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Comparative In Vitro Metabolism of the Cannabinoids

D. J. HARVEY AND N. K. BROWN

University Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT, UK

HARVEY, D. J. AND N. K. BROWN. *Comparative in vitro metabolism of the cannabinoids*. PHARMACOL BIOCHEM BEHAV **40**(3) 533–540, 1991.—The metabolism of delta-9-tetrahydrocannabinol (delta-9-THC), delta-8-THC, delta-11-THC, cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), cannabigerol (CBG) and the *equatorial*-isomer of hexahydrocannabinol (HHC) was studied in microsomal preparations obtained from rats, mice, guinea pigs, rabbits, hamsters, gerbils and a cat. Identification of metabolites was by GC/MS and quantification by gas chromatography. Major metabolites were monohydroxylated compounds but the pattern of hydroxylation varied considerably between the species, no doubt reflecting the variable nature of the cytochrome P-450 mixed-function oxidases. Although the primary carbon allylic to the endocyclic double bond of tricyclic cannabinoids was usually the major site of attack, the 4′ (side-chain, omega-1 position) and the terpene ring were usually favoured by the cat and hamster respectively. The guinea pig generally produced more metabolites hydroxylated in the side-chain (all positions) than did the other species. The results from HHC were very similar to those from THC, namely hydroxylation at C-11 in most species, and the production of high concentrations of 8alpha-hydroxy-HHC in the mouse and 8beta-hydroxy-HHC in the hamster. As this molecule lacks the double bond of the THCs and, hence, the allylic nature of C-11 and C-8, the results suggest that it is the orientation of the molecule to the active site of the cytochrome P-450 mixed-function oxidase rather than the reactivity of the C-H bond that governs the position of hydroxylation.

Cannabichromene Cannabidiol Cannabigerol Cannabinol Cat Gerbil Guinea pig Hamster
Hexahydrocannabinol Liver Mass spectrometry Microsomes Mouse Rabbit Rat
Tetrahydrocannabinol

ALTHOUGH metabolism of most major cannabinoids has been well studied (10,23), both in vivo and in vitro, there is little information on comparative metabolism in different species as different investigators have used a variety of media in which to identify the metabolites. The major initial biotransformation pathways shown by cannabinoids are hydroxylations at a variety of sites, catalysed by cytochrome P-450 mixed-function oxidases. These oxidases are known to vary considerably between species and, thus, will produce a different pattern of cannabinoid hydroxylation. Several hydroxy metabolites of cannabinoids are psychoactive (31) and it is, thus, important to know the hydroxylation pattern produced by any species in which pharmacological testing is carried out. This paper compares the in vitro microsomal metabolism of eight major cannabinoids, delta-9-tetrahydrocannabinol (delta-9-THC, I), delta-8-THC (II), delta-11-THC (III), cannabidiol (CBD, IV), cannabinol (CBN, V), cannabichromene (CBC, VI), cannabigerol (CBG, VII) and the C-9-*equatorial*-isomer of hexahydrocannabinol (HHC, VIII) (Fig. 1) in rat, rabbit, mouse, guinea pig, hamster, gerbil and cat under the same experimental conditions in order to quantify differences in metabolism and give some information on the nature of the P-450 isozymes.

METHOD

Materials

All eight cannabinoids were obtained from the National Institute on Drug Abuse (NIDA) and were examined for purity by GLC [trimethylsilyl (TMS) derivative]. All samples were within the range 97–99% pure. The following hydroxylated cannabinoids were also obtained from NIDA and were used as reference compounds for metabolite identification: 1′-, 2′-, 3′-, 4′-, 5′-, 8alpha-, 8beta- and 11-hydroxy-delta-9-THC, 7alpha-, 7beta- and 11-hydroxy-delta-8-THC. Allylic hydroxy metabolites of CBC (17) and CBG (11) were synthesised by selenium dioxide oxidation of CBC followed by reduction with lithium aluminium hydride as described earlier. Epoxide and epoxy-diol metabolites of the three THCs were synthesised by reaction of their acetates with m-chloroperbenzoic acid to give the epoxide, followed by perchloric acid-catalysed epoxide ring opening to give the diol (7,22).

Animals

All animals were male except for one rabbit which was only used for metabolism studies with CBD and are listed in Table 1 together with details of cytochrome P-450 and protein content.

Preparation of Microsomes

Animals were killed by cervical dislocation and the livers were removed. These were homogenized in phosphate-buffered saline (pH 7.4) equal to 3× the weight of liver at 4°C. The homogenate was centrifuged at 20,000 × g for 30 min and the supernatant was centrifuged for a further 60 min at 105,000 × g. The crude microsomal pellet was resuspended in phosphate-

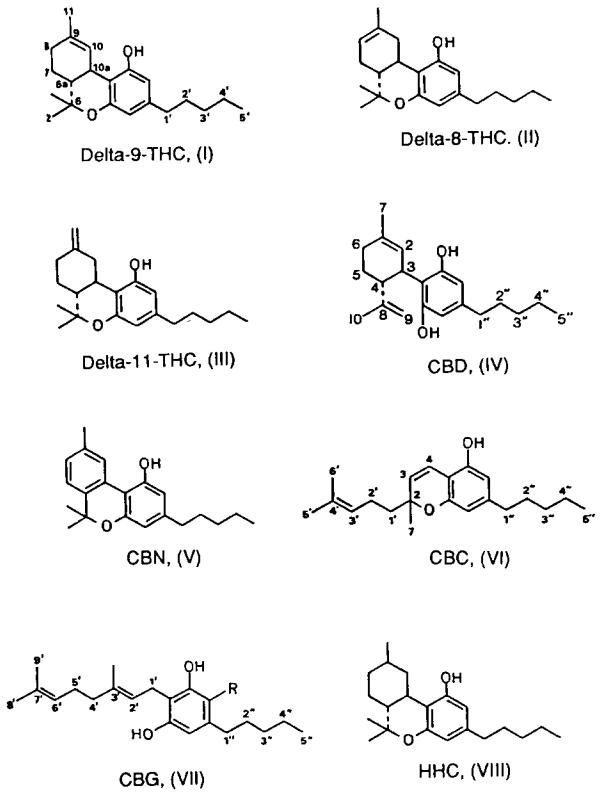


FIG. 1. Structures of the cannabinoids.

buffered saline and either used directly for metabolism studies or stored at −70°C. Protein content was measured by the method of Lowry et al. (24) and cytochrome P-450 by the method described by Omura and Sato (30). The specific P-450 content is listed in Table 1. Duplicate preparations were made from all animals except hamster, gerbil and cat.

Microsomal Incubations

The cannabinoid (0.5 mg) in ethanol (5 μl) was added to a mixture of NADP (0.8 mM), glucose-6-phosphate (40 mM) and magnesium chloride (10 mM) at 4°C. The microsomes (1 ml) were added, followed by glucose-6-phosphate dehydrogenase (20 unit) and the volume was made up to 10 ml with phosphate-buffered saline (pH 7.4). The mixture was bubbled with a mixture of oxygen (95%) and carbon dioxide (5%) for 1 min and then incubated in a shaking water bath for 60 min at 37°C.

Extraction of Metabolites

Metabolites were extracted from the cooled microsomal incubations with ethyl acetate (3 × 10 ml) with centrifugation at 2000 rpm (1450 × g) to aid separation of the two layers, and concentrated by chromatography on Sephadex LH-20 (5 g packed into a 1 cm diameter column) in chloroform and chloroform:methanol mixtures as described earlier (19). The metabolite-containing fraction, which was eluted with 35–70 ml of chloroform and 50 ml of a 10% mixture of methanol in chloroform, was concentrated under reduced pressure and redissolved in ethyl acetate (1.0 ml) for storage. Aliquots (0.1 ml) of this solution were converted into derivatives for GC/MS as described below.

Preparation of Derivatives

TMS derivatives. The sample was heated with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA, 10 μl) for 10 min at 60°C.

[$^2H_9$]TMS derivatives (25). These were prepared as for the TMS derivatives with [$^2H_{18}$]bis-trimethylsilylacetamide replacing the BSTFA.

Catalytic hydrogenation. The reference cannabinoid or extracted microsomal mixture, dissolved in ethanol, was added to a suspension of rhodium on alumina (Aldrich Chemical Co.) in ethanol (1 ml) contained in a 3.5 ml vial that had been filled with hydrogen (12). The vial was shaken for 10 min (12) or overnight (metabolite-containing fraction), centrifuged to precipitate the catalyst and the ethanolic solution was removed. This was evaporated to dryness with a nitrogen stream and the reduced metabolites were converted into TMS and [$^2H_9$]TMS derivatives as described above.

Gas Chromatography

GLC retention data of the major metabolites and peak areas for quantification were measured with a Hewlett-Packard 5890A gas chromatograph fitted with a 50 m × 0.3 mm OV-1 bonded phase fused silica capillary column (film thickness 0.52 μm). Helium at 2 ml/min was used as the carrier gas with a split ratio of 10:1. The injector and detector (FID) temperatures were both

TABLE 1

ANIMALS, PROTEIN AND CYTOCHROME P-450 CONCENTRATIONS IN THE INCUBATION MIXTURES

Animal	Weight	No. Used	Protein/1 ml Incubation Mixture	P-450 in 1 ml Incubation Mixture	P-450/mg Protein
Mouse (Charles River CD-1)	23–25 g	160	32.7 mg	39 nmol	1.19 nmol
Rat (Wistar)	200 g	8	20.3	16	0.79
Guinea Pig (Dunkin-Hartley)	400 g	5	36.8	33	0.90
Rabbit (New Zealand White)	2.5–3 kg	3	58.4	58	0.99
(female)	3.5 kg	3			1.22
Hamster (Syrian long haired)	60 g	10	29.2	32	1.09
Gerbil (Mongolian)	40 g	10	20.7	8.6	0.42
Cat (Oxford inbred)	2.5 kg	1	66.5	15.6	0.23

TABLE 2

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF MONOHYDROXY METABOLITES FORMED BY INCUBATION OF DELTA-9-THC WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster	Gerbil	Cat
1'-OH	—	2	5	3	—	—	6
2'-OH	—	—	4	—	—	—	—
3'-OH	—	1	30	2	—	—	7
4'-OH	—	1	1	2.5	—	—	51
5'-OH	—	—	11	2.5	—	—	7
8alpha-OH	31	3	3	1	2	2	7
8beta-OH	9	1	4	1	93	—	—
11-OH	60	92	42	88	5	98	22

300°C and the column oven was temperature programmed from 200 to 350°C at 2°C/min.

GC/MS

System 1: VG 12B single focusing mass spectrometer interfaced via a glass jet separator to a Varian 2440 gas chromatograph which was fitted with a 2 m × 2 mm (i.d.) glass column packed with 3% SE-30 on 100–120 mesh Gas Chrom Q (Applied Science Laboratories, State College, PA). Helium at 30 ml/min was used as the carrier gas and the injector, separator and mass spectrometer ion source temperatures were 300, 300, and 260°C respectively. The column oven was temperature programmed at 2°/min from 180° to 300°. Other operating conditions for electron-impact mass spectrometry were: accelerating voltage, 2.4 kV; trap current, 100 μA; electron energy, 25 eV; scan speed, 3 s/decade. Spectra were acquired with a VG 2050 data system and processed with a linked VG 11:250 data system. System 2: VG 70/70F double focusing mass spectrometer connected to a Varian 2440 gas chromatograph. The column was a 30 m × 0.2 mm OV-1 bonded-phase fused silica capillary terminating 1 cm inside the ion source. Helium at 1 ml/min (measured in the absence of the vacuum) was used as the carrier gas. The injector was an SGE split/splitless system used in the split mode with a split ratio of 10:1 and the column oven was temperature-programmed from 220°C to 300° at 2°/min. Operating conditions were: injector, transfer line and ion source temperatures, 300, 300, and 280°C respectively; electron energy, 70 eV; trap current, 1 mA; accelerating voltage, 4 kV. The instrument was scanned repetitively at 1 s/decade under the control of a VG 11:250 data system and spectra were acquired and processed with the same data system.

RESULTS AND DISCUSSION

Identification of Metabolites

Metabolites were identified by GC/MS as TMS and [$^2H_9$]TMS derivatives and by comparison with reference compounds where available and with literature data (1, 6, 8, 9). In addition, metabolites of the various THCs were hydrogenated to hydroxyhexahydrocannabinols (HHCs) and their spectra (TMS derivatives) compared with those of reference hydroxy-HHCs synthesised from the hydroxy-THCs (12). The GLC peaks attributed to these metabolites in the gas chromatograms from the various species were absent from the profiles from control animals not treated with the drug. The same metabolites were present in most species but their relative abundance varied considerably. Concentrations of the metabolites were measured by GLC and are expressed for each cannabinoid as a percentage of the total recovered material as determined by measurement of peak area. Absolute concentrations were not measured. Results from the duplicate experiments were in close agreement.

Delta-9-THC

The relative concentrations of the metabolites are listed in Table 2. It is clear that for all species except hamster and cat, the major metabolite was 11-hydroxy-delta-9-THC. Hydroxylation in this position is normally regarded as the major metabolic route for this cannabinoid in most species, including man (34,40), but the results demonstrate that this is not always the case. In the hamster, the major site attacked was 8beta whereas in the

TABLE 3

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF MONOHYDROXY METABOLITES FORMED BY INCUBATION OF DELTA-8-THC WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster
1'-OH	1	12	8	—	2
2'-OH	—	—	—	—	3
3'-OH	—	11	4	4	3
4'-OH	2	3	22	13	2
5'-OH	—	—	17	3	—
7alpha-OH	4	2	—	—	2
7beta-OH	8	7	7	2	82
11-OH	85	65	42	78	5

TABLE 4

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF METABOLITES FORMED BY INCUBATION OF DELTA-11-THC WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster	Gerbil	Cat
1′-OH	—	1	9	T	T	4	16
2′-OH	—	T	T	—	—	—	—
3′-OH	—	2	40	5	8	2	2
4′-OH	T	T	26	6	T	6	8
5′-OH	—	1	5	T	—	T	—
8alpha-OH	59	29	5	9	17	—	—
8beta-OH	19	35	4	10	45	19	65
10-OH	4	T	—	—	—	1	—
11-OH	T	—	—	8	T	20	—
1,11-Di-OH	18	32	11	62	30	48	9

T = Trace.

cat it was 4′. Side-chain hydroxylation also occurred in rat, guinea pig, and rabbit, and was particularly prominent in the guinea pig. This is in agreement with earlier work using whole liver preparations (21). It is significant that 5′-hydroxy-delta-9-THC was abundant in the guinea pig; this is the first intermediate in beta-oxidation and, in vivo, the guinea pig has been shown to produce considerable amounts of beta-oxidised metabolites (21) unlike species such as the mouse which, as shown by the present study, does not produce measurable concentrations of side-chain hydroxy metabolites.

The mouse, on the other hand, produced the highest concentration of 8alpha-hydroxy-delta-9-THC, again in agreement with previous work in this species (19,27). Marriage and Harvey (27) have investigated the cytochrome P-450 system with respect to its ability to hydroxylate delta-9-THC in more detail in this species and have shown that several of the isozymes, partially purified by chromatofocusing (26), were capable of producing both 11- and 8alpha-hydroxy-delta-9-THC. Some of the minor enzymes produced low concentrations of metabolites hydroxylated in the side-chain and at two more, unidentified, positions. However, the concentrations were so low that they would have been masked by the two major metabolites in whole microsomal preparations, as found in the present study.

Widman et al. (36) have shown that in a perfused liver experiment in dog, the major metabolite is 8beta-delta-9-THC with only a small proportion of the 11-hydroxy metabolite. This trend for 8-hydroxylation to be preferred in dog is reflected by urinary metabolites of CBD where hydroxylation in this position is present in the major metabolites (32). In a separate study in dogs (36), it has been shown that the perfused dog lung produces a different range of monohydroxy metabolites with higher concentrations of 3′- and 4′-hydroxy-delta-9-THC than that seen from the liver. This is further evidence for the differential hydroxylation properties shown by cytochrome P-450 as those isozymes in the lung are known to be different from those in liver. The brain has also been shown to produce a different range of metabolites from those found in liver (33); the major metabolite in the rat is 4′-hydroxy-delta-9-THC whereas in mouse, guinea pig and rabbit, both 4′- and 5′-hydroxy-delta-9-THC are formed.

Delta-8-THC

The metabolic pattern generated from delta-8-THC by five of the seven species is shown in Table 3. This showed similarities with that obtained from delta-9-THC in that 11-hydroxylation was the major metabolic route shown by all except the hamster which, again, produced the ring hydroxylated (7beta-delta-8-THC) compound as the major metabolite. It is significant that

TABLE 5

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF METABOLITES FORMED BY INCUBATION OF CBD WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster	Gerbil	Cat
1″-OH	3	T	6	T	6	T	10
2″-OH	T	T	6	1	3	4	—
3″-OH	8	6	18	5	4	9	7
4″-OH	24	21	32	14	16	37	24
5″-OH	T	T	6	7	9	9	2
6alpha-OH	16	11	13	2	10	12	44
6beta-OH	7	8	3	7	10	7	*
7-OH	42	49	11	64	19	16	13
10-OH	—	5	5	—	23	6	—

T = Trace.

*Stereochemistry not determined.

TABLE 6

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF METABOLITES FORMED BY INCUBATION OF CBN WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster	Gerbil	Cat
1'-OH	8	20	16	17	12	15	6
2'-OH	2	21	2	1	2	T	–
3'-OH	1	7	17	2	8	T	–
4'-OH	8	22	19	13	49	17	90
5'-OH	2	4	7	4	9	2	T
11-OH	79	26	39	63	20	66	4

T = Trace.

the hamster also hydroxylated delta-9-THC on the beta face of the terpene ring. Unfortunately, the cat was not studied because of a shortage of microsomes. 11-Hydroxylation has also been reported to produce the major metabolite from this cannabinoid in hepatic microsomal preparations from both man (34,41) and monkey (5,34). Maximum side-chain hydroxylation was again seen in the guinea pig.

Delta-11-THC

This cannabinoid showed the greatest variation in metabolism (see Table 4), possibly caused by the absence of the allylic site at C-11. However, the allylic site at C-8 was attacked by the mouse to give the alpha-hydroxy isomer (major metabolite) as from delta-9-THC. 8Beta-hydroxylation was prominent in the metabolic profiles of the other species and gave the major metabolite in rat, hamster and cat. Side-chain hydroxylation was again the main biotransformation pathway exhibited by the guinea pig.

No dihydroxy metabolites were found in this study, even though these have been reported by Binder and Barlarge (2) from a rat microsomal preparation. This, however, may be attributable to the use of phenobarbital-induced microsomes by these authors.

The exoxyclic double-bond of this cannabinoid appeared to be more prone to attack by epoxidation than the more hindered endocyclic bonds in the other two cannabinoids; this gave the corresponding epoxides and, following hydrolysis, the dihydrodiols (1,11-dihydroxy-HHCs). This was found as the major biotransformation pathway in rabbit and gerbil although it was also prominent in most other species. Rearrangement of the epoxide to HHC-11-al followed by reduction (18) appeared to give low concentrations of 11-hydroxy-HHC in rabbit and gerbil, the species utilizing the epoxide route to the greatest extent. Further details have been published (13).

TABLE 7

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF METABOLITES FORMED BY INCUBATION OF CBC WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster	Gerbil	Cat
2'-OH	14	–	10	–	–	13	–
5'-OH	24	65	57	39	35	23	7
6'-OH	22	20	18	17	8	11	2
1''-OH	4	2	2	12	7	5	16
2''-OH	2	–	–	–	2	–	12
3''-OH	3	2	4	12	3	2	4
4''-OH	17	9	9	15	5	3	9
5''-OH	4	2	–	5	2	–	4
Epoxide	10	–	–	–	10	12	42
3',4'-di-OH	–	–	–	–	28	31	4

Cannabidiol

Metabolites from this cannabinoid are listed in Table 5. The profile again showed considerable variability with 7-hydroxylation (equivalent to 11-hydroxylation in the other cannabinoids which are numbered differently) dominating the profile in mouse, rat and rabbit. Martin et al. (29) have observed a similar profile from the rat. 4''-Hydroxylation was dominant in the guinea pig and gerbil with the general high level of side-chain hydroxylation seen in the guinea pig reflecting that observed for the other cannabinoids. The cat again preferred ring hydroxylation at the allylic 6-position. It has long been suspected, but never observed, that CBD is hydroxylated at the other primary allylic position at C-10 and, indeed, this compound was found to be the major metabolite in the hamster. Once this observation had demonstrated where in the chromatogram this metabolite appeared, it was also found in low concentration in rat, guinea pig and gerbil (15).

Unlike the profiles obtained from the other cannabinoids, that from CBD contained several dihydroxy metabolites in low concentration [see (28)]. The positions substituted in these metabolites were the same as those found in the monohydroxy metabolites.

The study performed with microsomes obtained from an old female rabbit (14) showed low concentrations of dihydrodiol metabolites produced by hydroxylation of the delta-8-double bond. Their genesis undoubtedly involved prior epoxidation of the double bond and they were accompanied by analogues further hydroxylated at C-6, C-7 and in the side-chain. No evidence of epoxidation of the delta-1-double bond was found. However, Yamamoto et al. (37,38) have recently shown that this epoxide is unstable and decomposes to cannabielsoin, found as a microsomal metabolite in several species. No evidence of the presence of this compound was found in the present study.

Cannabinol

Metabolites are listed in Table 6. The profile from this cannabinoid was simpler than that observed for the other cannabinoids, partly on account of the absence of allylic ring-hydroxylation, thus, 11-hydroxylation dominated the profile from all species except hamster and cat where, once again, 4'-hydroxylation was preferred. Unlike metabolism of the other canna-

TABLE 8

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF METABOLITES FORMED BY INCUBATION OF CBG WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Cat
1''-OH	6	3	T	2	1
2''-OH	—	—	6	—	—
3''-OH	2	4	19	1	2
4''-OH	21	13	7	28	10
5''-OH	6	3	T	2	1
4'-OH	9	14	18	1	4
8'-OH	5	26	24	36	31
9'-OH	2	11	6	1	11
6',7'-Epoxide	46	15	14	29	9
6',7'-di-OH	3	—	6	—	T
6',7'-H_2	—	11	—	—	31

T = Trace.

binoids, considerable concentrations of 1'-hydroxylation were found in most profiles; this has also been observed in an earlier in vivo study using whole liver from rats (20). Once again, the guinea pig showed extensive side-chain hydroxylation. The results obtained in the present study were similar to those recently obtained for mouse, rat, guinea pig and rabbit by Yamamoto et al. (39). This study also reported microsomal metabolites from the dog; here 11-hydroxylation was again the major route (65%) with 1'-hydroxylation amounting to 22% of the total recovered metabolites. Low concentrations of 2'-, 3'- and 4'-hydroxy-CBN were also found. In an earlier study comparing metabolism in rat and rabbit (35), comparable profiles were found to those of the present study with the exception that no 1'-hydroxy-CBN was reported. For further details see (4).

Cannabichromene

Metabolite concentrations are reported in Table 7. Metabolites were difficult to identify as their mass spectra were dominated by the chromenyl ion produced by loss of the methylpentenyl chain. However, following hydrogenation over a rhodium/alumina catalyst, chromenyl ion formation was suppressed and fragmentation was redirected along pathways which gave ions diagnostic of the hydroxylation position (16). Metabolism of this cannabinoid showed some similarities and some differences to that of the compounds discussed so far. Allylic hydroxylation, predominantly at the primary carbon at C-5' and to a lesser extent at C-6', gave the major metabolites in all species except gerbil and cat where epoxidation of the delta-3'-double bond predominated (see Table 7). However, unlike the other cannabinoids, side-chain hydroxylation did not feature prominently in the profile produced by the guinea pig but was much more prominent in the mouse and rabbit.

The profiles obtained from the mouse, gerbil and cat contained several compounds that contained two phenolic hydroxy groups and appeared to have been formed by opening of the dihydropyran ring. However, these appeared to be chemical decomposition products as they were also formed from boiled microsomes; their structure were, therefore, not investigated further.

Cannabigerol

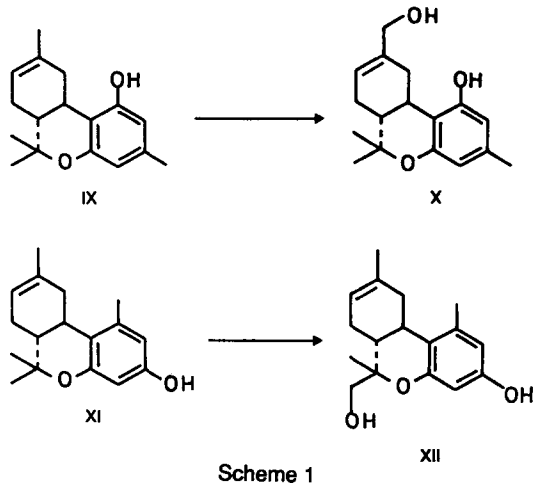
Metabolism of this cannabinoid, studied in five species, was similar to that of CBC (see Table 8) in that allylic hydroxylation at the terminal double bond of the C-10 chain produced the major metabolites in all except the mouse which preferred to form an epoxide at the terminal double bond. Metabolism by the guinea pig fitted the normal profile in that hydroxylation of the pentyl side-chain, particularly at C-3'' was prominent. In other species, particularly mouse, rat and rabbit, 4''-hydroxylation was the major biotransformation route undergone by this chain.

TABLE 9

RELATIVE CONCENTRATIONS (EXPRESSED AS A PERCENTAGE OF TOTAL RECOVERED METABOLITES) OF MONOHYDROXY METABOLITES FORMED BY INCUBATION OF HHC WITH HEPATIC MICROSOMES

Metabolite	Mouse	Rat	Guinea Pig	Rabbit	Hamster
1'-OH	—	5	18	3	2
3'-OH	—	T	10	5	2
4'-OH	—	4	25	10	7
5'-OH	3	—	4	3	3
Aromatic	—	T	7	6	2
8alpha-OH	49	43	—	12	20
8beta-OH	5	—	—	—	43
11-OH	43	48	36	61	21

T = Trace.



SCHEME 1. Formation of the major monohydroxy metabolites from methyl-delta-8-THC (IX) and from *abn*-methyl-delta-8-THC (XI).

The only other metabolite of note was a dihydro compound found in rat and cat; hydrogenation had occurred at the terminal double bond of the C-10 chain.

Hexahydrocannabinol

Metabolites produced from five of the species are listed in Table 9. The profiles were again very different from each other, but show the same general trends to those observed for the other cannabinoids. Thus, 11-hydroxylation dominated the profile from rat, rabbit and guinea pig and 8alpha-hydroxylation was very abundant in the mouse. Once again the hamster produced the 8beta-hydroxy metabolite as the major compound and the guinea pig produced substantial concentrations of side-chain hydroxylated metabolites.

Low concentrations of aromatic-hydroxylated metabolites were present in the extracts from several of the preparations. This is the first time that aromatic hydroxylation has been reported for the cannabinoids and the identification of these compounds will be the subject of a further communication.

CONCLUSIONS

These results exemplify the great differences in metabolism that can occur in different species on account of the different profiles of cytochrome P-450 isozymes. As most cannabinoids tend to have similar shapes, it is not surprising that the different compounds tend to show the same pattern of metabolism in a given species. Thus, the tricyclic cannabinoids (THCs and CBN) were extensively hydroxylated at C-11 in all species except cat and hamster. All cannabinoids except CBC were hydroxylated in all positions of the pentyl side-chain to the greatest extent by the guinea pig. Although the cat also preferred side-chain hydroxylation in many cases, this was invariably at C-4'. Hydroxylation of the terpene ring was common; however, whereas the mouse invariably preferred to hydroxylate the alpha face, the hamster preferred the beta face. This suggests that the fit of the molecule to the active site of the enzyme is critical in determining the stereochemistry of the product. It has been assumed above that hydroxylation at the allylic positions is dominant over that at other aliphatic positions in molecules such as the THCs because of greater chemical reactivity of the allylic C-H bonds. This, however, may be coincidence because when the equatorial isomer of HHC was metabolised by this microsomal system, it can be seen that the profile is very similar to that from THC (particularly the delta-9-isomer). Thus C-11 is usually the major site attacked and C-8 is the main site of ring hydroxylation. The stereochemistry of C-8 hydroxylation parallels that seen with THC namely 8alpha in the mouse and 8beta in species such as the hamster and guinea pig. The orientation of the molecule relative to the active site on the enzyme is possibly controlled by the phenolic group. Evidence for this is provided by an earlier study in the comparative metabolism of the normal (IX, Scheme 1) and abnormal (XI) positions of the THC (3). Here it was found that, although the normal isomer was metabolised much like the pentyl homologue, with the major attacked site being at C-11, the abnormal-isomer was mainly hydroxylated at one of the gem-methyl groups on the other side of the molecule, a site not metabolised in the normal-isomer.

It is hoped that further work will identify which P-450 isozyme is responsible for production of the individual hydroxy metabolites and how the molecules fit the active site to produce the individual metabolites.

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