Equilibria of Some α-Amino-acids containing Sulphur Atoms in the Chain with Metal(II) Ions. Part 2.† *SS'*-Methylenebis(L-cysteine) with Cobalt(II), Nickel(II), and Zinc(II) in Aqueous Solution

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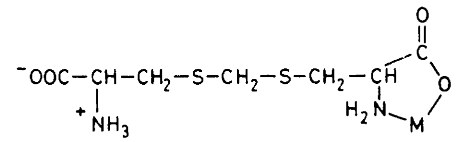
Studies of the complexes between bivalent metal ions Co^{2+}, Ni^{2+}, and Zn^{2+}, *SS'*-methylenebis(L-cysteine) (H_2L), and H^+ have been undertaken. The quantitative equilibrium investigations of H_2L with the above mentioned metal ions are described in detail. The proton ionization constants of the ligand have been determined in previous work, and the equilibrium constants of the corresponding proton, metal, and ligand ion association constants have been calculated from potentiometric data at 25 °C and I = 0.1 mol dm^{-3} (KCl). The ligand forms with metals the complexes $[Co(HL)]^+$, [CoL], $[CoL_2]^{2-}$; $[Ni(HL)]^+$, [NiL], $[NiL_2]^{2-}$; [ZnL], $[ZnL_2]^{2-}$, where the ligand L^{2-} = $^-O_2C(H_2N)CHCH_2SCH_2SCH_2CH(NH_2)CO_2^-$. The equilibrium constants follow the Irving–Williams series. All the complexed species very likely contain penta-atomic chelate rings of the glycine type. The probable structures of the chelated compounds formed in aqueous solution are deduced by structural analysis of the solid ligands, and their stability constants are compared with those of analogous chelated compounds. The H^+ of the protonated complexes is probably bound to the amine group not involved in the chelate ring. The importance of the protonated complexes in almost all systems studied has been demonstrated and the relative structures are discussed.

As a continuation of a research programme on the behaviour of sulphur-containing α-amino-acids,[1,2] both as acids [3] and as ligands,[4] the study of the complexing capacity of *SS'*-methylenebis(L-cysteine), $CH_2[SCH_2CH(NH_2)CO_2H]_2$ (djenkolic acid) (H_2L), with divalent metal ions has been carried out in aqueous solution. The problems raised by this ligand concern its capacity of binding to the metals through *N,O*-donor atoms, by analogy with other α-amino-acids, and the influence of the stability of the metal complexes on the decrease in basicity of the unco-ordinated amino-group. At intermediate pH values, the nitrogen-donor site, not implied in the co-ordination polyhedron, is generally able to co-ordinate with hydrogen ions by forming complexes of type $[M(HL)]^+$ (below) (*i.e.* those found in homocystine complexes [4] and generally in biological systems). This paper reports the results of a detailed study of the complexes of Co^{2+}, Ni^{2+}, and Zn^{2+} with *SS'*-methylenebis(L-cysteine) in aqueous solution.



EXPERIMENTAL

The amino-acid (H_2L) was employed without further purification (Found: C, 33.25; H, 5.60; N, 11.20; S, 24.80. Calc. for $C_7H_{14}N_2O_4S_2$: C, 33.05; H, 5.55; N, 11.00; S, 25.20%).

All the solutions of the reagents for potentiometric measurements were prepared and experimental titrations were carried out following the procedure previously described.[4-6] The concentrations of the starting solutions and pH ranges for each potentiometric titration are quoted in Table 1.

TABLE 1

Complex formation constant determinations. Initial solution compositions (T_M,T_H,T_L/mmol) and pH ranges for the titrations of djenkolic acid (H_2L) with divalent metal ions at 25 °C and I = 0.1 mol dm^{-3} (KCl)

Run	Ion	T_M	T_H	T_L	pH
1	Co^{2+}	0.210 999	0.844 122	0.211 019	2.49—9.62
2		0.040 020	0.800 551	0.200 128	2.47—8.13
3		0.037 736	0.756 865	0.188 687	2.51—9.63
4		0.047 157	0.756 678	0.188 647	2.52—9.62
5		0.064 945	0.781 882	0.194 899	2.50—9.56
6		0.110 758	0.888 658	0.221 517	2.45—9.44
7	Ni^{2+}	0.069 520	0.834 400	0.208 600	2.47—9.33
8		0.101 590	0.812 800	0.203 200	2.50—9.48
9		0.040 027	0.800 510	0.200 100	2.49—9.63
10		0.050 018	0.823 584	0.205 908	2.48—9.76
11		0.213 004	0.851 975	0.212 985	2.50—8.95
12	Zn^{2+}	0.039 895	0.798 350	0.199 027	2.48—9.00
13		0.050 617	0.809 632	0.202 369	2.48—9.50
14		0.063 915	0.773 638	0.193 405	2.52—9.24

RESULTS AND REFINEMENT

The proton ionization constants of the ligand H_2L have been determined in previous work.[3] The equilibrium and formation constants obtained for H_2L with different metal ions are reported in Table 2. The titration curves of the protonated ligand, H_4L^{2+}, alone and in the presence of Co^{2+}, Ni^{2+}, and Zn^{2+} are shown in Figure 1. In this diagram the titration curve of hydrochloric acid with K[OH] solution was included. The potentiometric titration curve of protonated *SS'*-methylenebis(L-cysteine), H_4L^{2+}, features a steep inflection at a = 3.5 {where a indicates the volume of base (K[OH]) added (in cm^3)} corresponding to the titration of the two carboxylic protons (strong acid). The two remaining protons ($-NH_3^+$) dissociate between a = 3.5 and 6.0.

Titration curves obtained with equal mixtures of djenkolic (H_2L) and hydrochloric acid with cobalt(II) and similar mixtures with nickel(II) and zinc(II) are superimposable with the titration curve of the free acid in the same conditions of concentration alone between a = 0 and 3.5. This behaviour implies that complexes of *SS'*-methylenebis(L-cysteine) with these metal ions are not present in appreciable

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† Part 1 is ref. 4.

concentrations until all the carboxylic protons have been neutralized. Between $a = 3.5$ and 6.0, complex formation occurs. This has the effect of depressing all the titration curves involving metal ions below that of the free acid.

TABLE 2

Cumulative and stepwise formation constants of djenkolic acid (H_2L) with Co^{II}, Ni^{II}, and Zn^{II} at 25 °C and $I = 0.1$ mol dm^{-3} (KCl). Standard deviations (σ values) are given in parentheses

	Co^{II}	Ni^{II}	Zn^{II}
$\log \beta_{111}$ [a]	12.82(2)	14.06(7)	
$\log \beta_{101}$	6.27(1)	8.66(2)	7.45(6)
$\log \beta_{102}$	8.79(4)	12.94(5)	11.46(9)
$\log K_2$	2.52(4)	4.28(5)	4.01(11)
$\log K_{111}{}^{101}$ [b]	6.55(2)	5.40(7)	
$\Sigma\Delta^2$ [c]	0.645 255 $\times 10^{-7}$	0.265 737 $\times 10^{-6}$	0.216 040 $\times 10^{-6}$
R [d]	0.003	0.008	0.009
N [e]	271	174	124

[a] $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$. [b] $\log K_{111}{}^{101} = \log\beta_{111} - \log \beta_{101}$. [c] $\Sigma\Delta^2 = \Sigma(\Delta C_M{}^2 + \Delta C_H{}^2 + \Delta C_L{}^2)$. [d] R factor $= \left[\frac{\Sigma_i(X_{o,i} - X_{c,i})^2}{\Sigma_i(X_{o,i})^2}\right]^{\frac{1}{2}}$, where $X_{o,i}$ and $X_{c,i}$ are observed and calculated quantities at point i. [e] N = Total number of data points used in the refinement.

By suitable use of the program MINIQUAD,[7] it is possible to obtain a high degree of discrimination in the selection of chemical species. This program minimizes the sum of the squares of the residuals, $\Sigma\Delta^2 = \Sigma(\Delta C_M{}^2 + \Delta C_H{}^2 + \Delta C_L{}^2)$, where C_M, C_H, and C_L are respectively the total molar metal, acid, and ligand concentrations. The inclusion of the hydrolyzed species $[ML(OH)_n]^{n-}$ and $[ML_2(OH)_n]^{(n+2)-}$ did not improve the estimated standard deviation (e.s.d.) and the estimated value of the equilibrium constants progressively decreased without convergence. Attempts to evaluate the stability constants for the minor species have resulted in this type of behaviour with MINIQUAD and similar programs. However, it was necessary in all metal–ligand systems studied, with the exception of Zn^{2+}, to introduce a protonated form of the complex, $[M(HL)]^+$, in order to minimize the estimated standard deviations. The best set of complexes was $[M(HL)]^+$, [ML], and $[ML_2]^{2-}$ for Co^{2+}; $[M(HL)]^+$, [ML], and $[ML_2]^{2-}$ for Ni^{2+}; and [ML] and $[ML_2]^{2-}$ for Zn^{2+}. Moreover, the computer program MINIQUAD was separately applied to the experimental data of each titration, and then the results ($\log \beta_{pqr}$ = cumulative stability constant $= [M_pH_qL_r]/[M]^p[H]^q[L]^r$) were averaged and assessed by using the computer program WGMEAN, based on the treatment of observation errors.[8] Values of the overall formation constants (Table 4) were obtained as weighted means of the values from a single titration. This procedure of refinement is recommended because it reveals the experimental errors, which are specific to each set of titration data, and gives a more reasonable measure of the dispersion of the errors.* A complete list of the experimental data is available as Supplementary Publication No. SUP 23068 (18 pp.).†

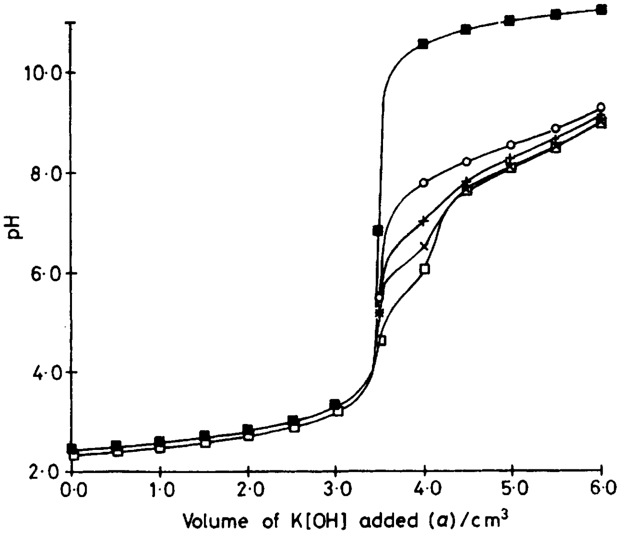

FIGURE 1 Titration curves as function of the volume of K[OH] added, calculated by HALTAFALL program,[9] equipped with PLOTTER Calcomp 936. Djenkolic acid, H_2L (○), with Co^{II} (+), Ni^{II} (□), Zn^{II} (×), and HCl–K[OH] (■). $C_L = 2.0 \times 10^{-3}$, $C_H = 8.684 \times 10^{-3}$, $C_{Co^{II}} = 4.014 \times 10^{-4}$, $C_{Ni^{II}} = 4.015 \times 10^{-4}$, $C_{Zn^{II}} = 4.002 \times 10^{-4}$, $C_{K[OH]} = 0.133\,4$ mol dm^{-3}; V_0 (initial volume of solutions) = 99.695 cm^3

TABLE 3

Comparison of equilibrium constants of equation (3) for Co^{2+} and Ni^{2+} metal ions with different α-amino-acids at 25 °C. Standard deviations (σ values) are given in parentheses

Ligand		Co^{II}	Ni^{II}
(A) [a]	$\log \beta_{111}$	12.30(2)	13.428(6)
	$\log \beta_{101}$	6.28(1)	8.159(3)
	$\log K$	6.02(2)	5.269(7)
(B) [b]	$\log \beta_{111}$	14.168(5)	15.255(8)
	$\log \beta_{101}$	5.01(1)	7.113(9)
	$\log K$	9.158(11)	8.142(12)
(C) [c]	$\log \beta_{111}$	14.505(4)	15.600(8)
	$\log \beta_{101}$		5.75(7)
	$\log K$		9.85(7)
(D) [d]	$\log \beta_{111}$	13.543(52)	14.327(10)
	$\log \beta_{101}$	5.759(25)	7.653(4)
	$\log K$	7.784(58)	6.674(11)
(E) [e]	$\log \beta_{111}$	12.82(2)	14.06(7)
	$\log \beta_{101}$	6.27(1)	8.66(2)
	$\log K$	6.55(2)	5.40(7)

[a] (A) = DL-2,3-Diaminopropanoic acid, ref. 10. [b] (B) = L-2,5-Diaminopentanoic acid (L-ornithine), ref. 10. [c] (C) = L-2,6-Diaminohexanoic acid (L-lysine), ref. 10. [d] (D) = DL-4,4′-Dithiobis(2-aminobutanoic acid) (homocystine), ref. 4. [e] (E) = *SS*′-Methylenebis(L-cysteine), present work.

TABLE 4

Cumulative formation constants of *SS*′-methylenebis(L-cysteine) with Co^{II}, Ni^{II}, and Zn^{II} obtained as weighted means of the values refined for each set of experimental data. Standard deviations (σ values) are given in parentheses

	Co^{II}	Ni^{II}	Zn^{II}
$\log \beta_{111}$	12.85(2)	14.10(5)	
$\log \beta_{101}$	6.28(1)	8.66(1)	7.28(4)
$\log \beta_{102}$	8.90(4)	12.98(6)	11.13(6)

DISCUSSION

Equilibria in aqueous solution at 25 °C and $I = 0.1$ mol dm^{-3} (KCl) between djenkolic acid (H_2L) and the

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* All the calculations were carried out on the computer CDC CYBER 70/76 of the Centro di Calcolo Interuniversitario dell'Italia Nord-Orientale (Casalecchio, Bologna), with the financial support from the University of Parma.

† For details see Notices to Authors No. 7, *J. Chem. Soc., Dalton Trans.*, 1980, Index issue.

divalent cations Co^{2+}, Ni^{2+}, and Zn^{2+} have been determined. Equilibria with Cu^{2+} cannot be studied because a wide number of equilibria are present and the treatment of the experimental data produces major problems in the species selection. The analysis and refinement of the formation constants of the complexes formed by H_2L with Co^{2+}, Ni^{2+}, and Zn^{2+} shows that the following species are present: $[Co(HL)]^+$, $[CoL]$, $[CoL_2]^{2-}$, $[Ni(HL)]^+$, $[NiL]$, $[NiL_2]^{2-}$, $[ZnL]$, $[ZnL_2]^{2-}$. The corrected potentiometric titration curves for the α-amino-acid with and without a nearly equivalent amount of the metal ions in the solution are given in Figure 1. The shapes of the curves clearly show the different types of observed reactions, involving the formation of simple mononuclear chelates, $[ML]$ and $[ML_2]^{2-}$, as well as monoprotonated chelate, $[M(HL)]^+$.

The most common initial reaction of the ligand H_4L^{2+} involving divalent transition-metal ions, after neutralization of the two carboxy-groups, is a one-proton displacement thus forming a monohydrogen

$$M^{2+} + H_2L \rightleftharpoons [M(HL)]^+ + H^+ \qquad (M = Co^{2+} \text{ and } Ni^{2+}) \qquad (1)$$

$$K_{111}^{021} = \frac{[M(HL)^+][H^+]}{[M^{2+}][H_2L]}$$

complex [equilibrium (1)]. This is generally followed by one subsequent dissociation reaction, in which one additional mole of base is required for each mole of metal chelate [equilibrium (2)]. In the cases of Co^{2+} and Ni^{2+}

$$[M(HL)]^+ \rightleftharpoons [ML] + H^+ \quad (M = Co^{2+} \text{ and } Ni^{2+}) \qquad (2)$$

$$K_{101}^{111} = \frac{[ML][H^+]}{[M(HL)^+]}$$

the buffer regions of the above reactions do not overlap, so that each reaction step is apparent in the titration curves, and equilibrium constants for each step may be calculated separately. Using the stability constants given in Table 2 and the proton ionization constants of the ligand under the same conditions, the percentage concentration of each of the complexes involving H^+, metal ions, and ligand has been calculated with the aid of the HALTAFALL computer program.[9] The results were reported by a PLOTTER Calcomp 936 on line to give profiles of the distribution of each complex species *versus* pH applicable to various concentrations of ligand and metal ion. The HALTAFALL program was adapted for a Control Data Corporation CYBER 70/76 and modified as follows. This program was completed from some subroutines written in Fortran VI in order to reproduce different diagram types, using the PLOTTER Calcomp 936. A typical species-distribution graph for djenkolic acid–metal ion mixture is shown for Co^{2+} (Figure 2), Ni^{2+} (Figure 3), and Zn^{2+} (Figure 4). Some general observations on the behaviour of the various systems are discussed as follows. (*a*) For the H_2L–Zn^{2+} system only mononuclear complexes are formed. The [ZnL] complex reaches a maximum concentration of 95.9% total metal at pH 7.00, and $[ZnL_2]^{2-}$ reaches a maximum of 90.7% at pH 9.50; (*b*) for the H_2L–Co^{2+} system three complexes were found: $[Co(HL)]^+$ reaches 21.4% total metal at pH 6.75, [CoL] reaches a peak of 93.9% at pH 8.05, and $[CoL_2]^{2-}$ reaches a maximum of 32.7% at pH 11.00; (*c*) for the H_2L–Ni^{2+} system the complexes were common to those of Co^{2+}: $[Ni(HL)]^+$ has a maximum of 22.9% at pH 5.62, [NiL] a maximum of 95.5% at pH 6.99, and $[NiL_2]^{2-}$ 95.4% at pH 11.00. These observations correlate well with the values for stability constants reported in Table 2. In particular the cumulative and stepwise formation constants for the 1 : 1 and 1 : 2 metal–djenkolic acid complexes follow the

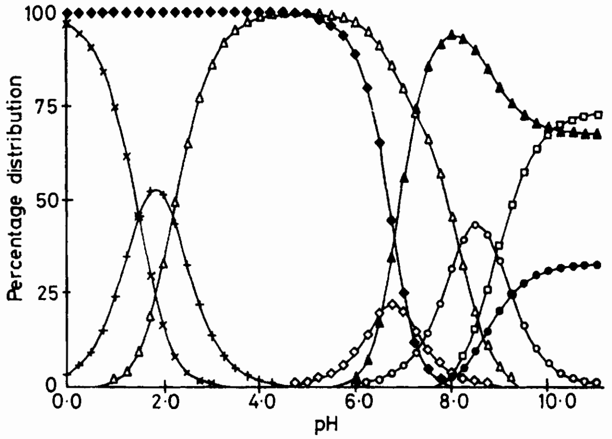


FIGURE 2 Typical distribution diagram for the system djenkolic acid–Co^{2+}; HL^- (○), H_2L (△), H_3L^+ (+), H_4L^{2+} (×), [ML] (▲), $[ML_2]^{2-}$ (●), $[M(HL)]^+$ (◇), L^{2-} (□), and M^{2+} (♦). The percentage of each species has been calculated from the data of a hypothetical solution of cobalt ions (0.000 401 4 mol dm^{-3}) and djenkolic acid (0.001 996 4 mol dm^{-3}) by HALTAFALL program,[9] equipped with PLOTTER Calcomp 936. The concentrations of the species not containing metal have been calculated as percentages of total ligand, those containing metal as percentages of total metal

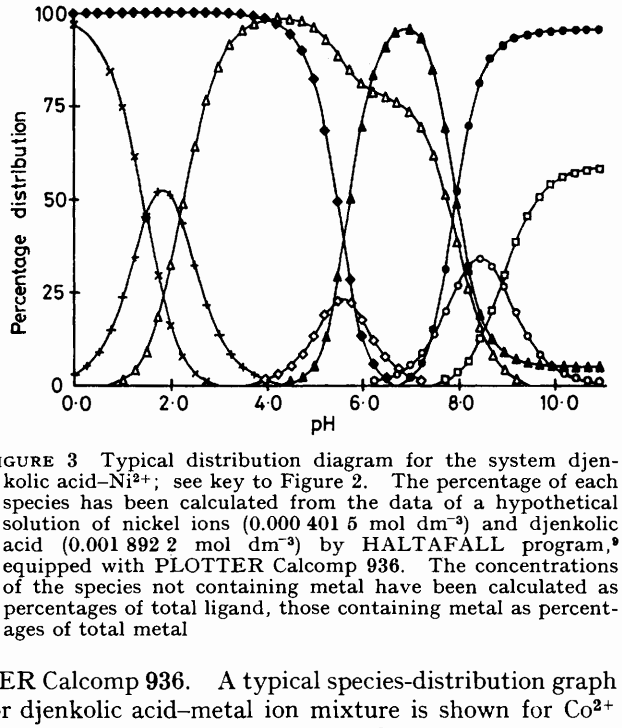


FIGURE 3 Typical distribution diagram for the system djenkolic acid–Ni^{2+}; see key to Figure 2. The percentage of each species has been calculated from the data of a hypothetical solution of nickel ions (0.000 401 5 mol dm^{-3}) and djenkolic acid (0.001 892 2 mol dm^{-3}) by HALTAFALL program,[9] equipped with PLOTTER Calcomp 936. The concentrations of the species not containing metal have been calculated as percentages of total ligand, those containing metal as percentages of total metal

Irving–Williams order. Because few tri- or tetradentate α-amino-acids, which contain two amino-groups and one or two carboxylic groups, are known, the stability constants obtained here have been compared to those of homocystine [4,4′-dithiobis(2-aminobutanoic acid), dtba] and other α-amino-acids (Table 3). The stoicheiometry and the cumulative stability constants of the complexes studied in the present work parallel those formed by dtba[4] with the same metal, except that the former complexes of Co^{2+} ([ML]) and Ni^{2+} ([ML] and $[ML_2]^{2-}$) are more strongly associated than the latter (Table 3). The increase of the stability constant values for djenkolic acid complexes can be explained by comparison of the several significant structural differences between djenkolic acid and dtba in the solid state.[1,2]

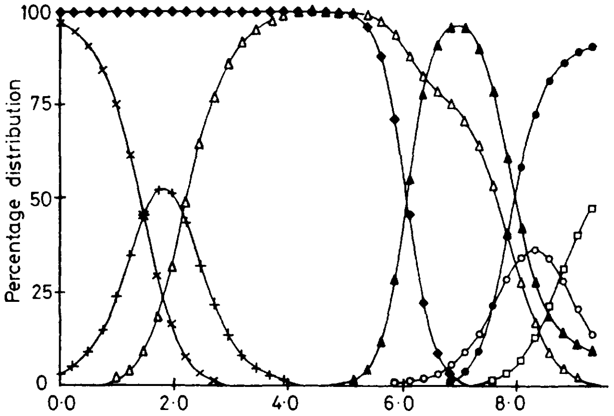
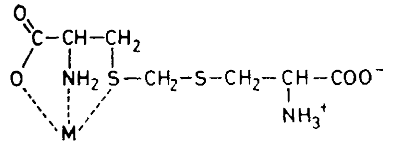
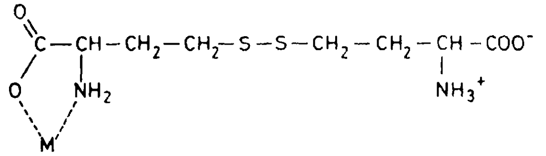


FIGURE 4 Typical distribution diagram for the system djenkolic acid–Zn^{2+}; see key to Figure 2. The percentage of each species has been calculated from the data of a solution of zinc ions (0.000 400 2 mol dm^{-3}) and djenkolic acid (0.001 996 4 mol dm^{-3}) by HALTAFALL program,[9] equipped with PLOTTER Calcomp 936. The concentrations of the species not containing metal have been calculated as percentages of total ligand, while those containing metal as percentages of total metal


(I)


(II)

The stereochemical and structural aspects, which depend upon the relative conformation of the donor atoms and are responsible for the differences in stability between djenkolic acid and dtba complexes can be illustrated for the complex [ML] by (I) and (II), in conformity with the structural information of the ligands in the solid state. This lower stability for the dtba complexes may be in part due to the glycine-like chelate, where the metal ion is probably bound through the carboxylate and α-amino-group only, (II). For djenkolic acid it is reasonable to suppose that this enhancement in stability of the metal complexes is the result of bonding in a glycine-like manner with, probably, some participation of the sulphur atom in the chain in the co-ordination, (I). The mode of bonding in the monoprotonated complexes of djenkolic acid (H_3L^+) is almost certainly glycine-like, with the proton residing on the amino-nitrogen atom not involved in the chelation and with participation of the sulphur atom to the co-ordination polyhedron.

Equilibrium constants for reaction (3) may be compared to those for the analogous process with some α-amino-acids, and results are reported in Table 3. First,

$$[ML] + H^+ \rightleftharpoons [M(HL)]^+ \qquad (3)$$

$$K = \frac{[M(HL)^+]}{[ML][H^+]} = \frac{\beta_{111}}{\beta_{101}}$$

the results suggest that the order of the stability constant (log K) for the various ligands quoted in Table 3 is in the sequence $K_{Co^{II}} > K_{Ni^{II}} > K_{Cu^{II}}$. Moreover, the decrease in basicity of the amino-group not chelated for these different ligands is influenced by the increasing stability of the corresponding complex formation [ML] (log β_{101}) for Co^{II} and Ni^{II}. The constants (K) for the equilibrium (3) form a sequence with the trend:[10] L-2,6-diaminohexanoic acid > L-2,5-diaminopentanoic acid > DL-4,4′-dithiobis(2-aminobutanoic acid) > *SS*′-methylenebis(L-cysteine) > DL-2,3-diaminopropanoic acid, for Co^{II} and Ni^{II}.

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