Synthesis and Reactions of Formylmethylcobalamin and of Related Compounds

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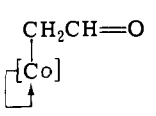
Abstract: The compound formylmethylcobalamin was prepared by the reaction of bromoacetaldehyde with vitamin B_{12s} and isolated in crystalline form. Formylmethylcobalamin was characterized by thin-layer chromatography, the uv-visible absorption spectrum, and by 220-MHz proton magnetic resonance measurements. Photolytic, hydrolytic, and thermolytic cobalt-carbon bond cleavage experiments provide additional proof of the structure of the compound. The synthesis of α-chloro- and α,α-dichloroformylmethylcobalamin is also described together with that of the diethylacetal of formylmethylcobalamin. Formylmethylcobalamin is stable in neutral aqueous solution but decomposes in acidic media according to the rate law: $-d[\text{formylmethylcobalamin}]/dt = [H^+][\text{formylmethylcobalamin}][(k_2K_a + k_2'[H^+])/(K_a + [H^+])]$. The values of the rate constants for "base on" (k_2), and "base off" (k_2') formylmethylcobalamin decomposition were found to be 4.3 and 0.30 $sec^{-1}\ M^{-1}$, respectively; the equilibrium constant K_a for the protonation of the coordinated 5,6-dimethylbenzimidazole is 6×10^{-4}. The acid-induced decomposition of the diethylacetal of formylmethylcobalamin and of related compounds was reinvestigated and fully elucidated. Conflicting reports in the literature concerning the stability and properties of formylmethylcobalamin and of its derivatives are explained on the basis of new experimental evidence and by comparison with formylmethylcobaloxime.

Similarities between the properties and reactions of organometallic derivatives of vitamin B_{12} and of those of model compounds such as the cobaloximes[1] are by now so well documented that it has become more interesting to examine significant differences between representatives of the two classes of compounds. The present paper deals with one such example, which recently gave rise to conflicting reports. The synthesis of formylmethylcobalamin, **1**, was claimed in 1973 independently by Silverman and Dolphin,[2]



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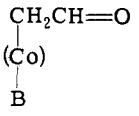

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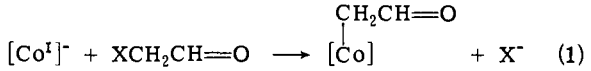
and in our laboratory.[3] The compound was initially of interest because it was considered to be an intermediate in the coenzyme B_{12} catalyzed enzymatic conversion of ethylene glycol to acetaldehyde. In spite of the apparent simplicity of the compound, its synthesis has remained doubtful since its properties as reported by the two groups of workers differed substantially. The formylmethylcobalamin described in ref 2 was found to be stable only in alkaline solution and was shown to decompose rapidly in neutral or acidic media. The formylmethylcobalamin obtained in our laboratory[3] possessed the stability of a typical substituted organocobalamin; its absorption spectrum was reported from measurements in pH 5.8 buffered aqueous solutions, thus under conditions where it should have undergone rapid decomposition if it had been identical with the compound described in ref 2. Consequently, questions were raised as to the authenticity of the compounds. However, the two "formylmethylcobalamins" described have thus far not been compared with each other since each group experienced difficulties in reproducing the findings of the other. After an initial failure[4] due to a lack of experimental detail in the early communications, all experimental observations of the authors of ref 2 have since been duplicated in our laboratory. In a later publication, the synthesis of the acid-labile formylmethylcobalamin by several different methods was delineated.[5] Due to its instability, the compound has apparently never been obtained in crystalline form; its characterization rests essentially on observations in solution. If formylmethylcobalamin is indeed as unstable as has been suggested,[2,5] the anomalous sensitivity of the Co-C bond in this compound must be explained in view of the observed stability of formylmethylcobaloxime, **2**. This compound was first described in 1967 and was at that time isolated without special precautions by the hydrolysis of its dimethylacetal.[6] Although diacetals of formylmethylcobalamin have also been synthesized, these were found to undergo acetal hydrolysis with simultaneous cleavage of the Co-C bond.[3,7] There is again considerable disagreement between the two groups of workers as to whether formylmethylcobalamin is an intermediate in the hydrolysis of the diacetals. Although a synthesis of the compound by the hydrolysis of the diacetals in weakly alkaline solution has been reported,[5] treatment of the diacetals with dilute acids indicated that the organocobalamins present in solution underwent complete decomposition.[3] It therefore became necessary to reinvestigate the reactions of the diacetals of formylmethylcobalamin as well. In the following we will show that our formylmethylcobalamin as well as α-chlorosubstituted derivatives thereof can be isolated and characterized. Formylmethylcobalamin possesses the properties as previously reported. We have also duplicated the experiments in ref 5 and have made a number of new observations which we feel resolve most if not all of the existing discrepancies.

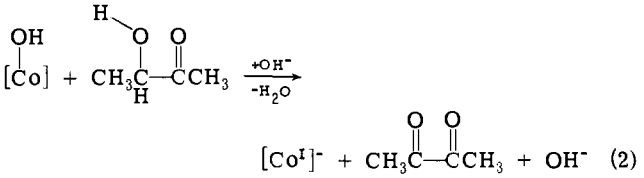
Results

Synthesis of Formylmethylcobalamin and of Related Compounds. The synthesis of **1** by the reaction of vitamin B_{12s} with haloacetaldehydes (eq 1) requires special precau-



$$[Co^I]^- + XCH_2CH{=}O \longrightarrow [Co]\text{–}CH_2CH{=}O + X^- \quad (1)$$

tions due to the reactivity of the CH=O moiety in halogenated derivatives of acetaldehyde.[3] It is convenient to employ alkaline 3-hydroxybutanone-2 as the reductant for the synthesis of vitamin B_{12s}. The reduction of hydroxocobalamin proceeds rapidly according to eq 2; a removal of the excess



$$[Co]\text{–}OH + CH_3C(H)(OH)\text{–}C(=O)CH_3 \xrightarrow[-H_2O]{+OH^-} [Co^I]^- + CH_3C(=O)\text{–}C(=O)CH_3 + OH^- \quad (2)$$

Table I. R_f and pK_a Values and Rate Constants (k_2) of Acid Decomposition of Formylmethylcobalamin and Related Compounds at 25°

Compound	R_f[a]	pK_a[b]	k_2, sec^{-1} M^{-1}
Formylmethylcobalamin	0.52	3.19	4.3 ± 0.5
Formylmethylcobinamide	(n.d.)	—	0.3 ± 0.05
α-Chloroformylmethyl-cobalamin	0.46	3.43	(7.0 ± 2) × 10^{-5}
α,α-Dichloroformyl-methylcobalamin	0.47	2.75	(8.9 ± 0.5) × 10^{-6}
Formylmethyl(aquo)-cobaloxime	(n.d.)	—	(2.6 ± 0.5) × 10^{-5}
β-Hydroxyethylcobalamin	0.43	3.15	(4.3 ± 0.5) × 10^{-3}
α-Chloro-β-hydroxyethyl-cobalamin	0.46	3.25	(1.0 ± 0.2) × 10^{-4}
α,α-Dichloro-β-hydroxy-ethylcobalamin	0.50	3.17	(6.7 ± 0.4) × 10^{-4}

[a] Chromatography on Brinkman or Kodak Cellulose plates with ascending phase 1-butanol:ethanol:H_2O (400:120:280) with 0.5% concentrated aqueous ammonia. [b] Protonation of coordinated 5,6-dimethylbenzimidazole, determined spectrophotometrically.

Table II. Observed and Calculated $t_{1/2}$ Times, Rate Constants, and Rates of Hydrolysis of Formylmethylcobalamin at Various pH Values at 25°

pH	$t_{1/2}$ times (min) Obsd	$t_{1/2}$ times (min) Calcd[a]	k_{obsd}[c]/(H+)	Rates (sec^{-1}) Obsd[b]	Rates (sec^{-1}) Calcd[a]
5.3	515	537	4.48	2.3 × 10^{-5}	2.15 × 10^{-5}
4.9	194	215	4.73	6.0 × 10^{-5}	5.36 × 10^{-5}
4.6	93.9	107	4.80	12.3 × 10^{-5}	10.80 × 10^{-5}
3.9	28.8	25.2	3.19	3.9 × 10^{-4}	4.48 × 10^{-4}
3.5	12.3	12.3	2.97	9.7 × 10^{-4}	9.37 × 10^{-4}
3.0	5.3	6.25	2.18	2.2 × 10^{-3}	1.85 × 10^{-3}
2.1	2.3	2.45	0.63	5.0 × 10^{-3}	4.72 × 10^{-3}

[a] Based on the rate law $d[B_{12a}]/dt = [H^+][\text{formylmethylcobalamin}]_T [(k_2K_a + k_2'(H^+))/((H^+) + K_a)]$; the values for the rate constants of the base-off (k_2') and base-on (k_2) hydrolysis of formylmethylcobalamin were determined to be 0.30 and 4.3 sec^{-1} M^{-1}, respectively; the former value was obtained from the hydrolysis of formylmethylcobinamide. $[\text{formylmethylcobalamin}]_T$ denotes the sum of the concentrations of base-on and base-off formylmethylcobalamin. [b] Average value at each pH. [c] In units of M^{-1} sec^{-1}.

of reducing agent prior to the alkylation is not necessary.[3,8,9] Formylmethylcobalamin was originally obtained by the reaction of vitamin B_{12s} both with chloro- and with bromoacetaldehyde.[3] The best yields of product were achieved with bromoacetaldehyde as the alkylating agent; the latter was also employed in the present study. Isolation of the organocobalamin from the reaction solutions was effected by phenol extraction and recrystallization from water–methanol or water–acetone mixtures.

The solid product proved to be stable on storage at 0° in the dark, was crystalline, and its absorption spectrum in pH 5.8 buffered aqueous solution was identical with that reported previously.[3] Thermolysis of the solid compound above 140° afforded acetaldehyde as the only volatile product derived from the cobalt-bound $CH_2CH{=}O$ moiety. Photolysis of neutral aqueous solutions produced hydroxocobalamin and acetaldehyde. The compound was shown to be pure by chromatographic analysis on cellulose plates with various solvent mixtures. The R_f value of 0.52 (with 1-butanol:ethanol:H_2O = 10:3:7 as the ascending phase containing 0.5% NH_4OH) was identical with that reported for Dolphin's[5] formylmethylcobalamin. Under the same conditions of chromatographic analysis, 2-hydroxyethylcobalamin has the R_f of 0.43 and was not detected in any of our preparations. The compound was identical with the product described as formylmethylcobalamin in ref 3; its pK_a for the displacement of the coordinated 5,6-dimethylbenzimidazole on protonation was redetermined and found to be 3.19, in essential accord with the value of 3.20 reported previously. The formylmethylcobalamin prepared according to eq 1 proved to be very stable in neutral aqueous solution but was found to decompose in acidic media to produce hydroxocobalamin and acetaldehyde. Rates of this reaction were determined under various conditions and will be described below.

Treating vitamin B_{12s} with α,α-dichloroacetaldehyde we also synthesized α-chloroformylmethylcobalamin; with chloral hydrate and vitamin B_{12s} the α,α-dichloroformylmethylcobalamin was obtained as well. Both compounds were isolated by phenol extraction and were recrystallized from water–methanol or water–acetone without decomposition. R_f and pK_a values of the compounds isolated are given in Table I together with the corresponding data for β-hydroxyethyl- and α-chloro-β-hydroxyethylcobalamin, which were synthesized for comparative purposes. Formylmethyl(pyridine)cobaloxime was prepared according to ref 6. The diethylacetals of formylmethylcobalamin and of α-chloroformylmethylcobalamin were synthesized by the reaction of vitamin B_{12s} with the diethylacetals of chloro- and of dichloroacetaldehyde, respectively. The diethylacetal of formylmethylcobalamin was was also prepared from ethyl vinyl ether and hydroxocobalamin in ethanol as described in ref 2, and the diethylacetals synthesized by the different methods were proved to be identical. According to ref 5, formylmethylcobalamin is formed if ethyl vinyl ether is treated with hydroxocobalamin in 95% ethanol containing a trace of triethylamine. The presence of a compound with the R_f value of 0.52 (1-butanol:ethanol:H_2O, 10:3:7, 0.5% NH_4OH) was confirmed by chromatographic analysis of the reaction solution. However, since the diethylacetal of formylmethylcobalamin is the by-product in this reaction pure formylmethylcobalamin is difficult to isolate by this method. There are other complications which shall be discussed later.

Acid-Induced Decomposition of Formylmethylcobalamin. Formylmethylcobalamin prepared according to eq 1 is stable in alkaline media but decomposes in acidic solution into hydroxocobalamin and acetaldehyde. The reaction was followed spectrophotometrically by measuring the rate of formation of hydroxocobalamin as a function of pH. Between pH 4 and 6, formylmethylcobalamin decomposes cleanly by a second-order reaction with k_2 = 4.3 ± 0.5 sec^{-1} M^{-1}. The calculated half-life is ca. 10^3 hr at pH 7, consistent with previous qualitative observations on the stability of this organocobalamin. The half-life at pH 5.8 is still approximately 24 hr and thus quite sufficient for measurements of the optical absorption spectrum. Below the pH of 4, the axially coordinated 5,6-dimethylbenzimidazole becomes protonated. This causes a decrease of the second-order rate constant. The observed $t_{1/2}$ times and rates at different pH's are in agreement with the calculated values (Table II). A typical example of a spectrophotometric rate determination is shown in Figure 1, and is consistent with the behavior of a pure compound.

Acid-Induced Decomposition of the Diethylacetal of Formylmethylcobalamin. The decomposition of the diethylacetal of formylmethylcobalamin was followed spectrophotometrically by measurement of the rate of hydroxocobalamin formation. Figure 2 shows that the decomposition occurs in three stages. The initial reaction proceeds with a second-order rate constant k_1 of 26000 sec^{-1} M^{-1}, identical with that reported in ref 3. Analysis of the reaction solutions by GLPC revealed that ethyl vinyl ether is formed during the initial rapid phase of the reaction; acetaldehyde is produced mainly during the slower process.

After a solution of the diethylacetal in aqueous pH 9.5

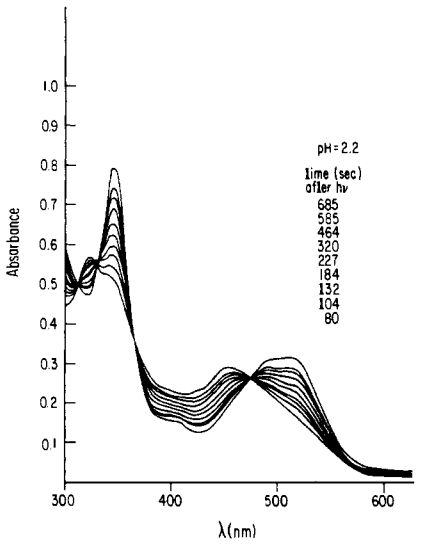


Figure 1. A repetitive scan of the visible spectrum of a solution of formylmethylcobalamin at pH 2.2 (phosphate buffer). The times of the scans (after the addition of formylmethylcobalamin to the buffer solution) are noted. The last scan refers to the t_∞ spectrum after photolysis. The peak at 337 nm is characteristic of vitamin B_{12a} and increases with time. At this pH isosbestic points occur in the visible spectrum at 309, 324, 352, and 480 nm.

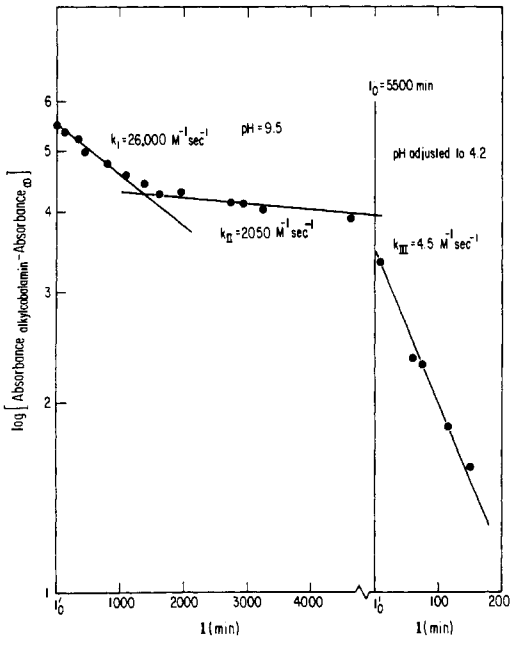


Figure 2. A logarithmic absorbance vs. time plot of the decomposition of formylmethylcobalamindiethylacetal at pH 9.5 (borate buffer). The decomposition was monitored by the change in the visible spectrum. The rate of appearance of vitamin B_{12a} was observed by the increase in the intensity of the characteristic 357 nm peak of the product. The rapid initial rate of decomposition (k_1 = 26000 sec^{-1} M^{-1}) was followed by a slower phase (k_{II} = 2050 sec^{-1} M^{-1}). After 3.5 days, glacial acetic acid was added to bring the pH to 4.2. The third phase of acid induced decomposition was monitored as before, the absorbance readings were corrected for dilution, and the second-order rate constant was calculated (k_{III} = 4.5 sec^{-1} M^{-1}). The t_∞ absorbance value was obtained by aerobic photolysis of the remainder of the alkylcobalamin to B_{12a}.

buffer was allowed to stand for 3.5 days at room temperature in the dark, the hydroxocobalamin formation was 78% complete. At this point the pH of the solution was lowered to 4.2 and the rate of decomposition of the organocobalamin remaining was determined. The second-order rate constant k_{III} for this reaction was 4.5 sec^{-1} M^{-1}, thus identical with that of formylmethylcobalamin. This result demonstrates that the diethylacetal undergoes partial hydrolysis to formylmethylcobalamin in alkaline solution, consistent with the claims of the authors of ref 5, except that the terminal product hydrolyzes at the rate identical with that of our compound.

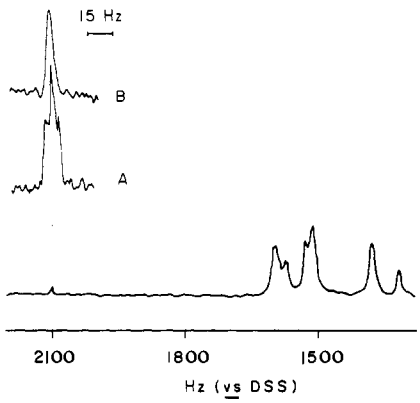


Figure 3. Downfield portion of the 220-MHz proton FT NMR spectrum of formylmethylcobalamin, in D_2O, pH 8.6. The spectrum represents the transform of 256 transients of free induction decay of the sample from a 15 μsec pulse. The inserts are the region around 2100 Hz (9.54 ppm) downfield from DSS, the internal standard, with increased spectral intensity. The A trace results from the aldehyde proton of formylmethylcobalamin. The triplet splitting of this signal is due to the two adjacent methylene protons. After standing for 4 days at this pH, these protons exchange with ^2H of the solvent and the splitting disappears as shown in B.

Acid-Induced Decomposition of Other Organocobalt Compounds. The observed acid lability of formylmethylcobalamin prompted us to study the behavior of the corresponding cobaloxime. This compound is more resistant to acid hydrolysis than the cobalamin but was found to undergo Co-C bond cleavage with acetaldehyde formation in 2-6 *N* HCl. The rates of acid decomposition of other compounds prepared for comparative purposes are summarized in Table I; all reactions were run under pseudo-first-order conditions at a constant pH.

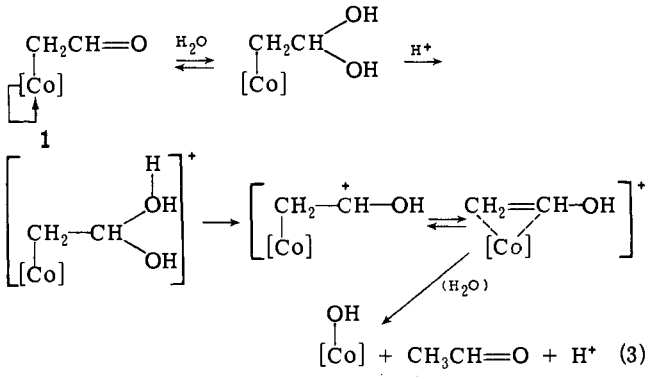
NMR Spectrum of Formylmethylcobalamin. The 220-MHz ^1H NMR spectrum of formylmethylcobalamin in D_2O shows the signal of the α-proton at 9.54 ppm (vs. DSS pH 8.6) (Figure 3), thus in the normal range for aldehyde protons. The spectrum was essentially the same as reported in ref 2. The signal showed the expected triplet with J = ~4 Hz and on prolonged standing isotopic exchange of the β-protons with deuterium causes changes, ultimately the appearance of a singlet. Photolysis of the solution of formylmethylcobalamin in D_2O caused the disappearance of the signal at 9.54 ppm with concomitant appearance of the signal of acetaldehyde (confirmed by coinjection). The internal standard, DDS (see above), is 2,2-dimethyl-2-silapentane 5-sulfonate, employed as the sodium salt.

Periodate Oxidation of 2,3-Dihydroxy-*n*-propylcobalamin. Silverman et al. briefly described the oxidation of 2,3-dihydroxy-*n*-propylcobalamin by periodate and reported that this yields formylmethylcobalamin.[5] However, the product considered to be formylmethylcobalamin was very acid sensitive. We have repeated this reaction at pH 9, 7, 6.5, and 4 and found that periodate induces the Co-C bond cleavage of 2,3-dihydroxy-*n*-propylcobalamin; the rate of this decomposition reaction is identical with that attributed to formylmethylcobalamin hydrolysis in ref 5. The yields of formylmethylcobalamin were very low in all of our experiments (<10% of the total amount of 2,3-dihydroxy-*n*-propylcobalamin, determined by thin-layer chromatography

and spectrophotometry of the reaction solutions); this product underwent acid hydrolysis at the same rate as reported for authentic formylmethylcobalamin (see Table II). We thus have found no contradiction in the behavior of formylmethylcobalamin synthesized by the two routes.

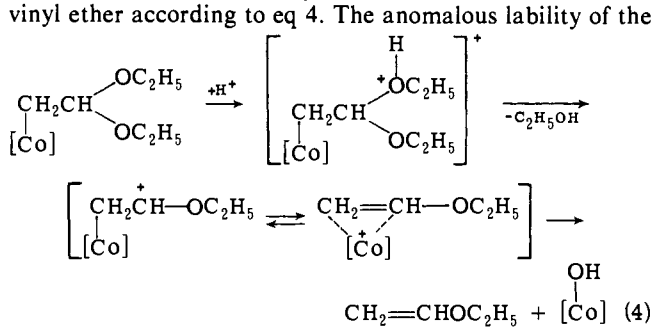
Discussion

The present work confirms that formylmethylcobalamin is a stable compound and that its decomposition is exceedingly slow in neutral solution at room temperature. Hydroxocobalamin and acetaldehyde are the products of the acid-induced Co-C bond cleavage which is best formulated according to eq 3. The cleavage of the Co-C bond in **1** is assumed to occur via the hydrate of **1**. Since β-hydroxyethylcobalt complexes undergo acid-induced Co-C cleavage as well, the analogous reaction of **1** thus is not unexpected. The same reaction has been observed with the cobaloxime derivative **2**. This compound is more acid-resistant than **1**, probably because of electronic rather than steric reasons. Extended MO calculations have shown that the cobalt atom in cobaloximes bears a higher positive charge than in corrins.[10] The Co-C bond cleavage according to eq 3, thus should be facilitated if the electron density on cobalt is high. The observed lower acid-sensitivity of α-chloro- and α,α-dichloroformylmethylcobalamin and of formylmethylcobinamide may be explained on this basis. In the Cl-substituted derivatives it is due to the inductively electron-withdrawing effect of chlorine; in the cobinamide the effective charge density on cobalt is probably lower due to the absence of the coordinated 5,6-dimethylbenzimidazole. In ref 5, the protolysis of **1** was formulated without invoking the hydration of the CH=O group. This mechanism must be considered as less likely in view of the known properties of aldehydes in aqueous media.



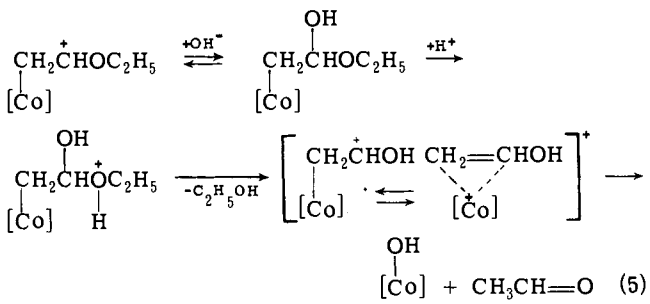
$$[\mathrm{Co}] + CH_3CH{=}O + H^+ \quad (3)$$

The acid-induced decomposition of diacetals of formylmethylcobalamin is accompanied by substantial Co-C bond cleavage, in contrast to the behavior of the corresponding cobaloxime derivatives, which undergo only acetal hydrolysis under similar conditions. The present work shows that the acid hydrolysis of the diethylacetal of formylmethylcobalamin occurs in at least two stages. During the rapid initial phase of the reaction, the diacetal undergoes Co-C bond cleavage to yield hydroxocobalamin, ethanol, and vinyl ether according to eq 4. The anomalous lability of the Co-C bond in the diacetals of formylmethylcobalamin is attributed to a steric effect due to the bulky $CH(OR)_2$ groups. The decomposition according to eq 4 is of interest in that it proceeds in the opposite direction of the vinyl ether–hydroxocobalamin synthesis reaction of Silverman and Dolphin.[2]

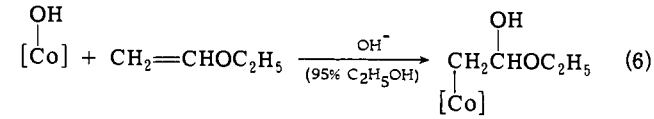


$$CH_2{=}CHOC_2H_5 + [\mathrm{Co}] \quad (4)$$

During the slower second phase of the acid-induced decomposition of the diacetal, the hemiacetal of formylmethylcobalamin is assumed to be the actually reacting species; its formation and subsequent Co-C bond cleavage are formulated in eq 5. The diethylacetal of formylmethylcobalamin undergoes slow hydrolysis in weakly alkaline aqueous solutions (pH 9.5), to yield 28% formylmethylcobalamin and 31% hemiacetal. The formation of formylmethylcobalamin under these conditions was previously postulated[2,5] and has now been confirmed. However, since the yields of formylmethylcobalamin are low and by-products are formed, this method is not very convenient for laboratory synthesis. Hydrolysis of the acetals under acidic conditions, furthermore, produces at best traces of formylmethylcobalamin, consistent with the original observations reported in ref 3. We may now proceed to explain the existing discrepancies between the properties of the two formylmethylcobalamins. It is clear that Silverman and Dolphin's compound is not formylmethylcobalamin, if only because of its anomalous NMR spectrum. The signal of the α-proton was assigned to a broad resonance at around 8.3 ppm, thus considerably more upfield than normally observed for aldehydes. This signal is absent in the NMR spectrum of our compound. Moreover, Silverman and Dolphin's compound undergoes acid decomposition far more rapidly than ours; the reported rate constant is equal to the rate constant observed by us for the second step in the acid decomposition of the diethylacetal, i.e., 2050 $sec^{-1}\ M^{-1}$. It is therefore concluded that this rate corresponds to the acid decomposition of the *hemiacetal of formylmethylcobalamin.* Since hemiacetals of formylmethylcobalamin are expected to be unstable, it is not surprizing that these compounds have as such not been characterized nor isolated in the solid state. Hemiacetals of formylmethylcobalamin rather than formylmethylcobalamin should also be the initial products of the reaction of hydroxocobalamin with alkyl vinyl ethers in aqueous alcoholic media (eq 6).



$$[\mathrm{Co}] + CH_3CH{=}O \quad (5)$$



$$[\mathrm{Co}] + CH_2{=}CHOC_2H_5 \xrightarrow[(95\%\ C_2H_5OH)]{OH^-} CH_2CHOC_2H_5\ (\mathrm{Co}) \quad (6)$$

In reaction eq 3-5 the products were assumed to form via π-complexes of vinyl ethers with hydroxocobalamin, in accord with ref 2 and 5. It is debatable whether these reactive intermediates are truly π-complexes, and we have questioned this formulation elsewhere.[4] The Co^{3+} ion in vitamin B_{12} in these reactions seems to function primarily as an electrophilic center just as in the known acid[11] catalyzed conversion of vinyl ethers to aldehydes or diacetals thereof. Although intermediate, bridged π-complexes of a proton

with the olefinic double bond can be written, the mechanism can equally well be formulated classically as shown in eq 7. The reactions of hydroxocobalamin with vinyl ethers

$$CH_2{=}CHOR \left(\longleftrightarrow \bar{C}H_2\overset{+}{C}HOR\right) \overset{\pm H^+}{\rightleftharpoons} CH_3\overset{+}{C}HOR \xrightarrow{+OH^-} CH_3\underset{OH}{C}HOR \xrightarrow[-ROH]{} CH_3CH{=}O \quad (7)$$

in anhydrous alcohols may therefore be written analogously (eq 8), or by a mechanism involving nucleophilic attack (eq 9). These mechanisms account for the available evidence as

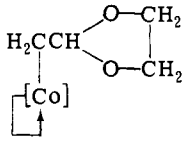
$$CH_2{=}CHOR + [Co^{3+}] \rightleftharpoons \underset{[Co]}{CH_2\overset{+}{C}HOR} \xrightarrow{+RO^-} \underset{[Co]}{CH_2CH(OR)_2} \quad (8)$$

$$CH_2{=}CHOR + OR^- \longrightarrow \bar{C}H_2CH(OR)_2 \xrightarrow[[Co^{3+}]]{} \underset{[Co]}{CH_2CH(OR)_2} \quad (9)$$

well as eq 3-5. Even the reported isomerization reactions[11,12] of alkylcarbethoxycobalt complexes[12,13] can be formulated without invoking π-complex organocobalt intermediates.

$$\underset{(Co)}{CH_2CH_2OCR}\overset{O}{\parallel} \xrightarrow[-RCO_2H]{+H^+} \left[\underset{(Co)}{CH_2\overset{+}{C}H_2} \rightleftharpoons \underset{(Co)}{\overset{+}{C}H_2CH_2}\right] \xrightarrow{+OR^-} \underset{(Co)}{ROCH_2CH_2} \quad (10)$$

Formylmethylcobalamin cannot be isolated from these reaction solutions; on phenol extraction, only hydroxocobalamin is obtained. Since the diethylacetal of formylmethylcobalamin undergoes Co-C bond cleavage in phenol, the same should be expected for the hemiacetal. The fact that a compound with the R_f of formylmethylcobalamin can be detected by chromatography on cellulose is not inconsistent with our interpretation. The chromatography is performed in an aqueous-alcoholic phase. Under these conditions hemiacetals or formylmethylcobalamin could form during the elution process, so that a distinction between the two compounds by chromatographic methods becomes difficult. The present work also explains the remaining conflicting data on the rates of hydrolysis of the diethylacetal of formylmethylcobalamin, formylmethylcobinamide, and of 1,3-dioxa-2-cyclopentylmethylcobalamin, **3**. The rate for the first compound was given as 2050 sec^{-1} M^{-1} and thus actually corresponds to the second stage of hydrolysis. The rate of decomposition of the diethylacetal of formylmethylcobinamide was reported[3] to be 26000 sec^{-1} M^{-1} and is very similar to the initial rapid stage of hydrolysis of the respective cobalamin derivative. The rate of decomposition of **3** was listed as 6 sec^{-1} M^{-1}, presumably because this compound underwent hydrolysis to formylmethylcobalamin on storage in alkaline solution (pH 9.5) prior to the kinetic measurements.



3

In summary, virtually all conflicting reports concerning the synthesis and properties of formylmethylcobalamin can now be explained. The compound is indeed as stable as expected for a β-substituted alkylcobalamin and as reported in ref 3. The diacetals and hemiacetals of formylmethylcobalamin are rapidly cleaved on protonation.[14,15] Compared to the corresponding cobaloxime derivatives, the cobalamins undergo Co-C bond cleavage more readily because of the higher charge density on cobalt and because the Co-C bond in cobaloximes is less subject to steric labilization than in the cobalamins. Addressing ourselves to the question whether formylmethylcobalamin is an intermediate in the enzymatic dehydration of ethyleneglycol we reemphasize that this compound is unlikely to undergo homolytic Co-C bond cleavage even under enzymatic conditions in view of its pronounced tendency to undergo heterolytic Co-C bond cleavage.[3] It has been argued[5] that the interaction of formylmethylcobalamin with the enzyme will modify its chemistry. To accomplish a genuinely homolytic cleavage of the Co-C bond it would be necessary to assume that the enzyme is capable of eliminating the polarity of the Co-C bond. This would seem to be unlikely if only for energetic reasons.

Table III. R_f Values of Formylmethylcobalamin in Three Different Solvent Systems, All Containing 0.5% of Concentrated NH_4OH

Solvent		R_f
1-Butanol:ethanol:H_2O	(10:3:7)	0.52
2-Butanol:methanol:H_2O	(55:15:30)	0.40
1-Butanol:isopropyl alcohol:H_2O	(7:6:7)	0.46

Experimental Section

Reagents and Starting Materials. Vitamin B_{12a} (hydroxocobalamin) and hydroxycobinamide were obtained from Merck Sharp and Dohme Research Laboratories, Rahway, N.J. Bromoacetaldehyde was prepared according to the method of Yanovskaya and Terent'ev from the Br_2 complex of *p*-dioxane and acetaldehyde.[16] Due to the thermal instability of bromoacetaldehyde it is preferable to avoid its distillation. For the synthesis of formylmethylcobalamin the bromoacetaldehyde was extracted with diethyl ether, and the extracts were neutralized with aqueous sodium carbonate prior to reaction with vitamin B_{12s}. Dichloroacetaldehyde was prepared from the commercially available diethylacetal by reaction with anhydrous oxalic acid in xylene at 70°, followed by distillation[17] (bp 88-90° (760 Torr)). α-Chloral, the diethylacetal of α-chloroacetaldehyde, and α,α-dichloro- and 1,1,1-trichloro-2-ethanol were commercial samples and used without prior purification.

Formylmethylcobalamin and Formylmethylcobinamide. One gram of hydroxocobalamin in a glass bottle of 300 ml capacity was dissolved in 200 ml of 2 *N* aqueous NaOH. The solution was purged with argon to free it from oxygen. Subsequently, 15 ml of an 85% aqueous solution of 3-hydroxybutanone-2 was added and the reaction vessel was closed with a rubber serum cap. The reduction of hydroxocobalamin to vitamin B_{12s} was complete within 30 min, standing at 27°C. At this point a solution of 5 g of freshly prepared bromoacetaldehyde in 50 ml of diethyl ether was injected. After 2 hr of standing at room temperature in the dark the excess of butanolone and of other organic reaction constituents was removed by extraction with diethyl ether. The remaining cherry red aqueous phase was subjected to phenol extraction. The formylmethylcobalamin was precipitated from the phenolic phase by the addition of acetone; chromatography in three different solvent systems revealed the presence of a major component at the R_f values given in Table III.

In all solvent systems traces of a product with R_f values corresponding to those of the diacetals of formylmethylcobalamin were observed; presumably, formation of the diacetals occurs during the chromatography. Solid formylmethylcobalamin was stored at 0° in the dark and exhibited the absorption spectrum typical of alkylcobalamins, i.e., peaks or shoulders (sh) at 283 nm (ϵ 14.4 × 10^3 M^{-1} cm^{-1}), 333 (ϵ 15.0 × 10^3), 365 (sh) (ϵ 12.5 × 10^3), 473 (sh) (ϵ 6.7 × 10^3), and 512 (ϵ 8.6 × 10^3), in aqueous solution at pH 6.9.

The extinction coefficients were determined after photolysis of the compound in solution and the addition of 1 ml of 0.1 *M* KCN, measuring the absorption of cyanocobalamin at 357 nm (ϵ 30.4 × 10^3). The thermolysis of formylmethylcobalamin was performed in argon-filled, rubber-serum capped test tubes of 15 ml capacity at temperatures not exceeding 160°. The gaseous decomposition product was identified as acetaldehyde by gas–liquid-phase chromatography (GLPC) and mass spectroscopy. The formation of acetaldehyde and of hydroxocobalamin on photolysis in neutral aqueous solution as well as on protolysis in acidic media was demonstrated by measurements of the optical absorption spectra and analysis of the gas phase by GLPC. In all cases the gas analyses were performed on a Varian Aerograph 1200 at the operating temperature of 200°, using a 6 ft × 0.25 in. i.d. copper tube filled with Varian Poropak Q resin, mesh-size 100–120. The identity of acetaldehyde was confirmed by cochromatography with an authentic sample.

Formylmethylcobinamide was prepared and isolated similarly as the corresponding cobalamin.

α-Chloro- and α,α-Dichloroformylmethylcobalamin. These compounds were prepared by the reaction of vitamin B_{12s} with dichloroacetaldehyde and chloral, respectively; the vitamin B_{12s} was generated as described above. The reaction products were extracted from the aqueous phase with phenol, precipitated with acetone, and recrystallized from methanol. The purity of the compounds isolated was checked by thin-layer chromatography, the observed R_f values are shown in Table I.

β-Hydroxyethylcobalamin and Related Compounds. β-Hydroxyethylcobalamin and the α-chloro and α,α-dichloro derivatives were prepared by the reaction of vitamin B_{12s} with chloroethanol, dichloroethanol, and trichloroethanol, respectively. The vitamin B_{12s} was generated from hydroxocobalamin with $NaBH_4$ in H_2O. The products were isolated by phenol extraction followed by precipitation with acetone. The purity of the compounds prepared was checked by thin-layer chromatography on cellulose plates, using 1-butanol:ethanol:H_2O = 400:120:280 as the ascending phase. The R_f values are given in Table I. The absorption spectra of the compounds were typical of alkylcobalamins. All compounds were found to be sensitive to acids as well as to alkali, in accord with known properties of β-hydroxyethylcobalamin and its derivatives.

Diethylacetal of Formylmethylcobalamin and of α-Chloroformylmethylcobalamin. A solution of 0.5 g of vitamin B_{12s} in 200 ml of 2 *N* NaOH was prepared as described for the synthesis of formylmethylcobalamin. Bromoacetaldehyde diethylacetal, 4 ml, was injected and the solution was allowed to stand for 30 min. The aqueous phase was first extracted with 250 ml of ether, and subsequently evaporated under reduced pressure in the dark to one-third of the original volume. The cobalamin was precipitated by the addition of acetone together with some inorganic salt which was not removed in order to maintain the organocobalamin in an alkaline environment. Crystalline diethylacetal of formylmethylcobalamin was synthesized from hydroxocobalamin and ethyl vinyl ether in absolute ethanol as described in ref 2 and 5. The diethylacetal of α-chloroformylmethylcobalamin was prepared by the reaction of vitamin B_{12s} with the diethylacetal of α,α-dichloroacetaldehyde and isolated by precipitation with acetone as described above.

Formylmethylcobaloxime. The formylmethylcobaloxime with pyridine as the axial base component was prepared by the hydrolysis of the diethylacetal, as described in ref 6. The aquo derivative of formylmethylcobalamin was generated in situ during the hydrolysis in 2–6 *N* HCl.

Hydrolysis of Formylmethylcobalamin and of Related Compounds. The acid-induced hydrolysis of formylmethylcobalamin was carried out in solutions containing 1% acetate or phosphate buffers in the pH range between 2 and 6. The rates of hydrolysis were determined by recording the optical absorption spectra and were found to be statistically significant (correlation coefficient >0.95). The t_∞ absorbance readings were obtained by photolyzing the solutions toward the end of the reaction. The terminal pH of the solutions was measured in all cases and was found to be unchanged within ±0.05 pH units. Several sharp isosbestic points[18] were observed in all cases (i.e., see Figure 1). The hydrolysis of the other compounds was studied similarly; the results are given in the text.

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References and Notes

(1) The following abbreviations are used: cobaloximes are bis(dimethylglyoximato)cobalt complexes used as vitamin B_{12} model compounds. The structure of the corrin moiety in vitamin B_{12} derivatives is symbolized by brackets, with inclusion of the axially coordinated 5,6-dimethylbenzimidazole symbolized by an arrow extending from the left bracket; the ligands in cobaloximes are abbreviated by parentheses. Additional trivial names are explained in the text. Adherence to nomenclature rules forces us to define the aldehyde proton in formylmethylcobalt complexes as α. On the other hand, bromoacetaldehyde, for example, is also designated as an α-derivative. This coincidence is mentioned to prevent confusion.
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