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Reactions of Vitamin B_{12r} with Organic Halides

Hans-Ulrich Blaser and Jack Halpern*

Contribution from the Department of Chemistry, The University of Chicago, Chicago, Illinois 60637. *Received August 27, 1979*

Abstract: Vitamin B_{12r} was found to react with certain organic halides (RX) according to the stoichiometry $2B_{12r} + RX + H_2O$ (or CH_3OH) $\rightarrow B_{12a} + R\text{-}B_{12} + X^-$ (where $B_{12a} = (H_2O)B_{12}^+$ + or $(CH_3OH)B_{12}^+$). For a variety of organic halides in methanol and of organic chlorides and bromides in water (0.5 M $KH_2PO_4/NaOH$, pH 7.0) the kinetics conformed to the second-order rate law $-d[B_{12r}]/dt = 2k_7[B_{12r}][RX]$. The results are interpreted in terms of a stepwise atom-transfer mechanism: $B_{12r} + RX \rightarrow X\text{-}B_{12} + R\cdot$ (rate determining); $B_{12r} + R\cdot \rightarrow R\text{-}B_{12}$ (fast); $X\text{-}B_{12} + H_2O$ (or CH_3OH) $\rightarrow B_{12a} + X^-$ (fast); in some cases, $R\text{-}B_{12}$ (or $R\cdot + B_{12r}$) $\rightarrow B_{12a}$ + unidentified products. Trends in the kinetic data, notably the dependence of k_7 on RX, are discussed. It is concluded that, contrary to earlier views, vitamin B_{12r} does react directly with organic halides. In aqueous solution, the reactions of vitamin B_{12r} with organic iodides, while conforming to the same stoichiometry, exhibited different kinetic behavior, corresponding to the third-order rate law $-d[B_{12r}]/dt = 2k_9[B_{12r}]^2[RX]$. No inhibition by vitamin B_{12a} was observed. The mechanism proposed for these reactions is $B_{12r} + RI \rightleftarrows [B_{12r}\cdot RI]$ (rapid equilibrium); $[B_{12r}\cdot RI] + B_{12r} \rightarrow R\text{-}B_{12} + B_{12a} + I^-$ (rate determining).

Introduction

Previous work in this laboratory has demonstrated that organic halides react with pentacyanocobaltate(II) and with cobalt(II) complexes of dimethylglyoxime and of Schiff's bases according to the stoichiometry and mechanism depicted by eq 1-3.[1-5] Since such low-spin cobalt complexes have been widely invoked as "models" or analogues of the corresponding vitamin B_{12} derivatives,[6,7] it was clearly of some interest to compare these systems to the reactions of vitamin B_{12r}, i.e., cob(II)alamin,[8] with organic halides.

$$L_5Co^{II} + RX \xrightarrow{k_1} L_5Co^{III}X + R\cdot \quad \text{(rate-determining)} \quad (1)$$

$$L_5Co^{II} + R\cdot \rightarrow L_5Co^{III}R \quad \text{(fast)} \quad (2)$$

$$2L_5Co^{II} + RX \rightarrow L_5Co^{III}X + L_5Co^{III}R \quad (3)$$

Several prior studies have led to the conclusion (or have assumed) that, in contrast to the behavior described above for other low-spin cobalt(II) complexes, vitamin B_{12r} does not react directly with organic halides.[9-11] The most extensive such study was described by Yamada et al.,[11] who confirmed the stoichiometry of eq 6, but concluded from indirect evidence (notably the influence of electrolytes on the rate) that the mechanism was that depicted by eq 4 and 5, i.e., disproportionation of vitamin B_{12r} to B_{12s} and B_{12a}, followed by the well-known alkylation of B_{12s} by the organic halide to form $R\text{-}B_{12}$.[12] This surprising discrepancy between the behavior of vitamin B_{12r} and other low-spin cobalt(II) compounds, which have been widely accepted as B_{12r} "models", suggested that a more thorough investigation of the reactions of vitamin B_{12r} with organic halides was warranted. The results of such an investigation of the products and kinetics of the reactions of vitamin B_{12r} with a variety of organic halides in methanol and aqueous solutions are reported in this paper.

$$2B_{12r} \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} B_{12s} + B_{12a} \quad (4)$$

$$B_{12s} + RX \xrightarrow{k_5} R\text{-}B_{12} + X^- \quad (5)$$

$$2B_{12r} + RX \rightarrow R\text{-}B_{12} + B_{12a} + X^- \quad (6)$$

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Also contributing to interest in the present studies is the role attributed to vitamin B_{12r} in the widely accepted mechanism of coenzyme B_{12} dependent rearrangements,[13,14] as well as the possible involvement of reduced forms of vitamin B_{12} in reactions with highly chlorinated hydrocarbons, including pesticides such as DDT.[15,16]

Experimental Section

Organic Halides. Methyl iodide was redistilled before use. The following compounds were recrystallized as indicated: *p*-nitrobenzyl iodide (from methanol), mp 124–127 °C; α-iodoacetamide (from benzene), mp 92–94 °C; trichloroacetamide (from water), mp 139–140 °C; α-iodoacetic acid (from benzene), mp 80–82 °C; α-bromoacetic acid (from hexane). The following esters were prepared from the corresponding acids and alcohols: methyl dichloroacetate; methyl, ethyl, and isopropyl trichloroacetate; methyl α-bromopropionate. Methyl α-iodoacetate was prepared from methyl α-chloroacetate and potassium iodide in acetone. Methyl α-bromoacetate (Pfaltz and Bauer) contained an unidentified highly reactive impurity which was removed by shaking for 2 days with a saturated solution of sodium carbonate, followed by distillation of the water-insoluble product. The following reagents were used as supplied: *p*-bromobenzyl bromide, mp 61–62 °C; *p*-cyanobenzyl bromide, mp 112–113 °C; *p*-nitrobenzyl bromide, (Aldrich), mp 98–100 °C; trichloroacetic acid (B. & A. reagent); dichloroacetic acid (Matheson Coleman and Bell); chloroform; carbon tetrachloride; 1,1,1-trichloroethane (Matheson Coleman and Bell).

Vitamin B_{12}. Cyanocobalamin (crystalline, Sigma) was the starting material for the synthesis of B_{12} derivatives.

Vitamin B_{12r} [Cob(II)alamin]. In order to obtain material that was free of excess reducing agent or inorganic salts, the following procedures were used to prepare vitamin B_{12r}. (a) Solid, amorphous vitamin B_{12r} was obtained by irradiation of methylcobalamin in 2-propanol and evaporation of the solvent.[11,17] (b) Crystalline vitamin B_{12r} was prepared as follows:[18] 200 mg (0.15 mmol) of hydroxocobalamin, 60 mL of methanol, and 25 mg of platinum oxide (Bishop, 83.5% Pt) were placed in a 500-mL Schlenck flask, connected via a medium fritted disk with a second Schlenck flask. The system was evacuated and filled with hydrogen and the reaction mixture was stirred for 20 min. The resulting yellow solution was filtered through the fritted disk and evaporated to dryness (0.05 Torr, 40 °C). The brown residue was dried for 2 h and then dissolved in 10 mL of deoxygenated water. Acetone (73 mL) was added followed, after 5 h at room temperature and 4 h at 5 °C, by further addition of 70 mL of acetone. The resulting solution was kept at 5 °C overnight and yielded 150 mg of brown-black, crystalline vitamin B_{12r} (75% yield, <5% B_{12a}). UV-visible spectrum (extinction coefficients, M^{-1} cm^{-1}, in parentheses): in water, found 310 nm (2.79×10^4), 405 (7.70×10^3), 473 (9.5×10^3) (lit.[19a] 311 nm (2.75×10^4), 402 (7.50×10^3), 473 (9.20×10^3)); in 1 M HCl, found 314 nm (2.38×10^4), 468 (1.17×10^4) (lit.[19a] 315 nm (2.43×10^4), 470 (1.10×10^4)).

Crystalline vitamin B_{12r} can be handled in air for short periods. Solutions thereof, especially in water at low pH, are very air sensitive and must be kept under nitrogen. Methanol solutions of vitamin B_{12r} decompose slowly (weeks) to give a yellow compound which is no longer oxidized by organic halides or air. This could be a cobalt(III) compound with a partially reduced chromophore (corrin).[20] The kinetic results were independent of the method of preparation of the vitamin B_{12r}.

Vitamin B_{12a} (hydroxocobalamin) was prepared from vitamin B_{12}.[21] pK_a (for H_2O-$B_{12}^+ \rightleftarrows$ HO-B_{12} + H^+) = 7.65 ± 0.1 (lit.[19b] 7.65).

Organocobalamins. Most of the organocobalamin reaction products were synthesized independently by the reactions of vitamin B_{12s} with the corresponding organic halides. The crude reaction products were purified by phenol extraction and recrystallized from aqueous acetone.[18] The following organocobalamins were prepared by the cited literature procedures (UV-visible spectra and R_f values were in agreement with literature data): methylcobalamin, from methyl iodide;[22] ethylcobalamin, from ethyl iodide;[22] trichloromethylcobalamin, from carbon tetrachloride.[15] The same procedures were used to prepare carboxymethylcobalamin from α-chloroacetic acid and methoxycarbonylmethylcobalamin from methyl α-chloroacetate [UV-visible spectra of both compounds (extinction coefficients, M^{-1} cm^{-1}, in parentheses): in water, 330 nm (1.42×10^4), 370 (1.34×10^4), 526 (8.25×10^3); in aqueous 1 M HCl, 325 nm (1.92×10^4), 425 (9.20×10^3), 456 (9.30×10^3)]. Carboxamidomethylcobalamin was prepared from α-iodoacetamide [UV-visible spectrum: in water, 330 nm (1.37×10^4), 372 (1.18×10^4), 529 (8.10×10^3); in aqueous 1 M HCl, 325 nm (1.72×10^4), 358 (1.19×10^4), 424 (8.80×10^3), 452 (8.50×10^3)]. Carboxychloromethylcobalamin and methoxycarbonylchloromethylcobalamin were prepared in low yield from dichloroacetic acid and methyl dichloroacetate; both compounds decomposed during phenol extraction [UV-visible spectra of both compounds: in water, 367 nm (1.51×10^4), 422 (5.90×10^3), 520 (8.20×10^3); in 1 M aqueous HCl, 322 nm (1.65×10^4), 350 (1.40×10^4), 407 (1.03×10^4). Benzylcobalamin and para-substituted derivatives thereof were too unstable for isolation.[23] The air-sensitive solutions formed by the reaction of vitamin B_{12s} with benzyl halides exhibited the following UV-visible spectra attributed to the corresponding organocobalamins: benzylcobalamin [in water, 337 nm (1.88×10^4), 500 (8.20×10^4); in aqueous 1 M HCl, 356 nm (1.85×10^4), 426 (1.22×10^4)]; *p*-bromobenzylcobalamin [in water, 340 nm (2.30×10^4), 438 (6.2×10^3), 501 (8.3×10^3); in aqueous 1 M HCl, 360 nm (2.23×10^4), 432 (1.53×10^4)]; *p*-cyanobenzylcobalamin [in water, 340 nm (2.46×10^4), 438 (6.5×10^3), 512 (8.0×10^3)]. Attempts to prepare methoxycarbonyldichloromethylcobalamin from methyl trichloroacetate were unsuccessful.

Buffer Solutions[24] (pH 1.5–5, $Na_2HCitrate$–HCl; pH 4.5–8, KH_2PO_4–NaOH). All chemicals were reagent grade. The pH of each solution was determined before and after reaction.

Chromatography. The following materials were used: cellulose thin layer sheets, 0.16 mm (Eastman); cellulose for thick layer plates, microcrystalline, Type 38 (Sigma); carboxymethylcellulose for column chromatography.

Characterization of Vitamin B_{12} Derivatives. UV-visible spectrophotometry and thin layer chromatography on cellulose were used to ascertain the identity of the various vitamin B_{12} derivatives. Comparisons with literature data were made whenever available. Concentrations were determined after conversion to dicyanocobalamin (standard absorption, ϵ_{368nm} 3.04×10^4 M^{-1} cm^{-1}).[25] Thin layer chromatography (TLC) on cellulose was performed with the following solvent systems: (a) 2-butanol, water-saturated, (b) 2-butanol–water–concentrated NH_3, 14.2:6:1; (c) 2-butanol–water–acetic acid, 100:50:1. Vitamin B_{12} (cyanocobalamin) was used as the reference compound for relative R_f values.

Characterization of Reaction Products. A. Isolation of Products. Isolable products were prepared using the following reaction conditions: [B_{12r}], 2.8×10^{-3} to 3.6×10^{-3} M; [RX], 0.2–0.7 M; reaction time, 2–15 h at room temperature. After completion of the reaction, the solvent and excess organic halide were removed by evaporation and the residue was chromatographed on a carboxymethylcellulose column. The organocobalamins were eluted with water and vitamin B_{12a} was eluted with aqueous 1 M HCl or NaCN solutions. The products were identified by comparison with the corresponding independently prepared authentic compounds (UV-visible spectra and TLC). Yields were determined spectrophotometrically after conversion to dicyanocobalamin.

B. Spectrophotometric Identification. Reactant concentrations were typically in the following ranges: [B_{12r}], 0.5×10^{-4} to 1.0×10^{-4} M; [RX], 0.01–0.5 M. The spectra of the reaction mixtures were quantitatively compared with the calculated spectra of the reaction products (R-B_{12} and B_{12a}) at pH 7 and in 1 M HCl. Typical results of such determinations are depicted in Figures 1 and 2. Discrepancies of 5–10% could be readily detected.

C. Difference spectra were measured in certain cases to detect small quantities of organocobalamins. Reactant concentrations corresponded to those in B. The difference spectra were determined on the product solutions, at pH <1, before and after irradiation which transformed the base-off organocobalamin to vitamin B_{12a}. These spectra provided some indication of the nature of the organocobalamin product and a rough estimate of its concentration. The results of the product determinations by these procedures are summarized in Table I.

Kinetic Measurements. All kinetic measurements were performed at 25 ± 1 °C in rubber serum capped quartz cells under nitrogen. Solvents and reagents were deoxygenated before use. The reactions were initiated by injecting the calculated amount of the organic halide or a solution thereof. The rate of reaction was measured spectrophotometrically in a Cary 14 spectrophotometer by monitoring either the formation of products (ca. 350 or 510–500 nm) or the disappearance of vitamin B_{12r} (460–470 nm). The reactions were studied in methanol

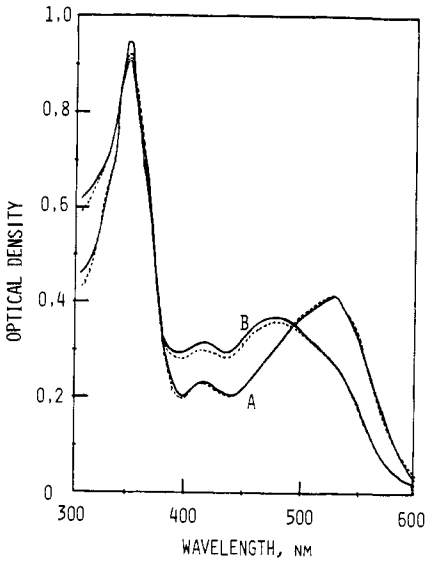

Figure 1. Reaction of vitamin B_{12r} (5.0×10^{-5} M) with excess $CH_2ICOOCH_3$ in aqueous solution. The solid curves are the experimental spectra determined after completion of the reaction. The dotted curves correspond to the calculated spectra of solutions containing 2.5×10^{-5} M vitamin B_{12a} and 2.5×10^{-5} M CH_3OOCCH_2-B_{12}: A, pH 7; B, 0.1 M HCl (1-cm light path).

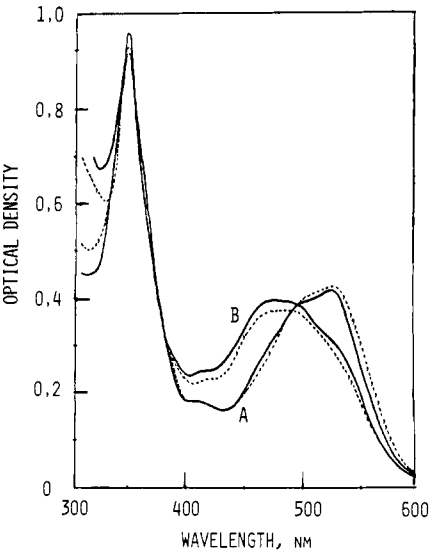

Figure 2. Reaction of vitamin B_{12r} (5.0×10^{-5} M) with excess CH_3I in aqueous solution. The solid curves are the experimental spectra measured after completion of the reaction. The dotted curves correspond to the calculated spectra of solutions containing 2.5×10^{-5} M vitamin B_{12a} and 2.5×10^{-5} M CH_3-B_{12}: A, pH 7; B, 0.1 M HCl (1-cm light path).

Table I. Products of Reactions of Vitamin B_{12r} with Organic Halides[a]

organic halide	solvent	R-B_{12}, %[b]			B_{12a}, %[b]	
		A	B	C	A	B
CH_3I	water		50			50
CCl_4, CCl_3Br	methanol			10-15	90-100	
$CHCl_2COOCH_3$	methanol	27			31	
$CHCl_2COO^-$	water		50			50
CCl_3COOCH_3	methanol	0			100	
CCl_2CONH_2	methanol	0			100	
CCl_3COO^-	water	0			100	
$CH_2BrCOOCH_3$	methanol	38	50		42	50
$CH_2BrCOOCH_3$	water		50			50
CH_2BrCOO^-	water		50			50
$CH_2ICOOCH_3$	methanol	30-35			45-60	
$CH_2ICOOCH_3$	water	36	50		38	50
CH_2ICONH_2	methanol	28			48	
CH_2ICONH_2	water		50			50
CH_2ICOO^-	water		50			50
p-$CNC_6H_4CH_2Br$	methanol		10-15		80-100	

[a] Method of estimation: (A) isolated product, yield determined spectrophotometrically; (B) from matching of spectra; (C) from difference spectra. [b] Yield based on initial vitamin B_{12r} concentration.

and in buffered aqueous solutions (0.5 M KH_2PO_4-NaOH).[24] Rate constants (k_{obsd}) were determined from plots of $\log |A_t - A_\infty|$ vs. time for pseudo-first-order reactions and of $|\Delta\epsilon|/|A_t - A_\infty|$ vs. time, for pseudo-second-order reactions (where A = absorbance and ϵ = extinction coefficient). $\Delta\epsilon$ ($=\Sigma\epsilon_{products} - \epsilon_{B_{12r}}$) was calculated from the known spectra of the organocobalamin, B_{12a} and B_{12r}. For the reaction of vitamin B_{12r} with $CH_2ICOOCH_3$, rates were determined at various pHs ranging from ca. 1.5 to 7, using the buffer systems described above. The results of the kinetic measurements are summarized in Table II. A detailed listing of rate constants is contained in the Appendixes (supplementary material).

Results

Stoichiometry and Products. The expected stoichiometry, corresponding to eq 6, was confirmed by isolation of products and by spectral matching, as described above. The results are summarized in Table I. While there was satisfactory accord with the stoichiometry of eq 6 for most of the reactions in aqueous solution, the results for methanol are less definitive. In some cases, notably at very low initial vitamin B_{12r} concentrations, the only cob(III)alamin detected was vitamin B_{12a}. Since the kinetics generally were well behaved, this may be due to fast *secondary* reactions of the initially produced R· radicals with the methanol solvent. Three classes of substrates warrant special comment.

A. CCl_4 and CCl_3Br. Although CCl_3-B_{12} is known[15] to be stable, only traces of it could be detected even at high B_{12r} concentrations. The high reactivity of CCl_3Br toward B_{12r} made it possible to perform a spectral titration which yielded an end point corresponding to $[B_{12r}]:[RX] = (2.3 \pm 0.2):1$, in reasonable agreement with eq 6.

B. Benzyl Halides. The instability of benzylcobalamin[23] precluded the isolation of organometallic products. The formation of benzylcobalamin was confirmed spectrophotometrically (see Experimental Section) but the estimated yields were consistently low.

C. Trichloroacetic Acid Derivatives. In all cases, in aqueous solution as well as in methanol, the only identifiable cobalamin product of reaction of these substrates with vitamin B_{12r} was vitamin B_{12a}. This may be due to the instability of the initially formed organocobalamin or unusually high reactivity of the intermediate $\dot{C}Cl_2COOH(R)$ radical. In accord with this, for the reaction of methyl trichloroacetate with vitamin B_{12r} in methanol 1 mol of $CHCl_2COOCH_3$ was detected (by GLC) as a product.

Kinetics. The following pattern of kinetic behavior was exhibited by the reactions of all the organic halides in methanol and by the chlorides and bromides in aqueous solution. For these reactions, the observed kinetics conformed to the second-order rate law corresponding to eq 7. Since RX was always in at least tenfold excess over vitamin B_{12r}, the observed kinetics were pseudo first order in accord with eq 8.

$$-d[B_{12r}]/dt = 2k_7[B_{12r}][RX] \quad (7)$$

$$-d \ln [B_{12r}]/dt = 2k_7[RX] = k_{7(obsd)} \quad (8)$$

Pseudo-first-order plots of $\log |A_t - A_\infty|$ vs. time were generally linear over several half-lives. Values of $k_{7(obsd)}$, derived from the slopes of such first-order plots, were generally reproducible to within 10%. The concentration of RX was typically varied by a factor of 5-20 to confirm the first-order dependence on the latter. In a few cases, where deviation from linearity of the first-order rate plots was significant, the initial

Table II. Summary of Kinetic Data for the Reactions of Vitamin B_{12r} with Organic Halides at 25 °C

organic halide	methanol soln k_7, M^{-1} s^{-1}	aqueous soln k_7, M^{-1} s^{-1}	aqueous soln k_9, M^{-2} s^{-1}
CCl_4	$(4.3 \pm 0.2) \times 10^{-2}$		
CCl_3Br	$(9.5 \pm 1.0) \times 10$		
CH_3CCl_3	$(5.4 \pm 1.0) \times 10^{-5}$		
$CHCl_2COOCH_3$	$(6.3 \pm 1.5) \times 10^{-4}$		
CCl_3COOCH_3	$(8.3 \pm 1.0) \times 10^{-1}$		
$CCl_3COOC_2H_5$	$(5.4 \pm 0.5) \times 10^{-1}$		
$CCl_3COOCH(CH_3)_2$	$(3.8 \pm 0.2) \times 10^{-1}$		
CCl_3CONH_2	$(1.6 \pm 0.2) \times 10^{-1}$		
$CH_2BrCOOCH_3$	$(6.4 \pm 1.0) \times 10^{-5}$	$(2.6 \pm 0.3) \times 10^{-3}$	
$CH_3CHBrCOOCH_3$	$(4.2 \pm 0.2) \times 10^{-2}$		
$CH_2ICOOCH_3$	$(4.3 \pm 0.4) \times 10^{-4}$		$(1.0 \pm 0.1) \times 10^4$ [a]
CH_2ICONH_2	$(9.0 \pm 1.5) \times 10^{-5}$		$(1.8 \pm 0.5) \times 10^2$
$C_6H_5CH_2Br$	$(1.0 \pm 0.2) \times 10^{-2}$		
p-$BrC_6H_4CH_2Br$	$(7.5 \pm 1.5) \times 10^{-3}$		
p-$CNC_6H_4CH_2Br$	$(1.4 \pm 0.1) \times 10^{-2}$		
p-$NO_2C_6H_4CH_2Br$	$(3.4 \pm 0.4) \times 10^{-2}$		
p-$NO_2C_6H_4CH_2I$	3.3 ± 0.2		
$CHCl_2COO^-$		$\sim 1 \times 10^{-4}$	
CCl_3COO^-		$(9 \pm 2) \times 10^{-2}$	
CH_2BrCOO^-		$(4.0 \pm 0.3) \times 10^{-4}$	
CH_3I			$(3.8 \pm 0.6) \times 10^4$ [a]
CH_2ICOO^-			$(1.4 \pm 0.3) \times 10$

[a] Rate unaffected by addition of up to 10^{-3} M vitamin B_{12a}.

concentration of B_{12r}, as well as of RX, was varied and the first-order dependence on each was confirmed from initial rate measurements. Rate constants are tabulated in Appendixes A and B and summarized in Table II.

The reactions of all the organic iodides with vitamin B_{12r} in *aqueous* solution (0.5 M KH_2PO_4-NaOH, pH 7) exhibited distinctively different kinetic behavior. For these reactions the observed kinetics conformed to the *third-order* rate law corresponding to eq 9 which, under the conditions of the measurements (with RX in at least tenfold excess over B_{12r}), reduces to the pseudo-second-order rate law, eq 10.

$$-d[B_{12r}]/dt = 2k_9[B_{12r}]^2[RX] \quad (9)$$

$$d([B_{12r}]^{-1})/dt = 2k_9[RX] = k_{9(obsd)} \quad (10)$$

The unexpected second-order dependence on $[B_{12r}]$ was confirmed in experiments in which the initial concentration of B_{12r}, as well as of RI, was varied. The pseudo-second-order rate plots of $[B_{12r}]^{-1}$ vs. time were generally linear over several half-lives. The values of $k_{9(obsd)}$, corresponding to the slopes of such plots, were generally reproducible to within 10%, although for the slowest reactions interference from autoxidation of B_{12r} occasionally was a complicating factor. Initial addition

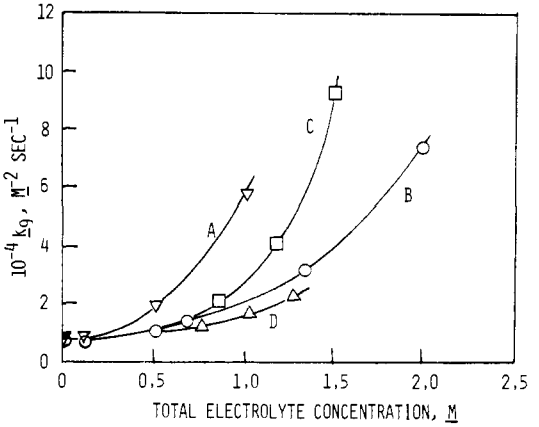

Figure 3. Influence of buffer concentration or added electrolyte on the rate of reaction of vitamin B_{12r} with $CH_2ICOOCH_3$ in aqueous solution at 25 °C: A, influence of added Na_2SO_4; B, influence of buffer concentration (KH_2PO_4-NaOH, pH 7); C, influence of Na_2SO_4 added to a 0.5 M buffer solution; D, influence of NaCl added to a 0.5 M buffer solution.

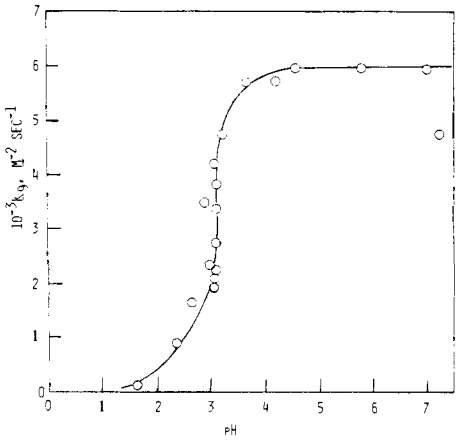

Figure 4. Influence of pH on the rate of reaction of vitamin B_{12r} with $CH_2ICOOCH_3$ in aqueous solutions containing 0.1 M buffer at 25 °C.

of vitamin B_{12a} to the reaction solutions, in up to tenfold excess over B_{12r}, was without effect on the rate. The rate constants for the reactions of organic iodides in aqueous solution are tabulated in Appendix C and summarized in Table II.

Influence of the Concentrations of Buffer and Other Electrolytes. Our results confirm the observations of Yamada et al.[11] that electrolytes accelerate the reactions of B_{12r} with most organic halides. The reactions with $CH_2ICOOCH_3$ and with CH_3I were particularly markedly affected by increases in the buffer concentration (KH_2PO_4-NaOH, pH 7) and by the addition of Na_2SO_4, while NaCl had a smaller effect (Figure 3). The influence of added Na_2SO_4 on the rates of reaction of $CH_2BrCOOCH_3$ and of CH_2ICOO^- was somewhat less marked, and the reaction of CCl_3COO^- was unaffected by addition of up to 0.8 M Na_2SO_4 (0.5 M buffer).

Influence of pH. Figure 4 depicts the influence of pH on k_9 for the reaction of $CH_2ICOOCH_3$ with vitamin B_{12r} in aqueous solution. The dependence has the form of a titration curve with a pK_a of about 3 which corresponds to that of the "base-on" + $H^+ \rightleftarrows$ "base-off" equilibrium for B_{12r}.[26] Accordingly, it is concluded that the "base-on" form of B_{12r} reacts with $CH_2ICOOCH_3$ at least 100 times more rapidly than the protonated "base-off" form. A qualitatively similar comparison was noted for the reaction of B_{12r} with CCl_4 in methanol.

Dependence of Reactivity on the Nature of the Organic Halide. The selection of organic halides encompassed by this study was dictated by considerations of solubility in methanol and water, availability, and stability toward solvolysis. The following trends are discernible and noteworthy. (a) k_7 in-

creases along the sequence RCl < RBr < RI as reflected in the following comparisons of k_7 values (M^{-1} s^{-1}) in methanol: CCl_4 (4.3×10^{-2}) < CCl_3Br (95); $CH_2ClCOOCH_3$ (too slow to measure) < $CH_2BrCOOCH_3$ (0.6×10^{-4}) < $CH_2ICOOCH_3$ (4.3×10^{-4}); etc. (b) k_7 increases along the sequence R_2CHX < $R_2C(CH_3)X$, e.g., $CHCl_3$ (too slow to measure) < CH_3CCl_3 (5.4×10^{-4}). (c) k_7 increases along the sequence R_2CHX < R_2CClX. Thus, in water, $CHCl_2COO^-$ ($\sim 1 \times 10^{-4}$) < CCl_3COO^- (9×10^{-2}). Correspondingly, in methanol, $CH_2ClCOOCH_3$ (too slow to measure) < $CHCl_2COOCH_3$ (6.3×10^{-4}) < CCl_3COOCH_3 (0.8). (d) C_6H_5, COOR, $CONH_2$, and COO^- substitutents, α to the halogen atom, markedly enhance the rates of reaction.

These trends are similar to those that have been identified for the reactions of other low-spin cobalt(II) compounds, notably $Co(CN)_5^{3-}$, with organic halides.[1-5]

Discussion

The course of reaction of vitamin B_{12r} with the organic halides which yield organocobalamin products (with the exception of organic iodides in aqueous solution, which are discussed separately below) appears to be similar to that found previously for other low-spin cobalt(II) complexes.[1-5] The results can be accommodated by the mechanism depicted by eq 11-13, involving rate-determining halogen abstraction by B_{12r} to give $X-B_{12}$ (which undergoes rapid solvolysis to form B_{12a} and X^-),[27] followed by rapid combination of the radical R· and B_{12r} to form $R-B_{12}$. The rapid occurrence of reaction 12 has been directly confirmed for the case R = CH_3· by flash photolysis experiments which yield a second-order rate constant of 3×10^9 M^{-1} s^{-1} for $CH_3· + B_{12r} \rightarrow CH_3-B_{12}$.[28]

$$B_{12r} + RX \xrightarrow{k_7} X-B_{12} + R· \quad (11)$$

$$B_{12r} + R· \xrightarrow{\text{fast}} R-B_{12} \quad (12)$$

$$XB_{12} + H_2O \text{ (or } CH_3OH) \xrightarrow{\text{fast}} B_{12a} + X^- \quad (13)$$

$$2B_{12r} + RX \rightarrow R-B_{12} + B_{12a} + X^- \quad (6)$$

Among the results that have been cited in support of such a mechanistic scheme, in the case of other low-spin cobalt(II) complexes,[1-5] are the following: (a) the second-order rate law, (b) the overall stoichiometry, and (c) the increases in reactivity along the sequences RCl < RBr < RI, R_2CHX < $R_2C(CH_3)X$, and R_2CHX < R_2CClX. These characteristic patterns are all observed for the reactions of vitamin B_{12r} and leave little doubt that, contrary to some earlier suggestions,[9,11] vitamin B_{12r} is indeed susceptible to direct reaction with, and alkylation by, organic halides.

As noted earlier, in a few instances (e.g., for R· = $\dot{C}Cl_3$ or $\dot{C}Cl_2COOCH_3$), especially in methanol at low B_{12r} concentrations, alternative reactions of R· apparently compete with reaction 12 leading, directly or indirectly, to the oxidation of B_{12r} to B_{12a} and the formation of RH. One possible such reaction scheme is depicted in eq 14 and 15.

$$R· + CH_3OH \rightarrow RH + CH_3O \quad (14)$$

$$CH_3O + B_{12r} \rightarrow CH_3O-B_{12}(\equiv B_{12a}) \quad (15)$$

As detailed above, the reactions of vitamin B_{12r} with organic iodides in aqueous solution, while conforming to the same stoichiometry as in methanol, exhibited quite different kinetic behavior corresponding to the third-order rate law of eq 9. This kinetic behavior clearly is incompatible with the mechanistic scheme of eq 11-13 involving a bimolecular rate-determining step. However, the results also are incompatible with the mechanism proposed by Yamada et al.[11] (eq 4 and 5) involving the disproportionation of B_{12r} to B_{12a} and B_{12s}, followed by alkylation of B_{12s}. Application of the steady-state approximation to B_{12s} yields the rate law corresponding to eq 16[29] which is incompatible with the observed kinetic behavior, notably the absence of any influence of added B_{12a} on the rate or departure from first-order dependence on [RX]. Furthermore, the recent demonstrations that the equilibrium constant for the disproportionation of vitamin B_{12r} according to eq 4 is only of the order of 10^{-15}-10^{-14} also rules out any significant contribution from this path.[29,30]

$$\frac{-d[B_{12r}]}{dt} = \frac{2k_4k_5[B_{12r}]^2[RX]}{k_{-4}[B_{12a}] + k_5[RX]} \quad (16)$$

A mechanism that is compatible with the observed rate law is that depicted by eq 17 and 18, involving the formation of a B_{12r} dimer which reacts with RX. Application of the steady-state approximation yields the rate law corresponding to eq 19, which reduces to the observed form (eq 9) under the limiting condition, $k_{-17} \gg k_{18}[RI]$. While such a mechanism cannot be discounted, the absence of independent evidence in other contexts for the formation of a B_{12r} dimer is disturbing. Furthermore, it is unclear (a) why vitamin B_{12r} should be activated by dimerization which would, on the contrary, be expected to hinder access of RX to the cobalt center, and (b) why such a mechanism should be selectively preferred for iodides relative to chlorides and bromides.

$$2B_{12r} \underset{k_{-17}}{\overset{k_{17}}{\rightleftharpoons}} [B_{12r}]_2 \quad (17)$$

$$[B_{12r}]_2 + RI \xrightarrow{k_{18}} R-B_{12} + B_{12a} + I^- \quad (18)$$

$$\frac{-d[B_{12r}]}{dt} = \frac{2k_{17}k_{18}[B_{12r}]^2[RI]}{k_{-17} + k_{18}[RI]} \quad (19)$$

Another mechanistic scheme, that also is consistent with the observed rate law and that we favor, is depicted by eq 20 and 21. This involves the formation of a B_{12r}·RI adduct which reacts with a second vitamin B_{12r} molecule. Application of the steady-state approximation to the intermediate adduct yields the rate law corresponding to eq 22 which reduces to eq 23 (i.e., the observed form) under the plausible limiting condition $k_{-20} \gg k_{21}[B_{12r}]$. According to this interpretation the difference between the behavior of iodides and that of chlorides and bromides may reflect the greater tendency of iodides to form adducts, owing to the significantly higher polarizability of iodine. This suggestion receives some support from the observation[31] that cob(II)yrinic acid heptamethyl ester forms a stable, structurally characterized complex of the formula [Co^{II}-I^--Co^{II}] and from our own qualitative observations that addition of ca. 0.1 M I^- (but not of Br^-) to an aqueous solution of vitamin B_{12r} results in a noticeable spectral change.

$$B_{12r} + RI \underset{k_{-20}}{\overset{k_{20}}{\rightleftharpoons}} B_{12r} \cdot RI \quad (20)$$

$$B_{12r} \cdot RI + B_{12r} \xrightarrow{k_{21}} R-B_{12} + B_{12a} + I^- \quad (21)$$

$$\frac{-d[B_{12r}]}{dt} = \frac{2k_{20}k_{21}[B_{12r}]^2[RI]}{k_{-20} + k_{21}[B_{12r}]} \quad (22)$$

$$-d[B_{12r}]/dt = 2(k_{20}k_{21}/k_{-20})[B_{12r}]^2[RI] \quad (23)$$

It is of interest to note that a third-order rate law, analogous to eq 9, also has been reported for the reactions of certain iron(II) porphyrin complexes with organic halides, and similarly interpreted in terms of an intermediate Fe^{II}·RX adduct.[32]

We are unable to offer a convincing explanation for the observed influence of added electrolytes on the rate, which was previously interpreted as providing support for the disproportionation mechanism.[11] The effect of electrolytes was most

Table III. Comparison of Reactivities of Various Cobalt(II) Complexes toward Organic Halides at 25 °C

cobalt(II) complex	solvent	second-order rate constant (k, M^{-1} s^{-1})[a]		
		p-$BrC_6H_4CH_2Br$	p-$CNC_6H_4CH_2Br$	p-$NO_2C_6H_4CH_2Br$
$[Co(CN)_5]^{3-}$ [b]	methanol–water (70:30)	7.5		1.0×10^2
$[Co(DH)_2(PPh_3)]$ [c]	benzene	6.1×10^{-2}	2.2×10^{-1}	3.7×10^{-1}
vitamin B_{12r} [d]	methanol	7.5×10^{-3}	1.4×10^{-2}	3.4×10^{-2}
[Co(saloph)py] [e]	methylene chloride		2.9×10^{-3}	5.5×10^{-3}

[a] Rate constant (k) defined by rate law $-d[Co(II)]/dt = 2k[Co(II)][RX]$. [b] From ref 2. [c] From ref 4. [d] This work. [e] From ref 5.

pronounced for neutral iodides, but still noticeable for anionic iodides and neutral bromides. This influence may reflect a "salting out" effect which enhances the rate of reaction by altering the activity of the organic halide.

The rates of reaction of organic halides with B_{12r} "model compounds", i.e., bis(dioximato)cobalt(II) and Schiff's base cobalt(II) complexes, have been found to exhibit a significant dependence on the nature of the axial ligand, reactivity generally increasing with basicity of the latter.[4,5] Our observation of a ca. 10^2-fold decrease in rate of reaction of vitamin B_{12r} when the intramolecularly coordinated 5,6-dimethylbenzimidazole ligand is substituted by water (i.e., in going from the "base-on" to the "base-off" form) is consistent with this trend and parallels the earlier observation that the rate constant for the reaction of bis(dimethylglyoximato)cobalt(II) with benzyl bromide decreases from 7×10^{-1} to 4×10^{-3} M^{-1} s^{-1} when axially coordinated 1-methylimidazole is replaced by water.[4]

Although differences in solvents and axial ligands complicate the comparison of vitamin B_{12r} with other cobalt(II) complexes, the data collected in Table III indicate that the reactivity of vitamin B_{12r} toward organic halides is considerably lower than that of pentacyanocobaltate(II) and lies somewhere between that of cobalt(II) complexes of dimethylglyoxime and Schiff's bases.

While the present study does confirm that there are indeed significant parallels between the reactivity patterns of vitamin B_{12r} and those of other low-spin cobalt(II) complexes that have been invoked as B_{12r} "models", it is noteworthy that some features of the behavior of vitamin B_{12r} (notably in its reactions with organic iodides in aqueous solution) are not reflected in the behavior of any of the model compounds that have been examined and were not anticipated on the basis of such model studies.

While the results of the present study do not contribute any new insights into the mechanism of coenzyme B_{12} dependent reactions, they afford a basis for the following comments. (a) Vitamin B_{12r}, one of the postulated intermediates in these reactions, is a stable, well-behaved species in aqueous or methanol solution with a moderate "radical-like" reactivity that has been identified as characteristic of low-spin cobalt(II) compounds.[33] (b) The protonated, base-off form of B_{12r} is considerably less reactive along these lines than the intact (i.e., base-on) form. (c) The isolation of alkylcobalamins from reaction mixtures containing coenzyme B_{12} and alkyl halides[10] cannot be taken as evidence of the involvement of B_{12s} since alkylcobalamins also are formed directly by the reactions of B_{12r} with alkyl halides. Thus, the observed effects of highly chlorinated organic compounds (e.g., DDT) on coenzyme B_{12} dependent processes has been interpreted in terms of the interaction of such compounds with vitamin B_{12s}.[16] Our results suggest that the effects may be due instead to interaction with vitamin B_{12r} which we have shown to be sufficiently reactive to reduce such compounds even under conditions (pH <5) where vitamin B_{12s} is very unstable.

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Supplementary Material Available: Appendixes A, B, and C listing rate constants for individual experiments (9 pages). Ordering information is given on any current masthead page.

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