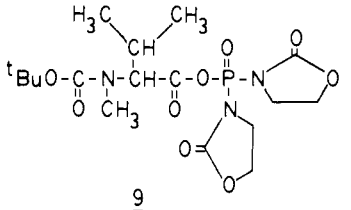
crude products were compared by HPLC. Under conditions that allowed complete resolution of the diastereomers,[15] essentially no racemization (<0.2%) was observed in either case.

Synthesis of a series of bis-*N*-methylated dipeptides by the above method indicated that steric hindrance in the carboxyl component plays a larger role than that in the nucleophile in determining yields. Compound **4**, in which branching of both the imino acid side chain and of the N-protecting group hinder access of the nucleophile to the initially formed anhydride **9**, is formed only



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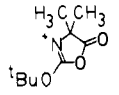
with difficulty (4 days, 36%). Reversal of the imino acids side chains, however, allowed facile synthesis of **5** (4 h, 80%). The effect of the N-protecting group is demonstrated by compounds **6** and **7**, where replacing the *tert*-butyl carbamate of **6** with the flatter and less hindered Fmoc[13,16] group resulted in a shorter reaction time and a higher yield (16 h, 66%, vs. 5 days, 32%). The main byproduct of the reactions leading to **4** and **6** has been identified as the symmetrical anhydride $(Boc\text{-}MeVal)_2O$ resulting from disproportionation of **9**.[17-19] We have found that, in these highly hindered systems, the symmetrical anhydride is unreactive toward the amine and represents a dead-end product.[20]

The utility of **1** as a coupling reagent appears to be quite general and not limited to *N*-methyl amino acids. Thus, condensation of *N*-Et-Ala-OBzl with Fmoc-Phe proceeded smoothly to yield **8** (overnight, 81%). Preliminary work in this laboratory has indicated that **1** may also be advantageously applied to the synthesis of larger N-alkylated peptides, as well as solution and solid-phase synthesis of non-N-alkylated peptides.[21] The only limitation we have encountered to date was in an attempted coupling of Boc-Aib[13] with Aib-OBzl, where only a small amount of product was formed. Presumably, the low yield was due to the "gem-dimethyl" effect[22] promoting intramolecular cyclization to the 5-oxo-Δ^2-oxazolinium cation **10** and subsequent decomposition,[9,23] since the starting acid was quantitatively consumed. In spite of this apparent[24] limitation, when compared with a wide range of other reagents, including several phosphorus-based compounds (BOP,[13,25] DPPA,[26] diphenylphosphoryl chloride[27]) which have been previously used for amide bond formation, compound **1** has proven to be the most useful in terms of yield, racemization levels, and convenience.[28] Further studies extending the applications of **1** toward solution and solid-phase syntheses of cyclosporin A and other hindered peptide systems are under way.



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Acknowledgment. This work was supported in part by a grant from the National Institutes of Health (AM 32007).

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Mechanism of Oxygen Atom Transfer from High Valent Iron Porphyrins to Olefins: Implications to the Biological Epoxidation of Olefins by Cytochrome P-450

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The mechanisms by which the cytochrome P-450 monooxygenase enzymes effect hydrocarbon oxygenation has inspired many studies of model metallaporphyrin catalysts.[1-3] The rate-limiting step for most of these oxygenations occurs prior to oxygen atom transfer from the active oxidant, a high-valent iron–oxo–heme complex, to the substrate so that kinetic studies of this most interesting but mysterious reaction have not been feasible. Only in the case of the manganese porphyrin catalyzed olefin epoxidation by hypochlorite (OCl^-) under phase-transfer conditions[4] has it been possible to obtain kinetic data for oxygen atom transfer from the metal to the olefin. Those kinetic studies revealed the presence of a reversibly formed intermediate which is in equilibrium with free substrate and a highly oxidized form of the catalyst.[5] By observing the kinetic behavior of different manganese porphyrin catalysts with a range of olefins, both individually and in direct competition, we obtained kinetic data which revealed that the equilibrium binding of the olefin to the oxidized porphyrin is sensitive to steric effects on both the porphyrin and the olefin but is little influenced by the electronic properties of the substrate.[6] Furthermore, the stereochemistry of the olefin and the epoxide are retained throughout most of these reactions. These results led us to speculate that the intermediate is a metallaoxetane rather than a charge-transfer complex. We now extend these studies to the more biologically relevant *iron* porphyrin catalyzed epoxidation reactions using a different oxygen atom transfer reagent.

Our present work was inspired by a report that pentafluoroiodosylbenzene (F_5PhIO) epoxidizes norbornene in the presence of iron(III) tetrakis(2,6-dichlorophenyl)porphrin chloride (Fe-

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Table I. Rate Data for the FePFPCl-Catalyzed Epoxidation of Olefins by F_5PhIO[a]

olefin	rate,[8b] turnovers/s[b]	selectivity, %[c]
cyclooctene	10.0	100
trans-β-methylstyrene	31.0[d]	100
cis-β-methylstyrene	17.4[e]	100
indene	8.8	52
cis-cyclodecene	7.9[e]	100
2-methyl-2-pentene	30.8	74
norbornene	0.15	100

[a] Experimental conditions: FePFPCl (5.08×10^{-5} mmol); F_5PhIO (0.24 mmol); olefin (0.3–3 mmol); CH_2Cl_2 solution (total volume = 0.6 mL); dodecane (GLC standard) (0.044 mmol); aliquots were taken periodically, quenched with Ph_3P, and analyzed by GC. [b] Rates are based on epoxide production; error is ±0.9 turnover/s, except for norbornene (±0.05 turnover/s). [c] mmol of epoxide/mmol of oxidant consumed (±5%). [d] Only *trans*-epoxide observed. [e] Only *cis*-epoxide observed.

TodCPPCl) at a rate *many* times greater than the analogous reaction in which iodosylbenzene is the terminal oxidant.[7] This implies that the rate-limiting step may be oxygen atom transfer from iron to the olefin rather than from F_5PhIO to iron. This supposition has been verified (vide infra).

Using iron(III) tetrakis(pentafluorophenyl)porphyrin chloride[8] (FePFPCl) as the catalyst, and F_5PhIO as the terminal oxidant suspended in CH_2Cl_2,[9] we have obtained well-behaved kinetic data for a variety of olefins (Table I). Experimental conditions ([FePFPCl] = 8.47×10^{-5} M) were carefully chosen so that the rate of substrate oxygenation is first order in catalyst, assuring that porphyrin dimerization is not a complicating factor in the kinetic analysis.[10] The rates are *independent of olefin concentration* (over the 0.5–3.0 M range examined), but *different olefins are epoxidized at different rates*, confirming that oxygen atom transfer from iron to the olefin is rate determining. These results can be accommodated by the mechanism outlined in Figure 1. We again postulate the presence of a metal–oxo–olefin intermediate, **2**, in rapid equilibrium with free olefin and the oxo compound[11] **1**, whose decomposition to epoxide is rate determining. Under conditions in which the catalyst is saturated with substrate, *the concentration of the intermediate*, **2** *is insensitive to the olefin concentration*, but the rate-determing (and product forming) step depends on the *nature* of the olefin. This mechanism further suggests that one olefin could competitively inhibit another olefin by forming an alternative intermediate,[12] **3** (Figure 1). The rate data shown in Figure 2 (upper plot) confirm this predicted result. Note that the addition of only 0.25% of norbornene (400:1), the slowest olefin, measurably inhibits the rate of cyclooctene epoxidation. This is consistent only with the presence of a reversibly formed oxo–olefin intermediate. Note also that the last data point of the uninhibited run evinces nearly 4000 catalyst turnovers. These competitive inhibition experiments can be recast in the form of a Dixon plot[13] (Figure 2, lower plot), from which it can be derived that the metal–oxo species **1** has a 300-fold higher affinity for norbornene than for cyclooctene.[13]

Competitive oxygenation experiments can be used to probe the nature of the iron–oxo–olefin intermediate.[14] Addition of 1 equiv of cyclooctene to a standard 2-methyl-2-pentene run resulted in a rate (13.4 turnovers/s, 43% of the uninhibited value) which shows that the catalyst has a slightly (2.6-fold) higher affinity for cyclooctene. The very similar binding affinities of a secondary and a tertiary alkene suggest that the oxo–olefin intermediate is not a simple olefin coordination complex. Alternatively, it has been proposed that the cytochrome P-450 catalyzed epoxidation of olefins proceeds via single-electron transfer from the alkene to the iron(V)–oxo porphyrin, generating an iron(IV)–oxo olefin π-radical cation pair which collapses to give the epoxide.[15,16] If such an intermediate were formed in our system, the catalyst should exhibit a much higher affinity for aryl-substituted olefins than simple alkyl-substituted alkenes, since the corresponding aryl-substituted radical cations are far more stable.[17] In fact, the rate of *cis*-β-methylstyrene oxide production was only 7.6 turnovers/s (44% of the uninhibited value), in the presence of 1 equiv of *cis*-cyclodecene, showing that the high-valent iron species **1** has about a 2.2-fold *lower* affinity for the aromatic olefin than cyclodecene. This rules out an olefin π-radical cation complex as the intermediate. To the extent to which this system serves as a model of P-450 itself, the presence of such intermediates in the biological reaction must be called into question.

In the absence of spectroscopic data, the nature of the iron–oxo–olefin intermediate cannot be assigned with confidence. However, the results presented here are in accord with the general conclusions reached for the manganese/OCl^- reaction, in which all of our data are consistent with the intermediacy of a metallaoxetane. We believe the analogous iron metallacycle **2** to be a reasonable candidate for the intermediate described here.[18]

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(8) (a) The FeTodCPPCl catalyst is remarkably stable toward oxidative degradation, which is a serious problem with most iron porphyrin catalysts. For this reason, we chose FePFPCl as our catalyst, since Chang has shown this ligand to be stable toward oxidation. Under our experimental conditions, porphyrin degradation is not observed. Chang, C. K.; Ebina, F. *J. Chem. Soc., Chem. Commun.* **1981**, 778–779. (b) In our hands the literature procedure[8c] for the synthesis of FePFPCl is ineffective. We have employed a modified method which affords a porphyrin that is pure by ^{19}F NMR and shows only one spot on TLC. Unfortunately, the compound tends to occlude hydrocarbons during attempts to recrystallize it. Therefore, the rates shown in Table I, and discussed in this report, are minimal values. We are working to obtain a catalyst with a higher "specific activity". Recently we have observed higher rates for each olefin employing a catalyst of higher purity (Kodadek, T.; Hampton, P., unpublished results). (c) Longo, F. R.; Finarelli, M. G.; Kim, J. B. *J. Heterocycl. Chem.* **1969**, *6*, 927–931.

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(10) The dependence of the rate on [FePFPCl] was found to vary from first order to zero order in experiments conducted at various catalyst concentrations. The zero-order behavior at high catalyst concentrations could be due either to porphyrin dimerization or starvation of the iron(III) porphyrin for the relatively insoluble oxidant. In any case, all experiments reported here were conducted at a sufficiently low catalyst concentration such that this is not a complicating factor.

(11) On the basis of precedent (see ref 1–3) the oxo complex is assumed to be two oxidation equivalents above the iron(III) state. No attempt has been made to elucidate the electronic state of this compound (i.e., iron(V) or iron(IV) porphyrin radical cation).

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(13) Dixon, M.; Webb, J. L. "Enzymes", 2nd ed.; Academic Press: New York, 1964; Chapter 8.

(14) From a competitive experiment with substrates A and B, relative K_M values (roughly an inverse binding constant) can be derived by using the equation

$$\frac{-dA/dt}{-dB/dt} = \frac{[A]}{[B]} \frac{V_{max}(A)K_M(B)}{V_{max}(B)K_M(A)}$$

See ref 6 for a detailed discussion of this method.

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(17) For example, the oxidation potentials of indene and 2-octene are 1.23 and 2.23 V, respectively (vs. Ag/Ag^+). Baizer, M. "Organic Electrochemistry"; Marcell-Dekker: New York, 1973.

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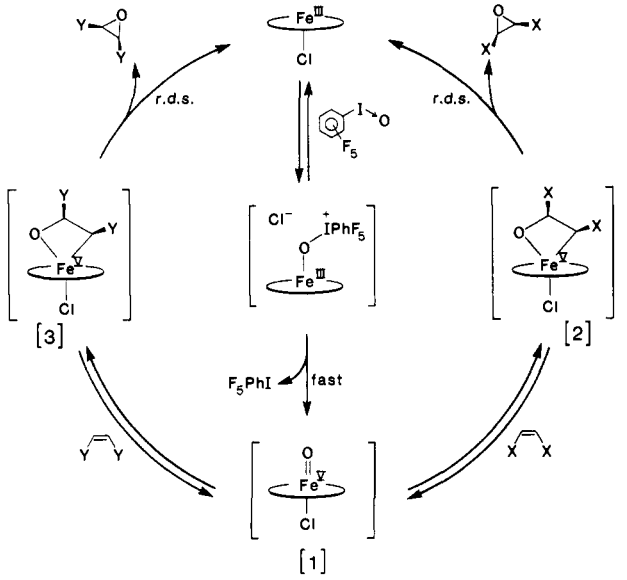

Figure 1. Proposed catalytic cycle for the competitive epoxidation between two different olefins.

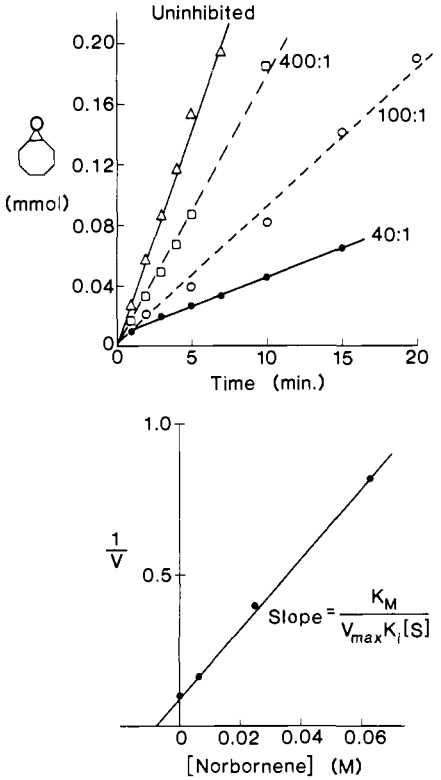

Figure 2. (Upper Graph) Cyclooctene oxide production vs. time for several cyclooctene/norbornene competitive epoxidation runs. The ratios shown are the initial cyclooctene:norbornene ratios. The concentration of cyclooctene was held constant while the amount of norbornene was varied. The total reaction volume was held constant (0.6 mL). (Lower Graph) Dixon plot of the results shown above. V = velocity; K_M = Michaelis constant; K_i = inhibition constant; [S] = cyclooctene concentration.

Attempts to characterize rigorously these intriguing species are ongoing.

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Solvent Water and the Biological Group-Transfer Potential of Phosphoric and Carboxylic Anhydrides

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Phosphoric anhydrides take part in many chemical and physical processes in living systems, and there are indications that carboxylic anhydrides may be formed as reactive intermediates during the action of certain enzymes.[1,2] These compounds have large negative group-transfer potentials in water.[3] Their reactions tend to occur in the active sites of enzymes where water is relatively scarce, and it would be of interest to know the extent to which solvent water affects their equilibria of hydrolysis. Large positive entropy changes that accompany hydrolysis of pyrophosphate derivatives suggest that solvation effects could play a major role in determining positions of equilibria of these reactions,[4] and molecular orbital calculations support this possibility.[5]

Effects of solvent water on these reactions could be evaluated directly if the affinities of anhydrides for water were available for comparison with those of the products of their hydrolysis. Unfortunately phosphoric anhydrides with dissociable protons, such as ATP, are too polar for direct measurement of their equilibria of distribution between water and nonpolar environments. In addition, acid anhydrides tend to be unstable in water. To circumvent these problems, we have measured apparent distributions of acetic anhydride and tetraethyl pyrophosphate at various concentrations between water and chloroform after timed intervals of mixing. Results were extrapolated back to the initial time of mixing, in order to obtain the distribution coefficient of the unhydrolyzed compound at the time of mixing. By comparison of the distribution coefficients of an anhydride and of reactant water with those of the acids produced by its hydrolysis, it should be possible to estimate the equilibrium constant for hydrolysis of the anhydride in wet chloroform (Scheme I).

Solutions of acetic anhydride or tetraethyl pyrophosphate (0.02–1.00 M in D_2O-saturated $CDCl_3$, 5 mL) were introduced into a Mixxor apparatus (Cole-Palmer Co., 10-mL capacity) along with $CDCl_3$-saturated D_2O (5 mL) containing 0.3 M KCl, these components having been previously adjusted to 20 °C in a water bath. Complete mixing was achieved in 15 s (eight strokes of the piston), and the phases were allowed to clear during the following 15 s. The aqueous phase was then removed for analysis. Tetraethyl pyrophosphate was determined in the D_2O phase using a Varian EM-390 NMR spectrometer (δ 1.5 for methyl groups), with pyrazine as an internal integration standard. Acetic anhydride was determined by allowing complete hydrolysis to occur in the aqueous phase over a period of 1 h. Acetic acid was then tritrated potentiometrically with standard KOH. These experiments were repeated after mixing intervals of 2, 5, 10, 15, and 20 min, and the results were extrapolated to obtain a value at zero time. Half-times for hydrolysis in water are approximately 7 h for tetraethyl phosphate[6] and 6 min for acetic anhydride.[7] Anhydrides favor the chloroform phase (see below), so that their effective half-lives were in fact longer under the conditions of the distribution experiments, approximately 2 h for acetic anhydride and 300 h for tetraethyl pyrophosphate.

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