Mixed Metal Complexes in Solution. Part 5.† Equilibrium and Conformational Study of Heterobinuclear Metal(II)–Isoserine Complexes in Aqueous Solution

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Simple and mixed-metal complexes of isoserine with zinc(II), cadmium(II), and cobalt(II) have been investigated in aqueous solution. Potentiometric data have shown the formation of dimeric species with two hydroxyl-deprotonated isoserine molecules. Proton n.m.r. measurements indicate that the statistical high stability of the mixed-metal dimers may be correlated with the favourable conformations permitted by the tetrahedral co-ordination of the zinc(II) ion.

In previous papers, we reported on the formation of hetero-binuclear complexes of transition-metal ions with ligands of biological interest such as histamine, histidine, citrate, and adenosine-5'-triphosphate.[1-4]

The present work deals with the simple and mixed-metal complexes formed by isoserine (3-amino-2-hydroxypropanoic acid), a β-hydroxylated amino acid, and some metal ions, namely cobalt(II), zinc(II), and cadmium(II).

Little exists in the literature on the behaviour of isoserine in aqueous solution; reported studies include the binary systems of cobalt(II), zinc(II), and nickel(II)[5] and also a dimeric species which has been reported[6] for the copper(II)–isoserine system.

Our interest in this ligand is related to its possible role in the genesis of the biological world. The experimental basis of this hypothesis lies in a series of investigations, carried out by means of contact glow discharge electrolysis. In fact, using aliphatic amines as starting materials, some researchers have been able to obtain some α-amino acids, present in biological systems, through τ- and δ-amino acids, such as isoserine, which apparently behave as unsubstitutable intermediate materials for the genesis of the biological world.[7-9] It is well known that in the prebiotic period the ligands, owing to their low concentration, should have been in the presence of an excess of metal ions; hence, the importance of studying interactions of these compounds with metal ions that might have played an essential role in the appearance of living organisms on our planet. In this connection, it is worth remembering that the co-ordination of a ligand to a metal ion may substantially affect its reactivity towards a given reagent.[10]

Experimental

Chemicals.—D,L-Isoserine was purchased from the Sigma Chemical Company and used without further purification. Its purity was checked by both acidimetric and alkalimetric titrations and was always greater than 99%.

Solutions of $M^{II}(NO_3)_2$ were standardized with ethylenediaminetetra-acetate according to Flaschka.[11] The ionic strength was adjusted to 0.1 mol dm⁻³ by adding KNO_3. Grade A glassware was used throughout.

E.m.f. Measurements.—Potentiometric measurements were carried out by using two semi-automatic instruments (Amel

232 dispenser coupled with an Analog Devices AD 2027B potentiometer) with glass electrodes (EIL or Ingold 201NS) and single calomels (Ingold).

Standard electrode potentials, $E°$, and protonation constants were determined by titrating a solution (25—50 cm³) containing HNO_3 and fully protonated D,L-isoserine, respectively, with KOH. The stability constants of simple complexes were computed from the data obtained by titrating solutions (25—50 cm³) containing M^{II} and isoserine with KOH; analogously, the stability constants of the mixed-metal complexes were determined by titrating solutions (25—50 cm³) containing M^{II}, $M^{II'}$, and isoserine with KOH. pH Values were reproducible within 0.01 units. Experimental details of the potentiometric measurements are given in Table 1. Other details were as previously described.[12-15]

Spectroscopy.—Electronic absorption spectra were recorded on an Optica CF4NI double-beam spectrophotometer, using 1-cm quartz cells. Proton n.m.r. spectra were obtained at 80 MHz on a Bruker WP-80 spectrometer at room temperature in the Fourier-transform mode. The concentrations of isoserine and the metal ions (in D_2O) were in the range 10—20 mmol dm⁻³. 2,2-Dimethyl-2-silapentanesulphonic acid was used as internal standard.

Calculations.—All calculations concerning the calibration of the electrodic system, the purity of the ligand, and the protonation constants were performed using the program ACBA[16] which refines the parameters of an acid–base titration by using a non-linear least-squares method minimizing the function $U = (v_{exp.} - v_{calc.})^2$, where v_i is the volume of the ith point. MINIQUAD 76A[17] was used to compute the stability constants of both the binary and ternary systems; this program minimizes the function $U = (T_{exp.} - T_{calc.})^2$, where T is the analytical concentration of the ith component. The residuals were analysed statistically according to the method proposed by Vacca et al.[18] Distribution diagrams were obtained by means of the computer program DISDI,[19] whereas the simulation of the titrations was performed by using the program EQUIL.[20]

Results and Discussion

Table 2 details the equilibrium constants concerning both the protonation and binary complex formation at 25 °C and $I = 0.1$ mol dm⁻³ (KNO_3). The data show that deprotonated dimers are the predominant species over a wide pH range under

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† Part 4 is ref. 4.

Table 1. Experimental details of potentiometric measurements at 25 °C and $I = 0.1$ mol dm^{-3}

M	M′	c_M/mmol dm^{-3}	$c_{M'}$/mmol dm^{-3}	c_L/mmol dm^{-3}	pH range	Number of titrations
Cd^{II}	Zn^{II}	1.5	—	4.4	2.3—11.0	3
		1.0	—	4.4	2.3—10.9	3
		2.5	—	5.5	2.5—10.0	3
		4.0	—	4.4	2.5—10.0	3
		3.0	3.1	6.0	2.3—11.0	3
		3.0	2.6	6.0	2.3—11.0	3
		4.0	4.0	8.0	2.3—11.0	3
		3.0	3.0	6.0	2.5—9.2	1
		3.5	3.6	7.0	2.5—9.2	1
		4.1	2.0	6.0	2.5—8.9	1
		5.0	3.0	8.0	2.2—8.9	2
Zn^{II}	Co^{II}	1.5	—	4.4	2.2—11.0	2
		1.0	—	4.4	2.3—11.0	3
		2.6	—	5.5	2.3—9.5	3
		4.0	—	4.0	2.4—9.0	3
		2.6	—	5.6	2.3—7.1	3
		3.0	3.2	6.1	2.4—8.8	3
		3.5	3.7	7.2	2.3—8.6	2
		4.0	4.0	8.1	2.3—8.1	3
		3.2	5.0	8.2	2.4—8.2	3
		2.5	4.7	7.3	2.3—8.4	2
Co^{II}	Cd^{II}	1.5	—	4.2	2.4—8.0	3
		2.5	—	4.4	2.3—7.9	3
		4.0	—	5.6	2.3—7.7	3
		4.4	—	4.7	2.4—7.1	3
		2.5	3.0	5.6	2.3—7.8	2
		2.0	3.5	6.0	2.4—7.9	2
		3.5	4.0	8.0	2.3—8.0	3
		3.2	3.5	6.7	2.2—7.9	3

Table 2. Stability constants for binary metal complexes of D,L-isoserine at 25 °C and $I = 0.1$ mol dm^{-3} *

Metal ion	log β_{111}	log β_{22-2}	log β_{12-2}
Cd^{II}	11.14(6)	−6.62(3)	−11.75(8)
Zn^{II}	11.03(5)	−3.50(3)	−9.1(1)
Co^{II}	10.4(2)	−5.61(4)	—

* D,L-Isoserine protonation constants: log K_1 = 9.14(2), log K_2 = 2.66(3); 3σ given in parentheses; $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$.

Table 3. Formation constants and log K_{mix} values for ternary complexes of D,L-isoserine at 25 °C and $I = 0.1$ mol dm^{-3} *

Metal ions	log β_{112-2}	log K_{mix}
Zn^{II}–Cd^{II}	−4.33(7)	1.46
Co^{II}–Cd^{II}	−4.97(7)	2.29
Co^{II}–Zn^{II}	−3.74(7)	1.63

* $\beta_{pqrs} = [M_pM'_qL_rH_s]/[M]^p[M']^q[L]^r[H]^s$; log K_{mix} = 2 log β_{112-2} − log β_{202-2} − log β_{022-2}.

our experimental conditions, the deprotonation occurring at the alcoholic oxygen, as already suggested.[5] At low pH (2.5—6.5), a protonated species is also formed. Its stability constant, similar for all three systems, suggests co-ordination through the ionized carboxylic and hydroxylic groups, with the consequent formation of a five-membered chelate ring; the amino group, still protonated over this pH range, is not involved in the co-ordination.

The results are in good agreement with those reported by other authors, with the exception of species 1 2 − 2, which was detected under our experimental conditions, most likely owing to the wider concentration range investigated.
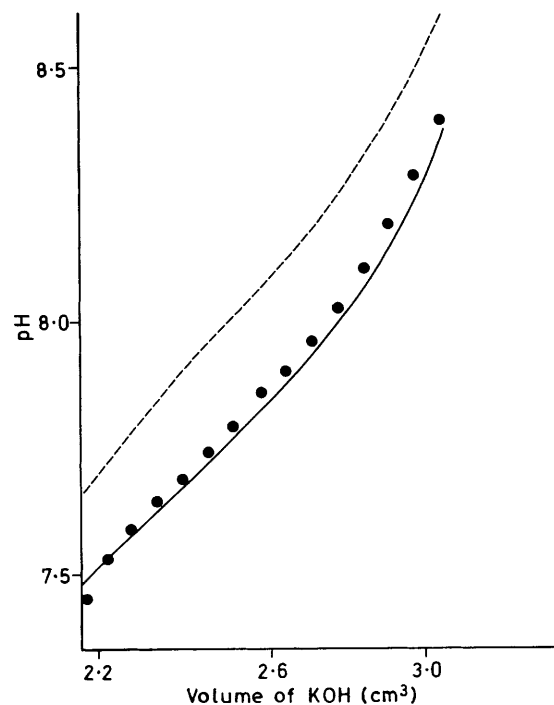

Figure 1. Graphical comparison between experimental potentiometric data (●) and calculated curves [the dashed line has been calculated by excluding and the full line by including the heterobinuclear complex for the cadmium(II)–cobalt(II) system]

Table 4. Chemical shifts (p.p.m.) and coupling constants (Hz) of D,L-isoserine (HL) species at 25 °C and $I = 0.1$ mol dm^{-3}

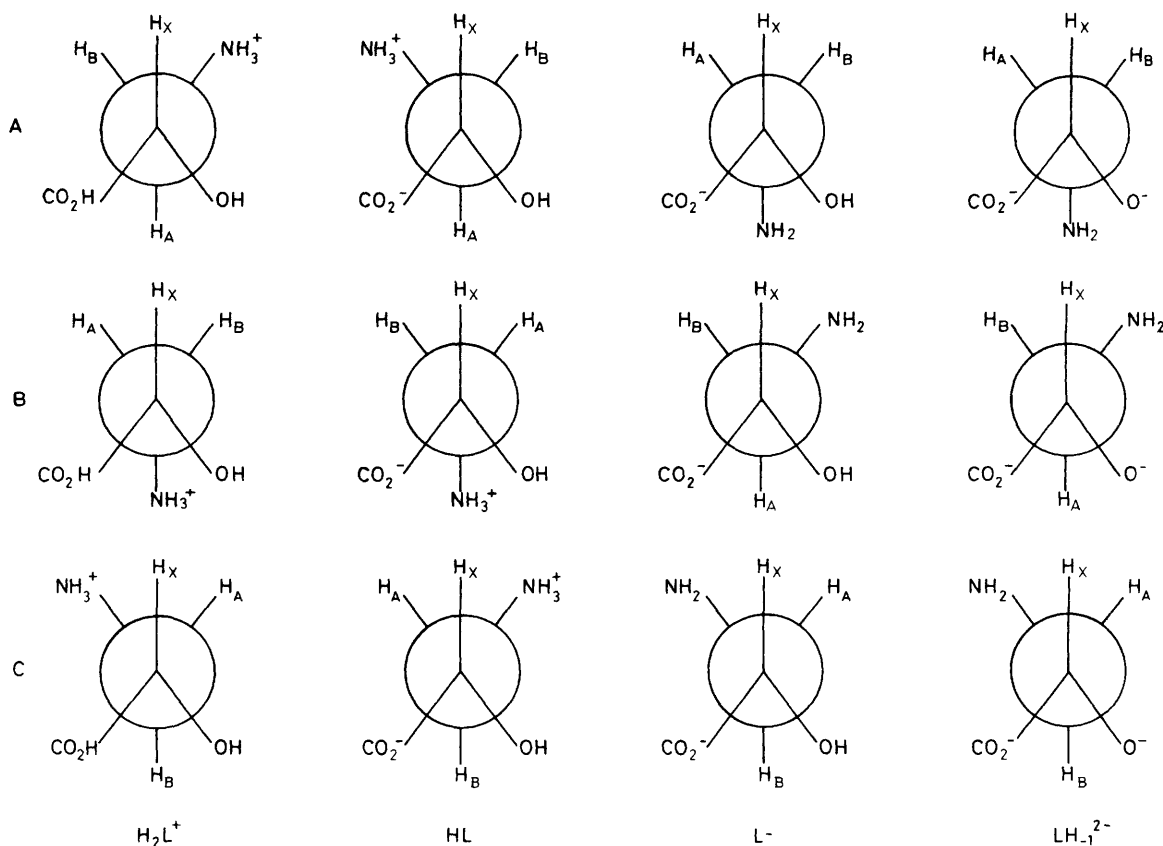| Species	δ_A	δ_B	δ_X	J_{AB}	J_{AX}	J_{BX}
$[Cd_2L_2H_{-2}]$	3.15	3.35	4.25	12.9	8.4	4.2
H_2L^+	3.27	3.46	4.56	13.6	8.6	3.9
HL	3.12	3.33	4.19	13.1	8.4	4.1
L^-	2.77	2.88	3.97	13.6	6.9	3.5
LH_{-1}^{2-}	2.61	2.72	3.80	13.1	6.8	3.2

Figure 2. The different rotamers for the isoserine species (populations: $p_A > p_B > p_c$)

Table 3 reports the formation constants for the mixed-metal complexes. In any of the investigated systems, the 1 1 2 − 2 species predominates. The experimental curve obtained for the cadmium(II)–cobalt(II)–isoserine system, together with two curves computed including or excluding the heterobinuclear species, are shown in Figure 1. Close agreement is observed when the mixed-metal complex is included.

The log K_{mix} values for the three ternary complexes, also reported in Table 3, provide a quantitative evaluation of the tendency to form a mixed-metal complex. The values obtained are far greater than those expected on a statistical basis only.

The homo- and hetero-binuclear complexes have also been investigated by spectroscopic techniques. The $\lambda_{max.}$ values obtained for the electronic spectra of the simple and mixed dimeric species of cobalt(II) ($[Co_2L_2H_{-2}]$, 553; $[CoZnL_2H_{-2}]$, 549; $[CoCdL_2H_{-2}]$, 556 nm), as well as $\varepsilon_{max.}$ values of <10 dm^3 mol^{-1} cm^{-1} for all three complexes, are consistent with an octahedral environment. Interestingly, the spectra are quite similar to one another, suggesting that the introduction of a heteroatom does not dramatically influence the cobalt(II) environment.

Table 4 shows the ^{1}H n.m.r. data concerning the stepwise protonation of isoserine as well as the dimeric cadmium(II) species. As expected, interaction of the isoserine with the proton or the metal ion results in a downfield shift. Generally, an ABX spectrum is observed. This is consistent with the diastereotopic nature of the two methylenic protons, due to the asymmetric α-carbon atom.[21]

Empirical methods have been developed in order to obtain conformational information from the coupling constants. They suffer a series of approximations; nevertheless, owing to the small uncertainties involved, it is assumed that they give populations of the single conformers with a reasonable confidence level. However, one has to consider that, owing to their empirical nature, they can be used only provided that the compounds belong to the same homogeneous class for which the proposed relationships have been developed. Surprisingly, some authors use relationships derived for α-amino acid zwitterions,[22,23] to study, for instance, dipeptides or even acidic or basic forms of amino acids, where the substituting groups no longer belong to the same class for which the relationships had been originally derived. In fact, the coupling constants depend
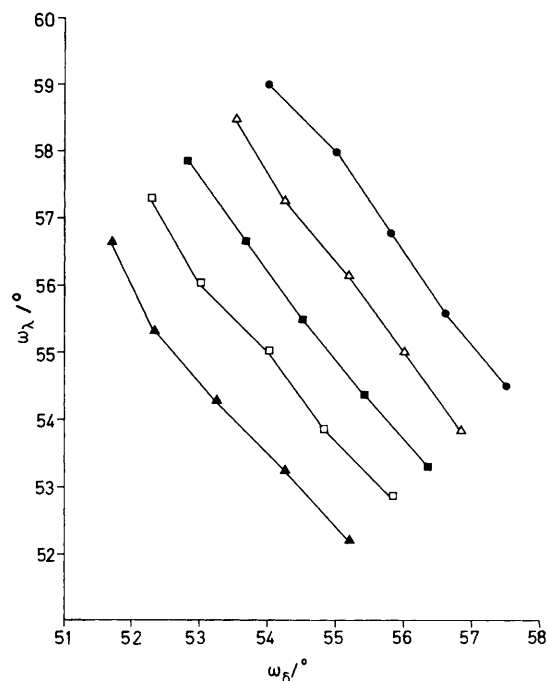
Figure 3. Values of ring puckering angles to obtain $\lambda : \delta$ 50:50 calculated for different values of Karplus constants ratio (α): $\alpha = 1.05$ (●), 1.10 ($\triangle$), 1.15 (■), 1.20 (□), or 1.25 (▲)

upon the dihedral angle between the protons as well as upon the electronegativity of the substituting groups, even if an explicit mathematical relationship correlating these parameters is not known. Moreover, as is the case for isoserine, the presence of charged groups is not easily accounted for.[24] For these reasons, no attempt has been made in the present investigation to estimate quantitatively the conformer populations.

It is possible, however, for each species of isoserine to predict qualitatively with reasonable confidence the order of population (*p*) of the possible staggered conformers (see Figure 2). The predicted order of stability is in qualitative agreement with the experimental data, as well as that reported for a similar compound, 3-amino-2-fluoropropionic acid.[24] The only exception is the zwitterion species, in which, in our opinion, the interaction between the charged groups is the preferred one. It must be pointed out that (*i*) for isoserine, the evidence obtained for 3-amino-2-fluoropropionic acid from the [19]F n.m.r. spectra is not available; (*ii*) the hydroxyl group cannot be assimilated to the fluorine atom because it is less capable of forming hydrogen bonds; and (*iii*) the hypothesis advanced[24] on the steric hindrance resulting from the hydration of the charged groups does not seem to take into account a decrease in hydration resulting from the interaction between the charged groups themselves.

With regard to diamagnetic dinuclear complexes, we were only able to analyse [$Cd_2L_2H_{-2}$]. In fact, the broad peaks* in the spectra of the dimeric zinc(II) species and the mixed zinc(II)–cadmium(II) complex preclude the possibility of obtaining chemical shifts and coupling constants of the isoserine protons.

In the discussion that follows an octahedral co-ordination of both cadmium(II) ions is assumed. Initially, one might envisage a situation in which both isoserine moieties are bound to each

metal ion through the amino nitrogen, the carboxylic oxygen, and the hydroxylic oxygen, which would act as a bridge. While such a complex should be highly stabilized through the formation of eight cadmium(II)–ligand bonds, the situation requires the formation of five 'fused together' chelate rings, one of which is four-membered. As a consequence, the resulting molecule would be very rigid and suffer different kinds of strain, due to the steric requirements of each ring. Therefore, such an hypothesis should be excluded.

If only one of the two hydroxylic oxygens bridges the two metal ions, there would be three chelate rings, two of which fused together. This should leave enough flexibility to the molecule. The Dreiding model shows that, of the two aminoalcoholic rings, one is in the λ-axial puckered form, while the other one is in the δ-equatorial form. This 'preferential' steric arrangement requires that the two co-ordinated isoserine moieties of the same complex molecule be of the same chirality. Other steric arrangements are in principle possible for the 'mixed' (L,D or D,L) complex, but they are unfavoured over the 'pure' (L,L or D,D) complex because the isoserine forms a total of six bonds.

We have carried out a treatment, according to Hawkins and Palmer,[25] of the experimental coupling constants of the [$Cd_2L_2H_{-2}$] species. The results have been summarized in graphical form in Figure 3, where for different values of the Karplus constants ratio (α), the ω_λ and ω_δ ring puckering angles satisfying a 50:50 $\lambda : \delta$ ratio are visualized. It can be seen that, in spite of the empirical nature of this treatment, values of ring puckering angles in the usual range for this kind of species (51—59°) have been obtained. Thus, this analysis is consistent with the n.m.r. findings, which indicate a 50:50 $\lambda : \delta$ ratio of the complexed isoserine moieties, at least as regards the 'pure' complexes.

With regard to the other two diamagnetic complexes, an analysis of the possible structures has been carried out, following the indications of Dreiding models and comparing the results thus obtained with those of the dimeric cadmium(II) species. This comparison helps understand the particularly high thermodynamic stability of [$CdZnL_2H_{-2}$] and [$Zn_2L_2H_{-2}$]. The reason for this is to be found in the preferential tendency of zinc(II) to give a tetrahedral co-ordination. The models show that the presence of a tetrahedral zinc(II) ion gives a more favourable molecular arrangement. This not only leads to an increased flexibility, but also, unlike the other dimeric complexes, implies that in addition to the 'pure' complex, the 'mixed' complexes may have a structure in which the two metal ions and isoserine form a total of seven bonds. An increase in stability is also suggested for the 'pure' complexes because the carboxylic group is equatorially substituted. This is known to be a more favourable disposition because the substituting group does not interact axially with the metal ion.

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* Although the potentiometric measurements leave no doubt as to the existence of an equilibrium reaction, this broadness indicates a slow, on the n.m.r. time-scale, exchange reaction, essentially concerning the two more abundant species in solution, *i.e.* the 'pure' and 'mixed' complexes.

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