Electron Spin Resonance Study of Copper(II) Complexes of X-Glycine and Glycyl-X Type Dipeptides, and Related Tripeptides. Variation of Co-ordination Modes with Ligand Excess and pH in Fluid and Frozen Aqueous Solutions

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Co-ordination modes for the various copper(II) complexes of glycine(Gly)-containing di- and tri-peptides (HL) with non-co-ordinating side-chains have been investigated. The e.s.r. spectra of predominant species at 1:1, 2:1, and 50:1 ligand:metal concentration ratios in the region pH ≈6—13 have been recorded in fluid and frozen aqueous solutions, and evaluated by computer simulation. The energies of the *d–d* electronic transitions have been determined by Gaussian analysis of the visible absorption spectra. Molecular-orbital coefficients characteristic of metal–ligand bonds for the various 1:1 and 1:2 complexes have been calculated assuming effective D_{4h} symmetry. At ligand excess in alkaline solution, the temperature strongly affects the chemical equilibria: low temperature promotes the formation of 1:2 complexes: $[Cu(LH_{-1})L]^-$ at pH ≈9, and $[Cu(LH_{-1})_2]^{2-}$ at pH ≈13 in the case of X-Gly type dipeptides. In the predominant isomers of these complexes one of the dipeptide molecules is co-ordinated equatorially through its amino nitrogen, deprotonated peptide nitrogen, and carboxylate oxygen atoms. The amino group of the other dipeptide occupies an axial position, while the fourth equatorial donor atom is either the peptide oxygen (pH ≈9) or the deprotonated peptide nitrogen (pH ≈13) of the second ligand. In the latter case, axial co-ordination of the second carboxylate group is also likely. Competition can be observed between the σ and π bonds in the equatorial plane on the one hand, and between the σ bonds of different symmetries on the other hand. The influence of the co-ordination modes, the type of ligand, and the temperature on the covalent character of the metal–ligand bonds is discussed.

E.s.r. spectroscopy has proved to be a useful tool for studying the complex equilibria in solutions of the copper(II) ion and various ligands of biological importance.[1–6] This method has been used for determining the equilibrium constants in liquid solutions.[1,2] E.s.r. spectra have provided information on the nature of the co-ordination in species predominating in different pH regions: computer simulation of the nitrogen superhyperfine structure has yielded the number of nitrogen atoms co-ordinated equatorially in the case of second-derivative spectra at room temperature[3,4] and multifrequency e.s.r. spectra at 77 K.[5] A systematic study of the anisotropic spectra of frozen solutions has afforded also further details of the co-ordination in a series of amino acid complexes,[6] including the covalent character of the metal–ligand bonds and small distortions in some cases. For most species, these results[6] are in good accordance with the co-ordination modes proposed by pH-metric, u.v.–visible, e.s.r., and n.m.r. relaxation studies in liquid solutions, suggesting that freezing does not alter significantly the co-ordination in these molecules.

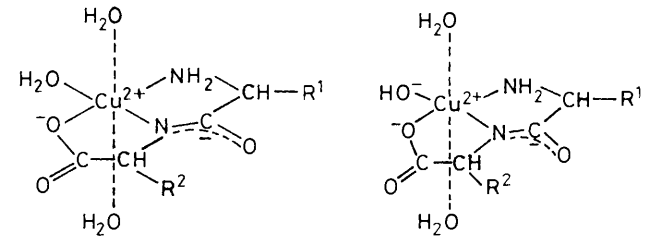
In the present work, various copper(II) complexes of di- and tri-peptides containing glycine residues, have been investigated. The e.s.r. parameters obtained by computer simulation of the spectra of fluid and frozen solutions, and the molecular orbital (m.o.) coefficients computed from the anisotropic e.s.r. data and the *d–d* electronic energies, are used to characterize the structure of different molecules. The effect of freezing and side-chain substituents on the co-ordination modes has been studied.

Experimental

Reagents and Solutions.—The dipeptide ligands contained glycine (Gly) as either a N- or C-terminal amino acid. Other amino acids were Gly, L-alanine (Ala), L-phenylalanine (Phe), and L-leucine (Leu). The complexes of L-prolylglycine (Pro-Gly), Gly-Gly-Gly, Ala-Gly-Gly, Gly-Gly-Phe, and Gly-Leu-Leu were also studied. The peptides in their zwitterionic form are symbolized by HL. The ligands were synthesized by treating the activated ester of N-terminal benzyloxycarbonyl acid, formed with *N*-hydroxysuccinimide, with the C-terminal amino acid of the dipeptide as described in the literature.[7] The peptides gave satisfactory analyses, and their purity was at least 95% by h.p.l.c. Reagents of analytical grade from Reanal (Hungary) were used both for syntheses and preparation of the solutions.

The copper(II) concentration of the solutions was 5 mmol dm^{-3}, and the ligand-to-metal concentration ratios were usually 1:1, 2:1, and 50:1. Optimum pH values in the region 6—13 for the formation of the different complexes were chosen according to literature data.[1,2,8] The pH was adjusted with NaOH to an accuracy of 0.01 pH unit using a Radelkis (Hungary) OP 211/1 pH-meter. The solutions also contained 5% (v/v) methanol to promote glass formation during freezing.

E.S.R. and Optical Measurements.—E.s.r. spectra were recorded at 77 and 288 K on a JEOL JES-FE3X spectrometer with 100-kHz field modulation using manganese(II)-doped MgO powder as field standard. They were evaluated on IBM



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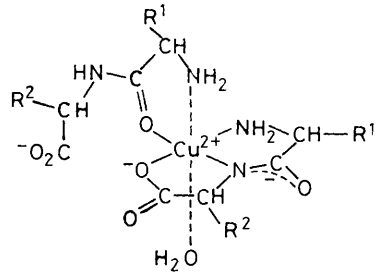

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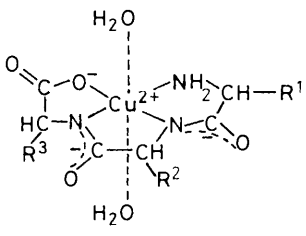

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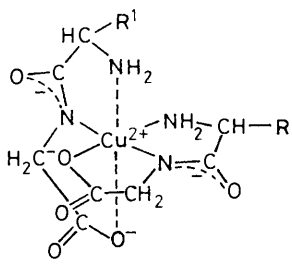

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360 compatible R-55 and PDP 11—equivalent EMU 11 computers with FORTRAN programs. In the case of isotropic spectra, the parameters g_0, hyperfine coupling constant (A_0), superhyperfine (s.h.f.) coupling constant (a_{N_0}), magnetic quantum number-dependent linewidth, and the number of equatorial nitrogen donor atoms were optimized. For simulation of the anisotropic spectra, the g, copper and nitrogen hyperfine, and quadrupole coupling tensors of axial symmetry, and orientation and magnetic quantum number-dependent linewidth were taken into account. The parameters were varied until the sum of the squares of differences between the calculated and measured intensities was minimized. With both types of spectra, the superposition of the spectra of isotopes ^{63}Cu and ^{65}Cu was calculated.

Electronic spectra were recorded between 450 and 900 nm on a Beckman DU spectrometer at 296 K, and at 273 K in some cases. The visible band was resolved into Gaussian components using a FORTRAN program run on the R-55 computer.

The e.s.r. and electronic spectral data were evaluated on the basis of the well known m.o. scheme, assuming an effective D_{4h} local symmetry around the copper(II). In the case of elongated octahedral geometry, the unpaired electron occupies the $\psi_{b_{1g}}$ antibonding orbital. The covalency of the in-plane σ, the in-plane π, and the out-of-plane π bond [the electron delocalization from the copper(II) $d_{x^2-y^2}$, d_{xy}, and $d_{xz,yz}$ orbitals, respectively] is characterized by α^2, β_1^2, and β^2, respectively.

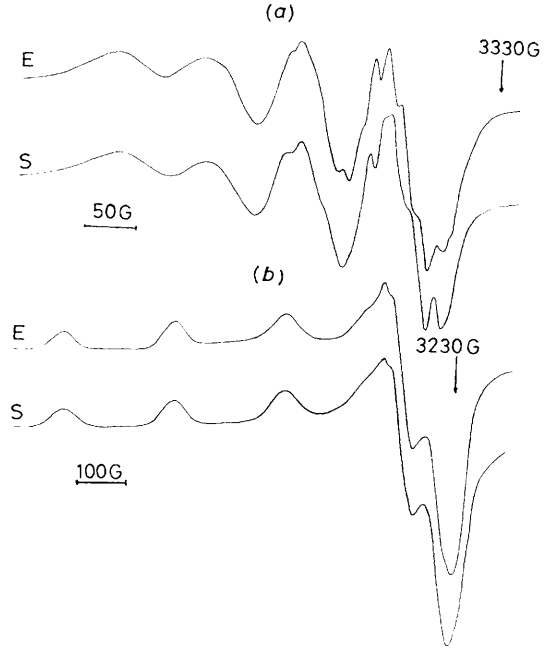

Figure 1. E.s.r. spectra of the [CuLH$_{-1}$] complex of L-alanylglycine (*a*) at 288 K. E = Experimental; S = simulated with g_0 = 2.116, $A_0(^{63}Cu)$ = 64.4 G, a_{N_0} = 16.5 and 10.7 G for the two non-equivalent nitrogen nuclei, and linewidths of 38, 24, 13, and 8 G in the sequence of increasing magnetic field; (*b*) at 77 K, S = simulated with the parameters in Table 1, and with linewidths of 14, 15, 17, and 20 G both in the parallel and the perpendicular region, in the sequence of increasing magnetic quantum number

The 4*s* σ bond [the electron density on the partially occupied 4*s* orbital of copper(II)] is characterized by the value of ε'^2. The calculation of the m.o. coefficients was based upon equations which relate the spin-Hamiltonian parameters to the covalency of the metal–ligand bonds.[9,10] Anisotropic e.s.r. parameters were determined by simulation of the spectra of frozen solutions, isotropic data were calculated as the arithmetic averages of the anisotropic values, and the *d*–*d* electronic energies were obtained from the visible absorption spectra. The whole calculation procedure was described in detail previously.[6]

The isotropic nitrogen s.h.f. coupling constant a_{N_0} was related to the s.h.f. coupling constants $a_{N_\parallel}$ and $a_{N_\perp}$ determined in the principal directions of the copper(II) g tensor according to equation (1).[6]

$$a_{N_0} = \tfrac{1}{3}[2a_{N_\parallel} + (2a_{N_\perp}{}^2 - a_{N_0}{}^2)^{\frac{1}{2}}] \quad (1)$$

Results and Discussion

E.S.R. Spectra and Co-ordination Modes of Predominant Complexes in Fluid and Frozen Solution.—At a 1 : 1 metal : ligand concentration ratio two species predominate in solutions of copper(II) and dipeptides with non-co-ordinating side-chains.[1,11,12] In slightly acidic and neutral media the complex [CuLH$_{-1}$] is formed, where the amino nitrogen, the deprotonated peptide nitrogen, one of the carboxylate oxygen atoms, and a water molecule are co-ordinated equatorially, while the two axial sites are occupied by water molecules [structure (**1**)]. A striking feature of the spectra of the fluid solution (Figure 1) is that the a_{N_0} values for the two nitrogen donor atoms are quite different. Probably, the a_{N_0} for the deprotonated peptide nitrogen is the larger, as a result of the greater *s* character of hybrid orbitals[13] and the particularly

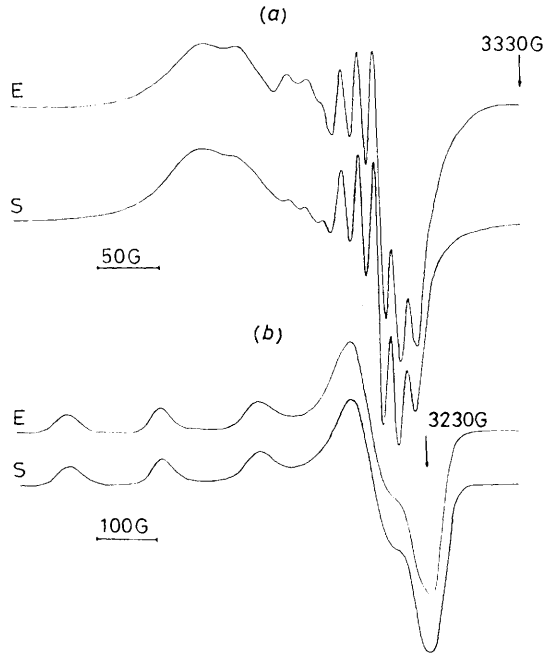

Figure 2. E.s.r. spectra of the $[Cu(LH_{-1})(OH)]^-$ complex of L-alanylglycine: (*a*) at 288 K, S = simulated with $g_0 = 2.114$, $A_0(^{63}Cu) = 40$ G, $a_{N_0} = 13$ G for the two equivalent nitrogen nuclei, and linewidths of 30, 20, 15, and 8.5 G in the sequence of increasing magnetic field; (*b*) at 77 K, S = simulated with the parameters in Table 1, and with linewidths of 13, 13, 17, and 20 G in the parallel and 14, 15, 17, and 20 G in the perpendicular region, in the sequence of increasing magnetic quantum number

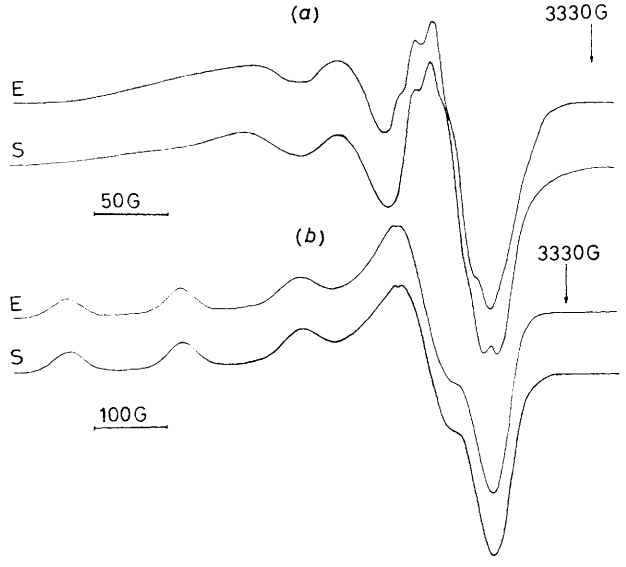

Figure 3. E.s.r. spectra of the $[Cu(LH_{-1})L]^-$ complex of glycylglycine: (*a*) at 288 K, S = simulated with $g_0 = 2.116$, $A_0(^{63}Cu) = 55$ G, $a_{N_0} = 13.5$ and 10.5 G for the two non-equivalent nitrogen nuclei, and linewidths of 80, 40, 24, and 10 G in the sequence of increasing magnetic field; (*b*) at 77 K, S = simulated with the parameters in Table 1, and with linewidths of 14, 15, 19, and 22 G in the parallel and 15, 15, 17, and 20 G in the perpendicular region, in the sequence of increasing magnetic quantum number

strong (short) bond with copper(II) as is shown by crystallographic studies.[14]

The species $[CuLH_{-1}]$ is replaced by $[Cu(LH_{-1})(OH)]^-$ at pH ≈9, where the water molecule in the fourth equatorial position is deprotonated [structure (**2**)]. This considerably modifies the e.s.r. parameters (Table 1). Surprisingly, the isotropic spectra show a notable reduction in the difference in s.h.f. coupling constants for the two nitrogen donors: good fitting can be achieved by assuming two equivalent nitrogen donor atoms in both the fluid and frozen state (Figure 2).

At ligand excess (over 2:1 dipeptide:metal concentration ratio), 1:2 complexes can also be formed. In the range pH ≈9—10 the species $[Cu(LH_{-1})L]^-$ predominates.[1,11,12] Even though this complex has three nitrogen donors (two amino and one deprotonated peptide nitrogen), e.s.r. spectra show only splittings of two non-equivalent nitrogens (Figure 3). McPhail and Goodman[4] have also found a s.h.f. pattern of two nitrogens in the case of second-derivative spectra of the Gly-Gly complex under similar experimental conditions. An increase in hyperfine coupling constants and a decrease in *g*-values, generally associated with an increase in the number of equatorial nitrogen atoms, was not observed in either liquid or frozen solution (Table 1). All these facts suggest that only two of the nitrogen atoms are bound in the equatorial plane, while the third occupies an axial position. Formation of this species from the complex $[CuLH_{-1}]$ is accompanied by an entropy decrease suggesting the formation of an additional chelate ring.[11] On the basis of this evidence, we propose structure (**3**) for the species $[Cu(LH_{-1})L]^-$, where the terdentate co-ordination of the 'first' ligand remains in the equatorial plane, while the fourth equatorial site and one of the axial sites are occupied by the peptide oxygen and the amino nitrogen atoms of the 'second' ligand, respectively, and a water molecule is bound in the second axial position. An isomer with three equatorial nitrogen atoms, however, seems to be present as a minor complex at least in the liquid phase, where the peptide oxygen and the amino nitrogen of the 'second' ligand are interchanged.[11] This is suggested by the $\lambda_{max.}$ values of the absorption maxima (see later), and, in our opinion, by the s.h.f. pattern[4] of the isotropic spectrum. In the second-derivative spectrum of the liquid solution, the high-field copper peak splits into a sextet of intensity ratio 1:3:5:5:3:1 which can be accounted for the superposition of two 1:2:3:2:1 quintets shifted by a_{N_0}, suggesting the presence of two isomers with two equatorial nitrogen atoms in equal concentrations; *i.e. cis–trans* isomerism may occur, as it has been described in ref. 4. Structure (**3**) does not allow for this kind of isomerism. If, however, an equilibrium occurs between an isomer with two and another with three equatorial nitrogen atoms, and their concentration ratio equals 3:1, the superposition of their multiplets shifted by a_{N_0} results in a septet of intensity ratio 1.3:3:5:4.3:3:1:0.3 which is closely similar to the above sextet.

Before discussing the co-ordination at high pH and excess of dipeptide ligands, it may be useful, for comparison, to deal with the structure of tripeptide complexes. The only predominant species over wide ranges of pH and ligand:metal concentration ratio is the complex $[CuLH_{-2}]^-$ of well known structure.[12] It has both of the peptide nitrogens deprotonated and bound to copper(II). The equatorial co-ordination is completed by a carboxylate oxygen and the amino nitrogen atom [structure (**4**)]. In accordance with expectation for three equatorial nitrogen donor atoms, larger hyperfine coupling constants and smaller *g* values are observed (Figure 4, Table 1). The difference in a_{N_0} values for the two tightly bound, deprotonated, peptide nitrogens and the amino nitrogen is considerable in fluid solution, similarly to that for the $[CuLH_{-1}]$ complexes of dipeptides.

According to equilibrium studies,[1,12] at high pH the hydroxide ions displace the 'second' ligand from the co-ordination sphere, and between pH ≈12 and 13 the species $[Cu(LH_{-1})(OH)]^-$ predominates even at high ligand:metal

Table 1. E.s.r. parameters obtained from anisotropic spectra of frozen solutions[a]

HL	Complex	$n_{N_{eq}}$[b]	$g_{\parallel}$[c]	$g_{\perp}$[c]	g_0[c]	$-A_{\parallel}$[d]	$-A_{\perp}$[d]	$-A_0$[d]	$a_{N_{\parallel}}$[e]	$a_{N_{\perp}}$[e]	a_{N_0}[e]
Gly-Gly	$[CuLH_{-1}]$	2	2.253	2.048	2.116	167	22	70.3	9	14	11.9
	$[Cu(LH_{-1})(OH)]^-$	2	2.245	2.044	2.111	148	23	64.7	9	14	11.9
	$[Cu(LH_{-1})L]^-$	2	2.234	2.047	2.109	154	24	67.3	10.5	15	13.1
	$[Cu(LH_{-1})_2]^{2-}$	3	2.206	2.037	2.093	184	20	74.7	11	14	12.8
Gly-Gly-Gly	$[Cu(LH_{-2})]^-$	3	2.204	2.037	2.093	189	23	78.3	12	13.5	13
Ala-Gly	$[CuLH_{-1}]$	2	2.245	2.050	2.115	165	21	69	10	13.5	12.1
	$[Cu(LH_{-1})(OH)]^-$	2	2.243	2.044	2.110	149	23	65	9	13.5	11.6
	$[Cu(LH_{-1})L]^-$	2	2.234	2.044	2.107	153	24	67	11	16	13.9
	$[Cu(LH_{-1})_2]^{2-}$	3	2.200	2.038	2.092	186	22	76.7	10	12	11.2
Ala-Gly-Gly	$[CuLH_{-2}]^-$	3	2.200	2.036	2.091	189	24	79	11	14	12.8
Gly-Ala	$[CuLH_{-1}]$	2	2.244	2.048	2.113	170	19	69.3	9	13.5	11.6
	$[Cu(LH_{-1})(OH]^-$	2	2.233	2.043	2.106	154	22	66	9	14	11.9
	$[Cu(LH_{-1})L]^-$	2	2.232	2.042	2.105	156	22	66.7	9	13.5	11.6
Phe-Gly	$[CuLH_{-1}]$	2	2.247	2.048	2.114	167	20	69	10	13	11.8
	$[Cu(LH_{-1})(OH)]^-$	2	2.230	2.043	2.105	156	23	67.3	9	13	11.3
	$[Cu(LH_{-1})L]^-$	2	2.228	2.047	2.107	157	22	67	11	15	13.4
	$[Cu(LH_{-1})_2]^{2-}$	3	2.200	2.038	2.092	183	15	71	11	16	13.9
Gly-Phe	$[CuLH_{-1}]$	2	2.241	2.048	2.112	170	18	68.7	10	14	12.4
	$[Cu(LH_{-1})(OH)]^-$	2	2.240	2.043	2.109	154	23	66.7	10	13	11.8
	$[Cu(LH_{-1})L]^-$	2	2.223	2.042	2.102	161	20	67	9	13	11.3
Gly-Gly-Phe	$[CuLH_{-2}]^-$	3	2.205	2.041	2.096	187	19	75	11	16	13.9
Leu-Gly	$[CuLH_{-1}]$	2	2.247	2.047	2.114	172	21	71.3	9	14	11.9
	$[Cu(LH_{-1})(OH)]^-$	2	2.242	2.045	2.111	149	24	65.7	9	14	11.9
	$[Cu(LH_{-1})L]^-$	2	2.230	2.043	2.105	155	23	67	9	14	11.9
Gly-Leu	$[CuLH_{-1}]$	2	2.247	2.046	2.113	170	19	69.3	10	13	11.8
	$[Cu(LH_{-1})(OH)]^-$	2	2.231	2.046	2.108	157	21	66.3	10	13	11.8
	$[Cu(LH_{-1})L]^-$	2	2.230	2.043	2.105	157	20	65.7	9	13	11.3
Gly-Leu-Leu	$[CuLH_{-2}]^-$	3	2.209	2.038	2.095	182	16	71.3	10	12.5	11.5
Pro-Gly	$[CuLH_{-1}]$	2	2.246	2.048	2.114	164	22	69.3	10	16	13.4
	$[Cu(LH_{-1})(OH)]^-$	2	2.244	2.044	2.111	149	21	63.7	10	13.5	12.1
	$[Cu(LH_{-1})L]^-$	2	2.236	2.043	2.107	150	25	66.7	10	15	12.9

[a] Coupling constants are given in G ($=10^{-4}$ T). Quadrupole coupling constant was 7 G in each case. [b] The number of equatorial nitrogen donor atoms. [c] Estimated error ± 0.001. [d] Data for the isotope ^{63}Cu. Estimated error ± 1 G. [e] Estimated error ± 0.5 G.

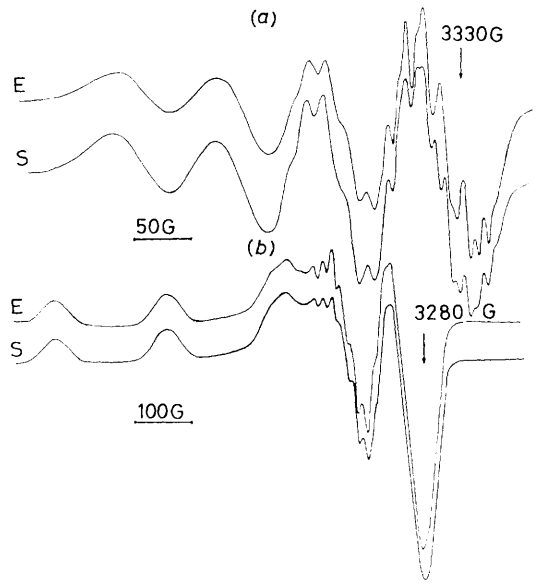

Figure 4. E.s.r. spectra of the $[CuLH_{-2}]^-$ complex of L-alanylglycylglycine: (*a*) at 288 K, S = simulated with $g_0 = 2.096$, $A_0(^{63}Cu) = 83.5$ G, $a_{N_0} = 15.65$ G for the two equivalent nitrogen nuclei and $a_{N_0} = 9.7$ G for the third, and with linewidths of 30, 20, 10, and 7 G in the sequence of increasing field; (*b*) at 77 K, S = simulated with the parameters in Table 1, and with linewidths of 9, 11, 14, and 18 G both in the parallel and the perpendicular region, in the sequence of increasing magnetic quantum number

concentration ratios in the case of dipeptides with non- or weakly co-ordinating side-chains. The ligand Gly-Gly, however, proved to be an exception: on increasing the ligand:metal concentration ratio to 1 000:1 between pH ≈ 12 and 13, a blue shift of the visible absorption band indicates that the complex $[Cu(LH_{-1})(OH)]^-$ has been replaced by a new species, $[Cu(LH_{-1})_2]^{2-}$.[15] Lately, Farkas and Kiss[8] have found this species also with the ligands serylglycine and threonylglycine by pH-metric and visible spectral studies. We show the e.s.r. spectra of the complex of Gly-Gly in Figure 5. Both of the peptide nitrogens are deprotonated, therefore a s.h.f. splitting of four nitrogens is expected. On the contrary, the best spectral fitting has been achieved with three nitrogen donor atoms at both 77 and 288 K (Figure 5, Table 1). Attempts to simulate the spectra with four nitrogen atoms led to square sums 5—10% larger than those for three nitrogens, and, in addition, too low values for the s.h.f. coupling constants have been obtained, though in the case of four nitrogen atoms a substantial increase in a_N values would be expected, as has been found by electron nuclear double resonance (ENDOR) spectroscopy for a large number of copper(II) complexes.[13] The changes in isotropic parameters correspond to the increase in number of equatorial nitrogen donors as compared to the former complexes of dipeptides. The values of A_0 and g_0 in fluid solution are close to those of tripeptides; the nitrogen atoms of different hybridization state, however, cannot be distinguished on the basis of their a_{N_0} values. In frozen solution the spectrum is similar to those of tripeptides in every respect (Table 1). On the basis of these facts, we propose structure (**5**) to describe the predominant co-ordination in the complex $[Cu(LH_{-1})_2]^{2-}$, where the terdentate equatorial ligation of the 'first' ligand remains, and

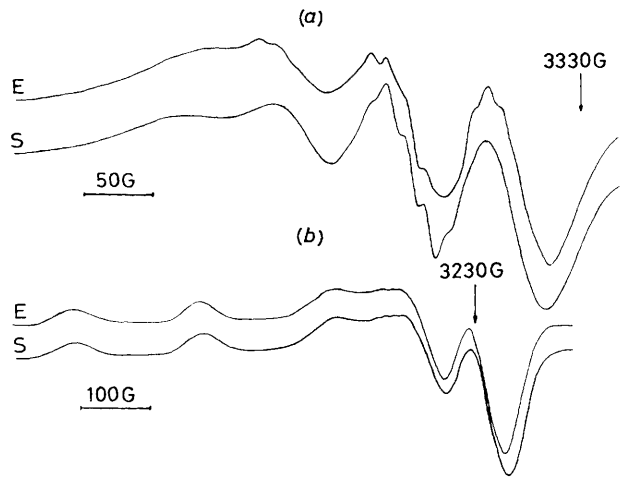

Figure 5. E.s.r. spectra of the $[Cu(LH_{-1})_2]^{2-}$ complex of glycylglycine: (*a*) at 288 K, S = the superposition of the spectra of $[Cu(LH_{-1})_2]^{2-}$ and $[Cu(LH_{-1})(OH)]^{-}$ in a ratio of 9:1, according to concentration distribution data in ref. 8; the parameters for the former complex are $g_0 = 2.102$, $A_0(^{63}Cu) = 67$ G, $a_{N_0} = 14$ G for the three equivalent nitrogen nuclei, and linewidths of 55, 30, 15, and 15 G in the sequence of increasing magnetic field; (*b*) at 77 K, S = simulated with parameters in Table 1, and with linewidths of 11, 11, 16, and 19 G in the parallel and 13, 14, 16, and 19 G in the perpendicular region, in the sequence of increasing magnetic quantum number

the 'second' ligand is bound equatorially through the deprotonated peptide nitrogen, while its amino nitrogen and carboxylate oxygen atoms occupy the axial positions.

The above spectra of the Gly-Gly complex at high pH and large ligand excess can serve as a clue to the interpretation of an interesting phenomenon, which, in turn, provides further evidence concerning the structure of the species $[Cu(LH_{-1})_2]^{2-}$. Recently, McPhail and Goodman[4] observed a drastic change upon freezing a solution of pH ≈12 which contained Gly-Gly and copper(II) in 5:1 concentration ratio: in fluid solution a complex of two-nitrogen s.h.f. pattern, probably $[Cu(LH_{-1})(OH)]^{-}$ predominates, while the spectrum of the frozen solution can be simulated well only if a three-nitrogen model is used. We have observed a similar change at a ligand:metal concentration ratio of 2:1 in the region pH ≈12—13. The fluid spectrum is the same as that at 1:1 metal:ligand concentration ratio, pH ≈12, *i.e.* it is undoubtedly the spectrum of the complex $[Cu(LH_{-1})(OH)]^{-}$, in accordance with equilibrium studies.[1,8,12] Surprisingly, after freezing, the spectrum markedly differs from that of the species $[Cu(LH_{-1})(OH)]^{-}$. Moreover, it is closely identical with the spectrum taken at large ligand excess (Figure 5). At the same time, another striking change can be observed: the colour has changed from blue to violet. The most likely explanation for these facts is that the species $[Cu(LH_{-1})_2]^{2-}$ replaces $[Cu(LH_{-1})(OH)]^{-}$ upon cooling to 77 K. This phenomenon can be observed also in the case of ligands Ala-Gly and Phe-Gly, while for Leu-Gly overlapping spectra are obtained which show features indicative of $[Cu(LH_{-1})_2]^{2-}$ as a minor component. This complex does not form in the case of Pro-Gly and dipeptides of type Gly-X.

The ligand Phe-Gly serves as an extreme precedent for the above phenomenon: the species $[Cu(LH_{-1})_2]^{2-}$ predominates at 77 K even at small (2:1) ligand excess, though it is not formed at all at 298 K up to a Phe-Gly:Cu^{II} concentration ratio of 50:1.[8] The question arises as to whether a decrease of ≈30 degrees in temperature (cooling from room temperature to ≈273 K, the usual freezing point of aqueous solutions) is enough to cause the enormous changes in complex equilibria at small ligand excess. It seems to be more likely that the concentration distribution of frozen solutions corresponds to a much lower temperature than ≈273 K. This assumption is supported by the fact that the visible spectra at 273 and 296 K, 2:1 ligand:metal concentration ratio, were found to be identical within experimental error, giving no evidence of any blue shift at 273 K, which would be associated with the formation of the complex $[Cu(LH_{-1})_2]^{2-}$. Overcooling of the solution may occur, *i.e.* some translational motion of molecules can take place below 273 K, which is necessary for combination of the 1:1 complex with the second ligand.

Information concerning the structure of $[Cu(LH_{-1})_2]^{2-}$ is implied by the fact that this species is highly favoured at low temperature. This suggests a considerable entropy decrease associated with the formation of this species. The proposed mode of co-ordination [structure (5)] is certainly accompanied by a notable stiffening of both ligands, and thus lower entropy, in contrast to the co-ordination of four equatorial nitrogen atoms, where the positions of the carboxylate groups are inappropriate for binding to copper(II). Our data do not suggest the presence of the latter molecule, which, however, cannot be excluded as a minor isomer at room temperature. In the case of Gly-Gly, the species $[Cu(LH_{-1})_2]^{2-}$ could be isolated in crystalline form, and then all nitrogen donors were bound equatorially.[16] Crystal-packing forces should play an important role in stabilizing this mode of co-ordination, however. Comparison of crystallographic data[14,16] shows that the bond between copper(II) and the deprotonated peptide nitrogen is much weaker when the dipeptide acts as a bidentate ligand, the carboxylate group taking no part in co-ordination: the Cu–N(peptide) bond of this complex is considerably longer (0.197 nm) than that for $[CuLH_{-1}]$ (0.188—0.193 nm), while the Cu–N(amino) bond length for the above Gly-Gly complex falls in the range for complexes $[CuLH_{-1}]$.

E.S.R. Parameters and Possible Distortions of Ligand Field.—It is of fundamental importance for the evaluation of electronic and e.s.r. spectral data whether the ligand field can be regarded as of effective D_{4h} symmetry or notable distortion occurs. A good fit of the experimental and calculated e.s.r. spectra could be achieved assuming axial g and A tensors. The linewidths in the perpendicular direction did not exceed 1.5 mT, which can mask a possible rhombic splitting of $g_\perp$ of not more than 0.01. This corresponds to a splitting of the E_g level of only a few hundred cm^{-1}. Thus 3*d*–4*s* orbital mixing should be negligible, if it occurs at all. The degree of tetrahedral distortion (3*d*–4*p* orbital mixing) can be characterized by the parameter $f = g_\parallel/A_\parallel$ cm,[17] which also depends on α^2 and the Fermi term (K).[18] At given values of the last two parameters, f increases with increasing tetrahedral distortion.[18] The f values for our complexes (113—145 cm) are below the upper limits for the square-planar geometry (130—150 cm at the corresponding α^2 and K values, see later), and hence the 3*d*–4*p* orbital mixing should make only a minor contribution to the changes in e.s.r. parameters. Thus it seems to be a reasonable assumption that the relatively simple relations derived for D_{4h} symmetry on the basis of simple ligand-field theory may give useful information on metal–ligand bonds for our series of complexes.

Visible Absorption Spectra.—Data on the ligand-field bands are compiled in Table 2. They are necessary for calculating the m.o. coefficients which characterize the metal–ligand bonds. The evaluation of the absorption spectra was based upon the following facts: circular dichroism studies show that each of the *d*–*d* electronic transitions occurs in the visible–near-i.r. region,[12] and the bands overlap; the e.s.r. data suggest no significant splitting of the E_g level (see above). Thus the visible band was resolved into three Gaussian components, which were assigned

Table 2. Absorption spectral data for the complexes

HL	Complex	$\nu_{max.}$[a] ($\varepsilon_{max.}$)[b]	Energy of Gaussian components[a,c] 1	2	3
Gly-Gly	$[CuLH_{-1}]$	15 700 (80)	14 797	15 717	16 984
	$[Cu(LH_{-1})(OH)]^-$	15 600 (77)	14 318	15 561	17 496
	$[Cu(LH_{-1})L]^-$	16 000 (81.5)	14 549	15 971	17 506
	$[Cu(LH_{-1})_2]^{2-}$	17 550 (98)	16 235	17 642	19 017
Gly-Gly-Gly	$[CuLH_{-2}]^-$	18 050 (120)	16 730	18 120	19 530
Ala-Gly	$[CuLH_{-1}]$	15 830 (86.5)	14 316	15 821	17 522
	$[Cu(LH_{-1})(OH)]^-$	15 650 (82)	14 471	15 669	17 725
	$[Cu(LH_{-1})L]^-$	15 930 (85)	14 449	15 929	17 716
	$[Cu(LH_{-1})_2]^{2-}$	17 800 (101)	16 500	17 850	19 500
Ala-Gly-Gly	$[CuLH_{-2}]^-$	18 250 (135)	16 912	18 298	19 824
Gly-Ala	$[CuLH_{-1}]$	15 730 (87)	14 608	15 662	17 128
	$[Cu(LH_{-1})(OH)]^-$	15 660 (80.5)	14 556	15 708	17 808
	$[Cu(LH_{-1})L]^-$	16 100 (87.5)	14 810	15 993	17 632
Phe-Gly	$[CuLH_{-1}]$	15 800 (86)	14 602	15 788	17 423
	$[Cu(LH_{-1})(OH)]^-$	15 830 (78)	14 571	15 807	17 739
	$[Cu(LH_{-1})L]^-$	16 050 (84.5)	14 720	16 023	17 776
	$[Cu(LH_{-1})_2]^{2-}$[d]				
Gly-Phe	$[CuLH_{-1}]$	15 870 (81.5)	14 475	15 935	17 599
	$[Cu(LH_{-1})(OH)]^-$	15 800 (74)	14 631	15 838	17 688
	$[Cu(LH_{-1})L]^-$	16 120 (83.5)	14 759	16 086	17 774
Gly-Gly-Phe	$[CuLH_{-2}]^-$	18 550 (126)	17 280	18 554	20 050
Leu-Gly	$[CuLH_{-1}]$	15 790 (83)	14 615	15 798	17 290
	$[Cu(LH_{-1})(OH)]^-$	15 720 (80)	14 551	15 697	17 654
	$[Cu(LH_{-1})L]^-$	16 070 (85)	14 715	16 031	17 809
Gly-Leu	$[CuLH_{-1}]$	15 790 (85)	14 609	15 788	17 243
	$[Cu(LH_{-1})(OH)]^-$	15 740 (81)	14 568	15 706	17 708
	$[Cu(LH_{-1})L]^-$	16 100 (83.5)	14 730	16 010	17 802
Gly-Leu-Leu	$[CuLH_{-2}]^-$	18 750 (146)	17 138	18 692	20 175
Pro-Gly	$[CuLH_{-1}]$	15 730 (98)	14 537	15 648	17 168
	$[Cu(LH_{-1})(OH)]^-$	15 650 (94)	14 636	15 715	17 830
	$[Cu(LH_{-1})L]^-$	15 870 (103)	14 759	15 926	17 815

[a] In cm^{-1}. [b] In $dm^3\ mol^{-1}\ cm^{-1}$. [c] Estimated error $\pm 70\ cm^{-1}$. [d] Data for the Gly-Gly-Phe complex were used.

according to the orbital-energy sequence for elongated octahedral complexes:[19] $A_{1g} \longleftarrow B_{1g} < B_{2g} \longleftarrow B_{1g} < E_g \longleftarrow B_{1g}$.

The energies of the absorption band maxima can provide information on the co-ordination modes, too. By multiple linear regression analysis of data for a large number of peptide complexes[12] a correlation has been recognized between the wavelength of the visible absorption maximum ($\lambda_{max.}$) and the nature and number of various donor atoms bound equatorially. Accepting this correlation as of wide validity, the calculated $\lambda_{max.}$ values can be used for checking the proposed structures. In the case of 1:1 complexes of dipeptides the absorption maxima are expected and have been found near 630 nm. Axial chelation generally gives to rise a small (5—15 nm) red shift of the absorption maximum.[6,12] Thus, for structure (**3**), $\lambda_{max.}$ values of $\gtrsim 630$ nm, while for the 'three-nitrogen' isomer of $[Cu(LH_{-1})L]^-$, $\lambda_{max.}$ values of $\gtrsim 580$ nm are expected. The measured $\lambda_{max.}$ values of 615—620 nm seem to support the coexistence of both isomers, with the 'two-nitrogen' molecules in larger concentration (see above). The equatorial co-ordination of the four possible nitrogen atoms in the complex $[Cu(LH_{-1})_2]^{2-}$ should result in a $\lambda_{max.}$ of ≈ 530 nm, not modified by axial chelation. On the contrary, $\lambda_{max.}$ has been found near 570 nm, in accordance with structure (**5**), for which $\lambda_{max.} \gtrsim 560$ nm is expected.

Metal–Ligand Bonds.—The m.o. coefficients for the complexes studied are shown in Table 3. Their values reflect a rather covalent character for each metal–ligand bond. An interesting correlation can be observed between α^2 and $A_{aniso} = |A_{\parallel} - A_0|$ [Figure 6(*a*)]. The anisotropy of the copper(II) hyperfine coupling tensor decreases as the delocalization of the unpaired electron from the $d_{x^2-y^2}$ orbital increases (*i.e.* α^2 decreases). It is striking that the data for $[Cu(LH_{-1})L]^-$ deviate only within experimental error from the straight line determined by the data for $[CuLH_{-1}]$ and $[Cu(LH_{-1})(OH)]^-$, while the complexes $[Cu(LH_{-1})_2]^{2-}$ form a separate group together with the tripeptide complexes. As A_{aniso} is determined by the *p* and *d* characters of the m.o. containing the unpaired electron, it depends not only on α^2, but also on the nature, number, and hybridization state of various donor atoms (for effective D_{4h} symmetry of our complexes, the equatorial donor atom set is the determinant). The above findings indicate the close similarity of the latter, directly donor atom-dependent contributions within the group of $[CuLH_{-1}]$, $[Cu(LH_{-1})(OH)]^-$, and $[Cu(LH_{-1})L]^-$ complexes on the one hand, and $[Cu(LH_{-1})_2]^{2-}$ and tripeptide complexes on the other hand, supporting structures (**3**) and (**5**).

The m.o. coefficients α^2 and ε'^2 also correlate with each other [Figure 6(*b*)]. This linear relationship reflects a competition between σ bonds of different symmetries.[10] It has been found to hold for several types of copper(II) complexes,[6,10] moreover it can be of diagnostic use for detecting the ligand field distortions.[10] Its existence supports the effective D_{4h} symmetry for the majority of our complexes. There are two compounds in the series, the $[Cu(LH_{-1})_2]^{2-}$ complex of Phe-Gly and the $[CuLH_{-2}]^-$ complex of Gly-Leu-Leu, for which a significant upward deviation occurs. This can be explained by a slight rhombic distortion (3*d*–4*s* orbital mixing).

A competition between the bonds in the equatorial plane can be observed, too. The decrease in electron delocalization from

Table 3. M.o. coefficients for the complexes

HL	Complex	M.o. coefficients * α^2	β_1^2	β^2	ε'^2
Gly-Gly	$[CuLH_{-1}]$	0.816	0.798	0.66	0.331
	$[Cu(LH_{-1})(OH)]^-$	0.739	0.854	0.70	0.305
	$[Cu(LH_{-1})L]^-$	0.739	0.840	0.74	0.287
	$[Cu(LH_{-1})_2]^{2-}$	0.815	0.743	0.58	0.360
Gly-Gly-Gly	$[CuLH_{-2}]^-$	0.819	0.749	0.59	0.346
Ala-Gly	$[CuLH_{-1}]$	0.800	0.797	0.72	0.327
	$[Cu(LH_{-1})(OH)]^-$	0.743	0.849	0.70	0.307
	$[Cu(LH_{-1})L]^-$	0.737	0.840	0.71	0.295
	$[Cu(LH_{-1})_2]^{2-}$	0.806	0.750	0.60	0.347
Ala-Gly-Gly	$[CuLH_{-2}]^-$	0.814	0.747	0.59	0.346
Gly-Ala	$[CuLH_{-1}]$	0.823	0.763	0.66	0.355
	$[Cu(LH_{-1})(OH)]^-$	0.746	0.816	0.69	0.319
	$[Cu(LH_{-1})L]^-$	0.752	0.817	0.66	0.323
Phe-Gly	$[CuLH_{-1}]$	0.814	0.787	0.67	0.345
	$[Cu(LH_{-1})(OH)]^-$	0.745	0.812	0.69	0.310
	$[Cu(LH_{-1})L]^-$	0.747	0.814	0.74	0.307
	$[Cu(LH_{-1})_2]^{2-}$	0.817	0.753	0.61	0.404
Gly-Phe	$[CuLH_{-1}]$	0.821	0.768	0.67	0.362
	$[Cu(LH_{-1})(OH)]^-$	0.753	0.836	0.68	0.313
	$[Cu(LH_{-1})L]^-$	0.762	0.784	0.66	0.342
Gly-Gly-Phe	$[CuLH_{-2}]^-$	0.824	0.763	0.66	0.368
Leu-Gly	$[CuLH_{-1}]$	0.828	0.772	0.65	0.344
	$[Cu(LH_{-1})(OH)]^-$	0.734	0.869	0.73	0.291
	$[Cu(LH_{-1})L]^-$	0.741	0.826	0.69	0.308
Gly-Leu	$[CuLH_{-1}]$	0.828	0.772	0.63	0.362
	$[Cu(LH_{-1})(OH)]^-$	0.755	0.799	0.72	0.322
	$[Cu(LH_{-1})L]^-$	0.758	0.806	0.68	0.339
Gly-Leu-Leu	$[CuLH_{-2}]^-$	0.824	0.782	0.62	0.399
Pro-Gly	$[CuLH_{-1}]$	0.796	0.796	0.68	0.324
	$[Cu(LH_{-1})(OH)]^-$	0.747	0.849	0.70	0.325
	$[Cu(LH_{-1})L]^-$	0.727	0.858	0.71	0.285

* Estimated errors ± 0.005; for β^2 ± 0.02.

the $d_{x^2-y^2}$ orbital (increase in α^2) is compensated by the increase in delocalization from the d_{xy} orbital (decrease in β_1^2), and *vice versa* [Figure 6(*c*)].

The m.o. coefficients for the various compounds are grouped according to the different kinds of molecules. The complexes of $[CuLH_{-1}]$ and $[Cu(LH_{-1})_2]^{2-}$ type, and those of the tripeptides, have relatively ionic in-plane σ bonds, which are compensated by 4*s* σ and in-plane π bonds of more covalent character (Table 3, Figure 6). For the complexes $[Cu(LH_{-1})(OH)]^-$ and $[Cu(LH_{-1})L]^-$, in turn, the in-plane σ bond is more, while the other bonds are less, covalent in character. There is a slight but significant difference between complexes of X-Gly and Gly-X type dipeptides: in the case of the former ligands, the ε'^2 values, *i.e.* the electron densities on the partially occupied 4*s* orbital of copper(II), are less. This correlates with the difference in basicities of the amino and peptide nitrogens between the two types of ligands: the p*K* values for the amino group and the values pK^{H}_{CuL} are smaller for the X-Gly dipeptides,[12] *i.e.* the nitrogen donor atoms of X-Gly ligands are less basic, which results in weaker 4*s* σ bonds.

The above conclusions concerning the metal–ligand bonds have been mainly based upon the e.s.r. data for frozen solutions. The question arises as to whether the bonding information holds for fluid solutions as well. Comparison of isotropic parameters obtained in the two different phases may help to answer, at least in part, this question. There are smaller or larger changes in these data: generally, g_0 and a_{N_0} are larger, while A_0 is smaller in fluid solutions (Figures 1—5, Table 1). As a result of changes in A_0 and g_0, the Fermi hyperfine contact term, *K*, is altered, generally reduced in liquid solution (except for the tripeptide complexes, where a slight increase can be observed). The dependence of *K* on α^2 is in the form of a curve with an extremum (maximum) between $\alpha^2 \approx 0.8$ and ≈ 0.9.[10] This can be used for estimating the change in the covalent character of the in-plane σ bond. The α^2 values for the complexes $[Cu(LH_{-1})(OH)]^-$ and $[Cu(LH_{-1})L]^-$ are small enough to fall in the rising part of the above curve, hence the reduced *K* probably indicates even smaller α^2 in fluid solutions, and is accompanied by a slight increase in a_{N_0} (larger unpaired spin density at the nitrogen nuclei). The complexes $[CuLH_{-1}]$ and $[Cu(LH_{-1})_2]^{2-}$, and those of tripeptides, fall near the maximum of the above-mentioned curve, therefore it is difficult to estimate the changes in α^2 from the small variations of *K*. The slight increase in a_{N_0} of fluid solutions, however, suggests a small increase in covalent character of the in-plane σ bond with these compounds as well. The other metal–ligand bonds probably have less covalent character in liquid solutions, corresponding to the above compensation mechanisms. The changes in m.o. coefficients are estimated to fall in the range of 0.01—0.1.

Conclusions

The most striking difference in the co-ordinating properties of X-Gly and Gly-X type dipeptides appears at ligand excess and high pH: the formation of species $[Cu(LH_{-1})_2]^{2-}$ occurs only the case of X-Gly type ligands at both room and low temperature. The formation of this complex is affected by the side-chain substituent, and is greatly influenced by temperature. Larger substituents of the N-terminal amino acid inhibit its formation at room temperature and near 273 K, at pH ≈13 up to a ligand:metal concentration ratio of 50:1, while with smaller side-chain substituents this species can be found in

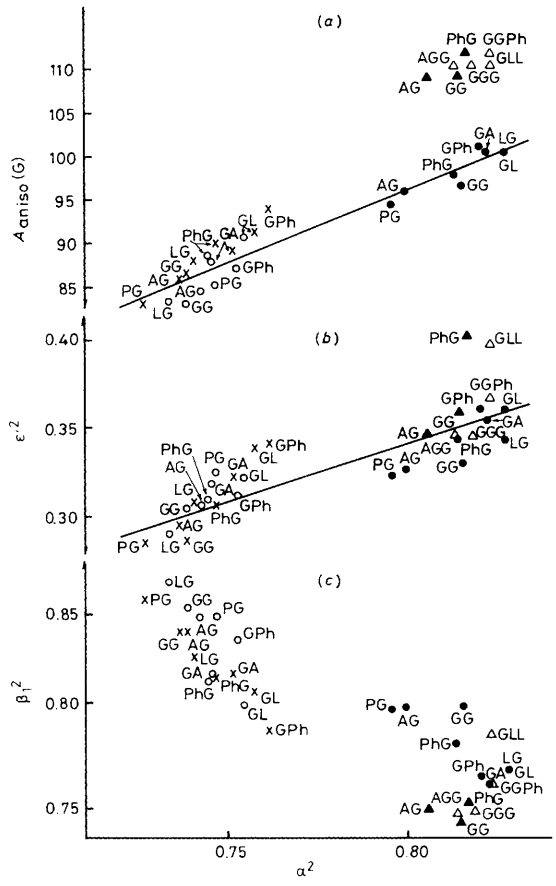

Figure 6. Correlations between e.s.r. and bonding parameters for the various copper(II)–di- and tri-peptide complexes; the ligands are symbolized by the first letters of the amino acids. Species $[CuLH_{-1}]$, (●), $[Cu(LH_{-1})(OH)]^-$ (○), $[Cu(LH_{-1})L]^-$ (×), $[Cu(LH_{-1})_2]^{2-}$ (▲), and tripeptide complexes (△). The solid lines represent the best linear fits to data points

liquid solutions at high ligand excess and pH ≈13. At small ligand excess and high pH, the complex $[Cu(LH_{-1})(OH)]^-$, predominant in fluid solutions, is replaced by $[Cu(LH_{-1})_2]^{2-}$ upon freezing for ligands of X-Gly type. This change is assumed to take place at much lower temperatures than 273 K.

Surprisingly, in the 1:2 complexes of dipeptides, the s.h.f. structure and other e.s.r. and visible spectral evidence indicate the equatorial co-ordination of less nitrogen atoms than the overall number of nitrogen donors, though isomers are likely to occur in small concentration, where all possible nitrogens are bound in equatorial positions. The following structures are proposed for the major isomers. The terdentate equatorial co-ordination of the 'first' ligand remains in both 1:2 complexes, while the fourth equatorial position is occupied by the donor atom from the peptide group of the 'second' dipeptide. This is either the peptide oxygen in the species $[Cu(LH_{-1})L]^-$ where the 'second' amino group is bound axially, or the deproponated peptide nitrogen in the complex $[Cu(LH_{-1})_2]^{2-}$ where the 'second' amino nitrogen and carboxylate oxygen atoms occupy axial positions.

E.s.r. data suggest neither 3*d*–4*s* nor 3*d*–4*p* orbital mixing, hence the effective D_{4h} symmetry for our complexes seems to be a reasonable assumption. The m.o. coefficients show the fairly covalent character of each metal–ligand bond. Compensation effects can be observed between the σ bonds of different symmetries on one hand, and between the σ and the π bond in the equatorial plane on the other hand. Smaller basicities of the amino and peptide nitrogens of X-Gly type dipeptides are manifested in lower electron density on the partially occupied 4*s* orbital of copper(II). Differences in the Fermi term and isotropic nitrogen s.h.f. coupling constant suggest slightly more covalent in-plane σ bonds in fluid than in frozen solutions.

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