

Experiment VI¹

Bioinorganic Model Compounds: Preparation and Properties of Cobaloxamines; Vitamin B₁₂ Model Compounds

Few stable organocobalt compounds were known before 1964 when it was discovered that a coenzyme of vitamin B₁₂ contained an adenosyl group bonded to cobalt by a cobalt-carbon single bond. This observation was extremely surprising since the coenzyme exists in a biological environment where it was susceptible to degradation by heat, air, and water. These are precisely the conditions which readily decomposed most of the known compounds containing a carbon-metal bond, organometallic compounds. The prevailing belief at this time was that organometallic compounds were very reactive, thermodynamically unstable, and could only be prepared in special cases. Today, thousands of organometallic compounds stable to the ambient are known.

About this time, work in Schrauzer's laboratories on bis(dimethylglyoximato)cobalt complexes, Figure 1, (termed "cobaloxamines") demonstrated that the cobalt atom, when surrounded by a planar array of nitrogen ligands, behaved similarly to cobalt in the macrocyclic corrin rings found in biological systems.² Schrauzer's compounds thus serve as models for the biological coenzyme. Among the various properties exhibited by cobaloxamines, it is particularly noteworthy that the +1, +2, and +3 oxidation states are all readily accessible and play an important role in their chemistry. The deep blue Co(I) species displays strong nucleophilic character and have been called "supernucleophiles" by Schrauzer. The Co(I) complexes are among the most powerful nucleophiles with a reactivity 10^7 times greater than iodide ion in classical S_N2 substitution reactions. In fact, one method for preparing organocobaloxamines involves the reaction of the Co(I) nucleophile with alkyl halides. It has been through the investigation of these remarkably stable alkyl-cobaloxamine model compounds that much has been learned about the catalytic role of vitamin B₁₂ coenzyme-dependent enzymes.

In this experiment you will synthesize and characterize methyl (pyridine) cobaloxamine. Bromo (pyridine) cobaloxamine will be prepared first and then converted to the alkyl cobaloxime via the nucleophilic Co(I) species. The Co(I) cobaloxamine is very air-sensitive and it will be generated using standard inorganic schlenk techniques. Brilliant color changes accompany this chemistry which serve as useful tools for characterization.

Figure 1.

Hazards

Methylene chloride (CAS No. 67-63-0): Methylene chloride (dichloromethane) is harmful if swallowed, inhaled, or absorbed through the skin. It is a possible carcinogen. ORL-HMN LDLo: 357 mg/kg. ORL-RAT LD50: 1600 mg/kg.

Tetrahydrofuran (CAS No. 109-99-9): THF may cause severe damage to the liver. The liquid is extremely flammable. On exposure to air, THF forms peroxides which can explode on contact with strong bases. ORL-RAT LD50: 2816 mg/kg.

Pyridine (CAS No. 110-86-1): Pyridine is harmful if swallowed, inhaled or absorbed through the skin. It has a noxious smell and is a general anesthetic. Dispense it only in the hood. Wash all utensils in contact with pyridine in the hood with acetone. ORL-RAT LD50:891 mg/kg.

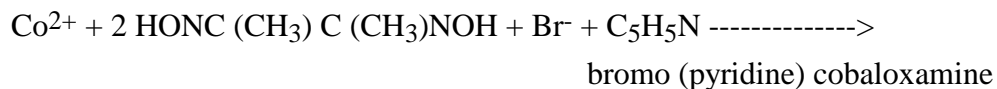
Cobalt (II) nitrate hexahydrate (CAS No. 10026-22-9): This compound is harmful if inhaled or swallowed. ORL-RAT LD50:691 mg/kg.

Sodium borohydride (CAS No. 16940-66-2): Sodium borohydride is a strong reducing agent which reacts violently with water. Avoid skin contact and keep in the hood.

The manipulation of inorganic compounds on a gas-vacuum manifold necessarily involves potentially dangerous procedures. The most common accident involves the implosion or explosion of glass. As always, it is imperative that laboratory safety glasses be worn at all times! Many accidents can be avoided by thoroughly checking glassware for star cracks before use. With careful laboratory technique these procedures are extremely safe.

Procedure

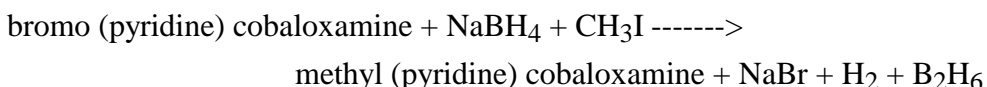
A. Preparation of Bromo (pyridine) cobaloxamine



[Figure 1.A.]

Dissolve 12.5 mmole of dimethylglyoxime in 30 mL of boiling 95% ethanol. Add to this mixture a solution of 6.6 mmol cobalt nitrate hexahydrate in 30 mL of hot ethanol. Boil the solution for about five minutes. Next, add a solution of 10 mmol NaBr in 10 mL of water and boil for another five minutes. Then, add 0.75 equivalents (with respect to Co) of pyridine as a 5 M pyridine solution in THF. Boil the solution for another five minutes, then cool in an ice bath to 20° C. Attach the aspirator hose, an aeration tube and a stopper to the flask. Bubble air through the solution for 20 minutes. During this time, set up the equipment needed for part B. After the twenty minutes is up, transfer the contents of the filter flask to a beaker and rinse the flask with ethanol. Collect the crystals by vacuum filtration. Wash the crystals with 5 mL portions of water and ethanol and finally a 10 mL portion of diethyl ether. Keep the crystals on the funnel until they are as dry as possible. Weigh the crystals on an analytical balance.

B. Methyl (pyridine) cobaloxamine



[Figure 1.B.]

Place 1.9 mmol of bromo (pyridine) cobaloxamine in a side-arm flask and add 10 mL of argon saturated methanol. Maintain an Ar atmosphere throughout the rest of the procedure. Weigh out 6.6 mmol of NaBH₄. Add approximately one half of the hydride to the suspension in small portions with stirring under a positive argon flow. The solution should be homogeneous and dark blue-black in color. Add 3.0 mmol of deoxygenated iodomethane and then the remaining portion of NaBH₄. The solution should be deep red-orange in color at this point. Allow the mixture to stir for 15 min under an argon ambient. If any blue color remains, add a small drop of the iodomethane solution. Pour the contents of the flask into 20 mL of very cold water in air. Collect the crystals and wash with 10 mL portions of water and petroleum ether. Recrystallize the product by dissolving it in a minimal amount of dichloromethane and reprecipitating it with petroleum ether. Collect and weigh the crystals. Place in a labeled vial. Next week characterize this complex by the following techniques: NMR spectroscopy, UV-Vis spectroscopy, melting point, and infra-red spectroscopy.

Current Research Efforts

The chemistry of organocobalt compounds as models for Vitamin B₁₂ continues to be an active area of research.³ The abundance and importance of metals in biology result in bioinorganic chemistry being perhaps the most rapidly growing area in science. The Johns Hopkins Chemistry Department is well represented in this exciting area of research.

Dr. Karlin's focus of interest is the coordination chemistry relevant to processes mediated by copper-containing proteins which utilize dioxygen or nitrogen oxides. Widely occurring and essential Cu enzymes function in O₂-transport, electron transfer, O₂-reduction, biological substrate oxygenation, and reduction of NO₂⁻ or N₂O. Dr. Karlin's main approach involves synthetic modeling (biomimetic chemistry) similar to today's experiment. A recent notable achievement includes the first structural determination of a reversibly bound O₂-complex of copper.

Dr. Miller's research combines the use of Nuclear Magnetic Resonance (NMR) and Electron Paramagnetic Resonance (EPR) spectroscopies to study metal-containing enzymes. Many of the most demanding reactions in biochemistry are catalyzed enzymes that employ metals in their active sites. The goal of this research program is to learn how proteins and bound metal sites interact with one another as well as how the protein controls the metal site's properties.

References

1. This experiment is adapted from *Chemistry 171L; Synthetic Chemistry Laboratory II*, Department of Chemistry, University of North Carolina at Chapel Hill, **1992**.
2. Schrauzer, G. N. *Acc. Chem. Res.* **1968**, *1*, 97, and references therein.
3. Toscano, P. J.; Marzilli, L. G. *Prog. Inorg. Chem.* **1984**, *31*, 105 and references therein.
4. For very readable reports of selected areas within bioinorganic chemistry see: *J. Chem. Ed.* **1985**, *62*, 917-1001.