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Metabolism of Δ^1-tetrahydrocannabinol by the isolated perfused dog lung. Comparison with *in vitro* liver metabolism

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The metabolism of (−)-Δ^1-tetrahydrocannabinol (Δ^1-THC) has been studied in the isolated perfused dog lung. After intravascular administration of [^{3}H]-Δ^1-THC there was an overall biotransformation of 12%. Two major metabolites were isolated and identified as 3″-hydroxy-Δ^1-THC and 4″-hydroxy-Δ^1-THC. 7-Hydroxy-Δ^1-THC was also present together with small amounts of 6α-hydroxy-Δ^1-THC and 6β-hydroxy-Δ^1-THC. An *in vitro* experiment using a dog liver microsomal preparation was also carried out and showed that the major metabolites were 6β-hydroxy-Δ^1-THC and 6α-hydroxy-Δ^1-THC. 7-Hydroxy-Δ^1-THC and 1,2-epoxy-hexahydrocannabinol were also isolated together with small amounts of 3″-hydroxy-Δ^1-THC and 4″-hydroxy-Δ^1-THC. The side-chain hydroxylated compounds are hitherto undescribed metabolites of Δ^1-THC.

(−)-Δ^1-Tetrahydrocannabinol (Δ^1-THC), the psychotomimetic compound of cannabis, is rapidly metabolized to more polar compounds in the liver of different species (*cf.* Mechoulam, McCallum & Burstein, 1975; Jones, Widman & others, 1974). The liver is considered to be the principal site for the transformation of THC, although extrahepatic metabolism might occur. Since the major route of cannabis administration in humans is by inhalation, it is important to know how the lung tissue affects the cannabinoids. Nakazawa & Costa (1971) have found that Δ^1-THC is enzymatically converted by rat lung. They also found by using thin-layer chromatography (t.l.c.) that the metabolite pattern of Δ^1-THC differed between lung and liver. So far no metabolites of Δ^1-THC have been isolated and reliably identified from lung tissue.

We have studied the metabolism of Δ^1-THC by lung tissue using isolated perfused dog lungs. The results have been compared with those obtained using dog liver microsomal preparations. The whole experiment, consisting of lung perfusion simultaneously carried out with liver incubation, was made in duplicate. Several metabolites from both tissues have been isolated and their structures determined. Two of the metabolites were side-chain hydroxylated compounds (3″-hydroxy-Δ^1-THC and 4″-hydroxy-Δ^1-THC) which had not been found previously.

MATERIALS AND METHODS

Metabolism of Δ^1-THC by isolated perfused dog lung. Lungs were excised from greyhounds and prepared for perfusion according to Briant, Blackwell & others (1973).

(−)-1″-[³H]-Δ¹-THC (9 mg, 1·7 mCi mmol^{-1}, over 96% pure by t.l.c. systems A and B and g.l.c., containing less than 1·5% CBN), was dissolved in 70% aqueous ethanol and administered intravascularly. At the end of the 2 h perfusion blood was collected and centrifuged. The plasma (155 ml) was extracted 3 times with 175 ml of 1·5% pentanol in redistilled light petroleum (b.p. 40–60°) which was followed by diethyl ether (4 × 175 ml). The dried petroleum and ether extracts were chromatographed on columns of Sephadex LH–20.

Lung tissue (173 g) was homogenized with distilled water. The homogenate (850 ml) was extracted with light petroleum (3 × 1200 ml) followed by diethyl ether (4 × 1200 ml). The dried extracts were chromatographed on Florisil followed by Sephadex LH–20 as described by Widman, Nordqvist & others (1974).

Metabolism of Δ¹-THC by dog liver supernatant. An enriched dog liver microsomal preparation, similar to the one reported earlier (Jones & others, 1974), was used. The liver (189 g) was homogenized with 210 ml of tris-KCl buffer pH 7·4. The homogenate was centrifuged at 10 000 *g* for 10 min, and the resulting supernatant (190 ml) was incubated at 37° for 2 h with [³H]-Δ¹-THC (27 mg, 0·59 mCi mmol^{-1}, dissolved in an emulsion of Tween 80). The incubation mixture also contained glucose-6-phosphate (1 g), NADP (150 mg), glucose-6-phosphate dehydrogenase (50 units), nicotinamide (300 mg) and $MgCl_2 \times 6H_2O$ (600 mg) dissolved in 100 ml of tris-KCl buffer.

The incubation mixture was extracted with light petroleum (3 × 300 ml) followed by diethyl ether (4 × 300 ml). The dried petroleum and ether extracts were chromatographed on Florisil and Sephadex LH–20.

Column chromatography. (*cf.* Widman & others, 1974). Separation of metabolites from lung and liver tissue was accomplished on Florisil (50 g). The following eluents of 250 ml were used: 20% v/v diethyl ether in light petroleum; 50% diethyl ether in light petroleum; 100% diethyl ether; 5% methanol in diethyl ether; and 100% methanol. The elution rate was 55 ml h^{-1}.

The extracts of the plasma and all the fractions from the Florisil columns eluted with 50% diethyl ether in light petroleum, 100% diethyl ether and some fractions eluted with 5% methanol in diethyl ether were further purified on Sephadex LH–20 (1 × 70 cm). The column was eluted with light petroleum–chloroform–ethanol (10:10:1). Fractions of 2 ml were collected at a rate of 10 ml h^{-1}.

Thin-layer chromatography. Δ¹-THC and its metabolites were analysed and purified on pre-coated Silica gel F plates (Merck) using the following systems: System A—solvent: diethyl ether–light petroleum (1:19), developed 2–3 times; System B—solvent: diethyl ether–light petroleum (1:4). Plates impregnated with 20% dimethylformamide in acetone. System C—solvent: diethyl ether–light petroleum (2:3). System D—solvent: diethyl ether–light petroleum (7:3). Spray reagent: 0·2% Fast Blue B salt (Merck) in 2N NaOH.

Liquid scintillation counting. Determination of radioactivity was carried out as reported earlier (Jones & others, 1974). Total plasma radioactivity was counted in Insta-Gel (Packard). Blood and tissue homogenates were solubilized before counting as described by Jones & others (1974).

Gas chromatography-mass spectrometry. For mass spectrometry (m.s.) an LKB 9000 gas chromatograph–mass spectrometer was used (2% SE–30 column Gas-Chrom Q, 210° 70 eV). To identify small amounts of side-chain hydroxylated metabolites mass spectra were also recorded for their trimethylsilyl ethers (*cf.* Binder,

Agurell & others, 1974). Conventional gas chromatography (g.l.c.) was carried out on a 2% SE-30 column/Gas-Chrom Q at 230° and 250°.

RESULTS

As the results from the duplicate experiments were similar, the figures will be given from one only.

Lung plasma metabolites. After 2 h of perfusion 89% of the radioactivity present in the plasma was extracted into light petroleum and 2% into diethyl ether. Unchanged Δ^1-THC counted for 88% of total radioactivity extracted. When the extracts were subjected to Sephadex LH–20, similar elution patterns were found for both extracts. The elution of radioactivity (petroleum extract) was as shown in Fig. 2A.

Fraction 1 was shown by t.l.c. (system C) to contain a mixture of metabolites contaminated with lipids and not further investigated. Fraction 2 was identified as unchanged Δ^1-THC by comparison with reference Δ^1-THC on t.l.c. systems A and B. Fraction 3 was shown by g.l.c. and t.l.c. (system B) to contain cannabinol (CBN) which could have originated as an impurity of the administered Δ^1-THC.

Fraction 4 was found to contain two compounds (I and II, Fig. 1) identified as 3″-hydroxy-Δ^1-THC and 4″-hydroxy-Δ^1-THC. The compounds were separated and purified by t.l.c. (system D).

3″-Hydroxy-Δ^1-THC (I): R_F (t.l.c. system D) 0·47; g.l.c. retention time 0·78 relative to 7-hydroxy-Δ^1-THC; m.s. *m/e* 330 (M^+, 19%), 315 (10), 258 (100), 247 (15), 243 (12) and 190 (6). Elution volume on Sephadex LH–20, t.l.c. and g.l.c. properties, as well as the mass spectrum (Binder & others, 1974) were close to those found for synthetic 3″-hydroxy-Δ^6-THC (Leander & Agurell, to be published).

4″-Hydroxy-Δ^1-THC (II): R_F (t.l.c. system D) 0·41; g.l.c. retention time 0·80 relative to 7-hydroxy-Δ^1-THC; m.s. *m/e* 330 (M^+, 87%), 315 (87), 287 (45), 258 (24),

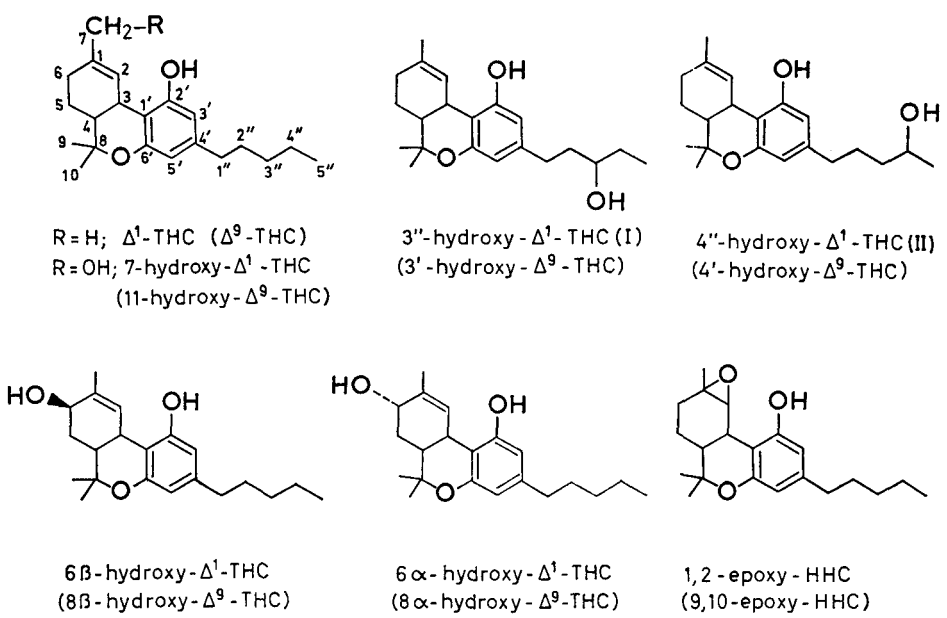

FIG. 1. Structures of Δ^1-THC and isolated metabolites. The monoterpenoid (Δ^1-THC) system of nomenclature is used in this paper; the dibenzopyran system (Δ^9-THC) is given in parentheses.

257 (21), 247 (100) and 243 (34). When silylated the following mass spectrum was obtained *m/e* 474 (M^+, 100%), 459 (67), 391 (29), 330 (75), 315 (21) and 117 (83).

Fraction 5 contained 7-hydroxy-Δ^1-THC and traces of 6β-hydroxy-Δ^1-THC and 6α-hydroxy-Δ^1-THC (Fig. 1). 7-Hydroxy-Δ^1-THC was identified by t.l.c., g.l.c., and m.s. comparison with an authentic sample. R_F values in t.l.c. systems C (developed twice) and D were 0·25 and 0·44, respectively. 6β-Hydroxy-Δ^1-THC and 6α-hydroxy-Δ^1-THC were identified by t.l.c. comparison with reference compounds using two systems. In system C (developed twice) the R_F's were 0·53 and 0·31, respectively, whereas in system D they were 0·57 and 0·43.

Lung tissue metabolites. When the lung tissue was extracted 76% of the radioactivity was recovered in light petroleum and 11% in diethyl ether. Unchanged Δ^1-THC was present in both extracts and made up for about 90% of all activity extracted from lung tissue. On the Florisil column, the mono-oxygenated metabolites (both extracts) were mainly distributed between the fractions eluted with 50% diethyl ether in light petroleum and 100% diethyl ether. The purification on Sephadex LH–20 of the 100% ether fraction from Florisil (ether extract) is shown in Fig. 2B.

Fraction 6 was isolated and identified a 3″-hydroxy-Δ^1-THC and 4″-hydroxy-Δ^1-THC.

Fraction 7 contained, like the corresponding fraction of the plasma extract (Fig. 2A), 7-hydroxy-Δ^1-THC and traces of 6β-hydroxy-Δ^1-THC and 6α-hydroxy-Δ^1-THC. There was an overlap of metabolites between the fractions on Florisil.

Liver metabolites. Of total activity present in the liver incubate 60% was extracted with light petroleum and 24% with diethyl ether. Metabolites were found in both extracts, and the overall conversion was about 25%. After Florisil chromatography

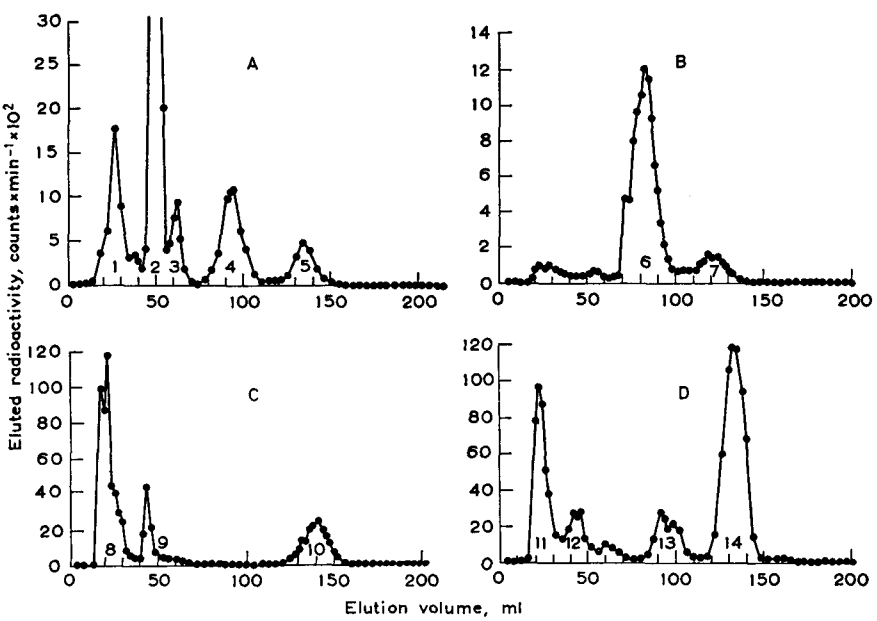

FIG. 2. Elution pattern on Sephadex LH–20 of metabolites of Δ^1-THC extracted from lung and liver tissues of dog (A) petroleum extract of lung circuit plasma; (B) 100% ether fraction (Florisil) of the ether extract of lung tissue; (C) 50% ether in petroleum fraction (Florisil) of the ether extract of liver; (D) 100% ether fraction (Florisil) of the ether extract of liver.

mono-oxygenated metabolites were mainly present in the fractions eluted with 50% diethyl ether in light petroleum and 100% diethyl ether.

The 50% ether in petroleum fractions of both petroleum and ether extracts showed similar elution patterns on Sephadex LH–20 (see Fig. 2C).

Fraction 8 contained a mixture of metabolites contaminated with lipids.

Fraction 9 was identified as 1,2-epoxy-hexahydrocannabinol (1,2-epoxy-HHC). R_F (t.l.c. systems C and D) 0·44 and 0·78; g.l.c. retention time 0·61 relative to 7-hydroxy-Δ^1-THC (silylated compounds); m.s. *m/e* 330 (M^+, 67%), 315 (48), 312 (24), 297 (24), 287 (52), 274 (93), 259 (100), 246 (79), 231 (50) and 193 (83). The metabolite showed the same elution volume on Sephadex LH–20 as synthetic 1,2α-epoxy-HHC. T.l.c. and g.l.c. properties as well as mass spectral data of the metabolite were also in agreement with those of 1,2α-epoxy-HHC (Sikemeier, 1968; Ohlsson & Leander, unpublished results).

Fraction 10 was identified as 6β-hydroxy-Δ^1-THC. R_F [t.l.c. systems C (developed twice) and D] 0·53 and 0·65; g.l.c. retention time 0·71 relative to 7-hydroxy-Δ^1-THC; m.s. *m/e* 330 (M^+, 37%), 312 (47), 297 (100), 295 (29), 271 (95), 257 (21), 231 (23) and 214 (46). The structure of the metabolite was confirmed by t.l.c., g.l.c., and m.s. comparison with an authentic sample.

When the 100% ether fraction from Florisil (ether extract) was purified on Sephadex, the pattern was as shown in Fig. 2D.

Fraction 11 was a mixture of metabolites contaminated with lipids.

Fraction 12 contained 1,2-epoxy-HHC.

Fraction 13 was identified as 3″-hydroxy-Δ^1-THC and 4″-hydroxy-Δ^1-THC.

Fraction 14 was found to contain three major compounds identified as 6β-hydroxy-Δ^1-THC, 6α-hydroxy-Δ^1-THC, and 7-hydroxy-Δ^1-THC.

The structures of 6β-hydroxy-Δ^1-THC and 7-hydroxy-Δ^1-THC were verified by comparison with references using t.l.c., g.l.c., and m.s. as described earlier (fractions 5 and 10).

6α-Hydroxy-Δ^1-THC was identified by t.l.c., g.l.c., and m.s. comparison with a reference compound. For t.l.c. properties, see fraction 5. G.l.c. gave a retention time of 0·73 relative to 7-hydroxy-Δ^1-THC and m.s. was in agreement with published data (Jones & others, 1974).

DISCUSSION

Side-chain hydroxylated metabolites. 1″-Hydroxy-Δ^6-THC, 2″-hydroxy-Δ^6-THC, 3″-hydroxy-Δ^6-THC and 5″-hydroxy-Δ^6-THC were synthesized (Leander, Agurell & others, unpublished results) and compared with the isolated metabolites I and II (Fig. 1) by t.l.c., g.l.c., and m.s.

By t.l.c. (system D) the R_F values of the metabolites I (0·47) and II (0·41) were close to 3″-hydroxy-Δ^6-THC (0·51) and 5″-hydroxy-Δ^6-THC (0·39).

G.l.c. gave a retention time for 1″-hydroxy-Δ^6-THC of 10·30 min, for 2″-hydroxy-Δ^6-THC 9·85 min, for 3″-hydroxy-Δ^6-THC 11·45 min, for 5″-hydroxy-Δ^6-THC 15.21 min, for metabolite I 12·50 min and for metabolite II 12·70 min.

The mass spectra of the metabolites (I and II) were compared with those of the synthetic compounds. All spectra showed the presence of the fragment *m/e* 247 which implied that there was a hydroxyl group located in the pentyl side chain (Budzikiewicz, Alpin & others, 1965).

Metabolite I and 3″-hydroxy-Δ^6-THC showed similar mass spectra with the base

peak at *m/e* 258 ($M^+ - C_4H_8O$). The mass spectra of silylated 3″-hydroxy-Δ⁶-THC and metabolite I, showing a major fragment *m/e* 330 ($M^+ - 144$), were in agreement (*cf.* Binder & others, 1974). Thus, the structure of metabolite I was determined to be 3″-hydroxy-Δ¹-THC.

Metabolite II gave a mass spectrum similar to that of 5″-hydroxy-Δ⁶-THC with the base peak at *m/e* 247. On the other hand, their trimethylsilyl ethers could be distinguished from each other. The presence of the fragment *m/e* 117 (α-cleavage between C–3″ and C–4″) in metabolite II showed hydroxylation of the metabolite in 4″-position (*cf.* Binder & others, 1974). After comparison (m.s., g.l.c., t.l.c.) with side-chain hydroxylated compounds of Δ⁶-THC, metabolite II was determined to be 4″-hydroxy-Δ¹-THC.

The synthetic side-chain hydroxylated compounds (1″-, 2″-, 3″-, and 5″-hydroxy-Δ⁶-THC) have been tested in rhesus monkeys for THC activity. 1″-Hydroxy-Δ⁶-THC was found to be essentially inactive while the other three compounds were shown to be as active as Δ⁶-THC (Mechoulam & others, 1975). Thus, one can assume 3″-hydroxy-Δ¹-THC and 4″-hydroxy-Δ¹-THC to be active metabolites.

Maynard, Gurny & others (1971) reported the structures of two metabolites with the hydroxylation in the 1″- and 3″-positions of the pentyl side chain of Δ⁶-THC using a dog (beagle) liver supernatant. Side-chain hydroxylated compounds of CBN have been found using rat and rabbit liver (Wall, 1971; Widman, Dahmén & others, 1975). Cannabidiol hydroxylated in the 3″-position has also been isolated using a rat liver supernatant (Nilsson, Agurell & others, 1973).

Comparison of metabolites formed by lung and liver. Lung tissue and plasma showed similar metabolite patterns. Two major metabolites were found, identified as 3″-hydroxy-Δ¹-THC and 4″-hydroxy-Δ¹-THC. After purification they accounted for 14 and 9%, respectively, of all metabolites extracted from lung and circuit plasma. 7-Hydroxy-Δ¹-THC yielded 5%, and 6α- and 6β-hydroxy-Δ¹-THC accounted for about 1% each.

The metabolites of Δ¹-THC formed by the isolated perfused dog lung were also produced by dog liver supernatant but in different proportions. The major metabolites formed by the liver were 6β-hydroxy-Δ¹-THC (8%) and 6α-hydroxy-Δ¹-THC (6%). 7-Hydroxy-Δ¹-THC made up for 2% while 3″-hydroxy-Δ¹-THC and 4″-hydroxy-Δ¹-THC accounted for about 1% each of total metabolites. In addition, 1,2-epoxy-HHC (2%) was produced by the liver.

Nakazawa & Costa (1971) suggested a qualitative difference between the lung and liver metabolism of Δ¹-THC in the rat. Our experiment, using dog, showed that the metabolites produced by the lung were also formed by the liver. The differences in metabolism between the two tissues were quantitative rather than qualitative according to the metabolites formed.

A high concentration of Δ¹-THC and its metabolites in lung tissue has been reported both after inhalation and intravenous administration (Agurell, Nilsson & others, 1970; Ho, Fritchie & others, 1970; Klausner & Dingell, 1971; Kennedy & Waddell, 1972; Freudenthal, Martin & Wall, 1972; Ryrfeldt, Ramsay & others, 1973; Willinsky, Kalant & others, 1974). As our study showed a significant metabolism of Δ¹-THC to several mono-hydroxylated compounds and since inhalation is a major route of cannabis administration, one has to consider the contribution of the lung to the overall metabolism of Δ¹-THC. The contribution of the metabolites formed by the lung towards pharmacological activity of Δ¹-THC in the dog is difficult to assess.

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