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Note

Identification of two *in vitro* metabolites of 3,4-methylenedioxyamphetamine by gas–liquid chromatography–mass spectrometry

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3,4-Methylenedioxyamphetamine (MDA) is chemically related to both mescaline and amphetamine, and although it is classified as a hallucinogen, some investigators have found the drug to possess some stimulant and minor sympathomimetic properties. Because of its widespread abuse on the street under such names as "Mellow Drug of America" or "Love Drug", metabolism studies were initiated. Benzyl methyl ketones and the corresponding oximes (*syn* and *anti*) have been isolated as metabolites of amphetamine, *p*-methoxyamphetamine and fenfluramine in *in vitro* metabolic studies with liver preparations (10,000 *g* supernatant, 20 min centrifugution) of various species[1,2]. These metabolites have also been identified in the urine of humans who were administered amphetamine[3–5]. It has also been demonstrated that these oximes are formed from the corresponding N-hydroxy derivatives of these amphetamines[2,6] by physical (*e.g.* heat), chemical and perhaps metabolic processes. This report describes the isolation and identification of two metabolites, namely 3,4-methylenedioxybenzyl methyl ketone (I) and 3,4-methylenedioxybenzyl methyl ketoxime (II), from hepatic preparations of guinea-pig and rabbit incubated with MDA.

MATERIALS AND METHODS

Chemicals and reagents

3,4-Methylenedioxybenzyl methyl ketone (I) was obtained from Aldrich (Milwaukee, Wisc., U.S.A.) and was purified by distillation under reduced pressure. Its purity was checked by gas–liquid chromatography (GLC). Diethyl ether was obtained from Mallinckrodt (St. Louis, Mo., U.S.A.) and was glass distilled before use. 3,4-Methylenedioxybenzyl methyl ketoxime (II) was synthesized as white needle-shaped crystals (m.p. 87°–89°) by reacting the corresponding ketone with hydroxylamine hydrochloride using pyridine as a catalyst. The oxime II gave C, H and N analyses consistent with its structure and gave a single peak when examined by GLC.

Gas–liquid chromatography

A Perkin-Elmer Model F 11 gas chromatograph equipped with a flame ionization detector and a Perkin-Elmer Model 56 recorder was employed. The chromatographic columns consisted of glass tubing 6 ft. × ¼ in. O.D. packed with 7.5%

Carbowax 20M on Chromosorb W (80–100 mesh) and glass tubing 6 ft. × ¼ in. O.D. packed with 5% OV-7 on Chromosorb W (80–100 mesh) supplied by Chromatographic Specialities (Brockville, Canada). Both columns were conditioned at appropriate temperatures for the respective phases with low nitrogen flow prior to use. The injection port, detector and column temperatures for the Carbowax 20M column were 190°, 180° and 150°, respectively. Nitrogen was used as a carrier gas at a flow-rate of 70 ml/min. When the OV-7 column was used, the injection port, detector and column tempeatures were 275°, 265° and 200°, respectively. Nitrogen at a flow-rate of 40 ml/min was employed as a carrier gas.

Gas–liquid chromatography–mass spectrometry (GLC–MS)

Mass spectra were recorded at 70 eV with a Hitachi Perkin-Elmer Model RMU-6L mass spectrometer with a Perkin-Elmer Model 900 gas chromatograph attached through a jet separator interphase.

Isolation of metabolic products

MDA (10 μmole/flask) was incubated with fortified 10,000 *g* (20 min centrifugation) liver preparations of guinea-pig, and rabbit, prepared according to the method of Beckett *et al.*[7]. The incubation mixture was adjusted to pH 7.2 with ammonia solution (5%) or with dilute acetic acid (3%), and extracted with freshly distilled diethyl ether (3 × 3 ml). The incubation mixture was then adjusted to pH 12 to 13 with 1 *N* NaOH and extracted again with freshly distilled diethyl ether. The neutral and basic ethereal extracts were concentrated under nitrogen to about 50 μl in each case before analysis.

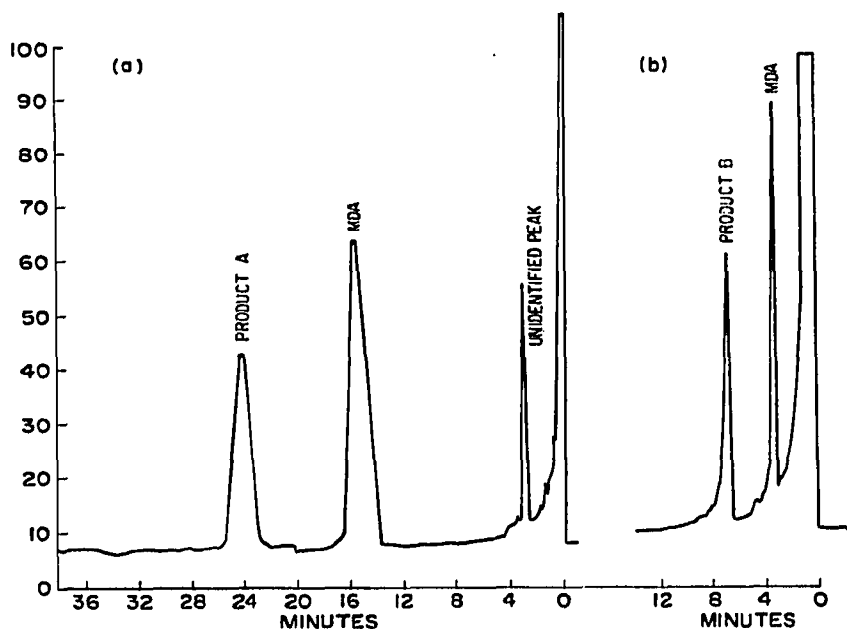

Fig. 1. Chromatograms of incubation mixture extracts. (a) Rabbit liver incubation extract (neutral). (b) Guinea-pig liver incubation extract (basic).

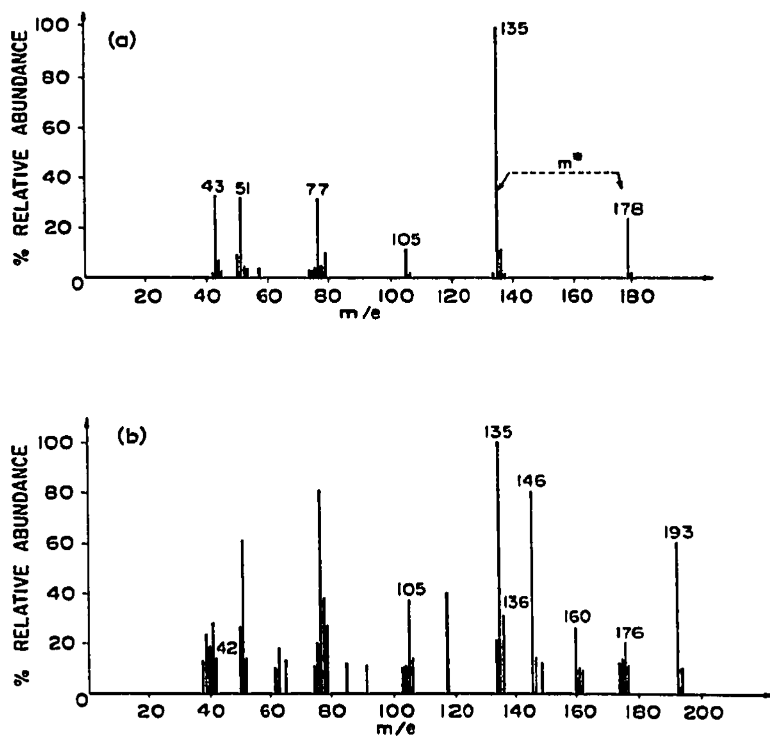

Fig. 2. GLC–MS of products A (a) and B (b).

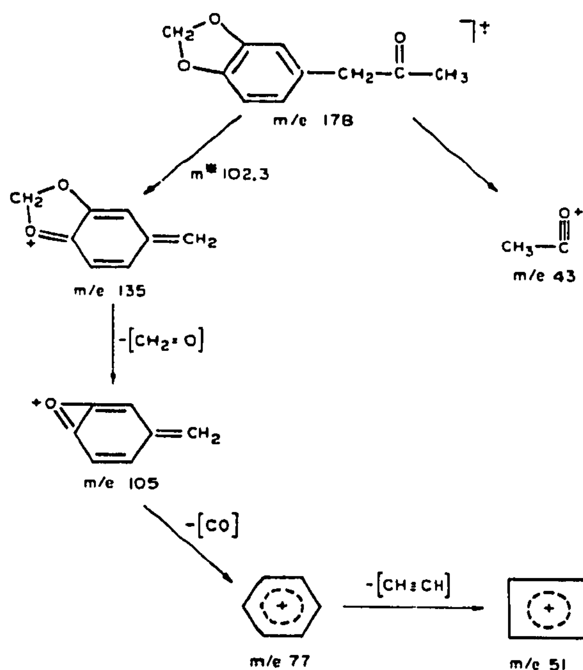

Fig. 3. Postulated mass spectral fragmentations of product A.

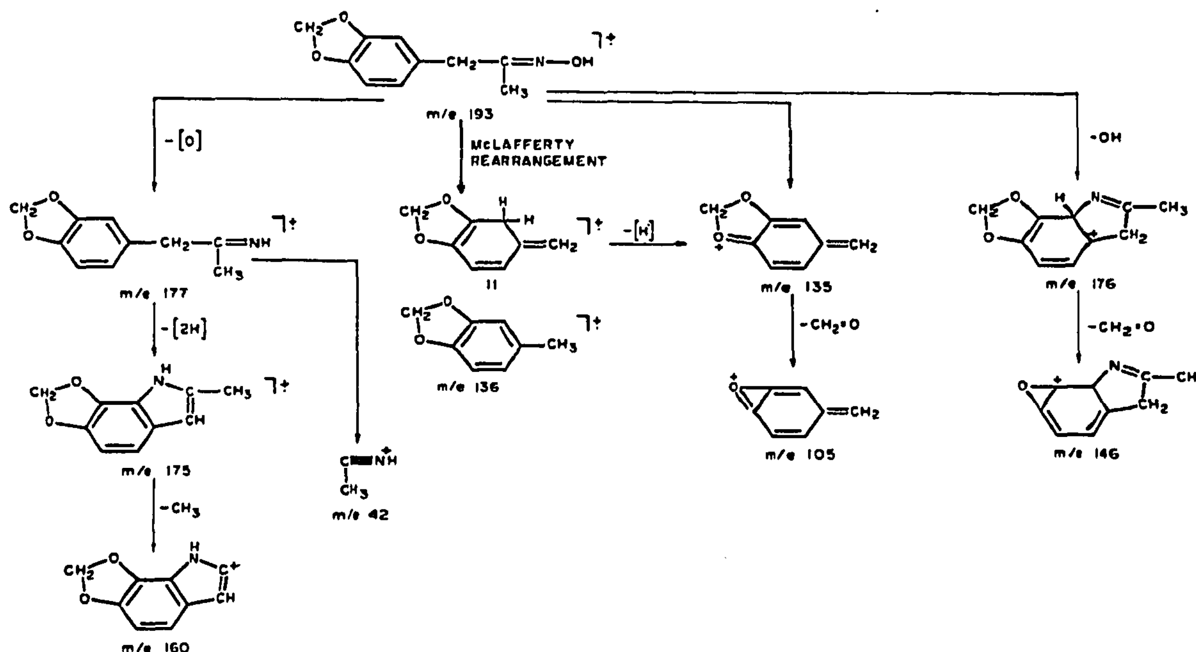

Fig. 4. Postulated mass spectral fragmentations of product B.

RESULTS AND DISCUSSION

Analysis of the neutral ethereal extract of the incubation mixture from guinea-pig and rabbit, on GLC using the Carbowax column, showed a peak (product A) other than MDA (Fig. 1a). This peak was not present in the blank incubation mixture obtained from each species. It had the same retention time as 3,4-methylenedioxy-benzyl methyl ketone (I) and its GLC–MS (Fig. 2a) was similar to that of the synthetic material. It had a molecular ion *m/e* 178 and a base peak at *m/e* 135. The metastable peak at *m/e* 102.3 indicates that the ion at *m/e* 135 is formed from the molecular ion. The ion at *m/e* 43 is diagnostic for ketones[8]. Structures have been postulated for other major diagnostic ions (Fig. 3). This evidence establishes that product A is 3,4-methyl-enedioxybenzyl methyl ketone.

Analysis of the basic ethereal extract from both species on GLC employing the OV-7 column showed a peak for product B other than MDA (Fig. 1b). This peak was absent when the blank ethereal extract (basic) was analysed under the same conditions. Product B had the same retention time as the synthetic oxime II. The GLC–MS (Fig. 2b) of product B was identical to that of synthetic oxime II. Both showed a molecular ion at *m/e* 193. Structures for other major ions have been postulated (Fig. 4). This evidence proves that product B has the structure 3,4-methylenedioxybenzyl methyl ketoxime.

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