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# Identification of metabolites of 3,4-methylenedioxymethamphetamine in rats*

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Liquid chromatography with electrochemical detection (LC/ECD) and gas chromatography/mass spectrometry (GC/MS) were used to identify metabolites of *N*-methyl-3,4-methylenedioxyamphetamine (MDMA) in samples of rat plasma and urine. Several potential metabolites, based on what is known about the metabolism of the desmethyl analog (i.e., MDA), were synthesized as standards to aid in the identification of the MDMA metabolites. MDA and *N*-methyl-1-(4-hydroxy-3-methoxyphenyl)-2-aminopropane (*3b*) were identified in urine by HPLC and confirmed by GC/MS. 1-(4-Hydroxy-3-methyoxyphenyl)2-aminopropane, (*3a*), *N*-methyl-1-(3-hydroxy-4-methoxyphenyl)-2-aminopropane (*2b*) and 1-(3,4-dihydroxyphenyl)-2-aminopropane (*4a*) were tentatively identified by LC/ECD but insufficient sample size precluded confirmation by mass spectrometry. MDA was also identified in brain and plasma extracts. Because MDA is a metabolite of MDMA in humans, and because it has been speculated that the neurotoxic effects of MDA and MDMA may be due to a metabolite, the results of the present study may ultimately aid our understanding of the neurotoxic mechanism of these drugs of abuse.

*Key words:* 3,4-methylenedioxyamphetamine; *N*-methyl-3,4-methylenedioxyamphetamine; metabolism; α-methyldopamine

## Introduction

3,4-Methylenedioxyamphetamine (MDA;*1a*) and its *N*-monomethyl analog, MDMA (*1b*) combine, to varying degrees, the structure and activity of amphetamine and mescaline. These agents are abused for their euphoric and emotive effects; the widespread abuse and number of fatalities reported (Cimbura, 1972; Lukaszewski, 1979; Poklis et al., 1979; Reed et al., 1972; Simpson and Rumack, 1981) for MDA have prompted research in the pharmacology (Glennon and Young, 1984a; Glennon and Young 1984b; Thiessen and Cook, 1973), toxicology (Nichols et al., 1975; Paton et al., 1975) and metabolism (Marquardt and Distefano, 1974; Marquardt et al., 1978; Midha, 1974; Midha et al., 1977; Midha et al., 1978) of this agent. After 1970 when MDA was classified as a Schedule I substance, its *N*-methyl derivative MDMA appeared on the street. Both isomers of MDA as well as the *S*(+)-isomer of MDMA produce a serotonin neurotoxicity characterized by a reduction of 5-HT, 5-hydroxyindoleacetic acid and 5-HT uptake sites in both rat and non-human primate brains (Ricaurte et al., 1985; Ricaurte et al, 1988; Ricaurte, 1989; Schmidt, 1987; Slikker et al., 1988; Stone et al., 1986). The Drug Abuse Warning Network (DAWN) and published case reports provide a means of assessing the acute toxic effects of these drugs in humans; however, the potential for long-term human neurotoxicity is difficult to determine

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(Ricaurte, 1989). Nevertheless, there is serious concern regarding the abuse of these agents (Peroutka et al., 1987; Price et al., 1989). The possibility that a metabolite of MDMA might be responsible for its neurotoxic effects prompted us several years ago to initiate an investigation of the metabolism of MDMA (Fitzgerald et al., 1988).

Since the metabolism of MDA had been previously investigated, we chose MDA as a model for the design and synthesis of potential metabolites of MDMA; that is, we synthesized the *N*-methyl analogs of metabolites that had already been identified for MDA. The synthesized compounds were then employed as standards to identify and confirm several previously unreported metabolites of MDMA. Two schemes were developed to extract basic and phenolic metabolites allowing for identification by LC/ECD and GC/MS. MDA and *N*-methyl-1-(4-hydroxy-3-methoxyphenyl)-2-aminopropane *3b* were identified in urine extracts from animals dosed with 40 mg/kg MDMA, by HPLC and confirmed by GC/MS. 1-(4-Hydroxy-3-methoxyphenyl)-2-aminopropane (*3a*), *N*-methyl-1-(3-hydroxy-4-methoxyphenyl)-2-aminopropane (*2b*) and 1-(3,4-dihydroxyphenyl)-2-aminopropane (*4a*) were tentatively identified by LC/ECD but insufficient quantities were present to obtain mass spectra for confirmation. MDA was also identified in rat plasma and brain extracts both by HPLC and GC/MS.

## Materials and methods

### *Chemicals*

Trifluoracetic anhydride (TFAA, 99 + %) was obtained from Aldrich Chemical Company. Organic solvents were HPLC grade and all other chemicals were reagent grade. 3,4-Dimethoxyphenylethylamine (DMPEA, *8*) and 3-hydroxy-4-methoxyphenethylamine (*9*), used as internal standards, were obtained from Aldrich Chemical company. The internal standards allowed calculations of relative retention times. MDA (*1a*), MDMA (*1b*), 1-(3-hydroxy-4-methoxyphenyl)-2-aminopropane (*2a*), *N*-methyl-1-(3-hydroxy-4-methoxyphenyl)-2-aminopropane (*2b*), 1-(4-hydroxy-3-methoxyphenyl)-2-aminopropane (*3a*), *N*-methyl-1-(4-hydroxy-3-methoxyphenyl)-2-aminopropane (*3b*), 1-(3,4-dihydroxyphenyl)-2-aminopropane (*4a*) and *N*-methyl-1-(3,4-dihydroxyphenyl)-2-aminopropane (*4b*) were synthesized as their racemic hydrochloride salts as described below. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Atlantic Microlab (Norcross, GA). Proton NMR spectra were recorded on a JEOL FX 90Q spectrometer, operating at 90 MHz and using tetramethylsilane as an internal standard. Infrared spectra were obtained on a Nicolet 5ZDX FT-IR spectrophotometer. The structures of 1—4 are shown in Fig. 1. Synthetic schemes are shown in Fig. 2.

### *Synthesis*

*(±)-1-(3-Hydroxy-4-methoxyphenyl)-2-aminopropane (2a).* Compound *2a* was prepared by the catalytic hydrogenolysis of 1-(3-benzyloxy-4-methoxyphenyl)-2-aminopropane hydrochloride (5) as we have previously reported (Glennon et al., 1980).

*(±)-N-Methyl-1-(3-hydroxy-4-methoxyphenyl)-2-aminopropane (2b).* A solution of (±)-1-(3-benzyloxy-4-methoxyphenyl)-2-aminopropane hydrochloride (*5*) (0.65 g) in water (50 ml) was basified to pH 10 with 10% aqueous NaOH solution and extracted with ether (3 × 30 ml). The combined ether portions were dried (magnesium sulfate) and evaporated to dryness under vacuum to give 0.55 g of the

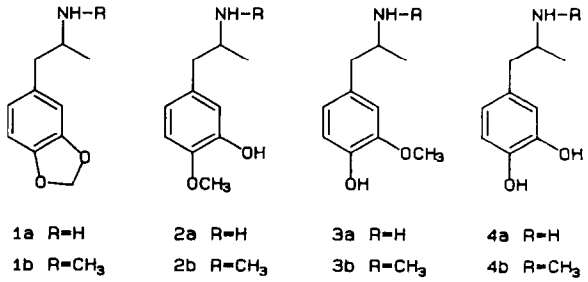


**Fig. 1.** Structures of MDA (*1a*), MDMA (*1b*), and potential metabolites.

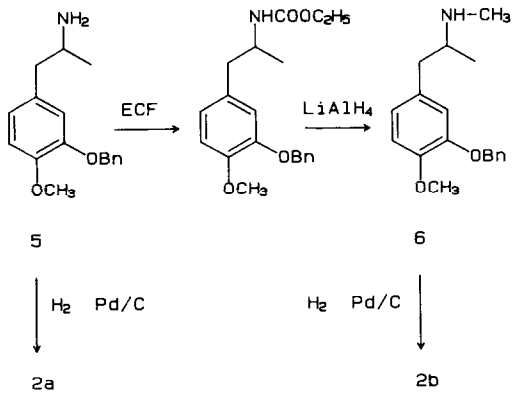


**Fig. 2.** Synthetic scheme for compounds *2a* and *2b*. *(ECF = Ethyl chloroformate; Bn = benzyl.)*

free base. A solution of ethyl chloroformate (ECF, 0.31 g) in tetrahydrofuran (10 ml) was added in a dropwise manner to a stirred solution of this free base and triethylamine (0.3 g) in tetrahydrofuran (30 ml) at room temperature. The reaction mixture was allowed to stir at room temperature overnight (16 h) and was filtered. The solid material was washed with warm tetrahydrofuran (20 ml) and the washing was added to the filtrate. The filtrate was evaporated to dryness under reduced pressure and the residue was dissolved in ether (100 ml); the ether solution was washed with 5% HCl (2 × 25 ml), water (2 × 10 ml), dried (magnesium sulfate) and evaporated to dryness to give 0.65 g (93%) of the crude carbamate, m.p. 81–83 °C. A solution of the carbamate (0.6 g, 1.75 mmol) in anhydrous ether (30 ml) was slowly added to a suspension of lithium aluminum hydride (0.1 g) in ether (20 ml) and the reaction mixture was heated at reflux for 20 h. The reaction mixture was cooled in an ice bath and water was slowly added until there was no further evolution of hydrogen. The suspension was filtered and the filtrate was dried (magnesium sulfate); HCl gas was bubbled through the solution until no further precipitation occurred. The crude product was collected by filtration and was recrystallized from a 2-propanol/ether mixture to afford 0.16 g (29%) of 1-(3-benzyloxy-4-methoxyphenyl)-2-aminopropane hydrochloride *6* as a white crystalline material, m.p. 143–144 °C. The benzyl protecting group was removed by treating a solution of the salt (0.15 g) in absolute ethanol (50 ml) with hydrogen gas (40 psig) in the presence of 10% Pd on carbon (70 mg) for 3 h. The product was recrystallized from 2-propanol to afford 0.095 g (88%) of *2b* as white crystals, m.p. 164–165 °C. $^1$HNMR (DMSO-$d_6$)$\delta$: 1.10(d, 3H, $CH_3$), 2.53 (s, 3H, *N*-$CH_3$), 3.15 (m, 2H, $CH_2$), 3.35 (m, 1H, CH) 3.74 (s, 3H, $OCH_3$), 6.57–6.90 (m, 3H, ArH), 9.01 (s, 1H, OH), 9.45 (brs, 2H, NH.HCl), FT-IR (KBr disc): 3310 (OH), 2931 ($-N^+H_2-$), 2797 (CH), 1590 (Ar C=C) cm$^{-1}$. Anal. calculated for $C_{11}H_{17}NO_2$.HCl: C, 57.02; H, 7.83, N, 6.04. Found: C, 57.09; H, 7.84; N, 6.00.

*(±)-1-(4-Hydroxy-3-methoxyphenyl)-2-aminopropane (3a).* This compound was prepared by the catalytic hydrogenolysis of 1-(3-methoxy-4-benzyloxyphenyl)-2-aminopropane hydrochloride (*7*) using a procedure we have previously reported (Glennon et al., 1980).

*(±)-N-Methyl-1-(4-hydroxy-3-methoxyphenyl)-2-aminopropane (3b).* Compound *3b* was prepared from 1-(3-methoxy-4-benzyloxyphenyl)-2-aminopropane hydrochloride (*7*) using the same procedure employed for the preparation of *2b*. Compound *7* (0.5 g of the free base; m.p. 61–63 °C) was acylated with ethyl chloroformate to afford the carbamate, m.p. 80 °C, in 79% yield. The carbamate (0.45 g) was reduced with lithium aluminum hydride to give 0.18 g (43%) of the benzyl-protected *N*-methyl amine hydrochloride m.p. 164–165 °C, of which 0.15 g was deprotected by catalytic hydrogenation to provide 0.1 g (93%) of *3b* as white crystals, m.p. 210–212 °C. $^1$HNMR (DMSO-$d_6$)$\delta$: 1.11 (d, 3H, $CH_3$), 2.52 (s, 3H, N–$CH_3$), 3.10 (m, 2H, $CH_2$), 3.35 (m, 1H, CH), 3.76 (s, 3H, $OCH_3$), 6.56–6.83 (m, 3H, ArH), 8.9 (s, OH), 9.17 (s, 2H, NH.HCl). FT-IR (KBr disc) 3170 (OH), 2943 ($-N^+H_2-$) 2776 (CH), 1605 (Ar C=C) cm$^{-1}$ Anal. calculated for $C_{11}H_{17}NO_2$.HCl: C, 57.02; H, 7.83; N, 6.04. Found: C, 56.90; H, 7.84; N, 6.03.

*(±)1-(3,4-Dihydroxyphenyl)-2-aminopropane (4a).* Compound *4a* was synthesized from 1-(3,4-dibenzyloxyphenyl)-2-aminopropane hydrochloride according to the method of (Marshall and

Castagnoli, 1973) except that the intermediate nitropropene was reduced with lithium aluminum hydride (in 70% yield) instead of alane (aluminum hydride). Compound *4a*, m.p. 191–192 °C (lit. (Marshall and Castagnoli, 1973) 192–194 °C) was a white crystalline product when freshly recrystallized from 2-propanol/anhydrous ether; however, upon standing, the material took on a gray palor.

*(±)-N-Methyl-1-(3,4-dihydroxyphenyl)-2-aminopropane (4b).* 1-(3,4-dibenzyloxyphenyl)-2-aminopropane (350 mg, 1 mmol) was acylated with ethyl chloroformate and the resulting carbamate (not isolated) was reduced with lithium aluminum hydride, using the same procedure employed for *2b* to give *N*-methyl-1-(3,4-dibenzyloxyphenyl)-2-aminopropane hydrochloride as a white crystalline material, m.p. 149–151 °C after recrystallization from a 2-propanol/anhydrous ether mixture. Catalytic hydrogenolysis afforded 45 mg (approx. 20% overall yield) of *4b* as white crystals after recrystallization from 2-propanol/anhydrous ether, m.p. 118–120 °C. As with *4a*, *4b* took on a light gray palor on standing. $^1$HNMR (DMSO-d6)$\delta$: 1.1 (d, 3H, $CH_3$), 2.55 (s, 3H, N–$CH_3$), 3.2–3.55 (m, 2H, $CH_2$), 3.6–4.0 (m, 1H, CH) 6.4–6.75 (m, 3H, ArH), 8.85 (s, 2H, NH.HCl). FT-IR (deposited film): 3190-3070 (OH), 2924 (–$N^+H_2$–), 2780 (CH), 1602 (Ar C = C) $cm^{-1}$. Anal. calculated for $C_{10}H_{15}NO_2$.HCl: C, 55.17; H, 7.41; N, 6.43. Found: C, 55.05; H, 7.42; N, 6.38.

*Animals and dosing*

Male Sprague–Dawley rats weighing between 250 and 350 g were used for this study. All rats received a single dose of 40 mg/kg MDMA.HCl subcutaneously. These doses were used in previous studies (e.g., Ricaurte et al., 1985). Two groups of animals were used. Both groups contained four MDMA-treated animals and two vehicle-treated animals. For plasma and tissue studies, rats were killed by decapitation 4 h after dosing. Urinary data were obtained by housing rats in metabolism cages and collecting 24-h urine specimens. Rats were given free access to food and water.

*Assay for basic compounds 1a and 1b*

To 0.5 ml of plasma, urine or tissue homogenates, DMPEA (500 ng) was added as the internal standard; the mixture was alkalinized with 0.5 ml of 2 N sodium hydroxide and extracted with 5 ml of hexane-isoamyl alcohol (99:1) by vortexing for 1 min. The mixture was centrifuged for 5 min, the organic layer transferred to another tube and reextracted into 0.5 ml of 10% formic acid. After centrifugation for 5 min, the upper organic layer was removed and discarded. The aqueous layer was alkalinized with 0.5 ml of sodium hydroxide (2 N) and extracted with 5 ml of hexane-isoamyl alcohol. The organic layer was evaporated in a water bath (60 °C) under nitrogen. The residue was dissolved in 100 $\mu$l of mobile phase for HPLC analysis or derivatized with TFAA as described below. Two mobile phases for basic metabolites consisted of 0.01 M phosphate buffer (pH = 7.1)/acetonitrile/methanol, 65:26:9; and 0.025 M ammonium acetate buffer (pH = 4.5)/acetonitrile/methanol, 50:33:17. A flow rate of 1.5 ml/min was used for the pH = 7.1 buffer and a flow rate of 0.8 ml/min was used for the pH = 4.5 buffer. Both mobile phases were used to separate MDA, MDMA and DMPEA on a cyano (15 cm × 0.46 cm i.d. × 5 $\mu$m) column using a Biorad UV detector set at 240 nm.

*Assay for compounds 2a, 2b, 3a and 3b*

3-Hydroxy-4-methoxyphenylethylamine (250 ng) was added as the internal standard to 0.5 ml of plasma or urine and the mixture was alkalinized with 0.5 ml of 5% sodium carbonate and extracted with 5 ml of a mixture of ethyl acetate/hexane/isoamyl alcohol (50:49.5:0.5). After centrifugation for 5 min the organic layer was separated and evaporated under nitrogen. The residue was dissolved in 100 $\mu$l of mobile phase for HPLC analysis or derivatized with TFAA as described below. The mobile phase for these phenolic metabolites consisted of a 0.25 M ammonium acetate buffer with 7.5% acetonitrile. A flow rate of 1.2 ml/min was used on a C-18 column (7.5 cm × 3 $\mu$m). A bioanalytical Systems 4B electrochemical detector with an

oxidation potential of 0.75 V provided good sensitivity for these compounds.

*Assay for 4a and 4b*

The same extraction procedure as for *2a*, *2b*, *3a* and *3b* was used except that samples were back extracted with 200 μl of 10% formic acid. The formic acid was evaporated to dryness and samples were reconstituted with mobile phase. The mobile phase consisted of 0.25 M triethylamine phosphate (pH = 3.0) containing 50 mg/l heptanesulfonic acid and 20 mg/l sodium octyl sulfate. The same HPLC system as for *2a*, *2b*, *3a* and *3b* was used except that the oxidation potential was 0.65 V and the flow rate was 1 ml/min.

*TFAA derivatization*

Dried extracts from either of the extraction assays were reconstituted with 50 μl of ethyl acetate and 50 μl of TFAA and heated at 60 °C 10 min. Samples were then evaporated under nitrogen, reconstituted with 25 μl of ethyl acetate and injected into the GC/MS. Alternatively, extracts can be derivatized on-column by reconstituting the initial extract with 25 μl of ethyl acetate and co-injecting 1 μl of ethyl acetate extract with 1 μl of TFAA.

*GC/MS methods*

A crosslinked methylsilicon column (12.5 m × 0.33 mm × 0.17 μm: length × i.d. × film thickness) effected separation for GC/MS analysis. Initial oven temperature was 150 °C, initial time was 4 min, temperature program rate was 20 °C/min and final temperature was 250 °C. The GC injector temperature was 250 °C. The mass spectrometer source temperature was 200 °C and the ionization potential was 70 eV.

## Results

*HPLC of 1a and 1b*

Compounds *8*, *1a* and *1b* are well separated on the cyano column using the mobile phase at pH 4.5 or pH 7.1. The plasma and urine blank from control untreated rats did not show any interfering peaks under our experimental conditions. A typical chromatogram using the mobile phase at pH = 4.5 of a standard and a plasma extract is shown in Fig. 3.

*HPLC of 2a, 2b, 3a and 3b*

Compounds *2a*, *2b*, *3a*, *3b* and *9* were all well separated on the C-18 column. Electrochemical detection provides high sensitivity for detection of these hydroxy compounds. A standard mixture containing *2a*, *2b*, *3a*, *3b* and an extract of a urine sample is shown in Fig. 4. This figure shows three peaks in the urine extract with retention times similar to standards of compounds *2b*, *3a* and *3b*. Blank urine did not show any peaks with these retention times.

*HPLC of 4a and 4b*

Compounds *4a* and *4b* eluted in the solvent peak when conditions for *2a*, *2b*, *3a* and *3b* were employed for urine samples, therefore we developed a separate system for chromatographing these compounds. Figure 5 shows a

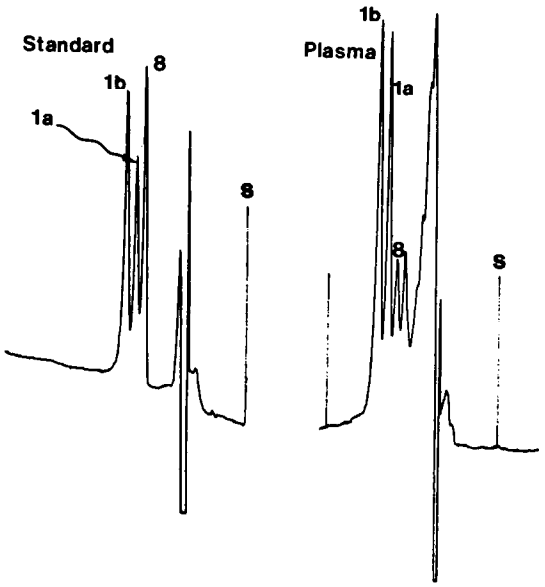


**Fig. 3.** HPLC of *1a*, *1b* and *8* in a plasma and a standard sample using the mobile phase of pH 4.5. The retention times of *1a*, *1b* and *8* are 4.2, 4.6 and 3.8 min, respectively. S represents the start of the chromatogram. See Methods section for chromatographic conditions.

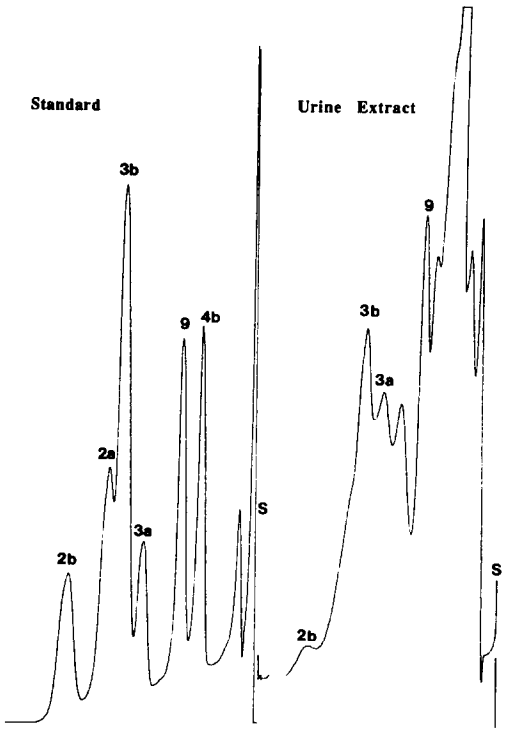


Fig. 4. HPLC of *2a*, *2b*, *3a*, *3b*, *4b* and *9* in a standard with retention times of 4.12, 5.26, 3.13, 3.70, 1.53 and 2.08 min, respectively. The chromatogram of the urine extract shows the tentative identification of *2b*, *3b* and *3a*. S represents the start of the chromatogram. See Methods section for chromatographic conditions.

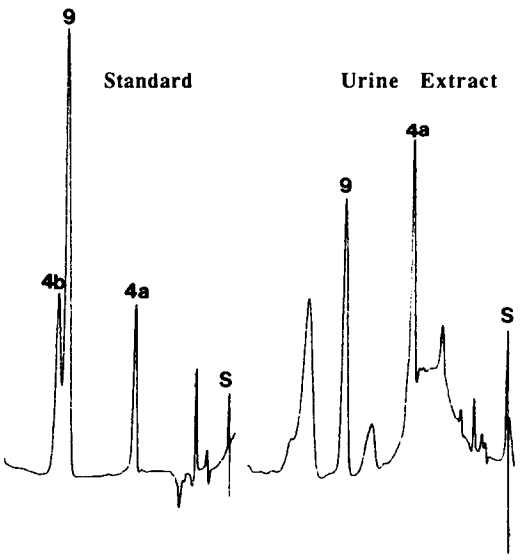


Fig. 5. HPLC of *4a*, *4b* and *9* in a standard extract with retention times of 4.10, 7.42 and 7.04 min, respectively. The chromatogram of the urine extract shows a peak with the same retention time as *4a*. S represents the start of the chromatogram.

standard mixture containing *9*, *4a*, *4b* and a urine extract. This figure shows the identification of *4a* by LC/EC in urine of a rat treated with MDMA.

### *GC/MS results*

The GC retention data and mass spectral data of major ion fragments of the TFAA derivatives of *1a*, *1b* and other synthetic compounds

**Table I.** The GC retention times and mass spectra of major ion fragments of synthetic compounds that were derivatized with TFAA. For structures and experimental conditions see text.

| Compound | Retention time (min) | Major ion fragments (% of base peak) |
|---|---|---|
| *1a* | 4.00 | 135 (100), 162 (24), 275 (5), 140 (6) |
| *1b* | 5.28 | 154 (100), 162 (64), 135 (65), 110 (36) |
| *2a* | 3.03 | 233 (100), 260 (85), 140 (62), 261 (15) |
| *2b* | 5.10 | 154 (100), 110 (30), 260 (15), 155 (6) |
| *3a* | 3.03 | 140 (100), 260 (49), 233 (10), 163 (9) |
| *3b* | 4.98 | 154 (100), 260 (19), 110 (17), 155 (7) |
| *4b* | 4.05 | 154 (100), 110 (25), 342 (9), 155 (5) |
| *8* | 4.90 | 151 (100), 164 (35), 107 (9), 277 (10) |

are presented in Table 1. Figure 6 shows a chromatogram of a TFAA derivatized basic urine extract. The larger peak (*1b*), corresponds to the retention time of MDMA and has a mass spectrum identical to the mass spectrum of MDMA standards treated with TFAA. The smaller peak in Fig. 6 has the same retention time as MDA standards and the mass spectrum of this peak is shown below the chromatogram and corresponds to the published spectrum of MDA derivatized with TFAA (Midha et al., 1976).

Using the extraction scheme for the phenolic compounds, followed by derivatization with TFAA, we identified a previously unreported metabolite of MDMA by GC/MS. Urine extracts from rats showed a peak with the exact retention time and mass spectrum of compound *3b* derivatized with TFAA. This confirmed the identification of *3b* by LC/EC.

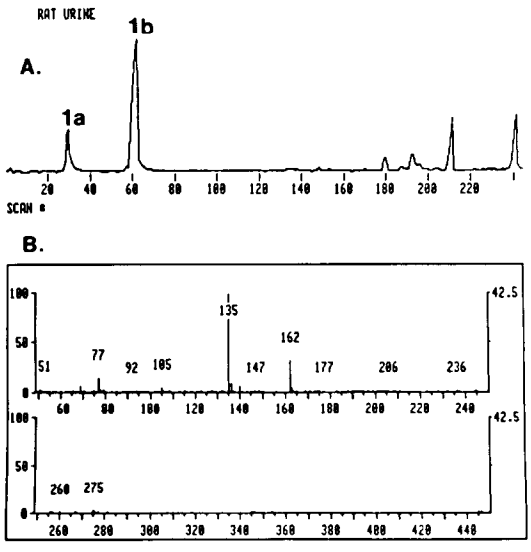


**Fig. 6.** Part A in this figure shows the gas chromatogram of a urine extract, derivatized with TFAA, from a rat dosed subcutaneously with 40 mg/kg MDMA. Peak *1b* had the same retention time (5.28 min) as a standard of MDMA treated with TFAA. The mass spectrum of *1a* is shown in B and corresponds to the published spectrum of MDA derivatized with TFAA.

## Discussion

We have described the synthesis of several possible metabolites of MDMA and have established the procedures for identification of these compounds using HPLC and GC/MS. The proposed metabolic scheme of MDMA (*1b*) is shown in Fig. 7. We identified *3b* as a metabolite of MDMA by LC/ECD and confirmed the structure by GC/MS. The *N*-demethylated analog of *3b*, compound *3a*, had previously been identified as a metabolite of MDA (Midha et al., 1977). We also identified *3a* as a metabolite in rats dosed with MDMA by LC/EC (see Fig. 4). Casida et al. (1966) have studied the metabolic fate of methylene-$^{14}$C-dioxyphenyl compounds, showing that the $^{14}$C label was recovered as formate, $CO_2$ and formaldehyde leaving the catechol of the parent compound. Compound *3b* is probably formed by dealkylation followed by *O*-methylation by catechol-*O*-methyltransferase. COMT preferentially alkylates the 3 position of endogenous catechols, but methylation at the 4 position is possible, depending on the hydrophilic nature of ring substituents (Creveling et al., 1970). Figure 4 shows a peak in the urine extract with retention time corresponding to *2b*, which is methylated at the 4 position.

Compound *4a*, the catechol corresponding to MDA, has been identified as a metabolite when rats are dosed with MDA (Midha et al., 1978). Using our methods *4a* was detected by LC/EC in urine of rats dosed with MDMA. We did not detect *4b*.

In a preliminary report, we had previously identified MDA as a metabolite of MDMA (Fitzgerald et al., 1988). Others have also identified MDA as a metabolite of MDMA but no data were presented on the methods used for its identification (Brady et al, 1986). We now present the MS confirmation that MDA is a metabolite of MDMA in rats and, further, that the 4-hydroxy-3-methoxy compound *3b* is a novel metabolite of MDMA. Evidence is also provided for the formation of other metabolites. The present study demonstrates that metabolism of MDMA in rats results in the formation of several phenolic products, includ-

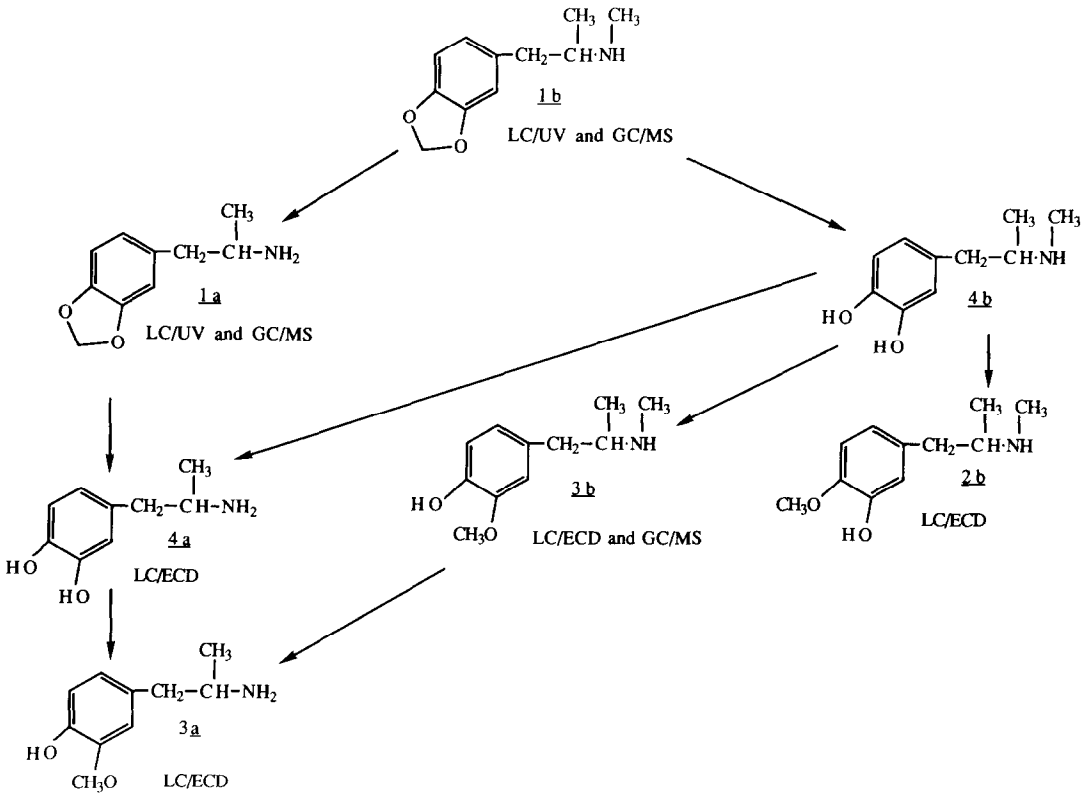


**Fig. 7.** Scheme showing the proposed metabolic scheme of MDMA and techniques used for identification.

ing α-methyldopamine (*4a*) which is a common metabolite of MDA and MDMA. Currently, there are two theories to account for the neurotoxicity of MDMA and related analogs: (a) released serotonin may be metabolized to a neurotoxic metabolite such as 5,6-dihydroxytryptamine and (b) MDMA may itself be metabolized to a neurotoxic metabolite (Fuller, 1989; Molliver et al., 1989). Although the mechanism of neurotoxicity is unknown and although several different mechanisms may be involved, the phenolic metabolites of MDMA are certainly candidates worthy of further investigation. For example, para hydroxylation of *4a* in vivo would give rise to the α-methyl derivative of a known neurotoxin, 6-hydroxydopamine. Some of the other phenolic metabolites may also be candidates for aromatic hydroxylation; furthermore, the methoxy metabolites might undergo *o*-demethylation in a manner that has been shown for other methoxyphenylisopropylamines to favor the *S*-isomer (e.g., Zweig and Castagnoli, 1977). Subsequent studies should focus on the neurotoxicity of these metabolites of MDMA.

MDA has recently been identified as a metabolite of MDMA in humans (Vereby et al., 1988). While this manuscript was in preparation, a group working independently identified and confirmed by GC/MS the metabolites which we had considered tentative and have also examined the metabolism of MDMA in a human subject (Lim and Foltz, 1988; Lim and Foltz, 1989). Since both species share at least part of the metabolic pathway and because an estimated 30,000 of doses of MDMA are taken each month

by humans (Ricaurte et al., 1988), it is important to continue to elucidate this metabolic pathway.

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