

Regular paper

Metabolism of 1,8-cineole by human cytochrome P450 enzymes: Identification of a new hydroxylated metabolite

Mike Duisken^a, Frank Sandner^a, Brunhilde Blömeke^b, Juliane Hollender^{a,*}

^aInstitute of Hygiene and Environmental Medicine, RWTH Aachen, Pauwelsstraße 30, 52074 Aachen, Germany

^bDepartment of Ecology and Ecotoxicology, Sickingerstr. 96, Wissenschaftspark Petrisberg Geb. 24, Trier, Germany

Received 26 April 2004; received in revised form 21 December 2004; accepted 21 December 2004

Available online 17 January 2005

Abstract

Human metabolism of the monoterpene cyclic ether 1,8-cineole was investigated in vitro and in vivo. In vitro, the biotransformation of 1,8-cineole was investigated by human liver microsomes and by recombinant cytochrome P450 enzymes coexpressed with human CYP-reductase in *Escherichia coli* cells. Besides the already described metabolite 2 α -hydroxy-1,8-cineole we found another metabolite produced at high rates. The structure was identified by a comparison of its mass spectrum and retention time with the reference compounds as 3 α -hydroxy-1,8-cineole. There was a clear correlation between the concentration of the metabolites, incubation time and enzyme content, respectively. CYP3A4/5 antibody significantly inhibited the 2 α - and 3 α -hydroxylation catalyzed by pooled human liver microsomes. Further kinetic analysis revealed that the Michaelis–Menten K_m and V_{max} for oxidation of 1,8-cineole in position three were 19 μ M and 64.5 nmol/min/nmol P450 for cytochrome P450 3A4, and 141 μ M and 10.9 nmol/min/nmol P450 for cytochrome P450 3A5, respectively. To our knowledge, this is the first time that 3 α -hydroxy-1,8-cineole is described as a human metabolite of 1,8-cineole. We confirmed these in vitro results by the investigation of human urine after the oral administration of cold medication containing 1,8-cineole. In human urine we found by GC-MS analysis the described metabolites, 2 α -hydroxy-1,8-cineole and 3 α -hydroxy-1,8-cineole.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Metabolism; 1,8-Cineole; Cytochrome P450; CYP3A; 2 α -Hydroxy-1,8-cineole; 3 α -Hydroxy-1,8-cineole

1. Introduction

Among numerous groups of naturally occurring compounds examined so far, monoterpenes are known as fragrances and flavouring agents. 1,8-Cineole, a monoterpene cyclic ether which is named eucalyptol, is widely distributed in plants and found in essential oils like *Eucalyptus polybractea*. It is extensively used for external application in pharmaceutical preparations, e.g., in nasal spray or as disinfectant [1]. Furthermore, it is used in cosmetics, for cough treatment, muscular pain, neurosis, rheumatism, asthma, and urinary stone [2]. Southwell et al. investigated the metabolism of 1,8-cineole in different insect species. Different metabolites such as 2 α - and

2 β -hydroxy-1,8-cineole, 3 α -hydroxy-1,8-cineole and 9-hydroxy-1,8-cineole as well as the diol 2 α ,9-dihydroxy-1,8-cineole were found [3]. After the administration of 1,8-cineole to rabbits, there were several alcohols and ketones identified in the urine such as 2 α / β -hydroxy-1,8-cineole, 3 α / β -hydroxy-1,8-cineole, and 3-oxo-1,8-cineole [4]. In addition, it was found that 1,8-cineole is metabolized to several cineolic acids, dihydroxycineoles and hydroxycineolic acids after feeding brushtail possums and male koalas [4–8]. The metabolism of 1,8-cineole was also investigated by liver microsomes of rat and humans as well as by recombinant cytochrome P450 enzymes in insect cells coexpressing human NADPH-P450 reductase. The results suggested that 1,8-cineole, which is oxidized in high rates to 2 α -hydroxy-1,8-cineole, is a substrate for CYP3A enzymes in rat and human liver microsomes [2]. So far, 2 α -hydroxy-1,8-cineole is the only known metabolite formed by human liver microsomes [9].

* Corresponding author. Tel.: +49 241 8088282; fax: +49 241 803388282.

E-mail address: juliane.hollender@post.rwth-aachen.de (J. Hollender).

CYP3A4 is the most abundant P450 enzyme in human liver, but is also expressed in extrahepatic tissues such as intestinal mucosa. CYP3A5 is also expressed in nonhepatic tissues and is the dominant CYP3A isoform in the kidney [10]. These two CYP3A enzymes metabolize similar compounds and exhibit high amino acid sequence identity (84%) [11]. Recently, CYP3A4 and CYP3A5 were both detected in human skin with CYP3A4 being more abundant than CYP3A5 [12].

In this study, we investigated the human metabolism of 1,8-cineole *in vitro* by recombinant cytochrome P450 enzymes and human liver microsomes. Furthermore, the human urine of volunteers ($n=3$) was examined after an oral administration of cold medication containing 1,8-cineole.

2. Materials and methods

2.1. Chemicals

1,8-Cineole was purchased from Fluka (Buchs, CH) and was used without further purification. The purity of this compound was >99% based on analysis with GC-MS. NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Roche (Basel, CH). Other reagents and chemicals were of the highest qualities commercially available. Authentic components, namely the hydroxy-1,8-cineole metabolites, were donated by Dr. Ray Carman, Department of Chemistry, University of Queensland, and by Dr. Ian Southwell, Wollongbar Agricultural Institute, Wollongbar.

2.2. Enzymes and antibody

Human liver microsomes were purchased from Xenotech (Kansas, USA). Recombinant human CYP3A4 and CYP3A5 and human CYP-reductase coexpressed in *Escherichia coli* were obtained from Cypex (Dundee, UK); the P450 amounts in the assays were chosen as recommended by the manufacturer. Purified inhibitory monoclonal antibody against human CYP3A4/5 with a protein concentration of 10 mg/ml was purchased from Xenotech (Kansas, USA).

2.3. GC/MS analysis

A Hewlett-Packard model 5860 Series II (Waldbronn, Germany) equipped with a Gerstel programmable temperature vaporizer (PTV)/Gerstel MPS large volume sampler (CIS 3, Gerstel, Mühlheim a. d. Ruhr, Germany) was combined with direct coupling to a Hewlett-Packard 5972 mass spectrometer. The metabolites were separated by a RTX-5SIL MS (Restek, Bad Homburg, Germany) silica capillary column (0.28 mm×30 m, 0.25 µm film thickness) using helium (at 0.7 ml/min) as carrier gas. The column temperature was increased from 37 to 200 °C at the rate of

6 °C/min and from 200 to 330 °C at a rate of 15 °C/min. 40 µl of the sample extracts was injected with an injection speed of 29 µl/min into the liner of the PTV containing silanized glass wool (93 mm×1 mm I.D., Gerstel) in the solvent vent mode with stop flow (vent flow, 200 ml/min helium). After purging of the organic solvent over 0.5 min at 20 °C and further 0.5 min isotherm, the injector was heated at 600 °C/min to 300 °C and was kept 2 min isotherm in the splitless mode. The GC-MS transfer line was held at 300 °C resulting in an ion source temperature of 180 °C, a quadrupole temperature of 180 °C and an ionization voltage of 70 eV. For detection, the scan mode (30–300 amu) was used.

2.4. Oxidation of 1,8-cineole by human liver microsomes, and human CYP3A4 and CYP3A5 enzymes

The oxidation of 1,8-cineole by human liver microsomes was determined as follows. 10 µl 1,8-cineole was dissolved in 10 ml of 100 mM potassium phosphate buffer (pH 7.4) and stirred vigorously before usage. Standard reaction mixtures containing human liver microsomes (0.4 mg of protein/ml) and 120 µM 1,8-cineole in a final volume of 500 µl 100 mM potassium phosphate buffer (pH 7.4) with a NADPH-generating system (0.1 mM NADP⁺, 0.8 mM glucose-6-phosphate and 1 unit of glucose-6-phosphate dehydrogenase/ml) were used. Incubations were carried out at 37 °C for various time points in an Eppendorf incubator (Hamburg, Germany), and the reaction was terminated by the addition of 1.0 ml ethylacetate. After additional 30 min extraction time and centrifugation for 10 min at 14,000 rpm (Eppendorf Centrifuge, Hamburg, Germany) the recovered organic phase was used for GC-MS analysis. The oxidation of 1,8-cineole with CYP3A4 (134 pmol/ml) and CYP3A5 (28 pmol/ml) was performed exactly as described above.

2.5. Urine collection from volunteers

Urine was collected from two males and one female after an oral intake of cold medication for several days. All volunteers gave informed consent to participate in the study. The urine was hydrolyzed enzymatically and the monoterpene metabolites were extracted by solid phase extraction (SPE) as described previously [13]. Samples were analyzed by GC-MS.

3. Results

3.1. Identification of metabolites from 1,8-cineole formed by human liver microsomes and recombinant human CYP3A4/3A5

Initially, the metabolism of 1,8-cineole was examined by human liver microsomes in the presence of a NADPH-generating system. The metabolites formed were analyzed

by GC-MS equipped with EI-MS. We found two main metabolites which were identified by their MS-spectra as 2 α -hydroxy-1,8-cineole and 3 α -hydroxy-1,8-cineole (Fig. 1). Component identification was made on the basis of mass spectral fragmentation, retention time comparison with authentic constituents, spiking with authentic constituents and mass spectral matching with commercial libraries (NIST, Wiley). Besides the chromatographic behaviour, in panel C (Fig. 1) the spectral data indicates the formation of a 3 α -hydroxylated metabolite. The molecular mass of the metabolite was increased by the introduction of an oxygen atom from 154 to 170 and the fragments 155 ($M^+ - CH_3$), 137 ($M^+ -$

$CH_5O = H_2O + CH_3$) and 127 ($M^+ - C_3H_7$) are described in the literature [3]. The chirality of the metabolites was not determined because quantities were too small for isolation and direct measurement and chiral GC have only been partially successful [3,14].

3.2. Influence of incubation time, P450 enzyme concentration and 1,8-cineole concentration on 3 α -hydroxylation of 1,8-cineole by human CYP3A4/CYP3A5

The formation of 3 α -hydroxy-1,8-cineole in the presence of human CYP3A4 and CYP3A5 with an NADPH-

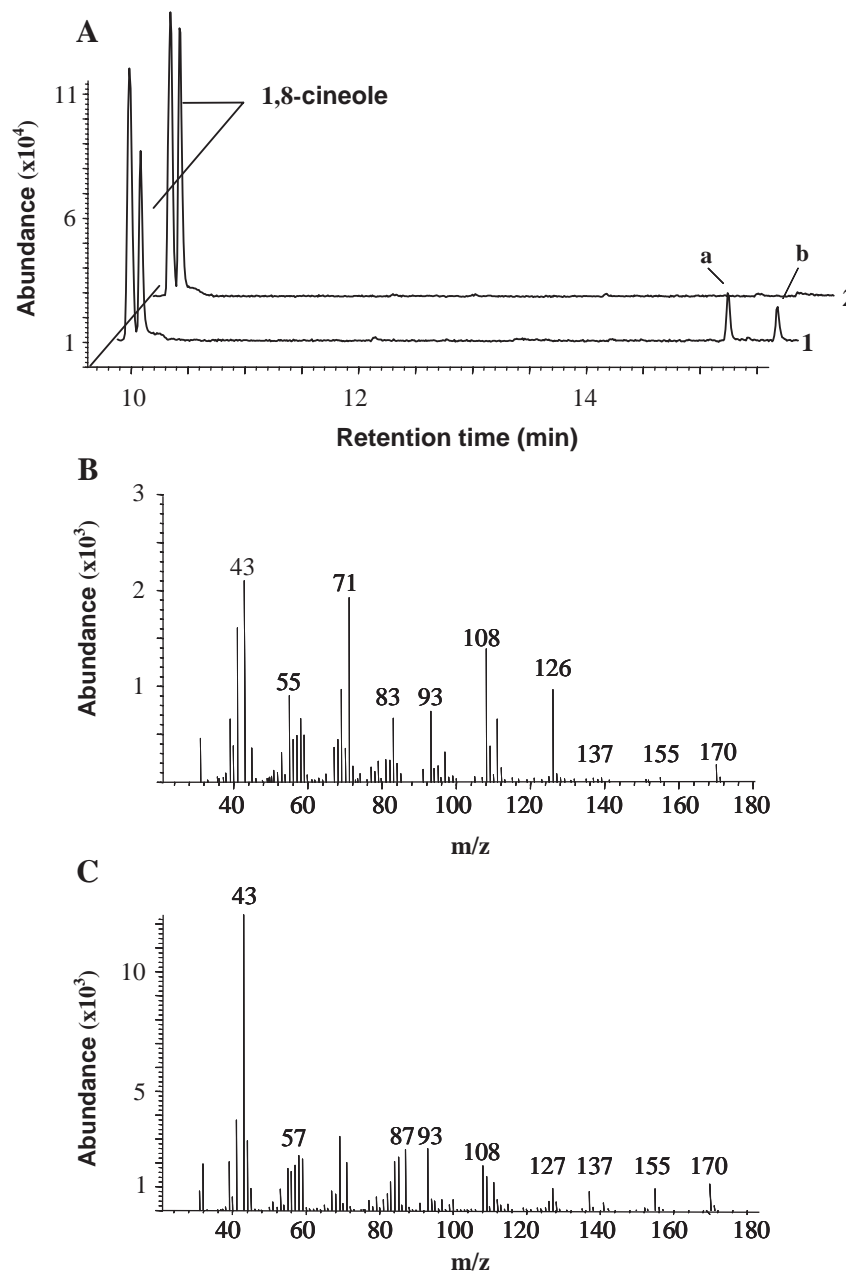


Fig. 1. GC-MS analysis of 1,8-cineole and its transformation products, 2 α -hydroxy-1,8-cineole (a) and 3 α -hydroxy-1,8-cineole (b). Panel A shows the GC-MS chromatogram of the compound 1,8-cineole with human liver microsomes in the presence of a NADPH-generating system (1) and without human liver microsomes (2). Panel B shows the full scan mass spectrum of 2 α -hydroxy-1,8-cineole. Panel C shows the full scan mass spectrum of 3 α -hydroxy-1,8-cineole.

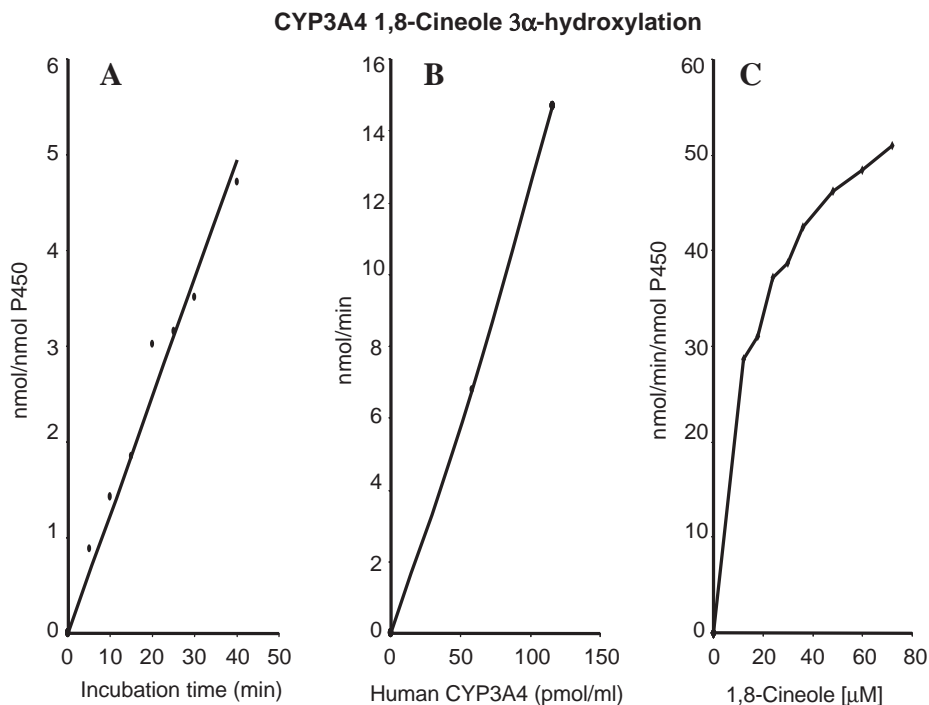


Fig. 2. Dependence on incubation time (A), enzyme contents (B) and 1,8-cineole concentrations (C) of 1,8-cineole 3 α -hydroxylation activities catalyzed by human CYP3A4. (A) Concentrations of 1,8-cineole and CYP3A4 were 120 μ M and 134 pmol/ml, respectively. (B) The incubation time and concentration of 1,8-cineole were 30 min and 120 μ M, respectively. (C) The incubation time and CYP3A4 concentration were 30 min and 134 pmol/ml, respectively.

generating system varied depending on incubation time, P450 concentration and substrate concentration (Figs. 2 and 3, respectively).

Using different incubation times, we found a linearity of the 1,8-cineole 3 α -hydroxylation activities catalyzed by human CYP3A4 and 3A5 up to 30 min. Increasing of the

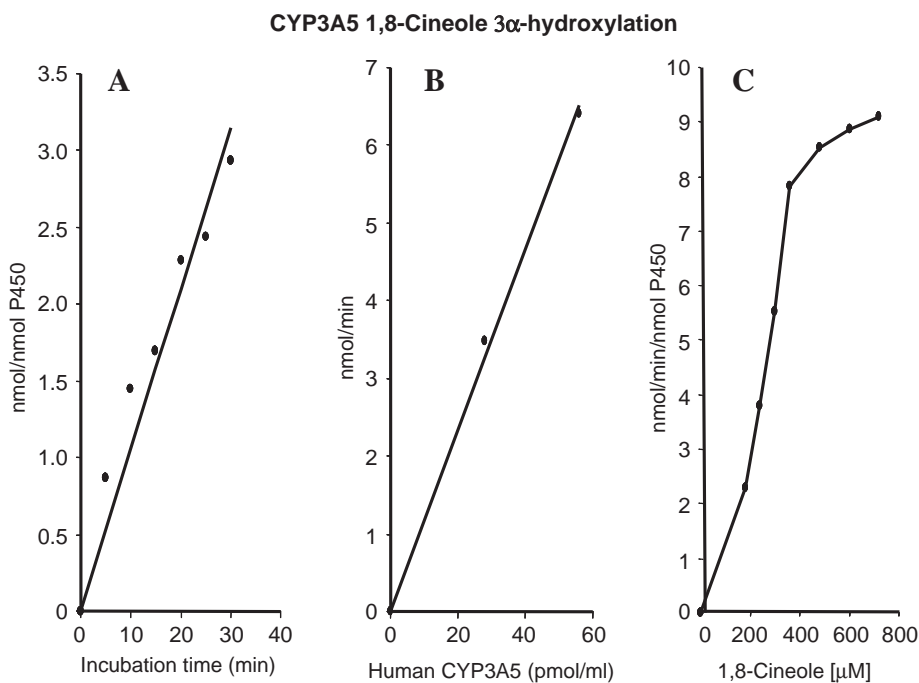


Fig. 3. Dependence on incubation time (A), enzyme contents (B) and 1,8-cineole concentrations (C) of 1,8-cineole 3 α -hydroxylation activities catalyzed by human CYP3A5. (A) The concentrations of 1,8-cineole and CYP3A5 were 1.2 mM and 28 pmol/ml, respectively. (B) The incubation time and concentration of 1,8-cineole were 30 min and 1.2 mM, respectively. (C) The incubation time and CYP3A5 concentration were 30 min and 28 pmol/ml, respectively.

Table 1

Kinetic analysis of the 2 α -hydroxylation and 3 α -hydroxylation of 1,8-cineole by human CYP3A4 and CYP3A5

Recombinant P450	K_m (μM)		V_{max} (nmol/min/nmol P450 $^{-1}$)		V_{max}/K_m (nM $^{-1}$ min $^{-1}$)	
	2 α -OH	3 α -OH	2 α -OH	3 α -OH	2 α -OH	3 α -OH
3A4	12	19	58.1	64.5	4.8	3.4
3A5	599	141	14.9	10.9	0.025	0.08

P450 enzyme concentration or the 1,8-cineole concentration lead to increasing 3 α -hydroxy-1,8-cineole formation. In the absence of human CYP3A4 and CYP3A5 we detected no 1,8-cineole hydroxylation at all.

3.3. Kinetic analysis of 1,8-cineole 3 α -hydroxylation by human CYP3A4/3A5

Kinetic analysis of the 1,8-cineole 3 α -hydroxylation catalyzed by human CYP3A4 and CYP3A5 were performed. The Michaelis–Menten K_m values for this reaction were 19 μM for human CYP3A4 and 141 μM for human CYP3A5, the V_{max} values were determined to be 64.5 nmol/min/nmol P450 for human CYP3A4 and 10.9 nmol/min/nmol P450 for human CYP3A5. Finally, the efficiencies of catalysis (V_{max}/K_m value) were 3.4 nM $^{-1}$ min $^{-1}$ for CYP3A4 and 0.08 nM $^{-1}$ min $^{-1}$ P450 for CYP3A5. For comparison the results for 1,8-cineole 2 α - and 3 α -hydroxylation are shown in Table 1.

3.4. Inhibition of 1,8-cineole 2 α - and 3 α -hydroxylation by human liver microsomes

Subsequently, we studied the 2 α - and 3 α -hydroxylation rates by human liver microsomes in the presence of a CYP3A inhibitor. CYP3A4/5 antibody inhibited the 2 α - and 3 α -hydroxylation (Fig. 4). The 3 α -hydroxylation was almost completely inhibited in the presence of 0.25 mg/ml CYP3A antiserum.

3.5. 1,8-Cineole 2 α - and 3 α -hydroxylation in vivo

To validate the in vitro findings we analyzed the urine of three volunteers (two males, one female) who had taken a cold medication containing 1,8-cineole for 5 days. Prior to the analysis by GC-MS the urine was prepared by solid phase extraction. Comparison of the resulting mass spectra from the peaks with the MS spectra library confirmed clearly the formation of the 2 α - and 3 α -hydroxylated 1,8-cineole metabolites (Fig. 5). No product formation was found in controls and before the intake of the medication (not shown). This was in concordance with our results using pooled human liver and CYP3A4 and CYP3A5, but in all urine samples the 2 α -hydroxy-1,8-cineol content was significantly higher than the 3 α -hydroxy-1,8-cineole content.

4. Discussion

Various monoterpenoids are metabolized by liver microsomes of different mammalian species. Limonene is attacked at the methyl group in the 7-position to form perillyl alcohol or at the 6-position on the ring system to form carveol [15,16]. The first human metabolite described for limonene was uroterpenol [17,18] which was formed also by the larvae of the common cutworm [19]. Furthermore, it has been shown that verbenone was catalyzed by human liver microsomes to 10-hydroxyverbenone [20].

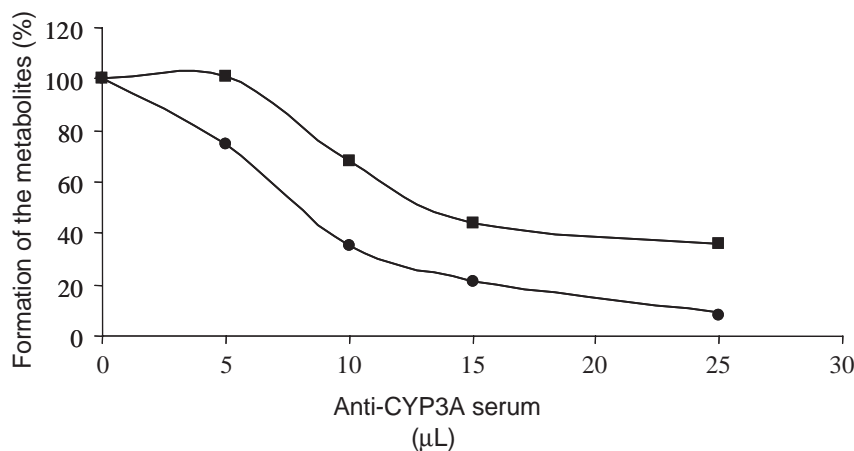


Fig. 4. Influence of Anti-CYP3A antibody on the 2 α -hydroxylation (■) and 3 α -hydroxylation (●) activities of 1,8-cineole in the presence of human liver microsomes.

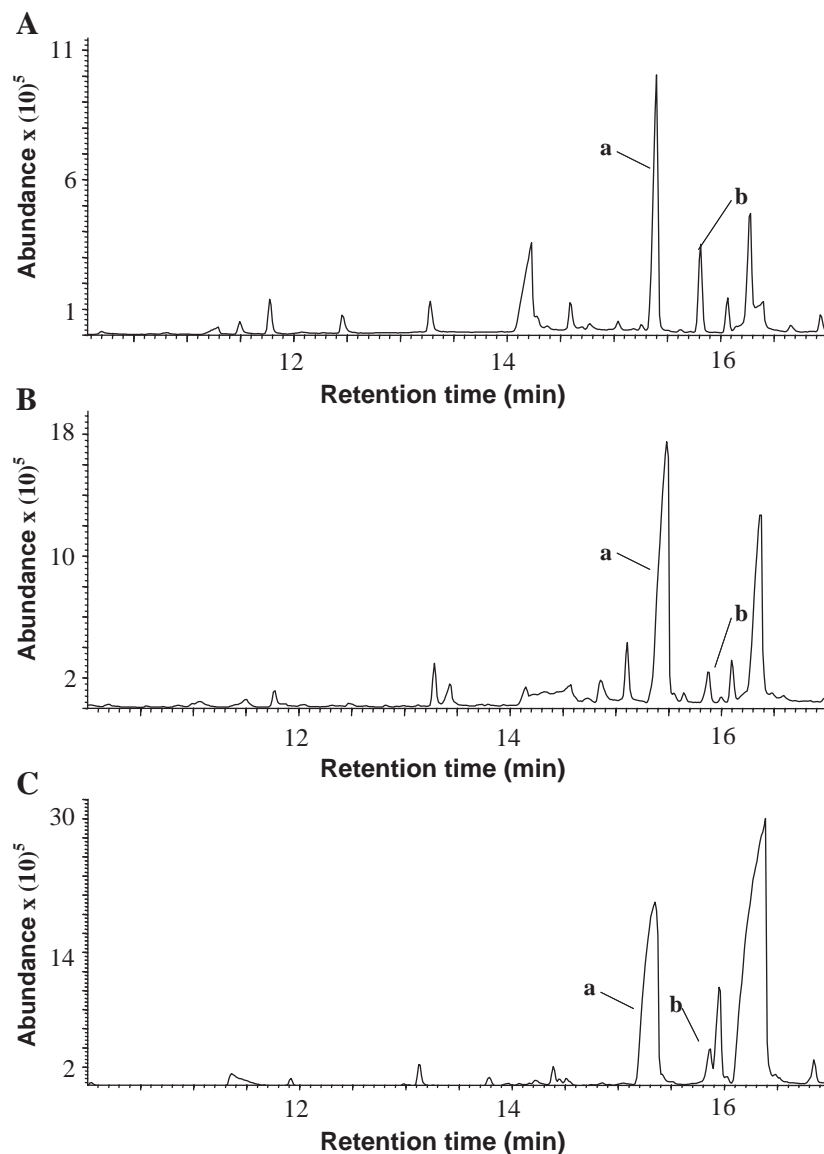
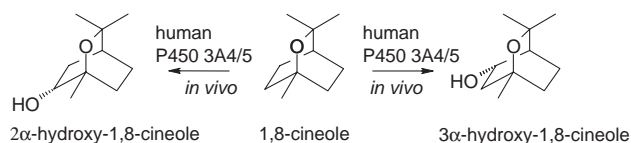


Fig. 5. Full scan GC-MS chromatograms of urine extracts from one female (C) and two males (A, B) after the oral administration of 1,8-cineole. The chromatographic peaks of 2 α -hydroxy-1,8-cineole (a) and 3 α -hydroxy-1,8-cineole (b) formed via the *in vivo* metabolism of 1,8-cineole are shown.

These results indicate that natural environmental compounds are differentially oxidized in several species. The structures of these chemicals determine which molecules are attacked at a definite position by P450 enzymes [21]. The metabolism of 1,8-cineole was studied previously in rats and rabbits. Two alcohols, namely 2-hydroxy-1,8-cineole and 3-hydroxy-1,8-cineole, were excreted in the urine [1,4]. More recently, Miyazawa et al. [2,9] reported that 1,8-cineole is metabolized by rat and human liver microsomes as well as by several recombinant cytochrome oxidases to only 2-hydroxy-1,8-cineole. In this study we demonstrated *in vitro* and *in vivo* that 1,8-cineole is converted to the expected 2 α -hydroxy-1,8-cineole but also to 3 α -hydroxy-1,8-cineole.

The following lines of evidence suggest that 1,8-cineole is also oxidized at the 3-position forming 3 α -hydroxy-1,8-cineole (Scheme 1). First, the incubation of 1,8-cineole with

human liver microsomes, human CYP3A4 and CYP3A5 revealed two metabolites which were identified by GC-MS as 2 α -hydroxy-1,8-cineole and 3 α -hydroxy-1,8-cineole. Second, anti-CYP3A4/5 antibody significantly inhibited the 1,8-cineole 3 α -hydroxylation and 2 α -hydroxylation activities catalyzed by human liver microsomes. Finally, both hydroxylated metabolites were detected in the urine of three persons after oral intake of medications containing



Scheme 1. Proposed metabolism of 1,8-cineole by human P450, human CYP3A4/CYP3A5 and *in vivo*.

1,8-cineole. To our knowledge, this is the first time that these hydroxylated 1,8-cineole metabolites were detected in human urine.

Miyazawa et al. [2] used ten different P450 enzymes expressed in insect cells to determine which P450 enzymes are the major catalysts for the 1,8-cineole hydroxylation. CYP3A4 had the highest activity for the 1,8-cineole 2-hydroxylation followed by CYP3A5 while the other enzymes exhibited only very low activities. Therefore, we investigated the 2- and 3-hydroxylation of 1,8-cineole by human CYP3A4 and CYP3A5. Interestingly, we found for CYP3A4 a higher 3 α -hydroxylation activity than 2 α -hydroxylation activity although Miyazawa et al. did not find hydroxylation in 3-position. However, we detected higher 2 α -hydroxylation activities with human liver microsomes and in vivo we found higher 2 α -hydroxy-1,8-cineole concentrations than 3 α -hydroxy-1,8-cineole concentrations. This suggests that other enzymes in the human body may be also catalysts of the 2-hydroxylation. The potent inhibition of the 2 α - and 3 α -hydroxylation of human microsomes by CYP3A antibody shows that both enzymes are major 1,8-cineole oxidizing enzymes in the human liver.

The V_{\max} value determined by Miyazawa et al. for 1,8-cineole 2-hydroxylation by recombinant human CYP3A4 was 48 nmol/min/nmol P450 [2]. This value is similar to the V_{\max} (58.1 nmol/min/nmol P450) determined in this study for CYP3A4 1,8-cineole 2 α -hydroxylation. For CYP3A5 1,8-cineole 2 α -hydroxylation we found a four-fold lower V_{\max} value (14.9 nmol/min/nmol P450) than for CYP3A4. Kinetic analysis of the 1,8-cineole 3 α -hydroxylation activities showed different K_m and V_{\max} values for human CYP3A4 and CYP3A5 presented in Table 1. It is likely that 1,8-cineole is more specific for CYP3A4 activity. The different K_m values for CYP3A5 catalyzed 2- and 3-hydroxylation are significant different. This is surprising because it is caused only by another position of the hydroxyl group at the ring system. The V_{\max}/K_m determined for CYP3A4 was 3.4 nM⁻¹ min⁻¹ (CYP3A5: 0.08 nM⁻¹ min⁻¹). In comparison to the V_{\max} values of other monoterpenoids metabolized by P450 enzymes the values described here for CYP3A4 are high. For example for the metabolism of limonene to carveol or perillyl alcohol the V_{\max} values were between 0.16 and 1.1 nmol/min/nmol P450 for recombinant human CYP3A4 [11,12].

In conclusion, the present study shows that 1,8-cineole is catalyzed by human CYP3A4 and CYP3A5 enzymes to 2 α -hydroxy-1,8-cineole and 3 α -hydroxy-1,8-cineole. Our findings show that in humans the ring carbon oxidation by cytochrome P450 enzymes is favored. The in vivo relevance is supported by the detection of both metabolites in the urine samples after an oral intake of 1,8-cineole. The metabolites can be used as urinary marker for intake of 1,8-cineole in the same way as verbenol for α -pinene exposure [13,22–24].

Acknowledgment

This work was supported by a grant from the Deutsche Forschungsgemeinschaft. We thank N. Fricke and M. Möller for technical assistance as well as W. Dott for helpful discussion. The authors are indebted to Dr. Ray Carman and Dr. Ian Southwell for providing the hydroxycineole samples and unpublished data.

References

- [1] K.M. Madyastha, A. Chadha, Metabolism of 1,8-cineole in rat: its effects on liver and lung microsomal cytochrome P-450 systems, *Bull. Environ. Contam. Toxicol.* 37 (1986) 759–766.
- [2] M. Miyazawa, M. Shindo, T. Shimada, 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 (2001) 200–205.
- [3] I.A. Southwell, M.F. Russell, C.D.A. Maddox, G.S. Wheeler, Differential metabolism of 1,8-cineole in insects, *J. Chem. Ecol.* 29 (2003) 83–94.
- [4] M. Miyazawa, H. Kameoka, K. Morinaga, K. Negoro, N. Mura, Hydroxycineole: four new metabolites of 1,8-cineole in rabbits, *J. Agric. Food Chem.* 37 (1989) 222–226.
- [5] R. Boyle, S. McLean, N.W. Davies, Biotransformation of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*), *Xenobiotica* 30 (2000) 915–932.
- [6] R. Boyle, S. McLean, W. Foley, N.W. Davies, E.J. Peacock, B. Moore, Metabolites of dietary 1,8-cineole in the male koala (*Phascolarctos cinereus*), *Comp. Biochem. Physiol., C Toxicol. Pharmacol.* 129 (2001) 385–395.
- [7] G.J. Pass, S. McLean, I. Stupans, N. Davies, Microsomal metabolism of the terpene 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*), koala (*Phascolarctos cinereus*), rat and human, *Xenobiotica* 31 (2001) 205–221.
- [8] R.M. Carman, A.C. Rayner, 2 α ,4-Dihydroxy-1,8-cineole. A new possum urinary metabolite, *Aust. J. Chem.* 47 (1994) 2087–2097.
- [9] M. Miyazawa, M. Shindo, Biotransformation of 1,8-cineole by human liver microsomes, *Nat. Prod. Lett.* 15 (2001) 49–53.
- [10] E.G. Schuetz, J.D. Schuetz, W.M. Grogan, A. Naray-Fejes-Toth, G. Fejes-Toth, J. Raucy, P. Guzelian, K. Gionela, C.O. Watlington, Expression of cytochrome P450 3A in amphibian, rat, and human kidney, *Arch. Biochem. Biophys.* 294 (1992) 206–214.
- [11] K.K. Khan, Y.Q. He, M.A. Correia, J.R. Halpert, Differential oxidation of mifepristone by cytochromes P450 3A4 and 3A5: selective inactivation of P450 3A4, *Drug Metab. Dispos.* 30 (2002) 985–990.
- [12] L.G. Yengi, Q. Xiang, J. Pan, J. Scatina, J. Kao, S.E. Ball, R. Fruncillo, G. Ferron, C. Roland Wolf, Quantitation of cytochrome P450 mRNA levels in human skin, *Anal. Biochem.* 316 (2003) 103–110.
- [13] F. Sandner, J. Fornara, W. Dott, J. Hollender, Sensitive biomonitoring of monoterpene exposure by gas chromatographic-mass spectrometric measurement of hydroxy terpenes in urine, *J. Chromatogr., B, Anal. Technol. Biomed. Life Sci.* 780 (2002) 225–230.
- [14] M.T. Fletcher, L.M. Lowe, W. Kitching, W.A. König, Chemistry of Leichhardt's grasshopper, *Petasida ephippigera*, and its host plants, *Pityrodia jamesii*, *P. ternifolia*, and *P. pungens*, *J. Chem. Ecol.* 26 (2000) 2275–2290.
- [15] M. Miyazawa, M. Shindo, T. Shimada, Metabolism of (+)- and (-)-limonenes to respective carveols and perillyl alcohols by CYP2C9 and CYP2C19 in human liver microsomes, *Drug Metab. Dispos.* 30 (2002) 602–607.
- [16] M. Miyazawa, M. Shindo, T. Shimada, 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 (2002) 15–20.
- [17] F.M. Dean, A.W. Price, A.P. Wade, G.S. Wilkinson, Uroterpenol beta-D-glucuronide, *J. Chem. Soc. [Perkin 1]* 19 (1967) 1893–1896.
- [18] A.P. Wade, G.S. Wilkinson, F.M. Dean, A.W. Price, Isolation, characterization and structure of uroterpenol, a monoterpene from human urine, *Biochem. J.* 101 (1966) 727–734.
- [19] M. Miyazawa, T. Wada, H. Kameoka, Biotransformation of (+)- and (–)-limonene by the larvae of common cutworm (*Spodoptera litura*), *J. Agric. Food Chem.* 46 (1998) 300–303.
- [20] M. Miyazawa, A. Sugie, M. Shindo, Biotransformation of (–)-verbenone by human liver microsomes, *Biosci. Biotechnol. Biochem.* 66 (2002) 2458–2460.
- [21] M. Miyazawa, A. Sugie, T. Shimada, Roles of human CYP2A6 and 2B6 and rat CYP2C11 and 2B1 in the 10-hydroxylation of (–)-verbenone by liver microsomes, *Drug Metab. Dispos.* 31 (2003) 1049–1053.
- [22] K. Eriksson, J.O. Levin, Identification of *cis*- and *trans*-verbenol in human urine after occupational exposure to terpenes, *Int. Arch. Occup. Environ. Health* 62 (1990) 379–383.
- [23] K. Eriksson, J.O. Levin, Gas chromatographic-mass spectrometric identification of metabolites from alpha-pinene in human urine after occupational exposure to sawing fumes, *J. Chromatogr., B, Biomed. Appl.* 677 (1996) 85–98.
- [24] K.A. Eriksson, J.O. Levin, T. Sandstrom, K. Lindstrom-Espeling, G. Linden, N.L. Stjernberg, Terpene exposure and respiratory effects among workers in Swedish joinery shops, *Scand. J. Work, Environ. Health* 23 (1997) 114–120.