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A Convenient and Rapid Synthesis of Mesohemin IX Dimethyl Ester Chloride

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Porphyrin and metalloporphyrin chemistry is an expanding research area. Tetraphenylporphyrin serves as an excellent synthetic model compound in many respects, but it is often desirable to work with species which more closely resemble the naturally-occurring porphyrins. Mesoporphyrin IX dimethyl ester is a convenient choice because of its greater stability over protoporphyrin IX [1], its synthetic simplicity, and its high solubility in non-aqueous solvents. The methods of Baker, *et al.* [2] or of Caughey, *et al.* [3] are acceptable for preparation of mesoporphyrin IX dimethyl ester [4]. However, we have modified the former procedure and extended it such that the mesohemin dimethyl ester can be obtained directly. Thus, assuming the iron derivative is desired, no re-metallation reaction is required. Although our modification of the method of Baker, *et al.* [2] is straightforward, this report describes the first detailed literature synthesis of mesohemin from protohemin without prior demetallation.

Materials

Protohemin chloride (bovine) was purchased from Aldrich Chemical Company. Platinum(IV) oxide catalyst ($PtO_2 \cdot H_2O$, 40 mesh) was obtained from Alfa Division of Ventron Corporation. Heptane, methanol, and methylene chloride were dried over calcium chloride, freshly distilled and stored over molecular sieves. Alumina (80–200 mesh, Fisher Scientific Company) was used as received as column chromatography adsorbent.

Synthesis Procedure

Protohemin (2.0 g) is dissolved in 750 ml of 0.02 *M* KOH solution in a one liter filtering flask which is magnetically stirred at a moderate rate. Platinum(IV) oxide catalyst (1.0 g) is added and the filtering flask is evacuated for several minutes using a water aspirator. Hydrogen gas (435 ml total) is introduced at one atmosphere pressure. If a gas buret is not available, an inverted chromatography column placed in a 1000 ml graduated cylinder filled with water is adequate. Once the gas buret empties, the same volume of nitrogen gas is placed in the buret to avoid a negative pressure in the flask. A purple tinge appears in the solution within two hours after the reaction is initiated. Hydrogenation is complete in 20 hours (room temperature), at which point the flask is sealed and placed in the refrigerator overnight. Decantation of the wine-colored solution allows separation of approximately 80% of the catalyst. Centrifugation of the decantate at 5000 rpm results in essentially complete removal of PtO_2. (The recovered catalyst should be washed and vacuum dried). Any traces of remaining catalyst will be removed in the course of purification. Acetic acid (1 *M*) is added until all the hemin has precipitated. The thick precipitate is then collected by centrifuge (up to 5000 RPM), washed with water, and dried. If vacuum drying is to be used, the voluminous wet product should first be frozen to prevent spattering. Methanol (500 ml), containing 5% sulfuric acid (w/v) is used to dissolve the dried solid, and the esterification reaction is carried out at room temperature 24 hours. Chloroform (150 ml) is added to the esterified mesohemin solution in a teflon-stoppered separatory funnel followed by water until the layers separate. Two smaller chloroform extractions provide complete transfer of the mesohemin dimethyl ester to the organic layer. The chloroform extract is washed with water and then with 2 *M* sodium carbonate solution.

Column Chromatography

Mesohemin dimethyl ester is in a volume of about 300 ml of chloroform at this point. The solution is placed on a dry column of alumina [5] (7 cm by 21 cm) and eluted with 1.5% methanol in methylene chloride. A very small, faint green band is eluted first (presumably ring modified material) followed by the major purple band of mesohemin dimethyl ester dimer. A small brown band remains at the top of the column (probably mono-esterified material). The dimer solution is filtered through a medium fritted glass funnel, and the volume is reduced by rotary evaporation to approximately 20 ml. Microcrystalline material (70% yield) may be obtained by the slow addition of heptane (*ca.* 50 ml) followed by reducing this volume to 20 ml and cooling. (Large needle-like crystals will result if the heptane addition is over the period of several days). If monomeric material is desired, the above methylene chloride solution of dimer (*ca.* 20 ml) is bubbled with HCl gas for 4–6 seconds followed by nitrogen gas for about 15

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minutes, and crystallization as above. (A dry atmosphere must be kept over the monomer solution or the bubbling of nitrogen will result in solution cooling and water condensation to yield dimer contamination).

Characterization

Mesohemin dimethyl ester as the chloride salt and as the μ-oxo-bridged dimer in methylene chloride solution exhibited characteristic absorption spectra [6, 7]. Homogeneity was demonstrated by thin layer chromatography of both species (on silica gel with 2.5% methanol in methylene chloride eluent). Cyclic voltammetric behavior was consistent with a single species in each case [8]. Iron analysis of the chloride monomer yielded 8.05% iron (8.07% calculated). Proton NMR examination of the low-spin bis-cyano monomeric complex is an excellent method for determining sample homogeneity as the hyperfine-shifted ring methyl resonances exhibit a characteristic downfield pattern [9]. Spectra were obtained on a Bruker HX-90E pulsed Fourier transform spectrometer operating at 90 MHz. A sample 0.015 *M* in monomer with a four-fold excess of potassium cyanide in methanol-d_4 was prepared with a TMS internal standard at 26 °C. Observation of only three downfield resonances at 16.8, 15.9, and 15.2 PPM with relative areas 1:1:2 confirms the presence of esterified mesohemin [9]. This species possesses the smallest spread of ring methyl resonances as compared to protoporphyrin and other 2,4-substituted derivatives [9]. It is expected that any other iron porphyrin impurity present in excess of about three percent would be discernible.

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