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Heteronuclear NMR Studies of Cobalamins. 10. ^{15}N NMR Studies of [^{15}N]Cyanocobalt Corrins[1]

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^{15}N NMR chemical shifts and ^{15}N-^{13}C coupling constants have been determined for cyanocobalamin (CNCbl) and several other monocyano and dicyanocobalt corrins enriched in ^{15}N or in ^{15}N and ^{13}C in the axial cyanide ligand(s). The ^{15}N chemical shift is found to be nearly as sensitive (ca. 75%) to changes in trans axial ligation as the ^{13}C chemical shift of ^{13}CN cyanocobalt corrins. All alterations in the cobalt coordination sphere have opposite effects on the ^{13}C and ^{15}N chemical shifts; i.e. there is a strictly inverse relationship between $\delta_{^{15}N}$ and $\delta_{^{13}C}$. This suggests that the resonance structure $Co^{+}{=}C{=}N^{-}$ indicative of $d\pi$-$p\pi$ metal-to-ligand back-bonding is important in these cobalt-cyanide complexes and that its contribution to the overall bonding is highly sensitive to trans axial ligation effects. ^{15}N chemical shifts and ^{15}N-^{13}C coupling constants of enriched, base-off CNCbl have also been studied as a function of acidity in sulfuric acid/water mixtures and have permitted an assignment of the previously demonstrated protonation of the axial cyanide ligand in such media. The results are only consistent with a conjugate acid species that is N-bound (rather than the normal C-bound) and protonated at the cyanide carbon.

Introduction

Over the past 16 years, heteronuclear NMR spectroscopy has seen increasing use as a means of studying the solution properties of vitamin B_{12} and its derivatives (see ref 2 and references therein for work prior to 1983). In recent years, increasing use has been made of ^{13}C NMR spectroscopy in studies of the solution chemistry of organocobalt corrinoids enriched in ^{13}C in the α-carbon of the organic ligand[3-5] and of natural-abundance ^{13}C NMR[6-11] spectroscopy including the unambiguous ^{13}C assignments of several 5′-deoxyadenosylcobamides.[8,10,11] ^{31}P NMR spectroscopy of cobalamins has also been exploited as a sensitive probe of nucleotide loop conformation[2,12,13] and has recently been used to probe the interaction of cobalamins with a vitamin B_{12} binding protein.[14]

However, to date, little ^{15}N NMR work has been reported on B_{12} derivatives.[15,16] This is undoubtedly due to the usual difficulties encountered in ^{15}N NMR spectroscopy, i.e. low natural abundance, low intrinsic sensitivity, and long relaxation times. Nonetheless, substantial work in related areas has capitalized on the advantages of ^{15}N NMR spectroscopy, i.e. a wide chemical shift range with high sensitivity to environmental and structural effects.[17-22] For instance, much work has been done with ^{15}N-enriched porphyrins.[23-29] Studies of cobalt(III)-amino acid complexes by ^{15}N NMR spectroscopy have been reported,[30,31] and ligand-exchange dynamics in Ni(II) and Co(II) complexes have been investigated by ^{15}N NMR line shape analysis.[32] In addition, ^{15}N-metal coupling constants have been used to investigate the electronic structure and ligating atom hybridization in both Rh(III)[33] and Pt(II)[34] complexes. ^{15}N NMR parameters have also been reported for both square-planar and tetrahedral tetracyanometal complexes.[35] Most importantly, for the current applications, ^{15}N chemical shifts for extensive series of substituted pentaamminecobalt(III)[36,37] and pentaamminerhodium(III)[38] complexes have been reported. These results show an extreme sensitivity of ^{15}N chemical shifts to ligand substitution, particularly in the trans position, where, for instance, the observed chemical shifts vary over 48 ppm in a series of 12 cobalt complexes.[37]

In addition, ^{15}N NMR spectroscopy has been successfully applied to a number of important problems in macromolecular biochemistry including protein conformation[39-42] and interactions in enzyme active sites.[43-49] Extensive ^{15}N NMR studies of ^{15}N-enriched cyano and isocyanide complexes of low-spin ferrihemes and ferriheme proteins have also been reported by Morishima and co-workers[50-54] and by Goff and co-workers,[55] including studies of intact erythrocytes. The cyanide nitrogen resonances of such complexes occur at very low field (as much as 1172 ppm downfield from free CN^{-}), presumably due to paramagnetic effects, and have been shown to be extremely sensitive to changes in the trans axial ligand and even to the structural differences between hemoglobin

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subunits and to species variation among hemoglobins.

One of our main interests in B_{12} heteronuclear NMR spectroscopy is the development of NMR probes to investigate the interaction of B_{12} derivatives with proteins. We have previously reported some success with ^{31}P NMR studies of cobalamins bound to a haptocorrin from chicken serum.[14] These studies showed that binding of cobalamins to this protein is accompanied by a change in nucleotide loop conformation, which could be due to steric compression of the axial Co–N bond.[2,12,13] We are consequently trying to develop NMR probes in the upper and/or lower axial ligand to further characterize the protein-bound cobalamins. Unfortunately, ^{13}C probes in the upper axial ligand are unlikely to be useful, since the ^{13}C resonances of cobalamins labeled at the α-carbon of the upper axial ligand are quite broad (8–23 Hz at 50 MHz),[1,2] presumably due to quadrupolar relaxation by the cobalt nucleus[56–58] ($I = {}^7/_2$). The anticipated further reduction of T_2 upon binding to a macromolecule with a long rotational correlation time would be expected to make such signals unobservable. Labeling at the β-carbon of an axial alkyl ligand would not be expected to yield a probe sufficiently sensitive to trans axial ligation to be particularly useful. We have consequently turned our attention to ^{15}N NMR spectroscopy particularly of ^{15}N-enriched cyanocobalt corrins, which hold several advantages over ^{13}C-labeled derivatives. Quadrupolar broadening of nitrogen by cobalt in such complexes is not expected to be a problem, and reduction of T_2 by reduced molecular motion in macromolecular complexes is not expected to lead to excessive line broadening, since T_2 values for such cyano nitrogens are normally quite long to begin with. Furthermore, reduction of nitrogen T_1 by macromolecular complexation provides the advantage of allowing more rapid pulse repetition to overcome the sensitivity problem.[21]

We have consequently undertaken an ^{15}N NMR study of ^{15}N-enriched and ^{15}N,^{13}C-enriched cyanocobalt corrins, which is the subject of this report. Only two previous reports of ^{15}N NMR analysis of B_{12} derivatives have appeared. Gust et al.[15] reported a single ^{15}N spectrum of cyanocobalamin (CNCbl) slightly enriched in ^{15}N in the cyanide nitrogen in which, in addition to the cyanide nitrogen resonance, resonances for all seven side chain amide nitrogens could be seen but the ring nitrogens were not observed. More recently, DiFeo et al.[16] successfully observed the side chain amide nitrogens of CNCbl at natural abundance by a polarization-transfer sequence and assigned four of these resonances by use of the cobalamin monocarboxylic acid analogues.

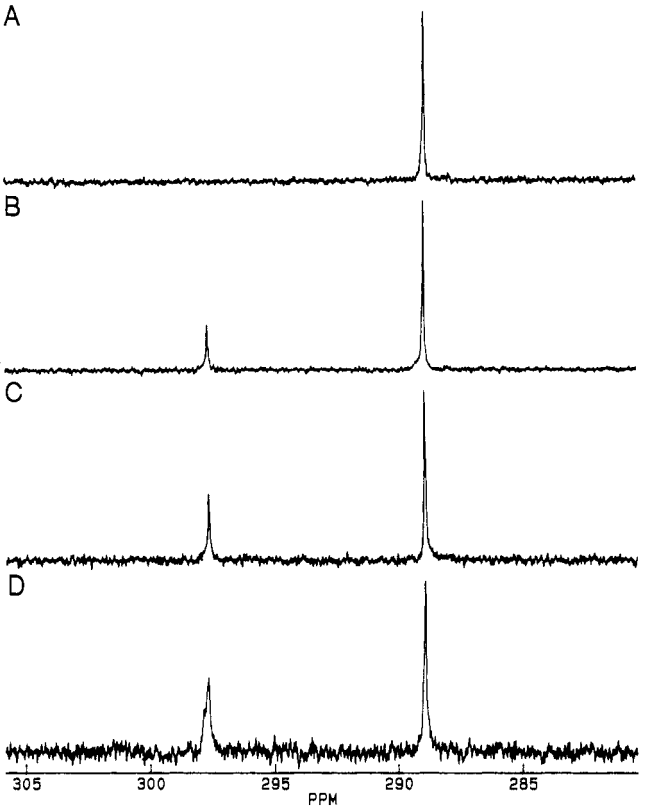

Figure 1. 30.4-MHz ^{15}N NMR spectra of C^{15}NCbl at 25 °C: (A) 10 mM in water; (B) 21 mM in 0.198 M H_2SO_4, $H = 0.592$ ($m^* = 0.919$, eq 1), $\alpha_{base\text{-}on} = 0.764$; (C) 13 mM in 0.301 M H_2SO_4, $H = 0.396$ ($m^* = 0.919$, eq 1), $\alpha_{base\text{-}on} = 0.667$; (D) 15 mM in 0.375 M H_2SO_4, $H = 0.287$ ($m^* = 0.919$, eq 1), $\alpha_{base\text{-}on} = 0.601$. Each spectrum represents ca. 20 000 accumulations.

Experimental Section

[^{15}N]Cyanocobalamin (C^{15}NCbl) and [^{13}C,^{15}N]cyanocobalamin (^{13}C^{15}NCbl) were prepared from aquocobalamin (H_2OCbl) and [^{15}N]-KCN (CIL, 96 atom % ^{15}N) and [^{13}C,^{15}N]NaCN (MSD, 99 atom % ^{13}C, 99 atom % ^{15}N), respectively, by the previously described procedure.[2] [^{13}C,^{15}N]Factor B (a mixture of diastereomeric cyanoaquocobinamides, i.e. α-(CN)-β-(H_2O)Cbi and α-(H_2O)-β-(CN)Cbi) was prepared by exchange of factor B with Na^{13}C^{15}N. Factor B (102.9 mg in 10 mL of water) was stirred briefly with a 4.9-fold molar excess of Na^{13}C^{15}N (24.0 mg) and then desalted by extraction through phenol. The doubly labeled dicyanocobinamide thus obtained was converted back to factor B by repeated acidification with 5 N acetic acid followed by evaporation to dryness. Acidities of H_2SO_4/H_2O mixtures were determined by titration as previously described.[2]

NMR samples contained 10–50 mM cobamides or 25 mM uncomplexed cyanide species in H_2O or in H_2SO_4/H_2O mixtures, in either 10- or 12-mm sample tubes containing D_2O in a concentric insert (Wilmad) to provide a ^{2}H-lock signal. Chemical shifts were referenced to external, neat CH_3NO_2 (also locked to D_2O) but were reported relative to NH_3(l) by using $\delta_{CH_3NO_2} = 380.23$ ppm relative to NH_3(l). As ^{13}C/^{12}C isotopic shifts of ^{15}N resonances in metal cyanide complexes have been reported to be quite small (≤0.1 ppm),[35] these effects have been ignored. ^{13}C–^{15}N coupling constants were determined by observing either ^{13}C or ^{15}N resonances, but in all cases, when the apparent line width exceeded 25% of the apparent spacing of the lines, the coupling constant, line width, and resonance frequency were determined by fitting the digitized data to the doublet line shape equation[59] via a nonlinear least-squares routine utilizing a simplex minimization algorithm.

^{15}N NMR spectra were obtained on either a Nicolet NT-200 wide-bore superconducting spectrometer (12-mm tubes) operating at 20.278

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Table I. ^{15}N and ^{13}C Chemical Shifts and ^{15}N–^{13}C Coupling Constants of ^{13}C- and ^{15}N-Enriched Cyanocobalt Corrins and Free Cyanide Species

compd[a]	$\delta_{^{15}N}$,[b] ppm	$\delta_{^{13}C}$,[c] ppm	$J_{^{15}N-^{13}C}$,[d] Hz
CNCbl, base-on	288.9	123.6	8.9
CNCbl, base-off	297.6	114.0	9.7
β-(CN)-α-(H_2O)Cbi	298.5	114.1	9.4
β-(H_2O)-α-(CN)Cbi	306.0	112.8	9.8
$(CN)_2$Cbl			
β-CN	277.6	141.5	6.5[e]
α-CN	284.4	140.2	6.7[f]
$(CN)_2$Cbi			
β-CN	277.4	141.6	4.7[g]
α-CN	283.3	140.6	5.3[h]
free CN^-	272.4	166.9	6.2[i]
free HCN	245.5	114.5	19.1[j]

[a] β signifies the "upper" axial ligand, and α, the "lower". [b] Relative to external NH_3(l) (all shifts downfield). [c] Reference 2; originally reported relative to external TMS, the values have been recalculated relative to external TSP. [d] The sign is probably negative for all compounds. [e] J_{CCoCN} = 3.0 Hz. [f] J_{CCoCN} = 2.9 Hz. [g] J_{CCoCN} = 4.7 Hz. [h] J_{CCoCN} = 4.6 Hz. [i] Literature value 6.1 Hz.[35] [j] Literature value 18.5 Hz.[61]

MHz or a Bruker MSL 300 superconducting spectrometer operating at 30.415 MHz. Typically, 30° tip angles were used with no ^1H decoupling and data were collected into 8K–32K data sets over sweep widths of 3000–5000 Hz. For cyanocobalt corrinoids in water, pulse repetition rates of 8 s proved necessary to avoid progressive saturation, but in H_2SO_4/H_2O mixtures when [H_2SO_4] exceeded about 4 M, spectra could be turned over much more rapidly (<2 s) without evidence of saturation. ^{13}C NMR spectra were observed on the Nicolet spectrometer at 50.312 MHz as described previously.[2]

Results

Figure 1 shows the ^{15}N NMR spectrum of $C^{15}N$Cbl in water (part A) and in increasing concentrations of sulfuric acid (parts B–D). The two resonances obviously represent the base-on species (δ = 288.9 ppm) and the base-off species (δ = 297.6 ppm), the exchange between the species being stopped on the NMR time scale as was also the case for the ^{13}C (at 50.312 MHz) and ^{31}P resonances (at 80.988 MHz) of base-on and base-off ^{13}CNCbl.[2] Similar spectra (not shown) were also obtained for $^{13}C^{15}N$Cbl, in which case each resonance was a doublet with $J_{^{15}N-^{13}C}$ = 8.9 Hz for the base-on species and 9.7 Hz for the base-off. The chemical shift and coupling constant data are collected in Table I. As the sign of $J_{^{13}C-^{15}N}$ for HCN[60] and CN^-[61] has been shown to be negative, all of the values determined here are probably negative as well.[35]

Assuming that the scanning parameters have been properly chosen such that neither resonance is partially saturated, the relative integrals of the base-on and base-off ^{15}N resonances should accurately reflect the relative proportions of the two species at each acidity. That this is the case is indicated in Figure 2, a plot of log [$(\alpha_{base\text{-}on})/(1 - \alpha_{base\text{-}on})$], where $\alpha_{base\text{-}on}$ is the fraction of the total integral found in the base-on resonance, vs H, the generalized acidity function due to Cox and Yates[62] (eq 1). The value of

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

the adjustable parameter, m^*, in eq 1 has been set to 0.919, the value previously found to be appropriate for the base-on/base-off protonic equilibrium from correlations of the ^{13}C and ^{31}P NMR integrals of ^{13}CNCbl with acidity.[2] The data in Figure 2 adequately (r^2 = 0.997) fit a straight line with slope 0.993 and give a value of $pK_{base\text{-}off}$ = 0.09 ± 0.01, in excellent agreement with the value of 0.11 ± 0.01 previously obtained.[2] It should be noted that data useful for the correlation in Figure 2 could only be obtained at acidities $H \geq 0$ ([H_2SO_4] ≤ 0.65 M). This is due to the fact that at higher acidities significant phosphodiester

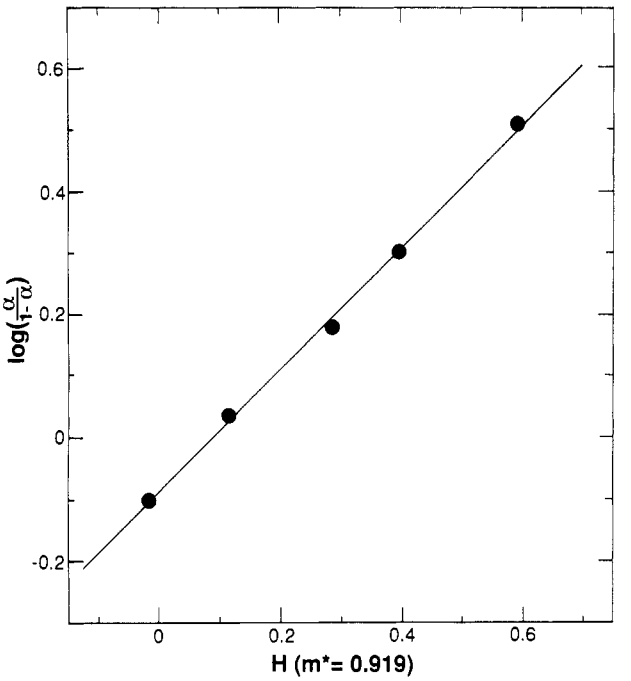

Figure 2. Plot of log [$\alpha_{base\text{-}on}/(1 - \alpha_{base\text{-}on})$], calculated from the relative integrals of the ^{15}N resonances of the base-on and base-off species of $C^{15}N$Cbl, vs H (at m^* = 0.919, eq 1). The solid line is a least-squares fit, slope = 0.993 ± 0.029, pK_a = 0.09 ± 0.01, and r^2 = 0.997.

hydrolysis occurs during the time required to obtain spectra of adequate signal-to-noise ratio (ca. 40 h).[2] As the ^{15}N resonances of the hydrolysis products (i.e. [^{15}N]cyanocobinamide and [^{15}N]cyanocobinamide phosphate)[2] are not cleanly resolvable from that of the base-off species, usable integrals of the species in equilibrium could not be determined.

The ^{15}N NMR spectra (not shown) of ^{13}C,^{15}N-enriched factor B, a mixture of the diasteromeric β-(CN)-α-(H_2O)Cbi and β-(H_2O)-α-(CN)Cbi,[2] consisted of two doublets, one at 298.5 ppm and one at 306.0 ppm. Because of the close proximity of the upfield resonance to that of base-off $C^{15}N$Cbl, this resonance has been assigned to the β-CN diastereomer and the lower field resonance to the α-CN diastereomer. The chemical shifts of these resonances display two interesting features. First, there is an 0.9 ppm difference in ^{15}N chemical shift between base-off $C^{15}N$Cbl and the β-diastereomer of $C^{15}N$Cbi, the latter resonance being the more downfield. We have previously noted[1] smaller but finite differences in ^{13}C chemical shifts between base-off cobalamins and the analogous β-cobinamides enriched in ^{13}C in the carbon bonded to cobalt, with the signed differences in chemical shift, $\Delta\delta$, being trended in the order R = $CH_3{}^{13}CH_2$ (0.3), $^{13}CH_3$ (0.08), $HOOC^{13}CH_2$ (–0.09), and $N^{13}C$ (–0.10). We have suggested that this effect is probably due to an influence of the pendant, but uncoordinated, nucleotide loop on the conformation of the corrin ring, as there is no evidence for an interaction of the nucleotide with the remainder of the structure in protonated, base-off cobalamins.[1,9] The current observations support this suggestion, and we note that in the case of the ^{15}N resonance this chemical shift difference is both much larger than and opposite in sign to the ^{13}C chemical shift difference, points to which we shall return shortly.

Figure 3A shows the ^{15}N NMR spectrum of $(C^{15}N)_2$Cbl formed by adding slightly less than 1 equiv of $KC^{15}N$ to $C^{15}N$Cbl. Two well-separated resonances are observed, and by analogy to the relative chemical shifts of the β-CN and α-CN diastereomers of factor B, the more downfield resonance in Figure 3A is assigned to the α-CN of $(C^{15}N)_2$Cbl. There are several important differences between the ^{15}N NMR and ^{13}C NMR spectra of $(CN)_2$Cbl. Our previous work with $(^{13}CN)_2$Cbl[2] showed that, at temperatures of 25 °C and above, a single, broad ^{13}C resonance is observed, which cleanly collapses to an AB quartet at 15 °C, with $\Delta\nu$ = 64.9 Hz and $J_{C\text{-}Co\text{-}C}$ = 56.6 Hz. Hence, exchange of

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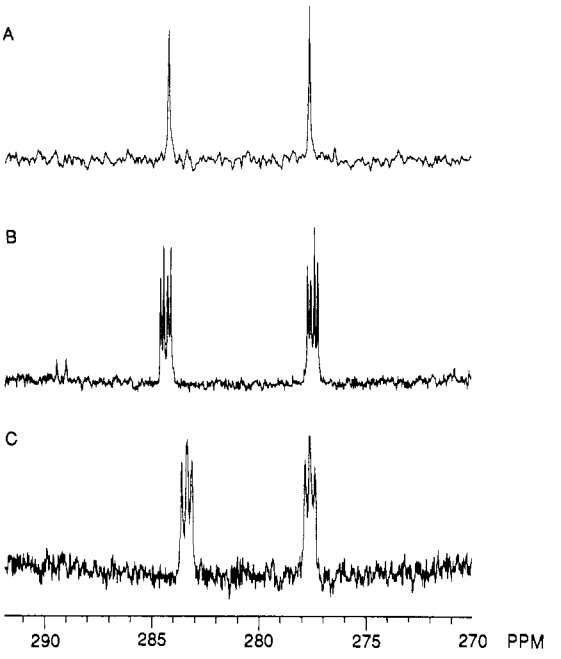

Figure 3. 20.3-MHz ^{15}N NMR spectra of ^{15}N- and ^{13}C,^{15}N-enriched dicyanocobalt corrins: (A) 50 mM $(C^{15}N)_2$Cbl; (B) 32 mM $(^{13}C^{15}N)_2$Cbl; (C) 27 mM $(^{13}C^{15}N)_2$Cbi.

the cyanide is reasonably fast on the ^{13}C NMR time scale (at 50.3 MHz) at room temperature. However, there is no evidence of exchange at these temperatures in the ^{15}N spectrum, nor is there any evidence of long-range ^{15}N–^{15}N coupling. We also note that the difference in nitrogen chemical shifts between the two cyanide ligands is both greatly enhanced and opposite in sign as compared to the difference in carbon chemical shift (Table I).

In situ generation of $(^{13}C^{15}N)_2$Cbl by addition of $Na^{13}C^{15}N$ to $^{13}C^{15}N$Cbl produces the spectrum shown in Figure 3B, in which each of the resonances is split into a doublet of doublets, with coupling constants of about 6.6 and 3.0 Hz (Table I). We assume that the larger coupling represents the one-bond ^{13}C–^{15}N coupling and the smaller a three-bond ^{13}C–Co–C–^{15}N coupling. Although the latter coupling (ca. 3 Hz) seems rather large, there are precedents for three-bond C–C–C–N couplings as large as 6.7 Hz in certain cyclic aliphatic amines.[63]

When $(^{13}C^{15}N)_2$Cbi is similarly generated from ^{13}C,^{15}N-enriched factor B and $Na^{13}C^{15}N$ (Figure 3C), the resonances appear to collapse to triplets and the more downfield (i.e., α) resonance is shifted 1.1 ppm to higher field (Table I). Close inspection of the downfield resonance shows evidence of splitting of the center line leading to coupling constants of 5.3 and 4.6 Hz, somewhat arbitrarily assigned to the one- and three-bond couplings, respectively. The upfield resonance is interpreted as a fortuitous triplet due to near-equivalence of the long- and short-range couplings (4.7 Hz). Interestingly, there is essentially no difference in either the ^{15}N or ^{13}C chemical shifts of the β-cyanide resonances of $(CN)_2$Cbl and $(CN)_2$Cbi (Table I), despite the observation of such differences between base-off cobalamins and cobinamides and the above discussion regarding the influence of the pendent, but uncoordinated, nucleotide loop on corrin ring conformation. In fact, it seems highly unlikely that there is any significant difference in corrin ring conformation between $(CN)_2$Cbl and $(CN)_2$Cbi, since a comparison of the natural-abundance ^{13}C spectra of these two species[9] has shown that the ^{13}C chemical shifts of every corrin ring carbon are virtually identical in $(CN)_2$Cbl and $(CN)_2$Cbi, suggesting that the macrocycle is locked into a rigid conformation when the cobalt is coordinated to two such strong-field ligands. Further comparison of the ^{13}C spectra of $(CN)_2$Cbl and $(CN)_2$Cbi did, however, reveal differences in the chemical shifts of one of the corrin side chains. Combined with observations of differences in ^{13}C chemical shift between the

(63) Reference 18, Table 151.

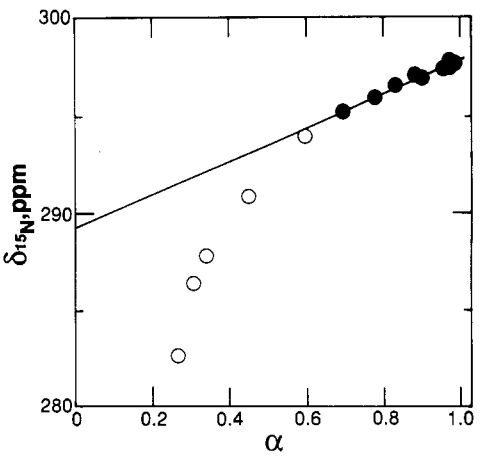

Figure 4. Plot of the ^{15}N chemical shifts of C^{15}NCbl in sulfuric acid/water mixtures vs α, the fraction of corrin ring C-10-deprotonated species calculated from $pK_a = -1.57$, and H (eq 1) at $m^* = 0.22$: (●) points used in linear regression ($N = 9$, $r^2 = 0.990$, slope = 8.58 ± 0.32 ppm, intercept = 289.27 ± 0.29 ppm); (○) points not used in linear regression.

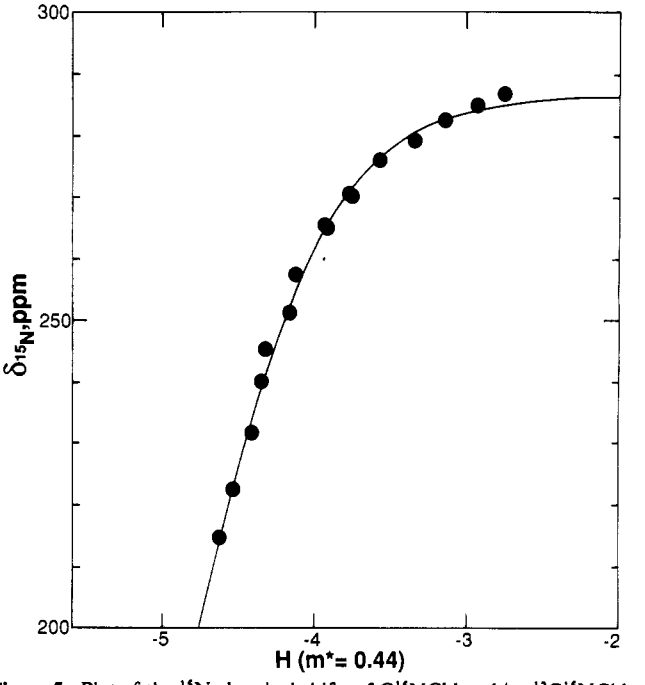

Figure 5. Plot of the ^{15}N chemical shifts of C^{15}NCbl and/or ^{13}C^{15}NCbl in sulfuric acid/water mixtures vs H (eq 1) at $m^* = 0.44$. The solid line is a nonlinear least-squares fit to a simple titration curve with $pK_a = -4.80$ and with 286.7 and 105.9 ppm as the base and acid end points, respectively.

pendent, uncoordinated, and unprotonated benzimidazole nucleotide in $(CN)_2$Cbl and the free nucleotide,[9] these results were interpreted to indicate that in the major solution species of $(CN)_2$Cbl there is a hydrogen-bonded interaction between the benzimidazole nitrogen (B3) and a side chain amide N–H. Further evidence for this so-called "tuck-in" species was subsequently obtained from a study of the thermodynamics of the base-on/base-off reaction of CH_3Cbl and $^-OOCCH_2$Cbl.[1] Presumably, it is the existence of the "tuck-in" species that explains the 1.1 ppm difference in ^{15}N chemical shift between the α-cyanide groups of $(CN)_2$Cbl and $(CN)_2$Cbi and, in fact, this observation should be taken as further evidence for the "tuck-in" species.

The ^{15}N resonance of base-off C^{15}NCbl was also found to be strongly acidity dependent. These data, depicted in Figures 4 and 5, will be discussed below.

Discussion

By far the most striking observation of the ^{15}N and ^{13}C chemical shift data of the cyanocobalt corrins (Table I) is the inverse

dependence of these chemical shifts upon changes in ligation. For instance, when free CN^- coordinates to cobalt to form base-on CNCbl, the ^{13}C chemical shift moves upfield 43.3 ppm (i.e., between the shifts of free CN^- and HCN) while the ^{15}N chemical shift moves *downfield* 16.5 ppm. In contrast, when CN^- "coordinates" to the hard Lewis acid, H^+, both the ^{13}C and ^{15}N resonances shift upfield. In a similar vein, conversion of base-on CNCbl to the base-off species (i.e., substitution of H_2O for dimethylbenzimidazole trans to cyanide) caused a 9.8 ppm upfield shift of the ^{13}C resonance but an 8.7 ppm *downfield* shift of the ^{15}N resonance. In fact, a plot (not shown) of the ^{15}N chemical shift vs the ^{13}C chemical shift of all eight cobalt-bound cyanide species in Table I shows a strictly inverse dependence and, interestingly, a reasonable linear ($r^2 = 0.89$) correlation with slope -0.73 ± 0.10; i.e. the ^{15}N chemical shift is nearly three-quarters as sensitive to changes in the cobalt coordination sphere as the ^{13}C resonance but in inverse fashion.

It is extremely difficult to envision any reasonable explanation for these striking results other than the existence of a $d\pi-p\pi$ back-bonded resonance species (**2** in eq 2), whose contribution

$$\underset{\mathbf{1}}{Co{-}C{\equiv}N} \leftrightarrow \underset{\mathbf{2}}{Co^{+}{=}C{=}N^{-}} \qquad (2)$$

to the overall structure is sensitive to changes elsewhere in the cobalt coordination sphere. That the nitrogen nucleus of **2** would be expected to resonate downfield from that of **1** (as required by the data) is anticipated due to both its increased negative charge density and considerations of the effect of the change in hybridization. Thus, the terminal nitrogen of diazo compounds ($RCH{=}N^+{=}N^-$), which can be considered to model the hybridization of species **2**, resonates downfield (range 364–447 ppm relative to $NH_3(l)$) from the terminal nitrogen of diazonium salts ($R{-}N^+{\equiv}N$, range 316–364 ppm).[64] Further suggestive evidence for the importance of species **2** in cyanocobalt corrins comes from the notorious lack of sensitivity of the ^{15}N chemical shift of organic nitriles ($RC{\equiv}N$) to the nature of the R group, the chemical shift range for a variety of such species (R = H, Cl, CH_3, CH_3CH_2, $CH_3CH_2CH_2$, $(CH_3)_2CH$, $(CH_3)_3C$) varying over only 15 ppm (δ = 236–251 ppm)[65] compared to a chemical shift range of 28.6 ppm shown in Table I. In addition we note, as above, that interaction of CN^- with the proton, a Lewis acid incapable of $d\pi$-donation, causes an upfield shift of *both* the ^{13}C and ^{15}N resonances while interaction with the cobalamin cobalt atom causes a downfield shift of the ^{15}N resonance.

In principle, it should be possible to infer additional evidence for the existence of resonance species **2** from the influence of trans ligand substitution on the coordinated cyanide ^{15}N chemical shift. Considering the separation of charge in **2**, good donor ligands would stabilize species **2** relative to poorer donors and shift the ^{15}N resonance downfield. However, axial ligands, which are also π-acceptors, would compete with cyanide for cobalt d-orbital electron density, destabilizing species **2**. Unfortunately, at this point it is impossible to quantitate the relative importance of these two effects on the stabilization of **2**. Of the three trans axial ligands represented in the current data, the donor strength clearly increases in the order OH_2 < dimethylbenzimidazole < CN^-. However, CN^- is (evidently) an excellent π-acceptor from cobalt, and dimethylbenzimidazole is at least a potential π-acceptor. Thus, the upfield shift of the β-CN ^{15}N resonance when H_2O is substituted by dimethylbenzimidazole as the trans axial ligand may indicate that dimethylbenzimidazole is indeed a π-acceptor in this situation and signify the importance of d-orbital competition in the stability of species **2**. Such competition would necessarily be inherent in the dicyanocobalt species, and the ^{15}N chemical shifts suggest that $d\pi-p\pi$ overlap is stronger between cobalt and the α-cyanide than between cobalt and the β-cyanide. This conclusion is supported by the fact that the ^{15}N resonance of the α-cyano nitrogen is at lower field than that of the β-cyano nitrogen in the two diastereomers of factor B and by the fact that removal of the trans d-orbital competitor (i.e., conversion of $(CN)_2Cbi$ to either of the two factor B diastereomers) causes a downfield shift of the α-cyanide ^{15}N resonance (22.7 ppm) larger than that of the β-cyanide ^{15}N resonance (21.1 ppm).

Some support for these ideas can be obtained from existing X-ray crystal structures of cyanocobalt corrinoids by examination of the cyanide C–N bond lengths. Structures are available for several base-on β-cyanocobamides including CNCbl (d_{C-N} = 1.11 Å for the "wet" structure),[66] cyano-13-epicobalamin (d_{C-N} = 1.13 Å),[67] and cyanocobalamin 5′-phosphate (d_{C-N} = 1.09 Å).[68] Unfortunately, no X-ray structures have appeared for any β-cyano-α-aquocobamides, but α-cyano-β-aquocobyrinic acid hexamide has d_{C-N} = 1.14 Å.[69] There, thus, appears to be a slight lengthening of the cyanide C–N bond in the α-position trans to H_2O, relative to the β-CN trans to benzimidazole, consistent with a larger contribution of resonance structure **2** and a downfield shift of the ^{15}N resonance in the former compared to the latter. Similarly, three X-ray structures have been reported for dicyanocobyrinic acid heptamethyl ester including the anhydrous species,[70] the monohydrate,[71] and a monosolvate of "2-propanol".[72,73] Two of the structures (both solvates) show a significant increase in the α-cyano C–N bond length relative to that of the β-cyano (1.171 Å vs 1.127 Å for the 2-propanol[71] solvate, 1.157 Å vs 1.144 Å for the hydrate), and the third shows an insignificant difference (the average values are 1.156 Å for the α-cyano and 1.136 Å for the β). Again, a slight lengthening of the α-cyano C–N bond is consistent with enhanced $d\pi-p\pi$ overlap of the α-position relative to the β-position and the greater downfield shift of the α-cyano ^{15}N resonance. Unfortunately, due to the size of these structures and the consequent relative uncertainty in bond lengths, support for π-bonding in such cyanocobalt corrins can only be considered suggestive. We are currently attempting to further investigate this phenomenon by ^{13}C and ^{15}N NMR studies in cyanocobaloximes.[74,75]

We have also attempted to use ^{15}N NMR spectroscopy to answer questions previously raised regarding the acid/base behavior of base-off CNCbl in H_2SO_4/H_2O mixtures.[2,5] The ^{13}C resonance of the base-off species of $^{13}CNCbl$ was found to undergo a large (ca. 16 ppm) upfield shift in H_2SO_4/H_2O mixtures, which appeared to follow a smooth titration curve when correlated to the Cox and Yates acidity function (eq 1) with $m^* = 0.25$.[2] This was taken to indicate that either the corrin ring or the axial cyanide ligand could be protonated in sufficiently acidic media. Subsequent work[5] showed that, in fact, both protonations occur and most likely overlap significantly. Thus, the base-off species of $^{13}CH_3Cbl$ and $CH_3{}^{13}CH_2Cbl$ (and CF_3Cbl by ^{19}F NMR spectroscopy), which cannot protonate at the β-ligand, were also shown to undergo acidity-dependent shifts of the ^{13}C resonance, although for all three compounds the resonances shifted *downfield* (2–3 ppm in the case of the ^{13}C-enriched species). This indicates that these compounds can indeed protonate on the corrin ring. For all three compounds, the acidity-dependent chemical shifts correlated well as single titrations with the Cox and Yates acidity function (eq 1), with an average value of $m^* = 0.22 \pm 0.04$. The pK_a values appeared to be independent of the alkyl ligand with an average

(64) Reference 18, Tables 134 and 135.
(65) Reference 18, Table 108.

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Table II. ^{15}N and ^{13}C Chemical Shifts and ^{15}N–^{13}C Coupling Constants for Protonated CNCbl Species and Free Cyanide Species

compd	$\delta_{^{15}N}$,[a] ppm	$\Delta\delta_{^{15}N}$,[b] ppm	$\delta_{^{13}C}$,[c] ppm	$\Delta\delta_{^{13}C}$,[b] ppm	J_{C-N},[d] Hz
CNCbl, base-off	297.6		114.0		9.7
CNCbl–H^{+}[e]	288.0[f]	−8.4			12.9
HCNCbl–H^{2+}[g]	105.9	−182.1			52.1
CN^-	272.4		166.9		6.2
HCN	245.5	−26.9	114.5	−52.4	19.1
$HCNH^+$	197.7	−47.8	104.2	−10.3	32.3

[a] Relative to external NH_3(l) (all shifts downfield). [b] Difference in chemical shifts between the fully protonated and deprotonated species. [c] Reference 5; relative to external TSP (all shifts downfield). [d] The sign is probably negative for all compounds. [e] Species protonated at corrin ring C-10. [f] Average of two values, 289.3 ppm (see Figure 4 and text) and 286.7 ppm (see Figure 5 and text). [g] Species protonated at both the corrin ring C-10 and the axial cyanide ligand.

value of −1.57 ± 0.05. Examination of the natural-abundance ^{13}C NMR spectrum of the corrin ring carbons of base-off CH_3Cbl at various acidities located the probable site of corrin ring protonation at C-10. It was consequently concluded that base-off CNCbl probably protonates both at the corrin C-10 and on the cyanide ligand, with the chemical shift effect of the two overlapping protonations being in opposite directions thus complicating the interpretation of the ^{13}C data for CNCbl substantially. Left unanswered was the question of the nature of the cyanide-ligand-protonated species: I.e., does cyanide remain C-bound and protonate at N or does it isomerize to an N-bound species with simultaneous protonation of C? We believe that our ^{15}N NMR measurements permit an answer to this question.

The ^{15}N resonance of base-off C^{15}NCbl undergoes a large, progressive upfield shift in H_2SO_4/H_2O mixtures of increasing acidity. The anticipated, overlappping protonations were subjected to the following, approximate treatment. Assuming that the corrin ring protonation of C-10 for base-off CNCbl is adequately represented by the average value of the pK_a observed for the other three cobalamins and that the same acidity function is followed, a plot of $\delta_{^{15}N}$ vs α (Figure 4) was constructed, where α represents the fraction of C-10-deprotonated species calculated from pK_a = −1.57 and the measured values of H (eq 1) by using m^* = 0.22. The plot is satisfactorily linear (N = 9, r^2 = 0.990) up to acidities of 5.43 M H_2SO_4 (H = −1.21, α = 0.70) with slope 8.58 ± 0.32 ppm and intercept 289.27 ± 0.29 ppm. At higher acidities, the chemical shifts begin to deviate seriously from the regression line (Figure 4), the obvious consequence of the onset of cyanide ligand protonation. Despite the rather long extrapolation required, the intercept of this regression line may be taken as one estimate of the chemical shift of C-10-protonated base-off CNCbl (Table II). The remainder of the data may be used to estimate the pK_a for cyanide ligand protonation of the C-10-protonated species as well as the chemical shift of the cyanide-ligand-protonated species as follows. Chemical shift data at [H_2SO_4] > 10.3 M (H(m^* = 0.22) < −1.9) are assumed to reflect only protonation of the cyanide ligand, since α (for C-10 protonation, Figure 4) is less than 0.3 at these acidities. Such data were fit to a titration curve by using a nonlinear least-squares routine with a simplex minimization algorithm and the Cox and Yates acidity function (eq 1). The value of m^* was varied so as to minimize the residuals from the fit, and such minimization occurred at m^* = 0.44. The resulting fit (Figure 5) gives pK = −4.80 and values of 286.7 and 105.9 ppm for the acid and base end points, respectively. The former value is a second estimate of the chemical shift of the C-10-protonated (but cyanide-deprotonated) species and agrees quite well with the previous estimate (289.3 ppm). The average value is listed in Table II. The acid end point shift, 105.9 ppm, is then an estimate of the chemical shift of the species protonated both at C-10 and on the axial cyanide. With this pK_a and acidity function (i.e., m^* = 0.44), the C–N coupling constants for the cyanide-protonated and deprotonated species could then be estimated by using six measured values at acidities ranging from 11.01 M H_2SO_4 (H = −2.93, m^* = 0.44) to 15.73 M H_2SO_4 (H = −4.33, m^* = 0.44 M). The values are listed in Table II. For comparison, we have also determined the ^{15}N chemical shift and C–N coupling constant of the conjugate acid of HCN. By use of the previously determined[5] pK_a (−2.61) and acidity function (m^* = 0.18), plots were constructed (not shown) of $\delta_{^{15}N}$ and J_{C-N} vs α_{HCN}, the fraction of deprotonated species, at acidities ranging from 0.75 M H_2SO_4 (H = 0.02) to 12.9 M H_2SO_4 (H = −2.09) for $\delta_{^{15}N}$ and 0.75 M H_2SO_4 to 16.9 M H_2SO_4 (H = −2.42) for J_{C-N}. Due to decomposition to formic acid and ammonium ion, it was difficult to observe the ^{15}N resonance at higher acidities and J_{C-N} was most often evaluated via doublet line shape fits of the ^{13}C resonances of the doubly labeled species (see Experimental Section), which were more easily observable. Each plot was satisfactorily linear (N = 13, r^2 = 0.996, intercept = 197.70 ± 0.76 ppm, slope = 47.80 ± 0.87 ppm for $\delta_{^{15}N}$; N = 10, r^2 = 0.996, intercept = 32.3 ± 0.3 Hz, slope = −13.1 ± 0.3 Hz for J_{C-N}), and the resulting chemical shift and coupling constant for the conjugate acid of HCN are listed in Table II.

The ^{13}C and ^{15}N chemical shifts of the various free cyanide species clearly reflect the sites of protonation. Protonation of CN^- at carbon causes a 52.4 ppm upfield shift of the carbon resonance but only a 26.9 ppm upfield shift of the nitrogen resonance. Subsequent protonation of HCN in strongly acidic H_2SO_4/H_2O mixtures causes only a 10.3 ppm upfield shift of the ^{13}C resonance but a 47.8 ppm upfield shift of the ^{15}N resonance and clearly occurs at nitrogen. The sequential protonations increase the ^{15}N–^{13}C coupling constant from 6.2 to 19.1 to 32.3 Hz. Protonation of the corrin ring of base-off CNCbl causes an 8.4 ppm upfield shift of the ^{15}N resonance. Assuming that such protonation shifts the ^{13}C resonance downfield, as is the case for $^{13}CH_3$Cbl and $CH_3{}^{13}CH_2$Cbl, there is again an inverse effect on the ^{13}C and ^{15}N resonances. Despite what must be rather large uncertainties in the ^{15}N chemical shift and ^{15}N–^{13}C coupling constant of the base-off, C-10- and cyanide-protonated species, it is clear that protonation of the cyanide ligand causes an upfield shift of well over 100 ppm, far in excess of the 47.8 ppm upfield shift upon N-protonation of HCN, and that the coupling constant of the cyanide-ligand-protonated species far exceeds that of $HCNH^+$. These results are incompatible with N-protonation of the cobalt-bound cyanide of CNCbl. We consequently conclude that protonation of the cyanide ligand of base-off CNCbl in strongly acidic media occurs with isomerization of the normally C-bound species to an N-liganded species with simultaneous protonation of the cyanide carbon. The very large upfield shift of the ^{15}N resonance accompanying this protonation seems less surprising in view of the fact that the ^{15}N resonances of isocyanides occur some 70–110 ppm upfield from those of nitriles (e.g., δ_{CH_3CN} = 244.4 vs δ_{CH_3NC} = 160.6, an upfield shift of 83.8 ppm).[76]

The observation of an HCN–cobalamin complex ligated via nitrogen has important consequences for the mechanism of ligand substitution reactions with cyanide. pH–rate profiles (corrected for all reactant ionizations) for substitution of H_2O by cyanide both in aquocobalamin[77,78] and in the ferric heme center of the heme-containing octapeptide from cytochrome c[79] always display a pH-independent region in acidic media, which has been assumed to indicate direct attack of HCN to form an N-bound intermediate that deprotonates and isomerizes to the stable, C-bound product. Our observations of an N-bound HCN-ligated cobalamin in strongly acidic media represent the first direct evidence that such species do indeed exist. Evidently, in such media this species is stabilized (i.e., from hydrolysis) by the extremely low activity of water[80,81] (e.g., at H = −4.4 (m^* = 0.44), where the cyanide ligand is only 30% protonated, a_{H_2O} = 3.8 × 10^{-4}).[82]

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Thus, ^{15}N NMR spectroscopy of cyanocobalt corrins enriched in ^{15}N in the axial cyanide proves to be both feasible and extremely instructive. Despite the relatively high sample concentrations required and the relatively long acquistion times needed, such measurements can be made and excellent spectra obtained. Further studies of the inverse dependence of ^{13}C and ^{15}N chemical shifts of cobalt-cyanide species are currently in progress in a simpler model system as well as attempts to observe the ^{15}N NMR spectrum of protein-bound cyanocobalamin.

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Photoelectron Spectroscopy of the Tin Dichalcogenides $SnS_{2-x}Se_x$ Intercalated with Cobaltocene

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Single crystals of the n-type semiconducting tin dichalcogenides $SnS_{2-x}Se_x$ (x = 0, 0.3, 0.5, 1.3, 1.85, 2), which have a two-dimensional layered structure, have been intercalated with cobaltocene to give the series of compounds $SnS_{2-x}Se_x(CoCp_2)_{0.33}$, where $Cp = \eta^5$-C_5H_5. Photoelectron spectroscopy has been employed to investigate the electronic changes upon intercalation, especially the electron transfer from the guest to the host. X-ray photoelectron spectroscopy (XPS) has revealed mixed oxidation states for both tin [Sn(II), Sn(IV)] and cobalt [Co(I), Co(II), Co(III)]. Of the three cobalt species observed by XPS, two have been unambiguously identified as $CoCp_2$ and $[CoCp_2]^+$, whereas the third cobalt species has only been tentatively assigned to a $Co(\eta^5$-$C_5H_5)(\eta^4$-$C_5H_5R)$ complex, in which cobalt is formally in the oxidation state Co(I). Ultraviolet photoelectron spectroscopy (UVPES) shows that the intercalates are either semiconducting (x = 0, 0.3, 0.5, 1.3) or metallic (x = 1.85, 2), whereas all the hosts are n-type semiconductors. An impurity-band model is presented as a possible qualitative explanation for this transition through the series.

Introduction

The series of compounds $SnS_{2-x}Se_x$ ($0 < x < 2$) belong to the diverse class of layered materials, which have created interest by virtue of their two-dimensional nature.[1] The crystal structure (space group $P\bar{3}m1$) is similar to that adopted by many transition-metal dichalcogenides (MX_2). It is based upon repeatedly stacked XMX lamellae bound together by van der Waals interactions between adjacent planes of hexagonally closed-packed chalcogenide atoms (X).[2] In SnX_2 the metal atoms are coordinated in nearly octahedral sites.

The process of intercalation by which sheets of guest molecules may be inserted into the interlamellar gaps of the host layered material has been the subject of much research.[3] For example, the search for novel battery systems has led to the development of high-energy-density storage batteries driven by the process of intercalation.[4] In addition, the intercalation of TaS_2 and $TaSe_2$ by a variety of guest species has led to the discovery of a new class of superconductors.[5]

The changes in electronic structure of layered systems upon intercalation can be investigated profitably by using photoelectron spectroscopy (PES).[6,7] For example, a semiconductor to metal transition is induced by the intercalation of K into WS_2.[8] Previous research has indicated that electron transfer occurs between the guest species and host lattice as a result of intercalation.[9] The changes in core- and valence-level electronic structure can provide information on this process and its consequences.[10]

Table I. Growth Conditions, Appearance, and c Spacing for $SnS_{2-x}Se_x$

compn	temp (T_1, T_2), °C	growth time, h	color	c spacing Å
SnS_2	685, 645	12	orange	5.928
$SnS_{1.79}Se_{0.21}$	670, 630	48	red	5.953
$SnS_{1.50}Se_{0.50}$	650, 610	48	dark red	6.008
$SnS_{0.72}Se_{1.28}$	620, 580	100	black	6.103
$SnS_{0.15}Se_{1.85}$	570, 530	50	black	6.136
$SnSe_2$	550, 510	72	black	6.141

The preparation of clean, undisturbed crystal surfaces can be achieved by cleavage of single crystals under ultrahigh vacuum (UHV) within the PES spectrometer. The synthesis of large single crystals (ca. 2 mm × 4 mm) of intercalated materials has often proved difficult. Given the poor kinetics of the general intercalation reaction,[3] the majority of intercalation reactions are only possible with finely powdered host compounds.

This paper describes first the preparation of the series of single crystals $SnS_{2-x}Se_x(CoCp_2)_{0.33}$ ($Cp = \eta^5$-C_5H_5, $0 < x < 2$), second their investigation using photoelectron spectroscopy, and third proposal of a qualitative impurity-band-model description of these materials. Cobaltocene intercalation into SnS_2 single crystals has previously been investigated by using X-ray photoelectron spectroscopy.[11] In addition, microcrystalline samples of $SnS_{2-x}Se_x$

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