Synthesis of N^{ε}-Hydroxy-L-lysine

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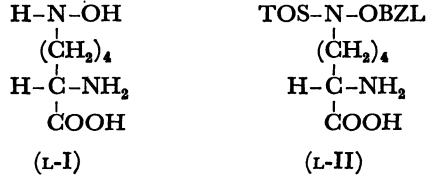
(Received April 22, 1974)

Optically active N^{ε}-hydroxy-L-lysine monohydrochloride (L-I·HCl) was synthesized from N^{ε}-tosyl-N^{ε}-benzyloxy-L-lysine (L-II) obtained by the procedure employed for the preparation of N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine. Removal of the protecting groups of L-II was carried out by the following two methods: a) treatment of L-II with boron tris(trifluoroacetate) in trifluoroacetic acid gave L-I in one step, which was isolated as 2-nitroindane-1,3-dione salt; b) deprotection of L-II *via* N^{ε}-benzyloxy-L-lysine (L-VIII), detosylation with hydrogen bromide in acetic acid and phenol, followed by debenzylation with hydrogenolysis, gave L-I·HCl having properties identical with those of the natural product obtained from mycobactine.

N^{ε}-Hydroxy-L-lysine (L-I) is an important intermediate in the synthesis of mycobactines, sexadentate chelating agents for ferric iron, which occur naturally and stimulate the growth of mycobacteria.

Snow reviewed the isolation and structual elucidation of L-I.[1] Its synthesis by various methods gave only the racemic form. Rogers and Neilands[2] prepared the N^{ε}-hydroxy-DL-lysine (DL-I) in the form of its 2-nitroindane-1,3-dione salt from the 5-(4-bromobutyl)-hydantoin *via* the corresponding nitro- and hydroxy-amino-hydantoin. Lancini, Lazzari, and Diena[3] obtained DL-I by the reaction of hydroxyamine with 5-(4-bromobutyl) hydantoin and the following hydrolysis with hydrochloric acid. Black, Brown, and Wade[4] suggested the preparation of DL-I·2 HCl by reacting diethyl 5-bromo-1-phthalimidopentane-1,1-dicarboxylate with benzaldoxime, followed by the transformation of the nitrone to the corresponding hydroxyamine hydrochloride, the final product being characterized only in solution. It was found that N^{ε}-hydroxy-DL-lysine (DL-I) was obtained from N^{α}-phthalyl-DL-lysine *via* the corresponding nitrone derivative.[5]

L-Isomer of I is a key compound for providing a route to the mycobactine. We wish to report on the synthesis of N^{ε}-hydroxy-L-lysine with the use of N^{ε}-tosyl-N^{ε}-benzyloxy-L-lysine (L-II), obtained by the procedure employed for the preparation of N^{δ}-tosyl-N^{δ}-benzyloxy-L-ornithine.[6] L-II is suitably protected at N^{ε}-position and therefore can be converted into N^{ε}-hydroxy-L-lysine (L-I) by removing the protecting groups under mild conditions without loss of optical purity.



(L-I) (L-II)

The sequence of reactions employed in the preparation of DL-II is given in Fig. 1.

O-Benzyl-*N*-tosylhydroxyamine (III)[6] was first haloalkylated with 1,4-dibromobutane to 4-(*N*-tosyl-*N*-benzyloxy)aminobutyl bromide (IV). This was allowed to react with diethyl sodio-acetamidomalonate, and the product (V) was refluxed with concentrated hydrochloric acid–acetic acid to yield the racemic N^{ε}-tosyl-N^{ε}-benzyloxy-DL-lysine (DL-II).

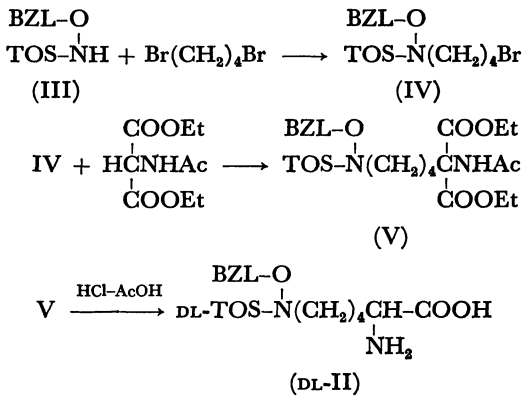


Fig. 1. Synthesis of N^{ε}-tosyl-N^{ε}-benzyloxy-DL-lysine (DL-II): BZL, benzyl; TOS, *p*-toluenesulfonyl; Ac, acetyl.

Optically active N^{ε}-tosyl-N^{ε}-benzyloxy-L-lysine(L-II) was obtained by the enzymatic resolution of N^{α}-acetyl-N^{ε}-tosyl-N^{ε}-benzyloxy-DL-lysine (DL-VI) *via* its anilide (L-VII) with aniline in the presence of papain (Fig. 2).

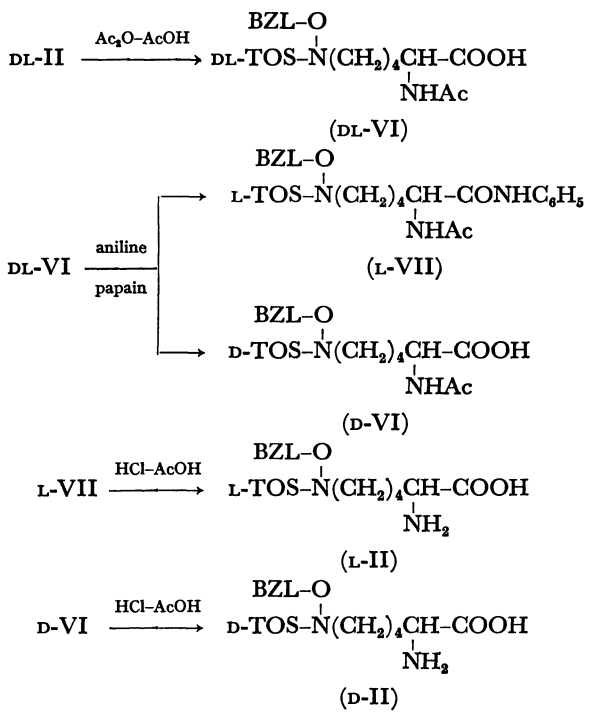


Fig. 2. Optical resolution of N^{α}-acetyl-N^{ε}-tosyl-N^{ε}-benzyloxy-DL-lysine (DL-VI).

The resulting N^{α}-acetyl-N^{ϵ}-tosyl-N^{ϵ}-benzyloxy-L-lysine anilide (L-VII) and N^{α}-acetyl-N^{ϵ}-tosyl-N^{ϵ}-benzyloxy-D-lysine (D-VI) showed optical rotations of $[\alpha]_D^{24}$ −48.6° and $[\alpha]_D^{22}$ −13.3° in chloroform solution. N^{ϵ}-Tosyl-N^{ϵ}-benzyloxy-L-lysine (L-II) and its D-isomer (D-II) prepared by acid hydrolysis of L-VII and D-VI showed optical rotations of $[\alpha]_D^{24}$ +18.4° and $[\alpha]_D^{22}$ −17.9° in acetic acid solution, respectively.

The optically active N^{ϵ}-protected hydroxylysine (L-II) thus obtained was deprotected *via* two different routes, a and b (Fig. 3), to N^{ϵ}-hydroxy-L-lysine (L-I) under mild conditions without racemization.

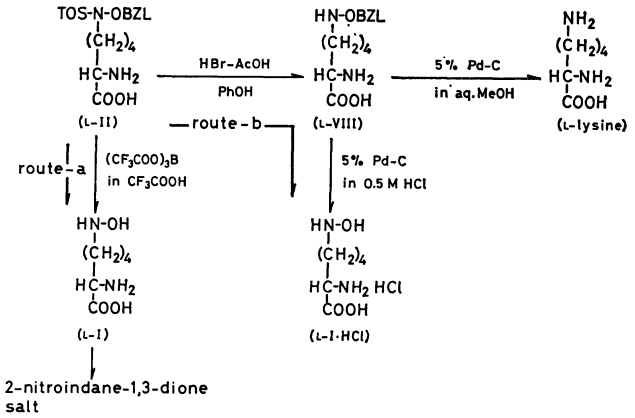


Fig. 3. Deprotection of N^{ϵ}-tosyl-N^{ϵ}-benzyloxy-L-lysine (L-II).

In route-a, the treatment of L-II with boron tris-(trifluoroacetate)[7] followed by neutralization of the product with aqueous ammonia afforded L-I as a colorless solid, mp 223—226 °C (decomp.) and $[\alpha]_D^{24}$ +24.2° (*c* 0.5, M hydrochloric acid) (lit,[8] mp 223—225 °C (decomp.) and $[\alpha]_D^{18}$ +23.9° (*c* 5.1, M hydrochloric acid)), in 58% yield. The N^{ϵ}-hydroxy-L-lysine (L-I) thus obtained was immediately transformed into 2-nitroindane-1,3-dione salt, because of the difficulty of preparing the L-I (HCl-free) in a state of reasonable purity; L-I was relatively unstable in the neutral and alkaline solution. The salt was obtained as pale brown crystals with melting point and the results of elemental analysis agreeing with those of the natural product reported by Snow.[8]

In route-b, the N^{ϵ}-protected hydroxylysine (L-II) was first detosylated with 36% hydrogen bomide in acetic acid in the presence of phenol and the resulting product (L-VIII) was hydrogenolyzed over 5% palladium on carbon in 0.5 M hydrochloric acid to afford the monohydrochloride (L-I·HCl) in 89% yield. In contrast, when the reduction procedure was carried out in a neutral solution *i.e.* in aqueous methanol, L-lysine was obtained in 51% yield. The monohydrochloride (L-I·HCl) could be precipitated from aqueous solution by addition of ethanol, because of the low solubility of L-I·HCl in aqueous ethanolic solution. The hydrochloride (L-I·HCl) thus obtained had mp 192—194 °C (decomp.) and $[\alpha]_D^{24}$ +25.5° in M hydrochloric acid. The hydroxyamine group was detected by the strong red coloration caused by treatment of the product with an alkaline solution of triphenyltetrazolium chloride. The structure of the synthetic L-I·HCl was confirmed by NMR and IR spectroscopy and elemental analysis. In particular, significant NMR-spectral features of L-I·HCl in trifluoroacetic acid corresponded with those of N^{δ}-hydroxy-L-ornithine monohydrochloride.[9]

The optical purity of L-II should be maintained in the reaction sequence, since L-lysine obtained by the hydrogenolysis of L-VIII in a neutral solution showed the same specific rotation as that of an authentic sample[10] and the synthetic specimens of L-I and L-I·HCl exhibited specific rotations identical with the corresponding data for the natural product.[8]

In view of the stability and yield of the desired product, we suggest that route-b (Fig. 3) is preferable for the preparation of N^{ϵ}-hydroxy-L-lysine (L-I).

Experimental

All melting points were determined with a Yanagimoto electric micromelting point apparatus and are uncorrected. Optical rotations were measured with a Yanagimoto automatic polarimeter OR-50. The nuclear magnetic resonance spectra were run on a Varian HA-100 High Resolution NMR spectrometer, using tetramethylsilane as an internal standard. Infrared spectra were recorded on a Hitachi EPI-G3 spectrophotometer as KBr disk.

O-Benzyl-N-tosylhydroxyamine (III). This compound was prepared by the procedure reported.[6]

4-(N-Tosyl-N-benzyloxy)aminobutyl Bromide (IV). To a solution of sodium (20.8 g, 0.902 mol) in ethanol (1.2 l) was added III (250 g, 0.902 mol) with vigorous stirring at 70 °C. After a clear solution was obtained, 1,4-dibromobutane (583 g, 2.70 mol) was added at once. The mixture was refluxed for 6 hr, and evaporated *in vacuo* to give oily material, which was extracted with ethyl acetate (1.4 l), the organic layer being thoroughly washed with water and dried over sodium sulfate. Evaporation of the solvent afforded IV as oily material, which was crystallized from ethyl acetate–*n*-hexane; yield 320 g (86%), mp 56—59 °C.

Found: C, 52.29; H, 5.45; N, 3.21%. Calcd for $C_{18}H_{22}NO_3SBr$: C, 52.43; H, 5.38; N, 3.40%.

Diethyl 5-(N-Tosyl-N-benzyloxy)amino-1-acetamidopentane-1,1-dicarboxylate (V). To a solution of sodium (15.1 g, 0.657 mol) in ethanol (1.0 l) was added diethyl acetamidomalonate (143 g, 0.657 mol) with stirring. After 5 min, IV (271 g, 0.657 mol) was added and refluxed for 8 hr. The solvent was then removed *in vacuo* and the residue was extracted with chloroform (1.2 l). The chloroform layer was successively washed with water, diluted hydrochloric acid and water, dried over sodium sulfate, and evaporated to yield a pale yellow syrup which gave a negative Beilstein test; yield 302 g (84%). Attempts to crystallize the syrup were unsuccessful and it was used for the next step without analysis or purification.

N^{ϵ}-Tosyl-N^{ϵ}-benzyloxy-DL-lysine (DL-II). A solution of V (275 g, 0.502 mol) in a mixture of acetic acid (2.4 l) and concentrated hydrochloric acid (800 ml) was heated under gentle reflux for 8 hr. The solution was evaporated *in vacuo* and 14% aqueous ammonia was added to the residue with ice-cooling. The resulting solid was collected by filtration, washed with water, and recrystallized from aqueous acetic acid; yield 147 g (72%) and mp 178—185 °C (decomp.).

Found: C, 58.87; H, 6.21; N, 6.96%. Calcd for $C_{20}H_{26}N_2O_5S$: C, 59.09; H, 6.45; N, 6.89%.

The product showed blue coloration on heating with an

aqueous solution of ninhydrin.

N^{α}-Acetyl-N^{ε}-tosyl-N^{ε}-benzyloxy-DL-lysine (DL-VI). Acetic anhydride (40 g, 0.392 mol) was slowly added to a stirred solution of DL-II (121 g, 0.297 mol) in acetic acid (600 ml). After being stirred for 12 hr at room temperature, the solvent was evaporated *in vacuo*. Water was added to decompose excess acetic anhydride and the resulting mixture was extracted with ethyl acetate (600 ml). The organic layer was washed with water and dried over sodium sulfate. The residue obtained on evaporation of the solvent was crystallized from ethyl acetate–petroleum ether to give DL-VI as an amorphous powder; yield 119 g, (89%) and mp 139—141 °C. Recrystallization from ethyl acetate caused no rise in melting point.

Found: C, 58.95; H, 6.47; N, 6.01%. Calcd for $C_{22}H_{28}N_2O_6S$: C, 58.91; H, 6.29; N, 6.25%.

Resolution of N^{α}-Acetyl-N^{ε}-tosyl-N^{ε}-benzyloxy-DL-lysine (DL-VI); N^{α}-Acetyl-N^{ε}-tosyl-N^{ε}-benzyloxy-L-lysine Anilide (L-VII). DL-Acetylamino acid (DL-VI) (44.8 g, 0.10 mol) was dissolved in 0.75 M sodium hydroxide (350 ml) and the solution was adjusted to pH 6.20 with 0.5 M citric acid. Papain (15 g) was added to the solution together with freshly distilled aniline (12 ml) and cysteine hydrochloride monohydrate (5.5 g). The mixture was incubated for 36 hr at 38 °C and the anilide deposited was collected by suction and washed with water, 7% aqueous ammonia and water. Recrystallization from methanol–water (3:1) gave clorless crystals; yield 23 g (88%), mp 137—139 °C and $[\alpha]_D^{24}$ −48.6° (*c* 1.0, chloroform).

Found: C, 64.27; H, 6.47; N, 7.84%. Calcd for $C_{28}H_{33}N_3O_5S$: C, 64.22; H, 6.35; N, 8.02%.

N^{α}-Acetyl-N^{ε}-tosyl-N^{ε}-benzyloxy-D-lysine (D-VI). The above filtrate combined with the washings adjusted to pH 2 with 5 M hydrochloric acid. The precipitate was then collected by suction, washed with water and dried. Recrystallization from ethyl acetate gave colorless crystals; yield 16 g (71%), mp 155—157 °C and $[\alpha]_D^{22}$ −13.3° (*c* 1.0, chloroform).

Found: C, 58.74; H, 6.31; N, 6.10%. Calcd for $C_{22}H_{28}N_2O_6S$: C, 58.91; H, 6.29; N, 6.25%.

N^{ε}-Tosyl-N^{ε}-benzyloxy-L-lysine (L-II). L-VII (17.0 g, 32.5 mmol) was dissolved in a mixture of acetic acid (130 ml) and concentrated hydrochloric acid (50 ml). After being refluxed for 6 hr, the solution was evaporated *in vacuo* and aqueous ammonia was added to the residue with ice-cooling. The deposits were collected by suction, washed with water and dried. Recrystallization from dilute acetic acid and treatment with hot methanol gave an amorphous powder; yield 11.4 g (86%), mp 204—207 °C (decomp.) and $[\alpha]_D^{24}$ +18.4° (*c* 1.0, acetic acid).

Found: C, 58.88; H, 6.46; N, 6.66%. Calcd for $C_{20}H_{26}N_2O_5S$: C, 59.09; H, 6.45; N, 6.89%.

N^{ε}-Tosyl-N^{ε}-benzyloxy-D-lysine (D-II). This was obtained from D-VI by the method employed for the preparation of L-II; yield 80%, mp 199—204 °C (decomp.) and $[\alpha]_D^{22}$ −17.9° (*c* 1.0, acetic acid).

Found: C, 58.80; H, 6.36; N, 6.87%. Calcd for $C_{20}H_{26}N_2O_5S$: C, 59.09; H, 6.45; N, 6.89%.

N^{ε}-Hydroxy-L-lysine (L-I) (prepared in route-a). L-II (10 g, 24.6 mmol) was added at 5 °C to a solution of boron tris(trifluoroacetate) prepared from boron tribromide (66 g, 263 mmol) in trifluoroacetic acid (150 ml). The reaction mixture was then stirred for 48 hr at room temperature and evaporated *in vacuo* below 30 °C. Methanol was added to the residue to decompose excess boron tris(trifluoroacetate) and the mixture was evaporated *in vacuo*. To the oily residue was added 6 M hydrochloric acid (250 ml) and the mixture was refluxed for 1 hr. Insoluble brown material was filtered off and the filtrate was concentrated *in vacuo*. Colorless crystals further precipitated were removed by suction and the filtrate was evaporated to dryness. The semi solid thus obtained was dissolved in ethanol (20 ml) and the solution was adjusted to pH 6—7 with 14% aqueous ammonia to yield a crude solid. The substance was reprecipitated from aqueous solution by adding ethanol and collected by suction. Procedures of neutralization with aqueous ammonia, reprecipitation, and collection by suction should be carried out as quickly as possible to avoid decomposition of hydroxyamine. L-I was obtained as an amorphous solid; yield 2.3 g (58%), mp 223—226 °C (decomp.) and $[\alpha]_D^{24}$ +24.2° (*c* 0.5, M hydrochloric acid).

Found: C, 45.38; H, 8.29; N, 17.19%. Calcd for $C_6H_{14}N_2O_3$: C, 44.43; H, 8.70; N, 17.27%.

The product showed a deep red coloration with alkaline triphenyltetrazolium chloride and an intense blue coloration with ninhydrin, and was negative to the Beilstein test. Successive purification caused the decomposition of hydroxyamine.

Preparation of 2-Nitroindane-1,3-dione Salt of L-I. The crystalline 2-nitroindane-1,3-dione salt was prepared by dissolution of L-I (100 mg) in hot water (7 ml) containing 2-nitroindane-1,3-dione (100 mg), followed by slow cooling of the resulting solution. The product (85 mg) was recrystallized twice from water to yield the salt as pale brown crystals; mp 217—218 °C (decomp.) (lit,[8] mp 217.0—217.5 °C (decomp.)).

Found: C, 50.91; H, 5.32; N, 11.75%. Calcd for $C_{15}H_{19}N_3O_7$: C, 50.98; H, 5.42; N, 11.89%.

N^{ε}-Benzyloxy-L-lysine (L-VIII). L-II (4.06 g, 10 mmol) was added at room temperature to a 20 ml solution of 36% hydrogen bromide in acetic acid and phenol (4 g) in a glass-stoppered bottle. After being stirred for 48 hr, the solution was evaporated *in vacuo* at a temperature below 35 °C. The residual syrup was extracted with water and the aqueous layer was washed repeatedly with ether, treated with activated carbon and evaporated *in vacuo* at a temperature below 35 °C. The residue was dissolved in water (5 ml) and adjusted to pH 7—8 with aqueous ammonia to yield a crude solid. Recrystallization from hot water gave L-VIII as a leaflet; yield 1.8 g (73%), mp 238—241 °C (decomp.) and $[\alpha]_D^{23}$ +18.1° (*c* 1.0, M hydrochloric acid).

Found: C, 61.83; H, 7.98; N, 11.21%. Calcd for $C_{13}H_{20}N_2O_3$: C, 61.88; H, 7.99; N, 11.11%.

The NMR spectrum in trifluoroacetic acid showed a multiplet at δ 1.90 (4H; β,γ-CH_2), a multiplet at δ 2.18 (2H; δ-CH_2), a multiplet at δ 3.51 (2H; ε-CH_2), a multiplet at δ 4.36 (1H; α-CH), a singlet at δ 5.19 (2H; BZL-CH_2), a sharp signal at δ 7.46 (8H; α-NH_3^+ and BZL-C_6H_5), and a broad signal at δ 9.57 (2H; ε-NH_2^+).

N^{ε}-Hydroxy-L-lysine Monohydrochloride (L-I·HCl) (prepared in route-b). A solution of L-VIII (500 mg, 1.98 mmol) in 0.5 M hydrochloric acid (20 ml) was hydrogenolyzed over 5% palladium on carbon (500 mg) at room temperature and atmospheric pressure for 24 hr. After the catalyst was removed by suction, the solution was evaporated to dryness. The residue was dissolved in water and again evaporated to dryness to eliminate excess hydrochloric acid and then dissolved in water (2 ml). The pH of this solution was brought to 4—5 by addition of pyridine. Addition of ethanol (4 ml) to the resulting solution gave a colorless solid, which was reprecipitated from aqueous solution by adding ethanol to afford L-I·HCl as an amorphous powder; yield 350 mg (89%), mp 192—194 °C (decomp.) and $[\alpha]_D^{24}$ +25.5° (*c* 0.22, M hydrochloric acid).

Found: C, 36.41; H, 7.61; N, 14.13%. Calcd for $C_6H_{15}N_2O_3Cl$: C, 36.27; H, 7.61; N, 14.10%.

The product showed a deep red coloration with alkaline triphenyltetrazolium chloride and a blue coloration with ninhydrin, and was positive to the Beilstein test.

Figure 4 shows the NMR spectrum of the synthesized L-I·HCl in trifluoroacetic acid with tetramethylsilane as an internal standard; δ 1.98 (4H; β,γ-CH_2), 2.27 (2H; δ-CH_2), 3.58 (2H; ε-CH_2), 4.44 (1H; α-CH), 7.53 (3H; α-NH_3^+), and 9.95 (2H; ε-NH_2^+).

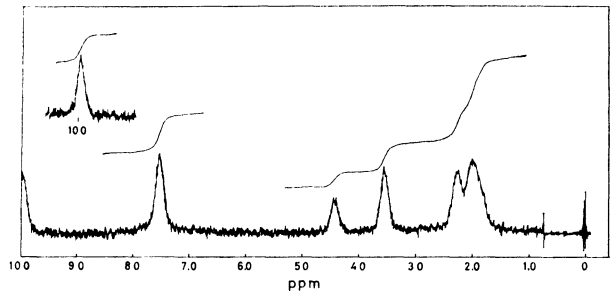


Fig. 4. NMR spectrum of N^{ε}-hydroxy-L-lysine monohydrochloride (L-I·HCl).

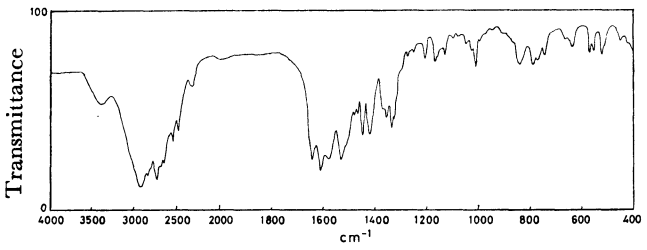


Fig. 5. Infrared spectrum of N^{ε}-hydroxy-L-lysine monohydrochloride (L-I·HCl).

A very close similarity to N^{δ}-hydroxy-L-ornithine monohydrochloride was observed in the IR spectrum (Fig. 5).

Hydrogenation of N^{ε}*-Benzyloxy-L-lysine (L-VIII) in a Neutral Solution.* A solution of L-VIII (200 mg, 0.79 mmol) in a mixture of methanol (10 ml) and water (5 ml) was hydrogenolyzed over 5% palladium on carbon (100 mg) at room temperature under atmospheric pressure for 48 hr. To the mixture was added M hydrochloric acid (5 ml) and the catalyst was filtered off. The filtrate was evaporated, adjusted to pH 5—6 by addition of pyridine and further evaporated. The residue solidified by addition of ethanol was reprecipitated from its aqueous solution by addition of ethanol to yield L-lysine monohydrochloride; yield 74 mg (51%), mp 259—263 °C (decomp., undepressed by admixture with an authentic sample[10]) and $[\alpha]_D^{22}$ +21.4° (*c* 0.38, M hydrochloric acid) (the authentic sample, $[\alpha]_D^{22}$ +20.7° (*c* 0.34, M hydrochloric acid)).

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10) This sample was purchased from Ajinomoto Co., Inc.