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# N-Trifluoroacetyl-L-prolylamine Derivatives for the Ultramicrodetermination of Amines by Mass Fragmentography

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The fragmentation of 21 kinds of biochemically important amines after leading to N-trifluoroacetyl-L-prolyl derivatives is investigated for the concurrent ultramicrodetermination or the selective identification of each amine. It was found that N-trifluoroacetyl L-prolylamine derivatives are useful for the concurrent determination of ultra micro-amounts (about $10^{-10}$ g level) of amines by mass fragmentography or the selective identification of amines by monitoring the each diagnostic peaks.

**Keywords**——N-trifluoroacetyl-L-prolylamine derivatives; mass spectrometry; mass chromatography; mass fragmentography; ultramicrodetermination of amines

In our preceding work,[2] examination of the mass spectra of N-trifluoroacetyl (TFA)-L-prolyl amino acid *n*-butyl esters indicated that these derivatives produced an intense ion at *m/e* 166 common to all amino acids or the ions specific to each amino acid, and we indicated the possibility of the concurrent ultramicrodetermination or the selective identification of amino acids using mass fragmentography by monitoring these ions.

A number of physiologically important amines have been successfully analysed by gas chromatography.[3] In the field of mass spectrometry, mass spectra of trimethylsilyl,[4] perfluoroacyl,[5] and N-TFA-L-prolyl derivatives of amines have been examined to date. Murano[6] examined the mass spectra of N-TFA-L-prolyl derivatives of 1-phenyl or naphthylalkylamines but he did not mention the possibility of ultramicroanalyses of these amines by mass fragmentography.

In the present work, 21 kinds of biochemically important amines, which had not hitherto been examined, were led to their N-TFA-L-prolyl derivatives and their mass spectra were measured by gas chromatography–mass spectrometry. The present paper also deals with the possibility of concurrent ultramicrodetermination or the selective identification of amines by mass fragmentography and mass chromatography.

## Experimental

**Apparatus and Conditions**——A Hitachi RMU-6MG mass spectrometer with 002 Datalizer using HITAC-10 computer was used. Operational conditions were the same as those reported previously.[2]

**Reagents and Chemicals**——All the solvents used were commercial special reagent grade. Amine salts were purchased from Tokyo Kasei Co. and Sigma Chemical Co. N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) and hypovials were purchased from Pierce Co. Pyridine was used after drying over NaOH pellets.

1) Location: *1–1, Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
2) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **25**, 285 (1977).
3) E.C. Horning, M.G. Horning, W.J.A. VandenHeuvel, K.L. Knox, B.G. Holmstedt, and C.J.W. Brooks, *Anal. Chem.*, **36**, 1546 (1964); S. Kawai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 699 (1968); A.G. Moffat and E.C. Horning, *Anal. Lett.*, **3**, 205 (1970); A.C. Moffat, E.C. Horning, S.B. Matin, and M. Rowland, *J. Chromatog.*, **66**, 255 (1972); S.B. Matin and M. Rowland, *J. Pharm. Sci.*, **61**, 1235 (1972).
4) F.P. Abramson, M.W. McCaman, and R.E. McCaman, *Anal. Biochem.*, **51**, 482 (1974).
5) E. Anggard and G. Sedvall, *Anal. Chem.*, **41**, 1250 (1969); S.H. Koslow, E. Cattabeni, and E. Costa, *Science*, **176**, 177 (1972); F. Karoum, F. Cattabeni, E. Costa, C.R.J. Ruthven, and M. Sandler, *Anal. Biochem.*, **47**, 550 (1972).
6) A. Murano, *Agr. Biol. Chem.* (Tokyo), **37**, 981 (1973).

**Preparation of 1-Amino-2-propanol and 3-Methylthiopropylamine**——These were prepared according to the literatures[7,8] by adding tetralin and tetralin peroxide respectively to threonine and methionine.

**Preparation of N-TFA-L-prolylamine Derivatives**——These amine derivatives were prepared in the same way as for amino acids.[2] In order to obtain a sharp and symmetrical peak in gas chromatography, N-TFA-L-prolyl derivatives of hydroxyamines and tryptamine were further trimethylsilylated with BSTFA with 1% TMCS.

## Result and Discussion

Table I shows the *m/e* values and relative intensities of peaks with 1st to 5th intensities (per cents of total ionization for base peaks and pattern coefficients for the others).

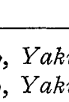
TABLE I. Five Peaks of Mass Spectra of N–TFA-L-prolylamine Derivatives
(The ions below *m/e* 40 were omitted.)

| Amine | Molecular ion | | 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* | % |
| Methylamine | 224 | — | 166 | 22.7 | 69 | 39.9 | 41 | 26.0 | 96 | 13.2 | 42 | 11.1 |
| Ethylamine | 238 | 5.8 | 166 | 14.8 | 167 | 69.4 | 69 | 52.9 | 139 | 47.5 | 41 | 39.5 |
| *n*-Propylamine | 252 | 5.3 | 166 | 15.6 | 167 | 89.9 | 41 | 45.3 | 139 | 44.8 | 69 | 38.1 |
| *n*-Butylamine | 266 | 3.4 | 167 | 18.1 | 166 | 99.3 | 41 | 43.7 | 139 | 40.2 | 69 | 27.0 |
| Isopropylamine | 252 | 1.3 | 167 | 16.8 | 166 | 73.8 | 41 | 46.6 | 139 | 48.3 | 69 | 42.3 |
| Isobutylamine | 266 | 2.5 | 166 | 19.5 | 167 | 80.3 | 41 | 45.6 | 139 | 31.7 | 69 | 26.6 |
| Isoamylamine | 280 | 5.1 | 167 | 17.6 | 166 | 92.8 | 41 | 42.2 | 139 | 36.2 | 69 | 31.6 |
| Pyrrolidine | 264 | 23.1 | 98 | 16.9 | 166 | 89.4 | 55 | 68.5 | 70 | 36.1 | 56 | 29.8 |
| Benzylamine | 300 | 9.2 | 166 | 13.9 | 106 | 93.6 | 91 | 63.9 | 167 | 61.1 | 139 | 32.3 |
| β-Phenylethylamine | 314 | 3.4 | 104 | 23.6 | 166 | 80.8 | 105 | 21.2 | 167 | 19.4 | 69 | 19.3 |
| 3-Methylthiopropyl-amine | 298 | 11.8 | 166 | 21.5 | 167 | 47.1 | 251 | 19.8 | 139 | 19.1 | 69 | 17.2 |
| Putrescine | 474 | 2.3 | 166 | 41.9 | 70 | 23.2 | 308 | 21.2 | 167 | 20.0 | 69 | 13.9 |
| Cadaverine | 488 | 3.3 | 166 | 30.5 | 322 | 21.5 | 70 | 16.5 | 41 | 15.6 | 69 | 14.0 |
| 1-Amino-2-propanol | 340 | — | 73 | 17.9 | 117 | 82.1 | 166 | 60.3 | 75 | 20.4 | 296 | 17.9 |
| Tyramine | 402 | 1.6 | 192 | 28.6 | 73 | 36.9 | 166 | 26.8 | 179 | 23.1 | 193 | 20.5 |
| Dopamine | 490 | — | 192 | 23.8 | 73 | 90.3 | 193 | 55.7 | 177 | 15.5 | 179 | 14.1 |
| 3-Methoxytyramine | 432 | 10.8 | 222 | 19.8 | 73 | 55.6 | 166 | 35.6 | 209 | 31.8 | 192 | 26.4 |
| Tryptamine | 425 | 14.2 | 202 | 22.3 | 73 | 84.6 | 215 | 69.8 | 166 | 18.7 | 203 | 18.2 |
| Serotonine | 513 | — | 73 | 22.5 | 215 | 77.3 | 202 | 64.0 | 216 | 30.8 | 203 | 13.5 |
| Octopamine | 490 | — | 267 | 25.7 | 73 | 76.6 | 268 | 26.2 | 166 | 23.0 | 269 | 11.4 |
| Ephedrine | 430 | — | 58 | 18.6 | 166 | 83.5 | 73 | 62.9 | 179 | 32.8 | 251 | 31.4 |

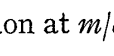
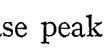
*a*) relative intensity (bease peak=100)
*b*) per cent of the total ionization over *m/e* 10

### N-TFA-L-prolylamines

Thirteen kinds of N-TFA-L-prolylamines (methylamine, ethylamine, *n*-propylamine, *n*-butylamine, isopropylamine, isobutylamine, isoamylamine, pyrrolidine, benzylamine, β-phenylethylamine, 3-methylthiopropylamine, putrescine and cadaverine) showed a peak with high intensity at *m/e* 166 or 167. Molecular ions were detected for all the amines except for methylamine, and it was especially strong for pyrrolidine, having a relative intensity of 23.1%.

As reported previously,[2] the base peak of N-TFA-L-prolyl proline *n*-butyl ester was detected at *m/e* 70 but for the corresponding amine, pyrrolidine, the base peak was appeared at *m/e* 98 (M–166,  ) and not appeared at *m/e* 70 (M–194, 36.1%). In addition, charac-

7) T. Suyama and S. Kanao, *Yakugaku Zasshi*, **85**, 531 (1965).
8) T. Suyama and S. Kanao, *Yakugaku Zasshi*, **84**, 1012 (1964).

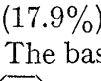
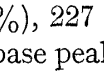
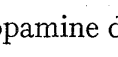
teristic ion was detected at *m/e* 126 (12.0%) for pyrrolidine derivative. Benzylamine derivative forms a characteristic ion at *m/e* 106 (  , 93.6%). The base peak of phenylalanine derivative[2] was detected at *m/e* 166 and the amine corresponding to it, β-phenylethylamine, showed the base peak at *m/e* 104 (  ). Characteristic peaks of N-TFA-L-prolyl-3-methylthiopropylamine were detected at *m/e* 251 (M-$SCH_3$, 19.8%), 132 (M-166, 7.6%), 104 (M-194, 3.8%), and 61 ($CH_3\overset{+}{S}CH_2$, 16.4%). Among these, peaks corresponding to *m/e* 251 and 132 were not detected in methionine derivative.[2]

In putrescine and cadaverine derivatives, the peak at *m/e* (M-166), which were observed for ornithine and lysine derivatives,[2] were also detected at *m/e* 308 (21.2%) and 322 (21.5%), respectively.

There was no notable difference in the fragmentation between N-TFA-L-prolyl derivatives of primary and secondary amines.

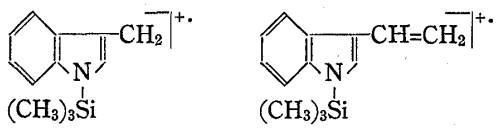
**Trimethylsilylated N-TFA-L-prolylamines**

The amines examined in this group were 1-amino-2-propanol, tyramine, dopamine, tryptamine, serotonine, octopamine, ephedrine, and 3-methoxytryptamine. The base peak of threonine derivative[2] was detected at *m/e* 117 but that of 1-amino-2-propanol derivative was observed at *m/e* 73 ($(CH_3)_3Si^+$), though the peak at *m/e* 117 had a high intensity. The characteristic peaks of 1-amino-2-propanol derivative were detected at *m/e* 325 (M-15, 7.7%), 296 (17.9%), 227 (12.6%) and 199 (7.5%).

The base peaks of tyramine and dopamine derivatives were detected at *m/e* 192 ($(CH_3)_3Si$–O–  ), that of 3-methoxytyramine derivative at *m/e* 222 ($(CH_3)_3Si$–O–  ), and that of octopamine derivative at *m/e* 267 ($(CH_3)_3Si$–O–  ). Other characteristic peaks were observed for tyramine and dopamine derivatives at *m/e* 179 and 177, and those of 3-methoxytyramine derivative at *m/e* 209 and 207.

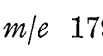
Among these four kinds of catecholamines, molecular ions were detected for tyramine and 3-methoxytyramine derivatives, in which one trimethylsilyl group was introduced, but the peaks at *m/e* (M-15) were not detected. On the other hand, molecular ions were not detected for dopamine and octopamine derivatives, in which two trimethylsilyl groups were introduced, and the peaks of *m/e* (M-15) were detected at *m/e* 475 (1.5%) and 475 (2.4%), respectively.

For tryptamine and serotonine derivatives, peaks of high intensity were detected commonly at *m/e* 73, 202 and 215, and the base peak was observed respectively at *m/e* 202 and 73. The structure of the ions at *m/e* 202 and 215 are assumed to be as shown below. Other characteristic fragmentation were observed as the same in the above four kinds of catecholamines; tryptamine derivative with introduction of one trimethylsilyl group showed molecular ion but not the ion at *m/e* (M-15), and serotonine derivative with introduction of two trimethylsilyl groups inversely did not show molecular ion but showed the ion at *m/e* 498 (M-15, 2.4%).




*m/e* 202 *m/e* 215

Chart 1

The base peak of ephedrine derivative appeared at *m/e* 58 ($\overset{+}{N}H{=}CH$, $CH_3$ $CH_3$)[9] and characteristic peaks were observed at *m/e* 179 (  , 32.8%) and 251 (M-179, 31.4%).

9) H.H. Fales, G.W.A. Milne, H.U. Winkler, H.D. Beckey, J.N. Damico, and R. Barron, *Anal. Chem.*, **47**, 207 (1975).

Although one trimethylsilyl group was introduced into ephedrine derivative, molecular ion was not detected and an ion was observed at *m/e* 415 (M-15, 3.1%).

## Mass Chromatography

Mass chromatograms of N-TFA-L-prolyl-3-methylthiopropylamine, -β-phenylethylamine, -tyramine, and -dopamine are shown in Fig. 1 and 2.

As shown in Fig. 1, it will be possible to selectively detect 3-methylthiopropylamine and β-phenylethylamine by monitoring the peaks common to both at *m/e* 139 and 166, and the characteristic ions for these two amines. Similarly, as shown in Fig. 2, the peaks for tyramine and dopamine, can be recognized by monitoring the ions characteristic to these amines at *m/e* 73, 166, 177, 179, 192, and 193.

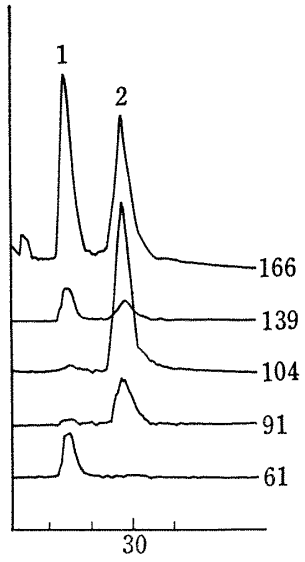



Fig. 1. Mass Chromatogram of N-TFA-L-prolyl Amines
1: 3-methylthiopropylamine
2: β-phenylethylamine

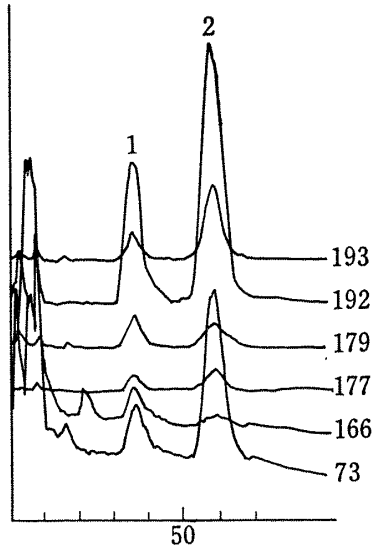



Fig. 2. Mass Chromatogram of Trimethylsilylated N-TFA-L-prolyl Amines
1: tyramine, 2: dopamine

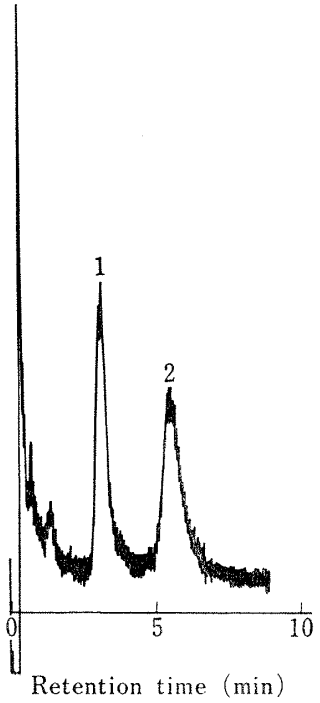



Fig. 3. Mass Fragmentogram of N-TFA-L-prolylamines
1: 3-methylthiopropylamine
2: β-phenylethylamine
column: glass, (50 cm × 3 mm i.d.), packed with modified OV-101[2] on Chromosorb W (AW)
column temperature: 135°
monitored ion: *m/e* 166
sample: about 2 ng of each amine injected

## Mass Fragmentography

In the previous work,[2] mass fragmentography was carried out on *n*-butyl esters of several N-TFA-L-prolyl-amino acids by monitoring the ion *m/e* 166, and it was found that they can be detected in approximately $10^{-10}$ g level. In the present work, mass fragmentography of N-TFA-L-prolyl derivatives of 3-methylthiopropylamine and β-phenylethylamine was carried out by monitoring the ion at *m/e* 166. Its chromatogram is shown in Fig. 3. Examination of the detection limit from the chromatogram showed that an ultramicroanalysis of these amines was possible at *ca.* $10^{-10}$ g level ($S/N=2$). The base peak of N-TFA-L-prolyl derivative of β-phenylethylamine was detected at *m/e* 104 and a higher sensitivity would be obtained if mass fragmentography is carried out at *m/e* 104.

Some of the amines examined in the present work showed a low relative intensity of the peak at *m/e* 166 (trimethylsilylated derivatives), contrary to the same derivatives of amino acid *n*-butyl esters. The peak at *m/e* 166 is observed in common to all the N-TFA-L-prolylamines, so that mass fragmentography by monitoring at *m/e* 166 should make it possible to carry out the concurrent ultramicrodetermination and general analysis of amines. In trimethylsilylated N-TFA-L-prolylamines except for tyramine and dopamine, the common base peaks were not observed, but mass fragmentography by monitoring the characteristic ions with high intensity for each of the amine derivatives would make it possible to identify each of the amines selectively and with a high sensitivity.

The experimental results indicated the full possibility for the ultramicrodetermination of amines if they were derived to N-TFA-L-prolyl derivatives. When mass fragmentography is carried out on N-TFA-L-prolyamine derivatives by monitoring the ion at *m/e* 166, amino acids will also be detected[2] if present, though their retention times in gas chromatography would be different. Further examinations are being made to develop a more rational derivatives for selective ultramicrodetermination of amines, and five kinds of derivatives (trimethylsilylated, benzoyl, pentafluorobenzoyl, TFA and N-TFA-L-4-thiazolidinecarbonyl derivatives) are now under examination.