upfield shift of the β-C's (C-2), unique for all known, related, oxygen-functionalized acetylenes ($C_\beta \equiv C_\alpha OR$: R = SO_2Ar;[5] R = Si≡;[11] R = Alkyl[12]). The mass spectra show appropriate molecular ions and fragmentation patterns. Moreover, phosphate esters **10** were independently prepared by the $(EtO)_2P(O)Cl$ trapping of the recently reported[14] alkynolate ions $(RC\equiv CO)^-Li^+$ and found to be identical in all respects. Hence, there is no doubt about the identity of these novel acetylenic esters.

Alkynyl benzoates **8** are reasonably stable, colorless liquids that decompose upon standing at room temperature over several days. The alkynyl diethyl phosphate esters **10** are also colorless liquids and even more stable than the corresponding benzoates but do undergo slow decomposition (over several weeks) upon standing neat at room temperature.

In summary, we have developed a simple, mild, general means of preparing novel alkynyl carboxylate and phosphate esters from readily available tricoordinate iodonium tosylate precursors. These new acetylenic esters have characteristic spectral properties consistent with their proposed structures and are isolable, reasonably stable, colorless liquids. The full scope of this methodology, along with the chemistry of these new esters, including the potential uses as possible enzyme-activated inhibitors,[14] are under active study and will be the subject of future papers.

Acknowledgment. Financial support by the National Cancer Institute of the NIH is greatly appreciated. M.B. is grateful to the HOECHST AG/W. Germany for providing a postdoctoral fellowship.

Registry No. 6a, 92473-47-7; **6b**, 92473-43-3; **8a**, 104911-35-5; **8b**, 104911-36-6; **8c**, 104911-37-7; **8d**, 104911-38-8; **9a**, 104911-39-9; **10a**, 104911-40-2; **10b**, 104911-41-3; $C_6H_5CO_2^-$, 766-76-7; p-$CH_3C_6H_4CO_2^-$, 5118-31-0; $(EtO)_2PO_2^-$, 48042-47-3.

(11) Maas, G.; Bruckmann, R. *J. Org. Chem.* **1985**, *50*, 2801.
(12) Levy, G. C.; Lighter, R. L.; Nelson, G. L. *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*; 2nd ed.; Wiley: New York, 1980; pp 90–95.
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Oxygen Activation by Metalloporphyrins Related to Peroxidase and Cytochrome P-450. Direct Observation of the O–O Bond Cleavage Step

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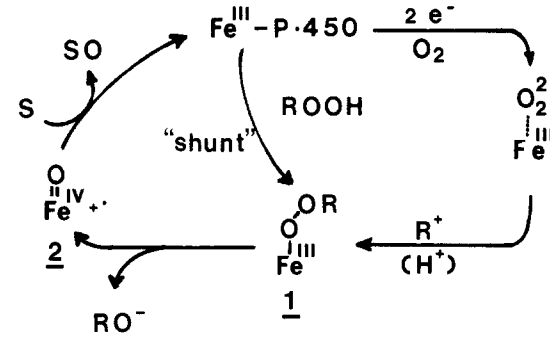
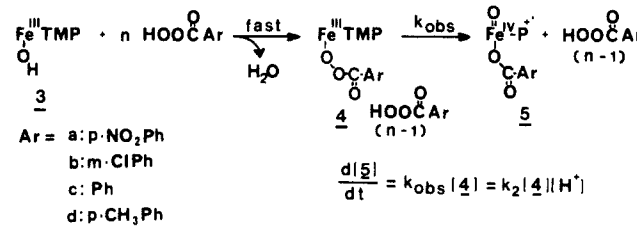
The reductive activation and transfer of dioxygen mediated by cytochrome P-450 is unique among the hemoproteins.[1] That two one-electron reductions are required for each cycle with dioxygen, and the apparent circumvention of this multistep process with exogenous peroxides,[2] has suggested the intermediacy of peroxyiron(III) species such as **1**.[1c,3] Heterolytic cleavage of the O–O bond in such a complex could give rise to an oxoiron(IV) porphyrin cation radical (**2**) (Scheme I). Support for this view derives from the oxidation of synthetic iron(III) porphyrins to

Scheme I



Scheme II



reactive complexes analogous to **2**.[4]

We describe here the formation of an (acylperoxy)iron(III) porphyrin complex analogous to **1** and its reaction to form an oxoiron(IV) porphyrin cation radical (**2**); the first direct observation of an iron-catalyzed O–O bond cleavage.

Attempts to follow the kinetics of the oxidation of chloro(5,10,15,20-tetramesitylporphyrinato)iron(III) [Fe^{III}(TMP)(Cl)] with peroxy acids at low temperature led to complicated sigmoidal rate profiles. By contrast the oxidation of *hydroxo* Fe(III)TMP was well behaved. Thus, Fe^{III}TMP(OH)[5] (**3**) was found to react with *p*-nitroperoxybenzoic acid instantaneously at −46 °C (1.48×10^{-5} M in CH_2Cl_2) to produce an intermediate (**4a**) which exhibited a visible spectrum typical of a five-coordinate, high-spin Fe(III) complex (λ_{max} 419, 508, 666, and 682 nm in CH_2Cl_2).[6] However, **4a** was not stable even under these mild conditions, and it decomposed to a bright green species **5a** (Figure 1). Intermediate **5a** was characterized as an oxoiron(IV) porphyrin cation radical (**2**) by comparison with authentic sample prepared by the reaction of Fe^{III}(TMP)(Cl) with mCPBA in CH_2Cl_2 at −50 °C.[7] Furthermore, **5a** reacted with added cyclooctene whereas [Fe^{III}(TMP·)(ClO_4)]$^+$ was stable under these conditions. The similarity of the visible spectrum of **4a** to that of Fe^{III}(TMP)(*p*-nitrobenzoate),[8] its facile conversion at low temperature to **5a**, and the 1.2:1 stoichiometry of its formation from Fe^{III}(TMP)(OH) are consistnet with an Fe^{III}(TMP)(peroxybenzoate) formulation. The corresponding (acylperoxy)*manganese*(III) porphyrin complex has been formed in the same way.[9]

The conversion of **4** to **5** could be monitored conveniently by observing changes in the visible spectrum upon the addition of at least 1.2 equiv of peroxy acid. Lines A and B in Figure 1 represent the time-dependent changes of absorbances at 418 and 363 nm upon the addition of 3 equiv of *p*-nitroperoxybenzoic acid to a CH_2Cl_2 solution of **3** (1.48×10^{-5} M) at −46 °C. Several clear isosbestic points were evident. The formation of **5** was found

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(3) (a) Sligar, S. G.; Kennedy, K. A.; Pearson, D. C. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1240. (b) White, R. E.; Sligar, S. G.; Coon, M. J. *Biol. Chem.* **1980**, *255*, 11108.
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(8) The authentic sample was prepared by the reaction of **3** and *p*-nitrobenzoic acid in CH_2Cl_2.
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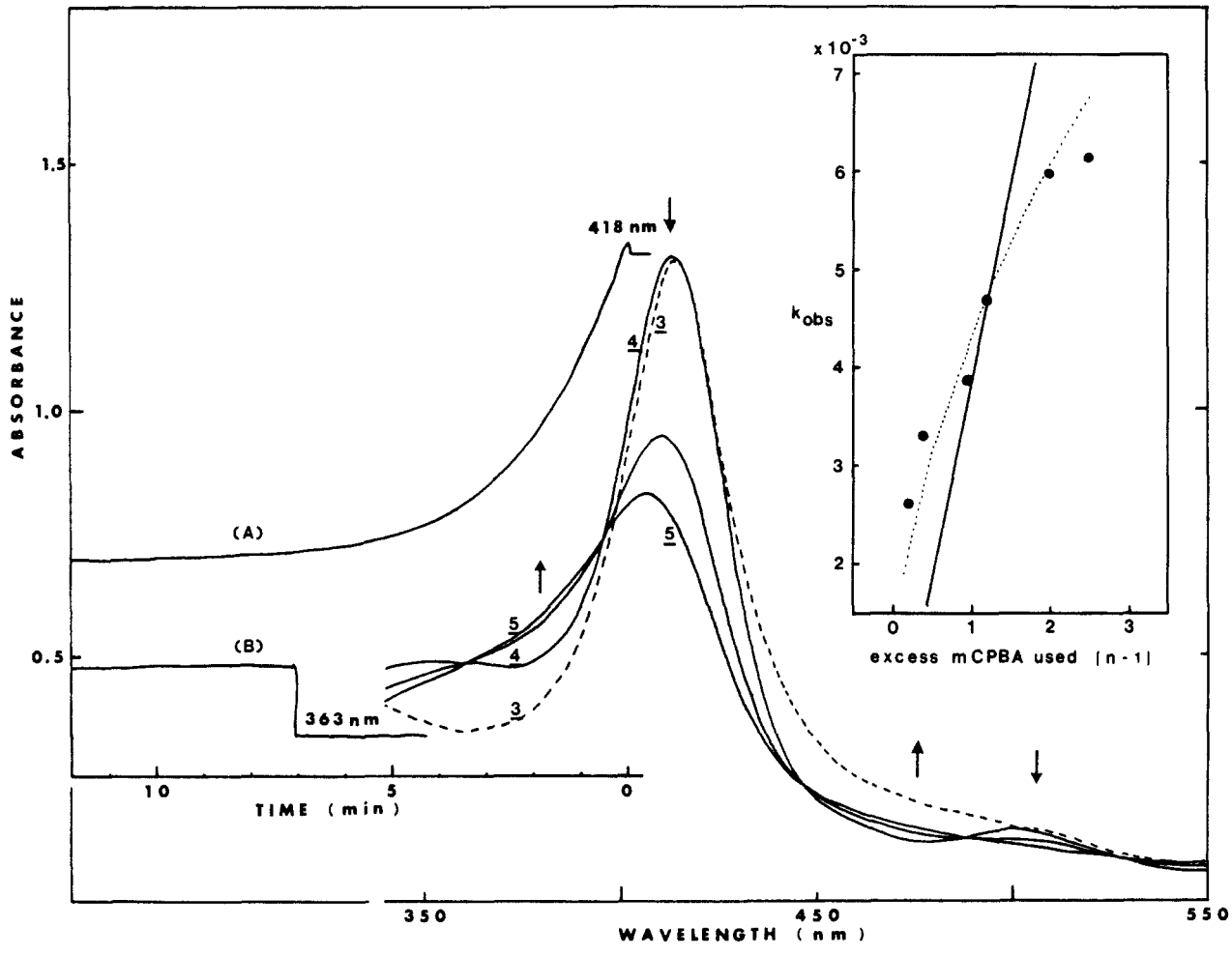

Figure 1. Visible spectral changes for the reaction of $Fe^{III}(TMP)(OH)$ (**3**) (1.48×10^{-5} M) and 3 equiv of *p*-nitroperoxybenzoic acid at −46 °C. $Fe^{III}(TMP)(OH)$ (---); **4**, immediately after the addition of pNPBA to **3**; **5**, final spectrum. Line A/B: Time course of absorbance changes at 418 and 363 nm, respectively. Inset: Effect of excess mCPBA for k_{obsd} at −48 °C; (—) calculated for first-order dependence on [mCPBA]; (···) calculated for half-order dependence on [mCPBA]; (●) observed.

to be accelerated by added benzoic acid or by excess peroxy acid. Good pseudo-first-order kinetics were observed for a variety of protic acids. However, as shown in Figure 1 (inset), the rate of formation of **5** (k_{obsd}) was not first-order in the concentration of excess peroxy acid. This observation is consistent with an *acid-catalyzed* O–O bond cleavage since $[H^+]$ can be shown to equal $K_a^{1/2}$[peroxy acid]$^{1/2}$. Thus, the overall reaction is as described in Scheme II. Ligand exchange of **3** to afford **4** consumes 1 equiv of protons producing H_2O. In the second reaction, protons from the remaining peroxy acid catalyze the decomposition of **4**. Traylor et al.[10] have described the catalytic decomposition of peroxy acids by iron(III) porphyrins to be general-base catalyzed in *buffered* aqueous methanol.

The nature of the O–O bond cleavage of the peroxyiron(III) intermediate in enzymes such as cytochrome P-450 and HRP is poorly understood. For example, HRP oxidizes substrates by utilizing (phenylperoxy)acetic acid *without* the formation of CO_2,[11] while some decarboxylation occurs with this peroxyacid with cytochrome-450.[12]

The rapid formation of an (acylperoxy)iron(III) species (**4**) by the addition of peroxy acids to the iron(III) *hydroxide* has allowed a direct monitoring of the effect of leaving group on the rate of O–O bond cleavage. In order to maintain approximately constant acidity, solutions were prepared with variable excesses of peroxy acid to compensate for the differences in pK_a.[13] Protons released upon the coordination of the peroxy acid are taken up with the hydroxo ligand to form water.

The observed rates of formation of **5** for a series of peroxy acids gave a good Hammett correlation ($\rho = 0.5$).[14] Similar values have been reported recently by Bruice et al.[15] ($\beta = -0.35$) and Traylor et al.[10] ($\beta = -0.24$) for the catalytic decomposition of peroxy acids by $Fe^{III}TPP(Cl)$, but ligand exchange is expected to be at least partially rate-limiting under these conditions. The acceleration by electron-withdrawing groups observed here indicates that the leaving ability of the corresponding benzoate dominates the rate, and that the nature of O–O bond cleavage process is heterocyclic under these acid-catalyzed conditions.[16] Furthermore, variations in the rate of formation of **5** with temperature gave a linear Arrhenius plot over the range of −30 to −50 °C. The results indicate an extraordinarily low enthalpy of activation and a large negative entropy of activation (E_a = 4.0

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(13) For example, 3.4 equiv of peroxy-*p*-toluic acid was employed to obtain an acidity equivalent to 2.3 equiv of peroxybenzoic acid according to the relationship

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

if $K_a[\text{HOOCOAr}] = K_a'[\text{HOOCOAr}']$. We have made the assumption that K_a/K_a' in water is about the same as that in methylene chloride. Thus $3.4K_{ap\text{-}me} = 2.3K_{aH}$. pK_a(peroxybenzoic acid) = $0.673pK_a$(benzoic acid) + 4.875. Blake, R. C., II; Coon, M. J. *J. Biochem.* **1980**, *255*, 4100.
(14) Substituent effects for peroxybenzoic acids at −48 °C: $k_{obsd} \times 10^3$, s^{-1} (equiv of peroxy acid): *p*-NO_2, 5.7 (1.8); *m*-Cl, 3.7 (2.4); H, 2.4 (3.3); *p*-Me, 2.0 (4.4); (phenylperoxy)acetic acid, 1.8 (4.0).
(15) Lee, W. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1985**, *107*, 513.
(16) Bruice, T. C. *J. Chem. Soc., Chem. Commun.* **1983**, 14.

Kcal/mol, $\Delta H^{\ddagger} = 3.6$ kcal/mol, and $-11 > \Delta S^{\ddagger} > -25$ eu. While a detailed analysis of these activation parameters must await further study, it is clear that there is very little intrinsic barrier to the heterolytic O–O bond cleavage in an iron(III) peroxybenzoate. The large negagive entropy term suggests an associative reaction which could involve positioning the necessary proton and perhaps coordination of a sixth ligand on the iron.

In summary, by direct observations of the primary steps, the mechanism of iron(III) porphyrin oxidation by peroxy acids to form the corresponding oxo iron(IV) porphyrin cation radical (i) has been shown to involve prior coordination of the peroxy acid. (ii) the nature of the O–O bond cleavage step for peroxyacyliron(III) benzoates is heterolytic and acid catalyzed.

In the accompanying paper,[17] formation of an iron(III) porphyrin N-oxide from the same peroxyiron(III) precursor (**4**) in non-polar solvents and in the absence of acid is proposed to result from a homolytic O-O bond cleavage in **4**.

Acknowledgment. Support of this research by the National Institutes of Health (GM 36298) and the National Science Foundation is gratefully acknowledged. Portions of this research were carried out at the University of Michigan.

Registry No. 3, 77439-20-4; **4** (Ar = CH_2Ph), 104619-66-1; **4a**, 104619-67-2; **4b**, 104463-57-2; **4c**, 104619-68-3; **4d**, 104619-69-4; **5** (Ar = CH_2Ph), 104619-70-7; **5a**, 104619-71-8; **5b**, 104619-72-9; **5c**, 104619-73-0; **5d**, 104619-74-1; HOOCO*p*-NO_2Ph, 943-39-5; HOOCO*n*-ClPh, 937-14-4; HOOCOPh, 93-59-4; HOOCO*p*-CH_3Ph, 937-21-3; $HOOCOCH_2Ph$, 19910-09-9; peroxidase, 9003-99-0; cytochrome P-450, 9035-51-2; oxygen, 7782-44-7.

(17) Groves, J. T.; Watanabe, Y., *J. Am. Chem. Soc.*, following paper in this issue.

Preparation and Characterization of an Iron(III) Porphyrin *N*-Oxide

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The nature of oxidized iron species in a variety of heme proteins has been clarified by the preparation of related synthetic iron porphyrin complexes. Thus, both an oxoiron(IV) porphyrin[1] and an oxoiron(IV) porphyrin cation radical[2] are now known. The latter of these is related to compound I of horse radish peroxidase and is the most attractive candidate for the ultimate oxidizing intermediate in the catalytic cycle of cytochrome P-450.[3] An alternative bridged iron porphyrin *N*-oxide has been suggested on the basis of the crystal structures of N-bridged iron porphyrin carbenes[4] and the existence of several metalloporphyrin *N*-oxides[5a-c] and an N-bridged nitrene.[5d] We describe here the preparation and characterization of the first iron porphyrin *N*-oxide and show that such species are chemically distinct from the formally isomeric oxoiron(IV) porphyrin cation radical complexes we have previously reported.

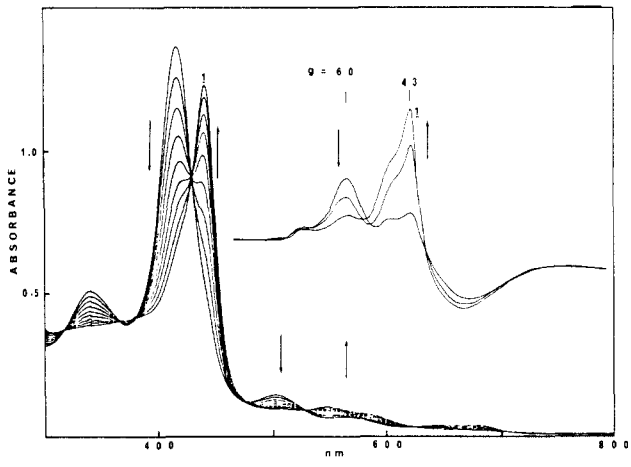

Figure 1. Visible spectral changes upon addition 0.25-equiv aliquots of mCPBA to Fe^{III}(TMP)(mCB) (1.27×10^{-5} M) in toluene at 0 °C. Inset, EPR spectral changes upon addition of mCPBA to Fe^{III}(TMP)(mCB) (5.3×10^{-4} M) to form **1**.

The slow addition of *m*-chloroperoxybenzoic acid (mCPBA) to a toluene solution of (5,10,15,20-tetramesitylporphyrinato)iron(III) *m*-chlorobenzoate [Fe^{III}(TMP)(mCB)] (ca. 10^{-4} M) at 0 °C gave a new species (**1**) with a dramatically red-shifted soret band (417 to 441 nm). The titration of Fe^{III}(TMP)(mCB) with mCPBA showed that 2 equiv of mCPBA were required to complete the formation of **1** (Figure 1). The EPR spectrum of Fe^{III}(TMP)(mCB) ($g = 6$) was replaced with strong new signals at $g = 4.3$ in the course of this titration (Figure 1 inset). The ^{1}H NMR spectrum of **1** at −50 °C 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 pyrrole protons were not evident. The visible spectrum of **1** was uneffected by added olefins even at room temperature.

The demetalation of **1** with acetic acid–HCl (4:1) was remarkably facile; complete in 60 s at 0 °C. Neutralization and thin-layer chromatography of the resulting green solution afforded H_2TMP *N*-oxide (**2**) in 25% yield and an equivalent amount of H_2TMP.[6] The structure of **2** was evident from its UV, ^{1}H NMR, and FAB mass spectra[7] and from its independent synthesis from H_2TMP.[5a] Complex **1** could not be prepared by the metalation of **2**.

The 20-nm red shift of the soret band of **1** and the facility of the demetalation reaction indicate that **1** has a substituent on the porphyrin pyrrole nitrogen.[8] The ^{1}H NMR spectrum of **1** supports a structure of *Cs* symmetry, and the meta protons are in a region typical for high-spin iron(III) porphyrins. The isolation of H_2TMP *N*-oxide (**2**) upon demetalation points strongly to an iron(III) porphyrin *N*-oxide structure for **1**. The $g = 4.3$ signal in the EPR of **1** is similar to that reported recently for an analogous

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(6) H_2TMP *N*-oxide (**2**) decomposed slowly to H_2TMP under these conditions and sometimes more than 80% of the recovered porphyrin was H_2TMP. The ratio of *N*-oxide and parent free base depended on the conditions of the dematalation reaction (cf. ref 5a).

(7) **2**: NMR (CD_2Cl_2) pyrrole, 8.53 (4 H, AB q, $J = 12.6$, 5.0 Hz) 8.40 (2 H, s) 7.49 (2 H, s); meta H, 7.36 (8 H, s); *p*-Me, 2.59 (12 H, s); *o*-Me, 1.88 (12 H, s), 1.86 (12 H, s); NH, 1.75 (2 H, s). UV (nm) (log ϵ) (toluene) 417 (5.17), 544 (3.77), 602 (3.78), 696 (3.28). IR (cm^{-1}, Nujol) 1273, 864 (N–O). Mass spectrum (FAB), *m/e* 798 (M^+, 72%), 782 (M^+ – O, base).

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