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A Convenient Small-Scale Synthesis of Protoporphyrin-IX-Dimethylester from Hemin

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There is at present extensive involvement of inorganic chemists in the chemistry of porphyrin and chlorin compounds. Questions pertinent to porphyrin structures of electronic ground and excited states, as well as the mechanism of energy transfer processes, arise from interest in porphyrin molecular properties. For these reasons we explore the presently described small-scale synthesis of protoporphyrin-IX-dimethylester (PP-DME), since this porphyrin and its metal substituted derivatives have been the subject of several studies.

Recently the crystal and molecular structure of protoporphyrin-IX-dimethylester (PP-DME) was determined [1]. Protoporphyrin-IX is a widely studied natural porphyrin due to its important role in various biological systems and because of its chemical reactivity. In particular, it is most susceptible to photooxidation at its two vinyl side-chains [2–4]. We report here a new detailed, small-scale synthesis procedure for protoporphyrin-IX-dimethylester which starts with conveniently available hemin (0.5–1 g). This procedure is in modification of the Grinstein method [5] which is reviewed in Falk's *Porphyrins and Metalloporphyrins* [6].

Early methods of isolation of protoporphyrin-IX (or its methyl ester analog) from whole-blood hemoglobin [5, 7, 8] are tedious and very time consuming. In the presently reported procedure the quantization of reagents is unique, and it differs from ordinary down-scaling of another procedure [9], which begins with a large quantity of hemin. The development of the column chromatography is also new in the use of alumina as the stationary phase. It is interesting that our yield of *ca.* 61% is close to the maximum possible (67%) in view of the existence of a hydrolysis equilibrium for Fischer esterification reactions [10]. The explicit synthesis procedure and characterization now follow.

Experimental

Reagents

Hemin (equine) was purchased from Sigma Chemical Company or K & K's ICN Pharmaceuticals, Inc. Pyridine (Fisher reag. grd.) was refluxed (16 hr) over NaOH pellets and distilled onto KOH pellets. Methanol (Fisher reag. grd.) was dried, freshly distilled [11] and stored over molecular sieves. $FeSO_4 \cdot 7H_2O$ (Matheson, Coleman & Bell) was powderized before usage. HCl gas (Air Products) was dried by bubbling through a conc. H_2SO_4 acid trap. $CHCl_3$ (Fisher reag. grd.) was washed with distilled water and dried over $CaCl_{2(s)}$ [11]. Benzene (Fisher reag. grd.) was freshly distilled onto molecular sieves. Alumina (Merck & Co., Inc.) was preheated in an oven (110 °C) before use as column chromatographic adsorbent.

Synthesis Procedure

Hemin (0.50 g) was dissolved in pyridine (2 ml) and the solution shaken vigorously for several minutes. Insoluble material was filtered off and the filtrate added to methanol (100 ml). Powderized $FeSO_4 \cdot 7H_2O$ (2 g) was then added to the solution. Next, dry HCl gas was vigorously bubbled through the reaction mixture (5–10 min) until the color became deep violet-red (PP-DME dication). The hot flask was cooled under tap water and the reaction mixture quenched with distilled water (500–600 ml). Three separate extractions with $CHCl_3$ (200 ml each time) were carried out. The combined $CHCl_3$ extracts were washed once with 2 *N* NH_4OH (400 ml) and then three times with distilled water (600 ml each time). The final volume of $CHCl_3$–ester solution was removed by reduced-pressure distillation on a Büchi Rotavapor-R evaporator; 3 ml of dry $CHCl_3$ was added to the residue.

Column Chromatography

A glass column (2.75 cm × 50 cm) was packed with an adsorbent slurry (300 ml Al_2O_3 and 200 ml benzene) and a thin layer of acid-washed (Ottawa) sand added to the top of the settled slurry. The column was also wrapped with Al foil to protect the photosensitive PP-DME from room lights. The 3 ml $CHCl_3$–ester sample was injected onto the column and elution with $CHCl_3$–benzene (1:9 v/v) caused moderate movement of the PP-DME (dark red-violet band), and this fraction (*ca.* 50 ml) was evaporated to dryness.

Recrystallization and Characterization

Dry $CHCl_3$ (10 ml) was added to the residue and the solution heated to boiling on a steam bath [12]. An equal volume of boiling methanol was added to the hot $CHCl_3$ mixture. Heating was continued until crystals formed on the surface of the solution upon slight cooling. At room temperature the crystals were filtered off and washed with dry, cold methanol (2 ml). Weight of recrystallized material: 0.276 g (61%); m.p.: 222–223 °C. *Electronic absorption spectra*

(in $CHCl_3$): Band I (629 nm), Ia (602 nm), II (575 nm), III (540 nm), IV (506 nm), Soret (407 nm).

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