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CATALYTIC ACTIVITY OF CYTOCHROME P450 ISOZYMES PURIFIED FROM RAT LIVER IN CONVERTING 11-OXO-Δ^8-TETRAHYDROCANNABINOL TO Δ^8-TETRAHYDROCANNABINOL-11-OIC ACID

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Abstract—Cytochrome P450 isozymes purified from rat hepatic microsomes were able to catalyse the oxidation of 11-oxo-Δ^8-tetrahydrocannabinol (11-oxo-Δ^8-THC) to Δ^8-THC-11-oic acid in the presence of NADPH, cytochrome P450 reductase and dilauroylphosphatidylcholine. The catalytic activities (nmol/min/nmol P450) of cytochrome P450s, UT-2 (IIC11), UT-4 (IIA2), UT-5 (IIC13), PB-1, PB-2 (IIC6), PB-4 (IIB1), MC-1 (IA2), MC-5 (IA1) and IF-3 (IIA1), were 0.69, 0.08, 0.07, 0.23, 0.46, 0.02, 0.06, 0.07 and 0.34, respectively, whereas the activities of cytochrome P450s, PB-5 (IIB2) and DM (IIE1), were less than 0.02 nmol/min/nmol P450. Cytochrome P450 IIC11 showed the highest catalytic activity of the cytochromes examined. The mechanism for the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid by cytochrome P450 IIC11 was established as being an oxygenation since one atom of oxygen-18 was exclusively incorporated into the carboxylic acid formed under $^{18}O_2$. The antibody raised to cytochrome P450 IIC11 inhibited by 60% the hepatic microsomal oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid in male rats. These results indicate that cytochrome P450 IIC11 is a major form of the cytochrome to catalyse the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid in the hepatic microsomes of male rats and that the oxidation of aldehyde to carboxylic acid is a catalytic activity common to most isozymes of P450.

Tetrahydrocannabinol (THC), a psychoactive component of marihuana, is metabolized mainly at the 11-position to form a carboxylic acid metabolite, THC-11-oic acid, as a final oxidation product [1–4]. The first oxidation step in the metabolism has been characterized as being the oxidation catalysed by cytochrome P450 to form 11-hydroxy-metabolite [5], which is further oxidized to THC-11-oic acid (carboxylic acid) through 11-oxo-THC (aldehyde) as an intermediate [6, 7]. Recently, we found that the final oxidation at the 11-position of THC may also be catalysed by hepatic microsomal cytochrome P450 of mice [8, 9], although this type of biological reaction is generally considered to be catalysed by other enzymes such as aldehyde dehydrogenase. We then reported that hepatic microsomal aldehyde oxygenase (MALDO) can catalyse the oxidation of various aldehydes to the corresponding carboxylic acids [10] and that cytochrome P450 MUT-2, purified from hepatic microsomes of male mice, is a major isozyme for catalysing the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid [11]. MALDO activity may be a common function of cytochrome P450s. This paper describes the catalytic activity of cytochrome P450s purified from rat hepatic microsomes in oxidizing 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid, and characterizes the cytochrome P450 that plays a major role in the reaction in hepatic microsomes of male rats.

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MATERIALS AND METHODS

Chemicals

NADP, NADPH and glucose-6-phosphate were purchased from Boehringer-Mannheim GmbH (Darmstadt, F.R.G.). Glucose-6-phosphate dehydrogenase (type V) and dilauroylphosphatidylcholine were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.); oxygen-18 gas (97 atom%) was from Amersham International (Amersham, U.K.); bis-trimethylsilyltrifluoroacetamide (BSFTA), trimethylsilylimidazole (TMSI), trimethylchlorosilane (TMCS) and heptafluorobutyric anhydride were from the Tokyo Kasei Kogyo Co. Ltd (Tokyo, Japan). Phenobarbital, 3-methylcholanthrene (3-MC) and other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan). 11-Oxo-Δ^8-THC [12], Δ^8-THC-11-oic acid [13] and 5′-nor-Δ^8-THC-4′-oic acid [14] were prepared by the previous methods.

Preparation of microsomes and antibody, and purification of cytochrome P450s

Male and female Sprague–Dawley rats (200–250 g) were used in the experiments. Phenobarbital (80 mg/kg/day) and 3-MC (40 mg/kg/day) were injected i.p. for 4 and 2 days, respectively. Hepatic microsomes were prepared by the method described previously [15]. Cytochrome P450s, UT-2 (IIC11), UT-4 (IIA2) and UT-5 (IIC13), were purified from untreated male rats [16]; P450s, MC-1 (IA2) and

MC-5 (IA1), were from 3-MC-treated male rats; P450s, PB-1* and PB-2 (IIC6), were from phenobarbital-treated male rats [16]. Cytochrome P450s, DM (IIE1) [18] and IF-3 (IIA1) [19], were purified from diabetic male rats and from immature female rats, respectively. Polyclonal antibody against the purified cytochrome P450 IIC11 was raised in female New Zealand White rabbits [20]. The IgG fraction from rabbit serum was obtained as described previously [20]. The antibody against cytochrome P450 IIC11 had some cross-reactivity with cytochrome P450 IIC6, amongst the cytochrome P450s examined in the present study [21]. NADPH-cytochrome P450 reductase was purified from microsomes of untreated male rats [22].

Measurement of MALDO activity

Microsomes. 11-Oxo-Δ^8-THC (50 μg) was incubated with hepatic microsomes (0.2 g liver equivalent) of untreated male rats, 0.5 mM NADP, 10 mM $MgCl_2$, 10 mM glucose-6-phosphate, 1 unit glucose-6-phosphate dehydrogenase and 100 mM sodium–potassium phosphate buffer (pH 7.4) to make a final volume of 1 mL. The mixture was incubated at 37° for 10 or 20 min and then extracted with 5 mL of ethyl acetate after addition of 5′-nor-Δ^8-THC-4′-oic acid as an internal standard. A portion of the extract was evaporated to dryness, and Δ^8-THC-11-oic acid formed was derivatized to methyl ester and heptafluorobutyrate as described previously [9]. Δ^8-THC-11-oic acid was determined by ECD-gas chromatography. The conditions were as follows: a Shimadzu GC-5A gas chromatograph equipped with an electron capture detector (^{63}Ni); column, 1% XE-60 on Chromosorb W (60–80 mesh, 3 mm × 2 m); column temperature, 195°; injection and detector temperature, 210°; carrier gas, N_2, 50 mL/min. The formation of Δ^8-THC-11-oic acid was linear under these conditions.

Reconstituted system. 11-Oxo-Δ^8-THC (20 μg) was incubated with purified cytochrome P450 (10–20 pmol), 0.33 units NADPH-cytochrome P450 reductase, 15 μg dilauroylphosphatidylcholine, 1 mM NADPH and 100 mM sodium–potassium phosphate buffer (pH 7.4) to make a final volume of 0.5 mL. The mixture was incubated at 37° for 30 or 60 min and then extracted with 5 mL of ethyl acetate, after addition of the same internal standard (0.5–1.0 μg) as described above. After evaporation of the solvent, the residue was dissolved in 0.1 mL of ethyl acetate. A portion (10 μL) of the solution was subjected to silica-gel thin-layer chromatography (TLC) using a solvent system of *n*-hexane: acetone: diethylamine (20:10:1) or of benzene: ethanol: diethylamine (20:10:1). 11-Oxo-Δ^8-THC and its metabolites were detected by spraying with 0.1% (w/v) aqueous solution of Fast Blue BB salt. Another portion (90 μL) of the solution was evaporated to dryness and the residue was methylated and then trimethylsilylated [8]. The carboxylic acid metabolite was determined by GC/MS with the measurement of abundance ratio in the molecular ions of TMS-Δ^8-THC-11-oic acid methyl ester (*m/z* 430) and TMS-5′-nor-Δ^8-THC-4′-oic acid methyl ester (*m/z* 416) as reported for the determination of Δ^9-THC-11-oic acid [23]. The experiment under $^{18}O_2$ (97 atom%) was performed as reported previously [8, 11]. GC/MS was carried out at 70 eV with a JEOL-GCG-06 gas chromatograph coupled with a JEOL JMS-DX 300 mass spectrometer and a JEOL-DA 5000 mass data system. The conditions were as follows: column, 2% OV-17 on Chromosorb W (60–80 mesh, 3 mm × 2 m); column temperature, 260°; ionizing current, 300 μA; carrier gas, He 40 mL/min.

RESULTS

Microsomal oxidation of 11-oxo-Δ^8-THC

Rat hepatic microsomes exhibited MALDO activity in the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid, as described in the case of mice [9]. The specific activity was 0.77 nmol Δ^8-THC-11-oic acid formed per min/mg (N = 4). Δ^8-THC-11-oic acid was not detected when the NADPH-generating system or the microsomes were omitted from the incubation system. NAD and NADP were not efficient cofactors for the microsomal oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid. The activity with NAD or NADP was less than 5% of that with the NADPH-generating system.

Formation of Δ^8-THC-11-oic acid by cytochrome P450 isozymes

Figure 1 illustrates the TLC profiles of ethyl acetate extracts from the reaction mixture of 11-oxo-Δ^8-THC with cytochrome P450 isozymes under the reconstituted system. Fast Blue BB salt-positive spots at origin suggest the formation of Δ^8-THC-11-oic acid. The spot at origin had the same mobility, R_f 0.32, as synthetic Δ^8-THC-11-oic acid, with a solvent system of benzene: ethanol: diethylamine (20:10:1). This result indicates that the carboxylic acid metabolite is formed by the incubation of the aldehyde with cytochrome P450s, IIC11, PB-1, IIC6 and IIA1. The other Fast Blue BB salt-positive spots, between R_f 0.2 and 0.4, suggest the formation of monohydroxylated metabolites. GC-MS analyses of these metabolites after methylation and trimethylsilylation established the structures of some monohydroxylated metabolites and Δ^8-THC-11-oic acid, as will be described in the next paragraph. Table 1 summarizes the catalytic activities of cytochrome P450 isozymes in oxidizing 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid in the reconstituted enzyme system. Among the cytochrome P450 isozymes examined, P450 IIC11 showed the highest activity, followed by P450 IIC6, P450 IIA1 and P450 PB-1. Other cytochrome P450s, IIA2, IIC13, IIB1, IA2 and IA1, had relatively low catalytic activities. Δ^8-THC-11-oic acid was not detectable in the extracts from the incubation mixture of 11-oxo-Δ^8-THC with cytochrome P450s, IIB2 and IIE1, under the conditions used. All of the cytochrome P450s that had higher catalytic activities were eluted in the pass-through fractions with an anion exchange HPLC [16, 19], suggesting that an ionic character may be

* No corresponding form to P450 PB-1 has been reported. This form of P450 could not be identified according to the nomenclature of Nebert *et al.* [17].

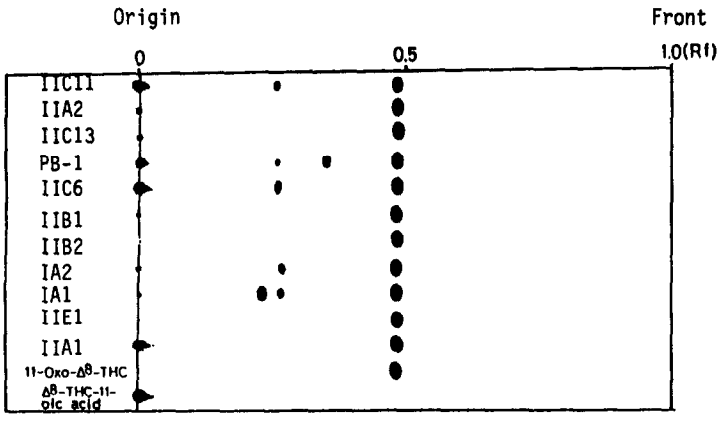

Fig. 1. Thin-layer chromatogram of 11-oxo-Δ^8-THC metabolites formed by cytochrome P450 isozymes. The solvent system was *n*-hexane : acetone : diethylamine (20 : 10 : 1). 11-Oxo-Δ^8-THC and its metabolites were detected as magenta-colored spots by spraying with 0.1% aqueous solution of Fast Blue BB salt.

Table 1. Catalytic activities of cytochrome P450 isozymes in the conversion of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid

Cytochrome P450 isozyme	Catalytic activity*	Other 11-oxo-Δ^8-THC metabolites formed‡
IIC11	0.69	4′-OH and 7 β-OH
IIA2	0.08	
IIC13	0.07	
PB-1	0.23	7α-OH and 7β-OH
IIC6	0.46	
IIB1	0.02	7β-OH
IIB2	ND†	
IA2	0.06	1′-OH
IA1	0.07	1′-OH and 3′-OH
IIE1	ND	
IIA1	0.34	

Incubations and analyses were carried out as described in Materials and Methods. Each result represents the mean of 2 to 4 incubations.

* nMol Δ^8-THC-11-oic acid formed per min/nmol P450.

† < 0.02.

‡ These metabolites were identified tentatively from their mass fragmentations.

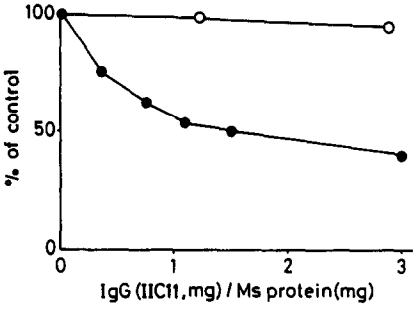

Fig. 2. Effect of antibody against P450 IIC11 on MALDO activity in oxidation of 11-oxo-Δ^8-THC in male rat liver. Open and closed circles indicate the addition of IgG from untreated- and cytochrome P450 IIC11 treated-rabbit serum, respectively. The indicated amount of IgG was preincubated with hepatic microsomes for 30 min at 37°. MALDO activity was assayed as described in Materials and Methods. The activity was expressed as a percentage of the control value (0.99 nmol Δ^8-THC-11-oic acid formed per min/mg protein).

an important factor for accepting 11-oxo-Δ^8-THC as a substrate.

Two monohydroxylated metabolites were formed by incubation of 11-oxo-Δ^8-THC with cytochrome P450 IIC11. They were identified tentatively from their mass spectra on the basis of fragment patterns of THC metabolites described by Binder *et al.* [24] and Harvey [25]. The molecular ion of these metabolites showed at m/z 488 and other characteristic ions at m/z 344, 117 and at m/z 303, suggesting that the two metabolites were 4′-OH- and 7β-OH-11-oxo-Δ^8-THC, respectively. 7β-OH-metabolite was also formed by cytochrome P450s, PB-1 and IIB1. Cytochrome P450 PB-1 formed an additional metabolite that was identified as 7α-OH-11-oxo-Δ^8-THC on the basis of its characteristic fragment ions at m/z 398 and 383 (M^+ −90 and M^+ −105) in the mass spectrum. Cytochrome P450s, MC-1 and MC-5, which are forms induced by 3-MC, formed monohydroxylated metabolites oxidized on the pentyl side chain at the 1′ and 3′ positions. The characteristic fragment ions of these metabolites were m/z 431 (M^+ −57, 1′-OH) and m/z 344 (M^+ −144, 3′-OH).

Inhibition with antibody

The inhibitory effect of antibody against purified cytochrome P450 IIC11 on the formation of Δ^8-THC-11-oic acid in the presence of hepatic microsomes of male rats is shown in Fig. 2. The formation of Δ^8-THC-11-oic acid was suppressed with increasing amounts of antibody. At a concentration ratio of 3 mg IgG/mg microsomal protein, the reaction was inhibited by 64%, while IgG from control rabbit did not inhibit the reaction at all.

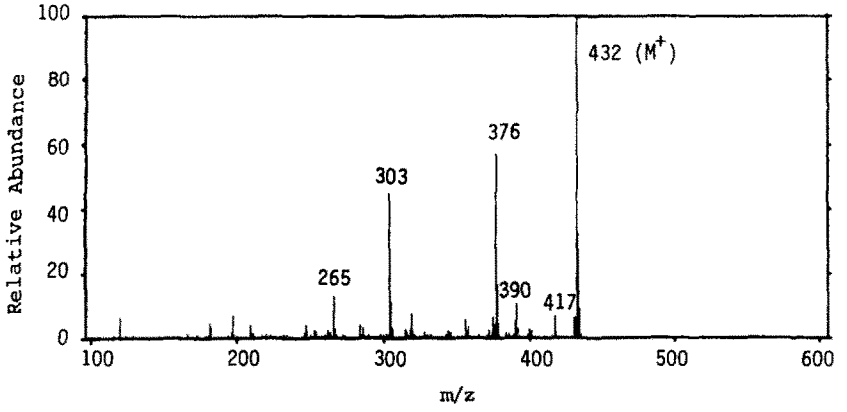

Fig. 3. Mass spectrum of Δ^8-THC-11-oic acid formed by P450 IIC11 under $^{18}O_2$ (97 atom%).

Incorporation of molecular oxygen into Δ^8-THC-11-oic acid

The oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid catalysed by P450 IIC11 was confirmed to be an oxygenation reaction. A mass spectrum of methyl ester and trimethylsilylated derivative of Δ^8-THC-11-oic acid formed under air showed a molecular ion at m/z 430 (relative intensity, 100) and other major fragment ions at m/z 374 (62), 303 (48) and 265 (14). The fragment ion at m/z 374 results from the loss of butylene by cleavage of the pentyl side chain, as suggested by Harvey [26]. Fragment ions at both m/z 303 and 265 involve the pentyl side chain and result from the loss of a carbon atom at the 11-position. Figure 3 summarizes a mass spectrum of the same derivative of the carboxylic acid metabolite formed under $^{18}O_2$ (97 atom%). A molecular ion at m/z 432 (100) and a fragment ion at m/z 376 (58) indicate that one atom of oxygen-18 was exclusively incorporated from molecular oxygen into the carboxylic acid metabolite formed.

DISCUSSION

Rat hepatic microsomes, like mouse hepatic microsomes [9], catalysed the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid. Rat hepatic microsomes are known to contain aldehyde dehydrogenase which is an enzyme well known to oxidize aldehyde [27, 28]. However, the NADPH-generating system was an essential requirement for the microsomal oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid, whereas aldehyde dehydrogenase requires NAD or NADP as a cofactor. In the present study, NAD- or NADP-dependent oxidation of 11-oxo-Δ^8-THC was negligible, indicating that MALDO is an enzyme distinct from aldehyde dehydrogenase and that 11-oxo-Δ^8-THC is not a substrate for aldehyde dehydrogenase in rat hepatic microsomes.

The oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid in rat hepatic microsomes could be catalysed by cytochrome P450 since some of the constitutive cytochrome P450s (IIC11, PB-1, IIC6) have a catalytic activity for the oxidation. Among them, cytochrome P450 IIC11, which is one of the major forms of cytochrome P450 expressed in hepatic microsomes of male rats [29], showed the highest catalytic activity. MALDO activity in adult male rat liver was suppressed more than 60% by the addition of the antibody against purified cytochrome P450 IIC11. These results suggest that cytochrome P450 IIC11 is a major isozyme responsible for the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid in the hepatic microsomes of male rats. Cytochrome PB-1, which is constitutively expressed in hepatic microsomes of male rats [30], may also participate partially in the formation of Δ^8-THC-11-oic acid in the microsomes. The mechanism for this aldehyde oxidation catalysed by cytochrome P450 IIC11 was oxygenation, since one atom of oxygen was incorporated from molecular oxygen into the carboxylic acid formed. The oxygenation mechanism has already been established for the same reaction catalysed by cytochrome P450 MUT-2 purified from hepatic microsomes of male mice [11].

We suggested previously that the oxidation of aldehyde to carboxylic acid is a common function of cytochrome P450s. In endogenous compounds, Soberman *et al.* [31] suggested that cytochrome $P450_{LTB}$, which catalyses the ω-oxidation of leucotriene B_4, could oxidize leucotriene B_4 aldehyde to its carboxylic acid. Recently, Gans and Werringloer [32] suggested that cytochrome P450 IIE1 has a catalytic activity in the oxidation of aliphatic aldehydes. Cytochrome P450 IIE1 showed negligible activity in oxidizing 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid in the present study. These findings indicate that cytochrome P450 isozymes show different catalytic activities with different aldehyde substrates.

The present study demonstrates that all the cytochrome P450s examined, except for cytochrome P450 IIB2 and IIE1, shows some catalytic activity in the oxidation of 11-oxo-Δ^8-THC to Δ^8-THC-11-oic acid. The results support the view that the catalytic activity of cytochrome P450 in oxidizing aldehydes to carboxylic acids is a common function of the enzyme.

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REFERENCES

1. Harvey DJ and Paton WDM, Identification of *in vivo* liver metabolites of Δ^6-tetrahydrocannabinol produced by the mouse. *Drug Metab Dispos* **8**: 178–186, 1980.
2. Watanabe K, Yamamoto I, Oguri K and Yoshimura H, Metabolic disposition of Δ^8-tetrahydrocannabinol and its active metabolites, 11-hydroxy-Δ^8-tetrahydrocannabinol and 11-oxo-Δ^8-tetrahydrocannabinol, in mice. *Drug Metab Dispos* **9**: 261–264, 1981.
3. Halldin MM, Carlsson S, Kanter SL, Widman M and Agurell S, Urinary metabolites of Δ^1-tetrahydrocannabinol. *Arzneim Forsch Drug Res* **32**: 764–768, 1982.
4. Wall ME, Sadler BM, Brine D, Taylor H and Perez-Reyes M, Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. *Clin Pharmacol Ther* **34**: 352–363, 1983.
5. Burstein S and Kupfer D, Hydroxylation of trans-Δ^1-tetrahydrocannabinol by hepatic microsomal oxygenase. *Ann NY Acad Sci* **191**: 61–72, 1971.
6. Ben-Zvi Z and Burstein S, 7-Oxo-Δ^1-tetrahydrocannabinol: a novel metabolite of Δ^1-tetrahydrocannabinol. *Res Commun Chem Pathol Pharmacol* **8**: 223–229, 1974.
7. Watanabe K, Yamamoto I, Oguri K and Yoshimura H, Microsomal oxygenase catalyzed oxidation of 11-hydroxy-Δ^8-tetrahydrocannabinol to 11-oxo-Δ^8-tetrahydrocannabinol. *Biochem Biophys Res Commun* **88**: 178–182, 1979.
8. Yamamoto I, Watanabe K, Narimatsu S and Yoshimura H, Oxygenation mechanism in the oxidation of xenobiotic aldehyde to carboxylic acid by mouse hepatic microsomes. *Biochem Biophys Res Commun* **153**: 779–782, 1988.
9. Watanabe K, Hirahashi N, Narimatsu S, Yamamoto I and Yoshimura H, Mouse hepatic microsomal enzyme that catalyzes oxidation of 11-oxo-Δ^8-tetrahydrocannabinol to Δ^8-tetrahydrocannabinol-11-oic acid. *Drug Metab Dispos* **19**: 218–221, 1991.
10. Watanabe K, Narimatsu S, Yamamoto I and Yoshimura H, Hepatic microsomal oxygenation of aldehydes to carboxylic acids. *Biochem Biophys Res Commun* **166**: 1308–1312, 1990.
11. Watanabe K, Narimatsu S, Yamamoto I and Yoshimura H, Oxygenation mechanism in conversion of aldehyde to carboxylic acid catalyzed by a cytochrome P450 isozyme. *J Biol Chem* **266**: 2709–2711, 1991.
12. Inayama S, Sawa A and Hosoya E, The oxidation of Δ^1- and Δ^6-tetrahydrocannabinol with selenium dioxide. *Chem Pharm Bull* **22**: 1519–1525, 1974.
13. Mechoulam R, Ben-Zvi Z, Agurell S, Nilsson IM, Nilsson JLG, Edery H and Grunfeld Y, Δ^6-Tetrahydrocannabinol-7-oic acid, a urinary metabolite: isolation and synthesis. *Experientia* **29**: 1193–1195, 1973.
14. Ohlsson A, Agurell S, Leander K, Dahmen J, Edery H, Porath G, Levy S and Mechoulam R, Synthesis and psychotropic activity of side-chain hydroxylated Δ^6-tetrahydrocannabinol metabolites. *Acta Pharm Suec* **16**: 21–33, 1979.
15. Watanabe K, Arai M, Narimatsu S, Yamamoto I and Yoshimura H, Effect of repeated administration of 11-hydroxy-Δ^8-tetrahydrocannabinol, an active metabolite of Δ^8-tetrahydrocannabinol, on the hepatic microsomal drug-metabolizing enzyme system of mice. *Biochem Pharmacol* **35**: 1861–1865, 1986.
16. Funae Y and Imaoka S, Simultaneous purification of multiple forms of rat liver microsomal cytochrome P-450 by high-performance liquid chromatography. *Biochim Biophys Acta* **842**: 119–132, 1985.
17. Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzales FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P450 gene subfamily: recommended nomenclature. *DNA* **6**: 1–11, 1987.
18. Funae Y, Imaoka S and Shimojo N, Purification and characterization of diabetes-inducible cytochrome P-450. *Biochem Int* **16**: 503–509, 1988.
19. Imaoka S, Kamataki T and Funae Y, Purification and characterization of six cytochrome P-450 from hepatic microsomes of immature female rats. *J Biochem* **102**: 843–851, 1987.
20. Narimatsu S, Watanabe K, Matsunaga T, Yamamoto I, Imaoka S, Funae Y and Yoshimura H, Inhibition of hepatic microsomal cytochrome P450 by cannabidiol in adult male rats. *Chem Pharm Bull* **38**: 1365–1368, 1990.
21. Imaoka S, Terano Yoshitake and Funae Y, Changes in the amount of cytochrome P450s in rat hepatic microsomes with starvation. *Arch Biochem Biophys* **278**: 168–178, 1990.
22. Yasukochi Y and Masters BSS, Some properties of a detergent-solubilized NADPH-cytochrome *c* (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344, 1976.
23. Yamamoto I, Watanabe K, Narimatsu S, Matsunaga T, Kijima T, Hiraiwa K and Yoshimura H, A GC-MS method for determination of Δ^9-tetrahydrocannabinol-11-oic acid in human urine. *Eisei Kagaku* **36**: 149–152, 1990.
24. Binder M, Agurell S, Leander K and Lindgren J-E, Zur identifikation potentieller metabolite von cannabis-inhalstoffen: kernresonanz- und massen spektroskopische untersuchungen. *Helv Chim Acta* **57**: 1626–1641, 1974.
25. Harvey DJ, The mass spectra of the trimethylsilyl derivatives of the hydroxy and acid metabolites of Δ^1- and Δ^6-tetrahydrocannabinol. *Biomed Mass Spectrom* **8**: 579–588, 1981.
26. Harvey DJ, The mass spectra of the trimethylsilyl derivatives of Δ^8- and Δ^9-tetrahydrocannabinol. *Biomed Mass Spectrom* **8**: 575–578, 1981.
27. Deitrich RA, Tissue and subcellular distribution of mammalian aldehyde-oxidizing capacity. *Biochem Pharmacol* **15**: 1911–1922, 1966.
28. Lindahl R and Evces S, Comparative subcellular distribution of aldehyde dehydrogenase in rat, mouse and rabbit liver. *Biochem Pharmacol* **33**: 3383–3389, 1984.
29. Kamataki T, Maeda K, Yamazoe Y, Nagai T and Kato R, Sex difference of cytochrome P-450 in the rat: purification, characterization, and quantification of constitutive forms of cytochrome P-450 from liver microsomes of male and female rats. *Arch Biochem Biophys* **225**: 758–770, 1983.
30. Imaoka S, Terano Y and Funae Y, Purification and characterization of two constitutive cytochrome P-450 (F-1 and F-2) from adult female rats: identification of P-450 F-1 as the phenobarbital-inducible cytochrome P-450 in male rat liver. *Biochim Biophys Acta* **916**: 358–367, 1987.
31. Soberman RJ, Sutyak JP, Okita RT, Wendelborn DF, Roberts II LJ and Austen KF, The identification and formation of 20-aldehyde leukotriene B_4. *J Biol Chem* **263**: 7996–8002, 1988.
32. Gans G and Werringloer J, NADPH-dependent metabolism of aldehydes catalyzed by liver microsomal cytochrome P-450. In: *Drug Metabolizing Enzymes: Genetics, Regulation and Toxicology* (Eds. Ingelman-Sundberg M, Gustafsson J-A and Orrenius S), p. 126. Stockholm Projeckygrupp AB, Stockholm, 1990.