Hypersensitive Radical Probe Studies of Chloroperoxidase-Catalyzed Hydroxylation Reactions

Patrick H. Toy,[†] Martin Newcomb,*[,†] and Lowell P. Hager[‡]

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, and Department of Biochemistry, University of Illinois, Urbana, Illinois 61801

Received February 19, 1998

The oxidation of hypersensitive radical probes by chloroperoxidase from *Caldariomyces fumago* (CPO) was studied in an attempt to "time" a putative radical intermediate. Oxidation of (*trans*-2-phenylcyclopropyl)methane, previously studied by Zaks and Dodds [Zaks, A., and Dodds, D. R. (1995) *J. Am. Chem. Soc.* **115**, 10419−10424] was reinvestigated. Unrearranged oxidation products were found as previously reported, and control experiments demonstrated that the cyclic alcohol from oxidation at the cyclopropylcarbinyl position, while subject to further oxidation, survives CPO oxidation as detectable species. However, in contrast to the report by Zaks and Dodds, the rearranged alcohol product expected from ring opening of a cyclopropylcarbinyl radical intermediate was shown to be unstable toward the enzyme oxidation reaction. Because of this instability, two new hypersensitive radical probes, (*trans*-2-phenylcyclopropyl)ethane and 2-(*trans*-2-phenylcyclopropyl)propane, and their potential cyclic and acyclic products from oxidation at the cyclopropylcarbinyl position were synthesized and tested. Oxidation of both of these probes at the cyclopropylcarbinyl position by CPO gave unrearranged alcohol products only, but control experiments again demonstrated that the rearranged alcohol products were unstable toward CPO oxidation conditions. From the combination of the probe and control studies, the lifetime of a putative radical intermediate must be less than 3 ps. Whereas the results are consistent with an insertion mechanism for production of alcohol product, they do not exclude a very short-lived intermediate.

Introduction

Chloroperoxidase from *Caldariomyces fumago* (CPO)[1] is unique among the known heme-containing peroxidase enzymes in that a thiolate from a protein cysteine serves as the fifth ligand to iron (*1*). Thus, the active site of CPO is related in gross architecture to those of the ubiquitous cytochrome P450 (P450) enzymes. CPO is also related in function to the P450 enzymes in that several of the oxidation reactions effected by CPO are similar to those of the P450 enzymes. The major difference between CPO and P450 enzymes is the source of oxygen in nature; CPO employs hydrogen peroxide, whereas P450 enzymes employ molecular oxygen and an ancillary reduction system that uses reducing equivalents from NAD(P)H (*2*). Nevertheless, because the reduction of molecular oxygen in the P450 enzymes gives the formal equivalent of hydrogen peroxide and given the similarity of the oxidation reactions effected by CPO and P450 enzymes, there is a good likelihood that a common oxidation mechanism exists for these enzymes. Accordingly, mechanistic studies of CPO oxidations could provide results that have a direct bearing on the mechanisms of P450 oxidations.

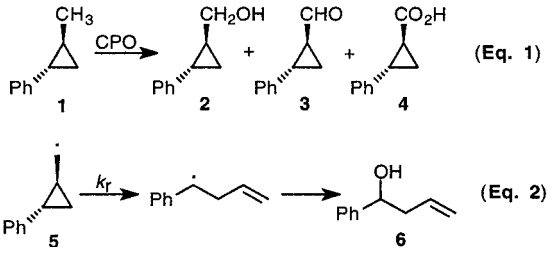
The consensus mechanism for hydrocarbon hydroxylation by P450 enzymes that evolved over the past 2 decades involves hydrogen abstraction by an "iron−oxo" intermediate followed by homolytic substitution of the radical at OH bound to iron (oxygen rebound step) (*3*−*5*). However, recent mechanistic studies of hydroxylation of hypersensitive radical probes by the rat liver isozyme P450 2B1 (CYP2B1) have indicated that the "radical" lifetime is too short for an intermediate and that the reaction proceeds by an insertion mechanism with a competing cation-forming pathway (*6*−*9*).

Although P450-catalyzed hydroxylations of hydrocarbons and the mechanism of this process have been widely studied, relatively few such studies have been performed with CPO. The first involved the hydroxylation of cyclohexene (*10*), and the others studied the hydroxylation of benzylic positions in substrates (*11*, *12*). One mechanistic study of CPO hydroxylation, reported in a paper focusing mainly on the use of CPO for stereoselective olefin epoxidations, resulted in the conclusion that this hydroxylation reaction involves an insertion process which occurs without the formation of a discrete radical intermediate (*11*). In that study, the radical probe **1** was hydroxylated to give cyclic alcohol **2**, aldehyde **3**, and acid **4** as the only products (eq 1). The authors reported that no rearranged alcohol product **6**, which would be produced from ring opening of the intermediate radical **5** (eq 2), was observed at a detection limit of at least 1 part in 100 and that compound **6** was stable to the reaction conditions.

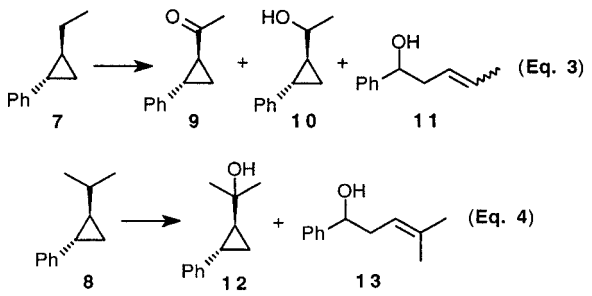
* Address correspondence to: Prof. Martin Newcomb. Tel: (313)-577-2782. Fax: (313)-577-8822. E-mail: men@chem.wayne.edu.
[†] Wayne State University.
[‡] University of Illinois.

[1] Abbreviations: CPO, chloroperoxidase; P450, cytochrome P450; MMO, methane monooxygenase; fs, femtosecond; HRMS, high-resolution mass spectrometry; ether, diethyl ether; LAH, lithium aluminum hydride; LHMDS, lithium bis(trimethylsilyl)amide.

S0893-228x(98)00029-0 CCC: $15.00
Published on Web 06/19/1998



The mechanistic study of CPO hydroxylation of probe **1** provided interesting results which are, nonetheless, not consistent with the kinetics of ring opening of radical **5**. The rate constant for rearrangement of this radical is so great ($k_r = 3 \times 10^{11}$ s^{-1} at ambient temperature) (*13*) that some rearranged product should have been observed even if **5** was only a component of the transition-state structure; this point is developed in the discussion below. In light of previous results using probe **1** in studies of the mechanisms of hydroxylation by P450 enzymes (*14*, *15*) and by methane monooxygenase (MMO) systems (*16*), we have reinvestigated the CPO hydroxylation of **1** in order to resolve this apparent dichotomy and to attempt to uncover mechanistic similarities between CPO hydroxylation reactions and those of P450 and MMO enzymes. We have also studied the CPO hydroxylation of two other hypersensitive radical probes, **7** and **8** (eqs 3 and 4), which have not previously been used to study enzymatic processes. The selection of probe **7**, previously used in a study of Gif oxidations (*17*), and probe **8** was based on the premise that oxidation at their cyclopropylcarbinyl positions would produce the analogous cyclic products (**9** and **10**, and **12**, respectively) as well as their ring-opened alcohols (**11** and **13**, respectively). We report that, whereas all three probes are oxidized by CPO, the rearranged alcohols **6**, **11**, and **13** are not stable to the reaction conditions. A very short maximum lifetime for a putative radical intermediate in CPO hydroxylation can be calculated from the results, but unequivocal conclusions regarding an insertion mechanism for hydroxylation cannot be made.



Experimental Procedures

Caution: *All synthetic reactions should be performed in properly ventilated hoods using published procedures for safe handling.*

General Methods and Materials. CPO from *C. fumago* was provided by Chirazyme. Commercially available starting materials and reagents were purchased from either Sigma or Aldrich Chemical Co. and were used as received. All moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Tetrahydrofuran (THF) and diethyl ether (ether) were distilled under a nitrogen atmosphere over sodium and benzophenone ketyl. Methylene chloride was distilled under a nitrogen atmosphere over phosphorus pentoxide. All substrates subjected to CPO oxidation were greater than 98% pure as determined by GC analysis.

Analytical Methods. NMR spectra[2] were acquired on a Varian Gemini 300 spectrometer. Quantitative GC analyses were performed with a Varian 3400 chromatograph equipped with a flame-ionization detector on a 15-m × 0.54-mm bonded phase Carbowax column (Alltech). GC/MS analyses were performed with a Hewlett-Packard model 5890 chromatograph interfaced to a Hewlett-Packard model 5971 mass selective detector on a 30-m × 0.25-mm capillary bonded phase Carbowax column (Alltech). HRMS (high-resolution mass spectrometry) analyses were performed by the Central Instrumentation Facility at Wayne State University (Detroit, MI). Melting points were determined using a Unimelt capillary melting point apparatus (Thomas-Hoover) and are uncorrected. Radial chromatography was performed on a Chromatotron model 7294T (Harrison Research Corp.) using plates coated with TLC grade silica gel with gypsum binder and fluorescent indicator (Merck).

Enzymatic Oxidation Reactions. The enzymatic oxidations of the substrates were performed as previously reported with the exception that the substrate was added without the aid of acetone (*18*). In a typical hydroxylation reaction, 1 mg (25 nmol) of CPO was dissolved in 4.5 mL of 100 mM citrate buffer (pH 5.0) in a 5-mL flask fitted with a magnetic stirrer and kept in a constant-temperature bath at 25 °C. The substrate (50 μmol) was added either neat or as a buffer solution, and this mixture was allowed to equilibrate for 10 min. The reaction was initiated by the addition of 0.75% hydrogen peroxide at a rate of 0.1 mL/h via a syringe pump. After a total of 0.5 mL of hydrogen peroxide solution had been added, the reaction mixture was extracted with CH_2Cl_2 (3 × 2 mL).

The combined organic phase was dried over anhydrous $MgSO_4$ and filtered through a plug of glass wool. An internal standard was added, and the solution was concentrated under a slow stream of nitrogen at room temperature to a final volume of approximately 0.2 mL. Quantitation of the product ratios was performed by GC, and product identities were determined by GC/MS by comparison with authentic samples using selected ion monitoring. Carboxylic acid **4** was analyzed as its methyl ester; thus, samples were treated with an ethereal solution of CH_2N_2 and allowed to stand in a fume hood overnight before analysis.

Control experiments to determine the stability of the rearranged products were performed as above with samples of the parent probe doped with small amounts of the putative alcohol product.

Methyl (*trans*-2-Phenylcyclopropyl)acetate (14). To a solution of (*trans*-2-phenylcyclopropyl)acetic acid (*13*) (6.00 g, 34.0 mmol) in CH_2Cl_2 (100 mL) was added a solution of CH_2N_2 in ether (*19*) until a slight yellow color persisted. The solution was stirred for 16 h and then was concentrated in vacuo. The crude product was chromatographed on silica gel (10% ethyl acetate in hexanes) to afford **14** (6.02 g, 31.6 mmol, 93%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.84 (1H, dt, $J_1 = 9.3$ Hz, $J_2 = 5.4$ Hz), 1.02 (1H, dt, $J_1 = 9.0$ Hz, $J_2 = 5.4$ Hz), 1.34−1.45 (1H, m), 1.77 (1H, dt, $J_1 = 9.3$ Hz, $J_2 = 5.1$ Hz), 2.31−2.51 (2H, m), 3.70 (3H, s), 7.07−7.30 (5H, m).

2-(*trans*-2-Phenylcyclopropyl)ethanol (15). To a solution of **14** (0.82 g, 4.31 mmol) in THF (25 mL) at 0 °C was added lithium aluminum hydride (LAH) (0.40 g, 10.5 mmol). The mixture was stirred under a nitrogen atmosphere for 2 h, and the reaction was quenched by the sequential addition of water (0.4 mL), 15% aqueous NaOH solution (0.4 mL), and water (1.2 mL). The resulting suspension was stirred at room temperature for an additional hour and then filtered and concentrated in vacuo. The crude product was purified by radial chromatography (40% ethyl acetate in hexanes) to afford **15** (0.56 g, 3.45 mmol, 80%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.83

[2] Compounds that were previously reported in the literature but were prepared by different methods and compounds which were isolated as mixtures of diastereomers were not necessarily characterized by ^{13}C NMR or HRMS.

(1H, ddd, $J_1 = 8.7$ Hz, $J_2 = 5.4$ Hz, $J_3 = 4.5$ Hz), 0.94 (1H, dt, $J_1 = 8.4$ Hz, $J_2 = 4.8$ Hz), 1.04−1.15 (1H, m), 1.46 (1H, bs), 1.58−1.75 (3H, m), 3.77 (2H, dd, $J_1 = 10.5$ Hz, $J_2 = 6.3$ Hz), 7.03−7.07 (2H, m), 7.14 (1H, tt, $J_1 = 6.9$ Hz, $J_2 = 1.2$ Hz), 7.22−7.28 (2H, m). ^{13}C NMR ($CDCl_3$): δ 15.6, 20.2, 22.8, 37.3, 62.8, 125.4, 125.6 (2C), 128.3 (2C), 143.4. HRMS: calcd for $C_{11}H_{14}O$, 162.1045; found, 162.1045.

(*trans*-2-Phenylcyclopropyl)ethane (7). A solution of **15** (0.41 g, 2.53 mmol) in THF (25 mL) under a nitrogen atmosphere was cooled to −30 °C. To this were added sequentially via syringe triethylamine (0.75 mL, 5.38 mmol) and methanesulfonyl chloride (0.25 mL, 3.23 mmol). The mixture was stirred at −30 °C for 30 min and then cooled to −78 °C. A solution of $LiBEt_3H$ (1.0 M in THF, 10.0 mL, 10.0 mmol) was added via syringe. The mixture was allowed to warm slowly to room temperature and was stirred for 16 h. The reaction was quenched by the addition of 30% H_2O_2 solution (5 mL) and 15% aqueous NaOH solution (5 mL). The resulting mixture was heated at reflux for 1 h and then cooled to room temperature. The organic layer was separated, and the aqueous layer was extracted with ether (3 × 25 mL). The combined organic phase was washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo at 0 °C. The crude product was purified by radial chromatography (pentane) to afford **7** (0.24 g, 1.64 mmol, 65%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.85 (1H, ddd, $J_1 = 8.7$ Hz, $J_2 = 5.7$ Hz, $J_3 = 4.8$ Hz), 0.96 (1H, dt, $J_1 = 8.4$ Hz, $J_2 = 5.1$ Hz), 1.06−1.13 (1H, m), 1.17 (3H, t, $J = 7.5$ Hz), 1.44−1.55 (1H, m), 1.49 (2H, q, $J = 7.2$ Hz), 1.71 (1H, dt, $J_1 = 8.7$ Hz, $J_2 = 4.5$ Hz), 7.13−7.24 (3H, m), 7.30−7.36 (2H, m). ^{13}C NMR ($CDCl_3$): δ 13.6, 16.1, 23.1, 25.6, 27.6, 125.2, 125.7 (2C), 128.3 (2C), 144.2. HRMS: calcd for $C_{11}H_{14}$, 146.1096; found, 146.1099.

2-(*trans*-2-Phenylcyclopropyl)propane (8). A solution of **14** (1.30 g, 6.83 mmol) in THF (50 mL) under a nitrogen atmosphere was cooled to −78 °C. To this was added a solution of lithium bis(trimethylsilyl)amide (LHMDS) (1.0 M in THF, 7.0 mL, 7.00 mmol). After 15 min, iodomethane (1.3 mL, 20.2 mmol) was added. The mixture was allowed to warm gradually to room temperature, and after 16 h, the reaction mixture was poured into saturated, aqueous NH_4Cl solution (100 mL) and extracted with ether (3 × 50 mL). The combined organic phase was washed with water (75 mL) and brine (75 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was chromatographed on silica gel (10% ethyl acetate in hexanes) to afford a mixture of diastereomers of methyl 2-(*trans*-2-phenylcyclopropyl)propionate (0.58 g, 2.84 mmol, 42%) as a clear, colorless oil.

To a solution of the above mixture of esters (0.58 g, 2.84 mmol) in THF (50 mL) at 0 °C was added LAH (0.20 g, 5.27 mmol). The mixture was stirred under a nitrogen atmosphere for 2 h, and the reaction was quenched by the sequential addition of water (0.2 mL), 15% aqueous NaOH solution (0.2 mL), and water (0.6 mL). The resulting suspension was stirred at room temperature for an additional hour and then filtered. Concentration of the filtrate in vacuo gave crude product which was purified by radial chromatography (40% ethyl acetate in hexanes) to afford 2-(*trans*-2-phenylcyclopropyl)-1-propanol (0.36 g, 2.04 mmol, 72%) as a clear, colorless oil. GC analysis showed this to be a 3:1 mixture of diastereomers. MS: calcd for $C_{12}H_{16}O$, 176; $(M)^+$ found at $m/z = 176$ for each isomer.

A solution of the above alcohols (0.35 g, 1.99 mmol) in THF (15 mL) under a nitrogen atmosphere was cooled to −30 °C. To this was added sequentially via syringe triethylamine (0.50 mL, 3.59 mmol) and methanesulfonyl chloride (0.18 mL, 2.33 mmol). The mixture was stirred at −30 °C for 30 min and then cooled to −78 °C. A solution of $LiBEt_3H$ (1.0 M in THF, 10.0 mL, 10.0 mmol) was added via syringe. The mixture was allowed to warm slowly to room temperature and was stirred for 16 h. The reaction was quenched by the addition of 30% H_2O_2 solution (5 mL) and 15% aqueous NaOH solution (5 mL). The resulting mixture was heated at reflux for 1 h and then cooled to room temperature. The organic layer was separated, and the aqueous layer was extracted with ether (3 × 25 mL). The combined organic phase was washed with brine (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo at 0 °C. The crude product was purified by radial chromatography (pentane) to afford **8** (0.19 g, 1.19 mmol, 60%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.76−0.88 (3H, m), 1.01−1.16 (1H, m), 1.02 (3H, d, $J = 4.5$ Hz), 1.03 (3H, d, $J = 3.9$ Hz), 1.66 (1H, dt, $J_1 = 8.7$ Hz, $J_2 = 4.8$ Hz), 7.05−7.16 (3H, m), 7.22−7.27 (2H, m). ^{13}C NMR ($CDCl_3$): δ 15.1, 21.8, 22.1, 22.4, 31.7, 33.5, 125.1, 125.7 (2C), 128.1 (2C), 144.1. HRMS: calcd for $C_{12}H_{16}$, 160.1252; found, 160.1255.

1-(*trans*-2-Phenylcyclopropyl)ethanone (9). A solution of *N*-methoxy-*N*-methyl-*trans*-2-phenylcyclopropanecarboxamide (*20*) (6.10 g, 29.7 mmol) in ether (250 mL) under a nitrogen atmosphere was cooled to 0 °C. To this was added a solution of methylmagnesium bromide (3.0 M in THF, 10.0 mL, 30.0 mmol). After 6 h, the reaction was quenched by pouring it into saturated aqueous NH_4Cl solution (250 mL), and then the mixture was extracted with ether (3 × 150 mL). The combined organic phase was washed with water (200 mL) and brine (200 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was chromatographed on silica gel (15% ethyl acetate in hexanes) to afford **9** (4.08 g, 25.5 mmol, 86%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.38 (1H, ddd, $J_1 = 8.4$ Hz, $J_2 = 6.9$ Hz, $J_3 = 4.5$ Hz), 1.68 (1H, ddd, $J_1 = 9.3$ Hz, $J_2 = 5.4$ Hz, $J_3 = 4.2$ Hz), 2.22 (1H, ddd, $J_1 = 8.4$ Hz, $J_2 = 5.4$ Hz, $J_3 = 4.2$ Hz), 2.31 (3H, s), 2.52 (1H, ddd, $J_1 = 9.0$ Hz, $J_2 = 6.6$ Hz, $J_3 = 4.2$ Hz), 7.07−7.11 (2H, m), 7.18−7.32 (3H, m). ^{13}C NMR ($CDCl_3$): δ 19.1, 28.9, 30.7, 32.8, 126.0 (2C), 126.5, 128.5 (2C), 140.3, 206.5. MS: calcd for $C_{11}H_{12}O$, 160; $(M)^+$ found at $m/z = 160$.

1-(*trans*-2-Phenylcyclopropyl)ethanol (10). To a solution of **9** (1.02 g, 6.37 mmol) in THF (30 mL) at 0 °C was added LAH (0.25 g, 6.59 mmol). The mixture was stirred under a nitrogen atmosphere for 2 h, and the reaction was quenched by the sequential addition of water (0.25 mL), 15% aqueous NaOH solution (0.25 mL), and water (0.75 mL). The resulting suspension was stirred at room temperature for 1 h and then filtered. The filtrate was concentrated in vacuo, and the resulting crude product was purified by radial chromatography (20% ethyl acetate in hexanes) to afford **10** (0.98 g, 6.04 mmol, 95%) as a clear, colorless oil. GC analysis showed this to be a 2:1 mixture of diastereomers. ^{1}H NMR ($CDCl_3$): δ 0.87−1.03 (2H, m), 1.24−12.31 (1H, m), 1.32 and 1.34 (3H, d, $J = 6.0$ Hz), 1.57 and 1.59 (1H, bs), 1.81 and 1.90 (1H, dt, $J_1 = 9.3$ Hz, $J_2 = 5.1$ Hz), 3.34−3.44 (1H, m), 7.05−7.09 (2H, m), 7.12−7.19 (1H, m), 7.23−7.29 (2H, m). MS: calcd for $C_{11}H_{14}O$, 162; $(M)^+$ found at $m/z = 162$ for each isomer.

(*E*)- and (*Z*)-1-Phenyl-3-penten-1-ol (11). A solution of **10** (0.43 g, 2.65 mmol) in THF (25 mL) under a nitrogen atmosphere was cooled to −30 °C. To this were added sequentially via syringe triethylamine (0.75 mL, 5.38 mmol) and methanesulfonyl chloride (0.25 mL, 3.23 mmol). After 1 h, water (15 mL) was added, and the reaction mixture was heated at reflux for 2 h. After cooling to room temperature, the reaction mixture was extracted with ether (3 × 25 mL). The combined organic phase was washed with saturated, aqueous $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over $MgSO_4$, and filtered. Concentration of the filtrate in vacuo gave crude product which was purified by radial chromatography (15% ethyl acetate in hexanes) to afford **11** (0.28 g, 1.73 mmol, 65%) as a clear, colorless oil. GC analysis showed this to be a 5:1 mixture of isomers. ^{1}H NMR ($CDCl_3$): δ 1.59−1.71 (3H, m), 2.01 (1H, bs), 2.34−2.63 (2H, m), 4.68 and 4.72 (1H, dd, $J_1 = 7.8$ Hz, $J_2 = 4.8$ Hz), 5.37−5.49 (1H, m), 5.55−5.68 (1H, m), 7.23−7.37 (5H, m). MS: calcd for $C_{11}H_{14}O$, 162; $(M)^+$ found at $m/z = 162$ for each isomer.

2-(*trans*-2-Phenylcyclopropyl)-2-propanol (12). A solution of **9** (1.71 g, 10.7 mmol) in ether (50 mL) under a nitrogen atmosphere was cooled to 0 °C. To this was added a solution of methylmagnesium bromide (3.0 M in THF, 4.0 mL, 12.0 mmol). After 6 h, the reaction was quenched by pouring it into saturated, aqueous NH_4Cl solution (100 mL), and the resulting

mixture was extracted with ether (3 × 75 mL). The combined organic phase was washed with water (75 mL) and brine (75 mL), dried over $MgSO_4$, and filtered. Concentration of the filtrate in vacuo gave crude product that was chromatographed on silica gel (20% ethyl acetate in hexanes) to afford **12** (1.49 g, 8.45 mmol, 79%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.85 (1H, dt, $J_1 = 8.7$ Hz, $J_2 = 4.8$ Hz), 1.01−1.07 (1H, m), 1.19 (1H, s), 1.23−1.30 (1H, m), 1.30 (6H, s), 1.94 (1H, dt, $J_1 = 8.7$ Hz, $J_2 = 5.1$ Hz), 7.07−7.18 (3H, m), 7.62 (2H, tt, $J_1 = 7.8$ Hz, $J_2 = 2.1$ Hz). ^{13}C NMR ($CDCl_3$): δ 11.6, 19.2, 28.9, 29.1, 34.1, 69.4, 125.4, 126.0 (2C), 128.3 (2C), 143.2. MS: calcd for $C_{12}H_{16}O$, 176; $(M)^+$ found at $m/z = 176$.

4-Methyl-1-phenyl-3-penten-1-ol (13). A solution of 2-phenyl-1,3-dithiane (2.95 g, 15.0 mmol) in THF (50 mL) under a nitrogen atmosphere was cooled to −20 °C, and BuLi (2.5 M in hexanes, 6.0 mL, 15.0 mmol) was added. After 1 h, the solution was cooled to −78 °C, and 1-bromo-3-methyl-2-butene (2.6 mL, 22.5 mmol) was added. The mixture was allowed to warm slowly to room temperature with stirring and was stirred for a total of 16 h. The reaction was quenched by pouring the mixture into saturated, aqueous NH_4Cl solution (100 mL). The mixture was extracted with ether (3 × 50 mL). The combined organic phase was washed with brine (75 mL), dried over $MgSO_4$, and filtered. Concentration in vacuo gave the crude product that was chromatographed on silica gel (10% ethyl acetate in hexanes) to afford 2-(3-methyl-2-butenyl)-2-phenyl-1,3-dithiane (3.41 g, 12.9 mmol, 86%) as a yellow oil.

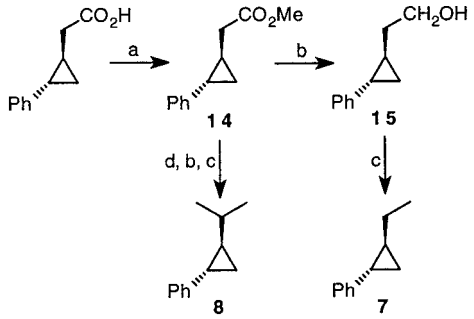
To a suspension of $CaCO_3$ (0.25 g, 2.49 mmol) and $Hg(ClO_4)_2 \cdot xH_2O$ (0.87 g, 2.18 mmol) in methanol (15 mL) was added a solution of the above dithiane (0.52 g, 1.97 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 10 min and then filtered through Celite. Solvent was removed in vacuo, and the crude product was chromatographed on silica gel (10% ethyl acetate in hexanes) to afford 4-methyl-1-phenyl-3-penten-1-one (0.28 g, 1.62 mmol, 82%) as a clear, colorless oil.

A solution of the above ketone (0.26 g, 1.49 mmol) in THF (25 mL) was cooled to 0 °C under a nitrogen atmosphere, and LAH (0.10 g, 2.64 mmol) was added. The mixture was stirred for 2 h, and the reaction was quenched by the sequential addition of water (0.1 mL), 15% aqueous NaOH solution (0.1 mL), and water (0.3 mL). The resulting mixture was stirred for 1 h and then filtered. Concentration of the filtrate in vacuo gave crude product that was purified by radial chromatography (10% ethyl acetate in hexanes) to afford **13** (0.25 g, 1.42 mmol, 95%) as a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.61 (3H, s), 1.73 (3H, s), 1.81 (1H, s), 2.37−2.55 (2H, m), 4.68 (1H, dd, $J_1 = 7.8$ Hz, $J_2 = 5.4$ Hz), 5.14−5.21 (1H, m), 7.24−7.38 (5H, m). MS: calcd for $C_{12}H_{16}O$, 176; $(M)^+$ found at $m/z = 176$.

Results

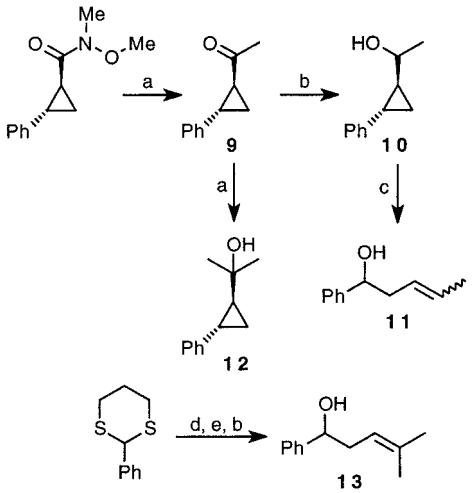
Syntheses of Probes and Putative Oxidation Products. The syntheses of probe **1** and its possible oxidation products have been described (*15*, *16*). Compounds **7** and **8** were reported previously (*21*, *22*), but the syntheses involved carbenoid additions to alkenes and did not give products in high diastereomeric purity. Therefore alternative syntheses, divergent from a common intermediate, were designed (Scheme 1) in which high diastereomeric purity was available in the starting material, *trans*-2-phenylcyclopropanecarboxylic acid. This acid was homologated to the corresponding (*trans*-2-phenylcyclopropyl)acetic acid by a literature method (*13*). Conversion of the acetic acid derivative to its methyl ester (**14**) (*23*) was achieved by treatment with diazomethane. Ester **14** proved to be a versatile intermediate for the synthesis of probes **7** and **8**. Reduction of **14** with LAH afforded alcohol **15** (*24*), which was reduced to hydrocarbon probe **7** by the sequence of methanesulfonation followed by hydride reduction. Ester **14** was converted

Scheme 1. Synthesis of Probe Substrates 7 and 8[a]



[a] (a) CH_2N_2, ether; (b) LAH, THF, 0 °C; (c) (i) MsCl, Et_3N, THF, −30 °C, (ii) $LiEt_3BH$, −78 °C; (d) (i) LHMDS, THF, −78 °C, (ii) MeI.

Scheme 2. Synthesis of Putative Oxidation Products 9−13[a]

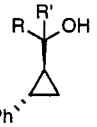
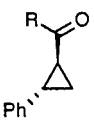
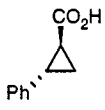
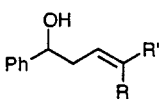


[a] (a) MeMgBr, ether, 0 °C; (b) LAH, THF, 0 °C; (c) (i) MsCl, Et_3N, THF, −30 °C, (ii) water reflux; (d) (i) BuLi, THF, −20 °C, (ii) prenyl bromide; (e) $Hg(ClO_4)_2 \cdot xH_2O$, $CaCO_3$, MeOH, CH_2Cl_2.

to probe **8** by a four-reaction sequence. It was first methylated at the α-position, to provide the proper carbon framework, by conversion to its lithium enolate by treatment with LHMDS and reaction of the enolate with iodomethane. This sequence gave a mixture of propionate diastereomers that were not separated but used directly since reduction of the ester group to a methyl group renders the cyclopropylcarbinyl position achiral. LAH reduction of the mixture of propionates gave the corresponding propanols as a 3:1 mixture of diastereomers. Conversion of the mixture of propanols to probe **8** was performed by the same two-step sequence as employed with **7**.

Synthesis of the putative oxidation products from **7** and **8** also utilized *trans*-2-phenylcyclopropanecarboxylic acid as the starting material (Scheme 2). The Weinreb amide of this acid (*20*) was the key intermediate for the formation of the cyclic products expected from both **7** and **8**. Treatment of this amide with methylmagnesium bromide afforded ethanone **9** (*25*). Reduction of **9** with LAH afforded a 2:1 mixture of alcohols **10** (*26*). Methanesulfonation of **10** followed by hydrolysis afforded a 5:1 mixture of ring-opened alcohols **11** (*27*). The cyclic hydroxylation product **12** (*28*) was prepared by methylmagnesium bromide addition to **9**. The acyclic hydroxylation product **13** proved to be rather difficult to synthesize. Attempts to prepare **13** by methods analogous to

Table 1. Products from CPO Oxidations with 25 nmol of Enzyme[a]

Substrate / Probe Reactions[b]					turnover[c]
1 (R, R' = H)	1.0	1.95	0.23	0	20
7 (R = Me, R' = H)	1.0	0		0	5
8 (R, R' = Me)	1.0			0	0.2
Control Reactions[d]					
6 (0.153 μmol)				<0.001 μmol	
6 (0.543 μmol)				<0.001 μmol	
1 + **6** (0.064 μmol)	1.0	0.6	n.d.[e]	<0.001 μmol	
1 + **6** (0.644 μmol)	1.0	1.0	n.d.[e]	0.038 μmol	

[a]50 μmol of probe substrate was employed; the amounts of alcohol **6** used are listed. [b]Relative yields from probes (average of 2 runs) are reported. [c]Number of enzyme turnovers. [d]Absolute yields of **6** are reported. [e]Not determined.

those used to prepare **11** proved futile, but it was available from a three-step sequence starting from 2-phenyl-1,3-dithiane by modification of a literature method (*29*). Alkylation of the dithiane with prenyl bromide provided the proper carbon skeleton. Removal of the dithioacetal proved to be difficult, but experiments showed that mercuric perchlorate was the reagent of choice for conversion to the corresponding ketone. This ketone was reduced with LAH to afford **13**.

CPO Oxidation Reactions. Probes **1**, **7**, and **8** were oxidized by CPO (Table 1). A dramatic steric effect in the hydroxylation reactions was apparent with the yields of oxidized products dropping precipitously as the steric bulk of the probe increased. This is unlike the case with the isozyme P450 2B1 where the same series of probes was found to react with only a minor decrease in yield with increase in steric bulk.[3] Nevertheless, hydroxylation products were identified from each of the probes. In the case of probe **1**, alcohol **2**, aldehyde **3**, and acid **4** were produced, but no ring-opened product **6** was observed. These results essentially reproduce those reported by Zaks and Dodds (*11*).

Reactions of probe **7** gave alcohol **10** (2.5:1 mixture of diastereomers) as the only identifiable products, and reactions of probe **8** gave only alcohol **12**. Due to the low yields of hydroxylation products and the presence of small amounts of unidentified volatile components from the enzyme reactions, the quantitative sensitivity of the analyses was compromised. However, the identities of products **10** and **12** were confirmed by GC/MS comparison to the respective authentic samples. In the case of probe **7**, the absence of ketone **9** and ring-opened alcohol **11** was firmly established by the absence of detectable GC peaks corresponding to those of the authentic samples. In a similar manner, no detectable GC peak corresponding to that of authentic ring-opened alcohol **13** was present from oxidation studies with probe **8**.

[3] Toy, P. H., Newcomb, M., and Hollenberg, P. F. Unpublished results.

Zaks and Dodds reported that ring-opened alcohol **6** from hydroxylation of probe **1** was stable to the enzyme oxidation conditions (*11*). We found in control experiments, however, that alcohol **6** was not stable (Table 1). CPO oxidations of small amounts of authentic **6** returned none of the alcohol. These experiments were designed such that the amount of alcohol **6** employed was approximately equal to the amount of ring-opened hydroxylation product expected if the ratio of unrearranged to rearranged products was similar to that observed in P450 hydroxylations of probe **1** (*14, 15*); specifically, the amounts of **6** were 0.3% and 1% of the amount of probe **1**. Further, when the CPO oxidation of probe **1** was repeated with a sample intentionally contaminated with 0.1% of alcohol **6**, none of the acyclic alcohol was observed by GC analysis. CPO oxidation of a sample of probe **1** containing 1.3% of alcohol **6** did yield a product mixture containing some **6**, but quantitative GC analysis showed that only about 5% of the initial amount of **6** was returned from this reaction. That the fully competent CPO enzyme system was responsible for destruction of alcohol **6** was demonstrated by control experiments in which **6** was subjected to enzyme in buffer (without H_2O_2) and to hydrogen peroxide in buffer (without enzyme); in both cases, **6** was returned quantitatively. In the discussion, we present a rationalization of the difference between our control experiments with **6** and the result reported by Zaks and Dodds (*11*).

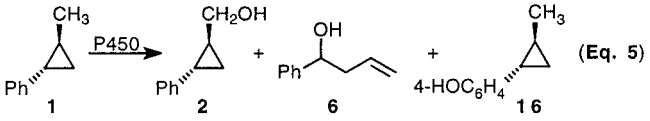
Another control experiment was designed to determine the stability of cyclic alcohol **2** toward CPO oxidation conditions. The production of aldehyde **3** and acid **4** in reactions of probe **1** clearly demonstrates that overoxidation occurs, but we wished to determine whether a significant portion of the initially formed alcohol **2** was further processed to unknown products. A sample of probe **1** doped with 0.2% of the perdeuteriophenyl analogue of alcohol **2** was subjected to CPO oxidation, and products **2** and **3** were analyzed by GC/MS. The total yield of **2** and **3** was about 1% of the initial amount of **1**,

consistent with the amounts of these products found from probe **1**. Alcohol **2** had a d_0/d_5 ratio of 2.5:1.0, and aldehyde **3** had a d_0/d_5 ratio of 1.4:1.0. The internal consistency of these values is excellent; because the average concentration of **2**-d_0 will be about one-half of the final concentration, the ratio of undeuterated to deuterated **2** should be about twice as great as the ratio for **3**. The internally consistent ratios of d_0- to d_5-products and the fact that the **2**-d_5 initially present was largely recovered as **2** or **3** demonstrate that little of the initially produced alcohol **2** was converted to unidentifiable products.

The observed instability of the ring-opened alcohol **6** was the impetus for attempting CPO oxidations of the methyl- and dimethyl-substituted analogues of probe **1**. Our hope was that the ring-opened alcohol products from probes **7** and **8** would be more stable than alcohol **6**. Unfortunately, control experiments demonstrated that they were not. Thus, CPO oxidations of small amounts of authentic alcohols **11** and **13** returned no detectable amounts of these alcohols.

Discussion

Probe **1** was previously used in mechanistic studies of hydroxylation reactions by microsomal cytochrome P450 from livers of phenobarbital-treated rats and the purified isozyme P450 2B1 (*14*, *15*) and by the MMO hydroxylase enzymes from *Methylococcus capsulatus* (Bath) and *Methylosinus trichosporium* OB3b (*16*). In those studies, probe **1** was oxidized to the cyclic alcohol **2**, ring-opened alcohol product **6**, and phenol **16** (eq 5). Two notable differences between CPO oxidation of **1** and oxidations by the other enzymes studied are the overoxidation of alcohol **2** by CPO, which has not been observed previously, and the absence of significant amounts of phenol **16** in the CPO oxidations.



The absence of phenol **16** is noteworthy. This could result from steric effects that prevent the appropriate orientation of the substrate in the CPO active site, and the dramatic reduction in the amounts of hydroxylation observed for the series of probes **1**, **7**, and **8**, which was not observed in P450 2B1 hydroxylations of the same series,[3] suggests that steric effects are important. It is possible, however, that the lack of production of phenol **16** is much more important from a mechanistic perspective. Vaz et al. recently reported that replacement of active-site threonine by alanine in two P450 isozymes results in an increase in epoxidation relative to allylic hydroxylation in oxidations of alkenes (*30*). This result indicates that two active electrophilic oxidants exist in P450: a hydroperoxy−iron species that is long-lived when threonine is not present in the active site and preferentially epoxidizes and the ultimate oxidant, taken to be iron−oxo, that preferentially hydroxylates. For probe **1**, phenol **16** is the "epoxidation" product, and we have found in unpublished work[4] that the ratio of phenol **16** to alcohols **2** and **6** increases for the same two P450 mutants reported by Vaz et al. (*30*). In the CPO oxidation reactions, a hydroperoxy−iron species will not be produced because the oxidizing equivalents are supplied by hydrogen peroxide, and the absence of phenol **16** is consistent with the premise that two electrophilic oxidants are present in P450 and the latter, the iron−oxo species, effects hydroxylations more efficiently than epoxidations.

Because alcohol **6** was shown to be stable in oxidations catalyzed by the P450 and MMO enzymes, the ratio of unrearranged alcohol product **2** to rearranged alcohol product **6** could be used with the known rate constant for ring opening of cyclopropylcarbinyl radical **5** to calculate a maximum lifetime for putative radical intermediates in the hydroxylation reactions. The lifetimes thus obtained for the MMO hydroxylase enzymes were <100 fs and ca. 150 fs for *M. capsulatus* (Bath) and *M. trichosporium* OB3b, respectively (*16*). For the P450 enzyme, the maximum lifetime for the putative radical intermediate calculated from studies with probe **1** was about 800 fs (*14*, *15*), but subsequent mechanistic studies of isozyme P450 2B1 employing other hypersensitive radical probes have suggested that an interfering cationic rearrangement occurs in P450 oxidations of probe **1** and that the "radical" has a much shorter lifetime, only about 70 fs (*6*, *7*).

The utility of the calibrated hypersensitive probes is apparent from the above results. An intermediate cannot have a lifetime of only 100 fs at ambient temperature because the maximum translation of a second-row atom in this period of time is only about 0.2 Å. Thus, the short periods have been ascribed to the "lifetimes" of the transition structures for insertion reactions that occur in a nonsynchronous manner. Further, the small distance over which an atom can translate in such a period has led to the conclusion that the carbon and oxygen atoms were nearly within bonding distance of one another at the instant of the insertion reaction.

Given the structural similarities of the active sites of the P450 enzymes and CPO, it is reasonable to assume that the mechanisms of the hydroxylation reactions catalyzed by P450 and CPO are similar. Thus, the report by Zaks and Dodds (*11*) that CPO oxidation of probe **1** gave only unrearranged oxidation products and that the rearranged alcohol was stable was surprising. On the basis of the results of P450 hydroxylations of **1** and other hypersensitive probes, some rearranged alcohol product **6** should have been observed even if an insertion mechanism operated.

Unfortunately, we found that alcohol **6** is not stable in the presence of CPO when the amount of **6** present was similar to the amounts formed as products in P450 oxidations of **1** (*14*, *15*). Details of the control experiments that led to the conclusion that **6** was stable were not reported (*11*), but we assume that the reactions involved relatively large amounts of **6**. Our previous studies have demonstrated that terminal alkenes and alkynes alkylate the heme in CPO (*31*), and it is possible that, in the control studies by Zaks and Dodds, the CPO enzyme was destroyed rapidly and little of the test substrate was consumed. In fact, if the amounts of **6** in the control reactions were similar to the amounts of other substrates used in that study, it is possible that the CPO consumed less than 1% of the rearranged alcohol on the

[4] Toy, P. H., Newcomb, M., Vaz, A. D. N., and Coon, M. J. Unpublished results.

basis of our observation that about 0.6 μmol of **6** was destroyed in the presence of 25 nmol of CPO.

The instability of the rearranged oxidation products toward CPO oxidation conditions severely limits the quantitative aspects of this probe study. We have demonstrated that most of the unrearranged oxidation product **2** survives in a detectable form (as **2**, **3**, or **4**), so the amounts of these products found in the CPO oxidation reactions of probe **1** can be used as yields without corrections. Further, we found that, when rearranged alcohol **6** was initially added to the reaction in an amount approximately equal to the amounts of unrearranged products ultimately observed, a detectable amount of **6** was found at the end of the reaction. Therefore, it appears to be safe to conclude that the amount of **6** produced in CPO oxidation of probe **1** was less than the total amount of products **2**−**4**. Accordingly, using the rate constant for rearrangement of cyclopropylcarbinyl radical **5** of 3×10^{11} s^{-1} at the reaction temperature (*13*), one calculates that the lifetime of a putative radical intermediate from probe **1** must be less than 3 ps. Due to the small turnover values for probes **7** and **8**, experiments with the corresponding rearranged alcohol products similar to those performed with **1** were not deemed productive, but the detection of unrearranged alcohol products in both cases requires that "radical" lifetimes were short.[5]

The maximum lifetime of 3 ps for putative radical **5** is short enough to preclude a freely diffusing radical intermediate because diffusional rate constants are only on the order of 10^{10} s^{-1}, but it is not short enough to preclude an intermediate that is trapped in a very rapid ($k > 3 \times 10^{11}$ s^{-1}) first-order or pseudo-first-order followup reaction. Unfortunately, one cannot determine whether the "oxygen rebound" event in the stepwise mechanism for P450 hydroxylation could occur with such a large rate constant because such a chemical reaction (homolytic displacement by an alkyl radical of a hydroxy group bound to a metal ion) is not calibrated. Such a bimolecular reaction would have to proceed with diffusion-controlled rates, indicating that the activation energy for the chemical reaction is smaller than that for the diffusional process, to support the abstraction/rebound mechanism for hydroxylation by CPO.

In the broader context of the mechanisms of hydroxylations catalyzed by iron-containing enzymes, the CPO oxidation studies lend some support to the notion that a common insertion mechanism is involved rather than the two-step hydrogen abstraction/oxygen rebound mechanism. With limited hypersensitive probe results, one could argue that undefined "steric effects" in the enzyme's active site had profound effects on the kinetics of the radical rearrangement reactions and vitiated the probe results, but this position has no experimental support and becomes increasingly less likely as studies with new enzymes and new probes appear. At the present time, maximum lifetimes for "radical" intermediates in the subpicosecond range have been found in hydroxylation studies[3] (*6*−*9*, *14*, *15*) of nine calibrated hypersensitive radical probes containing an aryl-substituted cyclopropylcarbinyl motif by the isozyme P450 2B1, and hydroxylations by the P450 isozymes 2B4 and 2E1 give similar results.[4] In the case of MMO hydroxylations, "radical" lifetimes of less than 200 fs have been implicated in studies of two MMO hydroxylase enzymes with probe **1** (*16*), and importantly, the same short lifetimes were found in oxidations of chiral ethane by the same enzymes (*32*, *33*). The conservative estimate that the lifetime of a putative radical intermediate in CPO hydroxylation is less than 3 ps provides no evidence against an insertion pathway for hydroxylation.

Acknowledgment. This research was supported in part by grants from the National Institutes of Health (GM-48722 to M.N., GM-07768 to L.P.H.).

References

(1) Sundaramoorthy, M., Terner, J., and Poulos, T. L. (1995) The crystal structure of chloroperoxidase: a heme peroxidase-cytochrome P450 functional hybrid. *Structure* **3**, 1367−77.

(2) Franssen, M. C. R., and van der Plas, H. C. (1992) Haloperoxidases: Their properties and their use in organic synthesis. *Adv. Appl. Microbiol.* **37**, 41−99.

(3) Groves, J. T., and Han, Y.-Z. (1995) Models and Mechanisms of Cytochrome P450 Action. In *Cytochrome P450 Structure, Mechanism and Biochemistry*, 2nd ed. (Ortiz de Montellano, P. R., Ed.) pp 3−48, Plenum, New York.

(4) Guengerich, F. P., and Macdonald, T. L. (1990) Mechanisms of cytochrome P-450 catalysis. *FASEB J.* **4**, 2453−2459.

(5) Woggon, W. D., and Fretz, H. (1992) Mechanisms of cytochrome P-450 catalyzed oxidations. In *Advances in Detailed Reaction Mechanisms* (Coxon, J. M., Ed.) Vol. 2, pp 111−147, JAI Press, Greenwich, CT.

(6) Newcomb, M., Le Tadic, M. H., Putt, D. A., and Hollenberg, P. F. (1995) An incredibly fast apparent oxygen rebound rate constant for hydrocarbon hydroxylation by cytochrome P-450 enzymes. *J. Am. Chem. Soc.* **117**, 3312−3313.

(7) Newcomb, M., Le Tadic-Biadatti, M. H., Chestney, D. L., Roberts, E. S., and Hollenberg, P. F. (1995) A nonsynchronous concerted mechanism for cytochrome P-450 catalyzed hydroxylation. *J. Am. Chem. Soc.* **117**, 12085−12091.

(8) Toy, P. H., Dhanabalasingam, B., Newcomb, M., Hanna, I. M., and Hollenberg, P. F. (1997) A substituted hypersensitive radical probe for enzyme-catalyzed hydroxylations: Synthesis of racemic and enantiomerically enriched forms and application in a cytochrome P450-catalyzed oxidation. *J. Org. Chem.* **62**, 9114−9122.

(9) Newcomb, M., and Le Tadic-Biadatti, M.-H. (1997) Hypersenstive probing for radicals in cytochrome P450 hydroxylations. In *Free Radicals in Biology and the Environment* (Minisci, F., Ed.) pp 91−108, Kluwer Academic Publishers, Dordrecht, The Netherlands.

(10) McCarthy, M.-B., and White, R. E. (1983) Functional differences between peroxidase compound I and the cytochrome P-450 reactive oxygen intermediate. *J. Biol. Chem.* **258**, 9153−9158.

(11) Zaks, A., and Dodds, D. R. (1995) Chloroperoxidase-catalyzed asymmetric oxidations: Substrate specificity and mechanistic study. *J. Am. Chem. Soc.* **117**, 10419−10424.

(12) Miller, V. P., Tschirretguth, R. A., and Ortiz de Montellano, P. R. (1995) Chloroperoxidase-catalyzed benzylic hydroxylation. *Arch. Biochem. Biophys.* **319**, 333−340.

(13) Newcomb, M., Johnson, C. C., Manek, M. B., and Varick, T. R. (1992) Picosecond radical kinetics. Ring openings of phenyl substituted cyclopropylcarbinyl radicals. *J. Am. Chem. Soc.* **114**, 10915−10921.

(14) Atkinson, J. K., and Ingold, K. U. (1993) Cytochrome P450 hydroxylation of hydrocarbons: variation in the rate of oxygen rebound using cyclopropyl radical clocks including two new ultrafast probes. *Biochemistry* **32**, 9209−9214.

(15) Atkinson, J. K., Hollenberg, P. F., Ingold, K. U., Johnson, C. C., Le Tadic, M.-H., Newcomb, M., and Putt, D. A. (1994) Cytochrome P450-catalyzed hydroxylation of hydrocarbons: kinetic deuterium isotope effects for the hydroxylation of an ultrafast radical clock. *Biochemistry* **33**, 10630−10637.

(16) Liu, K. E., Johnson, C. C., Newcomb, M., and Lippard, S. J. (1993) Radical clock substrate probes and kinetic isotope effect studies of the hydroxylation of hydrocarbons by methane monooxygenase. *J. Am. Chem. Soc.* **115**, 939−947.

[5] The rate constants for ring openings of the cyclopropylcarbinyl radicals corresponding to **7** and **8** are (1−2) $\times$ 10^{11} s^{-1} at ambient temperature. Choi, S.-Y., Toy, P. H., and Newcomb, M. Unpublished results.

(17) Newcomb, M., Simakov, P. A., and Park, S.-U. (1996) Hypersensitive radical probe studies of Gif oxidations. *Tetrahedron Lett.* **37**, 819−822.
(18) Allain, E. J., Hager, L. P., Deng, L., and Jacobsen, E. N. (1993) Highly enantioselective epoxidation of disubstituted alkenes with hydrogen peroxide catalyzed by chloroperoxidase. *J. Am. Chem. Soc.* **115**, 4415−4416.
(19) Vogel, A. (1989) *Vogel's Textbook of Practical Organic Chemistry*, 5th ed., p 432, Longman Scientific and Technical, Essex, England.
(20) Rodriques, K. E. (1991) A novel route to cyclopropyl ketones, aldehydes, and carboxylic acids. *Tetrahedron Lett.* **32**, 1275−1278.
(21) Harada, T., Katsuhira, T., Hattori, K., and Oku, A. (1993) Stereoselective carbon−carbon bond forming reaction of 1,1-dibromocyclopropanes via 1-halocyclopropylzincates. *J. Org. Chem.* **58**, 2958−2965.
(22) Brookhart, M., Humphrey, M. B., Kratzer, H. J., and Nelson, G. O. (1980) Reactions of η-$C_5H_5(CO)_2FeCHC_6H_5^+$ with alkenes and alkynes. Observation of efficient benzylidene-transfer reactions. *J. Am. Chem. Soc.* **102**, 7802−7803.
(23) Perkins, M. J., Peynircioglu, N. B., and Smith, B. V. (1978) The preparation and rates of deprotonation of some cyclopropylcarbinyl ketones. *J. Chem. Soc., Perkin Trans. 2* 1025−1033.
(24) Yamaguchi, M., and Hirao, I. (1984) An intramolecular nucleophilic substitution of oxetanes. *Tetrahedron Lett.* **25**, 4549−4552.
(25) DePuy, C. H., Dappen, G. M., Eilers, K. L., and Klein, R. A. (1964) The chemistry of cyclopropanols. II. Synthetic methods. *J. Org. Chem.* **29**, 2813−2815.
(26) Charette, A. B., and Marcoux, J.-F. (1996) Spectroscopic characterization of (iodomethyl)zinc reagents involved in stereoselective reactions: Spectroscopic evidence that $IZnCH_2I$ is not $Zn(CH_2I)_2 + ZnI_2$ in the presence of an ether. *J. Am. Chem. Soc.* **118**, 4539−4549.
(27) Iqbal, J., and Joseph, S. P. (1989) Cobalt mediated regioreversed addition of but-2-eneyltributylstannane to aldehydes. *Tetrahedron Lett.* **30**, 2421−2422.
(28) Mathias, R., and Weyerstahl, P. (1979) Darstellung und eigenschaften von 1,1-diiod- und 1-iod-1-x-cyclopropanen. *Chem. Ber.* **112**, 3041−3053.
(29) Padwa, A., Rodriguea, R., Tohidi, M., and Fukunaga, T. (1983) Intramolecular cycloaddition reactions of diazoalkenes. A theoretical prognosis of nitrene type behavior. *J. Am. Chem. Soc.* **105**, 933−943.
(30) Vaz, A. D. N., McGinnity, D. F., and Coon, M. J. (1998) Epoxidation of olefins by cytochrome P450: Evidence from site-specific mutagenesis for hydroperoxo-iron as an electrophilic oxidant. *Proc. Natl Acad. Sci. U.S.A.* **95**, 3555−3560.
(31) Dexter, A. F., and Hager, L. P. (1995) Transient heme *N*-alkylation of chloroperoxidase by terminal alkenes and alkynes. *J. Am. Chem. Soc.* **117**, 817−818.
(32) Priestley, N. D., Floss, H. G., Froland, W. A., Lipscomb, J. D., Williams, P. G., and Morimoto, H. (1992) Cryptic stereospecificity of methane monooxygenase. *J. Am. Chem. Soc.* **114**, 7561−7562.
(33) Valentine, A. M., Wilkinson, B., Liu, K. E., Komar-Panicucci, S., Priestley, N. D., Williams, P. G., Morimoto, H., Floss, H. G., and Lippard, S. J. (1997) Tritiated chiral alkanes as substrates for soluble methane monooxygenase from *Methylococcus capsulatus* (Bath): probes for the mechanism of hydroxylation. *J. Am. Chem. Soc.* **119**, 1818−1827.

TX9800295