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Electronic States of Iron Oxyporphyrin and Verdohemochrome Obtained by Coupled Oxidation of Iron Porphyrin

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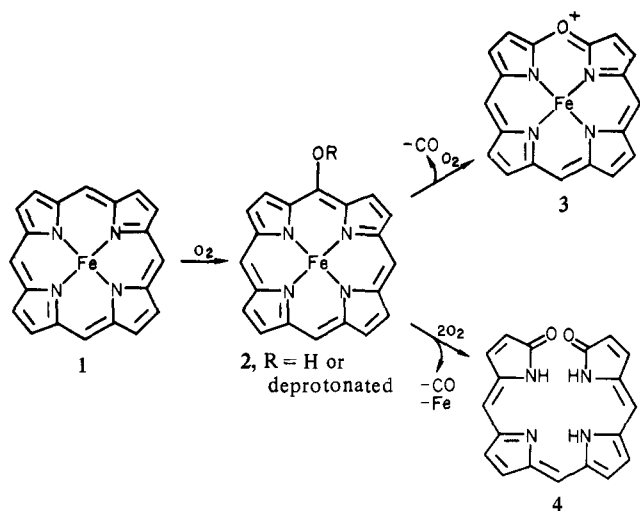
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Iron porphyrin (1) is oxidized by molecular oxygen or hydrogen peroxide to iron oxyporphyrin (2), which is further oxidized to verdohemochrome (3) by molecular oxygen.¹⁻⁵ Iron oxyporphyrin



(2) also has been detected as a primary product in the biological degradation of heme to biliverdin (4).^{6,7}

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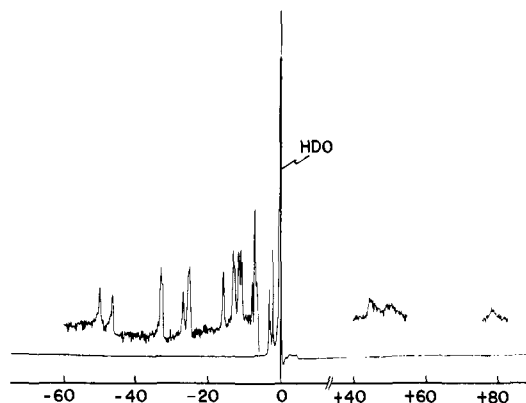
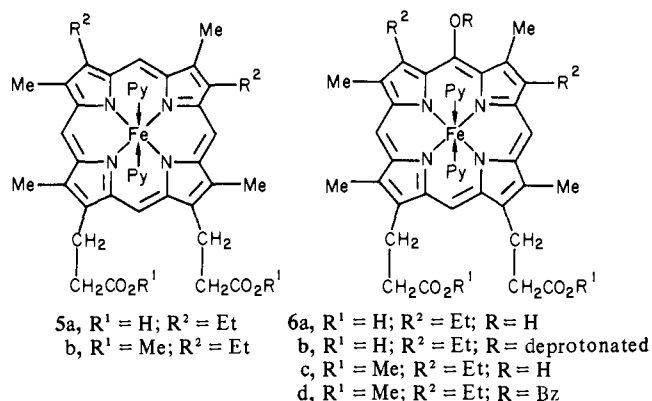


Figure 1. Iron oxymesoporphyrin (6b; ~2 mM) in 60% pyridine-*d* solution at room temperature. NMR spectrum was recorded with Varian HR-220/Nicolet TT-100 in a pulsed Fourier transfer mode.

The purpose of the present investigation is to elucidate the electronic and oxidation states of the iron of iron oxyporphyrin (2) and verdohemochrome (3) by using ESR, NMR, and Mössbauer techniques. We wish to report that the electronic state of iron oxyporphyrin 2 in pyridine is most likely low-spin Fe(I) and that of verdohemochrome low-spin Fe(II). Such assignments have not yet been made despite extensive studies carried out in the past decade.⁸⁻¹²

Iron oxymesoporphyrin 6a or 6b was prepared, according to



the method of Bonnett et al.⁵ with a minor modification, by reducing ferric mesoporphyrin dipyrindine 5a with ascorbate followed by addition of hydrogen peroxide. Compound 6b in a pyridine solution was stable under argon gas and showed Soret absorption band at 402 nm and a broad but significant band at 630 nm. This compound gave an anomalous ESR signal at $g_{\perp} = 2.30$ and $g_{\parallel} = 1.76$ at 77 K; the NMR spectrum of 6b was well resolved having the signal spreading from 10 to 50 ppm downfield from the HDO signal and exhibited signals locating at +45 to +80 ppm (Figure 1). This spectrum was entirely different from that of 5a. The Mössbauer spectra, however, showed no paramagnetic hyperfine interaction and the quadrupole splitting ($\Delta E_Q/(\text{mm s}^{-1}) = 1.18$ at 77 K and 1.15 at 4.2 K) and the isomer shift ($\delta\text{Fe}/(\text{mm s}^{-1}) = 0.37$ at 77 K and 0.49 at 4.2 K) resembled the corresponding parameters obtained with ferrous low-spin compounds.^{13,14}

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When oxygen was bubbled through a solution of iron oxymesoporphyrin for 1 min, verdohemochrome (λ_{\max} at 387, 493, 526 and 650 nm) was obtained. The anomalous ESR signals disappeared and no other signals were detected. The NMR spectrum was characteristic of a diamagnetic compound. These results and the quadrupole splitting, $\Delta E_Q/mms^{-1}$, of 1.24 at 77 K and 1.19 at 4.2 K as well as the isomer shift, $\delta Fe/mms^{-1}$, of 0.43 at 77 K and 0.44 at 4.2 K clearly indicated that verdohemochrome was low-spin Fe(II).

For quantitative preparation of iron oxymesoporphyrin an alternative procedure was undertaken, and the nature of the abnormal $g_{\perp} = 2.30$ signal was further investigated. This procedure involved the preparation of an intermediate **6d** by meso hydroxylation of pyridine mesohemochromogen dimethyl ester **5b**, followed by meso benzylation with benzoyl chloride, and a subsequent hydrolysis of the benzoyl and methyl esters with NaOH. The intermediate **6d** was extracted into a benzene layer, which was washed several times with 20% pyridine, 0.1 N HCl, and finally water and then evaporated to dryness in vacuo. The residue was dissolved in chloroform and chromatographed three times on a column of alumina oxide in chloroform-methanol (200:1, v/v) and then on LH-20 in chloroform-methanol (1:1, v/v). The chromatographic procedures were repeated until the purity criteria for **6d** were satisfied by TLC, HPLC, and spectroscopy in the visible region.

In order to facilitate hydrolysis of the compound **6d** and transfer of the hydrolysis product to an ESR tube under anaerobic conditions, a reaction vessel of a special design was employed. The vessel consisted of two side arms and a quartz ESR cell and was connected to a vacuum line through a glass taper joint. A stopcock below the joint aided isolation of the vessel from the vacuum line. One of the side arms contained **6d** in pyridine-sodium hydroxide and, when necessary, the other acid (barbituric acid or DCl). The reaction vessel was evacuated after several flushings with argon gas and removed from the vacuum line. After hydrolysis of both benzoyl and methyl esters of **6d** with NaOH at 70 °C for 1–1.5 h in the dark, the reaction mixture was transferred to the ESR cell. The ESR spectrum at 77 K exhibited a prominent signal at $g = 6.3$ (Figure 2A), which is characteristic of high-spin Fe(III) liganded with a hydroxy group. When the alkaline reaction mixture was allowed to contact with air by opening the stopcock of the reaction vessel, a new additional ESR species appeared showing a major radical signal at $g = 1.998$. This radical was spin trapped by *N-tert-butyl- α -phenylnitron* (BPN) (Figure 2B), and the spin adduct signal was analyzed in terms of the parameters $g = 2.006$, $a^N = 15.4$, and $a^H = 3.5$.¹⁵

On the contrary, when the hydrolysis product was brought to pH 9.5 by adding either barbituric acid or DCl anaerobically, the $g = 6.3$ signal disappeared, and concomitantly a strong and axially symmetric ESR spectrum with $g_{\perp} = 2.30$ and $g_{\parallel} = 1.76$ appeared as shown in Figure 2C. This spectral change is explained as due to the conversion of high-spin Fe(III) ($g = 6.3$) into a new iron electronic state ($g = 2.30$). In particular, the order of g anisotropy ($g_{\perp} > g_{\parallel}$) and the magnitude of the g value of 2.30 seem to be indicative of low-spin Fe(I) (d^7 , $S = 1/2$) with an unpaired electron in the d_{z^2} orbital. Indeed, it is known that Fe(I) of tetraphenylporphine generated by chemical or electrolytic reduction of tetraphenylporphine Fe(III) gives a similar spectrum ($g_{\perp} = 2.6$ – 2.26 and $g_{\parallel} = 1.93$); the variation of the g value as noted and the line shape are dependent on the nature of the solvent.^{16–18} Typical d^7 metal compounds with the $(d_{xz}, d_{yz}, d_{xy})^6(d_{z^2})^1$ electronic configuration such as low-spin Co(II)^{19,20} or Ni(III) tetra-

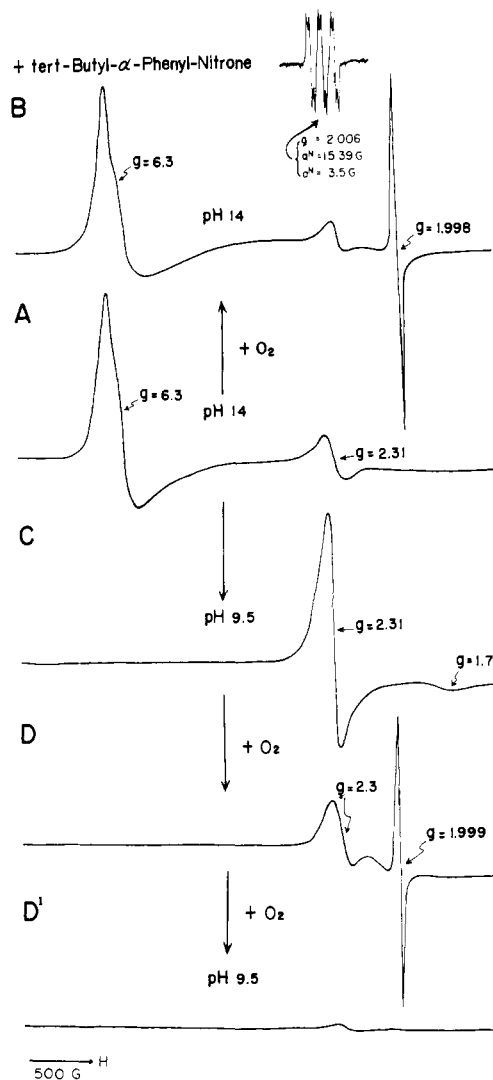
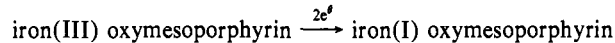


Figure 2. (A) Iron oxymesoporphyrin (**6b**; ~ 1 mM) in 60% aqueous pyridine solution containing 0.4 N NaOH; (B) air was bubbled through A and spin trapped with *tert-butyl- α -phenylnitron*; (C) pH of A was adjusted to 9.5 anaerobically by adding barbituric acid; (D) trace of air was introduced to C; (D') air was bubbled through D for another 1 min. ESR spectra were recorded with a JES-FE-3X spectrometer operating with 100-kHz magnetic field modulation.

phenylporphine²¹ also show the similar ESR signal near $g = 2.30$ and the same order of g anisotropy ($g_{\perp} > g_{\parallel}$) as described above. Consequently, a low-spin Fe(I) formulation seems to be the most reasonable spin/oxidation state assignment for the metal. This assignment requires that the Fe(III) of iron oxymesoporphyrin is reduced to Fe(I) through intramolecular transfer of two electrons even in the absence of any extraneous reducing reagent, as represented by the following equation.



Whether two electrons move from enolate **6b** to the iron or the first electron comes from the enolate and a second one from the porphyrin π -electron system is not yet clear. The Mössbauer data of iron oxymesoporphyrin, however, indicate the d^6 structure rather than d^7 for the iron, suggesting that an additional electron might have been taken up by the axial ligand. Such a mechanism is proposed for $Fe(CN)_5NO^{3-}$ to which the electronic configuration of $(d_{xz}, d_{yz}, d_{xy})^6d_{z^2}$ ligand rather than $(d_{xz}, d_{yz}, d_{xy})^6(d_{z^2})^1$ is assigned.²² A similar case was observed by Taube et al.²³ who interpreted

(15) These parameters seem to be close to those of $\cdot OR$ spin adduct of BPN. However whether the spin adduct is $O_2^{\cdot -}$ or $\cdot OR$ at the meso position or their mixture is not yet decided. We must determine the ESR spectra using $H_2^{17}O_2$. Verdohemochrome was not formed.

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their Mössbauer data of Lithium (iron) 4,5-(phthalocyanin)-tetrahydrofuran as indicative of a d^6 rather than d^7 iron. The NMR spectrum of **6b** was identical with that shown in Figure 1.

When the hydrolysis product at pH 9.5 was aerated for 1 min, the peaks at $g = 2.30$ and 1.76 disappeared (Figure 2D') with concomitant formation of ferrous low-spin verdohemochrome in a high yield. Thus the verdohemochrome formation from iron oxymesoporphyrin involves oxidation of Fe(I) to Fe(II) by molecular oxygen. This conclusion was also supported by the NMR and Mössbauer data. However, when the supply of oxygen was limited to a trace amount, the $g = 1.999$ signal appeared (Figure 2D), thus indicating the involvement of a free radical. Elucidation of the chemical nature of this radical (O_2^- or porphyrin π radical) is currently under way.

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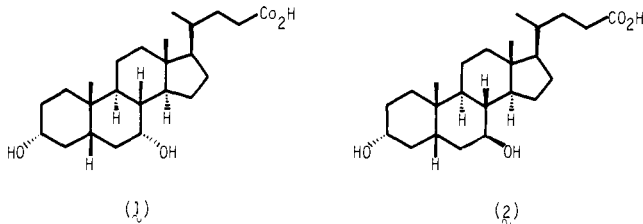
First Total Synthesis of (+)-Chenodeoxycholic Acid

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Chenodeoxycholic acid (**1**) is one of the two primary bile acids in man and recently has attracted much attention because of its clinical importance in the treatment of gallstones. Studies around the world, including countries where chenodeoxycholic acid (**1**) is now available for general medical use, have shown that about 60% of patients treated with chenodeoxycholic acid (**1**) have stone dissolution.¹⁻³ Ursodeoxycholic acid (**2**) has also been shown to



have almost the same activity as chenodeoxycholic acid for treatment of gallstones.⁴ These facts and the difficulties of obtaining a pure sample of chenodeoxycholic acid (**1**) by separating structurally closely related concomitants which prevent accurate biological evaluation of **1** prompted us to report the first, highly stereoselective total synthesis of (+)-chenodeoxycholic acid (**1**) in an optically pure form. One of the key strategies for this synthesis involved the use of olefinic benzocyclobutene **10** which has an α -acetoxy group on the cyclohexane ring to direct the stereochemical course of intramolecular cycloaddition of α -quinodimethane **11a** derived from thermolysis of **10** to form cis, anti, trans-D aromatic steroid **12** stereoselectively.⁵

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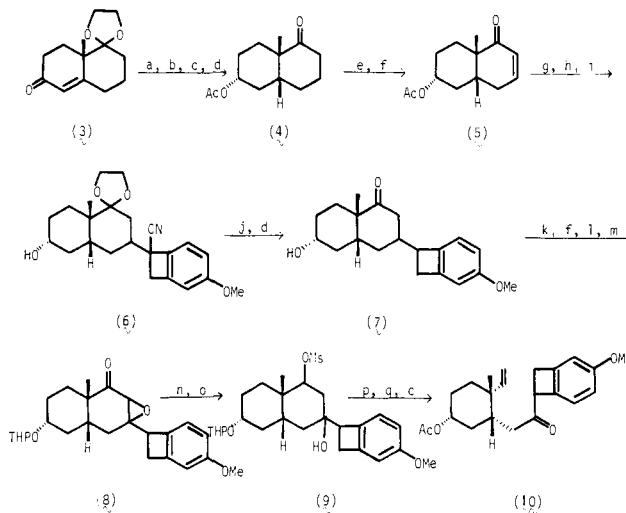
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Scheme I^a



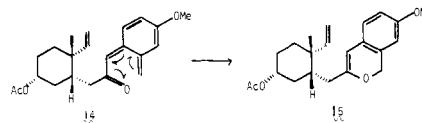
^a Reagents: (a) H_2 , Pd-C, EtOH, room temperature; (b) $NaBH_4$, MeOH, 0 °C; (c) Ac_2O , pyridine, room temperature; (d) 10% HCl, acetone, room temperature; (e) Br_2 , $CHCl_3$, room temperature; (f) LiBr, Li_2CO_3 , DMF, 125 °C; (g) 1-cyano-4-methoxybenzocyclobutene, $NaNH_2$, liq NH_3 , -78 °C; (h) $HOCH_2CH_2OH$, p -TsOH, C_6H_6 , reflux; (i) 5% NaOH, MeOH, room temperature; (j) Na, liq NH_3 , -78 °C; (k) pyridinium hydrobromide perbromide, $CHCl_3$, room temperature; (l) 30% H_2O_2 , 10% NaOH, MeOH, room temperature; (m) dihydropyran, p -TsOH, CH_2Cl_2 , room temperature; (n) $LiAlH_4$, THF, room temperature; (o) $MsCl$, pyridine, room temperature; (p) NaH, THF, reflux; (q) p -TsOH, room temperature.

The key intermediate, optically active [2-(benzocyclobutenyl)ethyl]cyclohexane **10**, was prepared from (8a*S*)-1,1-(1,2-ethylenedioxy)-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-oxonaphthalene (**3**) by the route shown in Scheme I.¹⁴ The optically active *cis*-octalone **5**, readily prepared in 56.2% overall yield from **3**, was converted into benzocyclobutene **7** in 83.7% overall yield from **5**, including Michael addition of 1-cyano-4-methoxybenzocyclobutene⁷ followed by reductive decyanation. The epoxide **8** derived in 73.5% overall yield from **7** in a usual manner was transformed into the key intermediate **10** in 52.2% overall yield from **8** via the fragmentation of hydroxy mesylate **9**. Thermolysis of **10** was conducted in boiling *o*-dichlorobenzene in a current of nitrogen for 45 min to afford *cis*, anti, trans-D aromatic steroid **12** stereoselectively in 42.7% yield.⁸ This was the first observation that the thermolysis of olefinic benzocyclobutene which has ethenyl and (benzocyclobutenyl)ethyl groups in *cis* relationship gave *cis*, anti, trans-fused steroidal compound stereoselectively. This stereoselectivity could be reasonably explained by the intervention

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ethenyl and (benzocyclobutenyl)ethyl groups, stereoselectivity of intramolecular cycloaddition reaction of α -quinodimethanes has been observed to give *trans*, anti, *trans*-fused steroidal compounds: (a) Kametani, T.; Suzuki, K.; Nemoto, H. *J. Chem. Soc., Chem. Commun.* 1979, 1127. *J. Org. Chem.* 1980, 45, 2204.

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