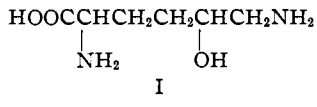
[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

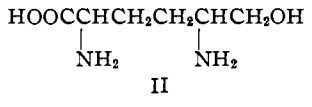
The Structure of Hydroxylysine

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

Van Slyke[2] proposed as probable structures for hydroxylysine either α,ϵ-diamino-δ-hydroxycaproic acid (I) or α,δ-diamino-ϵ-hydroxycaproic acid (II). Analyses indicated a hydroxylysine structure and a positive ninhydrin reaction showed it to be an α-amino acid. Periodic acid liberated one mole of ammonia and one mole of formaldehyde, indicating that the hydroxyl and one of the amino groups were on adjacent carbon atoms at the end of the chain. Van Slyke also interpreted the electrometric titration curve of hydroxylysine as evidence that the hydroxyl group was not adjacent to the α-amino group.



I



II

However, it should be noted that there is no direct chemical evidence establishing the structure of the carbon skeleton. Due to the difficulty of obtaining decisive evidence by degradation methods alone, we decided to employ a combination of degradation and synthesis. Because of its relationship to lysine, biochemical analogy favors formula I. This was proved to be the correct structure for hydroxylysine by conversion to methyl α,ϵ-diphthalimido-δ-keto-DL-caproate (IX), which was prepared independently for comparison by an unambiguous synthetic route.

The possible existence of four stereoisomers (two racemates) added to the difficulty of the synthesis and complicated the ultimate comparison of the synthetic and natural products. Racemization of the natural material for comparison purposes was not entirely satisfactory because this would also yield a mixture of two racemates.

To overcome the difficulties introduced by stereoisomerism, the synthetic work was directed toward the natural isomer itself. Here again, the similarities of the natural amino acids indicated that the use of an optically active natural amino acid as a starting material would yield a hydroxylysine which would at least have a configuration about the α-carbon atom identical with the natural form. Although optically active natural glutamic acid was used as the starting material racemization of various intermediates proved so troublesome that the synthesis was carried out with racemic intermediates. However, an indirect comparison of natural hydroxylysine with one of the synthetic intermediates (IX) possessing only one asymmetric center was possible, and the synthesis of this intermediate and its comparison with the product derived from hydroxylysine are described.

Phthaloyl-L-glutamic anhydride (III) was synthesized from L-glutamic acid (XII) without the intermediate isolation of phthaloyl-L-glutamic acid. Racemization of the anhydride was accomplished in good yield by a recrystallization from a xylene–acetic anhydride mixture. Direct reaction of the phthaloyl-DL-glutamic anhydride (III) with methanol yielded exclusively γ-methyl phthaloyl-DL-glutamate (XIII). The racemic ester was crystalline, but that prepared from optically active anhydride was an oil.

After considerable experimentation it was found that the desired α-methyl phthaloyl-DL-glutamate (IV) could be prepared by interaction of sodium methoxide in methanol and the anhydride (III). Some of the γ-ester (XIII) is produced simultaneously, but the desired α-ester is more insoluble in ether and can easily be obtained pure in a 45% yield. One of the procedures receiving consideration for the preparation of the α-ester (IV) was that involving conversion of the amide group of the methyl ester of N^{α}-phthaloyl-DL-glutamine (VIII) to the corresponding acid ester by treatment with nitrous acid. This glutamine ester was prepared by the action of diazomethane on N^{α}-phthaloyl-DL-glutamine (VII), obtained by the action of ammonia on phthaloyl-DL-glutamic anhydride (III). After the completion of this experimental work, a note appeared[3] reporting the synthesis of phthaloyl-L-glutamic anhydride, phthaloyl-DL-glutamic anhydride and N^{α}-phthaloylglutamine. Unfortunately no experimental details, yields or physical constants were included.

An interesting comparison can be made between phthaloylglutamic anhydride, carbobenzoxyglutamic anhydride, and acetylglutamic anhydride. Both phthaloylglutamic anhydride and acetylglutamic anhydride[4] yield a glutamine derivative on reaction with ammonia while carbobenzoxyglutamic anhydride gives an isoglutamine[5] derivative. With alcohols, phthaloylglutamic anhydride gives the γ-ester and with alkoxides it gives the α-ester, while carbobenzoxyglutamic anhydride yields the α-ester[6] with alcohols and approximately 30% of the γ-ester[7] with alkoxides.

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

(2) Van Slyke, Hiller, Dillon and MacFadyen, *Proc. Soc. Exp. Biol. Med.*, **38**, 548 (1938); Van Slyke, Hiller, MacFadyen, Hastings and Klemperer, *J. Biol. Chem.*, **133**, 287 (1940); Klemperer, Hastings and Van Slyke, *ibid.*, **143**, 433 (1942).

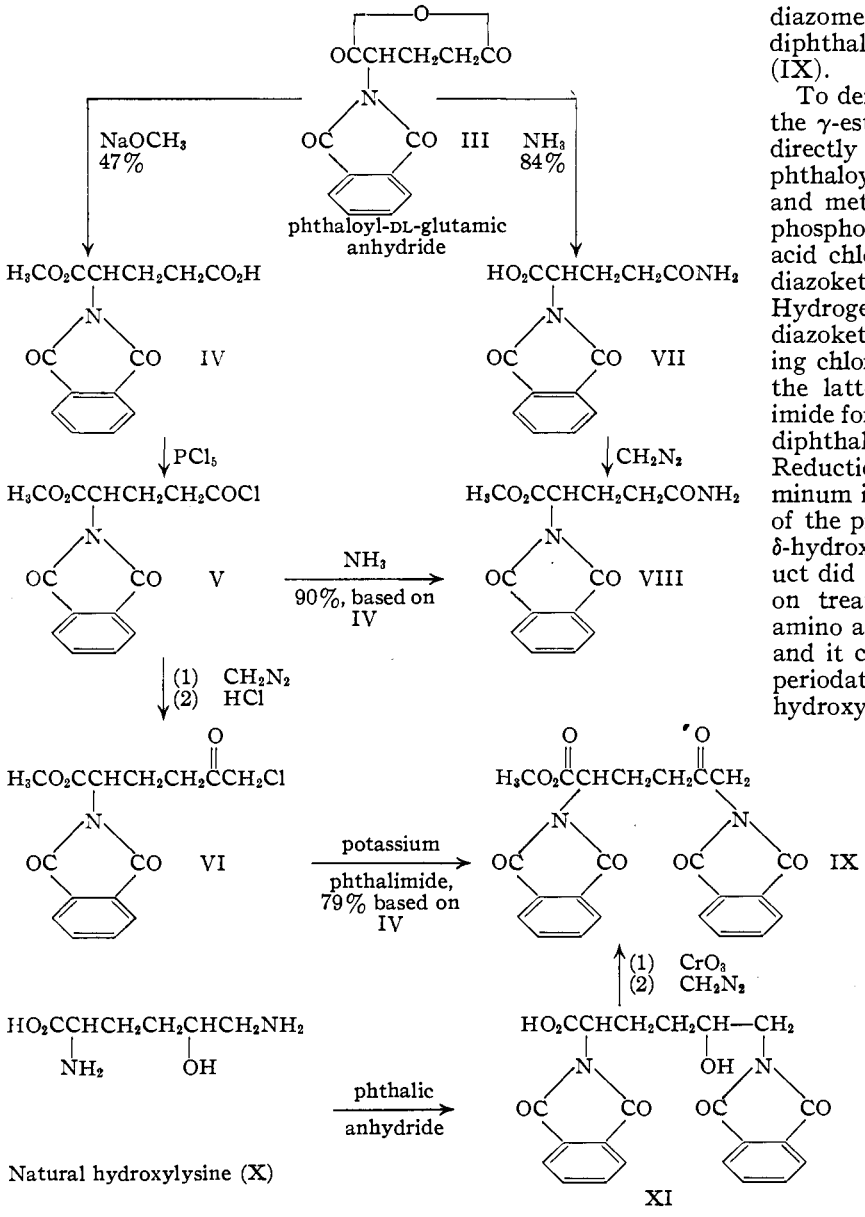
(3) Kidd and King, *Nature*, **162**, 776 (1948).

(4) Nicolet, THIS JOURNAL, **52**, 1192 (1930).

(5) Bergmann and Zervas, *Ber.*, **65**, 1192 (1932).

(6) Bergmann, Zervas and Salzmann, *Ber.*, **66**, 1288 (1933).

(7) Melville, Thesis, London, 1934 (reference in footnote of article by Neuberger, *Biochem. J.*, **30**, 2085 (1936)).



Phosphorus pentachloride converted α-methyl phthaloyl-DL-glutamate (IV) into the acid chloride V, diazomethane formed the diazoketone and the addition of hydrogen chloride led to crystalline methyl α-phthalimido-δ-keto-ε-chloro-DL-caproate (VI). Reaction of the latter compound with potassium phthalimide in dimethylformamide yielded methyl α,ε-diphthalimido-δ-keto-DL-caproate (IX). Compound IX was also prepared from natural hydroxylysine and a comparison of the two showed them to be identical.

Natural hydroxylysine (X), possessing a low specific rotation, was fused with phthalic anhydride to form the α,ε-diphthalimido-δ-hydroxycaproic acid (XI). Oxidation of the alcohol function with chromic anhydride and esterification with diazomethane formed methyl α,ε-diphthalimido-ε-keto-DL-caproate (IX).

To demonstrate conclusively that the γ-ester was the product formed directly from the interaction of phthaloyl-DL-glutamic anhydride and methanol, it was treated with phosphorus pentachloride and the acid chloride was converted into a diazoketone with diazomethane. Hydrogen chloride reacted with the diazoketone to form the corresponding chloroketone and interaction of the latter with potassium phthalimide formed crystalline methyl γ,ε-diphthalimido-δ-keto-DL-caproate. Reduction of this ketone with aluminum isopropoxide and hydrolysis of the product yielded γ,ε-diamino-δ-hydroxycaproic acid. This product did not liberate carbon dioxide on treatment with ninhydrin (α-amino acids evolve carbon dioxide) and it consumed significantly more periodate (1.6 moles) than natural 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]

Phthaloyl-DL-glutamic Anhydride[3] (III).—A mixture of 5.0 g. (0.034 mole) of L-glutamic acid and 5.0 g. (0.034 mole) of phthalic anhydride was heated at 140–150° for twenty minutes (140° was reached in about ten minutes). The clear melt was cooled to 100°, 6 ml. of acetic anhydride was added and the temperature was held at 100° for five minutes. Before cooling, 18 ml. of xylene was added and the mixture was then allowed to crystallize for sixteen hours at 0°. The product (5.1 g., 54.2%) melted at 199–201° with softening starting at 194°; $[\alpha]^{20}D$ −38.0° (3% solution in dioxane). The optical purity of this sample is not known.

To racemize the anhydride, 3.0 g. was dissolved in a mixture of 2.5 ml. of acetic anhydride and 7.5 ml. of xylene and the solution was heated under reflux for thirty minutes. After cooling to 0°, 2.8 g. (93.5%) of phthaloyl-DL-glutamic anhydride was collected by filtration.

A sample prepared for analysis by two further recrystallizations from the same solvent mixture melted at 204–205°, and the equivalent weight determined by titration with sodium hydroxide was 131 (calcd. 130).

Anal. Calcd. for $C_{13}H_9O_5N$: C, 60.24; H, 3.50; N, 5.40. Found: C, 60.38; H, 3.54; N, 5.41.

α-Methyl Phthaloyl-DL-glutamate (IV).—A solution of sodium methoxide in absolute methanol (475 ml., 0.420

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

N) was cooled to 5° and 51.0 g. of phthaloyl-DL-glutamic anhydride was added in ten separate portions as rapidly as the rate of solution of each portion permitted. After all the anhydride had been added, the methanol was removed by concentration under reduced pressure. The residue was dissolved in 200 ml. of water, acidified with hydrochloric acid, and extracted with 250 ml. and 100 ml. of chloroform. The combined extract was dried and concentrated to a viscous oil which was dissolved in 200 ml. of ether. After forty-eight hours at 25°, 26.7 g. (46.6%) of α-methyl phthaloyl-DL-glutamate (m. p. 150–151°) was obtained. Recrystallization from ethyl acetate and *t*-butyl alcohol gave a sample melting at 154–155°.

Anal. Calcd. for $C_{14}H_{13}O_6N$: C, 57.74; H, 4.50; N, 4.81. Found: C, 57.75; H, 4.59; N, 5.05.

By concentrating the ethereal mother liquor, there was obtained 7.5 g. of impure γ-methyl phthaloyl-DL-glutamate.

Methyl α-Phthalimido-δ-keto-ε-chloro-DL-caproate (VI).—To a suspension of 30 g. (0.103 mole) of α-methyl phthaloyl-DL-glutamate in 150 ml. of anhydrous ether was added 21.6 g. (0.103 mole) of phosphorus pentachloride. Both substances were insoluble in ether, but with stirring, a clear solution resulted in forty-five minutes. The ether was removed by distillation, 125 ml. of xylene added, and the solution concentrated to a viscous oil.

The α-methyl phthaloyl-DL-glutamyl chloride (V) was dissolved in 300 ml. of anhydrous ether and added to 410 ml. of a 0.75 *M* solution of diazomethane in ether at 5°. After forty-five minutes, gas evolution had ceased and the excess diazomethane was removed by distilling one-third of the solution on a steam-bath. A 35-ml. portion of a 3.8 *N* solution of anhydrous hydrogen chloride in anhydrous ether was added. There was an immediate evolution of gas. Fifteen minutes later the solution was decanted from a small amount of sticky brown gum and the ether was removed by concentration under reduced pressure. The residue crystallized readily, and after two washes with 200 ml. of petroleum ether, 31.8 g. of crude (m. p. 84–89°) methyl α-phthalimido-δ-keto-ε-chloro-DL-caproate was obtained.

This substance was very soluble in most organic solvents and a pure sample was prepared by successive recrystallizations from carbon tetrachloride and *t*-butyl alcohol; m. p. 92.6–93.4°.

Anal. Calcd. for $C_{15}H_{14}O_5NCl$: C, 55.65; H, 4.36; N, 4.33. Found: C, 55.61; H, 4.62; N, 4.49.

Methyl α,ε-Diphthalimido-δ-keto-DL-caproate (IX).—A solution of 27.0 g. (0.084 mole) of methyl α-phthalimido-δ-keto-ε-chloro-DL-caproate (crude) in 100 ml. of dimethylformamide (du Pont) and 16.2 g. (0.088 mole) of potassium phthalimide was stirred for thirty minutes. In five minutes, the slightly exothermic nature of the reaction had raised the temperature to 50°, from which point it slowly decreased. The reaction mixture was diluted with 750 ml. of chloroform and extracted with an equal volume of water, 0.1 *N* sodium hydroxide and water again. After drying, the chloroform solution was concentrated under reduced pressure and the warm residue was dissolved by adding slowly 500 ml. of ether. Rapid addition of the ether causes separation of the product as an oil which crystallizes in hard lumps. The ether-washed, colorless product weighed 30.0 g. (78.6% based on α-methyl phthaloyl-DL-glutamate); m. p. 143–144°. It is soluble in acetone, benzene, toluene, ethyl acetate, and hot alcohols. Recrystallization from methanol gave a product melting at 143.4–144.5°.

Anal. Calcd. for $C_{23}H_{18}O_7N_2$: C, 63.59; H, 4.19; N, 6.45. Found: C, 63.72; H, 4.33; N, 6.28.

Formation of Methyl α,ε-diphthalimido-δ-keto-DL-caproate (IX) from Natural Hydroxylysine.—To a suspension of 1.00 g. (0.005 mole) of optically inactive natural hydroxylysine hydrochloride[9] in 30 ml. of dioxane was added 3.60 ml. of 1.41 *N* sodium methoxide in methanol. The methanol and part of the dioxane were removed by concentration under reduced pressure. Pyridine (1.22 ml., 0.015 mole) and 1.5 g. (0.010 mole) of phthalic anhydride were added to the suspension and, after one hour the solvent was removed and the residue was heated at 150–175° for fifteen minutes under reduced pressure. A light yellow glass (2.4 g.) resulted which could be scraped from the flask and powdered. Trituration with water and drying gave 2.0 g. of white powder, which was insoluble in acid but soluble in warm dilute alkali.

One gram of the powder was oxidized by heating at 75° for ninety minutes with 10 ml. of 70% acetic acid containing 0.3 g. of chromic anhydride. The solution was concentrated to remove the acetic acid and the residue was treated with 100 ml. of an aqueous sodium bicarbonate solution. The precipitate was removed by filtration and, after acidification, the filtrate was extracted with eight 50 ml. portions of ethyl acetate. The combined extract was dried, concentrated to 30 ml., and an excess of diazomethane in ether was added to the solution. After one hour all the solvents were removed by concentration under reduced pressure. The residue was dissolved in 2 ml. of methanol and the solution was seeded. After storing for twenty-four hours at 0°, 0.20 g. of crude methyl α,ε-diphthalimido-δ-keto-DL-caproate was obtained. This product was successively recrystallized from *t*-butyl alcohol, xylene and isopropyl alcohol to obtain a pure sample, m. p. 143–144°. The melting point of a mixture with synthetic methyl α,ε-diphthalimido-δ-keto-DL-caproate (m. p. 143.4–144.5°) was not depressed.

Anal. Calcd. for $C_{23}H_{18}O_7N_2$: C, 63.59; H, 4.19; N, 6.45. Found: C, 63.71; H, 4.50; N, 6.71.

γ-Methyl Phthaloyl-DL-glutamate.—A 5-g. portion of phthaloyl-DL-glutamic anhydride was heated under reflux in 20 ml. of dry methanol for two hours. The methanol was removed by concentration under reduced pressure and the residue was dissolved in 25 ml. of hot toluene. After cooling at 0° for five hours, 5.2 g. (92.5%) of crystalline ester was collected. The equivalent weight was 287 (calcd. 291) and the melting point was 119.5–120.5°.

Anal. Calcd. for $C_{14}H_{13}O_6N$: C, 57.74; H, 4.50; N, 4.81. Found: C, 57.61; H, 4.50; N, 4.85.

γ-Methyl Phthaloyl-L-glutamate.—This ester was obtained from phthaloyl-L-glutamic anhydride in a manner similar to the preparation of the corresponding racemate. The optically active ester was obtained as an oil, soluble in toluene. The specific rotation was determined: $[\alpha]^{20}D$ −42.3° (3% solution in methanol); it is not known whether the sample was chemically or optically pure (eq. wt. calcd., 291; found, 299).

Methyl γ,ε-Diphthalimido-δ-keto-DL-caproate.—A mixture of 63.5 g. (0.22 mole) of γ-methyl phthaloyl-DL-glutamate and 45.8 g. (0.22 mole) of phosphorus pentachloride was stirred at room temperature in 300 ml. of anhydrous ether until a clear solution resulted (*ca.* one hour). The ether was removed under reduced pressure, 100 ml. of xylene was added to the residue, and the solution was again concentrated under reduced pressure. The light yellow residue of γ-methyl phthaloyl-DL-glutamyl chloride was dissolved in 300 ml. of anhydrous ether and added slowly to a cold solution of diazomethane (0.66 mole) in 1320 ml. of ether. A vigorous evolution of gas occurred and after thirty minutes the solution was evaporated on a steam-bath to 750 ml.

The solution of methyl γ-phthalimido-δ-keto-ε-diazo-DL-caproate was cooled to 0° and 75 ml. of a 3 *N* solution of anhydrous hydrogen chloride in anhydrous ether was added. A copious evolution of gas was observed and, after forty-five minutes, the solution was decanted from gummy material and concentrated to a residual oil weighing 72 g.

The oily residue of methyl α-phthalimido-δ-keto-ε-chloro-DL-caproate was dissolved in 75 ml. of xylene and heated at 140° for fifteen minutes with 40.4 g. (0.22 mole) of potassium phthalimide. The reaction mixture was cooled, diluted with 300 ml. of chloroform and filtered. Toluene (1000 ml.) was added to the filtrate and the solution was concentrated to 900 ml. to remove the chloroform.

(9) Sheehan and Bolhofer, THIS JOURNAL, **72**, 2466 (1950).

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.

Nα-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 Nα-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 Nα-Phthaloyl-DL-glutamine (VIII).

A. From Nα-Phthaloyl-DL-glutamine (VII).—A suspension of 7.0 g. (0.025 mole) of Nα-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 Nα-phthaloyl-DL-glutamine, m. p. 143–144°, was obtained. The melting point of a mixture with material prepared from Nα-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.

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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).