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On the Metabolism and the Toxicological Analysis of Methylenedioxyphenylalkylamine Designer Drugs by Gas Chromatography–Mass Spectrometry

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Summary: Designer drugs of the methylenedioxyphenylalkylamine type are increasingly abused. Studies on their metabolism in humans are necessary to develop a reliable gas chromatography–mass spectrometry (GC-MS) screening procedure. Such a method must allow their detection in urine for drug testing in clinical and forensic toxicology. Studies on racemic methylenedioxyamphetamine (MDA), methylenedioxymetamphetamine (MDMA), methylenedioxyethylamphetamine (MDE), benzodioxazolylbutanamine (BDB), and *N*-methylbenzodioxazolylbutanamine (MBDB) are presented. The metabolites were identified by GC-MS after enzymatic hydrolysis, isolation (pH 4.5 and 8–9), and derivatization (acetylation followed by methylation). The drugs undergo two overlapping metabolic pathways: *O*-dealkylation of the methylenedioxy group to dihydroxy derivatives followed by methylation of one of the hydroxy groups and successive degradation of the side chain to *N*-dealkyl and deaminooxo metabolites. MDA, MDMA, and MDE are subsequently metabolized to glycine conjugates of the corresponding 3,4-disubstituted benzoic acids. The hydroxy metabolites are excreted in a conjugated form. Based on these results, a GC-MS procedure was developed for simultaneous screening and identification of these designer drugs and/or their metabolites in urine after acid hydrolysis, isolation at pH 8–9, and acetylation. With use of mass chromatography with the most characteristic fragment ions *m/z* 58, 72, 86, 150, 162, 164, 176, and 178, the presence of the designer drugs was indicated and the peak underlying spectra could be identified by computerized comparison with reference spectra recorded during the presented studies. The procedure was suitable to detect an abuse of or an intoxication with the studied designer drugs (detection limit 5–50 ng/ml). **Key Words:** Designer drugs—Methylenedioxyphenylalkylamines—Metabolism—Analysis—Gas chromatography–mass spectrometry.

Designer drugs of the methylenedioxyphenylalkylamine type are widely abused as psychedelics. Methylenedioxyamphetamine [MDA; *R,S*-1-(3′,4′-methylenedioxyphenyl)-2-propanamine; "Love Pills"], methylenedioxymetamphetamine (MDMA; "Adam", "Ecstasy"), and methylenedioxyethylamphetamine (MDE; "Eve") as well as benzodioxazolylbutanamine [BDB; *R,S*-1-(1′,3′-benzodioxazol-5′-yl)-2-butanamine, *R,S*-1-(3′,4′-methylenedioxyphenyl)-2-butanamine] and *N*-methyl-benzodioxazolylbutanamine (MBDB) are able to enhance understanding, communicativeness, and empathy, almost without showing hallucinogenic effects (1,2). Nichols (3) described these substances as entactogens, a new drug class different from hallucinogenic phenylethylamines and phenylpropanamines (3). These substances have neurotoxic potency (4). The number of reports on abuse and intoxications with methylenedioxyamphetamine derivatives is increasing (5–11).

While the metabolism of MDA (12–14), MDMA

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(15–17), and MDE (18) has been investigated in rats, such studies in humans have not yet been published, with the exception of studies of MDMA (19). Studies on BDB and MBDB have not yet been reported.

Studies on the human metabolism of these designer drugs are performed for the following purposes: toxicological risk assessment and development of a gas chromatography–mass spectrometry (GC-MS) screening procedure. This procedure must allow the reliable detection of the drugs and/or their metabolites in urine specimens during clinical and forensic toxicological analysis.

The use of radioimmunoassay (20), enzyme immunoassay (21,22), and fluorescence polarization immunoassay (21,23) for screening of methylenedioxyamphetamine derivatives was studied. More specific procedures are, however, necessary for differentiation and positive identification of the amphetamines from nonscheduled drugs and some of their metabolites like, e.g., selegiline, with which the immunoassays also react (24). High performance liquid chromatographic procedures have been published (25–28), but their selectivity is not sufficient. GC-MS procedures have so far been published for the simultaneous detection of MDMA, amphetamine, and methamphetamine in urine (29,30). Noggle et al. (28) described problems arising in the mass spectral differentiation of underivatized MDMA and BDB.

In the following, results are presented on the metabolism and on the toxicological analysis of MDA, MDMA, MDE, BDB, MBDB, and their metabolites in urine by GC-MS.

MATERIALS AND METHODS

Urine Samples

Human urine samples were available from clinical and forensic cases. The exact amounts of the consumed drugs are not known. For the studies on MDE, samples were taken from healthy volunteers who ingested 140 mg of MDE while participating in a psychiatric study at the Department of Psychiatry, University of Freiburg, Germany (1). The reference substances of the designer drugs were synthesized by K.-A. Kovar (University of Tübingen, Germany).

Sample Preparation

Sample Preparation for Metabolism Studies

A 10-ml portion of urine was incubated at 37°C for 12 h with 100 μl of a mixture of glucuronidase and arylsulfatase, then adjusted to pH 8–9 and extracted with 10 ml of a mixture of dichloromethane/isopropanol/ethyl acetate (1:1:3). The organic layer was evaporated to dryness under a gentle nitrogen stream, and the residue was acetylated with 100 μl of acetic acid anhydride/pyridine (3:2) for 30 min at 60°C. After evaporation, the residue was dissolved in 100 μl of methanol. The same procedure with the exception of enzymatic hydrolysis was used to study whether MDE and its metabolites are excreted as enzyme-hydrolyzable conjugates. A further urine sample was prepared as described, but the extraction was performed at pH 4–5 with a mixture of ether/ethyl acetate (1:1) and the residue was methylated by diazomethane (31).

Sample Preparation for Systematic Toxicological Analysis

A 10-ml volume of urine was refluxed with 3 ml of 36% hydrochloric acid for 15 min. Following hydrolysis, the sample was basified with 4 ml of 10 mol/L aqueous sodium hydroxide, and the resulting solution was mixed with 10 ml of 30% aqueous ammonium sulfate to obtain a pH between 8 and 9. This solution was extracted and acetylated as already described.

GC-MS

A Hewlett-Packard (HP, Waldbronn, Germany) series 5890 gas chromatograph combined with an HP MSD series 5970 mass spectrometer and an HP MS ChemStation (DOS series) with HP G1034C software was used. The GC conditions were as follows: splitless injection mode; column, HP capillary (12 m × 0.2 mm I.D.), cross-linked methylsilicone, 330-nm film thickness; injection port temperature, 270°C; carrier gas, helium; flow rate, 1 ml/min; column temperature, programmed from 100 to 310°C, 20°C/min, initial time 3 min, final time 8 min; injection volume, 0.5–1 μl. The MS conditions were as follows: full scan mode; ionization energy, 70 eV; ion source temperature, 220°C; capillary direct interface heated at 260°C.

RESULTS AND DISCUSSION

The detected metabolites were identified by interpretation of the mass spectra of the postulated metabolites in correlation to that of the known parent compound according to the rules described by McLafferty and Turecek (32). As shown in Figs. 1

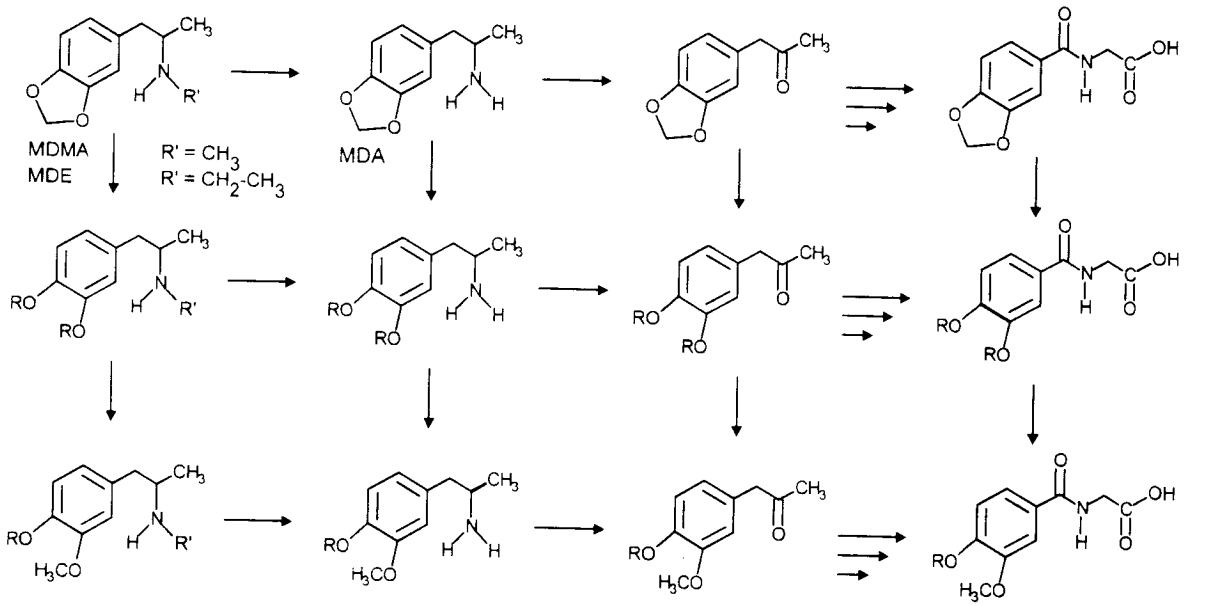

FIG. 1. Proposed scheme for the metabolism of the racemic methylenedioxyphenylpropanamine designer drugs MDA, MDMA, and MDE in humans. The hydroxy metabolites were also present as glucuronic acid and/or sulfate conjugates in urine. See text for abbreviations.

and 2, the methylenedioxyphenylalkylamine designer drugs undergo two overlapping metabolic pathways: *O*-dealkylation of the methylenedioxy group to dihydroxy derivatives followed by methylation of one of the hydroxy groups and successive degradation of the side chain to *N*-dealkyl and dea-

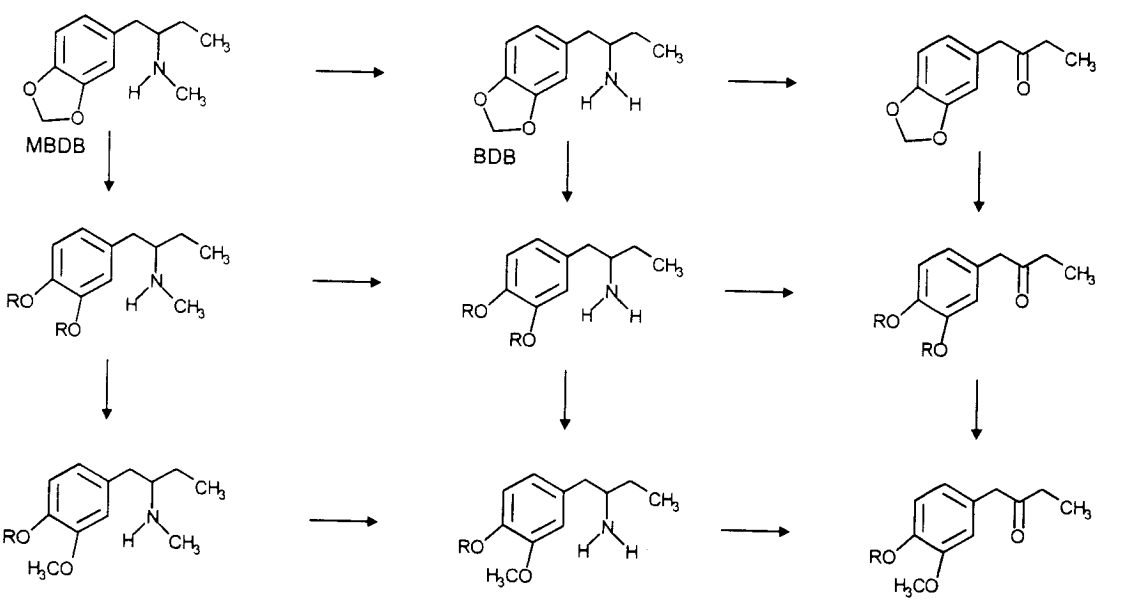

FIG. 2. Proposed scheme for the metabolism of the racemic methylenedioxyphenyl designer drugs BDB and MBDB in humans. The hydroxy metabolites were also present as glucuronic acid and/or sulfate conjugates in urine. See text for abbreviations.

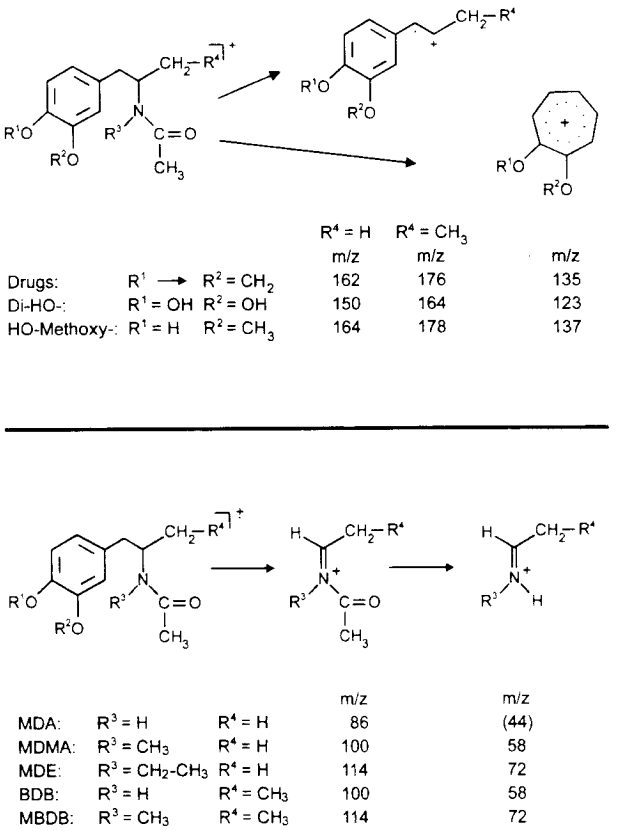

FIG. 3. Mass spectral fragmentation pattern of acetylated methylenedioxyphenylalkylamine designer drugs. The characteristic and predominant ions were selected for mass chromatography. MDA is also the *N*-dealkyl metabolite of MDMA and MDE; BDB is also the *N*-demethyl metabolite of MBDB. See text for abbreviations.

minooxo metabolites (33–35). In contrast to the butanamines BDB and MBDB, the propanamines MDA, MDMA, and MDE are additionally metabolized to glycine conjugates of the corresponding 3,4-disubstituted benzoic acids (hippuric acids) (33,34). However, it should not be overlooked that 3,4-dihydroxyhippuric acid and 3-methoxy-4-hydroxyhippuric acid can also be present in blank urine samples from a volunteer who has ingested benzoic acid, e.g., as a food preservative. This was described by Liebich and Foerst (36) and by Caldwell (37). The hydroxy metabolites of all the drugs were excreted in conjugated form. Detailed studies on the phase II metabolism of designer drugs are in progress (38).

Based on these results, a GC-MS procedure was developed for simultaneous screening and identification of these designer drugs and/or their metabolites in urine. For sample preparation, a procedure was used that has proved to be successful for several classes of drugs (39,40). Before isolation and derivatization, rapid acid hydrolysis was performed for cleavage of conjugates. This step was necessary because the hydroxy metabolites of the studied designer drugs are excreted in urine in a conjugated form. Since these metabolites are the only metabolites excreted in the late phase of excretion, the duration of detectability of the designer drugs can be extended. Derivatization was essential for sensitive detection of the polar drugs and their metabolites. For detection of the designer drugs and their metabolites, mass chromatography with the diagnostic ions *m/z* 58, 72, 86, 150, 162, 164, 176, and 178 was used. As shown in Fig. 3, these ions were selected for the characterization of the alkylamine side chains (58, 72, and 86 as products of a β-cleavage followed by onium reaction) and for the characterization of the ring systems (150, 162, 164, 176, and 178 as products of a McLafferty rearrangement). Five typical fragment ions and their relative abundances of the mass spectra used for the identification are given in Table 1. The full mass spectra are published elsewere (41–43). Generation of the mass chromatograms could be started by clicking the corresponding pull-down menu, which executes the user-defined macros. The identity of the peaks in the mass chromatograms was confirmed by computerized comparison of the peaks underlying full mass spectra with reference spectra recorded during the presented studies (43). Figure 4 shows the reconstructed mass chromatograms indicating the compounds that were identified in the urine of a patient who had ingested an unknown mixture of designer drugs. As shown, all the

TABLE 1. *Typical fragment ions and their relative abundances in mass spectra used for identification of designer drugs and their main metabolites*

Acetylated drug or metabolite					M^+
MDA	(44)[a]	86_{20}	135_{25}	162_{100}	221_7
MDMA	58_{100}	100_{20}	135_5	162_{20}	235_1
MDE	72_{100}	114_{20}	135_5	162_{40}	249_1
BDB	58_{100}	100_{20}	135_5	176_{25}	235_2
MBDB	72_{100}	114_{20}	135_5	176_{30}	249_1
MDA-M (di-HO-aryl-)	(44)[a]	86_{80}	123_{25}	150_{100}	293_1
MDMA-M (di-HO-aryl-)	58_{100}	100_{50}	123_{10}	150_{20}	307_1
MDE-M (di-HO-aryl-)	72_{100}	114_{40}	123_5	150_{10}	321_1
BDB-M (di-HO-aryl-)	58_{100}	100_{30}	123_5	164_{15}	307_1
MBDB-M (di-HO-aryl-)	72_{100}	114_{30}	123_5	164_{10}	321_1
MDA-M (HO-methoxy-aryl-)	(44)[a]	86_{30}	137_{20}	164_{100}	265_1
MDMA-M (HO-methoxy-aryl-)	58_{100}	100_{40}	137_5	164_{20}	279_1
MDE-M (HO-methoxy-aryl-)	72_{100}	114_{70}	137_5	164_{30}	293_1
BDB-M (HO-methoxy-aryl-)	58_{100}	100_{30}	137_{15}	178_{60}	279_1
MBDB-H (HO-methoxy-aryl-)	72_{100}	114_{40}	137_{10}	178_{25}	293_1

See text for abbreviations.
[a] Mass range for systematic toxicological analysis: *m/z* 50–550.

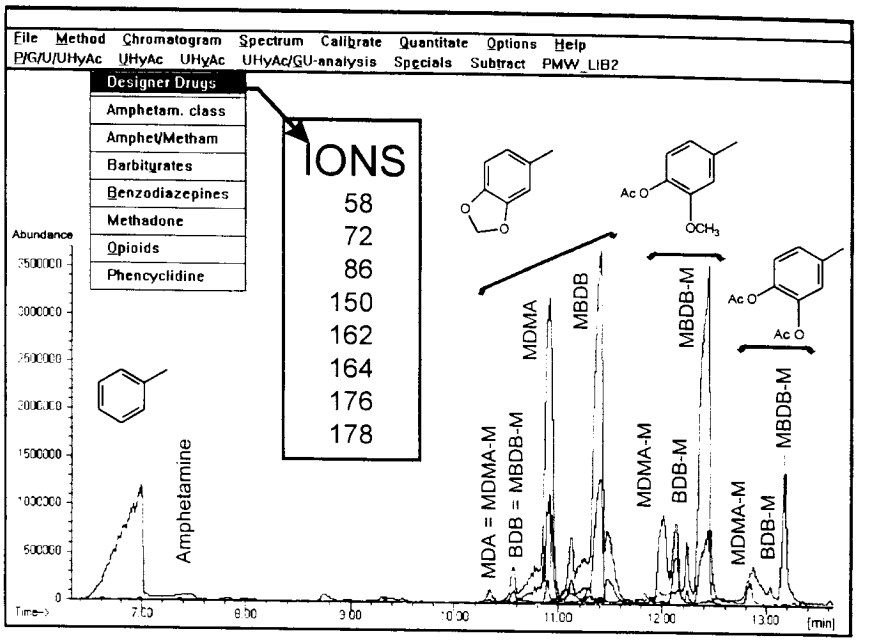

FIG. 4. Typical mass chromatograms with the ions *m/z* 58, 72, 86, 150, 162, 164, 176, and 178. They indicate the presence of amphetamine, MDMA, MBDB, and their main metabolites in an acetylated extract of a urine sample of a patient who had ingested an unknown mixture of designer drugs. The merged mass chromatograms can be differentiated by their colors on a color screen. See text for abbreviations.

drugs and their metabolites were sufficiently separated. Since their mass spectra are quite different, the given compounds could be confirmed. In contrast to the study of Noggle et al. (28), the acetylated spectra of MDMA and BDB can clearly be differentiated by computer library search, since the ion of the McLafferty rearrangement product (*m/z* 176) appears with a relative abundance of 25% in the spectrum of BDB. However, it cannot be differentiated whether the detected primary amines MDA and BDB were present as the *N*-dealkyl metabolites of MDMA, MDE, or MBDB or whether they were additionally taken. Since the ring *O*-dealkylation followed by methylation of the hydroxy group is the more prominent pathway than the side chain *N*-dealkylation, in the late phase of urinary excretion, the hydroxy methoxy metabolites of MDMA, MDE, and MBDB could be detected in contrast to the *N*-dealkyl metabolites. The hydroxy methoxy and the dihydroxy metabolite of MDE are common metabolites of ethylamphetamine. However, the detection of the parent drugs and/or further unique metabolites allowed the differentiation of the drugs. The limits of detection of the studied compounds ranged between 5 and 50 ng/ml of urine so that this procedure could also be used for the confirmation of positive immunoassay results (cutoff ~300 ng/ml). Interferences with other drugs or endogenous biomolecules are improbable since in our experience these compounds have different gas chromatographic and/or mass spectral properties (42).

The described analytical procedure is suitable to detect an abuse of or an intoxication with methylenedioxyphenylalkylamine designer drugs. This procedure has the further advantage that most of the toxicologically relevant drugs like barbiturates, benzodiazepines, opioids, analgesics, antidepressants, neuroleptics, antiparkinsonians, anticonvulsants, antihistamines, β-blockers, antiarrhythmics, and laxatives can simultaneously be detected (39,40) by starting the macro for generation of the corresponding selective mass chromatograms followed by library search of the peak underlying full mass spectra (43).

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