

Hydrolytic Dealkylation of Organocobaloximes Related to Coenzyme B<sub>12</sub>: Ligand Influences

Michael P. Jensen, Diana M. Zinkl, and Jack Halpern\*

Department of Chemistry, University of Chicago, 5735 South Ellis Avenue, Chicago, Illinois 60637

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The organocobaloxime complexes R–Co(DH)<sub>2</sub>(L) (R = 5'-deoxyadenosyl, Ado; 2',5'-dideoxyadenosyl, 2'dAdo; tetrahydrofurfuryl, THFF; L = 4-*tert*-butylpyridine, 4-*t*BuPy; OH<sub>2</sub>) were prepared and characterized (<sup>1</sup>H NMR, UV–vis spectroscopy). Rates of Co–C bond hydrolysis were determined as a function of temperature and pH (range 2.7–5.5), and products were identified. Rates of THFF–Co(DH)<sub>2</sub>(OH<sub>2</sub>) and Ado–Co(DH)<sub>2</sub>(OH<sub>2</sub>) dealkylation were fitted to a superposition of acid-dependent and -independent paths,  $k_{\text{obs}} = k_0 + k'[\text{H}^+]$ . Both pathways are hydrolytic, producing [Co<sup>III</sup>(DH)<sub>2</sub>(OH<sub>2</sub>)<sub>2</sub>]<sup>+</sup> and products derived from ring-opening protonation of the organic ligand (4-pentenol or adenine and 2,3-dihydroxy-4-pentalenol, respectively). For THFF–Co(DH)<sub>2</sub>(OH<sub>2</sub>),  $k_0 = 6.5 \times 10^{-6} \text{ s}^{-1}$  and  $k' = 1.2 \text{ M}^{-1} \text{ s}^{-1}$  at 50.4 °C; for Ado–Co(DH)<sub>2</sub>(OH<sub>2</sub>),  $k_0 = 1.9 \times 10^{-5} \text{ s}^{-1}$  and  $k' = 0.66 \text{ M}^{-1} \text{ s}^{-1}$  at 97.0 °C. In contrast, decomposition of 2'dAdo–Co(DH)<sub>2</sub>(OH<sub>2</sub>) proceeds predominantly by cleavage of the nucleosidyl glycosidic bond,  $k' = 0.34 \text{ M}^{-1} \text{ s}^{-1}$  at 60.2 °C. Finally, the use of [Co<sup>II</sup>(DH)<sub>2</sub>(OH<sub>2</sub>)<sub>2</sub>] as a radical trap in the kinetic determination of the Co–C bond dissociation enthalpy of coenzyme B<sub>12</sub> is assessed and validated.

## Introduction

Elucidation of the factors governing the reactivity of the cobalt–carbon bond of coenzyme B<sub>12</sub> and its analogues continues to warrant attention. Two distinct acid-catalyzed pathways of hydrolysis of such cobalt–carbon bonds are now known, both of which are significantly inhibited, when compared with selectively substituted analogues, by the hydroxyl and adenine substituents on the 5'-deoxyribofuranosyl ring of coenzyme B<sub>12</sub>.<sup>1</sup> Similar hydrolysis reactions are observed for a wide variety of β-substituted alkyl–metal complexes.<sup>2</sup> The research described in this paper was directed at examining the hydrolytic reactivities of 5'-deoxyadenosyl- and related organocobaloximes [R–Co(DH)<sub>2</sub>(OH<sub>2</sub>), DH<sub>2</sub> = dimethylglyoxime, R = 5'-deoxyadenosyl (Ado), 2',5'-dideoxyadenosyl (2'dAdo), and tetrahydrofurfuryl (THFF) (Scheme 1)] and comparing them with those of the corresponding organocobalamins reported earlier.<sup>1</sup>

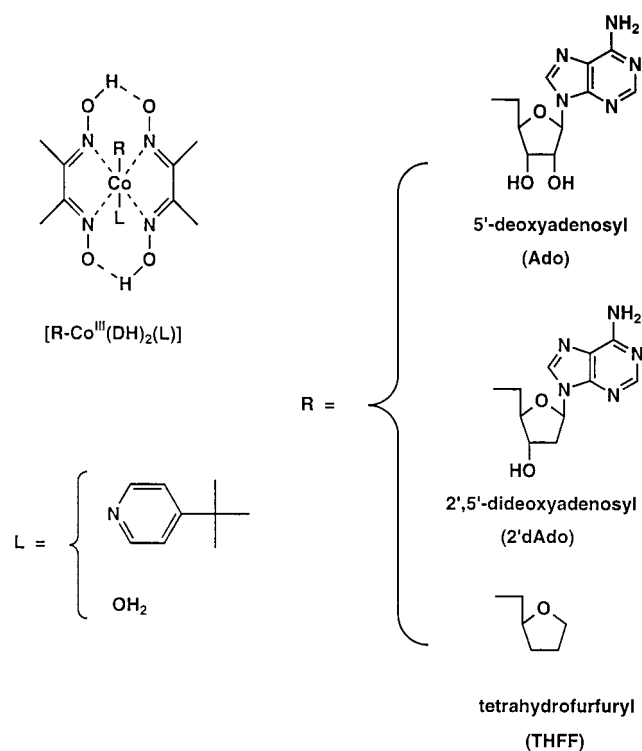
Organocobaloxime complexes have frequently been invoked as “models” for coenzyme B<sub>12</sub>,<sup>3</sup> and some information is available regarding their reactivity.<sup>4–14</sup> Alkyl complexes with β-alkoxide substituents, including Ado–Co(DH)<sub>2</sub>(OH<sub>2</sub>), typi-

cally undergo hydrolysis to generate equivalent amounts of alcohol or aldehyde, olefin, and cobalt(III).<sup>4–6,15</sup> Prior studies of the hydrolytic decomposition of Ado–Co(DH)<sub>2</sub>(OH<sub>2</sub>) were conducted at high acidities in order to achieve convenient rates at room temperature, precluding direct comparison with available corresponding data for Ado–B<sub>12</sub>, which were obtained at lower acidities.<sup>1,15–17</sup> The present study addresses this comparison by extending the range of measurements on the hydrolytic decomposition of Ado–Co(DH)<sub>2</sub>(OH<sub>2</sub>) to higher pH.

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## Scheme 1



Supporting evidence also is provided for the validity of an earlier kinetic determination of the Co–C bond dissociation enthalpy of coenzyme B<sub>12</sub>, in which [Co<sup>I</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>] was used to trap the Ado• radical.<sup>15</sup>

Portions of this work have been reported previously.<sup>16</sup>

## Experimental Section

**General Methodology.** The general experimental procedures have been described previously.<sup>1</sup> <sup>1</sup>H NMR spectra were referenced to residual organic solvent signals (CHCl<sub>3</sub> = 7.29 ppm, CHD<sub>2</sub>OD = 3.30 ppm, DMSO-*d*<sub>5</sub> = 2.49 ppm).

**Materials.** The following reagents were obtained from the indicated commercial sources: adenine, dimethylglyoxime, DL-glyceraldehyde, dimethyl sulfide, 4-*tert*-butyl pyridine (4-*t*BuPy), *p*-toluenesulfonyl chloride, 2'-deoxyadenosine, 5'-iodo-5'-deoxyadenosine, acetic acid-*d*<sub>4</sub>, and sodium acetate-*d*<sub>3</sub> were obtained from Aldrich; methanol-*d*<sub>4</sub>, deuterium oxide, and dimethyl sulfoxide-*d*<sub>6</sub>, all from Cambridge Isotope Labs; coenzyme B<sub>12</sub> from Sigma; tetrahydrofurfuryl bromide from Lancaster; and standard buffers from Fisher.

Literature syntheses were utilized to prepare 5'-tosyl-2'-deoxyadenosine,<sup>18</sup> [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>],<sup>19</sup> and [BrCo<sup>III</sup>(DH)<sub>2</sub>(4-*t*BuPy)].<sup>20</sup>

**Synthesis of Organocobalt Complexes.** Reliable routes to organocobaloximes by reaction of nucleophilic [Co<sup>I</sup>(DH)<sub>2</sub>]<sup>−</sup> complexes with tosylate esters and alkyl halides have been described previously,<sup>19,20</sup> including specific preparations of Ado–Co(DH)<sub>2</sub>(Py)<sup>5,15,21</sup> and THFF–Co(DH)<sub>2</sub>(Py).<sup>7c,8b</sup> These were employed with the modest adaptations described below.

**Synthesis of Ado–Co(DH)<sub>2</sub>(4-*t*BuPy).** [BrCo(DH)<sub>2</sub>(4-*t*BuPy)] (200 mg, 0.40 mmol), KOH (87 mg, 1.55 mmol), and NaBH<sub>4</sub> (30 mg, 0.79 mmol) were blended with a magnetic stirbar in a 100 mL Schlenk flask equipped with a gas-inlet sidearm. The flask was capped with a y-tube,

which was capped in turn with a rubber septum and with a tube containing 5'-iodo-5'-deoxyadenosine (183 mg, 0.49 mmol). The atmosphere was replaced three times with N<sub>2</sub>, and degassed MeOH (10.0 mL) was injected through the septum, with care taken not to moisten the iododeoxynucleoside sample. The mixture was stirred with the sidearm open to a purging manifold in order to relieve H<sub>2</sub> pressure. The initially brown solution turned dark blue over a 15 min period as reduction of cobaloxime progressed. The iodoadenosine sample was then tipped into the reaction mixture, and the solution color faded to orange-brown within seconds. The mixture was stirred for an additional 5 min, and the flask was opened to air. The solution was loaded onto a silica gel column (2 cm diameter × 20 cm length) and eluted with 1:1 MeOH/acetone, resulting in development of a golden yellow band of alkylated product ahead of brown unalkylated material. The yellow band was collected and evaporated to dryness. The resulting yellow oils were redissolved in MeOH (3.0 mL), and more 4-*tert*-butylpyridine was added (60 μL, 0.41 mmol). Water (4–7 volume equivalents) was added slowly until the shaken solution was persistently cloudy. The solution was allowed to stand at 4 °C to precipitate a bright yellow crystalline powder. The product was isolated by filtration, washed with cold water, and air-dried to constant weight. Yield: 80 mg (33%). The <sup>1</sup>H NMR (Figure 1) and UV–visible spectra were consistent with the assigned structure. UV–visible, λ<sub>max</sub> (ε, M<sup>−1</sup> cm<sup>−1</sup>): MeOH, 441 nm (780), 373 nm (1510, sh). <sup>1</sup>H NMR, CD<sub>3</sub>OD (intensity, multiplicity, apparent *J*, assignment): 8.36 ppm (2H, d, 6.7 Hz, py); 8.21 ppm (1H, s, H2 or H8); 8.19 ppm (1H, s, H8 or H2); 7.43 ppm (2H, d, 6.7 Hz, py); 5.85 ppm (1H, d, 5.5 Hz, H1'); 4.65 ppm (1H, "t", 5.3 Hz, H2'); 3.83 ppm (1H, "t", 4.3 and 4.8 Hz, H3'); 3.76 ppm (1H, d of "t", 9.8 and 3.4 Hz, H4'); 2.12 ppm (1H, "t", ±9.5 Hz, H5A'); 2.05 ppm (6H, s, DH<sub>A</sub>); 1.92 ppm (6H, s, DH<sub>B</sub>); 1.44 ppm (1H, d of d, 2.9 and −9.9 Hz, H5B'); 1.27 ppm (9H, s, *t*Bu); additional minor resonances due to ca. 8% solvolysis of Ado–Co(DH)<sub>2</sub>(CH<sub>3</sub>OH) and free 4-*t*BuPy.

**Synthesis of THFF–Co(DH)<sub>2</sub>(4-*t*BuPy).** Solid samples of [BrCo(DH)<sub>2</sub>(4-*t*BuPy)] (325 mg, 0.66 mmol), KOH (181 mg, 3.23 mmol), and NaBH<sub>4</sub> (59 mg, 1.56 mmol) were placed in a 100 mL Schlenk flask containing a magnetic stirbar. The flask was capped with a rubber septum, and the atmosphere was replaced three times with nitrogen. Degassed methanol (10.0 mL) was injected through the syringe, and the reaction was stirred 15 min with the sidearm open to a purging nitrogen manifold. Racemic tetrahydrofurfuryl bromide was injected into the dark blue solution, which immediately turned orange in color. The solution was stirred an additional 5 min, and acetone (1.0 mL) was added under air. The solution was charged onto a silica gel column; elution with 1:1 (v/v) MeOH/acetone developed a yellow band of alkylated material. This band was collected and evaporated to dryness on a rotary evaporator. The crude oils were redissolved in methanol (15 mL). Addition of water (45 mL) produced a cloudy solution, and yellow crystals formed upon standing at 4 °C. These were recovered by filtration and air-dried to constant weight. Yield: 88 mg (27%). <sup>1</sup>H NMR (Figure 1) and UV–visible spectra were consistent with the formulation. UV–visible, λ<sub>max</sub> (ε, M<sup>−1</sup> cm<sup>−1</sup>): MeOH, 433 nm (830), 386 nm (1820, sh). <sup>1</sup>H NMR, CD<sub>3</sub>OD (intensity, multiplicity, apparent *J*, assignment): 8.38 ppm (2H, d, 7.7 Hz, py); 7.45 ppm (2H, d, py); 3.7 ppm (1H, m); 3.5 ppm (2H, m); 2.14 ppm (6H, s, DH<sub>A</sub>); 2.13 ppm (6H, s, DH<sub>B</sub>); 1.8 ppm (4H, m); 1.27 ppm (9H, s, *t*Bu).

**Synthesis of 2'dAdo–Co(DH)<sub>2</sub>(4-*t*BuPy).** Samples of [BrCo(DH)<sub>2</sub>(4-*t*BuPy)] (207 mg, 0.42 mmol), 5'-tosyl-2'-deoxyadenosine (200 mg, 0.80 mmol), KOH (103 mg, 1.84 mmol), and NaBH<sub>4</sub> (48 mg, 1.27 mmol) were dissolved in degassed methanol (10 mL) under a nitrogen atmosphere. The solution was stirred for 2 h and then charged onto a silica gel column. A yellow band was eluted with 1:1 MeOH/acetone. This band was collected and evaporated to dryness to give the product as a crude yellow oil. UV–visible, λ<sub>max</sub>: MeOH, 427 nm, 372 nm.

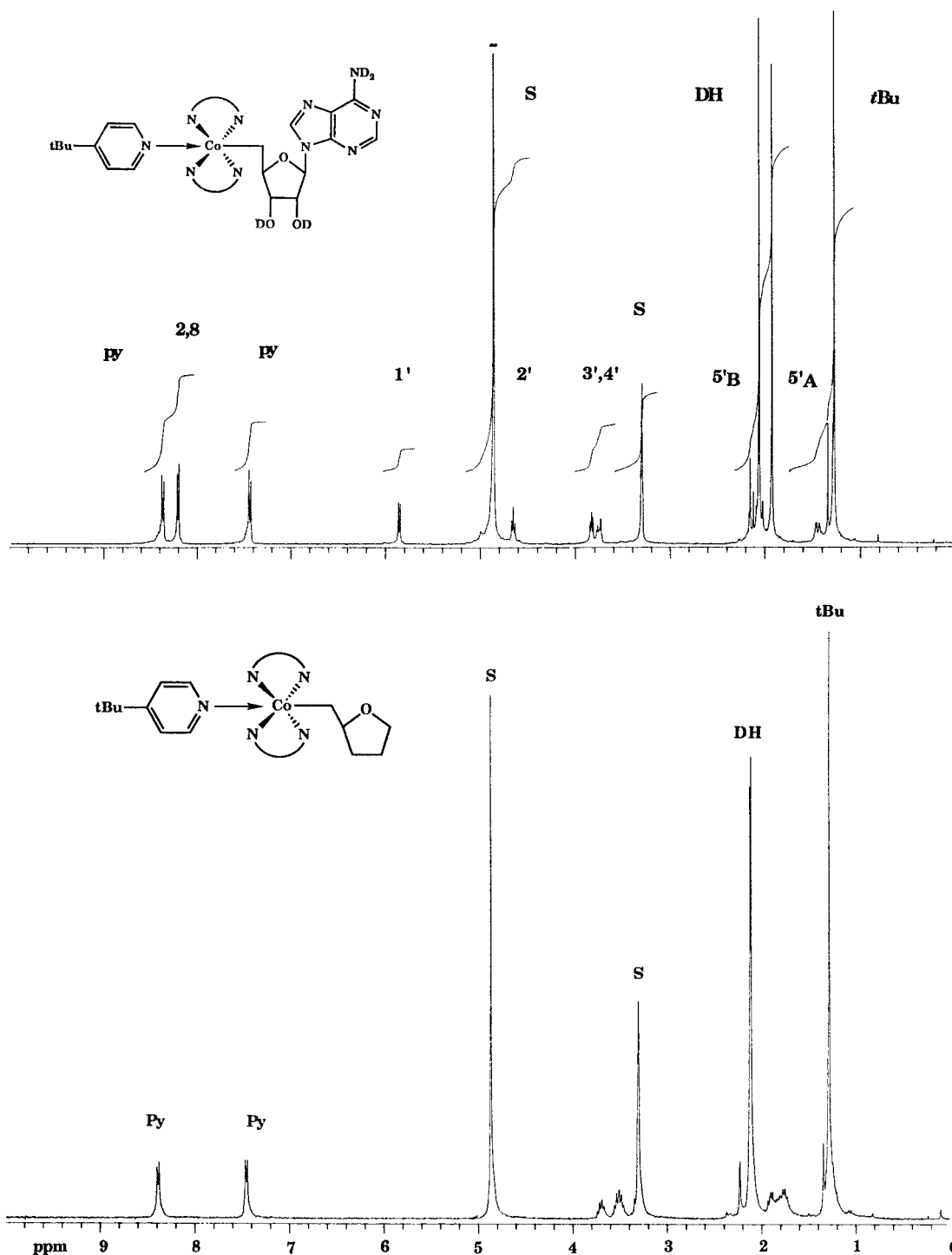
**Preparation of Ado–Co(DH)<sub>2</sub>(OH)<sub>2</sub>.** A sample of Ado–Co(DH)<sub>2</sub>(4-*t*BuPy) (20 mg) was dissolved in MeOH (3.0 mL). Water (3.0 mL) was added to the yellow solution, which was then extracted with aliquots (6 × 50 mL) of *n*-hexane. An emulsive layer rich in free 4-*tert*-butylpyridine formed between the aqueous and organic phases and was carefully separated and discarded. The empty separatory funnel was thoroughly washed with fresh methanol between extractions. A noticeable darkening of the aqueous layer signaled successful removal

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**Figure 1.**  $^1\text{H}$  NMR spectra of Ado-Co(DD) $_2$ (4-*t*BuPy) (top) and THFF-Co(DD) $_2$ (4-*t*BuPy) (bottom) in methanol- $d_4$  solutions.

of the axial base. Additional water (15 mL) was added to the aqueous phase without clouding, and methanol was removed by reduction of the solution volume to ca. 5 mL in a rotary evaporator. The concentrated aqueous solution deposited orange crystals upon standing, but these were quite insoluble in water without addition of a donor ligand. Therefore, the solution was diluted to desired working concentrations.  $^1\text{H}$  NMR and UV-visible spectra demonstrated that removal of the 4-*tert*-butylpyridine ligand had occurred. UV-visible,  $\lambda_{\text{max}}$  ( $\epsilon$ ,  $\text{M}^{-1}\text{cm}^{-1}$ ):  $\text{H}_2\text{O}$ , 443 nm (840), 372 nm (1240).  $^1\text{H}$  NMR,  $\text{CD}_3\text{OD}$  (intensity, multiplicity, apparent  $J$ , assignment): 8.19 (1H, s, H2 or H8); 8.17 (1H, s H8 or H2); 5.82 (1H, d, 5.7 Hz, H1'); 3.80 (1H, "t", 4.4 Hz, H3'); 3.52 (1H, d of "t", 9.7 Hz, H4'); 2.16 (6H, s,  $\text{DH}_A$ ); 2.01 (6H, s,  $\text{DH}_B$ ); 1.85 (1H, "t",  $\pm 9.5$  Hz,  $\text{H5}'_A$ ); 1.44 (1H, d of d,  $-9.9$  Hz,  $\text{H5}'_B$ ); H2' resonance obscured by residual HOD.

**Preparation of Other Organocobaloxime Aquo Complexes.** The procedure described for generation of Ado-Co(DH) $_2$ (OH) $_2$  stock solutions was used also to prepare the THFF and 2'dAdo complexes. Removal of the axial base was clearly indicated by changes in the visible spectrum analogous to those quantified for the 5'-deoxyadenosyl complex; therefore, further characterization of the aquo compounds was not pursued, and stock solutions were prepared by direct dilution of the aqueous phases after hexane extraction and methanol evaporation.

**Kinetic Measurements.** Aliquots of stock solutions of (aquo) organocobaloximes in neutral aqueous solutions (140–420  $\mu\text{M}$ ) were diluted 1:1 with citrate (0.2 M, pH 2.6–3.5) or acetate (0.10 M, pH 3.5–5.6) buffers. The solutions were degassed and loaded into Schlenk cuvettes under an active  $\text{N}_2$  purge. Samples were heated to obtain convenient reaction rates as described for the cobalamins,<sup>1</sup> either

**Table 1.** Fit of Observed Rates for Organocobaloxime Dealkylation

THFF–Co(DH) <sub>2</sub> (OH) <sub>2</sub> , 50.4 °C			
pH	10 <sup>5</sup> <i>k</i> <sub>obs</sub> , s <sup>-1</sup> <sup>a</sup>	10 <sup>5</sup> <i>k</i> <sub>obs</sub> , s <sup>-1</sup> <sup>b</sup>	10 <sup>5</sup> <i>k</i> <sub>calc</sub> , s <sup>-1</sup>
2.67		311	249
2.78		191	193
2.97		152	125
3.02		86.5	111
3.11		105	90.7
3.18		97.2	77.3
3.32		55.4	56.2
3.53	30.0	28.1	34.9
3.53		27.4	34.9
3.57	32.9	30.2	31.9
3.57	32.4	31.2	31.9
3.94	12.3		14.0
3.94		12.8	14.0
3.94		12.3	14.0
4.28	8.18		6.74
4.28		7.70	6.74
4.28		7.34	6.74
4.74	2.45		2.76
4.74	3.04		2.76
4.74		3.24	2.76
5.17	1.37		1.43
5.48	1.04		1.03

$k' = 1.16 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_0 = 6.50 \times 10^{-6} \text{ s}^{-1}$

Ado–Co(DH) <sub>2</sub> (OH) <sub>2</sub> , 97.0 °C		
pH	10 <sup>5</sup> <i>k</i> <sub>obs</sub> , s <sup>-1</sup>	10 <sup>5</sup> <i>k</i> <sub>calc</sub> , s <sup>-1</sup>
3.50	24.2	22.8
3.70	14.8	15.1
3.95	8.80	9.30
4.10	6.84	7.13
4.43	4.65	4.33
4.80	2.89	2.92

$k' = 6.62 \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_0 = 1.87 \times 10^{-5} \text{ s}^{-1}$

<sup>a</sup> Determined by [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> reduction/subtraction. <sup>b</sup> Determined from stable endpoint.

in the spectrophotometer cell block (<60 °C) or in an oil bath. Decompositions were monitored at 443 nm. Under conditions where the immediate [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> product was unstable to reduction on the time scale of the dealkylation reaction, the observed absorption values at 443 nm were corrected for the contribution from this species. This was done by multiplying the difference between recorded absorptions at 360 and 380 nm, observed to be negligible for organocobaloximes, by 0.854, the independently observed ratio of the absorption change between these wavelengths to that at 443 nm in the spectrum of exhaustively oxidized Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>. Under conditions where [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> was observed to be unstable to base hydrolysis,<sup>12</sup> glyceraldehyde was added to induce rapid reduction.<sup>11</sup> Addition of glyceraldehyde at low pH, where autoxidation is negligible, did not affect the observed rates, whether estimated (Table 1) by subtraction of [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> absorption (method A) or estimated by use of the stable endpoint (method B). The absorption value at 443 nm was further corrected for any precipitation of free DH<sub>2</sub> by monitoring the baseline absorption at 700 nm, where absorption by all cobaloxime complexes is negligible. The final, corrected absorption values gave excellent first-order plots in all cases.

**Identification of Ado–Co(DH)<sub>2</sub>(OH)<sub>2</sub> Decomposition Products.** Direct observation of decomposition products by <sup>1</sup>H NMR spectroscopy was not feasible, due to the inability to generate concentrated D<sub>2</sub>O solutions of the complex on a practical scale. Instead, the complex was decomposed in ordinary water buffered with acetate-*d*<sub>3</sub>, and the products were then extracted into deuterated organic solvents for NMR characterization. The formation of the predicted products was confirmed by this procedure, but quantitative yields could not be determined.

An aqueous solution of Ado–Co(DH)<sub>2</sub>(OH)<sub>2</sub> (5.8 mM) was diluted with four volume equivalents of aqueous (H<sub>2</sub>O) buffer solution (25 mM CD<sub>3</sub>CO<sub>2</sub><sup>-</sup>/CD<sub>3</sub>CO<sub>2</sub>D) and degassed with a stream of nitrogen. The solution was then transferred by cannula under N<sub>2</sub> into a 100 mL

Schlenk flask equipped with a sidearm and a high-vacuum valve. The flask was sealed and immersed in a 100 °C oil bath. The solution was heated for a period of time corresponding to six half-lives of the dealkylation reaction, as calculated from kinetic measurements. The resulting pale yellow-brown solution was cooled and poured into a round-bottom flask under air. The water was removed in a rotary evaporator, and the solids were dried under vacuum at 75 °C over P<sub>2</sub>O<sub>5</sub>. The dried, pale brown solids were extracted into DMSO-*d*<sub>6</sub>, and the <sup>1</sup>H NMR spectrum was recorded.

Isolation of purine product(s) was accomplished alternatively by extraction of the solids produced as above with EtOH. The extracts were loaded onto a silica gel column (2 cm diameter × 17 cm length), and 10.0 mL fractions were eluted with additional EtOH. UV absorbance was measured at 260 nm for each fraction; a single band was observed, and appropriate fractions were pooled. The solution was evaporated to dryness to give pale white solids, which were further dried over P<sub>2</sub>O<sub>5</sub> at 75 °C in vacuo. The dried solids were extracted completely into DMSO-*d*<sub>6</sub>, and the <sup>1</sup>H NMR spectrum of the resulting solution was recorded. Observed spectral features were identical to those of an authentic sample of adenine: 8.04 (1H, s); 8.00 (1H, s); 7.3 ppm (2H, br). A yield of 24% was calculated, based on integration before and after addition of a known quantity of authentic adenine, and also by total UV absorption at 260 nm of the eluted band. Furthermore, a yield of 90% relative to a control separation of adenine and air-oxidized Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub> also was calculated from UV absorption: λ<sub>max</sub>, nm (ε<sub>max</sub>, 10<sup>3</sup> M<sup>-1</sup> cm<sup>-1</sup>); 207 (22.0), 261 (14.0).

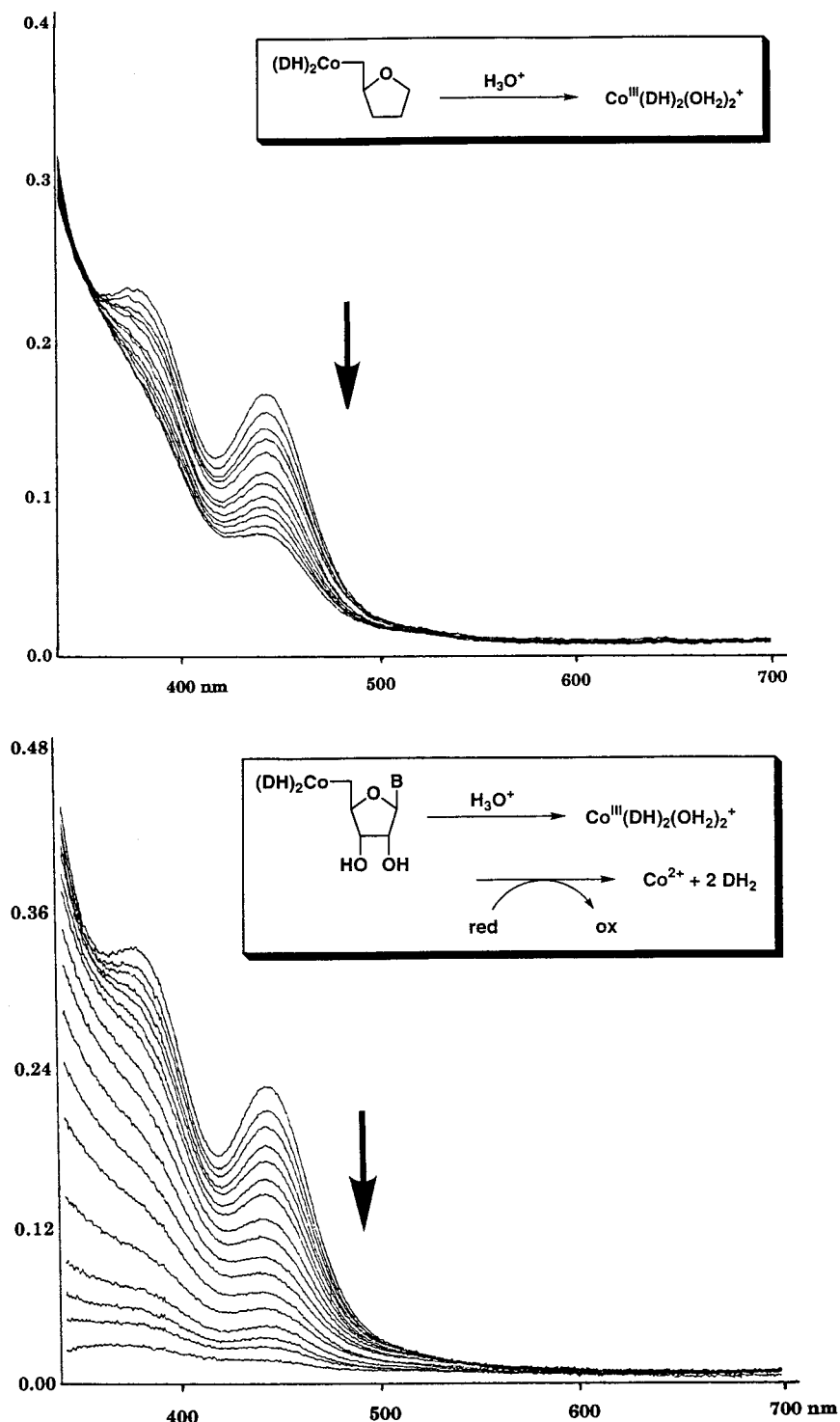
**Control Reductions of Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>.** Samples of Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>, 11–15 mg, were loaded into 100 mL round-bottom flasks and dissolved in ca. 50 mL of neutral water. The solutions were stirred until the initially orange-brown color faded to light yellow upon oxidation of the complex. Aliquots were then adjusted to pH 4.30 by addition of a volume equivalent of buffer solution (0.10 M acetate). Various reductants were added, notably glyceraldehyde and dimethylglyoxime, and aliquots were degassed with nitrogen and loaded into Schlenk cuvettes. The anaerobic samples were heated to 100 °C in an oil bath. Progress of the reductions was monitored periodically by UV–visible spectroscopy, after rapid quenching of the reaction by immersion of the cuvette into ice water.

## Results and Discussion

The organocobaloxime complexes identified in Scheme 1 were isolated as 4-*tert*-butylpyridine adducts following nucleophilic alkylation of [Co<sup>I</sup>(DH)<sub>2</sub>]<sup>-</sup>. The alkylations, particularly with tosylate esters, typically were more sluggish than those of cob(I)alamin, and yields of alkylated material were modest. Nevertheless, UV–visible spectra of isolated products were consistent with clean alkylation in each case, and the Ado–Co(DH)<sub>2</sub>(4-*t*BuPy) and THFF–Co(DH)<sub>2</sub>(4-*t*BuPy) complexes were rigorously characterized by <sup>1</sup>H NMR spectroscopy, Figure 1. The axial pyridine ligands were removed by repetitive hexane extraction of neutral aqueous solutions to yield solutions of the desired aquo complexes.

**Products.** Warming under moderately acidic conditions, ca. pH 2.7–5.5, resulted in thermolysis of the complexes. For THFF–Co(DH)<sub>2</sub>(OH)<sub>2</sub>, this was accompanied by a color change from golden yellow to yellow-brown, whereas initially yellow solutions of Ado–Co(DH)<sub>2</sub>(OH)<sub>2</sub> underwent bleaching when heated. The reactions were conveniently monitored by UV–visible spectroscopy near 50 and 97 °C, respectively (Figure 2). The spectroscopic results confirm that decomposition of THFF–Co(DH)<sub>2</sub>(OH)<sub>2</sub> proceeds through hydrolytic Co–C heterolysis to produce [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup>. On the other hand, while [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> also is the initial product of thermolysis of Ado–Co(DH)<sub>2</sub>(OH)<sub>2</sub>, further reduction, apparently by 2,3-dihydroxy-4-pentenal,<sup>11</sup> an expected coproduct, results in the release of free Co<sup>2+</sup> (aq) and 2 equiv of dimethylglyoxime.<sup>13,14</sup> The [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> concentration exhibits a





**Figure 2.** UV-visible spectral changes accompanying dealkylations of THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> at 50.4 °C, pH 4.28 (top), and Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> at 97.0 °C, pH 3.50 (bottom, observed at 20 °C).

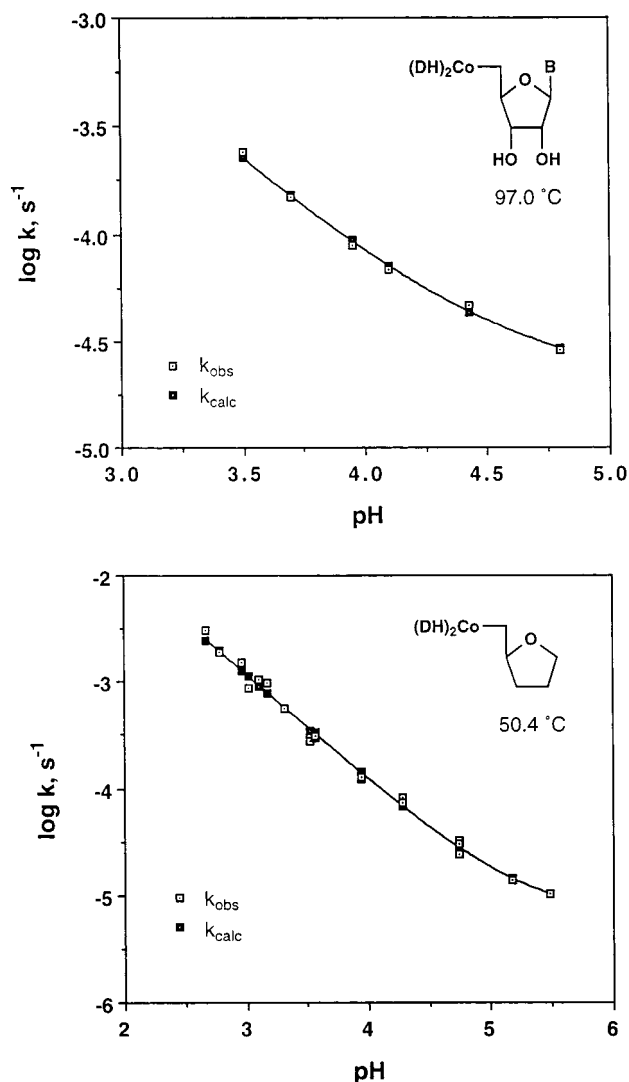
bell-shaped time dependence consistent with reduction by a species that accumulates as the reaction proceeds. Control experiments confirmed that [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup>, generated by aerobic oxidation of [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>], was rapidly reduced under N<sub>2</sub> at pH 4.3, 100 °C by glyceraldehyde, a close structural mimic of 2,3-dihydroxy-4-pentanal.<sup>17</sup> Addition of glyceraldehyde also resulted in reduction of the [Co<sup>III</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]<sup>+</sup> product of thermolysis of THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub>.

The organic products of decompositions of Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> and THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> (ca. pH 4.3) were consistent

with those obtained from acid-catalyzed hydrolysis of the corresponding organocobalamins.<sup>1</sup>

Following decomposition at pH 4.3, 50 °C, a solution of THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> was extracted with CDCl<sub>3</sub>; <sup>1</sup>H NMR spectroscopy confirmed the presence of 4-pentenol in the extracts.<sup>1</sup>

A sample of Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> was decomposed at 100 °C, pH 4.2, and the dried residue, following evaporation, was extracted with DMSO-*d*<sub>6</sub>. The resulting <sup>1</sup>H NMR spectrum, although broadened by the presence of paramagnetic Co<sup>2+</sup> ions,



**Figure 3.** Observed and calculated dealkylation rates as a function of pH for  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$  at  $97.0^\circ\text{C}$  (top) and  $\text{THFF-Co}(\text{DH})_2(\text{OH})_2$  at  $50.4^\circ\text{C}$  (bottom) in anaerobic solutions. Data are from Table 1.

exhibited two doublets at 5.22 ppm ( $^3J_{\text{HH}} = 17.3$  Hz) and at 5.08 ppm ( $^3J_{\text{HH}} = 10.5$  Hz) and a seven-peak multiplet (1:1:1:2:1:1:1) at 5.9 ppm ( $^3J_{\text{H}_3-\text{H}_4} = 5.2$  Hz). These features are nearly coincident with those of the spectrum of 2,3-dihydroxy-4-pentenal in  $\text{D}_2\text{O}$  produced by hydrolysis of coenzyme  $\text{B}_{12}$ .<sup>1</sup> The spectrum lacked a corresponding aldehyde resonance, as anticipated, due to oxidation by  $[\text{Co}^{\text{III}}(\text{DH})_2(\text{OH})_2]^{+}$ .<sup>11</sup> A large, isolated singlet peak at 1.89 ppm was due to intact dimethylglyoxime.

The spectrum also contained a large number of broad, uninterpretable peaks in the downfield aromatic region. The  $^1\text{H}$  NMR spectrum of a control sample containing partially oxidized  $\text{Co}^{\text{II}}(\text{DH})_2(\text{OH})_2$  and authentic adenine (1:1) in  $\text{DMSO}-d_6$  exhibited a similar envelope of broad peaks in the 6.0–8.2 ppm region; the features assigned to 2,3-dihydroxy-4-pentenoate were notably absent. Therefore, the envelope of peaks likely results from nonspecific binding of  $\text{Co}^{2+}$  ions to adenine.<sup>22</sup> A second sample of  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$  was decomposed under the same conditions, and the putative (adenine) $\cdot\text{Co}^{2+}$  complex was extracted into ethanol. The solubility of the complex was much higher than that observed for adenine alone. Adenine was

**Table 2.** Temperature Dependences for Dealkylation of Various Complexes at pH 4.30

$T, ^\circ\text{C}$	$10^5 k_{\text{obs}}, \text{s}^{-1}$
$\text{Ado-Co}(\text{DH})_2(\text{OH})_2$	
102.8	7.29
99.8	6.32
97.4	5.68
91.8	4.17
$\text{THFF-B}_{12}$	
39.8	97.3
29.0	47.4
20.0	24.4
10.0	10.4
$\text{THFF-Co}(\text{DH})_2(\text{OH})_2$	
64.2	54.3
56.6	19.2
50.4	8.18
	7.70
	7.34
43.0	2.48

separated from cobalt chromatographically in 23% yield (90% relative to a 1:1 control mixture of adenine and oxidized  $\text{Co}^{\text{II}}(\text{DH})_2(\text{OH})_2$ ) and characterized by  $^1\text{H}$  NMR spectroscopy. No other purine-containing product was evident.

**Kinetics.** Rates of decomposition of  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$  were measured at  $97.0^\circ\text{C}$  over the pH range 3.50–4.80, and of  $\text{THFF-Co}(\text{DH})_2(\text{OH})_2$  at  $50.4^\circ\text{C}$  over the pH range 2.67–5.48 (Figure 3 and Table 1). The reactions exhibited pseudo-first-order kinetics, the pH dependence of the pseudo-first-order rate constants conforming to  $k_{\text{obs}} = k_0 + k'[\text{H}^+]$ .

Extrapolated values of  $k_0$  are several orders of magnitude larger than those calculated from the measured second-order rate constants of the base-catalyzed hydrolysis of these complexes ( $1.6 \times 10^{-3} \text{M}^{-1} \text{s}^{-1}$  at  $27^\circ\text{C}$  for  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$  and  $9.5 \times 10^{-2} \text{M}^{-1} \text{s}^{-1}$  at  $57.5^\circ\text{C}$  for  $\text{THFF-Co}(\text{DH})_2(\text{OH})_2$ ).<sup>5,8b</sup> The close approaches of pH-dependent data for both complexes to the  $k_0$  limits rule out base-catalyzed (acid-suppressed) paths, as does failure to observe the 4',5'-anhydroadenosine and  $[\text{Co}^{\text{I}}(\text{DH})_2(\text{OH})_2]^-$  products reported for base hydrolysis of  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$ .<sup>4</sup>

The fitted  $k'$  value for  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$  ( $0.66 \text{M}^{-1} \text{s}^{-1}$  at  $97.0^\circ\text{C}$ ) is nearly identical to that of  $\text{Ado-B}_{12}$  ( $0.22 \text{M}^{-1} \text{s}^{-1}$  at  $90.0^\circ\text{C}$ ). This is in contrast to a previous report that at  $27^\circ\text{C}$  in 1.0 M HCl the cobaloxime decomposed some 30 times faster than  $\text{Ado-B}_{12}$  ( $2.2 \times 10^{-3} \text{s}^{-1}$  vs  $7.4 \times 10^{-5} \text{s}^{-1}$ ).<sup>5</sup> However, under the latter conditions,  $\text{Ado-B}_{12}$  is extensively protonated, protonation having been shown to reduce rate constants of organocobalamins (i.e.,  $k'$ ) by about 30-fold;<sup>1,23</sup> no slowing is noted for  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$  even at pH 3.50, where some protonation can be reasonably anticipated. By contrast,  $k'$  for  $\text{THFF-Co}(\text{DH})_2(\text{OH})_2$ ,  $1.2 \text{M}^{-1} \text{s}^{-1}$  at  $50.4^\circ\text{C}$ , is much lower than that for  $\text{THFF-B}_{12}$  ( $6.7 \text{M}^{-1} \text{s}^{-1}$  at  $20.0^\circ\text{C}$ ). The significance of these different patterns of acid-catalyzed hydrolysis of cobalamins and cobaloximes remains to be elucidated.

To explore this comparison further, the temperature dependences of the rates of dealkylation of  $\text{Ado-Co}(\text{DH})_2(\text{OH})_2$ ,  $\text{THFF-Co}(\text{DH})_2(\text{OH})_2$ , and  $\text{THFF-B}_{12}$  were determined at pH 4.30, over the temperature ranges  $91.8$ – $102.8^\circ\text{C}$ ,  $43.0$ – $64.2^\circ\text{C}$ , and  $10.0$ – $39.8^\circ\text{C}$ , respectively (Table 2). The contributions from the acid-catalyzed pathways to the overall decomposition rates of these complexes are approximately 64% (at  $97^\circ\text{C}$ ),

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(23) Brown, K. L.; Salmon, L.; Kirby, J. A. *Organometallics* **1992**, 11, 422.

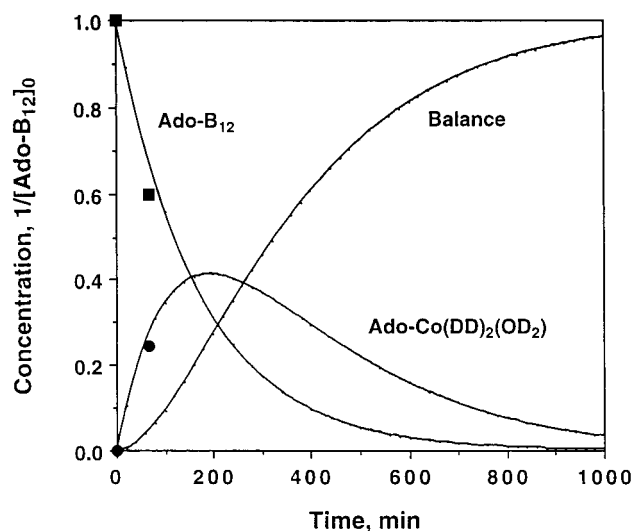
90% (at 54 °C), and 99% (at 20 °C), respectively. From the temperature dependences of the measured rates, the following apparent activation parameters were deduced: for Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub>,  $\Delta H^\ddagger_{\text{app}} = 13.2$  kcal/mol and  $\Delta S^\ddagger_{\text{app}} = -43$  cal/(mol K); for THFF-B<sub>12</sub>,  $\Delta H^\ddagger_{\text{app}} = 12.6$  kcal/mol and  $\Delta S^\ddagger_{\text{app}} = -32$  cal/(mol K); and for THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub>,  $\Delta H^\ddagger_{\text{app}} = 30.2$  kcal/mol and  $\Delta S^\ddagger_{\text{app}} = 16$  cal/(mol K). The strikingly different activation parameters for THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> compared with those for Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> and THFF-B<sub>12</sub> suggest that there may be detailed mechanistic differences among the acid-catalyzed hydrolyses of these complexes even though their overall features are similar. A possible contributor to these differences may be a greater degree of protonation of the alkyl ligand ring oxygen in THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub>, which lacks more basic sites (adenine or axial benzimidazole) that are present in the other complexes. However, because at least some of the apparent activation parameters in the comparison are composite values, the apparent differences should be interpreted with caution.

Not unexpectedly, there are similarities between the acid-catalyzed hydrolysis reactions of THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> and of HOCH<sub>2</sub>CH<sub>2</sub>-Co(DH)<sub>2</sub>(OH)<sub>2</sub>. For example, the rate of dealkylation of THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> at 25.0 °C, pH 4.30, extrapolated from the data in Table 2 is  $1.3 \times 10^{-6} \text{ s}^{-1}$ , corresponding to  $k' = 2.6 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$ , which is comparable to reported values of  $3.09 \times 10^{-2}$  and  $2.38 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$  for HOCH<sub>2</sub>CH<sub>2</sub>-Co(DH)<sub>2</sub>(OH)<sub>2</sub>.<sup>6</sup> The reported  $k'$  activation parameters for HOCH<sub>2</sub>CH<sub>2</sub>-Co(DH)<sub>2</sub>(OH)<sub>2</sub>,  $\Delta H^\ddagger = 28.4 \pm 0.5$  kcal/mol and  $\Delta S^\ddagger = 30 \pm 1$  cal/(mol K),<sup>6a</sup> are also similar to those for THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub>. As discussed previously, acid-catalyzed hydrolyses of simple  $\beta$ -substituted organocobaloximes, such as HOCH<sub>2</sub>CH<sub>2</sub>-Co(DH)<sub>2</sub>(OH)<sub>2</sub>, typically exhibit modest sensitivity to modification of the alkyl ligand.<sup>6</sup> Reaction rates apparently are limited by decomposition of a discrete, quenchable olefin intermediate present in minute steady-state concentrations.<sup>6,7</sup>

THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> and Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> also exhibit competitive acid-independent decomposition pathways. Co-C bonds of organocobaloximes are much stronger than those of cobalamins, and their homolysis rates are correspondingly much lower.<sup>10</sup> Yet the observed acid-independent limiting rate of decomposition of Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> is only ca. 4-fold lower than the homolysis rate of the predominantly base-on coenzyme at 97.0 °C, and that of THFF-Co(DH)<sub>2</sub>(OH)<sub>2</sub> is only about 12 times lower at 50 °C. These observations suggest that, whereas acid-independent  $k_0$  pathways of cobalamins correspond to Co-C bond homolysis,<sup>1</sup> those of the cobaloximes are dominated by uncatalyzed hydrolysis.

**2'dAdo-Co(DH)<sub>2</sub>(OH)<sub>2</sub>.** First-order plots for the dealkylation of 2'dAdo-Co(DH)<sub>2</sub>(OH)<sub>2</sub> at pH 4.28, 60.2 °C, also monitored spectrophotometrically, exhibited substantial initial curvature. The final limiting slope yielded a  $k'$  value of  $0.34 \text{ M}^{-1} \text{ s}^{-1}$ , close to the reported rate constant for hydrolysis of 2'-deoxyadenosine recorded under similar conditions.<sup>24</sup> This suggests that decomposition of this compound proceeds by glycosidic bond cleavage to give a 2,5-dideoxyribofuranosyl-cobaloxime intermediate. In view of the similarity of this observation to those previously reported for 2'dAdo-B<sub>12</sub>,<sup>1</sup> this reaction was not investigated in detail.

**Use of [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>] as a Free Radical Trap.** Assessment of the competition between homolysis and hydrolysis pathways of dealkylation of Ado-B<sub>12</sub> and Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> is relevant to the validity of an earlier study in which the Co-C

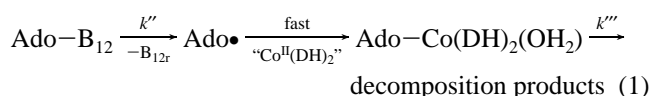


**Figure 4.** Calculated concentrations of Ado-B<sub>12</sub> and Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub>, and the ligand mass balance as a function of time during thermolysis of Ado-B<sub>12</sub> in the presence of a rate-limiting excess of [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>] radical trap at pH 4.3, 100.0 °C, calculated assuming quantitative and irreversible 5'-deoxyadenosyl radical transfer from B<sub>12</sub> to cobaloxime. Experimental data from Table 3, determined at pH 4.3, 100 °C for Ado-B<sub>12</sub> (■) and Ado-Co(DD)<sub>2</sub>(OD)<sub>2</sub> (●) are superimposed.<sup>15</sup>

bond dissociation enthalpy of Ado-B<sub>12</sub> was estimated from measurements of the kinetics of thermolysis of Ado-B<sub>12</sub> using [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>] as the Ado• radical trap.<sup>15</sup> The interpretation of these measurements is sensitive to contributions from hydrolytic decomposition of Ado-B<sub>12</sub>, (i.e., Co-C heterolysis), an upper limit to which may be deduced from the initial yield of Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> resulting from trapping of Ado• radicals by [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>]. The effectiveness of [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>] in such radical trapping was demonstrated by quantitative formation of neopentyl-Co(DH)<sub>2</sub>(OH)<sub>2</sub> and cyclopentylmethyl-Co(DH)<sub>2</sub>(OH)<sub>2</sub> when neopentyl-B<sub>12</sub> and cyclopentylmethyl-B<sub>12</sub> were decomposed in the presence of excess [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>], even under conditions (pH = 4.3) where the latter is largely dissociated into Co<sup>2+</sup> (aq) and DH<sub>2</sub>.<sup>14</sup>

Under the conditions (>90 °C, pH = 4.3) used to study the decomposition of Ado-B<sub>12</sub>, the Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> produced by trapping of Ado• by [Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub>] was hydrolytically unstable and was observed by <sup>1</sup>H NMR spectroscopy only as a transient intermediate.<sup>15</sup> Determination of the kinetics of decomposition of Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub>, as described below, permits estimations of the initial yield of the Ado• radical and, hence, of the contribution to the thermolysis of Ado-B<sub>12</sub> from the Co-C bond homolysis pathway, the magnitude of which has been the subject of earlier controversy.<sup>1,15,17</sup>

Rate constants for the decomposition of Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> ( $k''' = 7.6 \times 10^{-5} \text{ s}^{-1}$ ) and of Ado-B<sub>12</sub> with rate-limiting excess (3.0 mM) of Co<sup>II</sup>(DH)<sub>2</sub>(OH)<sub>2</sub> ( $k'' = 9.7 \times 10^{-5} \text{ s}^{-1}$ , compared with the previously reported value of  $10.0 \times 10^{-5} \text{ s}^{-1}$ )<sup>15</sup> were determined under the conditions of the earlier study (i.e., 100.0 °C, pH 4.30, 0.10 M acetate).<sup>15</sup> Using these values, normalized time-dependent concentrations of Ado-B<sub>12</sub> and Ado-Co(DH)<sub>2</sub>(OH)<sub>2</sub> were determined by fitting to the scheme of eq 1.



These were compared to previously reported concentrations determined after 65 min of reaction by <sup>1</sup>H NMR spectroscopy

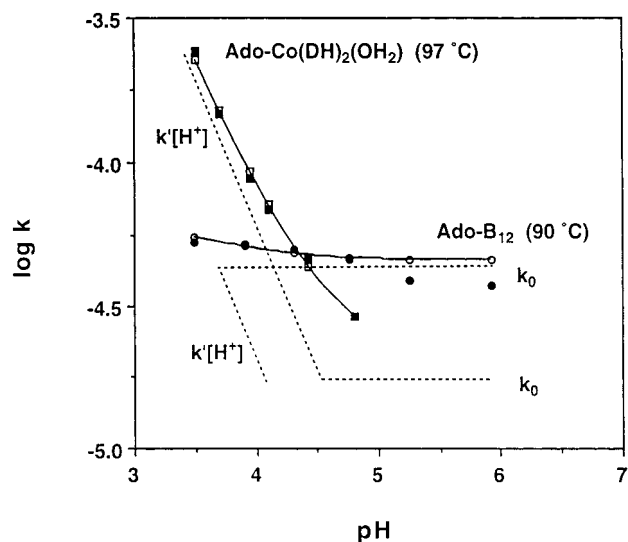
**Table 3.** Thermolysis of Ado-B<sub>12</sub> in the Presence of Excess [Co<sup>II</sup>(DD)<sub>2</sub>(OD<sub>2</sub>)]<sup>15</sup>

	[Ado-B <sub>12</sub> ] <sub>t</sub>	[Ado-Co(DD) <sub>2</sub> (OD <sub>2</sub> )] <sub>t</sub>	Σ(Ado)	ΣCo(DD) <sub>2</sub> (OD <sub>2</sub> )	ΣB <sub>12</sub>
mM, <i>t</i> = 0	2.80	0.0	2.80	2.9	2.80
mM, 65 m <sup>a</sup>	1.68	0.69	2.37	2.86	2.88
mM, calc	1.92	0.76	2.68		
% yield	87	91	88	99	101

<sup>a</sup> Determined by integration against internal standard after oxidation of Co<sup>II</sup> products, 100 °C, pD 4.3 (0.10 M CD<sub>3</sub>CO<sub>2</sub>D/CD<sub>3</sub>CO<sub>2</sub><sup>-</sup>).

(Figure 4 and Table 3).<sup>15</sup> The observed concentrations of Ado-B<sub>12</sub> and Ado-Co(DD)<sub>2</sub>(OD<sub>2</sub>) corresponded to 87% and 91%, respectively, of the calculated concentrations, with the two organocobalt compounds together accounting for 85% of the charged 5'-deoxyadenosyl ligand (88% after correction for decomposition of Ado-Co(DH)<sub>2</sub>(OH<sub>2</sub>)). The eventual products of the reaction sequence of eq 1 are predicted to be B<sub>12r</sub>, [Co<sup>III</sup>(DD)<sub>2</sub>(OD<sub>2</sub>)<sub>2</sub>]<sup>+</sup>, 2,3-dihydroxy-4-pentenal, and adenine, in agreement with an earlier report.<sup>17</sup>

These results constitute an unambiguous demonstration that the predominant decomposition pathway of Ado-B<sub>12</sub> at pD 4.3 involves net transfer of a 5'-deoxyadenosyl radical to Co<sup>II</sup>(DD)<sub>2</sub>(OD<sub>2</sub>)<sub>2</sub> in accord with eq 1 and consistent with our earlier report<sup>15</sup> of substantial inhibition of Ado-B<sub>12</sub> decomposition by B<sub>12r</sub> as predicted for a decomposition initiated by Co-C bond homolysis. Although Ado-B<sub>12</sub> and Ado-Co(DH)<sub>2</sub>(OH<sub>2</sub>) decompose with similar rates at pH 4.3, 100 °C, the kinetic pathways and reaction mechanisms clearly are quite different, Figure 5. Dealkylation of Ado-B<sub>12</sub> is substantially homolytic under these conditions, while that of Ado-Co(DH)<sub>2</sub>(OH<sub>2</sub>) is hydrolytic. The hydrolytic component of Ado-B<sub>12</sub> dealkylation under the conditions of the previously reported bond dissociation enthalpy measurement<sup>15</sup> is too small to be quantified reliably,



**Figure 5.** Comparison of Ado-B<sub>12</sub> and Ado-Co(DH)<sub>2</sub>(OH<sub>2</sub>) dealkylation rates as a function of pH in anaerobic solutions at 90.0 and 97.0 °C, respectively. Dashed lines approximate the limiting *k*<sub>0</sub> and *k'*[H<sup>+</sup>] contributions for each complex.<sup>1</sup>

and the correction of the observed activation parameters introduced by this contribution, accordingly, is expected to be small.

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