Methylcobalamin's Full- vs "Half"-Strength Cobalt-Carbon σ Bonds and Bond Dissociation Enthalpies: A $>10^{15}$ Co-CH_3 Homolysis Rate Enhancement following One-Antibonding-Electron Reduction of Methylcobalamin

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Abstract: Methylcobalamin (MeCbl, MeB_{12}) thermolyzed in ethylene glycol from 120 to 141 °C with 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) as a $Me^{\bullet}$ trap gives the homolysis products $Co^{II}B_{12r}$ and TEMPO-Me quantitatively. The 5,6-dimethylbenzimidazole axial-base-off–base-on equilibrium in ethylene glycol has an enthalpy change of −5.1 (± 2) kcal mol^{-1} and an entropy change of −10.5 (± 4) cal mol^{-1} K^{-1}, equilibrating between the 5,6-dimethylbenzimidazole-coordinated base-on form and the two distinct yet similar non-coordinated forms: the base-off and the so-called "tuck-in" forms. The MeB_{12} Co-CH_3 homolysis rates indicate an activation enthalpy of 41 ± 3 kcal mol^{-1}, an activation entropy of 24 ± 6 cal mol^{-1} K^{-1}, and an estimated methylcob(III)alamin Co-CH_3 bond dissociation energy of 37 ± 3 kcal mol^{-1}. This is the strongest Co-C cobamide bond measured. Comparison of the MeCbl homolysis rate constant ($10^{-19\pm4}$ s^{-1}) extrapolated to −30 °C with the known reduced-methylcobamide-radical-anion values indicates rate enhancements of $10^{22\pm4}$ (at −30 °C) following electrochemical reduction, or still over 10^{15} at 25 °C. Such reduction provides an antibonding electron which weakens the Co-C bond from 37 kcal mol^{-1} down to ca. 12 kcal mol^{-1}. These rate enhancements are greater than the analogous enzyme-induced Co-C cleavage rate enhancements in adenosylcobalamin (Coenzyme B_{12}, AdoCbl)-dependent enzymes. However, electron transfer is *not* predicted for the mechanism of any adenosylcobalamin-dependent or methylcobalamin-dependent enzymes.

Introduction

Methylcobalamin[1] (MeCbl, MeB_{12}, Figure 1) is one of nature's two biological alkylcobalamins.[2-4] Central to an improved understanding of the biological role of alkylcobalamins is characterization of the key Co-C bond therein, through demonstrated homolysis of this bond and measurement of the activation parameters and bond dissociation enthalpy (BDE) thereof.

While there is virtually universal agreement that Co-C bond homolysis is the key step in the 12 enzyme reactions which are dependent on adenosylcobalamin,[3] this mechanism is not thought to be viable for the four other enzyme reactions involving MeCbl [which instead are generally believed to involve one or more groups from the enzyme in bimolecular-type reactions (e.g., S_N2, S_H2, S_E2)]. The current research will show that the methyl group's bond to the Co(III) d^6 metal in MeCbl is strong enough to argue strongly against any simple homolysis mechanism for these enzyme reactions. The impossibility of the enzyme binding to the *methyl* group in MeCbl, analogous to the known binding of the 5'-deoxy-5'-adenosyl alkyl ligand in AdoCbl, is further indication that another mechanism for enzymic cleavage of the Co-CH_3 bond *of MeCbl* is necessary.

Another conceivable Co-C cleavage mechanism for MeCbl (or AdoCbl)[4] is one-electron reduction to form a radical anion in which the Co-C σ^* antibonding orbital has been populated, thereby forming a three-electron (or net "one"-bonding-electron) σ bond.[1] This mechanism should result in a greatly accelerated bond dissociation rate; to test the sufficiency of this mechanism, the necessary bond-dissociation–rate comparison between MeCbl and $MeCbl^{\bullet-}$ requires previously unavailable rate constants and activation parameters for the MeCbl homolysis reaction.[5]

This work provides the needed MeCbl data and constitutes the first quantitative comparison of the enormous difference between a normal and a half-strength metal–ligand bond.[1] The relevance of electron-transfer mechanisms to MeCbl (or AdoCbl) enzymes is discussed further below, as are relevant new insights on the mechanism of electrochemical cleavage of MeCbl and other cobamide (cobalamin and cobinamide) Co-C bonds.

The comparison of homolysis rates for reduced and unreduced cobamides may also be relevant to the stability of systems isoelectronic with reduced d^7 $(Co^{II}MeCbl)^{\bullet-}$, for example any possible d^7 Ni^{III}-CH_3 systems related to the function of co-factor F_{430}.[6] Furthermore, the fundamental[1,7] nature of this work should be significant for organometallic chemistry, where there is virtually no information on the strengths of M-L bonds in compounds differing only by the oxidation state of the metal,[8ab] nor on "19-electron" species.[9]

Experimental Section

General. Preparation, thermolysis, and photolysis of MeCbl and $Co^{II}B_{12r}$ solutions were done under N_2 as before.[3] Reactions were monitored by visible spectroscopy (Beckman DU-7 with Peltier temperature controller; 330–600 nm) in 1-cm Pyrex Schlenk[3] cuvettes. Photolysis of alkylcobalamin (MeCbl) solutions was prevented by either wrapping in foil or working in a hood under only red light (7.5 W). Ethylene glycol was distilled from 4 Å molecular sieves under reduced pressure, saturated with N_2, and stored in the drybox. 2,2,6,6-Tetramethylpiperidinyl-1-oxy (TEMPO; Aldrich) was sublimed before use (at room temperature to a water-cooled probe, under aspirator-reduced pressure), mp 39 °C (lit.[10] mp 37–39 °C).

Methylcobalamin. Using a modification of Dolphin's procedure,[11] to a flask (500 mL) was added a stir bar followed by HO-Cbl·HCl (Sigma, 98%, 0.253 g, 0.183 mmol), $Co(NO_3)_2 \cdot 6H_2O$ (7.25 mg, 0.0249 mmol,

(1) The homolysis of MeCbl and comparison to one-electron-reduction induced homolysis has been communicated in a preliminary form: Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1990**, *112*, 2419–2420.

(2) The other natural alkylcobalamin is 5'-deoxy-5'-adenosylcobalamin (Coenzyme-B_{12}, $AdoB_{12}$, AdoCbl), for which our group has previously[3] reported the Co-C homolysis parameters and noted the acceleration of homolysis by either enzymes[3] or an added electron.[4]

(3) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469–81. There is a minor typographical error on p 1478: AdoCbl in ethylene glycol is 45% base-on at 110 °C and only 39% base-on at 120 °C.

(4) Finke, R. G.; Martin, B. D. *J. Inorg. Biochem.* **1990**, *40*, 19–22.

(5) We note that it is appropriate to call the cleavage of a three-electron bond a "homolysis", as it is a cleavage of a bond with two electrons in a bonding orbital (i.e., containing a σ^2 configuration).

(6) See, for example: (a) Andrews, R. K.; Blakeley, R. L.; Zerner, B. Nickel and Its Role in Biology. In *Metal Ions in Biological Systems*; Sigel, H., Sigel, A., Eds.; Marcel Dekker: New York, 1988; pp 243–256. (b) Pfaltz, A.; Jaun, B.; Fässler, A.; Eschenmoser, A.; Jaenchen, R.; Gilles, H. H.; Diekert, G.; Thauer, R. K. *Helv. Chim. Acta* **1982**, *65*, 828–865.

(7) MeCbl is nearly ideal for this comparison, as its predominantly square-planar corrin ligand system minimizes non-axial distortions.

(8) Collman, J. P.; Hegedus, L. S.; Norton, J. R.; Finke, R. G. *Principles and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1987; (a) pp 259–260, (b) pp 244–246, (c) pp 314–315.

(9) Stiegman, A. E.; Tyler, D. R. *Comments Inorg. Chem.* **1986**, *5*, 215–245.

(10) Rozantzev, E. G.; Neiman, M. B. *Tetrahedron* **1964**, *20*, 131.

(11) Dolphin, D. *Methods Enzymol.* **1971**, *18*, *Part C*, 45.

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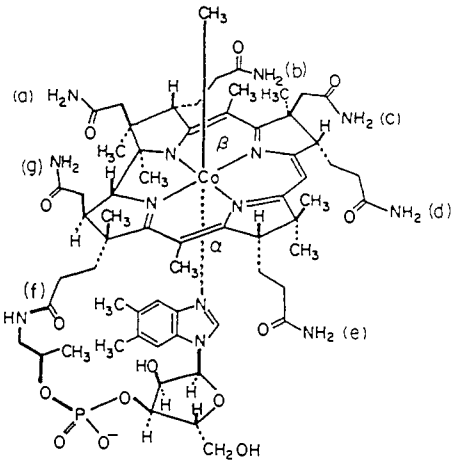


Figure 1. Structure of base-on methylcobalamin (MeCbl, MeB_{12}) with its a–g side chains. Methylcobinamide cation ($MeCbi^+$) has the axial base cleaved and removed at the O–PO_2R bond.

0.14 equiv), and H_2O (25 mL), and the solution was stirred under N_2 for 2 h. An aliquot (4.5 mL, 5.1 mmol, 28 equiv) of a degassed solution (1.1 M) of $NaBH_4$ (0.216 g, 5.71 mmol) in H_2O (5 mL) was syringed into the cobalt solution. The solution was stirred for 30 min, changing from red Co(III), to brown, to black with a purple tint. Under N_2, to this solution flask was added degassed MeI (0.75 mL, 1.71 g, 12.0 mmol, 66 equiv), and the solution was stirred for 1 h.

Following the method of Brown and Peck,[12] a desalting column (2 cm i.d., 9 cm high) was prepared from 33 g (dry) of Serva XAD-2 and the 0.13–0.15-mm particles were suspended in degassed CH_3CN (50% aqueous) and washed with water. SP-Sephadex-C50-120 (sulphopropyl groups on 40–120 μm cross-linked dextran beads) resin (1.8 g) was suspended (15×) in degassed water and the fines suctioned off the day before the acidic cation exchange column was prepared (2 cm × 18 cm high).

Under red light in air, the crude MeCbl solution was added (immediately after preparation) to NaCl in H_2O (100 mL) and then placed on the desalting column, eluting with water (ca. 90 min) until salt-free (by $AgNO_3$ test), and the red product solution was collected using 50% aqueous CH_3CN. The foil-wrapped solution was rotary evaporated to dryness, dissolved in water, and eluted down the Sephadex cation-exchange column, and the red band was collected giving the desired visible spectrum. The product was freeze-dried and collected, yielding MeCbl (202 mg isolated, 80% lit.[11] 91%). The apparently complete purity was verified by ^1H NMR [(D_2O, diagnostic peak assignments,[13] DSS ref) δ 7.185 (B7), 6.977 (B2), 6.281 (B4, R1), 5.913 (C10), 0.923 (12-β-CH_3), 0.469 (C1-CH_3), −0.100 (Co-CH_3)], by visible spectroscopy (ethylene glycol λ_{max} = 340, 376, 521 nm; λ_{min} = 364, 410 nm), and by HPLC (single peak at 17.8 min; eluant 20% MeCN/80% KH_2PO_4, 0.01 M aqueous buffer).

A Schlenk visible-spectroscopy cuvette[3] was loaded in the N_2 drybox with MeCbl in ethylene glycol solution (2.00 mL, 0.14 mM, 2.8×10^{-7} mol), and the spectrum was obtained at 5 °C intervals between 5.0 and 99.0 (±0.1) °C, equilibrating the temperature for 5–10 min between each spectrum. Between 25 and 75 °C, the density-corrected absorbance at 538 nm changed by 5.2%. As a check, the cell was then cooled back to 25.0 °C; the final spectrum was identical with the original.

(2,2,6,6-Tetramethylpiperidinyl-1-oxy)methyl (TEMPO-Me). In a modification of literature methods,[14,15] to a salt–ice cooled Schlenk round-bottom flask (250 mL) with stir bar and dropping addition funnel (125 mL) under N_2 were added colorless MeMgBr (10.0 mL, 30 mmol, 3.0 M in Et_2O, Aldrich) and Et_2O (10 mL, fresh can, N_2 saturated). The addition funnel was charged with a cooled, red solution of excess TEMPO (10.0 g, 64 mmol) in Et_2O (20 mL). Part of this was added dropwise over 3 h; additional Et_2O (15 mL) was added to the reaction to free the stir bar from the white precipitate which formed. When the orange color persisted in the reaction solution, TEMPO addition was stopped and the solution allowed to rise from an initial 14 °C to room temperature over 45 min, with a gradual loss of color. After the colorless solution was recooled to 0 °C, excess NH_4Cl (18.4 g, 0.338 mol, in 100 mL of H_2O at 0 °C) was added to neutralize any remaining Grignard, and the solution was shaken (turning the solution orange) and extracted; the upper, organic layer was washed (2 × 150 mL of H_2O) and dried ($MgSO_4$). After the supernatant solution was cooled overnight, it was pipetted into a flask (50 mL) and then degassed by aspirator. The solution (which can be stored under air, if necessary) was then distilled (65 °C, 9 mmHg), removing some colorless liquid (Et_2O by GC) from the solution, yielding a middle fraction which was then further distilled (at 70 °C, 1–2 mmHg). Gas chromatography (silica capillary column) of this red distillate showed TEMPO-Me, TEMPO-H (trace), and TEMPO•, in that order.

In a modification of Waddington's procedure[15] for neopentyl-TEMPO isolation, TEMPO-Me was isolated by preparative gas chromatography on a Varian 272010 Aerograph using He gas and thermal conductivity detection. The column was 5 ft × 0.25 in., the material 10% OV-1 60/80 Chromosorb W, oven isothermal 89 °C, injector temperature 205 °C, and detector temperature 220 °C. Aliquots of crude TEMPO/TEMPO-Me dark red liquid (50 μL) were injected, and after 3 min, droplets of the product (12 mg/injection, 26% by wt) were collected. At 6 min the TEMPO-Me abruptly terminated, the much darker TEMPO immediately started to come out (for 3.5 min), the collection vial was removed, and the outlet was connected by tubing to a fume hood. **Caution**: Vaporized TEMPO is harmful or toxic; traces of vapor cause headache, nausea, a bitter taste in the tongue, and olfactory damage.[16] The product collected from the crude mixture was a pale red; aliquots (50 μL) of this were reinjected as above, and the colorless pure TEMPO-Me (final yield 295 mg, 1.72 mmol, 6%, not allowing for undistilled product discarded) was isolated from the remaining impurities (mostly TEMPO). Re-injection of the purified TEMPO-Me gave a single GC peak.

The observed refractive index of TEMPO-Me is 1.4538 ± 0.0004 at 21.8 °C (lit.[17] 1.4548 at 20 °C corrected[18] to 1.4540 at 21.8 °C). Observed density is 0.92 g/mL. The VG-12-250 70-eV positive-ion mass spectrum of TEMPO-Me was *m/e* (rel intensity) 171 (7.0), 157 (13), 156 (100), 125 (5.5), 109 (22), 100 (10), 88 (38), 87 (10), 83 (11), 69 (48), 58 (12), 56 (35), 55 (40), 43 (12), 42 (29), 41 (50), 39 (15), in agreement with the literature.[19] NMR (CD_3CN) δ 3.55 (s, 3 H, OCH_3), 1.43 (s, 6 H, CH_2), 1.13 (s, 6 H, CH_3), 1.05 (s, 6 H, CH_3); NMR (C_6D_6) δ 3.56 (s, 3 H, OCH_3), 1.36 (dd, 6 H, J = 21, 3 Hz, CH_2), 1.22 (s, 6 H, CH_3), 1.15 (s, 6 H, CH_3); similar to the literature.[18-20]

MeCbl Thermolysis Kinetics. In a typical kinetics run, MeCbl (2.63 mg, 0.00196 mmol) and TEMPO (0.17959 g, 1.1494 mmol) were wrapped in foil, purged with N_2 for >5 min, and brought into the drybox. Ethylene glycol (10.0 mL each) was added, and solutions were stirred overnight, forming stock solutions of [MeCbl] = 1.96×10^{-4} M and [TEMPO] = 0.115 M. To foil-wrapped visible-spectroscopy Schlenk cuvettes were added TEMPO stock solution (0.200 mL, 0.0230 mmol) and MeCbl stock solution (2.00 mL, 0.000392 mmol), using foil-wrapped syringes. Typical final diluted solutions contained MeCbl (1.78×10^{-4} M, 0.000392 mmol, 0.526 mg) and TEMPO (10.4 mM, 0.0230 mmol, 3.58 mg, 59 equiv). After heating in a thermostated oil bath, cuvettes were cooled (from ≥120 °C) in ice-water (to quench the reaction) and then equilibrated at 25.0 °C for 5 min. After each thermolysis and observation, the cell would be wrapped in foil and heated further in the bath. Reactions were monitored at two MeCbl reactant wavelengths (521, 376 nm) and one $Co^{II}B_{12r}$ product wavelength (410 nm); the rates obtained from each wavelength agreed and were averaged together. Clean isosbestic points were observed at 335.8, 389.8, 485.4, and 583.2 nm.

Endpoint absorbances necessary for first-order kinetics were calculated by two methods: photolysis of MeCbl control solutions followed by calculating A_0/A_∞ ratios from spectral measurements; and computationally from the entire kinetic run, using a two-parameter exponential least-squares fitting routine (which optimized *only* the rate constant *and* the absorbance at infinite time). Endpoints and first-order behavior were established by the former method and confirmed by the latter.

The yield of the Co(II) product from MeCbl homolysis, $Co^{II}B_{12r}$, was estimated to be 82 ± 10% from absorbances at 474 and 404 nm in TEMPO/ethylene glycol/MeCbl thermolysis solutions, using absorptivities[21] of $\epsilon_{474} = 8.7 \times 10^3$ (±2%) M^{-1} cm^{-1} and $\epsilon_{404} = 6.9 \times 10^3$ (±2%)

(12) Brown, K. L.; Peck, S. Organocobalt Corrins. In *Organometallic Synthesis*; King, R. B., Eisch, J. J., Eds.; Vol. 4, 1988.
(13) Hensens, O. D.; Hill, H. A. O.; McClelland, C. E.; Williams, R. J. P. In B_{12}; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, p 474.
(14) Smith, B. L., Ph.D. Dissertation, University of Oregon, 1982, p 167.
(15) (a) See also footnote 42b in: (b) Waddington, M. D.; Finke, R. G., submitted for publication.
(16) Our observations are consistent with known reports: Aldrich Material Safety Data Sheet 2, 3217D, Jan 1991.
(17) The literature refractive index at 20 °C was corrected for temperature using the temperature dependence of isopropyl alcohol (−0.0008 from 20 to 21.8 °C) as a standard.
(18) Sholle, V. D.; Golubev, V. A.; Rozantsev, E. G. *Dokl. Acad. Nauk SSSR* **1971**, *200*, 137 (English version p 761).
(19) Whitesides, G. W.; Newirth, T. L. *J. Org. Chem.* **1975**, *40*, 3448.
(20) Kurumada, T.; Ohsawa, H.; Oda, O.; Fujita, T.; Toda, T.; Yoshioka, T. *J. Polym. Sci.* **1985**, *23*, 1477.

M^{-1} cm^{-1}. $Co^{II}B_{12r}$ decomposition was quantified using solutions of pure MeCbl and pure $Co^{II}B_{12r}$ (prepared in ethylene glycol from MeCbl by the photochemical method "a" of Blaser and Halpern[22]) with TEMPO in ethylene glycol, which were thermolyzed at the same temperature (129.9 and 142.5 °C for each pair) and monitored as above; decomposition of $Co^{II}B_{12r}$ became noticeable only after $1.5t_{1/2}$ of the parallel MeCbl thermolysis.[23]

The yield of TEMPO-Me was quantified using a HP5790 gas chromatograph with a DBWAX-30N capillary column (a Carbowax 20M analog, J&W Scientific, 30 m × 0.247 mm, film thickness 0.25 μm), flame ionization detection (215 °C, H_2 combustion, N_2 carrier gas), and a 50:1 nominal split-flow capillary injector system (200 °C). The oven was at 65 °C for 8 min and then increased at 30 °C/min to 215 °C for 4 min. A portion (0.78 mL) of the MeCbl/TEMPO/ethylene glycol solution thermolyzed at 142 °C was quantified by later addition of EtOH (1.00 μL) as an internal standard (calcd [EtOH] = 22.0 mM, initial [MeCbl] = 0.178 mM). The average area ratio of EtOH to TEMPO-Me signals observed was 39.4 ± 3.3 (5 runs). The relative response factor was determined from two TEMPO-Me/EtOH/TEMPO/ethylene glycol solutions independently prepared from authentic material ([TEMPO-Me] = 0.180 and 0.135 mM, [EtOH] = 0.891 and 0.67 mM, respectively). Area ratios of EtOH to TEMPO-Me for these solutions were 1.72 ± 0.35 (8 runs) and 1.57 ± 0.40 (3 runs), respectively, indicating the average relative response factor was 0.332 ± 0.053. The yield of TEMPO-Me at 142 °C was 0.185 ± 0.033 mM, or 104 ± 19%. Observed GC retention times (min): EtOH, 2.52; TEMPO-Me, 7.26; TEMPO, 12.0–12.5; ethylene glycol, 13.3.

MeCbl Photolysis. In ethylene glycol, MeCbl has relative maxima at 340, 376, and 521 nm and relative minima at 364 and 410 nm, within the observed region of 330–600 nm. In two separate experiments, solutions of MeCbl and TEMPO in ethylene glycol were sealed in visible spectroscopy cuvettes under N_2 and photolyzed to completion, forming[22] $Co^{II}B_{12r}$. Clean isosbestic points were visually observed at 335.8, 389.8, 485.4, and 583.2 nm. $Co^{II}B_{12r}$ (ethylene glycol) λ_{max} = 404, 473 nm, λ_{min} = 389, 423 nm. Observed A_0/A_∞ ratios (±1–3%), wavelengths (nm): 2.75, 521; 0.509, 410; 1.73, 376.

Protonation of MeCbl. To a Schlenk cuvette containing MeCbl (0.28 μmol, 0.14 mM in 2.00 mL ethylene glycol) was added 20 μL (0.168 mmol) of $H_3O^+BF_4^-$ (nominal 49% aqueous, 8.40 N measured), and the visible spectrum was taken at 5 °C intervals between 5.0 and 85 (±0.1) °C.

In a separate experiment, MeCbl (3.3 mL, 0.141 mM, 0.465 μmol in ethylene glycol) was scanned (330–600 nm) at 25.0 and 50.0 °C. To this diluted (1:100) $H_3O^+BF_4^-$ was added (4 μL, 0.34 μmol, 0.7 equiv), the 25.0 °C spectrum obtained, and more $H_3O^+BF_4^-$ added (16 μL more, 1.68 μmol total, 3.6 equiv). *The isosbestic points for 25 °C protonation did not approach those for the unprotonated axial-base-on–base-off thermal equilibrium.*

[MeCbi]$^+$[OAc]$^-$. The methylcobinamide acetate [Me-Cbi]$^+$[OAc]$^-$ aqueous solution, a gift from Prof. K. Brown,[12] was freeze-dried, yielding a fluffy solid (1.61 mg, 1.51 μmol of orange-red $MeCbi^+OAc^-$). Visible spectrum λ_{max}(water) = 352, 463 nm; λ_{min} = 347, 407 nm. $MeCbi^+OAc^-$ (0.73 mg, 0.69 μmol) was dissolved in ethylene glycol (4.90 mL) in the drybox, yielding a 0.14 mM solution. Visible spectrum (330–600 nm at 25.0 °C) λ_{max}(ethylene glycol) = 463, 369 (sh), 349 (sh) nm; λ_{min} = 408 nm. The $MeCbi^+OAc^-$ was then scanned at 5.0, 50.0, and 75.0 °C. After correcting spectra for density, they exhibited isosbestic points at 543, 458.6, and 372.9 nm. [Photolysis produced spectral changes indicating formation of one species (λ_{max}(ethylene glycol) = 467.5, 392 nm; λ_{min} = 419, 374 nm) and then its conversion into a third species (λ_{max}(ethylene glycol) = 467, 410 (sh) nm; λ_{min} = 383 nm).]

Results

MeCbl Thermolysis Products. Thermolysis of MeCbl (0.09–0.15 mM) with the nitroxide radical trap[3] TEMPO (7–43 mM) in ethylene glycol under N_2 (120.1–140.9 °C) displays visible spectra with clean isosbestic points and yields $Co^{II}B_{12r}$ (eq 1) and TEMPO-Me (eq 2). The average yield of $Co^{II}B_{12r}$ is 82 ± 10% by visible spectroscopy. The apparently low yield compared to previous[3] results is due to slow decomposition of $Co^{II}B_{12r}$ at the higher temperatures now necessary for MeCbl thermolysis. This Co(II) decomposition side-reaction was demonstrated and quantified[23] independently by thermolyses of authentic[22] $Co^{II}B_{12r}$ under the MeCbl reaction conditions (its decomposition rate constant[23] is 3×10^{-6} s^{-1} at 130 °C; $Co^{II}Cbi^+$ is similarly not completely stable at >105 °C).[24] Thus, the corrected *initial* $Co^{II}B_{12r}$ yield is essentially quantitative. Furthermore, from MeCbl thermolysis at 142.5 °C, the yield of TEMPO-Me is 104 ± 19%, based upon gas chromatography (calibrated with independently synthesized TEMPO-Me). Hence the homolysis and trapping reactions in eqs 1 and 2, respectively, have been established quantitatively within experimental error.

$$\text{MeCbl} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Co}^{II}\text{B}_{12r}^{\bullet} + \text{Me}^{\bullet} \quad (1)$$

$$\text{Me}^{\bullet} + \text{TEMPO} \rightarrow \text{TEMPO-Me} \quad (2)$$

Table I. Rates of MeCbl Thermolysis with TEMPO in Ethylene Glycol[a]

temp,[b] °C	$10^4 k_{obsd}$,[c] s^{-1}	$10^4 k_{h,on}$,[d] s^{-1}
140.9	2.64	3.70
135.0	1.04	1.42
135.0	1.10	1.50
135.0	1.10[e]	1.50[e]
129.9	0.639	0.855
120.1	0.179	0.231

[a] Rates (±8%) are averages of observed reactant and product rates at 521, 410, and 376 nm (see text). [TEMPO] = 6.7–18.3 mM unless otherwise noted. [b] ±0.2 °C. [c] Without axial-base corrections (see text). [d] With axial-base corrections of ΔH° = −5.1 kcal mol^{-1} and ΔS° = 10.5 cal mol^{-1} K^{-1} (see text). [e] [TEMPO] = 43.0 mM.

MeCbl Thermolysis Kinetics. Solutions of MeCbl and TEMPO in ethylene glycol have been thermolyzed at 120.1–140.9 °C and monitored by visible spectroscopy at 25.0 °C, yielding first-order rate constants (Table I). Infinity points are calculated from the observed initial absorbance and the observed ratio of A_0/A_∞ absorbances in separate MeCbl photolysis reactions; these points are verified by a two-parameter exponential least-squares curve-fitting routine (vide supra). The thermolysis rate constants at 135 °C are independent of the concentration of the TEMPO radical trap (at 6.7, 7.8, and 43.0 mM; 45–506 equiv).

MeCbl Axial-Base Equilibria. In order to calculate the fraction[25] of MeCbl with the 5,6-dimethylbenzimidazole base coordinated to the cobalt at the temperatures of thermolysis, the visible spectrum of MeCbl was obtained in ethylene glycol at 5 °C intervals between 5 and 99 °C.[26] The *density-normalized*[27,28] spectra of MeCbl in ethylene glycol at 5–99 °C have isosbestic points at 335, 383, 480, and 576 nm for the MeCbl equilibria perturbed by thermal change.

Models for Spectra of Base-Off MeCbl. Methylcobinamide[29] ($MeCbi^+OAc^-$) visible spectra have been obtained in water (in good agreement with the literature[12]) and in ethylene glycol at 5–95 °C (showing thermal isosbestic points at 373, 459, and 543 nm). Also obtained were the density-corrected spectra of MeCbl·H^+ (MeCbl protonated with $H_3O^+BF_4^-$, <1 to 600 equiv) in ethylene glycol at 5–85 °C, spectra which showed thermal isosbestic points at 378, 458, and 538 nm. The isosbestic points in the 25 °C spectrum for MeCbl·H^+ did not approach those for

(21) Hay, B. P., Ph.D. Dissertation, University of Oregon, 1986, p 117.
(22) Blaser, H.-U.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 1684.
(23) At 130 °C, $Co^{II}B_{12r}$ reacts at about 3×10^{-6} s^{-1}, which interferes with experimentally determined infinity points. However, decomposition of $Co^{II}B_{12r}$ is negligible within the first 1.5 half-lives of the thermolysis reaction; only these early data were used for kinetics.
(24) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012.
(25) For an explanation of cobalamin axial-base equilibria, see: Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3548–3555.
(26) (a) These base-on/"base-off" spectra exhibit *apparent* isosbestic points at 385, 470, and 580 nm *before* correction for density changes. Since density correction multiplies each point in a spectrum by the same factor, which changes smoothly with temperature, the density-correction process does not affect the existence (or number) of isosbestic points, but it does influence their final wavelengths. (b) See supplementary material.
(27) Each visible spectrum in ethylene glycol solution is multiplied by the density of ethylene glycol at 25.0 °C and divided by the linearly calculated density at the actual temperature, in order to correct for volumetric effects on concentration.
(28) Ethylene glycol densities are extrapolated from the following: *The Merck Index*, 10th ed.; Merck: Rahway, NJ, 1983; p 550.
(29) $MeCbi^+OAc^-$ was a gift from Professor K. Brown, prepared as described elsewhere.[12]

the unprotonated axial-base on–base-off thermal equilibrium of MeCbl, indicating the limitations of protonated MeCbl·H$^+$ as a model for the base-off MeCbl spectrum.

A reviewer has brought to our attention that although the MeCbi$^+$ and MeCbl·H$^+$ visible spectra are virtually identical above 300 nm in water, this is not true in ethylene glycol (for reasons that are not understood). In addition, the equilibrium responsible for the isosbestic temperature-dependent spectral changes for the nominally *base-off* MeCbi$^+$ and MeCbl·H$^+$ is also an unstudied and thus unresolved point; these changes may relate to the generally unresolved issue of 5- vs 6-coordinate (solvent coordinated) cobamides, to an unknown conformational isomer, or possibly to the presence of a so-called "tuck-in" form (vide infra) for the protonated axial base in MeCbl·H$^+$. Fortunately, however, these nominally base-off forms can be neglected for the purposes of the present work since they are expected to have base-off-like (slow) Co–Me homolysis rates (i.e. only pendant-base-on MeCbl is expected to contribute significantly to Co–Me homolysis, vide infra).

Discussion

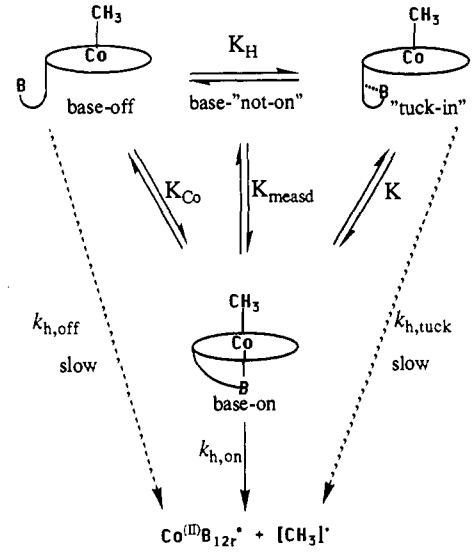
MeCbl Products and Stoichiometry. MeCbl thermolysis in ethylene glycol with TEMPO under N_2 gives the expected homolysis products, $Co^{II}B_{12r}$ and TEMPO-Me, cleanly and quantitatively within experimental error. In addition to the homolysis precedents of other alkylcobalamins (vide infra), MeCbl itself has been shown to undergo thermal homolysis in the absence of traps to give the methyl-radical products methane and ethane.[30] These results establish the necessary[31] condition that the reaction studied here is homolysis of the Co–CH_3 bond.

Co–CH_3 Homolysis Rate Constants and Activation Parameters. As in our adenosylcobamide homolysis studies,[3] kinetic studies of MeCbl homolysis gave reproducible rate constants of acceptable precision. Using the axial-base thermodynamic parameters obtained (as discussed below), the individual observed rate constants obtained can be corrected to reflect the rate of homolysis from the kinetically dominant, base-on form of MeCbl, yielding $k_{h,on}$ from each set of T and k_{obsd} pairs. From the Eyring plot of the corrected rate constants, the activation parameters (with 1 σ uncertainties) are $\Delta H^{\ddagger}_{h,on}$ = 41 ± 3 kcal mol^{-1} and $\Delta S^{\ddagger}_{h,on}$ = 24 ± 6 cal mol^{-1} K^{-1}.[32,33] Part of the uncertainty in our activation values (about 2 kcal mol^{-1} in $\Delta H^{\ddagger}$ and 4 cal mol^{-1} K^{-1} in $\Delta S^{\ddagger}$) is due to the axial-base equilibrium and part is from the Eyring plot (uncertainties of 1.8 kcal mol^{-1} in $\Delta H^{\ddagger}$ and 4.5 cal mol^{-1} K^{-1} in $\Delta S^{\ddagger}$). Thus, the effect of the axial-base uncertainty in $\Delta H°$ and $\Delta S°$ upon the error in $\Delta H^{\ddagger}_{h,on}$ and $\Delta S^{\ddagger}_{h,on}$ is relatively small and quite tolerable. (Our $\Delta H°$ and $\Delta S°$ are the measured values; i.e. they correspond to Brown's "complete scheme"[25] $\Delta H°_{measd}$ and $\Delta S°_{measd}$.)

The rate constants obtained at 135.0 °C indicate a zero-order dependence on the trap [TEMPO] between 6.67 and 43.0 mM. As the rate of loss of MeCbl and the rate of formation of $Co^{II}B_{12r}$ are independent of the trapping step, they represent the rates[34] of homolysis of the Co–C bond of MeCbl.

MeCbl Axial-Base Equilibria. As explained by Brown[25] (and discussed further in the supplementary material), alkylcobalamins equilibrate (at K_{measd}) between a base-on form (in which the 5,6-dimethylbenzimidazole nitrogen is coordinated to the cobalt, making Co–C homolysis relatively facile) and base-not-on forms (in which the cobalt is either solvated or five-coordinate, making homolysis relatively slow).[24,35-37] These "five-coordinate" forms include both the obvious base-off form and a "tuck-in" form[25] (the latter is *more* abundant, especially for MeCbl),[38] in which the 5,6-dimethylbenzimidazole is hydrogen bonded to a corrin side chain (but is not associated with cobalt), Scheme I. The newest evidence for the significance of the tuck-in form is the large disagreement between the MeCbl thermal isosbestic points and the base-off-model (MeCbi$^+$ and MeCbl·H$^+$) visible spectra reported herein.

Scheme I



The axial-base-off–base-on prior equilibrium must be quantitatively extrapolated to the thermolysis temperatures in order to get meaningful homolysis rate constants. The main equilibrium (eq 3, K_{measd}, to the base-on form) can be followed by visible spectroscopy at varying temperatures and in principle can be quantified.

$$\text{base-not-on MeCbl} \overset{K_{measd}}{\rightleftharpoons} \text{base-on MeCbl} \qquad (3)$$

(base-not-on means tuck-in *and* base-off)

However, quantitative measurement and extrapolation of the K_{measd} equilibrium has several problems, *especially for MeCbl*: (1) Uncertainties[39] about the pK_a in ethylene glycol of the pendant 5,6-dimethylbenzimidazole make use of the protonation equilibrium problematic. (2) Cobamide UV–visible extinction coefficients are, rigorously speaking, temperature-dependent (however, in *some* instances they can be taken as temperature-independent without introducing intolerable errors; see the supplementary material).[25,40] (3) The MeCbl axial-base equilibrium remains mostly base-on even at temperatures too high to study since Co–CH_3 homolysis begins (preventing measurement of the absorbance curve). (4) At least for MeCbl, the tuck-in form is spectroscopically slightly different from other base-off forms,[41] yet there is no rigorous

(30) Schrauzer, G. N.; Sibert, J. W.; Windgassen, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 6681–6688.
(31) Koenig, T. W.; Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1499.
(32) Interestingly,[33] the activation entropy of Me· dissociation is almost the same for MeCbl (24 eu) as for (MeCbi$^+$)$^{•-}$ (21 ± 3 eu).
(33) Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley-Interscience: New York, 1976; Chapter 3.
(34) (a) When extrapolated down to 110 °C, the observed rate constant (k_{obsd} = 5 (±1) × 10^{-6} s^{-1}; $t_{1/2}$ = 10^5 s) is consistent with the recent report of MeCbl homolysis in ethylene glycol with TEMPO at 110 °C (k_{obsd} = 9 × 10^{-6} s^{-1}) under various pressures: (b) Gamelkoorn, H. J.; de Bolster, M. W. G.; Balt, S., personal communication, 1989.
(35) (a) AdoCbi$^+$ homolyzes only 30 to 10$^{2±1}$ times slower[24] than AdoCbl[36] at 110 °C, so cobalamins and cobinamides are essentially the same *for the >10^{15} rate-enhancement comparisons made herein.* (b) Another estimate of the effect[24] of the axial base comes from comparing Lexa and Savéant's[37] homolysis rate constant for (MeCbl)$^{•-}$ at −30 °C (1200 s^{-1}) with that of reduced (MeCbi$^+$)$^{•-}$ (2.7 s^{-1}); this again indicates a *relatively* small effect due to the axial base (440× at −30 °C).
(36) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820.
(37) (a) Lexa, D.; Savéant, J.-M. *J. Am. Chem. Soc.* **1978**, *100*, 3220–3222. (b) Lexa, D.; Savéant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235–243.
(38) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2267–73.
(39) (a) Although reliable values for the pK_a of α-ribazole-3′-phosphate and α-ribazole are now available for aqueous solutions,[39b] that is not so in ethylene glycol. (b) Brown, K. L. *J. Am. Chem. Soc.* **1987**, *109*, 2277–2284. Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montejano, Y. D.; Jacobsen, D. W. *Inorg. Chem.* **1984**, *23*, 1463–1471.
(40) Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M. *J. Chem. Soc. A* **1968**, 2419–2428.

Table II. Dependence of MeCbl Homolysis Activation Parameters[a] upon the Thermodynamic Parameters of the MeCbl Axial-Base Equilibrium (Solvent = Ethylene Glycol)

equilibrium method	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, eu	$\Delta H^{\ddagger}_{on}$, kcal mol^{-1}	$\Delta S^{\ddagger}_{on}$, eu
none (minimum)[b]	$-\infty$	$+\infty$	40	20
MeCbl·H$^+$ (model actually used)[c]	−5.1	−10.5	41	24
probable upper limit[d]	−5.6	−13.3	42	27
"maximum"[e]	−6.7	−17.4	44	32
^{13}C NMR in H_2O[f]	−6.5	−14.0	41	25

[a] The estimated standard deviations are $\Delta H^{\ddagger}_{h,on} = \pm 2$ kcal mol^{-1} and $\Delta S^{\ddagger}_{h,on} = \pm 5$ eu (eu = cal mol^{-1} K^{-1}) for these activation parameters in ethylene glycol (calculated from the rate constants k_{obsd} derived using A_0/A_∞ infinity points at temperatures from 140.9 to 120.1 °C). [b] Minimum ϵ_{nol-on} (see text) by visible spectroscopy, equivalent to *no* axial-base correction. [c] Based on the unheated visible spectrum of MeCbl·H$_3$O$^+$ [assuming ϵ_{nol-on} (at 538 nm) = 2200 exactly]. [d] Probable upper limit to the axial-base correction (i.e., an assumption of $\epsilon_{nol-on} = 4000$ in the visible spectrum of base-not-on MeCbl), based on the maximum reasonable fit to MeCbl thermal isosbestic points (see text). [e] Approximate maximum axial-base correction (an assumption of $\epsilon_{nol-on} = 5000$ in the visible spectrum of base-not-on MeCbl), based on the maximum conceivable fit to MeCbl thermal isosbestic points (see text). [f] These axial base thermodynamic parameters, from the *aqueous* ^{13}C NMR studies by Brown[25] (with $I = 0$), *should only approximate* the values herein in ethylene glycol.

method of getting the necessary visible spectra for the individual base-not-on forms of MeCbl (needed for successful analysis of a *partial* absorbance vs temperature equilibrium curve).

We present herein an analysis of the axial-base equilibria which takes all these factors[25] into account, explicitly showing how the final $\Delta H^{\ddagger}_{h,on}$ and $\Delta S^{\ddagger}_{h,on}$ parameters and error bars are obtained. This is the first time in an alkylcobalamin-homolysis visible-spectroscopy study where all the complicating factors have been considered.

Temperature-dependent absorbances (A_T, from 5 to 99 °C) of MeCbl in ethylene glycol at 538 nm were fit to eq 4, using various

$$1/T = -[R \ln\{(A_T - [\text{MeCbl}]\cdot\epsilon_{not-on})/([\text{MeCbl}]\cdot\epsilon_{on} - A_T)\} - \Delta S°]/\Delta H° \quad (4)$$

estimates for ϵ_{not-on}, leading to values for ϵ_{on}, $\Delta H°$, and $\Delta S°$. It was assumed that the only[27] effect of temperature was a change in the relative amounts of base-on and base-not-on MeCbl (i.e., that K_H and ϵ_{not-on} are constant; see supplementary material),[42] as 538 nm is a region of significant MeCbl absorbance change with temperature, but is at or near isosbestic points for MeCbi$^+$ and MeCbl·H$^+$ thermal change in density-corrected spectra (see supplementary material).[15b]

By using the temperature vs absorbance data (538 nm), values of ϵ_{on}, $\Delta H°$, and $\Delta S°$ were calculated for various possible values of ϵ_{not-on}; the results ranged from a minimum of $\Delta H° = -4.8$ (±0.2) kcal mol^{-1} and $\Delta S° = -9.0$ (±0.6) cal mol^{-1} K^{-1} up to a realistic upper limit (i.e., for $\epsilon_{not-on} = 4000$) of $\Delta H° = -5.6$ (±0.2) kcal mol^{-1} and $\Delta S° = -13.3$ (±0.7) cal mol^{-1} K^{-1} (Table II). In the absence of a better method, an assumption of $\epsilon_{not-on} = 2200$ (based upon the ϵ_{off} spectrum of MeCbl·H$_3$O$^+$BF$_4^-$ in ethylene glycol at 25 °C) can be made and is illustrative; this assumption of $\epsilon_{not-on} = 2200$ is extremely close (at 538 nm for MeCbl) to that of using the cobinamide MeCbi$^+$ as a model, is well within the reasonable range for meeting MeCbl isosbestic points, and yields base-on–base-not-on thermodynamic equilibrium parameters and least-squares error bars (i.e. from the fit to eq 4) of $\Delta H° = -5.1$ (±0.2) kcal mol^{-1} and $\Delta S° = -10.5$ (±0.6) cal mol^{-1} K^{-1}.

A more rigorous treatment is to propagate an estimated ±1000 uncertainty in ϵ_{not-on} (2200 ± 1000); this yields a further 1.5 kcal mol^{-1} and 3 cal mol^{-1} K^{-1} of uncertainty so that the resultant axial-base equilibrium parameters and their uncertainties become $\Delta H° = -5.1$ (± 2) kcal mol^{-1} and $\Delta S° = -10.5$ (± 4) cal mol^{-1} K^{-1}. *Fortunately, the mathematics is such that this level of uncertainty in the base-off spectrum and thermodynamic parameters propagates a relatively small error into the desired final homolysis activation parameters, $\Delta H^{\ddagger}_{on}$ and $\Delta S^{\ddagger}_{on}$, as the last two columns of Table II demonstrate.* Furthermore, these axial-base estimates (in ethylene glycol) are within experimental error of those Brown[25] found by NMR for the base-on–base-elsewhere equilibria *in water* at zero ionic strength (Table II, last entry).[43] The above treatment is not to say, however, that more exact and precise measurements for MeCbl's axial-base and tuck-in equilibria in ethylene glycol are not desirable.

The MeCbl Bond Dissociation Enthalpy. By estimating the solvent-cage efficiency,[31] the Eyring activation parameters may be corrected for solvent-cage effects to yield a Co–CH$_3$ bond dissociation enthalpy (BDE). Applying the Eyring form of the usual empirical relationship[44-46] for the *change* in viscosity[47,48] (η) of the ethylene glycol solvent over 120–143 °C yields an enthalpy for viscous flow, $\Delta H^{\ddagger}_{\eta} = 4.0$ kcal mol^{-1}.[49] Next, the appropriate[50] recombination barrier correction[31,51] yields an estimate of the MeCbl Co–CH$_3$ bond dissociation enthalpy, 37 ± 3 kcal mol^{-1}.

Methylcobalamin homolysis activation parameters are also necessary for a more complete understanding of alkylcobalamins, especially for a comparison of Coenzyme-B$_{12}$ (AdoCbl) with the sterically extreme primary-alkylcobalamins methylcobalamin (nonbulky) and neopentylcobalamin (very bulky).[15b,52,53] X-ray diffraction of methyl- and adenosylcobalamins has shown that the Co–C bond length in MeCbl is 1.99 ± 0.02 Å,[54a] about the same as that in AdoCbl (at 2.05 Å).[55] On the basis of these *ground-state* structural determinations, it has been concluded that the Ado and Me alkyl groups are of similar steric bulk as alkylcobalamins,[54] a conclusion reinforced by NMR studies[54b] (where, again, no major corrin conformational changes are detected when comparing MeCbl to AdoCbl).

However, our MeCbl Co–CH$_3$ BDE measurement of 37 ± 3 kcal mol^{-1} is the largest solution-phase Co–C bond known,[56] larger than the 30 ± 2 kcal/mol BDE of AdoCbl.[3] It follows, then, that the BDE differences must reflect different *transition states* for

(41) (a) One wonders if it is just mere coincidence that the two biologically active alkylcobalamins (MeCbl and AdoCbl) appear[41b] to have the largest amounts[38,41b] of the tuck-in form. Assuming that they do,[41b] one speculative idea is that the tuck-in form is associated with an electronic and steric configuration in the cobamides which gives them the greatest ground-state *stability*, a feature necessary for biological transport of these cofactors. (b) A referee has questioned whether or not MeCbl and AdoCbl do in fact have the largest amount of the tuck-in form[38] and has noted that "the only two values of K_H that have been determined (CH$_3$Cbl and –O$_2$CCH$_2$Cbl) are essentially identical...so that, if anything, it looks like K_H may be largely independent of the axial ligands."

(42) Attempts at non-linear least-squares fitting of ϵ_{not-on} from 5–99 °C data always either diverged or converged to the imposed constraint limit. All fitting used T in degrees Kelvin.

(43) In Brown's complete scheme with the tuck-in form, K_{Co} is *not* the same as the base-on/base-not-on MeCbl equilibrium, K_{Measd} (with his $\Delta H°_{Measd} = -6.5$ for $I = 0$). Naturally, *both* measured constants also differ from values obtained in the solutions of high ionic strength, chosen by Brown, with $\Delta H°_{Co} = -7.9$ for $I = 1.0$ (see supplementary material).

(44) For this solvent (at 120–150 °C), Frenkel's "Eyring" form[45] of Guzmán's[46] "Andrade" equation is used to calculate $\Delta H^{\ddagger}_{\eta}$: $-R \ln(\eta/T) = \Delta H^{\ddagger}_{\eta}/T - \Delta S^{\ddagger}_{\eta}$.

(45) Frenkel, J. *Nature (London)* **1930**, *125*, 581–582.

(46) Guzmán, J. de *Anal. Soc. Espan. Fis. Quim.* **1913**, *11*, 353–362.

(47) Temperature (°C), viscosity (cP) data used: 119.6, 1.484; 131.1, 1.246; 140.7; 1.080; 149.6, 0.959.

(48) Thomas, L. H.; Meatyard, R.; Smith, H.; Davis, G. H. *J. Chem. Eng. Data* **1979**, *24*, 161–4.

(49) The activation enthalpy of viscous flow ($\Delta H^{\ddagger}_{\eta}$) depends only upon the *change* of viscosity with temperature and *not upon the magnitude* or units of the viscosity. For example, multiplying viscosities by 100 still gives $\Delta H^{\ddagger}_{\eta} = 4.0$ kcal mol^{-1}.

(50) Koenig, T. W.; Finke, R. G. *J. Am. Chem. Soc.* **1988**, *110*, 2657.

(51) An efficient cage ($F_c \simeq 1$) and BDE $\simeq \Delta H^{\ddagger}_{obsd}$(soln) – $F_c\Delta H^{\ddagger}_{\eta}$ are assumed.[31,50]

(52) Waddington, M. D.; Finke, R. G. 41st Northwest Regional ACS Meeting, June 16–18, 1986, Abstract No. 149.

(53) Kim, S.-H.; Chen, H. L.; Feilchenfeld, N.; Halpern, J. *J. Am. Chem. Soc.* **1988**, *110*, 3120–3126.

(54) (a) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729–38. (b) Brown, K. L.; Hakimi, J. M. *J. Am. Chem. Soc.* **1986**, *108*, 496.

(55) Glusker, J. P. In *B$_{12}$*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 3.

(56) Simões, J. A. M.; Beauchamp, J. L. *Chem. Rev.* **1990**, *90*, 629–688.

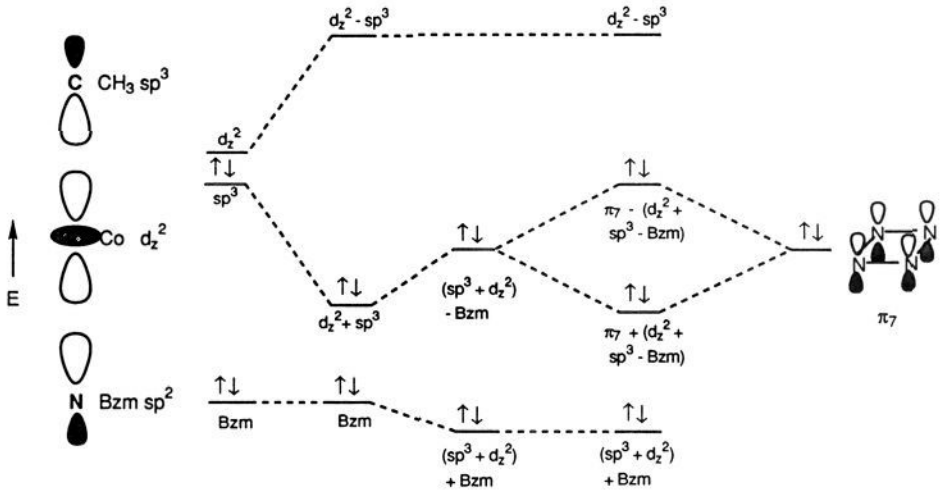


Figure 2. Molecular orbital diagram showing the σ^* LUMO created from the cobalt d_{z^2} and carbon sp^3 orbitals of an alkylcobalamin (see text), adopted with permission from ref 68. Bzm is 1,5,6-trimethylbenzimidazole.

homolysis in MeCbl and AdoCbl, perhaps due to different higher-temperature conformers for these two alkylcobalamins (that are closer to the (different) transition states for homolysis, but are different from the *similar* ground-state conformations detected by X-ray crystallography and NMR). Detecting and structurally characterizing these higher-temperature conformers of alkyl corrins, ones that look more like the transition state for Co–C homolysis, remains an unsolved problem in alkylcobalamin chemistry.

Our MeCbl BDE is lower than (but within 3 σ of) an earlier Co–C bond enthalpy *estimate* for MeCbl of 46 ± 3 kcal mol^{-1}, derived from photohomolysis threshold energies.[57] In another ligand system, Toscano recently examined alkyl dimethylglyoxime cobalt *model* complexes in bromoform, obtaining a methyl–cobalt bond energy of 33 ± 2 kcal mol^{-1}.[58] For *naked* Co–CH_3, Armentrout[59] obtained a gas-phase values of 45 ± 5 kcal mol^{-1}.

MO Diagram for Alkylcobinamides and Their One-Electron-Reduced Analogues. For alkylcobalamins (and presumably for all alkylcobamides), the lowest unoccupied molecular orbital (LUMO)[60] of alkylcobamides is the σ^* orbital along the Co–C bond perpendicular to the corrin system. This key LUMO is formed from interaction of the alkyl sp^3 orbital with the cobalt d_{z^2} orbital; the interactions of the corrin and the axial base do not change the relative order of the frontier orbitals.[1,61-64]

An electron added electrochemically or otherwise to this antibonding orbital (initially via corrin π^* and Co $d_{x^2-y^2}$ orbitals)[65] subsequently leads to loss of the alkyl radical and formation of $Co^{I}B_{12s}$ (eq 5). Note that the Co–CH_3 cleavage mechanism we expect for reduced alkylcobamides (eq 5, 6) differs from that

$$\text{reduced } (\text{Me–Co}^{\text{II}}\text{Cbl})^{\bullet -} \underset{}{\overset{k_5}{\rightleftharpoons}} (\text{Co}^{\text{I}}\text{B}_{12s})^- + \text{Me}^\bullet \qquad (5)$$

$$[\text{Me}^\bullet + \text{Trap} \rightarrow \text{Trap-Me}] \qquad (6)$$

presented in the electrochemical literature[37,66,67] by incorporating

Chart I

$\{\sigma\}^2$	$\{\sigma\}^2\{\sigma^*\}^1$
Me—— Co^{III}Cbl	(Me--------- $Co^{II}B_{12r})^{\bullet -}$
normal σ bond	weakened "one" $-e^-$ σ bond

reversible Co^{II}–CH_3 cleavage,[68] followed by $CH_3^\bullet$ *trapping*[69] if trap is added or if $H^\bullet$ donor solvents are present: $k_{obsd} = k_{h,apparent}$ = a composite (with the reverse of the initial Co–C cleavage step probably favored by the preferred base-off form[69b] of Co(I)). Hence, ideally, a radical trap should be deliberately added in such experiments to capture the $Me^\bullet$ (eq 6), although this is generally not done in the existing literature electrochemical studies of alkylcobamides. Nevertheless, the crucial result is that reduction of MeCbl enhances[67b] enormously the rate (k_5) of dissociation of this bond through the bond-weakening effect of the electron entering the antibonding σ^* LUMO (Figure 2).

These parallel reduced and non-reduced species homolyses permit comparison of normal vs "half"-strength M–C σ bonds (Chart I), as non-axial structural distortions and electronic rearrangements are minimized by the approximately square-planar corrin ligand, thus making it a near-ideal system to measure the full bond-weakening effect of one electron.[59,70]

Electrochemistry of Alkyl Cobamides. Lexa and Savéant have reported[37] electrochemically induced homolysis from reduced[71]

(57) Endicott, J. F.; Balakrishnan, K. P.; Wong, C.-L. *J. Am. Chem. Soc.* **1980**, *102*, 5519–26.
(58) Toscano, P. J.; Seligson, A. L.; Curran, M. T.; Skrobutt, A. T.; Sonnenberger, D. C. *Inorg. Chem.* **1989**, *28*, 166–168.
(59) Armentrout, P. B.; Georgiadis, R. *Polyhedron* **1988**, *7*, 1573–1581.
(60) Rubinson, K. A.; Parekh, H. V.; Itabashi, E.; Mark, H. B., Jr. *Inorg. Chem.* **1983**, *22*, 458–463.
(61) Salem, L.; Eisenstein, O.; Anh, N. T.; Burgi, H. B.; Devaquet, A.; Segal, G.; Veillard, A. *Nouv. J. Chim.* **1977**, *1*, 335–348, Figure 5.
(62) Christianson, D. W.; Lipscomb, W. N. *J. Am. Chem. Soc.* **1985**, *107*, 2682–2686.
(63) Mealli, C.; Sabat, M.; Marzilli, L. G. *J. Am. Chem. Soc.* **1987**, *109*, 1593–1594.
(64) Zhu, L.; Kostić, N. M. *Inorg. Chem.* **1987**, *26*, 4194–4197.
(65) Rao, D. N. R.; Symons, M. C. R. *J. Chem. Soc., Chem. Commun.* **1982**, 954–955.

(66) (a) MeCbl reduction required −1.5 V vs SCE in an aqueous buffer.[66b] (b) Rubinson, K. A.; Itabashi, E.; Mark, H. B., Jr. *Inorg. Chem.* **1982**, *21*, 3571–3573.
(67) (a) Birke observed reduction waves for MeCbl in aqueous solutions at $E_{1/2}$ = −1.2 and −1.5 V vs SCE, which he attributed to the base-off and base-on forms of MeCbl. (b) Kim, M.-H.; Birke, R. L. *J. Electroanal. Chem.* **1983**, *144*, 331–350.
(68) The trapping of an $R^\bullet$ by a *diamagnetic* metal is a little-recognized step that does, however, have precedent:[8c] Finke, R. G.; Keenan, S. R.; Watson, P. L. *Organometallics* **1989**, *8*, 263–277, especially p 269 and footnote 26.
(69) (a) This mechanism in eqs 5 and 6 clarifies several issues. It explains the observation of >97% H_3C–CH_3 following MeCbl electrochemical reduction in H_2O (with no trap).[66,67] It predicts the formation of CH_4 in reductions of MeCbl and $MeCbi^+$ in the $H^\bullet$ donor solvents propanol/DMF[3,37] (note that the prevention of recombination, eq 5, makes $k_{h,apparent} = k_{h,true}$ in this solvent mixture). This mechanism and its reversible Co–C cleavage step[68] (eq 5) also explains why $k_{h,apparent}$ is 10^4 slower in aqueous[66,67] and DMSO[37b] solvents with limited concentrations of radical traps or ineffective traps, for example $CH_3^\bullet$ self-trapping or 0.005% Triton X-100 surfactant. (This difference, noted earlier by two groups,[37b,67] *had been* explained as a $CH_3^\bullet$ solvation effect[67] instead of in terms of the mechanism in eqs 5 and 6.) Finally, this mechanism clarifies radical-cage effects and predicts that trapping of caged $CH_3^\bullet$ by the solvent-cage walls in DMF:propanol can lead to enhanced rates [in such efficient trapping (inefficient cage) cases, F_c tends toward zero].[31,50] (b) Lexa, D.; Savéant, J.-M. *J. Am. Chem. Soc.* **1976**, *98*, 2652.
(70) Upon reduction in a "naked" Co–Me system, for example, the unconstrained electron lone pairs would be free to rearrange relative to the Co–C bond.[59]

Table III. Comparison of Rate Constants as a Function of Temperature for Co–C Homolysis from Alkylcob(III)amides and Electrochemically Reduced Alkylcob(II)amides

cobamide	k_1, s^{-1}	(cobamide)$^{\bullet-}$	k_5, s^{-1}	T, °C	k_5/k_1
MeCbl[a]	$10^{-19\pm4}$	(MeCbl)$^{\bullet-}$[b]	1200	−30	$10^{22\pm4}$
MeCbl[a]	$10^{-19\pm4}$	(MeCbi)$^{\bullet}$[b]	3	−30	$>10^{19\pm4}$[c]
MeCbl[a]	$10^{-12\pm3}$	(MeCbi)$^{\bullet}$[b]	4400	25	$>10^{15\pm3}$[c]
AdoCbi$^+$[d]	$10^{-11\pm2}$	(MeCbi)$^{\bullet}$[b]	4400	25	$>10^{14\pm2}$[e]
AdoCbl[f]	$10^{-9\pm2}$	(AdoCbl)$^{\bullet-}$[g]	2700	25	$10^{12\pm2}$
MeCbl[a]	$10^{-12\pm3}$	(MeCbl)$^{\bullet-}$[g,h]	0.4	25	$\sim10^{11\pm3}$[h]

[a] In ethylene glycol.[1] [b] In DMF/propanol; [NBu_4BF_4] = 0.1–0.2 M.[37] [c] Note that this is a comparison between MeCbl and MeCbi$^+$ for reasons discussed in the text; even if this comparison is made at 90 °C, still $k_5/k_1 > 10^{13}$. [d] In pH 7 H_2O, [buffer] = 0.01 M.[24] [e] The k_5/k_1 ratio presumably would be even greater if the alkyls were the same: the solvent effect is unclear (see text). [f] In pH 7 H_2O, [buffer] = 0.01 M.[36] [g] In pH 12 H_2O,[67b] [buffer] = 0.5 M. [h] The lack of a R$^{\bullet}$ trap *in* H_2O decreases the *observed* (R–Cbl)$^{\bullet-}$ dissociation rate constant in this case (see text).

(MeCbl)$^{\bullet-}$ (at −30 °C, eqs 5 and 6) and reduced[37] MeCbi$^{\bullet}$ (from −20 to 19 °C), eq 7, in a 1:1 DMF–propanol mixture; their work

$$\text{reduced (Me–Co}^{\text{II}}\text{Cbi)}^{\bullet} \rightleftharpoons (\text{Co}^{\text{I}}\text{Cbi}) + \text{Me}^{\bullet} \qquad (7)$$

is more useful for the comparisons herein, in part[72] because the DMF–propanol is thought[37b,69] to serve as a H$^{\bullet}$ donor to trap Me$^{\bullet}$ as CH_4. Rubinson[66] and Birke[67] have reported similar reductive Co–C cleavages for MeCbl, but the single temperatures or different solvent[72,73] and lack of trap conditions[51,69] make their work less useful for comparison to our MeB_{12} Co–C thermolysis results in ethylene glycol.

Note that, in general, alkylcobinamides such as MeCbi$^+$ (or their reduced analogues such as MeCbi$^{\bullet}$) are more stable than alkylcobalamins like MeCbl (or their reduced analogues such as MeCbl$^{\bullet-}$) because the cobinamides lack the Co–C weakening axial 5,6-dimethylbenzimidazole base.[24] This is a key to why the rates of Co–C cleavage for reduced MeCbi$^{\bullet}$ (but not MeCbl$^{\bullet-}$) were slow enough to be measurable electrochemically even up to 19 °C.[37a]

One-Electron-Reduction Activation of MeCbi and Comparison to MeCbl Homolysis Data. Lexa and Savéant's temperature-dependent rate constant data[37] for reduced (MeCbi)$^{\bullet}$ (in DMF/1-propanol) indicate that $\Delta H^{\ddagger}_h = 19 \pm 1$ kcal mol^{-1} and that $\Delta S^{\ddagger}_h = 21 \pm 3$ cal mol^{-1} K^{-1}. Extrapolating[74,75] up to 37 °C using these activation parameters ($k_h = 10^{4\pm1}$ s^{-1}), electrochemically reduced cobinamide (MeCbi)$^{\bullet}$ has a homolysis activation barrier of $\Delta G^{\ddagger}_h = 12 \pm 2$ kcal mol^{-1} at 37 °C. For comparison, the methylcobalamin activation parameters measured as part of the present work indicate a homolysis activation barrier of $\Delta G^{\ddagger}_h = 34 \pm 3$ kcal mol^{-1} at 37 °C. Thus, one antibonding electron added to MeB_{12}'s Co–C bond weakens it by *more than* 21 ± 4 kcal mol^{-1} (>60%) (more than this amount since cobinamides have stronger Co–C bonds than cobalamins).[24,76] Table III compares the Co–C bond-homolysis rate constants of cobalt(III) alkylcobamides at different temperatures with those available[37,67b] for electrochemically reduced cobalt(II) alkylcobamide radical anions.

As can be seen from Table III, all of the reasonable comparisons of reduced and unreduced alkylcobamides show a rate enhancement upon reduction which is of the order (>10^{15}) found for MeCbl. Furthermore, the comparison of MeCbl and MeCbi$^+$ is valid here,[24,35] in the context of the total enhancement of >10^{15}, as it introduces relatively small errors of ca. <10^2.

A comparison[35] of activation parameters now available for $(\sigma)^2$ MeCbl and reduced $(\sigma)^2(\sigma^*)^1$ (MeCbi)$^{\bullet}$ (Table III) reveals that *an antibonding electron lowers the Co–C bond strength by more than half*, from 37 kcal mol^{-1} down[77,78] to ca. 12 kcal mol^{-1}. This finding, the first of its type for a solution-phase organometallic system, is consistent with the literature available on "half-bond strengths" of first- and second-row elements.[79–82]

Consideration of an Electron-Transfer Mechanism for Enzyme Activation of MeCbl or AdoCbl. While the free energy barrier for electrochemically reduced (MeCbi)$^{\bullet}$ homolysis is $\Delta G^{\ddagger}_{h,37C}$ = 12 kcal mol^{-1}, Richards' rate data[83] for the AdoCbl-dependent *enzyme* diol dehydratase yields a $\Delta G^{\ddagger}_{h,enz} \leq 14.5$ kcal mol^{-1}. That is, the Co–C homolysis activation barrier (and resultant rate enhancement) due to electrochemical reduction of either MeCbi$^+$ or[4] AdoCbl is lower (i.e. yields a greater rate enhancement)[3] than that due to an AdoCbl-dependent enzyme.[3,24,84]

This kinetic competence of electron-transfer-initiated homolysis *suggests* that an electron-transfer (et) process could conceivably account for the ca. 10^{12} *enzyme-acceleration* of homolytic cleavage of the Co–C bond of Coenzyme-B_{12} (AdoCbl). Furthermore, the notion of electron-transfer catalysis as possibly applied to MeCbl also follows from the recent report[85] of a reducing Fe_4S_4 cluster in a *Me*–corrinoid protein (*Clostridium thermoaceticum*); this cluster has an electrochemical potential previously estimated[86,87] at −0.6 ± 0.2 V and recently measured[88] to be −0.76 V (vs aqueous SCE).

However, we doubt[4] the relevance of electron transfer to either methylcobalamin- or adenosylcobalamin-dependent enzymes, for several reasons. First, and as others have previously noted,[89] such outer-sphere electron transfer to MeCbl is unlikely because of the extremely negative potentials required to reduce adenosylcobalamin (−1.2 to −1.6 V vs SCE),[37,61,62] even allowing for some

(71) (MeCbl) reduction required −1.6 V at −30 °C (vs SCE).[37]

(72) (a) The work[37] of Lexa and Savéant for (MeCbl)$^{\bullet-}$ (at −30 °C) in a 1:1 mix of DMF and 1-propanol was also selected (e.g. instead of Birke's studies in an aqueous medium[67]) since (i) the average dielectric constant of DMF:1-propanol (ϵ_{av} = 29) is closer to that of ethylene glycol (ϵ = 38) than is H_2O (ϵ = 78) and (ii), unlike H_2O, alcoholic solvents contain *both* hydrogen-bonding and non-bonding groups, as do proteins. (b) Kim and Birke[67] in effect note the possibility of differential solvation effects upon homolysis; these may not be negligible in their pH 12 high-dielectric high-ionic-strength aqueous solvent.[73]

(73) The (MeCbl)$^{\bullet-}$ Co–C cleavage rate constants in different solvent systems are composites that include differential solvation and probably cage effects.

(74) Caution should be used in MeCbl extrapolations down to −30 °C (150–175 deg differences), as activation parameters generally are found to be "constant" over only a small range (100 K).[75]

(75) Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley: New York, 1976; p 10.

(76) This is presumably due to a combination of energy-level changes in the ground state and the transition state.

(77) The Co–C homolysis enthalpy barrier is $\Delta H^{\ddagger}_h$ = 18.9 kcal mol^{-1} for (MeCbi$^+$)$^{\bullet-}$. The MeCbl case is approximated by subtracting 4.5 kcal mol^{-1} for the axial base contribution,[24] and the bond dissociation enthalpy is then obtained by subtracting $\Delta H^{\ddagger}_{\eta}$ (<2.3 kcal mol^{-1}, assuming $F_c \leq 1$),[51] which implies an *estimated* BDE for (MeCbl)$^{\bullet-}$ of 12 kcal mol^{-1}.

(78) Correcting for the difference in homolysis barriers for the presence of the axial base (based on AdoCbi$^+$ – AdoCbl)[24] gives $\Delta\Delta H^{\ddagger}_{h,base}$ = 4.5 kcal mol^{-1} and $\Delta\Delta S^{\ddagger}_{h,base}$ = 5 cal mol^{-1} K^{-1}. Thus, the approximate activation parameters for reduced (MeCbl)$^{\bullet-}$ homolysis should be given by $\Delta\Delta H^{\ddagger}_h$ = 18.9 − 4.5 = 14.4 kcal mol^{-1} and $\Delta\Delta S^{\ddagger}_h$ = 21 − 5 = 16 cal mol^{-1} K^{-1}.

(79) This bond weakening in a three-electron σ system is consistent with Baird's view[80] on overlap integrals, taking into account the large ionization-potential difference between CH_3 and Co(III). It is also consistent with calculations for reduction of IBr (from 42 down to 23 kcal mol^{-1}) and the weakness of the $H_3N{-}{-}{-}CH_3^{\bullet}$ three-electron system.[81]

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unknown enzyme effects on these potentials.[90] In principle, the electron-transfer process could explain the rapidity of cobalamin-dependent enzyme processes if one could find a biological electron source at a sufficiently reducing potential. However, in actual practice the natural ferredoxin proteins seem to be incapable of this. Second, no ferredoxin or other biological cofactor that could serve as a reductant[91,92] has been observed in *Coenzyme*-B_{12}-dependent rearrangement enzymes. Third, the $Co^{I}B_{12s}$ product predicted[93] by eq 5 is not observed in the holoenzyme; instead, $Co^{II}B_{12r}$ is observed[84,94] (leading one to have to further postulate biological electron-transfer *catalysis*[93]). Finally, the *coincidental* numerical equality of *Ado*Cbl homolysis rate enhancements ($10^{12\pm2}$; Table III) from the enzyme and from electrochemical reduction of enzyme-free AdoCbl actually is strong evidence *against* an electron-transfer mechanism. Any putative enzymic electron-transfer[8a] mechanism would require a rate enhancement for electrochemical-reduction homolysis *much greater* than that observed (10^{12}–10^{14}),[95,96] in order to compensate for the endoergic[95] electron-transfer prior equilibrium (required in the enzyme to overcome the reduction-potential gap).

Currently, the most satisfactory explanation of the enzymic Co–C activation mechanism[24] for AdoCbl is not electron transfer but rather involves using the intrinsic binding energy (of the apoenzyme for the B_{12} cofactor and the substrate) to distort and weaken the Co–C bond. The weight of the present evidence is that $(AdoCbl)^{\bullet-}$ and[97] $(MeCbl)^{\bullet-}$ species have no biological relevance. However, similar d^7 electronic configurations in other metal alkyl systems could make them extremely labile, notably any isoelectronic d^7 Ni^{III}–alkyl systems related to co-factor F_{430}.[6]

Conclusions

In conclusion, the following points result from this work. MeCbl thermolysis in ethylene glycol with TEMPO cleanly and quantitatively forms $Co^{II}B_{12r}$ and TEMPO-Me, consistent with and fully supportive of Co–CH_3 homolysis. The rate of MeCbl homolysis was established to be first order in MeCbl and independent of [TEMPO], indicating the reaction is not trap-induced. An estimate of the axial-base equilibrium thermodynamic parameters [$\Delta H^\circ = -5.1$ (±2) kcal mol^{-1} and $\Delta S^\circ = -10.5$ (±4) cal mol^{-1} K^{-1}] permits rate constants to be corrected to reflect homolysis from the active base-on form. These corrected rate constants give Co–C homolysis activation parameters of $\Delta H^{\ddagger}_{h,on} = 41 \pm 3$ kcal mol^{-1} and $\Delta S^{\ddagger}_{h,on} = 24 \pm 6$ cal mol^{-1} K^{-1} for MeCbl.

Subtracting an appropriate correction for the barrier to recombination (and assuming[31] the cage efficiency factor F_c is near unity,[51] and that differential solvation effects of the radical products are negligible) yielded an approximate bond dissociation enthalpy of 37 ± 3 kcal mol^{-1}. This is the strongest C–Co cobamide bond measured, larger than any known solution-phase Co–C bond enthalpy.

The antibonding effect of an extra electron in the σ^* LUMO along the C–Co bond greatly facilitates $CH_3^\bullet$ dissociation from MeCbl and promotes the key Co–C homolysis rate by a factor ($>10^{15}$) which is more than enzymes do for AdoCbl. However, the evidence points to the *ir*relevance of electron transfer for adenosyl- and methylcobalamin-dependent enzymes.

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Registry No. MeCbl, 13422-55-4; $Co^{II}B_{12r}$, 14463-33-3; TEMPO, 2564-83-2; TEMPO-Me, 34672-84-9; $MeCbl{\cdot}H^+$, 63911-11-5; methylcobinamide, 20313-07-9.

Supplementary Material Available: Additional background and discussion of the axial-base equilibria, including the "tuck-in" form and temperature-dependent absorptivities of alkylcobalamins (including detailed equations and discussions of the axial-base equilibria), experimental details, results, and discussion on temperature dependence of visible spectra of cobalamins, a listing of axial-base equilibrium constants and cobamide proportions at various temperatures, and two figures showing the Eyring plot and $MeCbi^+$, $MeCbl{\cdot}H^+$, and MeCbl visible spectra at various temperatures (14 pages). Ordering information is given on any current masthead page.

(90) An alternative possibility seems extremely unlikely: that protein-binding or other interactions could modify the energy of the σ^* orbital of the Co–CH_3 bond of MeCbl (by $\sim$0.7 V) enough to change the potential to that required for electron-transfer reduction of MeCbl within the holoenzyme.

(91) Reductants such as thioredoxin[92] are not involved in the B_{12}-dependent enzymic rearrangement reactions.

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(93) (a) Postulation of electron-transfer *catalysis*[8a] would predict the observed $Co^{II}B_{12r}$ final product. (b) To our knowledge such an (nonphotochemical)[93c] electron-transfer-*catalyzed* σ-bond cleavage is without precedent in biology. Here, this would require preferential protein binding of $Co^{II}B_{12r}$ (vs protein·$Co^{I}B_{12s}$) to provide the driving energy for protein·$Co^{I}B_{12s}$ oxidation (presumably through a protein conformational change). (c) Ono, N.; Tamura, R.; Kaji, A. *J. Am. Chem. Soc.* **1980**, *102*, 2851–2852.

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(96) A somewhat different way to express this argument is as follows. *If* an electron transfer prior equilibrium were operative, *then* $k_{obsd} = K_{eq}$(e.t.)·k_h(e.t.) $\geq 10^2$ s^{-1} (where 10^2 s^{-1} is the turnover rate for the enzyme diol dehydratase). A $\geq$0.5 V uphill electron transfer corresponds to a K_{eq}(e.t.) $\leq 10^{-9}$ which in turn leads to the physically unlikely (if not impossible) situation of k_h(e.t.) $\geq 10^{11}$ s^{-1}, a Co–C cleavage rate near (if not above) the vibrational time scale. The observed homolysis rate enhancement in such a situation for $MeCbl^{\bullet-}$ vs MeCbl would then be k_h(e.t.)/$k_h \geq 10^{11}/10^{-12}$ or 10^{23}, a value much greater than the observed 10^{12}–10^{14}.

(97) Ragsdale has concluded that heterolytic nucleophilic displacement is more likely to be relevant to at least one MeCbl-dependent enzyme than is electron transfer.[88]