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Chloroperoxidase-Catalyzed Asymmetric Oxidations: Substrate Specificity and Mechanistic Study

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Abstract: The substrate specificity of chloroperoxidase from *Caldaromyces fumago* in a number of halide-independent reactions was investigated and the ability of this enzyme to perform benzylic hydroxylations with high enantioselectivity is revealed. The substrate repertoire of chloroperoxidase is expanded and the enantioselectivity data for synthetically useful epoxidations are reported. The enzyme epoxidizes straight chain aliphatic and cyclic *cis*-olefins in a highly stereoselective manner favoring small unsubstituted substrates in which the double bond is not more than two carbon atoms from the terminal. The epoxidation of short-chain prochiral terminal dienes proceeds with high diastereoselectivity and moderate enantioselectivity, yielding monoepoxides exclusively. Unsubstituted straight-chain terminal olefins seven carbons or longer are epoxidized poorly. Aliphatic and aromatic alcohols are efficiently oxidized to aldehydes and acids. The utilization of radical probe substrate *trans*-2-phenyl-1-methylcyclopropane revealed that the mechanism of chloroperoxidase-catalyzed hydroxylation is incompatible with the existence of a discrete radical intermediate and most likely proceeds via transfer of the oxygen atom from the high valent iron oxo intermediate directly to the substrate.

Introduction

Chloroperoxidase produced by the filamentous fungus *Caldariomyces fumago* is a hemoprotein that catalyzes H_2O_2-dependent oxidations of inorganic and organic substrates. The protein has been crystallized[1,2] and shown to exist in solutions at neutral pH as a monomer with a MW of 42 000. It is heavily glycosylated (~25−30% by weight) and contains one ferriprotoporphyrin IX prosthetic group per subunit.[1] The central nitrogens of the porphyrin provide the four equatorial ligands to the iron species; the nature of the fifth axial ligand has been thoroughly investigated by EXAFS spectroscopy and by resonance Raman studies and shown to be sulfide derived from cysteine.[3]

The scope of reactions catalyzed by the chloroperoxidase is very broad[4] and can be divided into two categories, based on the dependence on halide ion. The halide-dependent reactions, such as halogenation of β-diketones[2] and halohydration[5−7] of olefins, are catalyzed by an acidic form of the enzyme. The halide-independent reactions include epoxidations of alkenes,[8−11] oxidations of sulfides to sulfoxides,[12] alcohols to aldehydes,[5,13]

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and amines to nitroso compounds,[14] disproportionations of peroxides,[15] peroxidative coupling of aromatics,[8] and N-dealkylations of alkylamines,[16] all of which are catalyzed by a neutral form of the enzyme.

Although the halogenation reaction was discovered several decades ago, its mechanism still remains controversial. It is generally agreed[17] that the first step in the catalytic cycle is a two-electron oxidation of the resting high-spin Fe^{III} enzyme by H_2O_2 leading to the formation of the species commonly called compound **I**. Although the formal oxidation state of compound **I** is Fe^{V}, experimental evidence indicates that it is likely to be an iron(IV) porphyrin radical cation in which an unpaired electron in the tetrapyrrole macrocycle is highly delocalized. In the halogenation pathway, compound **I** reacts directly with a halide anion to give an iron(III) hypohalite species. The exact details of the next steps are obscure but since halogenation is usually not regio- or stereoselective, in most cases it is unlikely to occur in the enzyme-active site. It is more likely that a molecule of hypohalous acid diffuses out of the active site and halogenates the olefin in the surrounding medium. However, the regio- and stereospecificity of some reactions, including bromination of 2,3-dehydrosialic acid,[18] indicate that the halogenation may also occur within the active site of chloroperoxidase.

The mechanisms of N-dealkylation and epoxidation are also not known with certainty. Ortiz de Montellano and co-workers have shown that epoxidation of *trans*-[1-^{2}H]styrene by chloroperoxidase proceeds without loss of stereochemistry and concluded that it occurs via a ferryl oxygen transfer mechanism[9] similar to oxidation of styrene by cytochrome P-450. In fact, chloroperoxidase strongly resembles cytochrome P-450 in all magnetic and spectral properties[19-22] and catalyzes a number of P-450-type monooxygenation reactions. Despite many similarities, chloroperoxidase can catalyze a number of reactions not typical for the cytochrome P-450 family of enzymes, a feature likely attributed to the differences in the heme environment of the enzymes. For example, the presence of a charged amino acid in the vicinity of the active site of chloroperoxidase influences ligand binding and, possibly, the catalysis.[23] It is thought that the more polar peroxidase-like heme environment enables the chloroperoxidase to catalyze peroxidative reactions.[9]

Although the synthetic utility of asymmetric epoxides is well recognized,[24] few studies of the chloroperoxidase-catalyzed epoxidations have been carried out. Neidelman and co-workers[10] were the first to qualitatively describe the reaction in 1986. Later, Ortiz de Montellano and co-workers showed that the epoxidation of *trans*-[1-^{2}H]styrene by the chloroperoxidase proceeded without detectable loss of stereochemistry.[9] Finally, Hager and Jacobsen[11] utilized chloroperoxidase for asymmetric epoxidation of a series of olefins, obtaining epoxides in varying yields (12−85%) and enantiomeric purities (ee 66−97%).

In this study we evaluated the substrate specificity and enantioselectivity of chloroperoxidase from *C. fumago* in the oxidation of olefins. We report here that in addition to epoxidation, the enzyme is capable of catalyzing allylic and benzylic hydroxylation of a number olefins with a high degree of stereoselectivity. The utilization of radical probe substrates revealed the key features of the mechanism of the hydroxylation reaction.

Results

The substrate specificity of the chloroperoxidase in the oxidation of alkenes, arylalkenes, and arylalkanes is illustrated in Table 1. The epoxidation of *cis*-2-heptene (**1**) resulted in the complete consumption of the starting material and the formation of the epoxide as the only product.[25] The enantiomeric excess of the epoxide determination by chiral GC was found to be 95%. The epoxidation of *cis*-3-heptene (**2**) also resulted in the formation of the epoxide with high enantiomeric purity. However, in this case the epoxidation was accompanied by the formation of two secondary allylic alcohols in amounts roughly equal to that of the epoxide. The epoxidation of *cis*-3-octene and *cis*-2-nonene (**3** and **4**) proceeded slower than that of either of the heptene substrates but also resulted in the formation of the corresponding epoxides of high enantiomeric purity (94−95% ee). *cis*-3-Nonene (**5**) was a very poor substrate: only 4% conversion was achieved in 5 h. No byproducts were formed with the substrates **1** and **3**−**5**.

Virtually no epoxidation occurred in the case of *trans*-olefins **6**, **7**, **9**, which have been previously reported to be poor substrates.[11] However, oxidation of *trans*-2-heptene (**6**) catalyzed by chloroperoxidase resulted in the formation of the aldehyde (hept-2-enal) in 35% yield. The oxidation of *trans*-3-heptene (**7**) was very slow. Mass spectrometry indicated that the small amount of product formed (6%) corresponded to secondary allylic alcohol(s); no further oxidation of the allylic alcohol to ketone was observed. In a manner similar to *trans*-3-heptene, *trans*-β-methylstyrene (**9**) was oxidized to cinnamaldehyde in 30% yield.

In the arylalkene series of substrates, styrene (**8**) was converted to a mixture that contained 40% styrene epoxide (ee 49%), 24% phenylacetaldehyde (formed most likely via 1,2-rearrangement of the protoporphyrin-bound intermediate),[9] and 4% phenylacetic acid. The enantioselectivity of styrene epoxidation was significantly lower than in the epoxidation of *trans*-[^{2}H]styrene that proceeded without detectable loss of stereochemistry.[9] α-Methylstyrene (**10**) was found to be a very reactive substrate. The olefin was efficiently converted to the corresponding epoxide which spontaneously hydrolyzed to 2-phenyl-1,2-propanediol. As in the case of styrene, this epoxidation was also accompanied by the formation of 1-methylphenylacetaldehyde, which was further oxidized to 1-methylphenylacetic acid.

The oxidation of 1-methylcyclohexene (**11**) resulted in the formation of a mixture of at least 4 compounds. The main product, identified as 1-methyl-1,2-dihydroxycyclohexane, resulted from hydrolysis of the enzymatically formed epoxide.

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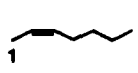
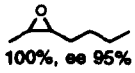
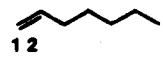
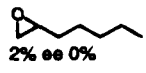
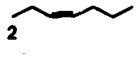
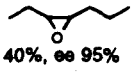
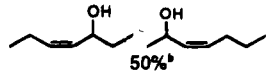
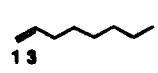
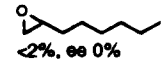
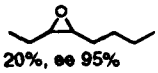
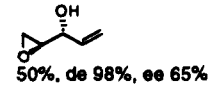
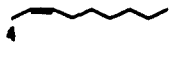
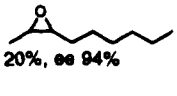
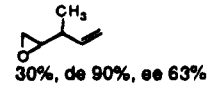
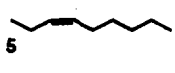
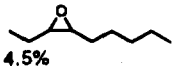
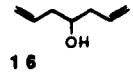
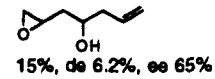
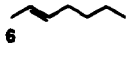
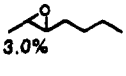
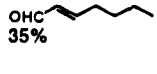
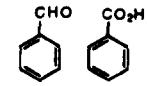
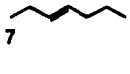
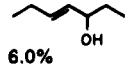
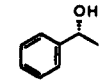
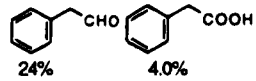
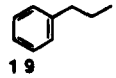
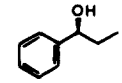
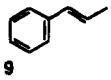
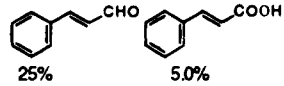
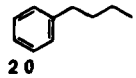
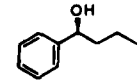
(25) Although no other products were detected only about 50% of the theoretical yield of the epoxide was obtained. The lower than expected yield of the epoxide was shown to be the result of substrate loss due to the high volatility of the water-insoluble olefin. This physical loss was further amplified by the evolution of oxygen bubbles formed by decomposition of hydrogen peroxide. In controlled experiments that did not contain the enzyme, similar losses of *cis*-2-heptene were observed.

Table 1. Chloroperoxidase-Catalyzed Oxidations

substrate	product/by-product[a]	substrate	product/by-product[a]
 1	 100%, ee 95%	 12	 2% ee 0%
 2	 40%, ee 95%	 50%[b]	 13	 <2%, ee 0%
 3	 20%, ee 95%	 14	 50%, de 98%, ee 65%
 4	 20%, ee 94%	 15	 30%, de 90%, ee 63%
 5	 4.5%	 16	 15%, de 6.2%, ee 65%
 6	 3.0%	 35%	 17	 85% 15%
 7	no epoxide is formed	 6.0%	 18	 20%, ee 97%
 8	 40%, ee 49%	 24% 4.0%	 19	 20%, ee 88%
 9	no epoxide is formed	 25% 5.0%	 20	 1.5 % ee 90%
 10	90%, ee 25%	6.5% 3.5%	21	no reaction
11	70%	30%[b]	22	no reaction

[a] As percent of medium composition after termination of reaction (in some oxidations the material balance was somewhat lower than expected due to volatility of substrates). [b] Combined yield; for conditions see Experimental Section.

The three other byproducts detected by mass spectrometry were formed as a result of chloroperoxidase-catalyzed hydroxylation of 1-methylcyclohexene followed by the oxidation of the allylic alcohols to the corresponding exo-cyclic aldehyde and the two possible α,β-unsaturated ketones. Although the hydroxylation of cyclohexene to the allylic cyclohexenol has been reported,[8] the oxidation of allylic alcohols to α,β-unsaturated ketones has not been previously observed.

In accord with the observation of Allain et al.,[11] terminal alkenes such as 1-heptene and 1-octene (**12**, **13**) were epoxidized very poorly and nonselectively. Surprisingly, the oxidation of shorter terminal olefins such as C-5 prochiral dienes **14** and **15** proceeded much more efficiently and resulted exclusively in the formation of monoepoxides. The oxidation of 3-hydroxy-1,4-pentadiene (**14**) proceeded with a high degree of diastereoselectivity (98%) and modest enantioselectivity (65%). The predominant product (2*S*,3*R*)-1,2-epoxy-4-penten-3-ol is an enantiomer of the epoxy alcohol produced via Sharpless epoxidation of the corresponding divinylcarbinol.[26] The substitution of the substrate's 3-hydroxyl with the methyl group **15** diminishes both conversion and diastereoselectivity of the reaction. This decrease is likely to result from the lower solubility of 3-methyl-1,4-pentadiene and its weaker propensity to bind to the enzyme. Moving the double bond away from the prochiral center (i.e. 1,6-heptadien-4-ol (**16**)) further decreases the diastereoselectivity of the epoxidation.

It is apparent from the above results that the chloroperoxidase catalyzes the epoxidation of a number of olefins with a high degree of enantio- and diastereoselectivity and that the reaction in some cases is accompanied by the formation of various allylic alcohols. Although the hydroxylation activity required to form allylic alcohols during the epoxidation of some olefins has been

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observed earlier,[8] the hydroxylation reaction has never been investigated in detail. Therefore, it was of great interest to separate the hydroxylation activity from epoxidation, and to study the former activity independently.

In order to simplify the experimental system by removing the possibility of epoxidation, the oxidation of a number of saturated alkylbenzenes was studied. The oxidation of toluene (**17**) resulted in its quantitative conversion to benzaldehyde and benzoic acid. Small amounts of benzyl alcohol detected during the early stages of the reaction disappeared due to oxidation to benzaldehyde. In fact, it was found in a separate experiment that the rate of oxidation of benzyl alcohol was at least five times faster than that of the hydroxylation of toluene.

Remarkably, the oxidation of ethylbenzene (**18**) resulted in the formation of 2-phenethyl alcohol in the (*R*) configuration with ee of 97%. Also surprising was the fact that the oxidation of propylbenzene (**19**) resulted in the alcohol product of the opposite stereochemistry, (*S*)-1-phenyl-1-propanol with an ee of 88% was produced. Although the hydroxylation of butylbenzene (**20**) was rather inefficient, the product had a good enantiomeric purity (ee 90%). α-Disubstituted (1-ethylpropyl)-benzene (**21**) and cumene (**22**) were not substrates for the chloroperoxidase.

The catalytic mechanism of the observed chloroperoxidase-catalyzed benzylic hydroxylation was then investigated. In order to determine whether the benzylic hydroxylation involved the formation of radical intermediates, a radical probe substrate, *trans*-2-phenyl-1-methylcyclopropane (**I**), was synthesized and subjected to hydroxylation in the presence of hydrogen peroxide and chloroperoxidase. The analysis of the products by GC revealed the formation of three products identified as *trans*-(2-phenylcyclopropyl)methanol (**III**), *trans*-(2-phenylcyclopropyl)-methanal (**VI**), and *trans*-2-phenylcyclopropanecarboxylic acid (**VII**) in a ratio of 1.0:8.0:1.4. The presence of the alcohol which would result from the rearrangement of a radical intermediate, 1-phenylbut-3-en-1-ol (**V**), was not detected. The possibility that alcohol **V** was originally formed but then degraded by further oxidation during the incubation with the chloroperoxidase was excluded by a control experiment in which a 10 mM solution of **V**, 0.5 mg/mL of chloroperoxidase, and H_2O_2 was incubated for 5 h. GC analysis revealed that under these conditions the alcohol **V** was stable and no products were formed. The quantitative analysis of the products of the chloroperoxidase-catalyzed hydroxylation of **V** revealed that the ratio of the products containing an intact cyclopropyl ring, **III**, **VI**, and **VII**, to the product **V** expected from rearrangement of a radical intermediate was at least 100:1.

Discussion

The recent discovery that chloroperoxidase is capable of catalyzing enantioselective epoxidation of alkenes[9,11] prompted us to investigate the substrate specificity of the enzyme in order ascertain its utility as a chiral catalyst. A great advantage of using chloroperoxidase as a catalyst stems from the simplicity of this reaction. In contrast to P-450 enzymes, chloroperoxidase utilizes hydrogen peroxide directly and does not require either electron transport proteins or cofactors.

Our results confirmed the earlier findings that chloroperoxidase from *C. fumago* catalyzes asymmetric epoxidations of a variety of *cis*-olefins resulting in the formation of highly enantiomerically enriched epoxides. The best substrates are short-chain alkenes that have the unsaturated carbon close to the terminal, such as *cis*-2-heptene and *cis*-2-octene. As the length of the alkyl chain is increased, and the double bond is moved further away from the terminal, the reactivity drops significantly. For example, the degree of conversion of *cis*-3-nonene is only a fraction of that of *cis*-2-heptene. Moreover, the oxidation of *cis*-3-olefins results in the formation of the corresponding allylic alcohols.

trans-Olefins are subject to only very limited epoxidation; instead they are efficiently hydroxylated in the allylic position and then further oxidized to aldehydes and ketones.

As expected,[11] terminal unsubstituted aliphatic olefins such as 1-heptene and 1-octene are very poor substrates. Conversely, aromatic "terminal olefin" substrates such as styrene and α-methylstyrene are efficiently converted to mixtures of the corresponding epoxides and aldehydes. The oxidation of short-chain prochiral terminal diolefins **14**–**16** resulted exclusively in the formation of monoepoxides with varying degree of diastereomeric and enantiomeric purity.

The most unexpected result of this study is the discovery of chloroperoxidase-catalyzed enantioselective benzylic hydroxylations. Although the range of substrates accepted by the enzyme is rather narrow, the enantioselectivity in some cases is very high. Realizing that only few olefins have been examined in this work, it is likely that the range of substrates for hydroxylations will be extended further.

The ability of chloroperoxidase from *C. fumago* to catalyze asymmetric epoxidations seems to be unique among peroxidative enzymes. Peroxidases from horseradish, soybean, *Basidomyces sp.*, and *Arthromyces ramosus* tested under the identical conditions were unable to carry out oxidation of *cis*-2-heptene to any extent.

Since the chloroperoxidase from *C. fumago* contains the ferriprotoporphyrin IX prosthetic group and has an active site resembling that of cytochrome P-450 it was of interest to evaluate the mechanism of chloroperoxidase hydroxylation and compare it to established earlier hydroxylations by P-450 enzymes. The recent studies of P-450-catalyzed hydroxylations favor a nonconcerted mechanism in which a high-valent oxo-ferryl species formed as a result of oxidation of Fe^{III} abstracts a hydrogen atom from the substrate. The formed hydrocarbon radical is then trapped by the ion-coordinated hydroxyl radical to give the hydroxylated product.[27–29] The strongest evidence for the existence of the radical intermediates during the hydroxylation process comes from the studies that utilize radical clock substrates. Upon hydrogen abstraction, these substrates form radicals which are known to re-arrange with rate constants as large as 4×10^{11} s^{-1} (Scheme 1). The formation of the rearranged products is considered to be good evidence for the nonconcerted mechanism that entails the existence of a discrete radical intermediate, which is subsequently quenched by the hydroxyl radical derived from the oxidized heme in the enzyme's active site.[30–35] The exclusive formation of the

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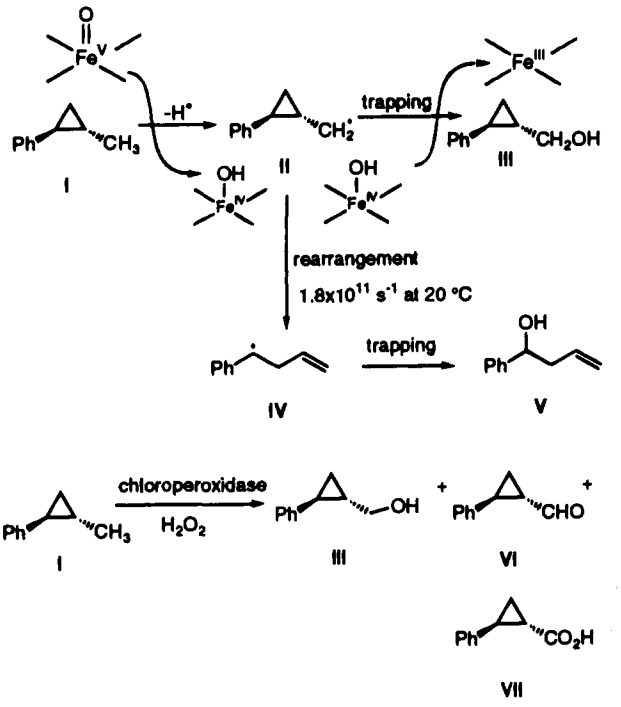
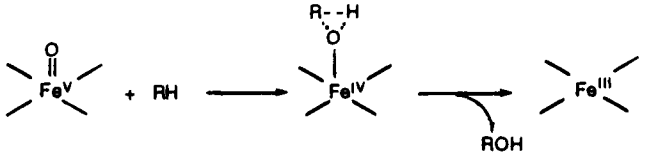
Scheme 1



unrearranged alcohols usually indicates that the hydroxylation proceeds via the concerted mechanism in which no discrete radical intermediate is generated. This mechanism can be visualized as the insertion of an oxygen atom into a C−H bond in the substrate molecule as illustrated in the scheme below:



In the case of radical probes which produce more stable, slowly rearranging radical intermediates, a fast quenching pathway may compete with the slow rearrangement pathway[33,36] resulting in appreciable amounts of unrearranged products, thus making the results ambiguous.

In order to circumvent this potential problem and determine whether substrate radicals are produced during hydroxylation by chloroperoxidase, hydroxylation of one of the fastest radical probe substrates, *trans*-2-phenyl-1-methylcyclopropane (**I**), was undertaken (Scheme 1). The incubation of 10 mM of **I** with chloroperoxidase in the presence of H_2O_2 resulted in the formation of the unrearranged cyclopropyl products **III**, **VI**, and **VII** in a ratio of 1.0:8.0:1.4. The presence of the rearranged allyl alcohol product **V** was not observed. Based on the detection limit of the analysis, the ratio of the unrearranged products to the rearranged product was estimated to be at least 100:1.

If the hydroxylation proceeds via a radical intermediate, in order to satisfy the experimentally observed product ratio the rate of quenching of the radical intermediate by delivery of a hydroxyl radical from the heme must greatly exceed the rate constant for rearrangement of the radical intermediate **II**. By making an assumption that the rearrangement of **II** is not significantly suppressed by the steric environment of the enzyme and considering that the rate constant for the rearrangement of **II** at 20 °C in aqueous solution is 1.8×10^{11} s^{-1},[37] the rate constant for transfer of the hydroxyl radical from the heme group required to satisfy the observed 100:1 product ratio must be approximately 1.8×10^{13} s^{-1}. The rate of rearrangement that involves a bond breaking cannot exceed the vibrational frequency of the bond being broken which is equal to

$$\nu = \kappa T/h$$

where κ is the Boltzmann constant, h is the Plank constant, and T is the absolute temperature. At 20 °C $\nu = 6.1 \times 10^{12}$ s^{-1}, approximately a factor of 3 too low to explain the observed product ratio. Hence, the mechanism of chloroperoxidase-catalyzed hydroxylation of **I** is incompatible with the existence of a discrete radical and therefore **I** is converted directly to the alcohol without passing through a radical intermediate. The proposed mechanism is similar to the hydroxylation catalyzed by P-450 monooxygenase from *M. capsulatus*.[33] In contrast, the hydroxylation of **I** catalyzed by a monooxygenase from *Pseudomonas oleovorans* proceeded through a nonconcerted radical-intermediate process.[31,32] A nonconcerted hydroxylation mechanism has also been proposed for microsomal cytochrome P-450.[30]

Conclusions

The scope of oxidations investigated in this study was intended to define the substrate specificity of chloroperoxidase from *C. fumago* and allow one to predict the outcome of new transformations with some degree of certainty. The enzyme epoxidizes straight chain aliphatic and cyclic[11] *cis*-olefins in a highly stereoselective manner. It favors small unsubstituted substrates with the unsaturation removed not more than 2 carbon atoms from the terminal. The enzyme catalyzes monoepoxidations of some short-chain terminal prochiral dienes with high diastereoselectivity and moderate enantioselectivity. However, the unsubstituted straight-chain terminal olefins 7 carbon units and longer are epoxidized poorly. Aliphatic and aromatic alcohols are efficiently oxidized to aldehydes and acids. Moreover, the enzyme catalyzes enantioselective benzylic hydroxylation of a number of substrates with high enantioselectivity.

Compared to other biological epoxidations catalyzed by cytochromes P-450,[38] microsomes,[39] and bacteria,[31,32,40-44] the chloroperoxidase from *C. fumago* affords a number of advantages. The enantioselectivity of the epoxidation is much higher than in most previously reported cases; the reaction is catalyzed by a single protein which does not require cofactors making the system easier to manipulate and products easier to recover. Continuous flow bioreactors have been developed which produce chloroperoxidase titers greater than 600 mg/L,[45] an amount of the enzyme sufficient to produce multigram quantities

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of epoxides. Also, an easy and efficient procedure for the protein purification on a large scale has also been reported.[46]

The new oxidation activities described here and those reported previously make it clear that the chloroperoxidase has high potential as a chiral catalyst.

Experimental Section

Materials and Methods. Chloroperoxidase from *C. fumago* with a specific activity of 1300−1400 IU/mg with monochlorodimedone as a substrate[1] was obtained as a 10-mg/mL solution from Chirazyme (Urbana, IL).

cis-2- and *cis*-3-octene, *cis*-2- and *cis*-3-nonene, and 1-phenyl-3-butene-1-ol were obtained from Wiley Organics (Coshocton, OH); styrene oxide was purchased from Eastman Kodak (Rochester, NY). All other racemic epoxides used for product identification and chiral analysis were prepared from the corresponding olefins and 3-chloroperoxybenzoic acid in CH_2Cl_2. Citric acid and 30% H_2O_2 were obtained from Fisher (Fair Lawn, NJ). *trans*-2-Phenylcyclopropanecarboxylic acid ethyl ester was obtained from Lancaster Synthesis Inc. (Windham, NH). All other chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI).

(2*R*,3*S*)-1,2-Epoxy-4-penten-3-ol used for the identification of the absolute stereochemistry of the enzymatically produced epoxide was synthesized via the Sharpless epoxidation with *tert*-butyl hydroperoxide in the presence of L-(+)-diisopropyltartrate and titanium isopropoxide.[26]

trans-(2-Phenylcyclopropyl)methanol (**III**) was synthesized via $LiAlH_4$ reduction of commercially available *trans*-2-phenylcyclopropanecarboxylic acid ethyl ester.[47] *trans*-(2-Phenylcyclopropyl)methanal (**VI**) was obtained by Swern oxidation[48] of **III** with oxaloyl chloride in CH_2Cl_2. *trans*-2-Phenyl-1-methylcyclopropane (**I**) was produced via a carbene insertion using *trans*-methyl-β-styrene and CH_2I_2 in the presence of Zn−Cu.[49] Zn−Cu couple was prepared by refluxing zinc dust with CuCl for 30 min in dry ether. Following the oxidation of the unreacted olefin with *m*-CPBA the product was purified by silica-gel chromatography with hexane as an eluent. *trans*-2-Phenylcyclopropanecarboxylic acid (**VII**) was obtained via pig liver esterase-catalyzed hydrolysis of *trans*-2-phenylcyclopropanecarboxylic acid ethyl ester in 100 mM KCl at pH 8.0.

Oxidation Reactions. The method of Allain *et al.*[11] was generally followed, but without the addition of acetone. Four substrates (**1**, **2**, **4**, **13**) from this reference were used to check the method reported here; results are compared in the table. In a typical oxidation reaction, 50 μmol of the substrate were added via the syringe through the septum into a 5-mL vial containing 4.5 mL of 100 mM citrate buffer at pH 5.0. The mixture was stirred with a magnetic stirrer for 10 min after which a 0.1-mL sample of the enzyme solution was added and the reaction was initiated by the addition of 3% H_2O_2 at the rate of 0.05 mL/h. Samples of 100 μL were withdrawn periodically, extracted with 1.0 mL of CH_2Cl_2, and analyzed by gas chromatography on a 30-m HP-5 (phenylmethyl silicone) column from Hewlett-Packard. After about 5 h the entire reaction was extracted with CH_2Cl_2, and the organic layer was dried, concentrated, and analyzed by GC-MS (Hewlett-Packard Model 5989 equipped with a 60-m HP-1 column).

Instrumentation. The enantiomeric purity of styrene oxide was determined by HPLC equipped with a diode-array detector on Chiralpack AS (Daicel Chemical Industries, LTD, Fort Lee, NJ) with 95% hexane and 5% isopropyl alcohol as the eluent column. The enantiomeric purity of 1,2-epoxy-4-penten-3-ol was determined by gas chromatography (N_2, 90 °C) on a 40-m Chiraldex A-TA column (Astec, Whippany, NJ). The purity of 1,2-epoxy-3-methyl-4-pentene was determined on a 40-m Chiraldex G-TA column (N_2, 40 °C). Only one diastereomer of 1,2-epoxy-6-hepten-4-ol was resolved on a 40-m Chiraldex BP-H column at 110 °C. The enantiomeric purity of the enzymatically produced 1-phenyl-1-ethanol, 1-phenyl-1-propanol, and 1-phenyl-1-butanol was determined by HPLC on Chiracell OB-H with the mobile phases hexane/isopropyl alcohol 96.5/3.5, 95/5, and 90/10, respectively. The absolute stereochemistry was determined by comparing the retention times for the products with that for the pure enantiomers obtained from Aldrich Chemical Co. Enantiomeric purity of all other epoxides was determined by GC on a 30-m Chiraldex G-PN column.

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