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METABOLISM OF RACEMIC 3,4-METHYLENEDIOXYETHYLAMPHETAMINE IN HUMANS

Isolation, Identification, Quantification, and Synthesis of Urinary Metabolites

HARTMUT K. ENSSLIN, HANS H. MAURER, EUPHROSYNE GOUZOULIS, LEOPOLD HERMLE, AND KARL-ARTUR KOVAR

Pharmaceutical Institute, University of Tuebingen (H.K.E., K.-A.K.), Institute of Pharmacology and Toxicology, University of Saarland (H.H.M.), Department of Psychiatry, University of Aachen, (E.G.), and Department of Psychiatry, Christophsbad, Goeppingen (L.H.)

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ABSTRACT:

Studies on the isolation, identification, quantification, and synthesis of the urinary metabolites of racemic 3,4-methylenedioxyethylamphetamine (MDE) in humans are presented. After oral administration of 140 mg of racemic MDE to healthy volunteers, the following phase I metabolites could be isolated and identified by GC/MS: unchanged racemic MDE (I), racemic 3,4-dihydroxyethylamphetamine (II), racemic 4-hydroxy-3-methoxyethylamphetamine (IIIa), racemic 3,4-methylenedioxyamphetamine (IV), racemic 3,4-dihydroxyamphetamine (V), racemic 4-hydroxy-3-methoxyamphetamine (VIa), methylenedioxyphenylacetone (VII), 3,4-dihydroxyphenylacetone (VIII), 4-hydroxy-3-methoxyphenylacetone (IXa), 3,4-methylenedioxyhippuric acid (X), and hydroxymethoxyhippuric acid (XII). The probable intermediate metabolite 3,4-dihydroxyhippuric acid (XI) could not be detected. Therefore, two overlapping phase I metabolic pathways for racemic MDE in humans could be postulated. The first and predominant pathway leads, *via* ring degradation by O-dealkylation, to the corresponding 3,4-dihydroxy metabolites, which are subsequently methylated at the hydroxyl group at position 3 of the aromatic ring. The second pathway leads, *via* side chain degradation by *N*-dealkylation, to the corresponding primary amines (IV, V, and VI). Oxidative *N*-deamination forms the substituted phenylacetones, which are degraded to the corresponding benzoic acids. This is followed by conjugation with glycine to form substituted hippurates. The structures of all of these metabolites were confirmed by chemical syntheses, which are described in this paper. All of the metabolites containing hydroxy groups are partly excreted in a conjugated form, because the amounts of these metabolites were much higher in urine extracts after enzymatic cleavage of conjugates. Quantification of the urinary excretion by HPLC revealed that 19% of the MDE dose was eliminated as I, 31.6% as IIIa, and 2.8% as IV within 32 hr.

Racemic MDE[1] (street name "Eve"), first described in 1980 (4, 5), is a designer drug with psychedelic properties. MDE and the homologous MDA ("love pills") and MDMA ("Adam" or "ecstasy") are able to enhance understanding, communicativeness, and empathy, almost without showing hallucinogenic effects. Nichols (6) described these substances in 1986 as entactogens, a new drug class different from hallucinogenic phenylethylamines and phenylpropaneamines. The use of entactogens for psychiatric exploration was discussed (7). Recently, the number of reports about abuse and intoxications with entactogens has increased (8–16). The substances have well-documented neurodegenerative effects on the central serotonergic system, which were studied in rats (17–23) and primates (24–28). The neurotoxic potency of MDE seems to be lower than that of MDA and MDMA (29–31). Because Molliver *et al.* (32) showed that no neurotoxic effects occurred after intracerebral application of MDMA, metabolites have been discussed as the neurotoxic compounds. Although the metabolism of MDE (33), MDA (34–36), and MDMA (37–39) has already been investigated in rats, as has that of MDMA in humans (40, 41), studies on the metabolism of MDE in humans have not yet been published. This paper describes qualitative and quantitative studies on the metabolism of MDE in humans using GC/MS and HPLC techniques. The detected metabolites were synthesized for confirmation of the structures and as standards for quantification.

[1] Abbreviations used are: MDE, 3,4-methylenedioxyethylamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; THF, tetrahydrofuran; DMSO, dimethylsulfoxide; EI, electron impact.

This paper is dedicated to Prof. Dr. Klaus Vianke, Homburg/Saar, Germany, on the occasion of his 60th birthday. Some of these results were reported at the annual meeting of the German Pharmaceutical Society in Berlin, Germany, October 1990 (1), and at the Symposium of the "Deutsche Gesellschaft für Suchtforschung," Blaubeuren, Germany, October 1991 (2), and they are part of the Ph.D. thesis of H.K.E. (3).

Send reprint requests to: Prof. Dr. Karl-Artur Kovar, Pharmazeutisches Institut der Eberhard-Karls-Universität Tübingen, Auf der Morgenstelle 8, D-72076

Materials and Methods

Materials. All chemicals (pro analysi or HPLC grade) and biochemicals used were obtained from Merck (Darmstadt, Germany), Boehringer Mannheim (Mannheim, Germany), or Aldrich (Steinheim, Germany). For synthesis of the amphetamine derivatives and the phenylacetone derivatives, the corresponding substituted benzaldehydes were used as precursors. For synthesis of 3,4-methylenedioxyhippuric acid (X) and 4-hydroxy-3-methoxyhippuric acid (XII), substituted benzoic acids were used as precursors. MDMA used as internal standard for the HPLC quantification was synthesized by Roesch (42).

Chemical Synthesis. The identity and the purity of all of the synthesized metabolites were confirmed by IR and ^{13}C-NMR spectroscopy and EI-MS after direct insertion (see *Apparatus*). The detailed spectral data are given below. *Synthesis of MDE (I) [85%, m.p. 186–187°C; Literature m.p. (4), 187–188°C] and MDA (IV) [88%, m.p. 201°C; Literature m.p. (4), 201–202°C].*

The synthesis of I was carried out according to the procedure of Braun *et al.*

crystals obtained with HCl gas were recrystallized from ethanol/diethyl ether (m.p. 201°C). IV was synthesized in the same manner but ammonium chloride was used instead of ethylammonium chloride.

Synthesis of Racemic 4-Hydroxy-3-methoxyethylamphetamine (IIIa) (65%, m.p. 146–147°C), Racemic 3-Hydroxy-4-methoxyethylamphetamine (IIIb) (60%, m.p. 171–174°C), and Racemic 3,4-Dibenzyloxyethylamphetamine (71%, m.p. 153–155°C). The syntheses were performed by modification of the procedure of Braun *et al.* (4). The precursors were dibenzyloxyphenylacetones, which were synthesized first.

Synthesis of Racemic 3,4-Dihydroxyethylamphetamine (II) (86%, m.p. 183–188°C). The synthesis was performed by catalytic hydrogenolysis of dibenzyloxyethylamphetamine hydrochloride, as described for VIb.

Synthesis of Racemic 4-Dihydroxyamphetamine Hydrochloride (V). The synthesis was carried out as described for VIb, by catalytic hydrogenolysis of 3,4-dibenzyloxyamphetamine hydrochloride [54%, m.p. 130–133°C; literature m.p. (43), 133–134°C], obtained from 3,4-benzyloxy-β-nitrostyrene (40%, m.p. 103–105°C) as described in ref. 44. The product was recrystallized with ethanol/diethyl ether from slightly gray-colored crystals melting at 195–197°C [literature m.p. (43), 192–194°C].

Synthesis of Racemic 4-Hydroxy-3-methoxyamphetamine (VIa). The intermediate 4-hydroxy-3-methoxy-β-methyl-nitrostyrene was synthesized according to the method of Gairaud and Lappin (44). The synthesis of VIa was carried out as described by Morgan and Beckett (45), with slight modifications. Ten millimoles of nitrostyrene in 50 ml of absolute THF were added dropwise into a suspension of 40 mmol of $LiAlH_4$ in 50 ml of THF. Decomposition of $LiAlH_4$ with ice water was followed by stirring for 12 hr at room temperature. The organic layer was separated. The aqueous solution was adjusted to pH 9–10 and extracted three times with 50 ml each of diethyl ether. THF and diethyl ether were combined and dried over Na_2SO_4. The organic solvent was removed by evaporation. HCl gas in etheral solution was used to form the hydrochlorides. Recrystallization with 2-propanol/diethyl ether yielded 20% [m.p. 259–264°C; literature m.p. (46), 259–260.5°C].

Synthesis of Racemic 3-Hydroxy-4-methoxyamphetamine Hydrochloride (VIb). This substance yielded white crystals [80%, m.p. 159–162°C; literature m.p. (38), 164–165°C] by hydrogenolysis of 3-benzyloxy-4-methoxyamphetamine hydrochloride, obtained from 3-benzyloxymethoxy-β-methylnitrostyrene in analogy to the modified procedure of Morgan and Beckett (45). Instead of benzene, 50 ml each of dry THF were used as organic solvent for 40 mmol of $LiAlH_4$ and for 10 mmol of nitrostyrene. After decomposition of $LiAlH_4$ with ice water, 50 ml of NaOH (10%) and 20 g of Celite were added. THF was separated and the filter cake was washed with 100 ml of diethyl ether. The combined organic fractions were dried over Na_2SO_4. After evaporation of the organic solvents, the residue was dissolved in 10 ml of diethyl ether and HCl gas was passed through the solution to form crystals of the hydrochloride. These were recrystallized with ethanol/diethyl ether (67%, m.p. 173–176°C). The catalytic hydrogenolysis was performed as described by Marshall and Castagnoli (43). In contrast to their method, room temperature and normal atmospheric pressure were sufficient for the catalytic hydrogenolysis.

Synthesis of 4-Hydroxy-3-methoxyphenylacetone (IXa) (40%, b.p. 93–94°C, 0.01 mbar), 3-Hydroxy-4-methoxyphenylacetone (IXb) (20%, b.p. 100°C, 0.015 mbar), and 3,4-Dibenzyloxyphenylacetone (52%, m.p. 240°C, 0.02 mbar). Syntheses were carried out in analogy to the procedure described by Morgan and Beckett (45).

Synthesis of 3,4-Dihydroxyphenylacetone (VIII) (80%, yellow-orange oil). Catalytic hydrogenolysis of 3,4-dibenzoyloxyphenylacetone was performed with the same modifications as described for VIb.

Synthesis of 3,4-Methylenedioxyhippuric Acid (X). This compound was synthesized according to the Schotten-Baumann reaction (47). The corresponding benzoylchloride (80%) was produced from a solution of 3,4-methylenebenzoic acid (12.5 g) and thionylchloride (70 g). The condensation with glycine was carried out with a modification of the procedure of Bayer *et al.* (48). The crude product (4 g) of the first step was dissolved in 20 ml of freshly distilled dioxane. This mixture was added dropwise to a cooled solution of 6.7 g of glycine in 20 ml of 3 M NaOH. The mixture was kept alkaline and was stirred for 2 hr. After addition of 20 ml of water, the pH was adjusted to pH 1 with concentrated hydrochloric acid. Then dioxane was evaporated. After recrystallization (in water) the yield was 35% (m.p. 187–192°C, white crystals).

Synthesis of 4-Hydroxy-3-methoxyhippuric Acid (XII) (60%, m.p. 103–107°C; Literature m.p. (40), 115°C). The synthesis was performed as described by Van Brussel and van Sumere (49).

IR, NMR, and MS Data for the Synthesized Compounds. *I-HCl.* IR: $\tilde{\nu}$/cm^{-1} 2973, 2941 (CH), 2787 (H_2C—O—CH_2), 1604, 1585, 1501, 1489 (C=C_{arom}), 1444 (δ-CH_3), 1246 (C—O—C), 1039 (O—C—O); ^{13}C-NMR (DMSO-d_6): δ 11.2 (q_d, J = 128 Hz, $NCH_2\underline{C}H_3$), 15.1 (q_d, J = 127 Hz, C-3), 38.1 (t, J = 130 Hz, C-1), 39.2 (t, J = 144 Hz, $N\underline{C}H_2CH_3$), 54.0 (d, J = 142 Hz, C-2), 100.9 (t, J = 175 Hz, $O\underline{C}H_2O$), 108.3 (d, J = 142 Hz, C-5'), 109.5 (dq_d, J = 163/6.7 Hz, C-2'), 122.4 (dq_d, J = 160/5.5 Hz, C-6'), 130.6 (sm, C-1'), 146.0 (sm, C-4'), 147.4 (sd, J = 6.7 Hz, C-3'); EI-MS of the free base: m/z 207 ($M^{+\cdot}$), 163 ($M-C_2H_6N$), 135 ($M-C_4H_8N$), 105 ($135-CH_2O$), 77 ($105-CO$), 72 ($M-C_3H_7O_2$, 100%).

II-HCl. IR: $\tilde{\nu}$/cm^{-1} 3420, 3284 (O—H), 1624, 1601, 1516 (C=C_{arom}), 1456 (δ-CH_3), 1181 (C—OH); ^{13}C-NMR (DMSO-d_6): δ 11.2 (q_d, J = 128 Hz, $NCH_2\underline{C}H_3$), 15.1 (q_d, J = 128 Hz, C-3), 37.9 (t, J = 128 Hz, C-1), 39.2 (t, J = 142 Hz, $N\underline{C}H_2CH_3$), 54.3 (d, J = 142 Hz, C-2), 115.8 (d, J = 158 Hz, C-5'), 116.7 (dd, J = 157/6.5 Hz, C-2'), 119.9 (dd, J = 158/5.5 Hz, C-6'), 127.5 (sd, J = 6.5 Hz, C-1'), 144.1 (st, J = 6.4 Hz, C-4'), 146.6 (sd, J = 4.3 Hz, C-3'); EI-MS of the free base: m/z 195 ($M^{+\cdot}$), 193 ($M-H_2$), 149 ($193-C_2H_6N$), 123 ($M-C_4H_{10}N$), 72 ($M-C_7H_7O_2$, 100%).

IIIa-HCl. IR: $\tilde{\nu}$/cm^{-1} 3149 (O—H), 2832 (O—CH_3), 1604, 1562, 1525 (C=C_{arom}), 1260 (C—O—Ph), 1156 (C—OH), 1035 (C—O—CH_3); ^{13}C-NMR (DMSO-d_6): δ 11.2 (q_d, J = 128 Hz, $NCH_2\underline{C}H_3$), 15.2 (q_d, J = 130 Hz, C-3), 38.1 (t, J = 127 Hz, C-1), 39.3 (t, J = 142 Hz, $N\underline{C}H_2CH_3$), 54.2 (d, J = 142 Hz, C-2), 55.7 (q_d, J = 144 Hz, 3'-$O\underline{C}H_3$), 113.4 (d, J = 156 Hz, C-2'), 115.5 (d, J = 158 Hz, C-5'), 121.5 (dd, J = 158/7.1 Hz, C-6'), 127.5 (sd, J = 7.0 Hz, C-1'), 145.4 (st, J = 7.2 Hz, C-4'), 147.6 (s, C-3'); EI-MS of the free base: m/z 209 ($M^{+\cdot}$), 164 ($M-C_2H_7N$), 122 ($164-C_3H_6$), 94 ($122-CO$), 137 ($M-C_4H_{10}N$), 72 ($M-C_8H_9O_2$, 100%).

IIIb-HCl. IR: $\tilde{\nu}$/cm^{-1} 3354 (O—H), 2845 (O—CH_3), 1622, 1591, 1510 (C=C_{arom}), 1246 (C—O—Ph), 1155 (C—OH), 1028 (C—O—CH_3); ^{13}C-NMR (DMSO-d_6): δ 11.2 (q_d, J = 128 Hz, $NCH_2\underline{C}H_3$), 15.0 (q_d, J = 130 Hz, C-3), 37.8 (t, J = 126 Hz, C-1), 39.2 (t, J = 143 Hz, $N\underline{C}H_2CH_3$), 54.2 (d, J = 144 Hz, C-2), 55.7 (q_d, J = 144 Hz, 4'-$O\underline{C}H_3$), 112.4 (d, J = 159 Hz, C-5'), 116.5 (d, J = 157 Hz, C-2'), 119.8 (dd, J = 159/7.1 Hz, C-6'), 129.3 (sd, J = 7.0 Hz, C-1'), 146.5 (s, C-4'), 146.6 (s, C-3'); EI-MS of the free base: m/z 209 ($M^{+\cdot}$), 164 ($M-C_2H_7N$), 122 ($164-C_3H_6$), 94 ($122-CO$), 137 ($M-C_4H_{10}N$), 72 ($M-C_8H_9O_2$, 100%).

IV-HCl. IR: $\tilde{\nu}$/cm^{-1} 2976, 2923 (CH), 2817 (H_2C—O—CH_2), 1610, 1582, 1502 (C=C_{arom}), 1443 (δ-CH_3), 1256 (C—O—C), 1042 (O—C—O); ^{13}C-NMR (DMSO-d_6): δ 17.4 (q_d, J = 127 Hz, C-3), 39.6 (t, J = 129 Hz, C-1), 48.2 (d, J = 139 Hz, C-2), 100.8 (t, J = 175 Hz, $O\underline{C}H_2O$), 108.3 (d, J = 165 Hz, C-5'), 109.5 (dq_d, J = 163/6.8 Hz, C-2'), 122.3 (dq_d, J = 160/5.9 Hz, C-6'), 130.6 (sd, J = 5.5 Hz, C-1'), 146.0 (st, J = 8.4 Hz, C-4'), 147.4 (sd, J = 4 Hz, C-3'); EI-MS of the free base: m/z 179 ($M^{+\cdot}$), 163 ($M-NH_2$), 136 ($M-C_2H_5N$, 100%), 135 ($M-C_2H_4N$), 105 ($135-CH_2O$), 77 ($105-CO$), 51 ($77-C_2H_2$).

V-HCl. IR: $\tilde{\nu}$/cm^{-1} 3404, 3354 (O—H), 1610, 1580, 1523, 1492 (C=C_{arom}), 1195 (C—OH); ^{13}C-NMR (DMSO-d_6): δ 17.4 (q_d, J = 127 Hz, C-3), 39.5 (t, J = 125 Hz, C-1), 48.4 (d, J = 141 Hz, C-2), 115.8 (d, J = 158 Hz, C-5'), 116.6 (d, J = 157 Hz, C-2'), 119.9 (dd, J = 159/6.0 Hz, C-6'), 127.5 (sd, J = 6.7 Hz, C-1'), 144.1 (s, C-4'), 145.2 (s, C-3'); EI-MS of the free base: m/z 167 ($M^{+\cdot}$), 166 ($M-H$), 165 ($M-H_2$), 149 ($165-NH_2$), 123 ($M-C_2H_5N$), 105 ($123-H_2O$), 77 ($105-CO$), 51 ($77-C_2H_2$, 100%).

VIa-HCl. IR: $\tilde{\nu}$/cm^{-1} 3227, 3190 (O—H), 2838 (O—CH_3), 1601, 1527, 1504 (C=C_{arom}), 1249 (C—O—Ph), 1153 (C—OH), 1036 (C—O—CH_3); ^{13}C-NMR (DMSO-d_6): δ 17.5 (q_d, J = 126 Hz, C-3), 39.6 (tm, J = 126 Hz, C-1), 48.3 (d, J = 144 Hz, C-2), 55.6 (q_d, J = 144 Hz, 3'-$O\underline{C}H_3$), 114.4 (dd, J = 156/7 Hz, C-2'), 115.6 (d, J = 158, C-5'), 121.5 (dd, J = 139/6 Hz, C-6'), 127.5 (sd, J = 7 Hz, C-1'), 145.4 (st, J = 7.3 Hz, C-4'), 147.6 (sd, J = 3 Hz, C-3'); EI-MS of the free base: m/z 181 ($M^{+\cdot}$), 164 ($M-C_2H_7N$), 122 ($164-C_3H_6$), 94 ($122-CO$), 138 ($M-C_2H_4N$, 100%), 137 ($M-C_2H_5N$), 107 ($137-CH_2O$), 77 ($107-CH_2O$).

VIb-HCl. IR: $\tilde{\nu}$/cm^{-1} 3387 (O—H), 2842 (O—CH_3), 1594, 1513, 1439 (C=C_{arom}), 1233 (C—O—Ph), 1136 (C—OH), 1026 (C—O—CH_3); ^{13}C-NMR (DMSO-d_6): δ 17.4 (q_d, J = 126 Hz, C-3), 39.6 (t, J = 128 Hz, C-1), 48.3 (d, J = 145 Hz, C-2), 55.6 (q_d, J = 144 Hz, 4'-$O\underline{C}H_3$), 112.4 (d, J = 159

Hz, C-5'), 116.4 (dd, J = 156/6.1 Hz, C-2'), 119.7 (dd, J = 158/4.8 Hz, C-6'), 129.3 (sd, J = 6.7 Hz, C-1'), 146.5/146.5 (s, C-4'/C-3'); EI-MS of the free base: m/z 181 ($M^{+\cdot}$), 164 ($M-C_2H_7N$), 122 ($164-C_3H_6$), 94 ($122-CO$), 138 ($M-C_2H_4N$, 100%), 137 ($M-C_3H_5N$), 107 ($137-CH_2O$), 77 ($107-CH_2O$).

VII. IR: ν/cm^{-1} 2780 ($H_2C-O-CH_2$), 1712 (C=O), 1608, 1503, 1490 ($C=C_{arom}$), 1249 (C—O—C), 1039 (O—C—O); ^{13}C-NMR ($CDCl_3$): δ 29.1 (q_d, J = 128 Hz, C-3), 50.4 (t, J = 127 Hz, C-1), 101.0 (t, J = 173 Hz, $O\underline{C}H_2O$), 108.4 (d, J = 165 Hz, C-5'), 109.8 (dq$_d$, J = 162/7.2 Hz, C-2'), 122.5 (dq$_d$, J = 160/5.9 Hz, C-6'), 127.8 (sq_d, J = 6.7 Hz, C-1'), 146.7 (st, J = 8.4 Hz, C-4'), 147.9 (s, C-3'), 206.8 (ss_6, J = 6.0 Hz, C-2); EI-MS: m/z 178 ($M^{+\cdot}$), 136 ($M-C_2H_2O$), 135 ($M-C_2H_3O$), 105 ($135-CH_2O$), 77 ($105-CO$), 51 ($77-C_2H_2$).

VIII. IR: ν/cm^{-1} 3337 (O—H), 1703 (C=O), 1606, 1522 ($C=C_{arom}$), 1197 (C—OH); ^{13}C-NMR ($CDCl_3$): δ 28.9 (q_d, J = 128 Hz, C-3), 50.5 (t, J = 128 Hz, C-1), 55.9 (q_d, J = 145 Hz, 3'-O$\underline{C}H_3$), 112.1 (dq$_d$, J = 156/6.8 Hz, C-2'), 114.8 (d, J = 160 Hz, C-5'), 122.2 (dq$_d$, J = 160/6.8 Hz, C-6'), 126.0 (sq_d, 7.6 Hz, C-1'), 144.9 (sm, J = 3.3 Hz, C-4'), 147.0 (sd, J = 5.0 Hz, C-3'), 207.6 (ss_6, J = 6.0 Hz, C-2); EI-MS: m/z 166 ($M^{+\cdot}$), 124 ($M-C_2H_2O$), 94 ($124-CH_2O$), 123 ($M-C_2H_3O$, 100%), ($123-H_2O$), 77 ($105-CO$), 51 ($77-C_2H_2$).

IXa. IR: ν/cm^{-1} 3419 (O—H), 2842 (O—CH_3), 1707 (C=O), 1602, 1516, 1464 ($C=C_{arom}$), 1237 (C—O—Ph), 1154 (C—OH), 1034 (C—O—CH_3); ^{13}C-NMR ($CDCl_3$): δ 28.6 (q_d, J = 128 Hz, C-3), 50.5 (t, J = 128 Hz, C-1), 55.9 (q_d, J = 145 Hz, 3'-O$\underline{C}H_3$), 112.1 (dq$_d$, J = 156/6.8 Hz, C-2'), 114.8 (d, J = 160 Hz, C-5'), 122.2 (dq$_d$, J = 160/6.8 Hz, C-6'), 126.0 (sq_d, 7.6 Hz, C-1'), 144.9 (sm, J = 3.3 Hz, C-4'), 147.0 (sd, J = 5.0 Hz, C-3'), 207.6 (ss_6, J = 6.0 Hz, C-2); EI-MS: m/z 180 ($M^{+\cdot}$), 138 ($M-C_2H_2O$), 137 ($M-C_2H_3O$, 100%), 107 ($137-CH_2O$), 77 ($107-CH_2O$), 122 ($M-C_3H_6O$), 94 ($122-CO$).

IXb. IR: ν/cm^{-1} 3412 (O—H), 2841 (O—CH_3), 1708 (C=O), 1609, 1591, 1512 ($C=C_{arom}$), 1241 (C—O—Ph), 1158 (C—OH), 1028 (C—O—CH_3); ^{13}C-NMR ($CDCl_3$): δ 29.1 (q_d, J = 128 Hz, C-3), 50.4 (t, J = 128 Hz, C-1), 56.0 (q_d, J = 145 Hz, 4'-O$\underline{C}H_3$), 111.0 (d, J = 159 Hz, C-5'), 115.7 (dq$_d$, J = 158/5.3 Hz, C-2'), 121.0 (dq$_d$, J = 160/6.0 Hz, C-6'), 127.4 (sq_d, 7.0 Hz, C-1'), 145.8 (s, C-4'/C-3'), 206.9 (ss_6, J = 5.6 Hz, C-2); EI-MS: m/z 180 ($M^{+\cdot}$), 138 ($M-C_2H_2O$), 137 ($M-C_2H_3O$, 100%), 107 ($137-CH_2O$), 77 ($107-CH_2O$), 122 ($M-C_3H_6O$), 94 ($122-CO$).

X. IR: ν/cm^{-1} 3389 (CO—NH), 2917 (O—CH_2—O), 1749 (COOH), 1633 (amide I), 1615, 1596, 1499 ($C=C_{arom}$), 1545 (amide II), 1246 (C—O—C), 1038 (O—C—O); ^{13}C-NMR (DMSO-d_6): δ 41.3 (t, J = 139 Hz, N$\underline{C}H_2$COOH), 101.7 (t, J = 176 Hz, $O\underline{C}H_2O$), 107.3 (dd, J = 165/7.7 Hz, C-2'), 107.9 (d, J = 166 Hz, C-5'), 122.3 (dd, J = 163/6.1 Hz, C-6'), 127.8 (sd, J = 7.4 Hz, C-1'), 147.4 (s, C-3'), 149.9 (st, J = 2.1 Hz, C-4'), 165.7 (sd, J = 3.6, CON), 171.5 (st, J = 5.5 Hz, $\underline{C}$OOH); EI-MS: m/z 223 ($M^{+\cdot}$), 178 ($M-CHO_2$), 149 ($M-C_2H_4NO_2$, 100%), 121 ($149-CO$), 91 ($121-CH_2O$), 63 ($91-CO$).

XII. IR: ν/cm^{-1} 3470 (OH), 3313 (CO—NH), 2858 (O—CH_3), 1746 ($COOH_{arom}$), 1620 (amide I), 1608, 1551, 1509 ($C=C_{arom}$), 1588 (amide II), 1284 (C—O—Ph), 1218 (C—OH), 1035 (C—OCH_3); ^{13}C-NMR (DMSO-d_6): δ 41.2 (t, J = 139 Hz, N$\underline{C}H_2$COOH), 55.6 (q_d, J = 145 Hz, 3'-O$\underline{C}H_3$), 111.3 (dd, J = 158/7.0 Hz, C-2'), 114.9 (d, J = 159 Hz, C-5'), 120.9 (dd, J = 161/7.1 Hz, C-6'), 124.9 (sd, J = 8.5 Hz, C-1'), 147.2 (s, C-3'), 149.7 (st, J = 5.0 Hz, C-4'), 166.3 (sd, J = 4.0, CON), 171.6 (st, J = 5.5 Hz, $\underline{C}$OOH); EI-MS: m/z 225 ($M^{+\cdot}$), 180 ($M-CO_2H$), 168 ($M-C_2H_2O_2$), 152 ($M-C_2H_3NO_2$), 124 ($152-CO$), 108 ($124-CH_4$), 151 ($M-C_2H_4NO_2$, 100%).

Drug Administration and Urine Sampling. Human urine was taken from healthy volunteers (psychiatrists or psychologists) who participated in a psychiatrists study at the Department of Psychiatry, University of Freiburg (Germany) (50, 51). A single oral dose of 140 mg of racemic MDE was administered to nine volunteers. Six volunteers collected urine samples in 4-hr fractions for 32 hr and three for 10 days. The latter samples were used only to determine the duration of detectability of the metabolites. All samples were stored at −20°C before analysis. Blank urine samples were taken from the volunteers before drug administration, to determine whether the urine samples were free of compounds interfering with MDE or its metabolites in the GC/MS and/or HPLC analysis.

incubated at 37°C for 24 hr with 100 μl of a mixture of glucuronidase and arylsulfatase (activity ratio, 1:2), adjusted to pH 8–9 with aqueous sodium hydrogen carbonate (30%), and extracted with 10 ml of a mixture of dichloromethane, isopropanol, and ethyl acetate (1:1:3, by volume). 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, by volume) for 30 min at 50°C. After evaporation, the residue was dissolved in 100 μl of methanol and 0.5 μl of this solution was injected into the GC system (52, 53). The same procedure, with the exception of enzymatic hydrolysis, was used to study whether MDE and its metabolites were excreted as enzyme-hydrolyzable conjugates. An additional urine sample was prepared as described above but the extraction was performed at pH 4–5 with a mixture of ether and ethyl acetate (1:1, by volume) and the residue was methylated with diazomethane (52).

Isolation of the Metabolites for HPLC Analysis. A 0.5-ml portion of urine was spiked with 100 μl of the internal standard (9 μg of MDMA-HCl in 100 μl of water), adjusted to pH 5 with 0.1 M acetate buffer, and incubated at 37°C for 24 hr with 100 μl of a mixture of glucuronidase and arylsulfatase (activity ratio, 1:2). After dilution with 2 ml of water, the sample was extracted on a solid-phase cation exchange column (Analytichem PRS Bond Elut, Varien, Harbor City). After rinsing with 1 bed volume of methanol, the column was conditioned with 1 bed volume of 0.1 M acetate buffer (pH 5). The sample was then drawn through the column at a flow rate of 1–2 ml/min. The column was washed with another 1 bed volume of 0.1 M sodium acetate buffer, dried for 5 min with maximum vacuum, washed with *n*-hexane, and finally dried for 5 min. The metabolites were eluted with four 500-μl portions of a mixture of methanol and 25% ammonium hydroxide (9:1). The eluate was evaporated under nitrogen at 50°C. The residue was dissolved in 200 μl of the HPLC eluent described below. The same procedure, with the exception of enzymatic hydrolysis, was used to study the fraction of conjugates.

Apparatus. *GC/MS.* A Hewlett-Packard (Hewlett-Packard, Waldbronn, Germany) series 5890 gas chromatograph, combined with a Hewlett-Packard MSD series 5970 mass spectrometer and a Hewlett-Packard MS ChemStation (DOS series) with Hewlett-Packard G1034C software, was used. The GC conditions were as follows: splitless injection mode; column, Hewlett-Packard 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 at 10°C/min; initial time, 3 min; final time, 8 min; injection volume, 0.5 μl. The MS conditions were as follows: scan mode; ionization energy, 70 eV; ion source temperature, 220°C; capillary direct interface heated to 260°C (52, 53).

HPLC. A Merck L-6200A HPLC gradient pump combined with a Merck LiChrospher 60 RP-select B column (5 μm, 125 × 4 mm), a programmable L-4250 UV/visible detector, and a D-2500 chromatointegrator were used (Merck, Darmstadt, Germany). The HPLC conditions were as follows: liquid phase, KH_2PO_4 buffer (2.7 g/1000 ml), adjusted to pH 3 with H_3PO_4/acetonitrile (92:8); detection, 278 nm; pressure, 82–83 bar; flow rate, 1.25 ml/min.

The mobile phase was modified for the separation of the isomers of VI, as follows: from 98% buffer/2% acetonitrile to 92% buffer/8% acetonitrile in 10 min, with no initial time but with isocratic elution from 10 min to 15 min.

IR. IR spectra of the synthesized metabolites were recorded on a Bruker FT-IR IFS 48 IR spectrometer.

NMR. ^{13}C-NMR spectra were recorded on a Bruker AC 250 (62.9 MHz) NMR spectrometer combined with a Bruker Aspect 3000 computer.

MS. EI mass spectra were recorded without derivatization, after direct insertion, on a Finnigan TSQ 70 mass spectrometer. The ion source temperature was 200°C and the ionization energy was 70 eV.

Quantification of the Metabolites in Urine. Metabolites I, IV, and IIIa were quantified, after isolation, by the HPLC method described above. The calibration curves were linear for the following ranges: I, 0.2–18.0 μg/0.5 ml of spiked urine; IIIa, 0.5–30.0 μg/0.5 ml; V, 0.1–6.0 μg/0.5 ml. Blank urine samples were spiked with the calibration standards. One hundred microliters of

TABLE 1

Calibration data for the HPLC quantification in urine (n = 12)

	I	IIIa	IV
Recovery (%)	81 (SD = 5.6)	90 (SD = 8.6)	81 (SD = 7.6)
Precision at LOQ (CV, %)[a]	12.0	2.7	12.1
Accuracy at LOQ (%)[b]	+17.2	+19.4	−19.7
LOQ in urine	0.2 μg/0.5 ml	0.5 μg/0.5 ml	0.1 μg/0.5 ml

[a] Precision at the limit of quantification (LOQ) must be less than 20% coefficient of variation (CV).

[b] Accuracy at the limit of quantification must be less than ±20%.

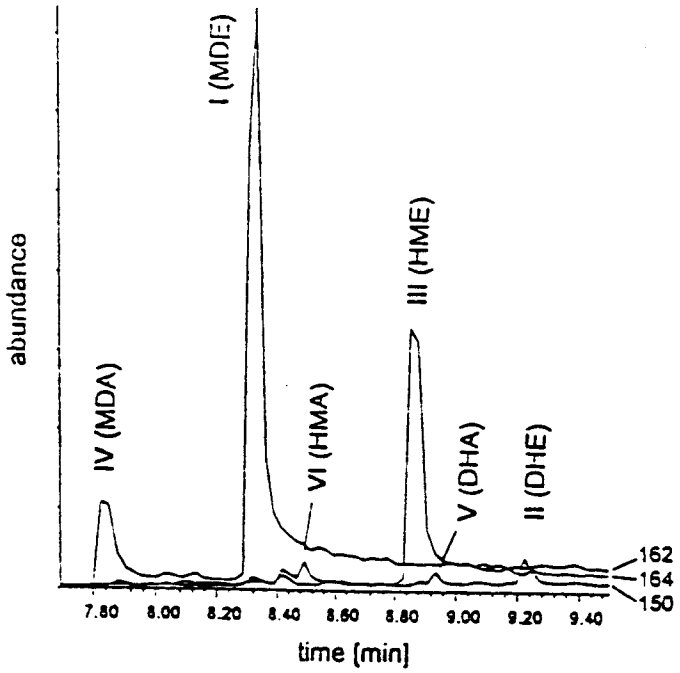
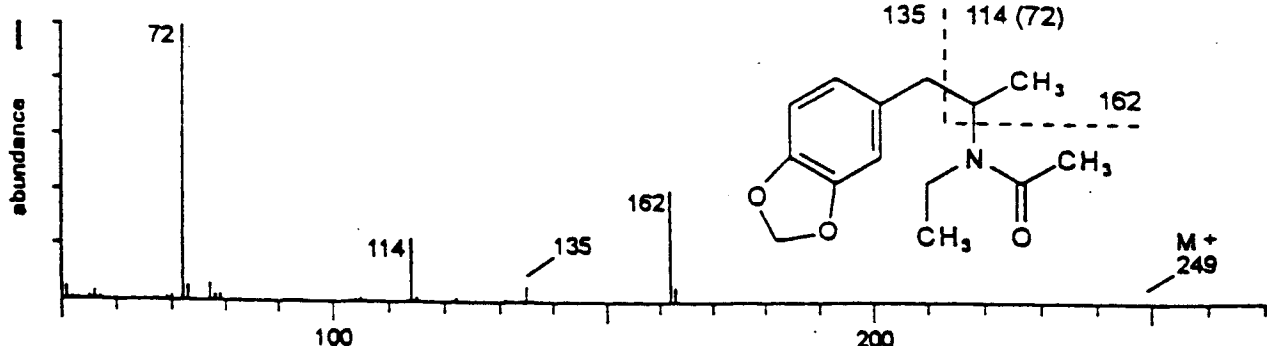
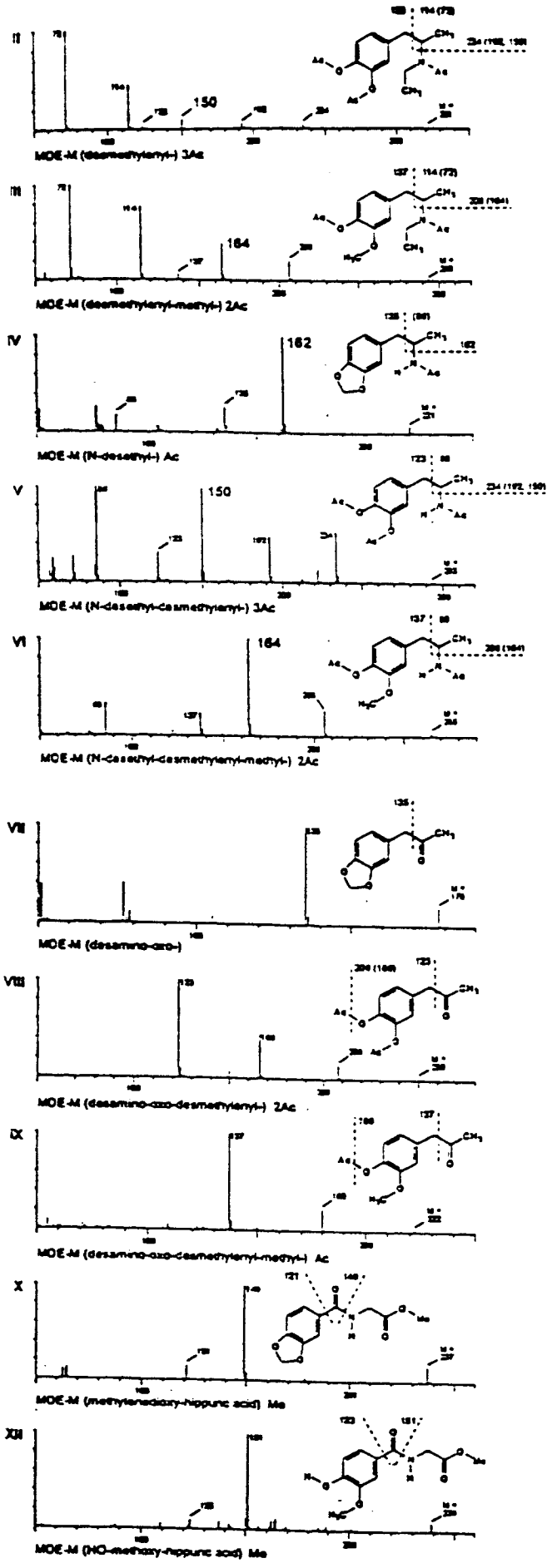


FIG. 1. *Typical mass chromatograms of the ions m/z 150, 162, and 164, indicating the presence of MDE and its main metabolites, recorded after enzymatic hydrolysis, extraction, and acetylation of a urine sample taken 24 hr after ingestion of 140 mg of racemic MDE.*

Results and Discussion

The MDE metabolites were first identified in urine, after cleavage of conjugates, extraction, and acetylation, by GC/MS. Mass chromatography with the masses *m/z* 162, 164, and 150 allowed monitoring of MDE and its main metabolites. In fig. 1, reconstructed mass chromatograms recorded from a urine sample taken 24 hr after ingestion of 140 mg of racemic MDE are shown. The given fragment masses were selected for screening because they correspond to the substituted benzyl fragments (McLafferty rearrangement products: *m/z* 162 for methylenedioxy, 164 for hydroxymethoxy, and 150 for dihydroxy derivatives) (figs. 2 and 3). Additional metabolites could be detected in other urine samples (VII, VIII, and IX) or after extraction at pH 4–5 followed by methylation (X and XII). The detected metabolites were first identified by interpretation of the mass spectra of the postulated metabolites in correlation with that of the known parent compound, according to the rules described by McLafferty and Turecek (55). In fig. 2 the mass spectrum of acetylated MDE and its proposed fragmentation pattern are shown. Fragment 162 corresponds to the McLafferty rearrangement product, fragment 135 to the substituted tropylium ion, and fragment 114 to the β-cleavage product of the side chain. Fragment 72 corresponds to the onium reaction product (deacetylated fragment). In fig. 3 the mass spectra, the proposed fragmentation patterns, and the structures of the acetylated or methylated metabolites of MDE are given. The numbers given in parentheses correspond to the mass to charge ratio of the deacetylated fragments. The interpretation of the spectra and the proposed structures were confirmed by synthesis of the corresponding metabolites and comparison of their chromatographic and mass spectral characteristics with those of the isolated metabolites. The structures of the metabolites III, VI, IX, and XII could not be unequivocally determined, because two isomers (3- or 4-methoxy) could have been formed. The GC retention times and the mass spectra of these were nearly identical. Using HPLC, the isomers in the urine extracts could be separated. Therefore, a urine extract from a volunteer was analyzed by HPLC directly (figs. 4 and 5, *traces A*), after spiking with the synthesized 4-hydroxy-3-methoxy isomer IIIa or VIa (figs. 4 and 5, *traces B*), and after spiking with the synthesized 3-hydroxy-4-methoxy isomer IIIb or VIb (figs. 4 and 5, *traces C*). As shown in figs. 4 and 5, only the 4-hydroxy-3-methoxy isomers of III and VI could be found in the authentic urine sample. This is in accordance with the metabolism of the catecholamines, which are also methylated in position 3 by catecholamine *O*-methyltransferase, but it is in contrast to the study of the metabolism of MDMA of Lim and Foltz (37), who found both isomers. The identity of the HPLC peaks in the urine extracts was confirmed by comparison of the UV spectra of the isolated and synthesized metabolites, which were recorded during a flow stop at the peak maximum. The differentiation of the isomers of IX and XII in urine was impossible, because the urinary concentrations of these metabolites were too low. However, it can be proposed that IX and XI are also the 4-hydroxy-3-methoxy isomers. 3,4-Dihydroxyhippuric acid (XI), which can be postulated as an intermediate metabolite, could not be found in human urine, whereas it could





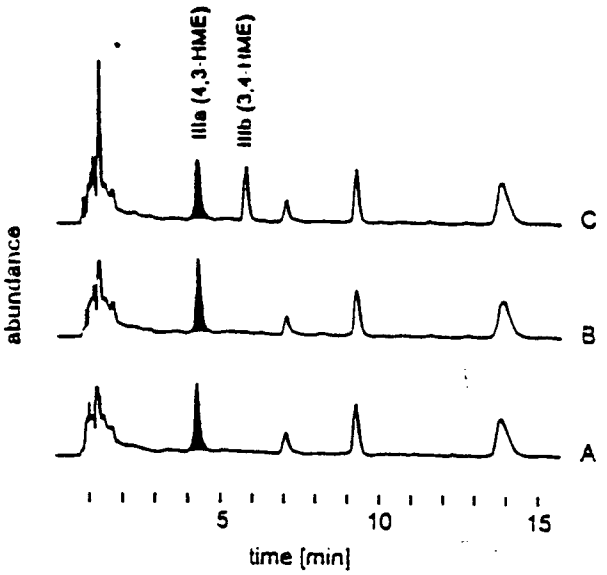


FIG. 4. *HPLC chromatograms of extracts from a urine sample taken 18 hr after ingestion of 140 mg of racemic MDE (A), from the same urine sample spiked with IIIa (B), and from the same urine sample spiked with IIIb (C).*

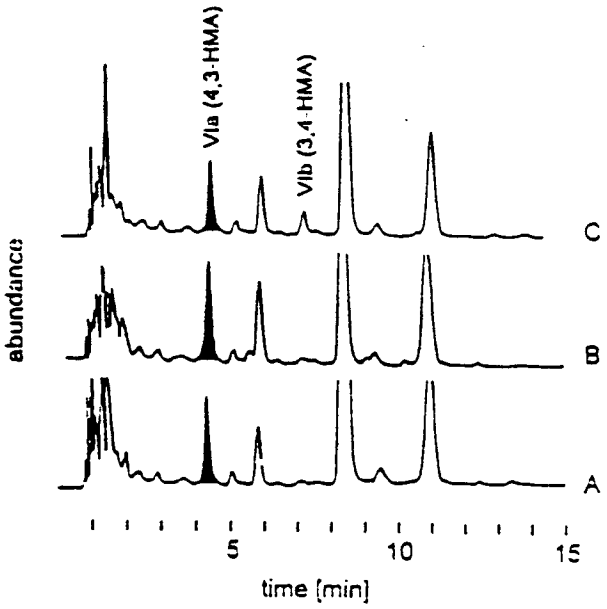


FIG. 5. *HPLC chromatograms of extracts from a urine sample taken 18 hr after ingestion of 140 mg of racemic MDE (A), from the same urine sample spiked with VIa (B), and from the same urine sample spiked with VIb (C).*

be found in rat urine (33). However, it should be noted that XI and XII could also be present in blank urine samples when the volunteer had ingested benzoic acid, *e.g.* as a food preservative. This was also described by Liebich and Foerst (56) and by Caldwell (57). The hydroxy metabolites were partly excreted as enzymatically cleavable conjugates, because the amounts of these metabolites were much higher in urine extracts after enzymatic cleavage of conjugates.

In summary, two overlapping metabolic pathways of racemic MDE in humans can be proposed, as shown in fig. 6. The first pathway

Fig. 3. *Mass spectra and structures of the acetylated (Ac) or methylated (Me) metabolites of MDE.*

The numbers of the spectra correspond to those of the compounds used in the text.

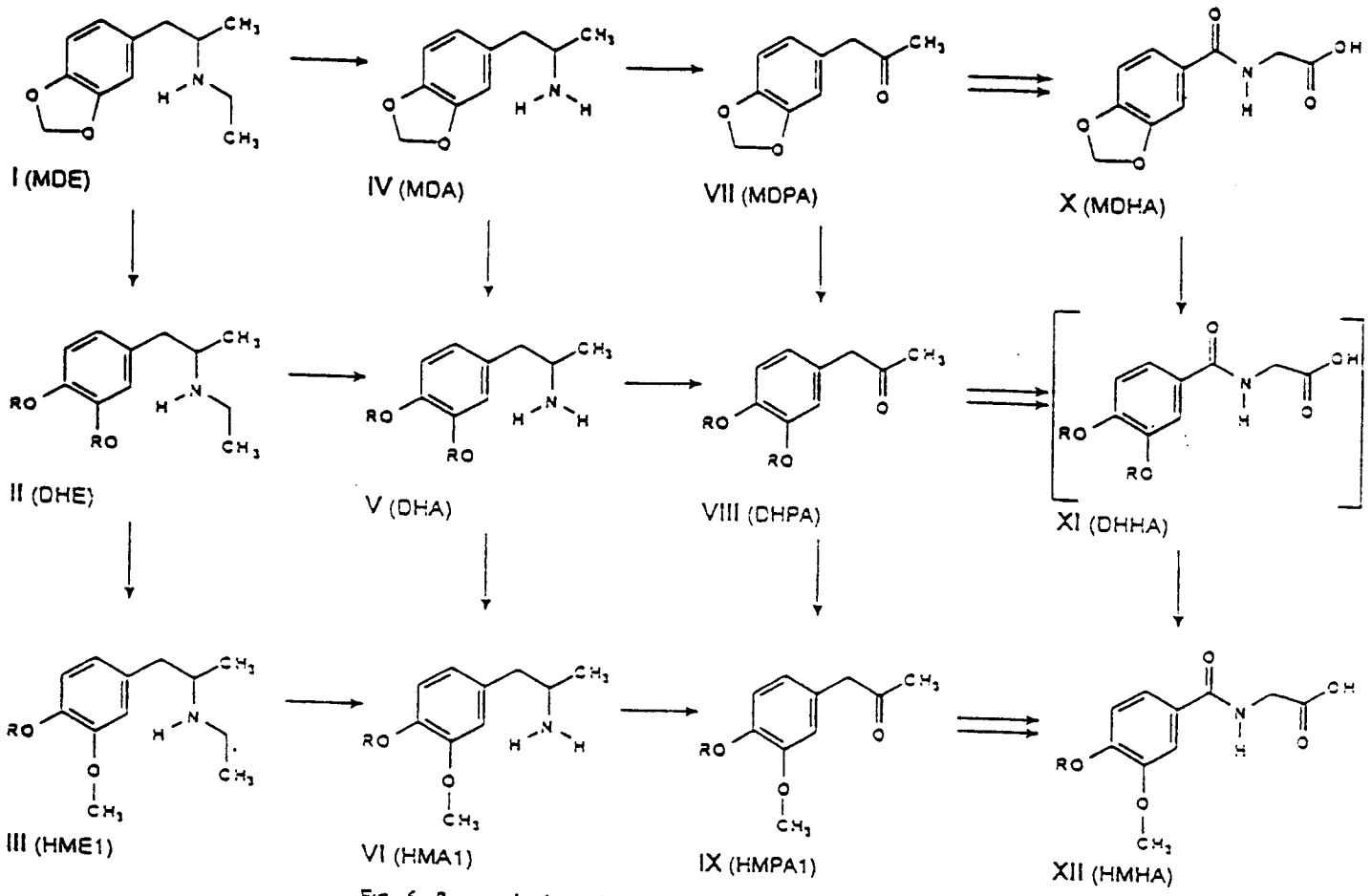


FIG. 6. *Proposed scheme for the metabolism of racemic MDE in humans.*

The metabolites II, III, V, VI, VIII, and IX were also present as glucuronic acid and/or sulfate conjugates in urine.

TABLE 2

Excretion pattern of racemic MDE and its main metabolites, calculated as their hydrochlorides, within 32 hr after administration

Volunteer	I·HCl		IIIa·HCl		IV·HCl	
	mg	*% of dose*	*mg*	*% of dose*	*mg*	*% of dose*
1	26.6	19.2	46.3	33.1	5.3	3.8
2	30.1	21.5	42.2	30.2	3.6	2.5
3	22.9	15.4	43.9	31.4	2.8	2.0
Mean	26.6	19.0	44.1	31.6	3.9	2.8
SD	3.61	2.55	2.06	1.46	1.28	0.92

IIIa was determined in its unconjugated form

leads, *via* ring degradation by *O*-dealkylation, to the corresponding 3,4-dihydroxy metabolites (II, V, and VIII), which are subsequently methylated at the hydroxy group at position 3 of the aromatic ring (III, VI, IX, and XII). The second pathway leads, *via* side chain degradation by *N*-dealkylation, to the corresponding primary amines (IV, V, and VI). Oxidative *N*-deamination forms the substituted phenylacetones (VII, VIII, and IX), which are degraded to the corresponding benzoic acids, followed by conjugation with glycine to form substituted hippurates [X, (XI), and XII]. The metabolites containing hydroxy groups are partly excreted in a conjugated form. Additional studies on the phase II metabolism of MDE in humans are in progress. Quantification by HPLC of the urinary excretion revealed that 19% of the MDE dose was eliminated as I, 31.6% as IIIa, and 2.8% as IV within 32 hr (table 2). Quantification of the other metabolites was not possible, because their concentrations were too low for precise HPLC determination. Metabolite IIIa was the only metabolite that could be detected by GC/MS up to 7 days. Because of the high excretion rate and the long duration of detectability of IIIa, it can be concluded that ring degradation *via* II to IIIa is the predominant pathway for MDE. Enantioselective studies on the metabolism of MDE in humans are in progress.

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