[Chem. Pharm. Bull. 27(6)1307—1315(1979)]

UDC 547.466.1.04.08 : 543.544.2.06

Gas Chromatography–Mass Spectrometry of Trimethylsilyl Derivatives of Amino Acids

Hiroshi Iwase, Yoko Takeuchi, and Asao Murai

Central Research Laboratories, Ajinomoto Co., Inc.[1]

(Received September 11, 1978)

The fragmentation of 58 N-trimethylsilyl amino acid trimethylsilyl esters, including those of α-, β-, γ-, and α-methyl-amino acids and related compounds, upon electron impact was investigated to provide a spectral basis for further studies of physiological samples, and to look into the possibility of selective identification and ultramicrodetermination of amino acids by mass fragmentography. It was found that the ion at *m/e* 73 or *m/e* (M-117) is the base peak common to most of the amino acids. The results obtained in this study suggest that this derivative can be used for the ultramicrodetermination and selective identification of amino acids by mass fragmentography because this derivative produces characteristic ions such as *m/e* (M-117), (M-15), 174, 218 and 232. The fragmentations of N-trimethylsilyl *n*-butyl esters and N-trimethylsilyl *l*-methyl esters of six amino acids were also studied.

Keywords——gas chromatography–mass spectrometry; amino acids; N-trimethylsilyl amino acid trimethylsilyl esters; N-trimethylsilyl amino acid *n*-butyl esters; N-trimethylsilyl amino acid *l*-menthyl esters

Gas chromatography of amino acids has been extensively studied during the past ten years. There have been numerous reports of suitable volatile derivatives for the analysis of amino acids by gas chromatography, and valuable reviews were published recently by Hušek and Macek,[2] and Nambara and Goto.[3]

Trimethylsilylation has been used not only in analyses of organic substances such as amino acids[4] and steroids,[5] but also for inorganic substances such as phosphoric acid[6] and inorganic anions[7] by gas chromatography. This is because of the attractiveness of the simple one-step derivative synthesis in which all of the active hydrogens of the samples are simultaneously derivatized. In addition, the resulting derivatives are thermally stable and sufficiently volatile to be readily gas chromatographed.

In the field of mass spectrometry, the mass spectra of trimethylsilylated phenylthiohydantoin amino acids[8] and N-trimethylsilyl (TMS) amino acid TMS esters[9] have been investigated. However, there have been relatively few studies on the mass spectra of many kinds of N–TMS amino acid TMS esters, N–TMS amino acid *n*-butyl esters and N–TMS amino acid *l*-menthyl esters.

Recent developments in gas chromatography–mass spectrometry (GC–MS) have facilitated the ultramicrodetermination of amino acids, amines and steroids in biological materials.

1) Location: *1-1, Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
2) P. Hušek and K. Macek, *J. Chromatogr.*, **113**, 139 (1975).
3) T. Nambara and J. Goto, *Bunseki kagaku*, **23**, 704 (1974).
4) C.W. Gehrke, H. Nakamoto, and R.W. Zumwalt, *J. Chromatogr.*, **45**, 24 (1969).
5) C.J.W. Brooks, W. Henderson, and G. Steel, *Biochimica et Biophysics Acta*, **296**, 431 (1973).
6) T. Hashizume and Y. Sasaki, *Anal. Biochem.*, **15**, 199 (1966).
7) W.C. Butts and W.T. Rainey, Jr., *Anal. Chem.*, **43**, 538 (1971).
8) R.E. Harman, J.L. Patterson, and W.J.A. VandenHeuvel, *Anal. Biochem.*, **25**, 452 (1968).
9) *a*) K. Bergström, J. Gurtler, and R. Blomstrand, *Anal. Biochem.*, **34**, 74 (1970), K. Bergström and J. Gurtler, *Acta Chemica Scandinavica*, **25**, 175 (1971), J. Marik, A. Capek, and J. Kralicek, *J. Chromatogr.*, **128**, 1 (1976); *b*) F.P. Abramson, M.W. McCaman and R.E. McCaman, *Anal. Biochem.*, **51**, 482 (1974).

Table I. Ten Mass Spectral Peaks of Trimethylsilylated

Amino acid	M^+		$(M-15)^+$		*m/e* 174	*m/e* 218	Base peak		2nd peak		3rd peak	
	m/e	%[a]	*m/e*	%	%	%	*m/e*	Σ_{10}%[b]	*m/e*	%	*m/e*	%
Gly	291	—	276	2.1	54.9	—	73	28.6	174	54.9	45	28.9
Sar	233	1.6	218	2.3	—	2.3	116	26.3	73	92.9	45	23.7
Ala	233	—	218	1.9	—	1.9	116	25.4	73	87.8	45	27.5
β-Ala	305	—	290	12.1	48.1	—	73	25.1	174	48.1	248	32.6
α-Aba	247	—	232	1.7	—	2.4	130	26.3	73	89.2	45	25.7
β-Aba	247	—	232	12.1	1.2	—	73	19.9	116	94.8	45	30.0
β-Aiba	247	—	232	1.3	—	3.6	102	28.9	73	67.8	45	16.9
γ-Aba	319	—	304	18.7	96.7	—	73	21.7	174	96.7	75	30.6
Nva	261	—	246	1.9	—	6.7	144	27.6	73	86.4	45	21.8
Val	261	—	246	1.7	—	16.3	73	23.5	144	89.5	45	25.6
Nle	275	—	260	2.2	—	3.5	158	31.8	73	72.4	45	15.4
Leu	275	—	260	1.3	—	2.7	158	32.3	73	73.0	45	17.0
Ile	275	—	260	—	—	16.5	158	27.3	73	90.0	45	20.6
tert-Leu	275	—	260	—	—	31.1	73	28.4	158	64.6	218	31.1
α-Cap	303	—	288	1.9	—	3.4	186	32.9	73	63.2	187	16.7
Ser	321	—	306	—	—	32.1	73	33.2	204	46.6	218	32.1
Thr	335	—	320	—	—	21.4	73	33.5	117	25.0	218	21.4
Pro	259	—	242	—	—	—	142	32.5	73	75.4	45	20.4
Pip	273	—	258	—	—	—	156	30.1	73	75.4	45	23.8
Hypro	347	—	332	—	—	—	73	35.8	230	50.3	140	21.2
Pga	273	—	258	5.6	—	—	73	22.9	156	77.0	45	32.0
Tca	277	—	262	—	—	—	73	32.4	160	67.8	45	24.4
AMCHA	301	—	286	4.2	—	—	102	39.0	73	47.8	75	14.7
Ana	281	—	266	50.0	—	—	73	34.1	266	50.0	45	27.6
Asp	349	—	344	1.4	—	3.8	73	31.5	232	42.6	100	18.6
Glu	363	—	348	4.2	1.3	—	73	25.6	246	53.8	75	24.2
Adi	377	2.7	362	6.5	—	2.5	73	22.5	260	52.4	55	30.2
Asn	348	—	333	1.3	—	3.8	73	33.1	116	29.9	75	19.9
Gln	362	—	347	2.2	—	—	73	28.9	156	47.2	75	28.6
Met	293	1.7	278	—	—	2.6	73	22.6	176	6.7	61	33.5
Eth	307	2.3	292	—	—	4.4	73	20.4	190	66.9	75	56.1
Met(O)	309	—	294	—	—	—	56	17.7	73	89.1	128	71.7
Met(O_2)	325	—	310	—	—	2.7	128	24.9	73	69.2	208	42.6
CySH	337	2.8	322	6.3	2.0	99.6	220	9.3	218	99.6	73	99.0
Cys	528	—	513	—	—	45.2	73	28.1	218	45.2	146	32.1
Pen	365	—	350	—	—	14.5	73	40.1	147	20.4	45	17.7
Pg	295	—	280	—	—	—	73	25.0	178	80.0	45	32.3
Phe	309	—	294	—	—	45.3	73	22.7	218	45.3	192	37.6
Tyr	397	—	382	1.6	—	63.7	73	32.0	218	63.7	45	16.6
DOPA	485	—	470	—	—	52.5	73	39.4	218	52.5	45	14.5
Trp	420	—	405	—	—	2.4	73	41.4	202	62.0	45	18.2
5H-Trp	508	—	493	—	—	—	73	39.7	290	48.4	291	20.6
Kyn	424	—	409	—	—	20.4	73	23.7	192	45.7	307	33.8
His	371	—	356	2.2	—	6.1	73	29.8	154	74.1	45	21.2
Orn	420	3.9	405	1.0	42.9	3.0	73	22.9	142	81.9	174	42.9
Lys	434	—	419	2.3	70.5	3.4	73	20.1	174	70.5	317	53.2

a) Relative intensity (base peak=100). *b*) Percent of the total ionization over *m/e* 10. *c*) The fragment ion

Abbreviations; Sar, sarcosine; Aba, amino-*n*-butyric acid; Aiba, amino-iso-butyric acid; Nva, norvaline; Nle, AMCHA, *trans*-4-(aminomethyl)-cyclohexane carboxylic acid; Ana, anthranilic acid; Adi, α-aminoadipic acid; Eth, 5-hydroxy-tryptophan; Kyn, kynurenine.

Amino Acids and Related Compounds

4th peak		5th peak		6th peak		7th peak		8th peak		9th peak		10th peak	
m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
59	19.1	147	16.0	43	13.9	175	13.0	86	12.0	74	9.0	248	8.3
147	18.0	43	15.1	59	14.8	117	11.7	75	9.5	42	9.4	74	8.0
43	15.0	59	14.5	147	13.9	117	12.4	75	11.4	74	8.4	44	8.4
45	28.0	147	23.2	75	18.4	59	18.2	86	14.8	100	12.8	43	12.1
59	13.7	131	13.0	43	12.4	147	12.0	75	11.7	74	8.6	44	5.7
75	25.1	100	22.9	59	19.9	43	17.2	147	16.4	117	13.4	232	12.1
147	13.6	75	13.5	59	10.0	103	9.3	43	9.2	81	8.3	176	7.3
147	28.3	45	21.7	175	17.5	59	16.2	86	15.3	74	9.2	176	8.0
145	13.2	59	12.2	147	10.3	75	10.4	43	10.1	74	8.2	218	6.7
43	16.5	218	16.3	59	13.1	75	13.0	147	12.4	145	12.3	74	10.6
159	14.5	59	10.2	29	8.3	74	7.9	147	7.3	75	7.2	43	5.9
159	14.5	59	11.1	147	8.4	43	7.7	41	7.4	75	7.3	74	7.1
218	16.5	29	14.8	159	14.1	41	11.2	147	10.1	59	9.0	75	8.9
45	20.5	41	12.4	75	11.0	29	11.0	100	10.4	57	9.9	147	9.3
45	11.8	59	8.5	43	8.5	75	7.6	147	6.6	41	6.5	188	5.2
45	20.2	100	13.0	147	11.7	205	9.1	75	8.3	74	7.9	59	6.4
45	19.0	219	18.5	101	9.8	57	9.4	75	9.2	147	8.8	74	8.6
143	14.0	43	10.3	59	8.1	75	7.9	147	7.0	74	6.7	72	5.1
157	14.8	59	11.5	43	10.9	75	8.9	74	7.6	55	5.3	58	5.1
45	19.1	75	13.2	232	10.7	74	7.8	147	6.9	59	6.6	43	6.3
75	20.9	43	18.3	147	14.5	157	10.7	74	10.5	59	10.0	58	9.0
75	11.3	43	10.5	59	9.6	74	8.6	161	7.9	147	7.9	162	6.2
103	10.2	59	7.9	45	7.8	30	6.2	74	5.9	147	5.1	286	4.2
75	14.1	43	13.3	267	11.5	134	9.8	74	8.4	59	4.9	147	3.9
45	18.1	75	15.6	147	11.8	74	11.6	43	9.8	233	9.4	59	6.3
45	20.3	128	18.8	147	15.6	74	11.6	84	11.2	247	11.1	56	9.0
75	28.1	128	19.5	45	19.6	217	15.3	147	15.2	74	14.8	261	13.5
45	15.8	231	14.3	132	12.6	74	10.3	147	9.0	188	8.3	100	7.1
155	23.1	45	17.6	245	16.4	147	13.2	74	11.7	157	6.7	128	6.2
45	31.5	128	28.9	75	19.9	43	13.0	59	12.5	74	11.5	47	10.0
128	34.6	45	30.6	29	22.1	47	13.3	59	12.4	43	11.0	191	9.8
75	35.1	45	25.5	130	18.9	74	17.7	447	13.1	52	11.1	147	11.0
56	23.2	75	19.5	202	13.6	45	12.6	129	12.1	130	9.5	147	8.3
45	86.1	100	73.8	147	42.5	40	41.1	221	20.9	75	38.3	59	33.9
147	16.5	100	16.4	45	12.6	75	11.4	219	9.5	74	9.0	115	8.0
218	14.5	75	9.4	74	8.4	291	6.4	59	6.4	219	6.3	101	5.8
43	16.9	179	13.8	59	11.5	75	11.3	74	11.0	147	8.4	44	7.9
45	22.5	91	18.3	100	12.5	147	12.2	75	10.3	74	9.1	43	8.0
219	13.5	75	10.0	179	9.8	91	9.5	74	9.4	147	7.8	280	7.0
267	10.4	219	10.2	74	8.4	75	7.3	179	6.2	100	5.7	220	4.5
203	12.8	74	8.1	75	7.9	43	5.4	291	3.9	44	3.7	147	3.0
45	14.3	75	9.3	74	8.9	292	8.2	218	6.9	147	4.3	202	3.1
75	24.7	218	20.4	45	18.2	425	11.2	147	11.0	308	8.5	193	8.1
254	17.3	155	14.7	74	9.4	75	7.6	43	7.3	182	7.0	218	6.1
143	11.0	74	10.8	147	10.7	45	10.3	59	9.2	200	8.5	86	7.9
156	27.0	318	19.0	128	17.8	175	16.5	147	11.8	230	11.4	86	7.9

(M-117) resulting from the loss of -COOTMS from the molecular ion is underlined.
norleucine; Cap, amino-*n*-caprylic acid; Pip, pipecolic acid; Pga, pyroglutamic acid; Tca, 4-thiazolidinecarboxylic acid; ethionine; Met(O), methionine sulfoxide; Met(O_2), methionine sulfone; Pen, penicillamine; Pg, phenylglycine; 5H-Trp,

Horning *et al.*[10] investigated the metabolic profiles of human adult and infant urinary compounds by GC–MS. Many human metabolic products are polyfunctional and not readily volatile, so it is necessary to carry out two or three successive or simultaneous reactions to obtain suitable derivatives. Mass fragmentography provides a useful procedure for the quantitative evaluation of low molecular metabolites at very low concentrations in blood or small samples of tissues such as those of a newborn baby or infant, because of its selective identification and capability of ultramicrodetermination. The selective identification and ultramicrodetermination of amino acids in the body are useful for the study of metabolites. In previous papers we reported that TMS derivatives are useful for the selective identification of ultramicroamounts of amino acids[11] and amines[12] using mass fragmentography. The use of TMS derivatives is advantageous for the analysis of amino acids by mass fragmentography because, as described above, the preparation of TMS derivatives is simple, TMS derivatives are thermally stable and sufficiently volatile, and the selective ultramicrodetermination of amino acids is possible. The present paper deals with the mass spectra of N–TMS amino acid TMS esters including α-, β-, γ-, and α-methyl-amino acids and other biologically important compounds, together with 6 kinds of N–TMS amino acid *n*-butyl esters and N–TMS amino acid *l*-menthyl esters to provide a spectral basis for further studies of physiological samples. In addition, the possibility of selective identification and ultramicrodetermination of amino acids by mass fragmentography was examined. A comparison was also made of the fragmentation features of TMS esters, *n*-butyl esters and *l*-menthyl esters of N–TMS amino acids.

Experimental

Apparatus and Conditions——A Hitachi RMU-6MG mass spectrometer was used with an 002 Datalizer coupled to a Hitac-10 computer. The operating conditions for this study were as described previously.[13] A glass column of 1 m×3 mm i.d. packed with 1.5% OV-101 on Diatoport S was employed for this study.

Reagents and Materials——Amino acids were obtained from Ajinomoto Co., Tokyo Kasei Co., and Sigma Chemical Co. Pyridine was used after drying over NaOH pellets. N,O-bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane and hypovials were purchased from Pierce Chemical Co.

Preparation of Trimethylsilylated Amino Acids——N-TMS amino acid TMS esters were obtained by the reported method.[4] N-TMS amino acid *n*-butyl esters and N-TMS amino acid *l*-menthyl esters were prepared by previously reported methods.[11]

Results and Discussion

Mass Spectra of N–TMS Amino Acid TMS Esters

Tables I and II show the *m/e* values and relative intensities of the peaks of 1st to 10 th intensities (per cent of total ionization for base peaks and pattern coefficients for the others).

Molecular Ions, *m/e* (M-15) and (M-117) Ions

Molecular ions were detected only for six α-amino acids (sarcosine, α-aminoadipic acid, methionine, ethionine, cysteine and ornithine), with 2.5% average relative intensities, and were not observed for β-, γ- and α-methyl-amino acids.

The ions at *m/e* (M-15) served as an indicator of the molecular weights of the trimethylsilylated compounds. The ions at *m/e* (M-15), which give prominent peaks for many trimethylsilylated derivatives, were detected for 32 kinds of amino acids, and this peak was especially strong for anthranillic acid, γ-amino-*n*-butyric acid, β-alanine and β-amino-*n*-butyric acid, with relative intensities of 50.0, 18.7, 12.1 and 12.1%, respectively. The (M-15) fragment

10) E.C. Horning and M.G. Horning, *J. Chromatogr. Sci.*, **9**, 129 (1971).
11) H. Iwase and A. Murai, *Anal. Biochem.*, **78**, 340 (1977).
12) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **25**, 3129 (1977).
13) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **25**, 285 (1977).

TABLE II. Ten Mass Spectral Peaks of N-TMS α-Methyl Amino Acid TMS Esters

Amino acid	M^+		$(M-15)^+$		*m/e* 174	*m/e* 232	Base peak		2nd peak		3rd peak		4th peak	
	m/e	%[a]	*m/e*	%	%	%	*m/e*	Σ_{10}%[b]	*m/e*	%	*m/e*	%	*m/e*	%
Ala	247	—	232	1.7	—	—	130	26.1	73	93.9	45	24.9	43	12.9
Aba	261	—	246	1.7	—	4.9	144	23.9	73	85.9	45	20.3	145	13.2
Val	275	—	260	—	—	23.6	73	27.6	158	86.2	232	23.6	45	21.1
Leu	289	—	274	1.8	5.8	—	172	26.9	73	75.3	45	18.2	173	15.1
Ile	289	—	274	1.5	4.6	21.6	73	23.5	172	75.4	29	24.2	232	21.6
Glu	377	—	362	3.4	—	—	73	29.5	260	50.8	75	21.9	45	19.9
Met	307	—	292	—	1.0	4.9	73	23.5	190	16.5	45	30.4	61	19.8
Tyr	411	—	396	—	—	60.7	73	31.5	232	60.7	114	17.1	45	14.0
DOPA	499	—	484	2.0	2.2	97.6	73	27.4	232	97.6	114	20.9	233	19.8
Trp	434	—	419	—	1.2	31.1	73	38.2	232	36.1	202	30.2	45	16.9
5H-Trp	522	—	507	—	—	23.3	73	38.9	290	48.4	232	23.3	291	14.6
Orn	434	—	419	—	1.2	5.7	73	24.6	156	58.9	84	31.8	45	17.2

Amino acid	5th peak		6th peak		7th peak		8th peak		9th peak		10th peak	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Ala	131	12.7	75	12.3	147	9.7	74	9.2	100	8.6	59	8.5
Aba	75	11.8	147	10.5	43	9.8	74	8.4	59	8.1	114	6.4
Val	114	12.9	43	11.3	75	11.2	159	10.7	147	9.6	74	9.1
Leu	147	9.4	75	9.3	100	9.1	74	8.6	43	8.4	59	7.6
Ile	45	21.5	41	16.9	114	14.0	173	11.7	75	11.2	57	10.7
Glu	147	11.6	98	11.2	74	11.2	261	10.8	170	6.5	142	6.3
Met	75	17.3	142	14.0	74	12.3	43	12.2	70	11.4	114	10.1
Tyr	75	12.5	233	12.0	74	9.3	42	8.4	147	8.0	43	5.5
DOPA	45	17.6	74	11.4	75	9.4	147	9.2	234	8.9	179	6.9
Trp	114	10.8	74	8.5	75	7.7	233	7.5	203	6.2	43	5.7
5H-Trp	45	12.1	114	10.6	74	7.6	75	5.9	147	5.1	292	4.5
Orn	74	15.8	245	15.1	102	9.6	157	8.7	59	8.7	114	6.9

a) Relative intensity (base peak=100).
b) Percent of the total ionization over *m/e* 10.
c) The fragment ion (M-117) resulting from loss of –COOTMS from the molecular ion is underlined.

ions of the other trimethylsilylated amino acids were observed with relative intensities of 1.5—6%. No peaks at *m/e* (M-15) were observed for the following 20 α-amino acids, branched side chain and trimethylsilylated side chain amino acids (isoleucine and *tert*-leucine, serine and threonine), sulfur-containing amino acids (methionine, ethionine, methionine sulfoxide, methionine sulfone, cystine, and penicillamine), cyclic amino acids (proline, pipecolic acid, hydroxyproline, and 4-thiazolidinecarboxylic acid), aromatic amino acids (phenylglycine, phenylalanine, 3,4-dihydroxyphenylalanine (DOPA), tryptophan, 5-hydroxy-tryptophan and kynurenine), and 6 kinds of α-methyl-amino (valine, methionine, tyrosine, tryptophan, 5-hydroxytryptophan and ornithine).

A base peak at *m/e* 73 was detected for 40 amino acids, (M-117) for 14 amino acids and other ions for 4 kinds of amino acids. The ions at *m/e* 73 and (M-117) were commonly observed with high relative intensities for many amino acids. Although the *m/e* 73 ion was observed with high intensities for β- and γ-amino acids, no *m/e* (M-117) fragment ions were detected for these amino acids. However, peaks corresponding to the (M-117) ions were detected

for β-alanine (*m/e* 174, M-131, 48.1%),[14] β-amino-*n*-butyric acid (*m/e* 116, M-131, 94.8%), β-aminoisobutyric acid (*m/e* 102, M-145, 100.0%) and γ-amino-*n*-butyric acid (*m/e* 174, M-145, 96.7%). The amino acids with low relative intensities of the ions at *m/e* (M-117) were sulfur-containing amino acids such as cysteine (*m/e* 411, 4.8%) and penicillamine (*m/e* 248, 4.5%), aromatic amino acids such as tyrosine (*m/e* 280, 7.0%) and DOPA (*m/e* 368, 3.5%), basic amino acids such as ornithine (*m/e* 303, 4.1%) and α-methyl-amino acids such as tyrosine (*m/e* 294, 5.3%) and DOPA (*m/e* 382, 3.2%). Ions at *m/e* (M-117) were not detected for *trans*-4-(aminomethyl)cyclohexane carboxylic acid, anthranillic acid, methionine sulfoxide, tryptophan, 5-hydroxy-tryptophan, α-methyl-tryptophan, α-methyl-5-hydroxy-tryptophan and α-methyl-ornithine.

m/e 174 Ion

In the previous paper,[12] we reported that the TMS amine derivative was useful for the determination of ultramicro-amounts (10^{-11} g level) of amines by mass fragmentography, monitoring the ion at *m/e* 174 $\left(\begin{matrix}\mathrm{TMS}\\ \mathrm{TMS}\end{matrix}\!\!>\overset{+}{\mathrm{N}}\!=\mathrm{CH_2}\right)$, which is a commonly produced intense fragmentation ion. When mass fragmentography is carried out on a TMS-amine derivative by monitoring the ion at *m/e* 174, other amino acids will also be detected if they also produce the intense ion at *m/e* 174, although their retention times will be different. Therefore, mass spectra of N–TMS amino acid TMS esters were recorded for the selective identification of amino acids and amines in samples by mass fragmentography. As can be seen in Tables I and II, the intense ion at *m/e* 174 was observed for glycine (54.9%), β-alanine (48.1%), γ-amino-*n*-butyric acid (96.7%), ornithine (42.9%) and lysine (70.5%), which possess the $\mathrm{R–CH_2NH_2}$ structure. In addition, these 5 amino acids also produced a prominent ion at *m/e* 86 ($(\mathrm{CH_3})_2\mathrm{Si}=\overset{+}{\mathrm{N}}=\mathrm{CH_2}$), with relative intensities of 12.0, 14.8, 15.3, 7.9, and 7.9%, respectively. The presence of both ions at *m/e* 174 and 86 may be diagnostic for the structure $\mathrm{R–CH_2NH_2}$. β-Amino-*n*-butyric acid, glutamic acid, cysteine, 5-hydroxy-tryptophan and α-methyl-amino acids (leucine, isoleucine, methionine, DOPA, and ornithine) also produce the *m/e* 174 ion, though the relative intensity is rather lower, *i.e.*, about 1—5%. The *m/e* 86 ion was observed for β-amino-*n*-butyric acid (1.8%), glutamic acid (1.0%) and cysteine (3.5%), but was not observed for 5-hydroxy-tryptophan and α-methyl-amino acids. Although Abramson *et al.*[9b] reported that amino acids possessing the $\mathrm{R–CH_2NH_2}$ structure produce an intense ion at *m/e* 174, β-aminoisobutyric acid cannot produce this ion. It is of interest that the relative intensity of the *m/e* 174 ion for ornithine (42.9%) is larger than that for α-methyl-ornithine (1.2%).

The intense ion at *m/e* 174 indicates a di-TMS ω-amino group in the mass spectra of glycine, β-alanine, γ-amino-*n*-butyric acid, ornithine and lysine. It should therefore be possible to selectively identify and ultramicrodetermine these five amino acids by mass fragmentography monitoring the intense ion at *m/e* 174.

m/e 218 and 232 Ions

The ion at *m/e* 218, which corresponds to loss of the side chain (R) of amino acids from the molecular ion, was observed with high intensity for valine (16.3%), isoleucine (16.5%), *tert*-leucine (31.1%), serine (39.1%), threonine (21.8%), sulfur-containing amino acids (cysteine (9.9%), cystine (45.2%), and penicillamine (14.5%)), and aromatic amino acids (phenylalanine (45.3%), tyrosine (63.7%), DOPA (52.7%) and kynurenine (20.4%)). A peak at *m/e* 232 corresponding to the ion at *m/e* 218 was observed for α-methyl-amino acids such as valine (23.1%), isoleucine (21.6%), tyrosine (60.7%), DOPA (97.6%) and 5-hydroxy-tryptophan (23.3%). Thus, it is concluded that amino acids, which have branched side chains,

14) Relative intensities of the ions are given in parentheses.

trimethylsilylated side chain, and aromatic rings, produce intense ions at *m/e* (M–R). The relative intensities of the peak at *m/e* 232 for α-methyl-amino acids were rather larger than those of the corresponding peak of α-amino acids. The specific ions at *m/e* 218 and 232 are useful for the selective identification and ultramicrodetermination of the above amino acids because TMS-amines do not produce these ions.

The Characteristic Ions of Each Amino Acid Derivative

We will report separately[15] on the specific fragment ions such as *m/e* 73, 75, 86 and 147 derived from the trimethylsilylated derivatives.

Aliphatic Amino Acids

Neutral Amino Acids (Glycine, β-Alanine and *tert*-Leucine)

As described above, glycine and β-alanine produce a specific ion at *m/e* 174. In addition, another characteristic ion at *m/e* 248 was also observed for these two amino acids, with relative intensities of 8.3% and 32.1%, respectively. However, γ-amino-*n*-butyric acid, ornithine and lysine, which produce the ion at *m/e* 174, do not produce the *m/e* 248 ion. The previous paper[13] reported that N-trifluoroacetyl (TFA)-L-prolyl-*tert*-leucine *n*-butyl ester produces an ion at *m/e* 57 (24.0%). The N–TMS *tert*-leucine TMS ester also produces the *m/e* 57 ion, with a relative intensity of 9.9%.

Hydroxy Amino Acids (Threonine and Hydroxyproline)

An intense ion at *m/e* 117 was observed for threonine. The relative intensity of the ion at *m/e* (M-117) for threonine was 21.4%. This may be considered as resulting from the loss of TMS carboxy group or TMS side chain from the molecular ion.

The relative intensity of the specific ion at *m/e* 140 from hydroxyproline was 21.2%.

Sulfur-Containing Amino Acids (Methionine, α-Methyl-methionine, Ethionine, Methionine Sulfoxide, Methionine Sulfone, Penicillamine, Cysteine and Cystine)

The characteristic ions observed for 8 sulfur-containing amino acids are summarized in Table III. The ions at *m/e* 47 ($CH_3\overset{+}{S}$) and 61 ($CH_3\overset{+}{S}CH_2$) are useful as structural indicators

Table III. Relative Intensities of Characteristic Ions for Sulfur-Containing Amino Acids

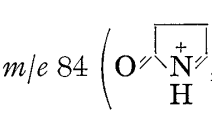
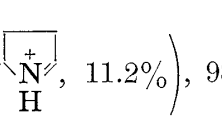
Ions	Met	MeMet	Eth	Met(O)	Met(O_2)	Pen	CySH	Cys
m/e 47	10.0%[a]	9.3%	13.3%	3.1%	2.6%	1.0%	7.5%	2.6%
m/e 61	33.5	19.8	8.0	10.0	2.0	—	3.5	—
m/e 75	19.9	17.3	56.1	35.1	19.5	9.4	38.3	11.4

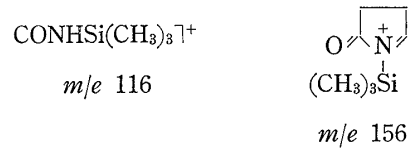
a) Relative intensity (base peak=100).
Abbreviations; Met, methionine; MeMet, α-methyl-methionine; Eth, ethionine; Met(O), methionine sulfoxide; Met(O_2), methionine sulfone; Pen, penicillamine; CySH, cysteine; Cys, cystine.

for sulfur-containing amino acids. The ion at *m/e* 75 from ethionine was attributed to the structures ($CH_3\overset{+}{S}CH_2CH_2$) and ($(CH_3)_2\overset{+}{S}OH$). Methionine sulfoxide and methionine sulfone produce common ions at *m/e* 56 and 128, having relative intensities of 17.7% and 23.2%, and 71.7% and 100.0%, respectively. The former amino acid also produces characteristic ions at *m/e* 130 (18.9%) and the latter also produces a specific ion at *m/e* 202 (13.6%). A diagnostic ion at *m/e* 291 (M-74, 6.4%) was observed for penicillamine, and ions at *m/e* 232 (5.3%) and 264 (5.6%) were also detected for cystine.

15) H. Iwase, Y. Takeuchi, and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **27**, 1009 (1979).

Acidic Amino Acids (Aspartic Acid, Glutamic Acid, α-Aminoadipic Acid, Asparagine, Glutamine and α-Methyl-glutamic Acid)

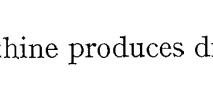
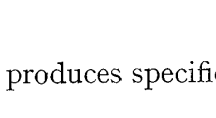
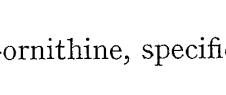
Glutamic acid, α-aminoadipic acid and α-methyl-glutamic acid produce specific ions at *m/e* 84  , 11.2%), 98  , 5.1%) and 98 , 11.2%), respectively. An ion at *m/e* 116 was observed for asparagine, with a relative intensity of 29.9%. Specific ions at *m/e* 156 and 84 were also detected for glutamine, with relative intensities of 47.2% and 2.3%, respectively. The structures of the ions at *m/e* 116 and 156 are assumed to be as follows. The ion at *m/e* 156 was also detected for glutamic acid, with a relative intensity of 7.2%. α-Methyl-glutamic acid and α-aminoadipic acid also produce an *m/e* 170 fragment ion, which corresponds to the *m/e* 156 fragment ion, with relative intensities of 6.5% and 3.4%, respectively.

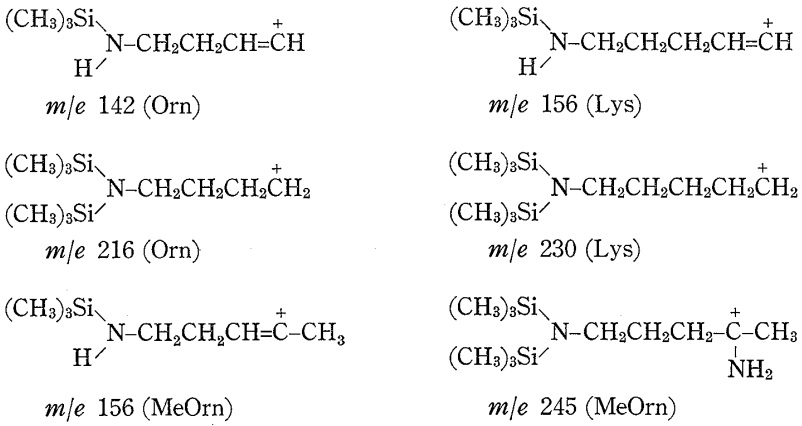


m/e 116 *m/e* 156

Chart 1

Basic Amino Acids (Ornithine, Lysine and α-Methyl-ornithine)

Ornithine produces diagnostic ions at *m/e* 70  , 1.2%), 142 (81.9%) and 216 (1.3%), and lysine produces specific ions at *m/e* 84  , 1.9%), 156 (27.0%) and 230 (11.4%). For α-methyl-ornithine, specific ions at *m/e* 83  , 31.8%), 156 (58.9%), and 245 (15.1 %) were also detected. The structures of these ions may be as follows.



m/e 142 (Orn) *m/e* 156 (Lys)

m/e 216 (Orn) *m/e* 230 (Lys)

m/e 156 (MeOrn) *m/e* 245 (MeOrn)

Chart 2

Aromatic Amino Acids (Phenylalanine, Tyrosine, DOPA, Tryptophan and 5-Hydroxy-tryptophan)

Characteristic ions, which correspond to the structure Ar–CH_2, were observed for aromatic amino acids such as phenylalanine (*m/e* 91, 18.3%), tyrosine (*m/e* 179, 9.8%), DOPA (*m/e* 267, 10.4%), tryptophan (*m/e* 202, 62.0%), 5-hydroxy-tryptophan (*m/e* 290, 67.1%), histidine (*m/e* 154, 74.1%), α-methyl-tyrosine (*m/e* 179, 6.9%), α-methyl-DOPA (*m/e* 267, 5.8%), α-methyl-tryptophan (*m/e* 202, 62.0%) and α-methyl-5-hydroxy-tryptophan (*m/e* 290, 48.4%). For kynurenine, an ion at *m/e* 192 was detected with the high relative intensity of 45.7%. The proposed structure of this ion is shown in Chart 3.

m/e 192

Chart 3

These results indicate that N–TMS amino acid TMS esters produce specific fragment ions with high relative intensities in their mass spectra. Accordingly, the selective identification and ultramicrodetermination of amino acids should be possible by monitoring the characteristic and intense ions such as *m/e* 218, 232, and (M-117) by mass fragmentography. The specific ions at *m/e* (R+29), (amine fragment ion; $R-CH=\overset{+}{N}H_2$), which were detected for N–TFA amino acid *n*-butyl esters[16] and the previously reported N–TFA-L-prolyl amino acid *n*-butyl esters,[13] were not observed for the N–TMS amino acid TMS esters studied here.

N–TMS Amino Acid *n*-Butyl Esters and N–TMS Amino Acid *l*-Menthyl Esters

Table IV shows the *m/e* values and relative intensities of peaks of the 1st to 6th intensities.

TABLE IV. Six Mass Spectral Peaks of N-TMS Amino Acid *n*-Butyl Esters and N-TMS Amino Acid *l*-Methyl Esters

Amino acid		M^+		$(M-15)^+$		*m/e* 174	Base peak		2nd peak		3rd peak		4th peak		5th peak		6th peak	
		m/e	%[a]	*m/e*	%	%	*m/e*	Σ_{10}%[b]	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
(1)[c]	Ala	217	—	202	2.1	7.0	116	8.6	73	99.2	29	97.4	118	78.0	117	67.8	41	60.3
	Val	245	—	230	—	15.6	144	18.9	73	99.2	29	64.6	146	62.8	202	59.9	145	39.0
	Leu	259	—	244	—	—	158	14.9	73	99.1	29	42.7	41	42.2	159	31.2	75	19.4
	Pro	243	—	228	—	—	142	19.0	73	99.2	29	38.2	41	29.7	143	26.4	45	20.6
	Asp	317	—	302	—	—	216	13.7	73	96.7	29	69.1	41	49.7	100	41.5	57	23.9
	Glu	331	—	316	—	7.2	84	12.8	231	99.7	73	69.4	41	50.4	29	53.6	75	24.4
(2)[d]	Ala	299	—	284	—	—	116	38.1	73	25.6	43	18.2	28	13.6	41	11.5	117	11.1
	Val	327	—	312	—	4.4	144	25.5	73	38.2	43	37.8	146	27.9	41	18.6	145	14.0
	Leu	341	—	326	—	—	158	34.9	73	28.0	43	26.9	28	21.9	159	13.7	41	13.4
	Pro	325	—	310	—	—	142	35.1	73	30.5	43	23.1	28	13.9	143	13.8	41	12.7
	Asp	481	—	466	—	—	160	19.4	298	64.6	55	32.3	83	30.2	73	27.6	43	26.8
	Glu	495	—	480	—	100.0	174	13.3	312	70.1	83	46.7	43	45.9	73	38.3	55	37.1

a) Relative intensity (base peak=100).
b) Percent of the total ionization over *m/e* 10.
(1) N-TMS amino acid *n*-butyl ester, *c*) The fragment ion (M-101) resulting from the loss of $-COOC_4H_9$ from the molecular ion is underlined.
(2) N-TMS amino acid *l*-menthyl esters, *d*) The fragment ion (M-183) resulting from the loss of $-COOC_{10}H_{19}$ from the molecular ion is underlined.

Molecular ions were not observed for these two N–TMS amino acid esters. A prominent peak at *m/e* (M-15) was detected only for N–TMS alanine *n*-butyl ester, with a relative intensity of 2.1%. As described above, 32 N–TMS amino acid TMS esters produce ions at *m/e* (M-15). Therefore, it may be considered that the (M-15) ions are specific for N–TMS amino acid TMS esters. An *m/e* 174 fragment ion was observed for N–TMS-alanine, -valine, -glutamic acid *n*-butyl esters, and N–TMS-valine and -glutamic acid *l*-menthyl esters.

The ion at *m/e* 174 was detected for many N–TMS amino acid esters. Accordingly, it must be considered in the selective identification and ultramicrodetermination of amino acids and amines, when mass fragmentography is carried out on TMS-amino acids and -amines by monitoring this ion, although the retention times will be different for different compounds.

It should be noted that the present method is advantageous for the estimation of small amounts of amino acids at low concentrations in samples compared to existing methods since the preparation of volatile N–TMS amino acid TMS esters is a simple one-step procedure, the resulting derivatives are thermally stable and the selective identification and ultramicrodetermination of amino acids are possible by mass fragmentography, monitoring diagnostic and intense ions. This may be of considerable value for metabolic studies of amino acids by GC–MS.

16) E. Gelpi, W.A. Koenig, J. Gibert, and J. Oro', *J. Chromatogr. Sci.*, **7**, 604 (1969).