mL, 10 mmol), 2.5 μmol of vitamin B_{12a} (0.5 mL of stock solution), 0.5 mL of V(III) stock solution, and 8.0 mL of 10% NaOH. The initial pH of this solution was 13.5. After deaeration with pure argon, the solution was electrolyzed as described above for 7 min, during which the conversion of vitamin B_{12} into carboxymethylcobalamin was essentially quantitative, as evidenced by TLC analysis of the phenol-extracted and ether/acetone precipitated cobalamin fractions. No yellow corrin oxidation products or methylcobalamin could be detected. The conversion of vitamin B_{12} to carboxymethylcobalamin was only 80% at pH 13.2 and fell to 35% at the pH of 12.6. At pH 11.2, the yield was only 6% after 10 min of electrolysis under otherwise identical conditions.

Synthesis of Carboxymethylcobalamin by the Use of Oxygen Gas or Air. Method D described above can be modified by bubbling oxygen or air through the reaction solution instead of electrolysis. After 7 min of reaction at the pH of 13.5, the yield of carboxymethylcobalamin was 99–100%, based on total vitamin B_{12}.

Preparative Synthesis of Carboxymethylcobalamin. Glacial acetic acid (0.57 mL, 10 mmol), a solution of 16.25 μmol (25 mg) of vitamin B_{12a} in 6.5 mL of water, 5.7 mL of a 10% aqueous solution of NaOH, and 0.5 mL of the V(III) stock solution were successively injected into a serum-capped, air-filled reaction bottle of 38-mL capacity, The bottle was gently shaken for 50 min at room temperature in the dark. The reaction solution was subsequently extracted with 10 g of phenol and the cobalamins precipitated with acetone/ether. Carboxymethylcobalamin was separated from unreacted vitamin B_{12a} and other byproducts by preparative TLC on cellulose plates. Carboxymethylcobalamin was isolated in 95% yield (based on vitamin B_{12a}); the remainders were side-chain-hydrolyzed carboxymethylcobalamin (1.7%) and unreacted hydroxocobalamin (3.3%).

Preparative Synthesis of Methylcobalamin from Acetic Acid. Glacial acetic acid (2 mL, 35 mmol) and 0.5 mL of a stock solution corresponding to 2.5 μmol (4.0 mg) were placed into an Erlenmeyer flask of 10-mL capacity which was equipped with a small magnetic stirrer bar and a silicone rubber seal with an argon inlet and outlet. The solution was deaerated by bubbling argon through the solution while stirring continued for 15 min. After deaeration, 0.5 mL of a solution of $FeSO_4$ in 1 N H_2SO_4 (200 μmol) and 0.5 mL of a solution of VCl_3 in 1 N HCl (200 μmol) were injected by means of a syringe. The reaction was started by the slow, dropwise addition of a 0.1% H_2O_2 solution. A total of 1 mL of this solution was added over the period of 15 min, during which the reaction mixture was stirred as vigorously as possible. The terminal pH was 1.6.

After completion of the addition of H_2O_2 the organocobalamins were isolated by phenol extraction and precipitation with ether/acetone. After separation by TLC on cellulose, methylcobalamin was isolated in 75.9% yield. Carboxymethylcobalamin was formed in 9.1% yield; unreacted vitamin B_{12} was present in 15% yield and recovered as B_{12a}. No yellow corrin oxidation products were detected.

Acknowledgments. This work was supported by Grant CHE 76-10890 of the National Science Foundation.

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(13) That vitamin B_{12r} can react with CH_3· and other radicals is well-known.[9a,14] Judging from the results of model experiments with other Co(II) chelates,[15] the rates of Co–C bond formation are high, with second-order rate constants in the other of $k_2 \sim 10^8\ M^{-1}\ s^{-1}$. Vitamin B_{12r} thus should be a very efficient radical scavenger, especially since the corrin ligand in addition is relatively more resistant against H abstraction by CH_3· radicals than a variety of other ligands of vitamin B_{12} model compounds.[14]
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Sterically Induced, Spontaneous Dealkylation of Secondary Alkylcobalamins Due to Axial Base Coordination and Conformational Changes of the Corrin Ligand[1]

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Abstract: The synthesis of various previously inaccessible secondary alkyl- and cycloalkylcobalamins by the reactions of olefins, alkyl iodides, and bromides with *hydridocobalamin* is described. The coordination of the axial 5,6-dimethylbenzimidazole ligand of alkylcobalamins is dependent on the steric bulk of the alkyl moiety and most secondary alkylcobalamins exist predominantly in the "base-off" form in neutral solution. Secondary and higher primary alkylcobalamins undergo sterically induced spontaneous dealkylation by way of syn β-elimination, the reverse of the reaction of hydridocobalamin with nonactivated olefins. They are generally more stable in acidic media, in which the axial base is protonated, while in neutral or alkaline solution axial coordination of this base causes a conformational change of the corrin ligand which accelerates the Co–C bond cleavage by orders of magnitude.

Whereas primary alkylcobalamins are as a rule reasonably stable compounds and hundreds of them have been prepared by many investigators since the early 1960s,[3] *secondary* alkylcobalamins seemed extremely labile and difficult to synthesize. This was attributed to steric restrictions imposed by the corrin ligand, since secondary alkyl derivatives of cob-

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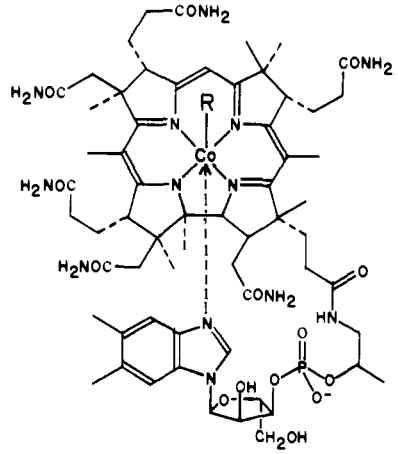


Figure 1. The structure of alkylcobalamins. Cobinamides lack the axial base and phosphoribosyl moieties.

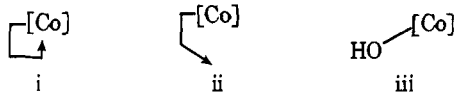
aloxime model compounds were readily accessible by conventional methods of synthesis.

Progress in our understanding of the factors which influence the stability of the Co-C bond in secondary alkyl derivatives of corrins began about 10 years ago in several laboratories. In 1968, Firth et al.[4] succeeded in preparing isopropyl*cobinamide* by the reaction of cobinamide(I) with isopropyl iodide. These authors were able to isolate the compound as a solid, but they also noted that it slowly decomposes in solution. They were unable to isolate isopropyl*cobalamin*, which appeared to be much less stable than the cobinamide. Shortly thereafter, Brodie[5] attempted the synthesis of cyclohexylcobalamin, but was successful only after he quaternized the 5,6-dimethylbenzimidazole ligand, thus preventing it from coordinating to the corrin-cobalt atom. Since both cyclohexyl- and *sec*-butylcobinamide were isolable, these observations suggested that the axial base in the cobalamins was a labilizing influence. It was assumed that its attachment to the cobalt atom induces a conformational change of the in-plane ligand which in turn causes the Co-C bond to become labilized.[6]

In 1970, work from our laboratory[6] showed that cyclohexylcobalamin can be prepared by alkylation of vitamin B_{12s} in methanol. The compound has a spectrum very similar to that of cyclohexylcobinamide, indicating that the axial base is not attached to cobalt. It is stable in acidic media but slowly decomposes in neutral or alkaline solution. In 1971, also from our laboratory, a new method of synthesis of organocobalamins via *hydridocobalamin*, the protonated form of vitamin B_{12s}, was reported.[7] It was shown that hydridocobalamin is formed if vitamin B_{12a} is reduced with zinc in anhydrous acetic acid, and that it can be alkylated with alkyl halides just like vitamin B_{12s}. Unlike vitamin B_{12s}, however, hydridocobalamin also reacts with normal, nonactivated olefins to yield alkylcobalamins, and several examples of this new synthesis of organocobalamins were given. Of particular interest are the reactions of hydridocobalamin with normal, nonactivated olefins and with secondary alkyl bromides or iodides, as these afforded the desired secondary alkylcobalamins. With propylene, for example, isopropylcobalamin was obtained in solution. It was stable under the conditions of synthesis because the 5,6-dimethylbenzimidazole ligand is protonated in the strongly acidic reaction solutions. Consistent with our earlier observations, this stabilizes the secondary organocobalamins sufficiently that they can be studied in solution and in many cases also isolated. In the present paper we describe the synthesis, properties, and some of the reactions of a number of secondary alkyl- and cycloalkylcobalamins. Observations on several alkylcobinamides, *n*-alkylcobalamins, and alkylcobaloximes will also be reported.

Nomenclature and Conventions

An abbreviated nomenclature will be used throughout this paper. Cobalamins will be represented by i in the "base-on" and by ii in the "base-off" forms, and cobinamides by iii. Vi-

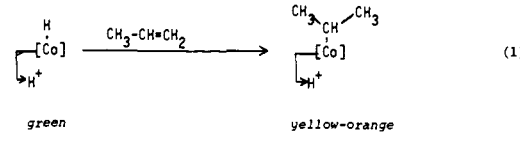


tamin B_{12a} is hydroxocobalamin, vitamin B_{12r} and B_{12s} are the Co(II) and Co(I) derivatives of cobalamin, and hydridocobalamin is the corresponding protonated base-off Brønsted acid of vitamin B_{12s}. The structure of an alkylcobalamin is shown in Figure 1.

Results

I. Preparation of Secondary Alkylcobalamins. Alkylcobalamins are commonly prepared by the alkylation of vitamin B_{12s} in neutral, alkaline, or weakly acidic media. The reactive species under these conditions is the unprotonated Co(I) "supernucleophile",[8] which reacts with alkyl halides by way of S_N2 substitution and also adds to activated olefins and other reactive bonds such as alkynes, epoxides, etc., but not with normal, nonactivated olefins. Vitamin B_{12s} generated in this manner also reacts with secondary alkyl iodides and bromides, but does not yield isolable organocobalamins.[8] Instead, a vitamin B_{12s} catalyzed evolution of olefins is observed.[9] From excess isopropyl bromide, for example, copious amounts of propylene are generated. In more acidic aqueous solutions, vitamin B_{12s} is rapidly oxidized by protons to vitamin B_{12r}.

The reduction of vitamin B_{12a} with zinc dust in anhydrous acetic acid, however, affords hydridocobalamin,[7] the Brønsted acid of vitamin B_{12s}, whose pK_a has been determined to be about 1.[10] Reaction of hydridocobalamin with propylene produces isopropylcobalamin in the protonated, base-off form, in terms of reaction eq 1.



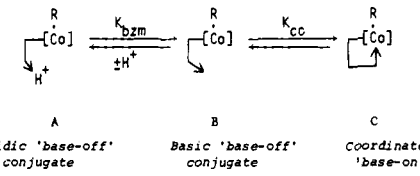
(1)

green yellow-orange

Alkylation of hydridocobalamin with secondary alkyl iodides is rapid and complete within seconds after addition; the corresponding reactions of secondary alkyl bromides may require minutes for completion, in accord with the known reactivity differences between alkyl iodides and bromides. The course of the alkylation reactions was followed by optical absorption spectroscopy; the purity of the alkylcobalt complexes was attested, where possible, by thin layer chromatography. Isolation of solid secondary alkylcobalamins was successful in several cases and is described in the Experimental Section.

In an attempt to synthesize *tert*-butylcobalamin, a rapid conversion of the green color of hydridocobalamin was observed upon the addition of *tert*-butyl bromide to yield a yellow-orange solution similar to that of secondary alkylcobalamins, but it rapidly reverted to green. Analysis of the gas phase indicated the presence of isobutane and isobutylene. The isobutane was the product of the B_{12}-independent reductive debromination of *tert*-butyl bromide with Zn/HOAc. Isobutylene, however, was formed only if vitamin B_{12} was also present. Its formation via an unstable *tert*-butylcobalamin can be rationalized but all attempts at isolation have thus far remained unsuccessful.

II. Properties of Secondary Alkylcobalamins. A. Optical Absorption Spectra. Secondary alkylcobalamins exhibit absorption spectra similar to alkylcobinamides and exist predominantly in the base-off form even in neutral aqueous solution. The possible species in solution are described by the equilibria in eq 2.

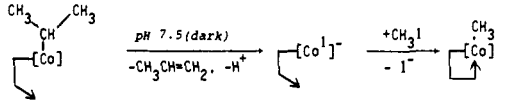


(2)

Forms A and B and the corresponding cobinamide may be assumed to have water in the sixth coordination position and are isospectral in the visible, differing characteristically from form C by a hypsochromic shift of the first inter-π transitions of the corrin ring, as follows from Figure 2.

While most primary alkylcobalamins exist mainly in the base-on form C in neutral solution ($K_{co} > 1$), all acyclic secondary alkylcobalamins are predominantly in the base-off form B ($K_{co} < 0.05$, estimated conservatively), as evidenced by their identical spectra in neutral and acidic solutions. Inspection of the spectra in Figure 2 shows that some weak bands in the neutral solution spectra of primary alkylcobalamins may be due to equilibrium amounts of base-off forms. The spectra of acyclic secondary alkylcobalamins, on the other hand, show no evidence of a contribution of base-on forms, and are all virtually identical with the spectrum of isopropylcobalamin. The neutral solution spectra of cycloalkylcobalamins, however, show an interesting progression from predominantly base-on, with increasing proportions of base-off forms, in going from cyclopropyl to cyclopentyl. Cyclohexylcobalamin and all cobalamins with larger alicyclic substituents directly attached to cobalt are completely base-off (see Figure 2). On the other hand, 2-norbornylcobalamin, prepared by the reaction of norbornene with hydridocobalamin, is partly base-on.

B. Stability in Solution and Products of Decomposition. A characteristic of all alkylcobalamins is their sensitivity to visible light, causing the cleavage of the Co–C bond. The secondary alkylcobalamins are significantly more light sensitive, photolyzing considerably faster than primary alkylcobalamins.[11] They should therefore be handled preferably in complete darkness. However, a spontaneous decomposition with Co–C bond cleavage occurs in solution even in the dark. In neutral or alkaline solutions, olefins and vitamin B_{12s} are formed under strictly anaerobic conditions. This was demonstrated by allowing isopropylcobalamin to decompose in the dark in the presence of methyl iodide. As expected, propylene appeared in the gas phase and a near-quantitative yield (95%, based on isopropylcobalamin) of methylcobalamin was obtained in terms of reaction eq 3.[12] Reaction eq 3 was complete in ca. 20



(3)

min, indicating that its rate is controlled by the spontaneous decomposition of isopropylcobalamin, whose half-life under these conditions is about 2.7 min.

As is well-known, higher alkylcobalamins with hydrogen in the β position decompose photochemically under anaerobic conditions to yield vitamin B_{12s} or hydridocobalamin and unrearranged olefins.[6,27b,42] At attempt was therefore made to show whether the undissociated form of hydridocobalamin is formed initially in the spontaneous dealkylation of a typical secondary alkylcobalamin. Cyclooctylcobalamin was selected and its decomposition was studied in DCl/D_2O under strictly anaerobic conditions. Analysis of the gas phase after completion of the reaction revealed the presence of HD and D_2 in the ratio of 0.12, a result consistent with the intramolecular syn elimination mechanism as shown in eq 4. We also investigated the decomposition of isopropylcobalamin-d_7 in aqueous HCl and similarly detected HD together with H_2. In both cases, the formation of HD suggests that undissociated hydridocobalamin (or deuteriocobalamin) is the first product of the spontaneous decomposition of secondary alkylcobalamins.

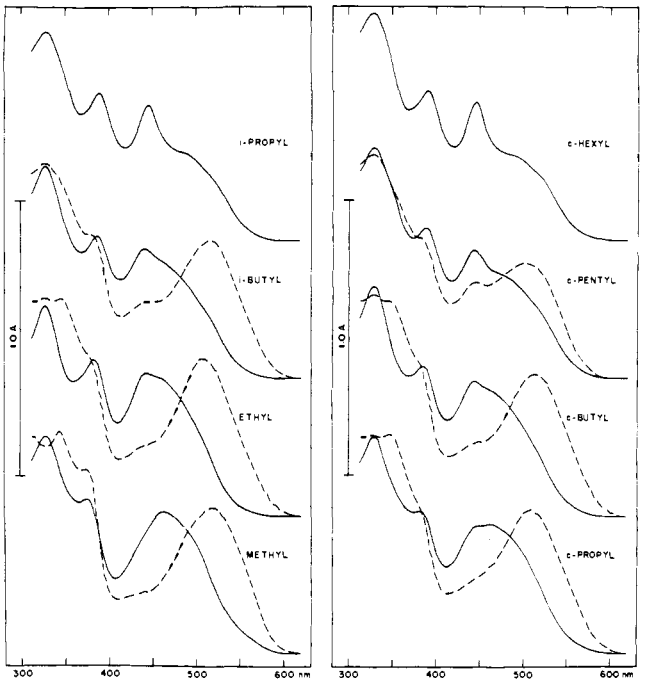
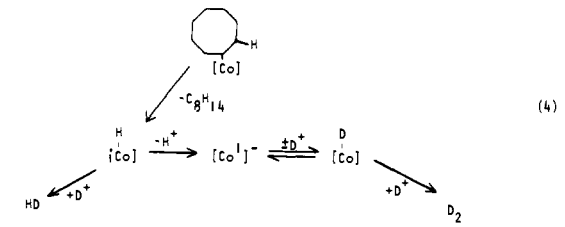


Figure 2. Absorption spectra of several acyclic and alicyclic alkylcobalamins in acidic (—, form A) and neutral (- - - -, forms B and C) aqueous solution, 46 μM.



(4)

III. Studies on Axial Base Coordination and Spontaneous Dealkylations. A. Isopropylcobalamin. 1. Dealkylation pH–Rate Profile. The spontaneous decomposition of isopropylcobalamin can be conveniently studied under aerobic conditions, measuring the formation of vitamin B_{12a} by the rapid oxidation of the primary decomposition product, hydridocobalamin or vitamin B_{12s}. At pH <9, the absorption spectra exhibit sharp isosbestic points; a typical result of one such measurement at pH 4.6 is shown in Figure 3. The isosbestic points appear at 318, 335, 371, and 475 nm. They are also sharp in experiments at higher pH, although shifted slightly bathochromically owing to the shift in the aquo–hydroxo equilibrium of vitamin B_{12a}. Above pH 9, the oxidation of vitamin B_{12r} to vitamin B_{12a} becomes too slow to allow aerobic spectrophotometric determination of dealkylation rates.

The first-order rate constants of decomposition of isopropylcobalamin (and of other alkylcobalamins described in this paper) were obtained by measuring the increasing absorbance at 350 nm (γ band of vitamin B_{12a}), with the t_∞ absorbance measured after photolysis of the reaction solution. They are given in Table I and shown graphically in Figure 4.

In neutral and alkaline solutions, isopropylcobalamin decomposition is pH and buffer *independent*. In strongly acidic solutions, the rates of decomposition are pH but not buffer independent and several orders slower than in neutral or alkaline media. Between those two extremes, the log (k_{obsd})–pH plot has the slope of 1.0. This behavior is best understood in terms of Scheme I, in which K_{bzm} and K_{co} are assumed to be established rapidly relative to the decomposition and oxidation

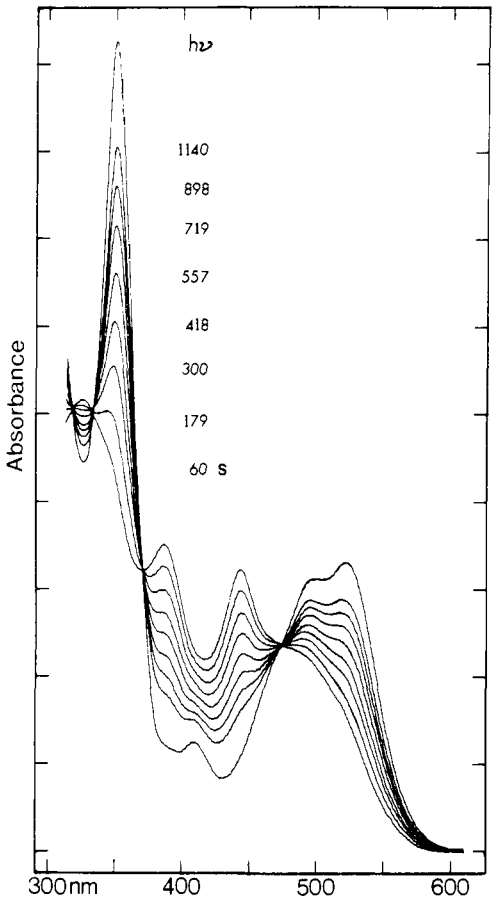
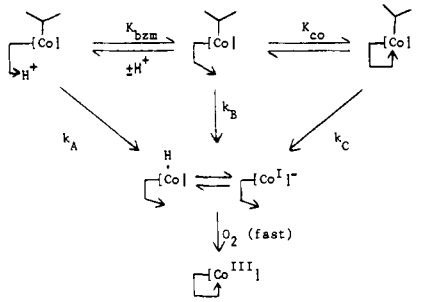


Figure 3. Repetitive spectral scan of the decomposition of isopropylcobalamin, 35 μM, to vitamin B_{12a} in aerobic 0.5 M acetate buffer, pH 4.6. Elapsed time since mixing was recorded at 350 nm. The final spectrum was obtained after 10-min photolysis.

Scheme I. Decomposition of Isopropylcobalamin



reactions.[13] From Scheme I, eq 5 has been derived:

$$k_{obsd} = k_A + \frac{k_C - k_B}{\frac{(H^+)}{K_{bzm}K_{co}} + \frac{1}{K_{co}} + 1} \quad (5)$$

As k_B is expected to be identical (or nearly so) with k_A, it is never an observed pathway, being insignificant relative to $K_{co}k_C$. Furthermore, for isopropylcobalamin, $K_{co} \ll 1$, and k_A is an insignificant term above pH ~2, reducing eq 5 to

$$k_{obsd} = \frac{K_{co}k_C}{\frac{(H^+)}{K_{bzm}} + 1} \quad (6)$$

The solid line in Figure 4 represents eq 6, using the values $K_{co}k_C = 4.2 \times 10^{-3}$ s^{-1} and $pK_{bzm} = 4.8$. The pK_a of 1-β-ribosyl-5,6-dimethylbenzimidazole is 4.70;[14] this value was adopted although it is recognized that cobalamins have an α-glycosidic linkage.

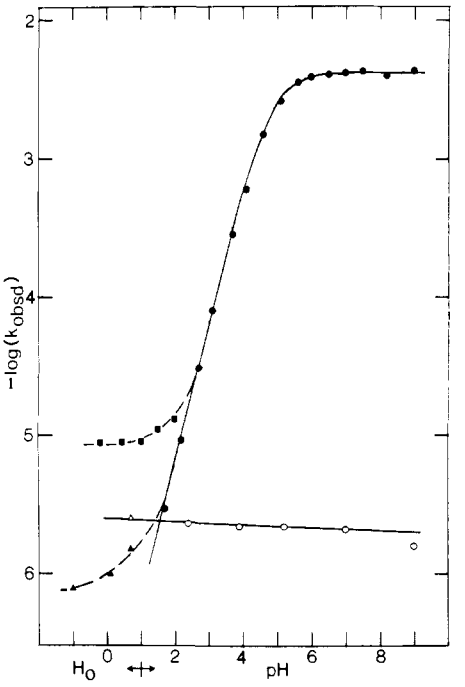


Figure 4. pH-rate profiles for the decompositions of isopropylcobalamin in buffers (●), hydrochloric acid (■), and phosphoric acid (▲), and isopropylcobinamide in buffers (○) and phosphoric acid (△).

Table I. Observed First-Order Rate Constants of Decomposition and Half-Lives of Isopropylcobalamin at Various pHs in Aqueous Media at 23 °C

pH	k_{obsd}, s^{-1}	$t_{1/2}$
9.0	4.3×10^{-3}	2.7 min
8.2	4.0×10^{-3}	2.9 min
7.5	4.3×10^{-3}	2.7 min
7.0	4.2×10^{-3}	2.8 min
6.5	4.1×10^{-3}	2.8 min
6.0	3.9×10^{-3}	3.0 min
5.6	3.6×10^{-3}	3.2 min
5.1	2.6×10^{-3}	4.4 min
4.6	1.5×10^{-3}	7.7 min
4.1	6.1×10^{-4}	19 min
3.7	2.8×10^{-4}	41 min
3.1	8.0×10^{-5}	2.4 h
2.7	3.1×10^{-5}	6.2 h
2.2	9.2×10^{-6}	21 h
1.7	2.9×10^{-6}	66 h
1 M HCl	8.8×10^{-6}	22 h
1 M H_3PO_4	1.5×10^{-6}	130 h

For alkylcobalamins such as isopropylcobalamin, in which the base-on form is not observable, the above mechanism is indistinguishable kinetically from ones in which the coordination of the axial base is rate determining, or occurs concerted with Co-C bond cleavage. Some alkylcobalamins, however, do have form C spectrally observable prior to decomposition. In these cases, which will be discussed below, the above simplifying assumption that $K_{co} \ll 1$ is no longer valid.

2. Propylene Production. The rate of propylene production from isopropylcobalamin in neutral solution was determined by GLC both aerobically and anaerobically. The rate constant of aerobic decomposition at pH 7 was measured as 3.7×10^{-3} s^{-1}, and anaerobically 4.0×10^{-3} s^{-1}, in satisfactory agreement with the more accurate spectrophotometrically determined rate constant of aerobic vitamin B_{12a} production from isopropylcobalamin (see Table II). Furthermore, the yield of propylene is undiminished under aerobic conditions. Thus, both vitamin B_{12a} and propylene production have the same rate-

Table II. Pseudo-First-Order Rate Constants for the Decomposition of Isopropylcobinamide in the Presence of Added Nitrogen Bases, 1.0 M[a]

base	pK_a[b]	k_{obsd}, s^{-1}	$t_{1/2}$, min
none		1.6×10^{-6}	7200
benzimidazole	5.5	1.0×10^{-4}	110
5,6-dimethylbenzimidazole	6.0	2.1×10^{-4}	55
imidazole	7.0	2.2×10^{-4}	53

[a] All measurements in the presence of nitrogen bases were performed in 80% ethanol, 20% 10 mM borate buffer (pH 9), saturated with gaseous oxygen to promote oxidation to cobinamide(III). Solvent in the absence of bases was aqueous pH 9, 10 mM borate. [b] In water. The pK_as of these bases are depressed in aqueous ethanol.[14]

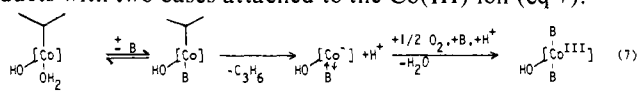
determining step, and oxygen is not involved in the decomposition before or during that step.

3. Effect of Silver Ions. In 10 mM $AgNO_3$ at pH 5.6, isopropylcobalamin decomposes approximately seven times slower than it does at the same pH in the absence of silver ions. Higher concentrations of silver ions depress the rate of decomposition even further. Silver ions are known to coordinate the axial base of primary alkylcobalamins, displacing it from cobalt.[15]

4. Deuterium Isotope Effect. (Isopropyl-d_7)cobalamin was prepared from isopropyl-d_7 bromide and hydridocobalamin and its rate of decomposition in pH 7 buffered solution was measured, giving a first-order rate constant of 1.4×10^{-3} s^{-1}. Thus, the β hydrogen is being eliminated in the rate-determining transition state. Assuming that the isotope effect on K_{Co} is negligible, this gives an isotope effect $k_{C(H)}/k_{C(D)}$ of 3.0, which is of the correct magnitude for a transition state having nonlinear hydrogen transfer, and typical of syn-elimination reactions.[16]

B. Isopropylcobinamide. The rate of decomposition of isopropylcobinamide was found to be essentially pH *independent* over the pH range in which the rates of decomposition of isopropylcobalamin span several orders of magnitude (Figure 4). The average first-order rate constant is 2.3×10^{-6} s^{-1}, a value very close to that of isopropylcobalamin in strongly acidic solutions (see Table I).

The visible absorption spectrum of isopropylcobinamde is unchanged in the presence of added imidazole, benzimidazole, or 5,6-dimethylbenzimidazole. Although binding of imidazole to primary alkylcobinamides has been observed,[17] cyclohexylcobinamide shows no spectrally detectable interaction with any nitrogen base.[5] The decomposition of cyclohexylcobalamin is, however, accelerated in the presence of excess imidazole.[6] The above-mentioned bases also significantly increase the rate of decomposition of isopropylcobinamide. Table II lists pseudo-first-order rate constants for base-induced dealkylations of isopropylcobinamide. Decompositions occurred with sharp isosbestic points and produced the cobinamide(III) adducts with two bases attached to the Co(III) ion (eq 7).



(7)

C. Higher Alicyclic Secondary Alkylcobalamins. 1. Decomposition Products. Table III lists the product distributions of olefins arising from the decompositions of three higher alicyclic secondary alkylcobalamins under various conditions. Several features are notable. First, 2-alkylcobalamins yield predominantly 1-olefin products. This is in contrast to the solvolysis of the corresponding tosylates (E_1 mechanism), for which 1-ene products are formed in minor amounts.[18] *trans*-2-Ene products are generally favored over *cis*-2-enes, less so in acidic than in neutral solution and more so as the steric bulk of the alkyl group increases. The product distributions from 3-pentylcobalamin are remarkably similar to those obtained from the pyrolytic syn eliminations of 3-pentyl-*S*-methylxanthate and 3-pentylacetate.[19]

Table III. Product Distributions for the Olefins Generated in the Decomposition of Three Acyclic Secondary Alkylcobalamins in Aqueous Solutions at 20 °C in the Dark

alkyl residue	conditions	products[a]	percent distribution
2-butyl	pH 7	1-C_4H_8	53
		2-*t*-C_4H_8	27
		2-*c*-C_4H_8	20
	1 N NaOH	1-C_4H_8	54
		2-*t*-C_4H_8	26
		2-*c*-C_4H_8	20
	1 N HCl	1-C_4H_8	55
		2-*t*-C_4H_8	22
		2-*c*-C_4H_8	23
2-pentyl	pH 7	1-C_5H_{10}	58
		2-*t*-C_5H_{10}	27
		2-*c*-C_5H_{10}	15
3-pentyl	pH 7	2-*t*-C_5H_{10}	69
		2-*c*-C_5H_{10}	31
	1 N HCl	2-*t*-C_5H_{10}	65
		2-*c*-C_5H_{10}	35

[a] Measured in gas phase; *t* = trans, *c* = cis.

Table IV. First-Order Rate Constants for the Spontaneous Decomposition of Acyclic Secondary Alkylcobalamins

alkyl group	conditions	k_{obsd}, s^{-1}	$t_{1/2}$
2-butyl	pH 7	7.3×10^{-3}	94 s
	1 M HCl	2.4×10^{-5}	8.1 h
2-pentyl	pH 7	5.6×10^{-3}	2.1 min
	1 M HCl	2.0×10^{-5}	9.6 h
2-octyl	pH 7	4.6×10^{-3}	2.5 min
3-pentyl	pH 7	2.2×10^{-1}	3.2 s
	1 M H_3PO_4	3.8×10^{-4}	30 min
	1 M HCl	3.5×10^{-4}	33 min
	1 M HCl	3.9×10^{-4}	30 min
3-hexyl	1 M HCl	4.7×10^{-4}	25 min
4-heptyl	pH 7	1.5×10^{-1}	4.5 s
	1 M HCl	4.4×10^{-4}	26 min
3-methyl-2-butyl pinacoyl	1 M HCl	1.1×10^{-3}	10.5 min
	conced HOAC	$>10^{-2}$	<1 min

Pinacoylcobalamin [$CH_3\dot{C}HC(CH_3)_3$ = pinacoyl] is so unstable that even its protonated base-off form cannot be separated from the acetic acid–methanol mixture in which it is prepared; its k_A must be $>10^{-2}$ s^{-1}. Analysis of the gas phase over a reaction mixture of hydridocobalamin and pinacoyl iodide revealed 3,3-dimethylbutene and tetramethylethylene in a mole ratio of 1.00:0.09. The gas phase over an identically prepared control mixture, lacking only vitamin B_{12}, contained only 4% as much 3,3-dimethylbutene, but an identical quantity of tetramethylethylene, which therefore must arise from the solvolysis of the iodide. Pinacoylcobalamin, thus, decomposes exclusively to the unrearranged olefin. For comparison, acid hydrolysis of pinacoyl alcohol yields approximately two-thirds of the rearranged olefin, while pyrolysis of the xanthate ester also gives exclusively unrearranged olefin.[20]

2. Rates of Decomposition. Table IV lists the first-order rate constants observed for the spontaneous dealkylations of higher acyclic secondary alkylcobalamins studied. Measurements were usually made at pH 7 to establish the neutral pH-independent rate ($K_{Co}k_C$) and in 1 M acid (HCl or H_3PO_4) to determine the protonated base-off rate (k_A). It is noteworthy that for a secondary alkylcobalamin such as 3-pentylcobalamin

Table V. Data on the Coordination of the Axial Base and the Rates of Spontaneous Dealkylation of Cyclic Secondary Alkylcobalamins

alkyl moiety	pK_a	K_{co}	decomposition rate		
			conditions	k_{obsd}, s^{-1}	$t_{1/2}$
cyclopropyl	3.71[a]	9[b]	pH 7		6 months[e]
cyclobutyl	3.83[a]	6[b]	pH 7		4-6 months[e]
cyclopentyl	4.50[c]	0.6[b]	pH 7	3.7×10^{-3}	3.1 min
			pH 4.93	2.8×10^{-3}	4.1 min
			pH 4.54	1.9×10^{-3}	6.1 min
			pH 4.15	1.1×10^{-3}	10.5 min
			pH 3.75	4.9×10^{-4}	24 min
			1 M H_3PO_4	2.4×10^{-6}	82 h
cyclohexyl	4.7[d]		pH 7	2.6×10^{-4}	44 min
			1 M H_3PO_4	1.2×10^{-7}	67 days
cycloheptyl	4.7[d]		1 M HCl	1.1×10^{-4}	102 min
cyclooctyl	4.7[d]		1 M HCl	4.3×10^{-4}	27 min
2-norbornyl		$\lesssim 0.5$[f]	pH 7	3.6×10^{-4}	32 min

[a] Determined by spectrophotometric titration (±0.05). [b] From eq 9, using $pK_{bzm} = 4.70$. [c] Determined kinetically. [d] Base-on form not spectrally observable in neutral solution, $pK_a = pK_{bzm}$. [e] For dealkylation; see text. [f] Estimated from neutral solution spectrum.

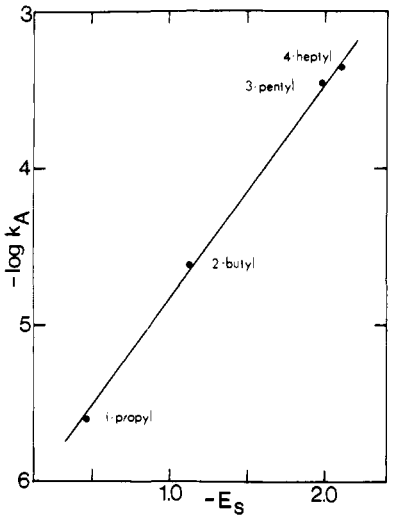


Figure 5. Correlation between log k_A and the Taft steric substituent parameter,[21] E_s, for acyclic secondary alkylcobalamins.

there is essentially no acid anion effect on k_A, and that the corresponding cobinamide decomposes at the same rate.

Further examination of the data reveals that the steric bulk of the alkyl moiety has a dramatic effect on the rate of decomposition. Generally, the effect on the protonated base-off rate (k_A) is greater than that on the neutral pH rate ($K_{co}k_C$), suggesting that a decrease in K_{co} partially offsets the increases in k_C. For none of the derivatives measured under both conditions, however, is K_{co} diminished to the point of making k_B a significant route of decomposition (assuming it to be of the same magnitude as k_A).

For the purpose of establishing trends, it is helpful to view the secondary alkylcobalt derivatives in terms of formula **1**.

1

Most critical in determining the rates of dealkylation is *branching at the β carbon.* In the series isopropyl, 2-butyl, 3-pentyl (R′, R″ = CH_3; R′ = CH_3, R″ = C_2H_5; R′, R″ = C_2H_5), the rates are increased by two orders of magnitude. Along the series isopropyl, 2-butyl, 3-methyl-2-butyl [R′, R″ = CH_3; R′ = CH_3, R″ = CH_2CH_3; R′ = CH_3, R″ = $CH(CH_3)_2$], k_C increases by three orders of magnitude, and the fourth member, pinacoyl [R′ = CH_3, R″ = $C(CH_3)_3$], decomposes so fast that a given hydridocobalamin solution consumes several moles of excess of pinacoyl iodide within 1 min. Furthermore, examination of the data for these two series reveals that each succeeding β-methyl substitution causes increasingly greater acceleration of the rate, in spite of the fact that the number of β hydrogens is decreasing.

Continued extension of the hydrocarbon chain past the γ carbon(s), however, no longer causes substantial rate increases. Such peripheral parts of the cobalt-bound substituent can easily swing away and thus reduce interactions with the corrin ring. Indeed, such extension in some cases even causes slight diminution of the rates of spontaneous decomposition, as exemplified by both neutral and acidic rates in the series isopropyl, 2-butyl, 2-pentyl, . . . , 2-octyl [R′, R″ = CH_3; R′ = CH_3, R″ = CH_2CH_3; R′ = CH_3, R″ = $CH_2CH_2CH_3$; . . . ; R′ = CH_3, R″ = $CH_2(CH_2)_4CH_3$]. The neutral rates for the series isopropyl, 3-pentyl, 4-heptyl (R′, R″ = CH_3; R′ = R″ = C_2H_5; R′ = R″ = $CH_2CH_2CH_3$), and the acidic rates for the series 2-pentyl, 3-hexyl, 4-heptyl [R′ = (in all cases) $CH_2CH_2CH_3$, R″ = CH_3, CH_2CH_3, $CH_2CH_2CH_3$], show the same trend of large increase followed by slight decrease. The acidic rates for the two series isopropyl, 3-pentyl, 4-heptyl, and 2-butyl, 3-pentyl, 3-hexyl (R′ = C_2H_5, R″ = CH_3, CH_2CH_3, $CH_2CH_2CH_3$), however, do show slight increases in the rate after 3-pentyl.

Figure 5 shows the remarkable correlation between the log k_A and the Taft steric substituent constant,[21] where available, for acyclic secondary alkylcobalamins. The neutral solution rates do not correlate quite as well, presumably owing to more complicated behavior arising out of competing steric effects on K_{co} (see below) and k_C.

D. Cyclic Secondary Alkylcobalamins. Table V summarizes the data collected for cyclic secondary alkylcobalamins. Cyclopropyl- and cyclobutylcobalamins have the properties and stability typical of primary alkylcobalamins and may also be prepared by the alkylation of vitamin B_{12s} under conventional conditions.

1. Coordination of the Axial Base. The coordination constant for the binding of the axial base of organocobalamins is critically affected by the nature of the organyl moiety. This is manifested in the pK_a for the protonation of the dimethylbenzimidazole; the greater the affinity for cobalt, the greater is this pK_a depressed below the value of pK_{bzm}. For relatively stable organocobalamins, the pK_a is conveniently measured by spectrophotometric titration in the region of the α,β bands, and is defined as

$$K_a = ([B] + [C])[H^+]/[A] \quad (8)$$

From the equilibria described by eq 2, one obtains

$$K_{co} = 10^{(pK_{bzm} - pK_a)} - 1 \quad (9)$$

The value of pK_{bzm} has traditionally been assigned that of 1-β-D-ribosyl-5,6-dimethylbenzimidazole, 4.70.[14]

Figure 2 shows the visible absorption spectra of the series cyclopropyl through cyclohexylcobalamin. Along this series, the neutral solution spectra show increasing contribution of the base-off α,β bands centered around 450 nm, caused by a decreasing K_{co}.

Cyclopropyl- and cyclobutylcobalamin, having the stability typical of primary alkylcobalamins, are amenable to spectrophotometric titration, and the pK_as and K_{co}'s thus determined are included in Table V. The values for cyclobutylcobalamin are in the range typical of *n*-alkylcobalamins, while cyclopropylcobalamin has a lower pK_a (higher K_{co}) than any unsubstituted alkylcobalamin except methylcobalamin.

Cyclopentylcobalamin has a far greater proportion of base-off form B present in neutral solution, as evidenced spectrally, but its greater instability prohibits spectrophotometric titration. Its pK_a was for this reason determined kinetically. For cobalamins where K_{co} is significant, and at pHs where k_A is insignificant, eq 5 reduces to

$$k_{obsd} = \frac{K_{co}k_C}{\frac{(H^+)}{K_{bzm}} + K_{co} + 1} \quad (10)$$

The pK_a reported in Table V was obtained by fitting the pH-rate data given to the equation

$$pH = \log [k_{obsd}/(k_{obsd(pH7)} - k_{obsd})] + pK_a \quad (11)$$

The base-on form C of cyclohexyl- and larger cyclic secondary alkyl cobalamins is not spectrally observable and K_{co} cannot be determined by these means. 2-Norbornylcobalamin has both unprotonated forms spectrally observable in neutral solution and is intermediate in behavior between cyclopentyl- and cyclohexylcobalamin.

2. Decomposition Rates. Table V also lists the first-order rate constants for the dealkylations of cyclic secondary alkylcobalamins. The approximate half-lives for cyclopropyl- and cyclobutylcobalamins were estimated from the increase of the intensity of the vitamin B_{12a} γ band, on storage of pH 7 buffered solutions under aerobic conditions in the dark. They may or may not represent decompositions by a mechanism analogous to the higher homologues, but are included to show the relative stabilities of these species.

Valuable mechanistic insight can be gained from the variation of reaction rate with ring size in a homologous alicyclic series. The Cope elimination of amine oxides has been used as a reference reaction of known syn-elimination mechanism for such studies.[22a] Figure 6 shows the log rate-ring size profile for k_A of cycloalkylcobalamins and for the Cope elimination of cycloalkylamine oxides.[22b] This profile has been attributed to the energies needed to achieve eclipsing and ring bond-angle enlargement (i.e., I-strain effects) in the transition state. The analogy is remarkable considering that the cobalamins have the added effect of release of steric repulsion of the alkyl moiety with the corrin ring, which increases monotonically (Taft E_s = −0.51, −0.79, and −1.10 for cyclopentyl, cyclohexyl, and cycloheptyl),[21] and which may be responsible for the less dramatic decrease in rate for cyclohexyl. The stability of cyclopropyl- and cyclobutylcobalamin is reasonable both in terms of I-strain and steric effects (cyclobutyl E_s = −0.06).

2-Norbornylcobalamin, prepared from norbornylene and hydridocobalamin, has identical spectra and decomposition rate with that prepared from *exo*-2-norbornyl bromide, indicating that both preparations produce the same isomeric species.

E. Primary Alkylcobalamins. 1. Coordination of the Axial Base. Figure 2 includes the UV-vis absorption spectra of several *primary* alkylcobalamins. In the order methyl, ethyl, isobutyl can be seen an increasing contribution of features of the "acidic-media" spectra to the "neutral-media" spectra, indicative of increasing proportions of base-off forms B present in neutral solution. Both inductive and steric effects are operable in determining the axial base coordination behavior of these cobalamins (see below). As in the alkyl substituent effects on the decomposition of secondary alkylcobalamins, extension of the hydrocarbon chain has only a minor effect on the coordination constant, relative to the importance of branching at the β-carbon atom. For the series propyl, isobutyl, neopentyl, the log K_{co} correlates with the Taft steric substituent constant with a correlation coefficient of $r = 0.9999$ ($p < 0.01$). This is also an order of increasing electron donation. However, the correlation with the Taft polar substituent constants is weak, with $r = 0.957$, $p = 0.09$.

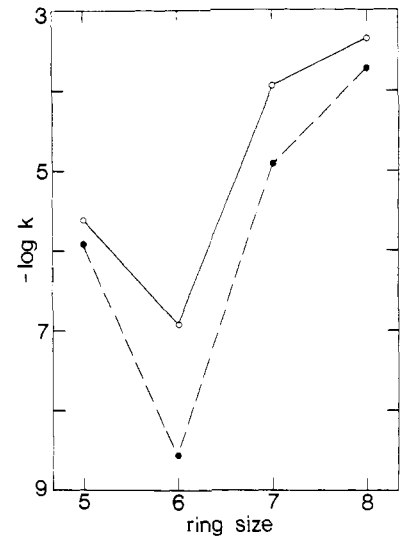


Figure 6. Rate-ring size profiles for the acidic media decompositions of cycloalkylcobalamins (—○—) and for the Cope elimination of cycloalkylamine oxides[22b] (- - -●- - -).

2. Spontaneous Dealkylation of Primary Alkylcobalamins. Although primary alkylcobalamins are normally considered as "stable", we have found that most are in fact metastable, dealkylating on storage in solution at room temperature in total darkness. Only methylcobalamin, with minimal steric requirements and lacking a β hydrogen, showed no discernible spectral changes on aerobic storage in pH 7 buffered solution in the dark for 6 months. Higher primary alkylcobalamins, however, underwent slow decomposition under these conditions. Approximate half-lives for the appearance on the vitamin B_{12a} γ band are as follows: methyl (∞), ethyl (6 months), *n*-propyl (4–5 months), *n*-butyl (6 months), *n*-octyl (11 months), isopentyl (years). Consistent with its relatively low K_{co}, isobutylcobalamin is considerably less stable, spontaneously dealkylating in neutral solution with a first-order $k_{obsd} = K_{co}k_C = 5.6 \times 10^{-7}\ s^{-1}$, $t_{1/2}$ = 14 days. It has far greater stability in acidic media. Neopentylcobalamin decomposes to vitamin B_{12a} in aerobic neutral solution even more rapidly ($t_{1/2} \simeq 30$ min). The series ethyl, *n*-propyl, isobutyl, neopentyl again demonstrates the critical effect of branching at the β carbon on labilizing the Co–C bond to spontaneous cleavage. Gas chromatographic analysis of the gas phases over neutral pH spontaneous decompositions of ethyl-, *n*-propyl-, *n*-butyl-, and isobutylcobalamins revealed olefins arising from β-elimination as the exclusive hydrocarbon products.

F. Alkylcobaloximes. Alkylcobaloximes are less susceptible to sterically mediated dealkylations than the cobalamins. Secondary alkylcobaloximes are accessible by conventional methods of synthesis. Reaction of cobaloxime(I) with *tert*-

Table VI. Measured pK_as and Calculated K_{co}'s for the Axial 5,6-Dimethylbenzimidazole of Primary Alkylcobalamins

alkyl moiety	pK_a	K_{co}[d]
methyl	2.72[a]	95
ethyl	3.87[a]	6
n-propyl	3.84[a]	6
n-butyl	3.93[b]	5
isobutyl	4.20[c]	2
neopentyl	4.55[c]	0.4

[a] From ref 23. [b] From ref 24. [c] Determined by spectrophotometric titration, ±0.05, this work. [d] Calculated from eq 9, with pK_{bzm} = 4.70.

butyl chloride, however, yields no isolable alkylcobaloxime, but evolves isobutylene.[26] Prompted by our experience with *n*-alkylcobalamins, we examined several alkyl(aquo)cobaloximes for their tendency to undergo spontaneous Co-C bond cleavage upon prolonged storage in solution in the dark. Over several months of dark storage in aqueous solution at room temperature, all the primary alkylcobaloximes studied, as well as isopropyl-, cyclopentyl-, and cyclohexylcobaloximes, showed no significant decomposition as measured by the intensity of the characteristic Co-C charge-transfer band. However, 2-butyl-, 2-pentyl-, and 3-pentylcobaloximes did show appreciable decomposition, increasing in this order. 3-Pentylcobaloxime has a half-life of about 1 week for the disappearance of its Co-C CT band. Although isopropylcobaloxime is stable at room temperature, it undergoes thermolysis at lower temperature than *n*-alkylcobaloximes.[27] A synthesis of a hydridocobaloxime by the thermal decomposition of isopropylcobaloxime [with $P(n\text{-}C_4H_9)_3$ as the axial base] has been described.[28]

Discussion

Our study demonstrates that secondary alkylcobalamins can be synthesized and are amenable to investigation. However, most of them exhibit a tendency to undergo spontaneous decomposition in solution. The axial base plays an important role in this decomposition, as does the corrin ring itself, whose flexibility is ultimately responsible for the intriguing phenomena observed.

Mechanism of Dealkylation. The evidence presented for dealkylation via syn β-elimination of olefins need not be repeated here. It should be pointed out, however, that this mechanism is also able to account for trends seen in the product distributions of secondary alkylcobalamin decompositions. For example, the hydrocarbon product ratios for the decomposition of isopropyl-, 2-butyl-, and 2-pentylcobalamin as given in Table VII show that elimination to the terminal olefin is favored over the formation of the 2-ene, and *trans*-2-ene over the cis isomer. These trends can be understood in terms of steric effects on the activation to the syn-elimination transition state. It can be readily envisaged that the transition state leading to the elimination of the 1-olefin is less subject to repulsive eclipsing interactions between the remaining alkyl substituents than the transition states giving rise to trans or cis 2-olefins; cis-olefin elimination is least favored of all. Furthermore, these differences increase with the increasing steric bulk of R‴ (Table VII). Such eclipsing effects also provide a rationale for the decreases in dealkylation rates which accompany extension of the alkyl chain beyond the γ carbon. β-Methyl branching, however, requires gauche methyl-corrin interactions, and, as expected, causes markedly increased decomposition rates.

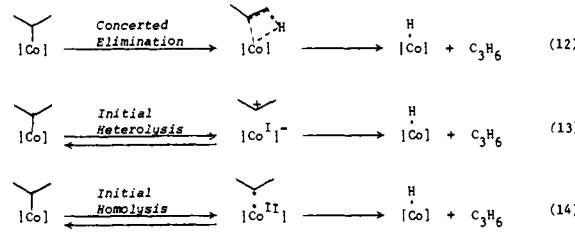
The similarity of the structure-stability trends for primary and secondary alkylcobalamins affirms that the elimination of olefins from primary alkylcobalamins also occurs by syn β-elimination. Indeed, the enhanced stability of the isopentyl

Table VII. Olefin Product Ratios from the Spontaneous Decomposition of Three Secondary Alkylcobalamins

alkyl moiety	R‴	elimination C_1/C_3 ratio	*trans*/*cis*-2-ene ratio
isopropyl	H	1.0	(1.0)
2-butyl	CH_3	1.1	1.3
2-pentyl	C_2H_5	1.4	1.8

derivative is reasonable in terms of the bulky isopropyl group on the β carbon preventing rotation to the eclipsed transition state.

Ultimate evidence for syn β-elimination would involve the characterization of the geometrical isomers arising from the dealkylation of diastereomeric alkylcobalamins of known absolute configuration at the α and β carbons. The question now remains whether the elimination occurs in a concerted manner (eq 12) or stepwise, i.e., via solvent-caged intermediates resulting from initial heterolytic or homolytic Co-C bond cleavage (eq 13 and 14). Pyrolytic syn β-eliminations are



(12) (13) (14)

generally considered as concerted heterolytic reactions with carbon leaving group bond breaking well advanced in the transition state.[29] This mechanism provides a plausible model for the spontaneous dealkylations of alkylcobalamins carrying hydrogen in the β position. The initial heterolysis of the Co-C bond is unlikely for energetic reasons. Initial homolytic-type labilization could occur but does not go as far as to yield solvent-dissociated radicals, as evidenced by the oxygen-insensitivity of the reaction. The photolysis of alkylcobalamins proceeds by a mechanism similar to eq 14,[6,27b,42] but is accelerated by oxygen.[24] The spontaneous elimination reaction must be distinguished from base-catalyzed dealkylations of certain substituted organocobalamins. These need not be discussed here in view of the virtual base independence of the spontaneous decomposition of isopropylcobinamide over a wide pH range.

Effect of the Axial Base. Our results demonstrate that the stability of secondary and other sterically bulky alkylcobalamins is critically dependent upon the ability of the axial base to coordinate to cobalt. When the axial base is absent or protected from coordination (e.g., by protonation or alkylation), the spontaneous dealkylation rates are decreased by orders of magnitude. This effect cannot be electronic as coordination of the nitrogen base increases the electron density on cobalt and thus disfavors activation to a transition state involving a shift of electron density to cobalt.

The origin of the acceleration of dealkylation by axial base coordination is evident from crystallographic data on corrinoids and related compounds.[30] In nickel corrin and cobyric acid, both lacking the axial base, the corrin ring system adopts a planar configuration.[30a] In cobalamin derivatives, however, contacts between the hydrogen atom on carbon B-4 of the benzimidazole and C-5 and C-6 of the corrin ring cause it to bend away from the axial base approximately through an axis containing cobalt and C-10 (see Figure 7). In wet vitamin B_{12} (cyanocobalamin),[30b] this bending amounts to a 19° angle between the two least-squares planes of the corrin ring. This steric interaction is also manifested in asymmetric binding of

the dimethylbenzimidazole, the Co-N(B-3)-C(B-2) and Co-N(B-3)-C(B-9) angles being 117 and 139°, respectively. In coenzyme B_{12},[30a] with a bulkier sixth axial ligand and a longer Co-N(B-3) bond, the corrin ring bend is reduced to 15°, and the angles around the coordinated nitrogen are changed to 123 and 132°. ^{13}C NMR studies suggest that these structural features are preserved in aqueous solution.[13b] We should also note that in the coenzyme the Co-C(α)-C(β) bond angle is 125°, perhaps attributable to repulsion with the corrin ring.

Thus, when the cobalt atom carries a bulky alkyl substituent, an upward bend of the corrin ligand caused by the coordination of the axial base increases repulsive steric interactions, and can be visualized to have a levering action leading to cleavage of the Co-C bond. We are thus dealing primarily with what could be called a "mechanochemical" effect.

It is of interest to note that added imidazole, benzimidazole, and 5,6-dimethylbenzimidazole all accelerate the rate of dealkylation of isopropylcobinamide to about the same extent. Reduced steric acceleration of the dealkylation step in the case of imidazole is apparently offset by greater coordination to cobalt due to its higher basicity and smaller steric requirements. Indeed, imidazole even accelerates the rate of dealkylation of cyclohexyl*cobalamin*.[6]

These conformational changes of the corrin ring must be distinguished from the effects of ligands and bases on iron in porphyrins.[31,32] In these, a movement of the iron out of the plane of the porphyrin is observed, causing diminished affinities for oxygen and carbon monoxide. Several authors have accordingly suggested that the cobalt atom of vitamin B_{12} may likewise move significantly out of the corrin ligand.[5,33] The extremely tight binding of cobalt in corrins as compared to iron in porphyrins argues strongly against a motility of cobalt. The corrin ring is also more flexible than the fully unsaturated porphyrin. It thus seems far more plausible to instead assume that the conformational changes in the corrins are due to bending of the corrin ring, without appreciable movement of the cobalt out of the plane of the corrin nitrogens.

Axial coordination is accompanied by a bathochromic shift of the first inter-π transition of the corrin chromophore, 7π-8π, due to higher energy of the 7π orbital. This orbital is delocalized over the whole corrin molecule, having contributions of not only the corrin π-electron system, but also of the cobalt atom ($3d_{z^2}$,4s,4p_z). On axial coordination, the stability of the axial Co-C bond is slightly decreased owing to the change in energy of 7π. This change is chemically of little consequence, because the energies involved are too small. Salem et al.,[33] however, have recently called 7π the "strain orbital" and formulated a hypothetical mechanism of coenzyme B_{12} enzymatic reactions on the basis of its energy changes with axial coordination and axial motion of Co out of the corrin. Our work demonstrates that the stability of the Co-C bond in unsubstituted alkylcobalamins is determined primarily by steric factors, secondarily by inductive electronic factors, and only slightly by electronic factors involving the vertical π-electron system.

Steric Effects of the Co-Alkyl Groups. If the corrin ligand system is flexible in both directions of the vertical axis, large alkyl substituents attached to cobalt should bend the corrin ring downward, resulting in cleavage of the coordinative bond between the axial base and the corrin cobalt atom. Depending on the degree of distortion, secondary alkylcobalamins may be entirely base-off or significantly base-on. Previously, variations in axial base coordination in cobalamins have been attributed to the σ-electron donating ability of the ligands in the trans position, with little contribution by steric factors.[34a] Indeed, the lack of axial base coordination in isopropyl- and cyclohexylcobalamins has been ascribed by some authors to the inductive effects of these groups.[34b] That this is the sole contributing effect is unlikely, considering the far smaller relative changes in base coordination with inductive effect seen among *n*-alkylcobalamins. Moreover, the cyclopentyl group is more electron donating than either isopropyl or cyclohexyl,[21] yet cyclopentylcobalamin has appreciable base-on character in neutral solution.

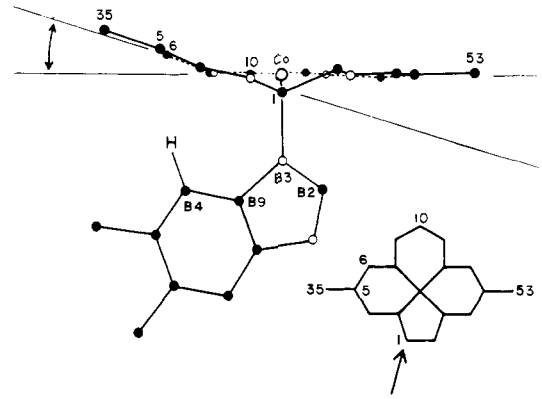


Figure 7. The axial base induced "bent-up" conformation of the corrin ligand in coenzyme B_{12}. Adapted from ref 30a.

A plot of log K_{Co} vs. Taft σ^* values reveals increasing deviations toward lower K_{Co} with increasing steric bulk of the alkyl groups. For example, isobutyl has an inductive effect between *n*-propyl and *n*-butyl, yet its cobalamin has a significantly lower K_{Co} than either of these. On the other hand, between methyl and ethyl, where steric requirements differ only slightly, the large difference in electron donation has the predominant effect on K_{Co}.

Among cycloalkylcobalamins, in the order cyclopropyl, cyclobutyl, cyclopentyl, both electron donation and steric bulk increase, causing decreasing K_{Co}'s. In changing from cyclopentyl to cyclohexyl, the increasing steric effect must dominate over the decreasing electron donation.

Evidence that such steric effects on axial base coordination are also operative in cobalamins lacking observable base-on form in neutral solution comes from examination of their dealkylation rates. Thus, Table IV reveals that the pH 7 dealkylation rates ($K_{Co}k_C$) of acyclic secondary alkylcobalamins are less sensitive to the steric bulk of the alkyl group than are the acidic solution rates (k_A). Owing to greater alkyl group–corrin ring steric interactions caused by coordination of the axial base, k_C is expected to be more sensitive to steric bulk than k_A. This effect must be offset by an opposing effect on K_{Co}. Acyclic secondary alkyl groups vary little in their inductive effect, and significantly in their steric effect.[21]

Conformational Equilibria in Alkylcorrinoids. Conformational changes of the corrin moiety were first discussed by Williams and his school,[4,35] who characterized the absorption spectra of corrinoids as ranging from "normal" to "anomalous", dicyanocobinamide and isopropylcobinamide being representative of the two extremes. Among alkylcobinamides, "anomalous" character increases in the order methyl, ethyl, isopropyl. It was furthermore shown that organocobinamides of "intermediate" character undergo temperature-dependent, reversible changes between the two extremes. The Oxford school proposed reversible equilibria involving dissociation of axial water, accompanied by conformational changes, to form five-coordinate species of "anomalous" character, favored by increasing temperature and electron donation by the axial ligand. Examining the temperature dependence of the ^{13}C NMR of ^{13}C-enriched organocorrinoids, Hogenkamp et al.[36] later concluded that these changes are best described as due to a conformational change of the corrin ligand, without invoking a change in coordination number. Brodie has assigned

coordinated water resonances in the ^1H NMR spectra of several alkylcobinamides in Me_2SO.[37]

According to the criteria of Firth et al.,[35] the alkylcobalamins of Figure 2 show increasing "anomalous" spectral character with increasing steric bulk of the alkyl group in the same manner as decreasing axial base coordination and increasing dealkylation rates. Thus, among the alkylcobalamins studied by us, increasing "anomalous" character appears to be associated with increasing bent-down distortion of the corrin ring. It may be assumed that the changes of the optical and NMR spectra of primary alkylcobalamins and cobinamides as a function of temperature are to a significant degree caused by conformational changes not unlike those induced by sterically demanding secondary or branched primary alkyl groups.

Biochemical Implications. Our study has shown that concentrated, heterolytic dealkylations of organocorrinoids can be induced under surprisingly mild conditions through conformational changes of the corrin ligand. These changes can result from the attachment of bases to cobalt or thermal motions of the corrin, and, because of close contacts with the cobalt-bound organic group, can exert a mechanochemical labilization. Conversely, the corrin ligand may undergo conformational changes in the opposite direction and thus accommodate bulky organic substituents. In biochemical reactions, such conformational changes could be enzymatically mediated and thus integrated with bond-forming and bond-breaking processes in the catalytic cycle.

Experimental Section

Materials. Vitamin B_{12a} (N.F., 82.5% hydroxocobalamin) and vitamin B_{12} (USP) were obtained from Merck Sharp and Dohme Research Laboratories, Rahway, N.J. Dicyanocobinamide was prepared from vitamin B_{12} according to Friedrich and Bernhauer[38] and converted to diaquocobinamide as described by Firth et al.[39] Argon was freed of oxygen by passing through a chromous or alkaline pyrogallol scrubber and dried with anhydrous calcium sulfate. Imidazole, benzimidazole, and 5,6-dimethylbenzimidazole were decolorized and recrystallized before use. DCl/D_2O was prepared from C_6H_6COCl and 99.8% D_2O. Alkylcobaloximes were prepared by standard methods.

Isomerically pure 2-pentyl iodide, 3-pentyl iodide, and 3-hexyl iodide were prepared from the corresponding alcohols via the tosylates by the method of Brown and Wheeler.[40] Neopentyl iodide, 3-methyl-2-butyl iodide, and 3,3-dimethyl-2-butyl iodide were prepared analogously by refluxing the tosylates with NaI in glyme. Other alkyl iodides, alkyl bromides, and olefins were commercial products used as received or dried and distilled as necessary. Secondary alkyl iodides were washed with aqueous $Na_2S_2O_3$ and distilled prior to use. The purity of the alkylating agents was checked by ^1H NMR. Isopropyl-d_7 bromide, 99 atom % D, was obtained from Merck Sharp and Dohme.

All other reagents and chemicals were used as received except where indicated.

Instrumentation. Visible absorption spectra were recorded on a Beckman DK-2A ratio recording spectrometer. Mass spectra were recorded on a LKB 9000 GC–mass spectrophotometer. Stopped-flow kinetic measurements were obtained on a Durrum-Gibson stopped-flow instrument. Hydrocarbons were determined on a Hewlett-Packard Model 700 gas chromatograph equipped with a 8 ft × 1/8 in. column of *n*-octane/Porasil C (GC Durapak, Waters Associates), with He as the carrier gas and FID detector. By variation of the carrier gas flow and the column temperature, satisfactory separation of each gas mixture studied was obtained. Identities of hydrocarbons were verified by cochromatography with authentic commercial samples. Photolyses of organocobalamins were performed at 15-cm distance from a 150-W floodlamp with air stream cooling.

Preparation of Alkylcobalamins. A. All primary alkylcobalamins, as well as cyclopropylcobalamin and cyclobutylcobalamin, were prepared by alkylation of vitamin B_{12s} by a modification of the alkaline acetoin method.[41] To 25 mg of vitamin B_{12a} in 3 mL of water in a 10-mL serum capped vial is added 0.5 mL of acetoin (85% aqueous solution) and the vial is flushed with argon. Injection of 1.0 mL of 6 N NaOH leads to the quantitative reduction of vitamin B_{12a} to vitamin B_{12s} within a few minutes. The alkylating agent (0.05 mL) is then injected and all further operations are performed in subdued laboratory light. The vial is shaken until the cherry-red color of the alkylcobalamin is obtained. The contents are then poured into a separatory funnel, diluted with water (20 mL), and neutralized with acetic acid. The cobalamins are extracted into a minimal volume (<5 mL) of 1:1 phenol–chloroform, precipitated by the addition of 10–20 volumes of ether, and collected by centrifugation. After redissolving in a minimal volume of methanol and reprecipitation with ether, the solid alkylcobalamin is dried under a stream of dry argon and stored in a foil-wrapped vial in a refrigerator. Isopropylcobinamide was prepared analogously, from diaquocobinamide.

B. Secondary alkylcobalamins were obtained by the reaction of hydridocobalamin with alkylating agents as follows. Vitamin B_{12a} (25 mg) is dissolved in 1 mL of glacial acetic acid in a 4-mL test tube. (The solvent is changed to 1:1 acetic acid–methanol for higher alkyl halides to improve solubility of the resulting cobalamins.) To the solution, 100 mg of oven-dried zinc dust is added and the tube serum capped and flushed with argon. Shaking produces green hydridocobalamin within a few minutes. (Hydridocobalamin is green in diffuse sunlight, but appears maroon in fluorescent light). Addition of a few drops of alkylating agent from a syringe needle, in the dark, produces the alkylcobalamin within a few minutes. The tube is centrifuged, and the supernatant added to 40 mL of dry ether, in which all supernatant components except the cobalamin, including Zn salts, are soluble. The precipitated alkylcobalamin is collected by centrifugation, washed several times with anhydrous ether, and dried in a stream of dry argon. Samples of the more stable derivatives, prepared in this manner, may be stored in foil-wrapped vials below 0 °C for months with little decomposition. Less stable derivatives are best prepared immediately prior to use, while the least stable ones are not amenable to isolation. For many experiments, the reaction supernatant is suitable for direct use.

Kinetic Measurements. A. Spontaneous Dealkylations with $t_{1/2} >$ 24 h. Solutions of the alkylcobalamin (>0.25 mM in 1 M acid or 0.1 M phosphate buffer, pH 7) were stored in foil-wrapped vials. At appropriate times, aliquots were diluted to spectrophotometric concentration and visible spectra recorded before and after photolysis. The photolyzed spectrum represents the infinite reaction corresponding to that particular time point. The first-order rate constants were determined from the slopes of plots of $\ln (1 - A_t/A_{h\nu})$ vs. time, where A_t is the absorbance at 350 nm before photolysis at time t. Analyzed in this manner, variations in the concentrations of measured solutions and in instrumental adjustments become inconsequential. Owing to the slow oxidation of cobinamide(II) to cobinamide(III) in neutral and alkaline solution, aliquots of these dealkylations were acidified 20 min before the spectra were recorded.

B. Dealkylations with $t_{1/2} < 24$ h. Supernatant solutions from hydridocobalamin reactions were prepared as above on a 1/5 to 1/10 scale. A fraction of a drop from a syringe needle was added to a cuvette of acid or buffer solution at ambient temperature and the visible spectrum recorded as a function of time. Sharp isosbestic points were observed. Toward the end of the reaction, the cuvette was photolyzed to obtain the infinite reaction spectrum. First-order rate constants were obtained from the least-squares slopes of $\ln (A_{h\nu} - A_t)$ vs. time (t) plots, the absorbance being that at 350 nm. Buffers were usually 0.5–1.0 M in phosphate, acetate, or borate. Solution pHs were checked at the completion of each kinetic run.

C. Stopped Flow Kinetics. Solutions of the alkylcobalamins were prepared in acetic acid–methanol as described above. An aliquot was diluted in 10 mN HCl and this solution loaded into one drive syringe. The other drive syringe (equal volume) was loaded with 0.5 M phosphate buffer, pH 7.0. Decay curves were monitored at 350 nm and first-order rate constants evaluated in the usual manner.

D. Isopropylcobinamide Dealkylations in the Presence of Added Bases. A fraction of a drop from a syringe needle of a concentrated solution of the cobinamide in ethanol was added to a serum-capped cuvette containing a 1.0 M solution of base in 80% ethanol, 20% 10 mM borate buffer, pH 9.0, which was flushed with oxygen, to promote oxidation to Co(III). Repetitive time scans gave sharp isosbestic points and the first-order rate constants were evaluated at the λ_{max} (γ band) of the resulting bis(base) adduct.

E. Propylene Evolution. Isopropylcobalamin (5 mg) was dissolved in 0.5 mL of 0.1 N HCl in a foil-wrapped serum-capped vial of 60-mL capacity. To initiate decomposition, 5.0 mL of 0.5 M buffer was rapidly injected. For anaerobic conditions, the vial and the buffer were

deaerated with argon first. The vial was continuously shaken during the reaction, and at appropriate time intervals 0.25-mL aliquots of the gas phase were removed with gas-lock syringes. Toward the end of the reaction, the foil was removed and the vial photolyzed to obtain the infinite reaction yield. The propylene content of the aliquots was analyzed by GLC and first-order rate constants were obtained in the usual manner.

Hydrocarbon Product Analyses. Solid alkylcobalamin (5-10 mg) was placed in a foil-wrapped, serum-capped vial of 25-mL capacity. Dealkylation was induced by the injection of 10 mL of the acid or buffer solution. After appropriate time interval, aliquots of the gas phase were analyzed by GLC.

Methyl Iodide Trapping Experiment. Isopropylcobalamin (5 mg) was placed in a foil-wrapped test tube which was serum capped and deaerated. Methyl iodide (0.2 mL) was added and the tube deaerated further to remove all oxygen. Deaerated 1:1 methanol-0.2 M phosphate buffer, pH 7.5 (0.5 mL) was then injected. After 20 min the reaction solution was cherry red owing to the formation of methylcobalamin. The product was isolated as a solid by phenol extraction from water and precipitation as described above. The yield was determined spectrophotometrically (95% bases on isopropylcobalamin) and the identity verified by TLC on cellulose with 10:3:7 (v) 1-butanol-ethanol-water as the eluent, and by cochromatography.

HD Evolution Experiment. Cyclooctylcobalamin (50 mg) was freshly prepared in CH_3CO_2D (ICN) from cyclooctyl bromide, isolated as a solid, and placed in a foil-wrapped, serum-capped vial. After careful deaeration by pyrogallol-scrubbed and KOH-dried argon, 3 mL of deaerated 1 M DCl in D_2O was injected. After decomposition was complete, the gas phase was analyzed by mass spectrometry, and showed a $HD^+:D_2^+$ peak ratio of 0.12. A control in which Zn was added to the acid gave $HD^+:D_2^+ = 0.02$. Decomposition of 4-heptylcobalamin in D_3PO_4/D_2O produced a similar proportion of HD. Likewise, decomposition of isopropylcobalamin-d_7 in 1 M HCl at 70 °C gave a mass 3:mass 2 ratio of 0.09.

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