0090-9556/78/0605-0507$02.00/0
DRUG METABOLISM AND DISPOSITION

Vol. 6, No. 5
Printed in U.S.A.

3,4,5-TRIMETHOXYBENZOIC ACID, A NEW MESCALINE METABOLITE IN HUMANS

LOTHAR DEMISCH, PETER KACZMARCZYK, AND NIKOLAUS SEILER[1]

Zentrum der Psychiatrie, Abteilung für Klinische Psychiatrie im Klinikum der J.W. Goethe-Universität[2] (L.D., P.K.), and Max-Planck-Institut für Hirnforschung, Arbeitsgruppe Neurochemie[2] (N.S.)

(Received February 20, 1978)

ABSTRACT

After ingestion of 400 mg of mescaline sulfate by human volunteers, 3,4,5-trimethoxybenzoic acid was isolated from urine and identified by gas chromatography-mass spectrometry. The amount of this anionic mescaline metabolite was found to be very low as compared with that of the well-known 3,4,5-trimethoxyphenylacetic acid. The significance of this finding is discussed.

Several studies have been published on mescaline metabolism in human subjects (1–6). The major route of degradation in man is *via* deamination to 3,4,5-trimethoxyphenylacetic acid (TMPAA).[3]

In previous papers it has been demonstrated that mescaline metabolism in brain is a more complex process than was generally believed (7–9). Beside N-acetylation and deamination to TMPAA, side-chain degradation occurs *in vivo* and *in vitro*, with formation of 3,4,5-trimethoxybenzoic acid (TMBA). In the present paper we describe the isolation and identification of this new mescaline metabolite from human urine.

Materials and Methods

Reagents and Radiochemicals. Mescaline sulfate, TMBA (E. Merck, Darmstadt, Germany) and 3,4,5-trimethoxyphenylacetic acid (Fluka, Buchs, Switzerland) were purchased. The acids were purified by recrystallization from water. 3,4,5-Trimethoxybenzoylglycine was prepared from glycine and the respective carboxylic acid chloride (10). N-Acetylmescaline was obtained by reaction of mescaline·HCl with acetic anhydride in the presence of pyridine. The acetylated amine was recrystallized from *n*-heptane. The purity of all reference compounds was checked by TLC, GLC, and MS.

3,4,5-Trimethoxy[2,6-^{3}H]phenylacetic acid (specific radioactivity, 50 Ci/mol) (^{3}H-TMPAA) was prepared as described previously for mescaline (11). [7-^{14}C]Benzoic acid (specific radioactivity, 7 Ci/mol) was obtained from New England Nuclear Corp. (Boston, Mass.).

Subjects and Urine Collection. Two male and two female volunteers, aged between 30 and 38 years, ingested orally a single dose of 400 mg of mescaline sulfate dissolved in 50 ml of water. The subjects had been free of drugs for at least 4 weeks and had fasted 6 hr before the experiment was started. Urine samples were collected before mescaline administration and during the following 24 hr. They were saturated with Na_2SO_4 and stored at −20°C until analyzed.

Isolation and Identification of the Acidic Metabolites. The 24 hr urinary output of each subject was adjusted to pH 1 with 3 N HCl and extracted two times with equal volumes of ethyl acetate. The combined ethyl acetate extracts were dried with Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml of 1 N KCl/HCl buffer, pH 1. This solution was subjected to column chromatography on kieselgur (Extrelut, E. Merck). The acidic fraction was eluted from the column with 40 ml of diethyl ether and evaporated to dryness. The methyl esters were prepared by reaction with diazomethane in diethyl ether. The reaction mixtures were evaporated to dryness with a stream of nitrogen and the residues dissolved in 1 ml of ethyl acetate. Aliquots (0.5 ml) of this solution were applied to thin-layer plates (200-μm thick silica gel G_{F254}, E. Merck). The methyl esters of TMBA, TMPAA, and of the glycine conjugate of TMBA were applied as reference compounds. The chromatograms were developed with ethyl acetate/cyclohexane (75:50, v/v). The spots of the reference compounds were visualized by means of fluorescence quenching (254 nm). The corresponding zones of the urinary derivatives were scraped off. Although this

This work was supported by a grant (BO 211/10) from the Deutsche Forschungsgemeinschaft.

[1] Present address: Merrell International, Strasbourg, France (see below).

[2] Frankfurt a.M., Germany.

[3] Abbreviations used are: TMPAA, 3,4,5-trimethoxyphenylacetic acid; TMBA, 3,4,5-trimethoxybenzoic acid; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; MS, mass spectrometry.

Send reprint requests to: Dr. Nikolaus Seiler, Centre de Recherche Merrell International, 16, rue d'Ankara, 67000 Strasbourg, France.

method does not separate TMPAA and TMBA, it is nevertheless essential for their separation from hippuric acid and other acid conjugates, which interfere with the GLC separation. (R_F values: TMPAA and TMBA, 0.6; hippuric acid and 3,4,5-trimethoxybenzoylglycine, 0.10). The silica gel was extracted twice with 3-ml portions of methanol. The combined extracts were evaporated and the residue was dissolved in 50 μl of ethyl acetate. Aliquots (1 μl) of these final solutions were used for GLC/MS. Recovery of the acids from urine was tested with [^{14}C]benzoic acid and ^{3}H-TMPAA; it was 70±5%.

GLC/MS was performed in a Varian model CH5 mass spectrometer to which a Varian gas chromatograph (model 2600) was attached. The chromatograph was equipped with a glass column (2 mm I.D. and 150 cm long) filled with 3% SE-30 on acid-washed Chromosorb (Serva, Heidelberg, Germany). The methyl esters of TMBA and TMPAA were separated isothermally at 135°C [retention times, 7.5 min (TMBA ester) and 9.5 min (TMPAA ester)]. The glycine conjugates were separated by use of a linear temperature gradient (190–220°C); retention time of 3,4,5-trimethoxybenzoylglycine was 6 min. The flow rate of helium was about 15 ml/min. The helium separator temperature was about 220°C; the electron energy was 70 eV. Mass spectra were recorded at the appearance of the peaks as monitored by the total ion current.

Results

In all urine samples collected after ingestion of mescaline, a small peak appeared 2 min before the appearance of the TMPAA peak, which showed the mass spectrum of fig. 1*B*. The two mass fragments most characteristic for TMBA methyl ester {*m/e* 226 (M^+) and 211 [$(M-CH_3)^+$]} were prominent, but the spectrum also contained peaks due to impurities (*m/e* 167 and 137). The TMBA spectrum (fig. 1*A*) was recorded from the peak eluted from the gas chromatograph under conditions similar to those of the analysis of the acidic fraction isolated from urine; it is therefore not a reference spectrum in the strict sense. Although the amount of TMPAA in urines is large after mescaline ingestion, the spectrum in fig. 1*B* does not contain any of the mass fragments typical for TMPAA methyl ester (figs. 1*C* and 1*D*); on the other hand, mass fragments at *m/e* 226 or 211 were never observed in urine samples which were collected before mescaline ingestion. Glycine conjugates of TMBA or TMPAA were not detected.

As determined by comparison with standard mixtures, the ratio TMBA/TMPAA was $< 10^{-4}$

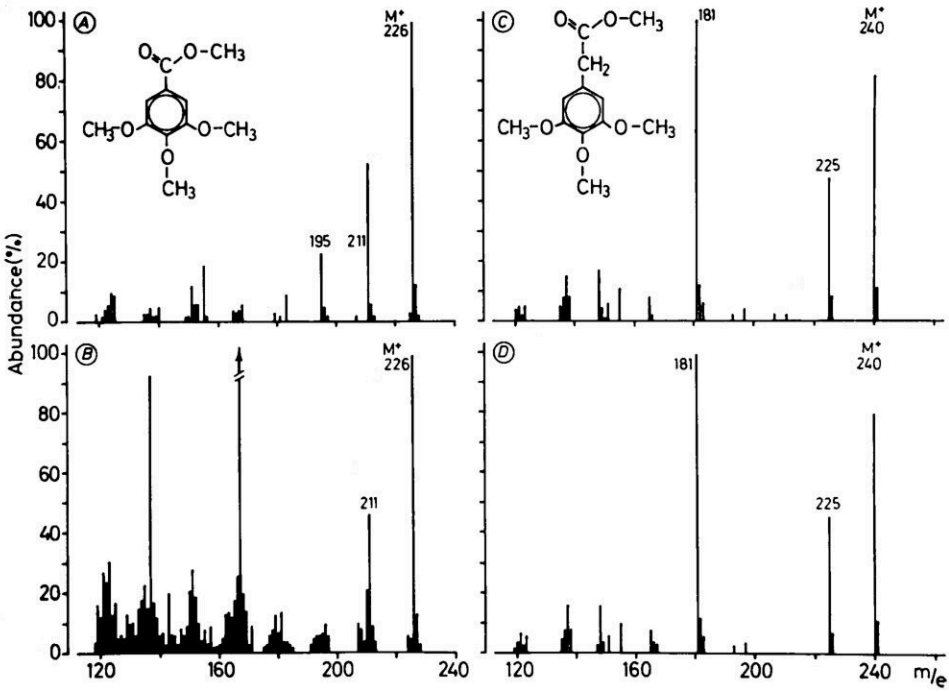

FIG. 1. *Mass spectra of urinary mescaline metabolites and of reference compounds.*

A, 3,4,5-trimethoxybenzoic acid methyl ester; *B*, methylated urinary metabolite of R_T 7.5 min; *C*, 3,4,5-trimethoxyphenylacetic acid methyl ester; *D*, methylated urinary metabolite of R_T 9.5 min.

for urine samples collected for 24 hr after administration of 400 mg of mescaline.

Discussion

All studies on mescaline metabolism indicate clearly that the bulk of mescaline in man is excreted unchanged. During the first 2 hr after mescaline ingestion, about 87% of the dose was excreted, of which 55–60% was unchanged; 27–30% corresponded to TMPAA (6). N-Acetyl-(3,4-dimethoxy-5-hydroxy)phenylethylamine (5%) and N-acetylmescaline (0.1%) have also been identified by chromatography. Harley-Mason *et al.* (3) reported the finding of small amounts (1–2% of the dose) of the glutamine conjugate of 3,4-dihydroxy-5-methoxyphenylacetic acid in human urine.

TMBA was first isolated from mouse brain and liver after injection of 120 mg/kg of [2,6-^{3}H]mescaline. The structure of this acid was identified after derivatization by MS. The enzyme system that catalyzes the oxidative deamination of mescaline to TMBA was localized in the nuclear and microsomal fractions of brain. It was inhibited by SKF 525-A, but not by mono- or diamine oxidase inhibitors (9). The occurrence of this acid in addition to TMPAA in human urine after ingestion of mescaline sulfate is now unequivocally established.

References

1. K. H. Slotta and J. Müller, *Hoppe Seyler's Z. Physiol. Chem.* **238,** 14 (1936).
2. D. Richter, *Biochem. J.* **32,** 1763 (1938).
3. J. Harley-Mason, A. H. Laird, and J. R. Smythies, *Confin. Neurol.* **18,** 152 (1958).
4. L. C. Mokrasch and J. Stevenson, *J. Nerv. Ment. Dis.* **129,** 177 (1959).
5. K. D. Charalampous, A. Orengo, K. E. Walker, and J. Kinross-Wright, *J Pharmacol. Exp. Ther.* **145,** 242 (1964).
6. K. D. Charalampous, K. E. Walker, and J. Kinross-Wright, *Psychopharmacologia* **9,** 48 (1966).
7. N. Seiler and L. Demisch, *Biochem. Pharmacol.* **23,** 259 (1974).
8. N. Seiler and L. Demisch, *Biochem. Pharmacol.* **23,** 273 (1974).
9. L. Demisch and N. Seiler, *Biochem. Pharmacol.* **24,** 575 (1975).
10. G. J. Shiple and C. P. Sherwin, *J. Biol. Chem.* **101,** 463 (1923).
11. N. Seiler, G. Werner, and K. H. Schmidt, *J. Labelled Compd.* **1,** 306 (1965).