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GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF TRIMETHYLSILYL DERIVATIVES OF ω-AMINO ACIDS

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SUMMARY

The silylation of C_4–C_{11} ω-amino acids and their hydrochlorides was carried out with N,O-bis(trimethylsilyl)acetamide. Di-TMS and tri-TMS derivatives were formed in the silylation of the hydrochlorides. The formation of the two derivatives is dependent on the reaction conditions, and the conditions under which only one derivative arises reproducibly, are reported. This finding may be utilized in the quantitative analysis of amino acids.

INTRODUCTION

In recent years, considerable attention has been paid to the analysis of the products of protein hydrolysis, and gas chromatography appeared to be suitable for this purpose. Amino acids are strongly polar compounds with very low volatility and, as with many other low-molecular-weight polyfunctional compounds, cannot be analyzed by gas chromatography (GC) without their prior conversion into suitable volatile derivatives[1]. The preparation of such derivatives should be as simple as possible, rapid, reproducible and give high yields. So far, almost 100 derivatization procedures have been described; for recent reviews see references 2 and 3.

One of the techniques applicable to the preparation of volatile amino acid derivatives is silylation. The theory of silylation was well explained by Pierce[4], together with description of several methods for the preparation of trimethylsilyl (TMS) derivatives. There are many silylation reagents available, and attention should be paid to their choice as well as that of the solvents that may be used for derivative preparation[5,6]. Strong TMS donors are now the most frequently used reagents, such as N,O-bis(trimethylsilyl)acetamide (BSA)[7–9], N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)[10–13] and trimethylsilylimidazole (TMSIM)[14,15]. In some reports,

the effectiveness of various silylation reagents is compared[14,15]. The conditions used in individual procedures are, however, different and therefore no general conclusions can be drawn. The fact that no generally applicable silylation procedure exists is a disadvantage as regards the preparation of volatile derivatives. Moreover, because of the ready hydrolysis of both silyl derivatives and silylation reagents, it is neccessary to exclude any moisture during the reaction.

The problems of the silylation of ω-amino acids has been far less studied. The reports published can be divided into two main classes. The first group include studies aimed at extending the general knowledge of silylation as a means of derivatization. Gehrke[17], for instance, studied the silylation of 20 protein amino acids with BSTFA in acetonitrile (ACN). He tried to find the optimal conditions for the formation of the derivatives of individual acids, including α-, β- and γ-aminobutyric acids. The same reagent and solvent were used by Bergström and Gütler[18] for the silylation of a large number of amino acids. For identification of the derivatives formed he used mass spectrometry, as did Ryhage[19,20]. Detailed studies on glycine showed that two derivatives were formed, the proportions of which changed with time. For the di-TMS and tri-TMS derivatives formed from both C_4 and C_6 amino acids, in mass spectrometry (MS) characteristic ions appeared to occur at $m/e = 102$ and 174, respectively. (The formation of these two ions from glycine was previously pointed out by Hils *et al.*[21], who had carried out silylation with N-trimethylsilyldiethylamine and identified the products by elemental analysis.) When the silylation was performed with the reagent alone in the absence of a solvent, only the di-TMS derivative was formed. In a subsequent paper, Bergström *et al.*[22] studied the conditions of the silylation of α- and ω-amino and α,ω-diamino acids and confirmed that more derivatives were formed from C_2–C_6 ω-amino acids. In their work, the conditions under which either the di-TMS or the tri-TMS derivative was formed were established.

The second group of papers include those for which the silylation is used as a technique for the analysis of polymeric compounds that contain sequences of ω-amino acids. The polymeric material is hydrolyzed to ω-amino acid hydrochlorides, which are subsequently silylated. Thus, Mori[23] performed silylation with BSA in ACN containing triethylamine (TEA). He followed the influence of the hydrochloric acid released on the course of whole derivatization process. Although he did not determine the degree of conversion, the reproducibility of the derivatization indicated that it proceeded virtually quantitatively. With the ω-amino acids studied (C_2–C_{12}), the formation of di-TMS derivatives is assumed. For instance, in the quantitative determination of nylon 66, a number of copolymers of cyclic lactams of ω-amino acids have been described[24]. ω-Amino acid hydrochlorides were silylated with an excess of BSA itself. As full details were not given, it is difficult to evaluate the results. Here also the formation of di-TMS derivatives is assumed.

We intended to prepare silyl derivatives of selected ω-amino acid hydrochlorides using BSA, with the aim of identifying the derivatives formed (some of them have not yet been reported), and further to examine known techniques and to find the optimal conditions for derivative preparation applicable to quantitative analysis. On the basis of our results, the analysis of polymeric materials of the polyamide type should be possible.

EXPERIMENTAL

Reagents and chemicals

N,O-Bis(trimethylsilyl)acetamide (BSA) was obtained from Pierce (Rockford, Ill., U.S.A.) and triethylamine (TEA) b.p. 89–90°, chromatographic standard from BASF (Ludwigshaven, G.F.R.).

The ω-amino acids 4-aminobutyric acid (Calbiochem, San Diego, Calif., U.S.A.), 6-aminocaproic acid (Silon, Planá nad Lužnicí, Czechoslovakia), 7-aminoenanthic acid (VNIIV, Moscow, U.S.S.R.), 8-amino caprylicacid (prepared according to Kubánek *et al.*[25]) and 11-aminoundecanoic acid (Fluka, Buchs, Switzerland) were purified by recrystallization from water–ethanol.

Preparation of derivatives

ω-Amino acid hydrochlorides. ω-Amino acids were converted into the corresponding hydrochlorides by treatment with 6 *N* hydrochloric acid. After the reaction, the solution was evaporated to dryness, then water was added to the dry residue, evaporation was repeated three times and the hydrochlorides were finally dried in a desiccator filled with solid potassium hydroxide under reduced pressure at 60°.

Silyl derivatives. ω-Amino acids or their hydrochlorides (5–10 mg) in closed vials were heated under nitrogen with the silylation reagent at 80° for 1–2 h, or alternatively, at 90° for 0.5–1 h. To permit the results to be compared with those reported in the literature, silylation was carried out at the concentrations of the reagents given later in Table II. Aliquots of the mixture (1 μl) were analyzed immediately after silylation and subsequently after standing for 24 h at 23–25°.

Equipment

A MAT-111 GC–MS system (Varian-MAT, Palo Alto, Calif., U.S.A.) was used for the analysis and identification. The following chromatographic conditions were used: glass column, 1.20 m × 3 mm I.D., containing 1% SE-30 on 100–120-mesh Chromosorb W, AW-DMCS, equipped for on-column injection; flow-rate of the carrier gas (helium), 18 ml/min; temperature of injection port, 190°; temperature of slit separator, 170°. The column oven was operated for 2.5 min isothermally at 90° and then programmed at 10°/min up to 180°. A built-in electron impact ionization detector (electron energy 20 eV) was employed for recording the gas chromatograms.

Mass spectrometry conditions. The following operating conditions were used: ionization source temperature, 200°; ionization energy, 80 eV; accelerating voltage, 820 V; trap current, 270 μA; resolving power, *ca.* 600. The separation efficiency of the column and the retention times of the individual derivatives are shown in Fig. 1 and Table I.

RESULTS AND DISCUSSION

We carried out the silylation of both free ω-amino acids and their hydrochlorides. Bergström and co-workers[18,22] found that silylation of free ω-amino acids with BSTFA in ACN gave rise to both di-TMS and tri-TMS derivatives, while if silylation is performed in absence of a solvent only the di-TMS derivative is formed. These

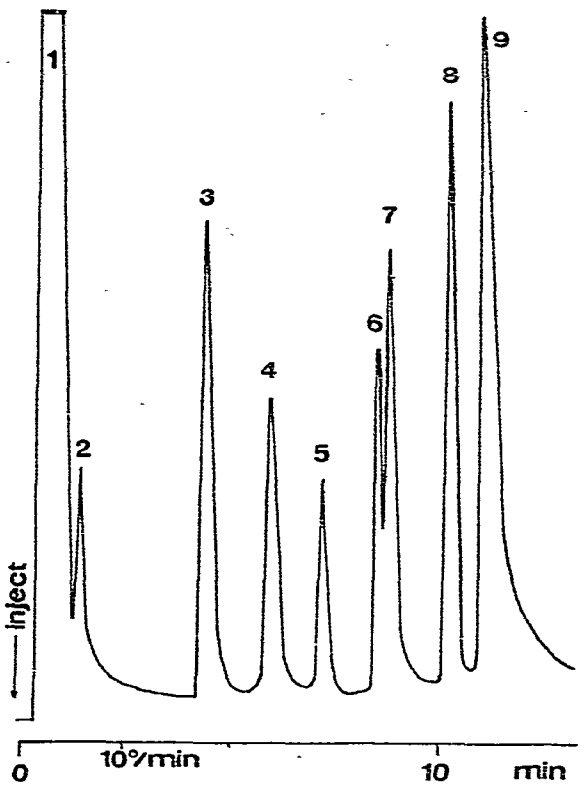


Fig. 1. GC separation of TMS derivatives of ω-amino acids. Glass column (1.2 m × 3 mm I.D.) containing 1% SE-30 on 100–120-mesh Chromosorb W, AW-DMCS. Initial temperature 90°, isothermal for 2.5 min, then programmed up to 180° at 10°/min. Carrier gas (helium) flow-rate: 18 ml/min. Peaks: 1, solvent; 2, di-TMS C_4; 3, tri-TMS C_4; 4, di-TMS C_6; 5, di-TMS C_7; 6, di-TMS C_8; 7, tri-TMS C_6; 8, tri-TMS C_7; 9, tri-TMS C_8.

TABLE I

RETENTION TIMES OF SILYL DERIVATIVES OF ω-AMINO ACIDS FOR A COLUMN MAINTAINED ISOTHERMALLY AT 90° FOR 2.5 min THEN PROGRAMMED UP TO 180° AT 10°/min

Compound (carbon number)	*Type of TMS derivative*	*Retention time (min)*
C_4	Di	1.35
C_4	Tri	4.35
C_6	Di	5.95
C_6	Tri	8.80
C_7	Di	7.55
C_7	Tri	10.50
C_8	Di	8.50
C_8	Tri	11.08
C_{11}	Di	5.85*
C_{11}	Tri	8.30*

* Column maintained isothermally at 150° for 2.5 min then programmed up to 180° at 10°/min.

TABLE II

SILYLATION OF C_4, C_6, C_7, C_8 AND C_{11} ω-AMINO ACIDS AND THEIR HYDROCHLORIDES UNDER VARIOUS EXPERIMENTAL CONDITIONS

The degree of silylation was evaluated according to the method of relative occurrence.

Compound silylated	*BSA (ml)*	*TEA (ml)*	*ACN (ml)*	*Immediately after reaction*		*After 24 h*	
				Di-TMS (%)	*Tri-TMS (%)*	*Di-TMS (%)*	*Tri-TMS (%)*
Hydrochloride	0.5	—	—	Trace	100	—	100
ω-Amino acid	0.5	—	—	100	—	100	—
Hydrochloride	0.3	0.1	—	25	75	6	94
ω-Amino acid	0.3	0.1	—	100	—	100*	Trace*
Hydrochloride	0.3	0.1	0.3	3	97	Trace	100
ω-Amino acid	0.3	0.1	0.3	94	6	25	75

* For the C_4 amino acid only, the proportions of the di-TMS and tri-TMS derivatives were 95% and 5%, respectively, after 24 h.

results are in excellent agreement with ours, obtained with BSA alone as reagent (see Table II). The silylation of hydrochlorides using BSA in the presence of ACN and TEA was carried out by Mori[23]. TEA is believed to function as both a trapping agent for hydrochloric acid and a catalyst of the silylation reaction. The formation of di-TMS derivatives is assumed without considering the possibility of secondary silylation on the nitrogen atom. We examined, under comparable conditions, both the silylation process and the resulting products and demonstrated the formation of both derivatives (Table II). The best results were achieved in the absence of a solvent (ACN) and TEA as under these conditions only trace amounts of di-TMS derivatives in comparison with a large excess of tri-TMS derivatives could be detected. During subsequent standing of the sample for 24 h at 23–25°, the trace amounts of di-TMS

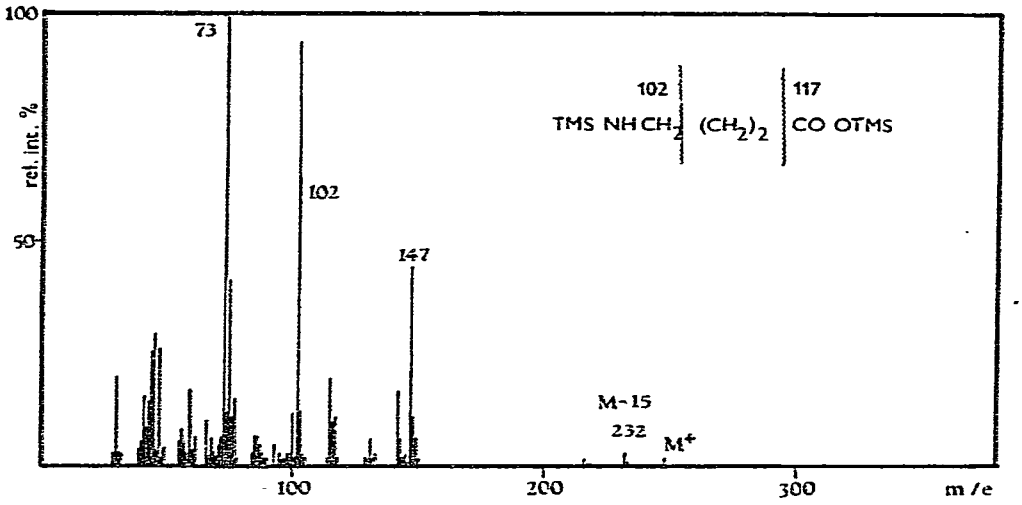


Fig. 2. Mass spectrum of di-TMS-4-aminobutyric acid. Temperature of ion source, 200°; ionization energy, 80 eV.

TABLE III

RELATIVE INTENSITIES OF MOLECULAR IONS OF SILYL DERIVATIVES OF ω-AMINO ACIDS

Compound (carbon number)	*Relative intensity of molecular ions (%)*	
	Di-TMS derivatives	*Tri-TMS derivatives*
C_4	0.15	0.05
C_6	0.80	0.10
C_7	1.60	0.20
C_8	0.40	0.30
C_{11}	0.50	0.20

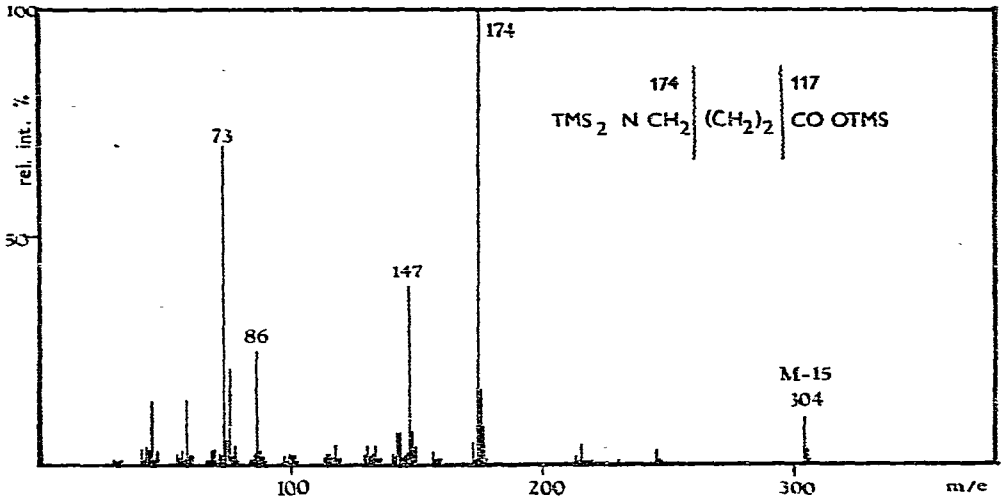


Fig. 3. As Fig. 2, for tri-TMS-4-aminobutyric acid.

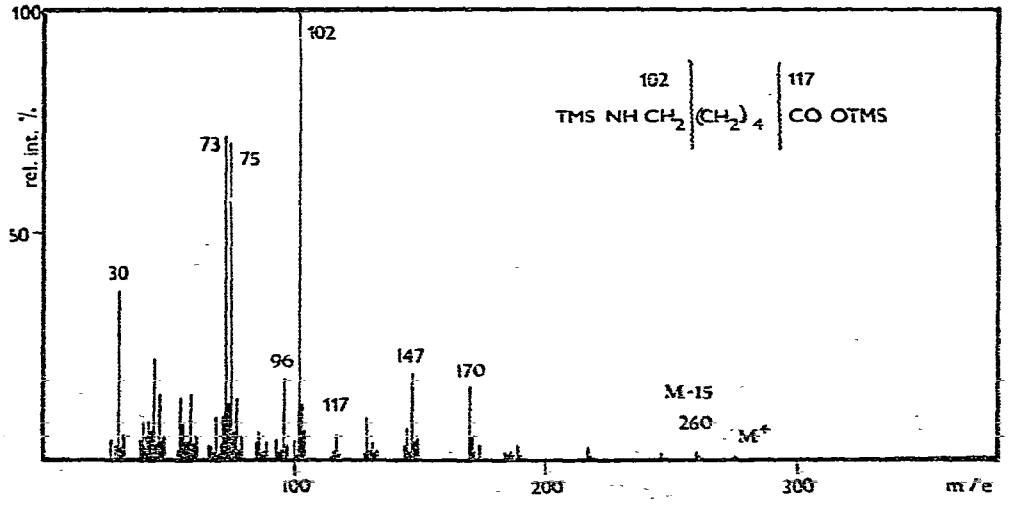


Fig. 4. As Fig. 2, for di-TMS-6-aminocaproic acid.

virtually disappeared and further changes in composition were not observed over the next 3 days (at 5°). The least promising method for quantitative analysis is silylation by means of BSA together with TEA.

With free ω-amino acids, TEA has the opposite effect, as faster formation of di-TMS derivatives than in BSA alone occurs. The addition of ACN, however, results in the formation of significant amounts of tri-TMS derivatives.

Tengler[24] carried out the silylation of the hydrochlorides of ω-amino acids with a large excess of BSA alone, and he assumed the formation of di-TMS derivatives. Under the conditions described in his work, the formation of di-TMS derivatives occurs to a negligible extent compared with tri-TMS derivatives (Table II). As it is possible by using the method described here (*i.e.*, by silylation in BSA alone at 90° for 0.5–1 h followed by standing for 24 h at 23–25°) to obtain only one derivative, *i.e.* tri-TMS derivative, reproducibly, we can utilize these findings for quantitative evaluation purposes. The results are summarized in Table II.

Mass spectra

The mass spectra of the di-TMS and tri-TMS derivatives of C_4, C_6, C_7, C_8 and C_{11} amino acids are shown in Figs. 2–11.

The molecular ions have low intensity in all instances, and more apparent are M—15 fragments, which are typical of most TMS derivatives[26,27]. The intensities of the molecular ions of the di-TMS derivatives are higher than those of the corresponding tri-TMS derivatives, as demonstrated in Table III. With all di-TMS derivatives the characteristic ion $m/e = 102$ occurs, representing the structure $TMS–\overset{+}{N}H=CH_2$. This structure has been confirmed as glycine, where a hydrogen atom on the carboxylic group was replaced with a methyl group. The characteristic ion in the spectra of tri-TMS derivatives is $m/e = 174$, representing the structure $(TMS)_2=\overset{+}{N}=CH_2$. In

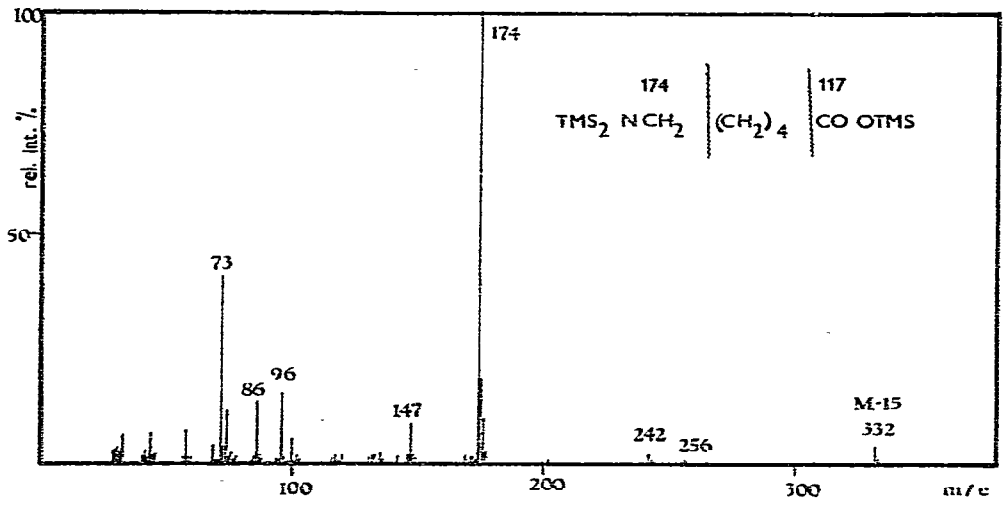
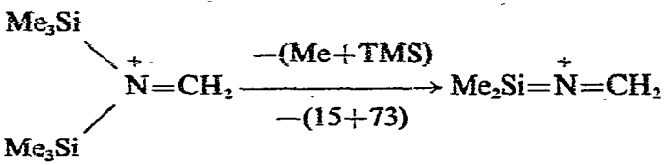


Fig. 5. As Fig. 2, for tri-TMS-6-aminocaproic acid.

addition, there is the predominant ion *m/e* = 86, arising by a metastable transition:



m/e 174 *m/e* 86

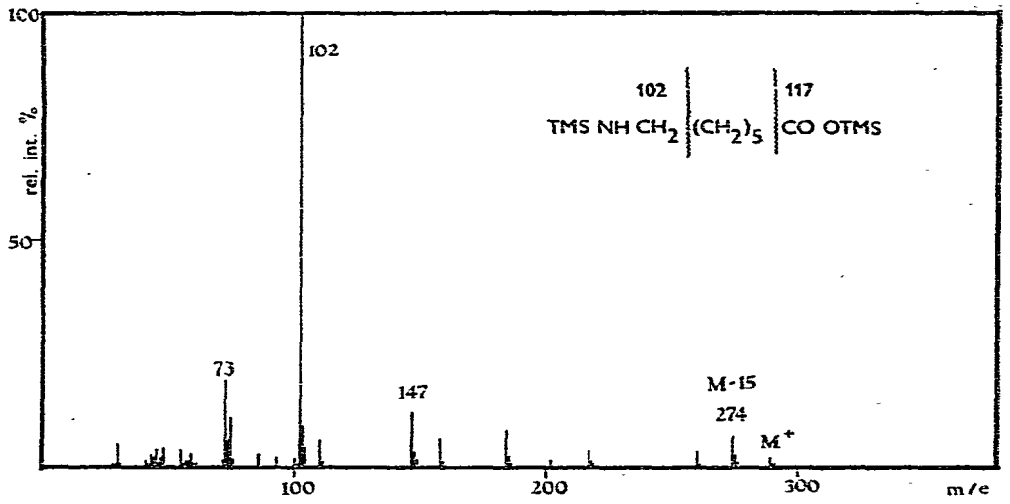


Fig. 6. As Fig. 2, for di-TMS-7-aminoenanthic acid.

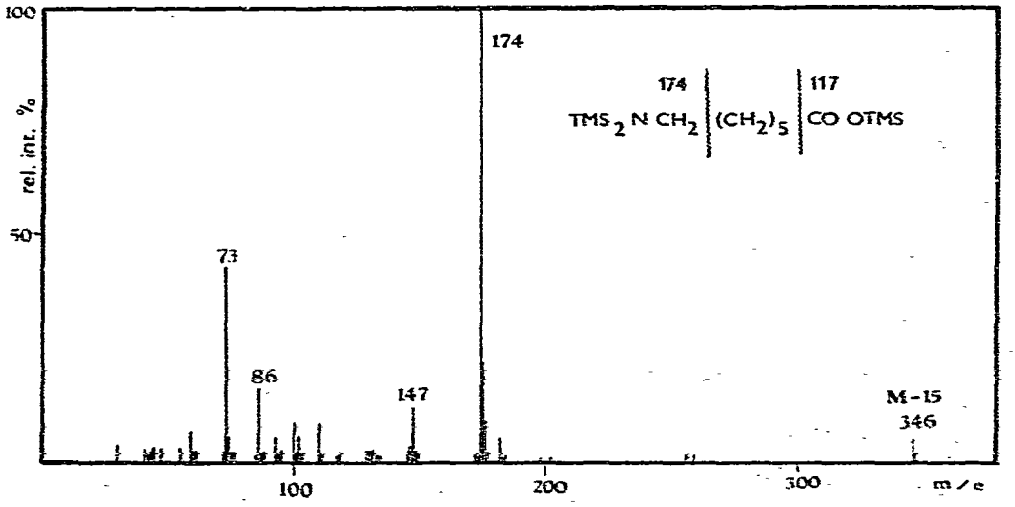


Fig. 7. As Fig. 2, for tri-TMS-7-aminoenanthic acid.

This structure was also found by Harman *et al.*[28]. The second transition represents fragmentation of *m/e* 174 to *m/e* 100 with the structure TMS–$\overset{+}{N}\equiv CH$, as described by Harman *et al.*[28] and Bergström and Gütler[18]. Both the *m/e* 102 and *m/e* 174 ions demonstrate the localization of silyl residues in the molecules of the derivatives. The low intensity of another possible product of α-cleavage, the ion *m/e* 117 with the structure $\overset{+}{O}\equiv CO$–TMS, may be explained by the formation of the high-density ion *m/e* 174, preferably carrying the charge[29,30]. The common ion in the spectra of both derivatives is *m/e* 147, found to be the fragment $Me_2Si\equiv\overset{+}{O}$–$SiMe_3$[27,31]. It arises by rearrangement of the TMS group of polysilyl derivatives and possesses a –O–TMS grouping. The relative intensity of the *m/e* 147 ion depends on the length of the amino acid chain. It decreases with length and, at the same time, the lower homologues show higher intensity as the di-TMS than as the tri-TMS derivatives.

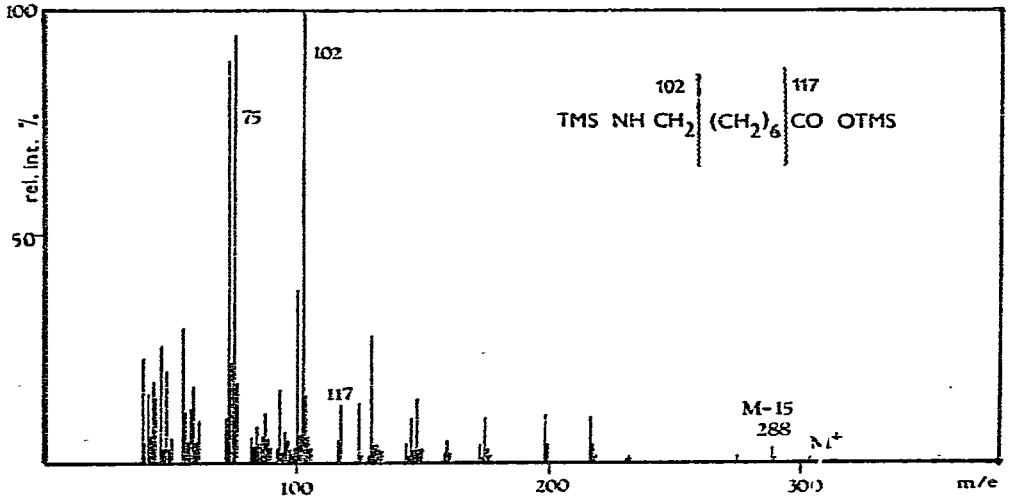


Fig. 8. As Fig. 2, for di-TMS-8-aminocaprylic acid.

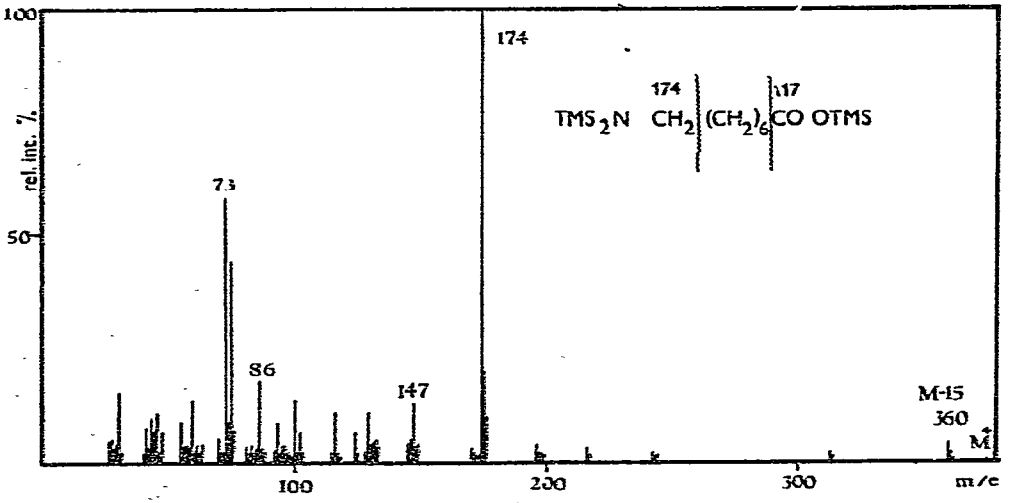


Fig. 9. As Fig. 2, for tri-TMS-8-aminocaprylic acid.

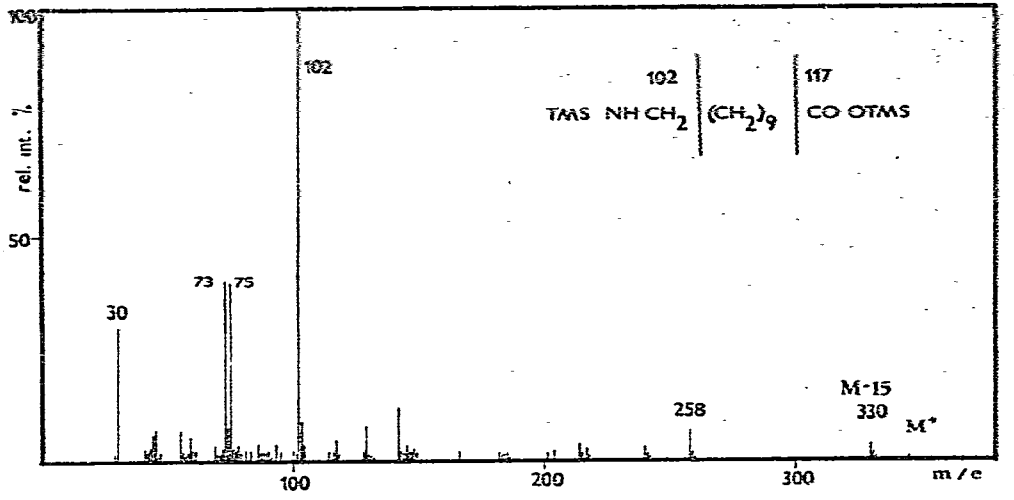


Fig. 10. As Fig. 2, for di-TMS-11-aminoundecanoic acid.

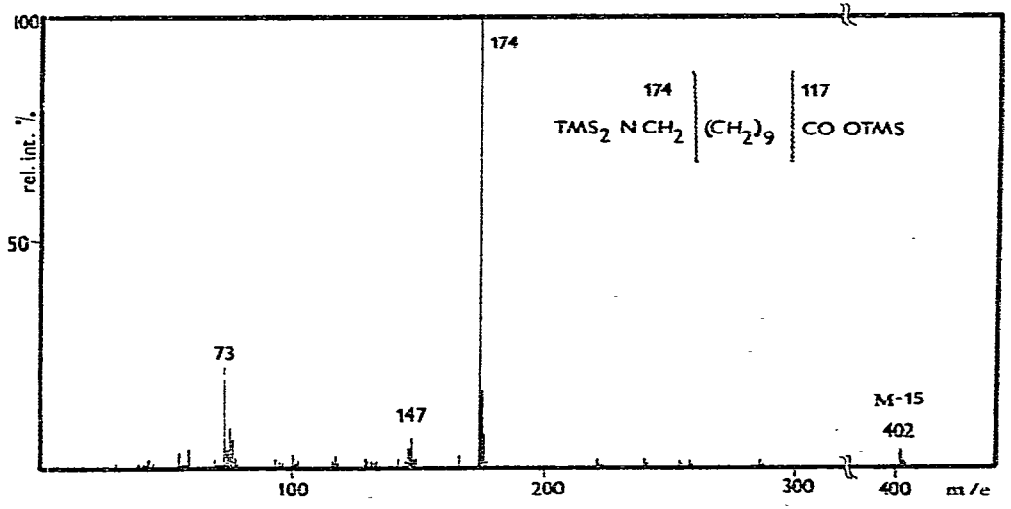


Fig. 11. As Fig. 2, for tri-TMS-11-aminoundecanoic acid.

CONCLUSION

Conditions for the silylation of ω-amino acid hydrochlorides with BSA applicable to quantitative analysis have been found. Under these conditions, silylation employing BSA alone yields tri-TMS derivatives, the structure of which has been confirmed by mass spectrometry. Because the silylation of the free amino acids with BSA under such conditions proceeds only as far as the di-TMS derivatives, it appears that the presence of hydrochloric acid is essential for the reaction, as it catalyzes the formation of the persilylated product.

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