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Chromatographic Methods for the Identification of the New Hallucinogen, 4-Methyl-2,5-dimethoxy-α-methylphenethylamine, and Related Drugs

BY K. GENEST AND D. W. HUGHES

(*Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, Canada*)

Thin-layer and gas-chromatographic methods are described for the detection of 4-methyl-2,5-dimethoxy-α-methylphenethylamine in the submicrogram range. Chromatographic criteria of identity for this new hallucinogen and for the related amines, amphetamine, methamphetamine and mescaline, and the hallucinogens, dimethyltryptamine and bufotenine, are also reported.

THE ever-increasing number of hallucinogens, and their expanding illicit use, make the need for reliable methods of identification imperative. While adequate methods are available for compounds such as LSD[1,2,3,4] and hallucinogens derived from natural products,[5,6,7] newer compounds in this group have not been covered as thoroughly. Recently, it was reported[8,9] that many persons who allegedly used a material known colloquially as STP suffered severe psychotomimetic reactions. Some samples, identified as STP, were shown by the U.S. Food and Drug Administration to contain 4-methyl-2,5-dimethoxy-α-methylphenethylamine. Chemically, this compound is closely related to the physiologically active amines, mescaline and amphetamine, and it has been proposed that it be included among the drugs controlled under the U.S. Drug Abuses Control Amendments of 1965. Establishment of identity for forensic purposes frequently requires the application of two or more different analytical techniques, and it is the purpose of this paper to report methods for the identification by thin-layer and gas chromatography of 4-methyl-2,5-dimethoxy-α-methylphenethylamine alone, and in the presence of amphetamine, methamphetamine, mescaline and two other hallucinogens, bufotenine and dimethyltryptamine.

EXPERIMENTAL

MATERIALS—

For thin-layer chromatography, solutions (1 μg per μl) of 4-methyl-2,5-dimethoxy-α-methylphenethylamine hydrochloride (Dow Chemical Company), DL-α-methylphenethylamine sulphate (amphetamine) (Smith, Kline and French Laboratories), *N*-α-dimethylphenethylamine hydrochloride (methamphetamine) (Abbott Laboratories), 3,4,5-trimethoxyphenethylamine sulphate (mescaline) (California Biochem.), *NN*-dimethyltryptamine (Koch-Light Laboratories Ltd.) and 5-hydroxy-*NN*-dimethyltryptamine (bufotenine) (California Biochem.) were used.

For gas chromatography, free bases were prepared from aqueous ammoniacal solutions of salts by exhaustive extraction with diethyl ether (benzene was used with mescaline). Solutions (1 μg per μl) of *NN*-dimethyltryptamine and bufotenine in methanol, and of mescaline in benzene, were used. All of the other bases were dissolved in diethyl ether.

THIN-LAYER CHROMATOGRAPHY

PROCEDURE—

Glass plates, 20 × 20 cm, were coated with 250-μ thick layers made from slurries of silica gel G (E. Merck) (30 g) and water (65 ml) or alumina G (E. Merck) (30 g) and water (55 ml), with the aid of a Desaga apparatus according to Stahl. The plates were activated before use at 110° C for 1 hour.

CHROMATOGRAPHIC SYSTEMS—

System I. Ethyl methyl ketone - dimethylformamide - ammonia solution (sp.gr. 0·90) (13 + 1·9 + 0·1) on silica gel G.
System II. Methanol - chloroform (1 + 1) on alumina G.
System III. Chloroform - methanol - acetic acid (75 + 20 + 5) on silica gel G.

DETECTION REAGENTS—

Reagent A—This consists of the reagents (i) sodium acetate (10 per cent. aqueous), (ii) 2,6-dibromo-*p*-benzoquinone-4-chlorimine (B.D.H. Ltd.) (1 per cent. in ethanol) and (iii) iodine crystals (2 g distributed in two small Petri dishes placed at the bottom of a thin-layer chromatographic tank. [(i) and (ii) remain usable for 5 days if kept in a refrigerator.]

The dried plates were sprayed lightly with (i), then immediately with (ii), carefully avoiding overspray. They were then placed in the iodine tank. Coloured spots appeared promptly. Plates chromatographed in system III were aerated until the acidic background had become dispelled and then sprayed twice with (i) before application of (ii) spray and exposure to iodine vapour.

p-*Dimethylaminobenzaldehyde.*[5]
Modified Procházka reagent.[10]
Potassium iodoplatinate.[11]

GAS CHROMATOGRAPHY

APPARATUS—

A Varian-Aerograph Gas Chromatograph, Model 1520-B, equipped with a Varian-Aerograph Recorder, Model 20, a disc integrator and a hydrogen flame-ionisation detector, was used. It was operated both isothermally and with a matrix multi-linear programmer. Stainless-steel columns, 5 feet × ⅛ inch, were packed with 5 per cent. silicone rubber S.E. 30 on 60 to 80-mesh Chromosorb W, or 3 per cent. silicone GE X.E 60 on 100 to 120-mesh Aeropak 30. The carrier gas used was nitrogen with a flow-rate of 25 ml per minute at 200° C. The injection port and detector were maintained at temperatures of 50° and 25° C higher, respectively, than that of the column. Gas flow-rates for the flame-ionisation detector were 25 ml per minute for hydrogen and 300 ml per minute for air. The X.E. 60 column was conditioned overnight at 240° C with 10 ml per minute gas flow. The S.E. 30 column was conditioned overnight at 250° C with no flow, then at 240° C with 25 ml per minute gas flow until a stable base-line was obtained.

PROCEDURE—

Samples (0·5 to 4 μl) were injected as free bases in diethyl ether, benzene or methanol solution. Salts of the bases and other solvents, such as chloroform, could also be used, but more frequent cleaning of the flame-ionisation detector was necessary with these. Retention times reported are averages (±0·1 minute) from six chromatograms of each compound.

REACTION WITH CARBON DISULPHIDE—

Ten to twenty micrograms of free base in diethyl ether (10 to 20 μl) (benzene was used for mescaline) were transferred into a small test-tube and an equal volume of carbon disulphide was added. The solution was left at room temperature (26° C) and, after timed intervals, or after 5 minutes with mixtures, 1 to 4 μl were injected into the gas chromatograph.

RESULTS AND DISCUSSION

THIN-LAYER CHROMATOGRAPHY—

4-Methyl-2,5-dimethoxy-α-methylphenethylamine could be separated from the related compounds in all of the systems (Table I). The low sensitivity of non-phenolic phenylamines towards general alkaloidal reagents, such as Dragendorff's or potassium iodoplatinate, or other non-specific reagents is well known,[10,12,13,14,15] and identification of mescaline has been considerably improved by application of a modified Procházka reagent and observation under ultraviolet light.[10] Strong fluorescence was also observed for the new hallucinogen with

TABLE I

R_F VALUES* IN THREE THIN-LAYER CHROMATOGRAPHIC SYSTEMS AND COLOUR WITH DETECTION REAGENT A

Compound	System I	System II	System III	Colour
Amphetamine	0·58	0·40	0·21	Violet
Methamphetamine	0·14	0·74	0·33	Yellow
4-Methyl-2,5-dimethoxy-α-methylphenethylamine	0·54	0·48	0·33	Yellow
Mescaline	0·49	0·24	0·20	Yellow
NN-Dimethyltryptamine	0·35	0·93	0·10	Orange - brown
Bufotenine	0·15	0·84	0·05	Dark grey

* Average (±0·02) of six measurements.

this reagent, but the reaction was less sensitive than for mescaline (Table II). The formation of isoquinoline derivatives, which has been suggested in the reaction of formalin with mescaline under the conditions of the thin-layer chromatographic spray,[10] could be impeded in 4-methyl-2,5-dimethoxy-α-methylphenethylamine, either because of steric hindrance caused by the α-methyl group or because of the electronic effect of the 5-methoxy group. Detection reagent A produced different colours with the various compounds (Table I) and was, further, the most sensitive detection reagent used in this investigation (Table II). Presence of the indole

TABLE II

LIMIT OF DETECTION (μg) FOR 4-METHYL-2,5-DIMETHOXY-α-METHYLPHENETHYLAMINE

Reagent	Thin-layer chromatographic system	μg
Reagent A	I	0·3
	II	0·03
Modified Procházka reagent	I	0·4
	II	0·1
Potassium iodoplatinate	I	2·0
	II	1·0

derivatives, *NN*-dimethyltryptamine and bufotenine, was ascertained by spraying with *p*-dimethylaminobenzaldehyde reagent. Other sprays tried, such as ninhydrin, Fast blue B, dansyl chloride, potassium permanganate, sodium tetraphenylborate - fisetin and *p*-nitroaniline, showed no advantages over those reported under Detection reagents.

TABLE III

GAS-CHROMATOGRAPHIC RETENTION TIMES (MINUTES) OF HALLUCINOGENS AND RELATED BASES ON TWO COLUMNS

	Columns: S.E. 30, 5 per cent.				Columns: X.E. 60, 3 per cent.				
Temperature, °C	130	180	205	220	110	150	180	210	240
Compounds									
Amphetamine	2·05	0·85	0·7	—	3·1	1·1	—	—	—
Methamphetamine	2·6	0·9	0·8	—	3·2	1·1	—	—	—
4-Methyl-2,5-dimethoxy-α-methylphenethylamine	8·2	2·2	1·1	—	>40	9·2	3·2	1·5	0·8
Mescaline	—	5·5	2·5	—	—	>40	9·0	3·0	1·2
NN-Dimethyltryptamine	—	6·7	3·4	2·1	—	—	12·5	4·5	1·85
Bufotenine	—	—	8·5	5·0	—	—	—	34·5	10·3

GAS CHROMATOGRAPHY—

Results of isothermal gas chromatography of the six compounds on 5 per cent. S.E. 30 and 3 per cent. X.E. 60 are shown in Table III. All of the compounds gave sharp peaks on both columns. For mixtures and temperature-programmed analyses the X.E. 60 column is

preferred. 4-Methyl-2,5-dimethoxy-α-methylphenethylamine is well separated from the other compounds (Fig. 1). Adequate separation of amphetamine and methamphetamine can be obtained on the S.E. 30 column at 130° C or on the same column with lower loads of liquid phase[16,17]; 0·02 μg of the new hallucinogen can be detected on both columns.

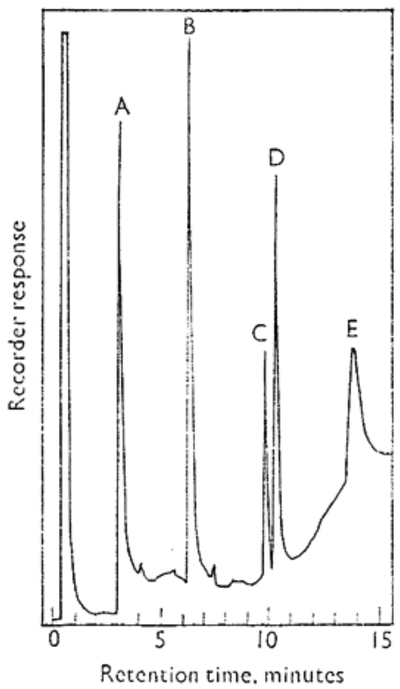

Fig. 1. Programmed gas chromatogram of a mixture of 1 μg each of amphetamine and methamphetamine (A), 2 μg of 4-methyl-2,5-dimethoxy-α-methylphenethylamine (B), 1 μg of mescaline (C), 2 μg of dimethyltryptamine (D) and 2 μg of bufotenine (E); 3 per cent. X.E. 60, single-column operation, flash heater at 220° C, flame-ionisation detector at 250° C, range 1, attenuation 64 and flow-rate of nitrogen, 25 ml per minute. Programme: initial temperature 100° C; 2/20° C; 3/hold; 4/30° C; 6/8° C; 12/15° C; 16/end (numbers before oblique represent minutes after injection, and those after represent the increase in temperature in degrees per minute)

Additional analytical information can be obtained by gas chromatography of derivatives produced by reaction of the primary bases with carbon disulphide at room temperature. Methamphetamine, *NN*-dimethyltryptamine and bufotenine show no reaction, but 4-methyl-2,5-dimethoxy-α-methylphenethylamine, mescaline and amphetamine, after brief treatment with carbon disulphide, yield a derivative generating a distinct peak with higher retention time on either column (Table IV). Formation of the derivative can also be achieved by "on-column reaction gas chromatography," *i.e.*, injection of 1 to 2 μg of the free base, followed, within 10 to 20 seconds, by 3 to 4 μl of carbon disulphide. As retention times obtained by this method are, however, often distorted and conversions incomplete, the procedure as described under Experimental is preferred. Differences were observed in the rate of conversion of the three amines that form derivatives with carbon disulphide. The reaction of mescaline is rapid, being more than 99 per cent. complete after 20 seconds. The reactions of amphetamine and 4-methyl-2,5-dimethoxy-α-methylphenethylamine with carbon disulphide

are slower. Approximate conversion rates were determined by comparing the integrated areas for the peaks of the free bases and their derivatives. In this manner, it was established that after 20 seconds, 2·5, 16 and 40 minutes, and 16 hours of contact with carbon disulphide, 51, 56, 71, 87 and 98 per cent. of amphetamine and 47, 53, 59, 67 and 99 per cent. of 4-methyl-2,5-dimethoxy-α-methylphenethylamine were converted into the corresponding derivatives.

TABLE IV

GAS-CHROMATOGRAPHIC RETENTION TIMES (MINUTES) OF ISOTHIOCYANATES ON TWO COLUMNS

	Columns								
	S.E. 30, 5 per cent.				X.E. 60, 3 per cent.				
Temperature, °C	130	180	205	220	110	150	180	210	240
Compounds									
Amphetamine - carbon disulphide	8·7	2·2	1·5	—	25·5	5·4	2·2	0·8	—
4-Methyl-2,5-dimethoxy-α-methylphenethylamine - carbon disulphide	—	10·7	5·0	—	—	—	11·2	4·0	1·8
Mescaline - carbon disulphide	—	15·1	6·9	—	—	—	36·8	10·2	3·1

The reaction of primary amines with carbon disulphide has been applied to the detection of amphetamine in urine,[18] and it has been shown that the product of this reaction is the mustard oil, α-methylphenethylisothiocyanate,[19] which is formed via the dithiocarbamic acid. Mescaline and 4-methyl-2,5-dimethoxy-α-methylphenethylamine probably follow an analogous course of reaction, and the formation of 2,4,5-trimethoxyphenethylisothiocyanate and 4-methyl-2,5-dimethoxy-α-methylphenethylisothiocyanate is likely under the experimental conditions.

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