

Cobaloximes: Models of Vitamin B₁₂

Note: This experiment requires one 3- or 4-hour laboratory period.

Cobalt is a trace element in living systems, and in humans is present at a concentration of only 10^{-8} M. This low concentration does not, however, indicate that cobalt is biologically unimportant: A deficiency of cobalt in the human diet leads to the fatal disorder pernicious anemia. In the body, cobalt is present in the family of coenzymes called cobalamins, one derivative of which is cyanocobalamin or vitamin B₁₂. Coenzymes are compounds that bind to proteins to form enzymes, nature's catalysts. As the first organometallic complexes found in nature, the cobalt-containing coenzymes have attracted much attention from both inorganic and biological chemists.

The structures of all cobalamins are similar and consist of a cobalt ion situated in a tetrazamacrocyclic ligand with trans imidazole (symbolized by L) and alkyl groups. The structure of methylcobalamin, in which the alkyl group is a methyl group, is shown in Figure 21-1.

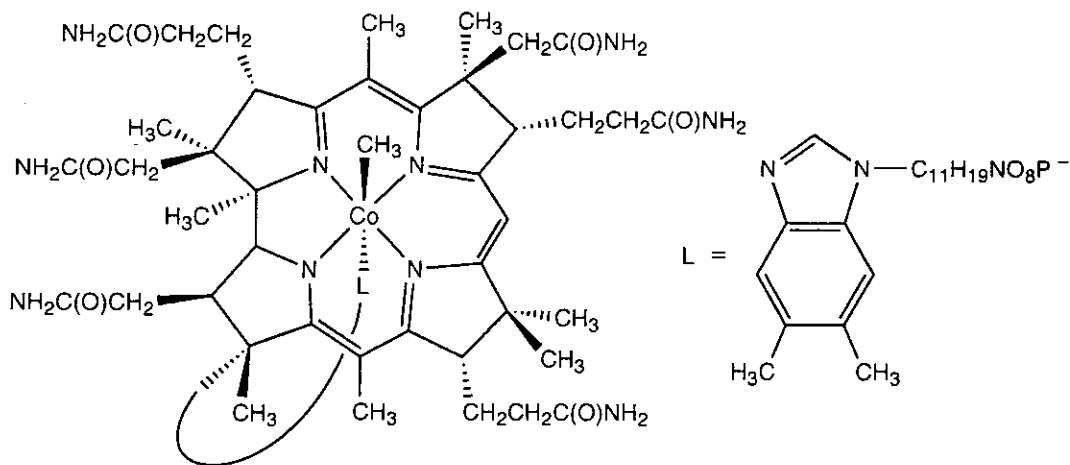
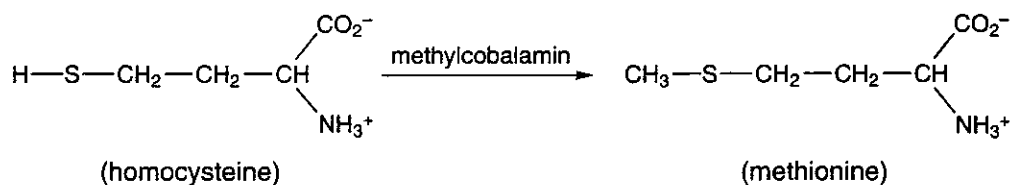


Figure 21-1
Structure of methyl cobalamin.

At various stages in the enzyme's catalytic cycle, the oxidation state of cobalt can be Co^{III} , Co^{II} , or Co^{I} . The macrocyclic ligand, called a corrin, resembles porphyrins (Experiment 23) except that the ring contains one less carbon atom and it is more reduced, consisting of only 13 contiguous sp^2 atoms. In the coenzyme, the Lewis base L is an N-substituted dimethylbenzimidazole. Recent evidence suggests that in the coenzyme-protein complex (holoenzyme) the Lewis base is sometimes replaced by the imidazole group of a histidine residue.

The most important component of the coenzyme is the cobalt-carbon bond, because this bond is cleaved during the enzyme's catalytic cycle. Cobalamin-based enzymes catalyze two kinds of transformations, depending on the identity of the alkyl ligand. When the alkyl group is methyl, the enzymes are involved in methylation reactions such as the biosynthesis of the amino acid methionine:



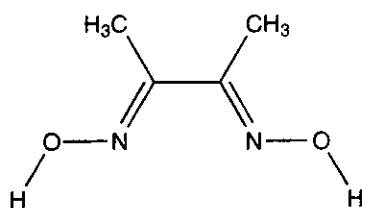
The occurrence of the poisonous substance methyl mercury in the environment has been attributed to the action of bacterial methylcobalamins on inorganic mercury salts. In the second class of cobalamin-based enzymes, in which the alkyl ligand is adenosyl, the enzyme catalyzes carbon skeletal rearrangements. For example, one adenosylcobalamin-based enzyme catalyzes the conversion of methylmalonate to succinate, a key step in the Krebs citric acid cycle:



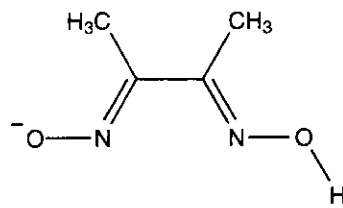
Unlike methylcobalamin (which serves as a source of CH_3^+), adenosylcobalamin initiates reactions by means of the reversible homolysis of the cobalt-adenosyl bond. The resulting carbon-based adenosyl radical abstracts a hydrogen atom from the substrate, thereby initiating the subsequent skeletal rearrangements.

The molecular complexity of the cobalamins makes it difficult to study the coordination chemistry of the cobalt center. Inorganic chemists, however, have found that many properties of these enzymes can be simulated using model compounds derived from fairly simple ligands. The most successful model complexes are known as the cobaloximes. In the cobaloximes, a pair of dimethylglyoximate ligands serves the function of the corrin macrocycle. Drawings of

dimethylglyoxime and its conjugate base, the dimethylglyoximate anion, are shown below:



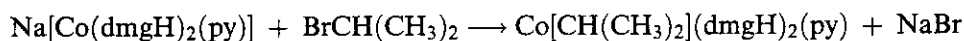
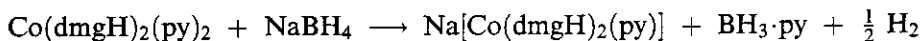
dimethylglyoxime
(dmgH₂)



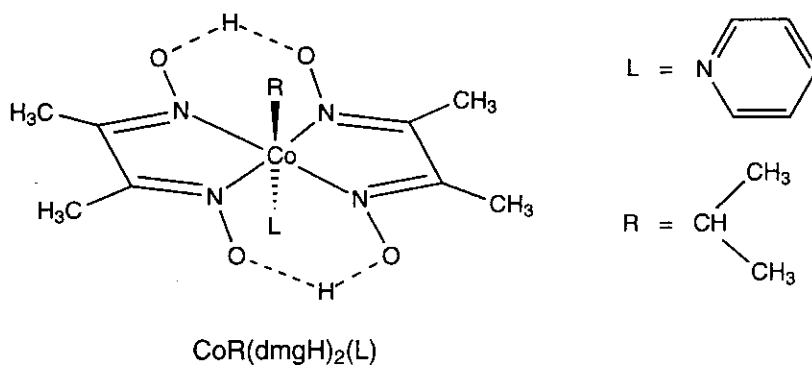
dimethylglyoximate
(dmgH⁻)

Two dimethylglyoximate (dmgH⁻) ligands can link through hydrogen bonds to form a planar anionic macrocycle that binds the cobalt through its four nitrogen atoms. An important feature of these compounds is that the nitrogen ligands are *sp*²-hybridized, in contrast to the *sp*³-hybridized amine ligands usually found in classical cobalt coordination compounds (see Experiments 13 and 14).

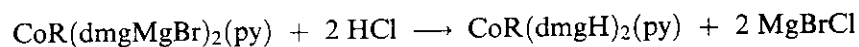
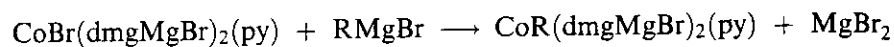
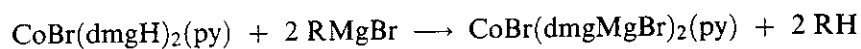
The present experiment begins with the preparation of Co(dmgh)₂(py)₂, where py = pyridine, although this cobalt(II) complex is not isolated. Reduction of this complex with BH₄⁻ produces the cobalt(I) derivative Co(dmgh)₂(py)⁻. This highly nucleophilic complex reacts with alkyl halides to give cobalt(III) alkyl complexes. In this experiment, you will use the alkylating agent isopropyl bromide (2-bromopropane):



The structure of the compound you will make is shown below:

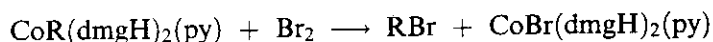


An alternative method for generating CoR(dmgh)₂(py) involves the reaction of CoBr(dmgh)₂(py) with Grignard reagents:



This method is less attractive because 3 mol of the Grignard reagent are required, the first two moles being consumed in the deprotonation of the acidic OH groups in the dmgH ligands.

In the second step of this experiment, you will use bromine to remove the alkyl group to give $\text{CoBr}(\text{dmgH})_2(\text{py})$:



EXPERIMENTAL PROCEDURE

Safety note: Pyridine, bromine, and bromoalkanes are toxic and should only be handled in a hood.

Isopropylbis(dimethylglyoximato)(pyridine)cobalt(III), $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$

The reaction apparatus consists of a 200-mL three-neck round-bottom flask and a pressure-equalizing addition funnel (Fig. 21-2). Fit one neck with a N_2 gas inlet

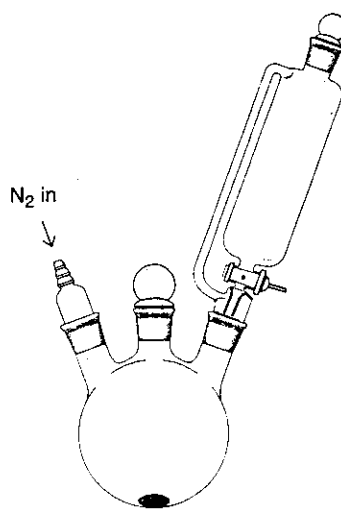


Figure 21-2

Apparatus for the synthesis of $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$.

but do not place the stopper in the central neck just yet. Lubricate all glass-to-glass joints with silicone grease. Charge the reaction flask with the following: a magnetic stir bar, 75 mL of methanol, 4 g (17 mmol) of $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$, and 4 g (34 mmol) of dimethylglyoxime. Charge the pressure-equalizing addition funnel with a solution of 1.25 g (31 mmol) of NaOH in 8 mL of water. While the methanol solution is stirring, flush the entire system with N_2 for several minutes. After reducing the N_2 flow, add the NaOH solution dropwise followed by 1.8 mL (1.75 g, 22 mmol) of pyridine. Stir the mixture for 20 min and then cool it in ice. While the reaction solution is cooling, prepare a solution of 1.5 g (33.5 mmol) of NaOH and 1.5 g (40 mmol) of NaBH_4 in 7 mL of H_2O . Add this NaBH_4 solution to the cold, stirred reaction mixture. The reaction mixture should become very dark as $\text{Co}(\text{dmgH})_2(\text{py})^-$ forms. Finally, add 2.25 mL (2.25 g, 18.3 mmol) of 2-bromopropane to the cold reaction solution, and then place a greased stopper in the central neck and turn off the N_2 flow.* After the mixture has stirred a further 40 min at 0°C , pour the reaction mixture into a beaker containing 75 mL of water. Collect the orange product by filtration (see Figure 13-1) and dry it under vacuum. Recrystallize your product by suspending the solid in 25 mL of hot (50°C) methanol in a 100-mL Erlenmeyer flask on a stirring hot plate. Add 5-mL portions of methanol until all of the solid dissolves. At this stage, slowly add 20 mL of water and then allow the solution to cool to room temperature. Collect the product by filtration. Record the yield. Measure the IR spectrum (see Experiment 19 for the preparation of a Nujol mull) and ^1H NMR spectrum of the product.

Bromobis(dimethylglyoximato)(pyridine)cobalt(III), $\text{CoBr}(\text{dmgH})_2(\text{py})$

In a three-neck flask equipped with a stir bar, dropping funnel, and a N_2 inlet, dissolve 0.75 g (1.8 mmol) of $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$ in 15 mL of acetic acid. Flush the flask with N_2 . Add dropwise a solution of 1.2 g (7.5 mmol) of Br_2 in 10 mL of acetic acid. Seal the flask with a stopper, and stir the mixture for a further 1.5 to 2 h. Filter off the green-brown solid product and dry it under vacuum. Determine the yield and record the IR spectrum of the product.

REPORT

Include the following:

1. Yields of $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$ and $\text{CoBr}(\text{dmgH})_2(\text{py})$.
2. The IR spectra of $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$ and $\text{CoBr}(\text{dmgH})_2(\text{py})$.
3. The ^1H NMR spectrum of $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$.

PROBLEMS

1. What is the valence electron count of the metal center in $\text{Co}[\text{CH}(\text{CH}_3)_2](\text{dmgH})_2(\text{py})$?

*The reagents CH_3I , $\text{C}_2\text{H}_5\text{I}$, or $\text{C}_6\text{H}_5\text{CH}_2\text{Cl}$ can be used in place of 2-bromopropane to make the corresponding methyl-, ethyl-, and benzylcobaloxime derivatives.

2. The square planar complex $\text{Ni}(\text{dmgH})_2$ reacts with two equivalents of BF_3 to give 2 equiv of HF and a complex whose ultraviolet-visible spectrum is similar to the starting complex. Suggest a structure for this product.
3. How do porphyrin and corrin ligands compare with respect to the ring size, formal charge, and degree of unsaturation?
4. It is speculated that cobalt is uniquely suited for its biological role in cobalamins due to the availability of three adjacent oxidation states, Co^{III} , Co^{II} , and Co^{I} . How does this situation compare with the oxidation states utilized by iron in nature? Give examples.
5. Many metalloenzymes have imidazole ligands. Which amino acid bears an imidazole substituent?

INDEPENDENT STUDIES

- A. Prepare $\text{CoCl}(\text{dmgH})_2(\text{py})$ and convert it to its phenyl derivative $\text{Co}(\text{C}_6\text{H}_5)(\text{dmgH})_2(\text{py})$. (Schrauzer, G. N. *Inorg. Synth.* **1968**, *11*, 61.)
- B. Prepare methylcobalamin from cyanocobalamin (vitamin B_{12}). (Dolphin, D.; Halko, D. J.; Silverman, R. B. *Inorg. Synth.* **1980**, *20*, 134.)
- C. Prepare cobaloxime complexes with 4-tert-butylpyridine. (Jameson, D. L.; Grzybowski, J. J.; Hammels, D. E.; Castellano, R. K.; Hoke, M. E.; Freed, K.; Basquill, S.; Mendel, A.; Shoemaker, W. J. *J. Chem. Educ.* **1998**, *75*, 447.)

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