Chem. Pharm. Bull. 29(3) 796—803 (1981)

The Use of a New Silylating Agent for Analysis of Catecholamines by Gas Chromatography-Mass Spectrometry

Hiroshi Miyazaki,*,[a] Masataka Ishibashi,[a] Kouwa Yamashita,[a] and Makoto Yakushiji[b]

Research Laboratories of Pharmaceutical Division, Nippon Kayaku Co.,[a] 3-31 Shimo, Kita-ku, Tokyo 115, Japan and Scientific Instrument Division, Dainiseikosha Co.,[b] 6-31 Kameido, Koto-ku, Tokyo 136, Japan

(Received August 13, 1980)

3-Methoxytyramine, dopamine, norepinephrine, epinephrine and 6-hydroxydopamine were derivatized to their N-trifluoroacetyl-O-dimethyl-*n*-propylsilyl ether or 2′-O-methyl-N-trifluoroacetyl-O-dimethyl-*n*-propylsilyl ether derivatives by treatment with trifluoroacetic anhydride, methanol and then dimethyl-*n*-propylsilyl imidazole. These derivatives could be separated completely by gas chromatography (GC) on a non-polar liquid stationary phase such as OV-101. In addition, these derivatives were very stable in benzene and ethyl acetate, and showed excellent stability during silica gel column chromatography in comparison with the corresponding trimethylsilyl ether derivatives. In GC-mass spectrometry (MS) of these derivatives, ammonia chemical ionization (CI)-MS provided the ion $[M+NH_4]^+$ as the base peak or an intense peak. The detection limit of the dopamine derivative in the CI mode with ammonia was 2 pg with S/N at 2:1.

Keywords——catecholamines; dimethyl-*n*-propylsilyl ethers; GC-EIMS; GC-CIMS; GC-CI-SIM; profile analysis; storage stability

Introduction

Much attention has been focused on the role of biologically active catecholamines *in vivo*. A number of microdetermination methods have been reported for investigations of the pharmacokinetics and distribution of these compounds in biological fluids and tissues. It has been considered that selected ion monitoring (SIM) is one of the best methods for trace determination of these amines with high specificity and high sensitivity. For this technique, it is essential to derivatize them to a thermally stable and volatile form. Several derivatization methods, such as perfluoroacylation,[1] trimethylsilylation,[2] N-trifluoroacetyl-O-trimethylsilylation,[3–5] and pentafluorobenzylimide-O-trimethylsilylation,[6] have been developed. Among these derivatives, the trifluoroacetates and the pentafluoropropionates of catecholamines have been widely used for gas chromatographic-mass spectrometric analyses because of their convenience. The use of pentafluoropropionate derivatives resulted in complete separation of norepinephrine and 6-hydroxydopamine[7,8] and in poor stability in organic solvents after derivatization.[1] The N-trifluoroacetylamido-O-trimethylsilyl ether (TFA–TMS) derivatives, which were developed to enhance the separation and stability of the catecholamine derivatives, have the disadvantage that by-product formation was observed during the process of derivatization at a very low concentration.[9,10]

Hosoda *et al.*[11] reported that steroids carrying phenolic *t*-butyldimethylsilyl (*t*-BDMS) ether group were more stable than those carrying the alcoholic ether group in acid, suggesting that the introduction of a *t*-BDMS ether group into catecholamines may improve their stability. In previous papers,[12,13] on the other hand, we have reported that the dimethylethylsilyl (DMES) and dimethyl-*n*-propylsilyl (DMnPS) ether derivatives of hydroxysteroids gave excellent results in gas chromatography-mass spectrometry.

The present paper deals with the gas chromatographic-mass spectrometric properties, the storage stabilities and the detection limits in gas chromatography-selected ion monitoring

of the DMnPS ether derivatives of N-trifluoroacetyl- and 2′-O-methyl-N-trifluoroacetylcatecholamines.

Experimental

Gas Chromatography——A Shimadzu GC-4BM gas chromatograph equipped with FID was employed. The column was 2 m×3 mm I.D. glass packed with 1.5% OV-101 (Ohio Valley Co., U.S.A.) on 80—100 mesh Gas Chrom Q (Applied Science Co., U.S.A.). The temperature of the column oven was maintained at 150—250° or was programmed from 180° at the rate of 5° min. The flow rate of the carrier gas (nitrogen) was 40 ml/min. The temperature of the injection port and detector was 270°.

Gas Chromatography-Mass Spectrometry——A DuPont 321 GC-MS system (Dimaspec®) equipped with a data processing system (DuPont 320) and an MID unit was employed. The column was 2 m×3 mm I.D. glass packed with 1.5% OV-101 on Gas Chrom Q. The temperature of the column oven was maintained at 180—250° or was programmed from 180° at the rate of 4° min. The flow rate of the carrier gas (helium) was 25 ml/min. The temperature of injection port and separator was 270°, and the ion source was kept at 290°. The ionization energy was 70 eV and the ionizing current was 1 mA for both electron impact ionization and chemical ionization (CI) modes. For CI mass spectrometry, methane, isobutane and ammonia were used as reagent gases. The pressure in the ion source housing was 3×10^{-5} torr to provide a suitable pressure in the ion chamber for CI conditions.

Samples and Reagents——The catecholamines used in this study were commercial products. Trimethylsilyl imidazole (TSIM) and trifluoroacetic anhydride (TFAAn) were purchased from Tokyo Kasei Kogyo Co., (Tokyo, Japan). Dimethyl-*n*-propylsilyl imidazole (DMnPSI) was prepared by the method described in our previous paper.[13] Trideuterated ammonia was purchased from Merck Sharp and Dohm (Japan) Co. (Tokyo, Japan).

Derivatization——The amines were converted into their N-trifluoroacetyl or 2′-O-methyl-N-trifluoroacetyl derivatives according to Änggard *et al.*,[14] and then silylated with TSIM or DMnPSI: Fifty to 100 μg each of amines was dissolved in 0.1 ml of a mixture of ethyl acetate and TFAAn (1: 1). After 30 min at room temperature, the solvent and reagent were evaporated off under reduced pressure. Methanol (1 ml) was added to the residue. The mixture was allowed to stand for 1 hr at room temperature then evaporated to dryness. The residue was dissolved in 0.2 ml of benzene or ethyl acetate and then silylated with 20 μl of TSIM or DMnPSI. These reactions are outlined in Chart I for dopamine and norepinephrine as representative compounds.

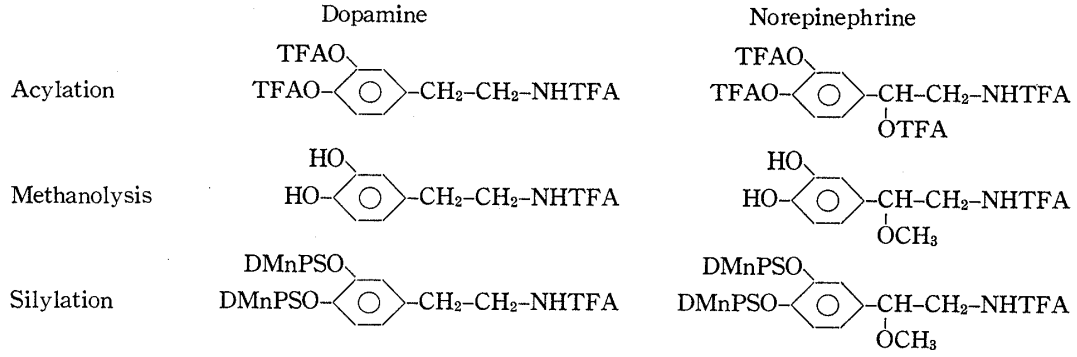

Chart 1

Results and Discussion

In this study, 3-methoxytyramine (3-MT), dopamine (DA), 6-hydroxydopamine (6-HDA), norepinephrine (NE) and epinephrine (E) were used as representative catecholamines. These amines were converted into the N-trifluoroacetyl-O-dimethyl-*n*-propylsilyl ether (TFA–DMnPS) derivatives by following the derivatization procedure described in "Experimental."

Figure 1 shows a gas chromatogram of the reaction product of NE after the above derivatization. This product exhibited a single, well-shaped peak on its gas chromatogram when analyzed on a non-polar liquid stationary phase such as OV-101, indicating that NE was converted almost quantitatively into the 2′-O-methyl-N-trifluoroacetyl-O-dimethyl-*n*-propylsilyl ether derivatives (TFA–DMnPS) derivative[15] without formation of by-products.

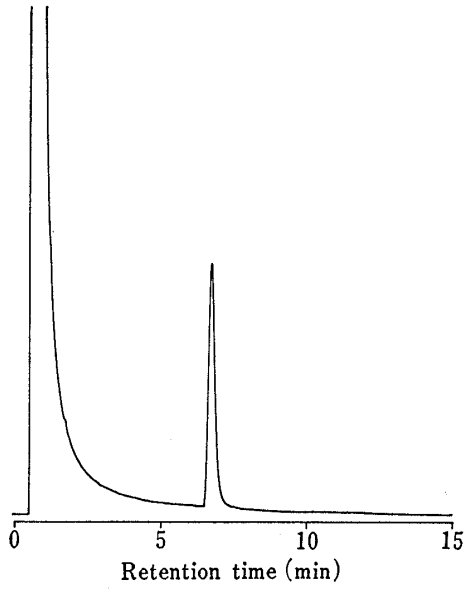

Fig. 1. Gas Chromatogram of the Reaction Product of Norepinephrine obtained by treatment with TFAAn, MeOH and then DMnPSI

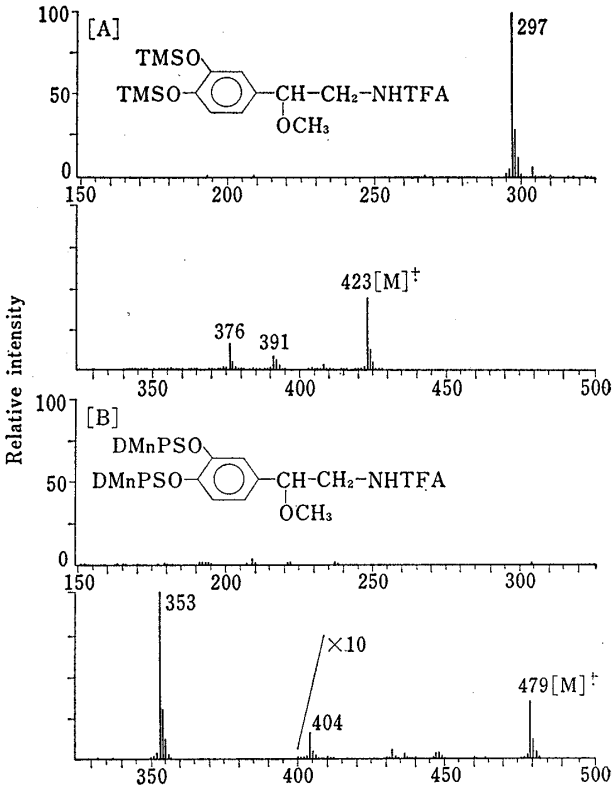

Fig. 2. Mass Spectra of the Norepinephrine TFA–TMS (A) and TFA–DMnPS (B) Derivatives

The mass spectrum of the NE TFA–DMnPS derivative is shown in Fig. 2, together with that of the corresponding trimethylsilyl ether (TMS) derivative.[4] The shifts of the molecular ion from m/z 423 to 479 and of a fragment ion from m/z 297 $[M-CH_2NHTFA]^+$ to 353 indicate the incorporation of the DMnPS group into NE. The mass fragmentation pattern of the DMnPS ether derivative is quite similar to that of the TMS ether except for the 28-mass -unit shift for each hydroxyl group in the underivatized amine.

Salient gas chromatographic (GC) and GC-mass spectrometric data are given in Table I. The structures of these catecholamine derivatives were supported by the observed molecular ion and characteristic fragment ions.

The mass spectra of the DMnPS ether derivatives have been characterized by the molecular ion $[M]^{+\cdot}$, and the characteristic fragment ions $[M-15]^+$ and $[M-43]^+$, as in the case of hydroxysteroid samples.[11,12] The ion cluster of $[M]^{+\cdot}$, $[M-15]^+$ and $[M-43]^+$ was also observed clearly in the mass spectra of the TFA–DMnPS derivatives of 3-MT, DA and 6-HDA. The molecular ion and the $[M-43]^+$ ion (corresponding to loss of the n-propyl group from the molecular ion)

Table I. GC and GC–MS Data for the Catecholamine TFA–DMnPS Derivatives

Catecholamine	MU value[a] (OV-101)	Molecular weight	Mass spectrometric data (Relative intensity %)[b]	
			Base peak	Other ions
3-Methoxytyramine	19.19	363	320$[M-43]^+$	126(42) 179(65) 192(58) 273(28) 363(23)
Dopamine	21.37	449	449$[M]^{+\cdot}$	193(20) 323(58) 324(20) 336(10) 406(60)
Norepinephrine	21.78	479	353$[M-126]^+$	479(2)
Epinephrine	22.39	493	353$[M-140]^+$	493(1)
6-Hydroxydopamine	24.48	565	565$[M]^{+\cdot}$	309(31) 397(16) 439(72) 452(9) 522(6)

a) MU values were determined at 200° with n-alkanes as reference compounds.
b) The mass spectra were obtained with a DuPont Dimaspec GC–MS system (DuPont 321) coupled to a data processing system.

appeared with very high intensity and either of these ions could be observed as a base peak, while the $[M-15]^+$ ion appeared at very low intensity. In the case of NE and E, a single prominent peak at m/z 353 $[M-(CH_2NHTFA)]^+$ or $[M-(CH_2NCH_3TFA)]^+$, produced by β-cleavage, was observed in their mass spectra. Even though the ion cluster was observed at very low intensity, it should be useful to confirm the presence of the DMnPS ether group in the derivatives.

The GC behaviour of the derivatives of catecholamines was investigated in order to determine optimum conditions for complete separation. Figure 3 illustrates the GC separation of catecholamine TFA–DMnPS derivatives. When analyzed on a non-polar liquid stationary phase such as OV-101, the representative catecholamine derivatives could be separated completely in the same manner as their TFA–TMS derivatives prepared by using TSIM instead of DMnPSI.[16]

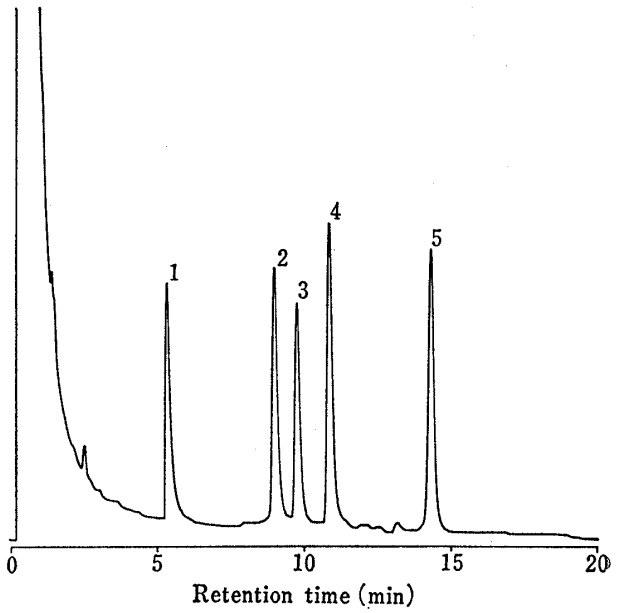

Fig. 3. Gas Chromatographic Separation of the Catecholamine TFA–DMnPS Derivatives; (1) 3-Methoxytyramine; (2) Dopamine; (3) Norepinephrine; (4) Epinephrine; (5) 6-Hydroxydopamine

As shown in Fig. 3, the TFA–DMnPS derivatives gave sharp, symmetrical peaks which were eluted in the order of 3-MT, DA, NE, E and 6-HDA in the gas chromatogram. The utility of these derivatives is clear from the excellent GC separation of NE and 6-HDA.

The storage stabilities of these TFA–DMnPS derivatives in benzene and ethyl acetate solution were compared with those of the TFA–TMS derivatives. The TFA–TMS and TFA–DMnPS derivatives of NE were diluted with dry benzene or ethyl acetate to 100 ng/ml and the residual amount of the derivatives in the solutions were determined by selected ion monitoring (SIM) of each of their base peaks with 2,4,2′,4′-tetrachlorobiphenyl as an internal standard. The results are shown in Fig. 4. The NE TFA–DMnPS derivative was very stable in benzene and ethyl acetate solutions for at least one week at room temperature. In contrast, the corresponding TFA–TMS derivative was relatively unstable and the residual amounts after one week at room temperature were less than 50% of the initial concentration.

The NE TFA–DMnPS derivative was chromatographed on silica gel with n-hexane and eluted with 10 ml of benzene–ether (95: 5). When ^{14}C-NE was used as a marker, as shown in Table II, 10 ng of NE was obtained with greater than 95% recovery, while the NE TFA–TMS derivative was decomposed and adsorbed on the silica gel. The stability of the NE TFA–DMnPS derivative during silica gel chromatography should be very useful for purification of catecholamines from extracts of biological fluids.

As mentioned above, the TFA–DMnPS derivatives of NE and E gave a low intensity for the ion cluster in the mass spectra, as was the case for the corresponding TFA–TMS derivatives,[5] whereas the other catecholamines such as 3-MT, DA and 6-HDA gave a base peak in the ion cluster.

Chemical ionization mass spectrometry (CIMS) offers significant advantages over electron impact ionization (EI) MS in sensitivity and in the formation of adduct ions. The ions formed from the compound to be analyzed are concentrated in the relatively high mass resion where the background contributions due to the column materials and other compounds in the samples

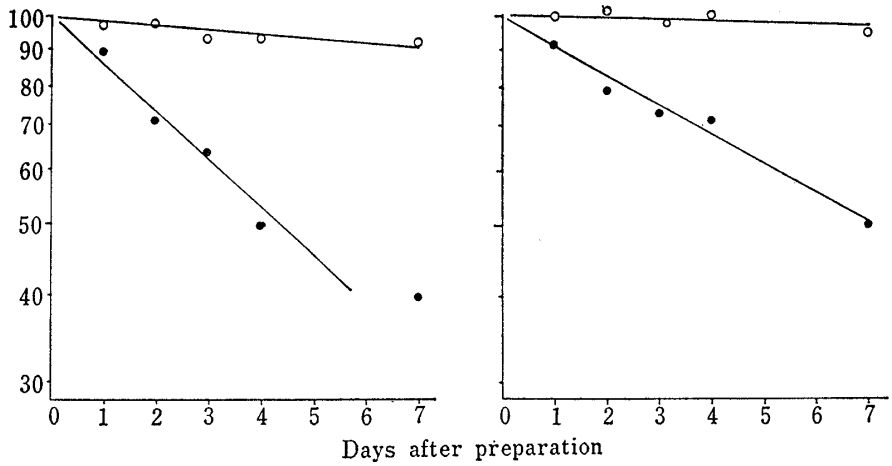

Fig. 4. Storage Stabilities of the NorepinephrineTFA–TMS (—●—) and TFA–DMnPS (—○—) Derivatives in benzene (left) and Ethyl Acetate (right)

Each of the derivatives (corresponding to 100 ng of norepinephrine) was dissolved in 1 ml of benzene or ethyl acetate and stored at room temperature.

Table II. Stability of the Norepinephrine TFA–TMS and TFA–DMnPS Derivatives on a Silica Gel Column

Amounts	Recovery	
	TFA–TMS	TFA–DMnPS
10 ng	—	More than 95%
50 ng	—	More than 95%
100 ng	—	More than 95%
1 μg	10—20%	More than 95%
10 μg	10—30%	More than 95%

Derivatives of ^{14}C-norepinephrine were chromatographed on a silica gel column [0.5×5 cm, Kiesel gel 60 (Merck AG)] with *n*-hexane and eluted with 10 ml of benzene–ether (95: 5).

are low. CIMS often affords an intense adduct ion when the compounds provide little or no molecular ion in EIMS, as in the cases of TFA–TMS derivatives and tetrakis-pentafluoropropionyl (perPFP) derivatives of NE and E.[5,8] This technique has been used for the microdetermination of biogenic amines in biological fluids.[17]

Figure 5 shows the CI mass spectra of the DA TFA–DMnPS derivative measured with methane, isobutane and ammonia as reagent gases, together with the EI mass spectrum. In EIMS, the DA TFA–DMnPS derivatives showed a considerable amount of informative fragmentation, major ions being produced by simple cleavage at *m/z* 449 $[M]^{+\cdot}$, 434 $[M-15]^+$, 406 $[M-43]^+$ and 323 $[M-126(CH_2NHTFA)]^+$.

The methane CI mass spectrum of the DA derivative clearly indicates some of the structural features of the compound, having peaks at $[M-15]^+$ and $[M-43]^+$, as in the EI mass spectrum. The $[M+H]^+$ ion observed at *m/z* 450 is accompanied by abundant ions due to collision stabilized complexes: $[M+C_2H_5]^+$ at *m/z* 478 and $[M+C_3H_5]^+$ at *m/z* 490. On the other hand, the CI mass spectra with isobutane and ammonia showed, as expected, relatively little fragmentation, and the adduct ion of $[M+H]^+$ (isobutane) or $[M+NH_4]^+$ (ammonia) was observed as the base peak, accompanied by the principal ^{13}C, ^{29}Si and ^{30}Si satellites at *m/z* 451, 452 and 453 for isobutane and *m/z* 468, 469 and 470 for ammonia.

As shown in Fig. 6, the methane and isobutane CI mass spectra of NE TFA–DMnPS derivative showed only one ion at *m/z* 448, which was thought to be formed by loss of methanol from the adduct ion $[M+H]^+$ at *m/z* 480. The ammonia CI mass spectrum showed an adduct

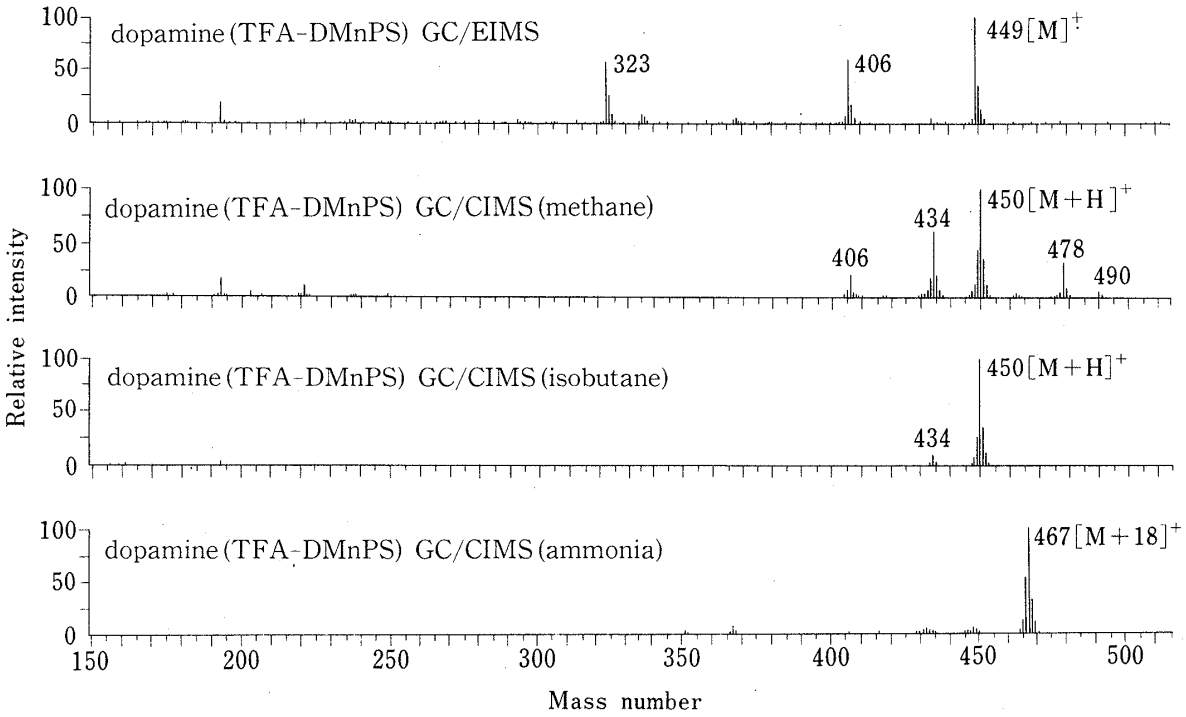

Fig. 5. Mass Spectra of the Dopamine TFA–DMnPS Derivative in the EI Mode (A) and CI Mode with Methane (B), Isobutane (C) and Ammonia (D)

ion of $[M+NH_4]^+$ at *m/z* 497 accompanied by the ion at *m/z* 448. Trideuterated ammonia ($[^2H_3]$ ammonia) was utilized as a reagent gas in order to clarify the formation of the ion at *m/z* 448. The adduct ion of the NE TFA–DMnPS derivative with $[^2H_3]$ ammonia was observed at *m/z* 502, suggesting that the active hydrogen atom in the trifluoroacetamide group was exchanged with deuterium and ionized with ND_4^+. In the case of the E TFA–DMnPS derivative, which had no active hydrogen atom in the molecule, deuterium exhange was not observed in the mass spectrum with $[^2H_3]$ ammonia as a reagent gas; the adduct ion was limited to a 4-mass-unit shift from *m/z* 511 in $[^2H_3]$ ammonia instead of ammonia. The intense fragment ion corresponding to the ion at *m/z* 448 in the NE TFA–DMnPS derivative was observed at

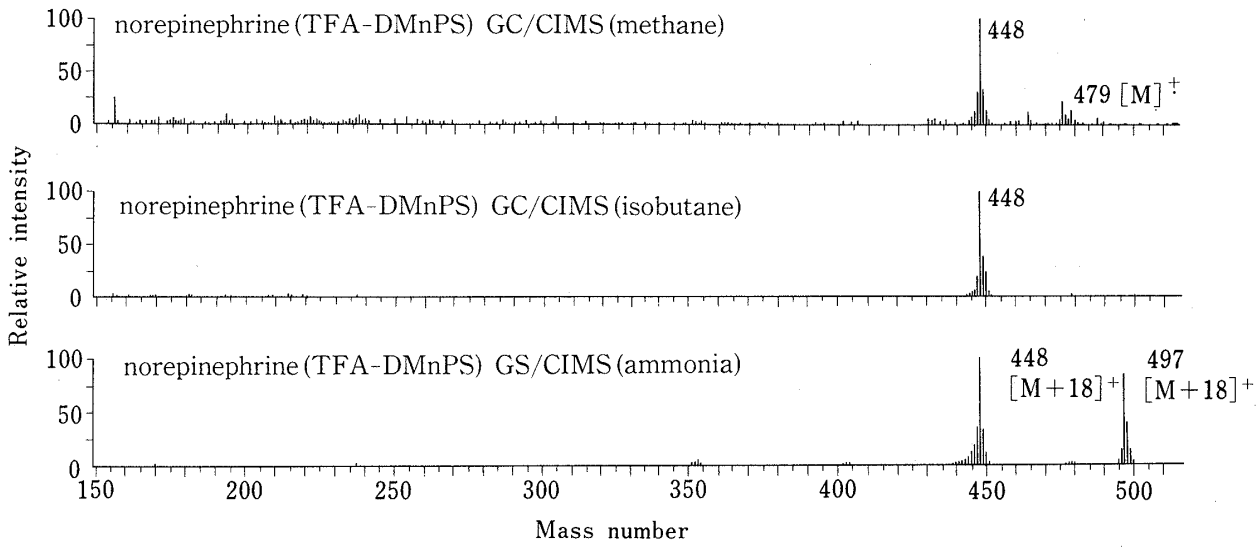

Fig. 6. CI Mass Spectra of the Norepinephrine TFA–DMnPS Derivatives with Methane (A), Isobutane (B) and Ammonia (C)

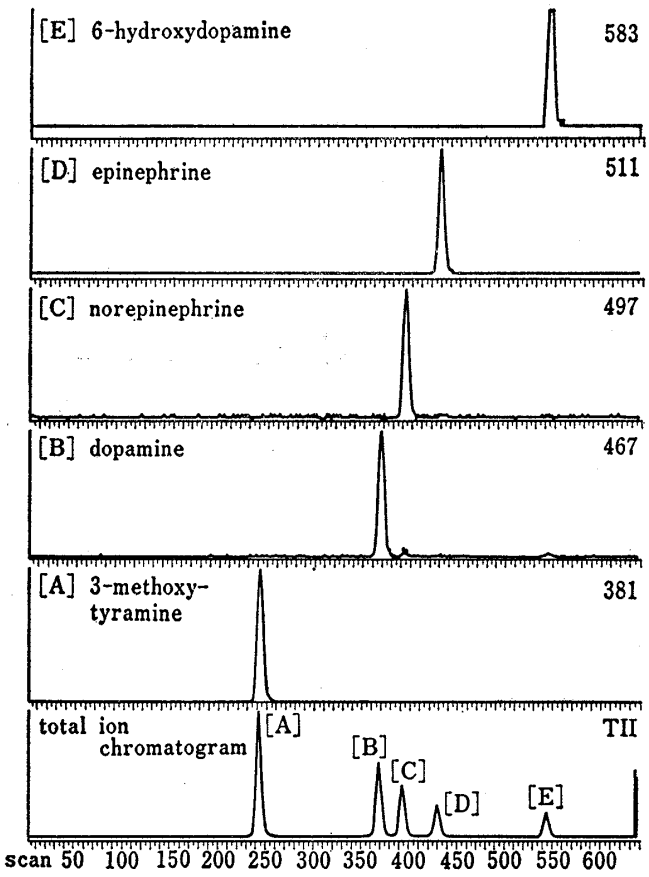

Fig. 7. Selected Ion Recording of the Catecholamine TFA–DMnPS Derivatives by Computer-Controlled SIM in CI (Ammonia) monitored at the Adduct Ion $[M+NH_4]^+$

m/z 449 due to the loss of 53-mass-unit (CH_3OND_4) from the ion $[(M+D-H)+ND_4]^+$ at m/z 502. This observation confirms that the ions at m/z 448 is produced by the loss of CH_3ONH_4 from the adduct ion $[M+NH_4]^+$.

Based on the these results, it appears that the ionization process, proton or ammonia ion transfer, occurs at the oxygen atom in the methoxyl group substituted at the β-position to the nitrogen atom. The ion of m/z 448 found to be the fragment formed by the elimination of CH_3OH or CH_3ONH_4 from the ion $[M+H]^+$ or $[M+NH_4]^+$. These elimination mechanisms were also supported by the loss of pentafluoropropionic acid from the molecule ion of the perPFP derivative in CIMS with isobutane and decadeuterated isobutane as reagent gases.[18,19]

In GC–CIMS with methane, isobutane and ammonia, only ammonia CIMS produces the adduct ion as the base peak or an intense peak for all of the catecholamine TFA–DMnPS derivatives. Figure 7 shows the results of selected ion recording of the catecholamine TFA–DMnPS derivatives by computer-controlled SIM in the CI mode with ammonia, by following the adduct ion $[M+NH_4]^+$: m/z at 381 (3–MT), 467 (DA), 497 (NE), 511 (E) and 583 (6-HDA).

SIM using an MID unit was used to determine the detection limits of these derivatives. In the case of DA, the detection limit was 2 pg with S/N 2: 1, and these was a good linear relationship between the peak area and the amount of DA in the range of 10—100 picograms.

Acknowledgement The authors are grateful to Dr. W. Tanaka, Research Laboratories of Pharmaceutical Division, Nippon Kayaku Co., and Dr. R. Hara, Scientific Instrument Division, Dainiseikosha Co., for their encouragement throughout this work, and to Dr. E.M. Chait, Scientific and Process Division of Instrument products, E.I. du Pont de Nemours and Co., for useful discussions.

References and Notes

1) E. Änggård and G. Sedvall, *Anal. Chem.*, **41**, 1250 (1969).
2) M.G. Horning, A.M. Moss, and E.C. Horning, *Biochem. Biophys. Acta*, **148**, 597 (1969).
3) M.G. Horning, A.M. Moss, E.A. Boucher, and E.C. Horning, *Anal. Letters*, **1**, 311 (1968).
4) M. Donike, *J. Chromatogr.*, **78**, 273 (1973).
5) M. Donike, *Chromatographia*, **7**, 651 (1974).
6) J-C. Lhuguenot and B.F. Maume, *J. Chromatogr. Sci.*, **12**, 411 (1974).
7) H. Ch. Crutius, M. Wolfensberger, B. Steinmann, U. Redweik, and J. Siegfried, *J. Chromatogr.*, **99**, 529 (1974).
8) H. Miyazaki, Y. Hashimoto, M. Iwanaga, and T. Kubodera, *J. Chromatogr.*, **99**, 575 (1974).
9) F. Karoum, F. Cattabeni, E. Costa, C.R.J. Ruthven, and M. Sandler, *Anal. Biochem.*, **47**, 550 (1972).
10) M. Claeys, M. Verzele, M. Vandewalle, J. Leysen, and P. Laduron, *Biomed. Mass Spectrum.*, **1**, 103

(1974).
11) H. Hosoda, K. Yamashita, H. Sagae, and T. Nambara, *Chem. Pharm. Bull.*, **23**, 2118 (1975).
12) H. Miyazaki, M. Shibashi, M. Itoh, and T. Nambara, *Chem. Pharm. Bull.*, **23**, 3033 (1975).
13) H. Miyazaki, M. Ishibashi, M. Itoh, and T. Nambara, *Biomed. Mass Spectrum.*, **4**, 23 (1977).
14) E. Änggård, G. Sedvall, and B. Sjoquist, *J. Chromatogr.*, **52**, 87 (1970).
15) The dimethyl-*n*-propylsilyl (or trimethylsilyl) ether derivative of 2′-methyl-N-trifluoroacetyl NE is designated simply as the TFA-DMnPS (or TFA-TMS) derivative in this paper.
16) H. Miyazaki, unpublished data.
17) Y. Hashimoto and H. Miyazaki, *J. Chromatogr.*, **168**, 59 (1979).
18) C.R. Freed, R.J. Weinkam, L.K. Melmon, and N. Castagnoli, *Anal. Biochem.*, **78** ,319 (1977).
19) Y. Hashimoto and H. Miyazaki, in preparation.