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Crystal Chemistry of Cobalamins. Structural Characterization of the Co−S Bond in Cobalamins

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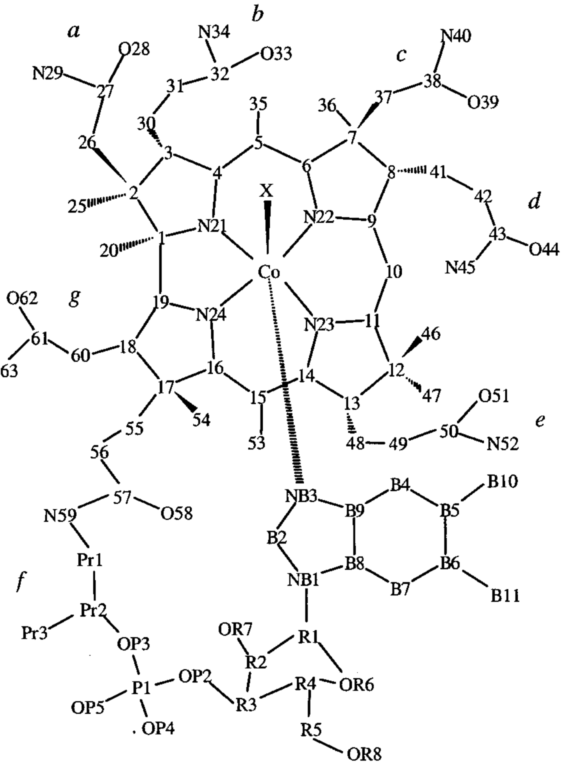
The first accurate structural characterization of the axial fragment in cobalamins, containing axial S ligand, is described and discussed. The preparations and the crystal structure determinations of two cobalamins, (SO_3Cbl)(NH_4)·$n$$H_2O$ (**1**) and [$(NH_2)_2$CSCbl]Cl·$n$$H_2O$ (**2**), based on synchrotron data collected at 100 K, are reported. The Co−S distances in **1** (2.231 (1) Å) and **2** (2.216(7) Å) are very close, whereas the trans Co−NB3 distance of 2.134(7) Å in **1** is significantly longer than that of 2.01(1) Å in **2**. This suggests that sulfite exerts a strong trans influence, close to that of a methyl group, whereas thiourea has a fairly weak trans influence, close to that of azide. An evaluation of the Co−NB3 and Co−SR distances, not yet experimentally determined, is given and allows us to suggest that the SR trans influence is greater than that of thiourea but significantly smaller than that of sulfite. These findings support the previous suggestion, based on structural and spectroscopic results for cobaloximes, that the thiolate ligation by homocysteine in MeCbl is unlikely to be an important step in the processes involving methionine synthase. Some features of the crystal chemistry, such as the crystal packing and the H-bond scheme among the crystallization water molecules, in **1** and **2**, are also discussed in relation to those of other cobalamins.

Introduction

Methylcobalamin (MeCbl) (Chart 1a) is the coenzyme of methionine synthase, which catalyzes the conversion of homocysteine to methionine.[1] Model chemistry suggests that homocysteinate reacts with MeCbl, with formation of a Co^{I}Cbl intermediate,[2] involving heterolytic cleavage and re-formation of the Co−C bond. To interpret the MeCbl function in the enzyme, several reactions of thiolates with organocobalt B_{12} model complexes were carried out, and among the proposed mechanisms, coordination at the Co trans to the alkyl group (thiolate ligation), with consequent weakening of the Co−C bond, was suggested. However, the reports of these reactions were controversial.[3] In addition, only two X-ray structural characterizations of the Co−SR bond have been reported for the cobaloxime model,[3,4] which supported spectroscopic data indicating that, in cobaloximes, thiolates have a moderate trans influence and, therefore, should be not able to influence the Co−C bond.[3] Alkanethiolate cobalamin complexes, RSCbl, are also of interest as analogues of the RS$^{\bullet}$ and Co^{II}Cbl radical pair involved[5] in the mechanism of the coenzyme B_{12} (adoCbl) dependent enzyme ribonucleotide reductase.[6] Hence, the knowledge of the properties and the behavior of the Co−SR bond should be of noticeable interest. However, despite numerous solution reports, only two thiolates have been isolated, the glutathionylcobalamin (GSCbl), where GS = γ-glutamylcysteinylglycine, characterized by EXAFS[7] and by NMR[8] and, very

Chart 1



X = (a) Me; (b) O_3S^{2-} (**1**), $(NH_2)_2$CS (**2**)

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Table 1. Crystal Data and Structure Refinement Details for **1** and **2**

	1	**2**
formula	$C_{62}H_{119.3}CoN_{14}O_{30.65}PS$	$C_{63}H_{112.8}ClCoN_{15}O_{24.4}PS$
fw	1673.27	1628.01
T, K	100(2)	100(2)
λ, Å	0.8	1.0
cryst system, space group	orthorhombic, $P2_12_12_1$	orthorhombic, $P2_12_12_1$
a, Å	16.005(5)	15.699(14)
b, Å	20.859(8)	22.32(2)
c, Å	24.328(4)	24.80(7)
V, Å^3	8122(4)	8693(27)
Z; D_{calcd}, Mg/m^3	4; 1.369	4; 1.244
μ, mm^{-1}	0.349	0.2870
F(000)	3578	3464
cryst size, mm	0.2 × 0.2 × 0.1	0.3 × 0.2 × 0.1
no. of reflns collected	12 404	7715
data/parameters	12 404/999	7388/791
GOF(F^2)	1.052	1.580
Final R indices [$I > 2\sigma(I)$]^a	R1 = 0.0535, wR2 = 0.1498	R1 = 0.1129, wR2 = 0.3183
R indices (all data)^a	R1 = 0.0581, wR2 = 0.1535	R1 = 0.1156, wR2 = 0.3265
abs structure parameter	−0.11(2)	0.092(16)

[a] $R1 = \sum||F_o| - |F_c||/\sum|F_o|$; $wR2 = [\sum w(|F_o| - |F_c|)^2/\sum|F_o|^2]^{1/2}$.

recently, the perfluorobenzenethiolate cobalamin, characterized by NMR.[9] The full X-ray structural characterization of GSCbl was attempted, but it was hampered by the severe disorder of the glutathionyl moiety.[9] Therefore, no detailed structural characterization of any Co−S bond in cobalamins has so far been available.[10,11] Recently, we described the accurate structural determination at 100 K of some cobalamins, XCbl·2LiCl (X = Cl, N_3), based on synchrotron diffraction data. Inclusion in the crystal of ions interacting with the amide side chains in the crystal structure allows a decrease in their conformational disorder.[12] This improves the crystallinity and leads to very accurate structural determinations in several cases. Previously, only two other very accurate structural determinations have been reported for [H_2OCbl](ClO_4)[13] and [H_2O(10-Cl-Cbl](ClO_4).[14] We have now applied this technique of crystallization to obtain single crystals of XCbl derivatives, where X is a S-coordinated ligand, such as thiourea, cysteinate, thiocyanate, and sulfite. The crystals were obtained by reaction in situ of (H_2OCbl)Cl with the appropriate S-containing reactant. Good-quality single crystals were obtained only for the sulfite derivative, (SO_3Cbl)(NH_4)·nH_2O (**1**), whose crystals however do not include electrolytes. In the case of the thiourea derivative, [$(NH_2)_2$CSCbl]Cl·$n$$H_2O$ (**2**), single crystals were also obtained, although the successive structural analysis showed that the crystal structure was affected by severe disorder. The preparations and the structural characterizations of **1** and **2**, based on synchrotron diffraction data collected at 100 K, are reported and discussed.

Experimental Section

Preparations. Commercial samples of (H_2OCbl)Cl, stated purity by manufacturer about 96%, were from Fluka. The other reactants were of reagent grade and were used without further purification. The complexes were prepared in situ. Their single crystals were grown by the hanging-drop method of vapor diffusion.

Complex 1. A 2 μL portion of an (H_2OCbl)Cl aqueous solution at 25 mg/mL was mixed with 2 μL of the precipitant solution containing LiCl and 0.06 M Na_2SO_3. Typical ranges for precipitants were 20−55% in $(NH_4)_2SO_4$ and 0−1.5 M in LiCl. Suitable single crystals were obtained only with 40% $(NH_4)_2SO_4$ and 1.5 M LiCl. Attempts either with only $(NH_4)_2SO_4$ or with PEG (with or without LiCl) were unsuccessful.

Complex 2. A 2 μL quantity of an (H_2OCbl)Cl aqueous solution at 25 mg/mL was mixed with 2 μL of the precipitant solution containing 0.12 M LiCl, $(NH_4)_2SO_4$, 20% glycerol, and 0.30 M thiourea. The concentration range for $(NH_4)_2SO_4$ was 40−50%, but suitable single crystals were obtained with the lowest concentration. Single crystals of another crystalline form of **2** were obtained by using only $(NH_4)_2SO_4$ as precipitant. However, their diffraction pattern was of lower quality, but good enough to carry out a preliminary analysis, which indicated differences in the corrin side chain conformations (vide infra). Attempts to improve the single-crystal quality are in progress.

X-ray Structural Determinations. The diffraction experiments were carried out at the X-ray diffraction beamline of the Elettra Synchrotron, Trieste, Italy, using the rotating-crystal method. Data were collected on a 345 mm MAR image plate with crystals mounted in loop and frozen to 100 K using a nitrogen-stream cryocooler. Crystallographic data for **1** and **2** are given in Table 1. The diffraction data were integrated using the program DENZO,[15] and reflections subsequently were scaled and merged using the program SCALEPACK.[15] Friedel equivalent reflections were not merged, and no absorption correction was applied. The phase problem was solved using as a starting model the coordinates of an isomorphous structure. The structural parameters were refined by full-matrix least-squares calculations on F^2 for all data using SHELXL-97.[16] Final R factors and refinement details are reported in Table 1.

Results and Discussion

Preparation of the Crystals. Following the previously described preparation of single crystals of XCbl·2LiCl (X = Cl, N_3),[12,17] many attempts to obtain single crystals of **1** by adding Na_2SO_3 to (H_2OCbl)Cl in the presence of LiCl and PEG400 did not lead to crystallization. Only when PEG400 was substituted by ammonium sulfate were single crystals, which

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[17] Furlan, M., Ph.D. Thesis, University of Trieste, 1999. This preparation method gives also high-quality crystals of XCbl·2LiCl (X = CN, NO_2) of CNCbl·KCl, which will be described elsewhere.

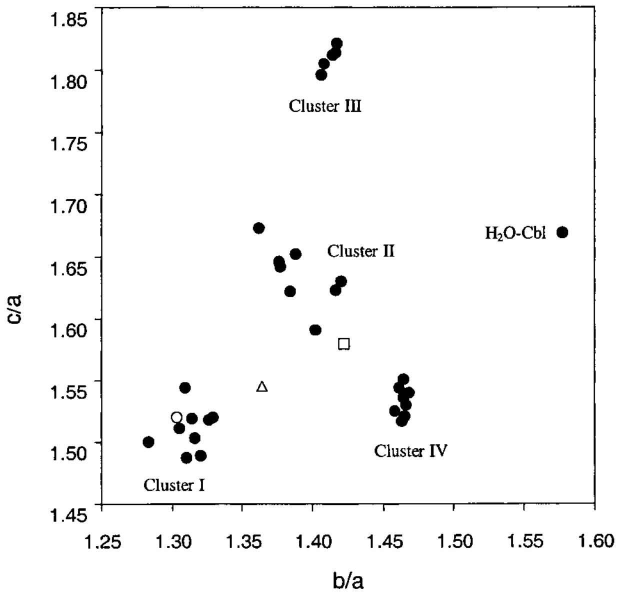

Figure 1. Scatterplot of *c*/*a* vs *b*/*a* for cobalamins. The open circle refers to **1**, the square to **2**, and the triangle to the other crystalline form of **2**.

however did not contain LiCl (vide infra), obtained under the conditions described in the Experimental Section. Single crystals of **2** were obtained by the previously described preparation,[12] but the successive crystal structure analysis showed that no LiCl was present in the crystal structure. Since attempts to obtain single crystals of any alkylcobalamin containing LiCl or other electrolytes in the crystal structure also failed,[12,17] we are inclined to think that, with this technique of crystallization, inclusion of electrolytes occurs only for *inorganic* neutral cobalamins, i.e. those with X = a monoanionic ligand, including cyanide,[17] but not when X = alkyl.

Description and Discussion of the Structures. According to Hodgkin et al.,[18] the crystals of cobalamins may be described as built up by stacking the same distorted close-packed layers of the roughly spherical molecules. More recently, Kratky et al.[11] showed that the vast part of the crystal structures of cobalamins, belonging to the $P2_12_12_1$ space group, can be assigned to three packing types, I−III, characterized by different values of the cell axis ratios *c*/*a* and *b*/*a* and differing only in the way the XCbl layers are stacked. Typically, CNCbl (dry)[18] belongs to type I, whereas CNCbl (wet)[19] belongs to type II and the B12 coenzyme (adoCbl)[20] belongs to type III. In addition, recent results showed[12] that cobalamins containing LiCl in the crystal belong to a new packing type, IV, characterized by very similar values of the *c*/*a* and *b*/*a* ratios.[17] A modification of the diagram of *c*/*a* versus *b*/*a* reported by Kratky and coworkers[11] is given in Figure 1, which shows the clusters I−IV for most of the cobalamins reported in CSDB and in refs 12 and 16. As already observed,[11] $[H_2OCbl](ClO_4)$ and MeCbl do not fit this scheme. The layers are packed in such a way as to form large cavities filled by water molecules and, if present, by molecules of an additional solvent and ions. Savage et al.[20] have shown that, in adoCbl·$n$$H_2O$, there are two symmetry-related cavities per unit cell. These authors fully described the cavity as made by a central channel running throughout the crystal, along a crystallographic 2-fold screw axis, and connected to side pockets, at intervals defined by the symmetry of that axis. An ordered H-bonded scheme of the water molecules characterizes the pocket, whereas the channel is occupied by a disordered pattern of the water molecules with fractional occupancy. Flattened cavities, even if they have differently shaped pockets, are formed also in the other packing types.[17] Furthermore, the X axial ligand protrudes into the channel. These features of cobalamin crystal chemistry and their relationship to cobalamin conformational properties (vide infra)[17] will be discussed in detail elsewhere. Crystals of **1** belong to a cluster of type I, whereas those of **2** belong to a cluster of type II.

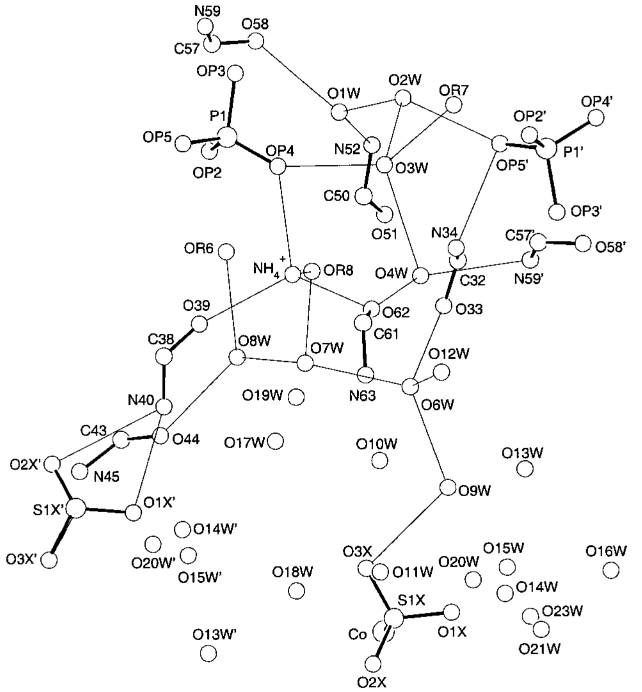

Figure 2. H-bond scheme within the pocket for **1**. The content of a portion of the channel, running approximately along the horizontal direction of the figure and containing the sulfite and the water molecules with fractional occupancy, is also shown.

The H-bond scheme within the cavity of **1** is shown in Figure 2, where the H-bonds are evidenced. The pocket is filled by the ammonium ion and by the full-occupancy water molecules O2W−O8W, while the channel is filled by water molecules with fractional occupancy and by the sulfite ligand and runs approximately along the horizontal direction in Figure 2. O9W, with 0.5 occupancy, is at the intersection between the pocket and the channel. The full-occupancy O1W molecule is at the boundary where the pocket contacts the channel of the other symmetry-related cavity. The water molecules have tetrahedral, trigonal, or pyramidal H-bond arrangements. They are held together and are associated with the surrounding N and O atoms of the corrin side chains by a well-ordered scheme of H-bond donors and acceptors (Table 2). The structure of the pocket, as well as the overall crystal packing of **1**, is very close to that of the isostructural CN−Cbl·KCl,[17] where the K^+ and Cl^- ions are located at the NH_4^+ and O4W sites of **1**, respectively. The assignment of the NH_4^+ ion, based on the refinement of the structure, is confirmed by its H-bond environment of O atoms of the *c*, *f*, and *g* side chains (Figure 2), characterized by N···O

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Table 2. H-Bond Schemes within the Pockets of **1** and **2**[a]

molecule	H-donors	H-acceptors
	Complex **1**	
O1W	**N52** (2.846(6) Å)	**O2W** (2.780(5) Å), **O58** (2.773(5) Å)
O2W	**O1W** (2.780(5) Å)	**O3W** (2.901(5) Å), **OP5** (2.772(5) Å)
O3W	**O2W** (2.901(5) Å), **O4W** (2.887(7) Å)	**OP4** (2.722(5) Å), **OR7** (2.748(5) Å)
O4W	**N59** (2.878(7) Å)	**O3W** (2.887(7) Å), **O62** (2.842(7) Å)
O6W	**O9W** (2.695(9) Å), *O12W* (2.71(1) Å)	**O7W** (2.713(7) Å), **O33** (2.673(7) Å)
O7W	**O6W** (2.713(7) Å)	**O8W** (2.724(7) Å), **OR8** (2.663(7) Å)
O8W	**O7W** (2.724(7) Å)	**O44** (2.754(6) Å), **OR6** (3.172(5) Å)
O9W	*OW13* (2.67(1) Å)	**O6W** (2.695(9) Å), **O3X** (2.965(9) Å)
	Complex **2**	
O3W	**O4W** (2.70(2) Å)	**OP4** (2.78(1) Å), **OR7** (2.75(1) Å)
O4W	**N59** (2.85(1) Å), **N34** (3.11(2) Å)	**O62** (2.83(2) Å), **O3W** (2.70(2) Å)
O5W	*N40A* (2.77(3) Å)	**OP4** (2.58(2) Å), **O62** (2.74(2) Å)

[a] Tentative assignments of H-bonds involving the water molecules (in bold characters) with full occupancy are given. The symbols of water molecules with fractional occupancy are in italic characters.

distances of about 2.8 Å and by a distorted pyramidal arrangement about NH_4^+ with O39, OP4, and O62 in the basal plane and OR8 at the apex. However, the alternative attribution of that position to an OH_3^+ species cannot be completely ruled out, although it is chemically unlikely.

The NH_2 group of the *c* chain makes two intramolecular H-bonds, one short with O1X (3.028 Å) and the other long with O2X (3.243 Å) of the SO_3 ligand (Figure 2). The H-bond involving the *c* side chain and the axial ligand is present in all the structures of types I and IV, where the *c* chain conformation is such as to direct the amidic group toward X. In cobalamins with crystal structures of types II and III, the intramolecular H-bond cannot be formed, since the *c* chain is oriented away from X.[17] Analogously, no intramolecular H-bond between OR8 of the *f* chain and O51 of the *e* chain, detected in type IV but not in the other type of structures,[12,17] is formed. The remaining amide chains are involved in H-bonds with the amide chains of the symmetry-related molecules of cobalamin and with water molecules.

The pocket structure in **2** is similar to that found[17] in structures of type II. Its content differs from that of **1** since the water molecule O5W is at the site occupied by NH_4^+ in **1** and forms an H-bond with N40. Unfortunately, the disorder affecting some water molecules within the pocket as well as the *a*, *c*, and *g* side chains and the CH_2OR8 group does not allow an accurate description of the H-bonding scheme. In fact, the O1W and O2W water molecules, with full occupancy in **1**, in **2** are found split over three and two sites, respectively. The ordered H-bond donor−acceptor scheme within the pocket is limited to the O3W−O5W water molecules with full occupancy and is given in Table 2. The *a*, *c*, and *g* chains exhibit a severe disorder, which was interpreted by assuming that each chain has two different conformations. In the orientation with the highest occupancy factor (o.f. = 0.7), the *c* chain points away from the axial thiourea ligand. In the other orientation (o.f. = 0.3), the *c* chain is directed toward the thiourea ligand, so that N40 is at H-bond distance (2.82(2) Å) from S. Detection of the *c* chain in two conformations, one typical of the structures of types II and III and the other typical of structures of types I and IV, may reflect the structure **2** position in the lower region of cluster type II in the scatterplot of Figure 1. The thiourea ligand undergoes a high thermal motion, which may be indicative of conformational disorder due to rotation about the Co−S bond, which cannot be resolved. It should be noted that the other crystalline form of **2** (see Experimental Section) is close to the type I cluster (Figure 1) and exhibits an intramolecular H-bond between S and the NH_2 group of the *c* side chain. The Cl^- ion, with o.f. = 0.7 is located at the intersection between the channel and the pocket and is H-bonded to N29 and O6W. Another site, H-bonded to N45 and O8W, was attributed to a Cl^- ion with o.f. = 0.3. The remaining corrin side chains are involved in H-bonds with the amide chains of the symmetry-related molecules of cobalamin and with water molecules.

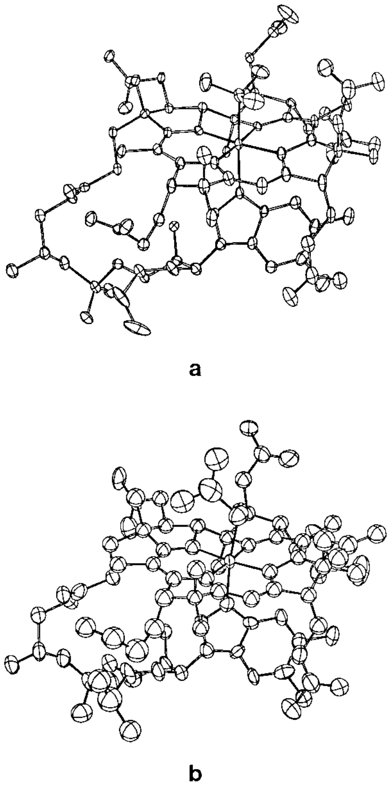

Figure 3. ORTEP drawings of **1** (a; 50% probability) and **2** (b; 30% probability). Conformations with the highest occupancy are shown for *a*, *c*, and *e* amide chains.

ORTEP[21] drawings of **1** and **2** are shown in Figure 3. The distortion of the corrin ring from planarity, as measured by the ϕ fold angle[10] (16.3(1)° in **1** and 14.9(3)° in **2**), is similar to those found in other cobalamins.[11] The ϕ angle is defined as the dihedral angle between the two planes passing through N21, C4,C5, C6, N22, C9, C10 and through C10, C11, N23, C14, C15, C16, N24, respectively. Some disorder was found to affect the C pyrrole ring in **2**. The coordination bond lengths in **1** and **2** are given in Table 3 and refer to the numbering in Chart 1.

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Table 3. Coordination Bond Lengths (Å) in **1** and **2**

	1	**2**		**1**	**2**
Co−S	2.231(1)	2.216(7)	Co−N22	1.913(2)	1.894(9)
Co−NB3	2.134(4)	2.01 (1)	Co−N23	1.890(3)	1.91(1)
Co−N21	1.870(3)	1.853(9)	Co−N24	1.886(4)	1.881(9)

Table 4. Axial Distances (Å) in Cobalamins and Cobaloximes

X	*T* (cobalamin), (K)	cobalamin		$XCo(DH)_2py$[a]	
		Co−X	Co−NB3	Co−X	Co−N(py)
H_2O	room[b]	1.952(2)	1.925(2)	1.916(3)	1.926(3)
Cl	100[c]	2.252(1)	1.981(3)	2.229(1)	1.959(2)
Cl	275[d]	2.265(2)	1.993(4)		
N_3	100[c]	1.980(3)	1.995(3)	1.950(2)	1.973(1)
N_3	275[d]	1.965(4)	2.010(3)		
N_3	295[d]	1.967(5)	2.010(4)		
SMe				2.286[e]	1.989(5)[e]
$(NH_2)_2CS$	100[f]	2.216(7)	2.01(1)		
CN	88[d]	1.886(4)	2.041(3)	1.937(2)	1.995(2)
O_3S	100[f]	2.231(1)	2.134(4)	2.225(2)[g] 2.207(7)[g]	2.111(7)[g] 2.105(9)[g]
Me	100[h]	1.972(6)	2.149(5)	1.998(5)	2.068(3)
ado	12[i]	2.02(1)	2.214(9)	2.015(2)[j]	2.072(2)[j]

[a] Data for cobaloximes are given in refs 24 and 25b, if not otherwise stated. [b] Reference 13. [c] Reference 12. [d] Reference 17. [e] Reference 4. [f] Present work. [g] Reference 22; the axial neutral ligand is NH_2Ph instead of py. [h] Unpublished data from this laboratory. [i] Reference 20; ado = 5-deoxy-5′-adenosyl. [j] Clegg, N.; Anderson, R. J.; Golding, B. T. *Acta Crystallogr*. **1989**, *C46*, 383; X = ribosyl.

As already observed in other cobalamins,[12] in **1** the mean value of the two Co−N equatorial distances involved in the five-membered ring of the equatorial moiety are shorter than that of the other two equatorial distances. The less accurate Co−N equatorial distances in **2** appear to follow the same trend. The axial fragment in **1** is characterized by $Co-SO_3$ and Co−NB3 distances of 2.231(1) and 2.134(4) Å, respectively. These figures should be compared with those of 2.35(3) and 2.16(4) Å, respectively, obtained from EXAFS analysis for the hydrogen sulfite cobalamin, HO_3SCbl.[7] The Co−NB3 distances are similar within the errors, whereas the Co−S distance in **1** is shorter by more than 0.1 Å. The sulfite geometry is characterized by S−O distances in the range 1.452(4)−1.472(4) Å and by approximately tetrahedral angles around S in the range 108.2(3)−111.9(3)°. In **2**, the Co−S and Co−NB3 distances are 2.216(7) and 2.01(1) Å, respectively. The Co−S bond lengths in **1** and **2** are very similar, within the experimental errors, while the Co−NB3 length is significantly longer in **1**. The latter observation indicates that sulfite exerts a trans influence stronger than that of thiourea. It has been observed[11,14] that the increase in the Co−NB3 bond length follows the decrease in the corrin fold angle. This correlation has been attributed to the release of the steric pressure of the benzimidazole residue on the corrin moiety.[11] This correlation does not appear to be followed in the case of **1** and **2**. The $Co-SO_3$ and the $Co-SC(NH_2)_2$ distances do not significantly differ from those of 2.225(2) and 2.207(7) Å reported for two independent structural determinations of the cobaloxime $[(O_3S)Co(DH)_2(NH_2Ph)]$, where DH = the monoanion of dimethylglyoxime.[22]

Estimate of the Geometry of the NB3−Co−SR Fragment. An interesting feature of the present work is the first accurate structural characterization of the geometry of the NB3−Co−S fragment in cobalamins. The $Co-SO_3$ and $Co-SC(NH_2)_2$ distances of 2.231(1) and 2.216(7) Å, respectively, are very similar and close to that found in the sulfite cobaloxime[22] but shorter than that of 2.286 Å found in the thiolate cobaloxime $[HgI_2][(MeS)Co(DH)_2py]$,[4] where py is a weak trans-influencing ligand. However, the latter Co−S distance could be lengthened by a further interaction of S with Hg.[3] This interaction may also decrease the trans-influence ability of MeS, which appears to be close to that of CN, as indicated by the similar values of the trans Co−py distances in the two cobaloximes (Table 4). The Co−S distances in **1** and in **2** are even shorter than that of 2.342(2) Å reported for the other thiolate cobaloxime $[(EtS)Co(DH)_2Me]^-$, where the lengthening was attributed to the strong trans influence of Me.[3] That the Co−S distances are lengthened in both thiolates with respect to a bond, *unperturbed* either by a trans influence or by an increase in the coordination number of S, is confirmed by the examination of the Co−SR distances reported in the literature.[23] An average value of 2.254(25) Å for 18 Co(II)− and Co(III)−S distances is calculated with the upper quartile, q_u, being 2.271 Å. q_u has the property that 25% of observations, presumably those involving Co(II), exceed q_u and 75% fall short of it. Therefore, it can be presumed that the Co(III)−SR distance is only slightly shorter than 2.25 Å. However, the availability of numerous structural data for the axial fragment in cobaloximes allows another way to estimate the *unperturbed* Co−SR bond length. In fact, it has been shown[24] that for the two series of cobaloximes $MeCo(DH)_2L$ (a) and $ClCo(DH)_2L$ (b) with several L, such as H_2O and N- and P-donor-containing ligands, the Co−L distances in series a, d_{Me}, exhibit a good linear relationship with their analogues in series b, d_{Cl}, according to the equation

$$d_{Me} = 0.989 d_{Cl} + 0.111 \qquad r = 0.997, \quad n = 10 \quad (1)$$

It was concluded[24] that a mean increase of about 0.1 Å in the Co−L distances should be expected in going from the weak (Cl) to the strong (Me) ligand, independent of the nature of L. Assuming that the trans influence of py is very close to that of Cl[25a] and that relation 1 holds also for thiolates, the *unperturbed* Co−SR distance in cobaloximes may be evaluated to be 2.26 Å, given $d_{Me} = 2.342$ Å.[3] This value is slightly larger than that found in the sulfite cobaloxime[22] and in **1**, and it is close to the mean value calculated for alkanethiolates.[23] The above discussion, although based on a few available structural data, suggests that the estimated Co−SR bond length, with small bulky R, in both cobaloximes and cobalamins should be close to that found in sulfite and thiourea complexes.

The axial distances in **1** and **2** are compared in Table 4 with their analogues reported for other cobalamins of comparable accuracy or with esd's on distances not larger than 0.01 Å. The distances in the X−Co−N fragment in the corresponding cobaloximes, $XCo(DH)_2py$, are also reported. As previously noticed,[12] the Co−NB3 distance increases by about 0.3 Å with an increase in the σ-donating ability of X. Comparison of the Co−NB3 distances shows that sulfite lengthens this bond by about 0.2 Å more than H_2O and by about 0.07 Å less than ado, determining a Co−NB3 distance very close to that of 2.149(5) Å in MeCbl (Table 4). Therefore, sulfite is a strong trans-influencing ligand similar to methyl. This is supported by the

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(25) (a) The Co−py distance is 1.950 Å in $[pyCo(DH)_2py]^+$ [24] and 1.959(2) Å in $ClCo(DH)_2py$.[25b] (b) Geremia, S.; Dreos, R.; Randaccio, L.; Tauzher, G.; Antolini, L. *Inorg. Chim. Acta* **1994**, *216*, 125.

Table 5. Axial Distances (Å) in Aniline Cobaloximes

	Co−X	Co−N
$[(NH_2Ph)Co(DH)_2(NH_2Ph)]^+$	2.009(5)	2.007(5)[a]
$ClCo(DH)_2(NH_2Ph)$	2.261(1)	2.019(2)[a]
$ICo(DH)_2(NH_2Ph)$	2.558(3)	2.053(8)[a]
$[O_3SCo(DH)_2(NH_2Ph)]^-$	2.225(2)	2.111(7)[b]
	2.207(7)	2.105(9)[b]
$MeCo(DH)_2(NH_2Ph)$	1.992(2)	2.129(1)[c]
$EtCo(DH)_2(NH_2Ph)$	2.030(3)	2.147(2)[d]
$[(EtO_2C)_2MeCCH_2]Co(NH_2Ph)$	2.035(2)	2.143(2)[c]
$(MeOCH_2)Co(DH)_2(NH_2Ph)$	2.013(4)	2.169(3)[d]
$^iPrCo(DH)_2(NH_2Ph)$	2.068(3)	2.177(2)[d]
$(adamantyl)Co(DH)_2(NH_2Ph)$	2.159(4)	2.214(4)[e]

[a] Reference 24. [b] Reference 22. [c] Geremia, S.; Mari, M.; Randaccio, L.; Zangrando, E. *J. Organomet. Chem*. **1991**, *408*, 95. [d] Marzilli, L. G.; Bayo, F.; Summers, M. F.; Thomas, L. B.; Zangrando, E.; Bresciani Pahor, N.; Mari, M.; Randaccio, L. *J. Am. Chem. Soc*. **1987**, *109*, 6045. [e] Bresciani Pahor, N.; Randaccio, L.; Zangrando, E.; Marzilli, L. G. *J. Chem. Soc., Dalton Trans*. **1989**, 1941.

SO_3^{2-} behavior in cobaloximes. The axial distances in several aniline cobaloximes are reported in Table 5, where X varies from weak trans-influencing ligands, such as Cl and NH_2Ph, to strong trans-influencing alkyl groups. The trend of the Co−NH_2Ph distance parallels that of the σ-donating ability of X, in a way similar to that shown for cobalamins and pyridine cobaloximes in Table 4, and it is scarcely affected by the bulk of X. The SO_3^{2-} position in the trend is close to that found in cobalamins. Therefore, it may be concluded that thiourea in cobalamins is a trans-influencing ligand largely weaker than sulfite and close to azide (Table 4), whereas thiolates are slightly stronger trans-influencing ligands than azide. In view of the greater sensitivity of the axial Co−N bond to the trans influence of X in cobalamins with respect to cobaloximes (Table 4), it might be expected that the Co−NB3 bond in RS-Cbl could be longer than that expected from the analysis of the cobaloxime analogues and possibly close to that found in CNCbl (Table 4). In fact, the relationship between Co−NB3 in cobalamins and Co−py in cobaloximes, illustrated in Figure 4 for several X ligands, is fairly linear with a correlation factor $r = 0.985$ and a slope of 1.816. A value of 2.034 Å for the Co−NB3 distance in thiolate cobalamins is calculated from the equation given in the caption of Figure 4. This value is not far from that of 2.041(3) Å found for CNCbl.[17] This evaluation is supported by EXAFS measurements, which suggested that the Co−NB3 distance in GSCbl is very similar to that in CNCbl.[7] However, we evaluate the Co−NB3 distance to be shorter by about 0.1 Å than that of 2.15(3) Å determined by the EXAFS analysis.[7]

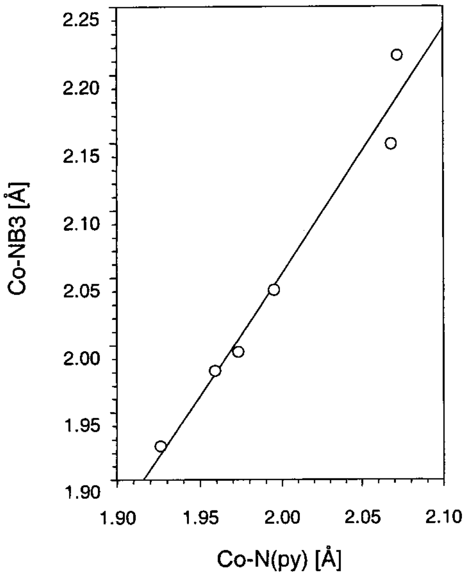

Figure 4. Correlation between the Co−N3 distances in cobalamins and the Co−N(py) distances in cobaloximes, represented by the equation Co−NB3 = 1.816Co−N(py) − 1.578, with $r = 0.985$ and $n = 6$. Data at 100 K have been chosen for cobalamins.

Although the structural analysis of GSCbl is not yet fully determined to give a reliable Co−NB3 distance,[9,26] the above hypothesis appears to give support to the conclusion,[3] based on structural and spectroscopic data for cobaloximes, that the thiolate ligation by homocysteine in MeCbl is unlikely to be an important step in the processes involving methionine synthase, since thiolates should exhibit a moderate trans influence also in cobalamins.

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Supporting Information Available: X-ray crystallographic files in CIF format and tables of crystallographic data, atomic coordinates, anisotropic thermal factors, calculated hydrogen atom coordinates, and bond lengths and angles. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) We are investigating how to improve the quality of the single crystals of this complex further, in collaboration with Professor R. G. Finke and his research group.