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An Improved Method for Synthesis of L-Homoglutamic Acid *via* Epsilon-*N*-deamination of L-Lysine Derivative

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A convenient procedure to prepare L-homoglutamic acid (L-2-aminoadipic acid; L-Aad) is described. Chlorination of ε-amino group in acetyl-L-lysine ethyl ester with *t*-butyl hypochlorite (*t*-BuOCl) followed by dehydrochlorination with pyridine gave ethyl 5-cyanovalerate which on acid hydrolysis afforded L-Aad in good yield.

2-Aminoadipic acid (Aad) is an intermediate in lysine metabolism and has been found in peptide antibiotics precursor of penicillin and cephalosporin biosynthesis. The absolute configuration of Aad from *penicillium chrysogenum* was determined to be the L-configuration by Chan *et al.*[1] Chemical synthesis of L-Aad has proceeded under optical resolution of DL-hydantoin derivative[2] or 2-(hydroxyimino)adipic ester.[3] In recent years Scott and Wilkinson[4] reported an alternative procedure based on the conversion of ε-amino group of N^{α}-benzyloxycarbonyl-L-lysine to the corresponding acid using NaOCl and a strong base, such as 1,8-diazabicyclo[5.4.0]undec-7-ene. L-Aad was obtained in 35% overall yield from the lysine derivative.

In a previous paper,[5] we reported a new method for the detection of intramolecularly hydrogen-bonded peptide NH by chlorine replacement reaction, which was initiated by adding a peptide to a solution containing Cl_2 or by adding *t*-BuOCl to a solution of peptide. The rate of chlorination measured by ^{1}H NMR showed that intramolecularly hydrogen-bonded peptide NH's were much more susceptible to the chlorination than solvent-exposed NH's in the peptide antibiotics, gramicidin S and tuberactinamin N. To make sure these new findings, substituent effects of alkyl (R-) or aryl (Ar-) group of CH_3CONHR (or -Ar) on H–Cl replacement reaction were also examined by ^{1}H NMR. The results well explained the differences in the reactivity of the amide NH protons: the more easily the amide nitrogen releases the proton (*viz.*, the more downfield NH proton signal appears), the more susceptible the NH proton is to chlorination. We postulated a mechanism to account for the H–Cl replacement reaction.[6]

We report here an improved method for the synthesis of L-Aad in optical form from N^{α}-acetyl-L-lysine ethyl ester by a deamination procedure. The procedure consists of chlorination of ε-amino group in the lysine derivative with *t*-BuOCl, dehydrochlorination of the *N,N*-dichloro amine derivative with pyridine, and hydrolysis of the nitrile. The overall yield was 60% from the lysine derivative. The present method provides a superior procedure for the synthesis of L-Aad to previously reported procedures[2–4] in high yield and in ease of operation.

Results and Discussion

Synthesis of L-*Glutamic Acid from* L-*Ornithine.* In the course of study on structure-activity relationship of gramicidin S, we found that amino acid analysis of the hydrolysis products of chlorinated gramicidin S gave three unknown peaks except ones of constituent amino acids in gramicidin S. Among the three peaks, one which appeared in the acidic region seemed to be glutamic acid derived from ornithine during chlorination of gramicidin S followed by acid hydrolysis. To confirm the results, we carried out chlorination of Ac-L-Orn-OEt and acid hydrolysis of the dichloro amine derivative. The preliminary experiments using the model compound, Ac-L-Orn-OEt, showed that the δ-NH_2 group was not susceptible to chlorination with Cl_2, but that in the case of *t*-BuOCl a chlorinated product having δ-NHCl or δ-NCl_2 group was detected by ^{1}H NMR and mass spectrometers. After a methanol solution of the dichloro amine derivative was tightly stoppered and stored in the dark for a month, the δ-NCl_2 group remained without decomposition, which was ascertained by appearance of its absorption band (310 nm, λ_{max}) in UV spectrum and by thin-layer chromatography. The dichloro amine derivative chlorinated with *t*-BuOCl was hydrolyzed with 6 mol dm^{-3} hydrochloric acid. Amino acid analysis of the products gave two peaks. One was ascertained to be ornithine and the other to be glutamic acid from the data of paper chromatography (PPC) and paper electrophoresis. In the above procedure, glutamic acid was obtained only in a small amount.

Nitrile synthesis from amine *via* *N,N*-dihalo amine followed by dehydrohalogenation was carried out by several procedures.[7–9] For example, the dehydrohalogenation was carried out by heating dihalo amine with cesium fluoride. In the conversion of 1,6-hexanediamine into adiponitrile the fluorinated derivative was successfully isolated.[9] Therefore, to obtain a high yield of glutamic acid, formation of nitrile by dehydrochlorination was carried out with triethylamine and pyridine. However, use of triethylamine as a base was no advantage to the yield of glutamic acid. On the other hand, application of excess pyridine gave a good yield of glutamic acid as a main product. Amino acid analysis and the value of $[\alpha]_D$ of the glutamic acid thus

Table 1. Reaction products of Ac-L-Lys-OEt with *t*-BuOCl under various conditions and acid hydrolysis of nitrile

Chlorination conditions		Chlorinated[a] NH/%		Acid hydrolysis of nitrile		
t-BuOCl[b]	time	α-NH	ε-NH_2	Aad[c]:	Lys:	insoluble
10 eq	3.5 h	60	100	15	20	65
10 eq	5 min	5	100	52	18	30
5 eq	5 min	0	100	75	7	18

a) Data from ^{1}H NMR. b) In MeOH at 0 °C and then room temperature. c) Weight ratio.

obtained were in fair agreement with those of an authentic sample. These results suggest that L-ornithine is converted into L-glutamic acid without racemization *via* the dichloro amine derivative in good yield.

Synthesis of L-Aad from L-Lysine. We are very interested in the preparation of L-homoglutamic acid (L-Aad) which is not easily available in large quantities. The above results prompted us to study for a convenient and practical synthesis of L-Aad from L-lysine derivative.

In order to find out suitable reaction conditions, chlorination of Ac-L-Lys-OEt (**2**) with *t*-BuOCl was carried out under various conditions shown in Table 1. The most satisfactory result was obtained by using 5 equivalents *t*-BuOCl for 5 min, and also acid hydrolysis of the nitrile derived from the dichloro amine under these conditions showed the best yield of L-Aad. After chlorination with *t*-BuOCl (5-equiv) in methanol for 5 min, the reaction mixture was concentrated *in vacuo* to give an oil (**3**). This oil was used in the next reaction without further purification because of its unstableness. The formation of the dichloro amine derivative was detected by ^{1}H NMR and UV spectra. As shown in Fig. 1, compound **3** was dehydrochlorinated by excess pyridine for 20 h at room temperature, the reaction mixture was concentrated *in vacuo*, and the oily residue was dissolved in chloroform. The solution was washed successively with 0.5 mol dm^{-3} sodium hydrogencarbonate, 0.5 mol dm^{-3} citric acid, and water to give the nitrile (**4**). The formation of **4** was detected by the 2260 cm^{-1} ν C≡N band of the product in the IR spectrum and further confirmed by ^{1}H NMR and mass spectra. The nitrile was refluxed with 6 mol dm^{-3} hydrochloric acid to yield L-Aad, lysine, and insoluble material. After filtering off the insoluble material, these products were subjected to ion-exchange column chromatography to afford L-Aad in 60% yield from Ac-L-Lys-OEt (**2**) which was easily obtained by catalytic hydrogenolysis of Ac-L-Lys(ε-Z)-OEt (**1**). Amino acid analysis, CD, and $[\alpha]_D$ data of the synthetic L-Aad (**5**) thus obtained were identical with those of an authentic sample.

By the above results, the deamination procedure proved to be a facile and convenient method for the synthesis of L-homoglutamic acid in optical form from L-lysine derivative.

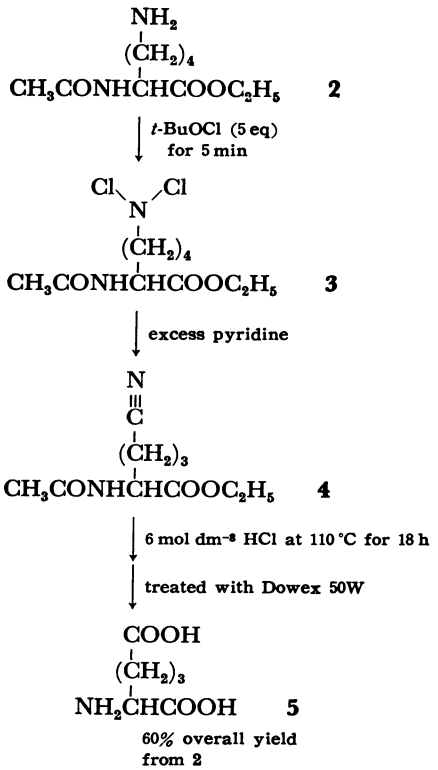

Fig. 1. Synthetic Procedure of L-2-Aminoadipic Acid.

Experimental

The melting point is uncorrected. All chemicals used for preparative experiments were of reagent grade and solvents were distilled before use. An authentic sample of L-Aad was obtained from Calbiochem-Behring Corp., La Jolla., Calf., U.S.A. Thin-layer chromatography was carried out on silica gel GF_{254} (Merck) with the following solvent system: R_f^1, $CHCl_3$-MeOH-AcOH (8:1:1, v/v). Compounds with protected amino groups were detected by spraying with 50% hydrochloric acid followed by ninhydrin. Chlorinated compounds were detected by spraying with the mixed solution (1:1, v/v) of 0.1 mol dm^{-3} potassium iodide in water and 2.1 mmol dm^{-3} *o*-tolidine in 2% AcOH. Paper chromatography was performed on Toyo Roshi No. 50 paper with the following solvent systems: R_f^2, *n*-BuOH-AcOH-H_2O (4:1:2, v/v) and R_f^3, *n*-BuOH-AcOH-pyridine-H_2O (4:1:1:2, v/v). Optical rotations were measured on a Yanagimoto polarimeter OR-50 and CD spectra with a Jasco J-20 spectropolarimeter. Mass spectra were taken on an ESCO EMD-05A and NMR spectra on a JEOL JNM-MH-100. Infrared spectra were recorded on a Hitachi 260-10 instrument using KCl plate. Amino acid analysis was carried out by amino acid analyzer model, JLC-6AH.

Ac-L-Lys(ε-Z)-OEt (1). To a solution of L-Lys(ε-Z)-OEt·*p*-TosOH[10] (7.21 g, 15 mmol) in pyridine (35 mL) was added acetic anhydride (7.1 mL, 75 mmol) at 0 °C. The mixture was stirred for 18 h at room temperature and then evap-

orated *in vacuo*. The residue was diluted with ethyl acetate (400 mL). The ethyl acetate solution was washed successively with 0.5 mol dm^{-3} citric acid, 0.5 mol dm^{-3} $NaHCO_3$, and water. The solution was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was obtained as an oil; yield 4.73 g (90%); R_f^1 0.65; ^1H NMR ($CDCl_3$): δ 7.32 (s, 5H, aromatic), 6.33 (d, 1H, -NH-), 5.00 (m, 1H, -NH-), 4.52 (q, 1H, -CH), 4.16 (q, 2H, $-CH_2-$), 3.16 (q, 2H, $-CH_2-$), 1.98 (s, 3H, $-CH_3$), 1.26 (t, 3H, $-CH_3$); Found: *m/z* 350. Calcd for $C_{18}H_{26}N_2O_5$: M, 350.

Ac-L-Lys-OEt (2). Compound **1** (3.8 g, 10.8 mmol) was hydrogenated in EtOH (35 mL) in the presence of palladium black for 4.5 h. The solution filtered from the catalyst was evaporated to dryness *in vacuo*. The oily product weighed 2.3 g (98%); R_f^2 0.57, R_f^3 0.61; ^1H NMR (DMSO-d_6): δ 8.38 (d, 1H, -NH-), 4.32 (m, 1H, -CH), 4.22 (q, 2H, $-CH_2-$), 2.60 (m, 2H, $-NH_2$), 1.91 (s, 3H, $-CH_3$), 1.23 (t, 3H, $-CH_3$); Found: *m/z* 216. Calcd for $C_{10}H_{20}N_2O_3$: M, 216.

N^{ε}-Dichloro-N^{α}-acetyl-L-lysine Ethyl Ester (3). To a solution of **2** (2.3 g, 10.6 mmol) in MeOH (30 mL) was added *t*-butyl hypochlorite (5.96 mL, 53 mmol) at 0 °C. After 5 min at room temperature, the solution was evaporated to dryness *in vacuo* at 5 °C. The compound was obtained as an oil. All experimental procedures were carried out in the dark; yield 3.0 g (99%); R_f^1 0.64; ^1H NMR (DMSO-d_6): δ 8.28 (d, 1H, -NH-), 4.10 (q, 2H, $-CH_2-$), 3.00 (t, 2H, $-CH_2-$), 1.88 (s, 3H, $-CH_3$), 1.20 (t, 3H, $-CH_3$).

Ethyl 2-Acetamido-5-cyanovalerate (4). To a solution of **3** (1.51 g, 5.3 mmol) in MeOH (15 mL) was added excess pyridine (93 mL) at 0 °C in the dark. After 20 h at room temperature, the reaction mixture was evaporated and dissolved in $CHCl_3$ (200 mL). The solution was washed with 0.5 mol dm^{-3} citric acid and aq NaCl, dried over anhydrous Na_2SO_4, and then concentrated under reduced pressure to an oil. The formation of nitrile **4** by dehydrochlorination was confirmed with the 2260 cm^{-1} ν C≡N band of the product in the IR spectrum. It weighed 1.04 g (93%); R_f^1 0.67; ^1H NMR ($CDCl_3$): δ 6.52 (d, 1H, -NH-), 4.60 (m, 1H, -CH), 4.22 (q, 2H, $-CH_2-$), 2.42 (t, 2H, $-CH_2-$), 2.04 (s, 3H, $-CH_3$), 1.31 (t, 3H, $-CH_3$); Found: *m/z* 212. Calcd for $C_{10}H_{16}N_2O_3$: M,212.

L-2-Aminoadipic Acid (5). Compound **4** (1.04 g, 4.9 mmol) was refluxed with 6 mol dm^{-3} HCl (150 mL) for 18 h. The cooled solution was evaporated to give an oil, which was dissolved in water (100 mL). After filtering off a small amount of insoluble material, the solution was applied on a column (2.1×25 cm) of Dowex 50 W (H^+ form). The column was washed with water (200 mL) and was eluted with 2 mol dm^{-3} aqueous ammonia (200 mL). The collected effluents were evaporated to leave an oily product, which was dissolved in water (90 mL). Chromatography of this solution with Dowex 50 W (NH_4^+ form) separated Aad by elution with water (200 mL) and lysine was successively recovered with 2 mol dm^{-3} aqueous ammonia (200 mL). The effluent containing Aad from the column (NH_4^+ form) was evaporated to a small volume and precipitated by the addition of EtOH. The crude product was dissolved in dil HCl and the solution was adjusted to pH 3.5 with 1 mol dm^{-3} aqueous ammonia and then crystallized by the addition of EtOH; yield 525 mg (60% from **2**); R_f^2 0.27, R_f^3 0.16; mp 199—200 °C; $[\alpha]_D^{21}$ +24.4° (*c* 2, 5 mol dm^{-3} HCl). Authentic L-Aad: R_f^2 0.27, R_f^3 0.16; mp 200—201 °C; $[\alpha]_D^{21}$ +24.6° (*c* 2, 5 mol dm^{-3} HCl). Amino acid analysis and CD spectrum of L-Aad were in fair agreement with the authentic L-Aad.

Found: C, 44.54; H, 7.07; N, 8.65%. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69%.

Electrophoresis. Electrophoresis of compound **5**, using Toyo Roshi No. 51A paper, was carried out with the following solvent systems: Ep^1, pyridine-AcOH-H_2O (1:10:89, v/v, pH 3.6) and Ep^2, pyridine-AcOH-H_2O (25:1:225, v/v, pH 6.5) at 500 V/30 cm for 2 h. Compound **5** and L-homoglutamic acid (Aad) showed the equal mobilities, which migrated towards the cathode, 0.4 cm in Ep^1 and the anode, 2.8 cm in Ep^2, respectively.

References

1) J. A. Chan, F. Huang, and C. J. Shin, *Biochemistry*, **15**, 177 (1976).
2) M. Takehara and R. Yoshida, *Nippon Kagaku Zasshi*, **90**, 101 (1969).
3) W. Dieckmann, *Chem. Ber.*, **38**, 1656 (1905).
4) A. I. Scott and T. J. Wilkinson, *Synth. Commun.*, **10**, 127 (1980).
5) M. Kondo, K. Okamoto, I. Nishi, M. Yamamoto, T. Kato, and N. Izumiya, *Chem. Lett.*, **1980**, 703.
6) M. Kondo, I. Nishi, K. Okamoto, T. Kato, and N. Izumiya, "PEPTIDES: Synthesis-Structure-Function," ed by D. H. Rich and E. Gross, Pierce Chemical Company, Rockford (1981), p. 291.
7) T. E. Stevens, *J. Org. Chem.*, **26**, 2531 (1961).
8) L. L. Jackson, G. N. R. Smart, and G. F. Wright, *J. Am. Chem. Soc.*, **69**, 1539 (1947).
9) C. M. Sharts, *J. Org. Chem.*, **33**, 1008 (1968).
10) T. Kato, S. Makisumi, M. Ohno, and N. Izumiya, *Nippon Kagaku Zasshi*, **83**, 1151 (1962).
11) Abbreviations for amino acids are according to the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972).