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Studies on the in vivo and in vitro metabolism of the novel psychoactive substance 3',4'-methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP)

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The in vivo and in vitro metabolism and the detectability in urine of 3',4'-methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP), a new pyrrolidinophenone-type designer drug, studied by GC-MS and LC-MS^n.

Meyer MR, Mauer S, Meyer GM, Dinger J, Klein B, Westphal F, Maurer HH.

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1 SUMMARY

3´,4´-methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP) is a new designer drug of the pyrrolidinophenone-type. This substance of abuse is used as a stimulating and performance-enhancing drug with similar effects than methylenedioxypyrovalerone (MDPV) or cocaine. MDPBP appeared on the worldwide drugs of abuse market during the last years and was seized in Germany for the first time in 2009. This drug can be purchased on the Internet as so called ‘bath salt’, ‘plant fertilizer’ or ‘NRG-1’.

The aim of the presented work was to study the phase I and phase II metabolism of MDPBP in rat urine and by single enzyme preparations. Therefore, rat urine samples were extracted with and without enzymatic hydrolysis and were derivatized afterwards. Metabolites were then separated and identified by gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS^n). All in all, 17 phase I and 7 phase II metabolites could be identified. The phase I steps were: demethylenation of the methylenedioxy moiety and methylation to the corresponding demethylenyl-methyl compounds, oxidative deamination to the corresponding 2´-oxo compounds, hydroxylation of the butyl side chain and oxidation to the corresponding ketone, hydroxylation of the phenyl ring, hydroxylation of the 2´-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactams, hydroxylation of the 2´-position of the pyrrolidine ring followed by ring opening to the respective aliphatic aldehyde and further oxidation to the carboxylic acid, degradation of the pyrrolidine ring to the corresponding primary amines and reduction of the keto group to the corresponding secondary alcohols. As phase II reactions, glucuronidation, methylation and sulfation were observed. The Cytochrome P450 (CYP) isoenzymes involved in the main metabolic step could be identified as CYP2C19 and CYP2D6. Additionally, CYP2D6 could be identified as the isoenzyme responsible for the main part of the total MDPBP CYP-dependent clearance.

The presented metabolism study demonstrated the extensive metabolism of MDPBP by the rat. Due to the enormous increase of new designer drugs and the corresponding health risks, it is an important issue to identify and to study new emerged substances. The presented study could therefore contribute to identification and detection of MDPBP by elucidating its metabolic pathways. Supposing similar kinetic processes in rats and

humans, this study could serve as a basis for developing suitable screening strategies for detection of a MDPBP intake.

2 ZUSAMMENFASSUNG

3´,4´-Methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP) ist eine neue Designerdroge vom Pyrrolidinophenon-Typ. Diese Droge findet Verwendung als Aufputschmittel oder wird zur Leistungsförderung missbraucht und hat ähnliche Wirkungen wie Methylenedioxy-pyrovalerone (MDPV) oder Kokain. MDPBP zählt zu den Neuerscheinungen der letzten Jahre auf dem weltweiten Dogenmarkt und wurde erstmals 2009 in Deutschland beschlagnahmt. Die Substanz kann im Internet erworben werden als sognannte „Badesalze“, „Pflanzendünger“ oder als „NRG-1“, welches oft eine Mixtur mit verschiedenen Cathinon-Derivaten ist.

Ziel dieser Studie ist die Identifizierung der Phase I und Phase II Stoffwechselprodukte von MDPBP in Rattenurin und mittels Enzym Experimenten. Die Aufarbeitung des Rattenurins erfolgte sowohl mit, als auch ohne Konjugatspaltung und anschließender Derivatisierung. Die Separation und Identifizierung der Metaboliten erfolgte unter Verwendung von Gaschromatographie-Massenspektrometrie (GC-MS) und Flüssigchromatographie-Massenspektrometrie ($LC\text{-}MS^n$). Nach entsprechender Probenvorbereitung und Versuchsdurchführung wurden 17 Phase I und 7 Phase II Metaboliten identifiziert. Die nachgewiesenen Phase I Stoffwechselwege waren folgende: Demethylenierung der Methylendioxybrücke und anschließende Methylierung zur korrespondierenden DemethylenylMethyl-Verbindung, oxidative Deaminierung zur 2´-oxo-Verbindung, Hydroxylierung der Butyl-Seitenkette und nachfolgende Oxidation zum entsprechenden Keton, Hydroxylierung des Phenylrings, Hydroxylierung der 2´-Position des Pyrrolidinrings gefolgt von Dehydrogenierung zum Laktam, Hydroxylierung der 2´-Position des Pyrrolidinrings mit anschließender Ringöffnung zum korrespondierenden Aldehyd und ferner Oxidation zur Carbonsäure, Dealkylierung zum primären Amin und Reduktion der Keto-Gruppe zum entsprechenden sekundären Alkohol. Als Phase II Reaktionen konnten Glucuronidierungen, Methylierungen und Sulfatierungen beobachtet werden. Des Weiteren konnte gezeigt werden, dass die Cytochrome P450 Isoenzyme CYP2D6 und CYP2C19 für die Hauptstoffwechselwege von MDPBP verantwortlich sind. Davon wiederum übernimmt CYP2D6 den größten Teil der CYP-abhängigen Metabolisierung von MDPBP.

Zusammenfassend lässt sich sagen, dass anhand dieser Studie nachgewiesen werden konnte, dass MDPBP von Ratten ausgiebig verstoffwechselt wird. Aufgrund der Tatsache, dass es zurzeit zu einem enormen Anstieg neuer Designerdrogen auf dem illegalen Drogenmarkt kommt und den damit einhergehenden gesundheitlichen Risiken, ist es von erheblicher Wichtigkeit diese zu identifizieren und deren Metabolismus zu studieren. Diese Arbeit konnte hierzu ihren Beitrag leisten, indem der Metabolismus von MDPBP aufgeklärt wurde und diese Droge somit mittels des Stoffwechselweges identifiziert und detektiert werden kann. In Annahme, dass die Kinetik im menschlichen Körper, derer in Ratten ähnelt, kann diese Doktorarbeit als Basis dienen, um eine MDPBP Einnahme detektieren zu können.

3 INTRODUCTION

3.1 Designer Drugs of the Pyrrolidinophenone-Type

After alpha-pyrrolidinophenone (PPP),[1, 2] 4´-methyl-alpha-pyrrolidinopropiophenone (MPPP),[1, 3] 4´-methyl-alpha-pyrrolidinohexanophenone (MPHP),[4] 4´-methoxy-alpha-pyrrolidinopropiophenone (MOPPP),[5] 3´4´-methylenedioxy-alpha-pyrrolidinopropiophenone (MDPPP),[6] 4´-methyl-alpha-pyrrolidinobutyrophenone (MPBP),[7] alpha-pyrrolidinovalerophenone (PVP),[8] and methylenedioxy-pyrovalerone (MDPV),[9] 4´-methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP) was the latest of the pyrrolidinophenones, which appeared on the illicit drug market in Germany. Their chemical structures are given in Fig. 1. So far MDPPP (1997), PPP (1998), MPPP (1999), and MDPV (2012) have been scheduled in the German Controlled Substances Act and their possession is prohibited.[8]

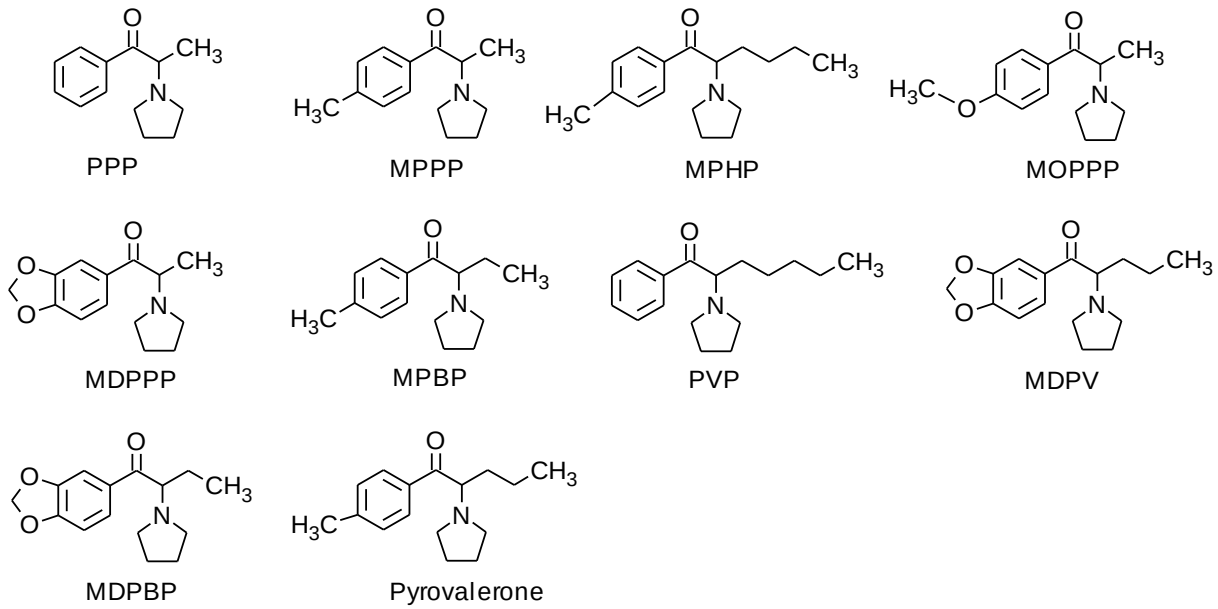

Figure 1: Chemical structures of pyrrolidinophenone-type designer drugs and of the psychostimulant pyrovalerone.

In Germany, MDPBP was first seized as a white powder mixed with lactose during a traffic control in 2009. Later analysis showed that the substance occurred in form of its hydrochloride (HCl).[10] MDPBP can be purchased via the Internet on different online shops as so called 'legal high', declared for example as 'bath salt', 'plant fertilizer' or 'NRG-1', which is a mixture with other drugs (Fig. 2). Due to the fact that these drugs of abuse are pharmacologically and toxicologically untested, the mode of action and adverse reactions could not be described in detail. Hence, only little information about application, dosage, pharmacological and toxicological effects of MDPBP is available so far. It is supposed to be taken nasally or orally like other pyrrolidinophenones, which have been distributed among drug abusers as powders, tablets or capsules.[11]

http://mcnewsreport.blogspot.de/2011/05/bath-salts-devil-is-in-details.html

Figure 2: Example for 'NRG-1' and so called 'bath salts' sold on the Internet.

Because of its close structural relation to pyrovalerone (Fig. 1), which was the first commercially available drug from the large group of alpha-pyrrolidinovalerophenones, it can be expected that application, dosage, pharmacological, and toxicological effects are similar. Pyrovalerone is a psychostimulant drug, which was synthesized and appeared on the market in the 1960s.[12-14] The mode of action of this drug is the release of dopamine and norepinephrine from the respective nerve terminals.[15, 16] This mode of action was first studied in animal experiments by Stille et al. in the early 1960s.[17] These authors compared pyrovalerone with amphetamine and reported that pyrovalerone had similar central stimulatory effects as amphetamine, but in contrast, less influence on locomotor activity and autonomous functions. The oral median lethal dose (LD_{50} value) of this substance in mice was reported to be 350 mg per kg body mass. Additionally, controlled human studies showed similar results.[13, 18] Further studies on pyrovalerone

analogues, concerning their biological activity on different transporters in 2006, revealed that these substances are inhibitors of dopamine and norepinephrine transporters with little effects on serotonin transport.[19] Furthermore, pyrovalerone was tested as a therapeutic drug for symptomatic therapy of chronic fatigue or lethargy.[12] But the abuse of intravenous drug use made it necessary to withdraw this substance from the market.[20]

3.2 Metabolism of Pyrrolidinophenones

Metabolism studies on novel drugs of abuse are important for clinical and forensic toxicology, workplace drug testing or doping control as prerequisite for thorough screening procedures. Concerning the fast moving development of new designer drugs, analytical data, such as clarifying drug metabolites and main metabolic steps, are essential to identify new emerging compounds. In case of PPP, MPPP, MPHP, MPBP, MOPPP, MDPPP, PVP and MDPV, the qualitative metabolism has been studied in animals, whereas in case of pyrovalerone metabolism studies have been performed in animals and also in human beings. So far, no data about the metabolites and main metabolic steps of MDPBP are available.

However, urine is used as biological sample for screening and identification of drugs or poisons, because it is easier available than other body fluids and usually contains a higher concentration of metabolites or the drug itself. Powerful methods for the identification of metabolites are GC-MS and LC-MS^n. They allow the simultaneous detection of many toxicants in biosamples in only one single step. Individual compounds can be identified by comparing full mass spectra with reference spectra.[21, 22, 23]

3.3 Aims of the Dissertation

1. Identification of the phase I metabolites of MDPBP in rat urine using GC-MS.
2. Confirmation of phase I and identification of phase II metabolites of MDPBP in rat urine using LC-MS^n.
3. Postulation of the metabolic pathways of MDPBP.
4. Elucidation of the CYP isoenzymes involved in the main metabolic reactions.

4 EXPERIMENTAL PROCEDURES/MATERIALS AND METHODS

4.1 Chemicals and Reagents

MDPBP-HCl was provided by the Landeskriminalamt Schleswig-Holstein (Kiel, Germany) for research purposes. Isolute HCX cartridges (130 mg, 3 mL) were obtained from Biotage (Uppsala, Sweden), nicotinamide adenine dinucleotide phosphate ($NADP^+$), Biomol (Hamburg, Germany) and isocitrate and isocitrate dehydrogenase from Sigma (Taufkirchen, Germany). Glucuronidase (EC No. 3.2.1.31) and arylsulfatase (EC No. 3.1.6.1) from Helix Pomatia L as well as all other chemicals and reagents were obtained from E. Merck (Darmstadt, Germany) and were of analytical grade. The following microsomes were from Gentest and delivered by NatuTec (Frankfurt/Main, Germany): baculovirus-infected insect cell microsomes (ICM) (Supersomes), containing 1 nmol/ml of human cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 (2 nmol/ml), CYP3A4, or CYP3A5 (2 nmol/ml) and pooled human liver microsomes (pHLM, 20 mg microsomal protein/ml, 420 pmol total CYP/mg protein). After delivery, the microsomes were thawed at 37°C, aliquoted, snap-frozen in liquid nitrogen and stored at -80°C until use.

4.2 Urine Samples

Studies were accomplished using urine of male rats (Wistar, Charles River, Sulzfleck, Germany) for toxicological diagnostic reasons. They were administered a single 20 mg/kg body mass dose of MDPBP for metabolism studies (identification of the metabolites) and a single of 1 mg/kg body mass dose for toxicological analysis (development of the screening procedure). The drug was applied in an aqueous suspension by gastric intubation. The rats were housed in metabolism cages for 24 h, having water ad libitum. Urine was collected separately from the feces over a 24 h period. All samples were directly analyzed and then stored at -20°C until further

analysis. Blank urine samples were collected before drug administration to check whether the samples were free of interfering compounds.

4.3 Sample Preparation

4.3.1 Sample preparation for the identification of the phase I metabolites by GC-MS

A 2.5 mL portion of urine was adjusted to pH 5.2 with acetic acid (1 M, approximately 50 µL) and incubated at 56°C for 1.5 h with a mixture (100 000 Fisherman u/mL) of glucuronidase and arylsulfatase. The urine samples were then diluted with 2.5 mL of water and loaded on solid phase extraction (SPE) Isolute HCX cartridges, 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 again with 1 mL of water. The retained non-basic compounds were first eluted into a 1.5 mL reaction vial with 1 mL of methanol (fraction 1), whereas the basic compounds were eluted in a second step into a different vial with 1 mL of a freshly prepared mixture of methanol/aqueous ammonia 32% (98:2 v/v; fraction 2). The eluates were gently evaporated to dryness under a stream of nitrogen at 56°C and then dissolved in 100 µL of methanol. Thereafter, the samples were divided into two aliquots and evaporated to dryness. One aliquot was dissolved in 50 µL methanol and one was derivatized according to published procedures for GC-MS.[23] Therefore, this aliquot was acetylated with 100 µL of acetic anhydride-pyridine mixture (3:2, v/v) for 5 min under microwave irradiation at about 440 W. After evaporation to dryness, the residue was dissolved in 50 µL of methanol. A 1 µL aliquot was injected into the GC-MS system.

4.3.2 Sample preparation for the identification of the phase I and II metabolites by LC-MS^n

SPE as described in 4.3.1 without derivatization was used for the confirmation of phase I metabolites. For identification of phase II metabolites, 1 mL of the urine samples was loaded on a Confirm C18 cartridge. Prior to sample load, the column was conditioned with 1 mL of methanol and 3 mL of water. After passage of the sample, the cartridge was washed with 1 mL of water. The retained compounds were eluted into a 1.5 mL reaction vial with a mixture of 1 mL of methanol and 200 µL of acetone. The samples were then gently evaporated to dryness under a stream of nitrogen at 56°C and reconstituted in a mixture of 10 mM ammonium formate buffer and acetonitrile (1:1 v/v).

4.4 Enzymatic Part

4.4.1 Microsomal incubations

In order to verify, which CYP isoenzymes are involved in the initial metabolic steps of MDPBP, microsomal incubations were performed at 37°C with 25 µM MDPBP with human liver microsomes (HLM), CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 or CYP3A5 for 30 min. Besides enzymes and substrate, incubation mixtures (final volume: 50 µL) consisted of 90 mM phosphate buffer (pH 7.4), 5 mM Mg^{2+}, 5 mM isocitrate, 1.2 mM $NADP^+$, 0.5 U/ml isocitrate dehydrogenase and 200 U/ml superoxide dismutase. Reactions were started by adding the ice-cold microsomes and stopped with 50 µl of an ice-cold mixture of acetonitrile and 0.1 µM internal standard diphenhydramine. The solution was centrifuged for 5 min at 14000 U/min. 60 µL of the supernatant was transferred to an autosampler vial and injected into LC-MS^n system for analysis. LC-MS^n conditions were chosen as described below.

4.4.2 Initial screening studies

The initial screening studies with the ten most abundant human hepatic CYP´s were performed with 25 µM of MDPBP and 50 pmol/mL of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, or CYP3A5 for 30 min.

4.4.3 Kinetic studies

Determination of kinetic constants of the initial metabolic step, the demethylenation of MDPBP, was derived from incubations with an incubation time of 10 min and 40 pmol/mL protein concentration for CYP2C19 and an incubation time of 30 min and 40 pmol/mL of protein concentration in case of CYP2D6. The described incubation conditions (incubation time and protein concentration) were chosen to be within a linear range of metabolite formation. The following substrate concentrations were used as provided in Table 1.

Table 1: Substrate concentrations used for microsomal incubations [µM].

CYP2D6	CYP2C19
1 5 10 50 100 125 250 500 750 1000	1 5 10 50 100 125 250 500 750 1000 1500 2000

Enzyme kinetic constants were estimated by non-linear curve-fitting using GraphPad Prism 5.00 software (San Diego, CA). The Michaelis-Menten equation (Eqn. (1)) was used to calculate apparent K_m values and V_{enzyme} values for single-enzyme systems.

$$V_{enzyme} = \frac{V_{max} \times [S]}{K_m + [S]} \qquad (1)$$

K_m is the substrate concentration at which the reaction rate is halfway maximal. Consequently, with the help of K_m values it is possible to make a statement about the affinity of the particular P450s substrates.

4.4.4 Metabolite quantification

Correct quantification of MDPBP-M (demethylenyl) in the incubation mixture was not possible, because there was no reference substance available. Instead, the peak area ratios of the respective target ions of MDPBP-M (demethylenyl) and its internal standard diphenhydramine were used.

4.4.5 Calculation of relative activity factors, contributions and percentages of net clearance

The relative activity factor (RAF) approach [24-26] was used to account for differences in functional levels of redox partners between the two enzyme sources. The turnover rates (TR) of CYP2C19 (probe substrate (PS) *S*-mephenytoin) and CYP2D6 (PS bufuralol) in insect cell microsomes (ICM) and human liver microsomes (HLM) were taken from the supplier's data sheets. The RAF´s were calculated according to equation (2).

$$RAF_{enzyme} = \frac{TR_{PS} \text{ in HLM}}{TR_{PS} \text{ in ICM}} \qquad (2)$$

The enzyme velocities V_{enzyme} (see equation (1)) for the respective metabolic reactions were calculated and then multiplied with the corresponding RAF leading to a value, which is defined as 'contribution'. The V_{max} and the K_m values (equation (1)) were obtained from the incubations with complementary deoxyribonucleic acid (cDNA)-expressed P450s.

$$contribution_{enzyme} = RAF_{enzyme} \times V_{enzyme} \qquad (3)$$

From these corrected activities (contributions) the percentages of net clearance by a particular P450 can be calculated according to equation (4):

$$\text{clearance}_{\text{enzyme}}\ [\%] = \frac{\text{contribution}_{\text{enzyme}}}{\sum \text{contribution}_{\text{enzyme}}} \times 100 \qquad (4)$$

4.5 GC-MS and LC-MS^n Settings

4.5.1 GC-MS apparatus

The MDPBP metabolites were separated and analyzed in urine extracts using a Hewlett Packard (HP, Agilent, Waldbronn, Germany) 5890 Series II gas chromatograph combined with a HP 5972 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 TG-1 MS Thermo Scientific (12 m × 0.2 mm ID), 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°C to 310°C at 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; electron ionization (EI) mode, ionization energy, 70 eV; capillary direct interface, heated at 280°C, positive ion chemical ionization (PICI) mode using methane; ionization energy, 230 eV; ion source temperature, 220°C; capillary direct interface, heated at 260°C.

4.5.2 LC-MS^n apparatus

For metabolite identification, the LC-MS^n gradient was programmed as described previously.[27, 28] MDPBP and their metabolites were analyzed using a ThermoFisher Scientific (TF, Dreieich, Germany) Accela LC system consisting of a degasser, a quaternary pump and a HTC PAL auto sampler (CTC Analytics AG, Zwingen, Switzerland), coupled to a TF Linear Ion Trap system equipped with a heated electrospray ionization II source. The LC conditions were as follows: TF Hypersil gold (10 x 2.1 mm, 1.9 µm), column temperature 35°C, and gradient elution with 10 mM aqueous ammonium formate buffer containing 0.1% (v/v) formic acid as mobile phase A and acetonitrile containing 0.1% (v/v) formic acid as mobile phase B. The flow rate was set to 0.5 mL/min and the gradient was programmed as follows: 0-1 min 98% A, 1-3 min to 90% A, 3-5 min to 85% A, 5-7.5 min to 80% A, 7.5-10 min to 75% A, 10-11.5 min to 65% A, 11.5-13 min to 65% A, 13-14.5 min to 50% A, 14.5 16 min to 40% A, 16-19 min to 0% A, 19-21 min hold 0% A. Column flushing and equilibration were performed in additional methods with a methanol/water (85:15, v/v) solution. The MS conditions were as follows: heated electron spray ionization (HESI), positive mode, sheath gas, nitrogen at a flow rate of 41.5 arbitrary units (AU), auxiliary gas, nitrogen at a flow rate of 27.5 AU, vaporizer temperature 300°C, spray voltage 2.00 kV; ion transfer capillary temperature 380°C capillary voltage 16 V; tube lens voltage 75 V.

Collision induced dissociation (CID)-MS/MS experiments were performed on precursor ions selected from MS1 using information-dependent acquisition (IDA): MS1 was performed in the full scan (FS) mode (*m/z* 100-800). MS2 and MS3 were performed in the CID mode: MS2 on the most intense signals from MS1; MS3 on the most and second most intense signals from MS2. Normalized collision energies were 35% and 40% in MS2 and MS3, respectively. Other settings were as follows: minimum signal threshold: MS1: 100 counts, MS2: 50 counts, isolation width, 1.5 u; activation Q, 0.25; activation time, 30 ms; dynamic exclusion mode, repeat counts 2, repeat duration 15 s, exclusion list 50, exclusion duration 15 s. For kinetic studies, collision-induced dissociation (CID)-MS^n experiments were performed on the following selected precursor ions from MS1 at *m/z* (u) 250, 256, 262, 276. The collision energies were the same as described before. For these studies, also the gradient was changed: 0-4 min from 98% A to 60% A, 4-4.01 min to 10% A, hold to 7 min, 7.01 min back to 98% A and hold for 3 min.

5 RESULTS AND DISCUSSION

5.1 Sample Preparation

The cleavage of conjugates was necessary before the extraction and GC-MS analysis in order not to neglect completely conjugated phase I metabolites and gentle enzymatic hydrolysis was used instead of acid hydrolysis. Enzymatic hydrolysis at elevated temperature (56°C) and short time (1.5 h) was previously successfully applied.[2, 5-8, 29]

Derivatization increased the sensitivity for detection of metabolites. In the present study acetylation was used in order to allow sensitive detection of possible primary or secondary amines and alcoholic and/or phenolic hydroxyl groups. This kind of derivatization has been provided to be useful in other metabolism studies and is favored for derivatization of metabolites with the above described chemical structures.[23, 30-32]

5.2. Identification of the Phase I Metabolites by GC-MS

The urinary metabolites of the substance MDPBP were identified by full-scan EI after GC separation.

The postulated structures of the metabolites were deduced from the fragments detected in the EI mode, which were interpreted in correlation to those of the parent compound according to the fragmentation patterns of other pyrrolidinophenones[7, 8, 29] and the general fragmentation rules described by e.g. McLafferty and Turecek[21] and Smith and Busch.[33] In order to verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded, but not shown in this study, because they contain strong molecular peaks (M^+H^+), in contrast to EI spectra. In addition, adduct ions typical for PICI with methane were produced ($M^+C_2H_5^+$, $M^+C_3H_5^+$). The gas chromatographic retention indices (RI) of the EI mass spectra were determined according to de-Zeeuw et al.[34] The EI mass spectra and the RI are shown in Fig. 3, the chemical structures and predominant fragmentation patterns of MDPBP and its metabolites in Fig. 4.

From the recorded mass spectra, the following metabolites (nos. in Fig. 3/4 are given in brackets) could be deduced from the acetylated and not acetylated urine extracts: MDPBP (1), MDPBP-M (deamino-oxo) (2), MDPBP-M (demethylenyl-methyl-deamino-oxo) (3), MDPBP-M (demethylenyl-deamino-oxo) (4), MDPBP-M (demethylenyl-methyl-deamino-oxo-dihydro) (5, 6), MDBPB-M (demethylenyl-deamino-oxo-dihydro) (7), MDPBP-M (demethylenyl-methyl-N,N-bis-dealkyl-HO-phenyl-dihydro) (10), MDPBP-M (demethylenyl-methyl-N,N-bis-dealkyl) (11), MDPBP-M (demethylenyl-methyl) (12, 13), MDPBP-M (pyrrolidinyl-oxo) (14), MDPBP-M (demethylenyl) (15), MDPBP-M (carboxy) (16), MDPBP-M (demethylenyl-methyl-pyrrolidinyl-oxo) (17), MDPBP-M (demethylenyl-methyl-HO-alkyl) (18, 20), MDPBP-M (demethylenyl-methyl-HO-phenyl) (19), MDPBP-M (HO-alkyl) (21), MDPBP-M (carboxy-oxo) (22) and MDPBP-M (demethylenyl-methyl-carboxy-oxo) (23). Metabolites nos. 5 and 6, 12 and 13, 18 and 20 were detected as diastereomers. The metabolite with the greatest abundance was supposed to be the 3´methylated compound (12) predicated on the assumption that the hydroxyl group at position 3´ was preferentially methylated by catechol-o-methyltransferase (COMT) as already described for related substances.[35]

Furthermore, the following artifacts were detected: MDPBP-M (demethylenyl-methyl-oxo) artifact (-4H) (8), MDPBP-M (demethylenyl-methyl) artifact (-4H) (9). This can be explained by the fact that the pyrrolidine ring could be twice oxidized, so that one or two double bounds were formed. Loss of two (artifact (-2H)) or four (artifact (-4H)) protons results in a decrease of the molecular mass of two or four units and corresponding ions.

PICI mass spectra with respective protonated molecular ions at acceptable abundance and the typical adduct ions could be detected for all metabolites excepting MDPBP-M (demethylenyl-deamino-oxo-dihydro) (7), MDPBP-M (demethylenyl-cmethyl-N,N-bis-dealkyl-HO-phenyl-dihydro) (10) and MDPBP-M (carboxy) (16). This could be explained by the fact that these metabolites were formed to minor dimension not sufficient for detection in PICI mode.

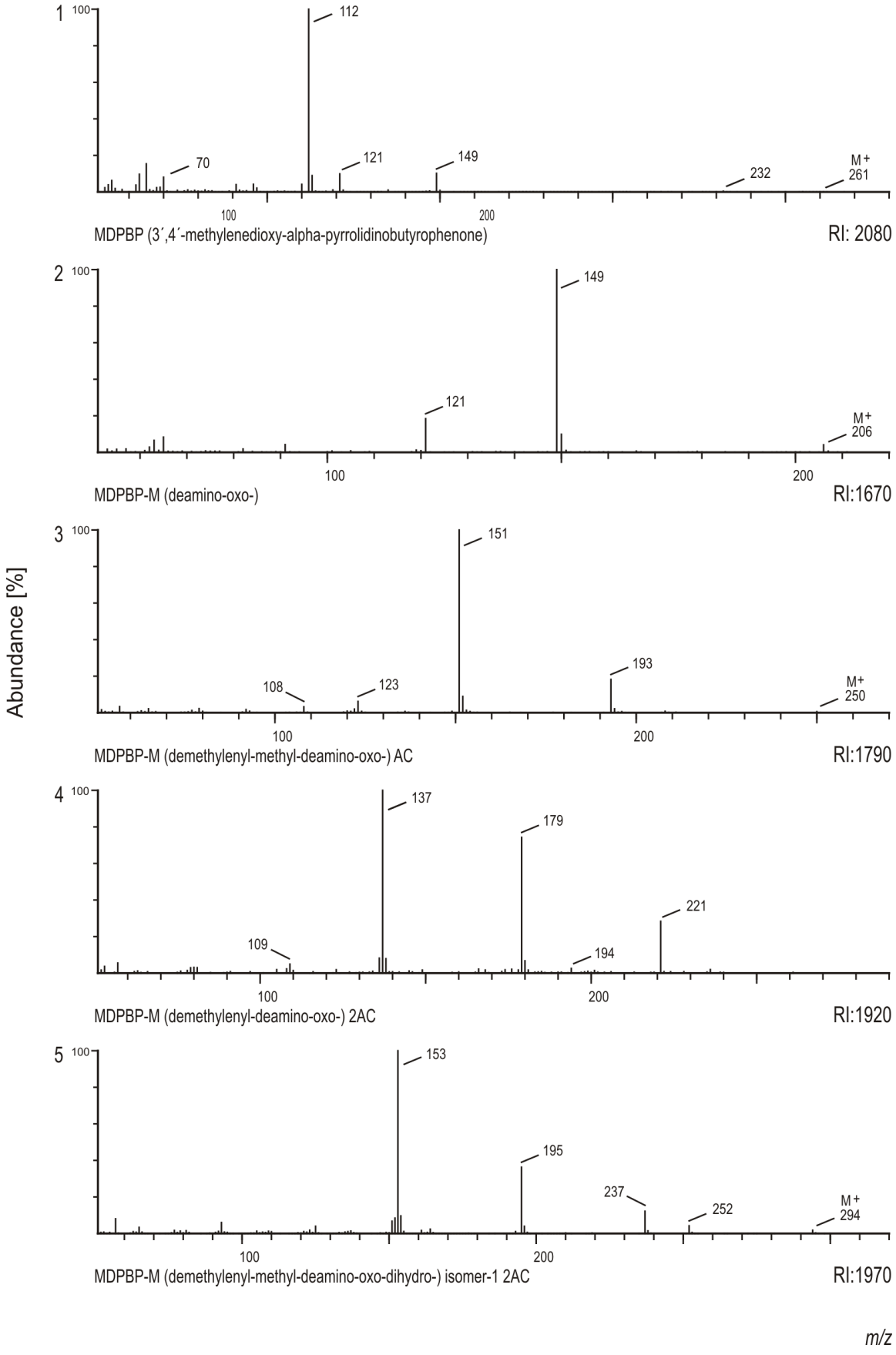

Figure 3: EI mass spectra and gas chromatographic retention indices (RI) of MDPBP and its phase I metabolites arranged according to their RI.

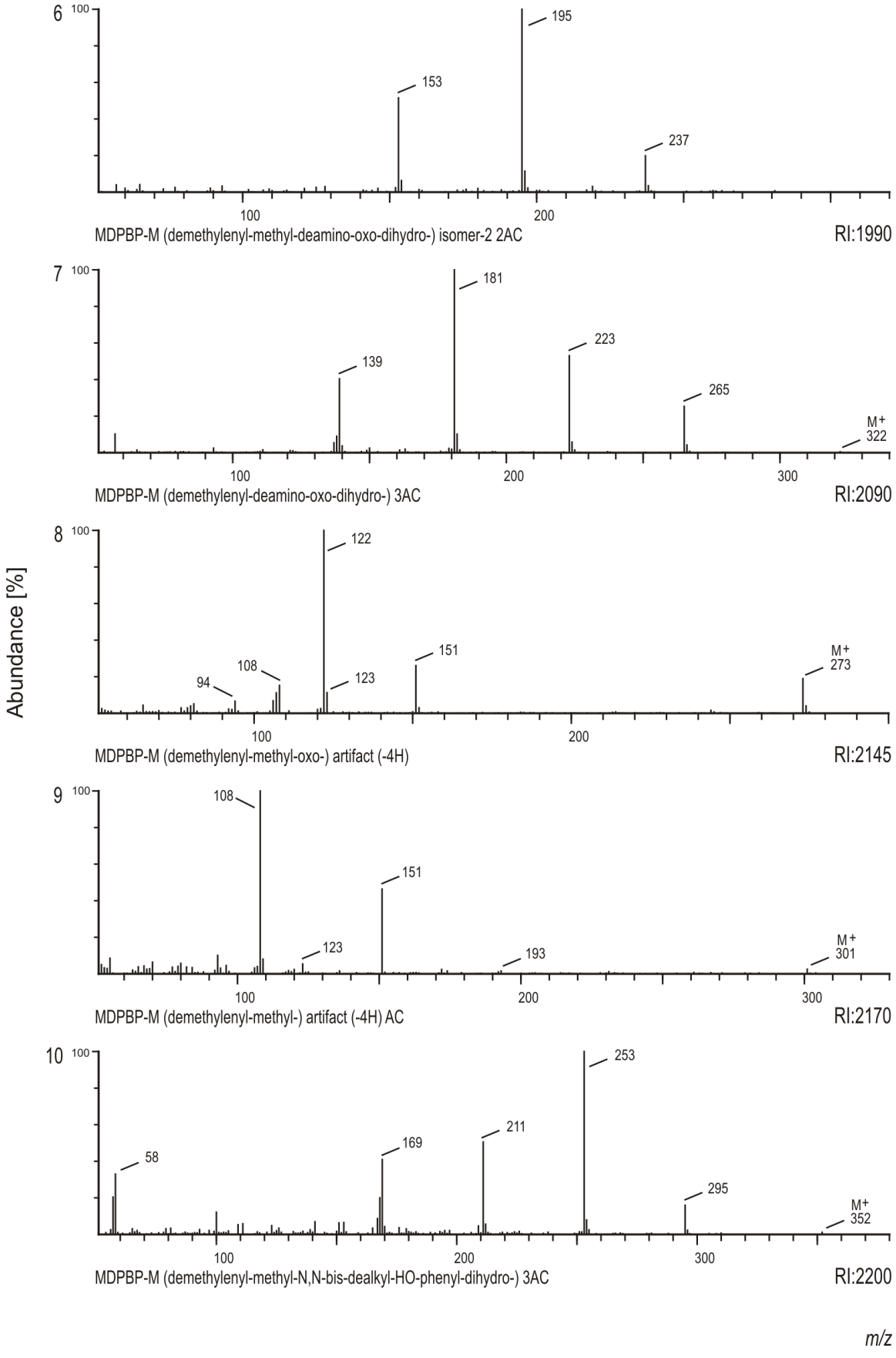

Figure 3: continued

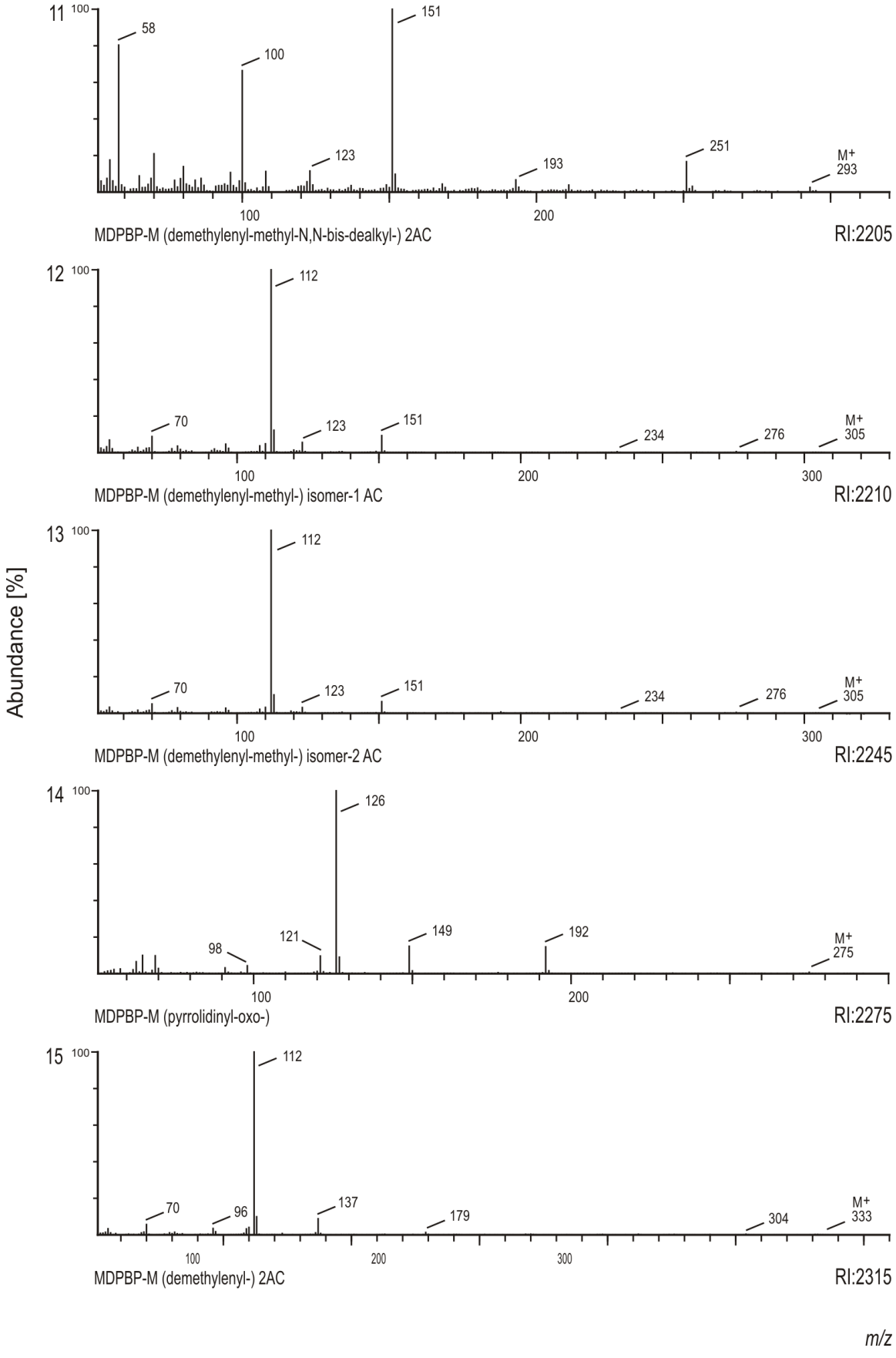

Figure 3: continued

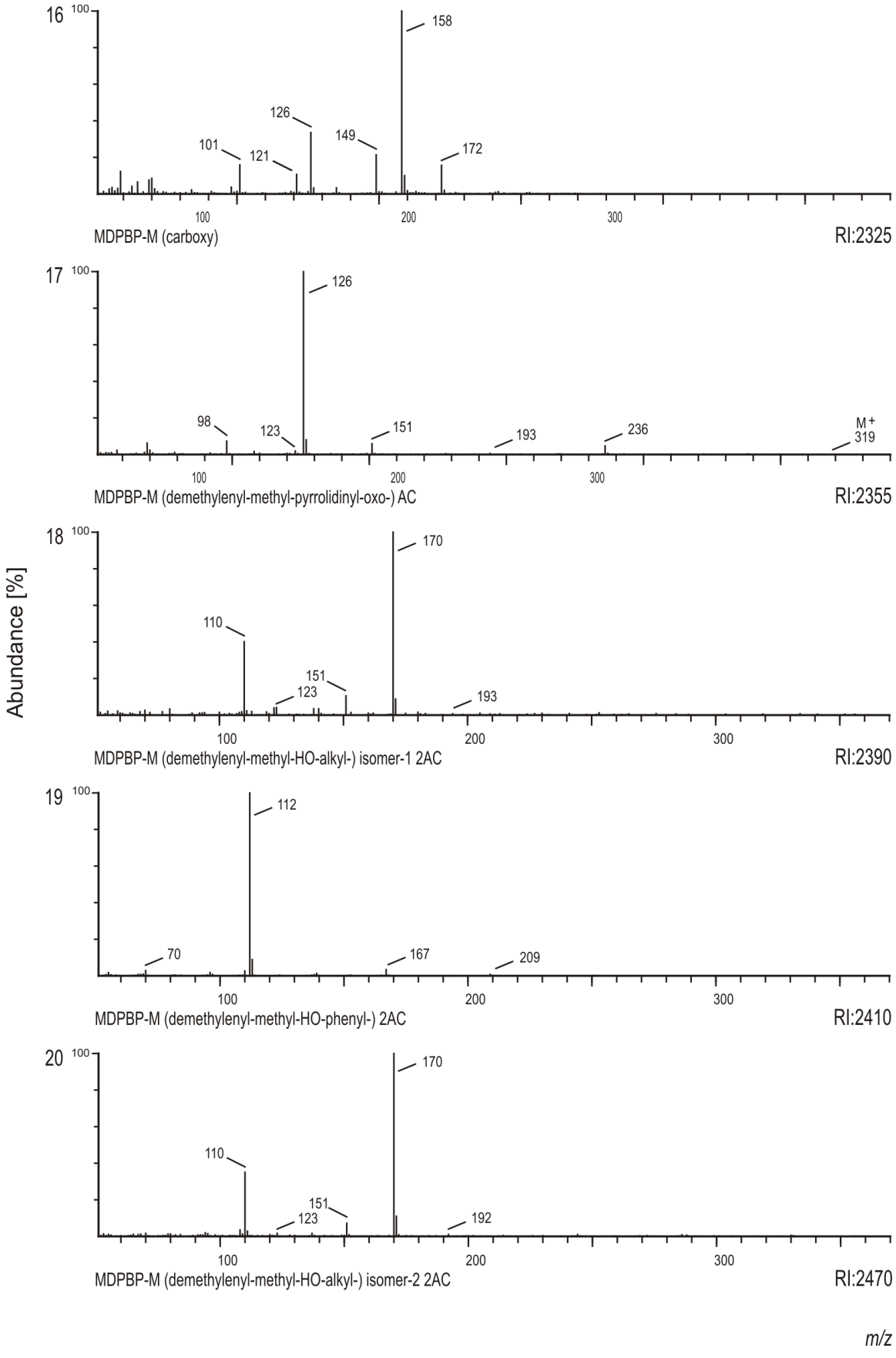

Figure 3: continued

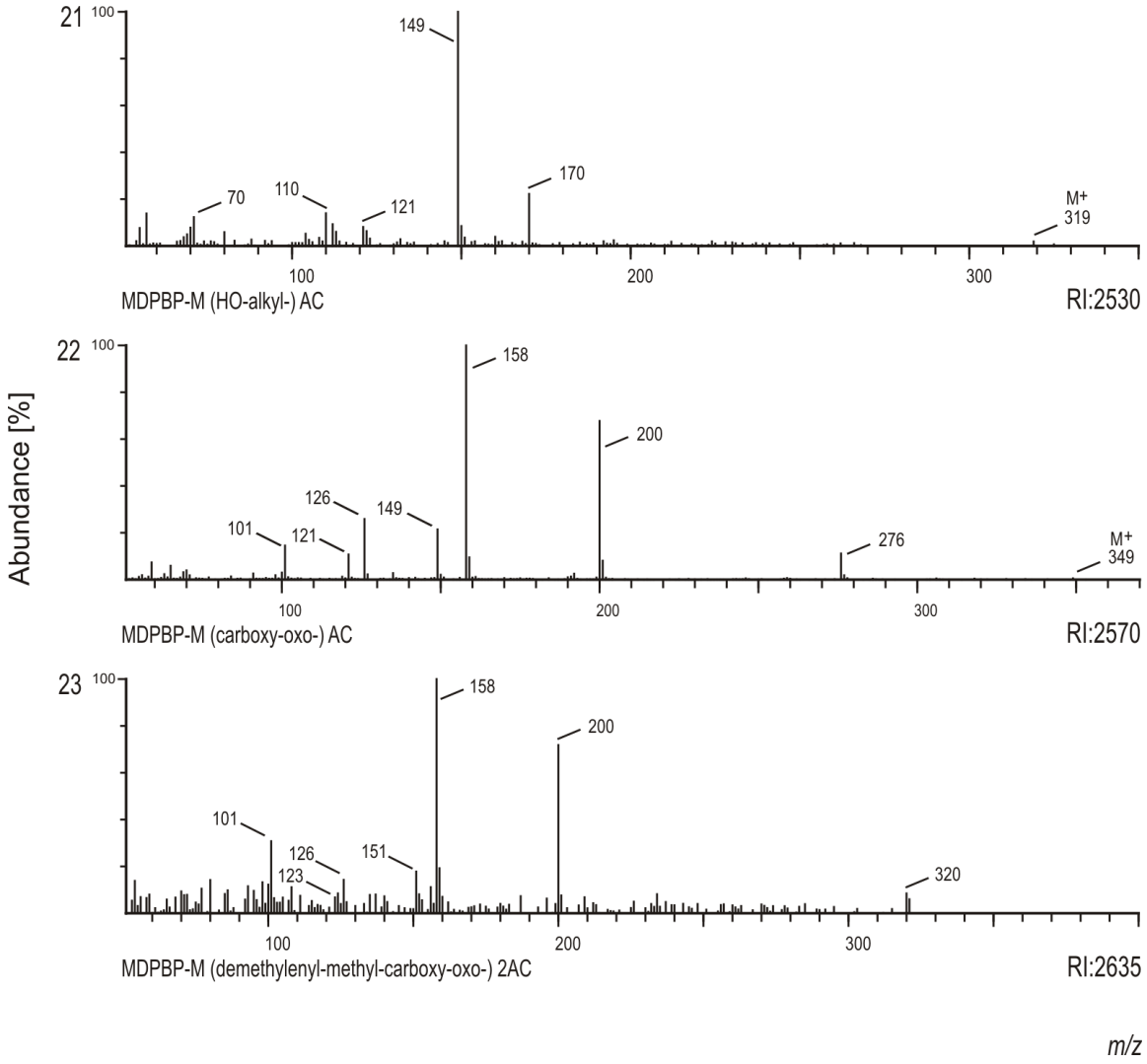

Figure 3: continued

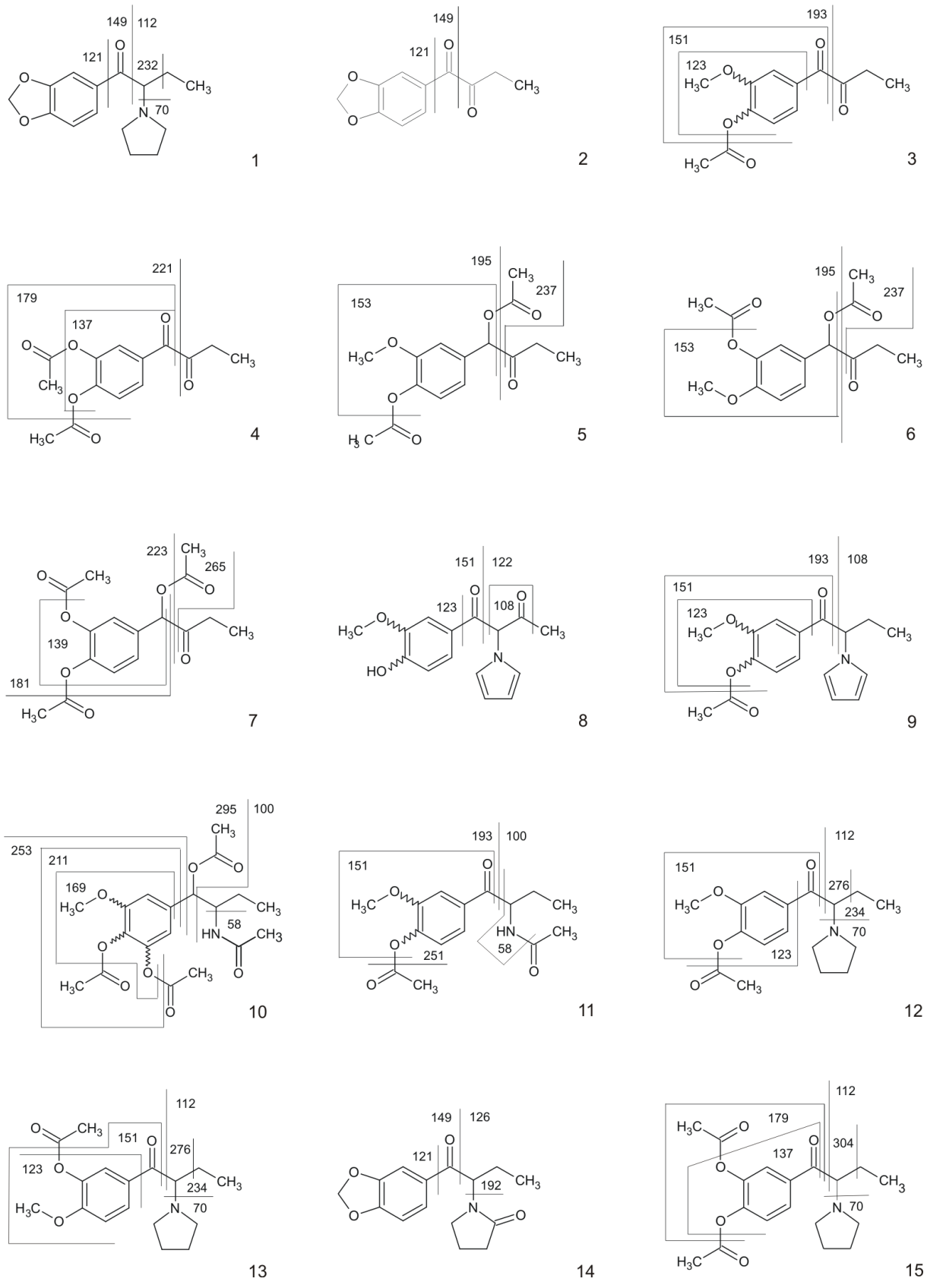

Figure 4: Proposed structures and predominant fragmentation patterns of MDPBP and its phase I metabolites.

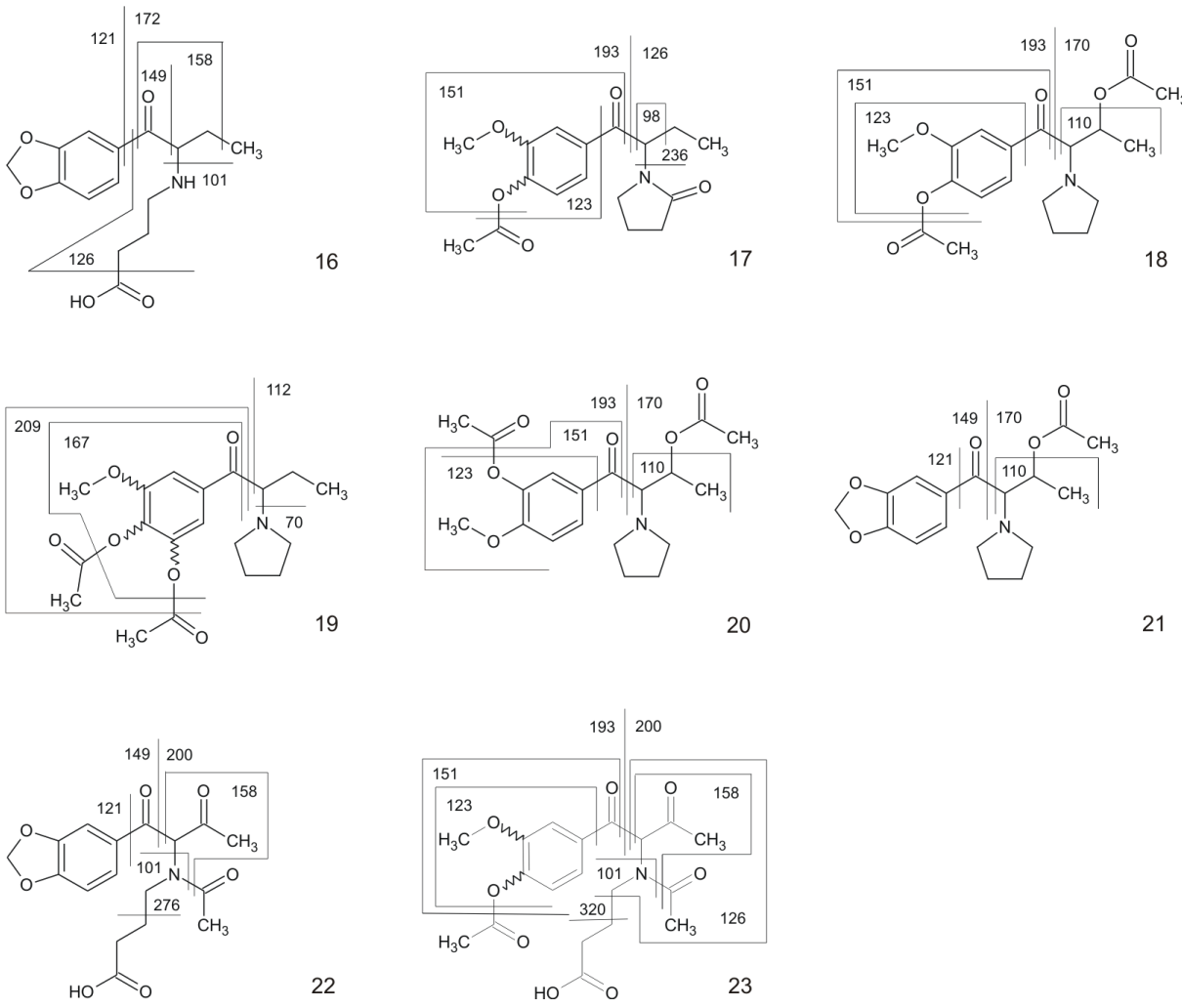

Figure 4: continued

5.3 Proposed Fragmentation Patterns for the Identification of the Phase I Metabolites by GC-MS

In the following, important fragmentation patterns of the EI mass spectra of MDPBP and its derivatized metabolites will be discussed in relation to the postulated metabolite structures depicted in Fig. 3 and Fig. 4. The numbers of the corresponding mass spectra in Fig. 3 and Fig. 4 are given in brackets.

The EI spectra showed comparable fragmentation characteristics like other alpha-pyrrolidinophenones.[7-9] Furthermore, the EI spectrum of the underivatized MDPBP was identical with the spectrum recorded by Westphal et al. (mass spectrum no. 1 in Fig. 3).[10]

The following fragmentation patterns could be observed (Fig. 5): alpha-cleavage between position one and two resulted in an ammonium ion with characteristic fragment ions at *m/z* 112 representing the base peak of the spectrum. Alternative alpha-cleavage between position one and two yielded to characteristic fragment ions at *m/z* 149, which are stabilized by mesomerism of the phenyl ring and the carbonyl moiety. A following CO loss led to characteristic fragment ions at ion *m/z* 121. The appearance of the fragment ions *m/z* 149 and *m/z* 121 in a mass chromatogram are indicating the presence of a methylenedioxybenzoyl moiety of the parent compound. Another alpha-cleavage reaction between position two and three yielded a methylenedioxybenzoyl-methylpyrrolidinyl cation with characteristic fragment ions at *m/z* 232. The pyrrolidine ring is represented by the fragment ions at *m/z* 70.

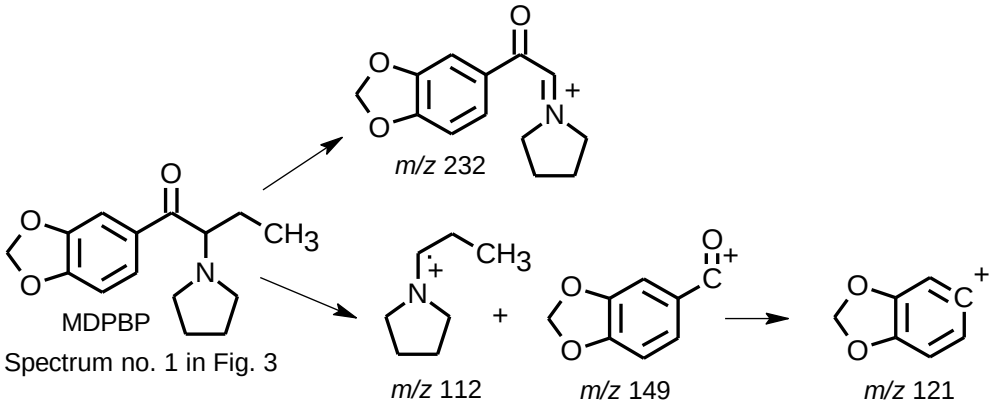

Figure 5: Characteristic EI fragmentation patterns of MDPBP.

In analogy to the parent compound, for all derivatized metabolites, an alpha-cleavage between position one and two could be noticed leading to characteristic fragment ions at *m/z* 112 (1, 12, 13, 15, 19), *m/z* 126 (14, 17), *m/z* 58 (2, 3, 4, 5, 6, 7), *m/z* 170 (18, 20, 21), *m/z* 200 (22, 23) or *m/z* 100 (10, 11). Alternative alpha-cleavage between position one and two yielded the following characteristic fragment ions at: *m/z* 149 (1, 2, 14, 16, 21, 22), *m/z* 137 (4, 15), *m/z* 151 (3, 11, 12, 13, 17, 18, 20, 23), *m/z* 153 (5, 6), *m/z* 139 (7) and *m/z* 167 (19). For acetylated metabolites, a shift of 42 u, 84 u (twice acetylated) or 126 u (triple acetylated) was observed.

The main metabolic steps are demethylenation of the methylenedioxybenzoyl moiety to the dihydroxy-metabolites followed by methylation. In analogy to the parent compound, an alpha cleavage between position one and two and a subsequent CO loss was observed in both derivatized metabolites (Fig. 6).

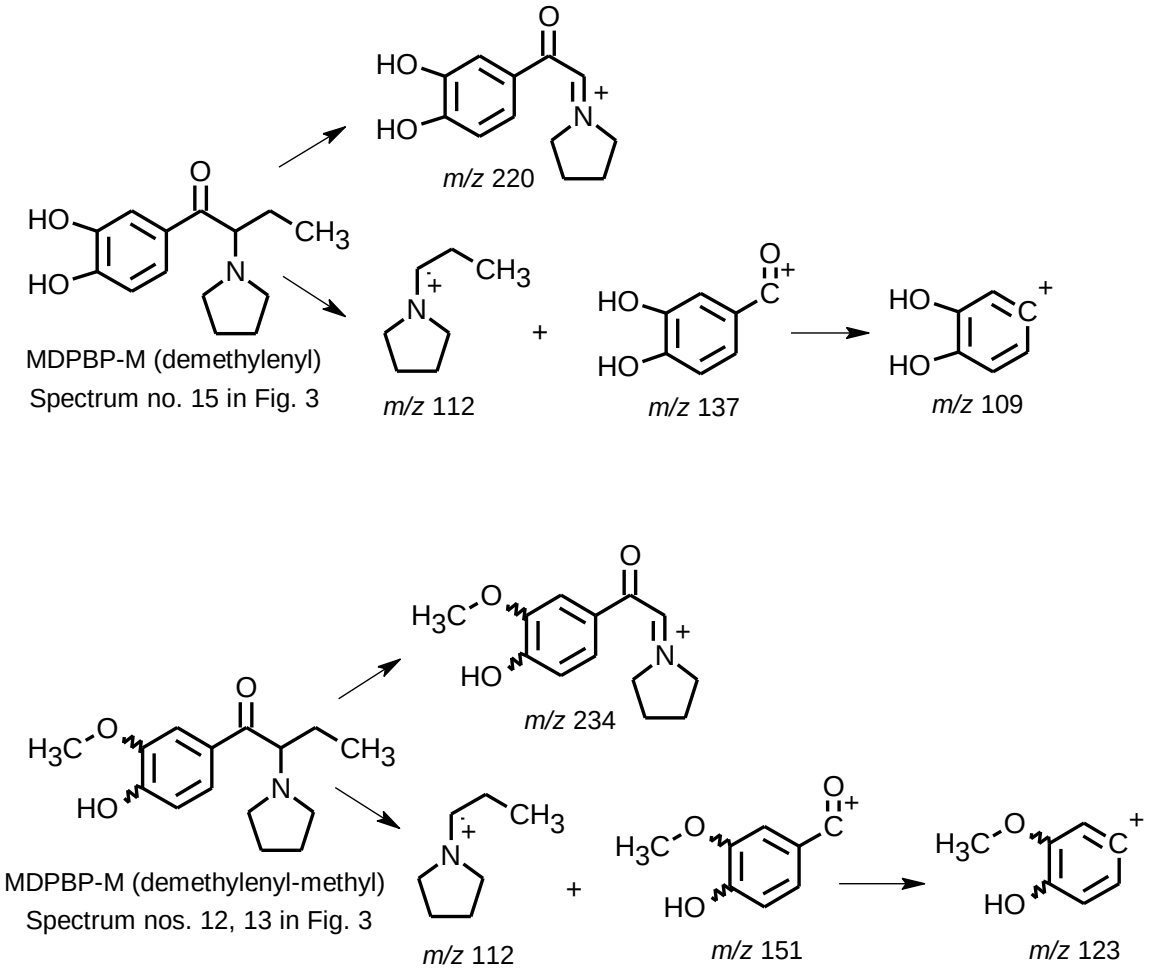

Figure 6: Characteristic EI fragmentation patterns of demethylenyl MDPBP (upper part) and demethylenyl-methyl MDPBP (lower part).

Demethylenation led to the metabolite MDPBP-M (demethylenyl) (mass spectrum no.15 in Fig. 3) with characteristic fragment ions at *m/z* 137 (alpha–cleavage) and characteristic fragment ions at *m/z* 109 (CO loss). Demethylenation and afterwards methylation yielded to MDPBP-M (demethylenyl-methyl) (mass spectra nos. 12, 13 in Fig. 3), which led to characteristic fragment ions at ion *m/z* 151 (alpha-cleavage) and fragment ions at *m/z* 123 (CO loss). So, appearances of the respective couples of ions in a mass chromatogram are indicating these metabolic steps. Because of the unaltered pyrrolidine ring in both metabolites, fragment ions at *m/z* 112 were still representing the base peak of the spectra. Both in acetylated and in not acetylated spectra two isomers of MDPBP-M (demethylenyl-methyl) could be identified (12, 13). They yielded almost the same mass spectra, but have different RI´s. However, it is not possible to

determine, which diastereomer belongs to which spectrum only based on fragmentation patterns. It could be supposed, that the diastereomer with greater abundance was the 3´-methylated, because former studies proved that this metabolic step is catalyzed by catechol-O-methyl transferase (COMT) leading to methylation of the hydroxyl groups in position 3´ preferentially.[8, 9]

Both metabolites could be derivatized by acetylation (Fig. 7). Via alpha-cleavage between position one and two in the acetylated metabolites, the following characteristic fragment ions were formed: *m/z* 193 (12, 13) in case of the demethylenyl-methyl-metabolites and *m/z* 221 in case of the demethylenyl-metabolites (15). Loss of acetyl moieties caused fragment ions at *m/z* 151 (12, 13), *m/z* 179 and *m/z* 137 (15).

In dependence of the above described alpha-cleavage between position two and three in the acetylated metabolites the following fragment ions could be detected: at *m/z* 276 (12, 13) and at *m/z* 304 (15). Loss of acetyl moieties led to fragment ions at *m/z* 234 (12, 13) and at *m/z* 262 (15).

The presence of fragment ions at *m/z* 276, 234, 193, and 151 indicates the acetylated type of MDPBP-M (demethylenyl-methyl) (12, 13) and the appearance of the fragment ions at *m/z* 304, 262, 221, 179 and 137 shows the twice acetylated type of MDPBP-M (demethylenyl) (15) (Fig. 7).

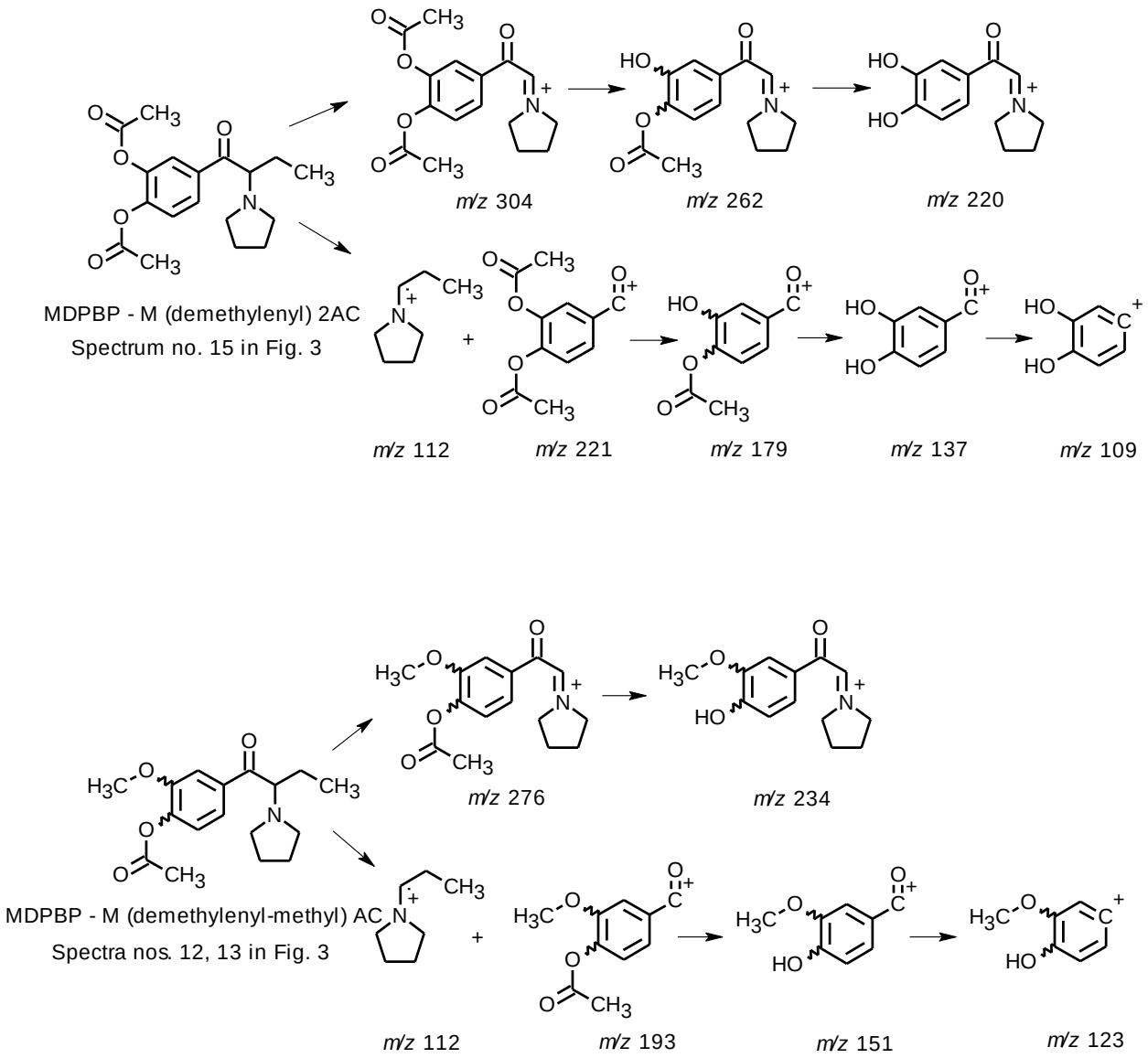

Figure 7: Characteristic EI fragmentation patterns of acetylated demethylenyl-methyl MDPBP (lower) and twice acetylated demethylenyl MDPBP (upper).

Another metabolic step was the oxidation of the pyrrolidine part or the butyl chain. The oxidation of the butyl-pyrrolidine moiety was represented by ions formed after alpha-cleavage between position one and two: fragment ions at *m/z* 126 (14, 17) for oxidation of the pyrrolidine part, which is depicted by a shift of 14 u (from fragment ions at *m/z* 112 to fragment ions at *m/z* 126), and fragment ions at *m/z* 170 (18, 20, 21) or fragment ions at *m/z* 200 (22, 23) for oxidation of the butyl chain. Subsequently, a neutral loss of acetic acid corresponding to 60 u led to fragment ions at *m/z* 110 (18, 20, 21). In case of oxidation of the pyrrolidine ring fragment ions at *m/z* 126 were representing the base peak of the mass spectra.

However, it must be mentioned that the exact position of the oxo group in pyrrolidinyl-oxo-metabolites could not be identified from the fragmentation patterns. In dependence on studies on the metabolism of other pyrrolidinophenones, the carbon atom was oxidized in position 2´ to lactam rather than in other positions, e.g. prolintane and nicotine are known for this.[2, 3, 5, 36-38]

From these data it could be deduced that metabolites 12, 13, 15 and 19 in Fig. 3 remained unchanged at the butyl-pyrrolidine moiety, which was in agreement with their spectra.

Further metabolic steps leading to carboxy-metabolites are the following: hydroxylation of the 2´-position of the pyrrolidine ring followed by ring opening to the respective aliphatic aldehyde that is further oxidized to the carboxylic acid (16, 22, 23) and finally degradation to primary amines (10, 11). These assumptions are in accordance with a similar metabolic pathway of phencyclidine or PVP, which was described by Holsztynska et al. and by Sauer et al.[8, 39] This perception approves again that the oxo group of the pyrrolidinyl-oxo-metabolites was indeed in position 2´, because such carboxy-metabolites could only be in the run if hydroxylation took place at this position. In case of carboxy-pyrrolidinyl-oxo-metabolites (22, 23), fragmentation was different from previously described ones. Alpha-cleavage between position one and two now resulted in the fragment ions at *m/z* 200 (22, 23). A shift of 42 u led to fragment ions at *m/z* 158, which implies a neutral loss of an acetyl group. This fragmentation suggested a ring opening of the pyrrolidine moiety leading to a carboxylic acid. After degradation to primary amine alpha-cleavage between position one and two yielded to fragment ions at *m/z* 100 (10, 11). A neutral loss of an acetyl group (shift of 42 u) led to the fragment ions at *m/z* 58. Similar metabolic steps were previously described for PVP and MDPPP.[8, 9]

Structurally related substances such as cathinones[40] are known for their dihydro-metabolites, which are additionally excreted in human beings to a remarkable extent. Such metabolites could also be detected in GC-MS analysis of MDPBP (5, 6, 7, 10). As only acetylated dihydro-metabolites could be observed, alpha-cleavage between position one and two led to fragment ions, which were shifted to 44 u, because of reduction of the keto compound to the secondary alcohol (2 u) and subsequent acetylation (42 u).

Oxidative deamination led to corresponding 2´-oxo compounds (2, 3, 4, 5, 6, 7). Because of losing pyrrolidine ring, alpha-cleavage between position one and two

resulted in fragment ions at *m/z* 58. Acetylation of these metabolites was not possible. Characteristically for these metabolites was the loss of basic compounds with the consequence that there was no more nitrogen for ionization. Therefore, these metabolites could only be detected by GC-MS, but not by LC-MS^n.

Other detected metabolites were formed by hydroxylation of the butyl chain or benzene ring. Based on the different fragmentation patterns, the detected hydroxyl-metabolites could be separated. Alpha-cleavage between position one and two of the acetylated HO-alkyl led to fragment ions at *m/z* 170 (18, 20, 21). A neutral loss of an acetic acid moiety yielded to fragment ions at *m/z* 110 (60 u shift). But the position of the hydroxyl group in the butyl side chain could not be exactly identified by fragmentation patterns. Similar problems had previously been described for other pyrrolidinophenones.[29] It was shown that short alkyl chains are most likely hydroxylated in position ω-1 at the penultimate carbon atom.[8] No acetylated HO-alkyl-metabolites could be found. Alpha cleavage between position one and two resulted in fragment ions at *m/z* 128, which were representing the base peak of the mass spectrum. Phenolic hydroxylation could also be detected (19). Alpha-cleavage between position one and two of the acetylated phenolic-metabolites resulted in the described fragment ions at *m/z* 112, which represented the base peak of the spectrum. Alternative alpha-cleavage at this position yielded to other fragmentation patterns than previously discussed. The following fragment ions were detected: at *m/z* 251, 209 and 167, which could be explained by loss of two acetyl moieties (42 u shift). Consequently, appearance of the respective ions allowed differentiation of HO-alkyl- and HO-phenyl-metabolites.

Moreover, the following artifacts were detected: MDPBP-M (demethylenyl-methyl) artifact (-4H) (9) and MDPBP-M (demethylenyl-methyl-oxo) artifact (-4H) (8). Alpha-cleavage between position one and two on the (-4H) artifacts led to fragment ions at *m/z* 108, which depicted the base peak of the spectrum (9). This phenomenon could be explained by building two double bounds into pyrrolidine ring, which resulted in a loss of four protons and causes the above described ion change of fragment ions at *m/z* 112 to fragment ions at *m/z* 108. Via the (-4H) artifact of MDPBP-M (demethylenyl-methyl-pyrrolidinyl-oxo) an oxo compound could be detected (8). Here, the oxo group could only be located at the butyl side chain and not at the pyrrolidine ring, because there was no more space for another compound.

5.4 Identification of the Phase II Metabolites by LC-MS^n

Glucuronides or sulfates were identified using LC-MS^n. The formation of the phase II metabolites could be confirmed by comparing the corresponding MS^n spectra of the phase II metabolites with the MS^{n-1} spectra of the phase I metabolites.

Using this approach, the following phase II metabolites (summarized also in Table 2) could be identified in rat urine (the numbers given in brackets correspond to those in Table 2): MDPBP-M (demethylenyl-glucuronide) (1, 2), MDPBP-M (demethylenyl-sulfate) (3), MDPBP-M (demethylenyl-methyl-pyrrolidinyl-oxo-glucuronide) (4), MDPBP-M (demethylenyl-methyl-glucuronide) (5, 6), MDPBP-M (demethylenyl-pyrrolidinyl-oxo-glucuronide) (7, 8), MDPBP-M (demethylenyl-methyl-HO-phenyl-glucuronide) (9, 10) and MDPBP-M (demethylenyl-methyl-HO-alkyl-glucuronide) (11).

Compound 1 and 2, 5 and 6, 7 and 8, 9 and 10 could be detected as diastereomers. However, the position of the glucuronic acid could not be exactly determined from fragmentation patterns and in case of diastereomers it was not possible to make a decision, which spectrum belongs to which diastereomer.

Table 2: Phase II metabolites of MDPBP with fragment ions of their MS1, MS2 and MS3 spectra and their nominal masses using LC-MS^n.

	Metabolite	MS	nominal mass
1	**MDPBP-M (demethylenyl-glucuronide) isomer-1**	MS1	426
		MS2 on 426	161, 179, 250
		MS3 on 250	72, 112, 123, 151, 161, 179, 250
		MS3 on 179	123, 133, 151
2	**MDPBP-M (demethylenyl-glucuronide) isomer-2**	MS1	426
		MS2 on 426	161, 179, 250
		MS3 on 250	72, 112, 123, 151, 161, 179, 250
		MS3 on 179	105, 123, 133, 151
3	**MDPBP-M (demethylenyl-sulfate)**	MS1	330
		MS2 on 330	179, 250
		MS3 on 250	72, 112, 123, 151, 161, 179, 250
		MS3 on 179	123, 151
4	**MDPBP-M (demethylenyl-methyl-pyrrolidinyl-oxo-glucuronide)**	MS1	454
		MS2 on 454	154, 278
		MS3 on 278	98, 126, 154, 193
		MS3 on 154	126
5	**MDPBP-M (demethylenyl-methyl-glucuronide) isomer-1**	MS1	440
		MS2 on 440	161, 193, 264
		MS3 on 264	112, 151, 161, 193, 264
		MS3 on 161	103, 133
6	**MDPBP-M (demethylenyl-methyl-glucuronide) isomer-2**	MS1	440
		MS2 on 440	161, 193, 264
		MS3 on 264	112, 151, 161, 193, 264
		MS3 on 193	161
7	**MDPBP-M (demethylenyl-pyrrolidinyl-oxo-glucuronide) isomer-1**	MS1	440
		MS2 on 440	126, 208, 264
		MS3 on 264	98, 126, 137, 154, 179, 264
		MS3 on 126	82, 98
8	**MDPBP-M (demethylenyl-pyrrolidinyl-oxo-glucuronide) isomer-2**	MS1	440
		MS2 on 440	126, 154, 264
		MS3 on 264	98, 126, 137, 154, 179, 264
		MS3 on 126	98
9	**MDPBP-M (demethylenyl-methyl-HO-phenyl-glucuronide) isomer-1**	MS1	456
		MS2 on 456	177, 209, 280
		MS3 on 280	112, 149, 167, 177, 209
		MS3 on 209	149, 177
10	**MDPBP-M (demethylenyl-methyl-HO-phenyl-glucuronide) isomer-2**	MS1	456
		MS2 on 456	177, 209, 280
		MS3 on 280	112, 149, 167, 177, 209
		MS3 on 209	177
11	**MDPBP-M (demethylenyl-methyl-HO-alkyl-glucuronide)**	MS1	456
		MS2 on 456	128, 177, 209, 280
		MS3 on 280	112, 128, 149, 161, 177, 209
		MS3 on 209	149, 177

5.5 Proposed Metabolic Pathways

By means of the identified and above described metabolites of MDPBP, the following partly overlapping pathways could be postulated (numbers in brackets correspond to those used in Fig. 3 and Fig. 4): Demethylenation of the methylenedioxy group (4, 7, 15), following by methylation of the dihydroxy-metabolites, most probably by COMT, to the corresponding demethylenyl-methyl compounds (3, 5, 6, 10, 11, 12, 13, 17, 18, 19, 20, 23), oxidative deamination to the corresponding 2´-oxo compounds (2, 3, 4, 5, 6, 7), hydroxylation of the butyl side chain (18, 20, 21) and oxidation to the corresponding ketones (22, 23), hydroxylation of the phenyl ring (10, 19), hydroxylation of the 2´-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactams (14, 17), hydroxylation of the 2´-position of the pyrrolidine ring followed by ring opening to the respective aliphatic aldehyde and further oxidation to the carboxylic acids (16, 22, 23), degradation of the pyrrolidine ring to the corresponding primary amines (10, 11) and reduction of the keto group to the corresponding secondary alcohols (5, 6, 7, 10). Metabolites 12, 13, 15, 17, 18, 19 and 20 were partly excreted as glucuronides and/or sulfates.

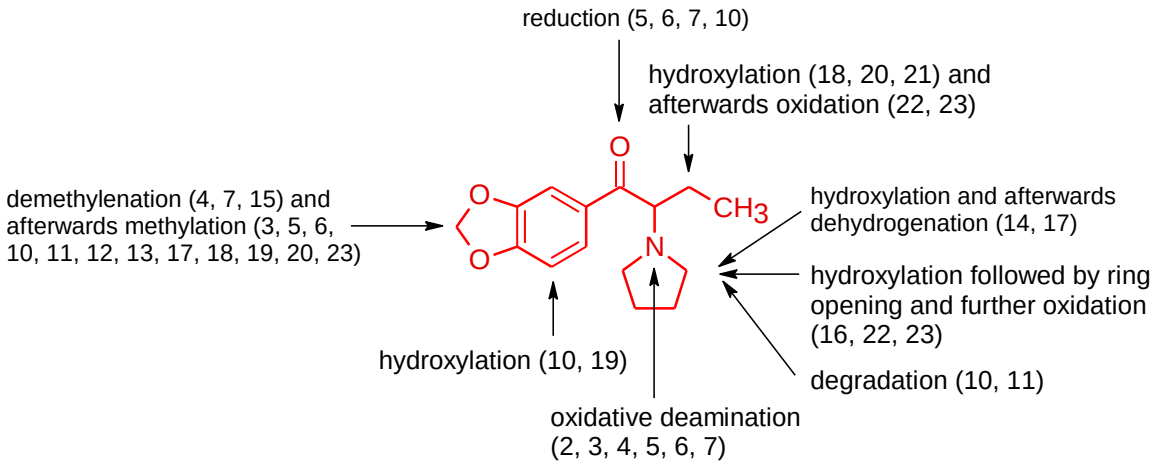

Figure 8: Proposed scheme for metabolism of MDPBP in rats. The numbering of the compounds corresponds to that of the mass spectra of the corresponding compound in Fig. 3.

5.6 Enzymatic Part

5.6.1 Initial CYP screening

The initial screening studies with the ten most abundant human hepatic CYP´s were used to identify their ability for catalyzing the formation of the initial metabolite, MDPBP-M (demethylenyl), in vitro. According to the supplier´s advice, the incubation conditions chosen were adequate to make a statement about the general involvement of a particular CYP enzyme. As shown in Fig. 9, the following CYP´s were in general capable to catalyze the demethylenation of MDPBP: CYP1A2, CYP2B6, CYP2C19, CYP2D6, CYP3A4 and CYP3A5.

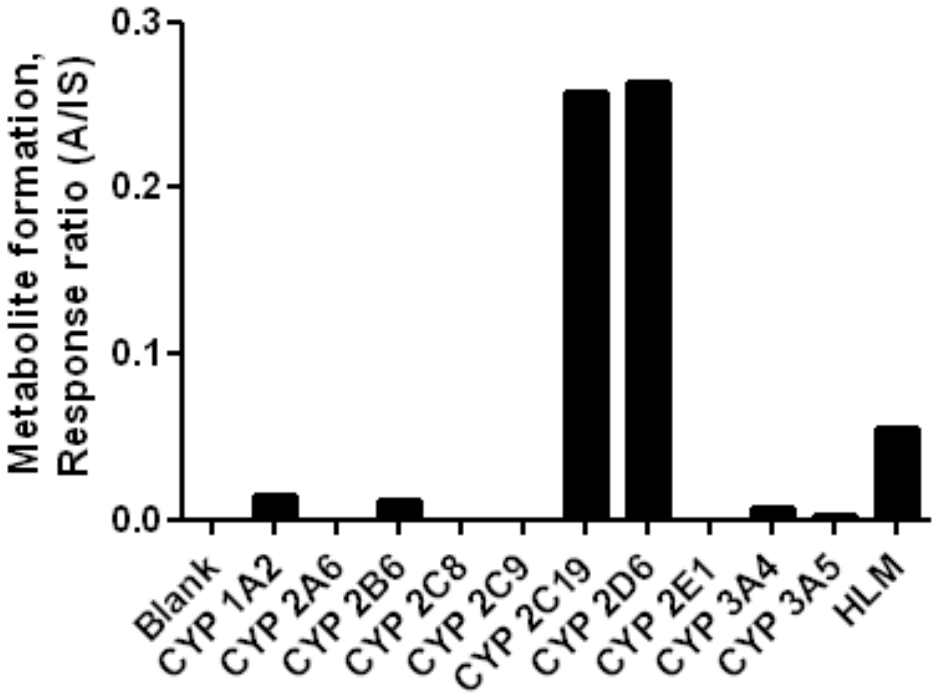

Figure 9: Metabolite formation (A/IS) of MDPBP-M (demethylenyl) formed after incubation of 25 µM substrate (37°C, 30 min) using human liver microsomes (50 mg protein/mL) and the ten most important recombinant CYP isoenzymes as enzyme source. Data are the average of two determinations.

5.6.2 Kinetic studies

The kinetic parameters were determined for the two most abundant CYP´s, which catalyze the demethylenation of MDPBP. The K_m values, which are representing the in vitro affinity of the particular P450s substrates and the V_{max} values are listed in Table 3. Both kinetic profiles fit well into Michaelis-Menten kinetics. In case of CYP1A2, CYP2B6, CYP3A4, and CYP3A5 activities were too low for calculation of kinetic parameters.

Table 3: Calculated K_m values (µM) and V_{max} values (au).

Enzyme	K_m for MDPBP-M (demethylenyl) formation	K_m for MDPBP-M (demethylenyl) formation
CYP2D6	12	4.25
CYP2C19	194	2

The net clearances for particular P450s at the four substance concentrations 0.1 µM, 1 µM, 10 µM and 100 µM were calculated to be 95%, 92%, 76% or 61% for CYP2D6 and 5%, 8%, 24% or 39% for CYP2C19. In conclusion, the net clearance data indicated that CYP2D6 was responsible for the main part of the total MDPBP CYP-dependent clearance and CYP2C19 is becoming more important at higher substrate concentrations.

5.6.3 Possible CYP interactions

Concerning the above described kinetic studies CYP2D6 and CYP2C19 turned out to be responsible for the initial metabolism step: the demethylenation of MDPBP. This is in accordance with previously published data of other pyrrolidinophenones with demethylenation as initial step like MDPV.[9] Therefore, it should be taken into account, that interactions might be possible between CYP2D6 or CYP2C19 and inhibitors of these enzymes, for example quinidine or 3´,4´-methylenedioxy-methamphetamine

(MDMA) (inhibitor of CYP2D6)[9, 41, 42] or fluconazole (inhibitor of CYP2C19).[9, 42] A common intake of such an inhibitor with MDPBP would lead to increased plasma concentrations and consequently increased adverse reactions. Additionally, genetic polymorphisms have to be taken into account and then have to be considered while interpreting plasma concentrations of the designer drug. CYP2D6 shows the largest variability in genetic polymorphism. This genetic basis of variability leads to different CYP2D6 functions: normal, reduced and non-existent, which causes a wide range from poor to ultra-rapid metabolizers.[43, 44]

Furthermore, inhibition studies are necessary to elucidate the inhibition potential of MDPBP. It was shown, that for example MDPV or MDMA, which are metabolized to a catechol by CYP2D6, are mechanism based inhibitors of CYP1A2 and CYP2D6.[9, 41] However, this question should be target of further studies.

6 CONCLUSIONS

The presented study about the new designer drug MDPBP demonstrates that this new drug was extensively metabolized by rats. Assuming similar kinetics between rats and humans, drug screening approaches should be focused on the metabolites and not on the parent compound. The metabolites showed similar fragmentation patterns than previous studies on alpha-pyrrolidinophenones. Both, GC-MS and LC-MS^n could be used for identifying phase I metabolites but GC-MS yielded better results and more metabolites could be found. Different CYP isoenzymes were found to be involved in the metabolism of MDPBP and these conclusions are in accordance with the results of other alpha-pyrrolidinophenone enzymatic studies.

7 REFERENCES

(1) Roesner P, Junge T, Fritschi G, Klein B, Thielert K, Kozlowski M: Neue synthetische Drogen: Piperazin-, Procyclidin- und alpha-Aminopropiophenonderivate. Toxichem Krimtech 1999; 66(2):81-90.

(2) Springer D, Fritschi G, Maurer HH: Metabolism of the new designer drug alpha-pyrrolidinopropiophenone (PPP) and the toxicological detection of PPP and 4'methyl-alpha-pyrrolidinopropiophenone (MPPP) studied in urine using gas chromatography-mass spectrometry. J Chromatogr B 2003; 796(2):253-266.

(3) Springer D, Peters FT, Fritschi G, Maurer HH: Studies on the metabolism and toxicological detection of the new designer drug 4'-methyl-alpha-pyrrolidinopropiophenone in urine using gas chromatography-mass spectrometry. J Chromatogr B 2002; 773(1):25-33.

(4) Springer D, Peters FT, Fritschi G, Maurer HH: GC-MS studies on the metabolism and toxicological analysis of the new pyrrolidino-hexanophenone designer drug 4'-methyl-alpha-pyrrolidinohexanophenone (MPHP); in: ed 14, 2002, p. 212.

(5) Springer D, Fritschi G, Maurer HH: Metabolism and toxicological detection of the new designer drug 4'-methoxy-a-pyrrolidinopropiophenone studied in rat urine using gas chromatography-mass spectrometry. J Chromatogr B 2003; 793(2):331-342.

(6) Springer D, Fritschi G, Maurer HH: Metabolism and toxicological detection of the new designer drug 3',4'-methylenedioxy-alpha-pyrrolidinopropiophenone studied in urine using gas chromatography-mass spectrometry. J Chromatogr B 2003; 793(2):377-388.

(7) Peters FT, Meyer MR, Fritschi G, Maurer HH: Studies on the metabolism and toxicological detection of the new designer drug 4'-methyl-alpha-pyrrolidinobutyrophenone (MPBP) in urine using gas chromatography-mass spectrometry. J Chromatogr B 2005; 824(1-2):81-91.

(8) Sauer C, Peters FT, Haas C, Meyer MR, Fritschi G, Maurer HH: New designer drug alpha-pyrrolidinovalerophenone (PVP): Studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. J Mass Spectrom 2009; 44(6):952-964.

(9) Meyer MR, Du P, Schuster F, Maurer HH: Studies on the metabolism of the alpha-pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) in rat urine and human liver microsomes using GC-MS and LC-high-resolution-MS and its detectability in urine by GC-MS. J Mass Spectrom 2010; 45(12):1426-1442.

(10) Westphal F, Junge T, Klein B, Fritschi G, Girreser U: Spectroscopic characterization of 3,4-methylenedioxypyrrolidinobutyrophenone: a new designer drug with alpha-pyrrolidinophenone structure. Forensic Sci Int 2011; 209(1-3):126-132.

(11) Westphal F, Junge T, Rosner P, Fritschi G, Klein B, Girreser U: Mass spectral and NMR spectral data of two new designer drugs with an alpha-aminophenone structure: 4'-Methyl-alpha-pyrrolidinohexanophenone and 4'-methyl-alpha-pyrrolidinobutyrophenone. Forensic Sci Int 2007; 169(1):32-42.

(12) Goldberg J, Gardos G, Cole JO: A controlled evaluation of pyrovalerone in chronically fatigued volunteers. Int Pharmacopsychiatry 1973; 8(1):60-69.

(13) Heimann H, Lukacs G: [Experimental psychological differentiation of the effect of 2 psychostimulants (F-1983 and amphetamine) in humans]. Psychopharmacologia 1965; 8(2):79-90.

(14) Soderholm S, Viukari NM, Rimon R: [The psychotropic effect of the Pyrovalerone (F-1983), placebo, the milieu therapy and mental condition of the patients in forensic psychiatry]. Nervenarzt 1976; 47(2):88-93.

(15) Fauquet JP, Morel E, Demarty C, Rapin JR: [Role of central catecholamines in the psychostimulant activity of pyrovalerone]. Arch Int Pharmacodyn Ther 1976; 224(2):325-337.

(16) Servin A, Fauquet JP, Jacquot C, Rapin JR: Effects of pyrovalerone on peripheral noradrenergic mechanisms. Biochem Pharmacol 1978; 27(12):1693-1694.

(17) Stille G, Ackermann H, Eichenberger E, Lauener H: [Comparative pharmacological study of a new central stimulant, 1-p-tolyl-1-oxo-2-pyrrolidino-n-pentane HCl]. Arzneim -Forsch 1963; 13:871-877.

(18) Holliday AR, Morris RB, Sharpley RP: Compound 84/F 1983 compared with D-amphetamine and placebo in regard to effects on human performance. Psychopharmacologia 1964; 6(3):192-200.

(19) Meltzer PC, Butler D, Deschamps JR, Madras BK: 1-(4-Methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (pyrovalerone) analogues: A promising class of monoamine uptake inhibitors. J Med Chem 2006; 49(4):1420-1432.

(20) Deniker P, Loo H, Cuche H, Roux JM: [Abuse of pyrovalerone by drug addicts]. Ann Med Psychol (Paris) 1975; 2(4):745-748.

(21) McLafferty FW, Turecek F: Interpretation of Mass Spectra, ed 4th. Mill Valley, CA, University Science Books, 1993.

(22) Roesner P: Mass Spectra of Designer Drugs (SpecInfo). New York NY, John Wiley & Sons, 2003.

(23) Maurer HH, Pfleger K, Weber AA: Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and their Metabolites, ed 4th Rev. Weinheim (Germany), Wiley-VCH, 2011.

(24) Clarke SE: In vitro assessment of human cytochrome P450. Xenobiotica 1998; 28(12):1167-1202.

(25) Crespi CL, Miller VP: The use of heterologously expressed drug metabolizing enzymes-state of the art and prospects for the future. Pharmacol Ther 1999; 84(2):121-131.

(26) Ewald AH, Ehlers D, Maurer HH: Metabolism and toxicological detection of the designer drug 4-chloro-2,5-dimethoxyamphetamine in rat urine using gas chromatography-mass spectrometry. Anal Bioanal Chem 2008; 390(7):1837-1842.

(27) Wissenbach DK, Meyer MR, Remane D, Weber AA, Maurer HH: Development of the first metabolite-based LC-MSn urine drug screening procedure - exemplified for antidepressants. Anal Bioanal Chem 2011; 400(1):79-88.

(28) Wissenbach DK, Meyer MR, Remane D, Philipp AA, Weber AA, Maurer HH: Drugs of abuse screening in urine as part of a metabolite-based LC-MS(n) screening concept. Anal Bioanal Chem 2011; 400(10):3481-3489.

(29) Springer D, Peters FT, Fritschi G, Maurer HH: New designer drug 4'-methyl-alpha-pyrrolidinohexanophenone: Studies on its metabolism and toxicological detection in urine using gas chromatography-mass spectrometry. J Chromatogr B 2003; 789(1):79-91.

(30) Staack RF, Fritschi G, Maurer HH: Studies on the metabolism and the toxicological analysis of the new piperazine-like designer drug N-benzylpiperazine in urine using gas chromatography-mass spectrometry. J Chromatogr B 2002; 773(1):35-46.

(31) Staack RF, Theobald DS, Paul LD, Springer D, Kraemer T, Maurer HH: In vivo metabolism of the new designer drug 1-(4-methoxyphenyl)piperazine (MeOPP) in rat and identification of the human cytochrome P450 enzymes responsible for the major metabolic step. Xenobiotica 2004; 34(2):179-192.

(32) Theobald DS, Fehn S, Maurer HH: New designer drug 2,5-dimethoxy-4-propylthiophenethylamine (2C-T-7): studies on its metabolism and toxicological detection in rat urine using gas chromatography/mass spectrometry. J Mass Spectrom 2005; 40(1):105-116.

(33) Smith RM, Busch KL: Understanding Mass Spectra - A Basic Approach. New York (NY), Wiley, 1999.

(34) de-Zeeuw RA, Franke JP, Maurer HH, Pfleger K: Gas Chromatographic Retention Indices of Toxicologically Relevant Substances and their Metabolites (Report of the DFG commission for clinical toxicological analysis, special issue of the TIAFT bulletin), ed 3rd. Weinheim, VCH publishers, 1992.

(35) Meyer MR, Maurer HH: Enantioselectivity in the methylation of the catecholic phase I metabolites of methylenedioxy designer drugs and their capability to inhibit catechol-O-methyltransferase-catalyzed dopamine 3-methylation. Chem Res Toxicol 2009; 22(6):1205-1211.

(36) Shin HS, Shin YS, Lee S, Park BB: Detection and identification of pyrovalerone and its hydroxylated metabolite in the rat. J Anal Toxicol 1996; 20(7):568-572.

(37) Springer D, Paul LD, Staack RF, Kraemer T, Maurer HH: Identification of cytochrome P450 enzymes involved in the metabolism of 3',4'-methylenedioxy-a-pyrrolidinopropiophenone (MDPPP), a designer drug, in human liver microsomes. Xenobiotica 2005; 35(3):227-237.

(38) Vickers S, Polsky SL: The biotransformation of nitrogen containing xenobiotics to lactams. Curr Drug Metab 2000; 1(4):357-389.

(39) Holsztynska EJ, Domino EF: Biotransformation of phencyclidine. Drug Metab Rev 1985; 16(3):285-320.

(40) Pokrajac M, Miljkovic B, Misailovic B: Mass spectrometric investigation of 2-aminopropiophenones and some of their metabolites. Rapid Commun Mass Spectrom 1991; 5(2):59-61.

(41) Heydari A, Yeo KR, Lennard MS, Ellis SW, Tucker GT, Rostami-Hodjegan A: Mechanism-based inactivation of CYP2D6 by methylenedioxymethamphetamine. Drug Metab Dispos 2004; 32(11):1213-1217.

(42) Indiana University Department of Medicine - Division of Clinical Pharmacology - Indianapolis: Drug Interactions - Cytochrome P450 System (http://medicine.iupui.edu/flockhart/). 2005.

(43) Ingelman-Sundberg M, Oscarson M, McLellan RA: Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. Trends Pharmacol Sci 1999; 20(8):342-349.

(44) Ingelman-Sundberg M: Polymorphism of cytochrome P450 and xenobiotic toxicity. Toxicology 2002; 181-182:447-452.

8 ABBREVIATIONS

AU	Arbitrary Units
cDNA	Complementary Deoxyribonucleic Acid
CID	Collision Induced Dissociation
COMT	Catechol-o-methyltransferase
CYP	Cytochrome P450
EI	Electron Ionization
FS	Full Scan
GC	Gas Chromatography
HCl	Hydrochloride
HESI	Heated Electron Spray Ionization
HLM	Human Liver Microsomes
HP	Hewlett Packard
HR	High Resolution
ICM	Insect Cell Microsomes
IS	Internal Standard
LC	Liquid Chromatography
LD50	Median Lethal Lose
MDPBP	3´,4´-methylenedioxy-alpha-pyrrolidinobutyrophenone
MDPPP	3´,4´-methylenedioxy-alpha-pyrrolidinopropiophenone
MDPV	Methylenedioxy-pyrovalerone
MDMA	3´,4´-methylenedioxy-methamphetamine
MOPPP	4´-methoxy-alpha-pyrrolidinopropiophenone
MPBP	4´-methyl-alpha-pyrrolidinobutyrophenone
MPHP	4´-methyl-alpha-pyrrolidinohexanophenone

MPPP	4´-methyl-alpha-pyrrolidinopropiophenone
MS	Mass Spectrometry
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NRG-1	Energy-1
pHLM	Pooled Human Liver Microsomes
PICI	Positive Ion Chemical Ionization
PPP	Alpha-pyrrolidinopropiophenone
PS	Probe Substrate
PVP	Alpha-pyrrolidinovalerophenone
RAF	Relative Aactivity Factor
RI	Retention Index
SPE	Solide Phase Extraction
TR	Turnover Rate

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