A Very Long Cobalt to Nitrogen Bond in a Coenzyme B_{12} Model. Relevance to the Role of the 5,6-Dimethylbenzimidazole in Co–C Bond Cleavage in Coenzyme B_{12}

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Abstract: The crystal and molecular structure of the complex *trans*-bis(dimethylglyoximato)isopropyl(2-aminopyridine)cobalt(III) is reported. The compound crystallizes in space group $P2_12_12_1$ with a = 27.83 (1) Å, b = 8.564 (6) Å, c = 8.198 (6) Å, V = 1958.1 Å^3, D_m = 1.45 g cm^{-3}, Z = 4, and D_c = 1.45 g cm^{-3}. A total of 2740 reflections were measured. The structure was solved with conventional Patterson and Fourier methods. Block-diagonal least-squares refinement led to a final R value of 0.033. The complex is pseudooctahedral with the dimethylglyoximato ligands occupying the four equatorial positions and the isopropyl ligand occupying one of the axial positions. The geometry of these ligands and their relationship to Co are very similar to those of the known pyridine analogue. *However*, the bond from Co to the endocyclic N of 2-aminopyridine is unusually long, namely 2.194 (4) Å. This long bond is believed to result from repulsion between the 2-amino group and the equatorial ligands. Another consequence of this interaction is the unsymmetrical nature of the Co–N–C angles, which are 129.7 (4)° for the 2-carbon and 115.7 (3)° for the 6-carbon. This angular distortion and the long Co–N bond are also found in coenzyme B_{12}. Rate studies with *trans*-alkylbis(dimethylglyoximato)(L)cobalt(III), in which 2-aminopyridine analogues (L) are exchanged by stronger ligands such as $P(OCH_3)_3$, can best be interpreted by the endocyclic binding mode of 2-aminopyridine in solution. In conjunction with literature on Co–C bond energies of related B_{12} models, the potential influence of the 5,6-dimethylbenzimidazole ligand in Co–C bond cleavage in coenzyme B_{12} dependent reactions is discussed.

Cobalt–carbon bond energies have recently been estimated for organocobalt complexes by both kinetic and equilibrium methods.[1-4] Using these bond energies combined with reasonable thermodynamic considerations, Halpern[1] has convincingly argued that the Co–C bond in coenzyme B_{12} (deoxyadenosylcobalamin) is relatively weak and only a small additional decrease in bond energy is needed to account for the rate of Co–C homolytic bond cleavage in enzymic processes dependent on coenzyme B_{12}.

Various spectroscopic and rate studies, which have often employed coenzyme analogues, have demonstrated that conformational changes occur in both the protein and the coenzyme, particularly on addition of substrate or substrate analogues to the holoenzyme.[5-9] This conformational change is generally thought to be associated with the Co–C bond cleavage process. Considerable speculation exists[10] as to the nature of the structural change, with emphasis having been placed on Co–C bond length, Co–C–C bond angle, and corrin ring conformational changes. The primary focus of most hypotheses has been the alkyl ligand (deoxyadenosyl) and its cis steric interaction with the equatorial macrocyclic corrin ring. The role of the 5,6-dimethylbenzimidazole in the sixth coordination position (trans to the deoxyadenosyl ligand) has been considered primarily in terms of its cis steric interaction with the corrin. The deformations thus induced in the corrin could lead to increased repulsive interactions between the corrin and the deoxyadenosyl moiety. We have termed this type of transmission of steric effects as a trans steric effect.

Very recently, Ng, Rempel, and Halpern[3] have established that Co–C bond energies can be substantially influenced by *electronic* effects of the ligand trans to the alkyl group in complexes of the type *trans*-4XpyCo(DH)$_2$CH(CH$_3$)C$_6$H$_5$, where DH = monoanion of dimethylglyoxime (bis(dioximato) complexes of this type are trivially named cobaloximes) and 4Xpy = pyridine or a 4-substituted pyridine. Since the 4-substituent is directed away from the Co, changes in X should involve minimal steric changes in the vicinity of the Co. These investigators also mentioned preliminary results for complexes containing 2-substituted pyridines, in which weaker than expected Co–C bond energies appeared likely.

We have been interested for some time in the structural properties of organocobalt complexes relevant to B_{12} biochemistry and, in particular, we have studied a number of sterically strained systems.[11-14] In view of the new observations from Halpern's laboratory on Co–C bond energies, an evaluation of the structural properties of 2-substituted pyridine–organocobalt complexes seemed merited. We therefore have prepared a number of these complexes, primarily with 2-aminopyridine (2NH$_2$py), and have structurally characterized 2NH$_2$pyCo(DH)$_2$-*i*-C$_3$H$_7$. This compound is remarkable in having a very long Co–N bond. This finding, in conjunction with the recent estimates of Co–C bond energies,[1-4] allows new insights into the relationship between coenzyme B_{12} structure and Co–C bond cleavage.

Experimental Section

Reagents. All reagents were from Aldrich except as noted. Tributylphosphine, trimethyl phosphite, 2-picoline, *N*-methylaniline, and aniline (Fisher) were distilled under vacuum before use. 4-Cyanopyridine, 2-aminopyridine, 3-aminopyridine, and 2-(benzylamino)pyridine

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Table I. Positional Parameters (X10⁴) of Non-Hydrogen Atoms of $2NH_2pyCo(DH)_2$-*i*-C_3H_7

atom	x	y	z
Co	1176 (0)	1875 (1)	104 (1)
O(1)	1909 (2)	4 (4)	−1390 (6)
O(2)	704 (2)	4550 (5)	−1187 (6)
O(3)	413 (2)	3659 (5)	1580 (6)
O(4)	1622 (1)	−878 (4)	1374 (5)
N(1)	1618 (2)	1289 (5)	−1536 (5)
N(2)	1043 (2)	3445 (5)	−1454 (5)
N(3)	714 (1)	2403 (5)	1697 (6)
N(4)	1289 (1)	234 (4)	1614 (5)
N(5)	1744 (2)	3279 (4)	1232 (5)
N(6)	1366 (2)	5740 (5)	1260 (8)
C(1)	1961 (3)	1817 (10)	−4263 (8)
C(2)	1635 (2)	2145 (7)	−2840 (7)
C(3)	1296 (2)	3435 (7)	−2784 (7)
C(4)	1240 (3)	4650 (9)	−4110 (9)
C(5)	302 (2)	1714 (8)	4279 (8)
C(6)	671 (2)	1488 (6)	2953 (6)
C(7)	1011 (2)	194 (6)	2900 (6)
C(8)	1044 (3)	−1069 (7)	4188 (8)
C(9)	681 (2)	515 (7)	−1192 (7)
C(10)	758 (3)	−1226 (7)	−1045 (8)
C(11)	164 (2)	932 (8)	−842 (9)
C(12)	2164 (2)	2499 (6)	1570 (7)
C(13)	2561 (2)	3126 (8)	2258 (7)
C(14)	2551 (2)	4688 (7)	2758 (8)
C(15)	2138 (2)	5523 (7)	2435 (8)
C(16)	1749 (2)	4822 (6)	1612 (7)

were treated with activated charcoal in CH_2Cl_2 and crystallized by the addition of petroleum ether. 2-(Methylamino)pyridine was recrystallized three times from the neat liquid at −5 °C. Reagent grade CH_2Cl_2 was used as the solvent in the rate determinations. All other materials were reagent grade and were used without further purification.

Instrumentation. The ligand exchange rates were monitored by using a Cary 14 spectrophotometer for the slow reactions ($k_{obsd} < 1.0\ s^{-1}$) and a Durrum-Gibson D-110 stopped-flow spectrophotometer for the fast reactions and competition ratio studies. Both instruments were equipped with thermostated compartments that maintained the reaction solution at 25.0 ± 0.04 °C. ^1H NMR spectral measurements were made on a Varian EM 390 spectrometer operating at 90 MHz.

Rate Measurements. The optimum wavelengths used to monitor the reaction rates were determined as described previously.[15] Suitable wavelengths were in the range 490–560 nm for the complexes studied. Neither the products nor the reactants exhibited photoinstability over the time period required for the measurements. Absorbance data were continually collected over at least 3 half-lives, with the final absorbance taken after ca. 10 half-lives.

Data Analysis. The experimental absorbance vs. time rate data were treated with the standard integrated expression for a first-order process using linear least-squares analysis.

The pseudo-first-order rate constants obtained from the slope of the least-squares fit of the raw data were then plotted as a $1/k_{obsd}$ vs. [L]/[L'] by a linear least-squares regression analysis (L = leaving and L' = entering ligands).[15] For systems undergoing reaction by an S_N1 LIM mechanism and exhibiting mass-law retardation, this analysis yields $1/k_1$ as the y intercept and k_{-1}/k_1k_2 as the slope. The rate constants are defined as follows:

$$ML \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M + L$$

$$M + L' \xrightarrow{k_2} ML'$$

Preparations: $2NH_2pyCo(DH)_2X$ (X = CH_3, CH_2CH_3, *i*-C_3H_7, *i*-C_4H_9, neo-C_5H_{11}, CH_2Br, CH_2CF_3). A mixture of finely ground $H_2OCo(DH)_2X$ (~0.3 g) and $2NH_2py$ (50% molar excess) in CH_2Cl_2 (20 mL) was stirred for 30–45 min. (The complex with X = CH_3 precipitated from the reaction mixture at this point. It was collected and washed as below for the other complexes.) The resulting solution was filtered and taken to dryness on a rotary evaporator. The residue was collected and washed with petroleum ether and ~5 mL of absolute diethyl ether. Compounds containing L = $PhNH_2$, $PhNHCH_3$, $2CH_3NHpy$, 2BzNHpy, $2CH_3py$, and $3NH_2py$ were prepared similarly.

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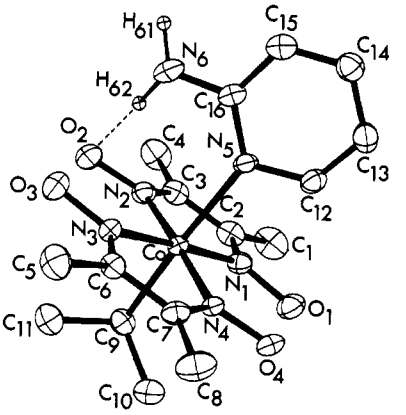

Figure 1. ORTEP drawing and numbering scheme for $2NH_2pyCo(DH)_2$-*i*-C_3H_7. The intramolecular hydrogen bonding is indicated by a broken line.

Table II. Selected Bond Lengths and Angles of $2NH_2pyCo(DH)_2$-*i*-C_3H_7

Bond Lengths, Å			
Co-N(1)	1.893 (5)	C(9)-C(10)	1.512 (8)
Co-N(2)	1.892 (4)	C(9)-C(11)	1.515 (9)
Co-N(3)	1.890 (4)	N(5)-C(12)	1.379 (7)
Co-N(4)	1.901 (4)	C(16)-N(5)	1.359 (6)
Co-N(5)	2.194 (4)	C(16)-N(6)	1.358 (8)
Co-C(9)	2.097 (6)		
Bond Angles, deg			
N(1)-Co-N(2)	80.6 (2)	N(4)-Co-N(5)	90.7 (2)
N(1)-Co-N(3)	177.5 (2)	N(4)-Co-C(9)	91.6 (2)
N(1)-Co-N(4)	99.1 (2)	N(5)-Co-C(9)	173.9 (2)
N(1)-Co-C(9)	85.5 (2)	Co-C(9)-C(10)	114.5 (4)
N(1)-Co-N(5)	88.5 (2)	Co-C(9)-C(11)	113.6 (4)
N(2)-Co-N(3)	99.4 (2)	C(10)-C(9)-C(11)	110.7 (5)
N(2)-Co-N(4)	177.3 (2)	C(15)-C(16)-N(6)	117.4 (5)
N(2)-Co-N(5)	92.1 (2)	N(5)-C(16)-N(6)	120.5 (5)
N(2)-Co-C(9)	85.7 (2)	Co-N(5)-C(12)	115.7 (3)
N(3)-Co-N(4)	80.9 (2)	Co-N(5)-C(16)	129.7 (4)
N(3)-Co-N(5)	94.0 (2)	C(12)-N(5)-C(16)	114.6 (4)
N(3)-Co-C(9)	92.0 (2)		

Yields: >50%. The complexes all gave satisfactory elemental analyses (Atlantic Microlabs, Table SVI).

Crystal Data. Crystals of $2NH_2pyCo(DH)_2$-*i*-C_3H_7 were obtained from an acetone/water mixture at 0–5 °C: $C_{16}H_{27}N_6O_4Co$, M_r = 426.4, orthorhombic, space group $P2_12_12_1$, a = 27.83 (1) Å, b = 8.564 (6) Å, c = 8.198 (6) Å, V = 1958.1 Å^3, D_m = 1.45 g/cm^{-3}, Z = 4, D_c = 1.45 g cm^{-3}, and λ(Mo Kα) = 0.7107 Å. Intensity data were collected on a Siemens AED computer-controlled diffractometer with θ–2θ scan technique up to θ = 28°. One standard reflection, measured every 100 reflections, did not show any significant variation throughout all the data collection. A total of 2740 reflections was collected, 1577 of which were independent and had $I > 3\sigma(I)$. The latter were corrected for Lorentz and polarization factors but not for absorption and used in the subsequent calculations ($0.03 < \mu_{cryst} < 0.04$ cm). The structure was solved by conventional Patterson and Fourier methods and refined by block-diagonal anisotropic least-squares methods to a final R value of 0.033. The contribution of the hydrogen atoms, held constant (B = 5 Å^2), was included in the final refinement. The final weighting scheme was $w = 1/(A + |F_o| + B|F_o|^2)$ where A = 12.6 and B = 0.016 were chosen so as to maintain $w(|F_o| - |F_c|)^2$ essentially constant over all ranges of $|F_o|$ and $(\sin\theta)/\lambda$. Atomic scattering factors were those given in ref 16. All the calculations were done by using the computer programs from X-Ray 70.[17] Final positional parameters are given in Table I for non-hydrogen atoms and supplementary table, SI, for hydrogen atoms.

Results

Description of the Structure. The numbering scheme of the atoms is given in Figure 1. The bond lengths and angles are given in Tables II and SII. The $2NH_2py$ ligand coordinates to cobalt through the N atom of the pyridine ring, occupying an axial

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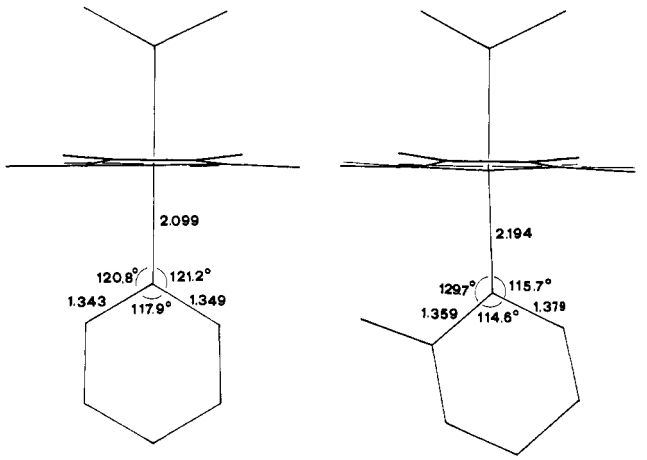

Figure 2. Side view of $2NH_2pyCo(DH)_2$-*i*-C_3H_7 (right) and of pyCo-$(DH)_2$-*i*-C_3H_7 (left) that permits comparison of bond lengths and bond angles of the neutral axial ligand. Esd's for the $2NH_2py$ and py compounds, respectively: 0.004 and 0.002 Å (Co–N bond); 0.006 and 0.004 Å (C–N bond); and 0.4° and 0.2° (bond angles).

position. The $Co(DH)_2$ unit appears to be relatively unaffected by steric strain, with normal bond lengths and angles that are very similar to those found for $pyCo(DH)_2CH_3$[18] and $pyCo(DH)_2$-*i*-C_3H_7.[12] The plane of the N(1), N(2) DH unit is nearly parallel to the plane of the equatorial N donors (coplanar within ±0.002 Å). The dihedral angle is 1.2°. The plane of the N(3), N(4) unit makes angles of 5.2° and 4.2°, respectively, with the above planes. The bending is toward the isopropyl group. The cobalt atom is displaced 0.044 Å from the N-donor plane toward the $2NH_2py$ ligand. The above values do not differ appreciably from those found in the py analogue.[12]

The $2NH_2pyCO$-*i*-C_3H_7 fragment is characterized by very long Co–C and Co–N bond lengths, which are 2.097 (6) and 2.194 (4) Å, respectively, with a C–Co–N angle of 173.9 (2)°. Thus the C(9) and N(5) donors are pushed away from N(3) and N(4), whereas the C(10) and C(11) atoms are above this side of the equatorial ligands in a manner analogous to that found in the py analogue.[12] The Co–N axial bond is ~0.1 Å longer than that found in the latter compound and is the longest bond so far reported for a vitamin B_{12} model. Correspondingly the $2NH_2py$ ligand is highly symmetric, with Co–N(5)–C(12) and Co–N-(5)–C(16) angles of 115.7 (3)° and 129.7 (4)°, respectively. As a consequence, the $2NH_2py$ ring is bent toward the side of O(1) and O(4) (Figure 2) in the plane normal to the 4-N equatorial donors. Such an asymmetry in the Co–N–C bond angles has already been found when xanthinato[7] and 3-benzyladenine[6] coordinate the $Co(DH)_2$ moiety, the angles being 132.9 (6)° and 124.5 (5)° in the former and 141.3 (5)° and 116.9 (5)° in the latter. The plane passing through the $2NH_2py$ atoms (coplanar within ±0.04 Å) makes a dihedral angle of 90° with the 4-N equatorial plane. The $2NH_2py$ ring is slightly rotated around the Co–N(5) bond toward the O(2) side, probably because of the hydrogen bond between O(2) and N(6) (see below). Furthermore, the bond lengths and angles involving N(5) appear slightly, but significantly, different from those reported for $pyCo(DH)_2$-*i*-C_3H_7. They are compared in Figure 2. The *i*-C_3H_7 group has C–Me bond lengths of 1.515 (9) and 1.512 (8) Å and a Me–C–Me angle of 110.7 (5)°, whereas the sum of the angles at C(9) is 338.2°. These results, which do not differ from those found in the py analogue, support the suggestion[12] that there is a flattening of the tetrahedron of C(9) with a consequent change in the hybridization of C(9) upon coordination.

The two acidic protons of the $(DH)_2$ ligands are found to be asymmetrically located between the dioxime oxygen atoms, each attached to one of the two DH ligands, namely to O(3) and O(1) atoms, respectively, whereas the Co–N (equatorial) bond lengths are very similar. These findings support[20] the formulation of the equatorial ligand as two monoanions. A hydrogen bond of 2.911 (7) Å is found between N(6) and O(2), one hydrogen of the NH_2 group being located nearly along the N(6)···O(2) direction. A longer hydrogen bond (2.988 (6) Å) may be hypothesized between the N(6) and O(4) atoms of the molecule at $x = 0$, $y = 1$, and $z = 0$. We note that the oxime oxygen atoms involved in hydrogen bonds do not bear the bridging H atoms.

Table III. Summary of Observed First-Order Rate Constants for the Axial Ligand Substitution of L from $LCo(DH)_2R$ by PBu_3[a]

R	$k_1(2NH_2py)$, s^{-1}	$k_1(4CNpy)$, s^{-1}	$k_1(2NH_2py)/k_1(4CNpy)$
CH_2CF_3	$2.4 \pm 0.1 \times 10^{-2}$	$2.7 \pm 0.2 \times 10^{-4}$	89
CH_2Br	$1.5 \pm 0.1 \times 10^{-1}$	$2.6 \pm 0.1 \times 10^{-3}$	58
CH_3	3.5 ± 0.1	$4.1 \pm 0.3 \times 10^{-2}$	81
C_2H_5	$5.8 \pm 0.3 \times 10^{1}$	$9.6 \pm 0.1 \times 10^{-1}$	60
i-C_4H_9	$1.0 \pm 0.1 \times 10^{2}$	1.4 ± 0.1	76

[a] Mean values ± standard deviations for at least three runs. PBu_3, 0.1 M; Co, ~0.01 M; 25 °C; CH_2Cl_2.

Table IV. Summary of Observed First-Order Rate Constants and Competition Ratios for the Axial Ligand Substitution of L from $LCo(DH)_2R$ by $P(OCH_3)_3$[a]

L	R	k_1, s^{-1}	k_{-1}/k_2	CR[b]
$2NH_2Py$	C_2H_5	$7.2 \pm 0.3 \times 10^{1}$	3.2	.31
$2CH_3NHPy$	C_2H_5	$7.7 \pm 0.3 \times 10^{1}$	*c*	
2BzNHPy	C_2H_5	$8.8 \pm 0.1 \times 10^{1}$	0.7[c]	1.4
$PhNH_2$	C_2H_5	$3.3 \pm 0.1 \times 10^{1}$	3.1	0.32
$PhNHCH_3$	C_2H_5	*d*		
$PhNHCH_3$	CH_2CF_3	$2.2 \pm 0.2 \times 10^{-1}$	1.8	0.56
$PhNH_2$	CH_2CF_3	$5.6 \pm 0.1 \times 10^{-3}$		
4CNPy	C_2H_5	$9.6 \pm 0.1 \times 10^{-1}$	1.0	1.0
$2CH_3Py$	CH_3	$1.5 \pm 0.2 \times 10^{2}$	0.8	1.3
$2NH_2Py$	*i*-C_4H_9	$1.0 \pm 0.1 \times 10^{2}$	3.2	0.31
$3NH_2Py$	C_2H_5		2.0[f]	0.50

[a] CH_2Cl_2, 25 °C. The rate constants and error limits are the mean values ± standard deviations for at least three runs with pseudo-first-order conditions of $P(OCH_3)_3$ (0.1 M) and cobaloxime (~0.01 M). The competition ratios were determined by least-squares analysis of rate data obtained under mass-law rate retardation conditions (0.01 M cobaloxime, 0.10 M $P(OCH_3)_3$, 0–0.5 M leaving ligand). [b] CR is the competition ratio expressed such that ligands which compete better than $P(OCH_3)_3$ will have ratios less than 1, in accord with previous studies.[15,21] [c] For $2CH_3NHpy$ and 2BzNHpy plots of $1/k_{obsd}$ is $[L]/[P(OCH_3)_3]$ exhibited upward curvature up to values of ~1 for this ratio. This curvature was approximately within the error limits for 2BzNHPy. [d] Too rapid for stopped-flow measurement. [e] This rate constant was obtained by extrapolation of rate data obtained under mass-law rate retardation conditions using excess $2CH_3Py$. [f] Obtained by displacement of 4CNPy by $3NH_2Py$ under mass-law rate retardation conditions using excess amounts of 4CNPy. The CR for CNpy is 1.

Ligand Exchange Rates. Since the Co–N bond found in $2NH_2pyCo(DH)_2$-*i*-C_3H_7 is so long and since the bonding mode of the $2NH_2py$ ligand in solution could be different than that in the solid, we decided to investigate the ligand exchange rates of $2NH_2py$ cobaloximes and determine the influence of a bulky group at the 2-position on the competition ratio. We have previously established that ligand exchange rates of cobaloximes proceed by an S_N1 LIM mechanism (see Experimental Section) and that the five-coordinate intermediate formed is "hot" and reacts almost nondiscriminately with incoming ligands; i.e., the competition ratio, k_{-1}/k_2, is ~1.[15,21] Bulky P-donor ligands are less effective competitors.[11] Since in the past this ratio was reported so that poor competitors had values greater than 1, we will continue this convention here.

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Table V. Comparison of the Geometry of the C-Co-N(axial) Grouping in $LCo(DH)_2R$ Complexes

L	R	Co-N-(axial), Å	Co-C, Å	L-Co-R, deg	d_{Co},[a] Å	α,[b] deg
py	N_3[c]	1.973 (5)	...	177.3 (2)	...	2.9
py	CH_3[d]	2.068 (3)	1.998 (5)	178.0 (2)	0.040	3.2
py	i-C_3H_7[e]	2.099 (2)	2.085 (3)	175.4 (1)	0.022	4.0
$2NH_2py$	i-C_3H_7[f]	2.194 (4)	2.097 (6)	173.9 (2)	0.044	5.2
NH_3	Cl[g]	1.965 (4)	...		0.005	3.7
$PhNH_2$	Cl[h]	2.019 (2)	...	178.4 (2)	...	...
$PhNH_2$	SO_3[i]	2.117 (7)	...	177.9 (4)	0.021	7.0

[a] Displacement of cobalt from the 4-N equatorial atoms. [b] Dihedral angle between the DH units. [c] Reference 23. [d] Reference 18. [e] Reference 12. [f] Present work. [g] Reference 24. [h] Reference 25. [i] Reference 26.

In our previous studies, we found that bulky P-donor ligands were particularly good leaving groups. For example, $(c\text{-}C_6H_{11})_3P$ is a very good leaving ligand whereas the small ligand of similar basicity, $(CH_3)_3P$, is a very poor leaving ligand.[11] Ligand exchange rates for $2NH_2py$, $pK_a \sim 6.7$, exceed those for 4CNpy, $pK_a \sim 1.9$, by a factor of ~50–100 (Table III). We attribute this increased reactivity to the steric effect of the amino group. Similarly, $2CH_3py$, which has an even bulkier axial ligand and which is incapable of H bonding to the equatorial ligand, is very reactive and is one of the best leaving groups we have studied thus far (Table IV). A comparison of the relative ligand exchange rates of $2NH_2py$ and 4CNpy progressing along the increasing trans effect series CH_2CF_3, CH_2Br, CH_3, C_2H_5, and i-C_4H_9 gives values 89, 58, 81, 60, and 76, respectively (Table III). Unfortunately, the i-C_3H_7 derivative was too reactive for us to determine ligand exchange rates by our procedures.

In contrast to our results with bulky phosphine ligands, the bulky $2NH_2py$ ligand was found to be a better competitor (CR <1) than nonsterically hindered ligands such as 4CNpy and $(CH_3O)_3P$ (Table IV). To assess the factors that may be contributing to this atypical result, we evaluated the competition ratio for ligands related to $2NH_2py$ (Table IV).

Discussion

Structure. The most interesting aspect of the structural determination of $2NH_2pyCo(DH)_2$-i-C_3H_7 is the long axial Co–N bond. The value of 2.194 (4) Å is not far from that of 2.23 Å[10] reported for the B_{12} coenzyme, and it is almost 0.1 Å longer than that of 2.099 (2) Å reported for $pyCo(DH)_2$-i-C_3H_7.[12] The relevance of this finding to B_{12} biochemistry will be discussed below.

The geometry of the C–Co–N(axial) group of $2NH_2pyCo(DH)_2$-i-C_3H_7 is compared with those in $pyCo(DH)_2X$ (X = N_3, CH_3, i-C_3H_7) in Table V. The Co–N(py) bond length increases by about 0.1 Å with increasing electronic trans influence of X, as follows: $N_3 << CH_3 < i$-C_3H_7. On the other hand, substitution of py by the bulkier $2NH_2py$ leads to a further increase of 0.1 Å. Since the electronic properties of the two pyridine ligands are similar, we believe that the further increase in Co–N bond length is actually due to the steric cis influence[11,22] of the $(DH)_2$ moiety on the $2NH_2py$ axial ligand that protrudes its 2-amino group into the equatorial ligand. This conclusion is also supported by the asymmetric bonding of the $2NH_2py$ ligand to cobalt and by variations in geometry of this ligand (see Results section).

The argument that the Co–N lengthening due to the steric cis influence appears to add to the lengthening due to the electronic trans influence has already been made for cobaloximes containing phosphine ligands.[11,22] Comparison of structural data available for other cobaloximes containing amine ligands, shown in Table V, also supports this hypothesis. In fact, the axial Co–N distance of 1.965 (4) Å in $NH_3Co(DH)_2Cl$[24] lengthens to 2.019 (2) Å in $PhNH_2Co(DH)_2Cl$[25] (steric cis influence) and 2.111 (7) Å in $[PhNH_2Co(DH)_2SO_3]$[26] (electronic trans influence). In addition, the Co–py distance is longer than the Co–NH_3 distance when py and NH_3 are both trans to weak trans-influencing ligands. This difference suggests that py has an appreciable bulk, since a shorter distance should be expected in the sp^2 py derivative than in sp^3 NH_3. In view of these considerations, two questions arise: First, why does $2NH_2py$ bind by the sterically more hindered endocyclic N rather than the less hindered exocyclic amino group? Second, how is $2NH_2py$ bound in solution? These questions are addressed in the next subsection.

The increased bulk of $2NH_2py$, as compared with that of py, does not appreciably affect the trans Co–C bond length. Also, despite the increasing bulk of the axial ligands along the series of Table IV, the geometry of the $Co(DH)_2$ unit remains relatively unaffected since the α and d values are very similar. Therefore, it appears that the steric repulsions between the equatorial and the axial ligands lead to the lengthening of the axial bonds and to distortions within the axial ligands.

Solution Studies. Although the solid-state structure of $2NH_2pyCo(DH)_2$-i-C_3H_7 is intriguing, this study was undertaken to seek a structural basis for the very weak Co–C bonds in ortho-substituted pyridine cobaloximes in solution.[1] Because of the potential ambidentate binding ability of $2NH_2py$, we wished to know the bonding mode in solution. We approached this problem using (a) NMR spectroscopy, (b) relative ligand-exchange rate studies on a series of $2NH_2pyCo(DH)_2X$ complexes, (c) evaluation of competition ratios for $2NH_2py$ and related ligands, and (d) evaluation of the exchange rates for $2NH_2py$ analogues.

We will not discuss the reuslts of our NMR spectroscopic studies (summarized in Table SV). The $2NH_2py$ is unsymmetrical and has a complex ^1H NMR spectrum. From a careful comparison of ^1H NMR shifts for complexes of this ligand with those of pyridine and aniline analogues, we concluded the differences in chemical shifts were insufficient to assign a bonding mode.

The relative reactivity (Table III) of the $2NH_2py$ and 4CNpy complexes did not change with the trans effect of the alkyl group. For the endocyclic bonding mode, one might have anticipated that as the trans influence of alkyl group (which parallels the trans effect) decreased, repulsion between the $2NH_2py$ and the equatorial dioximes would have increased, thereby leading to a higher relative rate of $2NH_2py$ dissociation for complexes with the poorer trans-effect alkyl groups. Although our series covered a range of $\sim 10^4$, we may not have reached the point where the relative rates would be affected. Alternatively, the $2NH_2py$ could be bound in solution via the $2NH_2$ group, in which case the results observed would have been expected.

In past studies, sterically hindered ligands, regardless of basicity, were found to be poor competitors (CR >1) for the putative five-coordinate intermediate in ligand exchange reactions of cobaloximes in noncoordinating solvents.[11,15,21] Nonhindered ligands were equally effective competitors (CR ~ 1) regardless of basicity. The endocyclic binding site of $2NH_2py$ is sterically hindered, and a CR >1 would be expected. Alternatively, the amino group is not hindered, and a CR ~1 would be expected. However, neither result was obtained. The CR for $2NH_2py$ was ~0.3 (Table IV). After examination of CR for a number of ligands containing NH groups, it became clear that, with a few exceptions, the values of CR <1 are characteristic of ligands with this group (Table IV). The origin of this low value of CR is not obvious and may relate to H bonding. However, the plots of $1/k_{obsd}$ vs. [L]/[L'] were typically linear and provided no evidence for any H-bonded species.[27]

Although all of the above studies of species in solution can equally well be interpreted via either binding mode, we believe that relative exchange rates of $2NH_2py$ analogues provide very

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strong evidence for endocyclic nitrogen coordination. From an examination of the ORTEP drawing in Figure 1, it is clear that an alkyl substituent in the amino group would be directed away from the equatorial plane and the remaining H or N could hydrogen bond to an oxime O. Except for the R group, the structures of $2NH_2$py and 2RNHpy cobaloximes should be nearly identical. Therefore, complexes of 2RNHpy ligands with endocyclic N-coordination would not be expected to have steric acceleration of the first (dissociative) step in the ligand exchange reaction. Additionally, the inductive effect of R on the basicity of the pyridine N would be slight. Therefore, for an endocyclic N-binding mode, $k_{obsd}(k_1)$ for ligand exchange should be very similar for 2RNHpy and $2NH_2$py for a given alkylcobaloxime. Indeed, for ethylcobaloximes, $k_{obsd}(k_1)$ for the $2NH_2$py and $2CH_3NH$py complexes were identical within experimental error (Table IV), and the k_{obsd} for the 2BzNHpy complex was only slightly greater. Aniline and *N*-methylaniline can coordinate only via an exocyclic N. Therefore, the consequences of alkyl group substitution on an exocyclic amino group can be determined. Aniline itself is a slightly worse leaving ligand than $2NH_2$py. *N*-Methylaniline is a much better leaving ligand than either aniline or $2NH_2$py. For ethylcobaloximes, *N*-methylaniline is too good a leaving ligand to be evaluated by our techniques but for F_3CCH_2 cobaloximes, it is almost 25 times better as a leaving ligand than aniline. Thus, we believe the similar rates for the 2RNHpy and $2NH_2$py cobaloximes can be rationalized reasonably only by involving endocyclic N-coordination.

For methylcobaloximes, the k_1 for py is $\sim 10^{-2}$ s^{-1}. Substitution of pyridine with an electron-withdrawing group (CN) at the 4-position increases k_1 by a factor of 4 to 5, whereas substitution with electron-donating groups at the 2-position increases k_1 by $\sim$350 times ($2NH_2$) and $\sim$1500 times ($2CH_3$) (Tables III and IV). Although we have been unsuccessful in obtaining high quality cobaloxime crystals with $2CH_3$py, this ligand must bind via the endocyclic N. The higher value of k_1 for $2NH_2$py is thus in the range expected for endocyclic N-coordination since the increase in k_1 must have a steric origin. The NH_2 group is a slightly smaller group than CH_3,[28] and the bulk of the NH_2 group is somewhat offset by H bonding to the oxime oxygens.

The ligand $3NH_2$py was too poor a leaving group for rate studies since it was not displaced completely by such good ligands as $P(OCH_3)_3$ and 1-methylimidazole. This ligand certainly binds via the pyridine N since the pyridine N is not hindered and is more basic than pyridine, whereas the amino group is less basic than aniline.

Pyridine ligands with a $2NH_2$ group and a substituent at the 6-position formed complexes that were too unstable for isolation. However, in situ preparation from the appropriate aquocobaloxime indicated, for example, that $2NH_2$,$6CH_3$py was $\sim$7 times better as a leaving ligand than $2NH_2$py. If both $2NH_2$py and $2NH_2$,$6CH_3$py are bound via the NH_2 group, then one expects that these ligands would have roughly equal leaving ability since the CH_3 is meta to the amino group and will not sterically favor dissociation.

In summary, none of the data would be best explained by invoking amino group binding by $2NH_2$py. Where clear differences in reactivity were seen, these differences strongly supported predominant endocyclic N-coordination of $2NH_2$py in solution, in agreement with the solid-state results. The results reported here do not rule out a fluxional process in which there is an equilibrium between the endocyclic N and the $2NH_2$ group binding for $2NH_2$py complexes.

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Relevance to B_{12} Biochemistry. In the introduction, we briefly reviewed evidence that conformational changes of B_{12} holoenzymes facilitate Co–C bond cleavage and that estimates of Co–C bond energies are consistent with this widely held view. Although it is clearly important to know the relationship between structure and Co–C bond energies, the only reliable correlations of steric effects and Co–C bond energy estimates come from Halpern's laboratory.[1-4] The demonstration that sterically hindered 2-substituted pyridines trans to the alkyl group in cobaloximes weaken Co–C bonds leaves open two possibilities. First, the Co–C bond could be weakened by the trans steric effect (see introduction) of the pyridine. Second, the Co–C bond could be weakened by an electronic steric effect—the trans ligand is prevented from forming an ideal bond with the metal. The pyridine would then be a poor electron donor. In the case of cobaloximes, it is known that, when weak electron donors occupy the trans position, Co–C bond energies are decreased. Our structural study of $2NH_2pyCo(DH)_2$-*i*-C_3H_7 clearly demonstrates that the latter effect occurs, at least in the solid state.

Appropriate interactions between the enzyme and coenzyme could lead to a lengthening of the Co–N(benzimidazole) bond in the conformational change observed for enzymic processes. Such a lengthening could occur even if there were also a simultaneous distortion of the macrocycle toward the deoxyadenosine (a cis steric effect). Spectroscopic and mechanistic evaluation of coenzyme analogues with modifications in the corrin amide side chains have led to the suggestion that the corrin macrocycle could be distorted by interaction of these side chains with the protein.[5-10] The structural and bond energy studies on cobaloximes reveal the possibility that a distortion of the corrin that increased steric interactions of the corrin and the benzimidazole would increase the Co–N(benzimidazole) bond length and would indirectly weaken the Co–C(deoxyadenosyl) bond. Unfortunately the limited data on Co–C bond energies, particularly for compounds that have been structurally characterized, make it impossible to select between the several reasonable alternative means by which the Co–C bond of coenzyme B_{12} is activated in B_{12} holoenzymes. More information is needed on the relationship of Co–C bond energies to structure.

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Registry No. $2NH_2pyCo(DH)_2CH_3$, 86941-99-3; $2NH_2pyCo(DH)_2CH_2CH_3$, 86942-00-9; $2NH_2pyCo(DH)_2$-*i*-C_3H_7, 86941-98-2; $2NH_2pyCo(DH)_2$-*i*-C_4H_9, 86942-01-0; $2NH_2pyCo(DH)_2$-neo-C_5H_{11}, 86942-02-1; $2NH_2pyCo(DH)_2CH_2Br$, 86942-03-2; $2NH_2pyCo(DH)_2CH_2CF_3$, 86942-04-3; $H_2OCo(DH)_2CH_3$, 25360-55-8; $H_2OCo(DH)_2CH_2CH_3$, 26025-30-9; $H_2OCo(DH)_2$-*i*-C_3H_7, 30974-89-1; $H_2OCo(DH)_2$-*i*-C_4H_9, 29131-78-0; $H_2OCo(DH)_2$-neo-C_5H_{11}, 43103-70-4; $H_2OCo(DH)_2CH_2Br$, 76550-22-6; $H_2OCo(DH)_2CH_2CF_3$, 57104-98-0; $4CNpyCo(DH)_2CH_2CF_3$, 86942-05-4; $4CNpyCo(DH)_2CH_2Br$, 64904-07-0; $4CNpyCo(DH)_2CH_3$, 38684-89-8; $4CNpyCo(DH)_2C_2H_5$, 52970-91-9; $4CNpyCo(DH)_2$-*i*-C_4H_9, 86942-06-5; $2CH_3NHpyCo(DH)_2C_2H_5$, 86942-07-6; $2BzNHpyCo(DH)_2C_2H_5$, 86942-08-7; $PhNH_2Co(DH)_2C_2H_5$, 26026-84-6; $PhNHCH_3Co(DH)_2C_2H_5$, 86942-09-8; $PhNHCH_3Co(DH)_2CH_2CF_3$, 86942-10-1; $PhNH_2Co(DH)_2CH_2CF_3$, 86942-11-2; $2CH_3pyCo(DH)_2CH_3$, 29130-81-2; $3NH_2pyCo(DH)_2C_2H_5$, 86942-12-3; $2NH_2py$, 504-29-0; 4CNpy, 100-48-1; PBu_3, 998-40-3; $P(OCH_3)_3$, 121-45-9; coenzyme B_{12}, 13870-90-1.

Supplementary Material Available: A listing of structure factors and tables of anisotropic temperature factors, hydrogen atom parameters, extensive bond length and bond angle data, ^{1}H NMR shifts, and elemental analyses (11 pages). Ordering information is given on any current mastehad page.