Inorg. Chem. **2005**, *44*, 1882–1889

Magnetic Resonance and Structural Investigations of (Monooxooctaethylchlorinato)iron(III) Chloride and Its Bis(imidazole) Complex

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Received July 9, 2004

(Monooxooctaethylchlorinato)iron(III) chloride, (oxo-OEC)FeCl, **1**, has been investigated by X-ray crystallography and by ^{1}H NMR spectroscopy. Its bis(imidazole-d_4) complex has been studied by multidimensional ^{1}H NMR and EPR spectroscopies, and the results are compared to those for the bis(Im-d_4) complex of (octaethylchlorinato)-iron(III) chloride, (OEC)FeCl, **2**. EPR and NMR results show that both [(oxo-OEC)Fe(Im-d_4)$_2$]Cl and [(OEC)Fe(Im-d_4)$_2$]Cl are low-spin Fe(III) complexes with $(d_{xy})^2(d_{xz},d_{yz})^3$ electronic ground states, both at 4.2 K (EPR spectra) and at ambient temperatures utilized for solution NMR studies. The pattern of chemical shifts of the pyrrole-CH_2 and *meso* protons are similar, with the 8,17-carbons having the largest and the 12,13-carbons having the smallest spin densities in each case, except that [(OEC)Fe(Im-d_4)$_2$]Cl has a slightly wider range of pyrrole-CH_2 chemical shifts and more resonances are observed for [(oxo-OEC)Fe(Im-d_4)$_2$]Cl due to its lower symmetry. Full proton resonance assignments for both complexes have been made from COSY, NOESY, and NOE difference experiments.

Introduction

Among the "green" hemes that catalyze biological reactions as diverse as assimilatory and dissimilatory nitrite and sulfite reductions, molecular oxygen reduction, and peroxide dismutation are heme d_1 of bacterial dissimilatory nitrite reductases[1−5] and siroheme of bacterial sulfite and nitrite reductases,[6−10] heme *d* of the cytochrome *bd* terminal oxidase from *Escherichia coli*,[11,12] and heme *d* of catalase HPII from *E. coli*.[13,14] In addition, the sulfhemes[15,16] and heme *s* (a formylhemin)[17] are also green in color, yet all of the macrocycles mentioned, all of which are sometimes described collectively as hydroporphyrins or reduced hemes, are uniquely different from each other. Some of the confusion regarding the classification of reduced hemes and expectations as to their electronic properties has been caused by the fact that hemes *d* and d_1 were once both thought to be chlorins. While this is indeed the case for heme *d* of the *E. coli* terminal oxidase[18,19] and the *E. coli* catalase HPII[20]

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10.1021/ic049088y CCC: $30.25
Published on Web 02/25/2005

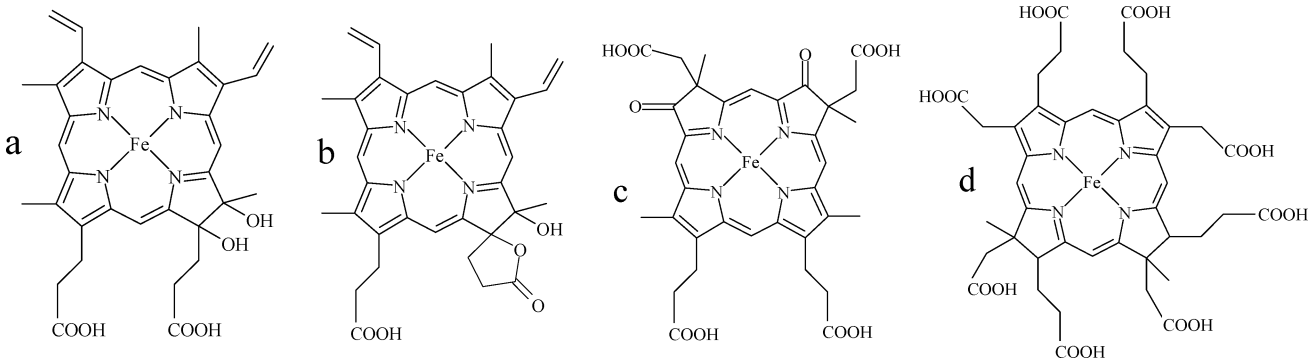

Figure 1. Structures of some naturally occurring hydroporphyrins: (a) heme *d* of bacterial cytochrome *bd* oxidase;[19] (b) heme *d* of *E. coli* catalase HPII;[20] (c) heme d_1 of bacterial cytochrome cd_1 dissimilatory nitrite reductases;[21] (d) siroheme of sulfite reductases.[22]

(although they differ in structure, in that the latter is the 6-propionate spirolactone of the former and the regioisomerism of the 5-hydroxy group differs), heme d_1 is now known to be a dioxoisobacteriochlorin (dioxo-*i*BC).[21] All of the heme *d*-named macrocycles, as well as siroheme, an iron isobacteriochlorin,[22,23] are shown in Figure 1.

Understanding the electronic properties of the iron(III) complexes of each of the individual "green" hemes is an important step in elucidation of their mechanisms of action. Hence, in an attempt to investigate the electronic properties of heme d_1, we have begun a study of the iron(III) complexes of monooxooctaethylchlorin (oxo-OEC) and dioxooctaethylisobacteriochlorin (dioxo-OE*i*BC), the latter of which should be a good model for the heme d_1 center. The synthesis of these macrocycles from octaethylporphyrin[24−26] and of derivatives of the natural porphyrins from hematoporphyrin[27] has been reported, as has the crystal structure of (oxo-OEC)FeCl[28] and (dioxo-OE*i*BC)FeCl.[29] In this work, a second molecular structure of (oxo-OEC)FeCl has been solved, and the ^{1}H NMR and EPR spectra of its bis(imidazole) complex are reported. The NMR and EPR spectra are compared to those of the bis(imidazole) complex of (octaethylchlorinato)iron(III), a macrocyclic complex of the same oxidation level.

Experimental Section

Materials and Methods. $OEPH_2$ was purchased from Mid-Century. The anhydrous iron(II) acetate (Aldrich) and *N*,*N*-dimethylformamide (DMF) (Aldrich) were used as received. Dinitrogen gas was purified using a BASF Catayst R3-11 column and anhydrous calcium sulfate (Drierite). All other reagents and solvents were obtained from Aldrich and used without further purification.

UV−vis spectra were obtained on a Perkin-Elmer Lambda 19 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Unity-300 NMR spectrometer or a Bruker DRX-500 spectrometer. The EPR spectra were obtained on a CW EPR spectrometer, ESP-300E (Bruker) operating at X-band, using 0.2 mW microwave power and a 100 kHz modulation amplitude of 2 G. A Systron-Donner microwave counter was used for measuring the frequency.

Synthesis. Chloroiron(III) Mono-Oxooctaethylchlorin (1). Pure oxo-$OECH_2$ (0.1 mmol), prepared and purified as previously described,[24−26] was dissolved in hot DMF (7 mL) under nitrogen. The solution was brought to reflux with stirring, 2 mmol of Fe(II) acetate and 2 mmol of NaCl were added, and the reaction was allowed to proceed. After 15 min, completion of the reaction was confirmed by UV−vis spectroscopy that showed that the bands of the free-base porphyrin had disappeared. The reaction flask was immersed in an ice−water bath, methylene chloride (25 mL) was added, and the mixture was washed with 3 × 25 mL of distilled water. The organic layer was dried over sodium sulfate, and then the solvent was evaporated. The iron oxo-OEC was separated from the small amount of free-base oxochlorin by chromatography on a 2 × 20 cm^2 column of silica gel (Baker no. 3405, 60−200 mesh), with 10:1 chloroform/methanol mobile phase. The solvent was removed from the heme-containing band under reduced pressure. The remaining solid was then redissolved in 25 mL of chloroform and treated with 100 mL of 1 M NaCl containing 120 mM HCl, to cleave any μ-oxo dimer product that had formed on the column. Removal of solvent and vacuum-drying yielded about 70% chloroiron(III) oxo-OEC, (oxo-OEC)FeCl.

The product was characterized by UV−vis and NMR spectroscopies, mass spectrometry, and X-ray crystallography. The UV−vis spectrum of pure (oxo-OEC)FeCl (**1**) in CH_2Cl_2 (λ_{max}: 384 (11.50 mM^{-1} cm^{-1}), 486 (1.581), 517 (sh, 1.40), 551 (1.205), 599 (2.19), 661 (0.436), and 744 nm (0.560); $A_{384}/A_{599} = 5.2$) resembles closely in wavelength maxima, general appearance, and relative intensities of the bands the spectrum for (OEC)FeCl, **2**, in C_6H_6 (λ_{max}: 377 (11.50), 473 (1.02), 511 (sh, 0.70), 559 (0.724), 602 (3.13), and 751 nm (0.323); $A_{377}/A_{602} = 3.7$)[30] and bears a marked resemblance to the spectrum of siroheme (λ_{max}: 376 and 594 nm; $A_{376}/A_{594} = 3.3$).[31] ^{1}H NMR data for the bis(imidazole-d_4) complex at 30 °C are provided in Table 1. The mass spectrum shows a peak at *m*/*z* 604.5 that corresponds to the [(oxo-OEC)Fe]$^+$ ion; the isotope pattern observed corresponds closely to that calculated.

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Table 1. Chemical Shifts of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl and [(OEC)Fe(Im-d_4)$_2$]Cl[a]

^{1}H shifts (ppm)		
[(oxo-OEC)Fe(Im-d_4)$_2$]$^+$	[(OEC)Fe(Im-d_4)$_2$]$^+$	assignment
19.7	26.0	8-CH_2 group (2H)
19.3	26.0	17-CH_2 (2H)
14.4	7.0	18-CH_2 (2H)
12.6	7.0	7-CH_2 (2H)
5.8	8.4	5-*meso* (1H)
4.4	3.5	10-*meso* (1H)
4.1	3.5	15-*meso* (1H)
3.5	8.4	20-*meso* (1H)
2.3−2.1[b]	2.9 (8, 17) 2.3 (7, 18)	7,8,17,18-CH_3 (12H)
1.9	−0.1	13-CH_2 (2H)
−0.4	−0.1	12-CH_2 (2H)
−1.2	−1.2	13-Me (3H)
−1.3	−1.2	12-Me (3H)
−2.3	−1.3	3-Me (6H)
−2.7	−5.0 (2, 3)	3-CH_2 (2H)
−4.4	−5.0 (2, 3)	3-CH_2 (2H)
	17.9	pyrroline-H (2H)

[a] In CD_2Cl_2 at 303 K. [b] Too crowded to make assignments.

(Octaethylchlorinato)iron(III) chloride ((OEC)FeCl) (2) was synthesized and purified as described previously.[30]

Crystallography. Crystals of (oxo-OEC)FeCl for X-ray investigations were grown using the following procedure. A small amount of **1** (10 mg) was dissolved in 0.2 mL of $CHCl_3$ (dried over molecular sieves and freshly distilled) in a thin glass tube and layered carefully with 0.2 mL of pure hexane or dodecane. The tube was covered with foil and stored in a dark place for several days. The dark-blue plate obtained from the chloroform/dodecane mixture having approximate dimensions of 0.02 × 0.13 × 0.22 mm^3 was mounted on a glass fiber in a random orientation. From the chloroform/hexanes mixture parallelepiped-like crystals were obtained. They had the same unit cell parameters as the first crystals, and both were very similar to those of Neal and co-workers.[28] Examination of the crystal on a Bruker SMART 1000 CCD detector X-ray diffractometer at 170(2) K and a power setting of 50 kV and 40 mA showed measurable diffraction to at least $\theta = 24.2035°$. Data were collected on the SMART1000 system using graphite-monochromated Mo Kα radiation ($\lambda = 0.710\ 73$ Å). The experimental details together with crystal data for **1** are given in Supporting Information Table S1.

NMR and EPR Spectroscopic Measurements. Samples for NMR studies were dissolved in CD_2Cl_2 (Cambridge Isotopes) to a concentration of ∼5 mM and placed in 5 mm NMR tubes. The solutions were not degassed. The complexes with the deuterated imidazole ligands were made by directly adding an excess of axial ligand ([(oxo-OEC)FeCl]:[imidazole] and [(OEC)FeCl]:[imidazole] ≈ 1:4, ∼20 mM Im-d_4) and then degassed three times with nitrogen before sealing the NMR tubes. NMR spectra were obtained on a Varian Unity 300 (COSY and NOESY) or a Bruker DRX 500 (NOE difference) spectrometers at 30 or 25 °C, respectively. The 2D spectra were recorded using 6−9 kHz spectral width, 512−1024 t_2 data points, 128 t_1 increments, and 512−1024 transients with relaxation delays ranging from 0.1 to 0.3 s and mixing time of 50 ms (NOESY). In the NOE difference experiments, a relaxation delay of 50−100 ms and a preirradiation time of 30−50 ms with an intermediate power level was usually used. The number of transients for each spectrum was 8192 or 16384 in an interleaved manner. The EPR samples were made in the same way as the NMR samples but in CH_2Cl_2. The EPR measurements were performed at 4.2 K using an Oxford continuous-flow cryostat, ESR 900.

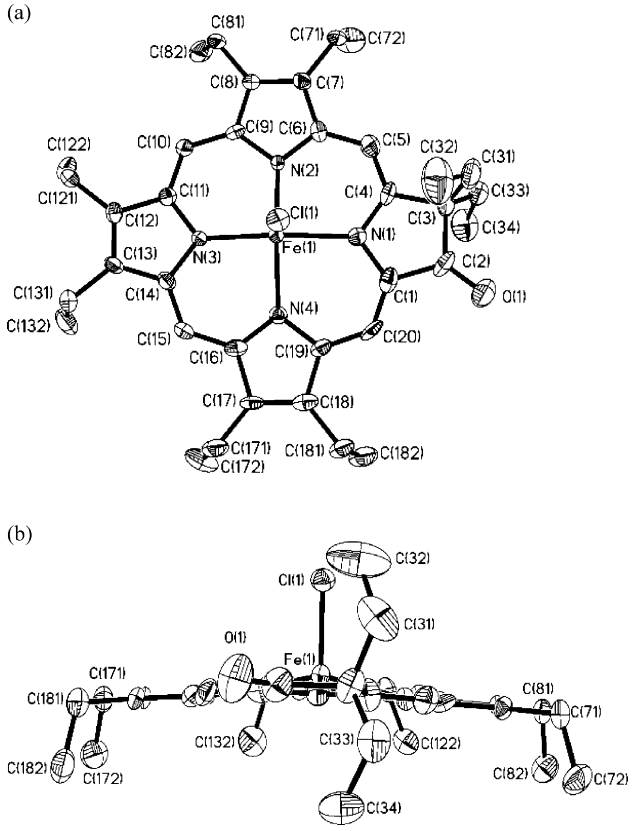


Figure 2. (a) ORTEP diagram of the macrocycle of **1** without H atoms for simplicity. The 50% probability surfaces are shown. (b) ORTEP diagram of the side view of the core. The Fe atom is 0.46 Å out of the 24 porphyrin mean plane and 0.45 Å out of the mean plane of the four nitrogens.

Results

Crystal Structure of (oxo-OEC)FeCl·CHCl$_3$. The molecular structure as well as the atom numbering scheme for **1** are presented in Figure 2a. Figure 2b shows the side view of **1**. The structure is very similar to that reported by Neal and co-workers for the methylene chloride solvate.[28] As reported previously,[28] the iron is 0.45 Å out of the plane of the four nitrogens; all ethyl groups (except one on the pyrroline ring) point in one direction, away from iron and chloride. Unlike (dioxo-OE*i*BC)FeCl,[29] which is domed, with all the C_β atoms except C2 lying below the plane of nitrogens, **1** adopts something close to a saddled conformation, with adjacent rings being displaced above and below the mean plane of the four nitrogens, as shown in Supporting Information Figure S1. The most distorted is the pyrroline ring that bears the carbonyl oxygen and the geminal diethyl group.

^{1}H NMR Spectroscopy of (oxo-OEC)FeCl. The ^{1}H NMR spectrum of **1**, shown in Figure 3, has many magnetically inequivalent protons for this type of complex because of its low symmetry (Chart 1). A total of 16 methylene, 8 methyl, and 4 *meso* proton resonances were expected. The highest resolution of signals was achieved at 40 °C. At other temperatures peaks either were broader or overlapped more extensively with each other. The methylene resonances of **1** at 40 °C are located downfield at 57.6, 45.9, 43.2, 42.6, 41.8, 40.7, 40.2, 39.5, 38.9, 38.6, 38.2, 14.5, and 14.1 ppm; these resonances were not assigned. The three broad upfield

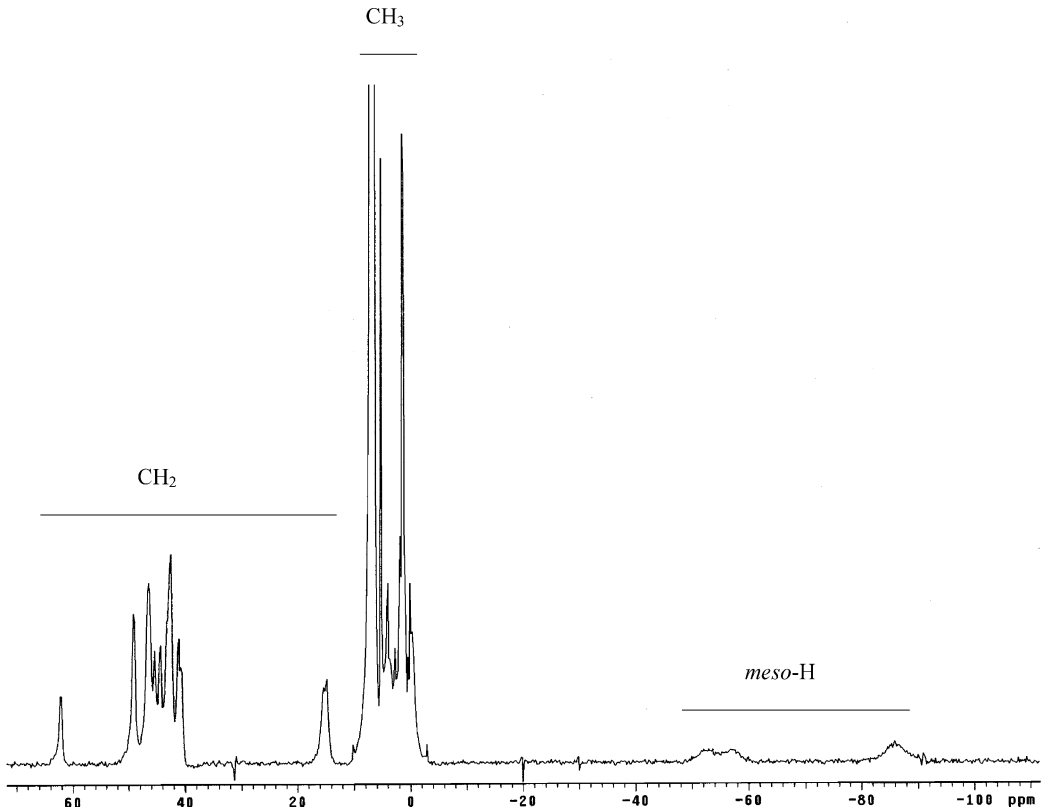
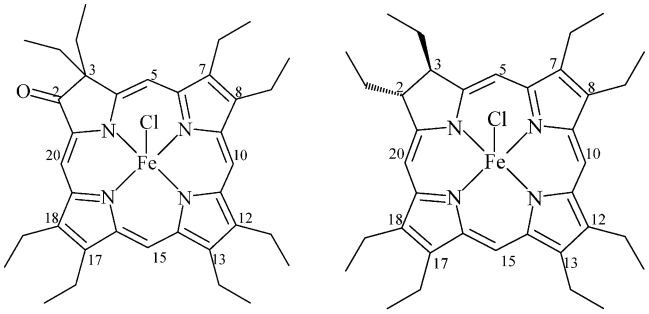


Figure 3. 1D proton NMR spectrum of (oxo-OEC)FeCl, recorded in CD_2Cl_2 at 23 °C.

Chart 1



1 (oxo-OEC)FeCl **2** (OEC)FeCl

resonances (−50.8 (1H), −53.8 (1H), and −81.7 ppm (2H)) are assigned to the four *meso* protons. The broad signal at 6.9 ppm corresponds to seven overlapping methyl groups. A narrow signal at 1.3 ppm is assigned to one CH_3 group. Other signals located between 0 and 5 ppm are due to impurities. The chemical shifts and pattern of the spectrum of **1** are quite similar to those of (OEC)FeCl, which has been investigated in detail in the accompanying paper,[32] indicating that **1** has the same electronic structure, that of HS Fe(III), $S = 5/2$.

^{1}H NMR Spectroscopy of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl. [(oxo-OEC)Fe(Im-d_4)$_2$]Cl has seven kinds of ethyl groups and four kinds of *meso* protons. Its ^{1}H spectrum is shown in Figure 4a, where it is compared to that of [(OEC)Fe-(Im-d_4)$_2$]Cl, discussed below, Figure 4b. For [(oxo-OEC)-Fe(Im-d_4)$_2$]Cl eight resonances are observed from the ethyl CH_2 groups (for the two ethyl groups at position 3, although they are chemically equivalent, the two geminal CH_2 protons in each ethyl group are not magnetically equivalent), seven resonances from the ethyl CH_3 groups, and four *meso*-H resonances. Complete peak assignments are given in Table 1. In the COSY spectrum of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl (Supporting Information Figure S2), for each ethyl group at position 7, 8, 12, 13, 17, and 18, only two pairs of cross-peaks, between the two CH_2 geminal protons and the CH_3 group within each ethyl group, are observed. The two ethyl groups at position 3, however, should give three pairs of cross-peaks, one between the two inequivalent CH_2 protons and two cross-peaks between each of them and the CH_3 group. Thus, the two peaks at −2.7 and −4.3 ppm can be assigned to the pyrrole-3-CH_2 protons. This assignment is consistent with the fact that the chemical shifts of pyrrole-3-CH_2 protons should have the smallest contact shift (compared to other pyrrole-CH_2 protons). The pyrrole-3-CH_2 groups are not directly attached to the aromatic ring of the macrocycle and hence are dominated by the negative pseudocontact (electron−nuclear dipolar) shift.[33] Several pairs of cross-peaks between two adjacent pyrrole-CH_2 groups and between CH_2 groups and the *meso* protons were observed in the NOESY spectrum (Figure 5). There are more cross-peaks covered by the large envelop in the diamagnetic region or by the noise. Therefore, NOESY results only give partial assignments of the pyrrole-CH_2 groups and *meso* protons.

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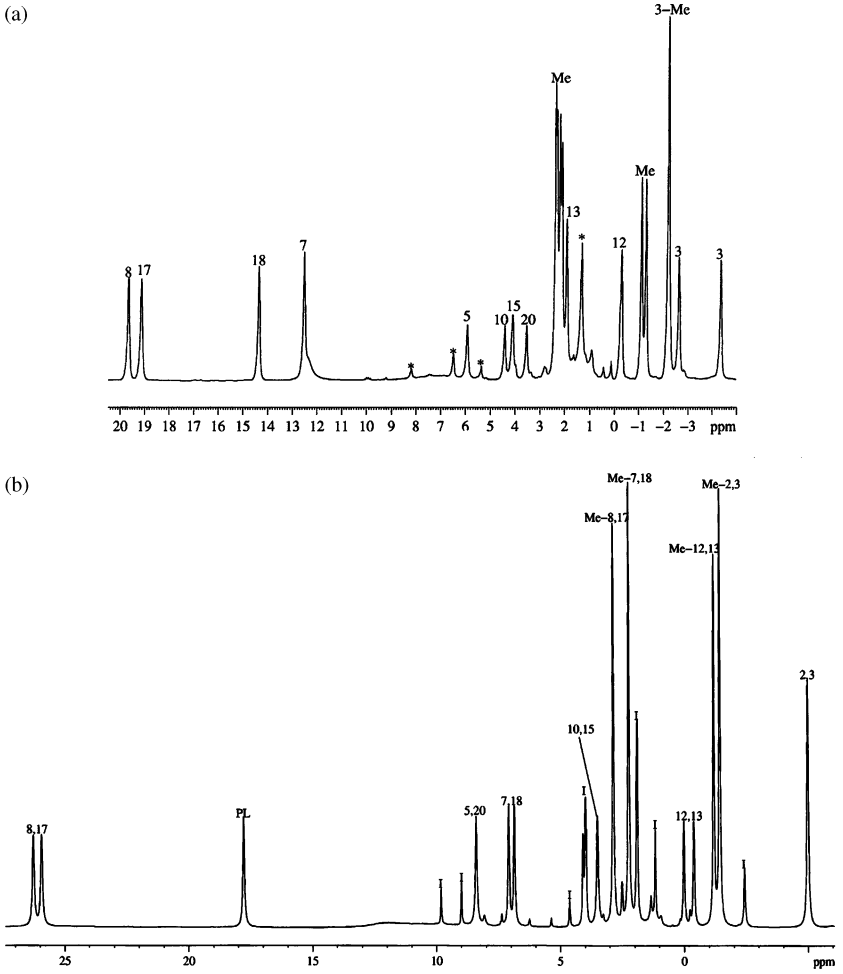

Figure 4. (a) ^{1}H spectrum of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl in CD_2Cl_2 at 30 °C: Me, methyl of the pyrrole ethyl group; numbers stand for the positions of the CH_2 groups and *meso* protons; asterisks indicate impurities. (b) ^{1}H spectrum of [(OEC)Fe(Im-d_4)$_2$]Cl in CD_2Cl_2 at 30 °C: I, impurity; Me, methyl of the pyrrole ethyl group; PL, pyrroline protons; numbers indicate the positions of the CH_2 groups and *meso* protons.

Full peak assignments were made from NOE difference experiments (Figure 6). For simplicity all CH_2 groups were assigned a number that corresponds to the number of the pyrrole β-C to which it is attached. In a typical NOE difference experiment, one proton resonance is irradiated and protons that are close in space to it give rise to positive peaks in the NMR spectrum. In Figure 6b, the *meso* proton resonance at 5.8 ppm was irradiated, giving two NOE signals, one from pyrrole-3-CH_2 and one from another pyrrole-CH_2 group at 12.5 ppm. According to the structure of **1**, only the *meso*-5-H can have an NOE with the pyrrole-3-CH_2 groups. Thus, the peak at 5.8 ppm should be from the *meso*-5-H and the pyrrole-CH_2 group at 12.5 ppm must be pyrrole-7-CH_2. From the NOESY spectrum, Figure 5, which shows cross-peaks between pyrrole-7-CH_2 and its adjacent CH_2 group (pyrrole-8-CH_2), the peak at 19.7 ppm is assigned to pyrrole-8-CH_2. In Figure 6c, the irradiated peak at 4.4 ppm that causes an NOE signal from the pyrrole-8-CH_2 group must be from the *meso*-10-H, and the other peak showing an NOE signal to *meso*-10-H can be assigned to pyrrole-12-CH_2. Although the NOE between the two pyrrole-CH_2 groups at positions 12 and 13 is invisible in the NOESY spectrum, it is detectable in the NOE difference spectra, when either of these two groups is irradiated (Figure 6f,g). Once the pyrrole-13-CH_2 group is assigned, the assignment of the *meso*-15-H can be made from the NOE between the pyrrole-13-CH_2 group and the *meso*-H at 4.1 ppm (Figure 6d). The other peak showing an NOE with the *meso*-15-H in Figure 6d is thus assigned to the pyrrole-17-CH_2 group. Since the pyrrole-17-CH_2 group shows cross-peaks with its adjacent CH_2 group (pyrrole-18-CH_2) in the NOESY spectrum (Figure 5), all pyrrole-CH_2 groups have now been assigned. Finally, the last *meso* proton, that whose resonance is at 3.5 ppm, the *meso*-20-H, which should have an NOE with the pyrrole-18-CH_2 group, can be assigned on the basis of the NOE shown in the difference spectrum (Figure 6e).

This assignment procedure can be briefly described by Scheme 1. In this scheme, the starting point is pyrrole-3-CH_2, whose assignment is known from the COSY spectrum and peak intensities. Then the assignments proceed clockwise around the pyrrole and *meso* positions of the oxochlorin ring shown in Chart 1. Each position is connected to the next position by NOEs detected in NOE difference spectra (*NOEdiff* in the scheme) or the NOESY spectrum. One of the findings of this work is that to observe the very weak NOEs of paramagnetic complexes, NOE difference experiments show an advantage over NOESY, because (1) they are steady-state experiments and suffer less than NOESY

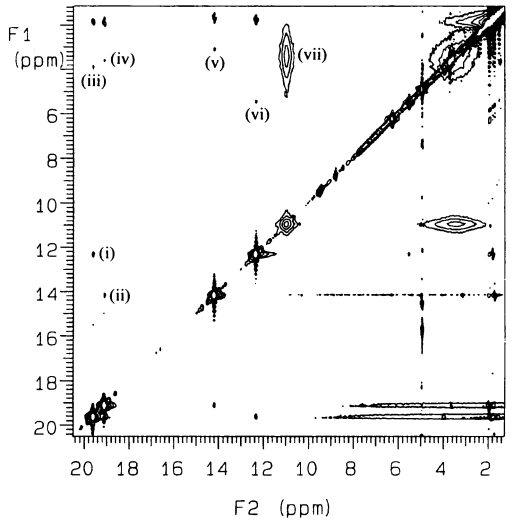

Figure 5. NOESY spectrum of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl. Several weak cross-peaks shown here are NOEs between (i) 7-CH_2 and 8-CH_2, (ii) 17-CH_2 and 18-CH_2, (iii) 8-CH_2 and *meso*-10-H, (iv) 17-CH_2 and *meso*-15-H, (v) 18-CH_2 and *meso*-20-H, and (vi) 7-CH_2 and *meso*-5-H. Note that some NOEs are invisible under the large envelope in the region of 4 to −4 ppm. The very broad cross-peaks vii are due to chemical exchange (the sign of these is opposite to that of NOEs) probably from traces of impurities, whose resonances were not observed in the 1D spectrum. Other cross-peaks (these stronger ones) are from the NOEs between CH_2 and CH_3 with the ethyl group, which were also observed in the COSY spectrum.

from the fast relaxation rates of the protons in the paramagnetic compound and (2) they are 1D experiments and thus larger numbers of transients (more than 10 000) can be obtained in a reasonable amount of time to improve the signal-to-noise ratio.

Scheme 1

pyrrole-3 —NOEdiff(*b*)→ *meso*-5 —NOEdiff(*b*)→ pyrrole-7 —NOESY→
pyrrole-8 —NOEdiff(*c*)→ *meso*-10 —NOEdiff(*c*)→ pyrrole-12 —NOEdiff(*f*,*g*)→
pyrrole-13 —NOEdiff(*d*)→ *meso*-15 —NOEdiff(*d*)→ pyrrole-17 —NOESY→
pyrrole-18 —NOEdiff(*e*)→ *meso*-20

^{1}H NMR Spectroscopy of [(OEC)Fe(Im-d_4)$_2$]Cl. Figure 4b shows the 1D spectrum of [(OEC)Fe(Im-d_4)$_2$]Cl. This complex has two kinds of *meso* protons and four kinds of ethyl groups, since it has C_2 symmetry along the axis that passes from the center of the pyrroline-2,3 bond to the center of the pyrrole-12,13 bond (Scheme 1). For each ethyl group, the two CH_2 protons are not equivalent due to the trans configuration of the two pyrroline protons. Thus, a total of eight resonances from CH_2 protons and two resonances from the *meso* protons were observed in the NMR spectrum of the low-spin complex. Partial assignments can be made from COSY (Supporting Information Figure S3) and NOE difference spectra (Supporting Information Figure S4), as detailed above for [(oxo-OEC)Fe(Im-d_4)$_2$]Cl. The chemical shifts of all proton resonances are summarized in Table 1, where they are compared to those of [(oxo-OEC)Fe(Im-d_4)$_2$]-Cl.

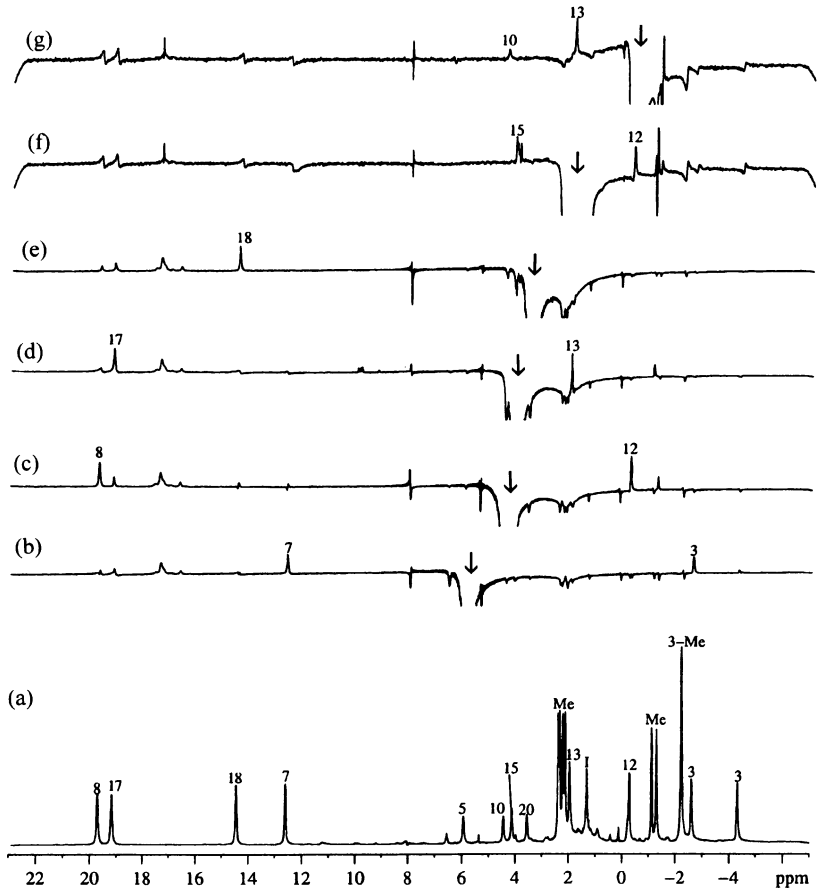

Figure 6. NOE difference spectra of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl: (a) control spectrum; (b−g) difference spectra. The arrows show the positions of irradiation in each case.

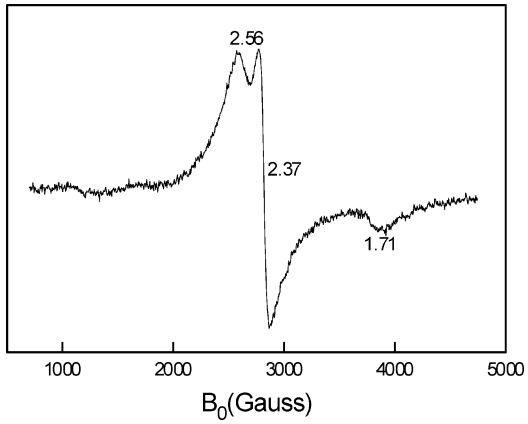

Figure 7. EPR spectrum of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl at 4 K.

EPR Spectroscopy of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl. The EPR spectrum (Figure 7) of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl (g = 2.56, 2.37, 1.71, $\Sigma g^2 = 15.10$, $V/\lambda = 3.14$, $\Delta/\lambda = 2.66$, $V/\Delta = 1.18$) is typical for a rhombic low-spin Fe(III) center.[33] It has g-values very similar to those of [(OEC)Fe(Im-d_4)$_2$]Cl (2.54, 2.39, 1.72, $\Sigma g^2 = 15.12$, $V/\lambda = 3.26$, $\Delta/\lambda = 2.51$, $V/\Delta = 1.30$)[32] and the tetraphenylchlorin analog [(TPC) Fe-(Im-d_4)$_2$]Cl (g = 2.49, 2.39, 1.75, $\Sigma g^2 = 14.97$, $V/\lambda = 3.59$, $\Delta/\lambda = 2.68$, $V/\Delta = 1.34$),[34] the latter of which has been shown by pulsed EPR techniques to have the $(d_{xy})^2(d_{xz},d_{yz})^3$ electronic ground state.[34] These EPR spectral results support the NMR data in confirming that the electronic ground state in both cases is $(d_{xy})^2(d_{xz},d_{yz})^3$, as is the case for [(TPC)Fe-(HIm)$_2$]$^+$ (which has similar g-values), and [(TPP)Fe-(HIm)$_2$]$^+$.[34]

Discussion

Our previous investigations of low-spin Fe(III) tetramesitylporphyrin complexes in which the axial pyridine ligand basicity and π donor−acceptor characteristics are varied systematically have shown that we can create a smooth shift in the position of the largest g-value signal to smaller values and that the pyrrole-H resonances shift smoothly from about −16 ppm to +2 ppm at 30 °C in the same order of ligand basicity.[35,36] We have shown that this smooth shift is indicative of a transition in electronic ground state from that usually assumed for low-spin Fe(III) hemes, i.e., $(d_{xy})^2(d_{xz},d_{yz})^3$, to the other possible ground state, $(d_{xz},d_{yz})^4(d_{xy})^1$.[35,37] The latter electron configuration has been suggested as the likely ground state of the reduced hemes, including low-spin Fe(III) chlorins,[30,38,39] sulfhemes,[15] and isobacteriochlorins.[30,40] However, we have shown that, in distinction to conclusions reached much earlier,[38,41] low-spin ferric tetraphenylchlorin complexes unambiguously have the more common $(d_{xy})^2(d_{xz},d_{yz})^3$ ground state, at least when bound to imidazole ligands, even though the EPR spectrum of the bis(imidazole) complex is much more compressed than that of its TPP or TMP counterpart.[34] In some low-spin complexes of reduced macrocycle ferrihemes the EPR spectra are axial, while in other cases they are rhombic but with small separation of the two largest g-values.[30,42] We have called these "near axial" or type III EPR signals[43] *if* it can be shown that g_{zz} is the smallest g-value or that NMR shifts are consistent with the metal d_{xy} orbital being used for spin delocalization to the $a_{2u}(\pi)$ porphyrin orbital. However, traditional methods of assigning the orbital of the unpaired electron, based on crystal field calculations that use the g-values of the complex,[34,41] have been shown to be unreliable,[44,45] so that either single-crystal EPR or pulsed EPR studies of frozen solutions of low-spin Fe(III) complexes of these macrocycles at very low temperatures and/or careful ^{1}H NMR investigations at ambient temperatures are required to determine unambiguously the orbital of the unpaired electron. Some ferric isobacteriochlorins have even been suggested to have a partial[46−48] or complete[49] Fe(II)P$^+$ electron configuration in the presence of π-acceptor axial ligands, i.e., a $(d_{xy},d_{xz},d_{yz})^6(\pi)^1$ ground state, and it has been suggested that an oxidized, $a_{1u}(\pi)$ cation radical state of Fe(III) siroheme may be accessible in the 6-electron reduction of SO_3^{2-} to S^{2-}.[22]

The ^{1}H NMR chemical shifts of the CH_2 resonances of [(oxo-OEC)Fe(Im-d_4)$_2$]Cl and [(OEC)Fe(Im-d_4)$_2$]Cl of this study range from 19.7 to −0.4 (average 14.1 ppm) and 26.0 to −0.1 (average 11.0 ppm), respectively, at 30 °C (Table 1). In comparison, the CH_2 resonance of [(OEP)Fe-(HIm)$_2$]Cl is found at 6.0 ppm at 29 °C.[50] To directly compare these pyrrole-CH_2 chemical shifts in terms of the approximate spin densities at the β-pyrrole carbons, it should be noted that the oxo-OEC and OEC rings have most of their spin density at six of the eight β-pyrrole positions and, thus, it is not surprising that the pyrrole-CH_2 resonances of the bis(imidazole) complexes of the latter two macrocycles have larger average chemical shifts than does the OEP complex. An approximate correction to allow proper comparison would

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be to reduce the average CH_2 shift of the two chlorin-based complexes to 3/4 the observed value, or 10.6 ppm for the oxo-OEC and 8.3 ppm for the OEC complex, as compared to 6.0 ppm for [(OEP)Fe(HIm)$_2$]Cl. With or even without this correction, we can see that the amount of spin delocalization to the β-pyrrole carbons is quite similar for the three complexes.

Likewise, for the *meso*-H shifts, the average values are 4.5, 6.0 (Table 1) and 3.0[50] ppm for the oxo-OEC, OEC, and OEP complexes, respectively, quite similar values as well. Both β-pyrrole-CH_2 and *meso*-H shifts are totally consistent with the $(d_{xy})^2(d_{xz},d_{yz})^3$ and totally inconsistent with the $(d_{xz},d_{yz})^4(d_{xy})^1$ electronic ground state, which would have very large negative (−37 ppm for [(OEP)Fe(*t*-BuNC)$_2$]$^+$ at 30 °C[51]) *meso*-H shifts and small (almost diamagnetic) shifts for the β-pyrrole-CH_2.[33,51,56] Thus, it is the *meso*-H shifts (or *meso*-phenyl-H shift patterns for *meso*-phenyl-substituted macrocycles[52]) that clearly define the electronic ground states of Fe(III) octaalkylporphyrinates and -chlorinates. NMR studies have also confirmed that the iron(III) tetraphenylchlorin,[53,54] octaethylchlorin,[32,51] and several naturally derived chlorins[55] have the common $(d_{xy})^2(d_{xz},d_{yz})^3$ electronic ground state when bound to imidazole and 4-(dimethylamino)-pyridine ligands but the less common $(d_{xz},d_{yz})^4(d_{xy})^1$ electronic ground state when bound to good π-accepting ligands such alkyl or aryl isocyanides,[32,53] as has been reported previously for (tetraphenylporphyrinato)iron(III) complexes with such ligands.[51,56] This was also clearly shown by a pulsed EPR investigation of the bis(phenyl isocyanide) complex of OEPFe^{III} that included ^{2}H-, ^{13}C-, and ^{15}N-labeled ligands, which has allowed clear determination of electronic ground state and the spin density on all ligand atoms of interest.[57]

The EPR *g*-values of the bis(imidazole) complexes of (oxo-OEC)Fe^{III} and (OEC)Fe^{III} are very similar to those of [(TPC)Fe(Im-d_4)$_2$]Cl,[34] which, as mentioned above, has been shown to have the $(d_{xy})^2(d_{xz},d_{yz})^3$ ground state, as does the TPP analogue.[34] Because these results are consistent with the NMR results, there is no need to investigate these complexes by pulsed EPR spectroscopy to confirm the assignment of the axes of the **g**-tensor. There is no question that the electronic ground state of the bis(imidazole) complexes of (oxo-OEC)Fe^{III} and (OEC)Fe^{III} are the same as those of the (TPC)Fe(III) and (TPP)Fe(III) bis(imidazole) complexes, i.e., $(d_{xy})^2(d_{xz},d_{yz})^3$. In comparison, the bis(*tert*-butyl isocyanide) complex of (OEC)Fe^{III} has been shown to have the $(d_{xz},d_{yz})^4(d_{xy})^1$ ground state[32] and the bis(cyanide) complexes of Fe(III) isobacteriochlorins and dioxoisobacteriochlorins are also believed to be $(d_{xz},d_{yz})^4(d_{xy})^1$ ground-state systems.[40,43] Further investigation of the low-spin iron(III) complexes of the latter macrocycles is under way in our laboratories.

In summary, both EPR and NMR results show that [(oxo-OEC)Fe(Im-d_4)$_2$]Cl and [(OEC)Fe(Im-d_4)$_2$]Cl are low-spin Fe(III) complexes, both with $(d_{xy})^2(d_{xz},d_{yz})^3$ ground states. The pattern of chemical shifts of the pyrrole-CH_2 and *meso* protons are similar (Table 1), except that [(OEC)Fe(Im-d_4)$_2$]-Cl has a slightly wider range of pyrrole-CH_2 chemical shifts and more resonances are observed for [(oxo-OEC)Fe-(Im-d_4)$_2$]Cl due to its lower symmetry. Full peak assignments have been made from COSY, NOESY, and NOE difference experiments.

Acknowledgment. The National Institutes of Health (Grant DK-31038 to F.A.W., Grant GM-33882 to A.M.S.) and National Science Foundation (Grant CHE9610374 to the University of Arizona Molecular Structure Laboratory) are gratefully acknowledged. This paper was written while F.A.W. was a Visiting Professor and Alexander von Humboldt Senior Awardee in Science in the Physics Institute of the University of Lübeck, and she wishes to thank Professor Alfred X. Trautwein for his hospitality and friendship.

Supporting Information Available: Figures S1−S4, showing spectra, and Table S1, listing X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

IC049088Y

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