Thermolysis of the Co–C Bond of Adenosylcobalamin. 2. Products, Kinetics, and Co–C Bond Dissociation Energy in Aqueous Solution

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Abstract: The reaction product, kinetic, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and Co–C bond dissociation energies (BDEs) are reported for the anaerobic, thermal decomposition of adenosylcobalamin ($AdoB_{12}$) in aqueous solution. These studies reveal that the reaction proceeds via two competing pathways, heterolytic Co–C bond cleavage to yield aquocobalamin, adenine, and a sugar residue and competing Co–C bond homolysis to give $Co^{II}B_{12}$ and 8,5′-anhydroadenosine. At pH 4.0, heterolysis is the major mode (88% at 85 °C) of decomposition, while at pH 7.0 homolysis dominates (90% at 85 °C). The temperature dependence of the rate of $AdoB_{12}$ Co–C bond homolysis in neutral H_2O was obtained from 85.0 to 110.0 °C, yielding $\Delta H^{\ddagger}_h = 31.8 \pm 0.7$ kcal/mol and $\Delta S^{\ddagger}_h = 6.8 \pm 1.0$ eu. These values are significantly different from previously reported aqueous (pH 4.3) values of $\Delta H^{\ddagger}_h = 26.3 \pm 0.6$ kcal/mol and $\Delta S^{\ddagger}_h = -6 \pm 2$ eu. The temperature dependence of the axial base equilibrium of $AdoB_{12}$ in neutral water was measured, yielding $\Delta H = -5.6 \pm 0.9$ kcal/mol and $\Delta S = -13 \pm 3$ eu. Combining the above results with other data yields an estimate of 30 ± 2 kcal/mol for the base-on Co–C BDE of $AdoB_{12}$ in water, in good agreement with our previously reported value of 31.5 ± 1.3 kcal/mol obtained in ethylene glycol.

Introduction

The Co–C bond in coenzyme B_{12} or adenosylcobalamin ($AdoB_{12}$) is unique among known natural products. Reversible homolysis of this bond is now thought to be the essential and probably the only role of the $AdoB_{12}$ cofactor,[1] but precedent for this initial homolysis step in vitro did not appear until the requisite radical trapping techniques were first developed by Halpern and co-workers,[2a,c] Schrauzer and Grate,[2b] Espenson et al.,[2d] and us.[3] In 1983 we reported the development of a nitroxide radical trapping technique,[3] the use of this method in obtaining reliable $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and Co–C bond dissociation energies (BDEs) in a close B_{12} mimic,[4] and preliminary evidence that the nitroxide technique also worked for B_{12} alkyls, specifically benzylcobalamine.[3,5] The nitroxide technique was the best trap to emerge from a several-year study[6] of traps such as $HMn(CO)_5$, *n*-BuSH, and O_2 and a consideration of traps used by others.[2] Ideally, one would like a trap, solvent, and other conditions where reversible homolysis can be demonstrated via an inverse Co(II) dependence *and* where a limiting rate with excess trap is observed, thereby demonstrating the absence of bimolecular, trap-induced reactions. As this manuscript will document, *an added important condition is the demonstration, when a rate-limiting excess of trap is used, of products that are consistent with homolysis.*

In early 1984 we reported the first product and kinetic study of the thermolysis of the Co–C bond in $AdoB_{12}$ using the nitroxide radical trapping technique in ethylene glycol.[7] The results of this study are summarized in Scheme I.[8] The anaerobic thermolysis of $AdoB_{12}$ quantitatively yields B_{12r} ($Co^{II}B_{12}$) with or without added 2,2,6,6-tetramethylpiperidinooxy (TEMPO). In the absence of TEMPO, two nucleoside products, 5′-deoxyadenosine (D-Ado) and 8,5′-anhydroadenosine (C-Ado for *cyclo*-Ado), were isolated and fully characterized (see Scheme I). In the presence of excess TEMPO, it was shown that the 5′-deoxy-5′-adenosyl radical (5′-Ado radical) was the common intermediate for both D-Ado and C-Ado. The stable and isolable, trapped 5′-Ado radical (T-Ado, Scheme I) was the only nucleoside product formed at high TEMPO concentration. By obtaining ±3% precision data in over 50 kinetic runs, the reaction was shown to be retarded 8% by the addition of $Co^{II}B_{12}$ and accelerated a maximum of 7% by the addition of TEMPO. These unusually small $Co^{II}B_{12}$ and TEMPO trap dependencies were shown to be due largely to the facile, cyclization "self-trapping" of the 5′-Ado radical to yield C-Ado (Scheme I). In accord with the rate law, eq 1 in Scheme I, a plot of $1/k_{obsd}$ vs. $[Co^{II}B_{12}]$ was linear and the observed rate constant reached the limiting value of k_h at $\geq 5 \times 10^{-3}$ M (50 equiv) TEMPO. Under these conditions of rate-determining free-radical formation, values of k_h were measured over a 30 °C range (90–120 °C) to yield $\Delta H^{\ddagger}_h = 30.6 \pm 0.3$ kcal/mol and $\Delta S^{\ddagger}_h = 2.9 \pm 0.7$ eu. From these and other results, the first *estimate* of the base-on $AdoB_{12}$ Co–C bond dissociation energy (BDE) of 31.5 ± 1.3 kcal/mol was obtained. Perhaps the most significant

(1) This is perhaps the key finding, one where the Oregon and Chicago labs agree,[1e,13a] inspite of the differences and details discussed herein. (a) Finke, R. G.; McKenna, W. P.; Schiraldi, D. A.; Smith, B. L.; Pierpont, C. *J. Am. Chem. Soc.* **1983**, *105*, 7592. (b) Finke, R. G.; Schiraldi, D. A. *J. Am. Chem. Soc.* **1983**, *105*, 7605. (c) Finke, R. G.; Schiraldi, D. A.; Mayer, B. J. *Coord. Chem. Rev.* **1984**, *54*, 1. (d) Halpern, J. *Science (Washington, D.C.)* **1985**, *227*, 869. (e) Wollowitz, S.; Halpern, J.; *J. Am. Chem. Soc.* **1984**, *106*, 8319.

(2) Scavengers used in B_{12} model studies[2a,c,d] and with B_{12} alkyls[2b] include R· reaction with Co(II),[2a] O_2,[2b] *n*-$C_8H_{17}SH$,[2c] and H_2O_2.[2d] (a) Halpern, J.; Ng, F. T. T.; Rempel, G. L. *J. Am. Chem. Soc.* **1979**, *101*, 7124. (b) Schrauzer, G. N.; Grate, J. H. *J. Am. Chem. Soc.* **1981**, *103*, 54. (c) Tsou, T. T.; Loots, M.; Halpern, J. *J. Am. Chem. Soc.* **1982**, *104*, 6231. (d) Gjerde, H. B.; Espenson, J. H. *Organometallics* **1982**, *1*, 435. See also reference 5.

(3) Finke, R. G.; Smith, B. L.; Mayer, B. J.; Molinero, A. A. *Inorg. Chem.* **1983**, *22*, 3677.

(4) Elliot, C. M.; Hershenhart, E.; Finke, R. G.; Smith, B. L. *J. Am. Chem. Soc.* **1981**, *103*, 5558.

(5) Usage of a nitroxide trap to study $PhCH_2Co(DMG\text{-}BF_2)_2OH_2$[5a] and benzylcobalamin[5b] has since appeared in the literature. (a) Bakac, A.; Espenson, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 5197. (b) Blau, R.; Espenson, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 3530.

(6) Smith, B. L. *Ph.D. Dissertation*, University of Oregon, 1982.

(7) (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3043. Correction published: Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1985**, *24*, 1278. (b) Several typesetting errors and one arithmetic error in this reference require correction. These errors in no way affect the interpretations of the kinetic data and/or the Co–C BDE: (i) The equation for the slope of the B_{12r} plot, designated as *m*, is incorrect twice on page 3042.[7a] The correct equation is found in ref 13c.[7a] (ii) The units of the slope of the $1/k_{obsd}$ vs. $[Co^{II}B_{12}]$ plot are M^{-1} s, not M^{-1}. (iii) The equation in ref 18 should read $d(\ln (k_{obsd}/T))/d(1/T) = d(\ln (k_2/T))/d(1/T) + d(\ln (K_2/(1+K_2))/d(1/T)$. (iv) The arithmetic error is in $\Delta S^{\ddagger}_2$ which should read 14 ± 1 eu, not 23 ± 1 eu. (c) We thank Prof. Jack Norton and his research group, Colorado State University, for bringing several of these errors to our attention.

(8) Due to the additional chemistry uncovered in this study and in the interest of clarity, we have adopted a new rate constant notation from that used in our preliminary communication:[7] the observed rate constant for *homolysis* is k_h (was k_{obsd}(TEMPO)), the rate constant for *base-on homolysis* is k_b(base-on) (was k_2), the rate constant for Co· + R· *recombination* is k_r (was k_{-2}), the rate constant for the *cyclization* of the 5′-Ado radical is k_c (was k_3), the rate constant for H· *abstraction* from ethylene glycol is k_a (was k_4), the rate constant for R· *trapping* by TEMPO is k_T (was k_5), and the equilibrium constant for the base-off to base-on *equilibrium* is K_{eq} (was K_1).

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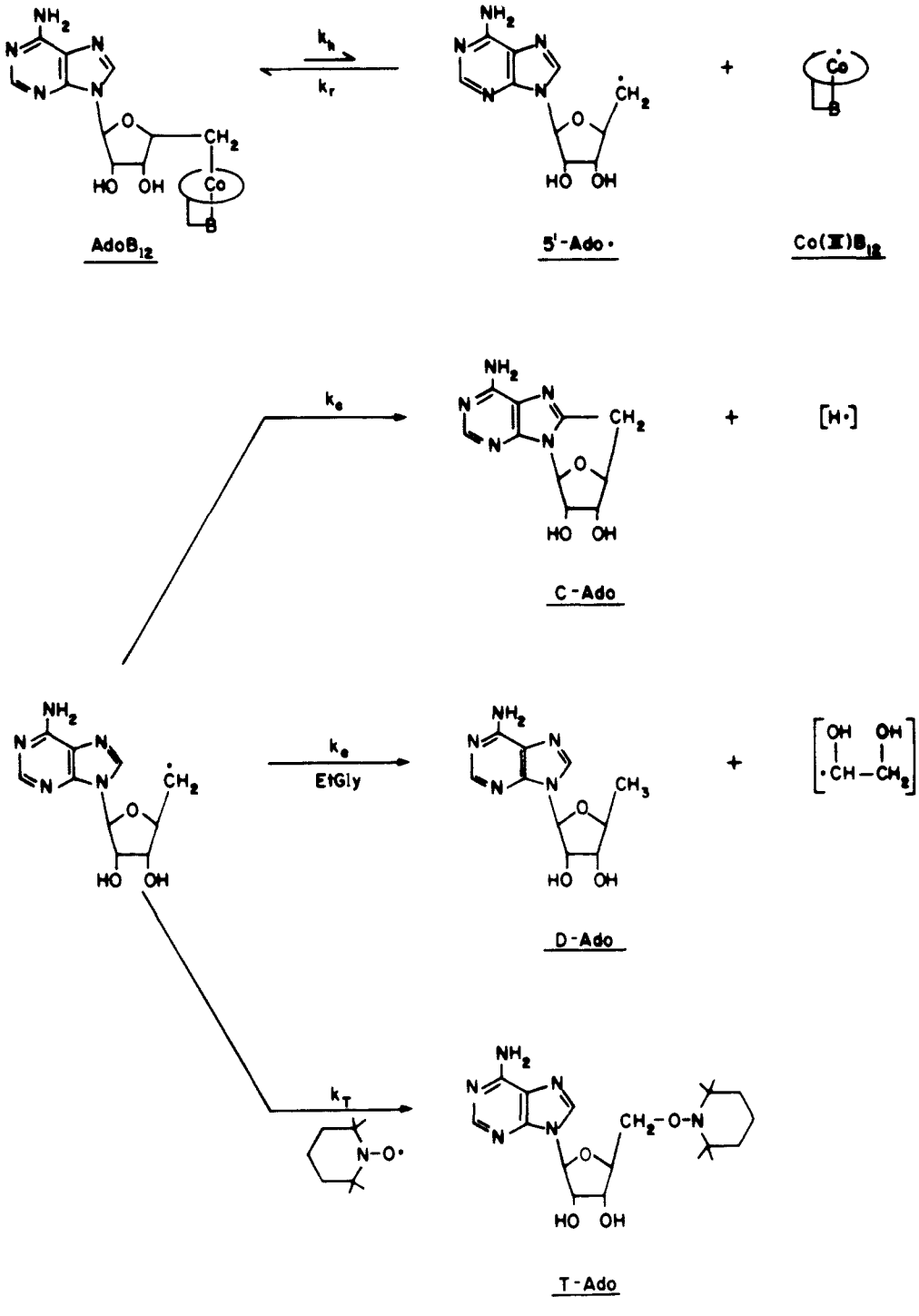
Scheme I



$$\text{Eq. 1} \qquad -\frac{d \ln [AdoB_{12}]}{dt} = k_{obs} = \left(\frac{k_c + k_e[EtGly] + k_T[TEMPO]}{k_c + k_e[EtGly] + k_T[TEMPO] + k_r[Co(II)B_{12}]}\right) \cdot k_h$$

finding was that the rate of $AdoB_{12}$ Co–C bond homolysis is $\geq 10^{10}$ faster in vivo than in vitro.[9] This finding has opened the door for further quantitative studies of axial base, corrin conformation, enzymic, and other factors contributing to this large $\geq 10^{10}$ activation of the Co–C bond of $AdoB_{12}$.

In later 1984, Halpern et al.[10] reported $AdoB_{12}$ thermolysis studies as summarized in Scheme II, using different experimental conditions of solvent (H_2O), pH (4.3), and a different radical

(9) (a) The apparent $\geq 10^{10}$ rate enhancement in vivo vs. in vitro cited earlier[7] is strengthened by the comparison of calculated in vitro rates ($k = 10^{-11}$–10^{-12} s^{-1} at 5 °C and 10^{-9}–10^{-10} s^{-1} at 25 °C) to the observed rates of Co–C bond homolysis in the enzyme systems diol dehydrase ($k \geq 2 \times 10^2$ s^{-1} at 5 °C)[9b] and ethanolamine deaminase ($k = 3 \times 10^2$ s^{-1} at 25 °C).[9c] The possibility of a chain mechanism where the Co–C bond is not remade and rebroken in each turnover is an intriguing possibility worth investigating.[47] (b) Valinsky, J. E.; Abeles, R. H.; Fee, J. A. *J. Am. Chem. Soc.* **1974**, *96*, 4709. (c) Holloway, M. R.; White, H. A.; Joblin, K. N.; Johnson, A. W.; Lappert, M. F.; Wallis, O. C. *Eur. J. Biochem.* **1978**, *82*, 143.

(10) Halpern, J.; Kim, S. H.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317. Correction published: Halpern, J.; Kim, S. H.; Leung, T. W. *J. Am. Chem. Soc.* **1985**, *107*, 2199.

Scheme II

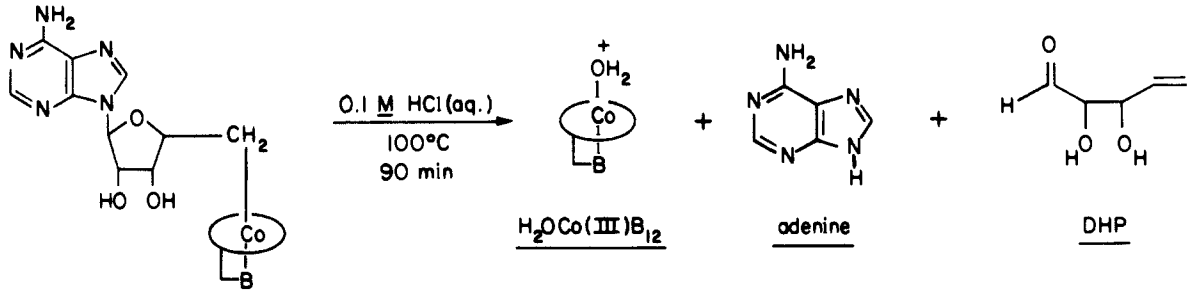
$$\text{AdoB}_{12} \underset{k_r}{\overset{k_h}{\rightleftharpoons}} \text{Co(II)} + 5'\text{-Ado}\bullet$$

$$5'\text{-Ado}\bullet + \bullet\text{Co(DMG)}_2 \underset{}{\overset{k_T}{\rightleftharpoons}} [\text{Ado-Co(DMG)}_2]$$

$$[\text{Ado-Co(DMG)}_2] \longrightarrow ??$$

$$\text{Eq. 2} \quad -\frac{d \ln [\text{AdoB}_{12}]}{dt} = k_{obs} = \left(\frac{k_T [\text{Co(II)(DMG)}_2]}{k_T [\text{Co(II)(DMG)}_2] + k_r [\text{Co(II)B}_{12}]} \right) \cdot k_h$$

Scheme III



scavenger, diaquoCo(II)cobaloxime ($Co^{II}(DMG)_2$ hereafter).[11] The stated basis of the trapping technique was the greater Co–C BDE of $AdoCo(DMG)_2OH_2$ compared to $AdoB_{12}$, but the reported control experiments showed that authentic samples of the putative $AdoCo(DMG)_2OH_2$ intermediate decomposed at a rate ($t_{1/2} \approx 1$ h at 100 °C) approximately twice the maximum rate ($t_{1/2} = 1.9$ h at 100 °C, pH 4.3 H_2O) at which it could have been formed. Thus, the claimed *quantitative* formation of the 5'-Ado radical in the presence of a rate-limiting excess of trap could not be confirmed due to the failure to detect appreciable amounts of $AdoCo(DMG)_2OH_2$. The products of the $AdoCo(DMG)_2OH_2$ decomposition were not reported. In fact, the only reported product was $Co^{II}B_{12}$, formed quantitatively in the presence of $\geq 10^{-3}$ M $Co^{II}(DMG)_2$. Nucleoside products or controls such as the products in the absence of $Co^{II}(DMG)_2$ were not reported. Kinetic studies were reported in accord with the proposed rate law (eq 2, Scheme II),[12] and limiting values of k_{obsd} were determined over a 19 °C temperature range to yield $\Delta H^{\ddagger}_{obsd} = 26.3 \pm 0.6$ kcal/mol, $\Delta S^{\ddagger}_{obsd} = -6 \pm 2$ eu, and a base-on Co–C BDE estimate of 26 ± 2 kcal/mol.

These aqueous activation parameters are significantly different from those obtained in ethylene glycol, $\Delta\Delta H^{\ddagger}_{obsd} = 4.3 \pm 0.7$ kcal/mol and $\Delta\Delta S^{\ddagger}_{obsd} = 9 \pm 2$ eu, with the difference in the activation enthalpies being the primary cause for the discrepancy between the resulting Co–C BDE estimates. These differences became a focal point, with the difference in solvents being cited as a possible cause for the variation in the activation parameters.[13b] This observed variation in activation parameters raises questions concerning the validity of the kinetic approach of obtaining BDEs, possibly suggesting that changes in experimental conditions such as solvent, pH, and radical scavenger might cause significant changes in the temperature dependence of the rate of Co–C bond homolysis and thus the apparent Co–C BDE. Because of this, we felt that an understanding of the factor(s) which gave rise to the differences in activation parameters could be of importance in providing an explanation of the 10^{10} rate acceleration in vivo, prompting us to take a careful look at the thermolysis of $AdoB_{12}$ in aqueous solution.

The acidic (pH 4.3) conditions used previously,[10] claimed as necessary to stabilize the $Co^{II}(DMG)_2$ radical trap, were an immediate concern to us due to literature demonstrating that heterolytic cleavage of the Co–C bond of $AdoB_{12}$ is a dominant reaction under acidic aqueous conditions.[14,15] For example,

(11) The $Co^{II}(DH)_2$ notation used in ref 10 has been previously employed by Halpern and co-workers to designate diaquoCo(II)cobaloxime.[11a,b] The acid-resistant BF_2-capped Co(II)cobaloxime[5a] is specifically not mentioned in ref 10. (a) Schneider, P. W.; Phelan, P. F.; Halpern, J. *J. Am. Chem. Soc.* **1969**, *91*, 77. (b) Halpern, J.; Phelan, P. F. *J. Am. Chem. Soc.* **1972**, *94*, 1881.

(12) The rate of $Co^{II}B_{12}$ formation was retarded by the addition of $Co^{II}B_{12}$ and accelerated by the addition of $Co^{II}(DMG)_2$ (see text). In accord with the proposed rate law, eq 2 in Scheme II, a plot of $1/k_{obsd}$ vs. $[Co^{II}B_{12}]$ was linear and the observed rate constant reached a limiting value at $\geq 10^{-3}$ M $Co^{II}(DMG)_2$. The results presented herein necessitate a reinterpretation of these kinetic results,[17b] which is impossible without a knowledge of the exact experimental conditions employed.[17c]

(13) (a) Stinson, S. C. *Chem. Eng. News* **1985**, *63*(7), 28. (b) See ref 18 in ref 10.

(14) (a) Barker, H. A.; Smyth, R. D.; Weissback, H.; Munch-Peterson, A.; Toohey, J. I.; Ladd, J. N.; Volcani, B. E.; Wilson, R. M., *J. Biol. Chem.* **1960**, *235*, 181. (b) Hogenkamp, H. P. C.; Barker, H. A. *J. Biol. Chem.* **1961**, *236*, 3097. (c) Johnson, A. W.; Shaw, N. *J. Chem. Soc.* **1962**, 4608. (d) Hogenkamp, H. P. C.; Oikawa, T. G. *J. Biol. Chem.* **1964**, *239*, 1911. (e) Schrauzer, G. N.; Sibert, J. W. *J. Am. Chem. Soc.* **1970**, *92*, 1022.

heating $AdoB_{12}$ for 90 min in aqueous 0.1 M HCl at 100 °C is known to give the quantitative formation of aquocobalamin ($H_2OCo^{III}B_{12}$), adenine, and 2,3-dihydroxy-4-pentenal (DHP) as shown in Scheme III.[14d] However, in these previous studies, there was no information concerning either the products or the kinetics of the acid heterolysis of $AdoB_{12}$ at a pH greater than 2. It was not possible, therefore, to rule out the presence of this heterolytic side reaction since the thermal stability of $H_2OCo^{III}B_{12}$, the expected corrin product, had not been reported under the conditions employed (pH 4.3, 84–103 °C, and $\geq 10^{-3}$ M $Co^{II}(DMG)_2$). An additional concern is that under acidic conditions, the literature indicates that $Co^{II}(DMG)_2$ is not stabilized but instead decomposes rapidly at room temperature to give aqueous Co^{2+} and protonated ligand, H-DMG (Scheme IV).[2d,16] According to Adin and Espenson,[16a] $Co^{II}(DMG)_2$ is hydrolyzed with a first-order half-life of 2.9 s at 25 °C in pH 4.9 acetate buffer (0.01 M total [acetate] and 0.1 M $LiClO_4$), 10^8 times faster than the decomposition of $AdoB_{12}$ at this temperature. Given the known instability of the $Co^{II}(DMG)_2$ scavenger and the possible interference of acid heterolysis, it seemed likely that the observed $\Delta\Delta H^{\ddagger}_h = 4.3 \pm 0.7$ kcal/mol and $\Delta\Delta S^{\ddagger}_h = 9 \pm 2$ eu differences did not arise from differences in solvent properties but rather from the use of acidic (pH 4.3 H_2O) vs. nonacidic (neat ethylene glycol) conditions.

Our approach to resolving the discrepancy between our results in ethylene glycol and those in aqueous, pH 4.3 solution was to investigate the $AdoB_{12}$ Co–C thermolysis in H_2O as a function of pH.[17] Herein we report the results of both product and kinetic studies of the aqueous thermolysis of $AdoB_{12}$ from pH 4 to 8. The product studies at pH 4.0 reveal that the reaction consists of 88% heterolysis and 12% homolysis at 85.0 °C. The product studies further reveal that even in pH 7.0 H_2O, 10% heterolysis obtains at 85.0 °C, which decreases to 3% at 110.0 °C. The kinetic studies in pH 7.0 H_2O yield $\Delta H^{\ddagger}_{obsd} = 30.9 \pm 0.5$ kcal/mol and $\Delta S^{\ddagger}_{obsd} = 4.8 \pm 0.8$ eu. These values have been corrected for heterolysis to yield the homolysis activation parameters in H_2O, $\Delta H^{\ddagger}_h = 31.8 \pm 0.7$ kcal/mol, $\Delta S^{\ddagger}_h = 6.8 \pm 1.0$ eu, and base-on $AdoB_{12}$ Co–C BDE = 30 ± 2 kcal/mol. These values show little dependence upon solvent in comparison to our previous values in ethylene glycol, $\Delta H^{\ddagger}_h = 30.6 \pm 0.3$ kcal/mol, $\Delta S^{\ddagger}_h = 2.9 \pm 0.7$ eu, and base-on BDE = 31.5 ± 1.3 kcal/mol. The results demonstrate that under the pH 4.3 conditions employed by others,[10] the observed differences in activation parameters can be accounted for by competing heterolysis.

Experimental Section

Adenosylcobalamin (≥98%), 5′-deoxyadenosine, adenine, and hydroxocobalamin hydrochloride (≥98%) were obtained from Sigma and used as received. Hydroxocobalamin was prepared by passing an aqueous solution of hydroxocobalamin hydrochloride down an Amberlite IRA-400 column in the basic form and recrystallizing from water/acetone. $Co^{II}B_{12}$ (B_{12r}),[18] diaquoCo(II)cobaloxime,[19] and 8,5′-anhydroadenosine[20] were obtained as crystalline solids by literature methods. TEMPO (Aldrich) was purified by sublimation (33 °C, water aspirator). Aqueous buffers, HOAc/NaOAc at pH 4–5 and H_3PO_4/K_3PO_4 at pH 6–8, at 0.01 ionic strength were prepared using house-distilled water. Proton NMR spectra were recorded as Me_2SO-d_6 solutions on a Nicolet NT-360 spectrometer. Visible spectra were recorded in 1-cm path length Pyrex, Schlenk cuvettes (1-cm path length Pyrex cuvettes, glass blown onto Teflon needle valves) on a Beckman DU-7 spectrophotometer thermostated at 25.0 ± 0.2 °C. HPLC analyses were carried out by using a Beckman chromatograph equipped with a Gilson Holochrome variable-wavelength detector on an Alltech 300- × 4.1-mm Versapack C-18 column. FAB mass spectrometry was provided by the Midwest Center for Mass Spectrometry (Lincoln, NE) using glycerol as a matrix.

Adenosylcobalamin solutions were prepared in a Vacuum Atmospheres glovebox (N_2, ≤2 ppm O_2) in solvents deoxygenated by three freeze/pump/thaw cycles. Kinetic samples were placed into Schlenk cuvettes. Samples for subsequent thermal decomposition product studies were placed into Carius tubes, frozen in liquid N_2, and flame sealed. During the handling of $AdoB_{12}$ samples, exposure to light was minimized by working under dim lighting and keeping vessels wrapped in aluminum foil. Thermal reactions were carried out in an oil bath, thermostated to ±0.2 °C.

Kinetics. The rate of the thermal reactions of (5–10) × 10^{-5} M $AdoB_{12}$ was monitored by visible spectroscopy. Thermolyses were carried out in Schlenk cuvettes in an oil bath at 85–110 °C, and spectra were recorded following rapid cooling to 25 °C to quench the reaction. (The Teflon needle valves on the Schlenk cuvettes permitted the use of temperatures above the boiling point of water, up to 110 °C.) After the mixture was scanned, the cuvette was returned to the oil bath for further reaction. The time uncertainties introduced by this method (≤1 min) were small compared with the half-life of the decomposition which ranged from 1 to 28 h over the temperature range used in these studies. Due to the presence of an $H_2OCo^{III}B_{12}$ intermediate formed in aqueous solution (vide infra), the disappearance of $AdoB_{12}$ was followed at 372 nm, an isosbestic point for the conversion of $Co^{II}B_{12}$ to $H_2OCo^{III}B_{12}$. Limiting values of k_{obsd} (±5%) reported for aqueous decompositions were obtained by using initial rate data, ≤25% reaction, where first-order plots were linear. When the reaction was monitored further, the plots are linear out to approximately one half-life but exhibit discernible curvature past this point due to competing recombination. (Typical kinetic plots documenting this are available as supplementary material (Figure A). Control experiments showed that (5–10) × 10^{-5} M $AdoB_{12}$ reactions in ethylene glycol from 90 to 120 °C followed by this method of initial rates gave rate constants which were the same (±5%) as the rate constants obtained in ethylene glycol in the presence of a limiting excess of TEMPO (≥50 equiv).[7]

Temperature Dependence of the Axial Base Equilibrium. A solution of 1 × 10^{-4} M $AdoB_{12}$ was put in a Schlenk cuvette, and spectra were recorded at 5-deg intervals from 15 to 70 °C. The absorbance values at 520 nm were corrected for changes in concentration due to volume expansion (of the observed 15% decrease in absorbance at 520 nm, 3% is due to thermal expansion) and the data were worked up as described previously.[21] During the curve fitting, ΔH and ΔS were constrained to be negative and $\epsilon_{base\text{-}on}$ and $\epsilon_{base\text{-}off}$ were constrained such that $\epsilon_{base\text{-}on} \geq Abs_{15°C}/[AdoB_{12}]_{total}$ and $\epsilon_{base\text{-}off} \leq Abs_{70°C}/[AdoB_{12}]_{total}$, i.e., constrained so that the absorbance values for the pure base-on and base-off forms serve as limits, as they must, for the observed absorbance range.

Isolation of 8,5′-Anhydroadenosine. Fifty milliliters of 1 × 10^{-3} M $AdoB_{12}$ in pH 7.0 H_2O was heated at 110 °C for 6 h. The solvent was removed at reduced pressure at 60 °C, and the residue was dried in vacuo for 2 h at 80 °C. The residue was taken up in absolute EtOH and introduced on a 4 × 1-cm silica gel column (Baker, 60–200 mesh). The

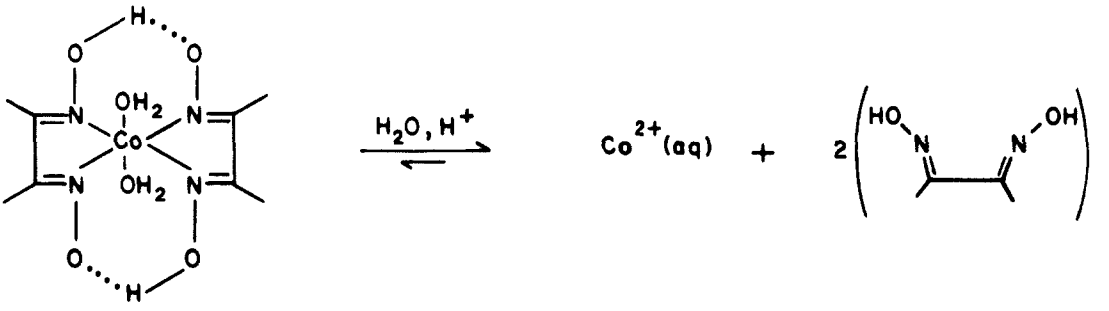
(15) Moreover, there is extensive literature[15a-j] on the acid instability of β-hydroxy-, β-alkoxy- ($AdoB_{12}$), β-[(acyloxy)alkyl]cobalt(III) complexes. (a) For a recent review see: Toscano, P. J.; Marzilli, L. G. *Progress in Inorganic Chemistry*"; Lippard, S. J., Ed; Wiley: New York, **1983**; Vol. 31, pp 166–168. (b) Golding, B. T.; Holland, H. L.; Horn, U.; Sakriker, S. *Angew. Chem., Int. Engl. Ed.* **1970**, *9*, 959. (c) Curzon, E. H.; Golding, B. T.; Wong, Y. *J. Chem. Soc., Chem. Commun.* **1982**, 63. (d) Brown, F. L.; Ingraham, L. L.; *J. Am. Chem. Soc.* **1974**, *96*, 7681. (e) Brown, K. L.; Ramamurthy, S. *Organometallics* **1982**, *1*, 413. (f) Brown, F. L.; Zahonyi-Budo, E. *J. Am. Chem. Soc.* **1982**, *104*, 4117. (g) Vickrey, T. M.; Wright, E. E.; Kok, R. A. *Inorg. Nucl. Lett.* **1979**, *15*, 317. (h) Vickrey, T. M.; Katz, R. N.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1975**, *97*, 7248. (i) Schrauzer, G. N.; Windgassen, R. J. *J. Am. Chem. Soc.* **1967**, *87*, 143. (j) Silverman, R. B.; Dolphin, D. *J. Am. Chem. Soc.* **1976**, *98*, 4633.

(16) (a) Adin, A.; Espenson, J. H. *Inorg. Chem.* **1972**, *11*, 686. (b) Alcock, N. W.; Atkins, M. P.; Golding, B. T.; Sellars, P. J. *J. Chem. Soc., Dalton Trans.* **1982**, 337.

(17) (a) We emphasize that our approach was to initiate our own, independent studies of the thermal reaction of $AdoB_{12}$ in H_2O using our own kinetic methods and not to attempt to repeat the exact experiments previously reported[10] nor to attempt to reinterpret in detail *all* of the previously reported observations.[12,17b] Our product and kinetic studies do cover a full range of pH (4–8) such that our results include the pH (4.3) used previously.[10] (b) Due to the acid instability of $Co^{II}(DMG)_2$ and the competing heterolysis reaction documented herein, the following require reinterpretation once the necessary data and full details of the exact experimental conditions[17c] employed previously[10] become available: (i) the linear $1/k_{obsd}$ vs. $1/[Co^{II}(DMG)_2]$ plot; (ii) the reported "k_{-1}/k_2" = 1.07 and what it is actually measuring; (iii) why small amounts of adenosylcobaloxime were apparently detected by NMR; (iv) what conditions were used that yielded neopentylcobaloxime and their relationship to the conditions used for $AdoB_{12}$; and (v) why 8,5′-anhydroadenosine was apparently not observed as a product. (c) We note, with the hope of avoiding controversy, that only the authors of the previous studies can explain completely[17b] their findings[10] as only they have access to the exact conditions employed. Specifically, the concentration of $AdoB_{12}$ used in either the kinetics studies or product studies, the concentration of the acetate buffer, the methods used to analyze for C-Ado (8,5′-anhydroadenosine), and the conditions under which neopentylcobaloxime was isolated as a reaction product were not reported.

(18) Blaser, H. U.; Halpern, J. *J. Am. Chem. Soc.* **1980**, *102*, 1684.
(19) Schrauzer, G. N. *Inorg. Synth.* **1968**, *11*, 61.
(20) Bernhauer, K. *Biochem. Z.* **1961**, *335*, 44.
(21) See ref 17 in ref 7.

Scheme IV



Co(II)(DMG)$_2$ H$^-$ DMG

column was eluted with absolute EtOH until the colored material was 1 cm from the bottom. The eluate was stripped to dryness on a rotary evaporator, leaving behind a white residue. This residue was taken up in Me_2SO-d_6 and exhibited an ^1H NMR identical with that of authentic 8,5′-anhydroadenosine:[22] ^1H NMR δ 8.08 (s, 1 H, H-2), 7.13 (s, 2 H, NH_2), 5.97 (s, 1 H, H-1′), 5.53 (d, 1 H, OH-3′), 5.24 (d, 1 H, OH-2′), 4.60 (d, 1 H, H-4′), 4.19 (d of d, 1 H, H-2′), 4.03 (d of d, 1 H, H-3′), 3.33 (d of d, 1 H, H-5′), 3.01 (d, 1 H, H-5′).

Isolation of Adenine. Fifty milliliters of 1×10^{-3} M $AdoB_{12}$ in pH 4.0 H_2O was heated at 85 °C for 48 h. The solution was neutralized to pH 7.0 by the addition of 0.1 M NaOH and then stripped to dryness at ≤40 °C. The residue was worked up in the same manner as the 8,5′-anhydroadenosine above. The resulting white residue gave an ^1H NMR spectrum identical with that of authentic adenine:[22] ^1H NMR δ 8.08 (s, 1 H), 8.06 (s, 1 H), 7.10 (br s, 3 H).

Preparation of 5′-Deoxy-5′-[(2,2,6,6-tetramethyl-1-piperidinyl)oxy]-adenosine. To a 500-mL round-bottomed flask were added 0.925 g (5.9×10^{-4} mol) of $AdoB_{12}$, 2.0 g (1.3×10^{-2} mol) of TEMPO, 200 mL of methanol, and a stir bar. The solution was degassed by bubbling with argon and photolyzed for 8 h at a distance of 60 cm from a 350-W tungsten lamp with vigorous magnetic stirring. After this time the solvent was removed by rotary evaporation. The residue was triturated with hexanes to remove excess TEMPO. The desired product was separated from the residue by column chromatography on silica gel as described above for 8,5′-anhydroadenosine. The resulting white solid was further purified by reprecipitation from absolute EtOH to yield 116 mg (49%): mp dec (vacuum-sealed capillary tube) 254–255 °C; FAB-MS (glycerol matrix) calcd molecular mass for $C_{19}H_{30}N_6O_4$ 406.4, found m/e M^+ ($+H^+$) 407; ^1H NMR δ 8.29 (s, 1 H, H-2 or H-8), 8.13 (s, 1 H, H-2 or H-8), 7.28 (s, 2 H, NH_2), 5.89 (d, 1 H, H-1′), 5.61 (d, 1 H, OH-2′), 5.26 (d, 1 H, OH-3′), 4.47 (m, 1 H, H-2′), 4.28 (m, 1 H, H-3′), 4.01 (q, 1 H, H-5′), 3.98 (m, 1 H, H-4′), 3.91 (q, 1 H, H-5′), 1.38 (m, 6 H, TEMPO CH_2's), 1.08 (s, 6 H, TEMPO CH_3's), 1.03 (s, 6 H, TEMPO CH_3's). Anal. Calcd for $C_{19}H_{30}N_6O_4$: C, 56.14; H, 7.44; N, 20.67. Found: C, 55.76; H, 7.44; N, 20.27.

HPLC Quantitation of the Nucleoside Products. (i) In H_2O. Samples for HPLC analysis were prepared at the same initial concentration of $AdoB_{12}$ used in the kinetic studies, i.e., 1×10^{-4} M in H_2O from pH 4 to 8 at pH 1 intervals. The samples were sealed under nitrogen in Carius tubes and heated in the dark for four or more half-lives at either 85 °C (60 h) or 110 °C (5 h). After this time the samples were opened to air and analyzed by HPLC. Base-line separation of adenine (12.5 min), 8,5′-anhydroadenosine (C-Ado) (17.5 min), and 5′-deoxyadenosine (D-Ado) (22.0 min) was obtained under the following conditions: column, Versapack C-18; flow rate, 1.0 mL/min; eluent, 15% CH_3OH/85% 0.01 M aqueous KH_2PO_4; sample size, 50 μL; temperature, ambient; wavelength, 260 nm. Under these conditions T-Ado and corrins are retained on the column but may easily be removed by rinsing with neat CH_3OH. When the eluent was changed to 70% CH_3OH/30% 0.01 M aqueous KH_2PO_4 and the other parameters were kept constant, it was possible to observe T-Ado (8.1 min). When authentic samples of each nucleoside mentioned above were used, the peak area was found to be linearly related (±5%) to concentration over the range of (1–20) $\times 10^{-5}$ M in every case, allowing for the quantitation of the nucleoside products derived from $AdoB_{12}$. Example HPLC traces are available as supplemental materials, Figure B.

(ii) In Ethylene Glycol. Samples for HPLC analysis were prepared at the same initial concentration used in the kinetic studies, i.e., 1×10^{-4} M in neat ethylene glycol in the presence of 0–10^{-2} M TEMPO. The samples were sealed under nitrogen in Schlenk cuvettes. In the absence of TEMPO, samples were heated for four or more half-lives at either 90 °C (65 h) or 110 °C (9 h). After this time the samples were opened by air and analyzed by HPLC as above.

Detection of Sugar Hydrolysis Product. Five milliliters of 5×10^{-3} M $AdoB_{12}$ in pH 4.0 (ionic strength 0.1) H_2O was heated at 85 °C for 48 h. Cation-exchange chromatography by the method of Hogenkamp and Barker[14b] was used to separate the sugar hydrolysis product(s) from the reaction mixture. The eluate gave, again following Hogenkamp and Barker, a positive test with orcinol/H_2SO_4[23] and Bial's reagent (orcinol/$FeCl_3$/HCl),[24] consistent with the presence of a reducing sugar. The yield was estimated to be 30 ± 10% by comparison to glyceraldehyde standards. The addition of 2,4-dinitrophenylhydrazine to the eluate resulted in the formation of an orange-red precipitate upon heating, consistent with the presence of an aldehyde. Due to the small scale of these experiments, we were not able to purify the DNP derivative for further characterization. The initial sugar hydrolysis product is known to be 2,3-dihydroxy-4-pentenal (DHP, Scheme I).[14b,25]

Nucleoside Stability Controls. The stability of the nucleoside derivatives C-Ado, D-Ado, and T-Ado to hydrolysis was investigated under reaction conditions used for $AdoB_{12}$. Three solutions, each containing 1×10^{-4} M of one nucleoside and 1 equiv of Co(II)B_{12} in pH 4.0 H_2O under nitrogen, were sealed in Carius tubes and heated for 60 h at 85.0 °C. After this time HPLC analysis revealed no reaction for C-Ado, ≤5% hydrolysis of D-Ado, and ≤5% hydrolysis of T-Ado.

Reduction of Aquocobalamin by Glyceraldehyde. As a model for the 2,3-dihydroxy-4-pentenal, 1 equiv of DL-glyceraldehyde was added to 1.0×10^{-4} M aquocobalamin in a Schlenk cuvette under nitrogen at both pH 4.0 and 7.0. When the solution was heated to 85 °C, aquocobalamin was cleanly reduced to $Co^{II}B_{12}$ with isosbestic points (observed at 25 °C) present at 338, 372, 488, and 572 nm.

Self-Reduction of Aquocobalamin. Heating just 1.0×10^{-4} M aquocobalamin in either pH 7.0 ($t_{1/2} \approx 2$ h) or 4.0 (≤10% after 9 h, 85 °C) H_2O under nitrogen results in the formation of a product with a spectrum identical with that of $Co^{II}B_{12}$, a result not previously reported in the literature. This clean self-reduction was monitored at 525 nm and gave the same isosbestic points observed above for the glyceraldehyde reduction. More concentrated samples, 20 mg/2 mL (7.4×10^{-3} M), were heated at 100 °C for 30 h at pH 4.0 and 7.0 H_2O. After this time the vessels were opened to air, and the corrins were precipitated from solution by the addition of 20 mL of acetone. Infrared spectra of the dried (2 h in vacuo at 80 °C) red-brown residues were obtained as KBr pellets. In the pH 7.0 case, the spectrum was almost identical with that of hydroxocobalamin. In the pH 4.0 case, a new band was present at 1773 cm^{-1}, suggestive of a γ-lactone carbonyl stretch.[26]

Stability of DiaquoCo(II)cobaloxime. In the glovebox, separate 16-mg samples of $Co^{II}(DMG)_2$ were added to separate 10.0-mL samples of pH 4.0 and 7.0 H_2O in 25-mL Erlenmeyer flasks. The flasks were then vigorously stirred for 5 min at 25 °C. After this time, the pH 7.0 sample was a clear, dark-orange solution, while the pH 4.0 sample was a light, yellow-brown solution and had a fine white precipitate present. Visible spectra of these samples in Schlenk cuvettes showed that the 460-nm band of the $Co^{II}(DMG)_2$ complex[13] was present at pH 7.0 but absent at pH 4.0. Heating the pH 4.0 sample at 85 °C for 1 h did not cause the appearance of the 460-nm band; i.e., this control shows that the reaction

(22) This and the other nucleoside products were quantified in separate HPLC experiments (see Experimental Section).

(23) Sorensen, M.; Haugaard, G. *Biochem. Z.* **1933**, *260*, 247.
(24) Miller, G. L.; Golder, R. H.; Miller, E. E. *Anal. Chem.* **1951**, *23*, 903.
(25) Saunders, R. M.; Ballou, C. E. *J. Org. Chem.* **1965**, *30*, 3219.
(26) γ-Lactones exhibit a carbonyl stretch in the range 1795–1760 cm^{-1}. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 3rd ed.; Wiley: New York, 1974.

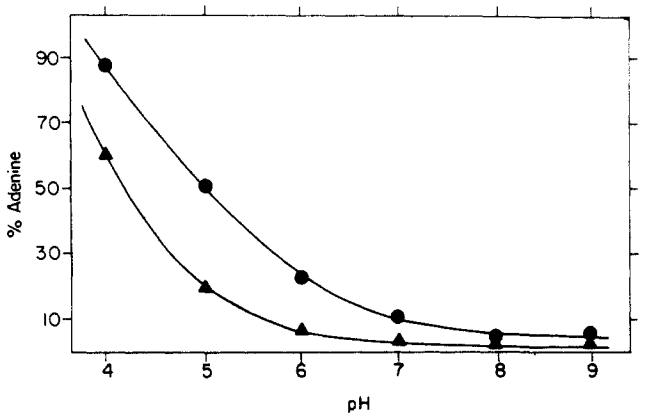


Figure 1. Plot of percent yield adenine from the decomposition of $AdoB_{12}$ in H_2O vs. pH at 85.0 °C (●) and at 110.0 °C (▲).

is not reversed at higher temperatures. The white precipitate formed in the pH 4.0 sample was removed by extraction with 5 mL of diethyl ether. Upon evaporation of the ether, a white residue was obtained which gave a single peak at 1.91 ppm (Me_2SO-d_6, ^1H NMR) identical with the spectrum of authentic dimethylglyoxime.

Reduction of Aquocobalamin by $Co^{II}(DMG)_2$. Hydrolysis Products. $H_2OCo^{III}B_{12}$ (1×10^{-4} M) was converted to $Co^{II}B_{12}$ by the reductant, 1.0×10^{-3} M $Co^{II}(DMG)_2$ (actually its' acid hydrolysis products, vide supra), under nitrogen in pH 4.0 H_2O in a Schlenk cuvette with a half-life of <1 h at 85 °C. In a separate experiment, it was shown that under the same conditions, 2×10^{-3} M dimethylglyoxime alone (a product of the hydrolysis of 1×10^{-3} M $Co^{II}(DMG)_2$) was able to reduce $H_2OCo^{III}B_{12}$ to $Co^{II}B_{12}$ with a half-life of <1 h.

Nucleoside Products in the Presence of $Co^{II}(DMG)_2$. Hydrolysis Products. A solution containing 1×10^{-4} M $AdoB_{12}$ and 1×10^{-3} M $Co^{II}(DMG)_2$ in pH 4.0 H_2O was sealed in a Carius tube under nitrogen. After heating for 60 h at 85 °C, the sample was analyzed by HPLC (vide supra). Both adenine (major) and 8,5′-anhydroadenosine (minor) were present, but no (≤5%) D-Ado (5′-deoxyadenosine) was detected.

Results

(A) Nucleoside Products. (i) In H_2O. Anaerobic solutions of 10^{-4} M $AdoB_{12}$ in pH 4.0–8.0 aqueous solution were heated in the dark for $\geq 4t_{1/2}$ at 85 °C (60 h) and 110 °C (5 h). After the solution was heated, nucleoside products were isolated, identified,[27] and, in separate experiments, quantified (±5%) by HPLC. The results are presented in Figure 1. Both C-Ado and adenine were found in all of the samples, and together they accounted for 95 (±5%)% of the nucleoside products. At pH 4.0 the yield of adenine, the expected nucleoside product of Co–C bond heterolysis, ranged from 88% at 85 °C to 60% at 110 °C. In addition, the analytical test used by Hogenkamp and Barker[14b] for the detection of the sugar heterolysis product, DHP, yielded positive results on an 85 °C sample. Upon comparison with glyceraldehyde standards, the overall yield of DHP was estimated to be 30 ± 10%. At pH 7.0, the 5′-Ado radical product C-Ado[28,29] is the main product,

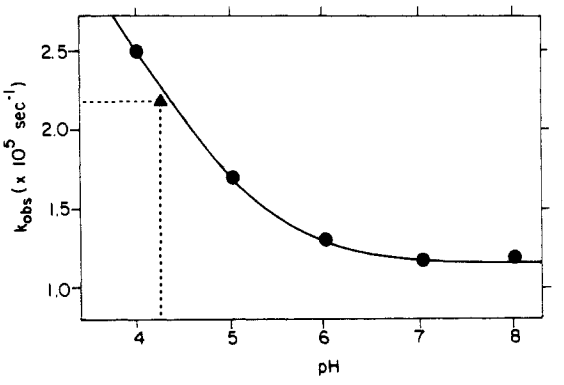


Figure 2. Plot of k_{obsd} for the decomposition of $AdoB_{12}$ in H_2O at 85.0 °C vs. pH. The ▲ indicates the 85.0 °C rate constant, obtained from the pH 4.3 $\Delta H^{\ddagger}_{obsd}$ and $\Delta S^{\ddagger}_{obsd}$ data from ref 10.

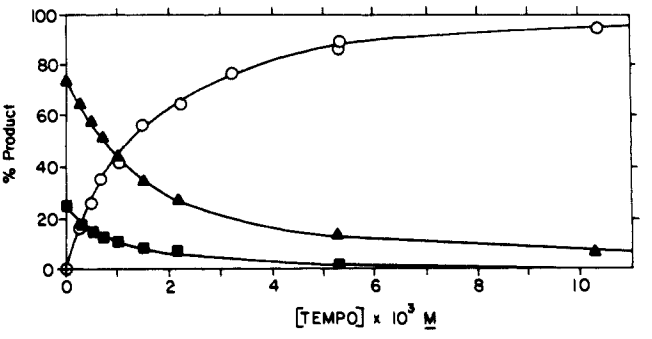


Figure 3. Plot of the percent yields of the nucleoside products from the decomposition of $AdoB_{12}$ (1.0×10^{-4} M) at 110 °C in ethylene glycol vs. [TEMPO]; C-Ado (▲), D-Ado (■), and T-Ado (○).

but some adenine is still present, ranging from 10% at 85 °C to 3% at 110 °C. Another point worth mentioning here is the close correspondence in the shape of the curve in Figure 1 to the one in Figure 2, describing the anomolous pH dependence of the *rate* of $AdoB_{12}$ decomposition in H_2O. This rate vs. pH dependence will be discussed in greater detail in the Kinetics section.

When the aqueous decompositions were carried in the presence of 100 equiv of TEMPO (conditions known to trap ≥95% of the 5′-Ado radical in the ethylene glycol (vide infra)), it was found that the yield of adenine was unaffected, while C-Ado yields were decreased to ≤5% with a corresponding appearence of T-Ado. Control experiments showed that both T-Ado and C-Ado are stable to hydrolysis under the reaction conditions (≤5% decomposition after 60 h, pH 4.0, 1 equiv of $Co^{II}B_{12}$, 85 °C). These results demonstrate that in aqueous solution the free 5′-Ado radical is an intermediate for C-Ado *but not for adenine*; i.e., these two products require two different, parallel kinetic pathways. Moreover, a control reaction showed that the addition of 10 equiv (10^{-3} M) of $Co^{II}(DMG)_2$ does not significantly alter the nucleoside product distribution in pH 4.0 H_2O; adenine (major) and C-Ado (minor) are the products at 85 °C (by HPLC) in this control experiment.

(ii) In Ethylene Glycol. The products in ethylene glycol have also been investigated by HPLC as part of our previous studies in this solvent.[7] The results are important for the present work because they provide quantitative evidence for the rapidity of the cyclization of the 5′-Ado radical.

In the absence of scavenger, C-Ado and D-Ado account for 95 (±5)% of the nucleoside products in ethylene glycol. Over the temperature range of 90–120 °C, the yield of C-Ado ranges from 73% to 78% and the yield of D-Ado ranges from 27% to 22%.[30] The effect of TEMPO on the product distribution in ethylene glycol was investigated at 110 °C, and the results are presented in Figure 3. As the concentration of TEMPO is increased, both

(27) At higher $AdoB_{12}$ concentration (10^{-3} M), both C-Ado and adenine were isolated from the reaction mixture and identified by ^1H NMR in comparison to authentic materials.

(28) The anaerobic photolysis of $AdoB_{12}$, known to generate the 5′-Ado radical,[28a] subsequently yields 8,5′-anhydroadenosine (C-Ado) in neutral aqueous solution. (a) Hogenkamp, H. P. C. *J. Biol. Chem.* **1963**, *238*, 477. (b) All other known examples of 8,5′-linkages in purine nucleosides and nucleotides are formed via radical intermediates.[28c-l] (c) Keck, K. *Z. Naturforsch.* **1968**, *238*, 1034. (d) Harper, P. J.; Hampton, A. *J. Org. Chem.* **1972**, *37*, 795. (e) Duong, K. N. V.; Gaudemer, A.; Johnson, M. D.; Quillivic, R.; Zylber, J. *Tetrahedron Lett.* **1975**, *34*, 2997. (f) Zylber, J.; Pontikis, R.; Merrien, A.; Merienne, C.; Baran-Marszak, M.; Gaudemer, A. *Tetrahedron* **1980**, *35*, 1579. (g) Mariaggi, N.; Cadet, J.; Teoule, R. *Tetrahedron* **1976**, *32*, 2385. (h) Matsuda, A.; Muneyama, K.; Nishida, T.; Sato, T.; Ueda, T. *Nucleic Acid Res.* **1976**, *3*, 3349. (i) Matsuda, A.; Tezuka, M.; Ueda, T. *Tetrahedron* **1978**, *34*, 2449.

(29) After the formation of the 8,5′ C–C bond, the cyclized Ado radical intermediate undergoes subsequent loss of a hydrogen atom from the C-8 position. This hydrogen loss could involve either H• abstraction by $Co^{II}B_{12}$ or a disproportionation of the type observed by Keck.[28c] These possibilities are under investigation.

(30) The previously reported nucleoside yields, ref 7, were based on isolated material.

C-Ado and D-Ado are decreased, consistent with and fully supportive of homolysis to the 5'-Ado radical as the precursor for both of these nucleoside products.

The observed TEMPO dependence in ethylene glycol is in quantitative accord with Scheme I. At ≥10 equiv of TEMPO, plots of [C-Ado] vs. [T-Ado]/[TEMPO] and [D-Ado] vs. [T-Ado]/[TEMPO] were linear with slopes of $k_c/k_T = (1.12 \pm 0.04) \times 10^{-3}$ M and k_a[ethylene glycol]$/k_T = (3.1 \pm 0.2) \times 10^{-4}$ M, respectively. With the reasonable if not excellent assumption that k_T falls within the known range of nitroxide R• trapping rate constants of $(3-5) \times 10^8$ M^{-1} s^{-1},[31] the first estimates of the rate constant for cyclization of the 5'-Ado radical, k_c (110 °C, ethylene glycol) $\approx 5 \times 10^5$ s^{-1}, and for 5'-Ado radical H• abstraction from ethylene glycol, k_a (110 °C, ethylene glycol) $\approx 7 \times 10^3$ M^{-1} s^{-1}, are obtained. Coupling these results with previously obtained $Co^{II}B_{12}$ dependence data[7] yields a rate constant for the recombination of $Co^{II}B_{12}$ and the 5'-Ado radical in ethylene glycol, k_r (110 °C, ethylene glycol) $\approx 3 \times 10^8$ M^{-1} s^{-1}.[32] Confidence in these rate constant estimates, all of which are relative to k_T, is fortified by the fact that they all fall within the known range of rate constants for similar processes.[33]

These values for k_c and k_r and thus for the ratio k_c/k_r[$Co^{II}B_{12}$] $= 1.66 \times 10^{-3}/[Co^{II}B_{12}]$ *prove* that the cyclization self-trapping reaction competes effectively with ≥98.5% of the Ado• + $Co^{II}B_{12}$ radical recombination if the condition [$Co^{II}B_{12}$] $\leq 2.5 \times 10^{-5}$ M is selected *in ethylene glycol*. In the Kinetics section these results will be combined with other data to show that the recombination reaction can also be minimized *in H_2O* under the conditions of an initial rate kinetic method.

(B) Corrin Products in H_2O. The thermal reactions of $AdoB_{12}$ were monitored by visible spectroscopy. In pH 4.0 H_2O at 100.0 °C where adenine is a major nucleoside product, the decomposition of $AdoB_{12}$ (Figure 4a) proceeded without isosbestic points, suggesting the presence of a second corrin product (an intermediate, vide infra). The appearance of a transitory peak at 351 nm, an intense absorption maximum for $H_2OCo^{III}B_{12}$,[34] strongly suggests the buildup *and decay* of an unstable $H_2OCo^{III}B_{12}$ intermediate, since the final spectrum is that of $Co^{II}B_{12}$. The situation is different in pH 7.0 H_2O at 100.0 °C where adenine is a minor product. Here the quantitative conversion of $AdoB_{12}$ to $Co^{II}B_{12}$ proceeds with the maintenance of four isosbestic points in the region of 330–800 nm (Figure 4b), a situation similar to our earlier studies in ethylene glycol (Figure 4c).

Although a $H_2OCo^{III}B_{12}$ intermediate was detected, the failure to detect the expected stoichiometric amounts of $H_2OCo^{III}B_{12}$ in aqueous solution led to control experiments demonstrating that under the conditions required to decompose $AdoB_{12}$, $H_2OCo^{III}B_{12}$ is not stable but rather reacts further to yield a product(s) which

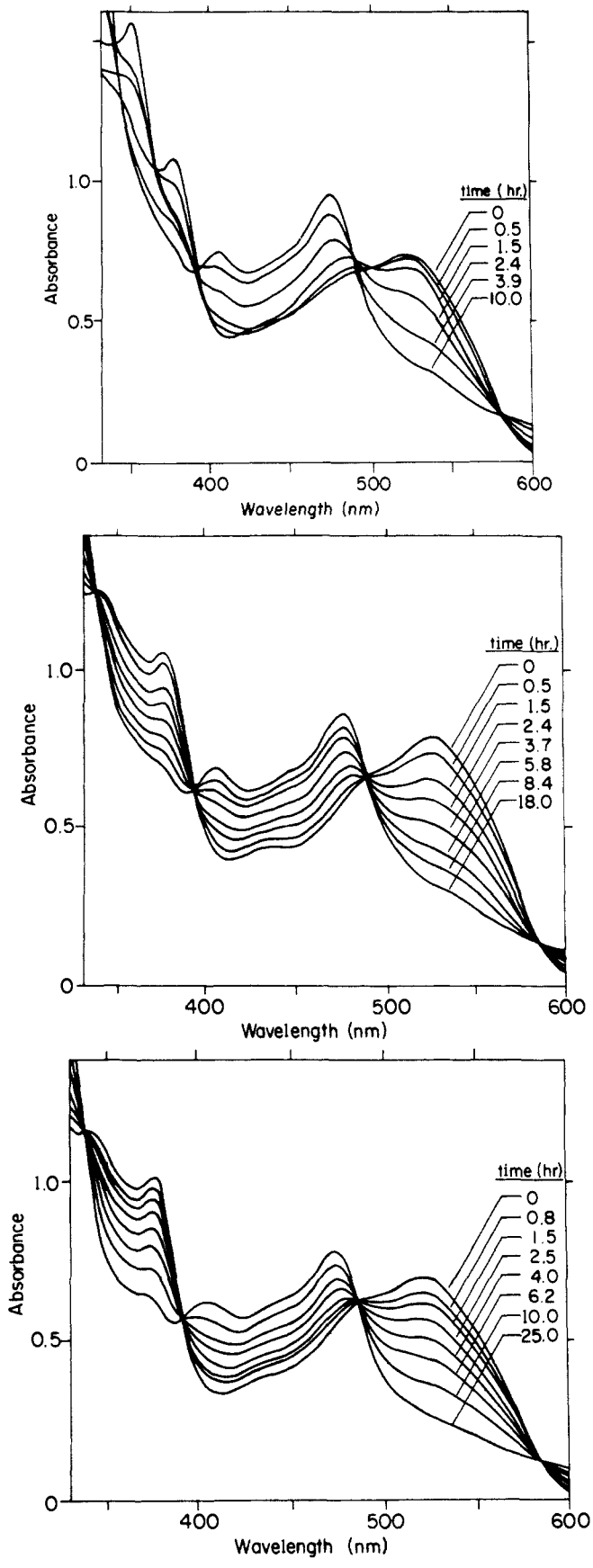


Figure 4. Visible spectra of the decomposition of $AdoB_{12}$ at 100.0 °C recorded as a function of time (spectra were recorded at 25.0 °C). (a, top) Reaction in pH 4.0 H_2O showing lack of isosbestic points. The peak at 351 nm, the absorption maximum for $H_2OCo^{III}B_{12}$, grows in and then decays. The final spectrum is that of $Co^{II}B_{12}$. (b, middle) Reaction in pH 7.0 H_2O showing the clean conversion of $AdoB_{12}$ to $Co^{II}B_{12}$ with isosbestic points at 339, 391, 493, and 583 nm. (c, bottom) The clean homolytic cleavage reaction in ethylene glycol with isosbestic points at 337, 390, 487, and 581 nm.

(31) (a) Schmid, P.; Ingold, K. U. *J. Am. Chem. Soc.* **1978**, *100*, 2493. (b) Nigam, S.; Asmus, K. D.; Willson, R. L. *J. Chem. Soc., Faraday Trans. 1* **1976**, 2324.

(32) The slope of the $1/k_{obsd}$ vs. [$Co^{II}B_{12}$] plot, equivalent to the ratio $k_r/(k_h(k_c + k_a$(ethylene glycol))), was found to be $(4.6 \pm 0.3) \times 10^6$ M^{-1} s.[7] With k_h, k_c, and k_a(ethylene glycol) in hand, a value of k_r in ethylene glycol is easily calculated.

(33) (a) The intramolecular radical cyclization at 110 °C occurs with a rate constant close to that of the 5-hexenyl radical, $\sim 10^6$ s^{-1} at 110 °C.[33b] Although this is the first quantitative estimate of the Ado• rate constant, the rapidity of the cyclization has been remarked upon previously.[33c,d] (b) Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317. (c) Brady, R. O.; Barker, H. A. *Biochem. Biophys. Res. Commun.* **1961**, *4*, 373. (d) Taylor, R. T.; Smucker, L.; Hanna, M. L.; Gill, J. *Arch. Biochem. Biophys.* **1973**, *156*, 521. (e) The rate constant for H• abstraction from ethylene glycol falls in the range observed for H• abstraction from alcohols by the methyl radical at 25 °C, 10^2–10^4 M^{-1} s^{-1}. Thomas, J. K. *J. Phys. Chem.* **1967**, *71*, 1919. (f) The value for k_r falls within the known range of R• + Co(II) recombination rate constants,[33g-m] $(0.05-2.0) \times 10^9$ M^{-1} s^{-1}. (g) Roche, T. S.; Endicott, J. F. *Inorg. Chem.* **1974**, *13*, 1575. (h) Endicott, J. F.; Ferraudi, G. J. *J. Am. Chem. Soc.* **1977**, *99*, 243. Their reported value for CH_3• + $Co^{II}B_{12}$ recombination in H_2O is $(1-2) \times 10^9$ M^{-1} s^{-1}. (i) Mok, C. Y.; Endicott, J. F. *J. Am. Chem. Soc.* **1977**, *99*, 1276; (j) **1977**, *100*, 123. (k) Tait, A. M.; Hoffman, M. Z.; Hayon, E. *Int. J. Radiat. Phys. Chem.* **1976**, *8*, 691. (l) Mulac, W. A.; Meyerstein, D. *J. Am. Chem. Soc.* **1982**, *104*, 4124. (m) Elroi, H.; Meyerstein, D. *J. Am. Chem. Soc.* **1978**, *100*, 5540.

(34) Hill, J. A.; Pratt, J. M.; Williams, R. J. P. *J. Theor. Biol.* **1962**, *3*, 423.

has a visible spectrum indistinguishable from that of $Co^{II}B_{12}$. Since 1 mmol each of $H_2OCo^{III}B_{12}$ and the aldehyde, 2,3-dihydroxy-4-pentenal (DHP) are produced initially from each millimole of $AdoB_{12}$ heterolysis, and since aldehydes are known to reduce $H_2OCo^{III}B_{12}$ to $Co^{II}B_{12}$,[35] control experiments were run using the 2,3-dihydroxy aldehyde, glyceraldehyde, as a model compound for DHP. At pH 4.0, 10^{-4} M $H_2OCo^{III}B_{12}$ was cleanly reduced to $Co^{II}B_{12}$ in the presence of 1 equiv of glyceraldehyde with a half-life of 3 h at 85 °C, ≈3× faster than the rate of the $AdoB_{12}$ thermal reaction under identical conditions. At pH 7.0, the rate of reduction was faster with a half-life of 10 min at 85.0 °C. These findings demonstrate that the reduction of $H_2OCo^{III}B_{12}$ by a 2,3-dihydroxy aldehyde can occur at rates kinetically competent to explain the appearance and subsequent decay of $H_2OCo^{III}B_{12}$ at pH 4.0 and the failure to observe $H_2OCo^{III}B_{12}$ at pH 7.0 during the aqueous $AdoB_{12}$ decompositions.

Interestingly, further controls revealed that merely heating 10^{-4} M $H_2OCo^{III}B_{12}$ in aqueous solution under nitrogen resulted in the clean conversion of $H_2OCo^{III}B_{12}$ to $Co^{II}B_{12}$ by visible spectroscopy.[36] At pH 4.0 this reduction is slow, <10% reaction over 9 h at 85.0 °C. However, at pH 7.0 this "self-reduction" alone is fast enough (half-life of 2 h at 85 °C) to account for the absence of $H_2OCo^{III}B_{12}$ during $AdoB_{12}$ decompositions in *neutral* aqueous solution.

Corrin Products when $Co^{II}(DMG)_2$ Is Added. Although the results herein show that the pH 4.0 H_2O thermal reaction of $AdoB_{12}$ yields both $Co^{II}B_{12}$ and $H_2OCo^{III}B_{12}$, others have reported that in the presence of $\geq 10^{-3}$M $Co^{II}(DMG)_2$, $Co^{II}B_{12}$ is the only corrin product.[10] These observations suggest that $Co^{II}(DMG)_2$, or actually its hydrolysis products (vide infra), is capable of reducing $H_2OCo^{III}B_{12}$. Control experiments show that 10^{-4} M $H_2OCo^{III}B_{12}$ is reduced to $Co^{II}B_{12}$ with a half-life of <1 h at 85 °C in the presence of either 10^{-3} M $Co^{II}(DMG)_2$ or 2×10^{-3} M dimethylglyoxime in pH 4.0 H_2O. This rate is clearly competent to effectively mask the formation of $H_2OCo^{III}B_{12}$ during the thermal reaction of $AdoB_{12}$, $t_{1/2}$ = 8.8 h at 85 °C in pH 4.0 H_2O.

The use of pH 4.3 acetate-buffered H_2O was claimed as necessary to stabilize the $Co^{II}(DMG)_2$ radical scavenger,[10] and a reference concerned with hydroxide ion attack upon $Co^{II}(DMG)_2$ was cited as precendent.[37] However, in addition to being base sensitive, $Co^{II}(DMG)_2$ is also known to be very acid sensitive,[2d,16] an observation confirmed herein. Control experiments demonstrated that $Co^{II}(DMG)_2$ decomposes immediately upon mixing with pH 4.0 acetate-buffered H_2O as shown in Scheme IV. We conclude that $Co^{II}(DMG)_2$ does not exist under the acidic pH 4.3 conditions previously employed to study the thermal reaction(s) of $AdoB_{12}$. In fact, instead of functioning as a radical scavenger, the redox chemistry of the dimethylglyoxime ligand and H_2O-$Co^{III}B_{12}$ serves to conceal the $AdoB_{12}$ heterolysis side reaction if one is looking only at the Co corrin products.

(C) Kinetics. As noted in the Introduction, the development of suitable trapping techniques is often the most difficult and the slowest step in kinetic studies of M–C bond thermolyses. Since we had ruled out the use of TEMPO *in* H_2O[38] as part of our earlier studies,[7] a suitable trapping technique for use in H_2O had to be developed. Because our product studies had already established that Ado• cyclization could effectively compete with recombination at low $[Co^{II}B_{12}]$,[39] $k_c/k_r[Co^{II}B_{12}] = 1.66 \times 10^{-3}[Co^{II}B_{12}]$ in ethylene glycol, it seemed likely that this situation would also obtain in aqueous solution. Furthermore, there should be only a small solvent effect on the cyclization step on going from ethylene glycol to H_2O so that k_c (110 °C, ethylene glycol) $\approx 5 \times 10^5$ s^{-1} should be approximately equal to k_c (110 °C, H_2O). Also, k_r (110 °C, ethylene glycol) $\approx 3 \times 10^8$ M^{-1} s^{-1} might increase in H_2O but not beyond the maximum value obtained to date for a $Co^{II}B_{12}$ + R• recombination *in* H_2O, $k_r \approx (1\text{–}2) \times 10^9$ M^{-1} s^{-1}.[33h] The result is the estimate that *in* H_2O, $k_c/k_r[Co^{II}B_{12}] \approx (2.5\text{–}5) \times 10^{-4}/[Co^{II}B_{12}]$; i.e., the estimated *limit* is 5%–10% recombination during initial rate measurements where $[Co^{II}B_{12}]$ remains ≤2.5 × 10^{-5} M.

Table I. Limiting Values of k_{obsd} for $AdoB_{12}$ Decompositions as a Function of Solvent

	k_{obsd}, × 10^5 s^{-1}		
temp, °C	EtGly[7]	pH 7.0 H_2O	pH 4.3 H_2O[10]
85.0	0.68	1.13	2.18[a]
90.0	1.22	2.01	3.68[a]
95.0	2.24	3.64	5.93
100.0	4.11	6.49	10.0
105.0	7.13	11.6	16.3[a]
110.0	12.1	20.4	26.1[a]
$\Delta H^{\ddagger}_{obsd}$, kcal/mol	30.6 + 0.3	30.9 + 0.4	26.3 + 0.6
$\Delta S^{\ddagger}_{obsd}$, eu	2.9 + 0.7	4.8 + 0.8	−6 + 2

[a] Values were calculated from the best fit activation parameters of ref 10; $\Delta H^{\ddagger}$ = 26.35 kcal/mol and $\Delta S^{\ddagger}$ = −6.65 eu.

Experimentally, it was found that initial rate plots are linear up to *at least* the first 25% reaction for $[AdoB_{12}]_{initial} \leq (5\text{–}10) \times 10^{-5}$ M in H_2O, i.e., when $[Co^{II}B_{12}] \leq 2.5 \times 10^{-5}$ M, and that the expected curvature is detectable after this point (see Experimental Section). The results demonstrate that minimal (≤5%) recombination occurs under the initial rate conditions. The consistency of the data, for example, of the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values in Table I (vide infra) also testifies to the validity of the initial rate method.

When the method of initial rates (≤25% reaction) and the conditions $[AdoB_{12}]_{initial} \leq (5\text{–}10) \times 10^{-5}$M were used, limiting values of the first-order rate constant for the decomposition of $AdoB_{12}$ in aqueous solution, k_{obsd}(±5%), were obtained. The temperature dependence of k_{obsd} was measured at 5-deg intervals from 85 to 110 °C in neutral H_2O (pH 7.0 phosphate buffer). The rate constants and activation parameters are presented in Table I which also includes our earlier ethylene glycol values and the reported pH 4.3 H_2O values. Plots of the data in Table I (ln (k_{obsd}/T) vs. $1/T$) are provided as supplementary material for the interested reader. The results clearly show that the ethylene glycol and pH 7.0 H_2O activation parameters are very similar, while the pH 4.3 H_2O data are significantly different. As for the small, 1.6× rate increase observed upon going from ethylene glycol to pH 7.0 H_2O, the data in Table I show it is caused by a small increase in $\Delta S^{\ddagger}_{obsd}$, while $\Delta H^{\ddagger}_{obsd}$ remains virtually unchanged.

Since the pH is the only difference between the two incongruous sets of H_2O data, values of k_{obsd} were measured at unit pH intervals from pH 4.0 to 8.0 at 85 °C. The results, presented in Figure

(35) Hohokabe, Y.; Yamazaki, N. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 2142.

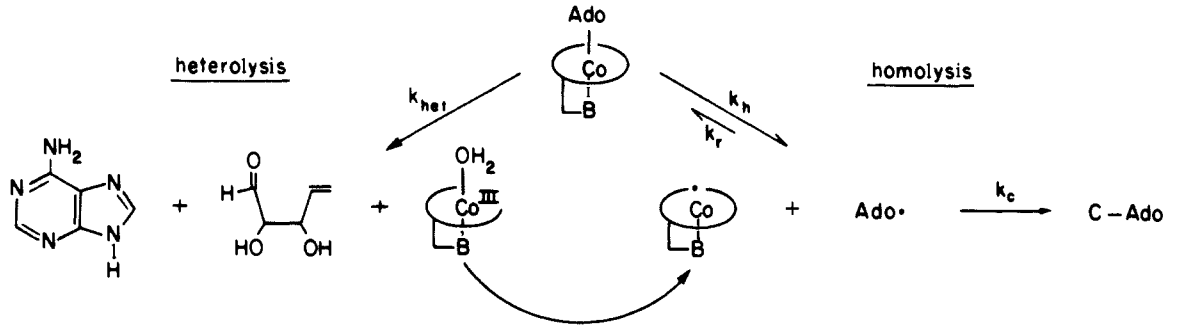
(36) $H_2OCo^{III}B_{12}$ is known to be a mild oxidant.[35,36a,b] In the presence of mild oxidizing agents Co corrins form C-side-chain lactams under basic conditions and C-side-chain lactones under acidic conditions.[36c–e] By UV–vis, and IR in the case of the lactam, these derivatives are not distinguishable from normal corrins. However, the C-side-chain lactone does give a prominent band in the IR at 1783 cm^{-1} for cyanocobalamin C-lactone[36d] and at 1805 cm^{-1} for dicyano-10-chlorocobyrinic acid C-lactone hexamethyl ester.[36e] The product formed upon heating anaerobic pH 4.0 H_2OB_{12} solutions exhibits a band at 1773 cm^{-1} and has been *tentatively* identified, solely on the basis of this band, as the C-side-chain lactone of aquocobalamin. At pH 7.0 the product lacks the lactone band and a speculative guess is that it is the C-side-chain. (a) Bayston, J. H.; Winfield, M. E. *J. Catal.* **1967**, *9*, 217. (b) Wan, T. S.; Fischli, A. *Helv. Chim. Acta* **1984**, *67*, 1461. (c) Bonnett, R. *In* B_{12}; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 1, pp 225–228. (d) Bonnett, R.; Cannon, J. R.; Clark, V. M.; Johnson, A. W.; Parker, L. F. J.; Smith, E. L.; Todd, A. *J. Chem. Soc.* **1957**, *1158*. (e) Gossauer, A.; Heiss, K. P.; Lass, H.; Inhoffen, H. H. *Liebigs Ann. Chem.* **1976**, *1150*.

(37) Simandi, L. I.; Nemeth, S.; Zahonyi-Budo, E. *Inorg. Chim. Acta* **1980**, *45*, L 143.

(38) (a) The TEMPO trapping method was not used since TEMPO is reduced by $Co^{II}B_{12}$ *in aqueous solution*[38c] at the elevated temperatures of $AdoB_{12}$ decompositions. This introduces uncertainty into the interpretation of the visible spectra. (b) This redox behavior has also been confirmed by Espenson et al. for TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidinooxy) and $Co^{II}B_{12}$.[5b] (c) However, this redox reaction does not occur in ethylene glycol. Two, possibly the main, reasons for this are that $Co^{II}B_{12}$ is a 0.2-V weaker reductant in alcohols than in water[38d] and that ethylene glycol is less acidic than H_2O and thus less able to protonate and stabilize the reduced nitroxide.[38e] (d) Faure, D.; Lexa, D.; Saveant, J. M. *J. Electroanal. Chem.* **1982**, *140*, 297. (e) See ref 3, ref 7.

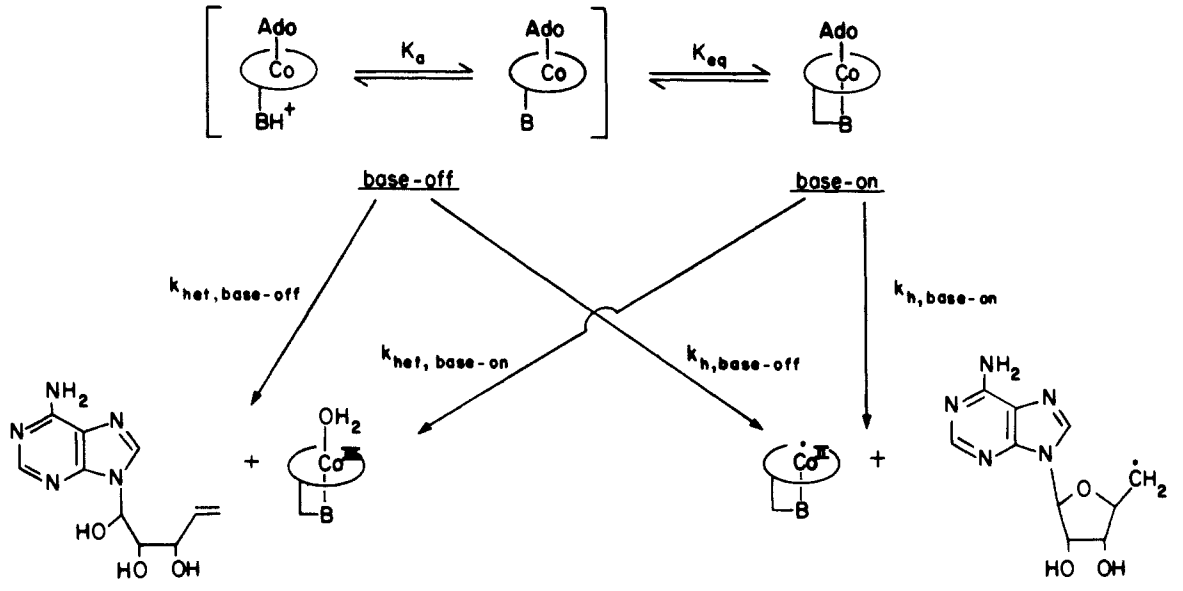
(39) The mechanism for pure homolytic cleavage of $AdoB_{12}$ with cyclization of the 5′-Ado radical competing with recombination yields the rate law $-d(\ln [Ado_{12}])/dt = k_{obsd} = k_h(k_c/(k_c + k_r[Co^{II}B_{12}]))$. At low $[Co^{II}B_{12}]$, $k_c \gg k_r[Co^{II}B_{12}]$ and $k_{obsd} \approx k_h$.

Scheme V



$$\text{Eq. 3} \qquad -\frac{d \ln [AdoB_{12}]}{dt} = k_{obs} = \left(\frac{k_c}{k_c + k_r[Co(II)B_{12}]}\right) k_h + k_{het}$$

Scheme VI



2 (vide supra), show the observed rate increase (of $AdoB_{12}$ disappearance) as the pH decreases. *This behavior is opposite to the known thermolysis chemistry of simple alkylcobalamins.* In every case studied to date, the observed rate of homolytic decomposition slows as the pH was decreased due to the displacement of the axial base equilibria to the less-reactive, base-off side.[40] In fact, ≈10% of the protonated base-off $AdoB_{12}$ (pK_a = 3.3–3.5, 25 °C)[41] is formed at pH 4.3, causing a corresponding decrease in the rate of homolysis. The data in Figure 2 established one other important point: that our initial rate method in pH 4.3 H_2O *yields the same value* (±5%) *of* k_{obsd} *as that reported previously*[10] *in the presence of excess* $Co^{II}(DMG)_2$. Coupling this kinetic result with the product studies reported herein demonstrates that competing heterolysis *quantitatively* accounts for the more rapid rates reported in pH 4.3 H_2O.

Discussion

The observed results greatly clarify the earlier, apparently discordant, ethylene glycol vs. water results and show that acidic water is, as expected on the basis of earlier literature,[14,15] an inferior solvent for the study of $AdoB_{12}$ Co–C bond homolysis due to competing heterolysis. At pH 4–8 over the temperature range of 85–110 °C, both adenine, a product of heterolytic Co–C bond cleavage, and C-Ado, a product of homolytic Co–C bond cleavage, are present. As expected, the 5′-Ado radical precursor of C-Ado is scavenged by TEMPO, while adenine yields are unaffected. The ratio of the adenine and C-Ado products is strongly influenced by pH at 85 °C, with more heterolysis (88% adenine) at pH 4.0 and less heterolysis (10% adenine) at pH 7.0. The corrin product of heterolysis, $H_2OCo^{III}B_{12}$ is observed but in less than stoichiometric amounts. Control experiments demonstrate that a 2,3-dihydroxy aldehyde is able to reduce $H_2OCo^{III}B_{12}$ to $Co^{II}B_{12}$ at kinetically competent rates. This observation and three tests for the presence of reducing sugars provided indirect evidence that the sugar heterolysis product, 2,3-dihydroxy-4-pentenal (DHP), is also produced in the reaction. The minimum mechanism required to account for the observed products is presented in Scheme V.

Scheme V is also consistent with and fully supported by the observed kinetic behavior within the pH range of 8–4. The presence of two parallel, first-order pathways is consistent with the observed overall first-order disappearance of $AdoB_{12}$. In accord with eq 3, $k_{obsd} = k_h + k_{het}$ under the conditions employed in this study of rate-determining homolysis and heterolysis. In order to account for the pH dependence of k_{obsd}, a more detailed scheme is required. Investigations of other alkylcobalamins have shown that the axial benzimidazole base exerts a strong influence on the stability of the trans Co–C bond.[40] Without exception the base-on form was found to be 100–1000 times more reactive toward

(40) (a) Grate, J. H.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1979**, *101*, 4601. (b) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2274. (c) Baldwin, D. A.; Betterton, E. A.; Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1985**, 1613.

(41) (a) Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485. (b) Broadie, J. D.; Poe, M. *Biochem.* **1972**, *11*, 2534. (c) Dolphin, D. H.; Johnson, A. W.; Rodrigo, R. *Ann. N. Y. Acad. Sci.* **1964**, *3*, 200.

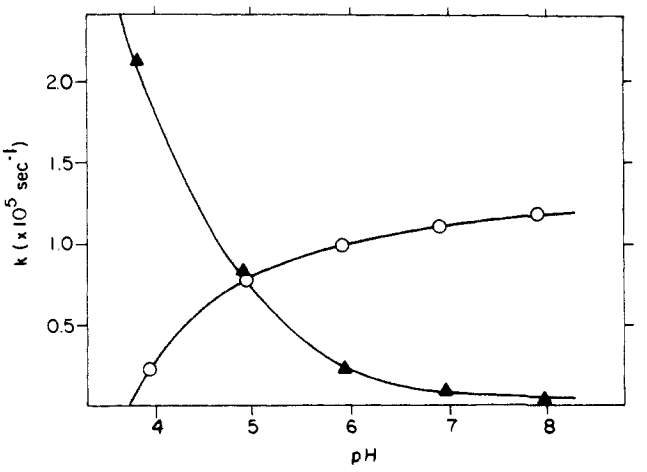


Figure 5. Plot of k_h and k_{het} components of k_{obsd} in H_2O at 85.0 °C vs. pH; ○ for k_h and ▲ for k_{het}.

homolysis than the base-off forms.[40] As shown in Scheme VI, lowering the pH of the solution would result in the shifting of the axial base equilibrium to the protonated base-off side, and thus a concomitant decrease in the rate of homolysis would be expected. The rate of heterolysis would also be expected to exhibit a pH dependence, but there are two opposing effects here. As the pH is lowered, greater amounts of base-off forms would tend to slow heterolysis, while greater amounts of protonation at the adenosyl moiety would enhance heterolysis. By combining the results of the kinetic and product studies reported herein, it is possible to estimate values of k_h and k_{het} as a function of pH.[42] The results (Figure 5) show that, as predicted, the rate of homolysis of the Co–C bond of $AdoB_{12}$ is retarded, while the rate of heterolysis is increased as the pH is decreased.

In pH 4.3 H_2O and in the absence of added scavenger, the method of initial rates employed herein yields the same (±5%) rate constants for the anaerobic thermal decomposition of $AdoB_{12}$ as were previously reported in pH 4.3 H_2O in the presence of (1–6) × 10^{-3} M $Co^{II}(DMG)_2$.[10] This demonstrates that the addition of $Co^{II}(DMG)_2$ and/or its' acid hydrolysis product does not influence the rate-determining step. *At this pH, product studies reveal that heterolysis accounts for 77% of the reaction at 85 °C and 45% of the reaction at 110 °C.* Therefore, it is not surprising that the activation parameters previously obtained in pH 4.3 H_2O reflect the presence of a bimolecular (H^+-dependent) heterolysis, $\Delta H^{\ddagger}_{obsd}$ = 26.6 ± 0.6 kcal/mol and $\Delta S^{\ddagger}_{obsd}$ = −6 ± 2 eu and that they are significantly lower than those obtained in ethylene glycol, $\Delta H^{\ddagger}_{obsd}$ = 30.6 ± 0.3 kcal/mol and $\Delta S^{\ddagger}_{obsd}$ = 2.9 ± 0.7 eu. The latter, ethylene glycol numbers were measured under conditions where homolysis was demonstrated, by both kinetic and product studies, to be the only mode of Co–C bond cleavage.[7] In water at pH 7.0, where heterolysis plays a 3–10% role in the reaction, the observed activation parameters are $\Delta H^{\ddagger}_{obsd}$ = 30.9 ± 0.5 kcal/mol and $\Delta S^{\ddagger}_{obsd}$ = 4.8 ± 0.8 eu. Corrections for 10% heterolysis at 85 °C and for 3% heterolysis at 110 °C yield the activation parameters of the Co–C bond homolysis of $AdoB_{12}$ in H_2O, 31.8 ± 0.7 kcal/mol for $\Delta H^{\ddagger}_h$ and 6.8 ± 1.0 eu for $\Delta S^{\ddagger}_h$.[43]

Because the binding of the appended, trans-axial benzimidazole base in B_{12} is thought to significantly influence the strength of the $AdoB_{12}$ Co–C bond,[2b,44] one of our research goals has been to obtain estimates of the Co–C BDE for both the base-on and base-off forms of $AdoB_{12}$. When the heterolysis pathways shown in Scheme VI are neglected, it can be shown that k_{obsd}(homolysis) = $k_h = [K_{eq}/(K_{eq}+1)]k_h$(base-on) + $[1/(K_{eq}+1)]k_h$(base-off). This equation illustrates why it is not yet possible to rigorously separate the contributions of the base-on and base-off forms in the observed rate of decomposition of $AdoB_{12}$ without an independent measurement of either k_h(base-on) or k_h(base-off). As mentioned earlier, however, the behavior of other alkylcobalamins[40] suggests that the base-on homolysis rate of $AdoB_{12}$ should be much faster than the base-off homolysis rate, i.e., k_h(base-on) ≫ k_h(base-off). If the assumption is made that the base-off form does not participate in the reaction, then k_{obsd}(homolysis) = $k_h = (K_{eq}/(K_{eq}+1))k_h$(base-on).[45] With this simplified kinetic scheme and equation, and given the temperature dependence of both k_h and K_{eq}, it is possible to calculate the activation parameters for k_h(base-on).

The required values of ΔH and ΔS for the base-off to base-on equilibrium of $AdoB_{12}$ in pH 7.0 H_2O were obtained by the same method used previously in ethylene glycol solvent.[21] The $AdoB_{12}$ visible spectrum was recorded at 5-deg intervals over the temperature range of 15–70 °C. Nonlinear regression curve fitting of the results to the appropriate equation[21] yields ΔH = −5.6 ± 0.9 kcal/mol and ΔS = −13 ± 3 eu for the base-off to base-on K_{eq} in neutral H_2O.[46] In the temperature range of interest (85–110 °C), the amount of base-on form varies from 79% (85 °C) to 69% (110 °C). Upon correcting the observed homolysis activation parameters, $\Delta H^{\ddagger}_h$ and $\Delta S^{\ddagger}_h$, for this temperature dependence of the axial base equilibrium, we obtain the *estimates* $\Delta H^{\ddagger}_h$(base-on) = 33 ± 2 kcal/mol and $\Delta S^{\ddagger}_h$(base-on) = 11 ± 3 eu, values which are, within error, identical with those obtained in ethylene glycol. As discussed elsewhere,[7] the Co–C BDE is given by BDE = $\Delta H^{\ddagger}_h$(base-on) − $\Delta H^{\ddagger}_r$, which is approximated by $\Delta H^{\ddagger}_h$(base-on) − (3 ± 1) kcal/mol, *yielding 30 ± 2 kcal/mol as the first reliable estimate* of the base-on Co–C BDE of $AdoB_{12}$ in aqueous solution. Comparison of this aqueous value to the 31.5 ± 1.3 kcal/mol value obtained in ethylene glycol demonstrates that the Co–C BDE is not significantly different in these solvents.

In future publications we will report an independent measurement of the rate constant for base-off homolysis (k_h(base-off), Scheme VI), thereby testing the assumption of kinetic nonparticipation of the base-off form used to obtain all $AdoB_{12}$ Co–C BDE estimates to date.[45] In addition to the axial base effects, we have initiated studies aimed at identifying and quantifying the steric, electronic, enzymic, and other effects[47] which are thought to contribute to the ≥10^{10} activation of the $AdoB_{12}$ Co–C bond in vivo.

Acknowledgment. Prof. T. Koenig and D. Tyler at Oregon and several colleagues at other institutions provided constructive criticism on this manuscript; their help is gratefully acknowledged. Financial support was provided by NIH Grant AM-26241. R.G.F. is a Dreyfus Teacher–Scholar (1982–1987) and a Guggenheim Fellow (1985–1986).

Registry No. $AdoB_{12}$, 13870-90-1; T-Ado, 89959-02-4; TEMPO, 2564-83-2.

Supplementary Material Available: Figure A consisting of typical kinetic plots, Figure B consisting of example HPLC traces for the aqueous nucleoside products, and Figure C consisting of ln (k_{obsd}/T) vs. $1/T$ plots of the data in Table I (3 pages). Ordering information is given on any current masthead page.

(42) Assuming $k_{obsd} = k_h + k_{het}$ yields k_h = (fraction of C-Ado)k_{obsd} and k_{het} = (fraction of adenine)k_{obsd}. This treatment is not rigorous due to the decreases in k_h as the [$Co^{II}B_{12}$] becomes appreciable; it yields slightly high values of k_{het} and low values of k_h. However, simulations show that the deviations do not significantly alter the observed trends shown in Figure 5.

(43) Assuming that $k_h \approx$ (fraction of C-Ado)k_{obsd}, the best-fit line through the ln (k_{obsd}/T) vs. $1/T$ plot was adjusted at the end points for 3% heterolysis at 110 °C and 10% heterolysis at 85 °C.

(44) (a) Summers, M. F.; Toscano, P. J.; Bresciani-Pahor, T.; Nardin, G.; Randaccio, L.; Marzilli, L. G. *J. Am. Chem. Soc.* **1983**, *105*, 6259. (b) Glusker, J. P. In B_{12}; Dolphin, D., Ed.; Wiley-Interscience: New York, 1982; Chapter 3. (c) Pratt, J. M. *J. Mol. Catal.* **1984**, *23*, 187 and references therein.

(45) The same assumption was used to obtain both of the previous estimates of the base-on Co–C BDE.[7,10]

(46) (a) The values of ΔH and ΔS obtained for the axial base equilibrium of $AdoB_{12}$ in neutral aqueous solution fall within the range of ΔH = 3–6 kcal/mol and ΔS = 8–16 eu reported for other alkylcobalamins in aqueous solution obtained by a similar technique.[46b] Values found in ethylene glycol were significantly different, with ΔH = −7.6 ± 0.2 kcal/mol and ΔS = −20.2 ± 0.7 eu.[7] (b) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2267.

(47) There is increasing evidence that a radical chain mechanism may be involved in at least some of the B_{12}-dependent reactions, a point we are currently investigating.