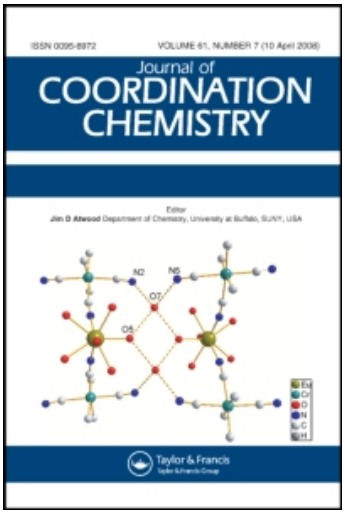


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

HISTIDINE AND HISTAMINE COMPLEXES OF COPPER AND ZINC

W. R. Walker[ab]; Yueh-Ho L. Shaw[ac]; Norman C. Li[a]
[a] Department of Chemistry, Duquesne University, Pittsburgh, Pa. [b] Department of Chemistry, The University of Newcastle, New South Wales, Australia [c] Yale University Medical School, New Haven, Conn

To cite this Article Walker, W. R. , Shaw, Yueh-Ho L. and Li, Norman C.(1973) 'HISTIDINE AND HISTAMINE COMPLEXES OF COPPER AND ZINC', Journal of Coordination Chemistry, 3: 1, 77 — 84
To link to this Article: DOI: 10.1080/00958977308073790
URL: http://dx.doi.org/10.1080/00958977308073790

J. Coord. Chem., 1973, Vol. 3, pp. 77–84

HISTIDINE AND HISTAMINE COMPLEXES OF COPPER AND ZINC†

W. R. WALKER,‡ YUEH-HO L. SHAW,§ and NORMAN C. LI

Department of Chemistry, Duquesne University, Pittsburgh, Pa. 15219

(*Received August* 14, 1972; *in final form Nov.* 1, 1973)

The mono-complexes Cu histamine Cl_2 and Cu histidine Cl_2 have been prepared for the first time and investigated. The rearrangement of the former in aqueous solution forming stable hydroxy-bridged binuclear copper(II) cations, with the release of protons, may be significant in an understanding of endogenous histamine activity.

Nmr data for zinc-histamine system show that when the amino group is protonated, the metal ion is coordinated at N(3). In the presence of zinc, copper(II) is coordinated at N(1) of histamine. For histidine, the line broadening of nmr signals by the paramagnetic copper(II) ion is not diagnostic of the sites of bonding.

Electronic spectra however, are informative and by studying the addition of imidazole to bis(glycinato)copper(II) in aqueous solution, which is a model for the Cu/histidine system, the following formation constant has been obtained: Cu(glycinate)$_2$ + imidazole = Cu(glycinate)$_2$·imidazole log K ~ 1.7. A spectrophotometric study of the copper(II) histidine system has shown that at *p*H 4.5–7.3 the Cu^{2+} has square-planar coordination with amino(N) and carboxyl(O) donor atoms with the pyridine(N) of the imidazole moiety interacting in the tetragonal site.

Experiments have also been carried out to see whether a recent claim for the non-enzymatic decarboxylation of aspartic acid to alanine was tenable. No evidence was forthcoming for this or for the decarboxylation of histidine to form histamine by heating Cu histidine Cl_2 (aq) to dryness in a vacuum oven.

INTRODUCTION

The interaction of copper and zinc with histamine and histidine has received particular attention recently. The physiological and pharmacological importance of histamine (which is formed by the *in vivo* decarboxylation of histidine) is reflected in its extensive literature. Indeed, chemical investigations of histidine often include histamine.

Recent reports on copper(II) histidine complexes include those by Meyer and Bauman,[1] Wilson, *et al.*,[2] and Sigel and McCormick.[3] These authors claim that the structure of the bis (histidine) copper(II) complex is "histamine-like", "glycine-like", or even both. A recent communication by Barnes and Pettit[4] suggests an interaction in the tetragonal sites between the imidazole nitrogen and copper which possesses square-planar coordination with the amino and carboxyl groups.

Copper(II) histamine complexes have been studied in solution by Mickel and Andrews[5] and more recently by Doran, *et al.*,[6] Perrin and Sharma,[7] Zarenbowitch,[8] Perrin, *et al.*,[9] Schubert, *et al.*,[10] Eilbeck, *et al.*,[11] and Beauchamp, *et al.*[12]. Perhaps of most significance are recent X-ray crystallographic structure determinations of bis (histamine) copper(II) tetrafluoroborate and perchlorate by Bonnet and Jeannin.[13,14] Interactions of zinc(II) with histamine and histidine have recently been discussed by various workers.[15–17]

The present work was carried out because of inconsistences in the literature. Another reason for investigating complexes of zinc and copper(II) with histidine and histamine was to see whether a recent claim by Marx *et al.*[18] concerning the non-enzymatic decarboxylation of aspartic acid, is tenable.

EXPERIMENTAL

Materials

L-histidine and L-histamine dihydrochloride were used as supplied by Merck (Germany) and L-histidine dihydrochloride by Mann Research. L-asparagine was supplied by Ajax Chemicals and L-alanine from Nutritional Biochemicals Corp.

Preparation of Compounds

Cu histamine Cl_2 was prepared by the method of Bridson and Walker[19] and was actually an attempt

† Supported by National Science Foundation, Grant GB-25117

‡ Department of Chemistry, The University of Newcastle, New South Wales, Australia

§ Yale University Medical School, New Haven, Conn.

to prepare Cu(histamine)$_2$Cl$_2$: a mixture of copper(II) hydroxide (1·0 g) and histamine dihydrochloride (3.5 g) in water (20 ml) was warmed on a water bath until all of the copper(II) hydroxide had reacted. The deep-blue solution was filtered, then left to stand. The green crystals which deposited after several days were filtered off and dried over P_2O_5 (vac.) Yield. 0.8 g.

Anal. Calcd. for $CuC_5H_9N_3Cl_2$: Cu 25.9; C, 24.5; H. 3.7; N, 17.5; Cl, 28.9. Found Cu, 25.2; C, 24.9; H, 3.9; N, 17.7; Cl, 29.1.

Cu histidine Cl$_2$ was prepared by mixing 25 ml ethanol solution containing $CuCl_2$ (2.7 g) with 50 ml aqueous solution containing histidine (3.1 g) and 10 M HCl (2 ml). Evaporation of the resultant blue solution finally yielded blue crystals which were recrystallized from water.

Anal. Calcd. for $CuC_6H_9N_3O_2Cl_2$: Cu, 21.9; Cl, 24.5. Found: Cu, 21.8; Cl, 24.0.

Attempts to prepare Cu (histidine)$_2$ Cl_2 by the addition of excess histidine to $CuCl_2$ in aqueous solution invariably resulted in the slow formation of dark-brown (almost black) products.

Attempts to prepare Zn (histamine)$_2$ Cl_2 and Zn histamine Cl_2 were unsuccessful. Bridson[20] had claimed preparation of the latter compound by the following method: A mixture of $Zn(OH)_2$ (0.5 g) and histamine dihydrochloride (1.84 g) in water (5 ml) was warmed on a water bath until all the $Zn(OH)_2$ had dissolved. The solution was then filtered and when NaOH (0.4 gm) in a little water was added, a white solid precipitated immediately. This was washed with water and dried P_2O_5 (vac.). Yield 0.3 g.

Anal. Calcd. for $ZnC_5H_9N_3Cl_2$; Zn, 26.4; Cl, 28.7; C, 24.3; H, 3.7; N, 17.0. Found: C, 24.7; H, 3.6; N, 17.2.

Because no metal and halide analyses were given for this "compound" the preparation was repeated on twice the scale. The white solid was dried over P_2O_5.

Anal. Found: Zn, 23.5; Cl, 7.4.

The material which Bridson[20] actually obtained was probably a mixture of $Zn(OH)_2$ and histamine hydrochloride.

Zn (histidine)$_2$.H$_2$O

$Zn(OH)_2$ (1 g) and L-histidine (3.1 g) were suspended in water (50 ml) and heated on the steam bath. After boiling on a hot plate, unreacted $Zn(OH)_2$ was filtered off and after reducing the volume to 18 ml, fine white crystals were obtained. These were dried over P_2O_5 (vac.).

Anal. Calcd. for $ZnC_{12}H_{18}N_6O_5$: Zn, 16.7. Found: Zn, 17.1.

Attempts to recrystallize this compound from water resulted in the precipitation of $Zn(OH)_2$ and release of histidine.

The following compounds were made for spectral studies:

Cu(glycine)$_2$.H$_2$O was prepared by reacting copper(II) acetate with glycine and recrystallized from water.

Anal. Calcd. for $CuC_4H_{10}N_2O_5$: Cu, 27.7. Found: Cu, 28.0.

Cu(L-asparagine)$_2$

L-asparagine (6.0 g) was dissolved in hot water (250 ml) and copper acetate monohydrate (4 g) in hot water (25 ml) was added. Mauve crystals appeared on cooling and after filtering off, these were washed with ethanol and dried over P_2O_5.

Anal. Calcd. for $CuC_8H_{14}N_4O_6$: Cu, 19.5. Found: Cu, 19.1.

Cu (L-alanine)$_2$

L-alanine (1.8 g) was dissolved in water (20 ml) and added to an aqueous solution of copper(II) acetate monohydrate (2 g) in water (60 ml). Evaporation down to a volume of 20 ml. resulted in the formation of deep-blue-violet crystals.

Anal. Calcd. for $CuC_6H_{12}N_2O_4$: Cu, 26.5. Found 25.7.

Cu aspartate.2H$_2$O

Copper(II) acetate monohydrate (4 g) was dissolved in hot water (25 ml) and filtered into a solution of L-aspartic acid (5.2 g) dissolved in 0.1 M NaOH (100 ml). The resultant pale-blue solid that precipitated was filtered off and washed well with water and then dried in vac. oven at $\sim 80°C$.

Anal. Calcd. for $CuC_4H_9NO_6$: Cu, 27.5. Found: Cu, 27.3%

INSTRUMENTAL

Nmr spectra were obtained with Varian A-60 nmr spectrometer at 60 MHz and at an ambient temperature of 33 $\pm$ 1°. Care was taken to keep the radiofrequency power level well below saturation and the field homogeneity such that a resolution of 0.3 Hz or better was attained. The chemical shifts of the important signals were measured with respect to 1% tetramethylsilane (TMS). The assignment of the histidine signals was according to

Carlson and Brown,[21] and of the histamine signals according to Varian NMR Spectra Catalog.[22]

Optical spectra were recorded with a Cary 14 recording spectrophotometer at room temperature. Quartz cells of 1, 5 and 10 cm were used depending on the solution concentrations. Measurements of *p*H were made with a Corning Model 10 *p*H meter with external electrodes. Only freshly prepared solutions were used.

RESULTS AND DISCUSSION

A. *Nature of Complexes*

Because it may be significant in understanding endogenous histamine activity, the mono-complex Cu histamine Cl_2 will be discussed first. It resulted from an attempt to make the bis-compound, Cu (histamine)$_2$ Cl_2, by reacting histamine dihydrochloride with copper(II) hydroxide. The possibility that this mono-complex might possess the "salt-like" structure [Cu(histamine)$_2$] [$CuCl_4$] has not been overlooked. Its diffuse reflectance spectrum, however, (Curve 5 of Fig. 1) shows a broad band centered around 680 nm. which is not indicative of a bis-complex. Thus the square-planar bis-complex, [Cu(en)$_2$] (ClO_4)$_2$ (en = ethylenediamine), absorbs at 520 nm.[20]

By using *p*H and conductimetric titrations with NaOH, the following rearrangement has been shown to occur in aqueous solution:[19]

$$2\,\text{Cuhistamine}\,Cl_2 \xrightarrow{H_2O} \text{Cu(histamine)}_2^{2+} + Cu^{2+} + 4Cl^- \rightleftarrows 2H_2O$$

$$[\text{histamine Cu}\langle^{OH}_{OH}\rangle\text{Cuhistamine}]^{2+} + 2H^+$$

I (I)

Curve 3 of Figure 1 is the spectrum of 10^{-3} M Cu histamine Cl_2 and curve 4 results after the addition of 2.5 moles of NaOH per mole of Cu histamine Cl_2. The absorption of 615 nm. is probably due to the binuclear-hydroxy-bridged species (I). This is analagous to the compound containing imidazole (instead of histamine) which, as reported by Bridson and Walker,[23] absorbs at 625 nm (ε 100). In calculating concentrations on the basis of a dimer, the value of ε must be doubled, so that ε for (I) is 98, not 49. Concerning the nature of the hydroxy-bridged copper(II) ion, it is relevant that the di-μ-hydroxy-bis (2,2′-bipyridyl) dicopper(II) ion absorbs at 620 nm (ε 105)[24] and its structure has been determined by Casey, *et al.*[25] Perrin and Sharma[7] have also shown that species (I) is the major hydrolyzed species present in copper(II) solutions containing 1:1 molar ratios of histamine. The fact that the mono-complex Cu histamine Cl_2 forms stable hydroxy-bridged cations with the release of protons may be significant in our understanding of endogenous histamine activity. It is to be noted that antihistamines such as antergan and benadryl, etc., contain tertiary amino groups that can remove protons.

The mono-complex Cu histidine Cl_2 merits discussion because it concerns the interaction of histidine with copper(II). It was made from acid solution as was Cu (histidine)$_2$ $(NO_3)_2 2H_2O$, the structure of which has been reported by Evertsson.[26] It must be emphasized that in this compound, coordination to copper is by the amino nitrogen and the carboxyl oxygen, in other words, coordination is "glycine-like".

In Figure 1, curve 1 shows that Cu histidine Cl_2 has λ_{max} 710 nm (ε 43). After the addition of NaOH

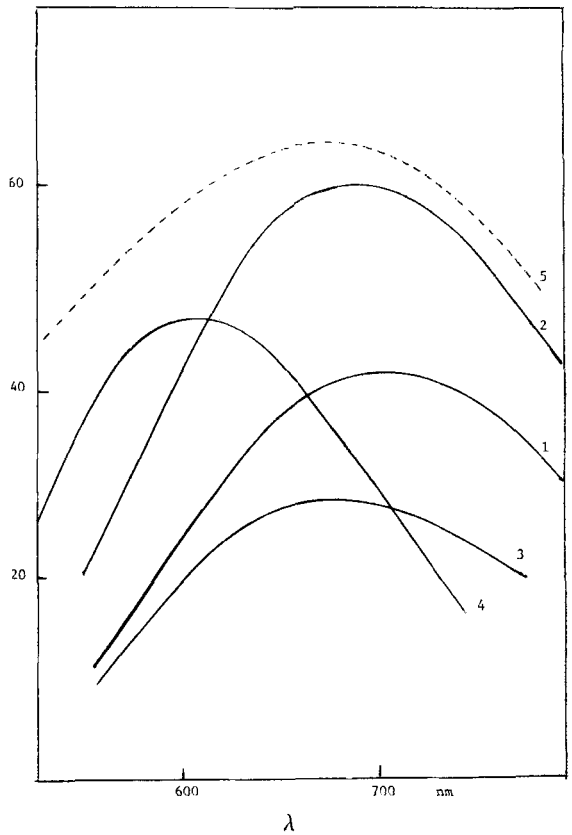


FIGURE 1 Spectra of mono-complexes: (1) 10^{-3}M Cu histidine Cl_2, (2) NaOH added (*p*H 5.9), (3) 10^{-3}M Cu histamine Cl_2, (4) NaOH added (*p*H 9.7), (5) solid Cu histamine Cl_2.

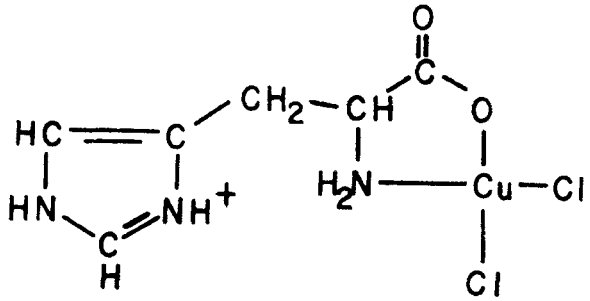
(*p*H changed to 5.9), the absorption shifts to 680 nm (ε 63) and this is shown in Figure 2. Since this blue complex was prepared from acid solution it may possess structure II or III.

The X-ray structure analysis of Zn(L-histidine)$_2$.H_2O was reported by Kretsinger, *et al.*[27], and that of Zn(D-L-histidine).5H_2O by Harding and Cole.[28] In the latter compound, four nitrogen atoms are tetrahedrally coordinated to zinc with the carboxyl groups loosely bound. The compound Zn(L-histidine)$_2$.H_2O, that was isolated in this investigation has been shown to rearrange in aqueous solution as follows:

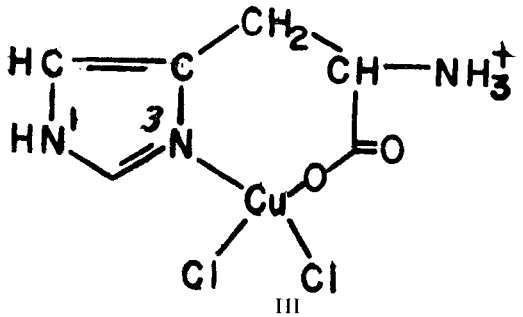
$$\text{Zn(histidine)}_2.H_2O \xrightarrow{H_2O} \text{Zn(OH)}_2\downarrow + 2\,\text{histidine} \qquad (2)$$

B. *Nmr Studies*

Table I shows that when 0.2 M $ZnCl_2$ was added to 0.2 M histamine (pD 6.3) the C_2H proton signal moves downfield 18 Hz, the C_5H proton signal moves only 7 Hz and the signal of the —CH_2CH_2— protons did not move. This could be explained by assuming that Zn^{2+} is coordinated at N(3) only, the amino nitrogen being protonated. Further evidence for this sort of coordination is afforded by the addition of Cu^{2+} to 0.2 M zinc chloride/histamine. The selective greater line broadening of C_2H and C_5H over that of —CH_2CH_2— indicates that Cu^{2+} bonds at N(1). Table I also shows that the addition of zinc chloride to histamine.2HCl has no effect on its nmr signals.



II



III

This is as expected, since both amino and imidazole nitrogens are protonated.

Nmr studies of the copper(II) histamine and the copper(II) histidine systems have been carried out by Sigel and McCormick.[3] On the basis of selective line broadening studies, they claimed that histidine coordinates to Cu^{2+} (aq.) in a "histamine-like" manner. According to them the CH proton adjacent to the amino group was broadened very strongly. However, as may be seen in Figure 2, all the proton signals of histidine are broadened by the paramagnetic copper(II). This could be taken as evidence for histidine acting as a tridentate ligand or that the line broadening of nmr signals may not be diagnostic of the binding sites. This opinion is shared by Wilson, *et al.*[2] concerning copper(II)-amino acid interactions.

The effect of acid on the nmr spectrum of histidine may also be seen in Figure 2. In histidine.2HCl both the amino nitrogen and the imidazole N(3) nitrogen are protonated, so that signals can be broadened only in the presence of high concentration of paramagnetic ions.

C. *Electronic Spectra*

Although nmr data may not be diagnostic of the bonding sites in the copper(II) histidine system, electronic spectra are informative. Figure 3 shows the absorption spectra of 0.001 M Cu^{2+} (aq.) to which increasing amounts of histidine have been added. The number of moles of histidine added (per mole of Cu^{2+}) is shown on each curve and the *p*H of each solution is given in parenthesis. These data may be interpreted by considering the ligand environment about the square-planar copper(II) ion as $Cu(N)_x(O)_y$ (where $x + y = 4$). The approach has been followed by various workers.[23, 29]

With no histidine added, the absorption at 760 nm is due to the Cu $(N)_0(O)_4$ chromophore of the aquated copper(II) ion. The addition of histidine may form species IV containing the $Cu(N)_2(O)_2$ chromophore absorbing at $\sim$ 630 nm. This may be compared to the spectrum of bis (glycinato) copper(II) which also possesses the $Cu(N)_2(O)_2$ chromophore and, as may be seen in Figure 4, has $\lambda_{max} \sim 630$ nm (ε 45). The bis-complexes Cu(alanine)$_2$ and Cu(asparagine)$_2$ absorb at $\sim$ 625 nm (ε 56) and 630 nm (ε 44) respectively.

It must be pointed out that the solutions of $CuCl_2$ containing excess histidine become brown on standing. The addition of NaOH to solutions of Cu(II): histidine (1:2) does not shift the observed maxima to longer wave lengths as claimed by

TABLE I

Nmr spectral data for histamine and its interaction with H^+, Zn^{2+} and Cu^{2+} in D_2O (All frequencies in Hz)

Solution	pD	C_2H	C_5H	$—CH_2—CH_2—$
0.2 M histamine	~8	484	441	~210
0.2 M histamine, 0.01 M $ZnCl_2$	7.4	489	443	~210
0.2 M histamine, 0.1 M $ZnCl_2$	6.4	502	448	~210
0.2 M histamine, 0.2 M $ZnCl_2$	6.3	502	448	~210
0.2 M histamine + 0.2 M $ZnCl_2$	6.3	502	448	~210
10^{-5} M $CuCl_2$ added	—	—no change—		
10^{-4} M $CuCl_2$ added	—	—broadened—		sharp
10^{-3} M $CuCl_2$ added	—	— flattened —		broadened
0.2 M histamine.2HCl	4.2	536	462	~220
0.2 M histamine.2HCl, 0.2 M $ZnCl_2$	—	536	462	~220

Sarkar and Wigfield[29] but, as may be seen in Table II to shorter wave length.

TABLE II

Spectral data for 0.001 M Cu^{2+}: histidine (1:2) solutions containing increasing amounts of NaOH measured as soon as possible after preparation. (Before adding NaOH λ_{max} = 630 nm, ε 36)

*p*H	4.3	5.6	10.2[a]	12.6[a]
λ_{max}	630	630	630	610
ζ	48	70	86	72

[a] Solutions become brown on standing with an intense charge-transfer band being present in the near UV.

To return to Figure 3, the addition of more histidine moves the absorption maxima to the red (645 nm) and when the histidine/copper ratio increased to 50 to 200, there is little further change in absorption. It is possible that at this stage, there may be the type of interaction suggested by Barnes and Pettit[4] and the existence of species V. Concerning this interaction between an imidazole N atom and the Cu^{2+} ion along the z axis, Barnes and Pettit[4] have stated "this will only be if either both ligands are of the same optical hand and are oriented *cis* on the copper, or are of opposite hand *trans* on the copper". It is relevant to note that

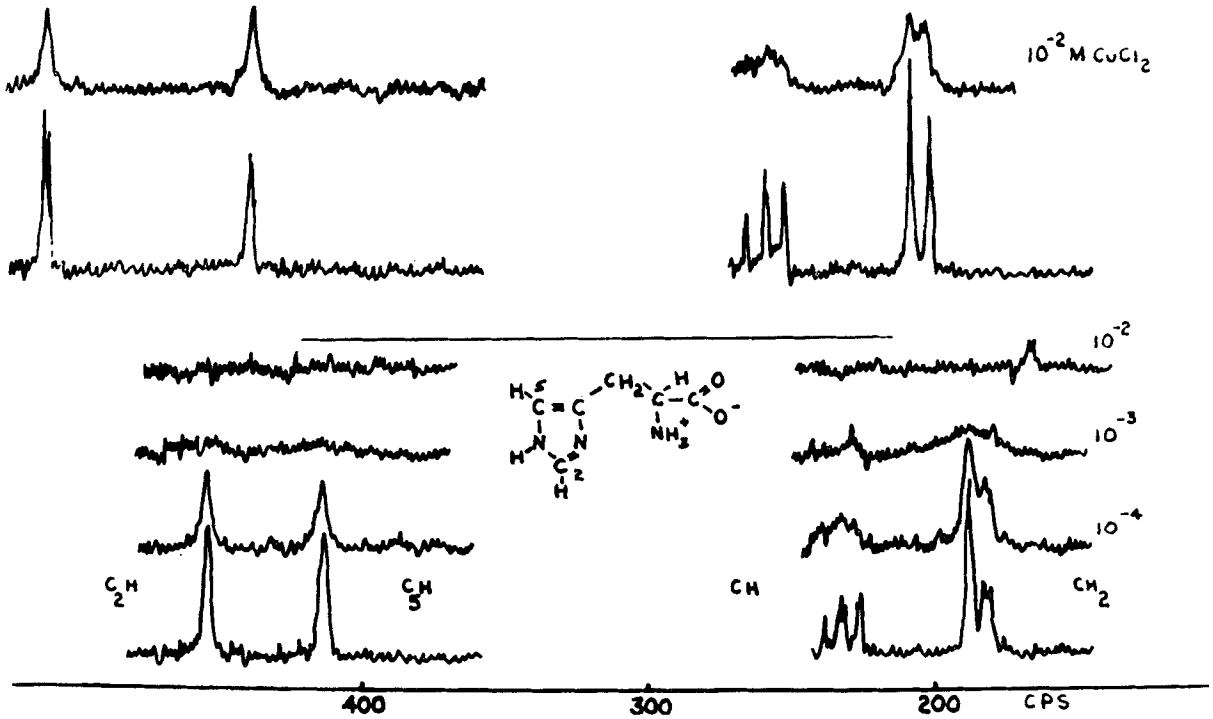


FIGURE 2 Effect of Cu^{2+} on nmr spectra of 0.18 M histidine (bottom four) and 0.18 M histidine dihydrochloride (top two) in D_2O.

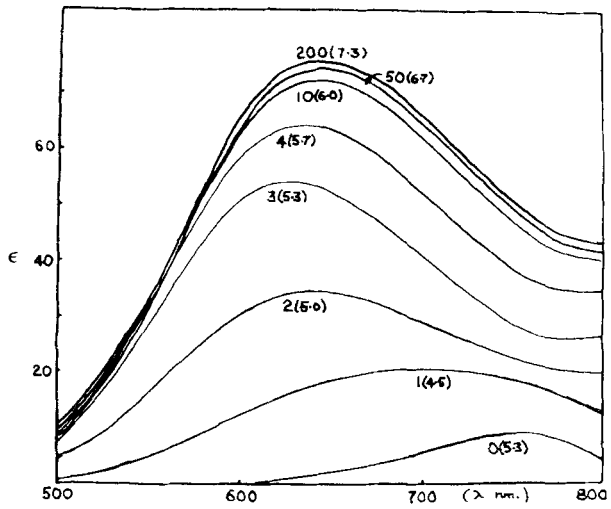


FIGURE 3 Spectra of 10^{-3}M CuCl solution containing increasing amounts of histidine. Numbers are mole ratios histidine/Cu^{2+}. *p*H of solutions in parenthesis.

X-ray crystallography has revealed that the structure of Co (L-histidine)$_2$[30] and Co(D-histidine)(L-histidine)[31] are radically different with the two imidazole groups *cis* and *trans*- respectively, to one another.

According to a report by Morris and Martin,[16] the *trans* disposition of groups would be favored in solutions of 2:1 complexes of bidentate amino acids and tetragonal metal ions such as copper(II). Since L-histidine has been used in this investigation and further, since species IV has the histidine ligands *trans* to each other, structure V is proposed. Here the pyridine nitrogen of one imidazole ring is interacting in one tetragonal site giving copper an essentially square-pyramidal configuration. Of course a third histidine molecule or a solvent molecule could interact in the other tetragonal site.

Coordination of the amino nitrogen and the carboxyl oxygen to copper(II) has been shown by Evertsson[26] to exist in bis (L-histidinato) copper(II) dinitrate dihydrate. This X-ray structure analysis is the only one so far reported for a bis (histidinato) copper(II) complex.

D. *The System Copper/Glycinate/Imidazole.*

To obtain further evidence for the presence of species V in aqueous solution, a spectral study was carried out in which increasing amounts of imidazole (HIm) was added to bis (glycinato) copper(II) monohydrate. This system was chosen because in solution the glycine complex is assumed to have structure VI (a $Cu(N)_2(O)_2$ environment) which Klotz, *et al.*,[32] have shown exists from *p*H 4 to *p*H 8. If imidazole interacts in the tetragonal sites,

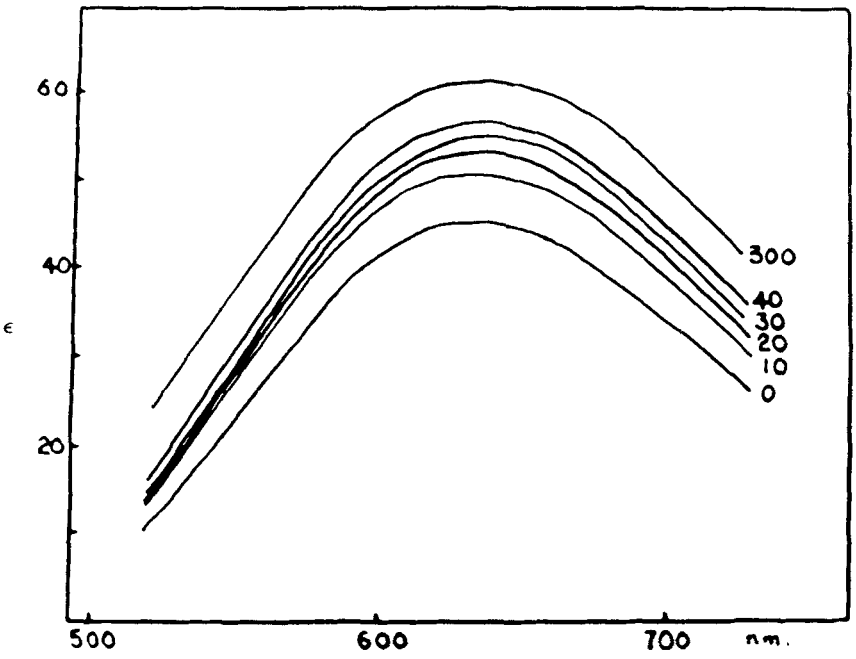
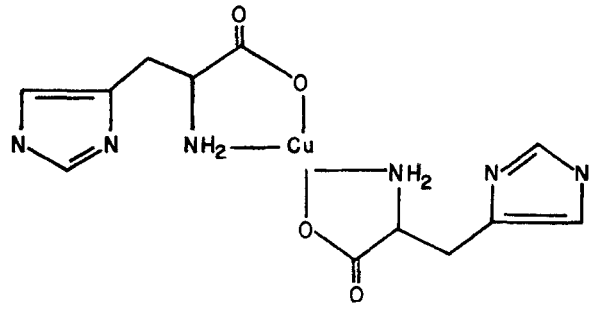
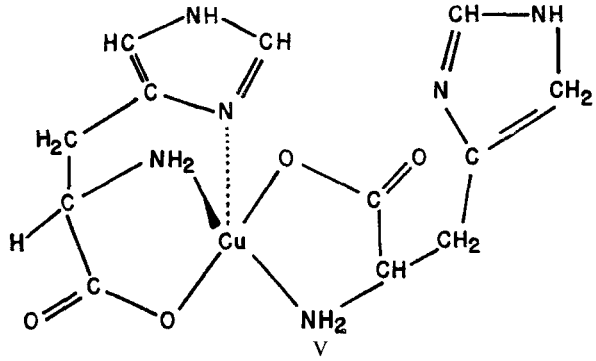


FIGURE 4 Spectra of 10^{-3}M Cu(glycinate)$_2$ containing increasing amounts of imidazole. Numbers are mole ratios imidazole: Cu(glycinate)$_2$.

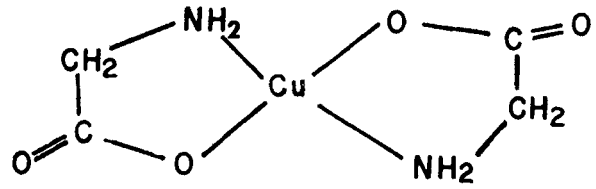
then the spectra should be similar to those of Figure 3. An inspection of Figure 4 shows striking resemblance. It has also been shown that the changes in the spectra on the addition of up to 300 moles of imidazole per mole of Cu(glycinate)$_2$ is not due to a change in *p*H. This was proved by adding NaOH to solutions containing 10 moles imidazole/mole Cu(glycinate)$_2$, giving *p*H 8.7 to 9.5.



IV



V



VI

Further evidence for the interaction of imidazole, [HIm], and Cu(glycinate)$_2$ was obtained by evaluating formation constants, according to the spectrophotometric method of Graddon and Watton[33] at a give wavelength:

$$[\text{Cu(glycinate)}_2(\text{HIm})] = \frac{\epsilon - \epsilon_0}{\epsilon_\infty - \epsilon_0} \times [\text{total copper}] \quad (3)$$

where ε_0 = molar absorptivity with no imidazole added

ε_∞ = molar absorptivity for 100% Cu(glycinate)$_2$ (HIm)

ε = a given value of molar absorptivity

It follows that

$$[\text{Cu(glycinate)}_2] = \frac{\epsilon_\infty - \epsilon}{\epsilon_\infty - \epsilon_0} \times [\text{total copper}] \quad (4)$$

$$\text{and } [\text{HIm}] = [\text{HIm}]_{\text{init.}} - [\text{Cu(glycinate)}_2(\text{HIm})] \quad (5)$$

The equilibrium constant is then given by

$$K_1 = \frac{[\text{Cu(glycinate)}_2(\text{HIm})]}{[\text{Cu(glycinate)}_2][\text{HIm}]} \quad (6)$$

In case a bis-imidazole complex is assumed to be formed, then

$$K_2 = \frac{[\text{Cu(glycinate)}_2(\text{HIm})_2]}{[\text{Cu(glycinate)}_2][\text{HIm}]^2} \quad (7)$$

$$\text{and } [\text{HIm}] = [\text{HIm}]_{\text{inst.}} - 2\,[\text{Cu(glycinate)}_2(\text{HIm})_2]. \quad (8)$$

Table III gives the values of log K_1 and log K_2, calculated by equations 6 and 7, respectively. It is seen that a consistent set of K values is obtained only for the reaction

$$\text{Cu(glycinate)}_2 + \text{HIm} = \text{Cu(glycinate)}_2(\text{HIm}) \quad (9)$$

with log $K_1 \sim 1.7$. This is much smaller than the value[34] of log K = 3.8 for the reaction

$$\text{Cu(glycylglycinate)} + \text{HIm} = \text{Cu(glycylglycinate)}_2(\text{HIm}) \quad (10)$$

TABLE III

Equilibrium Constants for the System of Cu(glycinate)$_2$ and Imidazole

$[\text{Cu(gly)}_2]_{\text{init.}}$	$[\text{HIm}]_{\text{init.}}$	ε640	log K_1	log K_2
1×10^{-3}M	0	45.0		
1×10^{-3}	1×10^{-3}	46.8	2.1	5.3
1×10^{-3}	5×10^{-3}	48.3	1.7	4.0
1×10^{-3}	10×10^{-3}	50.0	1.6	3.7
1×10^{-3}	20×10^{-3}	52.5	1.6	3.3
1×10^{-3}	30×10^{-3}	54.5	1.6	3.2
1×10^{-3}	40×10^{-3}	57.0	1.8	3.2
1×10^{-3}	∞ ($> 100 \times 10^{-3}$)	62.0		

When imidazole was added to solutions of Cu(alanine)$_2$ and also Cu(asparagine)$_2$, similar spectra to those of imidazole and Cu(glycinate)$_2$ system, Figure 4, were obtained. Because of the limited solubility, however, K values were not obtained.

(E) *Non-enzymatic decarboxylation*

In a recent communication Marx, *et al.*,[18] claimed to have produced alanine in 0.2% yield by

the non-enzymatic decarboxylation of aspartic acid. This was supposedly achieved by the evaporation to dryness of an aqueous solution of copper(II) sulfate and aspartic acid (1:4) and its subsequent heating to 100°C for four hours. Because of the biological importance of histidine and its decarboxylation to histamine, attempts were made to decarboxylate histidine and aspartic acid by heating their copper(II) complexes in a vacuum-oven.

Copper(II) aspartate dihydrate was in fact dried in a vacuum oven at 80°C and therefore does not decarboxylate on heating. By using infrared and electronic spectral studies, no evidence of the formation of alanine was obtained by heating aspartic acid in the presence of copper. The aspartic acid used by Marx *et al.* could have contained 0.2% alanine as impurity. By similar methods, no evidence was obtained for the decarboxylation of histidine to histamine by heating Cu Histidine Cl_2 (aq.) to dryness in a vacuum oven.

REFERENCES

1. J. L. Meyer and J. E. Bauman, *J. Amer. Chem. Soc.* **92,** 4210 (1970).
2. E. W. Wilson, M. H. Kasperian and R. B. Martin, *J. Amer. Chem. Soc.* **92,** 5365 (1970).
3. H. Sigel and D. B. McCormick, *J. Amer. Chem. Soc.* **93,** 2041 (1971).
4. D. S. Barnes and L. D. Pettit, *Chem. Commun.* 1000 (1970).
5. B. L. Mickel and A. C. Andrews, *J. Amer. Chem. Soc.* **77,** 5291 (1955).
6. M. A. Doran, S. Chabarek and A. E. Martell, *J. Amer. Chem. Soc.* **86,** 2129 (1964).
7. D. D. Perrin and V. S. Sharma, *J. Inorg. Nucl. Chem.* **28,** 1271 (1966).
8. J. Zarembowitch, *J. Chim. Phys.* **63,** 420 (1966).
9. D. D. Perrin, I. G. Sayce and V. S. Sharma, *J. Chem. Soc.* (A), 1755 (1967).
10. J. Schubert, V. S. Sharma, E. R. White and L. S. Bergelson, *J. Amer. Chem. Soc.* **90,** 4476 (1968).
11. W. J. Eilbeck, F. Holmes and T. W. Thomas, *J. Chem. Soc.* (A), 113 (1969).
12. A. L. Beauchamp, J. Israeli and H. Saulnier, *Can. J. Chem.* **47,** 1269 (1969).
13. J. J. Bonnet and Y. Jeannin, *C.R. Acad. Sci., Ser. C.* **270,** 1329 (1970).
14. J. J. Bonnet and Y. Jeannin, *Acta. Cryst.*, (B), **26,** 318 (1970).
15. D. R. Williams, *J. Chem. Soc.*, (A), 1550 (1970).
16. P. J. Morris and R. B. Martin, *J. Inorg. Nucl. Chem.* **32,** 2891 (1970).
17. B. Rao and H. B. Mathur, *J. Inorg. Nucl. Chem.* **33,** 809 (1971).
18. A. Marx, M. Sendrea and M. Petcovier, *Bull. Soc. Chim. Biol.* **52,** 353 (1970).
19. M. E. Bridson and W. R. Walker, unpublished results.
20. M. E. Bridson, Ph.D. Thesis, University of Newcastle, N.S.W., Australia, 1971.
21. R. H. Carlson and T. L. Brown, *Inorg. Chem.* **5,** 268 (1966).
22. N. S. Bhacca, L. F. Johnson and J. N. Shoolery, NMR Spectra Catalog, Analytical Instrument Division, Varian Associates, 1962.
23. M. E. Bridson and W. R. Walker, *Aust. J. Chem.* **23,** 1973 (1970).
24. C. M. Harris, E. Sinn, W. R. Walker, and P. R. Wolliams, *Aust. J. Chem.* **21,** 631 (1968).
25. A. T. Casey, B. F. Hoskins and F. D. Whillans, *Chem. Comm.* 904 (1970).
26. B. E. Evertsson. *Acta Cryst.* B **25,** 30 (1969).
27. R. H. Kretsinger, F. A. Cotton and R. F. Bryan, *Acta. Cryst.* **16,** 651 (1963).
28. M. M. Harding and S. J. Cole, *Acta. Cryst.* **16,** 643 (1963).
29. B. Sarkar and Y. Wigfield, *J. Biol. Chem.* **242,** 5575 (1967).
30. M. M. Harding and H. A. Long, *J. Chem. Soc.* (A), 2554 (1968).
31. R. Candlin and M. M. Harding, *J. Chem. Soc.* (A), 384 (1970).
32. I. M. Klotz, I. L. Fuller, J. M. Urguhart, *J. Phys. Chem. Colloid Chem.* **54,** 18 (1950).
33. D. P. Graddon and E. C. Watton, *J. Inorg. Nucl. Chem.* **21,** 49 (1961).
34. R. Driver and W. R. Walker, *Aust. J. Chem.* **21,** 671 (1968).