STUDY OF THE CONDITIONS OF OBTAINING THE COPPER COMPLEX OF THREONINE

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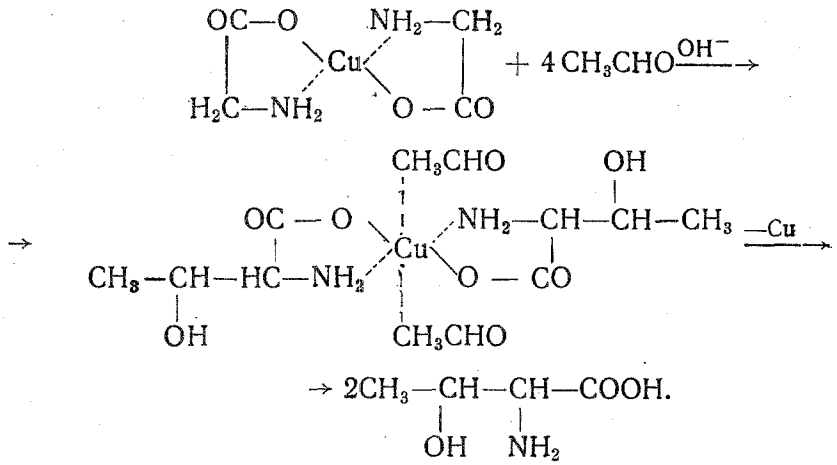
In syntheses of DL-threonine, in addition to the threo forms of α-amino-β-hydroxybutyric acid a considerable or even a major amount of the allo form is also produced. Of all the known methods, only the condensation of acetaldehyde with the copper complex of glycine in the presence of alkali [1] enables a predominating amount of the threo isomer to be obtained. The product of this reaction is a crystalline bis-acetaldehyde-bis-threoninate of copper [2] consisting of a mixture of diastereomeric forms:



Because of its high solubility in water, the copper complex of allothreonine can be removed by washing with cold water [3]. The isolation of the amino acid from the pure crystalline copper threoninate by means of hydrogen sulfide or cation exchangers is easy to carry out.

In the Akabori reaction, the complexed copper successfully combines the functions of activating the hydrogen atom in the methylene group and protecting the amino group from side reactions. It has been possible to use aldehydes, ketones, and keto acids in the reaction with copper glycinate and thus to obtain a number of important 2-amino-3-hydroxycarboxylic acids. This reaction, which is basically analogous to an aldol condensation, can be carried out not only with glycine but also with alanine, α-aminobutyric acid, and serine [4]. Active carbonyl compounds, formaldehyde, acetaldehyde, pyruvic acid, can react in a moderately alkaline medium with various α-amino acids in the presence of only catalytic amounts of copper [5].

The method mentioned is simple and gives high yields of threonine and, consequently, can be used industrially. The best catalysts for the condensation of copper glycinate with acetaldehyde are the alkali-metal hydroxides [1, 6]. Water [1, 6] and methanol [7] have been proposed as solvents. If methanol is used, the reaction forms a homogeneous solution of the complex free from the initial copper glycinate and the content of the threo isomer in the product increases considerably. When the reaction temperature is raised and the amount of alkali is increased, the yield of product falls. However, the published investigations [1-3, 6, 7] (mainly patent specifications) have not given a complete discussion of this important reaction. The optimum conditions for its performance have not been found.

We have investigated how the relative amounts of alkali, the concentration of the reactants, and the temperature affect the course of the reaction, the total yield of product, and the ratio of the threo and allo forms. In this way we have been able to draw up a general picture of the reaction which has enabled us to determine the best conditions for performing it.

In order to follow the formation and behavior of the threo and allo complexes during the reaction as a function of the alkalinity of the medium, we carried out a series of experiments with a gradually increasing amount of alkali (the temperature and the volume of solvent being constant). During the experiment samples were taken in which, after elimination of the solvent and removal of the copper, threonine and allothreonine were determined by paper chromatography and colorimetry. For each experiment we determined the time to achieve the maximum total yield (as a rule, it coincided with the time of formation of the maximum amount of threo form), the ratio of the threo and allo forms at this

time, and the ratio of the threo and allo forms after a period twice as long (Table 1).

It can be seen from Table 1 that an increase in the amount of alkali accelerates the condensation of acetaldehyde with copper glycinate. Under the mild conditions of experiment 1, the maximum amount of threo complex was obtained after 6 hr and then did not change. However, the total yield was then the maximum. The amount of the complex of allothreonine remained unchanged from the first samples to the end. No indications of the decomposition of the complexes under these conditions were found. If the amount of alkali was decreased further (less than 0.19 mole per mole of glycinate), the reaction did not go to completion in 25 hr and traces of glycine were always found in the product.

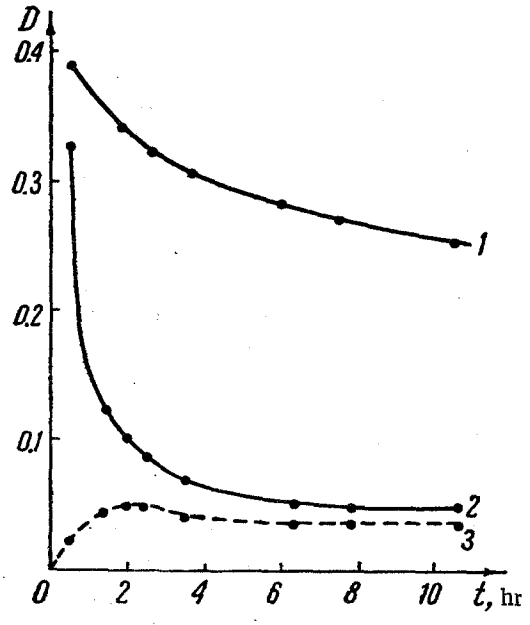
Table 1

Influence of the Amount of Alkali on the Reaction at 55° C

Experiment no.	KOH, mole/mole of glycinate	Time to reach the total maximum yield, hr	Ratio of threo and allo forms	Subsequent course of the reaction		
				time, hr	ratio of threo and allo forms	yield, % of maximum
1	0.207	6	3	12	3	100
2	0.283	3	3.8	6	4.3	85
3	0.305	2.5	3.7	5	4	90
4	0.381	~2	4.5	4	6.5	90
5	0.446	~1	5.9	~2	8	87

In a more highly alkaline medium (experiment 2), the maximum amount of threo form was found after 3.0 hr (simultaneously with the maximum total yield); in experiment 4 it was found after 1 hr 50 min, and then the amount of complex gradually decreased.

In a strongly alkaline medium, the formation and decomposition of complexes of the diastereomeric threonines takes place simultaneously. As a result, instead of a crystalline substance, a sticky mass is obtained, the isolation of the threonine is more difficult, and the total yield of product falls. However, the relative amount of the threo form in the product rises. Assuming that this phenomenon might be caused by the different stabilities of the copper complexes of threonine and allothreonine in an alkaline medium, we performed two experiments. The behavior of the copper complexes of DL-threonine and DL-allothreonine in strongly alkaline methanolic solutions with an excess of acetaldehyde was studied at 55° C. It was found that under such conditions both complexes decomposed but, as can be seen from the figure, this process takes place differently with the threo and the allo forms. The threo complex is stable in a more alkaline medium than is the allo complex and decomposes more slowly. A peculiarity of the behavior of the allo complex consists in the fact that on decomposition it is partially converted into the threo form (curve 3). When the excess of alkali has been completely consumed, the decomposition of the complex ceases and its amount remains constant. The decomposition products, which were detected on a chromatogram in the form of spots at the start increasing in size with time and which were stained by ninhydrin, were not identified.



Decomposition of the copper complexes of threonine and allothreonine in a strongly alkaline medium. 1) Decomposition of the complex of the threo form; 2) decomposition of the complex of the allo form; 3) conversion of the allo form into the threo form.

Consequently, an increase in the ratio of the threo and allo forms as the alkalinity of the medium is increased is explained by the lower stability of the complex of allothreonine in an alkaline medium, its decomposition, and its partial conversion into the threo form. An idea of the influence of the temperature and the solvent on the rate of the reaction and of the relative amounts of the complexes of the diastereomeric threonines formed can be gained from the data of Table 2.

These show that a change in the temperature has a marked effect on the rate of the reaction and, to some extent, on the ratio of the threo and allo forms. Under more severe conditions this ratio rises but the complex begins to decompose. The addition of

water to the reaction mixture moderates the reaction conditions, as is shown by a comparison of experiments 7 and 8, and 3 and 9. The rate of the reaction and the ratio of threo and allo forms decreases considerably. It is known that when the reaction is carried out in pure water the amount of the threo form never exceeds 2/3 of the mixture of diastereomers [1].

In addition, we found that when the concentration of the reactants is increased twofold, the reaction is accelerated almost twofold, while the ratio of the diastereromers does not change appreciably. In this case, indications of the decomposition of the complexes appear before the maximum yield is reached.

Thus, a careful study of the conditions of the Akabori reaction indicates that the best conditions for obtaining the maximum total yield of practically pure copper complexes of the diastereomeric threonines are the conditions of experiment 1 (see Table 1), under which there is no decomposition at all of the reaction product [8]. The conditions may be varied, taking the results presented above into account, according to the purpose in view.

Table 2

Influence of the Temperature and the Solvent on the Course of the Reaction

Experiment no.	KOH, mole/mole of glycinate	Temperature, °C	Solvent, ml	Time to reach the maximum yield, hr	Ratio of threo and allo forms	Further course of the reaction		
						time, hr	ratio of threo and allo forms	yield, % of the maximum
1	0.208	55	CH_3OH 100	6	3	12	3	100
6	0.208	60—62	The same	2.5	3.5	5	3.5	87
3	0.305	55	"	2.5	3.7	5	4	90
7	0.305	22	"	25	2.5	—	—	—
8	0.305	22	CH_3OH 80 CH_3OH 20	96—120	2.2	—	—	—
9	0.305	55	The same	7	2.8	—	—	—

Experimental

Performance of the reaction. The reaction was carried out in a two-necked flask with stirring by means of a powerful magnetic stirrer. A suspension of 3.75 g of copper glycinate [9] in methyl alcohol containing a definite amount of alkali was treated in drops with 8 ml of freshly distilled acetaldehyde. The temperature was kept constant. An average of 10–15 samples of the reaction mixture (2 ml each) was taken for each experiment. The reaction was generally complete after 6–9 hr.

Separation of the copper. The threonine (mixture of diastereomers) in the samples taken was isolated from the copper complex in the following way: The solvent from each sample was evaporated off slowly in vacuum, and the dry residue was dissolved in 2 ml of ammonia solution (1:1). Each of a number of test tubes was charged with 0.5 ml of Dowex 50 × 4 cation exchanger in the ammonium form and 0.1 ml of an ammoniacal solution of the sample. The copper was adsorbed on the resin and the colorless or faintly yellowish solution was decanted off and deposited on chromatographic paper with a standard capillary.

Chromatography. After the samples had been deposited on paper (Leningrad No. 2 Mill, grade M), chromatography was carried out by the descending method in the butan-1-ol–methyl ethyl ketone–concentrated ammonia solution–water (5:3:1:1) system [10] for 2 days. The chromatograms were dried in the air and heated at 60° C for 3–5 min. Then they were treated with a solution of ninhydrin in acetone [11]. Purple spots appeared on the dried and impregnated chromatograms. Each sample gave 2 spots corresponding to threonine (intense spot) and allothreonine (weaker spot). More or less intense spots may appear at the start depending on the conditions.

Colorimetry. The colored spots were cut out in the form of rectangles of similar dimensions equal to the dimensions of the largest spot. A rectangle of the same size was also cut out from the background. Each spot was extracted with 4 ml of methyl alcohol and the optical density of the resulting solutions was measured against the background in a FEK-N-57 photocolorimeter in a 3-mm cell at 536 mμ. From the results of a series of samples, curves of the change in the optical density with time were constructed which characterized the change in the amounts of threonine and allothreonine, the total yield, and the total ratio of the threo and allo forms during the reaction.

Production of a mixture of diastereomeric copper threoninates. The starting materials used were 3.75 g of copper glycinate, 0.19 g of caustic potash, 100 ml of methyl alcohol, and 8 ml of acetaldehyde. The experiment was carried

out for 6 hr at 55° C (experiment 1), and then 0.2 ml of acetic acid was added and the mixture was evaporated in vacuum. This gave 6.62–6.66 g (an almost quantitative yield) of a crystalline product containing the copper complexes of threonine and allothreonine in a ratio of 3:1, slightly contaminated with potassium acetate [8].

Stability of the copper complexes of threonine and allothreonine. A mixture of 0.3 g of the copper complex of threonine, 0.029 g of caustic potash, and 10 ml of methanol was stirred at 55° C. Samples were taken over 8 days and these were treated, chromatographed, and colorimetered as described above. An experiment with the copper complex of allothreonine was carried out similarly.

Summary

1. The influence of the temperature, the concentration of the reactants, and the amount of alkali on the reaction of the copper complex of glycine with acetaldehyde has been studied. The optimum conditions for carrying out the reaction have been found: ratio of alkali to glycinate of 2:10, temperature 55° C, reaction time 6 hr, excess of acetaldehyde, methanol as solvent.

2. The copper complex of threonine is more stable in an alkaline medium than the complex of allothreonine. The decomposition of the complex of the allo form is accompanied by its partial conversion into the threo form.

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