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THERMODYNAMICS OF ION ASSOCIATION

XXV. TRANSITION METAL COMPLEXES OF PROLINE AND HYDROXYPROLINE

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ABSTRACT

Calorimetric and potentiometric measurements have been made at 25°C of the formation of the mono- and bis-hydroxyproline complexes of cobalt(II), nickel(II), copper(II) and zinc(II) and the proline complexes of copper(II) and nickel(II). The data have been used to calculate ΔG, ΔH and ΔS values for both protonation and metal complexation and these are discussed in terms of the nature of the metal–ligand binding.

INTRODUCTION

The transition metal amino acid and peptide complexes are convenient models for the metal-binding sites on proteins and enzymes and many studies of solution equilibria between metal ions and ligands have, in recent years, been biochemically motivated. Although investigations have been made of the association constants of such reactions, comparatively few precise values are available for the heat content changes which accompany these reactions. Such values are of fundamental importance, because they can be used to give a direct measure of the bonding between metal ions and ligands and ultimately, to relate the strength of the chemical bonding to the nature of the reacting species. In addition, a knowledge of the heat content changes for reactions, together with corresponding equilibrium constants, permits the calculation of the entropy changes for these reactions. ΔS data provide additional insight into the interactions between the species and the solvent and the structures of the complex species formed in aqueous solutions.

Most ΔH values which have been reported for complex formation have been determined from the variation of the equilibrium constant with temperature; however, because of the limited temperature range available, and because of the frequently observed small variation of log K with temperature, the values obtained are subject to considerable uncertainty. Thus the direct calorimetric method has become the most reliable technique for determining heats associated with the formation of coordination complexes in solution.

In the present work, potentiometric and direct incremental titration calorimetric methods have been used for the determination of the free energy, enthalpy, and entropy changes associated with the interaction of transition metal ions with proline and hydroxyproline in aqueous solution. Trends in the thermodynamic functions reported for the formation of hydroxyproline complexes of Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} as well as Cu^{2+} and Ni^{2+} proline complexes are discussed.

EXPERIMENTAL

Materials

Grade A glassware and reagent grade chemicals were used, and solutions were prepared with doubly distilled water; carbon dioxide was excluded by bubbling with nitrogen. Hydroxyproline and proline, obtained from Nutritional Biochemical Corporation, were used without further purification. Copper(II), nickel(II), cobalt(II) and zinc(II) nitrate solutions were prepared from reagent grade chemicals and analyzed by EDTA complexometric titration, and also by passing through Dowex 50-W ion-exchange resin in the hydrogen form and titrating the liberated nitric acid with sodium hydroxide.

Potentiometric experiments

E.m.f. measurements were made at 25±0.02°C with cells of the type glass electrode/solution under study/satd. $KCl/Hg_2Cl_2, Hg$ using Beckman Type 39099-E-3 and 41263 glass electrodes, a Leeds and Northrop Type K3 potentiometer, and a Victoreen picometer (Model 474) as null detector; the e.m.f. reproducibility was ±0.1 mV. Each cell incorporated a pair of glass electrodes so that any irregularity in the behavior of one of them was immediately apparent. The electrode systems were standardized before and after each experiment with NBS standard buffer solutions prepared according to Bates[1]: 0.09 *M* potassium chloride; +0.01 *M* hydrochloric acid, pH 2.07; 0.05 *M* potassium hydrogen phthalate, pH 4.008; 0.025 potassium dihydrogen phosphate; +0.025 *M* disodium hydrogen phosphate, pH 6.865; and 0.01 *M* borax, pH 9.180. pH measurements were made in a nitrogen atmosphere in solutions containing the amino acid with or without added metal nitrate in 1:1 and 1:2 metal to ligand ratios; the solutions were titrated with carbon dioxide free sodium hydroxide solution. The metal nitrate concentrations were $\sim 5 \times 10^{-3}$ *M* and the concentration of amino acid ranged from $5\text{–}10 \times 10^{-3}$ *M*. Sufficient potassium nitrate or sodium perchlorate was added to maintain the ionic strength at 0.1±0.001 *M* during the entire titration. pH ($= -\log a_{H^+}$) values were converted to hydrogen ion concentrations by using a f_{H^+} activity coefficient value of 0.7815 calculated from the Davies equation[7].

Calorimetric measurements

The calorimeter assembly used for the heat measurements was similar to that described earlier[3]. In a typical experiment, the amino acid (proline or hydroxyproline) was weighed into the calorimeter together with an appropriate amount of metal nitrate solution. The ionic strength was adjusted to 0.1 *M* with potassium nitrate or sodium perchlorate. Sodium hydroxide solution was then added to achieve approximately 80% metal–ligand complexation. The heat of reaction accompanying the incremental addition of standard nitric acid was then measured. In order to correct for the concomittant protonation reactions the heats of protonation of the amino acids were determined independently by titrating the deprotonated ligand with nitric acid.

RESULTS AND DISCUSSION

Protonation reactions

The proton association reactions for proline and hydroxyproline (Fig. 1) are shown in Eqns. (1) and (2)

$$L^{-} + H^{+} \rightleftharpoons HL^{\pm} \qquad K_B \qquad (1)$$

$$HL^{\pm} + H^{+} \rightleftharpoons H_2L^{+} \qquad K_A \qquad (2)$$

Although the acid dissociation constants had been determined previously, it was necessary to determine the thermodynamic functions for the protonation of both proline and hydroxyproline under the present set of experimental conditions. The results summarized in Table I refer to the protonation of the amino group only (K_B; eqn. 1), however, since it is only necessary to consider reaction (1) under the conditions of the experiments reported in this paper.

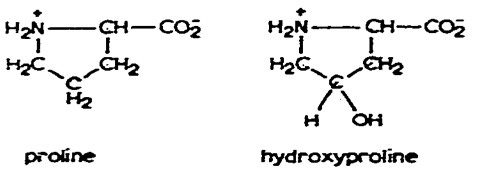

Fig. 1. Structural formula for proline and hydroxyproline.

The concentrations of the ionic species reported in Table I were calculated in the usual manner from the mass balance and electroneutrality expressions[4]. The mean of at least 25 determinations for each value of K_B were used to obtain the results of Table I.

TABLE I

THERMODYNAMICS OF PROTONATION OF PROLINE AND HYDROXYPROLINE WITH THE AMINE GROUP

Ligand	*log K_B*	*$-\Delta H$ kcal mole^{-1}*	*ΔS cal deg^{-1} mole^{-1}*
proline	10.41	11.35	9.4
hydroxyproline	9.46	10.34	8.5

The observed heat changes Q_{expt}, during the protonation titrations, corrected for heats of dilution, may be expressed in terms of the heats of formation, in solution, of the species represented by the subscripts in Eqn. (3).

$$Q_{expt} = Q_{H_2O} + Q_{HL} \tag{3}$$

The changes in the concentration of the ionic species from point to point in the enthalpy titrations were used to calculate the enthalpy changes from Eqns. (4–6).

$$Q_{H_2O} = \Delta H_{H_2O}(-\Delta[OH^-]) \tag{4}$$

$\Delta[OH^-]$ represents the change, in moles of hydroxide ion in solution, while ΔH_{H_2O} is the heat of formation of water (-13.49 kcal mole^{-1} at an ionic strength of 0.1 M, calculated from $\Delta H^{\circ}_{H_2O} = -13.34$ kcal mole^{-1} at $I \rightarrow 0$)[4].

$$Q_{HL} = \Delta H_{HL}(\Delta[HL]) \tag{5}$$

and

$$\Delta H_{HL} = \frac{Q_{expt} - Q_{H_2O}}{\Delta[HL]} \tag{6}$$

The enthalpy values listed in Table I represent the mean of at least 10 determinations of ΔH_{HL}. The heat change for reaction (2), ΔH_{H_2L}, was too small to be detected under the conditions of our experiments and could, therefore, be neglected.

The data in Table I indicates that the major contribution to ΔG for the protonation for both proline and hydroxyproline is made by the enthalpy term. The relatively large exothermicity reflects the high degree of covalency of the bond formation between nitrogen and proton. As would be expected, the ΔH_{HL} value is more negative for the more basic ligand, proline.

The values of the entropy of protonation are similar in magnitude to those observed for other amino acids[5], and may be understood in terms of the formation of zwitterion species. If the distance between the charged centers of the zwitterion is fairly small, then an effective intramolecular charge neutralization may occur with attendant release of electrostricted solvent and resultant positive entropy change.

It is somewhat surprising that proline is more basic than hydroxyproline since the inductive effect of a hydroxy group present on the pyrolidine ring would be expected to increase the basicity of the hydroxyproline secondary nitrogen. However, the same phenomenon does occur with the straight chain aliphatic amines with ethyl amine (pK 10.63, ΔH 13.63)[6] being more basic than enthanol amine (pK 9.45, ΔH 12.07)[7]; in each case the hydroxyl group is β to the amino nitrogen.

Metal-ligand complexation

In the presence of metal ions with a 1:1 and 1:2 metal to ligand ratio equilibria (7) and (8)

$$M^{2+} + L^- \rightleftarrows ML^+ \qquad K_1 \tag{7}$$

$$ML^+ + L^- \rightleftarrows ML_2 \qquad K_2 \tag{8}$$

must be taken into account. The corresponding equilibrium constants

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

and

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

were first determined under the conditions of the calorimetric experiments by titrating mixtures of metal nitrate and ligand with base.

The stability constants K_1 and K_2 were computed from the following equation[8].

$$\frac{\bar{n}}{(1-\bar{n})[L^-]} = -\frac{(\bar{n}-2)[L^-]}{(1-\bar{n})}K_1K_2+K_1 \tag{9}$$

The average number of protons bound per ligand ($\bar{n}_H$, Eqn. 10) is first determined

$$\bar{n}_H = \frac{K_B[H^+]+2K_AK_B[H^+]^2}{1+K_B[H^+]+K_AK_B[H^+]^2} \tag{10}$$

and then $\bar{n}$ and $[L^-]$ are calculated from the experimental data by Eqns. (11) and (12), respectively.

$$\bar{n} = \frac{\bar{n}_H T_L-(T_L-T_B-[H^+]+[OH^-])}{T_M\bar{n}_H} \tag{11}$$

$$[L^-] = \frac{T_L-\bar{n}T_M}{1+K_B[H^+]+K_AK_B[H^+]^2} \tag{12}$$

In these equations, T_M is the total metal ion and T_L the total ligand concentration, T_B the total concentration of base added, and $\bar{n}$ the formation function representing the average number of ligands bound per metal ion.

In the region $\bar{n} = 1$, these functions become indeterminate and data for which $1.1 > \bar{n} > 0.95$ were therefore neglected. $\bar{n}$ and $[L^-]$ values were determined at 20 or more points in the titration. A typical plot of $\frac{\bar{n}}{(1-\bar{n})[L^-]}$ versus $\frac{(\bar{n}-2)[L^-]}{(1-\bar{n})}$ is shown in Fig. 2. The lines were fitted to the data by a least squares procedure. The derived values for the association constants for all the metal–ligand systems are given in Table II.

The concentrations of ionic species in solution at each stage of the enthalpy titrations were calculated from Eqns. (12–15).

$$[M^{2+}] = \frac{2T_M-T_L+K_AK_B[H^+]^2[L^-]+K_B[H^+][L^-]+[L^-]}{2+K_1[L^-]} \tag{13}$$

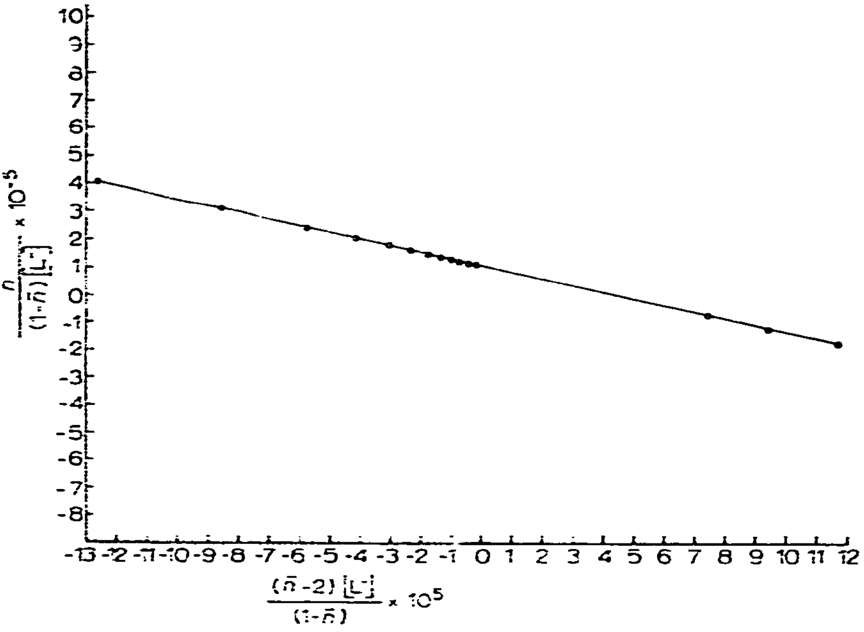

Fig. 2. The Rossotti and Rossotti plot for the zinc and hydroxyproline system.

$$[ML^+] = K_1[M^{2+}][L^-] \tag{14}$$

$$[ML_2] = K_L[ML^+][L^-] \tag{15}$$

The measured heat change Q_{expt} can be written

$$Q_{expt} = Q_{H_2O} + Q_{HL} + Q_{ML} + Q_{ML_2} \tag{16}$$

with Q_{H_2O} and Q_{HL} defined above.
Since

$$Q_{ML} = \Delta H_{ML}(-\Delta[M^{2+}]) \tag{17}$$

$$Q_{ML_2} = \Delta H_{ML_2}(-\Delta[M^{2+}]-\Delta[ML^+]) \tag{18}$$

equation (16) becomes

$$\frac{Q_{ML}+Q_{ML_2}}{\Delta[M^{2+}]} = \Delta H_{ML} + \Delta_{,}I_{ML_2} + \frac{\Delta[ML]}{\Delta[M^{2+}]}\Delta H_{ML_2} \tag{20}$$

Values of ΔH_{ML} and ΔH_{ML_2} were obtained by least squares from plots of $(Q_{ML}+Q_{ML_2})(\Delta[M^{2+}])^{-1}$ against $(\Delta[ML])(\Delta[M^{2+}])^{-1}$. A typical linear plot is shown in Fig. 3 with the thermodynamic functions for all the systems summarized in Table II.

Hydroxyproline complexes

Hydroxyproline, a mixed oxygen nitrogen chelator forms quite strong complexes with all four bivalent metal ions. The metal ions studied are borderline acceptors in the "soft" and "hard" acids and bases classification[9]. The appreciable contri-

TABLE II

THERMODYNAMICS OF COMPLEXATION OF PROLINE AND HYDROXYPROLINE

Reaction		pK	$-\Delta G$ *kcal mole*$^{-1}$	$-\Delta H$ *kcal mole*$^{-1}$	ΔS *cal deg*$^{-1}$ *mole*$^{-1}$	ΔH_e *kcal mole*$^{-1}$	$-\Delta H_c$ *kcal mole*$^{-1}$
Co+HOP[a]	⇄ Co(HOP)	4.83±0.01	6.59±0.01	3.3 ±0.2	11.0±0.5	1.5	4.8
Co(HOP)+HOP	⇄ $Co(HOP)_2$	4.05±0.01	5.52±0.01	3.36±0.08	7.2±0.3	1.2	4.6
Ni+HOP	⇄ Ni(HOP)	5.94±0.01	8.11±0.01	5.0 ±0.2	10.4±0.5	1.4	6.4
Ni(HOP)+HOP	⇄ $Ni(HOP)_2$	5.01±0.01	6.84±0.01	5.48±0.1	4.6±0.3	1.0	6.5
Cu+HOP	⇄ Cu(HOP)	8.38±0.01	11.43±0.01	6.9 ±0.2	15.2±0.5	1.8	8.7
Cu(HOP)+HOP	⇄ $Cu(HOP)_2$	7.04±0.01	9.60±0.01	7.8 ±0.1	6.0±0.5	1.1	8.9
Zn+HOP	⇄ Zn(HOP)	5.03±0.01	6.87±0.01	2.6 ±0.2	14.4±0.5	1.8	4.4
Zn(HOP)+HOP	⇄ $Zn(HOP)_2$	4.35±0.01	5.94±0.01	2.9 ±0.1	10.3±0.3	1.4	4.3
Ni+P[b]	⇄ NiP	5.95±0.01	8.09±0.01	5.7 ±0.2	8.1±0.3	1.3	7.0
NiP+P	⇄ NiP_2	4.95±0.01	6.73±0.01	6.1 ±0.2	2.0±0.5	0.8	6.9
Cu+P	⇄ CuP	8.84±0.01	11.95±0.01	7.4 ±0.2	15.3±0.5	1.8	9.2
CuP+P	⇄ CuP_2	7.44±0.01	10.22±0.01	7.7 ±0.2	8.4±0.3	1.3	9.0

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[a] Hydroxyproline. [b] Proline.

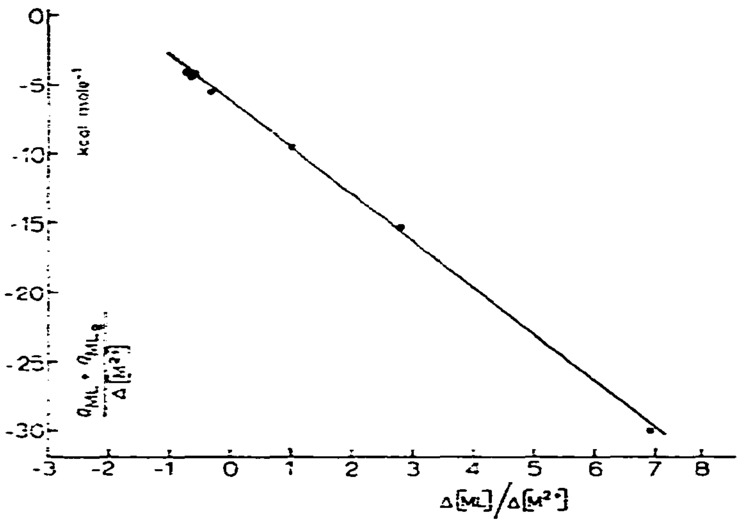

Fig. 3. Molar heat of complex formation of cobalt and hydroxyproline.

butions from both enthalpy ΔH and entropy ΔS terms to the driving force ΔG shown in Table II would, therefore, be expected. The enthalpy stabilization term can generally be attributed to the covalent bond formation between the metal ion and the nitrogen of the amino group. The positive entropy changes are due mainly to electrostatic interaction between the carboxylate group of the amino acid and the metal ion.

More detailed analyses of the enthalpy terms as described earlier by Degischer and Nancollas[10] reveal that the temperature-independent contribution, ΔH_c, is almost entirely responsible for the observed heat changes. This term represents the short-range or quantum-mechanical forces which are insensitive to the environment and can be considered to result primarily from the covalent nitrogen coordination. The temperature-dependent term, ΔH_e, although much smaller in magnitude for each of the hydroxyproline–metal interactions, nevertheless cannot be neglected. These values represent the net changes in the long-range electrostatic forces which are sensitive to the environment, in particular to the dielectric constant of the medium. ΔH_e would be expected to become more endothermic with increasing loss of bound solvent molecules (as reflected by increases in ΔS and this is seen to be the case in Table II). The release of solvent molecules from the coordination spheres of the metal ions is facilitated by charge neutralization which occurs in these systems via electrostatic interaction of the carboxyl group with the transition metal ions.

In addition to the covalent and electrostatic terms discussed above, it is necessary to consider the ligand field stabilization energy which is important in the interaction of ligands with metal ions having unfilled d orbitals. The resulting Irving–Williams[11] order would predict the following order of stability for the hydroxyproline complexes.

$Co^{2+} < Ni^{2+} < Cu^{2+} \gg Zn^{2+}$

The results of Table II clearly show that this is the case with both ΔG and ΔH following the predicted order.

Table II also shows that the heat changes for the second step, ΔH_2, are more exothermic for all the systems studied than for those of the first step, ΔH_1. The tendency of the successive heat changes to increase has been noted for many transition metal complexes and is generally ascribed to differences in the hydrational energies of the M^{2+} and ML^+ species[12].

The large positive entropy values observed for the metal–ligand interactions arise from both the net release of solvent molecules to the system accompanying the coordination of bidentate ligand molecules to the hydrated metal ion and, in addition, an increase in the randomness of the system upon complexation by the anionic ligand to the positive charged metal ion species. Charge neutralization occurs during the latter process which decreases the attraction of the polar water molecules for the central metal ion. The order $\Delta S_2 < \Delta S_1$ results from a greater disruption of the solvation sphere by the addition of the first ligand molecule to the very strongly hydrated "bare" metal ion than by the addition of the second molecule. The value of ΔS_2 for the nickel complexes appears to be anomalously low, however, suggesting a more ordered bis species. It is possible that a greater proportion of the dipolar *cis* isomer is formed, thus having a stronger orientation effect on the solvent molecules than for the other bis-hydroxyproline complexes.

The lower exothermicity exhibited by the zinc complexes can be adequately explained by the absence of ligand field stabilization energy of the d^{10} zinc ion. The relatively large positive entropy terms, however, may be due to structural effects and could represent the transition form an octahedral aqueous ion, $Zn(H_2O)_6^{2+}$ to a tetrahedral bis-hydroxyproline complex, $Zn(HyPro)_2$. This would liberate additional water molecules from the inner coordination sphere of the zinc ion as compared with the other metal–ligand complexes. These trends have been observed for other zinc complexes[13].

Proline complexes

It is seen in Table II that for the transition metal–proline complexes, ΔH_c is again the most important contributor to the total enthalpy of reaction. The values for successive additions of ligand are remarkably constant indicating that the energy involved in forming the covalent bonds is the same for the mono and bis complexes. The fact that ΔH_2 is greater than ΔH_1 is again primarily caused by the less endothermic contribution of the temperature-dependent term, ΔH_e, indicating that less bound solvent is released in the formation of the 2:1 ligand–metal complexes than for the 1:1 species. The corresponding smaller positive entropy change observed for the second step of the ligation reaction (*i.e.*, Eqn. 8) supports this conclusion.

In agreement with ligand field predictions, copper(II) forms more stable complexes with proline than does nickel(II) and this is reflected by the ΔH and ΔG values in Table II. The low ΔS_2 value obtained for nickel(II) may again be interpreted by assuming that a *cis* isomer is formed preferentially.

Copper proline complexes are somewhat more stable than those formed between hydroxyl proline and copper. The two sets of nickel species, however, have very similar ΔG values which is unexpected since the base strengths of the two ligands differ by 0.95 log K units and the heats of protonation by 1.0 kcal/mole (Table I). However, the heats of metal complexation follow the expected order with proline yielding more exothermic ΔH_1 values than hydroxyproline. This indicates that the observed similarity of the free energy terms is primarily due to compensating entropy changes. ΔS values for copper(II) interaction with proline and hydroxyproline are similar and the increased exothermicity of the proline reaction is reflected in the overall free energies of association. This trend of increasing enthalpy with increasing base strength of the ligands has also been shown in the thermodynamic properties for another series of structurally related amino acids[5].

REFERENCES

1 R. G. Bates, *Determination of pH*, Wiley, New York, 1963.
2 C. W. Davies, *Ion Association*, Butterworth, London, 1962.
3 A. B. Brunetti, M. C. Lim and G. H. Nancollas, *J. Amer. Chem. Soc.*, 90 (1968) 5120.
4 G. H. Nancollas, *Interactions in Electrolyte Solutions*, Elsevier, Amsterdam, 1966.
5 J. L. Meyer and J. E. Bauman Jr., *J. Chem. Eng. Data*, 15 (1970) 404.
6 A. G. Evans and S. D. Hamann, *Trans. Faraday Soc.*, 47 (1951) 34.
7 D. L. Levi, W. S. McEwan and J. H. Wolfenden, *J. Chem. Soc.*, (1949) 760.
8 F. J. C. Rossotti and H. Rossotti, *Determination of Stability Constants*, McGraw-Hill, New York, 1960.
9 R. G. Pearson, *J. Amer. Chem. Soc.*, 85 (1963) 3533.
10 G. Degischer and G. H. Nancollas, *J. Chem. Soc. (A)*, (1970) 1125.
11 H. Irving and R. J. P. Williams, *J. Chem. Soc.* (1953) 3192.
12 K. P. Anderson, D. A. Newell and R. M. Izatt, *Inorg. Chem.*, 5 (1966) 62.
13 M. Ciampolini, P. Paoletti and L. Sacconi, *J. Chem. Soc.*, (1961) 2994.