method[21] using redistilled thionyl chloride as solvent. The hexakis(thiocyanato)niobate(V) salts were prepared by using the method of Knox and Brown.[15]

The actual solutions that were used for spectroscopic study are described in Table I. All solutions were prepared as concentrated or saturated solutions with a niobium concentration in the range 0.5–1 M. The Cl:NCS:Nb ratios were generally 3:3:1, although these were varied from 5:1:1 to 1:5:1 in an attempt to detect additional resonance lines. All redistribution solutions, which were deep red in color, required one to several hours at room temperature to attain final equilibrium. Redistribution did not occur in 1,2-dichloroethane, and this was likely a function of the low dielectric constant of this solvent.

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Iron(II, III)–Chlorin and –Isobacteriochlorin Complexes. Models of the Heme Prosthetic Groups in Nitrite and Sulfite Reductases: Means of Formation and Spectroscopic and Redox Properties

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Abstract: Extensive series of iron(II, III)–hydroporphyrin complexes of the types Fe(P)L, $[Fe(P)LL']^{0,+}$, $[Fe(P)]_2O$, and Fe(P), with P = octaethylchlorin (OEC) and octaethylisobacteriochlorin (OEiBC) and L,L' = neutral or uninegative axial ligands, have been synthesized and isolated or generated in solution. Means of synthesis and reactivity properties of OEC and OEiBC complexes parallel those of octaethylporphyrin (OEP) complexes. This behavior, together with a detailed body of physicochemical properties (absorption, MCD, ^1H NMR, EPR, and infrared spectra and voltammetry), serves to identify all new complexes. Certain of the OEiBC species are pertinent as possible analogues of the siroheme prosthetic group of nitrite and sulfite reductases. Physicochemical properties of OEP, OEC, and OEiBC complexes at parity of axial ligation are compared in an attempt to identify any intrinsic features of isobacteriochlorin species that might render them particularly suitable for mediation of multielectron reductions of substrates as executed by siroheme enzymes. Properties such as Fe(III)/Fe(II) potentials and ν_{CO} of Fe(P)L(CO) and $Fe(P)(CO)_{1,2}$ were found to be nearly invariant to P, indicating little cis effect of these macrocycles, which are in different reduction levels. That property most dependent on macrocycle structure was found to be the potential for ring-based oxidation which increases in the order OEiBC < OEC < OEP. Comparative properties are discussed in some detail and are related to available information on sirohemes, including the question of axial ligaton in the native enzyme. This research affords the first comprehensive examination of the preparation and chemical, spectroscopic, and redox properties of iron(II, III)–hydroporphyrin complexes.

A substantial body of evidence now exists for a variety of heme-containing proteins and enzymes that demonstrates the presence of iron–hydroporphyrin prosthetic groups. Some dissimilatory nitrite reductases, which catalyze the reduction of NO_2^- to NO, contain heme *d*,[2-8] an iron–chlorin complex.[9] Assimilatory nitrite reductases as well as assimilatory and dissimilatory sulfite reductases, which catalyze the remarkable six-electron reductions $NO_2^- + 7H^+ + 6e^- \rightarrow NH_3 + 2H_2O$ and $SO_3^{2-} + 6H^+ + 6e^- \rightarrow S^{2-} + 3H_2O$, respectively, possess as a common prosthetic group an iron–isobacteriochlorin complex named siroheme.[10-14] This unit has been removed from several enzymes and examined separately.[11,13,15] It can be demetalated to afford the free macrocycle, sirohydrochlorin, for which structure **1** has been proven.[16] Spectroscopic studies of a number of enzymes,[12-14,17-20] especially of the assimilatory type, leave little doubt that siroheme is the substrate binding and activating site. Siroheme has also been detected in enzymes for which sulfide oxidase activity, i.e., the oxidation of sulfide to sulfite, has been proposed.[21]

Despite the seemingly obligatory presence of siroheme in the enzymes responsible for two of only three known six-electron

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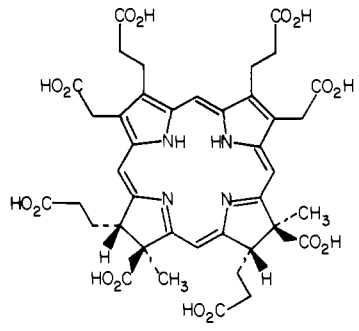

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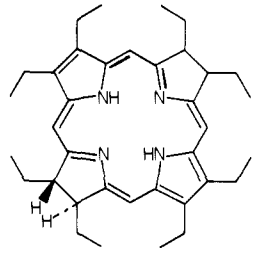

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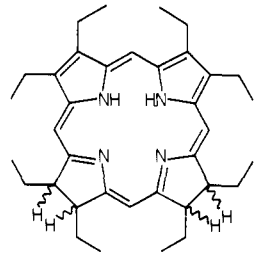

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reductions in biology, no evidence has yet been adduced as to the special competence of iron isobacteriochlorins (vs. other iron–tetrapyrrolic macrocycles) in mediating these reactions.[22] Indeed, given the ubiquity and diverse functions of unreduced heme groups in proteins and enzymes, one is led to question whether there exist any properties intrinsic to isobacteriochlorins and their metal complexes that would make them better suited to serve as a prosthetic group in nitrite or sulfite reductases than the corresponding complexes of other tetrapyrroles. The same point may be raised with regard to iron–chlorin prosthetic groups in dissimilatory nitrite reduction. Resolution of this question requires comparison of physicochemical and reactivity properties of porphyrins, appropriate hydroporphyrins, and their metal complexes—especially their Fe(II, III) complexes. In addition, comparison of spectroscopic properties of iron–chlorin and –isobacteriochlorin complexes with those of the respective heme *d*- and siroheme-containing enzymes could lead to identification, or at least a limitation of possibilities, of axial ligation in the various reaction states of the enzymes. This information, which would afford full identification of the iron coordination unit, is lacking for all reaction states at present and is essential to an ultimate description of enzymic action. Inasmuch as heme *d* and siroheme themselves are at present difficultly accessible in quantities required for extensive studies, an acceptable recourse is utilization of hydroporphyrins of corresponding oxidation levels and their iron complexes. However, the chemistry of such molecules, particularly of metallohydroporphyrins, is relatively poorly developed. Detailed studies of the synthesis and properties of isobacteriochlorins[23-27] have appeared only in the past 3 years, and reports of iron–isobacteriochlorin complexes[28,29] are no less recent.

In previous work we have examined spectroscopic and redox properties of octaethylchlorin (**2**, H_2(OEC)), octaethylisobacteriochlorin (**3**,[30] H_2(OEiBC)), and their Zn(II) complexes and compared them with those of octaethylporphyrin (H_2(OEP)) and its Zn(II) complex. It was found that OEiBC species are near-duplicate chromophores of the corresponding sirohydrochlorin species and that the most distinctive property of the former is the low potentials for ring oxidation compared to OEC and OEP species. Here we continue our investigations of octaethylhydroporphyrins and their complexes in the form of the first comprehensive study of the preparation and chemical, spectroscopic, and redox properties of $Fe^{II,III}$(OEC) and -(OEiBC) species. This study is a prerequisite not only to the desired comparisons above but also to a later examination of the interactions of these iron complexes with nitrite and sulfite in the presence of suitable electron carriers such as $[Fe_4S_4(SR)_4]^{3-}$ (synthetic analogues of the Fe–S prosthetic groups in these enzymes).

Experimental Section

Reagents and Solvents. Benzene, hexane, and toluene were purified by treatment with concentrated H_2SO_4, washed with water until the washings were neutral, dried over $CaCl_2$ and then over Linde 4A sieves, and distilled from sodium metal. Immediately before use THF was distilled from $LiAlH_4$, and methanol and ethanol were distilled from their respective magnesium alkoxides. Dichloromethane was distilled from CaH_2. *N*-methylimidazole was purified by treatment with CaH_2 followed by vacuum distillation. Pyridine was distilled and dried over 4A sieves. 2,6-Lutidine was passed through an alumina column and then distilled from $BF_3{\cdot}Et_2O$. 2,2,6,6-Tetramethylpiperidine and benzenethiol were distilled. *p*-Nitrobenzenethiol (technical grade) was purified[31] and *p*-(trifluoromethyl)benzenethiol was prepared[32] by literature methods. The disulfides of these thiols and of benzenethiol and *p*-chlorobenzenethiol were prepared by oxidation of the appropriate sodium thiolate with iodine in methanol and were purified by recrystallization from ethanol. The anhydrous compounds ferrous acetate,[33] basic ferric acetate,[34] and ferrous bromide[35] were prepared as described; a commercial sample (CERAC) of the latter compound was also used. (n-Bu_4N)(OPh) was isolated from the reaction of (n-Bu_4N)(OH) with phenol in water and dried under vacuum. Alumina (Woelm) used for chromatography of iron hydroporphyrins was activated and degassed by vacuum drying in a Schlenk flask at 110 °C for 48 h followed by multiple flushes with dinitrogen and reevacuations of the flask. It was stored under dinitrogen. Dinitrogen gas was purified by passage through columns of BASF Catalyst R3-11 (120 °C) and anhydrous calcium sulfate (Drierite). Carbon monoxide was purified by passage through columns of BASF Catalyst R3-11, Ascarite, and Drierite. $^{18}O_2$ (99.1% ^{18}O) was obtained from Prochem. Other reagents and solvents were the best available commercially and were used without further purification.

Preparation of Compounds. Iron–porphyrin and –hydroporphyrin complexes are light- and dioxygen-sensitive to varying degrees; consequently, these complexes were prepared and manipulated under an argon or a dinitrogen atmosphere. New iron–hydroporphyrin complexes were found not to crystallize well, presumably because of the existence of diastereomeric mixtures, and were isolated as lyophilized powders. Analytical data were not obtained for these compounds because of the insensitivity of analyses to the extent of ring reduction and, in some cases, because of the small quantities of material isolated. The collective physical properties described in the text suffice to identify new compounds and to limit impurity levels of other porphyrinoid materials to no more than a few percent. H_2(OEiBC)[23] and H_2(OEC)[36] were obtained

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by published methods. Hereafter P represents, as appropriate, any or all of the macrocycle dianions OEP^{2-}, OEC^{2-}, and $OEiBC^{2-}$.

Fe(P)Cl. Fe(OEP)Cl and Fe(OEC)Cl were prepared as described.[36] Fe(OEiBC)Cl was synthesized by using a modification of Fischer's metalation procedure.[37] H_2(OEiBC) (25 mg), 50 mg of $Fe(OAc)_2$, 40 mg of NaOAc, 25 mg of NaCl, and 15 mL of acetic acid were placed in a 50-mL Schlenk flask equipped with a stir bar. Stirring was initiated, and the solution was brought to a gentle reflux. As the insertion of iron proceeded, the color changed from purple to a dark blue-green, and all fluorescence was quenched. After 3 h at reflux temperature, the reaction was cooled. Solvent was removed under vacuum, and the resulting residue was washed five times with distilled water or until the washings no longer contained iron. The remaining solid was dried under vacuum; after being dried, the product was dissolved in benzene and lyophilized to afford a greenish black solid, Fe(OEiBC)Cl.

$[Fe(P)]_2O$. (a) The standard reaction conditions for $[Fe(OEP)]_2O$ and other μ-oxo compounds[38-40] afford the corresponding OEC and OEiBC complexes. A dichloromethane solution of Fe(P)Cl was treated with an excess of a 25% aqueous KOH solution in a Schlenk flask. After the mixture was stirred overnight, the KOH layer was removed and discarded. The organic layer was washed with distilled water until the washings were neutral. It was then dried over anhydrous sodium sulfate and filtered, and the solvent was removed under vacuum. Spectral examination of reaction products revealed in some preparations that oxidative dehydrogenation had occurred, resulting in impurity levels of μ-oxo complexes of the more oxidized macrocycle(s). Chromatography of a dichloromethane solution of the reaction products on basic alumina (Grade 3) separated all μ-oxo complexes from any other porphyrinoid species and separated $[Fe(OEP)]_2O$ from μ-oxo hydroporphyrin complexes. No separation was achieved between $[Fe(OEiBC)]_2O$ and $[Fe(OEC)]_2O$, the most probable impurity in the preparation of the former complex. Chromatographed solutions were taken to dryness under vacuum, the residue was dissolved in benzene, and the solution was lyophilized to afford $[Fe(P)]_2O$ as a brown (OEP), purple (OEC), or black (OEiBC) solid. Their solutions are red-green, green, and green-black in color, respectively. (b) Several milligrams of Fe(P) (vide infra) were dissolved in benzene. An immediate color change from that of the initial complex to that of the μ-oxo species ensued upon addition of several milliliters of air (or $^{18}O_2$) to the reaction flask. After 1 min the solution was frozen and the benzene was removed under vacuum to afford lyophilized μ-oxo complex. This method is applicable to P = OEP, OEC, and OEiBC.

Fe(P). (a) To a $\sim 10^{-3}$ M solution of $[Fe(P)]_2O$ in benzene was added a large excess (>200-fold) of ethanethiol, and the solution was allowed to stand overnight. With P = OEP purple crystals formed and were collected by filtration. In other cases all volatiles were removed under vacuum. The resulting solid was dissolved in benzene and lyophilized to afford a dark green or black powder for P = OEC or OEiBC, respectively. Solutions of Fe(P) are orange-red, green, or dark blue-green in color for P = OEP, OEC, and OEiBC, respectively. No ethanethiol was detectable in the ^1H NMR spectra of these compounds in C_6D_6.

(b) The direct Fe(II) metalation method[41] has been modified and applied to H_2(OEC) and H_2(OEiBC). To a mixture of 10 mg of H_2(P) and 20 mg of $FeBr_2$ in 5 mL of benzene was added 20 μL of 2,6-lutidine and 2 mL of THF. The reaction flask was placed in an 80 °C oil bath and stirring was initiated. After 6 h the reaction mixture was cooled and solvents were removed under vacuum. The residue was dissolved in a 2% methanol in benzene solution and was filtered through a 2-cm deep bed of degassed alumina. Elution with the same solvent was continued until the solution being collected was no longer highly colored. The collected solution was taken to dryness, and the solid was dissolved in benzene. Lyophilization of the benzene solution of the resulting solid afforded Fe(OEC) or Fe(OEiBC), by absorption spectrum identical with that obtained by method a.

Fe(P)L. Fe(OEP)OAc was prepared by metalation of H_2(OEP) with $Fe_3O(OAc)_7$ and was recrystallized from acetic acid.[31] Fe(OEP)OPh was prepared by the cleavage of $[Fe(OEP)]_2O$ with phenol in toluene.[31,38,40] Except for those described in the following section, spectroscopic samples of all other Fe(P)L or $Fe(P)L_2$ species were generated in solution by methods outlined in the text.

$Fe(P)L_2$, Fe(P)L(CO), and Fe(P)(CO). The same procedure was used to generate these species for visible and IR spectroscopy. Visible spectra were recorded by using $\sim 10^{-4}$ M benzene solutions in a Schlenk flask equipped with an optical cell (1-mm path). IR spectra were obtained by using $\sim 10^{-3}$ M dichloromethane solutions and a specially designed Schlenk IR liquid cell (0.2-mm path length). For generation of $Fe(P)L_2$ and Fe(P)L(CO) species (L = py, *N*-MeIm) a solution of Fe(P)Cl of appropriate concentration was prepared. The reaction was initiated by adding 200 equiv of L, 20 equiv of benzenethiol, and 2 equiv of triethylamine. The mixture was stirred until the formation of $Fe(P)L_2$ was complete, as judged by visible spectroscopy. This period was overnight for P = OEP and on the order of several hours for P = OEC and minutes for P = OEiBC. The hemochrome was converted to the carbonyl species by freezing the solution in liquid N_2 (to minimize solvent loss), evacuating the reaction vessel, and refilling it to 400 torr of pressure with CO. Fe(P)(L)(CO) formed immediately upon thawing. $Fe(P)(CO)_2$ and Fe(P)(CO) complexes were produced by distilling dichloromethane into the Schlenk IR cell reservoir containing solid Fe(P). The mixture was frozen (to prevent formation of Fe(P)Cl, observed at ambient temperature), and CO, typically at 300 torr, was admitted. The mixture was allowed to warm to room temperature and was transferred to the cell, and the IR spectrum was recorded.

Physical Measurements. All measurements were performed with exclusion of air and moisture. Absorption spectra were recorded in Cary Model 14, 17, and 219 spectrophotometers. MCD spectra were obtained with a JASCO Model J-40 spectrometer containing a 15.0-kG electromagnet with field direction parallel to the direction of light propagation. All visible and MCD spectra (excluding those in Figure 7) were recorded, normalized, smoothed, and manipulated on a Data-General Nova Model 840 computer. ^1H FT NMR spectra were obtained at 100 and 360 mHz by using Varian XL-100-15 and Bruker HXS-360 spectrometers, respectively; chemical shifts are referenced to Me_4Si internal standard. EPR measurements were made with a Varian E-12 spectrometer operating at X-band frequencies; sample temperatures were 80–85 K. Infrared spectra were measured by using a Nicolet Series 7000 FT spectrometer. Electrochemical measurements were performed as described;[23] the working electrode was a Pt disc and potentials are quoted vs. a saturated calomel electrode.

Results

Prior to 1979 the complexes Fe(OEC)X (X = Cl,[36,39] F, Br, I[39]) and $[Fe(OEC)]_2O$,[39] derived from the *trans*-chlorin **2**, had been prepared and isolated. Absorption spectral comparison of a species described by Eisner[42] as "iron octaethylchlorin" with that presented here for $[Fe(OEC)]_2O$ (vide infra) reveals that the former is actually the μ-oxo species. EPR properties of $[Fe(TPC)(Im)_2]^+$ and other low-spin complexes formed from *meso*-tetraphenylchlorin derivatives have been reported.[43] More recently, Richardson et al.[26] and Chang, Fajer, et al.[29] have examined redox and spectroscopic properties of Fe^{III}–Cl and several low-spin Fe(II) complexes of diastereomeric dimethyl-*gemini*-octaethylisobacteriochlorins, H_2(DMOEiBC).[27] We have reported redox potentials of Fe(OEiBC)Cl in relation to other members of the Fe(P)Cl series and partial EPR spectra of several Fe(OEiBC)(SR) species.[28] These and all other OEiBC complexes in this study consist of a mixture of two or more diastereomers owing to the presence of two diastereomers (C_2, C_s symmetries) of **3**.[23,24b] Amongst synthetic isobacteriochlorins substituted at pyrroline carbon atoms, only certain octamethyl derivatives have been prepared in isomerically pure form.[24ad] Their Fe complexes have not been reported. These macrocycles and H_2(DMeOEiBC), as sirohydrochlorin (**1**), carry two gem-disubstituted pyrroline rings and thus are resistant to oxidative dehydrogenation to chlorins or porphyrins. H_2(OEiBC) and its complexes are susceptible to such reactions, and formation of chlorin from the former has been observed under oxidizing conditions.[23] However, in the absence of dioxygen and under subdued illumination the Fe–OEiBC species examined here, whose macrocycle is prepared in one reaction step from H_2(OEP),[23] are adequately stable and conveniently accessible by the procedures described.

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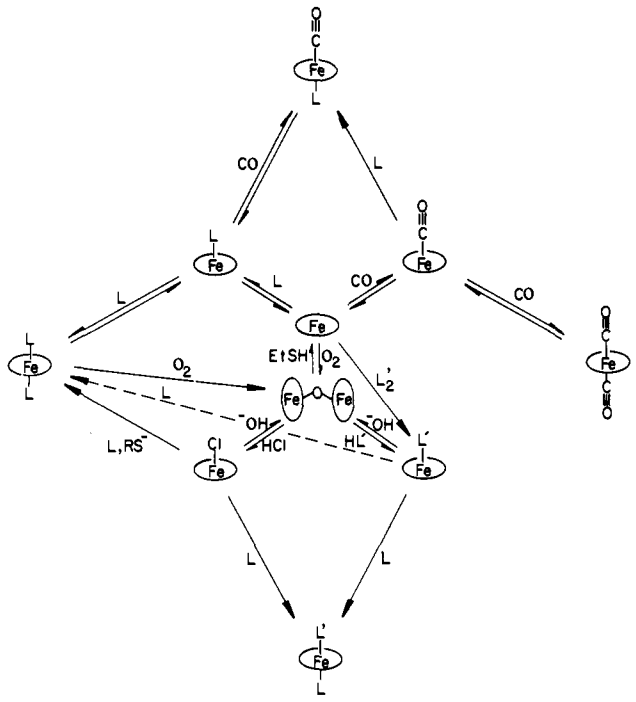

Figure 1. Generalized reaction scheme for $Fe^{II,III}(P)$ complexes: P = ⊖ (OEP, OEC, OEiBC); L and L′ are neutral or uninegative axial ligands. All species lying above and below the μ-oxo-iron(III) complex contain Fe(II) and Fe(III), respectively.

Preparation and Reactions of Fe(II,III)(OEC) and -(OEiBC) Complexes. The synthetic interrelationships of iron hydroporphyrins are set out in schematic form in Figure 1. Reactions of OEP, OEC, and OEiBC complexes differ less in kind than in degree, with the greatest difference resulting from the narrower range of conditions under which iron hydroporphyrins are stable. In addition, complexes of the three macrocycles differ in terms of possible isomer content. The two faces of OEP, *trans*-OEC, and C_2 OEiBC are equivalent, but the faces of C_s OEiBC are prochiral. This situation leads to the following enumeration of isomers in the OEiBC series of complexes: Fe(P) and $Fe(P)L_2$, 2; Fe(P)L and Fe(P)LL′, 3; $[Fe(P)]_2O$, 7. In the corresponding OEP and OEC series there is a single isomer of each species excepting $[Fe(OEC)]_2O$ for which there are two diastereomers. In this section the more important chemical interrelationships are briefly described. Physicochemical data supporting identification of reaction products are presented in subsequent sections.

The reactions in Figure 1 are analogous of those of iron porphyrins.[44] The usual entry to the manifold of iron–porphyrin complexes, viz., metalation of a free base at elevated temperatures to afford an iron(III) halide species, can be accomplished with the hydroporphyrins. With H_2(OEiBC), however, Fe(III) salts must be avoided for they cause oxidation, presumably to chlorin, at a rate competitive with metalation. Absorption spectra of three Fe(P)Cl complexes are compared in Figure 2. Base hydrolysis of these complexes affords the μ-oxo species $[Fe(P)]_2O$, whose spectra are shown in Figure 3. Occasional oxidative dehydrogenation of the hydroporphyrin complexes was observed in these reactions. Because $[Fe(OEC)]_2O$ and $[Fe(OEiBC)]_2O$ proved resistant to chromatographic separation (on basic alumina), their preparation, especially that of $[Fe(OEiBC)]_2O$, is better achieved by oxygenation of Fe(P) (vide infra).

The μ-oxo complexes are especially convenient for formation of five-coordinate Fe(III) species by generalized bridge cleavage reaction 1, which is well precedented with porphyrin complexes.[31,38-40,45] By this means a wide variety of species with L =

$$(P)Fe\text{–}O\text{–}Fe(P) + 2HL \rightarrow 2Fe(P)L + H_2O \qquad (1)$$

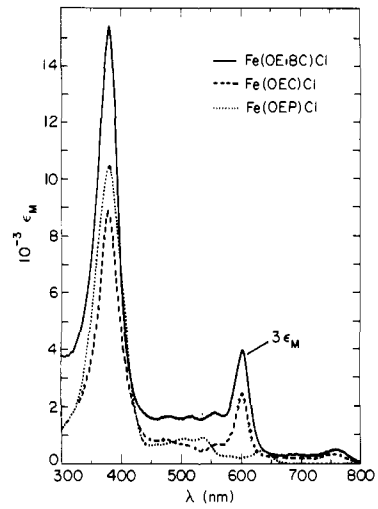

Figure 2. Absorption spectra of Fe(P)Cl complexes in dichloromethane solutions.

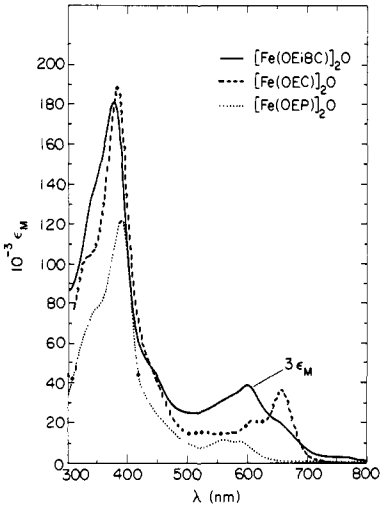

Figure 3. Absorption spectra of $[Fe(P)]_2O$ complexes in dichloromethane solutions.

OAc^-, RO^-, ArO^-, and ArS^- was generated and employed in the electrochemical and spectroscopic studies described below. The reactions (1) were conducted at ambient temperature in toluene or dichloromethane solution by using an ~10-fold excess of acid HL and were monitored spectrophotometrically. In every case isosbestic points were observed, inferring that clean conversion to a single product occurs. Reactions were found to be qualitatively faster in dichloromethane than in toluene and to increase in rate with an increase in the (aqueous) acidity of HL. Finally, $[Fe(OEP)]_2O$ reacted faster than the μ-oxo complex of either hydroporphyrin (of the two, $[Fe(OEC)]_2O$ reacted slightly faster), suggesting that the out-of-plane ethyl groups in the hydroporphyrins hinder access to the bridging oxygen. We found that reactions run in dichloromethane solution $\sim 10^{-4}$ M in μ-oxo complex with a tenfold excess (based on iron) of acidic reagent went to completion in an convenient period of time. Spectra of the generated complexes Fe(P)(OPh) and Fe(P)(SPh) are shown in Figures 4 and 5, respectively.

Based on the observations that reaction 1 when conducted with OEP[31] or hydroporphyrin μ-oxo complexes and *alkyl*thiols does not afford stable iron(III) thiolate species, a convenient synthesis of unligated Fe(P) has been developed. Treatment of a benzene solution of a μ-oxo complex with a large excess of ethanethiol results in crystallization of Fe(OEP) from the solution. Fe(OEC)

(44) J. W. Buchler in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, Chapter 5. J. W. Buchler in "The Porphyrins", Vol. I, D. Dolphin, Ed., Academic Press, New York, 1978, Chapter 10.

(45) J. P. Collman, T. N. Sorrell, and B. M. Hoffman, *J. Am. Chem. Soc.*, **97**, 913 (1975).

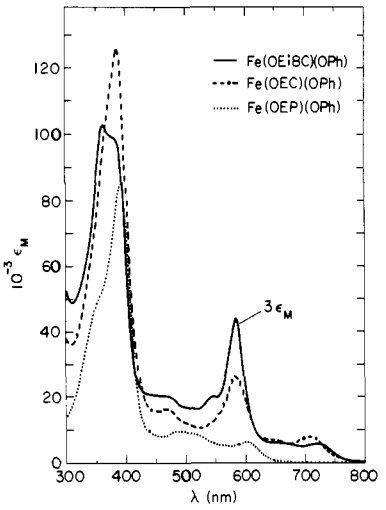

Figure 4. Absorption spectra of Fe(P)(OPh) complexes in dichloromethane solutions.

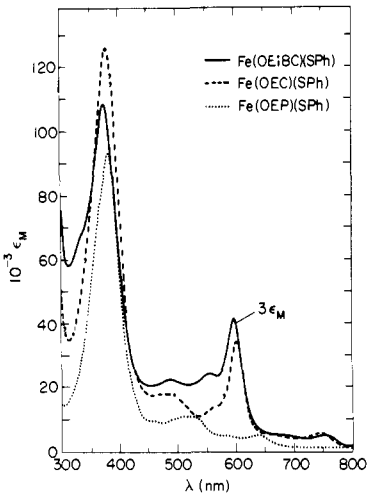

Figure 5. Absorption spectra of Fe(P)(SPh) complexes in dichloromethane solutions.

and Fe(OEiBC) are more soluble and were obtained as lyophilized solids. The complexes are presumably formed by reactions 2 and 3, in which the intermediate iron(III) alkylthiolate, proposed on

$$(P)Fe\text{–}O\text{–}Fe(P) + 2RSH \rightarrow 2Fe(P)(SR) + H_2O \quad (2)$$

$$Fe(P)(SR) \rightarrow Fe(P) + {}^1/_2RSSR \quad (3)$$

the basis of successful generation of analogous arylthiolate species, is unstable to autoreduction. Fe(OEC) and Fe(OEiBC) can also be readily obtained by direct metalation of the free-base macrocycles with $FeBr_2$ in the presence of a hindered base; this procedure is an extension of the method of Collman et al.[41] for Fe(II) metalation of porphyrins. For iron(II) hydroporphyrins these procedures are preferred to other methods of preparing iron(II) porphyrins such as reduction of Fe(III) complexes with $Cr(acac)_2$[46] or dithionite[47] and vacuum pyrolysis of hemochromes.[48] Reaction conditions are milder and isolation procedures are simpler and provide less opportunity for oxidation of the extremely reactive

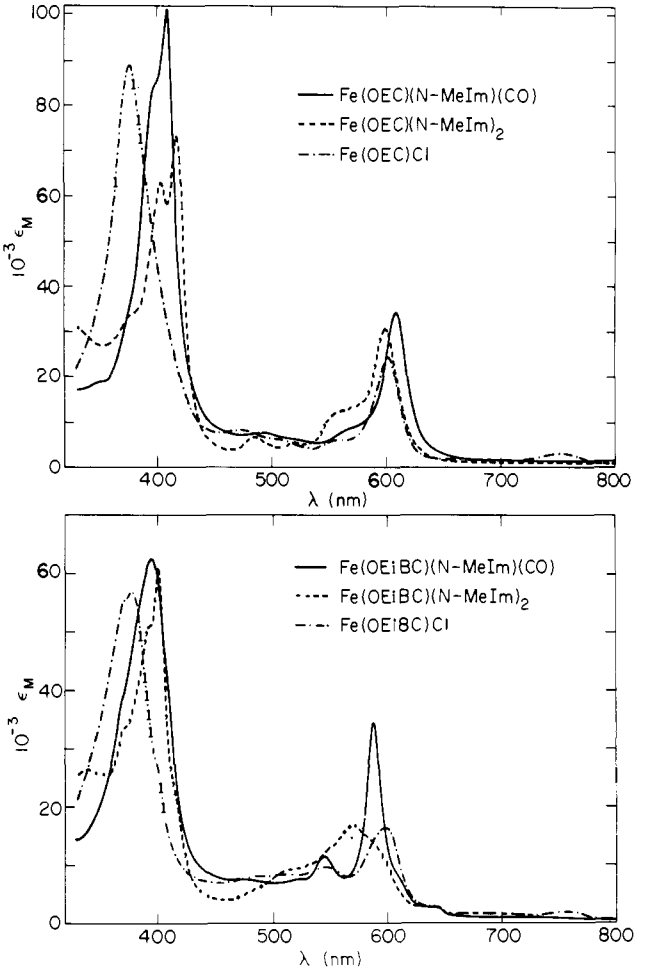

Figure 6. Absorption spectra demonstrating the conversion of Fe(P)Cl to Fe(P)(*N*-MeIm)$_2$ and the latter to Fe(P)(*N*-MeIm)(CO), reactions 5 and 6, in benzene solution at ambient temperature: upper, P = OEC; lower, P = OEiBC.

Fe(P) complexes. These complexes have proven synthetically useful. As with porphyrins,[44] iron(II) hydroporphyrins readily oxygenate to $[Fe(P)]_2O$ (reaction 4) at rates much faster than

$$2Fe(P) + {}^1/_2O_2 \rightarrow [Fe(P)]_2O \quad (4)$$

any further oxidation of the μ-oxo products. This reaction has been used to prepare ^{18}O-labeled species for infrared examination. The reverse of reaction 3 can be effected in the presence of excess disulfide. Aromatic disulfides, especially those containing electron-withdrawing substituents (e.g., $(p\text{-}CF_3C_6H_4S)_2$, $(p\text{-}O_2NC_6H_4S)_2$), present in moderate excess cause complete oxidation of Fe(P) to Fe(P)(SAr). As shown in Figure 1, Fe(P) complexes provide direct access to mono- and biscarbonyl complexes, by reaction 5, and to the hemochrome-type species $Fe(P)L_2$

$$Fe(P) \xrightarrow[K_1]{CO} Fe(P)(CO) \xrightarrow[K_2]{CO} Fe(P)(CO)_2 \quad (5)$$

and Fe(P)L(CO). The latter complexes are also conveniently prepared by use of earlier observations that both (alkylthiolato)- and (arylthiolato)iron(III)–porphyrin complexes tend to be unstable to autoreduction in the presence of nitrogeneous ligands L at ambient temperature.[31,45,49,50] Treatment of Fe(P)Cl with excess L = py or N-MeIm and benzenethiol, reactions 6 and 7, easily affords the desired compounds. Spectra of hydroporphyrin complexes with L = N-MeIm are set out in Figure 6.

(46) J. P. Collman, J. L. Hoard, N. Kim, G. Lang, and C. A. Reed, *J. Am. Chem. Soc.*, **97**, 2676 (1975).
(47) (a) D. Brault, M. Rougee, and M. Momenteau, *J. Chim. Phys. Phys.-Chim. Biol.*, **68**, 1621 (1971); (b) D. Brault and M. Rougee, *Biochemistry*, **13**, 4591 (1974); (c) T. Mincey and T. G. Traylor, *Bioinorg. Chem.*, **9**, 409 (1978).
(48) J. O. Alben, W. H. Fuchsman, C. A. Beaudreau, and W. S. Caughey, *Biochemistry*, **7**, 624 (1968); J. C. Maxwell and W. S. Caughey, *ibid.*, **15**, 338 (1976); D. Dolphin, J. R. Sams, T. B. Tsin, and K. L. Wong, *J. Am. Chem. Soc.*, **98**, 6970 (1976).
(49) H. H. Ruf and P. Wende, *J. Am. Chem. Soc.*, **99**, 5499 (1977); H. H. Ruf, P. Wende, and V. Ullrich, *J. Inorg. Biochem.*, **11**, 189 (1979).
(50) H. Ogoshi, H. Sugimoto, and Z. Yoshida, *Tetrahedron Lett.*, 2289 (1975).

$$Fe(P)Cl + 2L + PhSH + Et_3N \longrightarrow Fe(P)L_2 + \tfrac{1}{2}PhSSPh + Et_3NHCl \quad (6)$$

$$Fe(P)L_2 \xrightarrow{CO} Fe(P)L(CO) + L \quad (7)$$

Physicochemical Properties. Detailed in the following sections are the results of the first investigation of appreciable scope dealing with the spectroscopic and electrochemical properties of iron(II, III) hydroporphyrins. Corresponding information on OEP complexes, which as a group have been investigated in some detail previously, are included principally for purposes of comparison.

(a) Electronic Spectra. Spectra of analogous $Fe^{II,III}$OEP, -OEC, and -OEiBC complexes are presented in Figures 2–6 and serve as the simplest means of their identification. Quantitative data for these and other hydroporphyrin complexes of interest are collected in Table I. Results for the more familiar OEP complexes are restricted to the figures. Spectra of other Fe(II, III) complexes of OEP and related porphyrins, with the same or similar axial ligands in a number of cases, are available elsewhere.[31,38,40,44,47ab,50-52] With the exception of $Fe(P)L_2$ species (L = py, N-MeIm), porphyrin and hydroporphyrin complexes are differentiated by the appearance of a prominent and (usually) narrow visible band at ca. 550–600 nm in the spectra of the latter. Excluding $Fe(P)L_2$ and Fe(P)L(CO) species, the usual order of extinction coefficients of Soret and visible bands of analogous complexes is OEC > OEP > OEiBC. The order OEC > OEiBC holds in all cases. The ratio of intensities of the Soret band to the strongest visible band in analogous complexes is $\gtrsim$10 for OEP, $\gtrsim$4–5 for OEC, and $\lesssim$4 for OEiBC.

Inspection of results of the iron(III)–hydroporphyrin series Fe(P)L reveals several modest regularities with variant L and constant P. A progressive red shift of the prominent visible band occurs with increasing acidity of the conjugate acid of ligand L.[53,54] Thus in the OEiBC series band maxima of thiolate and phenolate species fall in the orders (8) and (9), respectively. Among the

$$SEt < SPh < SC_6H_4\text{-}4\text{-}NO_2 \quad (8)$$

$$OC_6H_3\text{-}3,4\text{-}(CH_3)_2 \lesssim OPh < OC_6H_4\text{-}3\text{-}NO_2 < OC_6H_4\text{-}4\text{-}NO_2 < OC_6H_3\text{-}2,4\text{-}(NO_2)_2 \quad (9)$$

ligands examined this behavior causes some overlap of band maxima of anionic oxygen-donor ligand (569–600 nm (OEC), 575–595 nm (OEiBC)) and thiolate complexes (602–605 nm (OEC), 590–599 nm (OEiBC)). However, it is always the case that thiolate complexes absorb at longer wavelengths than their oxygen analogues (e.g., Fe(OEiBC)(SPh) vs. Fe(OEiBC)(OPh)). The results in Table I[53] for both Soret and visible features make unlikely secure identification of the axial ligand(s) in oxidized (resting) forms of sulfite and nitrite reductases by absorption spectral criteria alone, a matter made evident by the rather small spectral changes among Fe(OEiBC)L complexes intended to simulate cysteinate, tyrosinate, and carboxylate ligation. The spectral data presented here should prove more valuable in identifying iron(II, III) chlorin vs. isobacteriochlorin prosthetic groups.

(b) MCD Spectra. MCD spectroscopy has proven of utility as an empirical diagnostic tool for deducing certain structural and electronic elements of heme (porphyrin) prosthetic groups. Examination of synthetic complexes has led to identification of a cysteinate axial ligand in the Fe(III) substrate-bound[55] and Fe(II) carbonyl[56] states of cytochrome P-450. In addition MCD spectroscopy has been shown capable of differentiating iron–porphyrin complexes possessing unlike oxidation and/or spin states.[41] In view of these results and the overlapping ranges of visible bands at nominal parity of axial ligand donor atoms, the MCD spectra of parallel series of Fe(P)L species have been obtained. Spectra obtained in the OEP series are very similar to those of protoporphyrin IX dimethylester (PPIXDME) complexes[51,55] and are not shown. Those in the OEC and OEiBC series with the biologically relevant ligands L = PhO^-, OAc^-, and PhS^- are presented in Figure 7. In the OEC series different axial ligation modes are distinguishable, especially PhS^- vs. oxygen ligands, principally by changes in the Soret region. Spectral differences in the porphyrin series, however, are more pronounced in that oxygen ligand complexes show roughly equal positive and negative ellipticities and thiolate complexes pronouncedly negative ellipticities in the Soret region.[51,55] On the other hand, the most conspicuous feature of Fe(OEiBC)L spectra is their similarity. In fact, the spectra from 300 to 750 nm are virtually independent of the axial ligand. This would seem to suggest a lack of interaction of the axial ligand with isobacteriochlorin π and π^* orbitals, at least for these high-spin Fe(III) complexes. Whether these observations hold true for isobacteriochlorin complexes in other oxidation and spin states remains to be established.

(c) Infrared Spectroscopy. (i) **$[Fe(P)]_2O$.** Owing to the importance of μ-oxo complexes as precursors in reactions 1 and 2, we have sought to characterize them by several techniques including IR spectroscopy. Spectra (1100–750 cm^{-1}) of $[Fe(P)]_2O$ complexes prepared by reaction 4 using natural abundance dioxygen and $^{18}O_2$ are given in Figure 8. Comparison of these spectra as well as those of the pairs Fe(P)Cl/$[Fe(P)]_2O$ revealed appreciable differences only in the 900–800 cm^{-1} region, where one or two broad bands corresponding to the Fe–O–Fe asymmetric stretch normally appear.[57,58] In this way the strong broad band of $[Fe(OEP)]_2O$ at 881 cm^{-1} (mull) was identified as ν(Fe–O–Fe), in reasonable agreement with the prior value of 870 cm^{-1} (KBr) assigned to this band.[38] With $[Fe(OEC)]_2O$, ν(Fe–O–Fe) appears to be broader and centered at 858 cm^{-1} (two partially resolved peaks at 865 and 851 cm^{-1}), roughly consistent with an earlier report.[39] In the ^{18}O complex this band shifts under the broad asymmetric feature at ~800 cm^{-1} found in the spectrum of both $[Fe(OEC)]_2O$ and Fe(OEC)Cl. The isotopic effect should cause a shift to lower energy of ~48 cm^{-1} and confirms the assignment. (A 45-cm^{-1} shift has been reported for the μ-oxo complex of protoporphyrin IX.[57a]) In the case of $[Fe(OEiBC)]_2O$ a composite absorption feature is observed at ~880–760 cm^{-1} whose breadth may be due in part to $\lesssim$7 isomers of this molecule. Comparison with the spectrum of Fe(OEiBC)Cl suggested that the μ-oxo complex has more absorption near ~860 cm^{-1}. The spectrum of the ^{18}O form of the latter confirms absorption at or near this frequency as corresponding to ν(Fe–O–Fe). Consequently, the IR results substantiate formulation of the hydroporphyrin species as μ-oxo complexes; $[Fe(OEP)]_2O$[38] is a well-authenticated compound.

(ii) **Fe(P)L(CO) and Fe(P)(CO).** Investigations of iron(II) porphyrin carbonyl complexes have led to the conclusion that the porphyrin structure, the identity of the ligand L trans to CO, and the medium all affect the Fe–CO bonding interactions.[59,60]

(51) T. Nozawa, S. Ookubo, and M. Hatano, *J. Inorg. Biochem.*, **12**, 253 (1980).

(52) (a) L. Edwards, D. Dolphin, and M. Gouterman, *J. Mol. Spectrosc.*, **35**, 90 (1970); (b) R. Bonnett and M. J. Dimsdale, *J. Chem. Soc., Perkin Trans. 1*, 2540 (1972).

(53) In addition to the complexes in Table I the following species were generated in dichloromethane solution by reaction 1: Fe(OEC)(OC_6H_3-2,4-$(NO_2)_2$) (600, 28); Fe(OEiBC)L, L = OC_6H_3-3,4-$(CH_3)_2$ (581, 11), OC_6H_4-3-NO_2 (589, 10), OC_6H_3-2,4-$(NO_2)_2$ (599, 10), SEt (590). Values of λ_{max} (nm) and ϵ (mM) for the most intense visible band are indicated. Fe(OEiBC)(SEt) was observed as a transient species prior to autoreduction (reaction 3).

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(55) J. H. Dawson, R. H. Holm, J. R. Trudell, G. Barth, R. E. Lindner, E. Bunnenberg, C. Djerassi, and S. C. Tang, *J. Am. Chem. Soc.*, **98**, 3707 (1976).

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(59) J. W. Buchler, W. Kokisch, and P. D. Smith, *Struct. Bonding (Berlin)*, **34**, 79 (1978).

Table I. Quantitative Absorption Spectral Data for Iron(II, III)-Chlorin and -Isobacteriochlorin Complexes

complex	solvent	λ_{max}, nm (ϵ, mM)[f]
Fe(OEC)Cl	C_6H_6	377 (89.0), 473 (7.9), 511 (sh, 5.4), 559 (5.6), 602 (24.2), 751 (2.5)
Fe(OEiBC)Cl	C_6H_6	377 (56.4), 475 (6.7), 512 (6.0), 554 (6.5), 601 (17.6), 684 (1.3), 756 (2.0)
Fe(OEiBC)Cl	CH_2Cl_2	377 (51.1), 474 (5.5), 511 (5.4), 554 (5.9), 603 (13.3), 688 (1.0), 758 (1.5)
Fe(OEC)(OAc)	CH_2Cl_2	375 (143), 466 (13.4), 506 (sh, 9.9), 554 (sh, 10.4), 596 (31.2), 743 (4.8)
Fe(OEiBC)(OAc)	CH_2Cl_2	332 (25.8), 372 (40.2), 512 (6.6), 549 (7.1), 595 (12.1), 678 (1.6), 748 (1.8)
Fe(OEC)(OEt)	CH_2Cl_2/EtOH[a]	342 (sh, 64.2), 386 (125), 459 (19.2), 569 (16.5), 663 (15.8)
Fe(OEiBC)(OEt)	CH_2Cl_2/EtOH[b]	357 (sh, 27.9), 379 (30.6), 575 (7.7)
Fe(OEC)(OPh)	CH_2Cl_2	385 (125), 467 (16.8), 582 (26.3), 708 (8.1)
Fe(OEiBC)(OPh)	CH_2Cl_2	361 (34.4), 378 (sh, 33.3), 459 (sh, 7.2), 541 (sh, 6.9), 582 (14.5), 655 (2.5), 719 (2.1)
Fe(OEC)(OC_6H_4-4-NO_2)[c]	CH_2Cl_2	377 (133),[d] 468 (15.0), 506 (12.0), 550 (sh, 11.0), 593 (27.9), 662 (3.6), 733 (5.1)
Fe(OEiBC)(OC_6H_4-4-NO_2)[c]	CH_2Cl_2	370 (40.2),[d] 479 (6.6), 509 (6.6), 547 (7.0), 593 (12.0), 674 (1.4), 742 (1.5)
$[Fe(OEC)]_2O$	CH_2Cl_2	330 (sh, 102), 380 (189), 526 (16.1), 605 (21.6), 655 (35.8)
$[Fe(OEiBC)]_2O$	CH_2Cl_2	341 (sh, 45.2), 376 (60.3), 432 (sh, 16.9), 601[e] (13.1), 662 (sh, 6.2)
$[Fe(OEiBC)]_2O$	C_6H_6	378 (77.4), 451 (sh, 19.0), 590[e] (18.0), 662 (sh, 8.7)
Fe(OEC)(SPh)	CH_2Cl_2	377 (125), 480 (18.1), 557 (sh, 13.6), 602 (34.4), 744 (5.9)
Fe(OEiBC)(SPh)	CH_2Cl_2	334 (sh, 22.5), 373 (36.8), 486 (7.3), 552 (8.0), 596 (14.0), 687 (1.5), 750 (1.7)
Fe(OEC)(SC_6H_4-4-NO_2)[c]	CH_2Cl_2	377 (127),[d] 470 (15.9), 512 (sh. 12.8), 566 (sh, 12.2), 605 (29.4), 681 (3.1), 756 (4.2)
Fe(OEiBC)(SC_6H_4-4-NO_2)[c]	CH_2Cl_2	374 (39.8),[d] 479 (6.4), 555 (7.8), 599 (11.5), 691 (1.3), 759 (1.5)
Fe(OEC)	C_6H_6	393 (90.3), 498 (9.2), 626 (23.3)
Fe(OEiBC)	C_6H_6	315 (sh), 339 (sh), 370 (31.7), 387 (33.6), 487 (sh), 517 (sh), 544 (sh), 573 (10.3), 616 (21.1)
Fe(OEC)$(py)_2$	C_6H_6	397 (66.3), 413 (87.1), 481 (14.1), 516 (9.4), 547 (14.3), 599 (26.3)
Fe(OEiBC)$(py)_2$	C_6H_6	368 (sh, 39.6), 390 (sh), 396 (51.4), 482 (sh, 9.8), 512 (sh), 530 (sh), 573 (15.5)
Fe(OEC)(N-MeIm)$_2$	C_6H_6	378 (34.3), 403 (64.0), 417 (74.3), 484 (6.7), 519 (5.4), 550 (sh, 11.3), 599 (31.2)
Fe(OEiBC)(N-MeIm)$_2$	C_6H_6	339 (26.2), 370 (sh, 33.4), 392 (sh, 50.6), 400 (60.6), 509 (8.7), 548 (12.8), 568 (16.7)
Fe(OEC)(py)(CO)	C_6H_6	394 (sh, 86.1), 406 (107), 491 (8.1), 565 (sh, 8.7), 609 (39.5)
Fe(OEiBC)(py)(CO)	C_6H_6	388 (51.9), 479 (7.8), 547 (11.2), 589 (30.5)
Fe(OEC)(N-MeIm)(CO)	C_6H_6	399 (sh, 86.0), 409 (101), 492 (7.6), 566 (sh, 8.5), 608 (34.9)
Fe(OEiBC)(N-MeIm)(CO)	C_6H_6	396 (62.4), 545 (11.2), 589 (34.7)

[a] 6.7:1 v/v. [b] 3:1 v/v. [c] Data corrected for background absorbance of excess phenol or thiol used to cleave precursor μ-oxo complex. [d] Values less well determined owing to background absorbance. [e] Very broad band. [f] Relative values are known to the precision indicated; absolute values are less well determined, being accurate only to several percent.

Variations in porphyrin structure which increase the basicity of the free-base porphyrin (a property bearing a rough inverse relationship to the π-acceptor capacity of the porphyrin[59]) increase the strength of the interactions—the "cis effect".[59] This is reflected in lower ν(CO) values and in increasing affinities for CO,[59-61] consistent with the π-acid character of CO. Similarly, variation of the trans ligand results in related changes, with a more basic axial ligand usually lowering ν(CO).[56,59,62]

In order to ascertain whether the nature of the macrocycle influences the effective electron density at the iron atoms sufficiently to be detectable by variations in ν(CO), we examined parallel series of Fe(P)L(CO) (L = py, *N*-MeIm, CO) and Fe(P)(CO) in dichloromethane after generation by reactions 5 and 7. Results are summarized in Table II. In the Fe(P)(N-base)(CO) series the more basic *N*-MeIm ligand lowers ν(CO) by 5–6 cm^{-1}, in agreement with earlier results for porphyrin complexes.[62a] At parity of axial ligand very little effect is observed upon varying the macrocycle; if anything, ν(CO) is about 3–4 cm^{-1} lower in OEP than in hydroporphyrin complexes. At p_{CO} = 300 torr all three Fe(P) species exhibit a band at 1951–1956 cm^{-1}, assigned to five-coordinate Fe(P)(CO). The OEC and OEiBC complexes show a narrower band at 2016 cm^{-1} that is attributed to the asymmetric stretch of Fe(P)$(CO)_2$. These assignments follow from increases in absorbance ratios $A_{1951-1956}/A_{2016}$ as p_{CO} was lowered and are consistent with those made for Fe(TPP)$(CO)_{1,2}$.[63] This ratio, at 300 torr of CO and constant Fe(P) concentration, decreases in the order OEP > OEiBC > OEC, showing that in reaction 5 K_2(OEC, OEiBC) > K_2(OEP). The absence of any significant macrocyclic cis effect in the Fe(P)$(CO)_{1,2}$

Table II. Carbonyl Stretching Frequencies of Fe(P)L(CO) Complexes in Dichloromethane Solutions

	ν_{CO},[a] cm^{-1}		
L/P	OEP	OEC	OEiBC
py	1959	1961	1962[b]
N-MeIm	1953	1956	1957
CO[c]	~2016[d]	2016	2016
(absent)[c]	1951	1951	1956

[a] Spectra obtained at 1-cm^{-1} resolution, p_{CO} = 300 torr, ~1 mM Fe(P). [b] Fe(DMOEiBC)(py)(CO): ν_{CO} 1970 cm^{-1}, CH_2Cl_2.[29a] [c] Fe(TPP)$(CO)_{1,2}$: ν_{CO} 1973, 2042 cm^{-1}, toluene.[63] [d] Very weak band at 300 torr of CO.

(60) W. S. Caughey, J. C. Maxwell, J. M. Thomas, D. H. O'Keeffe, and W. J. Wallace in "Metal-Ligand Interactions in Organic Chemistry and Biochemistry", Part 2, B. Pullman and N. Goldblum, Eds., D. Reidel Publishing Co., Dordrecht-Holland, 1977, p 131.

(61) (a) J. O. Alben and W. S. Caughey, *Biochemistry*, **7**, 175 (1968); (b) W. S. Caughey in "Inorganic Biochemistry", Vol. 2, G. L. Eichhorn, Ed., Elsevier, Amsterdam, 1973, Chapter 24.

(62) (a) W. S. Caughey, C. H. Barlow, D. H. O'Keeffe, and M. C. O'Toole, *Ann. N. Y. Acad. Sci.*, **206**, 296 (1973); (b) J. P. Collman and T. N. Sorrell, *J. Am. Chem. Soc.*, **97**, 4133 (1975).

(63) B. B. Wayland, L. F. Mehne, and J. Swartz, *J. Am. Chem. Soc.*, **100**, 2379 (1978).

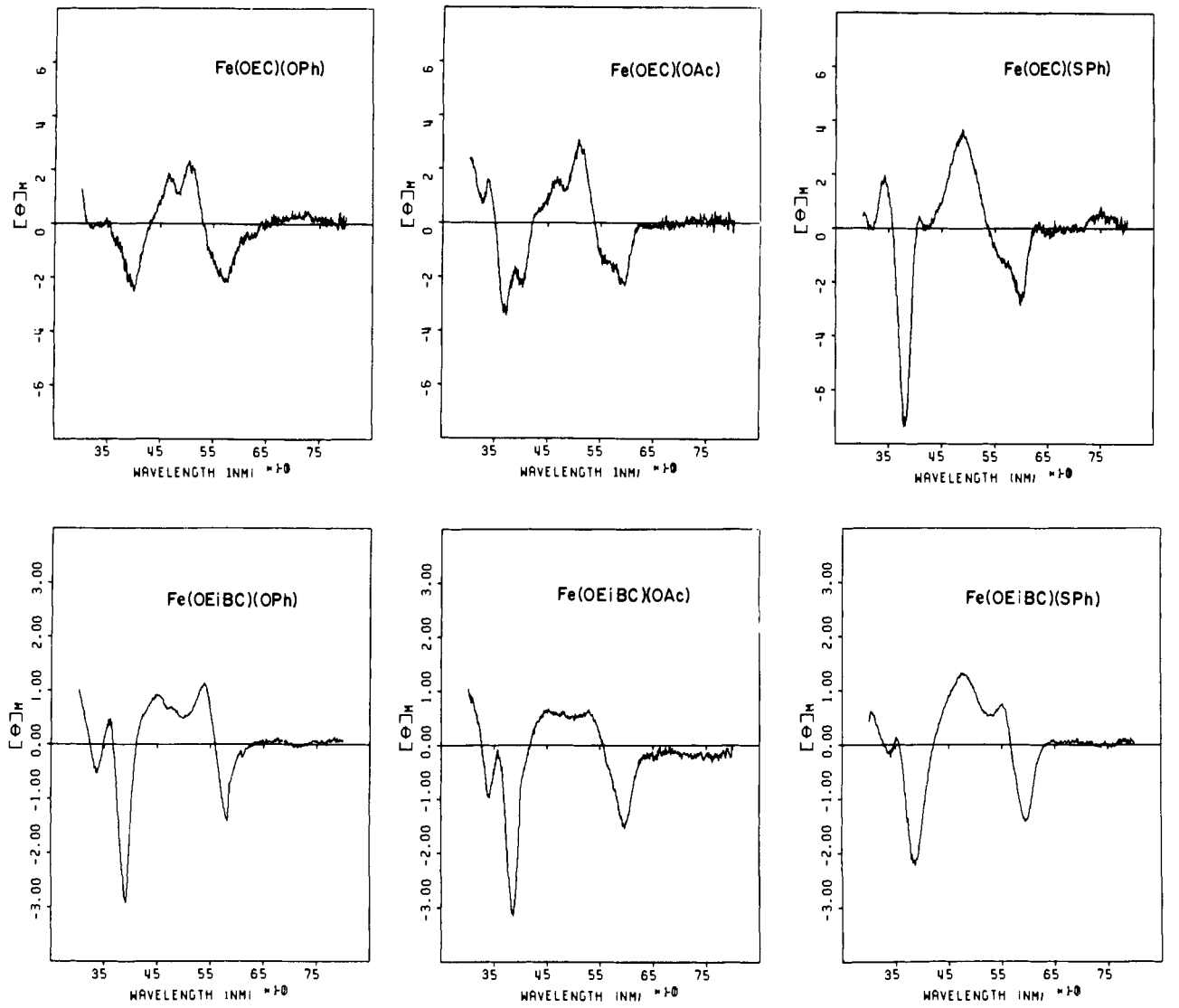

Figure 7. MCD spectra of Fe(OEC)L and Fe(OEiBC)L complexes in dichloromethane solutions: $[\theta]_M$ values are in units of deg cm^2 dmol^{-1} G^{-1} and the wavelength scale is in nm/10.

complexes as well is evidenced by only a 5-cm^{-1} spread in ν(CO) of the monocarbonyls and an essentially constant value for the biscarbonyls. Medium effects were not investigated other than to show that ν(CO) and the absorbance ratio of Fe(OEC)(CO)$_{1,2}$ were unchanged over the concentration range in Table II and that ν(CO) (1977 cm^{-1}) of Fe(PPIXDME)(py)(CO) did not vary over a 10-fold concentration range in bromoform solution. The latter complex is one used by Alben and Caughey[61a] in their series of 2,4-disubstituted porphyrin complexes that provides the best examples of a cis effect as manifested in ν(CO) changes.

(d) ^{1}H NMR Spectra. The spectra of paramagnetic iron(II, III) porphyrins,[64] including Fe(OEP)Cl,[65] [Fe(OEP)]$_2$O,[65] and Fe(OEP),[66] have been analyzed and found to be sensitive to oxidation and spin states of the iron atom. Spectra of corresponding hydroporphyrin complexes have not been described previously. Their determination and assignment in most cases has proven less than optimal because of solubility restrictions and, particularly in the OEiBC series, the presence of diastereomers and extensive magnetic inequivalencies therein. All spectra were obtained at 360 MHz and ~27 °C unless noted otherwise.

The spectrum of Fe(OEP)Cl in $CDCl_3$ consists of resonances at +56 (meso-H), ~−6.0 (CH_3), and −40 and −44 ppm (diastereotopic CH_aH_b).[67] In the same solvent Fe(OEC)Cl exhibits four meso-H signals (+46, +57, +83, +93 ppm), consistent with removal of the macrocycle C_2 axis upon metalation. About 20 of the remaining 26 signals were partially or fully resolved at 0 to −60 ppm. The spectrum of Fe(OEiBC)Cl in $CDCl_3$ contains at least four meso-H resonances in the +40 to +100 ppm range; some 30 of the remaining total of 80 signals (for three diastereomers) were observable at 0 to −90 ppm. [Fe(OEC)]$_2$O and [Fe(OEiBC)]$_2$O (two and seven possible diastereomers, respectively) afford spectra in $CDCl_3$ that are poorly resolved. However, all signals occurred at 0 to −10 ppm, consistent with the spectrum of [Fe(OEP)]$_2$O: −1.75 (CH_3), −5.10, −6.06 (diastereotopic CH_aH_b), −6.4 ppm[68] (meso-H).

Spectra of unligated iron(II) porphyrins are notable for their large isotropic shifts and narrow line widths.[64,66] This behavior extends to Fe(OEC) whose 100-MHz spectrum in toluene-d_8 at ~6 °C, containing also a few percent of Fe(OEP), is shown in Figure 9. The spectrum can be completely assigned and accords

(64) G. N. LaMar and F. A. Walker (Jensen) in "The Porphyrins", Vol. IV, Part B, D. Dolphin, Ed., Academic Press, New York, 1979, Chapter 2.
(65) G. N. LaMar, G. R. Eaton, R. H. Holm, and F. A. Walker, *J. Am. Chem. Soc.*, **95**, 63 (1973).
(66) H. Goff, G. N. LaMar, and C. A. Reed, *J. Am. Chem. Soc.*, **99**, 3641 (1977).
(67) In accordance with the usual convention for paramagnetic molecules shifts downfield and upfield of Me_4Si internal standard are designated − and +, respectively.
(68) This signal was obscured by overlap with methylene proton resonances in the original 100-MHz spectrum[65] but was detected in the 360-MHz spectrum.

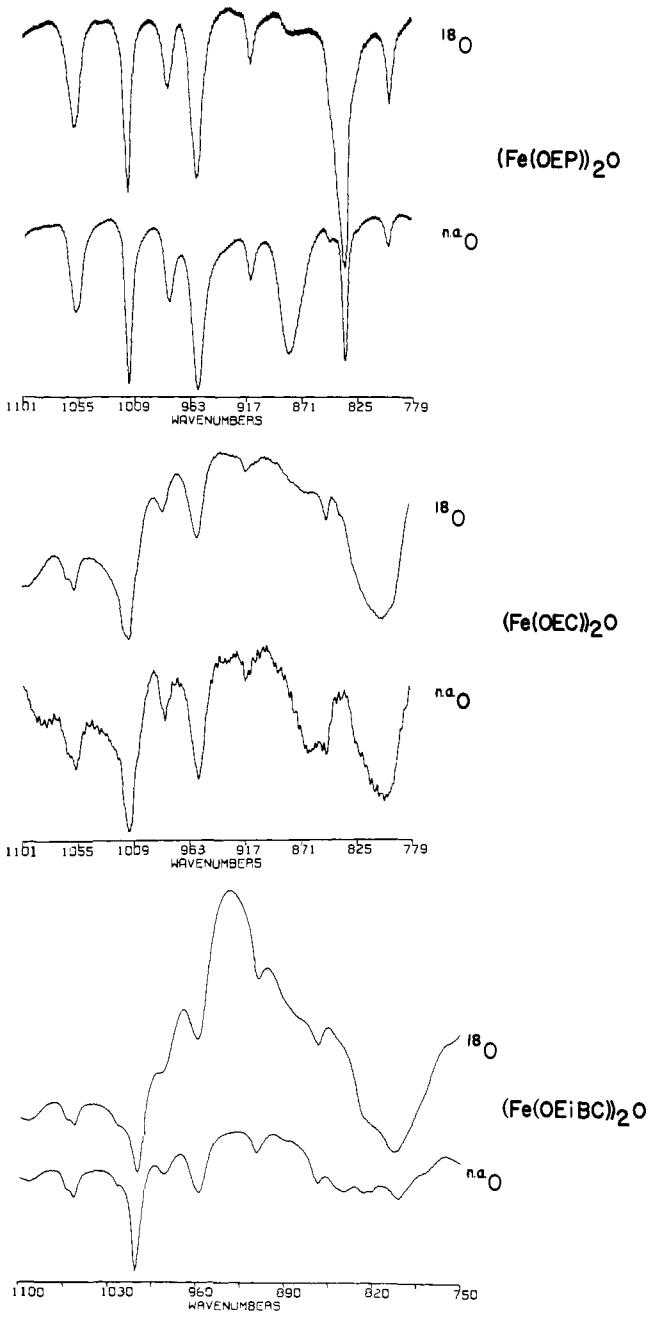

Figure 8. FT infrared spectra (1100–750 cm^{-1}) of natural abundance (^{na}O) and ^{18}O-substituted $[Fe(P)]_2O$ complexes in paraffin oil mulls.

with effective C_2 symmetry; a conspicuous feature is splitting of the diastereotopic methylene protons (CH_aH_b) of the pyrroline ring substituents by 6.30 ppm. On the basis of these results and spectral analysis of Fe(OEP), Fe(TPP), and derivatives of the latter,[64,66] it is virtually certain that Fe(OEC) possesses an $S = 1$ ground state and significant dipolar contributions to the isotropic shifts. Detailed magnetic susceptibility studies of Fe(TPP) reported recently[69] tend to confirm these conclusions from the earlier NMR analysis.[66] Spectra (100 MHz) of Fe(OEiBC) (two diastereomers) have proven to be much more complex and are not well resolved; consequently, they are not as useful for species identification. However, both hydroporphyrin complexes Fe(P) when treated with excess pyridine-d_5 in toluene-d_8 give ^1H NMR spectra (not shown) similar to those of the respective free-base macrocycles and ascribable to formation of the diamagnetic hemochromes $Fe(P)(py)_2$. The latter have also been spectrophotometrically detected as products of the similar reactions (6) (Table I). While additional experimentation will be required to secure full spectral assignments of iron(II, III) hydroporphyrins, the results presented here are sensibly consistent with proposed formulations of these complexes.

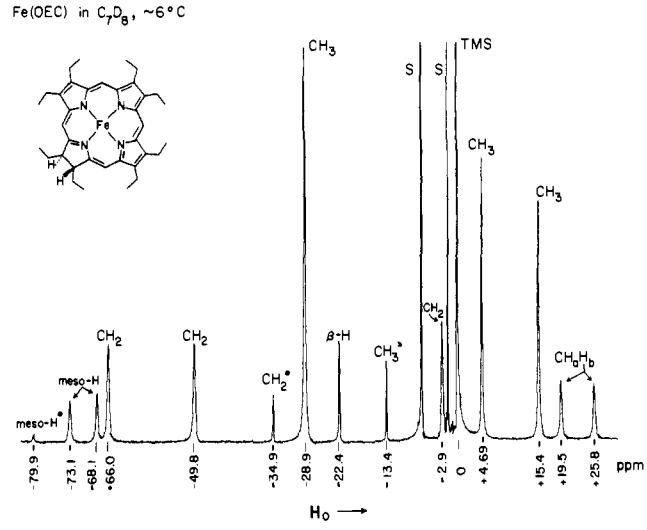

Figure 9. ^1H NMR spectrum (100 MHz) of Fe(OEC) in toluene-d_8 at ~6 °C. Signal assignments are indicated (S = solvent). Signals marked with an asterisk are due to Fe(OEP).

(e) **EPR Spectra.** Spectra of a variety of hydroporphyrin high-spin ($S = {}^5/_2$) Fe(P)L and low-spin ($S = {}^1/_2$) Fe(P)LL′ species have been examined in toluene glasses at 80–85 K in order to determine g values and delineate axial vs. rhombic spectral characteristics as dependent on macrocyclic and axial ligand structures. Spectral data for a large number of analogous synthetic porphyrins, including OEP complexes, are available elsewhere.[31,40,45,49,50]

(i) **Fe(P)L Species.** Spectra of species with L = Cl^-, OAc^-, ArO^-, and ArS^- in the OEC and OEiBC series have been determined. Acetate and phenolate complexes were generated by reaction 1; some representative spectra are shown in Figure 10. All hydroporphyrin Fe(P)Cl, Fe(P)(OAc), and Fe(P)(OAr) complexes, as their OEP counterparts, exhibit essentially axial spectra ($g_\perp = 6$, $g_\parallel = 2$),[70] apparent g values of the broad low-field and the weak high-field features being 6.0–6.2 and ~2.0, respectively. The spectrum of Fe(OEiBC)Cl was presented earlier[28] and is very similar to those in Figure 10. Fe(P)(SAr) complexes were generated by the reverse of reaction 3. Typically, 0.4 mL of a 0.5 mM solution of Fe(P) in toluene was treated with ~1 mg of disulfide. After the color change indicative of product formation was complete, the solution was separated from any undissolved disulfide and was frozen. Spectra of four OEC and OEiBC complexes are given in Figures 11 and 12, respectively. Because of the requirement of excess disulfide, this procedure[71] resulted in a mixture of Fe(P)(SAr) and low-spin species, formulated as Fe(P)(SAr)(ArSSAr). The spectra of both are readily identifiable. In marked contrast to the spectra of foregoing Fe(P)L complexes, all Fe(P)(SAr) spectra are markedly rhombic in character ($g \approx 7.1$–7.4, 4.4–4.9, 2.0). Apparent rhombic splittings tend to increase with decreasing acidity of ArSH, being largest for Fe(P)(SPh).

From the present results it is now evident that the lack of axial symmetry of the OEC and OEiBC ring systems itself does not afford resolvable rhombicity. Of the ligands examined here this property is manifested only by arylthiolate complexes.[72] Inasmuch

(69) P. D. W. Boyd, D. A. Buckingham, R. F. McMeeking, and S. Mitra, *Inorg. Chem.*, **18**, 3585 (1979).

(70) G. Palmer in "The Porphyrins", Vol. IV, Part B, D. Dolphin, Ed., Academic Press, New York, 1979, Chapter 6.

(71) For the purpose of obtaining EPR spectra free of other Fe(III) signals, both low- and high-spin, and for the prevention of appreciable autoreduction of the desired thiolate complex, this procedure proved superior to cleavage of μ-oxo complexes or to our earlier reactions of Fe(P)Cl and ArSH in the presence of a hindered base.[28] However, where comparisons could be made, all methods afforded the same Fe(P)(SAr) spectra.

Table III. EPR Data for Low-Spin Hydroporphyrin Fe(P)LL' Complexes in Toluene Glasses (80–85 K)

Fe(P)L	L'	g values
	S^-–Fe–N	
Fe(OEP)(SPh)[a]	*N*-MeIm	2.40, 2.26, 1.92
Fe(OEP)(SPh)[a]	py	2.43, 2.29, 1.90
Fe(OEC)(SPh)	*N*-MeIm	2.31, 1.94
Fe(OEC)(SPh)	*n*-$BuNH_2$	2.32, 2.26, 1.94
Fe(OEC)(SC_6H_4-4-NO_2)	*N*-MeIm	2.32, 1.93
Fe(OEC)(SC_6H_4-4-NO_2)	py	2.34, 1.92
Fe(OEC)(SC_6H_4-4-Cl)	*N*-MeIm	2.31, 1.93
Fe(OEC)(SC_6H_4-4-CF_3)	*N*-MeIm	2.32, 1.93
Fe(OEiBC)(SPh)	*N*-MeIm	2.30,[b] 1.94, (1.91)[c]
Fe(OEiBC)(SPh)	*n*-$BuNH_2$	2.32, 2.26, 1.93
Fe(OEiBC)(SC_6H_4-4-NO_2)	*N*-MeIm	2.32,[b] 1.92, (1.89)[c]
Fe(OEiBC)(SC_6H_4-4-Cl)	*N*-MeIm	2.31,[b] 1.93, (1.91)[c]
Fe(OEiBC)(SC_6H_4-4-CF_3)	*N*-MeIm	2.31,[b] 1.93, (1.90)[c]
	S^-–Fe–S	
Fe(OEP)(SPh)[a]	THT[e]	2.38, 2.27, 1.93
Fe(OEC)(SPh)	THT	2.29, 1.95
Fe(OEC)(SPh)	EtSH	2.30, 1.95
Fe(OEC)(SPh)	PhSH	2.29, 1.95
Fe(OEC)(SC_6H_4-4-NO_2)	$(4\text{-}NO_2\text{-}C_6H_4)_2S_2$	2.30, 1.94
Fe(OEC)(SC_6H_4-4-Cl)	$(4\text{-Cl-}C_6H_4)_2S_2$	2.30, 1.95
Fe(OEC)(SC_6H_4-4-CF_3)	$(4\text{-}CF_3\text{-}C_6H_4)_2S_2$	2.30, 1.94
Fe(OEiBC)(SC_6H_4-4-CF_3)	THT	2.30, 1.93
Fe(OEiBC)(SC_6H_4-4-Cl)	EtSH	2.30, 1.93
Fe(OEiBC)(SPh)	PhSH	2.31, 1.95
Fe(OEiBC)(SC_6H_4-4-NO_2)	$(4\text{-}NO_2\text{-}C_6H_4)_2S_2$	2.35, 2.25, 1.92/1.90[d]
Fe(OEiBC)(SC_6H_4-4-Cl)	$(4\text{-Cl-}C_6H_4)_2S_2$	2.32, 2.25, 1.92[b]
Fe(OEiBC)(SC_6H_4-4-CF_3)	$(4\text{-}CF_3\text{-}C_6H_4)_2S_2$	2.33, 2.24, 1.92[b]
	S^-–Fe–O	
Fe(OEP)(SPh)[a]	THF	2.35, 2.26, 1.95
Fe(OEC)(SPh)	2-MeTHF	2.29, 1.96
Fe(OEiBC)(SC_6H_4-4-NO_2)	2-MeTHF	(2.37),[c] 2.29, 1.95/1.92[d]
	O^-–Fe–N	
Fe(OEC)(OAc)	py[f]	2.38, 2.28, 1.58[g]
Fe(OEC)(OPh)	*N*-MeIm[h]	2.46, 2.25, 1.87
Fe(OEC)(OPh)	*n*-$BuNH_2$[i]	2.46, 2.25, 1.87
Fe(OEC)(OPh)	py[i]	2.47, 2.25, 1.85
Fe(OEiBC)(OAc)	*N*-MeIm[j]	2.44, 2.33, 1.71
Fe(OEiBC)(OPh)	*n*-$BuNH_2$[k]	2.47, 2.24, 1.86
	O^-–Fe–S	
Fe(OEC)(OPh)	EtSH[f,l]	2.33, 1.90
	N–Fe–N	
$[Fe(OEP)(N\text{-MeIm})_2]Cl$[a]		2.96, 2.25, 1.53
$[Fe(TPC)(Im)_2]^+$[m]		2.49, 2.39, 1.75
$[Fe(OEC)(N\text{-MeIm})_2]Cl$[n]		2.51, 2.37, 1.73
$[Fe(OEiBC)(N\text{-MeIm})_2]Cl$[k,n]		2.49, 2.37, 1.71

[a] Reference 31. [b] Presence of two or more species suggested by unresolved shoulder(s) near this feature (Figure 15). [c] Shoulder due to minority species. [d] Corresponding resonances of two low-spin species. [e] Tetrahydrothiophene. [f] Small extent of conversion to low-spin species. [g] Broad, weak signals. [h] Mixture of this species, $[Fe(OEC)(N\text{-MeIm})_2]^+$, and a rhombic high-spin species observed. [i] Mixture of this species and a majority high-spin component. [j] Observed species may be $[Fe(OEiBC)(N\text{-MeIm})_2]^+$. [k] Minority low-spin species also present. [l] Corresponding OEiBC species not observed. [m] Reference 43. [n] Formed in situ from Fe(P)Cl and excess *N*-MeIm.

as the spectra of porphyrin thiolate complexes are also rhombic (e.g., $g \approx 7.2, 4.7, 1.9$ for Fe(OEP)(SPh)[31]), it is concluded that, at least under the conditions employed, the structural element requisite to rhombic hydroporphyrin Fe(P)L spectra is axial thiolate ligation.

(72) Complexes of protoporphyrin IX dibutyl ester (PPIXDBE) with substituted phenolate ligands have been reported to have significantly rhombic EPR spectra.[40] It was not clear whether the rhombicity was due to some effect of the large ester groups and/or the use of ligands more basic than PhO^- itself. Some credence to the former possibility is provided by the rhombic spectrum of Fe(PPIXDBE)(OAc)[40] and the axial spectrum of Fe(PPIXDME)(OAc).[31] Our investigation of the series Fe(P)(OAr) with P = OEP and OEiBC and the ligands in series 9, plus $^-OC_6H_4$-2-NO_2, $^-OC_6H_3$-3,5-$(OMe)_2$, and $^-OC_6H_3$-2,6-$(OMe)_2$, gave axial spectra ($g \approx 6.0$–6.2, 2.0) in all cases except Fe(P)(OC_6H_3-2,6-$(OMe)_2$), whose spectrum revealed shoulders on the principal feature at $g \approx 6.2$. The latter may be a pathological example, however, because of the possibility of weak bonding of methoxyl groups to iron. A general feature of the spectra of Fe(P)(OAr) species is larger line width of the low-field resonance than found in Fe(P)Cl and Fe(P)(OAc) spectra.

(ii) Fe(P)LL' Species. These species, containing a variety of axial ligand combinations, were formed in toluene solution by addition of species L' to Fe(P)L complexes which had been previously generated by reactions 1 or 2 or the reverse of reaction 3. The resulting solutions were rapidly mixed and frozen and their EPR spectra, containing low-spin Fe(III) signals[70,73] of Fe(P)LL', were recorded. Hydroporphyrin Fe(P)(SAr) complexes, as their porphyrin analogues,[31] reacted nearly quantitatively with all added bases, including ligands as weak as disulfides, present in sufficient excess. When L' was a nitrogeneous base (*N*-MeIm, py, *n*-$BuNH_2$), very rapid freezing was necessary to prevent decay of the low-spin species by reaction 10, which has also been noted

$$Fe(P)(SAr)L' + L' \rightarrow Fe(P)L_2' + {}^1/_2ArSSAr \quad (10)$$

for porphyrin complexes. Phenolate complexes were partially

(73) M. Chevion, J. Peisach, and W. E. Blumberg, *J. Biol. Chem.*, **252**, 3637 (1977).

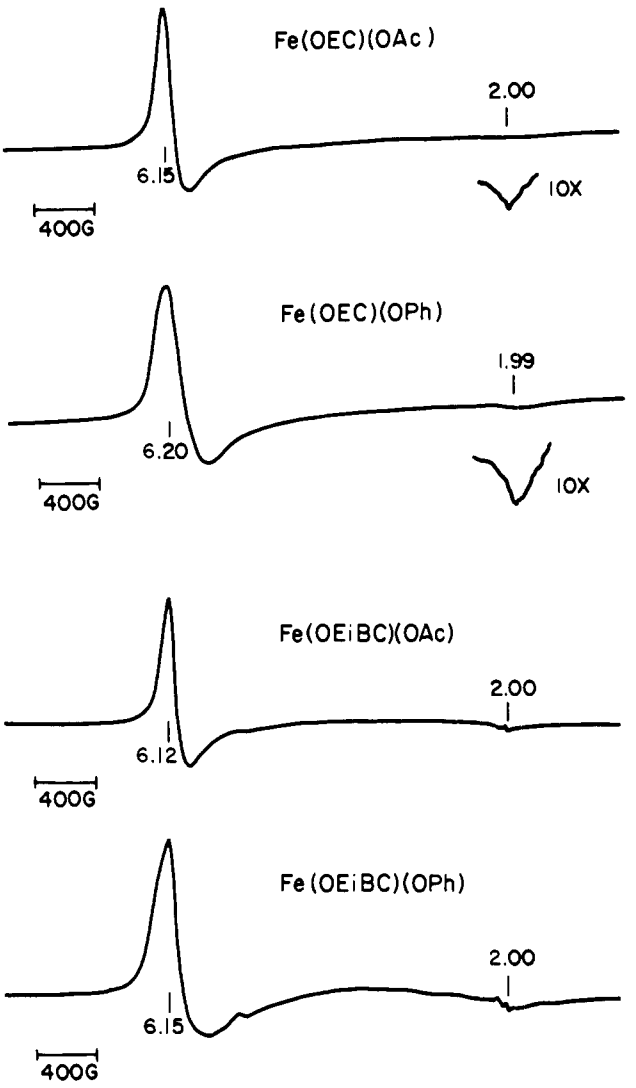

Figure 10. EPR spectra of high-spin hydroporphyrin Fe(P)L complexes (L = OAc^-, PhO^-) in toluene glasses at 80–85 K (apparent g values are indicated).

converted to low-spin species upon addition of N-bases and ethanethiol (OEC only). Weaker ligands such as 2-MeTHF and tetrahydrothiophene (THT) did not form detectable amounts of low-spin species with either phenolate or acetate complexes. EPR results, together with additional explanatory notes and some comparative data for Fe(OEP)LL′ complexes, are set out in Table III. Because at this stage low-spin species have been produced only from independently authenticated Fe(P)L complexes, not all combinations of axial ligands have yet been examined. Selected spectra are presented in Figures 13 and 14; spectra of Fe(P)(S-Ar)(ArSSAr) species, recorded in the presence of high-spin Fe(P)(SAr), are shown in Figures 11 and 12.

Table IV. Redox Potentials of Selected Iron–Porphyrin and –Hydroporphyrin Complexes in Dichloromethane Solutions

	$E_{1/2}$,[a] V			
complex	0/1−	1+/0	2+/1+	other
Fe(OEP)Cl[b-d]	−0.52[h]	1.01	1.39	
Fe(OEC)Cl	−0.44	0.72	1.24	
Fe(OEiBC)Cl	−0.45	0.43	1.00	
Fe(OEP)(OAc)[f]	−0.40	1.01	1.41	
Fe(OEC)(OAc)[f]	−0.34	0.69	1.15[i]	
Fe(OEiBC)(OAc)[f]	−0.35	0.42	1.08[i]	
$[Fe(OEP)]_2O$[e]	−1.34[h]	0.62	0.95	1.30 (3+/2+), 1.40 (4+/3+)
$[Fe(OEC)]_2O$	−1.32	0.33	0.65	1.20 (4+/2+)[g]
$[Fe(OEiBC)]_2O$	−1.37	0.04	0.38	1.02 (4+/2+)[g]
$Fe(OEP)(py)_2$[j]		−0.15[k]		
$Fe(OEC)(py)_2$[j]		−0.16	0.88[i]	
$Fe(OEiBC)(py)_2$[j]		−0.19	0.61[i]	

[a] $E_{1/2} = (E_{p,c} + E_{p,a})/2$, 100-mV/s scan rate, 0.05 M (n-Bu_4N)(ClO_4) supporting electrolyte. [b] Footnotes b-d refer to Fe(P)L. For oxidations $\Delta E_p \approx 80$–150 mV, $i_{p,c} \simeq i_{p,a}$. [c] Irreversible reductions, $E_{p,c}$ given. [d] See literature values in ref 29 and 79 for footnotes d and e. [d] −0.5, 0.96, 0.99, 1.40 V. [e] 0.66, 0.96 V. [f] Generated in situ. [g] $2e^-$ process. [h] Second reductions of Fe(P)L and $[Fe(P)]_2O$ (Fe(II) → Fe(I)[76]) not examined. [i] Irreversible oxidation, $E_{p,a}$ given. [j] Generated in 10:1 v/v CH_2Cl_2/py; $\Delta E_p \approx 90$–130 mV, $i_{p,c} \simeq i_{p,a}$, 50 mV/s. [k] Lit. −0.02,[29a] ~−0.13 V.[76c]

Several spectral features are noteworthy. At parity of L,L′ ligands g values of OEC and OEiBC species are virtually identical. More interesting are the observations that resolvable rhombicity occurs only (but not always) in the presence of at least one N-base ligand; further, the apparent extent of rhombicity is smaller for hydroporphyrin than for OEP and other porphyrin complexes with the same set of axial ligands, all of which evidence rhombic spectra.[31,49] Lastly, spectra of Fe(OEiBC)LL′ complexes formed with sterically more demanding ligands (L′ = N-MeIm, 2-MeTHF, ArSSAr) contain multiple features indicative of the presence of more than one low-spin species. These spectra are usually better resolved in the high-field than in the low-field region (cf. Figures 12 and 14). Spectra of corresponding Fe(OEC)LL′ complexes (one isomer) are those of a single axial or rhombic low-spin species. Multiple signals are ascribed to isomers of

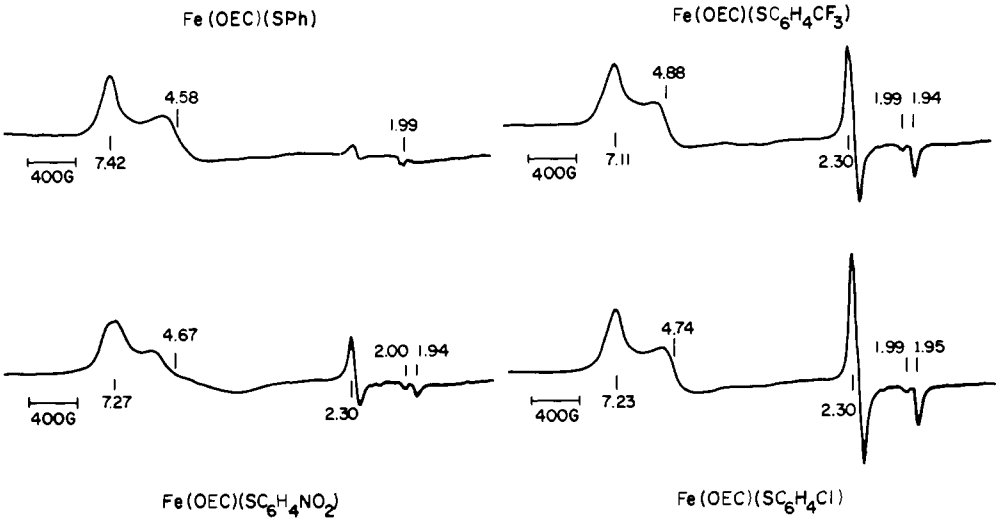

Figure 11. EPR spectra of mixtures of high-spin Fe(OEC)(SAr) and low-spin Fe(OEC)(SAr)(ArSSAr) species in toluene glasses at 80–85 K (apparent g values are indicated).

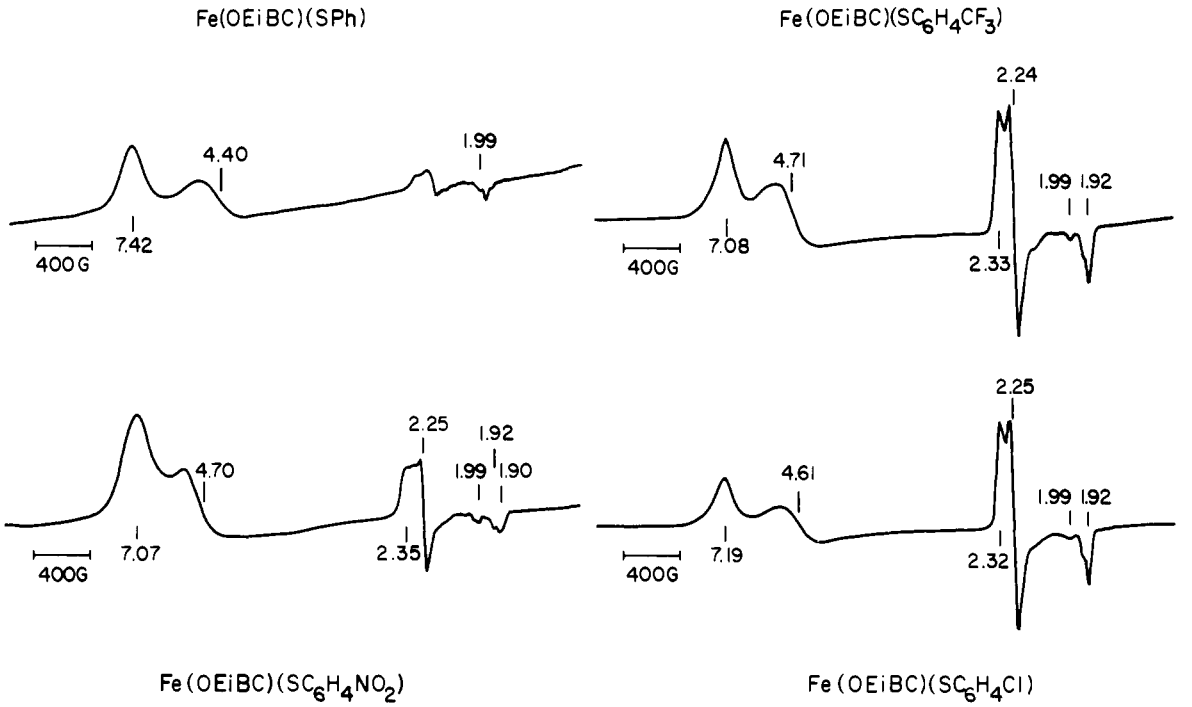

Figure 12. EPR spectra of mixtures of high-spin Fe(OEiBC)(SAr) and low-spin Fe(OEiBC)(SAr)(ArSSAr) species in toluene glasses at 80–85 K (apparent g values are indicated).

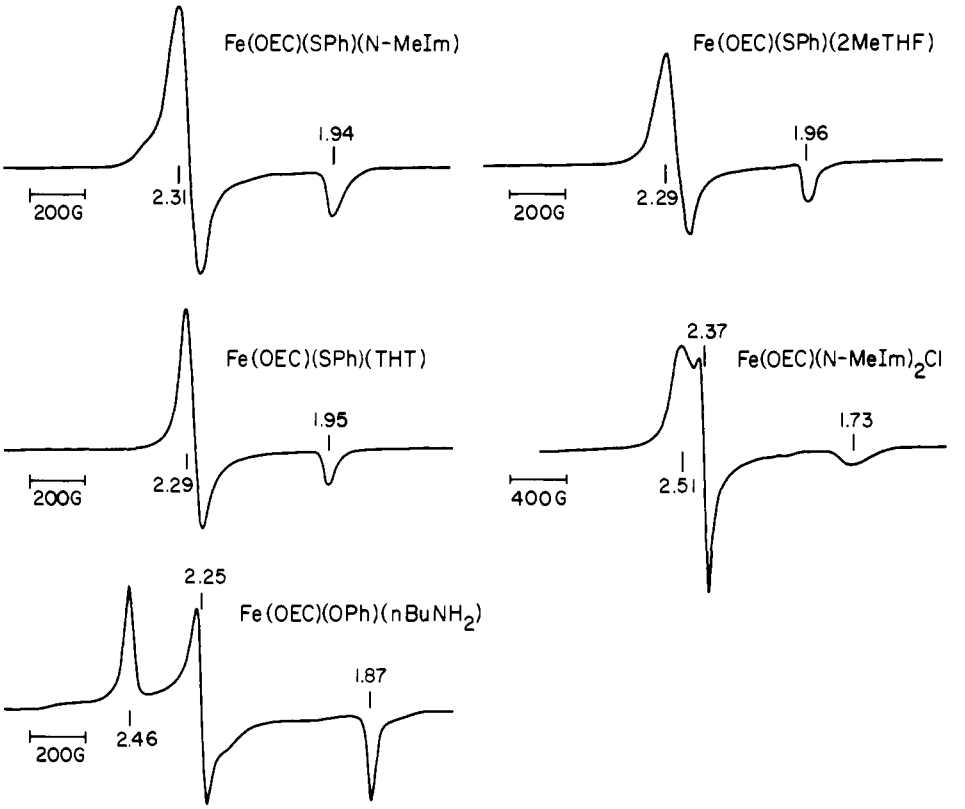

Figure 13. EPR spectra of selected low-spin Fe(OEC)LL′ species in toluene glasses at 80–85 K (g values are indicated).

Fe(OEiBC)LL′, for which a total of three was noted earlier.

(f) Voltammetry. Redox reactions of Fe(P)L (L = Cl^-, OAc^-), $[Fe(P)]_2$ and $Fe(P)(py)_2$ complexes were investigated in dichloromethane solutions by cyclic voltammetry. Potentials are collected in Table IV. The reaction patterns of hydroporphyrin Fe(III) species follow that of their OEP and other porphyrin counterparts,[74-76] viz., two oxidations at positive potentials and one or two reductions at negative potentials. Except for the hydroporphyrin acetates the first oxidations are chemically reversible ($i_{p,c} \simeq i_{p,a}$). Oxidation to the dications $[Fe(OEC)L]^{2+}$

(74) R. H. Felton in "The Porphyrins", Vol. V, D. Dolphin, Ed., Academic Press, New York, 1978, Chapter 3.

(75) R. H. Felton, G. S. Owen, D. Dolphin, and J. Fajer, *J. Am. Chem. Soc.*, **93**, 6332 (1971); R. H. Felton, G. S. Owen, D. Dolphin, A. Forman, D. C. Borg, and J. Fajer, *Ann. N.Y. Acad. Sci.*, **206**, 504 (1973).

(76) (a) K. M. Kadish, G. Larson, D. Lexa, and M. Momenteau, *J. Am. Chem. Soc.*, **97**, 282 (1975); (b) K. M. Kadish, M. M. Morrison, L. A. Constant, L. Dickens, and D. G. Davis, *ibid.*, **98**, 8387 (1976); (c) K. M. Kadish and L. A. Bottomley, *Inorg. Chem.*, **19**, 832 (1980).

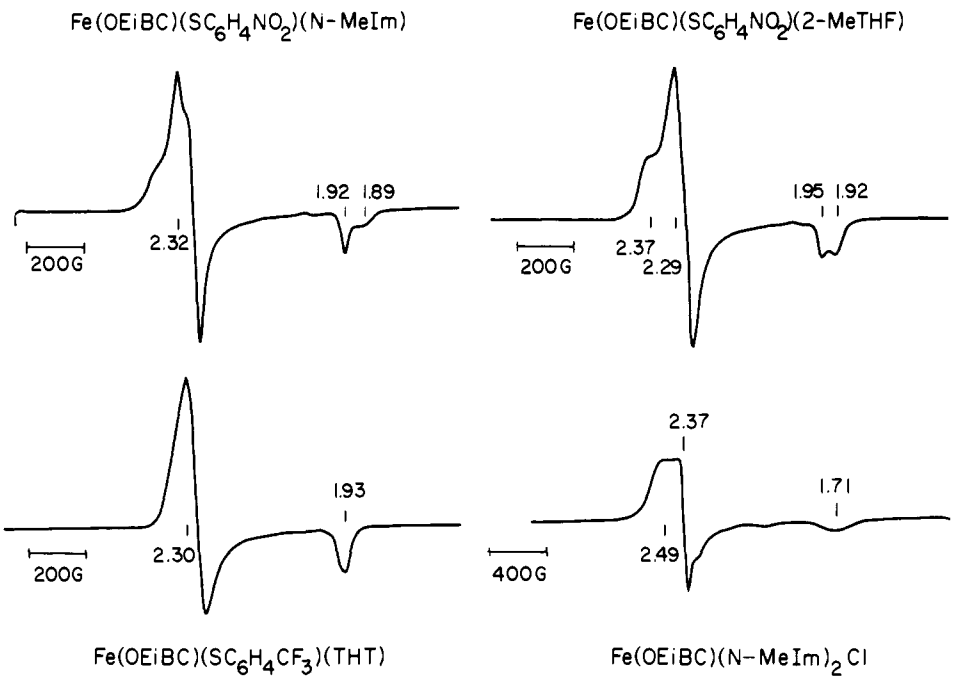

Figure 14. EPR spectra of selected low-spin Fe(OEiBC)LL' species in toluene glasses at 80–85 K (*g* values are indicated).

and $[Fe(OEiBC)L]^{2+}$ leads to some dehydrogenation, as shown by the appearance during repetitive scans of features due to OEP and OEC species, respectively. As seen in Figure 15 the μ-oxo complexes show additional oxidations, either two one-electron (OEP) or one two-electron processes (OEC, OEiBC) at $\gtrsim$+1 V. These reactions, which correspond to the second oxidations of Fe(P)L, provide further evidence for the μ-oxo structure. At potentials equal to or more positive than those of these oxidation processes repetitive scans reveal gradual dehydrogenation of the initial complexes. However, no appreciable quantities of mixed macrocycle species were present in the initial preparative samples used here and elsewhere; treatment of these species with HOAc afforded only the features due to the corresponding Fe(P)(OAc) complexes.

All oxidation processes occur at potentials which increase in the order (11), with incremental changes of 400–600 mV. H_2P

$$\text{OEiBC} < \text{OEC} < \text{OEP} \quad (11)$$

$$\text{DMOEiBC} < \text{etiochlorin I} < \text{OEP} \quad (12)$$

and Zn(P) follow the same sequence,[23] as do the free bases and Zn(II) and Fe^{III}–Cl complexes in series 12.[29] Potential differences for the latter species are substantial in both series and amount to 250–400 mV between adjacent members. In contrast, the irreversible first reductions of Fe(P)L span a range of 70 (L = Cl^-) and 50 mV (L = OAc^-). The first oxidations of $Fe(P)(py)_2$, also assigned as Fe(III)/Fe(II), are perfectly reversible chemically and cover a small range, 40 mV, as do the first reductions of $[Fe(P)]_2O$ (50 mV), which correspond to the same process.[76ab] Comparably small intervals have been observed for the first reductions of Fe–Cl and first oxidations of $Fe(py)_2$ complexes in series 12, with the reactions in both cases also being assigned as Fe(III)/Fe(II).[29]

Results presented here and elsewhere[23,25-29] lead to the following summary of redox properties: (i) in ligand-based processes corresponding to the formal change $P^{2-}/P^{-\cdot}$ isobacteriochlorin species are much more easily oxidized than are their chlorin and porphyrin counterparts, with potential differences of $\gtrsim$0.25 and $\gtrsim$0.5 V, respectively, being usual; (ii) in metal-centered Fe(III)/Fe(II) processes potentials exhibit a much smaller dependence on macrocycle than in i, with the ranges across series 11 and 12 being usually $\lesssim$50 mV. Statement i is based primarily on the behavior of free bases and Zn(II) complexes. Characterization of the electronic structures ($Fe^{IV}(P^{2-})$ or $Fe^{III}(P^{-\cdot})$) of the first oxidation products of Fe(P)L is beyond the scope of this investigation,[77]

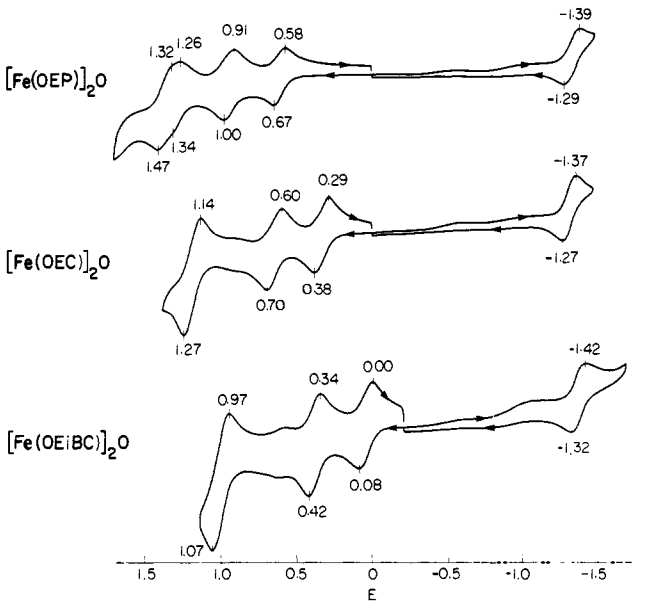

Figure 15. Cyclic voltammograms of $[Fe(P)]_2O$ complexes in dichloromethane solutions recorded at ~25 °C and 100 mV/s (peak potentials vs. SCE are indicated). The small waves preceding the major reductions presumably result from cleavage of μ-oxo complexes by acidic impurities in the solvent.

whose main purpose is an experimental determination of the effects of macrocycle structure on potentials. However, the parallel behavior of potentials of Fe(P)L and $[Fe(P)]_2O$ with those of H_2P and Zn(P) is strongly suggestive of first oxidations which are essentially ligand-based, a particularly likely event for isobacteriochlorin complexes owing to their intrinsically low potentials.

Discussion

During the course of this investigation of iron(II, III) hydroporphyrins some 34 and 37 new chlorin and isobacteriochlorin

(77) This matter has not been fully clarified for porphyrin Fe(P)L and $[Fe(P)]_2O$ complexes; cf. ref 74, 75, and R. G. Wollman and D. N. Hendrickson, *Inorg. Chem.*, **16**, 723 (1977); M. A. Phillippi and H. M. Goff, *J. Am. Chem. Soc.*, **101**, 7641 (1979); I. A. Cohen, *Struct. Bonding (Berlin)*, **40**, 1 (1980).

species, respectively, have been prepared and isolated or generated in solution and examined by one or more physical techniques. Other than for the species detected only by EPR in frozen solutions, the interlocking results from spectroscopic and electrochemical observations and reactivity properties suffice for the identification of the hydroporphyrin complexes schematically depicted in Figure 1. This body of information, together with that from related studies of etiochlorin I and isobacteriochlorin complexes of the DMOEiBC type,[26,29] substantially increases the knowledge of iron hydroporphyrins which should prove contributory to an ultimate interpretation of structure and function of these entities as catalytic sites in enzymes. In this context two main issues raised at the outset are considered. The first of these involves property comparisons of synthetic complexes and siroheme prosthetic groups in corresponding oxidation states in an attempt to deduce the nature of axial ligation in the enzymes. The second issue concerns an assessment of the effect(s) of macrocyclic structure on corresponding properties of porphyrin, chlorin, and isobacteriochlorin species with emphasis on uncovering any special feature(s) of hydroporphyrins which render them especially suitable for catalysis of multielectron reductions. As will be seen, neither of these issues can yet be satisfactorily dealt with, but certain possibilities can be foreclosed or rendered less likely than others. In the following sections emphasis is placed on isobacteriochlorins and siroheme enzymes; insufficient information has been collected on purified heme-*d* containing enzymes to make their inclusion of value at this stage.

Comparison with Siroheme Enzymes. When extracted from enzymes with acetone–HCl the siroheme chromophore exhibits a spectrum[11,15] (376, 594 nm, A_{376}/A_{594} = 3.3) in fairly good agreement with that of Fe(OEiBC)Cl in benzene (377, 601 nm, A_{377}/A_{601} = 3.2). Similarly, the spectra of extracted siroheme reduced in the presence of pyridine and CO[13] (558, 600 nm) and Fe(OEiBC)(py)(CO) in benzene (388, 547, 589 nm) are in reasonable correspondence but agree no less well with spectra in the absence of pyridine[15,78] (392, 553, 593 nm). Reduction in the presence of pyridine affords a chromophore (401, 520 (sh), 557 nm) which deviates somewhat more from its likely analogue, Fe(OEiBC)(py)$_2$ (Table I), than in the previous two cases. These observations, and others of band shape and relative intensity comparisons, indicate that Fe–OEiBC complexes are close but not exact duplicate chromophores of corresponding siroheme species.

In purified assimilatory enzymes the most intense visible band occurs in the ranges 572–580[12–15,79] and 584–595 nm[21,80–82] for oxidized (as isolated) nitrite and sulfite reductases, respectively. This form of the enzymes contains high-spin Fe(III)[19,81,83,84] and thus may have a Fe(P)L coordination unit. Spectral series 8 and 9 for Fe(OEiBC)L complexes offer reasonable ranges for visible bands arising from L = $-S^-$ and $-O^-$ coordination in the enzymes, with the variations in secondary ligand structure roughly simulating protein modulations (if any) of Fe–L bonding interactions.[31] On this basis thiolate ligation appears less likely than coordination by an anionic oxygen ligand in the nitrite enzymes. Imidazole binding cannot be excluded; no five-coordinate [Fe(OEiBC)(Im)]$^+$ species has yet been obtained. For sulfite reductases none of these ligation modes can be discounted from available Fe(OEiBC)L spectra. None of these spectra simulate those of the siroheme chromophores of the oxidized dissimilatory sulfite reductases desulforubidin and, especially, desulfoviridin,[11,84] which also contain high-spin Fe(III).[84–86] Another distinctive chromophore is the reduced enzyme–CO complex, whose principal visible bands fall at 585–590[12–14] and 593–602 nm[78,84,87] for nitrite and sulfite reductases, respectively. The 589-nm feature of Fe(OEiBC)(*N*-MeIm)(CO) in benzene is in fairly good agreement with the enzyme–CO species but, because Fe(OEiBC)L(CO) species with L = $-S^-$ and $-O^-$ ligands have not yet been generated, it is not decisive in identifying the axial ligand(s) in the enzymes.

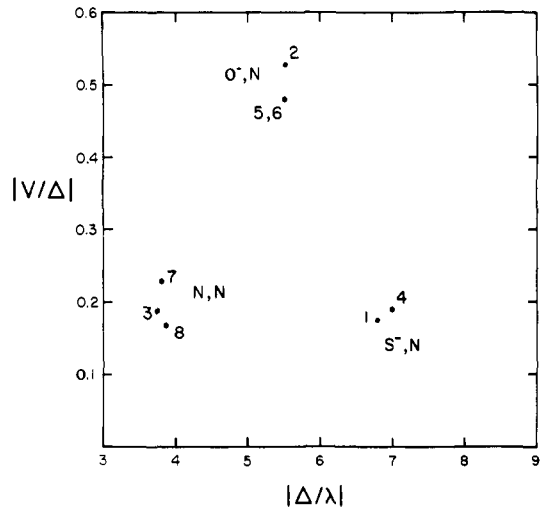

Figure 16. Plot of tetragonal (Δ) and rhombic (V) ligand field components of Fe(P)LL′ complexes with well-resolved rhombic spectra (Table III): P = OEiBC (1–3), OEC (4–7), TPC (8). λ is the spin–orbit coupling constant.

All assimilatory and dissimilatory siroheme enzymes as isolated exhibit rhombic high-spin Fe(III) EPR spectra.[19,81,83–86] Splittings of the g = 6 signal indicate that the percent rhombicity[88] $R \simeq$ 5–12%. Among the axial ligands in all three Fe(P)L series only thiolates invariably produce substantial rhombic splittings ($R \simeq$ 16–19%, Figures 10–12). These observations, while suggestive,[28] are by themselves not conclusive in respect of axial ligand identification in enzymes. Sources of rhombicity in high-spin protein sites have been considered,[89] and the greater sensitivity of high-spin vs. low-spin Fe(III) EPR spectra has been pointed out.[88] Environmental effects producing rhombic spectra of Fe(P)L species lacking thiolate ligands are documented.[90] Among proteins certain abnormal hemoglobin M mutants differing from normal hemoglobins by replacement of distal or proximal histidine by tyrosine show rhombic spectra.[91] Environmental effects aside, rhombic spectra have been reported for several synthetic complexes with L = ArO^-, OAc^-,[40,72] and EtO^-.[31]

EPR spectra of low-spin Fe(P)LL′ species have been examined by the truth diagram approach of Peisach and Blumberg.[70,73,92] The treatment is necessarily arbitrary because neither the signs nor the orientations of the principal **g** tensor components to a molecule-based axis system have been determined for any chlorin

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(82) M. Schedel and H. G. Trüper, *Biochim. Biophys. Acta*, **568**, 464 (1979).
(83) J. R. Lancaster, J. M. Vega, H. Kamin, N. R. Orme-Johnson, W. H. Orme-Johnson, R. J. Krueger, and L. M. Siegel, *J. Biol. Chem.*, **254**, 1268 (1979).
(84) M. J. Murphy, L. M. Siegel, H. Kamin, D. V. DerVartanian, J.-P. Lee, J. LeGall, and H. D. Peck, Jr., *Biochem. Biophys. Res. Commun.*, **54**, 82 (1973).
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(86) C. L. Liu, D. V. DerVartanian, and H. D. Peck, Jr., *Biochem. Biophys. Res. Commun.*, **91**, 962 (1979).
(87) K. Asada, G. Tamura, and R. S. Bandurski, *J. Biol. Chem.*, **244**, 4904 (1969); P. A. Trudinger, *J. Bacteriol.*, **104**, 158 (1970).
(88) J. Peisach and W. E. Blumberg in "Probes of Structure and Function of Macromolecules and Enzymes", Vol. II, B. Chance, T. Yonetani, and A. S. Mildvan, Eds., Academic Press, New York, 1971, pp 231–239.
(89) J. Peisach, C. A. Appleby, and W. E. Blumberg, *Arch. Biochem. Biophys.*, **150**, 725 (1972).
(90) Cf., e.g., M. Sato and H. Kon, *Inorg. Chem.*, **14**, 2016 (1975); M. Chikira, H. Kon, and K. M. Smith, *J. Chem. Soc., Dalton Trans.*, 526 (1980).
(91) A. Hayashi, T. Suzuki, A. Shimizu, H. Morimoto, and H. Watari, *Biochem. Biophys. Acta*, **147**, 407 (1967); H. Watari, K.-J. Hwang, K. Kimura, and K. Murase, *ibid.*, **120**, 131 (1966).
(92) W. E. Blumberg and J. Peisach, *Adv. Chem. Ser.*, **No. 100**, 271 (1971).

Table V. Summary of Comparative Physicochemical Properties of Synthetic Porphyrin and Hydroporphyrin Complexes: $Fe^{III}(P)L$ (1), $[Fe^{III}(P)]_2O$ (2), $Fe^{III}(P)LL'$ (3), $Fe^{II}(P)$ (4), $Fe^{II}(P)LL'$ (5), and $Fe^{II}(P)(CO)$ (6)

property/P	OEP[a]	OEC	OEiBC
Uv/vis[b]	1: (OAc^-, RO^-, ArO^-, RS^-, ArS^-)[c]	1: 375, 596 (OAc^-); 377–385, 592-600 (ArO^-); 377, 602-605 (ArS^-)	1: 372, 595 (OAc^-); 361–378, 582-599 (ArO^-); 373-374, 590-600 (RS^-)[d]
	2: 388, 563, 590	2: 380, 605	2: 376, 601
	4: 410, 562	4: 393, 626	4: 370, 387, 616
	5: 412, 548 (*N*-MeIm); 410, 528, 558 (*N*-MeIm, CO)	5: 403, 417, 599 (*N*-MeIm); 409, 608 (*N*-MeIm, CO)	5: 400, 568 (*N*-MeIm); 396, 589 (*N*-MeIm, CO)
	ϵ(Soret)/ϵ(vis): $\gtrsim$10	$\gtrsim$4-5	$\lesssim$4
	λ_{max} (nm, vis) follows series 8 and 9		
MCD	1: distinguishes L = ArS^- vs. OAc^-, ArO^-[e]		1: relatively insensitive to L = ArS^-, ArO^-, OAc^-
^{1}H NMR[g]	1: shifts −44 to +56 ppm[h]	1: shifts −60 to +93 ppm	1: shifts −80 to ~+100 ppm, 2–3 isomers
	2: antiferromagnetic, shifts 0 to −10 ppm;[h] contact shifts (1,2)	2: same[f]	2: same
	4: $S = 1$, shifts −80 to −13 ppm;[i] dipolar + contact shifts[j]	4: shifts −73 to +26 ppm,[i] probable $S = 1$ and dipolar + contact shifts	4: poorly resolved spectrum
EPR	1: high-spin, rhombic (ArS^-, RS^-),[k] $R \approx$ 12-28%;[l] ~axial (OAc^-, ArO^-, RO^-)[m]	1: same (ArS^-), $R \approx$ 14–19%; ~axial (OAc^-, ArO^-)	1: same
	3: low-spin, rhombic (all L,L' sets)[n]	3: same, rhombic (L = ArS^-, ArO^-; L' = $BuNH_2$; L,L' = N-MeIm); ~axial (other L,L' sets)	3: same [isomers detected (L' = *N*-MeIm, 2-MeTHF, $(ArS)_2$)]
IR	2: ν(Fe-O-Fe) 881 cm^{-1}	2: ~860 cm^{-1}	2: same
redox	5: (L = *N*-MeIm, py; L' = CO) / 6: } ν(CO) nearly independent of P ($\Delta k \lesssim 1\%$)		
	1 (L = Cl^-, OAc^-), 2: potentials for oxidation strongly P dependent and increase in the order OEiBC < OEC < OEP		
	1 (L = Cl^-, OAc^-), 2, 5 (L = L' = py): Fe(II)/Fe(III) potentials nearly invariant to P		

[a] Similar properties for other porphyrin complexes, cf. references. [b] Principal bands, nm. [c] For data cf. ref 31, 40, 50, and 51. [d] Includes transient EtS^- species. [e] Ref 51 and 55. [f] Refers to information in immediate left-hand column. [g] Paramagnetic species only. [h] Reference 65. [i] 6 °C. [j] Reference 66. [k] Reference 31, 45, and 50. [l] % rhombicity $R = 6.25\ (\Delta g)$.[89] [m] For exceptions cf. ref 72. [n] References 31, 45, 49, and 50.

or isobacteriochlorin complex. We have adopted the "proper" axis system and attendant g value signs deduced by Taylor[93] for $[Fe(TPC)(Im)_2]^+$ to the OEC complexes in Table III and, owing to the near identity of g values at parity of axial ligation, to the OEiBC complexes as well. This choice amounts to placing the tetragonal axis in the heme plane and satisfies two constraints of a proper axis system: $g_z + g_y - g_x$ is positive and the ratio of the rhombic and axial ligand field components $|V/\Delta| \leq 2/3$. The results, plotted in Figure 16 for complexes with well-resolved rhombic spectra, show separate regions for N,N, for O^-,N, and for S^-,N ligand sets. Similar treatment of the g values for low-spin forms of sulfite and nitrite reductases generated by addition of inorganic ligands to the high-spin forms[94] suggests that the two enzymes do not have a common native axial ligand, consistent with the spectral differences of the latter forms noted above. This approach may prove valuable in identifying native ligands when well-defined synthetic complexes containing the exogenous enzyme ligands are obtained. Few other well-characterized low-spin forms of the oxidized enzymes have been reported.[95]

Comparison of Iron Porphyrins and Hydroporphyrins. Properties of $Fe^{II,III}$OEP, -OEC, and -OEiBC complexes as obtained from application of six physicochemical techniques are summarized for convenient reference in Table V. Of these, the results from redox and IR spectroscopic studies are considered the more pertinent in seeking significant differences among the complexes related to their suitability as prosthetic groups in nitrite and sulfite reductases.

One possible role of the isobacteriochlorin macrocycle could be to impart greater reducing power to Fe(II). The first reduction potentials of the Fe(P)L species vary only slightly, however. This behavior can be rationalized by assuming that the well-established d orbital energy order for iron(II, III) porphyrins[64,69,70] holds in hydroporphyrins (using for convenience the same axis system), inasmuch as these reductions would then involve addition of an electron to the largely nonbonding d_{xy} orbital of the high-spin d^5 configuration.[97] Although these reductions are presumably related to the first reductions of siroheme in oxidized enzymes,[99] they are not germane to substrate reducing power. The situation with a low-spin d^6 substrate-bound complex is different in that substrate reduction would remove electron(s) from Fe(II) $d\pi$-type orbitals which are mixed with the HF π MO of each macrocycle, whose potentials differ largely across series 11. A preferred role for isobacteriochlorin vs. chlorin or porphyrin in imparting greater reducing power to Fe(II) should then imply an increase in electron density at Fe in low-spin d^6 complexes. In pursuing this matter we have employed two experimental probes: oxidation potentials of $Fe(P)(py)_2$ (Table IV) and ν(CO) of Fe(P)L(CO) and $Fe(P)(CO)_{1,2}$ complexes using CO as a "pseudosubstrate" in order to assess the relative ability of the heme groups to transfer electron density to the LUMO of a substrate. The EPR properties of the closely related $[Fe(P)(N\text{-MeIm})_2]^+$ complexes (Table III) leave little doubt that these potentials correspond to metal-centered

(93) C. P. S. Taylor, *Biochim. Biophys. Acta*, **491**, 137 (1977).
(94) J. R. Lancaster, Jr., J. C. Salerno, L. M. Siegel, and H. Kamin, *Biochemistry*, in press.
(95) Analysis of the g values of the dissimilatory nitrite reductase of *T. denitrificans* in the isolated low-spin form[96] (heme *d*) places it in the N,N region of Figure 17, suggesting two imidazole axial ligands.
(96) J. LeGall, W. J. Payne, T. V. Morgan, and D. V. DerVartanian, *Biochem. Biophys. Res. Commun.*, **87**, 355 (1979).
(97) A different measure of the similarity of metal orbitals lacking π symmetry is the near invariance of g values and Cu hyperfine splittings in the series Cu(P), P = OEP, OEC, OEiBC;[23,98] TPP, TPiBC;[26] PPIXDME, sirohydrochlorin.[10]
(98) J. H. Fuhrhop, P. K. W. Wasser, J. Subramian, and U. Schroder, *Justus Liebigs Ann. Chem.*, 1450 (1974).
(99) Potential data for this reaction are limited, with estimates being $\leq$−310[85] and −345[18] mV and −50[100] and −120[19] mV vs. SHE for sulfite and nitrite reductases, respectively.
(100) M. L. Stoller, R. Malkin, and D. B. Knaff, *FEBS Lett.*, **81**, 271 (1977).

processes. In the latter approach one envisions a situation in which both P and L transfer electron density to Fe, which is back-bonded to the CO π^* MO. The observed ν(CO) values differ little at constant axial ligand or in its absence and in each series correspond to a difference in CO stretching force constants, $\Delta k \lesssim 1\%$. Thus, by this type of measure at least, the three-ring systems reveal only very slightly different cis effects influencing electron density transferable to axial ligand π^* orbitals; if anything, OEiBC species are marginally less effective than OEP species in this regard. These findings and the near constancy of $[Fe(P)(py)_2]^{0,+}$ potentials, which span an interval of only 40 mV (with $Fe(OEiBC)(py)_2$ marginally the most reducing), indicate that Fe–P orbital interactions are insufficiently extensive to afford an experimentally decisive dependence of electron density at Fe upon macrocycle. In Fe(P)L(CO) species this situation could arise from the energy order HF π MO > Fe dπ, indicated by MO calculations[29b] and supported by the observation that oxidation of Fe(DMOEiBC)(py)(CO) isomers does not result in loss of CO but instead produces ligand-based radicals.[26,29] Oxidation of two enzymes in their reduced siroheme–CO forms is reported to result in loss of CO,[14,78] suggesting the reverse energy order. Unless the energy levels differ significantly between enzyme-bound siroheme and the models, these results are modest evidence against imidazole ligation in the Fe(II) state of either enzyme. Furthermore, they suggest that different axial ligand(s) may alter the order and energy separation of these orbitals and, as one consequence, the extent of orbital mixing. Thus the possibility remains that, given the proper axial ligand(s), isobacteriochlorins might increase the available electron density at Fe(II).

Of all comparative properties none is more prominent than the markedly greater ease of oxidation of iron(III) isobacteriochlorins (series 11 and 12); indeed, for ligand-based oxidations this must be considered an intrinsic property for all isobacteriochlorins. The foregoing observations have suggested to us, and to others using similar complexes of series 12 and related macrocycles,[26,29] that a functional role of reduced siroheme could entail an effective two-electron transfer to bound substrate S. Such a process would involve both Fe and ring oxidation and could have two limiting descriptions, e.g., reactions 13 and 14.[101] Some precedent for

$$Fe^{II}(P^{2-})L(S) \rightleftharpoons Fe^{II}(P^{-\bullet})L(S^{-}) \rightleftharpoons Fe^{III}(P^{-\bullet})L(S^{2-}) \quad (13)$$

$$Fe^{II}(P^{2-})L(S) \rightleftharpoons Fe^{III}(P^{2-})L(S^{-}) \rightleftharpoons Fe^{III}(P^{-\bullet})L(S^{2-}) \quad (14)$$

reaction 13 is found in the ligand-based oxidation of Fe(DMOEiBC)(py)(CO). The loss of CO upon oxidation of enzyme does not necessarily rule out the intervention of a iron(II) siroheme radical species during substrate reduction inasmuch as different axial ligands, including substrate, may alter the energy order of Fe dπ and macrocycle HF π orbitals. Other than observing that $Fe(OEiBC)(py)_2$ undergoes a second (irreversible and presumably ligand-based) oxidation at +0.61 V, reaction 14 is difficult to test at present. While reactions 13 and 14 or related formulations deserve further consideration, no evidence currently exists that necessitates or even implies that siroheme ring oxidation occurs during the course of enzyme action.

In summary, this investigation, as well as complementary studies by others,[26,29] has uncovered only one markedly different comparative property of iron(II, III) isobacteriochlorins that might be of physiological relevance—the much lower potentials for reversible ligand-based oxidation. The properties investigated (Table V), with the exception of redox behavior, are "static" spectroscopic features. It is entirely possible that significant differences amongst Fe complexes of the three macrocycle types may be more evident in dynamic properties related to kinetics and equilibria of their reactions. Certain observations, as yet incomplete but to be pursued, suggest that this may be the case. Metalation of free bases and the autoreduction reaction 6 occur in the rate order OEiBC $\gg$ OEC > OEP, and, as noted earlier, K_2(OEiBC, OEC) > K_2(OEP) for reaction 5. In assessing the results of this investigation, which is an example of the synthetic analogue approach to the study of protein prosthetic groups,[102] we emphasize that well-selected analogues convey only the intrinsic properties of the prosthetic groups, i.e., those unmodulated by protein influences. Comparison of the spectral data of free and enzyme-bound sirohemes reveal small but definite differences. At this relatively early stage of development of siroheme enzymes it remains to be learned whether, in particular, reaction properties of the enzymes can be approached by the analogues.

Lastly, the placement of sirohydrochlorin as a biosynthetic precursor to corrins,[18,103] and the determination that corrin biosynthesis is more ancient than heme biosynthesis,[104] suggests that sirohydrochlorin is indeed archaic. This raises several questions. Is it possible that siroheme-containing enzymes developed at a time when only isobacteriochlorins were available?[105] Could the central importance of these enzymes to the metabolic cycles of the biosphere have led to a strong pressure for their strict evolutionary conservation? If so, it is conceivable that the presence of siroheme in these enzymes merely represents a happenstance of, or is a "lock-in"[18] in further, evolution rather than selection of the prosthetic group most suited for the chemical task at hand. It is our view that these considerations, not being amenable to direct resolution, are best addressed by further seeking a possible special competence of iron isobacteriochlorins, in conjunction with electron carriers such as $[Fe_4S_4(SR)_4]^{3-}$,[106] as catalysts of multielectron reductions.

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(105) Porphyrinogens are biosynthetic precursors to isobacteriochlorins and as such should be even more archaic. However, recent results[24b-d] show that porphyrinogens, in the presence of metal salts, are converted to metalloisobacteriochlorins. Thus, metalloporphyrinogens would probably have been unavailable to the enzymes.

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