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Communications

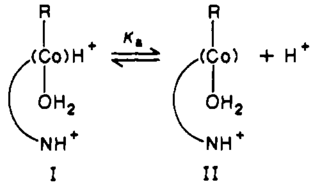
Heteronuclear NMR Studies of Cobalamins. 7. Protonation of the Corrin Ring in Sulfuric Acid/Water Mixtures[1]

Sir:

In an earlier communication[2] it was reported that the ^{13}C chemical shift of base-off cyanocobalamin, enriched in ^{13}C in the axial cyanide ligand ($^{13}CNCbl$) undergoes an upfield shift in sulfuric acid/water mixtures that correlates well with the Cox and Yates generalized acidity function[3] (eq 1, where C_{H^+} is the

$$-H = m^*X + \log C_{H^+} \qquad (1)$$

concentration of hydrogen ion, X is the so-called "excess acidity", and m^* is an adjustable parameter presumably reflecting the solution demands of the protonated species under investigation) to give a pK_a of −1.87 at $m^* = 0.25$.[4] At that time, we were unable to distinguish between the possibilities of reversible protonation of coordinated cyanide[5] and reversible protonation of the corrin (eq 2).[7] We now report further studies of this phenomenon using additional organocobalamins with NMR active nuclei in the organic axial ligand in which the organic ligand cannot undergo protonation.



(2)

The ^{13}C chemical shift of the base-off forms of methycobalamin enriched in ^{13}C in the axial methyl carbon ($^{13}CH_3Cbl$) and of ethylcobalamin enriched in ^{13}C in the α-carbon of the organic ligand ($CH_3{}^{13}CH_2Cbl$) as well as the ^{19}F chemical shift of base-off trifluoromethylcobalamin (CF_3Cbl) were all found to undergo changes in sulfuric acid/water mixtures (Figure 1) that were well correlated by the generalized acidity function (eq 1). The apparent pK_a and m^* values thus obtained are collected in Table I, along with the values previously obtained for $^{13}CNCbl$.[2] Unfortunately, CF_2HCbl apparently decomposes in sulfuric acid (multiple resonances were observed), and the change in ^{19}F chemical shift of CF_3CH_2Cbl in acid was too small to be useful. We note that all of these protonations (Table I) follow acidity functions characterized by similarly (and probably identical) small values of m^*[4] ($m^* = 0.22 \pm 0.04$) and that the range of pK_a values observed is very narrow considering the large differences in inductive effect of the upper axial ligand of this collection of cobalamins.[8,11] The

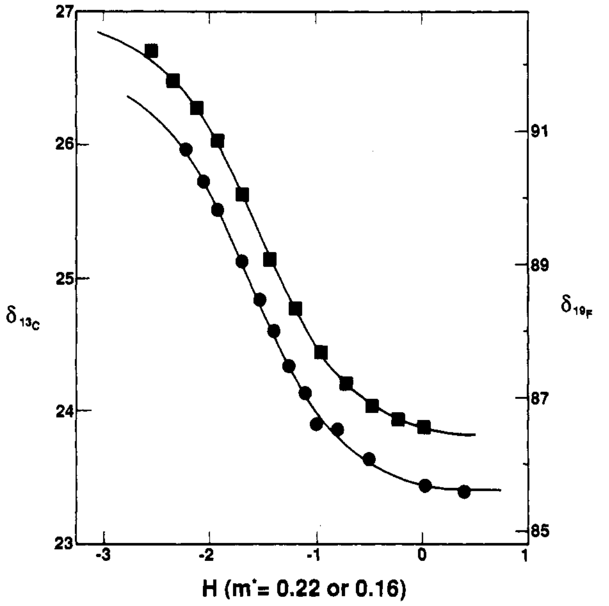


Figure 1. Dependence of the ^{13}C chemical shift of $CH_3{}^{13}CH_2Cbl$ (●) (left ordinate, $m^* = 0.22$) and the ^{19}F chemical shift of CF_3Cbl (■) (right ordinate, $m^* = 0.16$) on acidity, expressed via the generalized acidity function (eq 1). The solid lines are calculated for simple titrations according to eq 2, using the chemical shifts and m^* values listed in Table I.

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(1) Part 6: Brown, K. L. *J. Am. Chem. Soc.* **1987**, *109*, 2277–2284.

(2) Brown, K. L.; Hakimi, J. M. *Inorg. Chem.* **1984**, *23*, 1756–1764.

(3) Cox, R. A.; Yates, K. *J. Am. Chem. Soc.* **1978**, *100*, 3861–3867.

(4) This value of m^* produces a rather extraordinary acidity function that rises very slowly with sulfuric acid concentration.

(5) This was originally formulated[2] as C-protonation of C-coordinated cyanide. However, other possibilities include simultaneous isomerization of such a species to an N-coordinated, C-protonated species,[6] or N-protonation of C-coordinated cyanide.

(6) Reenstra, W. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 5780–5791.

(7) An alternative explanation involving reversible formation of a pentacoordinate cyanocobalt corrin species due to the reduced activity of water in such acidic mixtures should probably be rejected because of the unrealistically low value for the equilibrium constant for water addition to such a species (4.1)[2] obtained by this treatment. Further evidence against such an assignment is presented herein.

(8) For example, the pK_a's for protonation and displacement of the axial benzimidazole ligand (p$K_{base-off}$) at 25°C for these cobalamins are 4.16 (CH_3CH_2Cbl),[9] 2.89 (CH_3Cbl),[10] 1.44 (CF_3Cbl),[10] and 0.10 ($CNCbl$).[2]

(9) Brown, K. L. *Inorg. Chem.* **1986**, *25*, 3111–3113.

(10) Brown, K. L.; Hakimi, J. M.; Nuss, D. M.; Montijano, Y. D.; Jacobson, D. W. *Inorg. Chem.* **1984**, *23*, 1463–1471.

(11) This observation effectively rules out the possibility that the acidity dependence of these NMR resonances is due to formation of pentacoordinate species in this medium.[2,7] For instance, the binding constant for cyanide ion trans to these ligands in the revelant cobinamides varies by at least 6 orders of magnitude.[12]

(12) Pratt, J. M. *Inorganic Chemistry of Vitamin* B_{12}; Academic: New York, 1972; p 164.

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Table I. Chemical Shifts, pK_a's, and Acidity Function Behavior of Cobalamins and Cyanide Species at 25 ± 1 °C

compd	pK_a	m^{*a}	$\delta_{base}{}^{b}$	$\Delta\delta^{c}$
^{13}CNCbl^{d}	−1.87	0.25	113.98^{e}	−16.21
$^{13}CH_3Cbl$	−1.54	0.24	1.52	2.31
$CH_3{}^{13}CH_2Cbl$	−1.62	0.16	23.38	3.21
CF_3Cbl	−1.54	0.22	86.39^{f}	6.25
$^{13}CN^-$	9.04^{g}		166.98	−51.90
$H^{13}CN$	−2.61	0.18	114.54^{h}	−10.37

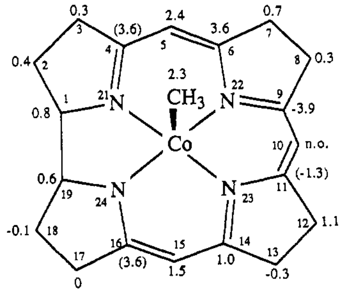
^{a}Equation 1. ^{b}Chemical shift of the base-off, but otherwise unprotonated species of cobalamin (i.e. II, in eq 2). For the last two compounds, δ_{base} is for $^{13}CN^-$ and $H^{13}CN$, respectively. Except as noted, carbon chemical shifts were measured at 50.311 MHz relative to *p*-dioxane (external reference in concentric insert) but are reported relative to TSP. ^{c}Difference in chemical shift between the fully protonated and deprotonated forms ($\delta_I - \delta_{II}$ (eq 2) for the cobalamins). ^{d}Reference 2. ^{e}Originally measured relative to external TMS, but reported here relative to TSP. ^{f}^{19}F chemical shift (188.238 MHz) relative to external monofluorobenzene. ^{g}Measurements in 1.0 M aqueous KCl. ^{h}J_{HC} = 269.0 Hz for $H^{13}CN$ and 297.4 Hz for the fully protonated species.

observation of such acidity-dependent behavior for $^{13}CH_3Cbl$, $CH_3{}^{13}CH_2Cbl$, and CF_3Cbl clearly indicates that these cobalamins must be undergoing protonation of the corrin (eq 2) in these media. There is, however, a significant difference in the acidity-dependent NMR behavior of ^{13}CNCbl and the other alkylcobalamins. While corrin protonation of the latter is accompanied by relatively small (ca. 2–3 ppm for ^{13}C, 6 ppm for ^{19}F) downfield shifts of the axial NMR resonance, ^{13}CNCbl protonation produces a comparatively large (16 ppm) upfield shift of its ^{13}C resonance (Table I). This suggests that ^{13}CNCbl undergoes closely overlapping protonations both in the corrin ring and on the axial cyanide ligand with the much larger upfield chemical shift effect of the latter protonation completely masking the smaller downfield chemical shift of the former protonation.

In order to further investigate this possibility, the NMR consequences of protonation of $^{13}CN^-$ and $H^{13}CN$ were investigated. In base (pH 10.92), $K^{13}CN$ had a single, relatively narrow NMR resonance of half-width 2.9 Hz at 166.98 ppm, while at neutral pH (6.85), a single line (without proton decoupling) was observed at 115.08 ppm with a half-width of 7.7 Hz; i.e., protonation of $^{13}CN^-$ causes an upfield shift of 51.90 ppm (Table I). At all intermediate pH's (7.84–9.94) a single, greatly broadened (half-width as large as 84.7 Hz) resonance of intermediate chemical shift was observed, indicating that exchange between $H^{13}CN$ and $^{13}CN^-$ is relatively slow on the NMR time scale. However, the observed chemical shifts are obviously properly weighted average values as the data produced a smooth titration curve (not shown) with pK_a = 9.04, which is in excellent agreement with a literature value[6] (by potentiometric titration) of 9.0. In dilute sulfuric acid (0.723 M, H = 0.05 at m^* = 0.18) the proton-coupled ^{13}C resonance of $H^{13}CN$ was a doublet (J_{HC} = 269.0 Hz) at 114.54 ppm that collapsed to a singlet upon proton noise decoupling. With increasing acidity this doublet shifted upfield, producing a smooth titration curve (not shown) with pK_a = −2.61 at m^* = 0.18 and (extrapolated) values of 104.17 ppm and 297.4 Hz for the chemical shift and coupling constant of the fully protonated species (Table I). Thus, protonation of $H^{13}CN$ (presumably at nitrogen) causes an upfield shift of the ^{13}C resonance of 10.37 ppm. These results indicate that the pK_a, acidity function (i.e. m^* value), and chemical shift displacement for ^{13}CNCbl protonation are prefectly reasonable for axial cyanide ligand protonation, which apparently overlaps with and completely obscures corrin ring protonation.

We have also investigated the effects of these protonations on the UV–visible spectra of cobalamins. The base-off forms of the simple alkylcobalamins, as typified by CH_3Cbl, undergo surprisingly small changes in electronic spectrum in sulfuric acid/water mixtures. These consist of a minor decrease in absorptivity of the visible (α and β) bands with a slight shift of the β band to shorter wavelength (463–460 nm), a very small decrease in absorptivity of the γ band with a shift to longer wavelength (376–382 nm), and a slight decrease in absorptivity of the first UV band and a slight shift to longer wavelength (305–308 nm). The previously noted[13] small shifts of the UV bands attributable to the axial nucleotide (287 and 277 nm) to longer wavelengths (289 and 280 nm) are clearly attributable to protonation of the detached, cationic dimethylbenzimidazolium nucleotide at N-1, as previously seen in the free nucleoside (α-ribazole).[10] Much more significant spectral changes occur when base-off CNCbl is protonated in sulfuric acid/water mixtures. These include a large decrease in absorption of the α band (which becomes a shoulder) and a shift of the β band from 497 to 478 nm, and a large decrease in absorption of the γ band (355 nm) accompanied by a slight shift to longer wavelength (358 nm). Thus, the significant differences in the electronic spectral effect of protonation of the base-off RCbl's and CNCbl in sulfuric acid/water mixtures agree with the conclusions arrived at above, from NMR considerations, concerning the differences in protonation behavior. Furthermore, the significant changes in the α and β bands (which are quite sensitive to changes in axial ligation) of base-off CNCbl in such media suggest that a change in liganding atom may well occur upon protonation. Thus, the cyanide-protonated species formed from base-off CNCbl in sulfuric acid may well be C-protonated and N-coordinated.

We have also attempted to assign the site of corrin ring protonation by natural-abundance ^{13}C NMR of unlabeled CH_3Cbl in sulfuric acid/water mixtures. Assignment of the resonances is somewhat complicated as many resonances are shifted in such acidic media, particularly in the downfield region where the side-chain carbonyls (which must certainly protonate) are located. However, many of the corrin nucleus resonances occur in relatively uncrowded regions of the spectrum, are shifted only a few ppm, and, hence, are readily assignable by comparison to the ^{13}C spectra of base-off CH_3Cbl in less acidic media[1] and methyl cobinamide and the completely assigned ^{13}C spectrum of base-off 5′-deoxyadmosylcobalamin (AdoCbl).[14] In addition, even the most downfield of the corrin resonances (C-4, C-11, and C-16) can be assigned at least tentatively by carefully following the changes in chemical shift of the downfield resonances in spectra recorded at increasing acidities (H = −1.36, 40% protonated; H = −1.46, 46% protonated; H = −2.10, 78% protonated). The results are shown in structure III, as the signed difference in chemical shift between the fully protonated species (calculated from the spectra of partly protonated samples) and the deprotonated but base-off species, where the values for tentatively assigned resonances are given in parentheses. Within the resonating system the carbon resonances neighboring C-10 are shifted upfield while those progressively further away are shifted progressively more downfield in a nearly symmetrical pattern. Carbons not in the resonating system generally undergo much smaller shifts. However, the C-10 resonance could not be discerned at any acidity. No resonances occurred within ±5 ppm of the position of the C-10 resonance in base-off CH_3Cbl (98.23 ppm), and no resonances that could not be assigned definitely to other carbon atoms occured within



III

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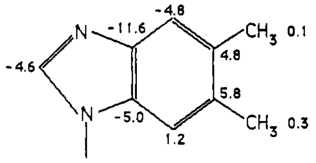
[13] Hayward, G. C.; Hill, H. A. O.; Pratt, J. M.; Vanston, N. J.; Williams, R. J. P. *J. Chem. Soc.* **1965**, 6485–6493.

[14] Bax, A.; Marzilli, L. G.; Summers, M. F. *J. Am. Chem. Soc.* **1987**, *109*, 566–574.

±20 ppm of this location. Decreasing the tip angle and increasing the delay between pulses (to allow for a slowly relaxing nucleus) also failed to resolve any resonance assignable to C-10.

The chemical shift pattern shown in III is quite reminiscent of that previously observed[15] for protonation of the 5,6-dimethylbenzimidazole moiety of the detached axial nuceloside (α-ribazole, IV) at N-3, except that in this case the shifts are more pronounced in this aromatic species. This similarity in chemical shift patterns suggests that protonation of the corrin nucleus at N-22 or N-23 should be considered. This possibility can be quickly dismissed, however, as it is extremely unlikely that the pK_a's for protonation at N-22 or N-23 would be so insensitive to the nature of the upper axial ligand (Table I) or that such protonation would follow an acidity function with such a small m^* value.[3,10] Thus, protonation of the corrin in aqueous sulfuric acid at C-10 seems most likely both because of the pattern of chemical shift changes (III) and our inability to observe a C-10 resonance, which is presumably broadened greatly by exchange as was the case for partial protonation of $^{13}CN^-$. In addition, protonation at C-10 is consistent with and explains previous observations of hydrogen-deuterium exchange of the C-10 proton in acidic, deuteriated media.[16,17]



IV

Considering the known electrophilic reactivity of the corrin C-10,[18-20] Pratt[21] has previously raised intriguing questions concerning this position including the reasons why it remains unmethylated in biosynthesis (in contrast to C-5 and C-15) and whether maintenance of an unmethylated bridging carbon has important chemical and/or biochemical consequences. Some attempts to answer these questions have already been made, but with conflicting results. Thus, while both the 10-chloro and 10-bromo derivatives of AdoCbl are active as coenzymes for bacterial diol dehydrase[22] (with K_m's virtually identical with that of AdoCbl and activities of 40% and 20% of the natural coenzyme for the chloro and bromo derivatives, respectively), the 10-chloro derivative is completely inactive with glutamate mutase.[23] Obviously much remains to be learned about this interesting biochemical system.

Acknowledgment. This work was supported by The Robert A. Welch Foundation, Houston, TX, Grant No. Y-749.

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(16) Bonnett, R.; Redman, D. G. *Proc. R. Soc. London, A* **1965**, *288*, 342-343.

(17) Hill, H. A. O; Mann, B. E.; Pratt, J. M.; Williams, R. J. P. *J. Chem. Soc. A.* **1968**, 564-567.

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(20) Wagner, F. *Proc. R. Soc. London, A.* **1965**, *288*, 344-347.

(21) Reference 12; p 281.

(22) Tamao, Y.; Morikawa, Y.; Shimizu, S.; Fukui, S. *Biochim. Biophys. Acta* **1968**, *151*, 260-266.

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Synthesis and Coordination Chemistry of Poly(4-vinyl-4′-methyl-2,2′-bipyridine) Films on Electrode Surfaces

Sir:

Reductive electrochemical polymerization of vinyl-containing transition metal complexes has provided a convenient preparative route to redox-active, thin polymeric films on metallic and semiconductor electrodes.[1] However, the absence of a variety of preparative strategies, along with redox instabilities during the electropolymerization, limits the generality of this approach.[2] Here we describe a significant advance in the underlying preparative chemistry of chemically modified electrodes that is based on octahedral $Zn(vbpy)_3^{2+}$ and the square-planar complexes $[M(vbpy)(COD)]^+$ (vbpy is 4-vinyl-4′-methyl-2,2′-bipyridine; COD is 1,5-cyclooctadiene; M is Rh(I) or Ir(I)).[3] In both cases the metal ions are relatively labile and can be removed to give metal ion free films that have *different* coordination chemistries. Alternatively, the metal ions can be displaced by using suitable metal precursors to give redox-active films containing different metal ions. Our approach differs from those of previous studies in that it emphasizes preparative chemistry at the polymer electrode/solution interface.

The preparations of $Zn(vbpy)_3^{2+}$ and $[M(vbpy)(COD)]^+$ are straightforward or follow from literature procedures.[4a-c] The complexes have been characterized by elemental analyses and ^1H NMR spectroscopy.[4d]

Thin polymeric films of poly-$[Zn(vbpy)_3]^{2+}$ are prepared from the monomer by reductive electropolymerization by using potential scans from −0.8 to −1.5 V (vs the NaCl saturated calomel electrode, SSCE) in 0.2 M tetra-*n*-butylammonium hexafluorophosphate (TBAH)/CH_3CN on Pt-button, glassy-carbon-button, or planar Au/polyester electrodes. Under similar scanning conditions in fresh electrolyte, poly-$[Zn(vbpy)_3]^{2+}$ exhibits sequential bpy-based reductions at $E_{p,c} = -1.48$ and −1.61 V, with the corresponding oxidations being at $E_{p,a} = -1.40$ and −1.57 V. Typical surface coverages, which were estimated by the integrated peak areas, are ca. 10^{-8}–10^{-7} mol/cm^2 for ca. 0.12-cm^2 Pt-disk electrodes.[5] Reductive cycling past −1.75 V results in rapid

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(1) (a) Abruña, H. D.; Denisevich, P.; Umana, M.; Meyer, T. J.; Murray, R. W. *J. Am. Chem. Soc.* **1981**, *103*, 1. (b) Murray, R. W. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Dekker: New York, 1984; Vol. 13, and references therein.

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(3) Note, for example, for relevant catalytic applications, the following references on hydrogenation and transfer hydrogenation: (a) Mestroni, G.; Zassinovich, G.; Camus, A. *J. Organomet. Chem.* **1977**, *140*, 63. (b) Camus, A.; Mestroni, G.; Zassinovich, G. *J. Mol. Catal.* **1979**, *6*, 231. (c) Mestroni, G.; Zassinovich, G.; Camus, A.; Martinelli, F. *J. Organomet. Chem.* **1980**, *198*, 87. (d) Martinelli, F.; Mestroni, G.; Camus, A.; Zassinovich, G. *Ibid.* **1981**, *220*, 383.

(4) (a) Cocevar, C.; Mestroni, G.; Camus, A. *J. Organomet. Chem.* **1972**, *35*, 389. Mestroni, G.; Camus, A.; Zassinovich, G. *Ibid.* **1974**, *73*, 119. (b) Preparation of $[Zn(vbpy)_3][PF_6]_2$: On a ca. 0.5 mM scale the Zn complex was prepared by heating at reflux a 3:1 ratio mixture of vbpy and $ZnCl_2$ in a convenient volume of reagent grade MeOH for 30 min. After this time the reaction mixture was cooled and a solution of NH_4PF_6 in MeOH was added to precipitate the white $[Zn(vbpy)_3][PF_6]_2$ product. Purification was achieved by precipitation from CH_3CN with Et_2O. (c) The vbpy ligand can now be prepared in large quantities (10-20 g) by a slight modification of the published procedure, i.e.: Abruña, H. D.; Breikss, A. I.; Collum, D. B. *Inorg. Chem.* **1985**, *24*, 988. (d) For example, in the ^1H NMR spectrum of $[Rh(vbpy)(COD)][PF_6]$ in CD_3CN the vbpy ring and vinylic proton resonances (δ) are found at 8.21 (d, 1), 7.73 (m 2), 7.55 (d, 1), 7.42 (d, 1), 6.86 (d of d, 1), 6.33 (d, 1), and 5.79 (d, 1); the Me group is found at 2.48 (s, 3), and the COD protons are found at 4.54 (br s, 4), 2.58 (br s, 4), 2.13 (m, 4). Anal. Calcd for $Zn(vbpy)_3(PF_6)$: C, 49.63; H, 3.82; N, 8.91. Found: C, 49.22; H, 3.73; N, 8.75.

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