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Characterization of the butyl homologues of Δ^1-tetrahydrocannabinol, cannabinol and cannabidiol in samples of cannabis by combined gas chromatography and mass spectrometry

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The butyl homologues of Δ^1-tetrahydrocannabinol, Δ^1-tetrahydrocannabinolic acid, cannabinol and cannabidiol have been identified in several samples of cannabis. 8 samples contained Δ^1-tetrahydrocannabinolic acid, one sample contained cannabinol and one sample contained both cannabinol and cannabidiol. Separation by gas chromatography and identification by gas chromotography-mass spectrometry was achieved by the preparation of trimethylsilyl, d_9-trimethylsilyl, triethylsilyl and tri-n-propylsilyl derivatives.

The characterization of the C_4'-propyl (Vollner, Bieniek & Korte, 1969; Gill, 1971; Merkus, 1971; Vree, Breimer & others, 1971, 1972a; Fetterman & Turner, 1972) and C_4'-methyl analogues of the cannabinoids (Vree, Breimer & others, 1972b) suggests the presence of other related homologous series in samples of *Cannabis sativa* L. Hydrocarbon chains such as are found in these compounds are synthesized biochemically from acetate residues and, consequently, members of such series usually differ by two methylene groups. However, these series are frequently accompanied by small amounts of the homologues differing by only one methylene group, as has recently been shown for the gingerols (Masada, Inoue & others, 1973) and for hydrocarbons in cannabis itself (De Zeeuw, Wijsbeek & Malingré, 1973). It might therefore be anticipated that, although cannabinoids containing odd carbon chains predominate, the compounds with even carbon chains may also be present. No examples of such compounds have yet been reported.

During a recent investigation of a number of cannabis samples with a combined gas chromatograph-mass spectrometer-computer system, a small gas chromatographic peak was observed in one sample midway between the peaks produced by propyl-Δ^1-tetrahydrocannabinolic acid (Pr-THC-acid) and THC-acid itself. As the mass spectrum of this compound contained an abundant ion at *m/e* 473, there was a strong possibility that it was produced by the butyl homologue of Δ^1-THC-acid. Δ^1-THC-acid and Pr-Δ^1-THC-acid produce abundant (100%) ions at *m/e* 459 and *m/e* 487 respectively. The sample and several others were therefore investigated more fully and the presence of butyl-Δ^1-THC, butyl-cannabinol and butyl-cannabidiol were confirmed.

MATERIALS AND METHODS

Cannabis samples. Sample 1. Cannabis resin obtained from police seizures. Sample 2. An old sample of 'Tincture of Indian hemp'. The exact age was unknown but bottles of other drugs from the same manufacturers obtained with this sample were dated 1837–1845. Samples 3–10. Cannabis resin and leaves obtained from police seizure.

Extraction of cannabinoids. Samples (about 50 mg) were crushed in a mortar and stood in ethyl acetate (10 ml) for about 1 h. The solid material was filtered off, washed with ethyl acetate and the solvent was removed from the combined ethyl acetate extract and washings under reduced pressure followed by a stream of nitrogen. The residue was converted into derivatives for analysis by gas chromatography and mass spectrometry as described below. Liquid (tincture) samples (0·1 ml) were evaporated to dryness with a stream of nitrogen before derivative formation.

Decarboxylation. The cannabis sample (about 50 mg) was placed in a screw top (35 ml) vial which was then filled with nitrogen. This was heated in an oven at 110° for 90 min. The cannabinoids were then extracted as described above.

Preparation of derivatives

(a) *Trimethylsilyl (TMS) derivatives.* Acetonitrile (0·1 ml), *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA, 0·1 ml) and trimethylchlorosilane (TMCS, 0·05 ml) were added to the cannabis sample extracted as described above. Following dissolution of the resin, the samples were allowed to stand at room temperature for 30 min to complete the formation of the derivatives. 0·2-1 μl samples were then examined by gas chromatography and combined gas chromatography–mass spectrometry.

(b) *d_9-TMS derivatives.* These were prepared as described above by substituting d_{18}-bis (trimethylsilyl)-acetamide (d_{18}-BSA) for the BSTFA and using a trace of TMCS as a catalyst.

(c) *Triethylsilyl derivatives.* A solution of triethylchlorosilane (0·1 ml), pyridine (0·2 ml) and triethylamine (0·05 ml) was prepared as described previously (Harvey & Paton, 1975). This was added to the cannabis sample and, following dissolution, the mixture was allowed to stand at room temperature for 30 min.

(d) *Tri-n-propylsilyl derivatives.* These were prepared as for the triethylsilyl derivatives by substituting tri-*n*-propylchlorosilane for the triethylchorosilane.

Gas chromatography. Samples were examined with a Varian 2400 gas chromatograph fitted with dual flame ionization detectors and 2 m × 2 mm (i.d.) glass columns packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories, State College, Pennsylvania, U.S.A.). Nitrogen was used as the carrier gas and the column oven was programmed linearly over the range 100–330° at 4° min^{-1}. The injection and detector bath temperatures were maintained at 280 and 300° respectively. Methylene unit values are listed in Tables 1 and 2.

Mass spectrometry. Mass spectra were recorded with a VG micromass 12B electron impact mass spectrometer interfaced via a glass jet separator to a Varian 2400 gas chromatograph fitted with a similar (SE-30) column to that described above. Helium at 30 ml min^{-1} was used as the carrier gas. The column oven was programmed at 2° min^{-1} over the range 170–280° with an injector temperature of 280°. The inlet line, separator and ion source temperatures were maintained at 230, 230 and 260° respectively. 25 eV mass spectra were recorded using an ionizing current of 100 μA and an accelerating voltage of 2·5 KV giving an upper mass limit of 630 and a resolution of 1000. The mass spectrometer was interfaced to a VG Data System Ltd. computer system type 2040 and this was set to scan the mass spectrometer at 3 s per decade from high to low mass over the mass range *m/e*

Table 1. *Methylene unit values and salient mass spectral data for the derivatives of the homologous Δ¹-THC's.*

Compound	Concentration*	Deriv.	MU	Mol wt	[M−15]⁺	Ion *c*
				372	357	289
Me-Δ¹-THC	·0087	Et_3Si	23·21	(100)**	(95)	(59)
				414	399	331
		Pr_3Si	24·58†	(100)	(62)	(27)
				358	343	275
Pr-Δ¹-THC	·076	TMS	21·70	(100)	(95)	(49)
				367	352	284
		d_9-TMS	—	(92)	(100)	(54)
				400	385	317
		Et_3Si	24·65	(100)	(71)	(34)
				442	427	359
		Pr_3Si	25·90	(100)	(46)	(24)
				414	399	331
Bu-Δ¹-THC	·0078	Et_3Si	25·48	(100)	(79)	(31)
				456	441	373
		Pr_3Si	26·80	(100)	(56)	(26)
				386	371	303
Δ¹-THC	6·45	TMS	23·50	(100)	(94)	(33)
				395	380	312
		d_9-TMS	—	(100)	(81)	(31)
				428	413	345
		Et_3Si	26·35	(100)	(69)	(85)
				470	455	387
		Pr_3Si	27·60	(100)	(39)	(23)
				446	431	363
Me-Δ¹-THC-acid	—	TMS	—	NS‡	(100)	NS
				464	449	381
		d_9-TMS	—	NS	(100)	NS
				474	459	391
Pr-Δ¹-THC-acid	—	TMS	24·99	(3·7)	(100)	(2·9)
				492	474	409
		d_9-TMS	—	(3)	(100)	(1·5)
				488	473	405
Bu-Δ¹-THC-acid	—	TMS	25·72	(9)	(100)	NS
				506	488	423
		d_9-TMS	—	NS	(100)	NS
				502	487	419
Δ¹-THC-acid		TMS	26·52	(5)	(100)	(2·5)
				520	502	437
		d_9-TMS	—	(3·7)	(100)	(1·6)

* As a percentage of the total cannabinoid content of sample 1. ** Relative intensitites are given in parentheses. † Peak contained several components. ‡ NS = not seen.

Table 2. *Methylene unit values and salient mass spectral data for the SiR_3 derivatives of the homologous cannabinols.*

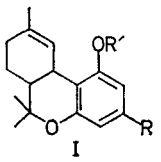
Compound	Concentration*	Deriv.	MU	Mol wt	[M−15]⁺
				326	311
Me-CBN	0·6	TMS	20·95	(14)	(100)
				368	353
		Et_3Si	23·90	(18)	(100)
				410	395
		Pr_3Si	25·39†	(16)	(100)
				354	339
Pr-CBN	6	TMS	22·42	(14)	(100)
				396	381
		Et_3Si	25·30	(17)	(100)
				438	423
		Pr_3Si	26·76	(18)	(100)
				368	353
Bu-CBN	0·2	TMS	23·36	(14)	(100)
				410	395
		Et_3Si	26·27	(16·5)	(100)
				452	437
		Pr_3Si	27·65	(20)	(100)
				382	367
CBN	100	TMS	24·30	(14)	(100)
				424	409
		Et_3Si	27·25	(18)	(100)
				466	451
		Pr_3Si	28·55	(20)	(100)

* As percentage of CBN in sample 2. † Peak contained several components.

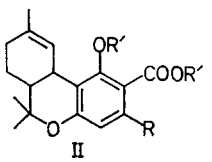
630–*m/e* 40 with an inter-scan delay of 2 s. This resulted in the spectrometer magnet cycling every 6·5 s to produce 360 mass spectra in a g.l.c. run. Spectra were recorded on an ASR 33 teletype, a VT8e visual display unit and a Bryans 26000 analogue XY plotter.

RESULTS AND DISCUSSION

Fig. 1 shows the gas chromatogram of the TMS derivative of the cannabis sample (1) in which butyl-Δ^1-THC-acid was first observed. The peaks were identified by gas chromatography and mass spectrometry, and are listed in the figure legend; Table 1 gives the methylene unit values and salient mass spectral information for the homologous Δ^1-THC's (I a-d) and their acids (II a-d).



I



II

a R = Me R′ = H, $SiMe_3$, $SiEt_3$ or $SiPr_3$
b R = Pr
c R = Bu
d R = Pen

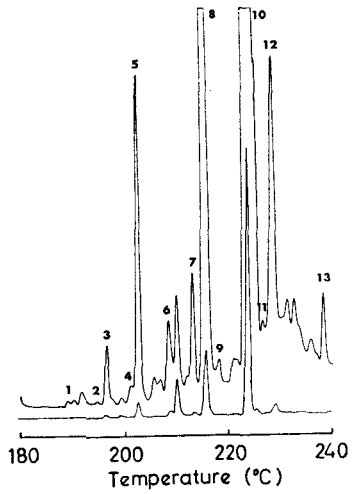
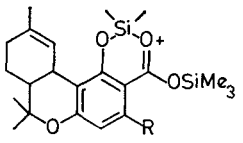


FIG. 1. Gas chromatogram of the TMS derivatives of cannabis sample 1. The experimental conditions are given in the experimental section. Peaks identified are: 1, Propyl-Δ^1-tetrahydrocannabinol; 2, propyl-cannabinol; 3, cannabidiol; 4, cannabichromene; 5, Δ^1-tetrahydrocannabinol; 6, cannabinol; 7, propyl-Δ^1-tetrahydrocannabinolic acid; 8, cannabidiolic acid; 9, butyl-Δ^1-tetrahydrocannabinolic acid; 10, Δ^1-tetrahydrocannabinolic acid; 11, cannabichromenic acid; 12, cannabinolic acid. 13, n-nonacosane.

The major constituent was Δ^1-THC-acid with all of the other cannabinoids present in much lower concentrations. Unfortunately the geographical origin and age of this sample were unknown although from its appearance and cannabinoid concentration it was probably of recent origin.

The presence of butyl-Δ^1-THC-acid was first suspected when an abundant ion of mass 473 appeared in the interscan report during a g.l.c.-m.s. run with the data system set to scan every 6·5 s. A corresponding ion at *m/e* 488 $[M - 18]^+$ in the spectrum of the d_9-TMS derivative (McCloskey, Stillwell & Lawson, 1968) indicated the presence of two TMS groups. A plot of methylene unit value against alkyl chain length for the propyl, suspected butyl and pentyl homologues of Δ^1-THC acid was found to be a straight line, strongly suggesting the butyl structure for this compound. The concentration of butyl-Δ^1-THC acid was very low and consequently a definitive mass spectrum could not be obtained; Δ^1-THC acids in any case have rather uninformative mass spectra, dominated by a very abundant $[M - 15]^+$ ion (*a*) with all other ions less than 5% relative abundance.



(*a*)

All ions except $[M - 15]^+$ for Bu-Δ^1-THC-acid were partially obscured by other ions in the spectrum. The very high abundance of $[M - 15]^+$ was thought to be the result of ring formation (*a*) following loss of a TMS methyl group ($[M - 18]^+$ in the spectrum of the d_9 derivative).

The tetrahydrocannabinols themselves exhibit much more informative spectra and so this cannabis sample was decarboxylated by heating at 110° for 90 min and re-examined as its TMS derivative. Δ^1-THC and its propyl homologue were identified but a large peak produced by cannabidiol obscured the possible presence of butyl-Δ^1-THC. To remove the cannabidiol peak from this region of the chromatogram, the triethylsilyl and tri-n-propylsilyl derivatives were prepared. These derivatives, as previously described (Harvey & Paton, 1975) have the effect of introducing a greater retention increment shift in the dihydroxy compounds such as cannabidiol, than in mono-hydroxy compounds, thus removing their peaks to a higher temperature region of the chromatogram (Fig. 2). Examination of these samples by

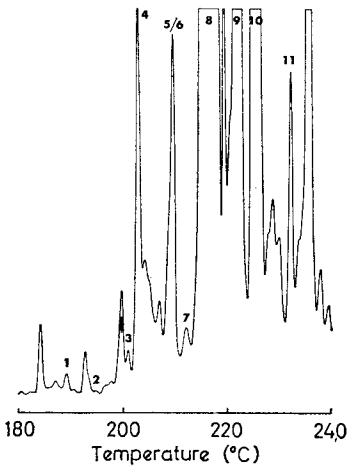
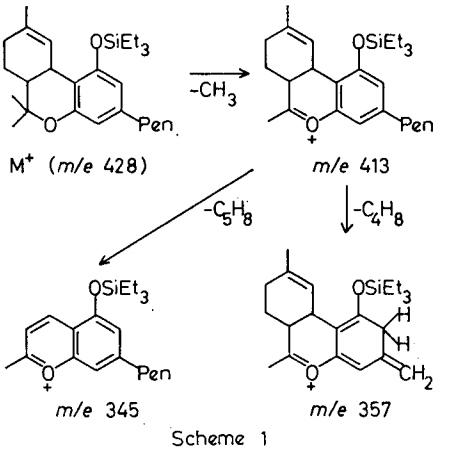


FIG. 2. Gas chromatogram of the triethylsilyl derivatives of the decarboxylated cannabis sample 1. Separation conditions are given in the experimental section. Peaks identified include: 1, methyl-Δ^1-tetrahydrocannabinol; 2, methylcannabinol; 3, propylcannabichromene; 4, propyl-Δ^1-tetrahydrocannabinol; 5/6, propylcannabinol and butyl-Δ^1-tetrahydrocannabinol; 7, cannabichromene; 8, Δ^1-tetrahydrocannabinol; 9, cannabinol; 10, cannabidiol; 1, n-nonacosane.

g.l.c.–m.s. confirmed the presence of butyl-Δ^1-THC. Three other homologues were present; Δ^1-THC itself, propyl-Δ^1-THC and methyl-Δ^1-THC. Plots of methylene unit value against chain length for the pentyl, butyl and propyl homologues lay on straight lines (Fig. 3) for each derivative; methyl-Δ^1-THC in all cases fell slightly above the line but this is not uncommon for the first member of a homologous series. Both methylene unit values and mass spectra of the different silyl derivatives showed the presence of one derivatized hydroxyl function. The mass spectra of both the triethyl and tri-n-propylsilyl derivatives of butyl-Δ^1-THC were contaminated with ions from other compounds and column 'bleed' but by substracting these by means of the data system, a suitable spectrum (for the triethylsilyl derivatives) was obtained. The similarity with the spectra of the other three homologues is striking. The spectra (Budzikiewicz, Aplin & others, 1965; Claussen & Korte, 1966) for the four tri-n-alkylsilyl homologues are given in Table 1. In all cases the molecular ion was the most abundant and this fragmented as shown in Scheme 1 (Et_3Si deriv. of Δ^1-THC. Ionic structures are *m/e* 413, *b; m/e* 345, *c; m/e* 357, *d*).

Ions M^+, *b* and *c* retained the intact aliphatic chain and shifted in the spectra of the various homologues, ion *d* had lost part of this chain and consequently did not shift (*m/e* 357 for the tri-



Scheme 1

ethylsilyl derivatives and *m/e* 399 for the tri-*n*-propyl silyl derivatives). Fragmentation of these compounds was mainly initiated by charge localization on the heterocyclic oxygen atom (Harvey & Paton, 1975) and was largely independent of the silyl substituent. The relative abundance of each ion in the various spectra was equivalent. The relative concentration of the 4 homologous Δ^1-THC's are listed in Table 1.

Other cannabinoids having a butyl side-chain were not observed in this sample but this was hardly surprising in view of the very low relative concentration of the other propyl and pentyl cannabinoids when compared with Pr-THC and THC respectively.

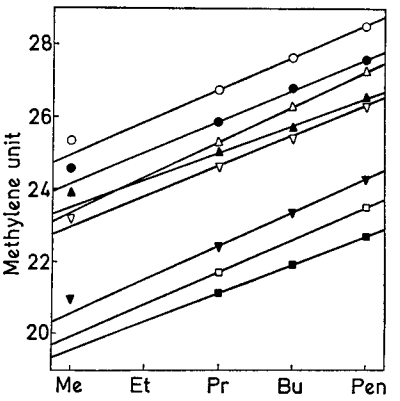


FIG. 3. Plots of methylene unit value against alkyl chain length for the silyl derivatives of the butyl-cannabinoids. ▲–TMS derivatives of the tetrahydrocannabinolic acids. □–TMS derivatives of the Δ^1-tetrahydrocannabinols. ▽–Et_3Si derivatives of the Δ^1-tetrahydrocannabinols. ●–Pr_3Si derivatives of the Δ^1-tetrahydrocannabinols. ▼–TMS derivatives of the cannabiols. △–Et_3Si derivatives of the cannabinols. ○–Pr_3Si derivatives of the cannabinols. ■–TMS derivatives of the cannabidiols. The lines representing each derivative of any particular cannabinoid are parallel to each other but not parallel to the lines of the other compounds.

In general the relative amounts of the individual cannabinoids are similar for each homologous series and thus the very low concentration of butyl-Δ^1-THC (Table 1) would result in the ions from any other butyl-cannabinoid being obscured by other ions from the sample. Consequently additional samples with higher relative concentrations of the other cannabinoids were examined in the hope of finding additional members of the butyl series.

Cannabis sample 2 was a very old (possibly 130 years) sample of 'tincture of Indian hemp' and was found to contain a very high proportion of cannabinol (Fig. 4). This was most probably due to ageing and thus other homologous series of cannabinoids would be expected largely as the corresponding cannabinols. Examination of the TMS derivatives of this sample revealed the presence of the known methyl and propyl-cannabinols in addition to cannabinol itself and a small amount of butyl-cannabinol. As with the Δ^1-THC's, a plot of the pentyl, butyl, and propyl homologues was a straight line (Fig. 3) for the three derivatives (TMS, tri-ethylsilyl and tri-n-propylsilyl) and the mass spectra of butyl-cannabinol derivatives were similar to the spectra of the other homologues (Table 2) with the appropriate mass shifts. Although the spectra of the derivatives of butyl-cannabinol were contaminated with other ions, single ion chromatograms showed coincidence of M^+ and $[M - 15]^+$ for all derivatives but not for most other ions, and spectrum subtraction enabled fairly pure spectra to be obtained. The relative concentration of the various homologous cannabinols are given in Table 2. Me, Pr and Pen-cannabinol were measured from their g.l.c. peak heights; butyl-cannabinol was estimated by comparing the heights of $[M - 15]^+$ of both Me-CBN and Bu-CBN with *m/e* 207, 'bleed' peak in the spectrum of constant height and thus a suitable internal standard.

Cannabis sample 3 contained high concentrations of cannabidiol and cannabinol. Examination of its TMS derivative enabled butyl-cannabidiol to be identified by its methylene unit and mass spectrum (Table 3). This sample also contained butyl-cannabinol showing that this compound is a naturally occurring cannabinoid and not solely the result of ageing as might be concluded from sample 2. The ratios of the concentration of butyl-CBD and butyl-CBN were equivalent to the ratios of CBD and CBN themselves.

Table 3. *Methylene unit values and salient mass spectral data for the TMS derivatives of the homologous cannabidiols.*

Compound	MU	M^+	Base	$[M-15]^+$
		402		387
Me-CBD	—	NS	334	NS
		430		415
Pr-CBD	21·10	(11)	362	(6·6)
		444		429
Bu-CBD	21·90	(10·5)	376	NS
		458		443
CBD	22·70	(10)	390	(5)

NS = Not seen.

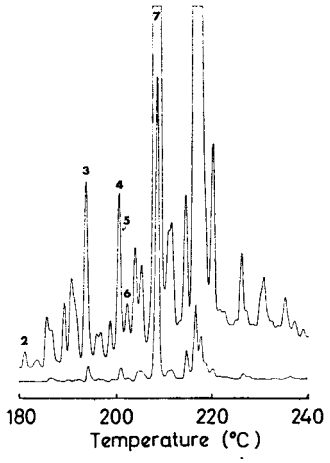


FIG. 4. Gas chromatogram of the TMS derivatives of cannabis sample 2 (Indian hemp). The separation conditions are given in the experimental section. Peaks were identified as: 1, palmitic acid; 2, methyl-cannabinol; 3, propyl-cannabinol; 4, cannabichromene; 5, butyl-cannabinol; 6, Δ^1-tetrahydrocannabinol; 7, cannabinol. The upper trace is magnified × 10.

The identification of three butyl-cannabinoids in three unrelated samples suggested that these homologues might occur more widely. Seven other samples with high THC-acid content were examined and butyl-Δ^1-THC-acid was found in low concentration in all of them. Methyl and propyl homologues were also present, the methyl series in low relative concentration and the propyl series varying from about the concentration of the methyl homologues to nearly as high as that of the pentyl-cannabinoids. Ethyl and higher homologues were not detected in any of the samples using the g.l.c.–m.s. computer system described above.

The low concentration of the butyl-homologues will almost certainly mean a very small contribution to the overall biological activity of cannabis samples. As the butyl homologues of synthetic Δ^3-isomers generally exhibit a lower activity than the pentyl

compounds (Adams, Loewe & others, 1941) it is probable that the butyl homologues of Δ^1-THC will also be less active.

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