THE CHELATION OF METAL IONS BY DIPEPTIDES AND RELATED SUBSTANCES

PART 5.—CUPRIC COMPLEXES OF SARCOSYL AND LEUCYL LIGANDS

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The cupric complexes of some sarcosyl and leucyl ligands have been investigated by potentiometric methods employing the glass electrode. When the ligand/metal ratio is 2/1, the two acid ionizations per metal occur from the complexes of sarcosine amide, sarcosine methylamide, sarcosyl-L-leucine, leucine amide, leucylglycine and leucylglycylglycine, but not from the complexes of sarcosine, sarcosylsarcosine, sarcosine dimethylamide or leucine. When the ligand/metal ratio is 1/1, two acid ionizations per metal occur from the complexes of the peptides other than sarcosylsarcosine, but not from the complexes of the amino acid amides. The stability and acid dissociation constants of the complexes are reported. With the exception of sarcosine dimethylamide, the amino acid amides and the tripeptide, leucylglycylglycine, give the biuret reaction, the nature of which is discussed.

The Cu(II) complexes of amino acids, peptides and related substances were discussed in previous articles in this series [1, 2] and have been the subject of a recent review.[3] Acid dissociations from the cupric complexes of di- and tripeptides were first demonstrated by Dobbie and Kermack,[4, 5] and the reactions which occur have been interpreted in terms of the following equilibria: [2, 4, 5]

(*a*) for a mixture equimolar with respect to peptide and Cu(II),

$$Cu^{2+} + L^- \overset{K_1}{\rightleftharpoons} CuL^+, \qquad \text{(i)}$$

$$CuL^+ \overset{K_c}{\rightleftharpoons} CuL + H^+, \qquad \text{(ii)}$$

$$CuL \overset{K_{c'}}{\rightleftharpoons} CuL^- + H^+. \qquad \text{(iii)}$$

(*b*) for a mixture in which the molar ratio peptide/Cu(II) is 2 or more,

$$Cu^{2+} + L^- \overset{K_1}{\rightleftharpoons} CuL^+, \qquad \text{(i)}$$

$$CuL^+ \overset{K_c}{\rightleftharpoons} CuL + H^+, \qquad \text{(ii)}$$

$$CuL + L^- \overset{K_2}{\rightleftharpoons} CuL_2^-, \qquad \text{(iv)}$$

$$CuL_2^- \overset{K_{c''}}{\rightleftharpoons} CuL_2^{2-} + H^+. \qquad \text{(v)}$$

Datta and Rabin [1] showed that substitution of the peptide hydrogen in glycylglycine by a methyl group abolished the acid ionization (ii); it was concluded that this reaction involved the ionization of the peptide hydrogen atom. Reactions (iii) and (v) were attributed to acid ionizations from co-ordinated water molecules. The source of the acid ionizations from diglycylglycine + Cu(II) complexes is less certain since tripeptides in which the peptide hydrogen atoms are replaced by methyl groups have not been investigated. Diglycylglycine differs from glycylglycine in that the acid ionizations (iii) and (v) are accompanied by a change in colour from deep blue to purple; this change will be referred to as the biuret reaction.

The interactions of glycine amide with Cu(II) differ from those of glycylglycine and diglycylglycine in that no acid dissociations occur from the 1 : 1 complex,[1] but two acid ionizations per copper atom occur from the 2 : 1 complex. The following sequence of reactions has been suggested : [1]

$$Cu^{2+} + L \overset{K_1}{\rightleftharpoons} CuL^{2+}, \qquad \text{(vi)}$$

$$CuL^{2+} + L \overset{K_2}{\rightleftharpoons} CuL_2^{2+}, \qquad \text{(vii)}$$

$$CuL_2^{2+} \overset{K_c}{\rightleftharpoons} CuL_2^{+} + H^{+}, \qquad \text{(viii)}$$

$$CuL_2^{+} \overset{K_{c''}}{\rightleftharpoons} CuL_2 + H^{+}. \qquad \text{(ix)}$$

Reaction (ix) is accompanied by the formation of a biuret colour. Thus the reaction sequences for amides and tripeptides are different, but these ligands are similar in giving, under appropriate conditions, the biuret reaction. Dipeptides differ from tripeptides in failing to give the biuret reaction, but the reaction sequences for di- and tripeptides are the same. The Cu(II) complexes of amides and peptides of sarcosine and leucine have been investigated in order to elucidate the nature of the interactions.

EXPERIMENTAL

The sources of the ligands and the experimental procedure are as previously described.[6]

RESULTS

SARCOSINE AMIDE, SARCOSINE METHYLAMIDE AND LEUCINE AMIDE

The titration curves of sarcosine amide in the presence of Cu(II) are shown in fig. 1. When equimolar mixtures of the ligand hydrochloride and $CuCl_2$ were titrated with KOH, a precipitate of $Cu(OH)_2$ formed after the addition of 0·5 equivalents of alkali; thus the possibility that acid dissociations occurred from the 1 : 1 complex could not be investigated. When the molar ratio of ligand/metal is 2/1, four equivalents of alkali per Cu atom (i.e. 2 equivalents per ligand molecule) are required to titrate all the acid produced and no precipitation of $Cu(OH)_2$ was observed. Thus, under these conditions, two equivalents of acid per copper atom (i.e., one per ligand molecule) additional to those in the uncomplexed ligand are available for titration. During the titration of this additional acidity, a biuret colour was observed. Sarcosine methylamide and L-leucine amide showed identical behaviour; thus these three ligands are similar to glycine amide in their interactions with Cu(II). The scheme of complex formation is reactions (vi)-(ix); consistent values of equilibrium constants could not be computed on any other basis. The constants determined are defined thus:

$$K_a = \frac{[L][H^+]}{[L^+]}; \qquad K_1 = \frac{[CuL^{2+}]}{[Cu^{2+}][L]}; \qquad K_2 = \frac{[CuL_2^{2+}]}{[CuL^{2+}][L]};$$

$$K_c = \frac{[CuL_2^{+}][H^+]}{[CuL_2^{2+}]}; \qquad K_{c''} = \frac{[CuL_2][H^+]}{[CuL_2^{+}]}.$$

The equilibrium constants K_a, K_1 and K_2 were determined by methods previously described. For 1 : 1 ligand + Cu(II) mixtures, titration data before the onset of precipitation was employed; for 2 : 1 ligand + Cu(II) mixtures, data were used from the regions of the titration curves before the occurrence of additional acid dissociations.

The acid dissociation constants K_c and $K_{c''}$ were obtained from the titration curves of 2 : 1 ligand + Cu(II) mixtures. It was assumed that the 2 : 1 complex, CuL_2^{2+}, behaves as a dibasic acid. The method of Speakman [7] was employed to compute the constants from the data obtained in the region of the titration curve between the addition of two and four equivalents of alkali per copper atom. The concentrations of 1 : 1 complex and free ligand were assumed to be negligible.

The stability and acid dissociation constants of the Cu(II) complexes are shown in table 1.

SARCOSINE DIMETHYLAMIDE

Titration curves of this ligand in the presence and absence of cupric ions are shown in fig. 2, from which it is seen that no additional acid dissociations occur. The equilibria can be represented by eqn. (vi) and (vii) and the constants were computed in the usual manner (table 1).

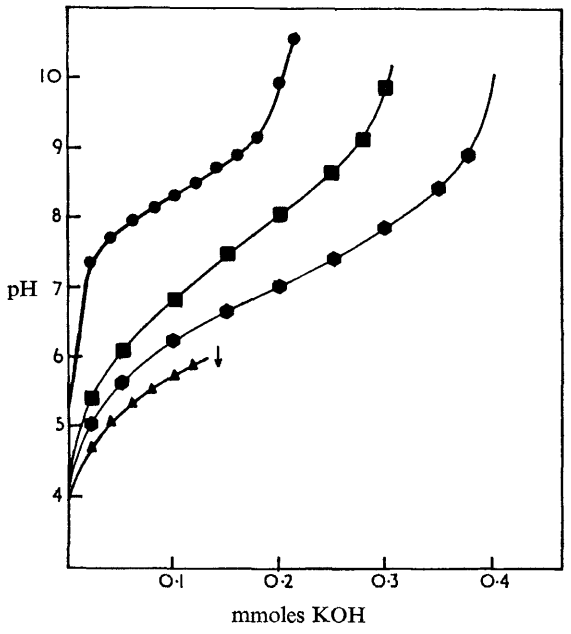


FIG. 1.—Titration curves of sarcosine amide (0·2 mmole in 20 ml water) alone and in the presence of $CuCl_2$.

—●—, ligand alone;
—▲—, ligand + 0·2 mmole $CuCl_2$;
—♦—, ligand + 0·1 mmole $CuCl_2$;
—■—, ligand + 0·05 mmole $CuCl_2$.

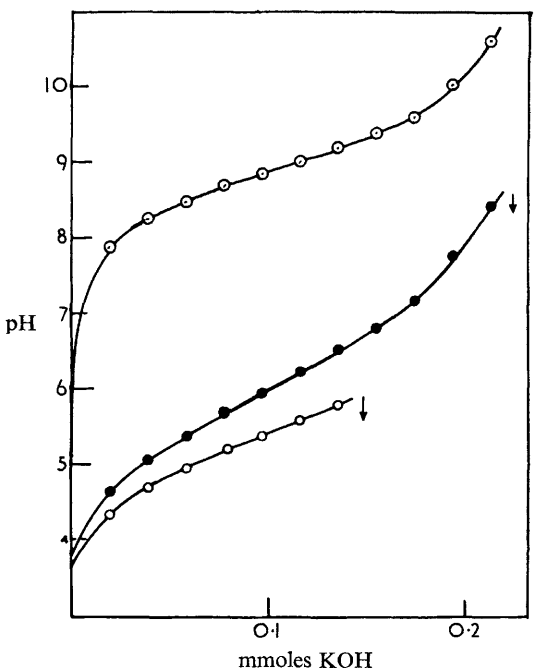


FIG. 2.—Titration curves of sarcosine dimethylamide (0·2 mmole in 20 ml water) alone and in the presence of $CuCl_2$.

—⊙—, ligand alone;
—○—, ligand + 0·2 mmole $CuCl_2$;
—●—, ligand + 0·1 mmole $CuCl_2$.

TABLE 1.—STABILITY AND ACID DISSOCIATION CONSTANTS OF CUPRIC COMPLEXES OF SOME AMINO ACID AMIDES AT 25°C

The initial ligand concentration was 0·01 M throughout. No activity coefficient corrections were made.

ligand	pK_a	$\log_{10} K_1$	$\log_{10} K_2$	pK_c	$pK_{c''}$	molar ratio [ligand]/[metal]
sarcosine amide	8·35	4·65	4·15	7·48	8·12	2 : 1
		4·72	4·23	*	*	1 : 1
sarcosine methylamide	8·28	4·79	4·00	7·76	8·90	2 : 1
		4·86	4·04	*	*	1 : 1
L-leucine amide	7·84	4·79	3·92	7·10	7·90	2 : 1
		4·67	3·95	*	*	1 : 1

* not measurable owing to precipitation of $Cu(OH)_2$.

SARCOSINE, LEUCINE AND SARCOSYLSARCOSINE

Titration of these ligands in the presence of cupric chloride in both 1 : 1 and 2 : 1 molar proportions showed the usual stepwise formation characteristic of simple α-amino acids [8] and the equilibria can be represented as follows:

$$Cu^{2+} + L^- \overset{K_1}{\rightleftharpoons} CuL^+, \qquad (x)$$

$$CuL^+ + L^- \overset{K_2}{\rightleftharpoons} CuL_2. \qquad (xi)$$

The constants determined are defined thus:

$$K_1 = \frac{[CuL^+]}{[Cu^{2+}][L^-]} \quad \text{and} \quad K_2 = \frac{[CuL_2]}{[CuL^+][L^-]}.$$

They were calculated as previously described [1] and are shown in table 2. No additional acid dissociations occurred for any of these ligands; hence sarcosylsarcosine resembles glycylsarcosine in its co-ordinating properties with Cu(II).

TABLE 2.—STABILITY CONSTANTS OF SOME CUPRIC COMPLEXES AT 25°

The initial ligand concentration was 0·01 M throughout. No activity coefficient corrections were made.

ligand	pK_a	$\log_{10} K_1$	$\log_{10} K_2$	molar ratio [ligand]/[metal]
sarcosine	10·09	7·85	6·61	2 : 1
		7·83	6·54	1 : 1
sarcosine dimethylamide	8·86	5·60	4·78	2 : 1
		5·60	4·94	1 : 1
sarcosylsarcosine	9·15	6·06	5·12	2 : 1
		6·01	5·17	1 : 1
L-leucine	9·77	8·37	6·98	2 : 1
		8·35	7·00	1 : 1

On titration of mixtures of leucine and cupric chloride (2 : 1 and 1 : 1) precipitates were formed in the pH region 4-5. These precipitates may be hydroxylated 1 : 1 complexes as described by Bretton. [9]

SARCOSYL-L-LEUCINE AND L-LEUCYLGLYCINE

Titration curves of sarcosyl-leucine in the presence of 0·5 and 1·0 molar equivalents of cupric chloride are shown in fig. 3. Titration curves of L-leucylglycine are similar and both ligands show the same behaviour as glycylglycine.[1, 4] The equilibria concerned are eqn. (i)-(v) and the constants determined are defined thus:

$$K_1 = \frac{[CuL^+]}{[Cu^{2+}][L^-]}; \qquad K_c = \frac{[CuL][H^+]}{[CuL^+]}; \qquad K_{c'} = \frac{[CuL^-][H^+]}{[CuL]};$$

$$K_2 = \frac{[CuL_2^-]}{[CuL][L^-]}; \qquad K_{c''} = \frac{[CuL_2^{2-}][H^+]}{[CuL_2]}$$

The method of Datta and Rabin [1] was used to calculate the constants K_1 and K_c from the titration data of mixtures of ligand and Cu(II) in equimolar proportions. As a check on the validity of the reaction scheme (i)-(v) these constants were also calculated from the data obtained from 2 : 1 ligand + Cu(II) mixtures in the region A′-B′ (fig. 3). The

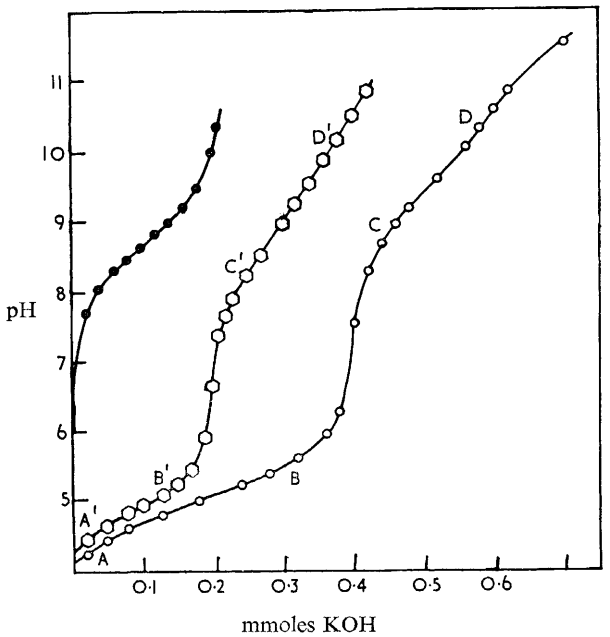


FIG. 3.—Titration curves of sarcosyl-L-leucine (0·2 mmole in 20 ml water) alone and in the presence of $CuCl_2$.

—●—, ligand alone; —○—, ligand + 0·2 mmole $CuCl_2$;
—◊—, ligand + 0·1 mmole $CuCl_2$.

equations of Datta and Rabin were modified to take account of the different molar proportions of ligand to metal. The equation relating K_1 and K_c to known or measured quantities under these conditions is

$$\frac{1}{K_1} = \frac{[A'\beta + 2[Cu_0](\beta - 1)][\beta(A'\delta - \epsilon[Cu_0]) + \delta(\beta - 1)[Cu_0]]}{[\beta\epsilon + \delta(\beta - 1)][2\epsilon[Cu_0] - A'\delta]},$$

where

$$A' = [Cl^-] - [K^+] - [H^+],$$
$$\beta = 1 + K_c/[H^+],$$
$$\delta = 1 + [H^+]/K_a + [H^+]^2/K_aK_b,$$
$$\epsilon = [H^+]/K_a + 2[H^+]^2/K_aK_b.$$

The method of handling this equation is essentially similar to that previously described,[1] except that a function ϕ_1 replaces ϕ, where

$$\phi_1 = \frac{A'(A'\delta - \epsilon[Cu_0])}{(2\epsilon[Cu_0] - A'\delta)}.$$

$\phi_1 \rightarrow 1/K_1$ as $1/[H^+] \rightarrow 0$. The procedure for determining the best values of K_1 and K_c commensurate with the data is identical with that employed for ligand + Cu(II) 1 : 1 mixtures.

Values of K_2 and K_c'' were obtained from analysis of the data in the region of the pH curve C′-D′ (fig. 3). In this region the equilibria to be considered are (iv) and (v)

and the following equations for conservation of ligand, metal and electroneutrality apply:

$$[L_0] = [L^{\pm}] + [L^-] + [CuL] + 2[CuL_2^=] + 2[CuL_2^{2-}], \quad \text{(xii)}$$

$$[Cu_0] = [CuL] + [CuL_2^=] + [CuL_2^{2-}], \quad \text{(xiii)}$$

$$[K^+] = [L^-] + [CuL_2^=] + 2[CuL_2^{2-}] + [Cl^-] + [OH^-]. \quad \text{(xiv)}$$

Combination of eqn. (xii), (xiii) and (xiv) gives the expression,

$$K_2 = \frac{([Cu_0] - B(1 + [H^+]/K_a))[(1 + K_{c'}/[H^+]) - (1 + [H^+]/K_a)(1 + 2K_{c''}/[H^+])]}{(1 + [H^+]/K_a)[B(1 + K_{c''}/[H^+]) - [Cu_0](1 + 2K_{c''}/[H^+])]^2},$$

where $$B = [K^+] - [Cl^-] - [OH^-].$$

The best values of K_2 and $K_{c''}$ were determined by the following method. A function ϕ_2 was defined thus:

$$\phi_2 = -\frac{[H^+][[Cu_0] - (1 + [H^+]/K_a)B]}{K_a(1 + [H^+]/K_a)(B - [Cu_0])^2}.$$

$\phi_2 \to K_2$ as $1/[H^+] \to 0$. An initial value of $\log K_2$ was obtained by plotting $\log \phi_2$ against $1/[H^+]$ and extrapolating to $1/[H^+] = 0$. An example of such a plot is shown in fig. 4. The procedure of minimizing the variances was used to obtain the final values

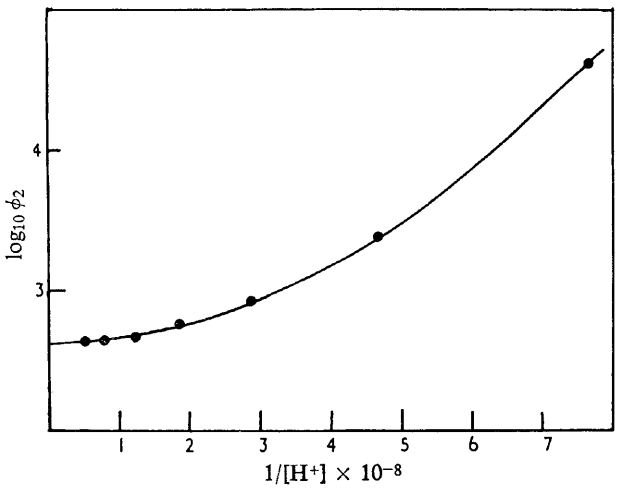


FIG. 4.—Example of the method used to obtain a preliminary value of $\log_{10}K_2$.

of K_2 and $K_{c''}$ as described [1] for K_1 and K_c. The minima in the plots of the variances of $K_{c''}$ against $\log_{10} K_2$ and the variances of K_2 against $pK_{c''}$ corresponded to the same values of K_2 and $K_{c''}$. This demonstrates that the method is internally consistent. Examples of these plots are shown in fig. 5.

TABLE 3.—STABILITY AND ACID DISSOCIATION CONSTANTS OF CUPRIC COMPLEXES OF SOME PEPTIDES AT 25°

The initial ligand concentration was 0·01 M throughout.
No activity coefficient corrections were made.

ligand	pK_a	$\log_{10} K_1$	$\log_{10} K_2$	pK_c	$pK_{c'}$	$pK_{c''}$	molar ratio [ligand]/[metal]
sarcosyl-L-leucine	8·64	5·32	2·61	4·35	—	9·18	2 : 1
		5·48	—	4·56	9·42	—	1 : 1
L-leucyl-glycine	8·25	5·29	2·59	3·83	—	9·40	2 : 1
		5·28	—	3·82	9·68	—	1 : 1
DL-leucyl-glycylglycine	7·94	4·72	3·82	4·70	—	7·85	2 : 1
		4·71	—	4·76	7·10	—	1 : 1

The constant $K_{c'}$ was calculated by the method previously described.[1] The values of $pK_{c'}$ were found to rise with increasing pH and can only be regarded as approximate. The stability and acid dissociation constants of the cupric dipeptide complexes are collected together in table 3.

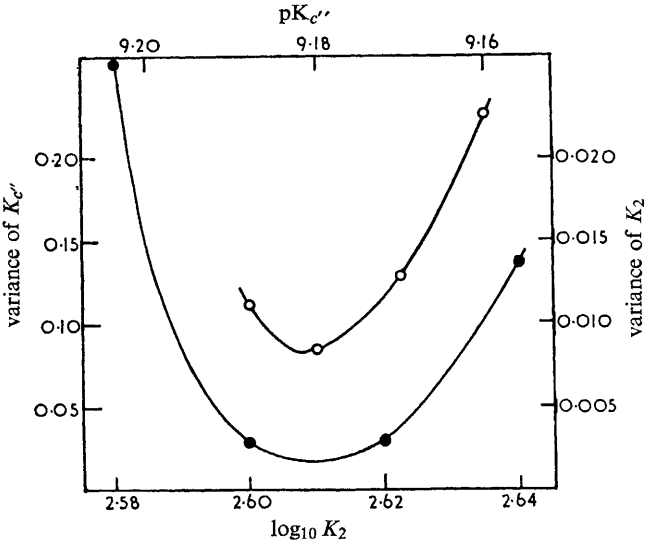


FIG. 5.—Examples of plots of variances in $K_{c''}$ against $\log_{10} K_2$ and variances in K_2 against $pK_{c''}$ for sarcosyl-L-leucine.

—○—, ordinate, variances in K_2; abscissa, $pK_{c''}$;
—●—, ordinate, variances in $K_{c''}$; abscissa, $\log_{10} K_2$.

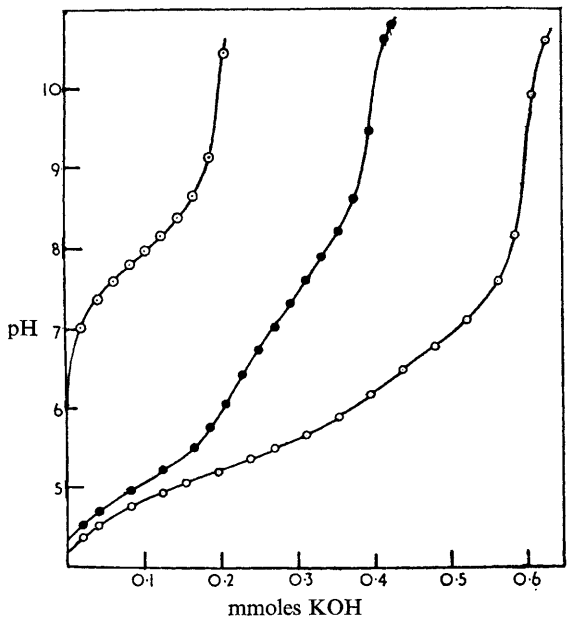


FIG. 6.—Titration curves of DL-leucylglycylglycine (0·2 mmole in 20 ml water) alone and in the presence of $CuCl_2$.

—⊙—, ligand alone; —○—, ligand + 0·2 mmole $CuCl_2$;
—●— ligand + 0·1 mmole $CuCl_2$.

DL-LEUCYLGLYCYLGLYCINE

The titration curves obtained for this ligand are shown in fig. 6. It can be seen that two additional equivalents of alkali per metal atom are required to titrate all the acid species produced from mixtures of ligand and metal in both 2 : 1 and 1 : 1 molar proportions. The equilibria involved are similar to the glycylglycine + Cu(II) system and were analyzed in terms of reactions (i)-(v); the order of magnitude of pK_c excludes a reaction sequence similar to that observed for the amides. The constants were computed by the methods described for sarcosyl-L-leucine. The addition of the final equivalent of alkali per copper atom was accompanied by the appearance of a biuret colour. The results obtained are shown in table 3; in this instance no variation of $K_{c'}$ with pH was observed.

DISCUSSION

Plots of $\log_{10}$ (stability constants) against pK (amino group) for the series of sarcosyl compounds investigated are shown in fig. (7). Similar plots are not shown for the leucyl series as, in this instance, only four ligands were investigated. Fig. 7 shows that an excellent straight line correlation exists between $\log_{10} K_1$ and pK_a,

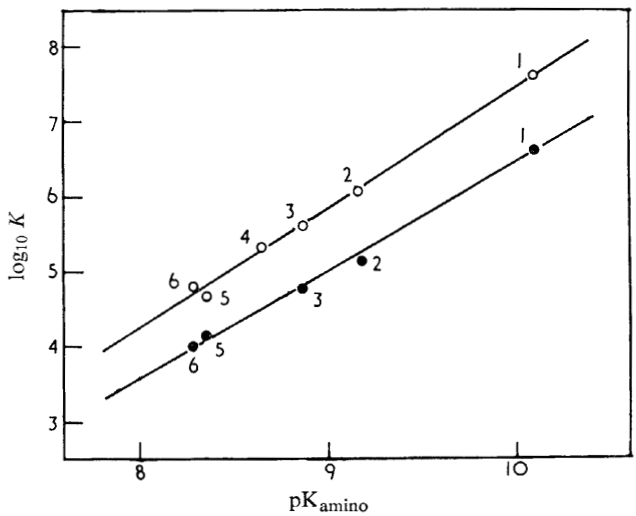
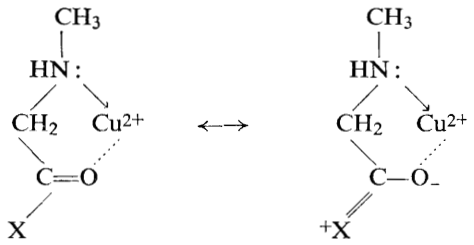


FIG. 7.—Correlation of stability constants with pK of amino group.
—○—, $\log_{10} K_1$; —●—, $\log_{10} K_2$.

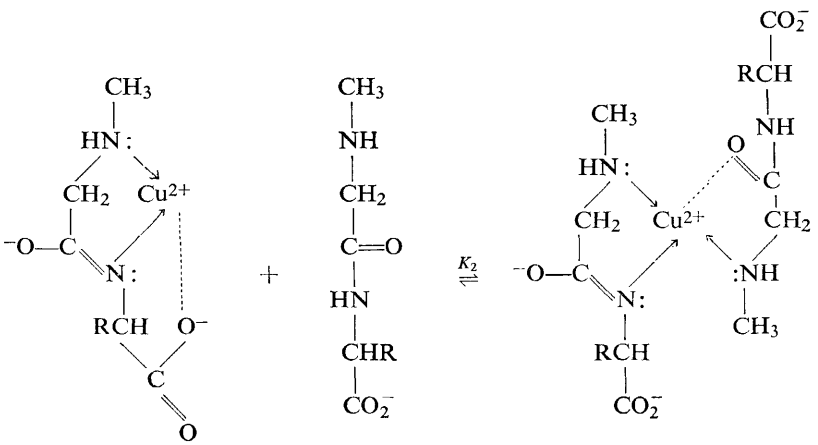
1. sarcosine; 2. sarcosylsarcosine; 3. sarcosine dimethylamide;
4. sarcosyl-L-leucine; 5. sarcosine amide; 6. sarcosine methylamide.

similar in every respect to the results previously obtained for a series of glycyl ligands.[2] This similarity allows the application of the same argument to deduce that the *initial* sites of bonding are the amino nitrogen atom and an oxygen atom thus:

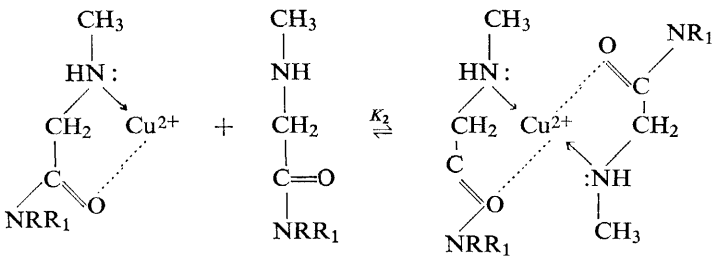


There is also an excellent straight line correlation between $\log_{10} K_2$ and pK_a, with the exception of sarcosyl-L-leucine for which $\log_{10} K_2$ is too low. This deviation, which is not observed for the other dipeptide investigated (sarcosyl-sarcosine), is probably due to a difference in the nature of the reactions leading to the formation of the 2 : 1 complexes. On the previous formulation [2] these reactions would be:

for sarcosyl-L-leucine:

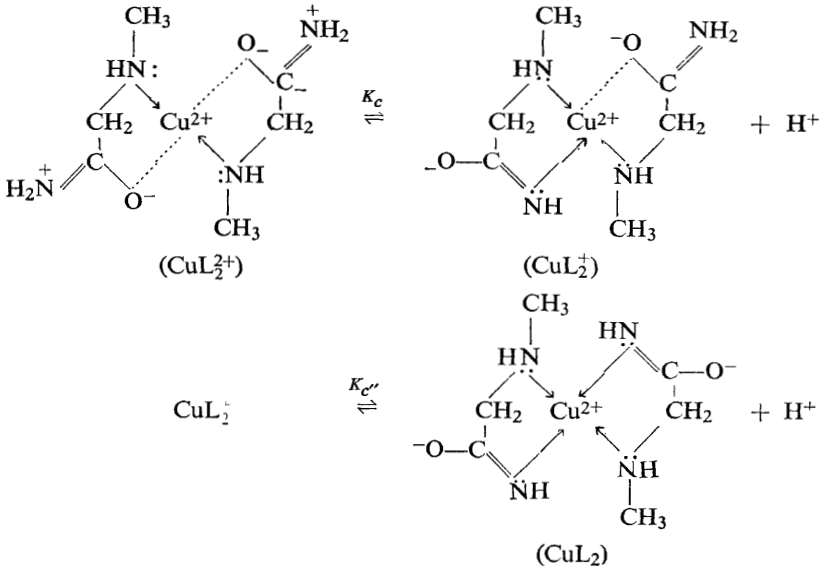


and for other sarcosyl ligands:



Additional acid ionizations have been shown to occur from the cupric complexes of sarcosine amide and sarcosine methylamide, but, unlike sarcosyl-L-leucine, the $\log_{10} K_2$ values for these complexes show the usual correlation with pK_a. This is consistent with the reaction sequence (vi)-(ix), in which all the additional acid ionizations occur from 2 : 1 ligand + Cu(II) complexes and none from the 1 : 1 complexes. No additional acid ionizations occur from the cupric complexes of sarcosine dimethylamide, which demonstrates unambiguously that the source of the acid ionization, K_c, from the other complexes is an amide hydrogen atom.

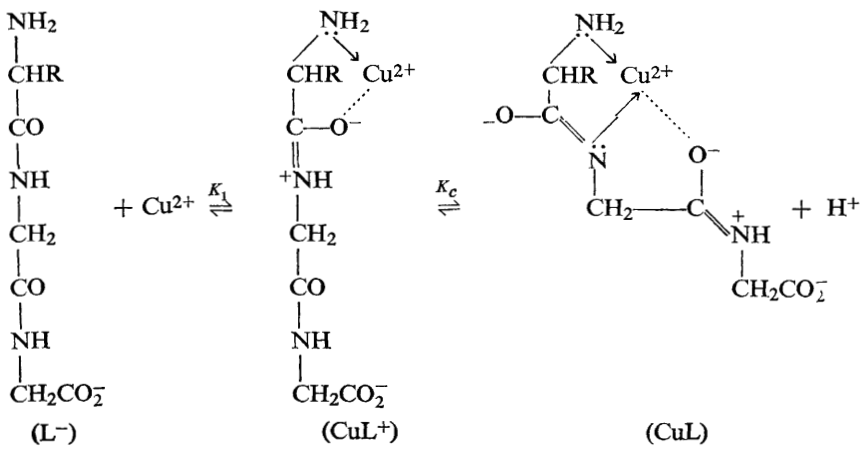
Titration data alone does not enable the source of the additional acid ionization of higher numerical pK value to be identified. However, an anhydrous red complex analyzing as Cu (leucineamide)$_2$ has been isolated; [10] the absence of water in this complex would preclude the possibility that the second additional acid ionization arises by the dissociation of a proton from a co-ordinated water molecule. The two additional acid ionizations cannot come from hydrogen atoms on the same amide nitrogen atom, since both ionizations occur in the sarcosine methylamide + copper (II) systen. From these data we conclude that the reactions involving additional acid ionizations from the amide + copper(II) complexes can be represented as follows:

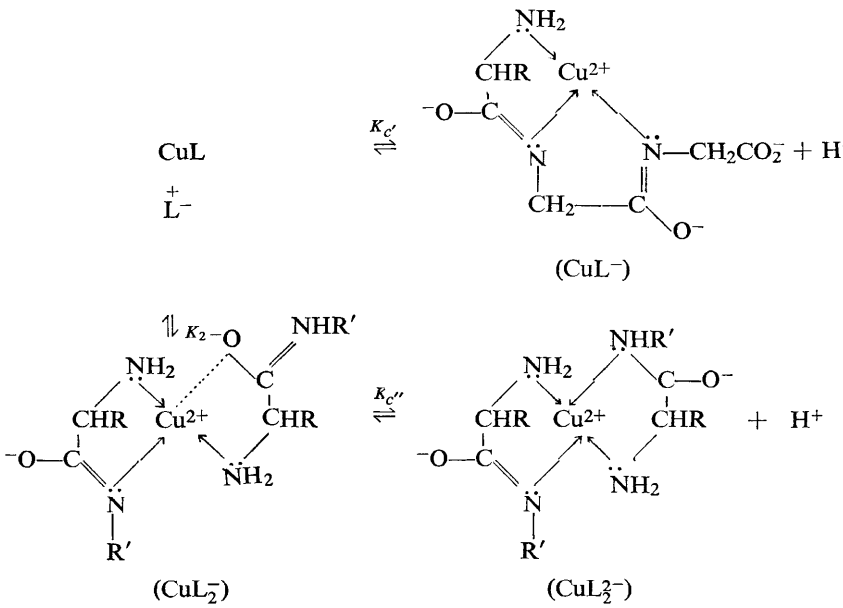


The biuret colour is probably associated with the formation of the complex CuL_2, which has two amide nitrogen atoms co-ordinated to copper(II) with the loss of two amide hydrogen atoms.

The absence of additional ionizations from the Cu(II) complexes of sarcosylsarcosine is in agreement with the earlier asignment of the source of these protons from dipeptide complexes.[2] Also the biuret colour has not been observed in the cupric complexes of any dipeptide, which is consistent with the suggestion [2] that reactions (iii) and (v), for dipeptides, involve the ionization of protons from co-ordinated water molecules. The reason for the difference between the cupric complexes of amino acid amides and those of dipeptides is not fully understood. It is possibly due to the presence of charged carboxyl groups near the sites of co-ordination in the dipeptides.

The leucylglycylglycine + copper system is similar to the diglycylglycine + copper system investigated by Dobbie and Kermack; [5] the cupric complexes of tripeptides resembling those of dipeptides as far as the reaction sequence (i)-(v) applies. The tripeptide complexes differ, however, from those of dipeptides in that reactions (iii) and (v) are accompanied by the appearance of a biuret colour; in this they resemble the amino acid amide complexes. Thus the structures of the cupric complexes of tripeptides, CuL^- and CuL_2^{2-}, are probably different from the corresponding complexes of dipeptides. It is suggested that the complexes formed in the interactions of tripeptides and Cu(II) are:





The biuret colur is associated with the formation of the complexes CuL^- and CuL_2^{2-}, which both contain two peptide nitrogen atoms from which protons have been dissociated co-ordinated to the Cu(II) atom.

This scheme, which is a combination of previous suggestions [2, 3, 5] is compatible with the determined values of the equilibrium constants, the absorption spectra of the complexes, and our knowledge of the structure of the cupric complexes of amino acid amides and dipeptides. The biuret colour occurs in complexes which have lost two peptide (or amide) hydrogen atoms, and in which one or more amino nitrogen atoms plus two or more peptide (or amide) nitrogen atoms are co-ordinated to a Cu(II) atom. The colour is not observed to occur in the dipeptide + copper systems, because for dipeptides the complexes CuL_2^{2-} are formed by the uptake of an hydroxyl ion,[2, 3] and not by the loss of a second peptide hydrogen atom.

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