Molecular Mechanics of Cobalt Corrinoids. 2. Structure of Alkylcobalamins and Thermolysis of the Cobalt−Carbon Bond

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Received July 7, 1994[⊗]

The structures of methyl-, (5′-deoxyadenosyl)-, benzyl-, and neopentylcobalamin were determined by molecular mechanics calculations using a force field developed specifically for the cobalt corrinoids. A grid search of the cobalamin ring side-chain orientations in MeCbl was used to identify their accessible conformations in the temperature range in which thermolysis of the alkylcobalamins have been studied. Rotation of the alkyl ligand in each complex was used to identify its possible orientations relative to the corrin ring. Thus, likely conformations of each complex were identified, and from these, the population-weighted Co−C bond lengths and Co−C−X (X = C, H) bond angles were determined. The steric strain induced in each complex on coordination of the alkyl ligand was investigated by determining the steric strain as a function of Co−C bond distance. The steric demands of the alkyl ligands were found to be Me < Bz ≈ Ado < Np, and the Co−C bond length and Co−C−X (X = H in MeCbl, X = C in AdoCbl, BzCbl, and NpCbl) angle increases with an increase in the steric demand. There is an inverse relationship between the bond dissociation energy for the Co−C bond and the steric strain in the complex and the Co−C bond length and the Co−C−C bond angle for MeCbl, AdoCbl, and NpCbl (but not BzCbl because of resonance stabilization of the incipient radical). This suggests that the structure of the ground state is influenced by the same factors that control the strength of the Co−C bond in alkylcobalamins. The implication of these findings for the AdoCbl-dependent enzymes is discussed.

It is generally accepted that the primary event in catalysis by (5′-deoxyadenosyl)cobalamin (AdoCbl)-requiring[1] enzymes involves the homolysis of the Co−C bond,[2] but the details of the mechanism, and in particular how the enzyme effects the tremendous rate enhancement observed, remain unclear. There is consequently considerable interest in understanding the factors which control the strength of the Co−C bond. Experimentally determined activation parameters for the Co−C bond homolysis in four alkylcobalamins, RCbls, are available (Table 1), but the crystal structures of only two of them, AdoCbl[3,4] and MeCbl,[5] have been reported.

Table 1. Activation Parameters for the Homolysis of the Co−C Bond in Alkylcobalamins

compound	$\Delta H_{\mathrm{on}}^{\ddagger}$ [a] (kcal mol^{-1})	$\Delta S_{\mathrm{on}}^{\ddagger}$ (cal mol^{-1} K^{-1})	$\Delta H^{\ddagger}_{\mathrm{Co-C}}$ (kcal mol^{-1})[b]	ref
MeCbl	41 ± 3[c]	24 ± 6[c]	37 ± 3	6
AdoCbl	33 ± 2[d]	11 ± 3[d]	30 ± 2	7
	34.5 ± 0.8[c]	14 ± 1[c]	32 ± 2	8
BzCbl	27 ± 1[d]	19 ± 3[d]	23 ± 1[e]	9
NpCbl	28.1 ± 0.2[d]	18.8 ± 0.6[d]	24.1 ± 0.3[f]	10

[a] For homolysis of the Co−C bond for the base-on form of RCbl. [b] The Co−C bond dissociation energy determined by the method of Finke et al.[11,12] [c] In ethylene glycol. [d] In aqueous solution. [e] From a plot of ln(η) against $1/T$, $\Delta H^{\ddagger}_{\eta} = 4.23 \pm 0.07$ kcal mol^{-1} in the temperature range 5−35 °C. [f] $\Delta H^{\ddagger}_{\eta} = 3.98 \pm 0.06$ kcal mol^{-1} in the range 15−40 °C.

In the absence of crystallographic information, molecular mechanics (MM) is potentially a very useful method for answering questions concerning molecular structure.[14] We have recently[15] extended the MM2 force field[16] specifically for studying the cobalt corrinoids. On the basis of 19 cobalt(III) corrinoid X-ray structures, the force field reproduced bond lengths, bond angles, and torsional angles to within 0.01 Å, 2.4°, and 4.2°, respectively—and hence within one standard deviation—of the mean of these parameters found in the solid

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[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1995.

(1) Abbreviations: Cbl, cobalamin; Ado, 5′-deoxyadenosyl; Np, neopentyl; Bz, benzyl; bzm, 5,6-dimethylbenzimidazole; AdePr, adenylpropyl.

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(12) The appropriate equation[11] is $\Delta H^{\ddagger}_{\mathrm{on}} = \Delta H^{\ddagger}_{\mathrm{Co-C}} + F_{\mathrm{c}}[\Delta H^{\ddagger}_{\mathrm{d}} - \Delta H^{\ddagger}_{\mathrm{c}}]$, where the last three terms refer to the cage efficiency and the activation enthalpies for diffusion of the two radicals away from the cage and their recombination in the cage, respectively. Assuming a robust cage, $\Delta H^{\ddagger}_{\mathrm{Co-C}} \approx \Delta H^{\ddagger}_{\mathrm{on}} - F_{\mathrm{c}}\Delta H^{\ddagger}_{\eta}$. F_{c} has been measured for AdoCbi^{+} to be $0.94 < F_{\mathrm{c}} < 1.0$; in other cases, F_{c} is assumed to be 0.75.[13] Similar equations exist for $\Delta S^{\ddagger}_{\mathrm{Co-C}}$.

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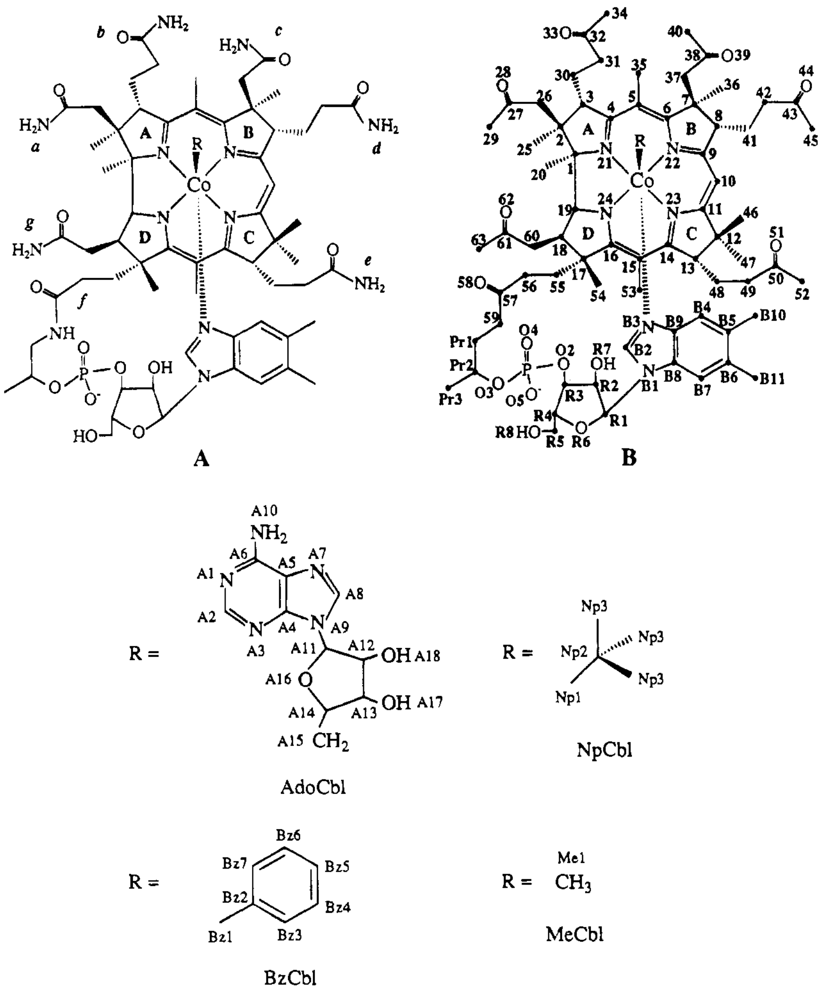

Figure 1. Cobalt corrinoids studied in this work and atom numbering used. In this standard view of the cobalamins, looking down on the upper (β) face, the C5, C10, and C15 bridging carbon atoms, and the C19–C1 bond define the "northern", "eastern", "southern", and "western" quadrants of the molecule, respectively. The four reduced pyrrole rings, clockwise from top left, are labeled A through D.

state. Using the force field, we verified that the axial ligand occupying the lower coordination site in the cobalamins, 5,6-dimethylbenzimidazole, has limited rotational freedom and is constrained by the downward-pointing *b* and *d* propionamide side chains of the corrin ring. Strain-energy profiles for rotation of the side chains of the corrin ring revealed several local energy minima and so explained the observed variability in the orientations of these side chains in the solid state. The known change in conformation which occurs in the C ring when the *e* side chain is epimerized from the lower to the upper face of the corrin ring in cyano-13-epicobalamin was correctly predicted. An analogous conformational change in the B ring in cyano-8-epicobalamin was predicted not to occur, and the epimerized *d* side chain was found to assume an equatorial orientation relative to the corrin ring. This was subsequently confirmed by an X-ray diffraction determination of the structure of this compound.[17] Parameters were also developed for alkylcobalamins which accurately reproduced the observed structure of methyl- and adenosylcobalamin.

Structural elements such as the Co–C bond length, $l_{\mathrm{Co-C}}$, and the Co–C–C bond angle, ϕ, depend on the orientation of the axial alkyl ligand.[15] It is by no means certain that the orientation of Ado observed in the solid state, for example,[3,4] persists in solution, especially at the high temperatures (85–110 °C)[7,8] needed to study the kinetics of its Co–C bond homolysis.[18] Indeed, there is NMR evidence[19] to show that at least one other orientation of Ado exists in solution at room temperature; in this, the Ado ligand has rotated by about 50° in a counterclockwise direction (when looking downward on the upper, or β, face of the molecule) from that observed crystallographically. Furthermore, because of the conformational flexibility of six of the seven side chains of the corrin ring in the cobalamins (the *f* side chain is constrained by the nucleotide loop), there is no guarantee, and indeed it is unlikely, that the orientation of these side chains observed in the solid state will persist in solution. Each of these side chains has three lowest-energy orientations relative to the corrin ring,[15] and there is only a small energy difference between them. Since the orientations of the side chains affect the conformation of the corrin ring, their orientations may indirectly affect the structural elements about the Co–C bond.

We report here the use of MM methods to predict the structures of the four alkylcobalamins listed in Table 1 (Figure 1), for which activation parameters for homolysis of the Co–C bond are available. We have identified the orientations of the side chains and the axial alkyl ligands which produce accessible conformations (i.e., > 0.1% of the population) in the temperature range in which the thermolysis was studied. The structures of

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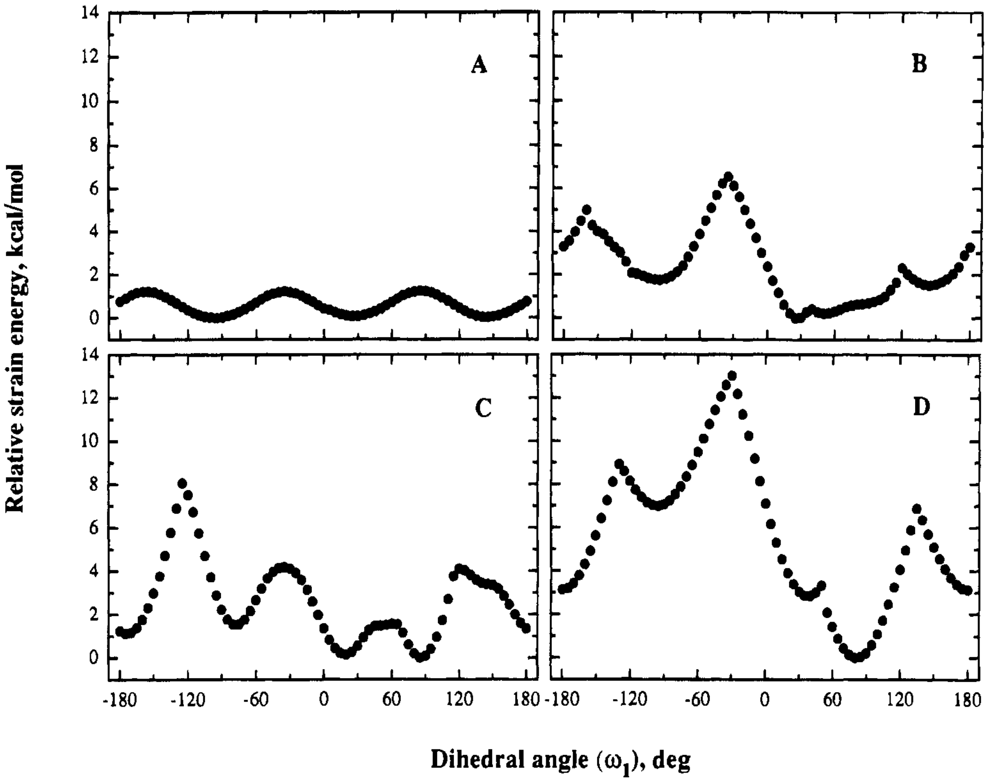

Figure 2. Strain energy for rotation of a coordinated alkyl ligand in alkylcobalamins, plotted relative the minimum strain energy found. (A) MeCbl. The dihedral angle refers to a N24−Co−C−H angle. (B) AdoCbl. The dihedral angle is the N24−Co−A15−A14 angle. The minimum conformation occurs when $\omega_1 = 25°$ (the crystallographically observed[4,5] orientations are between 11.6° [4] and 16.6° [5]). When $\omega_1 = 25°$, the ribose is near the C ring and the adenine is over C13−C14. Counterclockwise rotation to $\omega_1 = 90°$ brings the ribose over the C ring and adenine over C10. The barrier at $\omega_1 = 120°$ finds adenine over the *c* side chain. At 145°, the ribose is over the B ring and adenine is over the northern quadrant of the molecule. At −160°, ribose is over the northern quadrant and adenine is over the *a* side chain. At −100°, ribose is over the A ring and adenine is over the western quadrant. When $\omega_1 = -25°$, the ribose is near the D ring and adenine is near C54. (C) BzCbl, in which ω_1 is the N24−Co−Bz1−Bz2 angle. The four minima find the benzene ring of the Bz ligand over the southern ($\omega_1 = 25°$), eastern (85°), northern (±180°), and western (−80°) quadrants, respectively. (D) NpCbl. The overall minimum at ω_1 (N24−Co−Np1−Np2) = 80° has two of the Np3 methyl groups pointing downward toward the corrin ring and straddling the C46 methyl group. Clockwise rotation finds a minimum at ±180°, where these methyl groups straddle the C37 methylene of the *c* side chain, one at −80°, where they straddle the C26 methylene of the *a* side chain, and one at 35°, where the two methyl groups are between C46 and C54, with the Np2−Np3 bonds virtually eclipsing the N24−Co and N23−Co bonds.

the four alkylcobalamins, with the alkyl ligand placed in (but not constrained to) each of its minimum energy orientations and with the side chains in (but not constrained to) all their minimum energy orientations, have been determined. Population-weighted mean values of $l_{\text{Co-C}}$ and ϕ have been determined, and attempts have been made to correlate these structural elements with the available activation parameters for the homolysis of Co−C bonds in order to delineate features of the structure of the alkylcobalamins which may be important in controlling the strength of this bond.

Methods

The parameters for the MM force field for the cobalt corrinoids we have previously described,[15] developed using a modified[20] version of the MM program MM2(87),[16] were incorporated into the MM+ force field in HYPERCHEM (Hypercube, Inc., Waterloo, ON). The only difference in the functional forms between MM+ and MM2(87) lies in the treatment of the bond stretching term. In MM2(87), this is given as

$$E_{\text{str}} = 143.88 \sum {}^1/_2 k_s (l - l_0)^2 [1 + \text{CS}(l - l_0)]$$

where l_0 is the strain-free bond length, k_s is the bond-stretch force constant, CS is a cubic stretch term (= −2.0), and the numeric factor is a conversion factor into kcal mol^{-1}. At large values of l, the expression becomes repulsive and is therefore only suitable when the observed bond length, l, is not very much longer than l_0. In MM+, this problem has been overcome by using instead

$$E_{\text{str}} = 143.88 \sum {}^1/_2 k_s (l - l_0)^2 [1 + S(\text{CS})(l - l_0)]$$

where the switching function $S = 1$ if $(l - l_0) < \alpha$ and $S = 0$ if $(l - l_0) > \beta$, where $\alpha = -\text{CS}/3$ and $\beta = -4\text{CS}/3$; to avoid a discontinuity, $S = (\beta - (l - l_0))^2(\beta + 2(l - l_0) - 3\alpha)/(\beta - \alpha)^3$ when $\alpha \le (l - l_0) \le \beta$. Hence, at long bond distances, the equation becomes a quadratic, avoiding the repulsive behavior found in MM2(87). We verified that the bond stretching parameters used previously in MM2(87)[15] were appropriate in that the structures of AdoCbl, MeCbl, and CNCbl predicted with MM+ were virtually identical to the structures predicted using MM2(87). The convergence criterion used was <0.05 kcal mol^{-1} Å^{-1} in the rms gradient. The only changes which had to be made were to the parameters for the Co−C bond and the Co−N(bzm) bond. We previously used $l_0 = 1.960$, and $k_s = 1.740$ mdyn Å^{-1} to reproduce as best as possible the observed Co−C bond length in all alkylcobalamins whose structures have been reported; the values needed to effect the same task in MM+ were $l_0 = 1.900$ and $k_s = 1.650$ mdyn Å^{-1}. For the Co−N(bzm) bond, l_0 was increased from 2.010 to 2.160 Å to better reproduce the known Co-N(bzm) bond lengths in alkylcobalamins (viz., 2.234,[3] 2.243,[4] 2.237,[4] and 2.220 Å[4] in AdoCbl, 2.195 Å in MeCbl,[5] 2.211Å in AdePrCbl,[21] and 2.087 Å in 2,3-dihydroxypropylCbl;[22] average 2.187 Å); k_s remained unchanged at 1.80 mdyn Å^{-1}.

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We have previously shown[15] that each of the side chains, with the exception of the *f* side chain to which the nucleotide loop is attached, has three lowest energy conformations relative to the corrin ring. There are therefore 729 possible locally minimum energy structures. The energy of each of these in MeCbl was determined, and a Boltzmann distribution at 130 °C —representative of the temperature range in which the thermolysis of MeCbl has been studied—was performed; 32 structures (Table S1 of the supporting information) were identified which contained >0.1% of the population at this temperature. Since MeCbl contains the least sterically demanding alkyl ligand of the four complexes studied and the steric interaction between the alkyl ligand and the side chains will be smallest, it is therefore reasonable to assume that these 32 structures are the lowest energy structures available to any alkylcobalamin.

Steric barriers to the rotation of the alkyl ligand R in RCbls were examined by driving the Co-bound alkyl ligand through 360° in steps of 5° in the four complexes with the side chains set at, but not confined to, the orientations observed in the solid state in AdoCbl.[4] The dihedral angle, ω_1, chosen for this purpose was the N24–Co–C(alkyl)–X(alkyl) (X = C in Ado, Bz, and Np; X = H in Me). The strain-energy minima in the rotational profile of each of the four ligands were identified. Each ligand was set, but not constrained, to each of these locally minimum energy conformations, and the complex was energy minimized in each of the 32 lowest energy side-chain conformations identified for MeCbl. A Boltzmann distribution was then performed over all such energy-minimized conformations at a temperature representative of the temperature range at which the thermolysis of each complex has been studied; those conformations with >0.1% of the population were taken to be significant. The Co–C bond length and Co–C–X (X = H in MeCbl, X = C in MeCbl, BzCbl, and AdoCbl) bond angle for each complex were then determined as a population-weighted average.

The output files from MM+ for the energy-minimized structures were examined to determine the origin of the steric barriers to rotation of the alkyl ligand. Interactions for which there was a significant change[23] in the strain energy during the rotation of the alkyl ligand were identified. Since the total strain energy in any given molecule during an MM calculation will depend on the number and interrelationship of the atoms in that molecule, a direct comparison of the strain energies of different molecules is not meaningful. The strain energies were therefore recorded as ΔE_{str} values relative to the minimum found for each structure during rotation of the alkyl ligand. Similarly, the contribution to the strain energy of any given parameter (bond length, bond angle, torsional angle, van der Waals interaction, out-of-plane deformation, dipole interaction) was recorded as a ΔE value relative to the minimum value found for that parameter.

The strain energy due to coordination of the ligand in each alkylcobalamin was assessed by energy-minimizing structures with progressively longer Co–C bonds, achieved by changing the strain-free bond length, l_0, from 1.90 to 20.00 Å while maintaining the same stretching force constant.

The van der Waals volumes of the alkyl ligands were determined as previously described[24] with the atomic radii used in MM+. Unrestricted Hartree–Fock SCF–MO calculations were performed using the PM3 model[25] with HYPERCHEM.

Results

The variation in molecular strain energy as a function of the N24–Co–C(alkyl)–X (X = C in Ado, Bz, Np; X = H in Me) dihedral angle, ω_1, is shown in Figure 2 for the four compounds studied. The principal contributions to the strain-energy barriers to rotation of the alkyl ligands relative to the corrin ring were due to bond stretching, angle bending, torsional strain and van der Waals interactions;[26] only minor contributions arose from out-of-plane deformations of sp^2-hybridized atoms, stretch–bending deformations, and dipole interactions.

Table 2. Structural Parameters for Alkylcobalamins As Determined by Molecular Mechanics[a]

	MeCbl	AdoCbl	BzCbl	NpCbl
	Co–C Bond Length (Å)			
max	2.000	2.091	2.083	2.190
min	1.982	2.007	2.002	2.163
mean (σ)	1.988 (0.006)	2.023 (0.020)	2.018 (0.016)	2.171 (0.007)
	Co–C–C Bond Angle (deg)			
max		124.1	123.5	141.3
min		119.1	114.9	133.0
mean (σ)		121.6 (1.5)	117.0 (3.1)	136.7 (1.9)
	Co–C–H Bond Angle (deg)			
max	115.7	110.5	110.1	98.1
min	105.5	98.9	101.3	87.0
Mean (σ)	110.1 (0.1)	104.5 (3.2)	106.4 (1.3)	92.1 (1.9)
	Ligand van der Waals Volume (Å^3)			
	44.3	287.4	139.7	139.2
	Tilt Angle, θ (deg)[b]			
max[a]	85.5	88.5	86.9	84.5
min[a]	85.1	84.1	84.3	81.7
mean (σ)	85.3 (0.3)	85.9 (1.5)	85.9 (1.1)	83.4 (1.0)

[a] Weighted by population for all conformations found which contain >0.1% of the population. [b] Angle between the Co–C bond and the mean plane through the four corrin N donor atoms in the lowest energy conformation.

The rotational profile for coordinated Me in MeCbl (Figure 2A) shows three-fold periodicity as the three H atoms rotate over the macrocycle. The main contribution to the apparently small barriers to rotation are due to 1,4 van der Waals interactions between coordinated Me H atoms and the four corrin N donor atoms, although no single interaction is in itself very significant.[23]

As there is only a small energy difference between MeCbl structures where a C–H bond of the coordinated ligand and an equatorial Co–N bond are eclipsed and structures where they are staggered (Figure 2A), the methyl ligand was confined in both orientations and the side chains were placed in (but not confined to) the 32 lowest-energy conformations referred to above. After energy minimization of each of the 64 structures, a Bolzmann distribution at 130 °C (the thermolysis was studied between 120 and 140 °C[6]) was performed and 62 structures were found, each of which contained >0.1% of the population (Table S2 of the supporting information). The population-weighted average Co–C bond length and Co–C–H bond angles were determined from these structures, and the results are listed in Table 2.

The Co–C bond length depends on the orientations relative to the corrin ring of both the methyl ligand and the side chains. When the side chains are in the orientations found in AdoCbl, the Co–C bond undergoes a 3-fold periodic elongation from a minimum length of 1.984 Å to a maximum length of 1.991 Å as the ligand is rotated through 360° relative to the corrin ring. Depending on the orientation of the side chains, the bond length varies between 1.982 and 2.000 Å. The weighted average Co–C bond length is 1.988(6) Å. The lowest energy conformation of MeCbl predicted by the MM model finds the *c* side chain with a C8–C7–C37–C38 torsional angle of −67.6°; in the crystal structure, the value is 71.2°. The other side chains

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(23) Significant interactions were taken as those for which, during rotation of bound R, there was a > 0.5 kcal·mol^{-1} change in strain energy for that interaction.

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(26) van der Waals interactions in MM2(87) are either due to 1,4 interactions or other, more remote interactions; 1,2 and 1,3 interactions are not treated explicitly, being subsumed in bond-stretching and angle-bending terms.

are in the orientations observed in the solid state. The solid state conformation is predicted to be a 0.8 kcal mol^{-1} above the lowest energy conformation found. With the side chains in the orientations found in the crystal structure, the Co−C bond length was 1.982 Å. The crystallographically observed value in MeCbl is 1.99(1)Å;[5] a bond length of 1.998(5) Å is reported in methyl(pyridine)cobaloxime.[27] The Co−C−H bond angles vary between 115.7° and 105.5°, with a population-weighted average value of 110.1(1)°. The bond lengths, bond angles, and torsional angles for the remainder of the structure were within the previously observed deviations from the mean of the 19 crystal structures used in developing the force field.[15]

On the basis of an analysis of NMR observations of NOEs in AdoCbl at ambient temperature, Bax et al.[19] found significant cross peaks between the protons of A8 and C46; A11 and C54, C53, and C45; A14 and C19, C54, and C46; and A15 and C37, C26, and C19. They concluded that the Ado ligand exists in one of two conformations, the first corresponding to that observed crystallographically and the second in which Ado has rotated by 50−60° in a counterclockwise direction (as viewed from above) to lie over the "eastern" quadrant. The MM model (Figure 2B) is in agreement with these observations. Apparently, only a small barrier exists for the interconversion between the global energy minimum (which is the structure seen in the solid state) and a range of conformations where the the Ado ligand lies over the eastern portion of the molecule. Furthermore, only a relatively modest barrier exists to a further 50−60° counterclockwise rotation to bring the ligand over the "northern" quadrant. Yet another low-energy conformation occurs with the Ado ligand over the "western" quadrant of the molecule. We assumed that, in the temperature range in which homolysis of AdoCbl was studied (85−110 °C)[7,8] all four conformations are populated.

The Ado ligand was placed (but not constrained) to each of these four low-energy orientations relative to the corrin ring, and in each case, the structures with the side chains in (but not constrained to) the 32 low-energy conformations were energy minimized. A Boltzmann distrubution (100 °C) over all 128 conformations found 41 conformations containing >0.1% of the population (Table S2). The lowest energy conformation of AdoCbl found corresponds quite closely to the conformations observed crystallographically.[3,4] The Co−C bond length varied between 2.007 and 2.091 Å and the Co−C−C bond angle between 118.7° and 124.1° in the 41 significant conformations found. The population-weighted Co−C bond length is 2.023(20) Å (2.018 Å in the lowest energy conformation found; cf. 2.03(2) Å[3] and 1.98−2.04 Å[4] in the solid state). The mean Co−C−C bond angle is 121.6(1.5)° (123.4° in the lowest energy conformation; 125° [3] and 121−124° [4] in the solid state); the mean Co−C−H bond angle[28] is 104.5° (104° in the neutron-diffraction study[4]). There is therefore good agreement between the crystal structures of MeCbl and AdoCbl and the structures predicted by the MM model.

In AdoCbl, the strain-energy barriers to rotation of Ado relative to the corrin ring are due principally to angle-bending strain, torsional strain, and van der Waals interactions. There is considerable strain in the Co−A15−A14 bond angle in all orientations where the adenine lies over the A ring, western quadrant, or D ring. Angle bending within the ribose is significant as adenine passes over the *c* and *a* side chains. The major source of torsional strain occurs in the adenosyl ligand as it passes over the B, A, and D rings and in the corrin ring itself in the region between C11 and C16. Significant close contact is made between the α-H atoms (the H atoms of A15) and the corrin N donor atoms, in particular N22, N23, and N24. This interaction is similar to that seen in MeCbl but more significant because of the smaller Co−C−H bond angle (Table 2), forced by the presence of a *β*-C atom in the ligand. The most significant remote contacts occur between the upwardly projecting C19 hydrogen atom and the ribose oxygen atom A16, H(A14), and H(A15). Of the four "sentinel" carbon atoms of the corrin ring,[29] hydrogen atoms on C26 and C37, but not on C54 and C46, make significant contact with Ado during rotation. The presence of the bulky Ado ligand over the A ring, western quadrant, and D ring causes distortion in the corrin ring itself, and both H(C25) and H(C26) are induced into contact with H(C60) of the *g* side chain, attesting to the rather severe distortion which occurs in the corrin ring when Ado is over this region.

The side-chain orientations observed in the reported crystal structures[3,4] are predicted to be heavily favored. Of the 41 structures containing >0.1% of the population at 100 °C, all but 6, 4, 8, and 11 structures have, respectively, the same orientations of the *a, b, d,* and *g* side chains as found in the solid state, and all conformations have the same *e* side chain orientation. The most variable of the side chains is the *c* side chain, where all three orientations of the side chain are found with nearly equal frequency.

In BzCbl, there are four lowest energy orientations of the ligand relative to the corrin ring (Figure 2C); of these, the lowest two occur when the benzene ring of coordinated Bz is over the southern and eastern quadrants, respectively; these therefore resemble the conformations of AdoCbl observed by NMR, as mentioned above.

Searching the conformational space of BzCbl in analogous ways to the search done with MeCbl and AdoCbl revealed 41 structures with >0.1% of the population at 35 °C (Table S2). The lowest energy conformation finds the benzene ring just past methyl C46, over the "eastern" quadrant. The Co−C bond length varies between 2.002 and 2.083 Å, while the Co−C−C and Co−C−H bond angles vary between 114.9° and 123.5° and 101.3° and 110.1°, respectively. The population-weighted mean values for these parameters are 2.018 Å, 117.0°, and 106.4°, respectively.[30]

The Co−Bz1−Bz2 bond angle is a major contributor to the steric strain induced by rotation of Bz; there is also a significant contribution from the N21−Co−Bz1 bond, especially when Bz is over the A ring. Torsional strain is induced in the corrin ring, and close contact occurs between N22, N23, and N24, and the Bz1 hydrogen atoms. Remote contacts occur between H(C37), H(C26), H(C19), and C4 and the Bz ligand. Distortion induced in the corrin ring also brings H(C35) and H(C37) and H(C53) and H(C55), into contact, as well.

The rotational profile of NpCbl (Figure 2D) has already been discussed.[15] The lowest energy conformation in the rotational profile of the ligand finds two of the Np3 methyl groups pointing downward toward the corrin ring and straddling the C46 methyl

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(27) Bigotto, A.; Zangandro, E.; Randaccio, L. *J. Chem. Soc., Dalton Trans.* **1976**, 96.

(28) A force constant k_b = 0.105 mdyn Å rad^{-2} (cf., the default MM2 value of 0.360 mdyn Å rad^{-2} for as C−C−H bond angle, for example) is used in the force field to model the Co−C−H bond. This weak force constant was required to reproduce the Co−C−H bond angle of 104° observed in the neutron-diffraction structure of AdoCbl.[4]

(29) Glusker, J. P. In *B$_{12}$*, Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, p 23.

(30) It is difficult to gauge the reliability of these values. A crystal structure of BzCbl is not yet available, and a benzyl cobaloxime complex with benzyl *trans* to an aromic N donor appears not to have been reported. In benzyl(N-bound morpholine)cobaloxime,[31] the Co−C bond length is 2.11(1) Å. From the reported fractional coordinates, we calculate the Co−C−C bond to be 118.5°.

group. The conformational search (Table S2) found 53 conformations with >0.1% of the population at 40 °C (the thermolysis of NpCbl was studied at 5–45 °C);[10] all four orientations of the Np ligand are accessible at this temperature. The Co–C bond length varied between 2.190 and 2.163 Å, and the Co–C–C and Co–C–H bond angles varied between 141.3° and 133.0° and between 98.1° and 87.0°, respectively. The population-weighted averages of these parameters were 2.171(7) Å, 136.7(1.9)° and 92.1(1.9)°. Values of 2.060(6) Å and 130.3(4)° have been observed for the Co–C bond length and Co–C–C bond angle in neopentyl(pyridine)cobaloxime.[32] Distortion of the Co–Np1–H, N21–Co–Np1, B3–Co–Np1, N23–Co–C14, and N22–Co–Np1 bond angles are all significant contributors to the strain-energy barriers to rotation of bound Np, especially when the two downwardly projecting Np3 methyl groups are over the A ring, western quadrant, or D ring; this also induces torsional strain in the corrin ring. Close contact is made between H(Np1) and the corrin N donor atoms and between H(Np1) and H(Np3). There are many significant remote van der Waals interactions. The Np3 hydrogen atoms contact the ring carbon atoms C5, C6, C9, C11, and C14, the hydrogen atoms on C26, C37, C19, and C54, and the vertically projecting hydrogen atom of C19. Distortions in the corrin ring force close contacts between B3 and H(C20), H(19), and H(C26), and H(C37) and H(C35).

The tilt angle, θ, between the Co–C bond and the mean plane through the four corrin N donor atoms has been shown[33] to profoundly affect the Co–C overlap population, *e*, and hence the strength of the Co–C bond. Tilt angles were calculated for the structures corresponding to the minima and maxima in the strain-energy profiles for rotation of the alkyl ligands and are summarized in Table 2. There is no signficant difference in the average tilt angle for the four complexes.

The steric energy consequences of the coordination of the four alkyl ligands was assessed by energy minimizing the lowest energy conformation of each structure while the strain-free value, l_0, of the Co–C bond length was increased from 1.90 Å (used to model the ground state of these alkylcobalamins) to 20.00 Å. Because the complexes are not the same, the absolute strain energies cannot be directly compared. In Figure 3, the strain energy of each complex, relative to the minimum strain energy found for that complex, is plotted against l_0. In all cases, the strain energy initially decreases rapidly as l_0 is increased, reaches a minimum, and then increases and eventually levels off at long Co–C distances. The initial decrease is principally due to a decrease in bond-strain energy, with less significant contributions from angle-bending strain, torsional strain, and nonbonded interactions. At long (>ca. 4 Å) Co–C bond lengths, there is an increase in strain energy because of loss of favorable nonbonded interactions and a slight increase in strain due to stretch–bend interactions (Figure S1 of the supporting information shows these effects for MeCbl).

In MeCbl, the decrease in bond strain energy on elongation of the Co–C bond is principally due to relief of steric strain in the Co–Me1 bond and, less significantly, in Co–N21, Co–N22, Co–B3, and C1–N21 bonds. There are relatively minor changes in angle-bending strain, with some relief in, for example, the C1–C19–C18 and B3–B9–B4 angles, somewhat offset by small increases in the steric strain in the C1–C19–N24 and B1–B8–B7 angles. The relief of torsional strain is principally confined to the corrin ring. Nonbonded repulsions, in particular, interactions between Me1 of the coordinated methyl group and N22, N23, and N24, and, less significantly, between B3 and N21 and N22 and between one of the H atoms of the coordinated methyl group and N24, are initially relieved on elongation of the Co–C bond. As the Co–C bond is stretched beyond ≈4 Å, an increase in strain energy occurs due to stretch-bend terms and, more significantly, the loss of attractive nonbonded interactions, not only between the coordinated ligand and the corrin ring atoms (a small contributor in MeCbl but the principal contributor in the other complexes where the alkyl ligand is larger) but also between many atoms in the ring and the side chains.

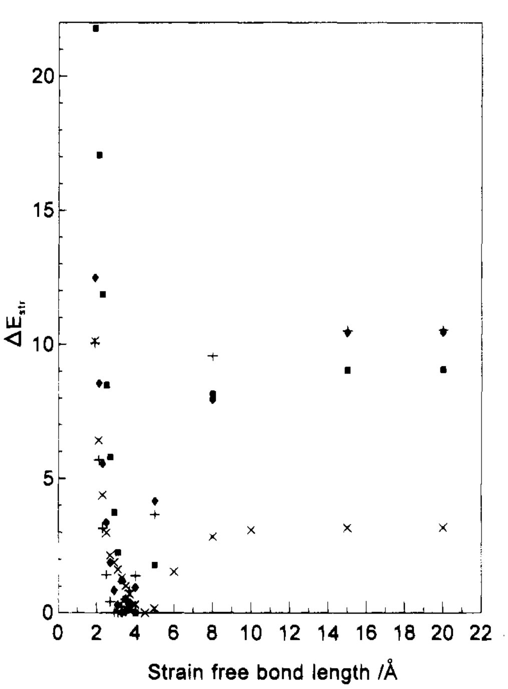

Figure 3. Differential strain energy (relative to the strain energy minimum for each compound) in MeCbl (×), AdoCbl (+), BzCbl (♦) and NpCbl(■) as a function of Co–C strain-free bond length.

Table 3. Steric Strain Relief in Alkylcobalamins on Conversion from the Ground State to the Transition State[a]

alkylcobalamin	[Co–C]‡ bond length[b] (Å)	ΔE_{str} (kcal/mol^{-1})
MeCbl	3.100	8.5
AdoCbl	3.092	10.5
BzCbl	3.109	12.2
NpCbl	3.140	20.0

[a] The strain-free Co–C bond distance in the transition state is assumed to be 3.1 Å. [b] The calculated Co–C bond distance in the transition state.

Discussion

The generally good agreement between the MM-predicted and, where available, the crystallographically-determined Co–C bond lengths, l_{Co-C}, and Co–C–C bond angles, ϕ, confirms the reliability of the force field. For the first time a method is available to seek correlations between experimental parameters for thermolysis of the Co–C bond and structural parameters involving these complexes.

The measurement of the temperature-dependence of the rate constants for thermolysis of the Co–C bond in the presence of a trap for the alkyl radical[8] and correction for the relatively inert base-off form lead to values for $\Delta H^{\ddagger}_{on}$ and $\Delta S^{\ddagger}_{on}$ for thermolysis of the base-on species of these RCbls. Finke et al.[11] have developed the appropriate radical cage chemistry to convert determined experimental $\Delta H^{\ddagger}_{on}$ values into $\Delta H^{\ddagger}_{Co-C}$

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(31) van Hooste, M.; Lenstra, A. T. H.; Kwiecinski, M.; Tyrilk, S. *Transition Met. Chem.* **1982**, *7*, 50.

(32) Bresciani-Pahor, N.; Randaccio, L.; Toscano, P. J.; Marzilli, L. G. *J. Chem. Soc., Dalton Trans.* **1982**, 567.

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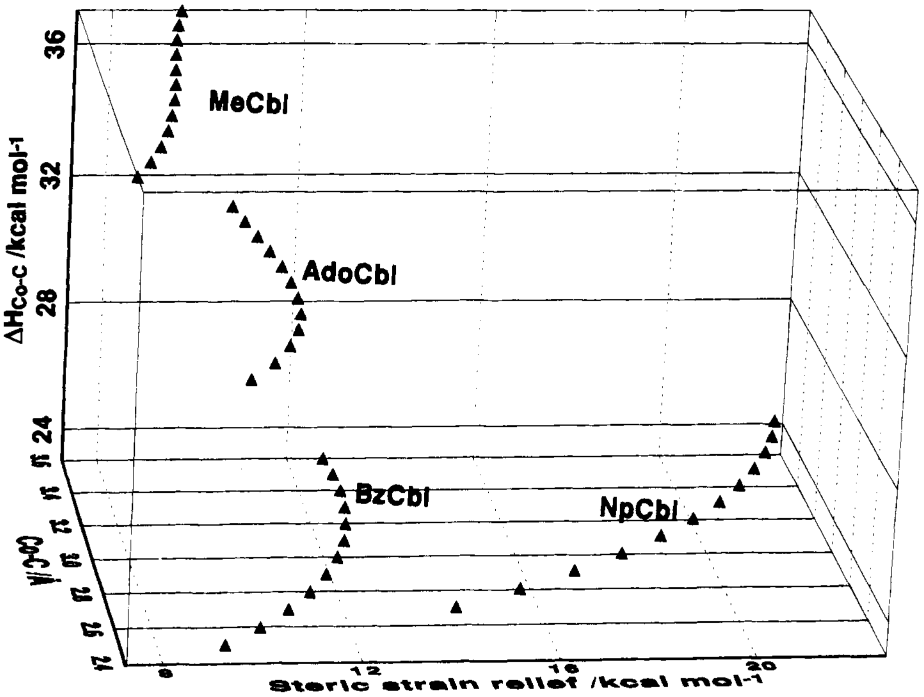

Figure 4. Relationship between the Co−C bond dissociation enthalpy, $\Delta H^{\ddagger}_{\mathrm{Co-C}}$, the strain-energy relief on passing from the ground to the transition state, and the Co−C distance in the transition state.

values for the bond dissociation energy of the Co−C bond.[12] These are listed in Table 1.

The position of the transition state for the thermolysis of the Co−C bond in the alkylcobalamins is unknown and, indeed, may vary from compound to compound. It is therefore difficult to decide on the appropriate value of the separation between the Co and C atoms and, hence, to determine the extent of strain-energy relief accompanying the approach to the transition state. *Ab initio* calculations of transition state geometries for C−H and O−H bond homolysis show that the ratio of the transition state internuclear distance to the ground state equilibrium distance is 1.6−1.7.[34] If we assume a similar value for the alkylcobalamins, then, in the transition state, an l_0 value of around 3.1 Å would be appropriate. The relief in strain energy in going from the ground state to the transition state can then be calculated, and the results are listed in Table 3. In Figure 4, the $\Delta H^{\ddagger}_{\mathrm{Co-C}}$ values are plotted as a function of the differential strain energy, ΔE_{str}, which accompanies the transition from the ground state to the transition state, and the Co−C distance, l_0, in the transition state. The figure shows that, irrespective of the exact position of the transition state, there is an inverse relationship between $\Delta H^{\ddagger}_{\mathrm{Co-C}}$ and the strain-energy relief, ΔE_{str}, for MeCbl, AdoCbl, and NpCbl, while the $\Delta H^{\ddagger}_{\mathrm{Co-C}}$ value for the Co−C bond in BzCbl is anomalously low.

Plots of $\Delta H^{\ddagger}_{\mathrm{Co-C}}$ against both the population-weighted Co−C bond length and the population-weighted Co−C−X bond angle, ϕ, are shown in Figure 5. The same inverse dependence between these parameters and the strength of the Co−C bond is observed, and the anomalously low bond energy of the Co−C bond in BzCbl is again clear. To determine whether these deviations for BzCbl were due to resonance stabilization of the emerging $\mathrm{PhCH_2^{\bullet}}$ radical in the transition state for BzCbl homolysis, semiempirical MO calculations for the lowest energy state of $\mathrm{PhCH_3}$ and $\mathrm{PhCH_2^{\bullet}}$ were performed; they gave $\Delta H^{\circ}_{\mathrm{f}}$ values of 13.9 and 39.5 kcal mol^{-1}, respectively. Hence $\Delta\Delta H^{\circ}_{\mathrm{f}}$(radical−hydrocarbon) = 25.6 kcal mol^{-1}. Similar calculations with the analogous saturated compounds $\mathrm{C_6H_{11}CH_3}$ and $\mathrm{C_6H_{11}CH_2^{\bullet}}$ gave $\Delta H^{\circ}_{\mathrm{f}} = -37.2$ and -3.7 kcal mol^{-1}, respectively, and $\Delta\Delta H^{\circ}_{\mathrm{f}} = 33.5$ kcal mol^{-1}. Hence, from the difference between the differentials of the saturated and aromatic compounds, $\Delta H^{\circ}_{\mathrm{f}}$ is lowered by 7.9 kcal mol^{-1} by resonance stabilization of the $\mathrm{PhCH_2^{\bullet}}$ radical. The additional stabilization of the transition state for BzCbl relative to the ground state (~4.7 kcal mol^{-1} in Figure 5A; ~5.3 kcal mol^{-1} in Figure 5B) is about 60% of the theoretically calculated resonance stabilization of

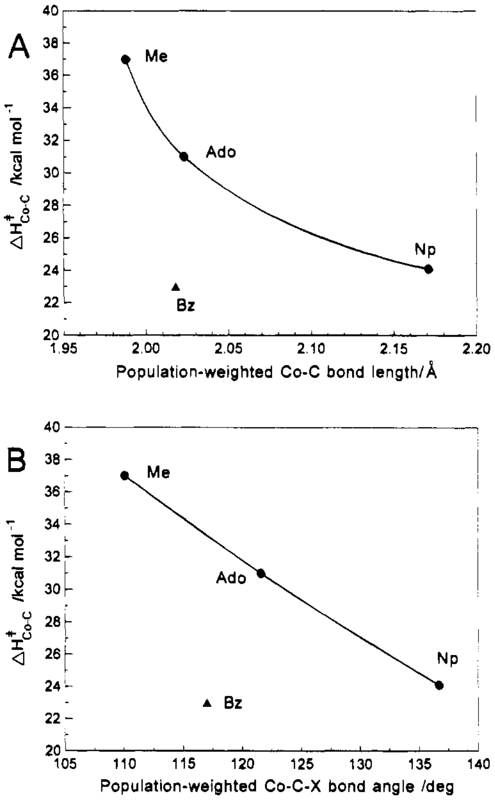

Figure 5. Relationship between (A) the population-weighted Co−C bond length and (B) the population-weighted Co−C−X (X = H in MeCBl, X = C in AdoCbl, BzCbl and NpCbl).

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(34) Saebo, S.; Radom, L.; Schaefer, H. F. *J. Chem. Phys.* **1983**, *78*, 845.

the PhCH$_2^\bullet$ radical; this may be an indication of the extent of the radical character in the emerging PhCH$_2^\bullet$ fragment in the transition state.

The present results suggest that binding of an alkyl ligand to cobalt in the cobalt corrinoids induces considerable steric strain. As the steric demands of the coordinated ligand increase (Me < Bz ≈ Ado < Np, Table 3), the Co−C bond length and Co−C−X (X = H in Me, X = C in Bz, Ado, Np) bond angle, ϕ, respond by increasing (Figure 5). The Co−C bond is essentially σ in nature[33,35] and could be expected to be sensitive to the Co−C bond length. However, both Zhu and Kostić,[33] and Christianson and Lipscomb[35] concluded that the strength of the Co−C bond in model complexes for RCbl is significantly more sensitive to angular distortion than to elongation. As mentioned above, we found no significant difference in the tilt angle, θ, and suggest that this is not an important factor in controlling the Co−C bond dissociation energy in these complexes.

From the reported[33] variation in Co−C overlap population, *e*, with ϕ, we calculate that an increase from 110.1° in MeCbl to 121.6° in AdoCbl (and neglecting an increase in bond length) results in a 24% decrease in *e*; from experiment, there is a 16% decrease in the measured bond-dissociation energy. Since the variation in *e* with ϕ was only reported up to 125° and extrapolations beyond this are not reliable, a value of *e* for NpCbl (ϕ = 136.7°) cannot be calculated but is likely to be very small. Nevertheless, the general trend is clear: steric hindrance on binding the alkyl ligand results in distortion in the ground state Co−C bond length and Co−C−X bond angle; this steric strain is relieved, or partially relieved, as the transition state is approached. This therefore suggests that the structure of the ground state is influenced by the same factors that control the strength of the Co−C bond in the alkylcobalamins.

Enzymatic activation of AdoCbl for Co−C bond homolysis by the enzyme ethanolamine deaminase at 25 °C leads to an approximately 10^{12}-fold rate enhancement relative to nonenzymatic thermolysis of AdoCbl,[7,8,36] requiring an approximately 16 kcal mol^{-1} lowering of the free energy of activation.[37] Recent work with side-chain-modified analogs[24] suggests that decreased ground state entropy due to enzymatically induced increases in steric restriction of side-chain thermal mobility might account for as much as one-third of the required reduction in activation free energy. The results of the current calculations suggest that the remainder could be obtained from perturbation of the Co−C bond in the ground state. Toraya et al.[38,39] have suggested that enzymatic activation of AdoCbl may be affected by angular bond strain induced by the enzyme "pulling" on the adenine moiety. The data of Figure 5 suggest that an increase in the Co−C−C bond angle to ~135° and a concomitant increase in Co−C bond length to ~2.17° could provide most of the additional 10 kcal mol^{-1} required.

The corrin ring has considerable flexibility as has been demonstrated by Glusker's analysis[40] of the X-ray structures of 13 corrinoids in which the corrin fold angle about the Co$\cdot\cdot$C10 axis varies by 20°. While many diverse mechanisms have been postulated for the enzymatic activation of AdoCbl, there is a widespread belief that steric effects induced by upward flexing of the corrin ring are an essential elements of such catalysis.[13,39c,41−47] Such an effect could presumably be caused by upward flexing of the corrin ring, increasing the steric contacts between the A15 H atoms and the corrin ring N donors. Considering the fact that we find the Co−C bond length and Co−C−C bond angle in AdoCbl to vary between 2.007 and 2.091 Å and between 118.7° and 124.1°, respectively, in the 41 conformations found which contain >0.1% of the population, an enzymatically induced increase in these parameters of the magnitude suggested above does not seem unreasonable.

Finally, we note that, not surprisingly, there is no correlation between $\Delta S^\ddagger$ and either $l_{\text{Co−C}}$ or ϕ. However, there is a linear correlation (not shown) between the size of the alkyl ligand, as measured by its van der Waals volume, and $\Delta S^\ddagger$ ($r^2 = 0.98$). As steric bulk increases, $\Delta S^\ddagger$ becomes less positive; in particular it is noteworthy that Bz and Np have virtually identical size, and virtually identical $\Delta S^\ddagger$ values. At present, we have no explanation for this observed trend. We hope to devise appropriate experiments to probe this question and to report the results elsewhere.

Acknowledgment. Much of this research was conducted at Mississippi State. H.M.M. thanks the University of the Witwatersrand for granting sabbatical leave, the Council of the University of the Witwatersrand for award of a Council Overseas Fellowship, and the Foundation for Research Development, Pretoria, for financial support. This research was supported by the National Institutes of General Medical Sciences through grant GM 48858 (to K.L.B.).

Supporting Information Available: Table S1, listing population distribution in MeCbl among accessible conformations as a function of side-chain orientations at 130 °C; Table S2, listing population distributions among accessible conformations in MeCbl, AdoCbl, BzCbl, and NpCbl; Figure S1, dependence of various components of strain energy in MeCbl on the Co−C strain-free bond distance (14 pages). Ordering information is given on any current masthead page.

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