Journal of Chromatography, 141 (1977) 355–375
Chromatographic Reviews

CHREV. 109

COMPLETE MASS SPECTRA OF THE PER-TRIMETHYLSILYLATED AMINO ACIDS

KENNETH R. LEIMER, ROY H. RICE and CHARLES W. GEHRKE

Department of Biochemistry and Experiment Station Chemical Laboratories, College of Agriculture, University of Missouri-Columbia, Columbia, Mo. 65201 (U.S.A.)

(Received May 25th, 1977)

CONTENTS

1. INTRODUCTION

Gas–liquid chromatography–mass spectrometry (GC–MS) has become an important method for the separation, identification and quantitation of the amino acids. The combination of GC-MS provides assurance as to the identification of the amino acids present in biological systems. This is based on experimental observations of GC retention data, MS structural confirmation, and elution band homogeneity. A comprehensive review on the GC of amino acids has recently been published by Hušek and Macek[1] (415 references).

The amino acids themselves are not amenable to GC analysis, but satisfactory volatility is obtained upon derivatization. The trimethylsilyl (TMS) derivative has received great interest due to the one-step derivatization as contrasted to multi-step procedures necessary for many other derivatives. Gehrke *et al.*[2,3] described the derivatization conditions and a single-column separation of the protein amino acids as the TMS derivative.

---

* Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 7862.

VandenHeuvel *et al.* reported on the GC and MS of deuterium-containing[4,5] and carbon-13 enriched[4–6] amino acids as their TMS derivatives. Through the use of the labelled amino acids, these authors were able to elucidate some fragmentation pathways for the TMS-amino acids, but no comprehensive study of these pathways was presented.

Bergström *et al.*[7,8] reported on the MS fragmentation patterns of α- and ω-amino acids and α,ω-diamino straight-chain carboxylic acids with 2 to 6 carbon atoms and the complete GC separation of the protein amino acids. This group found that some of these derivatives existed as their corresponding lactams and the fragmentation pattern of these compounds were discussed.

Laseter *et al.*[9] showed the application of the TMS derivative of amino acids to samples from botanical sources. This group used GC–MS to confirm the identity of the chromatographic peaks, and reported on the major mass fragments from each of the protein amino acids, but gave no comprehensive study on the fragmentation pathways.

Mitra *et al.*[10] recently reported on the use of the TMS derivative for GC–MS to study the metabolism of amino acids in germinating wheat seedlings grown in a medium containing large quantities of deuterium oxide. This group was able to relate deuterium incorporation to the metabolism of the individual amino acid.

Leimer *et al.*[11] reported recently on a comprehensive study of the complete MS fragmentation of the N-trifluoroacetyl-*n*-butyl ester derivatives of the amino acids. They have shown the importance of combined GC–MS methods for the identification of unexpected or unusual amino acids.

This investigation reports on a comprehensive study of the complete MS fragmentation pathways for the per-TMS derivatives of the amino acids and factors controlling multiple derivative formation.

2. EXPERIMENTAL

The TMS derivatives were prepared by heating the amino acids in acetonitrile–bis-TMS-trifluoroacetamide (1:1, v/v) at 150° for 15 min or $2^1/_2$ h as reported by Gehrke *et al.*[2,3].

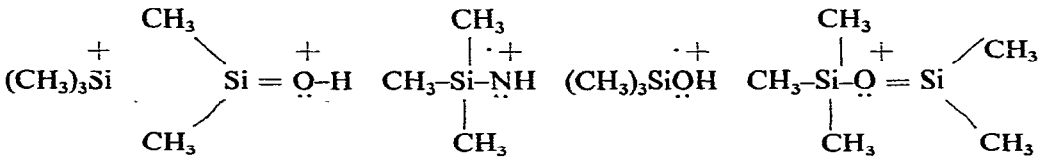
The GC–MS spectra were obtained using the methods reported by Leimer *et al.*[11].

All reagents were the same as previously reported[2,10].

3. RESULTS AND DISCUSSION

A. Aliphatic amino acids

In general, TMS derivatives of organic compounds upon electron impact yield ions which are characteristic of the presence of the TMS group. Listed below are the ions which are only characteristic of the TMS group and lend little to the structure elucidation.



Figs. 1–12 present the mass spectra for the TMS derivatives for glycine, alanine, valine, leucine, isoleucine, norleucine, α-aminobutyric acid, α-aminoisobutyric acid sarcosine, N-methylalanine, and N-methylleucine. Table 1 shows common high mass fragments observed for many of these amino acids, most of which do not contribute to the structural interpretation. The amino acids underwent surprisingly little other fragmentation. Fig. 13 presensts fragmentation pathways I which presents general fragmentation for the TMS derivative of the α-amino acids. The most characteristic fragments of the TMS α-amino acids are the M—15 (CH_3), M—43 (CH_3 + CO), M—117 (CO_2TMS) and M—R. The M—117 results from cleavage alpha to the carbonyl group and beta to the amino with charge retention on the amine fragment. This fragment is prominent due to resonance stabilization of the fragment ion and

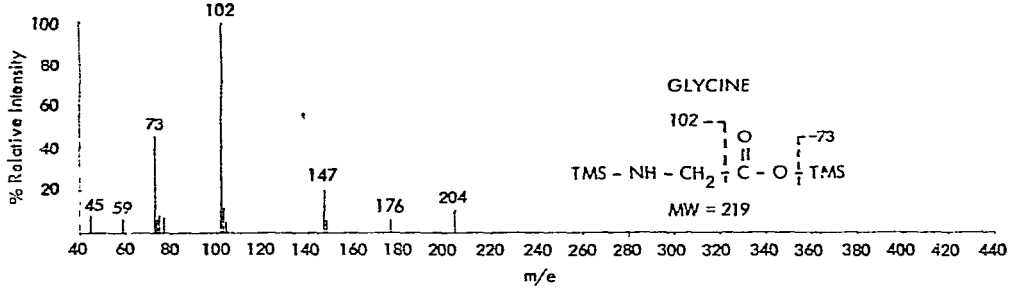


Fig. 1. Mass spectral fragmentation of N,O-di-TMS-glycine.

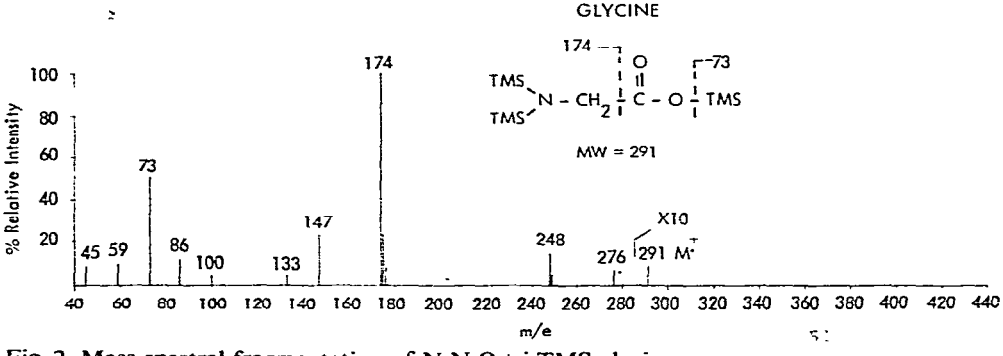


Fig. 2. Mass spectral fragmentation of N,N,O-tri-TMS-glycine.

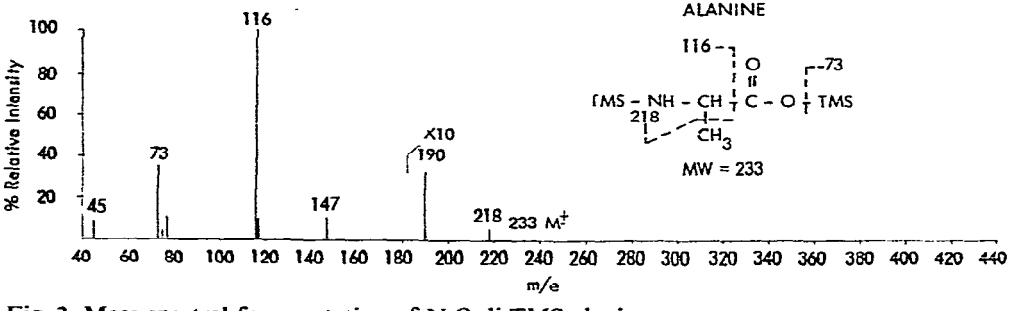


Fig. 3. Mass spectral fragmentation of N,O-di-TMS-alanine.

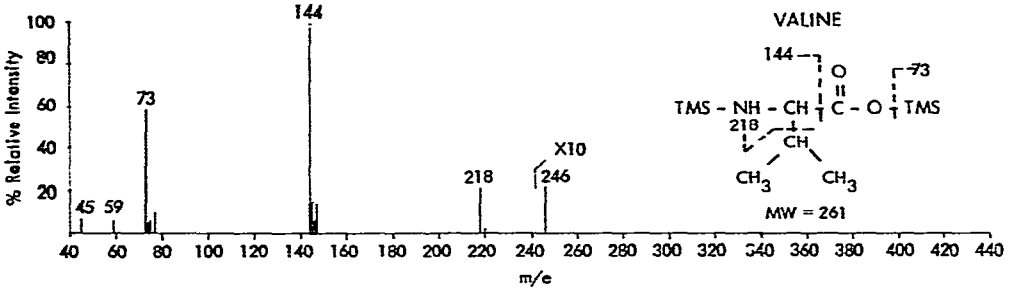


Fig. 4. Mass spectral fragmentation of N,O-di-TMS-valine.

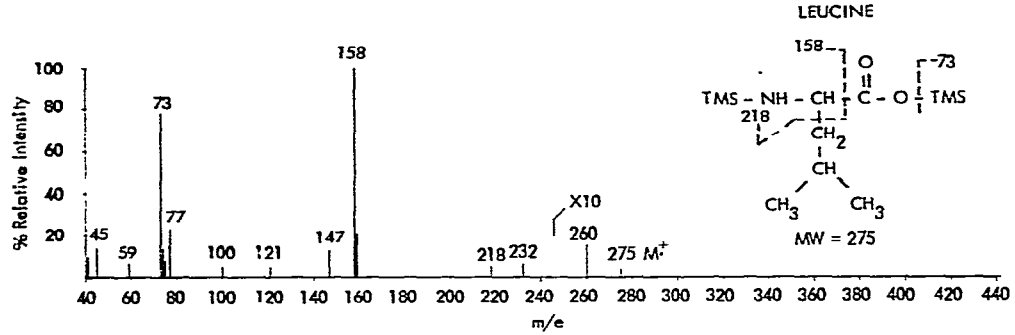


Fig. 5. Mass spectral fragmentation of N,O-di-TMS-leucine.

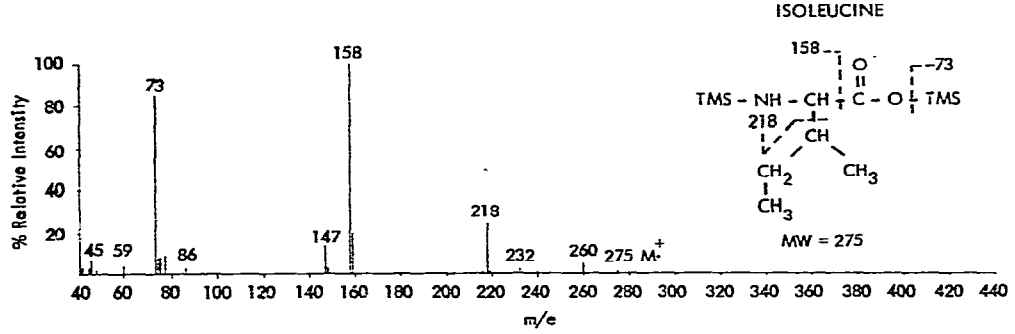


Fig. 6. Mass spectral fragmentation of N,O-di-TMS-isoleucine.

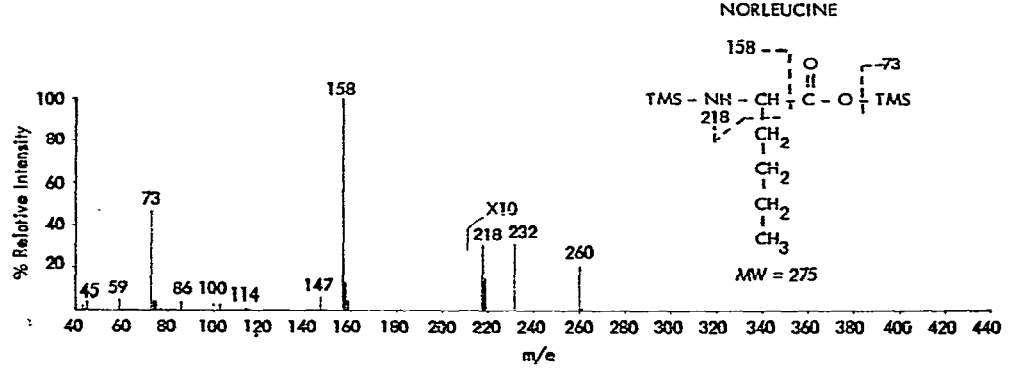


Fig. 7. Mass spectral fragmentation of N,O-di-TMS-norleucine.

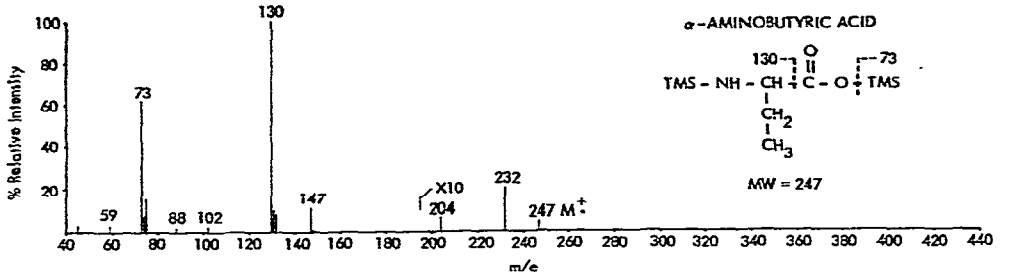


Fig. 8. Mass spectral fragmentation of N,O-di-TMS-α-aminobutyric acid.

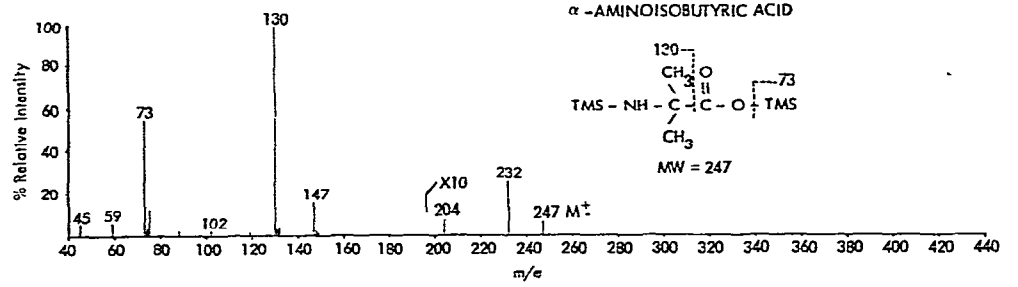


Fig. 9. Mass spectral fragmentation of N,O-di-TMS-α-aminoisobutyric acid.

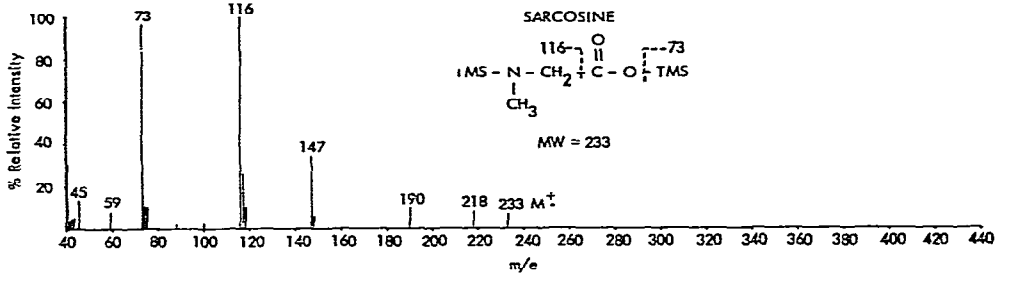


Fig. 10. Mass spectral fragmentation of N,O-di-TMS-sarcosine.

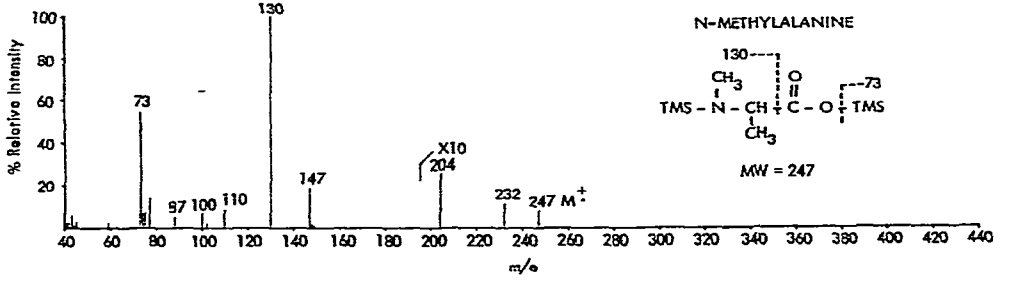


Fig. 11. Mass spectral fragmentation of N,O-di-TMS-N-methylalanine.

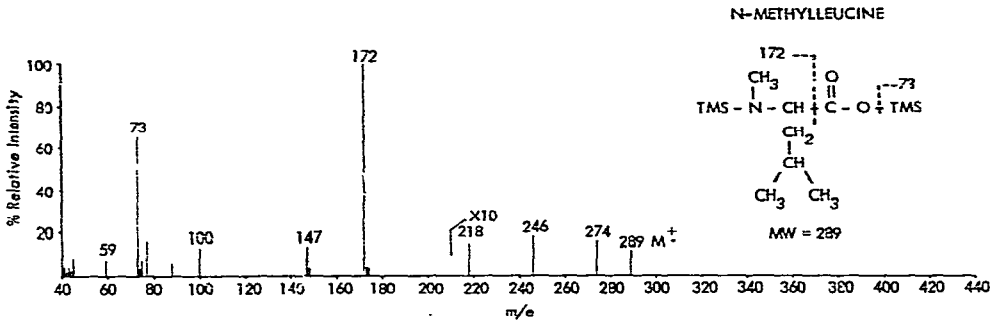


Fig. 12. Mass spectral fragmentation of N,O-di-TMS-N-methylleucine.

TABLE 1

SOME COMMON HIGH MASS FRAGMENT IONS OF THE PER-TRIMETHYLSILYLATED AMINO ACIDS

Fragmentation	*Origin*
M−15	M−CH_3
M−29	M−(CH_3 + CH_2)
M−43	M−(CH_3 + CO)
M−72	M−$(CH_3)_3$Si-H)
M−73	M−$(CH_3)_3$Si
M−87	M−CH_3($(CH_3)_3$Si−H)
M−89	M−$(CH_3)_3$SiO
M−90	M−$(CH_3)_3$SiOH
M−102	M−$(CH_3)_3$Si−CH_3−CH_2
M−104	M−CH_3−$(CH_3)_3$SiO
M−105	M−CH_3−$(CH_3)_3$SiOH
M−116	M−($(CH_3)_3SiCO_2$−H)
M−117	M−$(CH_3)_3SiCO_2H$
M−133	M−CH_3−CO−$(CH_3)_3$SiOH
M−162	M−$(CH_3)_3$Si−$(CH_3)_3$SiO
218	M−R (for α-amino acid)

the radical formed. The M—43 ion (Fig. 13), is produced by the loss of carbon monoxide from the M—15 as has been shown by the presence of a metastable ion for this transition. As shown by a metastable transition, the M—43 ion then fragments to form *m/e* 147 (structure IV in Fig. 13). The M—R (structure VII in Fig. 13), was usually present due to resonance stabilization of this ion by the amino group. A weak but discernible molecular ion was usually observed.

Figs. 14–19 present the mass spectra for the TMS derivatives for β-alanine, β-aminobutyric acid, β-aminoisobutyric acid and N-methyl-β-alanine. The fragmentation patterns for the β-amino acids are summarized in Fig. 20.

A comparison of the spectra of the α-amino acids with those of the β-amino acids showed a more favored cleavage of the 1,2 bond in the α-amino acids than in the β-amino acids. Cleavage of this bond is not favored in the β-amino acids due to the amino group being one more methylene group removed from the carbonyl than in the α-amino acids. An intense fragment for the β-amino acids is found for β-cleavage

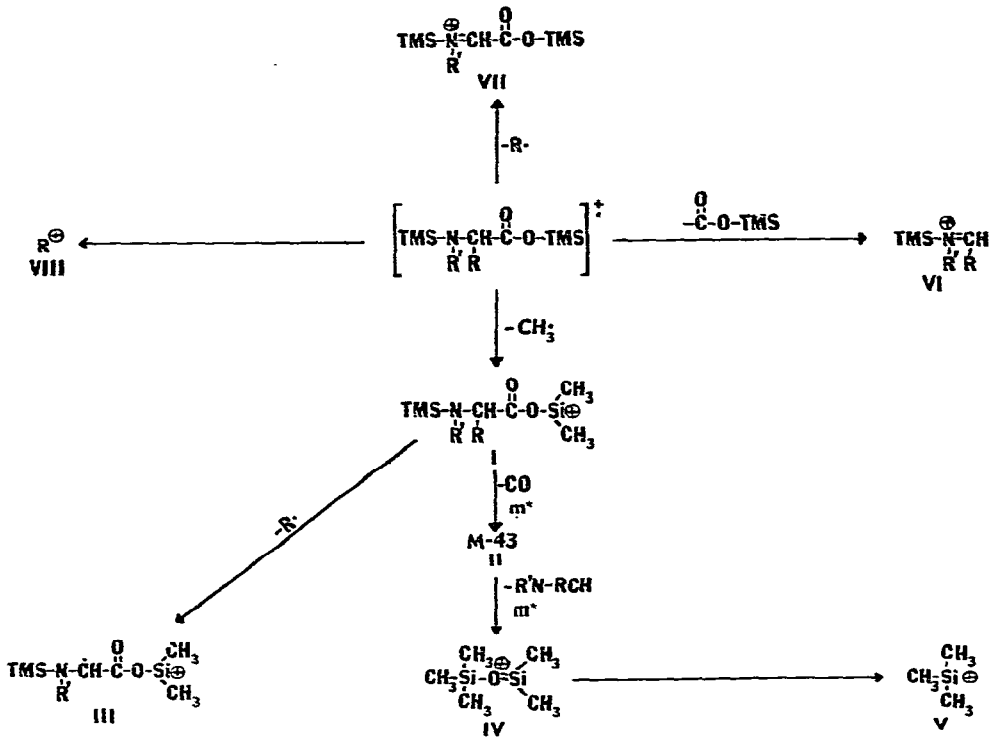


Fig. 13. Mass spectral fragmentation pathways of TMS-α-amino acids.

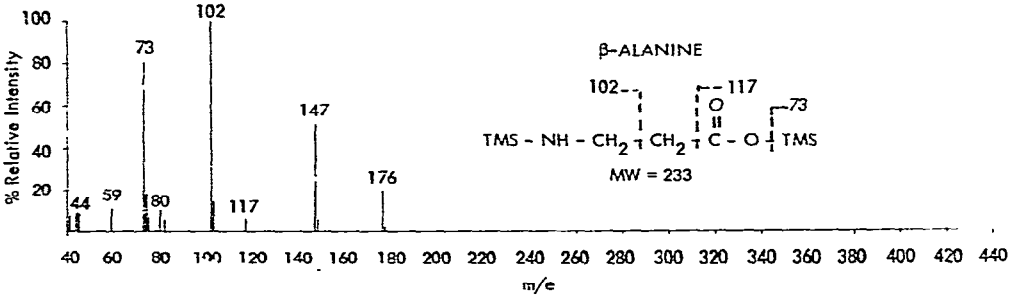


Fig. 14. Mass spectral fragmentation of N,O-di-TMS-β-alanine.

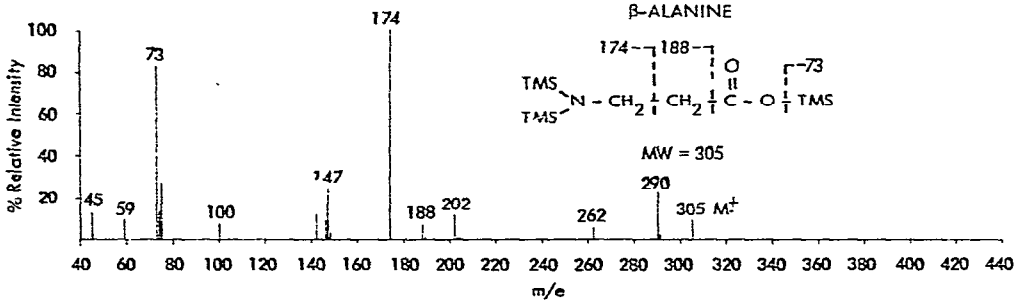


Fig. 15. Mass spectral fragmentation of N,N,O-tri-TMS-β-alanine.

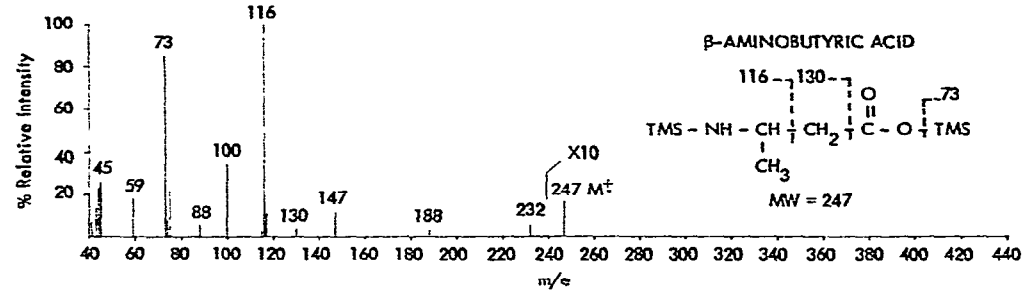


Fig. 16. Mass spectral fragmentation of N,O-di-TMS-β-aminobutyric acid.

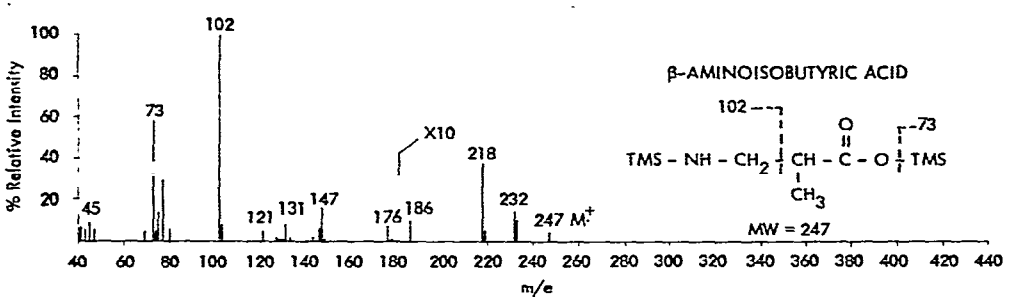


Fig. 17. Mass spectral fragmentation of N,O-di-TMS-β-aminoisobutyric acid.

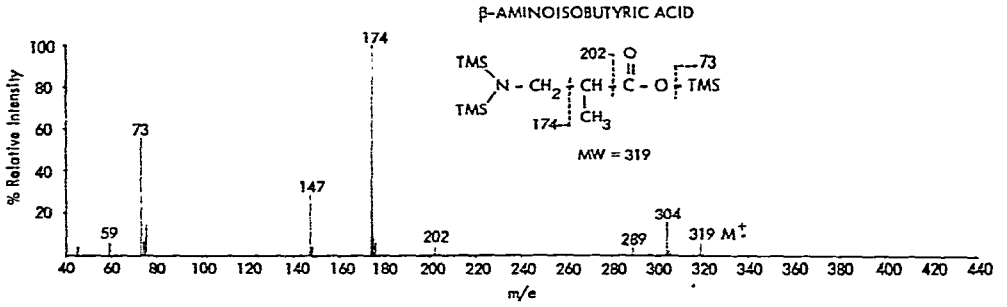


Fig. 18. Mass spectral fragmentation of N,N,O-tri-TMS-β-aminoisobutyric acid.

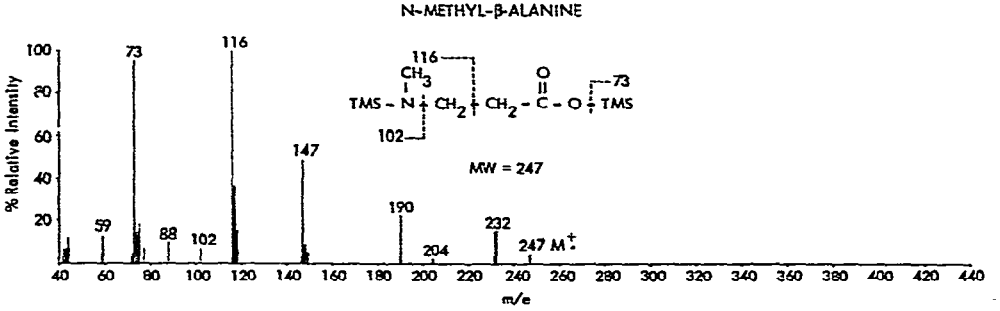


Fig. 19. Mass spectral fragmentation of N,O-di-TMS-N-methyl-β-alanine.

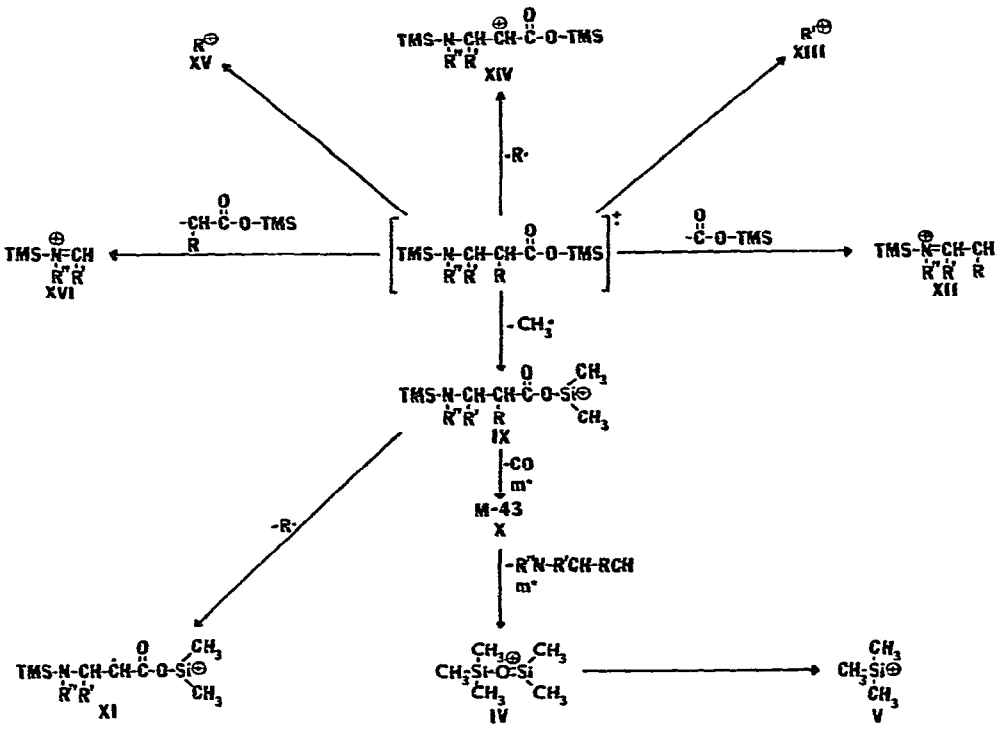


Fig. 20. Mass spectral fragmentation pathways of TMS-β-amino acids.

to the amino group with charge retention on the amine fragment (structure XVI in Fig. 20). The fragment resulting from the β-cleavage is dominant due to the resonance stabilization of the amine group. In general, the molecular ion appears to be more intense in the per-TMS β-amino acids than in the corresponding α-amino acids. Thus compare α-aminoisobutyric acid (Fig. 9) with β-aminoisobutyric acid (Figs. 17 and 18), with respect to the molecular ion intensity.

Figs. 21 and 22 present the mass spectra for the TMS derivatives of 4-aminobutyric acid. The dominant fragment observed for this amino acid was formed by cleavage beta to the amino group yielding *m/e* 102 for the di-TMS derivative and

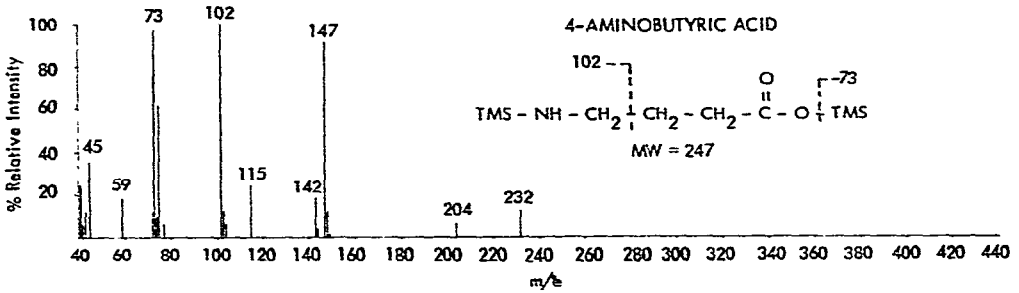


Fig. 21. Mass spectral fragmentation of N,O-di-TMS-4-aminobutyric acid.

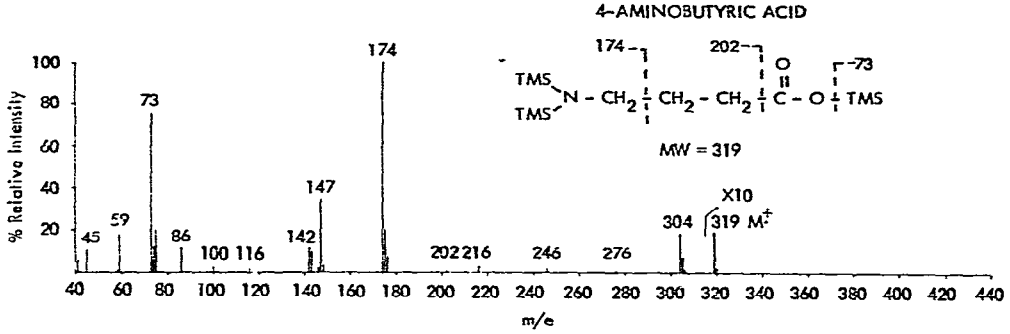


Fig. 22. Mass spectral fragmentation of N,N,O-tri-TMS-4-aminobutyric acid.

m/e 174 for the tri-TMS derivative with charge retention on the amine fragment as observed for the β-amino acids. Other characteristic fragments were M—15 and M—43. This example is insufficient to draw any additional conclusions about γ-amino acids.

Figs. 23 and 24 present the mass spectra of the TMS derivatives of 4-aminomethylcyclohexane carboxylic acid. These spectra are characterized by the molecular ion, M—15, and *m/e* 102 for the di-TMS derivative and *m/e* 174 for the tri-TMS derivative due to β-cleavage to the amino group. As has been observed for the β-amino acids and 4-aminobutyric acid, the M—117 (CO_2TMS) is of little importance

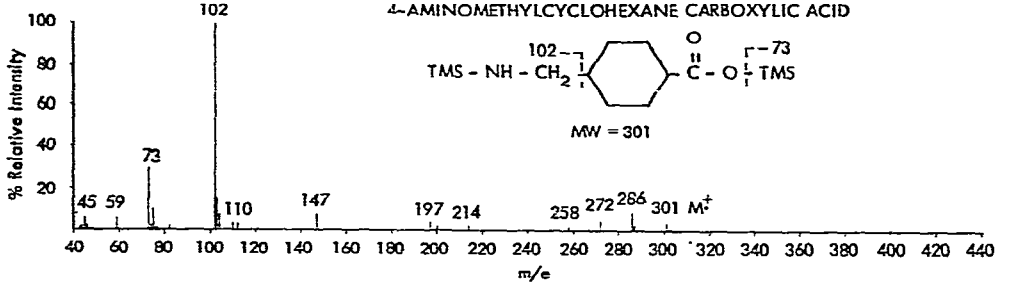


Fig. 23. Mass spectral fragmentation of N,O-di-TMS-4-aminomethylcyclohexane carboxylic acid.

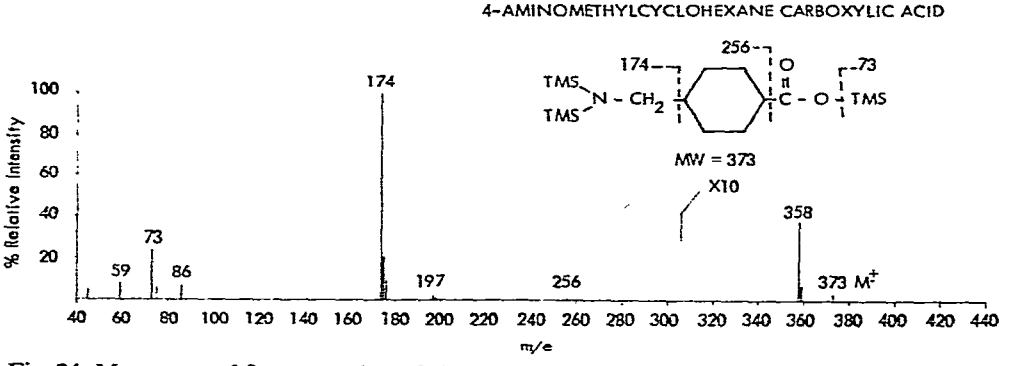


Fig. 24. Mass spectral fragmentation of N,N,O-tri-TMS-4-aminomethylcyclohexane carboxylic acid.

as compared to the α-amino acids. No loss of water from the molecular ion in the TMS derivative was observed as reported by Leimer *et al.*[11] for the N-trifluoroacetyl-*n*-butyl ester.

B. Acidic amino acids

Figs. 25–27 show the mass spectra for the TMS derivatives for aspartic acid, glutamic acid, and 2-aminoadipic acid. The fragments of these amino acids are easily explained with respect to Fig. 13 and Table 1. The most characteristic fragment for this group of amino acids is M—117 (CO_2TMS).

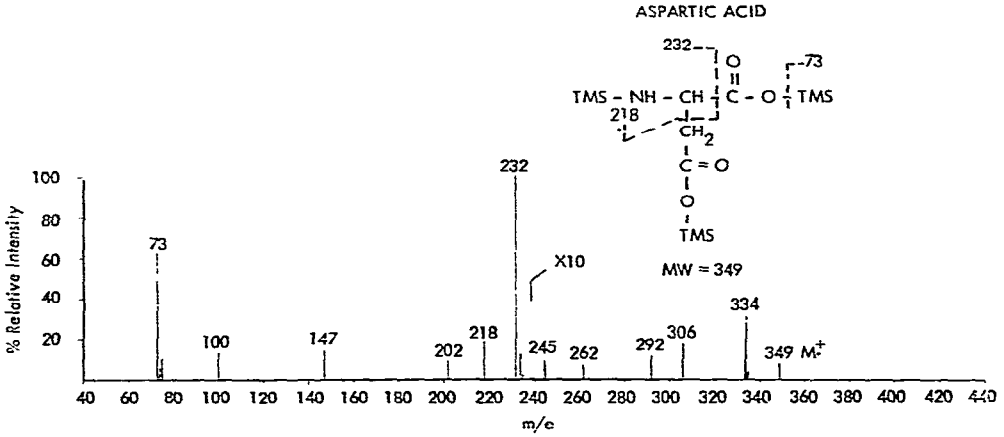


Fig. 25. Mass spectral fragmentation of N,O¹,O⁴-tri-TMS-aspartic acid.

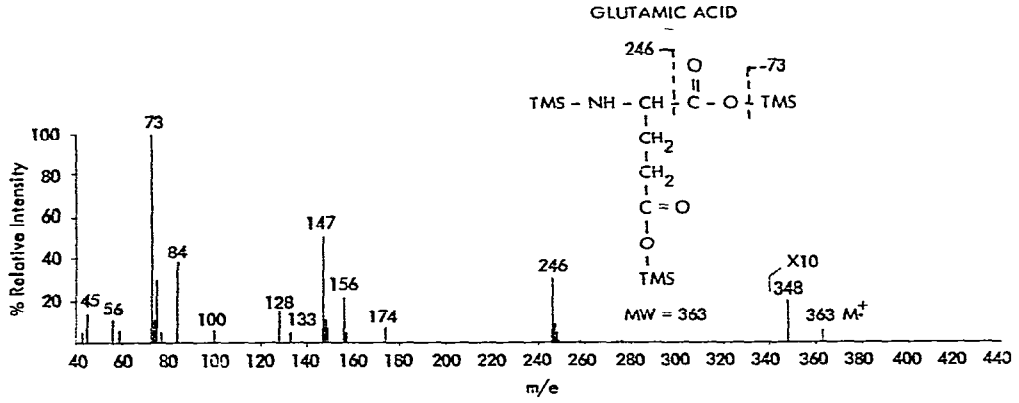


Fig. 26. Mass spectral fragmentation of N,O¹,O⁵-tri-TMS-glutamic acid.

C. Basic amino acids

Figs. 28–32 present the mass spectra for the TMS derivatives of lysine, ornithine, and ε-N-methyllysine. These spectra are characterized by the molecular ion, M—15, M—117, and cleavage beta to the ω-amino group with charge retention on

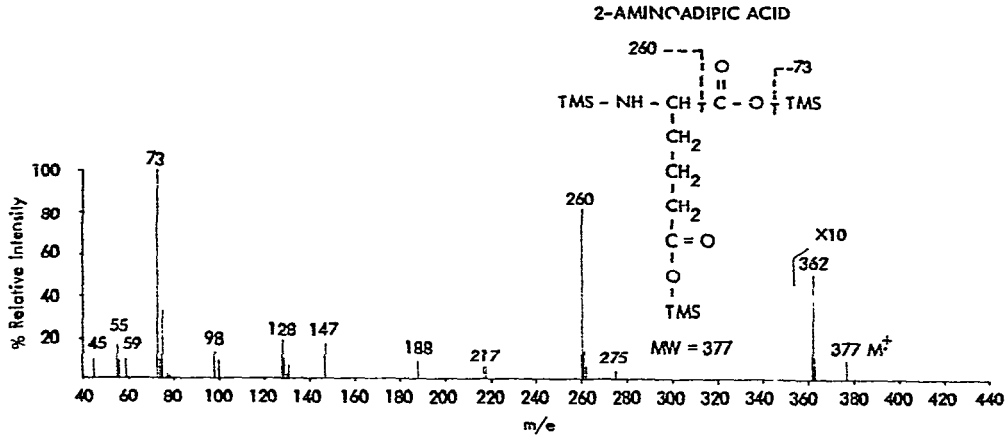


Fig. 27. Mass spectral fragmentation of N,O¹,O⁶-tri-TMS-2-aminoadipic acid.

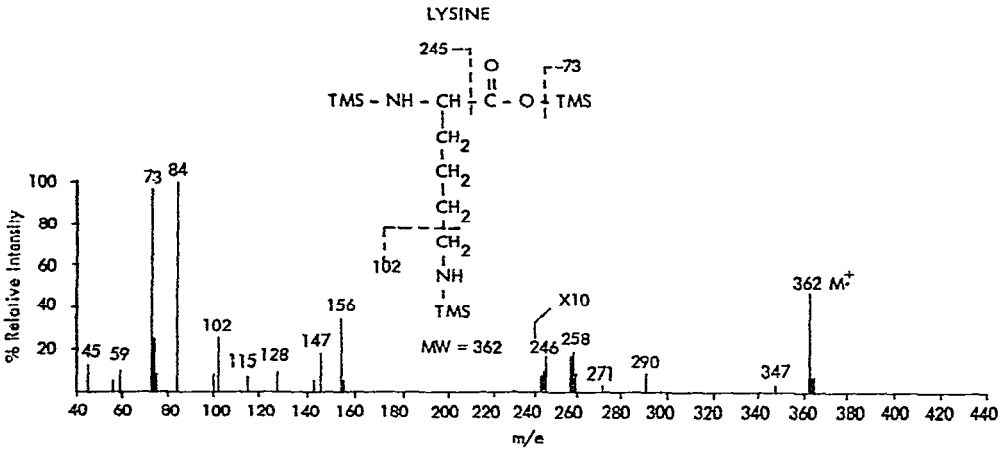


Fig. 28. Mass spectral fragmentation of N²,N⁶,O-tri-TMS-lysine.

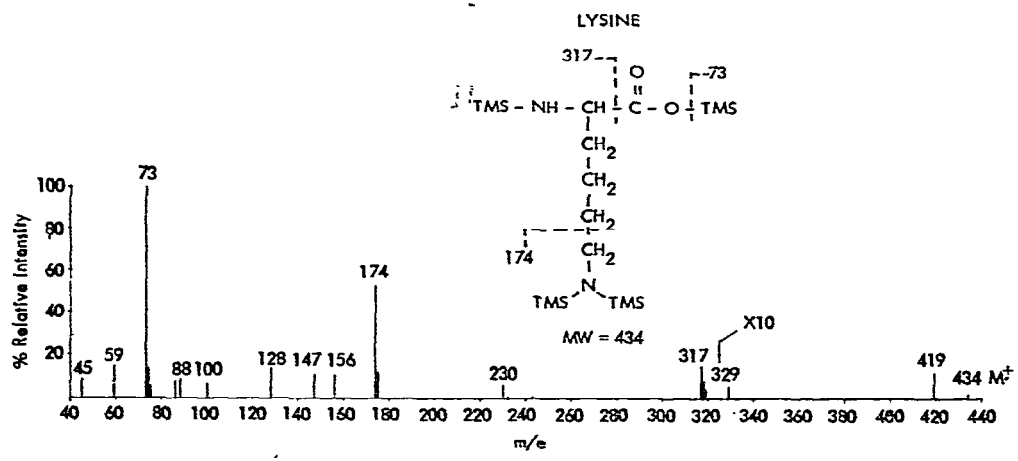


Fig. 29. Mass spectral fragmentation of N²,N⁶,N⁶,O-tetra-TMS-lysine.

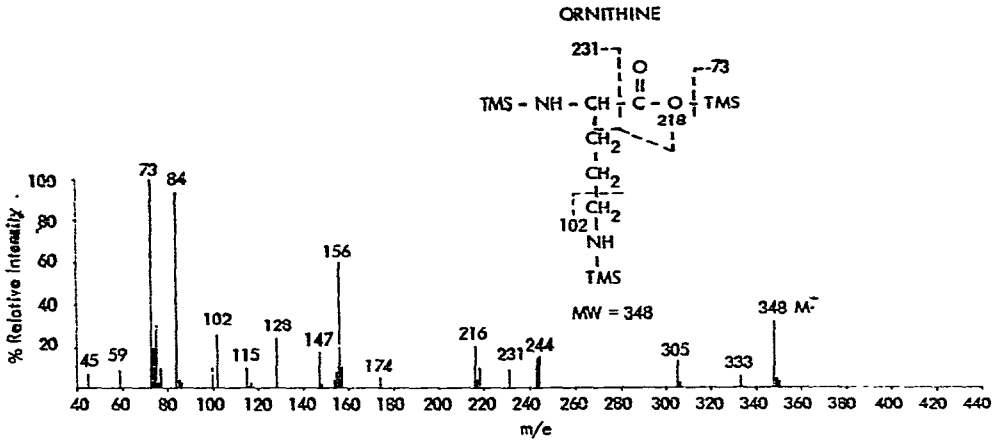


Fig. 30. Mass spectral fragmentation of N^2,N^5,O-tri-TMS-ornithine.

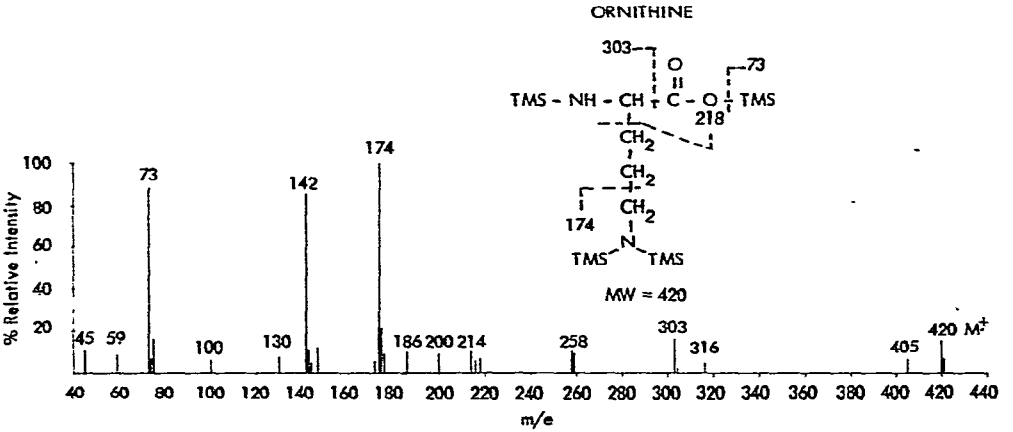


Fig. 31. Mass spectral fragmentation of N^2,N^5,N^5,O-tetra-TMS-ornithine.

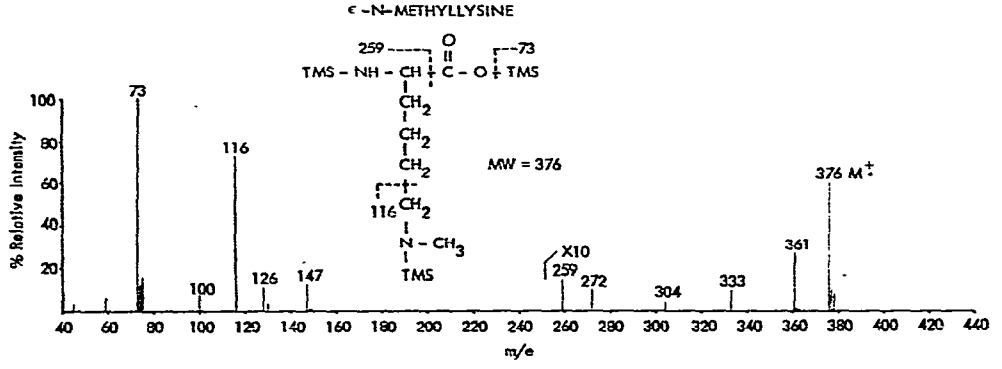


Fig. 32. Mass spectral fragmentation of N^2,N^6,O-tri-TMS-ε-N-methyllysine.

the amine fragment. One observes cleavage beta to the ε-amino group for ε-N-methyl-lysine which yields *m/e* 116 as a prominent ion rather than 102 or 174. This proves the location of the methyl group as being either on the ε-amino group or the terminal methylene group.

D. Aromatic amino acids

Figs. 33–38 present the mass spectra for the TMS derivatives of phenylalanine, tyrosine, tryptophan, histidine, 1-methylhistidine and 3-methylhistidine. These spectra are characterized by the fragments M—15, M—117, M—R and R. For the aromatic amino acids, the structurally most significant ion originated either from M—R or R. The ratio of these two fragments was quite interesting. This ratio (M—R/R) varied from 8:1 for phenylalanine to 1:20 for tryptophan. The controlling factors for these ratios are not understood at this time. The ratio observed for the TMS-phenylalanine is the inverse of that for the N-trifluoroacetyl-*n*-butyl ester of phenylalanine, whereas the ratio for the other aromatic TMS-amino acids follows the ratio observed for the N-trifluoroacetyl-*n*-butyl ester derivatives[11]. The aromatic residue (R) is the major fragment as compared to (M—R).

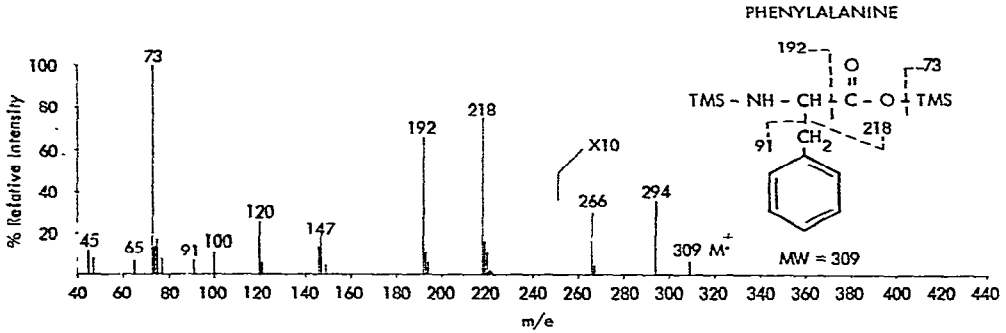


Fig. 33. Mass spectral fragmentation of N,O-di-TMS-phenylalanine.

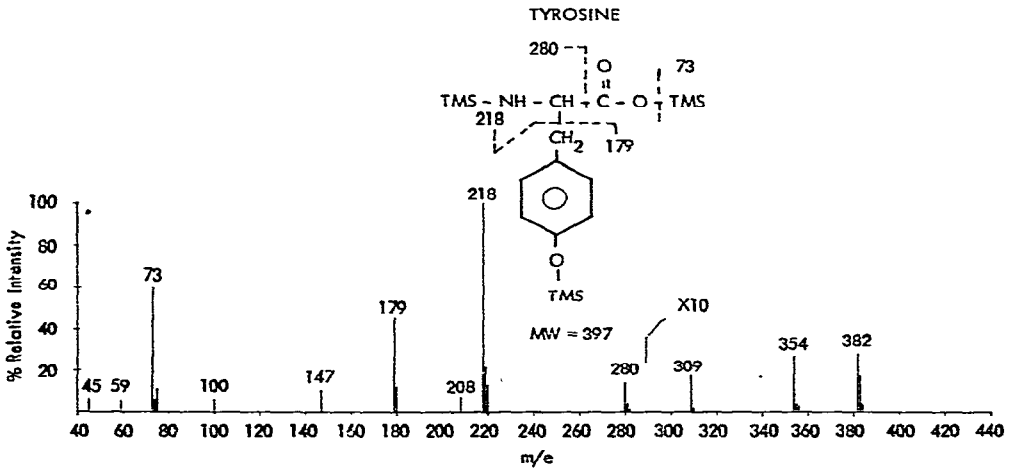


Fig. 34. Mass spectral fragmentation of N,O^1,$O^{4'}$-tri-TMS-tyrosine.

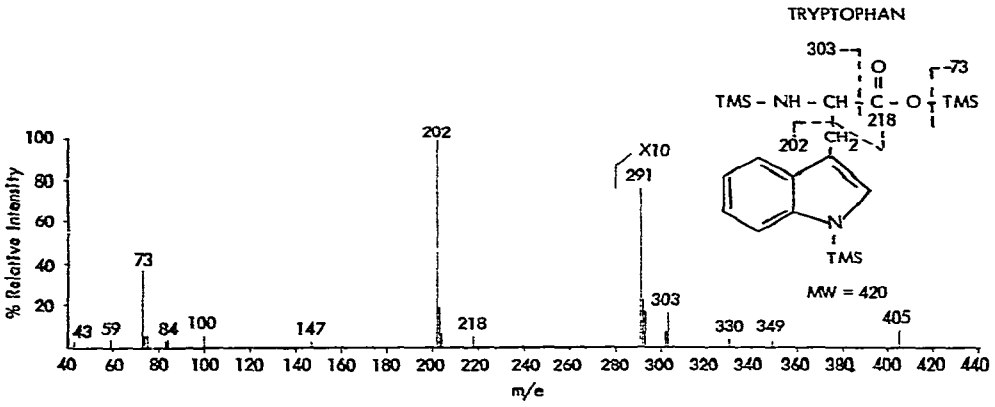


Fig. 35. Mass spectral fragmentation of N^2,$N^{5'}$,O-tri-TMS-tryptophan.

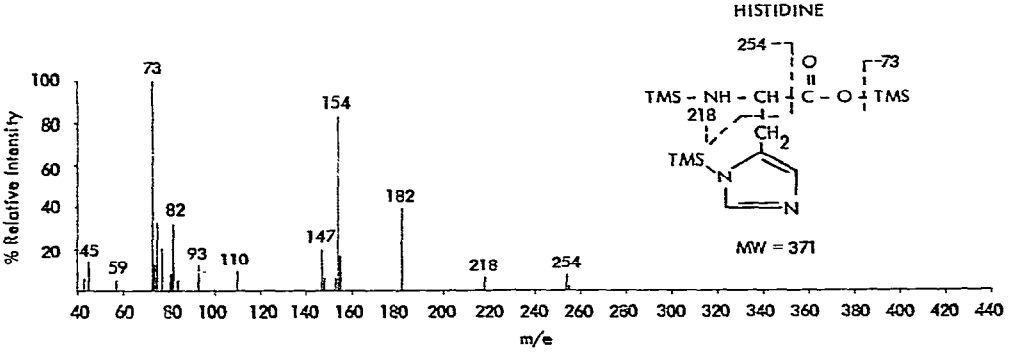


Fig. 36. Mass spectral fragmentation of $N^{1'}$,N^2,O-tri-TMS-histidine.

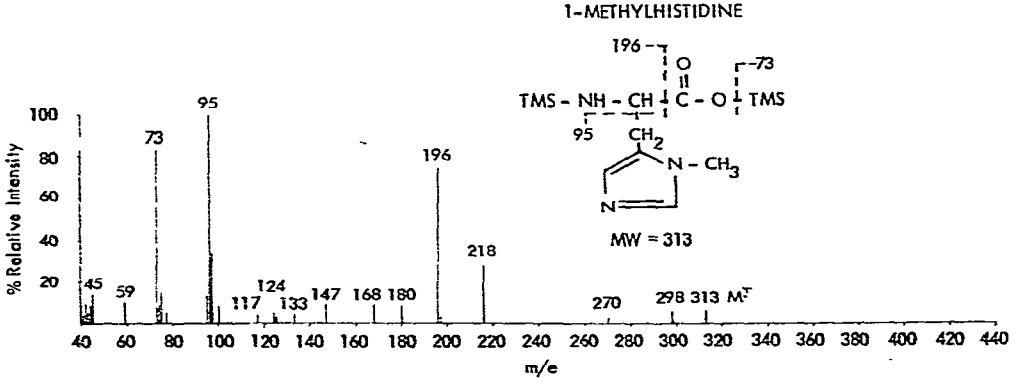


Fig. 37. Mass spectral fragmentation of N,O-di-TMS-1-methylhistidine.

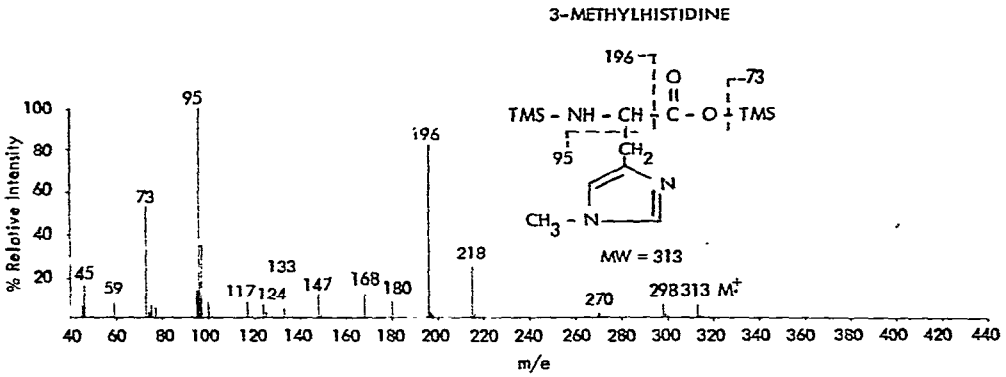


Fig. 38. Mass spectral fragmentation of N,O-di-TMS-3-methylhistidine.

E. Hydroxy amino acids

Figs. 39–41 present the mass spectra for the TMS derivatives for serine, threonine, and 6-hydroxy-2-aminohexanoic acid. These amino acids have the same characteristic fragments as those observed for the aliphatic α-amino acids presented in Fig. 13 and Table 1. The *m/e* 218 in serine (structure VII, Fig. 13), is prominent due to resonance stabilization of the amine fragment and of the radical due to the presence of the oxygen atom. This same ion is also prominent in threonine and is due to the above explanation plus the favorable cleavage of the 1,2 bond. The effect of the ether

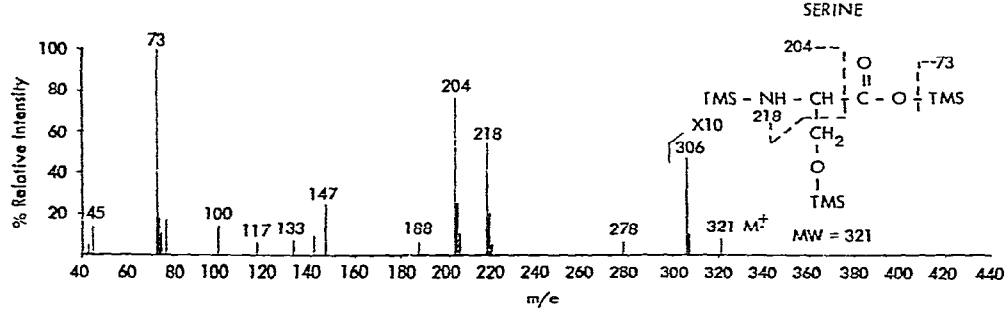


Fig. 39. Mass spectral fragmentation of N,O^1,O^3-tri-TMS-serine.

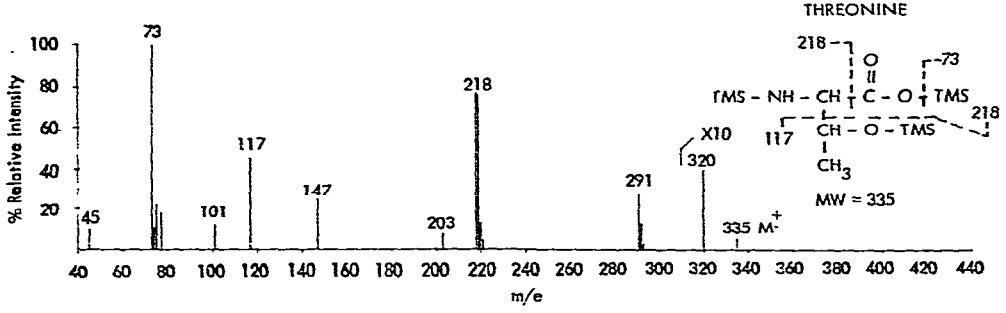


Fig. 40. Mass spectral fragmentation of N,O^1,O^3-tri-TMS-threonine.

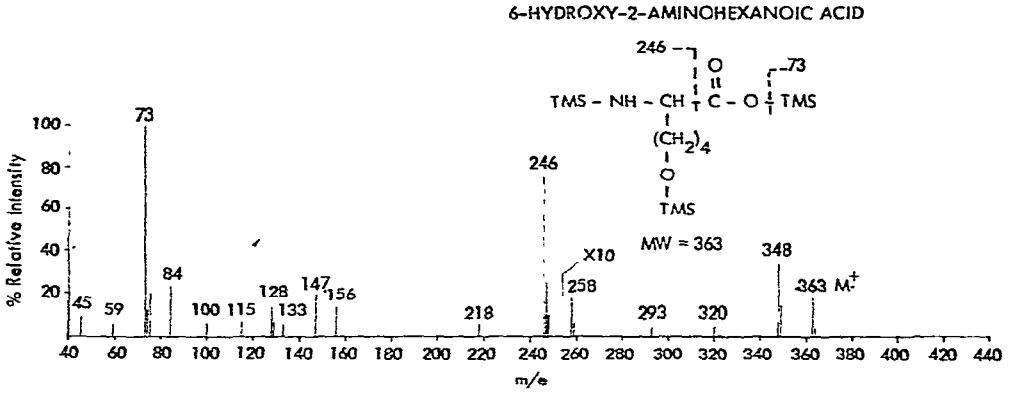


Fig. 41. Mass spectral fragmentation of N,O¹,O⁶-tri-TMS-6-hydroxy-2-amino-hexanoic acid.

linkage on the intensity of the *m/e* 218, M—R, is clearly shown by its small size in the mass spectrum of 6-hydroxy-2-aminohexanoic acid in which the ether linkage is removed by three more methylene groups.

F. Sulfur amino acids

Figs. 42–45 present the mass spectra for the TMS derivatives for methionine, cysteine, cystine, and 1-hydroxy-6-amino-3,4-dithiahexane-6-carboxylic acid. These amino acids have the same typical fragments as those observed for the aliphatic α-amino acids (Fig. 13 and Table 1). Additional fragments were observed due to the presence of the sulfur atom. Methionine shows fragments at *m/e* 47 and 61 originating from a cleavage of the carbon–sulfur bond and β-cleavage to the sulfur, respectively. Cystine showed cleavage of the carbon–sulfur and sulfur–sulfur bonds yielding characteristic ions of *m/e* 232 and 264, respectively. The TMS derivative of 1-hydroxy-6-amino-3,4-dithiahexane-6-carboxylic acid was cleaved at both carbon–sulfur bonds and at the sulfur–sulfur bond yielding *m/e* 232, 264 and 296. The *m/e* 296 ion may arise from either carbon–sulfur bond cleavage or the loss of the TMS-carboxyl group. High-resolution MS has shown that the ratio of these two *m/e* 296 fragments was approximately 1:15 for C–S/M—CO_2—TMS.

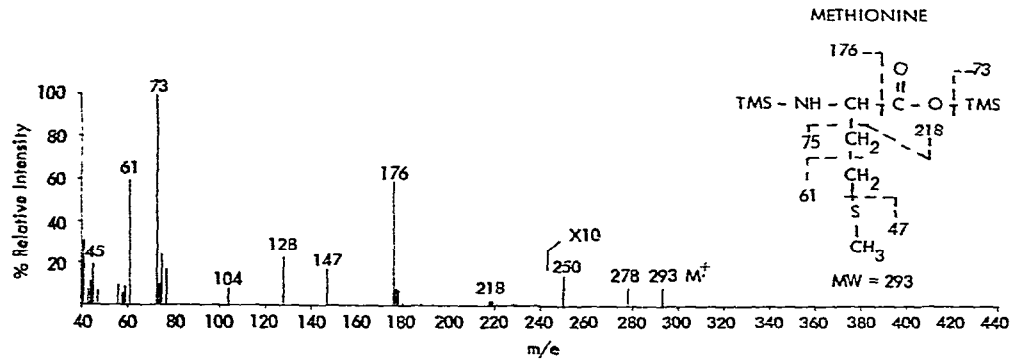


Fig. 42. Mass spectral fragmentation of N,O-di-TMS-methionine.

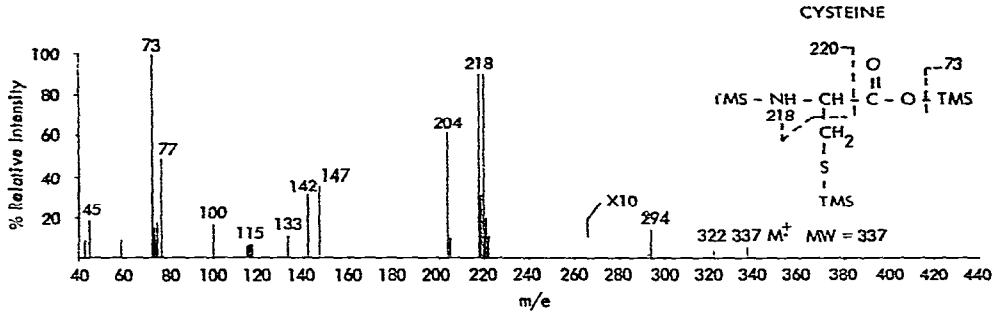


Fig. 43. Mass spectral fragmentation of N,O,S-tri-TMS-cysteine.

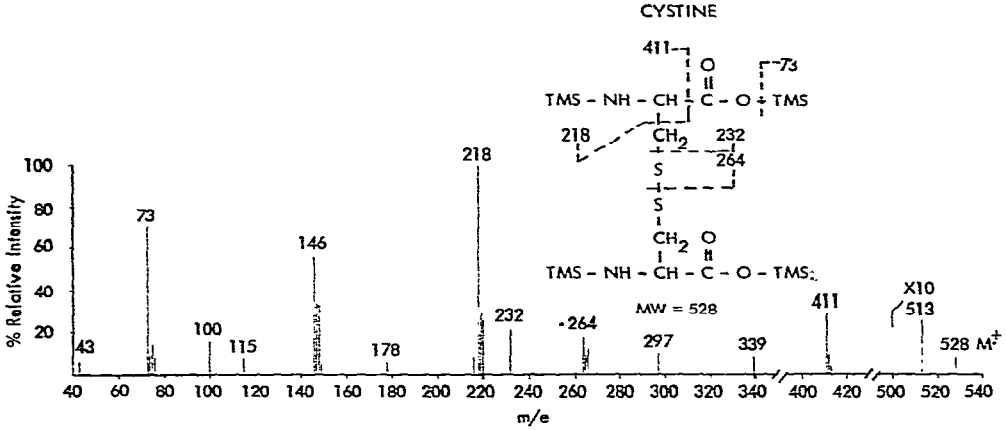


Fig. 44. Mass spectral fragmentation of N,N′,O,O′-tetra-TMS-cystine.

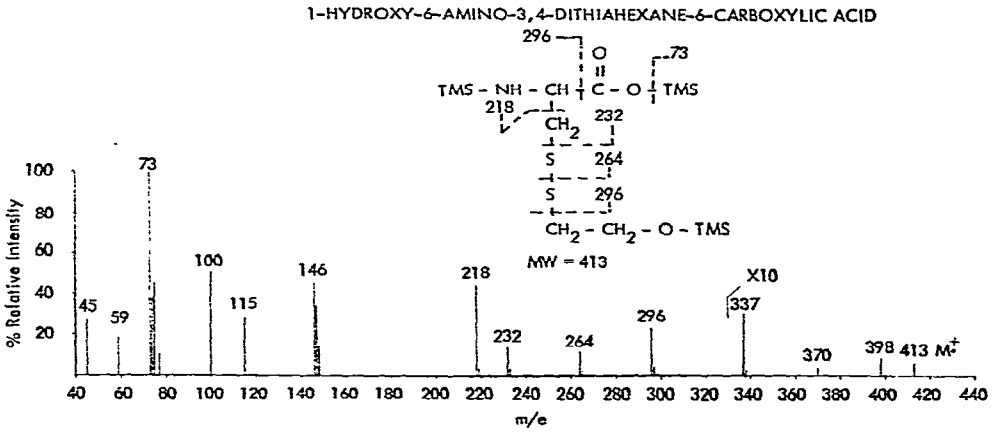


Fig. 45. Mass spectral fragmentation of N,O^1,O^6-tri-TMS-1-hydroxy-6-amino-3,4-dithiahexane-6-carboxylic acid.

G. Proline and hydroxyproline

Figs. 46 and 47 present the mass spectra for the TMS derivatives for proline and hydroxyproline. The fragment of these spectra showed the same fragment pattern as for the aliphatic α-amino acids (Fig. 13 and Table 1). The M—117 ion was particularly intense in these spectra due to cleavage yielding a secondary carbonium ion as well as being resonance stabilized by the amino group.

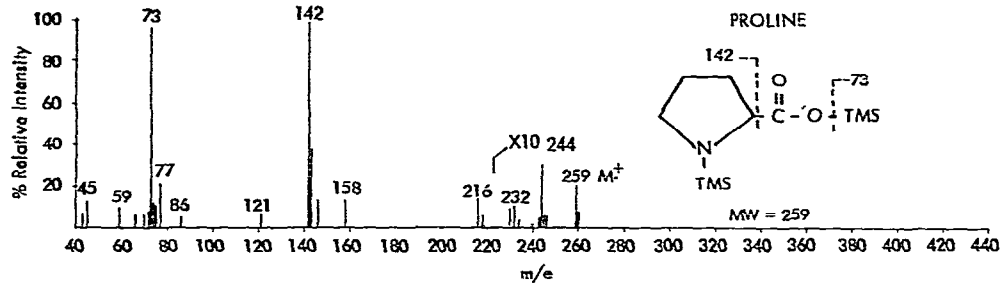


Fig. 46. Mass spectral fragmentation of N,O-di-TMS-proline.

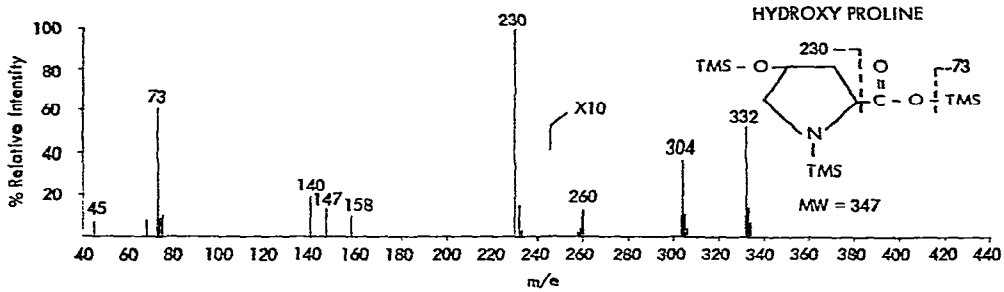


Fig. 47. Mass spectral fragmentation of N,O^1,$O^{4'}$-tri-TMS-hydroxyproline.

H. 2-Pyrrolidone carboxylic acid

The spectrum of the TMS derivative of 2-pyrrolidone carboxylic acid is presented in Fig. 48. This spectrum is characterized by a molecular ion, M—15, M—43, and M—117. This compound has been reported by Gehrke *et al.*[2] as Glu_2 in previous

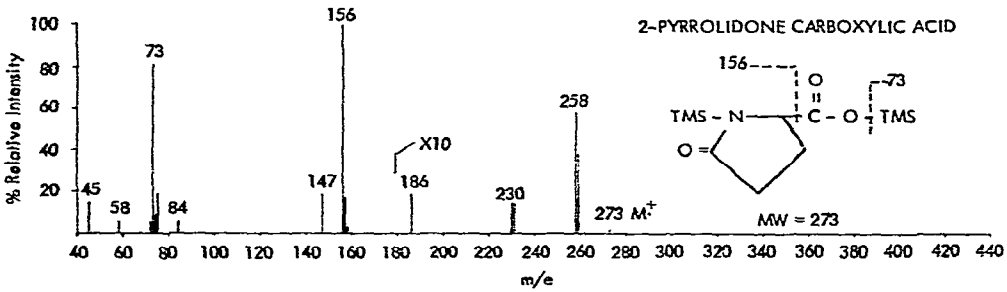


Fig. 48. Mass spectral fragmentation of N,O-di-TMS-2-pyrrolidone carboxylic acid.

work. The 2-pyrrolidone carboxylic acid was formed by cyclization of glutamic acid during the derivatization.

I. Arginine

The TMS derivative reported by Gehrke *et al.*[2] for arginine produced a mass spectrum identical with ornithine, containing four TMS groups. The assumption that arginine had been converted to ornithine during derivatization was confirmed by hydrolyzing the TMS derivative of arginine to form the free amino acid. The free amino acid was then derivatized as the N-trifluoroacetyl-*n*-butyl ester. The compound then showed identical mass spectrum and retention properties with N-trifluoroacetyl-*n*-butyl ester of ornithine. Arginine was heated at 150° in acetonitrile for $2^1/_2$ h and no ornithine was observed upon derivatization as the N-trifluoroacetyl-*n*-butyl ester. This suggested that the TMS derivative of arginine is not stable under the silylation conditions and fragments into ornithine and the TMS derivative of urea. The TMS derivative of urea would not be observed as it would not be resolved from the solvent.

J. Multiple TMS derivatives

Multiple TMS derivatives have been observed for glycine, β-alanine, β-aminoisobutyric acid, 4-aminobutyric acid, 4-aminomethylcyclohexane carboxylic acid, lysine, and ornithine under derivatization conditions used in this study. Steric hindrance of the amino group appears to be the controlling factor in the formation of multiple derivatives. The only α-amino group which is disilylated is glycine, which is the least hindered of the α-amino groups. Also comparison of alanine with β-alanine and α-aminoisobutyric acid with β-aminoisobutyric acid supported the assumption of steric hindrance as the controlling factor.

For those amino acids which do form multiple derivatives, the molecular ion in the mass spectrum appeared more intense for the more highly silylated derivative (*cf.* β-alanine, Figs. 14 and 15).

4. CONCLUSIONS

This study presents complete mass spectra and diagnostic criteria for the per-TMS derivatives of 46 amino acids. The aliphatic α-amino acids are characterized by spectra with a molecular ion, M—15 (CH_3), M—43 ($CH_3 + CO$), and M—117 (CO_2TMS) and the β-amino acids by more complex spectra with a more intense per-TMS molecular ion, M—15, M—43, a much weaker M—117, and β-cleavage to the amino group.

The addition of other functional groups to the aliphatic α-amino acids increase the complexity of the resulting spectra. Sulfhydryl and hydroxyl groups yield M—R of greater intensity than for the aliphatic α-amino acids. The disulfide group is characterized by C–S and S–S bond cleavage with charge retention on both fragments. The presence of an additional amino group results in β-cleavage to this group; and an aromatic substituent on the α-carbon gives a prominent fragment ion for R.

Mono- and disilylation of the amino group is controlled by the steric hindrance of this group. Multiple derivatives of arginine result from the conversion of arginine to ornithine during the trimethylsilylation step.

This research delineates the diagnostic criteria for distinguishing the TMS derivatives of the α- and β-amino acids from each other. The presence of other functional groups in the amino acids are readily identified by GC–MS.

5. ACKNOWLEDGEMENTS

The authors wish to thank Mr. Kenneth C. Kuo and Dr. Klaus O. Gerhardt for technical assistance and many useful discussions. The authors also wish to express their thanks and appreciation to Debi Whisenand and Pam Fitzgerald for their efforts in the preparation of the manuscript.

This study was supported in part by the National Institutes of Health, Contract No. NIH NO1 CM 12323.

6. SUMMARY

The complete mass spectra of the trimethylsilyl derivatives of 46 amino acids are presented. The spectra are discussed in terms of the common fragmentation pathways and factors controlling the fragment formation.

The aliphatic α-amino acids are characterized by a molecular ion, M—15 (CH_3), M—43 (CH_3 + CO), M—117 (CO_2TMS), and M—R. The β-amino acids are characterized by a more intense molecular ion, M—15, M—43, a small M—117, and presence of other functional groups results in more complex spectra. In sulfur amino acids, additional fragments were observed for C–S and S–S bond cleavage with charge retention on both fragments. For serine and threonine, hydroxyl groups caused more prominent cleavage of M—R than would otherwise be expected. Additional amino groups result in β-cleavage to this functional group. Aromatic substitution on the α-carbon results in the additional fragment R.

The spectra for 4-aminobutyric acid, and 4-aminomethylcyclohexane carboxylic acid are presented. These amino acids are characterized by a molecular ion, M—15, M—117, and β-cleavage to the amino group.

The trimethylsilyl derivative of arginine was found to be identical with that of ornithine. This transformation was shown to occur during the trimethylsilylation step.

The trimethylsilyl derivatives are particularly known for their multiple derivative formation. For amino acids, the multiple derivative formation is controlled by the steric hindrance of the amino group within the molecule.

REFERENCES

1 P. Hušek and K. Macek, *J. Chromatogr.*, 113 (1975) 139.
2 C. W. Gehrke, H. Nakamoto and R. W. Zumwalt, *J. Chromatogr.*, 45 (1969) 24.
3 C. W. Gehrke and K. R. Leimer, *J. Chromatogr.*, 57 (1971) 219.
4 W. J. A. VandenHeuvel, J. L. Smith, I. Putter and J. S. Cohen, *J. Chromatogr.*, 50 (1970) 405.
5 W. J. A. VandenHeuvel and J. L. Smith, *J. Chromatogr. Sci.*, 8 (1970) 567.
6 W. J. A. VandenHeuvel and J. S. Cohen, *Biochim. Biophys. Acta*, 208 (1970) 251.
7 K. Bergström, J. Gurthler and R. Blomstrand, *Anal. Biochem.*, 34 (1970) 74.
8 K. Bergström and J. Gurthler, *Acta Chem. Scand.*, 25 (1971) 175.
9 J. L. Laseter, J. D. Weete, A. Albert and C. H. Walkinshaw, *Anal. Lett.*, 4 (1971) 671.
10 R. Mitra, J. Burton and J. E. Varner, *Anal. Biochem.*, 70 (1976) 1.
11 K. R. Leimer, R. H. Rice and C. W. Gehrke, *J. Chromatogr.*, 141 (1977) 121.