Preparation of Homologs of L-2-Amino-5-(*p*-methoxyphenyl)pentanoic Acid

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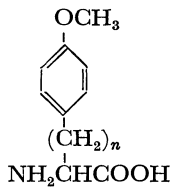
Lower and higher homologs, L-2-amino-4-(*p*-methoxyphenyl)butanoic acid (L-Amb) and L-2-amino-6-(*p*-methoxyphenyl)hexanoic acid (L-Amh), of L-2-amino-5-(*p*-methoxyphenyl)pentanoic acid were prepared by optical resolution of *N*-acetyl-DL-Amb and of *N*-acetyl-DL-Amh with *Aspergillus* acylase. *N*-Acetyl-DL-Amb or *N*-acetyl-DL-Amh was synthesized from [2-(*p*-methoxyphenyl)ethyl or butyl](acetamido)malonic acid by heating with *p*-xylene.

AM-Toxin I is a host specific phytotoxin, its structure being determined as *cyclo*(-L-Amp[1]-ΔAla[2]-L-Ala[3]-L-Hmb[4]-).[1] Shimohigashi *et al.* synthesized AM-toxin I[2] and its analog, [L-Tyr(Me)[1]]-AM-toxin,[3] and observed that [L-Tyr(Me)[1]]-AM-toxin showed extremely weak necrotic activity toward apple leaves. This finding implies that the methylene length of side chain in L-Amp affects the necrotic activity. Therefore, it is interesting to synthesize L-Amb or L-Amh, which is a lower or higher homolog of L-Amp (Fig. 1), in an attempt to prepare an AM-toxin analog containing an aromatic amino acid residue with a different methylene chain.

Only DL-Amb has been synthesized by Strecker's method,[4] but none of L-Amb, DL- and L-Amh have been prepared so far. This paper deals with the preparation of *N*-acetyl-DL-Amb (Ac-DL-**1a**) and *N*-acetyl-DL-Amh (Ac-DL-**1b**) and their optical resolution by acylase (Fig. 2). The procedure used for resolution is similar to that used for the preparation of L-Amp.[5] In the synthesis of diethyl [2-(*p*-methoxyphenyl)ethyl](acetamido)malonate (**3a**) from arylalkyl bromide (**2a**) and diethyl acetamidomalonate (**5**), we suffered from relatively low yield (27%). In contrast, diethyl [4-(*p*-methoxyphenyl)butyl](acetamido)-malonate (**3b**) in the present study and diethyl [3-(*p*-methoxyphenyl)propyl](acetamido)malonate reported in the previous paper[5] were synthesized in moderate yields of 66% and 64% from the corresponding bromide and the malonate (**5**), respectively. We found that the reaction mixture of the bromide (**2a**) and the malonate (**5**) contained a decarboxylated product (DL-**6**) besides the desired **3a**. The malonic acid derivative (**4a** or **4b**) was obtained in good yield from the diester derivative (**3a** or **3b**) by complete saponification with excess sodium hydroxide. The important intermediate Ac-DL-**1a** or Ac-DL-**1b** was prepared in good yield from **4a** or **4b** by heating in *p*-xylene.



	n
Tyr(Me)	1
Amb (**1a**)	2
Amp	3
Amh (**1b**)	4

Fig. 1. Structure of homologs of Amp.

Prior to the preparative resolution of Ac-DL-**1a** and Ac-DL-**1b** by *Aspergillus* acylase, the rates of hydrolysis of two substrates were determined using Ac-DL-Tyr-(Me) and Ac-DL-Amp as reference substrates, the results being shown in Table 1. It is observed that the rates of hydrolysis are decreased with the increase of the methylene length of side chain in aromatic amino acids. Although Ac-L-**1b** was less susceptible than Ac-L-Tyr(Me), the results indicate that optical resolution of Ac-DL-**1b** is achieved with an increased amount of the acylase. Ac-DL-**1a** or Ac-DL-**1b** was subjected to the action of the acylase, the desired L-amino acid (L-**1a** or L-**1b**) being obtained in a yield of 82% or 81% (Fig. 2). *N*-Acetyl-D-amino acid (Ac-D-**1a** or Ac-D-**1b**) was isolated from the mother liquor, and subsequently D-amino acid (D-**1a** or D-**1b**), which

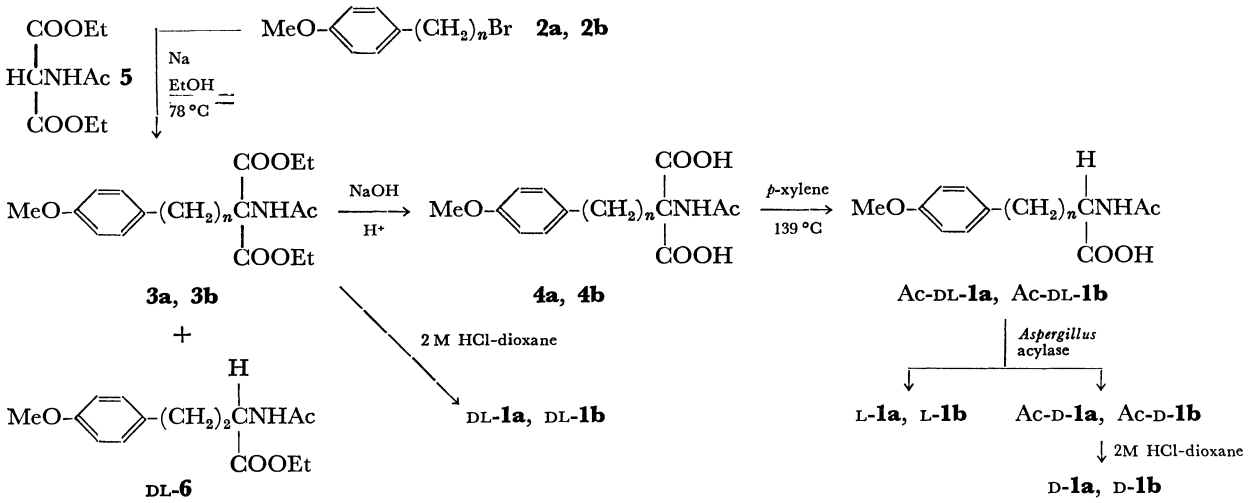


Fig. 2. Synthesis and resolution of *N*-acetyl-DL-amino acids (Ac-DL-**1a**, Ac-DL-**1b**). **a**: $n=2$, **b**: $n=4$.

Table 1. Proteolytic coefficients (C_0) of acetylamino acids by *Aspergillus* acylase

Substrate	C_0[a] at 0.01 M[b]
Ac-DL-Tyr(Me)	28 (100)[c]
Ac-DL-**1a**	24 (86)
Ac-DL-Amp	14 (50)
Ac-DL-**1b**	12 (43)

a) $C_0=K_0/E$ where $K_0=\%$ hydrolysis$\times$min^{-1} of $E=$mg protein $N\times$ml^{-1}; pH 7.3, temp 38 °C. b) Concentration of L-form of substrate. c) The values in the parentheses indicate the relative hydrolytic rate.

is also useful compound for synthesis of AM-toxin analogs, was prepared from Ac-D-**1a** or Ac-D-**1b** by heating in 6 M HCl.

Experimental

All the melting and boiling points are uncorrected. TLC was carried out on silica gel (Merck) with the following solvent systems: R_f^1, benzenea–cetone (1:1); R_f^2, 1-butanol–AcOH–pyridine–H_2O (4:1:1:2). Paper chromatography was carried out on Toyo Roshi No. 52 paper with the following solvent systems: R_f^3, the same solvent as used for R_f^2; R_f^4, 1-butanol–AcOH–pyridine–H_2O (15:3:10:12). The ratio in parenthesis is given in terms of volume. Powder of *Aspergillus* acylase was obtained from Kyowa Hakko Co., Ltd.

*2-(p-Methoxyphenyl)ethyl Bromide (**2a**).* 2-(*p*-Methoxyphenyl)ethanol (**7**) was synthesized following the literature;[6] yield, 76%; bp 121—125 °C/7 mmHg.† Compound **2a** was synthesized from **7** (0.69 mol) and PBr_3 (0.345 mol) by a slight modification of the literature;[7] yield, 77%; bp 122 °C/9 mmHg. In the literature,[7] PBr_5 had been used.

*4-(p-Methoxyphenyl)-1-butanol (**8**).* 4-Oxo-4-(*p*-methoxyphenyl)butanoic acid (**9**) was synthesized from anisole and succinic anhydride by a slight modification of the literature,[8] yield, 89%; mp 142—143 °C. 1-Nitropropane had been used as a solvent in the literature,[8] but anisole was used as a reagent and also as a solvent for the present synthesis of **9**. 4-(*p*-Methoxyphenyl)butanoic acid (**10**) was synthesized from **9** with amalgamated Zn following the literature;[9] yield, 88%; mp 60—61 °C. Compound **8** was synthesized from **10** following the literature.[10] However, the procedure will be described here because the description in the literature was not enough to carry out the experimental work. To a suspension of $LiAlH_4$ (4.75 g, 0.125 mol) in anhydrous ether (180 ml) was added portionwise **10** (19.4 g, 0.1 mol) in ether (450 ml) at 0 °C under stirring within 1 h. The mixture was stirred at 0 °C for another 4 h. Water (300 ml) was added to decompose an excess $LiAlH_4$. To the mixture was added 10% H_2SO_4 (150 ml) and insoluble material was filtered off. The ethereal layer was separated, and the aqueous layer was extracted with ether. The combined ethereal solution was washed with 2 M NaOH, water, and dried (Na_2SO_4). After removal of the solvent, distillation under reduced pressure gave **8**; yield, 13.2 g (73%); bp 135—140 °C/4 mmHg; mp 4 °C. Literature, mp 3—4 °C.[10]

*4-(p-Methoxyphenyl)butyl Bromide (**2b**).* To a solution of **8** (48.3 g, 0.268 mol) in CCl_4 (54 ml) was added dropwise PBr_3 (30.6 g, 0.113 mol) in CCl_4 (27 ml) at 50—55 °C under stirring within 4 h. The mixture was stirred at 40—50 °C for another 2 h and CCl_4 was evaporated. The residue was dissolved in ether, and the solution was washed with water, dried (Na_2SO_4), and evaporated. Then distillation under reduced pressure gave **2b**; yield, 56.3 g (86%); bp 125—130 °C/3 mmHg.

Found: C, 54.13; H, 6.34%. Calcd for $C_{11}H_{15}OBr$: C, 54.34; H, 6.22%.

*Diethyl [2-(p-Methoxyphenyl)ethyl](acetamido)malonate (**3a**).* To a solution of Na (14.4 g, 0.626 mol) in absolute EtOH (840 ml) was added diethyl acetamidomalonate (**5**) (114 g, 0.523 mol) under stirring. A solution of **2a** (113 g, 0.523 mol) in absolute EtOH (260 ml) was added to the previous solution within 1 h. The reaction mixture was stirred under reflux for 8 h, and the precipitate was filtered off and washed with EtOH. The combined filtrates were evaporated *in vacuo* to give an oil which was crystallized by the addition of water and petroleum ether. The product was recrystallized from EtOH–water; yield, 49.4 g (27%); mp 81—84 °C; R_f^1 0.72.

Found: C, 61.39; H, 7.12; N, 3.98%. Calcd for $C_{18}H_{25}O_6N$: C, 61.52; H, 7.17; N, 3.99%.

Ethyl DL-*2-Acetamido-4-(p-methoxyphenyl)butanoate (*DL-**6**).* This was obtained from **2a** (3.9 g, 18 mmol) and **5** (3.9 g, 18 mmol) as described for **3a** except that the reaction mixture was refluxed for 4 d; yield, 1.1 g (22%); mp 94—96 °C; R_f^1 0.66.

Found: C, 63.99; H, 7.49; N, 4.90%. Calcd for $C_{15}H_{21}O_4N$: C, 64.49; H, 7.58; N, 5.01%.

*Diethyl [4-(p-Methoxyphenyl)butyl](acetamido)malonate (**3b**).* This was prepared from **2b** (40.2 g, 0.165 mol) and **5** (35.8 g, 0.165 mol) as described for **3a**. The product was recrystallized from EtOH–water; yield, 41.0 g (66%); mp 37—40 °C; R_f^1 0.83.

Found: C, 63.01; H, 7.72; N, 3.70%. Calcd for $C_{20}H_{29}O_6N$: C, 63.30; H, 7.70; N, 3.69%.

*[2-(p-Methoxyphenyl)ethyl](acetamido)malonic Acid (**4a**).* A solution of **3a** (16.0 g, 46 mmol) and 2 M NaOH (230 ml) in EtOH (500 ml) was allowed to stand at 38 °C for 3 d until none of **3a** and the intermediate 2-acetamido-2-ethoxycarbonyl-4-(*p*-methoxyphenyl)butanoic acid (R_f^2 0.65) were detected in the reaction mixture. The solution was evaporated and acidified with 6 M HCl (138 ml). The liberated oil was extracted with EtOAc and the extract was washed with water, dried (Na_2SO_4), and evaporated. The residue was crystallized by the addition of ether and petroleum ether; yield, 10.5 g (78%); mp 124—126 °C; R_f^2 0.53. Compound **4a** was unstable; on being left to stand at room temperature for 10 d, purified crystals of **4a** gave a small amount of decarboxylated material (Ac-DL-**1a**). The values of elemental analysis of crystalline **4a** deviated from the calculated ones as **4a**.

*[4-(p-Methoxyphenyl)butyl](acetamido)malonic Acid (**4b**).* This was prepared from **3b** (8.1 g, 21.3 mmol) as described for **4a**; yield, 6.1 g (88%); mp 102—103 °C; R_f^2 0.66. Compound **4b** was unstable and its property was similar to **4a**.

DL-*2-Acetamido-4-(p-methoxyphenyl)butanoic Acid (Ac-*DL-***1a**).* Compound **4a** (3.0 g, 10.2 mmol) suspended in *p*-xylene (100 ml) was refluxed for 2 h. After being cooled to room temperature, crystals were collected and washed with petroleum ether; yield, 1.96 g (77%); mp 144—145 °C; R_f^2 0.73.

Found: C, 62.03; H, 6.86; N, 5.52%. Calcd for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.57%.

DL-*2-Acetamido-6-(p-methoxyphenyl)hexanoic Acid (Ac-*DL-***1b**).* This was prepared from **4b** (5.87 g, 18.2 mmol) as described for Ac-DL-**1a**; yield, 4.02 g (79%); mp 93—94 °C; R_f^2 0.83.

Found: C, 64.49; H, 7.57; N, 4.96%. Calcd for $C_{15}H_{21}O_4N$: C, 64.49; H, 7.58; N, 5.01%.

† 1 mmHg≈133.322 Pa.

DL-*2-Amino-4-(*p*-methoxyphenyl)butanoic Acid (DL-**1a**).* A suspension of Ac-DL-**1a** (100 mg, 0.4 mmol) in a mixture of 2 M HCl (20 ml) and dioxane (20 ml) was refluxed on an oil bath at 110 °C for 15 h. The solution was evaporated *in vacuo* several times by the addition of water. The residual solid was recrystallized from 1 M HCl–1 M aqueous Et_3N; yield, 70.3 mg (84%); mp 244—245 °C (dec); R_f^3 0.82, R_f^4 0.76. Literature, mp 245—246 °C.[4]

DL-*2-Amino-6-(*p*-methoxyphenyl)hexanoic Acid (DL-**1b**).* This was prepared from Ac-DL-**1b** (279 mg, 1 mmol) as described for DL-**1a**; yield, 205 mg (87%); mp 253—254 °C (dec); R_f^3 0.93, R_f^4 0.88.

Found: C, 65.69; H, 8.21; N, 5.82%. Calcd for $C_{13}H_{19}O_3N$: C, 65.80; H, 8.07; N, 5.90%.

Determination of Proteolytic Coefficient (C_0) by Acylase. In a 2-ml flask, an *N*-acetyl-DL-amino acid (0.04 mmol) and 0.2 M NaOH (0.2 ml) were added. Further, 1/7 M Na barbital buffer (1 ml) of pH 7.5, 1/40 M $CoCl_2$ (0.1 ml), and an aqueous enzyme solution (0.2 ml) containing the acylase (0.2 mg) were added. The solution was made up to 2.0 ml with the buffer. 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 a Hitachi amino acid analyzer KLA-5: column, 0.6 cm × 10 cm; buffer, standard 0.2 M Na citrate at pH 5.28. The hydrolysis of substrates follows zero-order kinetics within experimental error, the results being shown in Table 1.

*Resolution of Ac-DL-**1a**.* L-**1a**: Ac-DL-**1a** (2.51 g, 10 mmol) was dissolved in 0.0125 M aqueous Et_3N (800 ml), the pH being adjusted to 7.5 with AcOH. To the solution were added the acylase (50 mg), 1/80 M $CoCl_2$ (100 ml), and water (100 ml). The solution was left to stand at 38 °C for 5 d, the pH being adjusted occasionally to 7.5. The precipitate formed was collected by filtration (later, the precipitate was subjected to recrystallization). The pH of the combined filtrates was adjusted to 5 with 1 M HCl. A small amount of Norit was added, and the mixture was maintained at 50—60 °C for 10 min. The filtrate was concentrated to a small volume, and its pH was adjusted to 3 with 1 M HCl. The oil separated was extracted with EtOAc and the extract was washed with water (the extract was used for the preparation of Ac-D-**1a**). The aqueous layers were applied to a column of Dowex 50 (H^+ form), and the column was washed with water and eluted with 2 M NH_4OH. The eluate was evaporated, and the residue was combined with the above precipitate. Then, the product was recrystallized from dilute HCl–Et_3N–acetone; yield, 0.86 g (82%); mp 268—271 °C (dec); $[\alpha]_D^{20}$ +42.2 (*c* 0.2, 5 M HCl); R_f^3 0.82, R_f^4 0.76.

Found: C, 62.81; H, 7.44; N, 6.76%. Calcd for $C_{11}H_{15}O_3N$: C, 63.14; H, 7.23; N, 6.69%.

*Ac-D-**1a***: The extract with EtOAc obtained above was dried (Na_2SO_4) and evaporated. The residual solid was recrystallized from EtOH–water; yield, 1.07 g (85%); mp 172—173 °C; $[\alpha]_D^{20}$ −22.5 (*c* 1, EtOH); R_f^2 0.73.

Found: C, 62.05; H, 6.95; N, 5.32%. Calcd for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.57%.

D-**1a**: This was prepared from Ac-D-**1a** (0.50 g, 2 mmol) as described for DL-**1a**; yield, 0.34 g (81%); mp 268—270 °C (dec); $[\alpha]_D^{20}$ −41.8 (*c* 0.2, 5 M HCl); R_f^3 0.82, R_f^4 0.76.

Found: C, 63.21; H, 7.26; N, 6.42%. Calcd for $C_{11}H_{15}O_3N$: C, 63.14; H, 7.23; N, 6.69%.

*Resolution of Ac-DL-**1b**.* L-**1b**: Ac-DL-**1b** (2.79 g, 10 mmol) was treated with the acylase as mentioned above. Amino acid L-**1b** was obtained from the reaction mixture; yield, 0.96 g (81%); mp 230—233 °C (dec); $[\alpha]_D^{20}$ +31.1 (*c* 0.1, 5 M HCl); R_f^3 0.93, R_f^4 0.88.

Found: C, 65.62; H, 8.12; N, 5.85%. Calcd for $C_{13}H_{19}O_3N$: C, 65.80; H, 8.07; N, 5.90%.

*Ac-D-**1b***: The extract with EtOAc was treated as described for Ac-D-**1a**: yield, 1.15 g (82%); mp 134—135 °C; $[\alpha]_D^{20}$ −13.4 (*c* 1, EtOH); R_f^2 0.83.

Found: C, 64.35; H, 7.65; N, 4.82%. Calcd for $C_{15}H_{21}O_4N$: C, 64.49; H, 7.58; N, 5.01%.

D-**1b**: This was prepared from Ac-D-**1b** (0.56 g, 2 mmol); yield, 0.44 g (93%); mp 234—236 °C (dec); $[\alpha]_D^{20}$ −30.6 (*c* 0.1, 5 M HCl); R_f^3 0.93, R_f^4 0.88.

Found: C, 65.84; H, 8.04; N, 5.77%. Calcd for $C_{13}H_{19}O_3N$: C, 65.80; H, 8.07; N, 5.90%.

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References

1) Abbreviation used: Ac, acetyl; Amb, 2-amino-4-(*p*-methoxyphenyl)butanoic acid; Amh, 2-amino-6-(*p*-methoxyphenyl)hexanoic acid; Amp, 2-amino-5-(*p*-methoxyphenyl)-pentanoic acid; ⊿Ala, dehydroalanine; Hmb, 2-hydroxy-3-(methyl)butanoic acid; Tyr(Me), *O*-methyltyrosine.

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