Electron Spin Resonance Study of Copper(II)–Amino-acid Complexes: Evidence for *cis* and *trans* Isomers and the Structures of Copper(II)–Histidinate Complexes in Aqueous Solution

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Electron spin resonance spectra are reported for copper(II)–amino-acid complexes $[CuL]^{n+}$ and $[CuL_2]^{m+}$ (for 12 α-amino-acids) and $[Cu(HL)L]^+$ (HL = L-histidine). Buffer solutions of aqueous copper(II) and ligand were prepared to give optimum concentrations of these complexes. Isotropic g and A values are in the ranges 2.144—2.157 and 5.5—6.5 mT respectively for $[CuL]^{n+}$ and 2.118—2.127 and 6.4—7.1 mT respectively for $[CuL_2]^{m+}$. The high-field absorption for $[CuL_2]^{m+}$ complexes showed nitrogen hyperfine structure, which for ^{63}Cu and solvent D_2O could be resolved in the second derivative into two overlapping quintet components characteristic of *cis* (*N, N, O*$^-$, *O*$^-$) and *trans* (*N, O*$^-$, *N, O*$^-$) isomers respectively. Spectra have been fitted to this model by computer simulation. From observed nitrogen hyperfine structure, and by comparison with spectra of other amino-acid complexes and that of bis(histamine)copper(II), it is deduced that the histidinate complex $[Cu(HL)L]^+$ has three nitrogen atoms co-ordinated to copper in the planar co-ordination sites, whereas $[CuL_2]$ appears to consist of a mixture of structures having three and four nitrogen atoms bound to the copper.

RECENT e.s.r. work on the uptake of copper(II) by wheat roots has established that the copper(II) which is adsorbed from solution is complexed by amino-acids.[1,2] The present study has been undertaken to characterise the e.s.r. spectra of the 1 : 1 and 1 : 2 complexes formed in aqueous solution between copper(II) and a selection of amino-acids found in growing wheat roots, *viz.* glycine, α-alanine, valine, leucine, lysine, asparagine, aspartic acid, glutamine, glutamic acid, phenylalanine, proline, and histidine.[3,4]

Many workers have studied the e.s.r. spectra of copper(II)–amino-acid complexes as dilute powders,[5,6] glasses,[5,7,8] and frozen solutions.[5,9–12] However, such spectra do not generally reveal the nitrogen super-hyperfine structure shown by solution spectra,[13] which can indicate the number of nitrogen atoms co-ordinated to the copper(II) ion. In the present work the isotope ^{63}Cu has been used along with solvent D_2O in order to obtain maximum resolution of any nitrogen super-hyperfine structure. By recording second-derivative spectra, this structure has been used to characterize the nature of the co-ordination to copper of the various α-amino-acids, and also the amine, histamine, for comparison with histidine.

EXPERIMENTAL

The amino-acids, L-glutamine (Cambrian Chemical Co.), glycine and L-lysine monohydrochloride (L. Light and Co.), L-proline, L-phenylalanine, L-alanine, L-histidine monohydrate, L-asparagine, L-glutamic acid, L-aspartic acid, and DL-valine (B.D.H. Biochemicals), and the amine, histamine dihydrochloride (Koch-Light), were used without further purification.

A FORTRAN computer program was used to calculate the percentage distribution of copper(II), amongst the complexes [CuL], $[CuL_2]$, [Cu(HL)], [Cu(HL)L], $Cu^{2+}(aq)$, $[Cu(OH)]^+$, and $[Cu_2(OH)_2]^{2+}$ as a function of $p[H^+]$ ($= -\log_{10}[H^+]$) for given metal : amino-acid (H_nL) ratios (the overall charges on the amino-acid complexes depend on the acid used and have been omitted for convenience). Literature values for the ligand protonation constants,[14] β_i, complex formation constants,[14] K_i, and copper(II) hydrolysis constants [15] were used in the calculations; values of β_i and K_i were chosen for 25 °C and 0.10 mol dm^{-3} $K[NO_3]$ where possible. Acidified solutions of A.R. grade copper(II) chloride (5×10^{-3} mol dm^{-3}) and amino-acids (M : H_nL = 1 : 1, 1 : 2, and 1 : 5) in $K[NO_3]$ buffers (I = 0.1 mol dm^{-3}) were titrated with Na[OH] (*ca.* 1 mol dm^{-3}) to $p[H^+]$ values corresponding to maximum concentrations of [CuL], $[CuL_2]$, or [Cu(HL)L]. Values of pH were measured with an EIL 7300 pH meter coupled with EIL all-purpose glass and calomel electrodes. The measured pH was converted to $p[H^+]$ by the use of the relationship derived from phthalate buffers in 0.10 mol dm^{-3} $K[NO_3]$, pH (measured) = 1.012 $p[H^+]$ + 0.037.[16]

Solutions of complexes $[^{63}CuL_2]$ were prepared in D_2O under an N_2 atmosphere from weighed quantities of ^{63}Cu-$[SO_4]$ and ligand. The solutions (1.0 cm^3) were titrated (Alga micrometer syringe) with Na[OD] (0.62 mol dm^{-3}) to complete the reaction at pH 6.5—7.0.

$$Cu^{2+} + 2H_nL + 2nOH^- \longrightarrow [CuL_2] + 2nH_2O$$

Electron spin resonance spectra were recorded on a Varian E104 spectrometer operating at 9.51 MHz. The field strength was checked against strong pitch and aqueous manganese(II) as calibrants.

Visible absorption spectra were recorded for solutions in 1-cm cells using a Unicam SP700 spectrophotometer.

RESULTS AND DISCUSSION

Amino-acid Complexes $[CuL]^{n+}$ *and* $[CuL_2]^{m+}$.—Calculations of the percentage distribution of copper amongst the various complexes, illustrated in Figure 1 for the copper–glutamic acid system, show that it is possible to obtain solutions where the copper is present entirely as the bis complex $[CuL_2]^{m+}$, but that the mono-complex $[CuL]^{m+}$ is always accompanied by other species. Electron spin resonance spectra of solutions prepared to contain the maximum amounts of $[CuL]^{n+}$ and $[CuL_2]^{m+}$ are shown in Figure 2 for the glutamine system. The isotropic spectra consist of four ($2I$ + 1)

absorption peaks (as first derivatives) arising from coupling of the electron spin dipole ($S = \frac{1}{2}$) with the copper nuclear spin dipole ($I = \frac{3}{2}$). The spectrum in Figure 2(*a*), nominally from $[CuL]^{n+}$, shows a small high-field component arising from the presence of a small amount of $[CuL_2]^{m+}$; also the shape of the low-field peak is affected by the presence of some $Cu^{2+}(aq)$, which in solution at room temperature gives a single broad peak. The spectrum in Figure 2(*b*) arises entirely from the $[CuL_2]^{m+}$ species.

The spectra in Figure 2 indicate that distinctive e.s.r. parameters characterize the mono and bis complexes of a given ligand and Table 1 reports the isotropic g and A values ($g_{iso.}$ and $A_{iso.}$ respectively) for the $[CuL]^{n+}$ and $[CuL_2]^{m+}$ α-amino-acid complexes. For $[CuL]^{n+}$ most of the $g_{iso.}$ values are in the range 2.148 to 2.152, with a distinctively low value for histidine of 2.144, and high value for glycine of 2.157. The $A_{iso.}$ values are in the range 5.5 to 6.5 mT. For the $[CuL_2]^{m+}$ complexes $g_{iso.} = 2.118$ to 2.127 and $A_{iso.} = 6.4$ to 7.1 mT. Dezor [17] has reported somewhat different values for $g_{iso.}$ and $A_{iso.}$ for the glycinate complexes $[CuL]^{+}$ (2.148 and 5.14 mT respectively) and $[CuL_2]$ (2.125 and 6.25 mT respectively). Misra and Sharma [5] have reported values of $g_{iso.} = 2.148$ and 2.121 for unbuffered solutions, which (we infer) contained the

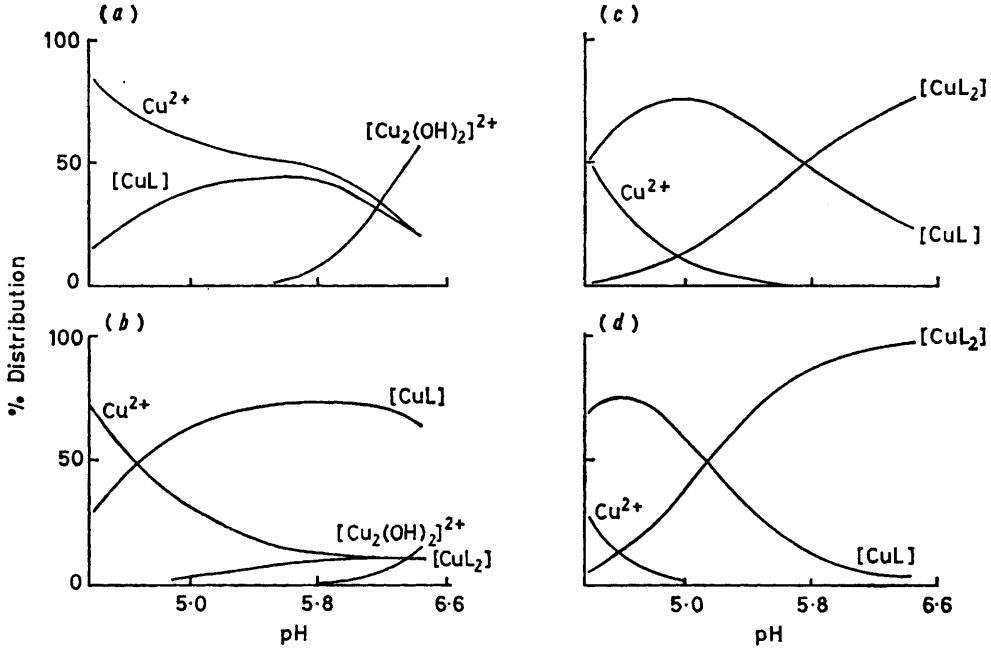

FIGURE 1 Variation of composition of copper(II)–glutamic acid solutions with pH for copper(II) concentrations of 5.0×10^{-3} mol dm^{-3} and Cu : L ratios of (*a*) 2 : 1, (*b*) 1 : 1, (*c*) 1 : 2, and (*d*) 1 : 5

TABLE 1

E.s.r. and visible absorption parameters for copper(II) amino-acid complexes in aqueous solution ($I = 0.10$ mol dm^{-3}, $K[NO_3]$) and in D_2O (^{63}Cu) *

	$[CuL]^{n+}$			$[CuL_2]^{m+}$				
				natural Cu		^{63}Cu		
Ligand	$g_{iso.}$	$A_{iso.}$/mT	$\lambda_{max.}$/nm	$g_{iso.}$	$A_{iso.}$/mT	$g_{iso.}$	$A_{iso.}$/mT	$\lambda_{max.}$/nm
Glutamic acid	2.152	5.6	723	2.122	7.1	2.121	6.85	620
Asparagine	2.149	5.5	731	2.121	6.9	2.123	6.7	613
Proline	2.148	6.0	725	2.118	7.0	2.120	6.8	604
Glycine	2.157	5.8	728	2.126	6.7	2.127	6.6	619
Leucine	n.d.	n.d.	n.d.	n.d.	n.d.	2.123	6.8	n.d.
Phenylalanine	n.d.	n.d.	n.d.	n.d.	n.d.	2.125	6.8	n.d.
Aspartic acid	2.149	5.6	729	2.120	6.7	2.126	6.4	621
Lysine	n.d.	n.d.	n.d.	n.d.	n.d.	2.126	6.7	n.d.
Valine	2.151	6.0	n.d.	2.122	7.0	2.120	6.65	609
Histidine	2.144	6.5	695	2.119	7.1	2.119_5	7.0	642
Glutamine	2.151	5.9	715	2.123	7.0	2.123	6.75	610
Alanine	2.149	5.9	731	2.119	6.9	2.123	6.7	612
(Histamine)	2.145	6.8	690	n.d.	n.d.	2.110_5	7.7	604

* Values are considered accurate to ±0.001 and A values to ±0.02 mT; n.d. = not determined.

mono(L-glutamate) and bis(DL-aspartate) complexes respectively. For the bis(histidinate) complex $[CuL_2]$, Sarker *et al.*[18] reported $g_{iso.} = 2.122$ and $A_{iso.} = 7.00 \pm 0.2$ mT (M : L = 1 : 10; pH = 7.4). No nitrogen superhyperfine structure was reported in any of these works.

For ligands other than histidine the visible absorption spectra are consistent with two nitrogen atoms co-ordinated in $[CuL_2]^{m+}$ ($\lambda_{max.}$ 604—621 nm) and one nitrogen co-ordinated in $[CuL]^{n+}$ ($\lambda_{max.}$ 715—731 nm).

Nitrogen Hyperfine Structure: $[CuL_2]$ *Complexes.*—For several of the ligands studied the high-field absorption showed poorly defined superhyperfine structure in the first-derivative spectra as shown for the bis(glutamate)

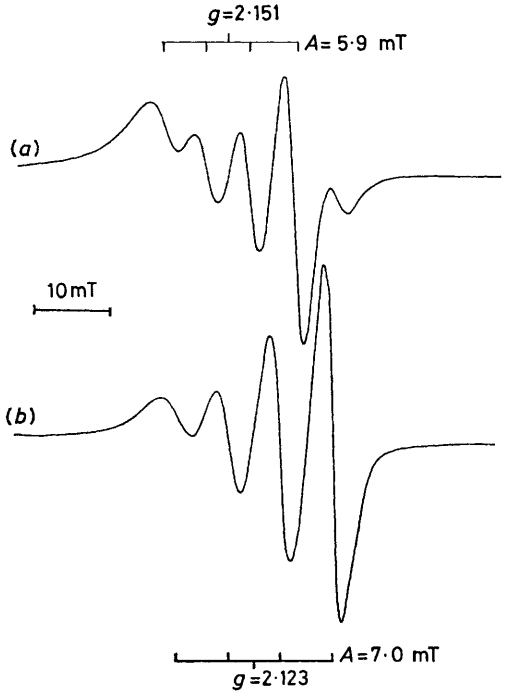

FIGURE 2 First-derivative e.s.r. spectra of copper–glutamine complexes: (*a*) $[CuL]^+$ and (*b*) $[CuL_2]$

complex in Figure 3(*a*). This structure may be ascribed to the effects of (*i*) nitrogen superhyperfine splitting, and (*ii*) the presence of two copper isotopes, ^{63}Cu (69.1%) and ^{65}Cu (30.9%), which have slightly different magnetic moments and for which the outermost peaks will differ by *ca.* 0.7 mT for $A_{iso.} = 7.0$ mT. By use of $^{63}Cu[SO_4]$ the nitrogen superhyperfine structure was enhanced and the peak widths reduced [Figure 3(*b*)]. Further improvements in resolution were obtained by using solvent D_2O [Figure 3(*c*)]. Resolution of this superhyperfine structure was further enhanced in second-derivative spectra, as shown in Figure 4(*a*) for the high-field copper absorption peak of the bis(L-asparagine) complex. (No superhyperfine structure was observed on the other three copper hyperfine peaks.) This spectrum is characterized by an asymmetric septet, as are those for the L-glutamate, L-glutamine, L-alanine, L-proline, L-valine, L-leucine, and L-phenylalanine complexes. For aspartic acid the two low-field components of the septet were incompletely resolved, while for glycine a single component with a shoulder at high field was observed.

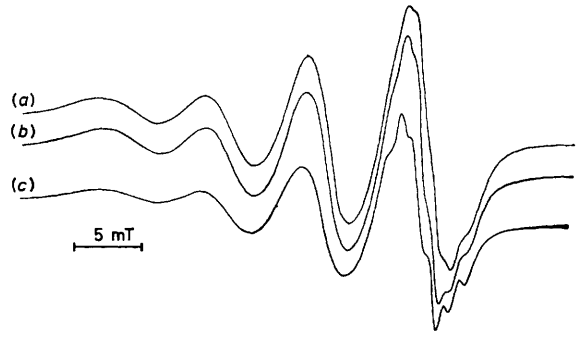

FIGURE 3 First-derivative e.s.r. spectra of the bis(glutamate)-copper(II) complex using (*a*) natural copper and solvent H_2O, (*b*) ^{63}Cu isotope and solvent H_2O, and (*c*) ^{63}Cu isotope and solvent D_2O

The superhyperfine structure may be interpreted in terms of two overlapping quintets, each with intensity ratio 1 : 2 : 3 : 2 : 1, arising from (an equilibrium mixture of) *cis* and *trans* bis(ligand) complexes, each complex having two nitrogen atoms ($I = 1$) and two oxygen atoms co-ordinated to the metal ion. Spectra simulated for this model are in close agreement with observed spectra, as illustrated in Figure 4(*b*). In each case the nitrogen superhyperfine coupling constant was slightly

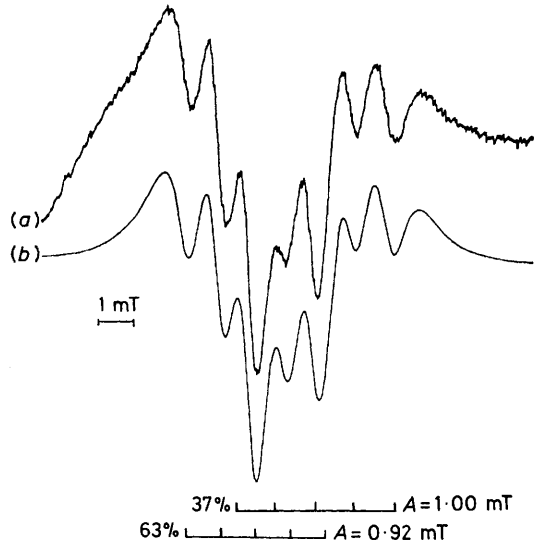

FIGURE 4 (*a*) Second-derivative e.s.r. spectrum of the high-field absorption peaks for the bis(asparagine)copper(II) complex using ^{63}Cu and solvent D_2O. (*b*) Computer simulation of the above spectrum using two quintets with relative peak areas of 1 : 2 : 3 : 2 : 1 and Lorentzian line shapes. Positions and relative amounts are shown in the accompanying stick diagram

larger for the higher-field quintet than for that at lower field. The latter quintet is assigned to the *trans* isomer in which there will be a smaller net electron delocalisation onto the nitrogen atoms, in accord with the *trans*

effect. The ratio of *cis* to *trans* co-ordination can, therefore, be determined from the spectra and is given in Table 2 along with the values of the ^{14}N superhyperfine splitting for each species.

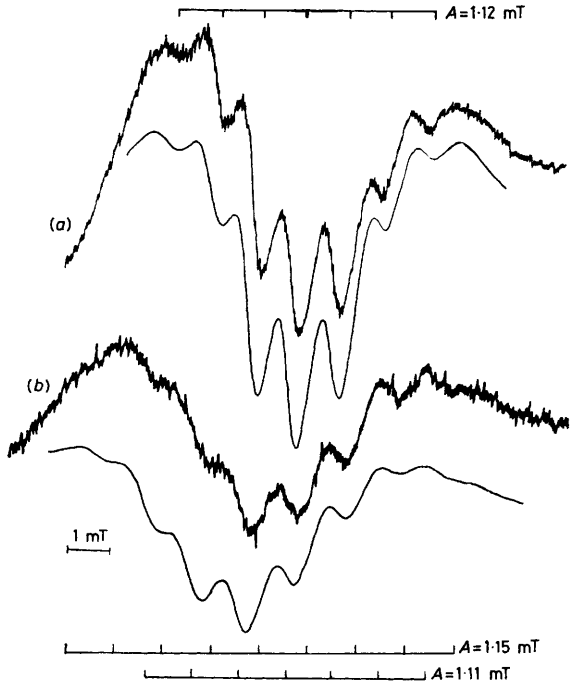

FIGURE 5 Second derivative of the high-field copper absorption in the e.s.r. spectra of ^{63}Cu–histidine complexes in D_2O. (*a*) [Cu(HL)L] spectrum along with a computer simulation assuming a septet of peaks with relative intensities 1 : 3 : 6 : 7 : 6 : 3 : 1 and positions shown in the stick diagram, and (*b*) [CuL_2] spectrum, with a computer simulation assuming a mixture of equal amounts of a septet (as above) and a nonet with relative intensities 1 : 4 : 10 : 16 : 19 : 16 : 10 : 4 : 1 having peaks in the positions in the stick diagram

Zand and Palmer [8] observed that copper(II)–amino-acid complexes, for which single-crystal *X*-ray structure analyses had been reported, were all *trans* except for the glycinate (for which a second uncharacterized form is also known).[18] Also, Gillard *et al.*[19] noted that the *trans* bis(α-alaninato)-complex converts slowly to the *cis* isomer by prolonged equilibration in water, suggesting that the configuration adopted in the solid state may be determined by kinetic rather than thermodynamic factors. The present work indicates that both *cis* and *trans* isomers exist in aqueous solution and it is of interest that the glycinate complex alone shows no nitrogen superhyperfine structure: for this ligand the interconversion of *cis* and *trans* isomers may be rapid with respect to the e.s.r. time scale.

Histidine Complexes $[CuL]^+$, $[Cu(HL)L]^+$, *and* $[CuL_2]$.—The structures of the copper(II)–histidine complexes are of considerable interest, because of their importance in blood plasma [20] and because of the complexities introduced by combining a metal ion favouring four-co-ordination with a potentially tridentate ligand.

Different workers have used potentiometric, calorimetric, colorimetric, crystallographic, n.m.r., and e.s.r. techniques to study these complexes. Single-crystal *X*-ray structure analysis has established that the cation $[Cu(HL)_2]^{2+}$ (crystallised as the nitrate salt from solution at pH 3.7) has two (amino) nitrogen atoms and two carboxylate oxygen atoms co-ordinated to the copper atom, with water molecules occupying the *trans*-planar sites; the (protonated) imidazole amino-group is not co-ordinated to copper.[21] The complex [Cu(L-histidinate)-(D-histidinate)] has, in the solid state, four nitrogen atoms co-ordinated in the square plane with the carboxylate groups hydrogen bonded to water molecules co-ordinated in the *trans*-planar sites.[22] However the physiologically important complexes $[Cu(HL)L]^+$ and $[CuL_2]$, HL = L-histidine, have not been crystallised from aqueous solution and diverse inferences have been made about the structures of these cations in solution, as reviewed by Sigel.[23] Much inference has been made from chemical and physical measurements providing 'indirect' evidence, *e.g.* oxidation of the imidazole group by

TABLE 2

^{14}N superhyperfine coupling constants and relative amounts of *cis* and *trans* isomers for copper(II)–bis(amino-acid) complexes in aqueous solution with estimated standard deviations in parentheses

	cis isomer			*trans* isomer		
Ligand	$A(^{14}N)$/mT	'*g*' [a]	%	$A(^{14}N)$/mT	'*g*' [a]	%
Glutamic acid	1.06(4)	2.060(2)	38(2)	0.90(4)	2.069(2)	62(2)
Asparagine	1.00(4)	2.061(2)	37(2)	0.92(4)	2.071(2)	63(2)
Proline	1.07(5)	2.057(2)	40(3)	0.95(5)	2.068(2)	60(3)
Glycine	No ^{14}N structure observed					
Leucine	1.02(4)	2.061(2)	38(2)	0.89(4)	2.071(2)	62(2)
Aspartic acid	1.06(5)	2.063(2)	42(3)	1.00(5)	2.076(2)	58(3)
Lysine	1.00(5)	2.058(2)	33(3)	0.92(5)	2.068(2)	67(3)
Valine	1.00(4)	2.057(2)	37(2)	0.92(4)	2.068(2)	63(2)
Glutamine	1.00(4)	2.060(2)	31(3)	0.90(4)	2.069(2)	69(3)
Alanine	1.00(4)	2.059(2)	40(2)	0.85(4)	2.071(2)	60(2)
Histidine Cu(HL)L [b]	1.12(1)	2.054(1)	100			
Histidine CuL_2 [c]	(*i*) 1.11(3)	2.055(2)	50(5)	(*ii*) 1.15(3)	2.051(2)	50(5)
(Histamine) [d]	1.27(1)	2.049(1)	100			

[a] '*g*' represents the *g* value at the centre of the ^{14}N superhyperfine structure on the high-field Cu component. [b] The spectrum of [Cu(HL)L] for HL = L-histidine consists of seven peaks representing three equivalent N atoms. [c] The spectrum of $[CuL_2]$ for H_2L = L-histidine consists of two components having (*i*) three and (*ii*) four equivalent N atoms. [d] The spectrum of $[Cu(histamine)_2]$ has four equivalent N atoms.

H_2O_2,[24] and calorimetric [25] and potentiometric [23,26] measurements respectively; in contrast, e.s.r. measurements on complexes in solution can provide direct evidence for the number of nitrogen atoms co-ordinated to copper(II).

In interpreting the e.s.r. spectra for copper(II) histidine complexes it is necessary to note the distinctive spectroscopic parameters for α-amino-carboxylate complexes (Table 1), diamine complexes {$[Cu(en)]^{2+}$ (en = ethylenediamine), $g_{iso.} = 2.132$; $[Cu(en)_2]^{2+}$, $g_{iso.} = 2.101$},[27] and mono- and bis-(histamine) complexes ($g_{iso.} = 2.145$, $A_{iso.} = 6.8 \pm 0.2$ mT; $g_{iso.} = 2.110_5$, $A_{iso.} = 7.7 \pm 0.1$ mT). It is also noted that, although $g_{iso.}$ for copper(II) is affected to different extents by co-ordination of primary amino- and imidazole-nitrogen atoms, the nitrogen hyperfine structure observed for $[Cu(histamine)_2]^{2+}$ is consistent with four equivalent (or near-equivalent) nitrogen atoms co-ordinated to the copper (Figure 7).

The present work provides the following information on the co-ordination of L-histidine to copper(II). (*i*) The complex $[CuL]^+$ probably has histidine co-ordinated with amino- and imidazole-nitrogen atoms in a square-planar arrangement, because, although the value of $g_{iso.}$ of 2.144 is only slightly smaller than those for the other α-amino-carboxylate complexes, it is very similar to that for the mono(histamine) complex, in which two N atoms are co-ordinated to the copper. The spectra for none of these complexes display nitrogen superhyperfine structure so a more positive assignment of the structure cannot be made. However, a histamine-like structure for $[Cu(histidinate)]^+$ is consistent with the observations that $-\Delta H$ and log K for formation of this complex are similar to the values for $[Cu(histamine)]^{2+}$ and significantly higher than those for the phenylalanine complex.[25] The 1 : 1 Cu^{2+}–histamine complex and 1 : 1 Cu^{2+}–histidine complex also have similar visible absorption spectra (Table 1).

(*ii*) The complex $[Cu(HL)L]^+$ probably has three nitrogen atoms strongly co-ordinated to the copper atom. This is established by the symmetric septet (1 : 3 : 6 : 7 : 6 : 3 : 1) due to nitrogen superhyperfine

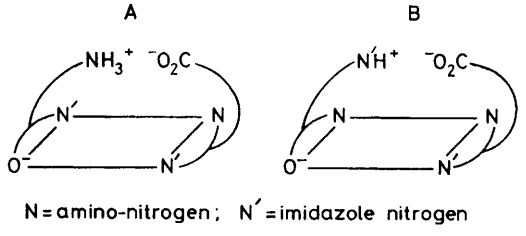

FIGURE 6 Two possible structures for the $[Cu(HL)L]^+$ copper–histidine complex

coupling on the high-field ^{63}Cu hyperfine peak, as observed in the second-derivative spectrum [Figure 5(*a*)]. Two possible structures for the $[Cu(HL)L]^+$ complex are shown in Figure 6, both being stabilized by hydrogen bonding between ammonium and carboxylate. By reference to the known structure of the cation $[Cu(HL)_2]^{2+}$ in the solid state and the known stabilities of five- and six-membered chelate rings, it may be deduced that B is the more probable structure. Structure A was suggested on the basis of thermodynamic data, but without reference to hydrogen bonding. Hydrogen bonding may, however, contribute to the marked stability of $[Cu(HL)L]^+$ with respect to $[CuL_2]$ and $[Cu(HL)_2]^{2+}$; log K for the reaction $[CuL_2] + [Cu(HL)_2]^{2+} \longrightarrow 2[Cu(HL)L]^+$ is 2.06, considerably greater than the statistical value of 0.3.[22]

(*iii*) For $[CuL_2]$, the nitrogen superhyperfine structure

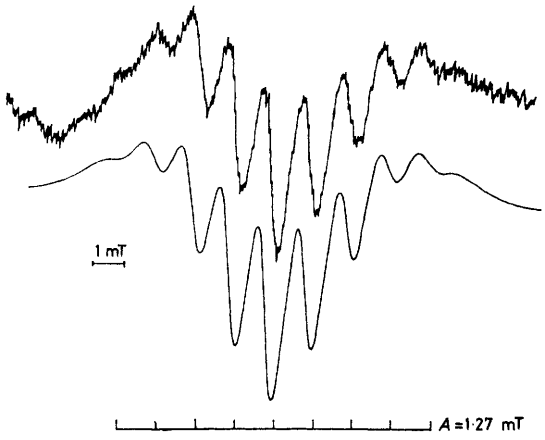

FIGURE 7 Second derivative of the high-field copper absorption of the e.s.r. spectrum of the $[^{63}Cu(histamine)_2]^{2+}$ complex in D_2O along with a simulation which assumes the presence of nine peaks of relative intensities 1 : 4 : 10 : 16 : 19 : 16 : 10 : 4 : 1 and whose positions are indicated in the accompanying stick digram

observed on the high-field copper resonance [Figure 5(*b*)] is *asymmetric*. This is in contrast to the *symmetric* multiplets observed for both $[Cu(HL)L]^+$, which has three nitrogen atoms co-ordinated, and $[Cu(histamine)_2]^{2+}$ (Figure 7) which has four nitrogen atoms and produces a 1 : 4 : 10 : 16 : 19 : 16 : 10 : 4 : 1 structure on the high-field copper peak with $A_{iso.}(N) = 1.27$ mT. The overall spectrum, although broader, bears a superficial resemblance to the spectra of the bis(α-amino-carboxylate) complexes. However, several workers have suggested bis(histamine) structures for $[CuL_2]$ or an equilibrium mixture of structures having respectively three and four nitrogen atoms co-ordinated.[23-25] Such structures would be consistent with the observed stability, which is significantly higher than that for a bis(α-amino-carboxylate) complex.[25] A computer simulation using a mixture of seven- and nine-peak components essentially reproduces the experimental spectrum [Figure 5(*b*)], thus indicating the possibility of an equilibrium between structures having three and four N atoms, respectively, co-ordinated to the copper.

Conclusions.—Mono- and bis-(α-amino-carboxylate)-copper(II) complexes can be readily distinguished on the basis of their e.s.r. g and hyperfine A values. The observation of N-superhyperfine splitting has shown that the bis complexes exist in solution as an equilibrium mixture of structures having two N and two O atoms

co-ordinated in *cis* and *trans* arrangements respectively. Also, the structure of the histidine complex $[Cu(HL)L]^+$ has three N atoms co-ordinated to Cu, whereas the $[CuL_2]$ complex may exist in solution as an equilibrium mixture of structures having three and four N atoms co-ordinated to the Cu.

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