Designer drugs 2,5-dimethoxy-4-bromo-amphetamine (DOB) and 2,5-dimethoxy-4-bromo-methamphetamine (MDOB): studies on their metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques†

Andreas H. Ewald,1 Giselher Fritschi,2 Wolf-Rainer Bork3 and Hans H. Maurer1∗

1 Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Saarland, D-66421 Homburg (Saar), Germany
2 Hessisches Landeskriminalamt, D-65187 Wiesbaden, Germany
3 Landeskriminalamt Berlin, D-12101 Berlin, Germany

Received 29 September 2005; Accepted 31 December 2005

Studies are described on the metabolism and the toxicological analysis of the amphetamine-derived designer drug 2,5-dimethoxy-4-bromo-amphetamine (DOB) and its corresponding N-methyl analogue 2,5-dimethoxy-4-bromo-methamphetamine (MDOB) in rat urine using gas chromatographic/mass spectrometric techniques. The identified metabolites indicated that DOB was metabolized by O-demethylation followed by oxidative deamination to the corresponding ketone as well as deamination followed by reduction to the corresponding alcohol. Other metabolic pathways were O,O-bisdemethylation or hydroxylation of the side chain followed by O-demethylation and deamination to the corresponding alcohol. The expected oxo compound after deamination could not be detected. All metabolites carrying hydroxy groups were found to be partly excreted in the conjugated form. MDOB underwent O-demethylation, O,O-bisdemethylation, or hydroxylation of the side chain followed by O-demethylation. Additional N-demethylation to DOB occurred, including the above-mentioned metabolites. Again, all metabolites carrying hydroxy groups were found to be partly 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 of an intake of a dose of DOB and MDOB in rat urine that corresponds to a common drug user’s dose. Assuming a similar metabolism, the described STA procedure in human urine should be suitable as proof of an intake of DOB and MDOB. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: DOB; MDOB; metabolism; GC/MS; urinalysis

INTRODUCTION

Amphetamine (AM) and a wide variety of its derivatives such as methamphetamine (MA), 3,4-methylenedioxy-amphetamine (MDA), 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy), 3,4-methylene-dioxy-N-ethylamphetamine (MDEA), 1-(1',3'-benzodioxol-5'-yl)-2-butanamine (BDB), and N-methyl-benzodioxolylbutanamine (MBDB), are widely abused recreational drugs. In addition to these classical stimulants, dimethoxylated AMs such as 4-bromo-2,5-dimethoxy-amphetamine (DOB), its N-methyl analogue 4-bromo-2,5-dimethoxymethamphetamine (MDOB), 2,5-dimethoxy-4-iodo-amphetamine (DOI), or 2,5-dimethoxy-4-methyl-amphetamine (DOM), have appeared in the illicit drug market. These drugs have psychoactive properties.1,2 Structure–activity relationship studies revealed that the optimal hallucinogen-like activity was caused by the primary amine functionality separated from the phenyl ring by two carbon atoms, the presence of methoxy groups in position 2 and 5 of the aromatic ring, and a hydrophobic 4-substituent (alkyl, halogen, etc.).3 The methyl moiety in the α-position to the nitrogen atom is reported to be responsible for increased in vivo potency and duration of action.3

DOB was first synthesized by Alexander Shulgin, who also gave qualitative and quantitative comments on its effects in his book Pihkal.4 DOB was described to bind selectively to central 5-HT2 binding sites5 and to lead to vaso spasms.6 It has surfaced in the illicit drug market in the form of tablets or blotters.2,7 The street names of DOB were Golden...
Eagle or LSD-25. DOB became quite popular as a drug of abuse, especially in Australia and Italy.\textsuperscript{7,8} Some intoxications related to DOB have also been reported.\textsuperscript{9–11} Therefore, it was scheduled in the lists of controlled substances of many countries as well as by the UN in 1971. Only very few data are available for MDOB. It was first synthesized also by Alexander Shulgin,\textsuperscript{4} and described to possess 1 order of magnitude lower affinity to 5-HT\textsubscript{2} receptor binding sites than DOB.\textsuperscript{11}

Although some studies have been published on the metabolism of the often-abused amphetamine-derived designer drugs,\textsuperscript{12,13} the metabolism of DOB and MDOB has not been systematically studied so far. Only in a case report by Alexander Shulgin,\textsuperscript{4} and described to possess 1 order of magnitude lower affinity to 5-HT\textsubscript{2} receptor binding sites than DOB.\textsuperscript{11}

The investigations were performed using the urine of male rats (Wistar, Ch. River, Sulzflleck, Germany) for toxicological screening procedures, especially in urine, to have the knowledge of the metabolism of the compounds in question. Furthermore, data on the metabolism are needed for toxicological risk assessment because the metabolites may play a major role in the toxicity of a drug. Therefore, the aim of the study presented here was to identify DOB and MDOB metabolites in rat urine using GC/MS in the electron ionization (EI) and positive-ion chemical ionization (PICI) modes. In addition, the detectability of DOB, MDOB, and their metabolites within the authors' systematic toxicological analysis (STA) procedure in urine by GC/MS was studied.\textsuperscript{15–18}

**EXPERIMENTAL**

**Chemicals and reagents**

DOB was provided by Hessisches Landeskriminalamt (Wiesbaden, Germany) and MDOB by Landeskriminalamt Berlin (Berlin, Germany) for research purposes. All other chemicals and biochemicals were obtained from Merck (Darmstadt, Germany) and were of analytical grade.

**Urinary samples**

The investigations were performed using the urine of male rats (Wistar, Ch. River, Sulzflleck, Germany) for toxicological diagnostic reasons, according to the corresponding German law. They were administered a single 5 mg/kg body mass (BM) dose of DOB or a single 10 mg/kg BM dose of MDOB for metabolism studies by gastric intubation. For toxicological detectability studies, 0.05 mg/kg BM or 0.1 mg/kg BM dose, respectively, were administered to the rats (n = 2 for each dose, respectively). Urine was collected separately from the feces over a 24-h period. All samples were directly analyzed. Blank rat urine samples had been collected before drug administration to check whether they were free of interfering compounds.

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

A 5-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 100 μl 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 adjusted to pH 6–9 with 1 ml of 37% hydrochloric acid, 2 ml of 2.3 mol/l aqueous ammonium sulfate and 1.5 ml of 10 mol/l aqueous sodium hydroxide, and extracted with 5 ml of a dichloromethane–isopropanol–ethyl acetate mixture (1:1:3; v/v/v). After phase separation by centrifugation, the organic layer was evaporated to dryness under reduced pressure at 70 °C. The residue was either dissolved in 100 μl of methanol or was derivatized by acetylation with 100 μl of an acetic anhydride–pyridine mixture (3:2; v/v) for 5 min under microwave irradiation at about 440 W. After careful evaporation, the residue was dissolved in 100 μl of methanol. Aliquots (2 μl) of the derivatized extracts were injected into the GC/MS.

Another urine sample was worked up as described above, but the pH was adjusted to 4–5 with acetic acid prior to extraction. After reconstitution of the extraction residue in 50 μl of methanol, methylation was conducted with 50 μl of a solution of diazomethane in diethyl ether, synthesized according to the procedure of McKay et al.\textsuperscript{19} The reaction vials were sealed and left at room temperature for 30 min. Thereafter, the mixture was once again carefully evaporated to dryness under a stream of nitrogen, acetylated as described above, and finally redissolved in 50 μl of methanol. Again, 2 μl were injected into the GC/MS.

**Sample preparation for STA by GC/MS**

A 5-ml portion of urine was worked up as described for 4-MTA\textsuperscript{16} or 2C-T-7.\textsuperscript{17} After cleavage of the conjugates by acid hydrolysis and liquid–liquid extraction as described above, the sample was derivatized by acetylation. Aliquots (2 μl) of the derivatized extracts were injected into the GC/MS.

**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 a HP 5988B MS Engine 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 × 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 30/°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; PIC1 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 × 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 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.

**RESULTS AND DISCUSSION**

**Metabolism studies**

The urinary metabolites of DOB and MDOB were separated by GC and identified by EI MS and PICI MS after gentle enzymatic hydrolysis, extraction, and with or without acetylation. Acetylation was chosen as the derivatization step, because in the authors’ experience it is a versatile method for elucidation of the structures of metabolites.15–18 Furthermore, acetylation is the standard derivatization step in the authors’ STA. However, when using acetylation as derivatization procedure, physiologically N-acetylated metabolites cannot be differentiated from acetyl derivatives. For this particular question, the presence of N-acetylated metabolites was excluded in urine extracts after trifluoroacetylation (for experimental details see Ref. 17). For detection of possible acidic metabolites, the enzymatically cleaved urine samples were extracted at acidic pH followed by methylation and acetylation.

The postulated structures of the (derivatized) metabolites were deduced from the fragments detected in the EI mode, which were interpreted in correlation with those of the parent compound according to the rules described by e.g. McLafferty and Turecek21 and Smith and Busch.22 In order to verify the molecular mass of the postulated metabolites, PICI mass spectra were recorded, because they contain abundant peaks of the protonated molecule [M + H]⁺ with adduct ions typical for PICI using methane as reagent gas, [M + C₂H₅]⁺ and [M + C₃H₅]⁺. The EI and PICI mass spectra, the retention indices (RI), the structures, and predominant fragmentation patterns of DOB, MDOB, and their metabolites with or without derivatization are shown in Fig. 1. The number of the respective EI and PICI mass spectra in Fig. 1 are given in brackets. In the acetylated or native extract of the urine sample of the rat that was administered DOB, the following compounds could be identified: N-acetyl-DOB (no. 1), 1-(4-bromo-2,5-dimethoxyphenyl)acetone (no. 2), 1-(acetoxy-4-bromo-methoxy-phenyl)acetone (no. 3), 2-(4-bromo-2,5-dimethoxyphenyl)-1-methyl ethyl acetate (no. 4), 1-(acetoxy-4-bromo-methoxyphenyl)propionate-1,2-diyldiacetate (no. 5), two isomers of N-acetyl-acetoxy-4-bromo-methoxy AM (nos 6 and 8), 2-(acetylamino)-1-(4-bromo-2,5-dimethoxyphenyl)propyl acetate (no. 7), N-acetyl-2,5-diacetoxy-4-bromo AM (no. 9), and 2-(acetylamino)-1-(acetoxy-4-bromo-methoxyphenyl)propyl acetate (no. 10).

In the acetylated urine extract of the rat which was administered MDOB, the following compounds could be identified: N-acetyl-MDOB (no. 11), N-acetyl-DOB (no. 1), 1-(4-bromo-2,5-dimethoxyphenyl)acetone (no. 2), 1-(acetoxy-4-bromo-methoxyphenyl)acetone (no. 3), 2-(4-bromo-2,5-dimethoxyphenyl)-1-methyl ethyl acetate (no. 4), 1-(acetoxy-4-bromo-methoxyphenyl)propane-1,2-diyldiacetate (no. 5), two isomers of N-acetyl-acetoxy-4-bromo-methoxy AM (nos 6 and 8), 2-(acetylamino)-1-(4-bromo-2,5-dimethoxyphenyl)propyl acetate (no. 10), two isomers of N-acetyl-acetoxy-4-bromo-methoxy MA (nos. 12 and 13), N-acetyl-4-bromo-2,5-diacetoxy MA (no. 14), 2-(acetylmethylamino)-1-(4-bromo-2,5-dimethoxyphenyl)propyl acetate (no. 15), and 2-(acetyl(methyl) amino)-1-(acetoxy-4-bromo-methoxyphenyl)propyl acetate (no. 16). Unfortunately, PICI spectra of metabolite 5 and 9 could not be recorded because their concentrations might have been too low. No further metabolites could be identified in the acidic extracted urine sample.

In the following, possible fragmentation patterns of acetylated DOB, MDOB, and their postulated metabolites are discussed. The numbers of the respective mass spectra in Fig. 1 are given in brackets. N-Acetyl-DOB (no. 1) showed a molecular ion at m/z 315. Loss of acetamide may lead to a fragment ion of m/z 256. Benzyl cleavage may lead to fragment ions m/z 229 and m/z 86, and additional neutral loss of CH₂O or loss of two methyl moieties may lead to ion m/z 199. All fragment ions m/z 86 resulted from benzyl cleavage of the acetylated nitrogen-containing side chain of DOB or some of its metabolites, and are not mentioned in the following discussions. 1-(4-Bromo-2,5-dimethoxyphenyl)acetone (no. 2) showed a molecular ion of m/z 272 and a fragment ion of m/z 229 after benzyl cleavage, and additional loss of one methyl group may lead to m/z 214. 1-(Acetoxy-4-bromo-methoxyphenyl) acetone (no. 3) showed a molecular ion of m/z 300. Loss of the acetyl moiety may lead to the fragment ion m/z 258 and additional benzyl cleavage may lead to m/z 215. 2-(4-Bromo-2,5-dimethoxyphenyl)-1-methylethyl acetate (no. 4) showed a molecular ion of m/z 316. Loss of the acetyl moiety may lead to the fragment ion m/z 274. Benzyl cleavage and additional loss of one methyl moiety may lead to m/z 214. Loss of the side chain and one methoxy group may lead to m/z 186. 1-(Acetoxy-4-bromo-methoxyphenyl)propane-1,2-diyl...
diacetate (no. 5) showed a molecular ion of $m/z$ 402. Loss of an acetyl moiety leads to $m/z$ 360. Further loss of acetic acid may lead to $m/z$ 300. Benzyl cleavage and additional loss of an acetyl moiety may lead to $m/z$ 273 and finally benzyl cleavage with additional loss of two acetyl moieties may lead to $m/z$ 231. The 4-acetyl-acetoxy-4-bromo-methoxy AM isomers 1 and 2 (nos. 6 and 8) showed molecular ions of $m/z$ 343. Loss of an acetyl moiety may lead to a fragment ion $m/z$ 301. Loss of acetamide may lead to $m/z$ 284 and additional loss of the remaining acetyl moiety may lead to $m/z$ 242. Benzyl cleavage and loss of the acetyl moiety may lead to the fragment ions $m/z$ 215. The exact position of the metabolically formed hydroxy group could not be determined by means of GC/MS. This is indicated.
by the wavy binding lines in the molecular structures in Figs 1–3. However, since two isomers of this and some later compounds could be detected, it can be concluded that both methoxy groups were demethylated. 2-(Acetylamino)-1-(4-bromo-2,5-dimethoxyphenyl)propyl acetate (no. 7) showed a molecular ion of m/z 373. Loss of acetic acid may lead to m/z 313 and additional loss of the acetyl moiety may lead to m/z 271. N-Acetyl-4-bromo-2,5-diacetoxy AM (no. 9) showed a molecular ion of m/z 371. Loss of one acetyl moiety may lead to m/z 329 whereas loss of two acetyl moieties may lead to m/z 287. Loss of acetamide may lead to m/z 312, additional loss of one acetyl moiety may lead to m/z 270, and additional loss of two acetyl moieties may lead to m/z 228. Finally, the fragment ion m/z 201 may result after benzyl cleavage and loss of two acetyl moieties. 2-(Acetylamino)-1-(acetoxy-4-bromo-methoxyphenyl)propyl acetate (no. 10) showed a molecular ion of m/z 401. Loss of one and two acetyl moieties may lead to m/z 359 and m/z 317, respectively. Additional loss of acetamide may lead to the fragment ion m/z 258. N-Acetyl-MDOB (no. 11) showed a molecular ion of m/z 329. Loss of methylacetamide may lead to m/z 256. Benzyl cleavage may lead to m/z 229 and m/z 100, and additional loss of the methyl moieties of the two methoxy groups may lead to the ion m/z 199. Loss of the acetyl moiety of the fragment ion m/z 100 may lead to m/z 58. All fragment ions of m/z 100 and m/z 58 resulted always after benzyl cleavage from the acetylated side chain of MDOB containing methylated

Figure 1. (Continued).
nitrogen and are not mentioned in the following discussions of the metabolites of MDOB; but they are shown in Fig. 1. The N-acetyl-acetoxy-4-bromo-methoxy MA isomers 1 and 2 (nos. 12 and 13) showed molecular ions of m/z 357. Loss of an acetyl moiety may lead to a fragment ion m/z 315. Loss of methylacetamide may lead to m/z 284 and additional loss of the acetyl moiety may lead to m/z 242. Benzyl cleavage and loss of the acetyl moiety may lead to fragment ion m/z 215. N-Acetyl-4-bromo-2,5-diaceetoxy MA (no. 14) showed a molecular ion of m/z 385. Loss of one acetyl moiety may lead to m/z 343. Loss of methylacetamide may lead to m/z 312, and additional loss of one or two acetyl moieties may lead to m/z 270 or m/z 228, respectively. Benzyl cleavage and loss of the two acetyl moieties may lead to the fragment ion m/z 201. 2-(Acetyl(methyl)amino)-1-(4-bromo-2,5-dimethoxyphenyl)propyl acetate (no. 15) showed a molecular ion of m/z 387. Loss of methylacetamide may lead to m/z 314, and an additional loss of the acetyl moiety may lead to m/z 272. Neutral loss of CH2O or loss of the two methyl moieties of the fragment ion m/z 314 may lead to m/z 284, additional loss of the acetyl moiety may lead to m/z 242, and the subsequent benzyl cleavage may lead to m/z 215. 2-(Acetyl(methyl)amino)-1-(acetoxy-4-bromo-methoxyphenyl)propyl acetate (no. 16) showed a molecular ion of m/z 415. Loss of an acetyl moiety may lead to m/z 373. Loss of methylacetamide of fragment ion m/z 373 may lead to m/z 300 and additional loss of the remaining acetyl moiety may lead to m/z 258.
On the basis of these identified compounds, the following metabolic pathways of DOB, shown in Fig. 2, could be postulated: DOB undergoes single or double O-demethylation, oxidative deamination followed by reduction to the corresponding alcohol, hydroxylation of the side chain, and finally combinations of these steps. With the exception of the deamino-oxo metabolite (no. 2 in Fig. 2), all metabolites were partly conjugated by glucuronidation and/or sulfation. The metabolic pathways of MDOB are shown in Fig. 3 and are as follows: MDOB undergoes single or double O-demethylation, N-demethylation to DOB followed by oxidative deamination and reduction to the corresponding alcohol, hydroxylation of the side chain, and finally combinations of these steps. With the exception of the metabolites 7 and 9 of DOB, all other DOB metabolites were also formed by MDOB. Again, with the exception of the deamino-oxo metabolite, all metabolites were partly conjugated by glucuronidation and/or sulfation. Such a
conjugation was concluded because the peak areas of these metabolites were more abundant after glucuronidase and sulfatase hydrolysis.

Detection by GC/MS within the STA

As already discussed elsewhere, the authors' STA procedure has proved to be suitable for many different drug classes and allows a very comprehensive urine screening. After STA work-up, the extraction efficacy (mean \( \pm SD \), \( n = 5 \)) was determined to be 83 \( \pm 24\% \) and 107 \( \pm 14\% \) at 1000 ng/ml for DOB and MDOB, respectively. DOB, MDOB, and their metabolites were separated by GC and identified by a full-scan EI MS. Mass chromatography with the ions \( m/z \) 242, 86, 284, 256, and 315 was used to indicate the presence of acetylated DOB and its O-demethyl isomers. Mass chromatography with the ions \( m/z \) 58, 100, 256, 242, and 284 was used to indicate the presence of acetylated MDOB and its O-demethyl isomers. Figures 4
and 5 show typical reconstructed mass chromatograms with the given ions of acetylated extracts of rat urine samples collected over 24 h after application of 0.05 mg/kg BM of DOB or 0.1 mg/kg BM of MDOB, respectively. These doses corresponded to common users’ doses of about 1–3 mg or 6–8 mg, respectively. The identity of peaks in the mass chromatograms was confirmed by computerized comparison of the underlying mass spectrum with the reference spectra recorded during this study (Fig. 1). Although interference from other drugs cannot be entirely excluded, they are rather unlikely, because their mass spectra and/or their RIs should be different. As already mentioned, all DOB metabolites – with the exception of the metabolites 7 and 9 – were found also in urine after administration of MDOB. However, an intake of MDOB can be differentiated via its unique metabolites 11–16, all containing the N-methyl
Figure 3. Proposed scheme for the metabolism of MDOB in rats. The compounds in square brackets are assumed intermediates. The numbers correspond to those of the spectra, structures, and peaks shown in Figs 1, 2, 4, and 5.

Figure 4. Typical reconstructed mass chromatograms with the given ions of an acetylated extract of a rat urine sample collected over 24 h after intake of 0.05 mg/kg BM of DOB (a). They indicate the presence of DOB and its metabolites. EI mass spectrum underlying peak 6 in (b) is identified as an O-demethyl DOB isomer.

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moiety. The RIs were recorded during the GC/MS procedure and calculated in correlation with the Kovats’ indices of the components of a standard solution of typical drugs, which was measured daily for testing the GC/MS performance.

The limit of detection of DOB was 20 ng/ml and that of MDOB was 10 ng/ml (signal-to-noise \( S/N > 3 \)). Because of the lack of authentic human urine samples, a comparison of the metabolites found in rat and human urine after administration of DOB or MDOB was not yet possible. However, detection of an O-demethyl metabolite of DOB has been reported also in human urine. Other studies showed a good correspondence between the rat model and humans.

CONCLUSIONS

The metabolism studies presented here showed that the amphetamine-derived designer drugs DOB and MDOB were mainly metabolized by single or double O-demethylation. This was confirmed for DOB in humans. The authors’ STA procedure allowed the detection in rat urine of an intake of a dose of DOB and/or MDOB that corresponds to common drug users’ doses. As suggested in a case report, the target analytes in human urine after DOB consumption are the same as in rat urine, so that the described STA procedure should be suitable as proof of intake of DOB. Assuming similar metabolisms of DOB and MDOB, the described STA procedure of human urine should also be suitable as proof of intake of MDOB.

Acknowledgements

The authors wish to thank Dr. Frank T. Peters, Jochen Beyer, Christoph Bauer, Denis S. Theobald, Gabriele Ulrich, and Armin A. Weber for their support.

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