussing of the mass spectrometer was achieved as follows. The magnet was set to record *m/z* 371, the $[M\text{-}CH_3]^+$ ion, with the accelerating voltage at its normal setting of 4.0 kV. This voltage was then raised to 4.16 kV to bring the ion at *m/z* 371, formed from *m/z* 386 ($M^{+\cdot}$) in the first field-free region, into focus. Ions at *m/z* 371 formed from endogenous lipids do not, in general, originate from a species of mass 386 and so they are not transmitted through the electrostatic analyzer under these conditions. As these are the ions causing the high background noise, the selectivity and hence sensitivity of the system were increased considerably. Residual interfering ions from fatty acids were eliminated by methylation of the sample with diazomethane prior to TMS formation, leaving an almost clean background against which delta-9-THC could be measured

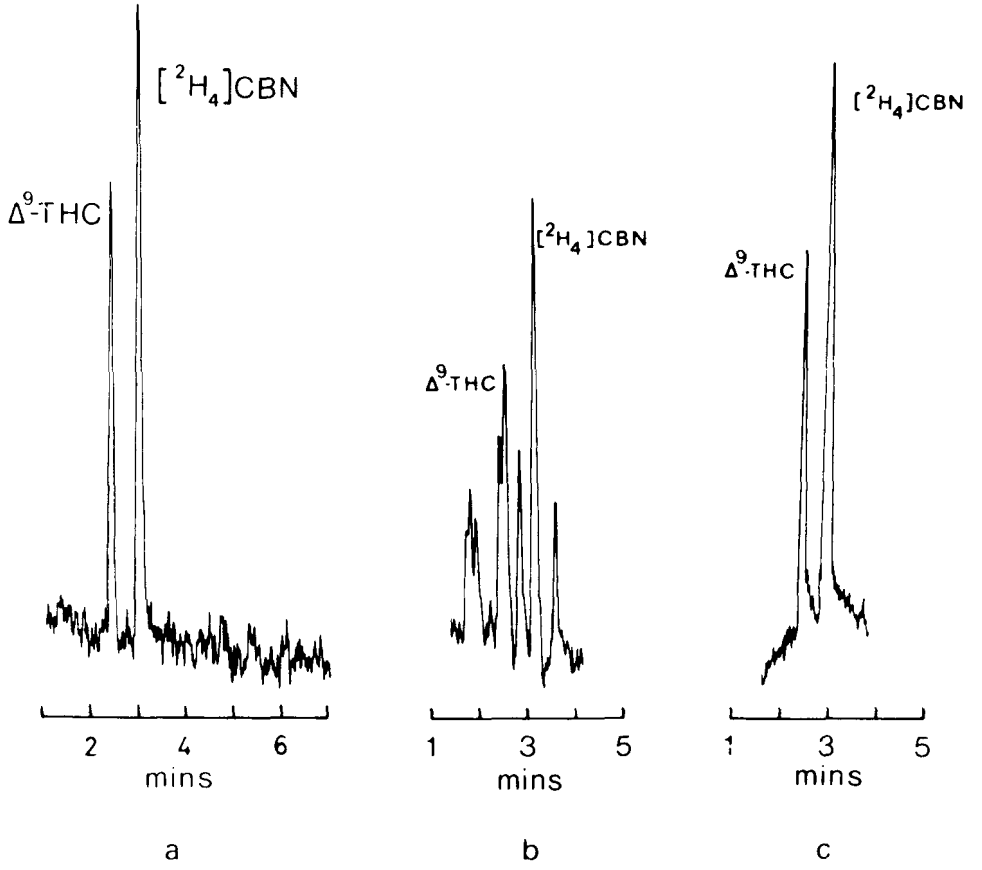


Figure 28. Chromatograms obtained by metastable ion monitoring of the TMS derivatives of (a) 0.01 ng of delta-9-THC and [1′,1′,2′,2′,$^2H_4$]CBN, (b) the same compounds extracted from rabbit plasma (recovery 70%) and (c) the same compounds extracted from plasma and methylated with diazomethane before formation of the TMS derivatives. (Reproduced with permission from Academic Press Ltd.)

to 0.005 ng/mL (Fig. 28). The internal standard was [1′,1′,2′,2′-$^2H_4$]CBN, a compound giving a metastable ion at the same mass as that from THC and which could be separated by GLC.

The method was used to study the pharmacokinetics of delta-9-THC in rabbits (239–242) and mice (243), to measure the distribution of the drug in the eye (244), where it has been shown to be effective in glaucoma by reducing intraocular pressure, and also to measure partition into membranes (245), the proposed major site of action of the drug. In rabbits, the drug was shown to have a half-life of between 30 and 66 h after a single dose and to accumulate in tissues to give a longer half-life of 83 h after repeated administration (Fig. 29). For measurements of delta-9-THC in tissues, an additional cleanup stage using Sephadex LH-20 column chromatography is necessary before derivatization (241). The sensitivity of the method is high enough to

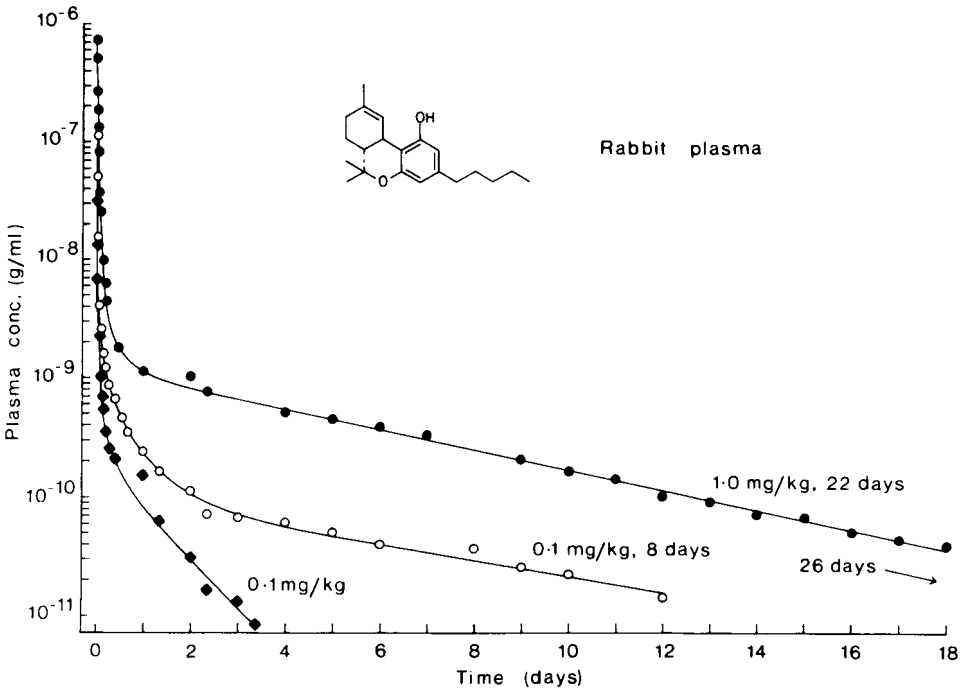


Figure 29. Plots of the concentration of delta-9-THC in rabbit plasma after a single 0.1 mg/kg intravenous dose (♦), after 8 daily injections of the same dose (○) and after 22 doses of the drug at a concentration of 1.0 mg/kg (●). (Reproduced with permission from Elsevier Scientific Publishing Co. Ltd.)

enable the drug to be measured in plasma for 10 days after a single dose of 1 mg/kg.

Extension of the method to the measurement of 11-hydroxy-delta-9-THC and delta-9-THC-11-oic acid required field switching as different metastable ions were present for each compound (246). This was achieved on a VG 7070F mass spectrometer by keeping the accelerating voltage constant at 4.0 kV, setting the ESA by means of the 8-channel peak selector to the value determined by M_2/M_1 where M_1 and M_2 are the masses of the parent and daughter ions, respectively, and setting the magnetic field manually to the value given by M_2^2/M_1. Field switching was required between the elution of each peak. The compounds were extracted from plasma with ethyl acetate, isolated from co-extracted lipids by chromatography on a short Sephadex LH-20 column and converted into methyl ester: TMS derivatives. [$^2H_4$]CBN was again used as the internal standard. Detection limits are: delta-9-THC, 0.005 ng; 11-hydroxy-delta-9-THC, 0.01 ng; delta-9-THC-11-oic acid, 0.005 ng.

Another technique frequently used for increasing the sensitivity of GC/MS assays is to operate the mass spectrometer at high resolution in order to exclude interfering ions of the same nominal mass but of different elemental composition from those being measured. Valentine et al. (247) isolated delta-9-THC by using an HPLC column of silica gel and have detected the drug in plasma to 2.5 ng/ml with [$^2H_3$]delta-9-THC as the internal standard. The

double-focussing mass spectrometer was adjusted to a resolution of 2500, and ions at *m/z* 314 and 317 were monitored. The same authors (248) later used the same method for the analysis of delta-9-THC in human breath following trapping of the compound with either a polyethylene foam wafer or with a cryogenic trap containing ethanol.

The measurement of delta-9-THC in breath by mass spectrometry was also reported by Manolis and co-workers (249). A number of absorbents such as Tenax-GC were used but all gave poor recovery. Measurement was by EI GC/MS of the pentafluoropropionyl derivative by selectively scanning the ions at *m/z* 297, 417, and 460 ($M^{+\cdot}$). Although the drug could be measured for up to 12 min after smoking, there was no correlation between the concentrations found in breath and blood, suggesting that what was being measured was residual unabsorbed drug in the oral cavity and lungs.

VI. FORENSIC ASPECTS

The cost of GC/MS equipment restricts the application of this technique in forensic studies, and it is mainly used as a confirmatory method. Two aspects of forensic analysis are appropriate for study by mass spectrometry; the examination of confiscated drug material both to confirm its identity and to determine its origin, and to confirm presumptive use by analysis of urine or blood samples. Analysis of confiscated material is usually performed by simple color tests such as reaction with Fast Blue B, microscopic examination of cystolith hairs, and TLC; these observations generally provide all of the evidence needed. Legal aspects of Cannabis analysis relating to the law in Britain were recently discussed by Baker and Phillips (250).

Many applications of conventional MS or GC/MS can be found in the literature, but a few reports have appeared of more specific methods aimed at rapid analysis or at the confirmation of cannabinoids at very low concentration. For example, Liu and co-workers (251) introduced samples directly into the mass spectrometer and measured the major ions from THC, CBD, and CBN at four electron energies. Regression analysis of the results was used to determine if Cannabis was present. Vree et al. (252) reported that the presence of *abnormal* isomers of cannabinoids, of the type shown in structure **11**, can often be used to determine if a suspect sample is of synthetic rather than natural origin; these isomers do not occur naturally. Plasma chromatography of cannabinoids was recently described for low level detection of cannabinoids (253,254). In the positive mobility spectrum, 10 ng of the drug could be detected; no negative mobility spectra could be obtained.

Analysis of cannabinoids in biological media to confirm Cannabis use is more demanding and needs the sensitivity of radioimmunoassay or GC/MS. Several GC/MS methods designed mainly for forensic detection have been published. Thus Pirl, Papa, and Spikes (255) extracted delta-9-THC, CBN, and CBD from postmortem samples with chloroform and purified the extract by paper chromatography. Eight ions were monitored, and the method was thus able to give partial mass spectra, which were useful for forensic confirmation of the presence of cannabinoids. The detection limit was 0.5 ng/mL.

Hattori and co-workers (256) measured the drug in urine and plasma to less than 1 ng/mL with tetraphenylethylene as the internal standard, and Semkin et al. (257) extracted the drug from lung tissue with ethanol and benzene for measurement by EI GC/MS using a 3% SE-30 column.

A different approach to detection and quantification was taken by Green (258,259). Cannabinoids extracted from urine were converted into TMS derivatives and examined with a computer-based GC/MS system by using probability based matching. Ions from delta-9-THC and several of its metabolites were matched against library spectra by using a reverse-search procedure. Although the sensitivity was not as great as could be obtained by other GC/MS techniques, the method allowed unambiguous identification of several metabolites in a single experiment.

It is now generally recognized that measurement of delta-9-THC-11-oic acid is of more use forensically than measurement of delta-9-THC itself. An early method for detection of this metabolite was reported by Agurell's group (260,261) and based on a modification of the original method for delta-9-THC measurement. The metabolite was converted into the methyl ester-TMS ether and measured by using packed column GC/MS and monitoring the ions at *m/z* 374, 415, and 430. A combination of GC/MS and the TLC method described by Kaistha and Tadrus (262) was used by Nakamura et al. (263). The sample was detected as its methyl ester, and the background level was stated to be low. ElSohly et al. (264,265) compared HPLC, GC/FID, GC/ECD, and GC/MS methods for the measurement of this metabolite with GC/MS employing EI being used as the reference technique. In general, good correlation was found between the values given by the various techniques with GC/MS being the most sensitive with a detection limit of 1 ng/mL.

Because of the selectivity of GC/MS techniques, several laboratories have evaluated other methods using GC/MS as the reference. Yeager et al. (266) used Detrick and Foltz's method (234) to validate a radioimmunoassay technique for the measurement of delta-9-THC in hemolyzed blood. Moffat and co-workers (267,268) compared their RIA and HPLC/RIA technique with a GC/MS method involving isolation of the cannabinoids by HPLC and measurement in the nonderivatized state with [5′,5′,5′-$^2H_3$]delta-9-THC as the internal standard. Good correlation was found between the HPLC/RIA and GC/MS methods, but the simple RIA technique gave consistently high readings because of cross-reactivity between metabolites and the anti-serum. These metabolites have been identified by using GC/MS as delta-9-THC-11-oic acid and an unidentified conjugate (269). Law, Pocock, and Moffat (270) found that 25% of measurements using homogenous enzyme immunoassay (EMIT) give false negative results and also commented that the method is more expensive than RIA. In another comparison of GC/MS with a radioimmunoassay and an EMIT method for cannabinoid analysis (271), correlation was found to be "far from adequate." Several samples providing positive results by EMIT had undetectible levels of cannabinoids by GC/MS even after enzymatic hydrolysis. Similarly, some samples producing negative results by EMIT contained measurable amounts of cannabinoids when examined by GC/MS.

In another related study (272) a comparison was made between ^{3}H- and ^{125}I-radioimmunoassay and GC/MS. It was found that serum is a better matrix than blood for the determination of cannabinoids, but that the three methods give different quantitative results. This difference was ascribed to calibration problems with the radioimmunoassays. Clearly, results from immunoassay techniques, particularly when low levels of delta-9-THC or its metabolites are involved, must still be treated with some caution. As concentrations of delta-9-THC itself are very low in urine, analysis of the major acid metabolite is preferred and has been discussed earlier in this paper. However, because of rapid metabolism and uptake of the drug into tissues, it is difficult to determine the time of drug use from single measurements of either delta-9-THC or its metabolites in blood or urine. More definitive results were obtained by the use of multiple measurements and the determination of metabolite ratios and their variation with time (273), but the situation is still far from satisfactory.

VII. CONCLUSIONS

From the above account, it can be seen that mass spectrometry has played a major role in the structural elucidation of many natural cannabinoids and their metabolites. Fragmentation mechanisms leading to the major ions in the mass spectra of these compounds are now well understood, but more work remains to be done on the less significant compounds. In quantitative analysis, the most urgent requirement is still higher sensitivity as the pharmacokinetics of the drug are still not fully understood in humans. Accumulation of the drug in adipose tissue and cell membranes and its slow release to give substantial background levels in blood, and thus in brain, would appear to be the main hazard associated with this drug, and more sensitive analytical techniques are required to study these processes fully. Possibly the use of multi-sector instruments with their ability to provide extreme selectivity will eventually yield this information and provide the basis for a rational explanation of the danger of prolonged cannabis use.

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