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Gas chromatographic–mass spectrometric identification of metabolites from α-pinene in human urine after occupational exposure to sawing fumes

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Abstract

Three metabolites from α-pinene (two diols and an alcohol with an aldehyde group) have been identified in human urine after occupational exposure to sawing fumes from pine. Urine was enzymatically hydrolyzed, cleaned up on a C_{18} micro-column and the metabolites were identified by GC–MS using electron impact (70 eV) and chemical ionization with ammonia or isobutane as the reagent gas. Analysis of underivatized metabolites was performed using a capillary column with a semi-polar phase and trimethylsilylated derivatives were analyzed using a non-polar phase.

Keywords: α-Pinene; Monoterpenes

1. Introduction

Occupational exposure to the monoterpenes α-pinene, β-pinene and Δ^3-carene occurs in saw mills and joinery shops as these substances are the main constituents in sawing fumes from pine (*Pinus sylvestris*) [1]. Personal exposure to these substances may be assessed by air-sampling using diffusive samplers, followed by GC analysis of the monoterpenes [2,3]. The present exposure limit in Sweden for total as well as for individual monoterpenes is 150 mg m^{-3} [4], and this exposure limit is often exceeded [1,5]. The air-sampling procedure measures the concentration of the monoterpenes in the breathing zone of the worker, but not the personal uptake of the substances, as an individual's uptake of an air pollutant may be influenced by lung ventilation (work load), fat solubility of the substance(s), genetic differences, sex, etc. [6]. Biological monitoring, i.e., identification and quantification of a substance itself and/or its metabolites in biological fluids like blood or urine, is thus an attractive alternative to air sampling, as this method measures the individual's uptake of an air pollutant.

trans-Verbenol, myrtenol and myrtenic acid have been identified as metabolites from α-pinene by GC–MS in urine from rabbits that were administered the monoterpene in a water suspension through a stomach tube [7]. In the brushtail possum (*Trichosurus vulpecula*) *trans*-verbenol (faeces) and

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myrtenic acid (urine) were identified as metabolites from α-pinene, after feeding the animal with bread soaked with this monoterpene [8]. Borneol, myrtenol and bornylacetate have been identified as metabolites from α-pinene in human urine after acute poisoning with pine oil [9].

We have recently identified *cis*- and *trans*-verbenol as metabolites from α-pinene in urine from workers exposed to sawing fumes containing α-pinene, β-pinene and Δ^3-carene, but borneol or bornylacetate was not detected. However, traces of myrtenol were found [1]. We have studied the elimination kinetics of the verbenols in humans, after exposure in an exposure chamber, to the optical isomers (+)- and (−)-α-pinene, respectively [10]. During the GC analysis of urine samples from the exposed individuals, we detected at least three other components besides the verbenols that are suspected of being metabolites from α-pinene. These components were confirmed in urine samples from workers exposed to sawing fumes from pine.

In this paper, we describe the identification by GC–MS of these additional metabolites. The mass spectrometric determinations of the metabolites and their trimethylsilyl derivatives have been performed by electron impact (EI) at 70 eV and chemical ionization (CI) with ammonia (NH_3) or isobutane (i-C_4H_{10}) as the reagent gases. This investigation on terpene metabolism in man was performed in order to study the possibility of using biological monitoring for occupational exposure assessment in connection with terpene exposure.

2. Experimental

2.1. Urine samples

Urine samples were collected immediately after the end of the chamber exposure or after a work shift of 8–9 h at a saw mill. The urine samples from chamber-exposed individuals were analyzed within one month, whilst the samples from saw mill workers were analyzed the next day. The samples were kept at −20°C until analysis. Urine samples (10 ml) were submitted to enzymatic hydrolysis for 24 h at 37°C. After this treatment, the sample was cleaned up on a C_{18} micro column (Sep-Pak, Millipore, MA, USA), and the substances were eluted with 10 ml of *n*-heptane. The "work up" procedure has been described in detail elsewere [1]. A 2-μl sample of the eluate was submitted for analysis on a gas chromatograph–mass spectrometer.

2.2. Derivatization

A 2-ml volume of the *n*-heptane eluate was reduced to dryness in a glass vial under helium gas at room temperature. A 500-μl volume of BSA [N,O-bis(trimethylsilyl)acetamide; purum] (Fluka Chemie, Buchs, Switzerland) in pyridine (1:1, v/v) was added to the residue, and the silylation reaction was performed over 24 h at 60°C. The silylated metabolites were analyzed directly after derivatization.

2.3. Gas chromatography–mass spectrometry

For the separation of the underivatized α-pinene metabolites, we used a Hewlett-Packard Model 5890 gas chromatograph (Hewlett-Packard, Amsterdam, Netherlands) equipped with a SP-1000 capillary column (25 m × 0.32 mm I.D., 0.15 μm phase layer thickness). Helium was used as the carrier gas at an inlet pressure of 7 bar, and the following temperature programme was used; 60°C for 2 min, 6°C min^{-1} to 200°C and finally 15 min at 200°C. Separation of the trimethylsilylated substances was performed on a 30 m × 0.32 mm I.D. SE-30 capillary column (0.25 μm phase thickness) with helium as the carrier gas (inlet pressure 7 bar). The temperature programme was as follows: 40°C for 2 min, 3°C min^{-1} to 200°C and at 200°C for 15 min.

The capillary columns were connected to a JEOL double-focusing mass spectrometer JMS-SX102 and it worked under the following conditions:

Electron impact

The inlet temperature of the direct GC–MS interface was 250°C and the temperature in the ion source was 200°C. The scan range was 50–300 m.u., scan slope 1.0, resolving power 1000, acceleration voltage 10.0 kV DAC^{-1} and cycle time 0.8 s.

Chemical ionization

The conditions were the same as those used during electron impact (EI) ionization except that the scan

range used was 50–400 m.u., and the temperature in the ion source was 180°C.

3. Results and discussion

3.1. Determination of the molecular mass of the metabolites of α-pinene

A gas chromatogram from the analysis of a urine sample from an α-pinene chamber-exposed individual is illustrated in Fig. 1. The previously identified verbenol isomers are marked in the chromatogram. The determination of the molecular mass of the three additional metabolites, marked 1, 2 and 3, respectively, in Fig. 1, was of primary interest to us. None of these components were detected in gas chromatograms of urine samples from unexposed individuals, but, as stated in the Introduction, were confirmed in urine samples from workers exposed to sawing fumes from pine during a work shift.

The metabolites were subjected to mass spectrometric analysis in the EI mode at 70 eV. The resultant spectra of the α-pinene metabolites are shown in Fig. 2, Fig. 3a and Fig. 4a, respectively. Each one of these spectra contains fragment ions at *m/z* 79, 93, 107 and 121 (C_nH_{2n}–5), indicating that the substances are terpenes or terpene derivatives [11]. They also show an ion of relatively low abundance at 168, 168 and 166 respectively, possibly representing the molecular ion $[M]^+$ of the individual substances. The ions may, however, correspond to a fragment ion resulting from the loss of a neutral fragment from the molecular ion, which itself may be absent in the spectra, due to its instability under the experimental conditions used. Electron impact studies of terpenes and terpene derivatives have been shown to produce spectra with no molecular ion or a molecular ion of low abundance [12].

Chemical ionization (CI), with isobutane or ammonia as the reagent gas, have earlier been used to determine the molecular mass and to investigate the ionization and fragmentation behaviour of mono- and sesqui-terpene derivatives such as alchols and esters. Depending on the proton affinity (PA) of the terpene derivative either a protonated molecule or a collision stabilized ion was produced [13–20].

The PA values of our compounds were not known

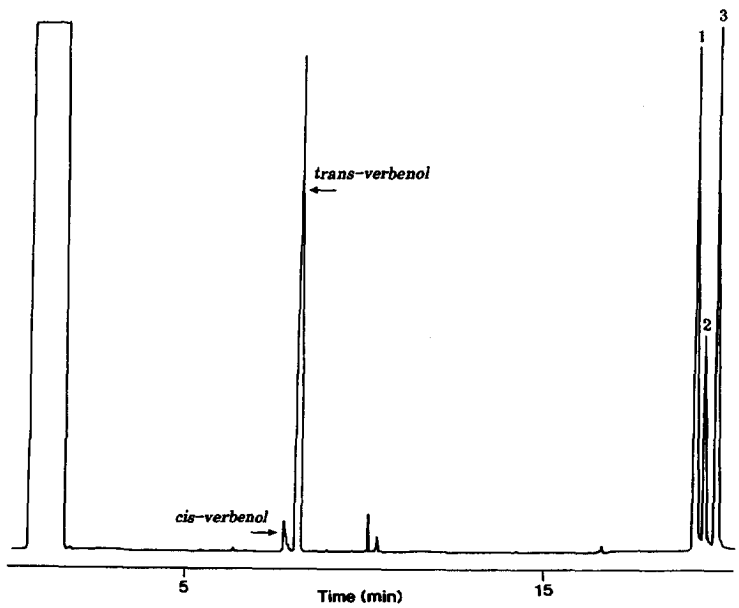

Fig. 1. GC analyses of a urine sample from an α-pinene-exposed individual. Peaks 1, 2 and 3 represent the metabolites identified in this paper.

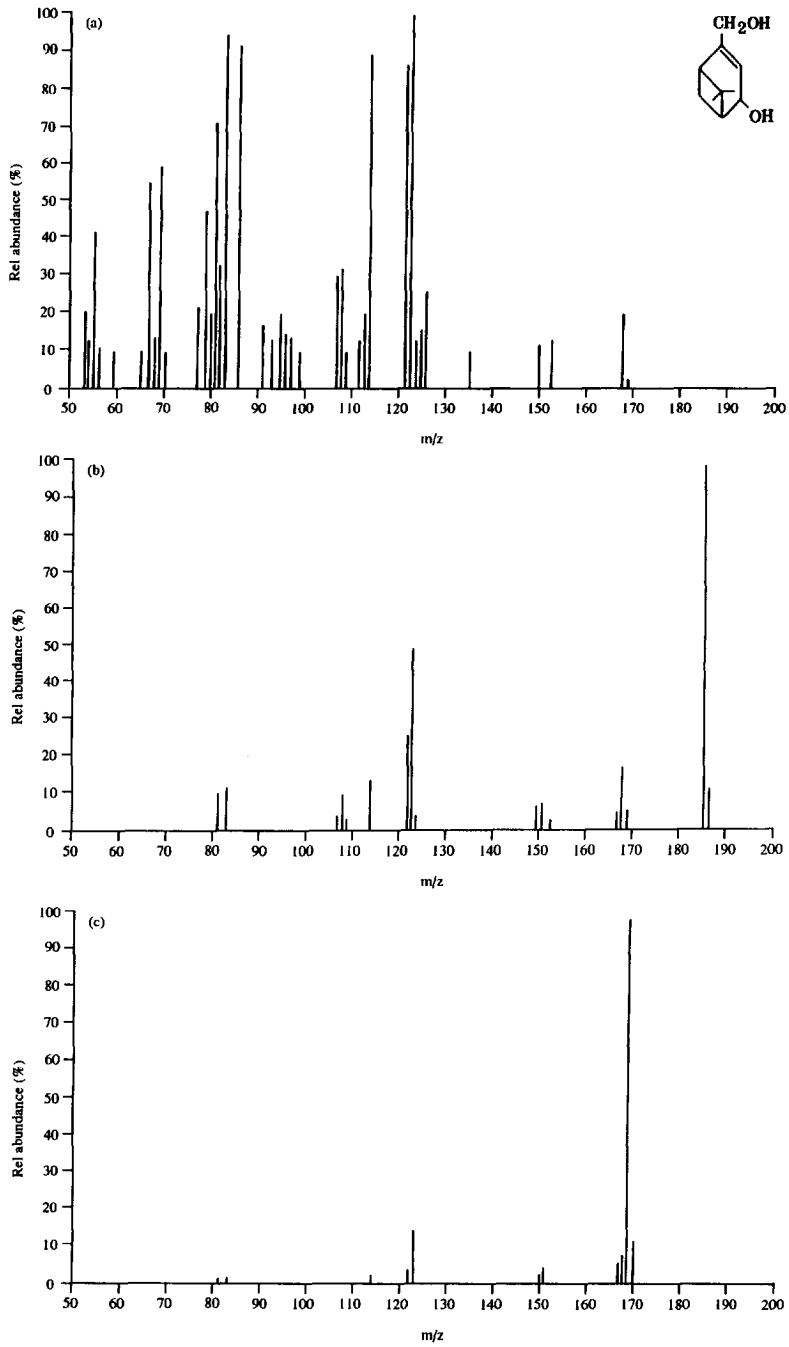

Fig. 2. (a) EI mass spectrum, (b) CI (NH_3) mass spectrum and (c) CI (isobutane) mass spectrum of metabolite 1.

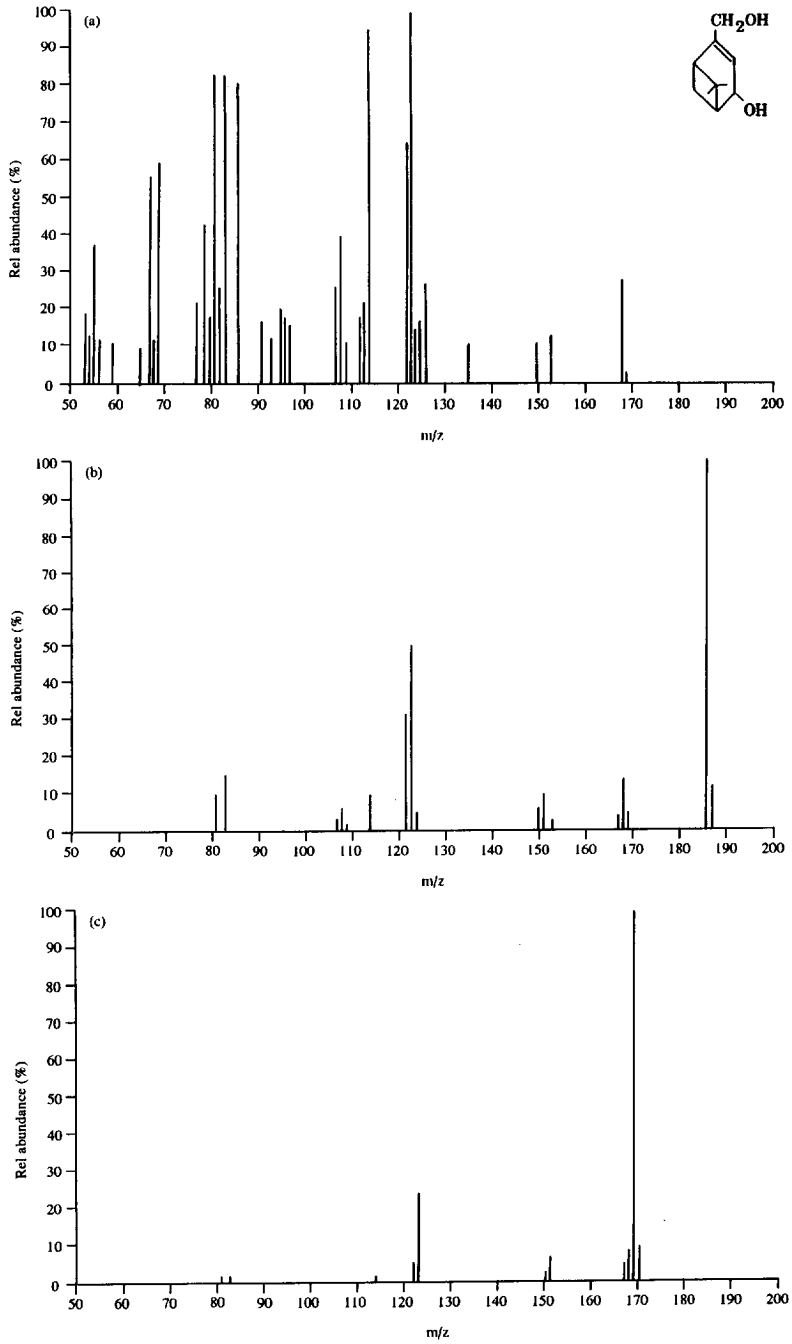

Fig. 3. (a) EI mass spectrum, (b) CI (NH_3) mass spectrum and (c) CI (isobutane) mass spectrum of metabolite 2.

to us, but we expected them to have PA values in the same range as the terpene derivatives investigated recently [13]. Thus, we decided to use isobutane or ammonia as the reagent gas for the determination of the molecular mass of the metabolites. The ammonia CI spectra of the α-pinene metabolites show base

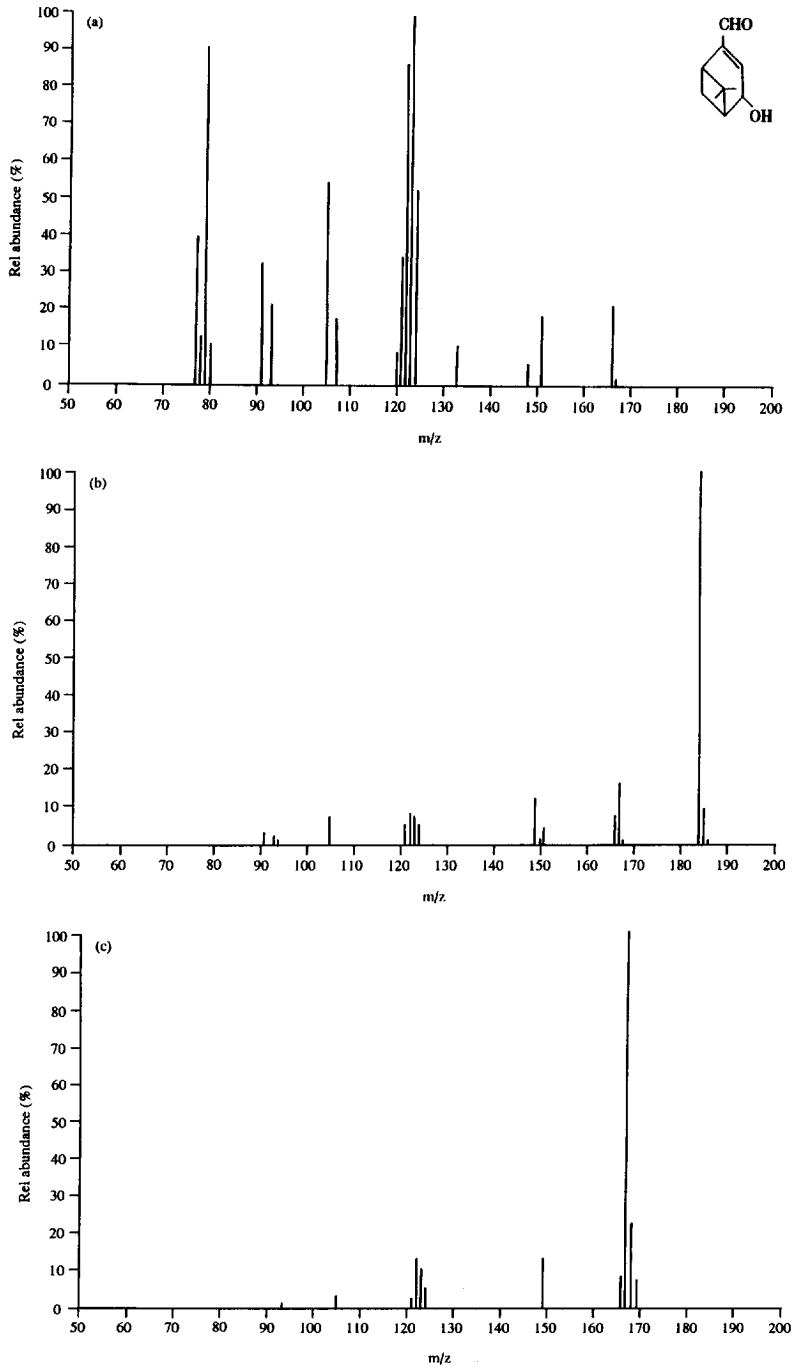

Fig. 4. (a) EI mass spectrum, (b) CI (NH_3) mass spectrum and (c) CI (isobutane) mass spectrum of metabolite 3.

peaks at 186, 186 and 184, respectively (Fig. 2b, Fig. 3b, Fig. 4b), corresponding to a collision stabilized ion of $[M+NH_4]^+$.

The CI i-C_4H_{10} spectra show base peaks at 169, 169 and 167, respectively which are shown in Fig. 2c, Fig. 3c and Fig. 4c. This ion corresponds to a protonated molecule $[M+H]^+$.

In conclusion, the results from the CI determinations show that the base peaks of 168, 168 and 166 in the EI spectra of the metabolites represent the molecular ion $[M]^+$.

3.2. Interpretation of electron impact spectra

The spectral pattern of the substances produced during the electron impact mode indicates that the metabolites undergo substantial rearrangements during the ionization process. Below, some fragmentation pathways producing some of the ions of the stereo-isomeric diols and the alcohol–aldehyde metabolite are discussed (Fig. 5).

Hydrogen transfer to the saturated oxygen atom of a hydroxyl group, and the elimination of a neutral molecule of water, gives ion *m/z* 150 and *m/z* 148, respectively. Elimination of a neutral CH_3 fragment from *m/z* 150 and *m/z* 148 produces ion *m/z* 135 and *m/z* 133, respectively.

Elimination of a $CH_3^{\cdot}$ radical from $[M]^+$ yields ion *m/z* 153 and *m/z* 151, respectively. The opening of the four-membered ring produces ion **a**. A reaction of *cis*-verbenol following electron bombardment, that produces a similar ion has been suggested earlier [21]. A hydrogen rearrangement (rH) within this ion produces ion **b**.

The loss of a neutral molecule of water from ion **a** and the elimination of a neutral C_3H_6 fragment produces the fragment ion *m/z* 108 and *m/z* 106, respectively. The loss of a neutral C_3H_6 fragment produces the fragment ion *m/z* 126 and *m/z* 124, respectively.

The fragment ions *m/z* 107 and *m/z* 105, *m/z* 125 and *m/z* 123 are produced from ion **b** in a similar way but with a loss of a neutral fragment of C_3H_7 instead of a C_3H_6 fragment.

The fragment ion *m/z* 123 and *m/z* 121 respectively, may result from ion **a** by the loss of a neutral molecule of water, a methyl shift of one of the CH_3 groups of the isopropyl group to a carbon atom

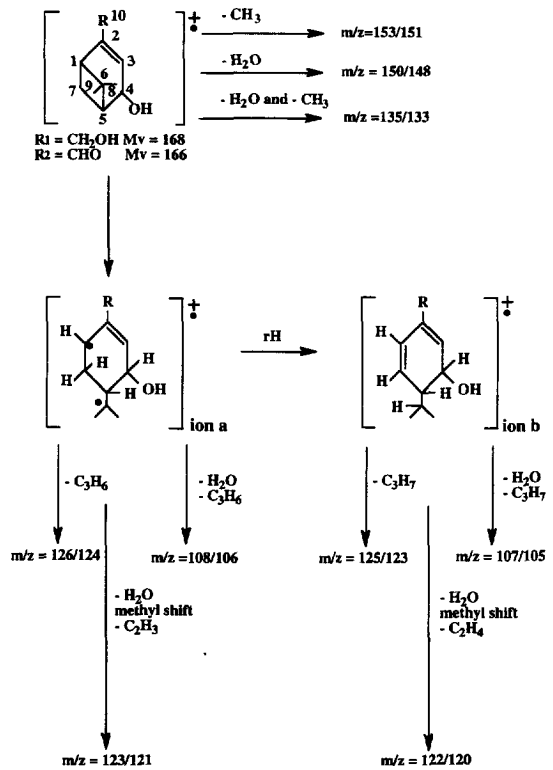

Fig. 5. Proposed EI fragmentation pathways of the diols and the alcohol–aldehyde metabolite.

within the six membered ring and with the elimination of a neutral C_2H_3 fragment. In a similar way, the fragment ion *m/z* 122 and *m/z* 120, respectively, from ion **b** are explained, but with a loss of a C_2H_4 fragment instead of a C_2H_3 fragment. Ion *m/z* 124 and *m/z* 122 may be explained by a loss of a hydrogen from ion *m/z* 125 and *m/z* 123, respectively. Elimination of the CH_2OH and the CHO group from ion 123 and 121 respectively, followed by the loss of a hydrogen, produces *m/z* 91 which probably is the tropylium ion $[C_7H_7]^+$.

Differentiation of the previously identified *cis*- and *trans*-isomers of verbenol under EI conditions was not permitted because of the extreme similarity between the spectral pattern of these substances. Comparison of the retention times of standard substances with the retention indices of the metabolites, at the GC conditions used, made it possible to distinguish the verbenols from each other [1]. The spectral pattern of the diols following EI is also very

similar, indicating that the substances are *cis/trans*-isomers. Standard substances of these metabolites have not been available. Thus, we have not been able to distinguish the stereoisomers of the diols from each other by using the retention indices of the metabolites.

During the mass spectrometric determinations of the urine samples, we could not detect any other substance with a spectral pattern similar to the alcohol–aldehyde metabolite, indicating that this substance does not exist as a *cis/trans*-isomeric metabolite in humans. Steric hindrance within one of the stereo-isomers of the diol, suggested to be the precursor of the alcohol–aldehyde metabolite, is one possible explanation.

Three different terpene diols and their corresponding glucosides have been identified in extracts of nettle roots (*Urtica dioica*) by GC–MS [22]. One of the compounds identified, with a molecular mass of 168, was a diol with α-pinene as the suggested precursor. The hydroxyl groups of the metabolite were suggested to be positioned at carbon atoms number 9 and 10, respectively. (See Fig. 5 for the American Chemical Society numbering of the basic carbon skeleton of the monoterpene [23].) This is also a possible structure of the terpene diols identified by us. The mass spectrometric determination (EI; 70 eV) of the nettle root metabolite was performed on a double focusing instrument, producing a spectrum different from the EI spectra of the diols identified in this work; for instance, the ions *m/z* 126, 125, 124 and 123, respectively, were not present. Electron impact determinations of identical substances performed on different instruments, but under similar experimental conditions, produce spectra containing the same fragment ions, but the relative abundancies of the ions may differ. Thus, the absence of the ions mentioned above in the EI spectrum of the diol from the nettle root strongly indicates that this substance does not have the same molecular structure as the diols identified by us.

3.3. Chemical ionization spectra of the metabolites

Proton transfer, hydride abstraction, dehydrogenation of a protonated ion molecule, hydroxyl group abstraction, dehydration of $[M+H]^+$, substitution of the hydroxyl group with the corresponding base of the reagent gas are major types of reactions and fragmentations of alcohols under CI using ammonia or isobutane as the reagent gas [17,18]

The ions 168, 168 and 166, respectively, in the isobutane CI spectra of the α-pinene metabolites (Fig. 2c, Fig. 3c, Fig. 4c) are probably the result of a hydride abstraction of the protonated ion. Dehydrogenation of $[M+H]^+$ gives the ion 167 in the diol spectra. The ions 151, 151 and 149, respectively, correspond to a hydroxyl group abstraction of the molecular ion, and/or the dehydration of the protonated molecule. These fragmentations have been shown earlier in chemical ionization of terpene alcohols using isobutane as the reagent gas [13]. Loss of alkenes from ions 151, 151 and 149, respectively, may explain the remaining ions in the isobutane CI spectra of the metabolites.

A proton transfer reaction produces the ion 169, 169 and 167, respectively, and a hydride abstraction yields the ion 167, 167 and 165 respectively, all found in the ammonia CI spectra of the substances (Fig. 2b–4b).

We also found the ion 168, 168 and 166, respectively. This ion can be the $[M]^+$ of the corresponding substance, but could also be the species $[M+NH_4-H_2O]^+$, described as a major fragmentation reaction for various alcohols in CI with isobutane as the reagent gas [18]. When interpretating ammonia CI spectra of different monoterpene alcohols, the corresponding ion was found and, by high resolution mass spectrometry, using linalol as a test compound, it was verified that this peak was exclusively the species $[M+NH_4-H_2O]^+$ [13]. Thus, we suggest that 168, 168 and 166 represents the species $[M+NH_4-H_2O]^+$ in the CI spectra of the α-pinene metabolites.

The ion 151, 151 and 149 respectively, correspond to a hydroxyl group abstraction, the ion 150, 150 and 148 respectively, represents the dehydration of $[M]^+$. The remaining fragment ions in the spectra may be explained by loss of alkenes from $[M]^+$.

Returning to the EI spectra in Fig. 2, Fig. 3a and Fig. 4a we observe the ions 150, 150 and 148 respectively, corresponding to a $[M-18]^+$ ion. The loss of H_2O as a neutral fragment is commonly seen in the EI mass spectra of alcohols but also in higher molecular mass aldehydes, ketones and ethers [11]. Thus, the results from the EI and CI determinations indicate that the three additional metabolites from

α-pinene are *cis/trans*-isomers of a diol and an aldehyde with a hydroxyl group.

Differentiation of stereoisomers of terpene alcohols and terpene acetates using CI, by changing ion source temperature or gas pressure of the reactant gas (i-C_4H_{10}) in the ion source, has been used successfully earlier [13,20]. However, availability of standard substances is needed to perform such studies. We did not have access to standard substances of the metabolites studied, and thus we could not use this technique to distinguish the isomers from each other.

3.4. Electron impact and chemical ionization spectra of derivatized metabolites

By silylating hydroxyl group(s), it is possible to use the increase in molecular mass to obtain information about the number of hydroxyl groups present in the molecule of interest. We silylated the metabolites using N,O-bis(trimethylsilyl)acetamide (BSA) and pyridine (1:1, v/v) and subjected the derivatives to EI.

The spectra show an ion, possibly the molecular ion, at *m/z* 240, 240 and 238, respectively (Fig. 6, Fig. 7a and Fig. 8a). In EI spectra of compounds with two or more trimethylsilylated groups, the ion *m/z* 147 ($[(CH_3)_2Si{=}O{-}Si(CH_3)_3]^+$), is often present [24]. This ion could not be found in the EI spectra of the diols or the alcohol–aldehyde metabolite, thus indicating that only one of the hydroxyl groups in the diols is silylated. Steric hindrance is one possible explanation for this. Electron impact of trimethylsilyl derivatives often produces a parent ion of low abundance, but in addition, the loss of a neutral CH_3-fragment producing a $[M-15]^+$ ion, often of higher abundance than the parent ion, is commonly seen in the EI spectra produced [24]. In

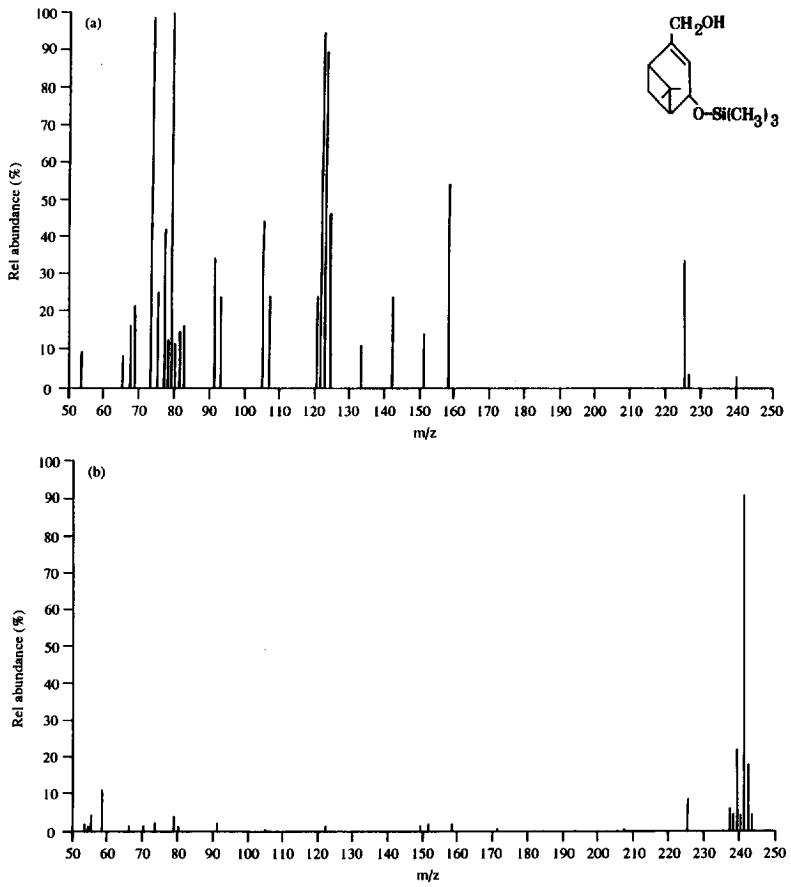

Fig. 6. (a) EI mass spectrum and (b) CI (isobutane) mass spectrum of the trimethylsilylated derivative of metabolite 1.

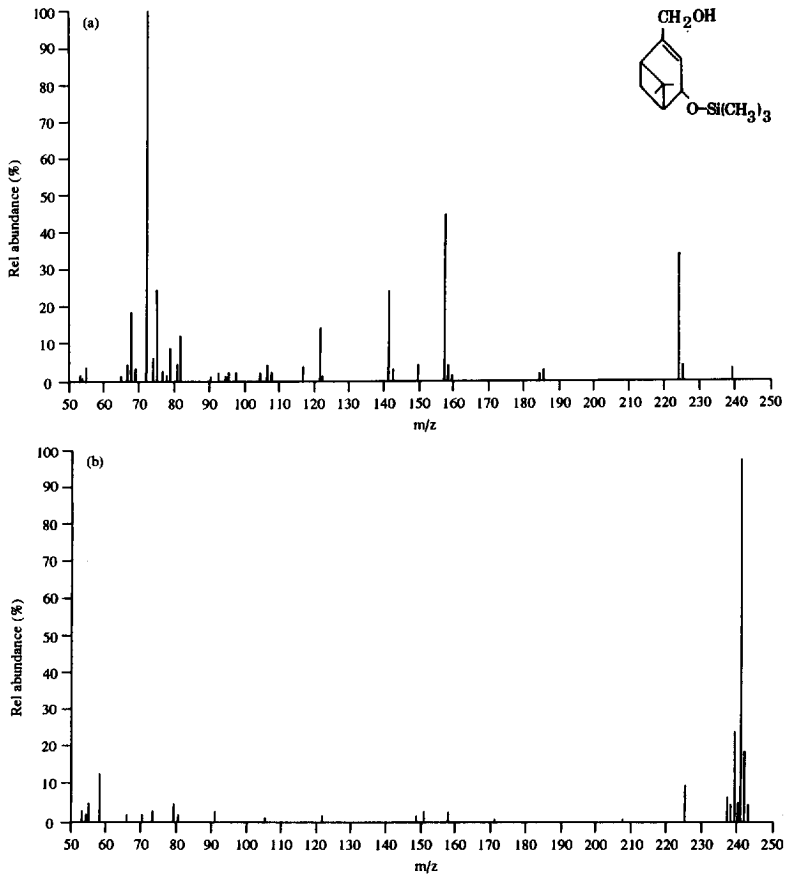

Fig. 7. (a) EI mass spectrum and (b) CI (isobutane) mass spectrum of the trimethylsilylated derivative of metabolite 2.

the EI spectra of the diols the ion m/z = $[225]^+$ and m/z = $[223]^+$ in the alcohol–aldehyde metabolite corresponds to a $[M-15]^+$ ion indicating that the parent ion of 240, 240 and 238 represents the molecular ion $[M]^+$.

To determine the molecular mass of the derivatized metabolites we subjected the metabolites to CI and used i-C_4H_{10} as the reagent gas. The resulting spectra are shown in Fig. 6b, Fig. 7b and Fig. 8b. The base peak is m/z = $[241]^+$ in the diols and m/z = $[239]^+$ in the alcohol–aldehyde metabolite, corresponding to a protonated molecule, indicating that the ion of 240, 240 and 238, present in the EI spectra represents the molecular ion of the derivatives.

A hydroxyl group, present in substances such as alcohols, directs the loss of water following electron bombardment. Silylation of a compound with a hydroxyl group forms an ether, and the loss of water following EI is thus suppressed. The absence of ion m/z = $[220]^+$ ($[M-18]^+$) in the spectrum of the alcohol–aldehyde metabolite strongly indicates that the hydroxyl group of the alcohol–aldehyde metabolite is masked by the derivatization reaction. The absence of the corresponding $[M-18]^+$ ion (m/z = $[222]^+$) in the spectra of the diols indicates that the hydroxyl group silylated in these metabolites directs the loss of water following electron impact of the underivatized compound. In nearly every EI spectra of trimethylsilylated compounds, the ion m/z 73 $[(CH_3)_3Si]^+$ (often the base peak) together with m/z 75 $[HO{=}Si(CH_3)_2]^+$ is present [24]. These species are observed in the EI spectra of the silylated α-pinene metabolites, confirming that silylation of a hydroxyl group has been performed.

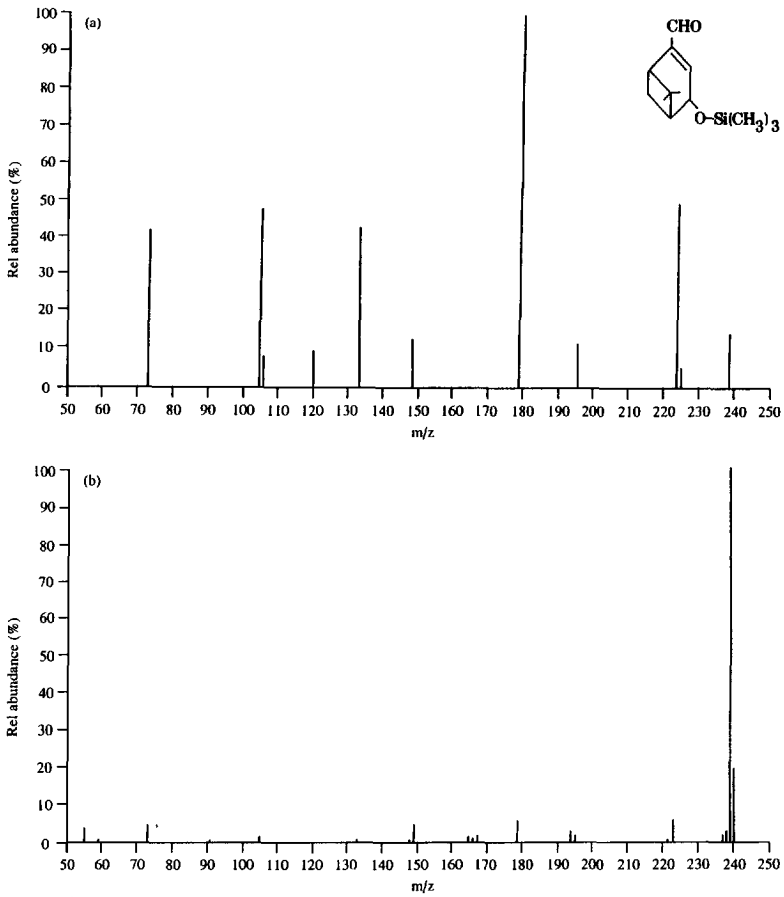

Fig. 8. (a) EI mass spectrum and (b) CI (isobutane) mass spectrum of the trimethylsilylated derivative of metabolite 3.

In the work by Kraus and Spiteller [22], the diol from the root extract was silylated using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), a silylation reagent of similar silylating power to BSA [25]. The EI spectrum of the silylated compound showed an ion at m/z = $[147]^+$, probably of the structure ($[(CH_3)_2Si{=}O{-}Si(CH_3)_3]^+$). The steric hindrance within the terpene diol identified in the root extract, is probably less than within the diol molecule identified by us, thus producing a molecule with two $(CH_3)_3SiO$ groups after silylation. This further indicates that the diol identified in urine samples from exposed saw mill workers, and from α-pinene chamber-exposed individuals, is not identical to the diol identified in root extracts from the stinging nettle.

The EI spectra of the silylated diols show a fragment ion at m/z 166 (Fig. 6 and Fig. 7a). This ion is suggested to be the precursor to the fragment ions m/z 151, m/z 148, m/z 133, m/z 124, m/z 123, m/z 122, m/z 121, m/z 106 and m/z 105, respectively. The precursor and the fragment ions are, in our opinion, produced in the following way:

A neutral fragment of mass 73 $[Si(CH_3)_3]$ is eliminated from the molecular ion $[M]^+$, a McLafferty rearrangement of a hydrogen to the oxygen atom and the elimination of a hydrogen produces the ion m/z 166. Similar to the fragmentation pathway of the underivatized alcohol–aldehyde metabolite (Fig. 5), this fragment ion may exist in two different configurations. The suggested fragmentation pathway yielding m/z=166 is shown in detail in Fig. 9. Thus, the fragment ions m/z 151, 148, 133, 124, 123, 122, 121, 120, 106 and 105 are produced from ion m/z =

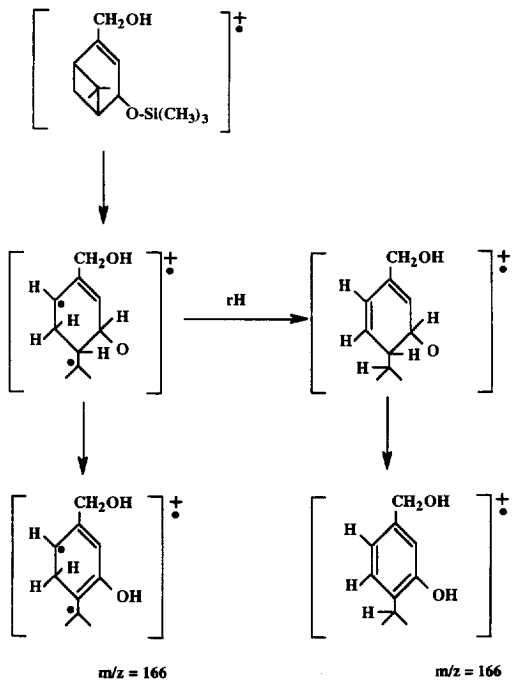

Fig. 9. Suggested fragmentations of the silylated diol yielding fragment ion m/z 166. From this fragment ion m/z 151, 148, 133, 124, 123, 122, 121, 106 and 105 are produced.

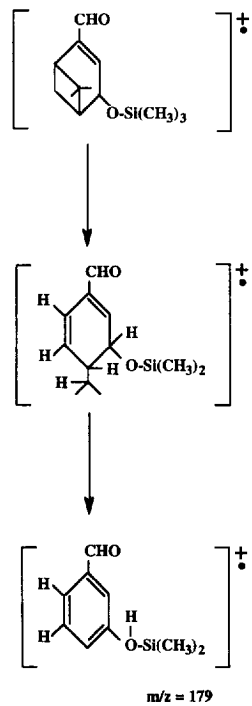

Fig. 10. Fragmentations of the silylated alcohol–aldehyde metabolite producing the ion of the highest relative abundance in the EI spectrum.

$[166]^+$ by a fragmentation pathway similar to that of the underivatized alcohol–aldehyde metabolite discussed above.

The silylated alcohol–aldehyde metabolite spectrum shows a base peak at m/z = $[179]^+$ (Fig. 8a). This fragment ion is suggested to have been produced from the molecular ion $[M]^+$ by the loss of a neutral CH_3 fragment from the $(CH_3)_3SiO$ group, a McLafferty rearrangement of a hydrogen atom to the oxygen atom of the $O–Si(CH_3)_2$ group, the loss of a hydrogen atom and the loss of a neutral C_3H_7 fragment. The suggested fragmentations are shown in Fig. 10. The fragment ion m/z 148 is produced by a loss of the neutral fragments CH_3 and $HO=Si(CH_3)_2$ from the molecular ion $[M]^+$. Ion m/z 133 is produced by $[M-15-75-15]^+$, ion m/z 106 by $[M-15-75-42\ (C_3H_6)]^+$ and ion m/z 105 by $[M-15-75-43\ (C_3H_7)]^+$, respectively. The fragments C_3H_6 and C_3H_7 are produced in a similar way to the fragmentations shown in Fig. 6 and Fig. 9, respectively.

The fragmentation pathways of the underivatized diols and the alcohol–aldehyde metabolite under electron impact conditions are similar (Fig. 5). Introduction of a trimethylsilyl group to these substances produces electron impact spectra which are different from each other. Different functional groups, within molecules of similar structure, often show different spectral patterns following electron impact [11]. This is one possible explanation of why the trimethylsilylated metabolites show dissimilar fragmentation pathways, compared to the underivatized metabolites.

4. Conclusions

The electron impact and chemical ionization mass spectrometric determinations of the three metabolites from α-pinene presented in this work show that the molecular masses are 168, 168 and 166, respectively. The molecular mass of the substances correspond to the molecular mass of two diols and an alcohol–

aldehyde metabolite. The diols are suggested to be the result of an hydroxylation of the verbenols, earlier identified by us as metabolites from α-pinene. Oxidation of the primary alcohol group in the diol yields the alcohol–aldehyde metabolite, suggested to have a *trans*-configuration. A proposed metabolic pathway of α-pinene in humans is presented in Fig. 11.

Electron impact (70 eV) of the diols yields spectra with strong similarities, indicating that they, like the verbenols, are *cis*/*trans*-isomers. The alcohol–aldehyde metabolite does not exist as a stereoisomer. The diols are suggested to be the precursor of this metabolite and probably, steric hindrance within one of the stereoisomers inhibits further metabolic conversion of that particular isomer.

Chemical ionization of the compounds using ammonia or isobutane as the reagent gas produces spectra with fragment ions, confirming that the metabolites are two diols and an alcohol–aldehyde.

The suggested configurations of the metabolites are confirmed by the fragmentations of the underivatized and trimethylsilylated substances. Trimethylsilylation of the compounds verifies that the metabolites are hydroxylated compounds.

Saw mill workers handling pine (*Pinus sylvestris*) are exposed to sawing fumes containing the monoterpenes α-pinene, β-pinene and Δ^3-carene. Further studies must be performed to show if quantification of the substances identified can be used to evaluate the personal exposure to sawing fumes or other vapours containing α-pinene during a work shift.

Note. All mass spectra have been redrawn for comparison purposes. The original spectra may be obtained by request.

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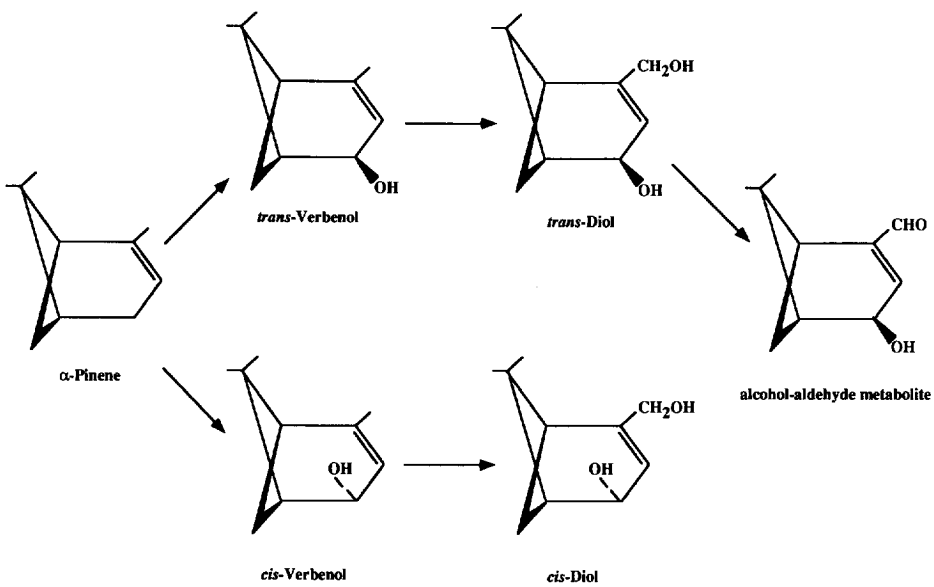

Fig. 11. Suggested metabolic conversion of α-pinene in humans.

nowledged for supplying us with urine samples from chamber-exposed individuals.

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