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Acidic metabolites of Δ^1-tetrahydrocannabinol isolated from rabbit urine

MARIANNE NORDQVIST*, JAN-ERIK LINDGREN† AND STIG AGURELL†‡

‡*Department of Pharmacognosy, Faculty of Pharmacy, BMC, Box 579, S-751 23 Uppsala, Sweden,*
†*Astra Läkemedel AB, S-151 85 Södertälje, Sweden*

The in vivo metabolism of Δ^1-tetrahydrocannabinol (Δ^1-THC) was investigated in the rabbit after i.v. administration. Thirteen acidic metabolites were isolated from rabbit urine and identified by gas chromatography–mass spectrometry and by proton magnetic resonance spectroscopy. One additional metabolite was tentatively identified. All but three were new metabolites and all but one were oxidized in the pentyl side chain. The metabolites included dicarboxylic acids, monocarboxylic acids and mono- or dihydroxylated derivatives thereof. However, the dicarboxylic acid metabolites were the most prominent.

Previous in vitro studies on the metabolism of Δ^1-THC, the main psychoactive principle of cannabis, have revealed the primary modes of biotransformation (Agurell et al 1972; Burstein 1973). These involve allylic hydroxylation to 6α-, 6β- or 7-hydroxy-Δ^1-THC (Ben-Zvi et al 1974a,b; Jones et al 1974; Wall et al 1970; Wall 1971; Nilsson et al 1970), hydroxylation of the pentyl side chain (Widman et al 1975; Wall & Brine 1976a) and epoxidation of the Δ^1-double bond (Gurny et al 1972; Ben-Zvi & Burstein 1975; Widman et al 1975). These primary metabolites are further conjugated, e.g. to β-glucuronic acid (Widman 1975), sulphate (Agurell et al 1972) or fatty acids (Leighty 1973; Leighty et al 1976) or oxidized to aldehydes (Ben-Zvi & Burstein 1974), ketones (Gurny et al 1972; Jones et al 1974) and carboxylic acids.

Several acidic metabolites of Δ^1-THC have recently been characterized from in vivo investigations. They are monocarboxylic acids (Wall et al 1976a,b; Harvey et al 1977a; Martin et al 1976), aromatized (Ben-Zvi et al 1974, 1976) and hydrogenated monocarboxylic acids (Harvey et al 1977b), mono- and dihydroxylated monocarboxylic acids (Burstein et al 1972; Harvey et al 1977a) and a dicarboxylic acid (Nordqvist et al 1974). This paper reports the structures of major nonconjugated metabolites of Δ^1-THC present as highly oxidized metabolites in rabbit urine after i.v. administration. All but one of the acids were oxidized in the side chain, which was predominantly shortened.

* Present address: Bldg. 4, Rm. 220, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.
‡ Correspondence

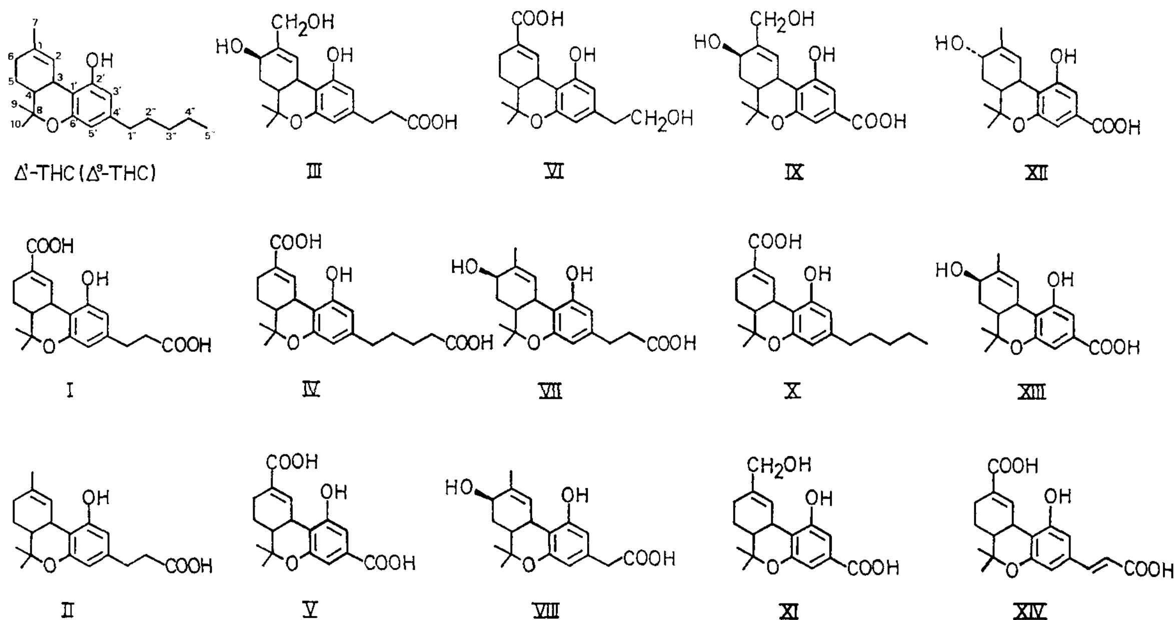
MATERIALS AND METHODS

(−)-Δ^1-Tetrahydro-[3′,5′-^{14}C]cannabinol (110 mg; specific activity 14·6 μCi m mol^{-1}) in 70% aqueous ethanol was administered to four female New Zealand white rabbits (2 kg) via an ear vein for five days. The chemical and radiochemical purity of the sample was greater than 95% according to g.l.c. and t.l.c. (Silica gel plates, diethyl ether–light petroleum (b.p. 40–60° C) 1 : 19, developed three times). Urine was collected for six days.

Radioactivity was assayed in a Packard Tri Carb Model B 2450 spectrometer with external standardization. The scintillation fluid for urine aliquots was Insta-Gel. For non-aqueous samples a solution of Permablend III (Packard) in toluene was used.

Isolation of metabolites

The collected urine (3 litres) was filtered through Celite, adjusted to pH 6 with phosphoric acid and passed through a column of Amberlite XAD-2 resin (Rohm and Haas Co.) 4·5 × 55 cm. The column was washed with 3 litres water before eluting the metabolites with 3 litres methanol (Nilsson et al 1973). The methanol was evaporated under vacuum. The pH of the residual aqueous mixture was adjusted to 8 with saturated aqueous sodium bicarbonate before extraction with equal volumes of diethyl ether (3 × 150 ml) to remove non-acidic constituents. The aqueous phase was then acidified to pH 3 with phosphoric acid and extracted with diethyl ether (3 × 150 ml). The acids present in the ether extracts were re-extracted (Widman et al 1974) into 5% aqueous sodium bicarbonate followed by acidification and renewed transfer into diethyl



Structures of Δ^1-THC and isolated metabolites. The monoterpenoid (Δ^1-THC) system of nomenclature is illustrated.

ether (3 × 450 ml). The final ether extract was dried over sodium sulphate, the solvent evaporated under vacuum and the residue dissolved in 5% aqueous sodium bicarbonate (30 ml). After the pH was adjusted to 6, the acids were absorbed on a column of Amberlite XAD-2 resin (2 × 40 cm) and salts were removed with water (500 ml). A rough separation of acids was obtained by elution with 350 ml aqueous ammonia (ammonia fraction) followed by 500 ml methanol (methanol fraction). The metabolites eluted with 10% aqueous ammonia (Fig. 1) were re-extracted into diethyl ether at pH 3, and applied to a column of Sephadex G-25 Superfine (1·8 × 185 cm) as described by Nordqvist et al (1974). The methanol fraction was applied to a separate column (1·8 × 165 cm). The acids of both fractions were eluted with 0·1 M ammonium bicarbonate (1% butanol added) at an elution rate of 4·5 ml h^{-1}. Fractions of 4·5 ml were collected and those of high radioactivity (Fig. 1) were extracted with diethyl ether at pH 3. Further isolation and purification was accomplished on Sephadex LH-20 columns after esterification (Nilsson et al 1973) and by t.l.c.

Identification methods

T.l.c. was on precoated Silica gel F plates (Merck, 0·25 mm) with 0·2% Fast Blue B salt in 2N sodium hydroxide as visualizing agent. The solvent system was diethyl ether–light petroleum (7 : 3), if not stated otherwise. For preparative use the plates were pre-washed with benzene–methanol (1 : 1). Metabolites were extracted from the Silica gel with methanol–dichloromethane (1 : 9). Redistilled solvents were used.

Conventional g.l.c. was carried out at 250 °C using a Varian Aerograph Model 2100 (2% SE-30 column/Gas-Chrom Q). For mass spectrometry (m.s.) an LKB 9000 gas chromatograph-mass spectrometer was used (3% SE-30 column/Gas-Chrom Q). Mass spectra were recorded at 20 and 70 eV. ^{1}H-Nuclear magnetic resonance (p.m.r.) spectra were recorded with a Varian 100 MHz instrument (Fourier transform). The solvent was $CDCl_3$ for methyl esters and CD_3OD for non-derivatized sample.

Whenever possible the isolated metabolites were compared with reference samples. (For synthesis of reference acids in the Δ^6-series carrying a carboxylic function in positions 1″- to 5″ of the side chain see Ohlsson, Leander et al, to be published). 7-Hydroxy-Δ^1-THC, 6α,7-dihydroxy- Δ^1-THC and Δ^1-THC-7-oic acid were supplied by the National Institute of Drug Abuse. Acids were esterified with diazomethane. Reductions were performed with lithium aluminium hydride ($LiAlH_4$) and lithium aluminium deuteride ($LiAlD_4$) in dry diethyl ether. Silyl derivatives were formed with *N,O*-bis-(trimethylsilyl)-acetamide in dry acetonitrile kept at 60 °C for 15 min.

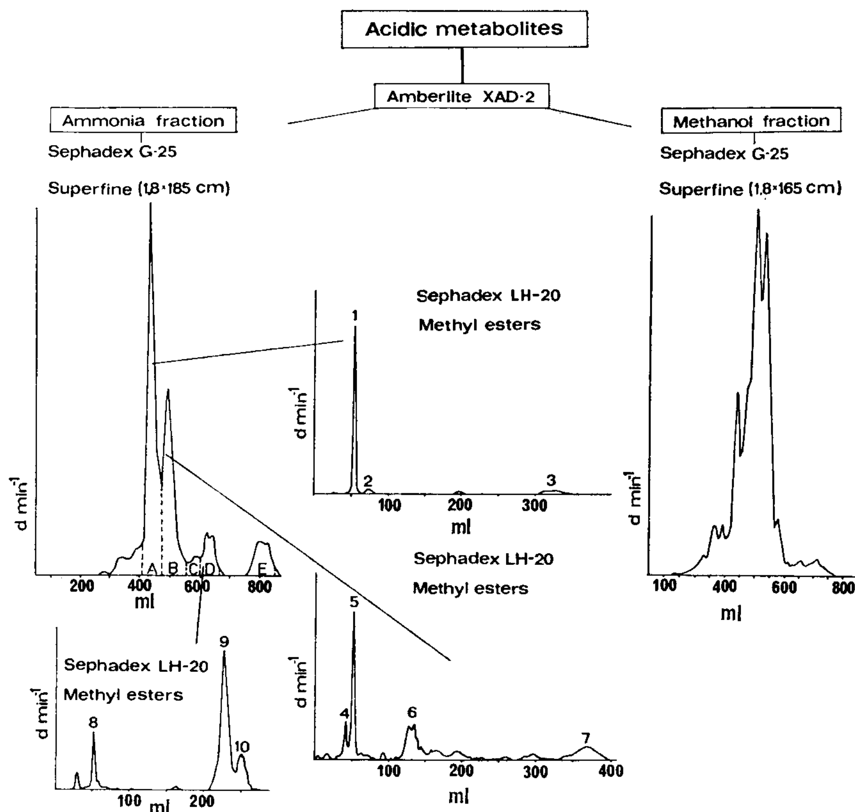


FIG. 1. Scheme for preliminary purification, extraction and separation of acidic metabolites of Δ^1-THC in rabbit urine. The acids extracted into diethyl ether at pH 3 were eluted from a column of Amberlite XAD-2 resin (2 × 40 cm) with 10% aqueous ammonia (350 ml) followed by methanol (500 ml). The ammonia fraction was further separated into fractions A-E on a Sephadex G-25 column with 0·1 M ammonium bicarbonate as eluant. Final purification by column chromatography was accomplished on Sephadex LH-20 after esterification and with light petroleum–chloroform–ethanol (10 : 10 : 1) as eluant. Peaks 1–10 are discussed in this paper. The percentage figures are calculated on the total radioactivity excreted in urine.

RESULTS AND DISCUSSION

Excretion of radioactivity

During the period of collection 39% of the injected ^{14}C-Δ^1-THC was recovered in the rabbit urine (cf. Agurell et al 1970; Nordqvist et al 1974). Urinary radioactivity was extracted into diethyl ether at pH 8 and 3 (Fig. 1). No unchanged Δ^1-THC (<1%) could be detected in the pH 8 extract by t.l.c. (diethyl ether–light petroleum 7 : 3). The acids re-extracted at pH 3 were separated into the ammonia and the methanol fraction and chromatographed on Sephadex columns (Fig. 1). The isolation and identification of metabolites only from the ammonia fraction will be described.

Metabolites of the ammonia fraction

Fractions A, B, D and E were subjected to column chromatography on Sephadex LH-20 after previous esterification. The eluant used for fraction A, B and D was light petroleum–chloroform–ethanol (10 : 10 : 1). Fraction E was eluted with methanol–chloroform (1 : 1). Peaks eluted from Sephadex LH-20 were further purified by t.l.c. before structure elucidation by p.m.r. and m.s. and comparisons with reference compounds using g.l.c. and t.l.c.

Peak 1 (5·8% of the radioactivity in urine) was methyl 4″,5″-bisnor-Δ^1-THC-7,3″-dioate (I, R_F = 0·66, Rt = 8·65 min), previously reported (Nordqvist et al 1974).

Peak 2 (0·2%) contained methyl esters of three metabolites (R_F = 0·34, 0·38, 0·43), one of which was tentatively identified as 4″,5″-bisnor-Δ^1-THC-3″-oic acid (II) from the m.s. of its silylated ester (Table 1). Although the ion *m/e* 315 was missing (cf. Martin et al 1976), the metabolite seemed to undergo benzylic cleavage with and without proton transfer, *m/e* 330 and 329 respectively.

Peak 3 (0·5%) was the methyl ester of 6β,7-dihydroxy-4″,5″-bisnor-Δ^1-THC-3″-oic acid (III, R_F = 0·08, Rt = 8·85 min) from the m.s. of its silylated methyl

ester (Table 1). The base peak and the next most abundant ion in the m.s. derived from loss of -CH_2OT and loss of both -CH_2OT and -HOT, respectively. The latter fragment ion forms the base peak in the m.s. of silylated 6β,7-dihydroxy-Δ^1-THC (Wall et al 1976a,b). Silylated 6α,7-dihydroxy-Δ^1-THC exhibited loss of -HOT as the dominant fragmentation route. Loss of -CH_2OT was 6%, and loss of -HOT, -CH_3 5%. Also p.m.r. data (Table 2) were in agreement with that of a 6β,7-dihydroxylated cannabinoid (Wall et al 1970; Pitt et al 1975).

Peak 4 was predominantly the methyl ester of Δ^1-THC-7,5″-dioic acid (IV, 0·3% of urinary radioactivity, R_F = 0·68, Rt = 15·35 min). The m.s. of the silylated derivative (Table 1) differed from I in that the fragment ions were all 28 m.u. higher except for *m/e* 359 deriving from cleavage of the side chain. The p.m.r. spectrum (Table 2) differed from I only in the C-1″ protons.

Peak 5 contained a major metabolite 2″,3″,4″,5″-tetranor-Δ^1-THC-7,1″-dioic acid (V, 1,4%, R_F = 0·68, Rt = 6·20 min). The mass spectrum (Table 1) indicated that the molecular weight of V was 28 and 56 mass units lower than I and IV respectively. The aromatic protons were deshielded 0·83 and 0·91 p.p.m. compared to the C-5′ and C-3′ protons in IV (Table 2). This suggested a carbomethoxy function in the C-4′ position, giving rise to a sharp 3H singlet at δ 3·89, a downfield shift of 0·22 p.p.m. compared to the side chain ester protons of IV. The C-1 location of the other methyl ester was evident from the sharp 3H singlet at δ 3·77 as in I and IV.

Peak 6 was separated by preparative t.l.c. into three components (VI-VIII). The most polar compound was the methyl ester of 2″-hydroxy-3″,4″,5″-trisnor-Δ^1-THC-7-oic acid (VI, 0·3%, R_F = 0·28, Rt = 5·00 min). The m.s. of its silylated derivative (Table 1) indicated the C-1 position for the ester function, further supported by the m.s. (20 eV) of the silylated primary alcohol obtained by reduction with $LiAlD_4$ [*m/e* 522 (M^+, 10), 507 (7), 432 (6, -HOT), 417 (100, -CD_2OT)]. The loss of -CD_2OT is analogous to loss of -CH_2OT in silylated 7-hydroxy-Δ^1-THC (Wall et al 1976a,b), although the equivalent loss of -HOT and -CH_2 is also possible. Comparison with silylated 3″,4″,5″-trisnor-Δ^6-THC-2″-oic methyl ester (Ohlsson et al private communication) showed the following fragment ions at 20 eV: *m/e* 388 (M^+, 100), 373 (9), 345 (16), 329 (8), 320 (19), 315 (3), 305 (88), 285 (4). Here the loss of 59 m.u. was of low abundance, thus a side chain position for the ester function in the methyl ester of VI is less probable. In the case of 2″-hydroxy-3″,4″,5″-trisnor-Δ^6-THC [*m/e* 432 (M^+, 100), 417 (21), 389 (11), 373 (5), 364 (11), 349 (65), 342 (5), 329 (2); 20 eV] small losses of both -CH_2OT and -HOT were evident whereas for the Δ^1-isomer a higher loss of -HOT (55%) is reported (Martin et al 1976). This was in agreement with the fragmentation of the silylated methyl ester of VI. The structure of VI was further confirmed by p.m.r. (Table 2) with the absence of a methylene signal at δ 3·86 (as in 7-hydroxy-Δ^1-THC; Razdan et al 1973) and the prominent shift of the vinylic proton from δ 6·33 as in Δ^1-THC (Archer et al 1970) to δ 7·95 in VI.

The second peak 6 metabolite (VII, 0·2%, R_F = 0·53, Rt = 4·60 min) was assigned to 6β-hydroxy-4″,5″-bisnor-Δ^1-THC-3″-oic acid by comparison with the least polar metabolite from peak 6 (VIII, 0·3%, R_F = 0·59, Rt = 4·50 min). Metabolite VIII was assigned the structure 6β-hydroxy-3″,4″,5″-trisnor-Δ^1-THC-2″-oic acid (for data see Tables 1 and 2). The C-1 methyl group of Δ^1-THC was intact in the methyl ester of VIII appearing as a singlet at δ 1·74, a small downfield shift compared to Δ^1-THC (Wall 1971). The α-hydrogen on C-6 appeared at δ 4·05 as reported for 6β-hydroxy-Δ^1-THC (Ben-Zvi et al 1971; Wall 1971). The downfield shift of the C-3′ and C-5′ aromatic protons reflected the proximity of the ester function. Further evidence for a 2″-oic ester was the 2 H singlet at δ 3·47 from the benzylic protons. The base peaks in the m.s. of silylated methyl esters of VII and VIII derived from loss of 131 m.u., fragmentation diagnostic of silylated 6β-

Table 1. M.s. fragment ions diagnostic of acidic metabolites of Δ^1-THC.

	M.s. ions[a]												
Metabolite[b]	M^+	-CH_3	-OCH_3	-C_3H_7	-$COOCH_3$	Benz[c]	Benz (H)[d]	SC[e] or -CH_3, Benz (H)[d]	-HOT[f]	-HOT, -CH_3[f]	-CH_2OT[f]	-CH_2OT, -HOT[f]	−131
I	446 (100)	431 (44)	415 (16)	403 (7)	387 (78)	—	—	359 (6)	—	—	—	—	—
II	402 (100)	387 (48)	371 (12)	—	—	329 (29)	330 (19)	—	—	—	—	—	—
III	578 (43)	563 (20)	547 (8)	—	—	—	—	—	488 (43)	473 (40)	475 (100)	385 (76)	447 (24)
IV	474 (100)	459 (34)	443 (18)	431 (5)	415 (60)	—	374 (6)	359 (14)	—	—	—	—	—
V	418 (100)	403 (76)	387 (14)	375 (12)	359 (59)	—	—	359 (59)	—	—	—	—	—
VI	476 (100)	461 (61)	445 (6)	433 (6)	417 (80)	373 (6)	—	—	386 (39)	371 (8)	373 (6)	—	—
VII	490 (36)	475 (45)	459 (6)	—	—	417 (2)	—	—	400 (10)	385 (10)	—	—	359 (100)
VIII	476 (32)	461 (30)	—	—	417 (4)	417 (4)	—	403 (5)	386 (23)	371 (26)	—	—	345 (100)
IX	550 (36)	535 (25)	—	—	—	—	—	—	460 (42)	445 (44)	447 (100)	357 (78)	419 (31)
X	430 (91)	415 (60)	—	387 (3)	371 (100)	373 (23)	374 (23)	359 (21)	—	—	—	—	?
XI	462 (6)	447 (7)	431 (2)	—	403 (1)	—	—	403 (1)	372 (5)	357 (9)	359 (100)	—	331 (2)
XI[g]	520 (10)	505 (13)	—	—	—	—	—	—	430 (9)	—	417 (100)	—	—
XII	462 (1)	447 (5)	431 (1)	—	—	—	—	—	372 (100)	357 (20)	—	—	331 (27)
XIII	462 (11)	447 (2)	431 (3)	—	?	—	—	—	372 (49)	357 (100)	—	—	331 (14)
XIV	444 (100)	429 (48)	—	401 (9)	385 (64)	—	—	—	—	—	—	—	—

[a] 20 eV. [b] Trimethylsilylderivative of the methyl ester. [c] Benzylic cleavage of the pentyl side chain.
[d] Benzylic cleavage with proton transfer. [e] Loss of the entire pentyl side chain. [f] T = $Si(CH_3)_3$. [g] TMS derivative of the acid.

Table 2. P.m.r. data of methyl esters of acidic metabolites of Δ^1-THC[a].

Resonancing proton attached to: Metabolite	C-2	C-3	C-4	C-5	C-6	C-7	C-9	C-10	C-5′	C-3′	C-1″	C-2″	C7-O_2C[c]	C1″-O_2C[c]	C2″-O_2C[c]	C3″-O_2C[c]
I	7·98d	3·36br.d.	1·66m	2·0m	2·5m		1·43s	1·10s	6·26d	6·19d	2·7m		3·74s			3·69s
III	6·97d	3·17br.d.		2·0m	4·44d	4·29s	1·46s	1·12s	6·30d	6·15d	2·7m					3·69s
IV	7·96d	3·36br.d.	1·66m	2·0m	2·5m		1·43s	1·10s	6·27d	6·13d			3·74s			
V	8·01d	3·43br.d.	1·66m	2·0m	2·55m		1·45s	1·11s	7·10d	7·04d			3·77s	3·89s		
VI	7·95d						1·45s	1·13s	6·33d	6·20d	2·72t	3·83t	3·74s			
VIII	6·91d				4·05s	1·74s	1·71s	1·20s	6·37d	6·29d	3·47s				3·69s	
XI[b]	6·75br.s.	3·12	1·7	2·0m	2·3m	3·95s	1·42s	1·09s	6·95d	6·91d						
XIII					4·03br.s.	1·76s	1·64s	1·21s	7·15d	7·02d				3·89s		
XIV	7·93m			2·03d	2·54m		1·47s	1·13s	6·65d	6·44d	7·52d	6·34d	3·75s			3·81s

[a] Chemical shifts in ppm in deuterochloroform with tetramethylsilane as internal standard.
[b] Not derivatized metabolite.
[c] Methyl ester protons.

hydroxy-Δ^1-THC (Wall et al 1976a). The corresponding fragment 59 m.u. was lost from the non-silylated 6β-hydroxy-Δ^1-THC (Widman et al 1975) and esters of VII and VIII with only the phenolic groups silylated. The fully silylated methyl esters lost -HOT with and without an additional loss of -CH_3 to the same extent, which also indicated a 6β-hydroxylation (Wall et al 1976a,b; Harvey & Paton 1976). The fragment ions of the mono-silylated esters that derived from losses of both -H_2O and -CH_3 were of higher abundances than those formed by loss of -H_2O only. This is in contrast to 6α-hydroxy-Δ^1-THC (cf. Jones et al 1974). Benzylic cleavage with loss of the ester function was present in both VII and VIII. A low intensity ion due to loss of -$COOCH_3$ was seen only in VIII, further supporting a side chain position for the ester group.

Peak 7 was the methyl ester of a single metabolite (IX, 0·2%, R_F = 0·09, Rt = 5·25 min), 6β,7-dihydroxy-2″,3″,4″,5″-tetranor-Δ^1-THC-1″-oic acid. The structure followed by comparison of the m.s. of its silylated methyl ester with that of III (Table 1), the difference of 28 m.u. being equivalent to the side chain of IX being shorter by two carbon atoms. In fraction C the major components were identical to those in fraction D, XIII being the dominant metabolite.

Peak 8 was shown to be identical with an authentic specimen of methyl Δ^1-THC-7-oate (X, <0·1%, R_F = 0·89, Rt = 5·10 min) (Table 1).

Peak 9 contained methyl esters of three metabolites (XI-XIII) separated by preparative t.l.c. The most polar metabolite (XI, 0·8%, R_F = 0·37, Rt = 4·00 min) was 7-hydroxy-2″,3″,4″,5″-tetranor-Δ^1-THC-1″-oic acid. The dominant m.s. fragmentation route (Table 1) gave loss of -CH_2OT, a strong indication of a hydroxymethyl group on C-1 (Harvey & Paton 1976; Wall et al 1976a,b). The loss of -$COOCH_3$ was minor. The structure was conclusively established by p.m.r. (Table 2). A 2H singlet was recorded at δ 3·95 but no 3H singlet at δ 1·69 from a methyl group at C-1. The vinylic proton at C-2 showed up as a broad singlet at δ 6·75, a downfield shift compared to Δ^1-THC. These shifts were in good agreement with those of 7-hydroxy-Δ^1-THC (Nilsson et al 1970; Wall et al 1970). The aromatic protons were as in V strongly shifted downfield.

M.s. of silylated methyl ester of XII (<0·2%, R_F = 0·48, Rt = 3·00 min) was characterized by a dominant loss of -HOT (base peak), a strong indication of 6α-hydroxylation (Wall et al 1976a,b; Harvey & Paton 1976). The structural assignment to 6α-hydroxy-2″,3″,-4″,5″-tetranor-Δ^1-THC-1″ oic acid was further supported by m.s. comparisons with XI and XIII. Their R_F values were also in consistency with those of the series 6α, 6β, and 7-hydroxy-Δ^1-THC (Fonseka et al 1976).

The least polar metabolite from peak 9 (XIII, 0·6%, R_F = 0·61, Rt = 3·25 min) was 6β-hydroxy-2″,3″,4″,5″-tetranor-Δ^1-THC-1″-oic acid. The m.s. fragments of its nonsilylated ester [*m/e* 318 (M^+, 47), 303 (12), 300 (26), 287 (19), 285 (94), 283 (77), 275 (7), 259 (28), 234 (23), 219 (100), 181 (21); 70 eV] were in agreement with those of 6β-hydroxy-Δ^1-THC (Widman et al 1975). When silylated (Table 1) it fragmented like VII and VIII except for the lack of benzylic cleavage. The intensities were however different with loss of -CH_3 and -HOT forming the base peak. But, the fragment ions of both VII and VIII occurred at different abundances at different m.s. runs. In the other m.s. of silylated methyl ester of VIII loss of -CH_3 and -HOT resulted in the base peak [*m/e* 476 (M^+, 18), 461 (4), 417 (4), 403 (5), 386 (68), 371 (100), 369 (18), 345 (12), 305 (28); 20 eV]. This was in good agreement with the corresponding m.s. of XIII, a homologue to VIII with a 14 m.u. shorter side chain. The protons in their terpene moieties exhibited similar p.m.r. shifts (Table 2). The aromatic protons in XIII showed a downfield shift of the same magnitude as in V, 0·9 p.p.m. compared to 6β-hydroxy-Δ^1-THC (Wall 1971). Methyl ester protons of XIII and V also resonanced at the same shift.

Peak 10 contained metabolite XI (t.l.c. and m.s.).

Fraction E contained a major metabolite, methyl 1″,2″-dehydro-4″,5″-bis-nor-Δ^1-THC-7,3″-dioate (XIV, 1·4%, R_F = 0·66), which was isolated by preparative t.l.c. The m.s. (Table 1) indicated that it was a homologue of I with the molecular weight 2 m.u. lower. The p.m.r. (Table 2) showed no signal from a C-1 methyl group but two 3H singlets at δ 3·81 and δ 3·75 indicating a dimethyl ester, one at C-1. There was no change at C-9 or C-10, but the aromatic protons were shifted downfield, revealing a side chain position of the other ester function. An AB-system was formed by two protons. The 1H doublets centered at δ 7·52 and δ6·34 showed a coupling constant (J = 16 Hz) typical for *trans* olefinic protons. The strong deshielding may be explained by an α,β-unsaturated carbonyl function conjugated to the aromatic ring.

CONCLUSION

It has previously been suggested that allylic hydroxylation of Δ^1-THC at C-7 and further biotransformation to the 7-oic acid is prominent in the rabbit (Burstein et al 1972; Nordqvist et al 1974), rat (Harvey et al 1977a), rhesus monkey (Ben-Zvi et al 1976), man (Wall et al 1976b) and mouse (Harvey et al 1977a). Present results support the view that major

metabolites of Δ^1-THC in rabbit urine are compounds with a 7-hydroxy or 7-carboxy function. Compared with man (Wall et al 1976b), the amount of Δ^1-THC-7-oic acid (X) is, however, low. Preferentially, the side chain is also hydroxylated, further oxidized or shortened. Dicarboxylic acid metabolites predominate with 4″, 5″-bisnor-Δ^1-THC-7,3″-dioic acid (I) (Nordqvist et al 1974) as the major non-conjugated metabolite. Dicarboxylic acid metabolites of any cannabinoid other than Δ^1-THC have so far not been found in any species and those of Δ^1-THC only in the rabbit.

Allylic attack at C-6 yields mainly the 6β-hydroxy derivatives of monocarboxylic acids. Two 6β,7-dihydroxylated acid metabolites were identified (III, IX). Neither ketones nor aldehydes from oxidized allylic hydroxyl groups were detected, nor was there evidence of a hydrogenated acid as reported for the mouse (Harvey et al 1977b). Aromatization of the terpene ring reported in the case of the rhesus monkey (Ben-Zvi et al 1976) was not noted.

The pentyl side chain appears to be more easily oxidized in the rabbit than in man, mouse and rat, where monocarboxylic acids with a 7-oic function seem to be major metabolites of Δ^1-THC (Wall et al 1976a,b; Harvey et al 1977a), of cannabidiol (CBD) (Martin et al 1977) or of cannabinol (CBN) (Burstein & Varanelli 1975; Yisak et al 1978). Side chain hydroxylation is known to occur in the rabbit (Burstein et al 1972), dog (Widman et al 1975), rat (Widman et al 1974; Harvey et al 1977a; Yisak et al 1978), guinea-pig and mouse (Harvey et al 1977a; Martin et al 1977). The occurrence of hydroxylated 7-oic acids in the methanol fraction of rabbit urine is under investigation. An unusual metabolite of Δ^1-THC was identified as 2″-hydroxy-3″,4″,5″-trisnor-Δ^1-THC-7-oic acid (VI).

Acidic metabolites of Δ^1-THC with degraded side chains have earlier been reported in the rabbit (Nordqvist et al 1974; Martin et al 1976), rhesus monkey (Ben-Zvi et al 1976), guinea-pig, mouse and rat (Harvey et al 1977a) and of CBD and CBN in the mouse (Burstein & Varanelli 1975; Martin et al 1977). These side-chain carboxylic acids might be formed by initial hydroxylation followed by oxidation via a keto derivative to the acid. The metabolism of CBN in the rat leads to a side-chain keto derivative of CBN-7-oic (Yisak et al 1978). Another route of formation could involve the β-oxidation enzyme system. The occurrence of e.g. the dicarboxylic metabolites as homologues suggests such degradation of the pentyl side chain. A possible precursor to 2″,3″,4″,5″-tetranor-Δ^1-THC-7,1″-dioic acid (V) was identified as the side-chain unsaturated 1″,2″-dehydro-4″,5″-bisnor-Δ^1-THC-7,3″-dioic acid (XIV). This could be formed by the activity of dehydrogenase enzymes on the major metabolite 4″,5″-bisnor-Δ^1-THC-7,3″-dioic acid (I).

It remains to be clarified whether the polar acidic metabolites of Δ^1-THC described in this paper also are formed in man. The major part of excreted metabolites of Δ^1-THC in human urine is reported to be polar acids (Wall et al 1976b), so far not identified.

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