CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 25, No. 12 December 1977

Regular Articles

[Chem. Pharm. Bull. 25(12)3129—3136(1977)]

UDC 547.298.1.08 : 543.51.061

On the Derivatives for the Ultramicrodetermination of Amines by Mass Fragmentography

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(Received January 19, 1977)

The fragmentation of five derivatives of isobutylamine, 3-methylthiopropylamine, β-phenylethylamine, tyramine and dopamine upon electron impact is studied for the concurrent ultramicrodetermination and the selective identification of amines by mass fragmentography. The results obtained in this study indicated that trimethylsilyl derivative was the most preferable for the concurrent ultramicrodetermination of amines because this derivative produced the common intense ion at *m/e* 174, which is specific for amines. Using mass fragmentography monitored at *m/e* 174, about 10^{-11} g of amine can be detected.

Keywords——mass spectrometry; mass chromatography; mass fragmentography; ultramicrodetermination of amines; trimethylsilylated amines; N-trifluoroacetyl-L-4-thiazolidinecarbonylamines; benzoylamines; pentafluorobenzoylamines; trifluoroacetylamines

In 1968, Hammar, *et al.*[2] first used the term "mass fragmentography" in the study of the metabolism of chloropromazine. This method has since been used more widely in the analyses of ultramicro-amounts of amino acids,[3] amines,[4] steroids[5] and drugs[6] because this technique is not only very sensitive but also highly specific for qualitative and quantitative analyses.

In the previous papers,[7] we have reported that N-trifluoroacetyl (TFA)-L-prolyl derivatives of amino acids and amines show the common intense peak at *m/e* 166 in their mass spectra and are useful for the concurrent ultramicro-amounts (detection limit: *ca.* 10^{-10} g level) of amino acids and amines. In addition, we have suggested the possibility of the selective identification of these compounds by mass fragmentography or mass chromatography moni-

1) Location: *1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
2) C.-G. Hammar, B. Holmstedt, and R. Ryhage, *Anal. Biochem.*, **25**, 532 (1968).
3) R.E. Summons, W.E. Perelra, W.E. Reynolds, T.C. Rindfleisch, and A.M. Duffield, *Anal. Chem.*, **46**, 582 (1974).
4) *a*) S.H. Koslow, F. Cattabeni, and E. Costa, *Science*, **176**, 177 (1972); *b*) F. Karoum, F. Cattabeni, E. Costa, C.R.J. Ruthven, and M. Sandler, *Anal. Biochem.*, **47**, 550 (1972); *c*) F.P. Abramson, M.W. McCaman, and R.E. McCaman, *Anal. Biochem.*, **51**, 482 (1974).
5) B.F. Maume and J.A. Luyten, *J. Chromatogr. Sci.*, **11**, 607 (1973).
6) H. Miyazaki and H. Abuki, *Chem. Pharm. Bull.* (Tokyo), **24**, 2572 (1976).
7) *a*) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **25**, 285 (1977); *b*) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **25**, 1215 (1977).

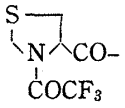
tored the ion at *m/e* 166 or other diagnostic ions. Although the retention times are different, both amino acids and amines can be detected when mass fragmentography is carried out at *m/e* 166. In this study, we examined five derivatives (N-TFA-L-4-thiazolidinecarbonyl, TFA, benzoyl, pentafluorobenzoyl (PFB) and trimethylsilyl (TMS)) of the five amines (isobutylamine, 3-methylthiopropylamine, β-phenylethylamine, tyramine and dopamine) in order to find more preferable derivatives than N-TFA-L-prolyl derivative[7b] for the ultramicrodetermination and selective identification of amines by mass fragmentography or mass chromatography.

Experimental

Reagents——All solvents used in this study were of analytical reagent grade. Amine hydrochlorides and benzoyl chloride were purchased from Tokyo Kasei Co. Trifluoroacetic anhydride, pentafluorobenzoyl chloride, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and hypovials were obtained from Pierce Chemical Co. Pyridine was used after drying over NaOH pellets.

Derivatives——3-Methylthiopropylamine was prepared according to the literature[8] by adding tetraline and tetraline peroxide to methionine. N-TFA-L-4-thiazolidinecarbonyl, TFA, TMS, benzoyl and PFB amine derivatives were prepared in the same ways as for amino acids.[9]

Apparatus——A Hitachi RMU-6MG mass spectrometer with an 002 Datalizer using HITAC-10 computer was used. The operational conditions for this study were the same as described previously.[7a]

Table I. Fragmentation Patterns of N-TFA-L-4-thiazolidinecarbonylamine Derivatives 
(The ions below *m/e* 40 were omitted.)

	$M^{+\cdot}$		Base peak		2nd peak		3rd peak		4th peak		5th peak	
	m/e	%[a]	*m/e*	$\sum_{10}$%[b]	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Isobutylamine	284	—	101	12.2	87	93.3	184	62.5	69	52.7	59	51.6
3-Methylthiopropylamine	316	3.0	160	8.1	159	72.4	41	71.8	61	71.2	112	65.0
β-Phenylethylamine	332	—	104	12.7	176	75.2	105	64.8	91	41.2	184	46.0
Tyramine[c]	420	—	192	31.7	73	61.6	193	28.6	179	16.1	177	11.3
Dopamine[c]	508	—	73	23.9	280	86.0	281	23.3	267	15.5	179	14.8

a) Relative intensity (base peak=100).
b) Per cent of the total ionization over *m/e* 10.
c) *o*-Trimethylsilylated.

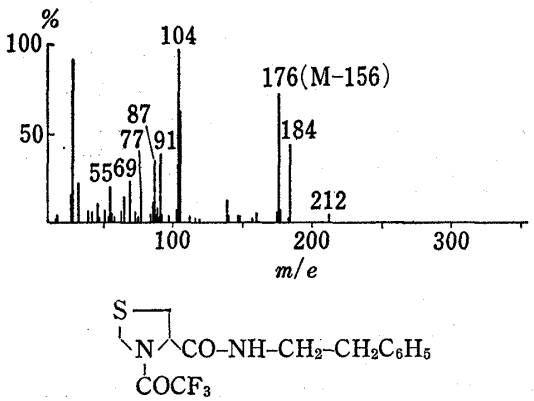


Fig. 1. Mass Spectrum of N-TFA-L-4-thiazolidinecarbonyl-β-phenylethylamine

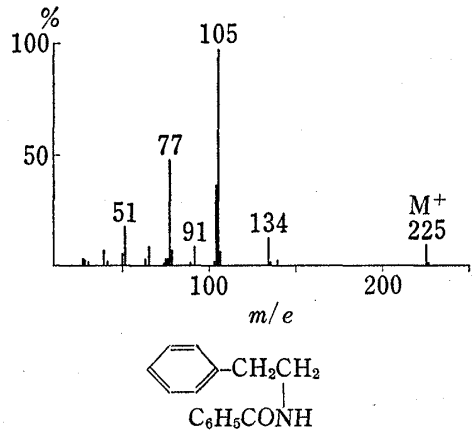


Fig. 2. Mass Spectrum of Benzoyl-β-phenylethylamine

8) T. Suyama and S. Kanao, *Yakugaku Zasshi*, **84**, 1012 (1964).
9) H. Iwase and A. Murai, *Anal. Biochem.*, **78**, 340 (1977).

Table II. Fragmentation Patterns of Benzoylamine Derivatives C_6H_5CO–

	M+·		Base peak		2nd peak		3rd peak		4th peak		5th peak	
	m/e	%	*m/e*	Σ_{10}%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Isobutylamine	177	12.2	105	30.9	77	43.7	51	31.7	122	15.4	41	12.5
3-Methylthiopropylamine	209	3.1	105	20.9	77	77.9	162	40.9	134	40.2	51	31.6
β-Phenylethylamine	225	9.9	105	32.6	77	50.4	104	39.7	51	19.6	134	14.2
Tyramine[a)]	313	—	105	44.3	77	55.9	51	21.9	106	9.1	226	7.3
Dopamine[a)]	401	—	105	44.9	77	38.2	303	15.7	51	10.8	106	8.7

a) *o*-Trimethylsilylated.

Table III. Fragmentation Patterns of PFB-amine Derivatives C_6F_5CO–

	N+·		Base peak		2nd peak		3rd peak		4th peak		5th peak	
	m/e	%	*m/e*	Σ_{10}%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Isobutylamine	267	5.1	195	31.0	167	22.3	41	23.7	212	18.7	117	13.2
3-Methylthiopropylamine	299	5.1	195	21.2	252	32.3	167	26.9	61	23.7	206	20.5
β-Phenylethylamine	315	1.4	104	25.9	195	86.0	91	45.6	65	22.8	167	18.8
Tyramine[a)]	403	—	195	36.6	314	26.3	167	18.6	196	9.8	117	5.7
Dopamine[a)]	491	—	195	36.6	314	25.7	167	15.6	196	7.2	117	5.0

a) *o*-Trimethylsilylated.

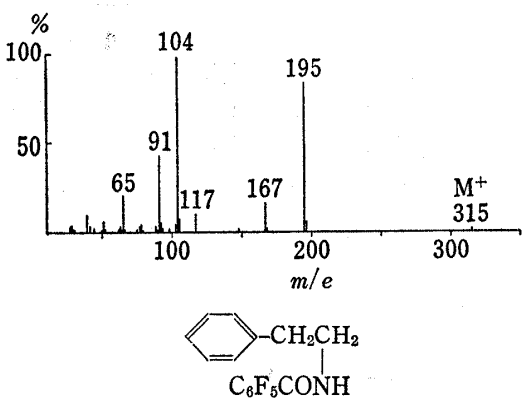


Fig. 3. Mass Spectrum of PFB-β-phenylethylamine

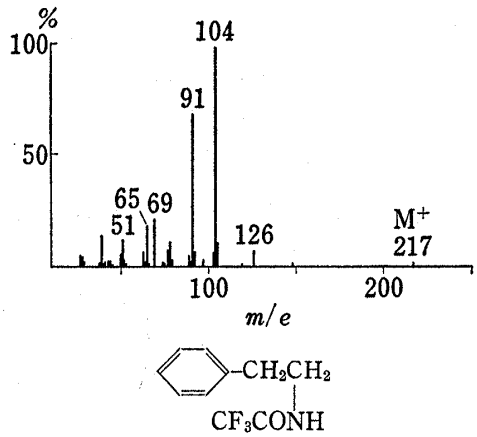


Fig. 4. Mass Spetcrum of TFA-β-phenylethylamine

Table IV. Fragmentation Patterns of TFA Derivatives CF_3CO–

	M+·		Base peak		2nd peak		3rd peak		4th peak		5th peak	
	m/e	%	*m/e*	Σ_{10}%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Isobutylamine	169	—	41	12.9	43	75.7	127	75.1	56	73.0	69	68.1
3-Methylthiopropylamine	201	45.4	61	8.7	69	82.0	41	73.2	45	67.9	154	54.8
β-Phenylethylamine	217	1.6	104	27.7	91	70.5	69	23.1	65	20.2	105	12.8
Tyramine	329	—	69	18.8	216	88.4	78	28.2	203	26.2	126	25.2
Dopamime	441	—	69	19.4	328	77.1	126	67.4	77	25.0	78	24.3

Table V. Fragmentation Patterns of Trimethylsilylated Amine Derivatives $(CH_3)_3Si$-

	$M^{+\cdot}$		Base peak		2nd peak		3rd peak		4th peak		5th peak	
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Isobutylamine	217	—	43	19.6	174	81.0	73	69.9	45	23.1	59	21.1
3-Methylthiopropylamine	249	7.2	174	20.0	73	84.5	59	36.4	86	33.4	45	22.4
β-Phenylethylamine	265	—	174	27.3	73	68.5	91	50.5	86	26.3	59	20.6
Tyramine[a)]	353	—	174	27.7	73	99.4	175	22.6	86	16.2	59	14.8
Dopamine[a)]	441	—	174	38.4	73	77.1	175	18.6	86	8.8	45	8.2

a) *o*-Trimethylsilylated.

Results

As a preliminary examination to mass fragmentography, the mass spectra of derivatives of five amines were studied. The *m/e* values and relative intensities of peaks with 1st to 5th intensities (per cent of total ionization for base peaks and pattern coefficients for the others) are shown in Tables I—V. The mass spectra of five different derivatives of β-phenylethylamine are also given in Figs. 1—5.

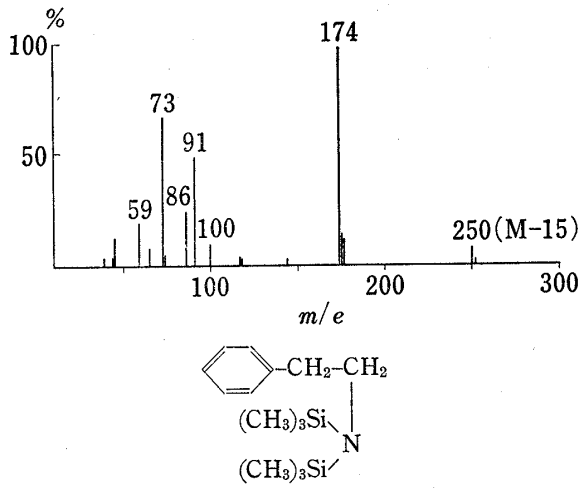
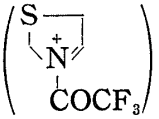
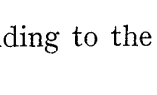
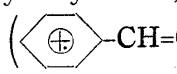
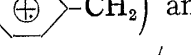
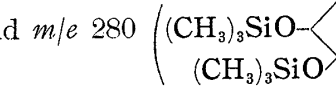


Fig. 5. Mass Spectrum of Trimethylsilylated β-Phenylethylamine

N-TFA-L-4-thiazolidinecarbonyl Derivative

N-TFA-L-4-thiazolidinecarbonyl derivatives of tyramine and dopamine were further trimethylsilylated with BSTFA with 1% TMCS to obtain the sharp peaks in gas chromatography. Although the structure of this derivative is similar to that of the N-TFA-L-prolyl derivative,[7b] the fragmentation features of two derivatives are rather different. This derivative produces the specific ion at *m/e* 184  for isobutylamine, 3-methylthiopropylamine and β-phenylethylamine, corresponding to the ion at *m/e* 166  of the N-TFA-L-prolyl derivative.[7b] Molecular ion was detected only for 3-methylthiopropylamine, having the relative intensity of 3%. The base peaks of isobutylamine, 3-methylthiopropylamine, β-phenylethylamine, tyramine and dopamine derivatives exist at *m/e* 101 (M—183), 160 (M—156), 104  , 192 and 73 ($(CH_3)_3Si^+$), respectively. The specific ions were observed at *m/e* 61 $(CH_3\overset{+}{S}CH_2)$ for 3-methylthiopropylamine, *m/e* 91  and 176 (M—156) for β-phenylethylamine, *m/e* 179, 177 and 73 for tyramine, and *m/e* 280  , 267 and 179 for dopamine.

Benzoyl and PFB Derivatives

Tyramine and dopamine were converted to benzoyl and PFB derivatives, which were further trimethylsilylated with BSTFA with 1% TMCS in order to obtain the definite peaks. Benzoyl derivatives produce the common characteristic base peak at *m/e* 105 ($C_6H_5CO^+$) and specific common peak at *m/e* 77 ($C_6H_5^+$). PFB derivatives produce the base peak at *m/e* 104 for β-phenylethylamine and *m/e* 195 ($C_6F_5CO^+$) for other four PFB amine derivatives and also produce the specific common ion at *m/e* 167 ($C_6F_5^+$). Both derivatives produce the molecular ions except for the trimethylsilylated derivatives. The sharp and symmetrical peaks were not obtained for both benzoyl and PFB derivatives in gas chromatograms and many unknown peaks, which had the common ions at *m/e* 105 or 195 in their mass spectra, were also observed in the gas chromatograms of these derivatives.

TFA Derivative

As can be seen in Table IV, this derivative does not produce the common base peak. The ion at *m/e* 126 ($CF_3C\overset{+}{O}NH{=}CH_2$) was detected for TFA-isobutylamine (53.8%), TFA-3-methylthiopropylamine (33.6%), TFA-β-phenylethylamine (8.5%), TFA-tyramine (25.2%) and TFA-dopamine (67.4%). Molecular ions were detected for 3-methylthiopropylamine derivative (45.4%) and β-phenylethylamine derivative (1.6%). TFA-3-methylthiopropylamine produces the base peak at *m/e* 61, TFA-tyramine and TFA-dopamine produce the common base peak at *m/e* 69, and characteristic peaks at *m/e* 216 (88.4%) and 328 (77.1%) which are formed by the loss of CF_3COO group from molecular ions. TFA-β-phenylethylamine produces the specific base peak at *m/e* 104, and the characteristic ions at *m/e* 91 and 65.

TMS Derivative

Although the fragmentation of this derivative has been reported,[4c] the spectra of this derivative were recorded and comparison was made with other four derivatives examined in this study for the selective and concurrent ultramicrodetermination of amines. TMS derivatives showed the common trimethylsilylated ions at *m/e* 45 ($(CH_3)\overset{+}{S}iH_2$), *m/e* 59 ($(CH_3)_2\overset{+}{S}iH$), 73 ($(CH_3)_3\overset{+}{S}i$), 86 ($(CH_3)_2Si{=}\overset{+}{N}{=}CH_2$), 100 ($(CH_3)_3Si{=}\overset{+}{N}{=}CH$) and 174 $\left(\begin{matrix}(CH_3)_3Si\\(CH_3)_3Si\end{matrix}\right)\!\!>\overset{+}{N}{=}CH_2$. Molecular ion was detected for 3-methylthiopropylamine derivative, having the relative intensity of 7.2%. TMS derivative produces the base peak at *m/e* 43 for isobutylamine and *m/e* 174 for 3-methylthiopropylamine, β-phenylethylamine, tyramine and dopamine. The diagnostic ion at *m/e* (M−15) appears for isobutylamine (*m/e* 202, 16.4%), 3-methylthiopropylamine (*m/e* 234, 11.7%), β-phenylethylamine (*m/e* 250, 7.8%), tyramine (*m/e* 238, 8.8%) and dopamine (*m/e* 426, 3.8%). This derivative shows a high abundance of a common fragment ion at *m/e* 174.

Table VI. Base Peak of Different Derivatives of Amines

	1		2		3		4		5		6	
	m/e	Σ_{10}%	*m/e*	Σ_{10}%	*m/e*	Σ_{10}%	*m/e*	Σ_{10}%	*m/e*	Σ_{10}%	*m/e*	Σ_{10}%
Isobutylamine	166	19.5	101	12.2	105	30.9	195	31.0	41	12.9	43	19.6
3-Methylthiopropylamine	166	21.5	160	8.1	105	20.9	195	21.2	61	8.7	174	20.0
β-Phenylethylamine	104	23.6	104	12.7	105	32.6	104	25.9	104	27.7	174	27.3
Tyramine	192	28.6	192	31.7	105	44.3	195	36.6	69	18.8	174	27.7
Dopamine	192	23.8	73	23.9	105	44.9	195	36.6	69	19.4	174	38.4

(1) N-TFA-L-prolyl derivatives,[7b] (2) N-TFA-L-thiazolidinecarbonyl derivatives, (3) benzoyl derivatives, (4) PFB derivatives, (5) TFA derivatives, (6) trimethylsilylated derivatives.

Discussion

The *m/e* values and $\sum_{10}\%$ values of the base peaks of six kinds of amine derivatives including the previous reported N-TFA-L-prolyl derivative[7b] are summarized in Table VI.

Data in Table VI and Tables I—V show that N-TFA-L-prolyl derivative,[7b] benzoyl, PFB and TMS derivatives are considered as the preferred derivatives for the concurrent ultramicrodetermination of amines by mass fragmentography because these four derivatives produce the common intense fragment ions at *m/e* 166, 105, 195 and 174, respectively. Benzoyl and PFB derivatives, however, were not useful for the ultramicrodetermination of amines because these derivatives did not show the sharp and symmetrical peaks in gas chromatograms. TFA and N-TFA-L-4-thiazolidinecarbonyl derivatives were not useful for the concurrent determination of amines because these derivatives did not produce the common intense ions. However, these derivatives are useful for the selective identification of amines by mass fragmentography monitored the each diagnostic ion. The previous papers[7] reported that both amino acids and amines after leading to N-TFA-L-prolyl derivatives were eluted in mass fragmentograms monitored the ion at *m/e* 166, although their retention times are different. From the point of view mentioned above, TMS derivative is the most preferable one for the concurrent ultramicrodetermination and selective identification of amines because this derivative produces the characteristic intense peak at *m/e* 174, which is specific for amines. In addition, the ion at *m/e* 174 is not detected in the TMS derivatives of the most of amino acids[4c] and the preparation of TMS derivative is simpler than that of the other derivatives. Fig. 6 and Fig. 7 show the selective identification of trimethylsilylated 3-methylthiopropylamine, β-phenylethylamine, tyramine and dopamine by mass chromatography.

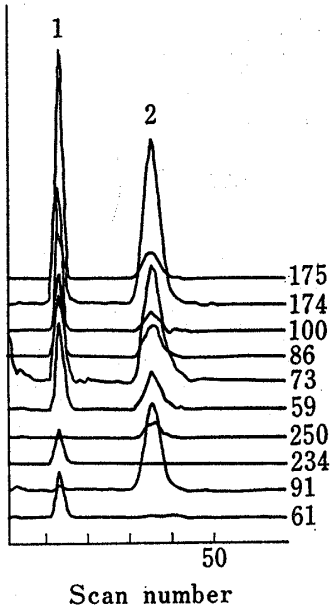


Fig. 6. Mass Chromatogram of Trimethylsilylated Amines

1, 3-methylthiopropylamine.
2, β-phenylethylamine.

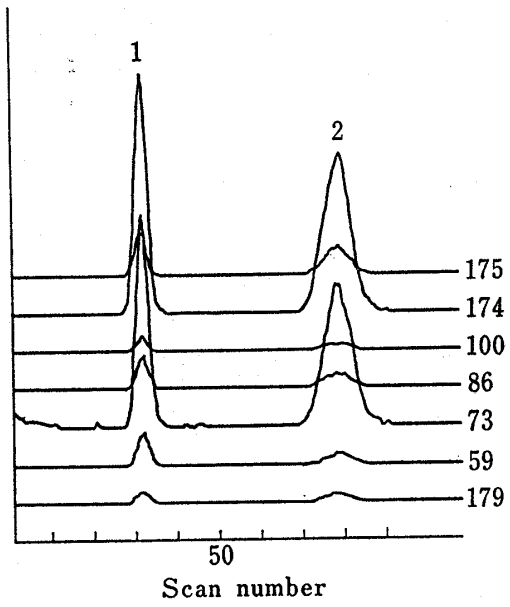


Fig. 7. Mass Chromatogram of Trimethylsilylated Amines

1, tyramine.
2, dopamine.

Fig. 6 shows a possibility for the selective identification of 3-methylthiopropylamine and β-phenylethylamine by monitoring the common peaks at *m/e* 175, 174, 100, 86, 73, 59 and diagnostic ions at *m/e* 250 (M—15) and 91 for β-phenylethylamine, *m/e* 234 (M—15) and 61 for 3-methylthiopropylamine. Similarly, as can be seen in Fig. 7, tyramine and dopamine are

identified by monitoring the ions at *m/e* 175, 174, 100, 86, 73, 59 and specific ion at *m/e* 179 for hydroxyamines.

Fig. 8 and Fig. 9 also show the ultramicrodetermination of the trimethylsilylated amines (3-methylthiopropylamine, β-phenylethylamine, tyramine and dopamine) by mass fragmentography monitored the ion at *m/e* 174.

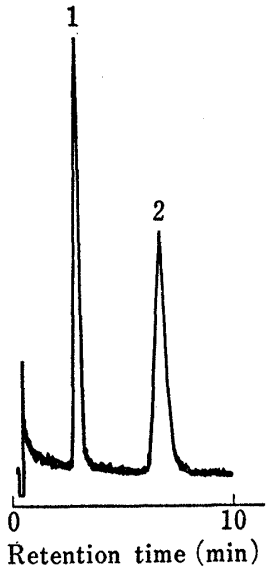


Fig. 8. Mass Fragmentogram of Trimethylsilylated Amines

1, 3-methylthiopropylamine. 2, β-phenylethylamine.
Column: glass, (1 m×3 mm i.d.), packed with 1.5% OV-101 on 80—100 mesh Diatoport S.
Column temperature: 90°.
Monitored ion: *m/e* 174.
Sample, 2.5 ng of each amine injected.

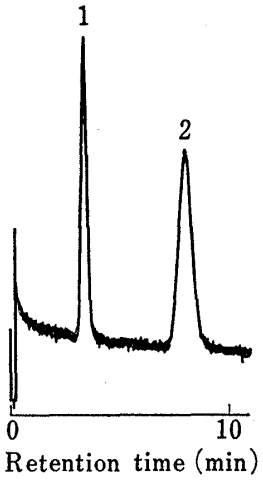


Fig. 9. Mass Fragmentogram of Trimethylsilylated Amines

1, tyramine. 3, dopamine.
Column: glass, (1 m×3 mm i.d.), packed with 1.5% OV-101 on 80—100 mesh Diatopot S.
Column temperature: 140°.
Monitored ion: *m/e* 174.
Sample, 2.5 ng of each amine injected.

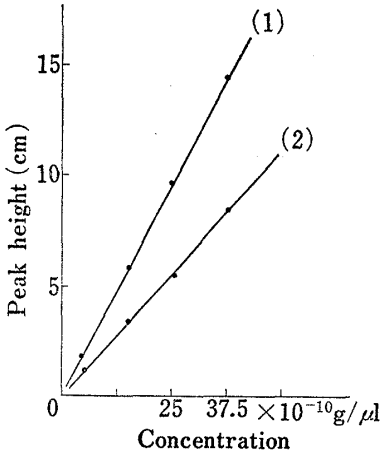


Fig. 10. Calibration Curve Showing Peak Height Mass Fragment Ionic Intensity plotted against Concentration of Trimethylsilylated Amines

The mass spectrometer was focused on the base peak at *m/e* 174.
(1); 3-methylthiopropylamine,
(2); β-phenylethylamine.

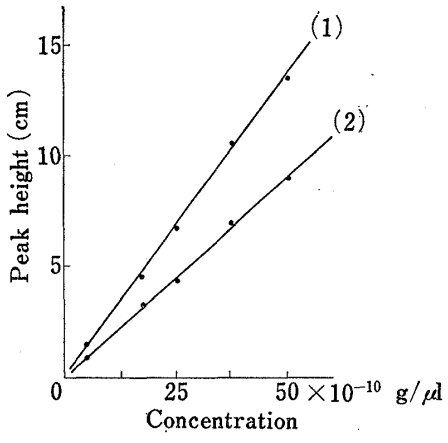


Fig. 11. Calibration Curve Showing Peak Height Mass Fragment Ionic Intensity plotted against Concentration of Trimethylsilylated Amines

The mass spectrometer was focused on the base peak at *m/e* 174.
(1); tyramine, (2); dopamine.

This method has a sufficient sensitivity for the ultramicrodetermination of the amines tested. After *ca.* 40 pico gram (10^{-11} g level) of each trimethylsilylated amine was subjected to mass fragmentography, the peaks on the chromatograms were sharp and sufficient intensity ($S/N=2$). The preceding paper[7b] reported that the detection limit of N–TFA-L-prolyl derivative was about 10^{-10} g level. Therefore, TMS derivative was superior to N–TFA-L-prolyl derivative for the purpose of ultramicrodetermination. Some of the calibration curves are shown in Fig. 11 and Fig. 12. The calibration curves were linear in the range of 5 to 50 $\times 10^{-10}$ g/μl.