BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 40 2826—2829 (1967)

Reactions of Metal Chelates. I. The Reactions of Copper(II) Chelates of Some β-Diketones and Their Related Compounds with Glycine, Diglycine and Triglycine

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(Received June 19, 1967)

The reactions of copper(II) chelates of β-diketones such as acetylacetone, benzoylacetone, dibenzoylmethane and benzoylacetaldehyde and of chelates of their related compounds such as salicylaldehyde and 2-hydroxyacetophenone with glycine or glycylglycine were investigated. Only bis(salicylaldehydato)copper(II) and bis(benzoylacetaldehydato)copper(II) reacted with glycine to form the respective glycinato-Schiff base complexes, whereas the other chelates did not react with glycine under the same condition. On the other hand, all the chelates except bis(dibenzoylmethanato)copper(II) reacted with glycylglycine to form the respective glycylglycinato-Schiff base complexes. The reaction at pH 4.5 between bis(salicylaldehydato)copper(II) and triglycine resulted in the hydrolytic cleavage of peptide bond, producing the salicylideneglycylglycinatocuprate(II) and free glycine. As the results of this investigation, five new copper(II) chelates were synthesized and characterized.

In an earlier paper from this laboratory it was reported that bis(salicylaldehydato)copper(II) reacted readily with glycine or glycylglycine to form salicylidene-glycinato- or -glycylglycinato-chelate, respectively.[1] In an attempt to find out a convenient metal chelate by which the hydrolytic cleavage of peptide bonds is successfully achieved, the same type of reactions of a series of copper(II) chelates of β-diketones and their related compounds were investigated as the preliminary work. Consequently, it has been disclosed that there exist distinct differences between the reactivities of those chelates. The copper(II) chelates employed in this work were of acetylacetone (I), benzoylacetone(II), dibenzoylmethane (III), benzoylacetaldehyde (IV) salicylaldehyde (V), 2-hydroxyacetophenone (VI) and of ethylacetoacetate (VII). Among these, only aldehyde chelates, IV and V, reacted readily with glycine to form the respective glycine-Schiff base complexes, whereas the ketone chelates did not react with glycine under the same condition. In a more severe condition, I, II and VI disclosed the tendency to give the bis (glycinato)-copper(II) which was supposed to be the resultant compound of the substitution reaction; even in such a condition, however, no glycine-Schiff base complex was obtained, showing a marked contrast with the reactivity of IV and V. No essential difference was, on the other hand, observed in the cases of the reactions of glycylglycine with those copper(II) chelates, I, II, IV, V and VI. The reaction products here were the respective glycylglycine-Schiff base chelates. Differing from those described above, the copper(II) chelate of dibenzoylmethane, III, reacted neither with glycine nor with glycylglycine at all. Further the copper-(II) chelate of ethylacetoacetate, VII, was substituted easily both by glycine and glycylglycine to give the bis(glycinato)copper(II) and the glycylglycinatoaquocopper(II), respectively.

All the results are summarized in Fig. 1. Inspection of Fig. 1 reveals that there are distinct differences in the reactivities of the copper(II) chelates depending upon the structure of the adjacent group of the carbonyl >C=O in coordination. Figure 1 implies that the reactivity to form the respective Schiff base chelates decreases in the order –H, $-CH_3$, $-C_6H_5$. Though it is not always easy to explain this order, the finding itself may be useful in understanding the non-enzymatic transamination reaction catalyzed by metals and pyridoxal, which is quite similar to the salicylaldehyde. In that reaction the function of the aldehyde group in the pyridoxal molecule has so far been elucidated only from its Schiff base formation with amino acids. Accordingly, there has been no positive reason why it must not be a ketone instead of aldehyde. However, we have now pertinent reason that the metal chelate capable of forming Schiff base complexes with amino acids are only aldehyde chelates. Biological significance of the aldehyde group in the pyridoxal molecule, which has been well-known as the coenzyme of the various transaminases might be understood in such a way.

As the result of this investigation five new copper(II) chelates have been prepared (VIII, X, XI, XII and XIV). Among those only VIII

1) A. Nakahara, This Bulletin, **32**, 1195 (1959).

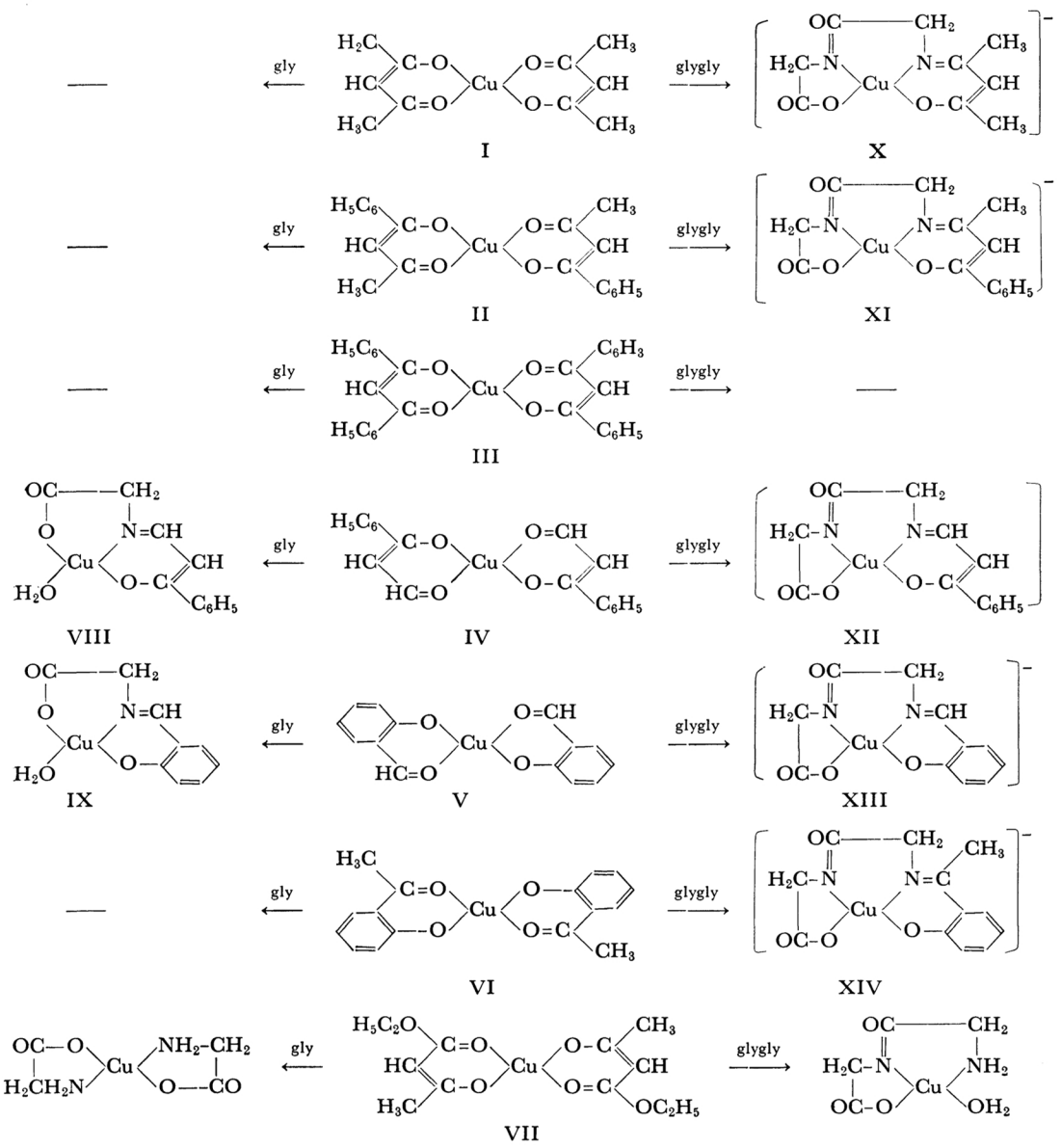
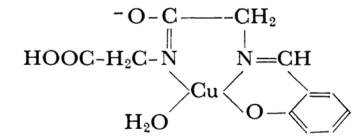


Fig. 1. The reactions of copper(II) chelates of β-diketones and their related compounds with glycine and glycylglycine: —— indicates that no corresponding reaction occurs.

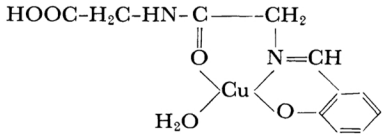
appears green and is a glycine-Schiff base chelate, in which the Schiff base is probably coordinated around copper(II) as a terdentate ligand as that in *N*-salicylideneglycinatoaquocopper(II).[1,2] All the other chelates, X, XI, XII and XIV, appear blue- or red-purple and are glycylglycine-Schiff base chelates, in which the respective Schiff base ligands are probably coordinated around copper(II) as quadridentate ligands as that in *N*-salicylideneglycylglycinatocuprate(II).[1] The glycylglycine-Schiff base chelates are fairly stable in the crystalline state, do not decompose below 220°C and are capable of being recrystallized from water without hydrolytic decompositions. The glycine-Schiff base chelate is, on the other hand, not very stable, being decomposed at 187°C and more or less accompanied by hydrolytic decomposition when it is treated in hot aqueous media. Although the bluish purple aqueous solution of a glycylglycine-Schiff base chelate becomes blue or even green upon the addition of dilute acetic acid, it is not supposed as due to an entirely irreversible change in the structure, since the original

2) G. L. Eichhorn and N. D. Marchand, *J. Am. Chem. Soc.*, **78**, 2688 (1956).

purple color is mostly recovered by readjusting the pH of the solution. The color change may be explained by taking into account a little change at the fifth or sixth coordination site of the copper(II). However, there is also a possibility of occurring a partial dissociation of the glycylglycine-Schiff base ligand as illustrated in XV or XVI.



XV



XVI

The hydrolytic cleavage of the peptide bond in glycylglycine moiety, which is observed when the chelate XIII is treated at low pH, and especially at high temperature, is understood from the structure XV or more likely from XVI.

The reactions between bis(salicylaldehydato)-copper(II), V, and triglycine have also been investigated. In this case the reaction products are the *N*-salicylideneglycylglycinatocuprate(II) and free glycine, suggesting that triglycine is hydrolyzed into glycylglycine and glycine. The coordination compounds which hydrolyze amino acid residues from peptide chains through a mechanism involving chelation of the terminal amino acid was already reported by Collman *et al.*[3,4] Those complexes were the *cis*-hydroxyaquobis(ethylenediamine)cobalt(III) and β-hydroxyaquotriethylenetetraminecobalt(III). The peptide hydrolyses carried out by those complexes were reported to be specific for *N*-terminal amino acid residues. On the other hand, the hydrolytic reaction by the present complex, V, might be considered to be specific for *N*-terminal dipeptide residues. The direct evidence for this consideration will be given, for instance, from a similar hydrolytic reaction of alanylglycylglycine or of glycylglycylalanine instead of triglycine. This work is now under way.

Experimental

The Preparation of Copper(II) Chelates of β-Diketones and Their Related Compounds. The bis(acetylacetonato)copper(II), I, bis(benzoylacetonato)-copper(II), II, bis(dibenzoylmethanato)copper(II), III, bis(benzoylacetaldehydato)copper(II), IV, bis(salicylaldehydato)copper(II), V, bis(2-hydroxyacetophenonato)copper(II), VI, and bis(ethylacetoacetato)copper-(II), VII, were prepared from copper(II) acetate and the respective carbonyl compounds in aqueous ethanol, and purified by recrystallization from ethanol, chloroform, acetone or other ordinary organic solvents. The respective chelates were identified on the basis of the elemental analyses and their infrared spectra.

The Preparation of the Copper(II) Chelate, VIII. To a 6 g of finely powdered bis(benzoylacetaldehydato)copper(II) in 100 m*l* of ethanol was added 1.2 g of glycine in 50 m*l* of water. The mixture was stirred and heated at about 65°C for about one hour; hereupon, a dark green solution was obtained. After it had been filtered and cooled, small green platelets were deposited from the filtrate. These were filtered by suction, and were recrystallized from water. Mp 187—188°C (decomposition). The elemental analysis of the dehydrated complex was as follows:

Found: C, 49.72; H, 3.54; N, 5.10%. Calcd for $[Cu(C_{11}H_9NO_3)]$: C, 49.53; H, 3.38; N, 5.25%.

The Copper(II) Chelate, IX. The method of preparation of this complex was already reported in a previous paper,[1] but in this work the compound was obtained as the resultant compound of the reaction between bis(salicylaldehydato)copper(II) and glycine, in an attempt to compare its reactivity with that of other β-diketone-copper(II) chelates. The procedure was exactly the same as that in the preparation of VIII. The complex was identified on the basis of the elemental analysis and its infrared spectrum.

The Copper(II) Chelate, X. To a one-to-one mixture of bis(acetylacetonato)copper(II) and glycylglycine hydrochloride in aqueous ethanol (one-to-two by volume mixture) was slowly added an equimolar amount of sodium carbonate. The reaction mixture was then stirred and heated at about 65°C for eight hours. After it had been cooled, the unreacted insoluble bis(acetylacetonato)copper(II) was separated by filtration. Then the filtrate was concentrated *in vacuo* to give a purple crystalline substance. This was recrystallized from water by adding a large quantity of acetone. The final pure crystal appeared to be purple needles and decomposed at 250°C.

Found: H_2O, 21.90%. Calcd for $Na[Cu(C_9H_{11}N_2O_4)]\cdot 4.5H_2O$: H_2O, 21.40%.

The analytical data for the dehydrated complex were as follows:

Found: C, 36.03; H, 3.91; N, 9.34%. Calcd for $Na[Cu(C_9H_{11}N_2O_4)]$: C, 36.30; H, 3.69; N, 9.41%.

The Copper(II) Chelate, XI. The same procedure as that employed in the preparation of X was applied in this case. However, the alkali used here was not sodium carbonate but potassium carbonate. The final pure complex appeared to be purple needles and decomposed at 244°C. The elemental analysis was performed as to the dehydrated compound.

Found: C, 45.27; H, 3.96; N, 7.48%. Calcd for $K[Cu(C_{14}H_{13}N_2O_4)]$: C, 44.73; H, 3.46; N, 7.46%.

The Copper(II) Chelate, XII. Exactly the same procedure as that in the preparation of XI was applied in this case. However, since the bis(benzoylacetaldehydato)copper(II) was more reactive than the other copper(II)-diketone chelates, the reaction was completed within one hour. The recrystallization of the complex was attained by using water and ethanol. Purple needles, mp 222°C (decomposition).

3) J. P. Collman and D. A. Buckingham, *ibid.*, **85**, 3039 (1963).

4) D. A. Buckingham, J. P. Collman, D. A. R. Happer and L. G. Marzilli, *ibid.*, **89**, 1082 (1967).

Found: C, 41.09; H, 3.37; N, 7.92%. Calcd for $K[Cu(C_{13}H_{11}N_2O_4)]\cdot H_2O$: C, 41.10; H, 3.42; N, 7.38%.

The Copper(II) Chelate, XIII. The method of preparation was reported in the previous paper.[1] However, the compound was obtained through the reaction between bis(salicylaldehydato)copper(II) and glycylglycine according to the direction employed in the preparation of XII. The complex was identified on the basis of the elemental analysis and its infrared spectrum.

The Copper(II) Chelate, XIV. The same procedure as that employed in the preparation of XI was applied for preparing this compound. The crystal appeared to be blue-purple needles and decomposes at 326°C. The elemental analysis of the dehydrated compound was as follows:

Found: C, 40.78; H, 3.10; N, 8.06%. Calcd for $K[Cu(C_{12}H_{11}N_2O_4)]$: C, 41.19; H, 3.14; N, 8.01%.

The Reaction between the Copper(II) Chelates, I, II, III, VI and VII with Glycine. The reaction of these chelates with glycine did not give the respective glycine-Schiff base chelates, though the almost same procedure as that described in the preparation of VIII was applied. The unreacted respective β-diketone-chelates were recovered. In a more severe condition (for example, the reaction at 75°C), I, II and VI disclosed the tendency to give the bis(glycinato)copper(II) instead of the respective glycine-Schiff base chelates. The chelate VII gave, on the other hand, easily the bis(glycinato)copper(II) even in a relatively mild condition. The identification of bis(glycinato)copper-(II) and the respective unreacted chelates was attained on the basis of the respective infrared spectra.

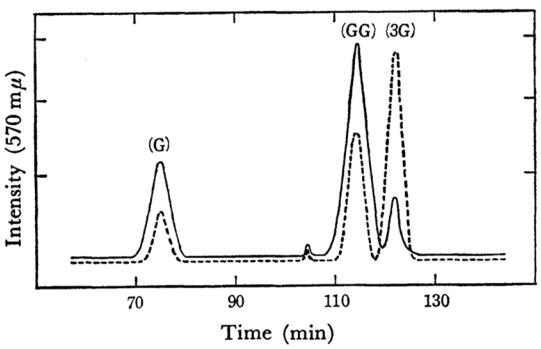


Fig. 2. The results of analyses of glycine (G), glycylglycine (GG) and triglycine (3G): ----, the standard sample of G, GG and 3G; ——, the reaction products of triglycine with bis(salicylaldehydato)copper(II).

The Reaction between the Copper(II) Chelates, III and VII with Glycylglycine. The reaction of these chelates with glycylglycine resulted in the recovery of III, or the formation of glycylglycinatoaquocopper(II) under the same condition as employed in the preparation of X. The resultant compounds were respectively identified by their infrared spectra.

The Reaction between Bis(salicylaldehydato)-copper(II) and Triglycine. A two-to-one mixture of bis(salicylaldehydato)copper(II) and triglycine in aqueous ethanol (one-to-one by volume mixture) was adjusted to pH 4.5. The mixture was then stirred and heated at about 65°C for three hours. After it had been cooled, hydrogen sulfide was bubbled through the mixture until all the copper(II) precipitated as copper-(II) sulfide. This was then filtered and diluted, and was analyzed by an amino acid analyzer. A typical result recorded by an amino acid analyzer (Yanagimoto LC-5) is illustrated in Fig. 2. The validity of the assignment of peaks for glycine, diglycine and triglycine were confirmed by using the respective standard samples. Thus the present result indicates that bis(salicylaldehydato)copper(II) is effective for the hydrolytic cleavage of peptide bonds. Though the same reaction was investigated in the absence of bis(salicylaldehydato)-copper(II), no remarkable hydrolysis was observed.

A part of the expenses for this work was defrayed by a grant from the Ministry of Education, to which the authors' thanks are due.