Coenzyme B_{12} Model Studies. Equilibria and Kinetics of Axial Ligation of Substituted Alkylcobaloximes by Pyridine in Aqueous Solution[1,2]

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Abstract: A series of 11 substituted alkylcobaloximes ($\sigma^* = -0.19$ to $+1.30$) has been synthesized and characterized chemically and spectrophotometrically where R = isopropyl, ethyl, methyl, 2-phenethyl, 3-cyanopropyl, 2-methoxyethyl, 1-propenyl, iodomethyl, 2,2,2-trifluoroethyl, chloromethyl, and cyanomethyl. The kinetics and equilibria for axial ligation of pyridine to the neutral and anionic alkylcobaloximes have been measured as well as the proton dissociation constants for both the alkylcobaloximes and the alkyl(pyridine)cobaloximes. There are substantial influences of substitution at the alkyl position of alkylcobaloximes upon processes trans to it, for example, the rates of pyridine dissociation from neutral and anionic alkyl(pyridine)cobaloximes and the rates of pyridine association with anionic alkyl(hydroxo)cobaloximes (ρ^* values of -4.1, -5.6, and -5.4, respectively). In contrast to the above *linear* free energy correlations, rate and equilibrium constants for pyridine ligation to *neutral* alkylcobaloximes and proton dissociation constants for alkylcobaloximes are *nonlinearly* correlated with polar substituent constants, σ^*. These observations of *nonlinear* free-energy correlations provide one of the three lines of evidence that suggest that alkylcobaloximes in aqueous solution exist as equilibrium mixtures of hexacoordinate *and* pentacoordinate species, and that there is a progressive shift of the ratio of penta/hexacoordinate species from ~ 1 to $\ll 1$ with increasing electron withdrawal inductive effect exerted through the alkyl substituent. The remaining two lines of evidence are: (i) the changes in the visible spectra of alkylcobaloximes through the series from electron-donating to electron-withdrawing substituents resemble the spectral changes of an electron-donating substituted alkylcobaloximes upon axial ligation with pyridine; and (ii) the much greater temperature dependence of the visible spectra of electron-donating than of electron-withdrawing substituted alkylcobaloximes. Based on the assumption that the aforementioned *nonlinearity* in the correlations is attributable to the communicating $K_{cw} = [RCo(D_2H_2)]/[RCo(D_2H_2)(HOH)]$ equilibrium, estimates of K_{cw} have been obtained for the substituted alkylcobaloximes and the K_{cw} values correlated with σ^*. Data are presented that are consistent with the suggestion that dissociative mechanisms are operative for the ligation reactions and the tentative conclusion that the anionic complexes are alkyl(hydroxo)cobaloximes, formed formally by proton dissociation from the axial rather than the equatorial ligand system.

In earlier studies,[4,5] we reported the kinetics and equilibria for the binding of various nitrogen and sulfur ligands to the coenzyme B_{12} model compound, methylcobaloxime.[6] From these studies numerous interesting questions arose concerning the mechanism of axial ligand substitution, the relative importance of metal-to-ligand π donation, the assignment of the alkaline proton dissociation site for methylcobaloxime, and the possible existence of the stable pentacoordinate species, $CH_3Co(D_2H_2)$, in aqueous solution. In an attempt to gain further insight into the answers to these questions, we have extended our earlier studies to include the binding of a single ligand, pyridine, to a series of 11 substituted alkylcobaloximes, including several which have not been previously reported, covering a wide range of electronic inductive effect ($\sigma^* = -0.19$ for isopropyl to $\sigma^* = +1.30$ for cyanomethyl).[7] We herein report the results of studies of the reaction shown in eq 1:

$$\text{alkylcobaloxime} + \text{pyr} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{alkyl(pyridine)cobaloxime} \quad (1)$$

which suggest (i) that a dissociative mechanism is operative in the ligation reactions, (ii) that the anionic complexes are alkyl(hydroxo)cobaloximes, and (iii) that neutral alkyl(aquo)cobaloximes are in equilibrium with some sort of pentacoordinate species in aqueous solution.

Experimental Section

Materials. Alkyl halides were obtained in the highest purity commercially available and were redistilled prior to their employment as alkylating agents except for methylene chloride (Fisher Certified) and 1,1,1-trifluoro-2-bromoethane (Columbia) which were used without further purification. Dimethylglyoxime, cobaltous chloride, sodium borohydride, sodium hydroxide, methanol, buffer components, and inorganic salts were obtained in the highest purity available commercially and used without further purification.

Alkyl(aquo)cobaloximes were in general synthesized by a slight modification of the procedure of Schrauzer[8] which was followed through the isolation of the alkylated dimethyl sulfide complexes, $RCo(D_2H_2)(CH_3SCH_3)$, but these complexes were not hydrolyzed by boiling aqueous suspensions due to significant pyrolysis of some of the derivatives at 100°. Instead, hydrolyses were effected by evaporation of aqueous suspensions of dimethyl sulfide complexes on a rotary flash evaporator in dim light. The time required for complete hydrolysis (generally reliably determined by smelling the suspension and confirmed by ^1H NMR analysis) varied from about an hour for complexes of electron-donating alkyl groups to several days for complexes of extremely electron-withdrawing alkyl groups.

The product of a synthesis employing a cis–trans mixture of 1-bromo-1-propene as the alkylating agent was a mixture of *cis*- and *trans*-1-propenyl(aquo)cobaloxime based upon the ^1H NMR spectrum, and this result is in agreement with the observations of other investigators on the reactions of cobaloximes (I) with *cis*- and *trans*-1-halo-1-alkenes.[9]

The yields of $ICH_2Co(D_2H_2)(HOH)$ and $ClCH_2Co(D_2H_2)(HOH)$ were quite small utilizing the above synthesis procedure. Therefore, a modification of a procedure reported by Schrauzer[10] was utilized for the synthesis of $ClCH_2Co(D_2H_2)(HOH)$ in dim light as follows.

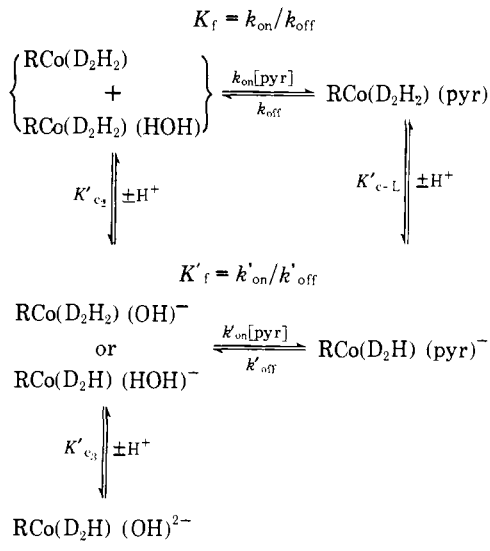
Cobaltous chloride hexahydrate (23.8 g, 0.1 mol) plus dimethylglyoxime (23.2 g, 0.2 mol) were stirred in 250 ml of methanol under a stream of O_2-free argon.[11] Following an addition of 8.0 g (0.2 mol) of NaOH in 30 ml of deaerated water, the suspension was stirred for 15 min to allow the formation of the cobalt(II) dimers $[Co^{II}(D_2H_2)(HOH)]_2$. Methylene chloride (20.8 ml, 0.5 mol) and NaOH (4.0 g, 0.1 mol, in 15 ml of deaerated water) were added, and the reaction mixture was placed on Parr hydrogenation apparatus and shaken under 30 psi H_2 in a stream of warm air (re-

Table IV. Equilibrium Constants for Pyridine Ligation with and Proton Dissociation from Alkylcobaloximes in Aqueous Solution, 25.0°, Ionic Strength 1.0 *M*

R	σ^*[a]	K_f, M^{-1}[b]	K_f, M^{-1}[c]	K_f, M^{-1}[d]	pK'_{c_2}	pK'_{c-L}	K'_f, M^{-1}[e]	K_{cw}[k]
Isopropyl	−0.19	484 ± 18 506 ± 22	450 ± 32	480 ±14	13.28 ± 0.02	13.93 ± 0.18	106 ± 45	3.9
Ethyl	−0.10	885 ± 35	685 ± 23	785 ± 21	12.97 ± 0.02	13.88 ± 0.14	96.8 ± 30.4	3.0
Methyl	0	2040 ± 191[h] 2320 ± 93	2100 ± 83	2150 ± 76	12.68 ± 0.02	13.61 ± 0.09	252 ± 53	2.2
Phenethyl	+0.08	1360 ± 160	1380 ± 61	1370 ± 86	12.78 ± 0.02	13.56 ± 0.14	227 ± 73	1.7
3-Cyanopropyl	+0.17[f]	1170 ± 63 1270 ± 69	1120 ± 46	1190 ± 35	12.22 ± 0.04	13.37 ± 0.09	82.9 ± 19.0	1.3
2-Methoxyethyl	+0.19[g]	1420 ± 65 1450 ± 71	1380 ± 82	1420 ± 42	12.19 ± 0.02			1.2
1-Propenyl	+0.36	1650 ± 84	1440 ± 47	1550 ± 48	12.02 ± 0.01[i]	13.39 ± 0.08	66.5 ± 13.2	0.74
Iodomethyl	+0.85	2600 ± 198	2680 ± 105	2640 ± 112	11.72 ± 0.12			0.17
2,2,2-Trifluoroethyl	+0.92	2960 ± 145 2250 ± 247	2850 ± 253	2690 ± 127	10.96 ± 0.01[i]	12.30 ± 0.01	122 ± 7	0.14
Chloromethyl	+1.05	2630 ± 230 2380 ± 73	1850 ± 70	2290 ± 84	11.95 ± 0.02			0.09
Cyanomethyl	+1.30	4530 ± 220	5640 ± 95	5090 ± 120	10.56 ± 0.01[j]	11.77 ± 0.01	311 ± 14	0.04

[a] See ref. 7. [b] Spectrophotometric determinations. [c] Calculated as $K_f = k_{on}/k_{off}$. [d] Average values from *b* and *c*. [e] Calculated as $K'_f = K'_{c-L}K_f/K'_{c_2}$. [f] Calculated from σ^*(3-cyanopropyl) = σ^*(cyanomethyl)/(2.76)^2. [g] Calculated from σ^*(2-methoxyethyl) = σ^*(methoxymethyl)/2.76. [h] Reference 5. [i] Average of two spectrophotometric determinations. [j] $pK'_{c_3} = 14.16 \pm 0.04$. [k] Calculated from $\log K_{cw} = \rho^*_{K_{cw}}\sigma^* + \log K^\circ_{cw}$.

Scheme I



action mixture temperature ca. 40°). After 4 hours the calculated amount of H_2 had been taken up. The reaction mixture was filtered, flash evaporated to ca. 100 ml, diluted with 200 ml of water, and placed in the cold overnight for crystallization. The product was recrystallized from warm methanol–water and dried in air.

Pyridine was obtained in the highest purity commercially available, refluxed over solid KOH for several hours, redistilled monthly,[12] and stored in dark bottles over molecular sieve (Linde type 4A). Deionized water of greater than 5×10^5 Ω cm specific resistance was used throughout, and ionic strength was maintained with KCl at 1.0 *M*.

Methods. Measurements of pH and uv and visible spectra were obtained as described previously.[4]

Alkylcobaloximes were characterized by electronic absorption spectra, photolability monitored spectrophotometrically,[4] NMR spectra,[13] and elemental analysis (Table I).[14] The purity of the products was further confirmed by the migration of the compounds as single spots in both methanol and acetone on Silica Gel F-254 thin layer chromatography plates (Table I).

Apparent equilibrium constants (K_f) (Scheme I) for the formation of $RCo(D_2H_2)(pyr)$ from alkylcobaloxime and pyridine (pyr) (eq 2)

$$K_f = [\text{alkyl(pyridine)cobaloxime}]/[\text{alkylcobaloxime}][\text{pyr}]_{free} \quad (2)$$

were determined spectrophotometrically as described previously,[5,15] generally at pH 8.4, in 0.1 *M* borate. Additional values of K_f for all complexes were calculated from the values of the apparent rate constants for pyridine association (k_{on}) and dissociation (k_{off}) measured independently (see below) and the relation $K_f = k_{on}/k_{off}$.

Values for the proton dissociation constants (K'_{c_2}) (Scheme I) for the series of alkylcobaloximes and for the proton dissociation constant for monoanionic cyanomethylcobaloxime (K'_{c_3}) were also determined spectrophotometrically.[4,15,16]

The proton dissociation constants (K'_{c-L}) for the alkyl(pyridine)cobaloximes (as well as the ligand dissociation rate constants k_{off} and k'_{off} for the neutral and anionic pyridine complexes, respectively) were determined by the pH dependence of the ligand dissociation rates in alkali (see method IV ref 5) for the complexes isopropyl, ethyl, phenethyl, and 3-cyanopropyl (using a Gilford 2000 recording spectrophotometer and a dilution stopped-flow mixing device, when necessary). For the 2,2,2-trifluoroethyl- and cyanomethyl(pyridine)cobaloximes, alkaline decomposition[17] of the alkylcobaloximes at rates comparable to the pyridine dissociation rates prevented the use of this technique. However, the slow ligand dissociation rates permitted the direct spectrophotometric titration of these pyridine complexes, which were generated in situ in solutions containing alkylcobaloxime and sufficient pyridine to maintain the complexes >99% as the pyridine complexes (as calculated from the measured values of K_f, Table IV). Measurements of the absorbance of aliquots of such solutions at various alkaline pH values could always be made rapidly enough so that ligand dissociation was negligible. The constant, K'_{c-L}, could not be evaluated by either method for $ClCH_2Co(D_2H_2)(pyr)$ and $ICH_2Co(D_2H_2)(pyr)$ due to the rapid rates of alkaline decomposition of the corresponding aquo complexes (see below and ref 17) and the rapid ligand dissociation rates from the pyridine complexes.

Apparent equilibrium constants K'_f for the binding of pyridine to the anions of the alkylcobaloximes 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).

Kinetic runs were performed as before[4] under pseudo-first-order conditions with [pyr] at least tenfold greater than the [alkylcobaloxime]. The pH was maintained with 0.1 *M* phosphate, borate, acetate, and formate buffers, or KOH. No significant buffer catalysis of these ligation reactions has been detected.[4] Rapid kinetic measurements were made on a stopped-flow apparatus with either a 1:1 or a 40:1 dilution mixer on a Gilford 2000 recording spectrophotometer with thermostated cell compartments maintained at 25.0 ± 0.1°. Suitable wavelengths for reaction progress measurements and observed first-order rate constants (k_{obsd}) were determined as described previously.[4,5,18] The kinetics of ligation were studied at pH values at least 2 units below the pK'_{c_2} value for proton dissociation from alkylcobaloxime and at least 2 units above

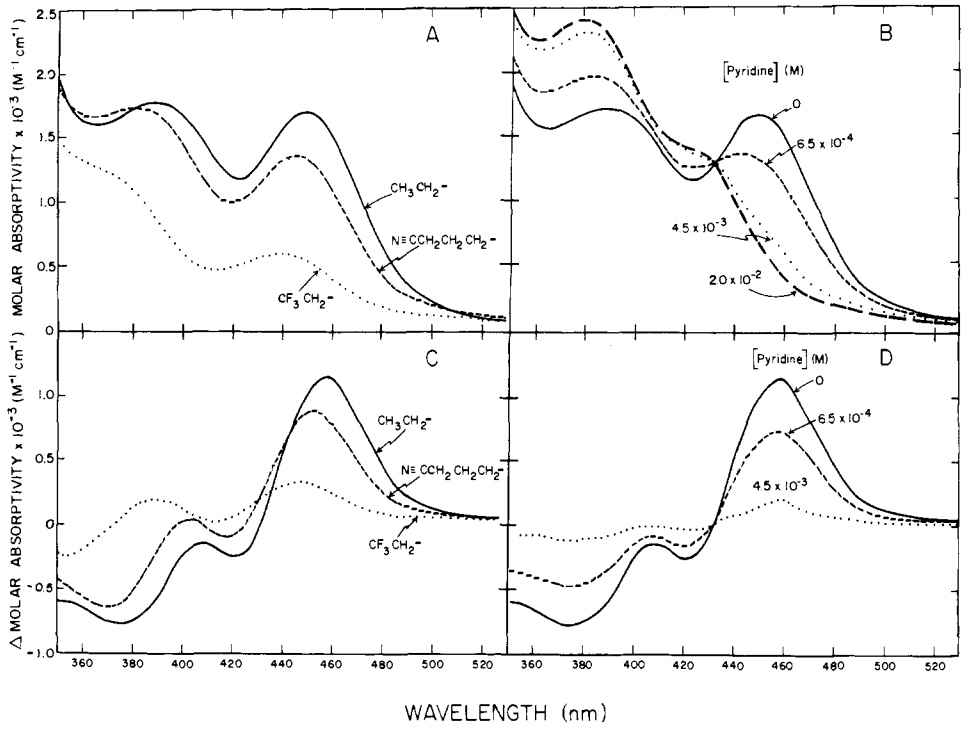


Figure 1. Visible spectra of alkylcobaloximes and $RCo(D_2H_2)(pyr)$, pH 6.5. (A) Spectra of ethylcobaloxime (—), cyanopropylcobaloxime (- - -), and trifluoroethylcobaloxime (•••). (B) spectra of ethylcobaloxime with various concentrations of added pyridine: [pyr] = 0 (—); [pyr] = 6.5×10^{-4} *M* (- - -); [pyr] = 4.5×10^{-3} *M* (•••); [pyr] = 0.02 *M* (-- -- --). (C) Difference spectra for alkylcobaloxime–alkyl(pyridine)cobaloxime: ethyl- (—), 3-cyanopropyl- (- - -), 2,2,2-trifluoroethyl- (•••). (D) Difference spectra for ethylcobaloxime + $[pyr]_x$-ethyl(pyridine)cobaloxime; pyridine concentration (pyr) as in part B.

the pK'_a value of 5.51[4] for the pyridinium ion (i.e., pH 8.4). Apparent second-order rate constants (k_{on}) for ligation were obtained from the slopes of graphs of k_{obsd} vs. [pyr] determined by the least-squares method (eq 3)

$$k_{obsd} = k_{on}[pyr] + k_{off} \quad (3)$$

and for the isopropyl derivative, the k_{off} value was reliably determined from the ordinate intercept. The plots of k_{obsd} vs. [pyr] were linear with standard deviations of <3.6% of the slope.[19] The values for the ligand dissociation rate constants for neutral and anionic pyridine complexes, k_{off} and k'_{off}, respectively, were more generally obtained from the pH dependence of ligand dissociation rates in alkali (see above). For the pyridine complexes of the 2,2,2-trifluoroethyl, cyanomethyl, chloromethyl, and iodomethyl derivatives, values of k_{off} were determined by diluting stock solutions of preformed $RCo(D_2H_2)(pyr)$ in methanol into aqueous solutions buffered at pH values between 3.5 and 5.5 (with concentrations and pH values arranged so that the ligand dissociation reactions proceeded >90% to completion following dilution) and measuring the time dependent absorbance changes at suitable uv wavelengths determined as described previously.[5] The value of k_{off} determined by the dilution method for the isopropyl derivatives was in satisfactory agreement with that determined from eq 3 (see above).

Values of k'_{on}, the ligation rate constant for binding of pyridine to anionic alkylcobaloximes, were calculated from the relation $k'_{on} = k'_{off}K'_f$.[5]

Relaxation measurements employed an electrical discharge temperature jump perturbation instrument type SPA 7, Messanlagen Studien Gesellschaft mbH, Gottingen, Germany (from 5.5 to 9.1° and 15 to 20°). The isopropyl- and ethylcobaloximes ($6–7 \times 10^{-4}$ *M*) were each studied at pH 6.5 in 0.1 *M* potassium phosphate, ionic strength 0.1 *M* at two wavelengths, 460 and 510 nm for the former compound and 450 and 485 nm for the latter compound on 1–5 mV/division scales.

Results

Spectral Data. The electronic spectra of the alkylcobaloximes for electron-donating alkyl groups are characterized by two visible bands of nearly equal molar absorptivity at ca. 440–462 and ca. 384–390 nm (Figure 1A, *solid line*) as well as two or more shoulders in the uv region and a low wavelength uv peak (not depicted). The progression to the more electron-withdrawing alkyl groups is generally associated with decreases in molar absorptivity values for both visible absorbance bands and, less consistently, shifts of both visible bands to shorter wavelengths (Figure 1A, *dashed* and *dotted lines*). The wave lengths and molar absorptivity values of the visible bands of the alkylcobaloximes and alkyl(pyridine)cobaloximes and the main uv band of the alkylcobaloximes in aqueous solution are given in Tables II and III.[14]

Equilibria. The equilibrium constants for the binding of pyridine to the neutral and anionic alkylcobaloximes as well as the equilibrium constants for the proton dissociations from the alkylcobaloximes and the alkyl(pyridine)cobaloximes are compiled in Table IV. The pK'_{c-L} value for $CH_3OCH_2CH_2Co(D_2H_2)(pyr)$ could not be measured since the rate of ligand dissociation from this complex showed no significant pH dependence. For the mixture of *cis*- and *trans*-1-propenylcobaloxime (see above), spectral evidence suggests the existence of two distinct proton dissociation constants (at about 11.7 and 12.2), but there is no evidence of multiple binding constants for the formation of the pyridine complexes. Titration data of sufficient accuracy to separate these two very closely overlapping proton dissociation constants could not be obtained.

Kinetics. The pyridine association and dissociation rate constants for both the neutral (k_{on} and k_{off}) and anionic (k'_{on} and k'_{off}) alkylcobaloximes are contained in Table V.

In the measurements of k_{on} (see Experimental Section), the plots of k_{obsd} vs. [pyr] showed no significant deviations from linearity at increasing pyridine concentration (i.e., there was no evidence of saturation kinetics) up to a total pyridine concentration of 0.1 *M* (see Experimental Section) for any of the alkylcobaloximes.

Table V. Rate Constants for Pyridine Association and Dissociation with Alkylcobaloximes in Aqueous Solution, 25.0°, Ionic Strength 1.0 *M*

R	σ^*[a]	k_{on}, M^{-1} sec^{-1}	k_{off}, sec^{-1}	k'_{off}, sec^{-1}	k'_{on}, M^{-1} sec^{-1} [b]
Isopropyl	−0.19	$1.49 \pm 0.04 \times 10^4$	$3.31 \pm 0.22 \times 10^1$ [c]	$1.14 \pm 0.16 \times 10^2$	$1.21 \pm 0.54 \times 10^4$
Ethyl	−0.10	$1.17 \pm 0.02 \times 10^3$	1.71 ± 0.05	4.93 ± 0.51	$4.77 \pm 1.60 \times 10^2$
Methyl	0	$1.16 \pm 0.03 \times 10^2$	$5.52 \pm 0.15 \times 10^{-2}$ [d]	$1.63 \pm 0.10 \times 10^{-1}$ [d]	$4.11 \pm 0.90 \times 10^1$
Phenethyl	+0.08	$1.22 \pm 0.04 \times 10^3$	$8.83 \pm 0.22 \times 10^{-1}$	1.76 ± 0.11	$4.00 \pm 1.31 \times 10^2$
3-Cyanopropyl	+0.17 [e]	$1.64 \pm 0.01 \times 10^2$	$1.47 \pm 0.06 \times 10^{-1}$	$4.11 \pm 0.20 \times 10^{-1}$	$3.41 \pm 0.80 \times 10^1$
2-Methoxyethyl	+0.19 [e]	$1.65 \pm 0.02 \times 10^2$	$1.20 \pm 0.07 \times 10^{-1}$		
1-Propenyl	+0.36	$2.34 \pm 0.02 \times 10^1$	$1.62 \pm 0.05 \times 10^{-2}$	$4.88 \pm 0.24 \times 10^{-2}$	3.25 ± 0.66
Iodomethyl	+0.85	4.20 ± 0.04	$1.57 \pm 0.06 \times 10^{-3}$		
2,2,2-Trifluoroethyl	+0.92	$4.56 \pm 0.07 \times 10^{-1}$	$1.60 \pm 0.14 \times 10^{-4}$		
Chloromethyl	+1.05	$1.06 \pm 0.02 \times 10^1$	$5.73 \pm 0.20 \times 10^{-3}$		
Cyanomethyl	+1.30	$5.59 \pm 0.08 \times 10^{-2}$	$9.92 \pm 0.09 \times 10^{-6}$		

[a] See ref 7. [b] Calculated from $k'_{on} = k'_{off}K'_f$. [c] Average of two values (see Experimental Section). [d] Reference 5. [e] See Table IV, footnotes *f* and *g*.

The traces of absorbance vs. time for both ligand association and dissociation reactions for the 1-propenyl complex were distinctly biphasic, suggesting different rate constants for the cis and trans configurations. No attempt was made to analyze the rapid phases of these traces, and the values for rate constants for this complex represent data from the slower phases only.

No relaxation spectra were obtained for the isopropyl- and ethylcobaloximes that were distinct from the dead time of the instrument (~5 μsec) at 9 or 20°.

The values for the thermodynamic and kinetic parameters for the anionic complexes are significantly less well determined (see Tables IV and V) than those for the neutral complexes for the reasons previously stated (see ref 5, ref 21).

Discussion

Deviant Complexes. The data for the methyl-, chloromethyl-, and iodomethylcobaloximes deviate from the data of the other substituted alkylcobaloximes on the free-energy correlations to be discussed below. This situation is similar to that of substituted methylcobalamins which deviate from correlations involving other substituted alkylcobalamins.[21] Whether the basis of these deviations is steric[13,22-36,38a] or otherwise is unclear at present, and these complexes will be omitted from further discussion. Such steric effects have been assumed to cause a displacement of the cobalt atom from the equatorial ligand plane,[24,37] a configuration which is also associated with the formation of stable pentacoordinate alkylcobalt complexes.[24,32,38b]

A steric effect may be present for the *cis*-1-alkenylcobaloximes and the findings of biphasic kinetic data for ligand association and dissociation with the cis–trans mixture of 1-propenylcobaloximes is in accord with the presence of hindered and unhindered complexes. Consequently, the more rapid phase in both cases was assigned to the hindered cis configuration (not analyzed), and the slower phases were assigned to the trans configuration for inclusion in Table V.

Evidence for Pentacoordinate Alkylcobaloximes. We have previously discussed the possibility of the existence of stable pentacoordinate complexes among the alkylcobaloximes.[4,5] There is considerable evidence in the recent literature that this is the case for the alkylcobaltcorrins[38b-41] and an ever increasing body of evidence for pentacoordinate alkylcobalt complexes among several B_{12} model chelate systems.[32a,37,42-44]

Other authors have also suggested the possible existence of pentacoordinate species for the alkylcobaloximes[45,46] despite the conclusion by Calligaris et al.[35] that the bis(dimethylglyoximato) equatorial ligand sytem cannot stabilize carbon–cobalt bonds in the absence of a sixth axial ligand. Dehydrated complexes have been formed from alkyl(aquo)cobaloximes by azeotropic distillation of benzene suspensions or by heating in vacuo at 80–100° [27,47] and appear to be dimers in both nonaqueous solvents and in the solid state.[27,48,49] However, dimers are unlikely to occur in coordinating solvents, and this unlikeliness is supported by our observations that, for the alkylcobaloxime series studied, the visible spectra *and* measured K_f values are independent of the concentration of alkylcobaloximes over the ranges of 5.0×10^{-6} to 1.0×10^{-2} and 5.0×10^{-5} to 1.5×10^{-3} *M*, respectively.[50]

In coordinating solvents "pentacoordinate" species are unlikely to be truly pentacoordinate since the cobalt atom will be solvated by one or more solvent molecules at the open axial ligand position. Hence such species are perhaps better described as outer sphere complexes involving pentacoordinate alkylcobalt complexes and solvent. Nevertheless, whatever their exact form, it is only necessary for such species to be thermodynamically distinct from the conventional hexacoordinate species (i.e., separated by a potential energy barrier) to be theoretically detectable. For convenience these species will continue to be referred to as pentacoordinate. To the extent that these systems involve something resembling two isomeric species rapidly interconverting, it has proved extremely difficult prior to these studies to detect and measure this equilibrium. Nonetheless, three lines of evidence from this work lend support for the occurrence of some kind of thermodynamically distinct pentacoordinate species of alkylcobaloxime in aqueous solution.

(1) Spectral Characteristics of the Alkylcobaloxime Series. The changes in the visible spectra of the alkylcobaloximes on proceeding through the series from electron-donating to electron-withdrawing alkyl groups (Figure 1A) are similar to the spectral changes observed upon addition of varying amounts of pyridine to an electron-donating alkylcobaloxime (Figure 1B for ethylcobaloxime plus pyridine).

Since virtually identical visible spectral changes are seen when various nitrogen and sulfur ligands are added to methylcobaloxime,[4,5] it appears that the various axial liganded alkylcobaloximes exhibit similar spectra for a given alkylcobaloxime regardless of the nature of the liganding atom. The progressively more minor changes in the difference spectra (Figure 1C) between reactant alkylcobaloxime and product, $RCo(D_2H_2)(pyr)$, in aqueous solution, with increasingly electron-withdrawing alkyl substituent, may then reflect differences in the state of ligation of the reactant alkylcobaloxime by solvent. Such a situation would obtain if electron-donating substituted alkylcobaloximes were largely unliganded (pentacoordinate) and electron-withdrawing substituted alkylcobaloximes were largely liganded (hexacoordinate) by solvent in aqueous solution, in which case the greatest *net* change in axial ligation in forming the

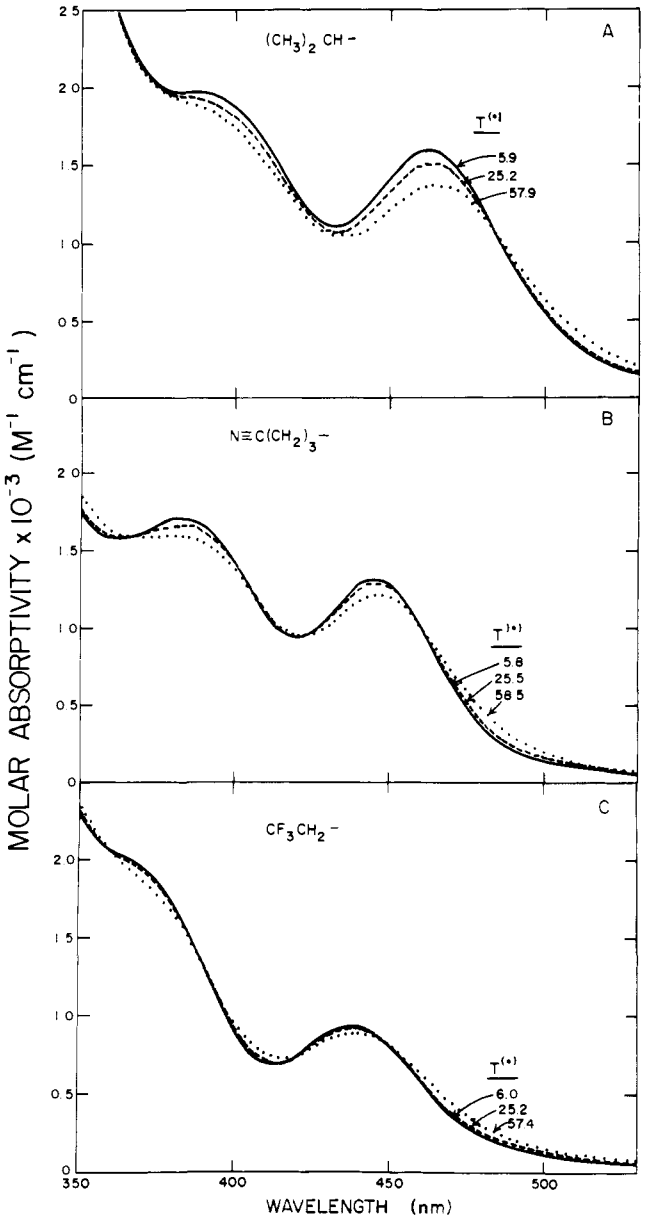


Figure 2. Temperature dependence of the visible spectra of representative alkylcobaloximes, pH 6.5: (A) isopropylcobaloxime, 5.9° (—), 25.2° (- - -), 57.9° (···); (B) cyanopropylcobaloxime, 5.8° (—), 25.5° (- - -), 58.5° (···); (C) trifluoroethylcobaloxime, 6.0° (—), 25.2° (- - -), 57.4° (···).

pyridine complex would occur with the electron-donating substituted alkylcobaloximes (e.g., ethylcobaloxime in Figure 1C).

This point is illustrated for ethylcobaloxime by the progression to more minor spectral changes in the difference spectra between solutions of ethylcobaloxime of increasing extents of pyridine ligation and totally pyridine liganded ethylcobaloxime (Figure 1D). The minor noncorrespondences between these two sets of difference spectra (Figures 1C and 1D) may be ascribed to minor and incompletely cancelled dissimilarities in the electron inductive effects of the alkyl groups on the alkylcobaloxime spectra and the spectrum of its pyridine complex. These data suggest that there is a progressive shift of a ligation equilibrium (with solvent, eq 4) through the series of increasingly electron-withdrawing substituted alkylcobaloximes in aqueous solution that is similar to the progressive shift of the ligation equilibrium to form the pyridine complex caused by the addition of increasing concentrations of pyridine to ethylcobaloxime.

$$RCo(D_2H_2)(HOH) \underset{k_{-1}}{\overset{K_{cw}}{\underset{k_1}{\rightleftharpoons}}} RCo(D_2H_2) \qquad (4)$$

$$K_{cw} = [RCo(D_2H_2)]/[RCo(D_2H_2)(HOH)] = k_1/k_{-1}$$

Schrauzer and coworkers[38a] have assigned the lower energy visible electronic absorption band of the alkylcobaloximes to an electronic transition between the highest bonding and lowest antibonding axial bond molecular orbitals, and thus the spectral changes shown in Figure 1A and the decrease in K_{cw} values with increasing electron withdrawal effect are consistent with shifts in an axial ligation equilibrium (eq 4).

(2) Temperature Dependence of the Spectra for the Alkylcobaloxime Series. The temperature dependence of the spectra of three representative alkylcobaloximes, isopropyl ($\sigma^* = -0.19$), 3-cyanopropyl ($\sigma^* = +0.17$), and 2,2,2-trifluoroethyl ($\sigma^* = +0.92$), in aqueous solution over the temperature range from ~6 to ~58° shows some degree of band sharpening on cooling but, in addition, shows clear isosbestic points as well as a shift of the lowest energy band to longer wavelengths on warming (Figure 2). These latter two effects appear to be clearly indicative of a temperature dependent shift of a chemical equilibrium rather than simple temperature effects on electronic transitions. Furthermore, the magnitude of the temperature dependent spectral changes over the given temperature range can be seen to decrease markedly with increasing electron withdrawal by the alkyl group as would be expected if, in this temperature range, the most electron-withdrawing substituted compounds are predominantly hexacoordinate. These effects are extremely similar to those described by Hill and coworkers[40,41] for the temperature dependence of the spectra of alkylcobinamides and have been assigned by these workers to temperature dependent shifts of the hexacoordinate-pentacoordinate equilibrium, with pentacoordinate species being relatively more stable at the higher temperature.

Attempts to measure the rate of equilibration of the penta- and hexacoordinate species at 20 and 9° for several of the alkylcobaloximes by means of temperature-jump relaxation spectrophotometry at several wavelengths were unsuccessful presumably due to the fact that the rate of equilibration exceeded the instrument dead time (~5 μsec). This allows a lower limit to be placed on the rate of equilibration, $k = k_1 + k_{-1}$ of approximately 2×10^5 sec^{-1}.

(3) Nonlinear Free Energy Relationships. The equilibria involving the ligation of $RCo(D_2H_2)(OH^-)$ by pyridine or the proton dissociations from $RCo(D_2H_2)(pyr)$, K'_f and $K'_{c\text{-}L}$ (Scheme I), and the kinetics of pyridine association with and dissociation from anionic complexes, k'_{on} and k'_{off} (Scheme I), as well as pyridine dissociation from $RCo(D_2H_2)(pyr)$, k_{off}, are all expected to be independent of whether neutral alkylcobaloximes actually exist as hexacoordinate and/or pentacoordinate species in aqueous solution since the latter species are not involved per se in these reactions.

The ρ^*-σ^* correlations for the polar influences upon these equilibria, $K'_{c\text{-}L}$ and K'_f, are depicted in Figure 3 and the correlations appear to be *linear* with ρ^* values of -1.46 ± 0.08 and $+0.14 \pm 0.17$, respectively. The ρ^*-σ^* correlations for k'_{on} and k'_{off} but especially for the more extensive data covering a greater range of σ^* values for k_{off} are also *linear* with ρ^* values of -5.90 ± 1.03, -5.57 ± 0.83, and -4.13 ± 0.28, respectively (Figure 4).

In contrast, equilibria and kinetics that involve neutral alkylcobaloximes per se in aqueous solution are expected to be sensitive to the existence of a significant fraction of reactant as the pentacoordinate complex and to changes in this fraction through the series as polar substituent effects vary. Thus, a variation in K_{cw} values from <1 to >1 through the

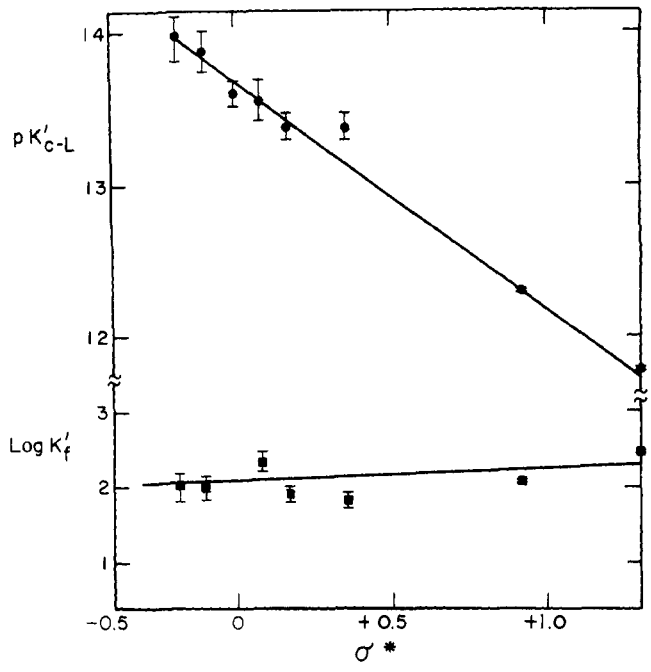


Figure 3. Dependence of log K'_f (■) and $pK'_{c\text{-}L}$ (●) for the binding of pyridine to anionic alkylcobaloxime and for the alkaline proton dissociation from $RCo(D_2H_2)(pyr)$, respectively, upon polar substituent constant, σ^*. *Solid lines* are calculated from the following equations and parameters obtained by least-squares methods contained in Table VI: $\log K'_f = \rho^*\sigma^* + \log K'^\circ_f$ and $pK'_{c\text{-}L} = \rho^*\sigma^* + pK'^\circ_{c\text{-}L}$. The error bars represent ±1 standard deviation if depicted or fall within the symbols.

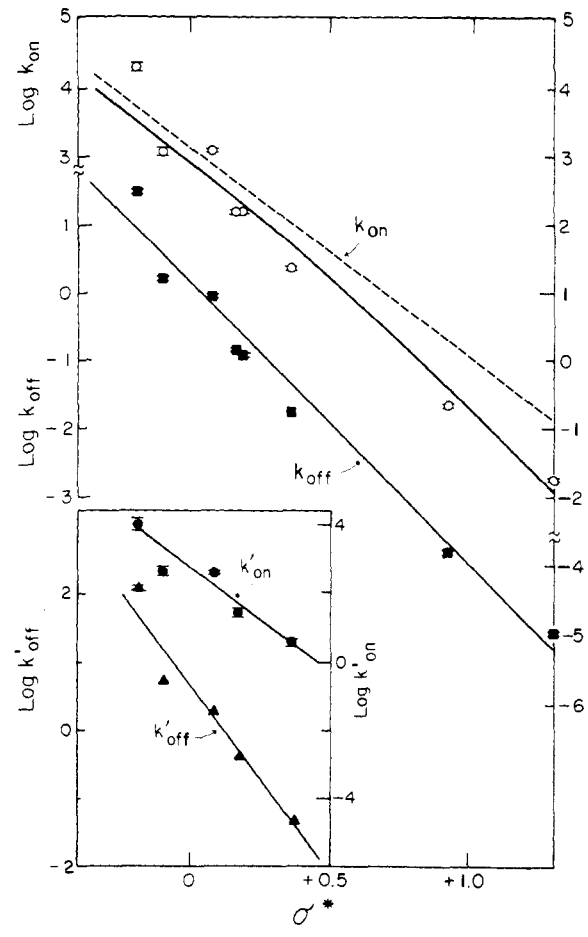


Figure 4. Dependence of log k_{on} (○), log k'_{on} (●), log k_{off} (■), and log k'_{off} (▲) for rates of ligand association to neutral and anionic alkylcobaloxime and rates of ligand dissociation from $RCo(D_2H_2)(pyr)$ and $RCo(D_2H)(pyr)^-$, respectively. The lower three *solid lines* are calculated from the following equations and the parameters contained in Table VI: $\log k_{off} = \rho^*\sigma^* + \log k^\circ_{off}$, $\log k'_{on} = \rho^*\sigma^* + \log k'^\circ_{on}$, $\log k'_{off} = \rho^*\sigma^* + \log k'^\circ_{off}$. The *dashed line* is calculated from the following equation and parameters contained in Table VI without consideration of the hexa- and pentacoordinate equilibrium described by K_{cw}: $\log k_{on} = \rho^*_{k_2}\sigma^* + \log k^\circ_2$. The uppermost *solid line* is calculated from the following equation which includes the hexacoordinate-pentacoordinate equilibrium described by K_{cw} and the parameters contained in Table VI, $\log k_{on} = \rho^*_{k_2}\sigma^* + \log k^\circ_2 - \gamma$, where $\gamma = \log [1 + 10^{-(\rho^*_{K_{cw}}\sigma^* + \log K^\circ_{cw})}]$. The standard deviations for the individual points fall within the symbols unless otherwise depicted.

Table VI. Linear Free Energy Correlations with Polar Substituent Constants (σ^*) for the Equilibria and Kinetics of Pyridine Ligation to Neutral and Anionic Alkylcobaloximes[a]

Reaction[b]	Slope ρ^*	Intercept Value	Intercept Term
K_L	+1.4	3.2	$\log K^\circ_L$
K_{OH}	−2.7	12.6	$pK^\circ_{OH} + pK_w$
$K'_{c\text{-}L}$	−1.46 ± 0.08	13.69 ± 0.05	$\log K^\circ_{c\text{-}L}$
K'_f	+0.14 ± 0.17	2.1 ± 0.1	$\log K'^\circ_f$
k_2	−2.8	3.2	$\log k^\circ_2$
k_{off}	−4.13 ± 0.28	0.08 ± 0.16	$\log k^\circ_{off}$
k'_{on}	−5.90 ± 1.03	2.66 ± 0.21	$\log k'^\circ_{on}$
k'_{off}	−5.57 ± 0.83	0.62 ± 0.17	$\log k'^\circ_{off}$
K_{cw}	−1.30	0.34	$\log K^\circ_{cw}$

[a] 25°, ionic strength 1.0 *M*. [b] The reaction constants are defined by Scheme I and eq 5, 6, and 9.

series is expected to be manifest in *nonlinear* ρ^*-σ^* correlations. For example, under circumstances in which a significant fraction of the total alkylcobaloxime exists as a hexacoordinate species, the apparent binding constant for pyridine ligation to alkylcobaloxime, K_f, is given by eq 5 and involves the equilibrium constants for the two separate reactions, first, loss of water from the axial position to form the pentacoordinate species (K_{cw}) and second, pyridine coordination to the axial position of the pentacoordinate species (K_L)

$$RCo(D_2H_2)(HOH) \underset{k_{-1}}{\overset{K_{cw}}{\rightleftharpoons}} RCo(D_2H_2) \underset{k_{-2}}{\overset{K_L[L]}{\rightleftharpoons}} RCo(D_2H_2)(L) \quad (5a)$$

$$K_f = [RCo(D_2H_2)(L)]/([RCo(D_2H_2)(HOH)] + [RCo(D_2H_2)])[L] = K_L\{K_{cw}/(K_{cw} + 1)\} \quad (5b)$$

where $K_L = [RCo(D_2H_2)(L)]/[RCo(D_2H_2)][L] = k_2/k_{-2}$. For extremely electron-withdrawing alkyl groups where $K_{cw} \ll 1$, $K_f = K_{cw}K_L$ and for extremely electron-donating alkyl groups where $K_{cw} \gg 1$, $K_f = K_L$. The complete equation describing the dependence of log K_f upon σ^* is eq 6

$$\log K_f = \rho^*_{K_L}\sigma^* + \log K^\circ_L - \gamma \quad (6)$$

derived from the expression for K_f given above (eq 5b) and the relations $\log K_L = \rho^*_{K_L}\sigma^* + \log K^\circ_L$, $\log K_{cw} = \rho^*_{K_{cw}}\sigma^* + \log K^\circ_{cw}$, and $\gamma = \log\{1 + 10^{-(\rho^*_{K_{cw}}\sigma^* + \log K^\circ_{cw})}\}$. Equation 6 describes a curved line with assymptotes, eq 7 and 8

$$\log K_f = (\rho^*_{K_L} + \rho^*_{K_{cw}})\sigma^* + \log K^\circ_L + \log K^\circ_{cw} \quad (7)$$

$$\log K_f = \rho^*_{K_L}\sigma^* + \log K^\circ_L \quad (8)$$

for extremely electron-withdrawing and extremely electron-donating alkyl groups, respectively. Similar derivations yield eq 9b-d for the other ρ^*-σ^* correlations based upon the following relations $K'_{c_2} = K_{OH}K_w\{K_{cw}/(K_{cw} + 1)\}$,

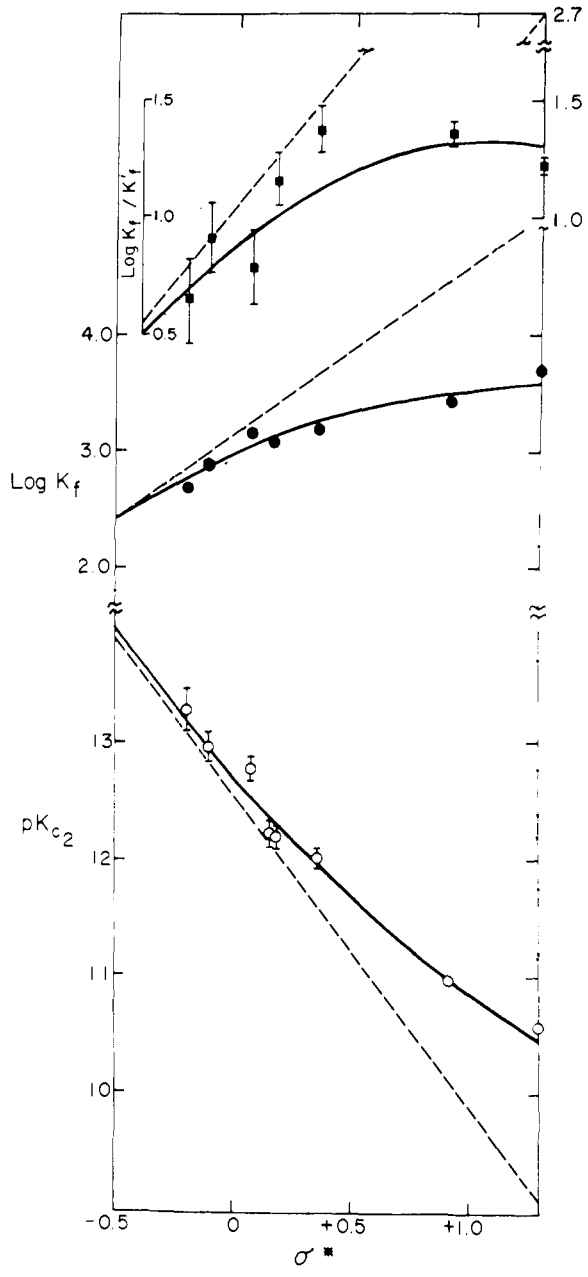


Figure 5. Dependence of log K_f (●), log (K_f/K'_f) (■), and pK'_{c_2} (○) for the binding of pyridine to neutral (K_f) and anionic (K'_f) alkylcobaloxime and for the proton dissociation from alkylcobaloxime upon polar substituent constant, σ^*. The *dashed lines* are calculated from the following equations and the parameters contained in Table VI without consideration of the hexa- and pentacoordinate equilibrium described by K_{cw}: log $(K_f/K'_f) = \rho^*\sigma^* + \log (K^\circ_L/K'^\circ_f)$, log $K_f = \rho^*_{K_L}\sigma^* + \log K^\circ_L$, p$K'_{c_2} = \rho^*_{K_{OH}}\sigma^* + pK^\circ_{OH} + pK_w$. The *solid lines* are calculated from the following equations which include the hexacoordinate-pentacoordinate equilibrium described by K_{cw}: log $(K_f/K'_f) = \rho^*\sigma^* + \log (K^\circ_L/K'^\circ_f) - \gamma$, log $K_f = \rho^*_{K_L}\sigma^* + \log K^\circ_L - \gamma$, p$K'_{c_2} = \rho^*_{K_{OH}}\sigma^* + pK^\circ_{OH} + pK_w + \gamma$. γ values are obtained as described in legend to Figure 4. The ρ^* value for the log (K_f/K'_f) correlation is $\rho^*_{K_L} - \rho^*_{K'_f} = +1.26$. The error bars represent ±1 standard deviation.

$K_{OH} = [RCo(D_2H_2)(OH^-)]/[RCo(D_2H_2)][OH^-]$, $K_w = 10^{-14}$ (eq 9a), and $k_{on} = k_2\{K_{cw}/(K_{cw} + 1)\}$ (eq 5a).

$$RCo(D_2H_2)(HOH) \overset{K_{cw}}{\rightleftharpoons} RCo(D_2H_2) \overset{K_{OH}\ +OH^-}{\rightleftharpoons} RCo(D_2H_2)(OH^-) \quad (9a)$$

$$pK'_{c_2} = \rho^*_{K_{OH}}\sigma^* + pK^\circ_{OH} + \gamma + pK_w \quad (9b)$$

$$\log (K_f/K'_f) = (\rho^*_{K_L} - \rho^*_{K'_f})\sigma^* + (\log K^\circ_L/K'^\circ_f) - \gamma \quad (9c)$$

$$\log k_{on} = \rho^*_{k_2}\sigma^* + \log k^\circ_2 - \gamma \quad (9d)$$

The *nonlinear* ρ^*-σ^* correlations for the equilibria described by K_f and K_{c_2} (Scheme I) are depicted in Figure 5, and the nonlinearity is more dramatically shown in the ρ^*-σ^* correlation for log K_f/K'_f (in which nonpolar substituent effects tend to be cancelled out). The nonlinearity clearly falls beyond the experimental error limits represented by the bars (±1 sd) in Figure 5 and contrasts sharply with the linear correlations contained in Figure 3. The situation is similar for the kinetic data and, while the kinetic data for ligation of pyridine to alkylcobaloximes, k_{on}, are less satisfactory than the equilibrium data for demonstrating this effect, the ρ^*-σ^* correlation for k_{on} appears to be *nonlinear* and as such is consistent with the suggestion that pentacoordinate species are present and must be taken into account. If these nonlinearities as exemplified by compounds with σ^* values <0.3 compared with those with σ^* values > 0.3 in Figures 4 and 5 are attributable to the changing values of the K_{cw} equilibrium, as formulated in eq 5, 6, and 9, the estimates of the appropriate limiting slopes and intercepts contained in Table VI result. It must, however, be stressed that these values are crude estimates and have been used only to demonstrate that the calculated correlations in Figures 4 and 5 (solid lines), based on these estimates and eq 5, 6, and 9, adequately describe the non-linear behavior of the data. Further experiments at other temperatures, which may be expected to alter K_{cw}, are in progress.

Assignment of the Site of Alkaline Proton Dissociation from Alkylcobaloximes. While it is clear that the alkyl(pyridine)cobaloximes must dissociate a proton from the equatorial ligand system (eq 10)

$$RCo(D_2H_2)(pyr) \overset{K'_{c\text{-}L}}{\rightleftharpoons} RCo(D_2H)(pyr)^- + H^+ \quad (10)$$

the alkyl(aquo)cobaloximes may form anionic conjugate bases either in this fashion or by axial ligation of hydroxide ion (eq 9).

Proton dissociations for complexes of the type XCo(D_2H_2)(HOH) (where X is not an alkyl group) have been assigned on the basis of expected spectral changes in the visible region for axial water proton dissociation and changes in uv absorption (shift of peaks from about 245–250 to 260 nm, due to a $\pi \rightarrow \pi^*$ transition in the equatorial dimethylglyoxime ligands) for equatorial ligand proton dissociation.[51-53] We have observed that the formation of the monoanionic alkylcobaloximes is accompanied by spectral changes mainly in the visible region, which are not unlike the spectral changes observed on binding an axial ligand to alkylcobaloxime, while the trifluoroethyl- and cyanomethylcobaloximes show a second proton dissociation, pK'_{c_3}, associated with large uv spectral changes on going from the mono- to the dianionic forms (i.e., a shift of the uv band to longer wavelengths and a large increase in molar absorptivity at 260–270 nm) with little or no further change in the visible region. Similarly, the spectral change on proton dissociation from the pyridine complexes, $RCo(D_2H_2)(pyr)$, which can only undergo equatorial proton dissociation, while not insignificant in the visible region, is much larger in the uv range, again being characterized by a shift of the uv peak to longer wavelengths and a large increase in molar absorptivity in the 270–280 nm region. Thus, these spectral observations allow a tentative assignment of the monoanionic alkylcobaloximes as $RCo(D_2H_2)(OH^-)$ and as such is consistent with the small apparent dependence of K'_f upon

σ^* value (Figure 3) due to the competition between OH^- and pyridine for $RCo(D_2H_2)$.

Mechanism of Ligand Substitution. Neutral Complexes. In view of the evidence presented above for the existence of pentacoordinate alkylcobaloximes and the recent ligation kinetic studies of others both on alkyl cobalt complexes with other equatorial ligand systems[43] and on cobaloxime(III) complexes,[23,54,55] an SN1 mechanism (eq 5a) appears to be operative. Recent observations of Tauzher et al.[56] leading to the conclusion that $R[Co(DOH)(DO)(pn)]HOH^+$ undergoes ligand substitution by aromatic incoming ligands via an I_d type mechanism do not seem to be relevant to the present system since we find no saturation of ligand substitution rate constant with increasing [pyr] such as is seen by the above authors. Since an SN1 mechanism only has meaning if the alkylcobaloxime exists substantially as $RCo(D_2H_2)(HOH)$ (i.e., $K_{cw} = k_1/k_{-1} < 1$), for such compounds $k_1 < k_{-1}$ (eq 5a). In view of the temperature-jump experiments which show $k_1 + k_{-1} > 2 \times 10^5$ sec^{-1}, then $k_{-1} > 10^5$ sec^{-1} and any values of $k_2 < 2 \times 10^4$ M^{-1} sec^{-1} would be consistent with failure to observe saturation kinetics at experimentally attainable pyridine concentrations.[61]

Sakurai et al.[57] determined the $\Delta S^\dagger$ to be 19 ± 1.5 eu for the substitution of pyridine for water in $CH_3Co(D_2H_2)(HOH)$ which may be compared with the value $\Delta S^\dagger = -9 \pm 4$ eu for the substitution of NCS^- for HOH in $NO_2Co(D_2H_2)(HOH)$.[58] Although data for methyl derivative is frequently somewhat deviant on various correlations (see above), such a large positive entropy of activation would certainly be consistent with the SN1 process of eq 5a. The ρ^* value of −2.80 indicating that the pyridine association rate constants (k_2) are *aided* by electron *donating* substituents may be due to a significant contribution of π bonding in the transition states for ligand association involving pyridine[4,5] and further experiments with alkylamines, purely σ donors, would be desirable.

The correlation of log k_{off} with σ^* value (Figure 4, $\rho^* = -4.13 \pm 0.28$) indicates a strong *trans* effect of the alkyl group on ligand dissociation rate. A similar strong *trans* effect on the rate of ligand dissociation of HOH from $R[Co(DOH)(DO)(pn)]HOH^+$ has been demonstrated by Costa et al.[43] in 1% aqueous acetone.

Mechanism of Ligand Substitution. Anionic Complexes. The choice between SN1 and I_d mechanisms for the substitution of pyridine for OH^- in the anionic alkylcobaloximes cannot be made on the basis of the present data. Similarly, the apparently more negative value of ρ^* (−5.90 ± 1.03) for the ligand association rate constants (k'_{on}) in the anionic complexes as compared with the ρ^* value (−2.8) for the ligand association rate constants (k_2) for the neutral complexes cannot be reasonably interpreted without definite assignments of the mechanisms of these reactions (especially in view of the fact that they could proceed via different mechanisms).

The limited data for the rates of ligand dissociation from the anionic pyridine complexes, $RCo(D_2H)(pyr)^-$, (Figure 4, solid symbols), show a value of ρ^* (−5.57 ± 0.83) for ligand dissociation which is not substantially different from the ρ^* value (−4.13 ± 0.28) for ligand dissociation from the neutral complexes. As expected[5] the rate of ligand dissociation from the anionic complexes is always greater than that from the neutral complexes. The growing body of evidence for significant metal-to-ligand π-donation among the alkyl cobalt complexes[4,5,33,57,59,60] suggests that the apparent similarity of ρ^* values for the k_{off} and k'_{off} correlations may indicate that the decrease in Co–L bond stability due to the increased negative charge on the cobalt atom in the anionic complexes (a σ bonding effect) is approximately offset by the increase in π-interactions made possible by this increase in electron density on cobalt.

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Supplementary Material Available. Tables I, II, and III will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-7338.

References and Notes

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(2) Abbreviations: $R[Co(DOH)(DO)(pn)]HOH^+$ = alkyl(aquo)-1,3-bis(biacetylmonoximimino)propanecobalt monocation, pyr = pyridine, $RCo(D_2H_2)(HOH)$-substituted alkyl(aquo)bis(dimethylglyoximato)cobalt = alkyl(aquo)cobaloxime, $RCo(D_2H_2)(pyr)$-substituted alkyl(pyridine)bis(dimethylglyoximato)cobalt = alkyl(pyridine)cobaloxime.
(3) National Institutes of Health Postdoctoral Trainee, 1972–1973. Department of Chemistry, The University of Texas at Arlington, Arlington, Texas 76019.
(4) K. L. Brown and R. G. Kallen, *J. Am. Chem. Soc.*, **94**, 1894 (1972).
(5) K. L. Brown, D. Chernoff, D. J. Keljo, and R. G. Kallen, *J. Am. Chem. Soc.*, **94**, 6697 (1972).
(6) See G. N. Schrauzer, *Acc. Chem. Res.*, **1**, 97 (1968), and references therein for a review of the chemistry of the cobaloximes.
(7) R. W. Taft in "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, N.Y., 1956, Chapter 13.
(8) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **89**, 3607 (1967).
(9) (a) K. N. Van Duong and A. Gaudemer, *J. Organomet. Chem.*, **22**, 473 (1970); (b) A. N. Nesmeyanov, M. I. Rybinskaya, and T. V. Popova, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, 1982 (1969).
(10) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **89**, 143 (1967).
(11) L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948).
(12) D. D. Perrin, W. L. F. Armarego, and D. R. Perrin, "Purification of Laboratory Chemicals", Pergamon Press, Elmsford, N.Y., 1966, p 248.
(13) C. J. Cooksey, D. Dodd, C. Gatford, M. D. Johnson, G. J. Lewis, and D. M. Titchmarsh, *J. Chem. Soc., Perkin Trans. 2*, 655 (1972).
(14) See paragraph at end of this paper concerning supplementary material.
(15) R. O. Viale and R. G. Kallen, *Arch. Biochem. Biophys.*, **146**, 271 (1971).
(16) Both the trifluoroethyl- and $NCCH_2Co(D_2H_2)HOH$ derivatives gave spectrophotometric evidence for an additional proton dissociation at higher pH values, but alkaline decomposition[17] precluded a determination of the pK'_{c_3} value for the former compound.
(17) Unpublished observations. All alkyl(aquo)cobaloximes show identical spectral changes in strong alkali due to cleavage of the carbon–cobalt bond. Most such cleavages are quite slow (half-times from ca. 1 to 7 hr at pH 14) allowing measurement of some of the kinetic and thermodynamic parameters for the anionic complexes. However, the monohalomethyl(aquo)cobaloximes decompose with half-times of ~10 sec at pH 14.
(18) R. G. Kallen, *J. Am. Chem. Soc.*, **93**, 6236 (1971).
(19) A correction for an apparent self-association of pyridine[20] had little effect (<10%) on the slope and standard deviation of the slope.
(20) (a) A. R. Fersht and W. P. Jencks, *J. Am. Chem. Soc.*, **92**, 5432 (1970); (b) A. J. Kirby and W. P. Jencks, *ibid.*, **87**, 3209 (1965).
(21) H. P. C. Hogenkamp, J. E. Rush, and C. A. Swenson, *J. Biol. Chem.*, **240**, 3641 (1965).
(22) J. Halpern and P. F. Phelan, *J. Am. Chem. Soc.*, **94**, 1881 (1972).
(23) G. Costa, G. Tauzher, and A. Puxeddu, *Inorg. Chim. Acta*, **3**, 41 (1969).
(24) J. D. Brodie, *Proc. Natl. Acad. Sci. U.S.A.*, **62**, 461 (1969).
(25) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **89**, 1999 (1967).
(26) C. Giannotti, C. Fontaine, and A. Gaudemer, *J. Organomet. Chem.*, **39**, 381 (1972).
(27) A. W. Herlinger and T. L. Brown, *J. Am. Chem. Soc.*, **94**, 388 (1972).
(28) E. A. Parfenov and A. M. Yurkevich, *J. Gen. Chem. USSR* (*Engl. Transl.*), **42**, 470 (1972).
(29) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **88**, 3738 (1966).
(30) (a) The possibility that steric interactions in the chloromethyl and iodomethyl derivatives contribute to their deviations in linear free energy

correlations is supported by the following observations: (i) the standard synthetic procedures produce atypically small yields of these complexes (see Experimental Section); (ii) alkaline alkyl–Co bond cleavage rates for the monohalomethylcobaloximes are orders of magnitude faster than those for all other alkylcobaloximes;[17] and (iii) the ligand association and dissociation rate constants for these complexes are significantly larger than those of other alkyl derivatives as is expected if dissociative mechanisms are in play[30b] (see below) for sterically hindered complexes. Inspection of the covalent radii for these halogens (0.99 Å for Cl, 1.33 Å for I, compared with 0.32 Å for H in CH_4)[31] shows that the size of these substituents is approaching the lengths of the carbon–cobalt bonds which run from about 1.94 to 2.05 Å for numerous alkylcobalt complexes.[32-36] (b) F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions", 2nd ed, Wiley, New York, N.Y., 1967, p 387.

(31) L. Pauling, "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, New York, N.Y., 1966, p 224.
(32) (a) S. Brückner, M. Calligaris, G. Nardin, and L. Randaccio, *Inorg. Chim. Acta*, **3**, 308 (1969); (b) M. Calligaris, G. Nardin, and L. Randaccio, *Inorg. Nucl. Chem. Lett.*, **8**, 477 (1972).
(33) S. Brückner, M. Calligaris, G. Nardin, and L. Randaccio, *Inorg. Chim. Acta*, **2**, 416 (1968).
(34) P. G. Lenhert, *Chem. Commun.*, 980 (1967).
(35) M. Calligaris, D. Minichelli, G. Nardin, and L. Randaccio, *J. Chem. Soc., A*, 2720 (1971).
(36) M. Calligaris, G. Nardin, and L. Randaccio, *J. Chem. Soc., Dalton Trans.*, 1433 (1972).
(37) G. Costa, *Pure Appl. Chem.*, **30**, 335 (1972).
(38) (a) G. N. Schrauzer, L. P. Lee, and J. W. Sibert, *J. Am. Chem. Soc.*, **92**, 2997 (1970); (b) H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *Discuss. Faraday Soc.*, **47**, 165 (1969).
(39) R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, and R. G. Thorp, *Chem. Commun.*, 1013 (1967).
(40) H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *Chem. Br.*, **5**, 156 (1969).
(41) R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp, and R. J. P. Williams, *J. Chem. Soc. A*, 2419 (1968).
(42) A. Bigotto, G. Costa, G. Mestroni, G. Pellizer, A. Puxeddu, E. Reisenhofer, L. Stefani, and G. Tauzher, *Inorg. Chim. Acta Rev.*, **41**, 4 (1970).
(43) G. Costa, G. Mestroni, G. Tauzher, D. M. Goddall, and H. A. O. Hill, *Chem. Commun.*, 34 (1970).
(44) G. Costa, G. Mestroni, G. Tauzher, and L. Stefani, *J. Organomet. Chem.*, **6**, 181 (1966); G. Costa and G. Mestroni, *ibid.*, **11**, 325 (1968); G. Costa, G. Mestroni, and L. Stefani, *ibid.*, **7**, 493 (1967); G. Costa, G. Mestroni, and G. Pellizer, *ibid.*, **11**, 333 (1968).
(45) C. Giannotti, B. Septe, and D. Benlian, *J. Organomet. Chem.*, **39**, 5c (1972).
(46) G. Costa, G. Tauzher, and A. Puxeddu, *Inorg. Chim. Acta*, **3**, 41 (1969).
(47) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **88**, 3738 (1966).
(48) L. M. Ludwick, and T. L. Brown, *J. Am. Chem. Soc.*, **91**, 5188 (1969).
(49) T. L. Brown, L. M. Ludwick, and R. S. Stewart, *J. Am. Chem. Soc.*, **94**, 384 (1972).
(50) For ligation by L in a system in which dimers (D) of the alkylcobaloxime monomers (M) occur

$$D \underset{K_d}{\rightleftharpoons} M + M \overset{K_x}{\underset{\pm L}{\rightleftharpoons}} ML$$

the apparent equilibrium constant for ligand association (K_f) is given by

$$K_f = \frac{2K_x}{1 - b \pm (b^2 + 2K_d C_T)^{1/2}}$$

where $K_d = [M]^2/[D]$, $K_x = [ML]/[M][L]$, $b = (1 + K_x[L])/2$, and C_T is the total alkylcobaloxime concentration. The K_f values therefore depend upon the total alkylcobaloxime concentrations employed.
(51) A. V. Ablov and M. P. Filippov, *Zh. Neorg. Khim.*, **5**, 2717 (1960).
(52) B. A. Borykin, *Zh. Neorg. Khim.*, **17**, 169 (1972).
(53) G. P. Syrtsova, *Zh. Neorg. Khim.*, **17**, 172 (1972).
(54) J. E. Earley and J. G. Zimmerman, *Inorg. Nucl. Chem. Lett.*, **8**, 687 (1972).
(55) J. Zsako, Z. Finta, and C. S. Varhelyi, *J. Inorg. Nucl. Chem.*, **34**, 2887 (1972).
(56) G. Tauzher, R. Dreos, and G. Costa, *J. Chem. Soc., Chem. Commun.*, 413 (1973).
(57) T. Sakurai, J. P. Fox, and L. L. Ingraham, *Inorg. Chem.*, **10**, 1105 (1971).
(58) D. N. Hague and J. Halpern, *Inorg. Chem.*, **6**, 2059 (1967).
(59) R. B. Silverman, D. Dolphin, and B. M. Babior, *J. Am. Chem. Soc.*, **94**, 4028 (1972).
(60) H. A. O. Hill, K. G. Morallee, F. Cernivez, and G. Pellizer, *J. Am. Chem. Soc.*, **94**, 277 (1972).
(61) An exact treatment of the kinetics of SN1 mechanism of eq 5a with regard to the dependence of the observed k_{on} values upon pyridine concentration under circumstances in which "saturation kinetics" is observed will be included in a future publication. In this instance, "saturation kinetics" is the term applied to concave downward plots of k_{on} vs. [L] i.e., the progression from first-order to zero-order dependence upon the concentration of [L] of k_{on}. Such "saturation kinetics" reflect a change in rate-determining step from ligation of pentacoordinate alkylcobaloxime by L, k_2, to rate-determining dehydration of the RCo(D_2H_2)(HOH) species, k_1 (eq 5a): P. S. Tobias and R. G. Kallen, unpublished observations.

Substrate Induced p*K* Perturbations with Chymotrypsin and the Possible Significance of Nonproductive Binding[1]

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Abstract: The binding of the substrates *N*-acetyl-L-tyrosine *p*-chloroanilide and *N*-formyl-L-phenylalanine formylhydrazide to chymotrypsin is accompanied by a significant perturbation of the p*K* of a group on the enzyme. This perturbation does not occur with the closely related nonsubstrate derivatives *N*-formyl-D-phenylalanine formylhydrazide, *N*-formyl-*N*-methyl-L-phenylalanine formylhydrazide, *N*-acetyl-D-tyrosine *p*-chloroanilide, and *N*-acetyl-*N*-methyl-L-tyrosine *p*-chloroanilide. These results are consistent with a hypothesis that a component of the catalytic process can contribute to the binding of substrates. The recent suggestion that the nature of the aniline moiety in acylamino acid anilide substrates influences the apparent binding (K_m) by contributing to nonproductive binding could not be substantiated. It was found that at pH 7.13 *N*-acetyl-D-tyrosine *p*-chloroanilide, a compound which is especially suited for the proposed nonproductive binding mode, is not bound as well as is the corresponding L substrate. The rate of reaction of formylphenylalanylchymotrypsin with formylhydrazine and of acetyltyrosylchymotrypsin with *p*-chloroaniline and ammonia has the same pH dependence as that for hydrolysis of these acyl enzymes. These results rule out a previously proposed mechanism in which there is a pH-induced change in rate-determining step. The results are also not in accord with a mechanism suggested to account for the pH-dependent ^{15}N isotope effect seen in the hydrolysis of acetyl-L-tryptophan amide.

It has been found with chymotrypsin that with a number of substrates with a weakly basic amine leaving group the K_m is pH dependent in the neutral pH range.[2] To determine whether this behavior is a reflection of the binding process being influenced by the pH-dependent catalysis or by pH-dependent nonproductive binding, we have studied the pH dependence of the binding of nonsubstrate analogues of the compounds for which the unusual pH effect is observed. We also report results bearing on a proposed change in rate-determining step in the catalytic mechanism.[2c,3]