Stability Constants and Relaxation Spectra of L-Proline and L-Hydroxyproline Metal Complexes

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Complex formation between L-proline and L-hydroxyproline and the ions Co^{2+}, Ni^{2+}, and Cu^{2+} has been studied at 25 °C and ionic strength 0·1 M (KNO_3) from equilibrium and kinetic standpoints. A potentiometric method was used to determine the following stoicheiometric stability constants: proline: Ni^{2+}, log K_1 = 6·15, log K_2 = 5·13; Co^{2+}, log K_1 = 5·05, log K_2 = 4·22; hydroxyproline: Co^{2+}, log K_1 = 4·81, log K_2 = 3·81. (Literature values for Cu^{2+}–proline and Cu^{2+}– and Ni^{2+}–hydroxyproline complexes were confirmed.) Temperature-jump studies were used to determine rate constants for the process $ML_{n-1} + L \rightleftharpoons ML_n$, where M and L are metal ion and ligand respectively and n = 1 or 2. The dissociation rate constants (*i.e.*, the rate constant for $ML_n \rightarrow ML_{n-1} + L$), k_{-n}, can be calculated from the relation $K_n = k_n/k_{-n}$; the directly measured k_n/l mol^{-1} s^{-1} for the process $ML_{n-1} + L \rightarrow ML_n$ are as follows: proline: Cu^{2+}, $k_1 = (2{\cdot}5 \pm 0{\cdot}2) \times 10^9$, $k_2 = (2{\cdot}7 \pm 0{\cdot}6) \times 10^8$; Ni^{2+}, $k_1 = (3{\cdot}4 \pm 0{\cdot}6) \times 10^4$, $k_2 = (8{\cdot}7 \pm 1{\cdot}3) \times 10^3$; Co^{2+}, $k_1 = (3{\cdot}5 \pm 0{\cdot}5) \times 10^5$, $k_2 = (9{\cdot}6 \pm 0{\cdot}7) \times 10^5$; hydroxyproline: Cu^{2+}, $k_1 = (7{\cdot}4 \pm 0{\cdot}4) \times 10^8$, $k_2 = (2{\cdot}8 \pm 0{\cdot}6) \times 10^8$; Ni^{2+}, $k_1 = (1{\cdot}2 \pm 0{\cdot}3) \times 10^4$, $k_2 = (1{\cdot}8 \pm 0{\cdot}1) \times 10^4$; Co^{2+}, $k_1 = (7{\cdot}0 \pm 1{\cdot}4) \times 10^4$, $k_2 = (9{\cdot}0 \pm 0{\cdot}4) \times 10^5$. It is concluded that proline behaves as a 'normal' amino-acid; hydroxyproline forms complexes less readily. As these results cannot be explained on the basis of the inductive effect of the substituted hydroxy-group, another effect must play a role in these reactions. Hydrogen-bond formation between co-ordinated water molecules of the metal ion and the hydroxy-group of the ligand could provide an explanation for these observations.

FAST-REACTION techniques, such as temperature-jump and stopped-flow methods, have been widely used in determining rate constants of metal-complex formation.[1-3] In most such studies, rate constants for the formation of monosubstituted complexes are independent of ligand characteristics.[4] The results are

[1] M. Eigen and L. De Maeyer in 'Techniques of Organic Chemistry,' ed. A. Weissberger, Wiley, New York, 1963, vol. VIII, part 2, p. 895.

[2] G. G. Hammes and J. I. Steinfeld, *J. Amer. Chem. Soc.*, 1962, **84**, 4639.

[3] J. C. Cassatt and R. G. Wilkins, *J. Amer. Chem. Soc.*, 1968, **90**, 6045.

[4] M. Eigen and R. G. Wilkins, 'Mechanisms of Inorganic Reactions,' Adv. Chem. Ser. 49, Amer. Chem. Soc., Washington, D.C., 1965.

consistent with the assumption that complex formation is a multistep mechanism in which loss of the first water molecule from the inner co-ordination sphere is the rate-determining step.

For bivalent transition-metal complexes, ligands such as cysteine,[5] α-alanine,[6,7] α-aminobutyric acid,[8] and glycine [2,9] are found to follow the normal substitution reaction mechanism. However, exceptions to this mechanism have been observed in the following systems: β-alanine,[6,7] β-aminobutyric acid,[8] malonate,[10] and histidine,[7] in which six- or seven-membered rings are formed and the ring closure is the rate-determining step.

Serine (β-hydroxyalanine),[11] an amino-acid with a β-hydroxyl group, has a normal mechanism for complex formation. On the other hand, L-Dopa (3,4-dihydroxyphenylalanine),[12] which has two hydroxyl groups on the phenyl moiety, is quite different in this respect, reducing the complexation rate with copper(II) below the normal value. The present work is concerned with the kinetics of formation of proline and hydroxyproline complexes of the ions Ni^{2+}, Co^{2+}, and Cu^{2+}. The study was carried out to examine the effect of chelate-ring geometry and ligand hydroxyl group on the rate of complex formation. It was necessary to determine some stability constants. All relevant equilibrium data are in Table 1.

TABLE 1

Equilibrium constants

(*a*) Proline and hydroxyproline

Ligand	Metal ion	log K_a	log K_b	log K_1	log K_2	log β_2	Ref.
Proline		1·93	10·68				*a*
	Cu^{2+}			8·92	7·66		*b*
	Ni^{2+}			6·15	5·13	11·28	Present study
						11·30	*a*
	Co^{2+}			5·05	4·22	9·27	Present study
						9·30	*a*
Hydroxyproline		1·80	9·47				Present study
	Cu^{2+}			8·33	6·96	15·29	*c*
	Ni^{2+}			5·92	4·84	10·76	*c*
	Co^{2+}			4·81	3·81	8·62	Present study

(*b*) Indicator

Indicator	K_d/l mol^{-1}	Ref.
Phenol Red	$1·26 \times 10^{-8}$	*d*
Bromothymol Blue	$7·94 \times 10^{-7}$	*d*
Bromochlorophenol Blue	$1·0 \times 10^{-4}$	*e*

[a] A. Albert, *Biochem. J.*, 1950, **47**, 531. [b] R. D. Gillard, H. M. Irving, R. M. Parkins, N. C. Payne, and L. D. Pettit, *J. Chem. Soc.* (*A*), 1966, 1159. [c] N. C. Li, E. Doddy, and J. M. White, *J. Amer. Chem. Soc.*, 1958, **80**, 5901. [d] I. M. Kolthoff, *J. Phys. Chem.*, 1930, **34**, 1446. [e] F. P. Cavasino, *J. Phys. Chem.*, 1969, **69**, 4380.

EXPERIMENTAL

Doubly distilled water was used throughout. Fisher reagent grade nitrates of potassium, nickel(II), and cobalt(II), and Baker reagent grade copper(II) nitrate, as well as other reagent grade chemicals, were used without further purification. L-Proline and hydroxy-L-proline were from Schwarz Mann Co. The indicators were Aldrich Phenol Red, Fisher Bromothymol Blue, and Matheson Coleman Bromochlorophenol Blue.

Stock solutions of the metal nitrate, ligand, and indicator were prepared by weight. The concentrations of the nitrate stock solutions were determined by passing a solution down a cation exchanger (Dowex 50) and titrating the liberated acid with standard sodium hydroxide solution. Solutions to be studied were prepared either by dissolving weighed amounts of solid materials in deaerated, distilled water or by mixing the desired amounts of stock solutions in 100 ml volumetric flasks. The final pH was adjusted by dropwise addition of NaOH and/or HNO_3 to $\pm 0·01$ pH unit measured with a Radiometer pH meter.

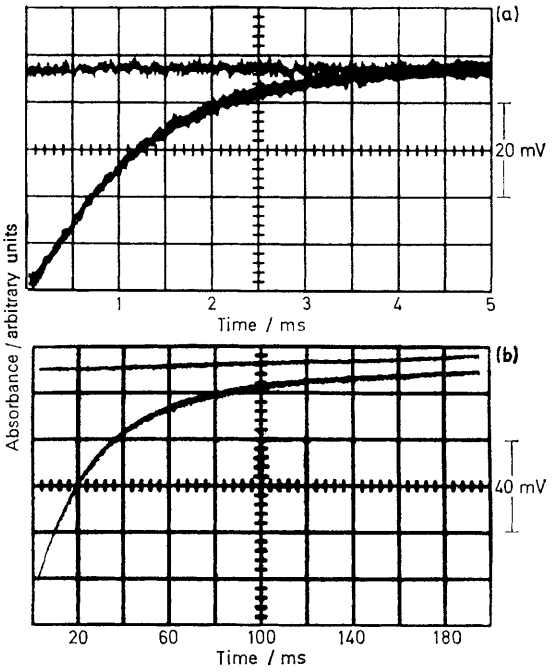

FIGURE 1 Oscilloscope traces of temperature-jump experiments (oscilloscope sensitivity is indicated on the right). (*a*) Co–proline showing one relaxation effect: $[Co]_0 = 4·79 \times 10^{-3}$M, $[L]_0 = 3·51 \times 10^{-3}$M, pH = 7·85; (*b*) Ni–hydroxyproline showing two relaxation effects: $[Ni]_0 = 5·00 \times 10^{-3}$M, $[L]_0 = 2·00 \times 10^{-2}$M, pH = 8·27

The ionic strength I was brought to 0·1M with KNO_3. The temperature was 25 ± 1 °C for all experiments. Details of the temperature-jump apparatus have been described.[13] Blank experiments with metal ion (2×10^{-3}M) and indicator, and with ligands and indicator, showed no discernible relaxation effects.

[5] G. Davies, K. Kustin, and R. F. Pasternack, *Trans. Faraday Soc.*, 1968, **64**, 1006.
[6] K. Kustin, R. F. Pasternack, and E. Weinstock, *J. Amer. Chem. Soc.*, 1966, **88**, 4610.
[7] W. B. Makinen, A. F. Pearlmutter, and J. E. Stuehr, *J. Amer. Chem. Soc.*, 1969, **91**, 4083.
[8] A. Kowalak, K. Kustin, R. F. Pasternack, and S. Petrucci, *J. Amer. Chem. Soc.*, 1967, **89**, 3126.
[9] G. Davies, K. Kustin, and R. F. Pasternack, *Inorg. Chem.*, 1969, **8**, 1535.
[10] U. Nickel, H. Hoffmann, and W. Jaenicke, *Ber. Bunsengesellschaft. Phys. Chem.*, 1968, **72**, 526.
[11] R. L. Karpel, K. Kustin, and R. F. Pasternack, *Biochim. Biophys. Acta*, 1969, **177**, 434.
[12] R. L. Karpel, K. Kustin, and R. F. Pasternack, *J. Amer. Chem. Soc.*, 1971, **93**, 1085.
[13] P. Hurwitz and K. Kustin, *Inorg. Chem.*, 1964, **3**, 823.

Each relaxation time represents an average of at least three photographic determinations. For consecutive formation of two complexes there should be two relaxation times.[2] However, for the copper(II) and cobalt(II) complexes under study only one relaxation time was found. This observation may be due to one or both of the following reasons: the other relaxation time lies outside the time range for which the temperature-jump technique is applicable, or the other effect is too small to be measured. If only one relaxation effect is observed [Figure 1(*a*)] the relative error is within ±10%. Like Ni^{2+}–arginine,[14] Ni^{2+}–proline and –hydroxyproline complexes show multiple effects in some of the experiments [Figure 1(*b*)]. The 'slow' relaxation time is always quite slow and, owing to the onset of convection, difficult to measure accurately as a determination of the base-line of the exponential relaxation is precluded [see Figure 1(*b*)]. In order to determine the 'fast' relaxation time, an arbitrary base-line was drawn to make a best straight-line fit in the semilogarithmic plot. The relative error for these experiments is *ca.* ±20%.

In determining the stability constants for complex formation, a double-walled cell of capacity 200 ml was used. The temperature of the solutions was kept at 25 ± 0·1 °C by circulating constant-temperature water from a thermostat through the wall of the cell. The glass electrode was calibrated with standard buffer solution and checked after each titration. The hydrogen-ion concentrations in the solution were measured under nitrogen with a pH meter.

RESULTS

Stability Constants.—If we assume that only two complexes are formed, the equilibria between the different species can be expressed as in Scheme 1, where H_2L^+, HL,

$$HL + H^+ \rightleftharpoons H_2L^+ \qquad K_a = \frac{[H_2L^+]}{[HL]\,[H^+]}$$

$$L^- + H^+ \rightleftharpoons HL \qquad K_b = \frac{[HL]}{[L^-]\,[H^+]}$$

$$M^{2+} + L^- \rightleftharpoons ML^+ \qquad K_1 = \frac{[ML^+]}{[M^{2+}]\,[L^-]}$$

$$ML^+ + L^- \rightleftharpoons ML_2 \qquad K_2 = \frac{[ML_2]}{[ML^+]\,[L^-]}$$

SCHEME 1

and L^- are the protonated, neutral, and anionic forms of the ligand, M^{2+} is the free metal ion and ML^+ and ML_2 are the complex species; the ligand proton association constants are K_a and K_b and K_1 and K_2 represent stepwise equilibrium constants for complex formation. Values of K_1 and K_2 can be determined from a knowledge of $[L^-]$, the concentration of free chelating species, and $\bar{n}$, the average number of ligand molecules bound to metal ions, by equations (1) and (2) (where T_L is total ligand concentration, and T_M is total metal concentration). Equation (2) can

$$[L^-] = \frac{T_L - [NaOH] - [H^+] + [OH^-]}{[H^+]/K_b + 2[H^+]^2/K_aK_b} \quad (1)$$

$$\bar{n} = (T_L - [L^-] - [HL] - [H_2L^+])/T_M = \frac{K_1[L^-] + 2K_1K_2[L^-]^2}{1 + K_1[L^-] + K_1K_2[L^-]^2} \quad (2)$$

be rewritten in the form (3).

$$\frac{\bar{n}}{(1-\bar{n})[L^-]} = \frac{(\bar{n}-2)[L^-]}{(\bar{n}-1)} K_1K_2 + K_1 \quad (3)$$

Plots of $\bar{n}/\{(1-\bar{n})[L^-]\}$ against $\{(\bar{n}-2)[L^-]\}/(\bar{n}-1)$ are shown in Figures 2 and 3, approximate value of K_1 and K_2 being obtained from the slopes and intercepts. A linear least-squares analysis was performed with the aid of a PDP-10 computer to evaluate more accurately the values of K_1 and K_2. Results so obtained are in Table 1; values have a relative error of ±2%.

Relaxation Spectra.—Concentrations of the various species were calculated by means of a Newton–Raphson iteration

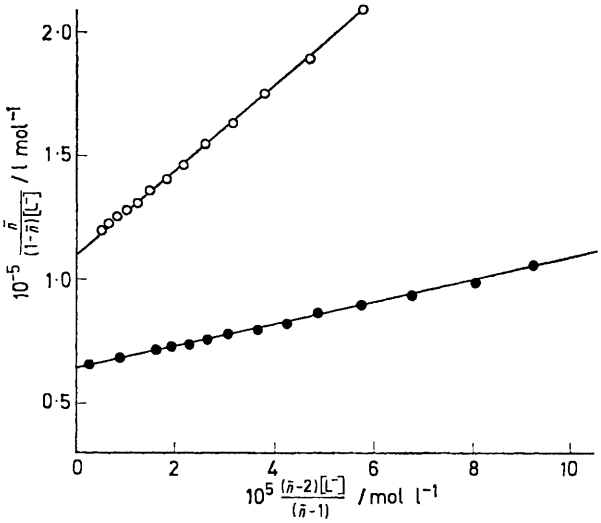

FIGURE 2 Cobalt(II)–proline and hydroxyproline complexation. (○), $[Co]_0 = 4{\cdot}19 \times 10^{-3}$M, $[L]_0 = 8{\cdot}30 \times 10^{-3}$M (proline); (●), $[Co]_0 = 2{\cdot}81 \times 10^{-3}$M, $[L]_0 = 5{\cdot}67 \times 10^{-3}$M (hydroxyproline)

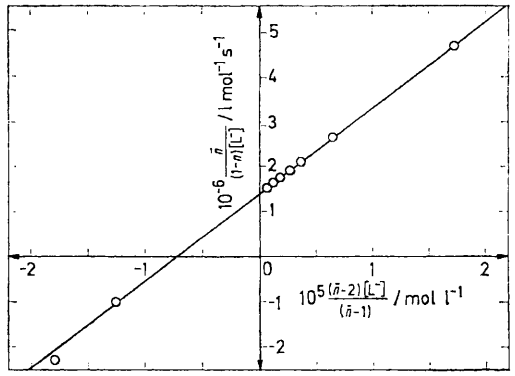

FIGURE 3 Nickel(II)–proline complexation. $[Ni]_0 = 2{\cdot}82 \times 10^{-3}$M, $[L]_0 = 6{\cdot}35 \times 10^{-3}$M

method. Some of the stability constants (Table 1) used in this calculation have been measured at an ionic strength (0·16M) different from that of our temperature-jump experiments (I = 0·1M). However, the modest variation of amino-acid equilibrium constants with ionic strength is within the deviation of our experimental results.

Experiments have been carried out at a variety of hydrogen-ion concentrations [pH ranged from 3·4 to 4·7 for copper(II) complexes, from 6·8 to 8·5 for nickel(II) and cobalt(II) complexes]. The lack of a dependence of the rate constants on pH showed that reaction pathways *via* hydrolytic species of the type MOH^+ and other protonated

[14] G. Davies, K. Kustin, and R. F. Pasternack, *Internat. J. Chem. Kinetics*, 1969, **1**, 45.

forms of the ligand can be neglected. For proline and hydroxyproline complexation with Cu^{2+}, Ni^{2+}, and Co^{2+}, up to two complexes with the anionic form of the ligand may be present. As in previous studies, it was assumed that only the anionic amino-acids are the attacking forms.[2,6,7,9,11,15]

The relaxation processes are then explained in terms of Scheme 2, where $n = 1$ or 2 and L represents the anionic

$$ML_{n-1} + L \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} ML_n \quad K_n = k_n/k_{-n}$$

SCHEME 2

form of the ligand (charges have been omitted for simplicity). The general treatment put forth by Hammes and Steinfeld [2] has been applied in calculating the relaxation time, τ, from the quadratic equation (4), the roots of which are given in equation (5), where a_{ij} is a function of the rate constants,

$$\tau^{-2} - (a_{11} + a_{22})\tau^{-1} + (a_{11}a_{22} - a_{12}a_{21}) = 0 \quad (4)$$

$$(1/\tau_{\pm}) = \tfrac{1}{2}\{(a_{11} + a_{22}) \pm \sqrt{[(a_{11} + a_{22})^2 - 4(a_{11}a_{22} - a_{12}a_{21})]}\} \quad (5)$$

the concentrations of the species in solution, and the equilibrium constants of the coupled fast reactions (6)—(8).

$$H^+ + L^- \rightleftharpoons HL \quad (6)$$

$$HL + H^+ \rightleftharpoons H_2L^+ \quad (7)$$

$$H^+ + In^- \rightleftharpoons HIn \quad (8)$$

A non-linear least-squares routine based on equation (4) was carried out to calculate the forward and reverse rate constants. As shown in equations (4) and (5) the observed relaxation time τ_{obs} may correspond to either root, τ_+ or τ_-. In the calculation, no assumption was made as to the assignment of the observed relaxation time. The calculated rate constants k_n and k_{-n} were then fed into a program based on equation (5) to calculate τ_+ and τ_-. Comparisons of the calculated relaxation time τ_{calc} and that observed, τ_{obs}, are given in Table 2. Most of the τ_{obs} values correspond to τ_+. Only in one case ($[Ni^{2+}]_0 = 2 \times 10^{-3}$M, $[\text{hydroxyproline}]_0 = 2 \times 10^{-2}$M) was the calculated τ_- related to τ_{obs}. For the nickel(II) complexes, even in some experiments, the oscillographic traces showed two relaxation effects, but owing to the difficulties in the determination of the slower one, τ_-, only the calculated τ_+ values are reported.

The slower relaxation time was often unexpectedly slow in comparison with similar systems.[11,14] It was, in fact, considerably longer than the convectionally produced dissipation of the temperature gradient between the heated portion of the solution and the rest of the cell, *viz.*, *ca.* 2 s. The limiting case of this occurrence, *i.e.* $\tau_- \longrightarrow \infty$, is discussed in the Appendix.

Rate constants determined in this study are in Table 3; the relative error does not exceed ±25%. Specific error limits for the separately determined constants are also shown.

DISCUSSION

Stability Constants.—Potentiometric titration results show that only two complexes, ML and ML_2, are formed under the conditions of study. As shown in Figures 2 and 3, good straight lines are obtained from the plot of

[15] K. Kustin and R. F. Pasternack, *J. Phys. Chem.*, 1969, **73**, 1.

TABLE 2

Relaxation spectra

(*a*) Proline complexes

$10^3[M^{2+}]_0$/M	$10^3[HL]_0$/M	pH	τ_{obs}/ms	τ_{calc}/ms
Cu^{2+}				
2·79	3·39	4·29	20	21
4·98	5·35	4·31	9·4	10
4·72	7·52	4·01	19	23
2·34	2·47	4·36	20	23
3·44	4·84	4·40	10	10
2·23	4·51	3·80	89	88
3·48	6·11	4·49	7·3	7·3
1·84	5·77	4·30	21	22
4·28	5·83	4·32	14	11
1·19	1·61	4·33	58	51
1·07	2·03	4·69	17	16
1·23	4·17	4·63	17	13
Ni^{2+}				
4·21	5·84	7·80	0·070	0·062
3·93	5·74	7·85	0·061	0·088
4·55	8·76	8·08	0·072	0·064
5·21	10·50	8·40	0·069	0·069
2·91	6·74	8·41	0·11	0·12
1·12	3·96	8·42	0·27	0·30
1·84	5·58	8·42	0·19	0·20
5·40	5·67	6·87	0·08	0·08
6·50	2·22	6·90	0·19	0·15
5·88	3·46	6·79	0·12	0·12
Co^{2+}				
3·83	4·62	7·85	1·1	1·1
3·72	4·98	8·02	0·86	0·88
2·04	3·79	8·25	1·3	1·3
1·15	1·91	8·40	2·3	2·8
4·79	3·51	7·85	1·1	1·1
4·58	6·58	8·02	0·63	0·63
9·59	2·57	8·08	2·7	3·7
3·79	2·64	7·64	1·9	2·1

(*b*) Hydroxyproline complexes

Cu^{2+}				
10·0	10·0	3·90	8·5	6·0
10·0	2·5	4·47	4·5	4·3
15·0	5·0	3·40	31	27
7·50	7·50	3·86	9·4	9·3
3·00	9·00	3·94	1·4	1·1
5·00	5·02	4·03	7·1	9·0
10·0	5·09	4·02	6·0	7·2
10·0	2·37	4·01	12	13
7·50	4·47	4·01	7·6	9·0
4·00	8·40	4·05	7·7	6·9
Ni^{2+}				
5·00	10·0	7·29	0·029	0·030
3·00	10·0	7·63	0·095	0·079
2·00	10·0	8·30	0·15	0·14
5·00	20·0	8·27	0·083	0·092
5·00	20·0	7·12	0·062	0·046
10·0	20·0	8·39	0·039	0·039
2·00	20·0	8·34	0·13	0·10
2·00	10·0	8·28	0·16	0·14
Co^{2+}				
3·75	5·99	7·69	0·49	0·50
2·08	3·57	7·95	0·98	0·84
1·97	2·27	7·81	0·79	1·10
3·46	6·40	7·75	0·54	0·52
2·78	3·92	8·32	0·70	0·63
2·45	2·78	7·85	0·74	0·88
1·30	1·54	8·49	1·30	1·30
1·03	3·09	6·83	0·68	0·63
4·10	2·70	7·05	0·80	1·1
0·965	1·42	7·36	2·3	2·9
1·17	2·99	7·76	1·8	1·4
1·67	5·17	7·28	1·2	1·1

TABLE 3

Rate constants of metal complexation at 25 °C and $I = 0{\cdot}1$M

Metal	n	Proline	Hydroxyproline
		k_n/l mol^{-1} s^{-1}	
Cu^{2+}	1	$(2{\cdot}5 \pm 0{\cdot}2) \times 10^9$	$(7{\cdot}4 \pm 0{\cdot}4) \times 10^8$
	2	$(2{\cdot}7 \pm 0{\cdot}6) \times 10^8$	$(2{\cdot}8 \pm 0{\cdot}6) \times 10^8$
Ni^{2+}	1	$(3{\cdot}4 \pm 0{\cdot}6) \times 10^4$	$(1{\cdot}2 \pm 0{\cdot}3) \times 10^4$
	2	$(8{\cdot}7 \pm 1{\cdot}3) \times 10^3$	$(1{\cdot}8 \pm 0{\cdot}1) \times 10^4$
Co^{2+}	1	$(3{\cdot}5 \pm 0{\cdot}5) \times 10^5$	$(7{\cdot}0 \pm 1{\cdot}4) \times 10^4$
	2	$(9{\cdot}6 \pm 0{\cdot}7) \times 10^5$	$(9{\cdot}0 \pm 0{\cdot}4) \times 10^5$
		k_{-n} [a]/s^{-1}	
Cu^{2+}	1	3·0	3·1
	2	5·9	2·8
Ni^{2+}	1	0·024	0·014
	2	0·064	0·25
Co^{2+}	1	3·14	1·2
	2	39	135

[a] Error limits for the dissociation constants are not reported as these are calculated from the relation $k_{-n} = k_n/K_n$.

$\bar{n}/\{(1 - \bar{n})[L^-]\}$ against $\{(\bar{n} - 2)[L^-]\}/(\bar{n} - 1)$. For proline complexes, the overall stability constants, log β_2, are in good agreement with those of Albert (Table 1, ref. *a*). For both types of complex, the order of metals found is in agreement with Mellor and Maley's series of bivalent metals,[16] namely $Cu^{2+} > Ni^{2+} > Co^{2+}$.

In the complexations of amino-acids with metal ions, one of the factors which governs their stability is the basic ionization constant of the ligand. The protonation constant of proline (log K_b 10·6) is higher than that of hydroxyproline (log K_b 9·47), which is reflected in the higher stability constants of proline complexes. The relatively low value of this constant for complex formation between metal ion and hydroxyproline may then be ascribed to either the inductive effect or internal hydrogen-bond formation. Depression of the stability constant by the substituted hydroxy-group also has been found in studies of 3,4-dihydroxyphenylalanine (Dopa) [17] and 3,4-dihydroxyphenylglycine (Dopg) [18] studies.

Reaction Mechanism.—The detailed multistep mechanism of complex formation between first-transition 2+ ions with bidentate ligands has been described.[4,19,20] With most five-membered ring-forming bidentate ligands, the ring-closure step is more rapid than elimination of the first water molecule. This mechanism of complex formation is called normal substitution. On the other hand, if the ring closure is slower than water release and is the rate-determining step, then the observed rate constant will be less than normal. This case has been termed sterically controlled substitution (SCS). In Table 4 we summarize the results of a variety of ligand complexations for both normal and sterically controlled substitution. It appears that formation of most of the five-membered ring systems follow normal substitution, while six- and seven-membered-ring formation with amino-acid ligands shows the sterically controlled substitution mechanism.

Let us consider the observed values of k_1, which are $2{\cdot}5 \times 10^9$, $3{\cdot}4 \times 10^4$, and $3{\cdot}5 \times 10^5$ l mol^{-1} s^{-1} for Cu^{2+}–, Ni^{2+}–, and Co^{2+}–proline complex formation, respectively. By comparison with Table 4, it is seen that the reaction rates for this first complex are rather similar to those for amino-acids attacking as the −1 species.

TABLE 4

Selected complexation rate constants for Cu^{2+}, Ni^{2+}, and Co^{2+}

Ligand	Charge of attacking form	k_1 / l mol^{-1} s^{-1}	k_2 / l mol^{-1} s^{-1}	Ref.
(*a*) Normal substitution				
Cu^{2+}				
Phenylalanine	−1	$1{\cdot}2 \times 10^9$	3×10^8	12
Serine	−1	$2{\cdot}5 \times 10^9$	5×10^8	11
α-Alanine	−1	$1{\cdot}3 \times 10^9$	$1{\cdot}5 \times 10^8$	7
Ni^{2+}				
Malonic acid	−2	$7{\cdot}0 \times 10^4$		21
α-Alanine	−1	$2{\cdot}0 \times 10^4$	$4{\cdot}0 \times 10^4$	6
L-Cysteine	−1	$1{\cdot}5 \times 10^4$	$4{\cdot}4 \times 10^4$	5
Glycylglycine	−1	$2{\cdot}1 \times 10^4$	$4{\cdot}0 \times 10^3$	2
Imidazole	0	$5{\cdot}0 \times 10^3$	$4{\cdot}3 \times 10^3$	2
Ammonia	0	$3{\cdot}3 \times 10^3$		22
1,10-Phenanthroline	0	$3{\cdot}9 \times 10^3$		23
Co^{2+}				
Malonic acid	−2	9×10^6		21
L-Cysteine	−2	$5{\cdot}6 \times 10^6$	$1{\cdot}5 \times 10^6$	5
α-Alanine	−1	$6{\cdot}0 \times 10^5$	$8{\cdot}0 \times 10^5$	6
L-Cysteine	−1	$5{\cdot}6 \times 10^5$	$1{\cdot}5 \times 10^6$	5
L-Glycine	−1	$4{\cdot}6 \times 10^5$	$2{\cdot}2 \times 10^6$	2
Imidazole	0	$1{\cdot}3 \times 10^5$	$1{\cdot}1 \times 10^5$	2
Ammonia	0	$9{\cdot}5 \times 10^4$		22
1,10-Phenanthroline	0	$1{\cdot}4 \times 10^5$		23
(*b*) Sterically controlled substitution				
Cu^{2+}				
β-Alanine	−1	$2{\cdot}0 \times 10^8$	$8{\cdot}0 \times 10^6$	7
Histidine	0	$1{\cdot}3 \times 10^7$	$3{\cdot}0 \times 10^6$	7
Ni^{2+}				
β-Alanine	−1	$1{\cdot}0 \times 10^4$	$6{\cdot}9 \times 10^3$	6
β-Aminobutyric acid	−1	$4{\cdot}0 \times 10^3$	$8{\cdot}0 \times 10^3$	8
Co^{2+}				
β-Alanine	−1	$7{\cdot}5 \times 10^4$	$8{\cdot}6 \times 10^4$	6
β-Aminobutyric acid	−1	$2{\cdot}0 \times 10^4$	$7{\cdot}0 \times 10^4$	8

Like most amino-acids, it is assumed that proline forms a five-membered chelate and bonding occurs between the carboxylate and α-amine nitrogen. It is therefore reasonable to conclude that, within experimental error, complex formation of proline is 'normal'. Tables 3

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and 4 show that hydroxyproline is among the less-reactive ligands. The k_1 value obtained is $7{\cdot}4 \times 10^8$ l mol^{-1} s^{-1} for Cu^{2+}, $1{\cdot}2 \times 10^4$ l mol^{-1} s^{-1} for Ni^{2+}, and 7×10^4 l mol^{-1} s^{-1} for Co^{2+}. In particular, the forward rate constants of the first hydroxyproline complexes are between $\frac{1}{3}$ and $\frac{1}{5}$ of those of the proline complexes.

The basicity of hydroxyproline is low in comparison with that of proline, consistent with the lower stability constant. Similar results are observed in the study of 3,4-dihydroxyphenylalanine in which two hydroxy-groups are substituted in the phenyl group of phenylalanine. On the other hand, although serine also has a hydroxy-group in the β-position, and the stability constant is lower than that of α-alanine, the forward rate constant is normal. If the weakening of the metal–ligand bond is caused by withdrawal of negative charge away from the amino-group by the hydroxy-group, then the complex-dissociation process would therefore be facilitated; hence the dissociation rate constants would be expected to be larger and the stability constants smaller. This effect is true for serine complexes. For hydroxyproline and 3,4-dihydroxyphenylalanine, the reverse rate constants are smaller than those of the corresponding unsubstituted ligands (proline and phenylalanine). Therefore, it does not seem reasonable to assume that the depression of the stability and forward rate constants in these systems is related to the inductive effect of the substituted hydroxy-group.

In hydroxyproline, the co-ordinated water molecules of the metal ion may be hydrogen-bonded to the hydroxy-group of the ligand. The ensuing orientation, which is unfavourable for complex formation, will effectively reduce the solid angle available for successful reaction; thus the forward rate constant will be decreased. Formation of the hydrogen bond probably will also affect the dissociation process. The hydrogen bonding between the ligand and water molecules will make it relatively more difficult to break up the complex, producing a relative decrease in the reverse rate constant.

The quantity k_2/k_1 for several ligands with Ni^{2+} takes the values: glycine, 4·0; α-alanine, 2·0; L-cysteine, 3·0; α-aminobutric acid, 1·5; proline 0·3; and hydroxyproline, 1·5, whereas for Co^{2+} values are: α-alanine, 1·3; L-cysteine, 2·7; glycine, 4·8; proline, 2·7; and hydroxyproline, 12·8. The low value of k_2 in comparison with k_1 for Ni^{2+}–proline complexes is exceptional. For Ni^{2+}–hydroxyproline, Co^{2+}–proline, and Co^{2+}–hydroxyproline complexes the relative increase in k_2 is similar to that found previously.[6,9,11,14]

One characteristic of copper(II) complex formation is that ligand penetration rate constants are decreased after substitution of the first chelating ring.[7,12] This special characteristic is also exhibited with proline and hydroxyproline. The decrease in the second forward rate constant is probably due to Jahn–Teller distortion of the d^9 Cu^{2+} ion. Two water molecules in the axial positions are farther away from the central ion and thus more labile than the other molecules. In addition, it is believed that there is an inversion process by which equatorial and axial positions can be exchanged. This interchange process can be hindered by the presence of a bidentate ligand on the metal ion and hence slow down attack of a second ligand to form CuL_2.

APPENDIX

To understand the anomalously long relaxation time observed in some of the nickel(II) complex relaxation spectra, we first consider the properties of an amino-acid ligand. Generally, only the negative form of the ligand attacks the metal ion; paths involving neutral, zwitterionic amino-acids do not contribute to the rate.[3] Therefore, studies at $[H^+]$ values below the second ionization constant of the ligand ensure that $[\overline{M}]' \text{ and } [\overline{ML}]' \gg [\overline{L}]$ (where the bar over the symbol denotes equilibrium and the prime means that the concentration has been corrected for the presence of rapidly established equilibria). Amino-acid–nickel(II) complexes are quite stable; thus at moderate $[\overline{M}]$ (say 10^{-2}M) the approximation $k_n[\overline{ML}_n] \gg k_{-n}$ holds.

Linearized rate equations for the formation of complexes ML and ML_2 under the above conditions can be written as (9) and (10), where $x_1 = \delta[M]$ and $x_2 = \delta[ML_2]$. Setting $k_1[\overline{M}]' = a$ and $k_2[\overline{ML}]' = b$ we obtain

$$-dx_1/dt = k_1[\overline{M}]'x_1 - k_1[\overline{M}]'x_2 \quad (9)$$

$$-dx_2/dt = -k_2[\overline{ML}]'x_1 + k_2[\overline{ML}]'x_2 \quad (10)$$

equations (11) and (12). Note that det A, given by relation (13), indicates a linearly *dependent* set of differential equations. Relaxation times are found by

$$-dx_1/dt = ax_1 - ax_2 \quad (11)$$

$$-dx_2/dt = -bx_1 + bx_2 \quad (12)$$

solving the secular determinant. Let $\lambda = 1/\tau$, then we obtain relation (14) or (15), where I is the unit matrix, and thence relations (16)—(18).

$$\det A = \det \begin{bmatrix} a & -a \\ -b & b \end{bmatrix} = 0 \quad (13)$$

$$\begin{vmatrix} a-\lambda & -a \\ -b & b-\lambda \end{vmatrix} = 0 \quad (14)$$

$$|A - \lambda I| = 0 \quad (15)$$

$$\lambda^2 - (a+b)\lambda = 0 \quad (16)$$

$$\lambda = 0,\ \lambda = a + b \quad (17)$$

$$1/\tau = 0,\ 1/\tau = k_1[\overline{M}]' + k_2[\overline{ML}]' \quad (18)$$

This system of reactions possesses only a single finite relaxation time. The meaning of this result will become more apparent when the equations are transformed into a new set in the normal concentration variables y_i. The set of simultaneous linear differential equations above can be written in matrix notation as (19). To find the

$$d\boldsymbol{X}/dt = A\boldsymbol{X} \quad (19)$$

normal concentration variables we wish to find a vector $\boldsymbol{Y}$, related to $\boldsymbol{X}$ by a similarity transformation in such a

way that equation (20) holds where Λ is a diagonal

$$d\boldsymbol{Y}/dt = \Lambda\boldsymbol{Y} \quad (20)$$

matrix. The relation between $\boldsymbol{Y}$ and $\boldsymbol{X}$ is defined by a matrix M so that $\boldsymbol{Y} = M\boldsymbol{X}$; M is the transformation matrix from vector space (x_1x_2) to vector space (y_1y_2). It can be shown that equation (21) holds. The M

$$MA = \Lambda M \quad (21)$$

matrix is constructed from the eigenvectors corresponding to the individual eigenvalues (22) and (23).

$$M = \begin{bmatrix} -1 & 1 \\ \frac{b}{a} & 1 \end{bmatrix} \quad (22)$$

$$M^{-1} = \begin{bmatrix} \frac{-a}{a+b} & \frac{a}{a+b} \\ \frac{b}{a+b} & \frac{a}{a+b} \end{bmatrix} \quad (23)$$

If y_i is a normal relaxation co-ordinate then we obtain equation (24), where $\boldsymbol{Y} = M\boldsymbol{X}$, and find equation

$$d\boldsymbol{Y}/dt = \Lambda\boldsymbol{Y} \quad (24)$$

$$\begin{vmatrix} y_1 \\ y_2 \end{vmatrix} = \begin{bmatrix} -1 & 1 \\ b/a & 1 \end{bmatrix} \begin{vmatrix} x_1 \\ x_2 \end{vmatrix} \quad (25)$$

(25). The individual normal co-ordinates are given by (26) and (27).

$$y_1 = -x_1 + x_2 \quad (26)$$

where $\tau_1 = 1/(k_1[\overline{M}]' + k_2[\overline{ML}]')$

$$y_2 = \frac{b}{a}x_1 + x_2 \quad (27)$$

where $\tau_2 = \infty$

A simple interpretation of $\tau_2 \longrightarrow \infty$ may be made by assuming a reasonable set of clarifying conditions. Suppose that the enthalpies of reaction for both steps $M + L \rightleftharpoons ML$ and $ML + L \rightleftharpoons ML_2$ are of the same sign. Then the change in [M] will be opposite in sign to that in $[ML_2]$. As a result, y_1 may be positive or negative under these conditions, but never zero; it is a sum, never a difference, of the absolute values of the concentration changes. The same is not true for y_2 which is always a difference of concentration changes. Under these conditions, y_2 may be zero, according to the quantity b/a and absolute values of the concentration changes in the individual reaction steps.

We acknowledge support from the National Institute of General Medical Sciences and thank Mr. Mort Barr for suggestions.

[2/1478 *Received, 26th June,* 1972]