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Note

Separation and identification of trimethylsilyl derivatives of tyramines and methoxytyramines by gas–liquid chromatography*

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The main objective of the present study was to develop a gas chromatographic (GC) method for the identification of the tyramine hydrolysation products of cyclopeptide alkaloids. Cyclopeptide alkaloids (more than 70) contain either 13-, 14- or 15-membered cyclic systems[1,2]. In addition to two amino acids, the 13-membered rings contain β-(2-methoxy-5-hydroxy)styrylamine groups, whereas 14-membered rings are characterized by *p*-hydroxystyrylamine. Total hydrolysis[3] of the hydrogenated alkaloids releases β-(2-methoxy-5-hydroxyphenyl)ethylamine (13-membered ring) and β-(4-hydroxyphenyl)ethylamine (14-membered ring), together with the corresponding amino acids.

The present study was to find out a GC method for the separation of a number of tyramines in order to identify those which are already known and those which may be present in the cyclopeptide alkaloids. GC has been used to separate phenolic, catecholic and related biogenic amines as the free amines[4], but more usually after derivative formation. Trifluoroacetyl (TFA)[5–10] and especially pentafluoropropionyl (PFP) and heptafluorobutyryl (HFB)[9–16] derivatives show excellent electron affinities for electron-capture detection. Isothiocyanate[17] (NCS) and N-2,4-dinitrophenyl (DNP)[18] derivatives and pentafluorobenzaldehyde (PFB)–amine condensation products[19] have also been used for GC. Acetylations[20–22] and silylations have been widely used for flame ionization detection. Trimethylsilylations were carried out with hexamethyldisilazane (HMDA)[23–25], with the system bis(trimethylsilyl)acetamide (BSA) and trimethylsilylchlorosilane (TMCS)[26–28], and with N-trimethylsilylimidazole (TMS–Im)[22,26]. For the present separation, all of the methods cited above were tested, with the exception of PFP and HFB derivatives (since no electron-capture detector was available).

p-Tyramine and 3-methoxy-*p*-tyramine have been separated as the diacetyl[20], as the di-TFA[7] and as the O-TMS, N-PFB derivatives[19]. Separation of the two isomeric monomethyl ethers of dopamine could not be achieved by Moffat, whereas Narasimhachari and Vouros[17] were successful using O-TMS–NCS derivatives. Edwards and Blau[18] have reported the separation of 3-methoxy-*p*-, 3-methoxy-*o*- and 3-methoxy-*m*-tyramine as the O-TMS,N-DNP derivatives.

* Devoted to Prof. Dr. Eugen Müller with best wishes for his 70th birthday.

EXPERIMENTAL

Standard amines

β-Phenylethylamine (1) was obtained from Aldrich (Milwaukee, Wisc., U.S.A.) and *p*-tyramine (2) was from J. T. Baker (Gross Gerau, G.F.R.). *m*-Tyramine (3) was synthesized by decarboxylation of D,L-*m*-tyrosine in boiling fluorene under a stream of nitrogen gas[29]. *o*-Tyramine (4), 3-methoxy-*p*-tyramine (5), 4-methoxy-*m*-tyramine (6), 6-methoxy-*m*-tyramine (7), 3-methoxy-*o*-tyramine (8) and 5-methoxy-*o*-tyramine (9) were prepared from the corresponding benzaldehydes by use of nitromethane[30–32] and subsequent reduction to phenethylamines[30,33].

Derivative formation

The hydroxyl groups of the standard compounds were converted into trimethylsilyl ethers and the primary amino groups to tertiary N,N-bis-(trimethylsilyl)-amino groups. In all of the steps, precautions were taken to avoid the presence of moisture. 1 mg of the amine or amine hydrochloride was placed in a 1-ml reaction vial which was sealed with a Mininert valve (Pierce, Rotterdam, The Netherlands). Through the silicone septum of the valve, 400 μl of dry acetonitrile, 200 μl of N,O-BSA (E. Merck, Darmstadt, G.F.R.), 50 μl of TMCS (freshly destilled) and 1 μl of water were added by means of a microlitre syringe. The resulting solution was heated to 90° for 30 min. Aliquot portions for GC analysis were taken directly from the vial.

Gas chromatography

All of the investigations were made on a Pye Unicam Series 104 chromatograph (packed column) and a Carlo Erba Model G.I. chromatograph (capillary column), both instruments being fitted with flame ionization detectors. The following conditions and stationary phases were used. (a) A glass column (2 m × 4 mm I.D.) was packed with chromosorb W HP (80–100 mesh) coated with 3% silicone OV-101. Carrier gas (nitrogen) flow-rate, 30 ml/min. Oven temperature, isothermal at 160°. Injection block temperature, 190°. (b) A capillary glass column (50 m × 0.2 mm I.D.) was coated with silicone OV-101. Carrier gas (nitrogen) pressure, 1.8 atm. Oven temperature, isothermal at 170°. Injection block temperature, 190°.

Cyclopeptide alkaloids

The alkaloids were hydrogenated in methanol over a palladium–carbon catalyst for 4–8 h, and the purified dihydro products were subsequently hydrolyzed with 20% sodium hydroxide as described by Zbiral *et al.*[3] (basic hydrolysis does not affect the methoxy groups). After adjusting the solutions to pH 8, the samples were evaporated under a stream of nitrogen gas. The tyramines were extracted with ethanol and then silylated.

RESULTS AND DISCUSSION

As GC reference compounds *o*-, *m*- and *p*-tyramine and five isomeric methoxytyramines were used.

Silylation of the tyramines (see refs. 26 and 28) yielded single O,N,N-tris(tri-

TABLE I

RELATIVE RETENTION DATA FOR THE O,N,N-TRIS(TRIMETHYLSILYL)DERIVATIVES

Relative retention = (retention time for the derivative)/(retention time of the derivative of *o*-tyramine). The retention times of the derivative of *o*-tyramine were 18 and 8.5 min at 160 and 180° on 2 m of OV-101, and 15 min at 170° on 50 m of OV-101.

No.	*Compound*	*2 m OV-101*		*50 m OV-101*
		160°	*180°*	*170°*
2	*p*-Tyramine	1.37	1.28	1.25
3	*m*-Tyramine	1.11	1.08	1.08
4	*o*-Tyramine	1.00	1.00	1.00
5	3-Methoxy-*p*-tyramine	2.28	2.00	1.88
6	4-Methoxy-*m*-tyramine	2.19	1.90	1.78
7	6-Methoxy-*m*-tyramine	2.19	1.90	1.81
8	3-Methoxy-*o*-tyramine	1.86	1.69	1.60
9	3-Methoxy-*o*-tyramine	2.28	2.00	1.85

methylsilyl) derivatives of high stability (slow hydrolysis in solution and no on-column decomposition) which formed sharp symmetrical peaks without tailing.

The selectivities of the stationary phases GE SE-30, OV-101, PPG 2025, DC QF-1 and GE XE-60 were evaluated in runs with temperature programming. OV-101 had the most suitable properties.

o-Tyramine was used as the internal standard since the parent compound, β-phenylethylamine, that was first chosen resulted in interference with the solvent peak. The derivatives were characterized by their relative retentions, retention times being measured from the point of injection (Table I).

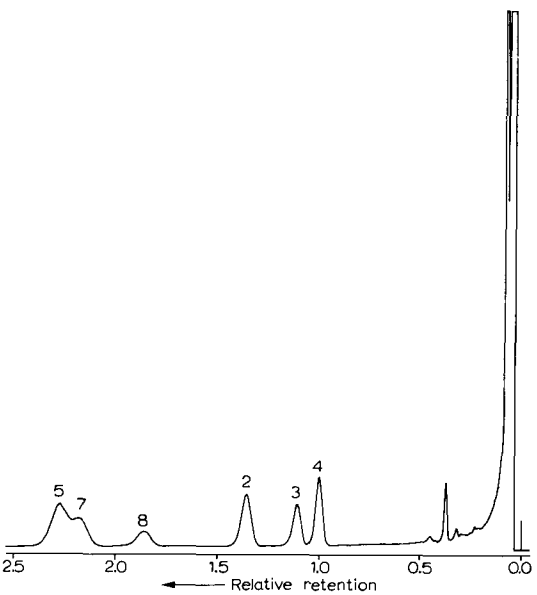

Fig. 1. GC separation of tyramines as their O,N,N-tris(trimethylsilyl) derivatives on a 3% OV-101 column (2 m × 4 mm), isothermal at 160°. The peak numbers correspond to Table I.

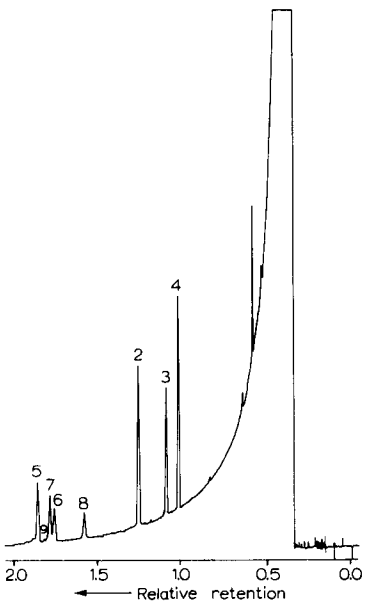

Fig. 2. GC separation of tyramines as their O,N,N-tris(trimethylsilyl) derivatives on an OV-101 capillary column (50 m × 0.2 mm), isothermal at 170°. The peak numbers correspond to Table I.

Chromatograms obtained on both packed and capillary columns showed a satisfactory separation of the compounds 1–4 and 8. Compounds 6 and 7 and 5 and 9 had very similar retention values on packed columns. The first peak to be eluted (pair 6 + 7) appeared as a shoulder on the second peak (pair 5 + 9) with an overlapping of 75%. With capillary columns, however, good separation efficiencies were achieved for these pairs without interference from each other or from the solvent peak (Figs. 1 and 2).

Identifications from dihydropeptide alkaloids

Integerressine (integerrine type, 14-membered cyclic system[1]) and ziziphine-A (ziziphine-A type, 13-membered system[1]) were chosen in order to examine the applicability of the method of analysis to determinations in these biological tissues. Identification of the phenethylamines was achieved: (a) by comparing the relative retentions with the standard values. (On capillary columns the result was definite.) (b) with simultaneous administration of the mixture of all of the standard amines, when the peaks of compound 2 (with integerressine) and of compound 7 (with ziziphine-A) were distinctly elevated.

False chromatographic results may arise for the following reasons. Partial hydrolysis of the silylated derivatives can occur due to the presence of moisture or to water in the BSA solution. This hydrolysis reaction can be reversed by increasing the BSA concentration. Partial decomposition (irreversible) may also occur on storing the samples at room temperature or at 5° for more than 4 or 5 days. However, silylated amino acids originating from the alkaloid hydrolyzate do not cause interference since they have significantly lower retention times.

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