Near-Infrared Fourier Transform Raman Spectroscopy of Photolabile Organocobalt B_{12} and Model Compounds. 3. Vibrational Assessment of Factors Affecting the Co–C Bond in Models

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Abstract: Near-infrared Fourier transform (FT)-Raman spectra have been measured for a large number of B_{12} model compounds containing the $(DH)_2$ equatorial ligand system (DH = monoanion of dimethylglyoxime) in order to assess the importance of various factors (viz., trans electronic effect, trans steric effect, environmental effect) that influence the Co–C bond stretch. The Co–CH_3 stretching mode of these B_{12} models in the solid state is generally detected as a very intense and sharp Raman line at ca. 500 cm^{-1}, which exhibits a frequency decrease of 2–27 cm^{-1} in chloroform solution. Comparison of FT-Raman results with X-ray structural data indicates the existence of structural differences between the solid state and solution. It is suggested that the flexible $Co(DH)_2$ unit is bent from planarity more in solution than in the solid. Such a conformational distortion should lead to a weakening of the Co–C bond. Although there is a well-established relationship between the Co–C bond cleavage rate of (4-X-pyridine)$Co(DH)_2R$ complexes and 4-X-pyridine basicity, FT-Raman studies of a series of (4-X-pyridine)$Co(DH)_2CH_3$ compounds (X = H, cyano, *tert*-butyl, and NMe_2) in chloroform unambiguously reveal the absence of a trans electronic influence on the Co–C stretching frequency in the ground state. A similar study of a series of $PR_3Co(DH)_2CH_3$ complexes (R = methyl, *n*-butyl, phenyl, and cyclohexyl) confirms the presence of a trans steric influence. The observed trans steric influence in the ground state, however, is not large enough to account for the differences in Co–C bond cleavage rates for related B_{12} models with bulky axial alkyl groups. These findings suggest that the energetics of the transition state/ground state properties not directly related to Co–C bond strength are important. Since the $\nu_{(Co-C)}$ stretch in these models is similar to that in the coenzyme, methyl B_{12}, we discuss the possibility that the B_{12}-dependent enzymes enhance the Co–C bond cleavage rate by lowering the overall energy of the transition state, rather than by significantly weakening the Co–C bond in the ground state.

Introduction

A key feature in the most widely accepted mechanism of coenzyme B_{12} dependent rearrangements is the homolytic cleavage of the coenzyme's cobalt–carbon bond.[1] In order to achieve a better understanding of the relationship between structure and Co–C bond stability, extensive investigations have been carried out on both biologically active cobalamins[2] and B_{12} model compounds.[2,3] Spectroscopic and kinetic studies of naturally occurring and chemically modified cobalamins have been interpreted to suggest that both coenzyme B_{12} and the protein undergo substantial conformational changes upon addition of the substrate (or analogues) to the holoenzyme.[4] Such conformational changes are thought to induce structural distortions that trigger the Co–C bond cleavage. The exact nature of the structural distortions, however, remains unclear. The flexibility of the corrin and its butterfly conformation,[5] the role of the axial base and side chains in inducing this conformation,[4,6] and the angular distortion of the Co–C–C group[6a,7] have been suggested as key variables in the conformational distortion picture. Previous investigations of B_{12} model complexes with kinetic, spectroscopic, and crystallographic methods have also provided valuable insight into factors influencing the Co–C bond.[3,6,8] The most extensively studied "cobaloxime" models are of the type $LCo(DH)_2R$, where L and R are neutral and mononegative monodentate axial ligands, respectively, and DH is the monoanion of dimethylglyoxime. Figure 1 schematically shows the structures of coenzyme B_{12} (adenosylcobalamin, $AdoB_{12}$) and the model compounds $LCo(DH)_2R$.

Halpern and co-workers[9] have estimated the Co–C bond dissociation energies (BDE's) for systematically related organocobalt complexes using both kinetic and equilibrium methods and have also studied the effect of ligands trans to the alkyl ligand on the

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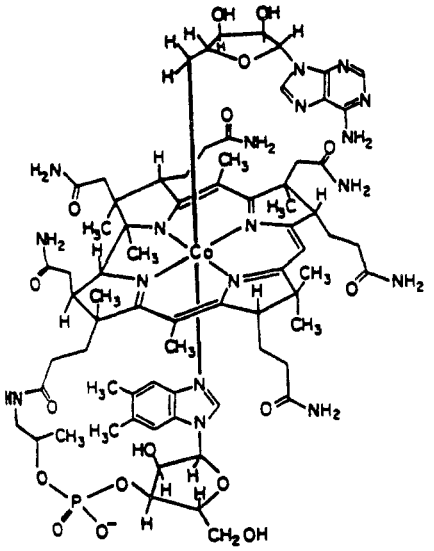
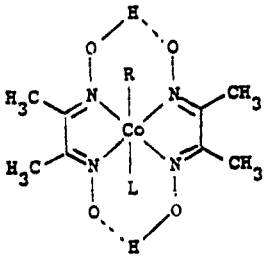


(a) Coenzyme B_{12}



(b) $LCo(DH)_2R$

Figure 1. Structures of (a) coenzyme B_{12} (adenosylcobalamin) and (b) model compound $LCo(DH)_2R$.

Co–C BDE. While the measurement of BDE's is essential for gaining a deeper understanding of B_{12}-dependent processes, kinetic and equilibrium methods do not differentiate the effects of systematic changes on the energetics of the ground state from those of the transition and product states. The transition state and product state energies are similar since, in general, reformation of the Co–C bond from Co(II) and R• is a diffusion-controlled process.[1,9] However, for B_{12}-dependent processes, only the cleavage rates are accessible and the BDE's are not so readily estimated. Progress toward the eventual elucidation of mechanisms of the B_{12}-dependent catalysis has been severely impeded by an incomplete knowledge of the factors contributing to Co–C bond homolysis, by the current unavailability of structural data for the B_{12}-dependent proteins, and by the lack of a physical technique capable of providing detailed information about the Co–C moiety in both the solid state and solution.

We have recently reported the first application of near-IR excited Fourier transform Raman spectroscopy to the study of photolabile organocobalt B_{12} model compounds[10] as well as biologically active cobalamins.[11] In comparison with the conventional Raman method,[12] the attractiveness of the FT-Raman technique arises from two main features:[13] first, the use of near-IR laser excitation (1.064 μm) precludes electronic absorption and thus completely eliminates the Co–C bond photolysis and fluorescence interference problems encountered previously in resonance Raman studies of related organocobalt compounds;[14] second, the use of a Michelson interferometer for detection affords superior spectral resolution, frequency accuracy, and relatively high energy throughput.[15] It was demonstrated that this newly developed technique is capable of providing rich vibrational information on the Co–C moiety, the axial ligands, and the equatorial ligand system in both the solid state and solution.[10,11]

Unlike previous kinetic and equilibrium methods,[9] the Co–C bond probed by Raman spectroscopy reflects solely ground-state properties. This technique also appears to have a great potential for investigating the properties of the Co–C bond in holoenzyme–B_{12} coenzyme complexes. Such an undertaking would be highly significant since the B_{12}-dependent enzymes are generally too large and too complex for NMR 3-D spectroscopic structural techniques to be readily applied. The crystallization and structural characterization of B_{12}-dependent enzymes have never been reported. Furthermore, such solution and solid-state structural methods are unlikely to provide highly accurate data on the Co–C bond but, instead, will provide information on the interaction of the coenzyme and the proteins.

In this paper, we focus our attention on a systematic investigation of factors that influence the Co–C stretching frequency by studying a large number of cobaloximes. These B_{12} models are known to have shorter Co–L bonds as compared to cobalamins,[3] but they do have relevance to cobalamins in the following aspects: (1) the conformational distortion of the $Co(DH)_2$ unit leads to increased Co–C bond lengths, and the basis of such an effect has been convincingly shown to be steric rather than electronic;[3,5,6,8] (2) cobaloximes appear to respond structurally to changes in the electronic and steric properties of axial ligands[3,5,6,8,16] in an analogous manner to cobalamins; and (3) the $\nu_{(Co-C)}$ stretch in these model compounds has a very similar value to that in the coenzyme, methyl B_{12}.[11]

In particular, we have investigated the influences of the environment and the electronic and steric properties of the trans ligand L on the Co–C vibrational frequency. Comparison between solid-state and solution vibrational data clearly indicates the sensitive nature of the Co–C bond to environment. For example, our results suggest that conformational distortions of the equatorial $Co(DH)_2$ are modulated by the crystal packing forces and that this conformation is generally more distorted from planarity in solution than in the solid state. In chloroform solution, the Co–C stretching frequency is influenced by the steric bulk of L but, for closely related L, not by its electronic properties.

Experimental Section

Materials. The preparation and characterization of the B_{12} model complexes, $LCo(DH)_2R$ (R = alkyl ligand, L = N- or P-donor ligand), have already been reported.[3b,8,16] These materials are stable indefinitely

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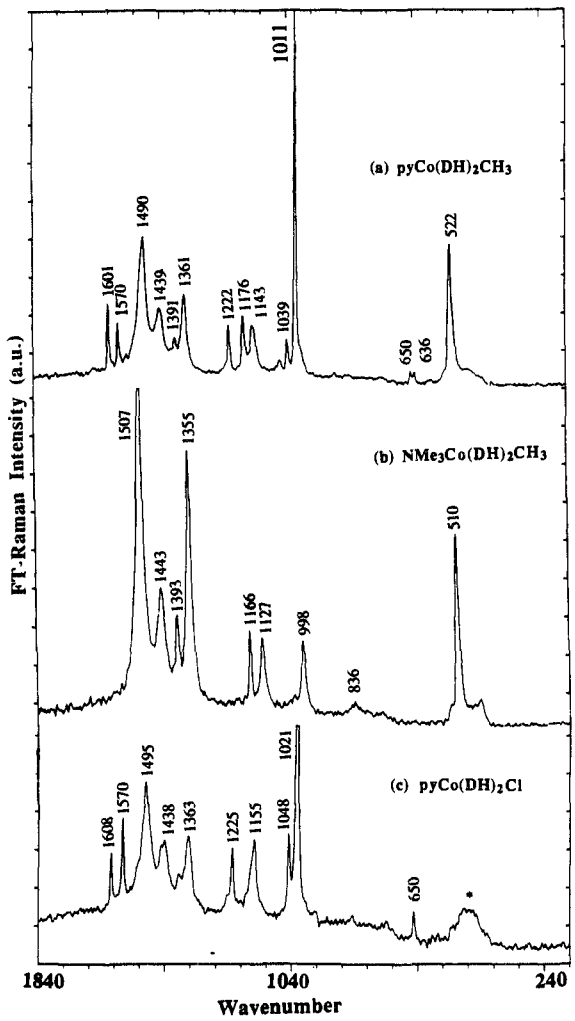

Figure 2. Solid-state FT-Raman spectra of (a) $pyCo(DH)_2CH_3$, (b) $NMe_3Co(DH)_2CH_3$, and (c) $pyCo(DH)_2Cl$. Data acquisition conditions: excitation wavelength = 1.064 μm; laser power = 1.0 W; spectral resolution = 4.0 cm^{-1}; data accumulation time = 3.5 min (100 coadded scans). The asterisk identifies instrumental artifact.

when stored in the dark in a freezer. Sample purity was confirmed by ^1H NMR spectroscopy.

FT-Raman Spectroscopy. FT-Raman spectra were obtained by using a Bomem DA3.02 spectrophotometer equipped with a highly sensitive, liquid nitrogen cooled InGaAs detector. The strong Rayleigh component was effectively removed by employing three dielectric optical filters positioned at 5° with respect to the collected light beam normal. Near-IR excitation at 1.064 μm was provided by a Quantronix CW Nd:YAG laser. A DEC minicomputer with high speed vector processor and mass storage capability was used to process acquired data. FT-Raman spectra for solid samples were recorded via 180° scattering with use of two sapphire plates to hold approximately 1 mg of the material, while liquid samples (normally 0.1 mL of saturated cobaloxime chloroform/methylene chloride solutions) were put in a rectangular quartz tube for data acquisition.

Results

Solid-State Data. In Figure 2 are shown high-quality FT-Raman spectra obtained for $pyCo(DH)_2CH_3$, $pyCo(DH)_2Cl$, and $NMe_3Co(DH)_2CH_3$ in the solid state. In a previous report,[10] we have conclusively assigned the intense 522-cm^{-1} line in the FT-Raman spectrum of solid $pyCo(DH)_2CH_3$ to the Co–C stretching mode on the basis of its sensitive nature to axial methyl deuteration (492 cm^{-1} for $pyCo(DH)_2CD_3$) and its disappearance in the FT-Raman spectra of cobaloximes lacking the Co–C bond, such as $pyCo(DH)_2Cl$. In general, the Raman-active Co–CH_3 vibration gives rise to a characteristically intense and sharp line around 500 cm^{-1}, and its relative intensity is modulated by the nature of the trans ligand.[17] Other vibrational modes associated with the Co–CH_3 moiety are detected at 2897 cm^{-1} (CH_3 stretching) and 1176 cm^{-1} (CH_3 bending). Comparison of the FT-Raman spectra in Figure 2 also shows that the technique of near-IR FT-Raman is capable of providing rich vibrational information regarding the axial base ligand and the equatorial ligand system. Clearly, the FT-Raman spectrum of $NMe_3Co(DH)_2CH_3$ lacks the prominent pyridine internal vibrational lines that are present in the spectra of $pyCo(DH)_2CH_3$ (located at 1601, 1570, 1222, 1039, 1011, 650 cm^{-1}) and $pyCo(DH)_2Cl$ (located at 1608, 1570, 1225, 1048, 1021, 650 cm^{-1}). It is interesting that replacement of the axial methyl with the poorly electron-donating Cl^- not only leads to frequency changes of the axial pyridine vibrational modes (trans influence) but also affects the DH vibrational frequencies (located at 1490, 1439, 1391, 1361, 1143 cm^{-1} for $pyCo(DH)_2CH_3$ and at 1495, 1438, 1393, 1363, 1155 cm^{-1} for $pyCo(DH)_2Cl$). FT-Raman detection of axial base vibrational modes is further demonstrated

Table I. Co–C, DH, and Axial Ligand Vibrational Frequencies of B_{12} Models $LCo(DH)_2R$ (L = N- or P-Donor) in the Solid State[a]

L/R	$\nu_{(Co-C)}$ (cm^{-1})	DH vibrations (cm^{-1})	L vibrations (cm^{-1})
py/CH_3	522	1490, 1439, 1391 1361, 1143	1601, 1570, 1222 1011, 650, 636
py/CD_3	492	1490, 1439, 1391 1361, 1143	1601, 1570, 1222 1011, 650, 636
py/Cl		1495, 1438, 1363 1155	1608, 1570, 1225 1048, 1021, 650
4-*t*-Bu-py/CH_3	506	1491, 1444, 1389 1358, 1146	1617, 1574, 1235, 1204 1131, 1065, 1025, 730
4-CNpy/CH_3	512	1493, 1440, 1386 1360, 1140	2248, 1609, 1197, 1071 1015
4-NMe_2py/CH_3	514	1485, 1440, 1386 1357, 1140	1619, 1241, 1069 1011, 950, 762
3,5-Lut/CH_3	512	1492, 1438, 1387 1364, 1144	1596, 1509, 1459 1158, 1033, 759
1-MeImd/CH_3	520	1499, 1436, 1391 1353, 1133	1374, 1289, 1239, 1106 1083, 1031, 996, 946, 853 672
Me_3BZM/CH_3	508	1493, 1438, 1387 1362, 1143	1586, 1517, 1455, 1420 1387, 1362, 1345, 1276 737, 485
NMe_3/CH_3	510	1507, 1443, 1393 1355, 998	1127
PMe_3/CH_3	498	1484, 1447, 1391 1362, 996	1413, 1127, 950, 743 674
PBu_3/CH_3	491	1478, 1447, 1391 1360	1414, 1128, 1094, 1052 996, 892
$P(OCH_3)_3$/CH_3	510	1507, 1447, 1393 1355	1129, 998, 749, 645
PPh_3/CH_3	491	1482, 1438, 1361 1134	1586, 1575, 1180, 1160 1095, 1029, 1001, 683
PPh_3/Cl		1488, 1441, 1359	1586, 1197, 1166, 1154 1090, 1029, 1006, 996
$PcHex_3$/CH_3	505	1472, 1443, 1360 1131	1291, 1270, 1198, 1079 1056, 1046, 1029, 1000 849, 817, 728, 707, 583
PPh_2cHex/CH_3	493	1482, 1438, 1360 1141	mixure of Ph and cHex vibrational modes
$PPhcHex_2$/CH_3	491	1482, 1447, 1393 1360	mixture of Ph and cHex vibrational modes
$P(CH_2CH_2CN)_3$/CH_3	498	1484, 1438, 1362	2247, 1420, 1308, 1297, 1251, 1227, 1131, 1006

[a] Abbreviations: py = pyridine; 4-*t*-Bu-py = 4-*tert*-butylpyridine; 4-CNpy = 4-cyanopyridine; 4-NMe_2py = 4-(dimethylamino)pyridine; 3,5-Lut = 3,5-lutidine; 1-MeImd = 1-methylimidazolew; Me_3BZm = 1,5,6-trimethylbenzimidazole; NMe_3 = trimethylamine; PMe_3 = trimethylphosphine; PBu_3 = tri(*n*-butyl)phosphine; $P(OCH_3)_3$ = trimethyl phosphite; PPh_3 = triphenylphosphine; cHX = cyclohexyl; $PcHex_3$ = tricyclohexylphosphine; PPh_2cHex = cyclohexyldiphenylphosphine; $PPhcHex_2$ = dicyclohexylphenylphosphine; $P(CH_2CH_2CN)_3$ = tris(2-cyanoethyl)phosphine.

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(17) The broad feature at ca. 475 cm^{-1} in the FT-Raman spectrum of $pyCo(DH)_2Cl$ was an instrumental artifact since the same broad band was observed for solid KBr, which, by itself, does not exhibit any vibrational bands.

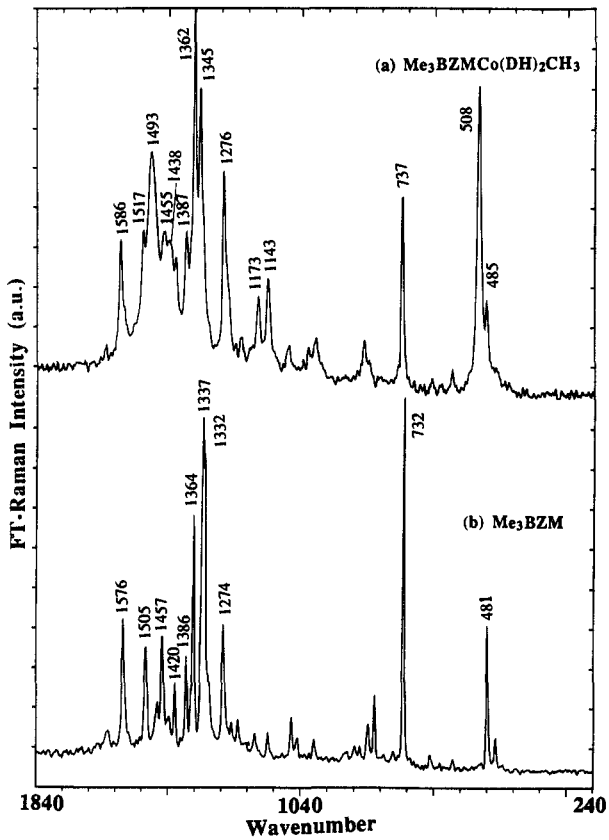

Figure 3. Comparison of solid-state FT-Raman spectra of (a) $Me_3BZMCo(DH)_2CH_3$ and (b) Me_3BZM (where Me_3BZM = 1,5,6-trimethylbenzimidazole). Data acquisition conditions were the same as in Figure 2.

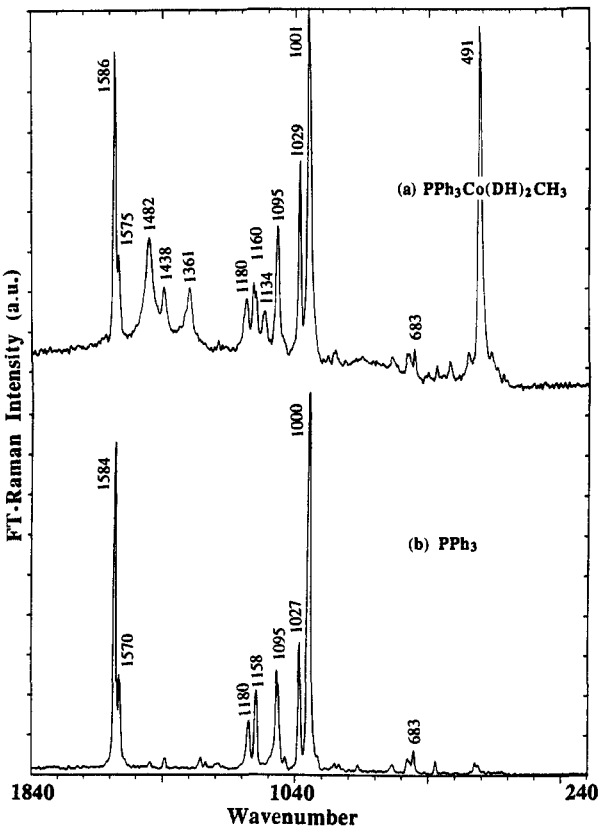

Figure 4. Comparison of solid-state FT-Raman spectra of (a) $PPh_3Co(DH)_2CH_3$ and (b) the axial ligand PPh_3. Data acquisition conditions were the same as in Figure 2.

in Figure 3, which compares FT-Raman spectra of pure 1,5,6-trimethylbenzimidazole (Me_3BZM) and $Me_3BZMCo(DH)_2CH_3$. This neutral ligand is an appropriate model for the benzimidazole present in the naturally occurring B_{12} coenzyme.[8e] It is clear that Raman lines at 1455, 1362, 1276, 737, and 485 cm^{-1} in the cobaloxime spectrum originate from Me_3BZM internal vibrations. Table I summarizes the solid-state FT-Raman data obtained for B_{12} model compounds of the type $LCo(DH)_2R$ (L = N- or P-donor).

For cobaloximes containing P-donor axial bases, the Raman-active Co–C stretching mode is also detected as a very intense and sharp line around 500 cm^{-1}. In Figure 4 are shown solid-state FT-Raman spectra of $PPh_3Co(DH)_2CH_3$ and the P-donor ligand, PPh_3. As in the case of N-donor compounds, the FT-Raman spectra of P-donor cobaloximes also provide detailed information about the Co–C moiety, the equatorial ligands, as well as the axial base ligand. Intense Raman lines at 1586, 1575, 1095, 1029, and 1001 cm^{-1} arise from triphenylphosphine vibrations as evidenced by the appearance of almost identical Raman lines in the spectrum of pure PPh_3 crystals. Interestingly, upon coordination of the phosphorus atom to cobalt, the phenyl vibrational modes exhibit a small but finite frequency upshift of 1–5 cm^{-1}. While this wavenumber change is considerably smaller than the frequency increase (up to 20 cm^{-1}) observed between coordinated and free pyridine (992 cm^{-1} for neat pyridine), this observation points to the influence of the P–Co bond on the vibrational properties of phenyl groups.

Solution Data. As noted earlier, one significant advantage of nonresonant FT-Raman spectroscopy is its ability to provide equally rich structural information in both the solid state and solution. Hence, solution and solid-state properties of various B_{12} models can be investigated by using the same technique, avoiding uncertainties involved in comparing X-ray crystal data and solution kinetic/NMR results.[3] In Figure 5 are shown the Co–C stretching frequencies of a series of 4-substituted pyridine $Co(DH)_2CH_3$ compounds in chloroform solution. The rationale for selecting

Table II. Comparison of Co–C Stretching Frequencies in Organocobalt B_{12} Complexes of the Type $LCo(DH)_2R$ in the Solid State and Solution

L/R	solid (cm^{-1})	$CHCl_3$ (cm^{-1})	solid–$CHCl_3$ (cm^{-1})	D_e(Co–C) ratio[a] $(L/CH_3)/(py/CH_3)$
py/CH_3	522	504[b]	18	1.00
py/CD_3	492	477	15	
4-*t*-Bu-py/CH_3	506	504	2	
4-$CNpy/CH_3$	512	504	8	
4-NMe_2py/CH_3	514	504	10	
2-$MeNHpy/CH_3$	516	489	27	0.94
1-$MeImd/CH_3$	520	508	12	1.02
Me_3BZM/CH_3	508	506	2	1.01
$PhNH_2/CH_3$	510	502	8	0.99
PMe_3/CH_3	498	495[c]	3	0.96
PBu_3/CH_3	491	489[c]	2	0.94
PPh_3/CH_3	491	487[c]	4	0.93
$PcHex_3/CH_3$	505	481[c]	24	0.91
PPh_2cHex/CH_3	493	485	8	0.92
$PPhcHex_2/CH_3$	491	485	6	0.92
$P(CH_2CH_2CN)_3/CH_3$	498	485	13	0.92

[a] By definition, D_e includes the zero-point energy ($^1/_2\ h\nu$)) while the bond dissociation energy (BDE) does not. In practice, D_e is usually taken as BDE. [b] 507 cm^{-1} in CH_2Cl_2. [c] Same value in CH_2Cl_2.

4-X-pyridines as the trans ligand is that changes of the substituent at the 4-position should induce minimal steric changes in the vicinity of the Co since the 4-substituent is directed away from the Co center. The basicity of the 4-substituted pyridine, on the other hand, varies significantly as judged by the pK_a values of 1.86 for 4-cyanopyridine and 9.12 for 4-aminopyridine.[18] Therefore, the trans electronic effect on the Co–C bond in the ground state can be assessed by examining the Co–C stretching frequency. In order to investigate the trans effect, a series of cobaloximes with trialkylphosphine axial bases was selected. The FT-Raman spectra obtained in chloroform solution are illustrated in Figure 6. Since these PR_3 ligands (R = alkyl group) are known to have similar electron-donating abilities,[19] the observed changes of the Co–C

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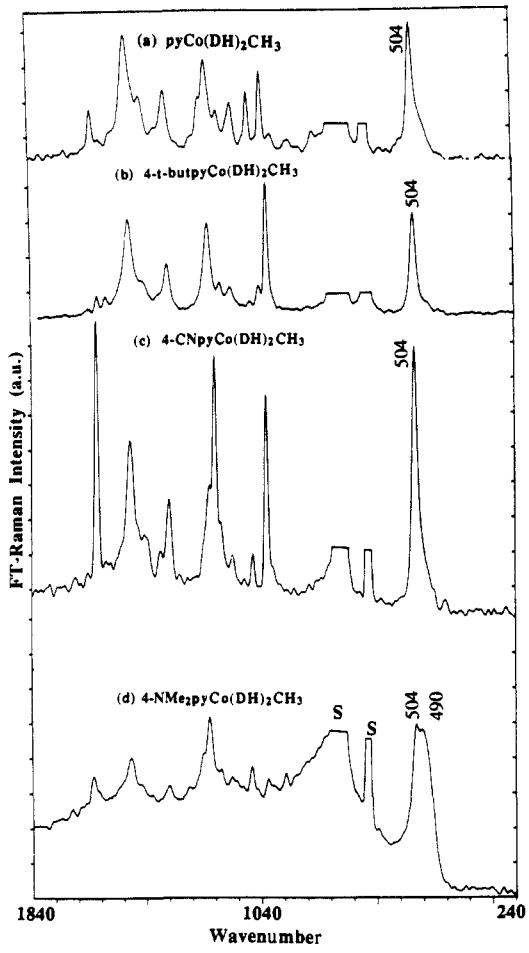

Figure 5. Solution FT-Raman spectra of (4-X-py)$Co(DH)_2CH_3$ in chloroform (X = H, *tert*-butyl, CN, and NMe_2). Data acquisition conditions: excitation wavelength = 1.064 μm; laser power = 1.5 W; spectral resolution = 4.0 cm^{-1}; data accumulation time = 7.0 min (200 coadded scans). S: solvent Raman lines. Note the appearance of a doublet at ca. 500 cm^{-1} in the spectrum of $NMe_2pyCo(DH)_2CH_3$. The assignment of the 490-cm^{-1} Raman line remains unclear at the present time.

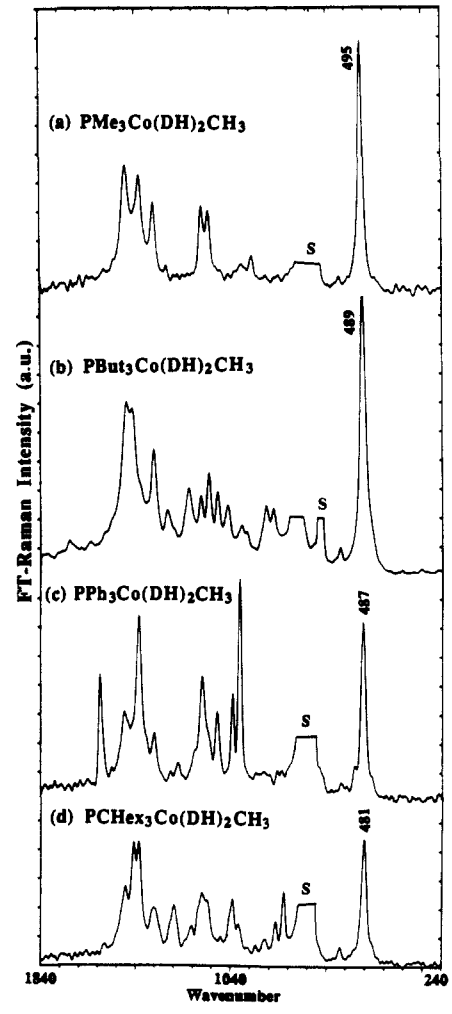

Figure 6. Solution FT-Raman spectra of $PR_3Co(DH)_2CH_3$ in chloroform (R = methyl, *n*-butyl, phenyl, and cyclohexyl). Data acquisition parameters as in Figure 5. S: solvent Raman lines.

stretching frequency are primarily attributable to the trans steric effect. The Co–C stretching frequencies recorded for various organocobalt B_{12} models in the solid state and in solution are compared in Table II.

Discussion

Methodology. Although the feasibility of Fourier transform Raman spectroscopy was demonstrated 25 years ago,[20] only recently have the potential benefits of performing Raman spectroscopy in the near-infrared region with an FT spectrophotometer been established.[13] It is thus important to discuss the advantages and limitations of this new technique in the context of physical methods that have been employed in B_{12} biochemistry.

X-ray crystallography has been particularly useful in investigating the relationship between Co–C bond stability/lability and structural parameters such as bond length and bond angle.[1a,3] Extensive X-ray data exist for a large number of B_{12} model compounds.[3] Crystal structures are also available for two biologically active cobalamins: adenosylcobalamin, solved by Lenhert and Hodgkin;[21] and methylcobalamin, solved by Rossi, Glusker, Marzilli, and co-workers.[22] However, the X-ray technique is restricted to the study of solid samples and the structural results become less accurate as the size and complexity of the molecule increase. Furthermore, relatively few cobalamins have been crystallized to X-ray standards.

NMR spectroscopy and kinetic methods are well-suited for studying the solution properties of cobalamins[22,23] and model compounds.[3] Correlations have been found interrelating the ^{13}C and ^1H chemical shifts, Co–L dissociation rates, and Co–L bond distances.[3,24] The disadvantages of NMR spectroscopy are that solid-state NMR spectra for complex molecules (viz., B_{12} models) are difficult to interpret and that NMR chemical shifts do not reflect directly the Co–C bond energy.[25] Kinetic methods, on the other hand, suffer from the drawback that dissociation of the axial alkyl ligand in cobaloximes is usually too slow to be measured.[3]

Vibrational spectroscopic techniques (IR and Raman) have also been applied to study cobalamins and models,[26] but the Co–C

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stretching vibration appears to be IR-active. The reported far-IR absorption band at ~324 cm^{-1} is related to the Co–C moiety,[26a] but it definitely does not arise from the Co–C stretching mode.[27] Raman spectroscopy provides vibrational information complementary to the IR absorption technique, and thus the Co–C stretching vibration is expected to be Raman-active. However, the utility of laser excited Raman spectroscopy in elucidating the nature of the Co–C bond in B_{12} coenzyme and model compounds has been severely hindered by the photolability of the organometallic Co–C bond.[28] While the rapid-flow technique was demonstrated to be effective in mitigating the photolysis problem,[14c] the resulting resonance Raman spectra of organocobalamins yielded no information about the Co–C bond, nor any internal axial ligand vibrations.

Through the elimination of Co–C bond photolysis and fluorescence interference previously encountered with visible/near-UV excitations,[14] the technique of near-IR excited FT-Raman spectroscopy appears to be particularly well-suited for studying organocobalamins and model complexes. As demonstrated by the results presented above, detailed information about the Co–C bond, the axial ligands, as well as the equatorial ligand is obtainable in both the solid state and solution. The disadvantage of this technique lies in the intrinsically low efficiency associated with the nonresonant Raman scattering process.[12] Due to its fourth power dependence on excitation energy, the Raman scattering efficiency at 1.064 μm is ca. 18 times lower than that at 514.5-nm excitation. Consequently, high excitation powers (0.5–2.0 W) and concentrated samples are required for obtaining high-quality FT-Raman spectra.

Structural Differences between the Solid State and Solution. Although X-ray structural data have frequently been compared with solution NMR and kinetic results,[3] we have found significant differences between the solid-state and solution FT-Raman spectra of B_{12} model compounds. Particularly striking is the frequency decrease of the Co–C stretching mode upon dissolving in chloroform/methylene chloride solution. It is clear from the results in Table II that weakening of the Co–C bond in solution is a general phenomenon for all the cobaloximes investigated in this study. $PcHex_3Co(DH)_2CH_3$ exhibits a large $\nu_{(Co-C)}$ decrease of 24 cm^{-1} in chloroform, while a decrease of only 2 cm^{-1} is detected for 4-*t*-Bu-py$Co(DH)_2CH_3$, $Me_3BZMCo(DH)_2CH_3$, and $PBu_3Co(DH)_2CH_3$ (see Table II). It is conceivable that intermolecular interactions of solvent molecules with the Co–CH_3 moiety may lead to vibrational frequency changes of the Co–C stretch. Such an effect would be expected to produce comparable frequency shifts for the structurally similar compounds py$Co(DH)_2CH_3$ and 4-*t*-Bu-py$Co(DH)2CH_3$. However, the observed 18-cm^{-1} decrease for the former contrasts sharply with the 2-cm^{-1} decrease for the latter.

Convincing evidence has been presented by Marzilli, Randaccio, and co-workers[6,8] that the DH cobaloximes often exhibit an upwardly bent conformation (butterfly conformation). The extent of such a conformational distortion from planarity is measured by the dihedral angle (α) between the DH planes of the $Co(DH)_2$ unit and the displacement (*d*) of Co from the 4-N equatorial plane. Since the Co–C bond length has been demonstrated to be most responsive to conformational distortions of the $Co(DH)_2$ unit[8] and both the bulkiness of the axial ligands and crystal packing forces influence the α and *d* values,[24b] the stretching frequency downshift of the Co–C bond in solution is probably indicative of conformational differences of the $Co(DH)_2$ unit between solid state and solution. As judged by the trend that the Co–C stretching mode always exhibits a lower frequency in solution, we suggest that, in every case, the equatorial ligand system is distorted further from planarity (i.e., increased α) in solution. This explanation is supported by several lines of spectroscopic and X-ray crystallographic evidence. (1) The crystal structures of $PPh_3Co(DH)_2$-*i*-C_3H_7 and $PPh_3Co(DH)_2$-2-C_4H_9 are considerably different, as revealed by the Co–C bond lengths and α bending angles (2.22 Å and 14° for the former, 2.085 Å and 4° for the latter), but their solution properties are almost identical,[8b,24b] revealing the presence of a crystal lattice effect on conformational distortions. (2) A conformational change is also expected to influence the Co–L bond. Indeed, the axial pyridine ν_1 mode in py$Co(DH)_2CH_3$ changes from 1011 cm^{-1} in the solid state to 1014 cm^{-1} in chloroform solution. This vibrational mode is known to be sensitive to the M–N (pyridine) coordination bond.[29] (3) As compared to the corrin ring in cobalamins and the $Co(DH)_2$ unit in B_{12} models, the porphyrin macrocycle is considerably more rigid and permits much less conformational distortion from planarity.[30] Indeed, a resonance Raman study of pyFe(TPP)CO (TPP = tetraphenylporphyrin) reveals no differences in the stretching frequency of the Fe–C bond between solid state and solution.[31] It is also interesting to note that, while the solid-state Co–CH_3 stretch in py$Co(DH)_2CH_3$ is significantly higher (by 16 cm^{-1}) than that in 4-*t*-Bu-py$Co(DH)_2CH_3$, the DH vibrational modes in these two molecules differ only by 1–5 cm^{-1}. The equatorial DH modes thus appear to be less sensitive to conformational distortions when compared to the Co–C stretching mode.

A survey of the solution and solid-state data in Table II indicates that (a) large frequency downshifts are generally associated with cobaloximes bearing a smaller axial ligand such as pyridine, 4-cyanopyridine, and 1-imidazole and (b) compounds bearing a highly bulky axial ligand such as triphenylphosphine usually exhibit much smaller frequency changes. It is well established that the bulkiness of axial ligands strongly affects the equatorial $Co(DH)_2$ conformation. In fact, X-ray studies[3] have shown that the $Co(DH)_2$ unit is significantly distorted from planarity in the crystals of $LCo(DH)_2CH_3$ (L = bulky ligand). Thus the apparent correlation of $\nu_{(Co-C)}$ stretching frequency changes with the axial base size suggests that the equatorial $Co(DH)_2$ unit can easily change from a near-planar conformation to a highly distorted one in the absence of crystal packing forces (e.g., in solution), but further conformational distortions may be difficult for an already bent $Co(DH)_2$ system.

A quantitative relationship between the $Co(DH)_2$ bending angle and the Co–C stretching frequency cannot be established at the present time, since it requires preparation of a specific B_{12} model in different crystal forms, each having a different $Co(DH)_2$ bending angle. Nevertheless, it is interesting to compare the X-ray structures of two closely related compounds, $LCo(DH)_2CH_3$ (L = PPh_3 and $PcHex_3$), with their $\nu_{(Co-C)}$ values in the solid state and in solution. The α and *d* values are 14° and 0.11 Å for $PPh_3Co(DH)_2CH_3$[8b] and 12° and 0.12 Å for $P(cHex)_3Co(DH)_2CH_3$.[8f] but the former shows a $\nu_{(Co-C)}$ decrease of only 3 cm^{-1} in solution while the latter exhibits a large $\nu_{(Co-C)}$ downshift of 24 cm^{-1}. This anomalous result seems to indicate that $Co(DH)_2$ conformational distortions in solution are considerably more pronounced for the tricyclohexylphosphine compound than for the triphenylphosphine one.

There is strong evidence that $PcHex_3$ is bulkier than PPh_3. Analysis of $PR_3Co(DH)_2(CH_3OH)^+$ proton NMR spectral shifts in CH_3OH solution reveals that the major determinant of the shifts, particularly the methyl signal of coordinated CH_3OH, is the size of PR_3.[32] This correlation provides clear support for the finding that $PcHex_3$ is larger. Likewise, in the series $PR_3Co(DH)_2CH(CH_3)C_6H_5$,[9] the Co–C bond cleavage rates appear to parallel the Tolman cone angle. Both the NMR shifts and Co–C

(27) The far-IR absorption band observed at ca. 324 cm^{-1} in alkylcobaloximes should not be assigned to the Co–C stretching mode since (a) this value is too low compared to the Co–C stretching frequencies (~500 cm^{-1}) found in other organocobalt complexes and (b) its isotopic shift (6 cm^{-1}, CH_3 vs CD_3) is significantly smaller than that expected for a stretching mode (ca. 36 cm^{-1}). A recent report by Ervin et al. (see ref 14d) also indicates that this far-IR band does not originate from the Co–C stretching mode.

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dissociation rates were attributed to distortions of the $Co(DH)_2$ equatorial moiety which would affect Co anisotropy and nonbonded repulsions, respectively. Thus, it seems likely that the X-ray structure of the PPh_3 complex closely resembles that in solution but that the X-ray crystal structure of the $PcHex_3$ complex is not an adequate reflection of the solution structure.

Trans Electronic Influence. Employing kinetic and equilibrium methods, Halpern and co-workers[9] have demonstrated that Co–C BDE's can be substantially affected by electronic properties of the trans ligand L in complexes of the type 4-X-pyCo$(DH)_2$CH-$(CH_3)C_6H_5$, where 4-X-py = pyridine or 4-substituted pyridines. Replacement of 4-cyanopyridine with the more basic 4-aminopyridine was found to produce a BDE increase of ca. 3.5 kcal/mol, and a linear correlation was found between the Co–C BDE and pK_a value of L.[1b] In contrast, solution FT-Raman results shown in Figure 5 unambiguously reveal the absence of trans electronic effect on the Co–C stretching frequency. This finding is in agreement with an approximate ab initio study of geometrical deformations, which also shows the absence of any major electronic effect.[7b] Furthermore, X-ray structural studies of Marzilli, Randaccio, and co-workers[8] have provided clear evidence that the effect of the trans ligand on the Co–C bond length is steric and not electronic in nature. These seemingly incongruous results can be reconciled by the fact that while BDE's depend on the energetics of both the ground and the product states, FT-Raman and X-ray methods probe solely ground-state properties of a molecule. Thus, the trans electronic effect on Co–C BDE, as measured by Halpern et al.,[9] must be associated with the product and/or ground-state properties of the reactants not directly related to Co–C bond strength. Indeed, Halpern[9] suggested that the more basic Xpy ligands should stabilize the Co(III) oxidation state over the Co(II) oxidation state better than the less basic Xpy ligands. Our findings are in agreement with this suggestion.

Trans Steric Influence. The importance of steric influence on Co–C bond lengths has been demonstrated convincingly by the results of X-ray structural determinations on a series of LCo-$(DH)_2R$ compounds.[3,8] Significant lengthening of the Co–C bond is found with increasing steric bulk of R and L.[3,8] In line with the X-ray data, Halpern et al.[9] have reported the trans steric effect on Co–C bond dissociation energy in solution. However, when $R = CH_3$, there is no strong evidence for a steric effect, although Co–C bond lengths are slightly longer for P-donor L compared to N-donor L.[3a] The FT-Raman results obtained for a series of $PR_3Co(DH)_2CH_3$ compounds in chloroform provide evidence that the Co–C bond strength in the ground state is influenced by the steric bulk of the trans ligand L, even for $R = CH_3$.

Quantitative determination of the Co–C bond energy from its stretching frequency is possible but requires an explicit knowledge of its potential function. If the Co–C bond in these cobaloximes exhibits, a priori, the most commonly used Morse potential curve[33]

$$U_{(r)} = D_e\{1 - \exp[-a(r - r_e)]\}^2$$

(where $U_{(r)}$ = potential energy, D_e = bond energy, usually taken as the bond dissociation energy, r = bond length, r_e = bond length at equilibrium, a = constant), then the Co–C bond's harmonic force constant (κ) is equal to the second derivative of $U_{(r)}$ at $r = r_e$, i.e., $\kappa = 2D_e a^2$. By using the conventional formula relating force constant (κ) and vibrational frequency (ν), the following relationship is readily derived:

$$\nu/\nu' = (\kappa/\kappa')^{1/2} = (D_e/D'_e)^{1/2}$$

Thus, the Co–C bond energy ratios for these B_{12} models can be calculated by using their solution Co–C stretching frequencies (see Table II).

The Co–C BDE for pyCo$(DH)_2CH_3$ has recently been measured (33.1 kcal/mol) via solution thermochemical methods.[34] On the basis of this value, the BDE difference between PBu_3Co-$(DH)_2CH_3$ and $PPh_3Co(DH)_2CH_3$ is calculated to be approximately 0.3 kcal/mol, which is substantially lower than the measured BDE difference of ca. 4.0 kcal/mol between closely related cobaloximes, L$Co(DH)_2CH(CH_3)C_6H_5$ (L = PPh_3 and PBu_3).[1b] Despite the crude nature of this calculation, an important point is that for this series of compounds the difference in Co–C BDE does not appear to reflect differences localized in this bond, but rather, distributed over many chemical bonds in the pertinent molecules. Analogous to our conclusion on the trans electronic effect, the steric bulk of the trans ligand may exert a strong effect on the energetics of the products. Thus, our FT-Raman study of both trans electronic and steric effects suggests that an investigation of cobalt(II) products is highly merited.

Comparison with Resonance Raman Results of Ligand Binding in Porphyrins. The principal function of coenzyme B_{12} is that of an organic free radical carrier in much the same way as myoglobin/hemoglobin reversibly bind and release molecular oxygen.[1,9] Fulfillment of these parallel functions by the alternative choices of cobalt/corrin and iron/porphyrin is both intriguing and important.[30]

In view of the valuable insights into the control mechanism of heme reactivity that have come from resonance Raman investigations of the Fe–porphyrin system,[35] we now compare the FT-Raman results of B_{12} models with resonance Raman data of porphyrins. (1) Significant differences in Co–C stretching frequency are noted for B_{12} models between the solid state and solution, whereas the $\nu_{(Fe-C)}$ frequency in pyFe(TPP)CO is essentially identical with that in the solid state and in benzene solution.[31] This is attributable to the fact that the $Co(DH)_2$ unit in B_{12} models is considerably more flexible than that in the porphyrin system, and thus more conformational distortions are permitted for the cobaloximes in solution. A recent study of porphyrin and corrin macrocycles by Halpern and Geno[30] also indicates the importance of the corrin's flexibility to its function in vitamin B_{12}. (2) In the porphyrin system, an unusually strong Fe–C bond is found when the trans Fe–ligand bond is weak or absent.[36] In contrast, the presence of a weak Co–L bond is known to labilize the Co–C bond.[6d,e] (3) FT-Raman studies of trialkylphosphine complexes show the decrease of $\nu_{(Co-C)}$ with increasing steric bulk of the trans ligand, whereas in the porphyrin system a tension on the trans Fe–imidazole bond increases the Fe–C bond strength.[36] This is consistent with the widely held view that Co–C bond length increases are due to sterically induced distortions of the $Co(DH)_2$ unit rather than the electronic properties of the trans L ligand.[8] (4) Distortions of the Fe–C–O linkage from linear geometry in sterically hindered heme complexes have been shown to increase the Fe–C stretching frequency but decrease the CO binding affinity.[37] Similarly, distortion of the Co–C–C bond angle away from the normal tetrahedral value has been implicated as an important controller of Co–C bond stability.[6a] However, it remains unclear whether the Co–C stretching frequency (ground state properties) exhibits any dependence on the angular distortion of the Co–C–C moiety in B_{12} models. (5) In parallel with resonance Raman studies of ligand binding affinity in the Fe–porphyrin system,[36] the Fe–C/Co–C bond dissociation rates are influenced by both the metal–ligand bond strength and structural changes in other parts of the molecule. In this regard, the knowledge of protein control of heme reactivity may prove to be instructive in elucidating the enzymatic cleavage mechanism of the Co–C bond.

New Insight into the Co–C Bond Homolytic Cleavage. The most widely accepted proposal that could account for the enzymatic enhancement of the Co–C cleavage rate (by $\sim 10^{12}$-fold) involves ground state distortions of the corrin macrocycle.[1-4,30] In this proposal, the protein interactions with the amide side chains induce a butterfly conformational distortion of the corrin ring in an "upward" direction toward the 5′-deoxyadenosyl group. The

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consequent repulsive interactions facilitate the "lift-off" of the group. There is no direct evidence for a corrin ring distortion beyond the normal puckered conformation during an enzymic process or in an isolated cobalamin,[5,22] although such distortions have been well documented in model compounds.[3]

Data presented in this paper are limited to the nonbulky CH_3 ligand in model compounds. These results demonstrate that the electronic effect of the L ligand on Co–C BDE is not directly manifested in the ground state Co–C bond strength. Although the data provide evidence for a steric influence of L on Co–CH_3 bond strength, the effect is small, albeit based on a crude calculation. Taken together, our results do suggest that the differences in BDE's (and, hence, cleavage rates) in model compounds may be more reflective of differences in the product state (and, hence, the transition state) than differences in the ground state. Models such as those studied in this paper have played an essential role in the development of concepts used to understand B_{12} biochemistry. Therefore, it is gratifying that the $\nu_{(Co-C)}$ in methyl B_{12}[11] of 500 cm^{-1} is similar to the values found here. Recently, the Co–CH_3 BDE has been reported as 37 kcal/mol.[42] This value is slightly greater than that in $pyCo(DH)_2CH_3$.[34] In view of the similar $\nu_{(Co-C)}$ values, the result is consistent with the importance of transition state effects on rates.

It is interesting to assess evidence for corrin distortion in enzymic processes to determine if only a ground state effect can reasonably account for the acceleration of the Co–C bond cleavage rate. Prior to such an assessment, we consider the bulk of the 5′-deoxyadenosyl ligand. Is it more like CH_3 or $C(CH_3)H(C_6H_5)$? A recent comparison of the corrin ring conformation in coenzyme B_{12} with that in methyl B_{12} reveals little difference.[22] Recently, the structure of B_{12r} (the five-coordinate Co(II) species formed by homolysis) was reported and the cobalamin conformation is strikingly similar to those of the two coenzymes.[38] The 5′-deoxyadenosyl ligand does not appear to influence corrin ring pucker significantly. These findings are consistent with model studies which reveal that the major influences of R bulk are increases in Co–C bond length and Co–C–C bond angles.[3] These parameters in $AdoB_{12}$ are similar to the values of an ethyl group in model compounds.[3,5]

Several lines of evidence from studies of coenzyme analogues have led to the hypothesis that corrin distortions in the ground state are important in enzymic processes. Key elements favoring this hypothesis are that modification of the amide side chains reduces coenzyme activity and that a ring is needed between adenine and the Co center.[4,39,40] These results have been interpreted to mean that amide side chain interactions with the protein lead to the distortion that induces repulsions with the (ribose) ring. However, it is also clear that adenine interacts with the protein.[4,40] Furthermore, the adenine N7 and 6 NH_2 group appear to be particularly important.[40] Acceleration of Co–C bond cleavage is small when the alkyl group lacks an adenine, even if the alkyl group is bulky.[41] Can this evidence be interpreted in another way?

If the transition state resembles the $Co^{II}B_{12}$/Ado• intermediate state, then the cleavage will be accelerated by stabilizing this state. Such a stable state probably requires good H-bonding interactions of the protein with the amide side chains and the adenine ring. Formation of this intermediate would require the correct positioning of the intact coenzyme in the holoenzyme, prior to cleavage. It is conceivable that modifying the side chains or the ring in the intervening link between Co and adenine will compromise this positioning and lower or destroy activity. If this positioning is important, the similar corrin conformation of $AdoB_{12}$ and B_{12r} is reasonable.[38] Furthermore, the axial Co–N bond is shorter in the Co(II) derivative since the Co has moved out of the plane of the four corrin N's. This shortened bond should stabilize the Co(II) state and facilitate homolysis as we have argued previously on the basis of model studies.[43] Later studies on B_{12} derivatives have also stressed the need to refocus attention onto the transition state.[1d,38] Thus, it is not necessary to invoke corrin distortions to explain the results with coenzyme analogues. However, it is clear that a direct measure of Co–C bond strength in a holoenzyme is desirable to distinguish between these two alternative hypotheses or to determine whether both hypotheses have elements of truth.

Summary

Our results with Co–CH_3 compounds suggest that the differences in Co–C BDE do not appear to be significantly concentrated in differences in intrinsic Co–C bond strength in the ground state.[44] The BDE differences may be (a) primarily a product state effect, (b) distributed throughout the molecule, or (c) a combination of (a) and (b). In the cleanest comparison possible for $XpyCo(DH)_2R$ compounds, the new near-IR FT-Raman data support the Halpern suggestion that the more basic Xpy ligands stabilize the Co–CH_3 bond by favoring the III over the II oxidation state. These published studies on models as well as the model studies performed here, when combined with studies on cobalamin structures and on the influence of coenzyme analogues on enzymic processes, are consistent with the interpretation that transition state effects dominate Co–C cleavage rate in B_{12} holoenzymes. However, we cannot rule out the widely held hypothesis that ground state steric effects are important, since the $\nu_{(Co-C)}$ stretch does exhibit a small dependence on steric influences of L. Insufficient data on Co–CH_3 BDE's exist to determine the quantitative relationship with $\nu_{(Co-C)}$. Lattice forces in the solid state also appear to alter the $\nu_{(Co-C)}$ bond stretch slightly. The near-IR FT-Raman technique continues to be a promising new tool for studying light-sensitive organocobalt systems.

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