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COMMUNICATIONS

Identification of metabolites of Δ^1- and $\Delta^{1(6)}$-tetrahydrocannabinol containing a reduced double bond

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Recent studies on the metabolism of Δ^1- and $\Delta^{1(6)}$-tetrahydrocannabinol (THC) have disclosed a number of biotransformation pathways. These include allylic (Ben-Zvi, Mechoulam & Burstein, 1970; Nilsson, Agurell & others, 1970; Wall, Brine & others, 1970) and aliphatic (side-chain) hydroxylations (Agurell, Binder & others, 1976), oxidations to acids (Mechoulam, Ben-Zvi & others, 1973, Nordqvist, Agurell & others, 1974; Martin, Harvey & Paton, 1976), aldehydes (Ben-Zvi & Burstein, 1974), and ketones (Gurny, Maynard & others, 1972; Jones, Widman & Agurell, 1974), conjugation with fatty acids (Leighty, Fentiman & Foltz, 1976) or β-glucuronic acid (Harvey, Martin & Paton, 1977a), and expoxidation of the double bond (Gurny & others, 1972; Ben-Zvi & Burstein, 1975). These transformations can occur singly to produce monosubstituted metabolites or in combination to form a variety of polysubstituted compounds such as diols, triols, hydroxyacids or hydroxyketones (Wall, 1971; Burstein, Rosenfeld & Wittstruck, 1972; Harvey & Paton, 1976a,b). We now report a new metabolic pathway, the reduction of the terpene double bond in both Δ^1- and $\Delta^{1(6)}$-THC to give a series of substituted hexahydrocannabinols.

Mice (male, Charles River CD1, 23 g) were treated with either Δ^1-THC or $\Delta^{1(6)}$-THC (100 mg kg^{-1}, i.p.) suspended in Tween 80 and isotonic saline administered at 26 h and 2 h (Δ^1-THC) or at 1 h (Δ^1- and $\Delta^{1(6)}$-THC) before death by stunning and decapitation. The livers were removed, homogenized in isotonic saline (10 ml) and the metabolites were extracted with ethyl acetate (3 × 10 ml). Metabolites were separated from endogenous lipids by chromatography on Sephadex LH-20 in chloroform and chloroform–methanol mixtures as previously described (Harvey & Paton, 1976a,b) and the metabolite fractions (4 and 5) were converted into derivatives for examination by combined gas liquid chromatography - mass spectrometry (g.c.-ms). The g.l.c. column (3% SE-30) was temperature programmed at 2° min^{-1} from 170° and 25 eV mass spectra (V.G. Micromass 12B) were recorded repetitively from 190° at 3 s $decade^{-1}$ with a 2 s inter-scan delay, using a V.G. 2040 data system.

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The *in vivo* liver metabolites of Δ^1-THC in the mouse have been reported (Harvey & Paton, 1976 a,b; Harvey, Martin & Paton, 1977b); those formed by Δ^1-THC are under investigation but are similar in that the 7-hydroxy and 7-carboxylic acid metabolites, together with several of their hydroxylated derivatives are abundant.

Two substituted hexahydrocannabinols with a molecular weight of 490 were observed in the gas chromatograms of the TMS derivative of the main metabolite fraction (fraction 5) from both Δ^1- and $\Delta^{1(6)}$-THC treated mice. These compounds contained two TMS groups (18 a.m.u. shift of the molecular ions in the [$^2H_9$] TMS spectra; McCloskey, Stillwell & Lawson, 1968), formed monomethyl esters with diazomethane, and eluted from the g.l.c. column just ahead of the THC-7-oic acid metabolites ($M^{+\cdot}$, *m/e* 488). These compounds thus appeared to be dihydro-acids and their presence in the metabolic profiles of both Δ^1- and $\Delta^{1(6)}$-THC treated mice was consistent with reduction of the terpene double bond and the formation of two compounds epimeric about C_1. This was confirmed by synthesis of both isomers from an authentic sample of $\Delta^{1(6)}$-THC-7-oic acid (kindly supplied by Professor R. Mechoulam as the acetate derivative of its methyl ester). Hydrogenation of the derivative (10% Pd on charcoal in ethanol for 20 min) gave a mixture of epimeric hexahydrocannabinol (HHC)-7-oic acids as their methyl ester, acetate derivatives, separable by g.l.c. and in the ratio of 3:1 with the major isomer eluting first. A high resolution mass measurement [$M^{+\cdot}$, *m/e* 402·2397, ($C_{24}H_{34}O_5$: 402·2403)] confirmed the incorporation of two hydrogen atoms and proton magnetic resonance spectroscopy (90 MHz, Fourier transform, Bruker WH90) of the mixture in C_5D_5N showed the absence of the olefinic proton. Hydrolysis of this mixture with sodium hydroxide gave the free acids and the g.c.-ms properties of their TMS derivatives (Table 1) were identical with those of the metabolites.

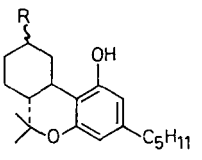
Although the nmr spectrum of the methyl ester - acetate derivative clearly showed the presence of the two isomers (methyl ester protons at δ 3·69 and δ 3·67 for the major and minor isomers respectively), information on the stereochemistry was not definitive. The H_3 proton signal whose shift was used by Archer, Boyd &

Table 1. *G.c.-ms data for the 7-substituted hexahydrocannabinols.*

Compound and derivative	Rt (min)	MU[a]	$M^{+\cdot}$	Base	$[M-15]^+$	$[M-56]^{+\cdot}$	$[M-7\text{-Sub}]^+$
1. HHC-7-oic acid (axial)							
TMS	24·5	26·62	490[b] (100)	490	475 (26)	434 (30)	372 (29)
Me-TMS	22·55	—	432 (100)	432	417 (7)	376 (73)	372 (18)
Me-OAc	—[c]	26·49	402 (28)	360	387 (0)	346 (0)	342 (3)
2. HHC-7-oic acid (equat.)							
TMS	25·5	27·0	490 (100)	490	475 (12)	434 (39)	372 (4)
Me-TMS	23·2	—	432 (100)	432	417 —	376 (80)	372 —
Me-OAc	—	26·68	402 (27)	360	387 (0)	346 (2)	342 (1)
3. 7-OH-HHC (equat.)							
TMS	23·1	26·28	476 (100)	476	461 (5)	420 (31)	372 (0)
4. 7-OH-HHC (axial)							
TMS	24·35	26·46	476 (100)	476	461 (6)	420 (20)	372 (10)

[a] Methylene unit (3% SE-30).
[b] *m/e* and (relative intensity).
[c] Not recorded under the same conditions.

others (1970) and by Gaoni & Mechoulam (1966) to determine the stereochemistry of a mixture of the two synthetic HHC's was not resolved from the acetate methyl (δ 2·51) or 1″-hydrogen triplet. However, the axial 10 methyl signal of the major isomeric HHC acid was observed at a higher field (δ1·20) than the corresponding signal from HHC itself, suggesting that the major isomer contained an axial 1-carboxymethyl group. This stereochemistry was finally confirmed by observation of the ratio of the axial (1) and equatorial (2) isomers produced with continued reaction of the free $\Delta^{1(6)}$-THC-7-oic acid with hydrogen over the Pd/charcoal catalyst. Initially the isomer with the shorter g.l.c. retention time predominated, but after about 1 h, complete conversion to the second isomer had occurred. This isomer being thermodynamically more stable must contain an equatorial acid group. Thus, the first isomer to elute from the g.l.c. column (the major metabolite) must contain an axial carboxyl group (1).



1 R = α-COOH
2 R = β-COOH
3 R = β-CH_2OH

The low resolution mass spectra of the TMS derivatives of these acids (Table 1) was fully consistent with this structural assignment. The major (axial) isomer (1) produced an abundant loss of 118 a.m.u. which was shown to be the elimination of the carboxy-TMS group with a hydrogen. Only the axial isomer has a suitably positioned hydrogen ($H_{3\alpha}$) to undergo abstraction in this way.

A third substituted hexahydrocannabinol was present in the monohydroxy metabolite fraction (fraction 4) from the livers of the $\Delta^{1(6)}$-THC treated mice. Its TMS derivative eluted just ahead (23·1 min) of the major mono-hydroxy metabolite, 7-hydroxy-$\Delta^{1(6)}$-THC (24·0 min) and the structure of 7-hydroxy-HHC (3) was indicated by the 2 a.m.u. increase in the $M^{+\cdot}$, $[M-15]^+$, and $[M-56]^{+\cdot}$ ions over those of 7-hydroxy-$\Delta^{1(6)}$-THC (Table 1). A high resolution mass measurement (probe, Micromass 70/70F) taken of the entire underivatized metabolite fraction confirmed the elemental composition [$M^{+\cdot}$, *m/e* 332·2345, ($C_{21}H_{32}O_3$: 332·2350)]. In addition, the compound formed a *bis*-TMS derivative and did not react with diazomethane. Confirmation of the structure was provided by lithium aluminium deuteride reduction of the two HHC-7-oic acids synthesized above. This gave the two isomeric alcohols [high resolution measurement of $M^{+\cdot}$, *m/e* 334·2480, ($C_{21}H_{30}{}^2H_2O_3$: 334·2474)]. The first isomer to elute from the g.l.c. column had identical g.l.c. properties to those of the metabolite except for the $^2H_2$ incorporation (Table 1). As this compound was produced by reduction of the second HHC-7-oic acid (shown by the ratio of peak areas), the metabolite must contain an equatorial hydroxy-methyl group. The second (axial) 7-hydroxy-HHC was not found as a metabolite, but its presence may have been masked by the large peak produced by 7-hydroxy-$\Delta^{1(6)}$-THC whose second isotope ions were coincident in mass with the 7-hydroxy-HHC ions. No 7-hydroxy-HHC's were found as metabolites of Δ^1-THC but this may have been the result of the different method of drug treatment.

Three hydroxy-substitued HHC-7-oic acids [$M^{+\cdot}$, *m/e* 578 (TMS derivative); *m/e* 530 (Me-TMS derivative)] were also present in fraction 5 but the position of the additional hydroxyl group has not yet been determined because the amounts of the compounds were small and they co-chromatographed with more abundant metabolites. The most likely site for this hydroxy group is the pentyl side-chain.

The concentrations of all these metabolites were low and amounted to about 1–2% of the total recovered metabolites in fractions 4 and 5. However, because of the large number of metabolites present, the concentration of the axial HHC-7-oic acid of about 5% that of the major metabolite ($\Delta^{1(6)}$-THC-7-oic acid) was comparable with those of several of the other metabolites. Each hydroxy-HHC-7-oic acid was present at about twice this concentration.

The metabolic saturation of double bonds is a fairly

common reaction in terpene (Williams, 1959) and steroids (Fotherby & James, 1972), but at the enzyme level does not seem to be well documented for other drugs (Hutson, 1970). In the case of the cannabinoids it would appear that the presence of a β-oxygen function may be necessary for saturation of the double bond to occur as no unsubstituted HHC was found in any of the liver fractions. As the latter compound does not contain a double bond, allylic hydroxylation is prevented; thus, if formed, HHC would not be expected to undergo complete metabolic transformation to further hydroxylated or oxidized products and should therefore be observed.

Edery, Grunfeld & others (1971) have reported that both the axial and equatorial isomers of HHC and the equatorial methyl isomer of 6β-hydroxy-HHC are active in the dog ataxia test. Equatorial HHC has an activity which is similar to that of $\Delta^{1(6)}$-THC but the axial isomer is less active. It is probable, therefore, that the equatorial isomer of 7-hydroxy-HHC, reported here as a metabolite of $\Delta^{1(6)}$-THC, would also be active, although somewhat less so than 7-hydroxy-THC.

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