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Biotransformation of deramciclane in primary hepatocytes of rat, mouse, rabbit, dog and human

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Abbreviations:

deramciclane: (1R,2S,4R)-(-)-2-phenyl-2-(2'-dimethylamino-ethoxy)-1,7,7-trimethyl-bicyclo[2.2.1]heptane

N-desmethyl-deramciclane (M4): (1R,2S,4R)-(-)-2-phenyl-2-(2'-methylamino-ethoxy)-1,7,7-trimethyl-bicyclo[2.2.1]heptane

9-hydroxy-deramciclane (M5): (1R,2S,4R,7R)-2-(2'-dimethylamino-ethoxy)-2-phenyl-7-(hydroxy-methyl)-1,7-dimethyl-bicyclo[2.2.1]heptane

deramciclane N-oxide: (1R,2S,4R)-(-)-2-phenyl-2-(2'-dimethylamino-ethoxy)-1,7,7-trimethyl-bicyclo[2.2.1]heptane-N-oxide

hydroxy-deramciclane II (M6): (1R,2S,4R)-(-)-2-phenyl-2-(2'-dimethylamino-ethoxy)-5 or 6-hydroxy-1,7,7-trimethyl-bicyclo[2.2.1]heptane

N-desmethyl 9-hydroxy-deramciclane (M2): (1R,2S,4R,7R)-2-(2'-methyl-amino-ethoxy)-2-phenyl-7-(hydroxy-methyl)-1,7-dimethyl-bicyclo[2.2.1]heptane

N-desmethyl hydroxy-deramciclane II (M3): (1R,2S,4R)-(-)-2-phenyl-2-(2'-methylamino-ethoxy)-5 or 6-hydroxy-1,7,7-trimethyl-bicyclo[2.2.1]heptane

phenylborneol (M8): (1R,2S,4R)-(-)-2-hydroxy-2-phenyl-1,7,7-trimethyl-bicyclo[2.2.1] heptane

phenylbornylene: (1S,4R)-2-phenyl-1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ene

phenylcamphene: 3,3-dimethyl-2-methylene-1-phenyl-bicyclo[2.2.1]heptane

4”-hydroxy-deramciclane: (1R,2S,4R)-(-)-2-(4”-hydroxy-phenyl)-2-(2’-dimethyl-amino-ethoxy)-1,7,7-trimethyl-bicyclo[2.2.1]heptane

CID: collision-induced decomposition

FAB: fast-atom bombardment

MIKES: mass-analyzed ion kinetic energy spectrometry

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ABSTRACT:

Metabolic fate of deramciclane [(1R,2S,4R)-(-)-2-phenyl-2-(2'-dimethylamino-ethoxy)-1,7,7-trimethyl-bicyclo[2.2.1]heptane], a new anxiolytic drug-candidate, has been determined in rat, mouse, rabbit, dog and human hepatocytes. Rat and rabbit cells were the most active, whereas the rate of metabolism was quite slow in human hepatocytes. During biotransformation, deramciclane underwent side chain modification and oxidation at several positions of the molecule. The side chain modification led to the formation of N-desmethyl deramciclane and phenyborneol. The oxidation of deramciclane resulted in several hydroxy-, carboxy- and N-oxide derivatives. The hydroxlation took place at primary or secondary carbons of the camphor ring as well as at the side chain, furthermore, dihydroxylated derivatives were also found. The side chain modified metabolites were also oxidized to hydroxy- or carboxy-derivatives. Conjugation of phase I metabolites, as a route of elimination, was also observed in rat, rabbit and dog hepatocytes. Although there were some species differences in biotransformation of deramciclane, it was concluded that phase I metabolism in human liver cells seemed to be similar to the metabolism in the hepatocytes isolated from rat. With careful approach, the rat model may be considered to be predictive for human metabolism of deramciclane.

Introduction

The early knowledge of routes by which a new drug-candidate may be metabolized is important for the interpretation of pharmacological and toxicological data obtained in drug development studies. Furthermore, information on species differences in the rates and pathways of metabolism is of great interest and will help to select the laboratory animal species most suitable to study the toxic properties of drugs (Gillette, 1995; Vermeulen, 1996). Primary hepatocytes in metabolic studies can offer a simple and useful model, since they retain most of the metabolic capabilities of the intact liver and provide an opportunity to study the biotransformation of novel drugs at a very early stage in drug development process (Maurel, 1996). The potential advantages of such an approach include the relatively high amounts of metabolites formed during the incubation, the easy extraction procedure of the metabolites from the medium and the cells and the high purity for further analysis.

In the last few years, efforts have been made to develop new non-benzodiazepine type anxiolytics with minimal risk of sedative and muscle relaxant side effects in the therapeutic dose range. Deramciclane developed by EGIS Pharmaceuticals Ltd. (Budapest, Hungary), is a novel potential anxiolytic agent that is more effective than diazepam or chlordiazepoxide (Gacsályi et al., 1988; Berényi et al., 1990). Deramciclane has been shown to be a potent and relatively specific 5-HT2A/2C receptor antagonist in receptor binding and functional studies (Palvimaki et al., 1998). Its anticonvulsant activity is exerted via inhibition of synaptosomal γ-aminobutyric acid uptake (Kovács et al., 1989). Our work demonstrates the similarities and differences in biotransformation of deramciclane by comparing catalytic activities of rat, mouse, rabbit, dog and human hepatocytes.

Materials and Methods

Chemicals. Deramciclane and the putative metabolites: N-desmethyl-deramciclane, 9-hydroxy-deramciclane, deramciclane N-oxide, N-desmethyl 9-hydroxy-deramciclane, phenylborneol, phenylbornylene, phenylcamphene and 4"-hydroxy-deramciclane were provided by EGIS Pharmaceuticals Ltd. (Budapest, Hungary). [^{14}C]-deramciclane labelled on the phenyl-ring (1.3394 GBq/mmol) was synthesized at the Chemical Research Center of the Hungarian Academy of Sciences (Budapest, Hungary). Collagenase, HEPES and Williams' medium E were the products of Sigma Chemie GmbH (Deisenhofen, Germany). All other chemicals for hepatocyte isolation, β-glucuronidase / arylsulfatase and ethyl acetate were purchased from Merck (Darmstadt, Germany). Triethylamine was obtained from Loba Feinchemie (Fischamend, Austria). Methanol, dichloromethane, butanol, n-hexan and chloroform were obtained from ChemoLab (Budapest, Hungary) as chromatography-grade products. All other chemicals were obtained from Reanal (Budapest, Hungary).

Isolation and culture of hepatocytes. Experiments were carried out by using hepatocytes prepared from male Wistar rats (240-250 g), NMRI mice (30-35 g) (ToxiCoop Safety Toxicological Study Center, Budapest, Hungary), New Zeland rabbits (3.8-4.3 kg) and Beagle dogs (9.5-11.0 kg) (Institute for Drug Research Ltd., Budapest, Hungary). Human livers were obtained from kidney transplant donors at the Transplantation and Surgery Clinic, Semmelweis University Budapest (Hungary). Permission of the Local Research Ethics Committee was obtained to use human tissues. The liver cells were isolated by the method of Bayliss and Skett (1996). Hepatocytes having viability better than 90% as determined by trypan blue exclusion (Berry et al., 1991), were used in the experiments. The cells were plated at a density of $4x10^6$ cells/dish onto 60-mm plastic dishes precoated with collagen in medium described by Ferrini et al. (1998).

Biotransformation of deramciclane. Hepatocytes were maintained in primary culture for 24 hr in culture medium containing 50 μM deramciclane (266,4 MBq/mmol). After the incubation period, the cells and extracellular medium were separated and the metabolites were extracted with dichloromethane. Conjugates of deramciclane metabolites formed in hepatocytes were subjected to enzymic hydrolysis by β-glucuronidase/arylsulfatase in 0.1 M Na-acetate buffer (pH 4.4) and then the metabolites were extracted with dichloromethane. The organic phases were evaporated to dryness in vacuo and the residue was resolved in methanol. The radioactivity of the samples at each stage was followed by liquid scintillation techniques (LKB 1217 Rackbeta type, Wallac, Turku, Finland). Radioactivity recoveries for the extractions ranged from 89% to 99%. The extracts of the cells or the extracellular medium (before and after enzymic hydrolysis) were analyzed by thin layer chromatography on 0.2-mm-thick DC-Alufolien Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany) using three different delivery systems: i) ethyl acetate/triethylamine (25:1, v/v), ii) butanol/acetic acid/water (4:1:1, v/v) and iii) n-hexan/chloroform (1:1, v/v). The amounts of deramciclane and its metabolites were calculated on the basis of digital autoradiography by a Berthold digital autoradiograph type LB-287 (EG&G, Berthold Technologies, Bad Wildbad, Germany). The metabolites were identified on the basis of R_f values on thin layer compared with the authentic standards or on the basis of mass spectrometric and infrared spectroscopic analyses. The spots containing the metabolites were directly transferred to the mass spectrometer (Ludányi et al., 1997) or they were eluted from thin layer plates with methanol, the samples were evaporated and subjected to spectroscopic analysis.

Mass spectrometric analysis. Attempts were made to identify the chemical structures of separated and eluted metabolites by mass spectrometric analysis using a reverse geometry VG-ZAB-2SEQ type mass spectrometer (Manchester, England). 30 kV Cs^+ ions were used in

fast atom bombardment (FAB) ionization mode and the matrix was glycerol. Tandem mass spectra were observed by the mass-analyzed ion kinetic energy spectrometry (MIKES) technique combined with collision-induced decomposition (CID) using argon collision gas at a pressure corresponding to 50% main beam transmission. The resolution of exact mass measurements was 10 000. Some samples separated by thin layer chromatography were further analyzed by gas chromatography/mass spectrometry (GC-MS) with the following GC-experimental conditions: HP-5890 GC instrument equipped with a capillary column (25m x 0.25 mm x 0.25 μm Cp-Sil 8), He carrier gas (2 ml/min). In these experiments electron impact ionization (EI) was performed at 70 eV electron energy and at 200°C source temperature.

Infrared spectroscopic analysis. For the on line GC-IR investigations of the metabolites, a NICOLET 170 SX Fourier-transform spectrometer connected to a Varian 3700 type gas chromatograph was used. The experimental parameters were as follows: Ge/KBr beamsplitter, quartz light-pipe (150 mm x 0.5 mm), He carrier gas (6ml/min), HP-1 column (10 m, 0.53 mm).

Results

Biotransformation of deramciclane. *In vitro* metabolism of deramciclane was studied in primary hepatocytes isolated from rat, mouse, rabbit, dog and human. The metabolites formed during the 24-hr incubation period were extracted from the cells or from the extracellular matrix and analyzed by thin layer chromatography and autoradiography. Table 1 summarizes the amounts of deramciclane and its metabolites with their structures, chromatographic properties, MS data (molecular ion and fragment ions) and the species which were able to produce the given metabolites from deramciclane.

The most extensive metabolism of deramciclane was observed in liver cells of rat and rabbit. After the 24-hr incubation period, deramciclane could be detected merely in trace (less than 10% of deramciclane amount present at 0 min) in the cells or in the extracellular medium. The rate of biotransformation was much slower in mouse and dog hepatocytes; about 40% and 50% of deramciclane amount remained unchanged. Human liver cells were much less active than the hepatocytes from any animal species investigated.

Fig. 1 displays metabolic profile found in extracts of rat hepatocytes and extracellular medium. Metabolites were separated on thin layer plates with ethyl acetate/triethylamine (25:1, v/v) delivery system and were detected by digital autoradiography. Although the metabolites were identified on the basis of R_f values compared with the authentic standards, the final evidence for the structures was provided by mass spectrometric analysis. Demethylation of deramciclane resulted in the formation of N-desmethyl deramciclane (M4), which was found mainly inside the cells because of its lipophilicity. The main metabolite produced by rat liver cells was 9-hydroxy-deramciclane (M5), which was eliminated primarily as glucuronide. Other hydroxylated metabolites: hydroxy-deramciclane II (M6, hydroxy-group was located at 5 or 6 position of camphor ring), N-desmethyl 9-hydroxy-

deramciclane (M2), N-desmethyl hydroxy-deramciclane II (M3) were also detected as minor metabolites as well as phenylborneol and its hydroxylated derivatives (M8).

Metabolites produced by mouse or rabbit liver cells were similar to those formed by rat hepatocytes. However, glucuronide formation as the route of deramciclane metabolism was not detected at all in mouse cells (total radioactivity could be extracted without enzymic hydrolysis), whereas rabbit hepatocytes eliminated hydroxylated metabolites mainly as glucuronide conjugated form. Some differences could be observed in the formation of metabolites by dog hepatocytes. N-desmethyl deramciclane (M4) was detected as the major metabolite formed by dog hepatocytes. Although hydroxylated derivatives of deramciclane (9-hydroxy-deramciclane, M5; hydroxy-deramciclane II, M6; N-desmethyl hydroxy-deramciclane II, M3) were produced, their amounts were estimated to be small. Phenylborneol (M8) was also detected in trace. Neither free nor conjugated form of N-desmethyl 9-hydroxy-deramciclane (M2) was produced in detectable amount by dog liver cells.

Metabolic profile found in human cells missed some of deramciclane derivatives that were detected in hepatocytes of other species. On the other hand, none of the metabolites could be considered as the major one, all of them were produced at similar rate. Hydroxylation and side chain modification resulted in hydroxy-deramciclane II (M6), N-desmethyl deramciclane (M4) and phenylborneol (M8). 9-Hydroxy-deramciclane (M5) and N-desmethyl 9-hydroxy-deramciclane (M2) were not produced in detectable amount. Whereas N-desmethyl hydroxy-deramciclane II (M3) was detected in trace in the extracts of extracellular liquid. Conjugation of phase I metabolites was not observed at all.

Characterization of deramciclane metabolites. Mass spectrum of deramciclane obtained by FAB-MS technique displayed a very abundant peak of protonated molecular ion (MH^+) at m/z 302 and a main fragment peak at m/z 213 characteristic of deramciclane

skeleton. In CID spectrum of the protonated molecular ion, two peaks were observed at m/z 213 and 90 (Table 1). These peaks were formed by the cleavage of dimethylamino-ethanol side chain, where both sides of the molecule were protonated. These complementary fragments were proved to be the most useful both for structural characterization of deramciclane derivatives and for analytical purposes.

N-desmethyl deramciclane was present in hepatocytes of all species investigated. Chromatographic and mass spectrometric behaviour of the demethylated derivative was identical to that of the authentic reference compound.

Several hydroxylated derivatives of deramciclane were isolated mainly from the extracellular matrix in free or conjugated forms. Although the protonated molecular ion at m/z 318 was characteristic of all hydroxy-deramciclane compounds, three types could be distinguished by MS/MS(CID) analysis. In the case of two types, the hydroxy-group was located at the camphor ring, which was confirmed by the presence of fragment ion at m/z 229 (cleavage of dimethylamino-ethanol side chain). The water loss from the m/z 229 (resulted in m/z 211) indicated the location of the hydroxy-group at the camphor and not at the aromatic ring. In the case of the third type of hydroxy-deramciclane compounds, the masses of the CID fragments indicated hydroxylation at the dimethylamino-ethanol side chain. Finally, it must be mentioned that hydroxylation on the 4”-position of the phenyl-ring was ruled out on the basis of chromatographic properties of the authentic reference compound.

i) Type I called primary hydroxylated derivatives, had the hydroxy-group at one of the primary carbons of the camphor ring (Fig. 2). CID-spectrum of m/z 318 with fragments at m/z 229, 211, 185, 171 and 143 confirmed that the hydroxy-group was likely to locate at 8 or 9 position. 10 position was not probable due to the assumed fragmentation route. Further evidence for the structure was provided by infrared spectroscopic analysis (Fig. 3). Infrared spectrum of the metabolite with an R_f of 0.059 in butanol/acetic acid/ water (4:1:1, v/v) was

identical to that of 9-hydroxy-deramciclane authentic reference compound. From these results, it was concluded that the spot with an R_f of 0.135 contained 8-hydroxy-deramciclane.

ii) Type II of hydroxy-deramciclane metabolites involves the compounds whose one of the secondary carbon atoms of the camphor ring was hydroxylated (Fig. 4). CID-spectrum of m/z 318 with fragments at m/z 229, 169 and 156 predicted that the hydroxy-group was likely to locate at 5 or 6 position.

iii) Type III was proved to have the hydroxy-group at the dimethylamino-ethanol side chain of deramciclane. Fragmentation of the molecular ion (MH^+ at m/z 318) was different from that of the previous two types. The fragment at m/z 213 indicated that the camphor ring was intact, whereas the side chain was likely to contain the hydroxy-group. It was also confirmed by the presence of complementary fragment at m/z 106. The hydroxy-group was supposed to be located at one of the carbons of the side chain. The oxidation of any of the methyl-groups on the nitrogen would lead to the formation of formaldehyde (and N-desmethyl deramciclane) and the methyl-hydroxy-intermediate could not be demonstrated (Hollenberg, 1992; Guengerich, 1993). The oxidation of the carbon (2') closest to the nitrogen (between the nitrogen and oxygen) is also doubtful, since it would result in desamination of deramciclane (Guengerich, 1996). We think that the only point, where the hydroxy-group can be located, is the carbon (1') closest to the oxygen forming the structure of 1'-hydroxy-deramciclane. It should be noted that the mobility of deramciclane N-oxide authentic standard (its MH^+ was also at m/z 318) in butanol/acetic acid/water (4:1:1, v/v) was different (higher).

Rat and human hepatocytes were proved to be able to produce deramciclane N-oxide. Chromatographic and mass spectrometric properties of the isolated metabolite was identical to that of authentic reference compound. The chromatographic mobility of deramciclane N-oxide standard was rather slow in ethyl acetate/triethylamine (25:1, v/v), - it ran close to the

start point (M1 spot of Fig. 1), - but it could be separated on thin layer plate in butanol/acetic acid/water (4:1:1, v/v). The results of MS/MS analysis (MH^+ at m/z 318 and fragments at m/z 213 and 106) provided the evidence that the skeleton of deramciclane remained unchanged, whereas the side chain involved one more oxygen suspected to form N-oxide.

Hydroxylation of N-desmethyl deramciclane also occurred at several positions of the molecule. The structures of N-desmethyl 8- or 9-hydroxy-deramciclane were determined by MS/MS after isolation from thin layer. Thin layer or gas chromatographic properties and MS behaviour of N-desmethyl 9-hydroxy-deramciclane metabolite (R_f: 0.115 in butanol/acetic acid/water) were identical to that of the authentic reference compound. Thus the spot with an R_f of 0.262 was supposed to contain N-desmethyl 8-hydroxy-deramciclane. GC-MS analysis of the extracts proved the presence of one more N-desmethyl hydroxy-deramciclane metabolite that was produced by the hepatocytes of all species investigated. The results of MS measurement made it clear that N-desmethyl-deramciclane underwent hydroxylation at one of the secondary carbons of the camphor ring.

Among hydroxylated derivatives of deramciclane, there were two dihydroxylated metabolites with different chromatographic mobilities in butanol/acetic acid/water, but identical mass spectrometric properties (Table 1). The results of MS/MS analysis supported the assumption that one of the hydroxy-group was located at the camphor ring, whereas the other was at the side chain. The exact mass measurement also confirmed the structure of dihydroxy-deramciclane metabolites as $C_{20}H_{32}O_3N$ (MH^+ = 334.2382) and of the side chain as $C_4H_{12}O_2N$ (m/z 106 fragment = 106.0868).

Using butanol/acetic acid/water solvent delivery system, two carboxy-derivatives of deramciclane could be separated, which were produced by oxidation of one of the methyl-groups of the camphor ring to carboxylic acid. Furthermore, N-demethylation and oxidation to carboxylic acid resulted in N-desmethyl carboxy-deramciclane with the molecular ion

(MH^+) at m/z 318 and fragment ions at m/z 243 and 76 (as a result of the cleavage of monomethyl amino-ethanol side chain). Although it was identical to the molecular ion of hydroxy-deramciclane derivatives, they could be distinguish on the basis of CID-spectra.

The cleavage of the total side chain of deramciclane resulted in the formation of phenylborneol as it was identified on the basis of chromathograhic properties of the authentic standard. Although GC-MS analysis determined its structure as phenylcamphene and phenylbornylene that might be due to the chemical modification of phenylborneol (loss of water) during the isolation from thin layer plate or GC-procedures. Since the authentic reference compounds for phenylborneol, phenylbornylene and phenylcamphene had the same R_f values in both mobile phases used, the correct separation had to be developed in n-hexan/chloroform (1:1, v/v). The radiodensitogram confirmed that the metabolite produced during deramciclane biotransformation was neither phenylbornylene nor phenylcamphene, but most probably phenylborneol. Spot with an R_f of 0.720 (in butanol/acetic acid/water) also contained hydroxy-phenylborneol as a product of rat hepatocytes.

Discussion

Deramciclane was metabolized at highly different rates in hepatocytes from various species examined. Rat and rabbit cells were the most active, whereas dog and mouse hepatocytes exhibited reduced biotransformation rates. The extent of deramciclane metabolism was much lower in human liver cells compared with the cells from laboratory animal species. A comparative pharmacokinetic study on orally administered deramciclane in rat, rabbit, dog and man also revealed considerable species differences regarding the elimination of the compound. Due to intensive metabolism, the elimination half-life was found to be significantly lower in rats and rabbits (4.05 and 5.77 hr, respectively) than in dogs and man (12.0 and 31.61 hr, respectively) (Klebovich et al., 1998).

The proposed metabolic scheme for biotransformation of deramciclane based on the results of this study is depicted in Fig. 5. Oxidative metabolism of deramciclane occurred on the ring of camphor and/or on the side chain, but the aromatic ring remained unchanged. Primary routes of metabolism in all species were N-demethylation and hydroxylation of the molecule. N-Demethylation does not result in pharmacological deactivation of deramciclane. N-Desmethyl-deramciclane has been reported to have receptor binding characteristics and pharmacological activity similar to the parent compound. Previous studies proved that N-desmethyl deramciclane was also formed *in vivo* in several animal species (Kanerva et al., 1998; Nemes et al., 2000; Magyar et al., 2002) and in man (Huupponen et al., 2004).

Both deramciclane and N-desmethyl-deramciclane were subjected to hydroxylation at different positions of the camphor part or the side chain. Camphor is known to undergo oxidative metabolism to form hydroxy-derivatives (Leibman and Ortiz, 1973; White et al., 1984; Sariaslani et al., 1990). In the case of deramciclane, several positional isomers of hydroxy-metabolites were also identified. 9-Hydroxy-deramciclane was produced in detectable amount by the hepatocytes from all species investigated except for human.

Hydroxylation of the camphor ring at 9 position was considered to be one of the major routes of deramciclane metabolism in rat, mouse and rabbit hepatocytes, whereas dog cells formed 9-hydroxy-deramciclane as a minor metabolite. In contrast, 8-hydroxy-deramciclane was detected only in trace and solely in rat liver cells. As it is often observed with metabolites hydroxylated at primary carbon atoms of a xenobiotic, further oxidation to carboxylic acids was also observed in the case of deramciclane. From the fact that carboxy-deramciclane was produced by human hepatocytes, the formation of 9- or 8-hydroxy-deramciclane as an intermediate metabolite was also supposed. Hydroxylation at one of the secondary carbon atoms (at 5 or 6 position) of the camphor ring also occurred. One of the hydroxy-deramciclane II metabolites – supposed to be 5-hydroxy-deramciclane - was produced by the hepatocytes from all species, while 6-hydroxy-derivative was formed only in rat cells. The major metabolite, N-desmethyl deramciclane was subjected to further hydroxylation at primary or secondary carbon atoms of the camphor ring. Further oxidation of N-desmethyl deramciclane also resulted in the formation of N-desmethyl carboxy-deramciclane in rat and rabbit hepatocytes, possibly via the intermediate metabolites, N-desmethyl 9- or 8-hydroxy-deramciclane.

Our results showed that hydroxylation at 1’ position of the side chain of deramciclane also occurred in rat and human cells. Furthermore, dihydroxylated derivatives with one hydroxy-group at the side chain and the other at the camphor ring were also found in rat, rabbit and dog hepatocytes, but not in human. Oxidation of the side chain at the nitrogen led to the formation of considerable amount of deramciclane N-oxide in rat and human cells. It should be noted that sequential metabolism of N-oxide was not observed. Phenylborneol due to the cleavage of the total side chain of deramciclane was formed as a minor metabolite in hepatocytes isolated from all species investigated. However, water-loss leading to phenylbornylene and phenylcamphene was excluded in all species. The amount of

phenylborneol can be supposed to be reduced by sequential metabolism to hydroxy-phenylborneols. Hydroxy-phenylborneol formation was proved in rat hepatocytes.

Glucuronide conjugation of hydroxy-deramciclane derivatives seemed to be facile in rat, rabbit and dog liver cells. However, there was no indication for the presence of glucuronide conjugates in mouse, or human hepatocytes.

One of the main goals of comparative metabolic studies is considered to be their appropriateness for selecting the best animal model(s) for preclinical toxicology studies on the basis of qualitative similarity in metabolic profile to human. The profiles of deramciclane metabolites in the hepatocytes isolated from several laboratory animals and human were not exactly the same across species. Although the authors highlighted species differences in deramciclane metabolism, it was concluded that phase I metabolism in human liver cells seemed to be similar to the metabolism in rat hepatocytes. Phase I metabolites isolated and identified from human hepatocytes were also produced by rat cells. With careful approach, the rat model may be considered to be predictive for human metabolism of deramciclane.

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Footnotes:

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Legends for figures

FIG. 1. *Deramciclane metabolites produced by rat hepatocytes.*

After 24hr incubation period, the extracts of the cells (a) and extracellular matrix (b) were analyzed by thin layer chromatography with the mobile phase of ethyl acetate/triethylamine (25:1, v/v).

FIG. 2. *Mass spectrometric behaviour of 9-hydroxy-deramciclane.*

a: FAB spectrum; b: CID spectrum of MH+ at m/z 318

FIG. 3. *Infrared spectrum of 9-hydroxy-deramciclane metabolite (a) and authentic reference compound (b) in vapour phase.*

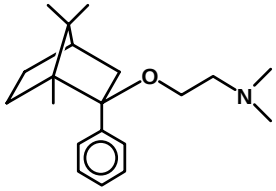
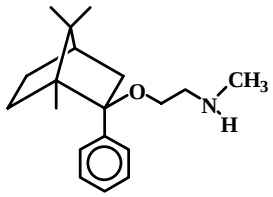
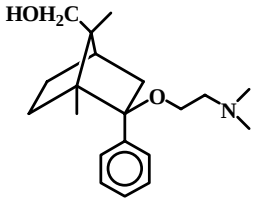
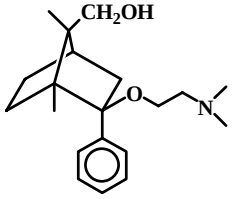
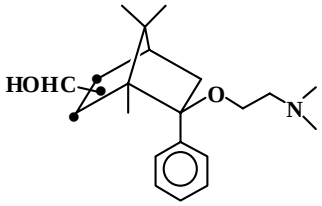
FIG. 4. *Mass spectrometric behaviour of hydroxy-deramciclane II.*

a: FAB spectrum; b: CID spectrum of MH+ at m/z 318

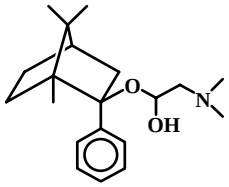
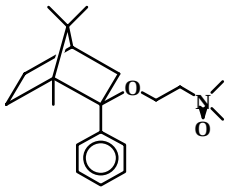
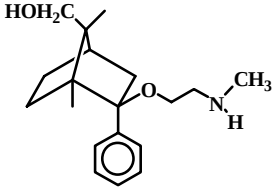
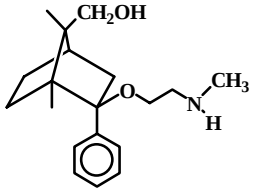
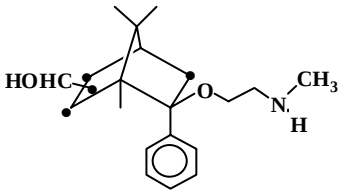
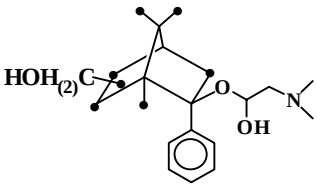
FIG. 5. *Proposed metabolic scheme for deramciclane.*

TABLE 1

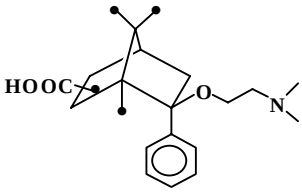
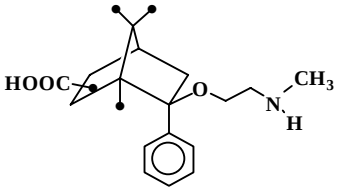
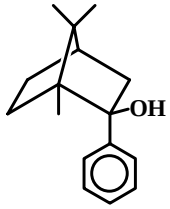
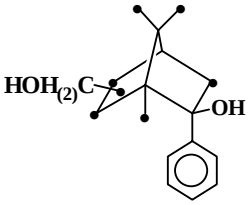
Chemical structures, R_f values and MS data for deramciclane and its metabolites produced by hepatocytes from various species

Metabolite[a]	Formed by the hepatocytes (%)		R_f[b]	Molecular ion[c]	Fragment ions
Deramciclane (FAB, MS/MS) 	Rat	8.67±1.59[d]	0.200	302(+)	213, 90
	Mouse	37.82±2.49			
	Rabbit	4.51±1.91			
	Dog	47.67±10.57			
	Human	64.73±0.164			
N-desmethyl deramciclane (FAB, MS/MS) 	Rat	18.15±3.82	0.370	288(+)	213
	Mouse	24.50±4.28			
	Rabbit	7.00±0.554			
	Dog	27.12±7.66			
	Human	4.55±4.83			
9-hydroxy-deramciclane (FAB, MS/MS) 	Rat	28.44±3.57 (3:1)[e]	0.059	318(+)	229, 211, 185, 171, 143, 90
	Mouse	10.92±3.73			
	Rabbit	24.25±3.74 (2:3)			
	Dog	0.57±0.519			
8-hydroxy-deramciclane (FAB, MS/MS) 	Rat	<2.00	0.135	318(+)	229, 211, 185, 171, 143, 90
hydroxy-deramciclane IIa (FAB, MS/MS)	Rat	6.10±1.74	0.088	318(+)	229, 169, 156, 90
	Mouse	1.65±1.06			
	Rabbit	12.54±0.57 (1:0)			
	Dog	3.67±2.09 (1:1)			
	Human	1.16±0.39	0.115	318(+)	229, 169, 156, 90
hydroxy-deramciclane IIb (FAB, MS/MS) 	Rat	<2.00			

(Continued)

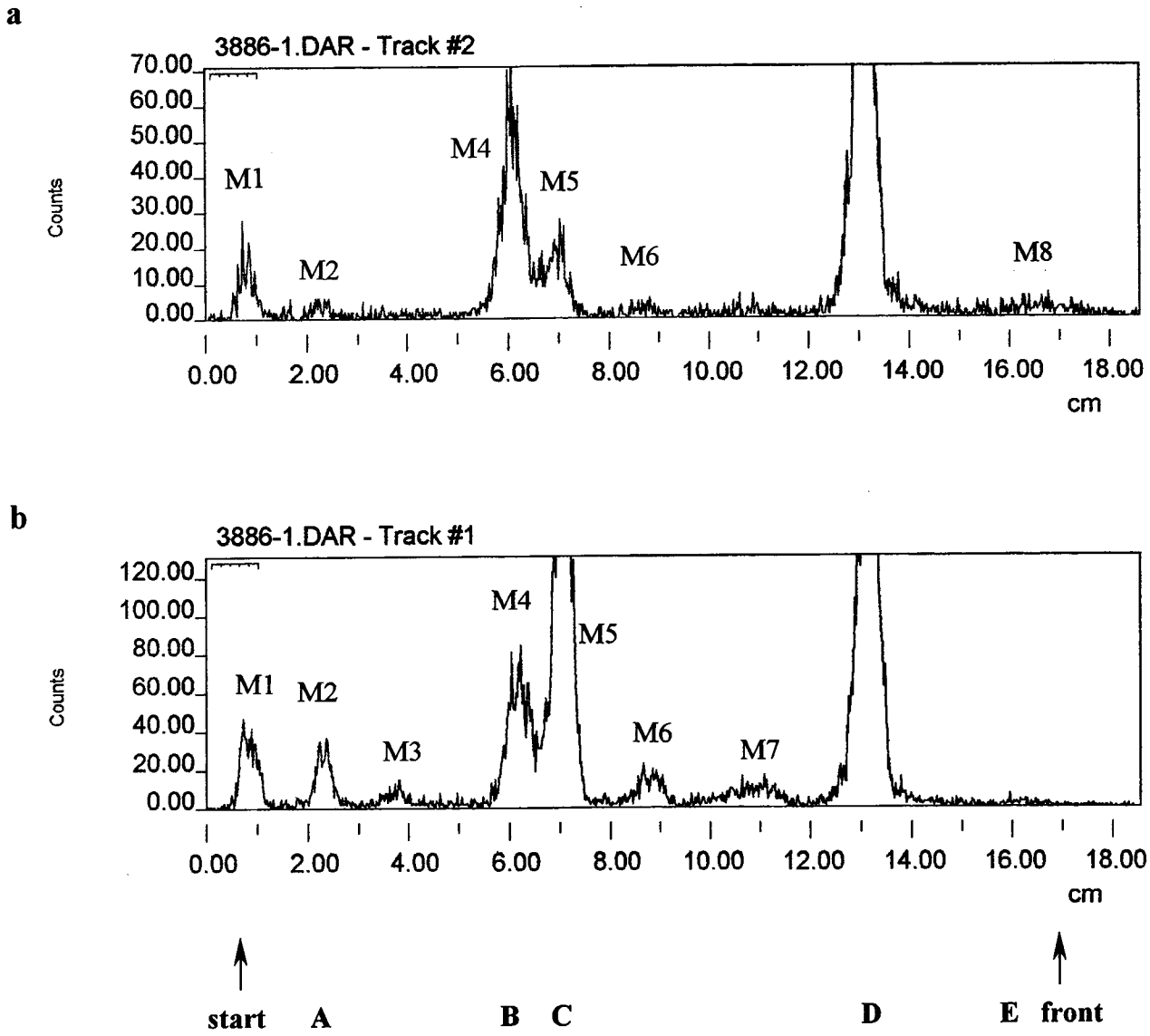
Metabolite[a]	Formed by the hepatocytes (%)		R_f[b]	Molecular ion[c]	Fragment ions
1'-hydroxy-deramciclane (FAB, MS/MS) 	Rat Human	1.71±0.70 1.71±0.67	0.200	318(+)	213, 106
deramciclane N-oxide (FAB, MS/MS) 	Rat Human	4.42±2.53 1.23±0.59	0.338	318(+)	213, 157, 106
N-desmethyl 9-hydroxy-deramciclane (FAB, MS/MS) 	Rat Mouse Rabbit	5.57±3.05 (3:2) 1.92±0.72 23.09±3.33 (2:3)	0.115	304(+)	229, 211, 185, 171, 143, 76
N-desmethyl 8-hydroxy-deramciclane (FAB, MS/MS) 	Rat Rabbit	<2.00 <2.00	0.262	304(+)	229, 211, 185, 171, 143, 76
N-desmethyl hydroxy-deramciclane II (GC-MS) 	Rat Mouse Rabbit Dog Human	1.66±1.78 1.77±1.21 8.03±3.04 (2:3) 1.16±0.32 <2.00		303( )	229, 211, 58, 44
dihydroxy-deramciclane a (FAB, MS/MS)	Rabbit	<2.00	0.115	334(+)	229, 211, 106
dihydroxy-deramciclane b (FAB, MS/MS) 	Rat Rabbit Dog	<2.00 <2.00 <2.00	0.241	334(+)	229, 211, 106

(Continued)

Metabolite[a]	Formed by the hepatocytes (%)		R_f[b]	Molecular ion[c]	Fragment ions
carboxy-deramciclane a (FAB, MS/MS)	Rat Human	<2.00 <2.00	0.200	332(+)	243, 197, 90
carboxy-deramciclane b (FAB, MS/MS)	Rat Rabbit	<2.00 <2.00	0.241	332(+)	243, 197, 90
					
N-desmethyl carboxy-deramciclane (FAB, MS/MS)	Rat Rabbit	<2.00 <2.00	0.294	318(+)	243, 197, 76
					
phenylborneol (GC-MS)	Rat Mouse Rabbit Dog Human	3.66±3.72 (3:2) 1.30±1.04 7.76±3.92 (2:3) 1.91±0.21 (4:1) 0.97±0.22	0.720	230( )[f]	197, 184, 169
					
hydroxy-phenylborneol (GC-MS)	Rat	<2.00	0.720	244( )[g]	197, 169
					

[a] The method of identification: FAB, MS/MS(CID) or GC-MS(EI).
[b] R_f values on thin layer developed in butanol/acetic acid/water (4:1:1, v/v).
[c] Molecular ion: MH^+ (FAB) in (+) ion mode or M^+ (GC-MS) in ( ) ion mode.
[d] The values represent the amount of parent compound remained unchanged during the 24-hr incubation period and the relative amounts of metabolite formed in 24 hr. They are expressed as mean±SD (N=3).
[e] The values in brackets represent the ratio of glucuronide conjugate and phase I metabolite.
[f] Phenylcamphene and phenylbornylene (M^+=212) were identified by GC-MS instead of phenylborneol (M^+=230).
[g] Hydroxy-phenylcamphene and hydroxy-phenylbornylene (M^+=228) were identified by GC-MS instead of hydroxy-phenylborneol (M^+=244).

Figure 1



authentic references:
A: N-desmethyl 9-hydroxy-deramciclane
B: N-desmethyl deramciclane
C: 9-hydroxy-deramciclane
D: deramciclane
E: phenylborneol

Figure 2

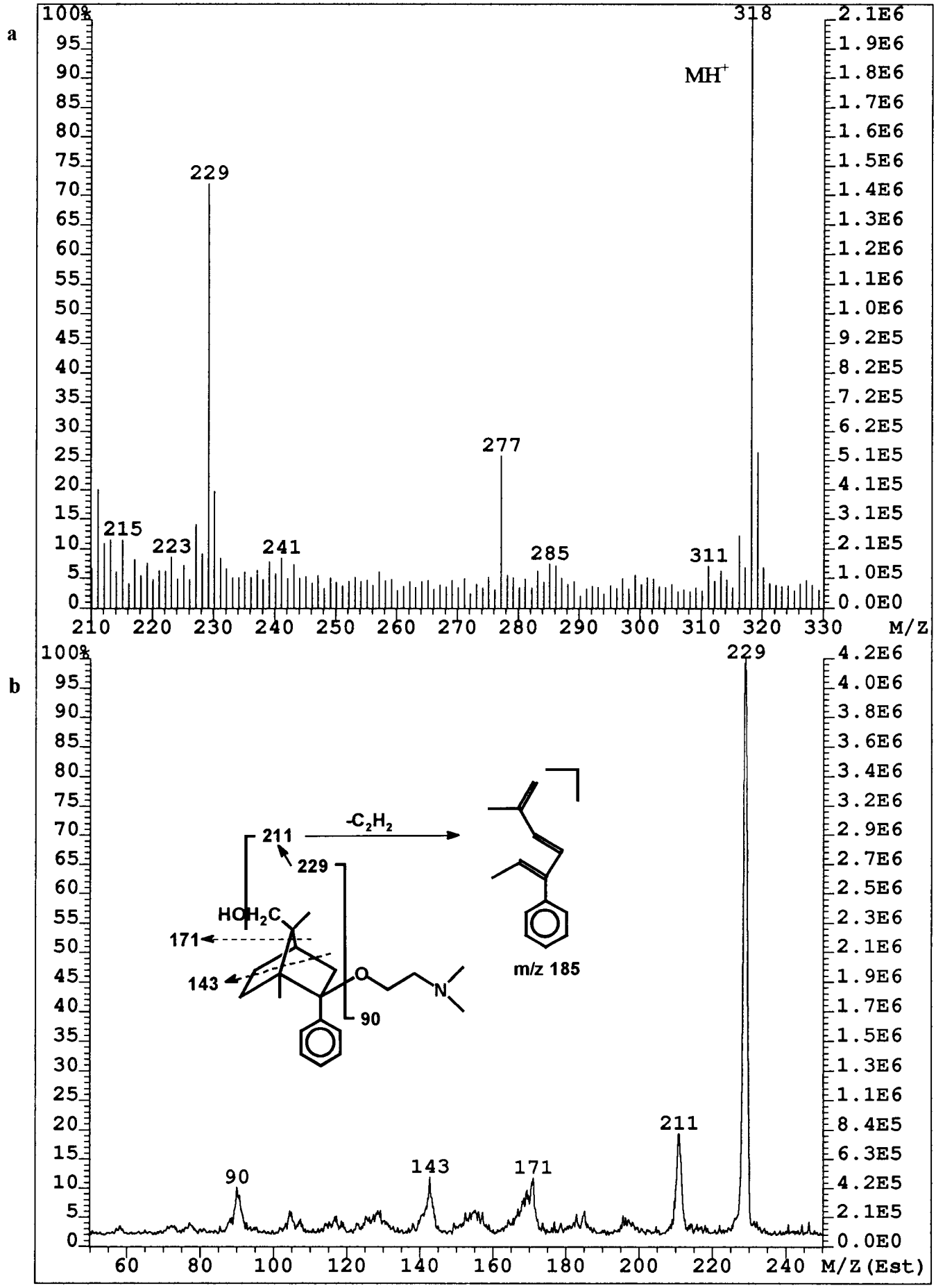
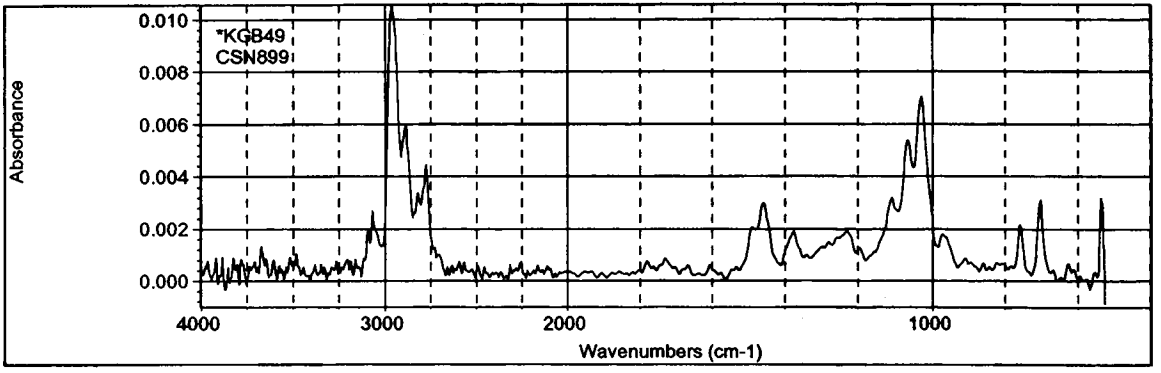

a
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
318
MH+
229
277
215
223
241
285
311
2.1E6
1.9E6
1.8E6
1.7E6
1.6E6
1.5E6
1.4E6
1.3E6
1.2E6
1.1E6
1.0E6
9.2E5
8.2E5
7.2E5
6.2E5
5.1E5
4.1E5
3.1E5
2.1E5
1.0E5
0.0E0
210 220 230 240 250 260 270 280 290 300 310 320 330 M/Z
b
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
229
211
-C2H2
229
HOH2C
171
143
90
O
N
m/z 185
211
90
143
171
4.2E6
4.0E6
3.8E6
3.6E6
3.4E6
3.2E6
2.9E6
2.7E6
2.5E6
2.3E6
2.1E6
1.9E6
1.7E6
1.5E6
1.3E6
1.1E6
8.4E5
6.3E5
4.2E5
2.1E5
0.0E0
60 80 100 120 140 160 180 200 220 240 M/Z(Est)

Figure 3


*KGB49
CSN899
Absorbance
0.010
0.008
0.006
0.004
0.002
0.000
4000
3000
2000
1000
Wavenumbers (cm-1)

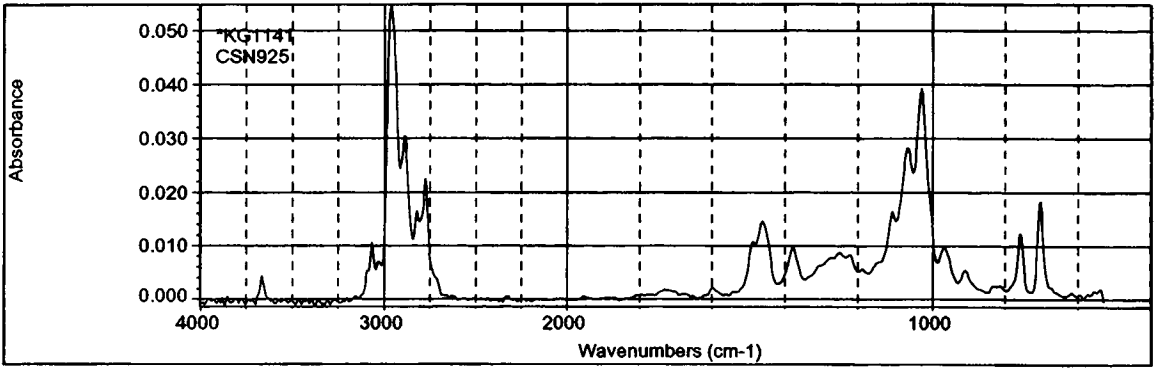

*KG1141
CSN925
Absorbance
0.050
0.040
0.030
0.020
0.010
0.000
4000
3000
2000
1000
Wavenumbers (cm-1)

Figure 4

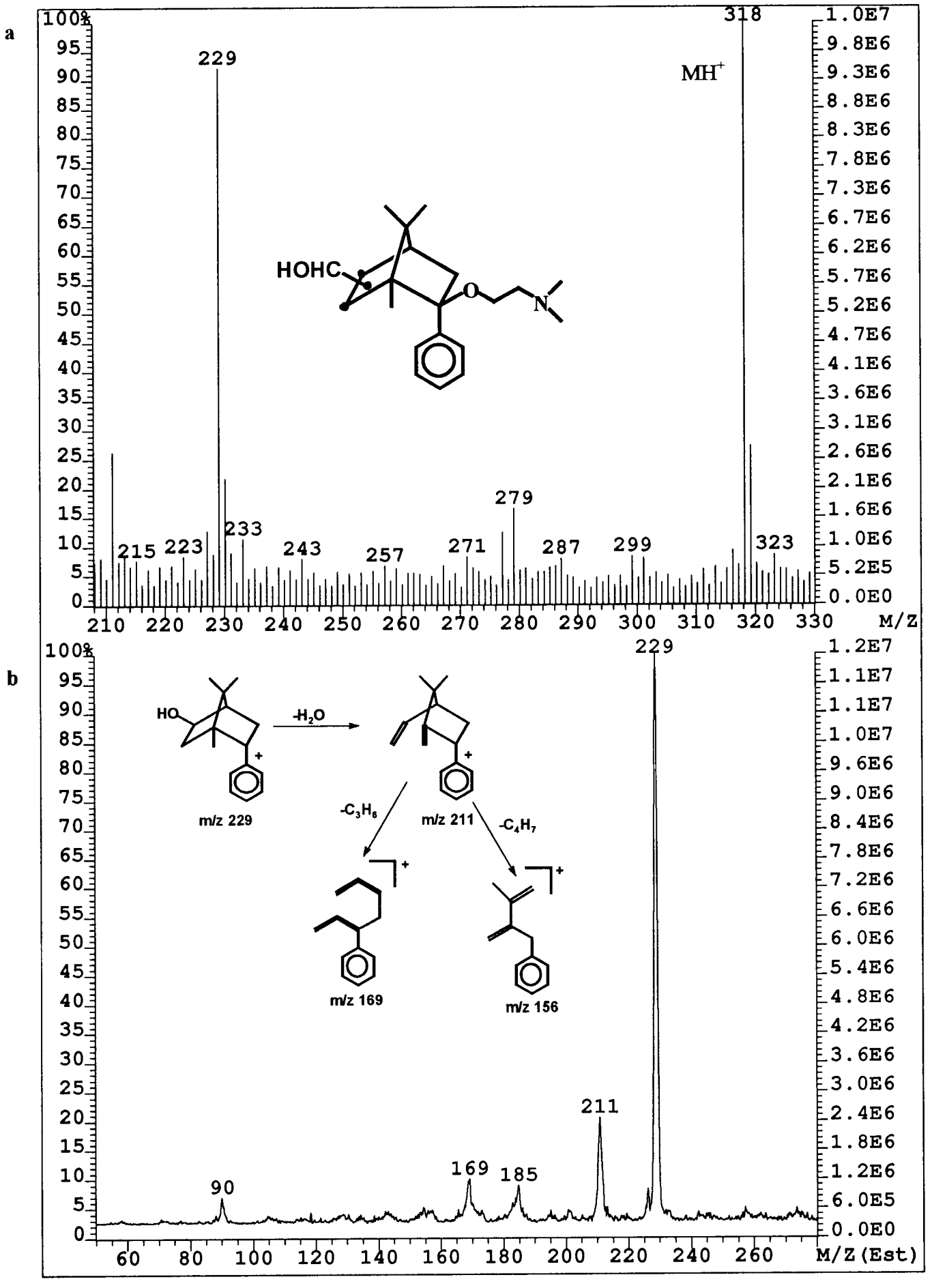

a
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
229
318
MH+
HOHC
O
N
279
233
215
223
243
257
271
287
299
323
1.0E7
9.8E6
9.3E6
8.8E6
8.3E6
7.8E6
7.3E6
6.7E6
6.2E6
5.7E6
5.2E6
4.7E6
4.1E6
3.6E6
3.1E6
2.6E6
2.1E6
1.6E6
1.0E6
5.2E5
0.0E0
210 220 230 240 250 260 270 280 290 300 310 320 330
M/Z
b
100%
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
HO
-H2O
m/z 229
-C3H6
m/z 211
-C4H7
m/z 169
m/z 156
229
211
169
185
90
1.2E7
1.1E7
1.1E7
1.0E7
9.6E6
9.0E6
8.4E6
7.8E6
7.2E6
6.6E6
6.0E6
5.4E6
4.8E6
4.2E6
3.6E6
3.0E6
2.4E6
1.8E6
1.2E6
6.0E5
0.0E0
60 80 100 120 140 160 180 200 220 240 260
M/Z(Est)

Figure 5