The Acid-Catalyzed Decomposition of Phenacylcobalamin: Evidence for the Formation of an Enol–Co(III) π-Complex Intermediate†

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ABSTRACT: Phenacylcobalamin has been synthesized and characterized by thin-layer chromatography and uv-visible spectroscopy, as well as identification of the cobalt-containing and organic products of its cleavage in acid and base and by aerobic photolysis. The major organic product from all three cleavage reactions is acetophenone and the cobalt-containing product is aquacobalamin (or hydroxocobalamin, its conjugate base). In aqueous acidic solution (pH 0 to 7.3, ionic strength 1.0 M, and 25.0 °C), the kinetics of the formation of aquacobalamin are biphasic representing the linear sum of two exponential terms. The pH dependence of the first-order rate constant of both phases shows a first-order dependence on proton concentration but with an inflection point at pH 3.55 for the faster phase and at pH 4.03 for the slower phase. This behavior is interpreted in terms of the specific acid catalyzed formation of an intermediate from both "base on" and "base off" phenacylcobalamin with different second-order rate constants for each form, followed by an intermediate decomposition step with a similar formal mechanism. The nature of the intermediate is discussed and it is concluded to be a π-complex between cob(III)alamin and the enol of acetophenone.

Recent investigations of elimination reactions of organocobalt complexes which form Co(III) salts and olefinic organic products have shown that such reactions proceed via intermediate, cationic species believed to be Co(III)–olefin π-complexes (Golding et al., 1970; Parfenov and Yurkevich, 1972; Silverman et al., 1972; Brown and Ingraham, 1974). These novel π-complexes have been proposed as intermediates in adenosylcobalamin mediated enzymatic rearrangements including the diol dehydrase catalyzed rearrangement of simple diols (Silverman and Dolphin, 1973). Recently the acid-catalyzed rearrangement of a β-hydroxyalkylcobaloxime[1] via such a π-complex intermediate was demonstrated (Brown and Ingraham, 1974). However, since the diol dehydrase mechanism based on such π-complexes requires the formation of an enol–Co(III) π-complex, rearrangement models proceeding via simple olefin–Co(III) π-complexes seem inadequate. Consequently an organo-cobalt elimination reaction, which leads to the formation of a stable organic ketone and which might be expected to proceed via intermediate enol formation, has been studied in order to seek evidence for the formation of a π-complex between a Co(III) salt and the emerging enol. The synthesis and aqueous acidic decomposition of the β-keto organocobalamin, phenacylcobalamin, are the subjects of this report.

Experimental Procedure

Materials. Hydroxocobalamin was from Sigma. Acetophenone and styrene (Aldrich, 99%) were used without further purification. Phenacyl bromide was recrystallized from aqueous ethanol. Organic solvents, sodium borohydride, mercuric oxide, iodine, inorganic salts and acids, and buffer salts were obtained in the highest purity commercially available and used without further purification. Deionized water of $>2 \times 10^5$ Ω-cm specific resistance was used throughout.

α-Methoxystyrene was synthesized by the procedure of Tiffeneau (Tiffeneau, 1907) as modified by Winstein and Ingraham (Winstein and Ingraham, 1955).

Phenacylcobalamin was synthesized as follows: a 400-mg sample of hydroxocobalamin was dissolved in 20 ml of 95% ethanol. The solution was stirred under argon for 1 h in a 100-ml three-neck round-bottomed flask. Powdered sodium borohydride (400 mg) was added and the reaction system closed. After the reduction of hydroxocobalamin to cob(I)alamin was complete (15 min), a solution of 500 mg of phenacyl bromide in 10 ml of ethanol (also deoxygenated with argon for 1 h) was added from a pressure equalizing addition funnel. The solution changed immediately from greyish green to red. The mixture was stirred for an additional 15 min and filtered to remove salts. The ethanol was removed by flash evaporation and the dried product dissolved in 20 ml of 0.1 M pH 8 phosphate buffer. This solution was extracted four times with 20 ml of ether to remove organic contaminants. The product was recovered by lyophilization.

Methods. Thin-layer chromatography was performed on silica gel F sheets (Baker) in four different solvent systems: water saturated with *sec*-butyl alcohol; *n*-butyl alcohol, isopropyl alcohol, water (10:7:10); *n*-butyl alcohol, acetic acid, water (4:1:5); and isobutyl alcohol, acetic acid, water (100:3:50). Phenacylcobalamin cleavage products in acid and base and by aerobic photolysis were characterized by thin-layer chromatography (cobalt-containing products) and by GLC analysis (organic products) on a Varian Aerograph Series 1200 gas chromatograph equipped with 5 ft × ⅛ in. FFAP column (10% on 60–80 mesh Chromosorb N), a 5 ft × ⅛ in. Porapak Q column (80–100 mesh), or a 7 ft × ⅛ in.

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[1] Cobaloximes are bis(dimethylglyoximato)cobalt complexes which serve as models for the cobalt-corrins. For a review of their chemistry, see G. N. Schrauzer, *Acc. Chem. Res. 1*, 97 (1968).

Carbowax 20M column (5% on 70–80 mesh Chromosorb G), using a variety of conditions. In addition the organic products of the acid-catalyzed cleavage reaction were analyzed by mass spectroscopy on a Finnigan Model 3200 GC/MS system equipped with a 5 ft × ⅛ in. 5% Carbowax 20M column.

Aqueous solutions of phenacylcobalamin were prepared fresh daily or stored frozen (−20 °C) for a maximum of 3 days. Uv and visible spectra were recorded on a Cary 14 spectrophotometer. The concentration of phenacylcobalamin in reaction mixtures was determined by the absorbance of the product of its acidic (or photolytic) decomposition (aquacobalamin) at 351 nm [ϵ_{351} 2.42 × 10^4 M^{-1} cm^{-1}; lit. 2.02 × 10^4 M^{-1} cm^{-1} (Dolphin and Johnson, (1965))]. All operations involving phenacylcobalamin were performed in dim light, and its solutions were protected from light by aluminum foil.

Kinetic measurements were made on a Gilford 2400 spectrophotometer or a Durrum Model D-110 stopped-flow spectrophotometer by recording the time dependence of the increase in absorbance at 351 nm due to the formation of aquacobalamin of solutions which were generally ca. 2.9 × 10^{-5} M in phenacylcobalamin, pH maintained with phosphate, acetate, or formate buffers (at either 0.05 M or varying from 0.033 to 0.5 M) or HNO_3 or HCl, ionic strength maintained at 1.0 M with KNO_3 or KCl, and 25.0 ± 0.2 °C. Rate constants from the biphasic traces thus obtained were analyzed by plotting log ($OD_\infty - OD_t$) vs. time (after correcting for a small, linear end-point drift[2]). The slope of the linear portion of this plot (i.e., at times such that $t > 5T_{1/2}$ of the first phase) was taken as the first-order rate constant for the slower phase, k_2. The linear portion was then extrapolated to time zero and a replot of the log of the difference between the extrapolated absorbance and the absorbance at time t (log [$OD_{extrap} - OD_t$]) vs. time yielded a second straight line whose slope was taken as the first-order rate constant for the faster phase, k_1. All such reactions were run in triplicate.

Samples for GLC, mass spectral, or GC–MS analysis were generally prepared at ca. 2 × 10^{-3} M phenacylcobalamin (or acetophenone) buffered at pH ca. 5.2 with potassium acetate buffer, 0.05 M, ionic strength maintained at 1.0 M with KNO_3, in 1.7% enriched ^{18}O water (Weizmann Institute) for isotope exchange experiments or in plain water for product determinations, generally in 1.0-ml volume. Organic products were extracted into 1 ml of ether which was over one chip of "Linde" type 4A molecular sieve. Ether extracts were generally concentrated by evaporation before analysis.

Isotope incorporation from ^{18}O enriched water was determined by mass spectral analysis on a CEC 21-104 mass spectrometer or on a Finnigan Model 3200 GC–MS system equipped as above and operated in the mass fragmentography mode. For the latter analysis, fragments of acetophenone were collected at *m/e* 120 (molecular ion) and 122, and at 105 ($\phi - CO^+$) and 107 and abundances determined by computer integration of the mass fragmentograms.

Acid decomposition of phenacylcobalamin in methanol was accomplished by addition of 0.5 ml of 0.1 N methanolic HNO_3 to a solution (1.8 ml) of phenacylcobalamin (ca. 1 × 10^{-3} M) in absolute methanol. After 1 h the solution was neutralized with methanolic NaOH, concentrated by rotary flash evaporation, and analyzed by GLC on the FFAP column described above.

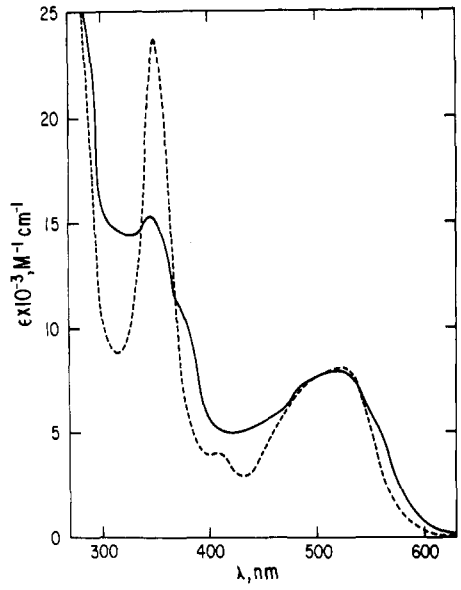


FIGURE 1: Spectra of phenacylcobalamin, at pH 7.14 (—) and after 19 h incubation at pH 5.22 (- - -), ionic strength 1.0 M in KNO_3.

Results

Characterization of Phenacylcobalamin and its Decomposition Products. Phenacylcobalamin behaves like an alkylcobalamin on silica gel thin layers in four different solvent systems. It is, however, contaminated with a small amount of unalkylated hydroxocobalamin (as revealed by thin-layer chromatography) and with phosphate salts. Attempts to remove these impurities by phenol extraction or chromatography on carboxymethyl-cellulose were unsuccessful due to the instability of phenacylcobalamin under these conditions.

The visible spectrum of the material (Figure 1) is typical of organocobalamins (Pratt, 1972) with a γ band (shoulder) at ca. 375 nm. Upon incubation at acidic pH, the spectrum changes to that of aquacobalamin (Figure 1). Incubation in base produces the spectrum of hydroxocobalamin (not shown) and aerobic photolysis changes the spectrum to that of either aquacobalamin or hydroxocobalamin (or a mixture of the two) depending on the pH. The cobalamin product from all three types of cleavage reactions was further identified by thin-layer chromatography. The organic product from all three reactions was positively identified as acetophenone by co-chromatography with authentic acetophenone on GLC on three different supports. In addition, the product from acid-catalyzed decomposition at pH 5.1 was identified as acetophenone by its mass spectrum which was identical with that of authentic acetophenone. Benzaldehyde and 1-phenylethanol were identified as minor products under the same conditions by their mass spectra.

Kinetics of the Acid-Catalyzed Decomposition. The kinetics of the formation of aquacobalamin from phenacylcobalamin at all pH's between 0 and 7.3 are very distinctly biphasic (Figure 2A). The analysis of a sample trace (Figure 2B) shows both phases are simple exponentials suggesting a series of two first-order steps:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \qquad (1)$$

[2] The absorbance of such solutions continues to increase slowly and approximately linearly even after 7 half-times of the slower phase. The rate of endpoint drift never exceeded 0.5% of the total absorbance change of the reaction per half-time of the slower phase.

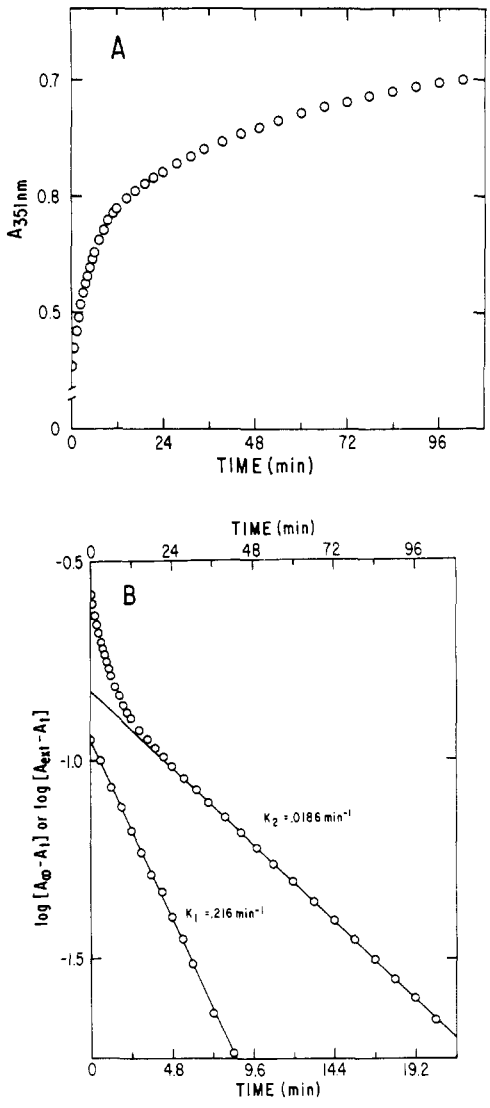


FIGURE 2: (A) Graph of absorbance at 351 nm vs. time for phenacylcobalamin decomposition at pH 5.86, ionic strength 1.0 M (KCl), 25.0 °C. (B) Analysis of the kinetic trace of part A. (Upper plot) Log [$A_\infty - A_t$] vs. time (upper time scale). (Lower plot) Replot of log [$A_{extrap} - A_t$] vs. time (lower time scale). See text.

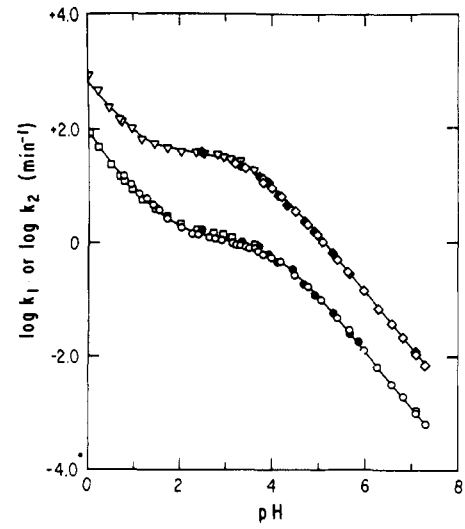


FIGURE 3: Graph of log k_1 (upper) and log k_2 (lower) vs. pH for phenacylcobalamin decomposition, ionic strength 1.0 M (maintained with inert electrolyte, as indicated), 25.0 °C. (○) k_2, KNO_3; (□) k_2, KNO_3, stopped-flow; (●) k_2, KCl; (◇) k_1, KNO_3; (▽) k_1, KNO_3, stopped-flow; (◆) k_1, KCl

(See Frost and Pearson (1961) for a description of such kinetics). Despite the slow end point drift[2], it was always possible to obtain three half-times of linear data for each phase. Furthermore, at each pH a minimum of three samples was run and the rate constants from each phase were averaged to yield values of k_1 (faster phase) and k_2 (slower phase). The average percent standard deviation over all pH's was 3.9% for k_1 and 3.6% for k_2. It should be pointed out that the analysis of biphasic kinetics presented here (which is similar to that employed by Fersht and Jencks (1969, 1970)) will only yield accurate results when the rate constant for the faster phase exceeds that for the slower phase by approximately fivefold. In the present study, the ratio k_1/k_2 varied from ca. 9 to 26.

The pH dependences of k_1 and k_2 are shown in Figure 3. Each is characterized by a first-order dependence on proton activity complicated by an inflection in the region between pH 1 and 4. No buffer catalysis of either k_1 or k_2 was detected in the range 0.033 to 0.5 M buffer for acetate, formate, or phosphate buffers of several compositions. No difference in either k_1 or k_2 was observed when KCl was substituted for KNO_3 as the inert electrolyte at numerous pH's between 2.50 and 5.90 (Figure 3).

^{18}O and Methanol Incorporation. Table I shows the results of several experiments and controls on the incorporation of ^{18}O from ^{18}O-enriched water into acetophenone produced from phenacylcobalamin decomposition in aqueous acid. At pH 1.2 acetophenone undergoes exchange with solvent oxygen with a rate constant of about 2×10^{-3} min^{-1} (ca. 90% exchange in 20 h). At pH's near 5.15 (0.05 M acetate buffer) the exchange is about 5 to 9% complete in either the presence or absence of 2×10^{-3} M aquacobalamin in 70 to 100 min. When phenacylcobalamin is decomposed under similar conditions for 30 or 70 min, the acetophenone produced shows only about 7% incorporation of ^{18}O (i.e., incorporation is no higher than the controls).

When phenacylcobalamin was decomposed in methanol by addition of nitric acid, no α-methoxystyrene could be detected in the reaction mixture by GLC on 10% FFAP although authentic acetophenone and α-methoxystyrene were easily separable on this support.

Discussion

The occurrence of clearly biphasic kinetics under all reaction conditions studied (Figure 2) demands eq 1 as a minimal scheme for phenacylcobalamin decomposition; i.e., there must be at least one intermediate which accumulates in spectrophotometrically significant amounts at all pH's. The complete equation for the time dependence of absorbance at a given wavelength for the minimal scheme of eq 1 is given in eq 2:

$$OD_t = A_0 \left\{ \epsilon_A e^{-k_1 t} + \frac{\epsilon_B k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) + \epsilon_C \left[1 + \frac{1}{k_1 - k_2}(k_2 e^{-k_1 t} - k_1 e^{-k_2 t}) \right] \right\} \quad (2)$$

where OD_t is the absorbance at time t, A_0 is the initial concentration of the reactant, and ϵ_A, ϵ_B, and ϵ_C are the molar absorptivities of the species A, B, and C at the wavelength of interest. Unfortunately, as has been pointed out (Fersht and Jencks, 1970), such equations are functionally symmet-

Table I: Exchange of Acetophenone Oxygen with 1.7% Enriched ^{18}O Water, Ionic Strength 1.0 M.

Concentration of Reactants (M)						
Acetophenone	Aquacobalamin	Phenacylcobalamin	pH	Time	Enrichment (%)	% Exchange
2.00×10^{-2}			1.23	18:10	1.51	89.3
2.08×10^{-3}			1.22	21:50	1.55	91.3
2.00×10^{-2}			5.14	1:47	0.08	4.90
2.00×10^{-2}			5.15	41:40	1.06	62.6
2.00×10^{-3}	2.00×10^{-3}		5.13	1:25	0.028	1.64[a]
2.00×10^{-3}	2.00×10^{-3}		5.22	1:15	0.151	8.87
		2.00×10^{-3}	5.18	1:10	0.129	7.62
		2.00×10^{-3}	5.18	0:30	0.121	7.12

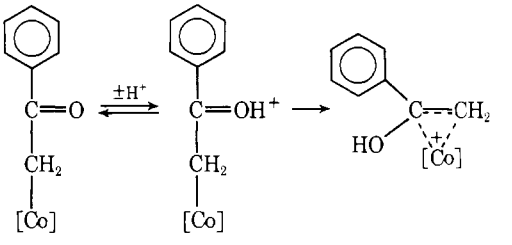
[a] Run in unenriched water.

ric with respect to the interchange of k_1 and k_2; i.e., it is not possible to assign rate constants to particular spectrophotometric phases a priori. Since A_0, ϵ_A, and ϵ_C are known, it is possible to calculate ϵ_B for both possible assignments of k_1 and k_2. The values thus obtained for ϵ_B for a typical calculation at pH 5.86 and λ 351 nm ($A_0 = 2.93 \times 10^{-5}$ M, $\epsilon_C = 2.42 \times 10^4$ M^{-1} cm^{-1}, $\epsilon_A = 1.55 \times 10^4$ M^{-1} cm^{-1}, $k_1 = 0.309$ min^{-1}, and $k_2 = 0.0273$ min^{-1}, or vice versa) are 2.03×10^4 M^{-1} cm^{-1} for $k_1 > k_2$ and 7.03×10^4 M^{-1} cm^{-1} for $k_2 > k_1$. Unfortunately neither of these values lies sufficiently far outside the range of molar absorptivities for known cobalt-corrin complexes (Pratt, 1972; Dunne, 1971) to exclude either assignment on this basis. Interestingly, careful spectrophotometric observations on the time dependence of the spectral change for this reaction in the region of λ 400 to 600 nm and 300 to 400 nm (not shown) show that the points of spectral crossing between phenacylcobalamin and aquacobalamin (Figure 1) at λ 337 and 369 nm are actually tight isosbestic points within the limits of resolution of the spectrophotometer (Cary 14) and those at λ 500 and 538 nm are nearly isosbestic. While the latter two "near-isosbestic" points are not surprising in view of the magnitude of the spectral changes in the region of 480 to 540 nm, the occurrence of isosbestic points at 337 and 369 nm, where the spectral changes in the surrounding region are large, is unexpected where accumulation of an intermediate is mandated by the occurrence of distinctly biphasic kinetics. By use of eq 2 it is easy to show that the appearance of isosbestic points concomitant with a biphasic, monotonically increasing dependence of absorbance on time can only occur when A and B are spectrally very similar in the wavelength region of isosbesticity. This implies that in this region at least, the intermediate is spectrally similar to alkylcobalamins and distinct from aquacobalamin.

There are very few reasonable candidates for the intermediate in this reaction. The carbonyl-protonated conjugate acid of phenacylcobalamin can readily be dismissed as a possibility. The pK_a of the conjugate acid of acetophenone is -6.2 and ranges from -7.0 to -4.7 for several ring-substituted analogues (Arnett, 1963). Although little is known about the electronic and steric effects of chelated cobalt centers on covalently bound alkyl groups, it is known that carboxymethylcobalamin is less acidic than acetic acid by more than two orders of magnitude (Walker et al., 1974). This phenomenon is similar to the so-called β effect seen in numerous other organometallic compounds (Green, 1968). It may be due to hydrogen-bonding interactions between acetamide side chains of the corrin ring and the carboxyl moiety, as has been suggested (Walker et al., 1974; Hogenkamp et al., 1965) or to σ–π conjugation (Traylor et al., 1972). Whatever the mechanism, it is clear that the acidities of functional groups β to the cobalt atom in organocobalamins may be considerably lower than the noncobalt-substituted analogues. However, even if the pK_a of the carbonyl-protonated conjugate acid of phenacylcobalamin were as high as -2.0 (i.e., four orders of magnitude less acidic than the conjugate acid of acetophenone), it would not be possible for spectrophotometrically significant amounts of this species to accumulate at pH's >7.0 as required by the data. Furthermore, it can easily be shown that the rate of proton transfer from hydronium ions to phenacylcobalamin even at pH 7 would be far too fast to account for either of the spectrophotometric phases measured here (Eigen, 1964).

It is also unlikely that the intermediate is "base off" phenacylcobalamin.[3] The pK_a's for "base off" forms of numerous alkylcobalamins range from about 2.2 to 4.0 (Pratt, 1972; and Hogenkamp et al., 1965). Again, spectrophotometrically significant amounts of this intermediate could not accumulate at pH's > 7.0. In addition the rates of formation of "base off" alkylcobalamins are also too fast to account for the formation of the intermediate in this reaction (H. P. C. Hogenkamp, personal communication).

The only remaining and the most likely, candidate for the spectrophotometrically active intermediate in phenacylcobalamin acidolysis is a π-complex between cob(III)alamin and the enol of acetophenone generated in an acid-catalyzed step as shown below:



Ample evidence for the formation of such π-complexes with simple olefins (Brown and Ingraham, 1974; Golding et al., 1970; Parfenov and Yurkevich, 1972; Silverman et al., 1972; Parfenov et al., 1972) as well as with electron-rich olefins (Silverman and Dolphin, 1973; Silverman and Dol-

[3] "Base off" phenacylcobalamin is Coα-aqua-Coβ-phenacyl(cobalamin) (see IUPAC-IUB Commission on Biomedical Nomenclature, *Biochemistry 13*, 1555 (1974)). It results from the dissociation of axial 5,6-dimethylbenzimidazole concomitant with its protonation. The open axial ligand position is assumed to be filled by water.

Table II: Kinetic and Equilibrium Parameters for the Acid-Catalyzed Decomposition of Phenacylcobalamin, Ionic Strength 1.0 M, 25.0 °C, and Hydroxyethylcobalamin.

Reaction	$k_{Bz}^{H^+}$ (M^{-1} min^{-1})	$k_{HOH}^{H^+}$ (M^{-1} min^{-1})	pK_a
Phenacylcobalamin (faster phase)	$1.39 \pm 0.01 \times 10^5$	$6.58 \pm 0.13 \times 10^2$	3.55 ± 0.01
Phenacylcobalamin (slower phase)	$1.22 \pm 0.01 \times 10^4$	$8.54 \pm 0.12 \times 10^1$	4.03 ± 0.01
Hydroxyethylcobalamin[a]	7.00	0.114	2.82
Hydroxyethylcobalamin[b]	7.35 ± 0.22	0.230 ± 0.007	2.98 ± 0.03

[a] Dunne (1971). Data not subjected to statistical fit. [b] Data of Dunne (1971), subjected to least-squares fit to eq 3 by simplex method. See text.

phin, 1974; Parfenov et al., 1974; Chervyakova et al., 1974; Parfenov et al., 1973) has been obtained. It in fact appears that such π-complexes are more stable with electron-rich olefins (such as the enol of acetophenone) than with simple olefins in view of the fact that electron-rich olefins will alkylate Co(III) complexes in the presence of ambient nucleophiles (Silverman and Dolphin, 1973; Silverman and Dolphin, 1974; Parfenov et al., 1974; Chervyakova et al., 1974) while simple olefins will not (Silverman and Dolphin, 1973; K. L. Brown and L. L. Ingraham, unpublished experiments).

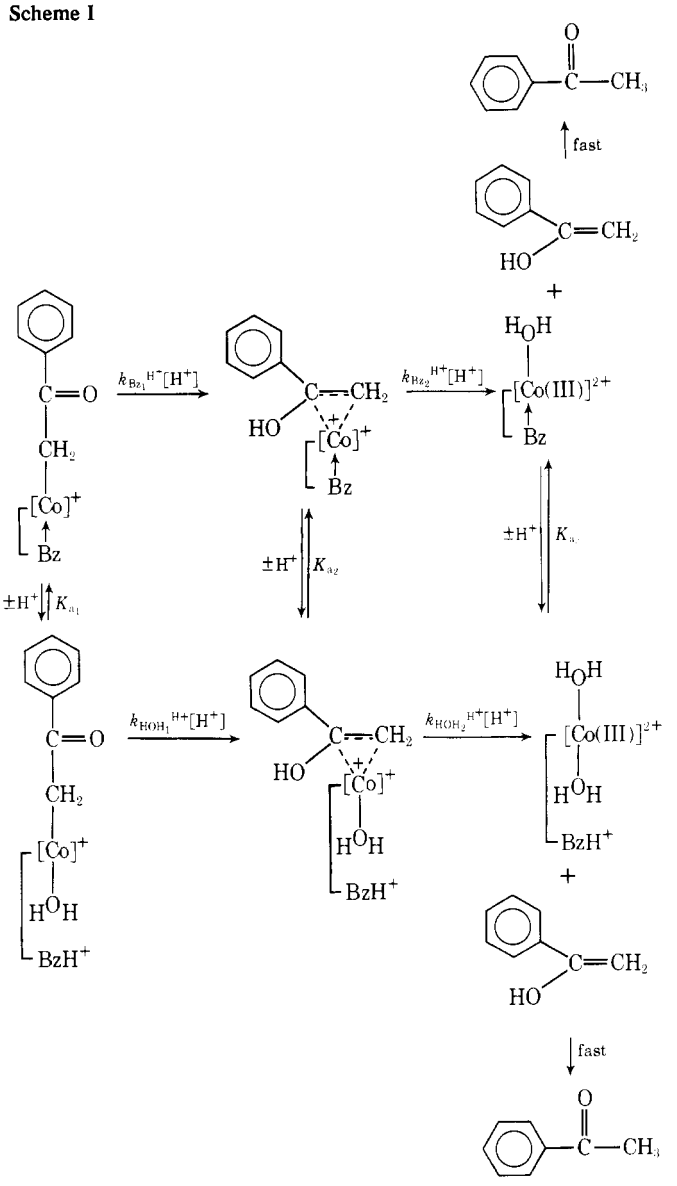
The pH dependencies of both k_1 and k_2 (Figure 3) are very similar to the pH dependence of the rate constant for the acid-catalyzed decomposition of hydroxyethylcobalamin (Dunne, 1971) which was ascribed to the occurrence of specific acid catalyzed pathways for both the "base on" and "base off" cobalamin, with different second-order rate constants for each species. Application of the same principle to the present system leads to Scheme I. Note that pK_{a_3} has been determined to be -2.4 (Hayward et al., 1965). Assuming the protonic equilibria and ligand exchanges represented by K_{a_1} and K_{a_2} are at equilibrium, the rate law of eq 3 applies to either step:

$$k = \frac{k_{HOH}^{H^+}[H^+]^2 + k_{Bz}^{H^+}K_a[H^+]}{K_a + [H^+]} \qquad (3)$$

The solid lines in Figure 3 were calculated from eq 3 and the appropriate parameters (Table II) which were obtained by a nonlinear least-squares fit of the data to eq 3 utilizing a simplex minimizing routine (Nelder and Mead, 1965). The fact that the rates of both phases are unchanged by substituting KCl or KNO_3 as inert electrolyte (Figure 3 and Results) supports the assignment of the "base off" species as aquo complexes. The fit values for pK_{a_1} and pK_{a_2} (Table II) are reasonable values for alkylcobalamins and suggest that the cob(III)alamin-enol π-complex behaves like an alkylcobalamin in this respect; i.e., its pK_a (either pK_{a_1} or pK_{a_2}) is much higher than that of aquacobalamin (pK_{a_3} = -2.4, Hayward et al., 1965).

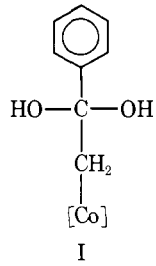
The rate and equilibrium parameters for the decomposition of phenacylcobalamin in acid can be compared with those for hydroxyethylcobalamin decomposition (Dunne, 1971) also included in Table II. Interestingly, Dunne found only single exponential kinetics for hydroxyethylcobalamin decomposition between pH 0 and 4 and the observed rate constants are between two and three orders of magnitude slower than the slowest of the phases in the present work. While an ethylene-cob(III)alamin π-complex intermediate would be expected to intervene in hydroxyethylcobalamin acidolysis, it is possible that its decomposition is so fast relative to its rate of formation that it cannot be detected. This interpretation would contrast with the cobaloximes where π-complex dissociation was shown to be rate determining in the acidic decomposition of β-hydroxypropylcobaloximes (Brown and Ingraham, 1974).

Scheme I



Although the chemistry of Co(III)-olefin π-complexes is poorly understood, several instances of rapid addition of ambient nucleophiles (alcohols or water) to form organocobalt derivatives are known both for simple olefins (Brown and Ingraham, 1974; Silverman et al., 1972; Golding et al., 1970; Golding and Sakrikar, 1972; Parfenov et al., 1972; Parfenov et al., 1973) as well as electron-rich olefins (Silverman and Dolphin, 1973; Silverman and Dolphin, 1974; Parfenov et al., 1974) for both the cobaloxime and cobalamin systems. Consequently, we expected that the π-complex

between cob(III)alamin and the enol of acetophenone would rapidly add water to form, after loss of a proton, the geminal diolic alkylcobalamin shown below:



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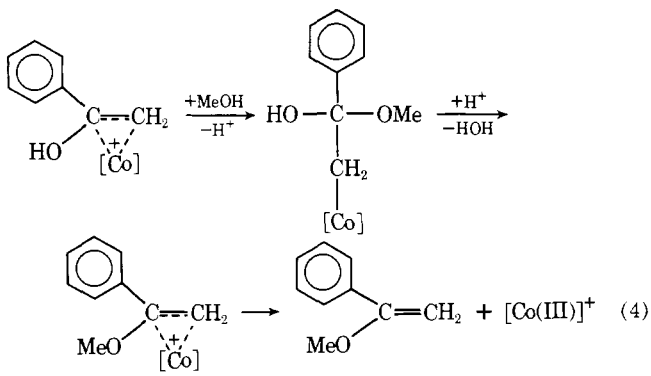
This would provide an explanation for the fact that both spectrophotometric phases of phenacylcobalamin decomposition are specific acid catalyzed (since the diolic species I will be acid labile) as well as be consistent with the above mentioned conclusion that the intermediate must be spectrally similar to starting material. Unfortunately, the experiments summarized in Table I show that the net formation of acetophenone from phenacylcobalamin in aqueous acid proceeds *without* incorporation of solvent oxygen into acetophenone. Attempts to add methanol to the π-complex by decomposition in acidic methanol, which would be expected to yield some α-methoxystyrene via the route shown in eq 4



(4)

were also unsuccessful. These experiments demonstrate that for reasons that are not understood the cob(III)alamin–acetophenone enol π-complex is not as electrophilic toward oxygen nucleophiles as was expected.

The failure to detect any diolic intermediate (I) also leaves us unable to adequately explain the fact that both of the spectrophotometric phases of phenacylcobalamin decomposition are acid catalyzed. Although it is clear that protonation of the enol portion of the dicationic ("base on") or tricationic ("base off") cob(III)alamin–enol π-complex (Scheme I) would be expected to greatly enhance the rate of dissociation of enol from the cobalt center, it is not clear where such protonation might occur. This dilemma probably reflects the general lack of knowledge of the chemistry of Co(III)–olefin π-complexes and a better understanding of this mechanism will have to await further studies of these interesting species.

Finally, it must be noted that the appearance of minor amounts of benzaldehyde and 1-phenylethanol among the reaction products of the acidic decomposition of phenacylcobalamin (Results) indicates that the decomposition of the Co(III)–enol π-complex is probably more complicated than the simple model presented here. In particular the formation of these minor products may indicate that a pathway involving electron transfer between the cobalt center and the enol contributes to some extent to the overall formation of stable products.

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