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Substituent Effect Analysis in Alkylcobalamins. Correlation of the ^{15}N NMR Chemical Shifts of the Axial Nucleotide and Corrin Ring N22 and N23 via a Multiparameter Substituent Effect Equation

Kenneth L. Brown* and Daniel R. Evans

Department of Chemistry, Box CH, Mississippi State University, Mississippi State, Mississippi 39762

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By use of the ^{1}H, ^{15}N HMQC experiment, optimized for a ^{1}H–^{15}N coupling of about 7 Hz, the ^{15}N NMR resonances of the axial nucleotide nitrogens (B1 and B3) and two of the corrin ring nitrogens (N22 and N23) have been observed for a series of 12 alkylcobalamins of the type YCH_2Cbl. The B3, N22, and N23 chemical shifts are well correlated by a multiparameter substituent effect equation using traditional inductive, resonance, and steric substituent constants for the substituent, Y. The success of this approach suggests the importance of hyperconjugative delocalization of electron density between the metal center and the Y substituent and demonstrates the applicability of the classical Taft E_s steric substituent constants to alkylcobalamins. Together with a similar correlation of the intramolecular equilibrium constant for the base-on/base-off reaction of these YCH_2Cbl's, the data have been used to estimate the inductive, resonance, and steric substituent constants for the Y moiety of (5′-deoxyadenosyl)cobalamin (coenzyme B_{12}).

Introduction

Substituent effect analysis in organometallic chemistry has had a long and varied development. As originally pointed out by Marzilli et al.,[1] such analyses have generally fallen into three classes. One group of studies have viewed the metal center as a substituent of the organic moiety.[2–6] Such studies provide useful predictive capability for the chemical and spectroscopic properties of the organic ligand and valuable information as to how the structure of the metal center affects the metal's electronic properties. A second group of studies has correlated the properties of arylmetal complexes with parameters characterizing the substituent on the aromatic ligand.[7–9] These studies have provided important insights into metal–ligand bonding in complexes of this type. Finally, a number of attempts have been made to correlate the properties of organometallic complexes with the substituent parameters of the organic ligand.[1,10–14] This last approach is of great interest, because of both the potential of predicting the properties of complexes with new organic ligands and the potential for probing the nature of the carbon–metal bond.

Recently, substantial progress has been made in understanding the influence of the organic ligand on the properties of alkylcobaloximes of the type $YCH_2Co(D_2H_2)L$,[15] by use of multiparameter substituent effect equations in which the substituent is considered to be the group Y.[1,11c] Earlier use[11c] of eq 1,[16,17] where

$$Q_Y = \rho_I\sigma_I + \rho_R\sigma_R + \rho_S\sigma S + C \qquad (1)$$

Q_Y is a kinetic, equilibrium, or spectroscopic quantity of the $YCH_2Co(D_2H_2)L$ complex, σ_I, σ_R, and σ_S are inductive, resonance, and steric substituent constants, respectively, characteristic of the group Y, ρ_I, ρ_R, and ρ_S are the susceptibilities of the investigated quantity to inductive, resonance, and steric effects, respectively, and C is a constant, has led to excellent correlations of spectroscopic (NMR), thermodynamic, and kinetic data for such complexes.[11c] These correlations utilized standard substituent constants widely applicable in organic substituent effect analysis, thus relating substituent effects in these organometallic compounds to the vast variety of data available for organic substituent effects. Importantly, the success of the application of this method to $YCH_2Co(D_2H_2)L$ complexes, as well as the results of these correlations, has shown that the "resonance" effect is quite important in such complexes, often nearly as important as the inductive effect when the Y substituent is one methylene group

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(15) Abbreviations: $RCo(D_2H_2)L$, alkyl(ligand)cobaloxime = alkyl(ligand)bis(dimethylglyoximato)cobalt(III); $RCbi^+$, alkylcobinamide; R(CN)Cbi, alkyl(cyano)cobinamide; RCbl, alkylcobalamin, Bzm, 5,6-dimethylbenzimidazole; CNCbl, cyanocobalamin (vitamin B_{12}); AdoCbl, (5′-deoxyadenosyl)cobalamin (coenzyme B_{12}).

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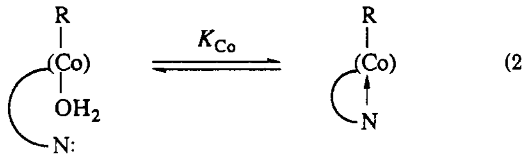
removed from the metal atom. This resonance effect has been interpreted as being indicative of significant hyperconjugation in these complexes, even in situations where exalted hyperconjugation ($\sigma \rightarrow \pi$ conjugation) is not possible.[11c] Unlike the latter phenomenon, the hyperconjugation revealed by this method can involve either acceptance of electron density from, or donation of electron density to, the cobalt center. In addition, this earlier work showed that steric effects of the axial organic ligand, while often quite small, were nonetheless significant, since inclusion of the third term in eq 1 generally gave rise to significant improvements in the correlations.

An attempt has also been made to apply this method to substituent effect analysis in organocobalt corrinoids, however with significantly less success.[18] The problem here has been in obtaining sets of data for a sufficient number of compounds in which the correlatable quantity varies substantially across the series. For instance, while the binding constant for the cyanide ion trans to the orgnaic ligand in alkylcobinamides[19] ($RCbi^+$)[15] varied by 4.8 log units between $CH_3CH_2Cbi^+$ and $NCCH_2Cbi^+$, many R(CN)Cbi derivatives were unstable, spontaneously dealkylating in the dark, thus limiting the number of complexes for which data could be obtained.[18] While other ligands produced stable alkylcobinamide adducts, the binding constants were low and variability across a series of $RCbi^+$'s was very small.[18] A resonably successful correlation ($r^2 = 0.93$) using eq 1 was obtained for the intramolecular equilibrium between the base-on and base-off species of alkylcobalamins (RCbl)[15] (eq 2). However, while



(2)

the values for this equilibrium constant vary by 6.8 log units across a series of spanning $C_6H_5CH_2Cbl$[20] to H_2OCbl^+,[21] they vary by only 2.85 log units across the series of accessible YCH_2-Cbl complexes.[18]

We have consequently investigated the possibility of the use of NMR spectroscopic properties of YCH_2Cbl's to obtain data sets suitable for testing the applicability of eq 1 to alkylcobalt corrinoids. However, while the ^{13}C chemical shifts of the axial benzimidazole ligand (Bzm) of base-on cobalamins vary by as much as 3 ppm across a series of compounds including CNCbl and H_2OCbl^+,[22] restricting the series to YCH_2Cbl's reduces the variability to <1 ppm at every Bzm carbon. Similarly, comparison of the ^{13}C chemical shifts of the corrin ring carbons of AdoCbl[15,23] and CNCbl[24,25] shows variations of as much as 3–4 ppm at C4, C9, and C16 (Figure 1), but YCH_2Cbl complexes show at most a 1.7 ppm variation (at C9).[26] As a result, the possibility of using the ^{15}N NMR chemical shifts of the B3 (i.e., coordinated) nitrogen of the axial nucleotide (Figure 1) to test the applicability of eq 1 to YCH_2Cbl complexes has become attractive. Observations of the B3 and B1 axial nucleotide ^{15}N resonances of cobalamins, using CNCbl enriched in ^{15}N at these positions, showed that coordination of the nucleotide to the metal center in CNCbl changes the B3 ^{15}N chemical shift by 44.6 ppm.[27] Moreover, a method was recently devised[28] for observation of these resonances at natural abundance by indirect detection utilizing the heteronuclear multiple-quantum coherence (HMQC)[29,30] experiment with the coupling delay set to maximize the transfer function for a 7-Hz coupling, i.e., the approximate value of the two-bond coupling constants for the B2 proton and the B1 and B3 nitrogens.[27] In addition, this HMQC method also permits observation of the ^{15}N resonances of the corrin ring N22 and N23 nitrogens which are apparently coupled significantly to the C10 hydrogen (Figure 1). Preliminary results[28] have shown that the chemical shift of the B3 resonance varies by 81.4 ppm across the series CH_3Cbl, CNCBl, H_2OCbl^+ and that the chemical shifts of the N22 and N23 resonances vary by 22.1 and 19.3 ppm, respectively, across the same series. These results suggested that the ^{15}N chemical shift differences among a series of YCH_2Cbl complexes might provide excellent data sets for substituent effect analysis. We now report the observation of the B1, B3, N22, and N23 ^{15}N NMR resonances and the successful application of eq 1 for the correlation of the chemical shifts of three of these resonances with standard substituent constants.

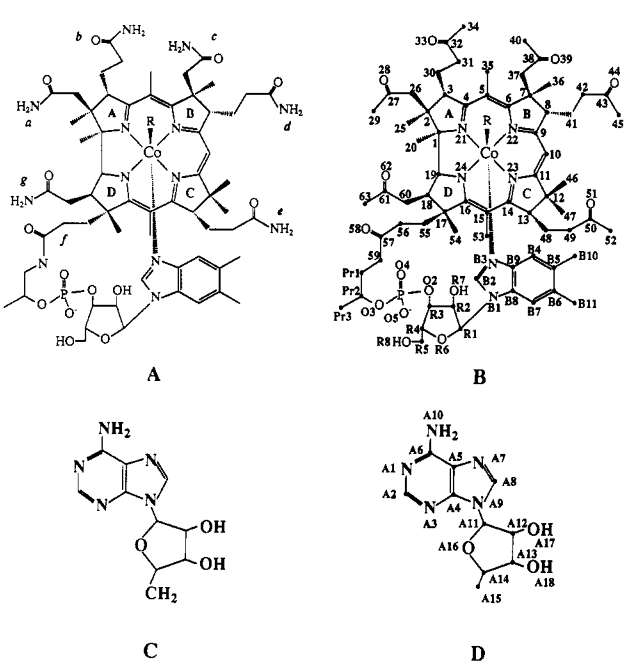


Figure 1. (A, B) Structure and numbering scheme for an alkylcobalamin (RCbl). (C, D) Structure and numbering scheme for the 5′-deoxyadenosyl (Ado) ligand of AdoCbl.

Experimental Section

Alkylcobalamins were prepared and characterized (by UV–vis, HPLC and, in some cases, ^{13}C NMR and FAB MS) as described previously (AdoCbl,[31] CH_3CH_2Cbl,[32] $CH_3CH_2CH_2Cbl$, $NC(CH_2)Cbl$, CH_3Cbl, $CH_3OOCCH_2CH_2Cbl$, CH_3OOCCH_2Cbl, and CF_3CH_2Cbl,[11d] $(CH_3)_2CHOCH_2CH_2Cbl$, $CH_3OCH_2CH_2Cbl$, and $C_6H_5OCH_2CH_2Cbl$,[33] $ClCH_2$-Cbl,[18] and $NCCH_2Cbl$[21]). NMR samples (0.5 mL in 5-mm tubes) contained 10–50 mM RCbl in DMSO-d_6 plus TSP as an internal ^1H chemical shift reference, except for $NCCH_2Cbl$, which was observed in D_2O, and CH_3CH_2Cbl, $CH_3CH_2CH_2Cbl$, and $CH_3OOCCH_2CH_2Cbl$, which were observed in D_2O containing 15% (v/v) CD_3CN. ^{15}N chemical shifts were referenced to external CH_3NO_2 but are reported relative to

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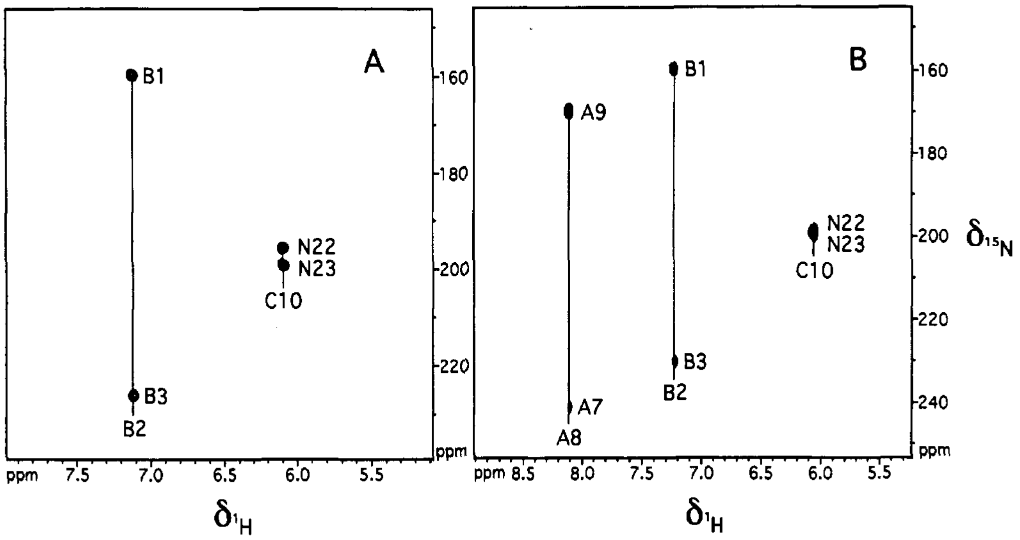

Figure 2. ^{1}H, ^{15}N HMQC spectra, optimized for a ^{1}H–^{15}N coupling of ca. 7 Hz, of $C_6H_5OCH_2CH_23Cbl$ (A) and AdoCbl (B) in DMSO-d_6.

NH_3(l) using $\delta_{CH_3NO_2}$ = 380.32 ppm.[34] ^{1}H, ^{15}N HMQC spectra were obtained on a Bruker AMX 300 NMR spectrometer operating at 300.136 MHz (^{1}H) and 30.413 MHz (^{15}N). Data were collected into a 512 × 256 data matrix using 512 scans per t_1 value, preceded by 4 dummy scans. The sweep widths were 7604.4 and 5404.5 Hz in the F1 and F2 dimensions, respectively, and the H–X coupling delay was 68 ms. The data were zero-filled to a 512 × 512 matrix and processed with Gaussian line broadening of –5 Hz.

Table 1. ^{15}N NMR Chemical Shifts of the B1, B3, N22, and N23 Nitrogens of Base-On Cobalamins[a]

	$\delta_{^{15}N}$,[b] ppm				$\delta_{^1H}$,[c] ppm	
compound	B1	B3	N22	N23	B2H	C10H
AdoCbl[d]	159.7	229.9	199.1	199.1	7.22	6.04
CH_3CH_2Cbl[e]	154.9	229.4	199.9	205.3	7.07	6.10
$CH_3CH_2CH_2Cbl$[e]	154.7	229.2	200.2	205.3	7.03	6.07
$NC(CH_2)_3Cbl$	159.5	228.2	197.7	202.2	7.13	6.07
$(CH_3)_2CHOCH_2CH_2Cbl$	159.9	228.0	196.1	200.4	7.15	6.11
$CH_3OCH_2CH_2Cbl$	159.6	227.3	195.7	200.1	7.09	6.06
$C_6H_5OCH_2CH_2Cbl$	159.8	226.2	195.7	199.1	7.12	6.10
CH_3Cbl[f]	159.5	225.0	196.0	198.8	7.04	5.90
$CH_3OOCCH_2CH_2Cbl$[e]	155.4	223.6	195.7	201.4	6.91	5.96
$ClCH_2Cbl$	160.0	216.7	193.9	193.9	7.07	5.95
CH_3OOCCH_2Cbl	160.7	215.4	193.0	193.0	7.00	5.99
CF_3CH_2Cbl	160.7	215.2	190.5	190.5	7.02	5.95
$NCCH_2Cbl$[g]	157.2	207.7	186.3	186.3	7.03	6.04
CNCbl[f]	163.2	188.3	173.4	176.4	6.97	5.93
H_2OCbl^+[f]	166.0	143.6	173.9	179.5	6.72	6.00

[a] At 27 °C in DMSO-d_6, except as noted. [b] ^{15}N Chemical shifts were determined relative to external CH_3NO_2 but are reported relative to NH_3(l) using $\delta_{CH_3NO_2}$ = 380.32 ppm.[34] [c] ^{1}H chemical shifts are relative to internal TSP. [d] Additional crosspeaks are observed at 8.08 and 169.6 ppm (A8H–A9) and 8.08 and 241.9 ppm (A8H–A7). [e] In 15% CD_3CN/D_2O. [f] Reference 28. [g] In D_2O.

Results

For all of the RCbl's except AdoCbl, the ^{1}H, ^{15}N HMQC spectra obtained by maximizing the transfer function for ca. 7-Hz couplings consisted of four (or three) crosspeaks, as shown in Figure 2A, for example, for $C_6H_5OCH_2CH_2Cbl$, two occurring at a ^{1}H chemical shift near 7 ppm and two (or one) occurring at a ^{1}H chemical shift near 6 ppm. When only one crosspeak appeared near 6 ppm, it was of very high intensity and clearly represented the superposition of the two crosspeaks often found at this ^{1}H shift. As discussed previously,[28] these crosspeaks are due to the B2H–B1 and B2H–B3 couplings and to the C10H–N22 and C10H–N23 couplings, respectively. The values of the chemical shifts of these protons and nitrogen resonances for the 12 compounds studied here are collected in Table 1, along with the values previously reported[28] for CH_3Cbl, CNCbl, and H_2OCbl^+. The axial nucleotide ^{15}N resonances of CNCbl were originally assigned by analogy to other nitrogen heterocycles and by observation of the ^{15}N line widths in the direct-detected ^{15}N NMR spectra of [B1,B3-$^{15}N_2$]CNCbl.[27] Thus, the broader, more downfield resonance of the pair was assigned to the B3 nitrogen due to the anticipated effect of the cobalt atom's quadrupolar relaxation on the T_2 relaxation rate of the coordinated nitrogen. The results reported herein (Table 1) confirm these assignments in that the chemical shift of the resonance assigned to the cobalt-coordinated nitrogen (B3) varies by 86.3 ppm across the series from AdoCbl to H_2OCbl^+, while that assigned to the *N*-glycosidic nitrogen (B1) of the axial nucleotide varies by only 11.3 ppm. In every case except H_2OCbl^+, the B3 nitrogen exhibits the more downfield resonance of the pair. The ^{15}N resonances of the corrin ring nitrogens N22 and N23 for CNCbl were previously assigned by observation of the influence of structural modifications to the corrin macrocycle on the chemical shifts of the two nitrogens coupled to C10H. For the remainder of the compounds, these assignments have been made by analogy to CNCbl; i.e., when resolved, the more upfield resonance is assigned to N22 and the more downfield to N23. All of these assignments are in accord with those recently made by Hollenstein and Stupperich[35] for the CNCbl analog cyano(5-hydroxybenzimidazoyl)cobamide.

For AdoCbl, two additional crosspeaks occurred in the ^{1}H, ^{15}N HMQC spectrum at the proton chemical shift of 8.08 ppm (Figure 2B). These are evidently due to ^{1}H–^{15}N couplings in the adenine moiety of the 5′-deoxyadenosyl ligand. From the published ^{1}H NMR assignments of AdoCbl in D_2O,[23] we conclude that these crosspeaks arise from couplings between the A8 hydrogen and the A7 and A9 nitrogens (Figure 1). By analogy to the assignments of the free-base, detached nucleoside α-ribazole,[28] we assign the more upfield of these two Ado resonances (169.6 ppm) to A9 and the more downfield (241.9 ppm) to A7.

For $NCCH_2Cbl$ and $CH_3OOCCH_2CH_2Cbl$, poor solubility prevented the observation of the ^{1}H, ^{15}N HMQC spectrum in DMSO-d_6. Instead, these spectra were observed in D_2O and in 15% (v/v) CD_3CN in D_2O, respectively. Previous results[27,28] have demonstrated that there is essentially no difference in chemical shift for the B1 and B3 nitrogens in DMSO-d_6 or in D_2O, as might be anticipated since these nitrogens would not be expected to be solvated in base-on Cbl's. For CH_3CH_2Cbl and $CH_3CH_2CH_2Cbl$, the B2H–B1 and B2H–B3 crosspeaks could

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Table 2. Substituent Constants Used for Correlations via Eq 1

Y	$\sigma_I{}^a$	$\sigma_R{}^a$	$\sigma_R{}^{-1a}$	$\sigma_{R^+}{}^a$	$E_s{}^{(Y)\,b}$	$E_s{}^{(YCH_2)\,c}$
CH_3	0.01	–0.18	–0.18	–0.32	–1.24	–0.07
CH_3CH_2	0.00	–0.15	–0.19	–0.30	–1.31	–0.36
$NCCH_2CH_2$	0.09[d]	–0.12[d]	–0.05[d]	–0.10[d]	–2.19	–0.50
$(CH_3)_2CHOCH_2$	0.10[d]	–0.11[d]	–0.04[d]	–0.08[d]	–1.61	–0.78
CH_3OCH_2	0.13	–0.12	–0.04[d]	–0.18	–1.55	–0.77
$C_6H_5OCH_2$	0.08	–0.10	–0.04[d]	–0.11[d]	–1.86	–0.87
H	0.03	0.00	0.00	0.00	0.00	0.00
CH_3OOCCH_2	0.19[d]	–0.10[d]	–0.03[d]	–0.07[d]	*e*	–0.94
Cl	0.42	–0.19	–0.23	–0.31	–0.97	–0.24
CH_3OOC	0.34	0.11	0.41	0.15	*e*	*e*
CF_3	0.38	0.16	0.27	0.23	–2.40	*e*
NC	0.51	0.15	0.49	0.15	–0.51	–1.04

[a] Taken from ref 17, except as noted. [b] Taft steric substituent constant[38] for the substituent Y. The values are from ref 17. [c] Taft steric substituent constant[38] for the substituent YCH_2. The values[17] have been renormalized to $E_s{}^{(YCH_2)} = 0$ for $YCH_2 = CH_3$. [d] Taken, or calculated, from ref 37. [e] Value not available.

not be observed in the HMQC spectra in DMSO-d_6, possibly because of exchange broadening since these are the only two compounds for which significant (although small) amounts of the base-off species exist, at least in neutral aqueous solution.[32] For both compounds, however, the B1 and B3 crosspeaks were readily observed in 15% CD_3CN/D_2O solution.

The data in Table 1 show the exquisite sensitivity of the chemical shift of the B3 nitrogen of base-on cobalamins to the nature of the trans axial ligand. Even when the series is restricted to compounds of the type YCH_2Cbl (i.e., CNCbl and H_2OCbl^+ are omitted), the B3 chemical shift varies by 22.2 ppm. The N22 and N23 chemical shifts show somewhat less variation among the YCH_2Cbl's (13.9 and 19.0 ppm, respectively) but still depend rather strongly on the nature of Y. The chemical shift of the B1 nitrogen is considerably less sensitive to Y (6.0 ppm) as might be expected.

Correlations of these ^{15}N chemical shifts were attempted using the inductive (σ_I) and resonance (σ_R, σ_{R^-}, σ_{R^+}) parameters recently tabulated by Hansch et al.[36] For substituents for which values were not available from this source, the substituent constants were taken from the extensive listings of Charton.[37] For steric substituent constants (σ_S), the E_s values of Taft[38] were taken from the extensive tabulation of Unger and Hansch.[17] Correlations were not attempted using the modified E_s' steric substituent parameter of Dubois et al.,[39,40] since values were not available for a sufficient number of compounds. Charton's steric parameter, ν,[16] was not used in any correlations since it has been shown to be linearly related to the Taft's E_s.[41] The values of the various substituent constants used are given in Table 2.

For each ^{15}N resonance, correlations were attempted using only the inductive term in eq 1, using the inductive and resonance terms, and using the complete equation including the steric term. For correlations including the resonance term, all three of the resonance substituent constants, σ_R, σ_{R^-}, and σ_{R^+}, were used, and for correlations using the complete equation, the Taft steric substituent constant, $E_s{}^{(Y)}$, for the substituent Y, was used as well as that for the alkyl group, YCH_2 ($E_s{}^{(YCH_2)}$). Both the coefficient of determination, r^2, and the f statistic[42] have been used to judge the goodness of fit, using Topsom's criterion[43] that $0.1 < f < 0.2$ is an acceptable fit, while $f < 0.1$ is an excellent fit.

The best correlations obtained with one, two, or all three terms from eq 1 are summarized in Table 3. For the B3 chemical shifts, a fair correlation ($f = 0.29$) is obtained using the inductive term only. The correlation is significantly improved (to $f = 0.19$) by inclusion of the resonance term, with σ_R giving the best correlation. Inclusion of the steric term improves the correlation to excellent ($f = 0.09$), with the σ_R resonance constant again giving the best fit. While the best correlation was obtained using $E_s{}^{(Y)}$, the $E_s{}^{(YCH_2)}$ parameters give nearly as good a fit. For the best correlation, the relative sensitivity of the B3 chemical shift to inductive, resonance, and steric effects decreases in the order $|\rho_I| > |\rho_R| > |\rho_S|$ with ρ_R/ρ_I being 0.478 and ρ_S/ρ_I being 0.0557.

No resonable correlations could be obtained for the B1 chemical shifts using any number of terms or any combination of substituent constants. However, very good correlations were obtained for both the N22 and N23 chemical shifts. Both of these chemical shifts correlated poorly ($r^2 < 0.9$) with the inductive parameter alone but produced fair correlations ($0.92 < r^2 < 0.96$) when the resonance term was added, again with σ_R giving the best correlations. Inclusion of the steric term significantly improved both correlations. For the N22 chemical shifts, the $E_s{}^{(Y)}$ steric parameter gave essentially equally good fits with σ_R and σ_{R^-}, while correlation with the $E_s{}^{(YCH_2)}$ parameters and σ_R gave a slightly better fit. The magnitude of the resonance effect for the N22 correlations is similar to that of the inductive effect ($\rho_R/\rho_I = 0.75$–1.20), while the steric effect is again much smaller ($\rho_S/\rho_I \approx 0.03$). For the N23 chemical shifts, $E_s{}^{(Y)}$ gave the best fits, but correlations were about equally as good with σ_R or σ_{R^+}.

As previously discussed,[11c,18] these correlations via eq 1 can be visualized by use of the two-dimensional transformation of eq 3, where $\bar{\sigma}$ and $\bar{\rho}$ are defined in eqs 4–7. Such two-dimensional

$$Q_Y = \bar{\sigma}\bar{\rho} + C \qquad (3)$$

$$\bar{\sigma} = (\sigma_I + \lambda_1\sigma_R + \lambda_2\sigma_S)/(1 + |\lambda_1| + |\lambda_2|) \qquad (4)$$

$$\bar{\rho} = \rho_I(1 + |\lambda_1| + |\lambda_2|) \qquad (5)$$

$$\lambda_1 = \rho_R/\rho_I \qquad (6)$$

$$\lambda_2 = \rho_S/\rho_I \qquad (7)$$

representations of the correlations of the B3, N22, and N23 chemical shifts with σ_I, σ_R, and $E_s{}^{(Y)}$ are shown in Figures 3 and 4.

Discussion

The data in Table 1 clearly represent good data sets for testing the applicability of eq 1 to the properties of YCH_2Cbl complexes. There is adequate variation in the inductive effect of the Y substituent (σ_I varies by 0.51) and in its resonance properties (σ_R varies by 0.35), although additional compounds with resonance-withdrawing substituents would be desirable. The steric bulk of Y also varies widely across the series ($\Delta E_s{}^{(Y)} = 2.4$). In addition, the variability of the chemical shift in each data set ($\Delta\delta_{^{15}N}$ = 22.2, 13.9, and 19.0 ppm for B3, N22, and N23, respectively) is sufficiently large both to inspire confidence in the meaningfulness of good correlations and to avoid the statistical artifacts inherent in data sets of limited variability.[42]

The success of the correlations of the B3, N22, and N23 chemical shifts (Figures 3 and 4) provides strong evidence for the applicability of the multiparameter substituent effect approach to the properties of YCH_2Cbl's. In each case, a poor correlation using only the inductive term is dramatically improved by inclusion of the resonance term. In addition, inclusion of the steric term

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Table 3. Results of the Correlations of ^{15}N Chemical Shift Data for YCH_2Cbl's via Eq 1

Q_Y[a]	R[b]	S[c]	ρ_I	ρ_R	ρ_S	C	N[d]	r^2	f
δ_{B3}			−38.7			230.0	12	0.918	0.29
δ_{B3}	σ_R		−32.4	−14.9		228.1	12	0.965	0.19
δ_{B3}	σ_R	$E_s^{(Y)}$	−31.8	−15.2	−1.77	226.0	10	0.991	0.09
δ_{B3}	σ_R	$E_s^{(YCH_2)}$	−34.6	−23.4	−3.22	225.8	10	0.989	0.10
δ_{N22}			−19.6			198.8	12	0.807	0.44
δ_{N22}	σ_R		−14.4	−12.7		197.2	12	0.922	0.28
δ_{N22}	σ_R	$E_s^{(Y)}$	−14.5	−14.9	−0.52	196.2	10	0.963	0.19
δ_{N22}	σ_{R^-}	$E_s^{(Y)}$	−12.8	−9.6	−0.40	196.9	10	0.969	0.18
δ_{N22}	σ_R	$E_s^{(YCH_2)}$	−14.7	−17.6	0.59	196.9	10	0.978	0.15
δ_{N23}			−30.9			203.9	12	0.855	0.38
δ_{N23}	σ_R		−23.5	−17.9		201.7	12	0.952	0.22
δ_{N23}	σ_R	$E_s^{(Y)}$	−23.9	−18.3	−0.86	200.2	10	0.984	0.13
δ_{N23}	σ_{R^+}	$E_s^{(Y)}$	−24.6	−12.0	−1.13	199.7	10	0.981	0.14

[a] Chemical shift correlated. [b] Resonance substituent constant used. [c] Steric substituent constant used. [d] Number of data points correlated.

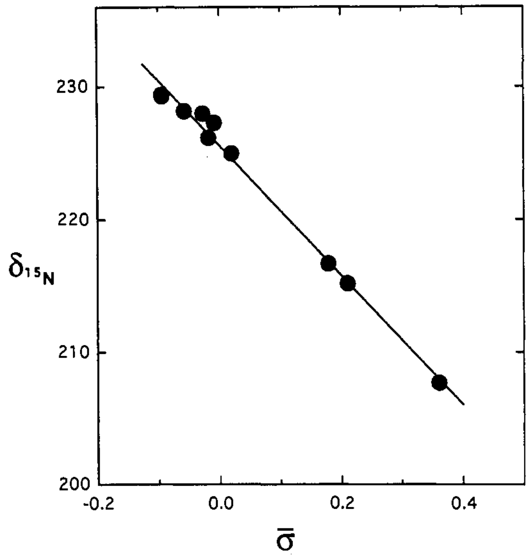


Figure 3. Plot of the ^{15}N chemical shift of the B3 nitrogen of YCH_2Cbl's vs $\bar{\sigma}$ (eq 4) according to eq 3. The value of $\bar{\rho}$ (−48.80) was calculated from eqs 5–7 and the data in Table 3.

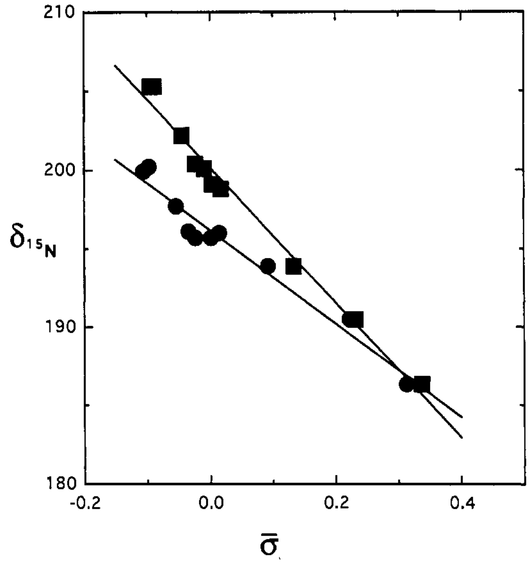
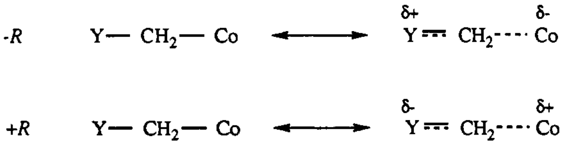


Figure 4. Plots of the ^{15}N chemical shifts of the N22 (●) and N23 (■) nitrogens of YCH_2Cbl's vs $\bar{\sigma}$ (eq 4) according to eq 3. The values of $\bar{\rho}$ (−29.88 for δ_{N22} and −43.03 for δ_{N23}) were calculated from eqs 5–7 and the data in Table 3.

provides further, significant improvement in each correlation. These observations have important consequences for understanding substituent effects and bonding in alkylcobalamins as discussed below.

Bonding Implications. First, and most important, the significance of the resonance term (Table 3) demonstrates that simple σ-orbital overlap does not represent an adequate description of the carbon–cobalt bond in these complexes. Marzilli et al.[1] originally attributed the "resonance" effect in $YCH_2Co(D_2H_2)L$ complexes to n → σ conjugation between lone pairs on Y and the Co–C bond. This cannot be the case here, as we have shown for such cobaloximes,[11c] since many of the YCH_2Cbl's successfully correlated here have no lone pairs on Y. In addition, such n → σ conjugation could only account for the "resonance" interactions of resonance donating (−*R*) substituents, while YCH_2Cbl complexes containing both resonance donating *and* resonance withdrawing (+*R*) substituents correlate well in this work. Similarly, $\sigma \rightarrow \pi$ conjugation (exhaulted hyperconjugation),[2,44–48] well-known to be responsible for the organometallic "β-effect"[4b,c,5d,e,h,11c,49,50] in both cobaloximes and cobalamins, cannot account for the "resonance" interactions of resonance donating (−*R*) substituents and requires a low-lying π-system involving the β atom of the organic ligand. The successful application of eq 1 to the YCH_2Cbl's, including compounds *without* Y lone pairs and *without* low-lying π-systems involving the β atom, but *including* compounds with both resonance-withdrawing and resonance-donating substituents, strongly suggests that a more general hyperconjugation phenomenon (eqs 8 and 9)is operative



$$-R \qquad Y\text{—}CH_2\text{—}Co \longleftrightarrow \overset{\delta+}{Y}\text{=}CH_2\cdots\overset{\delta-}{Co} \qquad (8)$$

$$+R \qquad Y\text{—}CH_2\text{—}Co \longleftrightarrow \overset{\delta-}{Y}\text{=}CH_2\cdots\overset{\delta+}{Co} \qquad (9)$$

here. In this bonding picture, hyperconjugative delocalization of electron density can occur by either donation or withdrawal of electron density by the Y substituent due to the metal atom's ability to stabilize a partial charge of either sign, and depending

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on the Y substituent's ability to accommodate a partial charge of opposite sign. Independent, and more direct, evidence of the existence of this type of hyperconjugation in YCH_2Cbl's is currently being sought.

The Steric Effect. Second, the steric effect of Y on the YCH_2-Cbl properties correlated here, though small, is clearly significant. In each case, the correlation is substantially improved by inclusion of the steric term. Importantly, these results show the adequacy of the Taft E_s steric substituent parameters for use with alkylcobalamins and obviate concerns raised earlier regarding the applicability of these parameters, originally derived from rate constants for acid-catalyzed hydrolysis of aliphatic esters,[38] to organometallic systems in general and to RCbl's in particular.[11d] Thus, despite the obvious differences in bonding geometry between the pseudooctahedral organometallic complexes at issue here and the aliphatic esters used to establish the E_s scale, the Taft steric substituent constants seem to adequately account for the steric effects in the YCH_2Cbl's. However, the magnitude of the steric effect in these correlations is similar to that previously observed for correlations of $YCH_2Co(D_2H_2)L$ properties.[11c] This is somewhat surprising in that steric crowding of the axial ligand positions of cobalamins by the corrin ring side chains might be expected to enhance the steric effects of Y substituents.

Interpretation of the Correlations. The structural determinants discussed above permit the following interpretation of the correlations described here (Table 3). Inductive withdrawal ($+I$) by the Y substituent in YCH_2Cbl's is seen to shift the B3 ^{15}N resonance upfield (i.e., $\rho_I < 0$). This can be interpreted as being due to a strengthening of the axial Co–N bond due to inductive withdrawal by Y, since coordination of the axial Bzm in CNCbl ($\delta_{B3} = 163.2$ ppm) causes a 69.1 ppm upfield shift of the B3 resonance of the base-off but Bzm-deprotonated $(CN)_2Cbl$ species ($\delta_{B3} = 232.3$ ppm).[27] Similarly, resonance withdrawal by Y ($+R$) also causes an upfield shift of the B3 resonance by strengthening the axial Co–N bond due to electron depletion from the metal atom (eq 9). Finally, an increase in steric bulk of the Y substituent causes the B3 resonance to shift downfield, indicating that the axial Co–N bond is weakened by bulky trans organic ligands. This important observation provides evidence of the widely held belief that steric effects can be transmitted from one axial ligand to the other in cobalamins, probably mediated by interactions with the corrin ring side chains and consequence changes in corrin ring pucker.

The N22 and N23 ^{15}N chemical shift correlations can also be understood in such terms by noting the effect of axial nucleotide coordination of these chemical shifts in the CH_3Cbl analog methyl-(5-hydroxybenzimidazoyl)cobamide, as recently determined by Hollenstein and Stupperich.[35] Thus, the conversion of the base-on species of this analog to the base-off, axial nucleotide-protonated species causes a downfield shift of both the N22 and N23 resonances.[35] Hence, inductive, hyperconjugative, and steric effects of the Y substituent on the N22 and N23 chemical shifts can be viewed as similar to their effects on B3, although other interpretations are, of course, possible.

Estimation of the Substituent Parameters for the Y Substituent of AdoCbl. The existence of three good correlations of YCH_2Cbl properties via eq 1 allows the possibility of estimating the inductive, resonance, and steric substituent parameters for the Y substituent of AdoCbl. Inspection of the ^{15}N chemical shift data (Table 1) shows that the B3 and N22 chemical shifts of AdoCbl are similar to those of CH_3CH_2Cbl and $CH_3CH_2CH_2Cbl$. However, the N23 resonance of AdoCbl is 6 ppm upfield from those of the latter two complexes. Inspection of Glusker's elegant stereo drawings of the X-ray structure of AdoCbl[51] shows that the adenine rings of the Ado ligand lie immediately above the N23 nitrogen, suggesting that the N23 resonance of this complex is anomalously shifted upfield by the magnetic anisotropy of the adenine moiety. This would clearly make the N23 chemical shift correlation unsuitable for estimation of the substituent parameters of the Ado ligand. We have consequently attempted to supply the needed third correlation by correlation of the values of log(K_{Co}) (eq 2) with the σ_I, σ_R, and $E_s^{(Y)}$ parameters given in Table 2 for the same 10 compounds for which the B3 and N22 chemical shifts were successfully correlated. As was previously the case,[18] the best correlation ($\rho_I = 2.72$, $\rho_R = 1.47$, $\rho_S = 0.29$) was only fair ($r^2 = 0.928$) and was obtained using the σ_{R^+} parameters. The limited range of the log(K_{Co}) values (2.45) for this data set may well be responsible for the modest precision of the fit. Nonetheless, if the difference between the σ_R and σ_{R^+} value for the Y substituent of AdoCbl is assumed to be small, this correlation may be used along with those of the B3 and N22 chemical shifts, and the value of K_{Co} (72.5) for AdoCbl (calculated from its on-off pK_a[21] as described previously[32]), to estimate the substituent parameters for this group. The resulting estimates are $\sigma_I = 0.06$, $\sigma_R = -0.20$, and $E_s^{(Y)} = -1.53$, suggesting that the Y group of AdoCbl is slightly inductively withdrawing (similar to $C_6H_5OCH_2$), significantly hyperconjugatively donating (similar to CH_3 and CH_3CH_2), and only about as bulky as CH_3OCH_2 (Table 2). While the last result may seem surprising, it may simply be an expression of the fact that, in AdoCbl, the Ado ligand is constrained to lie above the "southern" half of the macrocycle by the so-called sentinel methyl groups, C46 and C54 (Figure 1).[51] If it is correct that the Ado ligand is prevented from rotation about the Co–C bond by these sentinels in solution, its steric interactions with the upward projecting corrin ring side chains, and hence its apparent steric effect, would be expected to be considerably reduced from that expected due to its steric bulk.

In sum, the ^{15}N chemical shifts of the B3, N22, and N23 nitrogens of base-on cobalamins of the type YCH_2Cbl have been shown to give good to excellent correlations with the inductive, resonance, and steric substituent constants of the Y group via a multiparameter substituent effect equation (eq 1). Since both complexes in which Y does not have a lone pair and those which do not have a low-lying π-system involving the β atom of the organic ligand are well correlated, then n $\rightarrow$ σ and σ $\rightarrow$ π conjugation mechanisms cannot account for these significant "resonance" effects. Thus, the importance of the resonance effect in these correlations testifies to the importance of generalized hyperconjugation (eqs 8 and 9) in such complexes, which can occur with either resonance donation of electron density to or resonance withdrawal of electron density from the metal atom. Steric effects, while relatively small, are clearly important, as inclusion of the resonance term in the multiparameter substituent effect equation significantly improves these correlations. Importantly, the classical Taft E_s steric substituent parameter appears to adequately represent the steric influence of the organic ligand despite the fact that these constants were derived from measurements in an organic reaction system of radically different geometry from that of the YCH_2Cbl complexes.

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