

atoms O(3) and O(4), these distances being only 2.953 and 2.911 Å respectively.

The only short distance of approach between non-bonded atoms in the molecule is O(1) to O(2') = 2.549 Å. This may result in an internal hydrogen bond, but as the position of H(1) has not been determined experimentally, this can not yet be regarded as confirmed.

Fig. 2 shows the packing of the molecules in the unit cell.

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Crystallographic Studies of Metal–Peptide Complexes. IV. (Glycyl-L-histidinato)copper(II) Sesquihydrate

By J. F. BLOUNT, K. A. FRASER, H. C. FREEMAN, J. T. SZYMANSKI* AND C.-H. WANG†
School of Chemistry, University of Sydney, Sydney, Australia

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(Glycyl-L-histidinato)copper(II) sesquihydrate crystallizes from solution at pH 6.5. The blue crystals are tetragonal, space-group $P4_32_12$, with $a = 11.24 \pm 0.02$, $c = 17.84 \pm 0.04$ Å, $Z = 8$. The copper atom has coordination number 5. The four closest donor atoms, which have an approximately square arrangement about the copper, are the amino-, peptide- and imidazole-1- nitrogen atoms of one peptide molecule and a carboxyl oxygen atom of another. The fifth donor atom, completing a square-pyramidal arrangement, is the oxygen of a water molecule. The copper also interacts weakly with the second oxygen atom of the carboxyl group to which it is bonded. The additional semi-molecule of water indicated by the composition of the complex lies on a twofold axis and participates in the hydrogen-bond network. The structure was refined by full-matrix anisotropic least squares, the final reliability index R being 0.086 for the 1341 independent observed reflexions.

Introduction

This structure analysis is the fourth of a series‡ dealing with model compounds for metal–protein interaction (Freeman, Robinson & Schoone, 1964; Freeman, Schoone & Sime, 1965; Freeman & Taylor, 1965). The potential usefulness of structural information about this particular complex was suggested to us by the demonstration that, in solution, sperm-whale metmyoglobin binds copper(II) ion at the imidazole groups of histidine residues (Breslow & Gurd, 1963). This conclusion has already been supported by a study of the 1:1 Cu^{II}-metmyoglobin complex in the solid state (Banaszak, Kendrew & Watson, 1965). We therefore set out to make a detailed study of histidine–Cu^{II}

interaction in a much simpler system. Preliminary accounts of this work (Blount, Fraser, Freeman, Szymanski, Wang & Gurd, 1966) and of the closely related structure of (β -alanyl-L-histidinato)copper(II) dihydrate (Freeman & Szymanski, 1965) have been published.

Experimental

The complex was prepared from a solution of glycyl-L-histidine hydrochloride (0.001 mole) in 0.2M sodium hydroxide (5–6 ml). Freshly precipitated and washed copper(II) hydroxide (0.0015 mole) was added. The resulting violet solution was centrifuged, separated from undissolved copper hydroxide, and carefully titrated with 0.15M hydrochloric acid until the colour was blue (pH 6.5). Blue crystals separated overnight. The specimen used to record the crystallographic data had a square-pyramidal shape with curved faces.

Crystal data

$C_8H_{10}O_3N_4Cu \cdot 1\frac{1}{2}H_2O$ F.W. 300.8
Tetragonal, $a = 11.24 \pm 0.02$, $c = 17.84 \pm 0.04$ Å, $D_m = 1.72 \pm 0.02$, $D_x = 1.772$ g.cm⁻³, $U = 2253.9$ Å³, $Z = 8$, $F(000) = 1208$. $\lambda(\text{Cu } K\alpha_1) = 1.5405$ Å, $\lambda(\text{Cu } K\alpha_2) = 1.5443$ Å.

* Present address: Department of Chemistry, University of Toronto, Toronto 5, Canada.

† Permanent address: National Tsing Hua University, Hsinchu, Taiwan, China.

‡ The crystals were grown by H.C.F. while a guest at the University of Indiana, Indianapolis. The data were recorded and the intensities were measured in Sydney by J.T.S. and C.-H.W. respectively. The structure was solved independently by J.F.B. and K.A.F. in Sydney, and by C.-H.W. in Tokyo with the help of Dr Y. Saito. This paper is based on the Sydney refinement calculations.

Space group $P4_32_12$ or $P4_12_12$, determined from systematic absences ($00l$ present only for $l=4n$, $h00$ for $h=2n$); $P4_32_12$ confirmed by structure analysis and known configuration of L-histidine (Langenbeck, 1925).

The unit-cell dimensions were fitted by least squares to the values of $4 \sin^2 \theta / \lambda^2$ of 21 reflexions with $65^\circ < \theta < 76^\circ$, making allowance for absorption and eccentricity (Buerger, 1942). The spacings were measured on a Weissenberg photograph taken with Cu $K\alpha$ radiation and calibrated with aluminum powder diffraction lines. The probable errors quoted for a and c were taken arbitrarily as three times the standard deviations calculated from the least-squares matrix. Equi-inclination Weissenberg data were recorded about the a and c axes (Hkl for $0 \leq H \leq 10$, and $hk0$). The intensities were estimated visually, were corrected for the Lorentz and polarization factors but not for absorption, and were placed upon a common scale by comparisons of (i) symmetry-related reflexions recorded on different

layers and (ii) equivalent reflexions recorded about the two axes. A total of 1462 independent reflexions were estimated. Of these, 121 were unobservably weak reflexions which were assigned values of one-half the locally observed minimum intensity (Hamilton, 1955). Standard deviations were calculated for intensities for which multiple observations existed. After conversion of these intensities and their standard deviations to $|F_o|$, $\sigma(F_o)$ -values, the root mean squares $[\overline{\sigma^2(F_o)}]^\dagger$ were computed over ranges of 1.0 in $|F_o|$. Standard deviations read from a plot of these averaged values were used to assign weights $w=1/\sigma^2(F_o)$ in the subsequent refinement*.

* The $\sigma(F_o)$ were adequately represented as a function of $|F_o|$ by linear interpolation between the following values:

0.1864 $ F_o $ = 0	5	6	8	10	12	14	16	18
0.1864 $\sigma(F_o)$ = 0.44	0.26	0.30	0.40	0.53	0.66	0.85	1.10	1.55
0.1864 $ F_o $ = 20	25	30	40					
0.1864 $\sigma(F_o)$ = 2.30	2.86	3.15	3.65					

Table 1. Final positional parameters, their standard deviations (in parentheses) and shifts in the final least-squares cycle. The standard deviations σ and shifts Δ correspond to the least significant digits in the coordinates. e.s.d. = $[(\sigma_x^2 a^2 + \sigma_y^2 b^2 + \sigma_z^2 c^2)/3]^\dagger$

	$x(\sigma_x)\Delta_x$	$y(\sigma_y)\Delta_y$	$z(\sigma_z)\Delta_z$	e.s.d.
Cu	-0.0105 (1) 0	0.2827 (1) 0	0.1683 (1) 0	0.001 ₃ Å
N(1)	-0.0195 (7) 0	0.4624 (7) 0	0.1523 (5) 0	0.009
C(1)	0.0839 (9) 1	0.5210 (9) 1	0.1844 (7) 0	0.011
C(2)	0.1692 (9) 1	0.4357 (9) 2	0.2221 (6) 0	0.010
O(1)	0.2608 (6) 0	0.4784 (7) 0	0.2535 (5) 0	0.008
N(2)	0.1426 (7) 0	0.3223 (7) 1	0.2185 (5) 0	0.008
C(3)	0.2269 (8) 1	0.2386 (8) 1	0.2538 (6) 0	0.010
C(4)	0.3394 (8) 1	0.2208 (8) 1	0.2075 (6) 0	0.009
O(2)	0.4300 (6) 1	0.1827 (7) 0	0.2409 (4) 0	0.007
O(3)	0.3372 (5) 0	0.2469 (6) 0	0.1375 (4) 0	0.007
C(5)	0.1693 (9) 0	0.1184 (9) 1	0.2690 (6) 0	0.010
C(6)	0.1195 (9) 0	0.0613 (10) 1	0.1991 (6) 0	0.011
N(3)	0.0320 (8) 0	0.1127 (7) 1	0.1582 (5) 0	0.008
C(7)	0.0029 (10) 1	0.0410 (8) 0	0.0997 (7) 0	0.011
N(4)	0.0745 (8) 0	-0.0578 (8) 2	0.1040 (6) 0	0.009
C(8)	0.1482 (11) 0	-0.0448 (10) 1	0.1649 (8) 0	0.012
O(4)	-0.1316 (9) 1	0.3176 (8) 1	0.2809 (5) 0	0.009
O(5)	0.2790 (8) 1	0.7210 - -	0.2500 - -	0.009

Table 2. The final vibrational parameters and their estimated standard deviations (in parentheses) (all $\times 10^4$)

Anisotropic temperature factor: $\exp [-(h^2\beta_{11} + k^2\beta_{22} + l^2\beta_{33} + 2hk\beta_{12} + 2hl\beta_{13} + 2kl\beta_{23})]$

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Cu	29 (1)	40 (1)	30 (0)	-1 (1)	-3 (1)	-2 (1)
N(1)	34 (6)	28 (6)	44 (4)	2 (4)	-8 (4)	0 (4)
C(1)	48 (8)	39 (7)	42 (4)	-5 (6)	-11 (5)	1 (5)
C(2)	49 (8)	37 (7)	31 (4)	-6 (6)	-1 (4)	-4 (4)
O(1)	44 (6)	57 (6)	51 (3)	-3 (5)	-24 (4)	-13 (4)
N(2)	29 (6)	38 (6)	27 (3)	0 (5)	-4 (3)	-2 (3)
C(3)	41 (7)	51 (7)	27 (3)	6 (6)	-14 (4)	2 (4)
C(4)	35 (7)	35 (7)	30 (3)	-3 (6)	2 (4)	1 (4)
O(2)	40 (5)	91 (8)	33 (3)	20 (5)	-2 (3)	7 (4)
O(3)	33 (5)	58 (6)	24 (2)	5 (4)	1 (3)	1 (3)
C(5)	56 (8)	44 (8)	31 (4)	-6 (6)	3 (5)	3 (4)
C(6)	45 (8)	52 (8)	33 (4)	-10 (6)	5 (5)	4 (5)
N(3)	59 (8)	31 (6)	39 (4)	15 (5)	5 (4)	-7 (4)
C(7)	63 (9)	41 (7)	40 (4)	2 (7)	8 (6)	-9 (4)
N(4)	49 (7)	43 (6)	45 (4)	5 (6)	6 (5)	-6 (4)
C(8)	77 (10)	55 (9)	41 (5)	15 (8)	-1 (6)	-9 (6)
O(4)	104 (9)	69 (7)	38 (3)	-9 (7)	19 (5)	-4 (4)
O(5)	58 (7)	58	202 (18)	25 (10)	-33 (10)	-33

Table 3. Observed and calculated structure amplitudes (x 10)

Table with multiple columns (H, K, L, FO, FC) and rows of numerical data. Includes a 'UNOS' label at the bottom right.

Structure solution and refinement

The position of the copper atom was established by means of a sharpened three-dimensional Patterson function using coefficients F_o^2/f_{Cu}^2 . The remaining atoms of the structure were all apparent in a single three-dimensional Fourier synthesis using phases calculated from the copper atom alone. A water molecule lying on a twofold axis was actually missed at this stage: it was later found to be represented by a peak half as high as those of the other atoms. The first structure-factor list had a reliability index $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$, of 0.27. The directness of the structure analysis was no doubt favoured by the acentric distribution of the eight heavy (Cu) atoms in the unit cell. Even though x_{Cu} was close to zero, no problem of false symmetry arose in the interpretation of the heavy-atom Fourier synthesis.

Three cycles of least-squares refinement with isotropic thermal parameters reduced R to 0.165. Insertion of the extra water (half-)molecule further improved the agreement to $R = 0.158$. Three cycles of full-matrix least-squares refinement with anisotropic thermal parameters brought the refinement to convergence, the quantity minimized being $\Sigma w(|F_o| - |F_c|)^2$. Unobserved reflexions were given zero weights in the refinement. The

final refinement criteria were: R (obs. F 's) = 0.086, R (all F 's) = 0.092, $\Sigma w\Delta^2 / (m - n) = 1.434$.

A Fourier ($F_o - F_c$) synthesis after the completion of the refinement had no positive excursions above the level of statistical significance (about $0.7 \text{ e.}\text{\AA}^{-3}$; average s.d. of electron density = $0.22 \text{ e.}\text{\AA}^{-3}$). Distorted peaks between 0.2 and $0.6 \text{ e.}\text{\AA}^{-3}$ were found at all the expected positions of hydrogen atoms but some spurious peaks as high as $0.4 \text{ e.}\text{\AA}^{-3}$ were also present. A negative annulus (about $-1 \text{ e.}\text{\AA}^{-3}$) surrounded the Cu atom at a radius of 0.7 \AA and in a plane normal to z . Further calculations to include the hydrogen atoms were therefore not made.

Standard scattering factors were used for carbon, nitrogen, oxygen (*International Tables for X-ray Crystallography*, 1962a) and Cu^+ (*International Tables for X-ray Crystallography*, 1962b). The curve for Cu^+ was corrected for the real part of the anomalous scattering by subtracting 2.1 electrons over the whole $\sin \theta$ range (Dauben & Templeton, 1955). The program used for the refinement calculations was ORFLS (Busing, Martin & Levy, 1962). The final positional and thermal parameters are listed in Tables 1 and 2, and the observed and calculated structure amplitudes in Table 3. The structure was actually solved and refined in space-group $P4_12_12$: this corresponded to the D isomer of

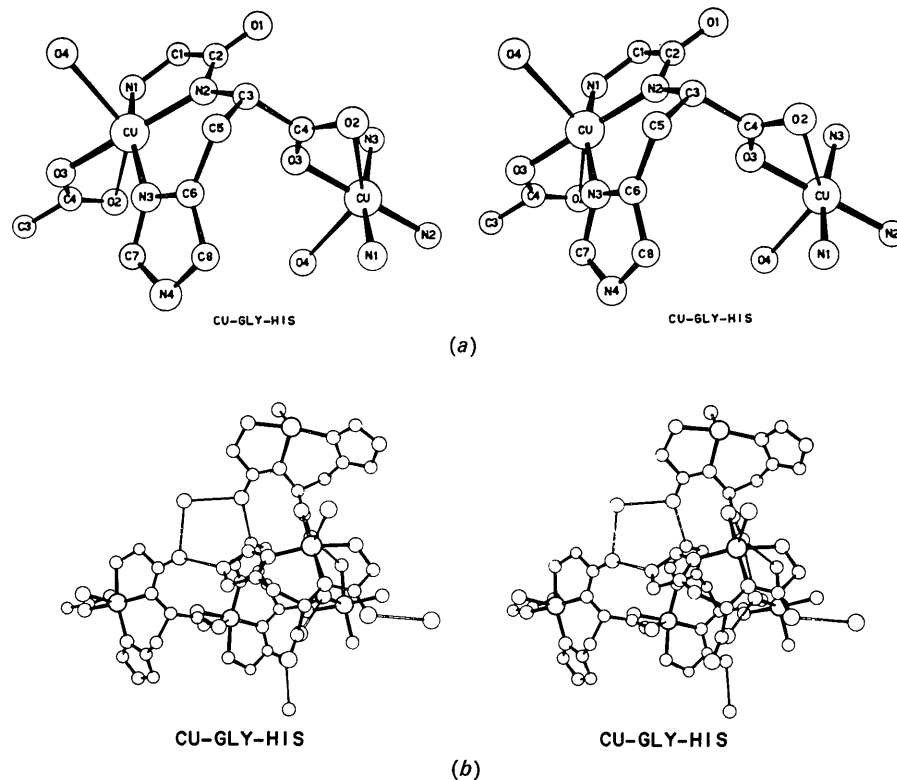
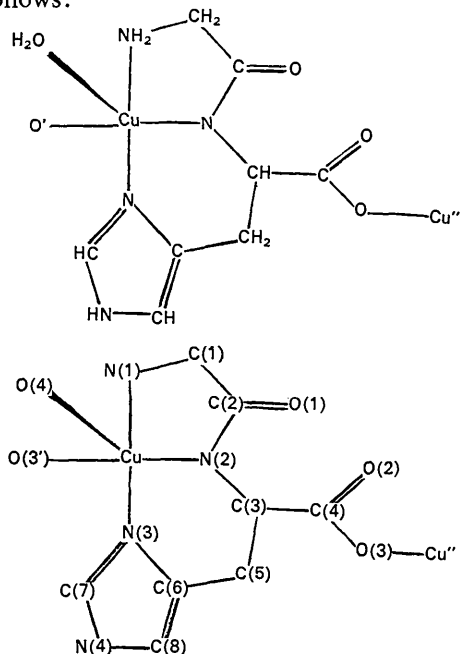


Fig. 1. (a) Stereoscopic view showing the coordination of two Cu^{II} atoms in (glycyl-L-histidinato)copper(II) sesquihydrate. (b) Stereoscopic view showing hydrogen bonding and packing of complexes and water molecules in (glycyl-L-histidinato)copper(II) sesquihydrate. Hydrogen bonds are represented by hollow bonds. A fourfold screw axis is parallel to the line of sight, a twofold screw axis runs from left to right, and the non-coordinated water molecules lie on twofold axes whose directions are at 45° to the directions of the other two types of axis.

the complex. Prior to the final structure-factor calculation, all parameters were converted to the enantiomorphous space-group $P4_32_12$ to which all Tables in this paper refer. The labelling of the dipeptide molecule is as follows:



Description of the structure

The structure consists of $-\text{Cu-peptide-Cu-peptide}-$ chains running parallel to the twofold screw axes at $(x, \frac{1}{4}, \frac{1}{8})$, $(\frac{1}{4}, y, \frac{3}{8})$, $(x, \frac{3}{4}, \frac{5}{8})$ and $(\frac{3}{4}, y, \frac{7}{8})$, respectively. Each dipeptide molecule therefore binds two copper atoms, adjacent $-(\text{Cu-peptide})-$ units being related by the screw operation. One such unit and its surroundings are shown in Fig. 1, and the dimensions are summarized in Fig. 2. A water molecule, whose oxygen atom is labelled O(5), lies on a twofold axis (accounting for the $\frac{1}{2}\text{H}_2\text{O}$ in the formula unit)*.

Environment of copper atom

The copper atom has coordination number 5 and a square-pyramidal environment. It is bound to one dipeptide molecule *via* the nitrogen atoms N(1), N(2) and N(3) of the amino, peptide and imidazole groups, and to a second dipeptide *via* the carboxylic oxygen atom O(3'). These four donor atoms all lie about 2 Å from the copper and form an approximate square around it. A fifth bond, perpendicular to this coordination square, joins the copper atom to the oxygen atom O(4) of a water molecule (2.46 Å). There is a sixth interaction, albeit a weak one (2.94 Å), between the copper atom and the second oxygen O(2') of the coordinated carboxyl group: slightly stronger interac-

tions of the same type, also lying at angles of about 50° from the bond directions for a regular octahedron, occur in the crystal structures of glutamatocopper(II) dihydrate (Gramaccioli & Marsh, 1966) and glycylglycylglycinatocopper(II) sesquihydrate (Freeman, Robinson & Schoone, 1964).

The atoms of the coordination square are not precisely co-planar. The deviations of N(1), N(2), N(3) and O(3') from their plane of best fit are between 0.12 and 0.13 Å (plane 5, Table 6) and are so directed that the configuration of these four atoms is a very flattened tetrahedron. The copper atom is displaced 0.17 Å out of this plane in the direction of the apical water molecule.

The copper-ligand bond-lengths and bond-angles are shown in Table 4, which also lists the lengths of bonds in comparable structures (Freeman, 1966). The only bond whose length lies significantly outside a previously recorded range is Cu-N(2). This bond is the first example of a bond between copper and a peptide nitrogen which is a member of a six-membered as well as of a five-membered chelate ring. Its abnormal length must be the result of the dual requirements, that strain in the chelate ring containing the imidazole group be minimized while the three bonds around the peptide nitrogen remain approximately coplanar. For the angles between the copper-ligand bonds it is more

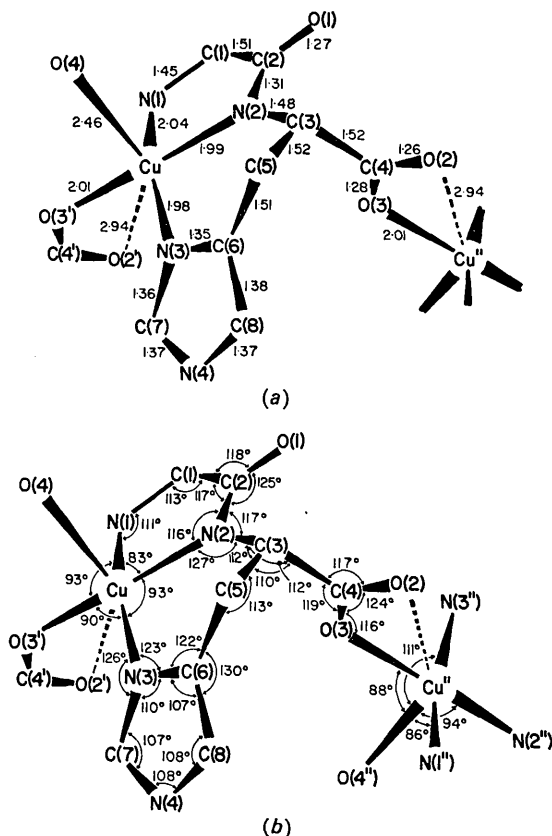


Fig. 2. (a) Bond lengths, and (b) bond angles in (glycyl-L-histidinato)copper(II) sesquihydrate.

* Since O(5) lies on the special position $x, 1-x, \frac{1}{2}$, no standard deviations or final shifts have been listed in Tables 1 and 2 for its constrained parameters y, z, β_{22} and β_{23} .

difficult to establish meaningful comparisons. The angle N(1)–Cu–N(2) has a value, 83.3°, in agreement with those usually found for N(amino)–Cu–N(peptide) angles in five-membered chelate rings (83–84°).

Geometry of the ligand molecule

The dimensions of the ligand molecule are listed in Table 5. Within the limits of precision and with only one exception (see below) all the bond-lengths and angles have values compatible with those in related structures. For the main peptide chain, the comparison values in Table 5 are taken from a recent review of the published structures of copper(II)–peptide complexes (Freeman, 1966). The dimensions of the histidine side-chain are compared with those of bis(DL-histidinato)-nickel(II) hydrate (Fraser & Harding, unpublished work).

The size of Cu–N(2)–C(3), 127.1°, lies at the limit of the normal range of values for this angle. Like the abnormal length of Cu–N(2), this is a result of the conflicting strains which have to be accommodated at N(2). In all previously recorded Cu^{II}–peptide complexes, any chelate rings in which the atoms corresponding to C(3) have been involved have been five-membered. In the present complex C(3) is in a six-membered ring, resulting in an increase of Cu–N(2)–C(3) at the expense of the other two angles at N(2). The other dimensional differences between the present complex and those chosen for comparison are all below the level of definite significance by the usual statistical criterion (Lipson & Cochran, 1953).

The peptide group C(1)C(2)O(1)N(2)C(3), the imidazole group C(5)C(6)N(3)C(7)N(4)C(8), and the carboxyl group C(3)C(4)O(2)O(3) are planar within the limits of accuracy (Table 6). The Cu atoms attached to these groups deviate by 0.08, 0.47 and 0.04 Å from their respective planes of best fit. Not only the Cu atom but also N(1) lies significantly out of the plane of the peptide group (plane 1, Table 6), so that the five-

membered chelate ring –CuN(1)C(1)C(2)N(2)– is slightly buckled. We note also that the atoms of the imidazole ring deviate by smaller amounts from the plane fitted to them alone (plane 3) than from the plane which includes C(5) (plane 2). The imidazole–Cu bond N(3)–Cu makes an angle of 14° with plane 2.

The conformation of the six-membered chelate ring between the peptide and imidazole nitrogen atoms is best described in terms of the dihedral angles between the planes intersecting in each bond. Looking along N(2)–C(3) and taking clockwise rotation as positive, the dihedral angle between the planes of the peptide group and N(2)C(3)C(5) is –19.1°. Continuing around the ring, the dihedral angles are +57.2° between N(2)C(3)C(5) and C(3)C(5)C(6), and –61.2° between C(3)C(5)C(6) and the imidazole ring, respectively. The angle between the planes of the imidazole ring and the coordination square is 33.0°. The plane of the carboxyl group lies at 89.6° and 83.8° from those of the coordination square and the peptide group.

Hydrogen bonds and non-bonded contacts

The hydrogen bonds formed by one complex have been listed in Table 7 and illustrated in Fig. 1. At five of the six atom-types which take part in the proposed hydrogen-bond network, there are unusual bond angles involving hydrogen bonds [e.g. C(2)–O(1)···N(4^{vi}), C(4)–O(2)···N(1^v), etc.]. We suggest that these are weak interactions in which the donor-hydrogen bonds lie about 20° away from the direct vectors.

The shortest non-bonded intermolecular contact in the structure (3.03 Å) is made by C(7) and O(3'). All other contacts exceed 3.2 Å.

Vibrational parameters

The thermal ellipsoids of all atoms except O(2) and O(4) lie with their principal axes at a mean angle of 13°, and always within 30°, of the z axis. The average r.m.s. components of thermal displacement are 0.25 Å

Table 4. Environment of Cu^{II} atom

Present work			Comparison values				
Bond	Length	$\sigma(l)$	Bond-type	Ave.	Range	No. in sample	Distribution
	<i>l</i>			<i>l</i>			
Cu–N(1)	2.043 Å	0.007 Å	Cu–NH ₂ –	2.01 Å	1.98–2.04 Å	12	10 complexes
Cu–N(2)	1.991	0.008	Cu–N(pept)	1.91	1.88–1.94	8	4 complexes
Cu–N(3)	1.977	0.008	Cu–N(im)	1.98	1.96–2.00	4	Cu(imidazole) ₂
Cu–O(3')	2.007	0.006	Cu–OCO–	1.97	1.91–2.04	12	9 complexes
Cu–O(4)	2.458	0.009	Cu–OH ₂	2.41	2.30–2.52	7	6 complexes
Cu–O(2')	2.943	0.008	Cu···O=CO–	2.70	2.59–2.82	2	See text

(b) Bond angles					
Angle	θ	$\sigma(\theta)$	Angle	θ	$\sigma(\theta)$
N(1)–Cu–N(3)	162.7	0.4	O(4)–Cu–O(3')	87.7	0.3
N(1)–Cu–O(3')	93.0	0.3	O(4)–Cu–O(2')	129.9	0.3
N(2)–Cu–N(3)	92.8	0.3	O(2')–Cu–N(1)	73.9	0.3
N(2)–Cu–O(3')	175.7	0.3	O(2')–Cu–N(2)	126.9	0.3
N(3)–Cu–O(3')	90.1	0.3	O(2')–Cu–N(3)	95.5	0.3
O(4)–Cu–N(1)	85.9	0.4	O(2')–Cu–O(3')	49.4	0.2
O(4)–Cu–N(2)	94.3	0.3			

along the major, and 0.17 Å along the two minor, principal axes of the ellipsoids. O(5) of the free water molecule has a displacement of 0.58 Å along its major principal axis and shows the most pronounced vibrational anisotropy.

Chemical significance

Comparison with related structures

This is the first detailed report of a structure analysis of a complex between a metal and a histidine-containing dipeptide. It is therefore interesting to establish the relationship of this complex to (i) bisimidazolocop-

per(II), in whose structure there are four crystallographically distinct types of imidazole nitrogen-copper bonds (Jarvis & Wells, 1960; Freeman, 1966); (ii) bis-L-histidinatozinc(II) dihydrate (Kretsinger, Cotton & Bryan, 1963), bis-DL-histidinatozinc(II) pentahydrate (Harding & Cole, 1963), and the bishistidinato complexes of nickel(II), cobalt(II) and cadmium(II) (Fraser, Long, Candlin & Harding, 1965); (iii) (β -alanyl-L-histidinato)copper(II) dihydrate (Freeman & Szymanski, 1965, and unpublished work); and (iv) glycyglycinatecopper(II) trihydrate (Strandberg, Lindqvist & Rosenstein, 1961), a copper(II)-glycyl-peptide complex in which a peptide nitrogen takes part in chelation.

Table 5. Dimensions of ligand molecule

Present work			(a) Bond lengths				
Bond	Length <i>l</i>	$\sigma(l)$	Bond-type	Comparison values			Distribution
				Mean <i>l</i>	Range	No. in sample	
N(1)-C(1)	1.45 Å	0.013 Å	CuNH ₂ -CH ₂ -	1.48 Å	1.44-52 Å	12	10 complexes
C(1)-C(2)	1.51	0.014	-CH ₂ -CO-	1.53	1.51-57	9	4
C(2)-O(1)	1.27	0.012	C=O(pept)	1.26	1.19-28	7	4
C(2)-N(2)	1.31	0.012	-OC-NCu-	1.30	1.29-32	6	3
N(2)-C(3)	1.48	0.012	-CuN-CH<	1.45	1.43-48	7	3
C(3)-C(4)	1.52	0.013	>CH-C(carbox)	1.52	1.49-53	13	11
C(4)-O(2)	1.26	0.012	-CO=O	1.23	1.21-25	13	11
C(4)-O(3)	1.28	0.012	-CO-O-	1.28	1.24-33	13	11
C(3)-C(5)	1.52	0.013					
C(5)-C(6)	1.51	0.015					
C(6)-N(3)	1.35	0.014		1.39			
N(3)-C(7)	1.36	0.014		1.33			
C(7)-N(4)	1.37	0.013		1.34			
N(4)-C(8)	1.37	0.016		1.36			
C(8)-C(6)	1.38	0.015		1.38			
			Corresponding bonds in bis(DL-histidinato)nickel(II) hydrate (s.d. ~ 0.016 Å).				

Present work			(b) Bond angles				
Angle	θ	$\sigma(\theta)$	Angle-type	Comparison values			Distribution
				Mean	Range	No. in sample	
Cu-N(1)-C(1)	110.7°	0.6°	Cu-N-CH ₂	111°	108-17°	11	9 complexes
N(1)-C(1)-C(2)	113.2	0.8	N-C-CO	110	108-16	15	7
C(1)-C(2)-O(1)	118.1	0.9	C-C=O	119	117-22	9	4
C(1)-C(2)-N(2)	116.8	0.9	C-CO-N<	115	113-7	9	4
O(1)-C(2)-N(2)	125.1	0.9	O=C-N<	126	123-8	9	4
C(2)-N(2)-Cu	115.9	0.7	OC-N-Cu	119	115-21	6	3
C(2)-N(2)-C(3)	117.0	0.8	OC-N-C	122	117-26	7	4
Cu-N(2)-C(3)	127.1	0.6	Cu-N-C	119	115-27	7	3
N(2)-C(3)-C(4)	112.6	0.8					
N(2)-C(3)-C(5)	111.7	0.8					
C(4)-C(3)-C(5)	109.5	0.8					
C(3)-C(4)-O(2)	117.4	0.9	C-C=O	120	116-26	11	
C(3)-C(4)-O(3)	118.8	0.8	C-CO-O-	118	113-21	11	
O(2)-C(4)-O(3)	123.7	0.9	O=C-O-	123	117-29	11	
C(4)-O(2)-Cu''	71.4	0.6					
C(4)-O(3)-Cu''	115.4	0.6	OC-O-Cu	120	114-40	10	
C(3)-C(5)-C(6)	112.9	0.9		113			
C(5)-C(6)-N(3)	122.2	1.0		124			
C(5)-C(6)-C(8)	130.3	1.1		130			
N(3)-C(6)-C(8)	107.4	1.0		107			
C(6)-N(3)-Cu	122.6	0.8		107			
C(6)-N(3)-C(7)	109.6	0.9		107			
Cu-N(3)-C(7)	125.8	0.7		110			
N(3)-C(7)-N(4)	107.2	1.0		108			
C(7)-N(4)-C(8)	108.1	0.9		108			
N(4)-C(8)-C(6)	107.5	1.0		107			
			Corresponding angles in bis(DL-histidinato)nickel(II) hydrate (e.s.d. ~ 1.5°).				

Table 6. *Deviations from planes of best fit*

Each plane is represented by $lX+mY+nZ+p=0$ where X, Y, Z are coordinates in Å, referred to the unit-cell axes. A deviation in parentheses indicates that the atom was *not* included in the least-squares computation.

Plane	Peptide	Imidazole		Carboxyl	Four nearest
	group	ring	ring	group	Cu-ligands
Coefficients	1	2	3	4	5
<i>l</i>	0.5096	0.6848	0.6813	-0.2791	0.4719
<i>m</i>	-0.0765	0.4655	0.4618	-0.9372	0.0467
<i>n</i>	-0.8570	-0.5607	-0.5679	-0.2090	-0.8804
<i>p</i>	2.7932	0.7564	0.7768	4.1697	2.3781
Deviations (Å)					
Cu	(-0.083)	(0.471)	(0.459)		(-0.172)
N(1)	(-0.045)				0.125
C(1)	0.007				
C(2)	-0.007				
O(1)	0				
N(2)	-0.007				-0.128
C(3)	0.007				
C(4)				-0.001	
O(2)				0.005	
O(3)				-0.002	
C(5)		-0.011	(-0.037)	-0.002	
C(6)		0.005	-0.007		
N(3)		0.009	0.004		0.122
C(7)		-0.004	0.002		
N(4)		-0.013	-0.006		
C(8)		0.013	0.008		
O(3')					-0.119
Cu''				(0.040)	

Table 7. *Hydrogen bonds*

Code for symmetry-related atoms

Superscript	Atom at:			Superscript	Atom at:		
none	<i>x</i>	<i>y</i>	<i>z</i>	v	$1-y$	$-x$	$\frac{1}{2}-z$
'	$-\frac{1}{2}+x$	$\frac{1}{2}-y$	$\frac{1}{2}-z$	vi	$-\frac{1}{2}+y$	$\frac{1}{2}-x$	$\frac{1}{2}+z$
''	$\frac{1}{2}+x$	$\frac{1}{2}-y$	$\frac{1}{2}-z$	vii	$\frac{1}{2}+y$	$\frac{1}{2}-x$	$\frac{1}{2}+z$
'''	$1-y$	$1-x$	$\frac{1}{2}-z$	viii	$\frac{1}{2}-y$	$\frac{1}{2}+x$	$-\frac{1}{2}+z$
iv	$-y$	$1-x$	$\frac{1}{2}-z$	ix	$\frac{1}{2}-y$	$-\frac{1}{2}+x$	$-\frac{1}{2}+z$

(a) Hydrogen bond-lengths

Bond	Bond	Length (<i>l</i>)
O(1)···H-O(5)	O(5)-H···O(1''')	2.73 Å
O(1)···H-N(4 ^{vii})	N(4)-H···O(1 ^{ix})	2.78
O(2)···H-O(4 ^v)	O(4)-H···O(2 ^{iv})	2.92
O(2)···H-N(1 ^v)	N(1)-H···O(2 ^{iv})	2.91
O(3)···H-O(4 ^{viii})	O(4)-H···O(3 ^{vi})	2.92

(b) Angles at hydrogen-bonded atoms

Atoms	Angle	Atoms	Angle
Cu—N(1)···O(2 ^{iv})	110.6°	C(4)—O(3)···O(4 ^{viii})	138.8°
C(1)—N(1)···O(2 ^{iv})	93.3	Cu''—O(3)···O(4 ^{viii})	105.2
C(2)—O(1)···O(5)	115.6	C(7)—N(4)···O(1 ^{ix})	115.6
C(2)—O(1)···N(4 ^{vii})	143.2	C(8)—N(4)···O(1 ^{ix})	135.8
O(5)···O(1)···N(4 ^{vii})	99.9		
C(4)—O(2)···N(1 ^v)	149.7	Cu—O(4)···O(2 ^{iv})	98.9
C(4)—O(2)···O(4 ^v)	142.4	Cu—O(4)···O(3 ^{vi})	134.2
N(1 ^v)···O(2)···O(4 ^v)	63.8	O(2 ^{iv})···O(4)···O(3 ^{vi})	125.0
N(1 ^v)···O(2)—Cu''	128.4		
O(4 ^v)···O(2)—Cu''	71.2	O(1)···O(5)···O(1''')	98.7

Appropriate abbreviations will be used in the following discussion.

In $\text{Cu}(\text{Im})_2$ there are two independent types of Cu atom. One of these is almost square-planar while the other has N-Cu-N angles between 96° and 140° . The Cu-N bond-lengths are 1.99–2.00 Å around the former and 1.96–1.97 Å about the latter. The Cu atom in $\text{Cu}(\text{Gly-His}) \cdot 1\frac{1}{2}\text{H}_2\text{O}$ lies between these two examples in the degree to which the arrangement of its four closest donor atoms is distorted from square-planar towards tetrahedral. The bond Cu-N(3) appropriately has a length (1.98 Å) between the two sets in $\text{Cu}(\text{Im})_2$. In both structures, too, the Cu atoms deviate from the imidazole planes [0.47 Å in $\text{Cu}(\text{Gly-His}) \cdot 1\frac{1}{2}\text{H}_2\text{O}$, 0.17–0.53 Å in $\text{Cu}(\text{Im})_2$], and in both cases steric strains are accommodated by rotating the imidazole rings about the Cu-N bonds. The angles between the normals to the least-squares planes of the imidazole rings and the four closest ligands are, respectively, 33° and $31\text{--}75^\circ$.

The same flexibility characterizes the imidazole-metal binding in the three bishistidinato-metal complexes for which completed structure refinements are now available [$\text{Zn}(\text{L-His})_2 \cdot 2\text{H}_2\text{O}$, $\text{Zn}(\text{DL-His})_2 \cdot 5\text{H}_2\text{O}$ and $\text{Ni}(\text{DL-His})_2 \cdot \text{H}_2\text{O}$]. In each of the three complexes the bond between the metal and the imidazole nitrogen is bent out of the plane of the ring, and the orientation of the imidazole plane seems to be determined more by external forces than by any interaction between the imidazole group and the metal atom.

With Zn^{II} and Ni^{II} – as well as with Cd^{II} and Co^{II} – histidine acts as a tridentate chelate, in which the terminal $-\text{NH}_2$ group, imidazole ring and $-\text{COO}^-$ are bound to the same metal atom. In each complex, the $-\text{COO}^-$ group forms the weakest of the three links with the metal, the bonds ranging from an unequivocal octahedral covalency in $\text{Ni}(\text{DL-His})_2 \cdot \text{H}_2\text{O}$ to a weak interaction in an irregular direction, stabilized by intramolecular $-\text{NH}_2 \dots \text{O}(\text{CO})-$ hydrogen-bonding, in $\text{Zn}(\text{L-His})_2 \cdot 2\text{H}_2\text{O}$. These carboxyl oxygen-metal bonds do not lie in the plane of the two nitrogen-metal bonds. Their formation is sterically possible because the tetrahedral geometry of the NH_2 -nitrogen atoms allows the carboxyl groups to swing within bonding distances of the metal.

In the present dipeptide complex, no rotation of the carboxyl group about C(3)–C(4) can bring it into the apical coordination position with respect to the Cu atom attached to the other three binding sites on the same molecule (see Fig. 1). Neither does it seem possible to achieve carboxyl-Cu bonding by any reasonable distortion. The direction of C(3)–C(4) is approximately determined once those of N(2)–C(3) and C(3)–C(5) are fixed. Of these, N(2)–C(3) has virtually no degree of freedom, since the three bonds at the trigonal peptide nitrogen atom N(2) lie in a plane determined primarily by the requirements of the stable five-membered chelate ring. The bond C(3)–C(5) is part of the six-membered chelate ring; its direction is fixed once

the conformation of the ring is decided. In this structure, C(3)–C(4) is in a 'quasi-axial' orientation with respect to the ring. An examination of an accurate model shows that in the alternative conformation of the ring C(3)–C(4) points in a quasi-equatorial direction so that the carboxyl group is even further away from the Cu atom. This alternative conformation, however, also brings C(4) to a distance smaller than 3 Å from O(1). This seems to be the decisive factor in determining the sense in which the ring is puckered.

Geometrically an acceptable alternative to amino/peptide/imidazole coordination would be to have the Cu^{II} atom bound to the terminal amino, peptide and carboxyl groups of the dipeptide molecule, as in $\text{Cu}(\text{GlyGly}) \cdot 3\text{H}_2\text{O}$. In this event, the imidazole instead of the carboxyl group could form a bond to a second Cu^{II} atom. This is exactly what happens in $\text{Cu}(\beta\text{Ala-His}) \cdot 2\text{H}_2\text{O}$. The ligand in this complex differs from glycyl-L-histidine only by having an additional CH_2 group between C(1) and C(2). Chelation by the three functional nitrogen atoms of this molecule would lead to the formation of two adjacent six-membered rings. This would introduce intolerable strains, especially at the trigonal peptide nitrogen atom. In $\text{Cu}(\beta\text{Ala-His}) \cdot 2\text{H}_2\text{O}$, a five-membered ring with the carboxyl group therefore replaces the less stable of the hypothetical six-membered rings (that involving the imidazole group). In the six-membered chelate ring, the internal angle at N(2) is close to 130° [cf. Cu–N(2)–C(3), above].

Comparison with deductions from solution data

Glycyl-L-histidine is one of a number of histidine-containing peptides whose behaviour in solution, both in the presence and absence of copper(II) ions, has been extensively investigated. The subject has been reviewed by Gurd & Bryce (1966). The conclusions drawn from potentiometric titrations (Martin & Edsall, 1960; Bryce, Roeske & Gurd, 1965) and supported by absorption spectra and ORD measurements (Bryce & Gurd, 1966) are (i) that the initial Cu^{II} -binding site in glycyl-L-histidine is the 1-nitrogen of the imidazole ring, and (ii) that between pH 6 and 8 the predominant species in solution has Cu^{II} coordinated with the three nitrogen atoms belonging, respectively, to the imidazole, peptide and amino groups. These conclusions are supported in all respects by the present structure analysis.

The tetrahedral distortion of the 'coordination square' of the Cu atom is in qualitative agreement with the predictions based on the optical rotatory dispersion data (Bryce & Gurd, 1966; Gurd & Bryce, 1966). These authors correctly realized the need for puckering of the chelate ring containing the imidazole group, and suggested that this ring has a conformation which brings the carboxyl group within interacting distance of the metal atom. The structure analysis confirms that the ring is puckered, but also shows that no rotation of the carboxyl group about C(3)–C(4) nor any reasonable distortion can bring O(3) into a chelating

position with respect to the Cu atom. The most probable influence favouring one conformation of the six-membered chelate ring is steric hindrance between C(4) and O(1) in the alternative conformer (see *Comparison with related structures*). The interaction between the Cu atom and the carboxyl group of a *neighbouring* complex in the crystal is presumably replaced in solution by a bond to a water molecule.

The same features of the structure explain the further observation of Bryce, Roeske & Gurd (1965), that the imidazole side chains in two appropriate tetrapeptides formed chelate rings involving peptide groups only on the NH₂-terminal side, and not on the COO⁻-terminal side. If the peptide chain in the present complex were continued on the COO⁻-terminal side, then the next peptide nitrogen atom would occupy a position similar to that of O(3), making bonding from it to the Cu impossible. A peptide group on the COO⁻-terminal side of a histidine residue can therefore participate in chelation only if the imidazole ring binds a *different* Cu atom.

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