Self-association of Organocobalamins in Aqueous Solution†

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The ^1H n.m.r. spectrum of base-on methylcobalamin (CH_3–B_{12}, base = terminal 5,6-dimethylbenzimidazole of a side chain) in neutral aqueous solution shows concentration-dependent shifts which conform to a monomer–dimer equilibrium. The shift changes on dimerization are characteristic of ring-current effects and support a structure in which the corrin rings are adjacent and roughly parallel-planar. This is also supported by the apparent steric effect of the organo-group on the equilibrium constant: CH_3– > CH_3CH_2–B_{12}, and $K = 0$ for n-C_3H_7– and 5′-deoxyadenosyl–B_{12}. The CH_3–B_{12} dimer is favoured by increasing [NaCl] (but not [$NaClO_4$]), and disfavoured by added miscible organic solvents. The temperature dependence of K yields $\Delta H = -13$ kcal mol^{-1} and $\Delta S = -33$ cal K^{-1} mol^{-1} at [NaCl] = 0. The energy of association originates at least in part in π–π interactions between corrin rings. Base-off CH_3–B_{12} in 1 mol dm^{-3} DCl gives a K value *ca.* 100-fold smaller than for the base-on form. The n.m.r. shifts indicate π–π bonding involving both 5,6-dimethylbenzimidazolium and corrin rings. The cobalamins H_2O–B_{12}^+ and CN–B_{12} show no observable tendency to self-associate, and H_2O–B_{12}^+, CN–B_{12}, n-C_3H_7–B_{12}, and 5′-deoxyadenosyl–B_{12} show no tendency to associate with base-on CH_3–B_{12}.

The evidence for reversible complexation of methylcobalamin (CH_3–B_{12}) with PtX_4^{2-} compounds in the transfer of a methyl group to PtX_4^{2-}–PtX_6^{2-} couples[1] prompted us to scrutinize the ^1H n.m.r. spectrum of CH_3–B_{12} with PtX_4^{2-} in aqueous solution. This led to the discovery of concentration-dependent shifts in the spectrum of CH_3–B_{12} alone. As the present report will document, these shifts indicate self-association between parallel-planar corrin ring systems. Similar interactions are well known among the metalloporphyrins.[2,3]

We undertook this study as part of a general program to examine weak bonding to the corrin ring of B_{12} compounds. This is potentially important to organo-group transfer and electron-transfer reactions of B_{12} compounds. For example, it has been suggested that cleavage of the C–Co bond by Pt^{II}–Pt^{IV},[1a] AuX_4^-,[4,5] and tetracyanoethylene[6] is triggered by electron transfer to the corrin-complexed reactant. It is also quite possible that weak interactions with the corrin ring play a role in the biological[7] and environmental[8,9] chemistry of B_{12} compounds.

Experimental

Methylcobalamin, aquocobalamin (H_2O–B_{12}^+), vitamin B_{12} (CN–B_{12}), and 5′-deoxyadenosylcobalamin (Ad–B_{12}) were purchased from Sigma Chemical Co. and used as received. Ethylcobalamin was synthesized.[10] n-Propylcobalamin was a gift from H. P. C. Hogenkamp.

Proton n.m.r. spectra were recorded on a Bruker 270-MHz pulse Fourier-transform spectrometer. The probe was thermostatted (±0.5 °C) and calibrated by reference to the chemical shift of the OH resonance of Bu^{t}OH. Solutions of the organocobalamins were prepared and handled under dim light. A typical experiment involved preparation of a concentrated solution in the medium of choice and successive dilution with additional medium. All shifts for base-on CH_3–B_{12} and the other cobalamins were referenced to internal sodium tetradeuterio-3-(trimethylsilyl)propionate (tsp) (1—10 mmol dm^{-3}). The chemical shift of internal tsp was found to be unchanged over the range of concentration used for the cobalamins when referenced to external 2% 1,4-dioxane in D_2O. External tsp in D_2O was used as a reference in the case of CH_3–B_{12} in 1 mol dm^{-3} DCl, since the chemical shift of tsp was not constant with changing CH_3–B_{12} concentration *vs.* external 1,4-dioxane in this medium.

The parameters δ_M, δ_D, and K that gave the best fit to equation (3) (see Results section) were determined using a computer grid search technique which sought to minimize the sum of the squares of the deviations (s.s.). The design of the program was as follows. First the smallest s.s. was picked from a three-dimensional parameter grid, which was bordered by selected parameter values and consisted of 6—10 divisions per parameter. The search was continued in subsequent grids. Each grid was centred on the parameter values corresponding to the smallest s.s. of the previous grid. The size of the subsequent grid was reduced by (*a*) 20, (*b*) 10, or (*c*) 0%, respectively, if the smallest s.s. of a given grid was (*a*) lower than that of the previous grid, (*b*) not lower than that of the previous grid, or (*c*) corresponded to one or more parameter values that fell on a border. In this way, iteration resulted in convergence to s.s.$_{min.}$. The results were independent of the initial parameter ranges, even if the best-fit parameter values lay outside these ranges. In some cases careful inspection of the error space by full printout of s.s. values confirmed the validity of the program design. The program is available from the authors.

Results

The ^1H n.m.r. spectrum of base-on CH_3–B_{12} in D_2O has been assigned.[11] We have found that the spectrum is concentration-dependent and is also sensitive to the addition of NaCl or miscible organic solvents. The largest shift is observed for the CH_3–Co peak. Figure 1(*a*) shows the concentration dependence of the CH_3–Co chemical shift at 0—5 mol dm^{-3} NaCl. A number of other hydrogens in the molecule also show concentration-dependent shifts which parallel that of CH_3–Co [Figure 1(*b*)].

The results can be interpreted in terms of a monomer–dimer equilibrium (1). The appropriate mathematical expression

$$\underset{M}{2\,CH_3\text{–}B_{12}} \xrightarrow{K} \underset{D}{(CH_3\text{–}B_{12})_2} \qquad (1)$$

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

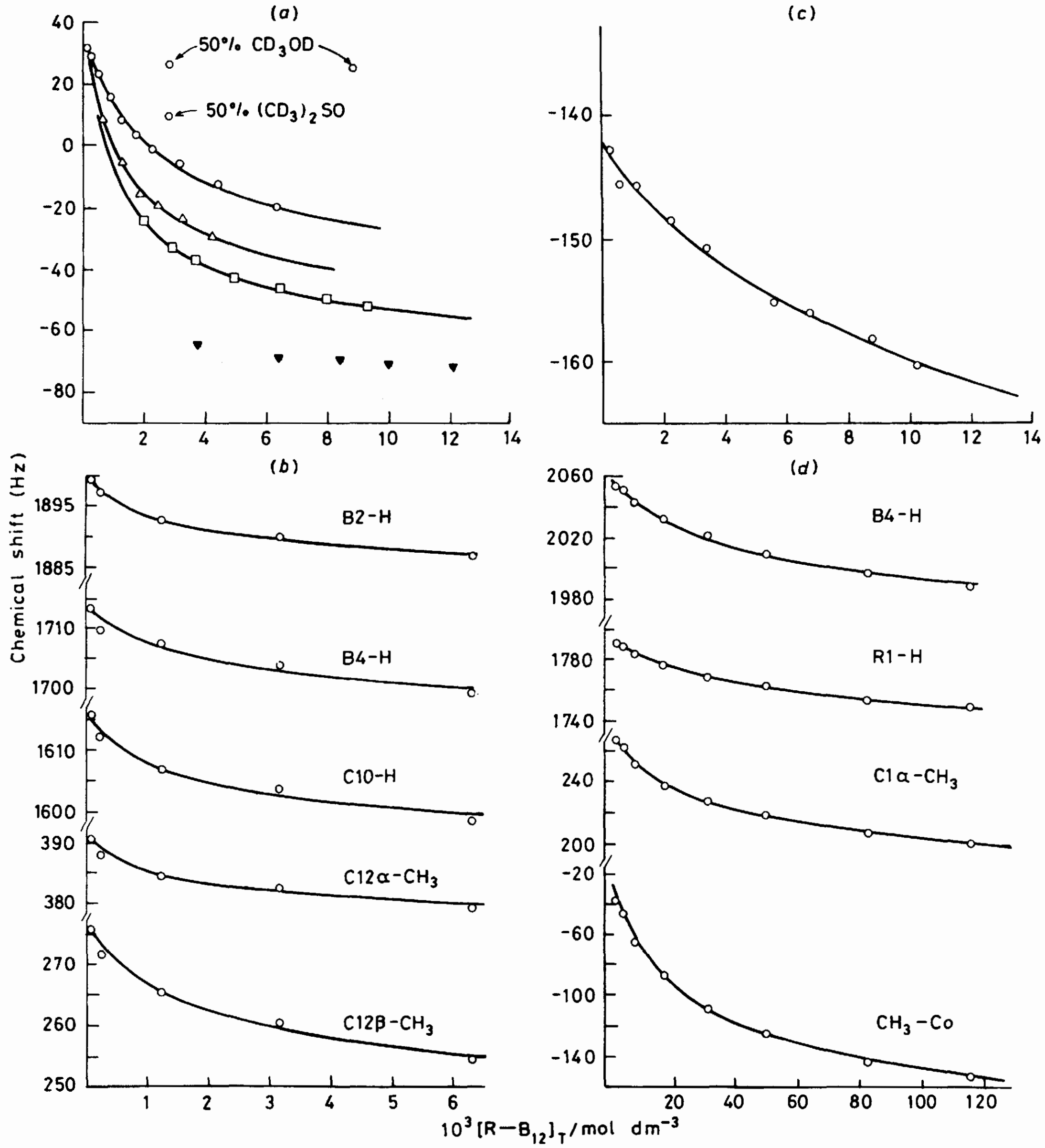


Figure 1. Concentration dependence of 270-MHz ^{1}H n.m.r. shifts for organocobalamins. Solid curves represent calculated best fit by equation (3). (*a*) CH_3–Co resonance of base-on CH_3–B_{12} at different [NaCl] [0 (○), 0.1 (△), 1.0 (□), and 2.5 mol dm^{-3} (▼)]. Top curve, unbuffered; all others, pH 7.2 (phosphate). (*b*) Other resonances of base-on CH_3–B_{12}; [NaCl] = 0, unbuffered. (*c*) CH_3CH$_2$Co resonance of base-on C_2H_5–B_{12}; [NaCl] = 0, unbuffered. (*d*) Resonances of base-off CH_3–B_{12}; 1.0 mol dm^{-3} DCl

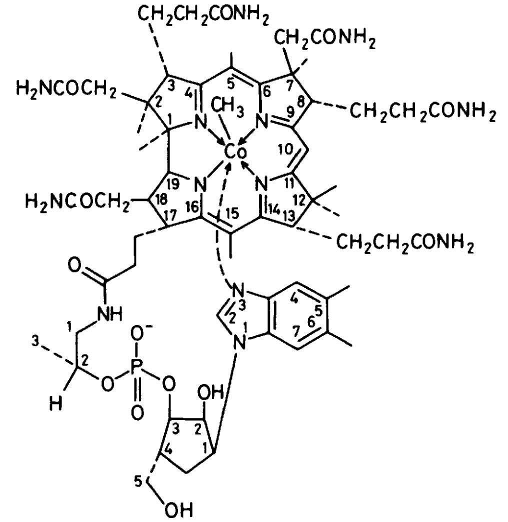
relating the observed shifts $\delta_{obs.}$ to the equilibrium constant K is given in equation (3). This is obtained from simultaneous solution of the mass balance, the equilibrium expression for equation (1), and the equation for the observed chemical shift of a nucleus undergoing rapid interconversion between two states [equation (2)] ($[B_{12}]_T$ = total cobalamin, δ_M and δ_D are the chemical shifts of the monomer and dimer, respectively).

$$\delta_{obs.} = \frac{[M]}{[B_{12}]_T}\cdot\delta_M + \frac{2[D]}{[B_{12}]_T}\cdot\delta_D \qquad (2)$$

$$\delta_{obs.} = \frac{(\delta_M - \delta_D)\{(1 + 8K[B_{12}]_T)^{\frac{1}{2}} - 1\}}{4K[B_{12}]_T} + \delta_D \qquad (3)$$

An iterative calculation was used to arrive at the best values of K, δ_M, and δ_D, minimizing the sum of the squares of ($\delta_{calc.} - \delta_{obs.}$). The results are given in Table 1 and the solid curves in Figure 1 represent the best fit by equation (3). Both Table 1 and Figure 1(*a*) show that dimer is favoured at increasing NaCl concentration. At [NaCl] = 0, K varied from 130 to 370 dm^3 mol^{-1} depending on which resonance was used for the determination. The value of K calculated from the CH_3–Co shift is likely to be closer to the true value because this resonance displays the largest shift difference between monomer and dimer and because more data were available for the calculation. With increasing NaCl concentration the sensitivity of the n.m.r. spectrometer decreased, compromising our ability to locate points in the curved portion (*i.e.* at low [CH_3–B_{13}]) of some plots in Figure 1(*a*). Thus, the absence of data below 3.8 mmol dm^{-3} CH_3–B_{12} at 2.5 mol dm^{-3} NaCl made it impossible accurately to calculate K at this salt concentration by our technique, but we estimate its value to be about (1—2) × 10^4 dm^3 mol^{-1}.

Table 1. Calculated chemical shifts and monomer–dimer equilibrium constants for organocobalamins in aqueous solutions



System	Resonance[a]	Shift[b]/p.p.m.		K/dm^3 mol^{-1}
		δ_D	δ_M	
Base-on CH_3–B_{12}: 0.0 mol dm^{-3} NaCl[c]	CH_3Co	−0.254	0.137	190
	C12β-CH_3	0.879	1.023	210
	C12α-CH_3	1.383	1.448	370
	C10-H	5.883	5.986	300
	B4-H	6.254	6.344	130
	B2-H	6.960	7.038	330
0.1 mol dm^{-3} NaCl[d]	CH_3–Co	−0.267	0.187	630
1.0 mol dm^{-3} NaCl[d]	CH_3–Co	−0.304	0.26	1 100
Base-off CH_3–B_{12}: 1.0 mol dm^{-3} DCl	CH_3–Co	−0.868	−0.109	16
	C1α-CH_3	0.558	1.006	15
	B4-H	7.059	7.615	6.6
	R1-H	6.289	6.638	7.2
Base-on C_2H_5–B_{12}: 0.0 mol dm^{-3} NaCl[c]	CH_3CH_2–Co	−0.764	−0.529	2.5

[a] C = Corrin, B = benzimidazole, R = ribose, and Pr = propanolamine. β Indicates the CH_3–Co side of the ring, α indicates the opposite side. [b] Base-off CH_3–B_{12} referenced to external tsp; all others to internal tsp. [c] Unbuffered. [d] Buffered at pH 7.2 with 0.1 mol dm^{-3} phosphate.

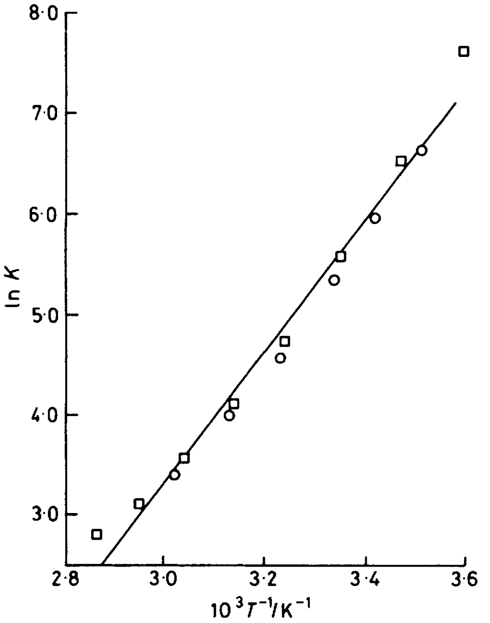


Figure 2. Van't Hoff plot for base-on CH_3–B_{12} in D_2O with K based on the CH_3–Co shift. Circles and squares represent separate experiments

Base-on C_2H_5–B_{12} in D_2O also shows evidence of dimerization. The concentration plot and fit by equation (3) for the CH_3CH_2–Co resonance in the absence of added NaCl is given in Figure 1(*c*) and K is listed in Table 1. The –CH_2–Co resonances at about 0.84 and 0.57 p.p.m. were not sufficiently resolved to be useful, but it was clear that they moved upfield with increasing concentration. The other hydrogens of base-on C_2H_5–B_{12} were shifted only very little.

The spectrum of CH_3–B_{12} in 1 mol dm^{-3} DCl is also concentration-dependent. Under these conditions the benzimidazole ligand is protonated and dissociated from the cobalt (*i.e.* base-off form). Values for several of the strongly shifted hydrogens are plotted as a function of concentration in Figure 1(*d*) along with the calculated best fits by equation (3). The calculated monomer–dimer equilibrium constants are listed in Table 1. Line broadening in the spectrum began to occur above 80 mmol dm^{-3}.

Monomer–dimer equilibrium constants for base-on CH_3–B_{12} were independent of whether the solutions were unbuffered or buffered at pH 7.2 with 0.1 mol dm^{-3} phosphate. The tendency toward dimerization found in chloride solutions was not observed for $NaClO_4$ media (0—5 mol dm^{-3}), in which no appreciable shifts occurred at constant total [CH_3–B_{12}] = 9.1 mmol dm^{-3}. The addition of CD_3OD or $(CD_3)_2SO$ to base-on CH_3–B_{12} solutions resulted in reversion of all resonances to values close to those of the monomer in D_2O. This is exemplified by the changes in the shift of CH_3–Co [Figure 1(*a*)]. The spectrum of CH_3–B_{12} in 50% CD_3OD in D_2O showed no significant concentration dependence above 16 °C, and the spectrum in 90% CD_3OD showed no concentration dependence to (at least) −6 °C. Thus, these solvents cause a breakdown of the dimer.

The temperature dependence of $\delta(CH_3$–Co) as a measure of the equilibrium constants for base-on CH_3–B_{12} generates the Van't Hoff plot in Figure 2. The least-squares fit of the data yields the thermodynamic parameters $\Delta H = -13$ kcal mol^{-1} and $\Delta S = -33$ cal K^{-1} mol^{-1} (14 points; correlation coefficient, $r^2 = 0.981$) at [NaCl] = 0. The noticeable curvature of the plot may indicate temperature-dependent changes in δ_M and δ_D operating simultaneously (see below).

Some other B_{12} derivatives were examined for self-association and association with CH_3–B_{12}. No significant concentration shifts (*i.e.* >3 Hz at 270 MHz) at 26 °C were observed for H_2O–B_{12}^+ (2.5—9.8), CN–B_{12} (2.3—9.3), Ad–B_{12} (3.8—11.5), or n-C_3H_7–B_{12} (4.8—14.4 mmol dm^{-3}). There is also no observable tendency for these cobalamins to associate with base-on CH_3–B_{12}; the spectrum of CH_3–B_{12} and each of the above cobalamins were unaffected in equimolar mixtures (≥4 mmol dm^{-3}).

Discussion

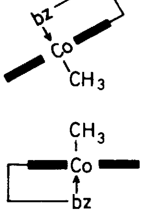
The chemical shift changes on passing from monomer to dimer for all of the assignable[11] resonances of base-on and base-off CH_3–B_{12} are given in Table 2. These shifts are extrapolated from changes in solutions containing mixtures of monomer and dimer. It was not possible to achieve both complete monomer and complete dimer formation by varying the total B_{12} concentration alone, due to solubility limits on the one hand and the detection limits of the instrument on the other. Although the dimer was favoured by increasing the concentration of NaCl or lowering the temperature, our studies indicate the existence of NaCl- and temperature-dependent shifts for many of the resonances. Table 1 shows the effect of NaCl on δ_D and δ_M of the CH_3–Co peak. Furthermore, CH_3–, H_2O–, n-C_3H_7–, CN–, and Ad–B_{12} show distinct (but not easily interpretable) chemical shift changes due to NaCl alone for some resonances. Other studies have suggested temperature-dependent conformational changes in B_{12} compounds which could (and do) affect chemical shifts.[11,12] The noticeable curvature in the Van't Hoff plot for base-on CH_3–B_{12} (Figure 2) may reflect such changes. To eliminate possible contributions from temperature or the medium, the shifts in Table 2 are reported for isothermal and constant-medium conditions.

Table 2. Concentration-dependent ^1H n.m.r. shifts ($\delta_D - \delta_M$/p.p.m.)[a] for methylcobalamin in D_2O at 26 °C

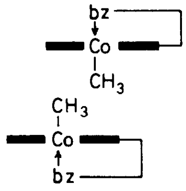
Resonance[b]	Base-on (unbuffered [NaCl] = 0)	Base-off (1.0 mol dm^{-3} DCl)
CH_3–Co	−0.391	−0.759
C1α-CH_3	−0.055	−0.448
C2α-CH_3[(a]	−0.029	−0.150
C17β-CH_3[(a]	−0.059	−0.140
C3β-H[#]	+0.118	
C8β-H[#]	+0.310	
C13β-H[#]	−0.224	
C5-CH_3	−0.043	−0.154
C7α-CH_3	−0.059	−0.218
C10-H	−0.100	(exchanges with D^+)
C12β-CH_3	−0.144	−0.145
C12α-CH_3	−0.065	−0.198
C15-CH_3	−0.067	−0.121
C18β-CH_2[•]	−0.016	
C2β-CH_2[•]	−0.037	
C19β-H	*ca.* 0	
B2-H	−0.078	−0.154
B4-H	−0.080	−0.556
B5-CH_3[$]	−0.031	−0.237
B6-CH_3[$]	−0.031	−0.290
B7-H	−0.016	−0.450
Pr1′-H	*ca.* 0	
Pr1″-H	*ca.* 0	
Pr2-H	0	
Pr3-CH_3	0	−0.094
R1-H	0	−0.349
R2-H	0	
R5′-H	0	
R5″-H	+0.028	

[a] Calculated (those resonances listed in Table 1) or extrapolated from monomer–dimer mixtures based on proportionality to the shift of CH_3–Co (all others). [b] See Table 1 for key. Ambiguous assignments are indicated by common symbols (see ref. 12).

Base-on Cobalamins.—Nearly all of the resonances of base-on CH_3–B_{12} are shifted upfield on dimerization. The largest shifts are found for the hydrogens on the periphery of the corrin ring and the benzimidazole group. The shifts of the ribose or propanolamine hydrogens, however, are quite small. This suggests a dimer structure in which the corrin rings are adjacent in a roughly parallel-planar arrangement; the upfield shifts thereby result from the mutual ring-current effect of the π-systems which are made up of the corrin ring and the metal atom.



(A)



(B)

Based on the pattern of shifts among the hydrogen atoms of base-on CH_3–B_{12} a structure in which the corrin rings are skewed (**A**), off-centre (**B**), or both is suggested. The equivalent dipole model for the magnetic field at a nucleus at polar co-ordinates R (r, θ) from the centre of a plane of a π system predicts a ring-current induced shift ($\Delta\delta$) proportional to $(1 - 3\cos^2\theta)/r^3$.[13] This is equivalent to a nodal cone extending from the π-system plane, where nuclei within the cone are shifted upfield and those outside are shifted downfield. A structure like (**A**) or (**B**) could thus account for the downfield shift of some corrin hydrogens on dimerization (*i.e.* two of the ambiguous trio at C3β, C8β, and C13β, see Table 1). Consistent with the inverse dependence of $\Delta\delta$ on distance from the corrin system of the adjacent CH_3–B_{12} unit are the following observations: (1) $\Delta\delta$ (C12β-CH_3) > $\Delta\delta$ (C12α-CH_3); (2) $\Delta\delta$ (B4-H) > $\Delta\delta$ (B7-H); (3) CH_3–Co displays the greatest shift of all; (4) the ribose and propanolamine hydrogens are little affected, consistent with their remoteness from the neighbouring corrin. An off-centre structure like (**B**) would circumvent some steric interaction between the organic ligands. A better determination of the geometry of the complex requires some knowledge about the magnetic ring-current properties of the corrin system through analysis of reference compounds.[3d,e,14]

A major contribution to dimer stability appears to be π–π bonding between adjacent corrin rings. Complexation by π–π bonding is well known for porphyrins and metalloporphyrins,[2,3] aromatic compounds,[13,14] and aromatic compounds with metalloporphyrins.[15] This would be the first evidence for such bonding among the corrinoids. The crystal structure of vitamin B_{12} (CN–B_{12}) shows no hint of such interaction.[16] The stabilization energy of a π–π complex is considered to originate in a charge-transfer interaction between polarizable π clouds.[14b,17] Among the metalloporphyrins the importance of polarizability of the π system is demonstrated by a correlation between the extent of self-aggregation and the extent of polarization between the metal atom and the porphyrin.[3f] The donor–acceptor character of the bond is demonstrated by the geometry of the complexes, wherein electron-rich pyrrole rings tend to overlap electron-poor rings,[3b,d,18] and by substituent effects.[3c] Hydrophobic forces may also contribute to complexation in aqueous solvents.[3a]

Factors which influence the stability of the organocobalamin dimer, steric hindrance, solvent properties, and electronic effects of the ligand, are in keeping with a π–π bonding interaction. Steric hindrance to approach is demonstrated by the following trend in K for R–B_{12}: R = CH_3 > CH_3CH_2 > $CH_3CH_2CH_2$, 5′-deoxyadenosyl ($K \approx 0$). The dimer is disfavoured by added organic cosolvents, which can be explained in terms of π–π bonding by a decrease in the dielectric constant resulting in a destabilization of the charge-transfer contribution to the wavefunction,[19–21] but favoured by added NaCl; porphyrins are known to aggregate in proportion to the ionic strength (chloride salts), presumably due to the screening of like charges on the monomeric units.[2] Curiously, $NaClO_4$ does not promote dimerization of base-on CH_3–B_{12}, for which we can offer no explanation.

The tendency for dimerization among these cobalamins depends critically on the ligand. The compounds H_2O–B_{12}^+/HO–B_{12}* and CN–B_{12} have no tendency to dimerize even at high [NaCl], nor do they complex with base-on CH_3–B_{12}. Possible explanations for this behaviour in terms of a π–π bond are: (1) the corrin ring in CH_3– and C_2H_5–B_{12} is more electron-rich[22] and therefore more polarizable, facilitating π–π bonding: (2) the H_2O–B_{12}^+ corrin–metal system carries an additional positive charge which inhibits association; (3) H_2O–B_{12}^+, HO–B_{12}, and CN–B_{12} monomers may be more highly solvated on the β side; (4) a 'hydrophobic' bond[23] contributes to the stability of the organo-B_{12} dimer.

Alternatively, dimer stability may originate in hydrogen bonds between amide groups on adjacent monomers. Amide-to-amide links are well known, for example in protein structure. It is difficult to rationalize certain of our observations by this mechanism alone. For example, 50% methanol completely disrupts the dimer above 16 °C, yet methanol is a weaker hydrogen bonder than water and would not be expected to compete favourably for hydrogen bonding to the amide groups. It is also difficult to explain the strong dependence on axial ligand for otherwise identical corrin structures in terms of hydrogen bonding. On the other hand, the ΔH value is at the high end of the range observed for π–π bonds in metalloporphyrins,[2] which suggests that hydrogen bonds may also be involved in the structure of the dimer.

Base-off CH_3–B_{12}.—Protonated base-off CH_3–B_{12} in 1 mol dm^{-3} DCl gives a monomer–dimer K that is smaller by a factor of *ca.* 10^2 than that of base-on CH_3–B_{12} in 1 mol dm^{-3} NaCl. The assignable resonances are all shifted upfield on dimerization (Table 2). The shifts are generally much greater than for base-on CH_3–B_{12}. The pattern is also quite different. One notable feature is that the benzimidazole hydrogens are strongly shifted, despite the fact that the nitrogen is protonated and the group is dissociated from the cobalt. This indicates that the benzimidazole group is positioned so as to be under the influence of ring-current effects. The near equivalency in the shifts of the B4-H and B7-H pair, and also the B5-H and B6-H pair, suggests a parallel-planar spatial arrangement of benzimidazole and the source of the ring-current magnetic field. This supports direct benzimidazole involvement in a π–π bond.

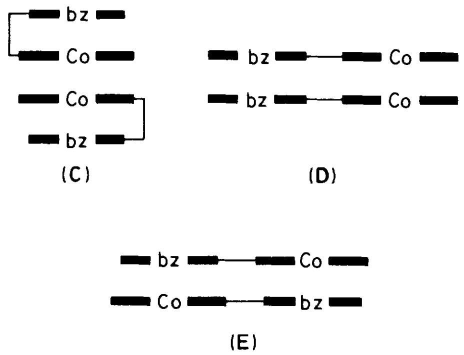


Figure 3. Possible structures of base-off CH_3–B_{12} dimer showing overlap among corrin and benzimidazolium π systems

The possible structures of the base-off dimer are shown in Figure 3. The relative benzimidazole:corrin ring shift ratios are considerably greater in the base-off than in the base-on CH_3–B_{12} dimer. For structure (**C**), similar ratios would be predicted if the benzimidazole–corrin overlap also exists in the corresponding monomer, for then the net influence on the benzimidazole hydrogens upon dimerization would be the remote presence of the corrin ring of the other CH_3–B_{12} unit. Structure (**C**) is, therefore, supported only if it is supposed that benzimidazole–corrin overlap is triggered by corrin–corrin overlap, and this does not seem credible. The results of this study do not offer a clear choice between (**D**) and (**E**). However, on the basis of donor–acceptor considerations pertaining to the energy of π–π bonds, wherein the positively charged benzimidazolium group is the acceptor and corrin the donor, structure (**E**) is more reasonable.

Other n.m.r. results relating to the structure of this dimer are noteworthy. First, the corrin α-CH_3 groups are more strongly shifted than the β-CH_3 groups, unlike the situation for base-on CH_3–B_{12} (*e.g.* C12 methyls); at the same time, however, the CH_3–Co resonance displays the largest shift. This is consistent, for any of the structures in Figure 3, with mixed α-side and β-side positioning of the opposing π system, with a further stipulation that β-side positioning is more distant, possibly due to the steric effects of the methyl ligand. Secondly, the ring-current shifts extend to the ribose (R1-H) and, to a lesser degree, the propanolamine (Pr3-CH_3) groups. Molecular models show that R1-H is sandwiched between ring systems in several of the possible conformations of (**C**)–(**E**) whereas Pr3-CH_3 is further away from an opposing π system.

Acknowledgements

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