Preparation of N^{α} -Acetylornithine

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A method for the preparation and purification of N^{α} -acetylornithine is described. The method involves the acetylation of ornithine rather than its N^{δ} -carbobenzyloxy derivative. Salts, diacetylornithine, and unreacted ornithine are removed with ion-exchange resin techniques. N^{δ} -Acetylornithine is then separated from N^{α} -acetyl-ornithine by adsorption of its copper complex on activated alumina.

INTRODUCTION

 N^{α} -Acetylornithine is an intermediate in the interconversion of glutamic acid and proline in *Escherichia coli* (1). The usual procedure for synthesis involves the formation of the N^{δ} -carbobenzyloxy derivative (1). We have developed an alternative method which does not include a carbobenzyloxy derivative.

Uncharged amino groups are acylated in preference to charged groups (2). Since it is known that an amino group adjacent to a carboxyl group is less basic than one farther away (3), it was thought that direct acetylation of ornithine might produce a significantly greater acetylation of the α amino group than of the δ -amino group.

When we found that acetylation of ornithine at pH 9 yielded five times as much α -acetylornithine as δ -acetylornithine, a practical method of preparing α -acetylornithine required only convenient methods of purification. α -Acetylornithine was purified by removal of unreacted ornithine and diacetylornithine with ion-exchange resins and adsorption of δ -acetylornithine as its copper complex on alumina.

EXPERIMENTAL

Synthesis of N^{α} -Acetylornithine

Dissolve 3.37 g. L-ornithine hydrochloride (20 mmoles) and 4.2 g. sodium bicarbonate (50

mmoles) in 50 ml. of cold water. While keeping the solution in an ice bath, add 2.36 ml. (2.56 g. = 25 mmoles) of acetic anhydride (acetyl chloride is unsatisfactory) continually during a 1- to 2-hr. period with constant stirring. After allowing the solution to stand overnight at 0°C, the pH is adjusted to 5.5 with acetic acid. A mild vacuum is placed on the solution to remove dissolved carbon dioxide, and the solution is transferred quantitatively to a column of 100 ml. of Dowex 50¹ (Dow Chemical Co., Midland, Mich.) in the hydrogen form (200-400 mesh) maintained at 4-6°C. (4). The resin is washed with 500 ml. of cold water and the wash discarded.

Place a column of carboxylic acid resin XE-64 (Rohm and Haas, Philadelphia, Pa.) in the hydrogen form (40 ml.) underneath the Dowex 50 column. Add 1 N ammonia until the effluent from the top column is alkaline; then wash with 200 ml. of cold water. If the effluent from the lower column should become alkaline, put the solution through a second column of carboxylic acid resin. The resultant solution contains only α - and δ -acetyl-ornithines, since anions do not adsorb on these resins while ornithine, ammonia, and other cations are retained.

Dry alumina (40 g. of activated alumina F20, Aluminum Company of America, East St. Louis, Ill.) is mixed with 10 g. of dry basic copper carbonate powder, and the mixture is packed into a

¹The mention of specific commercial products does not infer any recommendation or endorsement by the U. S. Department of Agriculture. Trade names and company names are used for convenience in specifying the type of product used dry state into a 4-cm. column (5). This column is placed over a 10 \times 1.5 cm. column of XE-64 in the hydrogen form. The solution of α - and δ -acetylornithines is poured through the two columns followed by 500 ml. of deionized water. The effluent solution and the wash containing α -acetylornithine are evaporated to dryness *in vacuo*. The δ -acetylornithine is adsorbed to the alumina as the copper complex and cannot be recovered conveniently.

After dissolving the residue in 3 ml. of hot water, 15 ml. of hot absolute methanol followed by 15 ml. of hot 95% ethanol are added. The solution is immediately filtered. α -Acetylornithine crystallizes out during standing at room temperature for several hours. Crystallization is complete after 18 hr. at 4°C. The colorless crystals are filtered and washed with cold absolute methanol. Crystallization may be repeated as above but usually is unnecessary.

The dry weight of crystals obtained in one experiment was 1.44 g. (44% of theory).

RESULTS

The elemental analysis of the synthetic material was 48.26% C, 8.19% H, and 15.99% N (theory 48.3% C, 8.05% H, and 16.1% N).

 α -Acetylornithine was synthesized by acetylation of δ -carbobenzyloxyornithine (6) according to the procedure of Neuberger and Sanger (7). The infrared spectrum of the compound prepared in this way was identical with that of the material obtained from the direct acetylation of ornithine.

The melting points of the two preparations were 226–227°C. (corr.), either alone or mixed, and the specific rotation was $(\alpha)_{D}^{25} = 6.5^{\circ}$ in water (C = 5). These values agree well with those of Vogel (1).

In the course of this work, it was found that α - and δ -acetylornithines can be readily separated by paper electrophoresis in 1 N acetic acid. This procedure also is a convenient method of separating α -amino acids from non- α -amino acids.

DISCUSSION

In order to learn more about the course of the reaction and to improve the yield of α -acetylornithine, quantitative data on the acetylated ornithines and unreacted ornithine were obtained. Unreacted ornithine was determined by Chinard's method (8). The effluent from the ion-exchange resins was assayed for diacetylornithine after hydrolysis to ornithine. Alpha- and δ acetylornithines were separated by onedirectional paper chromatography in butanol-acetic acid-water mixture (9:1:2.5). The amounts of the two monoacetylornithines were measured by developing the color with ninhydrin (9).

These analyses showed that 10.5 mmoles α -acetylornithine, 1.9 mmoles δ -acetylornithine were formed while 0.2 mmole ornithine was left. The high yield of diacetylornithine was undesirable, but attempts to improve the yield by varying the pH of the reaction mixture or the ratio of acetic anhydride to ornithine were unsuccesful.

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