The Ligand Substitution Reactions of Hydrophobic Vitamin B₁₂ Derivatives. Reaction of Cobyric Acid Heptapropyl Ester with Heterocyclic *N*-donor Ligands

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ABSTRACT

The hydrophobic cobyrinic acid heptapropyl ester corrinoids XCbs-Pr (axial ligand X = CN^-, SO_3^{2-}, CH_3^- and $CH_3CH_2^-$) have been prepared from vitamin B₁₂ by hydrolysis of the amide side chains and their conversion to propyl esters. Both the position of the γ-band and the general shape of the UV-visible spectra of these complexes show significant solvent dependence as the polarity of the solvent is varied. The equilibrium constants, *K,* for the reaction of five-membered heterocyclic nitrogenous bases (the azoles imidazole, pyrazole and 1,2,4-triazole) with displacement of coordinated H₂O in aquacyanocobyrinic acid heptapropyl ester, and coordination by the predominantly five-coordinate complexes sulphitocobyrinic acid heptapropyl ester, ethylcobyrinic acid heptapropyl ester and methylcobyrinic acid heptapropyl ester, have been determined spectrophotometrically at 25 °C in water, methanol, acetonitrile, ethyl acetate and toluene. Values of *K* are dependent on the identity of the *trans* ligand (X = CN^- > SO_3^{2-} > CH_3^- > $CH_3CH_2^-$); they increase with the basicity of the azole (pyrazole < 1,2,4-triazole < imidazole); and they increase as the solvent polarity increases (toluene < ethyl acetate < acetonitrile < methanol < H₂O). Molecular mechanics calculations suggest that these effects are largely electronic in origin.

KEYWORDS

Hydrophobic vitamin B₁₂, cobalt corrinoids, equilibrium constants, solvent polarity, *trans* influence.

1. Introduction†

Many studies on the ligand substitution reactions of hydrophilic cobalt (III) corrinoid complexes (vitamin B₁₂ derivatives) in aqueous solutions have been conducted over the past thirty years (see for example refs. 1–8). Very few studies have been performed in non-aqueous solvents. After converting the seven amide side chains of the naturally-occurring hydrophilic corrinoids into esters, the resulting compound, often referred to as the cobester (Fig. 1),[9] is soluble in a range of solvents of varying polarity, affording the possibility of determining the effect of the polarity of the solvent on the equilibrium constant for ligand substitution. There is a drawback, however. Since the corrin does not have a plane of symmetry, cleavage of the axial base 5,6-dimethylbenzimidazole from the corrin as is found in cyanocobalamin (CNCbl, vitamin B₁₂) itself and in aquacobalamin (H₂OCbl⁺) on forming a cobester means that diastereoisomerism can occur if the two axial ligands are not identical.[10–12] The 'upper', or β-face, of a corrinoid, with its upward projecting *a, c* and *g* acetamide (or ester) side chains, is less sterically hindered than the 'lower', or α-face, which is bracketed by the downward projecting *b, d* and *e* propionamides

and the secondary amide *f* side chain. The ratio between the two diastereomers of cyanoaqua complexes depends on a number of factors, including the presence or absence of the aminopropanol moiety in the *f* side chain of the corrin, and the number of carboxylic acid groups in the side chains of the corrin, presumably because of hydrogen bonding with coordinated H₂O.[13,14] The ratio α-cyano-β-aqua:α-aqua-β-cyano varies from about 2.5 to about 0.25; when all carboxylic acids are protonated (pH < 3), the α-cyano-β-aqua isomer is favoured (50–70 %), i.e. it appears that in the absence of hydrogen bonding moieties in the side chains, the sterically less demanding CN⁻ ligand tends to favour the more sterically crowded α face.

The properties of a corrin are affected by the solvent[15,16] as has been shown, for example, for the electronic spectra of (CN)₂Co(III) corrinoid and of (CN)₂Co(III)[17] tetrahydrocorrinoid complexes.[18] Markwell *et al.*[19] examined the effect of solvent polarity on the electronic absorption spectra of dicyanocobyrinic acid heptamethyl ester and found that the γ-band changed position from 374 nm in non-polar solvents (benzene and CCl₄), through 371 nm in more polar solvents (MeCN and ethyl acetate) to around 368 nm in protic solvents (H₂O and ROH), which changes were accompanied by increases in the CN stretching frequency. In the solid state, at least, one of the axial CN⁻ ligands appears to be hydrogen-bonded to an isopropanol solvent molecule. We suggest that these observations indicate that solvent polarity has a direct influence on the electronic structure of cobalt corrinoids, with polar solvents favouring bonding that is more ionic in nature between the metal and the axial ligand, while less polar solvents favour bonding that is
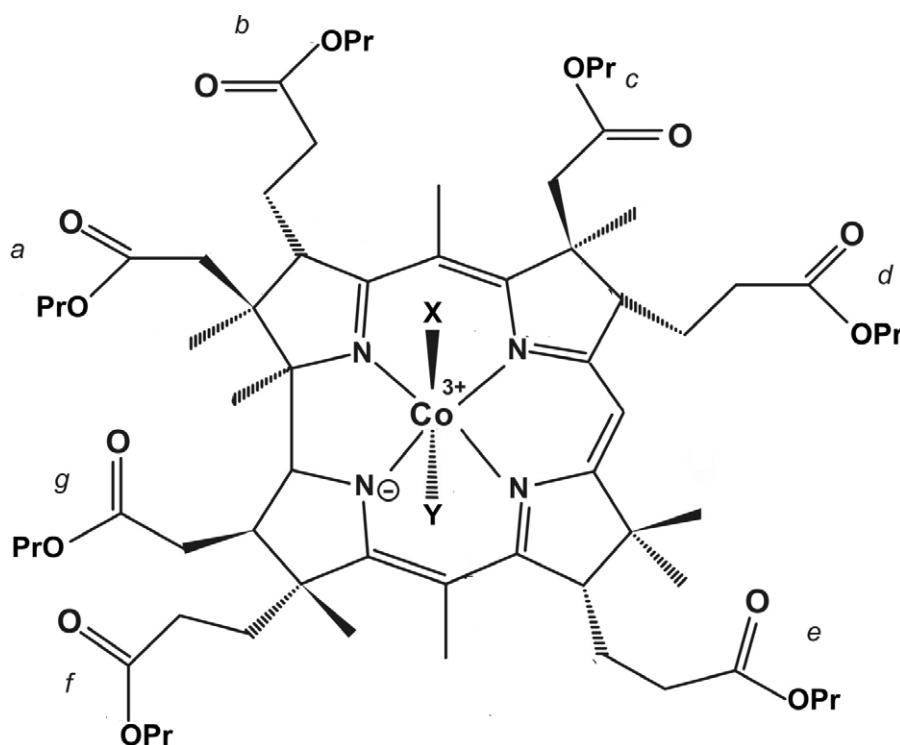
Figure 1 Cobyric acid heptapropyl ester. The face shown containing X as axial ligand is call the β face; the opposite face is referred to as the α face.

more covalent in nature, and a delocalization of electron density from the axial ligands onto the corrin ring (electronic communication between the axial ligands and the corrin delocalized electron system has been demonstrated).[20–23]

It is very likely that hydrogen bonds play an important role in the chemistry of the porphinoid complexes. They control the solubility of the cobalamins and are thought to play a key role in the way cobalamins interact with the protein in the B[12]-dependent enzymes and transport proteins.[24–26] Hydrogen bonding by aminoacid residues to the proximal His ligand of some haemoproteins, conferring on the proximal His ligand some imidazolate character, may be a way of controlling the properties of the iron porphyrin, and favouring, for example, the attainment of higher oxidation states in the catalytic cycle of the catalases and the peroxidases.[27,28]

The reactions of azoles with metallocorrins and metalloporphyrins are of interest because firstly, the imidazole ring of His occurs as a ligand in many haemoproteins[29] and secondly, because these five-membered heterocycles have been successfully used in several agricultural and medical fungicides, in which they inhibit steroid synthesis by acting as ligands to the Fe(III) porphyrins in P-450 enzymes.[30,31] The coordination chemistry of the azoles has also attracted significant interest because these compounds can be used to link studies on the metal binding properties of nitrogenous bases[32] with information on their proton affinities in the gas phase,[33,34] in aqueous solution and aprotic solvents[35,36] and on their H-bonding capabilities.[37] We have determined spectrophotometrically equilibrium constants for the coordination of pyridine and its 4-substituted derivatives by the five-coordinate sulphitocobyrinic acid heptamethyl ester (SCbs-Me) in six solvents[38] and found that the solvent polarity has an influence both on the UV-visible spectrum of SCbs-Me and on the equilibrium constants for the formation of the six-coordinate XSCbs-Me. We have now extended these studies to the reaction of the hydrophobic corrinoids XCbs-Pr (axial ligand X = CN$^-$, SO$_3^{2-}$, CH$_3^-$ and CH$_3$CH$_2^-$) with three azoles and report our results in this paper.

2. Results and Discussion

2.1. Electronic Spectra of Hydrophobic Corrinoids

Varying the polarity of the solvent is known to have a significant effect on the d-d transitions of the [Co(CN)$_6$]$^{3-}$ ion (the main transition shifts from 311 nm in H$_2$O, to 318 nm in MeCN and to 321 nm in DMSO) with accompanying changes in the stretching wavenumber of coordinated CN$^-$ (from 2127 cm^{-1} in H$_2$O to 2111 cm^{-1} in DMSO).[39] The observed effects are clearly due to direct interaction between the solvent and the coordinated cyanide in the hexacyanides. Similar effects are also observed in corrins. For example, titration of ACCbs-Me in CCl$_4$ with MeOH showed that one MeOH molecule hydrogen bonds to an axial ligand.[40] There is crystallographic evidence[19] that coordinated CN$^-$ in DCCbs is hydrogen-bonded to an isopropanol solvent molecule. Hydrogen bonding to CN$^-$ in DCCbs is associated with a shift in λ_γ from 374 nm (CCl$_4$) to 369.9 nm in *ca.* 20 % MeOH for one hydrogen bond ($K = 7$ dm^3 mol^{-1}) and to < 368 nm (in H$_2$O) on the formation of a second hydrogen bond. Similar effects were found with DCCbs-Pr, with the γ-band at 366 and 368 nm in water and acetonitrile, respectively, but at 370 nm in toluene. These changes are accompanied by a shift in intensity from the β to the α band in the visible region. We found in this work that while ACCbs-Pr (prepared in quantitative yield from DCCbs-Pr as described below) is only sparingly soluble in toluene, a sufficiently high concentration could be dissolved to conduct spectrophotometric titrations. Figure 2A shows that as the polarity of the solvent increases (toluene < acetonitrile < H$_2$O) the γ-band shifts to longer wavelength (352, 358 and 360 nm, respectively) while there is a concomitant increase in intensity of the longer wavelength band in the α,β region.

As might be expected, the UV-visible spectra of SCbs-Pr (Fig. 2B) are similar to those of SCbs-Me that we have reported previously.[38] Since all known six-coordinate corrinoids are red or purple with the $\alpha\beta$ bands above 500 nm, we assume that the spectra of the yellow complex (Fig. 2B) indicate that it is predominantly present as a five-coordinate species. The shift in the $\alpha\beta$
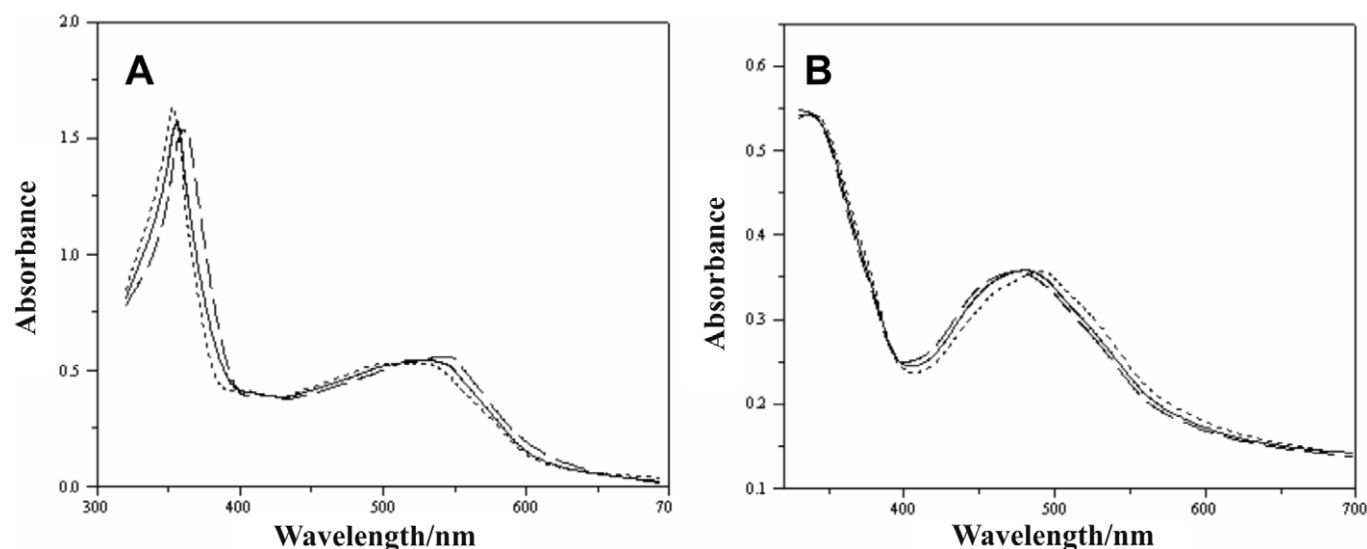
Figure 2 UV-visible spectra of (A) ACCbs-Pr and (B) SCbs-Pr in H_2O ($\cdots$), acetonitrile (—) and toluene (– – –).

bands to longer wavelength in aqueous solution (in contrast to the shift in these bands to shorter wavelength in the case of ACCbs-Pr) suggests that in this solvent some of the six-coordinate aqua-sulphito species is also present.

2.2. Equilibrium Studies

Our molecular modelling of ACCbs-Pr showed that the α-cyano-β-aqua complex is marginally more stable (by 1.2 kJ mol^{-1}) than its diastereomer; we therefore predict that the ratio of α-cyano-β-aqua:α-aqua-β-cyano isomers of ACCbs-Pr is 1.6. This observation is in general agreement with the experimental evidence[13,14] that, in the absence of hydrogen bonding effects (see Introduction), the sterically less demanding CN$^-$ ligand occupies the more sterically crowded α site. Interestingly, though (but it must be emphasized that the energy differences are small and that the modelling was performed *in vacuo* so that solvent effects are absent), we find that the reason for the small preference for the α-cyano-β-aqua isomer is largely due to more favourable torsions (by 1.6 kJ mol^{-1}) which is offset somewhat by less favourable non-bonded interactions (0.50 kJ mol^{-1}). We conclude that the log K values that we report in this work are composite values since the reactant ACCbs-Pr is expected to be an approximately 2:1 mixture of two diastereomers.

The reaction between a corrinoid complex and an azole, L, in which L displaces coordinated H_2O trans to the inert ligand, $X(X = CN^-)$ or occupies a trans position that is predominantly vacant because of the strong trans influence of X ($X = SO_3^{2-}$, CH_3^- and $CH_3CH_2^-$) can be represented by Eq. 1, in which the structure of Fig. 1 is represented schematically. This reaction between three azoles (imidazole (ImH), pyrazole (Pz) and 1,2,4-triazole (Tz)) and four hydrophobic corrinoids (ACCbs-Pr, SCbs-Pr, MeCbs-Pr and EtCbs-Pr) was studied in five different solvents (water, methanol, acetonitrile, ethyl acetate and toluene).
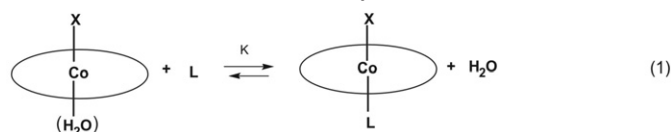
A preliminary examination of the reaction of XCbs-Pr in H_2O or in organic solvents showed that the equilibria are established rapidly with well-defined isosbestic points observed while scanning the spectrum in the 300–700 nm range (Fig. S1 of the Supplementary Material gives a typical example). The γ band of the

product (L)(CN)Cbs-Pr in H_2O is found at 358, 360 and 361.5 nm for L = Pz, Tz and ImH, respectively; hence the γ-band shifts to longer wavelength as the basicity of L increases. A similar trend was observed in other solvents with small changes in the position of the main absorption bands, for reasons discussed above.

2.3. Reactions with ACCbs-Pr

Figure 3, as an example, shows data obtained for the titration of ACCbs-Pr with Tz in aqueous solution. The solid line is a non-linear least squares fit of Eq. 2 (see below) to the data, from which the value of K can be obtained.

A plot of $\log[(A_0 - A_x)/(A_x - A_\infty)]$ against log [Tz] gave a good straight line (Fig. S2) with slope $n = 1.04 \pm 0.03$, confirming that a single Tz ligand is coordinated by Co(III) in the process shown in Fig. 3. Similar results were obtained with all other ligands in all solvents studied.

The values of log K were found to be 2.65 ± 0.02, 2.80 ± 0.02 and 3.57 ± 0.02 for the reactions of Pz, Tz and ImH with ACCbs-Pr in aqueous solution, respectively. Thus, log *K* increases as the basicity of the ligand increases (pK_a of Pz, Tz and ImH = 2.48, 2.6 and 7.24[41]). A linear dependence of log *K* on ligand p*K* is well-established in cob(III) alamin and iron(III) porphyrin chemistry.[42–44]
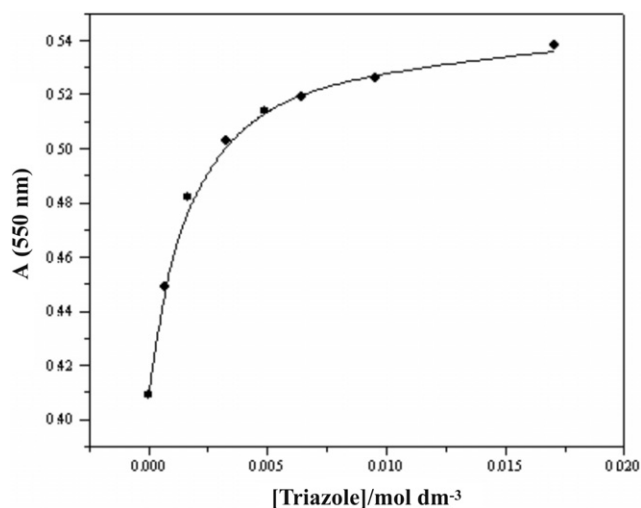

Figure 3 The titration of ACCbs-Pr with Tz in aqueous solution at pH 9 (25 °C, I = 0.1 mol dm^{-3}) in aqueous solution monitored at 550 nm.
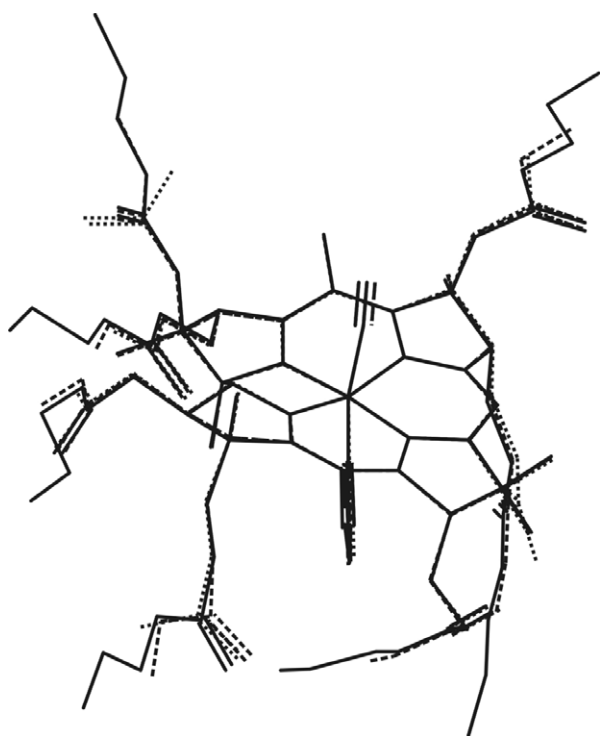
Figure 4 Overlay of the structures of (ImH)(CN)Cbs-Pr (—), (ImH)(CN) Cbs-Me (- - -) and (ImH)(CN)Cbi (· · ·) discovered by molecular dynamics/simulated annealing calculations.

Interestingly, we note that the values of log K for coordination of ImH to ACCbi (amide side chain),[45,46] ACCbs-Me (methyl ester side chain)[47] and ACCbs-Pr (propyl ester side chain) are 4.14, 3.95 and 3.57, respectively; this suggests that increasing the bulk of the side chains affects the ability of the metal to coordinate the ligand. However, molecular mechanics calculations of the (α-CN$^-$)(β-ImH) complexes *in vacuo* show that all three structures are very similar (Fig. 4), with the side-chains orientated away from the axial ligands. The change in strain energy for the reaction

ImH + (H$_2$O)(CN$^-$)Cbx → (ImH)(CN$^-$)Cbx + H$_2$O, x = i, s-Me, s-Pr
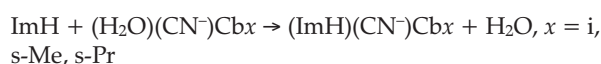
differs by <2 kJ mol^{-1} between the complexes (similar results were obtained for the diastereomeric (α-ImH)(β-CN$^-$) complexes). The axial bond lengths to CN$^-$ (1.925 ± 0.001 Å) and ImH (2.039 ± 0.001 Å) differ by at most 0.001 Å, as do the other bond lengths within the coordination sphere of the metal. All bond angles within the metal coordination sphere differ by < 1 °. This suggests, therefore, that differences in log K values are not a consequence of significant structural differences between the ground states of the complexes (assuming that the simulated annealing procedure found the energy minimum structures). It seems likely that many factors such as differential solvation of

the complexes and hindrance of approach by the incoming ligand to the metal coordination sphere by rotation of the side chains contribute to differences in log K values. We plan to investigate these factors fully, and will report on our findings elsewhere, but we note that others have attributed such differences to, for example, the nature of the microenvironment of the corrinoid in solution.[48,49]

Analogous spectrophotometric titrations were carried out in four other solvents. Similar spectral changes to those that occurred in aqueous solution were observed and values of K were determined as before. These are listed in Table 1.

Two trends emerge for log K values for the reaction of azoles with ACCbs-Pr. Firstly, log K increases as the basicity of the azole increases (Pz < Tz < ImH); secondly, log K increases as the polarity of the solvent increases; log K in toluene (E$_T$(30) = 33.9 < ethyl acetate, 38.1 < acetonitrile, 46 < methanol, 55.5 < H$_2$O, 63.1). These results are in broad agreement with Rillema's suggestions regarding the importance of dipole-solvent interactions in determining ligand-binding equilbria in non-aqueous solvents.[49]

2.4. Reactions with SCCbs-Pr, MeCbs-Pr and EtCbs-Pr

As with ACCbs-Pr, the three azoles reacted rapidly with SCbs-Pr, MeCbs-Pr and EtCbs-Pr in the five different solvents used during the course of this work, with well-defined isosbestic points. Typical spectral changes accompanying the spectrophotometric titration of SCbs-Pr with ImH in ethyl acetate, MeCbs-Pr with Pz and EtCbs-Pr with ImH (both in H$_2$O) are shown in Figs S3–S5 in the online Supplementary Material. Values of log K obtained as described above are listed in Table 1. Similar trends to those observed with ACCbs-Pr are evident: log K values increase with ligand basicity and with solvent polarity. The data also show that the values of log K for each of the three azoles depend on the nature of the *trans* ligand and vary in the order X = CN$^-$ > SO$_3^{2-}$ > CH$_3^-$ > CH$_3$CH$_2^-$; hence, as has been demonstrated before with other cobalt corrinoids, log K values decrease as the polarizability of the *trans* ligand increases.[9] This is clearly an electronic effect. To determine to what extent steric effects play a role, we examined the energy changes accompanying the reaction of the azoles with EtCbs-Pr using molecular mechanics methods.
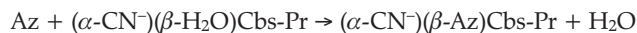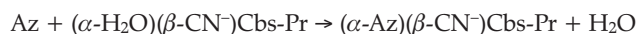
There is strong and long-standing evidence to suggest that an alkyl complex of an incomplete corrinoid (i.e. one lacking the 5,6-dimethylbenzimidazole base found in the cobalamins such as vitamin B$_{12}$ itself) exists predominantly, if not exclusively, as the β-alkyl complex;[9,50–52] the α position is either vacant or, in aqueous solution, occupied by H$_2$O (it is likely that the complexes exist as equilibrium mixtures of the five- and six-coordinate species). We found in the molecular mechanics modelling that α-Et-β-H$_2$OCbs-Pr is 6.03 kJ mol^{-1} higher in energy than α-H$_2$O-β-EtCbs-Pr, which means that at 25 °C the species is 92 % in the latter form. The difference in energy stems largely from

Table 1 Logarithms of the equilibrium constants (in dm^3 mol^{-1}) for the coordination of XCbs-Pr by azoles, L, in different solvents at 25 °C.

X	Solvent														
	Water L			Methanol L			Acetonitrile L			Ethyl acetate L			Toluene L		
	Pz	Tz	ImH	Pz	Tz	ImH	Pz	Tz	ImH	Pz	Tz	ImH	Pz	Tz	ImH
CN$^-$	2.65(2)	2.80(2)	3.57(2)	2.60(2)	2.77(2)	3.52(2)	2.42(2)	2.69(2)	3.45(2)	2.29(2)	2.53(2)	3.38(2)	2.03(3)	2.44(2)	3.30(2)
SO$_3^{2-}$	1.71(1)	1.97(1)	2.52(2)	1.56(1)	1.91(2)	2.39(2)	1.36(2)	1.69(2)	2.21(2)	1.08(3)	1.48(1)	2.03(2)	0.88(3)	1.15(3)	1.81(1)
CH$_3^-$	1.48(2)	1.64(2)	2.35(2)	1.34(2)	1.51(3)	2.21(2)	1.11(1)	1.41(2)	2.02(2)	0.90(2)	1.20(3)	1.72(2)	0.78(2)	0.95(2)	1.56(2)
CH$_3$CH$_2^-$	1.26(2)	1.43(2)	2.13(2)	1.15(3)	1.36(2)	1.91(2)	0.85(3)	1.25(2)	1.65(1)	0.60(2)	0.90(2)	1.32(2)	0.40(2)	0.72(2)	1.08(3)

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M.S.A. Hamza, M.A. Elawady and H.M. Marques,
S. Afr. J. Chem., 2008, **61**, 68–73,
<http://journals.sabinet.co.za/sajchem/>.

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more unfavourable non-bonded interactions in α-Et-β-H$_2$OCbs than in its diastereomer, confirming the view (see above) that the lower face is more sterically crowded. (A similar result is obtained if the complex is modelled as the five-coordinate alkyl complex; in that case the energy difference is 9.25 kJ mol^{-1} and the β-Et isomer is therefore predicted to account for some 98 % of the species.)

We examined the energy changes accompanying the reactions

$$Az + (\alpha\text{-H}_2O)(\beta\text{-CN}^-)Cbs\text{-Pr} \rightarrow (\alpha\text{-Az})(\beta\text{-CN}^-)Cbs\text{-Pr} + H_2O$$

$$Az + (\alpha\text{-CN}^-)(\beta\text{-H}_2O)Cbs\text{-Pr} \rightarrow (\alpha\text{-CN}^-)(\beta\text{-Az})Cbs\text{-Pr} + H_2O$$

where Az = ImH, Pz, Tz, and where in each case the two molecules were placed far apart (*ca.* 20 Å) so as not to interact significantly with each other. We then estimated log K_M, an equilibrium constant based on the equilibrium species distribution from the modelling, as log K_M = log[$\Sigma[x_{Prod}]/\Sigma[x_{React}]$] where x is the fraction of each species determined from a Boltzmann distribution at 25 °C (Table S1 in the Supplementary Material). The values of log K do not differ greatly amongst the three azoles (as might be anticipated given their similar molecular topology and considering that electronic factors are not explicitly taken into account in molecular modelling calculations). This confirms, therefore, that the observed variation in log K values (Table 1) is indeed largely electronic in origin.

3. Conclusions

This work provides further evidence that hydrogen bonding between axial ligands in Co(III) corrinoid systems and solvent molecules leads to small, but discernible, perturbations in the UV-visible spectra of these complexes.

The values of log K for the coordination of the three azoles were found to depend on the polarizability of the *trans* ligand (CN$^-$ > SO$_3^{2-}$ > CH$_3^-$ > CH$_3$CH$_2^-$). The values of log K increase as the basicity of the entering azole increases in all solvents studied (pyrazole < triazole < imidazole) and as the polarity of the solvent increases (toluene < ethyl acetate < acetonitrile < methanol < H$_2$O). Molecular mechanics calculations suggest that in the case of aquacyano-Cbs-Pr, both reactants and products exist as diastereomeric mixtures, while in the case of aquaethyl-Cbs-Pr, the alkyl ligand occupies virtually exclusively the upper (β) face of the corrin ring. The calculations also confirm that the dependence of log K on ligand basicity is largely electronic in origin.

4. Experimental

4.1. Materials

Vitamin B$_{12}$ (cyanocobalamin, Sigma-Aldrich, St Louis, MO, USA), and pyrazole, 1,2,4-triazole and imidazole (Sigma-Aldrich) were used as received. Phosphate and acetate (Merck, Darmstadt, Germany) were used for buffers in the pH range 5–9. All the solvents used were of analytical grade, supplied by Fisher (Loughborough, UK) or Merck and used as received.

4.2. Methods

All UV-visible spectrophotometric work was performed on a pre-calibrated single beam UV-visible spectrophotometer (Helios Beta, Unicam, Cambridge, UK) equipped with 1 cm pathlength quartz cuvettes thermostatted at 25.0 ± 0.1 °C with a water-circulating bath. All pH measurements were made on a E.D.T. model GP353 pH-mV meter (Dover, Kent, UK) equipped with an E.D.T. combination glass electrode with an accuracy of ± 0.01 pH units and calibrated against standard buffer solutions (pH 4 and 7).

4.3. Synthesis

Dicyanoheptapropyl cobester (DCCbs-Pr) was prepared and purified as described previously,[16,53] by refluxing vitamin B$_{12}$ in 1-propanol containing 1.0 mol dm^{-3} H$_2$SO$_4$ under N$_2$ for 4 days. The reaction mixture was concentrated, diluted with water, neutralized with NaHCO$_3$ and treated with excess KCN to give DCCbs-Pr. DCCbs-Pr was extracted first with CCl$_4$ then CH$_2$Cl$_2$ and the solvents were removed.

The aquacyanoheptapropyl cobester (ACCbs-Pr) was prepared as described previously for aquacyanocobinamide and aqua-cyanoheptamethyl cobester.[54] The dicyano cobester was dissolved in methanol to which CH$_3$COOH was added to adjust pH to *ca.* 3; a stream of nitrogen was passed through the solution for *ca.* 24 h to liberate HCN.

Diaquaheptapropyl cobester (DACbs-Pr) was prepared as has been previously described for diaquacobinamide.[55] A solution of ACCbs-Pr was adjusted to pH 2–3 with CH$_3$COOH and was poured into the space of an annular photolysis cell. Photolysis with a 60 W globe led to the liberation of HCN. After about 3 h the sample of DACbs-Pr was carefully neutralized with dilute sodium hydroxide.

Sulphitocyanoheptapropyl cobester (SCCbs-Pr) was prepared by reacting excess sodium sulphite with (DACbs-Pr) as described previously for sulphitocobinamide[55] and the analogous hepta-methyl ester.[38] The colour of the orange-red DACbs-Pr changed to yellow within a few seconds after adding sodium sulphite.

The methyl and ethyl cobyrinic acid heptapropyl esters were prepared as described previously[56] for methyl- and ethyl-cobinamide. DACbs-Pr was reduced by NaBH$_4$ under a N$_2$ atmosphere to give the yellow cob(II) ester which slowly changed to the grey-green cob(I) ester. Methyl iodide or ethyl iodide were added dropwise and the solution changed colour to yellow within a few minutes.

The purity of all compounds was checked by HPLC on a 4.6 × 250 mm TSK-Gel Silica-60 column using CH$_2$Cl$_2$:CH$_3$OH (97:3 v/v) as mobile phase with an elution rate of 1.0 cm^3 min^{-1}. Detection was by means of a UV-visible detector. The purity in all cases was >95 %.

4.4. Equilibrium Measurements

The hydrophobic corrinoids (ACCbs-Pr, SCbs-Pr, EtCbs-Pr and MeCbs-Pr, 1.0 × 10^{-5} mol dm^{-3}) dissolved in 2.5 cm^3 of either an acetate or phosphate buffer, ionic strength 0.1 mol dm^{-3}, or in an organic solvent, were placed in a cuvette in the thermostatted cell block of the spectrophotometer for 30 min. The solution was titrated by addition of small volumes of concentrated stock solution of an azole, prepared in the same solvent, using a Hamilton syringe. The reaction was followed by observing the change in absorbance at the wavelength that gave the greatest change during the course of the reaction. All titrations were carried out at least in duplicate. The values of the equilibrium constants, *K*, were obtained by fitting the absorbance versus concentration curve, after correction for dilution, to a binding hyperbola (Eq. 2, where A$_0$ and A$_\infty$ represent the absorbance at 0 % and 100 % formation of (L)(X)Cbs-Pr, respectively, and A$_x$ is the absorbance at any ligand concentration [L]) using standard non-linear least squares methods.

$$A_x = (A_0 + A_\infty K [L])/(1 + K[L]) \tag{2}$$

Since log K values are small, the assumption was made that [ligand]$_{total}$ = [ligand]$_{free}$. The linear plot obtained has a slope equal to the number of ligand molecules bound to the cobalt centre, and the intercept gives the value of log *K*. Analysis of the data was also carried out by plotting log[(A$_0$ − A$_x$)/(A$_x$ − A$_\infty$)]

against log[ligand]. The slope gives the number of ligands that coordinate.

4.5. Molecular Modelling

Molecular mechanics (MM) modelling was performed with the MM+ version of MM2[57] in the HYPERCHEM suite of programs.[58] The potential energy functions used have been listed previously.[59] The corrin core was modelled with the parameters that we have previously reported.[60–62] Standard MM2 parameters were used as required for modelling the ester side-chains and no MM+ parameters were used in the modelling. All dipole moments involving the metal ion were set to zero. The crystal structure of H_2OCbl^+ was suitably edited and used as the starting point for all calculations.[24] The conformational space of each molecule was explored using molecular dynamics simulations. In each case the molecule was heated from 0 K to 600 K over a period of 10 ps using a step size of 1 fs; this was followed by a dynamics run at 600 K for 100 ps, followed by a slow annealing to 0 K over 50 ps. The conformation discovered in each case was used for further calculations. The strain energy was minimized using firstly 1000 cycles of a steepest descent algorithm followed by a Polak-Ribiere conjugate gradient algorithm with a convergence criterion of r.m.s.d. < 0.04 kJ mol^{-1} Å^{-1} in the gradient. Minimization usually occurred within 500 cycles of the latter algorithm.

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