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Mixed Ligand Copper(II) Complexes of α-Amino Acids with Ligand-Ligand Interactions

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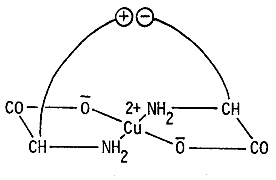
Synthetic and spectroscopic studies have been made on the ternary copper(II) complexes containing two optically active α-amino acids with charged groups in their side chains. Thus, for all combinations of ligands A and B, where A refers to aspartic or glutamic acid and B to arginine, lysine, or ornithine, the mixed ligand complexes, [Cu(L-A)(L-B)] and [Cu(D-A)(L-B)] except [Cu(L-aspartate)(L-ornithine)], were isolated as crystals. Infrared spectra of the isolated complexes suggested the presence of the geometrical isomers. By comparing the observed circular dichroism spectra of the ternary systems with the spectra estimated from those of the corresponding binary systems, the magnitude enhancements were detected for all the Cu(L-A)(L-B) systems, whereas no such enhancement was observed when one of the ligands was L-alanine or L-valine. The spectral behaviors as well as the solid complexes isolated have been interpreted as indicative of the existence of the intramolecular electrostatic bonding between the oppositely charged groups in the side chains of the ligands and their significance as an effective driving force leading to the ternary complex formation.

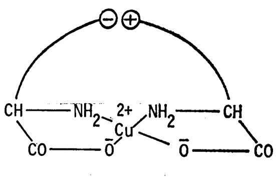
A mixed ligand metal complex is regarded as a simplified chemical model for the enzyme-metal-substrate complex formed in the course of the enzymatic reaction with a metal ion at the active center. As has been shown for the carboxypeptidase A–peptide complex by the X-ray studies,[1] the electrostatic bonding between the coordinated ligands is certainly one of the important driving forces leading to the formation of mixed ligand complexes. We reported in a previous paper[2] the synthetic and spectroscopic evidence, which suggests that such electrostatic or hydrogen bondings exist between the oppositely charged groups in the side chains of the two ligands in several copper(II)-containing ternary systems and that they affect the formation of mixed ligand complexes. Brookes and Pettit[3] recently showed by potentiometric studies that the ternary systems composed of copper(II), L- or D-histidine, and an amino acid with a positively charged side chain, such as L-arginine, L-lysine, and L-ornithine, exhibit appreciable stereoselectivity, which they ascribed to the electrostatic interaction between the positive group and the carboxylate oxygen of histidine in the systems with two L-ligands.

With a view to obtaining further information about the factors governing the mixed ligand complex formation and finding clues to the mechanism of the enzymatic reactions, we now extended the previous study to the copper(II) complexes of optically active α-amino acids and carried out synthetic and spectroscopic investigations on the possibilities of the electrostatic ligand-ligand interactions within the complex molecules. For the present purpose, we employed L- and D-glutamic acid (L- and D-Glu) and L- and D-aspartic acid (L- and D-Asp) as ligands with negatively charged side chains (referred to as A ligands hereinafter), and L-arginine (L-Arg), L-lysine (L-Lys), and L-ornithine (L-Orn) as ligands with positively charged side chains (referred to as B ligands). Based on the expected chelate structures **1** and **2**, which impose the steric and the mentioned electrostatic requirements on the formation of the ternary complex, Cu(A)(B), we attempted the isolation of the complexes with all combinations of A and B. The present paper deals with the synthesis of the chelates and the discussions of their structures and the intramolecular bondings.



Cu(L-A)(L-B)

1



Cu(D-A)(L-B)

2

Experimental

Materials. L-Arginine, L-lysine dihydrochloride, L-ornithine monohydrochloride, L- and D-glutamic acid, L- and D-aspartic acid, L-valine, and L-alanine were purchased from Nakarai Chemicals, Ltd. The optical purities of the enantiomers of glutamic and aspartic acids were checked by their specific rotations, $[\alpha]^{589}_{20}$, which were +11.2 and −10.9 (in H_2O) for L- and D-glutamic acid, respectively, and +25.1 and −22.1 (in 3M HCl) for L- and D-aspartic acid, respectively, indicating that the optical purity of D-aspartic acid was slightly lower.

All the chemicals used were of reagent grade or of highest grade available.

Preparation of Mixed Ligand Copper(II) Complexes. The complexes were prepared by essentially the same procedure as typically described below for [Cu(L-Glu)(L-Arg)]. Copper(II) perchlorate hexahydrate (1.85 g, 5 mmol), L-arginine (0.85 g, 5 mmol), and L-glutamic acid (0.74 g, 5 mmol) were dissolved in 30 ml of water, and the pH of the resulting solution was adjusted to *ca.* 9 with aqueous sodium hydroxide. The reaction mixture was stirred for 1 h at room temperature and concentrated *in vacuo* to a small volume at temperatures below 50 °C. Addition of methanol to the residue gave blue crystals, which were recrystallized from aqueous methanol–ethanol.

The pH values of the reaction mixtures containing L-ornithine or L-lysine as B ligands were adjusted to *ca.* 7.

The analytical data for the isolated complexes are shown in Table 1.

Measurements of Absorption and Circular Dichroism (CD) Spectra. The spectroscopic measurements were performed in water at room temperature for the systems with the Cu(II):

Table 1. Elemental analysis of mixed ligand copper(II) complexes of α-amino acids

Complex	Formula	Found (%)			Calcd (%)		
		C	H	N	C	H	N
[Cu(L-Glu)(L-Arg)]·2H_2O	$C_{11}H_{21}N_5O_6Cu\cdot 2H_2O$	31.45	5.64	16.77	31.54	6.02	16.72
[Cu(D-Glu)(L-Arg)]·H_2O	$C_{11}H_{21}N_5O_6Cu\cdot H_2O$	32.71	5.66	17.44	32.96	5.78	17.47
[Cu(L-Asp)(L-Arg)]·H_2O	$C_{10}H_{19}N_5O_6Cu\cdot H_2O$	30.77	5.59	18.17	31.05	5.47	18.11
[Cu(D-Asp)(L-Arg)]·1.5H_2O	$C_{10}H_{19}N_5O_6Cu\cdot 1.5H_2O$	30.10	5.47	17.49	30.34	5.60	17.69
[Cu(L-Glu)(L-Lys)]·1.5H_2O	$C_{11}H_{21}N_3O_6Cu\cdot 1.5H_2O$	34.52	5.97	11.19	34.59	6.34	11.01
[Cu(D-Glu)(L-Lys)]·H_2O	$C_{11}H_{21}N_3O_6Cu\cdot H_2O$	35.49	5.81	11.29	35.43	6.22	11.27
[Cu(L-Asp)(L-Lys)]·1.5H_2O	$C_{10}H_{19}N_3O_6Cu\cdot 1.5H_2O$	32.89	5·84	11.41	32.65	6.02	11.42
[Cu(D-Asp)(L-Lys)]·H_2O	$C_{10}H_{19}N_3O_6Cu\cdot H_2O$	33.51	5.79	11.90	33.66	5.90	11.71
[Cu(L-Glu)(L-Orn)]·2H_2O	$C_{10}H_{19}N_3O_6Cu\cdot 2H_2O$	32.05	5.63	11.28	31.87	6.14	11.15
[Cu(D-Glu)(L-Orn)]·2H_2O	$C_{10}H_{19}N_3O_6Cu\cdot 2H_2O$	31.85	5.71	11.45	31.87	6.14	11.15
[Cu(D-Asp)(L-Orn)]·2.5H_2O	$C_9H_{17}N_3O_6Cu\cdot 2.5H_2O$	28.92	5.39	11.34	29.07	5.97	11.30

A: B ratios of 1: 1: 1 (ternary systems) and 1: 2: 0 and 1: 0: 2 (binary systems) at pH 5—11 at a constant Cu(II) concentration of 5×10^{-3} M. The spectral samples were prepared from 0.1 M stock solutions of copper (II) perchlorate and the amino acids. The pH values, which were roughly adjusted with aqueous sodium hydroxide and dilute perchloric acid, were finally determined after the spectroscopic measurements. The ionic strength (μ) of the solutions was not adjusted at a constant value (μ=variable). Absorption spectra were recorded in the range 400—800 nm on a Union Giken SM-401 high sensitivity recording spectrophotometer. CD spectra were measured in a 1-cm path length quartz cell in the range 350—800 nm with a JASCO MOE-1 spectropolarimeter. Infrared spectra were obtained in the range 4000—650 cm^{-1} with a Hitachi EPI-S2 infrared spectrophotometer with the KBr disk method and in the range 700—200 cm^{-1} with a Hitachi EPI-L grating infrared spectrophotometer in the dry air with the Nujol mull method.

Results and Discussion

Preparation of Mixed Ligand Complexes. As shown in Table 1, all the expected ternary complexes except [Cu(L-Asp)(L-Orn)] were isolated as crystals in good yields. Although it was difficult to obtain pure crystals of [Cu(L-Asp)(L-Orn)], we may assume its formation from the analytical data.[4]

The structures of these complexes are expected from the space-filling models to be such as are expressed by **1** and **2**, which suggest that the (L-A)(L-B) pairs of ligands favor the *trans* structure around the central copper(II) ion (**1**), whereas the (D-A)(L-B) pairs prefer the *cis* structure (**2**). Both structures are more puckered and may be sterically less stable as compared with those without ligand-ligand interactions, but the synthetic results seem to indicate the existence of the electrostatic bondings, which supplement the steric disadvantage incurred. Although the complexes with pairs of A and B containing longer side chains, *e.g.* Glu–Arg and Glu–Lys, were isolated easily, the total length of the two side chains may not be the sole factor affecting the isolation, because [Cu(L-Glu)(L-Orn)] was more easily isolated than [Cu(L-Asp)(L-Lys)] which has the same side chain length as the former. The difficulty encountered in isolation of [Cu(L-Asp)(L-Orn)] with the shortest chain length nevertheless suggests that the chain lengths as well as the solubility and the *cis-trans* geometry around the copper(II) ion exert influence over the formation and crystallization of the complexes.

IR Spectra and cis-trans *Isomerism.* Figure 1 shows the IR spectra of the mixed ligand complexes in the range 4000—650 cm^{-1}. The spectral patterns in the fingerprint region are different from those of the corresponding binary complexes. Subtle spectral differences in this region are also detected between the Cu(L-A)(L-B) and Cu(D-A)(L-B) series with all combinations

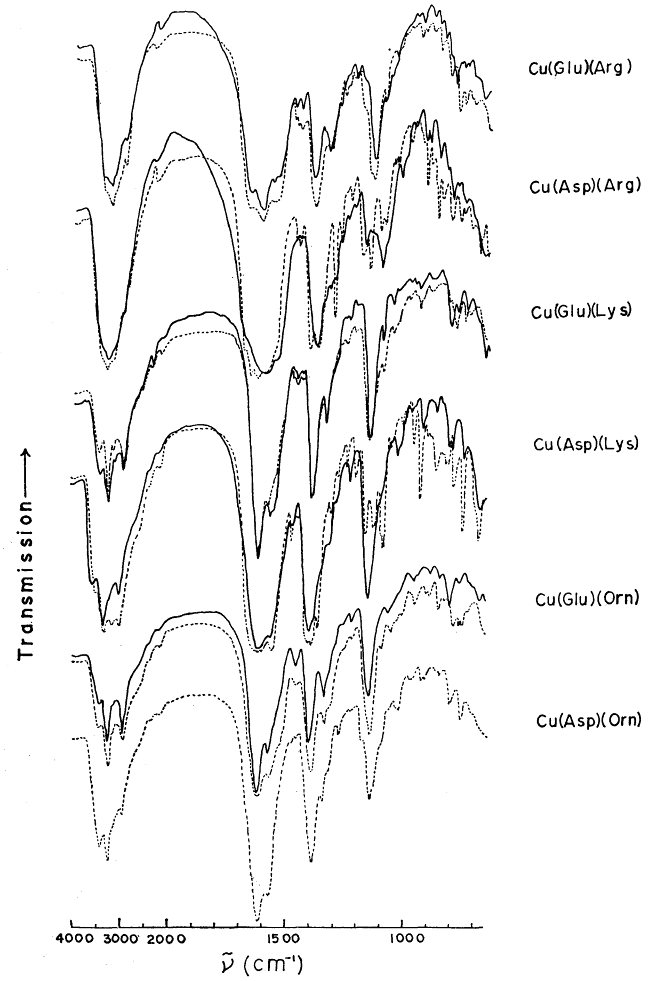


Fig. 1. Infrared spectra of copper(II) complexes in the range 4000—650 cm^{-1}.
Cu(L-A)(L-B)—; Cu(D-A)(L-B)---.

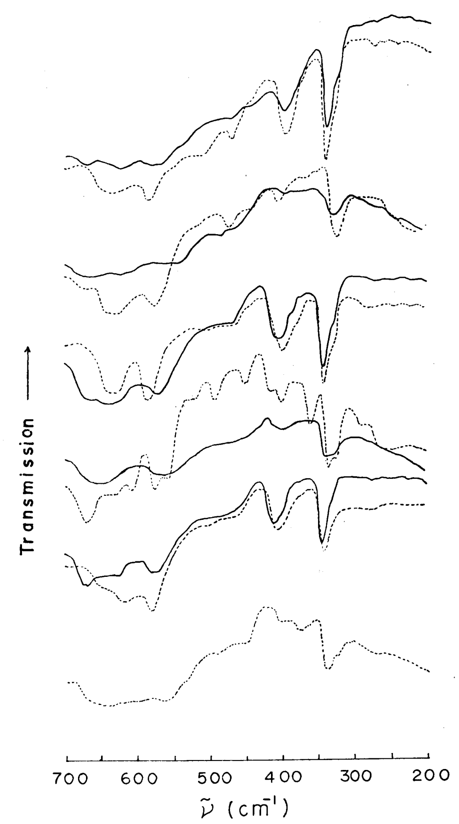


Fig. 2. Infrared spectra of copper(II) complexes in the range 700—200 cm^{-1}.
Cu(L-A)(L-B)—; Cu(D-A)(L-B)---.

of A and B except the Asp-Orn pair, which may reflect the structural differences as illustrated by **1** and **2**.

Geometrical isomerism has been known to give rise to characteristic bands due to metal-ligand bonds in the range below 700 cm^{-1}.[5] Thus, the spectral patterns of *cis*- and *trans*-[Cu(glycine)$_2$] in this region[6] are considerably different from each other. We see from Fig. 2 that at 700—500 cm^{-1} and 400—330 cm^{-1} the complexes with (L-A)(L-B) pairs give the spectra, which are typical of the A ligands and differ from those exhibited by the complexes [Cu(D-A)(L-B)]. This suggests the presence of the *cis-trans* isomers expressed by **1** and **2**, and since the side chains in these systems are comparable, the isomerism in turn points to the intramolecular ligand-ligand bonding. The stereoselectivity as observed for the histidine-containing ternary copper(II) complexes[3] would implicate the preference of one geometrical isomer over the other in the present ternary systems.

Absorption and CD Spectra and Interpretation of the CD Magnitudes. The CD spectra obtained for each of the systems at various pH values between 5 and 11 were satisfactorily reproducible. The spectral data are summarized in Table 2 and some examples of the curves are depicted in Figs. 3 and 4 (solid lines). The CD magnitudes of the ternary systems increase with pH, indicating that the mixed ligand complexes are the major species present at pH 9—10. The systems with L-Orn show marked spectral changes at pH>8 owing to the apical coordination by the δ-amino group,[2,7,8] while the other systems including those with Asp show

TABLE 2. RELATIVE CD MAGNITUDE IN WATER (μ=variable)[a)]

Complex	pH	$\Delta\varepsilon_{\max}$ (Found)	Relative magnitude	$\lambda_{\max}$ (nm) Found	$\lambda_{\max}$ (nm) Calcd[b)]
[Cu(L-Val)(L-Arg)]	7.2	0.19	1.0	590	590
	8.6	0.19	1.0	580—590	590
[Cu(L-Val)(L-Glu)]	7.4	0.19	1.0	600	600
	8.8	0.19	1.0	600	600
[Cu(L-Ala)(L-Arg)]	9.4	0.10	1.0	610	600
[Cu(L-Ala)(L-Glu)]	7.8	0.10	1.0	610	610
[Cu(L-Ala)(L-Asp)]	9.3	0.06	1.0	630	630
[Cu(L-Glu)(L-Arg)]	7.3	0.14	1.4	600	610
	8.5	0.15	1.3	600	600
[Cu(L-Glu)(L-Lys)]	7.3	0.13	1.2	600	610
	9.0	0.13	1.1	600	600
[Cu(L-Glu)(L-Orn)]	7.5	0.13	1.4	600	600
[Cu(L-Asp)(L-Arg)]	7.2	0.09	1.4	630	630
	9.5	0.09	1.3	630	630
[Cu(L-Asp)(L-Lys)]	7.2	0.08	1.2	640	630—640
	9.6	0.08	1.1	640—650	630—640
[Cu(L-Asp)(L-Orn)]	7.1	0.07	1.5	640	640

a) Relative CD magnitude refers to $\Delta\varepsilon_{\max}$ (found)/$\Delta\varepsilon_{\max}$ (calcd). b) The approximate maximum wavelength estimated from the CD spectral curves.

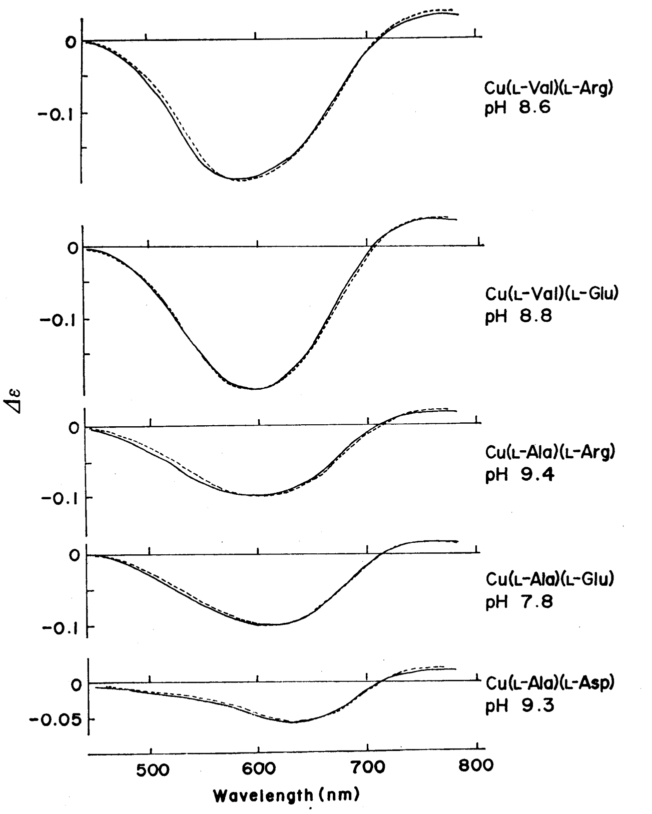


Fig. 3. Circular dichroism curves of ternary copper(II) systems without electrostatic ligand-ligand interactions.
Experimental curves:—, estimated curves:---.

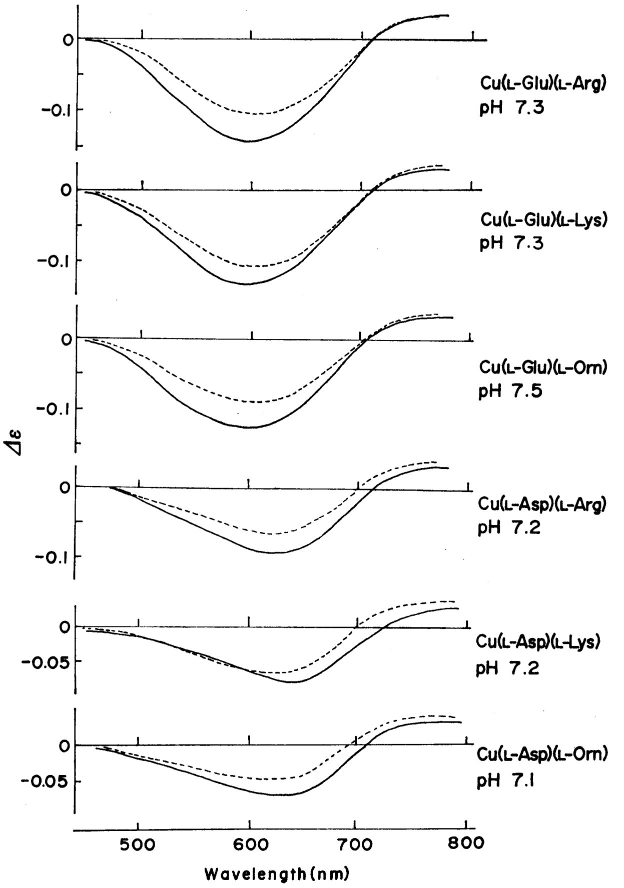


Fig. 4. Circular dichroism curves of ternary copper(II) systems containing ligands with oppositely charged side chains.
Experimental curves:—
Estimated curves:---.

moderate changes.

On the assumption that the CD magnitude is an additive function under the present conditions, we estimated the theoretical CD curves for the ternary systems (broken lines in Figs. 3 and 4) by summing up half the magnitude ($1/2\Delta\varepsilon$) of CuA$_2$ and of CuB$_2$ at every wavelength. As is apparent from Fig. 3, the L-Val- or L-Ala-containing ternary systems, where no intramolecular ligand-ligand interaction is expected, exhibit excellent fits of the calculated curves to the experimental ones. On the other hand, serious deviations of the curves are observed most remarkably for the systems with two L-ligands each of which have an oppositely charged side chain that can form intramolecular electrostatic bonds (Fig. 4). Since the spectra were obtained for 5×10^{-3} M solutions, these observations may imply that such discrepancies arise from the intramolecular bonds, which cause fixation of the complex structures and reduce the freedom of motion of the side chains. Regarding the Cu(D-A)(L-B) systems, the experimental curves do not deviate greatly from the expected ones. Because the absolute values of $\Delta\varepsilon$ for these systems are much smaller than those for the Cu(L-A)(L-B) systems and the deviations are roughly in the range of experimental errors, it is difficult to find any significant difference between them.

Inspection of the curve fit of the L-Orn-containing systems was made at pH 7—8, because, as mentioned earlier, they exhibit spectral changes at pH>8 due to apical coordination. These systems give anomalous CD curves which are difficult to interpret, and further investigations will be necessary.

The observed CD magnitudes may be explained by the hexadecant (D_{4h}) rule,[9,10] according to which the side chains of the L-ligands in [Cu(L-A)(L-B)] with the *trans* geometry (**1**) are located in the sectors with negative sign, and this configuration may contribute to the observed magnitude enhancement. Although the complex formation in the binary systems are supposed to be nearly complete at pH 9—10[11] from the pH dependence of the CD magnitude, the above argument is contingent on the species distributions in the ternary systems at equilibrium and a more quantitative comparison of the CD magnitudes should be made from the study of the solution equilibria.

The absorption spectral data in the visible region are

TABLE 3. ABSORPTION SPECTRAL DATA

Complex	pH	λ_{max} (nm)	ε	Remarks
[Cu(L-Glu)(L-Arg)]	5.2	670	36	
	7.3	625	57	
	8.5	622	56	
[Cu(D-Glu)(L-Arg)]	5.4	651	55	
	7.3	626	58	
	9.4	(620)[a]	(60)[a]	turbid
[Cu(L-Glu)(L-Lys)]	5.8	677	35	
	7.3	626	53	
	9.0	(622)	(56)	turbid
[Cu(D-Glu)(L-Lys)]	5.5	659	39	
	7.4	625	52	
	9.3	(623)	(56)	turbid
[Cu(L-Glu)(L-Orn)]	5.2	660	39	
	7.5	625	56	
	9.2	626	59	
[Cu(D-Glu)(L-Orn)]	5.5	643	44	
	7.8	620	57	
	9.3	626	59	
[Cu(L-Asp)(L-Arg)]	5.3	(622)	(40)	cryst[b]
	7.2	633	53	
	9.5	(630)	(55)	ppt[c]
[Cu(D-Asp)(L-Arg)]	5.5	(654)	(38)	cryst
	7.3	634	53	
	9.8	(641)	(54)	ppt
[Cu(L-Asp)(L-Lys)]	5.3	659	37	
	7.2	632	50	
	9.6	630	52	
[Cu(D-Asp)(L-Lys)]	5.7	651	40	
	7.4	633	49	
	9.7	(629)	(55)	turbid
[Cu(L-Asp)(L-Orn)]	7.1	630	51	
	9.4	630	54	
	10.8	634	56	
[Cu(D-Asp)(L-Orn)]	7.3	634	51	
	9.4	630	54	
	10.8	636	57	

a) Numbers in parentheses are approximate values.
b) Crystals separated. c) A precipitate was formed.

listed in Table 3, which indicates that the peaks of the ternary systems are between those of the corresponding binary systems. The maximum wavelengths and the ε values do not explicitly reflect the effects of the ligand-ligand interactions and the differences due to L- and D-enantiomers, although there are observed slight disagreements in the shapes of the curves. This may not be unexpected, because all the chelate rings of the systems in consideration have the same structures around the copper(II) ion and the ε values of the absorption spectra are more than a hundred times greater than the $\Delta\varepsilon$ values of the CD spectra.

As regards α-amino acids as ligands, only a small number of mixed ligand copper(II) complexes have so far been reported,[12] and we may take this as an indication that isolation of such labile complexes is not always feasible under normal conditions but requires additional factors favoring or stabilizing ternary systems. The present results of the synthesis and the relevant CD spectral information strongly suggest the existence of the ligand-ligand bonding and lead us to the conclusion that such intramolecular bondings serve as an effective driving force for the mixed ligand complex formation.

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