Identification of new *in vivo* side-chain acid metabolites of Δ^1-tetrahydrocannabinol

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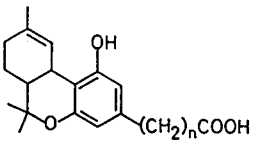
Previous studies have shown that liver microsomal enzymes metabolize Δ^1-tetrahydrocannabinol (Δ^1-THC) by several alternative routes. Recently, considerable interest has been focused on the identification of highly oxidized metabolites such as Δ^1-THC-7-oic acid (Wall, Brine & Perez-Reyes, 1973), its monohydroxy (Burstein, Rosenfeld & Wittstruck, 1972) and dihydroxy derivatives (Harvey & Paton, 1976), and 4″,5″-bisnor-Δ^1-THC-7,3″-dioic acid (7,3″-dioic acid; Nordqvist, Agurell & others, 1974). This communication reports the identification of a series of homologous side-chain acids that are formed *in vivo* by different species.

Δ^1-THC was suspended in Tween 80–saline and administered intraperitoneally to two Duncan-Hartley guinea-pigs (400 g), four Charles River CD-1 mice (25 g), and one Chinchilla cross rabbit (2·6 kg). Each animal received doses of 100 mg kg^{-1} 26 and 2 h before death. Livers were removed and homogenized in 5 volumes of saline. Metabolites were extracted from the homogenate with three portions of redistilled ethyl acetate (twice the homogenate volume) at neutral pH (reduction in pH did not improve extraction efficiency). The extracts were dried over magnesium sulphate, and the solvent was removed under vacuum. The residue was dissolved in redistilled chloroform, and an aliquot representing 1·5–2·0 g of liver was chromatographed on a Sephadex LH-20 column (5 g, 1 × 25 cm). Δ^1-THC and monohydroxylated metabolites were eluted with chloroform (70 ml) and more polar metabolites with 10% methanol–chloroform (50 ml).

Samples and reference standards were converted into trimethylsilyl (TMS), deuterated-TMS (d_9-TMS; McCloskey, Stillwell & Lawson, 1968), and methyl ester-phenolic TMS (Me/TMS) derivatives for gas chromatography-mass spectrometry (g.c.-m.s.) as described by Harvey & Paton (1976). The g.c.-m.s. data were obtained using a V.G. Micromass 12B mass spectrometer interfaced with a Varian 2400 g.c. The g.c. column was 3% SE-30 on 100–120 mesh Gas-Chrom Q, and the column temperature was programmed from 170 to 270° at 2° min^{-1}. Mass spectra (m.s.) were recorded at 25 eV with an ionizing current of 100 μA and an accelerating voltage of 2·5 kV. A VG Data System Ltd computer system type 2040 enabled the mass spectrometer to acquire sequential spectra by repetitively scanning at 3 s a decade with an inter-scan delay of 2 s.

Analysis of the TMS derivative of the 10% methanol–chloroform fraction from the guinea-pig revealed the presence of three unidentified acid metabolites whose



I. n = 2; 4″,5″-bisnor-Δ^1-THC-3″-oic acid (3″-acid).
II. n = 1; 3″, 4″, 5″-trisnor-Δ^1-THC-2″-oic acid (2″-acid).
III. n = 3; 5″-nor-Δ^1-THC-4″-oic acid (4″-acid).

retention times (Rt) were considerably shorter than that of the TMS derivative of Δ^1-THC-7-oic acid (Rt 25·67 min). One of these (metabolite I) was the major Δ^1-THC metabolite in this fraction. It had an Rt of 20·17 min and a molecular ion (M^+) at *m/e* 460 (d_9-TMS, 478 which indicated two TMS groups). Methylation of the metabolite with diazomethane for 1 min before TMS formation reduced the M^+ to *m/e* 402 thus indicating the presence of a carboxylic acid group. As shown in Table 1, loss of 118, 127 or 60 atomic mass units was consistent with M^+-HCOOTMS, M^+-HCOO-d_9-TMS, or M^+-$HCOOCH_3$ respectively. Comparison of the m.s. to that of Δ^1-THC-7-oic acid (M^+ at *m/e*, 488) revealed that metabolite I had lost the equivalent of two methylene groups. Despite this loss, the monoterpene moiety remained unchanged as evidenced by the presence of both *m/e* 417 (loss of an isopropyl group; C-8, 9, 10) and *m/e* 377 (loss of C_5H_8 along with one of the gem dimethyl groups) as described by Budzikiewicz, Aplin & others (1965). The m.s. of all derivatives exhibited a prominent ion at *m/e* 315 which suggested loss of the entire side chain. Based on these data, metabolite I was assigned the structure of 4″,5″-bisnor-Δ^1-THC-3″-oic acid (3″-acid). Further evidence was provided by the $LiAlD_4$ reduction of the 3″-acid to the corresponding alcohol (Rt 16·9 min) which was in turn compared to the synthetic reference compound 3″-hydroxy-4″,5″-bisnor-Δ^6-THC (Rt 18·2 min) (Lawrence, 1974). The TMS derivatives of both compounds were characterized by M^+, $[M-15]^+$, $[M-C_5H_8-CH_3]^+$, and a benzylic cleavage with a hydrogen transfer (deuterium transfer in the reduced compound). Formation of the latter ion (base peak) as described by Binder, Agurell & others (1974) was supported by the deuterium transfer.

Metabolite II (TMS derivative had Rt 16·42 min, M^+ at *m/e* 446; Me/TMS derivative had M^+ at *m/e* 388) was also found to be an acid, but it contained one less methylene group than the 3″-acid (I). The spectra of its TMS and d_9-TMS derivatives were similar to those of the 3″-acid; they contained abundant losses of HCOOTMS and HCOOd_9-TMS, respectively, and ions

* Correspondence.

Table 1. *M.s. characteristics for derivatives of the side-chain carboxylic acids.*

	Relative intensities								
	Metabolite I (3″ acid)			Metabolite II (2″ acid)			Metabolite III (4″ acid)		
Ion	TMS 100	d_9-TMS 100	Me/TMS 100	TMS 100	d_9-TMS 100	Me/TMS 100	TMS 100	d_9-TMS 100	Me/TMS 100
M^+	(*m/e* 460)	(*m/e* 478)	(*m/e* 402)	(*m/e* 446)	(*m/e* 464)	(*m/e* 388)	(*m/e* 474)	(*m/e* 492)	(*m/e* 416)
$[M-CH_3]^+$	64	53	72	78	44	73	>100*	>100*	76
$[M-OCH_3]^+$	—	—	7	—	—	—	—	—	48
$[M-C_3H_7]^+$	14	12	23	19	16	31	26*	16	32
$[M-C_5H_8-CH_3]^+$	19	15	31	26	18	67	14	26	72
[M-HCOOR]+ †	22	23	5	48	44	—	18	11	—
[M-HCOOR -CH_3]+	3	4	3	—	—	—	18	18	72
$[M-C_5H_8-CH_3-HCOOR]^+$	2	2	4	—	—	—	10	13*	—
[M-side-chain]+ (*m/e* 315)	22	25	39	32	21	86	56	56	76

* Relative intensity exaggerated by an interfering ion.
† R = TMS, d_9-TMS or Me.

showing that the monoterpene moiety remained intact ($[M-C_3H_5]^+$ and $[M-C_5H_8-CH_3]^+$). These data along with the fragmentation involving loss of the side chain showed that the carboxylic acid group was in the side chain. Metabolite II was thus identified as 3″,4″,5″-trisnor-Δ^1-THC-2″-oic acid (2″-acid). The 2″-acid was reduced with $LiAlD_4$ to the alcohol whose TMS derivative gave M^+ at *m/e* 434 (100%), $[M-CH_3]^+$ (88), $[M-TMSOH]^+$ (55), $[M-TMSOH-CH_3]^+$ (66) and [M-side chain]+ (26) which was consistent with the 2″-hydroxy-3″,4″,5″-trisnor-Δ^1-THC structure.

The m.s. of the three derivatives of metabolite III (Table 1) indicated that it was also a side-chain acid but that it had one more methylene group than the 3″-acid (I). It was thus assigned the structure of 5″-nor-Δ^1-THC-4″-oic acid (4″-acid), and this was supported by its g.c. retention time (23·10 min). Reduction of the 4″-acid with $LiAlD_4$ gave the appropriate alcohol as shown by the m.s. of the TMS derivative M^+ at *m/e* 462 (100), $[M-15]^+$ (67), $[M-C_3H_7]^+$ (30), $[M-C_5H_8-CH_3]^+$ (35), $[M-CH_2CH_2CD_2OTMS]^+$ (77) and [M-side-chain]+ (52).

In the guinea-pig the 3″-acid was a major metabolite, whereas the 2″-acid and 4″-acid were formed to a minor extent. Only trace amounts of the 3″-acid were found in the rabbit, while the other acids were not detected. However, a metabolite was found that was tentatively identified as 2″,3″,4″,5″-tetranor-Δ^1-THC-1″-oic acid based on the m.s. of the TMS and Me/TMS derivatives. The m.s. of the TMS derivative gave the following: M^+ at *m/e* 432 (100), $[M-CH_3]^+$ (50), $[M-C_3H_7]^+$ (21), $[M-C_5H_8-CH_3]^+$ (19) and [M-side-chain]+ (58). Of all the side-chain acids thus far identified, only the 3″-acid could be detected in mouse liver.

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