[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Chemical Preparation of L-Ornithine from L-Arginine[1]

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A method is described for the preparation of L-ornithine from L-arginine by hydrolysis with barium hydroxide and direct precipitation of the ornithine as the monohydrochloride.

In connection with another problem large quantities of L-ornithine were required. A survey of the literature revealed no simple procedure for the preparation of L-ornithine by the chemical hydrolysis of L-arginine.

The main difficulties encountered by the earlier workers involved racemization—the stronger the alkali and the longer the treatment the greater the racemization—and the isolation of the ornithine. Thus, whereas Schulze and Winterstein[2] prepared L-ornithine in low yield by boiling L-arginine with baryta water for an hour, other workers, using longer periods of hydrolysis in 5 *N* potassium hydroxide[3] or sodium hydroxide,[4,5] have demonstrated[3,4] and proved by isolation[5,6] that almost quantitative yields of ornithine may be obtained; however, under such conditions the ornithine is completely racemized.

L-Ornithine has been isolated from the reaction mixture as ornithuric acid[2] and as the salicylidene derivative of the barium salt.[7] In these procedures still further losses occur in converting the derivative to ornithine.

We wish to report a convenient preparation of L-ornithine that involves the hydrolysis of L-arginine by means of barium hydroxide, precipitation of the barium as barium sulfate and direct precipitation of L-ornithine monohydrochloride by the addition of ethanol to the proper concentration. A careful study of the hydrolysis of arginine was made to determine the conditions giving maximum conversion to ornithine coupled with minimum racemization of the product. It was discovered that by using 2.5 to 5 moles of barium hydroxide per mole of arginine and a minimum hydrolysis time of 2 hours yields of 60–75% of L-ornithine hydrochloride could be obtained. Under these conditions some racemization occurs since the mother liquors yield small amounts of partially racemized ornithine, but by controlling the quantity of alcohol added, the amount of the racemic form precipitated can be minimized.[8] After one recrystallization from aqueous alcohol, the L-ornithine hydrochloride thus obtained was found to be 99% optically pure.[9]

A separation of the ornithine and citrulline in the mother liquor was effected, albeit in low yield, by passing the mother liquor, after removal of the alcohol and dilution with water, over a column of Amberlite IRC-50 resin (Li phase) buffered at *p*H 7.

The citrulline was recovered by eluting with water until a negative ureido test was obtained, while further elution with dilute hydrochloric acid liberated the ornithine. The recovered ornithine, after recrystallization to constant melting point, was found to consist of a mixture of the L- and DL-forms, whereas the citrulline, after one recrystallization, consisted of essentially pure L-form.

D-Ornithine hydrochloride was prepared in the same manner from D-arginine hydrochloride. The "crude" D-ornithine hydrochloride, precipitated directly from the solution without further purification, was 98.5% optically pure.[9] This material and its derivatives had the same melting points and properties, except for opposite signs of rotation, as the corresponding derivatives of L-ornithine.

Experimental

Preparation of L-Ornithine Monohydrochloride.—Barium hydroxide octahydrate (160 g., 0.51 mole) was added to a solution of L-arginine hydrochloride (40 g., 0.19 mole) in 1 l. of water, and the solution was boiled under reflux for 2 hours. The reaction mixture was then cooled and acidified (*p*H 1.5 using pHydrion paper) with 6 *N* sulfuric acid. The resulting precipitate of barium sulfate was removed by filtration through a pad of Celite and the filtrate was concentrated on the water pump to a volume of about 150 ml. The solution was adjusted to a *p*H of 7–7.2 with a concentrated solution of barium hydroxide. After removal of the barium sulfate, the filtrate was acidified (*p*H 4–4.5) with 3 *N* hydrochloric acid and concentrated on the water pump to a volume of about 150 ml. Warm ethanol (350–400 ml.)[10] was added to the warm aqueous solution of the amino acid and the solution was allowed to stand at 5° for 18 hours. The white product was collected, washed with ethanol and dried in a vacuum desiccator. The yield of L-ornithine monohydrochloride was 24 g. (75%), m.p. 230–232° dec.; $\alpha^{26}D$ +17.1°[11,12] (4.91% solution in 3 *N* hydrochloric acid).

Separation of Citrulline and Ornithine.—The mother liquor was concentrated to remove the alcohol, diluted with water to a volume of 175 ml. and then passed over a column of Amberlite IRC-50 resin (Li phase) buffered at a *p*H of 7. After a positive ninhydrin test was obtained, 25-ml. fractions were collected until a negative ureido test resulted, water being used as the eluant. The solution, about 250 ml., was concentrated under reduced pressure to a volume

(1) The authors wish to express their thanks to the Abbott Laboratories, Eli Lilly and Company, and the Upjohn Company for a generous grant in support of this work.

(2) E. Schulze and E. Winterstein, *Hoppe-Seylers Z. physiol. Chem.*, **26**, 1 (1898–1899).

(3) D. D. Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911).

(4) R. H. A. Plimmer, *Biochem. J. (London)*, **10**, 115 (1916).

(5) W. R. Boon and W. Robson, *ibid.*, **29**, 2684 (1935).

(6) P. B. Hamilton and R. A. Anderson, *Biochem. Preparations*, **3**, 96 (1953).

(7) M. Bergmann and L. Zervas, *Hoppe-Seylers Z. physiol. Chem.*, **152**, 282 (1926).

(8) Some citrulline and unhydrolyzed arginine may also be precipitated by excessive amounts of alcohol.

(9) The authors are greatly indebted to Dr. Jesse P. Greenstein for determining the optical purity of several samples of L- and D-ornithine hydrochloride by the use of D-amino acid oxidase and L-ornithine decarboxylase and for generously supplying us with a large sample of D-arginine hydrochloride.

(10) Addition of larger volumes of alcohol increases the yield but the material is less pure as evidenced by a positive ureido test (W. R. Fearon, *Biochem. J. (London)*, **33**, 902 (1939)), (indicating the presence of citrulline), and a lower melting point.

(11) The rotation is calculated on the basis of ornithine dihydrochloride.

(12) L-Ornithine dihydrochloride is reported to have a rotation of +16.7° (A. Hunter, *Biochem. J. (London)*, **33**, 27 (1939)) and +16.5° (L. Levintow and J. P. Greenstein, *J. Biol. Chem.*, **188**, 643 (1951).

of 30 ml., diluted with alcohol until cloudy and allowed to stand at 5° for 48 hours. The white precipitate was collected and dried. It weighed 1.34 g. and melted at 214–216° dec.; $\alpha^{24}D$ +15.5° (4.78% solution in 3 *N* hydrochloric acid). Recrystallization from aqueous alcohol yielded 0.44 g. of material melting at 219–220° dec.; $\alpha^{21}D$ +22.6°[13,14] (4.44% solution in 3 *N* hydrochloric acid). A paper chromatogram revealed only the presence of citrulline.

To recover the ornithine the column was eluted with 1 *N* hydrochloric acid until a negative ninhydrin test resulted. The solution, after concentrating under reduced pressure, diluting with water and then concentrating again to remove excess acid, was treated with Amberlite IR-45-OH^- resin until the *p*H became 3.5–4.0. After removing and washing the resin, the solution was again concentrated to a volume of about 40 ml., diluted with alcohol until cloudy and allowed to stand at 5° for 48 hours. The white product that was collected weighed 1.92 g. and melted at 226–227° dec.; $\alpha^{25}D$ +10.8° (4.86% solution in 3 *N* hydrochloric acid). Recrystallization from water gave 1.47 g. of material with the same melting point and rotation. A paper chromatogram revealed only the presence of ornithine.

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(13) The rotation is calculated on the basis of citrulline as the free base.

(14) Values of +17.9° (Hunter), +24.2° (Levintow and Greenstein, ref. 12) and +21° (Hamilton and Anderson, *Biochem. Preparations*, **3**, 102 (1953)) have been reported.

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The Biosynthesis of Valine[1]

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Yeast was grown on glucose in the presence of tracer amounts of C^{14}-labeled substances, and valine was isolated from hydrolysates of the cell proteins. The C^{14}-distribution in valines isolated from yeast grown in the presence of labeled lactates indicated that pyruvate was the sole source of valine carbons; the carboxyl furnishing the carboxyl, the α-carbon supplying carbons 2 and 3, and the β-carbon furnishing the methyl carbons of valine. Distribution of acetate and glycine carbons in valine was in accord with their prior conversion to pyruvate by known processes. A mechanism for valine biosynthesis was suggested, involving the following steps: (1) decarboxylation of pyruvate to acetaldehyde; (2) condensation of acetaldehyde and pyruvate to yield acetolactic acid; (3) migration of a methyl carbon from carbon 2 of the pyruvate moiety to carbon 1 of the acetaldehyde moiety of acetolactate to yield the keto analog of valine.

In a preliminary communication,[3] data were reported which led us to the hypothesis that the carbon chain of valine arises exclusively from pyruvic acid carbons. A mechanism was formulated involving a ketol condensation between acetaldehyde and pyruvate to yield α-acetolactic acid, followed by an intramolecular migration of a methyl carbon. In the present report a more detailed description of these experiments is given, together with additional data supporting this concept.

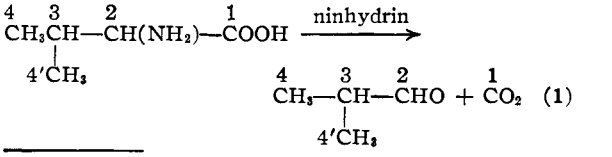
Experimental

The method of approach in these isotopic studies has been described previously.[4,5] Briefly summarized, it involves the growth of a strain of *Torulopsis utilis* on glucose as essentially the sole source of carbon, together with tracer quantities of labeled compounds. The cells are harvested, washed and hydrolyzed, and the amino acids are separated by column chromatography according to the procedure of Moore and Stein.[6] Details of the cultivation of the organism, radioactivity assays and other experimental procedures are given in previous publications.[4,5]

Isolation of Valine.—The solutions containing the neutral amino acids, from which arginine and lysine had been removed by previously described methods,[6,7] were treated with several 50-ml. portions of a solution of amyl alcohol–ethanol–ether to remove excess phosphotungstic acid. The aqueous solution was then neutralized to a *p*H 5 to 6 with a saturated solution of $Ba(OH)_2$ and was evaporated to dryness under reduced pressure. The residue was taken up in 20 ml. of 1.5 *N* HCl and the solution placed on a column, 4 cm. in diameter and 120 cm. long, containing Dowex 50 (200–500 mesh) previously treated with 2 *N* NaOH and washed several times with 1.5 *N* HCl. The amino acids were washed into the column with three or four 30-ml. portions of 1.5 *N* HCl, and the column was eluted at the rate of 50 ml. per hour with HCl of the same concentration. The various 15-ml. fractions collected with the aid of a Technicon fraction cutter were each tested individually by paper chromatography. Those fractions containing valine were combined and evaporated to dryness under reduced pressure.

In more recent experiments, the protein hydrolysates were submitted directly to the Neuberg purification method,[8] and the basic, acidic and neutral amino acids were separated directly, without recourse to chemical precipitation. This was achieved by placing the mixture of amino acids on a 4 × 15 cm. column of Dowex 50. Elution with HCl of successively higher normalities (1.5, 2.5 and 4 *N*) produced simple mixtures, each containing three or four amino acids. Separation of these mixtures into the individual amino acids was accomplished by chromatography on the 120 cm. column of Dowex 50 as described previously. In this way, chromatographically pure valine samples, weighing from 50–120 mg., were isolated from mixtures representing approximately 4 to 8 g. of yeast cells.

The Degradation of Isotopically Labeled Valine.—The distribution of activity among the carbon atoms of valine was determined by means of the following series of reactions



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(2) Post Doctoral Fellow of the National Institutes of Health, Department of Health, Education, and Welfare. This work constitutes part of a thesis submitted by Murray Strassman to the Graduate School of Temple University in partial fulfillment of the requirements for the Ph.D. degree.

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(8) C. Neuberg and J. Kerb, *Biochem. Z.*, **40**, 498 (1912).