REACTION OF THE COPPER COMPLEX OF L-ALANINE WITH ACETALDEHYDE AND THE MECHANISM OF THE AKABORI REACTION

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Complexing involving a nitrogen atom directly bound to the CH group is known to increase the acidity of the latter. When it was found that the copper complex of glycine can be converted to threonine by reacting it with acetaldehyde (the Akabori reaction) [1], a great deal of research was undertaken on electrophilic substitution in the α-CH groups of metallic complexes of the amino acids. These investigations revealed certain special features in the structure and reactivity of cobalt and, to a lesser extent, copper complexes of the amino acids. It was established that the Akabori reaction was a general reaction of copper and cobalt complexes of amino acids (glycine and alanine) with carbonyl compounds (aliphatic aldehydes and ketones and aromatic aldehydes) [2].

The data on the influence of the amount of alkali on the condensation rate of copper glycinate with acetaldehyde published in our previous report on the conditions for the Akabori reaction [3] are in agreement with the hypothesis that a CH-acid participates in this reaction. Buckingham [4] investigated deuteron exchange for the α-CH proton and racemization in coordinated L-alanine and L-valine in cobalt complexes by the NMR method and showed that the proton-exchange and racemization rates are equal for an amino acid complex. This apparently results from an S_E1 process, i.e., formation of an intermediate carbanion during racemization and hydrogen exchange.

The difficulties involved in studying the reversible reaction of copper complexes of the amino acids with aldehydes are due to the fact that several parallel reactions take place simultaneously, including reduction of the copper to the metallic state and autocondensation of the aldehydes [3, 5]. Moreover, the NMR method cannot be used for studying electrophilic hydrogen replacement in copper complex. All these factors hamper quantitative study of the reactions of amino acid copper complexes.

We set out to study the mechanism of the Akabori reaction by investigating the reaction of the copper complex of optically active L-alanine with acetaldehyde in an alkaline medium and analyzing the course of the reaction by gas–liquid chromatography (GLC) and polarimetry. Investigation of complex racemization by GLC and polarimetry showed that noticeable racemization of the L-alanine copper complex began only at a pH above 11.5 and had a very low rate. Almost no racemization of L-alanine was observed in the same alkaline medium, but rapid racemization of the L-alanine copper complex occurred in the presence of acetaldehyde (Figs. 1 and 2). The α-methylthreonine formed during the reaction of the L-alanine copper complex with acetaldehyde was always racemic, even during the initial stages of the reaction, where the racemization of the initial alininate was slight. The rate of α-methylthreonine formation, like the racemization rate of the unreacted L-alanine copper complex, was always higher than the racemization rate for the L-alanine copper complex in the absense of acetaldehyde.

The results obtained can be interpreted in the following manner: 1) an intermediate product having a higher C–H acidity than the initial complex is formed when an amino acid copper complex is reacted with acetaldehyde; 2) this intermediate product is apparently common to racemization and formation of a C–C bond.

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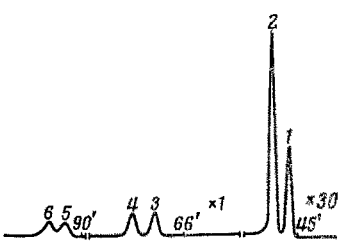


Fig. 1

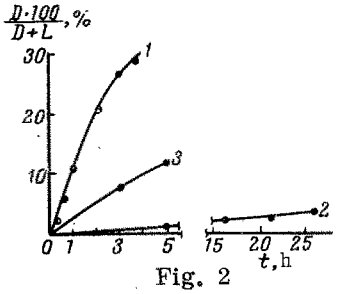


Fig. 2

Fig. 1. Chromatogram of mixture of d-sec-butyl esters of N-trifluoroacetyl alanine and methylthreonine derivatives: 1) D-alanine; 2) L-alanine; 3) D-threomethylthreonine; 4) L-threomethyl alanine; 5) D-allomethylthreonine; 6) L-allomethylthreonine.

Fig. 2. Influence of acetaldehyde on racemization of L-alanine copper complex in alkaline medium (GLC data). 1) racemization with CuL-ala_2 : KOH : CH_3CHO ratio of 1 : 1 : 5 in 50 ml of aqueous solution at 40°, L-alanine copper complex concentration of 0.02 mole/liter, acetaldehyde concentration of 0.1 mole/liter, and racemization in absence of acetaldehyde; 2) CuL-ala_2 : KOH = 1 : 1 in 50 ml of aqueous solution at 40°, L-alanine copper complex concentration of 0.02 mole/liter; 3) CuL-ala_2 : KOH = 1 : 1 in 50 ml of aqueous solution at 55°, L-alanine copper complex concentration of 0.036 mole/liter.

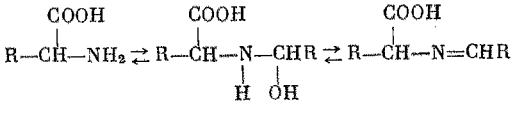
The hypothesis that an intermediate product is formed was confirmed by the dependence of the optical activity of the reaction mixture on time and the acetaldehyde concentration (Fig. 3).

The optical-activity maximum exhibited by the solution in an alkaline medium may have been due to accumulation of this intermediate product. The observed increase in optical activity did not result from formation of α-methylthreonine, since a similar rise in optical activity was observed in a neutral medium, where this compound is not formed; moreover, the α-methylthreonine formed in an alkaline medium is always racemized, as was pointed out above.

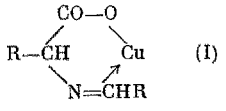
As for the structure of the intermediate product, it can be assumed from the data in the literature [6] that it is a copper complex of a Schiff base. Such complexes of aldehydes with amino acids having a Schiff-base structure are known for copper complexes of salicylaldehyde with glycine [7-9] and of acetaldehyde with threonine [10].

It is also known [7] that the equilibrium between an amino acid and an imine

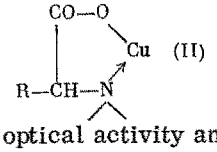
Scheme 1



is shifted to the right; complexing with copper is stabilized by the imino form (I):



(I)



(II)

Formation of Schiff-base complexes causes an increase in optical activity and a change in the dispersion of optical rotation for solutions [7, 11]. It can therefore be stated that the observed increase in rotation for the L-alanine copper complex solution was caused by establishment of this equilibrium (scheme 1). The limiting value of α_t/α_0 in a neutral solution (see Fig. 3) corresponds to the equilibrium imino form (I) concentration under the conditions in question, while the process is complicated by racemization and reactions involving formation of a C–C bond in an alkaline medium. It can be presumed that proton mobility in the

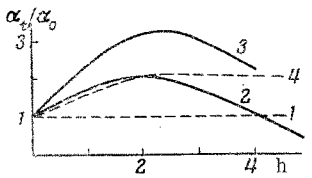


Fig. 3

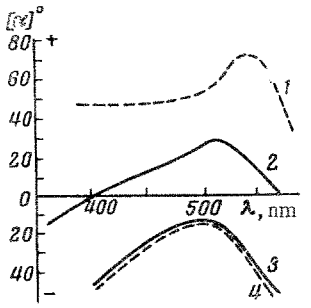


Fig. 4

Fig. 3. Change in optical rotation during reaction of L-alanine copper complex with acetaldehyde (50°, $\lambda = 500$ mμ). 1) Without acetaldehyde; L-alanine copper complex concentration of $5 \cdot 10^{-3}$ mole/liter, pH 9.2; 2) acetaldehyde concentration of $2 \cdot 10^{-2}$ mole/liter, L-alanine copper complex concentration of $5 \cdot 10^{-3}$ mole/liter, pH 9.2; 3) acetaldehyde concentration of $1 \cdot 10^{-1}$ mole/liter, L-alanine copper complex concentration of $5 \cdot 10^{-3}$ mole/liter, pH 9.2; 4) acetaldehyde concentration of $1 \cdot 10^{-1}$ mole/liter, L-alanine copper complex concentration of $5 \cdot 10^{-3}$ mole/liter, pH 7.3.

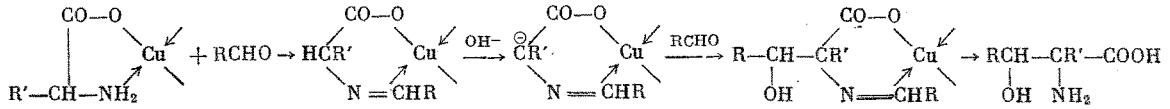
Fig. 4. Dispersion of optical rotation for copper complexes of L-alanine and L-N, N-dimethyl alanine (the specific rotation was calculated for 1 g of amino acid in solution). 1) L-alanine copper complex; 2) L-alanine copper complex after heating at 50° for 2 h with fivefold excess of acetaldehyde at pH 9.2; 3) L-N,N-dimethylalanine after heating for 2 h with tenfold excess of acetaldehyde at pH 9.2.

α-CH group depends on the manner in which complexing with the copper occurs. This mobility is evidently higher in a π-donor system like (I) than in p-donor systems of type (II). Actually, it has been established [9] that the copper complex of salicylaldehyde with glycine reacts very readily with carbonyl compounds, even in a neutral medium. These data support our hypothesis that both racemization in the presence of acetaldehyde and the Akabori reaction involve an imine as an intermediate product.

We found confirmation of this view in studying the copper complex of L-N, N-dimethylalanine. The presence of acetaldehyde did not alter the optical properties of the complex in solution under conditions where it was stable (pH < 10.3; Fig. 4). (A decrease in optical activity resulting from breakdown of the amino acid by fragmentation was observed in more alkaline media.)

It is significant that the copper complex of L-N, N-dimethylalanine does not undergo racemization or form N, N-dimethyl-2-methylthreonine. We were unable to find the latter compound in the reaction mixture over the pH range 7.8-11 by paper chromatography or other analytic procedures.

Imine formation was apparently a necessary intermediate stage in the Akabori reaction:



EXPERIMENTAL

The amino acid esters for gas–liquid chromatography were produced in the following manner: 10 mg of amino acid and 0.5 ml of secondary butanol were saturated with dry HCl for 1 h. The excess alcohol was distilled off under vacuum, absolute benzene or chloroform (1 ml) was added, and the mixture was again vacuum-distilled. The dry residue was flooded with 1 ml of trichloroacetic anhydride and left to stand overnight at room temperature. After the trichloroacetic anhydride was driven off, the residue was dissolved in benzene or chloroform.

Gas chromatography was carried out in a capillary column 90 m long with an inside diameter of 0.5 mm; we employed a neopentylglycol adipinate phase at a pressure of 0.3 atm and a temperature of 105-110°. The detector was of the flame-ionization type and the carrier gas was nitrogen.

Paper chromatography (after removal of the copper from the solution with hydrogen sulfide) was carried out by the descending method in a solvent consisting of pyridine and water in a ratio of 65 : 35, with Whatman No. 41 paper and an exposure time of 2.5 h. The developer was a solution of 0.5 g of ninhydrin in a mixture of 95 ml of acetone, 1 ml of CH_3COOH, and 4 ml of H_2O. The R_f of the alanine was 0.61, that of the methylthreonine was 0.72, and that of the N, N-dimethylalanine was 0.78.

The pH of the reaction mixture was measured with a Radiometer PHM-4c pH-meter. The dispersion of optical rotation was investigated with a JASCO ORD UV-5 spectrophotopolarimeter in a controlled-temperature cell with quartz windows.

The L-alanine copper complex was produced from analytically pure copper bicarbonate and chemically pure L-alanine. According to the GLC data [12], the optical purity of the α-alanine was 99.5%. The α-methylthreonine was produced by a somewhat modified version of the method of Mix et al. [13]: a mixture of 0.01 M copper alaninate, 10 ml of 1 N KOH, and 1.35 g of freshly distilled acetaldehyde in 5 ml of water was agitated at 0° for 1.5 h, another portion of the same amount of aldehyde solution was added, and agitation was continued at 40° for 3.5 h. Paper chromatography showed that a small amount of alanine remained in the reaction mixture as an impurity. The subsequent treatment of the mixture was the same as that described by Mix et al. [13]. The α-methylthreonine yield was about 55%. The alanine impurity disappeared after two recrystallizations from aqueous alcohol; the decomposition point of the resultant compound was 233-235°. Observed: 44.96, 44.85% C; 8.25, 8.20% H; 10.40, 10.31% N. $C_5H_{11}NO_3$. Calculated: 45.10% C; 8.33% H; 10.52% N.

Figure 5 shows a gas chromatogram of the methylthreonine obtained (using a steel capillary column 90 m long with an inside diameter of 0.5 mm at a pressure of 0.43 atm and a temperature of 110°, with a flame-ionization detector and nitrogen as the carrier gas). The α-methylthreonine formed consisted of a mixture of the threo- and allo-forms, with a considerable preponderance of the former.

We studied the racemization of L-alanine and the Akabori reaction with $2 \cdot 10^{-2}$ M copper L-alaninate solutions in water, methanol, and acetonitrile at 40° with different medium alkalinities and acetaldehyde concentrations.

Example. Chilled solutions of 0.001 M L-Cu-Ala_2, 0.001 M KOH, and 0.05 M freshly prepared acetaldehyde were mixed in a 50-ml measuring flask. The contents of the flask were quickly transferred to a thermostatted reaction vessel fitted with a mixer. Five or six samples of the reaction mixture were taken during the experiment. Each sample was diluted with water and acidified with acetic acid; the solvent and unreacted aldehyde were driven off in a rotary evaporator. The residue was dissolved in water and again acidified with CH_3COOH and the copper was removed with hydrogen sulfide. The solution was transferred to a column (0.9 cm in diameter and 10 cm long) of Dowex 50 ×8.400 mesh cationite (H form) and washed with water to remove the CH_3COOH and H_2S. The amino acid was diluted from the column with 3 N ammonia. The eluate was evaporated and the solid residue was dried by distillation with benzene. The samples were analyzed by paper and gas chromatography.

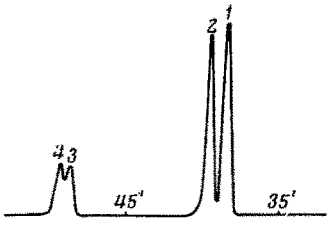


Fig. 5. Chromatograms of mixture of d-sec-butyl esters of N-trifluoroacetylmethylthreonine. 1) D-threomethylthreonine; 2) L-threomethylthreonine; 3) D-allomethylthreonine; 4) L-allomethylthreonine.

Figure 1 shows a typical sample chromatogram. The same procedure was employed to investigate the racemization of the L-alanine copper complex under similar conditions in an alkaline solution, but with acetaldehyde.

In order to determine the change in the ratio of the D- and L-forms of alanine in the reaction mixture from sample to sample during the experiment, we measured the ratio of the areas of the D- and L-alanine peaks in the chromatograms. Figure 2 shows the rate of D-alanine formation under the conditions of the Akabori reaction (curve 1) and in the same alkaline medium but without the aldehyde (2); curve 3 represents the rate of D-alanine formation under more severe conditions than for curve 2, also in the absence of aldehyde.

The L-N, N-dimethylalanine was produced by room-temperature hydrogenation of a mixture of formaldehyde and L-alanine with a catalyst consisting of 10% Pd on activated charcoal [14]. The reaction required 50-55 h with an equimolar ratio of L-alanine and Pd and a twofold excess of formaldehyde. We checked for complete conversion of the alanine by paper chromatography and from the disappearance of coloration in the benzidine reaction [15]. After reduction was complete, the reaction mixture was heated almost to the boiling point, the catalyst was removed by hot filtration, and the solution was vacuum-evaporated. The resultant oily yellow mass was reprecipitated from absolute ethanol with absolute ether. We obtained slightly yellowish, very hygroscopic crystals with a melting point of 200-203°. Observed: 11.59% N. $C_5H_{11}NO_2$. Calculated: 11.96% N.

CONCLUSIONS

1. The Akabori reaction involves a stage in which an intermediate imine copper complex is formed from acetaldehyde and copper alaninate.

2. The intermediate complex has a higher CH acidity than the initial alanine copper complex.

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