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Crystal Structures of Mixed Ligand Copper(II) Complexes Containing L-Amino Acids. III. Aqua(L-histidinato)(L-serinato)copper(II) Trihydrate

Yoshio SASADA,* Akio TAKENAKA, and Toshio FURUYA†

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227

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The copper(II) complex containing L-histidine and L-serine crystallizes from an aqueous solution. Crystals of aqua(L-histidinato)(L-serinato)copper(II) trihydrate are monoclinic, space group $P2_1$, $a=11.046(2)$, $b=7.315(1)$, $c=9.737(1)$ Å, $\beta=101.34(2)°$ and $Z=2$. A square-planar coordination around copper consists of α-amino nitrogen and δ-nitrogen atoms in histidine and α-amino nitrogen and α-carboxyl oxygen atoms in serine, the two amino groups being in *cis* position. The fifth coordination site is occupied by a water molecule, and the carboxyl oxygen atom of serine in the adjacent complex makes a weak bond with copper to complete a distorted octahedron. The chelating scheme and overall crystal structure are the same as those of (L-alaninato)aqua(L-histidinato)copper(II) trihydrate (**1**). Crystallization water molecules are situated on the fixed positions in the void space surrounded by the columns of the complexes, but the hydroxyl group of serine takes two sites randomly. The common chelating scheme in the present crystal and **1** is in correspondence with the observation that serine and alanine are of moderate cooperativity with histidine in forming the ternary complexes.

Although most of the copper ions in human serum are bound unexchangeably to ceruloplasmin, the remaining copper ions (10^{-6} mol dm^{-3}) form complexes with albumine, some amino acids and others in complicated equilibria. Binary and ternary complexes, such as L-glutaminatocopper(II) and (L-histidinato)(L-threoninato)copper(II), can be separated by chromatography, and are supposed to be mediators in copper transport between blood and tissues.[1] Neumann and Sass-Kortsak[2] suggested that asparagine, glutamine, and threonine exhibit significant cooperativity with histidine in forming ternary complexes, while alanine, valine, serine, and glutamic acid have weaker effects. Computer simulation also indicated that the content of copper complexes containing histidine is relatively high, although some details are controversial.[3,4] In order to establish the structural basis of these observations, several attempts to synthesize ternary complexes and to determine their geometry have been made, but only a few has been successful.[5]

We have previously reported the structures of (L-asparaginato)(L-histidinato)copper(II) and its hydrate[6] and also (L-alaninato)aqua(L-histidinato)copper(II) trihydrate,[7] (hereafter these complexes are abbreviated as, *e.g.*, Cu(asn his) by using the three-letter code of amino acids). In the former complex, histidine behaves as a terdentate ligand, while in the latter it is a bidentate ligand. It seems that the second amino acid has an effect on the character of the histidine ligand. As found by Freeman *et al.*,[5] Cu(his thr) has the same structure as Cu(asn his). It is interesting to examine whether histidine is a terdentate or bidentate ligand when the second amino acid is serine, which is an amino acid closely related to threonine. We have succeeded in growing suitable crystals of aqua(L-histidinato)(L-serinato)copper(II) trihydrate and have determined its crystal structure by X-ray analysis. The present paper deals with the structure of this complex and discusses the three-dimensional architectures of such ternary complexes.

† On leave from Product Development Laboratories, Yamanouchi Pharmaceutical Co., Ltd., 1-1-8, Azusawa, Itabashi-ku, Tokyo 174

Experimental

Suitable blue single crystals were obtained from aqueous solution placed under an atmosphere of acetone vapour. Preliminary Weissenberg photographs indicated a monoclinic space group $P2_1$ or $P2_1/m$. Since this complex was optically active, the former space group was chosen. Accurate unit cell dimensions were determined by least-squares fits from 2θ values of 20 reflexions measured on a Rigaku four-circle diffractometer. Crystal data are as follows: $C_9H_{16}O_6N_4Cu\cdot 3H_2O$, $P2_1$, $a=11.046(2)$, $b=7.315(1)$, $c=9.737(1)$ Å, $\beta=101.34(2)°$, $Z=2$, $D_m=1.67$, $D_x=1.67$ g cm^{-3}.

Since the crystals gradually deteriorated in air, they were sealed in Lindemann-glass capillaries. Intensity measurements were carried out on a Rigaku four-circle diffractometer, graphite-monochromated Mo $K\alpha$ radiation ($\lambda=0.71073$ Å) being used. Intensities of independent reflexions were collected within the range of $2°<2\theta<55°$. An ω-2θ scan mode was applied with scanning rate of 4° (2θ) min^{-1}. The crystal used had dimensions of $0.6\times0.4\times0.5$ mm^3. Five standard reflexions monitored showed no significant change in intensity. Out of 1917 reflexions measured, 123 reflexions had zero intensity ($I<0$). The observational threshold value, $F_{\rm llm}$, was 1.65. The standard deviations were estimated by the equation, $\sigma^2(F_o)=\sigma_p^2(F_o)+q|F_o|^2$, where $\sigma_p(F_o)$ was evaluated by counting statistics and q (1.22×10^{-5}) was derived from the variations of the monitored reflexions. No correction for absorption was made.

Structure Determination

Although it was expected from the similarity of the unit cell dimensions that the crystal structure of the present complex is close to that of Cu(ala his), the structure determination was made by the straightforward application of the heavy atom method. The first Fourier synthesis based on phases of the copper atom revealed the positions of the three nitrogen and one oxygen atoms that were directly coordinated to copper. Successive Fourier and difference syntheses followed by least-squares refinement led to the location of the other non-hydrogen atoms and to the disordering of the hydroxyl oxygen atom of serine into two sites. Occupancies at

TABLE 1. ATOMIC COORDINATES AND EQUIVALENT ISOTROPIC TEMPERATURE FACTORS

The e.s. d.'s in parentheses refer to last digits. $B_{eq}=8\pi^2(U_1+U_2+U_3)/3$, where U_i's are principal components of the mean square displacement matrix. Values in < > are anisotropicities defined by $\{\sum(B_{eq}-8\pi^2U_i)^2/3\}^{1/2}$.

Atom	x	y	z	B_{eq}/Å^2
Cu	0.19651(6)	0.2500(3)	0.15170(7)	2.71<135>
C(1)	0.4971(7)	0.354(1)	0.5050(7)	3.0<8>
C(2)	0.4369(5)	0.3193(9)	0.3519(6)	2.2<3>
C(3)	0.4791(6)	0.447(1)	0.2511(6)	3.1<15>
C(4)	0.4405(5)	0.396(1)	0.1010(6)	2.4<5>
C(5)	0.3173(5)	0.304(1)	-0.0911(6)	2.6<7>
C(6)	0.4983(7)	0.423(1)	-0.0079(7)	2.7<9>
N(1)	0.2988(4)	0.3228(8)	0.3322(5)	3.1<10>
N(2)	0.3236(4)	0.3242(8)	0.0455(4)	2.4<8>
N(3)	0.4227(4)	0.3631(8)	-0.1266(5)	2.7<10>
O(1)	0.4303(4)	0.3762(8)	0.5925(4)	4.2<19>
O(2)	0.6119(4)	0.3544(9)	0.5341(5)	5.5<33>
C(11)	-0.0303(5)	0.1548(9)	-0.0112(6)	2.6<5>
C(12)	-0.0604(5)	0.150(1)	0.1368(6)	2.8<10>
C(13)	-0.1768(5)	0.259(2)	0.1393(7)	5.3<38>
N(11)	0.0468(4)	0.215(1)	0.2405(5)	3.2<16>
O(11)	0.0802(3)	0.1919(7)	-0.0211(4)	3.2<13>
O(12)	-0.1121(4)	0.1245(8)	-0.1129(4)	3.8<14>
O(13A)	-0.1960(6)	0.299(2)	0.2676(8)	5.5<32>
O(13B)	-0.167(1)	0.405(2)	0.194(2)	5.5<31>
O(1')	0.2720(4)	-0.0625(8)	0.1997(4)	3.9<8>
O(2')	-0.0107(7)	0.212(3)	0.6481(7)	16.5<112>
O(3')	0.1764(6)	0.042(2)	0.5073(7)	13.0<92>
O(4')	0.1760(9)	0.484(2)	0.5787(8)	18.5<136>

these two sites were estimated to be 0.6 and 0.4, respectively, from a difference map constructed at a later stage. Structural parameters were refined by the block-diagonal least-squares method: the quantity minimized was $\sum w(|F_o|-|F_c|)^2$ with $w=1/\sigma^2(F_o)$. Zero reflexions were taken in the least-squares by assuming $F_o=F_{lim}$ and $w=w(F_{lim})$. Hydrogen atoms found on a difference map were included in the calculation. Atomic scattering factors were taken from Ref. 8. The R value dropped to 0.057 for 1767 reflexions with $|F_o|>3\sigma(F_o)$. The final atomic coordinates and equivalent temperature factors are listed in Table 1.[9]

Results and Discussion

Molecular Structure. The geometry of the molecule and the numbering system are presented in Fig. 1, where the bond lengths and angles are also shown.

A square-planar coordination around copper is formed by the chelation of histidine and serine; α-amino nitrogen and imidazole δ-nitrogen atoms in the former and α-amino nitrogen and α-carboxyl oxygen atoms in the latter participate in coordination. Two α-amino nitrogen atoms are in a *cis* position, as found in the mixed ligand copper complexes already reported.[5-7] An apical coordination site is occupied by the water molecule, and the carboxyl oxygen atom in the adjacent complex makes a weak bond to copper from the direction opposite to the water ligand, to complete a distorted octahedron. This coordination scheme is the same as that found in (L-alaninato)aqua(L-histidinato)copper(II) trihydrate;[7] histidine behaves as a bidentate ligand. Since the value of pK_a of serine is 2.20,[10] slightly smaller than that of alanine, the explanation for modifying the ligating nature of histidine, proposed in the previous paper,[7] could be applied to the present complex.

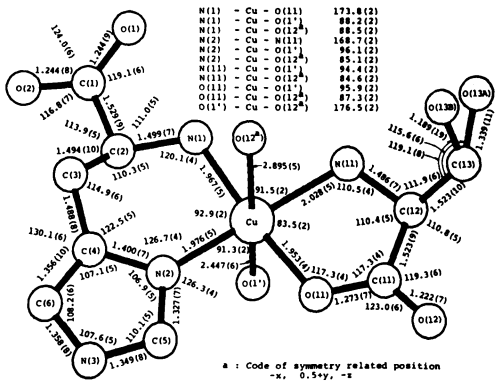

Fig. 1. Bond lengths (l/Å) and angles (ϕ/°) in aqua(L-histidinato)(L-serinato)copper(II). Estimated standard deviations in the last digits are in parentheses.

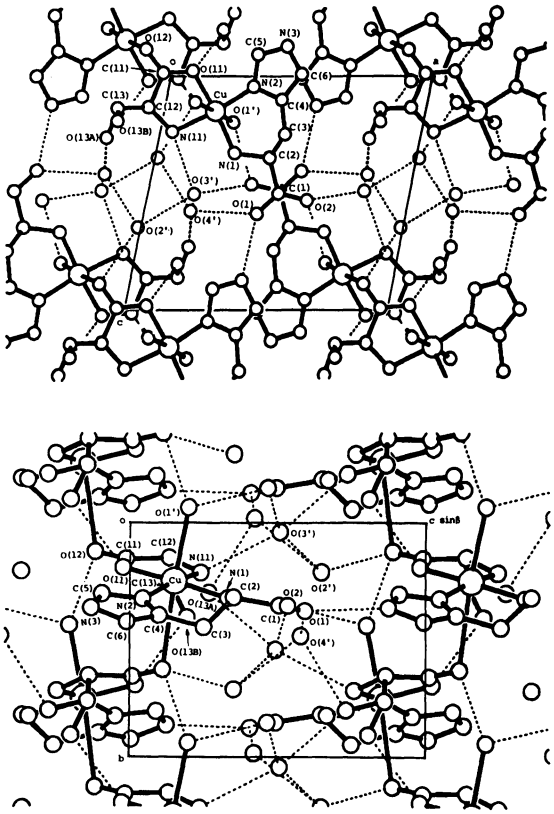

Fig. 2. Crystal structure of aqua(L-histidinato)(L-serinato)copper(II) trihydrate projected along **b** (upper) and **a** (lower).

According to Neumann and Sass-Kortsak,[2] alanine and serine belong to the class of moderate cooperativity with histidine in forming the mixed ligand copper complexes, while asparagine and threonine belong to a class of significant cooperativity. The present serial studies, in addition to the result by Freeman *et al.*,[5] have established that two different chelation modes correspond to the cooperativity classes. These observations reinforce the idea that one of the structural bases for the cooperativity is the chelating mode of histidine.

Coordination distances Cu–N(1), Cu–N(2), Cu–N(11), and Cu–O(11) are 1.967(5), 1.976(5), 2.028(6), and 1.953(4) Å, respectively. The former two Cu–N

TABLE 2. HYDROGEN BOND GEOMETRY

Donor (D)	Acceptor (A)	Site of A[a)]	l(D···A) Å	ϕ(D–H···A) °
N(3)	O(1)	f	2.755(7)	176(6)
N(11)	O(3′)	o	2.99(1)	128(4)
O(13A)	O(4′)	d	2.74(1)	162(10)
O(13B)	O(11)	a	2.97(1)	113.0(9)[b)]
O(1′)	O(12)	b	2.913(7)	163(6)
O(1′)	O(2)	c	2.726(7)	169(6)
O(2′)	O(12)	e	2.85(1)	166(8)
O(2′)	O(3′)	o	2.97(2)	118(8)[c)]
O(2′)	O(4′)	d	3.07(2)	122(8)[c)]
O(3′)	O(2)	c	2.81(1)	157(10)
O(3′)	O(13A)	d	2.79(1)	147(8)
O(4′)	O(1)	o	2.89(1)	140(9)

a) Site is expressed by symmetry code. o: x, y, z; a: $-x$, $1/2+y$, $-z$; b: $-x$, $-1/2+y$, $-z$; c: $1-x$, $-1/2+y$, $1-z$; d: $-x$, $-1/2+y$, $1-z$; e: $x, y, -1+z$; f: $x, y, -1+z$. Donor atom at o. b) Since the position of hydrogen atom attached to O(13B) is not certain, this is an angle of C(13)-O(13B)···O(11). c) Probably bifurcated hydrogen bond.

distances are slightly shorter than the values (1.98···2.04 Å) reported for rectangular coordination complexes.[11] But these distances, except for Cu–N(1), and two apical coordination distances for Cu–O(12[a]) and Cu–O(1′) seem to be within the fluctuation range found in the related mixed ligand copper complexes.[6,7]

The copper atom is shifted from the mean basal coordination plane toward the apical O(1′) by 0.118(7) Å. The dihedral angle between the planes, N(1)–Cu–N(2) and N(11)–Cu–O(11), is 10.9(2)° (Λ configuration).

The imidazole ring is approximately planar, with maximum deviations of −0.016(6) for C(4) and +0.015(7) Å for C(5). Its mean plane makes a dihedral angle of 2.4(3)° with the plane of N(1)–Cu–N(2).

Crystal Structure. Crystal structures projected along the b and a axes are shown in Fig. 2. As expected from the similarity of the unit cell dimensions, the principal frame of the crystal structure is the same as that of Cu(ala his). The carboxyl oxygen atom, O(12), is weakly coordinated to copper in the complex at $(-x, -1/2+y, -z)$, so that a column extending along the b axis is constructed. ε-Nitrogen atom of the imidazole ring is hydrogen-bonded to the carboxyl oxygen, O(1), in the complex at $(x, y, -1+z)$. These hydrogen bonds connect together the columns above-mentioned, so that a void space extending along the b axis is left, in which three crystallization water molecules and serine side chain are occluded.

While three water molecules are distributed among seven sites randomly in the void space of Cu(ala his), those in the present crystal occupy fixed positions. The serine hydroxyl oxygen atom makes two kinds of hydrogen bonds, by taking two different sites: O(13A) at one site with occupancy of 0.6 links to the water molecule, O(4′), and O(13B) at another site with occupancy of 0.4 is connected to the carboxyl oxygen atom, O(11) in the complex at $(-x, 1/2+y, -z)$.

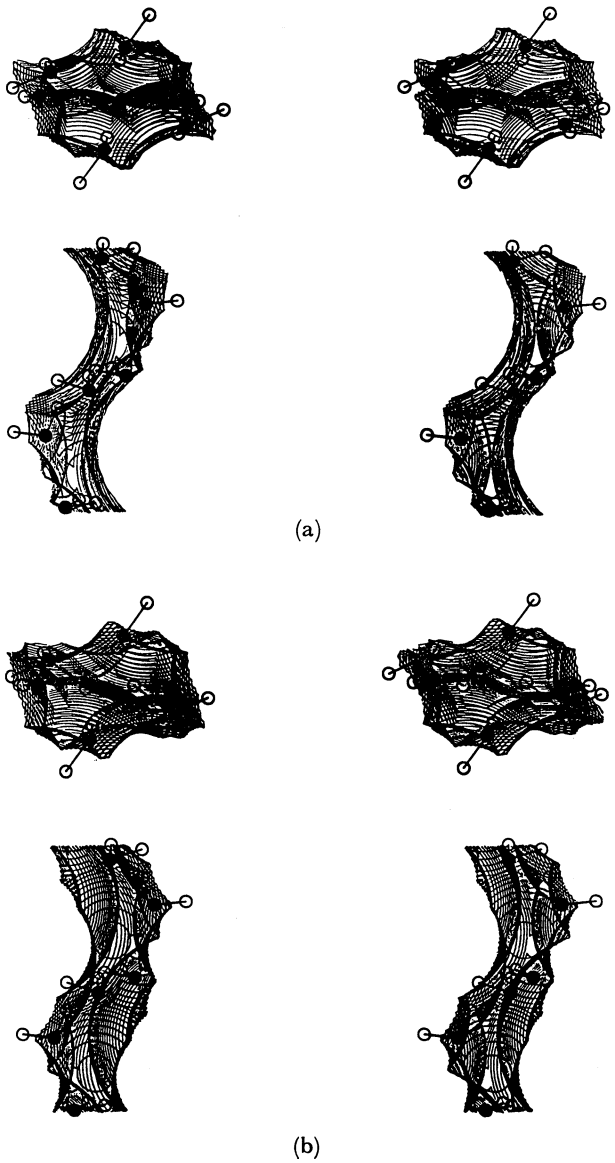

Fig. 3. Cavity for three water molecules viewed along **b** (upper) and **a** (lower), a) when the hydroxyl group of serine takes the site A, b) when the hydroxyl group of serine takes the site B.

Details of these hydrogen bonds are listed in Table 2.

In order to clarify the feature of arrangement of three water molecules, we have drawn the "cavity" for waters, which is defined as a closed space in which the atomic centres of the occluded molecules can be situated (for details of the cavity, see Ref. 12). Taking into account the hydrogen bond formation between water and its surrounding molecules, the radii allotted for hydrogen bond donor and acceptor atoms, O and N, were assumed to be 2.70 and 2.85 Å, respectively (hydrogen atoms attached to these atoms are neglected). Figure 3 shows the cavity viewed along the a and b axes, where the positions of the three water molecules are indicated. As seen from Fig. 3a, the oxygen atoms of water, consisting a triangle, fit very well with the cavity. Hydrogen atoms of water may protrude from the cavity, because the radii of O and N are taken as hydrogen bond distances. Figure 3b indicates the cavity for the case in which the

serine hydroxyl group takes a site with low occupancy. The fit of the water triangle into the cavity is also good. Disordering of serine O(13) gives rise to a slight change for the shape of the cavity, but this does not affect the position and orientation of the triangle. The cavity in the crystal of Cu(ala his) was also drawn, and the shape is nearly the same as that in the present case.

The volumes of the cavities were calculated in the way proposed in the previous paper[12] to be 12.94 Å^3 for the present complex and 17.22 Å^3 for Cu(ala his). The larger cavity of the latter allows the disordering of three water molecules, as discussed in Ref. 7.

Since the side chain of alanine is hydrophobic, while that of seine is hydrophilic, one might expect different crystal structures for Cu(ala his) and Cu(his ser). It is interesting that completely the same building principle applies to those two ternary complexes, perhaps owing to the common chelating mode of the mixed amino acids.

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References

1) B. Sarkar and T. P. A. Kruck, "The Biochemistry of Copper," ed by J. Peisach, P. Aisen, and W. E. Blumberg, Academic Press, New York (1966), p. 183.

2) P. Z. Neumann and A. Sass-Kortsak, *J. Clin. Invest.*, **46**, 646 (1967).

3) P. M. May, P. W. Linder, and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, **1977**, 588.

4) R. P. Agarwal and D. D. Perrin, *J. Chem. Soc., Dalton Trans.*, **1975**, 268.

5) H. C. Freeman, J. M. Guss, M. J. Healy, R. P. Martin, C. E. Nockolds, and B. Sarkar, *Chem. Commun.*, **1967**, 225.

6) T. Ono, H. Shimanouchi, Y. Sasada, T. Sakurai, O. Yamauchi, and A. Nakahara, *Bull. Chem. Soc. Jpn.*, **52**, 2229 (1979).

7) T. Ono and Y. Sasada, *Bull. Chem. Soc. Jpn.*, **54**, 90 (1981).

8) "International Tables for X-Ray Crystallography," The Kynoch Press, Birmingham (1974), Vol. IV, p. 72.

9) Tables of anisotropic thermal parameters for non-hydrogen atoms, of coordinates and temperature factors for hydrogen atoms, and of the observed and calculated structure factors are kept in the office of the Chemical Society of Japan (Document No. 8318).

10) "Lange's Handbook of Chemistry," Eleventh ed, ed by J. A. Dean, McGraw-Hill, New York (1972), p. 5—37.

11) H. C. Freeman, *Adv. Protein Chem.*, **22**, 257 (1967).

12) Y. Ohashi, A. Uchida, Y. Sasada, and Y. Ohgo, *Acta, Crystallogr., Sect. B*, **39**, 54 (1983).