Co-ordination Properties of *N*-Protected Amino-acids. Solution and Solid-state Behaviour of Bis(benzyloxycarbonyl-L-tryptophanato)-nickel(II) and its Mixed Complexes

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Polarographic measurements show that nickel(II) ion and benzyloxycarbonyl-L-tryptophan (Z-L-tryptophan) form complexes in methanol with 1 : 2 and 1 : 4 metal-to-ligand ratios, depending on the ligand concentration. A complete scheme of the reactions which take place in solution and at the electrode is proposed. The 1 : 2 complex $[NiL_2]\cdot 2H_2O$ and some amine adducts have been obtained in the solid state. In the solids the amino-acid is co-ordinated only through the carboxylate group.

BEING particularly interested in the donor properties of *N*-protected amino-acids, where the protecting group is an acyl or a benzoyl group,[1-3] in this paper we extend our previous investigation by studying the co-ordination ability of a benzyloxycarbonyl-protected amino-acid, benzyloxycarbonyl-L-tryptophan (Z-trp, its anion being Z-trpO), towards the nickel(II) ion in solution and in the solid state. The effects of additional bases in axial positions in the amino-acid co-ordination sphere are also investigated.

Further interest derives from the fact that the nickel(II) ion has been found to favour peptide group co-ordination[4] and, as has recently been suggested, from the fact that the nickel(II) ion may also have a biological role in animals.[5]

EXPERIMENTAL

All the reagents were reagent grade and used as received.

Preparation of the $[Ni(Z\text{-}trpO)_2]\cdot 2H_2O$ *Complex.*—The compound was obtained by adding a methanolic nickel(II) perchlorate hexahydrate solution (2×10^{-2} mol) to a methanolic solution of ligand (4×10^{-2} mol) neutralised with a stoicheiometric amount of K[OH]. The precipitated potassium perchlorate was filtered off and the green solution was slowly concentrated until a small volume was obtained. By adding water and stirring vigorously a green powder separated, which was dried over P_2O_5 in vacuum, m.p. 168—172 °C (decomp.), yield *ca.* 85% (Found: C, 59.3; H, 5.00; N, 7.45. Calc. for $C_{38}H_{38}N_4NiO_{10}$: C, 59.3; H, 5.00; N, 7.30%).

Preparation of the Adducts.—All the adducts were prepared in *ca.* 65—80% yield by adding Et_2O to an ethanolic solution of $[Ni(Z\text{-}trpO)_2]\cdot 2H_2O$ and the corresponding amine in a 1 : 2 molar ratio. $[Ni(Z\text{-}trpO)_2(py)_2]\cdot 2H_2O$ (py = pyridine): green, m.p. 132—136 °C (decomp.) (Found: C, 62.2; H, 4.95; N, 9.10. Calc. for $C_{48}H_{48}N_6NiO_{10}$: C, 62.15; H, 5.20; N, 9.05%). $[Ni(Z\text{-}trpO)_2(imH)_2]\cdot 2H_2O$ (imH = imidazole): green, m.p. 128—132 °C (decomp.) (Found: C, 58.1; H, 5.15; N, 12.9. Calc. for $C_{44}H_{46}N_8NiO_{10}$: C, 58.35; H, 5.10; N, 12.4%). $[Ni(Z\text{-}trpO)_2(mim)_2]\cdot 2H_2O$ (mim = 2-methylimidazole): green, m.p. 133—137 °C (decomp.) (Found: C, 59.1; H, 5.35; N, 11.65. Calc. for $C_{46}H_{50}N_8NiO_{10}$: C, 59.15; H, 5.40; N, 12.0%). $[Ni(Z\text{-}trpO)_2(phen)]$ (phen = 1,10-phenanthroline): green, m.p. 151—155 °C (decomp.) (Found: C, 65.45; H, 4.85; N, 9.75. Calc. for $C_{50}H_{42}N_6NiO_8$: C, 65.7; H, 4.65; N, 9.20%).

Physical Measurements.—The i.r. spectra of the complexes in KBr or Nujol mull on KBr pellets (250—4 000 cm^{-1}) were recorded with a Perkin-Elmer 180 spectrophotometer. The room-temperature electronic spectra of the solid compounds were recorded as mull transmission spectra with a Shimadzu MPS 50L spectrophotometer. The room-temperature magnetic moments were measured by the Gouy method using $[Ni(H_2NCH_2CH_2NH_2)_3(S_2O_3)]$ as calibrant and correcting for diamagnetism with the appropriate Pascal constants.

Polarographic Measurements.—For the study of the system in methanolic solution, anhydrous methanol (C. Erba RSE) was used, and the salts were recrystallised from this solvent before use. Sodium perchlorate (0.1 mol dm^{-3}) was used as base electrolyte and the ionic strength of the solution was kept constant (0.1 mol dm^{-3}). The polarographic measurements were performed by adding accurately weighed and varying amounts of the amino-acid dissolved in anhydrous methanol containing an equimolar amount of Na[OH] to a 5×10^{-4} mol dm^{-3} nickel(II) perchlorate hexahydrate methanolic solution. The polarographic measurements were carried out using an Amel Multipolarograph model 471 at 25 ± 0.5 °C and at a drop time of 2.0 s; a saturated Ag|AgCl, KCl electrode was used as a reference in methanolic solution. All half-wave potential values refer to the saturated calomel electrode (s.c.e.).

Analyses.—Carbon, hydrogen, and nitrogen were analysed by Mr. G. Pistoni, using a C. Erba Elemental Analyser Mod. 1106.

RESULTS AND DISCUSSION

Behaviour of the Complexes in Methanolic Solution.—In methanolic solution the $[(Ni(Z\text{-}trpO)_2]\cdot 2H_2O$ complex and the Ni^{2+}–Z-L-tryptophanate system at various ligand concentrations have been polarographically examined to evaluate the type, number, and stability constants of the complexes, and to determine the range of concentration in which the $[NiL_2]$ complex prevails.

The polarographic curve for $[NiL_2]\cdot 2H_2O$ dissolved in methanol presents only a well defined reduction wave. The analysis of the wave at various dropping times and concentrations of the solid complex shows that the reduction is a quasi-reversible, two-electron, diffusion-controlled process. Its electrochemical parameters obtained are $(E_{\frac{1}{2}})_{irr.} = -0.95$ V (*versus* s.c.e.), $i_d = 1.7$ μA, and $\alpha n = 0.67$ [$(E_{\frac{1}{2}})_{irr.}$ is the value of the potential calculated by extrapolating at $\log i/(i_d - i_\lambda) = 0$ the upper part of the curve obtained from the logarithmic analysis of the wave; this value corresponds to the value of $E_{\frac{1}{2}}$ when the process is totally irreversible; i = current; i_d = diffusion current; α = charge-transfer coefficient; n = number of electrons].

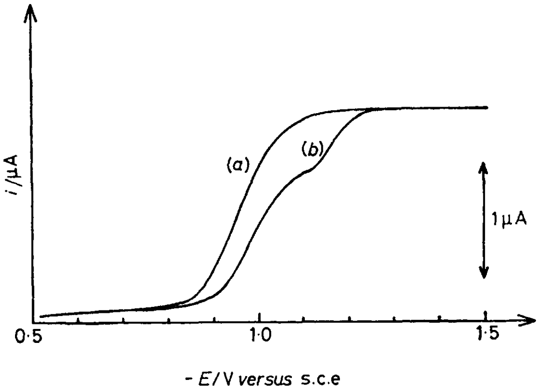
TABLE 1

Polarographic parameters of the nickel(II)–Z-L-tryptophanate system ($[Ni^{2+}] = 5 \times 10^{-4}$ mol dm^{-3}; c_L = total ligand concentration; 25 °C; M = metal ion; I and II = first and second waves; l = limiting)

$c_L \times 10^3$/ moldm^{-3}	$-(E_{\frac{1}{2}})^{I}$/V	$-(E_{\frac{1}{2}})^{II}$/V	i_l^{I}/μA	i_l^{II}/μA	$\frac{i_l^{I}}{i_l^{II}}$	αn^{I}	αn^{II}
1	0.96_0		1.7_5			0.67	
2	0.97_7	1.17_5	1.3_7	0.3_8	3.6	0.67	
3	0.97_8	1.17_0	1.2_1	0.5_4	2.2	0.67	1.10
5	0.99_0	1.20_0	1.0_2	0.7_3	1.6	0.67	0.71
6	1.00_5	1.20_0	0.9_0	0.8_5	1.1	0.67	0.65
8	1.02_0	1.22_0	0.8_0	0.9_5	0.8	0.67	0.65
10	1.03_5	1.23_0	0.7_5	1.0_0	0.7	0.67	0.65

At $c_L = 0$, $-(E_{\frac{1}{2}}^{M}) = 0.89$ V and $i_d^{M} = 1.75$ μA.

Typical current–voltage curves of the Ni^{2+}–Z-L-tryptophanate system at different ligand concentrations are reported in the Figure. With a constant metal ion concentration (5×10^{-4} mol dm^{-3}) and a ligand concentration below 10^{-3} mol dm^{-3}, a single wave is observed at about -0.9 V; above 2×10^{-3} mol dm^{-3} ligand concentration, a second wave appears at about -1.1 V. The electrochemical parameters obtained from the analyses of the waves are reported in Table 1. The ratio of the limiting currents of the two waves decreases on increasing ligand concentration, but their sum remains constant and has the same i_d value as that determined for the $[NiL_2]$ complex at the same concentration. To determine the characteristics of the species present in solution, the two waves were separately examined.



Current–voltage curves obtained for the Ni^{2+}–Z–L–tryptophanate system corresponding to metal to ligand molar ratios of (*a*) 1 : 2 and (*b*) 1 : 6

Analysis of the first wave. The polarographic parameters determined by the analysis of the first wave are reported in Table 2. Since the $(E_{\frac{1}{2}})_{irr.}$ and αn values of the first wave, determined when the $[Ni^{2+}]/c_L$ ratio is 0.5, are very similar to the values already obtained for the reduction of the $[NiL_2]\cdot 2H_2O$ complex, and since αn does not change, and $E_{\frac{1}{2}}$ and $(E_{\frac{1}{2}})_{irr.}$ values weakly decrease at increasing ligand concentration (Tables 1 and 2), it is possible to suggest that the first wave always corresponds to the reduction of the same complex species that is reduced as in equation (1).

$$[NiL_2] + Hg + 2e^- \rightleftharpoons Ni(Hg) + 2L^- \qquad (1)$$

Since the reduction process, which is diffusion controlled, is quasi-reversible, the reduction mechanism can be determined and the overall stability constant value [3] can be calculated by using the Ayabe–Matsuda [6] and De Ford–Hume [7,8] methods. The value of $\log \beta_2$, reported in Table 2, has been calculated by using the value of E_{Ni}^{0} corresponding to the ratio $[Ni^{II}]/[Ni(metal)]$,[9] since values of $[Ni^{II}]/[Ni(Hg)]$ are not available in the literature and the $(E_{\frac{1}{2}})_{[Ni^{II}]/[Ni(Hg)]}$ values in methanol correspond to a total irreversible process.

TABLE 2

Polarographic parameters and q and $\log \beta_2$ values determined for the first wave ($[Ni^{2+}] = 5 \times 10^{-4}$ mol dm^{-3}; c_L = total ligand concentration)

$c_L \times 10^3$/ mol dm^{-3}	$-(E_{\frac{1}{2}})_{irr.}$/V *versus* s.c.e.	αn	$-(E_{\frac{1}{2}})_{rev.}$[a]/V *versus* s.c.e.	$f(c_L)$ [b]
1	0.96_0	0.67	0.88_5	14.42
2	0.97_7	0.67	0.90_3	15.40
3	0.97_8	0.67	0.91_2	15.30
5	0.99_0	0.67	0.91_6	16.00
6	1.00_5	0.67	0.92_3	16.20
		q	2	2
		$\log \beta_2$	19.01	20.6

[a] See ref. 6. [b] See ref. 10.

The slope of the linear plot of $(E_{\frac{1}{2}})_{rev.}$ values *versus* $\log c_L$ (c_L = total ligand concentration) confirms that two ligand molecules are co-ordinated with the metal ion.

The number of co-ordinated ligand molecules and $\log \beta_2$ was further calculated using equation (2),[10] where

$$\log \beta_2 + q \log c_L = \log\left\{\frac{i_d - i}{i} - \left[\frac{c_L(0,t_1)}{c_L}\right]^{q-p} \exp \frac{\alpha n F}{RT}\left[E - (E_{\frac{1}{2}})_{irr.}\right]\right\} - \frac{nF}{2.3RT}(E - E_{Ni}^{0}) = f(c_L) \qquad (2)$$

c_L = total ligand concentration, $c_L(0,t_1)$ = ligand concentration at the electrode surface at time t_1, q = number of ligand molecules, and p = number of co-ordinated ligand molecules of the complex reduced at the electrode.

This equation, here used for quasi-reversible processes, is derived from a general expression for the current–voltage characteristic, which is valid for all the degrees of reversibilities and ligand concentrations. The parameters reported in Table 2 were calculated assuming that the ligand concentration at the electrode surface, $c_L(0,t_1)$, is equal to the total concentration of the ligand, and the value of $(q-p)$ can be considered equal to zero because $(E_{\frac{1}{2}})_{\text{irr.}}$ is nearly independent of the ligand concentration. The good agreement between the results obtained with the two methods confirms the reliability of the data and supports the validity of the methods themselves.

Analysis of the second wave. When ligand concentration is greater than 3×10^{-3} mol dm^{-3} a second wave appears and its i_l value increases at increasing c_L, but the total limiting current is constant, then the current of the first wave decreases when the current of the second one increases. The study of the waves on changing the dropping time has shown that each wave is diffusion controlled ($i_d/h^{\frac{1}{2}}$ is constant; h is the height of the mercury head); thus the current ratio may depend on a kinetic step of the complex formation reaction occurring in the bulk of the solution. The analysis of the second wave shows that its reduction process is quasi-reversible, then the same methods and considerations, described above, are applied.

TABLE 3

Polarographic parameters and q and log β_4 values determined for the second wave ($[Ni^{2+}] = \cdot 5 \times 10^{-4}$ mol dm^{-3}; c_L = total ligand concentration)

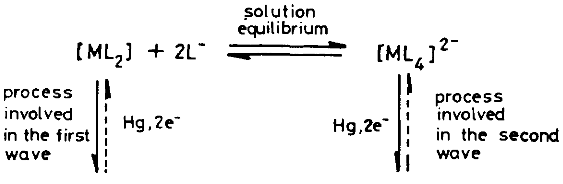
$c_L \times 10^3$/ mol dm^{-3}	$-(E_{\frac{1}{2}})_{\text{irr.}}$/V *versus* s.c.e.	αn	$-(E_{\frac{1}{2}})_{\text{rev.}}$ [a]/V *versus* s.c.e.	$f(c_L)$ [b]
3	1.18_5	1.10	1.15_0	22.32
5	1.20_0	0.71	1.17_0	23.11
6	1.20_0	0.65	1.17_6	23.48
8	1.22_0	0.65	1.18_8	23.67
		q	4	4
		log β_4	32.0	32.6

[a] See ref. 6. [b] See ref. 10.

The results, reported in Table 3, show that four ligand molecules are involved in the formation of the complex that is reduced at the electrode. The stoicheiometry of the metal complex was also determined using the method of molar ratio,[11] *i.e.* the current values are plotted against the molar ratio of ligand to metal ion: the slope of the curves changes before and after the equivalent point. Then the mechanism (3) for the second reduction can be proposed.

$$[NiL_4]^{2-} + Hg + 2e^- \rightleftharpoons Ni(Hg) + 4L^- \quad (3)$$

Moreover, the current values of the two waves and their ratios (Table 1) indicate that the four-co-ordinated species is formed from the two-co-ordinated one. Therefore the study of the system suggests the scheme of reactions (shown below) which take place in solution and at the electrode.



SCHEME

The $[ML_4]^{2-}$ species prevails over $[ML_2]$ when the ligand to metal molar ratio is greater than 6.

The values of log β_2 and log β_4 suffer from the error due to the difference $E^0_{[Ni^{II}]/[Ni(Hg)]} - E^0_{[Ni^{II}]/[Ni(metal)]}$, as discussed above; nevertheless log K = log β_4 − log β_2 = log β_4/β_2 = *ca.* 12 is independent of that difference and may be considered the real constant of the equilibrium taking place in solution. The high value of β_4 may be presumably an effect of the solvent methanol.

Behaviour of the Complexes in the Solid State.—To establish unambiguously the groups on the ligand involved in the metal co-ordination, we have also examined the behaviour of the nickel(II)–Z-L-tryptophanate complexes in the solid state.

In the reaction between the nickel(II) and Z-L-tryptophanate ions only one compound, having a metal to ligand molar ratio of 1 : 2, is separated, as expected from the results of the solution study reported above.

The identity of the complex in solution and in the solid state is also demonstrated by the similarity of their electronic spectra and by the molecular weight measurements in nitrobenzene (Found: M = 760; calc. for $[ML_2]\cdot 2H_2O$: 769.05). The room-temperature electronic and magnetic properties (Table 4) are typical of six-co-ordinated nickel(II). The calculated ligand-field parameters (Table 4) closely resemble those found for

TABLE 4

Room-temperature magnetic moments, electronic spectra, and ligand-field parameters (cm^{-1}) for the solid complexes

	$\mu_{\text{eff.}}$/ B.M. [a]	ν_1		ν_2	ν_3	Dq [b]	B [b]	β [b,c]	Dq [d]	B [d]	β [c,d]
$[Ni(Z\text{-}trpO)_2]\cdot 2H_2O$	3.14	8 620	13 330 (sh)	14 820	25 510	860	965	0.91	900	980	0.84
$[Ni(Z\text{-}trpO)_2(imH)_2]\cdot 2H_2O$	3.25	9 260		15 920	26 460	925	970	0.92	980	860	0.81
$[Ni(Z\text{-}trpO)_2(mim)_2]\cdot 2H_2O$	3.22	8 890		15 380	25 970	890	980	0.93	940	870	0.82
$[Ni(Z\text{-}trpO)_2(py)_2]\cdot 2H_2O$	3.10	9 350		15 920	26 320 (sh)	935	945	0.89	985	850	0.80
$[Ni(Z\text{-}trpO)_2(phen)]$	2.87	10 870		17 790		1 090					

[a] 1 B.M. = 0.927×10^{-23} A m^2. [b] Calculated from ν_1, ν_2, and ν_3. [c] $\beta = B/B_0$; the value of the Racah parameter B_0 for the free nickel(II) ion is 1 056 cm^{-1} (see G. M. Cole and B. B. Garret, *Inorg. Chem.*, 1970, **9**, 1898). [d] Calculated from ν_2 and ν_3.

the NiO_6 chromophore[12-14] and for other nickel(II)–*N*-protected amino-acid complexes, where the protecting group is an acyl or a benzoyl group, previously examined.[15-19] Furthermore, the values of $E_{\frac{1}{2}}$ *versus* s.c.e. of 0.95 V and Dq of 8 600 cm^{-1} strongly agree with those found for the $[Ni(OH_2)_6]^{2+}$ system which are 1.00 V and 8 500 cm^{-1} respectively.[20] A ligand-field strength of the Z-L-tryptophanate ion similar to that of water and the great reactivity of the $[Ni(Z\text{-}trpO)_2]\cdot 2H_2O$ complex towards the amines with which it easily forms mixed complexes, reflecting the carboxylate ligand behaviour,[21-27] suggest that the amino-acid acts as a simple carboxylate ligand.

The *d–d* band positions and the ligand-field parameters of the mixed complexes (Table 4) are consistent with the presence of the NiO_4N_2 chromophore,[14] the amines being co-ordinated. The breadth of the low-energy band, and the ligand-field parameters, which are greater if calculated with the three *d–d* bands than with the sharp ν_2 and ν_3 bands,[28] indicate the presence of distortions from O_h symmetry in all the complexes.

The i.r. spectra of all the complexes present values of $\nu_{sym}(OCO)$ lower than 1 400 cm^{-1} and of a $\Delta\nu$ separation $[\nu_{asym}(OCO) - \nu_{sym}(OCO)]$ of about 200 cm^{-1} in agreement with those found for the complexes of other *N*-protected amino-acids for which an essentially bidentate co-ordination through the carboxylate group has been structurally demonstrated.[29-32]

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