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Metabolism of (+)- and (-)-Menthols by CYP2A6 in Human Liver Microsomes

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Abstract: The *in vitro* metabolism of (+)-(1*S*,3*S*,4*R*) and (-)-(1*R*,3*R*,4*S*)-menthol enantiomers was examined by incubation with human liver microsomes, and the oxidative metabolites thus formed were analyzed using gas chromatography-mass spectrometry (GC-MS). The (+)- and (-)-menthols were found to be oxidized to the respective (+)-(1*S*,3*S*,4*S*)- and (-)-(1*R*,3*R*,4*R*)-*trans*-*p*-menthane-3,8-diol derivatives by human liver microsomal P450 enzymes. Cytochrome P450 (CYP) 2A6 was determined to be the major enzyme involved in the hydroxylation of (+)- and (-)-menthols by human liver microsomes on the basis of the following lines of evidence. First, of 11 recombinant human P450 enzymes tested, CYP2A6 catalyzed the oxidation of (+)- and (-)-menthols. Second, oxidation of (+)- and (-)-menthols was inhibited by (+)-menthofuran and anti-CYP2A6 antibody. Finally, (+)- and (-)-menthol activities were found to correlate with contents of CYP2A6 in liver microsomes of 9 human samples.

Key words: menthol, P450, CYP2A6, microsomes, human

1 INTRODUCTION

Menthol is a major component of various mint oils; it has a pleasant odor and taste and is widely used to flavor foods and oral pharmaceutical preparations. The concentration of menthol in essential oils of peppermint, spearmint, and other species has been reported to be~50%[1-3]. (-)-Menthol isomers (natural type) are more abundantly present in these plants than the racemic mixture and (+)-isomers (unnatural type). (-)-Menthol has been used as medicine and flavoring; however, (+)-menthol has been reported to be toxic[4]. P450 enzymes have been shown to detoxify and/or toxify these compounds to more polar and occasionally more reactive metabolites[5-9].

We have recently reported that larvae of the common cutworm (*Spodoptera litura*) convert menthol to 7-hydroxymenthol and 6-hydroxymenthol and that the soil-borne plant pathogenic fungi (*Rhizoctonia solani*) converts menthol to 6,8-dihydroxymenthol and 1-hydroxymenthol[10-12]. Additional studies have indicated that *Aspergillus niger* and *Penicillium* sp. convert menthol to hydrocarbons and several alcohols[13]. Metabolism of menthol has been studied using animal models in rats[14-16] and humans[17,18] both *in vivo* and *in vitro* and in monkeys

in vitro[18]. Menthol is an inhibitor of P450 2A6-mediated 7-hydroxylation of coumarin and the human liver microsomal oxidation of nicotine[18]; however, there are no detailed reports concerning menthol. Therefore, we examined whether the (+)- and (-)-menthols were converted by human liver microsomes and 11 recombinant human P450 enzymes *in vitro*.

We have previously reported that several monoterpenoids, including 1,8-cineole, 1,4-cineole, (+)- and (-)-limonenes, (-)-verbenone, (-)-menthone, (+)- and (-)-fenchones, and (-)-camphor were metabolized by P450 enzymes to their respective oxidation products in rat and human liver microsomes[19-28].

In the current study, we examined the oxidations of (+)-(1*S*,3*S*,4*R*)- and (-)-(1*R*,3*R*,4*S*)-menthols by P450 enzymes in liver microsomes prepared from different human samples and the metabolites formed were analyzed by GC-MS. We examined the metabolism of (+)- and (-)-menthols by human liver microsomes and recombinant human P450 enzymes expressed in insect cells expressing both human P450 and NADPH-P450 reducatase.

2 MATERIALS AND METHODS

2.1 Chemicals

(+)-(1*S*,3*S*,4*R*)- and (-)-(1*R*,3*R*,4*S*)-Menthols, and *trans-p*-menthane-3,8-diol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). (+)-Menthofuran was purchased from Fluka (Tokyo, Japan). NADP$^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich (St. Louise, MO, U.S.A.). Other reagents and chemicals used in this study were obtained from sources described previously or were of the highest quality commercially available[29].

2.2 Enzymes and antibody

Human liver microsomal samples were obtained from Gentest Co., Inc. (Woburn, MA, U.S.A.). Control serum, and mouse anti-human CYP2A6-specific monoclonal antibody and recombinant CYP1A1, 1A2, 2A6, 1B1, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 expressed in *Trichoplusia ni* cells infected with a baculovirus containing cDNA encoding human P450 and NADPH-P450 reductase, were obtained from Gentest Co., Inc. The P450 content of these systems was used according to the manufacturer's instructions. All samples and enzymes were stored at −80℃ until use.

2.3 Oxidation of (+)- and (-)-menthol enantiomers by human liver microsomes and by human P450 enzymes

Oxidation of (+)- and (-)-menthols by liver microsomes was determined as follows. The standard reaction mixture contained liver microsomes (0.1 mg of proteins/mL) or recombinant P450 (100 pmol/mL), and 200 μM (+)- and (-)-menthols in a final volume of 0.50 mL in 100 mM potassium phosphate buffer (pH 7.4) containing an NADPH-generating system (0.5 mM NADP$^+$, 5 mM glucose 6-phosphate, and 0.5 units of glucose 6-phosphate dehydrogenase/mL). Incubations were carried out at 37℃ for 60 min and terminated by the addition of 1.5 mL of dichloromethane. The mixtures were mixed vigorously and the extracts (organic layer) collected by centrifugation at 3,000 rpm for 5 min. The organic phase was transferred to an insert for identification of the metabolites by analysis with GC and GC-MS. The metabolic products were quantified on the basis of the relative peak areas of GC.

Electron ionization mass spectral data analysis (70 eV) was performed on a Hewlett-Packard model 5972 mass spectrometer connected to a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Atlanta, GA), equipped with a split injector and a DB-5 silica capillary column (30 m length, 0.25 mm i. d.), using helium (at 0.5 mL/min) as the carrier gas. Analysis was performed as the follows: The column temperature was programmed from 60℃ to 240℃ at the rate of 4℃ /min and then held at 240℃. The mass spectrometer was used in total ion chromatogram (TIC)

mode and the *m/z* values were monitored as follows: at t_R = 15.5 min, MS (*m/z*, relative intensity): 138 (M$^+$-H$_2$O, 21), 123 (32), 95 (69), 82 (36), 81 (80), 71 (100), 55 (46), 41 (60) ((+)-menthol); t_R = 22.1 min, MS (*m/z*, relative intensity): 157 (M$^+$-CH$_3$, 3), 154 (M$^+$-H$_2$O, 1), 139 (8), 121 (4), 96 (47), 81 (98), 59 (100), 54 (27), 43 (48) ((+)-*trans-p*-menthane-3,8-diol). The retention time and mass spectral data of the enantiomer (-)-menthol and its metabolite ((-)-*trans-p*-menthane-3,8-diol) were identical to those of (+)-menthol and its metabolite, respectively.

2.4 Inhibition experiments

We evaluated the inhibitory effects of known P450 enzyme-selective inhibitors on the metabolism of (+)- and (-)-menthols by human liver microsomes and recombinant P450 enzymes in order to identify which P450 enzymes were involved in each metabolic pathway. The inhibitors used in the present study were 2.5-10 μM (+)-menthofuran, a selective CYP2A6 inhibitor[30, 31] and 1-5 μL anti-CYP2A6.

2.5 Correlation test and kinetic analysis

Correlation between contents of CYP2A6 and rates of (+)- and (-)-menthol metabolite formations were compared in liver microsomes from 9 human samples.

Kinetic parameters for (+)- and (-)-menthol metabolites formation by microsomes were estimated using a computer program designed for nonlinear regression analysis. Substrate concentrations used for the analysis of metabolism of (+)- and (-)-menthols were 50, 100, 200, 400, and 600 μM.

3 RESULTS

3.1 Identification of (+)- and (-)-menthol metabolites on incubation with human liver microsomes

(+)-(1*S*,3*S*,4*R*) and (-)-(1*R*,3*R*,4*S*)-Menthol enantiomers were incubated with human liver microsomes in the presence of an NADPH-generating system and the products formed were analyzed by GC and GC-MS (Fig. 1). The mass spectrum data of (+)- and (-)-menthol-derived metabolites are presented in Fig. 1A. The mass spectral data of metabolites from (+)-menthol are consist with the reference compound (*trans-p*- menthane-3,8-diol). The mass data of metabolites from (-)-menthol were identical to those obtained from (+)-menthol. The enantiomers of metabolites were identified by their retention time on the chiral column, CHROMPACK WCOT fused silica CP-Cyclodextrin-β-236-M-19 (50 m length, 0.25 mm i.d.). On the basis of these data, it was concluded that the (+)- and (-)-menthol enantiomers were oxidized to respective (+)-(1*S*,3*S*,4*S*)- and (-)-(1*R*,3*R*,4*R*)-*trans-p*-menthane-3,8-diols by human liver microsomes.
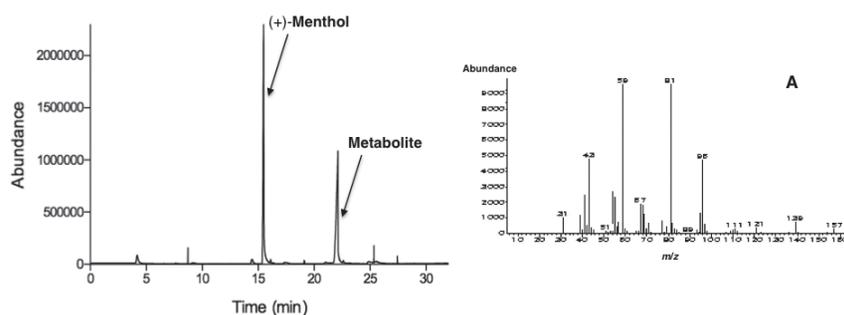
Fig. 1 GC-MS analysis of (+)-menthol and its metabolite.
Chromatographic peaks of the substrate (+)-menthol incubated with human liver microsomes in presence of an NADPH-generating system are shown. Panel A shows the full scan mass spectrum of (+) -*trans-p*-menthane-3,8-diol. GC-MS analysis of (-)-menthol and its metabolite reveals similar results.

Conversely, the reaction was not performed under conditions where (+)- and (-)-menthols were incubated without microsomes (data not shown).

3.2 Metabolism of (+)- and (-)-menthols by recombinant human P450 enzymes

We examined the metabolism of (+)- and (-)-menthols by recombinant systems containing 11 forms of human P450s and NADPH-P450 reductase expressed in *T. ni* cells. CYP2A6 catalyzed the metabolism of (+)- and (-)-menthols at significant levels whereas all other forms of P450 including CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were not active (Fig. 2).

3.3 Inhibition of (+)- and (-)-menthols oxidation by human liver microsomes using (+)-menthofuran and anti-CYP2A6

(+)-Menthofuran, a known inhibitor of CYP2A6-dependent catalytic activity, significantly inhibited metabolism of (+)- and (-)-menthols by human liver microsomes. Anti-CYP2A6 slightly inhibited formation of (+)- and (-)-menthols to (+)- and (-)-*trans-p*-menthane-3,8-diols, respectively (Fig. 3).

3.4 Correlation between contents of CYP2A6 and rates of (+)- and (-)-menthol oxidation by human liver microsomes

The above results indicate that CYP2A6 is an important enzyme in (+)- and (-)-menthol oxidation by human liver microsomes. Correlation between the contents of CYP2A6 and rates of formations of (+)- and (-)-*trans-p*-menthane-3,8-diols was examined in liver microsomes from 9 human samples. The mean level of CYP2A6 in the samples was estimated to be 12.5 ± 10.2 pmol/mg protein ($10.1\% \pm 6.7\%$ of total P450). We found a good correlations between CYP2A6 levels and formation of (+)- and (-)-*trans-p*-menthane-3,8-diols ($r = 0.91$ and 0.81, respectively) in these human samples (Fig. 4).
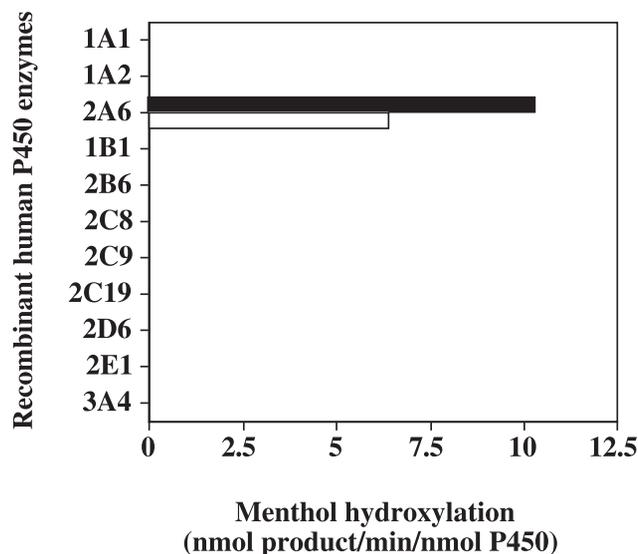

Fig. 2 Oxidation of (+)- and (-)-menthols to their respective 8-hydroxylated metabolites (■ and □) by recombinant human P450 enzymes expressed in *T. ni* cells. Substrate concentration was 200 µM.

3.5 Kinetic analysis

Kinetic analysis of (+)- and (-)-menthol oxidation activities were examined in human liver microsomes. The K_m and V_{max} values for the formation of (+)- and (-)-*trans-p*-menthane-3,8-diols by human liver microsomes were 42 µM and 0.12 nmol/min/nmol P450, and 45 µM and 0.08 nmol/min/nmol P450, respectively. Kinetic analysis was
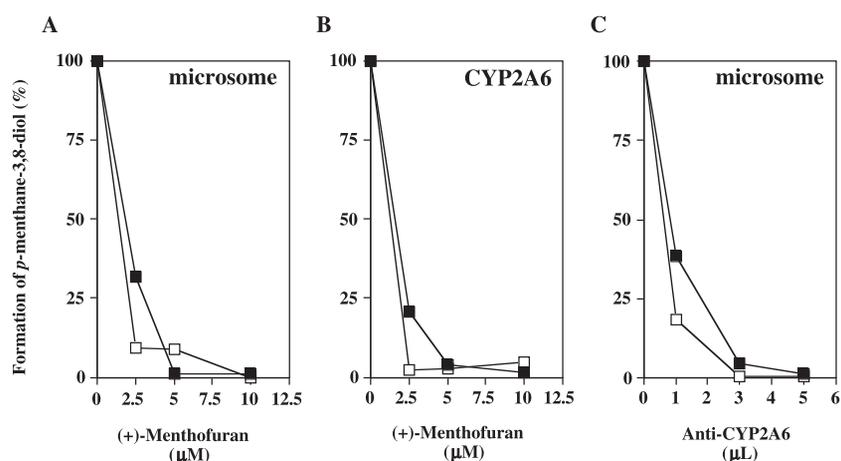
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Fig. 3 Effects of inhibitors (+)-menthofuran (A and B) and anti-CYP2A6 (C), on formation of (+)- and (-)-*trans-p*-menthane-3,8-diols (■ and □) by human liver microsomes and recombinant human CYP2A6. Substrate concentration was 200 μM.
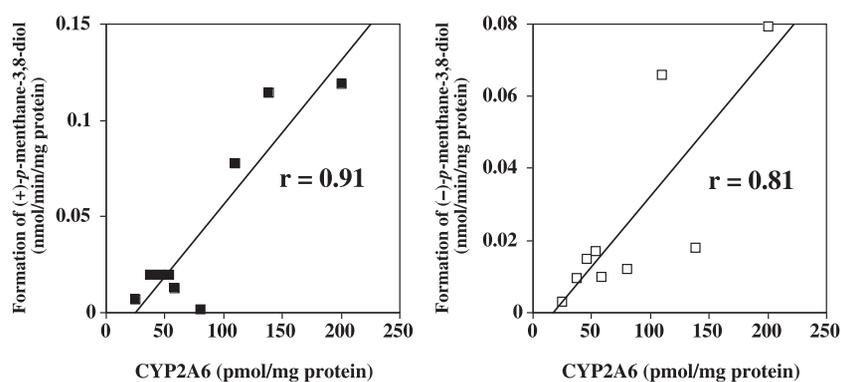

Fig. 4 Correlation between contents of CYP2A6 and catalytic activities of human liver microsomes in oxidation of (+)- and (-)-menthols (■ and □).

Table 1 Kinetic analysis of the oxidations of (+)- and (-)-menthols by human liver microsomes and recombinant human P450 enzymes expressed in *T. ni* cells.

| enzymes | oxidation of (+)- and (-)-menthols[a] | | | | | |
| | (+)-*p*-menthan-3,8-diol | | | (-)-*p*-menthan-3,8-diol | | |
	K_m (μM)	V_{max}	V_{max}/K_m	K_m (μM)	V_{max}	V_{max}/K_m
microsomes	42	0.12[b]	2.81[d]	45	0.08	1.77
CYP2A6	28	10.33[c]	369.8[e]	27	5.29	195.9

[a] Substrate concentrations used were 50, 100, 200, 400, and 600 μM.
[b] V_{max} expressed in nmol/min/ mg protein for human livermicrosomes.
[c] V_{max} expressed in nmol/min/ nmol P450 for recombinant CYP isozymes.
[d] Intrinsic clearance (V_{max}/K_m) expressed in nmol/min/mg protein for human liver microsomes.
[e] Intrinsic clearance (V_{max}/K_m) expressed in nmol/min/mg nmol P450 for recombinant CYP isozymes.

also performed for the oxidation of (+)- and (-)-menthols by recombinant CYP2A6. The K_m and V_{max} values for the formation of (+)- and (-)-*trans-p*-menthane-3,8-diols by CYP2A6 were 28 μM and 10.33 nmol/min/nmol P450, and 27 μM and 5.29 nmol/min/nmol P450, respectively (Table 1).

4 DISCUSSION

The metabolism of (+)-(1S,3S,4R) and (-)-(1R,3R,4S)-menthol enantiomers by human liver microsomal P450 enzymes was studied. The (+)- and (-)-menthols were converted to (+)-(1S,3S,4S)- and (-)-(1R,3R,4R)-*trans-p*-menthane-3,8-diols, respectively (**Fig. 5**). CYP2A6 was identified as the enzyme involved in formation of (+)- and (-)-*trans p*-menthane-3,8-diols by human liver microsomes based on the following evidence:Firstly, out of 11 recombinant human P450 enzymes tested, CYP2A6 catalyzed the oxidation of (+)- and (-)-menthols; secondly, oxidation of (+)- and (-)-menthols was inhibited by (+)-menthofuran and anti-CYP2A6 antibody; and finally, the activity of oxidation correlated with the content of CYP2A6 in liver microsomes of 9 human samples.

Kinetic analysis showed that the enzyme efficiencies (V_{max}/K_m ratio) for (+)- and (-)-*trans-p*-menthane-3,8-diols catalyzed by human liver microsomes were 2.81 and 1.77 ml/min/nmol protein, respectively. The (+)- and (-)-menthol 8-hydroxylations were catalyzed by CYP2A6, and the V_{max}/K_m values were 368.9 and 195.9 nmol/min/nmol P450, respectively. Both V_{max}/K_m values were 1.5- and 2.0-fold higher for the (+)-menthol 8-hydroxylaton by human liver microsomes and recombinant CYP2A6 than those for (-)-menthol. These results indicate that the substrate affinity of (+)-menthol is higher than that of (-)-menthol in human liver microsomes.

Various monoterpenoids, including menthol, have been reported to be biotransformed by several biocatalysts. We have previously shown that menthol is metabolized by *S. litura* to 7-hydroxymenthol and 6-hydroxymenthol, and by soil-borne plant pathogenic fungi (*R. solani*) to 6,8-dihydroxymenthol and 1-hydroxymenthol[10−12]. Additional studies have indicated that *A. niger* converts menthol to *cis-p*-menthane-7-ol, and limonene and thay *Penicillium* sp. converts menthol to *p*-cymene and γ-terpinene[13].

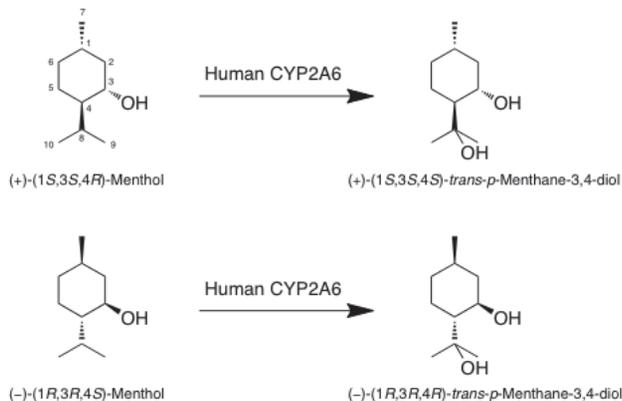Madhava *et al*. described the metabolism of menthol in rats[14]. Metabolism of (-)-menthol has been studied extensively in experimental animal models using rats. The investigations on intact rats show that menthol is rapidly glucuronidated and excreted in urine and faces. In studies using bile duct-cannulated rats it was shown that biliary excretion is rapid and extensive, and that menthol undergoes an intensive enterohepatic circulation. After cleavage of the glucuronide and reabsorption in the small intestine, it is further metabolized in the liver. It was proposed that the first step is hydroxylation at the C-8 position, followed by oxidation of the methyl group (C-7) to a carboxylic group, and then hydroxylation at the C-9 position. *p*-Menthane-3, 8-diol and 3,8-dihydroxy-*p*-menthane-7-carboxylic acid were identified as major metabolites (not quantitated) in the urine, and a fraction of these metabolites is excreted as glucuronides.

In this study, we found that CYP2A6 catalyzes the oxidation of (+)- and (-)-menthols at significant rates. This is in contrast with the results of studies in rats, which show menthol oxidation of C-8 position at high rates. Such species-related differences in the metabolism of xenobiotic chemicals by P450 enzymes are of interest to allow the extrapolation of biological and pharmacological data from experimental animals to humans.

In summary, the present results show that (+)-(1S,3S,4R) and (-)-(1R,3R,4S)-menthol enantiomers were oxidized to their respective (+)-(1S,3S,4S)- and (-)-(1R,3R,4R)-*trans-p*-menthane-3,8-diol derivatives in human liver microsomes. It is suggested that CYP2A6 is a principal enzyme in catalyzing (+)- and (-)-menthol 8-hydroxylations in human liver microsomes.

Fig.5 Metabolism of (+)- and (-)-menthols by human liver P450 enzymes.

References

1) Dimandja J. D.; Stanfill S. B.; Grainger J.; Patterson D. G. Jr. Application of comprehensive two-dimensional gas chromatography (GC × GC) to the qualitative analysis of essential oils. *J. High Resolut. Chrom.* **23**, 208-214 (2000).

2) Jirovetz, L.; Buchbauer, G.; Bail, S.; Denkova, Z.; Slavchev, A.; Stoyanova, A.; Schmidt, E.; Geissler, M. Antimicrobial activities of essential oils of mint and peppermint as well as some of their main compounds. *J. Essent. Oil Res.* **21**, 363-366 (2009).

3) Aziz, E. E.; Craker, L. E. Essential oil constituents of peppermint, pennyroyal, and apple mint grown in a desert agrosystem. *J. Herbs Spices & Medicinal Plants* **15**, 361-367 (2009).

4) Petrowitz, H. J. The effects of optical antipodes of menthol on termite species. *Naturwissenschaften* **67**, 43-44 (1980).

5) Guengerich F. P.; Shimada, T. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450

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enzymes. *Chem. Res. Toxicol.* **4**, 391-407（1991）.

6） Guengerich F. P. Human cytochrome P-450 enzymes. *Life Sci.* **50**, 1471-1478（1992）.

7） Gonzalez F. J. The molecular biology of cytochrome P450s. *Pharmacol. Rev.* **40**, 243-288（1989）.

8） Thomassen D.; Slattery J. T.; Nelson S. D. Contribution of menthofuran to the hepatotoxicity of pulegone: Assessment based on matched area under the curve and on matched time course. *J. Pharmacol. Exp. Ther.* **244**, 825-829（1988）.

9） Khojasteh-Bakht S. C.; Chen W.; Koenigs L. L.; Peter R. M.; Nelson S. D. Metabolism of（R）-（+）-pulegone and（R）-（+）-menthofuran by human liver cytochrome P-450s: Evidence for formation of a furan epoxide. *Drug Metab. Dispos.* **27**, 574-580（1999）.

10） Miyazawa M.; Kumagae S.; Kameoka H. Biotransformation of（+）- and（-）-menthol by the larvae of common cutworm（*Spodoptera litura*）. *J. Agric. Food Chem.* **47**, 3938-3940（1999）.

11） Miyazawa, M.; Kawazoe, H.; Hyakumachi, M. Biotransformation of *l*-menthol by soil-borne plant pathogenic fungi（*Rhizoctonia solani*）. *J. Chem. Technol. Biotechnol.* **77**, 21-24（2001）.

12） Miyazawa, M.; Kawazoe, H.; Hyakumachi, M. Biotransformation of *l*-menthol by twelve isolates of soil-borne plant pathogenic fungi（*Rhizoctonia solani*）and classification of fungi. *J. Chem. Technol. Biotechnol.* **78**, 620-625（2003）.

13） Esmaeili, A.; Sharafian, S.; Safaiyan, S.; Rezazadeh, S.; Rustaivan, A. Biotransformation of one monoterpene by sporulated surface cultures of *Aspergillus niger* and *Penicillium* sp. *Nat. Pro. Res.* **23**, 1058-1061（2009）.

14） Madyastha M. K.; Srivatsan V. Studies on the metabolism of *l*-menthol in rats. *Drug Metab. Dispos.* **16**, 765-772（1998）.

15） Austin, C. A.; Shephard, E. A.; Pike, S. F.; Rabin, B. R.; Phillips I. R. The effect of terpenoid compounds on cytochrome P-450 levels in rat liver. *Biochem. Pharmacol.* **37**, 2223-9（1988）.

16） Yamaguchi, T.; Caldwell, J.; Farmer P. B. Metabolic fate of [^{3}H]-*l*-menthol in the rat. *Drug Metab. Dispos.* **22**, 616-24（1994）.

17） Ayse, G.; Peyton, J. III.; Lisa, Y.; Neal, L. B. Disposition kinetics and effects of menthol. *Clin. Pharmacol. Ther.* **66**, 128-135（1999）.

18） James, M. M.; Keith, F.; Xiaodong, Z.; Scott, V. S.; John, R. C. Inhibition of human liver microsomal（S）-nicotine oxidation by（-）-menthol and analogues. *Chem. Res. Toxicol.* **16**, 988-993（2003）.

19） Miyazawa, M.; Shindo, M.; Shimada, T. Oxidation of 1, 8-cineole, the monoterpene cyclic ether originated from *Eucalyptus polybractea*, by cytochrome P450

3A enzymes in rat and human liver microsomes. *Drug Metab. Dispos.* **29**, 200-205（2001）.

20） Miyazawa, M.; Shindo, M.; Shimada, T. Roles of cytochrome P450 3A enzymes in the 2-hydroxylation of 1, 4-cineole, a monoterpene cyclic ether, by rat and human liver microsomes. *Xenobiotica* **31**, 713-723（2001）.

21） Miyazawa, M.; Shindo, M.; Shimada, T. Sex differences in the metabolism of（+）- and（-）-limonene enantiomers to carveol and perillyl alcohol derivatives by cytochrome P450 enzymes in rat liver microsomes. *Chem. Res. Toxicol.* **15**, 15-20（2002）.

22） Miyazawa, M.; Shindo, M.; Shimada, T. Metabolism of（+）- and（-）-limonenes to respective carveols and perillyl alcohols by CYP2C9 and CYP2C19 in human liver microsomes. *Drug Metab. Dispos.* **30**, 602-607（2002）.

23） Miyazawa, M.; Sugie, A.; Shindo, M. Biotransformation of（-）-verbenone by human liver microsomes. *Biosci. Biotechnol. Biochem.* **66**, 2458-2460（2002）.

24） Miyazawa, M.; Sugie, A.; Shimada, T. Roles of human CYP2A6 and 2B6 and rat CYP2C11 and 2B1 in the 10-hydroxylation of（-）-verbenone by liver microsomes. *Drug Metab. Dispos.* **31**, 1049-1053（2003）.

25） Miyazawa, M.; Nakanishi, K.; Biotransformation of（-）-menthone by human liver microsomes. *Biosci. Biotechnol. Biochem.* **70**, 1259-1261（2006）.

26） Miyazawa, M.; Gyoubu, K. Metabolism of（+）-fechone by CYP2A6 and CYP2B6 in human liver microsomes. *Biol. Pharml. Bull.* **29**, 2354-2358（2006）.

27） Miyazawa, M.; Gyoubu, K. Metabolism of（-）-fenchone by CYP2A6 and CYP2B6 in human liver microsomes. *Xenobiotica* **37**, 194-204（2007）.

28） Gyoubu, K.; Miyazawa, M. *In vitro* metabolism of（-）-campher using human liver microsomes. *Biol. Pharml. Bull.* **30**, 230-233（2007）.

29） Shimada, T.; Yamazaki, H.; Mimura, M.; Inui, Y.; Guengerich, F. P. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* **270**, 414-423（1994）.

30） Bourrie, M.; Meunier, V.; Berger, Y.; Fabre, G. Cytochrome P450 isoform inhibitors as a tool for the investigation of metabolic reactions catalyzed by human liver microsomes. *J. Pharmacol. Exp. Ther.* **277**, 321-332（1996）.

31） Khojasteh-Bakht, S. C.; Koenigs, L. L.; Peter, R. M.; Trager, W. F.; Nelson, S. D.（R）-（+）-menthofuran is a potent, mechanism-based inactivator of human liver cytochrome P450 2A6. *Drug Metab. Dispos.* **26**, 701-704（1998）.