Model Studies for Coenzyme B_{12} Dependent Enzyme-Catalyzed Rearrangements. Evidence for Cobalt(III)–Olefin π Complexes

Richard B. Silverman[1a] and D. Dolphin*[1b]

Contributions from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, and the Department of Chemistry, The University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada.
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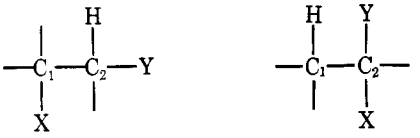
Abstract: Methanolysis of 2-acetoxyethyl-2-^{13}C-(pyridine)cobaloxime gave equal amounts of methoxyethyl-1-^{13}C- and -2-^{13}C-(pyridine)cobaloxime. This shows that during the solvolysis both carbon atoms, of the ligand bonded to cobalt, became equivalent, and the intermediate for this can be envisaged as an olefin π complex of cobalt. Further evidence for olefin π complexes of trivalent cobalt was obtained from the reaction between cob(III)alamins or cob(III)aloximes and vinyl ethers which gave the corresponding σ-bonded cobalt acetals. By involving such π complexes as intermediates of the reactions which are catalyzed by the B_{12} coenzyme, one can account for, and generalize, the overall enzymatic rearrangements.

Coenzyme B_{12} (Figure 1) has been reported as a required cofactor for ten different enzyme reactions.[2] These reactions can be categorized into four main reaction types according to the transformation which occurs during the conversion of substrate to product: they are elimination, carbon skeleton rearrangement, isomerization, and reduction. At first sight these reactions appear to be unrelated, but they all involve a net substrate rearrangement in which a hydrogen is interchanged with an alkyl, acyl, or electronegative group on an adjacent carbon atom.

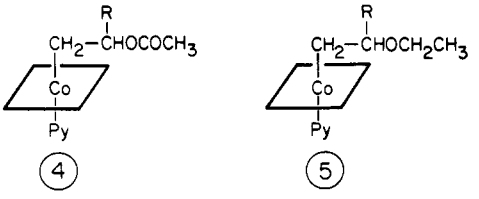


After homolytic cleavage of the Co–C bond of enzyme-bound coenzyme B_{12},[3,4] a hydrogen from C-2 of the substrate migrates to the coenzyme where it becomes equivalent with the two hydrogens at the C-5′ position of the coenzyme **1**, followed by a transfer of one of these three hydrogens back to C-1 of the substrate.[5]

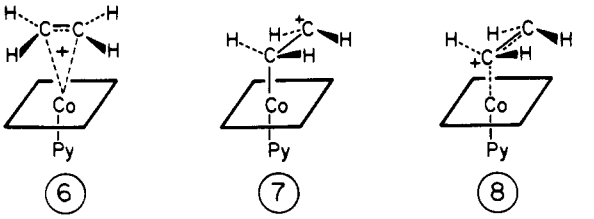
A reaction scheme has been presented[6] which summarizes much of our knowledge of these enzymatic reactions and is shown as an example in Scheme I using the dioldehydrase-mediated interconversion of ethylene glycol to acetaldehyde.

Numerous suggestions have been made concerning the mode of migration of the X group, but the mechanisms presented usually require many steps that are unrealistically high in energy and on occasion bear little relationship to the enzymology.[7-13]

In 1970, Golding et al.[14] reported that the ethanolysis of 2-acetoxyethyl-(**4**, R = H) and 2-acetoxypropyl(pyridine)-cobaloxime (**4**, R = CH_3) gave 2-ethoxyethyl- (**5**, R = H) and



2-ethoxypropyl(pyridine)cobaloxime (**5**, R = CH_3), respectively. The reactions followed first-order kinetics at a rate comparable to the rate of solvolysis of trityl acetate, and these data suggested a mechanism in which ionization to one of three



possible intermediates **6**, **7**, or **8** occurred in a rate-limiting step followed by rapid capture by solvent. If this attack by solvent on the intermediate π complex **6** were to occur at the carbon atom next to that at which the acetoxyl group was originally attached, a net rearrangement of cobalt to the adjacent carbon atom would be realized. Consequently, a reaction of this type could provide a reasonable pathway for the conversion of **3** to **2** in Scheme I.

Reversible $\sigma \rightleftarrows \pi$ rearrangements are well documented in organometallic chemistry[15] and solvolytic rate enhancement has been attributed to metal participation.[16,17] If the enhanced rates of solvolyses of 2-acetoxyalkylcobaloximes[14] are a result of cobalt atom assisted ionization, then at some point on the reaction coordinate, the cobalt would interact equally with the two carbon atoms to generate the π complex **6**. Although the isolation and characterization of a Co(III) π complex has never been reported, it has been suggested as an intermediate in a number of reactions involving cobalt–carbon bonds. Thus, acidification of potassium allylpentacyanocobaltate(III) to give propylene was believed to occur via the propylene π complex with pentacyanocobaltate(III) ion.[18] Likewise, protonation of $K_3[Co(CN)_5(CH_2COC_6H_5)]$ was assumed to lead to the π enol complex of acetophenone with the pentacyanocobaltate(III) ion.[18] The reactions of vinylcobalamin[19] and 2-hydroxyethylcobalamin[20] with acid produced ethylene and hydroxocobalamin, and it was suggested that both systems involved an ethylene π complex.

The purpose of our initial study was to provide evidence for the intermediacy of Co(III) π complexes in the chemistry of both cobalamins and cobaloximes. We envisaged that the π complex **6** might be generated by two independent routes: either by the loss of a nucleofugal group from a carbon β to the cobalt atom, as suggested by Golding's[14] work, or by the addition of an olefin to an unalkylated Co(III) complex (Scheme II).

In order to determine whether the interconversion of **6** to **9** proceeded via pathway a (Scheme II), we have examined the solvolysis of 2-acetoxyethyl-2-^{13}C-(pyridine)cobaloxime (**10**). If the alcoholysis to the 2-alkoxyethyl(pyridine)cobaloxime

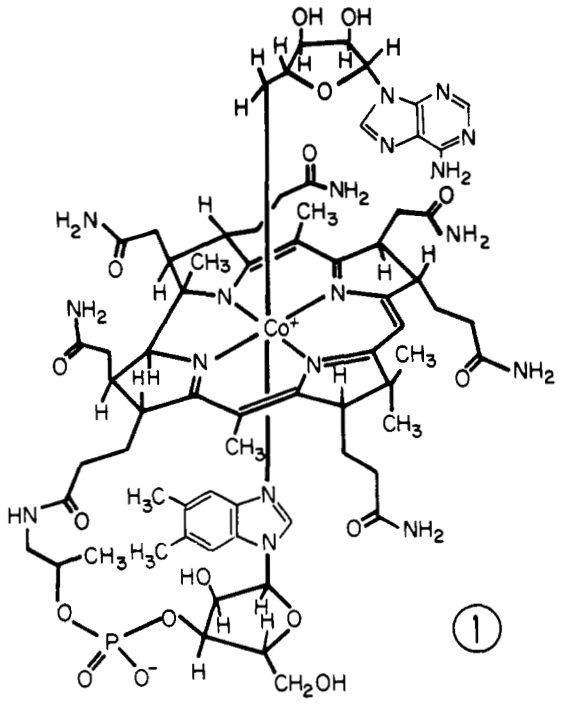


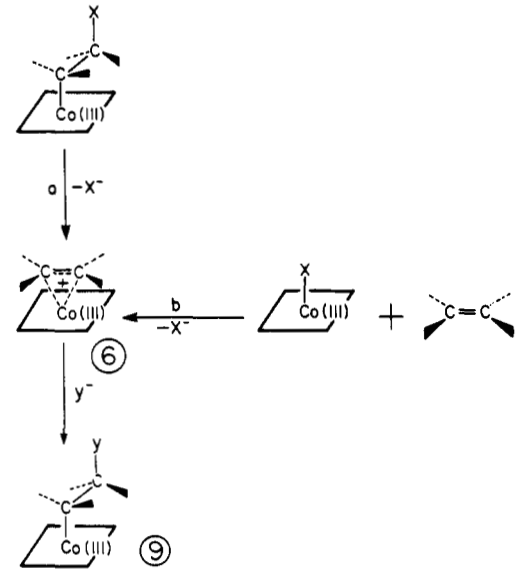
Figure 1. The vitamin B_{12} coenzyme.

proceeded via intermediate **7** or **8** then only 2-alkoxyethyl-*2*-^{13}C-(pyridine)cobaloxime (**11**) would be obtained, but if the reaction proceeded via the symmetrical π complex **6**, then a 50:50 mixture of **11** and 2-alkoxyethyl-*1*-^{13}C-(pyridine)cobaloxime (**12**) would result. Similarly if pathway b (Scheme II) occurs, then it should be possible to generate Co(III)–alkyl σ complexes via the olefin π complexes.

Results and Discussion

2-Acetoxyethyl-*2*-^{13}C-(pyridine)cobaloxime (**10**) was synthesized from carbon-^{13}C dioxide as shown in Scheme III. The 2-bromoethyl acetate-*1*-^{13}C contained 65–70 mol % ^{13}C

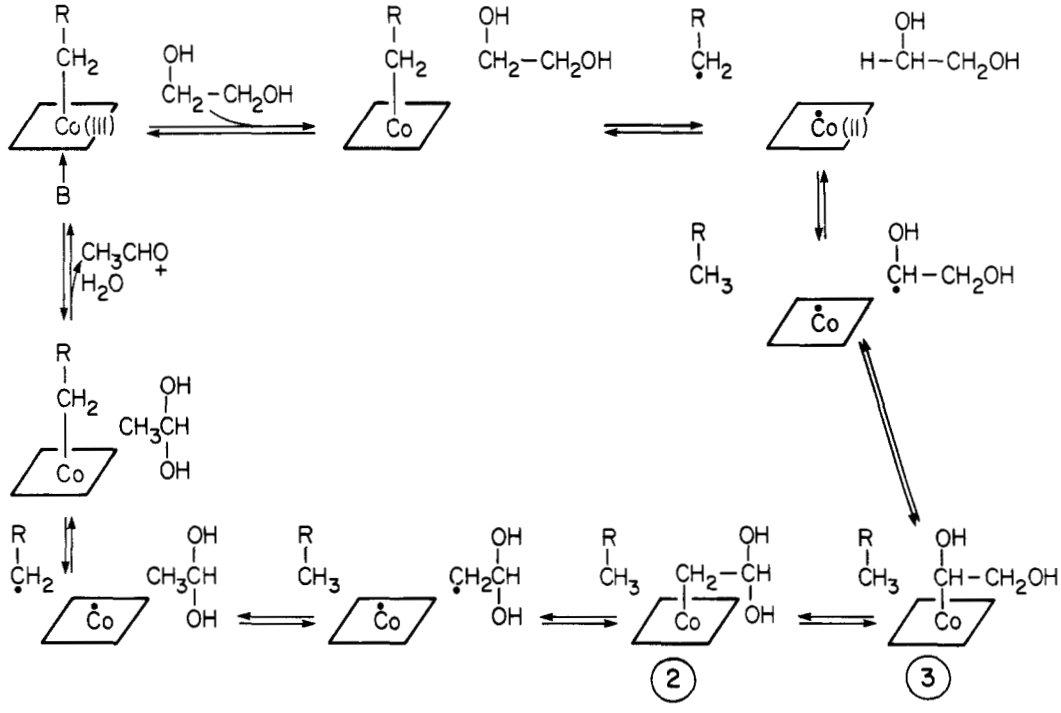
Scheme II



and thus the cobaloxime **10** was a 2:1 mixture of the ^{13}C- and ^{12}C-labeled complexes. The ^1H NMR spectrum of **10** (Figure 2) shows a triplet at 1.50 ppm (J_{H-H} = 8 Hz) with additional long-range coupling ($J_{^{13}CCH}$ = 2.5 Hz) for the C-1 protons, a triplet at 3.70 ppm (J_{H-H} = 8 Hz) for the C-2 protons on ^{12}C, and two ^{13}C satellite triplets at 2.96 and 4.44 ppm (J_{H-H} = 8 Hz; $J_{^{13}C-H}$ = 148 Hz) for the C-2 protons on ^{13}C. The acetoxylmethyl protons are at 1.94 ppm, the dimethylglyoxime methyl protons absorb at 2.16 ppm, and the pyridine protons are at their usual downfield positions.

Methanolysis of a suspension of the acetoxycobaloxime **10** for a period of 14 days at room temperature gave a product whose proton NMR spectrum (Figure 3) showed a triplet at 1.58 ppm (J_{H-H} = 9 Hz; $J_{^{13}CCH}$ = 2.5 Hz) for the C-1 protons on ^{12}C and two triplets at 0.90 and 2.26 ppm (J_{H-H} = 9 Hz; $J_{^{13}C-H}$ = 136 Hz) for the C-1 protons on ^{13}C. In addition to this, a triplet at 3.06 ppm (J_{H-H} = 9 Hz; $J_{^{13}CCH}$ = 2 Hz) for the C-2 protons on ^{12}C and two triplets at 2.36 and 3.76 ppm (J_{H-H} = 9 Hz; $J_{^{13}C-H}$ = 140 Hz) for the C-2 protons on ^{13}C

Scheme I



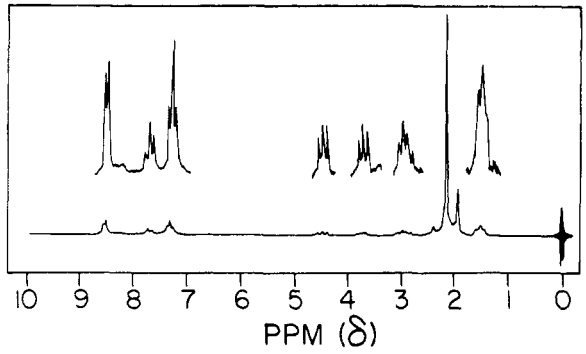


Figure 2. ^{1}H NMR spectrum (100 MHz) of 2-acetoxyethyl-*2*-^{13}*C*-(pyridine)cobaloxime (**10**) in $CDCl_3$.

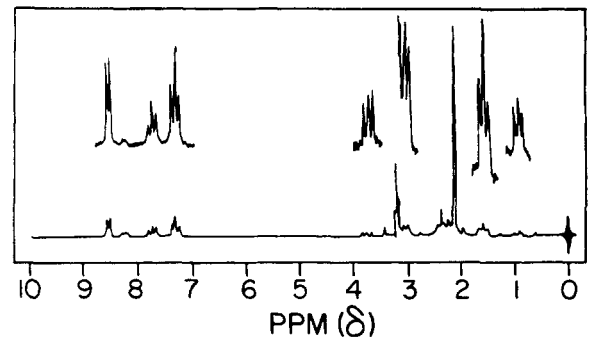


Figure 3. ^{1}H NMR spectrum (100 MHz) of methanolysis products **11** and **12** in $CDCl_3$.

Scheme III

$$^{13}CO_2 \xrightarrow[3.\ NaOH]{1.\ CH_3MgBr,\ 2.\ H_2SO_4} CH_3{}^{13}COONa \quad (1)$$

$$CH_3{}^{13}COONa + HCl \longrightarrow CH_3{}^{13}COOH \quad (2)$$

$$CH_3{}^{13}COOH + Br_2 \xrightarrow[(CH_3CO_2)O]{P} BrCH_2{}^{13}COOH \quad (3)$$

$$BrCH_2{}^{13}COOH + B_2H_6 \xrightarrow{THF} BrCH_2{}^{13}CH_2OH \quad (4)$$

$$BrCH_2{}^{13}CH_2OH + CH_3COCl \longrightarrow BrCH_2{}^{13}CH_2OCOCH_3 \quad (5)$$

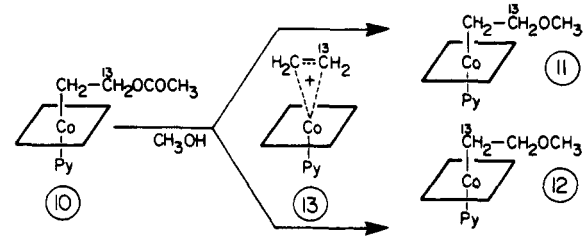
$$\begin{matrix} Cl \\ | \\ (CO) \\ | \\ Py \end{matrix} \xrightarrow[2.\ BrCH_2{}^{13}CH_2OCOCH_3]{1.\ NaBH_4} \begin{matrix} CH_2{}^{13}CH_2OCOCH_3 \\ | \\ (CO) \\ | \\ Py \end{matrix} \quad (6)$$

were observed. The two triplets of the $^{13}C_2$ protons and the triplet of the $^{12}C_2$ protons in **10** are all of equal intensity since there is twice as much $^{13}C_2$ as $^{12}C_2$ but the intensity of each $^{13}C_2$ proton triplet is halved as a result of ^{13}C coupling. However, in the solvolyzed products the four[21] ^{13}C proton triplets are of equal intensity, with each of the two ^{12}C proton triplets four times as intense as those of the ^{13}C. This can only occur when for either C_1 or C_2 there are twice as many ^{12}C protons as ^{13}C protons. Since the ^{13}C protons are split into two triplets, each ^{13}C proton triplet becomes one-fourth as intense as the ^{12}C proton triplets.

Mechanism of the Methanolysis. Randomization of label as a result of an intramolecular rearrangement of 2-acetoxyethyl-*2*-^{13}*C*- to 2-acetoxyethyl-*1*-^{13}*C*-(pyridine)cobaloxime was excluded when none of the latter compound was obtained after allowing a solution of the former to stand for 4 days in methylene chloride at room temperature. Scrambling of the label associated with exchange of methoxyl residues between 2-methoxyethyl-*2*-^{13}*C*-(pyridine)cobaloxime and solvent was likewise negated by showing that CD_3O- does not incorporate into 2-methoxyethyl(pyridine)cobaloxime in a reaction mixture containing equimolar amounts of the cobaloxime and acetic acid [a product from methanolysis of 2-acetoxyethyl-(pyridine)cobaloxime] in methanol-d_4 as solvent.

All of the above data are consistent with the intervention of an intermediate during the methanolysis in which the cobalt atom is bonded equally to both carbon atoms, and this intermediate can be envisaged as the π complex **13.** Quenching of such an intermediate by methanol at either of the two equivalent carbon atoms would produce a 50–50 mixture of 2-methoxyethyl-*1*-^{13}*C*-and 2-methoxyethyl-*2*-^{13}*C*-(pyridine)-cobaloxime and lead to the observed distribution of ^{13}C in the solvolysis product (Scheme IV).

Scheme IV



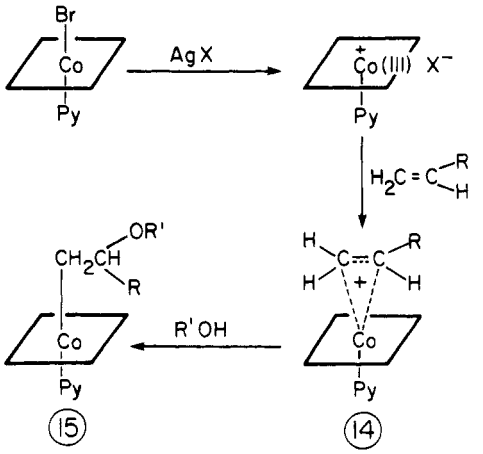
These experiments provided the first demonstration of a Co(III) $\sigma \rightleftarrows \pi$ rearrangement. Further evidence, reported by Golding,[22] for this mechanism is that the methanolysis of 2-acetoxyethyl-2-d_2-(pyridine)cobaloxime gave a 50–50 mixture of 2-methoxyethyl-*1*-d_2- and 2-methoxyethyl-*2*-d_2-(pyridine)cobaloxime.

Furthermore, solvolysis of 2-acetoxypropyl-2-(*S*)-(pyridine)cobaloxime with benzyl alcohol gave only 2-benzyloxypropyl-2-(*S*)-(pyridine)cobaloxime[22] indicating complete retention of configuration at C-2. The retention of configuration would arise from two inversions, i.e., cobalt atom assisted displacement of the acetoxyl group to give an optically active Co(III)–propylene π complex followed by nucleophilic attack at C-2 by benzyl alcohol to give the (*S*)-ether. In this case, however, there is no migration of the cobalt to the adjacent carbon atom since nucleophilic attack occurs only at C-2 as a result of better carbonium ion stabilization at the secondary rather than at the primary carbon atom to form the more stable primary cobaloxime rather than the secondary one. A similar preference for attack at the more stable carbonium ion was found by Parfenov et al.[23]

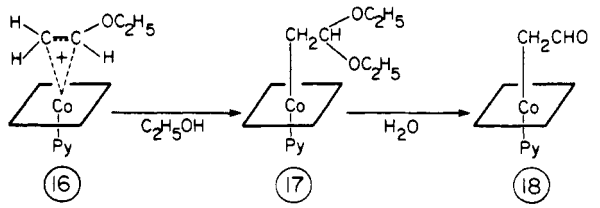
The initial strategy developed to determine whether Co(III) π complexes could be generated by the addition of olefins to cobalt(III) was to remove the halide ion from bromo(pyridine)cobaloxime with silver ion in an inert solvent, introduce the olefin, and then quench the incipient π complex **14,** if formed, with an alcohol to give the alkoxyethyl(pyridine)-cobaloxime (**15**) (Scheme V). Bromo(pyridine)cobaloxime has an NMR signal for the dimethylglyoximatomethyl protons at 2.38 ppm, whereas alkylated cobaloximes generally show that absorption between 2.05 and 2.25 ppm; thus a simple method for monitoring the reaction, directly in methylene chloride, was apparent.

Hydrocarbon Alkene Addition. No evidence for alkylated cobaloximes was found using ethylene, 2-methyl-1-butene, 3,3-dimethyl-1-butene, or 2-ethyl-1-butene under a variety of conditions: changing solvent, nucleophile, order of addition of reactants, stoichiometry of reagents, temperature, pressure, and neutralization base. This, however, was not too surprising since transition metal π complexes are, in general, most stable when the metal is in a low oxidation state and when the olefin is electron deficient.[24] The π complex we sought would contain

Scheme V



Scheme VI



cobalt in its highest normal oxidation state. This suggested that electron-rich olefins should be used, since we anticipated that the synergic bonding which stabilizes a complex between a transition metal in a low oxidation state (electron rich) and an electron-deficient olefin might also occur if the metal were in a high oxidation state (electron deficient) and the olefin electron rich (such as an enol ether).

Ethyl Vinyl Ether Addition. If the reaction between bromo(pyridine)cobaloxime and an electron-rich olefin, such as ethyl vinyl ether, were to give the π complex **16,** then it would be anticipated that an ambient nucleophile (e.g., ethanol) would attack only at the oxygen-bearing carbon atom (the stabilized carbonium ion center) of the complex to give the neutral acetal compound **17** (Scheme VI).

(a) With Prior Silver Ion Promoted Debromination of the Cobaloxime. When bromo(pyridine)cobaloxime, which was initially treated with silver trifluoromethanesulfonate and the precipitated silver bromide removed by filtration, was allowed to react with ethyl vinyl ether followed by ethanol and a base at −78°, a mixture of formylmethyl- and 2,2-diethoxyethyl(pyridine)cobaloxime (**18** and **17,** respectively) was obtained in a 2:1 ratio. The function of the base, added simultaneously with the alcohol, was to neutralize the trifluoromethanesulfonic acid generated during quenching and, therefore, should be nonnucleophilic. Triethylamine was most convenient, although in the initial experiments diisopropylethylamine was used.

(b) Without Prior Silver Ion Promoted Debromination. The axial ligands of cobalt complexes are in dissociation equilibrium.[25,26] Thus, it seemed reasonable that if ethyl vinyl ether, ethanol, and amine were in solution with bromo(pyridine)cobaloxime, an olefin complex could form in a slow equilibrium step after dissociation of bromide. If this intermediate were to then react at the β carbon with an ambient hydroxyl group, a Co(III)-alkyl complex would result, and, indeed, this reaction gave a quantitative yield of a mixture of 2,2-diethoxyethyl- and formylmethyl(pyridine)cobaloxime (**17** and **18**).

Even though absolute ethanol was used, a large amount (up to 50%) of formylmethyl(pyridine)cobaloxime was observed in the NMR spectrum[27] before workup. Since ethanol is in such a large excess and alcohol is supposedly a better nucleophile than water,[28] the thermodynamically more stable aldehyde must form in a step following acetal formation. To minimize the amount of aldehyde formed, various drying agents were employed. Satisfactory results were obtained when anhydrous potassium carbonate or Drierite (anhydrous calcium sulfate) was added to the cobaloxime solution prior to the introduction of the other reagents. When Drierite was used in the reaction mixture, an almost quantitative conversion to the diethylacetalcobaloxime was observed (determined by NMR). The usual workup procedures, however, caused partial hydrolysis of the acetal. The best procedure found to maximize the ratio of acetal to aldehyde was to stir the acetal–aldehyde mixture in deionized water (containing a trace of base). Although this promotes some hydrolysis of the acetal, the aldehyde is more soluble in water than the acetal and is removed. However, a few percent of the aldehyde complex was always observed by NMR. Attempts to recrystallize the impure acetal, using a variety of solvent systems, led to increased amounts of the aldehyde.

Separations of 2,2-diethoxyethyl-, formylmethyl-, and bromo(pyridine)cobaloxime by thin-layer and column chromatography also proved fruitless. TLC on silica gel, eluting with CH_2Cl_2 containing 1–5% pyridine, showed two clearly separated spots; however, the acetal decomposed smoothly to the aldehyde on this absorbant. Cellulose chromatography with benzene as eluent was excellent for separating alkylated from unalkylated cobaloximes but was not efficient enough to differentiate the acetal and the aldehyde.

Failure to find an adequate method of purification for 2,2-diethoxyethyl(pyridine)cobaloxime left only one alternative. The slightly impure product from the reaction of bromo(pyridine)cobaloxime and ethyl vinyl ether and ethanol was characterized by NMR and the product was then chromatographed on silica gel to give the aldehyde, which was readily recrystallized. The NMR and ir spectra, as well as the elemental analysis, were consistent for formylmethyl(pyridine)cobaloxime. The ir (KBr) carbonyl-stretching frequency for formylmethyl(pyridine)cobaloxime occurs at 1655 cm^{-1}. This is at a much lower frequency that that for acetaldehyde (1730 cm^{-1}) or α,β-unsaturated and aromatic aldehydes (1710–1685 cm^{-1}).[29] The weakening of the carbonyl bond appears to be a result of more than just an inductive effect of the more electropositive cobalt atom and probably arises from interaction of the Co d orbitals with the β-carbonyl group. This interaction at the β carbon could be responsible for the ease of solvolysis of groups on this carbon atom. Both the acetal and aldehyde cobaloximes were characterized by alternative syntheses using known routes.[30]

In all of the ethyl vinyl ether experiments with bromo(pyridine)cobaloxime, a large excess of the olefin was required to drive the reaction to completion. Even with a 50-fold excess, a small amount of unalkylated cobaloxime was observed in the NMR spectrum. Therefore, the reversibility of this reaction was tested. Equimolar amounts of 2,2-diethoxyethyl(pyridine)cobaloxime and triethylamine hydrobromide were dissolved in dry methylene chloride containing 0.5 equiv of triethylamine. After standing in the dark for 5 days, the NMR spectrum of an aliquot showed a mixture of starting cobaloxime, formylmethylcobaloxime, and unalkylated (presumably, bromo) cobaloxime in the ratio 27:18:55, respectively. A control, which contained everything but the triethylamine hydrobromide, produced no unalkylated cobaloxime and only 5% aldehyde. Formylmethyl(pyridine)cobaloxime, under the same conditions, was converted to only 9% unalkylated cobaloxime during that time. Thus the reaction of bromo(pyridine)cobaloxime with ethyl vinyl ether and ethanol is reversible.

2-Hydroxyethyl Vinyl Ether Addition to Bromo(pyridine)-cobaloxime. The instability of the 2,2-diethoxyethyl(pyridine)cobaloxime prevented us from isolating it in pure form.

Therefore, to assure formation of a more stable acetal, 2-hydroxyethyl vinyl ether was chosen as the olefin. An internal cyclization between the π complex and the β-hydroxyl group (**19** → **20**) would yield an ethylene glycol acetal of formylmethylcobaloxime. Internal cyclizations in five-membered rings can occur at a rate of more than 10^6 times as fast as an intermolecular nucleophilic reaction.[31-33] Moreover, ethylene glycol acetals are known to hydrolyze at rates which are slower than the corresponding diethyl acetals as a result of less favorable entropies of activation for the cyclic compounds.[34] Treatment of bromo(pyridine)cobaloxime with 2-hydroxyethyl vinyl ether in the presence of triethylamine gave a quantitative conversion to the cyclic acetal **20** (as shown by an NMR spectrum of the reaction mixture[35]). Normal workup procedure afforded relatively pure product which was stable to three recrystallizations and had NMR and ir spectra and elemental analysis consistent with the cyclic acetal complex. Independent synthesis of this compound by treatment of reduced cobaloxime [Co(I)] with the ethylene glycol acetal of bromoacetaldehyde[36] gave a compound with the identical spectroscopic properties as the one made by the olefin route.

When a solution of 1,3-dioxa-2-cyclopentylmethyl(pyridine)cobaloxime and triethylamine hydrobromide in dry CH_2Cl_2 containing 0.5 equiv of triethylamine was allowed to stand in the dark for 5 days, 1% or less of unalkylated cobaloxime was observed in the NMR spectrum of an aliquot. Therefore, unlike formation of the diethyl acetal cobaloxime, the reaction of bromo(pyridine)cobaloxime with 2-hydroxyethyl vinyl ether favors accumulation of product, which establishes the stability of the cyclic acetal cobaloxime relative to the diethyl acetal.

Addition of Other Olefins to Bromo(pyridine)cobaloxime. When 2-ethyl-1-butene or 3,3-dimethyl-1-butene was used as the olefin under normal reaction conditions, no Co-C bond formation occurred after 1 week. Likewise, the olefins did not add to the "cobaltium" ion (silver ion debrominated cobaloxime) at −78°. Apparently, the stability constants are much too low to observe products from an intermediate π complex.

Side Products from the Olefin Addition Reactions. Although the NMR spectrum of an aliquot of the reaction solutions showed quantitative conversions to the corresponding alkylcobaloximes, only 30% yield of the cyclic acetal cobaloxime and 50% yield of formylmethylcobaloxime (after silica gel catalyzed hydrolysis of the diethyl acetal) could be isolated. The remainder was water soluble, had a dimethylglyoximatomethyl proton absorption at the same frequency as the alkylated cobaloximes, and a strong resonance for a triethylammonium ion. The dimethylglyoximato bridge protons are quite acidic as shown by their absorption in the NMR at 18.2 ppm and it is therefore not surprising that excess triethylamine could deprotonate the dimethylglyoximato bridge and form a water-soluble triethylammonium salt.

It is generally assumed that cobaloximes provide good models for cobalamins. Nonetheless in order to establish that the same olefin π complex chemistry occurred with the cobalamins, it was necessary to show that they underwent the same chemistry with olefins as the cobaloximes.

2-Hydroxyethyl Vinyl Ether Addition to B_{12b}. 2-Hydroxyethyl vinyl ether was initially chosen as the olefin, since it is soluble in water, the solvent of choice for cobalamins. Moreover, an internal cyclization of the β-hydroxyl group of the incipient π complex might lead exclusively to acetal and if stable, no aldehyde would be obtained, even though the reactions were carried out in water. When hydroxocobalamin, in water, was treated with a 100-fold excess of 2-hydroxyethyl vinyl ether, in the presence of either sodium carbonate or triethylamine, the cyclic acetal 1,3-dioxa-2-cyclopentylmethylcobalamin was formed quantitatively.

The alkylation of hydroxocobalamin can be followed by observing the decrease in the optical absorption at 357 nm, the γ band[37] for hydroxocobalamin. It can also be followed by TLC on Brinkmann cellulose plates,[38] using 1-butanol–ethanol–water (10:3:7) containing 0.5% concentrated aqueous ammonia by volume as eluent;[39] the intensity of the red spot with R_f 0.22 (hydroxocobalamin) decreases and a new spot with R_f 0.56 (1,3-dioxa-2-cyclopentylmethylcobalamin) appears. This acetal had identical properties to the compound made from B_{12s} and 2-bromomethyl-1,3-dioxacyclopentane. Both gave the typical alkylcobalamin absorption spectrum, which upon photolysis produced the spectrum of the Co-C bond cleavage product, hydroxocobalamin; addition of excess sodium cyanide converted the compound in the light to dicyanocobalamin. When dilute acid was added to the alkylated cobalamin, in the dark, the spectrum of aquocobalamin was observed.

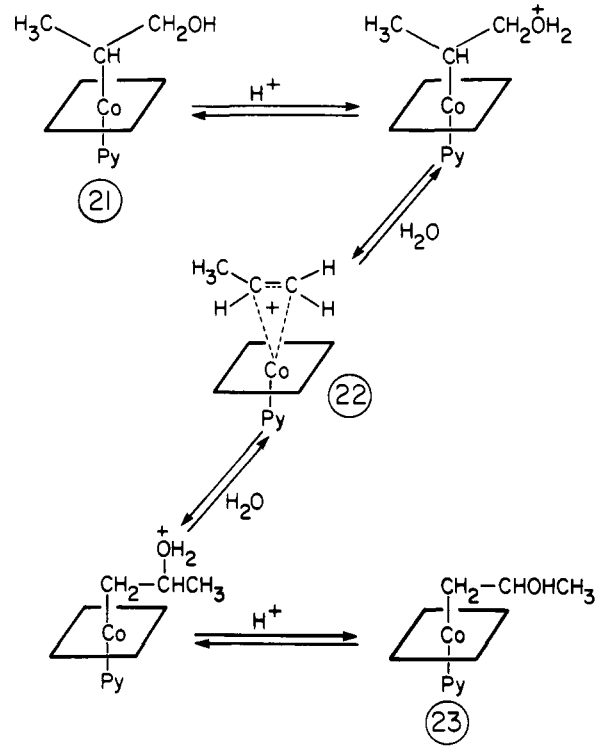
In water at pH 9.0,[40] hydrolysis of this acetal occurred. Three components were resolved by TLC:[38] hydroxocobalamin, starting material, and a compound with R_f 0.51. After about 2 days, the mixture contained the three compounds in a ratio of 50:25:25, respectively. The new component had the same R_f as formylmethylcobalamin prepared by the periodate cleavage of 2,3-dihydroxypropylcobalamin.[41] The conversion of the acetal to formylmethylcobalamin gives additional proof of the structural assignments. Formylmethylcobalamin then hydrolyzes further with Co-C bond fission to hydroxocobalamin.[41]

Ethyl Vinyl Ether Addition to B_{12b}. The reaction between hydroxocobalamin and ethyl vinyl ether in ethanol containing triethylamine gave a quantitative yield of the acetal, 2,2-diethyoxyethylcobalamin, which had the identical optical spectrum and TLC properties as the cobalamin prepared from B_{12s} and the corresponding bromoacetal. The olefin reaction was followed by the same techniques used for the 2-hydroxyethyl vinyl ether system. Recrystallization of the product from basic water–acetone caused slight hydrolysis to hydroxocobalamin and a trace of formylmethylcobalamin, once again demonstrating the instability of the diethyl acetal relative to the ethylene glycol acetal. When 60% ethanol was used as solvent, a mixture of mostly formylmethyl- and hydroxocobalamin with a little 2,2-diethoxyethylcobalamin was seen by TLC after 10 days. Using 95% ethanol, complete conversion to alkylated cobalamin was observed after 6 days, and TLC showed a 4:1 mixture of aldehyde–acetal.

Addition of Other Olefins to B_{12b}. No alkylation of hydroxocobalamin occurred when ethylene, 3,3-dimethyl-1-butene, or ethyl acrylate was used as the olefin, even after reaction periods of 1 month. There was no detectable reaction of ethylene (at 7 atm) with hydroxocobalamin in ethanol for 4 days. Acetaldehyde, assuming there is a finite equilibrium with vinyl alcohol under these conditions,[42] showed no apparent reaction after 2 weeks.

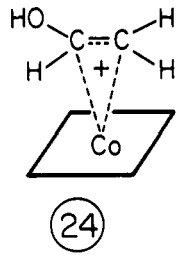
Proposed Mechanism of Action of Coenzyme B_{12} Dependent Enzymes. The above observations suggest that the reversible interconversion of Co(III)–alkyl σ complexes to Co(III)–olefin π complex is a facile process, especially when the olefinic component is electron rich as in the case of vinyl ethers. Additional evidence for such interconversions has recently been presented by Brown and Ingraham[43] who showed that β-

Scheme VII



hydroxyisopropyl(pyridine)cobaloxime (**21**) rearranges to β-hydroxy-*n*-propyl(pyridine)cobaloxime (**23**) under acid conditions and suggested that the reaction proceeded via the intermediacy of the olefin π complex **22** (Scheme VII).

It has been suggested[2] that the initial homolysis of the cobalt–carbon bond of the protein-bound B_{12} coenzyme initiates the transalkylation of the cobalt with the replacement of the 5′-deoxyadenosyl moiety by substrate (in the case of the enzymatic conversion of ethylene glycol to acetaldehyde this would generate intermediate **3**, Scheme I). Loss of the β-hydroxy group would generate the olefin π complex **24** and



readdition of the hydroxyl group to the other carbon atom would give the new σ complex **2** (Scheme I). The overall result of such a transformation would be the conversion of a substrate-bound σ-alkyl complex **3** to a product-bound σ-alkyl complex **2** via the olefin π complex **24.** A second transalkylation using 5′-deoxyadenosine could then convert **2** into acetaldehyde and regenerate the coenzyme (Scheme I).

The experimental observations described above are consistent with the intermediacy of cobalt(III)–olefin π complexes. By involving such π complexes as intermediates of the reactions which are catalyzed by the B_{12} coenzyme one can account for, and generalize, the overall rearrangements. It is interesting to speculate that the $\sigma \rightleftarrows \pi$ rearrangements of organometallic complexes, which have so dominated organometallic chemistry during the past two decades, may have been known to nature for a much longer time.

Experimental Section

Acetic Acid-*1*-^{13}C. Sodium acetate-*1*-^{13}C (3.61 g, 44 mmol), quantitatively prepared by the method of Lemmon[44] using ^{13}C-labeled carbon dioxide,[45] was hydrolyzed at 0° with 20% hydrochloric acid (8.2 ml, 53 mmol). The acidic solution was vigorously stirred with ether (125 ml) for 1 h, the ether layer was separated, and the aqueous phase extracted with ether (2 × 50 ml). The combined organic layers were dried over $MgSO_4$, filtered, and fractionally distilled until no more ether was collected. The remaining ether was then removed at 15° under reduced pressure leaving a clear yellow liquid (2.11 g, 81%): ^1H NMR δ^{neat} (Me_4Si, 60 MHz) 2.06 [s (11% ^{12}C), 0.33 H, and d (89% ^{13}C), $J_{^{13}CCH}$ = 7 Hz, 2.67 H], 10.95 (s, 1 H).

Bromoacetic Acid-*1*-^{13}C.[46] Acetic acid-*1*-^{13}C (1.15 ml, 0.02 mol) was stirred with acetic anhydride (0.19 ml, 0.002 mol) at 75° for 20 min and then cooled to room temperature. Red phosphorus (6.2 mg, 0.4 mmol) and bromine (1.2 ml, 0.022 mol) were added and the reaction mixture was stirred under an efficient condenser at 90–100° for 1.75 h, during which time the color lightened to orange. Additional bromine (0.1 ml) was added and heating continued for 1 h. Nitrogen was bubbled into the yellow solution, which, upon cooling, crystallized. The off-white crystals were dissolved in water (2 ml), extracted with ether (5 × 5 ml), and dried ($MgSO_4$). The ether was removed at 15° under reduced pressure and after azeotropic drying with benzene, pale yellow crystals (2.45 g, 88%) were obtained: ^1H NMR δ^{CDCl_3} (Me_4Si, 60 MHz) 3.93 [s (31% ^{12}C), 0.62 H, and d (69% ^{13}C), $J_{^{13}CCH}$ = 5 Hz, 1.38 H], 11.02 (s, 1 H).

2-Bromoethanol-*1*-^{13}C. To a stirred solution of bromoacetic acid-*1*-^{13}C (2.38 g, 17.0 mmol) in dry THF (20 ml) at 0° under nitrogen was added dropwise through a syringe a 1 M solution of diborane in THF (20.4 ml, 20.4 mmol). After stirring at 0° for 30 min and then at room temperature for 30 min, the product and excess diborane were hydrolyzed by the careful addition of 3 M HCl (15 ml). The reaction mixture was stirred at room temperature for 10 min and extracted with ether (2 × 50 ml, 5 × 30 ml), the combined ether extracts were dried ($MgSO_4$), and the solvent was removed by fractional distillation. The residual ether was removed at 15° under reduced pressure, affording a yellow liquid (1.7 g, 80%) containing some white particles, which were removed by filtration: ^1H NMR δ^{neat} (Me_4Si, 60 MHz) 3.46 [t (31% ^{12}C), J_{H-H} = 6 Hz, 0.62 H, and dt (69% ^{13}C), $J_{^{13}CCH}$ = 6 Hz, 1.38 H], 4.02 [t (31% ^{12}C), J_{H-H} = 6 Hz, 0.62 H, and dt (69% ^{13}C), $J_{^{13}CH}$ = 148 Hz, J_{H-H} = 6 Hz, 1.38 H], 5.59 (s, 1 H).

2-Bromoethyl Acetate-*1*-^{13}C. Acetyl chloride (2.4 ml, 34 mmol) was added dropwise through a syringe to stirred 2-bromoethanol-*1*-^{13}C (2.1 g, 17 mmol) under nitrogen. After the initial warming and gas evolution, the reaction mixture was stirred at room temperature under nitrogen for 2 h. The excess acetyl chloride was removed at reduced pressure and the dark yellow residue shaken with 10% aqueous sodium bicarbonate (5 ml). The basic mixture was extracted with ether (5 × 10 ml) and dried ($MgSO_4$), and the solvent removed, yielding the product as a golden liquid (2.21 g, 78%): ^1H NMR δ^{neat} (Me_4Si, 60 MHz) 2.07 (s, 3 H), 3.56 [t, (31% ^{12}C), J_{H-H} = 6 Hz, 0.62 H, and dt (69% ^{13}C), $J_{^{13}CCH}$ = 3 Hz, J_{H-H} = 6 Hz, 1.38 H], 4.36 [t, (31% ^{12}C), J_{H-H} = 6 Hz, 0.62 H, and dt (69% ^{13}C), $J_{^{13}C-H}$ = 152 Hz, J_{H-H} = 6 Hz, 1.38 H].

2-Acetoxyethyl-*2*-^{13}C-(pyridine)cobaloxime. Chloro(pyridine)-cobaloxime[36] (0.80 g, 2.0 mmol) was added to deaerated absolute ethanol (3 ml); then solid sodium borohydride (0.15 g, 4.0 mmol) was introduced under nitrogen. When the reaction mixture turned blue-green (ca. 10 min), 2-bromoethyl acetate-*1*-^{13}C (0.33 ml, 3.0 mmol) was injected. The reaction mixture turned brown and additional sodium borohydride (0.15 g, 4.0 mmol) was added. After cooling to room temperature, the mixture was poured onto ice water (6 ml). The orange-brown solid which precipitated was collected by suction filtration, washed with ice water, and air-dried. After extraction into chloroform and evaporation, the product was obtained as an orange powder (410 mg, 45%): ^1H NMR δ^{CDCl_3} (Me_4Si, 100 MHz) 1.50 [t, (31% ^{12}C), J_{H-H} = 8 Hz, 0.62 H, and dt (69% ^{13}C), $J_{^{13}CCH}$ = 2.5 Hz, J_{H-H} = 8 Hz, 1.38 H], 1.94 (s, 3 H), 2.16 (s, 12 H), 3.70 [t (31% ^{12}C), J_{H-H} = 8 Hz, 0.62 H, and dt (69% ^{13}C) $J_{^{13}C-H}$ = 148 Hz, J_{H-H} = 8 Hz, 1.38 H], 7.32, 7.77, 8.58 (3 m, 5 H).

Methanolysis of 2-Acetoxyethyl-*2*-^{13}C-(pyridine)cobaloxime. 2-Acetoxyethyl-*2*-^{13}C-(pyridine)cobaloxime (150 mg, 0.33 mmol) was stirred in absolute methanol (3 ml) in the dark for 14 days at room temperature. The solvent was removed and the orange-brown residue was washed with cold water (2 ml), affording the product as an orange solid (130 mg, 91%): ^1H NMR δ^{CDCl_3} (Me_4Si, 100 MHz) (this material contained 31% ^{12}C and 69% ^{13}C equally distributed between

the two cobalt-alkyl carbon atoms) 1.58 (t, J_{H-H} = 9 Hz, dt, $J_{^{13}C-H}$ = 136 Hz, J_{H-H} = 9 Hz, and dt, $J_{^{13}CCH}$ = 2.5 Hz, J_{H-H} = 9 Hz], 2.13 (s, 12 H), 3.06 [t, J_{H-H} = 9 Hz, dt, $J_{^{13}CCH}$ = 2 Hz, J_{H-H} = 9 Hz, and dt, $J_{^{13}C-H}$ = 140 Hz, J_{H-H} = 9 Hz], 3.23 (s, 3 H), 7.30, 7.73, 8.57 (3 m, 5 H).

Control Reaction for Possible Intramolecular Rearrangement of 2-Acetoxyethyl-2-^{13}C-(pyridine)cobaloxime. A solution of 2-acetoxyethyl-2-^{13}C-(pyridine)cobaloxime (100 mg) in dry methylene chloride (5 ml) was allowed to stand in the dark for 3.5 days. Evaporation of the solvent gave a yellow-orange solid having an NMR spectrum identical with the starting material.

Attempted Exchange of 2-Methoxyethyl(pyridine)cobaloxime with Methanol-d_4. 2-Methoxyethyl(pyridine)cobaloxime (220 mg, 0.5 mmol) was stirred with methanol-d_4 (3 ml) containing glacial acetic acid (30 μl, 0.5 mmol) in the dark at room temperature for 8 days. Upon evaporation of the solvent, a dark orange solid was obtained having an NMR spectrum identical with that of the starting material.

Silver Ion Debromination of Bromo(pyridine)cobaloxime and Subsequent Reaction with Ethyl Vinyl Ether and Ethanol. A solution of silver trifluoromethanesulfonate (0.26 g, 1.0 mmol) in dry THF-methylene chloride was added through a syringe to a stirred solution of bromo(pyridine)cobaloxime (0.49 g, 1.1 mmol) in dry deaerated methylene chloride (30 ml) under argon. A light solid precipitated almost immediately. After 10 min, the reaction mixture was filtered with positive argon pressure into an argon-flushed flask. The clear brown filtrate, under argon, was cooled to −78° and then ethyl vinyl ether (4.7 ml, 50 mmol) was added followed immediately by a solution of absolute ethanol (0.1 ml, 1.5 mmol) and diisopropylethylamine (0.26 g, 2.0 mmol, distilled from 1-naphthyl isocyanate) in dry methylene chloride (2 ml). The solvent was immediately removed by rotary evaporation; the brown residue was triturated with 5% aqueous sodium bicarbonate (5 ml), filtered, washed with water, and air-dried. The resulting orange-brown solid was dissolved in chloroform (25 ml), filtered, and evaporated to give an orange-brown solid, wt 260 mg. The NMR spectrum showed the product to be a mixture of formylmethyl-, 2,2-diethoxyethyl-, and unalkylated cobaloxime in the approximate ratio 60:30:10, respectively.

2,2-Diethoxyethyl(pyridine)cobaloxime. To a deaerated solution of bromo(pyridine)cobaloxime (0.9 g, 2.0 mmol) in dry CH_2Cl_2 (35 ml) over Drierite was syringed purified triethylamine (0.44 ml, 3.2 mmol), absolute ethanol (6 ml, 100 mmol), and then ethyl vinyl ether (10 ml, 100 mmol). The solution was allowed to stand sealed in the dark at room temperature until the reaction was complete[47] (about 3 days). The Drierite was removed by filtration and the solvent evaporated under reduced pressure, the temperature being kept below 35°. The yellow-brown residue was stirred in water (10 ml) containing a drop of triethylamine and filtered to give a yellow-orange solid (0.77 g). After recrystallization from CH_2Cl_2–cyclohexane, the orange crystals were stirred in water (10 ml) for 20 min, giving a yellow-orange solid, which was collected by filtration and air-dried: ^1H NMR δ^{CDCl_3} (Me_4Si) 1.09 (t, 6 H, J = 7 Hz), 1.55 (d, 2 H, J = 5 Hz), 2.13 (s, 12 H), 3.15–3.6 (m, 4 H), 4.27 (t, 1 H, J = 5 Hz), 7.32, 7.77, 8.58 (3 m, 5 H); ν^{KBr} 1770–1720 (OH), 1565 (C=N), 1235 and 1085 (NO), 1040 (CO), 520 cm^{-1} (CoN).

Formylmethyl(pyridine)cobaloxime. The above experiment was repeated (using half the quantities) and the yellow-brown solid obtained from evaporation of the solvent was dissolved in a minimum amount of CH_2Cl_2 and chromatographed on silica gel (Woelm activity II, 2.3 × 25 cm), eluting with CH_2Cl_2 containing 5% pyridine. The orange band was collected and the solvent evaporated. Recrystallization from CH_2Cl_2–cyclohexane afforded formylmethyl(pyridine)-cobaloxime as shiny orange crystals (0.24 g, 50%): ^1H NMR δ^{CDCl_3} (Me_4Si) 1.83 (d, 2 H, J = 5 Hz), 2.23 (s, 12 H), 7.32, 7.77, 8.50 (3 m, 5 H), 9.33 (t, 1 H, J = 5 Hz); ν^{KBr} 2820 and 2725 (CH), 1770–1720 (OH), 1655 (C=O), 1560 (C=N), 1238 and 1089 (CN), 515 cm^{-1} (CoN). Anal. Calcd for $C_{15}H_{22}CoN_5O_5$: C, 43.80; H, 5.39; N, 17.03. Found: C, 44.07; H, 5.49; N, 17.13.

1,3-Dioxa-2-cyclopentylmethyl(pyridine)cobaloxime. To a deaerated solution of bromo(pyridine)cobaloxime (224 mg, 0.5 mmol) in dry CH_2Cl_2 (8 ml) was syringed purified triethylamine (0.11 ml, 0.80 mmol) and then 2-hydroxyethyl vinyl ether[48] (1.9 ml, 25 mmol). The brown solution was allowed to stand in the dark at room temperature until the reaction was complete[49] (about 3 days). The solvent was removed under reduced pressure while the temperature was kept below 35° and the vinyl ether evaporated in vacuo. The residue was stirred in water (5 ml) containing a trace of triethylamine and filtered to give a yellow solid (73 mg, 33%). After recrystallization from CH_2Cl_2–cyclohexane, the product was obtained as shiny orange crystals: ^1H NMR δ^{CDCl_3} (Me_4Si) 1.46 (d, 2 H, J = 5 Hz), 2.14 (s, 12 H), 3.6–3.9 (m, 4 H), 4.79 (t, 1 H, J = 5 Hz), 7.32, 7.77, 8.58 (3 m, 5 H); ν^{KBr} 1790–1740 (OH), 1560 (C=N), 1233 and 1083 (NO), 1030 (CO), 517 cm^{-1} (CoN). Anal. Calcd for $C_{17}H_{26}CoN_5O_6$: C, 44.84; H, 5.76; N, 15.38. Found: C, 44.67; H, 5.67; N, 15.27.

Determination of Reversibility for the Reaction of Bromo(pyridine)cobaloxime with Vinyl Ethers. A solution of the appropriate acetal cobaloxime (0.5 mmol) and triethylamine hydrobromide (91 mg, 0.5 mmol) in dry CH_2Cl_2 (8 ml) containing triethylamine (35 μl, 0.25 mmol) was deaerated and then allowed to stand at room temperature in the dark for 5 days. The NMR spectrum of an aliquot was measured and the product ratios were calculated from the ratio of the resonance areas at 2.09, 2.10, 2.18, and 2.37 ppm (CH_2Cl_2) for the cyclic acetal-, diethyl acetal-, aldehyde-, and bromocobaloxime, respectively.

Hydroxocobalamin. A solution of methylcobalamin[50] (prepared from 1.7 g of cyanocobalamin) in water (ca. 1 l.) was placed in direct sunlight for 12 h[51] and then concentrated under reduced pressure to 5 ml. Acetone was added to the cloud point. After 2 days, shiny burgundy crystals (1.30 g) were obtained. A second crop of crystals (0.11 g) was recovered from the mother liquor.

1,3-Dioxa-2-cyclopentylmethylcobalamin. To a deaerated solution of hydroxocobalamin[52] (50 mg, 3.7 × 10^{-5} mol) in water (5 ml) was syringed triethylamine[53] (5–10 mg), followed by 2-hydroxyethyl vinyl ether[48] (0.25 ml, 3 × 10^{-3} mol). The red solution was allowed to stand in the dark until the reaction was complete[54] (about 2 days). The reaction solution was then concentrated; a drop of triethylamine[53] was added and then taken to the cloud point with acetone. After a day, bright red crystals were obtained (40 mg, 80%). TLC showed a single spot [R_f 0.56, cellulose, 1-butanol–ethanol–water (10.3:7) containing 0.5% concentrated aqueous ammonia by volume]: λ (H_2O–K_2CO_3) 265 nm (ε 22 900), 281 (21 900), 288 sh (19 900), 326 sh (13 700), 341 (14 600), 372 (12 900), 433 sh (5410), 490 sh (6980), 525 (8600).

2,2-Diethoxyethylcobalamin. To a deaerated solution of hydroxocobalamin (25 mg, 1.8 × 10^{-5} mol) in absolute ethanol (4 ml) was syringed triethylamine (1 drop), followed by ethyl vinyl ether (0.16 ml, 1.5 × 10^{-3} mol). The red solution was allowed to stand in the dark until the reaction was complete[55] (about 3 days). Fibrous red needles of 2,2-diethoxyethylcobalamin (20 mg, 80%), which had crystallized from the reaction solution, were collected by filtration:[56] λ (H_2O–K_2CO_3) 263 nm (ε 21 600), 281 (19 900), 288 sh (18 000), 323 (12 900), 338 (13 600), 373 (11 400), 432 sh (4640), 490 sh (6610), 525 (8420).

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References and Notes

(1) (a) National Institutes of Health Predoctoral Trainee, 1971–1974; Harvard University; (b) The University of British Columbia.
(2) R. H. Abeles and D. Dolphin, *Acc. Chem. Res.*, **9**, 114 (1976).
(3) J. E. Valinsky, R. H. Abeles, and A. S. Mildvan, *J. Biol. Chem.*, **249**, 2751 (1974).
(4) B. M. Babior, T. H. Moss, and D. C. Gould, *J. Biol. Chem.*, **247**, 4389 (1972).
(5) M. K. Essenberg, P. A. Frey, and R. H. Abeles, *J. Am. Chem. Soc.*, **93**, 1242 (1971).
(6) B. M. Babior, *J. Biol. Chem.*, **245**, 6125 (1970).
(7) H. R. Marston, J. A. Mills, and R. M. Smith, *Nature (London)*, **193**, 240 (1962).
(8) H. W. Whitlock, *Ann. N.Y. Acad. Sci.*, **112**, 721 (1964).
(9) L. L. Ingraham, *Ann. N.Y. Acad. Sci.*, **112**, 713 (1964).
(10) H. P. C. Hogenkamp, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **25**, 1623 (1966).
(11) J. Retey, A. Umani-Ronchi, J. Seibl, and D. Arigoni, *Experientia*, **22**, 502 (1966).
(12) R. G. Eager, B. G. Baltimore, M. M. Herbst, H. A. Barker, and J. H. Richards, *Biochemistry*, **11**, 253 (1972).
(13) P. Dowd and C. S. Nakagawa, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1173 (1972).
(14) B. T. Golding, H. L. Holland, U. Horn, and S. Sakrikar, *Angew. Chem., Int. Ed. Engl.*, **9**, 959 (1970).
(15) M. Tsutsui, M. N. Levy, A. Nakamura, M. Ichikawa, and K. Mori, "Introduction to Metal π-Complex Chemistry", Plenum Press, New York, N.Y., 1970.
(16) R. S. Bly and R. L. Veazey, *J. Am. Chem. Soc.*, **91**, 4221 (1969).
(17) G. Gokel, P. Hoffman, H. Klusacek, D. Marquarding, E. Ruch, and I. Ugi, *Angew. Chem., Int. Ed. Engl.*, **9**, 64 (1970).
(18) J. Kwiatek and J. K. Seyler, *J. Organometal. Chem.*, **3**, 433 (1965).
(19) G. C. Hayward, H. A. O. Hill, J. M. Pratt, N. J. Vanston, and R. J. P. Williams, *J. Chem. Soc.*, 6485 (1965).
(20) J. M. Pratt, "Inorganic Chemistry of Vitamin B_{12}", Academic Press, London, 1972, p 240.

(21) Only two (0.90 and 3.76 ppm) of the four ^{13}C triplets are completely resolved; the other two overlap at ca. 2.3 ppm. However, in a first-order spectrum of this type, the observation of one of the ^{13}C proton triplets characterizes both of them.
(22) B. T. Golding and S. Sakrikar, *J. Chem. Soc., Chem. Commun.*, 1183 (1972).
(23) E. A. Parfenov, T. G. Chervyakova, and A. M. Yurkevich, *J. Gen. Chem. USSR (Engl. Transl.)*, **42**, 2579 (1972).
(24) M. Herberhold, "Metal π-Complexes", Vol. II, Part I, Elsevier, New York, N.Y., 1972.
(25) D. Thusius, *J. Am. Chem. Soc.*, **93**, 2629 (1971).
(26) R. A. Firth, H. A. O. Hill, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 381 (1969).
(27) The percentages of products and starting material in the reaction solution can be estimated from the relative NMR peak areas of dimethylglyoximato methyl protons. These absorptions occur at 2.10, 2.18, and 2.37 ppm (in CH_2Cl_2) for the acetal, the aldehyde, and starting material, respectively.
(28) R. Breslow, R. Fairweather, and J. Keana, *J. Am. Chem. Soc.*, **89**, 2135 (1967).
(29) R. M. Silverstein and C. C. Bassler, "Spectrophotometric Identification or Organic Compounds", 2nd ed, Wiley, New York, N.Y., 1967, p 88.
(30) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **89**, 1999 (1967).
(31) E. S. Gould, "Mechanism and Structure in Organic Chemistry", Holt, Rinehart, and Winston, New York, N.Y., 1959.
(32) H. W. Heine, A. D. Miller, W. H. Barton, and R. W. Greiner, *J. Am. Chem. Soc.*, **75**, 4778 (1953).
(33) This, of course, assumes one can compare first- and second-order rate constants directly.
(34) T. H. Fife and L. K. Jao, *J. Org. Chem.*, **30**, 1492 (1965).
(35) The reaction can be followed by NMR, observing the decrease in the dimethylglyoxime methyl proton absorption at δ 2.37 and the increase at δ 2.09.
(36) By the procedure of H. A. O. Hill and K. G. Morallee, *J. Chem. Soc. A*, 554 (1969).
(37) The most intense band in the near-ultraviolet.
(38) Good results were obtained using Brinkmann cellulose plates (EM plates distributed by Brinkmann Instruments as Celplate-22, without indicator), precoated with microcrystalline cellulose.
(39) All of the cobalamins discussed in this paper can be cleanly separated using the prescribed solvent system. Satisfactory separations were also made with 1-butanol–2-propanol–water (7:6:7) and 2-butanol–methanol–water (55:15:30) both containing 0.5% concentrated aqueous ammonia.
(40) A standard 0.025 M borax–0.1 N HCl buffer was used: "Handbook of Chemistry and Physics", 46th ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1965, p D-73.
(41) R. B. Silverman, D. Dolphin, T. J. Carty, E. K. Krodel, and R. H. Abeles, *J. Am. Chem. Soc.*, **96**, 7096 (1974).
(42) If the enol of acetaldehyde exists at all, it is less than 1 part in ten million: A. Gero, *J. Org. Chem.*, **19**, 469 (1954).
(43) K. L. Brown and L. L. Ingraham, *J. Am. Chem. Soc.*, **96**, 7681 (1974).
(44) R. M. Lemmon in "Isotopic Carbon", M. Calvin, C. Heidelberger, J. C. Reid, B. M. Tolbert, P. F. Yankwich, Ed., Wiley, New York, N.Y., 1949, p 178.
(45) Purchased from Monsanto having 91% ^{13}C label.
(46) Variation of the procedure by G. A. Ropp, *J. Am. Chem. Soc.*, **72**, 4459 (1950).
(47) The reaction was followed by recording the NMR spectra of aliquots, observing the decrease in the dimethylglyoximato methyl protons' absorption at 2.37 ppm and the increase at 2.10 ppm.
(48) H. S. Hill and L. M. Pidgeon, *J. Am. Chem. Soc.*, **50**, 2718 (1928).
(49) The reaction was followed by NMR, observing the decrease in the dimethylglyoximato methyl protons' absorption at 2.37 ppm and the increase at 2.09 ppm.
(50) D. Dolphin, *Methods Enzymol.*, **18C**, 34 (1971).
(51) The photolysis is complete when all of the red color of an aliquot remains at the origin of a carboxymethylcellulose column after eluting with water.
(52) When cyanocobalamin was used, the product partially decomposed, presumably by cyanide ion dealkylation.
(53) Sodium or potassium carbonate can be substituted as the base.
(54) The reaction was followed, observing the decrease in the optical absorption at 357 nm or by TLC as described in the procedure (R_f 0.56 for the product, 0.22 for hydroxocobalamin).
(55) The reaction was followed by observing the decrease in the optical absorption at 357 nm or by TLC [appearance of a new spot with R_f 0.62, cellulose, *n*-BuOH–EtOH–H_2O (10:3:7) containing 0.5% concentrated aqueous ammonia].
(56) Recrystallization from water (containing Et_3N^{53})–acetone caused slight hydrolysis, giving a trace of formylmethylcobalamin and a small amount of hydroxocobalamin.

Model Studies for Coenzyme B_{12} Dependent Enzyme-Catalyzed Rearrangements. Kinetics and Mechanism of Decomposition of Formylmethylcobalamin and Its Acetals

Richard B. Silverman[1a] and D. Dolphin*[1b]

Contributions from the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, and the Department of Chemistry, The University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada.
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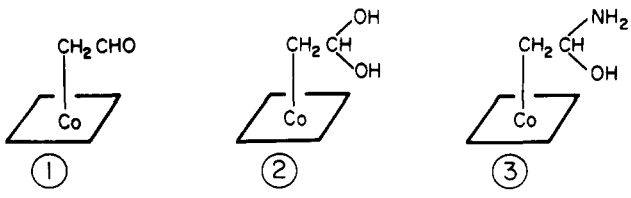
Abstract: Syntheses of formylmethylcobalamin and the kinetics and mechanism of decomposition of the aldehyde and its acetals are reported. Two different pathways were observed for the acid-catalyzed decomposition of the cobalamin acetals: direct cobalt–carbon bond cleavage to B_{12b} and a vinyl ether, and normal acetal hydrolysis to formylmethylcobalamin, followed by a rate-determining fission of the cobalt–carbon bond to B_{12b} and acetaldehyde. The rate law of decomposition of formylmethylcobalamin (A) is $d[A]/dt = -K[A][H_3O^+]$; the acid sensitivity and bimolecularity of the decomposition were rationalized as an initial protonation of the formyl carbonyl followed by a direct cleavage of the cobalt–carbon bond.

Formylmethylcobalamin (**1**), as its hydrated (**2**) or ammoniated (**3**) form, has been suggested as an intermediate in



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the enzymic conversion of ethylene glycol to acetaldehyde by dioldehydrase[2] and of ethanolamine to acetaldehyde by ethanolamine ammonia lyase.[3] Both of these enzymic reactions are vitamin B_{12} coenzyme dependent, and Abeles[2] has proposed the minimal mechanism described by the sequence outlined in Scheme I for the dioldehydrase reaction.

When acetaldehyde and ammonium ions were incubated with ethanolamine ammonia lyase and coenzyme B_{12}, hydrogen was transferred to acetaldehyde from the C-5′ of the coenzyme and the cobalt–carbon bond of the coenzyme dissociated. Omission of ammonium ion caused an acceleration of the cobalt–carbon bond dissociation, but no hydrogen was transferred from the coenzyme to acetaldehyde under these conditions.[3] Interpretation of the observations in the absence