Biochemical Pharmacology, Vol. 23, pp. 1163–1172, Pergamon Press, 1974. Printed in Great Britain.

BILIARY EXCRETION OF Δ^1-TETRAHYDROCANNABINOL AND ITS METABOLITES IN THE RAT*

MARIANNE WIDMAN, MARIANNE NORDQVIST, STIG AGURELL, JAN-ERIK LINDGREN† and FINN SANDBERG

Faculty of Pharmacy, Box 6804, S-113 86 Stockholm, Sweden

(*Received* 11 *July* 1973; *accepted* 15 *September* 1973)

Abstract—Δ^1-Tetrahydrocannabinol (Δ^1-THC) administered i.v. (1 mg/kg) to anaesthetized rats with cannulated bile ducts is rapidly eliminated as metabolites in the bile, 60–70 per cent in 6 hr. In comparison with the slow excretion via faeces an extensive enterohepatic circulation, which may be of toxicological importance, is indicated.

Unchanged Δ^1-THC and cannabinol are eliminated in low amounts (0·05–0·1 per cent) in the bile. A few per cent consists of two or more mono-oxygenated metabolites, neither of which is identical with 7-hydroxy-Δ^1-THC or 6-β-hydroxy-Δ^1-THC. The main part of the non-conjugated metabolites is present as compounds more polar than 7-hydroxy-Δ^1-THC and as carboxylic acids. These acids were more polar than Δ^1-THC-7-oic acid which could not be identified in a free or conjugated form.

In the rat about 60 per cent of the metabolites are eliminated as water-soluble conjugates. Hydrolysis with glucuronidase liberated aglycones which were mainly neutral whereas hydrolysis with alkali released neutral but also some acidic compounds. 7-Hydroxy-Δ^1-THC was identified as an aglycone of glucuronic acid and furthermore, three mono-oxygenated cannabinoids were isolated after hydrolysis and partially characterized.

PREVIOUS studies have shown that the psychotomimetically active compound (−)-Δ^1-tetrahydrocannabinol (I a; Δ^1-THC) is slowly and almost exclusively excreted via faeces in rats.[1–3] In man metabolites of Δ^1-THC are also predominantly eliminated through faeces.[4] The present study was undertaken to investigate the biliary excretion of Δ^1-THC in the rat after i.v. administration and to characterize the metabolic pattern.

EXPERIMENTAL

(−)-Δ^1-THC-1″-^{3}H was diluted with non-labelled material to a sp. act. of 1·3 μCi/mg.[5] It showed a chemical and radiochemical purity greater than 95 per cent according to TLC (System A and B) and GLC. The biological and chemical stability of the label in the side chain was satisfactory.[6]

Male rats (Sprague–Dawley, 300–350 g) were anaesthetized with pentobarbital (60 mg/kg i.p.). The bile duct was surgically exposed by a midline incision and cannulated with a polyethylene tubing (PE-10). The rectal temperature was maintained at 38° ± 1° with a heating lamp to prevent alteration in biliary flow due to hypothermia. Δ^1-THC-^{3}H dissolved in 50 μl of 70 per cent aqueous ethanol was injected into the femoral vein (1 mg/kg) and the bile was collected up to 6 hr after administration.

* Metabolism of Cannabis XX.

† Department of Toxicology, Swedish Medical Research Council, Karolinska Institute, S-104 01 Stockholm 60, Sweden.

Thin-layer chromatography. The following thin-layer systems were used:

System A. Silica gel GF plates impregnated with 20 per cent dimethylformamide in acetone. Solvent: diethyl ether–light petroleum (1:4).

System B. Silica gel GF plates. Solvent: diethyl ether–light petroleum (1:19) developed three times.

System C. Silica gel GF plates. Solvent: diethyl ether–light petroleum (1:1).

System D. Silica gel GF plates. Solvent: diethyl ether–light petroleum (7:3).

System E. Silica gel GF plates. Solvent: methanol–diethyl ether (1:99), developed twice.

System F. Silica gel GF plates. Solvent: acetone–chloroform (7:13).

System G. Aluminium oxide plates. Solvent: chloroform.

System H. Aluminium oxide plates. Solvent: methanol–chloroform (1:99), developed one time.

System I. Aluminium oxide plates. Solvent: methanol–chloroform (1:99), developed 3–4 times.

Determination of radioactivity. Radioactivity was assayed in a Packard Tri-Carb Model 3375 spectrometer with external standardization. To determine the excretion rate of Δ^1-THC and its metabolites bile samples of 50 μl from each collection period were mixed with 1 ml of water before adding scintillation medium (Instagel®, Packard Instrument Co.).

Excretion rate of Δ^1-THC. Five male rats were injected i.v. with Δ^1-THC-^{3}H (1 mg/kg) as described above. Bile samples were taken at 5, 10, 15, 30, 60, 90, 120 min and then at hourly intervals for the next four hours for determination of excreted radioactivity as shown by Fig. 1.

Preparative isolation of metabolites. The isolation and separation of metabolites are outlined in Figs. 2 and 3. Bile (Fig. 2; 2–1) was collected up to 6 hr from 25 rats injected i.v. with Δ^1-THC-^{3}H (1 mg/kg). After adjusting to pH 6 by addition of phos-

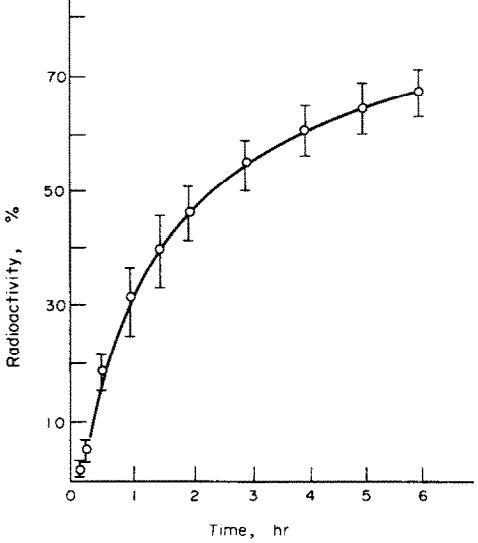


FIG. 1. Cumulative excretion of radioactivity in the bile after i.v. administration of Δ^1-THC-^{3}H to rats (1 mg/kg). Mean values + S.D. are given from five rats.

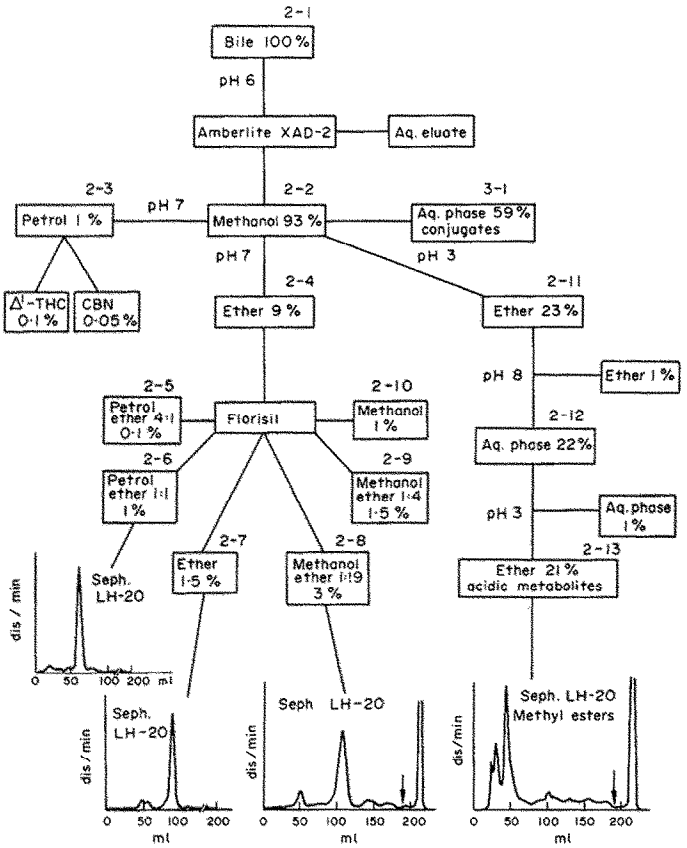
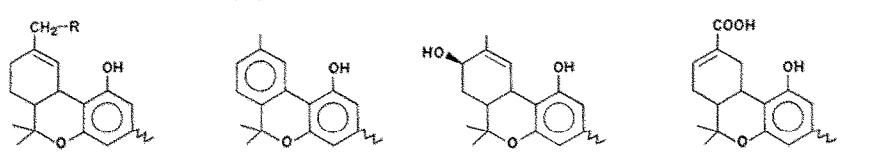


FIG. 2. Scheme for the separation of "non-conjugated" metabolites from the bile of rats. Δ^1-THC-^{3}H (1 mg/kg) was administered i.v. Changes of eluants are marked by arrows. In this and the following figure the abbreviation "petrol" refers to light petroleum (b.p. 40–60°).

phoric acid, the pooled bile (4·3 mg metabolites) was passed through an Amberlite XAD-2 column (2 × 100 cm). Inorganic materials were removed with 500 ml of water and the metabolites were eluted with 500 ml of methanol.

"*Non-conjugated metabolites*" (*Fig.* 2). The methanol fraction (2–2) was evaporated *in vacuo* and the residue dissolved in 30 ml of a phosphate buffer pH 7. This aqueous phase was first extracted (2–3) with 3 × 30 ml of light petroleum (b.p. 40–60°) and then with 3 × 30 ml of diethyl ether (2-4). Finally the water phase was adjusted to pH 3 and extracted with diethyl ether (3 × 60 ml) to remove less polar acids (2-11).

The light petroleum extract (2-3) was chromatographed on TLC[7] (System A) to confirm the presence of unchanged Δ^1-THC (I a) and cannabinol (II; CBN). Δ^1-THC and CBN were isolated by preparative TLC (System B).



(Ia) R = H; Δ^1-THC
(Ib) R = OH; 7-hydroxy-Δ^1-THC
(II) CBN
(III) 6β-hydroxy-Δ^1-THC
(IV) $\Delta^{1(6)}$-THC-7-oic acid

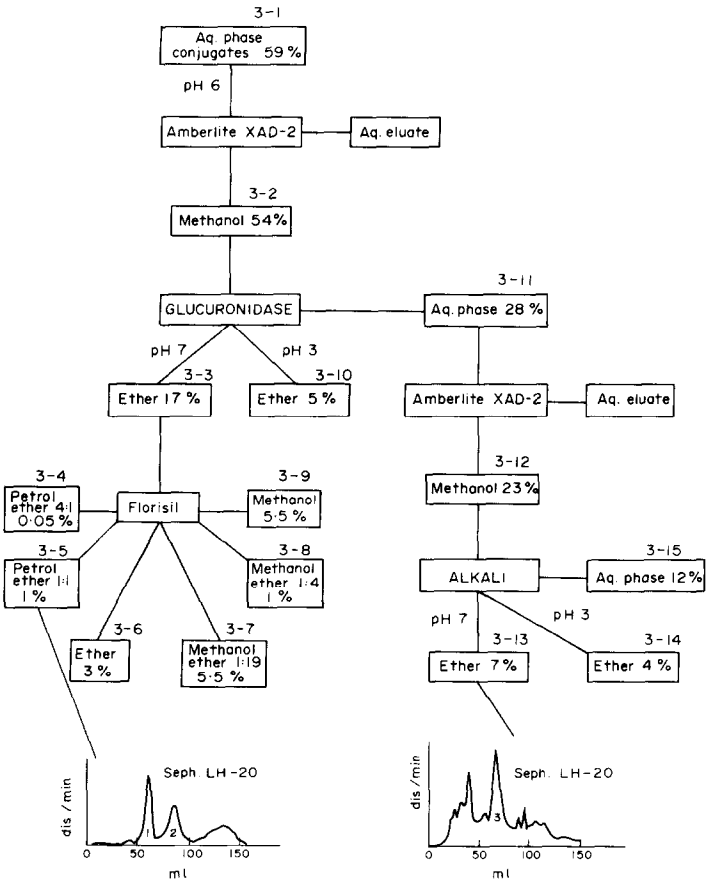


FIG. 3. Scheme for the separation of biliary metabolites liberated by enzymatic and or alkaline hydrolysis.

The ether extract (2–4) was chromatographed on a Florisil column (1 × 18 cm) eluting with 50 ml volumes of light petroleum–diethyl ether and solvents of increasing polarities as shown in Fig. 2. Fractions 2-6, 2-7 and 2-8 were separately chromatographed on Sephadex LH-20 (1 × 70 cm) with light petroleum–chloroform–absolute ethanol (10:10:1) as eluant.[8] To elute the total radioactivity of fraction 2-8 the eluant was changed to methanol–chloroform (1:1). Fractions of 2 ml were collected and an aliquot of each fraction was subjected to liquid scintillation counting.

The ether extract (2-11) containing acids was reextracted into 5 per cent aqueous sodium bicarbonate (2-12) followed by acidification and renewed transfer into ether (2-13). The acids were esterified with diazomethane and chromatographed on a Sephadex LH-20 column as described.

"*Conjugated metabolites*" (*Fig.* 3). The remaining aqueous phase (3-1) containing the more water soluble metabolites was passed (pH 6) through an Amberlite XAD-2 column to remove buffer salts. The methanol fraction (3-2) containing the metabolites was evaporated and dissolved in water. After buffering to pH 3·8 by addition of citrate–phosphate buffer, the aqueous phase was incubated with 280 mg β-glucuronidase type L-1 (900 units/mg, Sigma Chemical Co.) for 19 hr at 37°. Sulphatase

was inhibited by phosphate. Then the incubation mixture was buffered to pH 7 and extracted (3-3) with diethyl ether (3 × 50 ml). To remove acidic aglycones (3-10) the pH was adjusted to pH 3 followed by extraction with diethyl ether (3 × 100 ml). The combined ether extracts of the neutral aglycones (3-3) were evaporated to a small volume and chromatographed on a Florisil column (3-4 to 3-9) eluting with solvents of increasing polarities as shown in Fig. 3. Fractions 3-5 and 3-6 were further chromatographed on Sephadex LH-20 as described.

The remaining aqueous phase (3-11) was freed from buffer salts by purification on an Amberlite XAD-2 column, dissolved in a small volume of methanol and refluxed with 0·2 M sodium hydroxide in methanol for 2 hr under a stream of nitrogen.[9] After removal of methanol *in vacuo* the residue was dissolved in buffer and extracted at pH 7 (3-13) and pH 3 (3-14) with diethyl ether. The neutral aglycones (3-13) released by alkali were chromatographed on a Sephadex LH-20 column.

Mass fragmentographic identification. Mass fragmentography, as described by Hammar and Hessling,[10] was used to identify unchanged Δ^1-THC and some of its metabolites.

An LKB model 9000 gas chromatograph-mass spectrometer (LKB-Produkter, Bromma, Sweden) was used. Columns: 3% OV-17/Gas Chrom Q, 230° and 3% SE-30 Ultraphase/Gas Chrom Q, 230°.

RESULTS AND DISCUSSION

Previous studies have shown that Δ^1-THC is almost completely excreted as more polar metabolites in urine and faeces in the rat.[1-3] Also in man Δ^1-THC appears to be completely metabolized and, as in the rat, mainly eliminated via faeces.[4] The present study was designed to investigate the rate of elimination of Δ^1-THC and its metabolites by the liver into the bile as well as the pattern and possibly the identities of metabolites present in the bile.

Figure 1 shows the biliary excretion of Δ^1-THC and metabolites after i.v. injection (1 mg/kg) in five rats. The elimination was most rapid during the 1st hr after injection and within 2–3 hr 50 per cent of the dose was excreted. A number of experiments indicated that the elimination rate and metabolite pattern remained constant over a wide dose range (0·05–3 mg/kg). Pronounced biliary excretion of metabolites of Δ^1-THC in rat after i.p. administration was also found by Joachimoglu *et al.*[1]

Previously we have reported[2] that about half of the amount of Δ^1-THC and its metabolites are still present in the rat one week after i.v. administration of Δ^1-THC. Klausner *et al.*[3] likewise showed that only about 60 per cent of i.v. injected Δ^1-THC is excreted in urine and faeces in 5 days. In comparison with our biliary excretion data this indicates a pronounced enterohepatic circulation. This suggests that polar metabolites are repeatedly reabsorbed after elimination through the bile and indeed it has been shown that such metabolites occur at high levels both in blood and tissues.[6] These findings might be of considerable toxicological importance.

Metabolite pattern

Δ^1-THC is, as discussed later, almost entirely excreted as a variety of neutral, acidic and conjugated metabolites. The separation systems used for these metabolites and the percentage in each fraction of the total amount present in the bile are shown

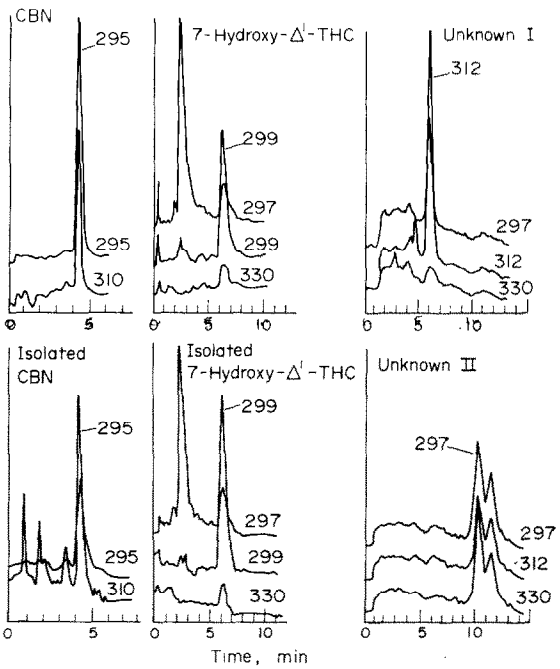


FIG. 4. Mass fragmentograms obtained from reference CBN and isolated CBN, reference 7-hydroxy-Δ^1-THC and the isolated aglycone 7-hydroxy-Δ^1-THC. The mass fragmentogram of Unknown I refers to peak 1 and Unknown II to peak 3 in Fig. 3.

in Fig. 2 ("non-conjugated metabolites") and Fig. 3 ("conjugated metabolites"). Here, also the designated fraction numbers are shown.

"*Non-conjugated metabolites*"

Neutral metabolites. The light petroleum extract (2-3) contained 1 per cent and the ether extract (2-4) 9 per cent of the total radioactivity excreted into the bile.

Small amounts of unchanged Δ^1-THC (I a; 0·1 per cent) and CBN (II; 0·05 per cent) were found in extract 2-3 according to TLC (System A). After purification by TLC (System B) the identities of Δ^1-THC and CBN (Fig. 4) were verified by mass fragmentography by comparison with synthetic compounds. The ion detector was used to record exclusively the intensities of the following three fragments in the mass spectrum of Δ^1-THC: *m/e* 314 (M^+), 299 and 271. Only two fragments were used for CBN: *m/e* 310 (M^+) and 295. In addition to TLC confirmation, both GLC retention times and the partial mass spectra of Δ^1-THC and CBN present in bile were in agreement with those of reference compounds.

The present results show that Δ^1-THC is indeed eliminated unchanged through the bile, although in very small amounts (about 0·1 per cent of given dose). However, Δ^1-THC is efficiently absorbed after oral administration[4] and the amounts present in faeces may therefore be expected to be even lower. CBN was identified as a metabolite and although it could conceivably occur at a low level (<1 per cent) in the administered Δ^1-THC, the similar concentrations of Δ^1-THC and CBN in the bile make it unlikely that contaminating CBN could significantly contribute to the CBN occurring in the bile. Neither is it plausible that CBN originates by microbial dehydrogenation of Δ^1-THC in the gut although our previous study[6] indicated that small

amounts of Δ¹-THC and metabolites are excreted in the mouse by the secretory part of stomach. Thus, CBN must be assumed to be a metabolic product of Δ¹-THC formed by dehydrogenation of Δ¹-THC and/or dehydration of oxygenated intermediates.

The "neutral" ether extract (2-4) was chromatographed on a Florisil column (Fig. 2). Fraction 2-6 showed one major peak at a retention volume of 60 ml when chromatographed on a Sephadex LH-20 column. This peak had a R_f value similar to that of 6β-hydroxy-Δ¹-THC (III) on TLC (System H). By mass fragmentography the three major ions (*m/e* 330 M^+, 312 and 297) in the mass spectrum of 6β-hydroxy-Δ¹-THC were recorded. The isolated compound, however, showed a GLC retention time (5·8 min) longer than that of 6β-hydroxy-Δ¹-THC (4·2 min). The intensities of the mass numbers of the two compounds were also different.

Fraction 2-7 was subjected to chromatography on a Sephadex LH-20 column. No detectable amount of 7-hydroxy-Δ¹-THC (I b) was found, but a major peak was observed with a smaller retention volume than 7-hydroxy-Δ¹-THC. According to TLC (System D) this isolated peak had a R_f value of 0·2, which was lower than that of 7-hydroxy-Δ¹-THC (0·4).

When fraction 2-8 was chromatographed on Sephadex, one major peak was found before the eluant was changed. This peak had a rentention volume close to that of the peak in fraction 2-7. TLC (System I) of the former peak revealed at least two metabolites, one with the same R_f value as the peak in fraction 2-7. The remaining radioactivity of fraction 2-8 as well as 2-9 and 2-10 contained more polar compounds according to TLC (System D and F).

In summary, non-conjugated 7-hydroxy-Δ¹-THC could not be identified in bile as a metabolite of Δ¹-THC in spite of the fact that it is a major metabolite of Δ¹-THC *in vitro* both in the rat[11] and in other species.[12,13] However, as judged by mass fragmentography, polarity on TLC and Florisil column at least two other mono-oxygenated metabolites of Δ¹-THC are eliminated in the bile of the rat.

Acidic metabolites. The "acidic" ether extract (2-11) contained 23 per cent of the total radioactivity excreted into the bile. After the purification step with 5 per cent sodium bicarbonate there remained 21 per cent of the activity. After methylation with diazomethane the acids were separated on a Sephadex LH-20 column. The elution pattern (Fig. 2) showed at least three major compounds before the change to the more polar eluant (methanol–chloroform, 1:1). No more than 0·1 per cent, if any, of the total excreted activity could be due to Δ¹-THC-7-oic acid as evidenced by TLC (System C; the compounds were methylated with diazomethane) with synthetic Δ¹⁽⁶⁾-THC-7-oic acid (IV) as reference compound. This is in agreement with an earlier study in the rabbit[14] where Δ¹-THC-7-oic acid could not be identified as a urinary metabolite of Δ¹-THC.

"*Conjugated metabolites*"

The aqueous phase 3-1 containing 59 per cent of total excreted activity was submitted to hydrolysis by enzymes and alkali. Table 1 shows the results of successive treatments of conjugates with sulphatase, glucuronidase and alkali in varying sequences. Small amounts, if any, of the conjugates were present as sulphates.* β-

* The designation as sulphate or glucuronide is based solely on the sensitivity of conjugates to corresponding enzymatic cleavage and their water solubility.

TABLE 1. PERCENTAGE OF CONJUGATED METABOLITES EXTRACTABLE WITH ETHER AT pH 7 AND pH 3 AFTER SUCCESSIVE TREATMENTS WITH ENZYMES AND ALKALI

Cleavage by enzymes and alkali	pH 7	pH 3	Cleavage by enzymes and alkali	pH 7	pH 3
Control	6	6	Sulphatase† Followed by	7	5
β-Glucuronidase* Followed by	29	8	β-Glucuronidase* Followed by	25	5
0·2 M NaOH	12	7	0·2 M NaOH	17	8
Cleavage by enzymes and alkali	pH 7	pH 3	Cleavage by enzymes and alkali	pH 7	pH 3
β-Glucuronidase‡ Followed by	24	7	0·2 M NaOH Followed by	27	14
0·2 M NaOH	21	8	β-Glucuronidase‡	12	3

The metabolites were purified on an Amberlite XAD-2 column between treatments. All percentage figures are mean values of two to four experiments, which gave very similar results. All enzymes were supplied by Sigma Chemical Co.

* β-Glucuronidase type L-1. Citrate–phosphate buffer pH 3·8

† Sulphatase type III. Citrate buffer pH 5.

‡ β-Glucuronidase type 1. Phosphate buffer pH 7.

Glucuronidase type L-1 split some 30 per cent of the conjugates and liberated mainly neutral aglycones. Alkaline hydrolysis released a similar amount of neutral metabolites as β-glucuronidase and also some bound acidic metabolites. Less than 0·01 per cent of Δ^1-THC or CBN was released on hydrolysis.

Neutral aglycones released by β-glucuronidase. The proportions of the neutral aglycones released by β-glucuronidase type L-1 after separation on a Florisil column are shown in Fig. 3.

Fraction 3-5 gave three peaks on Sephadex LH-20. Peak 1 (Fig. 3) moved like 6β-hydroxy-Δ^1-THC (III) on TLC (System H). Peak 2 had slightly higher R_f value than 7-hydroxy-Δ^1-THC as evidenced by TLC (System H). Peak 1 and 2 were also submitted to mass fragmentography. The mass fragmentogram of peak 1 (Fig. 4, unknown I) was compared to that of 6β-hydroxy-Δ^1-THC (*m/e* 330 M$^+$, 312 and 297). The retention time of peak 1 was longer and the intensities of the mass numbers were different from those of 6β-hydroxy-Δ^1-THC. Peak 2 gave mass fragmentograms with the mass numbers *m/e* 330, 299, 297 and 247. The presence of the fragment *m/e* 247 implied that a hydroxyl group was located in the pentyl side chain.[15] According to TLC and mass fragmentography these two peaks appeared to be mono-oxygenated Δ^1-THC derivatives. Furthermore, peak 2 was probably a side chain hydroxylated compound.

Fraction 3-6 gave at least two major peaks on the Sephadex LH-20 column before the change to the more polar eluant. One of these peaks with a retention volume of 135 ml consisted to more than 70 per cent of 7-hydroxy-Δ^1-THC (I b) as evidenced by two different TLC systems (System D and G). The mass fragmentogram of this peak (Fig. 4) was in agreement with that of 7-hydroxy-Δ^1-THC (*m/e* 330 M$^+$, 312 and 299).

The fractions 3-7, 3-8 and 3-9 gave on TLC (System E and F) indications of at least four metabolites more polar than 7-hydroxy-Δ^1-THC.

Neutral compounds released by alkali. Neutral compounds released by alkali were separated on Sephadex LH-20 (Fig. 3). Peak 3 had the same mobility as 6β-hydroxy-Δ^1-THC on TLC (System H) but was shown to be two other metabolites according to mass fragmentography (*m/e* 330, 312 and 297, Fig. 4, Unknown II). Both compounds had retention times longer (10·4 min and 11·4 min) than that of 6β-hydroxy-Δ^1-THC (4·1 min).

Acidic metabolites released by β-glucuronidase/alkali (3-10, 3-14). None of the liberated acidic metabolites behaved like $\Delta^{1(6)}$-THC-7-oic acid (IV) on TLC (System C; the compounds were methylated with diazomethane). The latter compound was identified in bound form as a minor metabolite of 7-hydroxy-$\Delta^{1(6)}$-THC in the rabbit.[16] Methylated acidic aglycones (3-10) released by glucuronidase showed a similar pattern as the free acidic metabolites on Sephadex LH-20.

CONCLUSIONS

The following main conclusions may be drawn from the present experiment, previous literature reports and the recent study of Turk *et al.*[17] The results show[2,3,17] that in the rat, the main route of elimination is via the faeces and that excretion is slow with some 60 per cent eliminated in 4 days,[17] 5 days[3] or 50 per cent in 1 week.[2] The main excretion is by way of the bile as also found by Turk *et al.*[17] and Joachimoglu *et al.*[1] The latter authors recovered about 30 per cent of the given dose in the bile of bile duct cannulated rats during 6 hr after i.p. administration of Δ^1-THC. Turk and co-workers[17] found 37 per cent during the same time after i.v. injection of Δ^1-THC to non-anaesthetized bile duct cannulated rats.

Our figures (60–70 per cent after i.v. administration to anaesthetized rats) are thus considerably higher, which may be due to different experimental design. Although the metabolism of Δ^1-THC under the present experimental conditions might differ from that in an intact animal, available data imply a pronounced biliary excretion.

Further there is general agreement that the Δ^1-THC metabolites occurring in the bile are polar metabolites. We have identified by TLC and mass fragmentography small amounts of Δ^1-THC and CBN in the bile and somewhat larger amounts of at least four monooxygenated metabolites of Δ^1-THC. 7-Hydroxy-Δ^1-THC—which, on the basis of TLC was assumed to occur in a free form in the bile[17]—was identified only after hydrolysis of the water-soluble conjugates. "Diacetyl-Δ^1-THC" the fully acetylated derivative of 7-hydroxy-Δ^1-THC, which was suggested[18] as a possible metabolite in bile was not identified in the present study. The predominant compounds in the bile are acids and water-soluble conjugates.

The elimination of metabolites in the bile is apparently not the rate-limiting step in the slow excretion of metabolites and thus it appears that metabolites are repeatedly reabsorbed after elimination in the bile. During such an enterohepatic circulation, the drug would be exposed to the intestinal microflora. The importance of these factors, reabsorption and microbial conversion of metabolites, needs further clarification. The recirculation is probably of more significance for the toxicological effects of the drug than for the prolongation of its psychotomimetic action.

Acknowledgements This work was supported by the Swedish Medical Research Council (03X-2724) and Försvarsmedicinska forskningsdelegationen and by the following grants to the department of toxicology: Swedish Medical Research Council 70E-3743, 14X-4041, the Wallenberg Foundation and NIMH-12007. The technical assistance of Mrs Cilly Stolt is greatly appreciated.

REFERENCES

1. G. JOACHIMOGLU, J. KIBURIS and C. MIRAS, *U.N. Secretariat Document* ST/SOA/SER. S/15 (1967).
2. S. AGURELL, I. M. NILSSON, A. OHLSSON and F. SANDBERG, *Biochem. Pharmac.* **18,** 1195 (1969).
3. H. A. KLAUSNER and J. V. DINGELL, *Life Sci.* **10,** 49 (1971).
4. L. LEMBERGER, J. AXELROD and I. J. KOPIN, *Ann. N.Y. Acad. Sci.* **191,** 142 (1971).
5. S. AGURELL, B. GUSTAFSSON, T. GOSZTONYI, K. HEDMAN and K. LEANDER, *Acta chem. scand.* **27,** 1090 (1973).
6. Å. RYRFELDT, C. H. RAMSAY, I. M. NILSSON, M. WIDMAN and S. AGURELL, *Acta pharm. suecica* **10,** 13 (1973).
7. F. KORTE and H. SIEPER, *J. Chromatogr.* **13,** 90 (1964).
8. E. ÄNGGÅRD and H. BERGKVIST, *J. Chromatogr.* **48,** 542 (1970).
9. S. BURSTEIN, J. ROSENFELD and T. WITTSTRUCK, *Science, N.Y.* **176,** 422 (1972).
10. C. G. HAMMAR and R. HESSLING, *Anal. Chem.* **43,** 298 (1971).
11. M. E. WALL, D. R. BRINE, G. A. BRINE, C. G. PITT, R. I. FREUDENTHAL and H. D. CHRISTENSEN, *J. Am. chem. Soc.* **92,** 3466 (1970).
12. I. M. NILSSON, S. AGURELL, J. L. G. NILSSON, A. OHLSSON, F. SANDBERG and M. WAHLQVIST, *Science, N.Y.* **168,** 1228 (1970).
13. H. D. CHRISTENSEN, R. J. FREUDENTHAL, J. T. GIDLEY, R. ROSENFELD, G. BOEGLI, L. TESTINO, D. R. BRINE, C. G. PITT and M. E. WALL, *Science, N.Y.* **172,** 165 (1971).
14. J. L. G. NILSSON, I. M. NILSSON, S. AGURELL, Z. BEN-ZVI and R. MECHOULAM, *Acta pharm. suecica* **9,** 215 (1972).
15. I. M. NILSSON, S. AGURELL, J. L. G. NILSSON, M. WIDMAN and K. LEANDER, *J. pharm. Pharmac.* **25,** 486 (1973).
16. I. M. NILSSON, S. AGURELL, J. L. G. NILSSON, A. OHLSSON, J.-E. LINDGREN and R. MECHOULAM, *Acta pharm. suecica* **10,** 97 (1973).
17. R. TURK, W. DEWEY and L. HARRIS, *J. Pharmaceut. Sci.* **62,** 737 (1973).
18. F. MIKES, A. HOFMANN and P. WASER, *Biochem. Pharmac.* **20,** 2469 (1971).