Synthesis of a Hemin with Structurally Equivalent Propionic Acid Side Chains: 2,4-Dimethyldeuterohemin IX Dimethyl Ester*

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Recent investigations concerning the structure of metalloporphyrin complexes and the nature of their interaction with proteins, for example [1] have emphasized the need for a simple symmetrical porphyrin, closely related to protoporphyrin, to which either one or two ligand bearing groups may be covalently attached. The familiar derivatives of protoporphyrin IX are unsuitable because the propionic acid groups, by which ligands are most conveniently attached by ester or amide linkage, are nonequivalent. Therefore synthetic derivatives having two different groups on the propionic acid side chains are composed of two structural isomers which cannot be easily separated. The presence of the two isomers was thought to be responsible, at least in part, for difficulties we have encountered in obtaining crystalline protoheme derivatives suitable for X-ray crystallography. In order to eliminate this problem, we developed an improved synthesis for 2,4-dimethyldeuteroporphyrin IX dimethyl ester (I).

Fischer and Jordan [2] first synthesized this compound starting from substituted pyrroles, a tedious procedure for any laboratory not already possessing the requisite pyrrole derivatives. A more convenient route, starting with protoporphyrin dimethyl ester is shown in Eq. (1). Porphyrin I also has been synthesized in low yield by Chang [3] starting from deuterohemin.

Protorporphyrin IX dimethyl ester (II) was first oxidized with osmium tetroxide to the bis-glycol (III) and subsequently with sodium periodate to 2,4-diformyldeuteroporphyrin IX dimethyl ester (IV) as described by Sparatore and Mauzerall [4]. Compound III was purified by column chromatography in chloroform methanol on silica gel (yield, 69%). Compound IV crystallized from the reaction mixture (yield, 80%) and was not further purified since only one component was evident on thin layer chromatography, its visible spectrum was identical to that reported [4] and it was found to slowly decompose on standing.

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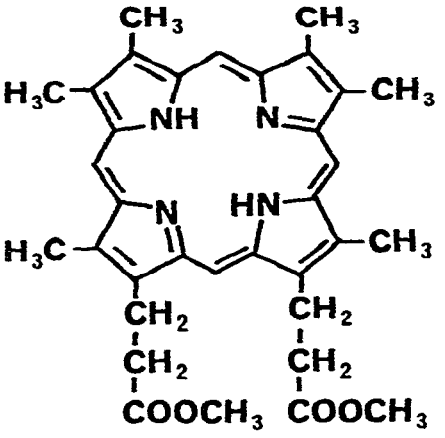
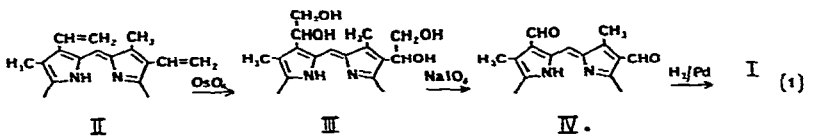


I

2,4-Dimethyldeuteroporphyrin IX dimethyl ester (I) was obtained by hydrogenation of IV with palladium black in anhydrous formic acid at 40 psi for 24 hr, essentially the same conditions employed by Fischer and Deilman [5] for the reduction of spirographis porphyrin. This reaction gave satisfactory yields (>50%) only at porphyrin concentrations less than 0.5 mg/ml. The product was isolated by dilution of the formic acid solution with water and extraction of the product into ether. Its final purification was accomplished by column chromatography on silica gel eluting with chloroform:methanol (50:1). The identity of 2,4-dimethyldeuteroporphyrin dimethyl ester was verified by its visible and nuclear magnetic resonance (nmr) spectra. The visible spectrum was identical to that reported for this compound by Fischer and Jordan [2] (maxima in $CHCl_3$ at 622, 569, 534, 493 nm with relative peak heights of 0.32, 0.52, 0.72, and 1.0, respectively), and very similar to the spectrum of mesoporphyrin dimethyl ester [6]. The nmr spectrum in $CDCl_3$ was characterized by signals at 9.95δ (methine), 4.38δ and 3.25δ (methylene), 3.65δ (ester methyls), 3.58δ and 3.49δ (ring methyls).

Iron was inserted into the porphyrin by a modification of the method of Warburg and Negelein [7]. The dipyridine hemochrome (1 M pyridine in benzene) was prepared by reduction of the hemin with CaH_2 and Pd as previously described [8]. The hemocrome spectrum (maxima at 547 and 517 nm) corresponds to that reported by Fischer and Jordan [2], and is nearly identical to the dipyridine mesohemochrome spectrum [6].

In order to prepare a derivative of I to which a single ligand bearing group could be attached easily, the monomethyl ester also was prepared. Partial hydrolysis of the diester was carried out in 6 N HCl as described by Asakura and Yonetani [9]. The reaction was terminated after 15 min by adding the HCl solution to solid $NaHCO_3$. The addition of excess tetrabutylammonium



hydroxide (25% in methanol) permitted extraction of all the porphyrin material into $CHCl_3$. After washing the $CHCl_3$ extract exhaustively with water the mono- and diacids precipitated overnight leaving unreacted diester in solution. The acids separated cleanly by chromatography on silica gel eluting with $CHCl_3$ containing increasing amounts of methanol.

It should be noted that the plane of symmetry possessed by 2,4-dimethyldeuterohemin renders the fifth and sixth coordination positions stereochemically equivalent. As a result, optical isomers of mixed hemo- and hemichromes (e.g., carboxyhemochrome, cyanide hemichrome) do not exist. However, when a ligand is covalently attached through a propionic acid side chain, in a manner analogous to compounds prepared by Chang and Traylor [1], Castro [10], van der Heijden et al. [11], and Warme and Hagar [12], the ligand can coordinate equally well with the Fe on either side of the porphyrin giving rise to a mixture of two enantiomers. The same applies to two attached ligands, whether or not they are the same. Although heme complexes derived from 2,4-dimethyldeuterohemin should be more readily crystallized than derivatives of proto or mesoheme, the crystals in some cases can be expected to be composed of a racemic mixture.

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