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HUMAN LIVER MICROSOMAL OXIDATION OF Δ^8-TETRAHYDROCANNABINOL

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Human liver microsomal oxidation products of Δ^8-tetrahydrocannabinol (THC) were isolated and identified by thin-layer chromatography, gas chromatography and gas chromatography-mass spectrometry. As the results, five monohydroxylated metabolites including 11-hydroxy-Δ^8-THC, and 8β,9α-dihydroxyhexahydrocannabinol were identified as trimethylsilyl derivatives. Isolation of the dihydroxylated metabolite suggests that epoxy metabolite(s) may be formed from Δ^8-THC in humans.

KEYWORDS ——— human liver microsomes; identification of metabolite; GC-MS; Δ^8-tetrahydrocannabinol; 11-hydroxy-Δ^8-tetrahydrocannabinol; 7α-hydroxy-Δ^8-tetrahydrocannabinol; 7β-hydroxy-Δ^8-tetrahydrocannabinol; 1'-hydroxy-Δ^8-tetrahydrocannabinol; 3'-hydroxy-Δ^8-tetrahydrocannabinol; 8β,9α-dihydroxyhexahydrocannabinol

Several workers have investigated the in vivo metabolism in humans of tetrahydrocannabinol (THC), a psychomimetic constituent of marihuana, using plasma, urine and feces.[1,2] However, knowledge of the in vitro metabolism in humans of THC has been very limited.[3] Halldin et al. very recently reported that Δ^9-THC was biotransformed to several metabolites by 10,000 x g supernatant of human liver.[4] We now wish to report identification of six metabolites of Δ^8-THC formed by human liver microsomes.

Δ^8-THC, 11-hydroxy-Δ^8-THC (11-OH-Δ^8-THC), 8α,9α- and 8β,9β-epoxyhexahydrocannabinol (EHHC) were prepared by the previous methods.[5-7] 8β,9α-Dihydroxyhexahydrocannabinol (diOH-HHC) was synthesized by the method of Ben-Zvi and Mechoulam.[8] The liver donor was a 48-years-old Japanese male who died in a traffic accident. The liver, 380 g, was excised 18 h after death, cut into small pieces (about 10 g each) and frozen at -80°C until use. A part of the liver (21.8 g) was homogenized in 44.0 ml of ice-cold 1.15 % KCl with a Polytron, and centrifuged at 9,000 x g for 20 min at 4°C. The supernatant was centrifuged twice at 105,000 x g for 60 min at 4°C. The pellet obtained was suspended in 11.0 ml of ice-cold 1.15 % KCl. Protein concentration was determined to be 16.5 mg/ml by Lowry's procedure.[9] Contents of cytochrome P-450 and P-420 determined by the method of Omura and Sato[10] were 0.130 and 0.361 nmol/mg protein, respectively.

Two ml of the microsomal suspension was added to incubation medium (36.0 ml) containing EDTA (360 μmol) along with other components necessary for NADPH genera-

tion, which were scaled up 4 times as described before.[11] After addition of Δ^8-THC (2.5 mg in 0.25 ml of ethanol), the reaction mixture was incubated at 37°C for 90 min. Metabolites in the mixture were then extracted 3 times with 40 ml of ethyl acetate and separated by the procedure described in the legend to Fig. 1. After evaporation of the solvent from each extract obtained, metabolites dissolved in 10 μl of acetone were trimethylsilylated with N,O-bis-(trimethylsilyl)acetamide (5 μl) and trimethylsilylimidazole (5 μl).[12] These trimethylsilyl (TMS) derivatives were then subjected to gas chromatography using a hydrogen flame ionization detector and gas chromatography-mass spectrometry under the same conditions described before, using glass columns of 1.5 % SE-30 on Chromosorb W.[11]

Only one peak corresponding to TMS-Δ^8-THC appeared on a gas chromatogram of Band I-extract. As with Band II-extract, there were five peaks, one of which appeared at the same retention time as that of authentic TMS-8β,9β-EHHC (t_R 7.2 min). All of them were, however, too small to be identified by GC-MS. In Band III-extract, two peaks, A (t_R 8.0 min) and B (t_R 10.1 min), were shown on a chromatogram. In addition, gas chromatogram of Band IV-extract showed three peaks, C, D and E (t_R 6.5, 10.6 and 12.4 min, respectively). Major fragment ions and their relative intensities of Peaks A, B, C and D are summarized in Table I. Peaks A, B, C and D were presumed to be 2TMS-7α-OH-Δ^8-THC, 2TMS-7β-OH-Δ^8-THC, 2TMS-1'-OH-Δ^8-THC and 2TMS-3'-OH-Δ^8-THC, respectively, because their fragmentations were in good agreement with those reported by Harvey.[13] Retention time and mass spectrum (Fig. 2) of Peak E was coincident with those of authentic 2TMS-11-OH-Δ^8-THC. On a gas chromatogram of Band V-extract, there appeared no peak except for that of 2TMS-11-OH-Δ^8-THC. Peak F (t_R 15.3 min) was shown on a chromatogram of Band VI-extract. By comparing the retention time and mass spectrum with those of an authentic sample, Peak F was found to be 3TMS-8β,9α-diOH-HHC (Fig. 2). Furthermore, there were a few peaks shown on a chromatogram of Band VII-extract, but they could not be identified because of interference by impurities located in this area.

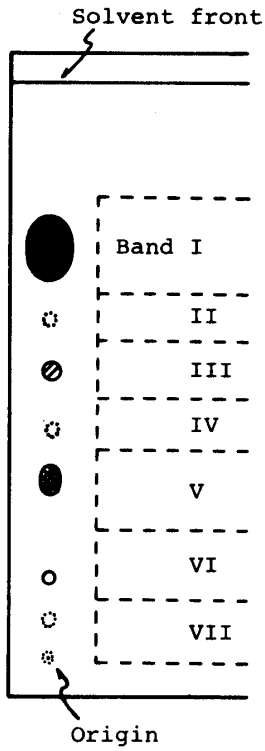

Fig. 1. Thin-Layer Chromatogram of Metabolites in AcOEt-Extract

The combined extract was dried over anhydrous sodium sulfate, and the solvent was evaporated under N_2 stream. The residue was dissolved in a small volume of chloroform, and spotted on a TLC plate coated with Wako gel B-5 (0.5 mm thickness, 20 x 20 cm). After spotting of a small amount of the solution on both edges of the plate, it was developed with a solvent system of n-hexane-acetone-diethylamine (20 : 10 : 1). Following the spots visualized on both edges by spraying of Fast Blue BB salt (1 w/v % in H_2O), seven bands were scraped off and metabolites located on Bands I, II, III, IV and V were extracted twice with 8 ml of $CHCl_3$-AcOEt (2 : 1). Metabolites on Bands VI and VII were extracted twice with 8 ml of $CHCl_3$-MeOH (4 : 1).

Table I. Mass Spectrometric Data for Peaks A, B, C and D

	Major fragment ions (m/z)
Peak A	474 (M^+, 11), 384 (32), 369 (100), 342 (10), 328 (21), 303 (24), 286 (16)
Peak B	474 (M^+, 43), 384 (41), 369 (19), 318 (10), 303 (100), 265 (21)
Peak C	474 (M^+, 46), 459 (5), 417 (100), 391 (5)
Peak D	474 (M^+, 26), 459 (7), 391 (10), 330 (100)

The spectra were recorded at 22 eV of ionizing voltage. Numbers in parentheses are relative intensities.

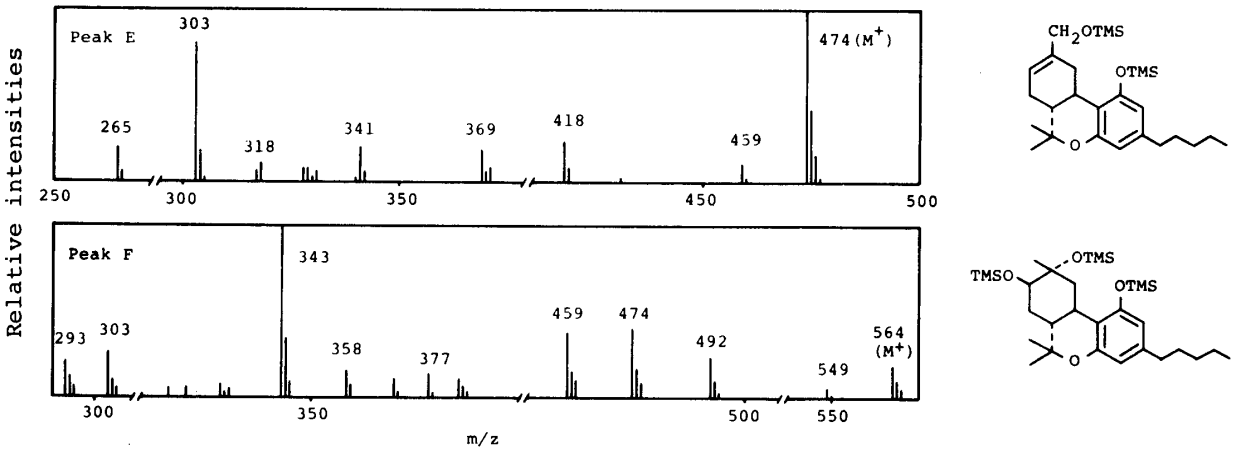

Fig. 2. Mass Spectra of Peaks E and F
The spectra were recorded at 22 eV of ionizing voltage.

Table II. Ratio of Peak Areas of Trimethylsilylated Metabolites on Gas Chromatogram

Metabolites	t_R (min)	Ratio (%)
11-OH-Δ^8-THC	12.4	100
Peak A (7α-OH-Δ^8-THC)*	8.0	44
Peak B (7β-OH-Δ^8-THC)*	10.1	15
Peak C (1'-OH-Δ^8-THC)*	6.5	3
Peak D (3'-OH-Δ^8-THC)*	10.6	2
8β,9α-diOH-HHC	15.3	18

* Presumed metabolites.

From these results, it was found that at least six metabolites including 11-OH-Δ^8-THC and 8β,9α-diOH-HHC were formed from Δ^8-THC by human liver microsomes. Then, ratio of every peak area of trimethylsilylated metabolites to that of 2TMS-11-OH-Δ^8-THC on the gas chromatograms was calculated (Table II). It is thought that these data approximately show a ratio of amount of each metabolite formed. It is noteworthy that a considerable amount of 8β,9α-diOH-HHC was formed under the present conditions. We recently found that both 8α,9α- and 8β,9β-EHHC are preferentially hydrolyzed to 8β,9α-diOH-HHC in the mouse both *in vitro* and *in vivo*.[14] On the basis of this finding, it is strongly suggested that a certain amount of 8α,9α- or 8β,9β-EHHC might be formed as an intermediate, and then hydrolyzed to 8β,9α-diOH-HHC by human liver microsomes. Authentic 8α,9α- and 8β,9β-EHHC had an Rf value (0.59) corresponding to Band II on a TLC plate under the condition used in this study. As mentioned above, a peak whose retention time agreed with that of authentic TMS-8β,9β-EHHC (t_R 7.2 min), but not with that of TMS-8α,9α-EHHC (t_R 6.7 min), was shown on the gas chromatogram of Band II-extract. We are now making efforts to identify the epoxy metabolite(s) using other human livers.

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