THE SYNTHESIS AND CHROMATOGRAPHY OF PEPTIDE NITRILES

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Abstract. Di- and tripeptide nitriles, glycylaminoacetonitrile (Gly-AAN), diglycylaminoacetonitrile (Gly-Gly-AAN), alanyl-α-aminopropionitrile (Ala-APN), and dialanyl-α-aminopropionitrile (Ala-Ala-APN) were synthesized first.

These peptide nitriles and related peptides and peptide amides were analyzed by means of ion-exchange chromatography. The every two diastereomers of dialanine, dialanine amide, and Ala-APN were separated into two peaks by using a pH 3.25 buffer as an eluent. The four isomers of trialanine, trialanine amide, and Ala-Ala-APN gave four, two, and one peak, respectively under the same conditions.

The trimethylsilyl derivatives of alanyl peptides and related compounds were analyzed by means of gas chromatography combined with mass-spectrometry. The parent (M^+ and/or M^+-15) and other mass numbers observed in their mass-spectra supported the introduction of various numbers of trimethylsilyl groups.

1. Introduction

The recent advances in the fields of chemical evolution and molecular astronomy are increasing the necessities of preparing the related compounds of α-aminonitriles. The following three kinds of products, peptides, peptide amides, and peptide nitriles may be formed when an α-aminonitrile polymerizes.

$$\text{H–(NH–CHR–CO)}_n\text{–NH–CHR–COOH} \quad \text{Peptide}$$

$$\text{H–(NH–CHR–CO)}_n\text{–NH–CHR–CONH}_2 \quad \text{Peptide amide}$$

$$\text{H–(NH–CHR–CO)}_n\text{–NH–CHR–CN} \quad \text{Peptide nitrile}$$

For the purpose of identification, these products should be compared with the standard samples. Ion-exchange chromatography of the prepared standard samples including peptides, peptide amides, and peptide nitriles (n = 1 and 2) was investigated. Furthermore, these standard samples were trimethylsilylated for gas chromatography combined with mass-spectrometry. The peptide nitriles (n = 1 and 2) and optically active α-aminonitriles were synthesized first by the present authors.

2. Preparation of Standard Samples and Related Compounds

Scheme 1 shows the synthetic route of DL-α-aminopropionitrile (APN). Ethylideniminopropionitrile prepared by the Strecker reaction was treated with a half mole of sulfuric acid to give the neutral sulfate of DL-APN.

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$$2CH_3CHO + NH_4Cl + NaCN \longrightarrow CH_3CH{=}NCH(CH_3)CN$$
$$\xrightarrow{1/2\ H_2SO_4} H_2NCH(CH_3)CN \cdot 1/2\ H_2SO_4 \quad \text{mp } 192\text{–}195\,^{\circ}C$$

SCHEME 1

o-Nitrophenylsulfenyl(Nps)-L-alanine amide was successfully dehydrated in cold $POCl_3$-pyridine to give Nps-L-APN. Nps-L-APN obtained was treated with dry HCl in ethyl acetate, to yield the hydrochloride of L-APN (Kawashiro *et al.*, 1976, 1977).

Scheme 2 shows the synthetic route of alanyl-α-aminopropionitrile (Ala-APN). Nps-dialanine amide was dehydrated in cold $POCl_3$-pyridine to give Nps-Ala-APN. The Nps group of the product was easily removed by the treatment with dry HCl in ethyl acetate and the hydrochloride of Ala-APN was obtained (Kawashiro *et al.*, 1975).

$$\text{Nps-Ala–Ala–NH}_2 \xrightarrow{POCl_3\text{-pyridine}} \text{Nps-Ala–APN}$$
$$\xrightarrow{HCl} \text{Ala–APN}\cdot\text{HCl} \quad \begin{matrix}\text{DL-DL} \\ \left.\begin{matrix}\text{L-DL} \\ \text{L-L}\end{matrix}\right\}\end{matrix} \quad \begin{matrix}\text{mp } 195\text{–}197\,^{\circ}C \\ \text{Hygroscopic}\end{matrix}$$

SCHEME 2

Scheme 3 shows the synthetic route of dialanyl-α-aminopropionitrile(Ala-Ala-APN). *t*-Butoxycarbonyl(Boc)-dialanine hydrazide was carefully converted into its azide by the Curtius method, and then coupled with APN in ethyl acetate. Boc-Ala-Ala-APN obtained was treated with trifluoroacetic acid and the Boc group was removed.

$$\text{Boc-Ala–Ala–NHNH}_2 \xrightarrow{HNO_2} \text{Boc-Ala–Ala–N}_3 \xrightarrow{APN}$$
$$\text{Boc-Ala–Ala–APN} \xrightarrow{CF_3COOH} \text{Ala–Ala–APN}\cdot CF_3COOH$$
$$\left.\begin{matrix}\text{DL-DL-DL, L-DL-L} \\ \text{L-L-DL, L-L-L}\end{matrix}\right\} \text{Hygroscopic}$$

SCHEME 3

Glycyl peptide nitriles, glycylaminoacetonitrile (Gly-AAN) and diglycylamino-acetonitrile (Gly-Gly-AAN) were synthesized by using trityl (Trt) as an N-protecting group. Trt-glycine *p*-nitrophenyl ester was coupled with aminoacetonitrile (AAN) in dimethylformamide to give Trt-Gly-AAN. The N-protected dipeptide nitrile was successfully detritylated by heating in 50%-aq. acetic acid to 90°C for 5 min to give the acetate of Gly-AAN (Kawashiro *et al.*, 1974). The acetate of Gly-Gly-AAN was synthesized similarly as shown in Scheme 4.

$$\text{Trt-Gly-ONp} + \text{Gly-AAN} \longrightarrow \text{Trt-Gly-Gly-AAN} \xrightarrow{50\%\text{-aq. AcOH}} \text{Gly-Gly-AAN}\cdot\text{AcOH} \quad \text{mp } 109\text{–}110\,^{\circ}\text{C}$$

Np: *p*-Nitrophenyl

SCHEME 4

Di- and tripeptides of glycine and alanine, and their amide derivatives were synthesized by the conventional method of peptide synthesis.

3. Ion-exchange Chromatography

Ion-exchange chromatography of the standard samples prepared above, peptides, peptide amides, and peptide nitriles, was carried out at a lower temperature of 30 °C to prevent

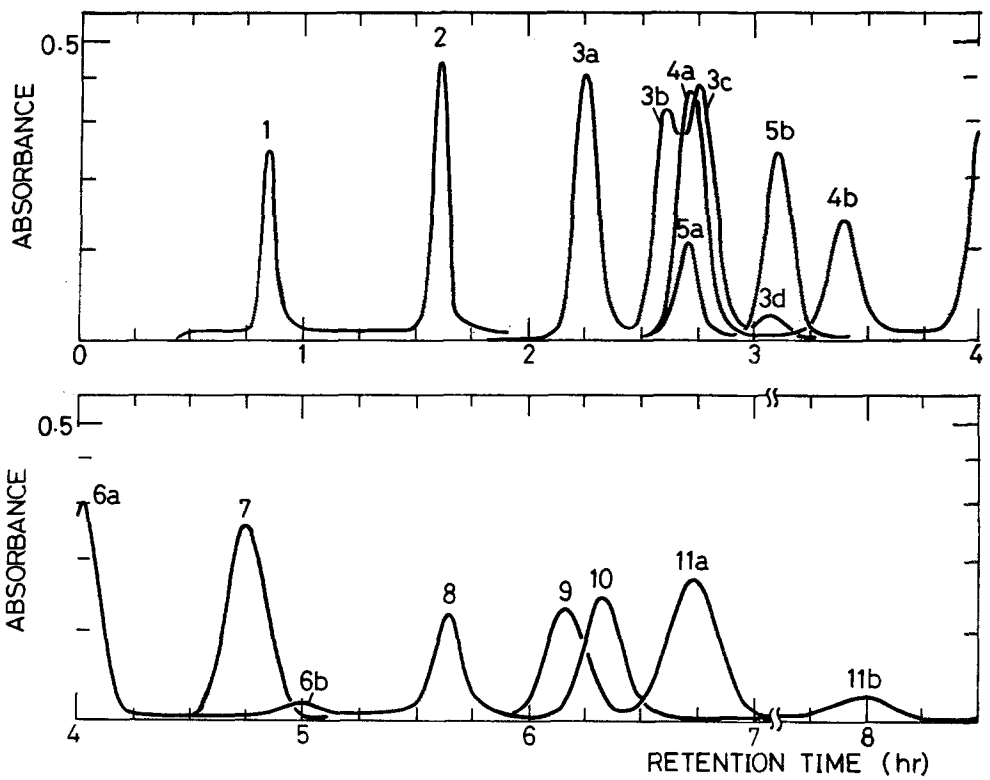


Fig. 1. Ion-exchange chromatogram of alanyl peptides and related compounds. SIBATA Amino acid analyzer, AA 600; column, 0.25 x 50 cm packed with Aminex A-4; flow rate, 6 ml hr^{-1}; jacket temperature, 30 °C; eluent, a pH 3.25 citrate buffer. 1, α,α'-Iminodipropionitrile; 2, Ala; 3, Ala-Ala-Ala (a, L-L-L; b, D-L-L; c, L-L-D; d, L-D-L); 4, Ala-Ala-Ala-NH_2 (a, L-D-L, D-L-L, and L-L-L; b, L-L-D); 5, Ala-Ala (a, L-D; b, L-L); 6, Ala-Ala-NH_2 (a, L-L; b, L-D); 7, Ala-Ala-APN; 8, NH_3; 9, Ala-NH_2; 10, α-Aminopropionitrile; 11, Ala-APN (a, L-D; b, L-L). Charge amount: Ala-Ala-Ala (DL-DL-DL), 0.4 μmol; Ala-Ala-Ala-NH_2 (DL-DL-DL), 0.2 μmol; Ala-Ala (DL-DL), 0.2 μmol; Ala-Ala-NH_2 (DL-DL), 0.2 μmol; Ala-Ala-APN (DL-DL-DL), 0.4 μmol; Ala-APN (DL-DL), 0.2 μmol; the others are 0.1 μmol.

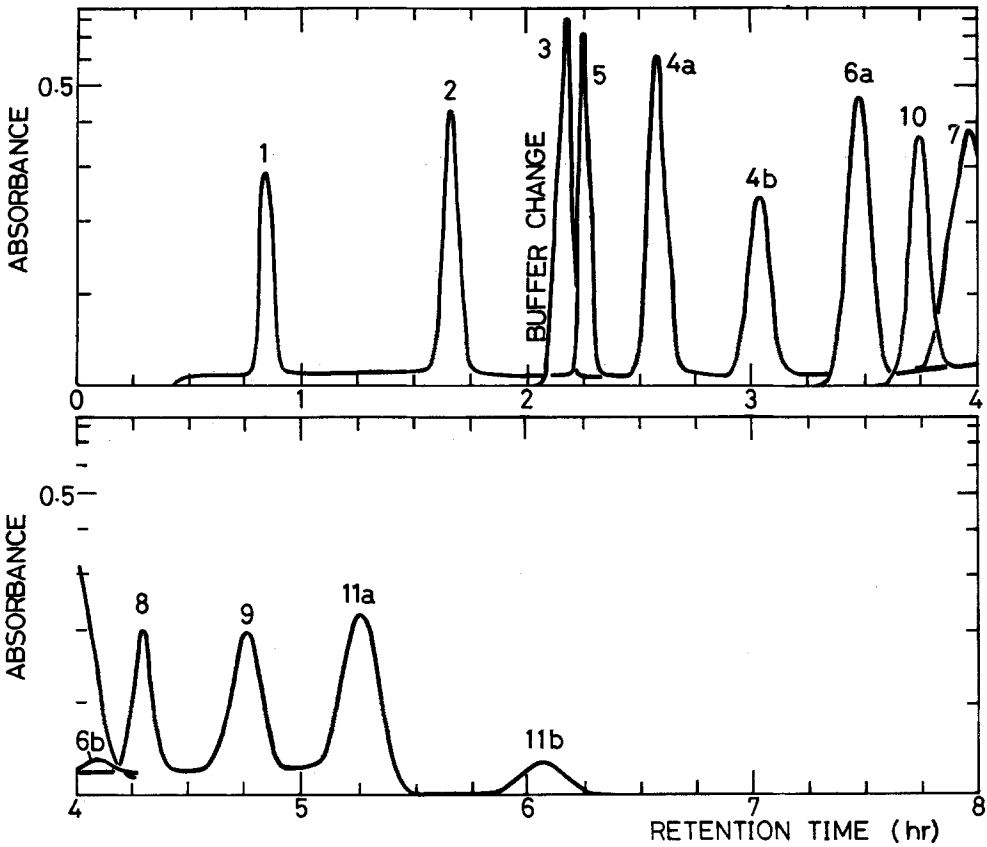


Fig. 2. Ion-exchange chromatogram of alanyl peptides and related compounds. Column, 0.25 x 50 cm; eluent, citrate buffers of pH 3.25 and pH 5.28. Thirty min after the elution began, the eluent was changed from a pH 3.25 buffer to a pH 5.28 buffer. Peak numbers are same as Figure 1. Charge amount: Ala-Ala-Ala-NH_2 (DL-DL-DL), 0.2 μmol; Ala-Ala-NH_2 (DL-DL), 0.2 μmol; Ala-Ala-APN (DL-DL-DL), 0.4 μmol; Ala-APN (DL-DL), 0.2 μmol; the others are 0.1 μmol.

hydrolysis of the peptide nitriles, without buffer change (a pH 3.25 citrate buffer only), and also with buffer change (citrate buffers of pH 3.25 and pH 5.28). When the eluent was changed from a pH 3.25 buffer to a pH 5.28 buffer 30 min after the beginning of the elution, the effect appeared near the retention time of 2 hr in the chromatogram.

Figure 1 shows the ion-exchange chromatogram of alanyl peptides and related compounds using a pH 3.25 buffer only. The every two diastereomers (L-L and L-D) of Ala-Ala, Ala-Ala-NH_2, and Ala-APN were separated into two peaks, the peak 5a (L-D) and 5b (L-L), the peak 6a (L-L) and 6b (L-D), and the peak 11a (L-D) and 11b (L-L), respectively. The four isomers of Ala-Ala-Ala gave four peaks, the peak 3a, 3b, 3c, and 3d corresponding to the L-L-L, D-L-L, L-L-D, and L-D-L isomer, respectively. The four isomers of Ala-Ala-Ala-NH_2 gave two peaks, the peak 4a including the three isomers (L-D-L, D-L-L, and L-L-L) and 4b corresponding to the L-L-D isomer. The four isomers (L-L-L, D-L-L, L-D-L, and L-L-D) of Ala-Ala-APN gave only one peak, the peak 7.

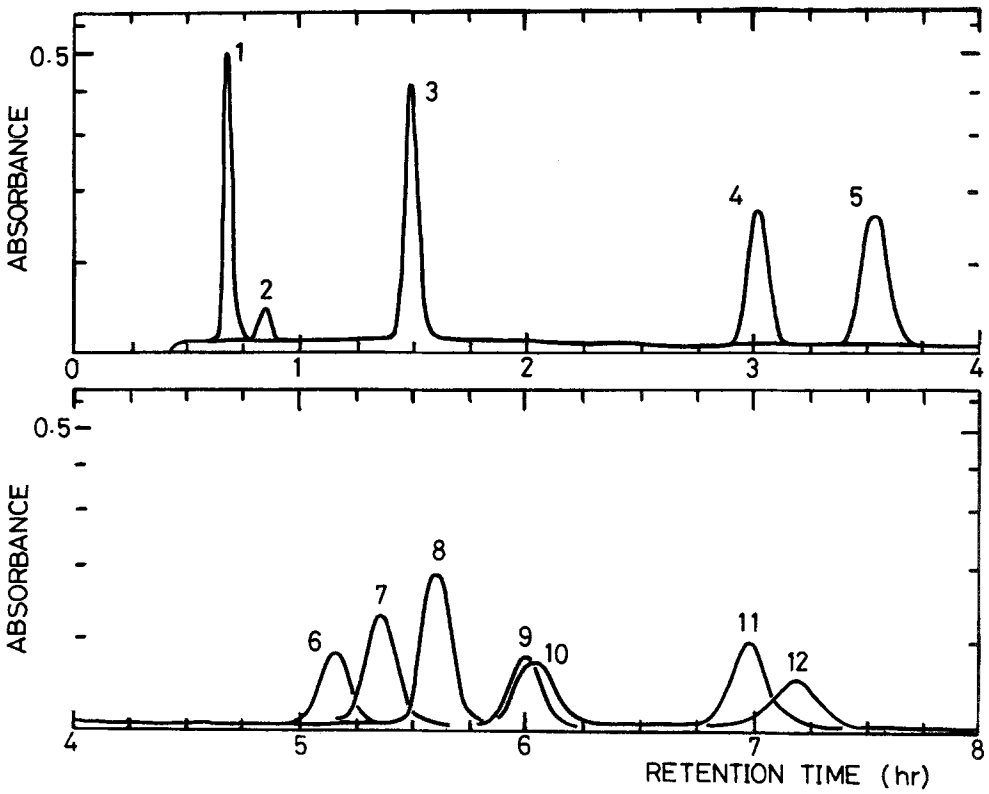


Fig. 3. Ion-exchange chromatogram of glycyl peptides and related compounds. Elution conditions, same as Figure 1. Charge amount of each sample is 0.1 μmol. 1, Iminodiacetic acid; 2, Iminodiacetonitrile; 3, Gly; 4, Gly-Gly; 5, Gly-Gly-Gly; 6, Gly-Gly-Gly-NH_2; 7, Gly-Gly-NH_2; 8, NH_3; 9, Gly-NH_2; 10, Aminoacetonitrile; 11, Gly-AAN; 12, Gly-Gly-AAN.

Figure 2 shows the ion-exchange chromatogram of the same compounds obtained with buffer change. The two diastereomers of Ala-Ala gave one peak, 5, and the four isomers of Ala-Ala-Ala gave also one peak, 3. The two peaks of Ala-Ala-Ala-NH_2, 4a (L-D-L, D-L-L, and L-L-L) and 4b (L-L-D), and the two peaks of Ala-APN, 11a (L-D) and 11b (L-L) became more apparent than in Figure 1. It is noted that the elution order of APN and NH_3 is reversed as compared with that of Figure 1.

Figure 3 shows the ion-exchange chromatogram (without buffer change) of glycyl peptides and related compounds. Iminodiacetic acid (peak 1), Iminodiacetonitrile (2), Gly (3), Gly-Gly (4), and Gly-Gly-Gly (5) were separated completely. Gly-Gly-Gly-NH_2 (6), Gly-Gly-NH_2 (7), and NH_3 (8) were separated poorly and Gly-NH_2 (9) and AAN (10) were not separated. Gly-AAN (11) and Gly-Gly-AAN (12) overlapped partially.

4. Gas Chromatography Combined with Mass-Spectrometry

Trimethylsilylation of alanyl peptides and related compounds was carried out according to the method of Gehrke *et al.* (Gehrke *et al.*, 1969). About 2–6 mg of each sample were

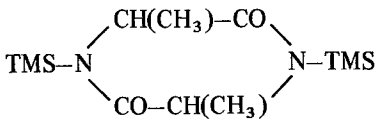
TABLE I
TMS derivatives of alanyl peptides and related compounds

Sample	Number of TMS introduced		M^+	M^+-15	Abundant mass numbers[a]				
DL-Ala anhydride	2		286	271	271	286	171	73	255
DL-Ala–DL-Ala	3	I	376	360	73	147	190	75	44
	3	II		362	188	189	190	361	147
	4		448		116	73	117	118	289
DL-Ala–DL-Ala–NH_2	3		375		116	73	117	171	118
DL-Ala–DL-APN	1		213	198	116	127	128	73	129
	2	I	285	270	116	73	117	155	100
	2	II	285	270	188	75	74	270	100
DL-Ala–DL-Ala–DL-Ala	3	I		432	188	73	189	190	147
	3	II		432	188	73	189	190	116
	4	I		503	147	73	324	281	75
	4	II		502	73	147	281	221	116
	4	III		503	147	221	73	281	355
	4	IV		503	147	221	281	355	73
DL-Ala–DL-Ala–DL-Ala–NH_2	3			432	257	73	258	116	327
	4	I		503	312	313	73	147	116
	4	II		503	221	147	73	355	281
	5	I		576	147	73	221	355	281
	5	II		576	147	221	355	73	281
	5	III		576	147	221	73	355	429
	5	IV		576	147	221	355	73	281
DL-Ala–DL-Ala–DL-APN	3	I	429		116	73	117	118	212
	3	II	429		116	187	143	216	99
	4			485	116	73	117	171	118

[a]Five abundant mass numbers are arranged in a way in which their relative abundances decrease.

suspended in acetonitrile (0.25 ml) followed by the addition of bis(trimethylsilyl)trifluoroacetamide (0.25 ml). The mixture was heated in an air bath to 130 °C for 15 min, and then a clear solution was obtained. The trimethylsilyl (TMS)-derivative obtained was analyzed by means of gas chromatography combined with mass spectrometry under the following conditions; column, 1.0 m x 2.0 mm I.D. glass packed with 1.5% OV 101 on H.P. Chromosorb G; carrier, He 23 ml $min.^{-1}$ The parent mass numbers (M^+ and/or M^+–15) and the most abundant five mass numbers of TMS derivatives are listed in Table 1.

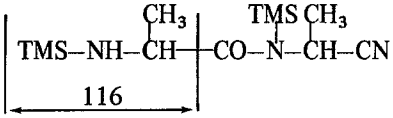
The TMS derivative of DL-alanine anhydride(cyclic dialanine) showed the abundant parent mass number 286, suggesting the trimethylsilylation of the peptide nitrogens:



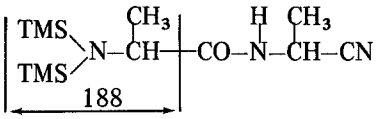
286

The di-TMS derivative of DL-Ala-DL-APN gave two structural isomers, I and II being

N-TMS-DL-Ala-*N'*-TMS-DL-APN, and *N,N*-di-TMS-DL-Ala-DL-APN, respectively:



(I)



(II)

However, it is not always true that *N*-(mono-TMS) derivatives and *N*-(di-TMS) derivatives show the mass number of 116 and 188, respectively. The heating method in the present work for trimethylsilylation may result in a higher temperature of the vial content than that of Gehrke *et al.* (Gehrke *et al.*, 1969) and may introduce more TMS groups. The samples having different charactors may require different conditions for trimethylsilylation and gas chromatography.

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