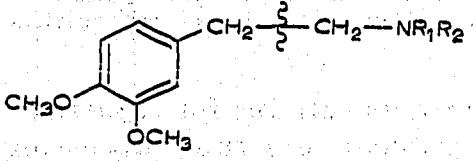
CHROM. 3601

Gas–liquid chromatography of the trimethylsilyl derivatives of several amines of biological interest

Recent efforts in our laboratory have been directed toward the identification and estimation of urinary amines in normal individuals and those suffering from Parkinson's disease, with special interest focused on 3,4-dimethoxyphenylethylamine (DMPEA) and tryptamine and the possible relationship of these substances to this disease[1]. Primary amines are often converted to less polar compounds to facilitate their successful gas–liquid chromatographic (GLC) analysis [2–10]. The availability of the powerful reagent *bis*-trimethylsilylacetamide (BSA)[10–12] led us to investigate trimethylsilylation as a method for derivatizing these two amines*.

Reaction of DMPEA with BSA in pyridine at room temperature for 10 min (or at 60° for 5 min) led to the formation of a new compound with a retention time somewhat more than twice that of the parent amine (Ia) with the non-polar or non-selective[13] stationary phase employed in this study. When DMPEA is allowed to react with BSA and trimethylchlorosilane in pyridine at 60° for 20 min a different derivative is formed with a retention time roughly three times that of the first derivative. On the basis of reaction conditions and retention behavior, it can be assumed that the faster moving trimethylsilylation product is the mono-TMSi DMPEA (Ib), whereas the more slowly eluted compound is the di-TMSi DMPEA (Ic). This has been confirmed by combined gas chromatography–mass spectrometry (GLC–MS), now widely acknowledged to be a powerful method of identification[8,10,12,14,15]. The mass spectrum of Ib exhibited *m/e* values expected for such a structure—molecular ion of 253 and electron impact-induced fragment arising from the side chain (by scission of the carbon–carbon bond) of 102. The corresponding values for Ic were 325 and 174, respectively, in complete agreement with the proposed structure.



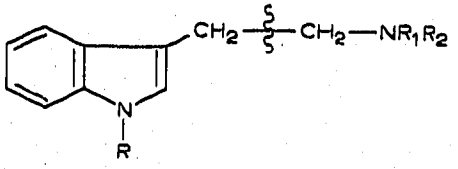
Ia $R_1 = R_2 = H$
Ib $R_1 = H, R_2 = (CH_3)_3Si$
Ic $R_1 = R_2 = (CH_3)_3Si$

Selective conversion of this primary amino group to either a mono- or di-TMSi derivative can thus be achieved by manipulation of reaction conditions.

The same kind of side chain occurs in tryptamine (IIa), but the presence of the indole imino group is a potentially complicating factor in forming derivatives of this compound. Ease of trimethylsilylation of the indole nitrogen was studied by subjecting N,N-dimethyltryptamine (IIb) to those reaction conditions which yield

* The reaction of catecholamines (including DMPEA) with several trimethylsilylating reagents has recently been reported[10].

the di-TMSi of DMPEA. GLC analysis indicated only a small conversion (< 10%) of N,N-dimethyltryptamine to a more slowly eluted compound—presumably the corresponding indole ring trimethylsilylated compound (IIc). The retention time increase (factor of 1.5) resulting from this functional group change is significantly smaller than those observed for trimethylsilylation of the amino group of DMPEA. When subjected to combined GLC–MS this compound (IIc) was observed to give *m/e* values at 260 (molecular ion), 202 (indole methylene fragment) and 58 (side chain fragment), all compatible with the proposed structure.



IIa $R = R_1 = R_2 = H$
IIb $R = H$, $R_1 = R_2 = CH_3$
IIc $R = (CH_3)_3Si$, $R_1 = R_2 = CH_3$
IId $R = R_1 = H$, $R_2 = CH_3$
IIe $R = H$, $R_1 = (CH_3)_3Si$, $R_2 = CH_3$
IIf $R = R_1 = (CH_3)_3Si$, $R_2 = CH_3$
IIg $R = R_1 = H$, $R_2 = (CH_3)_3Si$
IIh $R = H$, $R_1 = R_2 = (CH_3)_3Si$
IIi $R = R_1 = R_2 = (CH_3)_3Si$

Replacement of one of the methyl groups of N,N-dimethyltryptamine by a hydrogen atom gives N-methyltryptamine (IId), a compound which possesses a side chain secondary amino group*. The heating of N-methyltryptamine in BSA and pyridine for 5–10 min at 60°, or reaction with BSA and trimethylchlorosilane in pyridine at room temperature, leads to the production of a new compound (IIe) with a retention time twice that of the parent amine. Chromatography of the reaction mixture resulting from the addition of heat (60° for 20 min) to the second set of reaction conditions mentioned above discloses the presence of a small amount of a more slowly eluted compound (IIf), the retention time of which is 1.3 times that of IIe, the major component. These retention time changes suggest that the first trimethylsilylation product represents side chain substitution, and that it is reaction on the ring nitrogen atom which is more difficult to achieve. That this is the case was proven by combined GLC–MS analysis of the mixture of these two compounds. The more volatile component (IIe) exhibited *m/e* values of 246 (molecular ion), 130 (indole methylene fragment) and 116 (side chain fragment), whereas the less volatile component (IIf) is trimethylsilylated on the indole nitrogen atom as well (molecular ion, 318; indole methylene fragment, 202; side chain fragment also 116).

The silylation studies with DMPEA, N,N-dimethyltryptamine and N-methyltryptamine suggested that it should be possible to selectively prepare the mono-TMSi (side chain) derivative of tryptamine, but that successful selective transformation of tryptamine (IIa) to the di-TMSi (side chain) derivative might be more

* HORNING *et al.* have reported that in the catecholamine series secondary amines react very slowly if at all with BSA[10].

TABLE I

RETENTION BEHAVIOR OF 3,4-DIMETHOXYPHENYLETHYLAMINE, TRYPTAMINE, AND THEIR TRIMETHYLSILYL DERIVATIVES

Compound[a]	*Retention time*[b] *(relative to anthracene)*
Anthracene[c]	1.00
Ia 3,4-Dimethoxyphenylethylamine	0.32
Ib Mono-TMSi[d] 3,4-dimethoxyphenylethylamine	0.72
Ic Di-TMSi 3,4-dimethoxyphenylethylamine	2.36
IIa Tryptamine	0.78
IIb N,N-Dimethyltryptamine	0.95
IIc Mono-TMSi N,N-dimethyltryptamine	1.45
IId N-Methyltryptamine	0.92
IIe Mono-TMSi N-methyltryptamine	1.85
IIf Di-TMSi N-methyltryptamine	2.46
IIg Mono-TMSi tryptamine	1.50
IIh Di-TMSi tryptamine	5.03
IIi Tri-TMSi tryptamine	6.35

[a] Structures for the amines and their derivatives are given on pp. 354 and 355. Derivative formation [generally on 50 μg of amine; amounts of reagents as follows: bis-trimethylsilylacetamide (40 μl), pyridine (20 μl), trimethylchlorosilane (10 μl)] was carried out as described in the text. Bis-trimethylsilylacetamide and trimethylchlorosilane were obtained from Supelco, Inc., Bellefonte, Pa. (U.S.A.). The LKB Model 9000 was used for combined GLC–MS.

[b] Column conditions: 6 ft. × 4 mm I.D. glass U-tube; 5% F-60 (methylpolysiloxane containing a few percent of *p*-chlorophenyl groups) coated onto a 1% JXR (methylpolysiloxane) packing (80–100 mesh acid-washed and silanized Gas Chrom P); 165°; 13 p.s.i.; Barber–Colman Model 15 chromatograph equipped with a Lovelock argon ionization detector.

[c] Absolute retention time: 5.9 min.

[d] Trimethylsilyl.

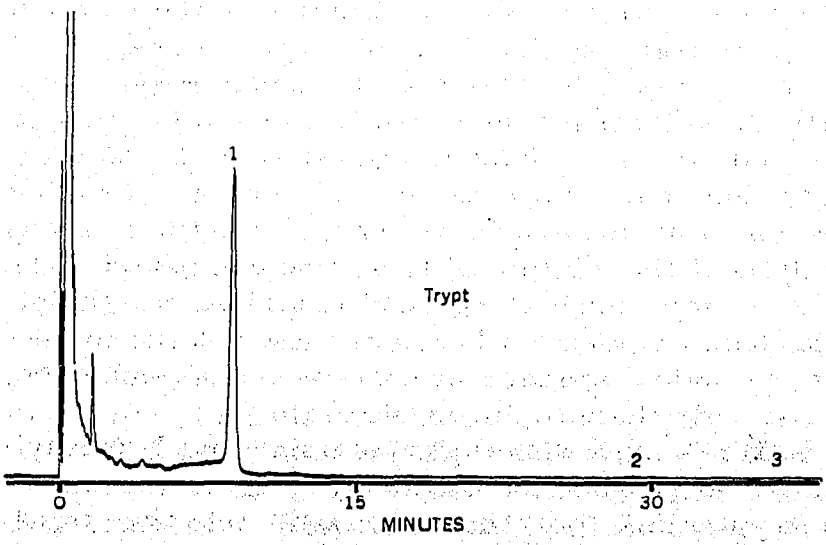


Fig. 1. Gas–liquid chromatogram obtained by analysis of an aliquot of the room temperature 10 min reaction mixture of tryptamine with bis-trimethylsilylacetamide in pyridine. The mono-trimethylsilyl derivative is at position 1; the di- and tri-derivatives, if present, would have appeared at positions 2 and 3, respectively. Column conditions are given in Table I.

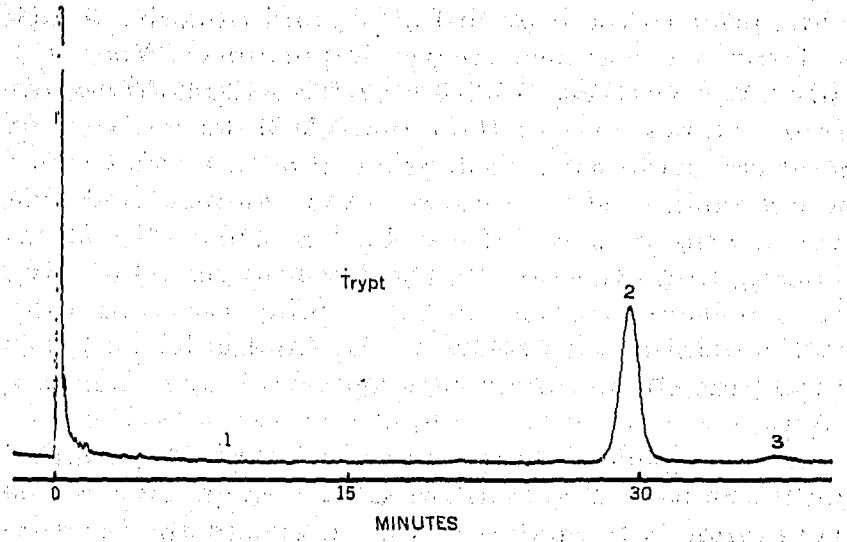


Fig. 2. Gas–liquid chromatogram obtained by analysis of an aliquot of the 60° 20 min reaction mixture of tryptamine with bis-trimethylsilylacetamide and trimethylchlorosilane in pyridine. The major peak (position 2) is the di-trimethylsilyl derivative; a trace of the tri derivative is at position 3; the mono derivative, if present, would have appeared at position 1. Column conditions are given in Table I.

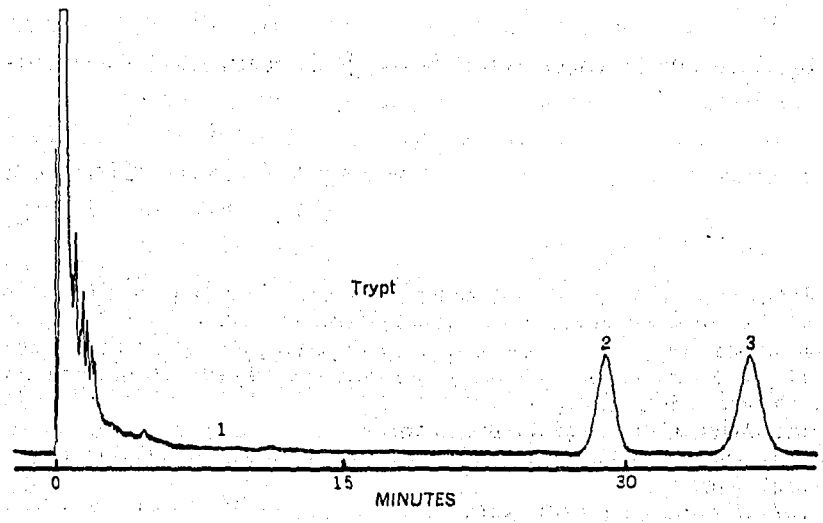


Fig. 3. Gas–liquid chromatogram obtained when a sample of tryptamine was treated as in Fig. 2, but with reaction time increased to 45 min. The major peaks are the di- and tri-TMSi derivatives (positions 2 and 3, respectively); no mono derivative (position 1) is present. Column conditions are given in Table I.

difficult because of the possibility of attack on the indole nitrogen atom to yield some of the tri-TMSi derivative. This is indeed what was observed. Reaction of tryptamine with BSA in pyridine for 10 min at room temperature (or at 60° for 5 min) leads to formation of a new compound (see Fig. 1) with a retention time twice that of the parent amine, a retention factor very similar to that observed for the mono trimethylsilylation of DMPEA. It is clear from the mass spectrum of this derivative (IIg) [*m/e* values of 232 (molecular ion), 130 (indole methylene fragment) and 102 (side chain fragment)] that it possesses a single TMSi group attached to the side chain nitrogen atom. Unfortunately this compound shows some indication of instability toward GLC con-

ditions (note the rise in the base line prior to the peak in Fig. 1), and thus the ability to selectively form the di-TMSi derivative assumes greater importance. When the reaction is carried out for 20 min at 60° with the addition of trimethylchlorosilane GLC analysis of the reaction mixture shows none of the mono-TMSi derivative, but rather a peak conforming to theoretical shape with a retention time three times that of IIg (suggesting that this new compound, IIh, contains a twice-substituted side chain) plus a trace of a still slower moving component (see Fig. 2). This late component, IIi, possesses a retention time 1.3 times that of IIh, the factor expected for ring substitution, and both of these retention behavior-based assumptions are fully supported by mass spectrometry. IIh exhibits *m/e* values of 304 (molecular ion), 130 (indole methylene fragment) and 174 (side chain fragment), whereas the corresponding values for IIi are 376, 202, and 174.

That reaction conditions for compounds containing a number of potential substitution sites must be carefully controlled is illustrated in Fig. 3. This is the chromatogram observed when tryptamine is treated as in Fig. 2, except the reaction is allowed to proceed for 45, rather than 20 min; the increase in the relative proportion of the tri-TMSi derivative of tryptamine is evident. This paper illustrates the value of combined GLC–MS in complementing GLC techniques in establishing reaction conditions appropriate for the preparation of derivatives suitable for the GLC of compounds of biological interest.

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