Monkey Hepatic Microsomal Alcohol Oxygenase: Purification and Characterization of a Cytochrome P450 Enzyme Catalyzing the Stereoselective Oxidation of 7α- and 7β-Hydroxy-Δ8-tetrahydrocannabinol to 7-Oxo-Δ8-tetrahydrocannabinol

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The formation of 7-oxo-Δ8-tetrahydrocannabinol (7-Oxo-Δ8-THC) from 7α- or 7β-hydroxy-Δ8-THC (7α- or 7β-OH-Δ8-THC) was found in hepatic microsomes of monkeys. The activity in 7β-OH-Δ8-THC was stereospecifically 2.5- to 4.6-fold higher than that from 7α-OH-Δ8-THC. The oxidative activities of 7α- and 7β-OH-Δ8-THC to 7-Oxo-Δ8-THC were inhibited to 35% and 10%, respectively, of the control value by the antibody against P450GPF-B (CYP3A3), a major enzyme responsible for the formation of 7-Oxo-Δ8-THC in guinea pigs. In the Lineweaver-Burk double-reciprocal plot analysis, testosterone 6β-hydroxylase activity was competitively inhibited by 7β-OH-Δ8-THC. Two cytochrome P450 enzymes, called P450JM-D and P450JM-E, were purified from hepatic microsomes of Japanese monkeys. P450JM-E, assumed to be CYP3A8, immunologically reacted with the antibody against P450GPF-B and showed high forming activity of 7-Oxo-Δ8-THC from 7α-OH-Δ8-THC. On the other hand, 7-Oxo-Δ8-THC forming activity of P450JM-D, assumed to be CYP2C, was less than 10% of that of P450JM-E (CYP3A8). Oxygen-18 (18O) derived from atmospheric oxygen was incorporated into about 40% of the corresponding ketone formed from 7α-OH-Δ8-THC or 8β-OH-Δ8-THC by P450JM-E (CYP3A8). The incorporation into the stable isotope into the oxidized metabolite from 7α-OH-Δ8-THC or 8α-OH-Δ8-THC was negligible. These results indicate that P450JM-E (CYP3A8) is a major enzyme of the oxidation of 7-OH-Δ8-THC in monkey hepatic microsomes. The oxidation mechanism may proceed as follows: the α- and β-epimers of 7-OH-Δ8-THC or 8-OH-Δ8-THC may be converted to ketone through dehydration of an enzyme-bound gem-diol by P450JM-E (CYP3A8), although this stereoselective dehydration differentiates between two epimers.

Key words 7-hydroxy-Δ4-tetrahydrocannabinol; microsomal alcohol oxygenase; CYP3A8; 7-oxo-Δ8-tetrahydrocannabinol; cytochrome P450; monkey liver

Tetrahydrocannabinol (THC) is the major psychoactive constituent of marijuana. It is well known that Δ8-THC is oxidized to numerous metabolites in the liver of various mammals including monkeys and humans.1,2 Most metabolites have been suggested to contribute to the psychoactivity of the parent compound. 7-Oxo-Δ8-THC shows almost equivalent pharmacological activity to Δ8-THC in mice, whereas 7α- and 7β-hydroxy-Δ8-THC (7α- and 7β-OH-Δ8-THC) were without significant activity.3 Several recent studies have clarified that cytochrome P450 (P450) plays a major role in the oxidation of THC.4,5 However, the metabolic reaction is complicated and the isomers responsible for specific metabolic reactions have not been completely elucidated. We have previously found that a guinea pig hepatic microsomal enzyme, called microsomal alcohol oxygenase (MA-CO), is able to oxidize 7-OH-Δ8-THC to 7-Oxo-Δ8-THC (Fig. 1), and have recently clarified that P450 isoforms belonging to the 3A subfamily are the major enzymes of 7-OH-Δ8-THC in guinea pigs (P450GPF-B),6 mice (CYP3A11),7 rats (CYP3A1 and CYP3A2),8 and humans (CYP3A4).9 CYP3A is a highly expressed enzyme in rodents, monkeys, and humans and it has been demonstrated to play a prominent role in the metabolism of many clinically important drugs.10 CYP3A shows selectivity for the chemical substituents rather than selectivity for substrates.11,12 For example, the allylic positions, in spite of structurally unrelated compounds, are the major site of oxidation catalyzed by CYP3A.17–20 Species-related differences exist in the enzymatic properties of P450 enzymes belonging to the same family or subfamily, but it is difficult to elucidate the properties of the enzyme responsible for a specific reaction across species.12,21 To our best knowledge, no detailed study has been reported with respect to the specific isoform(s) involved in the formation of 7-oxo-Δ8-THC from 7α- and 7β-OH-Δ8-THC in monkeys.

In the present study, we purified a P450 enzyme catalyzing the stereoselective oxidation of 7α- and 7β-OH-Δ8-THC to 7-Oxo-Δ8-THC from hepatic microsomes of Japanese monkeys and considered the oxidation mechanisms of 7-OH-Δ8-THC and 8-OH-Δ8-THC using oxygen-18 gas.

MATERIALS AND METHODS

Materials Sepharose 4B and 2′,5′-ADP-Sepharose 4B were from Pharmacia Fine Chemicals (Uppsala, Sweden); hydroxylapatite for an open column was from Bio-Rad (Richmond, CA, U.S.A.); preparative DEAE-5PW and hydroxylapatite columns for HPLC were from Tosoh (Tokyo, Japan), 18O2 (97 atom %) was from Amersham International plc (Buckinghamshire, U.K.). Emulgen 911 was kindly provided by Kao-Atlas Co. (Tokyo, Japan). 7α- and 7β-OH-Δ8-THC,22 7-Oxo-Δ8-THC,3 8α- and 8β-OH-Δ8-THC,23 8-Oxo-Δ8-THC,23 and 5′-nor-Δ8-THC-4′-oic acid24 were prepared by the methods previously reported. Purities of the cannabinoids were confirmed to be more than 98% by gas chromatography. Microsomal lipids were extracted from he-
Purification of P450 from Hepatic Microsomes of (7- and 10-Year-Old) Female Japanese Monkeys Microsomes were suspended in buffer A [0.1 M potassium phosphate buffer (pH 7.2) containing 20% glycerol, 1 mM EDTA, and 0.5 mM dithiothreitol]. Then 20% sodium cholate solution (pH 7.4) was added to a final concentration of 0.7%. This mixture was stirred for 30 min at 4°C to solubilize microsomes and the resulting suspension was centrifuged at 105000×g for 60 min.

The supernatant fraction of the cholate-solubilized hepatic microsomes was put on an ω-aminooctyl-Sepharose 4B column (4×30 cm) equilibrated with buffer A containing 0.5% sodium cholate. The column was washed with the equilibration buffer, and P450 was eluted with buffer A containing 0.4% sodium cholate and 0.1% Emulgen 911. The P450 fractions were pooled, concentrated with an ultrafiltration membrane (UK-50, Toyobo, Osaka, Japan), and dialyzed against 20 mM Tris-acetate buffer (pH 7.5) containing 20% glycerol and 0.4% Emulgen 911. The column chromatography was performed with a linear gradient of sodium acetate from 0 to 0.2 M in buffer B. The elution profiles of heme and protein were monitored at 417 and 244 nm, respectively, as described by Funae and Imaoka.26)

The immunologically reactive fractions with antibody against P450GPFB were dialyzed against buffer C [10 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol and 0.2% sodium cholate] and subjected to HPLC with a hydroxylapatite column, previously equilibrated with buffer C. P450 was eluted with a linear gradient of potassium phosphate buffer (pH 7.4) from 10 to 350 mM containing 20% glycerol, 0.2% sodium cholate, and 0.4% Emulgen 911. The fractions eluted with 125 to 185 mM potassium phosphate buffer were combined and concentrated by ultrafiltration. After dialysis against buffer D [5 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol, 1 mM EDTA, 0.1 mM dithiothreitol, and 0.4% Emulgen 911], the solution was applied to a CM-Sephadex C-50 column (1.2×30 cm), previously equilibrated with buffer D. P450 was eluted with 100 ml each of buffer D containing 5, 10, 20, 40, 80, or 160 mM potassium phosphate buffer. The fractions eluted with the 20 and 40 mM phosphate buffers were separately concentrated and dialyzed against buffer C. The dialyzed samples were subjected to HPLC with a hydroxylapatite column (0.75×7 cm) again, previously equilibrated with buffer C. P450 was eluted with a linear gradient of potassium phosphate buffer (pH 7.4) from 10 to 350 mM containing 20% glycerol, 0.2% sodium cholate, and 0.4% Emulgen 911. The fractions eluted with approximately 150 mM potassium phosphate buffer were electrophoretically homogeneous. The detergent was removed by using a small hydroxylapatite column as previously described.6)

Purification of Other Enzymes NADPH-cytochrome c (P450) reductase and cytochrome b₅ were purified from hepatic microsomes of male ddY mice (Hokuriku Experimental Animals Laboratory, Kanazawa, Japan) by the methods of Yasukochi and Masters,27) and Funae and Imaoka,26 respectively. One unit of the reductase was defined as the amount of reductase catalyzing the reduction of 1 μmol of cytochrome

![Diagram of microsomes](image)

Fig. 1. Oxidation of 7α- and 7β-OH-Δ⁸-THC to 7-Oxo-Δ⁸-THC by Hepatic Microsomes of Monkey Liver

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Fig. 1. Oxidation of 7α- and 7β-OH-Δ⁸-THC to 7-Oxo-Δ⁸-THC by Hepatic Microsomes of Monkey Liver
Measurement of Oxidative Activity  The formation of 7-Oxo-Δ⁴-THC was measured as previously described, except for the conditions in the reconstitution studies described below. 7-OH-Δ⁴-THC (12 μg) or 8-OH-Δ⁴-THC (12 μg) was incubated with purified P450 (25 pmol), 0.15 units of NADPH-cytochrome c (P450) reductase, 25 pmol of cytochrome b₅, 20 μg of microsomal lipids, 100 μg of sodium cholate, 1 mM NADPH, and 100 mM potassium phosphate buffer (pH 7.4) to make a final volume of 0.5 ml. The mixture was incubated at 37 °C for 20 min after preincubation at 37 °C for 2 min. Metabolites were extracted with ethyl acetate and analyzed by electron capture detector-gas chromatography or gas chromatography-mass spectrometry (GC-MS) as described previously.

The isotopic incorporation values were calculated using the following equation, where A was the ratio in relative intensities of ions at [(M + 2)+] to (M+) of the corresponding ketone formed from 7-hydroxy-Δ⁴-THC or 8-hydroxy-Δ⁴-THC under 18O₂. B was the ratio in relative intensities of ions at [(M + 2)+] to (M+) of the corresponding ketone formed from 7-hydroxy-Δ⁴-THC or 8-hydroxy-Δ⁴-THC under air, and C was atom % of 18O₂.

\[
\% \text{ of incorporation} = \frac{(A - B)}{(1 + A - B)} \times 1 \times 10^4
\]

The oxidative metabolism of testosterone was determined as described previously.

Other Methods  Polyclonal antibody against the purified P450 was raised in rabbits, and the IgG fraction from the serum was obtained by the previously reported method. Western blotting was performed as described previously. Protein concentration was estimated by the method of Lowry et al., using bovine serum albumin as a standard. P450 and cytochrome b₅ contents were determined by the methods of Omura and Takei, respectively. α-Aminoacyl-tRNA synthetase 4B was prepared as described previously. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli.

RESULTS

7-OH-Δ⁴-THC MALCO Activity in Monkey Liver

Table 1 shows the enzyme activities for the formation of 7-Oxo-Δ⁴-THC from 7α- and 7β-OH-Δ⁴-THC in hepatic microsomes of monkeys. There is no significant difference in the MALCO activities between male and female monkeys. The activity was stereoselective and the rate of conversion to 7-Oxo-Δ⁴-THC from 7β-OH-Δ⁴-THC was 2.5- to 4.6-fold higher than that from 7α-OH-Δ⁴-THC.

Effects of Antibody against P450GPF-B on 7OH-Δ⁴-THC MALCO Activity and Microsomal Testosterone Oxidation  Addition of the antibody against P450GPF-B to the incubation medium caused a dose-dependent suppression of the activities of both 7α- and 7β-OH-Δ⁴-THC MALCO in monkey liver (Fig. 2). When the anti-P450GPF-B IgG was added to a 3-fold amount of the microsomal protein, the MALCO activities were suppressed to about 35 and 10%, respectively, of the control values. The antibody also inhibited testosterone 6β-hydroxylation in the hepatic microsomes in the same manner as MALCO activity for 7-OH-Δ⁴-THC, especially the β-epimer (Fig. 2).

Effect of 7β-OH-Δ⁴-THC on Testosterone 6β-Hydroxylase Activity  To clarify the interaction between 7β-OH-Δ⁴-THC and testosterone in the substrate binding pocket or the active site of P450 belonging to the 3A subfamily, testosterone 6β-hydroxylase activity was determined in the presence and absence of different 7β-OH-Δ⁴-THC concentrations. The Lineweaver–Burk double-reciprocal plot in Fig. 3 shows that 7β-OH-Δ⁴-THC inhibits testosterone 6β-hydroxylase activity in competitive manner. The Kᵢ values with the addition of 0, 15, and 37.5 μM of 7β-OH-Δ⁴-THC were 0.23, 0.37, and 0.45 mM, respectively.

Purification of P450 from Hepatic Microsomes of Japanese Monkeys  On the basis of the above results, we carried out the purification of MALCO from hepatic microsomes of Japanese monkeys using the immunological cross-reaction with antibody against P450GPF-B as an indicator. The P450-containing fraction was eluted as one peak by washing the antibody against P450GPF-B (data not shown). The purified P450s showed a single protein band on SDS-PAGE, and the apparent molecular weights of P450JM-D and P450JM-E were isolated, although P450JM-D did not react with antibody against P450GPF-B (data not shown). The purified P450s showed a single protein band on SDS-PAGE, and the apparent molecular weights of P450JM-D and P450JM-E were estimated to be 52,200 and 51,800, respectively (Fig. 4). Both of the purified P450s had a specific content of 12.2 nmol/mg protein. The first 18 residues of the NH₂-terminal amino acid sequence of P450JM-D (M-D-S-L-V-V-L-V-L-X-L-S-X-L-L-L-S-L-W-R-Q) were the same as those of P450CMLd, which is an enzyme belonging to the CYP2C subfamily purified from hepatic microsomes of cynomolgus monkeys. The NH₂-terminal amino acid sequence of P450JM-E (M-D-L-I-P-D-L-A-V-E-T-W-L-L-L-A-V-T-L-V-L-L-Y-L-Y-G-T-H-S) was identical to that of a protein encoded by CYP3A8 cDNA.
Catalytic Properties of Purified P450

In the reconstituted system, we used a microsomal lipid system including cytochrome b₅ and sodium cholate as described previously.¹⁰)

The oxidative activities of 7α- and 7β-OH-Δ⁷-THC to 7-Oxo-Δ⁷-THC by P450JM-E were 1.11 and 6.33 nmol/min/nmol P450, respectively (Table 2). P450JM-E was also able to oxidize both 8α- and 8β-OH-Δ⁹-THC to 8-Oxo-Δ⁹-THC, although the oxidative activity of 8-OH-Δ⁹-THC was significantly lower than that of 7-OH-Δ⁷-THC. Under the same conditions, the 7-Oxo-Δ⁷-THC-forming activity of P450JM-E was 1.11 nmol/min/nmol P450.

Table 2. Catalytic Activities of P450JM-D and P450JM-E Purified from Hepatic Microsomes of Japanese Female Monkeys

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Metabolite</th>
<th>Activity (nmol/min/nmol P450)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7α-OH-Δ⁷-THC</td>
<td>7-Oxo-Δ⁷-THC</td>
<td>P450JM-D</td>
</tr>
<tr>
<td>7β-OH-Δ⁷-THC</td>
<td>7-Oxo-Δ⁷-THC</td>
<td>0.11</td>
</tr>
<tr>
<td>8α-OH-Δ⁹-THC</td>
<td>8-Oxo-Δ⁹-THC</td>
<td>0.01</td>
</tr>
<tr>
<td>8β-OH-Δ⁹-THC</td>
<td>8-Oxo-Δ⁹-THC</td>
<td>0.13</td>
</tr>
<tr>
<td>Testosterone</td>
<td>6β-OH-testosterone</td>
<td>1.02</td>
</tr>
<tr>
<td>Androstenedione</td>
<td></td>
<td>1.64</td>
</tr>
</tbody>
</table>

Enzyme activities were assayed as described in Materials and Methods. Each value represents the mean of duplicate determinations.
Table 3. Isotope Ratios in Molecular Ions of 7-Oxo-A\(^{\delta}\)-THC and 8-Oxo-A\(^{\delta}\)-THC Formed by P450JM-E under Oxygen-18 Gas

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative abundance of molecular ions (m/z 400 : 402)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Air (^{18})O</td>
</tr>
<tr>
<td>7(\alpha)-OH-A(^{\delta})-THC</td>
<td>100 : 15 100 : 70</td>
</tr>
<tr>
<td>7(\beta)-OH-A(^{\delta})-THC</td>
<td>100 : 16 100 : 33</td>
</tr>
<tr>
<td>8(\alpha)-OH-A(^{\delta})-THC</td>
<td>100 : 11 100 : 14</td>
</tr>
<tr>
<td>8(\beta)-OH-A(^{\delta})-THC</td>
<td>100 : 29 100 : 81</td>
</tr>
</tbody>
</table>

After incubation under \(^{18}\)O, 7-Oxo-A\(^{\delta}\)-THC formed was converted to the trimethylsilyl derivative and analyzed at an ionization energy of 70 eV on a JEOL DX-300 mass spectrometer.

D was less than 10% of that of P450JM-E. P450JM-E also showed high testosterone 6\(\beta\)-hydroxylase activity (9.21 nmol/min/nmol P450) in the reconstituted system. On the other hand, the androstenedione-forming activity of P450JM-D was more than 10-fold higher than that of P450JM-E.

**Incorporation of Atmospheric Oxygen into 7-Oxo-A\(^{\delta}\)-THC and 8-Oxo-A\(^{\delta}\)-THC**

7-OH-A\(^{\delta}\)-THC and 8-OH-A\(^{\delta}\)-THC were incubated with P450JM-E under an \(^{18}\)O\(_2\) gas phase, and the trimethylsilyl derivative of the metabolites was analyzed by GC-MS. Table 3 shows the relative intensities of molecular ions of the metabolites. The ratio in relative intensities of ions at m/z 402 to 400 of 7-Oxo-A\(^{\delta}\)-THC formed from 7\(\alpha\)-OH-A\(^{\delta}\)-THC was 0.70, showing that \(^{18}\)O derived from atmospheric oxygen molecule was incorporated into 37% of the oxidized metabolite. In the case of 7\(\beta\)-OH-A\(^{\delta}\)-THC, the ratio was 0.33, and \(^{18}\)O was incorporated into 15% of the metabolites. On the other hand, the ratios of 8-Oxo-A\(^{\delta}\)-THC formed from 8\(\alpha\)- and 8\(\beta\)-OH-A\(^{\delta}\)-THC were 0.14 and 0.81, and \(^{18}\)O was incorporated into 3% and 35%, respectively.

**DISCUSSION**

In the course of our metabolic studies on THC, we have found that oxidation at the C7-position of A\(^{\delta}\)-THC is a major metabolic pathway in hepatic microsomes of the guinea pig and that the oxidation of 7-OH-A\(^{\delta}\)-THC to 7-Oxo-A\(^{\delta}\)-THC may be catalyzed by P450, not dehydrogenase.\(^9\),\(^10\) We have also found that antibody against P450GPF-B purified from hepatic microsomes of guinea pigs as a major enzyme responsible for 7-OH-A\(^{\delta}\)-THC MALCO also inhibited MALCO activity in mouse, rat, monkey, and human livers.\(^10\) This result suggested that the protein immunologically related to P450GPF-B might be the principal enzyme involved in MALCO in various animal species.

We have previously reported that the MALCO activity for 7-OH-A\(^{\delta}\)-THC is stereoselective and the formation of 7-Oxo-A\(^{\delta}\)-THC from 7\(\beta\)-OH-A\(^{\delta}\)-THC in guinea pigs,\(^10\) mice,\(^11\) rats,\(^12\) and humans\(^13\) is 1.9-\(\times\), 2.0-\(\times\), 3.0-\(\times\), and 4.5-\(\times\), respectively, higher than that from 7\(\alpha\)-OH-A\(^{\delta}\)-THC. In the present study, the same stereoselectivity was also shown in hepatic microsomes of monkeys, and the MALCO activity for 7\(\beta\)-OH-A\(^{\delta}\)-THC was about 4-fold higher than that for 7\(\alpha\)-OH-A\(^{\delta}\)-THC. These results indicate that there is a species-related difference in the extent of stereoselectivity for the substrate.

Testosterone 6\(\beta\)-hydroxylase is thought to be one of specific reactions for the CYP3A enzyme in monkey liver.\(^37\),\(^38\) The MALCO activity for 7-OH-A\(^{\delta}\)-THC as well as testosterone 6\(\beta\)-hydroxylase activity was inhibited dose-dependently by antibody against P450GPF-B. Furthermore, in the Lineweaver-Burk double-reciprocal plot analysis, testosterone 6\(\beta\)-hydroxylase was competitively inhibited by the addition of 7\(\beta\)-OH-A\(^{\delta}\)-THC to the incubation mixture. These results suggest that the major enzyme of MALCO in monkey liver is P450 belonging to the 3A subfamily. 7\(\alpha\)-OH-A\(^{\delta}\)-THC MALCO activity, however, may be also catalyzed by other P450 enzymes together with CYP3A, since the activity inhibited by antibody against P450GPF-B was up to 35% of the control value. We purified two P450 enzymes, called P450JM-D and P450JM-E, from hepatic microsomes of Japanese monkeys. P450JM-E is assumed to be CYP3A8 and showed high forming activity of 7-Oxo-A\(^{\delta}\)-THC from 7\(\alpha\)- and 7\(\beta\)-OH-A\(^{\delta}\)-THC. On the other hand, the 7-Oxo-A\(^{\delta}\)-THC forming activity of P450JM-D was relatively low compared with that of P450JM-E. The testosterone 6\(\beta\)-hydroxylase activity of P450JM-E is considerably higher than that of CYP3A8 (P450CMC) purified from hepatic microsomes of cynomolgus monkey as reported by Ohmori et al.\(^37\) The reason may be attributable to the use of microsomal lipids instead of dilauroylphosphatidylcholine as the lipid in the reconstituted system of P450JM-E, because a number of studies have shown that the catalytic activities of CYP3A enzymes are very low in a reconstituted system using only dilauroylphosphatidylcholine as the lipid.\(^11\),\(^39\)—\(^41\) P450JM-E showed comparable activity to CYP3A1,\(^40\) CYP3A4,\(^41\) and CYP3A11\(^11\) for testosterone 6\(\beta\)-hydroxylase.

In the reconstituted system of P450JM-E, the MALCO activity for 7\(\beta\)-OH-A\(^{\delta}\)-THC was about 6-fold higher than that for 7\(\alpha\)-OH-A\(^{\delta}\)-THC. In our previous investigation, the formation of 7-Oxo-A\(^{\delta}\)-THC from 7\(\beta\)-OH-A\(^{\delta}\)-THC by P450GPF-B, P450MDX-B (CYP3A11), and CYP3A4 identified as the major enzyme of 7-OH-A\(^{\delta}\)-THC MALCO in guinea pigs,\(^10\) mice\(^11\) and humans\(^13\) was 1.7-\(\times\), 1.9-\(\times\), and 3.4-\(\times\), respectively, higher than that from 7\(\alpha\)-OH-A\(^{\delta}\)-THC. The stereoselectivity was consistent with the results using hepatic microsomes as described above. It is suggested that stereoselectivity for the substrates characterize the major P450 enzymes responsible for the formation of 7-Oxo-A\(^{\delta}\)-THC from 7-OH-A\(^{\delta}\)-THC.

When the substrates were incubated in the reconstituted system of P450JM-E under \(^{18}\)O\(_2\), \(^{18}\)O was incorporated into about 40% of the corresponding ketone formed from 7\(\alpha\)-OH-A\(^{\delta}\)-THC. This result indicates that the gem-diol pathway is operative in the oxidation of 7\(\alpha\)-OH-A\(^{\delta}\)-THC, whereas the preference for \(^{18}\)O release from the metabolic intermediate was observed. In the formation of 7-Oxo-A\(^{\delta}\)-THC from 7\(\beta\)-OH-A\(^{\delta}\)-THC, however, the degree of incorporation of \(^{18}\)O from molecular oxygen is only about 15\%. 8-OH-A\(^{\delta}\)-THC, an isomer of 7-OH-A\(^{\delta}\)-THC, was also oxidized to 8-Oxo-A\(^{\delta}\)-THC by P450JM-E. Interestingly, the behavior in incorporation of molecular oxygen into 8-Oxo-A\(^{\delta}\)-THC was different from that into 7-Oxo-A\(^{\delta}\)-THC, that is, the incorporation of \(^{18}\)O after the metabolism of the \(\alpha\)-epimer (8\(\alpha\)-OH-A\(^{\delta}\)-THC) is negligible. These results suggest that both the \(\alpha\)- and \(\beta\)-epimers of 7-OH-A\(^{\delta}\)-THC and 8-OH-A\(^{\delta}\)-THC are converted to the ketone through a dehydration of an enzyme-bound gem-diol, although the stereoselectivity of dehydration differentiates between two epimers.
Acknowledgements We thank Perkin Elmer Japan (Nagoya) for determination of the NH$_2$-terminal sequence of P450IM-D and P450IM-E. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by the Special Research Fund of Hokuriku University.

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