the same as those in the solid state when ligand dynamics are unimportant in the solution.

Conclusions

The present work yielded the following results. (1) ^{113}Cd chemical shifts of cadmium salts are very sensitive to the counteranions and the concentration. However, no effect is observed on $Cd(en)_3^{2+}$, in which no en is replaced by the anions. Thus, the effects of the counteranion and the concentration are attributable to the coordination of the counteranion to the cadmium. (2) ^{113}Cd nucleus deshielding of Cd complexes increases with increasing pK_a of the ligand, i.e., Cd-ligard σ bonding and the progression of ligand substitution. For example, $Cd(4\text{-Me-py})_4^{2+}$ exhibits a downfield shift of 8.8 ppm/pK_a unit. (3) As the chelate ring size of cadmium chelate compounds decreases from eight to five members, the ^{113}C nucleus of the compounds becomes more deshielded due to the decrease of chelate ring strain. (4) The exchange rates of the cadmium complexes with imidazole and pyridine and its derivatives were sufficiently reduced by cooling of the nonaqueous solution, such as ethanol, and all ^{113}Cd resonances of several species at equilibrium state can be clearly observed separately. (5) The chemical shifts of the solution NMR spectra of cadmium complexes with im, en, bpy, phen, and the derivatives agree well with those of the solid NMR spectra, indicating that both structures are substantially the same.

Acknowledgment. Thanks are due to Dr. T. Fujito and K. Deguchi of JEOL Ltd. for solid-state NMR measurements. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, No. 59540406.

Registry No. $[Cd(en)_3](ClO_4)_2$, 100515-34-2; $[Cd(en)_3](NO_3)_2$, 56123-55-8; $[Cd(en)_3]SO_4$, 41305-53-7; $[Cd(en)_3]Cl_2$, 15613-78-2; $[Cd(en)_3]Br_2$, 56123-54-7; $[Cd(en)_3]I_2$, 92798-55-5; $Cd(en)_3^{2+}$, 18153-92-9; $Cd(tn)_3^{2+}$, 66862-16-6; $Cd(tmd)_3^{2+}$, 100515-37-5; $Cd(CH_3NH(CH_2)_2NHCH_3)_3^{2+}$, 88425-83-6; $Cd(5\text{-}NO_2\text{-phen})_3^{2+}$, 100570-89-6; $Cd(5\text{-Cl-phen})_3^{2+}$, 100515-35-3; $Cd(phen)_3^{2+}$, 30261-46-2; $Cd(5\text{-Me-phen})_3^{2+}$, 37662-38-7; $Cd(2,9\text{-}Me_2\text{-phen})_3^{2+}$, 100570-90-9; $Cd(4,7\text{-}Me_2\text{-phen})_3^{2+}$, 38614-63-0; $Cd(bpy)_3^{2+}$, 18475-59-7; $Cd(4,4'\text{-}Me_2\text{-bpy})_3^{2+}$, 100515-36-4; ^{113}Cd, 14336-66-4.

Supplementary Material Available: Table S-I, showing NMR chemical shifts of cadmium salts at 23 °C, and Figure S-1, showing the effects of concentrations of $Cd(ClO_4)_2$ and ClO_4^- on the ^{113}Cd chemical shifts (2 pages). Ordering information is given on any current masthead page.

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Carbon-13 NMR Study of the Binding of Nitroprusside and Hexacyanoferrate(II) to Aquocobalamin, Vitamin B_{12a}

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Received October 23, 1985

High-frequency ^{13}C NMR spectroscopy using nitroprusside 90% enriched in ^{13}C shows that nitroprusside forms discrete 1:1 and 1:2 complexes in solution with aquocobalamin (vitamin B_{12a}). No binding occurs with cyanocobalamin (vitamin B_{12}), and it is concluded that the complexes contain Fe-C-N-Co fragments, involving in the 1:1 complex the axial cyano ligand and in the 1:2 complex a trans pair of equatorial cyano ligands. Similar complexes are formed between hexacyanoferrate(II) and aquocobalamin (but not cyanocobalamin), showing that the nitrosyl ligand is not crucial for complex formation. Similar, but much weaker, complexes are formed between nitroprusside or hexacyanoferrate(II) and the simpler complex aquomethylcobaloxime.

Introduction

Sodium nitroprusside, $Na_2[Fe(CN)_5NO]\cdot 2H_2O$, is a powerful hypotensive agent that is widely used in the treatment of severe hypertension, in the management of myocardial infarction, and in the induction of surgical hypotension.[1] Although nitroprusside is a valuable drug, there have been many reports[2,3] that the nitroprusside ion is metabolized in red blood cells with rapid release of cyanide into the bloodstream. However our own work[4-6] has cast considerable doubt on a number of earlier reports: we have found no decomposition of nitroprusside in whole blood[6] but find on the other hand that, under the analytical conditions normally employed for the determination of cyanide,[7] ready liberation of cyanide occurs from the primary photoproduct[8] of nitroprusside, the labile d^5 aqua ion $[Fe(CN)_5H_2O]^{2-}$. Furthermore, the photolysis of nitroprusside is rapid under normal lighting conditions.

As a possible antidote to potential cyanide poisoning induced by the administration of nitroprusside, independent of whether the cyanide is derived from a metabolic or a photochemical process, the use of aquocobalamin has been suggested[9] (this material is often referred to as hydroxocobalamin; but its pK_a is 8.1, and hence at physiological pH, it is primarily in the aquo form[10]). While some reports indicate that the administration of aquocobalamin alongside nitroprusside reduces levels of free cyanide in both red cells and plasma,[3,9,11] others suggest that aquocobalamin raises free cyanide.[12]

Preliminary experiments[13] have shown that aquocobalamin significantly influences the pharmacokinetics of the hypotensive action of nitroprusside, suggesting the possibility that aquocobalamin, far from merely being an antidote[9] to potential cyanide liberation from nitroprusside, actually interacts with the nitro-

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0020-1669/86/1325-0970$01.50/0

prusside. Such interactions in solution are ideal for study using ^{13}C NMR spectroscopy.

In the present paper we report the results of an NMR study of the interactions of nitroprusside and related cyanoferrates with aquocobalamin and cyanocobalamin. We have demonstrated previously[14] that, by use of 90% ^{13}C enrichment, it is possible to characterize unambiguously a wide range of cyanoferrate species in solution with high-frequency NMR.

Experimental Section

NMR Spectra. All NMR spectra were recorded in the FT mode, at 25 °C, on the Bruker WH-360 spectrometer of the Science and Engineering regional NMR service at the University of Edinburgh. The ^{13}C spectra were recorded at 90.56 MHz relative to external Me_4Si by using spectral widths of 12–14 kHz, typically with 1000–2000 scans, and a delay of 1.38 s between pulses of 3 μs; the ^{59}Co spectra were recorded at 85.45 MHz relative to external $K_3[Co(CN)_6]$ by using a spectral width of 125 kHz, 14000–20000 scans, and pulses of 4 μs.

All NMR spectra involving cobalamins and those of hexacyanoferrate/cobaloxime mixtures were recorded on solutions made up in deuterated phosphate buffer (pD 7.2); spectra of nitroprusside/cobaloxime mixture were recorded, for reasons of solubility, in methanol. Concentrations varied depending upon the exact stoichiometries employed, but the cyanoferrate concentration was usually 5.4×10^{-3} mol dm^{-3} (approximately 1.5%). In no case was it found necessary to use any relaxation agent. Experimental uncertainties in spectral parameters are ±0.1 ppm in chemical shifts and ±0.2 Hz in coupling constants. All spectral assignments were checked by spectral simulation, involving summation of the spectra due to $[Fe(^{13}CN)_5NO]^{2-}$ and both isomers of $[Fe(^{12}CN)(^{13}CN)_4NO]^{2-}$.

$Na[^{13}CN]$ (90% enriched) was purchased from MSD Isotopes, Inc., and was used as received. Samples of $Na_4[Fe(^{13}CN)_6]$ and $Na_2[Fe(^{13}CN)_5NO]\cdot 2H_2O$ were prepared as described previously.[14] Samples of $CH_3Co(DH)_2H_2O$ (DH = dimethylglyoxime) were prepared by using published procedures.[15] Cyanocobalamin and aquocobalamin were purchased from Sigma Chemical Co. Ltd. and were used as received.

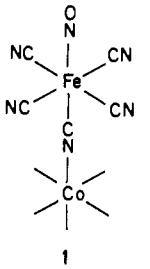
Results and Discussion

Reactions of Nitroprusside with Aquocobalamin. The nitroprusside ion has been shown by X-ray analysis to have C_{4v} symmetry,[16] and we have found[14] that when it is prepared from 90% enriched $Na[^{13}CN]$, the ^{13}C NMR spectrum is dominated by two isotopic species, $[Fe(^{13}CN)_5NO]^{2-}$ (59% abundant) and $[Fe(^{13}CN_{ax})(^{12}CN_{eq})(^{13}CN_{eq})_3NO]^{2-}$ (26.2% abundant). In the discussion that follows, we shall for the sake of convenience restrict attention solely to the species $[Fe(^{13}CN)_5NO]^{2-}$: all the spectra we observed contained contributions from both of the isotopic forms noted above, and the chemical shifts and coupling constants were independent of the isotopic form concerned, as found previously.[14] Hereafter in this paper, the term nitroprusside is taken to refer to the ion $[Fe(^{13}CN)_5NO]^{2-}$.

The ^{13}C NMR spectrum of the free nitroprusside ion is of AX_4 type, characterized in aqueous buffer by $\delta_A = +132.4$, $\delta_X = +134.4$, and $J_{AX} = 17.7$ Hz. When an equimolar quantity of aquocobalamin was added to nitroprusside, the spectrum was rapidly and completely replaced by a quite different AX_4 spectrum, characterized by $\delta_A = +143.3$, $\delta_X = +130.7$, and $J_{AX} = 18.9$ Hz, indicative of an intact pentacyanoferrate(II) species (species **1**) in a quite different environment. When a second molar equivalent of nitroprusside was added, the resulting spectrum was simply the summation of the two previous AX_4 spectra; there is no fast exchange on the NMR time scale between the two pentacyanoferrate species, $[Fe(CN)_5NO]^{2-}$ and **1**.

Upon addition of aquocobalamin to nitroprusside, the axial resonance, δ_X, moves to higher frequency by some 11 ppm, while the equatorial resonance, δ_X, moves to lower frequency by about 4 ppm. These changes are reminiscent of those observed[17,18] for the carbonyl resonances in Lewis acid adducts of polynuclear metal carbonyl species: the resonance of the carbonyl ligand that is coordinated at oxygen by the Lewis acid moves to much higher frequency, while the remaining carbonyl resonances generally move to slightly lower frequency.

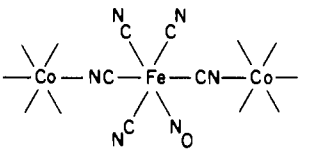
Consequently we assign to species **1** the constitution shown, where the axial cyano ligand of nitroprusside is coordinated via nitrogen to the cobalt(III) of the cobalamin.



1

The evidence that the binding site is at cobalt is 2-fold. First when cyanocobalamin, having no readily displaced axial ligand as in aquocobalamin, is added to nitroprusside even in mole ratios as high as Fe:Co = 1:4, the nitroprusside spectrum is wholly unperturbed. Second, although there are many potential sites in aquocobalamin at which the nitroprusside could be hydrogen-bonded, examination of the NMR spectra of mixtures of nitroprusside with a range of simple cobalt-free models, such as urea, malonamide, albumin, and polyasparagine, all showed that the spectrum was again entirely unaffected. The evidence that the binding of the nitroprusside is not specific to the nitrosyl ligand follows from a similar series of experiments using hexacyanoferrate(II) instead of nitroprusside, described below.

When nitroprusside is in molar excess over aquocobalamin, there is no exchange, on the NMR time scale, between free nitroprusside and bound nitroprusside. However, when a large excess of aquocobalamin was added to nitroprusside (molar ratio of Fe:Co ca. 1:5), the original AX_4 spectrum was replaced by a wholly new spectrum of exact AM_2X_2 type, characterized by $\delta_A = +123.6$, $\delta_M = +125.1$, and $\delta_X = +142.3$, with $J_{AM} = J_{AX} = 18.6$ Hz, and $J_{MX} = 13.0$ Hz. The A resonance is that of the unique axial cyano ligand, while the M and X resonances are those of two trans pairs of equatorial cyano ligands in the new species **2**. We assign to



2

species **2** the constitution shown, where the coordinated cyano ligands are those exhibiting the high-frequency shift of ca. 8 ppm, while the uncoordinated cyano ligands all exhibit a low-frequency shift (ca. 9 ppm for both equatorial and axial). The spectral data are summarized in Table I and show that the mean chemical shift moves to lower frequency on increased complexation by cobalamin. Again, the binding site is at cobalt, since a large molar excess of cyanocobalamin had no effect upon the spectrum of free nitroprusside.

When an additional $^1/_2$ molar equiv of aquocobalamin was added to preformed **1**, the spectrum was replaced by a summation of the spectra of **1** and **2**, indicating no fast exchange between **1** and **2**. Similarly, when approximately $^1/_2$ molar equiv of nitroprusside was added to preformed **2**, virtually all of the **2** was converted, within a few minutes, back to **1**, but by appropriate adjustment of the relative concentrations it is possible to obtain spectra comprising a summation of all three species, $[Fe(CN)_5NO]^{2-}$ and its 1:1 and 1:2 adducts with aquocobalamin, independent of one another (adducts described as *x*:*y* contain *x*Fe to *y*Co atoms, throughout this work). In none of these spectra is the line width perceptibly broadened in comparison with that observed for $[Fe(CN)_5NO]^{2-}$ alone, and hence we may place an approximate upper limit on the rates of any exchange processes involving $[Fe(CN)_5NO]^{2-}$, **1**, and **2** as 1 s^{-1}. However the ready

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Table I. Spectral Parameters for Complexes of $[Fe(CN)_5NO]^{2-}$ and $[Fe(CN)_6]^{4-}$ with Aquocobalamin (H_2O·Cb) and with $CH_3Co(DH)_2H_2O$ (H_2O·Cx)

		$\delta(^{13}C)$				coupling const/Hz		
complex	spectral type	A	M	X	mean	J_{AX}	J_{AM}	J_{MX}
$[Fe(CN)_5NO]^{2-}$	AX_4	132.4		134.4	134.0	17.7		
NP·Cb	AX_4	143.3		130.7	133.2	18.9		
NP·2Cb	AM_2X_2	123.6	125.1	142.3	131.7	18.6	18.6	13.0
$[Fe(CN)_6]^{4-}$	A_6	177.7			177.7			
$[Fe(CN)_6^{4-}$·Cb	*a*	174.1			174.1			
$[Fe(CN)_6]^{4-}$·2Cb	A_2X_4[b]	181.5		169.2	173.3	*b*		
$[Fe(CN)_5NO]^{2-}$[c]	AX_4	128.2		129.7	129.4	18.3		
NP·Cx[c]	AX_4	126.6		128.5	128.1	19.2		
$[Fe(CN)_6]^{4-}$·Cx	*a*	177.1			177.1			
$[Fe(CN)_6]^{4-}$·2Cx	A_2X_4[b]	178.5		173.9	174.8	*b*		

[a] See text. [b] No coupling resolved: see text. [c] Measured in methanol; all others in phosphate buffer.

conversion of **1** to **2** by addition of aquocobalamin and the reverse transformation by addition of further nitroprusside indicate reasonably mobile equilibria.

For binding in the 1:2 complex **2**, a pair of trans ligands must be employed, for steric reasons. That these are both cyano ligands provides a further indication that the nitrosyl ligand does not interact with the cobalt; perhaps the oxygen atom of the nitrosyl is insufficiently basic. Similarly, in the 1:1 complex **1**, the binding via the axial ligand suggests that the axial nitrogen is slightly more basic than the equatorial nitrogens. These two suggestions are both supported by EHMO calculations[19-21] on the isolated nitroprusside ion and on the isomeric adducts of nitroprusside with simple Lewis acids: the axial nitrogen is calculated both to be slightly more negatively charged (−1.06e vs. −1.05e) and to give a slightly more stable adduct with a proton than the equatorial nitrogens, whereas the oxygen is much less negatively charged (−0.50e) and gives an adduct with a proton of substantially higher energy.

Reaction of Hexacyanoferrate(II) with Aquocobalamin. The ^{13}C NMR spectrum of $[Fe(^{13}CN)_6]^{4-}$ in aqueous buffer consists of a singlet, having $\delta = +177.7$ and $\nu_{1/2} < 5$Hz: no additional absorptions were detected from $[Fe(^{12}CN)(^{13}CN)_5]^{4-}$ (present in 35.4% abundance when 90% ^{13}C enrichment is employed), and hence we conclude first that in $[Fe(^{12}CN)(^{13}CN)_5]^{4-}$ the ^{13}C nuclei cis and trans to ^{12}CN are effectively isochronous at 90.56 MHz, and second that the isotopic chemical shift between $[Fe(^{13}CN)_6]^{4-}$ and $[Fe(^{12}CN)(^{13}CN)_5]^{4-}$ is less than 0.05 ppm.

When an equimolar quantity of aquocobalamin was added to hexacyanoferrate(II), the spectrum contains two singlets of approximately equal intensity, one due to free $[Fe(^{13}CN)_6]^{4-}$ and a second having $\delta = +174.1$ and $\nu_{1/2} = 20$ Hz. When 2 molar equiv of the aquocobalamin was used, the resonance due to $[Fe(^{13}CN)_6]^{4-}$ was almost completely suppressed, that at $\delta = +174.1$ was still present, and two new resonances appeared at +169.2 and +181.5 ppm. At a 1:5 molar ratio, only the 169.2 and 181.5 ppm resonances remained. These last, in the approximate intensity ratio of 2:1, were both rather broad, unresolved multiplets from which no coupling data could be derived. When cyanocobalamin was used in place of aquocobalamin, no change was observed in the spectrum of $[Fe(CN)_6]^{4-}$ at any molar ratio.

While the spectra in the $[Fe(CN)_6]^{4-}$/aquocobalamin system do not allow definitive interpretation as for the nitroprusside analogues, it is clear that binding occurs and that this is prevented by blocking with cyanide the axial site at cobalt. Again it is probable that the new complexes contain Fe–C–N–Co fragments, but the absence of resolvable fine structure in the spectra does not allow positive identification of, for example, 1:1 or 1:2 complexes: if the tentative assignments of the two new species as 1:1 and 1:2 complexes are correct, then the ^{13}C chemical shifts of the cyano ligands (both the individual and the mean shift) exhibit the same trends upon complexation as those in the nitroprusside ion (see Table I).

It was noted earlier that although there was no exchange fast on the NMR time scale between free nitroprusside and its 1:1 and 1:2 complexes with cobalamin, the interconversion of these species upon change of the overall molar ratio of nitroprusside/aquocobalamin occurred readily within, at most, a few minutes. One possible interpretation of the observed spectra of the $[Fe(CN)_6]^{4-}$ complexes with cobalamin is that fluxional processes are now faster than in the $[Fe(CN)_5NO]^{2-}$ system. Fast site exchange, involving mobility of the cobalamin fragment over the six equivalent cyano ligands in $[Fe(CN)_6]^{4-}$ could account for the sharp singlet at δ +174.1, assigned to the 1:1 complex; similarly, the onset of site exchange in the 1:2 complex could account for the two broad unresolved absorptions, at $\delta = +169.2$ and +181.5, tentatively identified as the components of an A_2X_4 system. It must however, be emphasized that the occurrence of fluxionality in the $[Fe(CN)_6]^{4-}$ system is not proven and that the real significance of the $[Fe(CN)_6]^{4-}$ results is their demonstration that the nitrosyl ligand in nitroprusside is not crucial to the binding to cobalamin.

Reaction of Cyanide and Aquopentacyanoferrate(III) with Cobalamins. The reaction of cyanide (or hydrogen cyanide) with aquocobalamin at physiological pH is effectively irreversible, but fairly slow. For the formation of cyanocobalamin from aquocobalamin, Williams et al. reported[22] a formation constant, $K > 10^{12}$, but found, at concentrations of 2.5×10^{-5} mol dm^{-3} at pH 4.75, a half-life for cyanocobalamin formation of some $4^1/_2$ hours. A subsequent determination gave[23] $K = 1.2 \times 10^{14}$. The kinetics of the reactions of cyanide and hydrogen cyanide with aquocobalamin and cyanocobalamin were studied in great detail by Jencks;[10] although aquocobalamin reacts with cyanide to form both mono- and dicyano derivatives, the conjugate base, hydroxocobalamin, does not react at all.[24]

Consistent with the above, we observed by ^{13}C NMR spectroscopy that the reaction of [^{13}C]cyanide with aquocobalamin is slow. A freshly mixed solution, containing aquocobalamin and an excess of K[^{13}CN] in pH 7.2 buffer, showed a single ^{13}C resonance at +120.9 ppm, assignable to HCN. Subsequent rerecording of the spectrum of the cyanide/aquocobalamin solution over a period of several hours showed that the resonance at +120.9 ppm slowly decreased in intensity to be replaced by another resonance at +137.3 ppm, assignable[25] to dicyanocobalamin. We were unable to detect any chemical shift difference at 90.56 MHz between the two chemically distinct cyanide ligands, although the

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cyanide resonances for the two isomers, α and β, of aquocyanocobinamide exhibit a chemical shift difference of 1.1 ppm.

When the labile d^5 paramagnetic anion $[Fe(CN)_5H_2O]^{2-}$ was mixed with a 2-fold molar excess of aquocobalamin, no cyanide transfer to form cyano- or dicyanocobalamin was observed. The failure to observe any signal in the 137 ppm region cannot be ascribed to the paramagnetism of the aquapentacyanoferrate(III) ion since, although no resonance has been observed for this ion itself, control experiments on mixtures of aquapentacyanoferrate(III) and a range of diamagnetic cyanometalates showed that the spectra of these latter were not perturbed in any way by the paramagnetic ion, at least at the concentrations used in the present study.

Binding of Cyanoferrates to Other Cobalt Complexes. The binding of nitroprusside to cobalamin via a bridging cyano ligand Co–N–C–Fe is reminiscent of the intermediate isolated[26] from the redox reaction between $[Co(CN)_5]^{3-}$ and $[Fe(CN)_6]^{3-}$, of composition $[(NC)_5Co^{III}NCFe^{II}(CN)_5]^{6-}$: a similar intermediate, $[(edta)Co^{III}NCFe^{II}(CN)_5]^{5-}$ was subsequently detected[27] in the redox reaction of $[Co^{II}(edta)]^{2-}$ and $[Fe(CN)_6]^{3-}$. For an approximately square-pyramidal fragment $Co^{III}L_5$ containing spin-paired cobalt(III), both the crystal field model and the angular overlap model indicate that the LUMO is composed primarily of the cobalt d_{z^2} orbital, directed along the 4-fold symmetry axis. EHMO calculations[19-21] on the square-pyramidal $[Co^{III}(CN)_5]^{2-}$ fragment support this idea, although the LUMO is calculated to contain some admixture of $4p_z$ with $3d_{z^2}$.

Hence nucleophilic anions such as cyanoferrates should be expected to bind to other spin-paired Co(III) systems containing a readily displaceable water ligand. The ^{13}C NMR spectra of mixtures of either $[Fe(CN)_5NO]^{2-}$ or $[Fe(CN)_6]^{4-}$ with aquomethylcobaloxime $CH_3Co(DH)_2H_2O$ confirm this expectation, although the interaction is clearly much weaker than with aquocobalamin.

Spectra of the $[Fe(CN)_5NO]^{2-}$/cobaloxime system were all recorded in methanol as the solubility of the cobaloxime in phosphate buffer was very low. In methanol the spectrum of $[Fe(^{13}CN)_5NO]^{2-}$ is of AX_4 type, characterized by $\delta_A = +128.2$, $\delta_X = +129.7$, and $J_{AX} = 18.3$ Hz. Incremental addition of aliquots of $CH_3Co(DH)_2H_2O$ caused first the appearance of a second AX_4 spectrum having $\delta_A = +126.6$, $\delta_X = +128.5$, and $J_{AX} = 19.2$ Hz and eventually at high (>5:1) ratios of Co:Fe further very weak spectra, probably of the AM_2X_2 type. Even at such high ratios of Co:Fe, the majority of the nitroprusside is in the unbound form, indicative of very much weaker binding than occurs between nitroprusside and aquocobalamin. The new AX_4 spectrum is assigned, as previously, to a 1:1 adduct containing an Fe–C–N–Co bridge. Whereas for the aquocobalamin complexes the ^{13}C chemical shifts in the nitroprusside exhibit substantial changes upon complexation, the changes upon complexation to the cobaloxime are much smaller, again indicative of a much weaker interaction between the cobaloxime and nitroprusside than between the cobalamin and nitroprusside. Only weak indications were observed for the formation of a 1:2 complex.

As with the cobalamin complexes, the presence of the nitrosyl group in the nitroprusside ion is not crucial to the formation of complexes with $CH_3Co(DH)_2H_2O$. The spectrum of a 1:1 mixture of the cobaloxime and hexacyanoferrate(II) in phosphate buffer shows, in addition to uncomplexed $[Fe(^{13}CN)_6]^{4-}$ at $\delta = 177.7$, a second singlet having $\delta = +177.1$ of less than one-tenth the intensity of the original singlet. At high ratios (>5:1) of Co:Fe, two new weak resonances appeared, in an approximately 2:1 intensity ratio, at $\delta = +173.9$ and +178.5, respectively. If the new resonances represent 1:1 and 1:2 complexes, respectively (the absence of resolvable couplings prevents definitive interpretation), then the behavior of cyanoferrates with $CH_3Co(DH)_2H_2O$ is similar to that with aquocobalamin, except that the complexation is much weaker, (see Table I).

The ^{59}Co chemical shift of $CH_3Co(DH)_2H_2O$ in methanol was measured, relative to $K_3[Co(CN)_6]$, as +4270 ppm; when a 2-fold molar excess of $[Fe(CN)_5NO]^{2-}$ was added, this resonance was almost completely replaced by a new resonance at +3890 ppm, which from the ^{13}C spectra discussed above we assign to the 1:1 complex. We were unable to detect any ^{59}Co resonance in solutions either of aquocobalamin or of cyanocobalamin.

Concluding Remarks. We have shown that the nitroprusside anion binds to aquocobalamin, one of the proposed clinical antidotes to cyanide poisoning from nitroprusside metabolism, to form both 1:1 and 1:2 complexes: the nitrosyl ligand is not crucial to this complexing since hexacyanoferrate(II) also binds to aquocobalamin. In each case bridges of type Fe–C–N–Co are proposed. Such binding of cyanoferrates also occurs with the simple cobaloxime $CH_3Co(DH)_2H_2O$, but here the binding is very much weaker.

The formation of the 1:1 and 1:2 complexes reported here between nitroprusside and aquocobalamin has been observed at concentrations of nitroprusside somewhat higher than those (10^{-5} mol dm^{-3} or below) commonly used in clinical practice;[1] because of this, an investigation of the hypotensive behavior of these complexes is now in progress[13] and will be reported in due course.

Registry No. Cyanocobalamin, 68-19-9.

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