# Coenzyme $B_{12}$ Model Studies. Equilibria and Kinetics of Axial Ligation of Methylaquocobaloxime by Primary Amines and 4-Substituted Pyridines[1,2]

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*Received November 8, 1971*

**Abstract:** The ranges of equilibrium constants for the axial ligation reactions of methylaquocobaloxime at 25°, ionic strength 1.0 *M*, for a series of thiolate anions, 4-substituted pyridines, and aliphatic amines are $1\text{–}3 \times 10^5$, $0.03\text{–}1.1 \times 10^4$, and $0.05\text{–}4.3 \times 10^3$ $M^{-1}$, respectively. The dependence of the ligation equilibria upon ligand basicity within each series varies in the reverse order with $\beta$ values of 0.18, 0.21, and 0.38, respectively. The ranges of rate constants for axial ligation to methylaquocobaloxime are 4.3–7.5, 12.8–27.3, 49.6–55.2, and 92–200 $M^{-1}$ $\text{sec}^{-1}$ for $RNH_2$, $RS^-$, RSH, and X-py, respectively, and the dependence of these rate constants upon ligand basicity provides $\beta$ values less than 0.1 within each series. The kinetic studies are consistent with a mechanism for these ligation reactions which is SN1 in nature. These results support significant contributions of metal-to-ligand $\pi$ bonding to the stabilization of transition states and products in the thiol and pyridine series. From studies of the ligation reactions at high pH values, rate and equilibrium constants for ligation to anionic methylaquocobaloxime have been measured as well as the values for the constants for proton dissociation from the planar ligand system for the complexes, $Me(D_2H_2)L$ (L = $RNH_2$, X-py). It is proposed that considerations regarding the assignment for the site of proton dissociation from $Me(D_2H_2)HOH$ ($pK' = 12.68$) include the equatorial hydrogen-bonded proton.

The ligand substitution reactions of vitamin $B_{12}$, its derivatives,[4–8] and $B_{12}$ model compounds, the cobaloximes,[9,10] are of interest from the points of view of the mechanisms of inorganic ligand substitution reactions and the possibility that such reactions may also play an important role in coenzyme $B_{12}$ catalyzed reactions. Thus, despite some recent studies which have indicated that changes in the state of axial ligation of cobalamins may not occur during catalysis by some $B_{12}$-dependent enzymes,[11,12] other work has suggested that such ligation changes may indeed be important in the enzymatic reactions involving $B_{12}$.[13–17] We wish to report an extension of our earlier studies on the ligation reaction of thiols with methylaquocobaloxime[10] to a consideration of two series of nitrogen ligands: primary amines and 4-substituted pyridines (eq 1). This work has considered series of ligands with

$$\mathrm{Me(D_2H_2)HOH} + \mathrm{L} \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\rightleftarrows}} \mathrm{Me(D_2H_2)L} + \mathrm{HOH} \qquad (1)$$

biologically relevant functional groups in an attempt to evaluate the extent of metal-to-ligand $\pi$ donation in the metal–ligand bond and as a basis for studies of the kinetic trans effect in alkyl cobalt complexes to be reported separately. Equilibrium constants for the ligation of some amines to methylcobinamide have been reported by others.[13]

## Experimental Section

**Materials.** The synthesis of methylaquocobaloxime and the handling of solutions thereof have been described elsewhere.[10]

The free base primary amines, 4-substituted pyridines (py and Me-py), and ME were obtained commercially, redistilled, and stored in the dark under nitrogen at 4°. The hydrochloride of TFEA and the free bases of CN-py, CA-py, and A-py were recrystallized from the appropriate solvent systems and dried in air. Buffer components and inorganic salts were reagent grade and were used without further purification.

Solutions of amines and ME were prepared daily and handled as described elsewhere for ME.[10] Stock solutions of $Me(D_2H_2)HOH$ in methanol were used for several successive days and shown to be stable when protected from exposure to light by the criterion of spectral stability. Deionized water of greater than $5 \times 10^5$ ohms cm specific resistance was used throughout.

**Methods.** Proton dissociation constants ($pK_L'$) for ligands were determined potentiometrically or spectrophotometrically (CN-py, 275 nm) at 25°, ionic strength 1.0 *M*, maintained with KCl, and titration data were analyzed by a nonlinear least-squares method.[18]

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[1] This project was supported by the National Institutes of Health, U. S. Public Health Service, Grants GM 13,777 (R. G. K.), FR 15 (University of Pennsylvania Medical School Computer Facility), and FR 05415 (University of Pennsylvania School of Medicine).

[2] Abbreviations: A-py, 4-aminopyridine; CA-py, 4-carboxamidopyridine (isonicotinamide); CN-py, 4-cyanopyridine; DEA, 2,2-dimethoxyethylamine; ME, 2-mercaptoethanol; MEA, 2-methoxyethylamine; $Me(D_2H_2)HOH$, methylaquocobaloxime; Me-py, 4-methylpyridine (4-picoline); MPA, 3-methoxypropylamine; PA, propylamine; py, pyridine; TFEA, 2,2,2-trifluoroethylamine.

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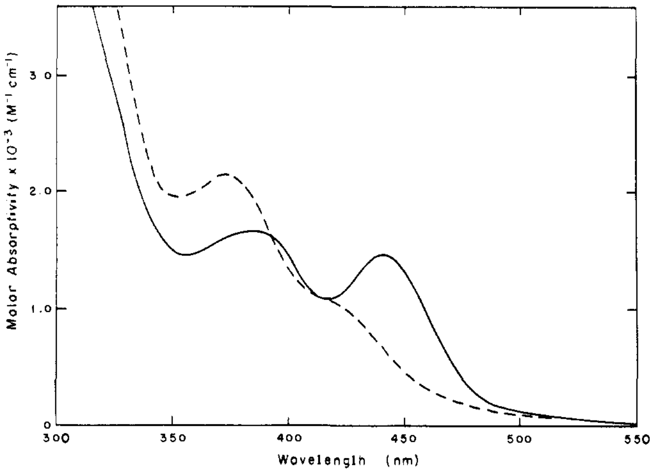

Figure 1. Spectra of $Me(D_2H_2)$ HOH (————) and $Me(D_2H_2)$-CA-py (--------). [Complex] = $3.33 \times 10^{-4}$ *M*, [CA-py] = 0.02 *M*, 0.1 *M* carbonate buffer, 35% free base, pH 9.65, ionic strength 1.0 *M*. Product formation was 95% complete.

Measurements of pH and recordings of uv and visible spectra were made as described elsewhere.[10]

The kinetic and thermodynamic constants for the ligation reactions of neutral and anionic methylaquocobaloxime (Scheme I)

**Scheme I**

$$\begin{tikzcd}
Me(D_2H_2)HOH \arrow[r, shift left, "K'_{c_2}"] \arrow[d, shift right, "\substack{k_{on}\\ +L}"'] & Me(D_2H)HOH^- + H^+ \arrow[l, shift left] \arrow[d, shift right, "\substack{k'_{on}\\ +L}"'] \\
Me(D_2H_2)L \arrow[r, shift left, "K'_{c-L}"] \arrow[u, shift right, "\substack{k_{off}\\ -L}"'] & Me(D_2H)L^- + H^+ \arrow[l, shift left] \arrow[u, shift right, "\substack{k'_{off}\\ -L}"']
\end{tikzcd}$$

$$k_{on}/k_{off} = K_f \qquad K'_f = k'_{on}/k'_{off}$$

were determined by the methods described below at ionic strength 1.0 *M* maintained with KCl.

**Method I. Spectrophotometric Determination of the Equilibrium Constant for Ligation, $K_f$.** The apparent equilibrium constants, $K_f^{app}$, for the formation of methylcobaloxime adducts from $Me(D_2H_2)HOH$ and ligand, L (eq 2), where $[L]_{free}$ is the equilibrium-

$$K_f^{app} = [Me(D_2H_2)L]/[Me(D_2H_2)HOH][L]_{free} \quad (2)$$

ligand concentration distributed over both ionization states, were determined by spectrophotometric measurements of solutions of $Me(D_2H_2)HOH$ (generally about $5 \times 10^{-4}$ *M*) at varying ligand concentrations at 440–445 nm (Figure 1). The data *at a given pH* were analyzed with a computer by a nonlinear least-squares program based upon eq 3,[18] where $A_0$ is the absorbance of $Me(D_2H_2)$-

$$A = A_0 - (\Delta\epsilon[Me(D_2H_2)HOH]_0[L]_{free})/([L]_{free} + 1/K_f^{app}) \quad (3)$$

HOH in the absence of added ligand, $\Delta\epsilon$ is the difference between the molar absorptivities of $Me(D_2H_2)HOH$ and $Me(D_2H_2)L$ at the wavelength used, and $[Me(D_2H_2)HOH]_0$ is the total concentration of cobalt-containing species. The $K_f^{app}$ values were determined at pH 11.07 for A-py and pH 9.5 for the remaining substituted pyridines; at these pH values the ligand was $\geqslant 98\%$ as the free base and $Me(D_2H_2)HOH$ was $<2\%$ ionized, and $K_f^{app} = K_f$ (Scheme I). A similar condition exists for TFEA at pH 8.5 but for the other primary amines determinations of $K_f^{app}$ were required at two or more pH values at which the ionization of the amines was incomplete (both $Me(D_2H_2)HOH$ and $Me(D_2H_2)L$ were $<2\%$ ionized). The values of the equilibrium constants for ligation with respect to free base amine were calculated from the relation $K_f = K_f^{app}/\alpha$ (where $\alpha$ is the fraction of total amine as the free base) and the values of $K_f$ thus obtained agreed to within 25% at worst and usually to within 10%.

**Method II. Direct Determination of Ligation Rates ($k_{on}$).** For the primary amines, the second-order rate constant for ligation to $Me(D_2H_2)HOH$ ($k_{on}$, Scheme I) was determined from absorbance measurements at 445 nm (Figure 1) obtained under pseudo-first-order conditions with ligand concentration in at least a tenfold excess over $Me(D_2H_2)HOH$ concentration. The pH was maintained with 0.1 *M* borate or potassium phosphate buffers in the same range as in method I. Reactions were initiated by adding a small volume (0.1–0.2 ml) of $Me(D_2H_2)HOH$ solution in methanol to cuvettes containing buffer, KCl, and ligand in the thermostated cell compartment of a Coleman-Hitachi Model 124 double-beam spectrophotometer maintained at 25.0 ± 0.1° (final reaction mixtures were 1.67% methanol in 3.0-ml total volume). The first-order rate constants ($k_{obsd}$) were obtained by the method previously described.[19] Second-order rate constants, $k_{on}^{app}$, for ligation were obtained from the slopes of graphs of $k_{obsd}$ *vs.* ligand concentration determined by the least-squares method (eq 4). The significant ordinate intercepts on such plots

$$k_{obsd} = k_{on}^{app}[L]_{total} + k_{off} \quad (4)$$

($k_{off}$), which were generally too small to be accurately determined, indicate incomplete conversion of reactants to products. The values for $k_{on}$ at two or more pH values were calculated from $k_{on} = k_{on}^{app}/\alpha$, where $\alpha$ is the fraction of total amine as the free base, and the agreement among $k_{on}$ values was within 15% at worst and usually better than 5%. The ligation rate constant, $k_{on}$, for TFEA was calculated from $K_f$ (method I) and $k_{off}$ (method IV, below), since recordings of absorbance *vs.* time for this amine deviated from first order for reasons which are not understood at present.

**Method III. Reaction of $Me(D_2H_2)L$ with ME (Determination of $k_{on}$ and $k_{off}$ for the Reaction of $Me(D_2H_2)HOH$ with L).** An alternative method for the determination of $k_{on}$ and $k_{off}$ for the substituted pyridines involved the reaction of $Me(D_2H_2)L$ with ME (eq 5a and 5b). $Me(D_2H_2)L$ was generated by dissolving sufficient $Me(D_2H_2)$-

$$Me(D_2H_2)L \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} Me(D_2H_2)HOH + L \quad (5a)$$

$$Me(D_2H_2)HOH + ME \underset{k_{-ME}}{\overset{k_{ME}}{\rightleftharpoons}} Me(D_2H_2)ME \quad (5b)$$

HOH and L in methanol to give >70% $Me(D_2H_2)L$ formation as calculated from the $K_f$ values (Table II). Reaction solutions contained buffer, EDTA[10] ($10^{-4}$ *M*), KCl, ligand (at a concentration at least tenfold > the final concentration of cobaloxime and sufficiently high to maintain >70% of the complex as the liganded species in the *absence* of ME), and various concentrations of ME (at least tenfold in excess over the final complex concentration and sufficiently high for >90% conversion of cobaloxime species to $Me(D_2H_2)ME$ at equilibrium).[10] Reactions were initiated by the addition of a 20–30-μl aliquot of stock solution of $Me(D_2H_2)L$ (final concentration of cobalt-containing species 5.0–6.67 × $10^{-5}$ *M*) to cuvettes containing the reaction mixtures (3.0 ml) situated within the spectrophotometer. Reaction progress was monitored by measuring the increase in absorbance at 325 nm due to the formation of $Me(D_2H_2)ME$.[10] The dependence of the observed pseudo-first-order rate constants, $k_{obsd}$, upon the concentrations of ME and L is given by the rate law of eq 6, obtained from eq 5a and

$$k_{obsd} = \frac{k_{off}[ME] + (k_{on}k_{-ME}/k_{ME})[L]}{[ME] + (k_{on}/k_{ME})[L]} \quad (6)$$

5b by application of the steady-state assumption to the concentration of $Me(D_2H_2)HOH$ when the concentrations of L and ME are in tenfold or greater excess over cobaloxime concentration. The scheme and rate law are similar to those considered by Haim, *et al.*,[20] for the reactions of $Co^{III}(CN)_5HOH^{2-}$ with various ligands. A plot of the $k_{obsd}$ values *vs.* ME concentration at a constant L concentration describes a rectangular hyperbola (Figure 2); the data were analyzed with a computer program[18] using a nonlinear least-squares method based upon eq 6 to obtain the parameters $k_{off}$ and $\{(k_{on}/k_{ME})[L]\}$ (the parameter $\{(k_{on}k_{-ME}/k_{ME})[L]\}$ was always too small to be distinguished from zero in the pH region of the present experiments (*cf.* ref 10)). The values for $k_{ME}$ can be calculated at any pH from the values for $k_A$, $k_B$, and $K_t'$[10] and eq 7, where $k_A$ and $k_B$ are the rate constants for ligation of

$$k_{ME} = k_A(1 - \alpha_t) + k_B\alpha_t \quad (7)$$

thiol and thiolate anion to $Me(D_2H_2)HOH$, respectively, $\alpha_t$ is the fraction of total ME as the free base, and $K_t'$ is the proton dissocia-

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Table I. Equilibrium and Kinetic Constants[a] for the Axial Ligation of Primary Amines to $Me(D_2H_2)HOH$, 25°, Ionic Strength 1.0 *M*

| Constant | Primary amine | | | | |
|---|---|---|---|---|---|
| | Trifluoroethylamine | Dimethoxyethylamine | Methoxyethylamine | Methoxypropylamine | Propylamine |
| $pK'_L$ | 5.68 | 8.72 | 9.68 | 10.33 | 10.80 |
| $K_f$ $(M^{-1})$[b] | 51.9 | $7.89 \times 10^2$ | $1.74 \times 10^3$ | $3.02 \times 10^3$ | $4.25 \times 10^3$ |
| $k_{on}$ $(M^{-1}\ sec^{-1})$[c] | 4.34[d] | 4.79 | 5.22 | 5.53 | 7.48 |
| $k_{off}$ $(sec^{-1})$[e] | $8.37 \times 10^{-2}$ | $5.91 \times 10^{-3}$ | $3.44 \times 10^{-3}$ | $2.08 \times 10^{-3}$ | $2.07 \times 10^{-3}$ |
| $K'_f$ $(M^{-1})$[f] | 4.22 | $2.39 \times 10^1$ | $5.31 \times 10^1$ | $1.20 \times 10^2$ | $1.51 \times 10^2$ |
| $k'_{on}$ $(M^{-1}\ sec^{-1})$[f] | 2.16 | 2.04 | 3.22 | 4.26 | 6.36 |
| $k'_{off}$ $(sec^{-1})$[e] | $5.13 \times 10^{-1}$ | $8.52 \times 10^{-2}$ | $6.06 \times 10^{-2}$ | $3.55 \times 10^{-2}$ | $4.21 \times 10^{-2}$ |
| $pK'_{c-L}$[e] | 13.77 | 14.20 | 14.21 | 14.08 | 14.13 |

[a] See Scheme I for definitions of the constants. [b] Average of two or more values determined by method I and one value calculated from the average value for $k_{on}$ (method II) and $k_{off}$ (method IV) and $K_f = k_{on}/k_{off}$. [c] Average of two or more values determined by method II. [d] This value was not determined directly but was calculated from $k_{off}$ and $K_f$. See Experimental Section. [e] Obtained from method IV. [f] Calculated by method V.

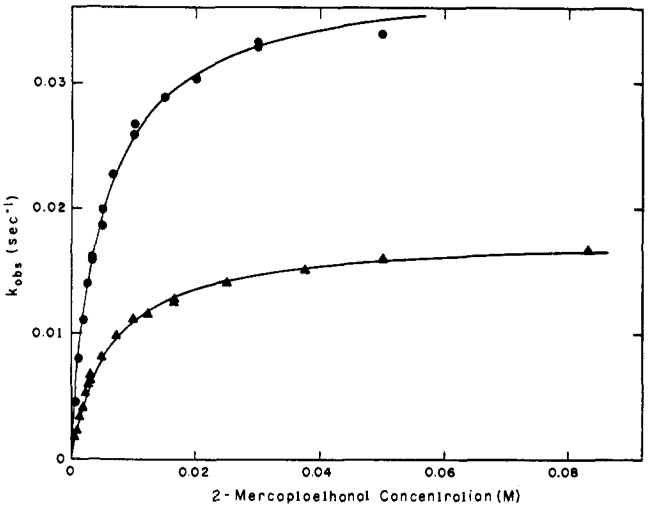

Figure 2. Reactions of methylaquocobaloxime with ligands: determination of $k_{on}$ and $k_{off}$ *via* reaction of $Me(D_2H_2)L$ with ME (method III), 25°, ionic strength 1.0 *M*. [Complex] = $6.67 \times 10^{-5}$ *M*. (●), $Me(D_2H_2)$Me-py + ME, pH 9.55 in 0.1 *M* carbonate buffer, 35% free base, [Me-py] = $1.50 \times 10^{-3}$ *M*, $k_{off}$ = 0.0384 sec$^{-1}$, $\{(k_{on}/k_{ME})\,[\text{Me-py}]\} = 4.95 \times 10^{-3}$ *M*; (▲), $Me(D_2H_2)$A-py + ME, pH 11.05 in 0.1 *M* phosphate buffer, 29.4% free base, [A-py] = $1.0 \times 10^{-3}$ *M*, $k_{off}$ = 0.0178 sec$^{-1}$, $\{(k_{on}/k_{ME})[\text{A-py}])\} = 6.36 \times 10^{-3}$ *M*. The *solid lines* were calculated from the above parameters obtained by a least-squares method and eq 6.

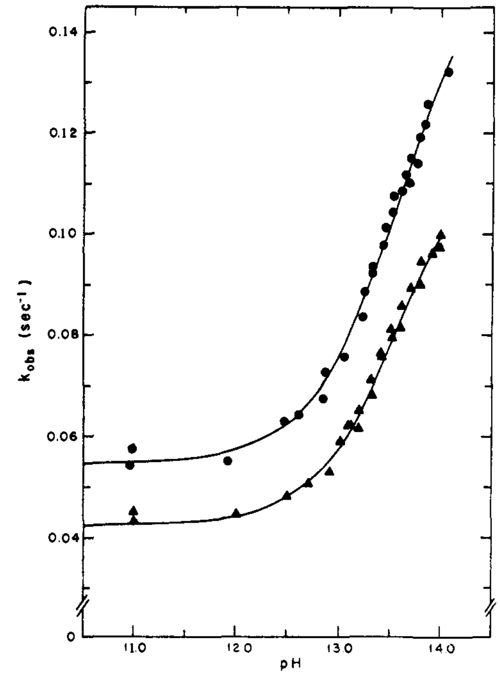

Figure 3. Proton dissociation constants for $Me(D_2H_2)L$: determination of $pK'_{c-L}$ by the pH dependence of $Me(D_2H_2)L$ ligand dissociation rates (method IV), 25°, ionic strength 1.0 *M*. (●), $Me(D_2H_2)$py ligand dissociation, final [complex] = $6.67 \times 10^{-5}$ *M*, $pK'_{c-L}$ = 13.61, $k_{off}$ = 0.0546 sec$^{-1}$, $k'_{off}$ = 0.163 sec$^{-1}$; (▲), $Me(D_2H_2)$Me-py ligand dissociation, final [complex] = $5.00 \times 10^{-5}$ *M*, $pK'_{c-L}$ = 13.65, $k_{off}$ = 0.0424 sec$^{-1}$, $k'_{off}$ = 0.126 sec$^{-1}$. The *solid lines* were calculated using the above parameters obtained by least-squares methods and eq 8.

tion constant for ME. The experiment allows calculation of $k_{on}$, $k_{off}$, and hence $K_f$ for the reaction of the ligand with $Me(D_2H_2)HOH$ (Scheme I). These experiments were performed at pH values at which the pyridines were >98% as the free base and $Me(D_2H_2)HOH$ was <1% ionized.

**Method IV. pH Dependence of the Ligand Dissociation Rates from $Me(D_2H_2)L$ (Determination of $k_{off}$, $k'_{off}$, and $pK'_{c-L}$).** The values for the proton dissociation constants for $Me(D_2H_2)L$, $pK'_{c-L}$ (Scheme I), were obtained from the pH dependence of the ligand dissociation rates of $Me(D_2H_2)L$ at various high pH values. $Me(D_2H_2)L$ was prepared as for method III by dissolving sufficient $Me(D_2H_2)HOH$ (0.004–0.02 *M*) and ligand (0.004–0.04 *M*) in methanol such that, upon dilution to 5.0–6.7 × $10^{-5}$ *M* cobalt-containing species, the breakdown reaction would proceed >90% to completion, as calculated from values of $K_f$, in order to make the contribution of $k_{on}^{app}[L]$ insignificant (eq 4). The appropriate wavelength for following the breakdown reaction was determined from the difference in the uv spectra of a solution of $1.0 \times 10^{-3}$ *M* $Me(D_2H_2)HOH$ with $1.0 \times 10^{-3}$ *M* ligand at 0.05-cm path length *prior to* and at 1.0-cm path length *following* 20-fold dilution into the appropriate buffer–KCl mixtures. The wavelengths for the studies of complexes with the substituted pyridines and primary aliphatic amines were 270 and 290 nm, respectively. The reactions were initiated and studies conducted as in methods II and III. Plots of $k_{obsd}$ *vs.* pH (Figure 3) follow *sigmoid* curves and although complete titration of the liganded complex, $Me(D_2H_2)L$, with alkali could not be attained due to the high values of $pK'_{c-L}$ (>13.5), the data were fit to eq 8 with a nonlinear least-squares computer

$$k_{obsd} = k_{off} + (k'_{off} - k_{off})\{1/(1 + a_{H^+}/K'_{c-L})\} \quad (8)$$

program[18] to obtain values for $K'_{c-L}$, $k_{off}$, and $k'_{off}$ (Scheme I). Such experiments could not be performed for CA-py or CN-py due to the large values of $k_{off}$ and the small difference between $k_{off}$ and $k'_{off}$ for these ligands, although an approximate value and certainly a lower limit for the value of $k'_{off}$ for CN-py was determined from the breakdown rate of $Me(D_2H_2)$CN-py at pH 13.98. The large $K_f$ value for formation of $Me(D_2H_2)$A-py required dilution of this complex to $8.0 \times 10^{-6}$ *M* in order to apply method IV and absorbance measurements were obtained with a Cary Model 14 recording spectrophotometer and 10-cm path length cells. For A-py the large $K_f$ value made it possible to determine the $pK'_{c-L}$ value from a direct spectrophotometric titration of the liganded complex at sufficiently high concentration of A-py (0.1 *M*) that the fraction of methylcobaloxime as the liganded species was >98% even in strong alkali. The values of $k_{off}$ for the primary amines in conjunction with the values of $k_{on}$ (method II) allowed the calculation of another value for $K_f$ (Scheme I) for each amine.

Table II. Equilibrium and Kinetic Constants[a] for the Axial Ligation of 4-Substituted Pyridines to $Me(D_2H_2)HOH$, 25°, Ionic Strength 1.0 *M*

| Constant | NC– | $H_2NCO$– | H– | $CH_3$– | $H_2N$- |
|---|---|---|---|---|---|
| | | | 4-Substituted pyridine | | |
| $pK'_L$ | 2.24 | 3.77 | 5.51 | 6.36 | 9.40 |
| $K_f$ $(M^{-1})$[b] | $3.29 \times 10^2$ | $8.03 \times 10^2$ | $2.04 \times 10^3$ | $3.25 \times 10^3$ | $1.11 \times 10^4$ |
| $k_{on}$ $(M^{-1}\ sec^{-1})$[c] | $9.21 \times 10^1$ | $1.17 \times 10^2$ | $1.14 \times 10^2$ | $1.28 \times 10^2$ | $1.96 \times 10^2$ |
| $k_{off}$ $(sec^{-1})$[d] | $2.91 \times 10^{-1}$ | $1.56 \times 10^{-1}$ | $5.52 \times 10^{-2}$ | $4.04 \times 10^{-2}$ | $1.94 \times 10^{-2}$ |
| $K'_f$ $(M^{-1})$[e] | | | $2.39 \times 10^2$ | $3.54 \times 10^2$ | $4.63 \times 10^2$ |
| $k'_{on}$ $(M^{-1}\ sec^{-1})$[e] | | | $3.90 \times 10^1$ | $4.46 \times 10^1$ | $5.83 \times 10^1$ |
| $k'_{off}$ $(sec^{-1})$[f] | $>4.78 \times 10^{-1}$[g] | | $1.63 \times 10^{-1}$ | $1.26 \times 10^{-1}$ | $1.26 \times 10^{-1}$ |
| $pK'_{c-L}$[f] | | | 13.61 | 13.65 | 14.06 |

[a] See Scheme I for definitions of the constants. [b] Average of one value determined by method I and one value calculated from $k_{on}$ and $k_{off}$ obtained in a single experiment by method III. [c] Average of one value determined by method III and one value calculated from the average value for $K_f$, the average value of $k_{off}$, and $k_{on} = k_{off}K_f$. [d] Average of one value determined by method III and one value determined by method IV. [e] Calculated by method V. [f] Obtained by method IV. [g] An approximate value and certainly a lower limit for $k'_{off}$ from measurements of the rate of dissociation of CN-py from $Me(D_2H_2)CN$-py at pH 13.98.

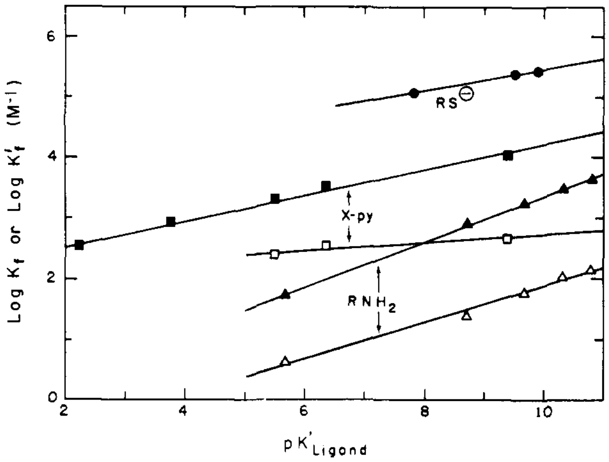

Figure 4. Dependence of equilibrium constants for ligation with neutral (solid symbols) and anionic (open symbols) methylaquocobaloxime upon the proton dissociation constant ($pK'_L$) for the conjugate acid of the ligand, 25°, ionic strength 1.0 *M*. (●), $RS^-$, slope = 0.18 ± 0.01; (■, □), X-py, slope = 0.21 ± 0.01 for $K_f$, slope = 0.06 ± 0.03 for $K'_f$; (▲, △), $RNH_2$, slope = 0.38 ± 0.01 for $K_f$, slope = 0.31 ± 0.03 for $K'_f$. The *solid lines* were calculated by a least-squares method.

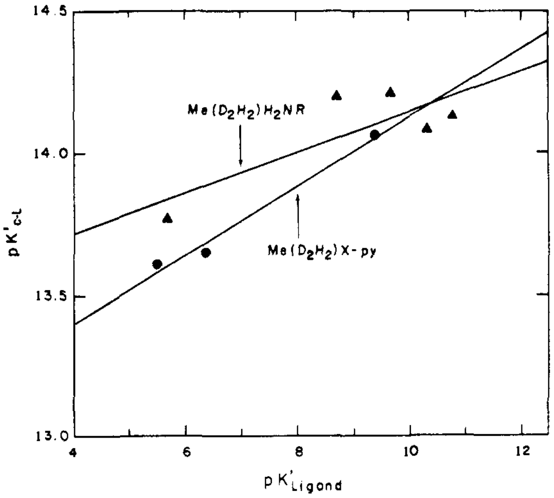

Figure 5. Dependence of proton dissociation constants for $Me(D_2H_2)L$ upon the proton dissociation constant ($pK'_L$) of the conjugate acid of the ligand, 25°, ionic strength 1.0 *M*. (●), $Me(D_2H_2)H_2NR$, slope = 0.07 ± 0.03; (▲) $Me(D_2H_2)$py-X, slope = 0.12 ± 0.02. The *solid lines* were calculated by a least-squares method.

**Method V. Calculation of $K'_f$ and $k'_{on}$.** The $K'_f$ values were calculated from the relation $K'_f = K'_{c-L}K_f/K'_{c_2}$ based on the cyclic nature of the equilibria (Scheme I), the data in Tables I and II, and the value $pK'_{c_2} = 12.68$.[10] Values for $k'_{on}$ were calculated from the values of $k'_{off}$ determined by method IV and $k'_{on} = k'_{off}K'_f$.

## Results

**Equilibrium Constants. Ligation Reactions of $Me(D_2H_2)HOH$.** The value of the equilibrium constants $K_f$ and $K'_f$ for the reaction of free base amine and pyridine ligands with neutral and anionic methylaquocobaloxime agree to within 15% among the various methods and several determinations for a given ligand (see Experimental Section and Tables I and II). Logarithmic plots of $K_f$ (for thiolate anions,[10] aliphatic amines, and pyridines) and $K'_f$ (for the latter two series of ligands) *vs.* $pK'$ value of the conjugate acid of the ligand are shown in Figure 4 (ligation of thiolate anions to anionic methylaquocobaloxime is insignificant).[10] The affinities for ligands to neutral methylaquocobaloxime increase in the order $RNH_2$ < X-py < $RS^-$ over a range of $K_f$ values 50 to $3 \times 10^5$ $M^{-1}$, but the slopes of the lines expressing the dependence of ligand affinity upon the $pK'$ value of the conjugate acid of the ligand within each series show the opposite trend ($\beta = 0.18 \pm 0.01$ for $RS^-$, $\beta = 0.21 \pm 0.01$ for X-py, and $\beta = 0.38 \pm 0.01$ for $RNH_2$). The affinity of anionic methylaquocobaloxime for ligands ($K'_f$) is between 9- and 33-fold less than the affinity of neutral methylaquocobaloxime. The dependence of $K'_f$ values upon the $pK'$ value of the conjugate acid of the ligand for $RNH_2$ ($\beta = 0.31 \pm 0.03$) is close to that for $K_f$ ($\beta = 0.38 \pm 0.01$), while within the limited data for substituted pyridines, the slope for the $K'_f$ correlation is significantly lower ($\beta = 0.06 \pm 0.03$)[21] than that for $K_f$ ($\beta = 0.21 \pm 0.01$).

**Proton Dissociation Constants for $Me(D_2H_2)L$.** The values for $pK'_{c-L}$ determined from the pH dependence of the apparent ligand dissociation rate constants (Table I for amines and Table II for pyridines) show little dependence upon the $pK'$ value of the conjugate acid of the ligand ($\beta = 0.07 \pm 0.03$ for the amines and $\beta =$

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[21] The parameters for the anionic methylaquocobaloxime are in general less well determined than those for the neutral species due to the large values for $pK'_{c-L}$ which prohibited titration of the complexes with alkali to greater than 50–70% completion. However, $k'_{off}$, the least well-determined constant in these experiments, was always measured with a standard deviation of less than 15% and frequently better than 10%.[18] The uncertainty in these constants is greatest for TFEA where the rapid rates of $Me(D_2H_2)L$ decomposition led to the least precision in the kinetic titration.

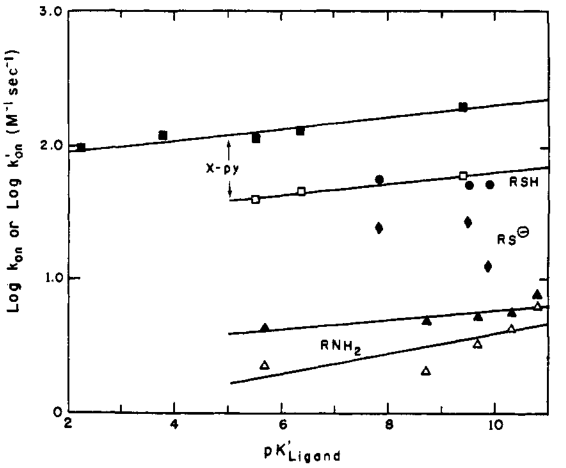

Figure 6. Dependence of ligation rate constants to neutral (solid symbols) and anionic (open symbols) methylaquocobaloxime upon the proton dissociation constant ($pK'_L$) of the conjugate acid of the ligand, 25°, ionic strength 1.0 *M*. (●), RSH;[22] (◆), $RS^-$; (■, □), X-py; (▲, △), $RNH_2$. All slopes are less than 0.1. The *solid lines* were calculated by a least-squares method.

0.12 ± 0.02 for the pyridines, Figure 5).[21] We estimate the error in these proton dissociation constants to be ±0.08 pH unit. For $Me(D_2H_2)A$-py it was possible to confirm the $pK'_{c-L}$ value obtained by the kinetic titration method with direct spectrophotometric titration in the presence of sufficient ligand to keep the complex >98% liganded as a consequence of the large values of $K_f$ and $K'_f$ for this complex. The two methods provided $pK'_{c-L}$ values of 14.05 and 14.07, respectively.

**Kinetics of Ligation to Methylaquocobaloxime.** The rate constants for ligation to both neutral and anionic methylaquocobaloxime are contained in Table I for the primary amines and in Table II for the substituted pyridines. For A-py, Me-py, and py the values of $k_{off}$ determined from direct ligand dissociation experiments (method IV) agreed to within 15% of the values obtained by reaction of $Me(D_2H_2)L$ with ME (method III). The dependencies of the rate constants for ligation to neutral methylaquocobaloxime upon the $pK'$ value of the conjugate acid of the ligand for the primary amines, substituted pyridines, thiolate anions, and neutral thiols[10,22] are shown in Figure 6, and while the magnitudes of the ligation rate constants for these series vary by more than 46-fold in the order $RNH_2$ < $RS^-$ < RSH < X-py, the slopes of the lines are uniformly small ($\beta < 0.1$). The rate constants $k'_{on}$ for ligation to anionic methylaquocobaloxime are smaller than $k_{on}$ for both the amines and the pyridines (thiolate anions show no significant reaction with anionic methylaquocobaloxime[10]) and the dependencies of $k'_{on}$ upon the $pK'$ value of the conjugate acid of the ligand yield $\beta$ values of less than 0.1 (Figure 6).

The amine ligands dissociate in general more slowly than do the pyridines from both the neutral and anionic complexes and the dependencies of the rate constants for ligand dissociation upon the $pK'$ value of the conjugate acid of the ligand are quite different ($\beta = -0.33 \pm 0.02$ for $RNH_2$, $\beta = -0.17 \pm 0.02$ for X-py for $k_{off}$; $\beta = -0.23 \pm 0.02$ for $RNH_2$, and $\beta = -0.08 \pm 0.03$ for X-py for $k'_{off}$, Figure 7).

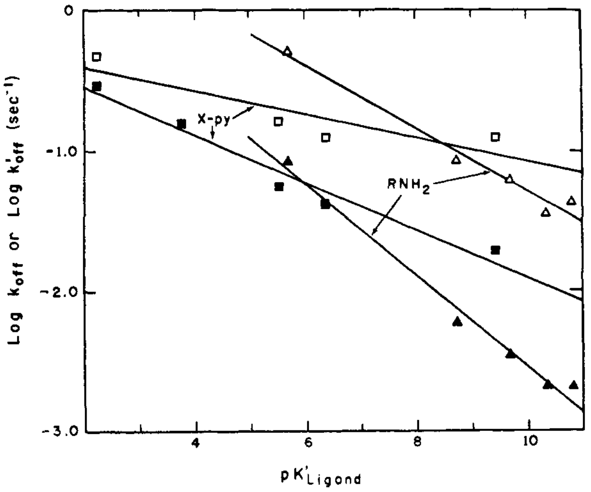

Figure 7. Dependence of the ligand dissociation rate constants from neutral (solid symbols) and anionic (open symbols) $Me(D_2H_2)L$ upon the proton dissociation constant ($pK_L'$) of the conjugate acid of the ligand, 25°, ionic strength 1.0 *M*. (■, □), X-py, slope = $-0.17 \pm 0.02$ for $k_{off}$, slope = $-0.08 \pm 0.03$ for $k'_{off}$; (▲, △), $RNH_2$, slope = $-0.33 \pm 0.02$ for $k_{off}$, slope = $-0.23 \pm 0.02$ for $k'_{off}$. The *solid lines* were calculated by a least-squares method.

## Discussion

**Ligation Equilibria of Methylaquocobaloxime.** A soft or class b character has been assigned to $Me(D_2H_2)HOH$,[10,24] cobaloximes (III),[25,26] vitamin $B_{12a}$,[4] and methylcobalt corrins[27] and is consistent with the observed greater ligand affinity of soft thiolate anions than the hard primary amines[28,29] to $Me(D_2H_2)HOH$ (Figure 4). In contrast, most Co(III) complexes (*e.g.*, $Co^{III}(NH_3)_5HOH^{3+}$, etc.) are hard acids[28] with the interesting exceptions of the soft acids, $Co(CN)_5HOH^{2-}$ [30] and $Co(NH_3)_5SO_3^+$ [31] (see below). It thus appears that the presence of one or more soft or unsaturated ligands is sufficient to confer the characteristic of softness to a cobalt complex. Furthermore, softness appears to be directly related to the ability of a cobalt complex to stabilize a carbon–cobalt bond as seen in the cobaloximes, the cobalt corrins, and even pentacyanocobaltate(III), the alkyl derivatives of which have been prepared by Halpern and Maher.[32]

The concept of metal-to-ligand π bonding will be invoked to explain both the order of strength of ligation ($RNH_2$ < X-py < $RS^-$) and the reverse order for the dependence of ligation strength upon ligand basicity (Figure 4). The order of $Me(D_2H_2)L$ stability is attrib-

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[22] While the values of the proton dissociation constants for the conjugate acids of neutral thiols have not been measured, they have been estimated to occur at $H_0 \sim -6$.[23] For simplicity the rate constants for the reaction of RSH with $Me(D_2H_2)HOH$ have been plotted *vs.* the $pK'$ for RSH.

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[29] W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 89–92.

[30] Reference 28, p 206.

[31] J. Halpern, R. A. Palmer, and L. M. Blakley, *J. Amer. Chem. Soc.*, **88**, 2877 (1966).

[32] J. Halpern and J. P. Maher, *ibid.*, **86**, 2311 (1964); **87**, 5361 (1965).

uted to the ability of thiols to accept electrons into relatively low-lying unfilled d orbitals while pyridines accept electrons into higher energy unfilled $\pi^*$-antibonding orbitals;[33,34] primary amines cannot accept electrons in either fashion. This ligand stability order is the same as the order previously established for the trans direction of substituents into the square-planar complexes of Pt[35] and for the stabililization of low-valence states of numerous transition metals,[36] processes which have been related to the $\pi$-accepting ability of the ligands. The reverse order for the dependence, $\beta$, of $Me(D_2H_2)L$ stability on ligand basicity among the three series of ligands ($\beta = 0.18$, 0.21, and 0.38, for thiolate anions, substituted pyridines, and primary amines, respectively) is not unexpected based on the following considerations: (i) an increase in basicity is associated with an increased ability for $\sigma$ donation for all ligands (note especially the greater dependence of $Me(D_2H_2)L$ stability on ligand basicity for the primary amines which are solely $\sigma$ donors); (ii) an increase in basicity is associated with a decreased ability for the X-py and thiolate anion ligands to function as $\pi$ acceptors (thus, as $\pi$ bonding becomes increasingly important, progressively lesser dependencies of $Me(D_2H_2)L$ stability upon ligand basicity are observed).

Metal-to-ligand $\pi$ bonding (i) has been firmly established in a number of transition metal complexes,[35-39] (ii) has been invoked for the cobalt corrins in low[40] oxidation states, for the alkylcobalt corrins,[14] for the ligation of pyridines to cobaloximes(III),[41] and for the ligation of various ligands to methylcobaloxime in nonaqueous solvents,[42] and (iii) is well substantiated in the complexes of olefins with cobaloximes(I)[43,44] and in the complexes of CO, $CH_3CN$, $CH_3NC$ with methylcobaloxime.[45]

Metal-to-ligand $\pi$ bonding in the transition state has been invoked previously in order to explain the increased reactivity of neutral thiol over thiolate anion[10] in the ligation reactions of $Me(D_2H_2)HOH$. The present evidence implicates significant contributions of this type of bonding in the ground state as well. An argument outlined by Jencks,[46] who has discounted the possibility that large differences in nucleophilicity when related to basicity can be attributed to differences in solvation of the nucleophile, can be extended such that differences in solvation of the ligands are unlikely to account for the large differences in the stabilities of the liganded complexes $Me(D_2H_2)L$ when related to basicity (Figure 4).

The equilibrium constants for ligation to anionic methylaquocobaloxime ($K'_f$) are significantly lower than those for binding to the neutral complex (Figure 4). The increased electron density on the cobalt atom upon loss of a proton from neutral $Me(D_2H_2)HOH$ to form an anionic complex apparently decreases the effectiveness of ligand electron donation and hence bonding to cobalt. The significantly lesser dependence of ligation affinity to anionic methylaquocobaloxime upon the p$K'$ value for the conjugate acid of the ligand for the pyridines ($\beta = 0.06 \pm 0.03$), the limited data notwithstanding, as compared to the primary amines ($\beta = 0.31 \pm 0.03$) may be yet another indication of the importance of metal-to-ligand $\pi$ donation. Thus, an increase in electron density on the cobalt atom (upon ionization to form the anionic conjugate base) would be expected to increase the effectiveness of such $\pi$ donation. This effect may then be responsible for the greater difference in $\beta$ values for the pyridines ($\Delta\beta = 0.21 - 0.06 = 0.15$) compared with the aliphatic amines ($\Delta\beta = 0.38 - 0.31 = 0.07$) for the dependence of the ligation equilibria on the p$K'$ of the conjugate acid of the ligand for the neutral and anionic complexes ($K_f$ *vs.* $K'_f$, Figure 4).

**Kinetics and Mechanism of Ligation to $Me(D_2H_2)$-HOH.** The rate constants for ligation ($k_{on}$, Tables I and II and Figure 6) indicate that $Me(D_2H_2)HOH$ is exceedingly reactive compared to ordinary Co(III) complexes[47-49] and the majority of cobaloximes(III), *e.g.*, $Co(D_2H_2)(NO_2)HOH$, etc.[25] Methylaquocobaloxime is, however, not as reactive as aquocobalamin[5,8] (by about an order of magnitude) and is only slightly more reactive than sulfitoaquocobaloxime, $Co(D_2H_2)(SO_3)HOH^-$.[26]

In comparisons between the alkylcobalt complexes and the usual Co(III) complexes there are $10^4$–$10^5$ increases in the rates of ligation (present data and ref 24, *cf.* ref 25) and substantial differences in the fraction of total complex as the pentacoordinate species (ref 50, *cf.* ref 48). These marked differences suggest that the common practice of considering alkylcobalt complexes to be Co(III) complexes with carbanion ligands is inappropriate and very probably misleading.[51]

The small dependence of $k_{on}$ upon ligand basicity (Figure 6) within each series of ligands ($\beta$ values $<0.1$) is clearly related to the fact that while the reacting complex is a soft acid, the reference acid (the proton) is hard. The rate constants are better correlated with the relative softness of the ligand among the three ligand series we have studied. The overall, rather small variation of ligation rates with ligand identity is probably indicative of an early transition state with a rather large ligand-to-metal bond distance.

The small differences in the rate of ligand substitution *despite* large differences in the stabilities of the

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**Table III.** Relative Ranges of Rate and Equilibrium Constants for the Ligation Reactions of Aquocobalamin, Cobaloximes(III), and $Me(D_2H_2)HOH$ at 25°

| Complex | Incoming ligands | Rel rate range | Rel equil range | $\frac{\text{Equil range}}{\text{rate range}}$ |
|---|---|---|---|---|
| Aquocobalamin[a] | $SCN^-$, $I^-$, $Br^-$, $N_3^-$, $NCO^-$, $S_2O_3^-$, $HSO_3^-$, $SO_3^{2-}$ | 13.5<br>(4.9)[a] | $2.4 \times 10^4$<br>($2.4 \times 10^4$)[a] | $1.8 \times 10^3$<br>($4.9 \times 10^3$)[a] |
| $Co(D_2H_2)(NO_2)HOH$[b] | $Cl^-$, $Br^-$, $N_3^-$, $NCS^-$, $HSO_3^-$ | $1.1 \times 10^2$ | $4.8 \times 10^2$ | 4.6 |
| $Co(D_2H_2)(I)HOH$[b] | $Cl^-$, $Br^-$, $NCS^-$, $HSO_3^-$ | 10.5 | $1.2 \times 10^3$ | $1.1 \times 10^2$ |
| $Me(D_2H_2)HOH$[c] | $RNH_2$, X-py, $RS^-$ | 45.0<br>(45.0)[d] | $5.3 \times 10^3$<br>($2.1 \times 10^2$)[d] | $1.2 \times 10^2$<br>(4.8)[d] |

[a] References 4, 8, and 53; values in parentheses refer to monoanions only. [b] Taken or calculated from ref 25. [c] This study and ref 10. [d] Values in parentheses refer to nitrogen ligands only.

ligation complexes for the usual Co(III) complexes[48] and aquocobalamin[8] have been taken to indicate a lack of significant activation of the transition state by the incoming ligand[47] and, conversely, domination of the transition state activation by the leaving ligand (*i.e.*, a dissociative interchange mechanism, $I_d$[52]). In other words, incoming ligand and leaving ligand (water) are at best loosely bound to cobalt in the transition state. A compilation (Table III) of the relative ranges for the rate and equilibrium constants for the ligation reactions of aquocobalamin,[4,8,53] some cobaloximes(III), and $Me(D_2H_2)HOH$ with various ligands shows that the equilibrium/rate range ratios for the cobaloximes(III) and $Me(D_2H_2)HOH$ are from one to three orders of magnitude smaller than the ratios for aquocobalamin (Table III). Thus, these kinetic and thermodynamic data do not compellingly argue in favor of an $I_d$ mechanism for the cobaloximes(III) and $Me(D_2H_2)HOH$ since the criteria of limited variation in rate despite large differences in the stability of the liganded complexes is not met. However, it is difficult to distinguish between a transition state in which both ligands are loosely bound (*i.e.*, an $I_d$ mechanism)

$$L \cdots Co \cdots HOH$$

and one in which the leaving ligand has completely dissociated (*i.e.*, an SN1 mechanism).[50]

$$L \cdots Co$$

The stability of pentacoordinate alkyl cobalt complexes[50] (*cf.* ref 48) and the evidence that both of the "deviant" soft Co(III) complexes, $Co(CN)_5HOH^{2-}$ and $Co(NH_3)_5SO^+{}_3$, undergo ligand substitution reactions *via* SN1 mechanisms[20,31] clearly favor this mechanism for ligation reactions of $Me(D_2H_2)HOH$. The correlation between the softness of a cobalt(III) complex and the stability of its pentacoordinate species permitting an SN1 mechanism for ligand substitution[54] and our evidence pointing to the soft character of $Me(D_2H_2)HOH$ supports the conclusion that the ligation reactions of $Me(D_2H_2)HOH$ probably proceed *via* an SN1 mechanism.

In the ligation reactions of $Me(D_2H_2)HOH$ (Figure 6), while the $\pi$-accepting ligands (X-py, $RS^-$, and RSH) react more rapidly than the purely $\sigma$ donors ($RNH_2$), the greater reactivity of the substituted pyridines than the thiolate anions or neutral thiols stands in contrast to the opposite reactivity order for these nucleophiles with various unsaturated carbon electrophiles.[55]

The increased electron density on cobalt in the anionic complexes is associated with decreased ligation affinity ($K'_f$ *vs.* $K_f$) that is the net result of both a decrease of ligation rate constant with anionic methylaquocobaloxime and an increase in ligand dissociation rate constant from liganded anionic complexes. The relatively smaller ratios of $k'_{off}/k_{off}$ of 3.0–6.5 for the pyridines compared to about 6–20 for the primary amines (Figure 7) may be yet another consequence of the $\pi$-acceptor properties of the pyridines and the $\pi$-donor ability of the cobalt atom; the latter should be increased by the increase in electron density on the cobalt atom in the methylaquocobaloxime anions.

**Proton Dissociation Constants of $Me(D_2H_2)HOH$ and $Me(D_2H_2)L$.** The loss of a proton from neutral $Me(D_2H_2)HOH$ to form an anionic species ($pK'_{c_2} = 12.68$, Scheme I) may be (a) from one of the H-bonded oxime protons in the planar, equatorial ligand system or (b) from the axial water ligand.

(a) The similar, alkaline proton dissociation constants of $Me(D_2H_2)L$ complexes of pyridine and aliphatic amines ($pK'_{c-L}$ values 13.6–14.2), of $Co(D_2H_2)(X)Y$ complexes (X, Y = $CN^-$, $NO_2^-$, $Br^-$, and $NH_3$, $pK'$ values 11.0–12.3),[56] and of $Co(D_2H_2)B_2$ complexes (B = substituted anilines and pyridines, $pK'$ values 7.0–9.0 for the pyridine complexes, 10.5–10.8 for the aniline complexes)[41] must represent the loss of a proton from the equatorial ligand system, an assignment which is clearly unambiguous in the many compounds devoid of dissociable protons on the axial ligands.

(b) On the other hand, the proton dissociations of aquocobalamin[5,57] and diaquocobinamide[58] with $pK'$ values of 7.55 and 6.0, respectively, have been assigned to the axial water ligand. The proton dissociations from $Co(D_2H_2)(B)HOH$ (B = anilines, pyridines,

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$NO_2^-$, and HOH) with p$K'$ values from 5.0 to 7.3[59] have also been assigned to the axial water ligand, in this case on the basis of indirect spectral evidence.

The alkaline proton dissociation has been assigned to the axial water ligand in the complexes $XCo(D_2H_2)$-HOH[24] where X = $NO_2^-$ (p$K'$ = 7.28),[59] $SO_2^{-2}$ (p$K'$ = 10.23),[26] and $CH_3$ (p$K'$ = 12.68) on the basis of the following approximate ratio of the rates of axial water replacement by $SCN^-$, $N_3^-$, and py: $NO_2^-/SO_3^{2-}/CH_3 = 1/10^4/10^5$.[24–26] The kinetic data, it is argued, reflect the strength of water bonding to cobalt and the acidity of the axial water ligand both of which decrease through the series $NO_2^-$, $SO_3^{2-}$, $CH_3$. However, the similarly alkaline proton dissociation constants for the complexes $Me(D_2H_2)L$ (Tables I and II) which, as noted above, must be assigned to the equatorial ligand system indicate that a similar assignment merits serious consideration for $Me(D_2H_2)HOH$ as well. Indeed, if methylcobalt complexes are predominately pentacoordinate in aqueous solution at room temperature,[50] then the proton dissociation from Me-$(D_2H_2)HOH$ *must* be assigned to the equatorial ligand system. For example, methylcobinamide, which has been shown to be predominately pentacoordinate in aqueous solution,[50] shows no evidence of proton dissociation up to pH 14.0.[58]

**Acknowledgment.** The authors acknowledge numerous helpful discussions with Dr. Richard O. Viale.

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# Kinetics of Protonation Reactions of Nickel(II) Peptide Complexes

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**Abstract**: Nickel tetraglycine, $Ni(H_{-3}GGGG)^{2-}$, and nickel triglycinamide, $Ni(H_{-2}GGGa)^-$, react with $H_3O^+$ with second-order rate constants ($M^{-1}$ sec$^{-1}$) of $7.1 \times 10^4$ and $3.3 \times 10^4$, respectively. The $Ni(H_{-3}GGGG)^{2-}$ and $[NiH_{-2}GGGa]^-$ reactions are not general-acid catalyzed while the reactions of nickel triglycine, $Ni(H_{-2}GGG)^-$, and the corresponding copper complex are subject to general-acid catalysis. Hydrogen carbonate ion is an effective general acid for the protonation of $Ni(H_{-2}GGG)^-$ and carbonate ion is an inhibitor. A general mechanism is proposed to account for the kinetics of the various protonation reactions of metal peptide complexes.

Tetraglycine, triglycine, and triglycinamide are known to ionize peptide and amide protons in the formation of yellow, diamagnetic, square-planar nickel(II) complexes.[1–6] The deprotonated nitrogen atoms become coordinated to nickel.[7] In this manner the triglycine anion ionizes two protons to give $Ni(H_{-2}GGG)^-$, while triglycinamide and the tetraglycine anion lose three protons to give $Ni(H_{-3}GGGa)^-$ and $Ni(H_{-3}GGGG)^{2-}$, respectively.[1,5,6,8]

The rates of the $H_3O^+$ reactions with the triglycine complexes, $Ni(H_{-2}GGG)^-$ and $Cu(H_{-2}GGG)^-$, are much slower than normal diffusion-controlled reactions.[5,6,9,10] A general-acid catalysis mechanism is observed in the reaction of other acids (HX) with these complexes.[5,10] The reactions are first order in the concentrations of the acids and first order in the concentration of the complex. The rate expression (eq 1) includes a solvent dissociation rate constant ($k_d$).

$$\text{rate} = (k_d + k_H[H_3O^+] + k_{HX}[HX])[M(H_{-2}GGG)^-] \quad (1)$$

Recently Wilkins and coworkers[6] have reported that the protonation rate of Ni(glycinamide-H)$_2$ was dependent on the concentration of buffer, excess ligand, and hydrogen ion as is the case with the triglycine complex, $Ni(H_{-2}GGG)^-$. However, they also reported that the tetraglycine complex, $Ni(H_{-3}GGGG)^{2-}$, undergoes a two-stage protonation at pH ~7 and that the rate constants are pH independent over some 0.5-unit range of pH. We disagree with these results for tetraglycine.

Ligand exchange reactions of $Ni(H_{-3}GGGG)^{2-}$ with polydentate amines have been reported[11] to take place in two measurable steps from pH 10.5 to 12. The first reaction supposedly produced yellow mixed complexes of unusual stoichiometries, such as (nickel tetraglycine)$_3$trien. Some of the peculiar reactions attributed to the tetraglycine complex of nickel may be due to other species produced in its spontaneous reaction with molecular oxygen.[12] We have found that under anaerobic conditions $Ni(H_{-3}GGGG)^{2-}$ reacts with trien (triethylenetetramine) without forming yellow mixed complexes and without undergoing the initial

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