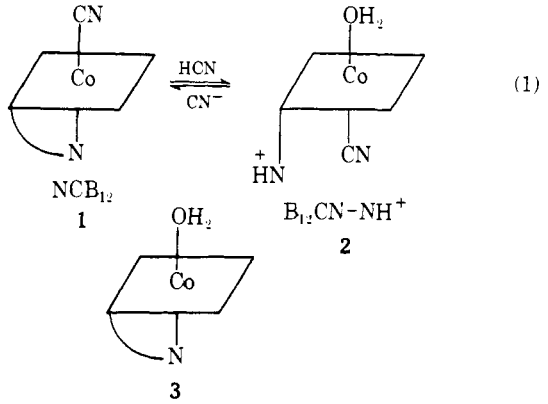
Reactions of Cyanide with Cobalamins[1]

William W. Reenstra and William P. Jencks*

Contribution No. 1273 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254. *Received March 26, 1979*

Abstract: Cyanide catalyzes the equilibration of α-aquo-β-cyanocobalamin and β-aquo-α-cyanocobalamin below pH 2 through the intermediate formation of α,β-dicyanocobalamin. Rate and equilibrium constants for the individual steps of the isomerization reaction were determined from the kinetics for equilibration and the rate constants for formation of the two isomers of cyanocobalamin from dicyanocobalamin. The rate of cyanide addition to cyanocobalamin becomes independent of cyanide concentration above a half-maximal value at 0.021 M CN^-, indicating a dissociative mechanism with a pH-independent rate constant of 0.042 s^{-1} for dimethylbenzimidazole dissociation. The faster dissociation in acid solutions suggests that cleavage of the Co–N bond is also catalyzed by acid. The addition of cyanide to aquocobalamin proceeds through addition of CN^- above pH 9 and a 3-fold slower addition of HCN between pH 6 and 8. Below pH 6 there is a change in rate-limiting step that is attributed to slow rotation of bound CN^-. A 35-fold difference between the rate constants at low and at high pH implicates a similar reaction sequence for the addition of cyanide to cyanocobalamin. The rates of cyanide addition to aquocobalamin and cyanocobalamin show positive deviations in hydrochloric acid (up to 3-fold in 1 M HCl) that reflect an unusual H_- acidity function for the ionization of HCN. All of the different kinetic patterns for the reactions of cyanide with cobalamin derivatives may be explained by a single underlying dissociative interchange mechanism.

We describe here an examination of the kinetics and mechanisms of addition reactions of cyanide to cyanocobalamin (vitamin B_{12}, **1**) and to aquocobalamin (**3**); see eq 1. The



(1)

former reaction was investigated following the observation of an unexpected, time-dependent change in the absorption spectrum of cyanocobalamin in the presence of cyanide at pH 0–2. This reaction proved to be a cyanide-catalyzed interconversion of the α and β isomers of cyanocobalamin (**1** and **2**, eq 1) that proceeds through α,β-dicyanocobalamin as an intermediate. In the course of the investigation several interesting features of cyanide addition reactions were demonstrated, including pathways for the addition of both CN^- and HCN, a change in rate-limiting step with decreasing pH, a slow dissociation of benzimidazole from cyanocobalamin at high pH, and an unusual acidity function for the ionization of hydrogen cyanide in hydrochloric acid solutions.

Experimental Section

Materials. Cyanocobalamin and hydroxycobalamin were gifts of Glaxo Laboratories. Organic reagents were purified and potassium cyanide was recrystallized prior to use.[2] Other inorganic reagents were used without further purification. Water was redistilled twice in glass. Solutions of hydrogen cyanide were prepared shortly before use by neutralizing aqueous potassium cyanide with 1 equiv or a small excess of hydrochloric acid (caution) and were stored in a stoppered flask.

Methods. All experiments were carried out at 25 °C and at ionic strength 1.0 M, maintained with potassium chloride. Determinations of pH were carried out after each run with a Radiometer 26 pH meter and a B electrode at 25 °C and ionic strength 1.0 M, after standardization of the pH meter with standard buffers or with 0.10 M hydrochloric acid at pH 1.10.[3] Reactions were followed spectrophotometrically at 560 or 580 nm, using 3.0-mL quartz cuvettes. Teflon-stoppered cuvettes were used for spectral measurements in the presence of cyanide and for kinetic runs with half-times longer than 1 min. Kinetic experiments were carried out under pseudo-first-order conditions in the presence of a large excess of hydrogen cyanide or potassium cyanide. Good first-order kinetics were observed for at least 3–4 half-times. Pseudo-first-order rate constants were obtained from plots of log $(A_\infty - A_t)$ or log $(A_t - A_\infty)$ against time.

A pK_a value of 0.38 was obtained for cyanocobalamin at ionic strength 1.0 M (KCl), 25 °C, by a spectrophotometric titration (560 nm) of 8×10^{-5} M cyanocobalamin at 11 concentrations of hydrochloric acid in the range 0.01–1.0 M. The same pK_a value was obtained based on measurements of the apparent pH values of the hydrochloric acid solutions. A pK_a of 0.1 has been reported previously for cyanocobalamin at low ionic strength; this pK_a involves dissociation from cobalt and protonation of the dimethylbenzimidazole group.[4] The pK_a of hydrogen cyanide was found to be 9.0 by titration at ionic strength 1.0 M and 25 °C.

Results

In the region of pH 0–2, cyanocobalamin undergoes a reaction in the presence of added cyanide that results in a time-dependent decrease in absorbance at 560 nm. At a given pH and cyanide concentration the absorbance change was found to follow accurate first-order kinetics; the pseudo-first-order rate constants increase linearly with the total concentration of added cyanide at a given pH value and ionic strength 1.0 M (KCl) (Figure 1). The observed rate constants increase with increasing pH (Figure 2), suggesting that cyanide anion may be the reactive species, but do not follow a simple rate law for a second order reaction of CN^- with cyanocobalamin, its conjugate acid, or both of these species.

The dependence on pH of the absorbance of cyanocobalamin extrapolated to zero time (closed circles) and at the end of the reaction (open circles) is shown in Figure 3A. Both of these curves follow the behavior expected for a simple acid dissociation reaction, and the slopes of plots of absorbance, A_{obsd}, against $(A_{NCB_{12}} - A_{obsd})/[H^+]$, where $A_{NCB_{12}}$ is the absorbance of NCB_{12} measured at high pH (Figure 3B), give apparent dissociation constants of $K_1 = 0.39$ M for the zero time absorbance and $K_P = 0.12$ M for the final absorbance of the products. Figure 3B also shows that the extrapolated absorbances at high acid concentration are the same for the reactants and products (as they are also at high pH, Figure 3A). The zero-time dissociation constant is the same, within experimental error, as the value of $K_1 = 0.42$ M ($pK_a = 0.38$) that was obtained from a spectrophotometric titration of cyanocobalamin at ionic strength 1.0 M in the absence of cyanide, and spectra of the reaction mixtures at zero time were

0002-7863/79/1501-5780$01.00/0

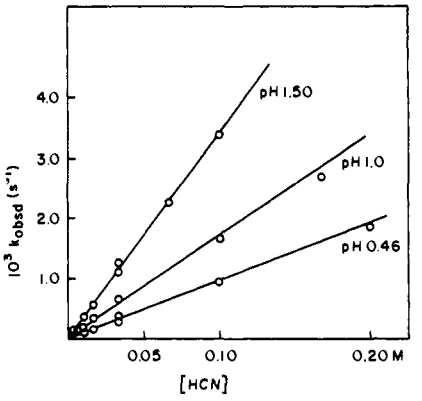


Figure 1. Dependence of the observed rate constants for the reaction of cyanocobalamin with cyanide on the concentration of hydrogen cyanide at pH 1.50, 1.0, and 0.46, 25 °C and ionic strength 1.0 M (KCl).

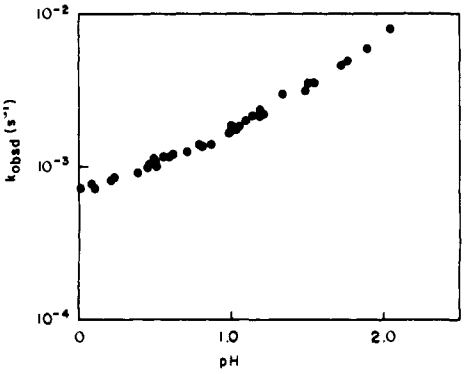


Figure 2. Dependence on pH of the observed rate constants for the reaction of cyanide with cyanocobalamin in 0.10 M hydrogen cyanide, 25 °C and ionic strength 1.0 M (KCl).

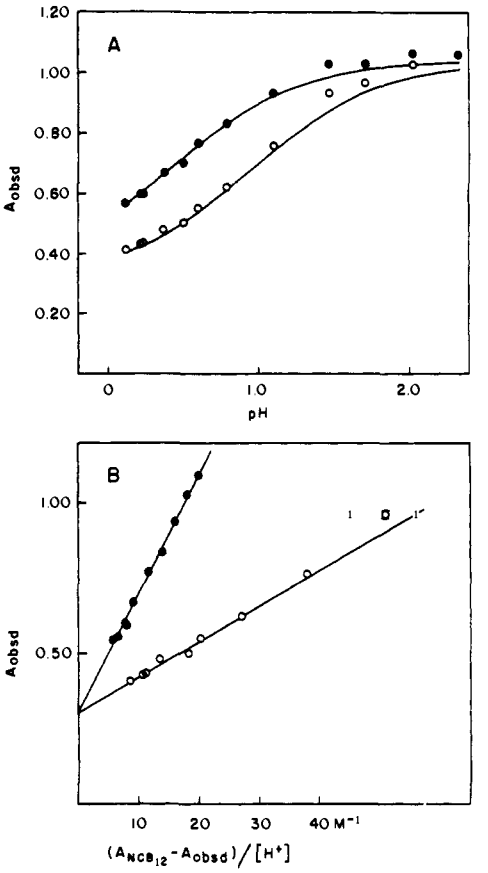


Figure 3. (A) Dependence on pH of the absorbance at 560 nm of a reaction mixture containing 1.2×10^{-4} M cyanocobalamin in 0.10 M hydrogen cyanide at (●) initial time, (○) infinite time, 25 °C and ionic strength 1.0 M (KCl). (B) A linear replot of the absorbance at 560 nm against the observed absorbance of 1.2×10^{-4} M NCB_{12} minus the observed absorbance of the reaction mixture, divided by the concentration of the proton, at (●) initial time and at (○) infinite time.

shown to be the same as the spectra of cyanocobalamin in the absence of cyanide at the same pH values. This shows that there is no detectable fast reaction of cyanide with cyanocobalamin prior to the observed first-order reaction.

Several observations show that the reaction is a cyanide-catalyzed, pH-dependent equilibration of two isomers of cyanocobalamin, each of which contains 1 equiv of cyanide, rather than an addition reaction of cyanide or some irreversible cyanide-induced reaction. First, the amount of the observed absorbance change at a given pH value was found to be independent of cyanide concentration, although the pseudo-first-order rate constants increase linearly with increasing cyanide concentration and have a zero intercept at zero cyanide concentration (Figure 1).

Second, the reaction was shown to proceed to a pH-dependent equilibrium position, rather than to completion, by the experiment described in Table I. Samples of cyanocobalamin in 0.1 M HCN were found to react at pH 0.52 (A) and pH 1.50 (C) with rate constants of 1.17×10^{-3} and 3.45×10^{-3} s^{-1}, respectively. When sample A was then brought to pH 1.52 (B) and sample C was brought to pH 0.51, (D), maintaining the concentration of HCN at 0.1 M, each sample underwent a second reaction, with rate constants of 3.47×10^{-3} and 1.10×10^{-3} s^{-1}, respectively. Thus, the observed rate constants at a given pH value are the same, irrespective of the composition of the reactants at the initial time. This is the behavior that is expected for a reaction that reaches an equilibrium position that depends only on the pH, in which case the observed first-order rate constant is equal to the sum of the forward and the reverse first-order rate constants and is independent of the composition of the reactants at zero time.[5] It is inconsistent with an irreversible reaction. Furthermore, comparison of the final absorbancies of samples A and D and of samples C and B (Table I) shows that the same final absorbance is reached at a given pH value, after allowing for the twofold dilution of samples B and D.

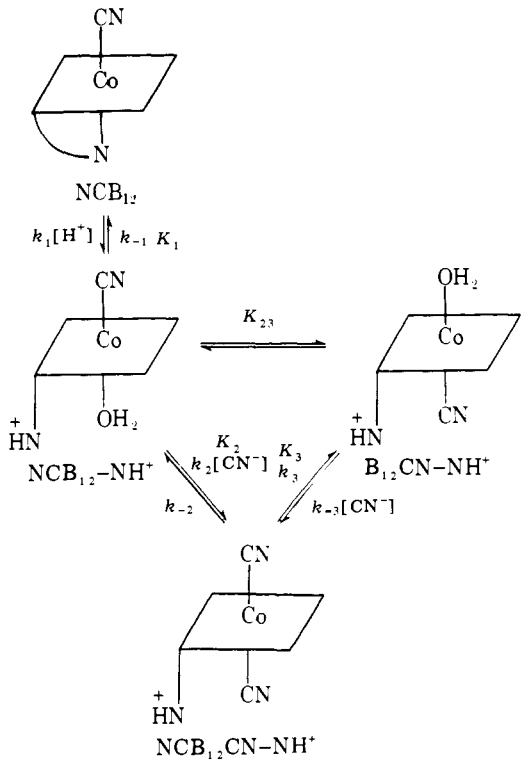
Third, the fact that the pseudo-first-order rate constants for the approach to equilibrium are first order with respect to cyanide at all pH values (Figure 1) means that both the forward and the reverse rate constants are first order in cyanide, because the observed rate constants are equal to the sum of the forward and reverse rate constants. Since the equilibrium position is independent of cyanide concentration, we conclude that the transition state must contain 2 equiv of cyanide, the reactants and products each contain 1 equiv of cyanide, and there is no significant accumulation of an intermediate containing 2 equiv of cyanide during the course of the reaction.

Table I. Equilibration of Cyanocobalamin with 0.1 M Hydrogen Cyanide and the Proton[a]

reaction[b]	pH	[HCN], M	$10^3 \times k_{obsd}$, s^{-1}	absorbance $t = 0$	absorbance $t = \infty$
A	0.52	0.10	1.17	1.40	0.99
B	1.52	0.10	3.47	0.75[c]	0.88[c]
C	1.50	0.10	3.45	1.97	1.76
D	0.51	0.10	1.10	0.64[c]	0.51[c]

[a] At 25 °C and ionic strength 1.0 M (KCl); followed at 560 nm. [b] The reactions are described in the text. [c] Diluted twofold compared with A and C.

Scheme I



These data suggest that the reaction is a cyanide-catalyzed equilibration between β-cyanocobalamin and β-aquo-α-cyanocobalamin proceeding through a dicyanocobalamin intermediate that is present at a steady-state concentration under the conditions of the experiment (Scheme I). The analogous isomerization of B_{12} derivatives lacking the benzimidazole group is known to be catalyzed by cyanide, presumably through the intermediate formation of the dicyano compound.[6] In the case of cyanocobalamin, isomerization requires removal of the dimethylbenzimidazole group from the α position of NCB_{12} by protonation to give NCB_{12}-NH^+, so that cyanide can add to form $NCB_{12}CN$-NH^+. This protonation also changes the overall equilibrium ratio of the α and β isomers, $[B_{12}CN\text{-}NH^+]/([NCB_{12}] + [NCB_{12}\text{-}NH^+])$ so that the $B_{12}CN$-NH^+ isomer can accumulate. At high acid concentration NCB_{12} is completely converted to the base-off species NCB_{12}-NH^+ and the equilibrium ratio becomes independent of pH (Figure 3B). The common intercepts for the absorbance of cyanocobalamin and the equilibrated reaction mixture at high acid concentration (Figure 3B) are consistent with the similar absorption spectra of α-aquo-β-cyanocobinamide and β-aquo-α-cyanocobinamide.[7]

The dissociation constant of protonated β-cyanocobalamin, K_1, and the equilibrium constant K_{23} (Scheme I) are related to the apparent dissociation constant of the equilibrated mixture of products, K_P, by

$$K_{23} = \frac{K_1 - K_P}{K_P}$$

and

$$K_P = \frac{[H^+][NCB_{12}]}{[NCB_{12}\text{-}NH^+] + [B_{12}CN\text{-}NH^+]}$$

The values of $K_1 = 0.42$ M and $K_P = 0.12$ M give $K_{23} = 2.5 \pm 0.2$.

The equilibrium constant K_{23} was also determined directly by taking advantage of the fact that a rapid change in pH gives a change in the initial absorbance of a mixture of the α and β isomers of cyanocobalamin that depends on the relative amounts of the two isomers. This occurs because only the β isomer gives an immediate spectral change due to the rapid and reversible addition of the dimethylbenzimidazole group to cobalt. A solution of 0.005 M cyanocobalamin was allowed to reach equilibrium in the presence of 0.1 M hydrogen cyanide and 0.97 M hydrochloric acid. Aliquots of this solution were diluted 30-fold into 0.97 M hydrochloric acid and into a series of solutions with final pH values in the range 0.49-1.96, and the initial absorbance was measured. The fraction of cyanocobalamin that was present as the β isomer in the equilibrated solution is given by

$$\frac{[NCB_{12}] + [NCB_{12}\text{-}NH^+]}{[NCB_{12}] + [NCB_{12}\text{-}NH^+] + [B_{12}CN\text{-}NH^+]} = \frac{\Delta A_{obsd}}{\Delta A_{calcd}} = \frac{(K_1/[H^+]) + 1}{(K_1/[H^+]) + 1 + K_{23}} \quad (2)$$

in which ΔA_{obsd} is the observed difference between the initial absorbance at a given pH value and in 0.97 M hydrochloric acid, and ΔA_{calcd} is the difference in the absorbance of the same concentration of β-cyanocobalamin under the same conditions. The results of 18 such pH-jump experiments gave a value of $K_{23} = 2.9 \pm 0.2$. The best value of K_{23} is taken to be 2.7.

The expulsion of cyanide from the dicyano intermediate, $NCB_{12}CN$-NH^+, was examined directly in order to determine the rate constants for the partitioning of this intermediate in the mechanism of Scheme I. The formation of $NCB_{12}CN$ from NCB_{12} becomes thermodynamically favorable at high pH and is essentially quantitative in the presence of 0.1 M potassium cyanide. The overall rate constant for expulsion of cyanide from $NCB_{12}CN$-NH^+, $k_{ov} = k_{-2} + k_3$, was determined by diluting a stock solution of $NCB_{12}CN$ in 0.1 M potassium cyanide 100-fold into a series of buffers at low pH values and following the change in absorbance at 580 nm. The value of k_{ov} was found to be 0.042 s^{-1} and is independent of pH over the range 0-2.0 (Figure 4). This rate constant is at least four times larger than the observed rate constants for the cyanide-catalyzed equilibration reaction.

The ratio k_3/k_{-2} was determined by measuring the product ratio from the decomposition of $NCB_{12}CN$ in acid by the method described above (eq 2). The rate constant ratio is equal to the initial ratio of products according to

$$\frac{k_3}{k_{-2}} = \frac{[B_{12}CN\text{-}NH^+]}{[NCB_{12}] + [NCB_{12}\text{-}NH^+]} \quad (3)$$

A solution of 5×10^{-3} M $NCB_{12}CN$ in 0.02 M potassium cyanide was diluted 10-fold into 1.0 M hydrochloric acid and was allowed to stand for 3 min. In this time the expulsion of 1 equiv of cyanide is complete ($t_{1/2} = 17$ s) and the cyanide-catalyzed isomerization of the initial products has proceeded less than 0.6%. Aliquots of this solution were then diluted 30-fold into 0.9 M hydrochloric acid, 0.1 M potassium chloride, and into a series of buffers to give final pH values between 0.75 and 1.92, and the initial differences in absorbance, ΔA_{obsd}, were measured. From the observed differences in absorbance and eq 2 and 3, the ratio k_3/k_{-2} was found to be 1.9 ± 0.2. This ratio and the value of $k_{ov} = k_3 + k_{-2}$ give $k_3 = 0.027$ s^{-1} and $k_{-2} = 0.014$ s^{-1}.

With this information it is possible to calculate all of the rate and equilibrium constants for the individual steps of the cyanide-catalyzed isomerization of protonated cyanocobalamin (Scheme I); the results are summarized in Table II. The observed pseudo-first-order rate constants for approach to equilibrium at each pH value were separated into the rate constants k_f and k_r for the forward and reverse reactions, respectively, from the relationships[5] $k_{obsd} = k_f + k_r$ and $K_{eq} = k_f/k_r$, in which K_{eq} is a dimensionless equilibrium constant that describes the equilibrium ratio of the total concentrations of the α and β isomers at a given pH value.

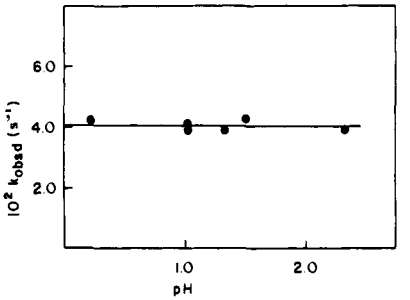


Figure 4. Dependence on pH of the observed rate constants for the loss of cyanide from $NCB_{12}CN\text{-}NH^+$ at 25 °C and ionic strength 1.0 M (KCl). The reaction was started by the addition of 0.03 mL of 0.005 M $NCB_{12}CN$ in 0.10 M potassium cyanide to buffer solutions.

Table II. Rate and Equilibrium Constants for the Equilibration of Cyanocobalamin with Cyanide[a]

term[b]	value
k_1, M^{-1} s^{-1}	>0.27
k_2, M^{-1} s^{-1}	$2.9 \pm 0.2 \times 10^6$
k_{-2}, s^{-1}	$1.4 \pm 0.1 \times 10^{-2}$
k_3, s^{-1}	$2.7 \pm 0.1 \times 10^{-2}$
k_{-3}, M^{-1} s^{-1}	$2.1 \pm 0.1 \times 10^6$
K_1, M	0.42
K_2, M^{-1} (k_2/k_{-2})	$2.1 \pm 0.3 \times 10^8$
K_3, M (k_3/k_{-3})	$1.3 \pm 0.1 \times 10^{-8}$
K_{23}	2.5 ± 0.2[c]
	2.9 ± 0.2[d]
	2.7[e]

[a] At 25 °C and ionic strength 1.0 M (KCl). [b] The constants are defined in Scheme I. [c] From the spectral data in Figure 3. [d] From direct measurement of the equilibrium. [e] Average value used in calculations.

$$K_{eq} = \frac{[B_{12}CN\text{-}NH^+]}{[NCB_{12}] + [NCB_{12}\text{-}NH^+]}$$

The value of K_{eq} at each pH value was calculated from

$$K_{eq} = K_{23}[H^+]/(K_1 + [H^+])$$

The values of k_f were then corrected by dividing by the fraction of cyanocobalamin present as $NCB_{12}\text{-}NH^+$ to give k_f', which is based on the concentration of this base-off species. No such correction is necessary for k_r because β-aquo-α-cyanocobalamin exists entirely in the form $B_{12}CN\text{-}NH^+$ under the conditions of the isomerization experiments. Values of $k_2 = 2.9 \times 10^6$ M^{-1} s^{-1} and $k_{-3} = 2.1 \times 10^6$ M^{-1} s^{-1} were then calculated from the steady-state equations for the rate constants in Scheme I,

$$k_f' = k_2k_3[CN^-]/(k_{-2} + k_3) \quad (4)$$

$$k_r = k_{-2}k_{-3}[CN^-]/(k_{-2} + k_3) \quad (5)$$

the values of k_{-2} and k_3 and the observed rate constants at pH values between 1.8 and 2.1. The equilibrium constants K_2 and K_3 were calculated from the ratios of the rate constants (Table II). The values of $K_2 = 2.1 \times 10^8$ M^{-1} and $K_3 = 1.3 \times 10^{-8}$ M are similar to the value of $K \sim 10^{-8}$ M for the dissociation of cyanide anion from dicyanocobinamide[8] (which has no benzimidazole group). These equilibrium constants show that the concentration of $NCB_{12}CN\text{-}NH^+$ is <4% of the total concentration of cyanocobalamins under the conditions of the kinetic experiments, which is consistent with the use of the steady-state approximation.

According to the mechanism of Scheme I, the values of log k_f' and log k_r should increase linearly with pH in the region pH 0–2 at constant hydrogen cyanide concentration, because

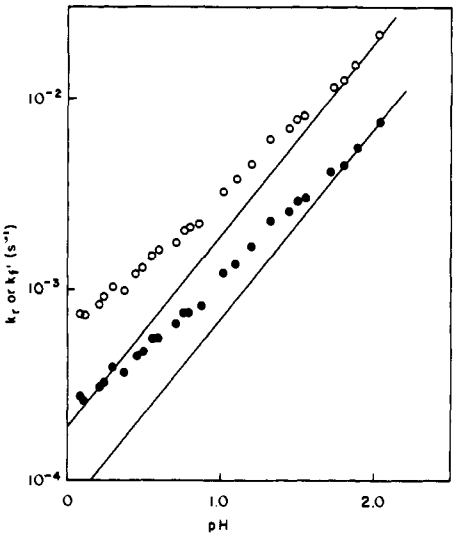
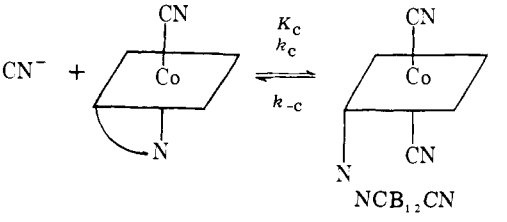


Figure 5. Dependence on pH of the forward, k_f' (○), and the reverse, k_r (●), first-order rate constants for the equilibration of cyanocobalamin with cyanide in 0.10 M hydrogen cyanide.

the isomerization rate depends on the concentration of cyanide anion. The data in Figure 5 show that the rate constants increase with increasing pH over this range, but there are positive deviations from the expected lines of slope 1.0 with increasing acidity that approach a factor of 3 for the rate constants at the lowest pH values. The deviations are several times larger than the estimated errors of the rate and equilibrium constants from which k_f' and k_r were obtained. They do not represent a contribution of an acid-catalyzed term to the isomerization reaction because any such acid catalysis should also be seen for the expulsion of cyanide from the $NCB_{12}CN\text{-}NH^+$ intermediate and the rate of this reaction is independent of pH in this pH region (Figure 4). They are attributed, therefore, to an activity coefficient or acidity function effect on the reaction that results from the substitution of hydrogen ions for potassium ions at ionic strength 1.0 M.

The Addition of Cyanide to Cyanocobalamin and Aquocobalamin. At high pH values the addition of cyanide to cyanocobalamin leads to the accumulation of $NCB_{12}CN$. The equilibrium constant for cyanide addition was found to be $K_c = 5 \times 10^3$ M^{-1} from measurements of the absorbance at 370



nm of 10 solutions of 1.1×10^{-5} M cyanocobalamin in the presence of 4×10^{-5} to 1×10^{-3} M and 0.1 M potassium cyanide in 0.2 M trifluoroethanol buffer at pH 11.70, 25 °C, and ionic strength 1.0 M (KCl). Values of $K_c = 10 \times 10^3$ M^{-1} and 6×10^3 M^{-1} under somewhat different conditions have been reported previously.[8,9]

The pseudo-first-order rate constants for the addition of cyanide anion to cyanocobalamin at pH 11.70 were determined by measuring the change in absorbance at 580 nm. The rate constants exhibit a leveling off with increasing cyanide concentration and approach a constant value that is independent of the concentration of cyanide. A plot of $1/k_{obsd}$ against $1/[CN^-]$ (Figure 6) gives a value of $k_4 = 0.042$ s^{-1} for the limiting, first-order rate constant at high cyanide concentration (from the reciprocal of the ordinate intercept) and $k_c = 2.0$

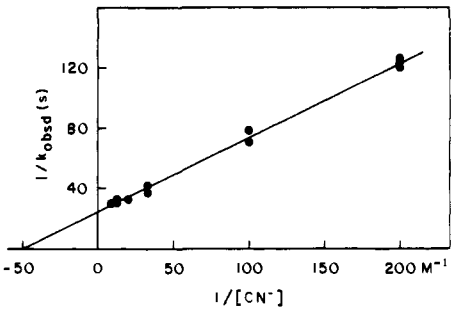


Figure 6. A double-reciprocal plot of the observed rate constants for cyanide anion addition to NCB_{12} against the concentration of cyanide anion in 0.33 M trifluoroethanol buffer at pH 11.70, 25 °C and ionic strength 1.0 M (KCl).

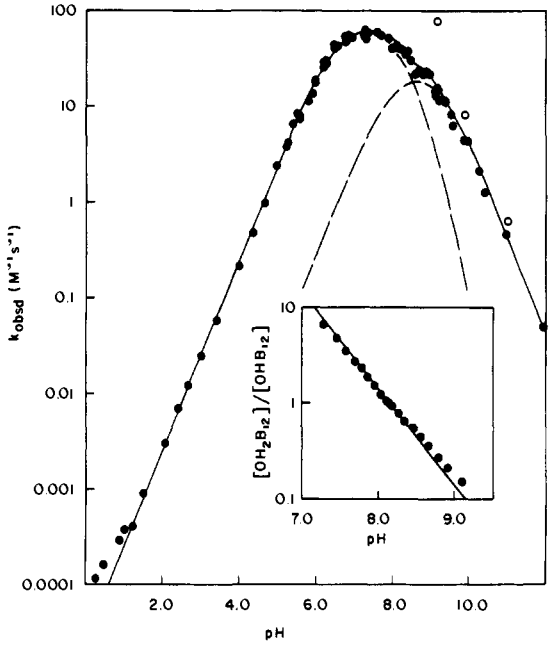


Figure 7. Dependence on pH of the observed second-order rate constants for the addition of cyanide to OH_2B_{12} at 25 °C and ionic strength 1.0 M (KCl). The open circles are data of Conn and Wartman.[12] The solid line is calculated from eq 10 and the dashed lines show the contributions of the two terms in eq 10. Insert: Dependence on pH of log ($[OH_2B_{12}]/[OHB_{12}]$) at 25 °C and ionic strength 1.0 M (KCl). Aquocobalamin, 0.012 M, was titrated with potassium hydroxide.

M^{-1} s^{-1} for the second-order rate constant at low cyanide concentration (from the reciprocal of the slope). A similar experiment in $HCN-CN^-$ buffers at pH 9.0 gave values of k_4 = 0.043 s^{-1} and k_c = 2.1 M^{-1} s^{-1}, demonstrating that these rate constants are independent of pH in this pH region. The absorbance changes in the kinetic runs were found to be independent of cyanide concentration; i.e., there is no accumulation of any intermediate complex with an absorbance different from that of cyanocobalamin at 580 nm.

In Figure 7 is shown the pH-rate profile for the addition of cyanide to aquocobalamin, based on total cyanide concentration (KCN + HCN). Titration of 0.012 M aquocobalamin with base gave pK_a = 8.1 at 25 °C, ionic strength 1.0 M (inset, Figure 7); pK_a values of 7.7, 7.8, and 7.6 have been reported previously at low (or unspecified) ionic strength.[4,10,11] Second-order rate constants expressed in terms of the addition of cyanide anion to aquocobalamin were obtained by dividing the apparent second-order rate constants (Figure 7) by the fraction of the cyanide present as CN^- and the fraction of the total cobalamin present as aquocobalamin (based on pK_a = 8.1; cyanide does not add to hydroxocobalamin[12]). These rate

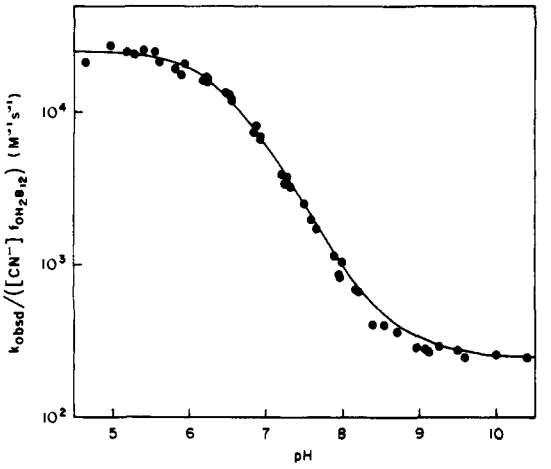


Figure 8. Dependence on pH of the corrected second-order rate constants, based on cyanide anion and aquocobalamin, for cyanide addition to aquocobalamin at 25 °C, ionic strength 1.0 M (KCl).

constants show a pH-independent region at high pH, an acid-catalyzed region between pH 6 and 9, and another pH-independent region at low pH (Figure 8).

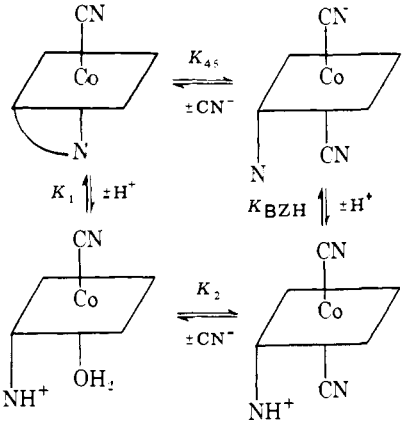
The observed rate constants for cyanide addition to aquocobalamin were found to be first order with respect to total cyanide concentration up to 0.04 M at pH 2.7, 0.003 M at pH 6.2, 0.0067 M at pH 8.9 and 0.1 M at pH 12.2. No indication of general acid–base catalysis was found with 0.07–0.94 M chloroacetate buffers, pH 2.7, with 0.5 and 1.0 M acetate and methoxyacetate buffers, 50% anion, and with 0.03–0.33 M trifluoroethanol buffers, pH 12.2. Rate constants determined in a series of 0.33 M cacodylate, phosphate, methylarsonate, ethylphosphonate, borate, and hexafluoroisopropyl alcohol buffers at different buffer ratios did not exhibit any deviations from a smooth pH-rate profile (Figure 7) that might be attributed to buffer catalysis. The addition of cyanide to aquocobalamin is essentially irreversible under the conditions of these experiments[4] and the absorbance extrapolated to zero time was shown to be independent of cyanide concentration at pH 6.2 and 8.8. At pH values below 2.0 the addition is followed by a rapid equilibration so that the observed product is a mixture of the α- and β-cyanocobalamins. At pH values above 2.0 the initial product was shown to be β-cyanocobalamin by examining the spectra of the reaction products. At high concentrations of cyanide anion, the final product is an equilibrium mixture of NCB_{12} and $NCB_{12}CN$. The addition of the second molecule of cyanide did not interfere with the rate measurements because the reactions were followed at 560 nm, an isosbestic point of NCB_{12} and $NCB_{12}CN$, and the second reaction was at least ten times slower than the initial reaction at pH values below 10. The rate constants obtained in this work are smaller than those measured previously by Conn and Wartman,[12] which are shown as the open circles in Figure 7. The difference between our value of k = 250 M^{-1} s^{-1} for the addition of cyanide anion at high pH and an earlier value[11,12] of k = 1500 M^{-1} s^{-1} is caused primarily by the different value of pK_a for aquocobalamin, which is itself probably the result of binding of chloride ion to aquocobalamin. A reported value[13] of K_{assoc} = 1.3 M^{-1} for such binding suggests that approximately half of the aquocobalamin is complexed with chloride ion in 1 M potassium chloride and correction for this complexation gives a pK_a of 7.7, which agrees with previously reported values.[4,10,11]

The rate constants in Figure 7 show a positive deviation from the line of slope 1.0 at low pH values. This deviation is similar to that observed for the addition of cyanide to cyanocobalamin in the isomerization reaction at low pH and is also attributed to an activity coefficient-acidity function effect.

Discussion

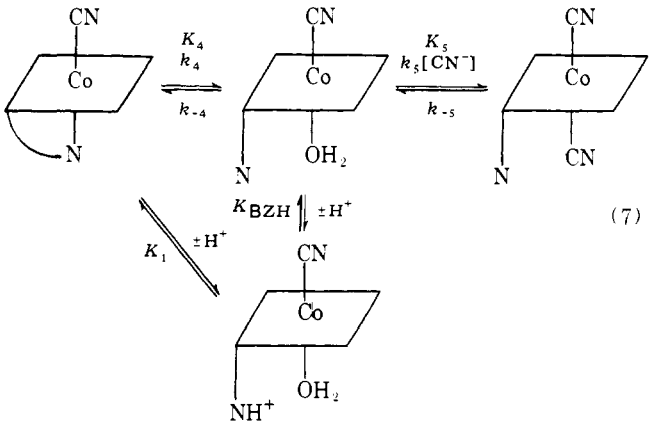
Isomerization of Cyanocobalamin. Equilibration of the α and β isomers of cyanocobalamin (Scheme I) occurs at pH values below 2.0 because the dimethylbenzimidazole (DMBz) group is removed and protonated in acid, so that cyanide can add to the α position. The equilibrium constants for the addition of cyanide anion to the α side of NCB_{12}-NH^+ and the β side of $B_{12}CN$-NH^+ are very similar, $K_2 = 2.1 \times 10^8\ M^{-1}$ and $1/K_3 = 0.8 \times 10^8\ M^{-1}$, respectively; the rate constants of $k_2 = 2.9 \times 10^6$ and $k_{-3} = 2.1 \times 10^6\ M^{-1}\ s^{-1}$ are also similar. Cyanide catalysis of this reaction is a manifestation of the labilizing influence of electron-donating ligands through the trans effect.[14] The rate of cyanide expulsion and isomerization from cyanocobalamin is negligible in the absence of added cyanide, as shown by the zero intercept in Figure 1, but the rate constants for cyanide expulsion from the α and β positions of $NCB_{12}CN$-NH^+ are 0.014 and 0.027 s^{-1}, respectively. Although the dicyanocobalamin intermediate does not accumulate to a significant extent during the isomerization reaction, it was possible to determine the individual rate and equilibrium constants for the reaction (Table II) by preparing this intermediate in alkaline solution and determining the rate constants for the expulsion of cyanide from the α and β positions under the conditions of the isomerization experiments.

The pK_a of the protonated dimethylbenzimidazole group of dicyanocobalamin, $NCB_{12}CN$-NH^+, is 5.0, based on the thermodynamic cycle of eq 6, the equilibrium constants in



(6)

Tables II and III, and $K_{BZH} = K_1K_{45}/K_2 = 10^{-5}$ M. This is in satisfactory agreement with the reported pK_a of 4.70 for 1-β-D-ribo-5,6-dimethylbenzimidazole at low ionic strength.[15,16] Assuming that the removal of cyanide from the α position does not affect the pK_a of the $DMBzH^+$ group in the base-off form, the equilibrium constant, K_4, for the dissociation of DMBz from cyanocobalamin is then $K_4 = K_{BZH}/K_1 = 2.4 \times 10^{-5}$. The value of K_5, for the addition of cyanide to the base-off species, is $K_5 = K_{45}/K_4 = 2.1 \times 10^8\ M^{-1}$, the same as K_2.[17] (See eq 7.) These equilibrium constants

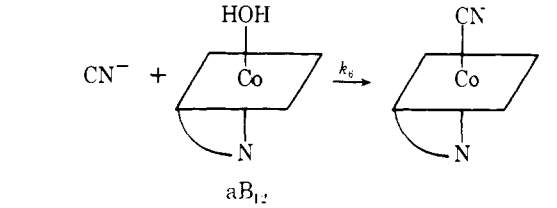


(7)

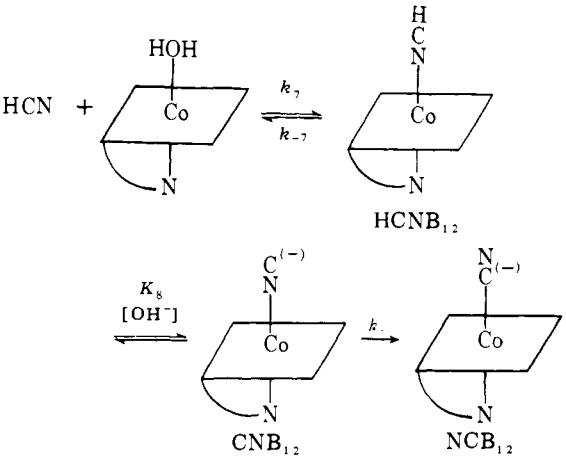
indicate that <5% of cyanocobalamin exists as the $NCB_{12}CN$-NH^+ intermediate under the conditions of the isomerization experiments.

Addition of Cyanide to Aquocobalamin. The pH–rate profile for the addition of cyanide anion to aquocobalamin (Figure 8) shows a pH-independent region above pH 9, an acid-catalyzed reaction at pH 6–9, and another pH-independent region below pH 6 that is 100-fold faster than the high pH region. Since the rate constants have been corrected for ionizations of the reactants, the break in this unusual curve at pH 6 must represent a change in rate-limiting step and the break at pH 9 must represent an additional reaction path.

These results are consistent with the mechanism of eq 8 and 9. According to this mechanism the pH-independent region



(8)



(9)

at high pH represents rate-limiting addition of cyanide anion (k_6, eq 8), the acid-catalyzed region below pH 9 represents rate-limiting addition of hydrogen cyanide to give an unstable intermediate with HCN bound to cobalt through the nitrogen atom, or possibly as a π complex (k_7, eq 9), and the pH-independent region at low pH represents rate-limiting isomerization of this intermediate to the stable carbon-bound species after loss of a proton (k_9, eq 9). The reaction at low pH corresponds to the pH-independent region of Figure 8, which is based on the concentration of CN^-, and to the base-catalyzed region of Figure 7, which is based on the concentration of HCN at low pH. The solid line in Figure 7 is calculated from the steady-state rate equation

$$v = k_6[CN^-][aB_{12}] + \frac{k_7K_8k_9[HCN][aB_{12}][OH^-]}{k_{-7} + K_8k_9[OH^-]} \quad (10)$$

based on eq 8 and 9, using $k_6 = 250\ M^{-1}\ s^{-1}$, $k_7 = 80\ M^{-1}\ s^{-1}$, and $k_7K_8k_9/k_{-7} = 2.5 \times 10^9\ M^{-2}\ s^{-1}$, and correcting the rate constants for the fractions of the reacting ionic species using $pK_a = 9.0$ for hydrogen cyanide and $pK_a = 8.1$ for aquocobalamin, aB_{12}. The line shows satisfactory agreement with the observed rate constants.

Precedent for binding of cyanide at more than one position, probably at the nitrogen as well as the carbon atom, is provided by the reported existence of cobaloxime dimers that are connected through a CN^- bridge[18] and of a methylcobaloxime complex with CH_3CN as the axial ligand.[19] The stable cyanide-containing product (cyanocobalamin) has been assigned a structure with the cyanide group bound end-on through carbon to cobalt.[20]

Margerum and Simandi have reported that both CN^- and HCN add to nickel–EDTA and that this reaction undergoes a similar change in rate-limiting step with decreasing pH.[21] The rate-limiting step at low pH was assigned to proton removal from the metal–HCN complex, based on the observation of catalysis by borate buffers. However, proton removal does not account for the change in rate-determining step in the reaction of cyanide with cobalamin. No buffer catalysis was detected for this reaction, although buffer bases as well as hydroxide ion should be able to catalyze proton removal. Furthermore, the predominant pathway for proton removal from an acid of $pK_a < 12$ at low pH values is expected to involve proton donation to water, rather than to hydroxide ion, which is inconsistent with the observed pH-dependence of the reaction.[22]

Rate-determining addition of the dipolar isomer of hydrogen cyanide, $HNC^{\pm}$, would account for the acid-catalyzed region at pH 6–9 if the rate constant for the addition of $HNC^{\pm}$ were much faster than that for addition of CN^-, but does not account for the change in rate-limiting step at lower pH values. Acid catalysis of water expulsion followed by addition of CN^- would satisfy the observed kinetics at pH 6–9, but microscopic reversibility requires that the addition of the small amount of H_3O^+ present at pH 6–9 would then represent the predominant path for the reverse, hydration reaction and would be faster than the addition of 55 M water, which does not seem possible. It is also difficult to account for the change in rate-limiting step at pH 6 by this mechanism.

Thusius[23] has observed a first-order relaxation of cobalamin in the presence of thiocyanate and proposed that this represents an isomerization between the isomers in which thiocyanate is bound through sulfur or nitrogen, with a rate constant of 40 s^{-1}.

It is probable that the rearrangement of cyanide occurs through a transition state (and possibly also an intermediate) that is stabilized by π bonding between cyanide and cobalt. The analogous rearrangement of isonitriles to nitriles occurs through a transition state with strong bonding to the migrating C≡N group, as demonstrated by the retention of stereochemistry of the R group of isonitriles, RNC, the absence of migration of the CN group to other positions on an aromatic ring or rearrangement of a cyclobutyl group during the isomerization, an entropy of activation near zero, and an almost negligible effect of polar substituents on the rate of isomerization.[24]

There is precedent for the addition of the protonated as well as the anionic species of ligands to cobalamin in the addition of hydrazoic acid and thiols,[11,25] but this is the first addition to cobalamin for which a change in rate-limiting step has been demonstrated. An isomerization step is not necessary to form a stable product with thiols or hydrazoic acid. The rate constant of $k_7 = 80\ M^{-1}\ s^{-1}$ for the addition of hydrogen cyanide to cobalamin is about one-third of the rate constant of $k_6 = 240\ s^{-1}$ for the addition of cyanide anion (Table III). A similar ratio of 1:4 has been reported for the addition of thiols and thiol anions to cobalamin,[25] but thiols add twice as fast as thiol anions to methylaquocobaloxime.[26]

The rate constants for the addition of cyanide at high and at low pH both follow the rate law $v = k[CN^-][\text{cobalamin}]$, as a consequence of the change in rate-limiting step, but the observed rate constant at low pH is 100-fold larger than the true rate constant for the addition of cyanide anion. Thus, caution should be exercised in comparing rate constants for the addition of different ligands under conditions in which one of the ligands is present largely as its conjugate acid and could undergo addition through an isomerization mechanism.

Addition of Cyanide to Cyanocobalamin. The change from first-order to zero-order dependence on the concentration of cyanide anion for the rate of addition of cyanide to cyanocobalamin at high pH (Figure 6) is evidence for a change in rate-determining step, from rate-determining cyanide addition to the base-off species of cyanocobalamin at low cyanide concentration (k_5, eq 7) to rate-determining dissociation of the DMBz ligand at high cyanide concentration (k_4, eq 7).[50] Formation of an outer sphere complex with cyanide is not a satisfactory explanation for the observed leveling off of the rate at high cyanide concentrations because such a complex would require an association constant of $K_{os} = 48\ M^{-1}$ to account for the data, which is at least an order of magnitude larger than would be expected for the formation of such a complex with a monoanion in water and is much larger than the values of $K_{os} = 0.12\text{–}1.8\ M^{-1}$ that have been observed for the formation of cobaloxime complexes with ligands containing hydrophobic, aromatic groups (no complexes were observed with small anionic ligands).[27] Furthermore, the observed rate constants for the addition of several anions to aquocobalamin are linear with increasing anion concentration up to 0.1–0.25 M, and up to 1.0 M for bromide anion.[28] The association constants for outer sphere complex formation with anions should be at least as large for aquocobalamin as for cyanocobalamin, which has one less positive charge. Rate-determining DMBz dissociation in a related reaction has been suggested previously, based on the similar first-order rate constants for cyanide addition to coenzyme B_{12} of 0.029 s^{-1} at pH 10.5, 0.1 M KCN, 25 °C, and 0.0083 s^{-1} at an unspecified pH, 0.01 M KCN, 27 °C.[29]

The rate constants $k_c = k_4k_5/k_{-4} = 2.0\ M^{-1}\ s^{-1}$ and $k_4 = 0.042\ s^{-1}$ (Table III) give the ratio $k_{-4}/k_5 = 0.021$ M, which corresponds to the concentration of cyanide anion at which the two steps are equally rate limiting and the rates of addition of cyanide anion and DMBz to cobalt are equal. Thus, the "effective molarity" for the intramolecular addition of DMBz relative to that of cyanide anion is only 0.021 M and, since the differences in the rate constants for bimolecular addition of different ligands to cobalamin are not large,[28] there is little advantage from intramolecularity in the addition of DMBz. This low effective molarity presumably reflects a small requirement for loss of entropy to form the loose transition state for ligand addition and the large number of rotational states that can be taken up by DMBz and the chain by which it is attached to the corrin ring; it may also reflect unfavorable nonbonding interactions for the addition of DMBz.

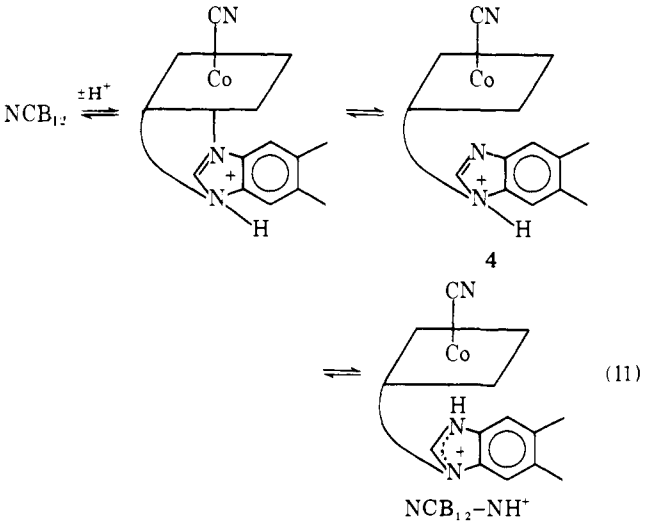
The limiting rate constant of $k_4 = 0.042\ s^{-1}$ for dissociation of the DMBz group at high pH is much slower than the formation of the protonated, base-off species $NCB_{12}\text{–}NH^+$ in acid, which occurs at a rate that is too fast to measure by ordinary techniques.[30] This suggests that dissociation of the DMBz group is subject to acid catalysis.[50] Since the lone pair electrons of the benzimidazole nitrogen atom are coordinated

Table III. Rate and Equilibrium Constants for the Addition of Cyanide Anion to Cyanocobalamin, NCB_{12}, and Aquocobalamin[a]

term[b]	value
k_4, s^{-1}	4.2×10^{-2}
k_{-4}, s^{-1}	1.8×10^{3}
k_5, $M^{-1}\ s^{-1}$	8.4×10^{4}
k_{-5}, s^{-1}	4.0×10^{-4}
k_6, $M^{-1}\ s^{-1}$	2.5×10^{2}
k_7, $M^{-1}\ s^{-1}$	80
K_{BZH}, M ($[H^+][NCB_{12}CN]/[NCB_{12}C\text{-}NH^+]$)	1.0×10^{-5} [c]
K_4 ($[NCB_{12}\text{-}N]/[NCB_{12}]$)	2.4×10^{-5} [d]
K_5, M^{-1} ($[NCB_{12}CN]/[CN^-][NCB_{12}\text{-}N]$)	2.1×10^{8}
K_{45}, M^{-1} ($[NCB_{12}CN]/[CN^-][NCB_{12}]$)	5×10^{3}

[a] All data at 25 °C and ionic strength 1.0 M (KCl). [b] Rate constants defined in eq 7-9. [c] From eq 6. [d] K_{BZH}/K_1.

to cobalt, a simple mechanism for this catalysis involves protonation of the nonliganded nitrogen atom to give the unstable protonated initial product **4** which rearranges rapidly to the



stable NCB_{12}–NH^+; the reverse, addition reaction of the DMBz group at pH values below 5 then involves an initial isomerization to **4**, followed by addition and deprotonation to give NCB_{12}. The dissociation of DMBz from methylcobalamin is catalyzed by mercuric ion[31] and the rapid dissociation of this DMBz group (k = 2000 s^{-1}) under conditions in which it is partially in the protonated, base-off form,[32] may represent an acid-catalyzed reaction. Part of the increase of 5×10^4-fold for this rate constant compared with that for cyanocobalamin at high pH could be accounted for by a larger kinetic trans effect of CH_3 compared with CN, but it is unlikely that this effect would be larger than the equilibrium trans effect, which is only 400-fold (this value is based on the ionization constants for protonation and dissociation of the DMBz group from methylcobalamin and cyanocobalamin[33]).

The rate constant for the addition of cyanide anion to the protonated base-off species NCB_{12}–NH^+ at low pH, $k_2 = 2.9 \times 10^6$ M^{-1} s^{-1}, is 35-fold larger than the rate constant for cyanide addition to the base-off species NCB_{12}-N at high pH, $k_5 = 8.4 \times 10^4$ M^{-1} s^{-1} (Tables II and III). Protonation of the DMBz group when it is dissociated from the cobalt atom would not be expected to have a significant effect on the rate of cyanide anion addition at ionic strength 1.0 M. The observation of these two different rates for the addition of cyanide anion at high and low pH requires that there must be an acid-catalyzed region of the pH-rate profile, similar to that in Figure 8, at an intermediate pH. Thus, the dependence on pH for the addition of cyanide to cyanocobalamin is essentially the same as for the addition to aquocobalamin and the rate-determining step at low pH may be assigned to isomerization of the N-liganded to the stable C-liganded species after addition and deprotonation of HCN in both reactions.

Acidity Function Effects. The observed rate constants for the addition of cyanide to cyanocobalamin and to aquocobalamin at low pH are faster than predicted for a rate law involving cyanide ion by factors of up to 3 (Figures 5 and 7). These deviations are attributed to an activity coefficient or acidity function effect on the ionization of hydrogen cyanide (which may be bound to cobalamin, eq 9) when the proton is substituted for potassium ion with increasing acid concentration at ionic strength 1.0 M. The facts that the equilibrium constants K_1 and K_P (and hence K_{23}) show no such deviations and that the rate constants for cyanide expulsion from $NCB_{12}CN$ show no dependence on pH in this pH region (Figure 4) show that the deviations are not caused by activity coefficient effects on dissociation of the cyanocobalamins nor

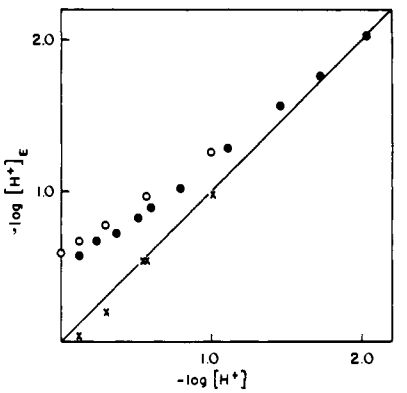


Figure 9. Dependence of the "effective concentration" of the proton for the equilibration of cyanocobalamin (●) on the stoichiometric concentration of the proton. Also shown are H_- (○) and H_0 (X) in hydrochloric acid.[34,35]

by a stabilization of the transition state for cyanide expulsion relative to $NCB_{12}CN$.

This behavior is unusual for activity coefficients in acid solutions because most acidity functions give rise to behavior corresponding to an acidity that is larger, rather than smaller, than expected from the stoichiometric concentration of acid. There is precedent for this kind of behavior in the H_- function for the ionization of chlorophenylphosphates and chlorophenylphosphonates in hydrochloric acid[34] and, in fact, cyanide addition to cyanocobalamin follows this acidity function fairly closely. The deviations from ideal behavior can be described in terms of an "effective concentration" of the proton, $[H^+]_E$, that is required to account for the observed behavior at a given stoichiometric concentration of acid. As shown in Figure 9, $[H^+]_E$ exhibits similar behavior for the H_- function and for hydrogen cyanide addition with increasing acidity, whereas the H_0 function[35] (crosses) shows a smaller deviation in the opposite direction.

This unusual acidity dependence may be accounted for by hydrogen bonding of the solvated proton to the anionic conjugate base of the acid, which will have the effect in this reaction of increasing the total concentration of cyanide anion at equilibrium above the amount that would be expected from the pH and the pK of HCN. An equation proposed by Hine[36] predicts an equilibrium constant of K_{AB} = 580 M^{-1} for the formation of such a hydrogen-bonded pair with CN^-, $[CN^- \cdots H_3O^+]$; this value is much larger than is needed to account for the data. The observed increase of only threefold in the apparent concentration of cyanide ion in 1 M hydrochloric acid may reflect a compensating increase in the activity coefficient of the proton, a relatively weak hydrogen-bonding ability of cyanide anion, and the use of a value of τ = 0.024 in the Hine equation that is too large for hydrogen bonding in water.[37] If the Hine equation holds generally, the rate constants for reactions of the conjugate base of a weak acid should frequently exhibit increases from such hydrogen bonding in acid solutions.

Mechanisms for Ligand Addition. The different modes of addition of cyanide to cobalamin described here illustrate how apparently different patterns of metal complex formation that are obtained in different circumstances can be explained by a single underlying reaction mechanism. We assume for the purposes of this discussion that the ground state of cobalamins in water is hexacoordinate. Although the existence of stable pentacoordinate cobalamins and alkylcobaloximes has been suggested, the available data now suggest that the stable form of cobalamins and most alkylcobaloximes in water is hexacoordinate.[38]

(1) The addition of cyanide anion to aquocobalamin is second order and probably occurs through the dissociative inter-

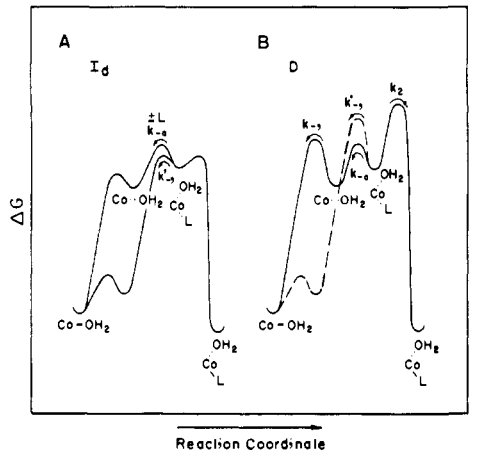
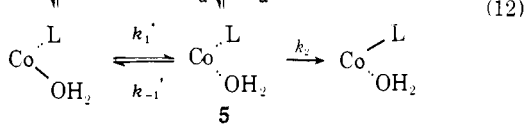


Figure 10. Gibbs energy diagrams to illustrate how the choice of a dissociative interchange, I_d, or a dissociative, D, mechanism in aqueous solution depends on the lifetime of the pentacoordinate intermediate.

change mechanism, I_d, that appears to be the predominant mechanism for the addition of ligands to octahedral metal complexes in aqueous solution, including cobalamins and cobaloximes.[39] A mechanism of this kind is consistent with the similar rate constants for the addition of ligands of widely varying affinity for the metal; for example, rate constants for the addition of a series of ligands to cobalamin[28] vary by a factor of only 14 when the equilibrium constants for addition vary by 2×10^4 and for a larger series of ligands the rate and equilibrium constants vary over ranges of approximately 10^2 and 10^{11}, respectively.[40]

The reaction coordinate-Gibbs free energy diagrams of Figure 10 provide a convenient way to illustrate how the lifetimes of intermediates determine the preferred reaction mechanism for ligand exchange.[41] The dissociative interchange mechanism for metal complex formation in aqueous solution is essentially the same as the preassociation mechanism that provides the lowest energy reaction pathway when the intermediate becomes sufficiently unstable in general acid–base catalyzed, carbonium, carbanion, and possibly metaphosphate-forming reactions; all of these reactions can be described by analogous Gibbs energy diagrams.[42] The formation of a metal-ligand complex, Co-L, from the octahedral aquo complex, Co-OH_2, ordinarily proceeds through the unstable pentacoordinate intermediate **5**.[39] This intermediate may be formed by either (a) a pure dissociative mechanism, D, in which the Co-OH_2 bond is broken (k_1, upper path of eq 12),



(12)

followed by encounter with the ligand to form **5** (k_a) and product formation (k_2) or (b) a dissociative interchange mechanism, I_d, with the initial formation of an outer sphere complex (K_{os}, lower path of eq 12), followed by breaking of the Co–OH_2 bond to form **5** (k_1') and product formation (k_2). The preferred mechanism is determined by the lifetime of the pentacoordinate intermediate–ligand complex, **5**, as shown in Figure 10. If this intermediate adds water, k_{-1}', faster than the ligand diffuses away from it, k_{-a}, the lowest energy pathway for its reversion to reactants proceeds through the outer sphere complex H_2O–Co · · L. The same pathway must then be the lowest energy route for the formation of the complex, as shown by the lower line for the I_d mechanism in Figure 10A. It follows that the I_d preassociation mechanism is preferred whenever the addition of all ligands to the pentacoordinate intermediate is diffusion controlled, i.e., when $k_{-1}' > k_{-a}$ and $k_2 > k_{-a}$. The dissociative pathway through k_1 and k_a is followed only when there is a relatively large barrier for the addition of water so that k_{-1}' (and k_{-1}) are slow relative to diffusion away of the ligand (k_{-a}). This pathway is shown by the solid line in Figure 10B; the dashed line shows the large barrier for k_{-1}'.

The diagram of Figure 10B illustrates the rather special requirements that must be met for a second-order type D reaction in aqueous solution. In order for the D mechanism to be favored over the I_d preassociation mechanism the addition of water to the pentacoordinate intermediate must be *slow* compared with k_{-a}, but in order for the reaction to be second order, with rate-limiting addition of the ligand, the addition of water (k_{-1}' and k_{-1}) must be *fast* compared with addition of the ligand through the k_2 step. This mechanism will not be common, because water is not an exceptionally reactive ligand toward most metals. Second-order kinetics requires that the metal have a low selectivity toward ligand addition so that the addition of 55 M water will be faster than the addition of ligand and the k_2 step is rate limiting, but such low selectivity is likely to be associated with a highly reactive intermediate that will react by an enforced preassociation mechanism, I_d. If the metal has a higher selectivity toward ligand addition, the barriers for the addition of water and the more reactive ligands to pentacoordinate metal will become comparable ($k_{-1} \sim k_2 k_a / k_{-a}$), the dissociation of water will become partly rate determining, and the reaction will no longer be second order. For this reason, a dissociative mechanism does not provide a satisfactory explanation for metal complex formation when the reactions are second order and the rate constants vary over a wide range. Strong evidence for a dissociative mechanism in water is provided by the observation of a change in rate-determining step with an approach to zero order dependence on ligand at ligand concentrations below those that would be expected to cause significant accumulation of an outer sphere complex, as in the addition of anions to $Co(CN)_5H_2O^{2-}$ and cobalt hematoporphyrin.[43,44] In contrast, the addition of ligands to aquocobalamin is first order in the ligand,[28] as expected for an I_d mechanism with a small outer sphere association constant, K_{os}.

A preassociation or dissociative interchange mechanism is enforced by the short lifetime of an unstable intermediate, so that $k_{-1}' > k_{-a}$. In carbon chemistry an even shorter lifetime of $\sim < 10^{-13}$ s, so that the "intermediate" does not exist, leads to an enforced concerted mechanism with significant electrophilic or nucleophilic assistance to the reaction by the final reactant,[42] but this is less common in metal ligand reactions because the available coordination sites and geometry are often not favorable for such assistance in the transition state. It is likely that the large differences in the rate constants for different ligands in the formation of some octahedral complexes do represent such nucleophilic assistance through a frontside displacement mechanism with an open, "exploded" transition state in which there is significant nucleophilic interaction of the incoming ligand with the metal before cleavage of the bond to the leaving water molecule is complete.[45] The species **5** then represents a transition state, rather than a discrete intermediate corresponding to a dip in the Gibbs energy profile. This may be described as an associative interchange mechanism, I_a.[39] Such nucleophilic assistance provides an explanation for the differences of almost 10^4 for the addition of different ligands to methylaquo-1,3-bis(biacetylmonoximeimino)propanatocobalt, for example,[27] and might account for the range of some 10–100-fold in the rate constants for addition of different ligands to aquocobalamin.[40]

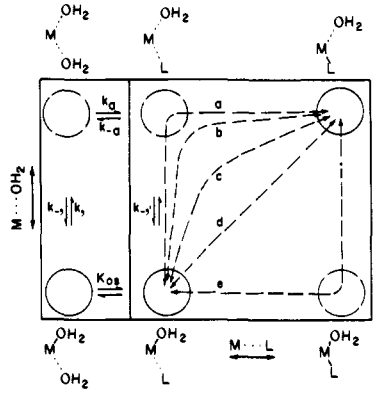


Figure 11. Three-dimensional reaction coordinate-energy diagram to illustrate different pathways for reactions of metal complexes. The contour lines are omitted.

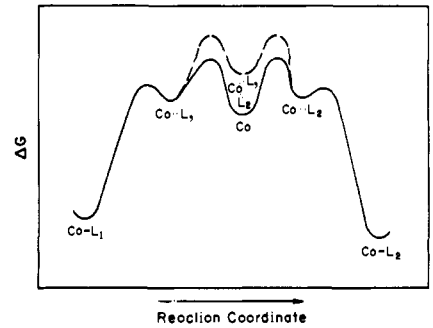


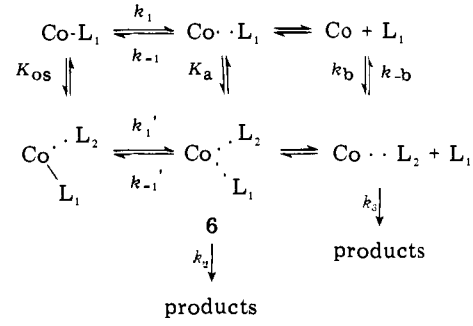
Figure 12. Gibbs energy diagram to show how a dissociative mechanism is favored over a dissociative interchange mechanism in a nonliganding solvent.

Even a small amount of nucleophilic assistance will give a significant rate acceleration in an enforced preassociation or dissociative interchange mechanism in a reactive solvent, because the incoming group must always be present in the rate-determining transition state. In order for nucleophilic assistance to be significant in a nonliganding solvent it must overcome the unfavorable entropy requirement for the inclusion of an additional molecule in the transition state and is, therefore, less likely to be significant.

The different pathways may be summarized with the three-dimensional reaction coordinate-energy diagram of Figure 11, with two axes representing bonding to the entering and leaving ligands and Gibbs energy in the third dimension.[46] Different reaction paths are indicated by the lines connecting the complexes in which a coordination site of the metal is bound to a ligand or to water; the energy contour lines have been omitted from Figure 11 for clarity. If the pentacoordinate intermediate, **5**, in the upper left corner of the square, has a sufficient lifetime to permit diffusional separation of ligands, the intermediate will equilibrate with the solvent and the reaction can proceed through the k_1 and k_a steps of a fully stepwise dissociative mechanism. If the lifetime is shorter, so that the intermediate adds water (k_{-1}') faster than L can diffuse away (k_{-a}), the reaction must proceed through an outer sphere complex and the dissociative interchange mechanism of path a. If the lifetime is still shorter, so that there is no barrier for the addition of water, or of ligand, the intermediate does not exist as a discrete species corresponding to a Gibbs energy well (dashed circle) and the reaction must proceed through a more-or-less concerted mechanism (path b). In this case, the transition state will involve some degree of bonding to the ligand and water, to the extent that this is compatible with the geometry and coordination number of the complex. In many octahedral substitution reactions the amount of bonding to the ligand is small and the transition state is close to the pentacoordinate species in the upper left corner. If an increase in coordination number in the transition state is feasible, the position of the transition state can shift diagonally in the direction of a conventional nucleophilic substitution reaction (paths c and d). Finally, the reaction can proceed in a stepwise, associative mechanism through an intermediate of increased coordination number if this species has an appreciable lifetime (path e, dashed circle in lower right corner).

It should be noted that there is no sharp line distinguishing the different mechanisms and reaction paths across the diagram when the intermediates have no significant lifetime; only a quantitative characterization as an I_d or I_a mechanism is possible, depending on the amount of nucleophilic assistance and the position of the transition state in the diagram. A clear-cut distinction between mechanisms may be made only with respect to (a) whether or not the intermediate has a significant lifetime, i.e., whether it represents a dip in the energy surface, and (b) whether an intermediate is formed via the k_1 or the k_1' pathway.

A dissociative mechanism will be favored in a nonliganding solvent, because an unstable pentacoordinate intermediate in such a solvent can have a lifetime that is sufficient to permit diffusion-controlled encounter with a new ligand. In sufficiently dilute solution the free pentacoordinate intermediate, Co, will always be of lower Gibbs energy than the ternary complex of the pentacoordinate intermediate with two ligands, **6** (eq 13), so that the lowest energy pathway will proceed



(13)

through this free intermediate and the two binary complexes Co··L_1 and Co··L_2, as shown in Figure 12. Pure dissociative mechanisms are, therefore, frequently observed in such solvents and have been clearly demonstrated for alkylcobaloximes.[47,48] For example, the rates of ligand exchange with a series of alkylcobaloximes in methylene chloride are independent of both the concentration and the nature of the incoming ligand and show competition ratios close to 1.0 for different ligands.[48] This suggests that the pentacoordinate intermediate is nondiscriminating and highly reactive, as would be expected if the reaction proceeds through an enforced I_d, preassociation mechanism in water.

(2) The addition of cyanide to cyanocobalamin, to give dicyanocobalamin (eq 7), is dissociative in the sense that the dissociation of DMBz is rate determining and the rate becomes zero order in cyanide at high cyanide concentration. However, if the addition of cyanide to aquocobalamin proceeds through an I_d mechanism that is enforced by the short lifetime of the intermediate, the exchange of DMB for cyanide must proceed through two dissociative interchange steps, as in other ligand exchange reactions of octahedral complexes, rather than through a pure dissociative mechanism.[49] The first step involves the dissociation of DMBz followed by rapid hydration of the pentacoordinate intermediate, according to the I_d mechanism of Figure 10A proceeding from right to left. This path is favored because the concentration of water is much higher than that of the ligand and, for a reaction in dilute

aqueous solution in which the I_d mechanism is enforced by the short lifetime of the pentacoordinate intermediate, outer sphere complexes and transition states containing water and a single ligand are of lower Gibbs energy than those containing two ligands (an exception to this generalization could occur at higher ligand concentrations when there is strong electrostatic stabilization of a complex with a 2+ or 3+ metal ion). The second step of cyanide addition then takes place through a second I_d mechanism that is described by the same diagram, proceeding from left to right. The rates of such reactions are usually zero order in the ligand undergoing addition, unless the aquo intermediate accumulates, the entering ligand forms a stable outer sphere complex, or the leaving ligand is added to cause inhibition by a mass law effect. In the cyanocobalamin reaction, the leaving DMBz group is attached by a covalent bridge to the rest of the molecule and undergoes readdition at low concentrations of cyanide, so that the reaction undergoes a change in rate-limiting step and changes from first order to zero order in cyanide as the cyanide concentration is increased (Figure 6).

The rate constant k_5 for the addition of cyanide to the base-off species of cyanocobalamin is 340 times larger than the rate constant k_6 for the addition of cyanide to aquocobalamin (Table III). This provides further evidence for the formation of an aquo, hexacoordinate intermediate because cyanide would be expected to increase the rate by accelerating the expulsion of water through a trans effect[14] in an I_d mechanism; it would not be expected to increase the rate of addition if there were no ligand in the trans position.

(3) Cyanide catalysis of the isomerization of cyanocobalamin is an example of nucleophilic catalysis that might be regarded as a simple associative displacement mechanism. The reaction is second order, proceeds through an intermediate containing two cyanide molecules, and involves the pushing out of one cyanide molecule by another, as a result of the trans effect. However, the reaction pathway proceeds through a series of steps, which can be explained by a common I_d mechanism to give this net displacement: first the dissociation of DMBz, then hydration, then cyanide addition (as described above) and finally dissociation of the trans cyanide ion and rehydration (Scheme I). The complete pathway at low pH proceeds through the addition of HCN followed by proton loss and N–C isomerization (eq 9); it presumably involves the reverse sequence for cyanide dissociation from the opposite side.

Thus, all of the different types of kinetic behavior described here can be satisfactorily explained by the basic dissociative interchange mechanism of eq 12 and Figure 10A; there may be a small component of nucleophilic participation by the incoming ligand in the transition state. The diagrams of Figure 10–12 illustrate how the mechanisms of dissociative metal–ligand reactions, like those of many carbon compounds, are determined in large part by (1) the lifetime of the unstable intermediate and (2) the presence or absence of a reactive solvent. In the presence of a liganding solvent a sufficiently short lifetime of the intermediate leads to an enforced I_d or preassociation, outer sphere mechanism, whereas in a nonliganding solvent a pure dissociative, D, mechanism is more likely to be followed.

References and Notes

(1) Supported in part by grants from the National Science Foundation (BG-31740) and the National Institutes of Health (GM20888 and 5-T01-GM00212; the latter to W.W.R.).
(2) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. "Purification of Laboratory Chemicals"; Pergamon: New York, 1966.
(3) Gold, V. "pH Measurements"; Methuen: London, 1956; p 119.
(4) Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485–6493.
(5) Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism"; Wiley: New York, 1953.
(6) Firth, R. A.; Hill, H. A. O.; Pratt, J. M.; Thorp, R. B. *J. Chem. Soc. A* **1968**, 453-456.
(7) Friedrich, W. *Z. Naturforsch. B* **1966**, *21*, 595–596.
(8) George, P.; Irvine, D. H.; Glauser, S. C. *Ann. N.Y. Acad. Sci.* **1960**, *88*, 393–415.
(9) Firth, R. A.; Hill, H. A. O.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1968**, 2428–2433.
(10) Hanania, G. I. H.; Irvine, D. H. *J. Chem. Soc.* **1964**, 5694–5697.
(11) Randall, W. C.; Alberty, R. A. *Biochemistry* **1967**, *6*, 1520–1525.
(12) Conn, J. B.; Wartman, T. G. *Science* **1952**, *115*, 72–73.
(13) Pratt, J. M.; Thorp, R. G. *J. Chem. Soc. A* **1966**, 187-191.
(14) Pratt, J. M. "Inorganic Chemistry of Vitamin B_{12}"; Academic Press: New York, 1972.
(15) Davies, M. T.; Mamalis, P.; Petrow, V.; Sturgeon, B. *J. Pharm. Pharmacol.* **1951**, *3*, 420–430.
(16) A small increase in pK_a is expected at higher ionic strength and the observed value agrees well with an estimated pK_a value of 5.0, based on a measured pK_a for benzimidazole of 5.77 at ionic strength 1.8 M, at 30 °C, and a difference of 0.78 unit in the pK_a values of benzimidazole and 1,β-D-ribo-5,6-dimethylbenzimidazole at low ionic strength.[15]
(17) The assumption that the presence of cyanide does not affect the pK_a of the base-off species of benzimidazole is equivalent to the assumption that protonation of benzimidazole in the base-off form does not affect the equilibrium constant for the addition of cyanide.
(18) Dodd, D.; Johnson, M. D. *J. Organometal. Chem.* **1973**, *52*, 45.
(19) Brown, T. L.; Ludwick, L. M.; Stewart, R. S. *J. Am. Chem. Soc.* **1972**, *94*, 384–388.
(20) Hodgkin, D. C.; Pickworth, J.; Robertson, J. H.; Prosen, R. J.; Sparks, R. A.; Trueblood, K. N. *Proc. R. Soc. London, Ser. A* **1959**, *251*, 306–352; Brink-Shoemaker, C.; Cruickshank, D. W. J.; Hodgkin, D. C.; Kamper, M. J.; Pilling, D. *Ibid.* **1964**, *278*, 1–26.
(21) Margerum, D. W.; Simandi, L. I. *Proc. Int. Conf. Coord. Chem. 9th*, **1966**, 371–374.
(22) The rate constant for the removal of a proton by water

$$AH \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} A^- + H^+$$

is $k_a = K_a k_{-a}$. Taking diffusion-controlled rate constants of 10^{10} M^{-1} s^{-1} for k_{-a} and for the rate constant for hydroxide-catalyzed proton removal, k_{OH}, the rate constants for removal of a proton by water and by hydroxide ion are equal at pH 2 for an acid of $pK_a = 12$ and at higher pH values for acids of $pK_a < 12$. Since the pK_a of the cyanide addition compound is expected to be less than that of HCN (the pK_a of imidazole is decreased by 2.2 units on binding to cobalamin[10]), this is inconsistent with the observed catalysis by hydroxide ion at low pH values (Figure 7).
(23) Thusius, D. *Chem. Commun.* **1968**, 1183–1184.
(24) Casanova, J., Jr.; Werner, N. D.; Schuster, R. E. *J. Org. Chem.* **1966**, *31*, 3473–3482.
(25) Nome, F.; Fendler, J. H. *J. Chem. Soc., Dalton Trans.* **1976**, 1212–1223.
(26) Brown, K. L.; Kallen, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 1894–1901.
(27) Tauzher, G.; Dreos, R.; Costa, G.; Green, M. *Chem. Commun.* **1973**, 413–414.
(28) Thusius, D. *J. Am. Chem. Soc.* **1971**, *93*, 2629–2635.
(29) Schrauzer, G. N.; Sibert, J. W. *J. Am. Chem. Soc.* **1970**, *92*, 1022-1030; Rudakova, I. P.; Pospelova, T. A.; Borodulina-Shvets, V. I.; Kurganov, B. I.; Yurkevich, A. M. *J. Organomet. Chem.* **1973**, *61*, 389–400.
(30) The rate constant for protonation of NCB_{12} in 0.89 M hydrochloric acid was found to be >0.36 s^{-1}. Cyanocobalamin is 68% in the protonated form, NCB_{12}-NH^+, under these conditions, which gives $k_1 > 0.27$ M^{-1} s^{-1} from $k_1[H^+] + k_{-1} > 0.36$ s^{-1} and $k_1/k_{-1} = 2.4$ M.
(31) DeSimone, R. E.; Penley, M. W.; Charbonneau, L.; Smith, S. G.; Wood, J. M.; Hill, H. A. O.; Pratt, J. M.; Ridsdale, S.; Williams, R. J. P. *Biochim. Biophys. Acta* **1973**, *304*, 851-863; Robinson, G. C.; Nome, F.; Fendler, J. H. *J. Am. Chem. Soc.* **1977**, *99*, 4969–4976.
(32) Milton, P. A.; Brown, T. L. *J. Am. Chem. Soc.* **1977**, *99*, 1390–1396.
(33) Reference 14, p 164.
(34) Phillips, J. N. *Aust. J. Chem.* **1961**, *14*, 183–189.
(35) Paul, M. A.; Long, F. A. *Chem. Rev.* **1957**, *57*, 1–45.
(36) Hine, J. *J. Am. Chem. Soc.* **1972**, *94*, 5766–5771.
(37) Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1978**, *100*, 1228–1235.
(38) Hogenkamp, H. P. C.; Vergamini, P. J.; Matwiyoff, N. A. *J. Chem. Soc., Dalton Trans.* **1975**, 2628–2633; Cockle, S. A.; Hensens, O. D.; Hill, H. A. O.; Williams, R. J. P. *Ibid.*, 2633–2634; Brown, K. L.: Awtrey, A. W. *Inorg. Chem.* **1978**, *17*, 111–119.
(39) Eigen, M.; Tamm, K. *Z. Elecktrochem.* **1962**, *66*, 107–121; Langford, C. H.; Gray, H. B. "Ligand Substitution Processes"; Benjamin: New York, 1965; Wilkins, R. G.; Eigen, M. *Adv. Chem. Ser.* **1965**, *49*, 55–67; Basolo, F.; Pearson, R. G. "Mechanisms of Inorganic Reactions", 2nd ed.; Wiley: New York, 1967; Crumbliss, A. L.; Wilmarth, W. K. *J. Am. Chem. Soc.* **1970**, *92*, 2593–2594; Espenson, J. H.; Russell, R. *Inorg. Chem.* **1974**, *13*, 7–11; Swaddle, T. W. *Coord. Chem. Rev.* **1974**, *14*, 217–268; Margerum, D. W.; Cayley, G. R.; Weatherburn, D. C.; Pagenkopf, G. K. *ACS Monogr.* **1978**, *No. 174*, 1–220.
(40) Poon, C. K. *Coord. Chem. Rev.* **1973**, *10*, 1–35.
(41) Langford, C. H. *Inorg. Chem.* **1965**, *4*, 265–266; Sutin, N. *Annu. Rev. Phys. Chem.* **1966**, *17*, 119–133.
(42) Jencks, W. P. *Acc. Chem. Res.* **1976**, *9*, 425–432; Young, P. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 8238–8248; Thibblin, A.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 4963–4973.
(43) Haim, A.; Wilmarth, W. K. *Inorg. Chem.* **1962**, *1*, 573–582, 583–586.
(44) Fleischer, E. B.; Jacobs, S.; Mestichelli, L. *J. Am. Chem. Soc.* **1968**, *90*, 2527–2531.
(45) Edwards, J. O. "Inorganic Reaction Mechanisms"; Benjamin: New York, 1964; p 100.
(46) More O'Ferrall, R. A. *J. Chem. Soc. B* **1970**, 274–277; Jencks, W. P. *Chem. Rev.* **1972**, *72*, 705–718; Jencks, D. A.; Jencks, W. P. *Ibid.* **1977**, *99*, 7948–7960.

(47) Guschl, R. J.; Stewart, R. S.; Brown, T. L. *Inorg. Chem.* **1974**, *13*, 417–422.
(48) Stewart, R. C.; Marzilli, L. G. *J. Am. Chem. Soc.* **1978**, *100*, 817-822.
(49) Pearson, R. G.; Moore, J. W. *Inorg. Chem.* **1964**, *3*, 1334–1336; Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry"; 3rd ed.; Interscience: New York, 1972; p 657.
(50) NOTE ADDED IN PROOF. It has recently been reported (de Tacconi, N. R.; Lexa, D.; Saveant, J. M. *J. Am. Chem. Soc.* **1979**, *101*, 467–473) that dissociation of the dimethylbenzimidazole group of aquocobalamin occurs with a pH-independent rate constant of $\sim 0.1\ s^{-1}$ at low pH values. Further experimental work is required to explain the apparent difference in the behavior of aquocobalamin and cyanocobalamin and the unexpectedly small difference between the pH-independent rate constants of $0.1\ s^{-1}$ for aquocobalamin and $0.042\ s^{-1}$ for cyanocobalamin; conclusions regarding the mechanism of DMBz dissociation must be regarded with reservation until these differences are explained.

Heat of Formation for *tert*-Butyl Cation in the Gas Phase

R. G. McLoughlin and J. C. Traeger*

Contribution from the Department of Physical Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia. *Received February 24, 1979*

Abstract: The appearance energies of $C_4H_9^+$ ions formed from isobutane and the *tert*-butyl halides have been measured by photoionization mass spectrometry. A heat of formation of 162.1 ± 0.8 kcal mol^{-1} has been derived for the *tert*-butyl cation in the gas phase. This result is discussed in terms of its use as a gas-phase proton affinity standard. A value of 7.6 ± 1.5 kcal mol^{-1} has been obtained for the *tert*-butyl radical heat of formation.

Introduction

In a recent photoelectron spectroscopic study of alkyl radicals,[1] it was proposed that the heat of formation for the *tert*-butyl cation is 162.9 kcal mol^{-1}, considerably less than the currently accepted value.[2] Since the proton affinity (PA) of isobutene is used as a reference for determining absolute proton affinities,[3-5] it is important that this standard, which is derived from $\Delta H_f((CH_3)_3C^+)$, be firmly established.

Previous estimates of the heat of formation of *tert*-butyl cation[2,3] have been derived from an ionization energy of the radical[6] which has since been shown[1,7] to be a vertical rather than an adiabatic value. In addition, the *tert*-butyl radical heat of formation used in the thermochemical calculation is not accurately known, with values ranging from 6.8 to 12.9 kcal mol^{-1}.[8-11] Houle and Beauchamp[1] have chosen Benson's value of 8.4 kcal mol^{-1} [9] which they support with experimental ionic equilibria data for benzyl cation reacting with *tert*-butyl chloride and *tert*-butyl bromide (loc. cit. ref 1). However, the combined uncertainty of $\geq \pm 3$ kcal mol^{-1} in the auxiliary thermochemical data used in the calculations does not satisfactorily resolve the question surrounding the heat of formation for *tert*-butyl radical. Moreover, the recent experiments of Rossi and Golden,[12] which propose an increase of 2 kcal mol^{-1} in the heat of formation for benzyl radical, and hence benzyl cation, cast further doubt on the *tert*-butyl radical heat of formation recommended by Houle and Beauchamp.[1] Ausloos and Lias[4,13] recognized the uncertainty in this direct method of calculating $\Delta H_f((CH_3)_3C^+)$ and preferred to base their PA calculations on a value of 168.2 kcal mol^{-1} derived from an appearance energy measurement for $C_4H_9^+$ from neopentane.[14]

In an attempt to clarify this situation, we have calculated a heat of formation for *tert*-butyl cation in the gas phase from several $C_4H_9^+$ appearance energy measurements using a photoionization mass spectrometer. Previous experience has shown that we are able to determine accurate heats of formation for ionic species,[15-18] provided that reliable supplementary thermochemical data are available. The series of compounds studied in the present work ($(CH_3)_3X$; X = Cl, Br, I, H) all have well-characterized heats of formation[19] as do the neutral species[20] ejected in the ionization-fragmentation process.

Experimental Section

The photoionization mass spectrometer has been described in detail elsewhere.[21] The photon source used in the present studies was the molecular hydrogen pseudocontinuum with an energy dispersion of 1.25 Å FWHM. All experiments were performed at ambient temperature (296 K). The compounds used were of high purity and showed no impurities of significance in their mass spectra.

Results and Discussion

The photoion yield curves corresponding to formation of $(CH_3)_3C^+$ from the four precursor molecules studied are shown in Figure 1, with the ion appearance energies, heats of formation, and auxiliary thermochemical data being summarized in Table I. The observed $C_4H_9^+$ ions may be inferred to have the *tert*-butyl cation structure as the calculated ionic heats of formation are all too low to be equated with other isomeric structures.[6]

For *tert*-butyl chloride and *tert*-butyl bromide, where no parent ions are observed, the ionization energy and corresponding $C_4H_9^+$ appearance energy are the same within experimental error. Because the fragmentation processes occur subsequent to initial ionization of the neutral precursor, the calculated $C_4H_9^+$ heats of formation will only represent an upper limit to $\Delta H_f((CH_3)_3C^+)$. This is also the case for *tert*-butyl iodide, even though there is a molecular ion observed in the mass spectrometer. The heat of formation calculated from the isobutane results should be representative of $\Delta H_f((CH_3)_3C^+)$ since the ionization energy is less than the appearance energy. Any excess energy involved in the process is expected to be negligible because of the small kinetic energy release (~ 0.007 eV)[22] associated with the corresponding metastable fragmentation.

As a consequence of the rapid fragmentation process following ionization of the *tert*-butyl halides, it is expected that there will be no observable kinetic shift. This is supported by the well-defined thresholds of the three photoion yield curves (Figure 1). The low-energy tail of each curve is consistent with the extent of expected hot band structure, i.e., ionization and subsequent fragmentation of thermally excited neutral precursor molecules. Following the theoretical studies of Chupka[23] and Guyon and Berkowitz,[24] a linear extrapolation of the ob-

0002-7863/79/1501-5791$01.00/0