Direct NMR Evidence Confirming Hyperconjugation in Alkylcobalamins: Secondary Deuterium Isotope Effects on the ^{15}N NMR Chemical Shift of the Axially Coordinated Nitrogen of Ethylcobalamin

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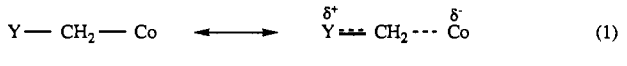
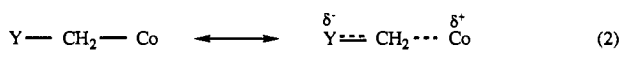
Introduction

Electron delocalization in carbon–metal bonding was recently demonstrated by the successful application of multiparameter substitution effect analysis to kinetic, thermodynamic, and spectroscopic properties of organocobaloximes of the type $YCH_2Co(D_2H_2)L$[1-3] and organocobalamins of the type YCH_2-Cbl.[1,4] In these systems, the "resonance" effect of the substituent, Y, is often as important as its inductive effect.[2-4] This has led to the conclusion[4] that classical hyperconjugation (eqs 1 and 2)



(1)



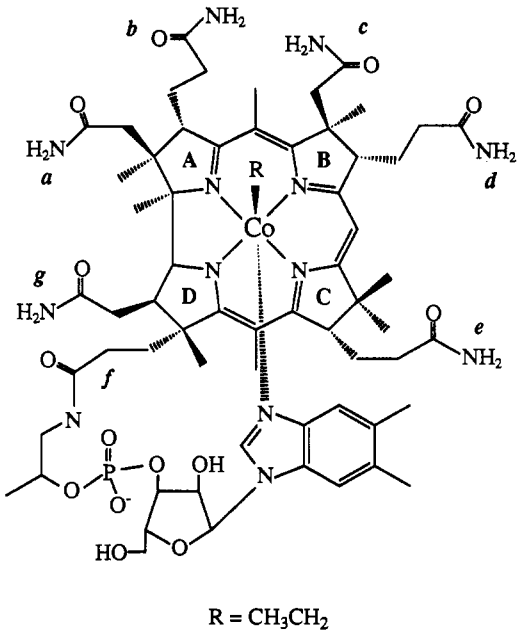
(2)

is a significant contributor to metal–carbon ligand bonding in these complexes. While the success of such correlations including an important "resonance" term provides indirect evidence for the existence of significant hyperconjugation in these complexes, direct evidence has been lacking. Such delocalization would be expected to give rise to significant secondary deuterium isotope effects if the β-hydrogens of RCbl's, such as ethylcobalamin (eq 3), are

(3)

substituted with deuterium, due to the anticipated loss of zero-point energy in the hyperconjugated species. Indeed, such secondary β-deuterium isotope effects have long been attributed to hyperconjugation.[5]

We now present direct evidence of such hyperconjugation in ethylcobalamin (Figure 1) in the form of a significant secondary β-deuterium isotope effect on the ^{15}N NMR chemical shift of the axially coordinated nitrogen atom. In addition, evidence is provided that this deuterium isotope effect is not due to an agostic



$R = CH_3CH_2$

Figure 1. Structure of ethylcobalamin (CH_3CH_2Cbl).

interaction between the β-methyl group of CH_3CH_2Cbl and the metal atom, despite the very high field proton chemical shift (–0.7 ppm) of this methyl.

The ^{15}N NMR chemical shifts of the coordinated nitrogen (B3) of the axial nucleotide of base-on RCbl's[4,6] are exquisitely sensitive to electronic effects of the trans ligand. Thus, as the trans ligand is altered from Ado[1] to H_2O, the B3 ^{15}N resonance shifts upfield by 86.3 ppm,[4,5] while the free energy for coordination of the pendent axial nucleotide becomes more negative by 7.9 kcal mol^{-1}.[7] This extreme sensitivity (10.9 ppm/kcal of binding free energy) suggests that very small changes in the binding constant for coordination of the axial nucleotide in RCbl's may be detectable using the B3 ^{15}N chemical shift.

Results and Discussion

The secondary β-deuterium isotope effect is demonstrated by the data in Table 1, which show that the ^{15}N resonance of the axially coordinated nitrogen in CD_3CH_2Cbl is shifted upfield 2.1 ppm relative to that of CH_3CH_2Cbl. Since hyperconjugation must be less important in the deuterated species, the metal center in CD_3CH_2Cbl should be more electrophilic (eq 3) and the binding constant for the axial nucleotide higher than that in the unlabeled complex. Thus, the direction of the B3 ^{15}N chemical shift perturbation is as anticipated if the hyperconjugated species is a significant contributor to the structure of CH_3CH_2Cbl. It is well-known that deuterium is more electropositive and thus more electron donating than hydrogen.[5a,b,g,9,10] The effect of deuterium substitution in CD_3CH_2Cbl should be to lower the binding constant for the axial nucleotide and to shift the B3 resonance downfield. This effect is clearly seen in the B3 ^{15}N chemical shift of CH_3-CD_2Cbl (Table 1), which is 0.7 ppm downfield from that of CH_3-

(1) Abbreviations: $RCo(D_2H_2)L$ = alkyl(ligand)bis(dimethylglyoximato)-cobalt(III) = alkyl(ligand)cobaloxime, RCbl = alkylcobalamin, AdoCbl = 5′-deoxyadenosylcobalamin (coenzyme B_{12}), $RCbi^+$ = alkylcobinamide. FB = cyanocobinamide, CNCbi, a mixture of α-H_2O-β-CN-Cbi and α-CN-β-H_2O-Cbi.

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Table 1. ^{15}N, ^{1}H, and ^{2}H NMR Data for Ethylcobalt Corrinoids and Their Deuterated Analogs[a]

	$\delta_{^{15}N}$, ppm[b]	$\delta_{^{1}H}$, ppm[c]	$^{1}J_{CH}$, Hz[c]	$\delta_{^{2}H}$, ppm[c]
CH_3CH_2Cbl[d]	227.7	−0.71	128[e]	
CDH_2CH_2Cbl[d]		−0.73	130[e]	−0.75
CD_2HCH_2Cbl[d]		−0.75[f]		
CD_3CH_2Cbl[d]	225.6			−0.78
CH_3CD_2Cbl[d]	228.4			
$CH_3CH_2Cbi^+$[g]		−1.06	129	
$CDH_2CH_2Cbi^+$[g]		−1.10	129	−1.09
$CD_2HCH_2Cbi^+$[g]		−1.10[f]		
$CD_3CH_2Cbi^+$[g]				−1.12
AdoCbl[h]	229.9			
CNCbl[i]	188.3			
H_2OCbl^+[i]	143.6			

[a] ^{15}N chemical shifts determined relative to external CH_3NO_2 but reported relative to NH_3(l) using $\delta_{CH_3NO_2} = 380.32$ ppm.[8] ^{1}H chemical shifts relative to internal TSP. ^{2}H chemical shifts measured relative to DMSO-d_6 (or natural-abundance DMSO-d_1) but reported relative to TSP. [b] Chemical shift of the axially coordinated nitrogen (B3), observed at natural abundance by inverse-detected ^{1}H, ^{15}N HMQC as previously described.[4,6] [c] Chemical shift or C–H coupling constant for the organic ligand β-methyl group. [d] In DMSO or DMSO-d_6, except as noted. [e] In 20% CD_3CN/D_2O. [f] Observed as the proton residual in the complex prepared from CD_3CH_2Br, 98 atom % D. [g] In D_2O. [h] Reference 4. [i] Reference 6.

CH_2Cbl. Thus, the upfield shift of the B3 resonance in CD_3CH_2Cbl cannot be due to the "inductive" effect of deuterium.

In addition, we note that significant hyperconjugation in CH_3CH_2Cbl (eq 3) would be expected to cause the β-methyl protons of the organic ligand to be acidic. Indeed, when the ^{1}H NMR spectrum of CH_3CH_2Cbl was observed in 0.1 M potassium deuterioxide in D_2O, the relative integral of the β methyl proton signal gradually disappeared, demonstrating an exchange with solvent deuterium with a half-time of 280 min. Thus, the β-methyl protons of CH_3CH_2Cbl are, in fact, significantly acidic.

Because of the high-field ^{1}H chemical shift of the β-methyl group of CH_3CH_2Cbl (−0.71 ppm) and the even higher field shift (−1.06 ppm) of this resonance in the axial nucleotide-free analog $CH_3CH_2Cbi^+$,[1] the possibility that an agostic interaction[11] of the β-methyl protons of CH_3CH_2Cbl with the metal ion might be responsible for the ^{15}N chemical shift secondary isotope effects as well as the acidity of the β-methyl protons must be considered. Such an interaction has been postulated to occur between the β protons and central cobalt atom of alkylcobalt porphyrins.[12] While in static agostic interactions ^{1}H NMR chemical shifts as high as −15 to −16 ppm have been reported and C–H coupling constants of 60–90 Hz are typically observed due to the reduced C–H bond order,[11,13] in fluxional systems such as agostic methyls, averaged chemical shifts are more moderate (+2.7 to −6.5 ppm) and the averaged value of $^{1}J_{CH}$ for the agostic (60–90 Hz) and the nonbridging hydrogens (as high as 140 Hz due to the increased s character for the nonbridging C–H's)[13,14] is often in the normal range (120–130 Hz) for a CH_3 group.[11,14] However, due to the smaller zero-point energy difference between D and H in the three-center bond relative to that in the nonbridging positions, there is a thermodynamic preference (0.1–0.2 kcal mol^{-1})[15] for hydrogen to occupy the bridging position. Thus, for agostic methyls, a decrease in ^{1}H chemical shift and $^{1}J_{CH}$ value with progressive deuteration is considered diagnostic of the agostic interaction.[11,13,14,15a,16] In addition, the ^{2}H resonances will occur downfield from the ^{1}H resonances and will shift downfield with progressive deuteration due to the preference for the lighter isotope in the agostic interaction.[16b,c] However, as seen in Table 1, neither the ^{1}H chemical shift, the C–H coupling constant, nor the ^{2}H chemical shift of the organic ligand methyl group of CH_3CH_2Cbl or $CH_3CH_2Cbi^+$ is significantly affected by progressive deuteration. We conclude that this methyl group is *not* agostic and that the secondary deuterium isotope effect on the ^{15}N resonance of the axially coordinated nitrogen of CH_3CH_2Cbl and significant acidity of the β-methyl group of the organic ligand must be due to hyperconjugative electron delocalization.

Exalted hyperconjugation (i.e., $\sigma \rightarrow \pi$ conjugation)[17] has previously been shown to be responsible for the organometallic "β-effect" in organocobalt complexes, observed as the weak acidity of 1-(carboxyalkyl)cobaloximes[17] and (carboxymethyl)cobalt corrinoids,[18] the anomalous ^{19}F chemical shifts of *p*-(fluorobenzyl)cobaloximes,[19] the anomalously low carbonyl stretching frequencies of (formylmethyl)cobaloximes,[20] and the upfield chemical shift of the ^{1}H NMR resonance of the aldehyde hydrogen of (formylmethyl)cobalamin.[21] However, since this type of electron delocalization requires a low-lying π system involving the β atom of the organic ligand and can only function to withdraw electron density from the metal center, $\sigma \rightarrow \pi$ conjugation cannot be operative in CH_3CH_2Cbl. Similarly, in their study of substituent effects in $YCH_2Co(D_2H_2)L$ complexes, Marzilli et al. attributed the substantial "resonance" effect of Y to n → σ conjugation from lone pairs on Y to the Co–C σ bond.[2] While such delocalization would indeed donate electron density to the metal center, the absence of a lone pair on the β atom of CH_3CH_2Cbl precludes this explanation for the delocalization observed here. Thus, the delocalization demonstrated in CH_3CH_2Cbl must be due to classical hyperconjugation which, due to the metal's ability to stabilize charge, is substantially enhanced in these organometallic systems.

Experimental Section

Deuterated ethyl bromides were from Cambridge Isotope Laboratories, and CNCbl was from Roussel. Factor B,[1,22,23] ethylcobalamins,[24,25] and ethylcobinamides[25] were prepared as described previously. 1D and 2D NMR spectra were obtained on GE QE 300 and Bruker AMX 300 NMR spectrometers, respectively. The ^{15}N NMR resonances of the axial nucleotide of ethylcobalamins were observed using an inverse-detected HMQC experiment maximized for the approximately 9-Hz coupling[26] as described previously.[6]

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