Syntheses of DL-Hydroxylysine and DL-Allohydroxylysine

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Although the separation of normal and allo form of DL-hydroxylysine derivatives were performed by Fones[1] through fractional recrystallizations of two diastereomeric α-chloroacetyl-ε-carbobenzoxy lactones and by Zahn and Zürn[2] in the same way through α, ε-dicarbobenzoxy lactones, none of them were considered satisfactory because of their poor yields. This paper describes the separation of α, ε-dicarbobenzoxyhydroxylysine lactones into the two diastereomers with good yield by a new method which was based on the fact that the normal form of dicarbobenzoxy lactone is saponified by alkali faster than its allo form.

For the synthesis of diastereomeric mixture of hydroxylysine hydrochlorides, the methods of the literatures[2-3] were slightly modified. To reduce an intermediate diethyl (γ-hydroxy-δ-nitro-*n*-butyl)-acetamidomalonate (I) to the δ-amino derivative, Van Zyl et al.[3] used platinum black as a catalyst and Zahn et al.[2] employed Raney-nickel catalyst under high pressure of hydrogen. However, both procedures seemed not to be practical for a large scale experiment. We studied with palladium catalyst on the reduction of the δ-nitro group in I in several different solvents and found acetic acid to be an excellent solvent. Some results were shown in Fig. 1. In this way, 300 g. of I were reduced to diethyl (γ-hydroxy-δ-amino-*n*-butyl)-acetamidomalonate which was then hydrolyzed with acid giving 158 g. of hydroxylysine hydrochloride. Thus, the yield was improved and the procedure was remarkably simplified. Chromatographic analysis[4] revealed that the ratio of normal to allo form of this synthetic hydroxylysine hydrochloride was 46 : 54. In the next steps of carbobenzoxylation and lactonization, although Zahn and Zürn[2] reported the synthesis of diastereomeric dicarbobenzoxy lactones with the yield of 61%, we improved the yield until 91% applying an azeotropic distillation technique with benzene for the completion of lactonization. For the separation of the diastereomers, a solution of dicarbobenzoxy lactones in dioxane was first treated with sodium hydroxide equivalent to an amount of normal form which was estimated on free hydroxylysine by chromatographic analysis. Sodium salt of dicarbobenzoxyhydroxylysine thus obtained by partial saponification gave pure normal dicarbobenzoxy lactone while unsaponified lactone extracted into ethyl acetate was purified, giving the pure allo form. The two diastereomers of hydroxylysine hydrobromide were obtained from the corresponding dicarbobenzoxy lactones by the treatment of hydrogen bromide in acetic acid, and their purities were ascertained by the elution analysis from a column of Dowex 50[4] (see Figs. 5, 6 and 7).

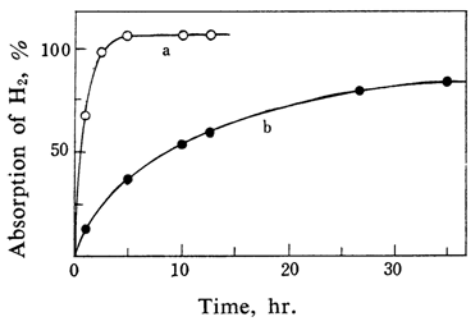


Fig. 1. Reduction of diethyl (γ-hydroxy-δ-nitro-*n*-butyl)-acetamidomalonate (I) : a, I (1 g.) and 10% Pd charcoal (0.2 g.) in acetic acid (10 ml.) ; b, I (1 g.) and 10% Pd charcoal (0.2 g.) in methanol (10 ml.) containing HCl equivalent to I.

The rates of saponification of the pure dicarbobenzoxy lactones were determined using a pH-Stat. As shown in Fig. 2, the normal

1) W. S. Fones, *J. Am. Chem. Soc.*, **75**, 4865 (1953).
2) H. Zahn and L. Zürn, *Chem. Ber.*, **91**, 1359 (1958).
3) G. Van Zyl, E. E. Van Tan Tamelen and G. D. Zuidema, *J. Am. Chem. Soc.*, **73**, 1765 (1951).
4) B. Witkop and T. Beiler, ibid., **78**, 2882 (1956).

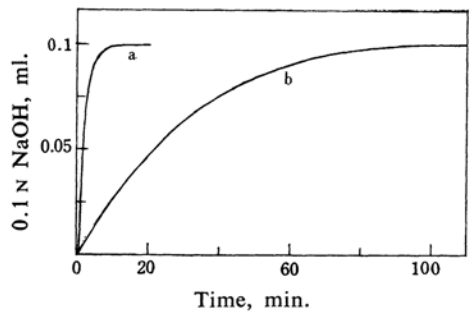
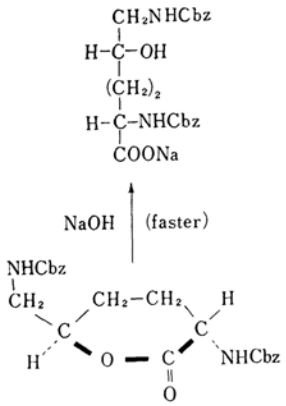
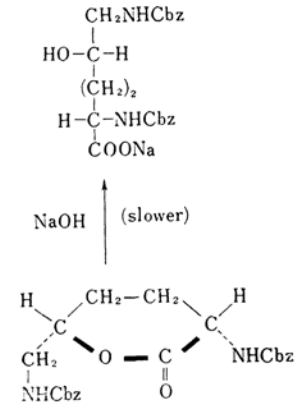


Fig. 2. Saponification of dicarbobenzoxyhydroxylysine lactones by pH-Stat: a, the normal lactone (III); b, the allo lactone (IV).



Hydroxylysine series



Allohydroxylysine series

Fig. 3. Configurations and correlations of hydroxylysine and allohydroxylysine derivatives.

form was saponified about 10 times faster than the allo form. Recently Witkop reported that the normal hydroxylysine and its lactone have erythro and trans configuration, respectively[5]. Taking this view into consideration, it is concluded that the lactone with trans configuration is easily saponified as shown in Fig. 3. In this connection, it should be noted that Wisiger reported that dibenzoyl derivative of L-allohydroxylysine was obtained as the lactone, whereas that of D-hydroxylysine as free acid[6]. In a preliminary experiment in this laboratory in collaboration with Dr. Bernhard Witkop, diastereomeric dicarbobenzoxy-DL-hydroxyornithine lactones, unlike the case of dicarbobenzoxyhydroxylysine lactones, could not be separated with the use of sodium hydroxide equivalent to an amount of the trans form.

Experimental

Diethyl (γ-Hydroxy-δ-nitro-*n*-butyl)-acetamidomalonate (I).—Diethyl acetamidomalonate (760 g., 3.5 mol.) and acrolein (257 ml., 3.86 mol.) were condensed in ethanol (1.4 l.) containing metallic sodium (1.1 g.)[7]. The solution containing γ-acetamido-γ,γ-diethoxycarbonylbutyraldehyde was added during 30 min. under stirring to an ice-cooled solution of nitromethane (0.88 l.), dioxane (0.088 l.) and 5 N sodium hydroxide (0.088 l.). After being stirred for 16 hr. at room temperature, 5 N sodium hydroxide (0.025 l.) was added and the stirring was continued for another 24 hr. After being neutralized with acetic acid (0.039 l.), the solution was evaporated in vacuo and the residue was added to a mixture of benzene (1.5 l.) and petroleum ether (0.15 l.). The mixture was allowed to stand for several days in a refrigerator to cause the separation of crystals which were filtered and washed with water. After being dried well and recrystallized from benzene (2.5 l.), it was dissolved in ethanol (1.5 l.) and the solution was evaporated in vacuo. The addition of water (1.5 l.) and the shaking of mixture caused the formation of crystals, yield 620 g. (53%), m. p. 68~71°C. This was saved for the next step. Two recrystallizations from aqueous ethanol gave an analytical sample, m. p. 76°C (Found: C, 46.70; H, 6.52; N, 8.34%). The reported values are m. p. 95.2~96°C[3] and 77°C[8].

Hydroxylysine Monohydrochloride (Diastereomeric Mixture).—I (300 g., 0.9 mol.) in acetic acid (1.5 l.) containing 10% palladium charcoal (30 g.) was reduced for 6 hr. at room temperature under 1 atm. of hydrogen in a 5 l. three-neck flask, fitted with a sealed stirrer, an inlet tube for hydrogen, and an outlet tube which was in contact with a surface of mercury. The catalyst was removed by filtration, the filtrate was added to concentrated hydrochloric acid (1.5 l.), and the solution was refluxed for 10 hr. It was evaporated to dryness in vacuo and the excess of acids was removed by repeated addition of water and evaporation. The residue was taken up in water, treated with triethylamine (0.4 l.) and the solution was evaporated to dryness in vacuo. After being washed with a mixture of ethanol (1.5 l.) and acetone (0.3 l.) by the method of decantation, the residue was taken

5) B. Witkop, *Experientia*, **12**, 372 (1956).

6) J. R. Wisiger, *J. Biol. Chem.*, **186**, 591 (1950).

7) O. A. Moe and D. T. Warner, *J. Am. Chem. Soc.*, **70**, 2763 (1948).

8) H. Zahn and E. Umlauf, *Z. physiol. Chem.*, **297**, 127 (1954).

up in water. The solution was decolorized with charcoal and concentrated in vacuo to a small volume. The addition of ethanol (1.8 l.) effected the precipitation of a powdery substance, yield 158 g. (88%). This was saved for the next step. The paper chromatography of this compound showed only one spot by means of the ninhydrin test with several solvent systems. As shown in Fig. 4, ion-exchange analysis of the compound gave a ratio, normal to allohydroxylysine, of 46 : 54. Two recrystallizations from water-ethanol gave a sample, m. p. 212~218°C (decomp.). (Found: C, 35.99; H, 7.72; N, 14.31%).

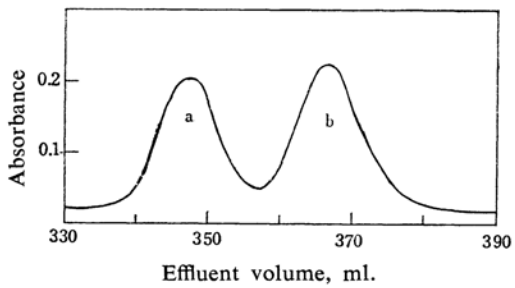


Fig. 4. Chromatography of diastereomeric hydroxylysine monohydrochloride: a, hydroxylysine; b, allohydroxylysine.

Chromatographic Assay of Normal and Allohydroxylysine. — The method of Stein and Moore as modified by Witkop and Beiler[4] was utilized to assay the composition of hydroxylysine. A 100× 0.9 cm. column of Dowex 50×8 (200~400 mesh) was treated with 0.1 M sodium citrate buffer of pH 3.4. An aqueous solution containing 1.0 mg. of hydroxylysine monohydrochloride was passed through the column and eluted with 0.1 M sodium phosphate buffer of pH 7.6. Slight pressure was applied to keep the flow rate at 4 ml. per hr. One-ml. samples were collected with an automatic fraction collector and analyzed by the ninhydrin method[9] at 570 mμ.

Dicarbobenzoxyhydroxylysine Lactone (Diastereomeric Mixture) (II).—The powdery hydroxylysine monohydrochloride (39.8 g., 0.2 mol.) obtained above was treated with carbobenzoxy chloride (89 ml., 0.52 mol.) and 2 N sodium hydroxide (0.52 l.) by Schotten-Baumann's procedure. An oil separated upon acidification with 5 N hydrochloric acid (0.21 l.) was extracted with chloroform. The chloroform solution was dried over sodium sulfate and evaporated in vacuo to an oil to which benzene (1 l.) was added. The mixture was heated under reflux for 3 hr., the liberated water being removed azeotropically using a Dean and Stark distilling apparatus[10]. After the filtration of some insoluble material, the filtrate was evaporated to an oil which was crystallized by the addition of petroleum ether. It was filtered and washed with petroleum ether, yield 75.4 g. (91%). This was used without further purification. We supposed that a ratio of normal to allo form in this compound should have been close to 46 : 54 because the yield of dicarbobenzoxy derivative from diastereomeric hydroxylysine, the ratio of which was 46 : 54, was excellent (91%).

Dicarbobenzoxy-DL-hydroxylysine Lactone (III). To a solution of II (41.2 g., 0.1 mol.) in dioxane (400 ml.) was added 1 N sodium hydroxide (46 ml.)* at room temperature. After standing for 1 hr., the solution was evaporated in vacuo to dryness. The residue was shaken with a mixture of ethyl acetate (600 ml.) and water (400 ml.) (the ethyl acetate layer was saved for the recovery of IV), the aqueous layer was acidified with 5 N hydrochloric acid (10 ml.) and an oil separated was extracted with ethyl acetate. The solution was dried over sodium sulfate and evaporated in vacuo to an oil to which benzene was added. The benzene solution was refluxed for 3 hr., the liberated water being removed azeotropically. Benzene was removed by evaporation in vacuo and the residue was recrystallized twice from ethyl acetate, yield 10.4 g. (55% based on the supposed amount (46%) of normal form in II), m. p. 135~136°C.

Found: C, 64.01; H, 5.97; N, 6.67. Calcd. for $C_{22}H_{24}O_6N_2$: C, 64.06; H, 5.87; N, 6.79%.

The reported value is m. p. 137~141°C[2].

Dicarbobenzoxy-DL-allohydroxylysine Lactone (IV).—After the ethyl acetate layer described above was kept in a refrigerator, crystals appearing were filtered and washed with cold ethyl acetate. The combined filtrate and washings were dried over sodium sulfate and evaporated in vacuo to dryness. The crystals and dried residue were recrystallized twice from ethanol, yield 17.8 g. (80% based on the supposed amount (54%) of allo form in II), m. p. 151°C.

Found: C, 63.96; H, 5.86; N, 6.74. Calcd. for $C_{22}H_{24}O_6N_2$: C, 64.06; H, 5.87; N, 6.79%.

The reported value is m. p. 154~155.5°C[2].

DL-Hydroxylysine Monohydrobromide (V). — A saturated solution of hydrogen bromide in acetic acid[11] (30 ml.) was added at room temperature to III (6.17 g., 0.015 mol.) which had been previously pulverized. III was dissolved within 2~3 min. and different crystals appeared after 5~6 min. The mixture was allowed to stand with occasional shakings for 1 hr., and then treated with ether (120 ml.) and petroleum ether (30 ml.). The crystals were filtered and washed with ether, but changed instantly to a gummy product because of its highly hygroscopic character. Second crop of the gummy product was obtained from the filtrate and washings by the addition of petroleum ether. The combined products were dissolved in water and triethylamine (9 ml.) was added**. The solution was evaporated in vacuo

9) H. Rosen, *Arch. Biochem. Biophys.*, **67**, 10 (1957).

10) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", J. Wiley & Sons, Inc., New York (1961), p. 940.

* In another run of the same experiment, a half mole of 1 N sodium hydroxide (50 ml.) was used, III and IV being obtained with the yields of 9.5 g. and 17.1 g., respectively.

11) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

** In another run of the same experiment, silver carbonate was added to the aqueous solution until pH 6.5 and pure DL-hydroxylysine monohydrobromide was obtained from the filtrate with a similar yield in the case of the use of triethylamine.

to dryness and the residue was filtered with the aid of ethanol. It was recrystallized from water (7 ml.)-ethanol (20 ml.), yield 3.17 g. (87%), m. p. 230~231°C (decomp.).

Found: C, 29.66: H, 6.31; N, 11.39. Calcd. for $C_6H_{15}O_3N_2Br$: C, 29.64; H, 6.22; N, 11.52%.

The product (1.0 mg.) was analyzed by the method of ion-exchange chromatography, the procedure being described before. The curves of absorbance of V itself and a mixture of V and L-allohydroxylysine monhydrochloride[12] were shown in Figs. 5 and 6, respectively. These results indicate that V is a pure normal form.

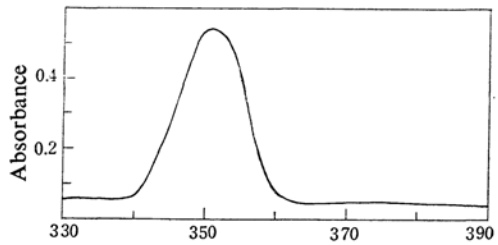


Fig. 5. Chromatography of DL-hydroxylysine monohydrobromide.

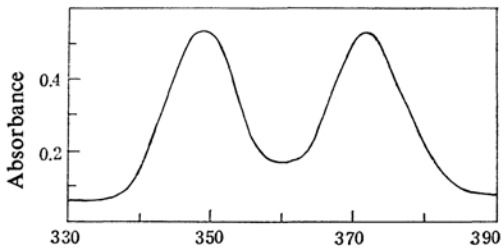


Fig. 6. Chromatography of a mixture of DL-hydroxylysine monohydrobromide and L-allohydroxylysine monohydrochloride.

DL-Allohydroxylysine Monohydrobromide (VI).—IV (6.17 g.) was treated with hydrogen bromide in acetic acid in the same way as described above. The yield of the recrystallized product was 2.61 g. (71%), m. p. 219°C (decomp.)

Found: C, 29.74; H, 6.26; N, 11.21. Calcd. for $C_6H_{15}O_3N_2Br$: C, 29.64; H, 6.22; N, 11.52%.

As shown in Fig. 7, the product is a pure allo form. Zahn and Zürn reported the preparation of DL-hydroxylysine and DL-allohydroxylysine monohydrochloride[2].

12) W. S. Fones, *Biochem. Preparation*, **8**, 62 (1961).

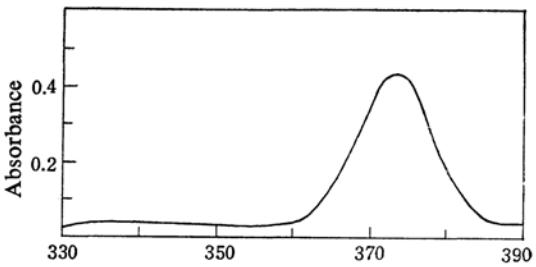


Fig. 7. Chromatography of DL-allohydroxylysine monohydrobromide.

Saponification of Dicarbobenzoxy Derivatives, III and IV.—A solution of III or IV (4.12 mg., 0.01 mmol.) in a mixture of dimethylformamide (1.5 ml.) and water (1.5 ml.) was saponified at 40°C with 0.1 N sodium hydroxide using Radiometer's pH-Stat (type TTTl) which was set at a constant pH of 10.0. The curves recorded automatically were shown in Fig. 2, indicating that III was saponified completely within about 10 min., while IV needed about 100 min. for complete saponification.

Summary

A diastereomeric mixture of DL-hydroxylysine hydrochlorides was obtained with a good yield through hydrogenation of diethyl (γ-hydroxy-δ-nitro-*n*-butyl)-acetamidomalonate with palladium catalyst in a solvent of acetic acid, which was followed by hydrolysis of the reduced product. Dicarbobenzoxy lactone was prepared with a yield of 91% from hydroxylysine hydrochloride obtained above. A solution of diastereomeric dicarbobenzoxy lactones in dioxane was treated with sodium hydroxide equivalent to the amount of normal form. The salt obtained after saponification converted into a pure normal dicarbobenzoxy lactone, a pure allo compound being isolated from the unsaponified portion. The two lactones were converted into chromatographically pure normal and allohydroxylysine monohydrobromides by the action of hydrogen bromide in acetic acid.

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