Placing hydroxide in the thermodynamic *trans* influence order of the cobalt corrinoids: equilibrium constants for the reaction of some ligands with aquahydroxocobinamide

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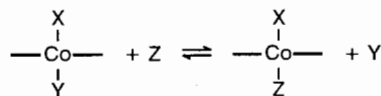*Department of Chemistry, Mississippi State University, Mississippi State, MS 39762 (USA)*

Abstract

Diaquacobinamide (DAC) was prepared by reducing aquacyanocobinamide (Factor B) with zinc and oxidising in aqueous HCl. The macroscopic acid dissociation constants pK_{Co1} and pK_{Co2} for coordinated H_2O in DAC were found spectrophotometrically to be 5.91 ± 0.04 and 10.30 ± 0.24 at 25 °C; from their temperature dependence it was found that $\Delta H = 45 \pm 4$ and 27 ± 2 kJ mol^{-1}, and $\Delta S = 40 \pm 14$ and -109 ± 8 J K^{-1} mol^{-1}, for pK_{Co1} and pK_{Co2}, respectively. Equilibrium constants for the reaction of the cobinamide with cyanide were determined in the pH range 8–12 at 25 °C, $\mu = 1.0$ M ($NaClO_4$). Two CN^- ligands bind to aquahydroxocobinamide and pH dependence of the observed equilibrium constants can be accounted for by assuming that both dihydroxocobinamide and hydroxocyanocobinamide are inert, while equilibrium constants for reaction of the labile species aquahydroxocobinamide and aquacyanocobinamide with HCN are insignificantly small compared to those for reaction with CN^- anion. A fit of the data shows that the macroscopic equilibrium constant, $\log \beta_2$, for reaction of $2CN^-$ with the two diastereomers of aquahydroxocobinamide to produce dicyanocobinamide is 19.0 ± 0.1. Hence the macroscopic $\log K$ for binding of CN^- to the two diastereomers of aquahydroxocobinamide is 11 since $\log K$ for binding of CN^- to aquacyanocobinamide is 8. Equilibrium constants for binding of azide, pyridine, *N*-methylimidazole and 3-amino-1-propanol to aquahydroxocobinamide were determined at 25 °C, $\mu = 1.0$ M ($NaClO_4$), pH 12. In these cases only a single ligand is bound; the macroscopic $\log K$ values are *N*-methylimidazole, 6.06 ± 0.04; 3-amino-1-propanol, 4.66 ± 0.04; pyridine, 4.19 ± 0.01; and azide, 3.45 ± 0.04. A comparison of the present results with others available shows that hydroxide is above water and 5,6-dimethylbenzimidazole, but below cyanide, sulfite and alkyl ligands in the *trans* influence order of the cobalt corrinoids.

Introduction

The *trans* influence is the effect which a ligand X, bound to a metal ion, has on the properties of the coordinated *trans* ligand, Y, and on the kinetics and thermodynamics of the replacement of Y by an incoming ligand, Z (eqn. (1))**. It is an established phenomenon in the chemistry of the cobalt corrinoids at the structural, the thermodynamic and the kinetic level (where it is usually referred to as a *trans* effect) [1].

$$\begin{matrix} X \\ | \\ -Co- \\ | \\ Y \end{matrix} + Z \rightleftharpoons \begin{matrix} X \\ | \\ -Co- \\ | \\ Z \end{matrix} + Y$$

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**For convenience, only the axial ligands are shown, and the overall charge is neglected.

$$K = \frac{[X-Co-Z][Y]}{[X-Co-Y][Z]} \tag{1}$$

In such complexes it has been established that for constant Z, $\log K$ decreases as X becomes a better σ-donor. For example, if Z = imidazole and Y = H_2O, we have the series (X, $\log K$): H_2O, 7.5 [2]; 5,6-dimethylbenzimidazole (dmbzim), 4.59 [3]; CN^-, 4.1 [2]; methyl, 1.0 [4]; and n-propyl, -1 [4]. A number of other such series has been established [1]. However, there appears to be little information where X = OH^-.

An examination of the effect of the *trans* ligand, X, on (i) the stretching frequency of coordinated cyanide, (ii) the equilibrium constant for replacement of H_2O in X-Co-H_2O by CN^-, and (iii) the rate of substitution of H_2O by CN^-, by Baldwin *et al.* [5] demonstrated that there is a correlation between the (i) structural, (ii) thermodynamic and (iii) kinetic *trans* influence.

Thus, the stretching frequencies for coordinated CN^- *trans* to dmbzim and OH^- are 2132 and 2131 cm^{-1}, respectively (cf. 2079 cm^{-1} for free CN^-); the equilibrium constants (log K) for binding of CN^- are 14.1 and c. 9, respectively; and the rate constants for substitution of H_2O by CN^- are c. 9×10^2 and 4.5×10^4 dm^3 mol^{-1} s^{-1}, respectively. These results suggest that OH^- has a stronger *trans* influence than dmbzim and is therefore above it in the *trans* influence series.

To obtain further evidence on the position of OH^- in the thermodynamic *trans* influence order, we have determined equilibrium constants for the binding of some neutral (pyridine, N-methylimidazole, 3-amino-1-propanol) and anionic (CN^-, N_3^-) ligands to aquahydroxocobinamide and report these results in this paper.

Experimental

Materials

Cyanocobalamin ($> 99\%$ pure, HPLC) was obtained from Roussel. Sodium cyanide, sodium azide, N-methylimidazole and pyridine were obtained from Merck, and 3-amino-1-propanol was obtained from Aldrich. All other reagents were of the highest purity available and all reagents were used as received. Water was purified by double distillation in an all-glass still and further purified by passage through a Millipore MilliQ system.

Preparation of diaquacobinamide (DAC)

Factor B* (50 mg, prepared by cerous hydroxide cleavage of B_{12} [6]) in 25 ml 5% NH_4Br in methanol was placed in a three-necked round bottom flask capped with serum stoppers and purged with Ar for 1 h. Zinc wool (500 mg, freshened with 2 M HCl) was added to the flask. Reduction was allowed to proceed for c. 1.5 h during which time the solution darkened. The reaction mixture was transferred by cannula into 100 ml of 1 M HCl and desalted by extraction into 1:1 phenol:chloroform (6×5 ml portions). The organic layer was washed with 1 M HCl (3×100 ml) and 200 ml ether and then back-extracted into water (5 ml portions). The ether was removed and the solution was concentrated on a rotary evaporator (50 °C). The pH was adjusted to 3 with 1 M KOH and the solution desalted as before. Typical yields of DAC were 35–40 mg (quantised spectrophotometrically by conversion to dicyanocobinamide, $\epsilon_{367} = 3.04 \times 10^4$ M^{-1} cm^{-1} [7]). While working with DAC, every care was taken to minimise its exposure to both air and light.

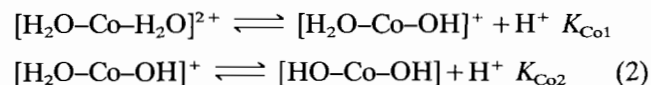*Factor B is a mixture of the diastereomers of aquacyanocobinamide, (α-CN-β-H_2O)cobinamide and (α-H_2O-β-CN)cobinamide.

Instrumentation

High performance liquid chromatography was performed on a SpectraPhysics SP8800 ternary gradient system coupled to a Linear 2000 variable wavelength spectrophotometric detector and a Varian 4290 integrator, or a Beckman System Gold HPLC system with a model 168 diode array detector. A Cary 2300 or a Cary 219 spectrophotometer was used for spectrophotometric work. The pH of solutions was measured using a Metrohm 605 pH meter and series 6.0210.100 glass electrode with NaCl internal solution (to prevent precipitation of $KClO_4$ in the porous junction) calibrated against standard buffers.

Methods

The acid dissociation constants for the two coordinated H_2O molecules in DAC** (pK_{Co1} and pK_{Co2}, eqn. (2)) were determined spectrophotometrically as follows.

$$[H_2O\text{–}Co\text{–}H_2O]^{2+} \rightleftharpoons [H_2O\text{–}Co\text{–}OH]^+ + H^+ \quad K_{Co1}$$

$$[H_2O\text{–}Co\text{–}OH]^+ \rightleftharpoons [HO\text{–}Co\text{–}OH] + H^+ \quad K_{Co2} \quad (2)$$

The pH of 30 ml of a 12.5 μM DAC solution containing 5 mM phosphate and $\mu = 1.0$ M ($NaClO_4$) in a stirred thermostatted cell was altered by addition of very small volumes of concentrated NaOH or $HClO_4$ such that dilution effects were negligible. At each suitable pH value, an aliquot was pumped through 0.8 mm diameter PTFE tubing into a 2 cm pathlength cuvette in the thermostatted cell block of the spectrophotometer by means of a peristaltic pump. The absorbance was measured at 278 and 344 nm (for pK_{Co1}), and at 348.5 nm and 535 nm (for pK_{Co2}), and the sample pumped back into the cell. Separate titrations were performed for determining pK_{Co1} and pK_{Co2}.

HPLC analyses were performed using a Brownlee Laboratories 5 μm C18 10 cm $\times$ 4.6 mm column (10 min linear ramp from 5% acetonitrile/95% 50 mM KH_2PO_4, pH 3, to 10% acetonitrile, then a 5 min ramp to 40% acetonitrile held for 5 min before returning to starting conditions over 2 min; flow rate 2 ml min^{-1}) or a Beckman Ultrasphere 5 μm C8 25 cm $\times$ 4.6 mm column (5% acetonitrile/95% 50 mM ammonium phosphate, pH 3, for 2 min, 12 min linear ramp to 30% acetonitrile, held for 3 min, and a return to starting conditions over 2 min; flow rate 2 ml min^{-1}). Semipreparative work for the attempted purification of DAC was done on a Brownlee Laboratories 20 μm C18 Aquapore 25 cm $\times$ 10 mm column (15 min ramp from 5% acetonitrile/95% phosphate buffer to 5.5% acetonitrile, followed by 1 min ramp to 40% acetonitrile

**Abbreviations used: DAC, diaquacobinamide; AHC, aquahydroxocobinamide; DHC, dihydroxocobinamide; ACC, aquacyanocobinamide (Factor B); HCC, hydroxocyanocobinamide; DCC, dicyanocobinamide.

held for 10 min before returning to starting conditions over 2 min; 5 ml min^{-1} flow rate).

Equilibrium constants were determined by adding aliquots of the appropriate ligand solution to c. 20 μM of the cobinamide in a 1.00 cm pathlength cell in the thermostatted cell holder of the spectrophotometer (25.0 ± 0.1 °C) and determining the change in absorbance at a selected wavelength (see Table 2). Absorbance readings were corrected for dilution effects, and the data were analysed as described below. Buffers used (0.1 M) were tris(hydroxymethyl-aminomethane)/HCl (pH 8–9.2); 3-[cyclohexylamino]-1-propane-sulfonic acid (CAPS) (pH 9.5–11.2); and Na_2HPO_4/Na_3PO_4 (pH 12).

Results and discussion

Preparation of DAC

Typically yields of 70–80% DAC from Factor B were obtained. Analysis by HPLC (Fig. 1) indicated a single major peak which integrated to between 80 and 95% of the total signal response, with minor peaks both before and after it. However, since there is considerable peak tailing on both C8 and C18, the integration may not be reliable.

Purification of DAC was attempted using semi-preparative HPLC. Using the elution profile describe above, two well-resolved peaks were obtained: DAC (retention time 11.5 min) and a peak containing Factor B and
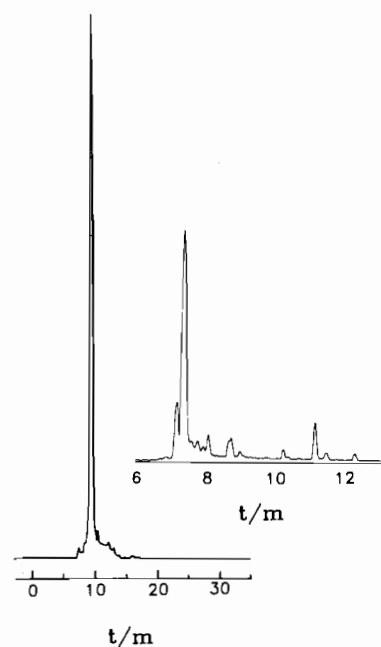

Fig. 1. HPLC of DAC on a C18 column. The major peak integrates to 95% with a retention time of 9.83 min. Similar results were obtained on C8 with a retention time of 7.33 min. See text for details of elution profiles. Inset: HPLC on a C8 column of DAC prepared by photolysis of methylcobinamide.

unidentified products of the reaction (retention time 18.5 min). The DAC fractions were collected and pooled, desalted and concentrated, and reanalysed. It was found that DAC decomposed during this attempted purification step with the DAC peak often no more than 70% of the total integrated signal. Multiple peaks due to unidentified decomposition products were seen by analytical HPLC, and co-eluted as the second peak in the semi-preparative HPLC procedure.

Attempts to produce DAC either by photolysis of methylcobinamide (see Fig. 1, inset) or, in our experience, by photolysis of Factor B as described by Baldwin et al. [8], yielded a heavily contaminated product.

We were never able to synthesise DAC with greater than about 95% purity. Attempts to purify it by semi-preparative chromatography were frustrated by its decomposition during the isolation procedure and we had to resort to using DAC contaminated to a small extent by side-products of the reaction. Nevertheless, the procedure for the preparation of DAC reported in this paper gives, in our experience, a significantly cleaner product than that obtained by other methods such as photolysis of methylcobinamide or photolysis of Factor B [8]. It appears, as others have also noted [9], that DAC is inherently unstable. Although we have not characterised the products into which it decomposes, their spectra are characteristic of the so-called 'stable yellow corrinoids' [1], cobalt corrinoids in which the corrin ring has been irreversibly modified. We routinely stored DAC at −20 °C under Ar and found there was no appreciable further decomposition for some weeks.

Acid dissociation constants of DAC

The pK_a values of DAC (eqn. (2)) have been reported by Baldwin et al.[8] as 5.9 ± 0.1 and 10.3 ± 0.2 and, more recently, by Ford et al. [9] as 5.40 ± 0.05 and 10.30 ± 0.07. The results we obtained, together with their temperature dependence, which appear not to have been reported before, are given in Table 1. Values of ΔH and ΔS were obtained from plots (not shown) of ln K against T^{-1}; values of the pK_as at 25 °C calculated from the ΔH and ΔS values are also listed in Table 1. The results obtained are in reasonable agreement with those of other workers. Since AHC, like Factor B, is expected to consist of a mixture of diastereomers depending on whether OH$^-$ is coordinated to Co via the upper or lower face of the corrin, pK_{Co1} and pK_{Co2} are both macroscopic acid dissociation constants; the microscopic constants are unknown.

Equilibrium constants

The spectra of AHC in the presence of varying amounts of a given ligand, L, displayed well-defined isosbestic points. The number of ligands binding to the metal centre, n, was determined from the slope of plots

TABLE 1. Acid dissociation constants and ΔH and ΔS of ionisation of diaquacobinamide (pK_{Co1}) and aquahydroxocobinamide (pK_{Co2}); $\mu = 1.0$ mol dm^{-3}, NaClO$_4$

	Temperature (°C)	pK_a[a]	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	pK_a^{25}[b]
pK_{Co1}	5.0	6.40 ± 0.36			
	15.0	6.14 ± 0.03			
	25.0	5.91 ± 0.04			
	35.0	5.55 ± 0.01	45 ± 4	40 ± 14	5.80
pK_{Co2}	10.4	10.60 ± 0.13			
	25.0	10.30 ± 0.24			
	45.0	10.14 ± 0.09	27 ± 2	−109 ± 8	10.43

[a]Average of determinations at 278 and 344 nm (pK_{Co1}) and at 348.5 nm and 535 nm (pK_{Co2}). [b]pK_a calculated at 25 °C from ΔH and ΔS.

of $\log[(A - A_0)/(A_\infty - A)]$ against $\log[\text{ligand}]$ where A_0 and A_∞ represent the absorbance at the monitoring wavelength corresponding to 0 and 100% complex formation. In the case of CN$^-$ two ligands were found to coordinate (Fig. 2(a), where $n = 2.1 \pm 0.1$); in all other cases, the slopes indicated the binding of a single ligand (see Table 2). The plot obtained for N$_3^-$
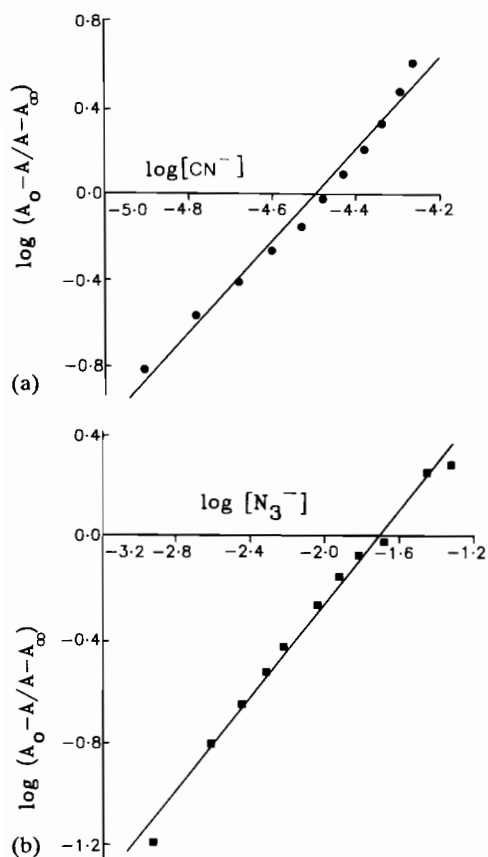

(a)

(b)

Fig. 2. Least-squares plots of $\log[(A - A_0)/(A_\infty - A)]$ against (a) $\log[\text{CN}^-]$ (slope = 2.1 ± 0.1) and (b) $\log[\text{N}_3^-]$ (slope = 0.91 ± 0.03) for binding of CN$^-$ and N$_3^-$ to aquahydroxocobinamide at pH 12.0, $\mu = 1.0$ M (NaClO$_4$). At this pH the cobinamide is present in solution principally as the dihydroxo species.

($n = 0.91 \pm 0.03$) is shown in Fig. 2(b) as an example. In principle, binding constants can be determined from the intercepts of such plots, but the results may be unreliable because, firstly, they are significantly affected by the value of A_0 and A_∞ chosen [10, 11]; whereas A_0 is known with reliability, A_∞ may not be if the equilibrium constant is relatively small. Secondly, the fraction of bound ligand, which may be a significant fraction of the total ligand added (see below) is not taken into account.

For reactions with $n = 1$, eqn. (3) is applicable.

$$\begin{array}{ccc} \text{HO}^- & & \text{HO}^- \\ | & & | \\ -\text{Co}- + Z & \rightleftharpoons & -\text{Co}- + \text{H}_2\text{O} \\ | & & | \\ \text{H}_2\text{O} & & Z \end{array} \qquad (3)$$

$$K = \frac{[\text{HO–Co–Z}]}{[\text{HO–Co–H}_2\text{O}][Z]} \qquad (4)$$

If A_0 and A_∞, respectively, represent the absorbance corresponding to 0 and 100% of complex formation at a fixed concentration of metal ion, then the absorbance, A, at any total ligand concentration $[Z]$ is given by eqn. (5).

$$A = \frac{A_0 + A_\infty K[Z]}{1 + K[Z]} \qquad (5)$$

For relatively small values of K and for relatively low $[\text{HO–Co–OH}_2]^+$, it follows that $[\text{HO–Co–Z}] \ll [Z]_{\text{total}}$ and $[Z]_{\text{free}} \approx [Z]_{\text{total}}$; eqn. (5) may then be fitted to the experimental data by non-linear least-squares methods with A_0, A_∞ and K as parameters. An example is shown in Fig. 3. Alternatively, since A_0 is known with certainty, this may be fixed. In the work reported here, it was found that if A_0 was allowed to vary, its value was not significantly different from the experimental value. Because of the inertness of bound hydroxide in the cobalamins (see below) the value of K determined in this fashion is a conditional binding constant, K_{exp}, as its value will depend on the pH at
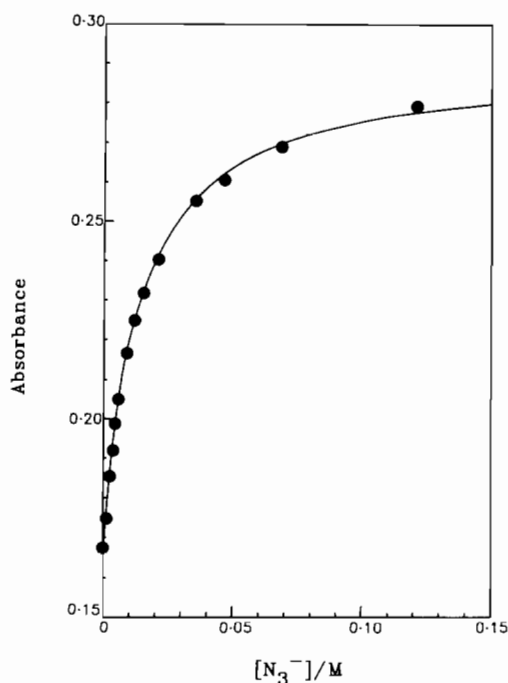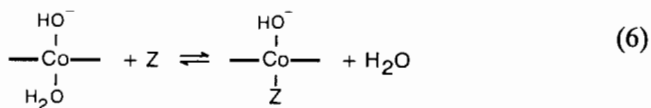
Fig. 3. Variation in absorbance at 370 nm on addition of N_3^- to aquahydroxocobinamide at 25 °C, $\mu = 1.00$ M, pH 12.0. The solid line is a fit of eqn. (5) to the data.

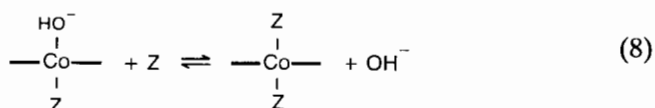which the determination was made; values of log K_{exp} obtained are listed in Table 2.

We attempted to determine log K at pH 12 for the binding of SO_3^{2-} to AHC, but found that the sulfite reacts with AHC to produce stable yellow corrinoids. This observation is in accord with Firth *et al.* [12], who estimated that log K for replacement of H_2O in DAC by SO_3^{2-} is ≈ 11. They observed that the treatment of AHC at pH 14–15 with a slight excess of Na_2SO_3 showed a rapid and reversible equilibration with a red complex and also slower, irreversible decomposition which prevented a quantitative study of the first equilibrium.

For reactions with $n = 2$, two modes of binding can be envisaged. The reaction may proceed through the formation of a mono-ligated intermediate which, at higher ligand concentrations, binds a second ligand to
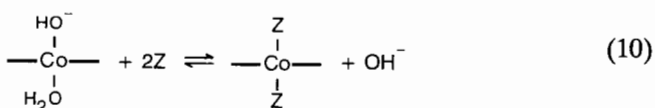
form the final bis-ligated species (eqns. (6–9)), or the binding constant for the second ligand may be of similar magnitude to, or larger than, the binding constant for the first ligand so that two ligands appear to bind simultaneously to the metal centre (eqns. (10–11)), as occurs, for example, in the binding of cyanide to iron porphyrins [10], and only an overall equilibrium constant, β_2, can be determined.

$$\underset{\underset{H_2O}{|}}{\overset{\overset{HO^-}{|}}{-Co-}} + Z \rightleftharpoons \underset{\underset{Z}{|}}{\overset{\overset{HO^-}{|}}{-Co-}} + H_2O \tag{6}$$

$$K_1 = \frac{[\text{HO–Co–Z}]}{[\text{HO–Co–H}_2\text{O}][Z]} \tag{7}$$

$$\underset{\underset{Z}{|}}{\overset{\overset{HO^-}{|}}{-Co-}} + Z \rightleftharpoons \underset{\underset{Z}{|}}{\overset{\overset{Z}{|}}{-Co-}} + OH^- \tag{8}$$

$$K_2 = \frac{[\text{Z–Co–Z}][\text{OH}^-]}{[\text{HO–Co–Z}][Z]} \tag{9}$$

$$\underset{\underset{H_2O}{|}}{\overset{\overset{HO^-}{|}}{-Co-}} + 2Z \rightleftharpoons \underset{\underset{Z}{|}}{\overset{\overset{Z}{|}}{-Co-}} + OH^- \tag{10}$$

$$\beta_2 = \frac{[\text{Z–Co–Z}][\text{OH}^-]}{[\text{HO–Co–H}_2\text{O}][Z]^2} \tag{11}$$

If the binding constants are large (as they are for CN^-) then the assumption that $[Z]_{\text{free}} \approx [Z]_{\text{total}}$ is no longer valid and expressions must be developed which relate the free ligand concentration to the total and bound ligand concentrations, respectively. The expressions are polynomials of the form of eqn. (12) where the coefficients for the expressions relevant to eqns. (9) and (11) are given in Table 3.

$$a[Z]_{\text{free}}^3 + b[Z]_{\text{free}}^2 + c[Z]_{\text{free}} + d = 0 \tag{12}$$

TABLE 2. Experimental data for the binding of ligands to aquahydroxocobinamide, pH 12.0, 25.0 °C, $\mu = 1.0$ M (NaClO$_4$)

Ligand	Monitoring wavelength (nm)	Isosbestic points (nm)	Ligand stoichiometry, n^a	$\log(K_{\text{exp}})^b$	$\log K^c$
Azide	370	543	0.91 ± 0.03	1.74 ± 0.04	3.45 ± 0.04
Pyridine	368	542	1.09 ± 0.03	2.48 ± 0.01	4.19 ± 0.01
N-Methylimidazole	365	391	0.94 ± 0.05	4.35 ± 0.04	6.06 ± 0.04
3-Amino-1-propanol	364	376	0.97 ± 0.05	2.95 ± 0.04	4.66 ± 0.04

^{a}The slope of the line $\log[(A - A_0)/(A_\infty - A)]$ against $\log[\text{ligand}]$. ^{b}Observed formation constants at pH 12. ^{c}Calculated using eqn. (9).

TABLE 3. Coefficients for eqn. (12) applicable for the cases where there is step-wise binding of two ligands to the metal centre (eqn. (9)) and the two ligands appear to bind simultaneously (eqn. (11))

Coefficient	Eqn. (9)	Eqn. (11)
a	$K_1 K_2$	β_2
b	$K_1 - K_1 K_2 [Z]_{total} - 2K_1 K_2 [M]_{total}$	$2[M]_{total}\beta_2 - \beta_2[Z]_{total}$
c	$1 - K_1[Z]_{total} + K_1[M]_{total}$	1
d	$-[Z]_{total}$	$-[Z]_{total}$

$[M]_{total}$ represents the sum of the concentrations of all metal ion complexes in solution. The cubic eqn. (12) can be solved by a variety of numerical methods; we employed an iterative procedure using Newton's method.

Equilibrium constants for binding of cyanide by aqua-hydroxocobinamide were determined as a function of pH between pH 8 and 12. At all pH values we obtained well-defined isosbestic points and the spectra suggest conversion from AHC to DCC without appreciable concentrations of ACC (or HCC) being present. Figure 4 shows an example of the spectral changes we observed. Well-defined isosbestic points occur at 310, 360, 397 and 533 nm.

We were unable to fit the data for titrations of AHC with cyanide using eqn. (9); the iterations were unstable and there was strong correlation between values of K_1 and K_2. Therefore we could only determine the overall stability constant for the binding of two ligands to AHC using eqn. (11). An example of a fit is shown in Fig. 5. The results are summarised in Table 4, and plotted in Fig. 6.
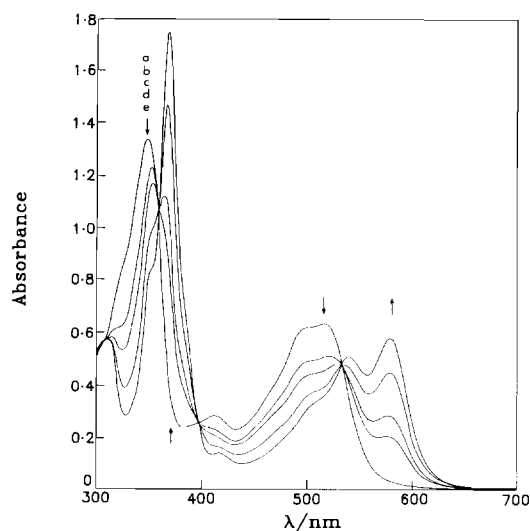

Fig. 4. Spectra of aquahydroxocobinamide in the presence of various concentrations of cyanide at pH 9.24, 25 °C, $\mu = 1.00$ M (NaClO$_4$); a: 57.6 μM AHC; b: +9.94 μM NaCN; c: +24.5 μM NaCN; d: +33.9 μM NaCN; e: +excess NaCN.
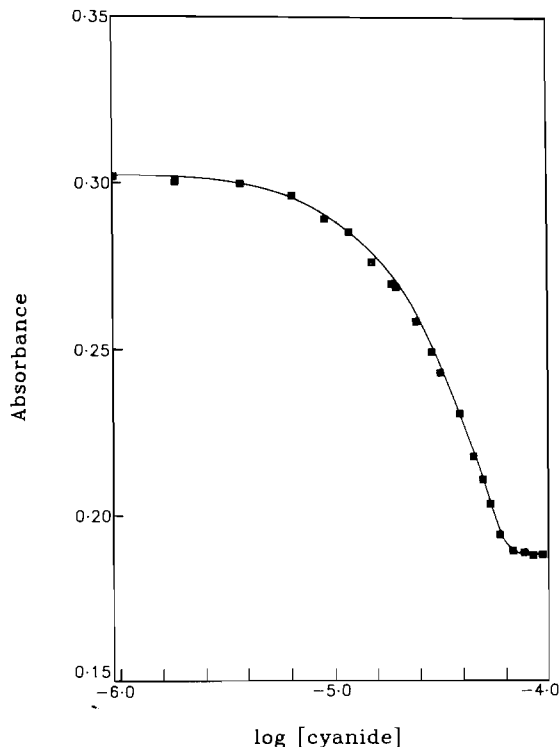

Fig. 5. Plot of absorbance at 518 nm against $\log[CN^-]_T$ for binding of cyanide by AHC at pH 9.24, 25 °C, $\mu = 1.00$ M (NaClO$_4$). The solid line is a fit of the data using eqn. (11).

TABLE 4. Values for $\log \beta_2^{obs}$ (eqn. (11)) as a function of pH for binding of cyanide by aquahydroxocobinamide at 25 °C, $\mu = 1.00$ M (NaClO$_4$)

pH	$\log \beta_2^{obs}$
8.16	16.5 ± 0.5
8.50	17.7 ± 0.4
9.24	18.4 ± 0.2
9.49	18.6 ± 0.2
10.07	18.7 ± 0.3
10.52	18.5 ± 0.5
11.20	18.2 ± 0.3
12.00	17.1 ± 1.3

Although the error on each equilibrium constant is large (spectrophotometric determinations of such large equilibrium constants are difficult), there is a clear
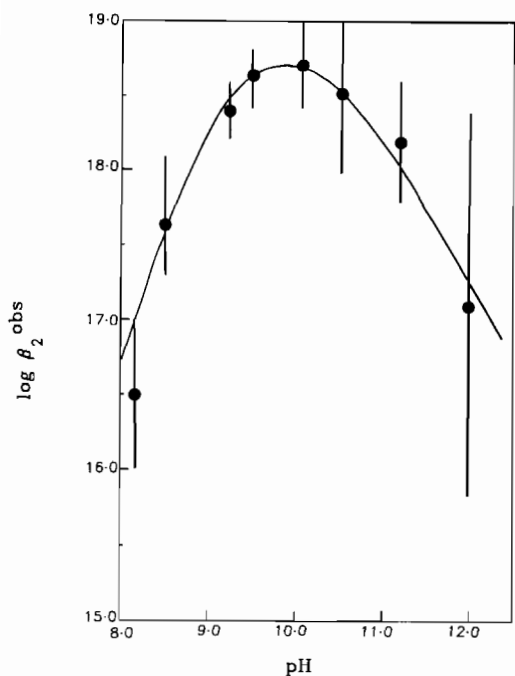
Fig. 6. Dependence of log β_2^{obs} (eqn. (11)) on pH for binding of cyanide by AHC. The solid line is a fit of eqn. (13) to the data and assumes that cobalt-bound hydroxide is inert. It was also assumed that equilibrium constants involving HCN are very much smaller than those involving CN^- and can be ignored. The overall stability constant, log $\beta_2 = 19.0 \pm 0.1$.

trend evident. The values of log β_2^{obs} increase between pH 8 and 10, and then decrease. The observed variation can be explained as follows. In the pH range 8–12, the cobinamide is present either as AHC or DHC. We assumed that cobalt-bound hydroxide is inert. Therefore, the inert species present are DHC and HCC, and the labile species are AHC and ACC (see Scheme 1). Furthermore, we assumed that equilibrium constants involving HCN are very much smaller than those involving CN^- and can be ignored. The acid dissociation constant for HCN, pK_L, is 9.04 at 25 °C [13]; the acid dissociation constant of ACC, pK_y, is 10.95 [14]; and log K_2 for binding of CN^- to ACC, as defined in Scheme 1, is 8 [15]. Accepting these approximations, it can be shown that the overall equilibrium constant, β_2, for binding of CN^- anion to AHC is given by eqn. (13).

$$\beta_2^{obs} = \frac{K_1 K_2 K_L^2 [H^+]}{(K_L + [H^+])^2 ([H^+] + K_{Co2})} \quad (13)$$

This equation was fitted to the experimental data with $(K_1 K_2)$ as the only adjustable parameter; the fit obtained, with $\log(K_1 K_2) = 19.0 \pm 0.1$ is shown in Fig. 6. The acceptable fit of the theoretical curve to the experimental data suggests (although does not prove) that the assumptions made are valid.
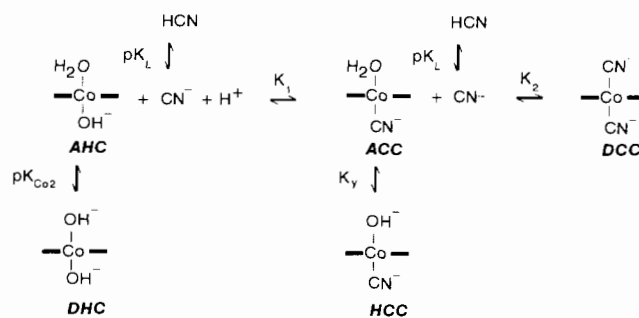
Scheme 1.

Since log $K_2 = 8$, we tentatively conclude that log K_1, for binding of a single CN^- anion to AHC, is 11. This may be compared to values of 14.1 for binding to aquacobalamin (i.e. trans to H_2O) [16], $\geqslant 14$ for binding to DAC [17], and 8 for binding to ACC [15]. The trans effect order as determined from equilibrium constants for binding of cyanide is therefore $H_2O \approx$ dmbzim $< OH^- < CN^-$.

Based on the results obtained with cyanide, we can therefore assume that cobalt-bound hydroxide is inert to substitution by other ligands. The values of log K_{exp} listed in Table 2 are therefore conditional stability constants since they will change with pH because of the inertness of DHC. A pH-independent log K value for the binding of a ligand, Z, to AHC can be obtained by using eqn. (14); log K values thus estimated are also listed in Table 2.

$$K = \frac{K_{exp}([H^+] + K_{Co2})}{[H^+]} \quad (14)$$

All equilibrium constants reported in this work are macroscopic equilibrium constants for binding of the incoming ligand, Z, to the two isomers of AHC expected to be in solution depending on whether OH^- is co-ordinated in the upper (β) or lower (α) face of the corrin ring. (It is known that the diastereomers in aquocyanocorrinoids are present in approximately a ratio of $\alpha:\beta = 2:1$ [18, 19]; it is also known that the microscopic equilibrium constants for binding of CN^- to the two diastereomers of AHC are similar [19].)

Further evidence for the placement of OH^- in the trans effect series is possible by considering the results for the other ligands, Z, used in this study. The values for the binding of a given ligand, Z, trans to a ligand X in the cobalt corrinoids are listed in Table 5 and the information is summarised as a scatter plot in Fig. 7. (Figure 7 presents visually the information of Table 5; the x-axis has no numerical value and the ligands are ordered on the basis of the average magnitude of the log K values for replacement of water by a variety of incoming ligands trans to the ligand on the x-axis.) These data therefore allow OH^- to be placed in the

TABLE 5. The thermodynamic *trans* effect in cobalamin chemistry. Stability constants (log K) are for the reaction $X-Co-H_2O + Z \rightleftharpoons X-Co-Z + H_2O$ and hence are a measure of the *trans* effect of X

Trans ligand X	Incoming ligand, Z			
	N_3^-	Pyridine	Imidazoles	Primary amines RNH_2
H_2O			imidazole 7.5[b]	R=H $\geqslant 9$[b]
dmbzim	4.9[e]		imidazole 4.59[f]	R=H 7[b] R=$CH_2CO_2^-$ 5.8[c]
OH^-	3.45[a]	4.19[a]	N-MeImidazole 6.06[a]	R=$(CH_2)_2CH_2OH$ 4.66[a]
CN^-	2.7[e]	2.6[b]	imidazole 4.1[g]	R=H 3.35[b]
SO_3^{2-}				R=H 0.6[b]
CH_3^-	< -1[e]	0.8[d]	imidazole 1.0[d] N-MeImidazole 0.7[d]	R=H -1[d] R=CH_2CH_2OH -1.5

[a]This work. [b]Ref. 2. [c]Ref. 20. [d]Ref. 4. [e]Ref. 12. [f]Ref. 3. [g]Ref. 21.
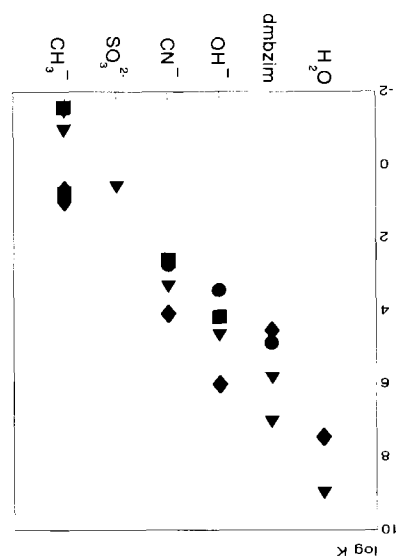

Fig. 7. The *trans* effect in cobalt corrinoids. Log K values for replacement of H_2O by azide (●), pyridine (■), imidazoles (♦) and primary amines (▲) *trans* to the ligand along the x-axis. The ordering of the ligands along the x-axis is on the basis of the average log K values.

trans effect series above H_2O and dmbzim, but below CN^-, SO_3^{2-} and an alkyl ligand such as methyl.

Acknowledgements

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