The strongest type of interaction should be the electrostatic attraction between the phosphate of 5′-GMP and the metal cation, and this alone might cause the observed effects. Ion pairing between $[Co(en)_3]^{3+}$ and HPO_4^{2-} in solution[48] and cation–phosphate interactions in solid $[Co(en)_3]_2[HPO_4]_3 \cdot 9H_2O$[49] occur via hydrogen bonding between the N–H protons of en and the phosphate oxygens. CPK models show that, although the 5′-GMP^{2-} ion is considerably larger than HPO_4^{2-}, if the 5′-GMP phosphate were the only site interacting with the cation, the guanine and ribose units would be sufficiently distant from the cation so as not to perturb it, and there should be no difference in the spectra of the HPO_4^{2-} and 5′-GMP^{2-} compounds. The significantly improved resolution of the en proton resonances in the NMR spectrum of $[Co(en)_3]_2[5'\text{-GMP}]_3$ suggests that additional interactions with 5′-GMP are present (Figure 4).

Changes in coupling constants occur as a result of changes in the geometry of the coupled nuclei. The most stable conformation of the ethylenediamine chelate ring is puckered, in which the CH_2 protons are gauche to each other.[50] The metal–H coupling constant is dependent on, among other factors, the metal–N–C–H dihedral angle,[51] and a flattening of the en chelate ring pucker is consistent with the experimentally observed decrease in the metal–CH_2 coupling constant. This makes the en NH_2 protons sterically more accessible to hydrogen bonding with the guanine N7. Erickson et al. have qualitatively correlated $^3J_{Pt-H}$ with the Pt–N–C–H dihedral angle for a series of Pt(II) amino acid complexes and concluded that the coupling constant decreases as the dihedral angle decreases from 180 to 0°.[52] The fact that the $^3J_{Pt-H}$ does not decrease with increasing concentration in $[Pt(en)_2]^{2+}$–HPO_4^{2-} solutions but does so in $[Pt(en)_2][5'\text{-GMP}]$ is further evidence for the persistence of cation–guanine base interactions in solution.

5′-GMP (β-D) stereoselectivity chooses the $[\Lambda\text{-}(+)\text{-}Co(en)_3]^{3+}$ enantiomer from the racemic reaction mixture on the basis of the lesser solubility of $[\Lambda\text{-}(+)\text{-}Co(en)_3]_2[5'\text{-GMP}]_3$. Each of the pure diastereomers exists, and they were readily prepared by starting with enantiomerically pure $[Co(en)_3]^{3+}$. CPK models show that the $[\Lambda\text{-}(+)\text{-}Co(en)_3]^{3+}$ ion can easily bond concurrently to the N7, C=O, and phosphate of the same 5′-GMP without any appreciable distortion of the most common 5′-GMP conformation (anti, gg). However, the $[\Delta\text{-}(-)\text{-}Co(en)_3]^{3+}$ enantiomer can only bind with difficulty to the phosphate when it is hydrogen-bonded to N7 and C=O. In this case, the orientation of the ethylene chain of en limits the accessibility of the nearest NH_2 group for hydrogen bonding with the phosphate, even with a bridging water present. The lesser solubility of $[\Lambda\text{-}(+)\text{-}Co(en)_3]_2[5'\text{-GMP}]_3$ may result in part from the three-site binding between the cation and 5′-GMP.

It has been shown that hydrogen bonds are formed between metal-coordinated amine ligands and the base of 5′-GMP in the solid compounds and that it is likely that they persist in solution, even in the competitive solvent water. Such interactions between nucleic acids and antitumor drugs or other metal complexes will increase the overall strength of the bonding and therefore lead to more effective control of certain reactions of nucleic acids.

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Effects of Axial Ligation on the Thermolysis of Benzyl- and Neopentylcobamides: Analysis of the "Base-On" Effect

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The nature of the "base-on" effect, in which the base-on species of thermally labile alkylcobalamins is found to be labilized by 10^2–10^3-fold relative to the base-off species or analogous alkylcobinamide, has been studied for benzyl- and neopentylcobalamin. Appropriate correction of the observed activation parameters of the alkylcobalamins in neutral solution for the occurrence of significant amounts of the base-off species shows that the base-on effect is entirely entropic for these sterically hindered alkylcobalamins; the enthalpies of activation of the base-on and base-off species are essentially identical. Studies of the effects of exogeneous ligands on the thermolysis of the alkylcobinamides show that, within an isosteric series of 4-substituted pyridines, the carbon–cobalt bond is stabilized by increasing basicity of the trans axial ligand. In addition, for all four of the organic nitrogen donors studied, the alkyl(ligand)cobinamides are of comparable reactivity to that of the base-on alkylcobalamin. Even azide is shown to cause a significant base-on effect. Taken together with data from the literature, these results suggest that the base-on effect is primarily steric in nature, but it is the steric consequence of the *presence* of a strongly donating axial ligand that is important rather than the ligand's steric bulk. With the aid of models, a picture of the steric activation of these alkylcobalamins for Co–C bond homolysis emerges in which steric crowding of the bulky organic ligand by the upward projecting acetamide side chains is the driving force for reaction. It is believed that in the base-off cobalamins (and in the cobinamides) the ground state is entropically stabilized by a distortion of the flexible corrin ring, which provides relief of the steric congestion. Tentative support for this idea is obtained from the thermodynamics of formation of the alkyl(ligand)cobinamide complexes.

Introduction

There remains great interest in the mechanism by which 5′-deoxyadenosylcobalamin (AdoCbl)-requiring enzymes activate AdoCbl by inducing homolysis of its carbon–cobalt bond. Hay and Finke's study[1,2] of the thermolysis of AdoCbl demonstrates that such enzymes can increase the rate of thermal homolysis by a factor of at least 10^{10} at 25 °C. Most[3] of the hypotheses for this mechanism invoke steric distortion of the coenzyme as the primary driving force promoting homolysis.[2,4–10] One such hy-

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pothesis, which has been referred to as the mechanochemical trigger mechanism,[10-12] envisions steric compression of the long (2.24 Å)[13] axial Co–N bond of AdoCbl as the primary steric event leading to Co–C bond fission. This is an attractive hypothesis on several grounds. It would provide a rationale for the cobalamin chelate being pentadentate as well as for the apparent steric bulk of its 5,6-dimethylbenzimidazole axial ligand. It receives support from evidence of the importance of steric effects at both the upper (β) and lower (α) axial ligand positions of cobalamins and for transmission of such steric effects from one axial ligand position to the other. This is presumably accomplished via the corrin side chains, the a, c, and g acetamide side chains projecting above the corrin ring, and the b, d, and e propionamides and the f nucleotide loop side chain projecting below the corrin ring, with the flexibility of the corrin macrocycle[12] permitting transmission of steric effects from one side to the other. Thus, the pK_a's for protonation and displacement of the axial benzimidazole ligand of alkylcobalamins (and hence the intrinsic affinity of this ligand for the cobalt center) are largely determined by steric effects of the organic ligand.[14,15] The mechanochemical trigger mechanism also provides a ready explanation of the failure of the AdoCbl-requiring enzyme ribonucleotide reductase to activate 5′-deoxyadenosylcobinamide ($AdoCbi^+$),[16] although there are conflicting reports in the earlier literature regarding the "partial activity" of $AdoCbi^+$ with other B_{12}-requiring enzymes.[16b-d]

The mechanochemical trigger mechanism would appear to receive prima facie support from what can be termed the "base-on" effect on the thermal homolysis of alkylcobalamins. This is the well-known labilization of the Co–C bond of sterically strained alkylcobalamins (i.e., secondary alkyl-, neopentyl-, and benzyl-Cbl)[14,17-23] and even the much less strained[13,24] AdoCbl[1,2,25] upon coordination of the axial benzimidazole ligand. Thus, the very slow ($T_{1/2}$ = 28 days) homolysis of neopentyl-Cbl in acidic solution (i.e., the base-off form) is increased over 500-fold in neutral solution.[18,21] While the base-on effect in other alkylcobalamins can be somewhat smaller (ca. 10^2), it is probably significantly larger than 500 for neopentyl-Cbl, since, as Schrauzer and Grate orginally pointed out,[21] the spectrum of this alkyl-Cbl in neutral solution strongly resembles that of a mixture of a base-on and a base-off alkyl-Cbl. That the neutral species of neopentyl-Cbl is substantially base-off was recently confirmed in our study of the interaction of benzyl-Cbl and neopentyl-Cbl with a vitamin B_{12} binding protein (haptocorrin) from chicken serum.[26] Cobalamins are known to bind to this protein nucleotide loop down,[11,27] and the haptocorrin fails to bind the base-off dicyanocobalamin.[27] The spectrum of the neopentyl-Cbl–haptocorrin complex is clearly base-on and confirms Schrauzer's estimate[21] that the neutral species of neopentyl-Cbl is over 50% base-off at 25 °C. Thus, the true base-on effect for neopentyl-Cbl is at least 10^3.

The nature of the base-on effect is unknown, but there are data in the literature which suggest that the electronic effect of substitution of a good donor, such as 5,6-dimethylbenzimidazole, for the weakly donating H_2O ligand presumed to be the trans ligand in the base-off alkylcobalamins should be to stabilize the Co–C bond. Thus, in the series of known X-ray structures of base-on RCbl's (R = Ado,[13,28] CH_3,[24] CN[29]) the Co–C bond lengths (2.04, 1.99, and 1.92 Å, respectively) decrease monotonically with the axial Co–N bond lengths (2.24, 2.19, and 1.97 Å, respectively), suggesting that increased axial Co–L interactions lead to increased trans axial Co–C interactions. A similar effect has been seen in organocobaloximes where long axial Co–N bonds have been associated with long and weak axial Co–C bonds.[10] More directly to the point, Halpern and co-workers[30,31] have shown that the enthalpy of activation for Co–C bond homolysis (and thus the Co–C bond dissociation energy) increases approximately linearly with increasing basicity in a series of isosteric α-(phenylethyl)-(4-substituted pyridyl)cobaloximes, directly demonstrating that strong trans axial ligation stabilizes Co–C bonds, at least in cobaloximes.

Such results suggest that the base-on effect in alkylcobaloximes should be largely steric. If this is the case, then the mechanochemical trigger mechanism is indeed supported by the base-on effect and remains a viable hypothesis for AdoCbl activation. Indirect evidence that this may be the case comes from Halpern and co-workers' studies of the effects of tertiary phosphine ligands on the thermal homolysis of benzylcobaloximes[32] and benzylcobalt octaethylporphyrin.[5] In the rigid porphyrin system, the enthalpy of activation for Co–C bond homolysis increased linearly with phosphine ligand basicity despite a substantial variation in cone size across the series of phosphine ligands. In the flexible cobaloxime system, the enthalpy of activation showed a linear, inverse dependence on phosphine cone angle despite the previous demonstration of the direct dependence of Co–C bond homolysis activation enthalpy on ligand basicity in an isosteric series of ligands.[30] The conclusion was that the steric effect of the phosphine ligands was readily transmitted to the trans axial position in the flexible cobaloximes and completely overwhelmed the electronic effect, while the rigid porphyrin system prevented

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transmission of the steric effect, allowing the electronic effect to dominate.

To date, no systematic study of the effects of axial ligands on the thermal Co–C bond homolysis of alkylcobalt corrins has appeared. In an attempt to delineate the relative importance of electronic and steric effects in the cobalt corrin system, we now report the results of such a study that does indeed provide substantial insight into the underlying factors leading to labilization of the Co–C bond in alkylcobalamins.

Experimental Section

H_2OCbl was obtained from Roussel Corp. Liquid ligands, pyridine (py), 4-methylpyridine (CH_3py), and aniline were redistilled and stored under argon. 4-Aminopyridine (NH_2py) was recrystallized from benzene/methanol, and imidazole (Im) was recrystallized from CCl_4 or benzene. Potassium azide was obtained in the highest purity commercially available and used without further purification. Factor B (cyanoaquocobinamide) was prepared as described previously[15] by the method of Renz.[33]

Benzyl-Cbl and neopentyl-Cbl were prepared from H_2OCbl by the method of Schrauzer and Grate.[21] Final purification was achieved by reversed-phase chromotography on Amberlite XAD-2 sorbent. Contaminating H_2OCbl was removed by elution with 10% acetonitrile/90% 10^{-2} M HCl, and the alkylcobalamins were eluted with 50% acetonitrile/50% 10^{-2} M HCl. The products were stored in solution in 10^{-2} M HCl (i.e., in the base-off form) at −20 °C. Neopentyl- and benzyl-Cbi^+ were similarly prepared by reducing factor B with zinc in 10% acetic acid, or with $NaBH_4$ under argon, and then alkylating with benzyl bromide or neopentyl iodide. After the sample was desalted by reversed-phase chromotography on Amberlite XAD-2 sorbent, final purification was effected by preparative HPLC on a 10 × 250 mm Beckman Ultrasphere C-8 column using the solvent system and gradient previously described[34] but at a flow rate of 7 mL/min. This procedure provided pure diaquacobinamide ($(H_2O)_2Cbi^{2+}$, T_r = 6.8 min) as well as benzyl-Cbi^+ (T_r = 13.4 min) and neopentyl-Cbi^+ (T_r = 14.2 min). The products were desalted by reversed-phase chromotography on Amberlite XAD-2 sorbent and stored in aqueous solution at −20 °C. All cobamides were quantitated spectrophotometrically by conversion to dicyanocobamides[35] as previously described.[15] Purity of all preparations was assessed by analytical HPLC[34] to be at least 95%.

Samples for anaerobic pyrolysis of alkylcobamides were prepared as described previously,[15] and products were identified by GC/MS on a Finnigan 4500 instrument equipped with a 6 ft × 2 mm Carbopak B/1% SP 1000 column. Spectrophotometric measurements were made on a Cary 219 recording spectrophotometer equipped with a thermostated cell block. Sample temperatures were monitored with a thermistor device (Yellow Springs Instruments) calibrated against NBS-calibrated thermometers. The pH was maintained with appropriate 0.1 M buffers (phosphate, chloroacetate, or acetate), or H_2SO_4, and ionic strength was maintained at 1.0 M with KCl. Thermal homolysis of RCba's was studied in air-saturated water without added radical traps, as dissolved O_2 has been shown to trap the radical products sufficiently rapidly to prevent recombination.[22] Samples (containing ca. 1.0×10^{-5} M alkylcobamide, 0.1 M buffer, KCl, and, where appropriate, ligand) were incubated in the thermostated cell block for at least 30 min before initiation of reactions by addition of a small amount of alkylcobamide solution. The pH of all samples was measured after completion of the reaction by using a Radiometer PHM 84 pH meter and a Radiometer Type C combined glass electrode with electrodes, samples, standards, and rinse water incubated at the measurement temperature. First-order rate constants were obtained from single-wavelength kinetic traces (at appropriate wavelengths as determined from scanning experiments) from the slopes of plots of $\ln |A_\infty - A_t|$ vs time by using standard linear regression analysis. For studies of the effects of axial ligands on RCbi thermolysis, py concentration varied from 0.5 to 3.5 M (pH 7.5), NH_2py varied from 0.04 to 0.53 M (pH 11.4), CH_3py varied from 0.1 to 1.2 M (pH 8.3), Im varied from 0.5 to 5.0 M (pH 11), and N_3^- varied from 0.2 to 0.72 M (pH 7.5). Anaerobic spectrophotometric observations were made in quartz cuvettes closed with serum stoppers (Suba-Seal). The cuvettes, containing buffer and, where appropriate, ligand and radical trap, were purged with argon for 2 h and the alkylcobamides transferred by cannula. For anaerobic reactions using the radical trap 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (4-HTMPO) kinetic determinations were made at wavelengths isosbestic for the reaction of Co^{II}-containing cobamides with 4-HTMPO.[22]

Equilibrium constants for binding of various ligands to CH_3Cbi were determined by spectrophotometric titration at 530 nm by incremental addition of neat ligands (or concentrated solutions of solid ligands) to a cuvette containing CH_3Cbi, appropriate buffer (0.1 M), and KCl (to maintain ionic strength at 1.0 M) with a Hamilton microliter syringe. After correction of absorbances for the volume change, the data were analyzed as previously described.[36] Concentration ranges for the various ligands were as follows: py, 0.01–1.8 M; CH_3py, 0.02–0.94 M; NH_2py, 1.3×10^{-3}–0.14 M; Im, 5.0×10^{-3}–0.71 M; N_3^-, 0.1–0.8 M.

Results

Benzyl- and neopentyl-Cbl and their respective cobinamides are well-known compounds previously reported by others.[14,18,22,23,37] In the current work, these complexes have been further characterized by mass spectral identification of the organic products produced upon their anaerobic pyrolysis, as has been done previously for organocobamides.[15,38,39] Benzylcobalamin (isolated as the chloride salt of its base-off species, benzyl-Cbl·Cl) and benzylcobinamide (isolated as the chloride salt, benzyl-Cbi·Cl) gave toluene, benzyl chloride, and bibenzyl in relative amounts that vary somewhat from sample to sample. Similarly, neopentyl-Cbl (again as the chloride salt of its base-off species) and neopentyl-Cbi·Cl gave neopentane, neopentyl chloride, and small amounts of 2,2,5,5-tetramethylhexane.

A question arises as to the nature of the alkylcobinamide species obtained, however, as it has been recently demonstrated that reductive alkylation of factor B can lead to a mixture of diastereomeric β-alkyl-Cbi^+ (with the organic ligand in the "upper" axial ligand position) and α-alkyl-Cbi^+ (with the organic ligand in the "lower" axial ligand position), whose composition can vary from 93% α-diastereomer (R = CF_3) to undetectable amounts (<2%) of α-diastereomer (R = CH_3CH_2).[38,39] This question can be answered by use of the UV/visible spectra of the alkyl-Cbi's. For the six known pairs of $RCbi^+$ diastereomers the electronic spectrum of the β-diastereomer has been shown to be identical (above 300 nm) with that of the base-off species of the analogous β-alkylcobalamin.[38,39] The spectra below 300 nm are complicated by the additional absorbance of the protonated benzimidazole nucleotide in the base-off cobalamin.[15] In contrast, the spectrum of the α-diastereomer of the $RCbi^+$ differs in molar absorptivity at all critical wavelengths and typically (except for R = CF_3) shows a substantial red shift (8–40 nm) of the longest wavelength band. Careful inspection of the high-performance liquid chromatograms of benzyl-Cbi^+ and neopentyl-Cbi^+ showed that each preparation migrated as a single, prominent band, with one to three very minor bands, none of which accounted for more than 2% of the total material. In contrast, the diastereomeric α- and β-RCbi's are readily separable on HPLC.[38,39] The UV/visible spectra of benzyl-Cbi^+ and base-off benzyl-Cbl (λ, nm (log ε): 428 (4.03), 355 (4.22), 305 (4.42)) were identical above 300 nm as were those of neopentyl-Cbi^+ and base-off neopentyl-Cbl (λ, nm (log ε): 436 (3.91), 389 (3.93), 304 (4.46)). We conclude that, as is the case with ethylcobinamide,[39] the products of reductive alkylation of factor B with benzyl bromide and neopentyl iodide are at least 98% β-diastereomer.

Rate constants for the thermal decomposition of benzyl-Cbl and neopentyl-Cbl in aerobic solution were determined as a function of pH in solutions buffered between pH 0 and 7.5 at several temperatures, and the data were fitted to a titration curve by a nonlinear least-squares method to provide values of $pK_{base\text{-}off}$, the apparent pK_a for the base-on/base-off reaction,[15] k_{off} (for the protonated, base-off species), and k_{neut} (for the neutral species). At temperatures below 25 °C for benzyl-Cbl (and at 25 °C for neopentyl-Cbl) the values of k_{off} were too low to be accurately measured, and so k_{off} was determined as the average of several values at pH 0 to 1.5 at more elevated temperatures to permit

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Table I. Rate Constants for the Thermal Homolysis of Benzyl- and Neopentyl-Cbl and Benzyl- and Neopentyl-Cbi$^+$ [a]

	R = benzyl				R = neopentyl			
T, °C	$pK_{base\text{-}off}$ [b]	10^4k_{neut}, [c] s^{-1}	10^4k_{off}, [d] s^{-1}	$10^4k_{H_2O}$, [e] s^{-1}	$pK_{base\text{-}off}$ [b]	10^3k_{neut}, [c] s^{-1}	10^5k_{off}, [d] s^{-1}	$10^5k_{H_2O}$, [e] s^{-1}
5	4.65 ± 0.03	0.823 ± 0.020						
15	4.56 ± 0.01	5.03 ± 0.11						
25	4.47 ± 0.02	22.1 ± 0.8	0.172 ± 0.010	0.147 ± 0.008	5.18 ± 0.01	0.112 ± 0.004		
35	4.39 ± 0.02	76.3 ± 5.0	0.775 ± 0.024	0.702 ± 0.018	5.04 ± 0.02	0.491 ± 0.015	0.100 ± 0.010	0.118 ± 0.006
45			2.95 ± 0.19	2.55 ± 0.22	4.91 ± 0.01	1.87 ± 0.05	0.569 ± 0.045	0.597 ± 0.073
55			11.7 ± 1.1	10.7 ± 0.9	4.81 ± 0.01	5.95 ± 0.25	2.30 ± 0.21	2.06 ± 0.10
65							8.70 ± 0.20	10.2 ± 0.3
$\Delta H^{\ddagger}$, [f] kcal mol^{-1}		25.1 ± 1.0	26.6 ± 0.4	26.9 ± 0.6		25.1 ± 0.5	30.0 ± 0.8	29.7 ± 1.3
		(24.6 ± 0.6[g])		(26.9 ± 0.5[g])		(23.4 ± 0.2[g]) (23.1 ± 0.7[h])		(32.1 ± 0.1[g])
$\Delta S^{\ddagger}$, [f] cal mol^{-1} K^{-1}		13.4 ± 3.4	8.8 ± 0.4	9.5 ± 1.9		7.9 ± 1.5	11.5 ± 2.6	10.8 ± 3.9
		(12.3 ± 2.0[g])		(9.2 ± 1.5[g])		(2.6 ± 0.1[g]) (2 ± 2[h])		(17.3 ± 0.4[g])

[a] Ionic strength 1.0 M (KCl). [b] Equation 1. [c] First-order rate constant for thermolysis of the neutral species of RCbl. [d] First-order rate constant for thermolysis of the protonated, base-off species of RCbl. [e] First-order rate constant for thermolysis of the RCbi$^+$. [f] From Eyring plots, Figures 1 and 2. [g] Reference 21. [h] Reference 37.

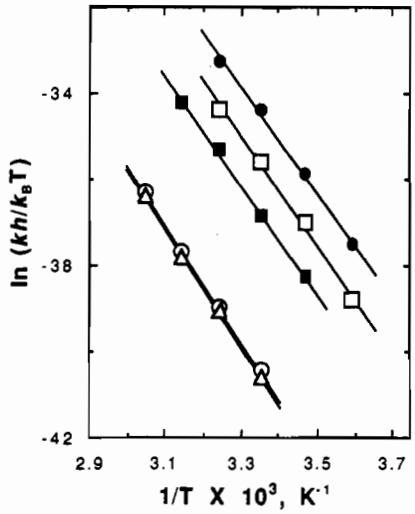

Figure 1. Eyring plots (ln (kh/k_BT) vs $1/T$, where h is Planck's constant and k_B is Boltzmann's constant) for the thermolysis of benzylcobamides: (□) neutral benzyl-Cbl; (○) base-off benzyl-Cbl; (△) behzyl-Cbi$^+$; (●) benzyl(py)Cbi$^+$; (■) benzyl(NH_2py)Cbi$^+$.

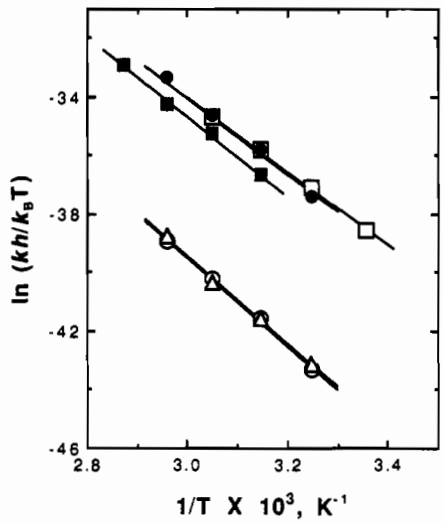

Figure 2. Eyring plots (ln (kh/k_BT) vs $1/T$, where h is Planck's constant and k_B is Boltzmann's constant) for the thermolysis of neopentylcobamides: (□) neutral neopentyl-Cbl; (○) base-off neopentyl-Cbl; (△) neopentyl-Cbi$^+$; (●) neopentyl(py)Cbi$^+$; (■) neopentyl(NH_2py)Cbi$^+$.

analysis of the activation parameters. These data are collected in Table I along with rate constants (k_{H_2O}) for the thermolysis of benzyl- and neopentyl-Cbi$^+$. Also collected in Table I are the enthalpies and entropies of activation of the neutral species, protonated base-off species, and cobinamides of both the benzyl- and neopentylcobamides, as determined from the slopes and intercepts of Eyring plots (Figures 1 and 2). The activation parameters for the neutral species of benzyl-Cbl and for benzyl-Cbi are in excellent agreement with those previously reported by Schrauzer and Grate,[21] but the activation parameters for the neutral species of neopentyl-Cbl and neopentyl-Cbi agree somewhat less well with those of Schrauzer and Grate[21] and Halpern et al.,[37] the principal disagreement being in the (generally less well determined) entropies of activation. These data represent the first ever determination of the activation parameters for the protonated, base-off species of thermally labile cobalamins, and the excellent agreement of these parameters with those for the cobinamides effectively demonstrates that the cobinamides are indeed good models for the thermal decomposition of base-off cobalamins despite NMR evidence that the presence of an uncoordinated nucleotide loop does affect the corrin ring conformation.[40,41] As anticipated, the data display the usual base-on effect, the neutral species of benzyl-Cbl being 100-fold more reactive than the base-off species (at 25 °C), while the neutral species of neopentyl-Cbl is 400-fold more reactive than the base-off species (at 35 °C).

The mechanism of the thermolysis of both neopentyl-Cbl[37] and benzyl-Cbl[22] has been firmly established to be carbon–cobalt bond homolysis. Thus, thermolysis is drastically slowed in the absence of oxygen (or other radical traps)[21,22,37] due to the high rate of recombination of the free-radical products (R$^{\bullet}$ and cob(II)alamin) in the absence of exogeneous scavengers. For both compounds, the anaerobic thermolysis is accelerated by added radical scavengers (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy[22] (4-HTMPO) or bis(dimethylglyoximato)cobalt(II)[37] ($Co(DH)_2$), and the rate of thermolysis becomes independent of scavenger concentration above a certain limiting concentration. For benzyl-Cbl, this scavenger concentration-independent first-order rate constant was identical with that observed in aerobic solution.[22] In addition, for both compounds, mass action retardation by cob(II)alamin has been demonstrated in anaerobic solution with radical traps. Thus, complications due to competing heterolysis, as in the thermolysis of AdoCbl and AdoCbi$^+$,[1,2,25] have been shown to be absent, the dissolved oxygen in air-saturated water has been shown to trap the radicals efficiently enough to ensure that carbon–cobalt bond homolysis is the rate-limiting step. Under aerobic conditions in water, we find the organic products from thermolysis of benzyl-Cbl, benzyl-Cbi$^+$, and benzyl-Cbi$^+$ in excess pyridine to be benzaldehyde and benzyl alcohol and those from neopentyl-Cbl,

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Table II. Rate Constants for Thermolysis Homolysis of Benzyl- and Neopentyl(ligand)cobinamides[a]

	R = benzyl, L					R = neopentyl, L	
T, °C	py	CH_3py	NH_2py	Im	N_3^-	py	NH_2py
5	$(2.9 \pm 0.6) \times 10^{-4}$						
15	$(1.5 \pm 0.2) \times 10^{-3}$		$(1.4 \pm 0.2) \times 10^{-4}$				
25	$(7.3 \pm 0.5) \times 10^{-3}$	$(3.2 \pm 0.2) \times 10^{-3}$	$(6.3 \pm 0.7) \times 10^{-4}$	$(6.9 \pm 1.3) \times 10^{-3}$	$(2.0 \pm 0.2) \times 10^{-4}$		
35	$(2.4 \pm 0.3) \times 10^{-2}$		$(3.0 \pm 0.4) \times 10^{-3}$			$(3.8 \pm 0.2) \times 10^{-4}$	
45			$(9.5 \pm 1.0) \times 10^{-3}$			$(1.9 \pm 0.3) \times 10^{-3}$	$(8.0 \pm 1.9) \times 10^{-4}$
55						$(6.2 \pm 0.9) \times 10^{-3}$	$(3.4 \pm 0.6) \times 10^{-3}$
65						$(2.3 \pm 0.3) \times 10^{-2}$	$(9.4 \pm 2.0) \times 10^{-3}$
75							$(3.7 \pm 0.7) \times 10^{-2}$
pK_a[b]	5.51[c]	6.36[c]	9.40[c]	7.24[d]	4.41[e]	5.51[c]	9.40[c]
$\Delta H^{\ddagger}$,[f] kcal mol^{-1}	25 ± 1		25 ± 1			27 ± 1	27 ± 1
$\Delta S^{\ddagger}$,[f] cal mol^{-1} K^{-1}	14 ± 3		12 ± 3			15 ± 3	12 ± 4

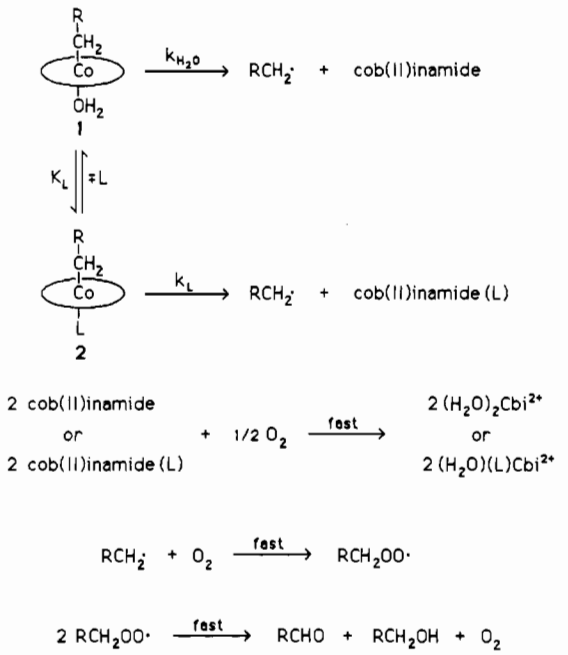
[a] Ionic strength 1.0 M (KCl). Values given are k_L (Scheme I), s^{-1}, for decomposition of R(L)Cbi. [b] pK_a of the conjugate acid of the ligand, L. [c] Reference 48. [d] Reference 49. [e] Reference 50. [f] From Eyring plots, Figures 1 and 2.

neopentyl-Cbi$^+$, and neopentyl-Cbi$^+$ in excess pyridine to be pivaldehyde, isobutene, and neopentane, in agreement with Blau and Espenson[22] and Schrauzer and Grate.[21] For aerobic thermolyses in buffered solution containing KCl, benzyl chloride and neopentyl chloride were also formed.

The possibility of competing heterolysis during the thermolysis of benzyl- and neopentyl-Cbi$^+$ and their complexes with added ligands (vide infra) was investigated by observing the effect of anaerobiosis on the thermolysis and the effect of the radical scavenger 4-HTMPO on the anaerobic decomposition rate. For decomposition in the presence of added ligand, the highest concentration of exogenous ligand observed aerobically (vide infra) was used. In the absence of oxygen, spectral changes for the alkylcobinamides with and without added ligands were extremely slow. If air was carefully excluded, spectra of samples that decomposed completely in minutes under aerobic conditions were essentially unchanged for several hours. Addition of 4-HTMPO to such anaerobic reactions mixtures (concentration range 2.0×10^{-3} to 2.5×10^{-2} M) restored the rate of spectral change to that observed in aerobic solution. At concentrations of 4-HTMPO of 4.0×10^{-3} M or above, the rate constants for decomposition were experimentally indistinguishable from those obtained in aerobic solution and were independent of the concentration of 4-HTMPO. In the case of the cobinamides and their complexes with the pyridine ligands, the final spectrum obtained in anaerobic solution with 4-HTMPO strongly resembled that of cob(II)inamide[42] or that of a mixture of base-on and base-off cob(II)alamin,[42-44] depending on the presence and concentration of added ligands. For the complexes of benzyl-Cbi$^+$ with imidazole and azide, the reaction of the cobalt(II) species with 4-HTMPO was sufficiently fast that the cobalt(II)-like spectrum could not be directly observed. The very slow spectral changes in anaerobic solution without 4-HTMPO could be used to estimate an upper limit for the extent of anaerobic decomposition in the absence of a radical trap. Anaerobiosis slowed the rate of thermal decomposition by 50- to 600-fold relative to aerobic decomposition or to anaerobic decomposition in the presence of 4-HTMPO. Thus, an upper limit of 2% heterolysis can be set for all thermolysis reactions studied.

Investigations of the effects of axial ligands on the thermolysis of alkylcobinamides are hampered by the relatively low affinity of organocobalt corrins for exogeneous ligands.[5,17,45-47] However, with use of relatively high concentrations of soluble ligands, demonstration of the effect of such ligands on RCbi$^+$ thermolysis is straightforward. For instance, when benzyl-Cbi$^+$ is added to solutions of imidazole (Im, 0.5–5.0 M) or N_3^- (0.2–0.8 M), the initial UV/visible spectrum is somewhat altered (by ligation) and thermolysis is evident from first-order time-dependent spectral changes with isosbestic points (349, 370, and 494 nm for Im; 324, 378, and 491 nm for N_3^-). The spectrum of the final product is identical with that obtained upon addition of $(H_2O)_2Cbi^{2+}$ to the same concentration of ligand (prominent γ-band at 359 nm (Im) or 355 nm (N_3^-)), consistent with the idea that the homolysis product cob(II)inamide is rapidly oxidized to cob(III)inamide under these conditions. Plots of the observed first-order rate constants for thermolysis vs ligand concentration (not shown) are monotonically increasing and show evidence of the expected "saturation" behavior. These data were analyzed according to Scheme I, in which **1** and **2** represent the alkylcobinamide and its ligand adduct, respectively, and K_L is defined in eq 1. Application of the law of mass action leads readily to eq 2, for the dependence of k_{obs} on [L]. The parameters are conveniently extracted by a plot of the reciprocal of $(k_{obs}-k_{H_2O})$ vs 1/[L] (eq

Scheme I



$$K_L = [2]/[1][L] \quad (1)$$

$$k_{obs} = (k_{H_2O} + k_L K_L[L])/(K_L[L] + 1) \quad (2)$$

(42) Brown, K. L.; Zou, X. Manuscript submitted to *Inorg. Chem.*
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(47) Firth, R. A.; Hill, H. A. O.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1969**, 381–386.

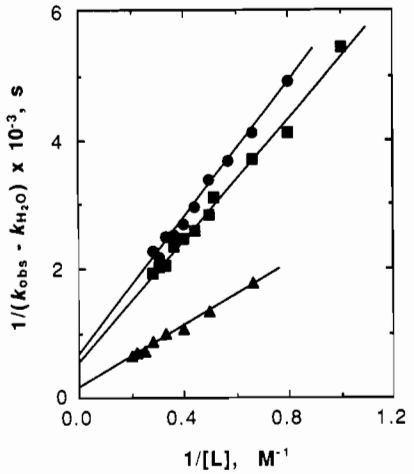

Figure 3. Plots of $1/(k_{obs} - k_{H_2O})$ vs $1/[L]$ according to eq 4 for thermolysis of alkylcobinamides in the presence of exogeneous ligand: (●) benzyl-Cbi$^+$ and py, 15 °C; (■) neopentyl-Cbi$^+$ and py, 45 °C; (▲) benzyl-Cbi$^+$ and Im, 25 °C.

3). Examples of such plots are shown in Figure 3, and the values of k_L and K_L for benzyl(Im)Cbi$^+$ and benzyl(N_3)Cbi are included in Tables II and III.

$$1/(k_{obs} - k_{H_2O}) = \{1/[K_L(k_L - k_{H_2O})]\}(1/[L]) + 1/(k_L - k_{H_2O}) \quad (3)$$

More complicated spectral changes ensue when benzyl-Cbi$^+$ is added to solutions of pyridine (py) as shown in Figure 4A for 0.5 M py at 25 °C. The initial spectrum of benzyl-Cbi$^+$ (dotted line) is only slightly perturbed by 0.5 M py (reflecting the partial conversion to benzyl(py)Cbi$^+$), and relatively rapid spectral changes occur (dashed lines) characterized by a decrease of absorbance between 290 and 355 nm, an increase between 355 and 380 nm with the formation of a prominent γ-band at 363 nm, a decrease in absorbance between 380 and 486 nm, and an increase above 486 nm, leading to formation of a typical α-band structure at long wavelengths. Isosbestic points occur at 355, 380, and 486 nm. These spectral changes strongly resemble those accompanying the decomposition of an organocobalt corrin to an inorganic cobalt(III) corrin, i.e., following the rapid oxidation of the initial cobalt(II) homolysis product. These changes are followed by much slower, secondary spectral changes (solid lines) with first-order time dependence ($T_{1/2}$ = 288 min) characterized by a large decrease in the γ-band absorbance at 363 nm, an increase in absorbance between 394 and 483 nm, and a decrease in the α-band absorbance above 483 nm, with isosbestic points at 318 and 483 nm. The slower, secondary spectral changes are exactly duplicated upon addition of $(H_2O)_2Cbi^{2+}$ (Figure 4B, dashed line) to a solution containing 0.5 M py at 25 °C. The initial spectrum generated strongly resembles that obtained at the end of the faster spectral changes in Figure 4A and apparently represents formation of a (py)$(H_2O)Cbi^{2+}$ adduct. This spectrum subsequently undergoes first-order time-dependent changes identical with those occurring during the slower changes in Figure 4A, with isosbestic points at 318, 394, and 483 nm with a half-time of 280 min. This suggests that the more rapid spectral changes occurring when benzyl-Cbi$^+$ is mixed with pyridine (Figure 4A, dashed lines), including the development of a prominent γ-band at 363 nm, represent Co–C bond cleavage leading, after rapid oxidation of the initially formed cobalt(II) homolysis product, to a (py)(H_2O)Cbi species, which subsequently undergoes a much slower chemical change. Proof that this is the case is provided by the experiment shown in Figure 4C. Here the experiment of Figure 4A was repeated except that, at the end of the second scan after mixing (i.e., the dashed lines), the sample was removed from the spectrophotometer and photolyzed with a 275-W tungsten lamp at a distance of 10 cm for 5 min. This procedure generates a spectrum (Figure 4C, dot–dash line) in which the primary

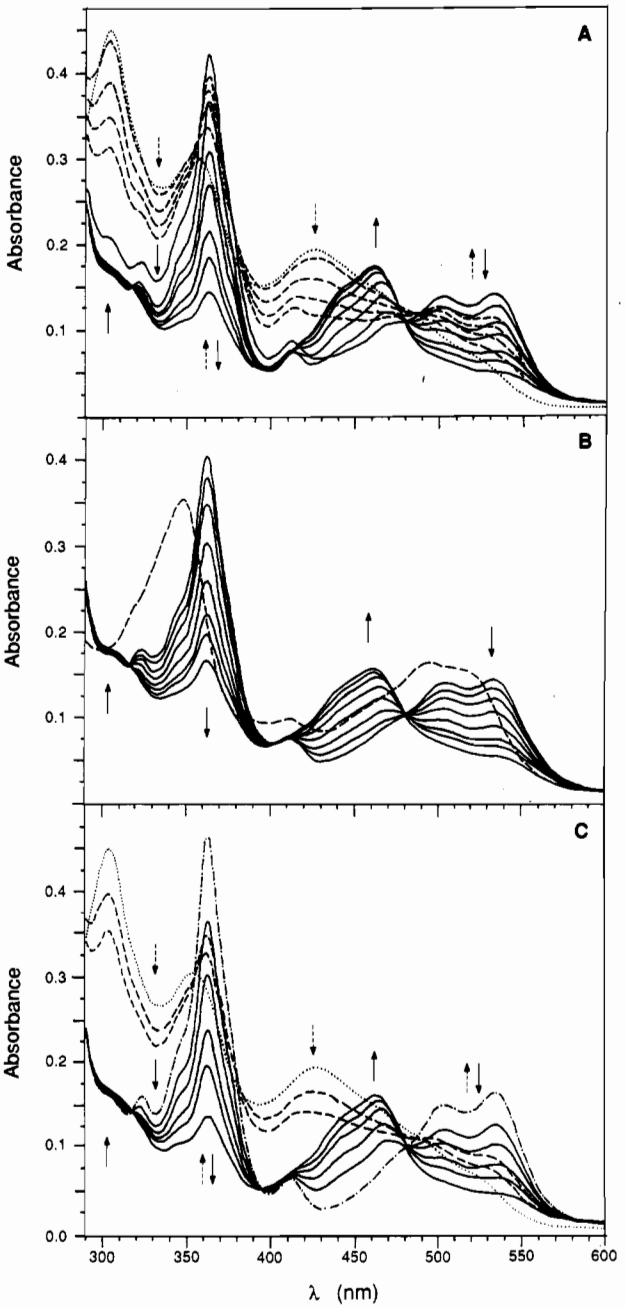

Figure 4. A: Time-dependent spectra of the decomposition of benzyl-Cbi$^+$ (1.9×10^{-5} M) in 0.5 M py, 0.1 M potassium phosphate buffer (pH 7.5, ionic strength 1.0 M (KCl), 25 °C). The dashed lines are spectra recorded immediately after mixing and at 13, 25, and 39 min thereafter. The solid lines are spectra taken at 99, 218, 339, 459, 639, 819, and 1299 min after mixing. The dotted line is the spectrum of 1.9×10^{-5} M benzyl-Cbi$^+$ in the absence of py. B: Time-dependent spectra of $(H_2O)_2Cbi^{2+}$ (1.9×10^{-5} M) in 0.5 M py, 0.1 M potassium phosphate buffer (pH 7.5, ionic strength 1.0 M (KCl), 25 °C). The solid lines are spectra recorded immediately after mixing and at 53, 123, 243, 423, 663, 933, and 1833 min thereafter. The dashed line is the spectrum of $(H_2O)_2Cbi^{2+}$ (1.9×10^{-5}) in the absence of py. C: Time-dependent spectra of the decomposition of benzyl-Cbi$^+$ (1.9×10^{-5} M) in 0.5 M pyridine, 0.5 M potassium phosphate buffer (pH 7.5, ionic strength 1.0 M (KCl), 25 °C). The dashed lines are spectra recorded immediately after mixing and at 12.9 min thereafter. The dot-dashed line is a spectrum recorded after the sample was photolyzed for 5 min (see text). The solid lines are spectra recorded at 138, 258, 438, 618, and 1278 min after mixing. The dotted line is the spectrum of benzyl-Cbi$^+$ (1.9×10^{-5} M) in the absence of py.

spectral changes of Figure 4A are prematurely completed, a spectrum identical with that initially formed upon addition of $(H_2O)_2Cbi^{2+}$ to py (Figure 4B). This spectrum subsequently decays (Figure 4C, solid lines) with a half-time of 281 min. This

Table III. Equilibrium Constants for Formation of Benzyl- and Neopentyl(ligand)cobinamides[a]

	R = benzyl L					R = neopentyl L	
T, °C	py	CH_3py	NH_2py	Im	N_3^-	py	NH_2py
5	0.12 ± 0.03						
15	0.12 ± 0.02		2.2 ± 0.2				
25	0.13 ± 0.01	0.75 ± 0.07	1.8 ± 0.2	0.061 ± 0.012	0.63 ± 0.05		
35	0.11 ± 0.03		1.8 ± 0.3			0.12 ± 0.01	
45			1.5 ± 0.2			0.11 ± 0.02	0.53 ± 0.05
55						0.11 ± 0.02	0.59 ± 0.10
65						0.12 ± 0.02	0.55 ± 0.10
75							0.42 ± 0.08
pK_a[b]	5.51[c]	6.36[c]	9.40[c]	7.24[d]	4.41[e]	5.51[c]	9.40[c]
ΔH_L, kcal mol^{-1}	−0.3 ± 0.5		−2.1 ± 0.5			0.0 ± 0.6	−1.6 ± 1.3
ΔS_L, cal mol^{-1} K^{-1}	−5.2 ± 2.0		−5.7 ± 1.6			−4.4 ± 1.8	−6.2 ± 4.0

[a] Ionic strength 1.0 M (KCl). Values given are K_L (eq 2), M^{-1}. [b] pK_a of the conjugate acid of the ligand, L. [c] Reference 48. [d] Reference 49. [e] Reference 50.

Table IV. Equilibrium Constants for Formation of Methyl(ligand)cobinamides[a]

	L				
T, °C	py	CH_3py	NH_2py	Im	N_3^-
15	9.62 ± 0.05		30.8 ± 0.2		
25	7.32 ± 0.05 (9.0,[b] 6.0[c])	11.5 ± 0.2	24.0 ± 0.1	8.01 ± 0.13 (11.0[c])	4.17 ± 0.09
35	6.29 ± 0.07		18.7 ± 0.3		
45	5.05 ± 0.03		12.7 ± 1.0		
55	4.18 ± 0.02				
pK_a[d]	5.51[e]	6.36[e]	9.40[e]	7.24[f]	4.41[g]
ΔH_L, kcal mol^{-1}	−3.82 ± 0.15		−5.27 ± 0.50		
ΔS_L, cal mol^{-1} K^{-1}	−8.8 ± 0.5		−11.4 ± 1.8		

[a] Ionic strength 1.0 M (KCl). Values given are K_L (eq 2), M^{-1}. [b] Reference 17. [c] Reference 45. [d] pK_a of the conjugate acid of the ligand, L. [e] Reference 48. [f] Reference 49. [g] Reference 50.

experiment effectively demonstrates that the more rapid spectral changes ensuing after mixing benzyl-Cbi$^+$ with py are associated with Co–C bond thermolysis. These secondary spectral changes were absent when benzyl-Cbi$^+$ was decomposed in excess pyridine anaerobically in the presence of 4-HTMPO. Under these conditions the complex decomposed cleanly to yield a final species whose spectrum strongly resembled that of base-on cob(II)alamin.

Similarly complicated spectral changes (not shown) occur when neopentyl-Cbi$^+$ is mixed with py in aerobic solution, except that the spectral changes associated with thermolysis are less well separated in the time domain from those associated with (py)(H_2O)Cbi^{2+} decay due to the slower rate of thermolysis of neopentylcobamides. The primary spectral changes were isosbestic at 349, 380, and 482 nm and led to the species (i.e., (py)(H_2O)Cbi^{2+}) with a γ-band at 363 nm. Both the character and rate of the slower spectral changes were identical with those shown in Figure 4. In addition, a photolysis experiment similar to that shown in Figure 4C demonstrated that Co–C bond homolysis was the primary spectral event and led, after air oxidation of the primary cobalt(II) product, to formation of (py)(H_2O)Cbi^{2+}. It was thus possible to monitor the thermolysis of benzyl-Cbi$^+$ and neopentyl-Cbi$^+$ in excess py by working at one of the isosbestic points for the slower spectral changes associated with the decay of (py)(H_2O)Cbi^{2+}. The most convenient wavelengths were 318 nm for benzyl-Cbi$^+$ and 394 nm for neopentyl-Cbi$^+$. Data collected in this manner obeyed eq 3 (see Figure 3), and led to the values of k_L and K_L for benzyl(py)Cbi$^+$ and neopentyl(py)Cbi$^+$ in Tables II and III.

Similar spectral changes occurred when benzyl-Cbi$^+$ was mixed with 4-methylpyridine (CH_3py). The more rapid changes (due to Co–C bond cleavage) were isosbestic at 355, 376, and 490 nm and led, after rapid air oxidation of the initially formed cobalt(II) species, to a (CH_3py)(H_2O)Cbi^{2+} species with a γ-band at 362.5 nm. This species decayed slowly with isosbestic points at 318, 416, and 483 nm, so that thermolysis kinetics could be conveniently monitored at 318 nm. No secondary spectral changes were detected for benzyl-Cbi$^+$ with 4-aminopyridine (NH_2py). The complex decomposed cleanly with isosbestic points at 349, 366, and 489 nm to form, after rapid cobalt(II) oxidation, a (NH_2py)(H_2O)Cbi^{2+} species with a prominent γ-band at 360 nm. Thermolysis kinetics were monitored at 319 nm. However, for neopentyl-Cbi$^+$ with NH_2py, very slow secondary spectral changes were observable at temperatures of 55 °C and above. The primary spectral changes were isosbestic at 489, 377, and 349 nm, leading to the (NH_2py)(H_2O)Cbi^{2+} species with a γ-band at 360 nm. The much slower secondary spectral changes were isosbestic at 304, 403, and 440 nm, and thermolysis kinetic data were collected at both 304 and 403 nm. The values of k_L and K_L for these complexes are also collected in Tables II and III.

For NH_2py it was possible to show that ligation must occur, as expected, via the endocyclic nitrogen by using aniline as a model for the exocyclic amino group of NH_2py. Thus, when benzyl-Cbi$^+$ was added to solutions (in 30% methanol) containing aniline in the concentration range 0–0.7 M, no change in the initial spectrum of benzyl-Cbi$^+$ occurred, nor was there any change in the rate of thermolysis. Similarly, aniline failed to perturb the spectrum of CH_3Cbi in the concentration range 0–0.78 M (also in 30% methanol). Since the exocyclic amino group of NH_2py must be substantially less basic than aniline, it seems reasonable to conclude that NH_2py coordinates via its aromatic nitrogen.

For neopentyl(NH_2py)Cbi$^+$, the relatively low values of K_L and the solubility of NH_2py limited the maximum extent of formation of this complex to about 25%. Consequently, the values of k_L and K_L for this complex are less well determined than the others. Eyring plots of k_L for the benzyl- and neopentyl(L)Cbi$^+$ complexes for L = py and NH_2py are shown in Figures 1 and 2, and the activation parameters are collected in Table II.

Because of the limited amount of data available in the literature for equilibium constants for addition of ligands to RCbi's, we have also measured values of K_L for β-CH_3Cbi$^+$ for L = py, CH_3py, NH_2py, Im, and N_3^-, as well as the temperature dependence of K_L for L = py and NH_2py, to permit comparison to the values of K_L for benzyl(L)Cbi$^+$ and neopentyl(L)Cbi$^+$ species. These values are listed in Table IV. Plots of ln K_L vs $1/T$ are shown

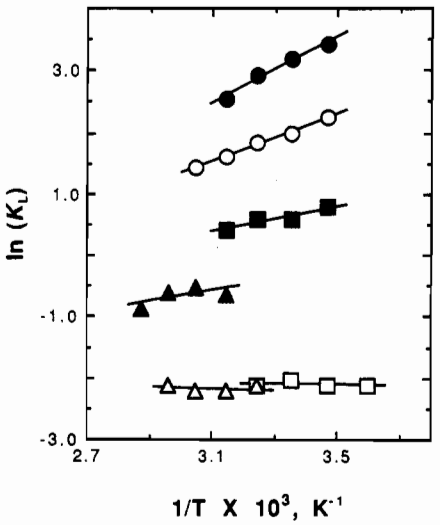

Figure 5. Plots of ln K_L (Scheme I and eq 2) vs $1/T$ for the formation of R(L)Cbi$^+$ complexes from RCbi$^+$ and exogeneous ligands, L: (○) CH_3(py)Cbi$^+$; (●) CH_3(NH_2py)Cbi$^+$; (□) benzyl(py)Cbi$^+$; (■) benzyl(NH_2py)Cbi$^+$; (△) neopentyl(py)Cbi$^+$; (▲) neopentyl(NH_2py)Cbi$^+$.

in Figure 5, and the resultant values of ΔH_L and ΔS_L are also collected in Table IV.

The data in Table II show that k_L for benzyl(Xpy)Cbi$^+$ complexes at 25 °C decreases in the order $k_{py} > k_{CH_3py} > k_{NH_2py}$ with the NH_2py complex being 11-fold less reactive than the py complex. A similar effect is seen in the neopentyl(Xpy)Cbi$^+$ complexes except that the NH_2py complex is only 2.4-fold less reactive than the py complex (at 45 °C). These results exactly parallel those previously reported by Halpern and co-workers[30,31] for α-(phenylethyl)(Xpy)cobaloximes. Most importantly, all of the Xpy complexes and the Im complex have k_L value which are comparable to the k_{on} value for the neutral species of the relevant alkylcobalamin. Even N_3^- shows a significant base-on effect on benzyl-Cbi$^+$ decomposition.

Values of K_L (Table IV) for β-CH_3Cbi$^+$ and py compare favorably to those previously reported by Brodie[17] (9 M^{-1}) and Pailes and Hogenkamp[45] (6 M^{-1}), and our value for CH_3Cbi$^+$ and Im agrees well with the value of Pailes and Hogenkamp[45] (11 M^{-1}). As previously seen with methylaquocobaloxime,[48] the values of K_L for the Xpy complexes of CH_3Cbi$^+$ increase with the basicity of the Xpy ligand, and this trend is also seen with the Xpy complexes of both benzyl-Cbi$^+$ and neopentyl-Cbi$^+$. Similarly, the enthalpies of formation of the CH_3(Xpy)Cbi$^+$ complexes (−3.8 kcal mol^{-1} for py and −5.3 kcal mol^{-1} for NH_2py) compare quite favorably to those previously reported[51] for substitution of H_2O by a primary amine ligand (dimethoxyethylamine) in a series of alkylaquocobaloximes (ΔH = −3.6 to −5.5 kcal mol^{-1}). In addition, the enthalpy and entropy of formation of the CH_3(Xpy)Cbi$^+$ complexes also compare favorably to those for formation of the base-on alkylcobalamins from the base-off but benzimidazole deprotonated species, where ΔH = −7.6 kcal mol^{-1} for all RCbl but ΔS varies from −4.7 to −24 eu with R.[40] However, the enthalpies for substitution of H_2O by Xpy trans to benzyl or neopentyl (Table III) deviate strongly from these precedents, the substitution being essentially isoenthalpic for L = py and only very slightly exothermic for L = NH_2py.

Discussion

As pointed out above, the mechanism of thermolysis of neopentyl-Cbl and benzyl-Cbl has been firmly established to be Co–C bond homolysis.[22,37] It seems extremely unlikely that this mechanism will be altered in benzyl-Cbi$^+$ and neopentyl-Cbi$^+$ or in complexes of these alkylcobinamides with other ligands. In the important case of AdoCbl[2] and AdoCbi$^+$,[25] Co–C bond heterolysis competes with homolysis (10% and ≤7%, respectively, at pH 7) because of the presence of a β-heteroatom substituent in the organic ligand, which permits a facile (and acid-catalyzed) elimination of a Co^{III} species. This is well-known chemistry in AdoCbl[52] and various β-hydroxy-, β-alkoxy-, and β-acyloxyalkylcobalt complexes.[53] The absence of such a β-heteroatom substituent in the benzyl- and neopentylcobamides precludes such a mode of Co–C cleavage.

Alkylcobalt complexes (particularly secondary alkylcobalt complexes) with organic ligand β-hydrogens are also well-known to undergo an apparent elimination of cobalt(I) species to form olefins.[20,21,37,54-57] Interestingly, Halpern and co-workers[31] have recently provided strong evidence that the formation of olefins in such systems is actually the result of rate-limiting Co–C bond homolysis followed by β-hydrogen atom transfer between the caged radical pair. In any case, the absence of organic ligand β-hydrogens in benzyl- and neopentylcobamides precludes this mode of thermolysis, and this was the impetus for Schrauzer and Grate's original studies of these compounds.[21]

The only remaining possibility for heterolytic Co–C bond cleavage would seem to be nucleophilic displacement of cob(I)inamides by exogeneous Lewis base ligands. However, cobalt(I) species are known to be highly nucleophilic[58-62] and there are no known instances of nucleophilic displacement of cobalt(I) species from organocobalt complexes, except by other cobalt(I) nucleophiles.[63-65] In addition, simple alkylcobalt model complexes are known to be indefinitely stable in high concentrations of Lewis base ligands, including highly nucleophilic thiolate anions.[48,51,66,67] The current demonstration of the inhibitory effect of anaerobiosis on the thermal decomposition of benzyl- and neopentyl-Cbi$^+$, with and without added ligands, and the restoration of thermolysis at a rate equivalent to that under aerobic conditions by addition of 4-HTMPO confirm that the mechanism of thermal Co–C bond cleavage in these cobamides is not changed by alteration of the axial ligand.

The nature of the slow, secondary spectral changes following R(Xpy)Cbi$^+$ aerobic thermolysis (and (Xpy)(H_2O)Cbi^{2+} formation from (H_2O)$_2$Cbi^{2+} and Xpy) is not at all clear. It seems unlikely that these spectral changes represent formation of the bis(pyridine) adduct, (Xpy)$_2$Cbi^{2+}, for several reasons. First, the extent of this spectral change does not depend on pyridine concentration although the rate does. Even at py concentrations as low as 0.01 M, the spectral change goes to completion. Thus, the binding constant for the second py to (py)(H_2O)Cbi^{2+} would have to be at least 10^3, and both this equilibrium constant and the slow rate of reaction would seem to be incompatible with what is known about cobalt corrins with two nitrogenous axial ligands.[68] Second,

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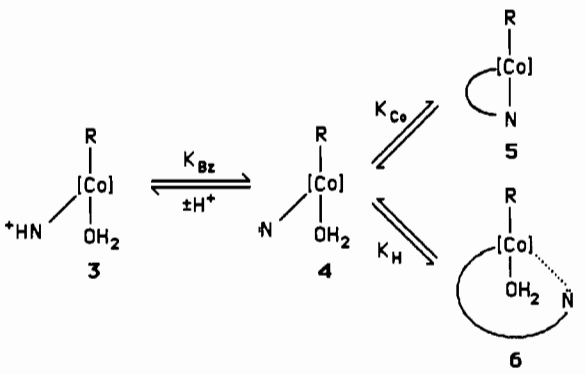
Hayward et al.[69] have reported spectral observation of (but unfortunately not the equilibrium constant for) the formation of the bis(adduct) of $(H_2O)_2Cbi^{2+}$ with Im. The γ-band of the bis adduct is reported to be red-shifted some 6 nm from that of $(Im)(H_2O)Cbi^{2+}$ and to have a higher molar absorptivity. The spectral changes shown in Figure 4 for $(py)(H_2O)Cbi^{2+}$ in excess py are of a radically different character. Third, the spectral changes shown in Figure 4B are not acid reversible. Acidification of the sample after cessation of the slow spectral changes (to pH 1.0 with H_2SO_4) causes a slight shift of the 363-nm band to shorter wavelengths, but no time-dependent evolution of the spectrum toward that of either $(py)(H_2O)Cbi^{2+}$ or $(H_2O)_2Cbi^{2+}$ occurs, even after 36 h. Thus, $(py)(H_2O)Cbi^{2+}$ appears to undergo a slow, irreversible reaction in excess py, the nature of which is obscure. This reaction apparently requires the Co^{III} oxidation state (and probably oxygen), since the secondary spectral changes are absent in the 4-HTMPO-promoted anaerobic decomposition of benzyl-Cbi^+ in excess pyridine.

As has been pointed out before,[1,2,26,37] observed values of rate constants for the thermolysis of alkyl-Cbl's in neutral solution do not represent true rate constants for the thermolysis of the base-on species, since RCbl's such as benzyl-Cbl and neopentyl-Cbl exist as a mixture of base-on and base-off species in neutral solution even at moderate temperatures. Halpern and co-workers[37] previously corrected values of k_{neut} for neopentyl-Cbl for the presence of the base-off species at neutral pH by use of a spectrophotometric method[1,2,4] for determining the enthalpy and entropy of the on-off reaction. In this method, the temperature-dependence of the absorbance of the neutral species at a visible wavelength sensitive to the on–off equilibrium is fitted to an appropriate equation relating absorbance to the enthalpy and entropy of the on–off reaction and the absorbances of the base-on and base-off species. We have previously expressed our concerns about the accuracy of this method[40] because of the complications of the temperature dependence of molar absorptivities (in the absence of thermally sensitive equilibria) and the known temperature dependence of the electronic spectra of alkylcobinamides[46] (and hence, presumably of base-off alkylcobalamins as well). This method seems particularly risky for neopentyl-Cbl, since temperature-dependent spectral measurements can only be made over a limited temperature range due to the thermal lability of this alkylcobalamin. We have consequently recently[26] attempted to correct the thermolysis rate constants for the neutral species of benzyl- and neopentyl-Cbl by estimation of the proportions of base-on and base-off species at neutral pH from comparisons of the spectra of the protonated, base-off species, and neutral species, and the complexes of these alkyl-Cbl's with a haptocorrin from chicken serum, the latter clearly being base-on. This spectral comparison showed that the neutral species of benzyl-Cbl is about 25% base-off and that of neopentyl-Cbl is about 60% base-off at 5 °C. These estimates are in very good agreement with those of Schrauzer and Grate.[21] It is then possible to estimate the relative proportions of base-on and base-off species at 25 °C by assuming that the enthalpy of the on–off reaction for these cobalamins is the same for that of other RCbl's ($\Delta H = -7.88 \pm 0.65$ kcal mol^{-1}, average of values for eight R for formation of the base-on species),[40] as this value has been shown to be independent of the organic ligand.[15,40] This method gives values of $\Delta H^\ddagger = 29$ kcal mol^{-1} and $\Delta S^\ddagger = 29$ eu for base-on benzyl-Cbl and $\Delta H^\ddagger = 30$ kcal mol^{-1} and $\Delta S^\ddagger = 28$ eu for base-on neopentyl-Cbl.[26] However, in light of the current data (as discussed below), the assumption that the enthalpy of formation of the base-on species for benzyl- and neopentyl-Cbl is the same as that for other RCbl's may not be valid.

The estimates of the relative proportions of on and off species at 5 °C permit a more detailed analysis based on the previously developed complete scheme (Scheme II) for the on–off equilibria,[40]

Scheme II



including the "tuck-in" species[40,70] (**6** in Scheme II) of the unprotonated, base-off form in which the benzimidazole N3 is hydrogen bonded to a side-chain amide NH. Application of the law of mass action leads readily to eq 4, where $K_{base\text{-}off}$ is defined in

$$K_{base\text{-}off} = (1 + K_H + K_{Co})K_{Bz} \quad (4)$$

$$K_{base\text{-}off} = ([4] + [5] + [6])[H^+]/[3] \quad (5)$$

eq 5. The ratio of the base-on to base-off species determined spectrophotometrically at 5 °C provides a value of K_{measd} (eqs 6 and 7, $K_{measd} = 3.0$ for benzyl-Cbl and 0.67 for neopentyl-Cbl),

$$K_{measd} = [5]/([4] + [6]) \quad (6)$$

$$K_{measd} = K_{Co}/(1 + K_H) \quad (7)$$

assuming that the visible spectra of the base-off (**4**) and tuck-in species (**6**) are not appreciably different. For benzyl-Cbl, from the measured value of $pK_{base\text{-}off}$ at 5 °C (Table I), K_{measd}, and the previously determined pK_a of α-ribazole as pK_{Bz},[15] a value of 3.4 can be calculated for K_H. This value is slightly lower than that previously determined for the tuck-in species of CH_3Cbl (4.2).[40] Assuming that the formation of the tuck-in species is isoenthalpic, as anticipated for formation of a hydrogen-bonded species in water and demonstrated for CH_3Cbl,[40] values of K_{Co} for benzyl-Cbl can be calculated at other temperatures by using the $pK_{base\text{-}off}$ values determined here and the previously determined temperature dependence of the pK_a of α-ribazole.[15] These values give $\Delta H_{Co} = -4.5 \pm 0.7$ kcal mol^{-1} and $\Delta S_{Co} = -11 \pm 2$ eu; i.e., the value for ΔH_{Co} is significantly less negative than ΔH_{Co} for other RCbl's. This treatment gives values for the activation parameters for base-on benzyl-Cbl of $\Delta H^\ddagger = 27 \pm 1$ kcal mol^{-1} and $\Delta S^\ddagger = 19 \pm 3$ eu. A similar treatment for neopentyl-Cbl (using a value of $pK_{base\text{-}off}$ 5.46, obtained by extrapolating a plot of ln $K_{base\text{-}off}$ vs $1/T$ to 5 °C) gives $K_H = 0.65$, significantly smaller than the value for CH_3Cbl, and yields $\Delta H_{Co} = -3.6 \pm 0.3$ kcal mol^{-1} and $\Delta S_{Co} = -13 \pm 1$ eu. Thus, the enthalpy of formation of the base-on species of neopentyl-Cbl deviates even further from the norm ($\Delta H_{Co} = -7.88 \pm 0.65$ kcal mol^{-1})[40] than the value for benzyl-Cbl. The possible cause of the low enthalpies of formation of the base-on species of benzyl- and neopentyl-Cbl and the low value of K_H for the latter species is discussed below. The corrected values for the activation parameters for base-on neopentyl-Cbl resulting from this treatment are $\Delta H^\ddagger = 28 \pm 1$ kcal mol^{-1} and $\Delta S^\ddagger = 21 \pm 1$ eu and are in excellent agreement with values obtainable by applying the thermodynamic parameters of Halpern and co-workers[37] for the on–off equilibrium of neopentyl-Cbl to our values of k_{neut} ($\Delta H^\ddagger = 29 \pm 1$ kcal mol^{-1} and $\Delta S^\ddagger = 22 \pm 1$ eu).[71] Most importantly, regardless of how the observed rate constants for the neutral species are corrected for the presence of the base-off species, comparison of the corrected activation parameters for the base-on species of neopentyl-Cbl and benzyl-Cbl to those of the base-off species and the cobinamide shows that the enthalpies of activation of the base-on and base-off species are nearly identical;

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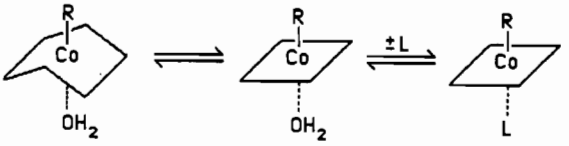
i.e., *the base-on effect is primarily, if not entirely, entropic in nature*, at least for these sterically strained alkylcobalamins.

The electronic effect of axial ligands on the thermolysis rate of alkylcobinamides can now, for the first time, be assessed by using the data in Table II. Despite the difficulties of measuring k_L and the inherently higher uncertainties in these values due to the relatively low values of K_L, it is quite clear from Table II that increasing donor strength in an isosteric series of trans axial ligands stabilizes the carbon–cobalt bond as is the case in organocobaloximes.[30,31] Although there appears to be some enthalpic component to this effect, the relatively greater uncertainties in these data suggest caution in overinterpreting the magnitudes of the activation parameters. The simple observation that increasing donor strength strengthens the trans axial Co–C bond again argues that the base-on effect cannot be primarily electronic; it must be primarily steric in nature. However, we note that the base-on effect of 5,6-dimethylbenzimidazole is not very different from the base-on effect of the less bulky 4-substituted pyridines and the even less bulky imidazole. Even the linear N_3^- has a significant base-on effect. Similarly, Nome et al.[23] have shown that CN^- is capable of displacing the benzimidazole ligand of benzyl-Cbl (as is the case for other RCbl's[70]) and increases the rate of Co–C bond homolysis about 5-fold above that of the neutral species (at 25 °C); i.e., benzyl(CN)Cbl is essentially as reactive as base-on benzyl-Cbl. Thus, while the base-on effect appears to be primarily a steric effect, it is not an effect of the steric bulk of the ligand per se but rather a steric consequence of the presence of a strongly donating axial ligand, all R(L)Cba's being substantially more reactive than the base-off species.

Thus, it is the base-off species that has the extraordinary reactivity, not the base-on species; i.e., the base-off species is substantially stabilized relative to the base-on and $R(L)Cba^+$ species. Considering the observations above in conjunction with inspection of models of benzyl- and neopentylcobamides, the following picture of the energetics of Co–C bond homolysis in sterically bulky alkylcobalamins emerges. Inspection of models shows that the steric bulk of the benzyl and neopentyl ligands substantially reduces the range of thermal motion accessible to the upward projecting a, c, and g acetamide side chains in the pseudooctahedral base-on species in which the corrin ring is relatively planar. Hence, the entropic nature of the driving force for homolysis is not due simply to the emerging separation of the products in the transition state but also to the dramatic increase in motional freedom of the acetamide side chains with the increase in Co–C separation. If, as originally intimated by Schrauzer and Grate (see Figure 5 in ref 21), in the base-off species (and in the cobinamide) the flexibility of the corrin permits adoption of a square-pyramidal conformation in which the corrin ring is bent back, away from the bulky alkyl ligand, steric suppression of acetamide side-chain motion is substantially decreased as clearly seen in models. Thus, in the base-off species, the entropy of the ground state is substantially raised by an increased range of side-chain thermal motion, and the relative increase in entropy as the transition state is approached is significantly reduced. This picture not only provides an explanation for the entropic nature of the base-on effect but may also explain the very low value of K_H for neopentyl-Cbl. Thus, a relatively more extreme square-pyramidal conformation in the base-off species of neopentyl-Cbl may prevent adoption of a favorable geometry for hydrogen bonding between benzimidazole and its side-chain partner. The more normal value of K_H for benzyl-Cbl would then suggest that the distortion of the corrin ring in the base-off species of benzyl-Cbl is less extreme, a conjecture that receives some support from these data, as discussed below. Given the strong dependence of hydrogen-bond strength on geometry, a relatively small difference in corrin conformation might well account for the observed difference in K_H.

It is tempting to suggest that such a conformational equilibrium in the base-off and cobinamide species is responsible for the very low binding constants (Table III) for the various ligands studied to benzyl- and neopentyl-Cbi^+ (Scheme III), as well as the low values of K_{Co} for benzyl- and neopentyl-Cbl. Thus, while $RCbi^+$

Scheme III



species are well-known to bind exogenous ligands weakly,[5,17,45-47] the affinity of pyridine for benzyl- and neopentyl-Cbi^+ is some 50 times lower (at 25 and 45 °C, respectively) than the affinity of pyridine for CH_3Cbi^+ (although most of the other comparisons possible in Table III and IV are less striking). In the absence of more extensive data on the trans effect of various organic ligands on ligand substitution equilibria in organocobamides, such an argument can only be considered speculative. However, the extremely low enthalpies of ligation for py and NH_2py with benzyl- and neopetnyl-Cbi^+ do seem to be significant. The known invariance of ΔH_{Co} for a wide variety of RCbl's[15,40] implies that the Co–O and Co–N bond enthalpies change by the same amount when the organic ligand is altered. If this is the case for other ligands as well, the values of ΔH_L for py and NH_2py binding to benzyl- and neopentyl-Cbi would be expected to be closer to the values of ΔH_L for py and NH_2py binding to CH_3Cbi^+. Instead, ΔH_L is significantly less negative for the binding of either of these ligands to benzyl- or neopentyl-Cbi^+ than to CH_3Cbi^+ ($\Delta\Delta H = \Delta H_L(RCbi^+) - \Delta H_L(CH_3Cbi^+)$; ca. 3.3 kcal for R = benzyl and ca. 3.8 kcal for R = neopentyl, for either L). This suggests that for each R there is a common equilibrium that competes with the ligand exchange equilibrium regardless of the nature of the ligand. This, of course, would be quite consistent with Scheme III. Similarly, the lowered enthalpies of formation of the base-on species for benzyl-Cbl ($\Delta\Delta H_{Co}$ = 3.4 kcal) and neopentyl-Cbl ($\Delta\Delta H_{Co}$ = 4.3 kcal) relative to those for unhindered RCbl's is consistent with ligation being preceded by an endothermic conformational equilibrium as depected in Scheme III. In both ligand systems (i.e., $RCbi^+$, K_L, and RCbl, K_{Co}) the putative conformational preequilibrium is more endothermic for R = neopentyl than for R = benzyl, suggesting that the corrin-distorted base-off conformation may indeed be more extreme for neopentyl-Cbl than for benzyl-Cbl. A more extreme geometry for base-off neopentyl-Cbl is, in fact, consistent with the substantially greater stabilization of the base-off species relative to the base-on species for neopetnyl-Cbl (ca. 3000–4000-fold, or 4.8 kcal) than for benzyl-Cbl (ca. 200–400-fold, or 3.4 kcal).

It is not at all clear why the conformational equilibrium proposed in Scheme III should lie substantially toward the square-pyramidal species when the axial ligand is H_2O, while for all nitrogenous ligands the pseudooctahedral conformation is preferred. The possibility must be considered that the base-off species of benzyl- and neopentyl-Cbl and the analogous cobinamides may, in fact, be pentacoordinate in aqueous solution. While confirmed examples (i.e., by X-ray crystallography) of five-coordinate organocobalt species are rare,[72,73] there is evidence for the existence of such species both in organocobalt model chelates[48,51,74-82] and

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in organocobalt corrins.[78,83-85] Indeed, AdoCbl^{+} [25] and both diastereomers of $CF_3CH_2Cbi^{+}$ [38] are known to be pentacoordinate in the vapor phase from mass spectral observations.

In conclusion, the base-on effect, at least in sterically hindered alkylcobalamins such as neopentyl- and benzyl-Cbl, is found to be a steric, rather than an electronic, effect. The higher reactivity of the base-on species is due to a substantial entropic stabilization of the base-off species, probably due to a conformational change of the corrin, reducing the steric interactions between the bulky organic group and the acetamide side chains. However, since sterically undemanding ligands produce R(L)Cbi^{+} complexes that are as reactive as the base-on species, the steric bulk of the axial ligand itself is not important in the base-on effect. Thus, the mechanochemical trigger mechanism of AdoCbl activation does not receive support from the existence of the base-on effect in benzyl- and neopentyl-Cbl. However, it remains possible that steric compression of the axial Co-N bond could play a role in enzymatic activation of AdoCbl if such compression were capable of causing an upward bending of the corrin ring intensifying the steric congestion between the acetamide side chains and the organic ligand. Indeed, the apparent flexibility of the corrin ring permits the persistance of such an hypothesis. Further enlightenment in this area requires the development of an experimental system in which probes of the axial Co-N bond length, the corrin ring conformation, and the mobility of the acetamide side chains can be monitored in complexes of cobalamins with proteins. Attempts to use NMR probes for these purposes are currently in progress.

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Heteronuclear NMR Studies of Cobalamins. 12. Further Studies of Dicyanocobamides and the Complete Proton, Carbon, and Amide Nitrogen NMR Assignments of Dicyanocobalamin[1,2]

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From a combination of ^{31}P-^{1}H chemical shift correlated, homonuclear *J*-correlated, absorption-mode hypercomplex NOE, hypercomplex homonuclear Hartmann-Hahn, ^{1}H-detected heteronuclear, and ^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence NMR spectroscopies, the complete ^{1}H and ^{13}C NMR assignments of dicyanocobalamin in D_2O have been made. From these proton assignments in conjunction with the NOESY map, the ^{1}H spectrum of dicyanocobalamin in DMSO-d_6 could be nearly completely assigned from a NOESY experiment in this solvent. In DMSO-d_6, the amide proton resonances are visible, and these could be unambiguously assigned from observation of numerous NOE's to side-chain methylene and corrin ring protons. Along with our previous determination of the amide ^{15}N chemical shifts and amide proton-nitrogen connectivities from ^{1}H-detected ^{1}H,^{15}N multiple-quantum coherence spectroscopy these assignments permitted, for the first time, the complete assignment of the amide ^{15}N resonances. Comparisons of the ^{13}C resonances of the nucleotides of dicyanocobalamin, the dicyano derivatives of the cobalamin b, d, and e monocarboxylate analogues, and the dicyano derivative of the C13 epimer of cobalamin among each other and with the free base of the detached nucleotide are consistent with the persistance of the previously postulated tuck-in species of base-off dicyanocobalamin, in which the benzimidazole nitrogen B3 is hydrogen bonded to a side-chain amide, in each of the above cobalamin analogues. These comparisons eliminate the b, d, and e amides as possible hydrogen-bond donors in the tuck-in species. Methylation of the benzimidazole B3 nitrogen was shown to prevent formation of the tuck-in species in the dicyano derivative of the trimethylbenzimidazolyl analogue by comparison of its ^{13}C spectrum to that of the detached, N-methylated nucleotide methyl ester. Taken together with previous ^{15}N NMR results, numerous NOE's observed between the benzimidazole B2, B4, and B7 protons and protons on the corrin side chains, ring and ring methyl groups strongly suggest that the g side-chain amide is the hydrogen-bond donor in the tuck-in species.

Introduction

In the last decade the use of modern NMR techniques and high-field spectrometers has given rise to complete ^{1}H and ^{13}C NMR assignments for a number of vitamin B_{12} derivatives including heptamethyl dicyanocobyrinate,[3,4] the base-on[5] and base-off[6] species of 5'-deoxyadenosylcobalamin, 5'-deoxyadenosylcobinamide,[7] and the b and e monocarboxylic acid derivatives of cyanocobalamin.[8] While such data have been used for biosynthetic studies,[3] for an analysis of the conformational consequences of the base-on/base-off reaction of AdoCbl,[6] and for positive identification of CNCbl analogues,[8] their potential use as probes of important conformational effects in the corrin

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(1) Part 11: Brown, K. L.; Brooks, H. B.; Zou, X.; Victor, M.; Ray, A.; Timkovich, R., *Inorg. Chem.* **1990**, *29*, 4841-4844.
(2) Abbreviations: CNCbl-b-COO^{-}, *Coα*-(α-5,6-dimethylbenzimidazolyl)-*Coβ*-cyanocobamic acid *a,c,d,e,g*-pentamide (cyanocobalamin *b*-monocarboxylic acid); CNCbl-d-COO^{-}, Coα-(α-5,6-dimethylbenzimidazolyl)-*Coβ*-cyanocobamic acid *a,b,c,e,g*-pentamide (cyanocobalamin *d*-monocarboxylic acid); CNCbl-e-COO^{-}, Coα-(α-5,6-dimethylbenzimidazolyl)-*Coβ*-cyanocobamic acid *a,b,c,e,g*-pentamide (cyanocobalamin *e*-monocarboxylic acid); $CNMe_3BzmCba$, *Coα*-(α-3,5,6-trimethylbenzimidazolyl)-*Coβ*-cyanocobamide; CN-13-epiCbl, cyano-13-epicobalamin; *N*-Me-α-ribazole 3'-P methyl ester, 1-α-D-ribofuranosyl-3,5,6-trimethylbenzimidazole 3'-phosphate methyl ester; AdoCbl, 5'-deoxyadenosylcobalamin; AdoCbi, 5'-deoxyadenosylcobinamide.
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