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GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC STUDIES ON THE METABOLISM AND PHARMACOKINETICS OF Δ^1-TETRAHYDROCANNABINOL IN THE RABBIT

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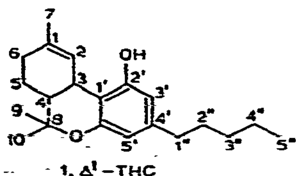
SUMMARY

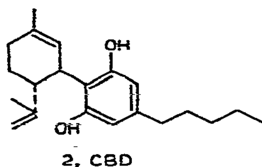
Gas chromatography–mass spectrometry has been used to investigate the *in vivo* hepatic metabolism of Δ^1-tetrahydrocannabinol (Δ^1-THC) in the New Zealand white rabbit. Sixteen metabolites were identified and shown to be present in different relative amounts compared with the hepatic metabolites of Δ^1-THC produced by other species. The metabolic profile was also different from that reported from rabbit urine particularly with regard to the lower relative concentrations of acidic metabolites in the liver. The pharmacokinetics of Δ^1-THC has been studied in the rabbit using the recently developed GC–MS method based on metastable ion monitoring. This revealed a terminal plasma Δ^1-THC half life ranging from 34.16 to 59.30 h (mean 46.75 h) after a single dose and a THC fat/plasma ratio of 10^3–10^4:1.

INTRODUCTION

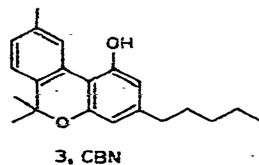
The metabolism of Δ^1-tetrahydrocannabinol (Δ^1-THC, 1), the major psychoactive principle of *Cannabis sativa* L. is complex and shows considerable species variability[1–3]. Major sites of attack are the allylic positions, 6 and 7 and the aliphatic carbons of the pentyl side-chain. The resulting hydroxy compounds are readily oxidized to aldehydes, ketones and acids and these can be excreted either free or as glucuronide conjugates[4]. Previous studies from this laboratory have concentrated on the structural determination of metabolites in tissues, particularly liver[3,5] rather than those in excreta as this gives a better measure of the molecular species present at the site of action of the drug. The results of these studies in mice, guinea pigs and rats have shown considerable species variation particularly with regard to the initial site of metabolic attack[6,7]. Comparative studies on hepatic metabolites in the rabbit are lacking even though this species has been used for teratological[8–10] and pharmacokinetic studies of Δ^1-THC[11]. In this paper we examine the hepatic metabolites of the drug in the New Zealand white rabbit using gas chromatographic–mass spectrometric (GC–MS) techniques comparable to those used in the early studies. Plasma and tissue



1, Δ^1-THC



2, CBD



3, CBN

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levels of the drug are also reported. These are studied by GC–MS using metastable ion monitoring[12].

Previous studies of the metabolism and excretion of Δ^1-THC by the rabbit have shown that the urinary route is preferred to the faecal route[13] in contrast to excretion by most other species where faecal elimination is usually dominant. Further studies have shown that these urinary metabolites are mainly acids, diacids and hydroxy acids with metabolic attack at C-6, C-7 and in the side-chain[14–17]. Nilsson *et al.*[18] have reported that 7-hydroxy-Δ^1-THC is a major metabolite formed by rabbit liver homogenates and further studies by Ben-Zvi and Burstein[19] on the same system have revealed the additional presence of 6α,7-dihydroxy-Δ^1-THC and hexahydrocannabinol-1α,2α-epoxide. The disposition of radiolabelled Δ^1-THC and some of its metabolites has recently been studied by Law[20] but the metabolites were not identified.

EXPERIMENTAL

Materials

Δ^1-THC was obtained from the National Institute on Drug Abuse. "Cannabis tincture" was obtained from W. Ransom (Hitchin) and its cannabinoid content was determined by gas–liquid chromatography (GLC) as described below.

Examination of the cannabis sample

The sample of "cannabis tincture" was evaporated to dryness (nitrogen stream) and converted into trimethylsilyl derivatives by reaction with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in acetonitrile (2:1:2) for 10 min at 60°C. 5α-Cholestane was used as the internal standard.

Quantitation of cannabinoids in the cannabis extract was performed by GLC using a Varian 2440 dual-column gas chromatograph fitted with flame ionization detectors and two 2 m × 2 mm I.D. glass columns packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.). The carrier gas was nitrogen at 30 ml min^{-1}, the column oven was temperature programmed from 150 to 300°C at 4°C min^{-1} and the injector and detector temperatures were 300°C.

Metabolism studies

Δ^1-THC (98% pure by GLC), suspended in Tween 80 and physiological saline were administered intraperitoneally to rabbits (male, New Zealand white, 2 kg) at a dose of 100 mg kg^{-1}. The animals were killed 1 h later, and the livers were removed and frozen. Metabolites were extracted from the homogenized livers with ethyl acetate and separated from neutral lipids by chromatography on Sephadex LH-20 (Table I), and converted into TMS, [$^2H_9$]TMS[21], methyl ester–TMS derivatives as described previously[5]. In addition, aliquots were reduced with lithium aluminium deuteride[22,23] and the resulting alcohols were examined by GC–MS as their TMS derivatives.

GC–MS of metabolites

GC–MS data from the metabolites were recorded with a VG Micromass 12B mass spectrometer interfaced to a VG 2040 data system and via a glass jet separator to a Varian 2440 gas chromatograph. The columns were 2 m × 2 mm glass packed with either 3% SE-30 or OV-17 on 100–120 mesh Gas-Chrom Q. Operating con-

TABLE I

FRACTIONATION OF THE CANNABINOIDS AND THEIR METABOLITES ON SEPHADEX LH-20

Fraction	*Solvent*	*Volume (ml)*	*Contents*
1	Chloroform	18	Triglycerides, cholesterol
2	Chloroform	8	Unchanged cannabinoids
3	Chloroform	10	Fatty acids, some CBN
4	Chloroform	35	Monohydroxy, ketohydroxy metabolites
5	Chloroform–methanol (4:1)	50	Polar metabolites

ditions were: column oven, temperature programmed from 150 to 300°C at 2°C min^{-1}; injector, separator and ion source temperatures, 300, 290 and 260°C, respectively; carrier gas, helium at 30 ml min^{-1}; electron energy 25 eV; trap current, 100 μA; accelerating voltage 2.5 kV; scan, 3 sec $decade^{-1}$, exponential.

Treatment of animals for pharmacokinetic studies

Δ^1-THC in Tween 80 and sterilized physiological saline was administered into the marginal ear vein of female New Zealand White rabbits (2 kg) at a dose of 1 or 0.1 mg kg^{-1}. Blood was collected into heparinized tubes from the other ear at intervals until Δ^1-THC could no longer be detected. This was centrifuged to obtain the plasma which was stored at −30°C until required. Two rabbits were also treated with 1.0 and 0.1 mg kg^{-1} Δ^1-THC equivalent of cannabis tincture. One month later the animals were treated again with the same dose daily for 8 days. One animal was treated for 22 days (1 mg kg^{-1}). Blood samples were collected as before commencing after the last dose.

Quantitation of Δ^1-THC in plasma and fat

[1″,1″,2″,2″-$^2H_4$]CBN was added to the plasma sample, the cannabinoids were extracted 3 times with hexane, blown to dryness, allowed to stand with ethereal diazomethane for 2 min and were converted into TMS derivatives using BSTFA. Full details have been published[12]. THC levels were measured using metastable ion monitoring as described below. For the measurement of levels in fat, the hexane extract was reconstituted in chloroform and chromatographed twice on Sephadex LH-20 in chloroform. The fraction eluting between 19 and 36 ml (fractions 2 and 3, Table I) was collected and derivatized as described above.

Metastable ion monitoring

This was performed with a VG Micromass 70-70F mass spectrometer interfaced via a glass jet separator to a Varian 2440 gas chromatograph fitted with a 2 m × 2 mm 3% SE-30 column as described above. Operating conditions were: column oven, 220°C; injector, separator and ion source temperatures, 300, 290 and 260°C, respectively; carrier gas, helium at 30 ml min^{-1}; electron energy, 70 eV; trap current, 1 mA; accelerating voltage, 4.16 kV; magnet set to record m/z 371.

RESULTS AND DISCUSSION

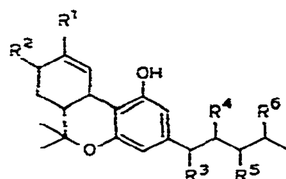
Techniques such as GLC and MS are ideal for studies of the metabolism and disposition of lipophilic drugs such as Δ^1-THC as they do not require isolation of the

metabolites prior to examination. The low concentrations of the compounds present in animal fluids and tissues, together with the presence of a relatively large number of compounds having similar structures, makes isolation impractical for all but the most abundant metabolites. The extraction method adopted for the present studies was designed to obtain metabolic fractions containing the largest possible range of metabolites with the minimum of "clean-up"; GLC was then used to separate these compounds and GC–MS was used for identification.

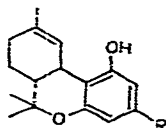
Extraction of the metabolites from 2-g samples of liver was achieved as described above. Spectra of the metabolites were compared with those of authentic samples where available (see Table II) and with published data. Functional groups were identified by the preparation of group-specific derivatives such as methyl esters for carboxylic acids and correlations between metabolites in different oxidation states (acids and ketones) were made by reduction with lithium aluminium deuteride[22,23]. The 16 metabolites identified are listed in Table II. All of these compounds have been

TABLE II

METABOLITES OF Δ^1-THC EXTRACTED FROM RABBIT LIVER



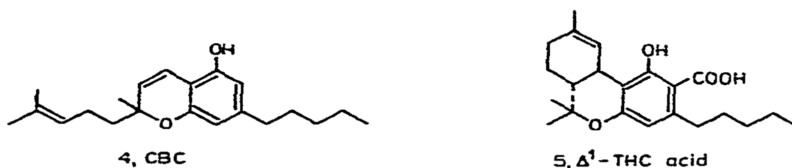
Metabolite	R^1	R^2	R^3	R^4	R^5	R^6	*Abundance**	*Lit. ref. for spectrum*
1″-OH-Δ^1-THC	H	H	OH	H	H	H	+	24, 25
3″-OH-Δ^1-THC	H	H	H	H	OH	H	+	24, 25
6α-OH-Δ^1-THC**	H	α-OH	H	H	H	H	+	25, 26
6β-OH-Δ^1-THC**	H	β-OH	H	H	H	H	+	25, 26
7-OH-Δ^1-THC**	CH_2OH	H	H	H	H	H	+++	25, 26
2″,7-di-OH-Δ^1-THC	CH_2OH	H	H	OH	H	H	+	26
3″,7-di-OH-Δ^1-THC	CH_2OH	H	H	H	OH	H	+	26
6α,7-di-OH-Δ^1-THC**	CH_2OH	α-OH	H	H	H	H	+	26
6β,7-di-OH-Δ^1-THC**	CH_2OH	β-OH	H	H	H	H	+	26
Δ^1-THC-7-oic acid**	COOH	H	H	H	H	H	+++	25, 26
6α-OH-Δ^1-THC-7-oic acid	COOH	α-OH	H	H	H	H	+	27, 28
2″-OH-Δ^1-THC-7-oic acid	COOH	H	H	OH	H	H	+	27, 28
3″-OH-Δ^1-THC-7-oic acid	COOH	H	H	H	OH	H	++	27, 28
4″-OH-Δ^1-THC-7-oic acid	COOH	H	H	H	H	OH	++	29



Metabolite	*R*	*Abundance**	*Lit. ref. for spectrum*
4″,5″-bis.nor-Δ^1-THC-3″-oic acid	C_2H_4COOH	++	2, 30
2″,3″,4″,5″-tetrakis.nor-Δ^1-THC-1″-oic acid	COOH	+	31

* + = trace metabolite; ++ = intermediate concentration; +++ = major metabolite. Accurate concentrations were not measured because of the lack of suitable standards.

** Authentic sample available.



observed as metabolites of Δ^1-THC in other species and the profile was intermediate between that observed for the mouse and guinea-pig. 7-Hydroxylation and oxidation to Δ^1-THC-7-oic acid was the major metabolic route in contrast to the results obtained by Nordqvist and co-workers[16,17] who have reported that in rabbit urine, 4″,5″-bis,nor-Δ^1-THC-3″-oic acid is the major metabolite. This is probably a reflection of the extent to which the 3″-acid is excreted into the urine rather than a difference in the overall profile. In our liver fractions, the 3″-oic acid was observed, but in much lower concentration than the 7-oic acid. It was accompanied by the 2″,3″,4″,5″-tetrakis,nor-Δ^1-THC-1″-oic acid. Other side-chain acids and their hydroxy and carboxy substituted derivatives reported by Nordqvist and co-workers were not observed in the liver, possibly as the result of their low concentration. In other respects the profiles were similar to those found by the other workers. Thus 6β-hydroxylation was preferred to 6α-hydroxylation although the amount of each metabolite was much lower than in the guinea-pig (Fig. 1). 6-Ketones were not detected. Hydroxylation of the side-chain was observed to a limited extent at positions 1″, 2″, 3″, and 4″. Table III shows a comparison of the *in vivo* hepatic metabolites of Δ^1-THC produced by the rabbit with those observed previously from mouse, rat and guinea pig.

Measurement of Δ^1-THC and its metabolites in plasma and tissues is difficult because of the low levels encountered and the presence of co-extracted lipid ma-

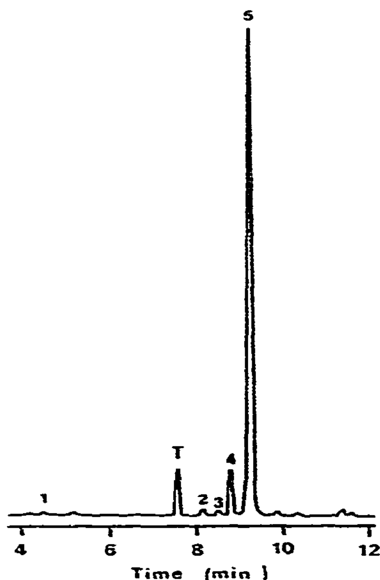


Fig. 1. Computer reprocessed total ion chromatogram [35] of the monohydroxy metabolite-containing fraction (fraction 4, Table I) from the livers of rabbits treated with Δ^1-THC. Peaks: 1 = 1″-hydroxy-Δ^1-THC; 2 = 6α-hydroxy-Δ^1-THC; 3 = 3″-hydroxy-Δ^1-THC; 4 = 6β-hydroxy-Δ^1-THC; 5 = 7-hydroxy-Δ^1-THC; T = tissue constituent.

TABLE III

SUMMARY OF THE MAJOR METABOLIC ROUTES FOR Δ^1-THC SHOWN BY FOUR SPECIES

+ → + + +. Increasing importance of metabolic route: —, not detected.

Metabolic route	*Mouse*	*Rat*	*Guinea-pig*	*Rabbit*
7-Hydroxylation	+ + +	+ + +	+ + +	+ + +
6α-Hydroxylation	+ +	+ +	+	+
6β-Hydroxylation	+	+	+ + +	+ +
1″-Hydroxylation	—	+	+	+
2″-Hydroxylation	+ +	+	+	+
3″-Hydroxylation	+ +	+ +	+	+
4″-Hydroxylation	+ +	+ +	+ +	+
7-Acid formation	+ + +	+ + +	+	+ + +
6-Ketone formation	+	+	+	—
β-Oxidation	+	+	+ + +	+ +

terial[32,33]. We have developed a rapid GC–MS assay for Δ^1-THC as its TMS derivative based on metastable ion monitoring of the $[M]^+$ (m/z 386) → $[M-CH_3]^+$ (m/z 371) transition using a double focussing mass spectrometer[12], as described in the experimental section. The method can measure Δ^1-THC to 5 pg/ml of plasma and has now been used to study the pharmacokinetics of Δ^1-THC in rabbits. Experiments were performed as described above and some typical results are presented in Fig. 2. These are in agreement with earlier results based on GLC and radiolabelling which indicate that Δ^1-THC accumulates extensively in the body[34,35]. Following a single 0.1 mg kg^{-1} dose, Δ^1-THC could be monitored for 4 days after administration. After an 8-day treatment with daily injections of the same dose, the drug could be monitored in plasma for 12 days and after a 22-day treatment with 1 mg kg^{-1} per day the drug could be monitored for 26 days. Terminal half lives were calculated using non-linear least squares regression analysis and ranged from 34.16 to 59.30 h (mean 46.75 h, n = 5) following a single dose and 77.94 to 120.02 h (mean 94.63 h, n = 5) following multiple doses. As this type of behaviour is typical of drugs which accumulate in

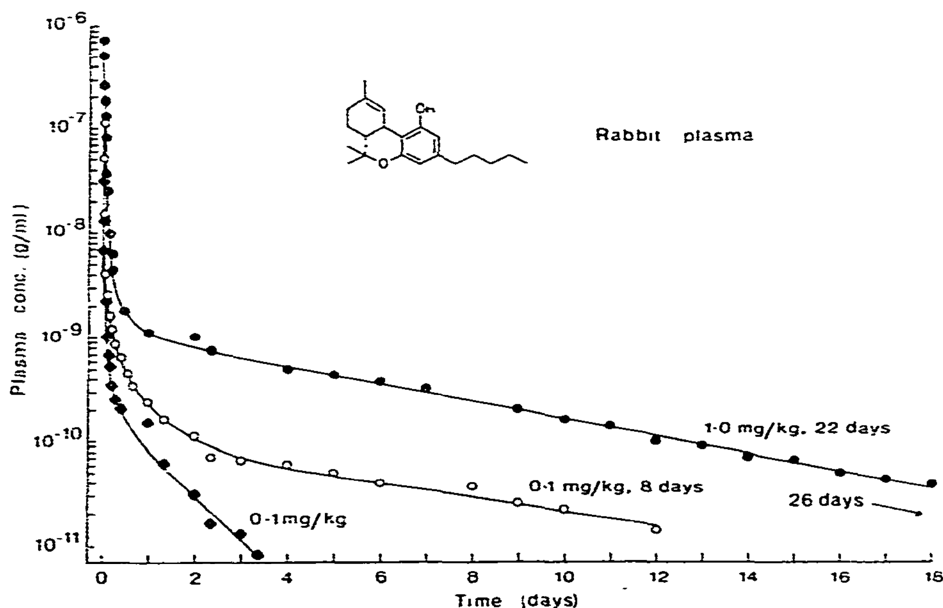


Fig. 2. Semi-logarithmic plot of the plasma levels of Δ^1-THC: time for rabbits dosed with: ◆, 0.1 mg kg^{-1}; ○, 0.1 mg kg^{-1} daily for 8 days; ●, 1.0 mg kg^{-1} daily for 22 days.

adipose tissue, the concentration of Δ^1-THC in fat was measured. Because of the large amount of triglyceride in the samples, these were chromatographed twice on Sephadex LH-20 prior to derivative formation. Concentrations in the region of 10^3 to 10^4 times the plasma concentration were found at various times after dosing and the decline in concentration matched the decline of the plasma Δ^1-THC levels. The concentration of Δ^1-THC in fat at 28 h after dosing with 1 mg/kg was 1161.0 ng/g and this fell to 36.4 ng/g at 240 h. In use, Δ^1-THC is usually present with other cannabinoids such as cannabidiol (CBD) and cannabinol (CBN) and the presence of these compounds could affect both the metabolism and distribution of the drug. Previous studies on the metabolism of a crude mixture of cannabinoids from "cannabis tincture" in the mouse suggests that the effects of the additional cannabinoids on the profile of hepatic metabolites is minimal[3]. The plasma levels of Δ^1-THC have now been measured in the rabbit treated with 1.0 an 0.1 mg/kg Δ^1-THC in cannabis tincture but no significant differences were found over the results from rabbits treated with pure Δ^1-THC. This is consistent with results recently obtained in other laboratories[36,37]. The composition of the "cannabis tincture" used in these experiments was determined by GLC and is shown in Table IV.

TABLE IV

CONCENTRATIONS OF THE MAJOR CANNABINOIDS PRESENT IN CANNABIS TINCTURE AS MEASURED BY GLC

Cannabinoid	*Quantity (mg/ml)*	*Relative amount (Δ^1-THC = 100)*
Pr-CBD	0.285**	20
Pr-Δ^1-THC	0.48	34
Pr-CBN	0.135	9.6
CBD	1.26	90
CBC*	0.28	20
Δ^1-THC	1.40	100
CBN	0.40	29

* Cannabichromene.

** Response factors of the propyl (Pr) cannabinoids were based on those of the pentyl homologues because of the lack of suitable standards.

CONCLUSIONS

GC–MS has proved to be a valuable method for analysis of cannabinoids and their metabolites. Identification of the metabolites can be accomplished at the microgram level by a combination of the use of group-specific derivatives, deuterium labelling and various GLC columns. The profiles obtained are complex and vary considerably with the animal species in which metabolism is studied. GC–MS using metastable ion monitoring has also provided the most sensitive method yet for the analysis of Δ^1-THC in body fluids and tissues and has enabled the pharmacokinetics of this compound to be elucidated. Results from these measurements have shown that despite its extensive metabolism, the psychoactive Δ^1-THC appears to be stored as such in a fatty reservoir, and to be slowly released over a prolonged period in a manner corresponding to its fat–water partition coefficient.

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