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Note

Excretion of methoxyphenamine and its metabolites in urine

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When the aromatic ring of phenethylamines is substituted by methoxy groups, very active substances with a hallucinatory effect arise. These drugs can become subject to abuse. The identification of these substances has been and continues to be discussed in many communications, especially the identification of 4-methoxy-amphetamine (PMA) and 2,4-dimethoxy-4-methyl-amphetamine (DOM). Metabolites of these drugs have been studied[1] and fatal cases after overdosage described[2].

The list of doping drugs includes phenethylamine substituted in the *ortho* position of the aromatic ring by a methoxy group. This drug is N,α-dimethyl-*o*-methoxyphenethylamine, its generic name being methoxyphenamine. It is produced under the name Orthoxine® (Upjohn, Caponago (Milan), Italy) and Mimexina® (Farmogal, Padova, Italy). Methoxyphenamine is an anorectic drug and bronchodilatans with psychostimulating effects. This substance was described first by Woodruff *et al.*[3]; it is easily soluble in water, ethanol and chloroform, and much less soluble in ether and benzene.

Proof of the parent substance can be shown by thin-layer chromatography (TLC) and gas–liquid chromatography (GLC)[4–6]. The metabolites of the drug were studied[7] by non-specific spectrophotometric methods using methyl orange.

EXPERIMENTAL

We met this substance during doping control, and performed some model experiments during the analysis of its metabolites. After administration of one tablet of Orthoxine (100 mg) to each of two adult subjects, samples of urine were collected for 60 h. The pH of urine ranged between 5.5 and 6.9. Samples of urine were made alkaline, then extracted and re-extracted with diethyl ether. The ether extracts were concentrated to 100 μl under nitrogen, and the concentrates were analyzed by TLC, GLC and gas chromatography–mass spectrometry.

Thin-layer chromatography

Silufol UV_{254}® (Kavalier n.p., Votice, Czechoslovakia) plates were used.

Solvent systems were: (A) methanol–ammonia (99:1); (B) chloroform–acetone–ammonia (50:50:1); and (C) *n*-butanol–formic acid–water (20:1:2).

Spray reagents for detection were: (a) Fast Blue B (0.5% in water); (b) bromo-

cresol green (0.1% in ethanol); (c) ninhydrin (0.2% in ethanol and glacial acetic acid); and (d) Marquis reagent.

Gas–liquid chromatography

Chrom 4 (Laboratorní přístroje, Prague, Czechoslovakia) gas chromatograph with flame ionization detector. The following columns and conditions were used.

Column I: metal tubing containing Chromaton N AW DMCS-treated, coated with 2% Carbowax 20M in 5% KOH. Oven temperature, 140°.

Column II: glass tubing containing Anachrom ABS DMCS-treated, coated with 3% SE-30. Oven temperature, 140°.

Column III: glass tubing containing Chromaton N AW DMCS-treated, coated with 1% OV-17. Oven temperature, 120°.

Column IV: glass tubing, containing HP Chrom G, coated with cyclohexanedimethanol succinate. Oven temperature, 120°.

Mass spectrometry

Mass spectra were recorded with a Finnigan Gas Chrom Peak Identifier Model 3000 gas chromatograph and mass spectrometer system, at an electron energy of 70 eV.

Column: glass tubing containing Gas-Chrom Q (60–80 mesh), coated with 3% OV-1. Oven temperature, 140°. The instrument was equipped with an all-glass separator.

RESULTS AND DISCUSSION

In system A two spots were obtained. One spot with R_F 0.20, detected with Fast Blue B, had a yellow colour, and was identical with its parent substance. The second spot with R_F 0.30 was reddish brown and changed gradually to yellow brown. This suggests the presence of two substances. Neither system B nor C produced any better separation; and more satisfying results were not obtained even when other special detection reagents were used.

On analyzing extracts by GLC, three separated peaks were obtained (Fig. 1). Separation of the parent substance and its metabolites is shown in Table I. Relative retention time was adjusted to nicotine standard.

After isolation of the spot with a lower R_F value in system A, a peak corresponding to methoxyphenamine was obtained by GLC. in columns I and II. From the second spot with a higher R_F two peaks were obtained, corresponding to metabolites 1 and 2. According to TLC and GLC it could be suggested that metabolite 1 was a dimethyl derivative of methoxyphenamine, which was identified by mass spectrometry. The spectrum of the parent substance (Fig. 2) revealed the base peak of *m/e* 58, molecular ion peaks M+1 and M−1 and significant fragment ions *m/e* 30 ($-\overset{+}{N}H-CH_3$) and *m/e* 149 ($M^+ - 30$). On the other hand, from the dimethyl derivative (Fig. 3) we found the base peak *m/e* 44, and molecular ion peak *m/e* 165, including M−1 and M+1. It is obvious that both spectra show also common fragments such as *m/e* 121, *m/e* 107, *m/e* 91, *m/e* 65.

The spectrum of the second metabolite (Fig. 4) shows a base peak *m/e* 58 and

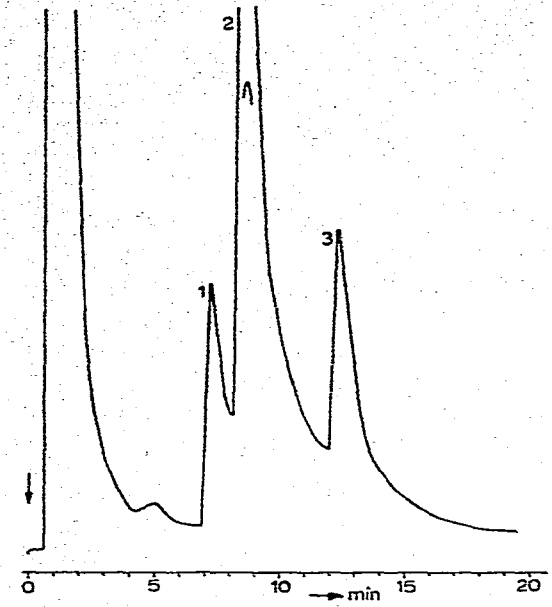

Fig. 1. Gas chromatogram of urine extract. 1, Metabolite 1 (dimethyl derivative of methoxyphenamine); 2, methoxyphenamine; 3, metabolite 2.

TABLE I

SEPARATION OF METHOXYPHENAMINE AND ITS METABOLITES

Reference retention times of nicotine: column I, 4.1 min; column II, 7.4 min; column III, 2.7 min; column IV, 5.5 min.

	Column I		*Column II*	*Column III*	*Column IV*
	Rel. retention time	*Retention index*	*Rel. retention time*	*Rel. retention time*	*Rel. retention time*
Methoxyphenamine	1.19	1796	1.17	1.22	1.27
Metabolite 1	1.22	1813	0.99	1.15	—*
Metabolite 2	6.02	2165	1.69	2.94	6.52

* Insensitive for primary amines.

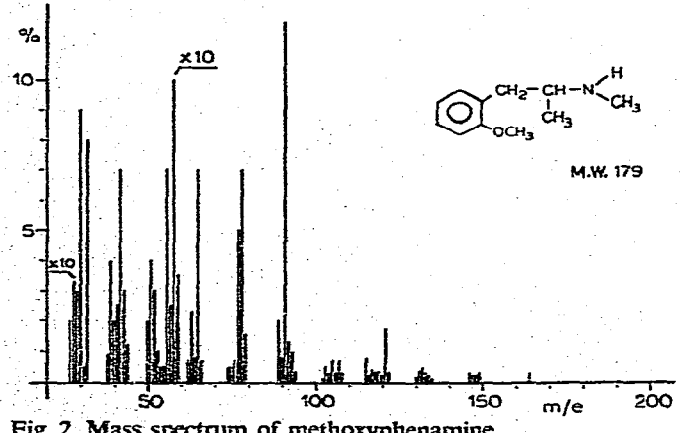

Fig. 2. Mass spectrum of methoxyphenamine.

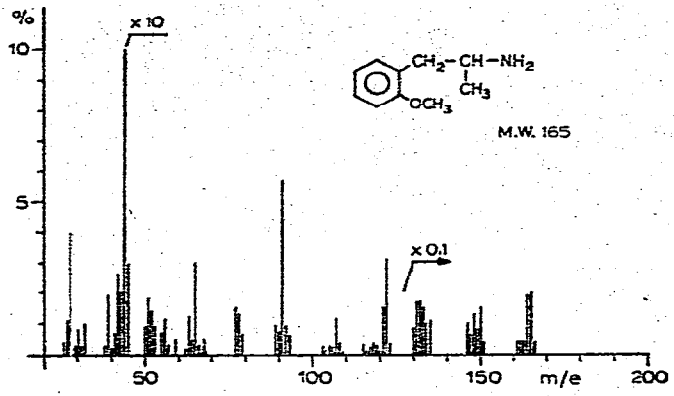

Fig. 3. Mass spectrum of metabolite 1 (dimethyl derivative of methoxyphenamine).

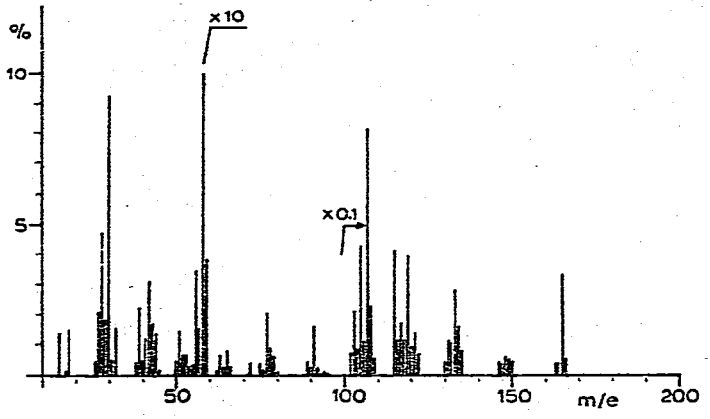

Fig. 4. Mass spectrum of metabolite 2.

other fragments close to the parent substance. According to Beckett and Al-Sarraj[8] a modified metabolic pathway of methoxyphenamine might be: methoxyphenamine → N-hydroxy-*o*-methoxyphenamine → 1-(*o*-methoxyphenyl)-propan-2-ol. The suggested pathway is still under investigation as is the unequivocal identification of metabolite 2.

REFERENCES

1 A. H. Beckett and K. K. Midha, *Xenobiotica*, 4 (1974) 297.
2 M. T. Barnhill, K. June, J. T. Mills, E. L. Slightom and B. A. Walker, *Bull. Int. Ass. Forensic Toxicol.*, 10 (1974) 19.
3 E. H. Woodruff, J. P. Zambory and W. E. Burt, *J. Amer. Chem. Soc.*, 62 (1940) 922.
4 A. H. Beckett, G. T. Turker and A. C. Moffat, *J. Pharm. Pharmacol.*, 19 (1967) 273.
5 G. F. Phillips and J. Gardiner, *J. Pharm. Pharmacol.*, 21 (1969) 793.
6 A. C. Moffat, *J. Chromatogr.*, 113 (1975) 69.
7 T. Otorii and T. Kojima, *Niigata Igakkai Zasshi*, 79 (1965) 625.
8 A. H. Beckett and S. Al-Sarraj, *J. Pharm. Pharmacol.*, 25 (1973) 328.