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N-Trifluoroacetyl-L-prolyl Amino Acid *n*-Butyl Ester Derivatives for the Ultramicrodetermination of Amino Acids by Mass Fragmentography

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The fragmentation of 46 kinds of N-trifluoroacetyl-L-prolyl amino acid *n*-butyl esters upon electron impact is investigated for the ultramicrodetermination of amino acids by mass fragmentography. It is found that ion at *m/e* 166 is the common and base peak for most of the amino acid. Characteristic ions for each α-amino acid are observed at *m/e* (M−101) or (R+29). Using mass fragmentography monitored at *m/e* 166, about 10^{-10} g of amino acids can be detected.

Keywords——mass spectrometry; mass chromatography; mass fragmentography; ultramicrodetermination of amino acids; resolution of racemic amine acids

Recent advance in gas chromatography–mass spectrometry combination makes it possible not only to characterize the molecular structure of micro-quantities but to determine the ultra-micro-amounts of substances. The latter is achieved by selective monitoring a characteristic fragment ion of gas chromatographic effluents to be analysed, referred as "mass fragmentography (MF)."

Numerous gas chromatographic investigations for the analysis of amino acids have been done up to the present and a well described review by Hušek and Macek[2)] was published recently. Many mass spectrometric investigations of amino acids as their ethyl esters,[3)] N-trifluoroacetyl (TFA) *n*-butyl esters,[4)] trimethylsilyl derivatives,[5)] and phenylthiohydantoin derivatives[6)] have also been reported.

It is not always true that the most suitable derivative for the gas chromatographic analysis is the most preferred one for the ultramicrodetermination of amino acids by MF. The desirable derivative for MF is the one that has a high intensity of fragment ion commonly appeared for all amino acids upon electron impact and that has a suitable volatility for gas chromatography. Generating a specific ion for a given amino acid is also highly advisable because it can be used for a diagnostic prove for the presence of given amino acids by MF or by mass chromatography (MC).

From the point of view stated above, the authors would like to recommend the N-TFA-L-prolyl amino acid ester derivatives, which have been investigated for the gas chromatographic resolution of racemic amino acids by Halpern and Westley[7)] and by the authors.[8)] The mass spectra and the fragmentation of N-TFA-dipeptide methyl esters were thoroughly studied by Weygand, *et al.*,[9)] but mass spectrometry of N-TFA-L-prolyl amino acid esters was not investigated.

1) Location: *1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
2) P. Hušek and K. Macek, *J. Chromatogr.*, **113**, 139 (1975).
3) K. Biemann, J. Seibl, and F. Gapp, *J. Am. Chem. Soc.*, **83**, 3795 (1961).
4) E. Gelpi, W.A. Koenig, J. Gibert, and J. Orò, *J. Chromatogr. Sci.*, **7**, 604 (1969).
5) K. Bergström, J. Gurtler, and R. Blomstrand, *Anal. Biochem.*, **34**, 74 (1970).
6) R.E. Harman, J.L. Patterson, and W.J.A. VandenHeuvel, *Anal. Biochem.*, **25**, 452 (1968).
7) B. Halpern and J.W. Westley, *Biochem. Biophys. Res. Commun.*, **19**, 361 (1965).
8) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **22**, 8 (1974); H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **22**, 2075 (1974).
9) F. Weygand, A. Prox, W. Koenig and H.H. Fessel, *Angew. Chem.*, **75**, 724 (1963).

The present paper deals with the mass spectral data of 46 kinds of N-TFA-L-prolyl amino acid *n*-butyl esters including α-, β-, γ-, and α-methyl-amino acids, the possibility of ultramicro-determination and the selective identification of amino acids by MF and MC. The present paper also deals with the resolution of racemic amino acids by MF.

Experimental

Reagents and Materials——All solvents used in this study were of reagent grade. Amino acids were obtained from Ajinomoto Co., Tokyo Kasei Co. and Sigma Chemical Co. Trifluoroacetic anhydride, BSTFA with 1% TMCS and hypovials were purchased from Pierce Chemical Co. The OV-101, Diatoport S and Chromosorb W (AW), which were used to prepare the chromatographic column, were obtained from Gas Kuro Kogyo Co., Hewiett-Packard Co. and Supelco. Inc., respectively.

Apparatus and Conditions——A Hitachi RMU-6MG mass spectrometer combined with 002 Datalizer using HITAC-10 computer for an on-line data processing was used. Ionization energy and accelerating voltage were 70 eV and 3000 V, respectively. The ion source temperature was 210°. An all-glass jet-type molecular separator was used for the gas chromatograph and mass spectrometer interface. To avoid the catalytic decomposition on metal surface, a minimum length (15 cm) of gold tubing was used to combine the column with the separator. Two glass columns, 50 cm×3 mm i.d. packed with 1.5% OV-101 on 60—80 mesh Diatoport S and 1 m×3 mm i.d. packed with modified OV-101 on 100—120 mesh Chromosorb W (AW) after thermal and solvent treatment by the method of Aue, *et al.*,[10] were employed. The former column was used for the separation of trimethylsilylated amino acid derivatives and the latter was used for the other amino acids.

Septums and O-rings were heated at 200° for 16 hours in an Abdelhalden apparatus connected to vaccum line with a cold trap (−78°). By this treatment, contaminant peaks from the septums or O-rings was eliminated.

Preparation of Amino Acid Derivatives——N-TFA-L-prolyl amino acid *n*-butyl ester derivatives were prepared by the previously reported method.[8] Hydroxy amino acids and tryptophan, after esterification and coupling with N-TFA-L-prolyl chloride, were trimethylsilylated for the good elution by gas chromatography.

Result and Discussion

Mass Spectra of N-TFA-L-prolyl Amino Acid *n*-Butyl Esters

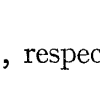
The *m/e* values, the relative intensities (in %) of the molecular ions and the first to tenth intense ions for each amino acid derivative are presented in Table I. For the base peaks the intensities were expressed by the relative abundances of the total ionization.

α-Amino Acids

The most intense ion of α-amino acid derivatives appears at *m/e* 166 with the exception of proline, pipecolic acid and trimethylsilylated amino acid derivatives other than serine and hydroxyproline derivatives. This ion must be araised from N-TFA-L-prolyl group, and the structure of this may be considered as follows;

m/e 166

Chart 1

The base peaks of proline and pipecolic acid derivatives exist at *m/e* 70  and *m/e* 84  , respectively. The relative intensity of the ion at *m/e* 166 from proline and pipecolic acid derivatives are 61.6 and 51.3%, respectively. For all the amino acid derivatives studied here the ion at *m/e* 166 exists at least within first to fourth intensity.

Characteristic ions for each amino acid derivative are generally present at *m/e* (M−101), (M−$COOC_4H_9$) and *m/e* (R+29), (amine fragment ion; R−$CH{=}NH_2^+$). Among natural com-

10) W.A. Aue, C.R. Hasting, and S. Kapila, *J. Chromatogr.*, **77**, 299 (1973).

TABLE I. Ten Peaks of Mass Spectra of N-TFA-L-prolyl Amino Acid *n*-Butyl Esters

Amino acid	M^+		Base peak		2nd peak		3rd peak		4th peak		5th peak		6th peak		7th peak		8th peak		9th peak		10th peak	
	m/e	%[a]	*m/e*	$\sum_{10}$%[b]	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Gly	324	6.5	166	27.8	167	34.6	41	31.1	29	23.7	69	17.7	27	14.1	139	11.9	39	11.9	96	9.9	57	9.7
Ala	338	4.2	166	24.4	167	61.1	41	21.6	237 M−101	18.3	29	18.0	194	17.3	139	16.1	28	15.9	44 R+29	14.5	69	11.8
α-Aba	352	3.5	166	21.6	167	67.2	58 R+29	31.9	41	25.8	251 M−101	24.4	29	21.0	139	16.4	194	14.6	69	13.1	28	11.7
Nval	366	2.9	166	19.6	167	68.8	72 R+29	41.3	28	39.8	265 M−101	29.9	29	26.3	41	25.1	139	17.2	194	13.0	70	11.6
Val	366	1.7	166	18.8	167	63.0	28	43.6	72 R+29	41.7	265 M−101	40.6	41	27.6	29	21.5	139	15.5	89	13.3	70	13.3
Nleu	380	3.4	166	19.1	167	76.0	86 R+29	47.1	279 M−101	34.8	41	28.9	29	24.7	139	15.8	28	14.9	194	13.2	69	12.3
Leu	380	1.1	166	18.9	167	69.6	86 R+29	50.2	279 M−101	37.6	41	31.1	28	19.7	29	18.4	43	15.8	139	14.3	194	14.2
Ile	380	1.9	166	17.0	167	60.0	279 M−101	42.8	41	37.5	86 R+29	36.6	29	30.9	28	23.2	69	17.1	194	14.9	57	14.5
tert-Leu	380	—	166	17.3	250	41.8	41	39.2	167	37.9	28	37.4	86 R+29	35.3	29	30.0	279 M−101	25.7	57	24.0	324	20.6
ω-Aca	408	1.3	166	20.6	167	67.2	114 R+29	39.0	41	30.5	307 M−101	28.0	29	21.8	43	19.5	139	13.2	70	12.3	55	11.9
Pro	364	—	70 R+28	22.8	28	86.6	166	61.6	41	23.6	170 M−194	19.3	114	17.5	263 M−101	15.9	29	14.7	69	13.4	27	10.0
Pip	378	—	84 R+28	24.0	166	51.3	128	32.0	277 M−101	19.6	41	18.7	28	16.7	29	15.2	184 M−194	13.4	55	13.2	69	9.9
PG	400	—	166	23.5	106 R+29	61.5	299 M−101	59.8	28	39.4	167	34.4	41	21.6	29	20.6	69	14.1	104	11.1	300	11.6
Phe	414	—	166	18.3	148	72.8	28	65.0	204	43.8	167	22.2	91	21.2	41	18.7	149	15.8	120 R+29	15.1	29	13.1
Asp	438	5.6	166	22.1	167	70.3	29	28.7	41	25.6	57	22.3	28	14.1	194	12.9	144 R+29	12.8	27	12.0	70	11.3
Glu	452	—	166	18.9	167	58.5	29	31.8	41	29.3	28	27.9	84	23.5	57	19.7	194	14.9	56	14.8	158 R+29	13.0
Met	398	5.0	166	18.7	28	50.4	250 S	30.1	61	29.8	41	27.6	324	23.7	29	22.8	167	20.8	263	17.9	194	11.3
Met(O)	414	—	166	19.9	41	30.0	61	27.5	250 S	25.1	29	20.7	324	19.9	167	17.9	263	15.7	56	15.2	69	13.6
Met(O_2)	430	2.8	166	23.1	167	63.6	41	33.4	29	32.6	56	28.3	57	15.3	69	14.0	139	12.7	28	10.6	250 S	9.7
Eth	412	2.7	166	17.8	250 S	34.4	75	33.9	29	27.6	41	26.3	324	24.9	167	20.9	263	17.8	28	16.9	56	15.1
CySH	370	1.7	166	25.3	167	28.3	41	25.0	69	17.5	29	13.8	57	15.3	69	14.0	139	12.7	28	10.6	53	7.2
Pen	398	—	166	17.8	250 S	42.4	29	35.5	41	33.0	28	29.5	167	24.0	324	17.0	70	15.9	55	15.0	73	13.3
Orn	574	4.5	166	20.6	70	75.4	114	28.8	263	25.8	41	20.4	167	19.9	139	10.8	71	10.4	194	10.0	408 M−166	9.3
Lys	588	3.3	166	20.9	84	42.0	70	39.8	167	19.6	277	18.9	41	18.0	128	17.8	139	10.1	114	9.9	96	9.4
Ser	426	—	166	23.3	73 TMS	45.9	41	29.8	29	21.2	167	18.3	69	13.9	27	11.3	96	9.6	57	6.5	53	7.5
Thr	440	—	117 R	14.6	166	98.4	73 TMS	88.7	41	34.2	396	28.8	29	28.4	75	25.9	167	15.1	69	14.7	57	12.2
Hypro	452	—	166	15.2	158 R+28	71.2	68	39.6	41	37.3	29	34.6	73 TMS	33.0	258 M−194	28.4	57	25.0	75	20.9	69	17.6
Tyr	502	—	179 R	21.8	73 TMS	54.3	292	51.3	166	39.8	180	16.4	41	16.1	29	14.0	293	12.1	237	11.3	69	6.9
Trp	525	—	202 R	27.1	73 TMS	55.3	166	36.8	130	35.4	203	19.1	41	10.6	29	9.3	69	6.0	74	5.4	244	5.3
DOPA	590	5.1	73 TMS	17.2	267 R	84.0	166	69.4	380	50.0	179	22.9	268	21.2	41	17.7	29	13.8	45	9.8	269	8.7
β-Ala	338	4.7	166	19.0	167	90.6	28	30.8	41	30.0	139	21.9	29	21.6	55	15.5	69	14.2	27	12.2	98	12.2
β-Aba	352	1.8	167	25.8	166	56.6	41	26.3	139	20.5	69	18.3	29	15.2	42	12.5	70	11.6	44	9.2	27	8.8
β-Aiba	352	4.3	167	19.7	166	84.0	41	34.9	69	25.7	139	21.6	29	19.1	28	18.2	42	12.9	27	11.4	57	10.1
γ-Aba	352	3.3	167	18.5	166	92.2	41	37.4	139	23.9	86	17.7	28	17.4	29	17.1	69	16.9	112	14.6	27	12.6
AMCHA	406	1.4	167	22.1	166	74.7	41	23.5	139	18.1	95	17.6	29	14.0	70	10.2	94	9.0	28	8.7	67	8.3
MeAla	352	—	58 R+43	13.1	167	89.2	166	73.4	41	60.7	29	43.3	42	40.7	251 M−101	33.5	27	26.8	57	26.7	69	25.8
MeAba	366	—	167	16.0	166	92.1	72 R+43	84.2	41	35.5	29	35.1	265 M−101	34.6	139	23.6	42	20.1	69	17.2	57	14.6
MeVal	380	—	166	15.9	86 R+43	72.5	167	62.4	279 M−101	42.6	41	35.0	42	26.3	70	24.5	139	17.0	43	16.0	69	15.6
MeLeu	394	—	167	14.7	166	86.8	100 R+43	86.5	293 M−101	41.6	241	40.8	57	21.4	29	21.3	139	20.2	42	18.8	43	17.8
MeIle	394	—	166	13.8	100 R+43	67.4	167	56.8	293 M−101	38.7	41	37.2	29	30.0	42	23.5	27	22.8	139	21.9	144	21.8
MeMet	412	—	166	11.8	264 S	74.1	144	63.9	61	54.1	41	44.5	338	40.6	167	40.2	70	39.2	118 R+43	26.2	29	21.1
MeGlu	466	1.1	166	14.3	167	67.7	98	54.4	41	42.4	172 R+43	39.1	29	26.1	365 M−101	22.2	70	19.5	99	19.4	57	18.5
MeOrn	588	1.0	166	18.2	84	73.3	277	41.2	167	23.5	41	22.3	128	21.9	70	18.7	139	11.8	422	11.2	69	10.2
MeTyr	516	—	179 R	24.0	166	48.0	73 TMS	44.3	306	30.2	180	18.9	41	15.5	29	13.1	308	8.1	69	7.0	42	6.8
MeTrp	539	—	202 R	22.5	73 TMS	88.1	42	29.8	203	24.3	29	22.5	130	18.0	41	14.2	45	13.7	57	11.1	74	10.7
MeDOPA	604	5.6	267 R	22.4	73 TMS	71.4	166	52.6	268	25.6	394	21.5	179	17.9	41	11.9	29	11.7	269	9.7	395	7.5

a) relative intensity (base peak=100)
b) per cent of the total ionization over *m/e* 10
abbreviations; S, characteristic peaks for sulfur-containing amino acids; R, ions corresponding to amino acid side chain; α-Aba, α-amino-*n*-butyric acid, Nval, norvaline; Nleu, norleucine; ω-Aca, ω-amino-*n*-caprylic acid; Pip, pipecolic acid; PG, phenylglycine; Met(O), methionine sulfoxide; Met(O_2), methionine sulfone; Eth, ethionine; Pen, penicillamine; β-Ala, β-alanine; β-Aba, β-amino-*n*-butyric acid; β-Aiba, β-amino-isobutyric acid; γ-Aba, γ-amino-*n*-butyric acid; AMCHA, *trans*-4-(aminomethyl)cyclohexane carboxylic acid; Me, α-methyl-

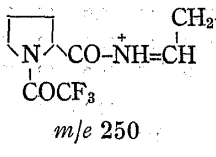
mon amino acid derivatives, however, glycine, cysteine, serine, tyrosine, tryptophan and lysine, do not produce these ions with the intensity larger than 2%. For the cyclic amino acid derivatives (proline, pipecolic acid and hydroxyproline), the ions at *m/e* (M−194), (N-TFA-L-prolyl group) which are not observed for the other amino acid derivatives exist at *m/e* 170 (19.3%), 184 (13.4%) and 258 (28.4%), respectively.

The characteristic ions for aromatic amino acid derivatives (phenylalanine, tyrosine, tryptophan, 3,4-dihydroxyphenylalanine (DOPA), α-methyl-tyrosine, α-methyl-tryptophan and α-methyl-DOPA) are listed in Table II.

Table II. *m/e* Value and Relative Intensity of Characteristic Ions for Aromatic Amino Acids

Ions	Phe	Tyr[a]	MeTyr[a]	DOPA[a]	MePODA[a]	Trp[a]	MeTrp[a]
Ar–CH=CX–$COOC_4H_9$[b]	204 (43.8)[c]	292 (51.3)	306 (30.2)	380 (50.0)	394 (21.5)	315 (4.4)	329 (2.4)
Ar–CH=CX–CO	131 (8.3)	219 (2.9)	233 (—)	307 (—)	311 (—)	242 (—)	256 (—)
Ar–CH_2	91 (21.2)	179 (100.0)	179 (100.0)	267 (84.0)	267 (100.0)	202 (100.0)	202 (100.0)

a) trimethylsilylated
b) α-amino acids: X=H, α-methyl-amino acids: X=CH_3
c) relative intensity, in %



m/e 250

(methionine; 30.1%, methionine sulfoxide; 25.1%, methionine sulfone; 9.7%, ethionine; 34.4%, penicillamine; 42.4%)

Chart 2

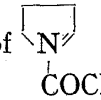
In trimethylsilylated derivatives of threonine, tyrosine, tryptophan, α-methyl-tyrosine, α-methyl-tryptophan and α-methyl-DOPA, the ions having the structures of trimethylsilylated side chain appear as the base peaks. Trimethylsilyl ion (*m/e* 73, $(CH_3)_3Si^+$) is also common intense ion for the trimethylsilylated amino acid derivatives.

Sulfur-containing amino acid derivatives do not generate the ions at (R+29) or (M−101) with relatively large intensities but generate common intense ion at *m/e* 250 except for cysteine. The structure of the ion is assumed to be as follows;

Glutamic acid derivative, though it has the common structure as illustrated above, does not produce this ion.

The acidic amino acid derivatives produce the base peak at *m/e* 166, and the fragmentation feature are similar to that of neutral amino acid derivatives. In addition, glutamic acid derivative generates the characteristic ions at *m/e* 296 (13.9%), 186 (9.3%) and 84 (23.5%).

General fragmentation pattern for α-amino acid derivatives are illustrated in Chart 3.

Basic amino acid derivatives, ornithine and lysine, produce M−166 ions (*m/e* 408 and 422, respectively) which are formed by the elimination of  radical from the molecular ions. These basic amino acid derivatives produce the characteristic ions at *m/e* 70, 114, 263, and *m/e* 84, 128, 277, respectively, with the relative intensities of about 20—75%. The fragmentation route of basic amino acid derivatives can be described as showed in Chart 4.

The base peak of these amino acid derivatives also exists at *m/e* 166.

The mass chromatogram of six amino acid derivatives, alanine, valine, leucine, proline, aspartic acid and glutamic acid, are shown in Fig. 1, in which the monitoring ions are selected at *m/e* 166 for the concurrent detection of the six amino acid derivatives at *m/e* (M−101), (R+28) and (R+29) for the selective identification of the amino acid derivatives.

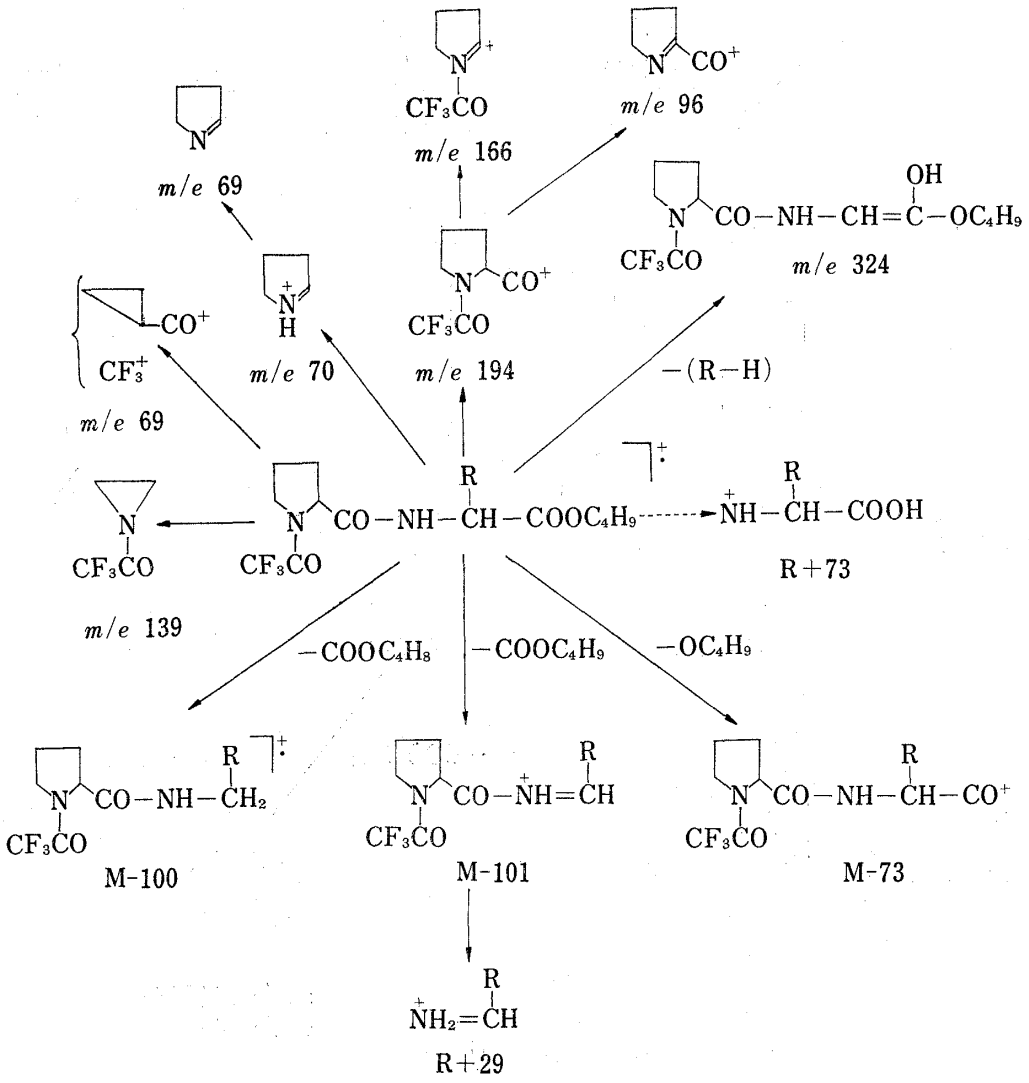


Chart 3. General Fragmentation Pattern for α-Amino Acid Derivatives

β- and γ-Amino Acids

The base peak of the β- and γ-amino acid derivatives studied (β-alanine, β-amino-*n*-butyric acid, β-aminoisobutyric acid and γ-amino-*n*-butyric acid) except for β-alanine exists at *m/e* 167. The ion at *m/e* 166 which is the base peak for most of α-amino acid derivatives exists as the second intense peak with the relative intensity of 60—90%. There exist no characteristic or diagnostic peaks among first to tenth intense ions in the spectra of β- and γ-amino acid derivatives studied.

The peak at *m/e* (M−101), which was formed by the loss of $COOC_4H_9$ group from the molecular ions of α-amino acid derivatives were not observed for the β- and γ-amino acid derivatives. In contrast, the ions at *m/e* (M−73), loss of OC_4H_9 group from the molecular ions), are observed in the spectra of β- and γ-amino acid derivatives, but are not detected in α-amino acid derivatives. These (M−73) ions, though their intensities are not so large, may be used for the diagnostic proves for β- and γ-amino acid derivatives.

α-Methyl-α-Amino Acids

The fragmentation pattern of α-methyl-α-amino acid derivatives is very similar to that of α-amino acid derivatives. The base peaks of α-methyl-amino acid derivatives always appear at *m/e* 166 or 167 with the exception of α-methyl-alanine and trimethylsilylated amino acid derivatives. The characteristic ions for neutral and acidic α-methyl-amino acid derivatives

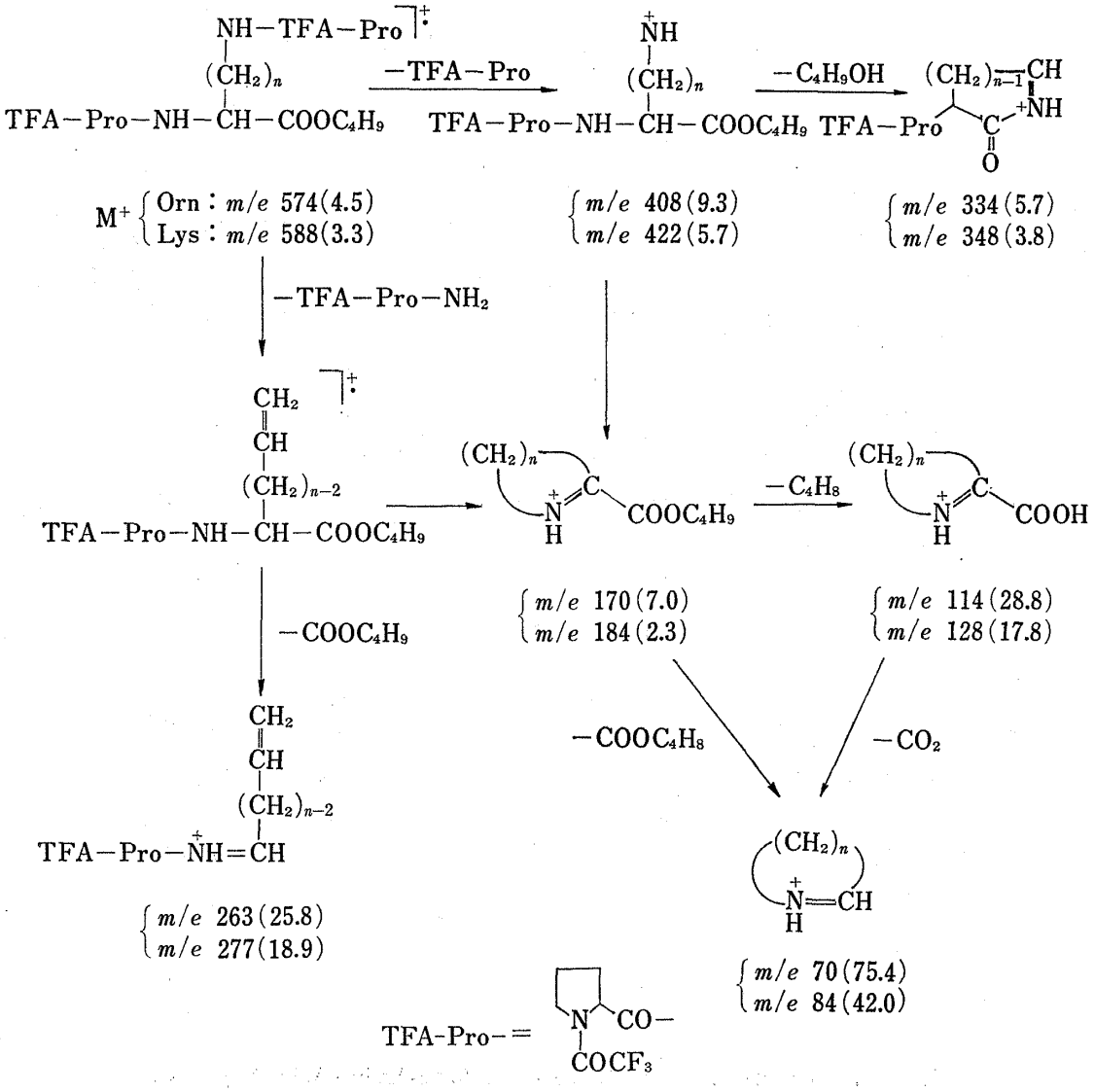


Chart 4. Typical Fragmentation of Basic Amino Acid Derivatives

(α-methyl-alanine, α-methyl-α-amino-*n*-butyric acid, α-methyl-valine, α-methyl-leucine, α-methyl-methionine, and α-methyl-glutamic acid) appear at *m/e* (M−101) and at *m/e* (R+43) that corresponds to amine fragment ions (R+29) of neutral α-amino acid derivatives. In trimethylsilylated α-methyl-amino acid derivatives (α-methyl-tyrosine, α-methyl-tryptophan and α-methyl-DOPA), the ions having the structures of trimethylsilylated side chain appear as the base peaks.

Mass Fragmentography of Ultramicroamounts of Amino Acids

Of the N-TFA-L-prolyl dipeptide-type derivatives, the ion at *m/e* 166 was always observed with a large intensity. In the case of α-amino acid derivatives with the exception of proline, pipecolic acid and trimethylsilylated amino acids other than serine and hydroxyproline, the ion at *m/e* 166 was the base peak. The relative intensities to the total ionization ($\sum_{10}$%) of this ion is average value of 18% for α-amino acid derivatives, 17% for β- and γ-amino acid derivatives. The latter amino acid derivatives showed lower $\sum_{10}$% value than that of corresponding to α-amino acid derivatives. Because of large intensities of the ion of trimethylsilylated side chain, the $\sum_{10}$% value of the ion at *m/e* 166 of tryptophan and hydroxy amino acid derivatives are rather lower, *i.e.*, about 13%.

For the purpose of sensitive and selective detection of amino acid derivatives by MF, it seems to be advantageous that the ion to be analysed, is in relatively higher mass region and has a even mass number. The ion at *m/e* 166 is satisfactory for this purpose.

MF of about 0.9 ng of L-alanine, L-valine, L-leucine, and L-proline monitoring at *m/e* 166 is shown in Fig. 2. The limit of detection of the amino acids, calculated from Fig. 2, is about 10^{-10} g level ($S/N=2$). As can be seen in Fig. 2, the concurrent ultramicrodetection of 4 amino acids was carried out by MF monitored at *m/e* 166.

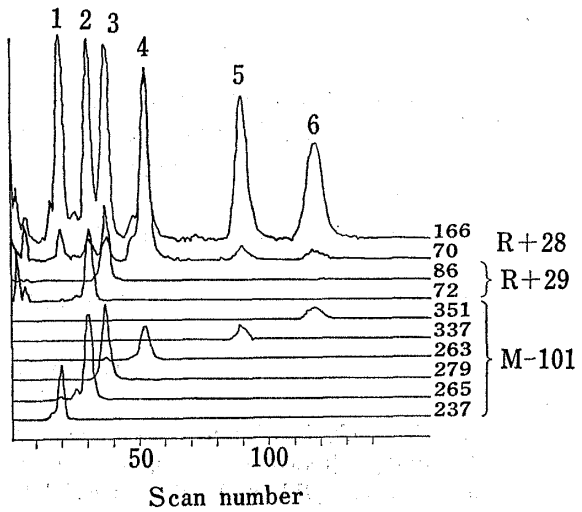


Fig. 1. Mass Chromatogram of N-TFA-L-prolyl Amino Acid *n*-Butyl Esters

1 alanine, 2 valine, 3 leucine, 4 proline, 5 aspartic acid, 6 glutamic acid
column, glass, (50 cm×3 mm I.D.), packed with modified OV-101 on 100—120 mesh Chromosorb W (AW)
column temperature, programmed from 140° to 170° at the rate of 5°/min
sample, about 500 ng each amino acid injected

Another merit of the N-TFA-L-prolyl amino acid *n*-butyl ester derivative is that this derivative is applicable to the mass fragmentographic resolution of the ultramicroamounts of racemic amino acids. Fig. 3 shows an example of the resolution of about 2.5 ng of DL-alanine, DL-valine, DL-leucine, DL-proline, DL-methionine, and DL-phenylalanine as their conversion to N-TFA-L-prolyl *n*-butyl esters by MF forcused at *m/e* 166.

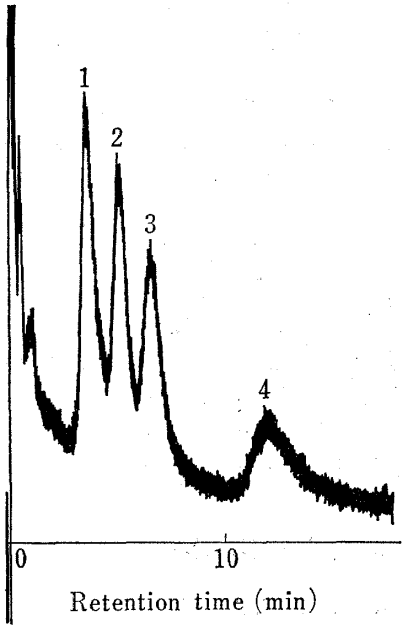


Fig. 2. Mass Fragmentogram of N-TFA-L-prolyl Amino Acid *n*-Butyl Esters

column, glass, (50 cm×3 mm i.d.), packed with modified OV-101 on 100—120 mesh Chromosorb W (AW); column temperature, 140°
monitored ion, *m/e* 166; Accelerating voltage' 3000 V
ionization energy, 70 eV sample, about 0.9 ng of each amino acid injected
1: alanine 3: leucine
2: valine 4: proline

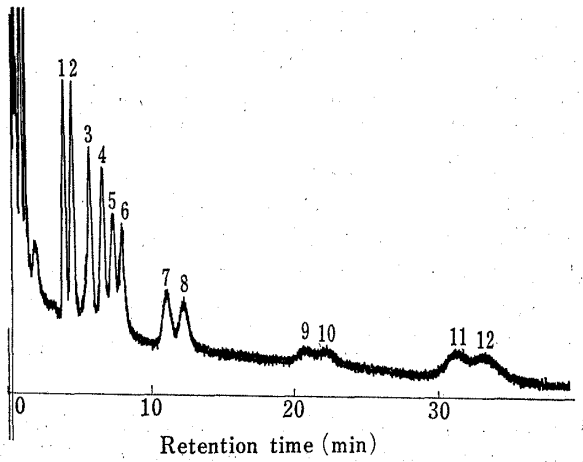


Fig. 3. Mass Fragmentogram of N-TFA-L-prolyl DL-Amino Acid *n*-Butyl Esters

column, glass, (1 m×3 mm i.d.), packed with 1.5% OV-101 on 100—120 mesh Chromosorb W (AW)
column temperature, 180°
monitored ion, *m/e* 166, accelerating voltage, 3000 V
ionization energy, 70 eV: sample, about 2.5 ng of each DL-amino acid injected
1: D-alanine 7: D-proline
2: L-alanine 8: L-proline
3: D-valine 9: D-methionine
4: L-valine 10: L-methionine
5: D-leucine 11: D-phenylalanine
6: L-leucine 12: L-phenylalanine