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Resolution of Amino Acids. XII. Preparation of L-2-Amino-5-arylpentanoic Acids, Constituent Amino Acids in AM-Toxins[1,2]

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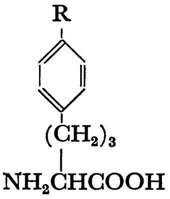
The L-forms of 2-amino-5-(*p*-methoxyphenyl)pentanoic acid (Amp), 2-amino-5-phenylpentanoic acid (App), and 2-amino-5-(*p*-hydroxyphenyl)pentanoic acid (Ahp) are constituent amino acids in AM-toxins. The *O*-methyl linkage of L-Amp was hydrolyzed gradually by heating with hydrochloric acid. A procedure without drastic acid treatment is given for the synthesis and resolution of Amp. Diethyl [3-(*p*-methoxyphenyl)propyl]acetamidomalonate (**3a**) was saponified to the corresponding malonic acid (**5a**), and **5a** was converted into acetyl-DL-Amp (DL-**6a**) by decarboxylation. The DL-**6a** and acetyl-DL-App were resolved by acylase to afford L-Amp and L-App, respectively. The L-Ahp was prepared from L-Amp by acid treatment.

A toxic substance isolated from *Alternaria mali* was named alternariolide by Okuno *et al.*[3] and AM-toxin I by Ueno *et al.*[4] They confirmed the structure to be a cyclic tetradepsipeptide which contains L-2-amino-5-(*p*-methoxyphenyl)pentanoic acid (L-Amp, L-**1a**) as a constituent.[3,4] Later Ueno *et al.* clarified the structures of AM-toxin II[4] and III,[5] indicating that II contains L-2-amino-5-phenylpentanoic acid (L-App, L-**1b**) and III L-2-amino-5-(*p*-hydroxyphenyl)pentanoic acid (L-Ahp, L-**1c**).[6] We undertook to synthesize AM-toxin I, II, and III with the intention of preparing three L-amino acids (L-**1a, b, c**). This paper deals with the synthesis of acetyl-DL-Amp and acetyl-DL-App, their subsequent optical resolution by acylase to afford L-Amp and L-App, and the preparation of L-Ahp from L-Amp by demethylation.



	R
Amp (**1a**)	OMe
App (**1b**)	H
Ahp (**1c**)	OH

Okuno *et al.* reported the synthesis of DL-Amp by Strecker method which involves heating with hydrochloric acid.[7] There has been no report on the synthesis of L-Amp. Ueno *et al.*[4] and Okuno *et al.*[7] described the isolation of L-Amp by heating AM-toxin I with 6 M hydrochloric acid at 110 °C for 20 h, but they reported neither occurrence nor isolation of Ahp in the acid hydrolysate. The results indicate that *O*-methyl linkage in Amp is quite stable during the course of acid treatment. Izumiya and Nagamatsu prepared D-tyrosine from *O*-methyl-D-tyrosine (*O*-Me-D-Tyr) by refluxing in 48% hydrobromic acid.[8] Hirota *et al.* also isolated D-tyrosine together with *O*-Me-D-Tyr by heating Cyl-2, a cyclic tetrapeptide containing *O*-Me-D-Tyr residue as a constituent,[9] with 6 M hydrochloric acid at 115 °C for 15 h. These reports stimulated us to study the stability of *O*-methyl group of Amp against acid treatment. Both L-Amp and *O*-Me-L-Tyr were heated with 6 M hydrochloric acid at 110 °C for certain intervals, and the resulting solution was subjected to an amino acid analysis for determination of the amount of Ahp or tyrosine. The result is shown in Fig. 1. Unexpectedly the rate of demethylation of L-Amp was slightly greater than that of *O*-Me-L-Tyr.

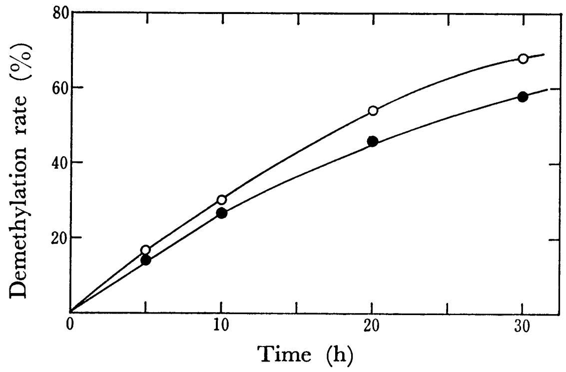
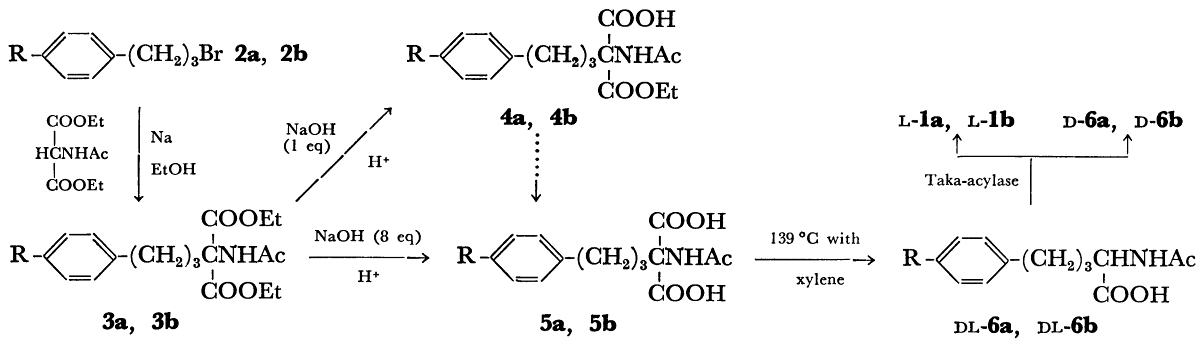


Fig. 1. Time course of demethylation of *O*-methyl-α-amino acids with 6 M HCl at 110 °C.
○: L-Amp, ●: *O*-Me-L-Tyr.



Scheme 1. Synthesis and resolution of acetyl-DL-amino acids (DL-**6a**, DL-**6b**).
a: R=OMe, **b**: R=H.

Table 1. Proteolytic coefficients (C_0) of acetylamino acids by Taka-acylase

Substrate	C_0[a)]	
	0.05M[b)]	0.01M[b)]
Ac-DL-Phe	1.21	2.26
Ac-DL-Amp (DL-**6a**)	0.32	1.19
Ac-DL-App (DL-**6b**)	0.23	1.70

a) $C_0=K_0/E$ where $K_0=\%$ hydrolysis × min^{-1} and $E=$ mg protein N × ml^{-1}; pH 7.3, temp 38 °C. b) Concentration of L-form of substrate.

For the synthesis and optical resolution of Amp, we designed a route which involves no heating with a mineral acid. As shown in Scheme 1, diethyl [3-(*p*-methoxyphenyl)propyl]acetamidomalonate (**3a**) was prepared from alkyl bromide (**2a**) and diethyl acetamidomalonate in a yield of 64%. When **3a** was subjected to saponification with excess sodium hydroxide at 38 °C, TLC indicated that **3a** rapidly turned to **4a**, and that **3a** or **4a** finally turned to [3-(*p*-methoxyphenyl)propyl]acetamidomalonic acid (**5a**) after ten days; **5a** was obtained from **3a** in a yield of 95%. Pure **4a** was easily prepared from **3a** by the use of one equivalent of sodium hydroxide. Acetyl-DL-Amp (DL-**6a**) was obtained from **5a** by heating in xylene in a yield of 95%. Andrako *et al.* prepared a series of alkylacetamidomalonic acids (**7**) from the corresponding diethyl malonates with sodium hydroxide at 100 °C. They used it for the next step without isolating **7**; a suspension of **7** in water was heated to give the corresponging acetyl-DL-amino acid.[10)] When we applied the procedure of Andrako *et al.* to **3a**, we could only isolate DL-**6a** in poor yield because of high hydrophobicity of a series of compounds (**3a, 5a**, DL-**6a**).

Acetyl-DL-App (DL-**6b**) was also prepared in good yield by decarboxylation of **5b** (Scheme 1). Nakashima *et al.* reported the preparation of DL-**6b** by acetylation of DL-App which was synthesized from **3b** with refluxing hydrochloric acid.[11)]

Prior to the preparative resolution of DL-**6a** and DL-**6b** by Taka-acylase, the rates of hydrolysis of two substrates were determined using acetyl-DL-Phe as a reference substrate. The results are given in Table 1. For the preparation of Taka-acylase from Takadiastase powder,[12)] the procedure of Masaki *et al.*[13)] was slightly modified. Although both L-**6a** and L-**6b** were less susceptible than Ac-L-Phe, the results indicate that optical resolution of DL-**6a** and DL-**6b** is achieved with a slightly increased amount of Taka-acylase. DL-**6a** or DL-**6b** was subjected to the action of Taka-acylase, the desired L-amino acid (L-**1a** or L-**1b**) being obtained in a yield of 83% or 82% (Scheme 1). Acetyl-D-amino acid (D-**6a** or D-**6b**) was isolated from the mother liquor after removal of L-amino acid. Nakashima *et al.* also isolated L-App from the reaction mixture of Ac-DL-App and aminoacylase.[11)]

For the preparation of L-Ahp, the L-Amp was heated with 6 M hydrochloric acid for 80 h (Fig. 1), L-Ahp being isolated in a yield of 82%. We suspected the occurrence of partial racemization in Ahp during prolonged heating with hydrochloric acid. For the detection of possible racemate in L-Ahp isolated, the method of Manning and Moore[14)] was applied with slight modification; benzyloxycarbonyl-L-leucine succinimide ester (Z-L-Leu-OSu) was used instead of L-leucine *N*-carboxyanhydride. DL-Ahp prepared from Ac-DL-Amp was coupled with Z-L-Leu-OSu, and the resulting Z-L-Leu-DL-Ahp was hydrogenated to afford L-Leu-DL-Ahp substance (**8**). In a similar manner, L-Leu-L-Ahp substance (**9**) was prepared from Z-L-Leu-OSu and the L-Ahp. A chromatogram of an amino acid analyzer is shown in Fig. 2. We assumed that a peptide showing peak C is L-Leu-L-Ahp while the one showing peak D is L-Leu-D-Ahp. Since **9** afforded peak C but no D, the L-Ahp is optically pure. In contrast, Manning and Moore reported that L-Leu-L-Tyr, which is a homolog of L-Leu-L-Ahp, is eluted more slowly than L-Leu-D-Tyr.[14)] We obtained the same results[14)] in the preparation of L-Leu-L-Tyr substance and L-Leu-DL-Tyr substance using Z-L-Leu-OSu, and subsequent chromatographic studies.

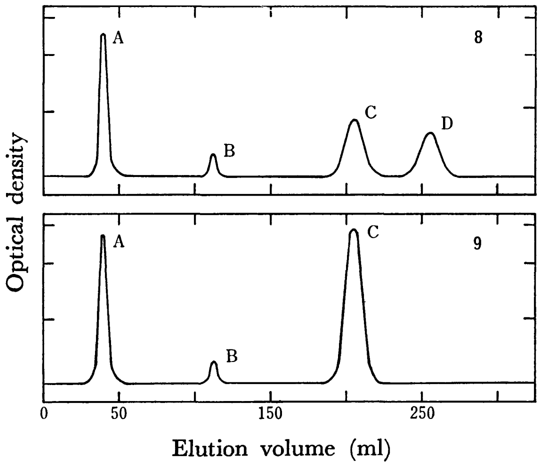


Fig. 2. Chromatogram of L-Leu-DL-Ahp substance (**8**) and L-Leu-L-Ahp substance (**9**). Buffer: pH 5.28 throughout. A: Leu, B: Ahp, C: L-Leu-L-Ahp, D: L-Leu-D-Ahp.

The synthesis of AM-toxin I by utilization of L-Amp has been reported.[2)]

Experimental

All the melting points are uncorrected. TLC was carried out on silica gel G (Merck) with the solvent system; R_f^1, *n*-BuOH(1-butanol)-AcOH-pyridine-H_2O (4:1:1:2,v/v). Paper chromatography was carried out on Toyo Roshi No. 51 with the solvent system: R_f^2, the same solvent as that used for TLC; R_f^3, *n*-BuOH–AcOH–H_2O (4:1:2, v/v). Amino acids and peptides were determined with a Hitachi amino acid analyzer KLA-5 with spherical resin under the following conditions: flow rate, 60 ml/h; jacket temperature, 55 °C. Specific rotation was measured with a Union high sensitivity polarimeter PM-71.

*3-(*p*-Methoxyphenyl)propyl Bromide (**2a**).* The procedure of Catch *et al.*[15)] was slightly modified as follows. Ethyl *p*-methoxycinnamate (65 g)[15)] in EtOH (300 ml) was treated with hydrogen in the presence of Pd black (5 g). Absorption of hydrogen (7.1 l) ceased after about 18 h.

The catalyst was removed and the filtrate was distilled under reduced pressure to afford ethyl 3-(*p*-methoxyphenyl)propionate (**10**) (59.7 g, 91%), bp 148—149 °C/8 mmHg. The **10** (59.3 g) was reduced with $LiAlH_4$ (6.88 g), and the resulting 3-(*p*-methoxyphenyl)-1-propanol (48.2 g, 90%) was treated with PBr_3 (33.1 g) and **2a** was distilled (bp 117—119 °C/5 mmHg).[15] Yield of **2a**, 49.8 g (75%).

3-Phenylpropyl Bromide (2b). The procedure of Norris *et al.*[16] was slightly modified as follows. A mixture of 3-phenyl-1-propanol (54.8 g) and 48% hydrobromic acid (192 ml) was refluxed for 36 h. After being cooled the solution was diluted with water, and extracted with ether. The extract was washed successively with 4% Na_2CO_3 solution and water, and then dried (Na_2SO_4). After removal of the solvent, distillation under reduced pressure gave **2b** (71.5 g, 89%), bp 84—85 °C/5 mmHg.

*Diethyl [3-(*p*-Methoxyphenyl)propyl]acetamidomalonate (3a).* To a solution of Na (2.08 g, 90.4 mmol) in absolute EtOH (70 ml) was added diethyl acetamidomalonate (16.4 g, 75.5 mmol) under stirring. A solution of **2a** (17.3 g, 75.5 mmol) in absolute EtOH (30 ml) was added within 30 min. The brown solution was stirred under reflux for 8 h. The precipitate filtered off was washed with EtOH. The combined filtrates were evaporated *in vacuo* to give an oil which crystallized with the addition of water. The crystals collected were washed successively with water and ether–petroleum ether (1:1, v/v); yield, 17.6 g (64%); mp 97—99 °C; R_f^1 0.94.

Found: C, 62.44; H, 7.33; N, 3.89%. Calcd for $C_{19}H_{27}O_6N$: C, 62.45; H, 7.45; N, 3.83%.

Diethyl (3-Phenylpropyl)acetamidomalonate (3b). This was prepared from **2b** (50 g, 251 mmol) as described for **3a**; yield, 65.0 g (77%); mp 86—87 °C; R_f^1 0.95 (Found: C, 64.47; H, 7.56; N, 4.18%). Andrako *et al.* synthesized **3b** with mp 87—88 °C in 14% yield by the same method.[10]

*2-Acetamido-2-ethoxycarbonyl-5-(*p*-methoxyphenyl)pentanoic Acid (4a).* To a solution of **3a** (730 mg, 2 mmol) in EtOH (8 ml) was added 1 M NaOH (2 ml) at 0 °C. After being left to stand for 18 h at room temperature, the solution was evaporated and acidified with 1 M HCl (2.2 ml). The oil separated was extracted with AcOEt. The extract was washed with water, dried and evaporated. The residual solid was recrystallized from AcOEt–ether–petroleum ether; yield, 517 mg (77%); mp 126 °C; R_f^1 0.73.

Found: C, 60.37; H, 6.93; N, 4.11%. Calcd for $C_{17}H_{23}O_6N$: C, 60.52; H, 6.87; N, 4.15%.

2-Acetamido-2-ethoxycarbonyl-5-phenylpentanoic Acid (4b). This was prepared from **3b** (1.68 g, 5 mmol) as described for **4a**; yield, 1.09 g (71%); mp 129—130 °C; R_f^1 0.79.

Found: C, 62.30; H, 6.88; N, 4.68%. Calcd for $C_{16}H_{21}O_5N$: C, 62.52; H, 6.88; N, 4.56%.

*[3-(*p*-Methoxyphenyl)propyl]acetamidomalonic Acid (5a).* To a solution of **3a** (14.2 g, 38.9 mmol) in MeOH (350 ml) was added 2 M NaOH (160 ml). The solution was allowed to stand at 38 °C for 10 days until no **3a** or **4a** was detected in the reaction mixture; TLC was used as a monitor. The solution was evaporated, and acidified with 6 M HCl (55 ml). The liberated oil was extracted with AcOEt. The extract was washed with water, dried and evaporated. The residual solid was recrystallized from AcOEt–ether–petroleum ether; yield, 11.4 g (95%); mp 109—110 °C; R_f^1 0.61. Compound **5a** is less stable than **5b**; on being left to stand at room temperature for several days, purified crystals of **5a** gave a small amount of decarboxylated material (DL-**6a**) as detected by TLC. The values of elemental analysis of **5a** deviated from the calculated ones.

(3-Phenylpropyl)acetamidomalonic Acid (5b). This was prepared from **3b** (16.8 g, 50 mmol) with 2 M NaOH (200 ml) as described for the preparation of **5a**. The rate of saponification of **3b** was slightly greater than that of **3a**. Yield, 12.6 g (90%); mp 119—120 °C; R_f^1 0.64.

Found: C, 60.19; H, 6.16; N, 5.03%. Calcd for $C_{14}H_{17}O_5N$: C, 60.20; H, 6.14; N, 5.02%.

Ac-DL-Amp (DL-6a). Compound **5a** (8.47 g, 27.4 mmol) suspended in *p*-xylene (100 ml) was refluxed for 2 h. After being cooled at 0 °C, crystals were collected and washed with petroleum ether; yield, 6.91 g (95%); mp 132—133 °C; R_f^1 0.79.

Found: C, 63.45; H, 7.24; N, 5.19%. Calcd for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28%.

Ac-DL-App (DL-6b). This was prepared from **5b** (12.0 g, 42.9 mmol) as described for DL-**6a**; yield, 9.10 g (90%); mp 148—149 °C; R_f^1 0.80 (Found: C, 66.47; H, 7.40; N, 5.82%). Reported value, mp 147—148 °C.[10]

Partially Purified Enzyme (Taka-acylase). Preparation of Taka-acylase: Takadiastase powder (100 g) was suspended in water (550 ml) and the insoluble material was filtered off at 0 °C. The filtrate was treated with $(NH_4)_2SO_4$(92 g, 0.26 saturation) and centrifuged (10000 rpm, 20 min) at 0 °C. The supernatant phase was brought to 0.85 saturation with $(NH_4)_2SO_4$ (209 g) and centrifuged (10000 rpm, 20 min) at 0 °C. The sediment was suspended in 30% acetone (200 ml) at −5 °C and the suspension was centrifuged (12000 rpm, 30 min). The sediment was dissolved in a small amount of water, dialyzed and lyophilized; yield of powder(Taka-acylase), 397 mg.

Determination of Proteolytic Coefficient (C_0) *by Taka-acylase:* In a 2-ml flask, an acetyl-DL-amino acid and one equivalent of 0.5 M NaOH were added. Further 0.1 M sodium barbital buffer (1 ml) of pH 7.3, 1/80 M $CoCl_2$ (0.2 ml), and an aqueous enzyme solution (0.2 ml) containing Taka-acylase (4 mg) were added. The solution was made up to 2.0 ml with water. The reaction mixture was incubated at 38 °C, aliquots being withdrawn at selected intervals. The rate of appearance of free amino acid was followed by the amino acid analyzer: column, 0.9×10 cm; buffer, standard 0.2 M sodium citrate at pH 5.28. It was found that the hydrolysis of substrates follows zero-order kinetics within experimental error. The result is shown in Table 1.

Resolution of Ac-DL-Amp (DL-6a). L-Amp (L-1a): DL-**6a** (5.31 g, 20 mmol) was dissolved in 0.2 M NaOH (110 ml), the pH being adjusted to 7.3 with AcOH. To the solution were added 128 mg of Taka-acylase in 0.2 M sodium acetate (20 ml) and 20 ml of 1/80 M $CoCl_2$. Water was added to bring the volume to 200 ml. After a few drops of toluene had been added, the solution was left to stand at 38 °C for 10 days. The precipitate formed was collected by filtration and washed with 100 ml of water (the precipitate was dissolved in a small amount of 1 M HCl and the solution was subjected to column chromatography). The pH of the combined filtrates was adjusted to 5 with 6 M HCl, a small amount of Norit was added, and the mixture was maintained at 50—60 °C for 10 min. The filtrate was evaporated to a small volume, its pH being adjusted to 3. The mixture was extracted with AcOEt, and the extract was washed with water (the extract was used for the preparation of Ac-D-Amp). The combined aqueous layers and the solution of the above precipitate in 1 M HCl were put on a column (2.2×20 cm) of Dowex 50×8 (H^+ form). The column was washed with water and eluted with 2 M NH_4OH (300 ml). The eluate was evaporated, and the residue was recrystallized from dilute HCl–Et_3N; yield, 1.85 g (83%); mp 232—234 °C (dec); $[\alpha]_D^{21}$ +31.8° (*c* 2.0, 5 M HCl–DMF);[17] R_f^2 0.88, R_f^3 0.76.

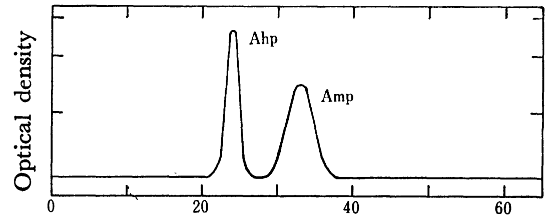


Fig. 3. Chromatogram of a solution after 20 h heating at 110 °C with 6 M HCl of L-Amp.

Found: C, 64.45; H, 7.60; N, 6.05%. Calcd for $C_{12}H_{17}O_3N$: C, 64.55; H, 7.68; N, 6.27%.

Ac-D-Amp (D-***6a***): The extract obtained above was dried (Na_2SO_4) and evaporated. Residual solid was recrystallized from EtOH–water; yield, 2.50 g (94%); mp 145 °C; $[\alpha]^{21}_D$ −16.1 ° (*c* 2.0, EtOH); R_f^1 0.79.

Found: C, 63.27; H, 7.18; N, 5.24%. Calcd for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28%.

*Resolution of Ac-DL-App (DL-**6b**).* L-*App (*L-***1b***)*: DL-**6b** (5.88 g, 25 mmol) was treated with Taka-acylase as mentioned above. L-App was obtained from the reaction mixture, and an extract with AcOEt was set aside for the next step. Yield, 1.98 g (82%); mp 227—229 °C (dec); $[\alpha]^{21}_D$ +33.5 ° (*c* 2.0, 5 M HCl–DMF); R_f^2 0.91, R_f^3 0.77 (Found: C, 68.29; H, 7.79; N, 7.24%). Reported value, $[\alpha]^{21}_D$ +25.7 ° (1 M HCl).11)

*Ac-D-App (*D-***6b****)*: The extract obtained above was treated as described for Ac-D-Amp; yield, 2.54 g (86%); mp 148—149 °C; $[\alpha]^{21}_D$ −11.0 ° (*c* 2.0, EtOH); R_f^1 0.80.

Found: C, 66.34; H, 7.29; N, 5.97%. Calcd for $C_{13}H_{17}O_3N$: C, 66.36; H, 7.28; N, 5.95%.

Demethylation of L-*Amp and O-Me-*L-*Tyr.* L-Amp (1.12 mg, 5 μmol) and O-Me-L-Tyr (0.98 mg, 5 μmol)8) were each heated in a sealed tube with 6 M HCl (1 ml) at 110 °C for certain intervals. A solution in the tube was subjected to amino acid analysis. Determination of the two components in the solution was carried out under the following conditions: column, 0.9×10 cm; buffer, standard 0.2 M citrate buffer at pH 5.28; effluent volume, 24 ml (Ahp) and 33 ml (Amp), 18 ml (Tyr) and 22 ml (*O*-Me-Tyr). The percentage of demethylation of these *O*-methyl-α-amino acids is plotted against time (Fig. 1), and the chromatogam of a solution after 20 h heating of L-Amp is shown in Fig. 3. Amp was converted completely into Ahp after heating for 70—80 h.

DL-*Ahp (*DL-***1c****)*. A suspension of DL-**6a** (220 mg, 0.83 mmol) in 6 M HCl (20 ml) was refluxed on an oil bath at 110 °C for 80 h. The solution was evaporated *in vacuo* several times by the addition of water. The residual solid was recrystallized from dilute HCl–Et_3N–EtOH; yield, 134 mg (77%); mp 272—273 °C (dec); R_f^2 0.78, R_f^3 0.66.

Found: C, 62.66; H, 7.21; N, 6.63%. Calcd for $C_{11}H_{15}O_3N$: C, 63.14; H, 7.23; N, 6.69%.

L-*Ahp* (L-***1c***). This was prepared from L-Amp (1.12 g, 5 mmol) as described above; yield, 0.86 g (82%); mp 242—243 °C (dec); $[\alpha]^{21}_D$ +36.1 ° (*c* 2.0, 5 M HCl–DMF); R_f^2 0.78, R_f^3 0.66.

Found: C, 62.65; H, 7.22; N, 6.50%. Calcd for $C_{11}H_{15}O_3N$: C, 63.14; H, 7.23; N, 6.69%.

Racemization Test of L-*Ahp.* L-*Leu-*DL-*Ahp Substance (**8**)*: To a solution of DL-Ahp (10 mg, 48 μmol), dioxane (1 ml), water (1 ml), and Et_3N (6.7 μl, 48 μmol) was added Z-L-Leu-OSu18) (26 mg, 72 μmol) in dioxane (1 ml) at 0 °C. The mixture was allowed to stand overnight at room temperature and then evaporated. The residue dissolved in MeOH–AcOH–H_2O (5:1:1, v/v) was hydrogenated in the presence of Pd black, and the filtrate was evaporated to dryness. The residue was designated **8**.

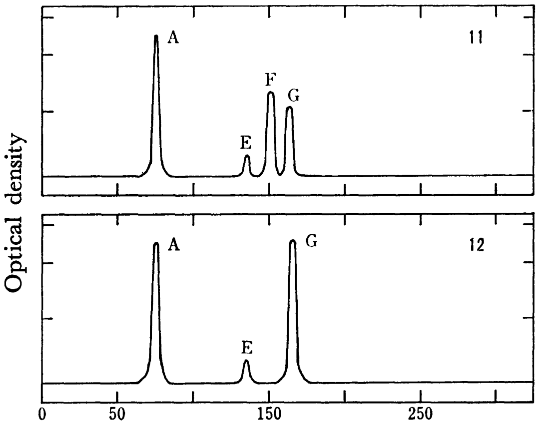


Fig. 4. Chromatogram of L-Leu-DL-Tyr substance (**11**) and L-Leu-L-Tyr substance (**12**). Buffer: pH 4.25 for 0—40 ml and pH 5.28 after 40 ml. A: Leu, E: Tyr, F: L-Leu-D-Tyr, G: L-Leu-L-Tyr.

L-*Leu-*L-*Ahp Substance (**9**)*: This was obtained from L-Ahp (10 mg, 48 μmol) as described above.

Chromatography with Amino Acid Analyzer: **8** and **9** were each dissolved in 0.2 M citrate buffer of pH 2.2, and an aliquot (1 μmol) of the solution was subjected to amino acid analysis. The following conditions were sufficient to permit the separation of two diastereomers; column, 0.9× 55 cm; buffer, the standard citrate at pH 5.28. The chromatograms are shown in Fig. 2.

Chromatography of L-*Leu-Tyr.* The L-Leu-DL-Tyr substance (**11**) and L-Leu-L-Tyr substance (**12**) were prepared as described for the preparation of **8** and **9**. **11** was eluted as one peak (effluent volume, 75 ml) which was faster than L-Leu-L-Ahp (effluent volume, 203 ml) when analysis was carried out under the same conditions for the separation of **8**. It was possible under the following conditions to separate two diastereomers in **11**; **11** was eluted with pH 4.25 buffer for 40 min, thereafter with pH 5.28 buffer. As shown in Fig. 4, L-Leu-L-Tyr substance (**12**) showed a peak of G, but the view that a peptide showing G might be *O*-leucyl peptide was not excluded. The possibility was eliminated by the following experiment. Z-L-Leu-OSu was coupled with *O*-benzyl-L-Tyr19) and the peptide substance obtained by hydrogenation gave a peak identical with that of G.

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toxin I and confirmed by several experiments that the Amp possesses an L-configuration. However, they reported no $[\alpha]_D$ because the amount of the L-Amp isolated was very small. For the L-configuration of App and Ahp in AM-toxin II and III, they gave no experimental evidence and made the assumption from an analogy of L-Amp in I.

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