1-Methyl-9-phenyl-Δ⁷-hexahydroindole (Vc).—A chilled solution of 1.42 g. of 9-phenyl-Δ⁷-hexahydroindole in 3 ml. of methyl ethyl ketone was treated with 0.9 g. of dimethyl sulfate. After 16 hours at room temperature, the mixture was refluxed for 1 hour, treated with 5 ml. of water, and refluxed for 3 hours more. Dilution with water and addition of alkali gave the amine which was extracted into ether and evaporatively distilled at 75–80° and 0.05–0.01 mm.; yield of colorless liquid, 1.35 g. (88%). In air the base quickly assumed a red color. The **picrate**, which was prepared in ether, formed yellow prismatic plates after several recrystallizations from methanol; m.p. 142–143°, depressed to 117–128° by admixture with the picrate of the starting amine.

Anal. Calcd. for $C_{21}H_{22}O_7N_4$: C, 57.0; H, 5.0; N, 12.7. Found: C, 57.3; H, 5.2; N, 12.4.

2-Oxo-1-phenylcyclohexane-ethylmethylbenzamide (IVd).—The benzamide obtained as a viscous liquid by a Schotten–Baumann reaction of Vc was converted into its **2,4-dinitrophenylhydrazone**, which after one recrystallization from acetic acid–methanol and three recrystallizations from benzene–petroleum ether formed rosettes of fine orange-yellow needles, m.p. 172–174°.

Anal. Calcd. for $C_{28}H_{29}O_6N_5$: C, 65.3; H, 5.6; N, 13.6. Found: C, 65.6; H, 5.7; N, 13.5.

2-Methoxyethyl-2-phenylcyclohexanone.—A solution of 20 g. of methyl cellosolve and 50 ml. of pyridine in 50 ml. of benzene was treated at 0° with 56 g. of benzenesulfonyl chloride. After 1.5 hours 100 ml. of ice-cold 6 *N* HCl was added to the cold mixture. The methoxyethylbenzenesulfonate was collected at 136–141° and 0.2 mm.; weight 35 g.

After the slow addition of a solution of 29.5 g. of 2-phenylcyclohexanone in 110 ml. of benzene to a stirred, refluxing suspension of 7.25 g. of finely divided sodamide in 100 ml. of benzene, the mixture was refluxed for 2.5 hours and then treated with 44 g. of methoxyethyl benzenesulfonate. After 12 hours of refluxing, 200 ml. of water was added, and the product which was isolated from the organic layer was refluxed with 100 ml. of acetic acid, 20 ml. of concentrated hydrochloric acid and 20 ml. of water for one-half hour in order to hydrolyze the excess alkylating agent. This procedure circumvented the difficulty which Newman and Magerlein[9] experienced in separating the product from the excess reagent (tosyl ester of ethyl cellosolve). The 2-methoxyethyl-2-phenylcyclohexanone was collected as a colorless viscous liquid at 138–140° and 0.5 mm.; weight 25.5 g. (65%). The **semicarbazone** crystallized from aqueous methanol in small colorless prisms; m.p. 185.5–186°.

Anal. Calcd. for $C_{16}H_{23}N_3O_2$: C, 66.4; H, 7.9; N, 14.5. Found: C, 66.6; H, 7.9; N, 14.1.

The 2,4-dinitrophenylhydrazone formed yellow needles after several recrystallizations from methanol; m.p. 133–134°.

Anal. Calcd. for $C_{21}H_{24}N_4O_5$: C, 61.2; H, 5.8. Found: C, 61.8; H, 5.4.

Summary

1-Phenylcyclohexane-ethylamine was prepared from 1-phenylcyclohexanepropionic acid by the Curtius reaction.

The Curtius reaction has been applied to the δ-keto acid, 2-oxo-1-phenylcyclohexanepropionic acid *via* the hydrazone hydrazide. The product of hydrolysis of the rearranged azide in acid solution was the cyclic amine 9-phenyl-Δ⁷-hexahydroindole. Various derivatives of the cyclic amine, of its N-methyl derivative, and of the aminoketones obtained by hydrolytic cleavage were prepared.

(9) Newman and Magerlein, THIS JOURNAL, **69**, 942 (1947).

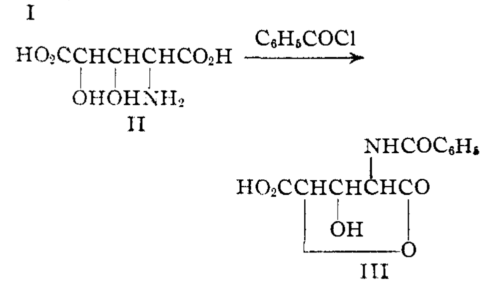
ANN ARBOR, MICHIGAN RECEIVED MAY 26, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY OF VANDERBILT UNIVERSITY SCHOOL OF MEDICINE AND THE DIVISION OF BIOCHEMISTRY OF THE UNIVERSITY OF ILLINOIS]

The Synthesis of β,γ-Dihydroxyglutamic Acid[1]

BY OSCAR TOUSTER AND H. E. CARTER

In recent years there has been considerable interest in the preparation of various derivatives of natural amino acids for possible use in antimetabolite studies. β-Hydroxyglutamic acid has been reported to be a potent competitor of glutamic acid in the nutrition of *Lactobacillus arabinosus*.[2] In connection with another problem, we prepared 3,5-dicarbethoxy-4-hydroxyisoxazole (I) from ethyl acetonedicarboxylate and amyl nitrite[3] and found that high pressure hydrogenation of the isoxazole and hydrolysis of the reduction product with hydrochloric acid yielded β,γ-dihydroxyglutamic acid (II). This is apparently the first report of the catalytic reduction of an isoxazole to a β-aminoalcohol, although Claisen[4] obtained β-imino- and β-aminoketones by the reductive cleavage of 3,5-dimethylisoxazole with sodium. The amino acid was isolated as the monoammonium salt and as the hydrochloride. The ammonium salt was characterized by elementary analyses, ammonium determination, and by quantitative study of its reaction with sodium metaperiodate. The structure of the amino acid was further established by its conversion to the N-benzoyl derivative (III), which existed in the expected lactone form, as indicated by elementary analyses, failure to react with sodium metaperiodate, and neutral equivalent. The lactone ring was opened readily by



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(2) Borek and Waelsch, *J. Biol. Chem.*, **177**, 135 (1949).

(3) Pechmann, *Ber.*, **24**, 857 (1891).

(4) Claisen, *ibid.*, **24**, 3900 (1891).

warm dilute sodium hydroxide. N-benzoyl-β,γ-dihydroxyglutamic acid lactone reacts rapidly with Nessler reagent, but the amino acid hydrochloride reacts only very slowly.[5]

We are indebted to Dr. Ernest Borek of the New York Psychiatric Institute for testing β,γ-dihydroxyglutamic acid for antimetabolite activity. The dihydroxy compound was found to have no inhibitory action on the growth of *Lactobacillus arabinosus* when used in thirty-eight times the molar concentration of glutamic acid. Under the same conditions, β-hydroxyglutamic acid causes complete inhibition of growth.[2]

Experimental

β,γ-Dihydroxyglutamic Acid (II).—Twenty grams of 3,5-dicarbethoxy-4-hydroxyisoxazole (I) was hydrogenated at a pressure of 3000 pounds, using 300 ml. of 95% ethanol as solvent, and Raney nickel as catalyst. The reduction was run at room temperature and took approximately five hours for completion. The solution was freed of catalyst and concentrated *in vacuo* to an orange-brown gum. The latter was dissolved in 120 ml. of 20% hydrochloric acid and hydrolysis was effected by refluxing the solution for three hours. The acid solution was concentrated *in vacuo* to a dark-brown residue which was dissolved in 100 ml. of water and decolorized with charcoal (Darco G-60). On evaporation of the solution there was obtained 13.17 g. of yellow, semicrystalline residue. This was dissolved in 19 ml. of water and the resulting solution brought to *p*H 5–6 with concentrated ammonium hydroxide. The monoammonium salt of β,γ-dihydroxyglutamic acid precipitated immediately. The cooled mixture was filtered and the precipitate washed with cold water, 50% acetone, acetone and ether. The white product weighed 5.27 g. (31% yield, based on amount of isoxazole reduced). Addition of acetone to the mother liquor and washings yielded only a small amount of solid (0.79 g.). The ammonium salt gave the following analysis after recrystallization from water.

Anal.[6] Calcd. for $C_5H_{12}O_6N_2$: C, 30.61; H, 6.17; N, 14.28; NH_4-N, 7.14. Found: C, 30.36; H, 5.86; N, 13.92(Kjeldahl); NH_4-N, 7.01.

Four moles of periodate were consumed in one hour, and none during the next twenty-four hours, when sodium metaperiodate was allowed to react with the compound.[7] The decomposition point varied with the melting point method employed. On a Fisher–Johns microblock, the decomposition range was 190–200°; in a capillary, the compound began to darken at 225° and decomposed sharply at 237° (cor.).

The hydrochloride of β,γ-dihydroxyglutamic acid was obtained by recrystallizing from concentrated hydrochloric acid a portion of the decolorized hydrolysis product. The hydrochloride was non-hygroscopic and did not have a sharp melting point.

Anal. Calcd. for $C_5H_{10}O_6NCl$: N, 6.50. Found: N, 6.55, 6.59 (Kjeldahl).

N-Benzoyl β,γ-Dihydroxyglutamic Acid Lactone (III).—A solution of 0.278 g. of monoammonium β,γ-dihydroxyglutamate in 1.75 ml. of 2 *N* sodium hydroxide was evaporated to dryness to remove ammonia. The residue was dissolved in 1 ml. of water, and 0.40 g. of benzoyl chloride and 2.85 ml. of 2 *N* sodium hydroxide were added in three portions.[8] After disappearance of the benzoyl chloride odor, concd. hydrochloric acid was added to precipitate benzoic acid, which was removed by filtration. The filtrate was evaporated to dryness and the residue, after thorough drying over phosphorus pentoxide, was extracted with two 75-ml. portions of boiling heptane to remove the remaining benzoic acid. The benzoyl derivative was extracted from the residue with dry ethanol. After evaporation, the derivative was dissolved in dry acetone and a trace of insoluble material removed by filtration. Evaporation of the acetone yielded an oily product which was crystallized from ethyl acetate. The yield of almost pure product was 100 mg. (28%). A yield of 34% was obtained when a much larger amount of amino acid was benzoylated under similar conditions. The benzoyl derivative melted at 196–198° (cor.) on a microblock after recrystallization from acetone–benzene.

Anal. Calcd. for $C_{12}H_{11}NO_6$: C, 54.33; H, 4.03; N, 5.49. Found: C, 54.34; H, 4.18; N, 5.28 (Dumas).

The substance did not react with sodium metaperiodate. With Nessler reagent it rapidly yielded an orange solution and then a gray precipitate. The equivalent weight obtained by permitting reaction of both the carboxyl and lactone groups was found to be 132 (calcd. 133).

Summary

β,γ-Dihydroxyglutamic acid has been synthesized from 3,5-dicarbethoxy-4-hydroxyisoxazole and converted to N-benzoyl-β,γ-dihydroxyglutamic acid lactone. The inability of the new amino acid to interfere appreciably with the utilization of glutamic acid by *Lactobacillus arabinosus* has been reported.

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(5) When the first sample of benzoyl derivative prepared gave a nitrogen analysis somewhat higher than the calculated value, the compound was tested for ammonia with Nessler reagent. An apparently positive test suggested that the high nitrogen content was due to incomplete removal of ammonia prior to benzoylation. It was later found, however, that analytically pure benzoyl derivative reacts rapidly with this reagent.

(6) Carbon–hydrogen and Dumas nitrogen analyses by Clark Microanalytical Laboratory, Urbana, Illinois. Kjeldahl nitrogen and ammonium determinations by Dr. Robert S. Pogrund.

(7) Periodate reactions were carried out by Miss Harriet B. Neville. Two moles of periodate apparently react rapidly with the amino acid, and the remainder of the periodate oxidizes the two moles of glyoxylic acid produced in the initial oxidation. Sprinson and Chargaff (*J. Biol. Chem.*, **164**, 433 (1946)) have shown that glyoxylic acid is oxidized by periodate in one hour.

(8) The reaction mixture gave a positive ninhydrin test in the presence of pyridine, even if a greater excess of benzoyl chloride was employed. This has also been observed by the authors in the benzoylation of glutamic acid and β-hydroxyglutamic acid in strong alkali. Free amino groups were not present, however, when the latter amino acid was benzoylated by the magnesium oxide procedure of Bergmann and Zervas (*Ber.*, **65B**, 1192 (1932)).