The Chemistry of Vitamin B_{12}. Part 29.[1] Co-ordination of Imidazoles and 1,2,4-Triazole by Aquacyanocobinamide

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Equilibrium constants K for the substitution of co-ordinated H_2O in the cobalt(III) corrinoid aquacyanocobinamide by imidazole, 1,2,4-triazole and other azoles have been determined in aqueous solution with I *ca.* 0.1 mol dm^{-3} at 25 °C by UV/VIS spectrophotometry. The results demonstrate that the azoles obey the linear free energy relationship $\log K = a\cdot pK + b$, where $a = 0.42$ and $b = 1.3$, over 12 pK units from 1,2,4-triazole (pK_b 2.3) to imidazolate (pK_a 14.3) and that determining the values of log K for just the four ligands imidazole, 1,2,4-triazole and their anionic forms (the latter *via* pK_c for deprotonation of the co-ordinated ligand) provides a general method for establishing the linear relationship and the values of a and b in labile complexes. It is shown that for kinetically inert complexes only the values of pK_c for deprotonation of co-ordinated imidazole and 1,2,4-triazole need to be determined in order to establish the linear relationship and to derive a value of a (but not of b), as in the case of $[Ru(NH_3)_5(azole)]^{3+}$ complexes where published values of pK_c can be used to derive a value of $a = 0.75$.

We have been studying the co-ordination of a range of nitrogen-containing bases by the water-soluble iron(III) porphyrin microperoxidase-8 (MP-8)[2–6] and cobalt(III) corrinoids.[7] Our main interest has been to establish the 'baselines' corresponding to the well-known linear relationship (1)[8] and to test for the occurrence of the so-called α effect.[9]

$$\log K = a\cdot pK + b \qquad (1)$$

We have now established the occurrence of the α-effect[5,6] and the existence of the linear relationship (1) for iron(III) MP-8 with primary amines[4] and with six-membered heterocycles (pyridines, pyrimidine and pyrazine)[5] and in the preceding paper[6] we have provided the first clear-cut demonstration with any metal ion both of relationship (1) for the five-membered azoles including imidazole (Him) and of systematic differences from the six-membered heterocycles. We have shown that a suitable span of pK values can be obtained by including, first, 1,2,4-triazole (Htz) and, secondly, the anionic forms of both Him and Htz (for pK values see Table 2 below), but the occurrence of other pH-dependent equilibria involving MP-8 in alkaline solution introduced sizeable errors into the values of log K for im$^-$ and tz$^-$. Confirmation with another metal ion, especially one which avoids such complications in alkaline solution, is clearly desirable.

Studies with iron(III) porphyrins and with cobalt(III) corrinoids are complementary. The cobalt corrinoids[10] allow one to study the effect of varying the Lewis acidity of the cobalt ion by changing the *trans* ligand but, although the basic corrin ring and its substituents (*i.e.* neglecting the nucleotide side-chain present in the cobalamin series) carry no functional group with pH-dependent equilibria which might interfere with the pH-dependent equilibria of the axial ligands, its crowded and non-planar structure might sterically hinder the co-ordination even of the unsubstituted py and Him. By contrast, the planar porphyrin ring in MP-8 offers minimal steric hindrance to any axial ligand but the second (proximal) axial ligand cannot readily be substituted and the pH-dependent equilibria of the terminal groups in the peptide side-chain introduce complications.

By comparing the published X-ray structural data of twelve corrinoids, Glusker and co-workers[11] have shown that the corrin ring can exhibit a variable degree of folding (2–22°) along the Co–C(10) line, thereby providing a mechanism for transmitting steric effects from one axial ligand to the other, and that this folding is greatest for the cobalamin series which possesses the bulky 5,6-dimethylbenzimidazole (dmbzim)† as one axial ligand. The effect of this folding can readily be seen by using the *trans* effect, which follows the order of increasing σ-donor power, *viz.* H_2O < dmbzim < CN^- < $HC{\equiv}C^-$ < $CH_2{=}CH^-$ < Me^-.[10a,12] The dependence of the values of log K for CN^- (with minimal steric requirements) and Him (the ligand of interest here) on the nature of the *trans*-ligand X are compared in Table 1 and Fig. 1. Diaquacobinamide (with X = H_2O) is not convenient for equilibrium studies because of kinetic slowness and the complexity of possible reactions (*cf.* refs. 7 and 14); we also find that the earlier qualitative value of log K of *ca.* 7 for the co-ordination of Him by the diaqua complex[14] needs to be corrected. Fig. 1 shows a linear relationship between the increase in log K with Him and with CN^- from X = Me through vinyl and acetylide to cyanide, *i.e.* any steric hindrance to the co-ordination of Him is insignificant in this region. For X = dmbzim, however, the observed value of log K for Him falls well below that expected by extrapolation of the linear portion,

Table 1 Effect of varying the *trans*-ligand X on the equilibrium constants K for the substitution of co-ordinated H_2O by CN^- and Him in cobalt(III) corrinoids

	log K	
trans-Ligand X	CN^-	Him
H_2O	16.6[a]	*ca.* 7.5[b]
dmbzim	14.1[c]	4.6[d,e]
CN^-	8.4[f]	4.1[g]
$HC{\equiv}C$	≥6.8[h]	3.4[h]
$CH_2{=}CH$	2.7[f,h]	1.3[i]
Me	2.1[f,h]	1.0,[j] 0.9[i,k]

[a] Ref. 13. [b] Ref. 14. [c] Ref. 15. [d] Ref. 16. [e] Ref. 17. [f] Ref. 12. [g] Ref. 18. [h] Ref. 19. [i] Ref. 20. [j] Ref. 21. [k] Ref. 22.

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† This is the base found in the nucleotide side-chain of cobalamins.

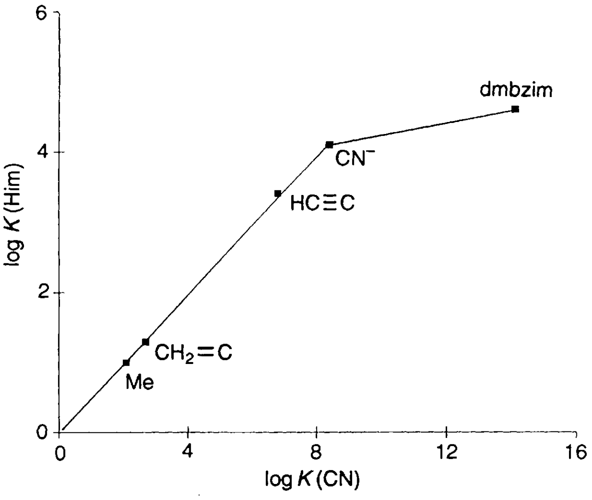

Fig. 1 Comparison of the effect of varying the *trans*-ligand X on log *K* for the co-ordination of Him (*y*-axis) and CN^- (*x*-axis). Data from Table 1

i.e. log *K* is significantly decreased exactly where the X-ray data show that steric hindrance to co-ordination will be greatest. Of the four corrinoids with X = Me, vinyl, acetylide and cyanide, the last is the easiest to prepare and gives the highest values of log *K* for co-ordination in the other axial position. Aquacyanocobinamide or Factor B, which possesses the basic corrin ring with seven amide side-chains, was therefore selected as the cobalt corrinoid with which to test and confirm equation (1) for the five-membered azoles.

Aquacyanocobinamide exists as an approximately equal mixture of the two isomers involving inversion of the axial ligands, which are fairly readily interconverted, especially in the presence of a trace of cyanide.[10b] It is therefore possible that a relatively bulky ligand such as Him may be preferentially co-ordinated in one of the two axial sites, but this possibility is ignored. The p*K* for conversion to the hydroxocyano complex is 11.0,[10c] and no other pH-dependent equilibria occur in the alkaline region. The cyanide is very firmly held with log *K ca.* 16 and the possibility of being displaced by a N-base can usually be ignored; because the Co–CN bond is light sensitive,[10d] however, reasonable precautions have to be taken against photolysis in acid solution where the rate of reformation of the Co–CN bond is severely reduced.

The aims of the paper are to demonstrate more unambiguously than was possible with MP-8 that im^- and tz^- can be treated as substituted azoles for the purposes of establishing the relationship in equation (1), to confirm that this relationship holds for the d^6 Co^{III} as well as the d^5 Fe^{III} ion, and to determine the relevant values of *a* and *b*. We also show that the relationship (1) can in principle be established and a value of *a* (but not of *b*) derived solely by determining values of the pK_c for proton loss from co-ordinated Him and Htz; this provides a method which could be applied to non-labile complexes where values of log *K* cannot readily be determined under equilibrium conditions.

In discussing recent work on proton affinities in the gas phase and in solution, Taft and co-workers commented[23] in 1988 that substituted pyridines had been the main subject of such studies during the previous decade but 'it is foreseeable that in the next decade azoles will replace pyridines as substrates for these investigations'. Likely developments can more readily be applied to co-ordination chemistry by establishing the main features of azoles as ligands towards metal ions.

Experimental

Materials.—Aquacyanocobinamide was prepared from cyanocobalamin (BDH) as previously described;[24] concentrations were determined in solution using the molar absorption coefficient of 2.4×10^4 dm^3 mol^{-1} cm^{-1} for the γ band (354 nm).[10e] 1,2,4-Triazole was obtained from Sigma, the other heterocycles from Aldrich; all were used as received.

Table 2 Equilibrium constants *K* for the substitution of co-ordinated H_2O in aquacyanocobinamide by azoles

Ligand	p*K*[a] of free base	log *K*[b]/ dm^3 mol^{-1}	λ_{max}[c]/ nm
Triazole	2.3	2.35	359.5
5-Chloro-*N*-methylimidazole	5.1	3.65	361
Imidazole	7.1	4.15	361
N-Methylimidazole	7.2	4.30	361
Triazolate	10.1	5.7	361.5
Imidazolate	14.3	7.25	363.5

[a] Values of p*K* taken from the preceding paper.[6] [b] Uncertainties in values of log *K* ±0.05 for neutral bases, ±0.2 for anionic tz^- and im^-. [c] Values ±0.5 nm.

Methods.—UV/VIS spectra were recorded and spectrophotometric titrations carried out on a Philips PU 8740 or 8720 spectrophotometer in cells of 1 cm pathlength (except where otherwise stated) thermostatted at 25 °C and containing *ca.* 5×10^{-5} mol dm^{-3} aqueous solutions of aquacyanocobinamide. For quantitative work the ionic strength was made *ca.* 0.1 mol dm^{-3} with 0.2 mol dm^{-3} phosphate buffers (pH *ca.* 8.5) or acetate buffer (pH 4.5). pH Measurements were made with a Hanna HI B417 pH meter and appropriate glass electrode, standardised at pH 4.00 and 9.00.

Results

The azole and azolate ligands studied here, together with their pK_a and pK_b values (taken from the preceding paper)[6] are listed in Table 2.

Preliminary experiments over the range of pH 4–10 and scanning the spectrum over the range 300–600 nm showed that all the bases reacted rapidly, reversibly and with good isosbestic points to give products with very similar spectra, and that the Htz complex showed a further reversible pH-dependent change involving a smaller change in spectrum with p*K ca.* 7 analogous to that observed for the Him complex with p*K ca.* 11 and assigned to proton loss from the co-ordinated azole ligand.[19] The spectra of cyanocobinamides with H_2O, Him and im^- as the second axial ligand are compared in Fig. 2. The wavelengths of the α, β and γ bands of these three species are 530, 498, 354 (H_2O), 550, 518, 361 (Him) and 559, 527, 363.5 nm (im^-) respectively.

Quantitative determinations of the equilibrium constants *K* were carried out by spectrophotometric titrations in duplicate experiments in acetate buffer (pH 4.5) for Htz or phosphate buffer (pH 8.5) for the other azoles, following the rise in absorption in the region of either the α- or γ-band of the product (*i.e. ca.* 550 or *ca.* 360 nm respectively, see Table 2 and Fig. 2). Analysis of the data confirmed a stoichiometry of 1.0 ± 0.1 base per Co in each case and gave the values of log *K* listed in Table 2.

The value of pK_c (for loss of the proton from the co-ordinated ligand) of the Him complex was determined in duplicate experiments by pH titration using concentrated NaOH solution from pH 8 to 13.5 in a 4 cm cell containing 1×10^{-5} mol dm^{-3} aquacyanocobinamide and (initially) with *I ca.* 0.1 mol dm^{-3}, following the rise in absorption at 565 nm (see Fig. 2). Using graphical extrapolation to obtain the initial (A_0) and final (A_∞) values of A_{565}, analysis of the changes in A_{565} (after correction for dilution) with pH corresponded to an equilibrium involving one proton with $pK_c = 11.2 \pm 0.1$ (see Fig. 3). Similar duplicate titrations were performed on the Htz complex from pH 4 to 11, following the rise in A_{555}, to give an equilibrium

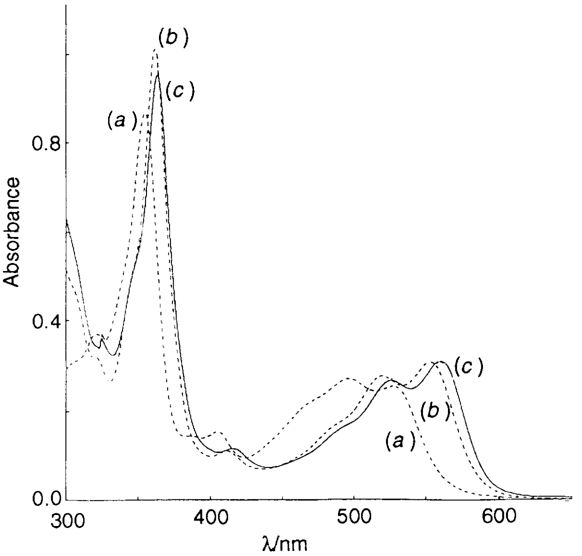

Fig. 2 Comparison of the UV/VIS spectra of cyanocobinamides where the second axial ligand is H_2O (*a*), Him (*b*) or im^- (*c*)

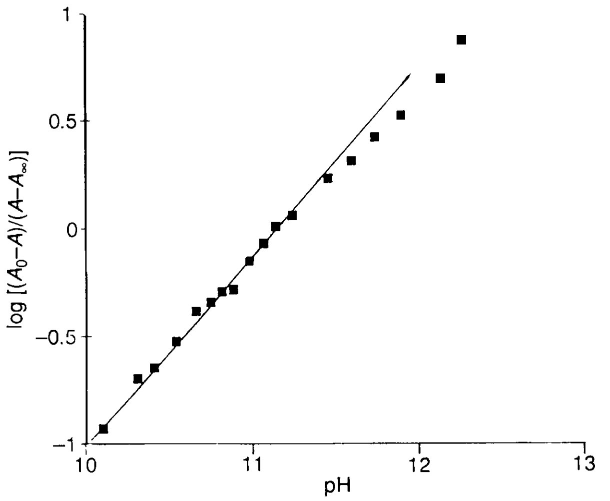

Fig. 3 Analysis of the pH titration of the Him complex of aquacyanocobinamide to establish the involvement of one proton (solid line corresponds to a slope of $n = 0.9$ protons) and the value of pK_c (=pH at $y = 0$)

involving one proton with $pK_c = 6.8 \pm 0.1$. The value of $\log K'$ (*i.e.* $\log K$ for the co-ordination of the conjugate base) was then obtained by using equation (2), together with the values of $\log K$

$$\log K' = \log K + pK_a - pK_c \qquad (2)$$

for the neutral azole and the pK_a for the free base listed in Table 2, to give the values of $\log K$ for im^- and tz^- listed in Table 2.

Qualitative tests were also carried out for the possible formation of bridged dimers involving the two end-members of the series, *viz.* Htz (which possesses two pyridinic N atoms which are both able to act as donor atoms) and im^- (Him can, of course, act as a bridging ligand only in its anionic form); varying the Co concentration over a 10-fold range (from low concentration in 4 cm cells to high concentration in 1 cm cells) under conditions of pH and ligand concentration expected to give *ca.* 50% formation of the complex in equilibrium with *ca.* 50% of the aqua or hydroxo complex (for Htz and im^-) led to no significant changes in the spectra, *i.e.* provided no evidence for a cobalt-dependent monomer–dimer equilibrium.

Discussion

The values of $\log K$ determined for the co-ordination of four uncharged azoles (by direct equilibrium studies) and the two azolate anions tz^- and im^- [indirectly from the pK_c for ionisation of the co-ordinated azole *via* equation (2)], together with the pK of the free base and the wavelengths of the main absorption band of the product are listed in Table 2. For reasons discussed in the preceding paper,[6] the values of $\log K$ and pK have not been corrected for so-called 'statistical factors'.[25,26] Our values of $\log K = 4.2$ for Him and $pK_c = 11.2$ for the Him complex should be compared with the previously reported values of 4.1 and 11.4[18] and the more qualitative values of 4.7 and *ca.* 11.[14] All these equilibria were rapidly established and reversible, good isosbestic points were observed, and no slow side-reactions were noted over the time-scale of the experiments. All the equilibria correspond to one ligand or one proton per Co. No evidence was found for any dimeric complex involving a bridging Htz or im^- ligand and the same was assumed for tz^-. All the products show remarkably similar UV/VIS spectra and the wavelength of the γ band moves from *ca.* 360 to *ca.* 363 nm when Htz is replaced by im^-, which is similar to the shift from 404 to 407 nm observed with the iron(III) porphyrin MP-8.[6]

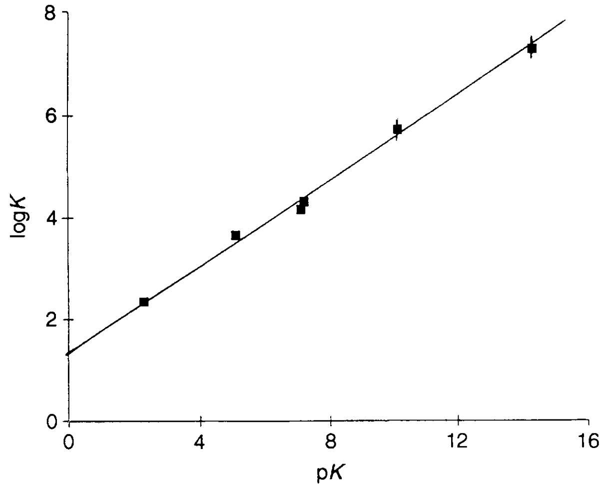

Fig. 4 Plot of $\log K$ (for co-ordination) *vs.* pK (for protonation of the free base) for azoles. Data from Table 2. The solid line corresponds to $\log K = 0.42\,pK + 1.3$

The values of $\log K$ for co-ordination to the Co^{III} ion are plotted against the pK of the free base towards the proton in Fig. 4. They show a good linear dependence of $\log K$ on pK in agreement with the relationship (1) where $a = 0.42$ and $b = 1.3$, compared to the values of $a = 0.34$ and $b = 0.21$ reported for MP-8 in the preceding paper.[6] They establish that the linear relationship (1) for five-membered azoles is not unique to the d^5 Fe^{III} ion.

The ligands in Table 2 and Fig. 4 therefore provide a series of sterically invariant probes for testing the relationship (1) for five-membered heterocycles with other metal ions in aqueous solution; in practice one need only use the two parent azoles (Htz and Him) and their derived anions, which together cover the whole range of 12 pK units from Htz (pK_b 2.3) to im^- (pK_a 14.4). In the present experiments with aquacyanocobinamide and MP-8 the bases *N*-methylimidazole and its 5-chloro analogue have served the purpose of testing the effects, firstly, of *N*-Me substitution (obviously minimal, as already found for protonation[27]) and, secondly, of conventional ring substitution. The interesting ligand 4(5)-nitroimidazole ($4NO_2$-Him) (*cf.* its effects on the stability of different valencies in the iron pentacyanide complexes[28]) with pK_b *ca.* −0.1 and pK_a *ca.* 9.2[29,30] is, unfortunately, too insoluble in its neutral form for general use in aqueous solution.[31] Where, however, azoles are studied in organic solvents and the anionic tz^- and im^- are inaccessible as ligands, the inclusion of 4-nitroimidazole could serve (if shown to belong to the linear sequence in Fig. 4) to increase the range of available azoles to give a span of over 7 pK units from Him (pK 7.1) down to 4NO$_2$-Him (pK −0.1).

It is possible to rearrange equations (1) and (2) to provide a method which is in principle applicable to substitution-inert complexes, where it is difficult or impossible to obtain values of log K under equilibrium conditions, and can provide values of the slope a (but not the intercept b) in the relationship (1). One can use equation (1) to replace log K_{HB} and log K_{B^-} in equation (2) to give equation (3) from which $pK_a - pK_c = a(pK_a - pK_b)$ which can be rearranged to give equation (4).

$$pK_c = (a{\cdot}pK_b + b) - (a{\cdot}pK_a + b) + pK_a = a(pK_b - pK_a) + pK_a \quad (3)$$

$$a = (pK_a - pK_c)/(pK_a - pK_b) \quad (4)$$

The value of a can then be derived, *via* the application of equation (4) and *assuming* the existence of the linear relationship (1), from the experimentally determined value of pK_c for a single complex containing an azole (*e.g.* Him), while the derivation of similar or identical values of a from the pK_c values for the pair of azole complexes (with Him and Htz) would provide direct evidence that the azoles do indeed obey relationship (1) for that particular metal ion.

The complexes $[Ru^{III}(NH_3)_5(HB)]^{3+}$ provide one of the few cases where pK_c values have been determined for both the Him and Htz derivatives. The values of $pK_c = 8.9$[32] and *ca.* 4.3[33] reported for the Him and Htz complexes in ionic strength $I < 3 \times 10^{-3}$ and 0.1 mol dm^{-3} respectively can be converted, using equation (4) and the values of pK_a and pK_b listed in Table 2, into values of $a = 0.75$ and 0.74. These values of a are self-consistent and, in spite of the difference in ionic strength, it seems reasonable to conclude that they demonstrate both the validity of equation (1) for azoles with this Ru^{III} ion and the utility of a method which can be applied to substitution-inert complexes.

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