Equilibria and Kinetics of Copper(II) Complex Formation of a Linear and of 13—15-Membered Macrocyclic Dioxo-tetra-amines

By **Mutsuo Kodama**, Department of Chemistry, College of General Education, Hirosaki University, Bunkyo, Hirosaki 036, Japan
Eiichi Kimura,* Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Kasumi, Hiroshima 734, Japan

Ligating properties of a linear (L^8) and of 13—15-membered cyclic dioxo-tetra-amines (L^5—L^7) (which possess two internal amide bonds) have been investigated with reference to structurally relevant tetra-amines (L^1—L^4) and the tripeptides glycylglycylglycine (L^9) and glycylglycylhistidine (L^{10}). The pH-metric titrations show that ionization of the two hydrogens (most likely at the two amide nitrogens) with concurrent 1 : 1 complex ($[MH_{-2}L]$) formation with Cu^{II} occurs at pH <7. The complex species $[ML]^{2+}$ and $[MH_{-1}L]^{+}$ are not found in the equilibria or kinetic studies. The macrocyclic effect and the cation–ring size selectivity observed for tetra-amines L^1—L^4 are retained for the dioxo-tetra-amines, as illustrated by logarithmic values of the cumulative formation constants $K_{CuH_{-2}L}$ (= $[CuH_{-2}L][H^+]^2/[Cu^{II}][L]$) of −2.2, 1.0, −4.5, and −5.1 (at *I* 0.2 mol dm^{-3} and 25 °C) for L^5, L^6, L^7, and L^8, respectively. The stability of the 14-membered L^6 complex surpasses that of the L^{10} complex. The kinetics have been measured for the $[CuH_{-2}L]$ formation (L = L^5 and L^6) in acetate buffers (4.8 < pH < 5.9 at *I* 0.2 mol dm^{-3} and 25 °C). The rate of reaction is expressed as $k_L[Cu(O_2CMe)^+][L] + k_{HL}[Cu(O_2CMe)^+][HL^+]$ for L^5 (where $k_L = 2.0 \times 10^7$ and $k_{HL} = 9.5 \times 10^2$ dm^3 mol^{-1} s^{-1}) and as $k_{HL}[Cu(O_2CMe)^+][HL^+]$ for L^6 (where $k_{HL} = 3.1 \times 10^3$ dm^3 mol^{-1} s^{-1}).

In earlier studies on complexation of tetra-amines L^1—L^4 it was demonstrated that the cyclization of linear L^4 dramatically enhances complex stability (' macrocyclic effect ') and that modification of the ring size of the macrocyclic ligands significantly alters the complexation selectivities.[1-5] We have now studied modified tetra-amines with two carbonyl functions, L^5—L^8. It was anticipated that such a modification of the donor groups might serve to increase the cation selectivities of macrocyclic tetra-amines, as found for macrocyclic hexaethers.[6]

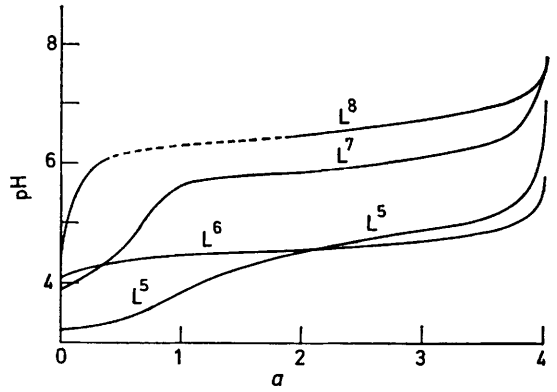


FIGURE 1 Titration curves of equimolar mixtures of diprotonated dioxo-tetra-amines and copper(II) ions with Na[OH]. With L^8, the solution becomes a little turbid in the region shown by the broken line

More interesting with the present dioxo-tetra-amines is the structural relevance to tripeptides, both potential quadridentate ligands featuring two isolated amides in between two terminal donor atoms. The concept of co-ordination of metal ions to the two amide nitrogens with displacement of the amide protons in square-planar geometries with tripeptides [7,8] and the linear dioxo-tetra-amine L^8 is well supported.[9-11] With the new ligands L^5—L^7 it was thought very likely that the steric requirements of their cyclic structures would further promote this type of co-ordination. Comparison of the present findings with those for the well studied tripeptides gly-gly-gly (L^9) [12,13] and gly-gly-his (L^{10}) [14,15] might shed more light on the properties of the new ligands, leading to exploration of their useful applications.

EXPERIMENTAL

Materials.—The macrocyclic dioxo-tetra-amines L^5—L^7 (as free forms) [16,17] and the linear L^8 (as 2HCl salt) [10] were prepared according to the literature procedures. The tripeptides L^9 and L^{10} were obtained from Peptide Institute Inc., Osaka, Japan. Sources of other reagents used were the same as those reported earlier.[2-5]

TABLE 1

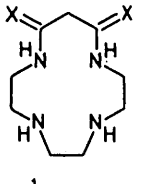
Comparison of dioxo-tetra-amine, tetra-amine, and tripeptide equilibrium constants [a]

Ligand	Protonation constant log K_1	Protonation constant log K_2	Complex formation constant log $K_{CuH_{-2}L}$	Complex formation constant log K_{CuL}
L^1 [b]	11.10	10.10		29.1
L^2 [c]	11.50	10.30		27.2
L^3 [d]	11.20	10.10		24.4
L^4 [e]	10.25	9.50		23.9
L^5	9.05 (5)	3.82 (5)	−2.2 (2)	
L^6	9.57 (5)	5.97 (5)	1.0 (1)	
L^7	9.40 (5)	6.52 (5)	−4.5 (4)	
L^8	9.08 (5)	8.82 (5)	−5.1 (5)	
L^9 [f]	7.90	3.27	−6.5	
L^{10}	8.22 [g]	6.87 [g]	−2.1 [h]	

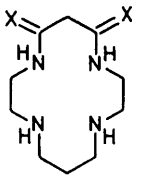
[a] At *I* 0.2 mol dm^{-3} and 25 °C, unless otherwise noted. Values in parentheses represent the standard deviation in the least significant digit. [b] Ref. 2. [c] Ref. 4. [d] Ref. 3. [e] D. C. Weatherburn, E. J. Billo, J. Jones, and D. W. Margerum, *Inorg. Chem.*, 1970, **9**, 1557. [f] Ref. 12. At *I* 0.1 mol dm^{-3} ($K[NO_3]$) and 24.9 °C. [g] Ref. 14. The second protonation occurs at the imidazole nitrogen. [h] Ref. 15; *I* 0.15 mol dm^{-3} and 37 °C.

Potentiometric Titrations.—Solutions (50 cm^3) containing L·2HCl (in the case of L^4; for L^5—L^7, 2 equivalents of

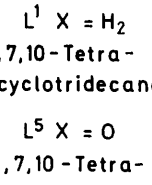
$HClO_4$ were added) (2.50×10^{-3} or 1.00×10^{-3} mol dm^{-3}) and M^{II} (1.00×10^{-3} mol dm^{-3}) were titrated with carbonate-free Na[OH] (0.100 mol dm^{-3}). Three titrations were



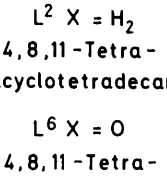
L^1 X = H_2
1,4,7,10-Tetra-azacyclotridecane



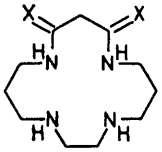
L^2 X = H_2
1,4,8,11-Tetra-azacyclotetradecane



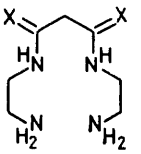
L^5 X = O
1,4,7,10-Tetra-azacyclotridecane-11,13-dione



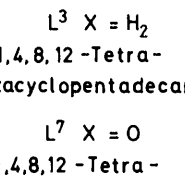
L^6 X = O
1,4,8,11-Tetra-azacyclotetradecane-12,14-dione



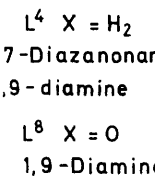
L^3 X = H_2
1,4,8,12-Tetra-azacyclopentadecane



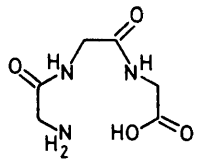
L^4 X = H_2
3,7-Diazanonane-1,9-diamine



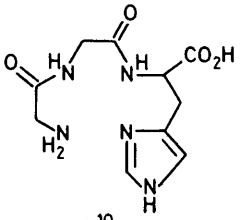
L^7 X = O
1,4,8,12-Tetra-azacyclopentadecane-9,11-dione



L^8 X = O
1,9-Diamino-3,7-diazanonane-4,6-dione



L^9
Glycylglycylglycine



L^{10}
Glycylglycylhistidine

conducted for each system. Typical titration curves for 1 : 1 Cu^{II} to $[H_2L]^{2+}$ solutions are shown in Figure 1. The pH values were read with an Orion 701 digital pH meter. Electrode response was standardized with buffer solutions at pH 4.01 and 6.86. All the measurements were performed at 25 ± 0.05 °C and I 0.2 mol dm^{-3} (adjusted with $Na[ClO_4]$). Under these conditions values of $-\log[H^+]$ were estimated by applying a correction of −0.13 pH unit to the meter reading.[18] The test solutions were protected from air by a stream of humidified purified nitrogen.

The ligand-protonation constants, expressed as shown in equations (1) and (2), were calculated from potentiometric measurements on metal-free systems. Values of K_i (mixed protonation constants) are listed in Table 1, and compared with those for tetra-amines.

Kinetic Measurements.—The complexation of the dioxo-ligands with Cu^{II} in acetate buffers was followed in a stopped-flow apparatus using the general approach outlined previously.[4] The reactions were monitored at 545 (L^5) and 525 nm (L^6) by measuring the increase in absorbance due to the formation of $[CuH_{-2}L]$. The rate law was determined by the initial-gradient method. Typical data are shown in Table 2.

TABLE 2

Initial-rate data for the reaction of Cu^{II} with L^5 and L^6 at 25 °C and I 0.2 mol dm^{-3} to give $[CuH_{-2}L]$

Initial concentration/mol dm^{-3}					
10^3 $[L]_T$	10^3 $[Cu]_T$	10^3 $[MeCO_2^-]$	pH	10^{-2} $k_{obs.}$ / dm^3 mol^{-1} s^{-1}	$\frac{K_{Cu(O_2CMe)}[MeCO_2^-]}{\beta_{O_2CMe}}$
L^5					
10.0	3.0	50	5.38	47.8	
10.0	10.0	50	5.38	47.1	
5.0	2.0	50	5.38	47.8	
5.6	5.0	30	5.18	26.9 (0.873)	0.871
5.6	5.0	50	5.18	30.8 (1.00)	1.00
5.6	5.0	75	5.18	32.6 (1.06)	1.06
5.6	5.0	100	5.18	33.4 (1.08)	1.07
5.6	5.0	120	5.18	33.0 (1.07)	1.07
L^6					
5.0	2.0	50	5.38	6.20	
10.0	10.0	50	5.38	6.15	
10.0	3.0	50	5.38	6.16	
5.0	5.0	30	5.18	3.29 (0.870)	0.871
5.0	5.0	50	5.18	3.78 (1.00)	1.00
5.0	5.0	75	5.18	4.01 (1.06)	1.06
5.0	5.0	100	5.18	4.05 (1.07)	1.07
5.0	5.0	120	5.18	4.04 (1.07)	1.07

CALCULATIONS

Equilibria of Complex Formation.—The following equilibria were found to take place in buffer regions of the titration curves (the data used for the following calculations were obtained at the titration point a, where $1.5 < a < 2.5$ for L^5, $0 < a < 1$ for L^6, $2.5 < a < 3.7$ for L^7, and $3.0 < a < 3.9$ for L^8). The sum of the hydrogen and sodium ion

$$L + H^+ \rightleftharpoons [HL]^+ \quad K_1 = [HL^+]/[L][H^+] \quad (1)$$

$$[HL]^+ + H^+ \rightleftharpoons [H_2L]^{2+} \quad K_2 = [H_2L^{2+}]/[HL^+][H^+] \quad (2)$$

$$M^{2+} + L \rightleftharpoons [MH_{-2}L] + 2H^+ \quad K_{MH_{-2}} = [MH_{-2}L][H^+]^2/[M^{2+}][L] \quad (3)$$

$$M^{2+} + OH^- \rightleftharpoons [M(OH)]^+ \quad K_{OH} = [M(OH)^+]/[M^{2+}][OH^-] \quad (4)$$

from Na[OH]) concentrations, α, at a is given by relation (5), and the total concentration of metal ions c_M and ligand c_L are expressed by (6) and (7).

$$\alpha = ac_M + [H^+] = 4[MH_{-2}L] + 2[L] + [HL^+] + [M(OH)^+] \quad (5)$$

$$c_M = [M^{2+}] + [MH_{-2}L] + [M(OH)^+] \quad (6)$$

$$c_L = [L]_F + [MH_{-2}L] \quad (7)$$

where $$[L]_F = [L] + [HL^+] + [H_2L^{2+}] \quad (8)$$

We define the symbols $(\alpha_H)_L$, β_H, and R as in (9)—(11).

$$(\alpha_H)_L = [L]_F/[L] = 1 + [H^+]K_1 + [H^+]^2K_1K_2 \quad (9)$$

$$\beta_H = 2 + [H^+]K_1 \quad (10)$$

$$R = K_{OH}[OH^-]/(1 + K_{OH}[OH^-]) \quad (11)$$

Then an appropriate combination of equations (1)—(11) yields the relations (12)—(15). Equation (15) can be

$$[L]_F = \frac{(\alpha_H)_L(4c_L - \alpha)}{(4 - R)(\alpha_H)_L - \beta_H} \quad (12)$$

$$[MH_{-2}L] = \frac{(\alpha_H)_L(\alpha - c_L R) - c_L\beta_H}{(4 - R)(\alpha_H)_L - \beta_H} \quad (13)$$

$$[M^{2+}] + [M(OH)^+] = \frac{[(4c_M - \alpha) - (c_M - c_L)R](\alpha_H)_L + (c_L - c_M)\beta_H}{(4 - R)(\alpha_H)_L - \beta_H} \quad (14)$$

$$K_{MH_{-2}L}[(4c_L - \alpha) + R(c_M - c_L)]\{[(4c_M - \alpha) - (c_M - c_L)R](\alpha_H)_L + (c_L - c_M)\beta_H\} = (1 + K_{OH}[OH^-])[(4 - R)(\alpha_H)_L - \beta_H][(\alpha_H)_L(\alpha - c_M R) - c_L\beta_H][H^+]^2 \quad (15)$$

simplified to (16) when R approximates to zero at pH <5.7 using $K_{OH} = 10^{6.06}$ for Cu^{2+}.[19] Equation (16) was used in

$$K_{MH_{-2}L}(4c_L - \alpha)[(4c_M - \alpha)(\alpha_H)_L + (c_L - c_M)\beta_H] = [4(\alpha_H)_L - \beta_H][\alpha(\alpha_H)_L - c_L\beta_H][H^+]^2 \quad (16)$$

the case of L^5 and L^6 (Figure 2) and equation (15) in the case of L^7 (see Figure 2) and L^8. The values of $K_{MH_{-2}L}$ were determined graphically from the gradients of the linear lines. All the results are listed in Table 1.

Kinetics of Complex Formation.—In acetate buffers the

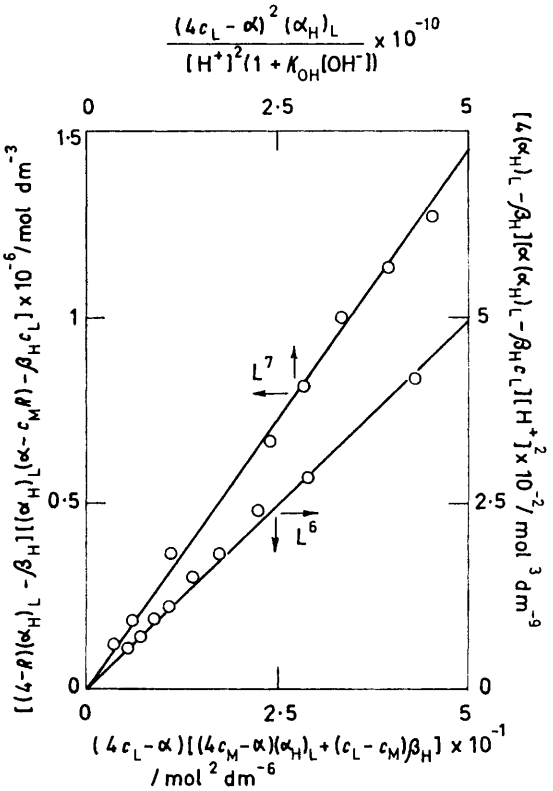


FIGURE 2 Plots of equation (16) for L^6 ($c_M = 1.00 \times 10^{-3}$ and $c_L = 2.50 \times 10^{-3}$ mol dm^{-3}) and of (15) for L^7 ($c_M = c_L = 2.00 \times 10^{-3}$ mol dm^{-3})

predominant ligand forms are $[HL]^+$ for L^5, and $[HL]^+$ and $[H_2L]^{2+}$ for L^6. Copper is present as Cu^{2+}(aq), $[Cu(O_2CMe)]^+$, and $Cu[O_2CMe]_2$. The hydrolysis of Cu^{2+}(aq) was neglected. At a given pH and $[MeCO_2^-]$, the observed rate

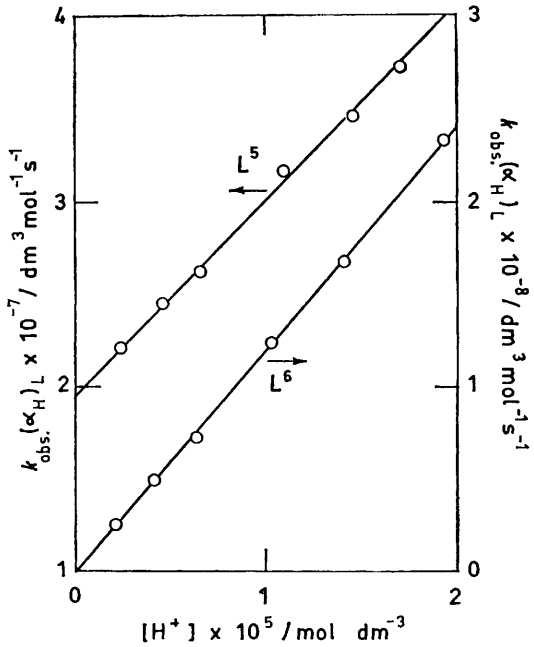


FIGURE 3 Plots of equation (19). Initial concentrations for both L^5 and L^6 are $[Cu^{II}]_T = [L]_T = 5.00 \times 10^{-3}$ and $[MeCO_2^-] = 0.10$ mol dm^{-3} at I 0.2 mol dm^{-3} and 25 °C

constants k_{obs} are first order in $[Cu^{II}]_T$ and first order in $[L]_T$. At constant pH, the $k_{obs.}$ values increase as $[MeCO_2^-]$ increases, in proportion to $K_{Cu(O_2CMe)}[MeCO_2^-]/\beta_{MeCO_2}$ (see Table 2), indicating that $[Cu(O_2CMe)]^+$ is a reactive species. Here, we may write the expressions (17) and (18). The values for $K_{Cu(O_2CMe)}$ (= 52.5 dm^3 mol^{-1})

$$\beta_{MeCO_2} = [Cu^{2+}]_F/[Cu^{2+}(aq)] = 1 + K_{Cu(O_2CMe)}[MeCO_2^-] + K_{Cu(O_2CMe)}K_{Cu(O_2CMe)_2}[MeCO_2^-]^2 \quad (17)$$

$$[Cu^{2+}]_F = [Cu^{2+}(aq)] + [Cu(O_2CMe)^+] + [Cu(O_2CMe)_2] \quad (18)$$

and $K_{Cu(O_2CMe)}K_{Cu(O_2CMe)_2}$ (= 93 dm^6 mol^{-2}) were taken from the literature.[20]

At a constant $[MeCO_2^-]$, $k_{obs.}$ increases as the pH increases for L^5 and L^6, according to relation (19). As

$$k_{obs.}(\alpha_H)_L = k_L + k_{HL}[H^+]K_1 \quad (19)$$

illustrated in Figure 3, plots of $k_{obs.}$ $(\alpha_H)_L$ against $[H^+]$ are linear with a finite (for L^5) and zero value (for L^6) of the intercept. The rate constants k_L and k_{HL} were thus determined graphically (Table 3).

TABLE 3

Rate constants (dm^3 mol^{-1} s^{-1}) for the reaction of various protonated forms of macrocyclic ligands with $[Cu(O_2CMe)]^+$ at I 0.2 mol dm^{-3} and 25 °C

L		$[HL]^+$	$[H_2L]^{2+}$
L^1 [a]		5.6×10^6	10
L^2 [b]		5.3×10^6	8
L^5	$(2.0 \pm 0.2) \times 10^7$	$(9.5 \pm 1.0) \times 10^2$	
L^6		$(3.1 \pm 0.3) \times 10^3$	

[a] Ref. 2. [b] Ref. 4.

DISCUSSION

Equilibrium Constants.—Incorporation of carbonyl functions at the α-carbon of two amine groups affects the basicities of the two other amine groups. A comparison of log K_1 in Table 1 shows that this effect is more marked in the cyclic than in the linear system, *e.g.* a decrease of 1.9 pH unit for L^2 going to L^6 as against 1.2 for L^4 going to L^8. Another comparison indicates a smaller increment brought about by the cyclization, *e.g.* 0.5 pH unit for L^8 going to L^6 as against 1.3 for L^4 going to L^2. These facts suggest extensive disruption of the intramolecular hydrogen bonding between nitrogen atoms which is characteristic of cyclic tetra-amines. The successive drop in log K_2 values as one goes from linear L^8 to L^7, L^6, and the smallest macrocycle L^5 reflects the decrease in flexibility of the conformation and/or a shortening of the distance between the free N and the NH^+ site.

Potentiometric titration of the dioxo-ligands in the presence of some bivalent metal ions indicated a metal-ion promoted ionization of two protons. Quantitatively, the most successful metal ion in promoting the deprotonation was Cu^{II} as shown in Figure 1.* With Zn^{II}, Cd^{II}, or Pb^{II} the dioxo-ligands underwent no deprotonation at pH <7. At pH >7 precipitation of hydrolyzed metal ions occurred. The different behaviour of the dioxo-ligands towards Cu^{II} and Zn^{II} at pH 4—7 is of considerable interest in comparison with the tetra-amines which indiscriminately sequester both metal ions at the corresponding pH.[2-5]

The titration data for Cu^{II} are in best agreement with the concomitant formation of $[CuH_{-2}L]$ complexes and thus the cumulative formation constants $K_{CuH_{-2}L}$ were determined. Calculations for the simultaneous or separate formation of $[CuL]^{2+}$ and $[CuH_{-1}L]^+$ were also made. However, the derived equations failed to fit the experimental data. It is thus concluded that neither $[CuL]^{2+}$ nor $[CuH_{-1}L]$ is formed in the buffer pH region. The formula $[MH_{-2}L]$ is consistent with co-ordination of the two amide nitrogens with displacement of the amide protons, and was previously assigned to the L^8 complexes isolable in alkaline solutions.[10] The binding of the Cu^{II} is assumed to take place first at the amine group, followed by immediate amide deprotonation and co-ordination. The latter co-operative action might occur more readily in the macrocyclic cavities, particularly in those having suitable sizes for the copper(II) ion. The observed complexation at low pH for L^5 and L^6 (see Figure 1) would thus be explained by their relatively small log K_2 values (*i.e.* free amines are available at low pH) as well as their favourable cavity size. The *d–d* transition in aqueous solutions occurs at 19 200, 19 600, and 19 300 cm^{-1}, respectively, for the L^5, L^6, and L^7 complexes, as compared with 19 400 cm^{-1} for the L^8 complex.[10] This lends support to an assignment of a similar complex configuration, *i.e.* a square-planar structure, for L^5 through L^8. The planar orientation and cavity size of $[H_{-2}L]^{2-}$ would permit the macrocyclic dioxo-ligands to chelate with small, in-plane, metal ions like Cu^{II}.

The cyclization effect and cation–ring size selectivity, properties associated with tetra-amine complexes,[1-5] are retained in the present dioxo-tetra-amine complexes (see Table 1): comparison of log $K_{CuH_{-2}L}$ values shows a macrocyclic effect of six orders of magnitude (L^6 *vs.* L^8) and the most favourable complexation (at constant pH) occurs with the 14-membered ring.

Comparison with Tripeptides L^9 *and* L^{10}.—Copper(II)-promoted amide deprotonation of peptides is well documented.[7,8] Tripeptides L^9 and L^{10} were chosen for the present comparison not only because of their structural similarities but also because of their current chemical and biological interest.[21-25] In addition to $[MH_{-2}L]$, tripeptides form $[ML]^{2+}$ and $[MH_{-1}L]^+$ complexes,[7] which is not the case for the dioxo-system. Presumably, the adoption of the peptide $[ML]^{2+}$ and $[MH_{-1}L]^+$ structures involving amide oxygen co-ordination is very unlikely.

At acid pH values, the complexation with Cu^{II} occurs more favourably for the macrocyclic dioxo-ligands L^5 and L^6 than for the tripeptides L^9 and L^{10}. This is shown by a calculation of the degree of dissociation (percentage in uncomplexed forms) at $[Cu^{II}] = [L] = 10^{-3}$ mol dm^{-3} and pH 5.50 using the K_1 and $K_{CuH_{-2}L}$ values in Table 1 : 7 (L^5), 1 (L^6), 51 (L^9), and 13 (L^{10}). It is to be noted that the complexed forms of peptides are $[CuL]^{2+}$, $[CuH_{-1}L]$,$^+$ and $[CuH_{-2}L]$, while only $[CuH_{-2}L]$ is found with the dioxo-ligands.

Calculations show that in the 1 : 1 metal to ligand solutions at pH 7 the $[CuH_{-2}L]$ complexes should be formed in practically 100% yield for L^6 and L^{10}. The relative complex stability was investigated by examining visible spectra of solutions of Cu^{II} containing equivalent ratios of $[L^6]$ and $[L^{10}]$ at pH 7.00 (phosphate buffer). For separate solutions, the absorption maximum occurs at 500 and 530 nm for $Cu–L^6$ and $Cu–L^{10}$, respectively. In a mixture of Cu^{II} and the two ligands the spectrum completely overlaps with that of the $Cu–L^6$ solution, thus demonstrating the overwhelmingly greater stability of the L^6 complex relative to that of L^{10}. The estimated

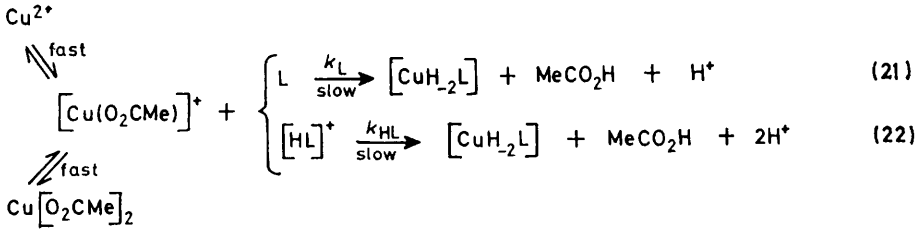
$$Cu–L^{10} + L^6 \rightleftharpoons Cu–L^6 + L^{10} \qquad (20)$$

equilibrium constant for (20) based on these calculations is *ca.* $10^{2.2}$.

The present findings may have a potential application in view of the fact that L^{10} is a molecule designed to mimic the copper(II)-specific transport site of serum albumin.[26-29]

Kinetics of Complex Formation.—In acetate buffers (4.8 < pH < 5.7), L^5 and L^6 form copper complexes while L^7 and L^8 do not. No intermediate complexes were detected in the course of the $[CuH_{-2}L]$ formation. At a given pH and $[MeCO_2^-]$, the observed rate of

* Cobalt(II) also promotes the deprotonation of L^6 with simultaneous complexation (although the reaction rate is slower) at higher pH. A similar treatment of the titration data at 2.0 (pH 7.2) < *a* <3.9 (pH 7.8) gave log $K_{CoH_{-2}L} = -8.4$. Measurements for linear L^8, however, were impossible due to precipitation of the hydrolyzed cobalt ion.



complexation is greater (*ca.* ten times) for L^5 than for L^6, probably due to the fact that the competitive proton affinity is lower for the smaller sized L^5.

A reaction scheme based on the kinetic results is as in (21) and (22). The reaction paths are not the same for L^5 and L^6. This is in contrast to the reaction of the tetra-amine homologues L^1 and L^2 which have a common rate law.[2,4] The ligand L^5 follows both (21) and (22): *e.g.* at pH 5.0, the k_L term contributes 65% and the k_{HL} term 35% to the overall reaction, whereas the reaction of L^6 proceeds mostly *via* (22). Hence, one may ascribe the observed slower complexation of L^6 relative to L^5 to the lack of the fast reaction route (21). In the reacting solutions of L^6, the amount of unprotonated relative to protonated L species is much less and the contribution of (21) would be undetectably small. All of this relates to the ring-size effect of the 13- and 14-membered macrocycles.

The rate of reaction of the unprotonated L^5 is comparable (allowing for charge differences) to those of monoprotonated L^1 and L^2 (see Table 3). A similar reaction mechanism might govern both cases. The much smaller rate constants for the reactions of monoprotonated L^5 and L^6 relative to L^1 and L^2 are understood in view of the number and basicity of the nitrogen atoms available for the initial Cu–N interaction.

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REFERENCES

[1] F. P. Hinz and D. W. Margerum, *J. Amer. Chem. Soc.*, 1974, **96**, 4993; *Inorg. Chem.*, 1974, **13**, 2941.
[2] M. Kodama and E. Kimura, *J.C.S. Chem. Comm.*, 1975, 896; *J.C.S. Dalton*, 1976, 1720.
[3] M. Kodama and E. Kimura, *J.C.S. Dalton*, 1976, 2341.
[4] M. Kodama and E. Kimura, *J.C.S. Dalton*, 1977, 1473.
[5] M. Kodama and E. Kimura, *J.C.S. Dalton*, 1977, 2269.
[6] R. M. Izatt, J. D. Lamb, G. E. Maas, R. E. Asay, J. S. Bradshaw, and J. J. Christensen, *J. Amer. Chem. Soc.*, 1977, **99**, 2365.
[7] H. C. Freeman, *Adv. Protein Chem.*, 1967, **22**, 257.
[8] D. W. Margerum and G. R. Dukes, ' Metal Ions in Biological Systems,' vol. 1, ed. H. Sigel, Marcel Dekker, New York, 1974, p. 157 and refs. therein.
[9] H. A. O. Hill and K. A. Raspin, *J. Chem. Soc.* (*A*), 1968, 3036; 1969, 619.
[10] H. Ojima and K. Yamada, *Nippon Kagaku Zasshi*, 1970, **91**, 49.
[11] A. Zuberbuhler, S. Fallab, and Th. Kaden, *Helv. Chim. Acta*, 1967, **50**, 889; 1968, **51**, 1798 and 1805.
[12] M. K. Kim and A. E. Martell, *J. Amer. Chem. Soc.*, 1966, **88**, 914.
[13] A. P. Brunetti, M. C. Lim, and G. H. Nancollas, *J. Amer. Chem. Soc.*, 1968, **90**, 5120.
[14] G. F. Bryce, R. W. Roeske, and F. R. N. Gurd, *J. Biol. Chem.*, 1965, **240**, 3837.
[15] R. P. Agarwal and D. D. Perrin, *J.C.S. Dalton*, 1977, 53 and refs. therein.
[16] I. Tabushi, Y. Taniguchi, and H. Kato, *Tetrahedron Letters*, 1977, 1049.
[17] H. Kato, Doctoral Thesis, Department of Pharmaceutical Sciences, Kyushu University, 1977.
[18] C. W. Davies, ' Ion Association,' Butterworths, Washington, D.C., 1962.
[19] C. W. Davies, *J. Chem. Soc.*, 1951, 1256. The value cited is corrected to I 0.2 mol dm^{-3}.
[20] N. Tanaka and K. Kato, *Bull. Chem. Soc. Japan*, 1960, **33**, 417, 1412.
[21] F. B. Bossu and D. W. Margerum, *J. Amer. Chem. Soc.*, 1976, **98**, 4003.
[22] L. F. Wong, J. C. Cooper, and D. W. Margerum, *J. Amer. Chem. Soc.*, 1976, **98**, 7268.
[23] T. P. A. Kruck, S. Lau, and B. Sarkar, *Canad. J. Chem.* 1976, **54**, 1300.
[24] N. Camerman, A. Camerman, and B. Sarkar, *Canad. J. Chem.*, 1976, **54**, 1309.
[25] F. P. Bossu, K. L. Chellappa, and D. W. Margerum, *J. Amer. Chem. Soc.*, 1977, **99**, 2195.
[26] T. Peters, jun., and F. A. Blumenstock *J. Biol. Chem.*, 1967, **242**, 1574.
[27] D. Breslow, *J. Biol. Chem.*, 1964, **239**, 3252.
[28] W. T. Shearer, R. A. Bradshaw, F. R. N. Gurd, and T. Peters, jun., *J. Biol. Chem.*, 1967, **242**, 5451.
[29] R. A. Bradshaw, W. T. Shearer, and F. R. N. Gurd, *J. Biol. Chem.*, 1968, **243**, 3817.