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COMPLETE MASS SPECTRA OF N-TRIFLUOROACETYL-*n*-BUTYL ESTERS OF AMINO ACIDS*

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1. INTRODUCTION

For the past two decades, cation-exchange chromatography has been the main method for the quantitative analysis of amino acids. In 1965, Lamkin and Gehrke[1] started a comprehensive study of the N-trifluoroacetyl-*n*-butyl (TAB) ester derivative for the gas–liquid chromatography (GLC) of the amino acids. Through the efforts of Gehrke and co-workers[2–6], GLC has become a routine analytical method for amino acids. This method has been applied world wide to a large variety of biological and geochemical samples. A comprehensive review on the gas chromatography of amino acids has recently been presented by Hušek and Macek[7] (415 references).

With mixtures of amino acids, ion-exchange or GLC retention data is insufficient for unequivocal identification of the amino acids present. One major advantage of the GLC method is the ease of interfacing the gas chromatograph with a mass spectrometer while ion exchange can not readily be interfaced with the mass spectrometer. The combination of gas chromatography–mass spectrometry (GC–MS) gives one, through the GLC retention data, MS structural confirmation and elution

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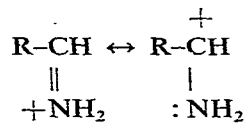
band homogeneity, a much greater assurance as to the identification of the amino acids present.

Since both protein and non-protein amino acids have been identified in biological systems[8], primitive and Jovian atmospheric experiments[9,10], and in the Murchison meteorite[11,12], a comprehensive study of the MS fragmentation and structural correlations of the amino acids would be advantageous. The use of GC–MS would permit one to identify the presence of an unusual or unexpected amino acid in biological and extraterrestrial samples. For example, Gehrke *et al.*[13] found a major peak in Apollo 11 through 17 samples with the retention characteristic of glycine, which by GC–MS was identified as di-*n*-butyloxalate.

Volatility of the amino acids for MS is obtained by blocking either one or both of the functional groups. Esterification and acylation are often the preferred methods to increase the volatility of amino acids for these investigations. Amino acid ethyl esters dissociate under electron impact to give simple spectra with the base peak in each corresponding to the resonance stabilized ion as described by Biemann *et al.*[14].



Andersson *et al.*[15] have studied the mass spectra of the N-acetyl amino acid methyl esters and have found that the acetyl group introduces more complex fragmentation patterns.

Manhas *et al.*[16] have reported on the unusual fragmentation of the N-trifluoroacetyl methyl and ethyl ester derivatives of some amino acids when compared to the N-acetyl methyl esters. This group delineated the role of the trifluoroacetyl group in some of these unexpected fragmentation pathways for this derivative through isotope labeling.

Gelpi *et al.*[17] have studied the GC–MS of the TAB esters of the protein amino acids with the exception of diacyl histidine and arginine. These authors present fragmentation pathways and partial spectra for the TAB derivatives obtained at 20 eV. In our laboratory, using 70 eV ionization potential, a number of significant differences have been observed in the spectra of these amino acids.

Lawless and Chadha[18] have studied the GC–MS of the C_3 and C_4 aliphatic amino acids as the TAB derivatives. They present spectra for a number of β-amino acids, N-substituted amino acids, and 4-aminobutyric acid, but do not give correlations of fragmentation and structure, in comparison with the protein amino acids.

Recently, Felker and Bandurski[19] reported on the GC–MS of 20 amino acids as the N(O)-perfluorobutyryl-O-isoamyl ester derivative. Their observed fragmentation pathways were in generally good agreement with those by Gelpi *et al.*[17] for the TAB derivatives, although there were a number of significant differences. Again, no comprehensive study was completed.

Previous authors[18,19] have reported that the various alkyl groups used for the esterification produced little change in the fragmentation patterns for the amino acids. For a comprehensive study of the structure and the fragmentation correlations,

it would be desirable to select the N-trifluoroacetyl-*n*-butyl esters as the derivative of choice, due to their state of development and wide use[5,6].

The subject of this investigation is the MS fragmentation of the TAB esters of the amino acids. A later paper will discuss the fragmentation patterns of the trimethylsilyl derivatives as another frequently used derivative.

2. EXPERIMENTAL

The TAB derivatives were prepared by the procedure of Kaiser *et al.*[6].

The GC–MS spectra were obtained on a CEC-21-110 mass spectrometer interfaced by a "Llewellyn type" silicone membrane separator to a Varian 1500 gas chromatograph. Nominal mass numbers were obtained by direct hook-up to a Jeolco JEC-6 Spectrum Computer. All elemental compositions reported in this investigation were verified with high resolution measurements by peak matching to known peaks in perfluorokerosene. The derivatives were chromatographed on a 1 m × 0.4 cm glass column containing 3% OV-101 on HP Chromosorb W with helium used as carrier gas. Other operational parameters were: injection port, 250°; molecular separator maintained at the approximate elution temperature of the amino acid; ion source, 250°; electron voltage, 70 eV; and scanning rate of approximately 8 sec per decade. All scanning was done at the apex of the peaks. No serious bias of relative intensity was observed due to changes in the concentration in the ion source during scanning, as the reproducibility was satisfactory.

3. RESULTS AND DISCUSSION

A. Aliphatic amino acids

Figs. 1–11 present the mass spectra for the TAB derivatives for glycine, alanine, valine, leucine, isoleucine, norleucine, α-aminobutyric acid, α-aminoisobutyric acid, sarcosine, N-methylalanine, and N-methylleucine. The basic fragmentation pathways for these amino acids may be summarized as in Fig. 12. The fragmentations are in general agreement with those presented by Gelpi *et al.*[17], however, some significant differences were observed.

The intensities of identical fragment ions for isoleucine, leucine, and norleucine differ sufficiently to distinguish these amino acids from one another. These differences

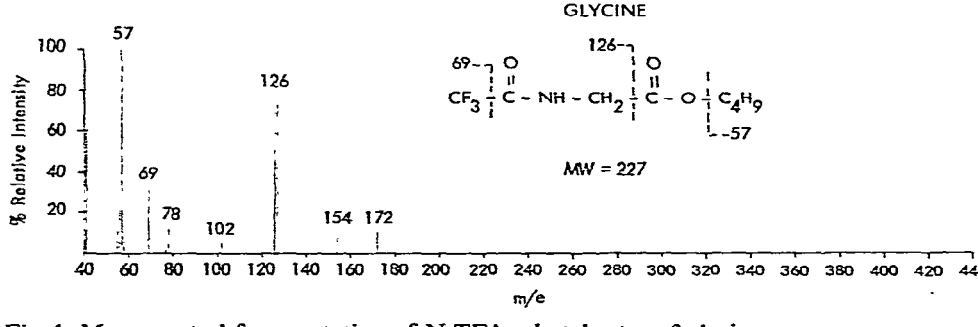


Fig. 1. Mass spectral fragmentation of N-TFA-*n*-butyl ester of glycine.

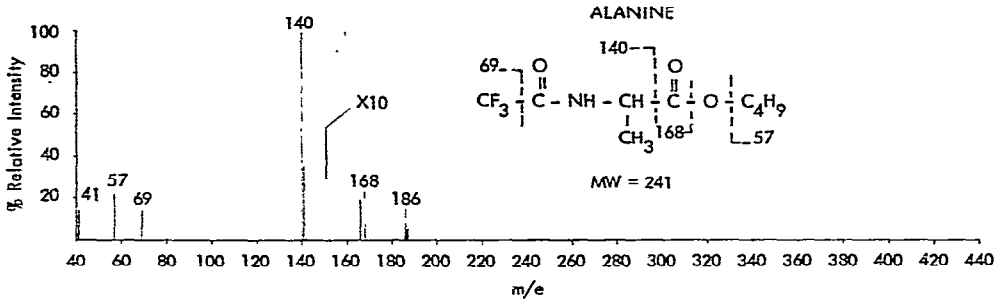


Fig. 2. Mass spectral fragmentation of N-TFA-*n*-butyl ester of alanine.

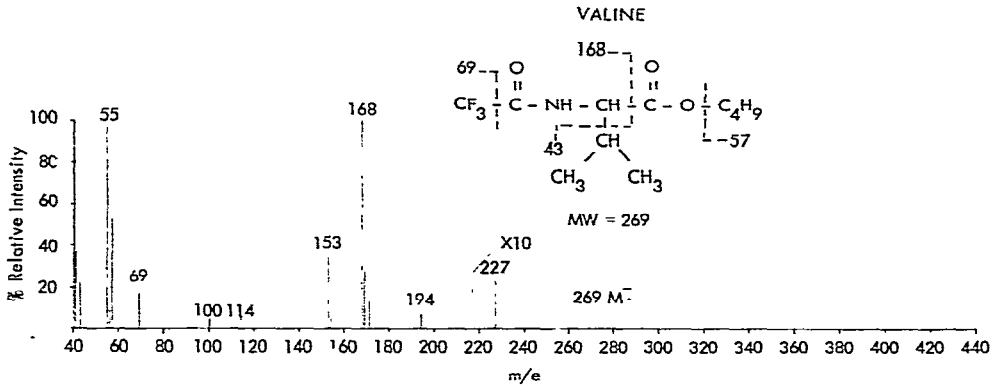


Fig. 3. Mass spectral fragmentation of N-TFA-*n*-butyl ester of valine.

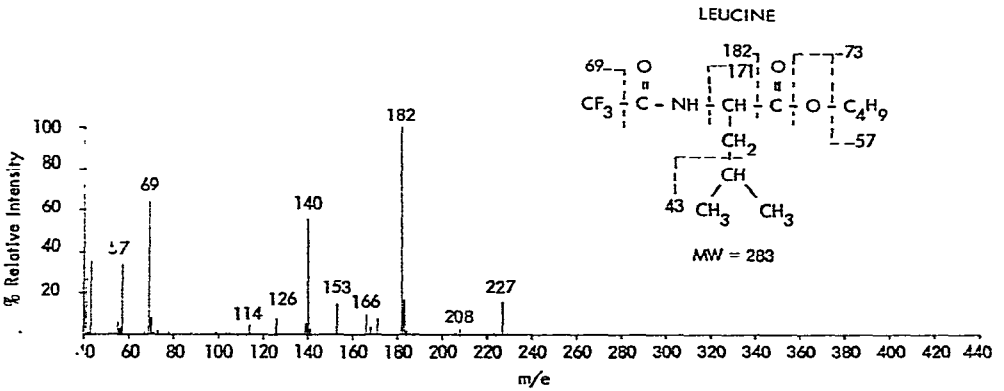


Fig. 4. Mass spectral fragmentation of N-TFA-*n*-butyl ester of leucine.

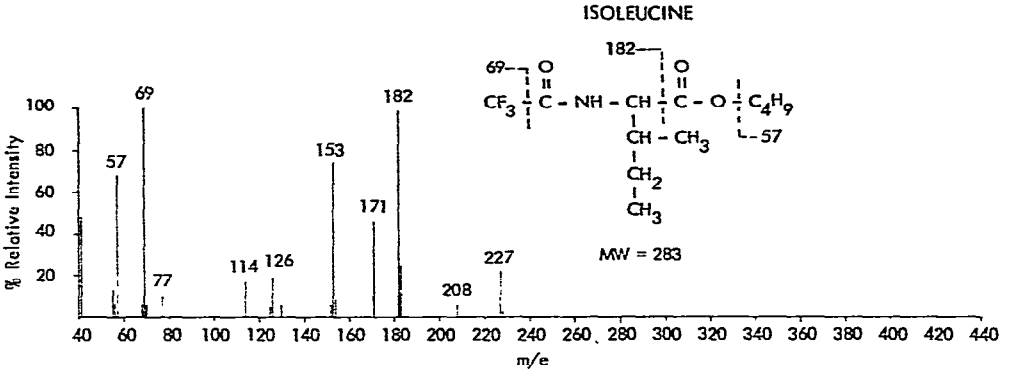


Fig. 5. Mass spectral fragmentation of N-TFA-*n*-butyl ester of isoleucine.

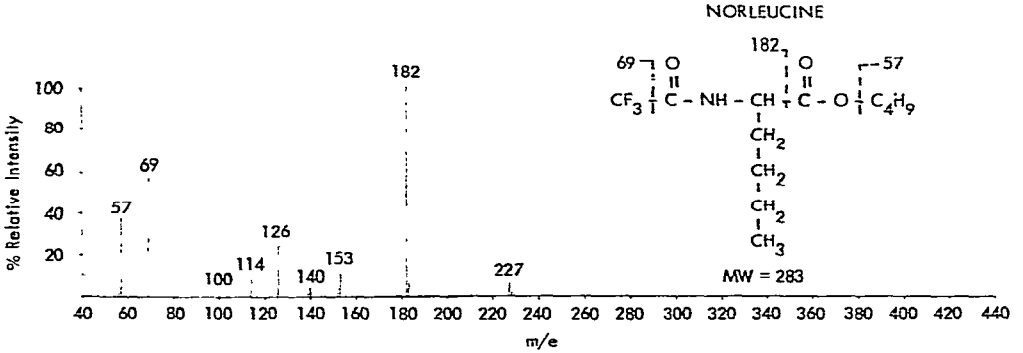


Fig. 6. Mass spectral fragmentation of N-TFA-*n*-butyl ester of norleucine.

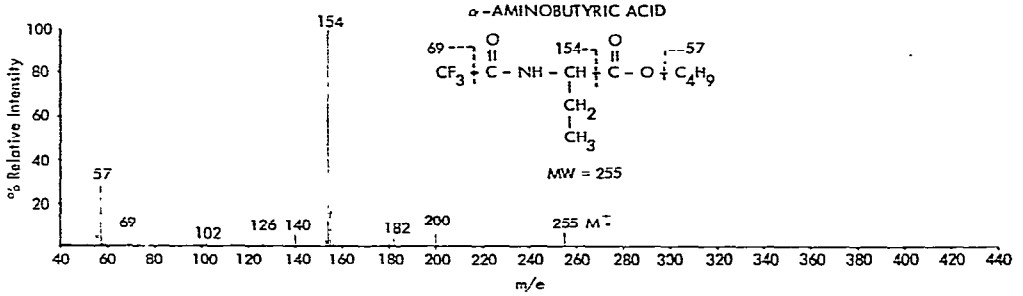


Fig. 7. Mass spectral fragmentation of N-TFA-*n*-butyl ester of α-aminobutyric acid.

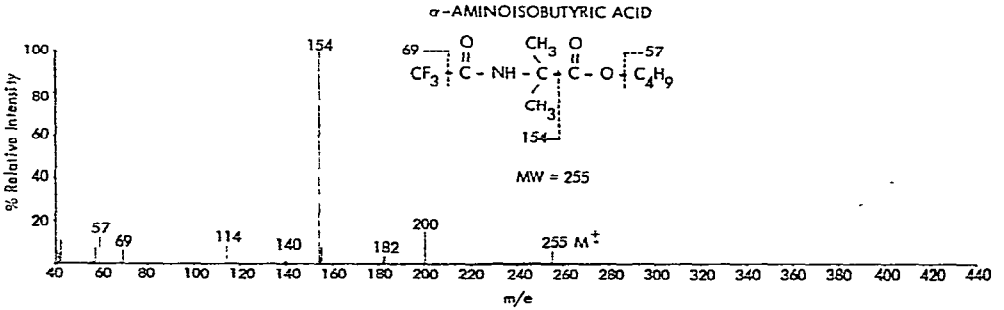


Fig. 8. Mass spectral fragmentation of N-TFA-*n*-butyl ester of α-aminoisobutyric acid.

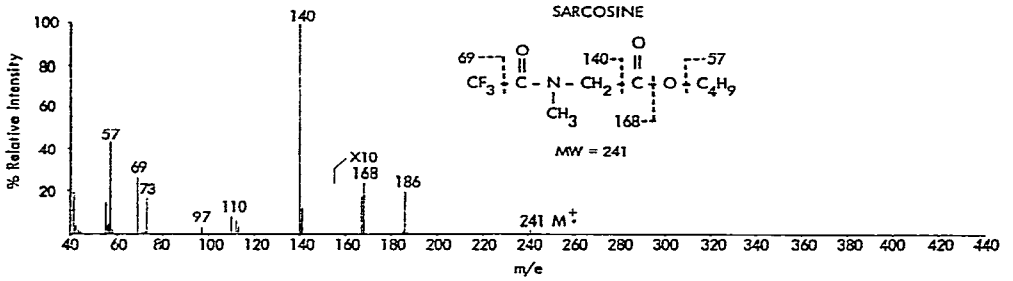


Fig. 9. Mass spectral fragmentation of N-TFA-*n*-butyl ester of sarcosine.

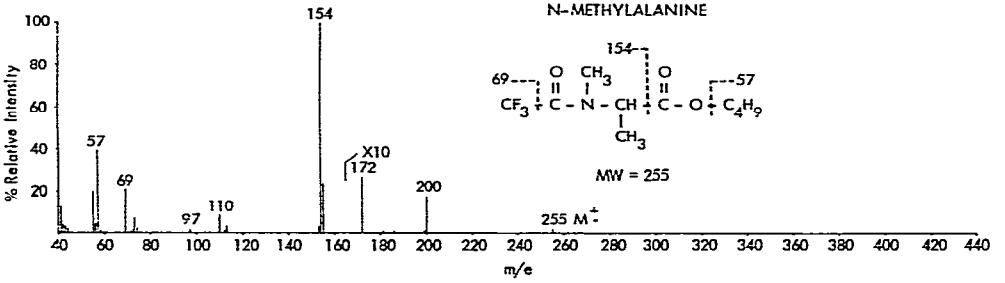


Fig. 10. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methylalanine.

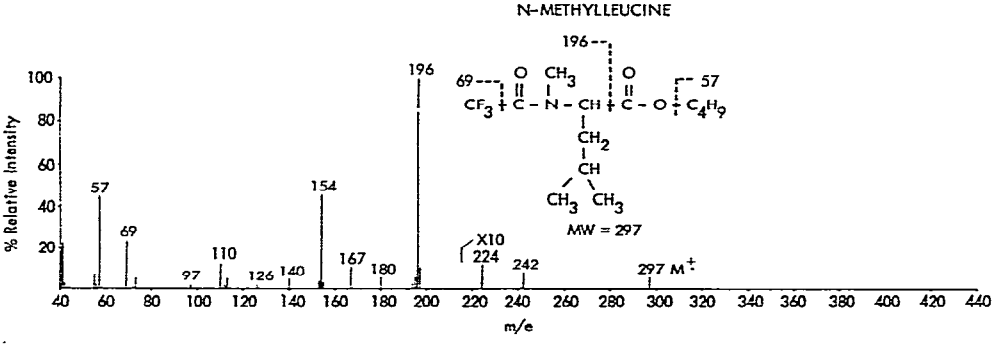


Fig. 11. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methylleucine.

arise from varying abilities of the leucines to yield primary and secondary radicals, and the resonance stabilization of the resulting fragment ions. Since neutral radicals have the following stability order: tertiary > secondary > primary, cleavage for isoleucine is favored between the 2 and 3 carbons while leucine will cleave between the 3 and 4 carbons. These fragmentations occur in conjunction with the elimination of a neutral species. Isoleucine eliminates a butoxy group or butene (mechanism presented below) group in conjunction with the cleavage between the 2 and 3 carbons yielding higher intensities for *m/e* 153 and 171 than do leucine and norleucine. Similarly, elimination of the secondary carbon in conjunction with the loss of a butoxycarbonyl group or butanol is preferred in leucine to yield *m/e* 140 and 166. The *m/e* 140 ion was not reported by Gelpi *et al.*[17], while it was the third strongest ion in our spectrum.

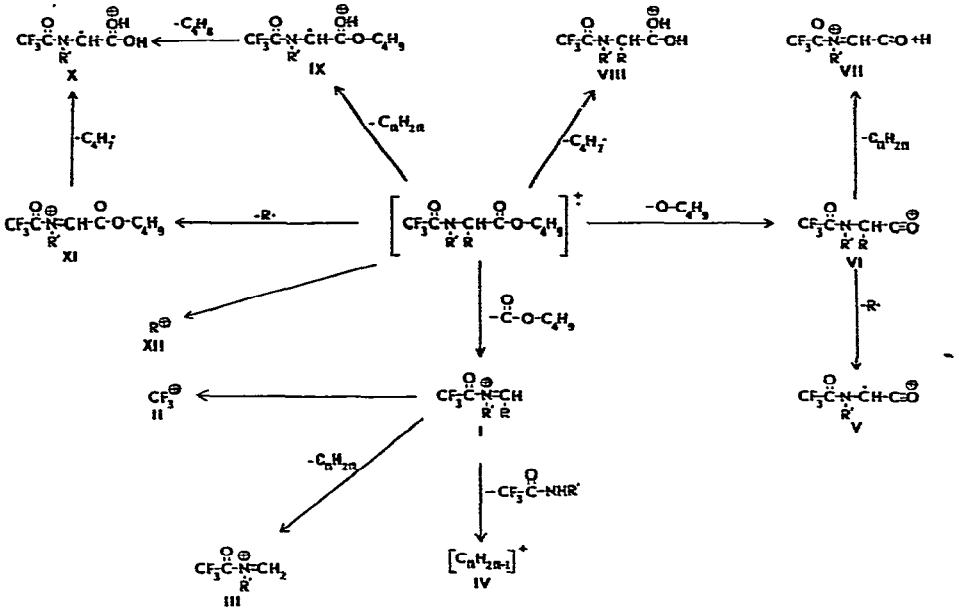
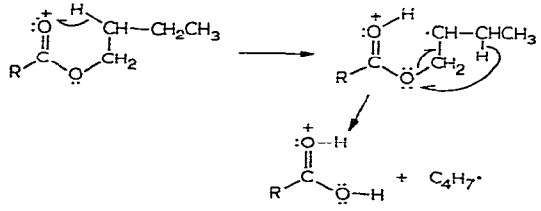


Fig. 12. Mass spectral fragmentation pathways of TAB α-amino acids.

The *m/e* 171, in the leucines, is also formed by α-cleavage to the amide nitrogen. This cleavage was found to be of minor importance by high resolution MS. No major fragments, besides the M—101 (*m/e* 182), were found for norleucine.

The formation of the M—55 (VIII in Fig. 12) is formed by the mechanism suggested by Benz and Biemann[21], which may be depicted as follows:



Whether this fragmentation occurs as a two-step process as depicted above or as a concerted mechanism is open to discussion.

The *m/e* 110, with elemental composition $C_3H_3NF_3$, was characteristic of the N-methylated α- and β-amino acids. The presence of this ion has not been observed in any non-N-methylated amino acid. The origin of this ion is not understood at this time. See sarcosine, N-methylalanine, and N-methylleucine, Figs. 9–11.

Figs. 13–18 present the mass spectra for the TAB derivatives for β-alanine, β-aminobutyric acid, β-aminoisobutyric acid, N-methyl-β-alanine, N-methyl-β-aminobutyric acid, and N-methyl-β-aminoisobutyric acid. Fig. 19 presents fragmentation

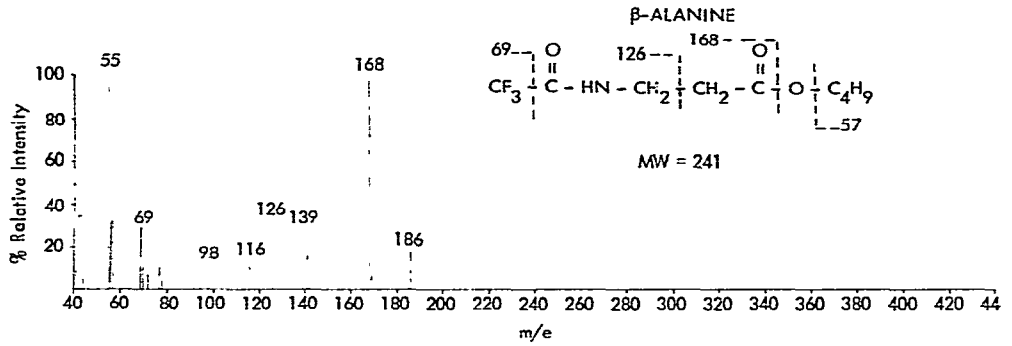


Fig. 13. Mass spectral fragmentation of N-TFA-*n*-butyl ester of *β*-alanine.

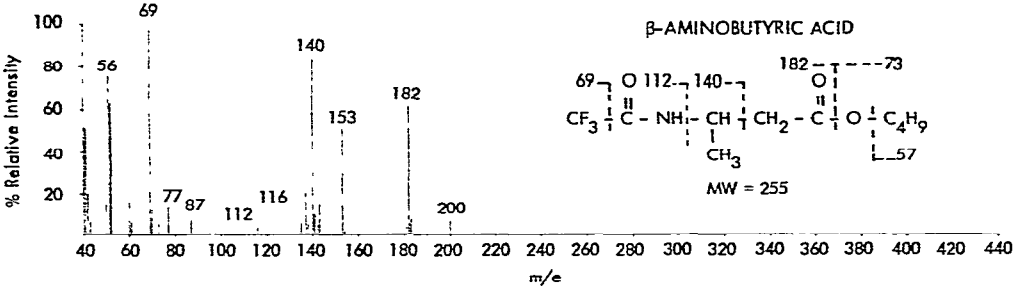


Fig. 14. Mass spectral fragmentation of N-TFA-*n*-butyl ester of *β*-aminobutyric acid.

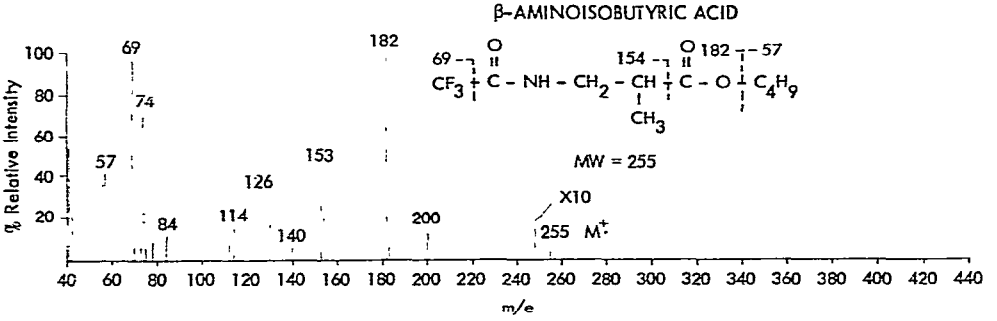


Fig. 15. Mass spectral fragmentation of N-TFA-*n*-butyl ester of *β*-aminoisobutyric acid.

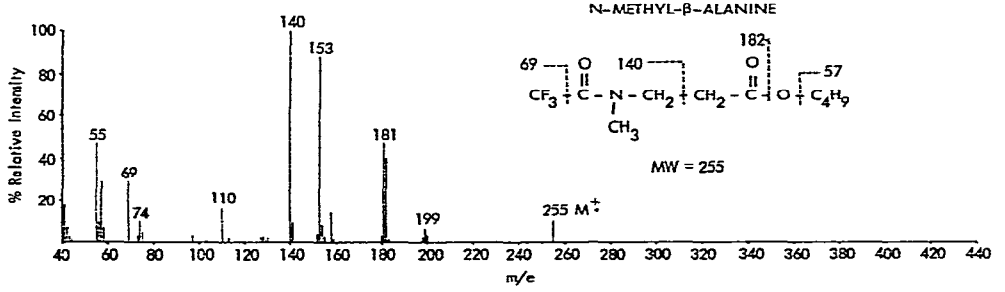


Fig. 16. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methyl-*β*-alanine.

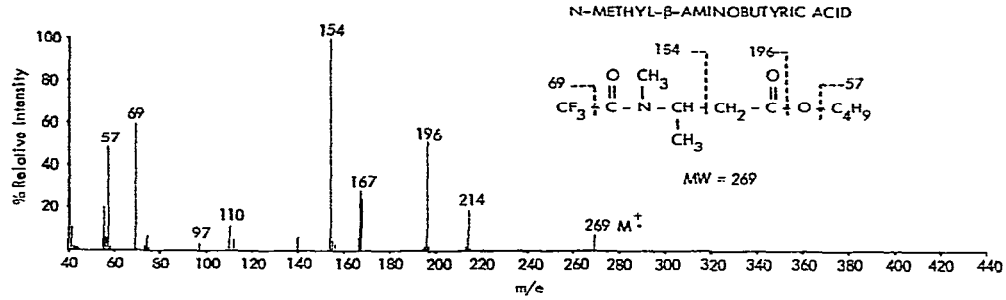


Fig. 17. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methyl-β-aminobutyric acid.

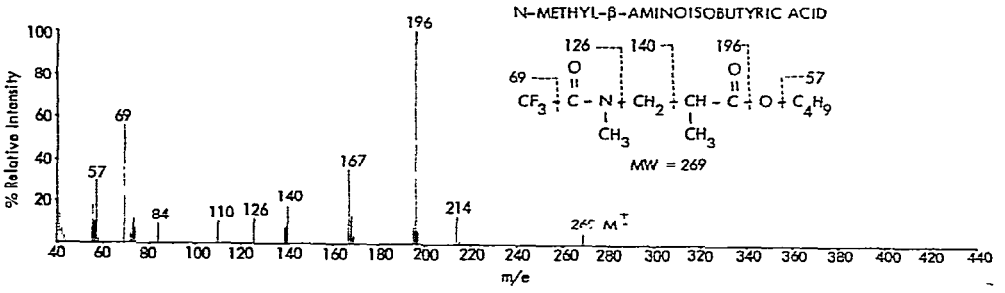


Fig. 18. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methyl-β-aminoisobutyric acid.

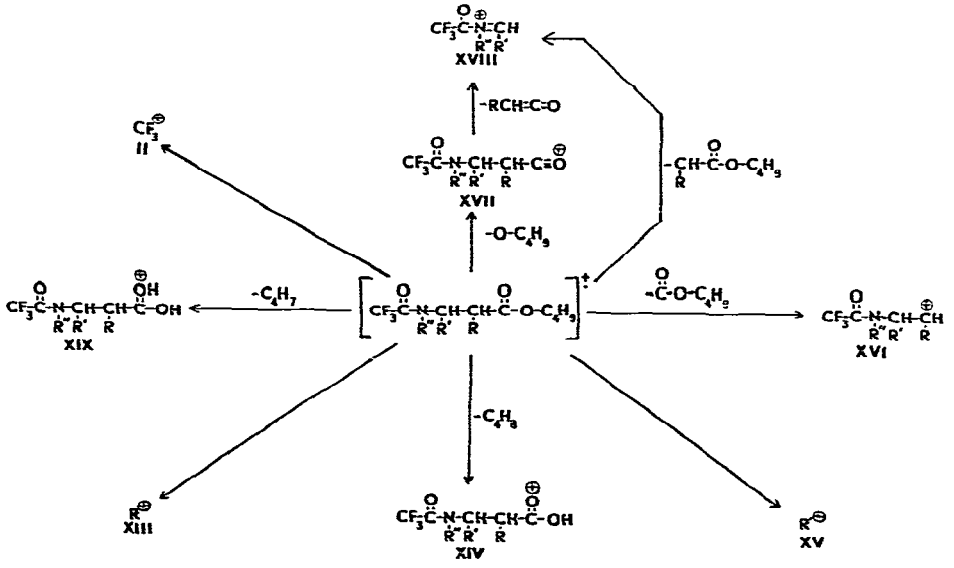


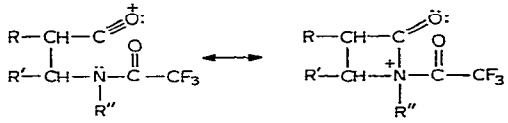
Fig. 19. Mass spectral fragmentation pathways of TAB β-amino acids.

pathways which explain the origin of many of the peaks for the β- and the N-methyl-β-amino acids. Some reasons for these fragmentation pathways will be discussed later.

Upon comparison of the spectra of the α-amino acids with the corresponding β-amino acids, one finds the molecular ion to be more intense in the β-amino acids. This is due to cleavage of the 1,2 bond in the α-amino acids, which is favored by the α-cleavage to a carbonyl group and β-cleavage to the amide nitrogen with charge retention on the amide fragment. The corresponding cleavage in the β-amino acids is not favored due to the additional methylene group between the carbonyl and the amide nitrogen.

The explanation also applies to the large M—101 (structure I in Fig. 12) in most α-amino acids, while this cleavage is of little importance in the β-amino acids. That this cleavage is more favored in the α-amino acids is demonstrated by the large percentage of the total ionization carried by the M—101 fragment. Compare alanine and β-alanine with respect to this cleavage, Figs. 2 and 13. With either α- or β-amino acids, this M—101 ion is further stabilized by substituents on the 2-carbon or on the nitrogen atom. Thus compare glycine with leucine and N-methylleucine, Figs. 1, 4, and 11. The β-amino acids in fact yield a M—102 (loss of butoxycarbonyl and a hydrogen) which is more intense than the M—101. An explanation for the stability of the M—102 in the β-amino acids has been offered by Lawless and Chadha[18], and earlier by Biemann *et al.*[14].

Relative to the α-amino acids, the β-amino acids show a more intense M—73. This ion originates from the loss of the butoxy group from the ester portion of the molecule (XVII in Fig. 19). Thus compare alanine, β-alanine, and N-methyl-β-alanine (Figs. 2, 13, and 16). This fragment ion is not intense in the α-amino acids, but its intensity in the β-amino acids is aided by stabilization of the charge as suggested by Lawless and Chadha[18] and depicted below:



This is a rather characteristic ion for noting the difference of an α- and β-amino acids.

The presence of an alkyl substituent on the nitrogen atom, or carbon atoms 2 or 3 in the β-amino acids increases the stability of ions such as structure XVIII in Fig. 19, resulting from β-cleavage to the amide. This is illustrated by comparing β-alanine with β-aminobutyric acid and N-methyl-β-alanine, Figs. 13, 14, and 16.

Figs. 20 and 21 present the fragmentation pattern for the TAB derivative of 4-aminobutyric acid and 4-N-methylaminobuturic acid. Fig. 22 shows the fragmentation pattern for 6-aminohexanoic acid. These three spectra are insufficient to draw any general conclusions except that the fragmentation patterns for these TAB amino acids are not as simple as for the α- and β-amino acids. Each of these spectra show cleavage β to the amide, α to the carbonyl group, and an M—73 ion (OC_4H_9) as characteristic fragments. These ions are the same characteristic ions as observed for the β-amino acids.

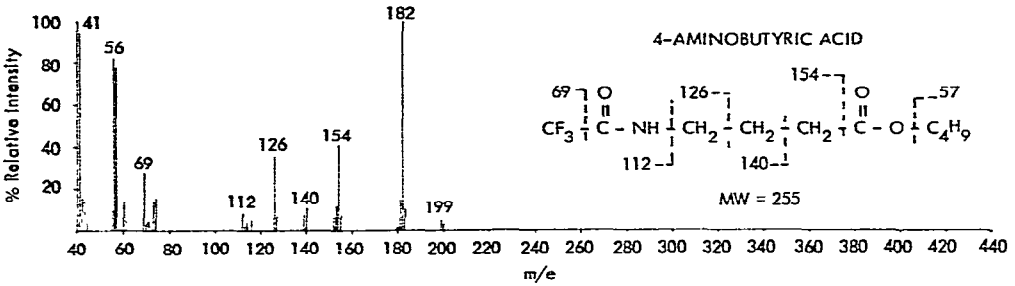


Fig. 20. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 4-aminobutyric acid.

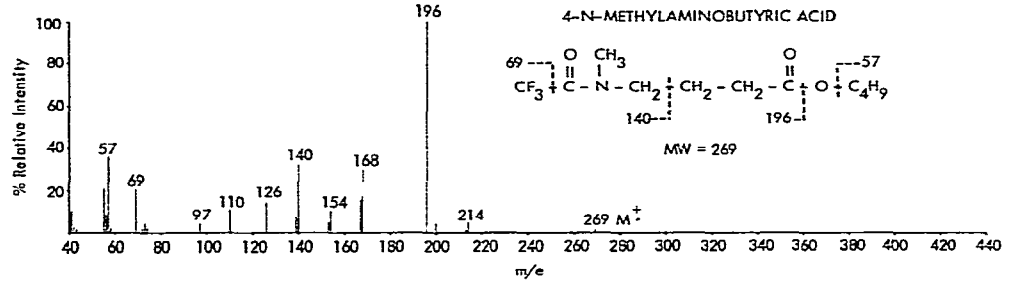


Fig. 21. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 4-N-methylaminobutyric acid.

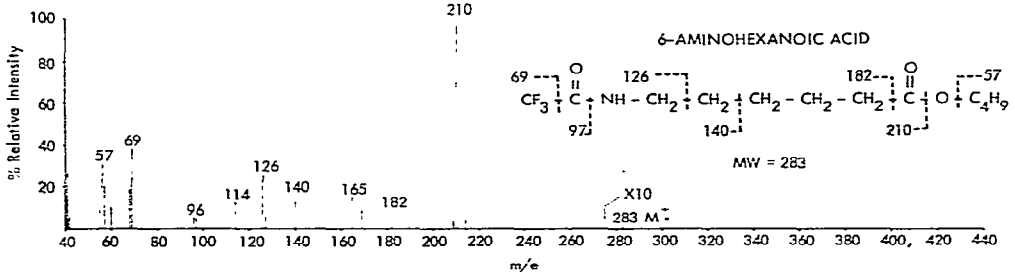


Fig. 22. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 6-aminohexanoic acid.

The 4-aminomethylcyclohexane carboxylic acid spectrum, Fig. 23, shows a very different fragmentation pattern than that observed for the other TAB aliphatic amino acids. The highest *m/e* observed was M—H_2O, which was confirmed by an exact mass measurement. The mechanism of this elimination is unknown. Other unusual ions are M—69 (CF_3) and M—126 (CF_3–CONH–CH_2). Additional characteristic ions were M—55 (C_4H_7), M—73 (OC_4H_9), M—101 ($CO_2C_4H_9$), and M—102 ($CO_2C_4H_9$+H). These fragments are in general the same as those observed for the β-amino acids, 4-aminobutyric acid, and 6-aminohexanoic acid.

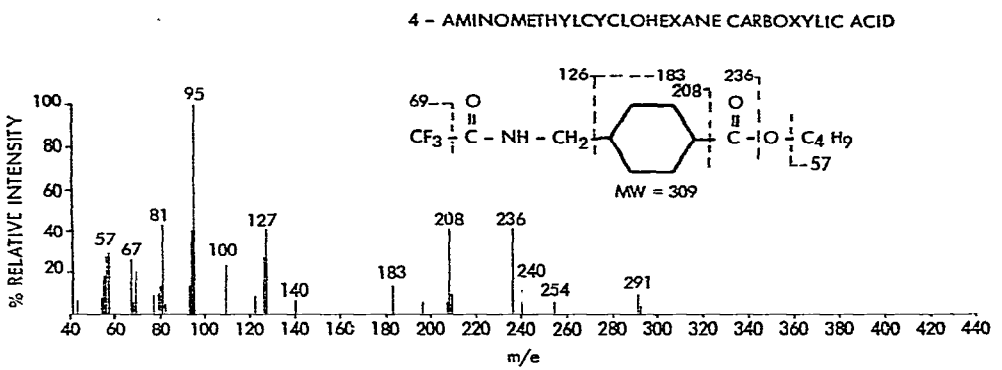


Fig. 23. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 4-aminomethylcyclohexane carboxylic acid.

B. Hydroxy amino acids

Figs. 24, 25, and 26 present the fragmentation patterns for the TAB derivative of serine, threonine, and 6-hydroxy-2-aminohexanoic acid. Our spectra for serine and threonine were in good agreement with those presented by Gelpi *et al.*[17] with the exception of a much more prominent ion for *m/e* 138 and 152, respectively. These intense ions at *m/e* 138 and 152 are also in good agreement with the work of Felker

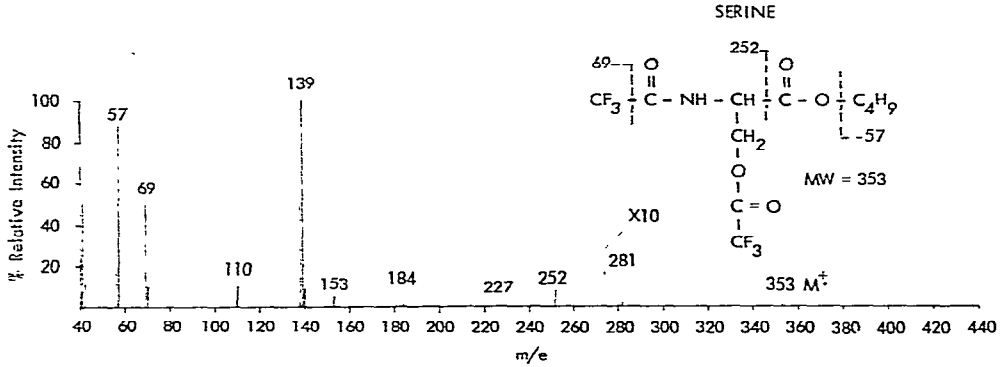


Fig. 24. Mass spectral fragmentation of N,O-di-TFA-*n*-butyl ester of serine.

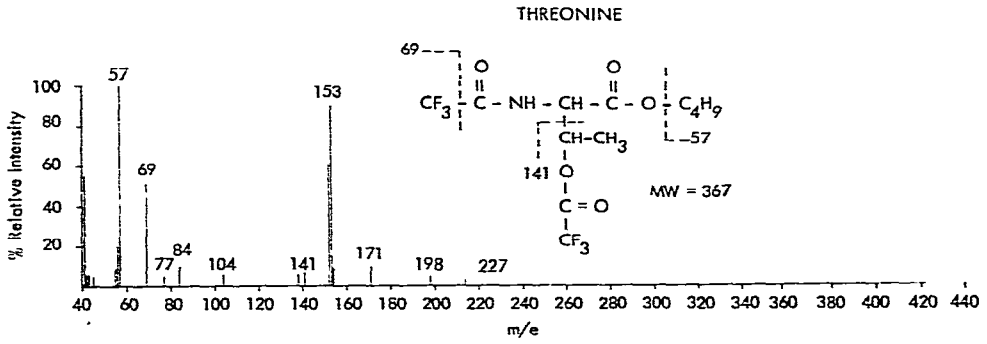


Fig. 25. Mass spectral fragmentation of N,O-di-TFA-*n*-butyl ester of threonine.

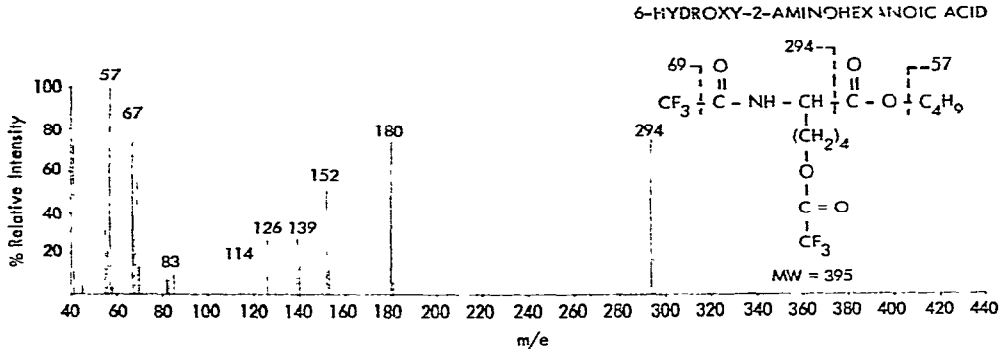


Fig. 26. Mass spectral fragmentation of N,O-di-TFA-*n*-butyl-6-hydroxy-2-aminohexanoic acid.

and Bandurski[19] for the N(O)-perfluorobutyryl-O-isoamyl esters, in which the corresponding ions were found as the base peaks. These ions correspond to the loss of the butoxycarbonyl group plus trifluoroacetic acid. For serine, a M—72 peak (*m/e* 281) is observed rather than an M—73 as reported by Gelpi *et al.*[17]. This ion must originate from a double hydrogen rearrangement from the ester portion of the molecule resulting in a loss of a neutral fragment with the elemental composition of C_4H_8O.

The fragmentation pattern for the TAB derivative of 6-hydroxy-2-aminohexanoic acid differed from that for serine and threonine. The elimination of the butoxycarbonyl group is much more prominent in this molecule (*m/e* 294). This loss plus the elimination of trifluoroacetic acid yields *m/e* 180 as a very diagnostic fragment. This might be explained as resulting from a McLafferty rearrangement involving the long alkyl chain. The McLafferty rearrangement is not possible for serine and threonine. The corresponding fragment for the loss of the butoxycarbonyl plus the trifluoroacetate group is of little prominence in this molecule as compared to serine and threonine.

C. Acidic amino acids

Figs. 27–30 present the mass spectra for the TAB derivative of aspartic acid, glutamic acid, 2-aminoadipic acid, and N-methylglutamic acid. A molecular ion was

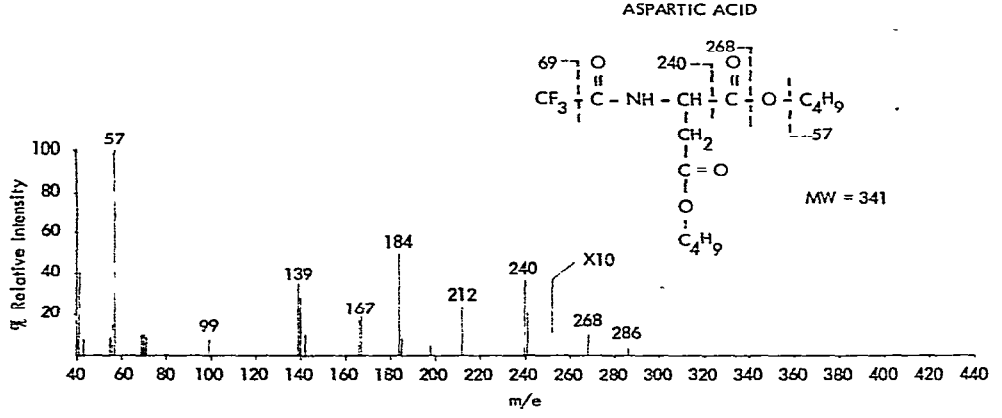


Fig. 27. Mass spectral fragmentation of N-TFA-*n*-butyl ester of aspartic acid.

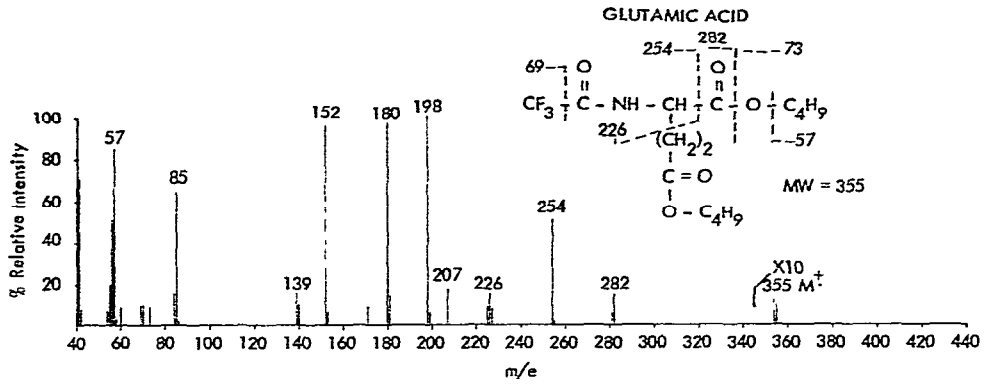


Fig. 28. Mass spectral fragmentation of N-TFA-*n*-butyl ester of glutamic acid.

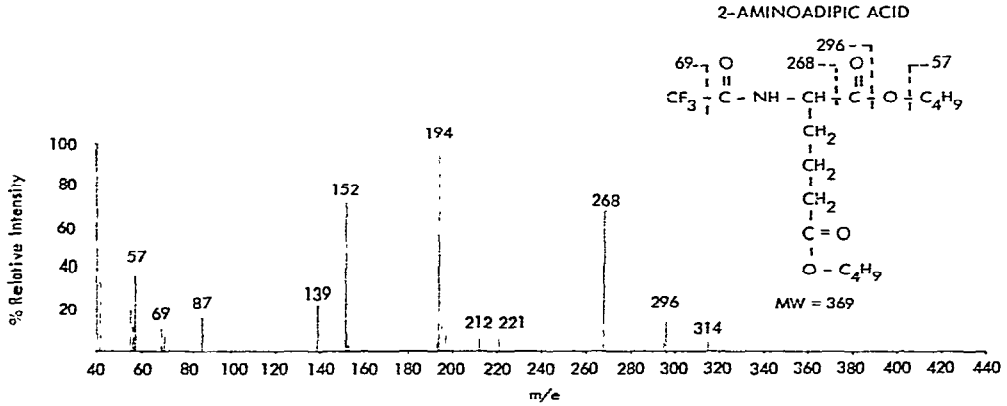


Fig. 29. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 2-aminoadipic acid.

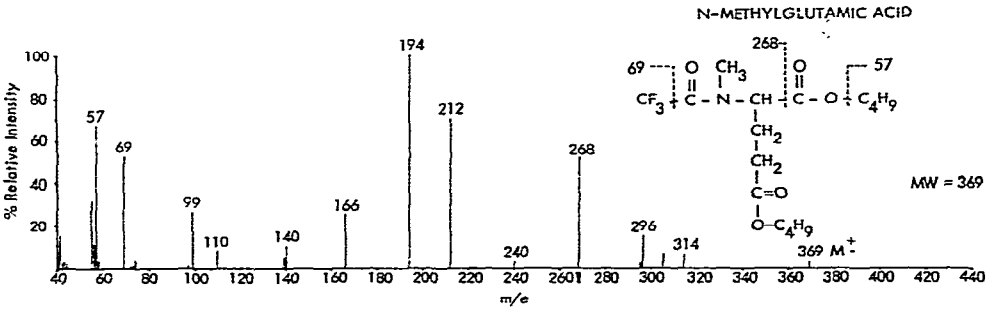


Fig. 30. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methylglutamic acid.

not observed for aspartic acid or 2-aminoadipic acid, while the molecular ion was 2% of the base peak for glutamic acid and 5% for N-methylglutamic acid. As with the α-amino acids, the most characteristic ions are the M—101 or the amide fragment (I in Fig. 12), M—55 (VIII in Fig. 12), M—157 (loss of a butoxycarbonyl group and butene from the ester portion of the molecule), and M—175 (loss of a butoxycarbonyl

group and butanol). Gelpi *et al.*[17] reported *m/e* 116, due to a McLafferty rearrangement, as being the characteristic ion for glutamic acid. This ion or the corresponding ion for N-methylglutamic acid and 2-aminoadipic acid was not observed in this study.

D. Basic aliphatic amino acids

Figs. 31–33 show the mass spectra of the TAB derivative of lysine, ornithine, and ε-N-methyllysine. The spectra for lysine and ornithine are characterized by the molecular ion, M—74 (loss of butanol), M—101 (I in Fig. 12), *m/e* 126 (β-cleavage to the ω-amide nitrogen), and M—214 (loss of a butoxycarbonyl group and trifluoroacetamide). The TAB derivative of ε-N-methyllysine shows the same losses for lysine and ornithine except that the M—214 is displaced by 14 mass units to M—228 showing the methyl group bonded to the ε-amide nitrogen. This loss is characteristic of the N-substituted ω-alkylamide group and permits the location of this substituent. As mentioned previously, the presence of the *m/e* 110 ($C_3H_3NF_3$) also shows the presence of N-methylation. Also present is the β-cleavage to the ε-amide nitrogen yielding *m/e* 140 rather than 126 for lysine and ornithine.

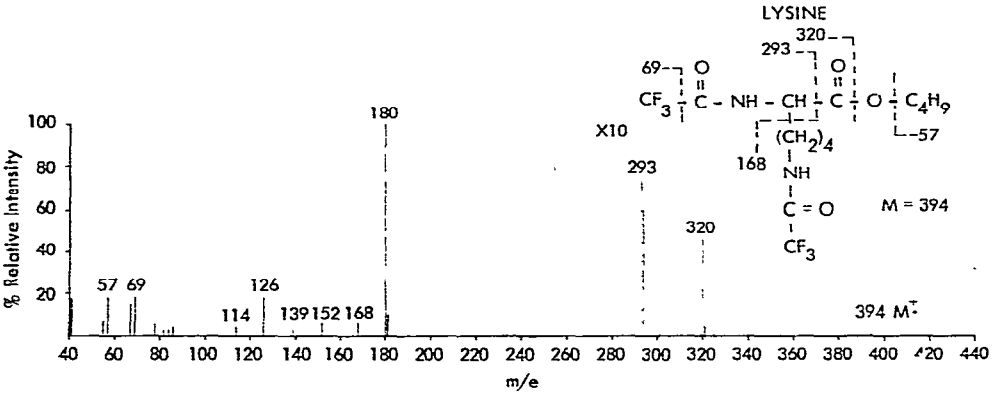


Fig. 31. Mass spectral fragmentation of N,N-di-TFA-*n*-butyl ester of lysine.

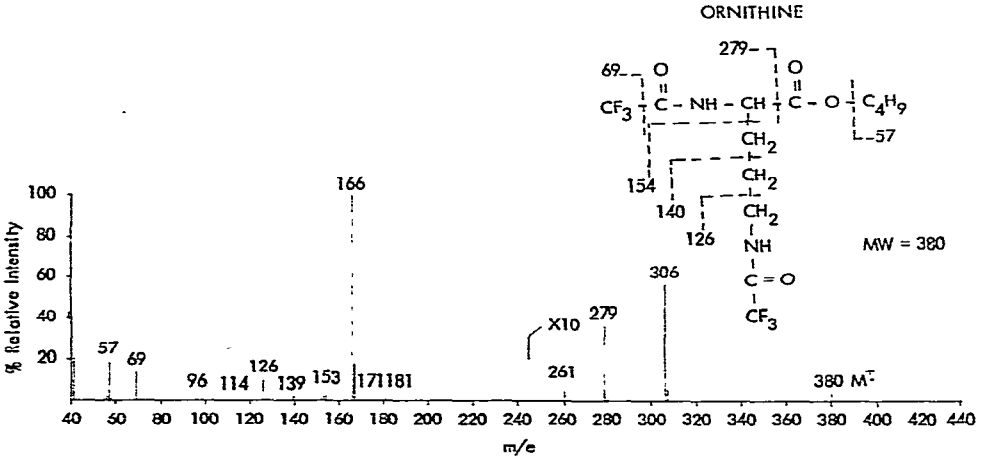


Fig. 32. Mass spectral fragmentation of N,N-di-TFA-*n*-butyl ester of ornithine.

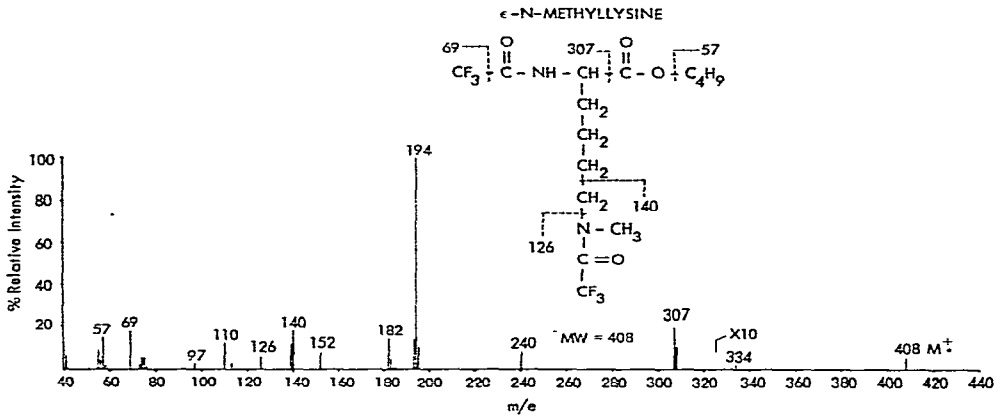


Fig. 33. Mass spectral fragmentation of N-TFA-*n*-butyl ester of ε-N-methyllysine.

E. Sulfur amino acids

Figs. 34–37 present the spectra for the TAB derivatives of methionine, cysteine, cystine, and 1-hydroxy-6-amino-3,4-dithiahexane-6-carboxylic acid.

The spectrum of methionine differs from that previously reported by Gelpi *et al.*[17] in that the same ions have quite different intensities. The *m/e* 227 ($M-CH_2=CHSCH_3$) resulting from a McLafferty rearrangement has been reported as the base peak; however, we found that this ion was only of 40% intensity. The ion *m/e* 61 ($CH_2=S-CH_3$) was observed as the base peak. This ion results from β-cleavage to the sulfur atom.

The cysteine spectrum shown is similar to that presented by Gelpi *et al.*[17]. We found the *m/e* 314 and 239 ion rather than 313 and 240 as they reported. The *m/e* 314 is due to a rearrangement in the formation of the M—55 ion (VIII in Fig. 12), while *m/e* 239 is explained by the loss of trifluorothioacetic acid (CF_3COSH). We did not observe an *m/e* 250 as previously reported. This is probably due to the higher 70 eV potential used in ionization.

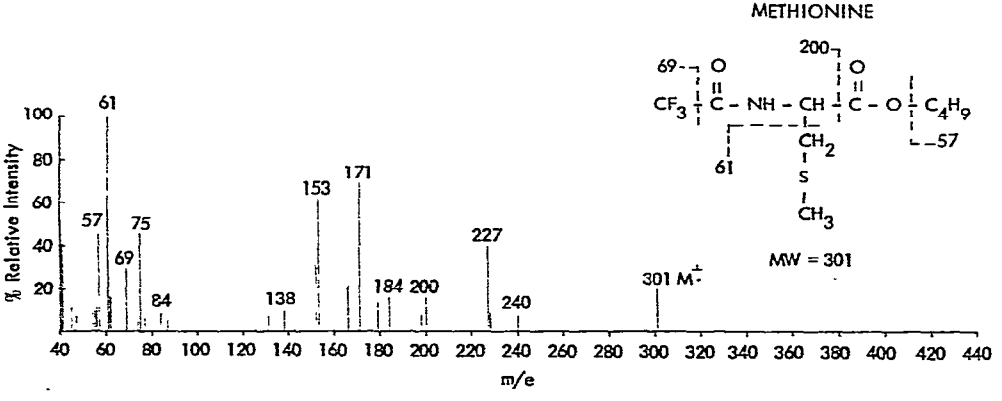


Fig. 34. Mass spectral fragmentation of N-TFA-*n*-butyl ester of methionine.

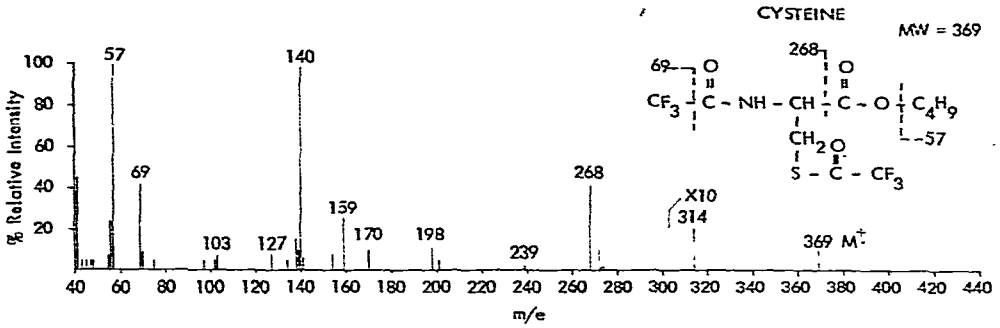


Fig. 35. Mass spectral fragmentation of N,S-di-TFA-*n*-butyl ester of cysteine.

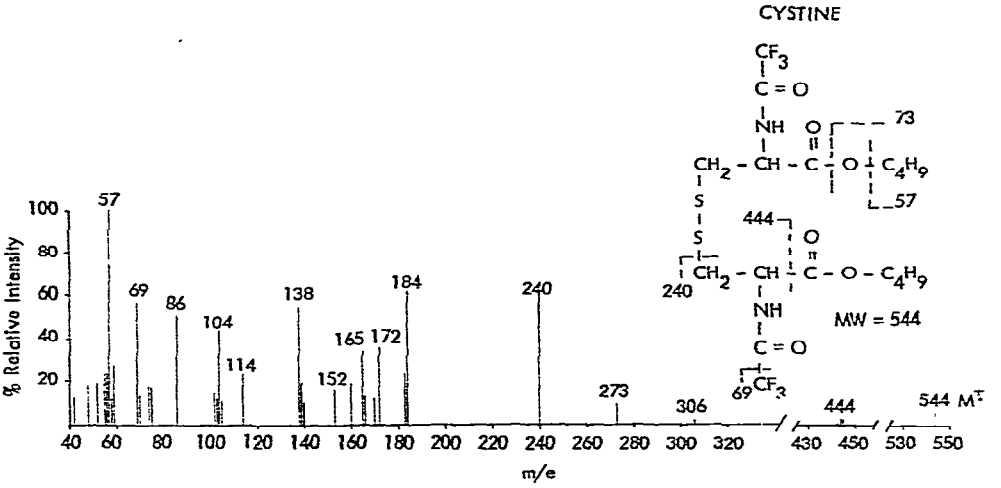


Fig. 36. Mass spectral fragmentation of N,N-di-TFA-*n*-butyl ester of cystine.

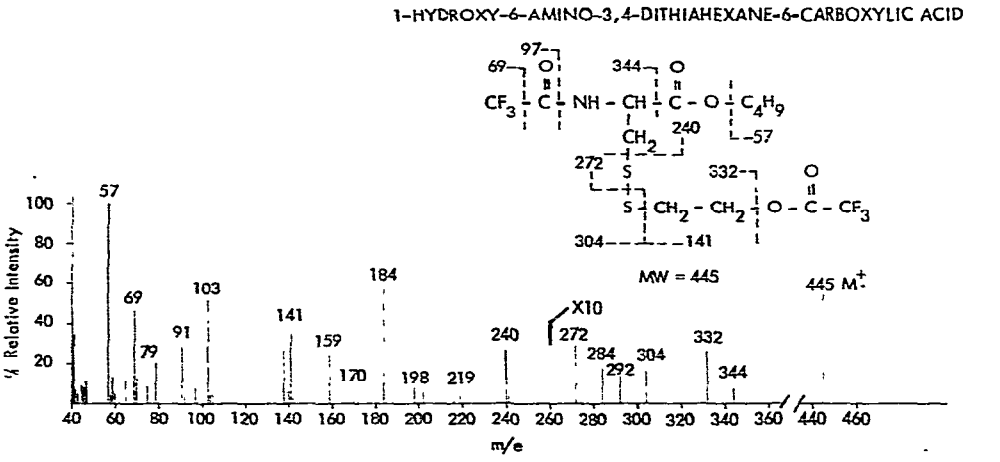


Fig. 37. Mass spectral fragmentation of N,O-di-TFA-*n*-butyl ester of 1-hydroxy-6-amino-3,4-dithiahexane-6-carboxylic acid.

The cystine spectrum is characterized by *m/e* 544 (molecular ion), 443 (loss of a butoxycarbonyl group), and 240 (cleavage of the carbon sulfur bond with charge retention on the carbon fragment). We did not observe *m/e* 272 as previously reported by Gelpi *et al.*[17], while observing *m/e* 273. This fragment must be due to cleavage of the sulfur–sulfur bond and a hydrogen rearrangement.

The 1-hydroxy-6-amino-3,4-dithiahexane-6-carboxylic acid was prepared by the method of Gehrke and Leimer[22]. The mass spectrum of this compound is characterized by the same fragmentations as observed for cystine. These ions are *m/e* 445 (molecular ion), *m/e* 344 (loss of the butoxycarbonyl group), *m/e* 240, 304, 141 (resulting from the cleavage of the carbon–sulfur bonds with charge retention on both fragments), and *m/e* 272 and 173 (resulting from the cleavage of the sulfur–sulfur bond).

F. Aromatic amino acids

Figs. 38–46 present the mass spectra for the TAB derivatives of phenylalanine, tyrosine, O-methyltyrosine, monoacylhistidine, diacylhistidine, tryptophan, 1-methylhistidine, 3-methylhistidine, and N-methylphenylalanine. While the fragmentation of

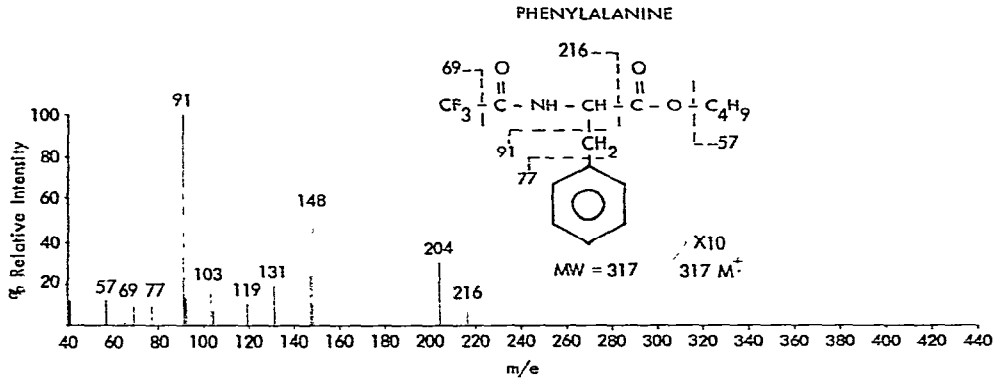


Fig. 38. Mass spectral fragmentation of N-TFA-*n*-butyl ester of phenylalanine.

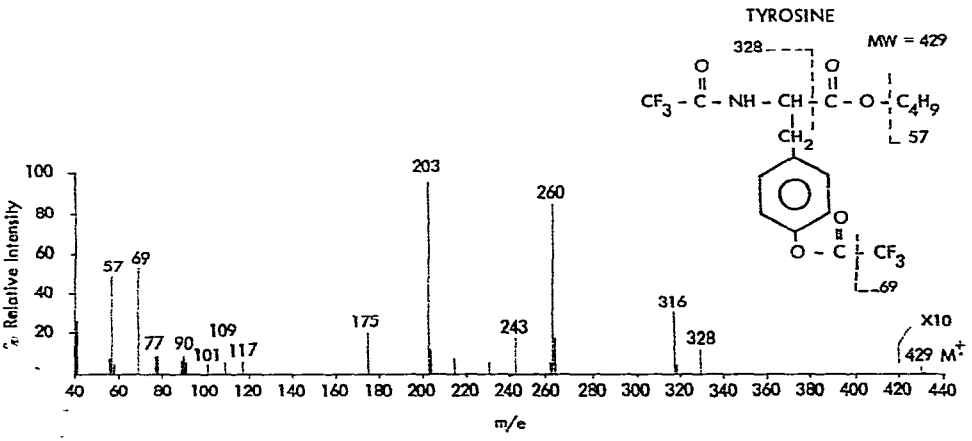


Fig. 39. Mass spectral fragmentation of N,O-di-TFA-*n*-butyl ester of tyrosine.

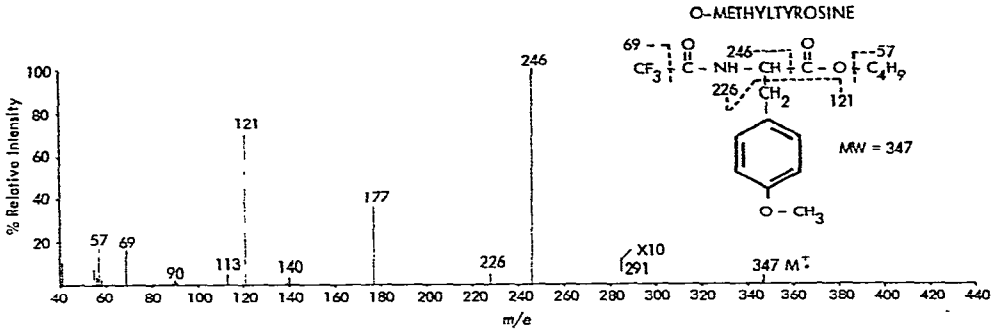


Fig. 40. Mass spectral fragmentation of N-TFA-*n*-butyl ester of O-methyltyrosine.

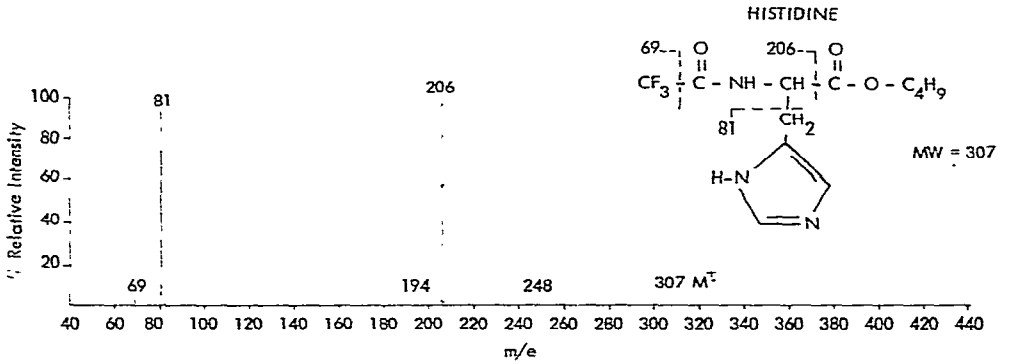


Fig. 41. Mass spectral fragmentation of N^{α}-TFA-*n*-butyl ester of histidine (monacyl).

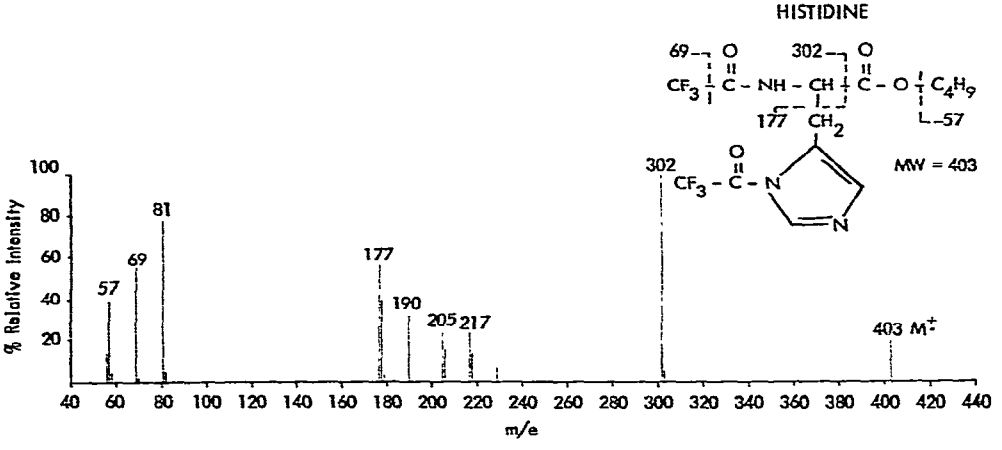


Fig. 42. Mass spectral fragmentation of N,N-di-TFA-*n*-butyl ester of histidine.

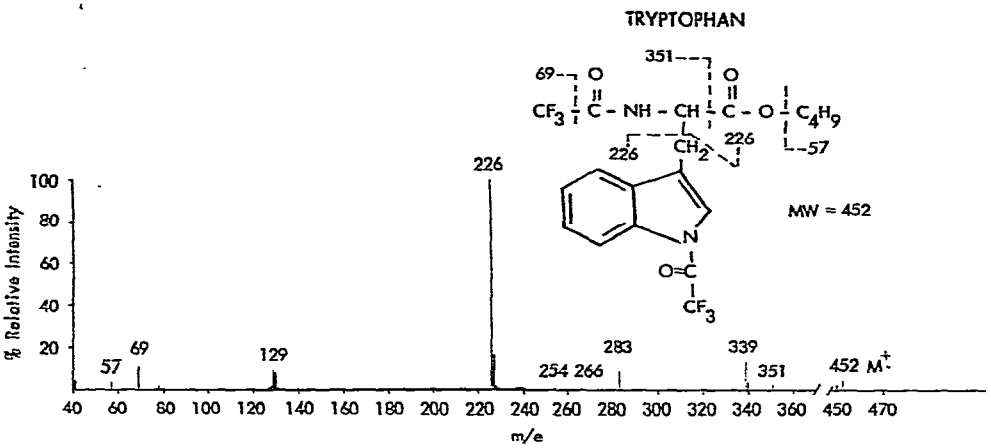


Fig. 43. Mass spectral fragmentation of N,N-di-TFA-*n*-butyl ester of tryptophan.

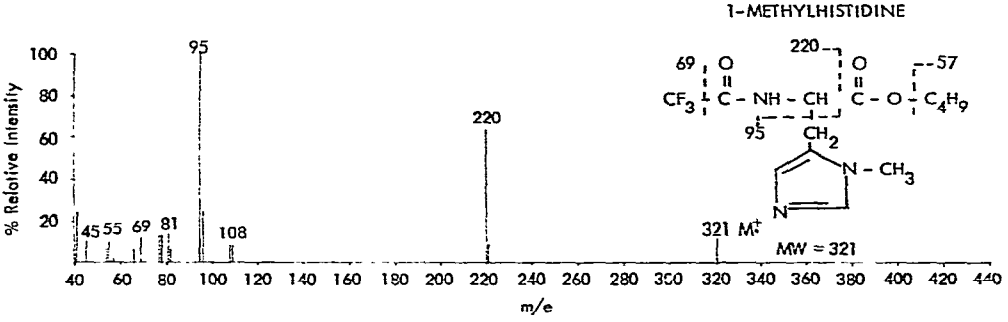


Fig. 44. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 1-methylhistidine.

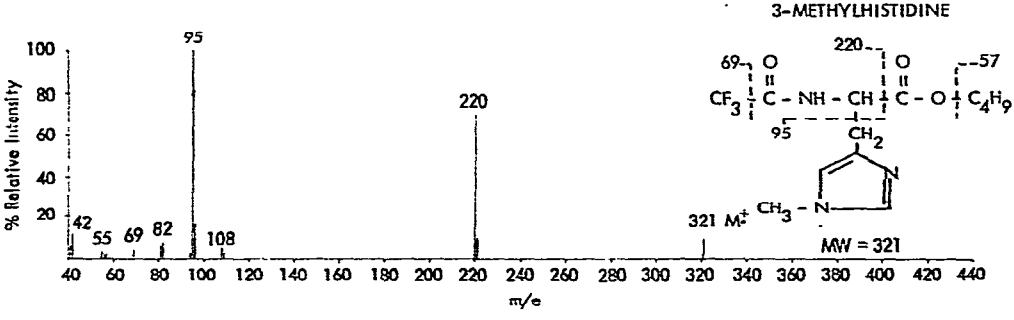


Fig. 45. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 3-methylhistidine.

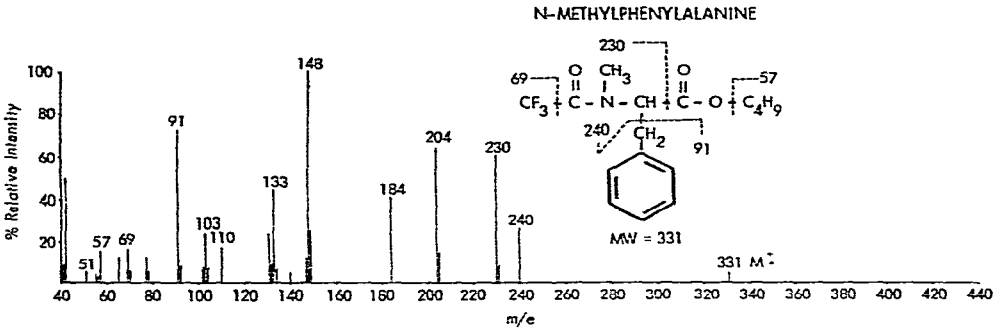


Fig. 46. Mass spectral fragmentation of N-TFA-*n*-butyl ester of N-methylphenylalanine.

the aromatic amino acids are very similar in this study with those of Gelpi *et al.*[17], the relative intensities are considerably different. Tyrosine exemplifies these differences with the *m/e* (intensity) and are reported here as 328 (16), 316 (25), 260 (85), and 203 (100). The analogous relative intensities reported by Gelpi *et al.*[17] are 328 (6), 316 (5), 260 (100), and 203 (54). In this study a much greater charge retention was observed on the aromatic portion of the molecule than has been previously reported. Our data is in general support of the aromatic amino acid fragmentation pathways presented by Gelpi *et al.*[17]. The spectrum of N-methylphenylalanine clearly shows the methyl group to be on the nitrogen due to the presence of the *m/e* 110 ($C_3H_3NF_3$) discussed earlier.

G. Proline and hydroxyproline

Figs. 47 and 48 show the complete mass spectra for the TAB derivatives of proline and hydroxyproline. Our spectrum for proline is dominated by *m/e* 166 (loss of a butoxycarbonyl group) as previously reported and a molecular ion at *m/e* 267. We have not observed ions at *m/e* 193, 194, and 211 as reported by Gelpi *et al.*[17], but we did observe an *m/e* 170 corresponding to the loss of the trifluoroacetyl group.

The spectrum for hydroxyproline is dominated by *m/e* 164, loss of a butoxycarbonyl group and trifluoroacetic acid, as observed for serine and threonine. Other characteristic fragments were observed at *m/e* 278 (M−101) and 379 (molecular ion).

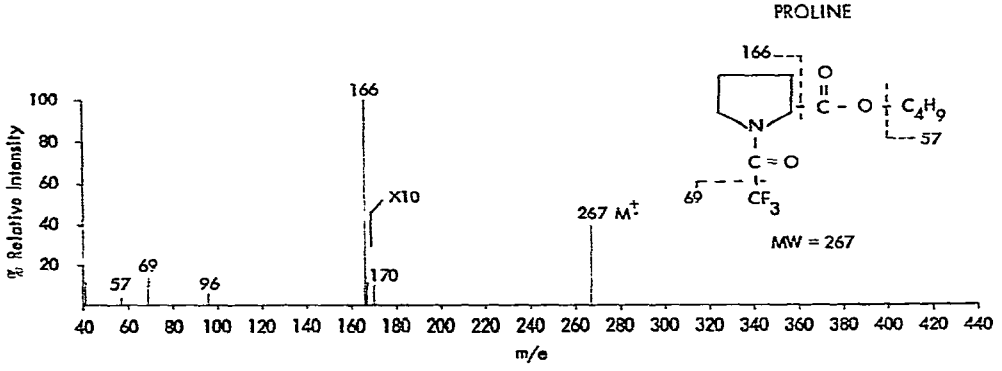


Fig. 47. Mass spectral fragmentation of N-TFA-*n*-butyl ester of proline.

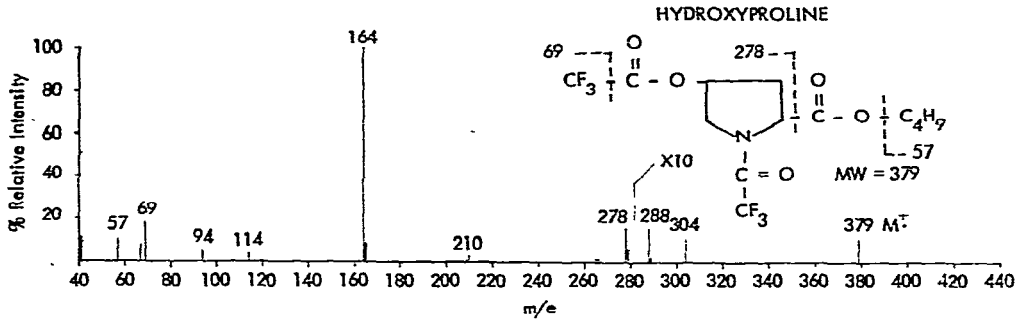


Fig. 48. Mass spectral fragmentation of N,O-di-TFA-*n*-butyl esters of hydroxyproline.

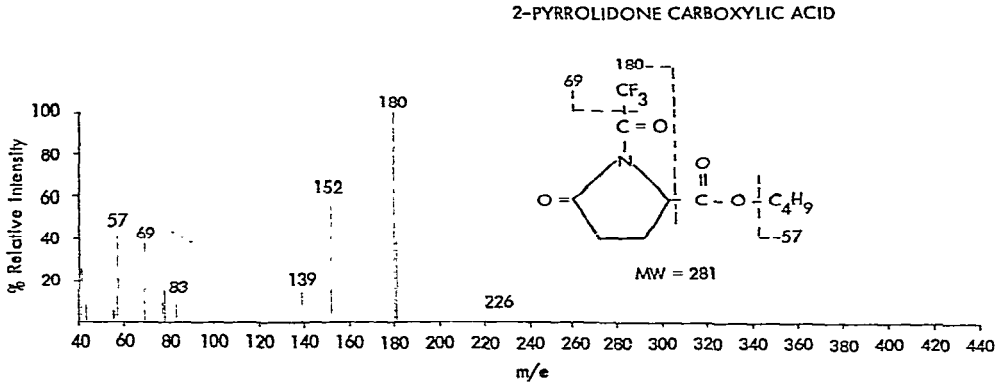


Fig. 49. Mass spectral fragmentation of N-TFA-*n*-butyl ester of 2-pyrrolidone carboxylic acid.

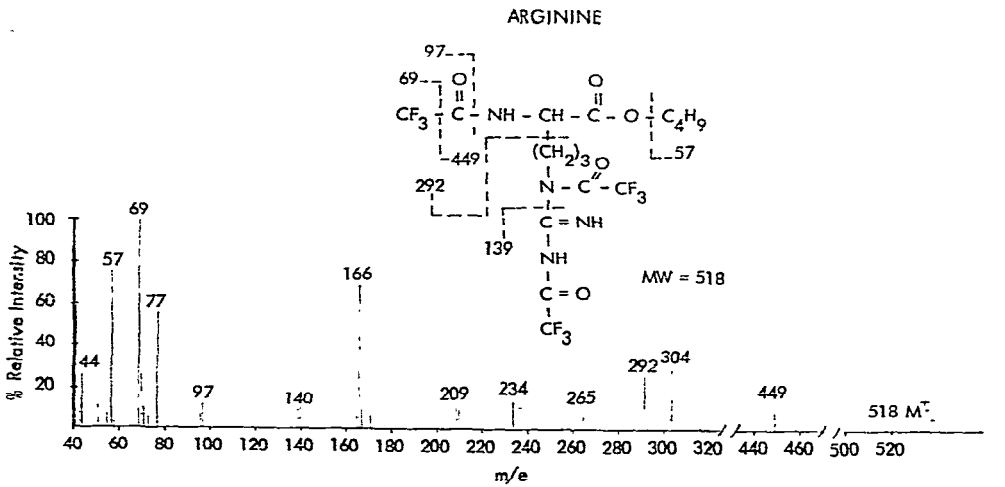


Fig. 50. Mass spectral fragmentation of N,N,N-tri-TFA-*n*-butyl ester of arginine.

H. 2-Pyrrolidone carboxylic acid

Fig. 49 presents the mass spectrum of the TAB derivative of 2-pyrrolidone carboxylic acid. This spectrum is characterized by *m/e* 180 (loss of a butoxycarbonyl group) and *m/e* 152 (loss of CF_3 and C_4H_8O from the ester portion of the molecule).

I. Arginine

Fig. 50 presents the mass spectrum of the TAB derivative for arginine. The spectrum shows the guanido group was diacylated and *m/e* 139 and 292 include the guanido group or a portion of it. This spectrum is characterized by *m/e* 518 (molecular ion), 449 (M—CF_3), and 304 (loss of a butoxycarbonyl group and trifluoroacetamide).

4. CONCLUSIONS

This study presents complete mass spectra and diagnostic criteria for the N-trifluoroacetyl-*n*-butyl ester derivatives of 48 amino acids. The α-amino acids are characterized by simple spectra with the molecular ion, M—55, and M—101 as the most structurally important ions. In contrast, the β-amino acids are characterized by a larger molecular ion, M—73, an M—102 more intense than the M—101, and β-cleavage to the amide group. The *m/e* 110 with elemental composition $C_3H_3NF_3$ is characteristic of the N-methylated amino acids. The presence of this ion has not been observed in any non-N-methylated amino acids.

The addition of other functional groups to the aliphatic α-amino acids such as sulfhydryl, disulfide, carboxyl, guanido, amino, or hydroxyl groups increase the complexity of the resulting mass spectrum. Sulfhydryl and hydroxy groups eliminate thiotrifluoroacetic acid and trifluoroacetic acid with other α-amino acid cleavages. The disulfide group is characterized by C–S and S–S bond cleavage with charge retention on both fragments. The presence of guanido and amino groups result in β-cleavage to these functional groups in the α-amino acids. The introduction of an additional carboxyl group results in 1,2-cleavage as the predominant fragmentation. Aromatic substituents on the α-carbon results in the fragment ions of R and M—R.

This research has delineated the diagnostic criteria for distinguishing the N-trifluoroacetyl-*n*-butyl ester of the α- and β-amino acids from each other, the presence of N-methylation, and other functional groups in the amino acids.

5. ACKNOWLEDGEMENTS

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6. SUMMARY

Complete mass spectra for 48 N-trifluoroacetyl-*n*-butyl esters of amino acids are presented. The spectra are discussed in terms of their common fragmentation pathways and factors controlling the formation of fragments.

The aliphatic α-amino acid TAB derivatives were generally characterized by a molecular ion, M—55 (C_4H_7), and M—101 (butoxy carbonyl). The addition of other functional groups to the aliphatic α-amino acids increases the complexity of the mass spectrum. The hydroxy and sulfhydryl amino acids are characterized by the loss of the butoxycarbonyl group plus trifluoroacetic acid or trifluorothioacetic acid. The dicarboxylic α-amino acids are characterized by a predominant M—101, while the basic amino acids have an additional fragment originating from β-cleavage to the ω-amide group. The sulfur amino acids have additional fragments due to cleavage of the carbon–sulfur and sulfur–sulfur bond with charge retention on either portion of the molecule.

Typical fragments for the aromatic α-amino acids were R, M−101, M−R, and the molecular ion. Proline and hydroxyproline were characterized by M−101, and the molecular ion. Hydroxyproline also gave M−215 as was observed for the hydroxy amino acids. Arginine was characterized by a molecular ion, M−CF_3, M−214 (butoxycarbonyl and trifluoroacetamide) and ions indicating di-trifluoroacetylation of the guanido group.

The β-amino acids gave more complex mass spectra than the α-amino acids, with typical ions being the molecular ion, M−73 (OC_4H_9), M−102, and M−(RCH−$CO_2C_4H_9$). In contrast to the α-amino acids, M−101 was of minor importance although usually present, while M−102 was larger than the M−101 ion. The ion M−73 was often base peak for the β-amino acids while not usually present in the α-amino acids.

Mass spectra of four additional amino acids, 2-pyrrolidone carboxylic acid, 4-aminobutyric acid, 4-N-methylaminobutyric acid, and 6-aminohexanoic acid are presented and discussed; these spectra were more complex than for the α- and β-amino acids.

The *m/e* 110 ($C_3H_3NF_3$) ion was characteristic of the N-methylated amino acids, including α, β, aromatic, and ω-N-methylated amino acids. The presence of this ion has not been observed in any non-N-methylated amino acids.

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