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Mass Spectra of Butyl Esters and *N*-Trifluoroacetyl Butyl Esters of Some Iminodicarboxylic Acids upon Electron Impact

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Mass spectra (electron impact at 20 eV) of butyl esters and *N*-trifluoroacetyl (TFA) butyl esters of some iminodicarboxylic acids (IDCAs) were determined by gas chromatography-mass spectrometry. The IDCAs included iminodiacetic acid (**1**), 2-(carboxymethylamino)propionic acid (**2**), 3-(carboxymethylamino)propionic acid (**3**), 2,2′-iminodipropionic acid (**4**), 2,3′-iminodipropionic acid (**5**), 3,3′-iminodipropionic acid (**6**), *N*-methyliminodiacetic acid (**7**), and nitrilotriacetic acid (**8**). The mass spectra of the butyl ester derivatives are simple, exhibiting the corresponding molecular (M^+) ions with the exception of **5**. The α,α'-IDCAs (**1**, **2**, **4**, **7**, and **8**) are characterized by M^+, M −101 ($COOC_4H_9$) (base peak), and M −157 ($COOC_4H_9+C_4H_8$) ions. The β,β'-IDCA (**6**) is characterized by a more complex spectrum with M^+, M −73 (OC_4H_9), M −115 ($CH_2COOC_4H_9$) (base peak), M −129 ($OC_4H_9+C_4H_9$), and M −171 ($CH_2COOC_4H_9+C_4H_9$) ions. The α,β'-IDCAs (**3** and **5**) in general show spectra in which both ions characteristic of α,α'- and β,β'-IDCAs are observed. The addition of TFA to the imino nitrogens of **1**—**6** increases complexity of the resulting spectra. In these instances, although M^+ ions are usually present, neither M −101 nor M −115 ions are base peaks. The fragmentation pathways of these two volatile derivatives of the IDCAs upon electron impact are discussed.

In connection with chemical evolution, Chadha *et al.*[1] applied a discharge through a simulated Jovian atmosphere. After a reaction mixture was hydrolyzed and derivatized, they identified amino acids such as glycine and alanine, and their *N*-methyl and *N*-acetic acid derivatives (iminodicarboxylic acids, IDCAs) by gas chromatography-mass spectrometry (GC-MS). This result demonstrates that non-protein amino acids such as *N*-alkyl amino acids and IDCAs can be formed along with protein amino acids when amino acid syntheses take place prebiologically from simple molecules or gaseous mixtures *via* a Strecker mechanism. The GC-MS has been shown to be the most suitable method for unequivocal identification of amino acids and related compounds by means of retention times and mass spectral confirmation.[2-5] In these studies, an *N*-trifluoroacetylated *s*-butyl,[2-4] butyl,[1] and isopropyl[5] ester derivatives were used for GC-MS analysis.

Electron impact (EI) mass spectra of *N*-trifluoroacetyl (TFA) butyl esters of protein amino acids[6,7] and certain non protein amino acids[7,8] including β- and γ-, and *N*-alkyl amino acids, have been determined by GC-MS. Recently, Cronin *et al.*[9] have studied GC-MS of the *N*-TFA *s*-butyl esters of five carbon β-, γ-, and δ-amino acids, and presented 70 eV spectra. However, little GC-MS information is available on IDCAs at present. This prompted us to investigate GC-MS of *N*-TFA butyl esters of some IDCAs, as shown in Fig. 1, for unequivocal identification of such IDCAs present in reaction mixtures of prebiological amino acid synthesis. The reason for the *N*-TFA butyl ester derivative to be chosen in the present study, is that not only the derivatives of amino acids have widely been used for GC,[10] but also their fragmentation pathways upon electron impact have been discussed in detail.[6,7,11] Furthermore, previous authors[6,8] have suggested that various alkyl groups used in the esterification gave almost the same fragmentation pathways for amino acids.

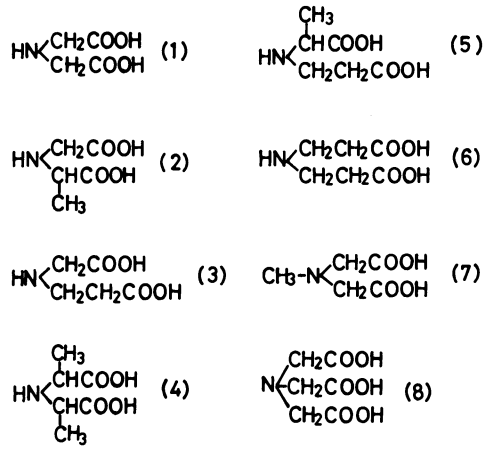


Fig. 1. Iminodicarboxylic acids.

In contrast with common amino acids, the NH of IDCAs is considered to be sterically hindered to some extent, so that trifluoroacetylation at room temperature was found to be incomplete in the case of 2,2′-iminodipropionic acid (**4**). Therefore, GC-MS of butyl ester derivatives was also studied.

The IDCAs studied in the present paper include iminodiacetic acid (**1**), 2-(carboxymethylamino)propionic acid (**2**), 3-(carboxymethylamino)propionic acid (**3**), **4**, 2,3′-iminodipropionic acid (**5**), 3,3′-iminodipropionic acid (**6**), *N*-methyliminodiacetic acid (**7**), and nitrilotriacetic acid (**8**). The structures of **1**—**8** are shown in Fig. 1. They are classified into three groups, α,α'-IDCAs (**1**, **2**, **4**, **7**, and **8**), α,β'-IDCAs (**3** and **5**), and β,β'-IDCA (**6**). Since **7** and **8** are *N*-substituted α,α'-IDCAs, only butyl ester derivatives were studied with them.

Results and Discussion

Gas Chromatography. Figure 2 shows a gas chromatographic separation of a mixture of the butyl esters of **1**—**8**. Although **1**, **2**, and **7** overlapped completely, separate experiments revealed that each IDCA except **4** gives a single GC peak. Compound **4** was found to give two GC peaks, which were evidenced by their mass spectra. This can be ascribed to the separation between diastereomers because **4** has two asymmetric carbon

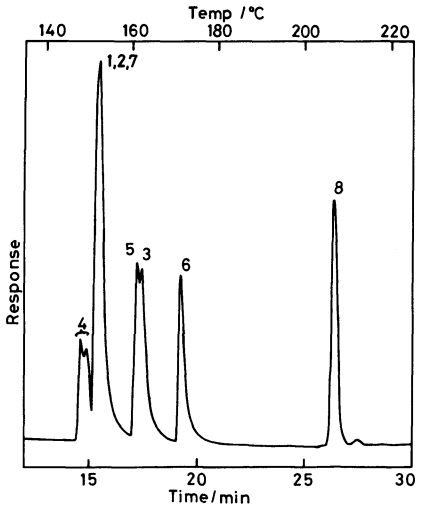


Fig. 2. Gas chromatogram of the butyl esters of **1**—**8**.
Injected: 3.12×10^{-3} μmol of each derivative.
Column: 1.5% OV-101 on Chromosorb G HP (100/120 mesh), 1 m×3 mm I.D. glass. Conditions: initial temperature, 100°C; initial hold, 5 min; program rate, 5°C/min; final temperature, 230°C. Sensitivity: 10×32.

atoms. Figure 3 shows a gas chromatographic separation of a mixture of the *N*-TFA butyl esters of **1**—**6**. Again, each derivative except **4** gave a single GC peak, while **4** gave two GC peaks due to the separation between diastereomers. As expected, the *N*-TFA butyl ester derivatives had somewhat longer retention times than the corresponding butyl ester derivatives.

Mass Spectra. *A) Butyl Ester Derivatives*: Figures 4a—c present the mass spectra of 2,2′-, 2,3′-, and 3,3′-iminodipropionic acid (**4**—**6**). Characteristic ions for **1**—**8** are listed in Table 1. The molecular ions (M^+) are present in all the spectra except **5**. The α,α′-IDCAs give very simple spectra upon electron impact. The base peaks are always M−101 ions which can originate from the following cleavage with charge retention on the nitrogen atoms:

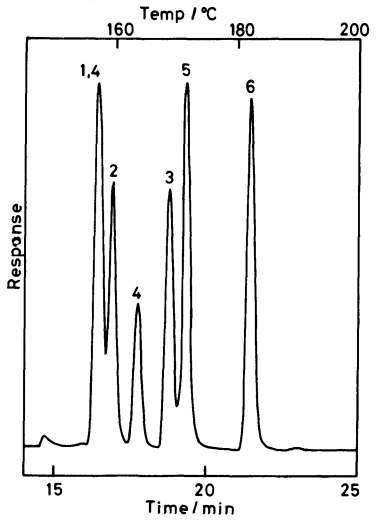


Fig. 3. Gas chromatogram of the *N*-TFA butyl esters of **1**—**6**.
Injected: 4.16×10^{-3} μmol of each derivative.
Conditions are the same as in Fig. 2. Sensitivity: 10×64.

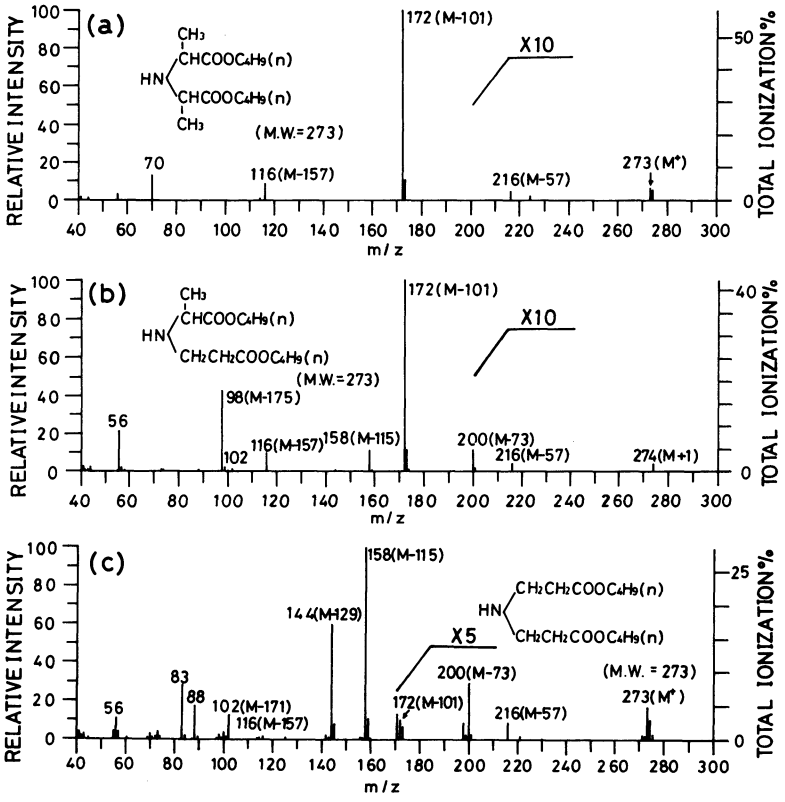
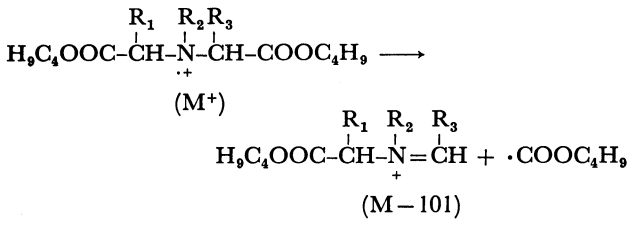


Fig. 4. Mass spectral fragmentations of the butyl esters of IDCAs. a: **4**, b: **5**, and c: **6**.

TABLE 1. SELECTED IONS FROM THE MASS SPECTRA OF THE BUTYL ESTERS OF **1**—**8**[a]

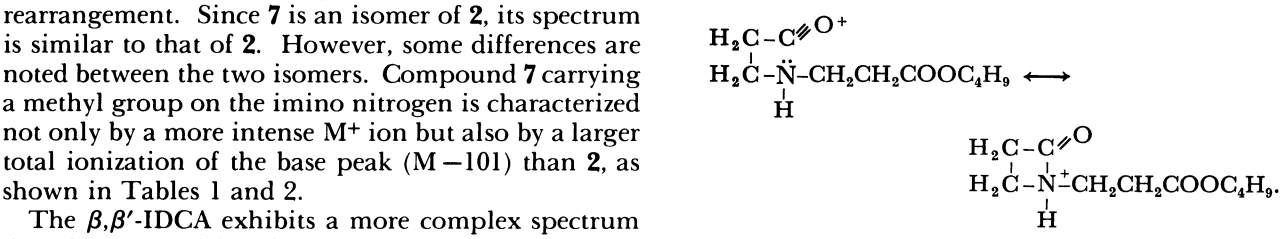
Parent IDCA	M+	M−57	M−73	M−101	M−115	M−129	M−157	M−171	Others
1	245	188	—	144	130	116	88	—	171, 56
	(10.6)	(3.9)		(100)	(0.2)	(0.2)	(31.2)		(6.3) (58.9)
2	259	202	—	158	—	130	102	—	56
	(0.3)	(0.8)		(100)		(0.6)	(43.4)		(25.8)
3	259	202	186	158	144	—	102	88	185, 84, 56
	(4.3)	(3.4)	(2.2)	(100)	(15.2)		(13.0)	(4.5)	(4.6) (38.9) (43.8)
4[b]	273	216	—	172	—	—	116	—	70
	(0.6)	(0.4)		(100)			(8.9)		(13.8)
5	—	216	200	172	158	—	116	102	98, 56
		(0.5)	(1.2)	(100)	(12.0)		(10.7)	(1.5)	(42.8) (22.0)
6	273	216	200	172	158	144	116	—	102, 88, 83
	(3.5)	(1.8)	(5.9)	(2.0)	(100)	(59.3)	(0.1)		(13.1) (17.5) (28.7)
7	259	202	—	158	—	—	102	—	—
	(6.0)	(0.8)		(100)			(13.1)		
8[c]	359	302	—	258	—	230	202	—	158
	(3.3)	(0.5)		(100)		(0.1)	(5.5)		(20.6)

a) Values in parentheses indicate relative intensity. b) The first gas chromatographic peak. c) A tributyl ester.



The predominance of the M−101 ($COOC_4H_9$) ion can be explained in terms of its increased stability due to resonance as described by Biemann *et al.*[12] Another prominent ion for the α,α'-IDCAs, the M−157 ion, results from the M−101 ion by the loss of 1-butene from the residual ester portion *via* a McLafferty rearrangement. Since **7** is an isomer of **2**, its spectrum is similar to that of **2**. However, some differences are noted between the two isomers. Compound **7** carrying a methyl group on the imino nitrogen is characterized not only by a more intense M^+ ion but also by a larger total ionization of the base peak (M−101) than **2**, as shown in Tables 1 and 2.

The β,β'-IDCA exhibits a more complex spectrum than the α,α'-IDCAs (Fig. 4c). The ion at m/z 158 (M−115) is a base peak, which arises from the M^+ ion by the loss of $CH_2COOC_4H_9$ due to alpha-beta bond cleavage (α-cleavage to the imino nitrogen). Because the M−115 ($CH_2COOC_4H_9$) ion resulting from the cleavage is very stable, the fragmentation losing $COOC_4H_9$ from the M^+ ion does not take place to any appreciable extent. Consequently, the M−101 ion typical of the α,α'-IDCAs is of minor importance in this case. The M−115 ion produces the M−171 ion at m/z 102 by the loss of 1-butene from the residual ester portion *via* a McLafferty rearrangement. An important M−73 ion results from the M^+ ion by the loss of OC_4H_9 from either of the two ester portions. This M−73 (OC_4H_9) ion is characteristic of the β,β'- and α,β'-IDCAs, namely those bearing a butoxycarbonyl group at the beta position to the imino nitrogen, and is not present in the spectra of the α,α'-IDCAs (Table 1). One possible explanation for the stability of the M−73 ion has been proposed by Lawless and Chadha[8] as follows:



However, the M−73 peaks observed for **3**, **5**, and **6** are much smaller than those of *N*-TFA-β-amino acid butyl esters reported by Lawless and Chadha.[8] This difference may be explained in terms of the stability of such a cyclized ion as shown above. In the *N*-TFA-β-amino acid butyl esters, the *N*-TFA group is considered to stabilize the resultant cyclized ions through electron-withdrawing. The prominent M−129 ion at m/z 144 originates from the loss of 1-butene from the residual ester portion of the M−73 ion *via* a McLafferty rearrangement. This M−129 ($OC_4H_9 + C_4H_8$) ion is missing in the spectra of the α,β'-IDCAs but present with a low intensity in those of certain α,α'- IDCAs (**1**, **2**, and **8**).

As shown in Fig. 4b, α,β'-IDCAs are characterized by spectra exhibiting both ions typical of the α,α'- and β,β'-IDCAs with the exception of the M−129 ion observed for **6**. However, base peaks are always M−101 and not M−115 ions, indicating that a butoxycarbonyl group (a), defined below, is more preferentially split

TABLE 2. TOTAL IONIZATION OF BASE PEAKS OF THE BUTYL ESTERS OF **1**—**8**

Parent IDCA	Base peak/m/z	Origin	Total ionization/%
1	144	M−101	20
2	158	M−101	38
3	158	M−101	20
4	172	M−101	60
5	172	M−101	42
6	158	M−115	29
7	158	M−101	66
8	258	M−101	42

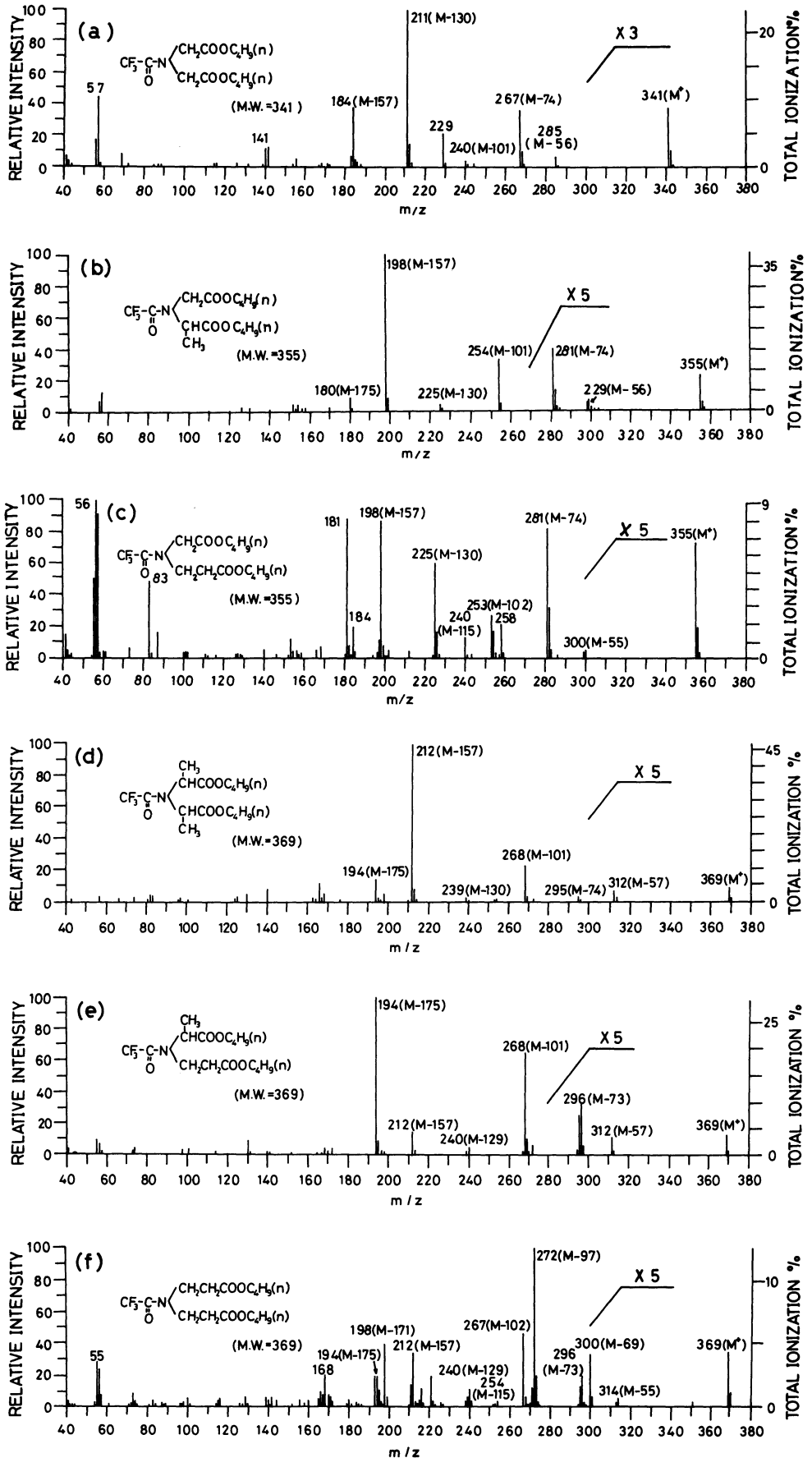
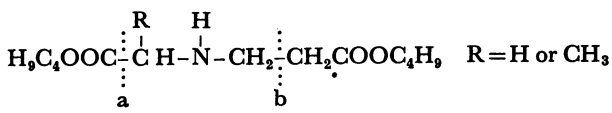
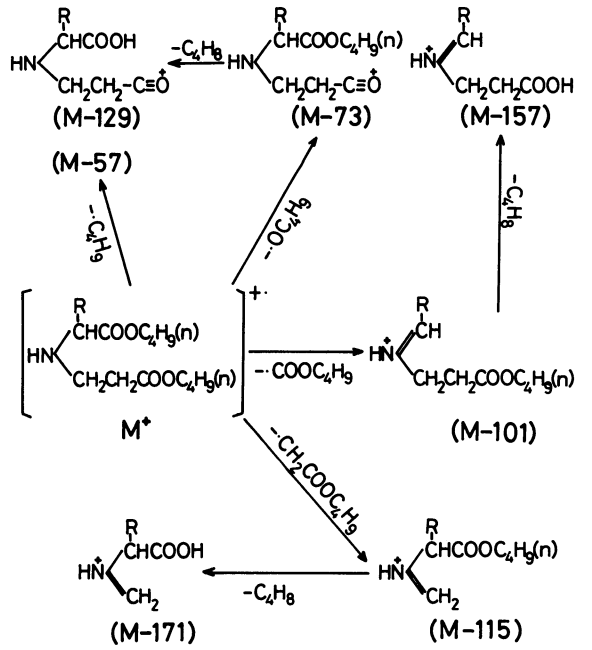


Fig. 5. Mass spectral fragmentations of the *N*-TFA butyl esters of IDCAs. a: **1**, b: **2**, c: **3**, d: **4**, e: **5**, and f: **6**.



off than a butoxycarbonylmethyl one (b), also defined above, by α-fission to the imino nitrogen atom. A prominent M−175 ion is observed for **3** and **5**, which corresponds to the one resulting from the M−157 ion by the loss of H_2O,[6] or from the M−101 ion by the loss of 1-butanol.[7] Fragmentation pathways for the α,β′-IDCAs are presented in Scheme 1 as an illustrative example, which involves both the pathways characteristic of α,α′- and β,β′-IDCAs.



Scheme 1. Fragmentation pathways of the butyl esters of the α,β′-IDCAs.

As shown in Table 1, the M−57 ion is consistently present in all the spectra, which arises from the loss of a butyl group from the corresponding M^+ ion. The M+1 ion is also observed for **1**—**8**, and in certain instances (**2** and **5**) this ion is more intense than the corresponding M^+ ion.

As shown in Table 2, the total ionization of the base peak (M−101) generally depends on the structure of IDCA. The IDCAs carrying a methyl group on an α-carbon or an imino nitrogen atom have larger total ionization.

In conclusion, the α,α′-IDCAs are characterized by the M^+, M−101 (base peak), and M−157 ions. The β,β′-IDCA is characterized by a more complex spectrum with the M^+, M−73, M−115 (base peak), M−129, and M−171 ions. The α,β′-IDCAs generally show spectra in which both ions characteristic of α,α′- and β,β′-IDCAs are present. The M−101 and not M−115 ion is a base peak for the α,β′-IDCAs.

B) N-TFA Butyl Ester Derivatives: Figures 5a—f show the mass spectra of *N*-TFA butyl esters of **1**—**6**. In general, each spectrum is more complex than that of the corresponding butyl ester derivative. However, M^+ ions are present in all the spectra. The M−101 (loss of a butoxycarbonyl) ion which is a base peak for the butyl ester derivatives with the exception of **6**, becomes less significant in these instances. This may be due to the fact that a strong electron-attractive TFA group attached to an imino nitrogen atom will decrease the stability of the ion. It is noted that the important ions, M−73 (loss of a butoxyl group), M−74 (loss of 1-butanol), M−129 (loss of a butoxyl group and 1-butene), and M−130 are consistently present in all the spectra. The M−130 ion is observed at *m/z* 225 for **3** and its elementary composition was determined to be $C_7H_6NO_4F_3$ by a high-resolution mass measurement, which corresponds to the loss of 1-butanol and 1-butene from the M^+ ion. Accordingly, the M−130 ion is considered to result from the M−74 ion by the loss of 1-butene *via* a McLafferty rearrangement. The M−74 ion arises from the loss of 1-butanol from either of the two ester portions of the M^+ ion. Gelpi *et al.*[6] have found, for the *N*-TFA butyl esters of aspartic and glutamic acids, that the cleavage of one of the butoxyl groups to give the M−73 ion is accompanied by removal of 1-butanol to give the M−74 ion. This fragmentation is significant especially for the *N*-TFA butyl esters of IDCAs, but not for the butyl ester derivatives.

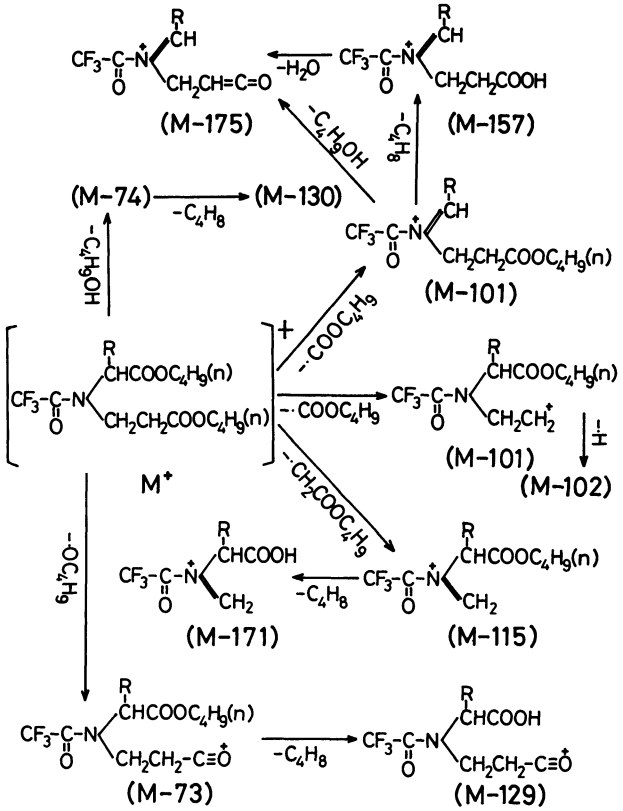
In the high mass region, two ions, M−55 and M−56, are usually present, although not as major peaks in any instances. The former ion may be explained in terms of a double hydrogen rearrangement. The latter ion results from the loss of 1-butene from the M^+ ion *via* a McLafferty rearrangement.

For the α,α′-IDCAs (**1**, **2**, and **4**), the M−74 and M−130 ions are more intense than the M−73 and M−129 ions, respectively. In the case of **1**, the M−130 ion at *m/z* 211 is a base peak. Additional characteristic ions are M−157 (loss of a butoxycarbonyl and 1-butene) and M−175 (loss of a butoxycarbonyl and 1-butanol). The former ion is a base peak for **2** and **4**.

In contrast, the β,β′-IDCA (**6**) shows a more complex spectrum (Fig. 5f) than the α,α′-IDCAs. The base peak at *m/z* 272 corresponds to a fragment, M−97 (CF_3CO), which was confirmed by a high-resolution mass measurement. Other unusual ions are M−69 (CF_3) at *m/z* 300 and M−102 ($COOC_4H_9$+H) at *m/z* 267, the elementary compositions of which were also confirmed by high-resolution mass measurements. The M−102 ion is more intense than the M−101. This fact is in good agreement with results obtained for β-amino acid derivatives,[7,8] since **6** is an *N*-substituted β-alanine. The stability of the M−102 ion for β-amino acids has been explained in terms of resonance by Lawless and Chadha.[8] Additional characteristic ions are M−115 (loss of a butoxycarbonylmethyl) at *m/z* 254 and M−171 (loss of a butoxycarbonylmethyl and 1-butene) at *m/z* 198. The characteristic ions of the α,α′-IDCAs such as the M−157 and M−175 ions are also observed. In contrast with the α,α′-IDCAs, the M−74 and M−130 ions are less intense than the M−73 and M−129 ions, respectively.

For the α,β′-IDCAs (**3** and **5**), fragmentations become more complicated. As shown in Fig. 5c, **3** exhibits a spectrum in which both ions characteristic of the α,α′- and β,β′-IDCAs are present. The elementary composition of an unusual ion at *m/z* 181 was determined to be $C_6H_6NO_2F_3$ by a high-resolution mass measurement, which corresponds to a fragment, M−174

($COOC_4H_9 + OC_4H_9$). The origin and structure of this ion are unkown. On the other hand, **5** exhibits an almost identical spectrum (Fig. 5e) with that of the corresponding α,α'-IDCA (**4**), except that the base peak is M−175 at m/z 194, and that the M−74 and M−130 ions are less intense than the M−73 and M−129 ions, respectively. Although the M−102 ion is present with a very low intensity, another ion typical of the β,β'-IDCA, M−115, is not observed. This fact indicates that either of the fragmentation characteristics of α,α'- or β,β'-IDCAs can take place predominantly depending on the structures of α,β'-IDCAs. Fragmentation pathways for the α,β'-IDCAs are presented in Scheme 2 as an illustrative example. The structures of the M−74 and M−130 ions are unknown.



Scheme 2. Fragmentation pathways of the *N*-TFA butyl esters of the α,β'-IDCAs.

Although fragmentation pathways for the *N*-TFA butyl esters of the IDCAs are more complicated than those for the butyl ester derivatives, it seems possible to identify unknown IDCAs by noting the characteristic ions described above.

Experimental

All the melting points were determined with a Yanagimoto micro melting point apparatus and were not corrected. ^1H NMR spectra were measured with a Hitachi R-24B spectrometer using DSS as an internal standard. Thin layer chromatography was carried out on silica gel 60 F_{254} (Merck) using the following solvent system: *n*-BuOH–AcOH–H_2O (4:1:1, v/v).

Preparation of IDCAs. Compound **1** was obtained from Wako Pure Chemical Industries, Ltd. and **8** was prepared by the method reported previously.[13]

2-(Carboxymethylamino)propionic Acid (2). Compound **2**·HCl was prepared from DL-alanine (5.6 g, 63 mmol) and chloroacetic acid (5.9 g, 62 mmol) in the presence of 4 M LiOH (1 M=1 mol dm^{-3}) (46.4 ml) according to the method of Okamoto *et al.*[14] The aqueous solution of **2**·HCl was poured into an ion-exchange column (2.0ϕ×100 cm, Amberlite CG 120 (H form)) and then **2** was eluted with H_2O. The fractions containing **2** were concentrated to give crystals of free **2**: these were recrystallized from H_2O-acetone: yield 2.47 g (27%); mp (decomp) 229—232° (lit,[15] 222—223 °C); R_f 0.04. Found: C, 40.55; H, 6.10; N, 9.52%. Calcd for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52%.

3-(Carboxymethylamino)propionic Acid (3). A solution of *N*-(2-cyanoethyl)glycine[16] (2.56 g, 20 mmol) in 6 M HCl (50 ml) was refluxed on a sand bath for 2 d. The reaction mixture was evaporated to dryness and the residue was dissolved in a minimum amount of H_2O. The solution was treated with Amberlite CG 120 (H form) (2.0ϕ×21 cm) by the method described for **2**. The fractions containing **3** were evaporated to dryness and the residue was recrystallized from H_2O–ethanol: yield 1.29 g (44%); mp (decomp) 195—198 °C; R_f 0.07; NMR (trifluoroacetic acid (TFA)) δ=7.86 (2H, br, NH_2^+), 4.22 (2H, t, J=4.7 Hz, N^+CH_2CO), 3.91—3.40 (2H, m, $N^+C\underline{H}_2CH_2$), and 3.10 (2H, t, J=5.4 Hz, $CH_2C\underline{H}_2CO$). Found: C, 40.64; H, 5.96; N, 9.32%. Calcd for $C_5H_9NO_4$: C, 40.82; H, 6.17; N, 9.52%.

2,2'-Iminodipropionic Acid (4). Compound **4**·HCl was prepared from DL-alanine (5.97 g, 67 mmol) and 2-bromopropionic acid (10.2 g, 67 mmol) in the presence of 1 M NaOH (250 ml) according to the procedure of Karrer and Appenzeller.[17] The free acid was obtained by ion-exchange chromatography and then recrystallized from H_2O–acetone: yield 5.09 g (47%); mp (decomp), 250—252 °C (lit,[18] 233—235 °C); R_f 0.12. Found: C, 44.72; H, 7.02; N, 8.71%. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69%.

2,3'-Iminodipropionic Acid (5). Compound **5** was prepared from *N*-(2-cyanoethyl)-DL-alanine[16] (2.84 g, 20 mmol) by the method described for **3**: yield 0.88 g (27%); mp (decomp) 200—203 °C; R_f 0.13; NMR (TFA) δ=7.75 (2H, br, NH_2^+), 4.64—4.04 (1H, m, CH), 3.92—3.34 (2H, m, N^+CH_2), 3.09 (2H, t, J=5.8 Hz, CH_2CO), and 1.81 (3H, d, J=7.3 Hz, CH_3). Found: C, 44.84; H, 6.69; N, 8.92%. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69%.

N-(2-Cyanoethyl)-β-alanine. This compound was prepared by the cyanoethylation of β-alanine (44.5 g, 0.55 mol) with acrylonitrile (29.2 g, 0.50 mol) according to the procedure of McKinney *et al.*[16] and recrystallized from H_2O–acetone: yield 38.1 g (52%); mp (decomp) 144—146 °C; R_f 0.19; NMR (TFA) δ=7.75 (2H, br, NH_2^+), 3.99—3.77 (4H, m, N^+CH_2), and 3.24—2.96 (4H, m, $CH_2CN + CH_2CO$). Found: C, 49.44; H, 7.38; N, 19.03%. Calcd for $C_6H_{10}N_2O_2 \cdot 1/4\ H_2O$: C, 49.14; H, 7.22; N, 19.10%.

3,3'-Iminodipropionic Acid (6). Compound **6** was prepared from *N*-(2-cyanoethyl)-β-alanine (2.84 g, 19.4 mmol) by the method described for **3**. After the hydrolyzate was poured into a column packed with Amberlite CG 120 (H form), the column was washed with H_2O to remove neutrals at first, and then the product was eluted with 2% aq pyridine: yield 1.98 g (63%); mp (decomp) 157—159 °C; R_f 0.11; NMR (TFA) δ=7.56 (2H, br, NH_2^+), 3.83—3.30 (4H, m, N^+CH_2), and 3.05 (4H, t, J=7.1 Hz, CH_2CO). Found: C, 44.49; H, 6.91; N, 8.72%. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69%.

N-Methyliminodiacetic Acid (7). Compound **7** was prepared from sarcosine (3.0 g, 34 mmol) and chloroacetic acid (3.1 g, 33 mmol) in the presence of 4 M LiOH (16.8 ml) by the method described for **2**. After being recrystallized from H_2O–acetone and dried in a vacuum desiccator at room temperature, the product was identified as a hemihydrate: yield

Table 3. Elution times and relative color constants of some IDCAs on ion-exchange chromatography

IDCA	Elution time/min	Relative color constant[a)]
1	42	0.99
2	42	0.32
5	64	0.009
3	66	0.30
Gly	94	1.00

a) Color constant of Gly : 87.6 μmol^{-1}.

2.50 g (49%); mp (decomp) 231—234 °C (lit,[19] 226—227 °C (free acid)); R_f 0.07; NMR (TFA) δ=8.40 (1H, br, NH^+), 4.38 (4H, s, CH_2CO), and 3.31 (3H, s, N^+CH_3). Found: C, 38.18; H, 6.29; N, 8.72%. Calcd for $C_5H_9NO_4 \cdot 1/2H_2O$: C, 38.46; H, 6.46; N, 8.97%.

Ion-exchange Chromatography. Ion-exchange chromatography was carried out with a Sibata amino acid analyzer AA-600 under the following conditions: Column, 0.25ϕ×50 cm packed with Aminex A-4 (Bio-Rad); flow rate of eluent (pH 3.25 citrate buffer, 0.2 M Na^+), 6 ml/h; flow rate of ninhydrin solution, 3 ml/h; jacket temperature, 30 °C. The time of heating in the reaction bath (100 °C) was designed to be 30 min. Each of the IDCAs was chromatographed separately, and its color constant was calculated by the common $H \times W$ method. Among the IDCAs, **4** and **6** were negative to color development with ninhydrin,[20] so that their elution times could not be determined. The ion-exchange chromatographic data are listed in Table 3. It was unsuccessful to separate a mixture of the IDCAs with the amino acid analyzer.

Derivatization for Gas Chromatography. For the preparation of butyl ester derivatives, each individual IDCA (0.1 mmol) was refluxed for 30 min with 1-butanol (5 ml) which was 5.2 M in dry HCl, and then complete solubilization was achieved. Excess reagent was distilled off at 50 °C under reduced pressure. The residual ester hydrochloride was dissolved in a minimum amount of H_2O. After being made alkaline with K_2CO_3, the solution was repeatedly extracted with CH_2Cl_2 (20 ml×4). The extracted solution was dried (Na_2SO_4), filtered, and then evaporated carefully to dryness at room temperature leaving an oil. The butyl ester derivative was taken up in CH_3NO_2 (2 ml), 1 μl portions of which were usually injected into the gas chromatographic column. For the preparation of *N*-TFA butyl ester derivatives, an ester hydrochloride prepared as above was suspended in CH_2Cl_2 (1 ml), to which $(CF_3CO)_2O$ (0.5 ml) was added. After being left overnight at room temperature, the reaction mixture was carefully evaporated to dryness, and then taken up in CH_3NO_2 (2 ml) for gas chromatography. When $(CF_3CO)_2O$ was added to the ester hydrochlorides of the *N*-substituted IDCAs (**7** and **8**), the corresponding butyl ester derivatives were liberated in the free state. In the case of **4**, trifluoroacetylation with $(CF_3CO)_2O$ at room temperature was incomplete due to steric hindrance. However, it was found that trifluoroacetylation was completed in 60 min at 100 °C; in this experiment 1 ml of $(CF_3CO)_2O$ was added.

Gas Chromatography. Gas chromatography was carried out with a Hitachi gas chromatograph 163 equipped with a flame ionization detector (H_2 flow rate, 35 ml/min). A single column technique was used. The conditions for gas chromatography were as follows: column, 1 m×3 mm I. D. glass packed with 1.5% OV-101 on Chromosorb G HP (100/120 mesh) (Shimadzu); injection port, 200 °C; flow rate of N_2 (carrier gas), 30 ml/min. The column oven was operated isothermally at 100 °C for 5 min after injection and then programmed to be heated up to 230 °C at a rate of 5 °C/min.

Gas Chromatography-Mass Spectrometry. Mass spectra were measured with a JEOL JMS-D 300 mass spectrometer, which was connected through a jet separator (glass) with a JGC-20 KP gas chromatograph. Each volatile derivative was chromatographed on a glass column (1 m×2 mm I. D.) containing 3% OV-101 on Chromosorb G HP (100/120 mesh) (Shimadzu) using He as carrier gas. The GC-MS combination was operated with the following parameters: injection port, 200 °C; separator, 250 °C; ionizing voltage, 20 V; ionizing current, 300 μA; accelerating voltage, 3.0 kV; ion source, 210 °C. The spectrometer was continuously scanned every 5 s.

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