Pharmacology Biochemistry & Behavior, Vol. 40, pp. 523–532. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Comparative Metabolism of Cannabidiol in Dog, Rat and Man

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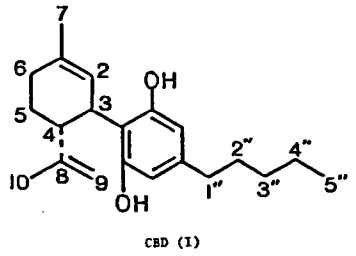
HARVEY, D. J., E. SAMARA AND R. MECHOULAM. *Comparative metabolism of cannabidiol in dog, rat and man.* PHARMACOL BIOCHEM BEHAV **40**(3) 523–532, 1991.—Urinary metabolites of cannabidiol (CBD) were extracted from human, dog and rat urine, concentrated by chromatography on Sephadex LH-20, and identified by GC/MS. Over 50 metabolites were identified with considerable species variation. CBD was excreted in substantial concentration from human urine, both in the free state and as its glucuronide. In dog, unusual glucoside conjugates of three metabolites (4''- and 5''-hydroxy and 6-oxo-CBD), not excreted in the unconjugated state, were found as the major metabolites at early times after drug administration. Other metabolites in all three species were mainly acids. Side-chain hydroxylated derivatives of CBD-7-oic acid were particularly abundant in human urine but much less so in dog. In the latter species the major oxidized metabolites were the products of beta-oxidation with further hydroxylation at C-6. A related, but undefined pathway, resulted in loss of three carbon atoms from the side-chain of CBD in man with the production of 2''-hydroxy-tris,nor-CBD-7-oic acid. Previous experiments indicate that 3'-hydroxy-metabolites are the precursors of compounds having this side-chain. Metabolism by the epoxide-diol pathway, resulting in dihydro-diol formation from the delta-8-double bond, gave metabolites in both dog and human urine. It was concluded that CBD could be used as a probe of the mechanism of several types of biotransformation, particularly those related to carboxylic acid metabolism, as intermediates of the type not usually seen with endogenous compounds were excreted in substantial concentration.

Acids Beta-oxidation Cannabidiol Conjugates Dog Epoxide-diol pathway Glucosides Glucuronides Hydroxylation Man Mass spectrometry Rat Urine

METABOLISM of CBD (I) has been studied in several animal species, particularly in vitro (9, 11, 12, 14, 16, 18, 22–24, 27) but there is little information available on urinary metabolites. This paper describes the identification by GC/MS of urinary metabolites of this drug in three species, compares urinary metabolites with those found from liver preparations and investigates mechanisms for the production of metabolites with oxidised side-chains.



CBD (I)

STRUCTURE I. CBD (I).

METHOD

Materials

CBD was obtained from the National Institute on Drug Abuse (NIDA) and from Makor Chemicals, Israel. Beta-glucuronidases and *N,O-bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Sigma Chemical Co., Poole, Dorset, UK. "Diazald," for the preparation of diazomethane, was obtained from Aldrich Chemical Co., Gillingham, Dorset, UK and methoxyamine hydrochloride from BDH Ltd, Poole, Dorset, UK.

Drug Treatment

Human. Urine (1.5 l) was collected over 24 h from a dystonic patient treated chronically with CBD (600 mg daily) at the Neurology Department, Hadassah-Hebrew University Hospital, Jerusalem (1).

Dogs. Three mongrel dogs (18–26 kg) were treated (in the Jerusalem Laboratories) with CBD (90 mg in 2 ml of 70% ethanol) by injection into the cephalic vein. Urine was collected via an indwelling catheter from the urinary bladder at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 22, 26 and 30 hours after drug administration was stored at −20°C.

Rats. Three male rats (250–350 g) were treated with CBD (5 mg in 0.5 ml of 70% ethanol) by injection into the tail vein. The rats were housed in metabolism cages and urine was collected at 6, 10, 20, and 26 hours after dosing.

Extraction of Metabolites

Metabolites were extracted from the urine (2 ml samples from dogs and rats) with ethyl acetate (3×2 ml for dogs and rats,

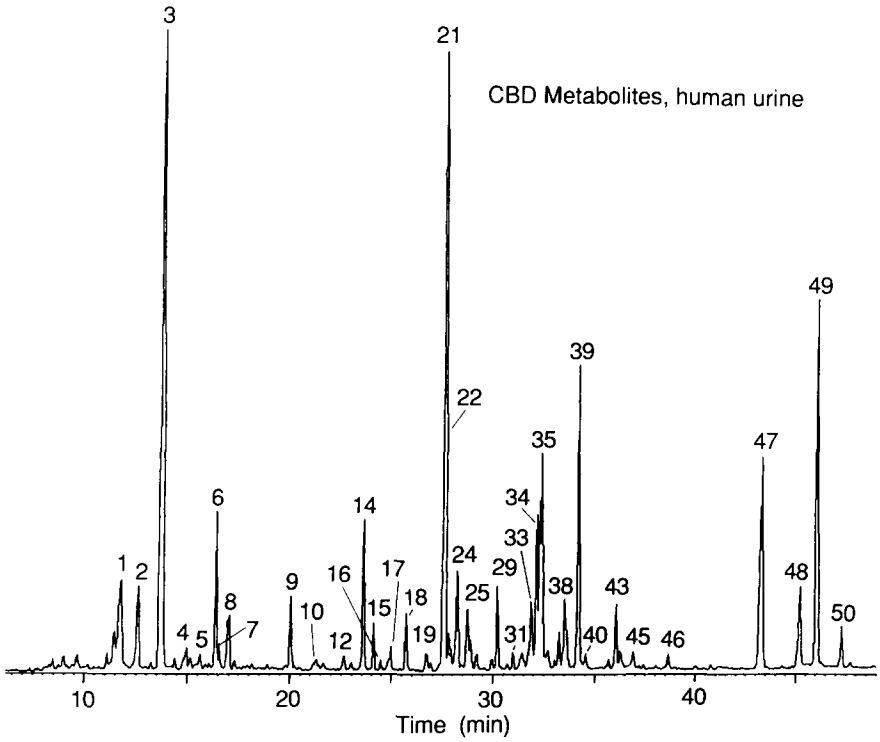


FIG. 1. Reconstructed ion chromatogram (m/z 330–700) of the metabolites of CBD (TMS derivatives) extracted from human urine. Separation was made with a 25 m × 0.2 mm OV-1 fused-silica capillary column operated as described in the Method section. Most peaks were produced by metabolites as identified in Tables 1–3 and in the text. Major peaks not identified are urinary constituents. Peak heights differ from those cited in the text as measured by GLC as the GLC and GC/MS (m/z 330–700) response factors differ.

300 ml for human), both before hydrolysis with beta-glucuronidase [type VII from E. coli, pH 6.8, and additionally with Type HP-2 (from *Helix pomatia*) at pH 5 for the dog urine] for 3 hours at 37°C. The solution was dried over $MgSO_4$ and evaporated to dryness. Half of the extract was derivatized for GC/MS as described below. The other half was chromatographed on Sephadex LH-20 (5 g packed into a 10 mm diameter column) in chloroform and chloroform:methanol mixtures as described earlier (15) in order to concentrate the metabolites. The fraction eluted with a 10% solution of methanol in chloroform was collected and evaporated to dryness under reduced pressure.

Preparation of Derivatives

TMS derivatives. Samples, of about 100 μg, of both the unhydrolysed and hydrolysed ethyl acetate extracts were heated with BSTFA (10 μl) for 10 min at 60°C.

[$^2H_9$]TMS derivatives (21). These were prepared as for the TMS derivatives with [$^2H_{18}$]*bis*-trimethylsilylacetamide replacing the BSTFA.

Methyl ester/TMS derivatives. Methanol (10 μl) was added to the dried sample from the ethyl acetate extract followed by a fresh, ethereal solution (0.5 ml) of diazomethane (prepared from Diazald). The mixture was stirred well and allowed to stand at room temperature for 2 min. The reagent and solvents were removed with a stream of nitrogen (the human sample was first centrifuged to remove the precipitate) and the residue was converted into TMS derivatives as described above.

Methyloxime/TMS derivatives. The dried sample was dissolved in pyridine (0.1 ml) and methoxyamine hydrochloride (about 100 μg) was added. The mixture was heated at 60°C for 1 h, cooled, diluted with water (1 ml) and the metabolites were extracted with ethyl acetate (3 × 1 ml). The combined ethyl acetate extracts were washed with water (1 ml) and saturated sodium chloride solution (2 × 1 ml) and evaporated to dryness with a stream of nitrogen. The residue was reacted with BSTFA as described above.

Reduction with lithium aluminium deuteride. An aliquot of the dried ethyl acetate extract from both hydrolysed and unhydrolysed samples was dissolved in dry (sodium) ether (1.0 ml) and refluxed with an excess of lithium alumnium deuteride for 1 h. The products were extracted with ethyl acetate after destruction of the excess of reagent with damp ether. The solution was then washed with water (1 ml) and satd. aqueous NaCl (2 × 1 ml), the solvent was removed with a stream of nitrogen and the residue was converted into TMS derivatives.

Gas Chromatography

GLC retention data and the relative concentration of the metabolites 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) (Hewlett-Packard). 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 300°C and the column oven was temperature programmed from 130°C to 350°C at 2°C/min. Data were recorded with a Servoscribe flat-bed recorder and with a Hewlett-Packard 3390A recording integrator.

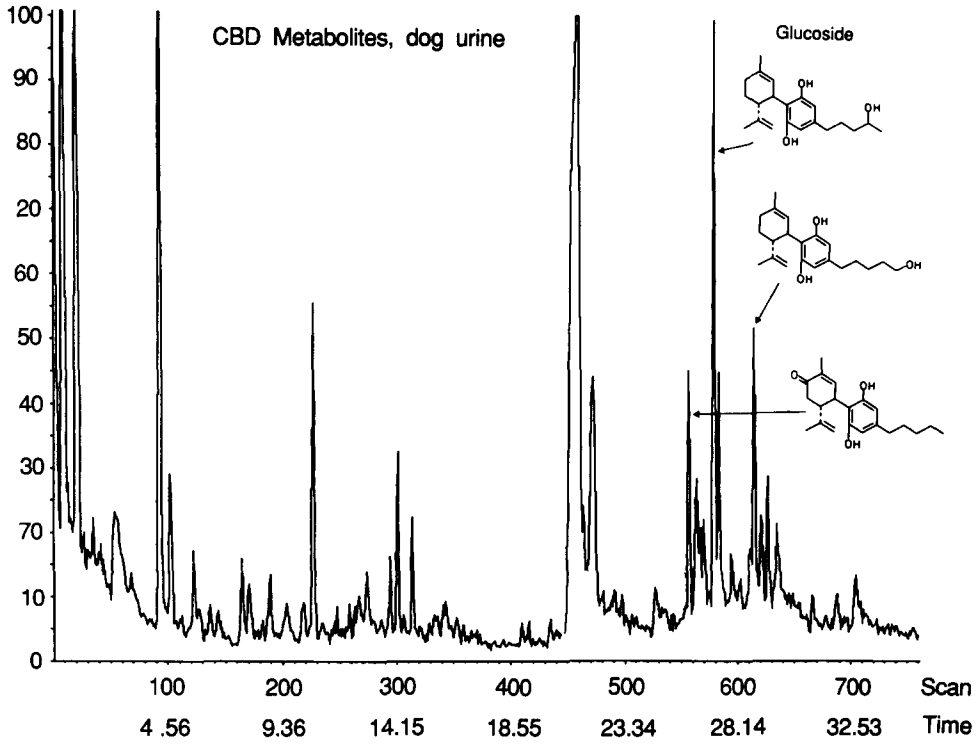


FIG. 2. Reconstructed ion chromatogram (m/z 330–700) of metabolites of CBD (TMS derivatives) from the dog taken 3 hours after drug administration showing the glucoside metabolites. Separation was made with a 25 m × 0.2 mm OV-1 fused-silica capillary column operated as described in the Method section. Most peaks were produced by metabolites as identified in Tables 1–3 and in the text. Major peaks not identified are urinary constituents.

GC/MS

GC/MS data were recorded with a VG 70/70F double focussing-mass spectrometer connected to a Varian 2440 gas chromatograph. The column was a 25 m × 0.2 mm DB-1 bonded-phase fused silica capillary (film thickness 0.33 μm) (J and W Scientific) 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. The column oven was temperature programmed from 220°C to 320°C at 2°/min. Other operating conditions were: injector, transfer line and ion source temperatures, 300, 300, and 250°C respectively, electron energy, 70 eV, trap current, 1 mA; accelerating voltage, 4 kV. An accelerating voltage of 3 kV was used to record spectra of the metabolites with molecular weights in excess of 700 Daltons. The instrument was scanned repetitively at 1 s/decade under the control of the VG 11:250 data system.

RESULTS

Identification of Metabolites

General. Few reference compounds are available for metabolites of CBD. Thus metabolites were identified by GC/MS using data from the literature and the spectra of different derivatives; these were used to identify the functional groups such as carboxylic acids (formation of a methyl ester). Full details have been published (6). The lithium aluminium deuteride reaction was used to correlate structures of metabolites in different oxidation states (alcohols, acids, etc.) as described earlier by reducing them to alcohols with diagnostic deuterium incorporation (17). Interpretation of the spectra of CBD metabolites was usually straightforward on account of the production of an abundant retro-Diels-Alder ion (usually the base peak) and a tropylium ion which located the metabolic changes to specific areas of the molecule [see (6)]. Six-, 11-, and side-chain hydroxylation was determined by the production of abundant diagnostic ions.

Metabolites Identified in Human Urine

Figure 1 shows the limited ion chromatogram of metabolites of CBD found in unhydrolysed human urine. Peaks are identified in Tables 1–3. Full details of the peak identification have been published (16) and only a summary of the findings will be reproduced here. One of the major excreted compounds (peak 3, 12.1% of the total excreted cannabinoids as determined by GLC) was identified as unmetabolized CBD (I) by the identity of its mass spectrum (TMS derivative) with that of an authentic sample. This compound was also excreted as its O-glucuronide (peak 49, 13.3%) which was identified by comparison of its mass spectrum (TMS and Me/TMS derivatives) with published data (14).

Other nonoxidized cannabinoids. The compounds producing peaks 6 (1.97%) and 8 (0.69%) were identified, by their mass spectra [TMS derivatives (6) and retention times], as delta-8- and delta-9-THC respectively and were presumably formed by cyclisation of CBD. The absence of metabolites of the THCs in the urine would suggest that cyclisation had occurred after excretion. The aromatic compound, cannabinol (CBN), was also identified as the compound producing peak 9 (0.6%).

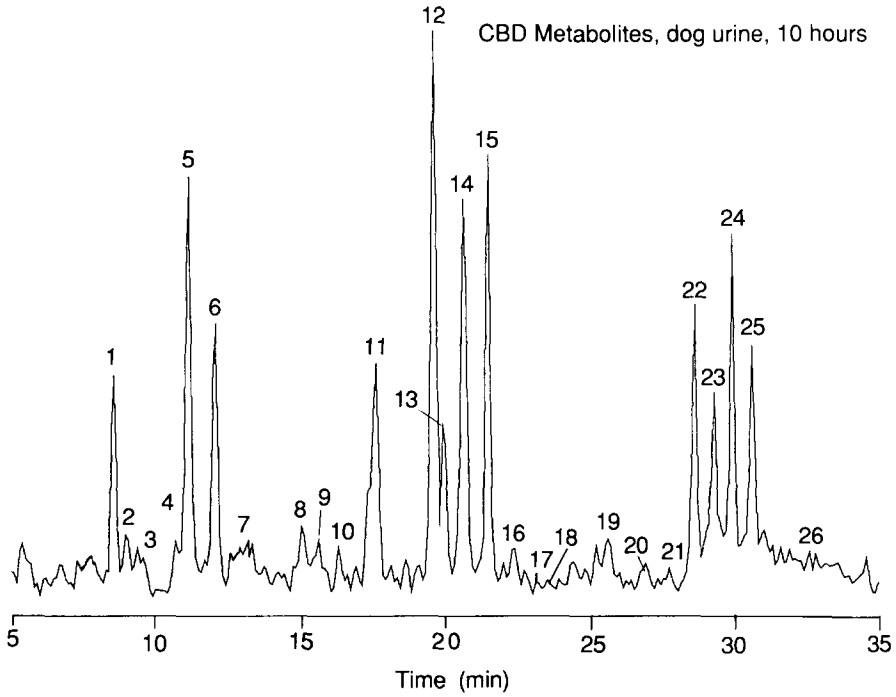


FIG. 3. Reconstructed ion chromatogram (m/z 330–700) of the metabolites (TMS derivatives) extracted from canine urine 10 hours after dosing. Separation was made with a 25 m × 0.2 mm OV-1 fused-silica capillary column operated as described in the Method section. Most peaks were produced by metabolites as identified in Tables 1–3 and in the text. Major peaks not identified are urinary constituents.

Oxidised metabolites.

Acids and hydroxy acids. Major metabolites in this sample were identified as side-chain hydroxylated derivatives (XII–XIX) of CBD-7-oic acid. The parent acid (XI) was identified as the compound giving peak 18. The resolution of the capillary column was such that the 1''-(XII, XIII), 2''-(XIV, XV) and 3''-hydroxy derivatives (XVI, XVII) were separated into two peaks corresponding, presumably, to the R and S forms of the alcohol. Other than one earlier report of the resolution of 1''-hydroxy-isomers (24), this is the first time that evidence has been presented to show that both R and S alcohols are produced metabolically. The compound producing peak 35 was identified as 10-hydroxy-CBD-7-oic acid (XX), the first example of a CBD metabolite hydroxylated in the isopropenyl chain.

The compound producing peak 14 was a carboxylic acid with a mass spectrum very much like that of CBD-7-oic acid except that all peaks were 4 units lower in mass. Its mass spectrum showed that the carboxylic acid group was located at C-7 and the mass of the tropylium ion (m/z 333) showed that the metabolic change had occurred in the side-chain. Corresponding compounds in dog urine (see below) contained a hydroxy group at C-6 and it was found that the metabolite was not reduced with lithium aluminium hydride. Although the metabolite was not identified, a structure with double unsaturation in the side-chain seems most likely. The metabolic production of aliphatic unsaturation is known to occur with other drugs such as valproic acid (26).

Compounds containing side-chains with reduced numbers of carbon atoms. Peaks 5 and 13 (Fig. 1) were mono-carboxylic acids that had lost 4 and 2 carbon atoms respectively from their side-chains to leave an acid group (compounds XXVI and XXVIII). Various 6- and 7-hydroxylated analogues of these acids were also identified in a similar manner and are listed in Table 2. The major metabolite (peak 21) had a molecular weight and reactivity towards diazomethane and [$^2H_{18}$]BSA indicating that it was a hydroxy-acid that had lost three carbon atoms from the side-chain. It was identified as 2''-hydroxy-*tris,nor*-CBD-7-oic acid (XL) and its 6- (XLII) and 7-hydroxy (XLI, peak 17) derivatives were also identified.

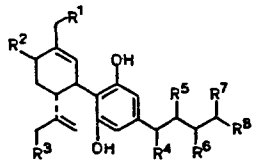
Metabolites produced by the epoxide-diol pathway. Two compounds of this type were found (peaks 37 and 43) They were formed by reaction of the delta-8-double bond to give a dihydro-diol structure (Table 3). Mass spectrometric fragmentation of these compounds differed from that of the metabolites discussed above in that fragmentation occurred more favourably from the dihydroxy-diol moiety than from the terpene ring (11). The compound producing peak 43 was an acid derivative of 8,9-dihydro-8,9-dihydroxy-CBD with the acid group at C-7 (XLIX) and the compound producing peak 37 was identified as its hydroxy analogue (XLIV).

Dog

Conjugates. Figure 2 shows a limited ion chromatogram (masses 300–700) of the metabolites present in the ethyl acetate extract of the unhydrolysed urine taken at 3 hours after dosing. The peaks eluting at around 30 min were also present in the fraction hydrolysed by beta-glucuronidase type VII but not in that hydrolysed by beta-glucuronidase type HP-2. This indicates that they are carbohydrate conjugates and this was confirmed by their mass spectra which showed typical sugar-derived ions (14,20). However, these were characteristic of a nonacidic sugar. The compounds did not react with diazomethane confirming the absence of an acidic group. They were confirmed as glucosides

TABLE 1

STRUCTURES AND QUANTITIES PRESENT IN URINE OF THE METABOLITES OF CBD CONTAINING AN INTACT SIDE-CHAIN



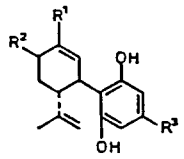
Compound	No.	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
7-OH	II	CH_2OH	H	H	H	H	H	H	CH_3
6-OH	III	CH_3	OH	H	H	H	H	H	CH_3
4″,6-di-OH	IV	CH_3	OH	H	H	H	H	OH	CH_3
4″,7-di-OH	V	CH_2OH	H	H	H	H	H	OH	CH_3
5″,6-di-OH	VI	CH_3	OH	H	H	H	H	H	CH_2OH
6,7-di-OH	VII	CH_2OH	OH	H	H	H	H	H	CH_3
2″,6,7-tri-OH	VIII	CH_2OH	OH	H	H	H	H	H	CH_2OH
4″,6,7-tri-OH	IX	CH_2OH	OH	H	H	H	H	OH	CH_3
5″,6,7-tri-OH	X	CH_2OH	OH	H	H	H	H	H	CH_2OH
7-COOH	XI	COOH	H	H	H	H	H	H	CH_3
1″-OH,7-COOH	XII	COOH	H	H	OH	H	H	H	CH_3
1″-OH,7-COOH	XIII	COOH	H	H	OH	H	H	H	CH_3
2″-OH,7-COOH	XIV	COOH	H	H	H	OH	H	H	CH_3
2″-OH,7-COOH	XV	COOH	H	H	H	OH	H	H	CH_3
3″-OH,7-COOH	XVI	COOH	H	H	H	H	OH	H	CH_3
3″-OH,7-COOH	XVII	COOH	H	H	H	H	OH	H	CH_3
4″-OH,7-COOH	XVIII	COOH	H	H	H	H	H	OH	CH_3
5″-OH,7-COOH	XIX	COOH	H	H	H	H	H	H	CH_2OH
10-OH,7-COOH	XX	COOH	H	OH	H	H	H	H	CH_3
7-OH,5″-COOH	XXI	CH_2OH	H	H	H	H	H	H	COOH
6-OH,5″-COOH	XXII	CH_3	OH	H	H	H	H	H	COOH
6-=O,5″-COOH	XXIII	CH_3	=O	H	H	H	H	H	COOH
6,7-di-OH,5″-COOH	XXIV	CH_2OH	OH	H	H	H	H	H	COOH
5″,7-di-COOH	XXV	COOH	H	H	H	H	H	H	COOH

Concentration			Peak		
Dog	Rat	Man	Fig. 4	Fig. 5	Fig. 1
+*	–	+	4	–	15
++	–	+	1	–	12
+++	+	–	15	8	–
+	–	–	17	–	–
+	–	–	18	–	–
+	–	–		–	–
++	–	–	19	–	–
+++	+	–	23	14	–
++	+	–		18	–
–	–	++	–	–	18
–	–	+++	–	–	21
–	–	++	–	–	23
+	–	+++	–	–	33
		+++	–	–	34
–	–	+++	–	–	37
–	–	+++	–	–	38
++	+++	+++	22	13	39
–	++	–	–	17	–
–	–	+++	–	–	35
+	–	+	26	–	44
++	–	+	24	–	41
++	–	–	25	–	–
+	–	+	33	–	–
++	+	+	32	21	–

*+++ = major metabolite; + = minor metabolite.

TABLE 2

STRUCTURES AND QUANTITIES PRESENT IN URINE OF THE METABOLITES OF CBD CONTAINING A SHORTENED SIDE-CHAIN



Compound	No.	R^1	R^2	R^3	Concentration			Peak	
					Dog	Rat	Man	Fig. 4	Fig. 5	Fig. 1
1″-COOH,2″,3″,4″,5″-*nor*	XXVI	CH_3	H	COOH	–	–	+	–	–	5
2″-COOH,3″,4″,5″-*nor*	XXVII	CH_3	H	CH_2COOH	–	–	+	–	–	
3″-COOH,4″,5″-*nor*	XXVIII	CH_3	H	$(CH_2)_2COOH$	–	–	+	–	–	13
4″-COOH,5″-*nor*	XXIX	CH_3	H	$(CH_2)_3COOH$	–	–	+	–	–	
6-OH-1″-COOH,2″,3″,4″,5″,-*nor*	XXX	CH_3	OH	COOH	+++	–	+	5	–	16
6,7-di-OH-1″-COOH,2″,3″,4″,5″-*nor*	XXXI	CH_2OH	OH	COOH	+	–	–		–	–
7-OH,1″-COOH,2″,3″,4″,5″-*nor*	XXXII	CH_2OH	H	COOH	–	++	+	–	1	17
6-OH,3″-COOH,4″,5″-*nor*	XXXIII	CH_3	OH	$(CH_2)_2COOH$	+++	+++	++	12	4	25
7-OH,3″-COOH,4″,5″-*nor*	XXXIV	CH_2OH	H	$(CH_2)_2COOH$	+	++	+++	16	6	29
6,7-di-OH,3″-COOH,4″,5″-*nor*	XXXV	CH_2OH	OH	$(CH_2)_2COOH$	+	++	–		12	–
6-=O,3″-COOH,4″,5″-*nor*	XXXVI	CH_3	=O	$(CH_2)_2COOH$	+++	–	–	14	–	–
3″,7-di-COOH,4″,5″-*nor*	XXXVII	COOH	H	$(CH_2)_2COOH$	++	+++	–	20	11	–
7-OH,4″-COOH,5″-*nor*	XXXVIII	CH_2OH	H	$(CH_2)_3COOH$	–	–	+	–	–	
6-OH,4″-COOH,5″-*nor*	XXXIX	CH_3	OH	$(CH_2)_3COOH$	–	–	++	–	–	32
2″-OH,7-COOH,3″,4″,5″-*nor*	XL	COOH	H	CH_2CH_2OH	–	++	+++	–	3	21
2″,7-di-OH,3″,4″,5″-*nor*	XLI	CH_2OH	H	CH_2CH_2OH	–	–	+	–	–	17
2″,6-di-OH,3″,4″,5″-*nor*	XLII	CH_3	OH	CH_2CH_2OH	–	–	+	–	–	

by the identification of glucose as its TMS derivative following GC/MS analysis of the aqueous fraction (27) resulting from hydrolysis with beta-glucuronidase Type HP-2. The compounds were identified as the glucosides of 4′′- and 5′′-hydroxy-CBD and 6-oxo-CBD, compounds not produced as metabolites in the unconjugated state. The formation, by TMS migration, of ions in their mass spectra (TMS derivatives) corresponding to the TMS derivatives of the unconjugated aglycones, confirmed the point of attachment of the sugar as the phenol group of CBD (20). These conjugates were not seen in samples taken at later times or in samples of urine from the other two species. Full details of the identification of these conjugates has been published (27).

Oxidized metabolites. Figure 3 shows the reconstructed ion chromatogram of metabolites extracted from a urine sample taken at 10 hours from one of the dogs. All three dogs gave metabolic profiles that were very similar. Most of the metabolites were acids as shown by their reaction with diazomethane and most of these were formed by beta-oxidation of the side-chain as shown by the masses of the tropylium ions. In addi-

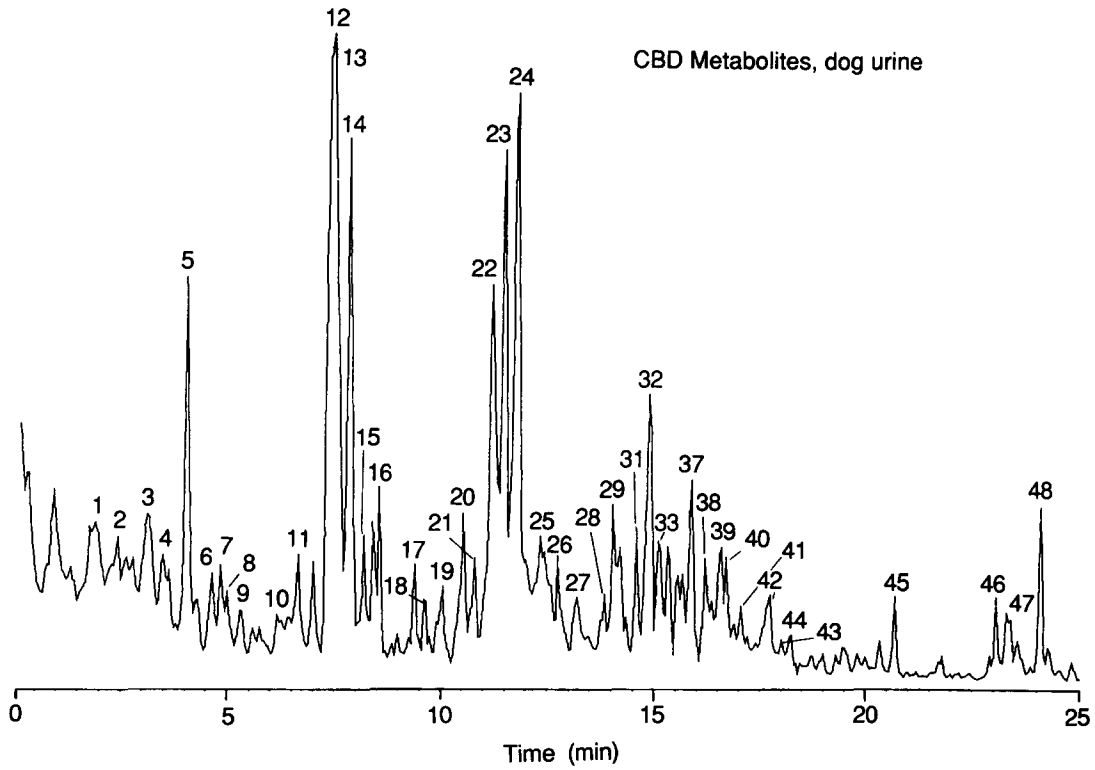
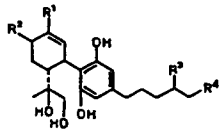


FIG. 4. Reconstructed ion chromatograph (m/z 330–700) of the metabolites (TMS derivatives) extracted from a sample of canine urine obtained by pooling an aliquot from each of the timed samples. Separation was made with a 25 m × 0.2 mm OV-1 fused-silica capillary column operated as described in the Method section. Most peaks were produced by metabolites as identified in Tables 1–3 and in the text. Major peaks not identified are urinary constituents.

TABLE 3

STRUCTURES OF THE 8,9-DIHYDRO,8,9-DIHYDROXY-METABOLITES OF CBD



Compound	No.	R^1	R^2	R^3	R^4
7,8,9-tri-OH	XLIII	CH_2OH	H	H	H
6,7,8,9-tetra-OH	XLIV	CH_2OH	OH	H	H
4″,6,8,9-tetra-OH	XLV	CH_3	OH	OH	H
5″,6,8,9-tetra-OH	XLVI	CH_3	OH	H	OH
4″,7,8,9-tetra-OH	XLVII	CH_2OH	OH	OH	H
5″,7,8,9-tetra-OH	XLVIII	CH_2OH	OH	H	OH
7-COOH,8,9-di-OH	XLIX	COOH	H	H	H

Concentration				Peak	
Dog	Rat	Man	Fig. 10,11	Fig. 12	Fig. 3
–	–	+	–	–	37
++	–	–	29	–	–
++	–	–	31	–	–
++	–	–	37	–	–
+++	–	–	32	–	–
+	–	–	38	–	–
–	–	++	–	–	43

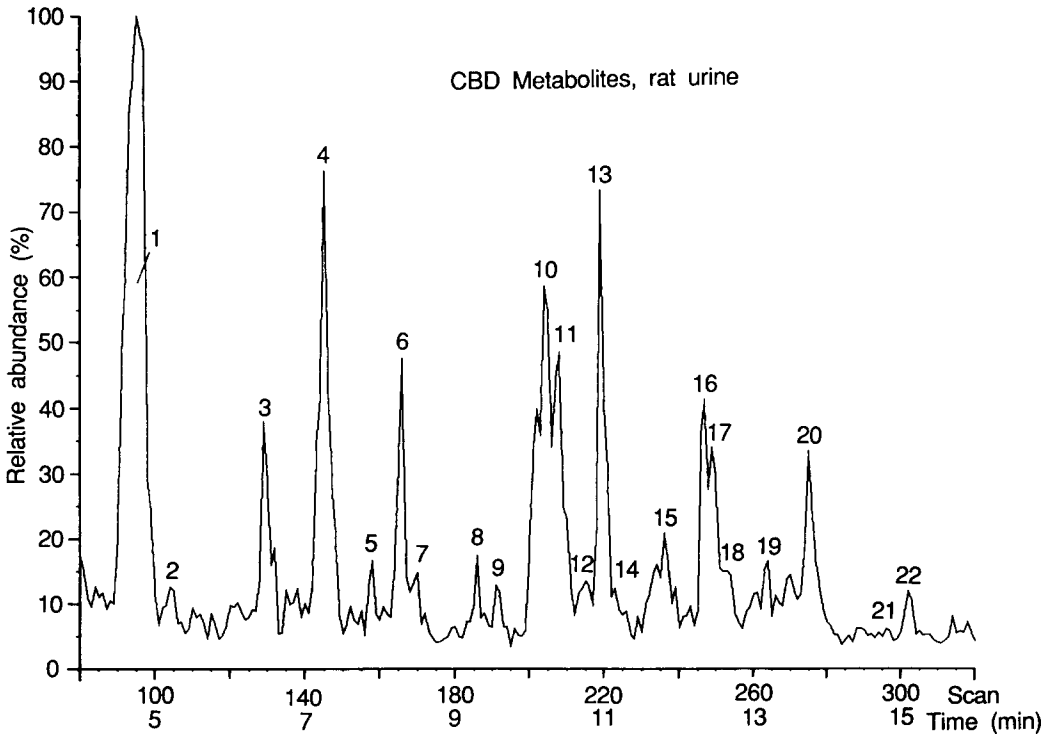


FIG. 5. Reconstructed ion chromatogram (m/z 330–700) of the metabolites (TMS derivatives) extracted from rat urine. Separation was made with a 25 m × 0.2 mm OV-1 fused-silica capillary column operated as described in the Method section. Most peaks were produced by metabolites as identified in Tables 1–3 and in the text. Major peaks not identified are urinary constituents.

tion, the majority of compounds contained a 6-hydroxy group (both alpha and beta isomers). 6-Oxo-CBD-4″,5″-*bis,nor*-CBD-3″-oic acid (XXXVI, Table 2) was also identified and its identity confirmed by preparation of the methyloxime derivative. Major identified compounds are listed in Table 2. Peak 15 was produced by 4″,6-dihydroxy-CBD (IV) and was the only compound whose peak intensity rose significantly after hydrolysis of the urine with beta-glucuronidase.

Metabolites produced by the epoxide-diol pathway. Figure 4 shows a chromatogram of metabolites obtained by combining aliquots of the urine samples taken at each time point. It contained, in addition to the compounds shown in Fig. 3, a series of metabolites (XLIV–XLVIII) formed by the epoxide-diol pathway involving the delta-8 double bond. These metabolites are listed in Table 3.

Rat. The reconstructed ion chromatogram of metabolites found in rat urine is shown in Fig. 5. No intact glucuronides or glucosides were found. The major metabolites were acids as shown by their reaction with diazomethane. Beta-oxidation was again prominent with most identified compounds containing hydroxylation at either C-6 or C-7. A larger proportion of metabolites was hydroxylated at C-7 than was found in the dog. Identified compounds are listed in Tables 1 and 2. Full details of the mass spectra will be published elsewhere.

DISCUSSION

Metabolism of CBD showed biotransformation routes reasonably typical for cannabinoids in general (9,18) with multiple hydroxylations, conjugations, oxidations to carboxylic acids and beta-oxidation reactions dominating the profile. Fifty-three metabolites were identified in the three species, but each individual species favoured different biotransformation pathways. Thus conjugates were not found in the rat urine, but a substantial proportion of the dose appeared as CBD glucuronide in human urine. In the dog, the glucoside conjugates of 4″- and 5″-hydroxy-CBD and 6-oxo-CBD, dominated the profile at early times. Glucose conjugates are unusual in mammalian systems and have only been identified on a few occasions [see (25) and citations therein]. It is possibly significant that in several of these earlier reports (2, 3, 25), most of these glucose conjugates were observed in the dog.

Most of the other metabolites were acids. These were of three types, hydroxy derivatives of CBD-7-oic acid (XII–XX), products of beta-oxidation, and related compounds with a degraded side-chain in which the carboxylic acid group was at C-7. The hydroxylated derivatives of CBD-7-oic acid were most numerous and abundant in human urine. The hydroxy group was found at each position of the side-chain and resolution on the fused-silica capillary column was sufficient to show that the 1″-, 2″- and 3″-hydroxy derivatives of CBD-7-oic acid were produced as R and S isomers. The abundance of 2″-hydroxy-CBD-7-oic acid (XIV, XV) was unusually high; in most species except the mouse, hydroxylation at C-2″ is usually a very minor biotransformation route for cannibinoids (22). Many similar acids have been found as metabolites of delta-9-THC in man (4,5).

In rat and dog urine, the major acids were products of beta-oxidation with metabolites formed by one stage of oxidation being the most common. In the dog, these were further hydroxylated mainly at C-6, whereas in rat and human, hydroxylation at C-7

was favoured. Products of two stages of beta-oxidation increased with time and there was also a greater tendency for oxidation of the 7-hydroxy group to a carboxylic acid with time in the rat. Thus the *bis*-acid, 4'',5''-*bis,nor*-CBD-3'',7-di-oic acid (XXX-VII) was reasonably abundant after 24 hours.

The third type of acid, containing a carboxylic acid at C-7 and a hydroxylated side-chain with a reduced number of carbon atoms, was particularly abundant in human urine with 2''-hydroxy-3'',4'',5''-*tris,nor*-CBD-7-oic acid (XL) being a major metabolite. These compounds were not products of the normal beta-oxidation pathway involving a 5'-hydroxy-intermediate as demonstrated by earlier work with delta-9-THC (13) which showed that metabolism of 5'-hydroxy-delta-9-THC and delta-9-THC-5'-oic acid, the normal intermediates in beta-oxidation, did not yield the metabolites with a hydroxy-side-chain. On the other hand, metabolism of 3'-hydroxy-delta-9-THC (7) did yield these products, whereas metabolism of 2'- (7) and 4'-hydroxy-delta-9-THC (8) did not. This strongly suggests that formation of 2''-hydroxy-3'',4'',5''-*tris,nor*-CBD-7-oic acid from CBD involves initial hydroxylation at C-3'', but the subsequent steps leading to loss of three carbon atoms from the side-chain have yet to be clarified. Supporting the intermediacy of 3''-hydroxy-CBD was the observation of its production in higher relative concentration in human urine than in the urine of dogs or rats. Another observation made during the study of the metabolism of 3'- and 4'-hydroxy-delta-9-THC was that 4',5'-*bis,nor*-delta-9-THC-3''-oic acid, and its hydroxylated derivatives were also formed as metabolites of these compounds. These are the products of normal beta-oxidation derived from 5'-hydroxy-delta-9-THC and the reactions demonstrated that there are several routes to these metabolites with shorter acids other than the classical beta-oxidation.

Another metabolic route seen in dog and human urine was epoxidation and subsequent hydroxylation of the delta-8-double bound to give 8,9-dihydro-8,9-dihydroxy metabolites. No metabolites were found in which epoxidation had occurred at the delta-1 bond probably because this epoxide appears to be unstable as recently demonstrated by Yamamoto et al. (28,29) who showed that it readily breaks down to give cannabielsoin. In human urine, these 8,9-dihydroxy-compounds were further biotransformed, particularly by acid formation, at C-7, whereas in the dog, hydroxylation and particularly dihydroxylation at C-6, C-7 or in the side-chain, was preferred.

CONCLUSIONS

These results highlight a number of features about drug metabolism and suggest ways in which CBD and other cannabinoids can give considerable information on biotransformation pathways. Firstly, although different metabolic profiles were observed from each animal, metabolism showed the same overall trends, namely hydroxylation, oxidation to carboxylic acids, beta-oxidation and related pathways, conjugation and epoxidation. Study of the relative extents to which these pathways differ, such as the positions of the molecule hydroxylated, can give valuable information on the different enzymes present in the various species. Cannabinoids are particularly valuable in this respect as they have multiple hydroxylation sites, all of which are attacked by cytochrome P-450.

Secondly, conjugation is extensive and can involve more than one sugar in a species-dependent manner. However, as pointed out in our original communication on glucose conjugates (27), the failure to observe these for some other compounds may be related to methodology; enzymatic hydrolysis by beta-glucuronidase from *H. pomatia*, and comparison of metabolic profiles with those without hydrolysis, is the usual method of inferring the presence of glucuronides. However, as shown here, glucosides are also hydrolysed by this enzyme and it is only with the more powerful technique of GC/MS and the examination of intact conjugates that the presence of these compounds can be conclusively demonstrated. Conjugation with fatty acids, although not observed here, was first observed with the cannabinoids (19) and provides a potent means of increasing lipophilicity and, hence, tissue accumulation.

Thirdly, because the cannabinoids are not ideal substrates for enzymes mediating beta-oxidation, reactions are slower and intermediates of the type found in this work are not usually seen with endogenous substrates. This has been used to show that the product of one stage of beta-oxidation in cannabinoids can occur, not only from the omega (5''-) hydroxy intermediate, but also from omega-1 (4''-hydroxy) and omega-2 (3''-hydroxy) intermediates. In the present study, metabolism of CBD has also shown a new product of a related oxidation mechanism, 2''-hydroxy-*tris,nor*-CBD-7-oic acid.

Finally, though again not shown here, the effect of lipophilicity changes on metabolism, and particularly on such factors as oxidation of alcohols to carboxylic acids (10), is easily studied as homologous cannabinoids are readily synthesised and undergo transformations similar to those of the naturally occurring compounds with C_5 side-chains.

ACKNOWLEDGEMENTS

This work was supported by NIDA (Grant No. DA04005). We thank Dr. R. L. Hawkes of NIDA for supplies of the reference cannabinoids. The mass spectrometric equipment was originally purchased with a grant from the Medical Research Council. Funds from the Wellcome Trust are also acknowledged.

REFERENCES

1. Consroe, P.; Snider, S. R. Therapeutic potential of cannabinoids in neurological disorders. In: Mechoulam, R., ed. Cannabinoids as therapeutic agents. Boca Raton, FL: CRC Press; 1986:21.
2. Duggan, D. E.; Baldwin, J. J.; Arison, B. H.; Rhodes, R. E. *N*-glucoside formation as a detoxification mechanism in mammals. J. Pharmacol. Exp. Ther. 190:563–569; 1974.
3. Hakusui, H.; Suzuki, W.; Takegoshi, T. Studies on pharmacokinetics and biotransformation of oxepinac in mouse, rat, dog, and man. Arzneimittelforschung 28:456–461; 1978.
4. Halldin, M. M.; Carlson, S.; Kanter, S. L.; Widman, M.; Agurell, S. Urinary metabolites of delta-1-tetrahydrocannabinol in man. Arzneimittelforschung 32:764–768; 1982.
5. Halldin, M. M.; Andersson, L. K. R.; Widman, M.; Hollister, L. E. Further urinary metabolites of delta-1-tetrahydrocannabinol in man. Arzneimittelforschung 32:1135–1138; 1982.
6. Harvey, D. J. Mass spectrometry of the cannabinoids and their metabolites. Mass Spectrom. Rev. 6:135–229; 1987.
7. Harvey, D. J. Further studies on the oxidative cleavage of the pentyl side-chain of cannabinoids: identification of new biotransformation pathways in the metabolism of 3''-hydroxy-delta-9-tetrahydrocannabinol by the mouse. Xenobiotica 19:1437–1447; 1989.
8. Harvey, D. J. Oxidative cleavage of the pentyl side-chain of cannabinoids; identification of new biotransformation pathways in the metabolism of 4''-hydroxy-delta-9-tetrahydrocannabinol by the mouse. Drug Metab. Dispos. 18:350–355; 1990.
9. Harvey, D. J. Metabolism and pharmacokinetics of the cannabinoids. Biochem. Physiol. Substance Abuse 3:279–365; 1991.
10. Harvey, D. J.; Brown, N. K. Metabolism of delta-8- and delta-9-tetrahydrocannabinol homologues in mice. In: Chesher, G.; Consroe, P.; Musty, R., eds. Marijuana: An international research report. Canberra: National Campaign Against Drug Abuse, Australian Government Printing Service; 1988:253–258.

11. Harvey, D. J.; Brown, N. K. In vitro metabolism of cannabidiol in the rabbit: Identification of seventeen new metabolites including thirteen dihydroxylated in the isopropenyl chain. Biomed. Environ. Mass Spectrom. 19:559–567; 1990.
12. Harvey, D. J.; Brown, N. K. In vitro metabolism of cannabidiol in seven common laboratory mammals. Res. Commun. Subst. Abuse 11:27–37; 1990.
13. Harvey, D. J.; Leuschner, J. T. A. Studies on the beta-oxidative metabolism of delta-1- and delta-6-tetrahydrocannabinol in the mouse: the in vivo biotransformation of metabolites oxidized in the side-chain. Drug Metab. Dispos. 13:215–219; 1985.
14. Harvey, D. J.; Martin, B. R.; Paton, W. D. M. Identification of the glucuronides of cannabidiol and hydroxycannabidiols in mouse liver. Biochem. Pharmacol. 25:2217–2219; 1976.
15. Harvey, D. J.; Martin, B. R.; Paton, W. D. M. Identification of di- and tri-substituted hydroxy and ketone metabolites of delta-1-tetrahydrocannabinol in mouse liver. J. Pharm. Pharmacol. 29:482–486; 1977.
16. Harvey, D. J.; Mechoulam, R. Metabolites of cannabidiol identified in human urine. Xenobiotica 20:303–320; 1990.
17. Harvey, D. J.; Paton, W. D. M. The use of deuterium labelling in structural and quantitative studies of tetrahydrocannabinol metabolites by mass spectrometry. Adv. Mass Spectrom. 8:1194–1203; 1980.
18. Harvey, D. J.; Paton, W. D. M. Metabolism of the cannabinoids. Rev. Biochem. Toxicol. 6:221–264; 1984.
19. Leighty, E. G.; Fentiman, A. F., Jr.; Foltz, R. L. Long-retained metabolites of delta-9- and delta-8-tetrahydrocannabinols identified as novel fatty acid conjugates. Res. Commun. Chem. Pathol. Pharmacol. 14:13–28; 1976.
20. Lyle, M. A.; Pallante, S.; Head, K.; Fenselau, C. Synthesis and characterization of glucuronides of cannabinol, cannabidiol, delta-9-tetrahydrocannabinol and delta-8-tetrahydrocannabinol. Biomed. Mass Spectrom. 4:190–196; 1977.
21. McCloskey, J. A.; Stillwell, R. N.; Lawson, A. M. Use of deuterium labelled trimethylsilyl derivatives in mass spectrometry. Anal. Chem. 40:233–236; 1968.
22. Martin, B.; Agurell, S.; Nordqvist, M.; Lindgren. J.-E. Dioxygenated metabolites of cannabidiol formed by a rat liver. J. Pharm. Pharmacol. 28:603–608; 1976.
23. Martin, B. R.; Harvey, D. J.; Paton, W. D. M. Biotransformation of cannabidiol in mice: identification of new acid metabolites. Drug Metab. Dispos. 5:259–267; 1977.
24. Martin, B.; Nordqvist, M.; Agurell, S.; Lindgren, J.-E.; Leander, K.; Binder, M. Identification of monohydroxylated metabolites of cannabidiol formed by a rat liver. J. Pharm. Pharmacol. 28:275–279; 1976.
25. Nakano, K.; Ando, H.; Sugawara, Y.; Ohashi, M.; Harigaya, S. Hopantenic acid beta-glucoside as a new urinary metabolite of calcium hopantenate in dogs. Drug Metab. Dispos. 14:740–745; 1986.
26. Rettenmeier, A. W.; Gordon, W. P.; Prickett, K. S.; Levy, R. H.; Lockard, J. S.; Thummel, K. E.; Baillie, T. A. Metabolic fate of valproic acid in the Rhesus monkey. Formation of a toxic metabolite, 3-*n*-propyl4-pentenoic acid. Drug Metab. Dispos. 14:443–453; 1986.
27. Samara, E.; Bialer, M.; Harvey, D. J. Identification of glucose conjugates as major urinary metabolites of cannabidiol in the dog. Xenobiotica 20:177–183; 1990.
28. Yamamoto, I.; Gohda, H.; Narimatsu, S.; Yoshimura, H. Identification of cannabielsoin, a new metabolite of cannabidiol formed by guinea pig hepatic microsomal enzymes and its pharmacological activity in mice. J. Pharmacobio-Dyn. 11;833–838: 1988.
29. Yamamoto, I.; Gohda, H.; Narimatsu, S.; Yoshimura, H. Mechanism of biological formation of cannabielsoin from cannabidiol in the guinea-pig, mouse, rat and rabbit. J. Pharmacobio-Dyn. 12:488–494; 1989.