Equilibrium and Structural Studies on Proton and Copper(II) Complexes of *N*-D-Gluconylglycine

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N-D-Gluconylglycine, a pseudopeptide derivative of glucono-1,5-lactone and glycine, was prepared and the equilibrium constants of its protonation and copper(II) co-ordination and the structures of the copper complexes formed were studied in aqueous solution by potentiometry, spectrophotometry, CD, EPR and ^{13}C NMR relaxation. The parent complexes formed in an acidic medium have low stabilities, characteristic of carboxylate co-ordination. In the range pH 5–9 the amide group and the 2-OH group of the ligand undergo deprotonation. In parallel with these processes, one ligand is replaced from the copper(II) co-ordination sphere. For the species MLH_{-2}, 3O,1N co-ordination in the equatorial plane is proposed. The EPR measurements indicate that dimeric species are also formed. At pH > 9, further base-consuming processes start as an indication of the deprotonation of other alcoholic hydroxy groups of the sugar moiety or the formation of mixed hydroxo complexes.

Glycoproteins play a prominent part in biological systems.[1,2] Pazur and Aronson[3] suggested that the role of the carbohydrate residues is to promote the transport of glycoproteins through cellular membranes. A second function relates to the immunological responses to these moieties, and a third may be the effect on the adhesion of the cells to one another. The co-ordination chemistry of amino acids, peptides and sugars has been reviewed,[4] their conjugates also being discussed.

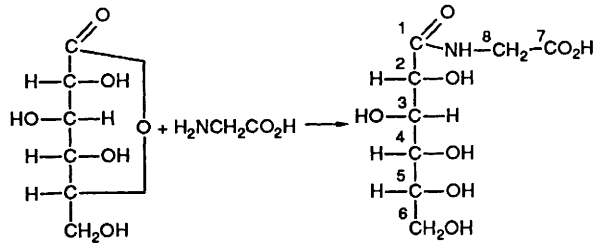
The interactions of divalent metal ions with 2-(polyhydroxyalkyl)thiazolidine-4-carboxylic acid derivatives were recently investigated.[5,6] These ligands were prepared through condensation of an aldose and L-cysteine. The analogous reactions with other amino acids give glycosylamino acids.[7] Hydrolysis of these compounds in aqueous solution, or the Amadori rearrangement, has hindered their co-ordination study in solution.[7-11] Several attempts were made by Lönngren *et al.*[12] to attach aldonic acid substituents to proteins through a pseudopeptide bond. Biondi *et al.*[13] prepared *N*-D-gluconylglycinated bovine pancreatic ribonuclease A and found that the modified enzyme binds the substrate slightly better and shows enhanced stability. Aldonamides were also formed in the Maillard reaction.

Consequently, although gluconamides are known, their complex formation properties remain relatively unexplored. Russian authors have reported qualitative paper chromatographic results on metal complexes of *N*-aldonylglycines.[14] Carbon-13 NMR measurements were carried out by Dill *et al.*[15] on different gluconamides in the presence of manganese(II) and gadolinium(III) ions. The results are in agreement with those of Angyal *et al.*[16]

In order to obtain more information on the complex formation behaviour of such ligands, we have prepared *N*-D-gluconylglycine [*N*-(2,3,4,5,6-pentahydroxyhexanoyl)glycine], and investigated its co-ordination by copper(II) ion by means of potentiometry, and VIS, CD, EPR and NMR spectroscopy.

Experimental

Materials.—All reagents used for the ligand preparation were Reanal products, except for D-glucono-1,5-lactone and glycine ethyl ester hydrochloride (Fluka). The concentration of the copper(II) perchlorate (Fluka) stock solution was determined complexometrically. The other chemicals used for measurements were Reanal p.a. products.



Scheme 1

For the synthesis of *N*-D-gluconylglycine we used the simple method reported by Schneider and Geyer,[17] with an equimolar mixture of D-glucono-1,5-lactone and sodium glycinate in a methanolic medium. The sodium salt was converted into the free acid through treatment with oxalic acid, or on Dowex 50 × $8H^+$ (Fluka) ion-exchange resin. The reaction route and the structure of ligand are depicted in Scheme 1.

We also used the method described by Biondi *et al.*[13] Instead of the sodium salt of glycine, its ethyl ester hydrochloride was added to a methanolic solution of D-glucono-1,5-lactone in the presence of triethylamine. After heating for 2 h, diethyl ether was added dropwise until the mixture became cloudy. The precipitated *N*-D-gluconylglycine ethyl ester was saponified with 1 mol dm^{-3} methanolic sodium hydroxide solution to yield the sodium salt of *N*-D-gluconylglycine. The product was identical to that obtained by the previous method [Found (Calc. for $C_8H_{15}NO_8$): C, 37.95 (37.95); H, 5.95 (5.95); N, 5.45 (5.55)%].

The molecular weight was found by titration with standard sodium hydroxide solution to be 254.2 (calc. 253.2). The ligand was characterized by Fourier-transform, ^1H and ^{13}C NMR methods. The IR spectrum displays the characteristic amide bonds at about 1640 and 1560 cm^{-1} and the carboxylate bond at above 1700 cm^{-1}. The ^{13}C NMR chemical shifts are listed in Table 2.

pH-*Metric Measurements.*—Both the protonation and the copper(II) co-ordination equilibria were investigated by

Table 1 Overall and stepwise protonation and copper(II) complex formation stability constants of *N*-D-gluconylglycine, absorption maxima and molar absorbances of individual species and p*K* values for the deprotonated species (r = 1–3)

Species	log β	log *K*	λ_{max}/nm	ε/dm^3 mol^{-1} cm^{-1}	$pK^{MLH_{-r}}_{MLH_{-r+1}}$
HL	3.39 ± 0.04	—	—	—	—
ML	1.94 ± 0.08	1.94	797 ± 5	21 ± 3	—
ML_2	3.51 ± 0.08	1.57	772 ± 3	34 ± 2	—
MLH_{-1}	−3.82 ± 0.19	7.99	696 ± 3	103 ± 3	5.76
MLH_{-2}	−9.63 ± 0.15	7.94	676 ± 1	109 ± 1	5.81
MLH_{-3}	−20.01 ± 0.16	3.37	667 ± 3	79 ± 3	10.38
$M_2L_2H_{-3}$	−10.20 ± 0.09	3.25	690 ± 4	112 ± 4	—
$M_2L_2H_{-4}$	−16.63 ± 0.11	2.63	686 ± 5	104 ± 4	—

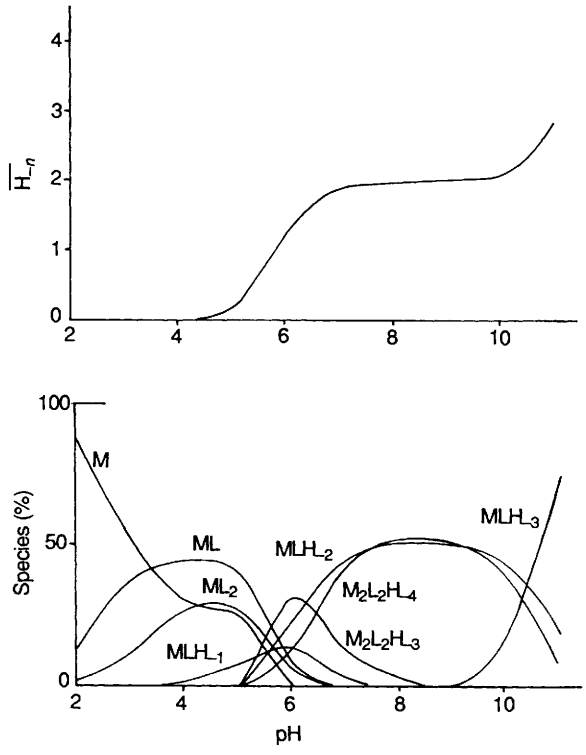


Fig. 1 Plot of $\overline{H}_{-n}$ *vs.* pH, together with the concentration distribution curves of the copper(II)–*N*-D-gluconylglycine system at a metal to ligand ratio of 1:8, [Cu] = 5.0 × 10^{-3} mol dm^{-3}

potentiometric titration in aqueous solution at constant ionic strength (0.1 mol dm^{-3} $NaClO_4$) at 298.0 ± 0.1 K. Details are given in our previous paper.[5] Before each measurement, the electrode system was calibrated in the following way. A mixture of Tris [tris(hydroxymethyl)methylamine] and strong acid ($HClO_4$) was titrated with sodium hydroxide. The protonation constant of the Tris was determined under identical conditions to be log K = 8.17 ± 0.01.

Both the protonation and complex formation constants were determined from five independent titrations with the aid of the computer program PSEQUAD.[18] The metal to ligand ratios were different in each case, varying from 1:5 to 1:15. The metal-ion concentration ranged from 2 × 10^{-3} to 10^{-2} mol dm^{-3}. The stability of the ligand was checked by back-titration from pH 11 to 2.

Visible Absorption and CD Spectra.—The VIS spectra were recorded with a Varian S634 spectrophotometer, and CD spectra with a Jobin-Yvon Mark VI spectropolarimeter in the wavelength interval from 300 to 850 nm.

NMR Spectroscopy.—The ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer at 100.12 MHz and 298 ± 2 K. The pH of solutions in deuteriated water was adjusted to 8.6 with NaOD. (This is the pH-meter reading in D_2O, uncorrected for the isotope effect.) All chemical shifts are given relative to sodium 4,4-dimethyl-4-silapentane-1-sulfonate; the internal reference used was dioxane (67.4 ppm from the sulfonate). Longitudinal relaxation times of ^{13}C nuclei were obtained from partially relaxed Fourier-transform spectra, using the 180°–τ–90° pulse sequence with the fast inversion-recovery variant.[19] Transverse relaxation times were obtained from line broadenings Δν (measured at half-width in the spectra). Specific relaxation rates $1/T_{ir}$ (i = 1 or 2; T_{iF} is the relaxation time in the

$$1/T_{ir} = [(1/T_i) - (1/T_{iF})]/P_M \quad (1)$$

free ligand) were obtained from the slope of a plot of the relaxation rates ($1/T_i$) as a function of the bound ligand, P_M = c_M/c_L. The ligand concentration c_L was 0.7 mol dm^{-3} for all measurements and the copper(II) concentration c_M varied in the interval 0–1.5 × 10^{-2} mol dm^{-3}.

EPR Measurements.—The EPR spectra were obtained with a JEOL-JES-FE 3X spectrometer in the X-band at 77 and 298 K with 100 kHz field modulation. Manganese(II)-doped MgO powder served as field standard. The EPR parameters were calculated with the program package described by Szabó-Plánka *et al.*[20]

Results and Discussion

Potentiometric Measurements.—The protonation and complex stability constants determined pH-metrically for *N*-D-gluconylglycine are listed in Table 1; log β_{CO_2H} is about one order higher than that for glycine. This is due to the presence of the amide group (Scheme 1) and therefore the hydrogen-bond network between the amino and carboxylic groups is distorted. The log β_{CO_2H} value of 3.39 agrees well with those of *N*-acetylglycine and *N*-benzoylglycine, 3.41 and 3.47, respectively.[21] This means that the −I property of the polyhydroxyalkyl chain has a small effect on the protonation constant of the carboxylate group of *N*-D-gluconylglycine. Deprotonation of the amide group in the absence of metal ion was not observable in the pH range (2–11) covered by our titrations.

In the copper(II)–*N*-D-gluconylglycine system at ligand to metal ratios above 5:1 no precipitation occurs in the pH range studied. In solution at pH 2–4 *N*-D-gluconylglycine forms the parent complexes ML and ML_2. The overall formation constants of these species are very similar to those of carboxylic acid complexes in which no other donor groups are present. This indicates that in this pH range the ligand co-ordinates to copper(II) only through the carboxylate group. The fairly small log β values allow only low accuracy in the calculations. For the overall formation constants and the stepwise complex stability constants see Table 1.

When the pH of the ligand solution is increased to above pH 5 in the presence of copper(II), further base-consuming processes start. The $\overline{H}_{-n}$ ($mol_{OH^-}/mol_{Cu^{2+}}$) *vs.* pH curve (shown in Fig. 1

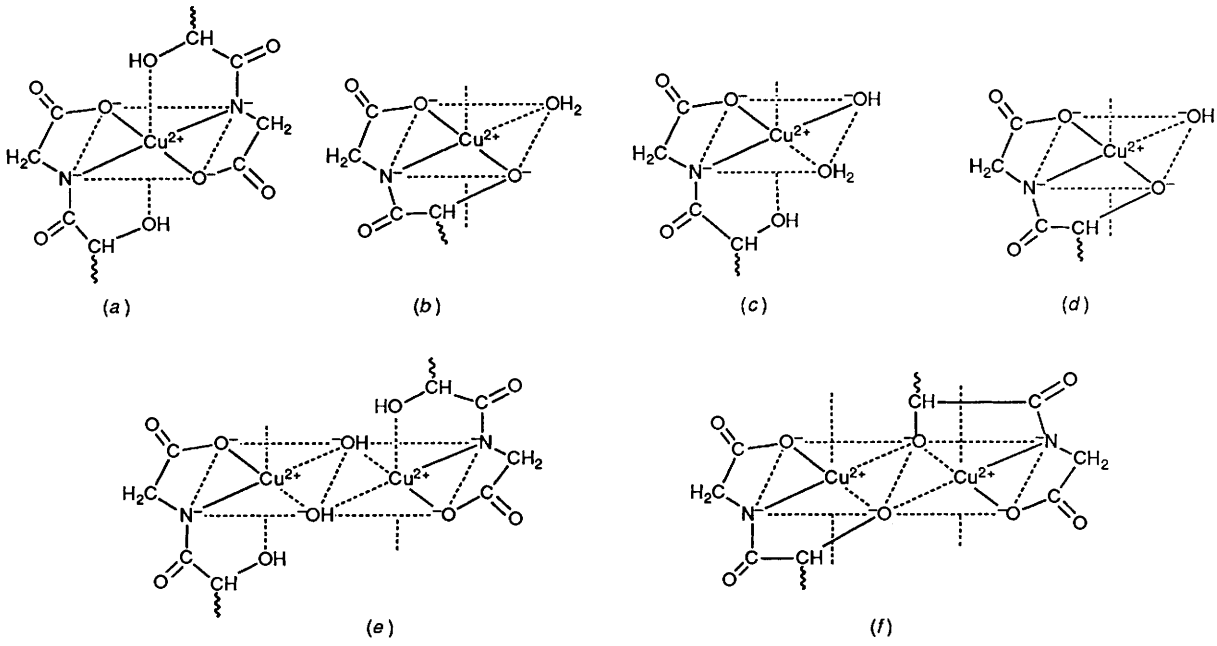


Fig. 2 Proposed structures for the species formed in the equilibrium system in the physiological and basic pH range

together with the distribution curves of the species existing in the equilibrium system) begins to increase at pH > 5 and attains in one step at about pH 7 a value of 2. This observation can be explained in several ways: (1) two equivalent protons are released through the deprotonation of co-ordinated water molecules, *i.e.* hydrolysis of the metal ion takes place; (2) the amide groups from both co-ordinated ligands are deprotonated; (3) one amide group and one hydroxy group on the sugar chain of the same ligand undergo deprotonation; (4) a mixed hydroxo complex is formed, with deprotonated amide and hydroxide-ion co-ordination.

It may be presumed that in the case of metal-ion hydrolysis the carboxylate co-ordination does not provide enough thermodynamic stability, and thus precipitation would occur, as in the case of *N*-acetylglycine.[22] It seems most likely that the amide groups release the protons, similarly as observed for several dipeptides.[4] Several authors[22,23] established that the metal ion needs a primary ligating site or anchor in order to chelate to the amide nitrogen by substitution of its proton. For *N*-D-gluconylglycine, carboxylate is the first group co-ordinated and deprotonation of the amide group results in a stable five-membered chelate ring. The latter type of complexation and the negative inductive effect of the polyhydroxyalkyl chain (which probably decreases the pK value of the amide group) might allow this process.

The alcoholic hydroxy groups are very weak acids, with pK > 12. In spite of this, Vicedomini[24] reported that in the gluconic acid–copper(II) system, where a five-membered chelate ring is formed, the alcoholic hydroxy group begins to deprotonate above pH 4, with $pK_{ML}^{MLH_{-1}} = 5.62$. On this basis, it may be assumed that, after deprotonation of the amide group, the 2-OH group is also deprotonated (see the pK values of the co-ordinated ligand in Table 1). Since the base-consumption is 2 equivalents per copper(II) ion, this latest process presumes the co-ordination of one organic ligand only, *i.e.* the second ligand leaves the co-ordination sphere. This can probably be explained in terms of steric effects, as in the case of *N*-dansylglycine (dansyl = 5-dimethylaminonaphtholene-1-sulfonyl)[25] and several dipeptides.[26] Since this process is not accompanied by the release of a hydrogen ion, it influences the experimental data to only a very slight extent. The values of $\log K_{MLH_{-1}} = 7.99$ and $\log K_{MLH_{-2}} = 7.94$ also suggest very stable co-ordination of the ligand through two fused five-membered chelate rings.

Finally, the deprotonation of one amide group and one co-ordinated water molecule may occur. The lower limit for the pK of $[Cu(OH)]^+$ is nearly 5.75, and for $Cu(OH)_2$ is 10.2.[27] The latter is very similar to $pK_{MLH_{-2}}^{MLH_{-3}} = 10.38$ in our system.

Parallel with the appearance of doubly deprotonated species, dimeric complex species are also formed (see the EPR measurements). Two copper(II) ions are probably bridged through one and two alcoholic hydroxy groups or hydroxide ions in the species $M_2L_2H_{-3}$ and $M_2L_2H_{-4}$, respectively. The proposed structures for possible species are depicted in Fig. 2.

(*a*) Overall formation processes

$$pM + qL \overset{\beta_{M_pL_qH_{-r}}}{\rightleftharpoons} M_pL_qH_{-r} + rH$$

or

$$pM + qL + rH_2O \overset{\beta_{M_pL_q(OH)_r}}{\rightleftharpoons} M_pL_q(OH)_r + rH$$

$$\beta_{M_pL_qH_{-r}} = \beta_{M_pL_q(OH)_r} = \frac{[M_pL_qH_{-r}][H]^r}{[M]^p[L]^q} = \frac{[M_pL_qH_{-r}]K_w^r}{[M]^p[L]^q[OH]^r}$$

(*b*) Formation of deprotonated species

$$MLH_{-r+1} + OH \overset{K_{MLH_{-r}}}{\rightleftharpoons} MLH_{-r} + H_2O$$

or

$$ML(OH)_{r-1} + OH \overset{K_{ML(OH)_r}}{\rightleftharpoons} ML(OH)_r$$

$$K_{MLH_{-r}} = K_{ML(OH)_r} = [MLH_{-r}]/[MLH_{-r+1}][OH]$$

(*c*) Deprotonation equilibria

$$MLH_{-r+1} \overset{K_{MLH_{-r+1}}^{MLH_{-r}}}{\rightleftharpoons} MLH_{-r} + H$$

or

$$ML(OH)_{r-1} + H_2O \overset{K_{ML(OH)_{r-1}}^{ML(OH)_r}}{\rightleftharpoons} ML(OH)_r + H$$

$$K_{MLH_{-r+1}}^{MLH_{-r}} = K_{ML(OH)_{r-1}}^{ML(OH)_r} = \frac{[MLH_{-r}]K_w}{[MLH_{-r+1}][OH]} = \frac{\beta_{MLH_{-r}}}{\beta_{MLH_{-r+1}}} = K_{MLH_{-r}}K_w$$

Scheme 2 Equilibrium processes in the copper(II)–*N*-D-gluconylglycine system and related stability constants where $K_w = 10^{-13.75}$ (ref. 28) and $r = 0$–3.

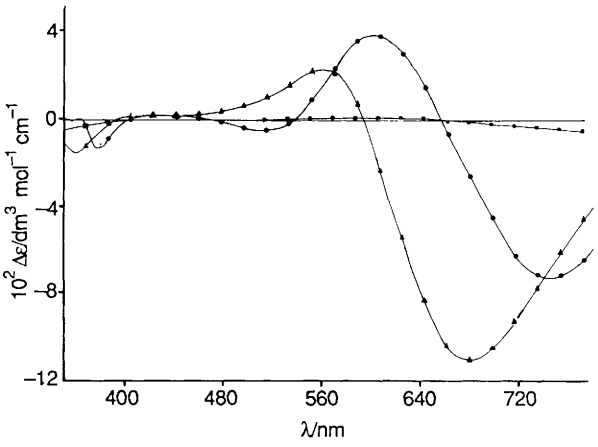


Fig. 3 The CD spectra of the copper(II)–*N*-D-gluconylglycine system at different pH values at [Cu] = 1.0×10^{-2} mol dm^{-3} and [L] = 1.0×10^{-1} mol dm^{-3}. pH 4.0 (■), 8.4 (●) and 10.5 (▲)

Table 2 Chemical shifts of metal-free *N*-D-gluconylglycine, and the longitudinal and transversal relaxation rates of ^{13}C nuclei in the presence of copper(II)

Atom	δ	T_r^{-1}/s^{-1}	T_{2r}^{-1}/s^{-1}
C(1)	175.02	48	1 141
C(2)	74.13	63	854
C(3)	71.16	38	819
C(4)	72.66	12	525
C(5)	71.93	8	378
C(6)	63.46	8	189
C(7)	177.41	85	4 228
C(8)	43.80	69	25 760

With further increase of the pH to above 9, the $\overline{H_{-n}}$ *vs.* pH curve rises further and the formation of new species is not complete until pH 11. This process could be ascribed, as discussed above, to the deprotonation of co-ordinated water or of a further alcoholic hydroxy group. On the basic of potentiometric results only it is not possible to distinguish between the above processes. Therefore, other spectroscopic methods were included in the structural study of the species formed.

The above processes are shown in Scheme 2.

Spectrophotometric Measurements.—Table 1 shows the λ_{max} values and molar absorbances determined by the PSEQUAD computer program for the copper(II)–*N*-D-gluconylglycine species present in solution at different pH. The shift in the absorption maximum to lower wavelengths with increasing pH demonstrates the increase in d–d transition energy. The value of λ_{max} = 676 nm at pH 8.13 is characteristic for copper(II) complexes formed by single-nitrogen co-ordination[22,25] and suggests that the species having the structures depicted in Fig. 2(*b*) or 2(*c*) predominate in solution. The theoretical calculated absorption maximum for the species in Fig. 2(*a*) would be at about 600 nm.[22] The spectrum of the copper(II)–*N*-D-gluconylglycine system at 1:1 molar ratio at pH 8.0 does not differ significantly from that obtained at higher L:M ratios, which supports the above hypothesis. With further increase in pH the decrease in the wavelength of the absorption maximum continued. At very high pH (> 12), λ_{max} attains a value of about 590 nm.

CD Measurements.—Circular dichroism spectroscopy proved to be suitable for investigation of the optically active species. The spectra obtained in the visible region are shown in Fig. 3. At pH about 4, as expected, practically no CD effect was observed. The fact that a CD spectrum could be obtained at pH 8.4 demonstrates that one of the alcoholic hydroxy groups is co-ordinated to the copper(II), since no other optically active part is present in the ligand.

A large number of measurements[29,30] led to the proposal that the co-ordination of one donor atom on a carbon atom having an *R* configuration caused two Cotton effects, a positive one at about 600 nm and a negative one at about 720 nm. Very similar spectra were obtained for the copper(II)–gluconic acid, –glucose and –galactosamine[31] systems. In these ligands the configuration of the carbon atom bearing the donor group is also *R*. These results therefore also indicate the co-ordination of an alcoholic hydroxy group, probably on C(2), at physiological pH values, suggesting the presence of the complex species with the structure shown in Fig. 2(*b*).

It was earlier found that the formation of mixed hydroxo complexes does not usually affect the shape of the CD spectrum. However, the CD curve obtained for the copper(II)–*N*-D-gluconylglycine system at pH 10–11 exhibits a similar course to that at pH 8.4, but the spectrum is shifted to lower wavelengths. This is probably due to the decomposition of dimeric species and to the formation of mixed hydroxo complexes.

NMR Relaxation Measurements.—Carbon relaxation in paramagnetic systems is widely used to obtain structural information on complexes of transition-metal ions. The paramagnetic relaxation enhancement is caused by a random variation of the nuclear–electron spin–spin interaction; the main features of this phenomenon have been described by Solomon[32] and by Bloembergen and Morgan.[33] Selective line-broadening measurement (enhancement in transversal relaxation times due to the paramagnetic metal-ion co-ordination) is a convenient means of gaining qualitative information about possible binding sites. In many cases, however, besides the dipolar mechanism, the scalar contribution to transversal relaxation is also important. The r^{-6} dependence appears only when the dipolar term is predominant and furnishes the basis for stating that lines from nuclei nearest to the paramagnetic ion are most broadened, equations (2) and (3) where τ_R is the reorientation

$$1/T_{2M} = 7a\tau_R r^{-6} \quad (2)$$

$$a = \gamma_I^2 g^2 \beta^2 S(S + 1)/15 \quad (3)$$

correlation time and *r* the distance between the paramagnetic ion and the measured nucleus.

Thus, in order to acquire a more detailed picture of the co-ordination the longitudinal (spin–lattice) relaxation of the ligand carbons were also measured, in which the dipolar term predominates in almost all cases; this interaction also contains an r^{-6} (metal ion–measured carbon) distance dependence [equation (4)]. Account is taken that the ligand exchange is fast

$$1/T_{1M} = 1/(T_{1r} + \tau_M) = 6a\tau_R r^{-6} \approx 1/T_{1r} \quad (4)$$

enough to make τ_M (the lifetime of the ligand in the complex) negligible with respect to T_{1r}, which is the case in general in copper(II) complexes.

The NMR relaxation measurements were performed in D_2O and with a high excess of ligand; the complex formed at pH 8.6 is the same as the monomeric species formed under the pH-metric measurement conditions. The chemical shifts and the longitudinal and transversal relaxation rates determined are listed in Table 2.

The ratios T_{1r}/T_{2r} for all carbon atoms were found to be higher than 7:6, which demonstrates the predominant role of the scalar mechanism in the transversal relaxation. On the other hand, the orders of magnitude of $1/T_{1r}$ and $1/T_{2r}$ agree quite well with each other, except in the case of C(8) (see Table 2). The very high value of $1/T_{2r}$ in this case suggests a much higher distribution of unpaired spin density on this carbon as compared with the others. The longitudinal relaxation rates reveal a better picture. The co-ordination of the carboxylate

Table 3 The EPR parameters for the predominant species at different pH values in the copper(II)–*N*-D-gluconylglycine system at a metal to ligand ratio of 1:5, $[Cu^{2+}] = 5.0 \times 10^{-3}$ mol dm^{-3}. Coupling constants in G (10^{-4} T)

pH	Species	$g_{\parallel}$	$g_{\perp}$	g_0	$A_{\parallel}$	$A_{\perp}$	A_0	$a_{N\parallel}$	$a_{N\perp}$	a_0
2.0	$M(H_2O)_6$	2.395	2.071	2.205	125	12.6	35.2	—	—	—
4.1	ML or ML_2	2.355	2.058	2.171	134	13.9	50.0	—	—	—
6.0	MLH_{-1}	2.291	2.045	2.159	160	15.2	56.9	12	12	12
8.4	MLH_{-2}	2.251	2.041	2.128	180	19.6	67.1	12	13	13
10.5	MLH_{-3}	2.250	2.045	2.122	173	18.8	37.4	13	14	12
>12	MLH_{-4}	2.215	2.036	2.100	191	23.9	73.4	14	14	13

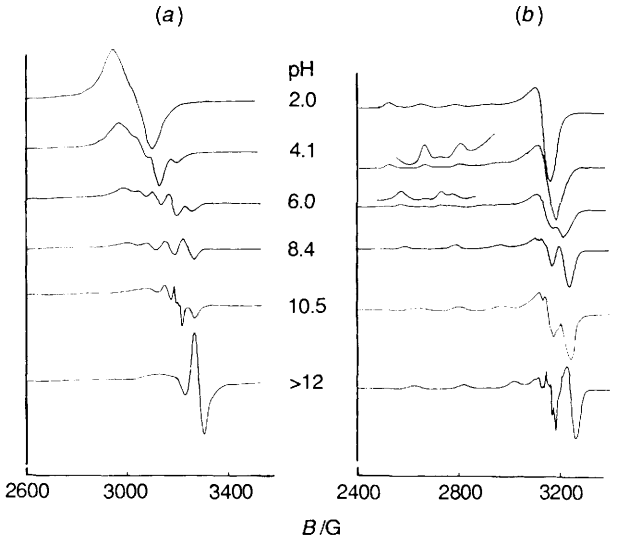


Fig. 4 The EPR spectra of the copper(II)–*N*-D-gluconylglycine system at different pH values at a metal to ligand ratio of 1:5 and [Cu] = 5.0×10^{-3} mol dm^{-3}. (*a*) 298, (*b*) 77 K; G = 10^{-4} T

group is evident from the data. For C(8) the same magnitude of $1/T_{1r}$ suggests co-ordination of the deprotonated peptide nitrogen. The high value of $1/T_{2r}$ for this carbon confirms this: the delocalization of a paramagnetic electron generally occurs *via* chemical bonds. Besides the average $1/T_{1r}$ values of C(1) and C(3), C(2) also has a higher longitudinal relaxation rate. This fact, in conjunction with the potentiometric results (two deprotonations per metal ion), indicates the co-ordination of the deprotonated hydroxy group on C(2). Deprotonation of this hydroxy group was also revealed in the copper(II)–gluconate system by combined potentiometric and NMR relaxation methods.[34]

Finally, as the relaxation rates show, C(4), C(5) and C(6) are far from the metal ion in the complex. Overall, therefore, our NMR relaxation measurements at $-\log[H^+] = 8.6$ suggest the co-ordination of carboxylate, deprotonated amide, and deprotonated alcoholic hydroxy groups, to form two five-membered chelate rings as shown in Fig. 2(*b*).

EPR Measurements.—This method is frequently used for the study of copper(II) complexes. We recorded EPR spectra at different pH values at room temperature and at 77 K. Several spectra are depicted in Fig. 4, and the determined EPR parameters are given in Table 3.

The spectra recorded at room temperature reflect the actual equilibria because shifts in these must be expected in consequence of the temperature dependence of the formation constants. However, in some cases the spectra obtained at liquid-nitrogen temperature may help towards an understanding of the isotropic spectra.

The spectrum at pH 2.0 does not differ significantly from the pattern typical for the hexaaquacopper(II) ion, with one broad band only. The parameters are very similar to published values.[35] With increase in pH to 4.1 the spectrum superimposed on the broad singlet shows that unco-ordinated copper(II) still exists at this pH. Nevertheless, the splitting of the spectrum indicates the start of complex formation. These lines probably belong to the species ML and ML_2. The spectrum at pH 6.0 reveals that the concentration of the species present in small amount at pH 4.1 is increased. The lines are shifted to higher magnetic fields. The parameters determined from both isotropic and anisotropic spectra are very similar to those of single-nitrogen-containing systems. Comparison with the distribution diagram (Fig. 1) may indicate that this species is MLH_{-1}.

The intensity of the spectrum is considerably decreased with increasing pH. This is probably due to the formation of dimeric species which are not EPR-detectable because of copper(II)–copper(II) antiferromagnetic interactions. For the copper(II) ion four lines would have been expected, but in the spectrum at pH 6.0 there is also a fifth line at higher magnetic fields, corresponding to another complex species, probably MLH_{-2}, which is the only EPR-detectable complex at pH 8.4.

In the spectra recorded at 77 K the lines due to the complexes ML and ML_2 as minor species could also be found. This pattern is well reflected in the distribution diagram, which shows that at pH 6 a large number of species are present. Owing to dimerization, the amplitude of the spectrum at pH 8.4 is small. Increase in the ligand–metal ratio results in an increase in amplitude, indicating that in the competition between deprotonation of the co-ordinated water molecule and of alcoholic hydroxy groups the latter becomes more important. This suggests that in the dimeric species the copper(II) ions are bridged *via* hydroxide ions [Fig. 2(*e*)]. The coupling constants determined for the species MLH_{-2} are higher than for MLH_{-1}. The increased hyperfine coupling constants and the decreased g values, *i.e.* the shift of the spectra to higher magnetic fields, means that the ligand field around the copper(II) ion is increased. This fact relates mainly to the increased number of nitrogen-donor atoms in the equatorial plane of the co-ordination sphere, but deprotonated alcoholic hydroxy groups can also cause this.[35,36] The EPR parameters are obtained through a computer simulation in which one nitrogen atom is assumed around the copper(II) ion as a best model, supporting the structure in Fig. 3(*b*) for the monomeric species MLH_{-2}.

At pH > 10 a new species appears. The increase in amplitude demonstrates the start of decomposition of dimeric species. An interesting feature of this spectrum is that the coupling constant is significantly decreased. Such an observation was likewise made for copper(II)–dipeptide systems in which mixed hydroxo complexes are formed.[20] In our case it suggests the structure in Fig. 2(*d*) for the species MLH_{-3}. This is in agreement with the results of the previous spectroscopic investigations. In this spectrum the triplet due to the superhyperfine splitting effect of one nitrogen is visible.

At very high pH (> 12) the spectrum is again shifted to higher magnetic fields. Only the last line in the spectrum is visible. Such a spectral pattern could be obtained when a bulky ligand chain surrounds the central ion, and consequently the rotation energy of the molecule decreases. If the energy of the rotation and the energy calculated from the distance between parallel and perpendicular lines are nearly equivalent, line broadening

occurs.[37] This phenomenon usually affects the lines at lower fields. The spectrum obtained at 77 K supports the explanation of the spectrum observed at room temperature. It is the spectrum of the new species alone with very high coupling constants [significantly different from that of the species $[Cu(OH)_4]^{2-}$, indicating that further deprotonated alcoholic hydroxy groups are probably co-ordinated to the copper(II) ion.

Conclusion

N-D-Gluconylglycine appears to be an efficient complex-forming ligand in the physiological pH range, through the deprotonation of amide and alcoholic hydroxy groups. The parallel formation of mixed hydroxo complexes and therefore of dimeric species also occurs. The deprotonation of the co-ordinated ligand and of water molecules cannot be easily distinguished. The copper(II)–*N*-D-gluconylglycine system is a very complicated one and requires further investigation.

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