A New Synthesis of Threonine

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Despite a little information available concerning the conversion of glycyl residue to threonyl or seryl residue[1-3], there is as yet no satisfactory method for the synthesis of threonine from glycine or glycyl peptides.

In this paper the authors present a new method for the synthesis of threonine from copper glycinate and acetaldehyde. The possibility for such a method has been suggested from the considerations that the methylene group in copper glycinate may be more active than that in free glycine and that the amino group in the copper salt may be protected from unfavorable side reactions. In the established method, the copper salt of glycine is subjected to the reaction with acetaldehyde in a weakly alkaline aqueous solution at 50–70° for 1–2 hours. After removal of copper and other inorganic salts, a mixture of threonine and allothreonine is obtained in a high yield. It seems likely that the reaction proceeds as shown in Fig. 1. The initial reaction of copper glycinate (I) with acetaldehyde appears to give rise to a coordination state (II) of copper threoninate, which is then transformed into a second coordination state (III). The overall reaction equilibrium is shifted in favor of the formation of threonine, since compound III seems to be more stable than compound II especially in alkaline media. These inferences have been drawn from the following observations: (1) while copper threoninate exhibits low pH values in aqueous solution (below 5), copper glycinate is neutral (pH 7.4), (2) the former is adsorbed on an anion exchange resin of OH-form, while the latter is not, and (3) the light absorption of the former is shifted considerably towards shorter wave-lengths as compared with that of the latter (see

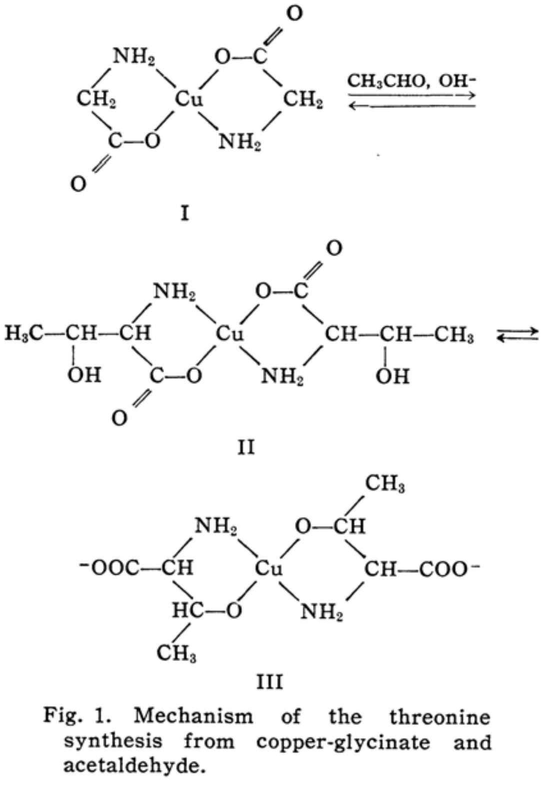

Fig. 1. Mechanism of the threonine synthesis from copper-glycinate and acetaldehyde.

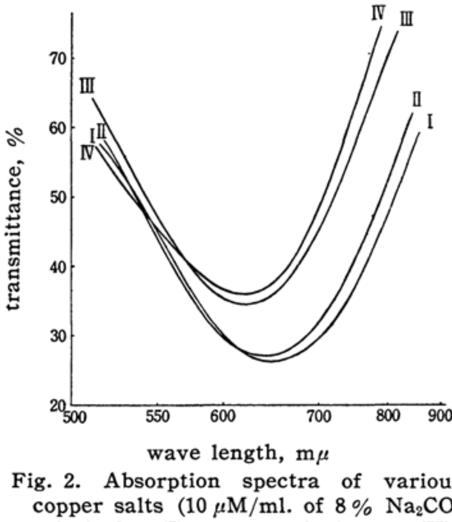

Fig. 2. Absorption spectra of various copper salts (10 μM/ml. of 8% Na_2CO_3 solution). (I), copper glycinate; (II), (I)+CH_3CHO at the beginning of the reaction; (III), (I)+CH_3CHO after the reaction; (IV) copper threoninate.

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[1] E. Snell, D. Metzler and J. Longenecker, *J. Am. Chem. Soc.*, **75**, 2786 (1953); ibid., **76**, 639 (1954).

[2] Th. Wieland and K. Dose, *Angew. Chem.*, **66**, 781 (1954).

[3] S. Akabori, K. Okawa and M. Sato, This Bulletin, **29**, 608 (1956).

Fig. 2). Copper threoninate is in fact bluish purple in color and resembles "biuret" or complexes of amino-alcohols in this respect.

It is expected from the structural formula of compound III that the threo-form of the copper salt may be stereochemically more stable than the allo-form. In fact it has been shown that the former is always produced predominantly over the latter (the ratio of threo/allo being more than 1.6), although the ratio may vary according to the conditions of the reaction. The results presented in this paper may suggest a possible mechanism of the biosynthesis of threonine*. Further experiments are now in progress to find out more favorable conditions for the selective preparation of the threo-form.

Experimental

Copper glycinate $(NH_2CH_2CO_2)_2Cu\cdot H_2O$.—This was prepared by the two following methods. (a) An aqueous solution of glycine was heated with basic cupric carbonate, the excess of the latter was filtered off from the hot solution, the filtrate concentrated in vacuo and cooled. Crystals of copper glycinate thus formed were collected, washed and dried. (b) Glycine (2 moles) was converted into sodium glycinate in aqueous solution and a saturated solution of cupric sulfate (1 mole) was added with stirring. Crystals of copper glycinate deposited were filtered off, washed three times with water and dried.

Copper threoninate.—This was prepared according to the method (a) described for copper glycinate.

Threonine.—A mixture of 115 g. of well-ground copper glycinate, 30 g. of anhydrous sodium carbonate, 370 ml. of water and 160 ml. of freshly distilled acetaldehyde was stirred under reflux on a water bath at 50° for 1 hour. After cooling, excess of acetaldehyde was removed by distillation. One liter of water and 700 ml. of concentrated aqueous ammonia (ca. 15 N) were then added and the solution was filtered. The filtrate was passed through a column of Dowex 50X 12 (H-form, 200-400 mesh, 10 cm × 20 cm) which had previously been percolated with 5 N aqueous ammonia. The effluent was concentrated in vacuo and treated with charcoal. The solution was again concentrated in vacuo until the crystallization of threonine began, and then about four volumes of methanol were added. After being kept in a refrigerator for 24 hours, the crystals consisting of both threonine and allo-threonine were collected, washed three times with methanol, and dried in vacuo. The yield was 70 g. From the mother liquor, 6 g of almost the same product was obtained. The total yield was 76 g., or 64 %.

The crystals thus obtained gave, on paper chromatograms, only two spots corresponding to threonine and allo-threonine, except for an insignificant spot due to glycine. Chromatographic analysis of the dinitrophenylated crystals using a column of Amberlite-IRC 50[4] showed that the ratio of threonine to allo-threonine was 1.8.

Anal. Found: C, 40.34; H, 7.14; N, 11.52. Calcd. for $C_4H_9O_3N$: C, 40.34; H, 7.56; N, 11.76%.

In another experiment, a mixture of 115 g. of copper glycinate, 29 g. of potassium carbonate, 230 ml. of water, and 116 ml. of freshly distilled acetaldehyde, was brought to react by heating at 70° for 2 hours. After acidification with hydrochloric acid, the reaction mixture was treated with hydrogen sulfide and filtered. The filtrate was treated with 400 g. of wet Dowex 50 (H-form), and after being freed from water the substanse was dissolved in absolute alcohol. After removal of the precipitated inorganic salts, the free amino acids were precipitated by the addition of aniline. By repeated recrystallization, 27 g. (22.7 %) of crystals (threo/allo = 2.4) was obtained. Further 5.5 g. (4.6 %) of crystals (threo/allo = 9) was recovered from the mother liquor. The total yield was 32.5 g. (27.3 %).

Summary

A new method is described for the synthesis of threonine from acetaldehyde and copper glycinate. This method gives a mixture of allo-threonine and threonine in a high yield (64 %).

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* After we completed this manuscript, we have become aware of a recent report by J. B. Gilbert (*J. Am. Chem. Soc.*, **79**, 2242 (1957)) which shows that a mixture of allo-threonine and threonine can be formed from acetaldehyde and glycine in the presence of an enzyme preparation from rat liver.

[4] T. Seki, to be published.