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A Novel Metabolite, an Oxepin Formed from Cannabidiol with Guinea-pig Hepatic Microsomes

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Abstract

The metabolic formation of an oxepin derivative, 3-pentyl-6, 7, 7a, 8, 9, 11a-hexahydro-1, 7-dihydroxy-7, 10-dimethyldibenzo-[b,d]-oxepin, from cannabidiol was studied in-vitro using guinea-pig hepatic microsomes.

The hepatic microsomes catalysed the formation of the metabolite from cannabidiol and 8*S*, 9-epoxycannabidiol in the presence of an NADPH-generating system and 3, 3, 3-trichloropropene-1, 2-oxide. 8*S*, 9-Epoxycannabidiol was thought to be an intermediate in the formation of the metabolite, which was identified by gas chromatography-mass spectrometry. The metabolite synthesized from 8*S*, 9-epoxycannabidiol diacetate exhibited catalepsy, hypothermia and pentobarbitone-induced sleep prolongation in mice, although the pharmacological effect was less potent than that of Δ^9-tetrahydrocannabinol.

Metabolism of cannabidiol, the major constituent of marihuana, has been extensively studied in-vitro (Harvey & Brown 1990a) and in-vivo (Martin et al 1976; Harvey et al 1980; Harvey & Mechoulam 1990). In spite of lacking psychoactivity, it has anticonvulsant (Dewey 1986) and barbiturate-induced sleep prolongation activities (Paton & Pertwee 1972). The latter effect is mainly caused by inhibition of hepatic metabolism of barbiturates, probably by active metabolites of cannabidiol formed in the liver (Karler et al 1979). There are many examples for inhibition of microsomal drug metabolism by active metabolites such as epoxides. It is, therefore, important to establish the formation of epoxy-intermediates in the metabolism of cannabidiol.

Recently, we reported that the formation of cannabielsoin as an in-vitro and in-vivo metabolite of cannabidiol, which might be formed through 1*S*, 2*R*-epoxycannabidiol as an intermediate (Yamamoto et al 1988, 1989). More recently, we found that an epoxide metabolite, 8*R*, 9-epoxycannabidinol was biotransformed to 6β-hydroxymethyl-Δ^9-tetrahydrocannabinol and 8, 9-dihydro-8, 9-dihydroxycannabidiol by hepatic microsomes of animals (Nagai et al 1993). We report here a further study on the metabolism of cannabidiol by hepatic microsomes.

Materials and Methods

Materials

NADP and glucose 6-phosphate were purchased from Boehringer Mannheim GmbH (Darmstadt, Germany); glucose 6-phosphate dehydrogenase (type V) was from Sigma Chemical Co. (St Louis, MO, USA); *N*, *O*-bis (trimethylsilyl) trifluoroacetamide (BSTFA), trimethylsilylimidazole and trimethylsilylchlorosilane and sodium pentobarbitone were from Tokyo Kasei Kogyo Co. (Tokyo, Japan); 3, 3, 3-trichloropropene-1, 2-oxide was from Aldrich Chemical Co. (Milwaukee, WI, USA). Cannabidiol was isolated and purified from cannabis leaves as described by Aramaki et al (1968). 8*S*, 9-Epoxycannabidiol diacetate was synthesized from cannabidiol (Nagai et al 1993). An authentic sample of the putative metabolite, 3-pentyl-6, 7, 7a, 8, 9, 11a-hexahydro-1, 7-dihydroxy-7, 10-dibenzo-[b, d]-oxepin (PHDO), was prepared as follows.

8*S*, 9-Epoxycannabidiol diacetate (31·0 mg, 94 μmol) was dissolved in 10 mL ethanol and to this solution, 1 mL 0·5 M NaOH was added. The mixture was stirred for 20 min at room temperature (21°C). The reaction mixture was extracted with diethylether. After evaporation of the solvent, PHDO (16·0 mg, 71 μmol) was purified by silica-gel column chromatography using a solvent system of *n*-hexane : ethyl acetate (4 : 1). NMR of PHDO ($CDCl_3$) δppm : 6·36, 6·26, (d, 2H, C-2 and C-4, J = 2 Hz), 6·17 (m, 1H, C-11), 4·85 (broad s, 1H, OH), 3·80, 3·40 (d, 2H, C-6, J = 12 Hz), 3·27 (dd, 1H, C-11a, $J_{11,11a} = 7$ Hz, $J_{11,7a} = 12$ Hz), 2·45 (t, 2H. C-1′, J = 7 Hz), 1·65 (s, 3H, C-10), 1·50 (s, 3H, C-7), 0·83 (t, 3H, C-5′). MS m/z : 330 (M^+, 72%), 299 (100%), 272 (31%), 231 (40%), 193 (19%).

Preparation of microsomes and metabolism of cannabinoids

Hepatic microsomes were prepared from male Hartley guinea-pigs (350–450 g) as described by Nagai et al (1993). A typical incubation mixture consisted of hepatic microsomes (equivalent to 0·1 g liver), 8*S*, 9-epoxycannabidiol diacetate (0·16 μmol), NADP (0·5 μmol), $MgCl_2$ (10 μmol), glucose 6-phosphate (5 μmol), glucose 6-phosphate dehydrogenase (1 unit) and potassium phosphate buffer (100 μmol, pH 7·4) to make a final volume of 1·0 mL. After incubation at 37°C for 20 min, metabolites were

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extracted with 5 mL ethyl acetate. The metabolism of cannabidiol was conducted in the incubation system, the scale of which was 10 times that for 8*S*, 9-epoxycannabidiol diacetate. The residue after evaporation of the organic solvent was subjected to preparative TLC with a solvent system of *n*-hexane : ethyl acetate (4 : 1). The zones of (R_f = 0–0·15 and 0·25) corresponding to dihydro-diol metabolite and PHDO, respectively, were scraped off and extracted with 5 mL $CHCl_3$: acetone (4 : 1). After evaporation of the solvent, the isolated metabolites were converted to trimethylsilyl derivatives with BSTFA, trimethylsilylimidazole and trimethlsilylchlorosilane. The metabolites were then analysed by gas chromatography-mass spectrometry (GC-MS) under the following conditions: JEOL JMS DX-300 mass spectrometer equipped with a JMS 5000 mass data system and connected to a column packed with 5% SE-30 on Chromosorb W (60–80 mesh, 3 mm i.d. × 2 m); column temperature 250°C; injection temperature, 270°C; carrier gas, He 40 mL min^{-1}; ionization current, 0·3 mA; ionization energy, 70 eV. The retention time of trimethylsilyl derivative of PHDO was 5·6 min under the above conditions.

Pharmacological experiments

Male ddN mice, 20–30 g, were obtained from Hokuriku Experimental Animal Laboratory (Kanazawa, Japan). Δ^9-Tetrahydrocannabinol and PHDO were suspended in physiological saline containing 1% Tween 80.

Catalepsy. Mice were separated into four groups, each of which consisted of eight animals, and injected intravenously with the cannabinoid at doses ranging from 3 to 15 mg kg^{-1}. Cataleptogenic effect was assessed by the simple bar test reported by Yoshimura et al (1978). The front paws of the mouse were placed on a horizontal bar (0·5 cm in diam. and 5 cm in height), forcing the mouse to stand on its hind legs. The cataleptogenic effect was regarded to be positive when the mouse maintained the abnormal position for more than 30 s.

Hypothermia. Each group, consisting of eight mice, was given intravenously PHDO (5 or 10 mg kg^{-1}), Δ^9-tetrahydrocannabinol (5 mg kg^{-1}) or the vehicle (10 mL kg^{-1}). The rectal temperatures of the mice were measured just before and at 10, 20, 30, 45, 60, 90 and 120 min after the injection.

Effect on pentobarbitone-induced sleep. Four groups of eight mice were injected intravenously with PHDO (5, 15 or 30 mg kg^{-1}) or the vehicle, and then given pentobarbitone (40 mg kg^{-1}, i.p.) 20 min later. The duration of loss of the righting reflex was measured as the sleeping time.

Statistics

The statistical significance of difference was calculated using Student's *t*-test.

Results and Discussion

Our previous study demonstrated that cannabidiol was bio-transformed to 6β-hydroxymethyl-Δ^9-tetrahydrocannabinol through 8*R*, 9-epoxycannabidiol by hepatic microsomes of mice (Nagai et al 1993). In the present study, 8*S*, 9-epoxycannabidiol diacetate was exclusively transformed by guinea-pig hepatic microsomes to a metabolite having an identical mass spectrum with that of 8, 9-dihydro-8, 9-dihydroxycannabidiol reported by Harvey & Brown (1990b). When trichloropropene oxide, an inhibitor of epoxide hydrolase, was added to the incubation mixture, another metabolite was identified by GC-MS. Fig. 1 shows mass chromatograms and the mass spectrum of the metabolite. The mass spectrum of a compo-

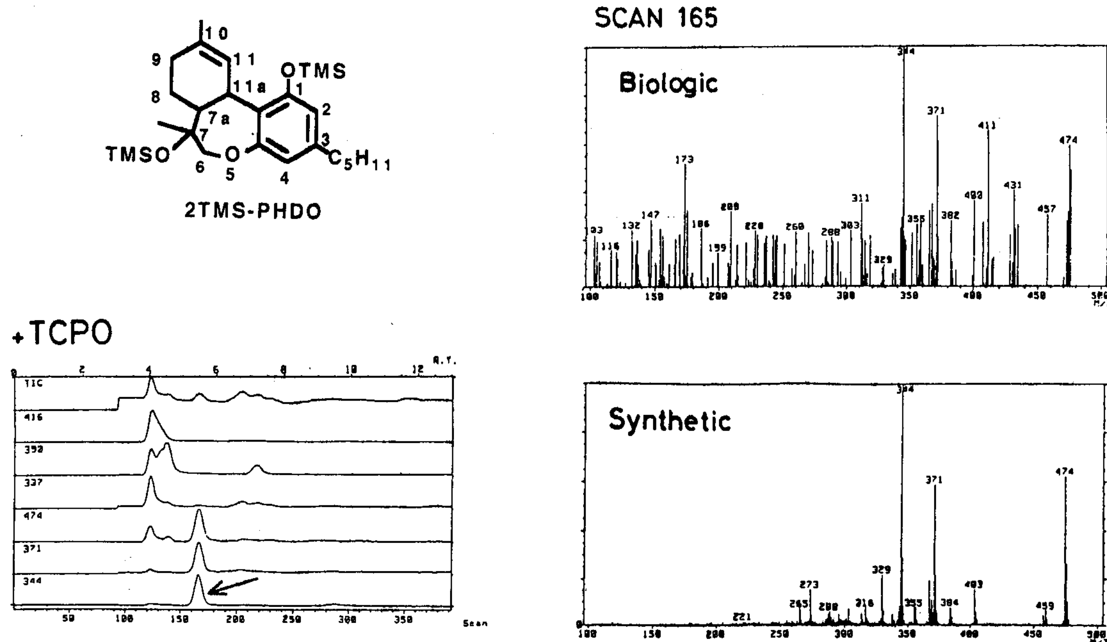

FIG. 1. GC-MS identification of PHDO as a metabolite of 8*S*, 9-epoxycannabidiol diacetate.

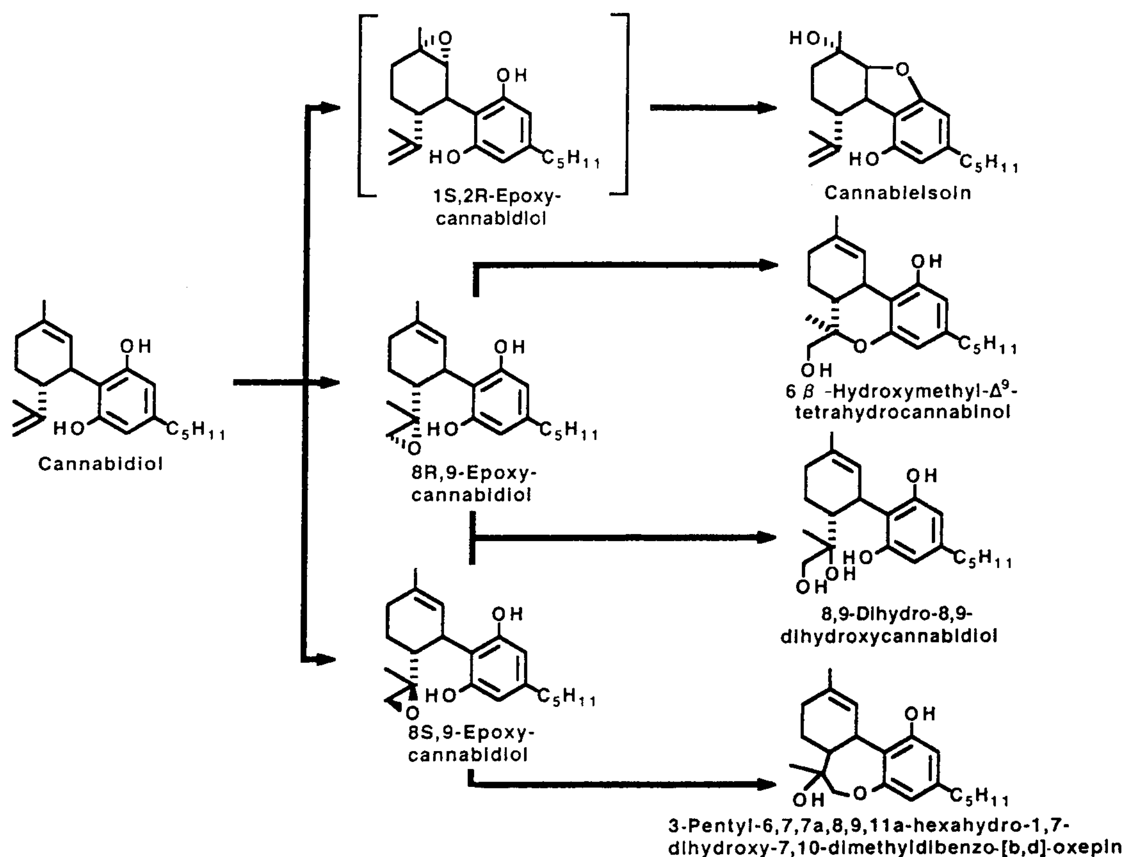

FIG. 2. Metabolic pathways of cannabidiol through the epoxidation.

nent (Rt 5·5 min) having mass ions of m/z 474, 371 and 344 was almost identical with that of the authentic PHDO derivative. A component around retention time of 4 min having mass ions of m/z 474, 416, 390 and 337 appears to be a trimethyl silyl derivative of 8*S*, 9-epoxycannabidiol. The present result and the previous findings indicate that 8*S*, 9- and 8*R*, 9-epoxycannabidiol were biotransformed to PHDO and 6β-hydroxymethyl-Δ^9-tetrahydrocannabinol, respectively, together with the dihydro-diol metabolite (Fig. 2). Cannabidiol was also transformed to PHDO by guinea-pig hepatic microsomes in the presence of an NADPH-generating system and trichloropropene oxide, although specific activity was less than 1 pmol min^{-1} (mg $protein^{-1}$). However, PHDO was not formed when boiled microsomes were used as the enzyme source or when the inhibitor or NADPH-generating system was omitted from the incubation mixture. These results suggest that the key step of PHDO formation is the formation of 8*S*, 9-epoxycannabidiol in the incubation system. This is the first example of cannabidiol being biotransformed to an oxepin derivative by the mammalian enzyme system.

Very few studies have been carried out to evaluate pharmacological effects of oxepin derivatives of cannabinoids. Jorapur et al (1985) synthesized two oxepin derivatives of Δ^9-tetrahydrocannabinol from cannabidiol. The derivatives showed relatively little hypothermia and spontaneous activity in mice, and behavioural effects in dogs. The present study demonstrated that PHDO exhibited some pharmacological effects. The ED50 value (mg kg^{-1}, i.v., and 95% confidence limit) of PHDO in the cataleptogenic test was 5·60 (3·98–7·90), half as potent as Δ^9-tetrahydrocannabinol reported by Narimatsu et al (1983).

The hypothermic effect of PHDO and Δ^9-tetrahydrocannabinol is illustrated in Fig. 3. The maximal hypothermia

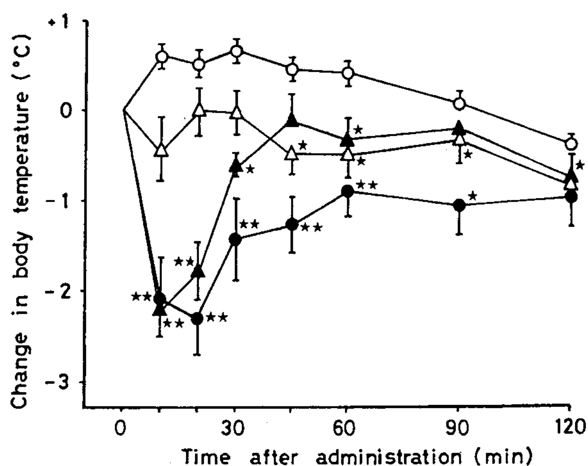

FIG. 3. Time course in hypothermic effect of PHDO and Δ^9-tetrahydrocannabinol. ○ Control; △ PHDO (5 mg kg^{-1}, i.v.); ▲ PHDO (10 mg kg^{-1}, i.v.); ● Δ^9-tetrahydrocannabinol (5 mg kg^{-1}, i.v.). *Significantly different from control $P < 0·05$. **Significantly different from control $P < 0·01$.

Table 1. Effect of PHDO on pentobarbitone-induced sleep time.

	Dose ($mg\,kg^{-1}$, i.v.)	Sleeping time (min)
Control	–	29 ± 2
PHDO	5	39 ± 3*
	15	55 ± 5**
	30	64 ± 2**

*Significantly different from control $P < 0.05$. **Significantly different from control $P < 0.01$.

was recorded at 10 min after the administration of PHDO ($10\,mg\,kg^{-1}$) and at 20 min after the injection of Δ^9-tetrahydrocannabinol ($5\,mg\,kg^{-1}$). The effect (−2·20°C) of PHDO was comparable with that (−2·30°C) of Δ^9-tetrahydrocannabinol ($5\,mg\,kg^{-1}$), indicating that PHDO is also less active than Δ^9-tetrahydrocannabinol in this test.

PHDO prolonged pentobarbitone-induced sleeping time dose-dependently (Table 1). At doses of 5, 15 and $30\,mg\,kg^{-1}$, pentobarbitone-induced sleeping time was significantly prolonged by 1·3, 1·8 and 2·1-fold, respectively, compared with that in vehicle-treated control.

The present study demonstrated that cannabidiol was biotransformed to a novel oxepin derivative of Δ^9-tetrahydrocannabinol through 8*S*, 9-epoxycannabidiol by guinea-pig hepatic microsomes, and that the oxepin had pharmacological effects half as potent as those of Δ^9-tetrahydrocannabinol.

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References

Aramaki, H., Tomiyasu, N., Yoshimura, H., Tsukamoto, H. (1968) Forensic chemical study on marihuana. I. A detection method of the principal constituents by thin-layer and gas chromatographies. Chem. Pharm. Bull. 16: 822–826

Dewey, W. L. (1986) Cannabinoid pharmacology. Pharmacol. Rev. 38: 151–178

Harvey, D. J., Brown, N. K. (1990a) In vitro metabolism of cannabidiol in seven common laboratory mammals. Res. Commun. Subst. Abuse. 11: 27–37

Harvey, D. J., Brown, N. K. (1990b) 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

Harvey, D. J., Mechoulam, R. (1990) Metabolites of cannabidiol identified in human urine. Xenobiotica 20: 303–320

Harvey, D. J., Martin, B. R., Paton, W. D. M. (1980) Identification of in vivo liver metabolites of Δ^1-tetrahydrocannabinol, cannabidiol, and cannabinol produced by the guinea-pig. J. Pharm. Pharmacol. 32: 267–271

Jorapur, V. S., Khalil, Z. H., Duffley, R. P., Razdan, R. K., Martin, B. R., Harris, L. S., Dewey, W. L. (1985) Hashish: synthesis and central nervous system activity of some novel analogues of cannabidiol and oxepin derivatives of Δ^9-tetrahydrocannabinol. J. Med. Chem. 28: 783–787

Karler, R., Sangdee, P., Turkanis, S. A., Borys, H. K. (1979) The pharmacokinetic fate of cannabidiol and its relationship to barbiturate sleep time. Biochem. Pharmacol. 28: 777–784

Martin, B., Nordqvist, M., Agurell, S., Lindgren, J.-E., Leander, K., Binder, M. (1976) Identification of monohydroxylated metabolites of cannabidiol formed by rat liver. J. Pharm. Pharmacol. 28: 275–279

Nagai, K., Watanabe, K., Narimatsu, S., Gohda, H., Matsunaga, T., Yamamoto, I., Yoshimura, H. (1993) In vitro metabolic formation of a new metabolite, 6β-hydroxymethyl-Δ^9-tetrahydrocannabinol from cannabidiol through an epoxide intermediate and its pharmacological effects on mice. Biol. Pharm. Bull. 16: 1008–1013

Narimatsu, S., Yamamoto, I., Watanabe, K., Yoshimura, H. (1983) 9 α, 10 α-Epoxyhexahydrocannabinol formation from Δ^9-tetrahydrocannabinol by liver microsomes of phenobarbital-treated mice and its pharmacological activities in mice. J. Pharmacobiodyn. 6: 558–564

Paton, W. D. M., Pertwee, R. G. (1972) Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. Br. J. Pharmacol. 44: 250–261

Yamamoto, I., Gohda, H., Narimatsu, S., Yoshimura, H. (1988) Identification of cannabielsoin, a new metabolite of cannabidiol formed by guinea-pig hepatic microsomal enzymes, and its pharmacological activity in mice. J. Pharmacobiodyn. 11: 833-838

Yamamoto, I., Gohda, H., Narimatsu, S., Yoshimura, H. (1989) Mechanism of biological formation of cannabielsoin from cannabidiol in the guinea-pig, mouse, rat and rabbit. J. Pharmacobiodyn. 12: 488–494

Yoshimura, H., Watanabe, K., Oguri, K., Fujiwara, M., Ueki, S. (1978) Synthesis and pharmacological activity of a phosphate ester of Δ^8-tetrahydrocannabinol. J. Med. Chem. 21: 1079–1081