The Chemistry of Vitamin B_{12}. Part 27.[1] The Cage Phosphite Ester 4-Ethyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane and the Anionic Dimethyl Phosphite as Ligands for Cobalt(III) Corrinoids

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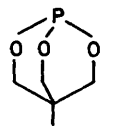
The equilibrium constant for the substitution of co-ordinated H_2O in aquocobalamin by 4-ethyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane (L^4) has been determined in aqueous solution at 25 °C by u.v.–visible spectrophotometry to be log K = 7.2; comparison with the estimated pK of 1.5 for the protonation of L^4 shows the marked preference of L^4 for Co^{III} over the proton. The cobinamide and acidified cobalamin (with protonated dbzm, the nucleotide base) containing anionic dimethyl phosphite (L^5) show a reversible temperature-dependent change in spectrum, ascribed to an equilibrium between five- and six-co-ordinate forms with axial ligands L^5-Co and L^5-Co-OH_2 respectively. Axial ligands L^4 and L^5 exert a similar *cis* effect (based on wavelength of the γ band), *viz.* $H_2O < dbzm < CN^- < SO_3^{2-} \approx L^4 \approx L^5 < HC{\equiv}C^- < CH_2CH^- < CH_3^-$, but a very different *trans* effect (based mainly on equilibrium constants), *viz.* $H_2O < dbzm < L^4 < CN^- < SO_3^{2-} \approx HC{\equiv}C^- < L^5 \approx CH_2{=}CH^- < CH_3^-$.

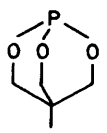
Steric effects can play an important role in metal–ligand interactions in Co^{III} corrinoids and these have been systematically studied with alkyl carbanions[2–4] and with the isoelectronic amines;[5] in both groups of ligands the absence of additional lone pairs on the tetrahedral donor atom simplifies the interpretation of the results. Distortion of the Co–C(alkyl) bond is associated with an increase in the thermodynamic *trans* effect of the alkyl ligand and in the lability of the Co–C bond, and probably occurs in the first step of the reactions of the B_{12}-dependent isomerase enzymes.[6]

Studies on the co-ordination of substituted amines show that the Co^{III} ion in aquocobalamin (B_{12a})† is more sensitive to steric effects than any metal ion previously studied.[5] It is therefore of interest to extend these studies to tetrahedral ligands with a heavier donor atom, *viz.* phosphines PR_3 and phosphite esters $P(OR)_3$ [R = Me (L^1) or Et (L^2)], L^3, L^4, and their derivatives $PR^1R^2O^-$ [R^1 = OMe, R^2 = OMe (L^5), F^- (L^6), or Ph (L^7); R^1 = R^2 = Ph (L^8)] and to compare them with the isoelectronic sulphite SO_3^{2-}, which has been fairly well studied.[7] N.m.r. studies (^1H, ^{13}C, ^{31}P) on cobaloximes suggest that, for both phosphines and phosphites, the effects of varying the ligand depend more on changes in steric than in electronic factors and that the effects of d_π–p_π bonding (donation of lone pairs from Co into the empty *d* orbitals on P) are insignificant.[8,9]

Neutral phosphite esters produce a very large crystal field (c.f.) splitting (for L^3 even greater than CN^-) with an accompanying large shift in the ^{59}Co n.m.r.;[10] phosphites are, in fact, the only unidentate ligands with a heavy donor atom which have been fully characterised with respect to their effect on the properties (*d–d* spectra and ^{59}Co n.m.r.) of the central Co^{III} ion.[11] No c.f. data are available for Co^{III} phosphine complexes, although ^{59}Co n.m.r. and n.q.r. data suggest that phosphines are similar to phosphites.[12] Studies on cobaloximes[8,13,14] and corrinoids[15,16] possessing various phosphite ligands have shown that anionic phosphites of types L^5—L^8, in contrast to the neutral types L^1—L^4, produce a marked lengthening of the Co–N bond in the *trans* position (*i.e.* a large structural *trans* effect)[8,13] and a large kinetic *trans* effect in the cobaloximes (with ligands such as L^5, L^7, and L^8 comparable to CH_3^-),[8,14] as well as a significant thermodynamic *trans* effect in the cobalamins (with L^5 approaching that of CH_3^-).[16] The phosphines, in comparison with the phosphites, show relatively small variations in *trans* effects.[8]



L^3



L^4

The importance of the phosphite ligands and their derivatives is that they provide a series of structurally related ligands with widely varying *trans* effect which may depend primarily on changes in steric factors. Values of the ligand cone angles,[17,18] half-neutralisation potentials in nitromethane,[19] and even gas-phase proton affinity constants[20] are available for certain neutral phosphite esters but not, unfortunately, for the anionic phosphites with high *trans* effect. A major gap in our knowledge is the absence of equilibrium constants for the co-ordination of any phosphite to any Co^{III} complex or, as far as we are aware, to any transition metal complex in aqueous solution.

We have therefore studied the co-ordination of the cage phosphite ester 4-ethyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]-octane (L^4) and the anionic dimethyl phosphite (L^5) by Co^{III} corrinoids in aqueous solution at 25 °C using u.v.–visible spectrophotometry. Ligands L^4 and L^5 were selected for study because (*i*) they are available commercially, (*ii*) L^4 is closely related to L^3 (not available commercially) with its very large c.f.

† Abbreviations: B_{12a} = aquo- and/or hydroxo-cobalamin, depending on pH; dbzm = the heterocyclic base (5,6-dimethylbenzimidazole) present in the cobalamin side-chain; dac = diaquo- and/or aquo-hydroxo-cobinamide, depending on the pH. N.B. The cobinamides lack the nucleotide side-chain ending in dbzm, which is present in the cobalamins such as cyanocobalamin (B_{12} itself).

Table 1. U.v.–visible spectra of corrinoids containing L^4 and L^5 as ligands

(*a*) Six-co-ordinate species

	λ/nm ($10^{-4}\varepsilon$/dm^3 mol^{-1} cm^{-1})					
			γ		β	α
L^4–Co–dbzm[a]	308	324	363.5 (2.8)	410	521 (0.70)	552 (0.74)
L^4–Co–OH_2[a]	305	319	354 (2.5)	404	474 (0.83)	530
L^4–Co–OH		324	360 (2.1)	410	518 (0.67)	544 (0.74)
L^4–Co–L^4			373		543	582
L^4–Co–CH_3		~373			~580	
(L^4)–Co–OH_2[b]	314		349 (2.0)	400	450 (0.76)	
L^5–Co–dbzm[c]	315	336	363.5 (2.1)	403	520 (0.80)	542 (0.81)
L^5-Co–CN	312		374	424	555	594

(*b*) Five-co-ordinate species

	λ/nm ($10^{-4}\varepsilon$/dm^3 mol^{-1} cm^{-1})			
L^5–Co[d]	316.5 (2.0)	346.5 (1.9)	404 (1.1)	437 (1.1)
L^5–Co[e]	316 (2.1)	346 (1.7)	402 (1.1)	437 (1.2)
(L^5)–Co[b]	302	345		446 (1.3)

[a] Spectrum shown in Figure 1. [b] Hydrolysis product; see text. [c] Spectrum shown in Figure 3. [d] Cobalamin at pH 1.0 (see Figure 3). [e] Cobinamide at pH 6.5 (see Figure 6).

Table 2. Comparison of the *cis* and *trans* effects of L^4, L^5, and other ligands in Co^{III} corrinoids (X–Co–Y)

Ligand X	γ Band (λ/nm) Y = dbzm[a]	γ Band (λ/nm) Y = CN^-[a]	p*K* (protonation) of dbzm in X–Co–dbzm[b]	p*K* (ionisation) of H_2O in X–Co–OH_2	log *K* (substitution) of H_2O by CN^- in X–Co–OH_2	k_2[c,d]/dm^3 mol^{-1} s^{-1}
H_2O	350	355	−2.4	6.0[b]	14.1[e]	$\leqslant 10^3$
dbzm		360.5		7.8[b]	$\geqslant 12$[b]	8.5×10^2
HO^-	357	362		10.3[f]	$\geqslant 9$[d]	1.9×10^4, 6.8×10^4
NC^-	360.5	368	0.1	11.0[b]	8[b]	2.1×10^5
$HC{\equiv}C^-$	367	377	0.7	13.0[g]	$\geqslant 6.8$[g]	3.6×10^6
$CH_2{=}CH^-$	372	393	2.0	15—15.5[g]	2.7[b]	$\geqslant 10^8$
CH_3^-	374	398	2.4	>15[b]	2.1[b]	$\geqslant 10^8$
SO_3^{2-}	364	372	2.0	14.7[b]	4.3[b]	$\geqslant 4 \times 10^6$[h]
L^4	363.5[h]		$\leqslant 1.1$[h]	10.7[h]		0.35×10^5, 1.1×10^5[h]
L^6	364[i]	374[i]	1.6[i]			
L^5	363.5[h,i]	374[h,i]	2.3[h]	>13[h]	4.1[h]	

[a] Ref. 7*a*, [b] Ref. 7*b*. [c] Rate constant for substitution of H_2O by CN^- in X–Co–OH. [d] Ref. 35. [e] Ref. 32. [f] Ref. 26. [g] Ref. 34. [h] This work. [i] Ref. 16.

splitting,[10,11] (*iii*) L^4 with its very small cone angle (101°)[17,18] should show maximum contrast in properties to ligands L^5 and L^6, both of which have previously been studied with corrinoids;[15,16] in addition, the crystal structure of a cobaloxime containing L^3 has recently been determined.[21,*]

The aim of this work is to characterise ligands L^4 and L^5 according to the parameters which are most readily studied with Co^{III} corrinoids, *viz.* (*i*) equilibrium constants for the substitution of co-ordinated H_2O, (*ii*) thermodynamic *trans* effect, (*iii*) kinetic *trans* effect, and (*iv*) ground-state *cis* effect (*viz.* effect on the π–π transitions of the corrin ring). To complete the comparison with SO_3^{2-} we have also studied the kinetic *trans* effect of co-ordinated sulphite.

* The corrinoids discussed here are identified by their axial ligands L^1—L^8; *e.g.* L^4–Co–OH_2 and L^5–Co.

The p*K* for protonation of L^4 has not been reported, but can be estimated as 1.5 (see Discussion section). It has been mentioned that L^4 is hydrolysed by water but no rate constants have been reported;[22] we have therefore made qualitative observations of the effect of pH on the rate of hydrolysis (see Results section). **CAUTION**: The phosphite L^4 is toxic[23] and must be handled with care. Dialkyl phosphites, including dimethyl phosphite, actually have the phosphonate structure $HPO(OR)_2$ with only a very small amount (<1 in 10^6) of the phosphite form $P(OR)_2OH$ present.[24] Dialkyl phosphites are very weak acids but are readily hydrolysed to give monoalkyl phosphites ($pK_a \approx 2$).[25]

Experimental

Materials.—Samples of vitamin B_{12a} were kindly given by Mr. A. P. Domleo of Glaxo-Allenbury (Pty) Ltd.; dac[26] was

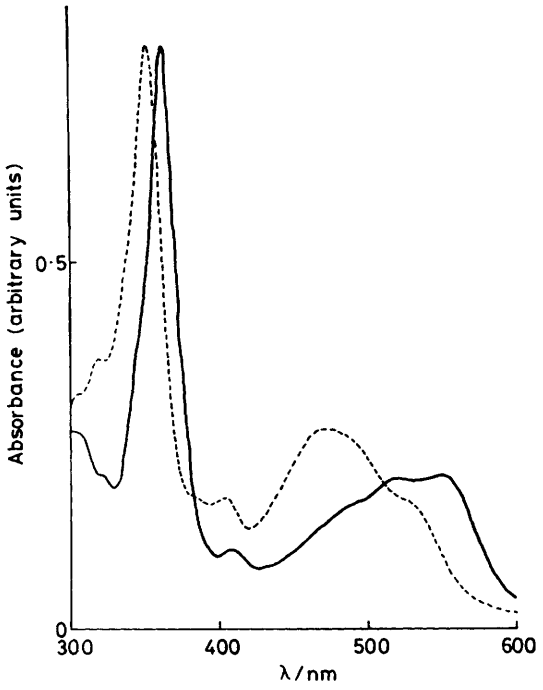


Figure 1. U.v.–visible spectra of 2.8 × 10^{-5} mol dm^{-3} L^4-Co-dbzm (——) and 3.2 × 10^{-5} mol dm^{-3} L^4-Co-OH_2 (– – – –) at pH 7.0

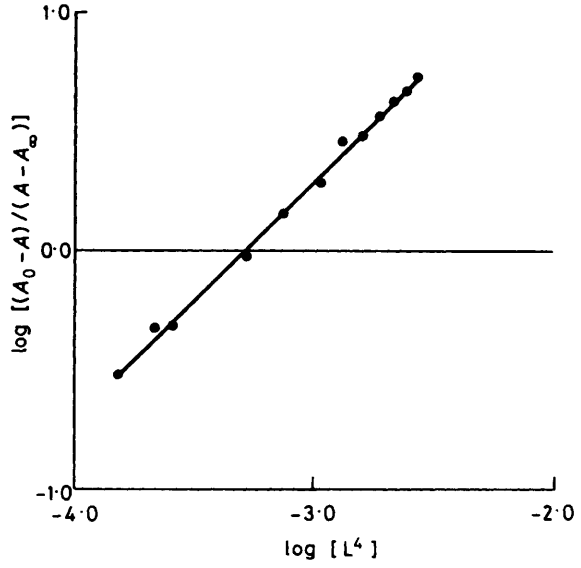


Figure 2. Evaluation of the experimental data for the equilibrium between B_{12a} and L^4 at pH 6.7 in the presence of 0.10 mol dm^{-3} sodium azide at 25 °C to give an apparent equilibrium constant K_{app} = 1.96 × 10^3 dm^3 mol^{-1}

prepared as described previously and by photolysis of methylcobinamide in methanol. Methylcobinamide was prepared as described previously.[27] 4-Ethyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane (Strem Chemicals Inc.) was used as obtained and stored at 5 °C. Dimethyl phosphite (Aldrich 99%) was used as obtained.

Methods.—U.v.–visible spectra were recorded with Jasco Uvidec-1 and Unicam SP8-100 spectrophotometers using 1-cm cells thermostatted at 25 °C. The concentrations of the corrinoids were determined after conversion to the dicyanide using ε_{367} = 3.04 × 10^4 dm^3 mol^{-1} cm^{-1}.[7a] The kinetics of ligand substitution were studied with a Durrum D-110 stopped-flow spectrophotometer thermostatted at 25 °C.

Thin-layer chromatography was carried out on cellulose (Merck, 0.1 mm, pre-coated) using s-butyl alcohol–water (9.5:4) (solvent I) and s-butyl alcohol–0.880 ammonia–water (9.5:0.675:4) (solvent III).[28]

A Metrohm EA 147 microglass electrode and a Metrohm pH-meter 532 were used for pH determinations. Sodium phosphate buffers (I = 0.2 mol dm^{-3}) were used for pH 5.4—7.0, sodium carbonate buffers (I = 0.2 mol dm^{-3}) for pH 9.0—11.0, 0.01 mol dm^{-3} sodium hydroxide with 0.19 mol dm^{-3} sodium perchlorate for pH 12.0, and 0.1 mol dm^{-3} sodium hydroxide with 0.1 mol dm^{-3} perchlorate for pH 13.0.

Results

Details of the spectra of corrinoids are given in Table 1 and relevant equilibrium and rate constants in Table 2.

Solubility of L^4.—By adding weighed amounts of solid L^4 until a permanent turbidity was obtained the solubility of L^4 was found to be 0.002 g cm^{-3} (0.01 mol dm^{-3}) in water and 0.13 g cm^{-3} (0.80 mol dm^{-3}) in methanol at 25 °C.

U.v.–Visible Spectra, Equilibria, and Reactions involving L^4.—L^4–Co–dbzm. Aqueous solutions of B_{12a} (red) react readily with an excess of L^4 over the range of pH 5—8, with reasonable isosbestic points, to give a product (also red) identified as L^4–Co–dbzm; the spectrum is given in Figure 1 and data are in Table 1. L^4–Co–dbzm is converted back to B_{12a} at pH < 3 and pH > 9.

The equilibrium constant for the co-ordination of L^4 by B_{12a}, which is very large, was determined in the presence of azide as a competing ligand. Solutions of B_{12a} (2.9 × 10^{-5} mol dm^{-3}) in sodium phosphate buffer (pH 6.7) and 0.10 mol dm^{-3} sodium azide (total I = 0.2 mol dm^{-3}) were allowed to equilibrate (15 h) with varying concentrations of L^4 (up to 2.7 × 10^{-3} mol dm^{-3}) at 25 °C and $A_{363.5}$ was measured. A plot of log $[(A_0 - A)/(A - A_\infty)]$ *vs.* log $[L^4]$ (see Figure 2) was linear with a slope of 0.99, indicating the binding of one molecule of L^4 per Co, and gave the apparent equilibrium constant K_{app} = 1.96 × 10^3 dm^3 mol^{-1}; correction for the partial formation of azidocobalamin (K = 7.2 × 10^4 dm^3 mol^{-1})[7b] and hydroxocobalamin (pK = 7.8)[7b] gave the true equilibrium constant for the substitution of co-ordinated H_2O in B_{12a} by L^4 as K = 1.5 × 10^7 dm^3 mol^{-1} (log K = 7.2).

An attempt was made to determine the pK_a for the protonation of L^4 in aqueous solution by studying the pH dependence of the rate of reaction (followed at 363 nm) of 6 × 10^{-5} mol dm^{-3} B_{12a} with 4 × 10^{-4} mol dm^{-3} L^4. The same initial rate was observed in deionised water and at pH 5 and 2.5, leading to complete formation of L^4–Co–dbzm at pH ≥ 5, *ca.* 50% at pH 2.5, but only *ca.* 4% at pH 2.1, *i.e.* the pK of L^4 is ≤ 2.5.

An attempt was made to determine the pK for the protonation of dbzm in L^4–Co–dbzm. If L^4–Co–dbzm is prepared in the presence of a large concentration of L^4 (0.01 mol dm^{-3}), then titration with dilute H_2SO_4 shows the apparent occurrence of a pH-dependent equilibrium with reasonable isosbestic points giving an orange-red product with a spectrum characterised by bands at 305, 318, 354 (γ) (ε 2.5 × 10^4 dm^3 mol^{-1} cm^{-1}), 405, 474 (0.81 × 10^4), and 530 nm.The spectrum is similar to that of L^4–Co–OH_2 (see Table 1). Analysis of the data gave an apparent pK of 1.1 with a slope of 1.2. The reaction is not reversible; raising the pH from −0.5 to +2.2, with cooling of the solution, merely caused complete decomposition to B_{12a}

within the time of the experiment (< 1 min). It is likely that the co-ordinated L^4 has been hydrolysed during acidification (see Discussion section). Rapid acidification to minimise the extent of hydrolysis of the co-ordinated ligand did not reveal any other $pK > 1$; one can therefore assume that the pK of the parent cobalamin L^4–Co–dbzm is < 1.1.

L^4–Co–OH_2. Neutral aqueous solutions of the orange-red dac react readily with L^4 to give L^4–Co–OH_2 (also orange-red; spectrum given in Figure 1 and data in Table 1) with, at the highest concentration of L^4 (0.01 mol dm^{-3}), a trace of L^4–Co–L^4. In methanol, increasing the concentration of L^4 to 0.80 mol dm^{-3} leads to substantial formation of the purple species L^4–Co–L^4 (spectroscopic data given in Table 1) due to the higher solubility of L^4. Aqueous solutions of L^4–Co–OH_2 are stable for 3—4 h over the pH range *ca.* 4—9. L^4–Co–OH_2, in neutral aqueous solution, is slowly converted to a yellow hydrolysis by-product, (L^4)–Co–OH_2, with bands at 314, 349, 400, and 450 nm (see Table 1); this yellow by-product was formed more rapidly with increasing temperature. No reversible changes were observed in the spectrum of L^4–Co–OH_2 on heating from 6 to 66 °C at pH 7.0.

It proved impossible to determine the formation constant of L^4–Co–OH_2 quantitatively because the obviously high formation constant required low concentrations of L^4, and hence long periods for equilibration, with consequent interference from the yellow by-product, but a minimum value of $\log K_{app} \geqslant 5$ at pH 7.0 could be estimated and correction for the pK_a of dac (5.9)[26] gives $\log K \geqslant 6$.

The cobalamin L^4–Co–OH_2 is converted back to dac at a rate increasing with pH ($t_{\frac{1}{2}} \approx 3$ min at pH 12.0 and complete conversion within 15 s at pH 13.0); a reddish complex (L^4–Co–OH, spectrum given in Table 1) can, however, be detected immediately after reacting dac with high concentrations (0.01 mol dm^{-3}) of L^4 at pH 12.0. The pK for the interconversion of L^4–Co–OH_2 and L^4–Co–OH was determined by adding dac (3.0×10^{-4} mol dm^{-3}, 0.2 cm^3) to buffer solutions (2.0 cm^3) of varying pH (7.0—12.0) and fixed ionic strength (I = 0.2 mol dm^{-3}) in the presence of a high concentration (0.01 mol dm^{-3}) of L^4. Superposition of spectra gave good isosbestic points; a plot of $\log [(A - A_0)/(A_\infty - A)]$ *vs.* pH was linear with a slope of 1.1, indicating that the equilibrium involved one proton, with $pK = 10.6 \pm 0.2$.

CH_3–Co–L^4. An aqueous solution of methylcobinamide shows no significant change in the spectrum on addition of L^4, even if the solution is saturated with L^4. In methanol, however, the addition of increasing amounts of L^4 causes the rise of a band at *ca.* 580 nm (see Table 1) which can be ascribed to the CH_3–Co–L^4 complex (*cf.* the band at 588 nm in CH_3–Co–CN); assuming that the molar absorption coefficient of the band at 580 nm is similar to that of the band at 588 nm of CH_3–Co–CN (*viz.* 0.07×10^4 dm^3 mol^{-1} cm^{-1})[7a] allows the estimation of A_{580} corresponding to 100% formation of CH_3–Co–L^4 and hence a value of $\log K \approx -0.5$ for the binding of L^4 by (mainly five-co-ordinate)[7b] methylcobinamide.

Attempts to form L^4–Co–CN. Aqueous solutions of dac were treated with varying concentrations of potassium cyanide and L^4 at different pH and the spectra scanned over the range 300—650 nm in order to find conditions for determining equilibrium constants for the reaction of L^4–Co–OH_2 with CN^- and/or NC–Co–OH_2 with L^4. However, the observed changes in the spectrum could not be interpreted in terms only of the expected three species (L^4–Co–OH_2, L^4–Co–CN, NC–Co–CN) and, in addition, the spectrum of the intermediate stage resembled that obtained by treating the yellow by-product from L^4–Co–OH_2 (see above) with CN^- (*viz.* bands at 365, 505, and 530 nm). It appears that CN^- may catalyse the hydrolysis of co-ordinated L^4; attempts to determine equilibrium constants were therefore abandoned.

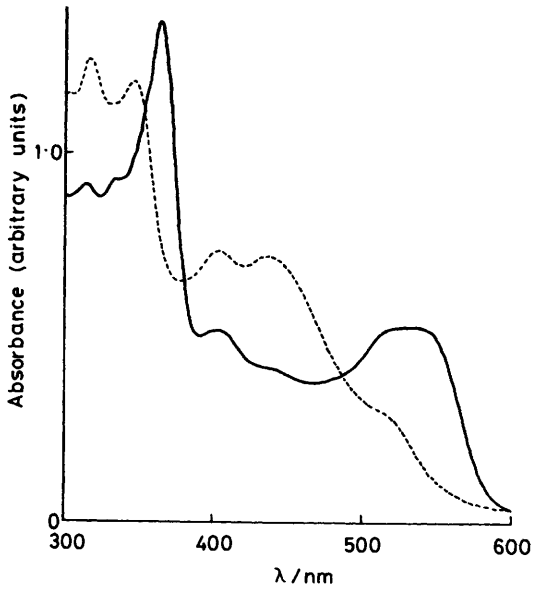


Figure 3. U.v.–visible spectra of 6.3×10^{-5} mol dm^{-3} dimethyl phosphitocobalamin at pH 7.0 (——) and pH 1.0 (– – – –) at 25 °C

Kinetics of Ligand Substitution.—L^4–Co–OH_2 *and* CN^-. An aqueous solution of dac was treated with a small amount of solid L^4 and the solution diluted with concentrated buffer to give a final concentration of 4×10^{-5} mol dm^{-3} L^4–Co–OH_2 (full formation checked from the spectrum) in 0.2 mol dm^{-3} sodium carbonate buffer (pH 9.2). The reaction kinetics were studied with concentrations of 10^{-3} and 10^{-4} mol dm^{-3} sodium cyanide and followed at 580 nm (the α band of NC–Co–CN). Similar biphasic traces were observed at both concentrations; analysis by curve-stripping using an estimated A_∞ for k_f gave fast and slow observed first-order rate constants, k_f(obs.) and k_s(obs.). Values of k_f(obs.) ≈ 4 and ≈ 43 s^{-1} were obtained with 10^{-4} and 10^{-3} mol dm^{-3} cyanide respectively, and k_s(obs.) ≈ 1 and ≈ 14.5 s^{-1} with 10^{-4} and 10^{-3} mol dm^{-3} cyanide respectively, *i.e.* both rate constants increase approximately linearly with cyanide concentration over a ten-fold range. After correcting for the dissociation of HCN ($pK = 9.3$)[29] and L^4–Co–OH_2 ($pK = 10.6$), this gives second-order rate constants of $k_f = 1.1 \times 10^5$ dm^3 mol^{-1} s^{-1} and $k_s = 3.5 \times 10^4$ dm^3 mol^{-1} s^{-1}.

SO_3–Co–OH_2 *and* CN^-. Sulphitocobinamide (1.0×10^{-5} mol dm^{-3}) was prepared by the addition of excess Na_2SO_3 to dac, followed by phenol–chloroform extraction,[7c] and reacted with 5×10^{-4} mol dm^{-3} sodium cyanide in 0.2 mol dm^{-3} sodium phosphate buffer (pH 8.0). The reaction kinetics were followed at 595 nm (the isosbestic point for the conversion of SO_3–Co–CN to NC–Co–CN) giving a rate constant, corrected for the pK of HCN,[29] $k \geqslant 4 \times 10^6$ dm^3 mol^{-1} s^{-1}.

U.v.–Visible Spectra, Equilibria, and Reactions involving L^5.—L^5–Co–dbzm. Vitamin B_{12a} reacts readily in methanol with an excess of L^5, with reasonable isosbestic points, to give the yellow base-off (*i.e.* acidified) form of dimethyl phosphitocobalamin [due to traces of the acidic hydrolysis product of L^5].[25] It was not possible to react L^5 with B_{12a} in aqueous solution as hydrolysis of L^5 occurs too rapidly at pH 1—12 but L^5–Co–dbzm in aqueous solution can be prepared by dissolving B_{12a} (2.8 mg) in dimethyl phosphite (0.6 cm^3), allowing to stand one week at room temperature, and diluting in water (1:40). Thin-layer chromatography shows a single red spot with a $R_{B_{12}}$ value (R_f value relative to that of B_{12} as a marker) of 1.24 in solvent I and 0.78 in solvent III.[28]

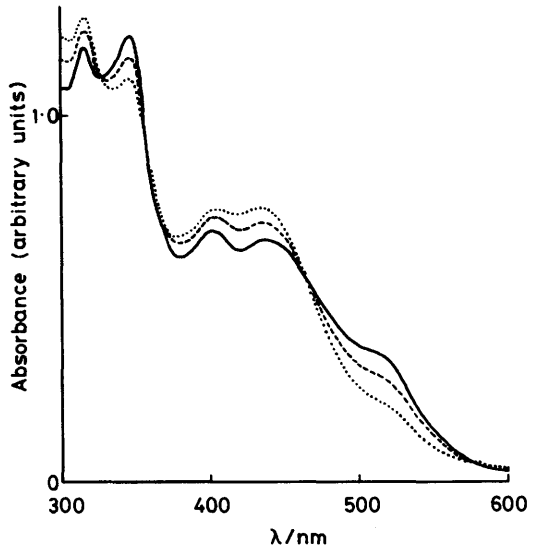


Figure 4. U.v.–visible spectra of 6.3×10^{-5} mol dm^{-3} dimethyl phosphitocobalamin at pH 1.0 at 6 (——), 35 (– – – –), and 66 °C (· · · ·)

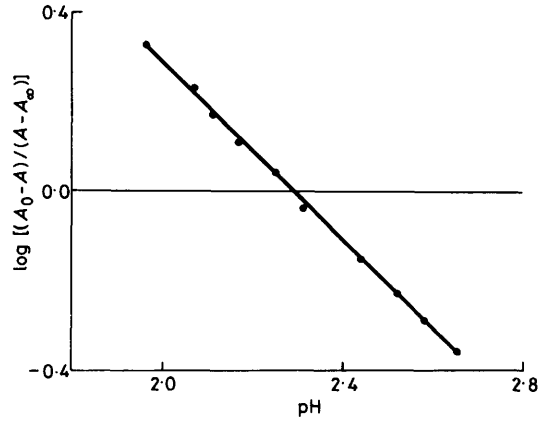


Figure 5. Evaluation of the experimental data for the protonation of the 5,6-dimethylbenzimidazole base in L^5-Co-dbzm at 25 °C to give pK = 2.29

The u.v.–visible spectrum (Figure 3, Table 1) of dimethyl phosphitocobalamin in both its base-on (red) and base-off (yellow) forms is identical to that reported for dimethyl phosphitocobalamin by Bieganowski and Friedrich.[16] The u.v.–visible spectrum of acidified dimethyl phosphitocobalamin, on heating at pH 1 from 6 to 66 °C, shows a reversible temperature-dependent equilibrium with very good isosbestic points (Figure 4) between a six-co-ordinate (red) L^5–Co–OH_2 and a five-co-ordinate (yellow) L^5–Co species (with the dbzm base protonated and not co-ordinated in both cases); L^5–Co predominates at all temperatures. No temperature-dependent equilibrium was observed on heating L^5–Co–dbzm from 25 to 66 °C at pH 7.0.

The pK for protonation of dbzm in dimethyl phosphitocobalamin was determined by titrating a 5.2×10^{-5} mol dm^{-3} solution of L^5–Co–dbzm in dilute aqueous sulphuric acid (pH 1.0) with dilute potassium hydroxide at 25 °C. Slight turbidity was observed near the end of the reaction. Good isosbestic points were observed and the reaction was reversible. The results were evaluated at $A_{363.5}$ by the method of Newton and Arcand.[30] A plot of log $[(A_\infty - A)/(A - A_0)]$ *vs.* pH gave a slope of 0.99 (one proton) and pK = 2.29 (Figure 5).

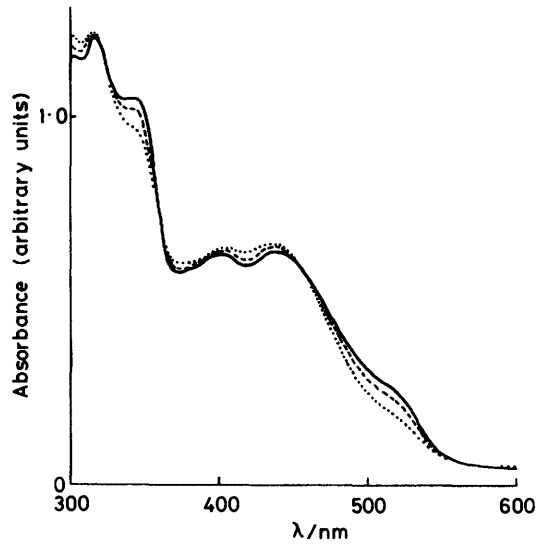


Figure 6. U.v.–visible spectrum of 5.1×10^{-5} mol dm^{-3} L^5-Co-OH_2 at pH 6.5 at 8 (——), 25 (– – – –), and 55 °C (· · · ·)

L^5–Co–OH_2. The cobinamide dac reacts with excess L^5 in methanol with good isosbestic points to give the yellow dimethyl phosphitocobinamide. Dimethyl phosphitocobinamide was prepared by reacting dac in methanol (1.4×10^{-4} mol dm^{-3}, 10 cm^3) with dimethyl phosphite (0.1 cm^3) for 15 h and diluting in water or buffer (1:10) and is stable in aqueous solution at pH 1—10 for at least 3 h.

Dimethyl phosphitocobinamide has a u.v.–visible spectrum (Table 1, Figure 6), at pH 1—12, very similar to that of the base-off form of dimethyl phosphitocobalamin. The u.v.–visible spectrum of dimethyl phosphitocobinamide, on heating from 6 to 55 °C, shows a reversible temperature-dependent equilibrium with very good isosbestic points between red six-co-ordinate L^5–Co–OH_2 and yellow five-co-ordinate L^5–Co at both pH 1.0 and 6.5. The yellow species predominates at both pH 1.0 and 6.5 and at all temperatures.

The spectrum of dimethyl phosphitocobinamide shows no pH changes up to pH 13 when irreversible decomposition occurs to give a yellow hydrolysis product, (L^5)–Co, with bands at 302, 345, and 446 nm (Table 1). The pK for the interconversion of the mixture L^5–Co–OH_2/L^5–Co and L^5–Co–OH must therefore be $\geqslant 13$.

L^5–Co–CN. The u.v.–visible spectrum of L^5–Co–CN is given in Table 1. The equilibrium constant for the reaction of L^5–Co–OH_2/L^5–Co with CN^- to give L^5–Co–CN was determined by titration of dimethyl phosphitocobinamide (1.4×10^{-5} mol dm^{-3}) in sodium carbonate buffer (pH 10.0, I = 0.2 mol dm^{-3}) with a 0.099 mol dm^{-3} aqueous solution of potassium cyanide, allowing sufficient time (10 min) for equilibrium after each addition. Reasonable isosbestic points were obtained. The results were evaluated at 375 nm. As A_∞ could not be obtained experimentally due to the formation of NC–Co–CN at high concentrations of CN^- the method of Newton and Arcand[30] was used. A plot of log $[(A_\infty - A)/(A - A_0)]$ *vs.* log $[CN^-]$ gave a slope of 1.00 (1 CN^- per Co) and log K = 4.07 after correction for the pK_a of HCN.[29]

Discussion

The solubility of L^4 in water with no added ions was found to be *ca.* 0.01 mol dm^{-3}. Binding constants for L^4, provided that they

are high as in the cobalamin and cobinamide, can therefore be determined in aqueous solution; lower binding constants necessitate the use of less polar solvents such as methanol in which the solubility of L^4 is increased to *ca.* 0.8 mol dm^{-3}. It has been reported[22] that acid causes the successive hydrolysis (rates not given) of the three C–O–P bonds of L^4 to give the trihydroxy compound and H_3PO_3. Using changes in the spectra of the corrinoids to monitor changes in the concentration of L^4 we find that hydrolysis of L^4 is promoted by acid and base. We find that L^4 is also decomposed while co-ordinated *via* P to Co, presumably by hydrolysis of one or more of the C–O–P bonds to P–OH and HO–C; decomposition occurs slowly in neutral aqueous solution but is accelerated by acid or cyanide. However, in spite of the problems of solubility and stability of L^4 in water, it is possible to study this very interesting ligand in aqueous solution.

The pK_a of L^3 [the methyl analogue of L^4] can be estimated as 1.5 by interpolation using the equation $pK_a(H_2O) = 10.12 - 0.0129 \times \Delta_{h.n.p.}(CH_3NO)$ and the value of 665 for $\Delta_{h.n.p.}$ (half-neutralisation potential) of L^3 in nitromethane,[19] and we assume that L^4 has the same pK_a value as L^3. We find, by studying the pH dependence of the rate of reaction of B_{12a} with L^4, that the pK_a of L^4 is $\leqslant 2.5$ (in agreement with the value found by interpolation).

The equilibrium constant for the substitution of co-ordinated H_2O by L^4 in B_{12a} was determined as 1.5×10^7 dm^3 mol^{-1} or $\log K = 7.2$. This appears to be the first equilibrium constant reported for the co-ordination of any phosphite ester or derivative with any transition metal ion in aqueous solution; values of $\log K \approx 2.5$ have, however, been reported for the binding of L^3 by bis(hexafluoroacetylacetonato)copper(II) in methylene chloride and *o*-dichlorobenzene.[31] Comparison with the calculated $pK_a = 1.5$ emphasises the marked preference ($7.2 - 1.5 = 5.7$) of L^4 for the Co^{III} ion in B_{12a} over the proton; *cf.* the similar preference (4.8) of cyanide for Co^{III} in B_{12a} ($\log K = 14.1$)[32] over the proton ($pK = 9.3$).[29] The spectrum of the cobalamin L^4–Co–dbzm is similar to those reported[16] for cobalamins with ligands L^5 and L^6.

The cobalamin L^4–Co–dbzm, on titration with acid, shows the apparent occurrence of a pH-dependent equilibrium with $pK = 1.1$. The reaction appears to be analogous to the reversible protonation (and displacement from co-ordination) of dbzm observed for many other cobalamins.[7b] In this case, however, the reaction is not reversible. Since all six-co-ordinate cobalamins undergo ligand substitution by a S_N1 mechanism with a forward rate constant for the substitution of co-ordinated H_2O in the range $k_{forward} = 10^2$—10^3 dm^3 mol^{-1} s^{-1},[33] a ligand such as L^4 with a binding constant $\log K = 7.2$ should be displaced by H_2O with a rate constant $k_{reverse}$ in the range 10^{-4}—10^{-5} s^{-1} (*i.e.* $t_{\frac{1}{2}} = 10^3$—10^4 s), which is incompatible with our results. The most likely explanation is that the co-ordinated L^4 has been hydrolysed during the course of acidification (*e.g.* by conversion of one of the P–O–C bonds to P–OH + HO–C) to give some other more labile ligand which still retains P as the donor atom. Since no other $pK > 1$ was observed it is assumed that the pK of the parent cobalamin L^4–Co–dbzm is < 1.1.

The identity of the cobinamide L^4–Co–OH_2 could not be established from the stoicheiometry of the reaction between dac and L^4 but follows from qualitative observations on the dependence of the three expected species (L^4–Co–OH_2, L^4–Co–OH, L^4–Co–L^4) on the experimental variables [pH, concentration of L^4] and also from the similarity of its absorption spectrum to those reported for the acidified, base-off forms of the cobalamins with ligand L^5 and L^6.[16] The spectrum of L^4–Co–OH_2 (Figure 1) is rather unusual in that it shows an intense γ band combined with an anomalous αβ region. The equilibrium constant for the substitution of H_2O by L^4 in dac is clearly very high ($\log K \geqslant 6$), but a quantitative determination was prevented by experimental difficulties.

It is expected that the equilibrium constant for the substitution of co-ordinated H_2O by L^4 should fall depending on the nature of the *trans* ligand, X, in the order observed for other ligands, *viz.* $H_2O >$ dbzm $> CN^- > \cdots > CH_3^-$.[7b] A low value of $\log K \approx -0.5$ was found for $X = CH_3^-$ in methanol and, because L^4 is uncharged, a similar value can be assumed in aqueous solution. The equilibrium constants for L^4 therefore fall (from $\log K = 7.2$ to ≈ -0.5) as the σ-donor power of X increases (from dbzm to CH_3^-). The fall is, in fact, very similar to that observed for NH_3 (*cf.* $\log K = 7$ and $\leqslant -1$ for X = dbzm and CH_3^- respectively)[7b] and provides further evidence (*cf.* refs. 8 and 9) against any significant effects of d_π–p_π bonding between the lone pairs on the Co and the unfilled 3*d* orbitals on P.

We have no evidence to show whether the observed biphasic character of the kinetics of reaction of L^4–Co–OH_2 with CN^- is caused by the presence of two axial isomers L^4–Co–OH_2 and H_2O–Co–L^4 (*cf.* the known isomers of NC–Co–OH_2 and H_2O–Co–CN)[34] with different reactivities or of H-bonded isomers (as in dac)[26] or, less probably, represents the two successive stages of the reaction L^4–Co–$OH_2 \xrightarrow{CN^-}$ L^4–Co–CN $\xrightarrow{CN^-}$ NC–Co–CN.

The position of L^4 in the kinetic *trans* effect order (see Table 2 and below) is not, however, affected by the observation of two rate constants. The low rate of reaction suggests that the complex is predominantly the six-co-ordinate L^4–Co–OH_2 and not the five-co-ordinate L^4–Co which would be expected to be extremely labile (*cf.* the five-co-ordinate CH_3–Co in Table 2).[35]

Since dimethyl phosphite contains only a little of the phosphite form in equilibrium with the phosphonate form[24] the reaction with B_{12a} and dac is rather slow. Dimethyl phosphitocobalamin and dimethyl phosphitocobinamide were therefore prepared in neat dimethyl phosphite and methanol respectively and diluted with deionised water or buffer; co-ordinated L^5 is stable enough to be studied in aqueous solution. Dimethyl phosphitocobinamide at pH $\geqslant 13$ shows irreversible decomposition to (L^5)–Co in which we presume that L^5 has been hydrolysed but still remains co-ordinated through the P atom.

The u.v.–visible spectrum of dimethyl phosphitocobalamin in both its base-on (L^5–Co–dbzm) and base-off (L^5–Co) form is identical to that obtained by Bieganowski and Friedrich,[16] who have also shown by electrophoresis that ligand L^5 contains one negative charge, *i.e.* is present as the anion.[16] The pK of L^5–Co–dbzm was determined at 25 °C as 2.29 and involved one proton; the difference from the pK value reported by Bieganowski and Friedrich (2.05)[16] can be ascribed to different experimental conditions and a different method of calculation. The identity of the cobinamide L^5–Co can be inferred from the similarity of its u.v.–visible spectrum to that of base-off dimethyl phosphitocobalamin. The identity of the cobinamide L^5–Co–CN can be inferred from the similarity of its u.v.–visible spectrum to that of the corresponding cobalamin.[16]

Both dimethyl phosphitocobinamide and acidified dimethyl phosphitocobalamin show very similar reversible temperature-dependent equilibria, between a red species at low temperatures and a yellow species at high temperatures, with the yellow species predominant at all temperatures measured (6—66 °C). By analogy with the similar temperature-dependent equilibria exhibited by organo- and sulphito-cobinamides[6,7] we conclude that the red and yellow forms are the six-co-ordinate L^5–Co–OH_2 and five-co-ordinate L^5–Co respectively. Data required to place L^4 and L^5 in the order of *cis* and *trans* effects have been included in Table 2 and can be summarised as follows.

cis Effect (measured by the wavelength of the γ-band in the u.v.–visible spectrum of X–Co–dbzm and X–Co–CN): H_2O < dbzm < CN^- < SO_3^{2-} ≈ L^4 ≈ L^5 < $HC{\equiv}C^-$ < $CH_2{=}CH^-$ < CH_3^-.

Thermodynamic *trans* effect (measured by the p*K* for the protonation of dbzm in X–Co–dbzm, p*K* for the ionisation of H_2O in X–Co–OH_2, and log *K* for the substitution of H_2O in X–Co–OH_2 by CN^-): H_2O < dbzm < L^4 < CN^- < SO_3^{2-} ≈ $HC{\equiv}C^-$ < L^5 ≈ $CH_2{=}CH^-$ < CH_3^-.

Kinetic *trans* effect (measured by the rate constant for the substitution of H_2O by CN^- in X–Co–OH_2): H_2O < dbzm < L^4 < CN^- < SO_3^{2-} ≈ $HC{\equiv}C^-$ ≈ $CH_2{=}CH^-$ < CH_3^-.

The following conclusions can be drawn. (*i*) Ligand L^4 is at the low end and ligand L^5 is at the high end of the thermodynamic *trans* effect series. (*ii*) Ligand L^4 occupies the same position as regards both its thermodynamic *trans* effect and its kinetic *trans* effect, *viz.* H_2O < dbzm < HO^- < L^4 < CN^- < SO_3^{2-} ≈ $HC{\equiv}C^-$. This is very similar to the order of the kinetic *trans* effect reported for $[Ru^{II}(NH_3)_5X]$ complexes, *viz.* NH_3 < HO^- < L^2 < CN^- < SO_3^{2-}.[36] (*iii*) All three phosphites [L^4—L^6] which have been studied as ligands for corrinoids show a similar *cis* effect (band at 364 nm), though there are significant differences in the relative intensities of the peaks, but a very different *trans* effect. (*iv*) Phosphite L^5 is similar to sulphite in both its *cis* effect and thermodynamic *trans* effect. It remains to be seen whether L^5 also has a high kinetic *trans* effect, as would be expected on the basis of its effect in the cobaloximes.[8,14]

These results show (*i*) that the correlation between *cis* and *trans* effects, which is observed for C-donor ligands (and probably other ligands with light donor atoms),[7a,b] breaks down for heavier donor atoms such as P and S, and (*ii*) that the phosphites provide a family of structurally related ligands with varying *trans* effect (between dbzm and CH_3^- in the *trans* effect series), which could prove useful in further probing the origin of the *trans* effect. Our present results provide the most extensive study yet reported of phosphites as ligands in aqueous solution.

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