Communications to the Editor

Co–C Homolysis and Bond Dissociation Energy Studies of Biological Alkylcobalamins: Methylcobalamin, Including a $\geq 10^{15}$ Co–CH_3 Homolysis Rate Enhancement at 25 °C following One-Electron Reduction

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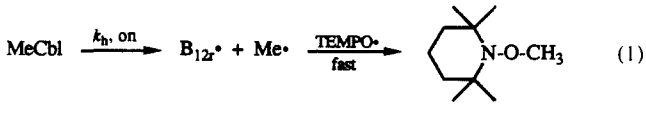
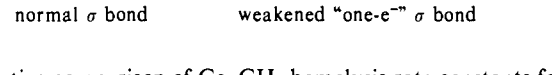
In seeking to extend knowledge[1-5] of the bond dissociation enthalpy (BDE) values of biologically important[6] alkylcobalamins,[7,8] we have determined the activation parameters for homolysis of the Co–C bond of methylcobalamin (MeCbl[9] or MeB_{12}), eq 1. These parameters and the Co–Me BDE accrue additional fundamental significance since they allow the first direct comparison of normal strength vs "half-strength" M–C σ bonds (Chart I). Note that MeCbl is nearly ideal for such a comparison, the MeCbl LUMO[10-12] being antibonding with respect to the key[13] Co–C bond, and the approximately square planar corrin system minimizing any other structural distortions following one-electron reduction.



(1)

Electrochemical reduction of MeCbl has been used to populate the Co––CH_3 σ^* orbital,[14-16] thereby generating the half-strength Co––CH_3 bond, which rapidly dissociates (eq 2). However, a

Chart I



quantitative comparison of Co–CH_3 homolysis rate constants for MeCbl (Co^{III}) vs $(MeCbl)^{\bullet-}$ (Co^{II})[16] was previously impossible due to the lack of MeCbl Co–C homolysis activation parameters. The rate enhancement of $>10^{15}$ at 25 °C which we now report quantifies the predictable[10,17-19] effect of a fundamental chemical process: partial bond breaking.

$$\text{MeCbl} + e^- \rightarrow (\text{MeCbl})^{\bullet-} \rightleftarrows (\text{Co}^{\text{I}}\text{B}_{12s})^- + \text{Me}^\bullet \xrightarrow{\text{trap}} \text{Me–Trap} \quad (2)$$

Upon thermolysis of pure MeCbl[20-22] (0.08–0.15 mM in ethylene glycol) with 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO•, 6.7–43 mM) as a CH_3• radical trap, the expected[1,23] homolysis products were produced quantitatively,[24] as shown by comparison to authentic[25] $Co^{II}B_{12r}$ and the authentic trapped alkyl TEMPO–Me[9,26] (eq 1).[8a] Standard[1,27] kinetic methods showed[28-30] the reaction to be first order in [MeCbl] and zero order in [TEMPO•]. The observed rate constants[31] were corrected[8a,32] for the ca. 20–30% of MeCbl lacking axial-benzimidazole-base coordination[33] to cobalt at these temperatures.[34] An Eyring plot gave[1,27] base-on 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} deg^{-1}.

The appropriate[2] cage chemistry correction[35,36] yields a Co–CH_3

(1) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469-1481 and references therein. 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.
(2) (a) Koenig, T. W.; Finke, R. G. *J. Am. Chem. Soc.* **1988**, *110*, 2657. (b) Koenig, T. W.; Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1499 and references therein.
(3) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1987**, *109*, 8012 and references therein.
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(5) Hay, B. P. Ph.D. Dissertation, University of Oregon, 1986.
(6) Wood, J. M. Mechanisms for B_{12}-Dependent Methyl Transfer. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 2, p 160.
(7) The only known Co–C bonds in Nature are those of MeCbl and AdoCbl; in addition to methylcobalamin[8a] and 5′-deoxy-5′-adenosylcobalamin (AdoCbl),[1,5] we have also studied in detail the thermolysis of neopentylcobalamin.[8b]
(8) (a) Martin, B. D.; Finke, R. G., manuscript in preparation. (b) Waddington, M. D.; Finke, R. G., unpublished results.
(9) Standard B_{12} nomemclature and abbreviations are used herein; see: Cohn, W. E. Nomenclature. In *B_{12}*; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, pp 17-22.
(10) 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.
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(13) In the case of AdoCbl, homolysis of the Co–C bond is the only—but key—role identified to date for this cofactor. Wang, Y.; Finke, R. G. *Inorg. Chem.* **1989**, *28*, 983-986 and references therein.
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(15) Rubinson, K. A.; Itabashi, E.; Mark, H. B., Jr. *Inorg. Chem.* **1982**, *21*, 3571-3573.
(16) (a) Lexa, D.; Savéant, J.-M. *J. Am. Chem. Soc.* **1978**, *100*, 3220. (b) Lexa, D.; Savéant, J.-M. *Acc. Chem. Res.* **1983**, *16*, 235-243.
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(19) Zhu, L.; Kostič, N. M. *Inorg. Chem.* **1987**, *26*, 4194-4197.
(20) MeCbl was synthesized by $NaBH_4$ reduction[21] and isolated (by Brown's chromatographic methods)[22] to >99% purity (by HPLC, ^{1}H NMR, and visible spectroscopy).
(21) Dolphin, D. *Methods Enzymol.* **1971**, *18*, 45.
(22) Brown, K. L.; Peck, S. Organocobalt Corrins. In *Organometallic Synthesis*; King, R. B., Eisch, J. J., Eds.; Vol. 4, in press.
(23) (a) Pyrolysis of *solid* MeCbl gives the methyl-radical products methane and ethane in roughly equal amounts: (b) Schrauzer, G. N.; Sibert, J. W.; Windgassen, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 6681-6688.
(24) At 142.5 °C, the GC yield of TEMPO-Me is 104 ± 19%; as expected,[8a] the visible spectroscopic yield of $Co^{II}B_{12r}$ is only[29] 82 ± 10%.
(25) Blaser, H.-U.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 1684.
(26) Smith, B. L. Ph.D. Dissertation, University of Oregon, 1982, p 167.
(27) Halpern, J. *Polyhedron* **1988**, *7*, 1483-1490 and references therein.
(28) (a) Loss of MeCbl and formation of B_{12r} were both monitored spectrophotometrically. The rate law is $-d(\ln [\text{MeCbl}])/dt = d(\ln [B_{12r}])/dt = k_{obsd}$. (b) Infinity absorbances were calculated from photolysis A_0/A_∞ ratios as before[1] and confirmed by two-parameter exponential fitting[30] of the data used ($<1.5t_{1/2}$), which showed little initial B_{12r} decomposition.
(29) The independently demonstrated high-temperature decomposition of a product (B_{12r}), along with the effect of the tuck-in form on the axial-base equilibrium determination, made this study[8a] the most difficult one of ours.[1-4]
(30) Gerhards, R.; Dietrich, W. *J. Magn. Reson.* **1976**, *23*, 21-29.
(31) (Average observed rate constants) × 10^4 (±8%; units s^{-1}) and temperatures (±0.2 °C) are as follows: 0.18 (120.1), 0.64 (129.9), 1.08 (135.0), 2.64 (140.9).
(32) (a) K_{measd} = [N-base-on MeCbl]/$\sum$[N-base-not-on MeCbl species]. (b) $k_{h,on} = k_{obsd}(K_{measd} + 1)/K_{measd}$ (assuming[40] $k_{h,on} \gg k_{h,off}$).[8a]
(33) Brown, K. L.; Peck-Siler, S. *Inorg. Chem.* **1988**, *27*, 3549.
(34) (a) In neutral ethylene glycol at zero ionic strength, density-corrected 538-nm absorbance data for MeCbl (from 5-99 °C) indicate[32a] axial base-coordination-equilibrium parameters of $\Delta H° = -5.1 \pm 0.7$ kcal mol^{-1} (maximum = -4.6) and $\Delta S° = -10.5 \pm 3.5$ eu (maximum = -8.4). (b) These parameters are within 2σ of Brown's[33] "complete scheme" ^{13}C NMR values *in* D_2O (I = 0): $\Delta H° = -6.5$ kcal mol^{-1} and $\Delta S° = -14.0$ eu. Either set of equilibrium parameters gives the same activation parameters.[8a] (c) The base-hydrogen-bonded tuck-in form[33] is chemically similar to the base-off form, but its visible spectrum differs significantly, as discussed elsewhere.[8a]

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bond dissociation enthalpy (BDE) estimate of 37 ± 3 kcal/mol. This is the highest[3,37] Co–C BDE yet measured,[38] slightly above Toscano's 33 ± 2 kcal/mol BDE for Me–Co(DH)$_2$py in bromoform.[39]

The activation parameters allow computation of a MeCbl Co–CH_3 homolysis rate constant at −30 °C of $k_{h,on} = 10^{-19\pm4}$ s^{-1}. This is the highest temperature at which the rate for (MeCbl)$^{\bullet-}$ homolysis is sufficiently slow[40] to be measurable electrochemically (rate constant = 1200 s^{-1} at −30 °C in DMF/1-propanol).[16] Comparing these two rate constants quantifies the *$10^{22\pm4}$ homolysis rate enhancement at −30 °C* due to the extra, Co−−CH_3 antibonding electron in (MeCbl)$^{\bullet-}$.

Informative rate comparisons at higher temperatures can be made if one compares MeCbl to methylcobinamide,[9] MeCbi$^+$ (the benzimidazole-base-free form of MeCbl; the lack of the axial base in MeCbi$^+$/MeCbi$^\bullet$ slows the Co–C cleavage rates enough to make them measurable electrochemically at 25 °C). Rigorously, the MeCbi$^+$/MeCbi$^\bullet$ electrochemical data[16] serve as a *lower limit*[40] to the rates for MeCbl$^{\bullet-}$ Co–CH_3 cleavage at other temperatures. That is, the rate enhancements that follow are *lower limits* to the true values. (If desired, the Co–C cleavage rates from MeCbi$^\bullet$ and MeCbl$^{\bullet-}$ can be taken as equivalent[40] within the estimated $\pm10^{2-3}$ error bars, and given the large rate enhancements observed.)

The electrochemically derived,[16] temperature-dependent MeCbi$^\bullet$ Co–CH_3 homolysis rates, k_h,[41] provide the activation parameters $\Delta H^\ddagger = 19$ (±1) kcal/mol and $\Delta S^\ddagger = 21$ (±3) eu. Hence at 25 °C the MeCbi$^\bullet$ k_h is 4400 s^{-1}, which, compared to our MeCbl $k_{h,on} = 10^{-12\pm3}$ s^{-1}, demonstrates a *rate enhancement of $>10^{15\pm3}$ at 25 °C*. The rate enhancement is still $>10^{13}$ or $>10^{11}$ at even 90 or 135 °C, respectively.

Comparing activation parameters for reduced $(\sigma)^2(\sigma^*)^1$ MeCbi$^\bullet$ and $(\sigma)^2$ MeCbl suggests that *an antibonding electron lowers the Co–C bond strength by more than half* (i.e., from 37 kcal mol^{-1} down to approximately[42-44] 12 kcal mol^{-1}). The effect of the M−−C antibonding electron—the first such measurement for any M–C/M–C$^{\bullet-}$ pair—is impressive.[42]

It is of interest to consider the possible biological relevance of this mechanism for greatly enhancing M–C cleavage. Extremely labile M–alkyls are hereby predicted for systems isoelectronic to d^7 Co(II)−−CH_3, notably any d^7 Ni(III)–alkyls related to cofactor F_{430}.[45] On the other hand, rather stable Co–methyl bonds (BDE = 37 kcal/mol) that *are not reducible by biological reductants*[46] are the apparent rule for d^6 Co–CH_3 corrinoids. This latter statement is supported by the work of Ragsdale and co-workers, who have recently tested for, but found no evidence of, reductive cleavage of a d^6 Co(III)–CH_3 bond in the corrinoid/4Fe-4S-containing protein which serves as the methyl carrier protein in the acetyl-CoA pathway of *Clostridium thermoaceticum*.[47] Perhaps it is the enormous stability difference between a d^7 Ni(III)–CH_3 and a d^6 Co(III)–CH_3 that Nature is exploiting.

Consistent with the above, the mechanism responsible for the observed enzymatic rate enhancement[1] of Co–C homolysis in AdoCbl probably does *not* involve (AdoCbl)$^{\bullet-}$.[6,46] Our reasoning behind this statement, and a parallel analysis of the rate enhancement following AdoCbl reduction, is presented elsewhere.[46]

Acknowledgment. We thank Prof. Stephen Ragsdale for a preprint,[47] Prof. Kenneth L. Brown for giving us his corrin purification procedures[22] prior to publication and for a gift of MeCbi$^+$, and Prof. Thomas W. Koenig at Oregon for helpful discussions. Financial support was provided by the NIH (Grant DK-26214).

Registry No. MeCbl, 13422-55-4; MeCbl$^{\bullet-}$, 67087-21-2; Co$^{II}B_{12r}$, 14463-33-3; TEMPO, 2564-83-2; TEMPO-Me, 34672-84-9.

(35) (a) An efficient cage ($F_c \simeq 1$) and BDE $\simeq \Delta H^\ddagger_{obsd}$(soln) − $F_c\Delta H^\ddagger_\eta$ are assumed.[2] (b) For this 0.96-1.5 cP viscosity[36] solvent (at 120–150 °C), the cage-escape diffusion barrier is approximated as $\Delta H^\ddagger_\eta$ (4.0 kcal mol^{-1}; calculated via the Frenkel form[35c] of Guzman's "Andrade" equation[2]). (c) Frenkel, J. *Nature (London)* **1930**, *125*, 581-582.

(36) Thomas, L. H.; Meatyard, R.; Smith, H.; Davis, G. H. *J. Chem. Eng. Data* **1979**, *24*, 161-164.

(37) The second highest Co–C BDE, that for AdoCbi$^+$,[3] is 34.5 ± 1.8 kcal mol^{-1}.

(38) (a) A previous estimate placed the MeCbl BDE at 46 ± 3 kcal mol^{-1}, based upon photohomolysis threshold energies and estimating $\Delta H^\ddagger_r$ as 2 kcal mol^{-1}. (b) Endicott, J. F.; Balakrishnan, K. P.; Wong, C.-L. *J. Am. Chem. Soc.* **1980**, *102*, 5519-5526.

(39) Toscano, P. J.; Seligson, A. L.; Curran, M. T.; Skrobutt, A. T.; Sonnenberger, D. C. *Inorg. Chem.* **1989**, *28*, 166-168.

(40) (a) Axial-base-on alkylcobalamins undergo Co–C homolysis faster than the corresponding base-off alkylcobalamins or benzimidazole-base-free alkylcobinamides.[1,3,4,40b] For example, AdoCbl[4,5] homolyzes 10^2 times faster than[3] AdoCbi$^+$ at room temperature. Furthermore, MeCbl$^{\bullet-}$ homolyzes 440 times faster than MeCbi$^\bullet$ at −30 °C (1200 s^{-1} and 2.7 s^{-1}, respectively).[16] (b) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 541-546.

(41) (a) The Co–CH_3 cleavage mechanism we expect for reduced alkylcorrins differs from that presented in the electrochemical literature[14-16] by incorporating *reversible* Co(II)−−CH_3 cleavage[41b] followed by $CH_3^\bullet$ trapping, Me[Co(II)corrin]$^{\bullet-}$ ⇌ Co(I)$^-$ + $CH_3^\bullet$, then $CH_3^\bullet$ + trap → CH_3-trap, k_{obsd} = $k_{h,apparent}$ = a composite (with the reverse of the first step probably favored by the preferred, base-off form[41c] of Co(I)). Fortunately, the solvent mixture DMF/1-propanol is apparently serving as a trap (a H$^\bullet$ source as previously noted),[16] thereby preventing Co(I) + Me$^\bullet$ recombination (and thus $k_{h,apparent} \approx k_{h,true} = k_h$ in DMF/1-propanol, but not in H_2O[14]). This mechanism, the evidence for it, and its implications will be discussed in a full paper.[8a] (b) The trapping of a R$^\bullet$ by a diamagnetic metal has precedent: 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; pp 314-315. Finke, R. G.; Keenan, S. R.; Watson, P. L. *Organometallics* **1989**, *8*, 263-277, especially p 269 and footnote 26. (c) Lexa, D.; Savéant, J.-M. *J. Am. Chem. Soc.* **1976**, *98*, 2652.

(42) Radical-cage effects,[2] although undoubtedly present in $k_{h,on}$ (F_c assumed $\simeq 1$)[35] and possibly $k_{h,apparent}$ (F_c assumed[43] $\simeq 1$, but arguably as small as $\simeq 0$),[41a,43] will not influence the conclusions in this paper (that are based on $>10^{15}$ rate differences) and are most likely negligible compared to the indicated $\pm10^{2-3}$ error bars.

(43) $\Delta H^\ddagger_h = 18.9$ kcal mol^{-1} for (MeCbi$^+$)$^{\bullet-}$ homolysis. Subtracting both 4.5 kcal mol^{-1} for the axial-base contribution[3,4] and $\Delta H^\ddagger_\eta$ (<2.3 kcal mol^{-1}; i.e., assuming $F_c \simeq 1$)[35] yields an *estimated* BDE for (MeCbl)$^{\bullet-}$ of 12 kcal mol^{-1}.

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(45) Leading references: (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.

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Long-Range Electron Transfer in Ruthenium-Modified Cytochrome *c*: Evaluation of Porphyrin–Ruthenium Electronic Couplings in the *Candida krusei* and Horse Heart Proteins

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Experiments in several laboratories have shown that electron transfer (ET) can take place at appreciable rates over long distances (>10 Å) in organic and inorganic molecules[1-6] and in

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(2) Closs, G. L.; Miller, J. R. *Science (Washington, D.C.)* **1988**, *240*, 440-447.

(3) (a) Oliver, A. M.; Craig, D. C.; Paddon-Row, M. N.; Kroon, J.; Verhoeven, J. W. *Chem. Phys. Lett.* **1988**, *150*, 366-373. (b) Oevering, H.; Paddon-Row, M. N.; Heppener, M.; Oliver, A. M.; Cotsaris, E.; Verhoeven, J. W.; Hush, N. S. *J. Am. Chem. Soc.* **1987**, *109*, 3258-3269.

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