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Trimethylsilylation of Amino Acids

II. Gas Chromatographic and Structural Studies on Trimethylsilyl Derivatives of Straight Chain Amino Acids

KURT BERGSTRÖM and JOSEF GÜRTLER

Department of Clinical Chemistry at Serafimerlasarettet, Karolinska Institutet, Stockholm, Sweden

Trimethylsilylation of α- and ω-amino acids and α,ω-diamino straight chain carboxylic acids with 2 to 6 carbon atoms using bis-(trimethylsilyl)trifluoroacetamide (BSTFA) has been studied. The amino acids, except for the α-amino acids, produce two or more TMS derivatives and the gas chromatographic retention times are given for the derivatives studied. Combined gas chromatography-mass spectrometry has been used for characterization of the derivatives. Some of the derivatives exist as their corresponding lactams and the fragmentation patterns of these compounds are also discussed. The presence of possible tautomeric isomers of the lactam TMS derivatives could not be excluded with certainty by low resolution mass spectrometry. The TMS derivatives of the diamino acid lactams exhibit characteristic ions such as M − 72, M − (2 × 72), M − 73, M − 87, and M − 89 and it has been proposed that these ions may be derived from the TMS-substituted ω-amino group. Further studies are necessary to elucidate these fragmentation processes. Preliminary applications based on these studies demonstrate the potential possibilities of the method for the analysis of, for example, protein hydrolysates.

In view of the importance of trimethylsilylation in gas chromatography, the conversion of amino acids to their TMS derivatives has been studied. In an ideal analytical method each amino acid would be converted quantitatively to a single derivative and separated from other derivatives on GLC. The potential advantages in speed, sensitivity and discriminating capacity of such a method are obvious. Many different amino acid derivatives have been tried [1–6] and the trifluoroacetylated amino acid butyl esters [5] have been the most extensively studied. Trimethylsilylation of amino acids has recently been used together with direct gas chromatography.[6,7] Both the conversion of the amino acids and the gas chromatographic separation is easier to perform with this technique than with the other ones used.

In a previous study of amino acids with BSTFA [7] it was found that some amino acids such as glycine and lysine could give rise to more than one deriva-

tive. Combined gas chromatography-mass spectrometry revealed that the silylation was more or less complete depending on the reaction conditions. The structural studies of some of these trimethylsilyl (TMS) derivatives prompted a more detailed survey of simple straight chain amino acid derivatives.

The α- and ω-amino and α,ω-diamino acids with 2 to 6 unbranched carbon atoms (Table 1) were silylated and studied with GLC and with combined gas

Table 1. Survey of three TMS amino acid series studied.

C atoms	α-Amino acid	ω-Amino acid	α,ω-Diamino acid
2	2-Aminoacetic acid (glycine)	2-Aminoacetic acid (glycine)	
3	2-Aminopropionic acid (α-alanine)	3-Aminopropionic acid (β-alanine)	2,3-Diaminopropionic acid
4	2-Aminobutyric acid	4-Aminobutyric acid (GABA)	2,4-Diaminobutyric acid
5	2-Aminovaleric acid (norvaline)	5-Aminovaleric acid	2,5-Diaminovaleric acid (ornithine)
6	2-Aminocaproic acid (norleucine)	6-Aminocaproic acid (EACA)	2,6-Diaminocaproic acid (lysine)

chromatography-mass spectrometry.[8,9] Relative retention times for methylene and TMS "units" were calculated and possible structures were discussed from the mass spectrometric data.

The reaction conditions worked out were used for some preliminary studies on the amino acid composition of protein hydrolysates *etc.* These will also be reported.

MATERIALS AND METHODS

Reagents. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Pierce Chem. Co., Rockford, Ill., USA. The amino acids and other chemicals used were of analytical grade.

Silylation. The reaction was performed in small sealed pyrex tubes under dry nitrogen with or without acetonitrile as a catalyst. After the heating period the sealed tube was kept for various lengths of time at room temperature. The conditions used to obtain the different derivatives are summarized in Table 2.

GLC separation was carried out on a Varian gas chromatograph, model 2100, fitted with flame ionization detectors. Single column technique was used and the U-shaped siliconized glass columns (370 cm × 4 mm i.d.) were packed with acid washed and siliconized Chromosorb W 80 – 100 mesh carrying 3 % (w/w) DC 550 as the stationary phase. Argon was used as carrier gas with a flow rate of 37 ml/min and the temperature was programmed from 60°C to 280°C at a rate of 10°C/min. 2 μl of the reaction mixture were usually injected and the sensitivity was 128 or 256×10^{-11} A/mV.

The characterization of the TMS derivatives was carried out with the combined GLC–MS instrument, LKB 9000, equipped with a column (270 cm × 4 mm i.d.) packed with 3 % DC 550 on acid washed and siliconized Chromosorb W, 80–100 mesh. Operating conditions when not otherwise stated were: temperature of the column: 60–250°C (programme rate: 10°C/min); of the ion source: 200–250°C and of the molecule separator: 210–250°C; the energy of the electrons: 70 eV. Ion peaks with an intensity of less than 5 % of the base peak were not included in the mass spectra presented.

RESULTS AND DISCUSSION

Gas chromatography

All amino acids studied (Table 1) except for α-amino acids (not including glycine, which also can be regarded as an ω-amino acid) were found to give two or more GLC peaks after silylation.

The retention times relative to phenanthrene are presented in Table 2 for the derivatives studied and Fig. 1 demonstrates the relative retention

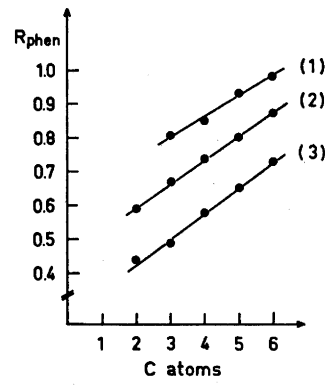


Fig. 1. Retention times relative to phenanthrene of three series of TMS amino acids plotted against the number of carbon atoms in the chain.
(1) Tetra-TMS α,ω-diamino acids.
(2) Tri-TMS ω-amino acids.
(3) Di-TMS ω-amino acids.

times of three series of di-, tri- and tetra-TMS derivatives, respectively. From these figures a mean "methylene unit" corresponding to a retention time of about 0.07 and a mean "TMS unit" with a retention time of about 0.15 relative to phenanthrene were calculated.

Some TMS amino acid derivatives exhibit retention times shorter than expected for a straight chain compound and these peaks could be related to their corresponding lactams (see below).

Silylation of arginine gave rise to GLC peaks with the same retention times as were obtained with ornithine (Fig. 2 B and C). Mass spectrometric analysis showed that these derivatives were identical to the TMS derivatives of ornithine. The expected urea split off from arginine could, however, not be found as its usual di-TMS compound in the gas chromatogram.

As has been shown earlier [7] the gas chromatographic separation of the common amino acid TMS derivatives is readily achieved. It was also possible to study for example ε-*N*-methyl lysine, an interesting amino acid derivative recently described [10,11] which might be of clinical diagnostic value. Its reten-

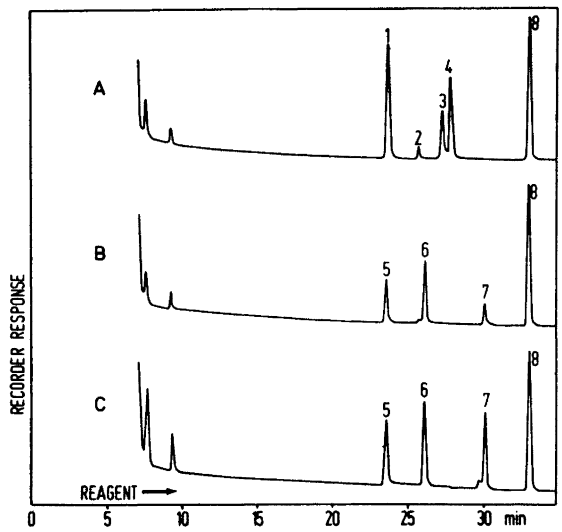


Fig. 2. Gas chromatogram of the silylation products of A, 2,4-diaminobutyric acid B, 2,5-diaminovaleric acid; C, arginine.

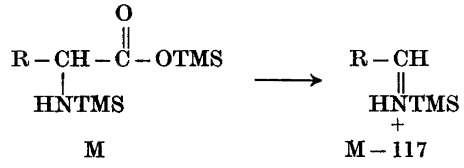
The GLC peaks represent: 1, di-TMS 2,4-diaminobutyric acid lactam; 2, tri-TMS 2,4-diaminobutyric acid lactam (not discussed dehydrogenated form); 3, tri-TMS 2,4-diaminobutyric acid lactam; 4, tetra-TMS 2,4-diaminobutyric acid lactam; 5, di-TMS 2,5-diaminovaleric acid lactam; 6, tri-TMS 2,5-diaminovaleric acid (ornithine); 7, tetra-TMS 2,5-diaminovaleric acid (ornithine); 8, phenanthrene (internal standard).

The conditions under which the reaction and gas chromatographic separation were performed are given in Table 2 and in the text.

tion time (0.88 for the tri-TMS ε-*N*-methyl lysine) does not interfere with the usual TMS amino acids. Using the regular Spackman, Moore and Stein technique [12] this amino acid has earlier been easily overlooked.

Mass spectra of straight chain TMS derivatives

Upon electron impact, all *α-amino acids* presented in Table 1 lose their silylated carboxyl, resulting in a base peak at M − 117.[7] An analogous fragmentation has been described by Andersson *et al.*[13]



The abundant siloxonium ion *m/e* 147 ($Me_2Si=\overset{+}{O}-SiMe_3$) indicates also the presence of an *O*-TMS ester in the molecule [7,14,15] and appears with a relative intensity of 10 − 30 %. The ion *m/e* 218 ($TMS-\overset{+}{N}H=CH-COOTMS$) is

present with an intensity of 3 – 7 % and is common for α-amino acids (containing an *N*- and *O*-TMS derivative, *cf.* Ref. 16). The M – 43 ion also appears in all of the α-amino acids studied and may be a rearranged ion. Metastable ions are found for the decomposition of M – 15 to M – 43 and further to *m/e* 147. The molecular and the M – 15 ion are also prominent peaks in all α-amino acids studied.

The *ω-amino acid series* forms at least two different TMS derivatives with molecular ions and M – 15 peaks indicating di- or tri-TMS derivatives. The characteristic ions *m/e* 102 and *m/e* 174 constitute the base peaks in these mass spectra representing the typical α-fission found in the *N*-mono-TMS and *N*-di-TMS ether, respectively:[7]

$$\underset{m/e\ 102}{TMS-\overset{+}{N}H=CH_2} \qquad \underset{m/e\ 174}{(TMS)_2=\overset{+}{N}=CH_2}$$

Except in glycine, M – 117 does not appear depending on the favoured α-fission, and the *m/e* 117 ($O=C=\overset{+}{O}-TMS$) appears only in low intensities because of a preferred charge localization to the α-amino nitrogen.[17] The ion *m/e* 117 is, however, somewhat more abundant in the mass spectra of di-TMS than of the tri-TMS derivatives. The disiloxonium ion *m/e* 147 exhibits similar behaviour. Its intensity is slightly higher for di- than for the fully silylated tri-TMS derivative. The 4-amino butyric acid and the 5-amino valeric acid yield beside the di- and tri-TMS also their corresponding lactams (see cyclic derivatives below).

The α,ω-diamino-TMS derivatives show more complex mass spectra. Great differences in fragmentation pattern have been obtained for tri- and tetra-TMS lysine in different ion source temperatures.[7] The tetra-TMS derivative of ornithine and its tri-silylated lactam also show considerable differences in their mass spectra recorded at ion source temperatures of 190°C and 250°C, respectively. Thermal instability demands a cold ion source [18] which is difficult to arrange for a GLC-MS instrument.

2,3-Diamino propionic acid forms two TMS derivatives which show only very weak molecular ions and M – 15 peaks in their high voltage (70 eV) mass spectra. By the use of 12 eV, these ions rise to about 3 % relative intensity. However, in the 70 eV spectra, characteristic ions such as *m/e* 102 appear, suggesting a mono substituted ω-aminogroup (the base peak for the tri-TMS derivative), and *m/e* 174 suggesting a di-substituted ω-amino group (the base peak for the tetra-TMS derivative). The tri-TMS derivative produces a *prominent M – 29 peak* (*m/e* 291) which becomes the base peak in the 12 eV spectrum. This ion was also found in the mass spectrum of di-TMS 4-amino butyric acid and the tetra-TMS 2,3-diamino propionic acid, suggesting the structure (II). None of the other α,ω-diamino acid TMS derivatives studied show this ion. A further ion which is found in the tetra-TMS derivative of this diamino acid is M – 101, which becomes the base peak in the 12 eV spectrum and which can be derived from the di-substituted ω-amino group (III). High resolution mass spectrometry or deuterium labelling must be used to evaluate this cleavage process.

$$R-NH-TMS \longrightarrow R-\overset{+}{N}H=SiH-CH_3 \qquad (II)$$
$$M-29 \ (m/e\ 291)$$

$$R-N(TMS)_2 \longrightarrow R-\overset{+}{N}H=SiH-CH_3 \qquad (III)$$
$$M-101 \ (m/e\ 291)$$

The 2,4-diamino butyric acid yields four different gas chromatographic peaks after silylation (Fig. 2 A). Three of these TMS derivatives seem to be cyclic products (with accompanying elimination of water) and will be discussed later (Fig. 2 A, peaks 1, 2, 3). The molecular ion (*m/e* 406) and the M−15

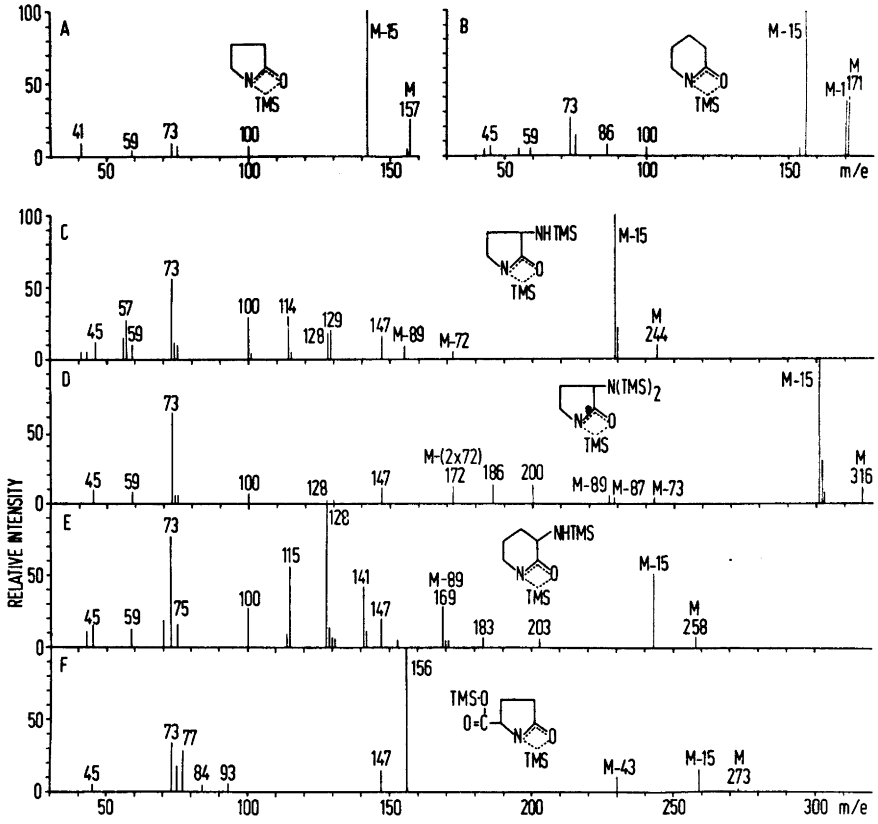


Fig. 3. Mass spectra of TMS derivatives of A, 4-aminobutyric acid lactam; B, 5-aminovaleric acid lactam; C, 2,4-diaminobutyric acid lactam (di-substituted); D, 2,4-diaminobutyric acid lactam (tri-substituted); E, 2,5-diaminovaleric acid lactam (di-substituted); F, pyroglutamic acid (di-substituted).

The temperature of the ion source was 200° for E and 250°C for the other derivatives. The electron energy was 70 eV and the temperature of the molecule separator was 210–250°C.

peak of the only straight chain TMS derivative obtained suggest a tetra-substituted compound with the base peak at *m/e* 174 indicating a di-TMS ω-amino group. In the 12 eV spectrum the molecular ion constitutes the base peak. The appearance of an ion M − 72 is surprising and may represent the loss of a TMS group, originating from a cyclization process. Such a mechanism may give rise to the ions M − 87 (*m/e* 319), M − 89 (*m/e* 317), *m/e* 128 and *m/e* 200, appearing also in the mass spectrum of the cyclic tri-TMS derivative (Fig. 3 D). They may be rearranged ions and will be discussed later. Of interest is the absence of the ion M − 117 identifying a silylated carboxyl.[7,13] This ion is also lacking in the tetra-TMS derivative of the 2,3-diamino propionic acid, but appears in all other diamino acids studied. The disappearance of this ion may confirm the assumed cyclization reaction. The ion *m/e* 218 and *m/e* 147 are, however, present as prominent peaks indicating the OTMS ester in the molecule. Another prominent ion is $M-(101+14)=m/e$ 291.

2,5-Diamino valeric acid forms two straight chain TMS derivatives. A cyclic di-TMS compound is also produced and will be discussed later. In contrast to the 2,4-diamino butyric acid TMS derivatives, the molecular ions appear in their 70 eV mass spectra as prominent peaks and suggest tri- and tetra-substituted derivatives.

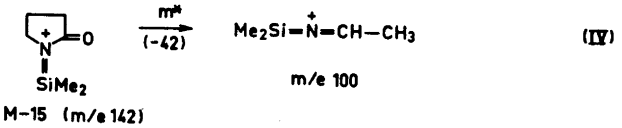
The tri-TMS compound exhibits ions such as *m/e* 218 and 102, indicating the structures $TMS-\overset{+}{N}H=CH-CO-OTMS$ (containing the α-amino group) and $TMS-\overset{+}{N}H=CH_2$ (α-cleavage product of the mono TMS substituted ω-amino group, respectively). The M − 117 ion suggests the loss of the silylated carboxyl. The base peaks are constituted by the rearranged ion *m/e* 147 ($DMS=\overset{+}{O}-TMS$) and by the $\overset{+}{Si}Me_3$ ion *m/e* 73. In the mass spectrum of the *tetra-TMS derivative* the *m/e* 174 appears with an intensity of 95 %. M − 117 and *m/e* 117 are also prominent peaks. The fragment *m/e* 102 is missing. The TMS derivatives of α,ω-diamino acids with four to six carbon atoms all show more complex fragmentation patterns (*cf.* Ref. 7) probably because of thermal instability and cyclization reactions.

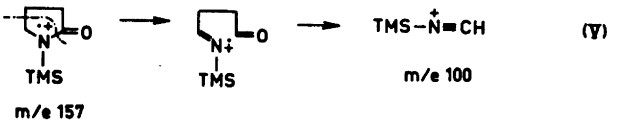
Mass spectra of the TMS lactams

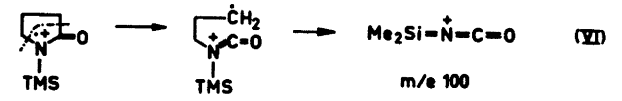
From the gas chromatographic analysis it is obvious that some of the TMS derivatives with shorter retention times may not be straight chain TMS compounds (Fig. 2 and Table 2). Mass spectrometric analysis shows evidence for the proposed lactam structure by the molecular ion which indicates elimination of water and further by the absence of the typical fragments of straight chain amino acid TMS derivatives[7] such as *m/e* 102 and 174 (mono- and di-substituted amino groups), M − 117[19] and *m/e* 117 (loss and appearance of a silylated carboxyl) and *m/e* 218[16] ($TMS-\overset{+}{N}H=CH-COOTMS$, typical for α-amino acid TMS derivatives). The mass spectrum of the 2-pyrrolidone TMS derivative is also identical with that of the TMS derivative of *4-amino butyric acid lactam*, produced under the silylation conditions used (Fig. 3 A). The base peak in this mass spectrum is constituted by the M − 15 peak and the molec-

ular ion has an intensity of 27 %. Other prominent peaks are *m/e* 59 and 73, derived from the silyl residue.[15,20]

For the fragment *m/e* 100, which has been described by Harman *et al.*[21] and by Bergström *et al.*,[7] three fragmentation processes can be proposed (IV, V, and IV).

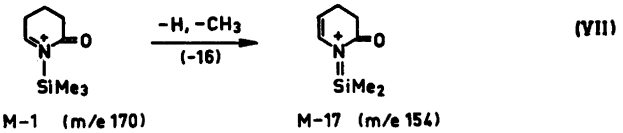






A metastable transition at *m/e* 70.4 support the decomposition of M − 15 to *m/e* 100 (IV). A second metastable ion at *m/e* 128.4 is also present, suggesting the decomposition of the molecular ion to M − 15.

The cyclic mono TMS derivative of 5-amino valeric acid exhibits fragmentation processes similar to that of the pyrrolidone TMS-ether (Fig. 3 B). Of interest is the M − 1 ion, which has almost the same intensity as the molecular ion. A metastable transition at *m/e* 169.0 is found for the decomposition of the molecular ion to M − 1. Another metastable ion at *m/e* 139.5 suggests the decomposition of the M − 1 ion to the M − 17 (*m/e* 154) and may be described by eqn. (VII).

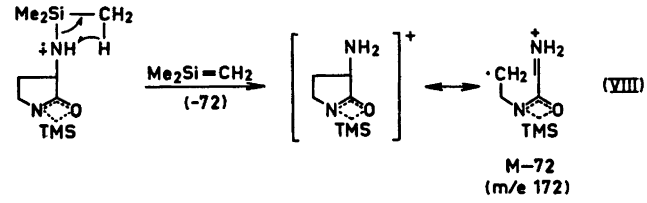


The *m/e* 86, with an intensity of 8 % may have the structure $H_2C=\overset{+}{N}=SiMe_2$ described by Harman *et al.*[21]

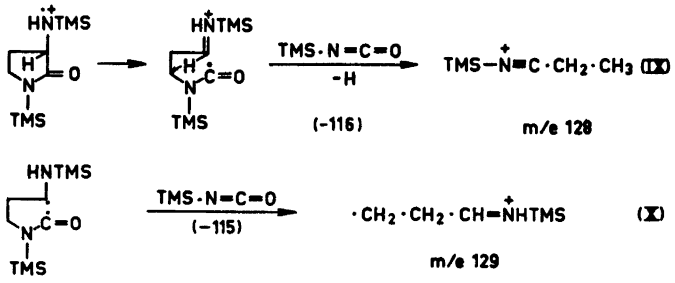
The 2,4-diamino butyric acid can form three cyclic derivatives with the elimination of water (Fig. 2, peaks 1, 2, and 3). Peak 2 with a molecular ion at *m/e* 242 may be a dehydrogenation product of peak 1 (M = 244) and appears only in small amounts. This derivative will not be further discussed. Peaks 1 and 3 correspond to a di- and a tri-TMS compound, respectively. Two positional isomers for these TMS derivatives can be assumed because of the possible tautomeric forms of the parent lactam. Both of the TMS derivatives show

a prominent peak at *m/e* 147 and a smaller one at *m/e* 75 in their mass spectra, which indicate the presence of an OTMS ester. The M − 72 (VIII) in the mass spectrum of the di-TMS derivative (Fig. 3 C) may correspond to the loss of a TMS residue with a fragmentation process analogous to that described by Budzikiewiez *et al.*[17] This ion does not give any information about the position of one of the two TMS groups, however.

The fragment *m/e* 172 also appears in Fig. 3 D as the M − (2 × 72) peak and a similar cleavage process may be assumed for that ion.

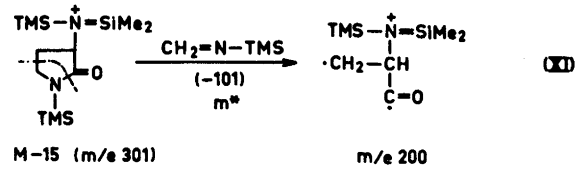


The ions *m/e* 128 and 129 are prominent peaks in Fig. 3 C but appear with only 4 and 2 % relative intensity, respectively, in Fig. 3 D. A fragmentation process assuming the presence of a TMS substituted lactam nitrogen with expulsion of a stable neutral fragment may be proposed by eqns. IX and X (*cf.* Ref. 17)



The pronounced *m/e* 114 and the smaller peak at *m/e* 115 in Fig. 3 C, which are lacking in Fig. 3 D can also be derived from the mono-TMS substituted amino group in a similar way as for IX and X. Also for this cleavage process, a substituted lactam nitrogen is more likely than an OTMS compound. The *m/e* 100, which appears in Fig. 3 C with 30 % relative intensity shows only 8 % in Fig. 3 D. Several fragmentation pathways may be active for that ion (see IV, V, VI above) but the higher intensity of this ion in Fig. 3 C may refer to a cleavage derived from the TMS substituted amino group. A metastable transition at *m/e* 78.1 in Fig. 3 C suggests the decomposition of the *m/e* 128 to *m/e* 100.

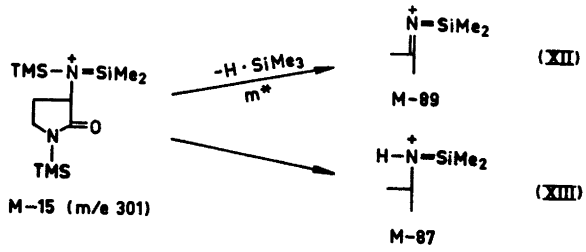
A pronounced metastable ion at *m/e* 132.9 appears in the mass spectrum of the tri-TMS derivative (Fig. 3 D) suggesting the decomposition of M − 15 (*m/e* 301) to *m/e* 200. Assuming a lactam TMS ether, the cleavage mode (XI) can be proposed.



(XI)

The ion *m/e* 200 is lacking in Fig. 3 C, which also suggests this process. The *m/e* 186 may be produced through a loss of a methylene from *m/e* 200.

Another pronounced *m** at *m/e* 171.2 (Fig. 3 D) suggests the decomposition of M – 15 (*m/e* 301) to M – 89 (*m/e* 227):



(XII)

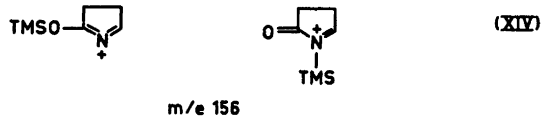
(XIII)

A similar process may also be proposed for the M – 87 (*m/e* 229) which appears in this mass spectrum. The *m/e* 147 appears with an intensity of 11 % indicating an *O*-TMS ester also in this compound.

The cyclic di-TMS derivative of ornithine exhibits considerable differences in the fragmentation pattern at different ion source temperatures indicating insufficient thermal stability. TMS-group interchange may also occur. The base peak at *m/e* 128 (Fig. 3 E) may originate from a fragmentation pathway similar to that described for *m/e* 128 (IX) in Fig. 3 C. The same would also be valid for the ions *m/e* 115 and *m/e* 100. For the generation of *m/e* 141 (M – 117) the expulsion of the neutral fragment TMS – NH – CHO would be the driving force resulting in a resonance stabilized ion $TMS-\overset{+}{N}H=CH-CH=CH-CH_2\cdot$. Of interest is the fragment *m/e* 75 ($H\overset{+}{O}=SiMe_2$) which increases considerably with increasing ion source temperature (190 to 250°C) indicating rearrangement reactions. The ion M – 89 (*m/e* 169) may result from the expulsion of a neutral fragment, $H_2N-SiMe_3$. High resolution mass spectrometry must be used for structure analysis of this ion.

It is obvious from this study that the mass spectra do not give clear information about the presence of either a lactam nitrogen TMS ether or an *O*-TMS ester. A TMS group interchange at high temperature [16] must be taken into consideration. Furthermore, the tautomeric lactam structure can produce isomers during the silylation. Mass spectrometric scanning of the ornithine GLC peak (Fig. 2 B, peak 5) indicated no heterogeneity. Glutamic acid also forms a cyclic di-TMS derivative, when it is silylated with BSTFA and acetonitrile. For comparison, the corresponding lactam, pyroglutamic acid, was silylated and the recorded mass spectrum was found to be identical with that

of the cyclic compound formed from the glutamic acid under the reaction conditions used. The mass spectrum (Fig. 3 F) is dominated by the M − 117 peak (loss of a silylated carboxyl) which may be derived from both possible isomers (XIV).



m/e 156 (XIV)

A metastable ion at *m/e* 205.0 indicates the decomposition of M − 15 to M − 43 (calc. $230^2/258 = 205$). Several fragmentation pathways are possible for the production of these ions and isotopic labelling must be used for studying these processes. Of interest is the absence of the *m/e* 100 with the possible structure of $SiMe_2=\overset{+}{N}=C=O$ (VI) which has been proposed for the 4-amino butyric acid TMS derivative and thus should account for a less favourable cleavage process for that ion (VI) in the mass spectra of the cyclic TMS derivatives.

The lack of M − 89 (Fig. 3 F) may indicate that this ion is derived from the TMS substituted amino group in Fig. 3 C, D, and E. This is furthermore confirmed by the mass spectra of the TMS derivatives of pyrrolidone and 5-amino valeric acid lactam (Fig. 3 A and B) in which this ion is also absent.

A pronounced *m** at *m/e* 34.2 suggests the decomposition of *m/e* 156 to the silyl ion *m/e* 73 ($\overset{+}{Si}Me_3$).

APPLICATIONS

The common amino acids, except for cystine, histidine, glutamine, and asparagine, have been studied with gas chromatography-mass spectrometry.[7] Suitable conditions for the silylation of protein hydrolysates have also been worked out, based upon this and previous studies, in regard to multiple forms of TMS derivatives. Preliminary applications of this method are presented in Table 3. It was also possible by direct silylation of lyophilized urine to quantitate glycine. This proved to be easier than by the use of conventional methods.[22]

Crystalline bovine serum albumin and a Bence-Jones protein (obtained by gel filtration of urine from a patient having an Ig G (κ) myeloma) were hydrolysed in 6 M HCl at 105°C for 48 h. The hydrolysate was dried and silylated with BSTFA in 100°C for 30 min. Tetra-TMS lysine and arginine (tetra-TMS ornithine) were obtained after silylation with acetonitrile as a catalyst in 150°C for 30 min. The GLC results were related to leucine and corrected for the yields obtained with a known amino acid standard solution.

As can be seen from Table 3 the amino acid composition of bovine serum albumin was similar to the data presented in the literature.[23] Further studies on, for example, histidine and arginine are, however, necessary. Light chain gamma globulins of λ and κ type differ considerably in their content of some

Table 2. Reaction conditions used for the silylation of the three amino acid series studied.

Number of C atoms	TMS derivative	Amount used, μg	BSTFA μl	CH_3CN μl	Temp. °C	Time min.	Kept at 23°C h	Rel. ret. time (phenanthrene)
			α-Amino acids					
2	di-	300	300	–	100	30	1	0.44
	tri-	300	150	150	150	30	4	0.59
3	di-	100	50	50	150	30	0.2	0.42
4	di-	120	50	50	150	15	1	0.47
5	di-	120	50	50	150	15	1	0.53
6	di-	150	50	50	150	15	1.5	0.59
			ω-Amino acids					
3	di-	70	100	–	150	15	0.2	0.49
	tri-	70	50	50	150	330	20	0.67
4	di-	140	100	–	150	15	24	0.58
	tri-	140	50	50	150	240	24	0.74
	lactam-mono-	140	50	50	150	60	0.2	0.52
5	di-	140	100	–	150	15	24	0.65
	tri-	110	50	50	150	90	24	0.80
	lactam-mono-	145	50	50	150	60	20	0.50
6	di-	200	200	–	100	30	1	0.73
	tri-	200	100	100	150	30	20	0.87
			α,ω-Diamino acids					
3	tri-	75	100	–	100	15	0.5	0.65
	tetra-	75	50	50	150	330	20	0.81
4	tetra-	130	50	50	150	60	18	0.85
	lactam-di-	130	100	–	150	60	20	0.72
	lactam-tri-	150	50	50	150	60	90	0.83
5	tri-	120	100	–	80	30	2	0.80
	tetra-	170	50	50	150	60	24	0.91
	lactam-di-	35	50	–	150	30	0.2	0.72
6	tri-	300	300	–	100	30	0.5	0.86
	tetra-	300	150	150	150	120	2	0.98

amino acids (alanine, glycine, isoleucine, methionine).[24,25] Thus it would be more accurate to determine the type of light chains by amino acid analysis. This is of interest, especially when studying the differences between the benign and the more malignant types of monoclonal hyper-gammaglobulinemias.

An easier and less expensive way of quantitative amino acid analysis is needed. Gas chromatography of trimethylsilyl derivatives is a very promising method for analysis of the common amino acids and of biologically interesting metabolites.

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Table 3. Preliminary results of the amino acid composition of bovine serum albumin and a Bence-Jones protein related to values obtained from the literature and recalculated in relation to leucine.

Amino Acid	Bovine serum albumin		Light chain gamma globulin				
	Literature values [23]	Found	Literature values [24,25]				Found
			λ Sh	λ Bo	λHa	κ Ag	
Ala	0.75	0.75	1.33	1.33	1.13	0.80	0.60
Gly (di-TMS)	0.26	0.26	1.13	1.17	1.13	0.87	0.82
Val	0.54	0.55	1.00	1.58	1.13	0.87	0.66
Leu (reference)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ile	0.21	0.21	0.33	0.25	0.27	0.60	0.48
Pro	0.44	0.44	0.93	1.25	1.00	0.73	0.67
Ser	0.43	0.39	1.87	2.83	2.13	1.87	1.30
Thr	0.52	0.49	1.27	1.58	1.27	1.33	0.84
Asp and Asn	0.88	0.84	1.00	1.42	0.87	1.33	1.07
Met	0.06	0.03	0.00	0.00	0.00	0.07	0.02
Glu and Gln (as tri-TMS Glu)	1.20	1.28	1.47	1.59	1.20	1.67	1.16
Phe	0.43	0.50	0.27	0.50	0.27	0.60	0.55
Tyr	0.30	0.29	0.60	0.83	0.67	0.60	0.46
His	0.27	0.02	0.27	0.25	0.27	0.20	0.00
Arg (as tetra-TMS Orn)	0.22	0.19	0.47	0.50	0.53	0.40	0.03
Lys (tetra-TMS)	0.94	0.84	0.73	0.92	0.67	0.87	0.63

REFERENCES

1. Weinstein, B. *Methods Biochem. Analy.* **14** (1966) 203.
2. Mc Bride, W. J. and Klingman, J. D. In Mattick and Szymanski, *Lectures on Gas Chromatography*, Plenum Press, New York 1966, p. 25.
3. Pisano, J. J. and Bronzert, T. J. *J. Biol. Chem.* **244** (1969) 5597.
4. Darbre, A. and Islam, A. *Biochem. J.* **106** (1968) 923.
5. Gehrke, C. W., Roach, D., Zumvalt, R. W., Stalling, D. L. and Wall, L. L. *Quantitative gas-liquid chromatography of amino acids in proteins and biological substances. Macro, semimicro and micro methods.* Anal. Biochem. Labs. Inc. 1908, Jackson Columbia, Miss. 65201, USA.
6. Stalling, D. L., Gehrke, C. W. and Zumvalt, R. W. *Biochem. Biophys. Res. Commun.* **31** (1968) 616.
7. Bergström, K., Gürtler, J. and Blomstrand, R. *Anal. Biochem.* **34** (1970) 74.
8. Ryhage, R. *Arkiv Kemi* **26** (1962) 185.
9. Ryhage, R. *Anal. Chem.* **36** (1964) 795.
10. Ambler, R. P. and Rees, M. W. *Nature* **184** (1959) 56.
11. Perry, T. L., Diamond, S. and Hansen, S. *Nature* **223** (1969) 668.
12. Spackman, D. H., Stein, W. H. and Moore, S. *Anal. Chem.* **30** (1958) 1190.
13. Andersson, C.-O., Ryhage, R., Ställberg-Stenhagen, S. and Stenhagen, E. *Arkiv Kemi* **19** (1961) 405.
14. Sweely, C. C., Elliot, W. H., Fries, J. and Ryhage, R. *Anal. Chem.* **38** (1966) 1549.
15. Richter, W., Vecchi, M., Vetter, W. and Walther, W. *Helv. Chim. Acta* **50** (1967) 364.

16. Pierce, A. E. *Silylation of organic compounds*, Pierce Chem. Co., Rockford, Ill., USA 1968.
17. Budzikiewiez, H., Djerassi, C. and Williams, D. H. *Mass spectrometry of organic compounds*, Holden-Day, San Francisco 1964.
18. Spiteller, G. and Spiteller-Friedmann, M. *Monatsh.* **94** (1963) 742.
19. Horning, M. G., Boucher, E. A., Moss, A. M. and Horning, E. C. *Anal. Letters* **1** (1968) 713.
20. Diekman, J., Thomson, J. B. and Djerassi, C. *J. Org. Chem.* **32** (1967) 3904.
21. Harman, R. E., Patterson, J. L. and Vanden Heuvel, W. J. A. *Anal. Biochem.* **25** (1968) 452.
22. Bergström, K. *To be published.*
23. Phelps, R. A. and Putnam, F. W. In Putnam, F. W. *The plasma proteins*, Academic, New York 1960, Vol. 1, p. 146.
24. Putnam, F. W., Shinoda, T., Titani, K. and Wikler, M. *Science* **157** (1967) 1050.
25. Titani, K., Whitley, E., Jr. and Putnam, F. W. *Science* **152** (1966) 1513.

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