for performing the elemental analyses for antimony. They are thankful to H. W. Patrick for his skillful technical assistance.

Registry No. $pyHSbBr_4$, 52279-30-8; 2-$MepyHSbBr_4$, 50284-20-3; 3-$MepyHSbBr_4$, 52279-31-9; 4-$MepyHSbBr_4$, 89438-19-7; 2,4,6-$Me_3pyHSbBr_4$, 89438-20-0; 2-Br(py)$HSbBr_4$, 89438-21-1; 2-Cl(py)$HSbBr_4$, 89438-22-2; $(3\text{-}MepyH)_2SbBr_5$, 89438-23-3; $(4\text{-}MepyH)_2SbBr_5$, 89438-24-4; $(2,4\text{-}Me_2pyH)_3Sb_2Br_9$, 89486-22-6; $(2,4,6\text{-}Me_3pyH)_3Sb_2Br_9$, 89438-25-5; $(2\text{-}Br(py)H)_2SbBr_5$, 89438-26-6; $(3\text{-}COOHpyH)_2SbBr_5$, 89438-27-7.

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Acid–Base Properties of α-Ribazole and the Thermodynamics of Dimethylbenzimidazole Association in Alkylcobalamins

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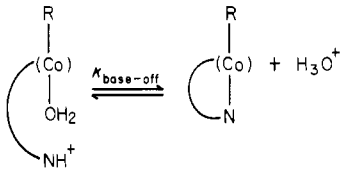
1-α-D-Ribofuranosyl-5,6-dimethylbenzimidazole (α-ribazole) has been prepared by cerous hydroxide catalyzed hydrolysis of cyanocobalamin, purified, and characterized by elemental analysis and ^1H and ^{13}C NMR and UV–visible spectroscopy. Values of the pK_a of N-3-protonated α-ribazole have been determined at several temperatures (ionic strength 1.0 M) and the pK_a of N-1-protonated α-ribazole has been estimated to be −7.2 from UV–visible spectral changes in sulfuric acid–water mixtures. Seven alkylcobalamins have been synthesized by standard reductive alkylation procedures and purified chromatographically. It has been found that reductive alkylation with CF_3Br produces mixtures of (trifluoromethyl)cobalamin and (difluoromethyl)cobalamin because the former is reductively converted to the latter by reducing agents commonly employed for reduction of cobalt(III) cobalamins to cob(I)alamin. The pK_a's for the base-on–base-off transition of these seven alkylcobalamins and methylcobalamin have been determined at the same temperatures as the α-ribazole pK_a's. From these values the apparent binding constants for ligation of the free-base benzimidazole nucleotide and the enthalpy and entropy changes for this ligand substitution have been calculated. The enthalpy change has been found to be quite insensitive to the nature of the organic ligand while the entropy change is quite sensitive. These results are discussed in terms of the probable importance of steric effects of the organic ligands on the base-on–base-off pK_a's of alkylcobalamins.

Introduction

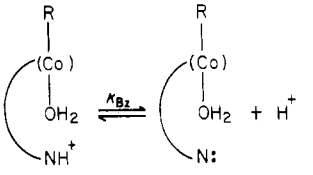
One of the most characteristic and perhaps one of the most thoroughly studied chemical properties of cobalamins is the so-called base-on–base-off reaction in which the axially coordinated dimethylbenzimidazole nucleotide is displayed by water and protonated (i.e., the reverse of eq 1). This reaction



(1)

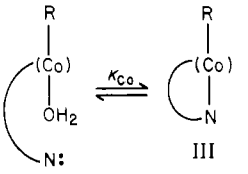
is sometimes referred to as the red–yellow shift due to the large changes in electronic spectrum attendant upon conversion of the base-on to the base-off form.[1] Values of $pK_{base\text{-}off}$ (eq 1) have been reported for a large number of cobalamins[2] and range from about 4.0 (*n*-heptyl(Cbl)[3] to −2.4 (H_2O(Cbl)[4]). Hogenkamp et al.[5] have shown that the values of $pK_{base\text{-}off}$ for most (but not all) of a series of alkylcobalamins can be successfully correlated with the Hammett σ_m substituent constant of the cobalt-bound alkyl group but that the values fall on two lines of approximately equal slope but different intercepts, one for substituted methylcobalamins and one for substituted ethylcobalamins. This observation has never been satisfactorily explained.

It is often pointed out that the base-on–base-off reaction of cobalamins (eq 1) may be viewed as the sum of two consecutive equilibria, i.e., the deprotonation of the benzimidazole-protonated base-off species (eq 2 and 3), and the substitution of free-base benzimidazole for water in the resulting deprotonated, base-off species (eq 4 and 5). Equation



I II (2)

$$K_{Bz} = [II][H^+]/[I] \quad (3)$$



II III (4)

$$K_{Co} = [III]/[II] \quad (5)$$

6 may then be derived (from consideration of the law of mass action), which relates $K_{base\text{-}off}$ to K_{Co} (eq 4 and 5) and K_{Bz} (eq 2 and 3) and allows calculation of K_{Co} from $K_{base\text{-}off}$ provided that a value of K_{Bz} is available. It is then often assumed that K_{Bz} for deprotonation of the base-off benzimidazolium species (I, eq 2) is approximately equivalent to that of the detached benzimidazolium ribonucleoside, i.e., the conjugate acid of 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole (or α-ribazole).

$$K_{base\text{-}off} = (1 + K_{Co})K_{Bz} \quad (6)$$

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Although the validity of this approximation is not yet clear, it seems reasonable given the remoteness of the substitution of the nucleoside ribose C-3 in the intact cobalamins from the site of benzimidazole protonation. It does, however, imply a lack of noncovalent interaction of the benzimidazole moiety with the remainder of the structure in both ionic species of bass-off cobalamins (I and II). Unfortunately, the value of the pK_a of α-ribazole has never been reported. The value of 4.70 (25 ± 1 °C) most often used in conjunction with eq 6 is that of the anomer 1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole.[6] This value seems quite low considering that these same authors found that the pK_a's of the conjugate acids of various N-1-substituted benzimidazoles vary by only ±0.25 pH unit from that of unsubstituted benzimidazole (pK_a = 5.48) while 5.6-dimethyl substitution causes an increase in pK_a to 5.98. This may imply substantial conformational effects on benzimidazolium acidity in benzimidazole nucleosides. We have consequently undertaken the preparation, purification, and characterization of α-ribazole. A thorough study of its acid–base properties along with those of several alkylcobalamins is the subject of this report.

Experimental Procedures

Materials. CH_3Cbl, $H_2O(Cbl)$, and CN(Cbl) were from Sigma and were used without further purification. All buffer components, inorganic salts and acids, chromatography solvents, alkylating agents, etc. were obtained in the highest purity commercially available and used without further purification except chloroacetic acid, which was dried in vacuo over P_2O_5, recrystallized from chloroform, and stored over desiccant. Glass-distilled deionized water was used throughout. SP-Sephadex (Sigma) was swelled and poured in water, rinsed with several column volumes of 0.5 M NaCl, and then water washed until the effluent was chloride free. Amberlite XAD-type 2 (Accurate Chemical and Scientific Corp., Hicksville, NY) was slurried and poured in 50% (v/v) acetonitrile and then washed with several column volumes of water before use.

α-Ribazole was prepared by cerous hydroxide catalyzed phosphodiester hydrolysis of CN(Cbl) by a modification of the method of Renz.[7] Factor B was removed from the hydrolyzate by extraction into phenol–chloroform (1:1) after the pH of the hydrolyzate had been adjusted to 3.5 with HCl. The remaining hydrolyzate containing α-ribazole was concentrated in vacuo and applied to a 3 × 30 cm column of AG50-X8, H^+ form. After the column was washed with water, α-ribazole was eluted with 0.5 M NH_3, concentrated, and crystallized from water at 0 °C. It was purified by repeated recrystallization from hot water. Anal. Calcd for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found (Galbraith Laboratories, Knoxville, TN): C, 60.20; H, 6.54; N, 9.91.

Alkylcobalamins were synthesized by reductive alkylation of $H_2O(Cbl)$ using sodium borohydride as the reducing agent and the appropriate alkylating agents (*n*-propyl bromide, 2,2,2-trifluoroethyl iodide, trifluorobromomethane, methyl bromoacetate, 4-bromobutyronitrile, methyl 3-bromopropionate, and difluorochloromethane) as follows: 2.0 g of $H_2O(Cbl)$ (1.49 mmol) was stirred in the dark in 100 mL of water under continuous argon purge for 1 h. A 0.25-g amount of $NaBH_4$ (0.026 mol) was dissolved in 2 mL of argon-purged water and injected by syringe into the reaction mixture. After 20 min of continuous purging and stirring the mixture had turned quite dark. A 5–10-fold molar excess of alkylating agent was then injected (either in neat form or as an aqueous solution in the minimal volume of agron-purged water), whereupon the mixture turned immediately red. After 10 min excess borohydride was destroyed by addition of 2 mL of acetone. The only exceptions to this procedure were for the gaseous alkylating agents CF_3Br, which was bubbled into the reaction solution for 30 min, and CF_2HCl, which was bubbled into the reaction solution for 1.5 h. Reaction mixtures were desalted by extraction through phenol, and the resulting aqueous solutions of cobalamins were concentrated to about 50 mL and applied to a 4 × 52 cm column of SP-Sephadex, sodium form, and eluted with water. After an initial faint pink band, the alkylcobalamins were eluted as a major red band.[8,9] $H_2O(Cbl)$, which remained on the column, was removed during regeneration by elution with 0.5 M NaCl. Final purification was affected by crystallization of the alkylcobalamins from concentrated aqueous solutions by addition of acetone. As $CH_3OOCCH_2CH_2Cbl$ proved unstable to the phenol extraction desalting procedure,[10] its preparation was repeated at smaller scale (0.25 g of $H_2O(Cbl)$) with desalting on Amberlite XAD.[11] The concentrated, aqueous reaction mixture was applied to a 2 × 20 cm column of Amberlite XAD and desalted by washing with several column volumes of water. The adsorbed cobalamins were then eluted with 50% (v/v) acetonitrile. Final purification was affected by chromatography on SP-Sephadex and crystallization from aqueous acetone. Yields of purified alkylcobalamins ranged from 80% ($CH_3CH_2CH_2Cbl$) to 20% ($CF_2H(Cbl)$).

A second preparation of CF_3Cbl with zinc and ammonium chloride as reducing agent was carried out as follows: 1.0 g of cyanocobalamin was dissolved in 100 mL of 10% NH_4Cl and stirred in the dark under continuous argon purge for 1 h. Reduction was initiated by addition of 10 g of zinc dust. After 1 h of vigorous stirring, CF_3Br was admitted to the argon gas stream and allowed to bubble through the reaction mixture for 30 min. The zinc dust was then removed by gravity filtration and the supernatant was reduced in volume to about 30 mL and applied to a 2 × 20 cm column of Amberlite XAD for desalting. The column was washed with 500 mL of water, by which time the effluent tested negative for chloride ion. The cobalamins were then removed by elution with 50% acetonitrile. After concentration, the mixture of cobalamins was applied to a 4 × 52 cm column of SP-Sephadex and eluted with water. After a minor red fraction, the major red fraction containing the organocobalamins was collected. As HPLC analysis (see below) showed this material to be greatly contamined with cyanocobalamin, it was purified by chromatography on a 1 × 54 cm column of Amberlite XAD, the CN(Cbl) eluting with 10% acetonitrile and the organocobalamins with 12–15% acetonitrile.

Resolution of the mixture of CF_3Cbl and $CF_2H(Cbl)$ obtained from the preparation with borohydride as reducing agent and CF_3Br as the alkylating agent was achieved by chromatography on Amberlite XAD. Prior to resolution the mixture was rechromatographed on SP-Sephadex to remove traces of $H_2O(Cbl)$ and some yellow impurities. In a typical run, 160 mg of the mixed solids was dissolved in about 50 mL of H_2O and concentrated to about 10 mL. This solution was applied to a 1 × 54 cm column of Amberlite XAD and eluted with 12% (v/v) acetonitrile. After 30 h of elution, the first fraction began to emerge. This fraction (F-1) was collected during 48 h of continuous elution, after which elution with 12% acetonitrile was continued for 15 h. This second fraction (F-2) was then eluted with 25% (v/v) acetonitrile (18 h). The average recovery of total cobalamins from three such runs was 88%, and the average ratio of F-2 to F-1 was 2.77 (by weight). Final purification of each of the pooled fractions was achieved by chromatography on SP-Sephadex and crystallization from aqueous acetone.

Conversion of CF_3Cbl to $CF_2H(Cbl)$ by treatment with sodium borohydride was carried out as follows: 108 mg of CF_3Cbl purified as above (i.e., Amberlite XAD F-2) was dissolved in 100 mL of 0.05 M potassium phosphate buffer, pH 7.4. A 0.189-g amount of $NaBH_4$ was then added, and the solution was stirred in the dark for 1.0 h. Following addition of 5 mL of acetone, the solution was reduced in volume to about 25 mL and desalted on Amberlite XAD, as above. $H_2O(Cbl)$ was then removed by chromatography on SP-Sephadex. The mixture of cobalamins that eluted from SP-Sephadex with water was separated by chromatography on a 1 × 54 cm column of Amberlite XAD. The mixed cobalamins (38.9 mg) were dissolved in about 10 mL of water and washed onto the column with water. Elution with 10% acetonitrile removed a minor yellow band. The first red band ($CF_2H(Cbl)$) was eluted with 12% acetonitrile, and the second red band (CF_3Cbl) was eluted with 25% acetonitrile. Both fractions were evaported to a small volume and diluted with acetone to precipitate the red solids.

Methods. All work with organocobalamins was performed in the dark, and solutions were covered with aluminum foil whenever practical. Ionic strength was maintained at 1.0 M with KCl. UV–visible spectra and single-wavelength absorbance measurements were

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obtained on a Cary 219 recording spectrophotometer whose cell block was maintained at the appropriate temperature (5.2, 15.1, 25.0, or 34.9 °C (all ±0.1 °C) as measured directly in a dummy cell with a thermistor device (Yellow Springs Instruments)) by a circulating water bath. pH measurements were made with a Radiometer PHM 64 pH meter with the electrode, samples, standards, and rinse water incubated at the appropriate temperature. NMR spectra were obtained on a Nicolet NT-200 wide-bore superconducting spectrometer (4.7 T) operating at 200.068 M Hz (^{1}H), 50.311 M Hz (^{13}C), or 188.238 M Hz (^{19}F) at 22 ± 2 °C. Paper chromatography was run on Whatman No. 1 in the ascending mode with use of the following solvent systems: I, butan-2-ol–water–ammonium hydroxide (50:36:14), II, *n*-butanol–ethanol–water (50:15:35); III, *n*-butanol–propan-2-ol–water (37:26:37);[12] IV, water-saturated butan-2-ol; V, *n*-butanol–water–ammonium hydroxide (100:36:14).[10] High-pressure liquid chromatography was performed on a Beckman Model 332 liquid chromatograph equipped with a Hitachi Model 100-40 wavelength detector (254 nm) and a 4.6 × 250 mm LiChrosorb C_8 column developing with a linear acetonitrile gradient from 5 to 30%.[13] A standard mixture of cobalamins was run before and after each set of samples and reliably gave the following retentions (R_f^{CN}, relative to cyanocobalamin): H_2O(Cbl) (0.88), HSO_3Cbl (0.90), CN(Cbl) (1.00), AdoCbl (1.08), CH_3Cbl (1.24). Mass spectral measurements were made on a Du Pont Model 321 GC/MS system equipped with a Riber 400 Model 1000 H data system and a 2 m × 3 mm Poropak Q column. CF_3Br analyses were performed at a column temperature of 50 °C and an ionizing potential of 70 eV.

Samples for anaerobic pyrolysis of (fluoromethyl)cobalamins (10 mg) were prepared in 1.0-mL Reactivials (Pierce) closed with Teflon Mininert valves (Pierce) as described previously.[14] They were pyrolyzed by immersion in a 225 °C oil bath for 5 min and cooled, and the gas space was sampled with a 500-μL gastight syringe. Analysis was performed on the GC/MS system using the Poropak Q column at 25 °C.

Alkylcobalamins were quantitated by conversion to dicyanocobalamin by exposure to light in the presence of 0.01 M KCN (0.01 M KOH) using $\epsilon_{368} = 30.4 \times 10^3$ M^{-1} cm^{-1}.[15] pK_a's of the conjugate acid of α-ribazole and p$K_{base\text{-}off}$'s of the alkylcobalamins were determined spectrophotometrically at 275 nm for α-ribazole and at the wavelength of maximal spectral change in the β region of the alkylcobalamin spectra (530–550 nm). Solutions (made in 3-mL, 1 cm path length quartz cells) contained chromophore, 0.1 M buffer (HCl, chloroacetate, acetate, or phosphate), and KCl (total ionic strength 1.0 M). They were incubated in the thermostated spectrophotometer cell compartment for at least 30 min prior to taking the absorbance measurement. Absorbance data were fit to eq 7 by

$$pH_x = pK_a + \log(|A_x - A_{AH}|/|A_{A^-} - A_x|) \quad (7)$$

the method of least squares, where A_x is the absorbance of the chromophore at pH_x, and A_{AH} and A_{A^-} are the titration end points (determined in duplicate), i.e., the absorbances at pH < pK_a − 2 and pH > pK_a + 2, respectively. The least-squares slopes of all such fits were within 2.5% of 1.00. In cases where the acid end point (i.e., A_{AH}) could not be reliably determined (e.g., CF_3Cbl, CF_2H(Cbl)), the data were fit to eq 8 in order to determine a value for A_{AH}, which was then used in conjunction with eq 7 to obtain the pK_a value.

$$|A_x - A_{A^-}| = |A_{AH} - A_{A^-}| - K_a|A_x - A_{A^-}|/[H^+] \quad (8)$$

Acidities of sulfuric acid–water mixtures were determined by duplicate titration of 100–500-μL aliquots of samples to a pH 7.0 end point with standard KOH. Values of H_0[16] were then determined from H_2SO_4 molarities with use of literature data,[17,18] with interpolation between data points as necessary.

Base-on alkylcobalamin photolysis rate constants were determined as follows: Solutions containing 4.15 × 10^{-5} M alkylcobalamin, 0.1 M phosphate buffer (pH 6.5), and KCl (ionic strength 1.0 M) were prepared in 1.0 cm path length quartz cuvettes and exposed to white light from a 275-W incandescent lamp at a distance of 75 cm. Periodically, the extent of conversion to aquocobalamin was determined by measurement of the absorbance at 351 nm (Cary 219 spectrophotometer). The only exception was for CF_3Cbl (3.88 × 10^{-5} M), for which conversion to aquocobalamin was determined by the decrease in absorbance at 358 nm. Semilogarithmic plots of the absorbance data, the slopes of which yielded values for k_{hv}, were linear for at least 3 half-times.

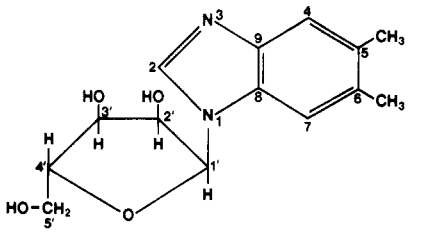

Figure 1. Structure and numbering scheme for 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole (α-ribazole).

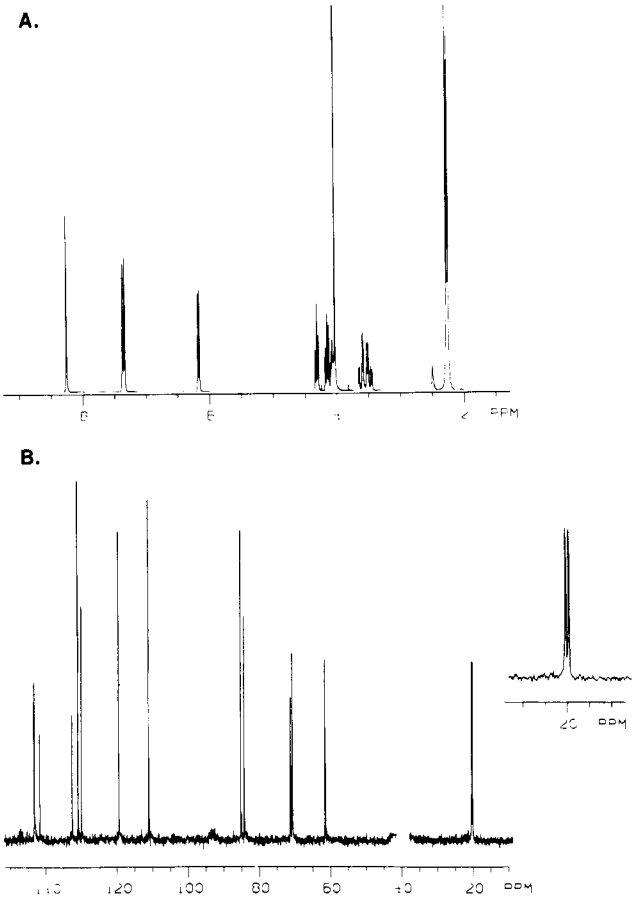

Figure 2. (A) ^{1}H NMR spectrum (200.068 MHz) of α-ribazole in Me_2SO-d_6. The H-4′ resonance is partially obscured by residual water in the solvent at 4.022 ppm. (B) ^{13}C NMR spectrum (50.311 MHz) of α-ribazole in Me_2SO-d_6. The solvent resonance at 39.5 ppm has been removed for clarity.

Results and Discussion

α-Ribazole. The structure and numbering scheme for α-ribazole are shown in Figure 1, and the ^{1}H and ^{13}C NMR spectra are shown in Figure 2. The ^{1}H NMR spectrum of α-ribazole is well resolved (Figure 2A) and, despite the somewhat complicated couplings of the ribose protons, has been essentially completely assigned by means of chemical shift, multiplicities, integration, and selective decoupling experiments including the diastereotopic C-5′ protons (Table I). Irradiation of the doublet at 6.170 ppm collapsed the multiplet at 4.306 ppm to a doublet from which the 2′–3′ coupling

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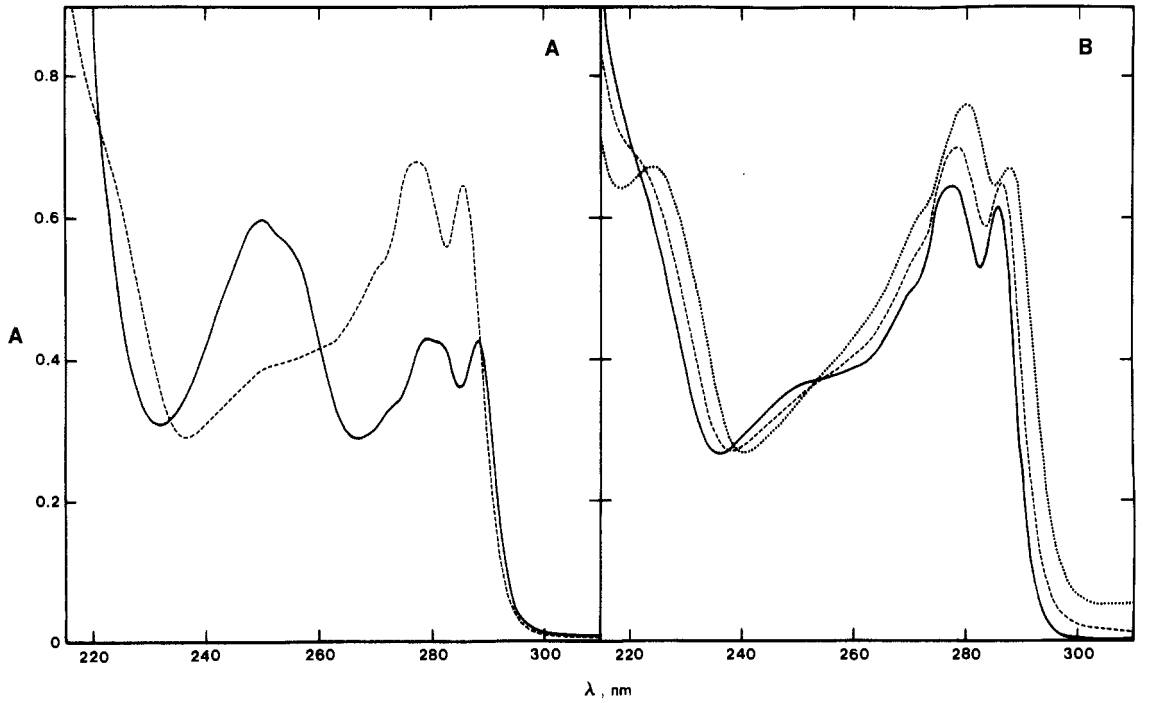

Figure 3. UV–visible spectra of α-ribazole, 7.55 × 10^{-5} M, at 25.0 °C: (A) pH 7.54 (—), pH 2.01 (---); (B) H_0 = 0.04 (—), H_0 = −3.72 (---), H = −8.32 (···).

Table I. ^{1}H and ^{13}C NMR Assignments for α-Ribazole[a]

group	δ^1H	coupling const, Hz	δ^{13}C
5-CH_3, 6-CH_3	2.270, 2.245	...	19.832, 20.138
2	8.255	...	142.957
4	7.366 or 7.336	...	119.179
5	...		130.631
6	...		129.661
7	7.366 or 7.336	...	110.833
8	...		132.281
9	...		141.452
1′	6.170	$J_{1'-2'}$ = 4.88	84.968
2′	4.306	$J_{2'-3'}$ = 4.88	70.508
3′	4.144	$J_{3'-4'}$ = 5.86	71.041
4′	4.068	$J_{4'-5'A}$ = 4.40 $J_{4'-5'B}$ = 2.93	83.998
5′A	3.424	$J_{5'A-5'B}$ = 12.20	61.288
5′B	3.663		

[a] In Me_2SO-d_6; all shifts reported from Me_4Si.

constant was obtained. Irradiation of the 4.306 ppm multiplet collapsed the 6.170 ppm doublet to a singlet and the 4.144 ppm multiplet to a doublet from which the 3′–4′ coupling constant was obtained. Irradiation of the 4.144 ppm multiplet collapsed the 4.306 ppm multiplet to a doublet from which the 1′–2′ coupling constant was obtained, and irradiation of the 4.068 ppm multiplet collapsed the multiplet at 3.40–3.65 ppm to an A–B quartet from which 5′A and 5′B chemical shifts and the 5′A–5′B coupling constant were calculated. Irradiation of the multiplet at 3.540 ppm collapsed the 4.068 ppm multiplet to a doublet from which the 3′–4′ coupling constant was obtained, and the 4′–5′A and 4′–5′B coupling constants were calculated from the coupled multiplet at 3.540 ppm. No unassignable peaks were found.

The proton noise-decoupled ^{13}C NMR spectrum of α-ribazole (Figure 2B) was cleanly resolved into 14 lines. Assignments (Table I) of the benzimidazole resonances were made by chemical shift and relative intensity and confirmed by observation of multiplicites due to residual C–H coupling during off-resonance proton noise decoupling. Ribose carbon

Table II. pK_a's of the Conjugate Acid of α-Ribazole at Various Temperatures (Ionic Strength 1.0 M)

temp, °C	pK_a	ΔH, kcal/mol	ΔS, eu
5.2 ± 0.1	5.90 ± 0.02		
15.1 ± 0.1	5.68 ± 0.01		
		+6.35 ± 0.44	−4.07 ± 1.51
25.0 ± 0.1	5.56 ± 0.01		
34.9 ± 0.1	5.40 ± 0.01		

assignments were made by chemical shift and selective continuous-wave irradiation of each of the ribose proton resonances and are strictly analogous to those of numerous other ribonucleosides and ribonucleotides.[19-21] These assignments were also in very good agreement with those of the nucleotide region of the ^{13}C NMR spectrum of CN(Cbl), which has recently been essentially completely assigned by Anton et al.[22] The only major difference is the ribose 2′- and 3′-carbons, whose resonances occur much closer together in CN(Cbl) (73.12 and 73.05 ppm) and have been assigned by these authors to the 3′- and 2′-carbons, respectively, on the basis of the observation of long-range C–P couplings in the intact nucleotide.

The acid–base behavior of α-ribazole has been investigated by UV–visible spectroscopy. Between pH 7.5 and 3.0 (Figure 3) major changes in the UV spectrum of α-ribazole are interpreted as resulting from protonation at N-3. Spectrophotometric titration at 275 nm and 25.0 °C gave a pK_a of 5.56 (Table II), a much more reasonable value than 4.70 considering the previously reported value of 5.98 for 5,6-dimethylbenzimidazole[6] and the observed effect of N-1 substitution, which changes the pK_a of benzimidazole by only ±0.25 pH unit (average ΔpK_a = 0.01 for seven N-1-substituted benzimidazoles). A value of 5.6 for the pK_a of α-ribazole was

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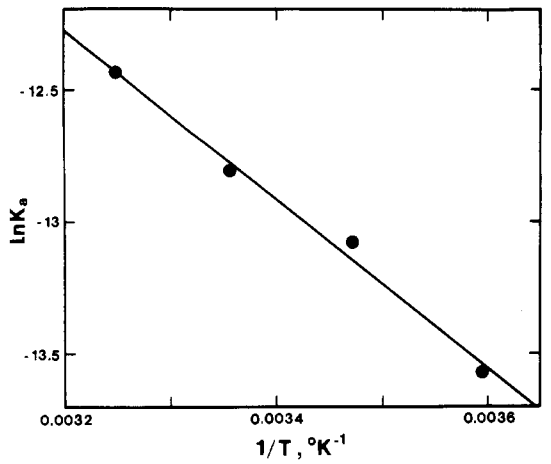

Figure 4. Plot of ln K_a for the conjugate acid of α-ribazole vs. $1/T$. The solid line is a least-squares fit: slope −3197 ± 223 K, intercept −2.05 ± 0.76.

previously reported by Jacobsen et al.[23] from pH-dependent fluorescence measurements. Spectrophotometric titration of α-ribazole was repeated at several other temperatures (Table II) so that the enthalpy and entropy of ionization could be calculated from a plot of ln K_a vs. $1/T$ (Figure 4). The values obtained (ΔH = +6.35 ± 0.44 kcal/mol, ΔS = −4.07 ± 1.51 eu) are in reasonable accord with the few existing literature values for 2-substituted benzimidazoles, which are characterized by relatively large positive enthalpies of ionization and relatively small positive or negative entropies of ionization.[24]

We have also investigated the acid–base behavior of α-ribazole in sulfuric acid–water mixtures as it has been suggested that protonation at N-1 may lead to specific-acid catalysis of benzimidazole dissociation in cyanocobalamin.[25] Relatively minor spectral changes are seen between H_0 = 0 and H_0 = −3.7 (Figure 3B), which can be attributed to protonation of the ribose hydroxyls.[26] Between H_0 = −5.0 and H_0 = −8.3 more major spectral changes occur, showing isosbestic points at 237 and 253 nm and a shift of a short-wavelength shoulder from 220 to 225 nm accompanied by a decrease in absorbance at 220 nm. At all H_0 values the observed spectrum was stable for at least 30 min. From the H_0-dependent spectral changes the pK_a for N-1-protonated α-ribazole was estimated to be −7.2.

Alkylcobalamins. All of the purified alkylcobalamins had electronic spectra that agreed well with those in the literature, where available,[5,10,27,28] and all underwent the expected red–yellow shift upon acidification with 0.1 N HCl. In addition, all of those materials were cleanly converted to aquocobalamin and/or hydroxocobalamin (depending on the pH) upon photolysis as demonstrated by UV–visible spectroscopy as well as HPLC analysis and all were converted to dicyanocobalamin upon incubation in excess cyanide and exposure to light. The latter reaction was used to quantitate cobalamins from the molar absorptivity of dicyanocobalamin at 368 nm (30.4×10^3 M^{-1} cm^{-1} [15]). In addition, all of the purified alkylcobalamins migrated as single spots in the various paper chromatograpy systems (Table III) and, more importantly, all save one migrated as single bands during HPLC analysis (Table III). The only exception was the "CF_3Cbl" preparation from CF_3Br under borohydride reduction, which showed two peaks, one at R_f^{CN} 1.20 and one at R_f^{CN} 1.25 at a ratio of about 1:2.24 (after correction for the differences in molar absorptivity at 254 nm). Both peaks were cleanly converted to hydroxocobalamin (R_f^{CN} = 0.88) upon exposure to visible light. Although the mobility of the slower moving band (1.25) is suspiciously close to that of CH_3Cbl (R_f^{CN} = 1.24), a coinjection showed them to be resolvable in this system.

Table III. Relative Mobilities of Alkylcobalamins on Paper Chromatography and HPLC[a]

	R_f^{CN} [a]					
compd	I	II	III	IV	V	HPLC
$CH_3CH_2CH_2Cbl$	1.91	1.35	1.41	1.90	1.87	1.35
CF_3CH_2Cbl	1.47	1.60	1.32	1.71	1.55	1.27
$NCCH_2CH_2CH_2Cbl$	1.25	1.10	1.14	1.09	0.97	1.22
CH_3OOCCH_2Cbl	1.19	1.63	1.40	1.28	1.92	1.13
$CH_3OOCCH_2CH_2Cbl$	dec	...	1.15	...	dec	1.22
CF_3Cbl	1.33	2.53	1.48	1.59	0.99	1.20, 1.25
$CF_2H(Cbl)$						1.20

[a] Mobilities are relative to CN(Cbl).

Preparative-scale resolution of the two apparent alkylcobalamins obtained from CF_3Br reaction with aquocobalamin after reduction with borohydride was achieved by slow elution of the mixture from a long Amberlite XAD column with 12% acetonitrile. The two materials were obtained in a ratio of 1:2.77 (by weight, average of four separate runs, the slower moving fraction being the larger). After final purification on SP-Sephadex each of the resolved cobalamins migrated as a single band in the HPLC assay—the larger, slower moving fraction having R_f^{CN} 1.25 and the smaller, faster moving fraction R_f^{CN} 1.20. ^{19}F NMR spectra in D_2O of the first Amberlite XAD fraction had a fluorine doublet at 18.261 ppm (downfield from external monofluorobenzene) with a coupling constant, J_{H-F}, of 53.2 Hz, while the second fraction had only a sharp singlet at 81.728 ppm (half-width 4.0 Hz). The unresolved mixture had both the downfield singlet and the upfield doublet in a molar ratio of 2.18, assuming the doublet has two fluorine atoms per mole and the singlet three. We have consequently assigned the less mobile species (R_f^{CN} 1.25) the structure CF_3Cbl and the more mobile (R_f^{CN} 1.20) the structure $CF_2H(Cbl)$. These assignments are in accordance with literature values for the difference in chemical shift ($\Delta\delta_{^{19}F}$) for various compounds of the type CF_3X and CF_2HX (50–62.7 ppm) and literature values for H–F geminal coupling constants for CF_2HX compounds (55–56 Hz).[29–34] It should be pointed out that these ^{19}F NMR results are in very poor agreement with those previously reported by Penley et al.,[27] who observed extremely broad ^{19}F resonances for these and other (fluoromethyl)cobalamins and -cobaloximes, reported no multiplicity for $CF_2H(Cbl)$, and reported an unlikely value of 117.8 ppm for the difference in ^{19}F chemical shift between CF_3Cbl and $CF_2H(Cbl)$. The reasons for these discrepancies are not at all clear. However, further confirmation of the identity of these two organocobalamins was obtained by observation of the gaseous products formed upon anaerobic pyrolysis. The material assigned the structure CF_3Cbl gave CF_3H as the only

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gaseous organic product detectable, while $CF_2H(Cbl)$ gave only CF_2H_2, both of the fluorocarbon products being positively identified by observation of their mass spectra. In addition, $CF_2H(Cbl)$ was independently synthesized from CF_2HCl and $H_2O(Cbl)$ with $NaBH_4$ as reducing agent. The alkylcobalamin product was in every respect identical with the more mobile species obtained in the alkylation with CF_3Br (i.e. $\delta_{^{19}F}$ = 18.246 ppm, J_{H-F} = 52.9 Hz, R_f^{CN}(HPLC) 1.20, identical electronic spectra).

At least three possibilities can be envisioned for the formation of $CF_2H(Cbl)$ in addition to CF_3Cbl during alkylation of Cob(I)alamin (from borohydride reduction) with CF_3Br: (1) the alkylating agent is contaminated with CF_2HBr; (2) under the reaction conditions the alkylating agent is partially transformed to a reagent (possibly difluorocarbene), which can react with reduced cobalamins to form CF_2HCbl; (3) under the reaction conditions, CF_3Cbl is converted to $CF_2H(Cbl)$. The first possibility was effectively eliminated by GC/MS analysis of the CF_3Br alkylating agent, which showed no evidence for the presence of CF_2HBr. The second possibility was not investigated when it was discovered that the third is evidently correct. Thus, while $CF_2H(Cbl)$ (R_f^{CN} 1.20) proved completely stable to treatment with 0.05 M $NaBH_4$ (in 0.05 M potassium phosphate buffer, pH 7.4) for 18 h in the dark, CF_3Cbl (R_f^{CN} 1.25) was completely decomosed after 1 h of this treatment into hydroxocobalamin (R_f^{CN} = 0.88, ca. 60%), an organocobalamin with R_f^{CN} 1.20 (i.e. $CF_2H(Cbl)$, ca. 20%), and an unidentified third cobalamin with R_f^{CN} 1.07 (ca. 20%). In a preparative-scale attempt to demonstrate this conversion, 108 mg of purified CF_3Cbl (R_f^{CN} 1.25) was stirred for 1 h with 0.05 M $NaBH_4$ in 0.05 M phosphate buffer, pH 7.4, in the dark. Evidently under these conditions the decomposition of CF_3Cbl was incomplete as the cobalamins that eluted from SP-Sephadex with water (about 40 mg) proved to be a mixture of CF_3Cbl (R_f^{CN} 1.25, ca. 60%). $CF_2H(Cbl)$ (R_f^{CN} 1.20, ca. 30%), and the unidentified cobalamin at R_f^{CN} 1.07 (ca. 10%). The remainder of the material (about 60 mg) was removed from SP-Sephadex with 0.5 M NaCl and proved to be hydroxocobalamin. The mixture of three cobalamins was resolved on Amberlite XAD, a minor yellow band (R_f^{CN} 1.07) eluting with 10% acetonitrile, followed by two red bands that were eluted with 12% and 25% acetonitrile, respectively. The first red band yielded 7.9 mg of red solid, which proved to be $CF_2H(Cbl)$ (i.e. R_f^{CN} 1.20, $\delta_{^{19}F}$ = 18.269 ppm, doublet, J_{H-F} = 53.7 Hz), while the second yielded 13.8 mg of CF_3Cbl (R_f^{CN} = 1.25, $\delta_{^{19}F}$ = 81.720 ppm, singlet, $w_{1/2}$ = 4.0 Hz).

Thinking that a hydride reducing agent was responsible for conversion of CF_3Cbl to $CF_2H(Cbl)$ under the synthesis conditions, we attempted a second preparation of CF_3Cbl by reductive alkylation of CN(Cbl) with CF_3Br using zinc and ammonium chloride as the reducing agent (i.e. the same conditions originally employed by Penley et al.[27]). The cobalamins that eluted from SP-Sephadex with water (0.614 g) proved to be substantially contaminated with cyanocobalamin (R_f^{CN} = 1.00). This material was removed by elution from Amberlite XAD with 10% acetonitrile. The organocobalamin fraction (275 mg) proved to be a mixture of 15% CF_3Cbl and 85% $CF_2H(Cbl)$ (by both HPLC and ^{19}F NMR), demonstrating that a hydride reducing agent is not required for the conversion.

Although several mechanisms can be envisioned for the conversion of CF_3Cbl to $CF_2H(Cbl)$ under the influence of such reducing agents, none seem very satisfactory considering experimental conditions. For instance, reductive C–Co cleavage of CF_3Cbl could lead to formation of cob(I)alamin and CF_3H. Presumably, the latter can alkylate reduced cobalt species, but the substantial dependence of the reactivity of alkyl halides with cob(I)alamin upon the nature of the halide leaving group (e.g. a nearly 4-order-of-magnitude decrease on going from CH_3Br to CH_3Cl[35]), coupled with the fact that the synthesis reaction mixtures were continually swept with argon/CF_3Br, makes this route seem unlikely. Another possibility might involve the thermally induced cleavage of CF_3Cbl to form difluorocarbene, which may then react with deduced cobalamins in water to form $CF_2H(Cbl)$. Although the known formation of difluorocarbene from pyrolysis of trimethyl(trifluoromethyl)tin[36,37] tends to support such a route, the apparent thermal stability of CF_3Cbl at room temperature would seem to refute it. Gaudemer and co-workers[38] have found a similar reductive lability of α-(haloalkyl)cobaloximes and found that (trifluoromethyl)cobaloxime was converted to methylcobaloxime (apparently via (fluoromethyl)- and (difluoromethyl)cobaloxime intermediates) when treated with $NaBH_4$.[39] These workers showed that carbon–cobalt bond cleavage did not occur during these conversions and that reduction of trifluorocobaloxime with $NaBH_4$ in CH_3OD led to formation of (trideuteriomethyl)cobaloxime. They consequently postulated a mechanism in which a two-electron reduction of the organocobalt complex followed by loss of α-halide leads to a carbene-like cobalt(III) complex, which then protonates to give the observed products. A similar mechanism may occur here with concomitant reductive carbon–cobalt bond cleavage (to give some HO(Cbl)) but with $CF_2H(Cbl)$ apparently being stable to further reduction.

Values of $pK_{base\text{-}off}$ (eq 1) have been determined for each of the eight alkylcobalamins at 5.2, 15.1, 25.0, and 34.9 °C and are listed in Table IV. Without exception, the temperature dependence of $pK_{base\text{-}off}$ for each R(Cbl) is extremely small, indicating that the enthalpy of the substitution of the free-base benzimidazole ligand for water (II → III, K_{Co}, eq 4 and 5) is nearly equal in magnitude but is of opposite sign to the enthalpy of ionization of the protonated, base-off alkylcobalamins (I → II, K_{Bz}, eq 2 and 3). If we make the common assumption that pK_{Bz} (eq 2 and 3) is equal to the pK_a of the conjugated acid of α-ribazole, then, using the values of this pK_a at the same temperatures (Table II), we may calculate values for the ligand substitution equilibrium constants (K_{Co}, eq 4 and 5) at each temperature, which are also given in Table IV. Unfortunately, given the difficulty of experimental determination of pK_{Bz}, it is currently impossible to evaluate the validity of this assumption. In theory, direct determination of pK_{Bz} should be possible from studies of the pH dependence of the rate of the base-on–base-off reaction of alkylcobalamins but such studies are hampered by the extremely high rates of these intramolecular reactions. For instance, Milton and Brown[40] have calculated a value of 2.09 × 10^3 s^{-1} (25 °C) for the first-order rate constant for benzimidazole dissociation from CH_3Cbl from line-shape analysis of the temperature dependence of the ^{13}C NMR resonance of $^{13}CH_3Cbl$. However, Reenstra and Jencks[25] have calculated a value of 5.0 for pK_{Bz} for base-off dicyanocobalamin (25 °C, 1.0 M ionic strength) from measurements of $pK_{base\text{-}off}$ (0.38) for CN(Cbl), equilibrium constants for addition of a second CN^- to base-on and base-off CN(Cbl), and the appropriate thermodynamic cycle. This value is substantially below that of 5.56 (Table II) currently reported for α-ribazole under the same conditions. More recently, Rubinson et al.[41] reported

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Table IV. $pK_{base\text{-}off}$ and K_{Co} Values for the Alkylcobalamins, R(Cbl), at Various Temperatures[a]

temp, °C		R = $CH_3(CH_2)_2$	CH_3	$N(CH_2)_3$	$CH_3OOC(CH_2)_2$	CF_3CH_2	CH_3OOCCH_2	CF_2H	CF_3
34.9	$pK_{base\text{-}off}$	4.12	2.92	3.56	3.37	2.69	2.44	2.15	1.48
	K_{Co}	1.81×10	3.00×10^2	6.90×10^1	1.06×10^2	5.19×10^2	9.17×10^2	1.77×10^3	8.37×10^3
25.0	$pK_{base\text{-}off}$	4.10	2.89	3.50	3.33	2.60	2.36	2.15	1.44
	K_{Co}	2.80×10	4.67×10^2	1.15×10^2	1.68×10^2	9.23×10^2	1.59×10^3	2.60×10^3	1.32×10^4
15.1	$pK_{base\text{-}off}$	4.12	2.86	3.48	3.30	2.59	2.31	2.13	1.41
	K_{Co}	3.56×10	6.56×10^2	1.57×10^2	2.38×10^2	1.24×10^3	2.37×10^3	3.52×10^3	1.85×10^4
5.2	$pK_{base\text{-}off}$	4.07	2.84	3.47	3.27	2.55	2.27	2.12	1.43
	K_{Co}	6.63×10	1.16×10^3	2.69×10^2	4.22×10^2	2.23×10^3	4.31×10^3	6.00×10^3	2.94×10^4
	ΔH_{Co},[b] kcal mol^{-1}	−7.12 ± 0.78	−7.49 ± 0.43	−7.55 ± 0.51	−7.75 ± 0.41	−8.02 ± 0.70	−8.62 ± 0.42	−6.82 ± 0.45	−7.04 ± 0.34
	ΔS_{Co},[b] eu	−17.4 ± 2.7	−13.0 ± 1.5	−16.1 ± 1.7	−16.9 ± 1.4	−13.5 ± 2.4	−14.6 ± 1.4	−7.32 ± 1.53	−4.86 ± 1.15
	σ^*[c]	−0.115	0	0.17	0.38	0.92	1.05	2.05	2.55
	E_s[d]	−0.36	0	−0.50	−0.90	−1.57	−0.66	−0.67	−1.16
	E_sR(Cbl)[e]	−1.13	0	−0.74	−0.74	−0.42	−0.28	−0.85	−0.53
	$10^2 k_{h\nu}$, s^{-1}	1.13	1.10	1.33	1.47	1.54	1.70	3.27	0.33

[a] All determinations at ionic strength 1.0 M (KCl). All $pK_{base\text{-}off}$ standard deviations were <0.01. [b] From plots of ln K_{Co} vs. $1/T$ (Figure 5). [c] Taken from ref 58 or calculated from ref 64. [d] Taken or estimated from ref 62 after renormalization to $E_s = 0$ for CH_3. [e] Calculated for R(Cbl)'s from eq 10 by using $\rho^* = 0.776$ and $\delta = 1.000$.

a value of 5.65 for pK_{Bz} for base-off cob(I)alamin (ionic strength 0.5 M, KCl) from pH-dependent spectroelectrochemical equilibrium measurements of aquocobalamin. Should this value turn out to be relevant to organocobalamins, it would represent a stunning confirmation of this assumption. Final evaluation of the legitimacy of this assumption will obviously have to await further experimental determinations of pK_{Bz} for alkylcobalamins. Nonetheless, if we accept the assumption, we can then use the calculated value of K_{Co} at each temperature to calculate the enthalpy (ΔH_{Co}) and entropy (ΔS_{Co}) changes associated with the ligand-exchange process of eq 4 from plots of ln K_{Co} vs. $1/T$ (Figure 5). The derived values are listed in Table IV. Surprisingly, the values of ΔH_{Co} are remarkably independent across the series of eight R(Cbl)'s, the average value being −7.55 ± 0.58 kcal mol^{-1} with six of the eight values falling within the mean plus or minus 1 standard deviation and all eight values falling within 90% confidence limits. Unfortunately, there is little literature data on the thermodynamics of ligand substitution reactions of cobalamins with which to compare the present results. However, our values of ΔH_{Co} compare favorable to the results of Eilbeck and West,[42] who reported values of −7.0 and −5.5 kcal mol^{-1} for the substitution of imidazole and 1-methylimidazole, respectively, for the upper axial water ligand of aquocobalamin. Of more direct relevance is the work of Chemaly and Pratt,[43] who estimated values of ΔH_{Co} and ΔS_{Co} for several alkylcobalamins from the temperature dependence of the electronic spectra of the base-on species in neutral, deionized water (i.e. at zero ionic strength). Although the values of ΔH_{Co} that these workers report for methyl-, ethyl-, and isobutylcobalamins (−3.6, −3.8, and −3.5 kcal mol^{-1}) are considerably smaller than our values, they are remarkably independent of the nature of the upper axial ligand. This apparent independence of the value of ΔH_{Co} of the organic ligand can be rationalized in terms of the ligand substitution process of eq 4, assuming that, over the temperature range studied, the base-off alkylcobalamins (II) exist predominately as the 6-coordinate aquo species as shown. Thus, the Co–O bond enthalpy of the base-off aquo species and the Co–N bond enthalpy of the base-on species (and any associated solvation enthalpy changes) must vary with the nature of the organic ligand in the same way so that the net difference for any

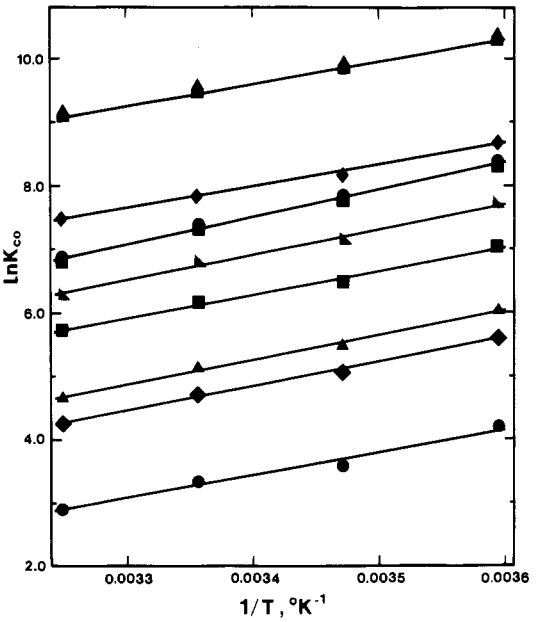

Figure 5. Plots of ln K_{Co} (eq 4–6) vs. $1/T$ for the R(Cbl)'s. The solid lines are least-squares fits from which the values of ΔH_{Co} and ΔS_{Co} (Table IV) have been determined (from top to bottom): CF_3Cbl, CF_2H(Cbl), CH_3OOCCH_2Cbl, CF_3CH_2Cbl, CH_3Cbl, $CH_3OOC(CH_2)_2$Cbl, $NC(CH_2)_3$Cbl, $CH_3CH_2CH_2$Cbl.

organocobalamin is about 7.5 kcal in favor of Co–N bond formation. This result is in line with what is known about the relatively poor affinity of oxygens donors for organocobalt centers compared to that of nitrogen donors.[44,45]

It should be pointed out that the nearly total lack of dependence of ΔH_{Co} on the nature of the organic ligand means that essentially all of the variability in the values of $pK_{base\text{-}off}$ (from 4.10 to 1.44 at 25 °C) across the series of alkylcobalamins is due to variations in ΔS_{Co} (Table IV). It should also be pointed out that this conclusion is not dependent on the assumption that pK_{Bz} is equal to the pK_a of the conjugate acid of α-ribazole but is due instead only to the observed independence of the values of $pK_{base\text{-}off}$ for each R(Cbl) on temperature. If pK_{Bz} is assumed to be 5.0 at 25 °C (i.e. the value reported by Reenstra and Jencks for dicyanocobalamin[25] and

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the upper limit of the values estimated by Chemaly and Pratt for various alkylcobalamins[43] and the enthalpy of ionization is assumed to be +6.35 kcal mol^{-1}, all eight values of ΔH_{Co} still fall within 90% confidence limits of the mean (−7.64 ± 0.56 kcal mol^{-1}) while the values of ΔS_{Co} become more negative by 0.4–4.7 eu.

The calculated values of ΔS_{Co} (Table IV) are startlingly large and negative with the exception of those for CF_2H(Cbl) and CF_3Cbl, and are, in general, in fairly good agreement with those of Chemaly and Pratt,[43] who reported values of −7.5, −10, and −9 eu for methyl-, ethyl-, and isobutylcobalamin, respectively. Considering that the conversion of II to III (eq 4) must be accompanied by a large increase in translational freedom of the water molecule coordinated to cobalt in the base-off species and one or more waters of solvation of the uncoordinated benzimidazole ligand, it is hard to believe that the decrease in rotational freedom of the nucleotide loop upon formation of the base-on species could possibly lead to such large net decreases in entropy. This suggests that formation of the base-on species is accompanied by a gross conformational reorganization of the molecule as a whole, leading to substantial decreases in rotational and fluxional freedom of the macrocycle and, most importantly, its peripheral side chains. X-ray crystal structures of base-on cobalamins[46-49] show that all of the peripheral acetamide side chains (a, c, and g) protrude above the corrin ring while the propionamide side chains (b, d, e, and f, the nucleotide loop side chain) protrude below the corrin ring and have led to the conclusion that the conformation of the corrin ring is largely determined by the need to reduce repulsions between the corrin substituents and between the corrin ring and the C-4 hydrogen of the benzimidazole nucleotide. This suggests that steric effects of the coordinated organic group may play a large role in determining ΔS_{Co} and hence K_{Co} and $pK_{base\text{-}off}$ for organocobalamins. There is also substantial experimental evidence[50] both for the importance of organic ligand steric effects on the labilization of the Co–C bond and for transmission of steric effects from the lower to the upper axial ligand position in that the C–Co bond of sterically strained organocobalamins (i.e. secondary organocobalamins and neopentyl- and benzylcobalamin) is substantially labilized by coordination of the axial benzimidazole.[51-57] Chemaly and Pratt[51] previously reached virtually the same conclusion, i.e., that $pK_{base\text{-}off}$ values of alkylcobalamins are largely determined by steric effects.

With these observations in mind, we have attempted to obtain linear free energy relationships of the K_{Co} values at 25 °C with parameters related to the organic ligands. Attempts to fit the K_{Co} data to a simple Taft equation (eq 9[58]), where

$$\log K_{Co} = \rho^*\sigma^* + \log K_{Co}^\circ \qquad (9)$$

σ^* is the electron inductive parameter of the alkyl group (Table IV), are largely unsuccessful. A fit to all eight data points gives $\rho^* = 0.786$, $\log K_{Co}^\circ = 2.07$, and a poor f value of 0.42 (f is the ratio of the root mean square of the deviations to the root mean square of the data values (after scaling to R = CH_3[59,60])), considering Topsom's criterion[60] of $f < 0.10$ for an excellent fit and $0.10 < f < 0.20$ for an acceptable fit. If the two points that deviate most from this correlation (R = CH_3 and $CH_3CH_2CH_2$) are omitted, the fit parameters remain essentially unchanged ($\rho^* = 0.788$, $\log K_{Co}^\circ = 2.06$) and the f value is improved to 0.26 but clearly still does not represent an acceptable fit. Interestingly, omitting the three organocobalamins with one-carbon alkyl ligands (CH_3Cbl, CF_2H-(Cbl), and CF_3Cbl) gives the best such correlation ($\rho^* = 1.43$, $\log K_{Co}^\circ = 1.69$, $f = 0.10$). These difficulties are reminiscent of those originally encountered by Hogenkamp et al.,[5] who attempted to fit $pK_{base\text{-}off}$ values for 10 organocobalamins to the Hammett σ_m substituent constant.

The previous dissociation of the importance of steric effects in determining $pK_{base\text{-}off}$ and hence K_{Co} values suggests that a modified Taft equation (eq 10[58,61]), in which K_{Co}° is the K_{Co}

$$\log (K_{Co}/K_{Co}^\circ) = \rho^*\sigma^* + \delta E_s \qquad (10)$$

value for CH_3Cbl, δ represents the susceptibility of the reaction to steric effects, and E_s is the substituent steric parameter, might provide better correlations. With use of the E_s values compiled by Unger and Hansch[62] (Table IV) a least-squares fit to eq 10 gave the values $\rho^* = 0.776$, $\delta = 0.589$, and $f = 0.56$. Interestingly, both ρ^* and δ have the expected signs (i.e., K_{Co} is increased by increasing electron withdrawal by the organic ligand but decreased by increased steric bulk) and the ρ^* value is unchanged from the simple correlation of all eight values without the steric term (i.e., eq 9). However, the f value is worse than that for the simple correlation based only on inductive effect. This is perhaps not surprising considering the fact that the E_s steric substituent constants were originally derived from correlations of the rates of acid-catalyzed hydrolysis of aliphatic esters, XCOOR, and presumably reflect the steric hindrance of the approach of a small nucleophile (i.e., H_2O) to the ester function by the substituent, X. It thus seems reasonable that such stric constants may have little or no relevance to the current situation, i.e., the steric effects of organic groups on formation of base-on organocobalamins. We consequently propose the use of eq 10 to *establish* a set of substituent constants, $E_s^{R(Cbl)}$, for use in correlations involving organocobalamins. This has been done by using the values 0.776 for ρ^* (i.e., the value obtained from fitting all eight K_{Co} values to either eq 9 or 10 using the Taft E_s values) and arbitrarily assigning a value of 1.00 to δ. The resulting $E_s^{R(Cbl)}$ constants are listed in Table IV. Obviously the utility of these constants must be ascertained by their ability to correlate R(Cbl) data totally independent of the current measurements of K_{Co}. Unfortunately, little such data are available in the literature for these organocobalamins. However, we have found that an equation analogous to eq 10 gives a good fit ($f = 0.12$, $\rho^* = 1.60$, $\delta = 0.72$) for a correlation of the energy of the γ-band electronic transition of the base-on organocobalamins (but *not* the base-off) relative to that of CH_3Cbl with use of the new $E_s^{R(Cbl)}$ constants. Even more encouraging is our attempt to correlate rate constants for base-on R(Cbl) photolysis reported by Hogenkamp et al.[5] Although only 4 of the 10 organocobalamins studied by these authors (R =

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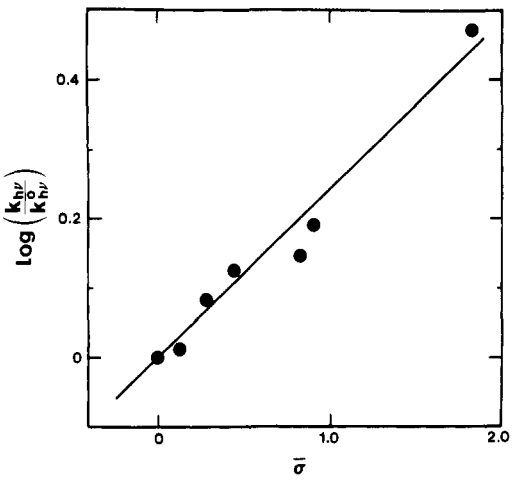

Figure 6. Derived, two-dimensional plot of the dual substituent parameter analysis of the rate constants for alkylcobalamin photolysis, $k_{h\nu}$, according to eq 12–15 ($\bar{\rho}$ = 0.242, λ = −0.239, f = 0.136).

CH_3, $CH_3CH_2CH_2$, CH_3OOCCH_2, and $CH_3OOCCH_2CH_2$) are included in our series, a fit of the logarithm of the ratio of the photolysis rate constant relative to that for CH_3Cbl gave an excellent fit (f = 0.05) with ρ^* = 0.31 and δ = −0.16, showing that both electron withdrawal *and* steric size increase the rate of photolytic C–Co cleavage.

This result was sufficiently encouraging to prompt us to measure photolysis rate constants for all eight of the organocobalamins in the present series. In agreement with Hogenkamp et al.,[5] we found the photolysis of the base-on R(Cbl)'s to be first order and obtained satisfactorily linear semilogarithmic plots for at least 3 half-times leading to the rate constants ($k_{h\nu}$) listed in Table IV. Inspection of these data shows that the rate constants for CF_3Cbl photolysis are anomalously low. Clearly any correlation in which both electron withdrawal and increased steric bulk will promote photolytic C–Co cleavage will demand that CF_3Cbl photolysis be faster than that of CH_3Cbl. The reasons for the anomalous photostability of CF_3Cbl are not at all clear. However, the other seven rate constants correlate well with eq 11, where $k_{h\nu}^{\circ}$

$$\log (k_{h\nu}/k_{h\nu}^{\circ}) = \rho^*\sigma^* + \delta E_s^{R(Cbl)} \quad (11)$$

is the photolysis rate constant for CH_3Cbl, giving ρ^* = 0.195, δ = −0.047, and f = 0.14, most of the deviation occurring in one data point (CF_3CH_2Cbl). This correlation is readily visualized with use of the graphical procedure of Wells et al.,[63] in which eq 11 is recast as eq 12

$$\log (k_{h\nu}/k_{h\nu}^{\circ}) = \bar{\sigma}\bar{\rho} \quad (12)$$

where

$$\bar{\rho} = \rho^* + |\delta| \quad (13)$$

$$\bar{\sigma} = (\sigma^* + \lambda E_s^{R(Cbl)})/(1 + |\lambda|) \quad (14)$$

and

$$\lambda = \delta/\rho^* \quad (15)$$

A plot of these data (excepting CF_3Cbl) according to eq 12–15 is shown in Figure 6 and gives some degree of confidence in the utility of the new $E_s^{R(Cbl)}$ values. However, further experimentation will be required to determine the general utility of this approach particularly in light of the failure of the $E_s^{R(Cbl)}$ value for CF_3Cbl.

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Registry No. α-Ribazole, 132-13-8; propyl bromide, 106-94-5; 2,2,2-trifluoroethyl iodide, 353-83-3; trifluorobromomethane, 75-63-8; methyl bromoacetate, 96-32-2; 4-bromobutyronitrile, 5332-06-9; methyl 3-bromopropionate, 3395-91-3; difluorochloromethane, 75-45-6; CNCbl, 68-19-9; H_2OCbl, 13422-52-1; $CH_3CH_2CH_2Cbl$, 13985-72-3; CF_3CH_2Cbl, 21180-98-3; $NCCH_2CH_2CH_2Cbl$, 89414-81-3; CH_3OOCCH_2Cbl, 15025-59-9; $CH_3OOCCH_2CH_2Cbl$, 14783-26-7; CF_3Cbl, 31532-05-5; CF_2HCbl, 28390-44-5.

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Stereochemical Aspects of the Thermal Substitution Reactions of Cobalt(III) Complexes

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On the basis of a simple ligand field model, the orbital and state correlation diagrams are calculated for the different five-coordinated structures intervening in the thermal substitution reactions of *cis*- and *trans*-$Co(en)_2AX^{n+}$ complexes (where X^- is the leaving ligand). Assuming a spin change along the reaction path of any stereomobile reaction, it is possible to derive general expressions showing the role of the inert ligand A in the activation energy. A comparison between theory and experiment reveals excellent agreement for the spontaneous and the induced aquations of *cis*- and *trans*-$Co(en)_2AX^{n+}$, the cis–trans isomerization of $Co(en)_2A(HO)^{m+}$, and the racemization of *cis*-$Co(en)_2A(H_2O)^{m+}$.

Introduction

In a previous paper,[1] it has been argued that any stereomobile reaction of a strong-field d^6 complex should be accompanied by a spin change somewhere along the reaction path. Here, we discuss a number of specific substitution reactions of $Co^{III}(en)_2AX^{n+}$ compounds in aqueous[2] solutions. By confining our attention to aqueous solutions—where the entering ligand E and the solvent molecule S are identical—we

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(2) We will restrict our attention exclusively to substitution reactions in acid solution. The corresponding base hydrolysis of the same complexes at higher pH apparently takes place by a D_{cb} mechanism, where one amine ligand loses a proton, giving rise to a strong π-donor amide. The presence of this second π ligand (in addition to A) modifies the picture to such an extent that significant changes can be expected, both in the kinetics and in the stereochemistry of these reactions.

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