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The Stability of Fused Rings in Metal Chelates. VII. Copper(II) Complexes of Schiff Bases Derived from 2-Acetylpyridine or Pyridine-2-aldehyde and Dipeptides Containing Glycine, β-Alanine and/or α-Aminoisobutyric Acid

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Eight new copper(II) complexes of Schiff bases derived from 2-acetylpyridine or pyridine-2-aldehyde and dipeptides containing glycine, β-alanine and/or α-aminoisobutyric acid have been prepared, and their relative stability was discussed on the basis of numerical data obtained by spectrophotometric, polarographic and other measurements. As a result, it was found that the stability of fused-ring systems decreases in the order 5-5-6-≃5-6-5->5-5-5->5-6-6-system (the first number represents the number of ring members containing pyridine derivatives and the second and third numbers represent those of ring members containing dipeptide moieties). The conclusion proposed previously that Schiff base chelates of aldehydes are generally less stable than those of corresponding ketones, has been proved to be valid also for the copper(II) chelates. It was found that transamination reactions take place in the copper(II) chelates of Schiff bases with pyridine-2-aldehyde and glycylglycine or glycyl-β-alanine.

In a previous paper,[1] it was pointed out that a fused-ring system with two six-membered rings is less stable than that with one six- and one five-membered ring or two five-membered rings in the case of copper(II) chelates of dipeptides. We also investigated the relative stability of copper(II) chelates with various multidentate aldehyde- and ketone-Schiff bases.[2,3]

As an extension of the investigations dealing with the relative stability of metal-multidentate-chelates, the isolation of eight copper(II) complexes of Schiff bases derived from 2-acetylpyridine or pyridine-2-aldehyde and dipeptides containing glycine and/or β-alanine have been performed. Structural formulae of these compounds are illustrated in Fig. 1, (I—VIII). This paper describes the preparations and the relative stabilities of the metal chelates. The temperature of decomposition, absorption maxima for the *d-d* transition band in aqueous solution and the half-wave potentials for the reduction of copper(II) at the dropping mercury electrode of the chelate compounds are tabulated in Tables 3 and 4. The relative stability of copper(II) chelates has been determined on the basis of the numerical data.

We have found that there occur transamination reactions in pyridine-2-aldehyde-glycylglycine- and pyridine-2-aldehyde-glycyl-β-alanine-Cu(II) systems. The correlation between the transamination reaction and the structure of fused-ring system is also described.

Copper(II) Complexes of Schiff Bases derived from 2-Acetylpyridine and Glycylglycine, Glycyl-β-alanine, β-Alanylglycine or β-Alanyl-β-alanine(I—IV). The new compounds have been prepared by the ordinary reaction[2-4] between equimolar amounts of each components. They have been concluded to have the structures, I—IV on the basis of analytical data and the quadricoordinating character of copper(II) complexes. As is clear from their structural formulae, the central copper(II) links, in all cases, to the Schiff base ligand through the pyridine-nitrogen, Schiff base-nitrogen, peptide-nitrogen and carboxylate-oxygen, forming copper(II) tetradentate chelates. Thus the four chelates I—IV differ from one another only in the size of ring containing the peptide moieties. The skeletal structure of I consists of 5-5-5-*1 membered fused rings, and in the same way II, III and IV consist of 5-5-6-, 5-6-5- and 5-6-6-membered fused rings, respectively. Table 3 shows that the polaro-

1) O. Yamauchi, Y. Hirano, Y. Nakao and A. Nakahara, *Can. J. Chem.*, **47**, 3441 (1969).

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3) Y. Nakao, N. Nonagase and A. Nakahara, *ibid.*, **42**, 452 (1969).

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*1 The abbreviation 5-5-5 indicates the fused-chelate-ring structure containing 5,5 and 5-membered rings in a counter-clockwise direction in the expression of structural formula I.

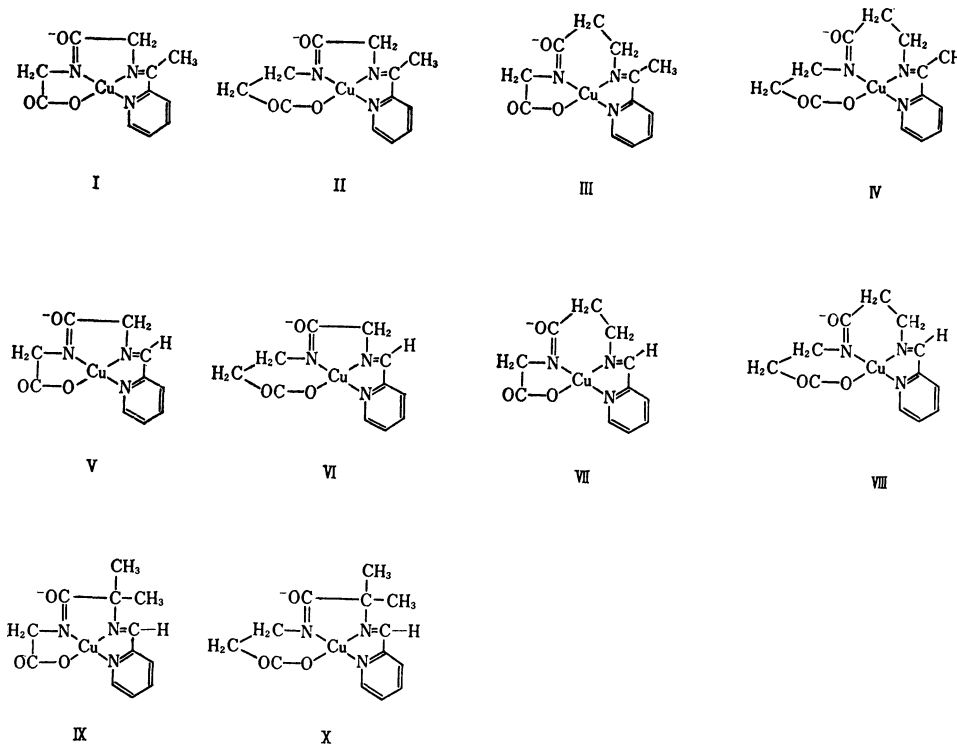

Fig. 1. Structural formulae of copper(II) chelates of aldehyde- and ketone-Schiff bases.

graphic half-wave potentials for the reduction of copper(II) in all the chelates(I—IV) are nearly independent of pH in the region 6–9.5. Only at about pH 11, the half-wave potential becomes slightly more negative. It can also be seen from Table 3 that there is no remarkable difference between the half-wave potential of chelates, II and III. On the other hand, the half-wave potential of I is a little less negative and that of IV is even less negative compared with those of II and III. It may be concluded from the above polarographic data that the stability of chelates I–IV decreases in the order II(5-5-6)≃III(5-6-5)> I(5-5-5)>IV(5-6-6). The numerical data for the decomposing temperatures and the maxima of absorption bands due to the ligand field splitting of *d*-orbitals also support the order in the stability of chelates I—IV. In the case of the copper(II) chelates of dipeptides (apart from the Schiff base chelates), copper(II) glycylglycinate is determined to be most stable[1]; but in complexes I—IV, copper(II)-2-acetylpyridine-glycylglycine fused-chelate ring system is less stable than either 2-acetylpyridine-glycyl-β-alanine- or -β-alanylglycine-Cu(II) systems. This is considered as due to the increasing strain in the 5-5-5-fused ring system.[4] It is of interest that the chelates produced from copper(II), 2-acetylpyridine and β-alanyl-β-alanine can easily be isolated as stable crystals, whereas the copper(II) β-alanyl-β-alaninate cannot be isolated.[1]

Copper(II) Complexes of Schiff Bases derived from Pyridine-2-aldehyde and Glycylglycine, Glycyl-β-alanine, β-Alanylglycine or β-Alanyl-β-alanine(V—VIII). Preparation of copper(II) complexes of Schiff bases containing pyridine-2-aldehyde instead of 2-acetylpyridine (V—VIII) was carried out. Isolation in the crystalline state was achieved only in cases of chelates with pyridine-2-aldehyde-β-alanylglycine(VII) and pyridine-2-aldehyde-β-alanyl-β-alanine (VIII). The half-wave potentials for the reduction of copper(II), temperatures of decomposition and absorption maxima in the visible region of the chelates VII—VIII are shown in Tables 3 and 4, from which a similar conclusion as in the preceding section is easily obtained: VII(5-6-5)>VIII(5-6-6). It can also be concluded from the comparison of III and VII, or IV and VIII, that if the skeletal structure is the same, ketone-Schiff base chelates are more stable than the corresponding aldehyde-Schiff base chelates(III>VII; IV>VIII). Apart from the relative stability of fused-chelate-rings, it has been found that transamination reactions take place as shown in Fig. 2 in the cases of copper(II) chelates with pyridine-2-aldehyde-glycylglycine- or pyridine-2-aldehyde-glycyl-β-alanine. Transamination has been observed by paper chromatographic test of the reaction solutions containing copper(II), pyridine-2-aldehyde, and glycylglycine or glycyl-β-alanine. Existence of 2-aminomethylpyridine in the paper chromatogram was distinctly observed

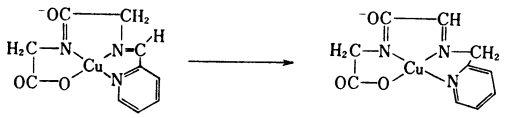

Fig. 2. The transamination reaction of pyridine-2-aldehydeglycine-Cu(II) system.

in the above reaction mixture as is clear from Fig. 2. On the other hand, no transamination was observed in the reaction solution for VII and VIII. Difficulty in isolating complexes V and VI might be elucidated by taking this tendency in transamination reaction into account. Investigation of transamination reactions between oligopeptides and various aldehydes is now under way.

Copper(II) Complexes of Schiff Bases derived from Pyridine-2-aldehyde and α-aminoisobutyrylglycine or α-Aminoisobutyryl-β-alanine(IX—X). In order to isolate copper(II) chelate which consists of 5-5-5- or 5-5-6-fused-ring system and at the same time possesses pyridine-2-aldehyde as carbonyl moiety in Schiff base ligand, the syntheses of pyridine-2-aldehyde-α-aminoisobutyrylglycine- and pyridine-2-aldehyde-α-aminoisobutyryl-β-alanine-copper(II) complexes, IX and X, have been carried out. As is clear from structural formulae IX—X, the transamination reaction cannot be expected in these systems. Thus it was possible to obtain both the chelates in crystalline state. Some properties of the compounds are tabulated in Tables 3 and 4. From the numerical data, it is concluded that X is a little more stable as compared to IX(X(5-5-6)> IX(5-5-5)). The conclusion is consistent with the result obtained through a comparison of I and II.

Experimental*2

Preparation of dipeptides. *Glycylglycine* and *glycyl-β-alanine* were prepared according to the diketopiperazine method[5,6] and chloroacetyl chloride method,[7] respectively.

Carbobenzoxy-β-alanine and -α-Aminoisobutyric Acid. There are several papers on the preparation of these compounds.[8,9] The present procedure is a slightly modified one by Winitz *et al.*[10] One-fifth mole each of β-alanine or α-aminoisobutyric acid were dissolved in 100 m*l* of 2N sodium hydroxide solution. To this was alternately added 52 m*l* of 4N NaOH and 34 m*l* of carbobenzoxy chloride over a period of 30—40 min, under constant stirring and cooling in an ice bath. After completion of the reaction, the reaction mixtures were extracted with ether and the aqueous fraction acidified with 6N HCl to pH 2—3. This was further extracted by ethyl acetate; the ethyl acetate fraction was dried over anhydrous magnesium sulfate. The filtrates were concentrated *in vacuo* to crystalline or oily residues. Recrystallization was carried out by using ethyl acetate and petroleum benzine.

β-Alanine Benzyl Ester p-Toluenesulfonate was prepared from β-alanine, benzyl alcohol and *p*-toluenesulfonic acid monohydrate according to the direction of Izumiya and Makisumi.[11]

Glycine Benzyl Ester p-Toluenesulfonate[12] was prepared by a similar procedure as that for the β-alanine derivative, except that chloroform was used as the solvent. The four materials described above were identified by comparing their melting points with those in literature.

Carbobenzoxy-β-alanylglycine Benzyl Ester. This was prepared in a similar procedure as that reported by Winitz *et al.*[10] To a solution of 15.6 g of carbobenzoxy-β-alanine, 23.6 g of glycine benzyl ester *p*-toluenesulfonate and 9.8 m*l* of triethylamine in 160 m*l* of chloroform was added 14.5 g of dicyclohexylcarbodiimide. The reaction mixture was stirred at 25°C overnight. After removal of white precipitated dicyclohexylurea, the filtrate was washed with water, dilute HCl, saturated $NaHCO_3$ aqueous solution and water. After the chloroform fraction had been dried over anhydrous magnesium sulfate, the filtrate was evaporated to dryness. The crystalline materials obtained were recrystallized from ethyl acetate and petroleum benzine. mp 107—109°C. Found: C, 64.57; H, 5.98; N, 7.97%. Calcd for $C_{20}H_{22}O_5N_2$: C, 64.84; H, 6.00; N, 7.56%.

Carbobenzoxy-β-alanyl-β-alanine Benzyl Ester. This was prepared from carbobenzoxy-β-alanine and β-alanine benzyl ester *p*-toluenesulfonate in the same way as described for carbobenzoxy-β-alanylglycine benzyl ester. mp 99—100°C.

Found: C, 65.96; H, 6.38; N, 7.32%. Calcd for $C_{21}H_{24}O_5N_2$: C, 65.60; H, 6.30; N, 7.29%.

Carbobenzoxy-α-aminoisobutyrylglycine Benzyl Ester and Carbobenzoxy-α-aminoisobutyryl-β-alanine Benzyl Ester were also prepared by the same method as described above. Z-α-NH_2-isoBu-GlyOBz. mp 91—92°C.

Found: C, 65.57; H, 6.45; N, 7.32%. Calcd for $C_{21}H_{24}O_5N_2$: C, 65.60; H, 6.30; N, 7.29%. Z-α-NH_2-isoBu-β-AlaOBz. mp 102—104°C.

Found: C, 66.32; H, 6.70; N, 6.90%. Calcd for $C_{22}H_{26}O_5N_2$: C, 66.30; H, 6.59; N, 7.03%.

β-Alanylglylcine, β-Alanyl-β-alanine, α-Aminoisobutyrylglycine and α-Aminoisobutyryl-β-alanine were obtained by catalytic reduction of the corresponding carbobenzoxypeptide benzyl ester using palladium black catalyst. In all cases, methanol containing a small volume of glacial acetic acid was used as solvent. The peptides obtained by the procedure described above were re-

*2 Melting points and decomposing temperatures were determined on a micro melting point apparatus and uncorrected.

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TABLE 1. ANALYTICAL DATA FOR [Cu 2-acetpy=peptide]*

Compound	C, %		H, %		N, %	
	Calcd	Found	Calcd	Found	Calcd	Found
[Cu 2-acetpy=Gly·Gly]·H_2O	42.00	41.72	4.13	4.35	13.35	13.38
[Cu 2-acetpy=Gly·β-Ala]·3.5H_2O	38.55	38.61	5.39	5.49	11.24	11.44
[Cu 2-acetpy=β-Ala·Gly]·3.5H_2O	38.55	38.66	5.39	5.31	11.24	11.76
[Cu 2-acetpy=β-Ala·β-Ala]·3H_2O	41.22	41.07	5.55	5.71	11.10	10.99

* 2-acetpy = 2-acetylpyridine

TABLE 2. ANALYTICAL DATA FOR [Cu py-2-al=peptide]*

Compound	C, %		H, %		N, %	
	Calcd	Found	Calcd	Found	Calcd	Found
[Cu py-2-al=β-Ala·Gly]·3H_2O	37.66	37.64	4.89	4.87	11.98	12.27
[Cu py-2-al=β-Ala·β-Ala]·4H_2O	37.64	37.53	5.53	5.07	10.98	11.12
[Cu py-2-al=α-AIBA·Gly]·2.5H_2O	40.51	40.94	5.06	4.91	11.81	11.96
[Cu py-2-al=α-AIBA·β-Ala]·3H_2O	41.22	40.82	5.55	5.66	11.10	11.02

* py-2-al = pyridine-2-aldehyde, α-AIBA·Gly = α-aminoisobutyrylglycine

crystallized from water and ethanol. β-Alanylglycine, β-alanyl-β-alanine and α-aminoisobutyrylglycine were identified by means of elementary analysis and melting points. Melting point and analytical data of α-aminoisobutyryl-β-alanine are as follows: mp 209—212°C. Found: C, 47.75; H, 8.29; N, 15.80%. Calcd for $C_7H_{14}O_3N_2$: C, 48.25; H, 8.12; N, 16.08%.

Preparation of Metal Chelates. *Copper(II) Chelates of Schiff Bases derived from 2-Acetylpyridine and Dipeptides (I—IV).* To a mixture of 0.005 mol each of 2-acetylpyridine and dipeptide in 10 m*l* of aqueous ethanol(one-to-one volume mixture) was added 0.005 mol of copper(II) acetate monohydrate. The pH-values of each reaction solution were adjusted to 9—11 by using concentrated NaOH solution. The reaction mixture was then stirred at room temperature for half an hour. It was allowed to stand for a few hours in a refrigerator; hereupon crystals were deposited. The products were filtered and recrystallized from aqueous ethanol(seven-to-three by volume mixture). The results of elementary analyses are shown in Table 1.

Copper(II) Chelate of Schiff Base derived from Pyridine-2-aldehyde and β-Alanylglycine (VII). Into 10 m*l* of a water : ethanol mixture(one-to-one by volume) were dissolved 0.37 g of β-alanylglycine and 0.27 g of pyridine-2-aldehyde, and then 0.5 g of copper(II) acetate monohydrate. The reaction mixture was adjusted to pH 8—9 by sodium ethoxide, and was then kept at room temperature for an hour under constant stirring. Bluish violet crystals were precipitated from the reaction solution. After it had been filtered, recrystallization from aqueous ethanol(one-to-one by volume) was carried out. Analytical data are given in Table 2.

Copper(II) Chelate of Schiff Base derived from Pyridine-2-aldehyde and β-Alanyl-β-alanine (VIII). A mixture of 0.40 g of β-alanyl-β-alanine and 0.27 g of pyridine-2-aldehyde was dissolved in 8 m*l* of aqueous ethanol (one-to-one by volume). To the solution was added 0.5 g of copper(II) acetate monohydrate, and the pH was adjusted to about 8—9 by powdered sodium carbonate. After it had been stirred at room temperature for one hour, the reaction mixture was allowed to stand overnight in a desiccator equipped with phosphorous pentoxide; thereupon blue crystals were obtained. The precipitates were collected by filtration and washed several times with an ethanol : acetone mixture (four-to-one by volume). Analytical data are given in Table 2.

Copper(II) Chelate of Schiff Base derived from Pyridine-2-aldehyde and α-Aminoisobutyrylglycine(IX). To a solution of 0.64 g of α-aminoisobutyrylglycine in 6 m*l* of a water : ethanol mixture (1 : 1 by volume) were added 0.43 g of pyridine-2-aldehyde and 0.80 g of cupric acetate monohydrate. The resulting mixture was adjusted to pH 7—8 by sodium hydroxide solution and stirred at 25°C for half an hour. After filtration, the filtrate was allowed to stand in a desiccator equipped with silica gel under reduced pressure until syrupy material was obtained. The material was recrystallized from ethanol containing a small amount of water. Analytical data are given in Table 2.

Copper(II) Chelate of Schiff Base derived from Pyridine 2-aldehyde and α-Aminoisobutyryl-β-alanine (X). A similar procedure to that in the preparation of complex IX was adopted. Compound X was isolated in crystalline state over silica gel. The crystal was washed by a water : ethanol mixture (1 : 4 by volume) and then with acetone. The result of elementary analysis is shown in Table 2.

Transamination Study. The following procedure was employed to estimate the transamination reaction in the fused-ring systems of chelates. To a mixture of 0.0025 mol each of pyridine-2-aldehyde and the dipeptide in 10 m*l* of aqueous ethanol (1 : 1 by volume mixture) was added 0.5 g (0.0025 mol) of copper(II) acetate monohydrate. The reaction mixture was adjusted to pH 9—11 by sodium hydroxide solution and was stirred at 25°C for an hour. After it had been filtered, cupric ion was precipitated as sulfide and filtered. The filtrate was used as a sample solution for the paper-chromatographic test for which the solvent system, *n*-butanol : acetic acid : water (4 : 2 : 1) and Toyo filter paper No. 50 were used. Spots of 2-aminomethylpyridine were detected by spraying a ninhydrine

TABLE 3. HALF-WAVE POTENTIALS OF [Cu 2-acetpy=dipeptide] AND [Cu py-2-al=dipeptide]

	Complex	pH	Half-wave potential, *vs.* SCE, 25°C
I.	[Cu 2-acetpy=Gly·Gly]	6.5	−0.34 V.
		9.5	−0.34
		11.0	−0.36
II.	[Cu 2-acetpy=Gly·β-Ala]	6.5	−0.40
		8.0	−0.40
		10.5	−0.40
III.	[Cu 2-acetpy=β-Ala·Gly]	6.0	−0.37
		9.0	−0.38
		10.5	−0.41
IV.	[Cu 2-acetpy=β-Ala·β-Ala]	7.5	−0.32
		9.5	−0.32
		11.0	−0.34
VII.	[Cu py-2-al=β-Ala·Gly]	6.6	−0.31
		9.0	−0.32
		11.0	−0.35
VIII.	[Cu py-2-al=β-Ala·β-Ala]	7.1	−0.25
		9.4	−0.25
		11.0	−0.26
IX.	[Cu py-2-al=α-AIBA·Gly]	6.0	−0·32
		10.1	−0.32
X.	[Cu py-2-al=α-AIBA·β-Ala]	5.6	−0.36
		11.0	−0.35

TABLE 4. PROPERTIES OF COPPER(II) SCHIFF BASE COMPLEXES

	Complex	Appearance of crystals	Absorp. max. ν_{max} 10^{13} sec^{-1} (log ε)	Decomp. temp.
I.	[Cu 2-acetpy=Gly·Gly]	violet	52.2 (2.21)	158—166°C
II.	[Cu 2-acetpy=Gly·β-Ala]	violet	52.6 (2.20)	203—212
III.	[Cu 2-acetpy=β-Ala·Gly]	red-violet	53.3 (1.89)	205—215
IV.	[Cu 2-acetpy=β-Ala·β-Ala]	blue-violet	51.2 (1.89)	150—158
VII.	[Cu py-2-al=β-Ala-Gly]	blue-violet	50.7 (1.79)	190—200
VIII.	[Cu py-2-al=β-Ala·β-Ala]	grey-violet	49.6 (1.89)	150—190
IX.	[Cu py-2-al=α-AIBA·Gly]	violet	52.0 (2.26)	249—260
X.	[Cu py-2-al=α-AIBA·β-Ala]	violet	52.7 (2.23)	193—196 (melt)

solution.

Spectroscopic Measurements. Visible absorption spectra were obtained with a Shimadzu Spectrophotometer MPS-50L and QR-50 at room temperature. Water was used as a solvent. The concentration of the solutions was 5×10^{-3} M. The numerical data for absorption band are listed in Table 3.

Polarographic Measurements. Measurements were carried out by the same method as described previously.[2] Water was used as a solvent. Concentration of the copper(II) complexes was 5×10^{-4} M. The supporting electrolyte used was 0.1 M potassium nitrate. Triton X-100 (0.002%) was used as a maximum suppressor. The pH-values of each solution were adjusted by potassium hydroxide solution.

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