NMR (C_6D_6) δ 2.14 (d, J = 5.2 Hz, 1 H), 1.93 (d, J_{HP} = 1.8 Hz, 15 H), 1.90 (s, 15 H), 1.18 (d, J = 6.4 Hz, 1 H); $^2H\{^1H\}$ NMR (C_6H_6) δ 1.29; $^{13}C\{^1H\}$ NMR (C_6D_6) δ 107.6 (d, J_{CP} = 9.8 Hz), 91.2 (d, J_{CP} = 2.6 Hz), 86.6 (s), 76.7 (s), 29.4 (s), 12.0 (s), 11.5 (s) (the phosphine carbons were not observed); $^{31}P\{^1H\}$ NMR (C_6D_6) δ −40.73 (s), −40.93;[29] MS, m/e 779 (M^+ base); UV (hexane) λ_{max} 266, 331, 500.

Thermolysis of 2-d_9. In the drybox 20 mg (0.026 mmol) of **2**-d_9 and 0.6 mL of C_6H_6 were added to an NMR tube that was fused to a ground glass joint. A Kontes vacuum stopcock was attached to the tube and the apparatus removed to the vacuum line and the solution degassed. The NMR tube was flame sealed under vacuum and the reaction mixture heated to 80 °C in an oil bath. After 4 h a new resonance appeared in the $^2H\{^1H\}$ NMR spectrum at δ 7.94 ppm. After an additional 4 h at 80 °C the NMR tube was cracked in the drybox, the volatile materials removed in vacuo, and then the residue dissolved in C_6D_6. ^1H NMR (C_6D_6) δ 5.53 (dt, J = 1.1, 6.5 Hz), 1.93 (d, J = 2 Hz), 1.90 (s), 1.29 (br d, J_{HP} = 9.4 Hz).[30]

The above product was again dissolved in C_6H_6 and heated to 100 °C for 4 h. New deuterium resonances in the $^2H\{^1H\}$ NMR spectrum appeared at δ 4.43, 2.39, 1.56, 1.05, −17.9 (d, J_{DP} = 5.7 Hz). After the solution was heated at 100 °C for an additional 4 h the solvent was once again changed to C_6D_6 and the ^1H NMR spectrum showed new proton resonances at δ 4.0 (br t, J = 6.7 Hz), 2.64 (d, J = 5.7 Hz), 1.64 (br s), 1.99 ns) for **7**-d_9.

(η^5-C_5Me_5)(η^2-CD_2=CD_2)Ir(η^1,η^3-C_3H_4)Ir(η^5-C_5Me_5) (**4**-d_4). The procedures for the synthesis of **4**-d_4 were identical with those given for **4**. ^1H NMR (C_6D_6) δ 7.10 (dt, J = 1, 5 Hz, 1 H), 5.28 (m, 1 H), 2.28 (br d, J = 5.4 Hz, 1 H), 1.83 (s, 15 H)8 1.77 (s, 15 H), 1.27 (d, J = 6.3 Hz); $^2H\{^1H\}$ NMR (C_6H_6) δ 1.6 (br s, 3D), 1.1 (br s, 1 D); MS, m/e 726, 694 (M^+, M^+ − C_2D_4 base).

Thermolysis of 4-d_4. A solution of **4**-d_4 in C_6D_6 was prepared in an NMR tube as described in the thermolysis of **2**-d_9 described above. The reaction materials were heated to 80 °C for 5 h, during which time no observable isotopic scrambling occurred by ^1H NMR spectrometry. Continued heating to 100 °C for 4 h and analysis by $^2H\{^1H\}$ NMR spectrometry in C_6H_6 showed the production of **9**-d_4. New resonances in the $^2H\{^1H\}$ NMR spectrum: δ 7.37, 3.34, 2.18 and broad peaks in the range 2-1 ppm.

Acknowledgment. This work was supported by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract DE-AC03-76SF000098. W.D.M. acknowledges the Organic Division of the American Chemical Society for a predoctoral fellowship. We are also grateful for a generous loan of $IrCl_3 \cdot nH_2O$ from Johnson-Matthey, Inc., and for helpful discussions with Thomas Foo.

Registry No. 1, 103619-65-4; **1**-d_6, 116323-57-0; **1**-d_{10}, 116323-58-1; **2**, 103639-16-3; **2**-d_9, 116323-59-2; **3**, 103619-69-8; **4**, 103619-70-1; **4**-d_4, 116323-60-5; **5**, 103619-71-2; **6**, 116323-61-6; **7**, 103619-66-5; **7**-d_9, 116323-62-7; **8**, 116323-63-8; **9**, 116323-64-9; **9**-d_4, 116323-65-0; **10**, 116323-66-1; **11**, 116323-67-2; (η^5-C_5Me_5)Ir(η^3-C_3H_5)H, 96427-40-6.

Supplementary Material Available: Tables of positional parameters and their estimated standard deviations, tables of general temperature factor expressions (B's), and root mean square amplitudes of thermal vibrations for **2** and **7** (10 pages); tables of F_o and F_c for the X-ray diffraction studies of complexes **2** and **7** (42 pages). Ordering information is given on any current masthead page. Full details of the X-ray diffraction study of complex **1** are given as supplementary material with the preliminary communication (ref 5c).

(29) We attribute the appearance of two resonances in the $^{31}P\{^1H\}$ NMR spectrum to be from **2**-d_9 and **2**-d_8; the difference in chemical shift is the result of slightly different isotopic perturbations on the phosphorous atom.

(30) This proton resonance corresponds to a small amount of hydrogen in the methyl groups of the phosphine ligand which originated from the α-allylic position in the starting material.

Reactive Iron Porphyrin Derivatives Related to the Catalytic Cycles of Cytochrome P-450 and Peroxidase. Studies of the Mechanism of Oxygen Activation

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Contribution from the Department of Chemistry, Princeton University, Princeton, New Jersey 08544. *Received March 14, 1988*

Abstract: The mechanism of oxidation of tetramesityliron(III) porphyrins [Fe^{III}TMP(X)] with peroxyacids has been examined. The reaction of Fe^{III}TMP(Cl) (**1**) with peroxyacids in methylene chloride at −46 °C afforded the corresponding oxoiron(IV) porphyrin cation radical [Fe^{IV}TMP$^{\cdot+}$(O)] (**3**). The kinetics of this process were complicated by an induction period that depended on the acidity of the peroxyacid used. By contrast, similar oxidation of Fe^{III}TMP(OH) gave evidence for rapid ligand metathesis to afford an acylperoxoiron(III) complex, Fe^{III}TMP(OOC(O)Ar) (**2**). The decomposition of **2** to form **3** was found to be first order in **2** and catalyzed by acid. Electron-withdrawing substituents on the aryl portion of the peroxyacid facilitated this reaction (ρ = +0.5). The temperature dependence between −32 and −48 °C indicated E_a = 4 ± 0.4 kcal/mol, $\Delta H^{\ddagger}$ = 3.6 ± 0.4 kcal/mol, and $\Delta S^{\ddagger} > -25$ eu. The oxidation of **1**-(*m*-chlorobenzoate) in toluene with peroxyacids afforded an iron(III) porphyrin *N*-oxide (**5**). The reaction required 2 equiv of peroxyacid and afforded 1 mol of a diacylperoxide. The presence of acid discouraged the formation of **5**. Substituent effects in the peroxyacid were the opposite for the formation of **5** (ρ = −0.4) than for the formation of **3**. The results indicated that there are competing homolytic and heterolytic O–O bond cleavage reactions for **2** mediated by iron(III).

Since the discovery of cytochrome P-450 monooxygenase[1] three decades ago, numerous attempts have been made to understand this unique enzyme.[2] The manipulation of molecular oxygen to afford an active oxygen species, the so called "oxenoid" (FeO^{3+}), by utilizing heme iron and reducing equivalents while generally accepted is still poorly understood in chemical terms. The initial steps in the catalytic cycle of cytochrome P-450 have been well established (Scheme I).[2c-e] The reaction sequence involves hydrophobic binding of the substrate close to the heme cofactor in the resting ferric state. Subsequent uptake of a single electron from an associated reductase enzyme and oxygen binding lead

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† Current address: School of Medicine, Keio University, Tokyo, Japan.

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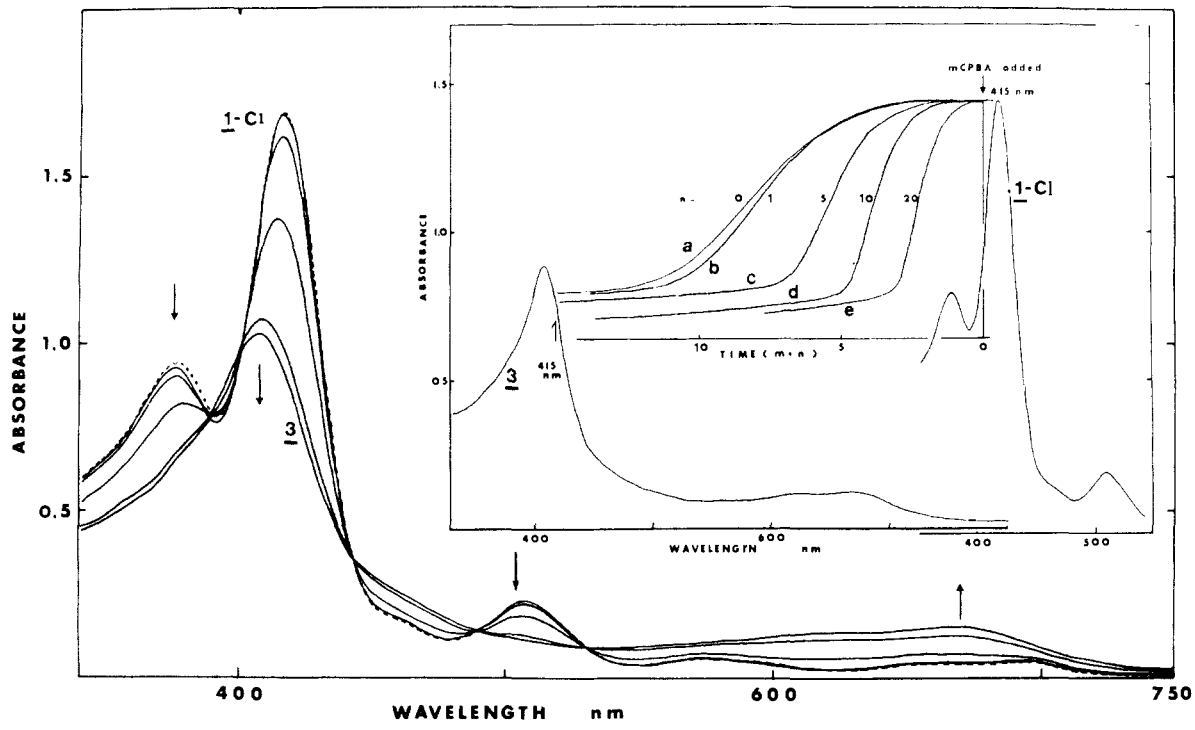
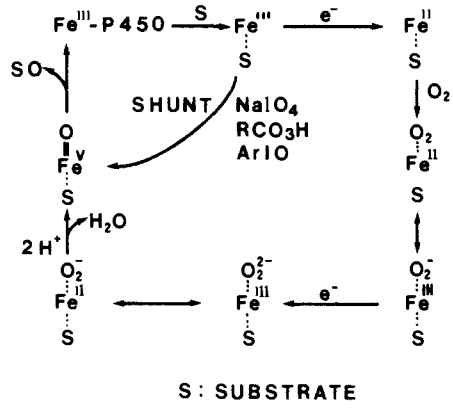


Figure 1. Visible spectral changes upon the addition of 4 equiv of mCPBA to $Fe^{III}TMP(Cl)$ (**1**-Cl) [1.22×10^{-5} M] (---) in methylene chloride at −46 °C at 2-min intervals (first scan was immediate after the addition of mCPBA). Inset: Effect of acid in the formation of **3**. Lines a–e are absorbance changes at 415 nm upon the addition of 3 equiv of mCPBA to the mixture of **1**-Cl [9.7×10^{-5} M] and *n* equiv of mCBA in methylene chloride at −46 °C.

Scheme I



to the formation of a ferrous–dioxygen complex or an equivalent superoxoferric complex. Capture of a second electron is thought to form a peroxoferric state (Scheme I). Synthetic superoxoferric and peroxoferric porphyrins have been characterized as stable species.[3,4] By contrast, the transformations in later steps in this process have not been observed directly and are not well understood.

Protonation (or, possibly, acylation[5]) of the peroxo oxygen followed by O–O bond cleavage to afford a species with a single oxygen bound to iron, the "oxenoid" intermediate, has not yet been observed. Cytochrome P-450 has been shown to catalyze monooxygenation reactions by utilizing oxidizing reagents such as alkyl hydroperoxides,[6] iodosylarenes,[7] peroxyacids,[8] and other oxene donors.[9]. This bypass of the usual oxygen reduction pathway is referred to in Scheme I as the peroxide shunt mechanism. Because of the similarity of horseradish peroxidase (HRP) and the peroxygenase function of cytochrome P-450, an oxoiron(IV) porphyrin π-cation radical, similar to the well characterized compound I of HRP,[10] is considered to be the most attractive candidate for the "oxenoid" intermediate in the cytochrome P-450 cycle. The preparation and characterization of synthetic oxoiron(IV) porphyrin π-cation radical complexes and the oxygen transfer reactions observed with these reactive intermediates encourage this argument.[11] Alternatively, a bridged iron porphyrin *N*-oxide has been suggested[12] as a candidate of the "oxenoid" intermediate based on the characterization of N-bridged iron porphyrin carbene adducts,[12,13] the existence of several metalloporphyrin *N*-oxides,[14] and a N-bridged nitrene.[15] Furthermore, MO calculations have suggested that iron(III) porphyrin *N*-oxides will be more stable than the isomeric oxoiron(IV) porphyrin cation radical.[16] In our previous studies,[17,18] we showed that acylper-

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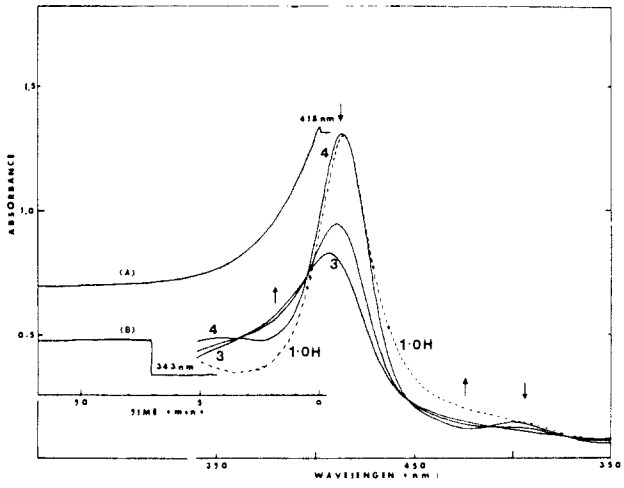

Figure 2. Visible spectral changes for the reaction of **1**-OH [1.48×10^{-5} M] (---) and 3 equiv of *p*-nitroperoxybenzoic acid. The spectrum of **4** was recorded immediately after the addition of *p*-nitroperoxybenzoic acid. Lines labeled A and B represent the time-dependent changes of absorbances at 418 and 363 nm.

oxoiron(III) porphyrins can be prepared and, depending on the reaction conditions, they can afford either an oxoiron(IV) porphyrin cation radical or an iron(III) porphyrin *N*-oxide. Thus, the addition of an acylperoxoiron(III) porphyrin to the family of observable iron porphyrin species now allows us to characterize all of the putative intermediates for the enzyme and for model systems. Furthermore, we are able to determine the detailed mechanisms of each primitive step in oxygen activation including the elusive O–O bond scission step by employing low-temperature spectroscopic techniques.

In this paper, we describe the conversion of an acylperoxoiron(III)-porphyrin to an oxoiron(IV)-porphyrin cation radical and an iron(III) porphyrin *N*-oxide by using (5,10,15,20-tetramesitylporphyrinato)iron(III) [Fe^{III}TMP] derivatives.

Results

Reaction of Fe^{III}TMP(Cl) (1-Cl) with Peroxyacids. The reaction of Fe^{III}TMP(Cl) (**1**-Cl) and *m*-chloroperoxybenzoic acid (mCPBA) took place in methylene chloride at −46 °C. The reaction profile was not as simple as expected for a one-to-one reaction between **1**-Cl and mCPBA, i.e., a significant induction period was followed by very fast disappearance of **1** to afford oxoiron(IV) porphyrin cation radical (**3**).[11] Figure 1 shows the visible spectra observed during the reaction recorded every 2 min after the addition of 4 equiv of mCPBA. It is clear that little change occurred during the initial few minutes followed by significant spectral changes without isobestic points between 400 and 420 nm. The effect of acid on this spectral change was examined. In the presence of various amounts of *m*-chlorobenzoic acid (mCBA), 3 equiv of mCPBA was introduced to a solution of **1**-Cl. As shown in Figure 1 (inset), the addition of acid had two obvious effects: (i) the induction period decreased with increasing acid concentration and (ii) the rate of the formation of **3** increased with increasing acid concentration, but finally saturation of this effect was observed. The simplest mechanism that explains these observations is as shown in eq 1, although we did not observe intermediate **2** under these conditions.

$$\underset{\text{1-Cl}}{\text{Fe}^{III}\text{TMP}(\text{Cl})} + \text{HO}_2\text{CAr} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\text{2}}{\text{Fe}^{III}\text{TMP}(\text{O}_2\text{CAr})} + \text{HCl} \xrightarrow{k_2} \underset{\text{3}}{\text{Fe}^{IV}\text{TMP}(\text{O}_2\text{CAr})} \quad (1)$$

Electron-withdrawing substituents on the aromatic ring of the peroxyacid were found to shorten the induction period and increase the rate of formation of **3**. Thus, the substituents affect both k_1 and k_2 and make it difficult to study the process **2** → **3** in eq 1 directly with chloride present.

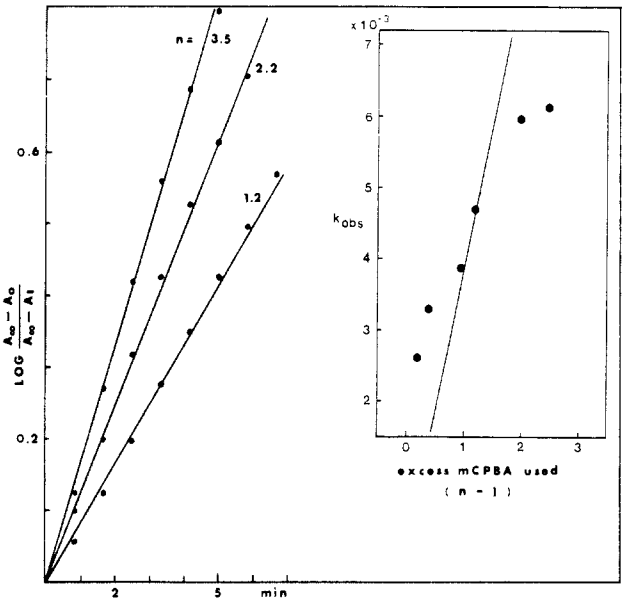

Figure 3. Plots of log $(A - A_0)/(A - A_t)$ at 415 nm vs time for the formation of **3** by the reaction of **1**-OH with *n* equiv of mCPBA in methylene chloride at −48 ± 0.5 °C. Inset: Effect of acid: ( ) observed; (—) calculated by eq 3, (...) calculated by eq 9.

Table I. The Effect of Peroxyacid Concentration on k_{obs}[a]

mCPBA added (*n* equiv)	*n* − 1	$10^3 k_{obs}$ (s^{-1})[b]
1.2	0.2	2.6
1.4	0.4	3.3
1.96	0.96	3.9
2.2	1.2	4.7
3.0	2.0	5.9
3.5	2.5	6.1

[a] For the oxidation of Fe^{III}TMP(OH) at −48 ± 0.5 °C in methylene chloride. [b] Correlation coefficients were >0.998.

Reaction of Fe^{III}TMP(OH) (1-OH) with Peroxybenzoic Acids. The reactions of **1**-OH with substituted peroxybenzoic acids were carried out under the conditions described above. As shown in Figure 2, the reaction to afford **3** was biphasic, namely, the instantaneous formation of intermediate **4** was observed followed by smooth isosbestic spectral changes to afford **3**, the spectrum of which was identical with that observed in the reaction of **1**-Cl and mCPBA. Traces A and B in Figure 2 show the time course of absorbance changes upon the addition of *p*-nitroperoxybenzoic acid to a methylene chloride solution of **1**-OH at −46 °C. The full spectrum of **4** in the reaction of **1**-OH with mCPBA was recorded at −45.8 °C immediately after the addition of mCPBA. For comparison an authentic sample of Fe^{III}TMP(*m*-chlorobenzoate) (**1**-mCB) was prepared by a similar reaction with excess mCBA at room temperature.

Both **1**-mCB and **4** displayed similar visible spectra; however, **4** decomposed to **3** even at low temperature. Furthermore, the reaction of **1**-OH with 1.2 equiv of *p*-nitroperoxybenzoic acid gave the same result. The similarity of the visible spectrum of **4** to that of the corresponding Fe^{III}TMP(benzoate), its facile conversion at low temperature to **3**, and the 1.2:1 stoichiometry of its formation from **1**-OH are consistent with an Fe^{III}TMP(peroxybenzoate) (**2**) formulation for **4**. The corresponding acylperoxo*manganese*(III) porphyrin complex has been formed in the same way.[19]

Kinetics in Methylene Chloride. (i) Effect of Acid. The decomposition of **2** (=**4**) to afford **3** was studied carefully by employing various amounts of mCPBA. In each case good first-order

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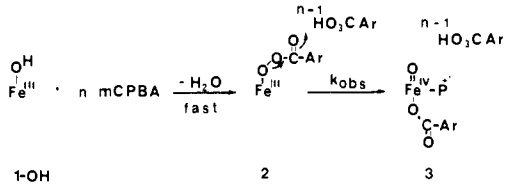
Table II. Temperature Effect on k_{obs}[a]

temp (K)[b]	$10^3 k_{obs}$ (s^{-1})[c]	
240.5	11.0	E_a = 4.03 + 0.44 kcal
239.4	10.0	$\Delta H^{\ddagger}_{230}$ = 3.57 + 0.44 kcal
234.2	8.2	$\Delta S^{\ddagger} > -25$ eu
228.7	6.6	
224.9	6.1	

[a] For the oxidation of Fe^{III}TMP(OH) with mCPBA. [b] Temperatures are ±0.5 °C. [c] Correlation coefficients were >0.994.

kinetics were obtained as shown in Figure 3. Larger amounts of mCPBA gave greater k_{obs} even though the reaction showed a first-order reaction profile. Data for the rate of formation of **3** are shown in both Table I and Figure 3 (inset). The solid line in Figure 3 (inset) shows the expected result for the general acid catalyzed reaction depicted in eq 2. The dotted line in Figure

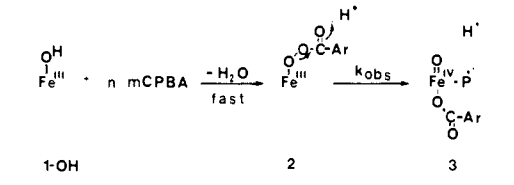


(2)

$$d[3]/dt = k_{obs}[2] = k_2[(n-1)\text{mCPBA}][2] \quad (3)$$

$$k_2[(n-1)\text{mCPBA}] = \text{constant}$$

3 (inset) represents the calculated result for the reaction catalyzed by acid [H^+] (eq 4), which is in good agreement with the experimental results. The rate expression derived in eq 5–9 indicates a half-order dependence in [peroxyacid] in this unbuffered medium.



(4)

$$\text{HOO-C(O)—Ar} \overset{K_a}{\rightleftharpoons} H^+ + {}^-\text{OO-C(O)-Ar} \quad (5)$$

$$d[3]/dt = k_{obs}[2] = k_2[H^+][2] \quad (6)$$

$$k_{obs} = k_2[H^+] = \text{constant} \quad (7)$$

$$K_a = [H^+][{}^-\text{OOC(O)Ar}]/[\text{HOOC(O)Ar}] = [H^+]^2/[\text{HOOC(O)Ar}] \quad (8)$$

$$d[3]/dt = k_2[2]K_a^{1/2}[\text{HOOC(O)Ar}]^{1/2} \quad (9)$$

(ii) Effect of Temperature. In order to determine activation parameters for O–O bond cleavage, the reaction of **1** with 3.5 equiv of mCPBA was examined at several temperatures over a range between −32.5 and −48.1 °C. A linear Arrhenius plot was obtained based on the results listed in Table II. The activation parameters were determined to be as follows: E_a = 4.0 ± 0.4 kcal/mol $\Delta H^{\ddagger}_{230}$ = 3.6 ± 0.4; $\Delta S^{\ddagger} > -25$ eu. The entropy of activation was calculated on the basis of the reasonable assumption that K_a in methylene chloride is smaller than that in aqueous solution at room temperature (3.8×10^{-8} M^{-1}).[20]

Reaction of Fe^{III}TMP(mCB) with mCPBA in Toluene. The reaction of **1**-mCB with mCPBA *in toluene* at 0 °C under *dilute conditions* (<10^{-4} M) afforded a new species (**5**) whose visible spectrum showed a red-shifted Soret band (441 nm) with respect to that of **1**-mCB (417 nm). The titration of **1**-mCB by mCPBA indicated that *2 equiv* of mCPBA was required to complete the formation of **5** (Figure 4). The reaction in the presence of acids

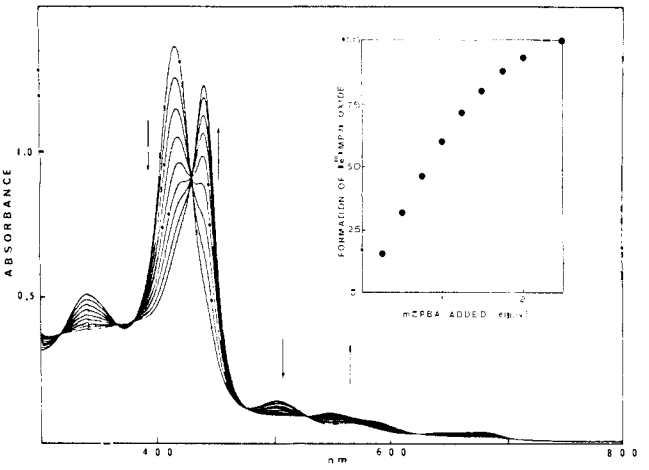


Figure 4. Visible spectral changes upon addition 0.25-equiv aliquots) of mCPBA to **1**-mCB [1.27×10^{-5} M] in toluene at 0 °C.

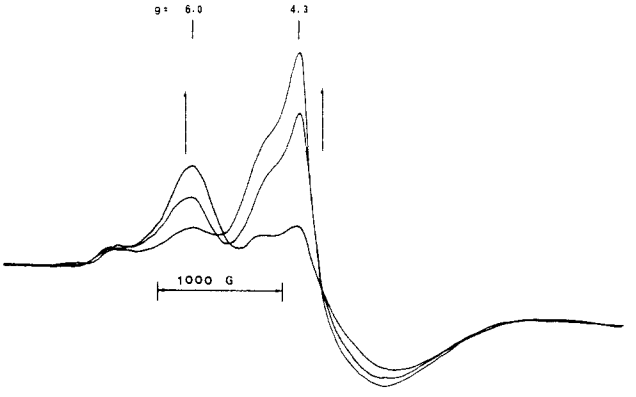


Figure 5. EPR spectral changes for the reaction of **1**-mCB and mCPBA in toluene.

such as mCBA discouraged the formation of **5**. Instead, decoloration of the porphyrin was observed even though **3** was stable under these conditions. The EPR spectrum of **1**-mCB (g = 6) was replaced with strong new signals at g = 4.3 in the course of this titration (Figure 5). The solution magnetic susceptibility of **5** obtained by the Evans method in toluene-d_8 at −25 °C was found to be 5.4 ± 0.1 μ_B.

The ^{1}H NMR spectrum of **5** at −50 °C in toluene-d_8 showed broad resonances for the *m*-mesityl protons at δ 17.2, 15.0, and 13.8 (relative intensity 1:2:1) and two *p*-methyl peaks (δ 4.2 and 3.9).

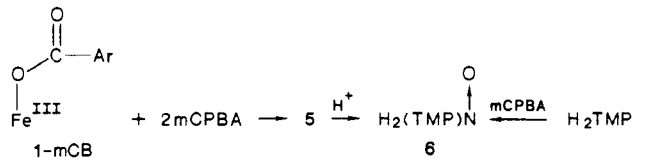
The demetalation of **5** with acetic acid–HCl (4:1) was achieved in less than 1 min at 0 °C. The color of the solution changed from reddish brown to green in this time. Neutralization, extraction, and thin-layer chromatography of the brown solution afforded H_2TMP *N*-oxide (**6**) in 25% yield and about the same amount of H_2TMP. The structure of **6** was confirmed by spectroscopic means and alternate synthesis. The ^{1}H NMR spectrum of **6** shows four different β-pyrrole resonances: δ 8.53 (4 H, ABq, J = 12.6, 5.0), 8.4 (2 H, s), and 7.49 (2 H, s). The FAB mass spectrum of **6** gave M^+ for H_2TMP *N*-oxide (m/z 798) with a base peak at m/z 782 (M^+ - O). N–O stretching[21] bands at 1273 and 864 cm^{-1} were observed in the FT-IR spectrum of **6**. The visible spectrum of purified **6** (417 nm) was the same as that obtained immediately after the demetalation of **5**. An authentic sample of **6** was prepared from H_2TMP according to the reported procedure of Andrews et al.[22]

Stoichiometry of the Reaction. Inspection of the FT-IR spectrum of the reaction mixture obtained upon the addition of

(20) k_{obs} = 6.1 × 10^{-3} (at 225 K) = $k_2K_a^{1/2}[H^+] < k_2(3.6 \times 10^{-8})^{1/2}(3.7 \times 10^{-5})^{1/2} = 1.15k_2 \times 10^{-6}$. Thus $k_2 > 5 \times 10^3$ mol^{-1} s^{-1} and $hk_2/kT = \exp((E_a/RT) - 1) = \exp(\Delta S/R)$. Therefore, $S > -25$ eu.

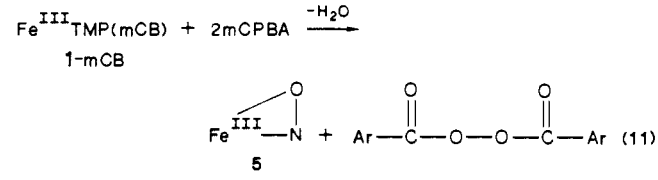
(21) Katritzky, A. R.; Lagowski, J. M. In *Chemistry of the Heterocyclic N-Oxide*; Academic Press: New York, 1971; Chapter 1.

(22) Andrews, L. E.; Bonnett, R.; Ridge, R. J.; Appelman, E. H. *J. Chem. Soc. Perkin Trans I* **1983**, *103*.



(10)

mCPBA to **1**-mCB showed a band for iron-coordinated *m*-chlorobenzoate (1660 cm^{-1}) and prominent peaks for 3,3′-dichlorodibenzoyl peroxide (1796, 1771, 1261, 1012 cm^{-1}). The intensity of these bands indicated that the diacyl peroxide had been produced in amounts equivalent of that of **5**. Additionally, no free *m*-chlorobenzoic acid was evident in the FT-IR spectrum and no mixed (unsymmetrical) diacyl peroxide was formed. When the reaction was performed with peroxybenzoic acid, dibenzoyl peroxide could be isolated from the reaction mixture. Accordingly, the stoichiometry for the formation of **5** is as described in eq 11.



(11)

Kinetics for the Formation of 5 in Toluene. The reaction of **1**-OH with 3.0 equiv of mCPBA in toluene was monitored by visible spectroscopy at −36 °C in toluene. The dotted line in Figure 6 shows **1**-OH and spectral changes of every 3 min after the addition of mCPBA. The spectrum of **7** was recorded immediately after the addition of mCPBA. The reaction is obviously biphasic, i.e., an instantaneous change from **1**-OH to a new high-spin iron(III) species (**7**) followed by a slow decomposition of **7** to afford **5**. The visible spectrum of **7** is quite similar to those of **2** in methylene chloride and **1**-mCB. Accordingly, we have assigned **7** as an acylperoxoiron^{III}TMP (**2**). The formation of the Fe(III) porphyrin *N*-oxide (**5**) from **7** (=**2**) was found to be kinetically well behaved, i.e., the reaction was first-order in iron porphyrin and in the excess peroxy acid remaining in the reaction solution.

Temperature Dependence. Second-order rate constants (k_2') for the reaction of **2** with mCPBA to afford **5** at several temperatures are listed in Table III. The kinetic parameters obtained were as follows: E_a = 11.1 ± 0.8 kcal; $\Delta H^{\ddagger}_{240}$ = 10.6 ± 0.8 kcal; $\Delta S^{\ddagger}$ = −10.6 ± 0.2 eu.

Substituent Effects on the Formation of 3 (k_2) and 5 (k_2'). The substituent effects on both k_2 and k_2' were examined at low temperature in methylene chloride and toluene, respectively.

In order to measure the relative rate constants for the formation of oxoiron(IV) porphyrin cation radical (**3**), solutions were prepared with variable excesses of peroxyacid to compensate for the differences in pK_a. For example, 4.4 equiv of peroxy-*p*-φtoluic acid was employed to obtain an acidity equivalent to 3.3 equiv of peroxybenzoic acid according to the relationships in eq 12 and 13 (also see eq 6 and 7). The ligand exchange of **1**-OH to afford

$$\frac{k_{obs}}{k_{obs}''} = \frac{k^2}{k_2''}\left[\frac{K_a[\text{HOOCOAr}]}{K_a''[\text{HOOCOAr}'']}\right]^{1/2} = \frac{k_2}{k_2''} \qquad (12)$$

$$K_a[\text{HOOCOAr}] = K_a''[\text{HOOCOAr}''] \qquad (13)$$

2 consumed 1 equiv of peroxyacid affording H_2O. Thus, in the second, slower reaction, the amount of peroxyacid remaining was 3.4 equiv for peroxy-*p*-toluic acid and 2.3 equiv of peroxybenzoic acid. We have assumed that K_a/K_a'' in water is about the same as that in methylene chloride. Therefore $(3.4)K_a(p\text{-Me}) = (2.3)K_a(\text{H})$, where K_a's of peroxybenzoic acids were calculated on the basis of the empirical relatioship in eq 14.[23] A Hammett

$$\text{p}K_a(\text{peroxybenzoic acid}) = 0.673\text{p}K_a(\text{benzoic acid}) + 4.875 \qquad (14)$$

(23) Blake, R. C., II; Coon, M. J. *J. Biol. Chem.* **1980**, *255*, 4100.

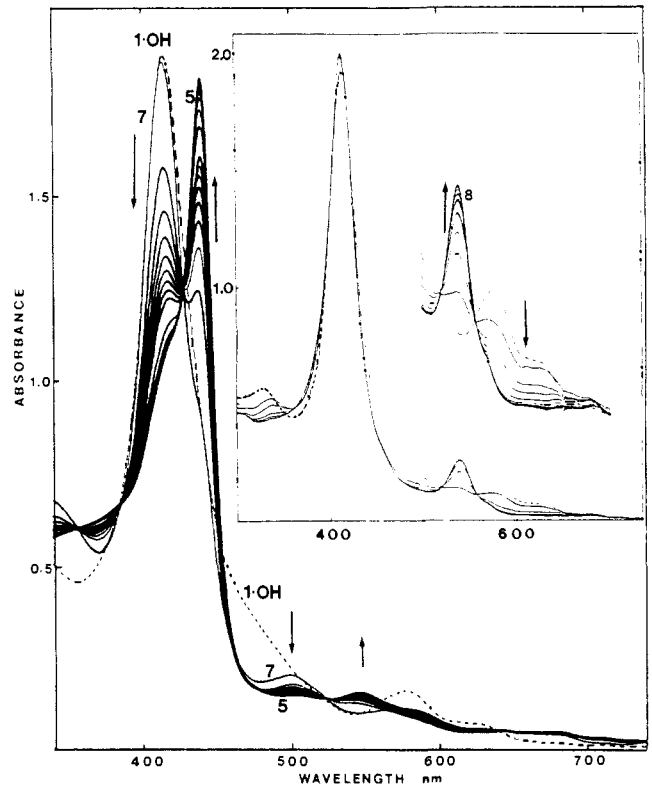


Figure 6. Time-dependent visible spectral changes in the reaction of **1**-OH [1.7 × 10^{-5} M] (---) and 3 equiv of mCPBA in toluene at −36 °C. Inset: Same reaction with 3.5 equiv of PPAA at −48 °C.

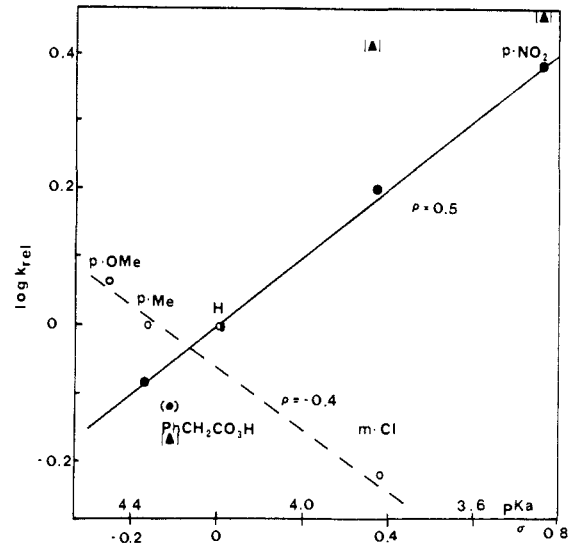


Figure 7. Hammett plots of k_{obs} (—) and k_2' (-·-).

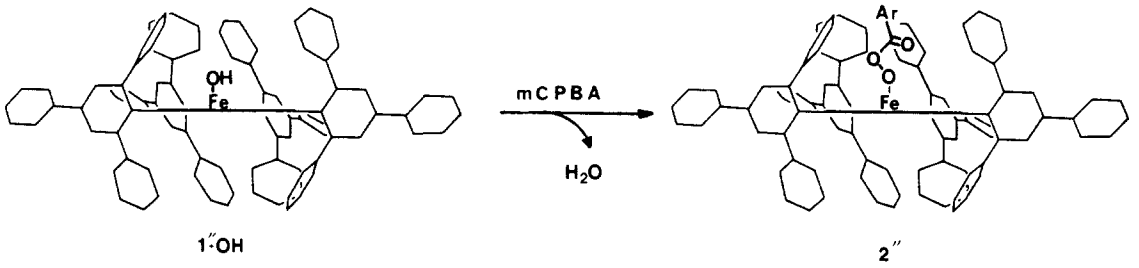
Table III. Second-Order Rate Constants for the Reaction of **1**-mCB with mCPBA in Toluene

temp (K)[a]	$10^{-1}k_2'$ (mol^{-1} s^{-1})	
243.7	7.4	
240.7	5.3	E_a = 11.1 + 0.8 kcal
237.2	3.6	$\Delta H^{\ddagger}_{240}$ = 10.6 + 0.8 kcal
235.8	3.7	$\Delta S^{\ddagger}$ = −10.6 + 0.2 eu
229.4	1.9	

[a] ±0.2 °C.

plot for log k_{rel} is shown in Figure 7 (solid line, ρ = +0.5). Values of k_2' for the reaction of **1**-OH and peroxybenzoic acid in toluene are listed in Table IV. Electron-donating groups were found to accelerate the reaction, whereas electron-withdrawing substituents retarded the formation of **5** (Figure 7, dashed line, ρ = −0.4).

Scheme II



1''-OH 2''

Table IV. Substituent Effects on k_{obs} and k_2'

substituent	$10^3 k_{obs}$ $(s^{-1})^a$	$10k_2'$ $(mol^{-1}\ s^{-1})^b$
p-NO2	5.7	
m-Cl	3.7	3.7
H	2.4	6.2
p-Me	2.0	6.0
p-MeO		6.9

[a] In methylene chloride at −48 ± 0.5 °C. [b] In toluene at −36.4 ± 0.3 °C.

Reaction of Fe^{III}TMP(OH) (1-OH) with Phenylperoxyacetic Acid. The reaction of **1**-OH with phenylperoxyacetic acid (PPAA) in toluene at −48 °C was monitored by visible spectroscopy (Figure 6, inset). Instead of the formation of **5**, the very distinctive visible spectrum of oxoFe^{IV}TMP (**8**) was observed.[25] Furthermore, toluene (63%), benzyl alcohol (19%), and benzaldehyde (11%) were recovered when this reaction was carried out in benzene at 5 °C, as expected for a reaction that generated phenylacetoxy radicals.[24]

Oxygen-18 Labeling Experiment. Peroxybenzoic acid having ^{18}O in carbonyl oxygen was prepared according to eq 15. The reaction of purified (tetrakis(2,6-dichlorophenyl)porphyrinato)-iron(III) *N*-oxide (**5'**), prepared from corresponding iron(III) porphyrin (**1'**) and ^{18}O-labeled peroxybenzoic acid, with triphenylphosphine was carried out in toluene at room temperature under nitrogen. Triphenylphosphine oxide was isolated as a sole product (64%). The mass spectrum of the triphenylphosphine oxide thus obtained showed only trace amounts of ^{18}O incorporation.

$$\mathrm{PhMgBr} + \mathrm{C^{18}O_2} \rightarrow \underset{(^{18}O_2:\ 95.9\%)}{\mathrm{PhC^{18}O_2H}} \xrightarrow[>99\%\ H_2O_2]{H^+} \underset{(^{18}O:\ 78.2\%)}{\mathrm{PhC(^{18}O)OOH}} \quad (15)$$

Discussion

The results described here have allowed the dissection of the oxidation of Fe(III) porphyrins into three reactions: ligand exchange to produce an acylperoxoiron(III) intermediate (**2**) and two different modes of O–O bond scission to produce either an oxoiron(IV) porphyrin π-cation radical (**3**) or an iron(III) porphyrin *N*-oxide (**5**).

The observation of a sigmoidal profile for the disappearance of the Soret band at 415 nm upon the addition of peroxyacid to **1**-Cl clearly indicates the presence of a pre-equilibrium in the reaction to afford **3** ([Fe(O)TMP]$^+$) (Figure 1). The zero-order kinetic dependence suggests that a steady-state concentration of an intermediate develops through the middle period of the reaction. Accordingly, the simplest description of the reaction would be as expressed in eq 1. Furthermore, k_2, the rate of O–O bond scission, must be equal to or greater than k_1, the rate of ligand metathesis, to have the observed sigmoidal curvature. The acceleration of the reaction and shortening of the induction period by acid (mCBA) indicates that at least the second step in eq 1 is acid catalyzed. Thus, with ligand exchange rate limiting, substituent effects on the peroxyacid cannot necessarily be equated directly with k_2. It is possible, however, that ligand exchange is facilitated in protic, buffered media.[26]

The presence of an induction period in the conversion of **1** to **3** suggests that ligand exchange is the rate-limiting step.[27] The detailed mechanism of the reaction of **1** with peroxybenzoic acid to give **3** has been illuminated by the observation of acylperoxo intermediate **2**. To prepare **2** in eq 1, the initial reaction between **1** and the peroxyacid had to be made irreversible and faster than the subsequent decomposition to form ferryl species **3**. Accordingly, the *hydroxo* iron porphyrin, Fe^{III}TMP(OH) (**1**-OH), was very useful as the starting material, since the reaction of **1**-OH with the peroxyacid was fast and did not produce an acidic proton. In fact, when *p*-nitroperoxybenzoic acid was added to a methylene chloride solution of **1**-OH at −46 °C, two successive spectral changes were evident: First, an instantaneous change from the visible spectrum of **1**-OH to that of a new species (**2**), which was typical for a high-spin iron(III) porphyrin[28] but different from either the hydroxide or alkoxide complex,[29a] was noted. Subsequently, a smooth, isosbestic spectral change to afford the oxoiron(IV) porphyrin cation radical (**3**) was discerned (Figure 2). The visible spectrum of **3** having a weak Soret band at 409 nm and a broad absorbance between 500 and 750 nm was identical with that of an authentic sample of **3** by the reaction of **1**-Cl with mCPBA.[11] The stoichiometry of the conversion of **1**-OH to **3** via **2** was evident from the observation that 1.2 equiv of *p*-nitroperoxybenzoic acid was sufficient to effect these changes. A crucial difference between **3** and Fe(III) porphyrin π-cation radicals is that the former reacted with olefins rapidly to form epoxides even at low temperature, whereas the latter were stable under these conditions.[30] The visible spectrum of **2** prepared by the reaction of **1**-OH with mCPBA was similar to that of **1**-mCB. Recently, we have prepared and characterized a stable sterically hindered acylperoxoiron(III) porphyrin analogous to **2** by this same ligand exchange procedure (Scheme II).[31] The coordination of the acylperoxo ligand in this case was discerned by the observation of the carbonyl stretching band in the IR spectrum at 1735 cm^{-1}. Thus, it seems clear that the Fe^{III}TMP case has behaved similarly. Methylperoxoiron(III) porphyrins have also been shown to have typical high-spin spectra.[29b]

The O–O bond cleavage of **2** to give **3** was found to be first order in **2** and half order in the stoichiometric excess of the

(24) The rate of decarboxylation for the phenylacetoxy radical is expected to be nearly diffusion controlled and may be concerted with its formation: Bartlett, P. D.; Hiatt, R. R.; cf. Braun, W. *J. Am. Chem. Soc.* **1958**, *80*, 1398.

(25) (a) Chin, D.-H.; La Mar, G. N.; Balch, A. L. *J. Am. Chem. Soc.* **1980**, *102*, 5947. (b) Groves, J. T.; Quinn, R.; McMurry, T. J.; Nakamura, M.; Lang, G.; Boso, B. *Ibid.* **1985**, *107*, 354. Schappacher, M.; Weiss, R.; Montiel-Montoya, R.; Trautwein, A.; Tabard, A. *Ibid.* **1985**, *107*, 3736. (d) Schappacher, M.; Chottard, G.; Weiss, R. *J. Chem. Soc., Chem. Commun.* **1986**, *93*.

(26) (a) Traylor, T. G.; Lee, W. A.; Stynes, D. V. *J. Am. Chem. Soc.* **1984**, *106*, 755. (b) Traylor, T. G.; Xu, F. *J. Am. Chem. Soc.* **1987**, *109*, 6201–6202. (c) Lee, W. A.; Bruice, T. C. *Ibid.* **1985**, *107*, 513. (d) Ostovic, D.; Knobler, C.; Bruice, T. C. *Ibid.* **1987**, *109*, 3444–3451. (e) Bruice, T. C.; Dicken, C. M.; Balasubramanian, P. N.; Woar, T. C.; Lu, F.-L. *Ibid.* **1987**, *109*, 3436–3443. (f) Bruice, T. C. *J. Chem. Soc., Chem. Commun.* **1983**, 14.

(27) Complicated spectral changes were observed for the reaction of both **1**-OH and **1**-Cl with mCPBa in methanol at ca. −50 °C.

(28) See: Scheidt, W. R.; Gouterman, M. In *Iron Porphyrins*; Lever, A. B. P., Gray, H. B., Eds.; Addison-Wesley Publishing Company: Reading, MA, 1983; Part I, p 89 and references therein.

(29) (a) Cheng, R.-J.; Latos-Grazynski, L.; Balch, A. L. *Inorg. Chem.* **1982**, *21*, 2412. (b) Arasasingham, R. D.; Balch, A. L.; Latos-Grazynski, L. *J. Am. Chem. Soc.* **1987**, *109*, 5846–5847.

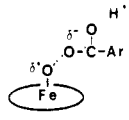
(30) Groves, J. T.; Watanabe, Y. *J. Am. Chem. Soc.* **1986**, *108*, 507.

(31) Groves, J. T.; Watanabe, Y. *Inorg. Chem.* **1987**, *26*, 785–786.

Table V. Estimated Entropy Values for the Reaction of **1**-OH with mCPBA

K_a (M^{-1})	k_2 (mol^{-1} s^{-1})	ΔS^* (eu)
3.8×10^{-8}	5×10^3	−25
3.8×10^{-12}	5×10^5	−20
3.8×10^{-16}	5×10^7	−16
3.8×10^{-20}	5×10^9	−11

peroxyacid (Figure 3). The reaction of **1**-Cl with peroxybenzoic acid (Figure 1, inset, and Figure 3) shows that acid (peroxybenzoic acid in this particular case) accelerated the decomposition of **2** with a first-order rate dependence on [**2**]. Accordingly, there was no consumption of acid during the formation of **3**. The observed effect of acid on the formation of **3** in this unbuffered medium is consistent with the rate expression in eq 9 (Figure 3, inset-chained line) although it is difficult to differentiate general acid and specific acid catalysis in this nonprotic medium. The interaction between the proton and the acylperoxoiron(III) complex (**2**) would favor charge development on the peroxy oxygen adjacent to iron as illustrated in eq 16 below. Therefore, under these conditions, heterolytic O–O bond cleavage to form **3** and benzoate is the favored process.



(16)

To elucidate the nature of the O–O bond cleavage step, several substituted peroxybenzoic acids were employed. The reactions of **1**-OH with peroxybenzoic acids were very similar, i.e., instantaneous formation of peroxo complex **2** followed by slow pseudo-first-order O–O bond scission to afford ferryl species **3** as the final product. To compare the substituent effect on k_2 instead of k_{obs}, which is a function of acid concentration (eq 6), we made the assumption that the relative dissociation constants (K_a's) of peroxybenzoic acids in methylene chloride are in the same relative order as in water, i.e., $K_a/K_a''(H_2O) = K_a/K_a''(CH_2Cl_2)$. Thus, when $K_a[HOOCOAr] = K_a''[HOOCOAr'']$, k_{obs}(rel) is the same as k_2(rel) due to eq 12. This treatment resulted in the linear Hammett plot shown in Figure 7 (solid line) and supports the approximate validity of the above assumption. The positive ρ (0.5) obtained for the decomposition of **2** is in good agreement with the result expected for heterolytic O–O bond cleavage of **2** to form **3**. Somewhat smaller values have been reported by Bruice[26c-e] and by Traylor[26a,b] for Fe(TPP)Cl in catalytic regimes. We have also examined the substituent effect on k_{obs} when 3 equiv of peroxybenzoic acids was used. The Hammett plot in this case was not linear; however, electron-withdrawing substituents gave greater k_{obs} ((Δ) in Figure 7). We have observed very similar substituent effects on the acid-catalyzed decomposition of acylperoxoMn^{III}TMP to form oxoMn^{V}TMP (ρ = 0.9) under acidic conditions.[32]

The activation energy calculated from k_{obs} for the conversion of **2** to **3** at various temperatures was very small with a large negative entropy. In Table V, estimated entropies for various dissociation constants (K_a) are listed. If the dissociation constant of mCPBA in methylene chloride at −40 °C is the same as that in water at room temperature ($K_a = 3.8 \times 10^{-8}$ M^{-1}), k_2 is 5×10^3 mol^{-1} s^{-1} and $\Delta S^* = -25$ eu, while $\Delta S^* = -16$ eu is obtained in the case of $K_a = 3.8 \times 10^{-16}$ M^{-1} with $k_2 = 5 \times 10^7$ mol^{-1} s^{-1}.

The very small activation enthalpy observed here for heterolytic cleavage of the O–O bond suggests a substantial interaction of the scissile bond with iron in the transition state. The large negative entropy term indicates significant reorganization and is consistent with an associative reaction. Positioning the necessary proton would certainly contribute to this entropy. Further, while the acylperoxoiron(III) complex is five coordinate and high-spin, coordination of a sixth ligand is an attractive explanation for the observed entropy of activation. The phases of pertinent orbitals

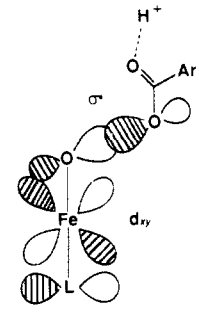


Figure 8. Idealized orbital interaction diagram for acylperoxoiron(III)–L complexes, where L is the sixth ligand to iron.

as shown in Figure 8 indicate that the iron d_{xz} and d_{yz} orbitals are favorably disposed to interact with the σ^* orbital of the bound peroxide. The maximum assistance for *heterolytic* O–O bond scission should derive from a doubly occupied $d_{xz(yz)}$ orbital. Coordination of the sixth ligand would be expected to favor the low-spin electronic configuration for iron(III). We suggest, therefore, that the transition state for heterolytic O–O bond scission may have the iron in a six-coordinate low-spin state.

When the peroxyacid oxidation of iron(III) porphyrins was carried out in toluene, completely different results were observed. The reaction of **1**-OH with peroxybenzoic acid at low temperature gave **2** just as the reaction in methylene chloride. However, instead of the formation of **3**, Fe^{III}TMP *N*-oxide (**5**) was obtained. This iron(III) *N*-oxide was much less reactive than **3** and was stable in the presence of olefins at 0 °C. The visible spectrum of **5** shows a Soret band red-shifted by 20 nm with respect to **1** as expected for N-substituted iron(III) porphyrins.[33] A strong $g = 4.3$ signal in the EPR suggests that **5** is a high spin ($S = {}^5/_2$) and of rhombic symmetry.[34] Recently, Mansuy et al. have reported that an iron(III) porphyrin complex with a vinylidene group inserted into an Fe–N bond has an intermediate spin state.[13b] By contrast, an iron(III) porphyrin complex with a nitrene moiety inserted into an Fe–N bond[15] displayed an EPR signal at $g = 4.3$ and a magnetic moment of 5.8 μ_B, similar to **5**, consistent with a rhombic, high-spin ($S = {}^5/_2$) Fe^{III} structure. The magnetic susceptibility for **5** measured by the Evans method in toluene at −25 °C, 5.4 ± 0.1 μ_B, and the EPR signal at $g = 4.3$ clearly indicate that **5** is also a high-spin Fe^{III} porphyrin with rhombic symmetry. Finally, demetalation of **5** gave conclusive evidence for the presence of *N*-oxide moiety. The FT-IR of the modified porphyrin ligand (**6**) showed two N–O stretching bands at 1273 and 864 cm^{-1}. The FAB mass spectrum of **6** showed a peak at m/z 798 (H_2TMP + 16) as a molecular ion peak (72%) along with H_2TMP peak at m/z 782 (100%). An authentic sample of H_2TMP *N*-oxide prepared by the method reported by Bonnett et al.[22] was identical with **6**. All these results definitely point to the formation of a high-spin Fe^{III}TMP *N*-oxide as the structure for **5**.

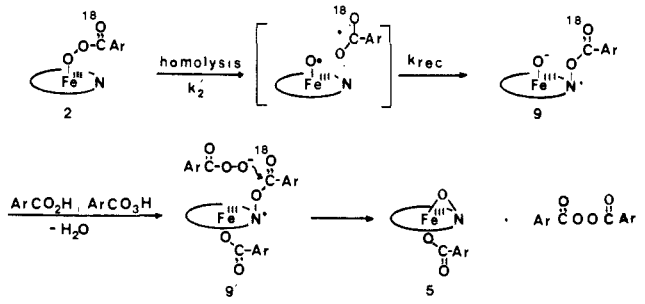
The titration of **1**-mCB with mCPBA (Figure 4) and subsequent product analysis have established the stoichiometry of the reaction to be as shown in eq 11. In contrast to the acceleration of the formation of **3** by acid in methylene chloride, the formation of **5** in toluene was diminished by mCBA and the brown color faded even at −45 °C. The rate of formation of **5** from **1** was similar to the rate of formation of **3**. However, *substituent effects on these two reactions were found to be completely opposite.* As mentioned above, the positive Hammett ρ (0.5) for the formation of **3** in methylene chloride can readily be associated with a heterolytic O–O bond cleavage in accord with the leaving ability

(32) Groves, J. T.; Watanabe, Y. *Inorg. Chem.* **1986**, *25*, 4808–4810.

(33) (a) Jackson, A. H. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. I, p 341. (b) Mansuy, D.; Battioni, J.-P.; Dupre, D.; Sartori, E.; Chottard, G. *J. Am. Chem. Soc.* **1982**, *104*, 6159.

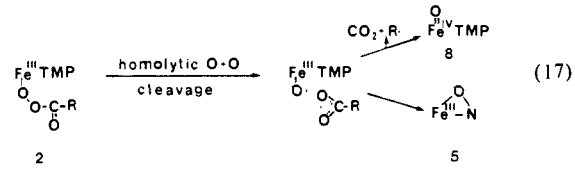
(34) (a) Palmer, G. In *Iron Porphyrins*; Lever, A. B. P., Gray, H., Eds.; Addison-Wesley Publishing Company; Reading, MA, 1983; Part II, p 43. (b) Reed, C. A.; Mashiko, T.; Bentley, S. P.; Kastner, M. E.; Scheidt, W. R.; Spatalian, K.; Lang, G. *J. Am. Chem. Soc.* **1979**, *101*, 2948. (c) Summerville, D. A.; Cohen, I. A.; Hatano, K.; Scheidt, W. R. *Inorg. Chem.* **1978**, *17*, 2906. (d) Masuda, H.; Taga, T.; osaki, K.; Sugimoto, H.; Yoshida, Z.; Ogoshi, H. *Ibid.* **1980**, *19*, 950.

Scheme III



of the corresponding benzoates.[26f] However, for the decomposition of **2** to afford **5** in toluene a different mechanism is required since electron-donating substituents accelerated the reaction. We have observed very similar results for the decomposition of acylperoxoMn^{III}TMP in the presence of hydroxide, which was attributed to a homolytic O–O bond cleavage reaction.[35] Furthermore, for the thermal decomposition of dibenzoyl peroxides, which are known to proceed via homolytic O–O bond cleavage, it has been found that electron-donating substituents accelerate the reaction due to the increase of electron–electron repulsion between two peroxy oxygens.[32]

Thus, the data support a homolytic or at least nonpolar transition state for O–O bond fission for the conversion of **2** to **5** and suggest the mechanism shown in Scheme III. The stoichiometric formation of dibenzoyl peroxide along with **5** and the consumption of exactly 2 equiv of peroxyacid suggest the formation of *N*-acyl intermediate **9**, which would be expected to acylate a peroxyacid to produce dibenzoyl peroxide.[36] That the reaction of **1**-OH with PPAA in toluene at −48 °C afforded an oxoiron(IV) porphyrin [Fe^{IV}TMP(O)] as the sole product is as expected for homolytic O–O bond fission and subsequent decarboxylation.[37] Decarboxylation of phenylacetoxy radical would be fast even at −30 °C (eq 17); however, the rate of reaction of PPAA was not found



(17)

to be unusually fast (Figure 7). The failure to observe any mixed diacyl peroxide appears to rule out mechanisms involving direct attack of the peroxy oxygen on the carbonyl carbon of a coordinated carboxylate since both benzoate and *m*-chlorobenzoate were present in similar concentrations. The activation parameters, particularly the entropy term (−10.6 ± 2 eu), are also consistent with a first-order process. The lack of ^{18}O-incorporation into **5** from the carbonyl oxygen of the peroxy acid is not readily accommodated by a *free* carboxy radical in Scheme III; however, if the pyrrole nitrogen interacts with the peroxo oxygen adjacent to the carbonyl group before O–O bond cleavage is complete as indicated, the observed ^{18}O-distribution can be accommodated. This observation is reminiscent of the Stevens rearrangement for which radical and concerted mechanisms appear to compete.[38]

Weiss et al. have recently shown that the reaction of peroxoiron(III) porphyrins with carbon dioxide afforded an oxoiron(IV) porphyrin.[25c,d] A homolytic O–O bond cleavage of an intermediate peroxycarbonate to give an oxoiron(IV) porphyrin (eq 18) is

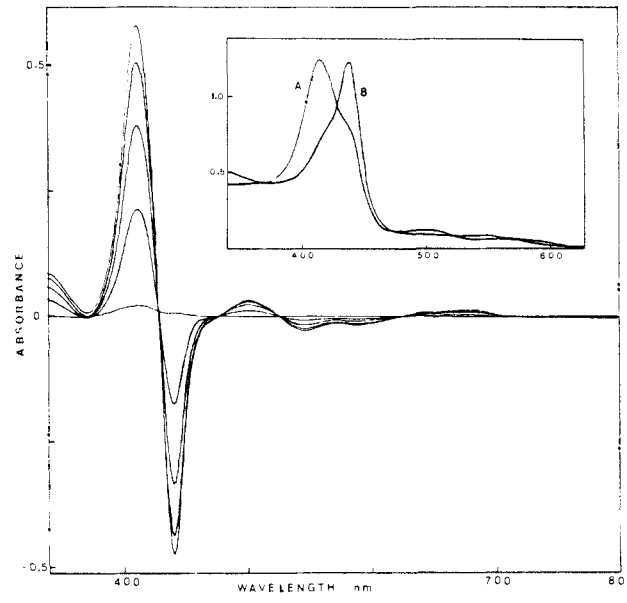
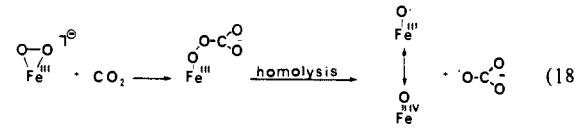


Figure 9. Difference spectral changes in the reaction of **1**-mCB [1.27 × 10^{-5} M] and 0.5 equiv aliquot additions of mCPBA at room temperature in toluene in the presence [sample side] and absence [reference side] of cyclooctene (15 μL/3 mL). Inset: After the addition of 2 equiv of mCPBA. A: Sample side. B: Reference side.

analogous to the homolytic peroxyacid reaction we have described here. Khenkin and Shteinman have suggested that the reaction



(18)

of peroxoiron(III) porphyrins with an acylating reagent in toluene–acetonitrile forms an acylperoxoiron(III) porphyrin intermediate that decomposes to form an iron(IV) porphyrin cation radical.[39] Furthermore, we have recently demonstrated the formation of acylperoxomanganese(III) porphyrins either in the reaction of Mn^{III}TMP hydroxide with mCPBA or peroxoMn^{III}TMP with acylating reagents.[19,32] The acylperoxoMn^{III} porphyrin decomposed to give oxoMn(V) species via heterolytic O–O bond cleavage under acidic conditions, whereas homolytic O–O bond cleavage was observed in the presence of hydroxide. Thus, these two different modes of O–O bond cleavage of acylperoxo metal porphyrin complexes appear to be general (Scheme IV).

The reaction of **1**-mCB with mCPBA in the presence of olefin under conditions favorable to the formation of **5** has also been examined. In the absence of olefin, only 2 mol of mCPBA were required to prepare **5**, whereas a large excess of mCPBA was consumed to prepare **5** in the presence of olefin. Figure 9 shows the difference spectral changes observed during the aliquot addition of mCPBA to **1**-mCB (total 2.5 equiv) in the presence (sample side) and absence (reference side) of cyclooctene. Obviously, the formation of **5** has been interrupted by the presence of the olefin. The addition of 6.8 equiv of mCPBA to a mixture of **1**-mCB and cyclooctene (large excess) was followed by visible spectroscopy. The spectrum of resulting solution indicated that most of the **1**-mCB was converted to **5**; however, cyclooctene oxide (46% based on mCPBA used) was also produced even though no epoxide was produced under these conditions without the porphyrin. The formation of epoxide under these conditions suggests that an intermediate in this reaction either reacts with olefins or catalyzes the epoxidation of olefins by peroxyacids. Accordingly, the reaction of peroxyacids with iron(III) porphyrins is more complex than has generally been assumed.

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(37) The decarboxylation of benzoyloxy radical is relatively slow at −30 °C (k = 4.0 s^{-1}) based on k = 1 × 10^4 s^{-1} at 60 °C and E_a = 14 kcal/mol. (a) Bevington, J. C.; Toole, J. *J. Polym. Sci.* **1958**, *28*, 413. (b) DeTar, D. F. *J. Am. Chem. Soc.* **1967**, *89*, 4058.

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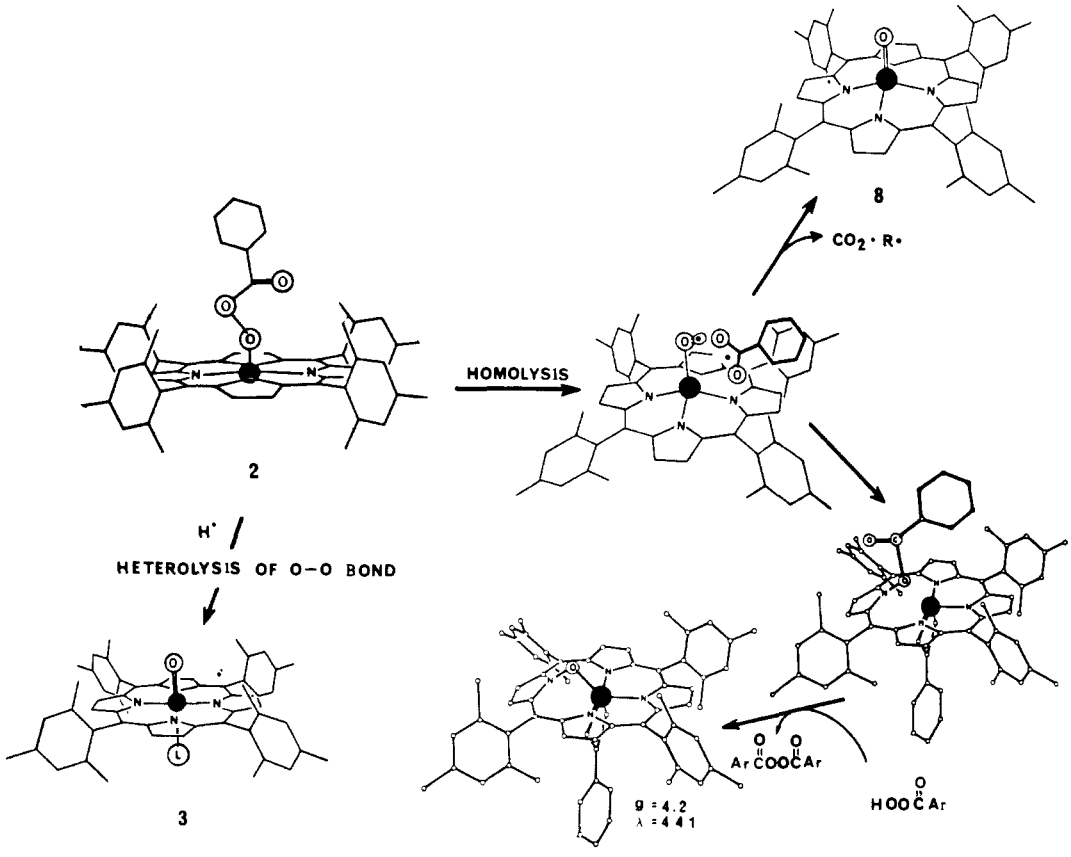
Scheme IV



Implications for Oxygen Activation by Cytochrome P-450. The reduction of iron(III) porphyrins is easily carried out either electrochemically or by reducing reagents to give iron(II) porphyrins.[40] Collman et al. have demonstrated that iron(II) porphyrins are able to bind molecular oxygen affording oxygenated species by using sterically protected picket fence porphyrins.[41] Further reduction of the iron–O_2 complex forms a peroxoiron(III) porphyrin,[4b] which also can be prepared by the reaction of superoxide with an iron(III) porphyrin.[4a] Sligar et al. have suggested the participation of an acylating reagent to activate peroxoiron(III) species in cytochrome P-450$_{cam}$ and proposed the formation of acylperoxoiron(III) intermediate before the formation of active species.[5] We have demonstrated here the formation of an acylperoxoiron(III) porphyrin (**2**) and shown that **2** decomposes by two distinct mechanisms: a heterolytic O–O bond fission to afford the oxoiron(IV) porphyrin cation radical (**3**) or a nonpolar, probably homolytic pathway to the iron(III) porphyrin *N*-oxide (**5**). The *N*-oxide **5** has been considered as an alternative candidate for the active oxygen species of cytochrome P-450.[12] In view of the results described here, particularly the lack of a reaction of **5** with olefins, the oxoiron(IV) porphyrin cation radical similar to compound I of peroxidase[42] is still the most attractive candidate for the active species in the cytochrome P-450 cycle. However, it remains to be seen what the reactive species is on the route to *N*-oxide **5**.

The oxidation of pyrogallol to purpurogallin catalyzed by hemeproteins such as cytochrome P-450 LM_2, LM_4, HRP, and chloroperoxidase has been examined.[43] When phenylperoxyacetic acid was employed as the oxidizing reagent, only cytochrome P-450 was found to produce purpurogallin *and* benzyl alcohol, the product resulting from the decarboxylation of an acyloxyl radical intermediate. Furthermore, in the presence of PPAA and a hydroxylatable substrate, cytochrome P-450 LM_2 catalyzed two reactions that proceeded concurrently, decarboxylation of PPAA and hydroxylation of the substrate.[44] Thus, there are two different modes of the O–O bond cleavage in the cytochrome P-450 system, while peroxidases catalyze the heterolytic O–O bond cleavage reaction exclusively. The recently published X-ray structure of cytochrome P-450$_{cam}$ indicates a nonpolar environment lacking acidic protons around the active site.[45] These are conditions that could allow competing homolytic and heterolytic pathways, consistent with results described here for the model system.

Conclusion

We have prepared and characterized three types of unstable, oxidized iron porphyrin complexes. Two of these, the acylperoxoiron(III) complex and the oxoiron(IV) porphyrin cation radical, have been postulated in the catalytic cycles of both cytochrome P-450 and horseradish peroxidase (Scheme IV). Heterolysis of the O–O bond in an acylperoxoiron(III) porphyrin complex has been shown to be acid catalyzed, affording a reactive oxoiron(IV) porphyrin cation radical **3**. Under neutral conditions O–O homolysis has been observed to form an iron(III) porphyrin *N*-oxide (**5**) that will not transfer its oxygen atom to olefins.

Experimental Section

Materials. Methylene chloride and toluene were distilled from CaH_2 and stored under N_2. mCPBA and *p*-nitroperoxybenzoic acid were purchased from Aldrich. Other substituted peroxybenzoic acids were

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prepared by literature procedures.[46] All peroxybenzoic acids were purified by washing with either phosphate buffer[47] or sodium bicarbonate solution. Methanesulfonic acid was distilled from phosphorous pentoxide. Highly concentrated hydrogen peroxide was prepared by the vacuum evaporation of water from aqueous H_2O_2 (30%) at 0 °C. Tetramesitylporphine [$TMPH_2$] was prepared by a modification of the published method.[48] Iron was inserted into $TMPH_2$ to form $Fe^{III}TMP(Cl)$ by a standard route.[49] $Fe^{III}TMP(OH)$ was prepared as described earlier.[29] Hydroxo(tetrakis(2,6-dichlorophenyl)porphyrinato)iron(III) was also prepared by a reported method.[50]

Preparation of ^{18}O-Labeled Peroxybenzoic Acid. An ether solution of phenylmagnesium bromide (1.5 M, 6 mL) was degassed several times (on a vacuum line) followed by the introduction of 100 mL of $C^{18}O_2$ (98% $^{18}O_2$, Cambridge Isotope Lab.). The resulting solution was stirred at −10 to −20 °C in a sealed tube in the dark for 20 h. Finally, the reaction was stopped by adding 6 N HCl–ice/CH_2Cl_2. ^{18}O-Labeled benzoic acid was recrystallized from *n*-pentane–CH_2Cl_2 to afford 370 mg of ^{18}O-labeled benzoic acid (95.9% $^{18}O_2$; $\nu_{C=^{18}O}$: 1971 cm^{-1}). To a solution of CH_2Cl_2 (0.6 mL) containing dry $MeSO_3H$ (0.9 mL) and ^{18}O-labeled peroxybenzoic acid (280 mg), 0.6 mL of highly concentrated H_2O_2 (99%) was added slowly at ca. 10 °C with stirring for 30 min and then an additional 2 h at room temperature. The resulting reaction mixture was treated with ice water and 260 mg of ^{18}O-labeled peroxybenzoic acid [PhC(^{18}O)OOH] ^{18}O: 78.2%; $\nu_{C=^{18}O}$: 1705 cm^{-1}) was obtained. The amount of ^{18}O incorporated was determined from the mass spectrum of benzoic acid.

Spectrophotometers. Proton NMR spectra were recorded on a Bruker WM-250 or a General Electric QE 300 spectrometer. Chemical shifts are reported relative to residual solvent resonances ($CHDCl_2$, δ 5.32; $PhCHD_2$, δ 2.09) relative to the tetramethylsilane. Visible spectra were obtained with a Cary 2390 spectrophotometer equipped with thermoelectric cold cells (Thermoelectrics Unlimited, Inc. Model CP-31). Temperatures reported were the that of the UV-cuvette wall measured by a digital thermocouple probe (Omega 450-AET). FT–IR spectra were recorded on a Nicolet FTIR-5DXB spectrophotometer. EPR spectra were obtained with a Varian E-12 spectrophotometer. Mass spectra (FAB, EI, and GC) were recorded on a Kratos MS50TC mass spectrometer with a Kratos HRGC/MS data system.

Reaction of $Fe^{III}TMP(X)$ (1) with Peroxybenzoic Acids. In a typical reaction, a methylene chloride solution of $Fe^{III}TMP(Cl)$ (1.0×10^{-5} M, 4 mL) in a 1-cm UV cuvette was placed in a cold cell of the UV–vis spectrophotometer. After equilibration to the desired temperature, 30–60 μL of methylene chloride containing several equivalents of the substituted peroxybenzoic acid was injected in one portion. The reaction was monitored by either recording repetitive scans or by recording absorption vs time at a fixed wavelength. Similar reactions were carried out with $Fe^{III}TMP(OH)$ ($1.2–1.4 \times 10^{-5}$ M) in methylene chloride. Reactions of $Fe^{III}TMP$ with substituted peroxybenzoic acids in toluene were carried out in the same way.

Preparation and Demetalation of $Fe^{III}TMP$ *N*-Oxide (5). To a stirred solution of $Fe^{III}TMP(mCB)$ (46 mg, 46 μmol) in toluene (500 mL) was slowly added a solution of mCPBA (20–30 mg, 0.12–0.17 mmol) in toluene (10 mL) at 0 °C. Completion of the reaction was monitored by observing changes in the UV–vis spectrum. A mixture of acetic acid–HCl (100–25 mL) was then added to the resulting solution at 0 °C. After complete color change from reddish brown to green, the solution was washed successively with water and saturated $NaHCO_3$ solution. The toluene phase was evaporated at 5 °C to a volume of 50 mL and the resulting solution was then submitted to column chromatography on silica gel. $TMPH_2$ was recovered at this stage (ca. 25%). Elution with ethyl acetate–benzene (1:15) gave 9–12 mg of $TMPH_2$ *N*-oxide (25–30%). ^1H NMR (CD_2Cl_2); pyrrole; δ 8.53 (4 H, ABq, J = 12.6, 5.0), 8.40 (2 H, s), 7.49 (2 H, s); meta-H: 7.36 (8 H, s); para-Me: 2.59 (12 H, s); ortho-Me: 1.88 (12 H, s), 1.86 (12 H, s); N-H: 1.75 (2 H, s). UV nm (log ϵ) in toluene: 417 (5.17), 544 (3.77), 602 (3.78), 696 (3.28). IR cm^{-1} (Nujol): 1273, 864. Mass spectrum (FAB): m/e 798 (M^+, 72%), 782 (M^+ − O, base).

Preparation of a NMR Sample of 5. A 25-mL round-bottom flask was charged with 1-mCB (4.6 mg, 4.6 μmol), sodium acetate (20 mg, 0.24 mmol), and toluene-d_8 (>99% *d*). The resulting solution was stirred at 0 °C and a 2.3 mM toluene-d_8 solution of mCPBA was added slowly until complete formation of **5** was evident in the visible spectrum. Filtration and evaporation of the solution at −25 °C reduced the volume to ca. 1 mL. The toluene solution was transferred to an NMR tube at −50 °C. ^1H NMR in toluene at −50 °C; meta-H: δ 17.2 (2 H, br s), 15.0 (4 H, br s), 13.8 (2 H, br s); para-Me: 4.2 (6 H, s), 3.9 (6 H, s).

Magnetic Susceptibility Measurements. Solution magnetic moments were measured by the Evans method[51] at −25 °C with toluene-d_8 and tetramethylsilane as the chemical shift references. After the measurement of **5**, the solution was warmed to room temperature to decompose **5** to the corresponding iron(III) *m*-chlorobenzoate. The resulting solution was then cooled −25 °C to obtain the magnetic susceptibility of $Fe^{III}TMP(mCB)$ (high-spin iron(III) (5.92 μ_B)).

EPR Measurements of 5. A toluene solution (10 mL) of 1-mCB (4.6 mg, 4.6 μmol) containing 20 mg of sodium acetate (0.24 mmol) was titrated with a 2.3 mM toluene solution of mCPBA up to 2.5 equiv. During the titration, aliquot samples were transferred to EPR tubes and frozen by liquid N_2 for the measurement. EPR spectra were obtained at −150 °C.

Reaction of (Tetrakis(2,6-dichlorophenyl)porphyrinato)iron(III) with ^{18}O-Labeled Peroxybenzoic Acid in Toluene. A toluene solution of hydroxo(tetrakis(2,6-dichlorophenyl)porphyrinato)iron(III) (44 mg in 50 mL) with 100 mg of CH_3CO_2Na (solid) was cooled to 5 °C and ^{18}O-labeled peroxybenzoic acid was then introduced slowly until the completion of the reaction as monitored by the visible spectrum. After removal of CH_3CO_2Na by filtration, the toluene solution was cooled to −78 °C and 150 mL of *n*-pentane was added to give precipitation of corresponding iron(III) *N*-oxide (**5′**). **5′** was collected by filtration at −30 °C and dissolved in toluene.

Oxygen in the resulting solution was purged by bubbling N_2 for 30 min at 0 °C. A large excess of triphenylphosphine was then introduced. After 20 h under N_2 the ^{18}O content of the resulting triphenylphosphine oxide was determined by GC mass spectroscopy. Only trace amounts of ^{18}O were observed.

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