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Gas Chromatography-Mass Spectrometry of Trimethylsilyl Derivatives of Some Iminodicarboxylic Acids

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Trimethylsilylation of eight iminodicarboxylic acids (IDCAs) with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or *N,O*-bis(trimethylsilyl)acetamide was studied under various reaction conditions. The IDCAs include iminodiacetic acid (**1**), 2- and 3-(carboxymethylamino)propionic acids (**2** and **3**), 2,2′-, 2,3′-, and 3,3′-iminodipropionic acids (**4**—**6**), *N*-methyliminodiacetic acid (**7**), and nitrilotriacetic acid (**8**). For **1**—**6**, both bis- and tris(trimethylsilyl) (TMS) derivatives were formed under usual silylating conditions, although the proportion of amounts of the two derivatives varied depending on reaction conditions. This double derivatization was due to the steric hindrance of *N*-substituent group. However, under mild silylating conditions (BSTFA alone, 120 °C, 30 min), **1**—**6** yielded only the di-TMS derivatives. Upon electron impact at 20 eV, both the di- and tri-TMS derivatives showed simple spectra with a molecular (M^+) ion and M −15 (loss of CH_3). The α,α'-IDCAs (**1**, **2**, **4**, **7**, and **8**) are characterized by fragments M −43 (loss of CH_3 and CO) and M −117 (loss of COOTMS) (base peak). On the other hand, the β,β'-IDCA (**6**) is characterized by fragments M −57 (loss of CH_3, CH_2, and CO) and prominent M −131 (loss of $CH_2COOTMS$). The α,β'-IDCAs (**3** and **5**) exhibit both the fragments characteristic of α,α'- and β,β'-IDCAs, M −43, M −57, M −117, and M −131. However, base beaks are usually the fragment M −117 and not M −131.

In recent years, gas chromatography-mass spectrometry (GC–MS) has become an important method for unequivocal identification of amino acids and related compounds. Amino acids themselves are nonvolatile and cannot be analyzed by GC without their prior conversion into suitable volatile derivatives. Preparations of a number of such volatile derivatives have been reported by many investigators, which have been reviewed by Hušek and Macek.[1] Of these volatile derivatives for GC, *N*-trifluoroacetyl (TFA) alkyl ester and trimethylsilyl (TMS) derivatives are used most widely at present.

In our previous paper,[2] we reported on mass spectra (electron impact at 20 eV) of both butyl and *N*-TFA butyl esters of some iminodicarboxylic acids (IDCAs) as shown in Fig. 1. The mass spectra of the butyl ester derivatives are rather simple, but those of the *N*-TFA butyl ester derivatives are very complicated due to the presence of rearrangement peaks. For this reason, we undertook a GC–MS study of TMS derivatives of IDCAs, which are expected to give much simpler spectra than the *N*-TFA butyl ester derivatives.

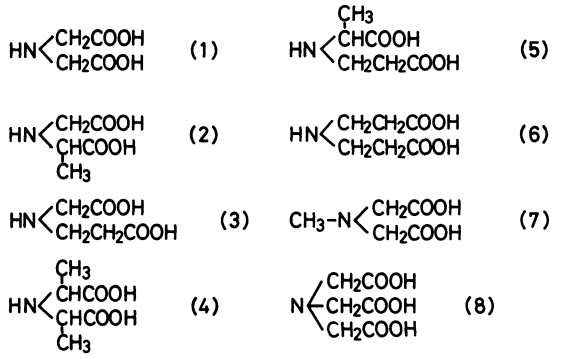


Fig. 1. Iminodicarboxylic acids.

TMS derivatives of amino acids have received great interest since Rühlmann and Giesecke[3] first reported on the preparation of some of them and their resolution by GC in 1961, because of the thermal stability and simplicity of preparation. In general, the TMS derivative can be prepared by use of a silylating reagent with heating (one-step derivatization), whereas multiple procedures are necessary for many other volatile derivatives such as the *N*-TFA butyl ester derivative. At present, many silylating reagents are easily available. Of these reagents, *N,O*-bis(trimethylsilyl)acetamide (BSA)[4] and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA)[5] are known to be strong TMS donors and have been used most frequently by many investigators. Gehrke *et al.*[6,7] reported on derivatization conditions using BSTFA and single-column resolution of protein amino acids for rapid and quantitative analysis of protein hydrolyzates.

The GC–MS of TMS derivatives of protein amino acids has been studied by several investigators[8–13] for unequivocal identification of amino acids present in biological samples. In most instances, mass spectra exhibited easily recognizable molecular (M^+) and M−15 ions, which are useful for molecular weight determination. However, double derivatization[8,12,13] due to steric hindrance has been found to take place for certain amino acids such as glycine and lysine.

The GC–MS of TMS derivatives of nonprotein amino acids[14–17] has far less been studied. Mařik *et al.*[16] studied silylation of some ω-amino acids with BSA in the presence of catalyst such as HCl. In most instances, both di- and tri-TMS derivatives were formed. However, they established the derivatization conditions under which only the tri-TMS derivative can be prepared.

This investigation reports on silylation of some IDCAs as shown in Fig. 1, with BSA or BSTFA under various conditions, and on mass spectra of the TMS derivatives obtained upon electron impact at 20 eV. The IDCAs include iminodiacetic acid (**1**), 2- and 3-(carboxymethylamino)propionic acids (**2** and **3**), 2,2′-2,3′-, and 3,3′-iminodipropionic acids (**4**—**6**), *N*-methyliminodiacetic acid (**7**), and nitrilotriacetic acid (**8**).

Results and Discussion

Trimethylsilylation. The silylation of the IDCAs was carried out under various conditions with or without solvent. In general, hydrogen atoms attached to heteroatoms (active hydrogens) can be replaced by TMS groups. In most instances, a suspended IDCA was dissolved into a reagent solution completely during silylation. This fact suggests that free IDCA remaining in the reaction mixture was of a negligible amount. For **1—6**, both di- and tri-TMS derivatives were found to be present by means of GC–MS, although the proportion of their amounts varied depending on silylating conditions. However, any GC peaks corresponding to a mono-TMS derivative could not be detected. These findings indicate that the silylation takes place in two steps, the first formation of di-TMS derivative and its successive conversion into tri-TMS derivative (*N*-TMS di-TMS ester). In the di-TMS derivative, the two TMS groups are considered to be present as silyl esters (di-TMS ester), because it is known that silylation of amines is more difficult than carboxyl groups.

Table 1 gives retention times of the two derivatives of the IDCAs on GC. As expected, the di-TMS derivative has a consistently shorter retention time than the corresponding tri-TMS derivative. Both the two derivatives gave sharp and symmetrical GC peaks on a glass column (1 m) packed with 1.5% OV-101 on Chromosorb G HP. Of these TMS derivatives, the tri-TMS derivative of **4** was found to give two GC peaks, which were evidenced on the basis of mass spectral data. This is ascribable to the separation between diastereomers, as **4** has two asymmetric carbons. However, such separation was not observed for the di-TMS derivative of **4**.

Table 1. Retention times of the TMS derivatives of **1—8** on GC[a]

Parent IDCA	Retention time/min	
	Di-TMS	Tri-TMS
1	10.2	12.5
2	10.0	13.3
3	12.1	15.2
4	9.6	14.2, 15.1
5	11.8	16.1
6	14.2	17.9
7	10.2	—
8	20.0[b]	—

a) Initial temp: 100°C. Initial hold: 5 min. Program rate: 5°C/min. Final temp: 230°C. Injector: 230°C. Column: 3 mmφ×1 m(glass). Packing: OV-101 1.5% on Chromosorb G HP (100/120 mesh). Retention time of phenanthrene: 17.0 min. b) A tri-TMS ester.

Table 2. Silylation of **1—6** in BSTFA-CH_3CN[a,b]

Parent IDCA	Immediately after silylation		After 1 d	
	Di-TMS/%	Tri-TMS/%	Di-TMS/%	Tri-TMS/%
1	2	98	2	98
2	10	90	7	93
3	11	89	10	90
4	79	21	78	22
5	82	18	56	44
6	12	88	8	92

a) Silylation was carried out at 140°C for 30 min (IDCA, 50 μmol; BSTFA, 0.25 ml; CH_3CN, 0.25 ml) and then the reaction mixture was allowed to stand at room temperature. b) Values indicate peak area ratios.

Table 2 gives the results of silylation of **1—6** in BSTFA-acetonitrile (1:1, v/v), which has been recommended by Gehrke *et al.*[5-7] for silylation of amino acids. Although the reaction conditions (140°C, 30 min) are rather severe, both the di- and tri-TMS derivatives were formed for **1—6**. It is well known that the proportion of the amounts of TMS derivatives varies with the standing time after silylation[8,16] when double derivatization occurs. Therefore, aliquots of the reaction mixture were analyzed by GC immediately after silylation and subsequently after standing for about 1 d at room temperature. A small amount of the di-TMS derivative (2%) still remains even for **1**, the imino group of which is least hindered of the IDCAs. The amounts of the tri-TMS derivatives are much smaller than those of the corresponding di-TMS derivatives for **4** and **5**, the imino groups of which are much more hindered than **1**. Under mild conditions (120°C, 30 min), **4** and **5** gave only the di-TMS derivatives immediately after silylation. These findings indicate that the conversion of the di-TMS derivatives into the corresponding tri-TMS derivatives is governed by the steric hindrance of *N*-substituted groups.

Gehrke and Leimer[18] studied the effect of different solvents on silylation of glycine with BSTFA. They found that polar solvents such as acetonitrile, *N,N*-dimethylformamide (DMF), and pyridine, gave both *N,O*-di-TMS and *N,N,O*-tri-TMS derivatives, whereas nonpolar solvents (dichloromethane, 1,2-dichloroethane, hexane, *etc.*) gave consistently only the di-TMS derivative, under the conditions studied. Table 3 presents the solvents used and their effects on silylation of **1** with BSA. Of these solvents, pyridine gives the highest yield of tri-TMS derivative. The amount of the derivative is 97% immediately after silylation and increases up to 98% after subsequent standing at room temperature for 1 d. It is interesting to note that tetrahydrofuran (THF) gives an extraordinarily high yield of tri-TMS derivative (93%) immediately after silylation, of the nonpolar solvents. In instances of nonpolar solvents such as 1,2-dichloroethane and hexane, as expected, the amount of tri-TMS derivative formed is rather small immediately after silylation, but it increases up to 95—97% on standing at room temperature for 1 d. The amount of tri-TMS derivative after 1 d is somewhat larger than that formed under comparable conditions in polar solvents such as acetonitrile and DMF, which have been used as preferable solvents most frequently. This complicated solvent effect cannot be explained in terms of the polarity of a solvent used, and other factors controlling silylation must be considered.

Figure 2 shows the catalytic effect of HCl on silylation of **1** using BSA or BSTFA in the absence of solvent. It is evident that the silylation is greatly enhanced by the presence of HCl as demonstrated by

Mařik *et al.*[16] for ω-amino acids. In the instance of the HCl salt of **1** and BSA (A), the amount of tri-TMS derivative is 96% immediately after silylation and then gradually increases up to 98% on standing for 3 d at room temperature. Further changes in composition were not observed over the next 4 d at room temperature. This finding indicates that the silylation reaches an equilibrium. As shown in Fig. 2, silylation in other three instances (B, C, and D) also has a tendency to proceed to an equilibrium slowly. These facts are consistent with results obtained by Bergström *et al.*,[8] that silylation of certain amino acids (glycine and lysine) proceeds to an equilibrium in which a few percent of a less silylated derivative still remains. Although Mařik *et al.*[16] have reported that only a tri-TMS derivative was obtained reproducibly in silylation of certain ω-amino acids under comparable conditions (*i.e.*, in silylation by BSA alone in the presence of HCl at 90 °C for 0.5—1 h followed by standing for 1 d at room temperature), we failed to find such conditions under which only the tri-TMS derivative can be formed for **1**—**6**. A comparison of A with C and of B with D in Fig. 2, indicates obviously that BSA is a stronger TMS donor than BSTFA.

The solvent effect on the silylation of **1**·HCl with BSA was also studied. The addition of pyridine accelerated the silylation to a slight extent. On the other hand, the addition of acetonitrile resulted in a decrease in the amount of the tri-TMS derivative ; this is consistent with results obtained by Mařik *et al.*[16] for the silylation of ω-amino acids. These findings suggest that pyridine is an effective solvent for silylation of hydrochlorides of IDCAs with BSA.

As described above, it seemed impossible to prepare only the tri-TMS derivative for **1**—**6** under the present conditions. Therefore, silylating conditions under which only the di-TMS derivative can be prepared selectively, were studied. When silylation was carried out with BSTFA alone at 120 °C for 30 min, only the di-TMS derivative was detected for **1**—**6** immediately after silylation. For **4**—**6**, the conversion into the tri-TMS derivative did not take place during subsequent standing at room temperature for several days. On the other hand, for **1**—**3** which have less bulky *N*-substituted groups, the formation of the tri-TMS derivative was observed as shown in Table 4. Therefore, when these IDCAs are analyzed as their di-TMS derivatives, the derivatized solution should be injected into a GC column as soon as possible after silylation. Under the same conditions, BSA was found to give both the

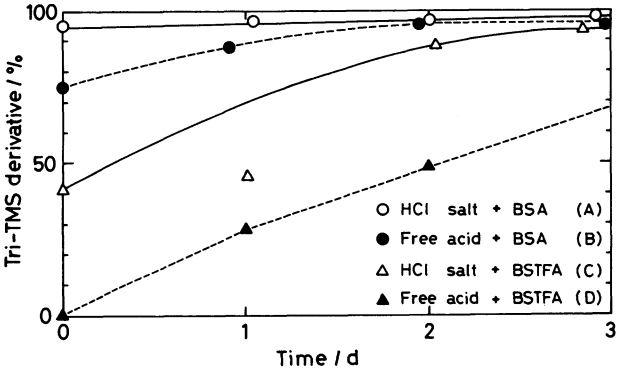


Fig. 2. Catalytic effect of HCl on silylation of **1**. Silylation was carried out at 120 °C for 30 min (**1** or **1**·HCl, 25 μmol; BSA or BSTFA, 0.25 ml) and then the reaction mixture was allowed to stand at room temperature.

Table 3. Solvent effect on silylation of **1** with BSA[a]

Solvent	Dielectric constant (20 °C)[b]	Immediately after silylation		After 1 d	
		Di-TMS/%	Tri-TMS/%	Di-TMS/%	Tri-TMS/%
CH_3CN	37.5	20	80	8	92
DMF	36.7[c]	9	91	10	90
Pyridine	12.3[c]	3	97	2	98
CH_2Cl-CH_2Cl	10.5	39	61	5	95
CH_2Cl_2	9.1	11	89	5	95
THF	7.6[c]	7	93	4	96
Hexane	1.9	28	72	3	97
None	—	25	75	12	88

a) Silylation was carried out at 120 °C for 30 min (**1**, 50 μmol; solvent, 0.25 ml; BSA, 0.25 ml) and then the reaction mixture was allowed to stand at room temperature. b) Cited from Ref. 19. c) At 25 °C.

Table 4. Silylation of **1**—**6** with BSTFA alone[a]

Parent IDCA	Immediately after silylation		After 1 d		After 2 d	
	Di-TMS/%	Tri-TMS/%	Di-TMS/%	Tri-TMS/%	Di-TMS/%	Tri-TMS/%
1	100	—	72	28	52	48
2	100	—	99	1	96	4
3	100	—	91	9	72	28
4	100	—	100	—	100	—
5	100	—	100	—	100	—
6	100	—	100	—	100	—

a) Silylation was carried out at 120 °C for 30 min (IDCA, 25 μmol; BSTFA, 0.25 ml) and then the reaction mixture was allowed to stand at room temperature. In the instances of **4** and **5**, 0.5 ml of BSTFA was used.

di- and tri-TMS derivatives for **1**—**3**, even immediately after silylation. This fact also indicates that BSA is a stronger silylating reagent than BSTFA.

Figure 3 shows the time course of the silylation of **2** with BSTFA alone at 120 °C, as a typical example. The peak area ratio of the di-TMS derivative to an internal standard (phenanthrene) becomes almost constant after reaction time of 20 min, and its conversion into the tri-TMS derivative was not observed. This fact indicates that **2** can be converted quantitatively into the di-TMS derivative under the silylating conditions (BSTFA alone, 120 °C, and 30 min) described above, and also that the amounts of free **2** and the mono-TMS derivative remaining in the reaction mixture are negligible.

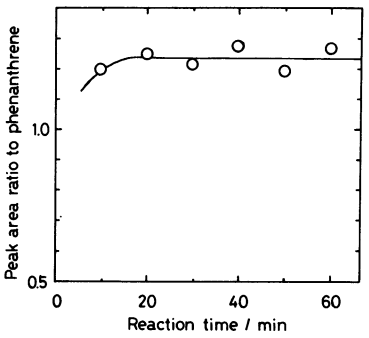
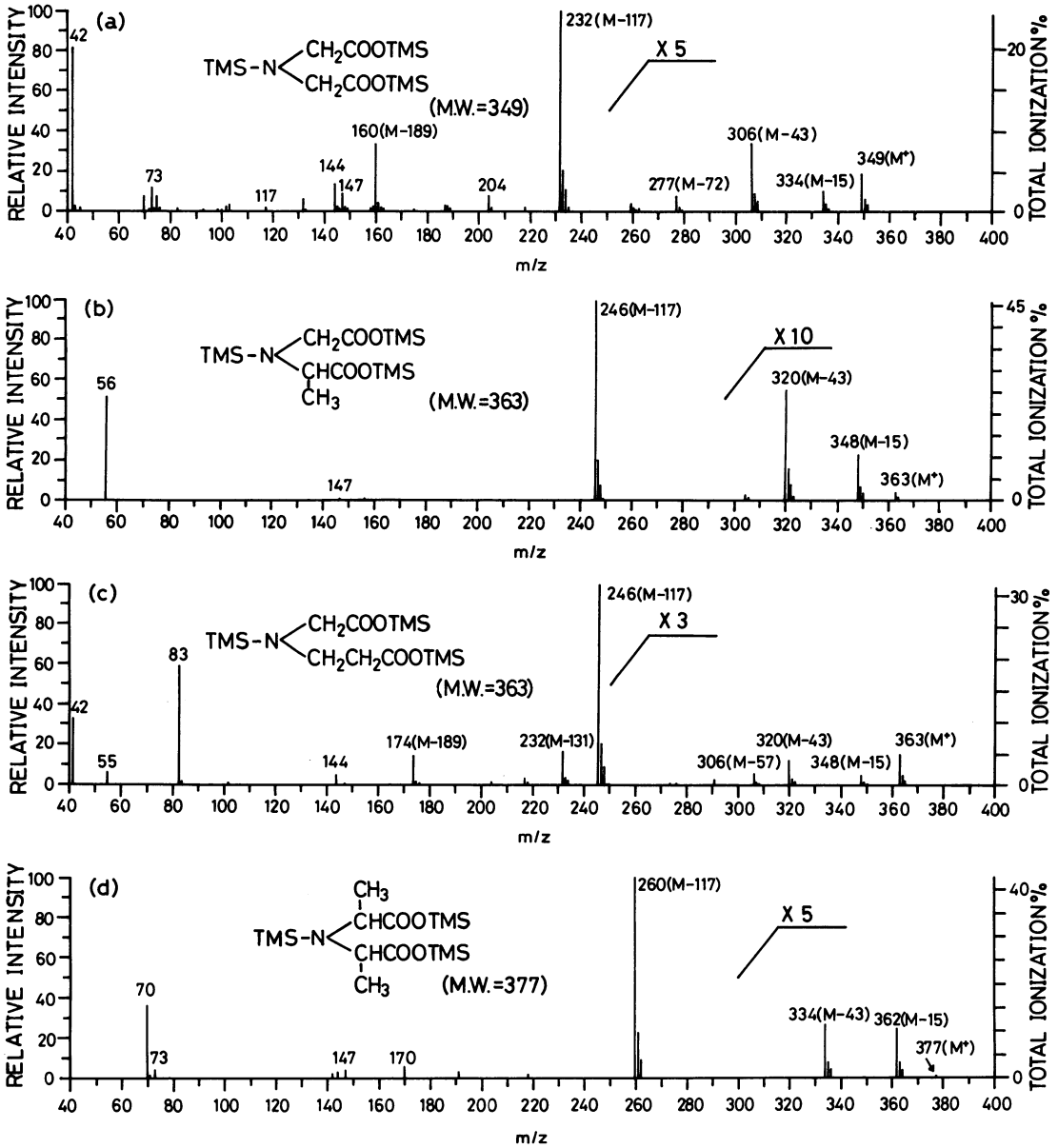


Fig. 3. Time course of the silylation of **2** with BSTFA alone at 120 °C (**2**, 25 mmol; BSTFA, 0.5 ml; phenanthrene, 1.8 mg).

As shown in Table 1, the GC separation of the di-TMS derivatives of the IDCAs is rather poor on the 1 m column. A longer column (2 m), however, showed an indication of partial decomposition of the TMS derivatives of **6** and **8** having longer retention times.

Mass Spectra. Since the IDCAs (**1**—**8**) are regarded as α- or β-amino acids, their TMS derivatives are expected to behave in the same manner as those of α- or β-amino acids upon electron impact. Compounds **7** and **8** are *N*-substituted IDCAs, so that only one derivative, a silyl ester, was formed for each compound.

A) Tri-TMS Derivatives (N-TMS TMS Esters): Figures 4a—f show the mass spectra of the tri-TMS deriv-



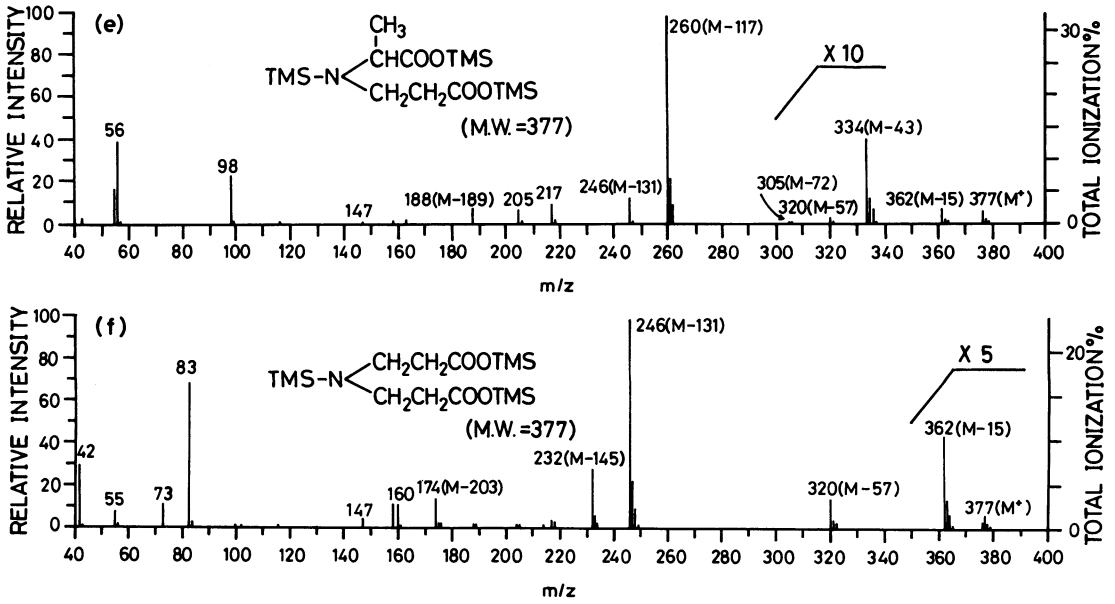
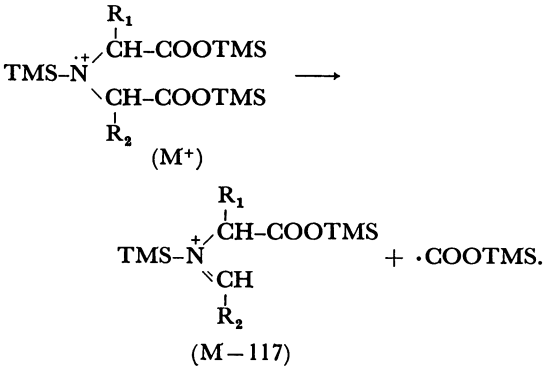


Fig. 4. Mass spectra of the tri-TMS derivatives of IDCAs.
a: **1**, b: **2**, c: **3**, d: **4**, e: **5**, and f: **6**.

atives of **1**—**6**. Both the M^+ and M−15 (CH_3) ions characteristic of TMS derivatives are present in all the spectra. These spectra are much simpler than those of the corresponding *N*-TFA butyl ester derivatives previously reported.[2]

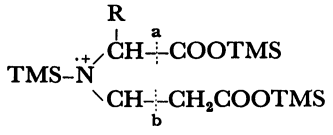
The α,α'-IDCAs (**1**, **2**, and **4**) exhibit characteristic ions such as M^+, M−15, M−43 (CH_3+CO), and M−117 (COOTMS). The M−117 ion constitutes a base peak, which results from the M^+ ion by the loss of a COOTMS group with charge retention on the imino nitrogen as follows:



This fragment is very prominent due to the resonance stabilization of the resultant ion. An analogous fragmentation has been described for α-amino acids.[8,13,14] The M−43 ion is difficult to be explained in terms of simple cleavage of the M^+ ion and may be a result of a certain rearrangement process.[14,17] This ion appears for the α,α'- and α,β'-IDCAs but not for the β,β'-IDCA (**6**), indicating the presence of an $\geq NCH_2COOTMS$ group in the molecule. Additional characteristic ions are observed at *m/z* 277 (M−72) and 160 (M−189) for **1**. The M−72 and M−189 ions may result from the M^+ and M−117 ions, respectively, by the loss of a neutral molecule, $CH_2{=}Si(CH_3)_2$, from the ester portion.[14]

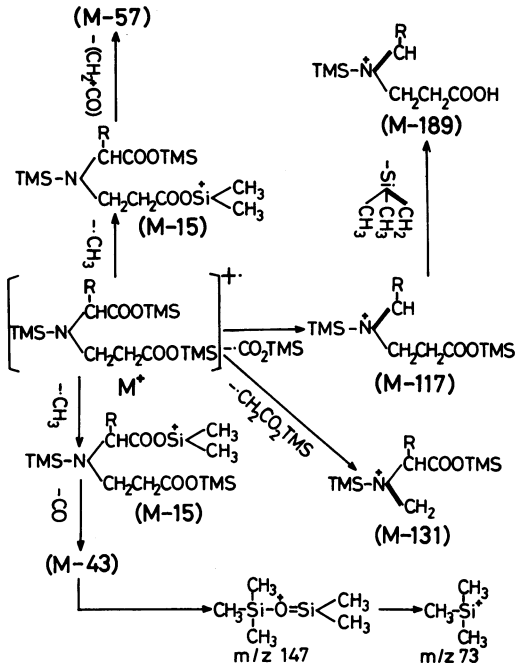
On the other hand, the β,β'-IDCA is characterized by the M^+, M−15, M−57 (CH_3+CH_2+CO), and M−131 ($CH_2COOTMS$) ions. The M−131 fragment at *m/z* 246 constitutes a base peak, which arises from the M^+ ion by the loss of a $CH_2COOTMS$ group due to the alpha-beta bond cleavage (α-cleavage) to the imino nitrogen. Because of the stability of this ion due to resonance, the fragmentation losing a COOTMS group which is typical of α,α'-IDCAs does not take place to any appreciable extent. This fragment then gives an M−203 ion at *m/z* 174 by loss of a neutral molecule, $CH_2{=}Si(CH_3)_2$, from the residual ester portion. The elementary composition of the M−57 ion was confirmed by a high-resolution mass measurement. This ion is observed for β,β'- and α,β'-IDCAs, but not for α,α'-IDCAs, indicating the presence of an $\geq NCH_2$-$CH_2COOTMS$ group in the molecule. The M−57 ion also is difficult to be explained in terms of simple cleavage of the M^+ ion as with the M−43 ion described for α,α'-IDCAs. It can therefore be presumed that a rearranged M−15 ion may break down to this ion by the loss of CH_2 and CO.

It is interesting to note that α,β'-IDCAs (**3** and **5**) are characterized by both the ions typical of α,α'- and β,β'-IDCAs. However, the M−117 ion constitutes a base peak as with α,α'-IDCAs. The M−131 ion which is a base peak for **6**, is much less intense than the M−117 ion. This fact indicates that a silylated carboxyl group (a), defined below, is more preferentially split off than



an *O*-silylated methylcarboxyl one (b), also defined above, due to α-cleavage to the imino nitrogen. Additional characteristic ions, the M−72 ($CH_2{=}Si(CH_3)_2$) and M−189 ($COOTMS+CH_2{=}Si(CH_3)_2$), appear for both **3** and **5**. The M−203 ($CH_2COOTMS+CH_2{=}Si(CH_3)_2$) ion

typical of **6**, however, is not observed in these instances. Scheme 1 presents the fragmentation pathways for α,β'-IDCAs as an illustrative example, which also involves the characteristics of both α,α'- and β,β'-IDCAs.



Scheme 1. Fragmentation pathways for the tri-TMS derivatives of α,β'-IDCAs.

B) Di-TMS Derivatives (TMS Esters): Figures 5a—c show the mass spectra of the di-TMS derivatives of 2,2′-, 2,3′-, and 3,3′-iminodipropionic acids (**4**—**6**). In general, the mass spectra of the di-TMS derivatives of **1**—**6** are more complex than those of the corresponding tri-TMS derivatives. For **1**—**6**, the absence of M−45 (COOH) ion indicates that the two TMS groups exist as silyl esters in the molecule, and therefore that the imino nitrogen is not silylated. Characteristic ions for **1**—**8** are listed in Table 5.

α,α'-IDCAs (**1**, **2**, **4**, **7**, and **8**) are characterized by the M^+, M−15, M−43, and M−117 (base peak) ions. On the other hand, β,β'-IDCA (**6**) exhibits a more complex spectrum with characteristic ions such as M^+, M−15, M−57, and M−131. The M−131 ion is very prominent but not a base peak. $\alpha;\beta'$-IDCAs (**3** and **5**) exhibit both the ions characteristic of α,α'- and β,β'-IDCAs. The M−117 ion constitutes a base peak in these instances. These facts indicate that the fragmentation pathways for the di-TMS derivatives are similar to those for the corresponding tri-TMS derivatives.

However, some differences are noted between these two TMS derivatives. The M+1 ion appears only for the di-TMS derivatives, the intensity of which is higher than the corresponding M^+ ion in certain instances (**4** and **5**). An ion *m/z* 172 observed for **4** (Fig. 5a), was confirmed to be M−133 (CH_3+CO+$(CH_3)_3SiOH$) by a high-resolution mass measurement. This fact suggests that this ion results from the M−43 ion by the loss of a $(CH_3)_3SiOH$ group. The M−133 ion is characteristic of α,α'-IDCAs, indicating the presence of an

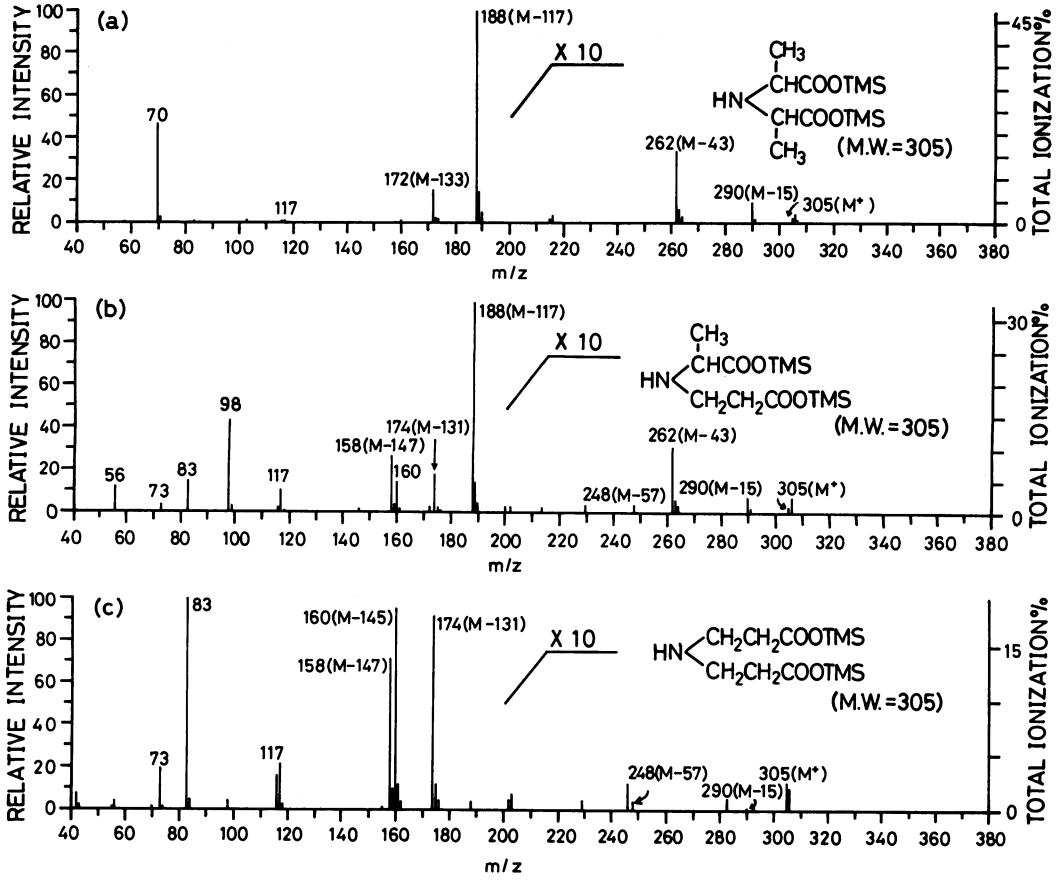


Fig. 5. Mass spectra of the di-TMS derivatives of IDCAs.
a: **4**, b: **5**, and c: **6**.

$>NCH_2COOTMS$ group in the molecule. This fragmentation has been described for TMS derivatives of amino acids and peptides.[13,20] On the other hand, two prominent unusual ions are observed at *m/z* 160 and 158 for **6** (Fig. 5c). The elementary composition of the latter ion was determined as $C_6H_{12}NO_2Si$ by a high-resolution mass measurement, which corresponds to M−147 (CH_3+CH_2+CO+$(CH_3)_3SiOH$). This fact suggests that the M−147 ion results from the M−57 ion by the loss of a $(CH_3)_3SiOH$ group as with the M−133 ion described for α,α'-IDCAs. The M−147 ion characteristic of β,β'-IDCA indicates the presence of an $>NCH_2CH_2COOTMS$ group in the molecule. Another prominent ion at *m/z* 160 was determined as $C_6H_{14}NO_2Si$ also by a high-resolution mass measurement, which corresponds to M−145 ($2CH_2$+COOTMS). This observation suggests that the M−145 ion results from the M^+ ion by the loss of a $CH_2CH_2COOTMS$ group *via* the N-C bond cleavage. The M−145 ion is characteristic of β,β'-IDCA and also observed at *m/z* 232 with a relative intensity of 29% for the tri-TMS derivative of **6** (Fig. 4f). As expected, α,β'-IDCAs (**3** and **5**) are characterized by the M−133, M−145, and M−147 ions.

Other characteristic ions such as M−89, M−90, M−102, M−103, M−104, and M−105 appear with a low intensity for the di-TMS derivatives. In the instance of **1**, however, the M−89 ion at *m/z* 188 is very prominent (Table 5). Of them, the M−89 and M−90 ions were determined to correspond to fragments M−$(CH_3)_3SiO$ and M−$(CH_3)_3SiOH$, respectively, by a high-resolution mass measurement. The characteristic ions described above have been observed for TMS derivatives of amino acids and peptides[13,20] except the M−103 ion. The disiloxynium ion ($(CH_3)_2Si{=}\dot{O}{-}Si(CH_3)_3$)[8,14,16] at *m/z* 147 indicating the presence of an -OTMS group, appears for all the tri-TMS derivatives, though as a minor peak. This ion is not observed for all the di-TMS derivatives. On the other hand, the ion *m/z* 117 with the structure $\dot{O}{\equiv}CO$-TMS[8,16] is observed for all the di-TMS derivatives but not for the tri-TMS derivatives except **1**. The ion at *m/z* 83 appears for most of the di-TMS derivatives with different intensities, which is a base peak for **6**. This ion is observed also for certain tri-TMS derivatives (**1**, **3**, and **6**).

As shown in Table 6, the total ionization of the base peak (M−117) for α,α'- and α,β'-IDCAs, generally depends on the structure of IDCA. The IDCAs with a methyl group on an α-carbon or an imino nitrogen atom have larger total ionization. Since **7** is an isomer of **2**, the mass spectra of their di-TMS derivatives resemble each other. However, **7** carrying a methyl group on its imino nitrogen is characterized not only by a more intense M^+ ion but also by a larger total ionization of the base peak (M−117) (Tables 5 and 6).

Previous investigators[13,16] have found that, for amino acids which do form double derivatives, the relative intensity of the M^+ ion was higher than that of more highly silylated derivatives, but this is not true for the TMS derivatives of **1**—**6**.

In the present study, the TMS derivatives of the IDCAs are shown to have advantages over the *N*-TFA butyl ester derivatives[2] in both the simplicity of preparation and the simple mass spectra containing the M^+, M−15, and additional characteristic ions. However, double derivatization was found to occur for **1**—**6**, which is often observed for TMS derivatives. This is possibly because of the bulkiness of TMS group. This double derivatization may be overcome by introducing TFA to the imino nitrogen instead of TMS group, which is less bulky and also known to be a good *N*-masking group for amino acids for GC

Table 6. Total ionization of base peaks of the di-TMS derivatives of **1**—**8**

Parent IDCA	Base peak/*m/z*	Origin	Total ionization/%
1	160	M−117	19
2	174	M−117	41
3	174	M−117	22
4	188	M−117	47
5	188	M−117	32
6	83		20
7	174	M−117	49
8[a]	290	M−117	22

a) A tri-TMS ester.

Table 5. Characteristic ions of the di-TMS derivatives of **1**—**8**[a]

Parent IDCA	M^+	M−15	M−43	M−57	M−117	M−131	M−133	M−147	Others
1	277	262	234	—	160	146	144	130	188[M−89], 98
	(4.0)	(2.9)	(4.8)		(100)	(3.9)	(29.1)	(1.0)	(84.9) (48.1)
2	291	276	248	—	174	160	158	144	83
	(0.5)	(0.5)	(3.4)		(100)	(7.4)	(20.6)	(3.7)	(14.4)
3	291	276	248	234	174	160	158	144	188[M−103], 83
	(2.9)	(0.9)	(3.2)	(2.7)	(100)	(38.3)	(13.4)	(34.5)	(19.8) (93.1)
4	305	290	262	—	188	—	172	—	70
	(0.2)	(1.0)	(3.4)		(100)		(15.2)		(46.5)
5	305	290	262	248	188	174	172	158	160[M−145], 98
	(0.2)	(0.7)	(3.1)	(0.3)	(100)	(18.7)	(2.0)	(27.4)	(14.8) (44.0)
6	305	290[b]	—	248	—	174	—	158	160[M−145], 83
	(1.2)			(0.4)		(92.0)		(71.4)	(95.4) (100)
7	291	276	248	—	174	160[b]	158[b]	—	103
	(4.2)	(1.5)	(5.5)		(100)				(11.6)
8[c]	407	392	364	—	290	276	274	—	232, 174, 103
	(7.8)	(14.6)	(8.5)		(100)	(2.9)	(1.4)		(25.9)(20.6)(26.2)

a) Values in parentheses indicate relative intensity. b) Relative intensity of this ion is less than 0.1%. c) A tri-TMS ester.

analysis. However, one-step derivatization of such *N*-TFA TMS esters of amino acids[21] is not established at present. Further study must be undertaken along this line.

Experimental

Materials. BSA and BSTFA were obtained from Wako Pure Chemical Industries, Ltd. and Nakarai Chemicals, Ltd., respectively. Acetonitrile, DMF, pyridine, dichloromethane, 1,2-dichloroethane, and hexane were obtained from Wako Pure Chemical Industries, Ltd. as S grade reagents and used as silylation solvents without further purification. Compounds **1**—**8** were the same as previously reported.[2]

Silylation. About 50 μmol of each IDCA was weighed into a 1 ml screw vial. Then, 0.25 ml of a silylating reagent and 0.25 ml of a solvent were added to the vial. It was closed tight with a silicone cap and then heated at different temperatures for different durations in a constant-temperature air bath (Yamato Drying-Oven, DX-58). When silylation was performed with a silylating reagent alone in the absence of solvent, about 25 μmol of each IDCA was heated with 0.25 or 0.50 ml of a silylating reagent. The hydrochloride of **1** was prepared by treating **1** with distilled 6 M HCl (1 M=1 mol dm^{-3}). After reaction, the solution was evaporated completely to dryness under reduced pressure. The hydrochloride obtained was purified by recrystallization. Usually, 1 μl portions of silylated solutions were injected into a GC column.

GC. GC was carried out with a Hitachi gas chromatograph 163 equipped with a flame ionization detector. The conditions were the same as previously reported.[2]

GC-MS. GC-MS was carried out with a JEOL JMS-D 300 mass spectrometer connected with a JGC-20 KP gas chromatograph and mass spectra upon electron impact at 20 eV were obtained. The GC-MS conditions were the same as previously reported.[2]

Note added in proof

Better GC separation of the di-TMS derivatives of the IDCAs was obtained when a fused silica capillary column (OV-101 FS-WCOT, 0.35 mmϕ×25 m, Gasukuro Kogyo Co., Ltd.) was used instead of the packed column. In this instance, the separation between diastereomers was observed for **4**. Decomposition of the di-TMS derivatives did not take place on the capillary column because of the absence of solid support.

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