

## *In Vivo* and *in Vitro* Metabolism of Cannabidiol Monomethyl Ether and Cannabidiol Dimethyl Ether in the Guinea Pig: On the Formation Mechanism of Cannabielsoin-Type Metabolite from Cannabidiol

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Oxidative metabolism of cannabidiol monomethyl ether (CBDM), one of the components of marihuana, was studied in the guinea pig. Cannabielsoin monomethyl ether (CBEM) was found to be formed with hepatic microsomes by gas chromatography-mass spectrometry (GC-MS). Experiments using various modifiers of enzymatic reaction suggested that, as in the case of cannabielsoin (CBE) formation from cannabidiol (CBD), CBEM was formed from CBDM by the monooxygenase system including cytochrome P450. When cannabidiol dimethyl ether (CBDD), in which phenolic hydroxyl groups of CBD are masked with methyl groups, was incubated with liver microsomes and an reduced nicotinamide adenine dinucleotide phosphate-generating system, 1*S*,2*R*-epoxy-CBDD was identified by GC-MS. The epoxy metabolite was also found in the liver of a guinea pig pretreated with CBDD (100 mg/kg, intraperitoneally) 1 h before sacrifice. Rate of 1*S*,2*R*-epoxide metabolism was slower than that of 1*R*,2*S*-epoxy-CBDD under the conditions, as in the microsomal oxidation of CBDD described above. These results indicate that 1*S*,2*R*-epoxides are formed from CBD, CBDM and CBDD and that the epoxides are quickly converted to elsoin-type metabolites in the cases of CBD and CBDM.

**Keywords** cannabidiol monomethyl ether; cannabidiol dimethyl ether; 1*S*,2*R*-epoxy-intermediate; cannabielsoin monomethyl ether; *in vitro* metabolism; *in vivo* metabolism; monooxygenase; elsoin-type metabolite

### Introduction

Cannabidiol (CBD), one of the major components of marihuana, is a promising anticonvulsant candidate.<sup>1,2</sup> Metabolism of CBD has been studied in various animal species.<sup>3-6</sup> Recently, we found cannabielsoin (CBE) as a novel metabolite of CBD in the guinea pig, and deduced that CBE might be biotransformed from CBD *via* 1*S*,2*R*-epoxy-CBD by the hepatic microsomal monooxygenase system including cytochrome P450.<sup>7,8</sup> Since the epoxides of tetrahydrocannabinols, psychoactive constituents of marihuana, were proved to be active metabolites,<sup>9-11</sup> it is important to further study the epoxide-forming pathways of CBD and the related compounds.

In the present study, we examined *in vitro* metabolism of CBD monomethyl ether (CBDM), which is also a marihuana component,<sup>12</sup> by hepatic microsomes of guinea pigs. Furthermore, in order to confirm our previous speculation, we conducted the identification of epoxy intermediate(s) formed from CBD dimethylether (CBDD) lacking free hydroxy groups.

### Materials and Methods

**Materials** Nicotinamide adenine dinucleotide (NAD) and its reduced form (NADH), nicotinamide adenine dinucleotide phosphate (NADP) and its reduced form (NADPH), and glucose-6-phosphate (G-6-P) were purchased from Boehringer Mannheim GmbH (Darmstadt, F. R. Germany); G-6-P dehydrogenase (type V, EC 1.1.1.49), Fast Blue BB salt, metyrapone and  $\alpha$ -naphthoflavone were from Sigma Chemical Co. (St. Louis, MO); silica gel (Wakogel B-5) for thin-layer chromatography (TLC) and *m*-chloroperbenzoic acid were from Wako Pure Chemical Co. (Osaka, Japan); trimethylsilylimidazole, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane were from Tokyo Kasei Co. (Tokyo, Japan); SKF 525-A was from Smith, Kline & French Lab. (Philadelphia, PA); 1,1,1-trichloropropene-2,3-oxide (TCPO) was from Aldrich Chemical Co. (Milwaukee, WI); Sephadex LH-20 was from Pharmacia (Uppsala, Sweden). CBD was isolated and purified from cannabis leaves by the method previously reported.<sup>13</sup> CBDM and CBDD were prepared by the methylation of CBD with iodomethane, and were isolated by silica gel column chromatography with a solvent system of *n*-hexane-benzene (8:1, v/v). Proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectrum of CBDM

[CDCl<sub>3</sub> ( $\delta$ ): 6.27 (2H, d,  $J$ =4.4 Hz, 3'- and 5'-H), 6.00 (1H, brs, 2'-OH), 5.36 (1H, s, 2-H), 4.42 (2H, d,  $J$ =9.6 Hz, 9-CH<sub>2</sub>), 3.71 (3H, s, 6'-O-CH<sub>3</sub>), 2.51 (2H, t,  $J$ =2.1 Hz, 1''-CH<sub>2</sub>), 1.77 (3H, s, 7-CH<sub>3</sub>), 1.62 (3H, s, 10-CH<sub>3</sub>), 0.89 (3H, t,  $J$ =7.5 Hz, 5''-CH<sub>3</sub>). <sup>1</sup>H-NMR spectrum of CBDD [CDCl<sub>3</sub> ( $\delta$ ): 6.45 (2H, s, 3'- and 5'-H), 5.34 (1H, s, 2-H), 4.55 (2H, s, 9-CH<sub>2</sub>), 3.87 (6H, s, 2'- and 6'-O-CH<sub>3</sub>), 2.65 (2H, t,  $J$ =7.5 Hz, 1''-CH<sub>2</sub>), 1.78 (3H, s, 7-CH<sub>3</sub>), 1.73 (3H, s, 10-CH<sub>3</sub>), 1.00 (3H, t,  $J$ =7.5 Hz, 5''-CH<sub>3</sub>). CBEM was also prepared from CBE in a similar manner except for using a solvent system of *n*-hexane-ethyl acetate (EtOAc)(8:1, v/v) in the column chromatography. <sup>1</sup>H-NMR spectrum of CBEM [CDCl<sub>3</sub> ( $\delta$ ): 6.15 (2H, d,  $J$ =5.2 Hz, 3'- and 5'-H), 4.32 (2H, d,  $J$ =7.4 Hz, 9-CH<sub>2</sub>), 3.97 (1H, d,  $J$ =4.4 Hz, 2-H), 3.51 (3H, s, 6'-O-CH<sub>3</sub>), 2.38 (2H, t,  $J$ =8.2 Hz, 1''-CH<sub>2</sub>), 1.65 (3H, s, 7-CH<sub>3</sub>), 1.34 (3H, s, 10-CH<sub>3</sub>), 0.76 (3H, t,  $J$ =8.4 Hz, 5''-CH<sub>3</sub>). 1*S*,2*R*-Epoxy-CBDD was prepared as follows; to CBDD (0.17 mmol) dissolved in 5 ml of dichloromethane, *m*-chloroperbenzoic acid (0.84 mmol) in 5 ml of dichloromethane was added at 0 °C. One hour later, the reaction was stopped by adding sodium sulfite. The reaction mixture was successively washed with 5% (w/v) aqueous sodium bicarbonate and water. The mixture was then extracted with EtOAc (100 ml  $\times$  2). The organic layer was evaporated *in vacuo* and the residue was applied to a silica gel column using a solvent system of *n*-hexane-EtOAc (8:1, v/v), giving 0.08 mmol of 1*S*,2*R*-epoxy-CBDD. <sup>1</sup>H-NMR of 1*S*,2*R*-epoxy CBDD [CDCl<sub>3</sub> ( $\delta$ ): 6.23 (2H, d,  $J$ =2.7 Hz, 3'- and 5'-H), 4.26 (2H, d,  $J$ =20.2 Hz, 9-H<sub>2</sub>), 3.75 (6H, d,  $J$ =9.5 Hz, 2'- and 6'-O-CH<sub>3</sub>), 2.93 (1H, s, 2-H), 2.55 (2H, d,  $J$ =4.1 Hz, 1''-CH<sub>2</sub>), 1.60 (3H, s, 10-CH<sub>3</sub>), 1.36 (3H, s, 7-CH<sub>3</sub>), 0.90 (3H, t,  $J$ =4.1 Hz, 5''-CH<sub>3</sub>). MS  $m/z$ : 358 (M<sup>+</sup>, 18% as relative intensity), 250 (51%), 221 (100%), 208 (14%). 1*R*,2*S*-Epoxy-CBDD was prepared as follows; 1*R*,2*S*-epoxy-CBD was obtained from CBD by the previous method,<sup>8</sup> and the epoxide (0.30 mmol) was methylated with iodomethane (6.06 mmol) in ethanol. The reaction medium was extracted with EtOAc (100 ml  $\times$  2), followed by evaporation. The residue was applied to a silica gel column using a solvent system of *n*-hexane-benzene (1:1, v/v), giving 0.06 mmol of 1*R*,2*S*-epoxy-CBDD. <sup>1</sup>H-NMR of 1*R*,2*S*-epoxy-CBDD [CDCl<sub>3</sub> ( $\delta$ ): 6.33 (2H, d,  $J$ =12.0 Hz, 3'- and 5'-H), 4.51 (2H, d,  $J$ =4.5 Hz, 9-CH<sub>2</sub>), 3.69 (6H, d,  $J$ =4.5 Hz, 2'- and 6'-O-CH<sub>3</sub>), 3.23 (1H, brs, 2-H), 2.51 (2H, t,  $J$ =6.0 Hz, 1''-CH<sub>2</sub>), 1.66 (3H, s, 10-CH<sub>3</sub>), 1.39 (3H, s, 7-CH<sub>3</sub>), 0.90 (3H, t,  $J$ =5.4 Hz, 5''-CH<sub>3</sub>). MS  $m/z$ : 358 (M<sup>+</sup>, 15%), 250 (6%), 221 (100%), 208 (72%).

<sup>1</sup>H-NMR spectra of the two isomeric CBDD epoxides were almost the same except for signals of a proton at the 2-position. Namely, a sharp singlet (1.5 Hz as a half-height value) for 1*S*,2*R*-epoxy-CBDD, and a broad singlet (3.6 Hz, as a half-height value) for 1*R*,2*S*-epoxy-CBDD appeared. This could have derived from a difference in angles between bincinal protons at the 2- and 3-positions of the structures (102° for 1*S*,2*R*-epoxide and 58° for 1*R*,2*S*-epoxide in their dreiding models).

**Animals** Male Hartley guinea pigs (350–400 g of body weight) were obtained from Hokuriku Experimental Animals Lab. (Kanazawa, Japan). The animals were kept in an air-conditioned room (23–24°C) with 12 h light and dark cycles, and were given food and water *ad libitum*.

**Conditions for Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)** GC: A Shimadzu GC-5A gas chromatograph equipped with a hydrogen flame ionization detector and a glass column packed with 2% OV-17 on Chromosorb W (60–80 mesh, 3 mm i.d. × 1.5 m) were used. Column temperature, 250°C; detector temperature, 270°C; carrier gas, N<sub>2</sub> 50 ml/min. GC-MS: a JEOL JMS-300 mass spectrometer equipped with a JEOL GCG-06 gas chromatograph and JEOL JMA-DA 5000 mass data system. Column; a glass column packed with 2% OV-17 on Chromosorb W (60–80 mesh, 3 mm i.d. × 1.5 m); column temperature, 250°C; injection port temperature, 270°C; carrier gas, He 40 ml/min; ionization current, 0.3 mA; ionization energy, 70 eV.

**Conditions for High Performance Liquid Chromatography (HPLC)** A Hitachi 655 type liquid chromatograph equipped with a 655 type variable wave length UV monitor, a 655-60 type data processor, and a DuPont Zorbax ODS column (4.6 mm i.d. × 15 cm) were used. A mobile phase, methanol-water-acetonitrile (80:10:10, v/v); flow rate, 1 ml/min; detection wave length, 280 nm. Retention times of 1S,2R- and 1R,2S-epoxy-CBDD were 8.1 and 8.4 min, respectively.

**In Vitro Metabolism of CBDM and CBDD** After starvation for 24 h, guinea pigs were killed by decapitation. Their livers were perfused with physiological saline, and microsomal fractions were prepared according to the previous method.<sup>7</sup> CBDM or CBDD (each 500 µg) was incubated with microsomes (equivalent to 1 g liver) in a medium consisting of NADP (5 µmol), G-6-P (50 µmol), MgCl<sub>2</sub> (90 µmol), nicotinamide (27 µmol), G-6-P dehydrogenase (5 units) and potassium phosphate buffer (100 mM, pH 7.4) to make a final volume of 9.0 ml. The medium was incubated at 37°C for 60 min. Metabolites formed were extracted with EtOAc (25 ml × 2), developed by silica gel TLC with a solvent system of *n*-hexane-acetone-diethylamine (20:10:1, v/v, for CBDM) and detected with Fast Blue BB salt or by GC for CBDD. The remaining samples were subjected to preparative TLC under similar conditions. CBDM metabolites separated by the TLC were converted to trimethylsilyl (TMS) derivatives as described previously,<sup>7</sup> and examined by GC and GC-MS. CBDD metabolites separated by the TLC were analyzed by GC and GC-MS without derivatization. The metabolite fractions of CBDD, except for epoxide, were then trimethylsilylated and reexamined by GC-MS. Trimethylsilylated metabolites of CBDM and CBDD were tentatively identified on the basis of their fragmentations in GC-MS by referring to the previous report on the GC-MS analysis of TMS derivatives of CBD metabolites.<sup>3</sup> Approximate amounts of the metabolites were calculated from peak areas on total ion or mass chromatograms.

To study the effects of various inhibitors on the CBEM formation from CBDM, the reaction mixture was extracted with EtOAc after incubation. The organic layer was evaporated *in vacuo*, and the residue was dissolved in 1 ml of *n*-hexane-EtOAc (4:1, v/v). The sample was applied to a small column of silica gel (1 g), and CBDM was eluted out with 4 ml of the same solvent. CBEM was then eluted out with 5 ml of EtOAc. Various inhibitors of cytochrome P450 (SKF 525-A and metyrapone<sup>13</sup>) or epoxide hydrolase

(TCPO<sup>14</sup>) were added to the incubation mixture at a concentration of 0.5 mM. Gas phase experiments were carried out by using Warburg-type reaction flasks.

**In Vivo Metabolism of CBDD** CBDD (100 mg/kg) suspended in physiological saline containing 1% (v/v) Tween 80 was intraperitoneally injected into a guinea pig (360 g of body weight). The animals was killed after 1 h, and its liver was removed and homogenized in three volumes of acetone with a Polytron (Kinematica GmbH, Luzern, Switzerland). The homogenate was shaken and centrifuged. The supernatant was removed and tissue pellet was extracted twice with acetone in the same manner. The combined acetone extract was evaporated *in vacuo*, and the residue was dissolved in 100 ml of EtOAc. The organic layer was washed three times with water, and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> followed by evaporation. The residue was dissolved in a small amount of CHCl<sub>3</sub>, and applied to a Sephadex LH-20 column (1 × 20 cm) equilibrated with CHCl<sub>3</sub>. Metabolites were eluted stepwise by increasing the concentration of methanol from 2 to 16% (v/v) in CHCl<sub>3</sub>. Effluents were subjected to GC and fractions containing a metabolite whose retention time was 6.9 min corresponding to that of synthetic 1S,2R-epoxy-CBDD were combined and examined by GC-MS.

**In Vitro Metabolism of 1S,2R- and 1R,2S-Epoxy-CBDD** Each epoxide (50 µg) was incubated with hepatic microsomes of the guinea pig and the NADPH-generating system at 37°C for 5, 10, 20, 30, and 60 min in a one-tenth scale of the *in vitro* metabolism of CBDM or CBDD described above. At various time points of the incubation, the reaction was stopped by adding EtOAc (5 ml) and shaking. A portion of the organic layer (4 ml) was evaporated. The residue was dissolved in 50 µl of ethanol, and an aliquot (1 µl) was subjected to HPLC. Amounts of substrates remaining after incubation were determined on the basis of the calibration curves. The calibration curves were made by adding known amounts of each epoxide to the reaction medium cooled in an ice-bath and by extracting with EtOAc as described above. Protein concentration was determined by the method of Lowry *et al.*<sup>15</sup>

**Statistics** Statistical significance of difference was calculated by Student's *t*-test.

## Results

**In Vitro Metabolism of CBDM** Among gas chromatographic peaks of trimethylsilylated metabolites of CBDM formed by incubation with guinea pig liver microsomes, a peak with a retention time of 3.4 min showed a molecular ion at *m/z* 416, and prominent fragment ions at *m/z* 333, 219, 197, 130 and 108 (a base ion) (Fig. 1). The retention time and fragment ions agreed with those of synthetic TMS-CBEM. In the control experiments under similar conditions using boiled microsomes, no peak corresponding to the authentic CBEM was seen on the chromatogram. Table I summarizes identified metabolites and their approximate amounts calculated from the peak areas on the

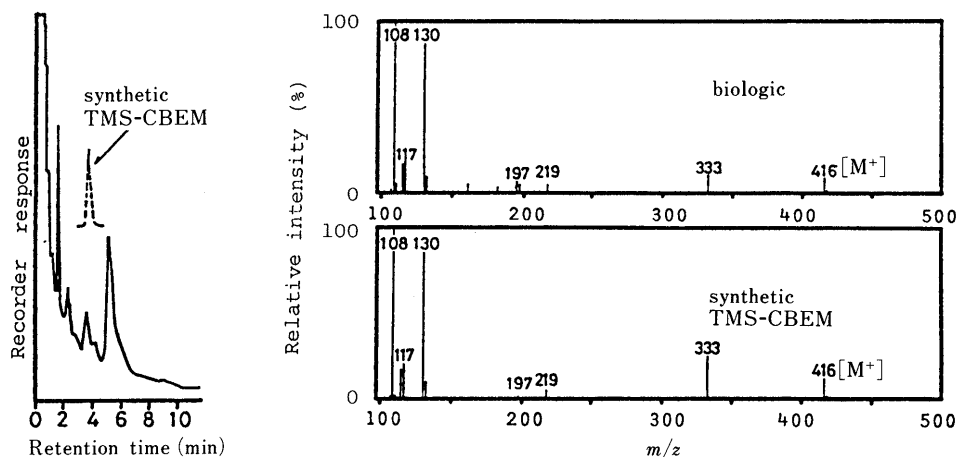


Fig. 1. Gas Chromatogram and Mass Spectra of Biological and Synthetic CBEM

Trimethylsilylated CBDM metabolites separated by preparative TLC were subjected to GC using a 2% OV-17 column (solid line). A peak of authentic CBEM appeared at 3.4 min (broken line). Detailed conditions in GC and GC-MS were described in Materials and Methods.

total ion chromatogram. The most abundant metabolite was 7-hydroxy-CBDM (7-OH-CBDM, 100%), followed by 6-OH-CBDM (96%), CBEM (7%), 6,7-diOH-CBDM (2%) and 7,2''-diOH-CBDM (2%). O-Demethylated CBDM, namely CBD, could not be found under these conditions.

TABLE I. CBDM Metabolites Formed by Liver Microsomes of Guinea Pigs

Metabolites	Ratio (%) <sup>a)</sup>
7-OH-CBDM	100
6-OH-CBDM <sup>b)</sup>	96
CBEM	7
6,7-DiOH-CBDM	2
7,2''-DiOH-CBDM	2

a) Ratio of peak areas on the total ion chromatogram in GC-MS. b) Mixture of 6 $\alpha$ - and 6 $\beta$ -OH-CBDM.

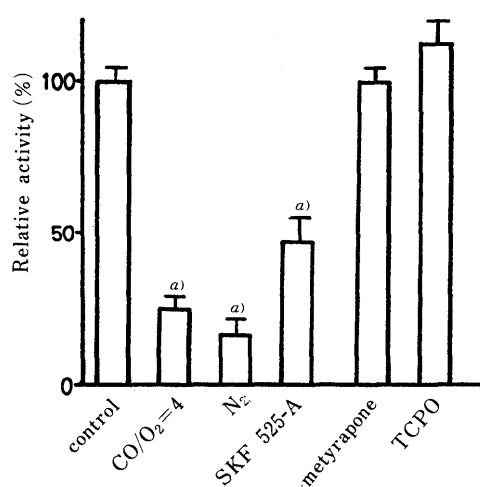


Fig. 2. Effects of Various Gas Phases and Inhibitors on CBEM Formation

Each Value represents the mean  $\pm$  S.E. of three determinations. Inhibitors were used at a concentration of 0.5 mM. CBDM was incubated under N<sub>2</sub> (100%) or CO (CO:O<sub>2</sub>=4:1) as a gas phase in a Warburg type reaction flask. The metabolites were separated by silica gel column chromatography, and subjected to GC. The conditions used are given in Materials and Methods. Significantly different from the control; a)  $p < 0.01$ .

### Properties of Enzyme(s) Involved in CBEM Formation

The nature of enzyme(s) catalyzing CBEM formation was examined by using liver microsomes of adult male guinea pigs. The results were almost the same as those obtained for CBE formation in the previous report,<sup>8)</sup> among four kinds of pyridine nucleotides examined, NADPH (0.5 mM) was the most effective as a cofactor in microsomal CBEM formation. NAD, NADH and NADP were much less effective as the cofactors (below 10% of the NADPH-dependent activity). Maximal activity was found when the pH of the reaction medium was around 7.5. Then CBDM was incubated for 10 min with microsomes (equivalent to 0.5 g liver, about 10 mg protein) and an NADPH-generating system in a final volume of 4.5 ml. These were found to be optimal conditions in a preliminary experiment. CBEM-forming activity was 7.3 pmol per min per mg protein. CBEM formation was not observed when CBDM was incubated in the reaction medium without the cofactor or microsomes. The CBEM formation significantly decreased under nitrogen (N<sub>2</sub>, 100%) by 16% or carbon monoxide (CO) (CO:O<sub>2</sub>=4:1) by 25% as a gas phase (Fig. 2). SKF 525-A (0.5 mM) significantly suppressed the microsomal CBEM formation by 48%, but metyrapone (0.5 mM) did not show any effect. On the other hand, TCPO (0.5 mM), an inhibitor of epoxide hydrolase, tended to enhance the activity.

**Identification of 1S,2R-Epoxy-CBDD** *In vitro* CBDD metabolite extracted with EtOAc (see Materials and Methods) was examined first by TLC, and then by GC-MS after conversion to TMS derivatives. A peak appeared with a retention time of 6.9 min which corresponded to that of synthetic 1S,2R-epoxy-CBDD on the gas chromatogram (Fig. 3). A molecular ion was seen at  $m/z$  358 and prominent fragment ions appeared at  $m/z$  290, 250, 221 (a base ion), 208, and 152. These data were in good agreement with those of the synthetic 1S,2R-epoxy-CBDD. Synthetic 1R,2S-epoxy-CBDD had a retention time of 6.8 min, close to that of 1S,2R-epoxide. However, the mass spectrum of the metabolite was different from that of 1R,2S-epoxide which showed fragment ions at  $m/z$  250 with lower relative

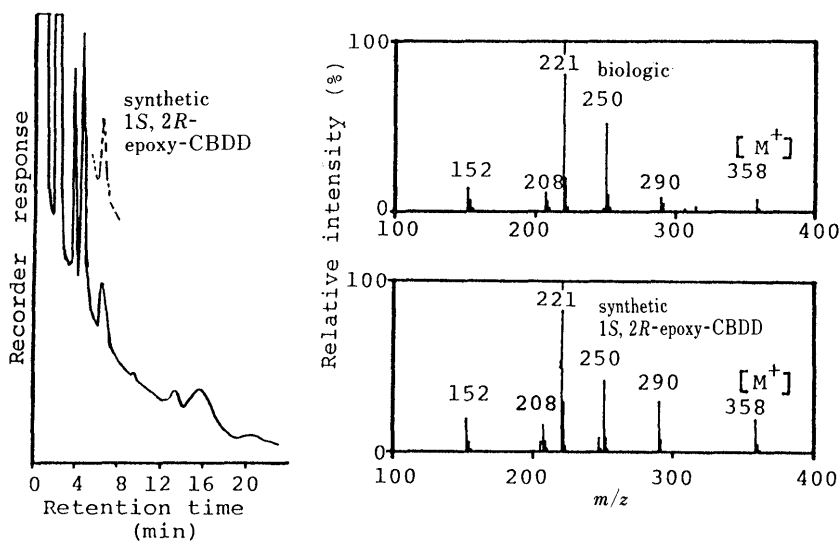


Fig. 3. Gas Chromatogram and Mass Spectra of Biological and Synthetic 1S,2R-Epoxy-CBDD

CBDD metabolites were separated by a Sephadex LH-20 column and subjected to GC using a 2% OV-17 column (solid line). A peak of authentic 1S,2R-epoxy-CBDD appeared at 6.9 min (broken line).

TABLE II. CBDD Metabolites Formed by Liver Microsomes of Guinea Pigs

Metabolites	Ratio (%) <sup>a)</sup>
4''-OH-CBDD	100
6-OH-CBDD <sup>b)</sup>	30
1''-OH-CBDD	4
1 <i>S</i> ,2 <i>R</i> -Epoxy-CBDD	1

a) Ratio of peak areas on the total ion chromatogram in GC-MS. b) Mixture of 6 $\alpha$ - and 6 $\beta$ -OH-CBDD.

intensity, and at  $m/z$  208 with higher relative intensity compared with those of 1*S*,2*R*-epoxy-CBDD (see Materials and Methods). In Table II, a ratio of the approximate amounts of metabolites identified is listed. The most abundant metabolite was 4''-OH-CBDD (100%), followed by 6-OH-CBDD (30%) and 1''-OH-CBDD (4%). *In vivo* metabolites extracted from the liver were applied to a column of Sephadex LH-20 and effluents were checked by GC. Combined fractions containing a metabolite with a retention time of 6.9 min (corresponding to that of the synthetic 1*S*,2*R*-epoxy-CBDD) were examined by GC-MS. The retention time (10.5 min) and profile of fragmentation of the metabolite in GC-MS agreed with those of the synthetic standard (data not shown).

***In Vitro* Metabolism of 1*S*,2*R*- and 1*R*,2*S*-Epoxy-CBDD** 1*S*,2*R*- or 1*R*,2*S*-epoxy-CBDD (each 50  $\mu$ g) was incubated with liver microsomes and the NADPH-generating system at pH 7.5 for 5 to 60 min. Amounts of the unchanged substrates at various time points of incubation were determined by HPLC. The amounts of 1*S*,2*R*-epoxy-CBDD were greater than those of 1*R*,2*S*-epoxy-CBDD at any time points determined, namely, from 5 to 60 min of incubation. After incubation for 60 min, 34  $\mu$ g (68% of the substrate added) of 1*S*,2*R*-epoxy-CBDD and 12  $\mu$ g (24%) of 1*R*,2*S*-epoxy-CBDD remained unmetabolized. Therefore, 1*R*,2*S*-epoxide was thought to be metabolized more easily than 1*S*,2*R*-epoxide under the conditions employed. Furthermore, in both cases none of the elsoin-type metabolites were identified.

## Discussion

We have previously reported that CBE was formed from CBD with the microsomal monooxygenase system including cytochrome P450 of guinea pig liver, and that 1*S*,2*R*-epoxide might be an intermediate of the CBE formation.<sup>7,8)</sup> In order to confirm this speculation, metabolism of CBDM, one of the constituents of marijuana, or of CBDD was investigated in the guinea pig in the present study. Emphasis was placed on the formation of elsoin-type metabolites and epoxides from these substrates. CBEM was found to be one of the metabolites of CBDM with hepatic microsomes of male guinea pigs. The CBEM formation was decreased with CO, N<sub>2</sub>, and SKF 525-A, but not with metyrapone. TCPO tended to enhance the activity. It is known that metyrapone does not inhibit all types of microsomal drug oxidations mediated by cytochrome P450.<sup>13)</sup> Therefore, the microsomal CBEM formation from CBDM may be catalyzed by P450 isozyme(s) which lacks sensitivity to metyrapone.

We thought that, as in the case of CBE formation from CBD, 1*S*,2*R*-epoxy-CBDM would be converted into CBEM by phenoxy anion at the 2'- or 6'-positions on the oxirane

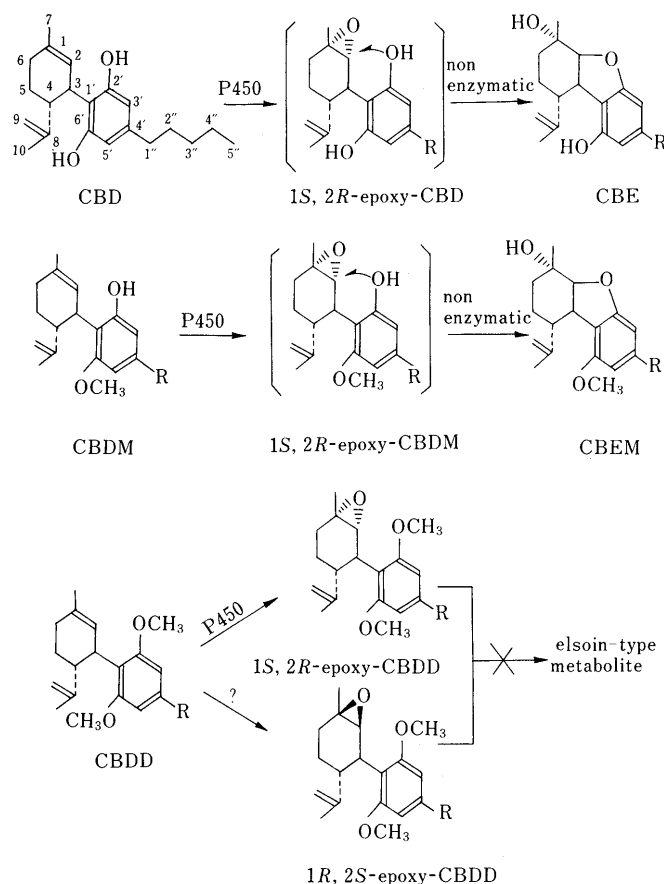


Fig. 4. Postulated Mechanism of Elsoin-Type Metabolite Formation  
P450: cytochrome P450. R: C<sub>5</sub>H<sub>11</sub>.

ring immediately after the epoxide formation. If that is the case, epoxide(s) can be identified as intermediate(s) when CBDD, a dimethyl ether of phenolic hydroxyl groups at the 2'- and 6'-positions of CBD, is used as a substrate. As anticipated, we could identify 1*S*,2*R*-epoxy-CBDD as one of the *in vitro* metabolites of CBDD. On the other hand, 1*R*,2*S*-epoxy-CBDD was not identified in the present study. When synthetic 1*S*,2*R*- or 1*R*,2*S*-epoxy-CBDD was thus incubated with microsomes under the conditions for oxidative metabolism of CBDD, 1*R*,2*S*-epoxide was metabolized more rapidly than the isomeric 1*S*,2*R*-epoxide. The results suggest that 1*R*,2*S*-epoxy-CBDD is better substrate than 1*S*,2*R*-epoxy-CBDD for the hepatic drug-metabolizing enzyme system or microsomal epoxide hydrolase. It may be one of reasons for identification of only 1*S*,2*R*-epoxy-CBDD in the present experiments, although there is a possibility that 1*S*,2*R*-epoxide is selectively formed from CBDD. In addition, the metabolic patterns of CBDM and CBDD were quite different from each other.

The results obtained here indicate that CBD and CBDM are biotransformed to 1*S*,2*R*-epoxides with the hepatic microsomal monooxygenase system including cytochrome P450, and the epoxides of CBD and CBDM are immediately converted to elsoin-type metabolites by the mechanism shown in Fig. 4.

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