Thermodynamic Considerations in Co-ordination. Part XII.[1] Potentiometric and Calorimetric Investigations of the Complexing between the Phenylalanate Anion and Iron(II), Iron(III), Cobalt(II), Nickel(II), and Copper(II)

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Formation constants for AB and A_2B (A = phenylalanate, B = Fe^{II} and Fe^{III}, Co^{II}, Ni^{II}, and Cu^{II}) and for $Fe^{II}A_3$, $Fe^{III}{}_2A_2(OH)_2$, and $Cu^{II}{}_2A_2(OH)_2$ are reported for I = 3·00M-$(Na^+)ClO_4^-$ at 25 °C and corresponding enthalpies and entropies of formation were determined calorimetrically for the Co, Ni, and Cu complexes listed above.

SINCE Schulze discovered phenylalanine in 1879, biochemical researches have generated waves of interest in the metal complexing properties of this amino-acid. However, when co-ordination chemists have attempted to quantify the solution chemistry of its complexes, they have usually been defeated by solubility problems (*viz.*, the relative sparseness of entries in ' Stability Constants ').[2]

Nevertheless, the recent interest in phenylalanine as an amino-acid involved in cancer promotion and therapy [3,4] and its structural relationship to ligands than we have previously studied (see histidine and tryptophan

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[1] Part XI, R. D. Graham, D. R. Williams, and P. A. Yeo, *J.C.S. Perkin II*, 1972, in the press.

[2] ' Stability Constants of Metal-ion Complexes,' eds. L. G. Sillén and A. E. Martell, *Chem. Soc. Special Publs.*, Nos. 17 (1964) and 25 (1971).

[3] D. R. Williams, ' Metals, Ligands and Cancer,' *Chem. Rev.*, 1972, **49**, 203.

[4] D. R. Williams, ' Anticancer drug design involving complexes of amino-acids and metal ions,' *Inorg. Chim. Acta Rev.*, 1972, **6**, in the press.

complexes in Parts VII—X[5-7]) has prompted us to examine anew the complexing properties of phenylalanine.

The ligand investigated was the L-form of the phenylalanate anion; the metal ions were those known to complex most strongly to amino-acids *in vivo*, namely, iron(II and III), cobalt(II), and copper(II) and also nickel(II) because, although the element is not necessary for human life, Fe, Co, and Ni constitute Group VIII of the first transition series. Complexes of metals from this group are currently undergoing clinical trials as anticancer therapeuticals.[3,4]

The approach was one of potentiometry, calorimetry [both in 3M-(Na)ClO_4 at 25 °C], and the analysis of solid complexes. Our aims were to measure the distribution of complexes present in metal ion–phenylalanine solutions, to determine the strengths of the bonds involved in these complexes, and to suggest structures for the complexes formed.

EXPERIMENTAL

Ligand.—Commercial L-phenylalanine [B.D.H. Biochemical grade; m.p. 284 °C (lit., 283—284 °C)] was dried and used without further purification (Found: C, 65·2; H, 6·9; N, 8·5. Calc. for $C_9H_{11}NO_2$: C, 65·4; H, 6·7; N, 8·5%).

Other Reagents.—Perchloric acid, sodium perchlorate, and sodium hydroxide were prepared as in ref. 6. Metal perchlorates were prepared from their hydrates (G. F. Smith Chemical Co.) with the exception of ferrous perchlorate which was prepared by dissolving iron sponge (Johnson Matthey Chemicals 'Specpure') in standard perchloric acid. Solutions were analysed for cation content by two independent methods and for acid content by Gran titrations.[8] All water used was deionised (Elgastat), boiled, and cooled by the passage of oxygen-free nitrogen. The resistivity was higher than 2 MΩ cm.

Formation Constant and Enthalpy Measurements.—Formation constants (β) were measured by glass-electrode potentiometry and the enthalpies of formation were measured by solution calorimetry. The approach was as described in refs. 5 and 6.

Data Analysis.—Initial formation constants were obtained graphically by use of the Fronaeus integral method [9] and final constants and standard deviations were computed by use of the SCOGS programme.[10] Calorimetric data were converted into enthalpies (ΔH°) and entropies (ΔS°) by use of the computer programmes described in ref. 6. These calculations require values for ΔH° and ΔS° of protonating phenylalanate and these were as previously communicated.[1] The data have been deposited in Supplementary Publication No. SUP 20464, 8 pp., 1 microfiche).*

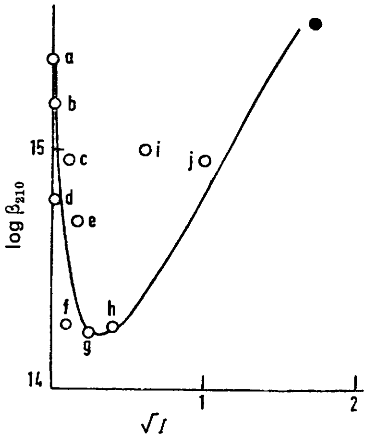
RESULTS AND DISCUSSION

The experimental data, the 3M-ClO_4^- metal hydrolysis constants as reviewed by Arnek,[11] and the known p*K*s and pK_w (9·610, 2·754, and 14·22 respectively) were used to compute the log (formation constants) for the metal complexes shown in Table 1. Formation constants have previously been reported for a range of ionic strengths (I):[12-22] our 3M-(Na)ClO_4 constants are higher than those obtained for lower I values but can be claimed to be in good agreement with most of them since plots of log β against $\sqrt{I}$ fit on a smooth Debye–Hückel-type curve (an example is given in the Figure).

TABLE 1

log β for the species $A_pB_qH_r$ for A = phenylalanate, B = metal ion, and H = H^+ (25 °C, I = 3·00M-ClO_4^-); [ ] are 3 × standard deviations in the computed log constants; n = number of experimental observations

B \ (p,q,r)	(1,1,0)	(2,1,0)	(3,1,0)	(2,2,−2)	n
Copper(II)	8·247[0·022]	15·549[0·022]		4·6[0·4]	108
Nickel(II)	5·353[0·026]	10·487[0·050]			84
Cobalt(II)	4·449[0·014]	8·439[0·037]			78
Iron(II)	3·736[0·009]	7·192[0·025]	10·7[0·2]		29
Iron(III)	10·39[0·04]	19·11[0·10]	26·0[0·7]	16·9[0·30]	44



log β_{210} for the copper(II) phenylalanate system plotted against $\sqrt{I}$. Letters = the following references, a = 12, b = 13, c = 14, d = e = 15, f = 16, g = 17, h = 18, i = 19, j = 20. The full symbol = present work

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[*] For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

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The constants β_1 and β_2 obey the Irving–Williams series and so it may be assumed that the bivalent metal ions are octahedrally surrounded by phenylalanato and by solvent donor atoms [with the exception of the bonds to copper(II) which are Jahn–Teller distorted] and that the amino-acid binding is glycine-like. Tris-complexes have been reported for other amino-acids,[5] but, for phenylalanine, the insolubility of the cobalt and nickel systems precluded our measurement of β values for such complexes.

Thermodynamic parameters for complex formation are shown in Table 2. Our $\Delta H°$ values are in general agreement with literature values, when allowance is made for ionic-strength variations.[13,18,22] There are, however, two exceptions: first our $\Delta H_2°$ (copper) is higher than the figures given in refs. 13 and 22 (58 *vs.* 49 kJ mol^{-1}) and secondly, although for nickel our $\Delta H_2°$ is close to that of Anderson *et al.*[22] ($I = 0$), the respective $\Delta H_1°$ and $\Delta H_{1,2}°$ values differ notably. However, they did report some uncertainty in splitting their $\Delta H_2°$ into its stepwise components.

TABLE 2

Gibbs free-energy changes, enthalpies, and entropies for the formation of metal(II) phenylalanate complexes at 25·0 °C, $I = 3{\cdot}00\text{M}\text{-}(\text{Na}^+)\text{ClO}_4^-$

	Copper(II)	Nickel(II)	Cobalt(II)
$-\Delta G_1°$/kJ mol^{-1}	47·09[0·12]	30·57[0·15]	25·40[0·08]
$-\Delta G_{1,2}°$/kJ mol^{-1}	41·69[0·12]	29·26[0·28]	22·78[0·20]
$-\Delta G_2°$/kJ mol^{-1}	88·78[0·12]	59·83[0·28]	48·18[0·20]
$-\Delta H_1°$/kJ mol^{-1}	19·18[1·80]	9·82[0·60]	5·30[0·80]
$-\Delta H_{1,2}°$/kJ mol^{-1}	39·17[4·00]	14·72[1·20]	8·06[1·61]
$-\Delta H_2°$/kJ mol^{-1}	58·35[4·00]	24·54[1·20]	13·36[1·61]
$\Delta S_1°$/J mol^{-1} K^{-1}	93·7[6·3]	69·6[2·5]	67·4[2·9]
$\Delta S_{1,2}°$/J mol^{-1} K^{-1}	8·4[13·8]	48·8[4·9]	49·4[6·0]
$\Delta S_2°$/J mol^{-1} K^{-1}	102·1[13·8]	118·4[4·9]	116·8[6·0]
n	55	30	28

For all systems studied, (i) $\Delta G°$ is a combination of enthalpy and entropy contributions in approximately equal quantities; (ii) $-\Delta H_1° < -\Delta H_{1,2}°$, an effect previously reported by Izatt *et al.*[23] and attributed to the large difference in hydrational energies between the aquated metal ion and the aquated 1 : 1 complex; (iii) $\Delta S_1° > \Delta S_{1,2}°$. Both $\Delta S_1°$ and $\Delta S_2°$ follow the pattern Cu > Ni > Co presumably because different numbers of water molecules are liberated during complex formation. Copper, having the smaller ionic radius, may be expected to have the larger spheres of hydration and, in addition, some $\Delta S°$ influence may arise from the Jahn–Teller effect.

As the free ligand concentration was increased, the nickel and cobalt(II) solutions began to precipitate at $\bar{Z} = 0{\cdot}8$. These solids analysed as dihydrated bis-complexes [Ni or $\text{Co}^{\text{II}}(\text{C}_9\text{H}_{10}\text{NO}_2)_2,2\text{H}_2\text{O}$] and, presumably, have such a high lattice energy that their precipitation excludes the possibility of forming soluble tris-complexes. Gergely *et al.* have also reported obtaining these solids.[13] By analogy with other amino-acids, the bis-complexes probably have *trans*-amino-acid anions slightly distorted out of an octahedral plane and two axial waters. Heating the solids *in vacuo* removed the $2\text{H}_2\text{O}$ and the ligand becomes terdentate in which the carboxylate group is both bidentate and bridging.[24]

In solution, protonated, hydrolysed, and polynuclear species have been reported for complexes of copper(II) and aliphatic amino-acids or related ligands.[7,25] Neither protonated nor polynuclear species could be detected during the present study but dimeric hydrolysis products were found for Cu^{II} and Fe^{III}. These complexes had compositions corresponding to $\text{Cu}^{\text{II}}{}_2(\text{phe})_2(\text{OH})_2$ and $\text{Fe}^{\text{III}}{}_2(\text{phe})_2(\text{OH})_2{}^{2-}$ respectively and can reasonably be expected to have structures related to their dihydroxy-bridged parents $\text{Cu}_2(\text{OH})_2{}^{2+}$ and $\text{Fe}_2(\text{OH})_2{}^{4+}$.[26]

Hydrolysed iron species are important intermediates for the *in vivo* storage of iron in the liver [27] and hydroxy-complexes of copper have a variety of biological roles.[28,29] Thus, it was interesting to find from our formation constants that for blood pH (7·4), 2% of phenylalanato-bound copper is in this dihydroxy-form.

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