J. Pharm. Pharmacol. 1980, 32: 267–271 Received March 15, 1979

Identification of in vivo liver metabolites of Δ^1-tetrahydrocannabinol, cannabidiol, and cannabinol produced by the guinea-pig

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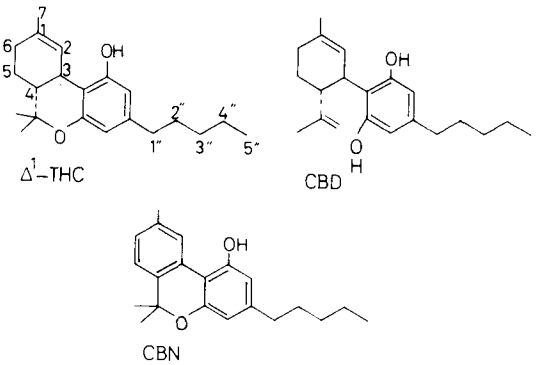
The metabolites of Δ^1-tetrahydrocannabinol (Δ^1-THC), cannabidiol (CBD), and cannabinol (CBN) produced in vivo by the guinea-pig have been studied by combined gas-liquid chromatography-mass spectrometry. 45 metabolites of Δ^1-THC were identified of which 12 have not been reported before. Several other metabolites were detected but not identified. The major metabolic routes involved allylic and aliphatic hydroxylations, oxidations to ketones and acids, oxidative degradation of the side-chain presumably by the β-oxidation pathway, and formation of glucuronide conjugates. Di- and tri-substituted metabolites were abundant. The metabolism differed considerably from that observed in mouse and rat in that 1″- and 6β-hydroxylation and oxidative degradation of the side-chain were major metabolic pathways. 1″-Hydroxy-Δ^1-THC was found as a pair of diasteroisomers. Similar metabolic pathways were observed with CBD; twenty metabolites were identified of which two were new. Only 6 metabolites of CBN were identified. These were mainly mono-substituted in the same positions as were observed with Δ^1-THC and CBD.

Extensive research in recent years has led to the elucidation of a number of the major biotranformation pathways involved in the metabolism of the cannabinoids (Mechoulam et al 1976; Harvey et al 1978). Metabolism has been studied in several species including rat, mouse, rabbit, dog and rhesus monkey, but less is known about metabolism by man. Metabolism by the guinea-pig has also received little attention although in some respects, such as its inability to synthesize ascorbic acid, the guinea-pig resembles man more closely than do many of the other species. Our initial studies with Δ^1-tetrahydrocannabinol (Δ^1-THC) (Martin et al 1976a; Harvey et al 1977a, 1978) have shown that the in vivo metabolites recovered from guinea-pig livers differ considerably from those found in species such as the mouse or rat. In particular, 6β- rather than 6α-hydroxylation seems to be favoured and abundant acid metabolites are produced by oxidative degradation of the side-chain (Martin et al 1976a). No reports have appeared on the metabolism by the guinea-pig of cannabidiol (CBD) or cannabinol (CBN), the other major cannabinoids of cannabis resin. In this paper, the in vivo liver metabolites produced by the guinea-pig from these three cannabinoids are examined and a number of new compounds are reported.



MATERIALS AND METHODS

Δ^1-THC (98% pure by g.l.c., remaining 2% was CBN) suspended in Tween-80 and 0·9% NaCl (saline) was administered intraperitoneally at a dose of 100 mg kg^{-1} to male Dunkin-Hartley guinea-pigs (450–500 g) at 1 h (n = 2), 2 h (n = 3), 3 h (n = 1) and 4 h (n = 1) before death by stunning and decapitation. CBD and CBN were administered similarly at 1 h and 2 h before death. Livers were removed, homogenized in saline and the metabolites were extracted with ethyl acetate, separated from neutral lipids by chromatography on Sephadex LH-20, converted into trimethylsilyl (TMS), [$^2H_9$]-TMS, methyl ester-TMS, or methyloxime-TMS derivatives and examined by g.l.c. and g.c.-m.s. as

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described previously (Harvey et al 1977a, b, 1978; Harvey & Paton 1976a, b). Mass spectral identification was based on comparison with synthetic samples where available or by the presence of diagnostic fragment ions indicative of substitution in the 6 and 7 positions (Wall & Brine 1976) or the 1″–5″ side chain positions (Wall & Brine 1976; Binder 1976; Binder et al 1974).

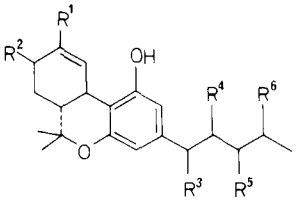
RESULTS AND DISCUSSION

Δ^1-*THC*

Seven monohydroxy metabolites were identified; these were substituted in the 1″-, 2″-, 3″-, 4″-, 6α-, 6β- and 7-positions (1–8, Table 1). The 6β- and 7-hydroxy compounds were major metabolites, others were present in low concentration. All except the 1″- and 2″-hydroxy compounds have been reported as metabolites of Δ^1-THC in other species. 1″-hydroxy-Δ^1-THC was present as diasteroisomers, separable on both SE-30 and OV-17 columns. These were present in unequal quantities but their absolute configurations have not yet been determined.

Thirteen dihydroxy metabolites (9–21) were identified; these contained hydroxy groups in the same positions as the mono-hydroxy metabolites. Of these, 6β, 7-dihydroxy-Δ^1-THC (10) was the major metabolite found in the liver. Its 6α-isomer (9) was present in low concentration. The high abundance of metabolites containing 6β rather than 6α-hydroxylation was one of the major differences between the metabolism of Δ^1-THC by the guinea-pig and that of the mouse or rat. The remaining dihydroxy metabolites were present in lower concentration. Four of these (11–14) contained hydroxylation in the 7-position accompanied by the second hydroxyl group in the 1″–4″ positions. A related series (15–18) contained 6β and 1″-4″-hydroxylation, their relative concentrations reflecting those of the parent monohydroxy metabolites. None of these 6β-substituted dihydroxy metabolites have been reported before. Their spectra were characterized by prominent $[M-131]^+$ ions at *m/z* 431 (Wall & Brine 1976). The other three dihydroxy metabolites (19–21) appeared to contain both hydroxyl groups in the side-chain. All produced base peaks at *m/z* 417 indicative of 1″-hydroxy substitution with loss of a side-chain fragment containing the second hydroxy group. G.l.c. retention increments and other ions in the spectra enabled this second hydroxyl group to be assigned to the 2″-, 3″- and 4″-positions in the three compounds. No dihydroxy metabolites in which both hydroxyl groups are in the side chain have been reported before.

Table 1. Structures of the metabolites of Δ^1-THC containing an intact side chain, extracted from guinea-pig liver.



Comp.	No.	R^1	R^2	R^3	R^4	R^5	R^6	Rel. concn
-OH-Δ^1-THC								
*1″ (peak 1)	1	CH_3	H	OH	H	H	H	+[a]
*1″ (peak 2)	2	CH_3	H	OH	H	H	H	+
2″	3	CH_3	H	H	OH	H	H	+
3″	4	CH_3	H	H	H	OH	H	2+
4″	5	CH_3	H	H	H	H	OH	+
6α	6	CH_3	α-OH	H	H	H	H	+
6β	7	CH_3	β-OH	H	H	H	H	3+
7	8	CH_2OH	H	H	H	H	H	4+
di-OH-Δ^1-THC								
6α,7	9	CH_2OH	α-OH	H	H	H	H	2+
6β,7	10	CH_2OH	β-OH	H	H	H	H	4+
1″,7	11	CH_2OH	H	OH	H	H	H	2+
2″,7	12	CH_2OH	H	H	OH	H	H	2+
3″,7	13	CH_2OH	H	H	H	OH	H	+
4″,7	14	CH_2OH	H	H	H	H	OH	2+
*1″,6β	15	CH_3	β-OH	OH	H	H	H	+
*2″,6β	16	CH_3	β-OH	H	OH	H	H	+
*3″,6β	17	CH_3	β-OH	H	H	OH	H	+
*4″,6β	18	CH_3	β-OH	H	H	H	OH	+
*1″,2″	19	CH_3	H	OH	OH	H	H	+
*1″,3″	20	CH_3	H	OH	H	OH	H	+
*1″,4″	21	CH_3	H	OH	H	H	OH	+
tri-OH-Δ^1-THC								
*1,6α,7	22	CH_2OH	α-OH	OH	H	H	H	+
2″,6α,7	23	CH_2OH	α-OH	H	OH	H	H	+
3″,6α,7	24	CH_2OH	α-OH	H	H	OH	H	+
4″,6α,7	25	CH_2OH	α-OH	H	H	H	OH	+
*1″,6β,7	26	CH_3OH	β-OH	OH	H	H	H	+
*2″,6β,7	27	CH_2OH	β-OH	H	OH	H	H	+
*3″,6β,7	28	CH_2OH	β-OH	H	H	OH	H	+
*4″,6β,7	29	CH_2OH	β-OH	H	H	H	OH	2+
hydroxy-Δ^1-THC-6-one								
3″	30	CH_3	= 0	H	H	OH	H	+
4″	31	CH_3	= 0	H	H	H	OH	+
7	32	CH_2OH	= 0	H	H	H	H	+
-THC-7-oic acid								
Δ^1	33	COOH	H	H	H	H	H	2+
1″-OH	34	COOH	H	OH	H	H	H	+
2″-OH	35	COOH	H	H	OH	H	H	2+
3″-OH	36	COOH	H	H	H	OH	H	2+
4″-OH	37	COOH	H	H	H	H	OH	2+
6α-OH	38	COOH	α-OH	H	H	H	H	+

(a) + → 4 increasing concentration.
* = new metabolite.

The positions of substitution in the eight trihydroxy metabolites (22–29) again paralleled those observed above. All were substituted at C-7 and C-6. Four (22–25) contained a 6α-hydroxy group (promi-

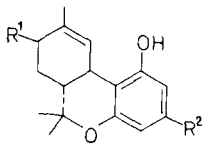
nent loss of TMSOH to give ions at *m/z* 560) whereas in the other four (26–29) the 6-hydroxyl group was β (prominent $[M-131]^+$ ions). The third hydroxyl group occupied one of the positions, 1″–4″ of the side-chain. All eight trihydroxy metabolites were present in low concentration. Again the compounds containing 6β-hydroxylation are new.

Most of the remaining metabolites were of similar structure to the above mono- and dihydroxy metabolites, but with one of the hydroxyl groups oxidized to a ketone (6-position) or an acid (7-position). Thus Δ^1-THC-7-oic acid (33, Wall & Brine 1976; Harvey & Paton 1976b) and its 6α, 1″-, 2″-, 3″- and 4″-monohydroxy derivatives (34–38) together with 3″-, 4″- and 7-hydroxy-Δ^1-THC-6-one (30–32) were identified. All of these compounds have been reported as metabolites of Δ^1-THC in other species (Harvey & Paton 1976b; Harvey et al 1977b, 1978; Burstein et al 1972). In the guinea-pig, unlike the other species, oxidation of the 6- and 7-hydroxy groups was a relatively minor process; Δ^1-THC-7-oic acid (33) and its 4″-hydroxy derivative (37) were the most abundant of these oxidized metabolites, but were present in only 20% of the concentration of the major metabolite, 6β, 7-dihydroxy-Δ^1-THC (10). In most other species, Δ^1-THC-7-oic acid is the major metabolite. Accurate measurements of tissue concentrations were not obtained because of the lack of suitable internal standards.

A major metabolic route, not yet seen in the mouse or rat, but reported to occur extensively in the rabbit (Nordqvist et al 1974, 1979a, b) involved β-oxidation of the side-chain and was represented by the four acids having a degraded side-chain consisting of COOH, CH_2COOH, C_2H_4COOH and C_3H_6 COOH groups (39–42, Table 2; Martin et al 1976a), and the hydroxy acid; 6β-hydroxy-4″,5-*bis*, *nor*-Δ^1-THC-7-oic acid (43, Nordqvist et al 1979a, b). The monocarboxylic acid, 4″,5″-*bis*, *nor*-Δ^1-THC-3″-oic acid (41, side chain = C_2H_4COOH) was the second most abundant metabolite found in liver; its concentration was nearly equal to that of 6β,7-dihydroxy-Δ^1-THC.

Two glucuronide conjugates, those of Δ^1-THC and of the major acid metabolite, 4″, 5″-*bis*, *nor*-Δ^1-THC-3″-oic acid were identified, the former by comparison with the reported spectrum of the Me-TMS derivative (Lyle et al 1977) and the latter by the presence, among others, of the glucuronic acid derived ions at *m/z* 317 (100%) and 217 (72%), and the aglycone ion at *m/z* 402 (81%) again in the spectrum of the Me-TMS derivative. The high abundance of the aglycone ions in both spectra indicated coupling of the glucuronic acid to the phenol group of the glucuronide (Billets et al 1973).

Table 2. Structures of the metabolites of Δ^1-THC containing a degraded side chain, extracted from guinea-pig liver.



Compound	No.	R^1	R^2	Rel. concn
Δ^1-THC-1″-oic acid	39	H	COOH	+[a]
Δ^1-THC-2″-oic acid	40	H	CH_2COOH	2+
Δ^1-THC-3″-oic acid	41	H	C_2H_4COOH	4+
Δ^1-THC-4″-oic acid	42	H	C_3H_6COOH	+
6β-OH-Δ^1-THC-3″-oic acid	43	β-OH	C_2H_4COOH	+

(a) + → 4 increasing concentration.

CBD

The time course for the metabolism of CBD appeared similar to that for Δ^1-THC; more polar metabolites were present after 2 h than at 1 h but after this the metabolic profile showed little change. The profile itself was very similar to that obtained for CBD in the mouse (Martin et al 1977). Of the twenty metabolites identified, only two, 6β-hydroxy-CBD-5″-oic acid and 6β-hydroxy-4″,5″-*bis*,*nor*-CBD-3″-oic acid have not been reported before. The spectra of their TMS derivatives were characterized by their base peaks at *m/z* 580 and 552 respectively ($[M-C_5H_8]^+$) and tropylium ions at *m/z* 439 (34%) and 411 (11%).

Six mono-hydroxy metabolites, 1″-, 3″-, 4″-, 6α-, 6β- and 7-hydroxy-CBD were identified by comparison with reported spectra (Martin et al 1976b). The two 6-hydroxy metabolites were better separated as TMS derivatives on OV-17 than on SE-30 and the 6β-isomer was observed in higher abundance than the 6α-isomer paralleling the metabolism of Δ^1-THC. 7-Hydroxy-CBD was the most abundant mono-hydroxy metabolite.

Other metabolic routes were similar to those observed with Δ^1-THC although no trihydroxy metabolites were observed. Dihydroxy metabolites included 6β,7-, 1″,7-, 3″,7-, 4″,7- and 4″,6β-dihydroxy-CBD (Martin et al 1976c) and the acids were represented by CBD-7-oic acid and its 4″-hydroxy derivative, the major metabolite; CBD-5″-oic acid and its 6- and 7-hydroxy derivatives; and 4″,5″-*bis*,

nor-CBD-3″-oic acid, a major metabolite, and its 6- and 7-hydroxy derivatives. In earlier reports (Martin et al 1976c, 1977) the stereochemistry at C-6 of 6,7-dihydroxy-CBD was not determined because of the similarity between the spectra of the TMS derivatives of the 6α- and 6β-isomers. On OV-17 we have found that the TMS derivative of 6,7-dihydroxy-CBD obtained from the guinea-pig eluted about 1 min earlier than that from the mouse and that its mass spectrum contained a weaker tropylium ion (13%) than that from the mouse (34%). As these differences are consistent with those found between 6α- and 6β-hydroxy-CBD it would appear that the guinea-pig produces mainly 6β,7-dihydroxy-CBD whereas the diol from the mouse has the 6α-structure. This correlates well with the guinea-pig's preference for 6β rather than 6α-hydroxylation. The remaining CBD metabolite was the glucuronide (Harvey et al 1977c) present in low concentration.

CBN

In contrast to the large number of metabolites found in the livers of guinea-pigs treated with Δ^1-THC or CBD, only six metabolites were found from CBN. These were 1″-, 3″-, 4″- and 7-hydroxy-CBN, 1″,7-dihydroxy-CBN and CBN-7-oic acid. All of these metabolites have been seen in other species (Widman et al 1971, 1975; Harvey et al 1977d; Fonseka & Widman 1977; Yisak et al 1977) and all were present in low concentration; the 1″- and 7-hydroxy-metabolites were the most abundant. No metabolites produced by oxidative degradation of the side-chain were found.

CONCLUSIONS

These results demonstrate that the guinea-pig, like other species, is able to metabolize Δ^1-THC rapidly and extensively. However, major differences in the preferred metabolic pathways were observed when the in vivo metabolites were compared with those obtained from the mouse (Charles River CD1) or rat. These were: the predominance of 6β-rather than 6α-hydroxylation, the high abundance of compounds containing acid groups in the side-chain and presumably produced by β-oxidation, the production of 1″-hydroxy derivatives, the higher abundance of monohydroxy metabolites with hydroxylation in the side-chain, the low abundance of 6-ketones, and the generally low abundance of Δ^1-THC-7-oic acid and its hydroxylated derivatives. In other respects the metabolism was very similar to that in other species with the major metabolic pathways being allylic hydroxylation in the 6- and 7-positions, aliphatic hydroxylation in the side-chain and oxidation of the allylic hydroxyls to ketones (6-position) or acids (7-position). Di- and tri-substituted metabolites appeared to be produced by combinations of all monohydroxylated species and their relative abundances tended to reflect the abundances of the monohydroxylated metabolites.

The metabolism of CBD was very similar to that of Δ^1-THC in that 6β-rather than 6α-hydroxylation was favoured and abundant metabolites were produced by oxidative degradation of the side-chain. No major differences were observed between the metabolites produced by the guinea-pig or mouse. CBN seemed to be metabolized more slowly than the other two cannabinoids to give very low concentrations of metabolites in the liver. With one exception these were all monosubstituted. However, the positions of side-chain hydroxylation were the same as with Δ^1-THC and CBD (1″, 3″ and 4″) but surprisingly, no side-chain acids were detected.

Acknowledgements

We thank Dr M. C. Braude for supplies through the MRC of Δ^1-THC, we are indebted for support by a Programme Research Grant from the MRC and a grant from the Wellcome Trust. We also thank Mr D. Perrin and Miss J. Hughes for expert technical assistance.

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