After standing for twenty-four hours, the gummy, flocculent material which separated was removed by filtration through Super-Cel. The filtrate was concentrated to 100 ml. and 49.3 g. of crude, crystalline methyl α,ϵ-diphthalimido-δ-keto-DL-caproate was obtained.

The crude product was dissolved in 150 ml. of chloroform and extracted twice with 100 ml. of cold 0.2 *N* sodium hydroxide and once with water. After being dried over sodium sulfate, the chloroform solution was concentrated to 80 ml. and 250 ml. of carbon tetrachloride was added. After twenty-four hours at 0°, 38.8 g. (40.7% based on γ-methyl phthaloyl-DL-glutamate) of purified methyl γ,ϵ-diphthalimido-δ-keto-DL-caproate was obtained. Recrystallization from xylene and ethyl alcohol yielded colorless crystals; m. p. 152–153°.

Anal. Calcd. for $C_{23}H_{18}O_7N_2$: C, 63.60; H, 4.18; N, 6.45. Found: C, 63.44; H, 4.40; N, 6.64.

γ,ϵ-Diamino-δ-hydroxycaproic Acid Monohydrochloride.—A solution of 34.0 g. (0.078 mole) of methyl γ,ϵ-diphthalimido-δ-keto-DL-caproate and 30.0 g. (0.145 mole) of aluminum isopropoxide in 150 ml. of anhydrous isopropyl alcohol was distilled slowly. The acetone formed in the reaction was separated by a distillation head of the type described in "Organic Reactions."[10] Fresh isopropyl alcohol was added from time to time to maintain a constant volume. After eighty hours, the test for acetone with a 2,4-dinitrophenylhydrazine reagent was negative.

The alcohol was removed by distillation under reduced pressure and the residue was treated with 350 ml. of cold 2 *N* hydrochloric acid and 200 ml. of chloroform. The layers were separated and the aqueous phase was extracted twice with 100 ml. of chloroform. The combined extract was dried and concentrated.

The residual oil was heated under reflux with 350 ml. of 6 *N* hydrochloric acid for ten hours. When the solution cooled, the phthalic acid which separated was removed by filtration and the filtrate was extracted with ether. The aqueous solution was concentrated to a glass weighing 22.5 g. A portion of this glass was purified through the phosphotungstate and converted to the monohydrochloride, which was obtained as a non-crystalline residue. This product did not liberate carbon dioxide in the presence of ninhydrin[11] and, when treated with sodium periodate, 1.6 moles was consumed (theory for hydroxylysine is 1 mole).

(10) "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 197–200.

(11) We are indebted to Dr. Torsti Salo for carrying out this determination.

N$^\alpha$-Phthaloyl-DL-glutamine[4] **(VII).**—Ten grams (0.039 mole) of phthaloyl-DL-glutamic anhydride was added to 85 ml. of 1 *N* ammonia in ethanol (made by adding concentrated ammonia to ethanol). Solution was rapid, and, after removal of the ethanol by concentration under reduced pressure, the residue was dissolved in 35 ml. of water. The solution was acidified with hydrochloric acid, cooled to 0°, and 9.0 g. (84.5%) of colorless crystalline N$^\alpha$-phthaloyl-DL-glutamine was obtained, m. p. 197–198°. The equivalent weight (calcd. 276) was 270. A pure sample (m. p. 203–204°) was obtained by recrystallization from water.

Anal. Calcd. for $C_{13}H_{12}O_5N_2$: C, 56.53; H, 4.38; N, 10.14. Found: C, 56.37; H, 4.68; N, 10.21.

Methyl Ester of N$^\alpha$-Phthaloyl-DL-glutamine (VIII).

A. From N$^\alpha$-Phthaloyl-DL-glutamine (VII).—A suspension of 7.0 g. (0.025 mole) of N$^\alpha$-phthaloyl-DL-glutamine in 25 ml. of methanol was treated with a twofold excess of diazomethane in ether. The solid completely dissolved and shortly thereafter crystals separated from the solution. After elimination of the excess diazomethane by boiling, the solution was cooled to 0° and 4.5 g. (61.2%) of product, m. p. 142–143°, was obtained. Recrystallizations from chloroform and isopropyl alcohol gave a pure sample, m. p. 145–146°.

Anal. Calcd. for $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.67; H, 4.87; N, 10.08.

B. From α-Methylphthaloyl-DL-glutamyl Chloride (V).—A solution in 10 ml. of ether of the acid chloride obtained from 1.0 g. of α-methylphthaloyl-DL-glutamic acid was added to 20 ml. of a 0.4 *N* solution of ammonia in anhydrous ether. The colorless precipitate was separated by filtration and dissolved in 20 ml. of water. On cooling, 0.90 g. (90.2%), based on α-methylphthaloyl-DL-glutamic acid) of the methyl ester of N$^\alpha$-phthaloyl-DL-glutamine, m. p. 143–144°, was obtained. The melting point of a mixture with material prepared from N$^\alpha$-phthaloyl-DL-glutamine was not depressed.

Summary

The structure of hydroxylysine has been shown to be α,ϵ-diamino-δ-hydroxycaproic acid by conversion to methyl α,ϵ-diphthalimido-δ-keto-DL-caproate, which was prepared for comparison from glutamic acid by an unambiguous synthesis.

CAMBRIDGE 39, MASSACHUSETTS
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

The Synthesis of Hydroxylysine

BY JOHN C. SHEEHAN AND WILLIAM A. BOLHOFER[1]

This paper reports the synthesis of hydroxylysine hydrochloride[2] (III) from glutamic acid *via* the intermediate methyl α,ϵ-diphthalimido-δ-keto-DL-caproate[3] (I). The reduction of the ketone I with aluminum isopropoxide and isopropyl alcohol gave a non-crystalline product which was hydrolyzed with hydrochloric acid. Pure hydroxylysine hydrochloride was isolated with the aid of phosphotungstic acid. The identity of natural and synthetic hydroxylysine was established by a comparison of the chemical and physical properties of the monohydrochlorides and the dipicrates.

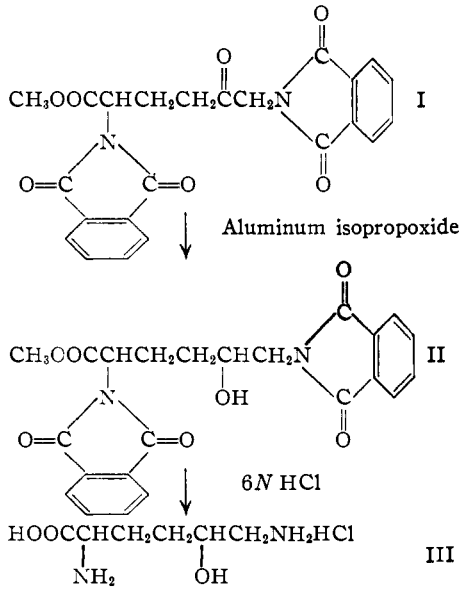
The synthetic hydroxylysine formed a dipicrate which existed in the characteristic two crystalline modifications similar to those described[4] for natural hydroxylysine dipicrate. The low-melting form melted somewhat higher than the low-melting natural hydroxylysine dipicrate, but it is quite possible that the latter does not consist of exactly similar proportions of the two possible racemates. The decomposition points of both the synthetic and natural dipicrates and monohydrochlorides

(1) Swift Amino Acid Fellow, 1947–1949.

(2) Since little is known regarding the stereoisomerism of hydroxylysine, this name will be applied throughout this report to any stereoisomer or mixture of stereoisomers of α,ϵ-diamino-δ-hydroxycaproic acid.

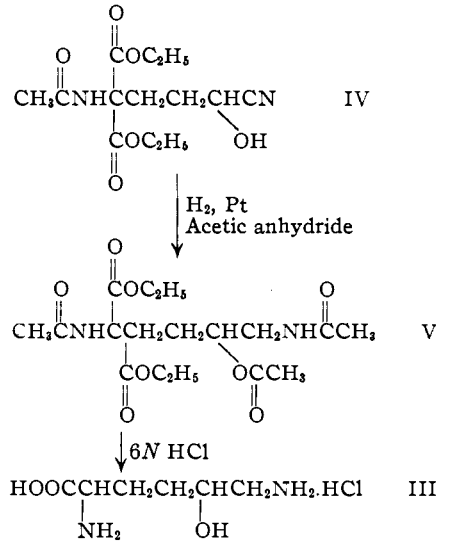
(3) Sheehan and Bolhofer, THIS JOURNAL, **72**, 2469 (1950).

(4) Sheehan and Bolhofer, *ibid.*, **72** 2466 (1950).



were identical. The ultimate and periodate analyses were in accord with the structures assigned.

When the synthesis of hydroxylysine was originally outlined, one of the alternate routes considered was a preparation from diethyl acetamidomalonate. Recently Warner and Moe[5] described the synthesis of lysine by this same route, differing only in the final step. Diethyl acetamidomalonate was added to acrolein, and the resulting aldehyde was converted to the cyanohydrin (IV) by the addition of hydrogen cyanide. Lysine was obtained by dehydration of the cyanohydrin (IV) and subsequent reduction of the unsaturated nitrile. It is evident that if the cyanohydrin (IV) is not dehydrated but reduced directly, an acetylated hydroxylysine derivative (V) will be formed. In the present work it was found that after hydrolysis and decarboxylation of the acetylated derivative (V) with 6*N* hydrochloric acid, periodate analysis indicated a 38% yield of hydroxylysine based on diethyl acetamidomalonate. Pure hydroxylysine hydrochloride (III) was obtained in low yield by direct crystallization. A dipicrate could be prepared which existed in the two characteristic modifications already observed with optically inactive synthetic and natural hydroxylysine.



(5) Warner and Moe, THIS JOURNAL, **70**, 3918 (1948).

The requirements for the acceptance of a compound as a protein amino acid were originally outlined by Schulze and Likiernik[6] and restated later in more explicit form by Vickery and Schmidt.[7] The fundamental criteria on which the acceptance of an amino acid should be based are: the isolation of the compound by some worker other than its discoverer; the establishment of its constitution by synthesis; and the demonstration of identity between the synthetic product and the racemized natural product or actual resolution of the synthetic product and preparation of the optically active natural isomer. These requirements now appear to have been fulfilled for hydroxylysine.

We wish to express our appreciation to Swift and Company for the support of a fellowship for one of us (W.A.B.).

Experimental[8]

α,ε-Diamino-δ-hydroxycaproic Acid Hydrochloride (Hydroxylysine Hydrochloride) (III). From Methyl α,ε-Diphthalimido-δ-keto-DL-caproate (I).—A solution of 20 g. of methyl α,ε-diphthalimido-δ-keto-DL-caproate[3] and 50 g. of aluminum isopropoxide in 300 ml. of isopropyl alcohol was heated at the boiling point and the vapors fractionated in a short, packed column. Isopropyl alcohol was allowed to distill at a slow rate until the final distillate gave a negative test for acetone with 2,4-dinitrophenylhydrazine (seventy-two hours). The solution was further concentrated under reduced pressure to a viscous oil which still contained some solvent. Chloroform (200 ml.) was added to the residue and the resulting solution was poured slowly with stirring into a mixture of 75 ml. of concentrated hydrochloric acid and 500 g. of ice. The chloroform layer was separated, washed with water, dried (Na_2SO_4) and concentrated.

The viscous residue (23 g.) was hydrolyzed by heating under reflux for eighteen hours with 200 ml. of 6 *N* hydrochloric acid. The clear solution was concentrated to about 25 ml. and then diluted to 75 ml. with water. Phthalic acid was removed by filtration, and a periodate analysis[9] of the filtrate indicated the presence of 4.12 g. of hydroxylysine hydrochloride (45.0%, based on methyl α,ε-diphthalimido-δ-keto-DL-caproate).

A. Purification by Crystallization of the Phosphotungstate.—The filtrate from the above reaction was diluted to 1600 ml. with boiling 0.25 *N* hydrochloric acid and a solution of 32 g. of phosphotungstic acid in 160 ml. of 0.25 *N* hydrochloric acid was added. A yellow precipitate formed immediately; the mixture was allowed to cool to 35°. The precipitate was removed by filtration and the filtrate was allowed to stand at 20° for twenty-four hours and at 0° for twenty-four hours. A first crop of colorless, glistening needles was collected by filtration but was not dried.

(6) Schulze and Likiernik, *Z. physiol. Chem.*, **17**, 513 (1893).

(7) Vickery and Schmidt, *Chem. Rev.*, **9**, 169 (1931).

(8) We are indebted to Mr. S. M. Nagy and his associates for the microanalyses. All melting points are corrected.

(9) Van Slyke, Hiller and MacFadyen, *J. Biol. Chem.*, **141**, 681 (1941).

The yellow precipitate was resuspended in the filtrate and the mixture was heated to boiling. After the solution had cooled to 35°, it was treated in the same manner as was used to obtain the first crop. The first and second crops were combined and on drying weighed 27.6 g.

Decomposition of the complex was accomplished by shaking with 200 ml. of 2 *N* hydrochloric acid and 200 ml. of a solution consisting of 1250 ml. of ether, 1000 ml. of amyl alcohol and 50 ml. of ethyl alcohol. After four extractions with 100 ml. of the organic solution, the aqueous solution was concentrated to a viscous residue. This was dissolved in 50 ml. of water and passed through a column of IR-4B resin (a periodate analysis at this point indicated the presence of 2.30 g. of hydroxylysine hydrochloride). The effluent was adjusted to *p*H 6.5–7.0 with hydrochloric acid and concentrated to a glass (2.6 g.). Crystallization was effected by dissolving the residue in 2 ml. of water and adding 50 ml. of methanol. After 16 hours at 0°, 1.75 g. of crystalline α,ε-diamino-δ-hydroxycaproic acid hydrochloride was obtained. By concentrating the mother liquor and adding 0.5 ml. of water and 25 ml. of methanol, an additional 0.2 g. of product was obtained. The overall yield of crystalline material, based on methyl α,ε-diphthalimido-δ-keto-DL-caproate, was 21.2%. The two crops were combined and recrystallized from 2 ml. of water and 30 ml. of methanol. The product decomposed at 215–220° and analysis with periodate indicated a purity of 100%.

Anal. Calcd. for $C_6H_{15}O_3N_2Cl$: C, 36.27; H, 7.61; N, 14.10. Found: C, 36.33; H, 7.90; N, 14.13.

A picrate, prepared by the method described for natural hydroxylysine picrate,[4] was found by periodate analysis to be a dipicrate. Two modifications were obtained: one which decomposed sharply at 195° without melting, and another which melted at 145–150°. On rubbing with a spatula, the latter form was converted to the modification which decomposed at 195°.

B. **Purification by Crystallization of the Picrate.**—The acid hydrolysis filtrate from the aluminum isopropoxide reduction of 1.0 g. of methyl α,ε-diphthalimido-δ-keto-DL-caproate, containing 0.206 g. of hydroxylysine hydrochloride, was decolorized (activated carbon) and concentrated to a dry glass. Five ml. of water was added and, after neutralization of the solution with lithium hydroxide, 0.5 g. of picric acid was added. After decantation of the warm (50°) aqueous solution from the insoluble tar which separated, 0.5 g. additional picric acid was added, and the solution was allowed to cool slowly. As soon as the light yellow crystals of hydroxylysine dipicrate started to appear, the excess picric acid was removed by centrifugation. The hydroxylysine dipicrate (0.35 g., 24.5%, based on methyl α,ε-diphthalimido-δ-keto-DL-caproate) was obtained by allowing the solution to cool to room temperature. After four recrystallizations from 2 ml. of water, and drying at room temperature, the product decomposed sharply at 195–200° without melting, and assayed 100% by the periodate method. This product was not converted to the hydrochloride. The low melting form of the dipicrate was not observed in this experiment.

α,ε-Diamino-δ-hydroxycaproic Acid Hydrochloride (Hydroxylysine Hydrochloride) (III). From the Cyanohydrin of γ-Acetamido-γ,γ-dicarbethoxybutyraldehyde (IV).—The platinum catalyst was prepared by reducing 0.2 g. of platinum oxide in 8 ml. of acetic anhydride. To this suspension was added 2.5 g. of the cyanohydrin of γ-acetamido-γ,γ-dicarbethoxybutyraldehyde[5] (non-crystalline) and the reduction was allowed to proceed at atmospheric pressure. The catalyst became poisoned rapidly and 0.2 g. of fresh platinum oxide was added. After reduction of the oxide, the rate of hydrogen consumption was 80 ml. per hour. This rate slowly decreased, and the reduction stopped when 382 ml. (theory, 410 ml.) of hydrogen had been taken up.

After removal of the catalyst by filtration, the filtrate was added to 50 ml. of 6 *N* hydrochloric acid and the solution heated under reflux for eighteen hours. Removal of the acid was effected by concentration under reduced pressure and the residue was dissolved in 20 ml. of water. The solution was passed through a column of Amberlite IR-4B ion-exchange resin and the effluent was adjusted to *p*H 6.5–7.0 with dilute hydrochloric acid. A titration with periodate showed the presence of 0.65 g. of hydroxylysine hydrochloride (37% based on diethyl acetamidomalonate).

The solution was concentrated to 2 ml. and 20 ml. of methanol was added. After the insoluble oil had separated the supernatant solution was decanted and concentrated to dryness. The residue was dissolved in 0.5 ml. of water and 10 ml. of methanol was added. Additional oil separated, and the clear supernatant solution was decanted and concentrated to 3 ml. in a stream of air. After cooling to 0°, 0.140 g. of crystalline α,ε-diamino-δ-hydroxycaproic acid hydrochloride (XV) separated (7% based on diethyl acetamidomalonate). Recrystallization from 0.15 ml. of water and 2.2 ml. of methanol gave a product which was 100% by the periodate analysis and which decomposed at 215–220°.

Anal. Calcd. for $C_6H_{15}O_3N_2Cl$: C, 36.27; H, 7.61; N, 14.10. Found: C, 36.33; H, 7.59; N, 14.19.

A dipicrate (determined by periodate analysis) prepared by the method used for natural hydroxylysine dipicrate, was found to exist in the two characteristic modifications. One form melted at 145–150° while the other decomposed sharply at 195°. The former could be converted to the latter by rubbing.

Summary

Hydroxylysine (α,ε-diamino-δ-hydroxycaproic acid) has been synthesized by two independent routes and shown to be identical to the racemized natural product.

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