

# New designer drug *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques

Christoph Sauer,<sup>1</sup> Frank T. Peters,<sup>1</sup> Roland F. Staack,<sup>1</sup> Giselher Fritschi<sup>2</sup> and Hans H. Maurer<sup>1\*</sup>

<sup>1</sup> Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany

<sup>2</sup> Hessisches Landeskriminalamt, D-65187 Wiesbaden, Germany

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Studies are described on the metabolism and toxicological detection of the phencyclidine-derived designer drug *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA) in rat urine using gas chromatographic/mass spectrometric techniques. The identified metabolites indicated that PCEPA was metabolized by *N*-dealkylation, *O*-deethylation partially followed by oxidation of the resulting alcohol to the corresponding carboxylic acid, hydroxylation of the cyclohexyl ring at different positions of PCEPA, *N*-dealkyl PCEPA, *O*-deethyl PCEPA, and of the corresponding carboxylic acids. Finally, aromatic hydroxylation of PCEPA, the corresponding carboxylic acids, and *O*-deethyl PCEPA, the latter partially followed by oxidation to the corresponding carboxylic acid and hydroxylation of the cyclohexyl ring could be observed. All metabolites were partially excreted in the conjugated form. The authors' systematic toxicological analysis (STA) procedure using full-scan GC/MS after acid hydrolysis, liquid-liquid extraction, and microwave-assisted acetylation allowed the detection in rat urine of an intake of a common drug users' dose of PCEPA. Assuming a similar metabolism in humans, the STA in human urine should be suitable as proof of intake of PCEPA. Copyright © 2006 John Wiley & Sons, Ltd.

**KEYWORDS:** *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine; PCEPA; metabolism; GC/MS; urinalysis

## INTRODUCTION

In the late 1990s, a considerable number of new synthetic drugs from various (new) drug classes were seized in the German federal state of Hesse and the surrounding federal states. One of these substances was *N*-(1-phenylcyclohexyl)-propanamine (PCPr), a phencyclidine-derived compound. In a short time, further members of this new class of phencyclidine-derived designer drugs appeared in the illicit drug market, namely, *N*-(1-phenylcyclohexyl)-3-methoxy-propanamine (PCMPA), *N*-(1-phenylcyclohexyl)-2-methoxy-ethanamine (PCMEA), and *N*-(1-phenylcyclohexyl)-2-ethoxyethanamine (PCEEA). The seized preparations contained either one compound alone or in mixtures with other designer drugs.<sup>1</sup> In expectation of its appearance on the illicit drug market, a further homologue, namely, *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine

(PCEPA), was synthesized as a reference substance for scientific purposes.

No information is available on the pharmacological properties of these compounds. However, owing to their structural similarities they might be assumed to be similar to phencyclidine or ketamine, both of which act as antagonists at *N*-methyl-D-aspartate (NMDA) receptors and have psychotomimetic as well as anesthetic properties.<sup>2</sup> Furthermore, it has been reported that (1-phenylcyclohexyl)-amine, a known metabolite of phencyclidine and a possible metabolite of the above-mentioned phencyclidine-derived compounds, produced a long-lasting, dose-dependent effect on the efflux of dopamine in rats.<sup>3</sup> Certainly, such pharmacological profiles would be in line with the abuse of the new phencyclidine-derived compounds as designer drugs. Anticonvulsant activity of (1-phenylcyclohexyl)-amine and some derivatives were reported by Thurkauf *et al.*<sup>4</sup>

At the current state of knowledge, there is no procedure for the detection of such drugs and/or their metabolites in body samples confirming their intake. However, for developing toxicological screening procedures in urine, it is

\*Correspondence to: Hans H. Maurer, Department of Experimental and Clinical Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany. E-mail: hans.maurer@uniklinikum-saarland.de.

a prerequisite to know the metabolism of the compounds in question, especially if they are excreted primarily or even exclusively in form of metabolites. Furthermore, data on the metabolism are needed for toxicological risk assessment, because the metabolites may play a major role in drug toxicity. Therefore, the aim of this study was to study the metabolic pathways of PCEPA using gas chromatography/mass spectrometry (GC/MS) with electron ionization (EI) and positive-ion chemical ionization (PCI). In addition, the detectability of PCEPA and its metabolites within the authors' systematic toxicological analysis (STA) procedure in urine by GC/MS was studied.<sup>5-9</sup>

## EXPERIMENTAL

### Chemicals and reagents

PCEPA HCl was provided by the Hessisches Landeskriminalamt (Wiesbaden, Germany). *N*-Methyl-bis-trifluoroacetamide (MBTFA) and *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA) were obtained from Fluka (Taufkirchen, Germany). Isolute Confirm HXC cartridges were obtained from Separtis (Grenzach-Wyhlen, Germany). All other chemicals and biochemicals were obtained from Merck (Darmstadt, Germany). All chemicals and biochemicals were of analytical grade.

### Urine samples

The investigations were performed using urine of male Wistar rats (Ch. River, Sulzflleck, Germany) for toxicological diagnostic reasons according to the corresponding German law. They were administered as a single 20 mg/kg body mass (BM) dose for metabolism studies or as a single 0.1 mg/kg BM dose for STA studies in aqueous suspension by gastric intubation ( $n = 3$  each). Urine was collected separately from the feces over a 24 h period. All samples were directly analyzed. Blank rat urine samples were collected before drug administration to check whether they were free of interfering compounds.

### Sample preparation for identification of metabolites by GC/MS

A 3-ml portion of urine was adjusted to pH 5.2 with acetic acid (1 mol/l) and incubated at 50 °C for 1.5 h with 150  $\mu$ l of a mixture (100 000 Fishman units per ml) of glucuronidase (EC no. 3.2.1.31) and arylsulfatase (EC no. 3.1.6.1) from *Helix pomatia*. Then the urine sample was loaded on an Isolute Confirm HXC cartridge (130 mg, 3 ml) previously conditioned with 1 ml of methanol and 1 ml of water. After passage of the sample, the cartridge was washed with 1 ml of water, 1 ml of 0.01 M hydrochloric acid, and 2 ml of methanol. Elution was performed using 1 ml of a freshly prepared mixture of methanol/aqueous ammonia (98:2; v/v). The organic layer was carefully evaporated to dryness at 56 °C under a stream of nitrogen. The residue was derivatized by one of the following procedures: Acetylation (AC) was conducted with 100  $\mu$ l of an acetic anhydride-pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 440 W. After careful evaporation of the excess reagent, the residue was dissolved in 100  $\mu$ l of methanol.

Trifluoroacetylation (TFA) was conducted with 100  $\mu$ l of MBTFA for 5 min under microwave irradiation at about 440 W. After careful evaporation of the excess reagent, the residue was dissolved in 100  $\mu$ l of ethyl acetate. Aliquots (2  $\mu$ l) of the acetylated or trifluoroacetylated extracts were injected into the GC/MS. Trimethylsilylation (TMS) was conducted with 100  $\mu$ l MSTFA on a heating block (60 °C, 30 min). Then, 2  $\mu$ l of this mixture was directly injected into the GC/MS apparatus without previous evaporation.

The same procedures, with the exception of enzymatic hydrolysis, were used to study whether the metabolites were excreted as glucuronide and/or as sulfate conjugates. Furthermore, 1 ml of blank rat urine and 1 ml of rat urine containing 100  $\mu$ l of a methanolic solution of PCEPA (0.01 mg/ml) were worked-up as described above, and 2  $\mu$ l of each was injected into the GC/MS apparatus in order to detect possible artifacts.

### Sample preparation for STA by GC/MS

A 5-ml portion of urine was worked-up as described for 4-MTA<sup>6</sup> or 2C-T-7.<sup>8</sup> After the cleavage of conjugates by acid hydrolysis and liquid-liquid extraction, the sample was derivatized by AC. Aliquots (2  $\mu$ l) of the derivatized extracts were injected into the GC/MS apparatus.

### GC/MS apparatus for identification of metabolites

The extracts were analyzed using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with an HP 5989B MS Engine mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software, version C03.00. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m  $\times$  0.2 mm i.d.), cross-linked methyl silicone, 330 nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate 1 ml/min; column temperature, programmed from 100 to 310 °C at the rate of 30 °C/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode,  $m/z$  50–800 u; EI mode, ionization energy, 70 eV; PCI mode using methane: ionization energy, 230 eV; ion source temperature, 220 °C; capillary direct interface, heated at 260 °C.

### GC/MS apparatus for STA

The extracts were analyzed using a Hewlett Packard (Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5972A MSD mass spectrometer and a HP MS ChemStation (DOS series) with HP G1034C software version C03.00. The GC conditions were as follows: splitless injection mode; column, HP-1 capillary (12 m  $\times$  0.2 mm i.d.), cross-linked methyl silicone, 330 nm film thickness; injection port temperature, 280 °C; carrier gas, helium; flow-rate 1 ml/min; column temperature, programmed from 100–310 °C at the rate of 30 °C/min, initial time 3 min, final time 8 min. The MS conditions were as follows: full-scan mode,  $m/z$  50–550 u; EI mode, ionization energy, 70 eV; ion source temperature, 220 °C; capillary direct interface, heated at 280 °C.

## GC/MS procedure for identification of metabolites and STA by GC/MS

PCEPA and its metabolites were separated by GC and identified by MS in the derivatized urine extracts. For toxicological detection of PCEPA and its metabolites, mass chromatography was used for extracting characteristic fragment ions from the total ion current. The following ions were used for this purpose:  $m/z$  232, 274, 273, and 290. These ions were selected from the mass spectra recorded during this study. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison of the mass spectra underlying the peaks (after background subtraction) with the recorded reference spectra.<sup>10</sup>

## RESULTS AND DISCUSSION

### Identification of the metabolites

The urinary metabolites of PCEPA were separated by GC and identified by EI MS and PICI MS after gentle enzymatic hydrolysis, extraction, and derivatization. Gentle enzymatic cleavage of conjugates was necessary before extraction in order not to miss conjugated metabolites. The described conjugate cleavage at an elevated temperature (56 °C) for a short period of time (1.5 h) had proven to yield similar results (i.e. same compounds detected at similar abundances) as the authors' standard conditions for metabolism studies (37 °C for 12 h). This is in accordance with other studies.<sup>11,12</sup>

The described mixed-mode solid phase extraction (SPE) had been used successfully in metabolism studies of other designer drugs in the authors' laboratory.<sup>6,11,13–17</sup> It has proved particularly suitable for the extraction of basic and amphoteric metabolites and yields clean extracts, thereby facilitating detection and identification of the metabolites. Regarding the structure of PCEPA, its metabolites were expected to be either basic or amphoteric, so SPE was preferred to the authors' liquid–liquid extraction procedure under alkaline or acidic conditions.<sup>8,18–21</sup>

Derivatization was needed to improve the GC properties of these relatively polar metabolites, thereby increasing the sensitivity of their detection. Acetylation has proved useful in metabolism studies.<sup>8,19,21,22</sup> It can be employed for derivatization of primary and secondary amino groups as well as alcoholic and/or phenolic hydroxy groups. In the authors' experience, the fragmentation patterns of the resulting derivatives in the EI mode are also easy to interpret facilitating the elucidation of metabolite structures.<sup>9,19,23–29</sup> Furthermore, AC is the standard derivatization step in the authors' STA, so for STA it was necessary to have the spectra of the acetylated metabolites. It was observed that under the described conditions the parent drug and its secondary amine metabolites were derivatized only to a minor extent at the amine moiety. Even when the amount of derivatization reagent and the reaction time were increased, the derivatization reaction remained incomplete at this position. This phenomenon might be explained by the steric hindrance from the neighboring phenyl and cyclohexyl rings and from the side chain. This effect might even be increased by formation of a hydrogen bond between the hydrogen of the secondary amine function and the ether oxygen of the side chain leading to a stable six-membered ring.

A disadvantage of using AC in metabolism studies is the problem that physiologically acetylated metabolites cannot be differentiated from acetyl derivatives. The presence of such physiologically acetylated metabolites was checked in urine extracts after TFA. Finally, TMS was necessary for the derivatization and structural elucidation of metabolites carrying carboxylic acid groups. Alternative methylation or ethylation as described in previously published metabolism studies was not applicable here, because they lead to structurally isomeric derivatives which could not be differentiated via their molecular masses and/or fragmentation patterns.<sup>11,17</sup>

The postulated structures of the (derivatized) metabolites were deduced from the fragments detected in the EI mode, which were interpreted in correlation with the fragmentation pattern of the parent compound as was described by Roesner *et al.*<sup>1</sup> Interpretation of the fragments was performed according to the rules described by e.g. McLafferty and Turecek<sup>30</sup> and Smith and Busch.<sup>31</sup> The EI mass spectra, the retention indices (RI), and the structures and predominant fragmentation patterns of PCEPA and its metabolites after derivatization are shown in Fig. 1. In order to verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded (spectra not shown), because they usually contain abundant peaks of the protonated molecule  $[M + H]^+$  with adduct ions typical for PICI using methane as the reagent gas  $[M + C_2H_5^+]$ ,  $[M + C_3H_5^+]$ . While all the recorded PICI mass spectra contained the respective protonated molecular ions at considerable abundances, the typical adduct ions were absent in about one-third of them (nos 1, 3–6, 8–13, 15, and 26–31). Possible explanations for this finding will be discussed below.

In the acetylated urine extract, PCEPA and the acetyl derivatives of the following metabolites (dehydrated compounds are artifacts formed during GC) could be identified. The numbers of EI spectra in Fig. 1 are given in brackets: PCEPA (1), *O*-deethyl PCEPA (2), *O*-deethyl-hydroxyphenyl PCEPA (3), *O*-deethyl-4'-hydroxy PCEPA isomer 1 (4), *O*-deethyl-4'-hydroxy PCEPA isomer 2 (5), *O*-deethyl-3'-hydroxy PCEPA (6), dehydrated *O*-deethyl-4'-hydroxy PCEPA (7), *O*-deethyl-3'-hydroxy-hydroxyphenyl PCEPA (8), *O*-deethyl-4'-hydroxy-hydroxyphenyl PCEPA (9), hydroxyphenyl PCEPA (10), 4'-hydroxy PCEPA isomer 1 (11), 4'-hydroxy PCEPA isomer 2 (12), 3'-hydroxy PCEPA (13), dehydrated 4'-hydroxy PCEPA (14), *N*-dealkyl PCEPA (15), *N*-dealkyl-4'-hydroxy PCEPA isomer 1 (16), *N*-dealkyl-4'-hydroxy PCEPA isomer 2 (17), *N*-dealkyl-3'-hydroxy PCEPA isomer 1 (18), *N*-dealkyl-3'-hydroxy PCEPA isomer 2 (19), dehydrated *N*-dealkyl-4'-hydroxy PCEPA (20), dehydrated carboxy PCEPA (21), dehydrated carboxy-4'-hydroxy PCEPA isomer 1 (22), dehydrated carboxy-4'-hydroxy- PCEPA isomer 2 (23), dehydrated carboxy-3'-hydroxy- PCEPA isomer 1 (24), and dehydrated carboxy-3'-hydroxy- PCEPA isomer 2 (25). In the trimethylsilylated urine extract, PCEPA and the trimethylsilyl derivatives of the following compounds could be additionally identified: carboxy PCEPA (26), carboxy-hydroxyphenyl PCEPA (27), *cis*-carboxy-4'-hydroxy PCEPA (28), *trans*-carboxy-4'-hydroxy PCEPA (29), carboxy-3'-hydroxy PCEPA

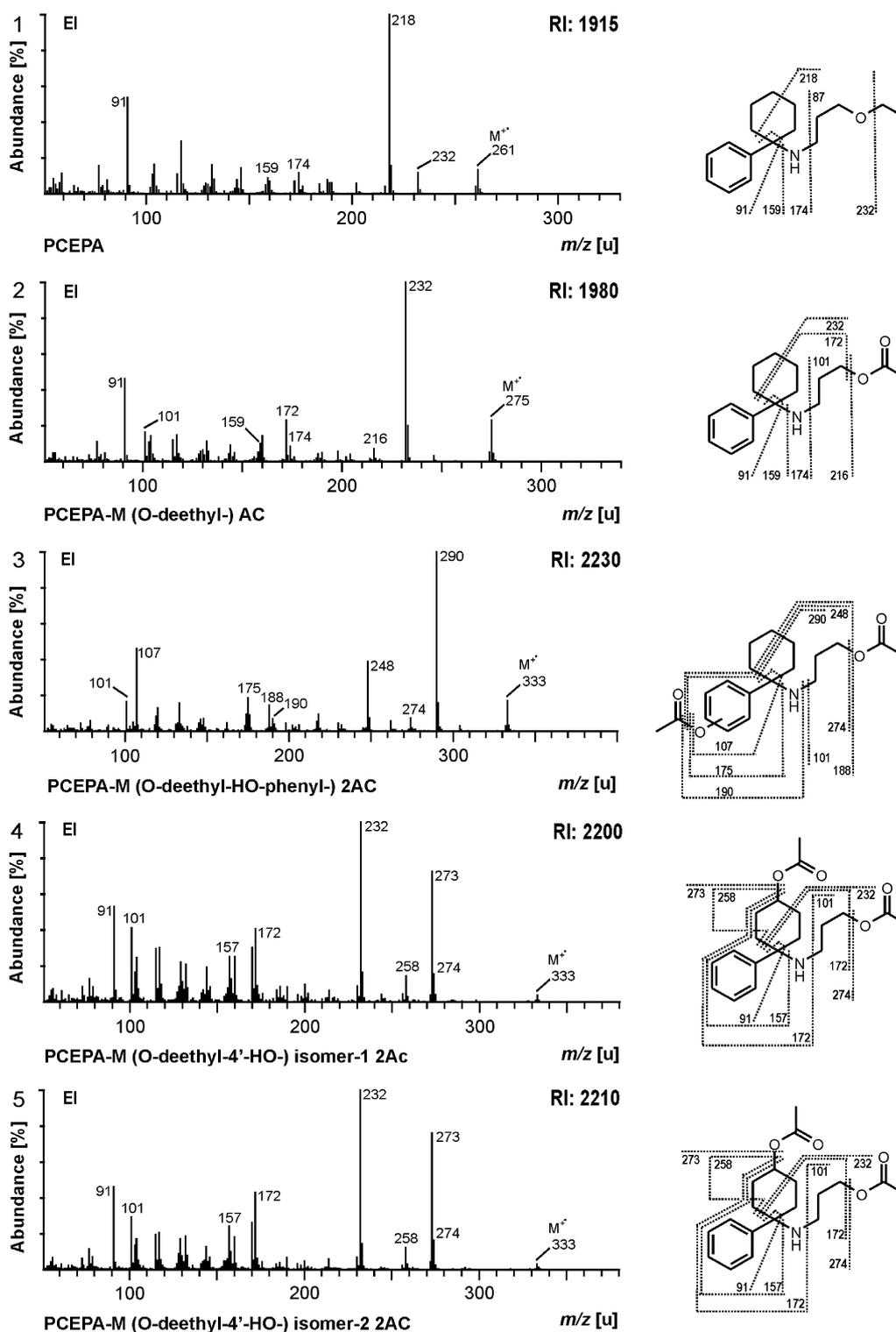


Figure 1. EI mass spectra, RIs, structures, and predominant fragmentation patterns of PCEPA and its metabolites after AC and TMS.

(30), carboxy-4'-hydroxy-hydroxyphenyl PCEPA (31), *cis*-O-deethyl-4'-hydroxy PCEPA (32), and *trans*-O-deethyl-4'-hydroxy PCEPA (33).

**Proposed fragmentation patterns**

In the following, possible fragmentation patterns of the EI mass spectra of PCEPA and its derivatized metabolites will be discussed in relation to the postulated metabolite structures depicted in Fig. 1. The numbers of the corresponding mass

spectra in Fig. 1 are given in brackets. PCEPA (1) and all its metabolites showed a cleavage between the nitrogen atom and the quaternary carbon atom of the phenyl cyclohexyl double ring system as depicted in Fig. 2, patterns A–C, cleavage I. This resulted in a tertiary carbenium ion further stabilized by mesomerism with the phenyl ring. In PCEPA (1) and its metabolites with an unchanged double ring system, the fragment had an *m/z* value of 159 (2, 26) or 158 for those with amide structure (15, 21; for explanation see below).

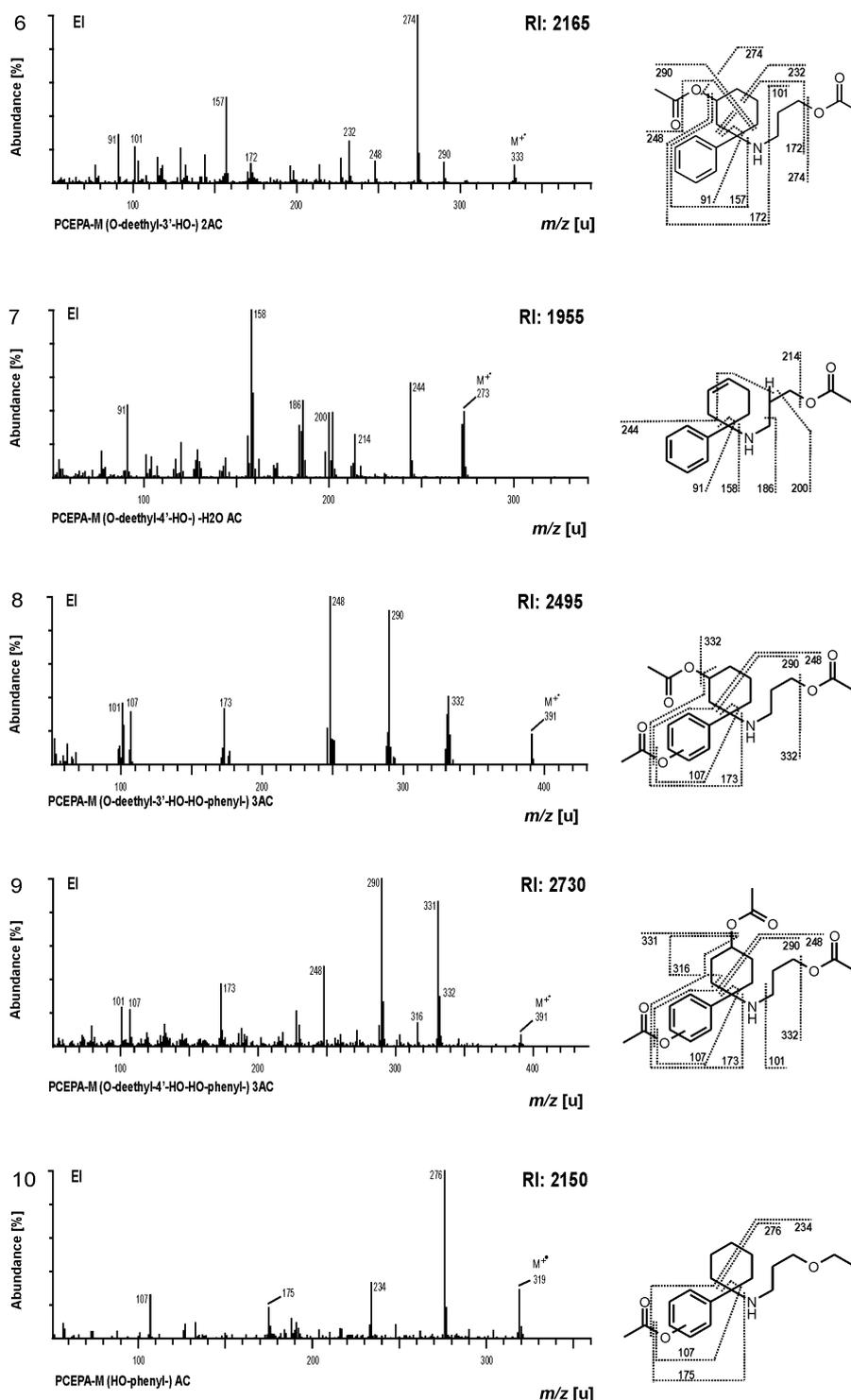


Figure 1. (Continued).

The hydroxyphenyl metabolites (3, 10) showed a respective fragment ion  $m/z$  175 after further neutral loss of the acetyl moiety, while 3'- and 4'-hydroxy metabolites showed a fragment  $m/z$  157 after further elimination of acetic acid (4–6, 11–13, 22–25) or  $m/z$  156 for those with amide structure (16–19; for explanation see below). In addition, a fragment ion  $m/z$  173 was observed for metabolites hydroxylated at both rings (8, 9) after loss of an acetyl moiety and elimination of acetic acid. Another common fragmentation pattern of PCEPA and all its metabolites was a benzyl-like cleavage

(Fig. 2(A)–(C), II) leading to formation of the tropylium ion  $m/z$  91 for all compounds with an unchanged aromatic ring and to a corresponding fragment ion  $m/z$  107 for all hydroxy-phenyl metabolites (3, 8, 9, 10) after the loss of the acetyl moiety. PCEPA (1) and all its metabolites containing a secondary amine but no hydroxy group at the cyclohexyl ring showed a  $\beta$ -cleavage of the cyclohexyl ring followed by rearrangement and loss of 43 u (Fig. 2(A), III) leading to fragment ions  $m/z$  218 (1),  $m/z$  232 (2),  $m/z$  290 (3), and  $m/z$  276 (10), which were always the base peaks of

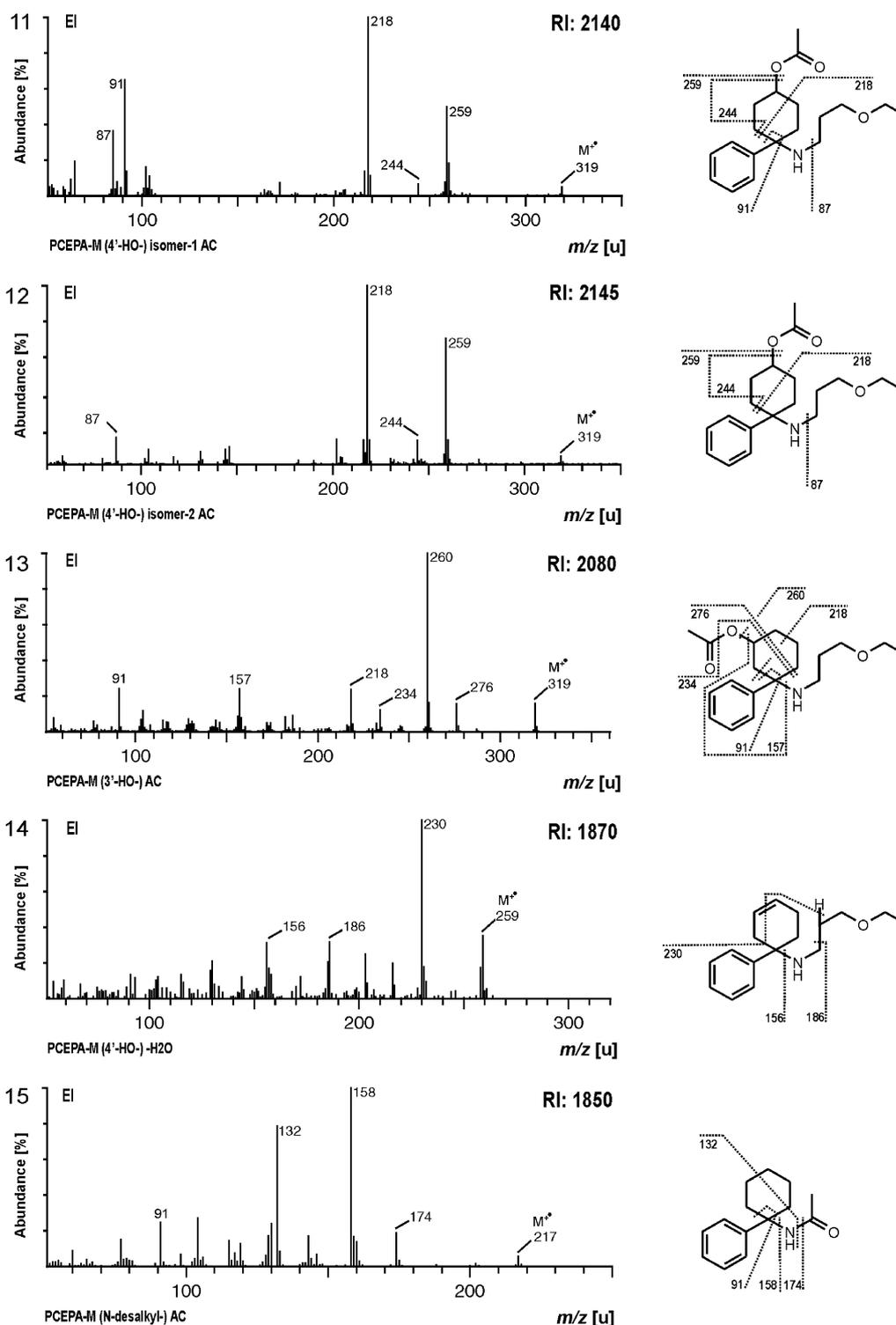


Figure 1. (Continued).

the respective EI mass spectra. The same  $\beta$ -cleavage was observed for the PCEPA metabolites containing a secondary amine and a hydroxylated cyclohexyl ring (Fig. 2(B) and (C), III). However, the neutral losses that followed after this cleavage depended on the position of the hydroxy group. In the case of the 3'-hydroxy metabolites (6, 13), two different positions of the  $\beta$ -cleavage were possible, as depicted in Fig. 2(B) and (C).  $\beta$ -Cleavage between position 1' and 6' and the above-mentioned rearrangement were followed by

two alternative fragmentation patterns. On the one hand, an acetylated enol-immonium cation with  $m/z$  290 (6) or  $m/z$  276 (13) was formed by homolytic cleavage between positions 3' and 4' leading to the above-mentioned loss of 43 u (Fig. 2(B), IV). This could be followed by an  $\alpha$ -cleavage resulting in a neutral loss of the acetyl moiety corresponding to 42 u and formation of an enol-immonium cation  $m/z$  248 (6) or  $m/z$  234 (13) stabilized by mesomerism (Fig. 2(B), V). On the other hand, homolytic cleavage of the carbon–oxygen

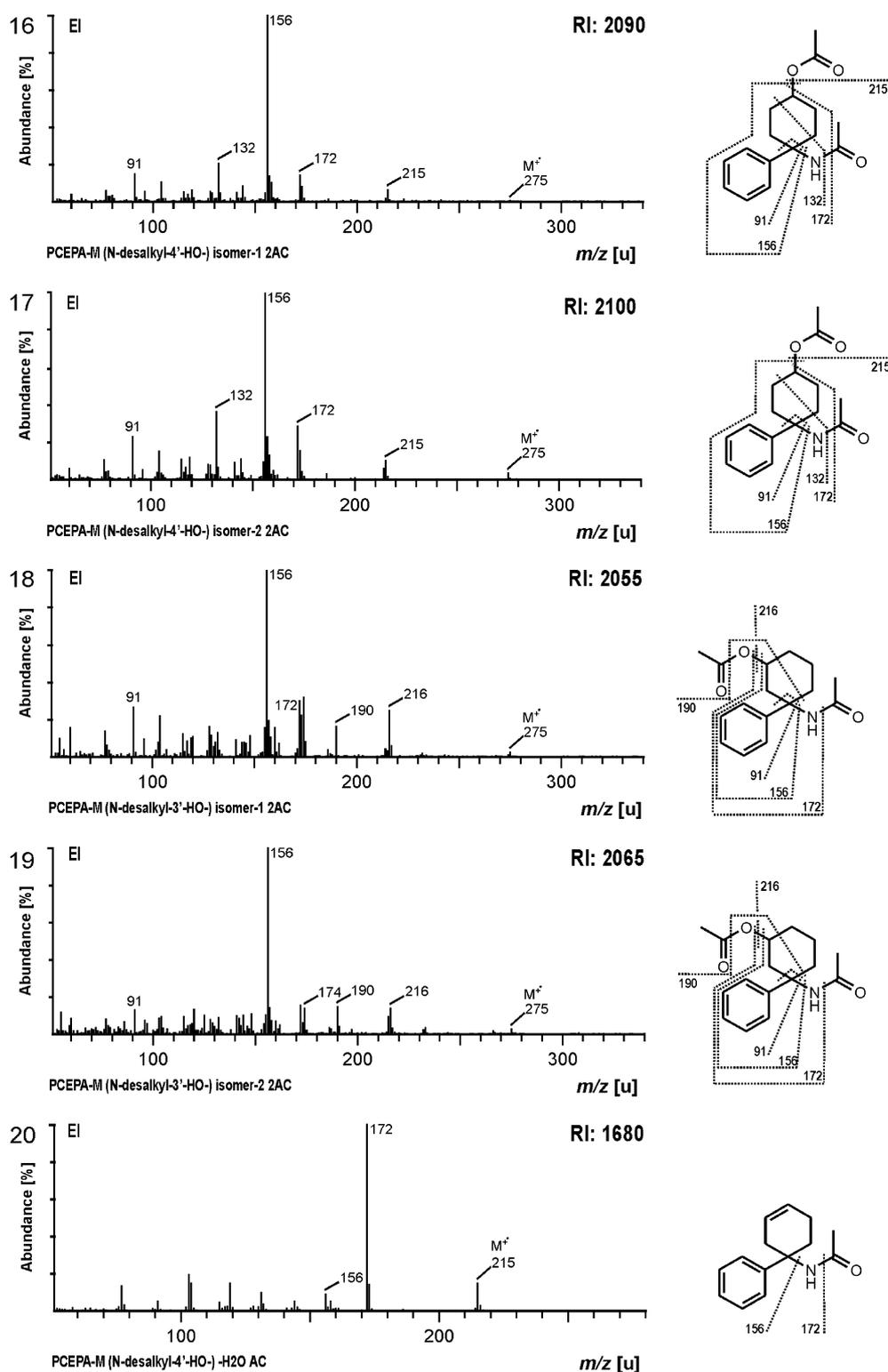


Figure 1. (Continued).

bond in position 3' leads to the loss of an acetyloxy radical corresponding to 59 u (Fig. 2(B), VI) resulting in fragment ions  $m/z$  274 (6) or  $m/z$  260 (13).  $\beta$ -Cleavage between position 1' and 2' (Fig. 2(B), III) leads to the above-mentioned rearrangement followed by a neutral loss of 101 u leading to fragment ions  $m/z$  232 (6) or  $m/z$  218 (13). Alternatively, the fragment ions  $m/z$  274 (6) or  $m/z$  260 (13) were formed by this  $\beta$ -cleavage directly followed by the homolytic cleavage

of the 3'-carbon–oxygen bond accompanied by a neutral loss of 59 u (Fig. 2(B), VII). In case of the 4'-hydroxy metabolites (4, 5, 11, 12),  $\beta$ -cleavage between positions 1' and 2' or between 1' and 6' and the above-mentioned rearrangement were followed by homolytic cleavages between positions 4' and 5' or between 3' and 4', respectively (Fig. 2(C), III). Since both cases led to the neutral loss of an acetoxy propyl radical, only a loss of 101 u and formation of fragment ions  $m/z$  232

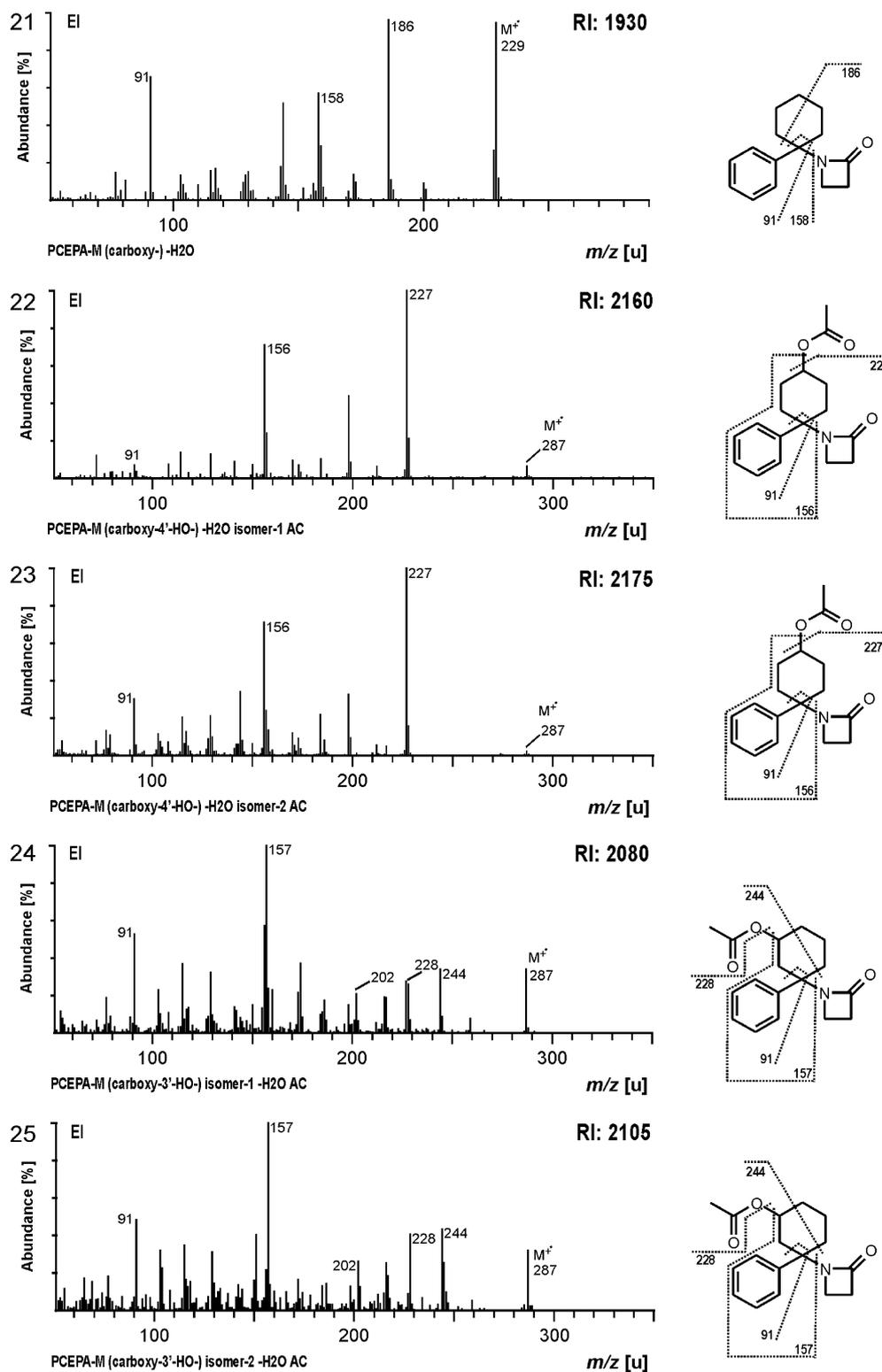


Figure 1. (Continued).

(4, 5) or  $m/z$  218 (11, 12) were observed. This was in contrast to the 3'-hydroxy metabolites, which additionally showed a neutral loss of 43 u, as described in the previous paragraph. Another important fragmentation pattern of the 4'-hydroxy metabolites was the elimination of acetic acid resulting in a loss of 60 u (Fig. 2(C), VIII) and formation of the fragment ions  $m/z$  273 (4, 5) or  $m/z$  259 (11, 12) with a double bond in the cyclohexyl ring. As has been described in the literature

for other cyclohexenyl rings,<sup>32,33</sup> this was followed by the loss of a methyl radical from the cyclohexyl ring (Fig. 2(C), IX), leading to the highly conjugated fragment ions  $m/z$  258 (4, 5) or  $m/z$  244 (11, 12). The latter two fragmentation patterns were not observed for the 3'-hydroxy metabolites, which instead showed a loss of an acetyloxyl radical of 59 u. This was followed by stabilization of the resulting carbenium ions under opening of the cyclohexyl ring and

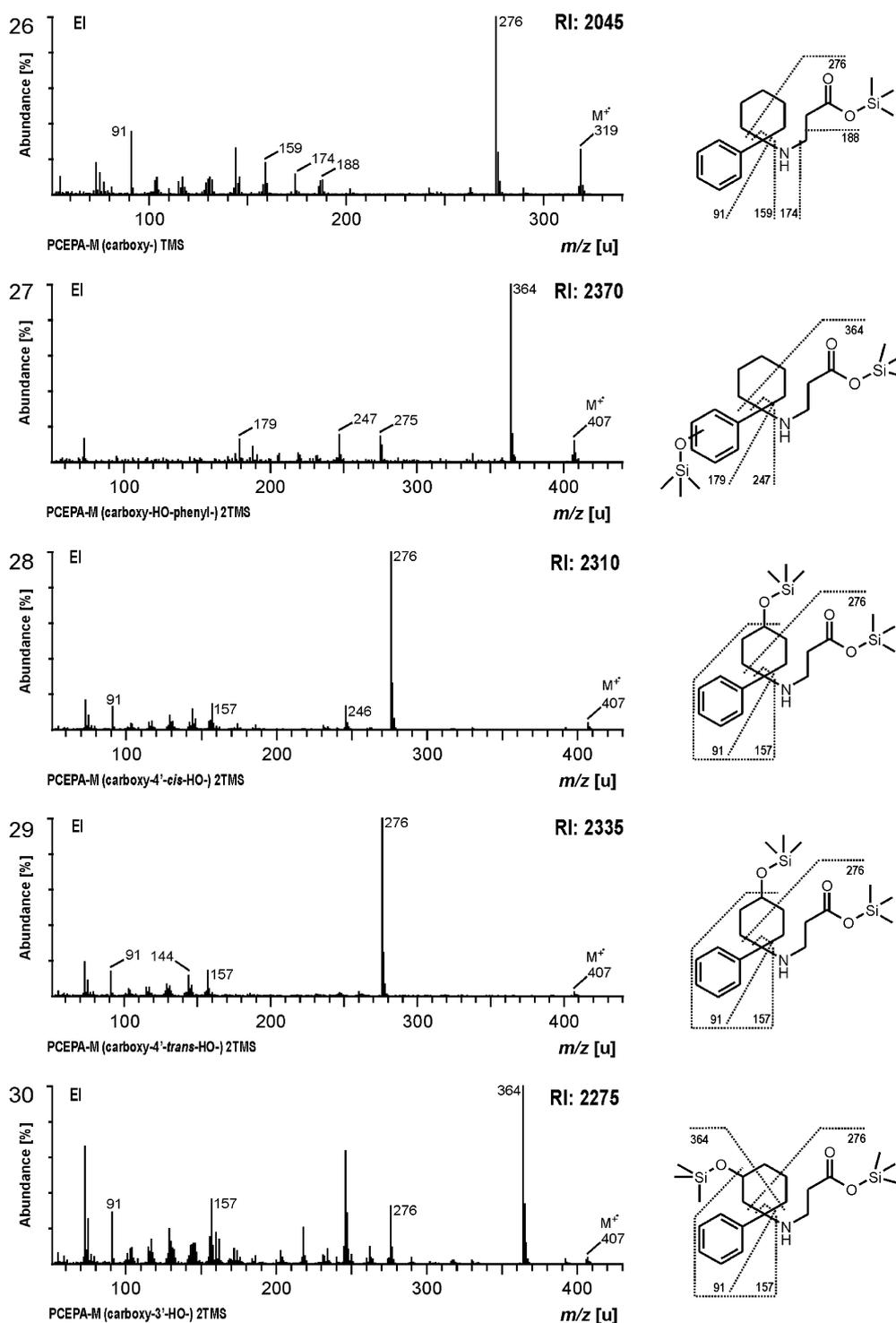


Figure 1. (Continued).

formation of immonium ions  $m/z$  274 (6) or  $m/z$  260 (13) (Fig. 2(B), VII), which are identical to those formed by  $\beta$ -cleavage and direct loss of an acetyloxyl radical as described in the previous paragraph. As depicted in Figs 3 and 4, respectively, the described differences in the fragmentation of the 3'- and 4'-hydroxylated metabolites allowed their differentiation and structural assignment by interpretation of the respective EI mass spectra. A similar differentiation of isomeric hydroxycyclohexyl metabolites has already been described for phencyclidine.<sup>34,35</sup>

PCEPA (1) and all its metabolites with an unchanged (10–13) or *O*-deethylated (2–6, 8, 9) side chain showed a cleavage between the nitrogen atom and position 1 of the side chain. The resulting fragment ions have an  $m/z$  value of 87 representing the unchanged side chain and  $m/z$  101 representing the *O*-deethylated side chain. The fragment ions representing the other part of the molecule (Fig. 2(A)–(C), X) were  $m/z$  174 in case of the unchanged double ring systems (1, 2),  $m/z$  190 for hydroxy-phenyl metabolites after loss of the acetyl moiety (3), and  $m/z$  172 for the 3'- or

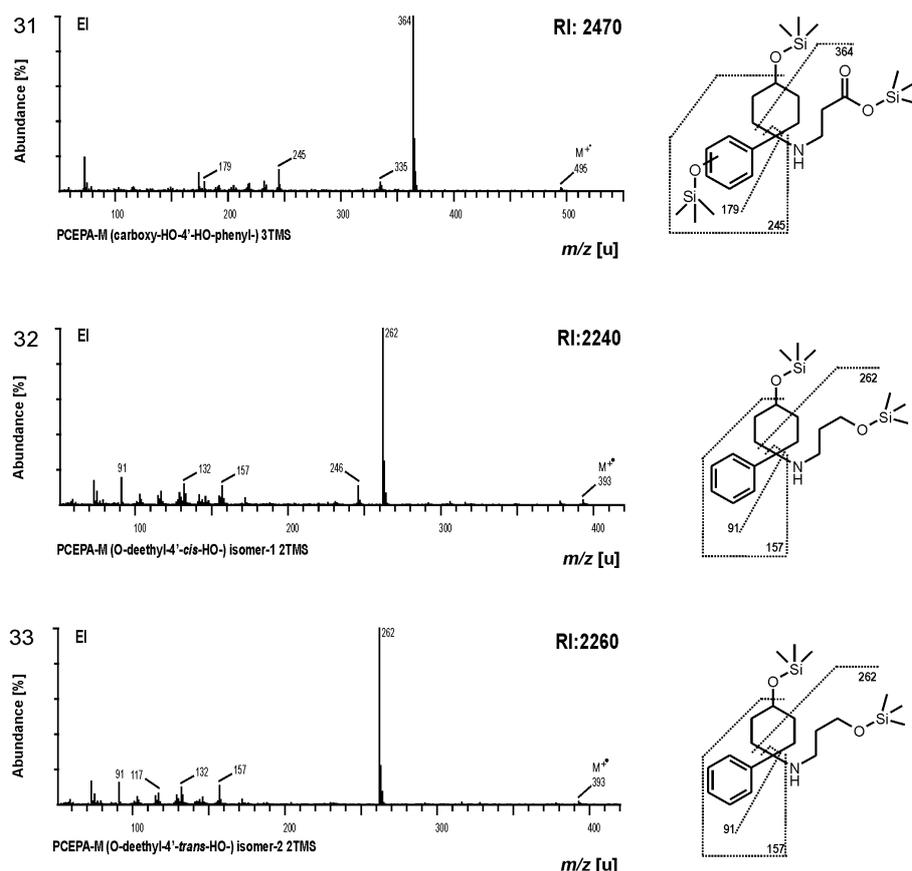


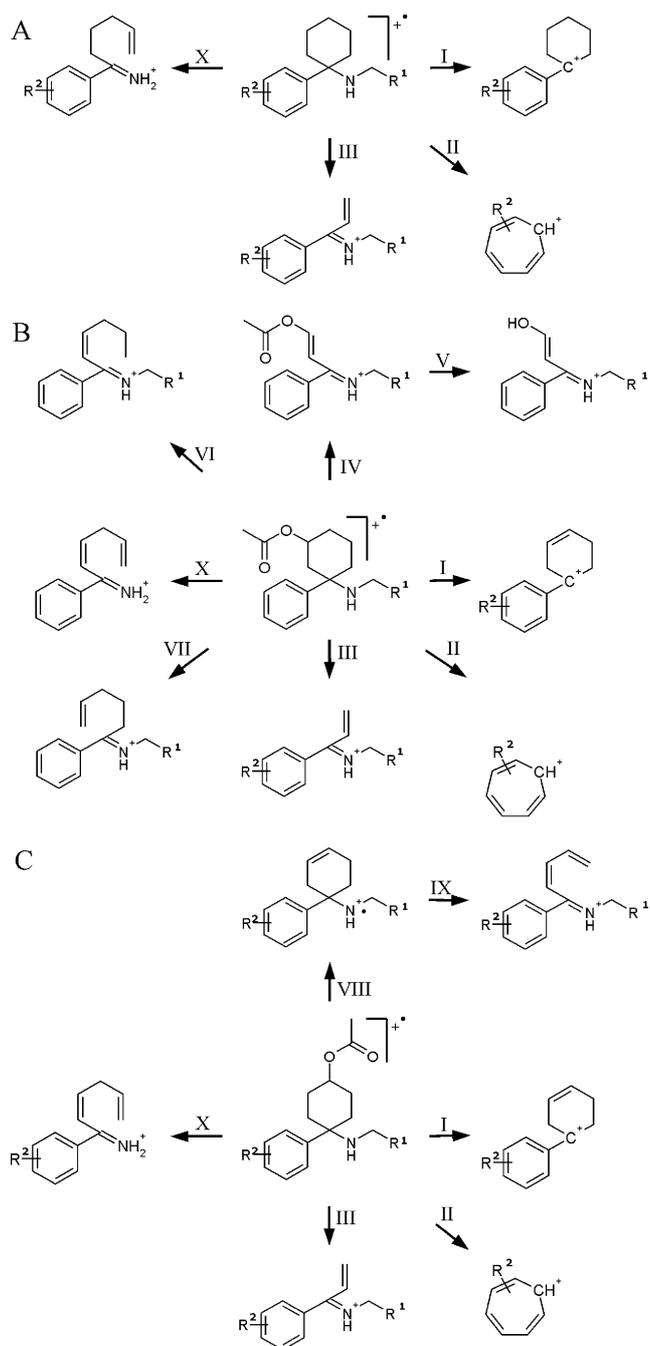
Figure 1. (Continued).

4'-hydroxy metabolites (4–6) after elimination of acetic acid. Alternatively, metabolites with an *O*-deethylated side chain could lose a neutral acetyloxyl radical corresponding to 59 u leading to a primary carbenium ion  $m/z$  216 in case of an unchanged double ring system (2),  $m/z$  274 in case of mono-hydroxylated double ring systems (3–6), or  $m/z$  332 in case of double hydroxylated double ring systems (8, 9). The primary carbenium ion could stabilize under the formation of a cyclobutyl ring with the nitrogen atom. After previous cleavage of the cyclohexyl ring and formation of the immonium cation described above, stabilization of this primary carbenium ion by ring formation is no longer possible because of the positively charged nitrogen atom. Therefore, the side chain fragmentation of the immonium cations resulting from *O*-deethylated metabolites (2–6) was characterized by elimination of acetic acid rather than by loss of an acetyloxyl radical. Thus, side chain fragmentation of immonium cations  $m/z$  232 (2, 4–6) and  $m/z$  248 (3) led to fragment ions  $m/z$  172 and  $m/z$  188, respectively.

In contrast to the metabolites discussed above, the *N*-dealkyl metabolites were easily acetylated at the primary amine moiety under the applied conditions. As expected, the resulting *N*-acetyl compounds showed a fragmentation pattern somewhat different from those described for the non-*N*-acetylated secondary amine compounds discussed above. One pathway started with migration of a hydrogen radical in position 2' to the nitrogen atom, which was followed by a cleavage between the nitrogen atom and the quaternary carbon atom of the phenylcyclohexyl double ring system. This resulted in a tertiary carbenium radical  $m/z$  158 (15)

for metabolites with an unchanged double ring system or  $m/z$  156 for 3'- and 4'-hydroxy metabolites after further elimination of acetic acid (16–19). These fragment ions were the base peaks of the respective EI mass spectra. A second pathway started with a cleavage between the acetyl moiety and the nitrogen atom corresponding to a loss of 43 u. This was followed by the opening of the cyclohexyl ring and rearrangement, resulting in the fragment ion  $m/z$  174 for the metabolite with an unchanged double ring system (15) and in the fragment ion  $m/z$  172 for the 3'- or 4'-hydroxy metabolites after further elimination of acetic acid (16–19). In the case of the *N*-dealkyl-3'-hydroxy metabolite, a  $\beta$ -cleavage of the cyclohexyl ring was also observed. This was either followed by rearrangement and loss of a propyl radical and the acetyl moiety from the oxygen at position 3' or by rearrangement and loss of an acetyloxyl radical. The former resulted in an *N*-acetylated enol-immonium ion  $m/z$  190 stabilized through extensive mesomerism from the enol oxygen to the amide oxygen (18, 19). The latter resulted in an acetylated immonium ion  $m/z$  216 which could in turn lose an acetyl moiety leading to an immonium ion  $m/z$  174 (18, 19). In case of the 4'-hydroxy metabolite, the characteristic loss of acetic acid was observed leading to  $m/z$  215 (16, 17). Finally, a fragment ion  $m/z$  132 was present in the EI mass spectra of all *N*-dealkyl metabolites, which might be an immonium ion with a structure similar to those mentioned above (16–19).

Other expected metabolites were the carboxylic acids formed by oxidation of the respective *O*-deethyl metabolites. In analogy to the previous metabolism studies of the authors'



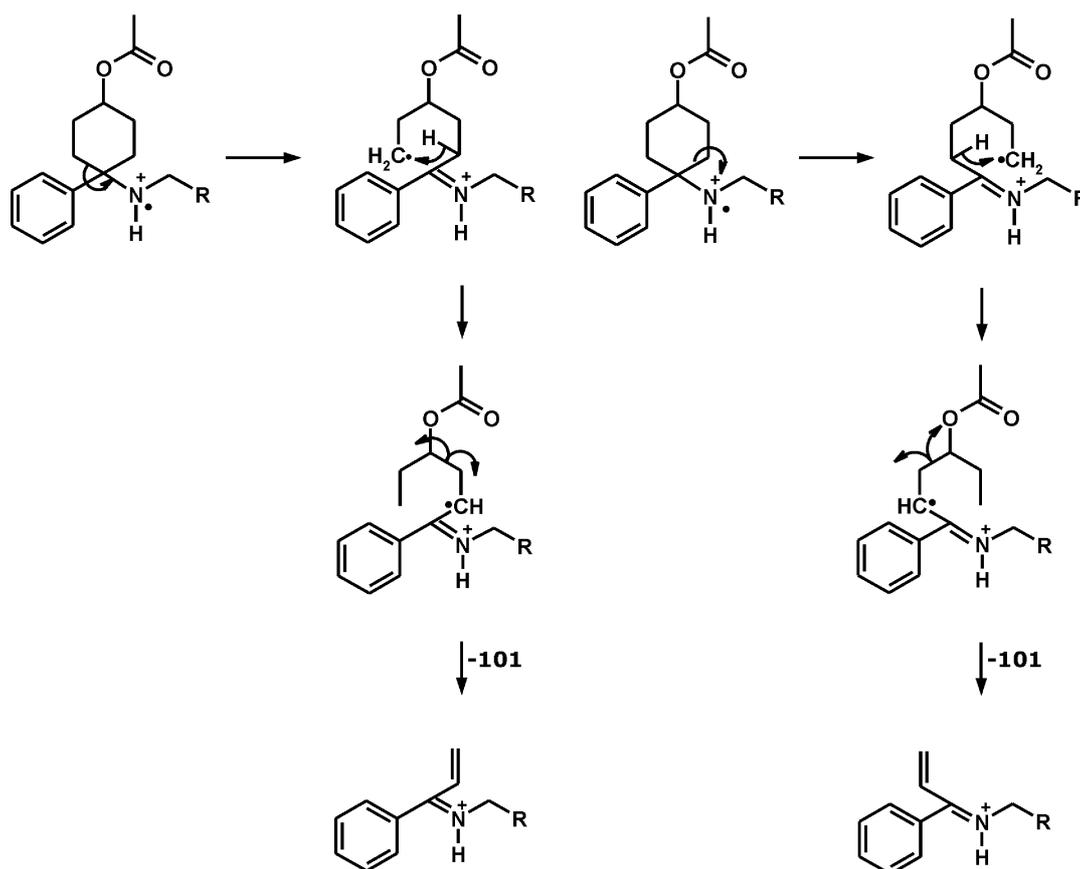
**Figure 2.** Proposed fragment ions characterizing the different metabolites.

working group, methylation and ethylation, alone and in combination with AC, were performed.<sup>11,33</sup> This led to derivatives that were likely attributable to these expected carboxylic acids, but were also positional isomers of other metabolite derivatives. Since the observed fragmentation patterns were also quite similar, unambiguous attribution of certain metabolite structures through interpretation of the EI mass spectra was not possible. A further indication for the formation of carboxylic acid metabolites was the detection of peaks in acetylated extracts, the mass spectra of which would be in line with  $\beta$ -lactams formed by intramolecular cyclization of the side chain after *O*-deethylation and oxidation (21–25). This cyclization should be favored by

elimination of the resulting water by acetic anhydride. Furthermore, from a sterical point of view, the carboxylic acid moiety would be in an ideal position to react with the amine function. The fragmentation patterns of these lactams were very similar to those of both the corresponding secondary amine metabolites and the amides. Those with an unchanged phenylcyclohexyl system showed the above-mentioned fragment ions  $m/z$  158 (21) as well as the characteristic loss of 43 u from the cyclohexyl ring leading to fragment ions  $m/z$  186 (21). The 3'- and 4'-hydroxy metabolites showed the above-mentioned fragment ions  $m/z$  156 (22) or  $m/z$  157 (23–25) after elimination of acetic acid. The 3'-hydroxy metabolite also showed the typical loss of 43 u leading to fragment ion  $m/z$  244 (24, 25) followed by a loss of an acetyl moiety corresponding to 42 u leading to fragment ion  $m/z$  202. Furthermore, the 3'-hydroxy metabolite showed the above-mentioned loss of 59 u, in this case leading to fragment ion  $m/z$  228 (24, 25), while in case of the 4'-hydroxy metabolite, elimination of acetic acid was observed leading to the fragment ion  $m/z$  227.

Even though the presence of the postulated lactams already indicated the presence of carboxy metabolites, silylation experiments were performed for confirmation. In the following, possible fragmentation patterns of PCEPA metabolites after silylation will be discussed in relation to the postulated metabolite structures. However, this discussion will be limited to the mass spectra of those metabolites that provided additional structural information. As before, the numbers of the corresponding mass spectra in Fig. 1 are given in brackets. The main fragmentation patterns of the silylated *O*-demethyl-carboxy metabolites were the same as those described in the previous paragraphs and the respective fragment ions confirmed their structures. Cleavage between the nitrogen atom and the quaternary carbon atom of the phenylcyclohexyl double ring system resulted in fragment ions  $m/z$  159 in carboxy PCEPA (26),  $m/z$  157 in metabolites hydroxylated at the cyclohexyl ring after the loss of trimethylsilanol corresponding to 90 u (28–30),  $m/z$  247 in hydroxyphenyl PCEPA (27), and  $m/z$  245 in carboxy-4'-hydroxy-hydroxyphenyl PCEPA (31). The benzyl-like cleavage led to the formation of tropylium ions with  $m/z$  91 for metabolites with an unchanged phenyl ring (26, 28–30) and  $m/z$  179 for silylated hydroxy-aryl metabolites (27, 31). Finally,  $\beta$ -cleavage of the cyclohexyl ring and rearrangement yielded fragment ions  $m/z$  276 for metabolites with an unchanged (26), 3'-(30), or 4'-hydroxylated (28, 29) cyclohexyl ring and  $m/z$  364 for metabolites with a hydroxylated phenyl ring (27, 31) or with a 3'-hydroxylated cyclohexyl ring (30).

A closer look at the structures of the 3'- and 4'-hydroxy metabolites shows that in both positions there is a possibility for *cis*–*trans* isomerism, as was previously described for the corresponding metabolites of phencyclidine.<sup>35</sup> In case of the 4'-hydroxylation, for each of the respective metabolites two peaks with virtually identical mass spectra were detected in the acetylated extracts. These were most likely attributable to corresponding *cis* and *trans* isomers. However, unambiguous assignment of these isomers to a certain peak was not possible owing to the similarity of the



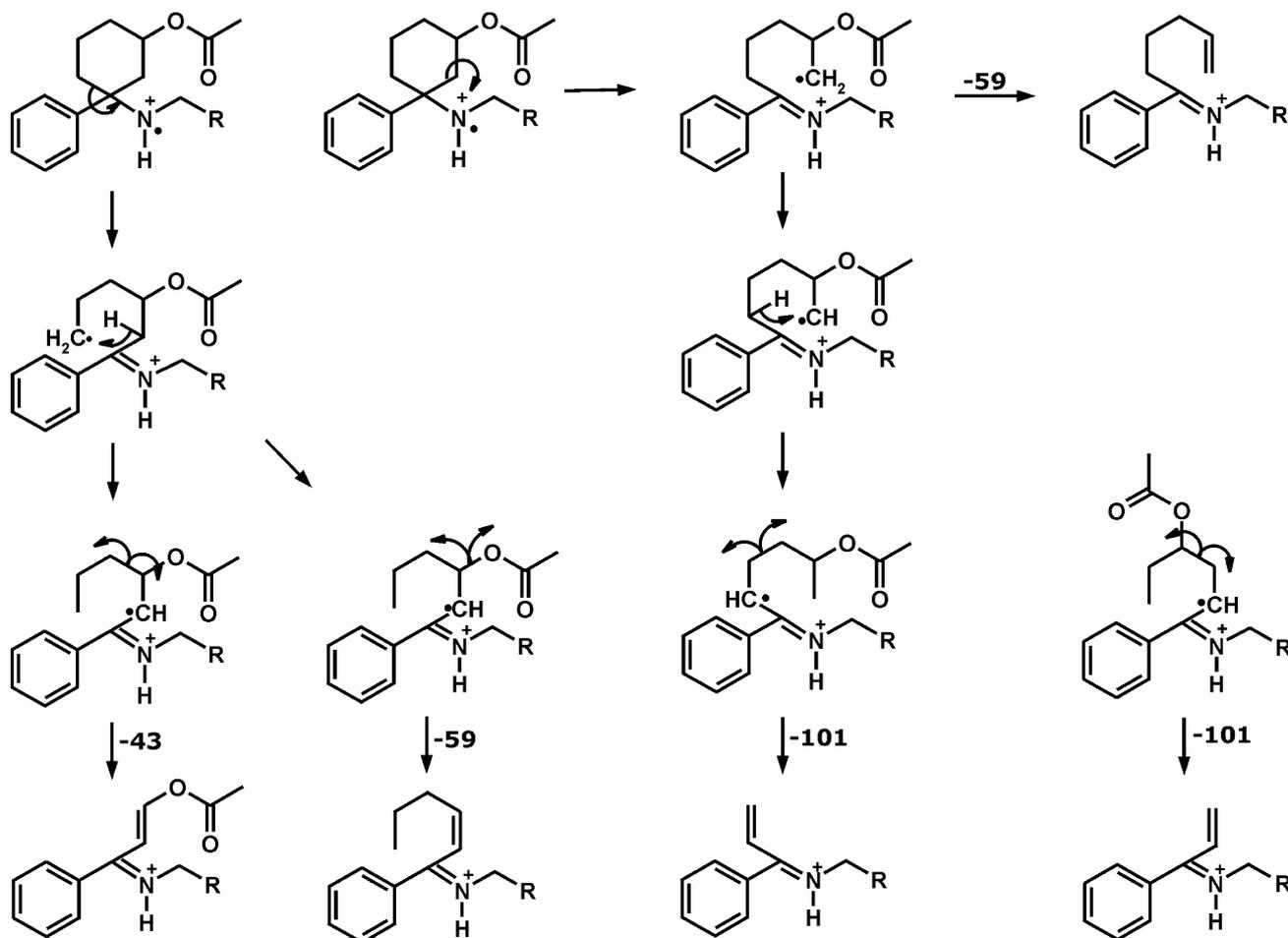
**Figure 3.** Fragmentation of the cyclohexyl ring after hydroxylation at position 4.

mass spectra, which is why the isomers are referred to as isomer 1 and isomer 2. In case of 3'-hydroxylation, two peaks with practically identical mass spectra were observed only for part of the metabolites, namely *N*-dealkyl-3'-hydroxy PCEPA (18, 19) and dehydrated 3'-hydroxy-carboxy PCEPA (24, 25). For the same reason as mentioned above, unambiguous stereochemical assignment was not possible, and the compounds were again referred to as isomer 1 and isomer 2. For the other 3'-hydroxy metabolites, only one peak each was observed, which might either be explained by the stereospecific formation of only one of the two possible diastereomers or by the formation of two diastereomers that can be separated neither under the applied chromatographic conditions nor when using a 30 m HP-5MS column and an oven temperature program with flatter gradients or even with constant temperature around the retention times of the respective peaks.

In the silylated extracts, two peaks were also detected for each of the 4'-hydroxy metabolites, which were again attributed to the *cis* and *trans* isomers. However, a closer look at the respective mass spectra (28, 29 and 32, 33) showed that their fragmentation patterns were slightly different. In both pairs, one of the mass spectra (28, 32) contained a fragment at  $m/z$  246, which was missing in the mass spectra of the respective isomers. Since the side chains of the two diastereomer pairs were different, the fragment ion  $m/z$  246 can relate only to the common 4'-hydroxylated double ring system. In a GC/MS study on phencyclidine, which carries only a piperidine ring instead of the *N*-alkyl side

chain of PCEPA, Gole *et al.*<sup>36</sup> have already described the fragment ion  $m/z$  246 to be typical for the *cis* isomer of 4'-hydroxy phencyclidine. In order to achieve stereochemical assignment of the isomer structures, they synthesized the *cis* and *trans* 4'-hydroxy-phencyclidine and recorded the respective EI mass spectra after silylation. They found that only the mass spectrum of the *cis* isomer contains the fragment ion  $m/z$  246. Considering the analogy between the fragmentation patterns described by Gole *et al.* and those described here, it could be concluded that mass spectra nos. 28 and 32 in Fig. 1 should correspond to the *cis* isomers of carboxy 4'-hydroxy PCEPA and *O*-deethyl-4'-hydroxy PCEPA, respectively, while mass spectra nos 29 and 33 corresponded to the respective *trans* isomers. A possible explanation for the differences in fragmentation of the *cis* and *trans* 4'-hydroxy isomers is illustrated in Fig. 5, using *O*-deethyl-4'-hydroxy PCEPA as an example. As shown in the left part of the figure, fragmentation of the *cis* isomer may start with the migration of a hydrogen radical from position 2' to the nitrogen atom of the amine moiety, as has been described for phencyclidine by Smith and Busch.<sup>27</sup> After the loss of the side chain as a neutral amine, the remaining tertiary carbenium ion with  $m/z$  246 can be stabilized by the formation of a six-membered ring with the oxygen atom in position 4'. As shown in the right part of the figure, this is not possible for the *trans* configuration. Therefore, this fragment is not observed in the mass spectra of the *trans* isomers.

As already mentioned, the PICI spectra of the parent drug PCEPA (1) and many of its metabolites (3–6, 8–13,



**Figure 4.** Fragmentation of the cyclohexyl ring after hydroxylation at position 3.

15, 26–33) contained the protonated molecular ions but not the adduct ions  $[M + C_2H_5]^+$  and  $[M + C_3H_5]^+$ , which are typically observed when using methane as the reagent gas. This phenomenon can be explained by the structures of the respective compounds. Since the mechanism of formation of the above-mentioned adducts is an electrophilic addition reaction, it requires a nucleophilic center.<sup>37,38</sup> On the basis of these considerations, the adduct formation of PCEPA and its metabolites would be expected to take place mainly at the nitrogen atom of the amine moiety. However, another prerequisite of adduct formation is sufficient steric expanse around the nucleophilic center. This second requirement is the most probable explanation for the absence of adduct formation of PCEPA and many of its metabolites. As already discussed in the context of AC, the amine moiety was sterically hindered by the neighboring phenyl and cyclohexyl rings. In PCEPA (1) and all its metabolites/artifacts with either an unchanged (10–14), *O*-deethylated (2–9), or *O*-deethylated and oxidized side chain (26–31), this was most probably enhanced by the formation of a hydrogen bond, similar to the one described above, leading to a six-membered ring. Indeed, with exception of the artifacts nos. 7 and 14 in Fig. 1, their PICI mass spectra showed the absence of adduct ions. Because the only differences between PCEPA (1) and artifact no. 14, and between *O*-deethyl PCEPA (2) and artifact no. 7 were double bonds in the cyclohexyl ring, these double bonds seem to serve as nucleophilic centers

for adduct formation.<sup>32</sup> The hypothesis of the absence of adduct formation because of sterical hindrance enhanced by formation of the above-mentioned hydrogen bonds is supported by the fact that adduct ions, though small, were present in the PICI mass spectra of *N*-dealkylated metabolites (16–20) and lactams formed from carboxy metabolites (21–25). Furthermore, the relatively abundant adduct ions in the PICI spectrum of artifact no. 20 support the assumption that a double bond in the cyclohexyl ring may serve as a nucleophilic center for adduct formation.

### Proposed metabolic pathways

On the basis of the identified compounds, the following metabolic pathways, shown in Fig. 6, could be postulated: *N*-dealkylation; *O*-deethylation partially followed by oxidation to the corresponding acid; hydroxylation of the cyclohexyl ring at different positions of PCEPA, *N*-dealkyl PCEPA, *O*-deethyl PCEPA, and of the corresponding acids; and finally, aromatic hydroxylation of PCEPA, the corresponding acids, and *O*-deethyl PCEPA, the latter partially followed by oxidation to the corresponding acid and hydroxylation of the cyclohexyl ring. After *O*-deethylation and oxidation the corresponding acid, the side chain of the acid could also be hydroxylated. Further possible pathways, which are not described in Fig. 6, are the following: *O*-deethylation (2) followed by *N*-dealkylation (15); *O*-deethylation followed by hydroxylation of the cyclohexyl ring (4–6) and

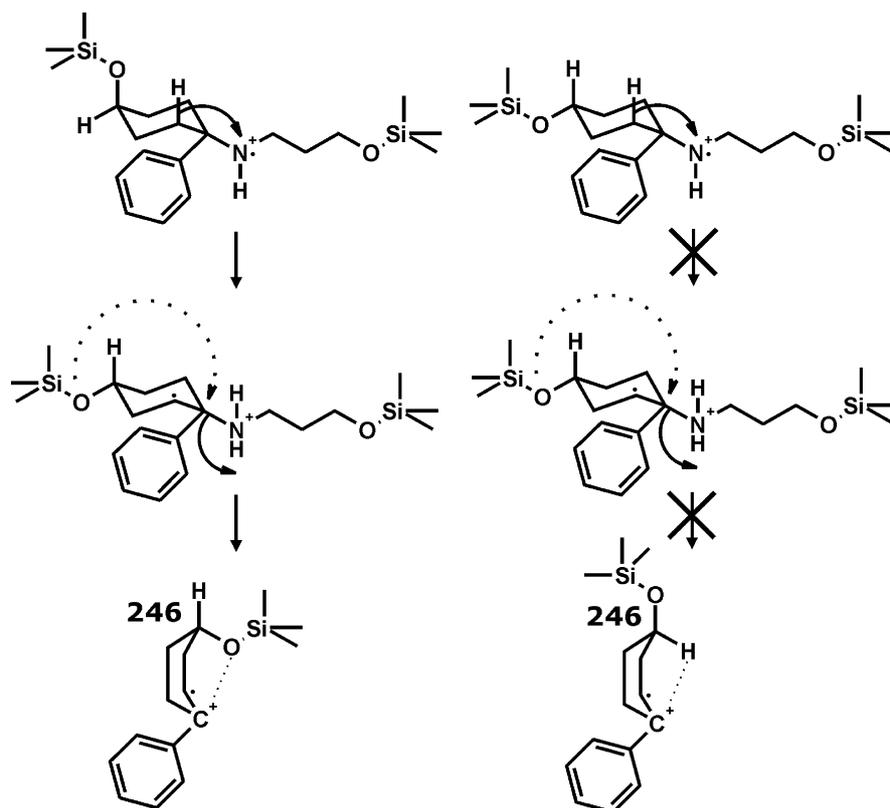


Figure 5. Influence of stereochemistry on fragmentation of *cis* and *trans* diastereomers.

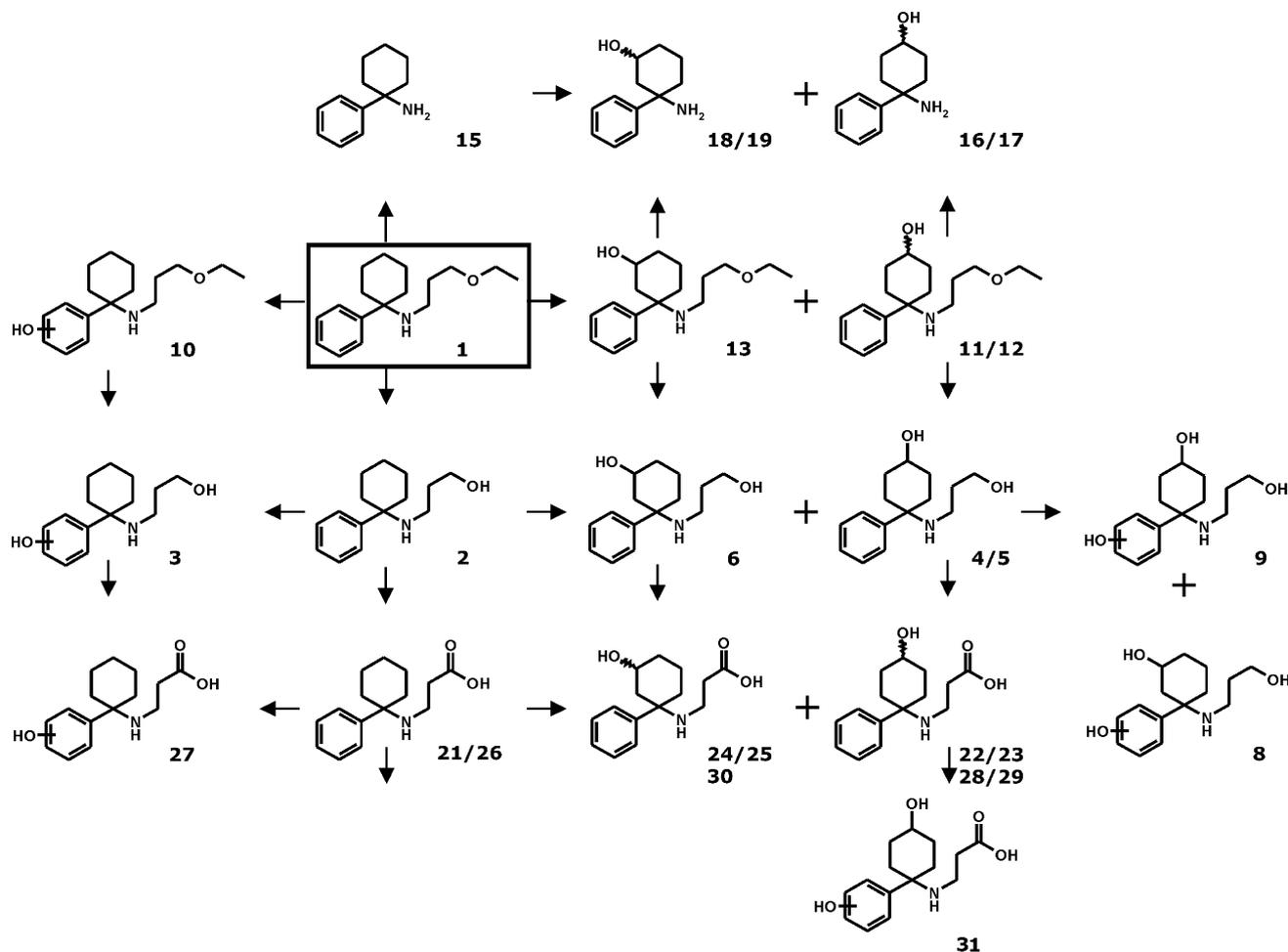
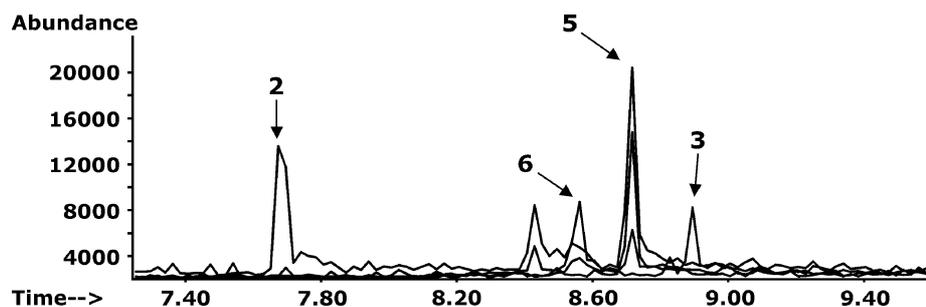


Figure 6. Metabolic pathways of PCEPA.



**Figure 7.** Typical reconstructed mass chromatograms with ions  $m/z$  232, 274, 273, and 290 of an acetylated extract of a rat urine sample collected over 24 h after application of 0.1 mg/kg BM of PCEPA which corresponded to a common drug users' dose of about 7 mg. The identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with the reference spectra.

*N*-dealkylation (16–19); *O*-deethylation followed by hydroxylation of the cyclohexyl ring and aromatic hydroxylation (8–9) and oxidation to the corresponding acid (32); and *O*-deethylation followed by oxidation to the corresponding acid and aromatic hydroxylation (27) followed by hydroxylation of the cyclohexyl ring (32). All metabolites were partially excreted in the conjugated form.

### Detection by GC/MS within the STA

The authors' STA procedure allowed the detection in urine of an intake of a dose of PCEPA that corresponds to a common drug users' dose. Figure 7 shows typical reconstructed mass chromatograms with the ions  $m/z$  232, 274, 273, and 290 from an acetylated extract of a rat urine sample collected over 24 h after application of a single dose of 0.1 mg/kg BM of PCEPA which corresponded to a dose of about 7 mg corresponding to that of seized tablets containing other phenacyclidine derivatives. They indicate *O*-deethyl PCEPA (2), *O*-deethyl-hydroxyphenyl PCEPA (3), *O*-deethyl-4'-hydroxy PCEPA isomer 2 (5) and *O*-deethyl-3'-hydroxy PCEPA (6). Their identity was confirmed by a computerized comparison of the underlying mass spectrum with the reference spectra recorded during this study.

### CONCLUSIONS

The metabolism studies presented here showed that the designer drug PCEPA was mainly metabolized by *O*-deethylation, hydroxylation of the cyclohexyl ring in different positions, hydroxylation of the phenyl ring, oxidation to the carboxylic acid, *N*-dealkylation, and combinations of these steps.

The authors' experience in metabolism and analytical studies on rats and humans support the assumption that the metabolites found in rat urine should also be present in human urine.<sup>5,19,39–41</sup> Therefore, it can be concluded that the procedure should also be applicable for human urine screening for PCEPA in clinical or forensic cases.

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