for ca. 5 min. in a 0.1 M $HClO_4/H_2O$ solution containing excess Ce^{IV}. Partial regeneration of the emission occurs on re-reduction of the redox sites in the film to Ru^{II} by electrochemical reduction—potentiostating at +0.5 V for ca. 30 min—or by soaking in a solution containing a chemical reductant—ca. 15 min in a 0.1 M $TEAP/CH_3CN$ solution containing excess $(bpy)_2Ru(py)Cl^+$ ($E^{\circ\prime}_{soln}$ = +0.79 V).

Conclusions

The results described here show that a variety of reagents can be incorporated into *p*-chlorosulfonated polystyrene films if the reagnet contains an appropriate site, preferably an amine group, because of the stability of the resulting sulfonamide link. In addition, the polystyrene films can be cast onto virtually any surface by evaporation. As a consequence, the results described here are notable since *they offer a broadly based, general approach to the controlled incorporation of redox sites and combinations of redox sites into the electrode–film interface.* As shown by electrochemical, spectral, and excited-state measurements, the bound redox sites retain many of the properties of their solution analogues.

Because of their properties, the films have found use in a number of applications, some of which are shown in summary form in Scheme I and which include the following: (1) the co-incorporation of two redox couples and of a potential excited state/quencher pair, $[(bpy)_2Ru(5\text{-}phenNH\text{-})^{2+}]/[(CH_3)_2(NC_6H_4NH\text{-})]$, (2) hydrolysis and subsequent binding of cationic complexes by ion exchange, (3) the preparation of films containing relatively strongly emitting excited states, and (4) the preparation of redox bilayers.

Acknowledgments are made to the Army Research Office—Durham under Grant No. DAAG29-790C-0044 for support of this research. The authors gratefully acknowledge the initial work on the polystyrene samples by Dr. George Samuels and the gift of chemicals from Ruth Freitag, Dr. Russ Schmehl, and Professor Daryle H. Busch.

Contribution from the Department of Chemistry, The University of Texas at Arlington, Arlington, Texas 76019

Heteronuclear NMR Studies of Cobalamins. 2. ^{13}C and ^{31}P NMR Studies of [^{13}C]Cyanocobalamin[1]

KENNETH L. BROWN* and JANETTE M. HAKIMI

Received August 16, 1983

NMR observations of [^{13}C]cyanocobalamin (cyano-labeled) in sulfuric acid–water mixtures at 25 °C show that both the base-on and base-off species may be observed, and quantitated, in both ^{13}C and ^{31}P NMR spectra. Observation of the ^{13}C resonance of the base-off species has permitted, for the first time, assignment of the two ^{13}C resonances observed upon addition of 1 equiv of $K^{13}CN$ to diaquocobinamide and, by analogy, the two resonances observed upon addition of 2 equiv of $K^{13}CN$ to both diaquocobinamide and aquocobalamin. Correlation of the base-off ^{31}P chemical shifts of cyanocobalamin with a generalized acidity function for sulfuric acid–water mixtures leads to values of −1.57 and −0.04 for the two macroscopic pK_a's for phosphodiester deprotonation. A similar study of methylcobalamin gave values of −1.59 and −0.01 for these two macroscopic pK_a's. An upfield shift of 35.68 Hz in the ^{31}P chemical shift upon displacement of the axial base of cyanocobalamin by protonation is observed and attributed to changes in phosphodiester conformation upon base displacement. Analysis of the change in the relative amounts of base-on and base-off species as a function of acidity shows that the base-on–base-off pK_a of cyanocobalamin is relatively independent of the state of protonation of the phosphodiester moieties and has a value of 0.11 ± 0.01. At higher acidities a change in the ^{13}C chemical shift of base-off [^{13}C]cyanocobalamin is observed, which can be attributed either to a reversible protonation of coordinated cyanide (pK_a = −1.87) or to reversible loss of water from the lower coordination position due to the depressed activity of water at such acidities.

Introduction

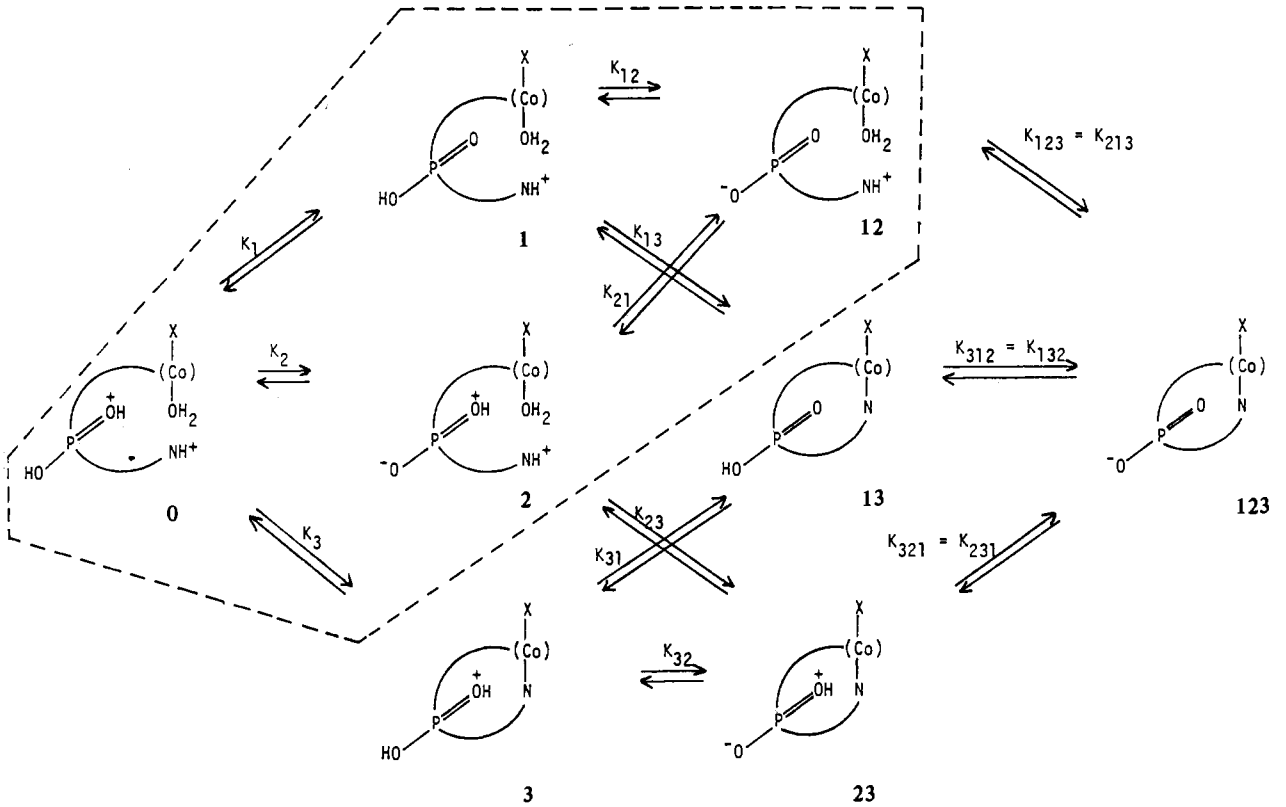
The coenzymatic forms of vitamin B_{12} are well-known to be involved in the catalysis of about 15 enzymic reactions in various organisms, including the 1,2-intramolecular rearrangements catalyzed by 5′-deoxyadenosylcobalamin (AdoCbl) requiring enzymes[2-5] and the methyl-transfer reactions catalyzed by methylcobalamin requiring enzymes.[2,5,6] As is the case with most coenzymes, the intimate details of the structure of cobalamins, well-known from X-ray crystallographic studies,[7-10] are difficult to relate directly to what is known of the function of cobalamin coenzymes.

As a sensitive probe of solution structure, nuclear magnetic resonance spectroscopy offers the possibility of understanding both the structure of cobalamins in functioning environments and the nature of the carbon–cobalt bond, the unique feature of the cobalamin coenzymes. Given the size of the structures, the ^1H NMR spectra of cobalamins are, of course, quite complex. Nonetheless, significant progress has been made in assigning the proton resonances and probing solution structure and dynamics via ^1H NMR,[11-16] particularly at very high field,

(1) For part 1, see ref 39a.
(2) Stadtmann, T. C. *Science (Washington, D.C.)* **1971**, *171*, 859-867.
(3) Babior, B. M. *Acc. Chem. Res.* **1975**, *8*, 376-384.
(4) Babior, B. M. In "Cobalamin"; Babior, B. M., Ed.; Wiley: New York, 1975; pp 141-214.
(5) Barker, H. A. *Annu. Rev. Biochem.* **1972**, *41*, 55-90.
(6) Stadtmann, T. C. In "Cobalamin"; Babior, B. M., Ed.; Wiley: New York, 1975; pp 111-140.
(7) Hodgkin, D. C. *Fortschr. Chem. Org. Naturst.* **1958**, *15*, 167-220.
(8) Hodgkin, D. S.: Lindsey, J.; Sparks, R. A.; Trueblood, K. N.; White, J. G. *Proc. R. Soc. London, Ser. A* **1962**, *266*, 494-517.
(9) Hodgkin, D. C. *Proc. R. Soc. London, Ser. A* **1965**, *288*, 294-305.
(10) Lenhert, P. G. *Proc. R. Soc. London, Ser. A* **1968**, *303*, 45-84.
(11) Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Williams, R. J. P. *J. Chem. Soc. A* **1968**, 564-567.
(12) Brodie, J. D.; Poe, M. *Biochemistry* **1971**, *10*, 914-922.
(13) Cockle, S. A.; Hill, H. A. O.; Williams, R. J. P.; Mann, B. E.; Pratt, J. M. *Biochim. Biophys. Acta* **1970**, *215*, 415-418.
(14) Hensens, O. D.; Hill, H. A. O.; Thornton, J.; Turner, A. M.; Williams, R. J. P. *Philos. Trans. R. Soc. London, Ser. B* **1976**, *273*, 353-357.
(15) Katada, M.; Tyagi, S.; Nath, A.; Peterson, R. L.; Gupta, R. K. *Biochim. Biophys. Acta* **1979**, *584*, 149-163.
(16) Milton, P. A.; Brown, T. L. *J. Am. Chem. Soc.* **1977**, *99*, 1390-1396.

0020-1669/84/1323-1756$01.50/0

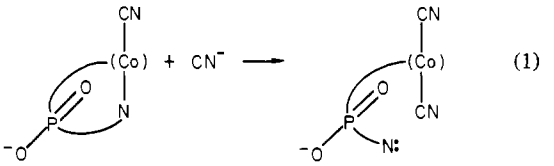
Scheme I



[a] Equilibrium constants subscripted $xx1$ are for P=OH$^+$ ionization, $xx2$ for P–OH ionization, and $xx3$ for benzimidazolium ionization and coordination to cobalt. The ionization scheme for the base-off phosphodiester is enclosed in the dashed line.

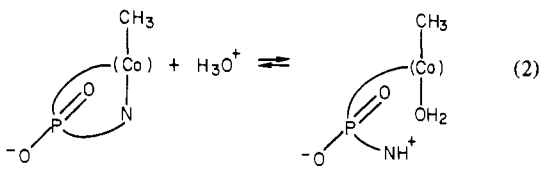
and via natural-abundance ^{13}C NMR,[17] culminating recently in the essentially complete assignment of the ^{13}C NMR spectrum of cyanocobalamin (CNCbl)[18] and both the ^{13}C and ^{1}H NMR spectra of heptamethyldicyanocobyrinate.[19] In addition, ^{13}C NMR studies of cobalamins and their derivatives selectively enriched with ^{13}C have provided new insights into structure–reactivity questions as well as the biosynthesis of vitamin B_{12}.[16,20-29]

Recently, Satterlee[30,31] and Mishra et al.[32] reported an upfield shift in the position of the ^{31}P NMR resonance of the phosphodiester moiety of the nucleotide loop of cyanocobalamin upon displacement of the axial base by excess cyanide (eq 1). We have been interested in this phenomenon not only



(1)

for its promise as a probe of the position of the nucleotide loop of cobalamins, which would presumably be independent of the state of ligation of the cobalt atoms and the electronic spectrum of the chromophore, but also because of substantial evidence that ^{31}P chemical shifts of phosphodiesters can serve as a sensitive probe of phosphodiester conformation.[32-38] While we have confirmed the upfield shift of the ^{31}P resonance of cyanocobalamin upon displacement of the axial base with excess cyanide (31.4 Hz at 81 MHz), we were unable to find any dependence of the ^{31}P chemical shift of methylcobalamin upon displacement of the axial base by protonation (eq 2)



(2)

although further lowering of the pH did cause a substantial upfield shift of the ^{31}P resonance due to protonation of the

(17) Ernst, L. *Liebigs Ann. Chem.* **1981**, 376–386.
(18) Anton, D. L.; Hogenkamp, H. P. C.; Walker, T. E.; Matwiyoff, N. A. *Biochemistry* **1982**, *21*, 2372–2378.
(19) Battersby, A. R.; Edington, C.; Fookes, C. J. R.; Hook, J. M. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2265–2277.
(20) Walker, T. E.; Hogenkamp, H. P. C.; Needham, T. E.; Matwiyoff, N. A. *J. Chem. Soc., Chem. Commun.* **1974**, 85–86.
(21) Scott, A. I.; Townsend, C. A.; Okada, K.; Kajiwara, M.; Cushley, R. J.; Whitman, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 8069–8080.
(22) Imfeld, M.; Townsend, C. A.; Arigoni, D. *J. Chem. Soc., Chem. Commun.* **1976**, 541–542.
(23) Battersby, A.; Hollenstein, R.; McDonald, E.; William, D. C. *J. Chem. Soc., Chem. Commun.* **1976**, 543–544.
(24) Scott, A. I.; Kajiwara, M.; Takahashi, T.; Armatase, I. M.; Denou, P.; Petrocine, D. *J. Chem. Soc., Chem. Commun.* **1976**, 544–546.
(25) Hogenkamp, H. P.; Tkachuck, R. D.; Grant, M. E.; Fuentes, R.; Matwiyoff, N. A. *Biochemistry* **1975**, *14*, 3707–3714.
(26) Hogenkamp, H. P.; Vergamin, P. J.; Matwiyoff, N. A. *J. Chem. Soc., Dalton Trans.* **1975**, 2628–2633.
(27) Hogenkamp, H. P.; Kohlmiller, N. A.; Howsinger, R.; Walker, T. E.; Matwiyoff, N. A. *J. Chem. Soc., Dalton Trans.* **1980**, 1668–1673.
(28) Needham, T. E.; Matwiyoff, N. A.; Walker, T. E.; Hogenkamp, H. P. C. *J. Am. Chem. Soc.* **1973**, *95*, 5019–5024.
(29) Anton, D. L.; Hogenkamp, H. P.; Walker, T. E.; Matwiyoff, N. A. *J. Am. Chem. Soc.* **1980**, *102*, 2215–2219.
(30) Satterlee, J. D. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 272–278.
(31) Satterlee, J. D. *Inorg. Chim. Acta* **1980**, *46*, 157–166.
(32) Mishra, P. K.; Gupta, R. K.; Goswami, P. C.; Venkatasubramanian, P. N.; Nath, A. *Biochim. Biophys. Acta* **1981**, *688*, 406–412.
(33) Gueron, M.; Shulman, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3482–3495.
(34) Patel, D. J. *Biopolymers* **1976**, *15*, 533–558.
(35) Patel, D. J.; Pardi, A.; Itakura, K. *Science (Washington, D.C.)* **1982**, *216*, 581–590.
(36) Gorenstein, D. G. *J. Am. Chem. Soc.* **1975**, *97*, 898–900.
(37) Gorenstein, D. G.; Kar, D. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 1073–1080.
(38) Gorenstein, D. G. *J. Am. Chem. Soc.* **1977**, *99*, 2254–2258.

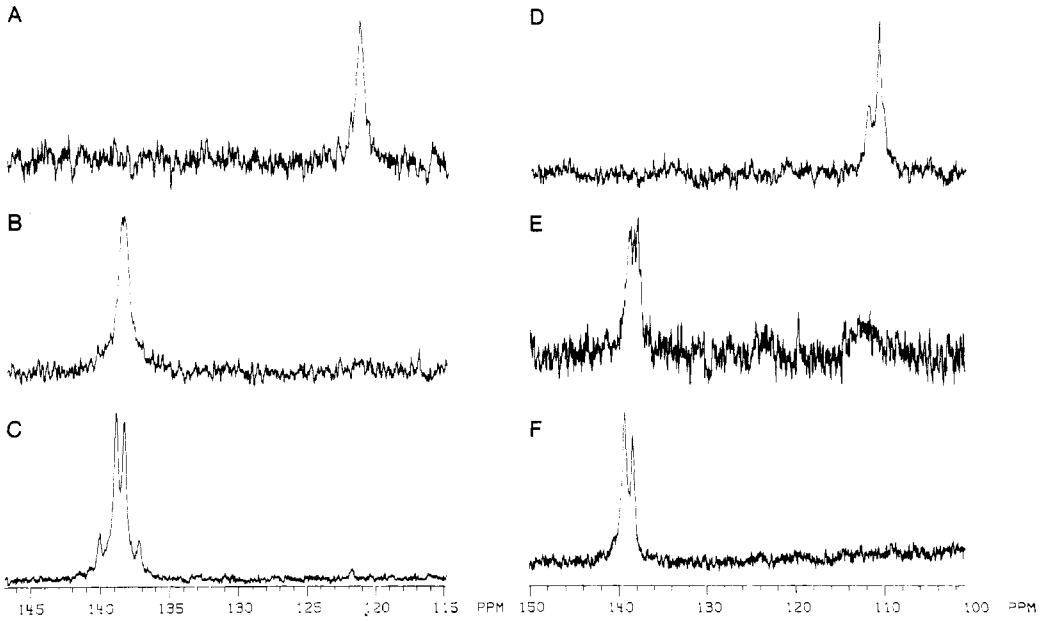


Figure 1. ^{13}C NMR spectra of aquocobalamin (H_2OCbl) and diaquocobinamide ($(H_2O)_2Cbi$) (0.01 M) plus added $K^{13}CN$ (chemical shifts relative to external Me_4Si): (A) H_2OCbl plus 1 equiv of $K^{13}CN$ at 34 °C, δ = 121.37, $w_{1/2}$ = 30 Hz; (B) H_2OCbl plus 2 equiv of $K^{13}CN$ at 34 °C, δ = 138.56, $w_{1/2}$ = 45.8 Hz; (C) same as (B) but at 15 °C, δ_A = 138.01, δ_B = 139.30, J_{C-Co-C} = 56.6 Hz, the four lines of the AB quartet occurring at 6905.12, 6961.01, 6990.42, and 7047.78 Hz; (D) $(H_2O)_2Cbi$ plus 1 equiv of $K^{13}CN$ at 25 °C, δ_1 = 110.61 (relative intensity 66.4%), δ_2 = 111.86 (relative intensity 33.6%), both $w_{1/2}$ ca. 20 Hz; (E) $(H_2O)_2Cbi$ plus 2 equiv of $K^{13}CN$ at 45 °C, δ = 138.42, $w_{1/2}$ ca. 78 Hz; (F) same as (E) but at 15 °C, δ_1 = 138.36, $w_{1/2}$ = 10.5 Hz, δ_2 = 139.35, $w_{1/2}$ = 15 Hz.

phosphodiester.[39a] We have consequently undertaken a more detailed study of the ^{31}P NMR properties of CNCbl and their dependence on the state of ligation of the nucleotide loop and the state of protonation of the phosphodiester moiety, which is the subject of this report. A preliminary account has been presented.[39b] Throughout this account the term "base-off ligandocobalamin" refers to the benzimidazole-protonated species (and phosphodiester-protonated conjugate acids, species **0**, **1**, **2**, and **12** in Scheme I) in which the benzimidazole nucleotide is not coordinated to cobalt and is presumably replaced by water. Dicyanocobalamin refers to the species generated by addition of excess CN^- to cyanocobalamin (eq 1) in which the dimethylbenzimidazole nucleotide is not coordinated to cobalt but remains unprotonated.

Experimental Procedures

Materials. Methylcobalamin, aquocobalamin, SP-Sephadex, and Sephadex G-10 were from Sigma, and $K^{13}CN$ (99% ^{13}C) was from Stohler Isotopes. Reagent-grade sulfuric acid was used without further purification, and glass-distilled, deionized water was used throughout.

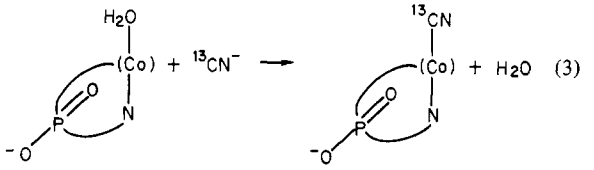
[^{13}C]Cyanocobalamin ($^{13}CNCbl$) was prepared as follows: 2.19 g (1.63 mmol) of aquocobalamin was stirred with 0.098 g (1.48 mmol) of $K^{13}CN$ in 100 mL of water for 1 h in the dark. The cobalamins were then desalted by extraction through phenol, and the resulting concentrated aqueous solution was applied to a 4 × 52 cm column of SP-Sephadex that had been swelled and poured in water. $^{13}CNCbl$ was eluted as a single red band with water[40,41] while excess aquocobalamin was removed by elution with 0.5 M NaCl. Diaquocobinamide ($(H_2O)_2Cbi$) was prepared by cerous hydroxide catalyzed hydrolysis of aquocobalamin by the procedure of Kerwer et al.[42] and purified by chromatography on Sephadex G-10.

Methods. ^{31}P NMR spectra were obtained on a Nicolet NT-200 wide-bore superconducting spectrometer operating at 4.7 T (observe frequency 80.988 MHz) as described previously,[39] except that samples were generally made 5.0 × 10^{-3}–1.0 × 10^{-2} M in cobalamins. Chemical shifts were determined relative to external 85% H_3PO_4. ^{13}C NMR spectra were obtained on the same instrument (observe frequency 50.312 MHz). A total of 8192 data points were collected over a 4000-Hz sweep width. Anywhere from 1000 to 50 000 transients were collected on 0.01 M samples with 7.5-μs 30° pulses and a cycle time of about 0.5 s. Generally speaking, signal-to-noise ratios were enhanced with use of exponential multiplication of the transient (line broadening 1–3 Hz). However, all line widths reported were obtained from spectra transformed without exponential multiplication. Chemical shifts were determined relative to external Me_4Si.

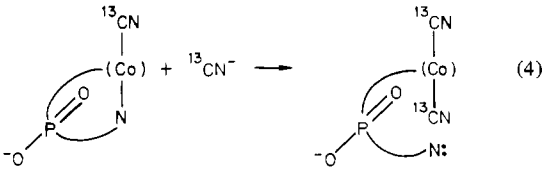
Acidities of sulfuric acid–water mixtures were determined by titration of duplicate aliquots (100–500 μL, with use of quantitative micro transfer pipets) of each NMR sample with standard KOH to a pH 7 end point (Radiometer PHM 64 pH meter). All such duplicate titrations agreed to within less than 1%. Values of various acidity functions were then obtained from the determined sulfuric acid molarities (or weight percent composition) from literature data, with use of linear interpolation between literature data points.

Results and Discussion

Addition of 1 equiv of $K^{13}CN$ to aquocobalamin (H_2OCbl) in water (eq 3) produces a ^{13}C NMR spectrum with a single resonance at 121.37 ppm and a line width of about 30 Hz (Figure 1A), in excellent agreement with the previous results of Satterlee.[31] Addition of a second equivalent of $K^{13}CN$ (eq



4) to form dicyanocobalamin ($(^{13}CN)_2Cbl$) produces, at 34 °C, a single broad ($w_{1/2}$ = 46 Hz) resonance at 138.56 ppm (Figure 1B), again in excellent agreement with Satterlee[31] and Needham et al.[28] However, when the sample is cooled to 15



(39) (a) Brown, K. L.; Hakimi, J. M. *Inorg. Chim. Acta* **1982**, *67*, L29–L31. (b) Brown, K. L.; Hakimi, J. M.; Mavynick, D. S. *Inorg. Chim. Acta* **1983**, *79*, 120–122.
(40) Tortolani, G.; Bianchini, P.; Mantovani, V. *J. Chromatogr.* **1970**, *53*, 577–579.
(41) Tortolani, G.; Mantovani, V. *J. Chromatogr.* **1974**, *92*, 201–206.
(42) Kerwer, S. S.; Mangum, J. H.; Scrigmeour, K. G.; Brodie, J. D.; Huennekens, F. M. *Arch. Biochem. Biophys.* **1966**, *116*, 305–318.

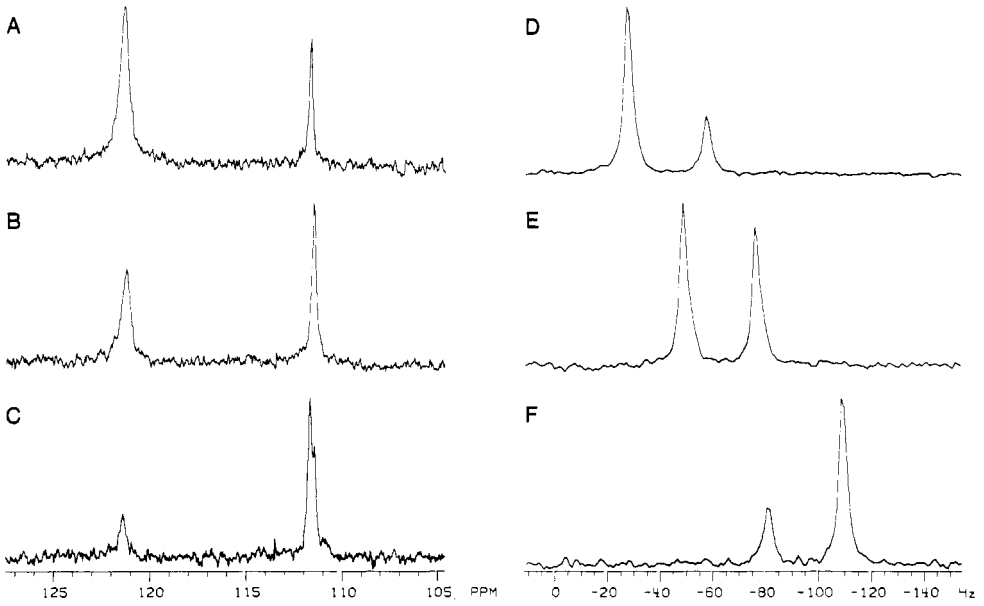
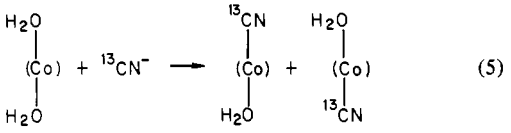


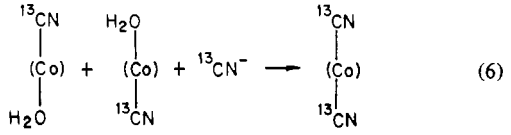
Figure 2. ^{13}C and ^{31}P NMR spectra of $^{13}CNCbl$ (0.01 M) in sulfuric acid–water mixtures at 25 °C (chemical shifts relative to external Me_4Si or 85% H_3PO_4): (A) ^{13}C NMR at $H_0 = 0.36$, $\delta_1 = 111.65$, $\delta_2 = 121.35$, $\alpha_{base\text{-}on} = 0.741$; (B) ^{13}C NMR at $H_0 = 0.03$, $\delta_1 = 111.49$, $\delta_2 = 121.29$, $\alpha_{base\text{-}on} = 0.518$; (C) ^{13}C NMR at $H_0 = -0.52$, $\delta_1 = 111.66$, $\delta_2 = 121.37$, $\alpha_{base\text{-}on} = 0.280$; (D) ^{31}P NMR at $H_0 = 0.36$, $\delta_1 = -57.80$ Hz, $\delta_2 = -27.84$ Hz, $\alpha_{base\text{-}on} = 0.753$; (E) ^{31}P NMR at $H_0 = 0.03$, $\delta_1 = -76.56$ Hz, $\delta_2 = -48.87$ Hz, $\alpha_{base\text{-}on} = 0.535$; (F) ^{31}P NMR at $H_0 = -0.52$, $\delta_1 = -109.65$ Hz, $\delta_2 = -81.30$ Hz, $\alpha_{base\text{-}on} = 0.290$.

°C, this spectrum is cleanly resolved into an AB quartet (Figure 1C) from which the chemical shifts 138.01 and 139.30 ppm and the C–Co–C coupling constant of 56.6 Hz can be extracted. This observation is reasonable, and expected, in that the magnetic environments of the two axial ligand positions of cobalt corrins have been shown to be nonequivalent (vide infra and Needham et al.[28]) and the exchange rate of the two axial cyanide ligands of $(CN)_2Cbl$ has been shown to be reasonably slow at 25 °C.[43] Also in agreement with Needham et al.[28] addition of 1 equiv of $K^{13}CN$ to diaquocobinamide ($(H_2O)_2Cbi$) (eq 5) leads to a ^{13}C NMR spectrum



(5)

(Figure 1D) with two closely spaced resonances, one at 111.86 ppm and one at 110.61 ppm in a ratio of about 1:2. Addition of a second equivalent of $K^{13}CN$ (eq 6) leads to an exceedingly broad resonance ($w_{1/2} \approx 78$ Hz at 45 °C) at 138.42 ppm (Figure 1E), which, when the sample is cooled to 15 °C, is resolved into singlets (Figure 1F) at 139.35 ppm ($w_{1/2} = 15$ Hz) and 138.36 ppm ($w_{1/2} = 10$ Hz), but no evidence of carbon–carbon spin coupling was found.



(6)

At all H_0 values[44-46] between +1.0 ($[H_2SO_4] = 0.0732$ M) and −0.5 ($[H_2SO_4] = 1.196$ M), the ^{13}C NMR spectrum of $^{13}CNCbl$ (Figure 2A–C) consists of two resonances: one at

Table I. ^{13}C Chemical Shifts of ^{13}C-Labeled Cyanocobalt Corrins

compd[a]	$\delta_{^{13}C}$[b]	$w_{1/2}$, Hz[c]
$^{13}CNCbl$, base-on	121.40[d]	23
$^{13}CNCbl$, base-off	111.77[e]	9
(β-^{13}CN)(α-H_2O)Cbi	111.86	20
(β-H_2O)(α-^{13}CN)Cbi	110.61	20
$(^{13}CN)_2Cbl$		
β-^{13}CN	139.30	
α-^{13}CN	138.01	
$(^{13}CN)_2Cbi$		
β-^{13}CN	139.35	15
α-^{13}CN	138.36	10

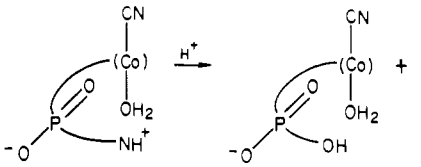
[a] β signifies the upper axial ligand and α the lower. [b] From external Me_4Si. [c] From Gaussian line fits of spectra transformed without exponential multiplication. [d] ±0.17 ppm, average of 15 observations at various acidities. [e] ±0.18 ppm, average of 10 observations at various acidities between $H_0 = +1.25$ and −0.01.

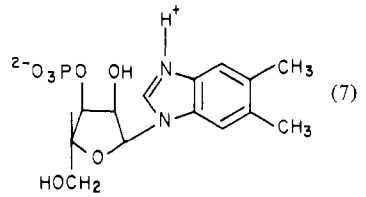
111.77 ± 0.18 ppm (average of 10 observations at various acidities) with a line width of 9 Hz and one at 121.40 ± 0.17 ppm (average of 15 observations at various acidities) with a line width of 23 Hz. As the intensity of the former increases, while that of the latter decreases with increasing acidity, they are assigned to the base-off and base-on species, respectively (Table I). No time dependence in the relative intensity of the two resonances is observed at any acidity, indicating that while exchange between the base-on and base-off species is slow on the NMR time scale, equilibrium is achieved rapidly on the time scale of sample preparation and observation. The significantly increased line widths of carbon atoms attached to cobalt (i.e., compare free $^{13}CN^-$, $\delta = 161.58$, $w_{1/2} = 5.0$ Hz) are generally attributed to quadrupolar relaxation of the cobalt-bonded spin by the ^{59}Co nucleus ($I = {}^7/_2$).[28,31,47] If this is indeed the case, the substantially narrower line width of base-off $^{13}CNCbl$ compared to that of the base-on species implies a significant increase in the carbon–cobalt bond length upon displacement of the axial base by protonation, although other explanations are possible. As the chemical shift of the base-off $^{13}CNCbl$ (111.77 ± 0.18 ppm) agrees precisely with

(43) Reenstra, W. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 5780–5791.
(44) Hammett, L. P.; Deyrup, A. J. *J. Am. Chem. Soc.* **1932**, *54*, 2721–2739.
(45) Bascombe, K. N.; Bell, R. P. *J. Chem. Soc.* **1959**, 1096–1104.
(46) Ryabova, R. S.; Medvetskaya, I. M.; Vinnik, M. T. *Zh. Fiz. Khim.* **1966**, *40*, 339–345.
(47) Coleman, V. M.; Taylor, L. T. *J. Inorg. Nucl. Chem.* **1981**, *43*, 3217–3219.

the more downfield of the two resonances observed for cyanoaquocobinamide (Figure 1D, δ = 111.86), the latter resonance can be assigned to the β-cyano-α-aquocobinamide isomer (i.e., cyanide in the upper axial ligand position) while the more upfield resonance (Figure 1D, δ = 110.61) is assigned to the β-aquo-α-cyanocobinamide isomer. By analogy, the more downfield of the two resonances of dicyanocobalamin (Figure 1C) and dicyanocobinamide (Figure 1F) at 15 °C can be assigned to the β-cyano ligand and the more upfield to the α-cyano ligand. These assignments, which have not been previously made since the base-off carbon resonance of ^{13}CNCbl has never been observed before, are summarized in Table I. Interestingly, in the formation of the two isomeric cyanoaquocobinamides by addition of K^{13}CN to diaquocobinamide (Figure 1D, eq 5), formation of the α-cyano isomer is favored over formation of the β-cyano isomer by about 2:1, presumably indicating greater steric crowding of the upper axial ligand position relative to that for the lower. A similar preference for ligation at the lower axial ligand position (trans to CN$^-$) has been shown by Reenstra and Jencks,[43] who obtained equilibrium constants of 2.1 × 10^8 M^{-1} for addition of a lower axial CN$^-$ to base-off β-cyano-α-aquocobalamin and 0.8 × 10^8 M^{-1} for addition of an upper axial CN$^-$ to base-off β-aquo-α-cyanocobalamin.

Similar to the ^{13}C NMR spectrum of ^{13}CNCbl in H_2SO_4–water mixtures, the ^{31}P NMR spectrum of CNCbl also shows two well-defined resonances separated by about 30 Hz ($w_{1/2}$ about 2 Hz) at all acidities between H_0 = +1.0 and −0.5 (Figure 2D–F) with the downfield member decreasing, while the upfield member increases in intensity with increasing acidity. Again, no time dependence in the relative intensity of the two resonances can be observed. By analogy with the ^{13}C spectra, the downfield resonance is assigned to the base-on species and the upfield resonance to the base-off. However, in this case the chemical shifts of both resonances move upfield with increasing acidity due to protonation of the phosphodiester moiety.[39a] In proton-coupled ^{31}P NMR spectra both of these phosphodiester resonances appear as symmetrical triplets (J_{H-P} ca. 8 Hz) due to spin coupling to the two structurally dissimilar but evidently magnetically equivalent hydrogens on either side of the phosphodiester moiety as previously discussed.[31,39a] At stronger acidities (H_0 less than −1.5) and/or longer scan times direct evidence of phosphodiester hydrolysis is observed in the time-dependent formation of two additional ^{31}P resonances downfield from the phosphodiester resonances of CNCbl, whose chemical shifts also move upfield with increasing acidity. For example, at H_0 = −2.44, where only the base-off phosphodiester resonance of CNCbl can be observed (δ = −188.5 Hz), two additional resonances of nearly equal intensity are observed at −118.2 Hz and −80.1 Hz whose combined intensity increases with time at the expense of the phosphodiester resonance at −188.5 Hz. In proton-coupled ^{31}P NMR spectra both of these resonances appear as symmetrical doublets (J_{H-P} ca. 8 Hz) as opposed to the triplets seen for the proton-coupled phosphodiester ^{31}P resonances. We can consequently assign these resonances to the two expected phosphomonoester intermediates in the net hydrolysis of the phosphodiester linkage (eq 7), i.e., cyanoaquocobinamide phosphate and α-ribazole 3′-phosphate, although we cannot tell which resonance is due to which phosphomonoester. At even longer scan times the intensity of both these resonances decreases with concomitant formation of a new resonance at even lower field (e.g., δ = +52.0 Hz at H_0 = −2.0) whose chemical shift is H_0 dependent and which appears as a singlet in both proton-coupled and proton-decoupled spectra, indicating formation of the final phosphorus-containing hydrolysis product, phosphoric acid (and/or its conjugate ionic forms depending on the acidity). In ^{13}C NMR spectra of ^{13}CNCbl, phosphodiester hydrolysis

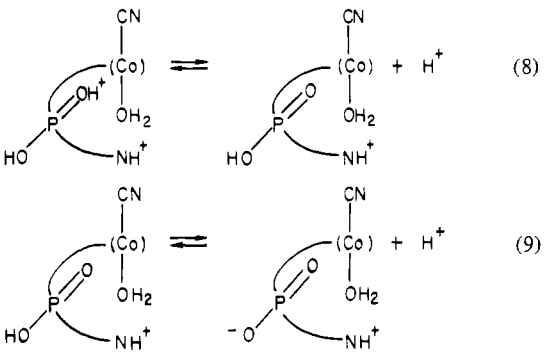




(7)

under the appropriate conditions is observed to cause an upfield shift of 0.20 ppm in the cyanide resonance of the base-off species (c.f. the upfield shoulder on the base-off ^{13}C resonance in Figure 2C, an overnight run at H_0 = −0.52).

In order to attempt to evaluate the pK_a's for ionization of the protonated phosphodiester moieties as well as for the base-on–base-off transition, we have studied the ^{13}C and ^{31}P NMR spectra of ^{13}CNCbl and CNCbl as a function of acidity. A plot (not shown) of the ^{31}P chemical shift of the base-off species ($\delta_{^{31}P}^{base\text{-}off}$), for which the most extensive data could be obtained, vs. H_0 clearly showed evidence of two sequential protonations, which must be assigned to the first (eq 8) and second (eq 9) phosphodiester ionizations. The complete



(8)

(9)

ionization scheme for CNCbl (Scheme I) thus includes eight microscopic species and 12 microscopic pK_a's from which three macroscopic pK_a's for overall ionization can be defined as in eq 10–15. As we can independently observe the ^{31}P resonance

$$G_1 = \frac{([\mathbf{1}] + [\mathbf{2}] + [\mathbf{3}])a_{H^+}}{[\mathbf{0}]} \quad (10)$$

$$G_1 = K_1 + K_2 + K_3 \quad (11)$$

$$G_2 = \frac{([\mathbf{12}] + [\mathbf{13}] + [\mathbf{23}])a_{H^+}}{[\mathbf{1}] + [\mathbf{2}] + [\mathbf{3}]} \quad (12)$$

$$G_2 = \frac{K_1K_{12} + K_1K_{13} + K_2K_{23}}{K_1 + K_2 + K_3} \quad (13)$$

$$G_3 = \frac{[\mathbf{123}]a_{H^+}}{[\mathbf{12}] + [\mathbf{13}] + [\mathbf{23}]} \quad (14)$$

$$G_3 = \frac{K_1K_{12}K_{123}}{K_1K_{12} + K_1K_{13} + K_2K_{23}} \quad (15)$$

of the base-off species (**0**, **1**, **2**, and **12** in Scheme I) and the base-on species (**3**, **13**, **23**, and **123**), we can also define two additional macroscopic pK_a's for base-off (eq 16–19) and base-on (eq 20–23) phosphodiester ionization. From the

$$G_4 = \frac{([1] + [2])a_{H^+}}{[0]} \quad (16)$$

$$G_4 = K_1 + K_2 \quad (17)$$

$$G_5 = \frac{[12]a_{H^+}}{[1] + [2]} \quad (18)$$

$$G_5 = K_2K_{21}/(K_1 + K_2) \quad (19)$$

$$G_6 = \frac{([13] + [23])a_{H^+}}{[3]} \quad (20)$$

$$G_6 = K_{31} + K_{32} \quad (21)$$

$$G_7 = \frac{[123]a_{H^+}}{[13] + [23]} \quad (22)$$

$$G_7 = K_{31}K_{312}/(K_{31} + K_{32}) \quad (23)$$

definitions of the macroscopic and microscopic pK_a's and the law of mass action, we can then derive equations for the dependence of the base-off (eq 24) and base-on (eq 25) ^{31}P

$$\delta_{^{31}P}{}^{\text{base-off}} = [\delta^{(0)} + (K_1\delta^{(1)} + K_2\delta^{(2)})/a_{H^+} + G_4G_5\delta^{(12)}/a_{H^+}{}^2]/[1 + G_4/a_{H^+} + G_4G_5/a_{H^+}{}^2] \quad (24)$$

$$\delta_{^{31}P}{}^{\text{base-on}} = [\delta^{(3)} + (K_{31}\delta^{(13)} + K_{32}\delta^{(23)})/a_{H^+} + G_6G_7\delta^{(123)}/a_{H^+}{}^2]/[1 + G_6/a_{H^+} + G_6G_7/a_{H^+}{}^2] \quad (25)$$

chemical shifts on the activity of hydrogen ion, where $\delta^{(0)}$, $\delta^{(1)}$, etc. represent the ^{31}P chemical shifts of the ionic species **1**, **2**, etc. (Scheme I). In addition, since we can evaluate the fraction of base-on species present at any acidity (i.e., $\alpha_{\text{base-on}}$) from integration of the base-on and base-off resonances in both the ^{13}C and the ^{31}P NMR spectra, we can also derive eq 26, which gives the dependence of $\alpha_{\text{base-on}}$ on the activity of hydrogen ion.

$$\alpha_{\text{base-on}} = \frac{K_3a_{H^+}{}^2 + (K_1K_{13} + K_2K_{23})a_{H^+} + G_1G_2G_3}{a_{H^+}{}^3 + G_1a_{H^+}{}^2 + G_1G_2a_{H^+} + G_1G_2G_3} \quad (26)$$

Attempts to fit the $\delta_{^{31}P}{}^{\text{base-off}}$ data to eq 24 by a nonlinear least-squares method using a simplex minimization algorithm and the Hammett acidity function, H_0, were unsuccessful, the fit being quite poor, particularly at higher acidities. This suggests that H_0 is not the appropriate acidity function for these ionizations and that one which rises less steeply with $[H_2SO_4]$ is required. This fit is considerably improved by use of the H_A (amide) acidity function,[48-51] which is known to be applicable to many compounds that protonate at doubly bonded oxygen, including ketones and conjugated carbonyl compounds,[52-54] secondary amides,[55] benzamides and phenylureas,[56] phosphinanilides,[57] and various phosphoryl and sulfinyl compounds.[58] The fit, however, is still badly trended at values of H_A less than −1.0. This situation is reminiscent of that described by Skvortsov et al.,[59] who studied the protonation of tertiary phosphine oxides by ^{1}H and ^{31}P NMR and found that while the ^{1}H NMR data were well fit by the H_A acidity function, the ^{31}P NMR data (for the same compounds) required a new acidity function (H_{PO}), which rises even more slowly with $[H_2SO_4]$ than does H_A. This suggests a rather strong dependence of ^{31}P NMR chemical shifts on solvation. While the use of this H_{PO} acidity improves the fit of the $\delta_{^{31}P}{}^{\text{base-off}}$ data to eq 24, an exact fit (Figure 3) can be obtained only by use of the Cox and Yates[60] generalized acidity function treatment (eq 27), in which C_{H^+} is the concentration of hydrogen ion, X is the so-called "excess acidity", and m^* is a parameter presumably reflecting the solvation demands of the protonated species under investigation.

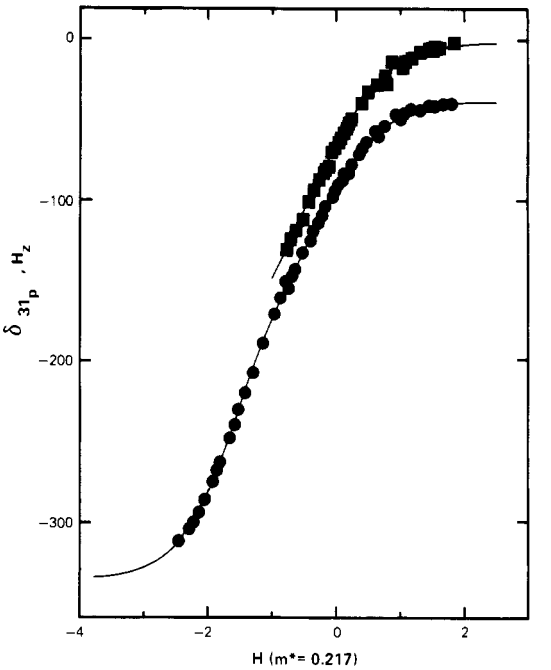


Figure 3. Plots of $\delta_{^{31}P}{}^{\text{base-off}}$ (●) and $\delta_{^{31}P}{}^{\text{base-on}}$ (■) for CNCbl at 25 °C vs. H (eq 27) at m^* = 0.217. The solid lines are nonlinear least-squares fits to eq 24 and 25, respectively. The final fit parameters were $\delta^{(0)}$ = −335.81 Hz, $K_1\delta^{(1)} + K_2\delta^{(2)}$ = −5418.4 Hz, $G_4G_5\delta^{(12)}$ = −1540.4 Hz, G_4 = 37.22, and G_4G_5 = 41.19 for the base-off correlation.

$$-H = m^*X + \log C_{H^+} \quad (27)$$

The procedure we used was to fit the data to eq 24 using the Cox and Yates acidity function (eq 27), varying the value of m^* and using the standard deviation of the fit as a "goodness-of-fit" criterion. The standard deviation was found to go through a rather sharp minimum at m^* = 0.217, producing the fit shown in Figure 3 (lower solid line). The final values of the five parameters in eq 24 were shown to be very insensitive to variations in the initial guesses of these parameters and gave values for the macroscopic pK_a's for phosphodiester deprotonation of the base-off species of pG_4 = −1.57 and pG_5 = −0.04 and chemical shifts of −335.81 Hz and −37.40 Hz for the base-off phosphodiester-protonated (**0** in Scheme I) and -deprotonated (**12** in Scheme I) species, respectively (Table II). For comparison, the ^{31}P chemical shift of dicyanocobalamin had previously been determined to be −37.59 Hz.[39a] A similar treatment (using eq 25 at m^* = 0.217) of the ^{31}P chemical shifts of the base-on species (Figure 3, upper solid line) does not yield reliable values for the phosphodiester pK_a's as this species can only be observed at acidities less than 3.14 M in H_2SO_4 ($\alpha_{\text{base-on}}$ is 0.033 at this acidity). Only the chemical shift of the base-on, phosphodiester-deprotonated species (**123** in Scheme I) is reliably determined to be −1.72 Hz. Hence, an upfield shift of 35.68 Hz is seen for the ^{31}P

(48) Yates, K.; Stevens, J. B.; Katritsky, A. R. *Can. J. Chem.* **1964**, *42*, 1957–1970.
(49) Yates, K.; Wai, H. *J. Am. Chem. Soc.* **1964**, *86*, 5408–5413.
(50) Yates, K.; Stevens, J. B. *Can. J. Chem.* **1965**, *43*, 529–537.
(51) Edward, J. T.; Wong, S. C. *Can. J. Chem.* **1977**, *55*, 2492–2494.
(52) Skvortsov, N. K.; Lizina, V. P.; Stepanyants, A. V.; Tereschchenko, G. F.; Ionin, B. I. *Zh. Obshch. Khim.* **1974**, *44*, 2293–2296.
(53) Zalewski, R. I.; Dunn, G. E. *Can. J. Chem.* **1968**, *46*, 2469–2470.
(54) Cook, K. L.; Waring, A. J. *J. Chem. Soc., Perkin Trans. 2* **1973**, 84–87.
(55) Farlow, D. W.; Moodie, R. B. *J. Chem. Soc. B* **1970**, 334–336.
(56) Barnett, J. W.; O'Connor, C. J. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1331–1333.
(57) Tysse, D. A.; Bauscher, L. P.; Haake, P. *J. Am. Chem. Soc.* **1973**, *95*, 8066–8072.
(58) Carci, R.; Levi, A.; Lucchini, V.; Scorrano, G. *J. Chem. Soc., Perkin Trans. 2* **1973**, 531–533.
(59) Skvortsov, N. K.; Tereshchenko, G. F.; Ionin, B. I.; Petrov, A. A. *Zh. Obshch. Khim.* **1973**, *43*, 981–987.
(60) Cox, R. A.; Yates, K. *J. Am. Chem. Soc.* **1978**, *100*, 3861–3867.

Table II. ^{31}P Chemical Shifts and Phosphodiester Macroscopic pK_a's for CNCbl and CH_3Cbl (25 ± 1 °C)

cobalamin	ionic species[a]	$\delta^{31}P$, Hz[b]	m^*[c]	pG_4[d]	pG_5[e]
CNCbl	0	−335.81	0.217	−1.57	−0.04
CNCbl	12	−37.40			
(CN_2)Cbl	base-off	−37.59[f]			
CNCbl	123	−1.72			
CH_3Cbl	0	−335.43	0.225	−1.62	−0.02
CH_3Cbl	12	−38.47			

[a] See Scheme I for definitions of the ionic species. [b] From external 85% H_3PO_4. Negative shifts are upfield from the reference. [c] Equation 27. [d] Defined in eq 16 and 17. [e] Defined in eq 18 and 19. [f] Reference 39.

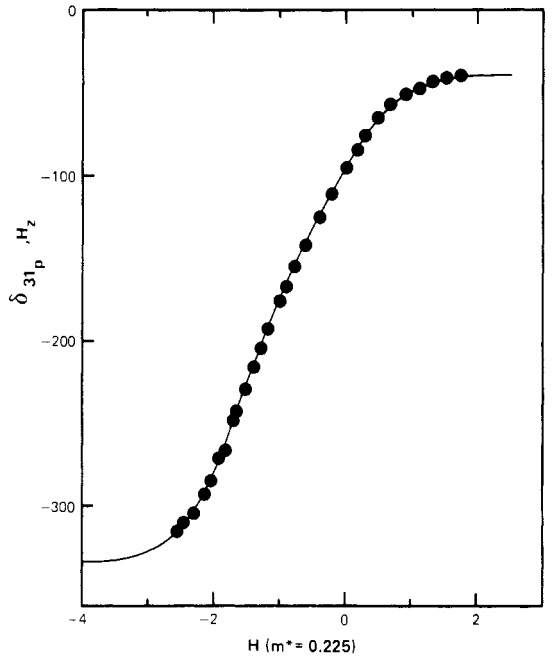


Figure 4. Plot of $\delta_{^{31}P}^{base\text{-}off}$ for CH_3Cbl at 25 °C vs. H (eq 27) at m^* = 0.225. The solid line is a nonlinear least-squares fit to eq 24 from which the final fit parameters were $\delta^{(0)} = -335.43$ Hz, $K_1\delta^{(1)} + K_2\delta^{(2)} = -6212.4$ Hz, $G_4G_5\delta^{(12)} = -1682.4$ Hz, $G_4 = 41.51$, and $G_4G_5 = 43.74$.

chemical shift of CNCbl upon displacement of the axial base by protonation (i.e., **123** → **12** in Scheme I) compared to that of 31.37 Hz previously determined for displacement of the axial base by excess cyanide.[39a]

As we had previously observed only an apparent single protonation of the phosphodiester moiety of CH_3Cbl,[39a] we have reexamined the ^{31}P chemical shift of CH_3Cbl as a function of acidity. The data (Figure 4) clearly show two protonations and give values of $pG_4 = -1.59$ and $pG_5 = -0.01$ when fit to eq 24 with $m^* = 0.217$ (eq 27). An independent minimization (Figure 4) of the standard deviation of the fit as a function of m^* had a minimum at $m^* = 0.225$ and gave the values $pG_4 = -1.62$ and $pG_5 = -0.02$ and −335.43 Hz and −38.47 Hz (Table II) for the chemical shifts of the base-off phosphodiester-protonated and -deprotonated species, respectively, suggesting that phosphodiester pK_a's and chemical shifts of base-off cobalamins are largely independent of the nature of the upper axial ligand.

We have also attempted to correlate $\alpha_{base\text{-}on}$ for CNCbl with acidity via eq 26 in order to attempt to determine the three macroscopic pK_a's for the overall ionization scheme, but with much less success. Here the standard deviations of fits of these data to eq 26 show very little dependence on m^* (eq 27) and the final parameter values are somewhat sensitive to the values of the initial guesses, indicating that the parameters are poorly determined despite the extensive data. At $m^* = 1.00$ (i.e., H_0) an excellent fit can be obtained (Figure 5) from which the reasonable values $pK_3 = 0.39$, $pG_1 = -2.69$, $pG_2 = -0.01$, and $pG_3 = 1.09$ can be extracted, but these must be considered provisional, at best, in light of the above. Interestingly, these data may be very successfully treated as the protonation of a single ionizable group, a plot of log $[\alpha_{base\text{-}on}/(1 - \alpha_{base\text{-}on})]$ vs. H at $m^* = 0.919$ (Figure 6) giving an excellent straight line with slope 0.993 ± 0.013 and an apparent pK_a of 0.11 ± 0.01. This presumably indicates that the base-on–base-off pK_a is fairly insensitive to the state of protonation of the phos-

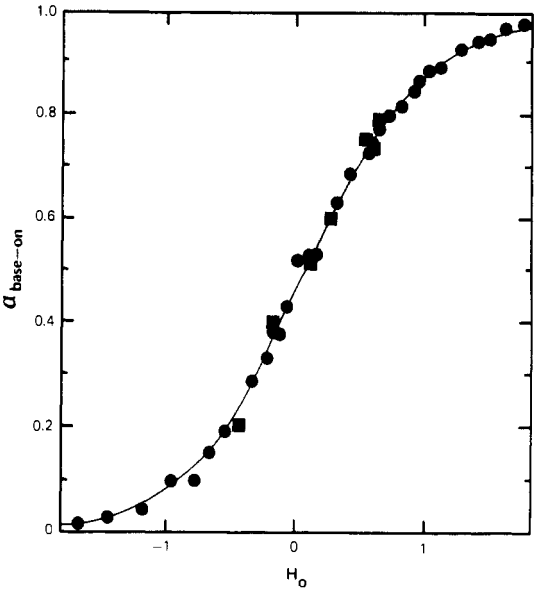


Figure 5. Plot of $\alpha_{base\text{-}on}$ obtained from integration of the ^{31}P resonances (●) and the ^{13}C resonances (■) of $^{13}CNCbl$ at 25 °C vs. H_0 (i.e., eq 27 at $m^* = 1.0$). The solid line is a least-squares fit to eq 26 for which the final fit parameters were $K_3 = 0.41$, $K_1K_{13} + K_2K_{23} = 435$, $G_1G_2G_3 = 40.6$, $G_1 = 492$, and $G_1G_2 = 502$.

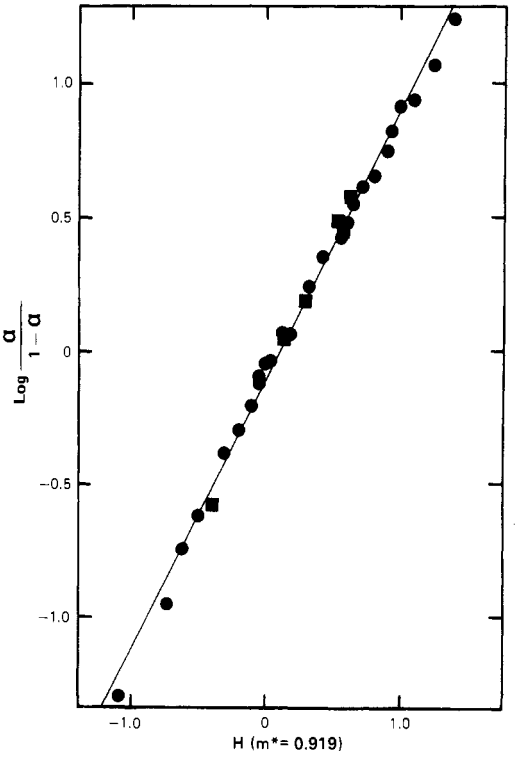
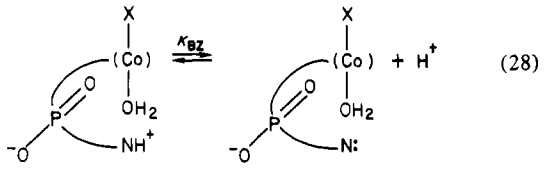


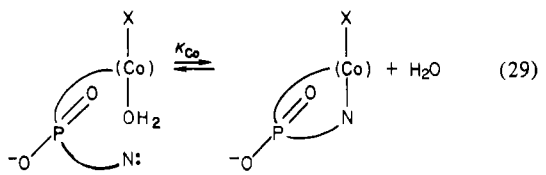
Figure 6. Plot of log $[\alpha_{base\text{-}on}/(1 - \alpha_{base\text{-}on})]$ from integration of the ^{31}P resonances (●) and ^{13}C resonances (■) of $^{13}CNCbl$ at 25 °C vs. H (eq 27) at $m^* = 0.919$. The solid line is a linear least-squares fit whose slope is 0.993 ± 0.013 and x intercept is 0.11 ± 0.01.

phodiester and that the value of 0.1 previously reported by Hayward et al.[61] from correlation of UV–visible spectral changes with H_0 is a good estimate of this pK_a. Reenstra and Jencks[43] recently reported a value of 0.38 for this pK_a at 25 °C and ionic strength 1.0 M.

The lack of an upfield shift of the phosphorus resonance of CH_3Cbl upon displacement of the axial base by protonation,[39a] in contrast to the case for CNCbl, must surely be due to differences in the magnetic environments of the phosphorus atoms of the base-on species, as the chemical shifts of the two base-off species (both phosphodiester protonated and deprotonated) are essentially identical (Table II). There is a considerable difference in the apparent affinity of the free-base benzimidazole ligand for the cobalt atom in these two cobalamins, which is amenable to quantitation. If we consider, as is frequently done, the overall base-on–base-off equilibrium (i.e., eq 2) to be the sum of two consecutive equilibria (eq 28 and 29), we can readily calculate this apparent affinity (K_{Co}



(28)



(29)

in eq 29) if we assume that (1) the value of K_{Bz} (eq 28) is independent of the nature of the upper axial ligand, X, and (2) the value of 5.0 for pK_{Bz} previously calculated by Reenstra and Jencks[43] is applicable. Using as our best estimate 0.11 for the overall base-on–base-off pK_a of CNCbl and 2.89 for this pK_a for CH_3Cbl,[39a] we can calculate values of K_{Co} (eq 29) of 1.3×10^2 and 7.81×10^4 for CH_3Cbl and CNCbl, respectively. Hence, the benzimidazole ligand is bound nearly 3 orders of magnitude more tightly in base-on CNCbl than in CH_3Cbl. Gorenstein and co-workers[36-38] have shown that phosphate ester ^{31}P NMR chemical shifts are largely determined by O–P–O bond angles and RO–P–OR torsion angles and that these bond and torsion angles are strongly coupled. It thus seems likely that the geometry about the phosphodiester phosphorus atom is distorted, relative to the base-off species, in the tightly coordinated base-on CNCbl but is not distorted in the much more loosely coordinated base-on CH_3Cbl. Thus, displacement of the axial benzimidazole either with cyanide ion or by protonation releases the distortion and causes the observed change in ^{31}P chemical shift. In the more loosely coordinated CH_3Cbl the phosphodiester conformation apparently is the same for the base-on and base-off species. Some support for the idea of a difference in base-on phosphodiester conformation between CNCbl and an alkylcobalamin can be obtained from existing X-ray crystal structures of CNCbl[8] and 5′-deoxyadenosylcobalamin[10] as a model alkylcobalamin since the structure of CH_3Cbl has not yet been reported. The observed angles are respectively, for 5′-deoxyadenosylcobalamin and CNCbl, −74 and −60° for the (isopropanolamine)C–O–P–O torsion angles and 172 and 157° for the O–P–O–C(ribose) torsion angles, showing an apparently significant difference in phosphodiester conformation between the two base-on cobalamins.

At H_0 values below −0.5 (i.e., $[H_2SO_4] > 1.2$ M) we have also observed an acidity-dependent upfield shift of the ^{13}C

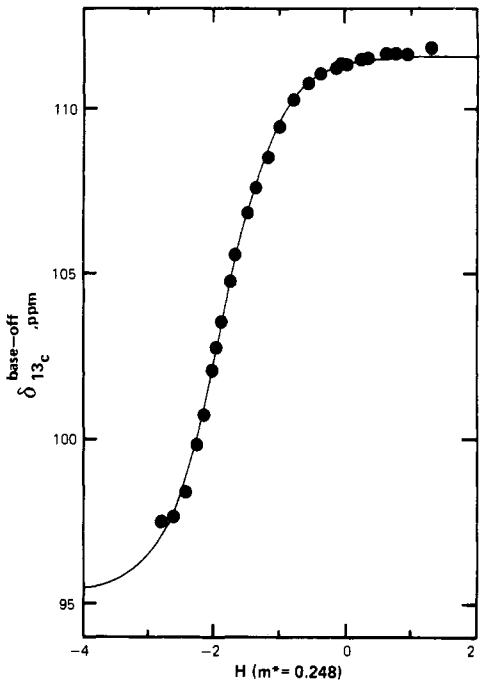
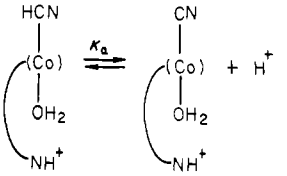


Figure 7. Plot of $\delta_{^{13}C}^{base\text{-}off}$ for $^{13}CNCbl$ at 25 °C vs. H (eq 27) at $m^* = 0.248$. The solid line is a least-squares fit to eq 31 from which the values p$K_a = -1.87$, $\delta_{^{13}C}^{(I)} = 95.39$, and $\delta_{^{13}C}^{(II)} = 111.60$ were obtained.

resonance of base-off $^{13}CNCbl$ (Figure 7). This effect could be characterized as a reversible protonation of the coordinated cyanide ligand (eq 30 and 31) or possibly of the corrin macrocycle.

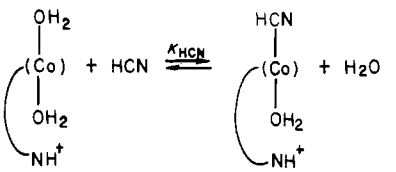


(30)

I II

$$\delta_{^{13}C}^{base\text{-}off} = \frac{K_a\delta_{^{13}C}^{(II)} + a_{H^+}\delta_{^{13}C}^{(I)}}{K_a + a_{H^+}} \quad (31)$$

A fit of these data to eq 31 using the Cox and Yates acidity function[60] (eq 27) and varying m^* produced a best fit at $m^* = 0.248$ (Figure 7) and gave the values p$K_a = -1.87$, $\delta_{^{13}C}^{(I)} = 95.39$, and $\delta_{^{13}C}^{(II)} = 111.60$. If this interpretation is correct, we can estimate the binding constant for HCN to base-off aquocobalamin (eq 32) from the thermodynamic cycle in-



(32)

cluding eq 32, eq 30, the ionization of HCN (p$K_a = 9.0$[43]), and the equilibrium constant for replacement of one aquo ligand of base-off H_2OCbl by CN^- (assumed to be equal to the equilibrium constant for replacement of one aquo ligand of diaquocobinamide by cyanide, estimated to be $>10^{14}$ by Hayward et al.[61]). The value obtained is $K_{HCN} > 1350$ M^{-1} (eq 32), indicating little or no dissociation of coordinated HCN would occur under our conditions (i.e., $[^{13}CNCbl] = 0.01$ M) in agreement with our failure to detect any time-dependent change in the ^{13}C chemical shift of base-off $^{13}CNCbl$ at these acidities (other than that connected with the 0.2 ppm upfield

(61) Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485–6493.

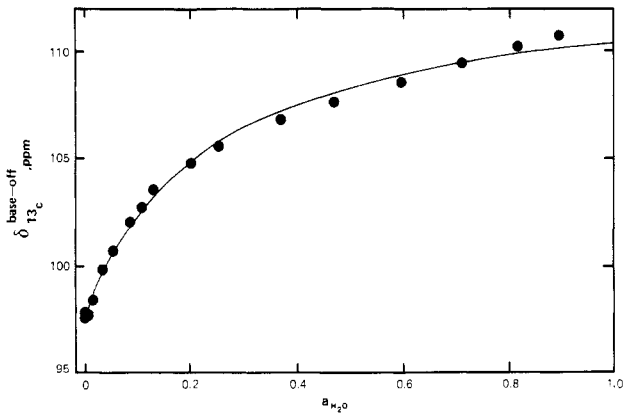
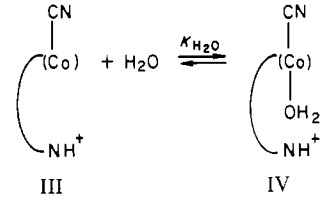


Figure 8. Plot of $\delta_{^{13}C}^{base\text{-}off}$ for ^{13}CNCbl at 25 °C vs. the activity of water in the aqueous sulfuric acid solvent. The solid line is a least-squares fit to eq 34 from which the values of $K_{H_2O} = 4.13$, $\delta_{^{13}C}^{(III)} = 97.60$, and $\delta_{^{13}C}^{(IV)} = 113.58$ were obtained.

shift due to phosphodiester hydrolysis).

An alternative explanation for the changes in base-off ^{13}C chemical shift of ^{13}CNCbl at H_0 below −0.5 involves the reversible formation of a pentacoordinate cyanocobalt corrin species (eq 33, 34) due to the reduced activity of water in such acidic mixtures.[49,62] Spectroscopic evidence of various kinds has previously been presented for the existence of such 5-coordinate–6-coordinate equilibria in cobalt corrins.[63-66] A fit of these data to eq 34 (Figure 8) yields the values $K_{H_2O} = 4.13$, $\delta_{^{13}C}^{(III)} = 97.60$, and $\delta_{^{13}C}^{(IV)} = 113.58$. It should be pointed out that although this fit is clearly trended, the fit of these data to eq 31 (Figure 7) is similarly trended although it is more difficult to see due to the plot vs. logarithm of titrant concentration. In either case, some trending of these fits must be expected since it seems unlikely that either K_a in eq 30 or K_{H_2O} in eq 33 would be completely independent of the phosphodiester protonations occurring in this same acidity range. Nonetheless, the value of K_{H_2O} (4.13) is surprisingly low and implies that, at 25 °C in unit activity water, base-off CNCbl is some 20% 5-coordinate. While it is difficult to believe this assignment, it cannot be ruled out a priori on the basis of the evidence at hand. Additional experimentation with other cobalamins in aqueous sulfuric acid is currently in progress to attempt to resolve this question.



(33)

$$\delta_{^{13}C}^{base\text{-}off} = \frac{\delta_{^{13}C}^{(III)} + \delta_{^{13}C}^{(IV)} K_{H_2O} a_{H_2O}}{1 + K_{H_2O} a_{H_2O}} \qquad (34)$$

Acknowledgment. This research was supported by the Robert A. Welch Foundation, Houston, TX (Grant No. Y-749), and the Organized Research Fund of The University of Texas at Arlington. The authors are extremely grateful to Prof. Dennis S. Marynick (UTA) for supplying the nonlinear least-squares routine and assisting in its use.

Registry No. ^{13}CNCbl (base-on), 89975-17-7; ^{13}CNCbl (base-off), 89959-47-7; ($^{1}\beta$-^{13}CN)(α-H_2O)Cbi, 90026-99-6; (β-H_2O)(α-^{13}CN)Cbi, 89959-48-8; $(^{13}CN)_2$Cbl, 89959-49-9; $(^{13}CN)_2$Cbi, 89959-50-2; CH_3Cbl, 13422-55-4; $(H_2O)_2$Cbi, 15259-55-9.

(62) Wyatt, P. A. H. *Discuss. Faraday Soc.* **1957**, *24*, 162-170.
(63) Hill, H. A. O.; Pratt, J. M.; Williams, R. J. P. *Discuss. Faraday Soc.* **1969**, *47*, 165-171.
(64) Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Thorp, R. G. *Chem. Commun.* **1967**, 1013-1014.
(65) Hill, H. A. O.; Pratt, J. M.; Williams, R. J. P. *Chem. Br.* **1969**, *5*, 156-161.
(66) Firth, R. A.; Hill, H. A. O.; Mann, B. E.; Pratt, J. M.; Thorp, R. G.; Williams, R. J. P. *J. Chem. Soc. A* **1968**, 2419-2428.

Contribution from the Department of Chemistry, University of California at Davis, Davis, California 95616

Influence of Ligand Structure on the Mechanism of Oxidation of Dithioethers by Copper(II). X-ray Crystal Structure of Bis(3-hydroxy-1,5-dithiacyclooctane)copper(II) Perchlorate

W. K. MUSKER,* M. M. OLMSTEAD, and R. M. KESSLER

Received August 16, 1983

Although 1,5-dithiacyclooctane (1,5-DTCO) rapidly reduces copper(II) perchlorate, substitution of a hydroxyl group at the 3-position of the eight-membered ring (3-hydroxy-1,5-dithiacyclooctane, 3-OH-1,5-DTCO) gives a facially coordinating ligand that completely inhibits reduction of copper(II). The crystal structure of $Cu(3\text{-}OH\text{-}1,5\text{-}DTCO)_2(ClO_4)_2$ is also reported: space group $P2_1/c$, $a = 6.811$ (3) Å, $b = 9.225$ (4) Å, $c = 16.575$ (5) Å, $\beta = 90.54$ (3)°, $Z = 2$. On the basis of these and other observations a mechanism that accounts for the differences in the ability of various thioether ligands to reduce copper(II) perchlorate is proposed. This mechanism depends on a linear Cu–S···S arrangement of orbitals, which facilitates ligand to metal electron transfer and leads, in turn, to metal–ligand bond cleavage.

Introduction

Our interest in the coordination and redox chemistry of copper complexes of dithioethers arose from the observation that the eight-membered ring dithioether 1,5-dithiacyclooctane (1,5-DTCO) causes a rapid reduction of copper(II) perchlorate in methanol at room temperature, whereas the six-membered ring dithioether 1,4-dithiane (1,4-DT) causes *no* reduction of copper(II) perchlorate even in refluxing methanol. This facile reduction is not due to any destabilization of a planar copper(II) complex as noted for 2,9-dimethyl-*o*-phenanthroline complexes[1] since planar bis complexes of eight-membered mesocycles are well-known.[2,3] Because of the behavior of

(1) James, B. R.; Williams, R. J. P. *J. Chem. Soc.* **1961**, 2007.

0020-1669/84/1323-1764$01.50/0