The Chemistry of Vitamin B_{12}. Part 22.[1] Steric Effects in the Co-ordination of Amines by Cobalt(III) Corrinoids †

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The following equilibrium constants (given as $\log_{10}K/dm^3\ mol^{-1}$) have been determined by spectrophotometry for the substitution in cobalt(III) corrinoids of co-ordinated H_2O by various amines B with different *trans* ligands X in aqueous solution (I = 0.2 mol dm^{-3}) at 25 °C: X = 5,6-dimethylbenzimidazole (dbzm), B = NH_2Me (*ca.* 6), $NHMe_2$ (3.4), NMe_3 ($\leq$1), NH_2Et (5.3), NH_2Pr^i and NH_2Bu^t (both $\leq$1); X = CN^-, B = NH_2Me (3.4), $NHMe_2$(2.0), NMe_3(*ca.* 0); X = vinyl, B = NH_3 (−0.06), NH_2Me (+0.04), $NHMe_2$ and NMe_3 (both ≤ -1). These results show that steric effects increase in importance as the donor power of the *trans* ligand X decreases (from vinyl through CN^- to dbzm) and that significant steric effects are caused by substitution on the β (C) atom as well as on the α (N) atom.

The amines provide a family of ligands which can be used to probe steric (and, to a lesser extent, electronic) factors in metal–ligand bonding. We have been studying steric effects in alkyl-corrinoids [2–4] because of their relevance to the mechanism of action of the vitamin B_{12} coenzymes in the isomerase and ribonucleotide reductase reactions and to provide support for our proposal [5] that the protein labilises the Co–C bond in the coenzyme through steric distortion of the Co–C–C bond angle, caused by a conformation change in the protein and induced by the binding of the substrate to the active site; the evidence has recently been reviewed.[6] We have now extended our studies to include amines, since amines and alkyls are both co-ordinated through a tetrahedral atom and parallels can be expected between steric effects in the two series. We report here the determination by u.v.–visible spectrophotometry of equilibrium constants for the substitution of co-ordinated H_2O in cobalt(III) corrinoids by various amines in aqueous solution at 25 °C.

Our aim has been to provide answers to the following questions concerning equilibrium constants for the co-ordination of amines. (1) What is the effect of substitution on the α (*viz.* N) and β (*viz.* C) atoms (by studying the series from NH_3 to NMe_3 and NH_2Me to NH_2Bu^t)? (2) Does the magnitude of any steric repulsion vary with the nature of the *trans* ligand X? (3) How does the cobalt(III) ion compare with other metal ions in its sensitivity to steric effects? We have determined most of the equilibrium constants with X = 5,6-dimethylbenzimidazole (dbzm) (*i.e.* with aquocobalamin or B_{12a}) but, in order to answer question (2), we have determined a few additional constants with X = CN^- and vinyl. Equilibrium constants have previously been reported [7a] for the co-ordination of NH_3 with X = H_2O, dbzm, CN^-, methyl, and SO_3^{2-}, of glycine with X = dbzm, and of piperidine and ethanolamine with X = methyl, but not for any of the above-mentioned alkylamines.

Experimental complications (see Results section) arise from (*i*) competition between amines and HO^- as ligands, (*ii*) slow rates of equilibration with X = dbzm and H_2O, and (*iii*) irreversible formation of so-called ' stable yellow corrinoids ',[7b] whose structure is now known.[8] Difficulties of interpretation arise because the corrin ring lacks a plane of symmetry and isomers are possible when the two axial ligands are different. Cyanoaquocobinamide (Factor B) exists as an approximately equal mixture of the two isomers which are fairly readily interconverted;[7c] it is therefore possible that a sterically hindered amine may be preferentially co-ordinated in one of the two axial sites, but this possibility is here ignored. In aquocobalamin the nucleotide base occupies the lower (or α) site and the amines can therefore only occupy the upper (β) site. Vinylcobinamide appears to exist as a single isomer since no t.l.c. evidence for two isomers has been reported (contrast ethynylaquocobinamide [1]), and we assume that vinyl, like the simple alkyls,[7d] occupies the upper site. It exists in aqueous solution as a mixture of *ca.* 30% of the six-co-ordinate vinylaquo-complex and *ca.* 70% of the five-co-ordinate vinyl complex,[7e] but the existence of the five-co-ordinate form is ignored here. We have concentrated on aquocobalamin (X = dbzm), first, because steric factors increase in importance as X is varied in the order vinyl < CN^- < dbzm < H_2O (see Discussion section) but experimental difficulties prevented studies with diaquocobinamide (see Results section) and, secondly, to ensure a direct comparison between steric effects in amine and alkyl ligands (both in the upper position).

Equilibrium constants for the co-ordination of NH_3 have been reported for many metals in aqueous solution [9,10] but constants for the co-ordination of a series of related amines (*e.g.* NH_3 to NMe_3 or NH_2Me to NH_2Bu^t) are virtually confined to large metal ions with a low co-ordination number (of two or four), *viz.* Ag^I,[9–12] $HgMe^+$,[13] and *trans*-[$PtCl_2(C_2H_4)(H_2O)$] [14] (see Table 1). For transition-metal ions of the first row, where the smaller ionic radius and higher co-ordination number are expected to lead to more pronounced steric effects, the only relevant data reported are values of log K_1 for the nickel(II) ion which were determined indirectly by a kinetic method (see Table 1); [15] values have also been reported for the co-ordination of three different amines with Cu^{II}.[16–18] Consideration of the relative ionic radii, charges, and co-ordination numbers suggests that steric effects may be greater for cobalt(III) complexes than for those of any other metal ion yet studied. This is also suggested by the *X*-ray data; compare the Co–N–C bond angles of 121, 122, 123, 124, and 124.5° in [$Co(NH_2Me)_5Cl][NO_3]_2$[19] and the Co–C–C bond angle of 122° in [CoEt(Hdmg)$_2$(py)] [Hdmg = dimethylglyoximate(1−), py = pyridine],[20] where steric effects must be minimal for the amine and alkyl series respectively yet the formally ' tetrahedral ' bond angles are all significantly larger than the trigonal bond angle. We therefore need to know how to distinguish steric from other (*e.g.* inductive) effects of varying alkyl substitution in the above-mentioned two series.

There is now a reasonable understanding of some of the factors which determine the observed variation of donor strength of amines towards Lewis acids in different environ-

† *Non-S.I. unit employed:* cal = 4.184 J.

Table 1. Equilibrium constants for the protonation (pK) or co-ordination (log K_1) of amines in aqueous solution

	Lewis acid					
Amine	H^+	Ag^+		$HgMe^+$	Pt^{II} [a]	Ni^{2+} [b]
NH_3	9.25	3.3		7.25	7.8	2.80
NH_2Me	10.70	3.5 [c]		7.57	8.6	2.23
$NHMe_2$	10.80	2.7 [c]		6.76	8.0	1.47
NMe_3	9.80	1.6 [c]		5.05	*ca.* 5.5	
NH_2Me	10.70		3.06	7.57		2.23
NH_2Et	10.7		3.44	7.64		1.81
NH_2Pr^i	10.67		3.64	7.56		1.56
NH_2Bu^t	10.69		3.69	7.52		
I/mol dm^{-3}	mostly 0.2	various	0.1	not kept constant	0.2	1.0
θ_C/°C	25	25	25	25	23	25
Ref.	10, 11	9, 10	12	13	14	15

[a] *trans*-$[PtCl_2(C_2H_4)(H_2O)]$. [b] Values determined indirectly from kinetic data. [c] $(\log \beta_2)/2$ (no values of $\log K_1$ available).

ments, from the proton in the gas phase to metal ions in aqueous solution. The effects of β-substitution are of particular interest because they are easier to interpret and also because of the marked effects of β-substitution in alkylcorrinoids; neopentylcobalamin ($Co-CH_2CMe_3$), for example, undergoes homolytic fission of the Co–C bond even at room temperature and provides a protein-free model for labilisation of the Co–C bond in the coenzyme.[4] We therefore discuss the effects of β-substitution (*i.e.* NH_2Me to NH_2Bu^t) with Lewis acids where steric repulsion is, first, minimal (*viz.* H^+) and, secondly, significant (BMe_3).

The proton affinities of amines in the gas phase rise regularly from NH_2Me to NH_2Bu^t with an overall change of 7—8 kcal mol^{-1} in the negative free energy.[21-23] It has been estimated that the inductive effect provides only the minor (*ca.* 2 kcal mol^{-1}) contribution to the greater proton affinity of NH_2Bu^t, while the charge-induced dipole stabilisation of the protonated ion provides the major (*ca.* 6 kcal mol^{-1}) contribution, *i.e.* the polarisability effects of the alkyl substituents dominate the inductive effects in the gas phase.[22,24] In aqueous solution, however, the pK values are almost identical (see Table 1). Calculations support the conclusion that the main effect of aquation is to disperse the positive charge of the cation to the solvent molecules; this reduces the polarisability effect to almost zero and the inductive effect to *ca.* 1 kcal mol^{-1} and thereby virtually eliminates the difference between NH_2Me and NH_2Bu^t which was observed in the gas phase.[22,25] By contrast, the stability of adducts with BMe_3 in the gas phase at 100 °C decreases steadily from NH_2Me to NH_2Bu^t with an overall change of $\Delta G = 4.1$ kcal mol^{-1} or $\log_{10}K = 2.4$,[26] which can clearly be ascribed to the predominance of steric over polarisability effects. One would predict that a change from the gas phase to aqueous solution, which would nullify the polarisability but not the steric effects, would lead to a much larger decrease in the same order; experimental verification is, of course, prevented by hydrolysis of the adducts. The effects of α-substitution on the stability of the BMe_3 adduct are, rather surprisingly, less than those of β-substitution, but this is clearly due to the opposing changes in steric and inductive effects; *cf.* the following equilibrium constants ($\log_{10}K$) for the formation of adducts between BMe_3 in the gas phase and NH_3 (−0.66), NH_2Me (+1.46), $NHMe_2$ (+1.67), and NMe_3 (+0.33).[26,27]

It therefore appears that the effects of β-substitution on equilibria with metal ions in solution will be determined almost entirely by steric effects and, conversely, that the extent of reduction of the equilibrium constant by increasing β-substitution may provide a sensitive indicator of the importance of steric repulsion between the metal ion and the ligand. This is supported by the fact that for the series NH_2Me to NH_2Bu^t the binding constants show little variation for those Lewis acids where steric effects are expected to be small, *viz.* Ag^+ and $HgMe^+$ as well as H^+ in aqueous solution; a significant decrease has been reported only for Ni^{2+} (see Table 1). The effects of α-substitution, on the other hand, are more complex [25] and the maximum binding constant in aqueous solution may be found with any one of the members of the series NH_3 to NMe_3 (see Table 1); *cf.* also the movement of the position of maximum pK on changing the solvent.[28]

The above data provide the background against which to evaluate the observed equilibrium constants for the co-ordination of amines by cobalt(III) corrinoids. The possible role of contact between the γ atoms of the ligand and the corrin side-chains is considered in the Discussion section.

Experimental

Materials.—Samples of vitamins B_{12} and B_{12a} (cyano- and aquo-cobalamin) were kindly donated by Mr. Domleo of Glaxo-Allenbury (Pty) Ltd. Cyanocobalamin was converted into cyanoaquocobinamide (Factor B) and subsequently into vinylcobinamide as previously described.[29] The amines were obtained and purified as follows. Methylamine (Merck) and $NHMe_2$ (Hopkins and Williams) were treated with HCl to precipitate the hydrochloride, which was washed with $CHCl_3$ (former only) and recrystallised three times from EtOH,[30] and the amine then distilled from NaOH at room temperature and collected at −77 °C. Trimethylamine (B.D.H.) was distilled from $PhSO_2Cl$ at room temperature (Hinsberg method).[31] Ethylamine (Riedel-de-Haen) was distilled from NaOH at room temperature. Isopropylamine and NH_2Bu^t (both Merck, for synthesis) and NH_3 (Merck, for analysis) were used as received. The concentration of the amines was determined by back-titration using H_2SO_4 and NaOH. Water purified by the Millipore Milli-Q system was used throughout.

Analysis of Amines.—The purified amines were analysed for low concentrations of other amines, in particular of the less sterically hindered amines with higher binding constants (see Table 4), except where no co-ordination was observed (*e.g.*

Table 2. Analysis of the amines

Amine	% impurity (w/v)				
	NH_3	NH_2Me	$NHMe_2$	NMe_3	NH_2Et
NH_2Me	<0.1	—	6×10^{-2}	$<1 \times 10^{-3}$	$<1 \times 10^{-3}$
$NHMe_2$	<0.1	6×10^{-3}	—	$<1 \times 10^{-3}$	$<1 \times 10^{-3}$
NMe_3	<0.1	1×10^{-4}	1×10^{-2}	—	$<1 \times 10^{-3}$
NH_2Et	<0.1	3×10^{-3}	$<1 \times 10^{-2}$	$<1 \times 10^{-2}$	—

Table 3. Determination of equilibrium constants for the substitution of co-ordinated H_2O by amines in cobalt(III) corrinoids with various *trans* ligands X; experimental conditions and results. All experiments at 25 °C with 2×10^{-5}–7×10^{-5} mol dm^{-3} Co, amine concentration and pH as given below, and $I = 0.2$ mol dm^{-3} (except with NH_2Pr^i and NH_2Bu^t)

X	Amine	$\lambda_{max.}$ of product (nm)	pH [a]	[Amine] [b]/ mol dm^{-3}	λ used (nm)	t [c]/ h	K [d]/ dm^3 mol^{-1}
dbzm	NH_3	357, 514, 540					
	NH_2Me	359, 513, 542	9.14	0—0.12	359	12	*ca.* 3×10^6
	$NHMe_2$	357, 508, 534	9.10	0—0.2	357	1.5	$(2.8 \pm 0.8) \times 10^3$
	NMe_3		9.10	0—0.5		1.5	⩽20
	NH_2Et	360, 514, 541	9.14	0—0.014	358	15	*ca.* 2×10^5
	NH_2Pr^i		10.5	2.3		2	⩽10
	NH_2Bu^t		10.5	2.0		2	⩽10
CN^-	NH_3	361, 520, 553					
	NH_2Me	361, 526, 560	8.84	0—0.1	560	t_1	$(2.50 \pm 0.1) \times 10^3$
	$NHMe_2$	360, 525, 558	11.97	0—0.5	560	t_1	$(1.01 \pm 0.1) \times 10^3$
	NMe_3	*e*	9.10	0—0.5		t_1	*ca.* 1
vinyl	NH_3	366, 509, 561	12.94	0—3	550	t_1	0.88 ± 0.05
	NH_2Me	*e*	12.94	0—0.63	560	t_1	1.1 ± 0.1
	$NHMe_2$		12.94	1.1		0.5	⩽0.1
	NMe_3		12.94	0.5		0.5	⩽0.2

[a] pH 10.5, unbuffered solutions containing *ca.* 20% (v/v) amine; all other solutions buffered (compositions given in Experimental section). [b] Total amine added (free, protonated, and co-ordinated). [c] Time required or allowed for the various solutions to equilibrate; t_1 indicates titration of a single solution with rapid equilibration. [d] Defined in equation (1). [e] Degree of formation too low for accurate estimation of band positions.

with NH_2Pr^i). Ammonia was determined by the method of Gove *et al.*[30] and the amines by using a Packard 427 gas chromatograph operated isothermally (90 °C) with a glass column (length 1.8 m, internal diameter 3 mm) packed with GP Cardopack B, 4% CW20M, 0.8% KOH (Supelco Inc.) and with a carrier-gas (N_2) flow rate of 23 cm^3 min^{-1}. Prior to use, the column was conditioned overnight at 200 °C followed by the injection of twenty 1-μl portions of CO_2-free distilled water over a period of 30 min. When contamination of the packing occurred, this was overcome by raising the oven temperature to 200 °C and injecting 25×1 μl water followed by 10×1 μl concentrated NH_3 solution and 25×1 μl water, all at 10—15 s intervals; the ammonia, which was not registered by the flame-ionisation detector, rapidly cleared the column of residual amines. The following approximate retention times were found on injecting 1-μl samples: NH_2Me, 1 min; $NHMe_2$, 2.5 min; NMe_3, 3 min; NH_2Et, 1.5 min. In addition, a peak due to ethanol, which was carried over from the recrystallisation of the hydrochloride salts, was sometimes found with a retention time of *ca.* 4 min. The maximum levels of impurities determined by these methods are given in Table 2.

Buffers.—The following buffer solutions,[32] all with $I = 0.2$ mol dm^{-3}, were used to provide the pH values finally selected (see Table 3) for quantitative studies: pH 8.84, 9.10, and 9.14, all $NaHCO_3$–Na_2CO_3; pH 11.97, 0.01 mol dm^{-3} NaOH + 0.19 mol dm^{-3} $NaClO_4$; pH 12.94, 0.1 mol dm^{-3} NaOH + 0.1 mol dm^{-3} $NaClO_4$. The required solutions of amines in buffers were prepared by titrating the pure amine to approximately the desired pH with ice-cold $HClO_4$ and then diluting with the appropriate concentrated buffer solution; the final pH was checked with a Metrohm EM 147 micro glass electrode. The electrode was calibrated with B.D.H. buffers of pH 7.00 and 10.00.

U.v.-Visible Spectra.—These were recorded with a JASCO UVIDEC-1 spectrophotometer using 1-cm cells thermostatted at 25 °C.

Evaluation of Data.—We wish to obtain the equilibrium constant K defined by equation (1), where B is the unprotonated amine; only the axial ligand undergoing substitution is indicated and concentrations (not activities) are used. Since many of the experiments were carried out at a pH above the pK of the aquo-complex and below the pK of the amine, the equilibrium constant determined directly from the experimental data involves Co–OH and HB^+ as well as Co–OH_2 and B. The true concentration of the free base was therefore calculated from the known pK of the base and the pH of the buffered solution (both given in Table 3) to give $K_{obs.}$ [equation (2)], and K then derived by the use of equation (3), where pK_a represents the pK of the Co–OH_2 complex.

$$K = [Co\text{–}B]/[Co\text{–}OH_2][B] \quad (1)$$

$$K_{obs.} = [Co\text{–}B]/([Co\text{–}OH_2] + [Co\text{–}OH])[B] \quad (2)$$

$$K = K_{obs.}(1 + 10^{pH-pK_a}) \quad (3)$$

Results

All equilibrium constants were determined in aqueous solution with $I = 0.2$ mol dm^{-3} at 25 °C by u.v.–visible spectrophotometry. Preliminary experiments (with scanning of the spectra from 300 to 650 nm) were carried out to check for the occurrence of reversible equilibria, irreversible side-reactions, and isosbestic points and to select the conditions for quantitative studies at a fixed wavelength. Although the main (γ) band of hydroxocobalamin occurs at a wavelength (357 nm) similar to those of the amine complexes (356—359 nm, see Table 3), the former has an anomalously low absorbance with a pronounced shoulder at *ca.* 325 nm, while all the amine-cobalamins detected here have a γ band with more normal intensity; see Figure 1 of ref. 33 for a comparison of the spectra of hydroxo- and ammonia-cobalamin.

Experimental difficulties increase with the nature of X in the order vinyl < CN^- < dbzm < H_2O for both thermodynamic and kinetic reasons. The pK values for the formation of the hydroxo-complex decrease with X in the order X = vinyl (15—15.5),[1] CN^- (11.0),[1] dbzm (7.8),[7f] and H_2O (5.9) [34] and cross the pK values for the amines (10—11) and NH_3 (9.25). The rates of substitution of co-ordinated H_2O also decrease regularly in the same order, while the co-ordinated HO^- is essentially inert towards ligand substitution.[35] Vinylcobinamide therefore presents no problem since the possible co-ordination of amines can be studied in a region of pH (*ca.* 13) where they are present entirely as the free base and suffer no competition from the co-ordination of HO^-; in addition, the parent vinylcobinamide is kinetically very labile [36] and present mainly as the five-co-ordinate complex.[7e] No real problems were encountered with cyanoaquocobinamide either. For X = dbzm, however, competition between the amine and HO^- for the cobalt ion and between the proton and the cobalt ion for the amine is serious at all pH values and explains the high minimum value (≤ +1) reported in several cases where no reaction was observed (see Table 3); the experimental conditions chosen therefore represent a compromise between conflicting demands. Attempts to study the co-ordination of amines by diaquocobinamide were abandoned because of the slow rate of co-ordination with X = H_2O and because of the complex set of equilibria observed (six permutations of H_2O, HO^-, and amine as the two axial ligands) when one axial ligand is not fixed; *cf.* the study of the equilibrium between diaquocobinamide and NH_3.[35]

Further complications due to side-reactions may occur as the rate of co-ordination falls. It is known that amines such as 4-methylpyridine and ethylamine will slowly and irreversibly attack the corrin ring to form stable yellow corrinoids.[7b,8] Our preliminary experiments showed that $NHMe_2$ will also slowly attack the corrin ring of all three corrinoids (X = dbzm, CN^-, or vinyl) at comparable rates, as shown in the case of aquocobalamin by the fall in absorbance in the αβ and γ regions to leave bands at *ca.* 320 and 470 nm. This side-reaction caused a problem only in the case of aquocobalamin and $NHMe_2$; when solutions were left to equilibrate the absorbance at 357 nm, A_{357}, first rose (co-ordination of $NHMe_2$) and then much more slowly fell again (destruction of the corrin ring). The final 'equilibrium' values of A_{357} (= A in next paragraph) were obtained by adjusting A to maximise the linear portion (always ≥2 half-lives) of the pseudo-first-order rate plot.

The corrinoids, amines, and experimental conditions finally chosen, together with the experimental results, are given in Table 3. Reasonable isosbestic points were observed for all the equilibria studied. The method involved either direct titration of the corrinoid with the amine inside the spectrophotometer cell with correction for dilution (X = CN^- or vinyl), or allowing separate solutions with different amine concentrations to come to equilibrium (X = dbzm); the approximate time required (for equilibration) or allowed (where no reaction was observed) is given in Table 3. The end-point corresponding to complete formation of the amine complex (*i.e.* A_∞) was observed directly only in the cases of vitamin B_{12a} with NH_2Me and NH_2Et and the cyanide complex with $NHMe_2$. In all other cases the changes in absorbance with concentration were evaluated according to the method of Newton and Arcand [37] to give the value of A_∞. Plots of log ($|A - A_0|/|A_\infty - A|$) against log [B] were then used to confirm that only one molecule of B was co-ordinated per atom of Co (slope of 1.0 obtained in all cases) and to derive values of $K_{obs.}$ and then K (see Experimental section), using the pK values for the amines listed in Table 1 and the values of pK_a = 7.8 and 11.0 for the aquo–hydroxo-equilibrium with X = dbzm and CN^- respectively.[7f]

Table 4. Summary of equilibrium constants for the substitution of co-ordinated H_2O by amines in cobalt(III) corrinoids with various *trans* ligands X

Amine	log K/dm^3 mol^{-1} for X = dbzm	CN^-	CH_2=CH
NH_3	7 *	3.35 *	−0.06
NH_2Me	*ca.* 6	3.40	+0.04
$NHMe_2$	3.4	2.0	≤ −1
NMe_3	≤ +1	*ca.* 0	≤ −1
NH_2Me	*ca.* 6		
NH_2Et	5.3		
NH_2Pr^i	≤ +1		
NH_2Bu^t	≤ +1		

* Ref. 7*a* (I not held constant). All other values from Table 3 (I = 0.2 mol dm^{-3}).

Discussion

The experimental conditions and results are given in Table 3 and the equilibrium constants, defined by equation (1), are summarised in Table 4 for comparison with the data in Table 1. The inclusion of maximum values of log K, where no co-ordination was actually observed, requires justification. Since vinylcobinamide is present mainly as the five-co-ordinate complex,[7e] exhibits a second-order rate constant for the co-ordination of cyanide which is close (≥10^8 dm^3 mol^{-1} s^{-1}) to the diffusion-controlled limit,[36] and can be studied above the pK of the amine, there is little doubt that failure to observe the co-ordination of $NHMe_2$ or NMe_3 can be ascribed to thermodynamic rather than kinetic factors. In the case of aquocobalamin, kinetic studies have shown that the rate constants for the substitution of co-ordinated H_2O are virtually independent of the nature of the incoming ligand, as expected for a dissociative mechanism; [38,39] in addition, the cobalt(II) cobalamin (B_{12r}) can react with the neopentyl radical,[4] and the cobalt(I) complex (B_{12s}) with neopentyl bromide,[2] to give the $Co-CH_2CMe_3$ complex which is isostructural with the expected $Co-NH_2CMe_3$ complex. We conclude that failure to observe the co-ordination of NH_2Pr^i, NH_2Bu^t, and NMe_3 by aquocobalamin is due to a low equilibrium constant and not to a low rate constant.

The data of Table 4 show that for aquocobalamin (X = dbzm) the value of the equilibrium constant is markedly decreased by substitution on either the N or the C atom. In the light of the analysis given in the Introduction these effects must be ascribed to the steric, and not to the inductive, effects of increasing alkyl substitution; the inductive effects of β-

substitution are negligible, while those of α-substitution are significant but eventually overwhelmed by the steric effects. As expected for strong steric repulsion around small donor and acceptor atoms (see Introduction), the sensitivity of the equilibrium constant to β-substitution appears to be at least as great as its sensitivity to α-substitution. One need only compare the data for NH_3 to $NHMe_2$ in Tables 1 and 4 to see that aquocobalamin is more sensitive to steric effects than any metal ion previously studied. This agrees with the marked steric distortion of the 'tetrahedral' Co–N–C and Co–C–C bond angles in cobalt(III) complexes revealed by *X*-ray analysis (see Introduction).

Previously reported data on the co-ordination of NH_3 show that the equilibrium constants (given as log *K* in parentheses and not corrected for the presence of five-co-ordinate forms) for the substitution of co-ordinated H_2O by NH_3, like those for other ligands, increase as the *trans* ligand X is varied in the order X = methyl (−1), vinyl (0, see Table 3), CN^- (3), dbzm (7), and H_2O (≥9).[7f] Since varying X in this order is almost certainly accompanied by an increase in the residual positive charge on the cobalt and by a decrease in the Co–N bond length, one would expect steric factors to become more critical towards the X = H_2O end of the *trans*-effect order. The data of Table 4 show that steric effects do indeed become more important as X is changed from CN^- to dbzm. One would therefore predict steric factors to be even greater for X = H_2O but verification was, unfortunately, prevented by experimental difficulties (see Results section). We have also attempted to investigate the converse thermodynamic *trans* effect of various amines with differing steric requirements on equilibria in the *trans* position by studying their effect on the p*K* for displacement and protonation of dbzm in the cobalamins, *i.e.* on the equilibrium constants for the substitution of co-ordinated H_2O by dbzm. This approach was, however, prevented by complications due to the occurrence of other reactions and equilibria (*e.g.* loss of co-ordinated amine, hydrolysis of the nucleotide side-chain) under the acid conditions required (pH < 0).

The effect of the co-ordinated amine on the wavelength of the γ band, which represents a ground-state *cis* effect of the amine ligand, provides some additional information on the nature of the Co–N(amine) bond, even though the range of data available is rather limited (see Table 3). Previous work has shown that, to a first approximation, the γ band moves to longer wavelength as the σ-donor power of the axial ligand increases and that steric hindrance in nitrogenous bases reduces the shift to longer wavelength; compare the wavelength (in nm) of the γ band in the cobalamins with H_2O (350), 4-methylpyridine (361), 2-methylpyridine (356), imidazole (358), and benzimidazole (354).[7g] Our present results (see Table 3) show little variation in wavelength where there is little variation in the magnitude of the equilibrium constants, *i.e.* with X = CN^- and vinyl. With X = dbzm, however, we observe the order NH_3 (356) < NH_2Me (359), which can probably be ascribed primarily to changes in the inductive effect, and NH_2Me (359) > $NHMe_2$ (357), which presumably reflects steric hindrance leading to a poorer overlap between the Co and N atomic orbitals and hence to a smaller degree of electron donation from the amine.

Finally, we need to reassess whether any of the observed effects of β-substitution in amine and/or alkyl ligands could be due to interaction between the β or γ atoms on the axial ligand and the upward-projecting side-chains of the corrin ring. We focus attention here on the alkyl ligands, with particular reference to comparing Co–Et (*cf.* the central position of NH_2Me in the present studies) with Co–Pr^i and Co–CH_2CMe_3 (to exemplify the effects of α- and β-substitution respectively) and to comparing corrinoids with non-corrinoids. We showed that the replacement of Et by Pr^i or neopentyl in corrinoids led to similar large changes in, for example, the u.v.–visible spectra (ground-state *cis* effect) and equilibria in the *trans* position (thermodynamic *trans* effect) as well as the lability of the Co–C bond itself.[2–4] In view of the contact, revealed by *X*-ray analysis of the vitamin B_{12} coenzyme, between the O atom of the ribose ring of the ligand (which should be comparable to a γ-C atom in Co–CH_2CMe_3) and the corrin ring between C(14) and C(15),[40] we suggested that a ligand such as neopentyl would experience considerable steric interaction with the side-chains on the corrin ring and that this would in turn serve to increase the Co–C–C bond angle.[2] However, *X*-ray analysis of the complexes [CoR(Hdmg)$_2$(py)] subsequently provided direct evidence for considerable steric distortion with R = neopentyl (Co–C 2.060 Å, Co–C–C 130°)[41] as well as Pr^i (Co–C 2.085 Å, Co–C–C 114°),[42] compared to Et (Co–C 2.065 Å, Co–C–C 122°)[20] and Me (Co–C 1.998 Å),[43] while the bond-dissociation energies D_{Co-R} (given in parentheses in kcal mol^{-1}) calculated for the complexes [Co–R(saloph)(py)] [saloph = *o*-phenylenebis(salicylideneiminate) (2−)] by an indirect kinetic method fall from R = Pr^n (25) to Pr^i (20) and even further with neopentyl (18).[44] It is clear that β-substitution can have marked steric effects in organocobalt(III) complexes even with planar equatorial ligands, as well as in adducts with BMe_3 (see Introduction); *cf.* also the anomalously low M–CH_2CMe_3 bond dissociation energies found for M = Ti, Zr, and Hf.[45] This supports our conclusion that the failure to observe the co-ordination of, for example, NH_2Bu^t is due to thermodynamic, and not kinetic, reasons and also strongly suggests that any contact between the γ atoms of the axial ligand and substituents on the corrin ring plays only a minor role in the observed effects of β-substitution in both amine and alkyl ligands in cobalt(III) corrinoids. The origin and transmission of these steric effects will be discussed again in a later paper.

The present work provides the most extensive set of data on the co-ordination of amines by any transition metal of the first row and also appears to be the first attempt to correlate steric effects in analogous amine and alkyl complexes for any metal.

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