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The Use Haloperoxidases in Organic Synthesis: Selected Reactions of Oxidation, Epoxydation and Sulfoxidation

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*This paper is fondly dedicated to Professor **Genrih A. Tolstikov** of the Novosibirsk Institute of Organic Chemistry on the occasion of his 70th birthday.*

Abstract

Haloperoxidases are ubiquitous metalloenzymes that catalyse a variety of enantioselective oxygen-transfer reactions with hydrogen peroxide or alkylperoxides. Haloperoxidases are enzymes which catalyze the reaction of oxidation, epoxidation and sulfoxidation by hydrogen peroxide. These enzymes usually contain the FeHeme moiety or vanadium as an essential constituent at their active site, however, a few haloperoxidases which lack a metal cofactor are known. This review will examine the reactivity of the different haloperoxidases, particularly the mechanism of oxidation by hydrogen peroxide, and the mechanism of oxidation and sulfoxidation, including the newly reported regioselectivity and enantioselectivity of the haloperoxidases. The structure of chloroperoxidase, the vanadium active site and the role of critical amino acid side chains for catalysis and functional biomimetic systems, with specific relevance to the mechanism of the haloperoxidase enzymes. Advances have recently been made in using them to prepare, under controlled conditions, chiral organic molecules that are valuable for the synthesis of a wide range of useful compounds. The application of biocatalytic methods in asymmetric organic synthesis is of great interest as an alternative to chemical procedures employing chiral auxiliaries. Asymmetric oxidation of prochiral sulfides to yield optically active sulfoxides has been performed by many different techniques yielding varying enantiomeric excess values. Oxygenated metabolites are compounds that are commonly found in nature and they are produced by many different organisms. The oxygen atom is incorporated into organic compounds by enzyme-catalyzed reactions with oxygen ions as the oxygen source. For over 40 years haloperoxidases were thought to be responsible for the incorporation of mainly halogen atoms into organic molecules. However, haloperoxidases lack substrate specificity and regioselectivity, and the connection of haloperoxidases with the in vivo formation of oxygenated as well as halometabolites has been demonstrated. Recently, molecular genetic investigations showed that, at least in bacteria, fungi, and other organisms a different class of halogenases is involved in halo- and oxygenated metabolite formation. These halogenases were found to require $FADH_2$, which can be produced from FAD and NADH by unspecific flavin reductases. The $FADH_2$-dependent halogenases and haloperoxidases show substrate specificity and regioselectivity, and their genes have been detected in many halometabolite-producing organisms, suggesting that this type of halogenating enzymes constitutes the major source for halo- and oxygenated metabolite formation in bacteria and also in other organisms. Distribution of haloperoxidases in nature also is demonstrated in this brief review.

Introduction

Biological systems have evolved haloperoxidase enzymes to catalyze the oxidation of chloride, bromide and iodide by hydrogen peroxide. Recent reviews have been published that deal with various aspects and activity of these enzymes [1-11]. Three classes of haloperoxidases have been identified. The first is a class of enzymes found in bacteria without a prosthetic group [12,13]. The second is heme-containing peroxidases such as chloro-peroxidae (CPO) first discovered in the marine fungus *Caldariomyces fumago* in 1966 [14], myeloperoxidase, eosinophil peroxidase and lactoperoxidase from mammalian systems [15]. The third class of haloperoxidases is va-

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nadium-containing peroxidases that require a vadanade ion (VO_4^{-3}). Vanadium peroxidase was fist discovered in the brown alga *Ascophyllum nodosum* in 1984 [16] but simce then it is also been found in lichen [17] and in fungi [18].

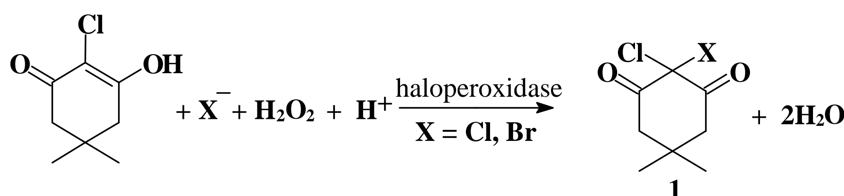
The preparation of chiral compounds in non-racemic form is a goal of great interest in organic synthesis, due to the large application that these compounds have in several fields, such as in medicinal chemistry [19]. Interest in this field has been directed toward the use of biocatalysis for regio- and stereoselective discrimination of alcohol functions so as to achieve polyhydroxylated compounds in enantiopure form [20-24]. The enantioselective direct introduction of oxygen onto olefins with biocatalysis by haloperoxidases, in oxygenase-type reactions, is very useful and effective for this purpose [25]. In particular the use of *Caldariomyces fumago* CPO is especially advantageous, since this usually involves peroxides (H_2O_2 or ROOH), without requiring expensive cofactors. Moreover, due to its broad substrate acceptance, this CPO has great synthetic potential and has allowed the stereoselective epoxidation and the hydroxylation of a wide range of olefins in satisfactory yield and with high enantiomeric excess [26-31].

Current interest in catalytic oxidative transformations in industry is goverened by two major issues: the first one is the replacement of oxidations which use a stoichiometric amount of heavy metal salts by catalytic processes using hydrogen peroxide or oxygen as the oxidant. A second major issue is the need for high chemo-, regio- or enantioselectivies in order to improve chemical yields, to minimize waste streams and to avoid enantiomeric ballast. Haloperoxidases are potentially suitable biocatalysts for meeting these two goals.

This review will examine the reactivity of the haloperoxidases, particularly the mechanism of oxidation by hydrogen peroxide, and the mechanism of oxidation, epoxidation and sulfoxidation, including the newly reported regioselectivity and enantioselectivity of the vanadium haloperoxidases. This is the first review which combines oxidation, epoxidation, and sulfoxidation catalysed by haloperoxidases isolated from different natural sources.

Oxidation reactions catalyzed by chloro- and bromoperoxidases

The classic organic substrate used to evaluate and compare haloperoxidases from different sources is monochlorodimedone **1** (2-chloro-5,5-dimethyl-1,3-dimedone) (Scheme 1). **1** has been used to investigate the enzyme kinetic mechanism of the vanadium haloperoxidases [33-35].

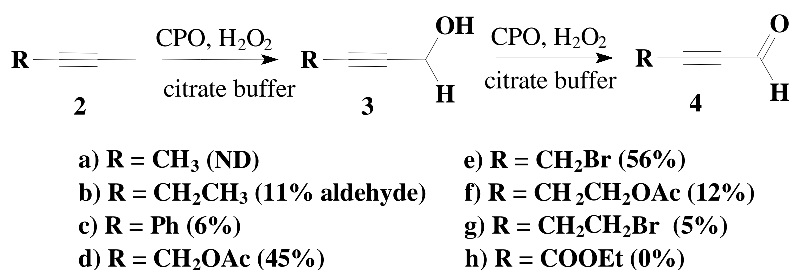


Scheme 1

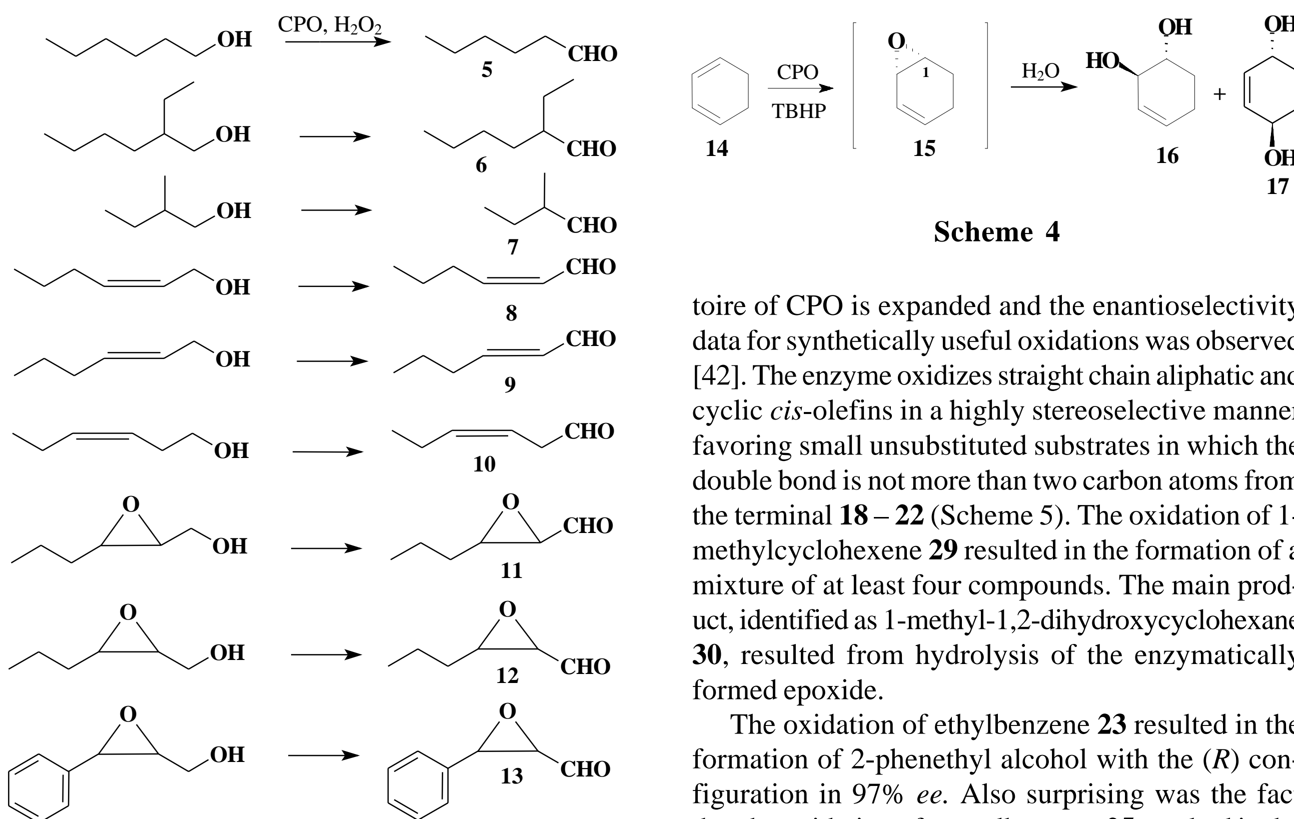
Chiral propargylic alcohols are important building blocks for the enantioselective synthesis of complex molecules, in particular, biologically active compounds [36]. It was found that CPO catalyzed the oxidation of 2-alkynes **2** to aldehydes **4** in the presence of hydrogen peroxide or *t*-butyl hydroperoxide as shown below (Scheme 2). The CPO propargylic oxidation of alkynes to aldehydes procceds *via* an alcohol intermediate **3**. When propargylic alcohols were incubated with CPO in the presence of H_2O_2 [37], it was observed that the alcohols were completely and rapidly converted to aldehydes (92 – 95%). Also it was reported that CPO catalyzed highly enantioselective propargylic hydroxylations [38].

CPO from the fungus *Caldariomyces fumago* also catalyzed the oxidation of primary alcohols selectivity to the corresponding aldehydes **5** – **13** in a biphasic systems of hexane or ethyl acetate and a buffer (pH = 5.0) (Scheme 3) [39,40]. The *cis* to *trans* isomerization in the case of *cis*-2-hexenal was also observed.

Asymmetric oxidation of prochiral 1,3-cyclohexadiene is catalyzed by a CPO from *Caldariomyces fumago* [41]. The process occurs enantio-



Scheme 2



Scheme 3

Scheme 4

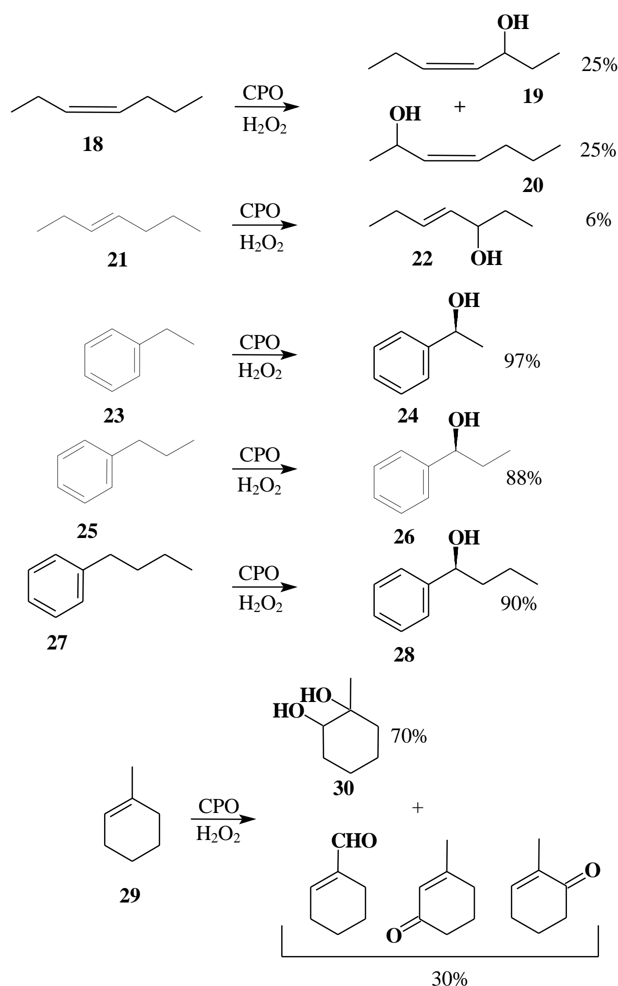
selectively and furnishes the non-racemic *trans* diols 1,2- and 1,4-dihydroxycyclohexene, (-)- **16** and (+)- **17**, in good yield (Scheme 4). The oxidation of cyclohexadiene **14** was carried out in citrate buffer (0.1 M, pH = 5). Although cyclohexadiene oxide 15 has not been detected n the reaction mixture the, it is plausible that in the first step in the mechanism involving CPO and tundergoes fast nucleophilic attack by water, with partial rearrangement, giving the *trans*-diols **16** and **17**, respectively.

The substrate specificity of CPO from *Caldaromyces fumago* in a number of halide-independent reactions has been investigated and the ability of this enzyme to perform benzylic hydroxylations with high enantioselectivity is revealed. The substrate repertoire of CPO is expanded and the enantioselectivity data for synthetically useful oxidations was observed [42]. The enzyme oxidizes 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 **18** – **22** (Scheme 5). The oxidation of 1-methylcyclohexene **29** resulted in the formation of a mixture of at least four compounds. The main product, identified as 1-methyl-1,2-dihydroxycyclohexane **30**, resulted from hydrolysis of the enzymatically formed epoxide.

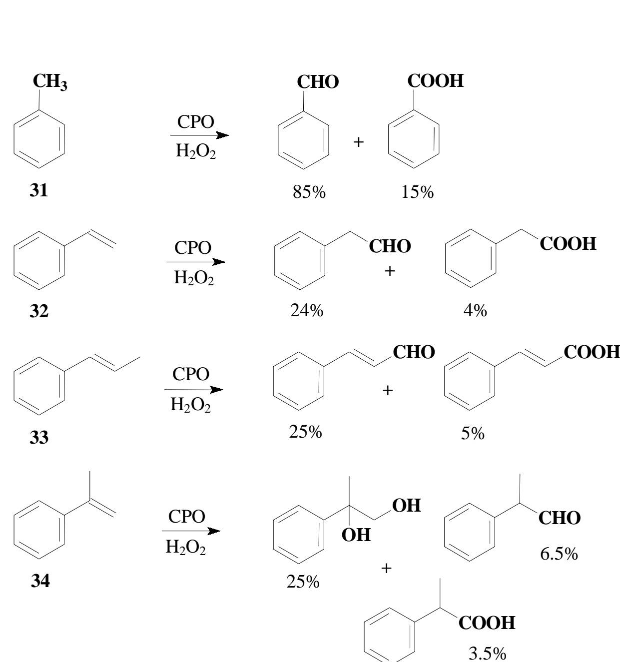
The oxidation of ethylbenzene **23** resulted in the formation of 2-phenethyl alcohol with the (*R*) configuration in 97% *ee.* Also surprising was the fact that the oxidation of propylbenzene **25** resulted in the alcohol product with the opposite stereochemistry, (*S*)-1-phenyl-1-propanol, with an *ee* of 88%. Although the hydroxylation of butylbenzene **27** was rather inefficient, the product had a good enantiomeric purity (*ee* 90%).

Oxidation of aromatic compounds **31** – **34** has been also reported [31,42]. The oxidation of toluene **31** 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 that the rate of oxidation of benzyl alcohol was at least five times faster than that of the hydroxylation of toluene.

In the arylalkene series of substrates, styrene **32** was converted to a mixture that contained 24% phenylacetaldehyde (formed most likely via 1,2-re-



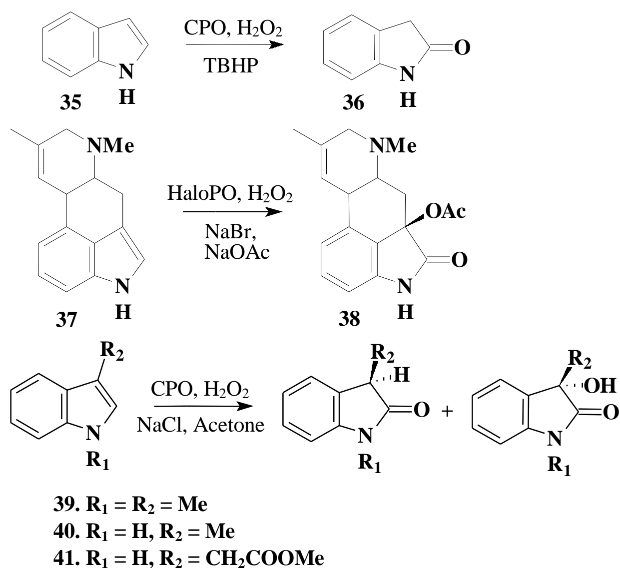
Scheme 5



Scheme 6

arrangement of the protoporphyrin-bound intermediate) [43], 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 [43]. α-Methylstyrene **34** was found to be a very reactive substrate. The olefin was efficiently converted to the corresponding epoxide which spontaneously hydrolyzed to 2-phenyl-l,2-propanediol (Scheme 6). As in the case of styrene, this epoxidation was also accompanied by the formation of l-methylphenylacetaldehyde, which was further oxidized to l-methylphenylacetic acid [42].

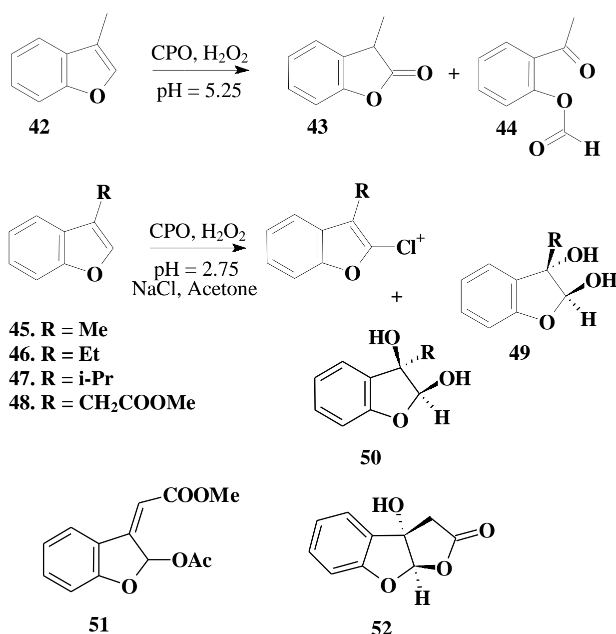
CPO from *Caldariomyces fumago* was used in the oxidation of indole **35** to lactone **36** (Scheme 7) [44]. The same reaction was demonstrated by Kren et al. [45] as an unusual double oxidation catalyzed by CPO during a study of the metabolism of ergot alkaloids **37**. The oxidation of the indole derivatives **39** – **41** by CPO from *Caldariomyces fumago* has been investigated. Under conditions in which inactivation of CPO was minimised by the presence of chloride and hydrogen peroxide [46], the oxidation products of N-unsubstituted indoles tautomerised to give the corresponding lactam.



Scheme 7

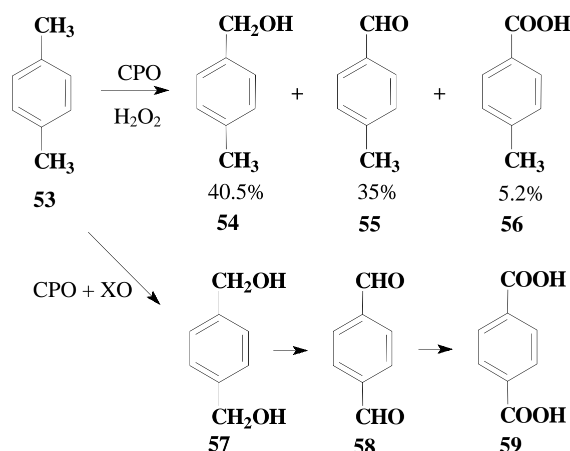
3-alkyl benzofurans **42** - **45** gave 2,3-diols **46** and **47** as initial products. The diols (predominantly *trans)* formed from the benzofurans were sufficiently stable for isolation [46]. Under conditions where catalase activity was high, the predominant products from benzofurans were heterocyclic ring cleaved compounds

such as the ketoformate **44** (Scheme 8). However, at mildly acidic pH, in the presence of acetone and with careful control of both enzyme and hydrogen peroxide concentration, it was possible to isolate significant quantities of 1,2-diol oxidation products of benzofurans (derivatives **45 – 48**) by extraction of the reaction mixture with ethyl acetate. When dichloromethane was used for extraction, the major product was the lactone **43**, presumably derived by acid-catalysed dehydration of the diol, and the ketoformate **44**. In the case of the 3-isopropylbenzofuran **47**, it is noteworthy that only the *trans* isomer could be isolated, as both diol and diacetate, probably because of the larger size of the isopropyl group compared with methyl. In the case of the benzofuran 3-acetic-acid derivative **48**, two of the isolated products **51** and **52** presumably derive from the initial diol that was formed by CPO-catalysed oxidation, followed by dehydration or intramolecular nucleophilic attack.



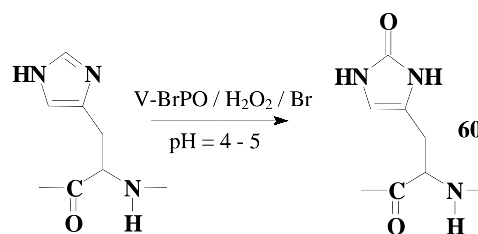
Scheme 8

CPO from *Caldariomyces fumago* was used to oxidize *p*-xylene **53**. However, only one of the two aromatic methyl groups was oxidized and forms 4-methylbenzyl alcohol **54**, *p*-tolualdehyde **55**, and *p*-toluic acid **56** (Scheme 9). Investigation of numerous peroxidase and oxidase enzyme systems has shown that the route from 1,4-benzenedimethanol **57** to terephthalic acid **59** is most efficient with a combination of two enzymes, CPO and xanthine oxidase. Oxidation of **53** to a mixture of predominantly terepthaldicarboxaldehyde, 4-carboxy-benzaldehyde, and 4-hydroxymethylbenz-aldehyde was carried out by CPO with the continuous addition of hydrogen peroxide as an oxidant. Subsequent addition of XO resulted in a 65% yield of terephthalic acid **59** [47].



Scheme 9

Vanadium bromoperoxidases (V-BrPO) are all acidic proteins [48,49] with very similar amino acid composition [50], molecular weight, charge (pH = 4-5), and vanadium content. Bromoperoxidase activity has been observed in nearly 100 marine algae [51]. Bromoperoxidase activity was most prevalent in red and green algae, *i.e.* 76% and 71%, respectively. Bromoperoxidase (V-BrPO) isolated from the brown marine alga *Ascophyllum nodosum* under low pH provided 2-oxohistidine **60** in the presence of hydrogen peroxide (Scheme 10). The inactivation and 2-oxohistidine formation are not the result of oxidation by singlet oxygen produced by V-BrPO, since they do not occur under conditions in which V-BrPO produces singlet oxygen quantitatively [52].

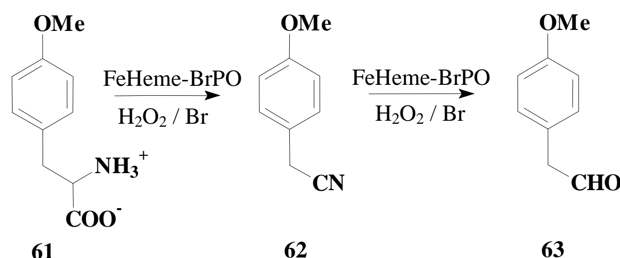


Scheme 10

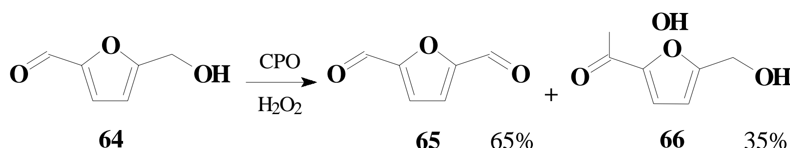
Another bromoperoxidase (FeHeme-BrPO) from the green alga *Penicillus capitalus* has been shown to catalyse the conversion of α-amino acids and peptides to the decarboxylated nitriles and aldehydes [53].

Thus, **61** was converted to *p*-methyloxyphenylacetonitrile **62**, and then to *p*-methoxyphenyl-acetoaldehyde **63** (Scheme 11).

5-Hydroxymethyl-furfural **64** was oxidized by a CPO from *C. fumago* to corresponding carboxylic acid **66,** but the major compound was found **65** [54]. Oxidation of the aldehyde to acid proceeds via direct oxygen transfer as indicated by complete incorporation of $H_2{}^{18}O_2$ (Scheme 12).

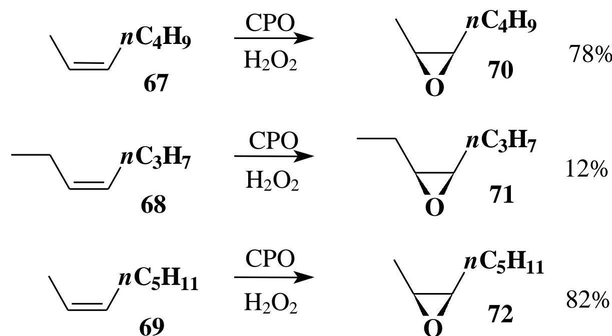


Scheme 11



Scheme 12

Epoxidation reactions



Scheme 13

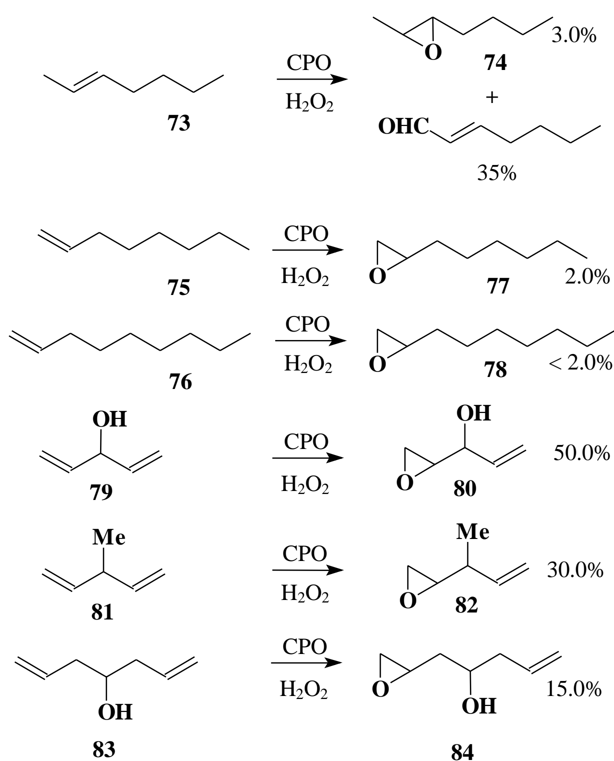
Asymmetric epoxidation is of fundamental importance not only from the synthetic point of view but also in biological systems. Optically active epoxides are very useful chiral synthons because they can give bifunctional compounds through stereospecific ring opening [55]. Native horseradish peroxidase usually does not catalyse the epoxidation reaction, whereas various mutants (F41L, F41T, F41A, H42V) lead to optically active styrene oxide derivatives [56,57]. The synthetic importance of this reaction is limited by the formation of large amounts of rearranged aldehydes as byproducts. Similar results are obtained in the epoxidation of styrenes catalysed by cytochrome-c peroxidases [58].

The CPO-catalysed epoxidation recently discovered by Colonna et al. [29] and Allain et al. [30] proceeds in high chemical and optical yields. Highly enantioselective epoxidation of the disubstituted alkenes **67** – **69** with hydrogen peroxide catalyzed by CPO provided the *R* epoxides **70** – **72** preferentially. All the data support the view of oxygen delivery from the ferryl oxygen directly to the substrates (Scheme 13).

Excellent enantioselectivity is observed in the CPO-catalysed epoxidation of short-chain *cis* alkenes with a chain length of nine or fewer carbon atoms, except for monosubstituted olefins, which often function as reversible suicide inhibitors of the enzyme [30,31,59]. *Trans* olefins **73** are highly unreactive substrates , *i.e.*,**74** is obtained in only 3% yield, [31] and terminal alkenes lead to heme alkylation and subsequent enzyme deactivation [60]. The epoxidation reaction can be optimized by using branched 1-alkenes [42].

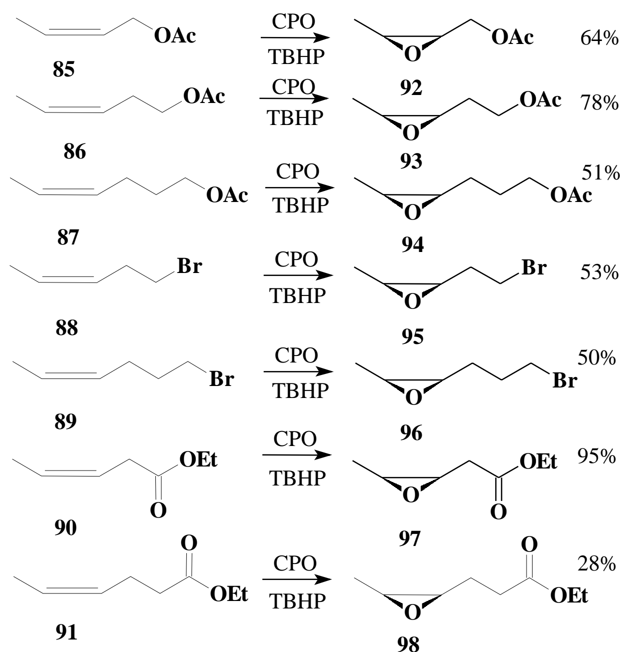
According to Allain et al. [30], terminal alkenes such as 1-heptene and 1-octene **73,75** were epoxidized very poorly and nonselectively (Scheme 14). Surprisingly, the oxidation of shorter terminal olefins such as C-5 prochiral dienes **79** and **81** proceeded much more efficiently and resulted exclusively in the formation of monoepoxides. The oxidation of 3-hydroxy-1-1,4-pentadiene **79** 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 [61]. The substitution of the substrate's 3-hydroxyl with the methyl group as in **81** 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 **83**) further decreases the diastereoselectivity of the epoxidation. It is apparent from the above results that the CPO 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.



Scheme 14

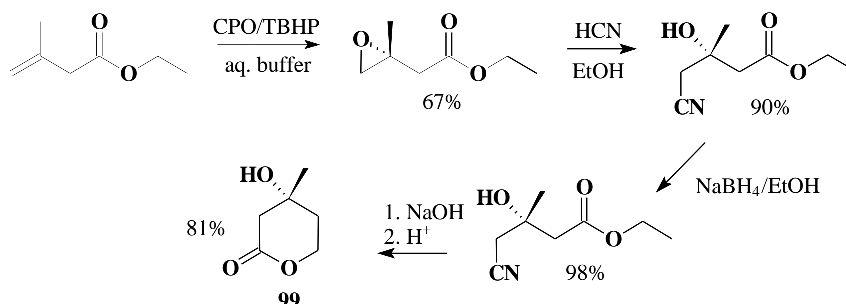
Asymmetric epoxidation of functionalized *cis*-2-alkenes **85** – **91** catalyzed by CPO using *tert*-butyl hydroperoxide (TBHP) as terminal oxidant to form the corresponding epoxides **92** – **98** (Scheme 15) [26]. Especially in large-scale reactions, the use of *tert*-butyl-OOH appears to be more effective than H_2O_2 because CPO is relatively sensitive to H_2O_2, losing activity rapidly in the presence of excess reagent.



Scheme 15

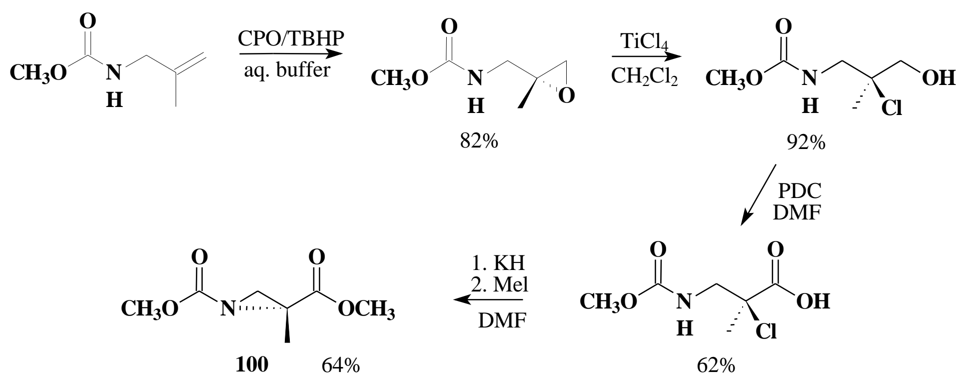
An important application of CPO as an enantioselective epoxidation catalyst is the efficient synthesis of (*R*)-(-)-mevalonolactone **99** (Scheme 16) [59]. A survey of the literature revealed that prior methods required many steps to produce the lactone, in low overall yield, with moderate enantiomeric excess, required expensive starting materials, or various combinations thereof. Meanwhile, a retrosynthetic analysis starting with an appropriately functionalized epoxide provided confidence that CPO could rescue the situation if used in the key stereogenic step.

Another more recently completed synthesis is depicted in Scheme 17. Again the epoxide is generated in high yield with conversion to (*R*)-dimethyl-2-



Scheme 16

methylaziridine-1,2-dicarboxylate **100** which may serve as a synthon for β-methylamino acids [62].



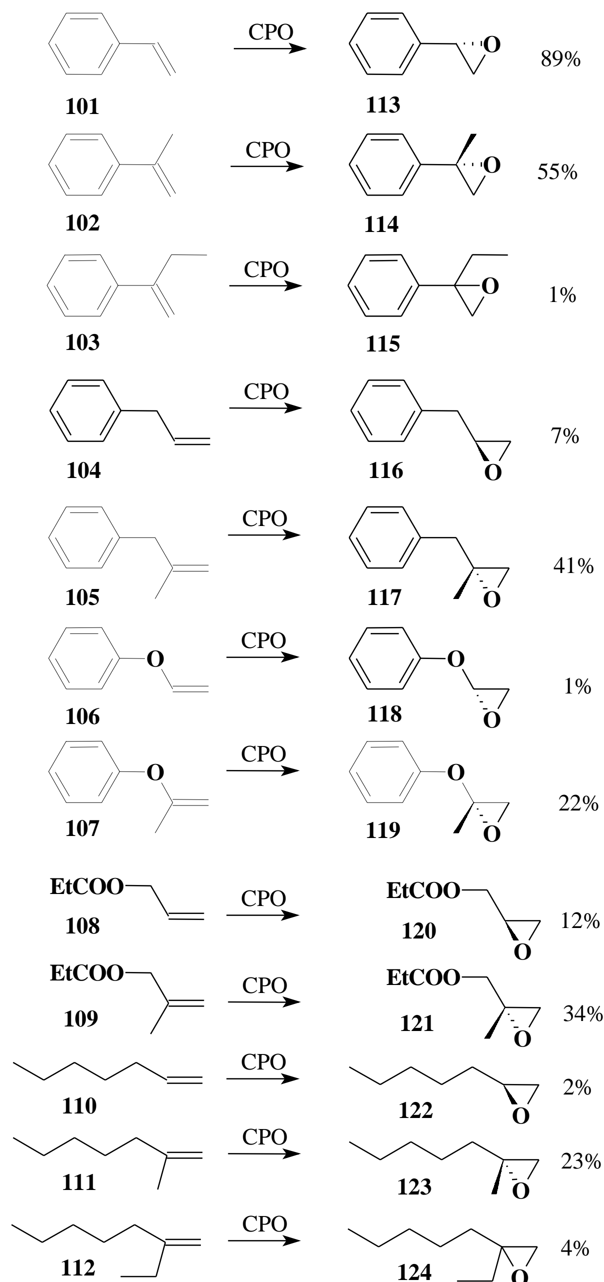
Scheme 17

Epoxidation of several monosubstituted olefins with CPO (Scheme 18, entries **101**, **104**, **106**, **108**, **110**) under conditions similar to those employed previously for *cis*-2-alkenes gave low catalytic turnovers (mol of epoxide / mol of enzyme), with poor to moderate enantioselectivities. While the highest turnover and enantioselectivity were obtained with styrene; in accordance with a previously published report [29], the ee was only moderate. Enzymatic oxidation of the remaining four monosubstituted olefins led to the formation of the green enzyme species similar to that previously reported for allylbenzene accompanied by low yields of epoxide with inferior enantioselectivity (10-46%).

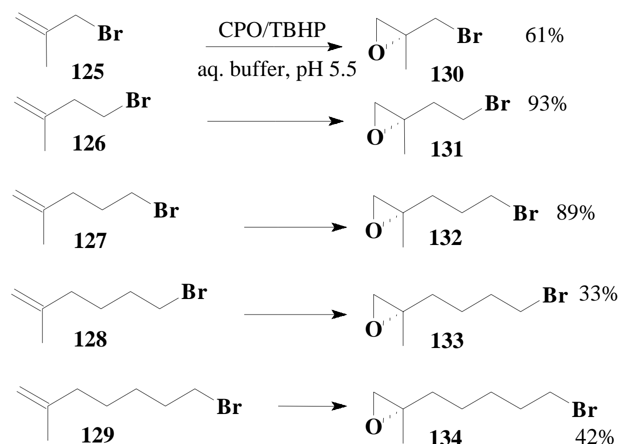
By contrast, epoxidation of matched 2-methylalkenes (Scheme 18, entries **102**, **105, 107**, **109**, **111**) showed a dramatic increase in both turnover and enantioselectivity. For the matched pair, allyl and methallyl propionate, an increase in catalytic turnovers of **103** with several orders of magnitude could be observed as a consequence of substitution of the double bond. Further, while the epoxidation of allyl propionate with CPO leads rapidly to the formation of an inactive green enzyme derivative, the formation of such a species during the epoxidation of methallyl propionate could not be detected. At the same time, the enantioselectivity increased from 24% with allyl propionate **108** to 94% with methallyl propionate **109**. Similar increases in both turnover and enantioselectivity were observed for each pair of matched olefins, except for styrene, in which substitution of the double bond led to a decrease in the turnover number accompanied by an increase in enantioselectivity.

High epoxidation enantioselectivity was observed in the former case, but the catalytic turnover declined for both to a level similar to those observed for monosubstituted alkenes **103** and **112**. For ethyl-substituted terminal olefins, it appears that the greater steric size begins to limit access of the olefin to the active site. Steric exclusion of the olefin from the active site is expected to promote catalase activity when peroxide is added to the enzyme reaction, leading to oxidative destruction of the catalytic heme in a formally alkene-independent process, and hence to reduction of epoxidation turnovers. Conversely, addition of facile olefin substrates to the reaction should act to protect the enzyme from autoxidative inactivation. It appears probable that this effect accounts for an almost linear increase in turnover number observed when the initial concentrations of facile substrates such as methallyl propionate are increased.

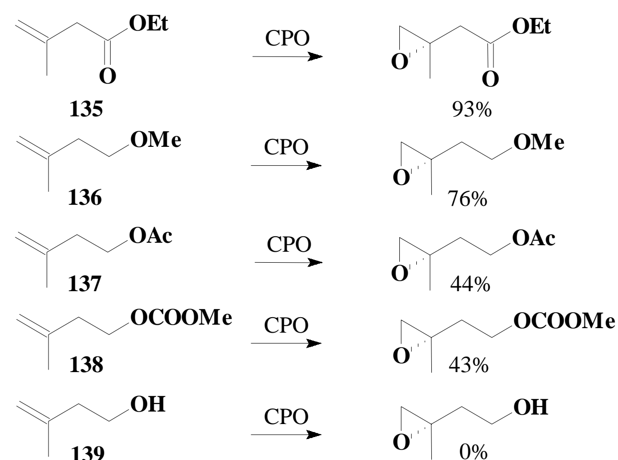
CPO mediated epoxidation of ω-bromo-2-methylalkenes **125** – **129** with enantioselectivity as a function of chain length to corresponding epoxides **130** – **134** was investigated [28]. ω-Bromo-2-methyl-1-alkenes were epoxidized under identical condition by using CPO from *C. fumago.* In all cases the predominant enantiomer produced was of the (*R*)-configuration, except 3-bromo-2-methylpropene oxide **126** which was predominantly *S* only because of priority switch. The enantiomer of this latter compound was synthesized from commercially available (*S*)-methylglycidol to confirm its tereochemistry. Substrate selectivity was approached by observing the effect of chain length of ω-bromo-2-methylalkenes on substrate conversion. Entries **125** and **126** in Scheme 19 were entirely converted to products **130** and **131**,



Scheme 18



Scheme 19



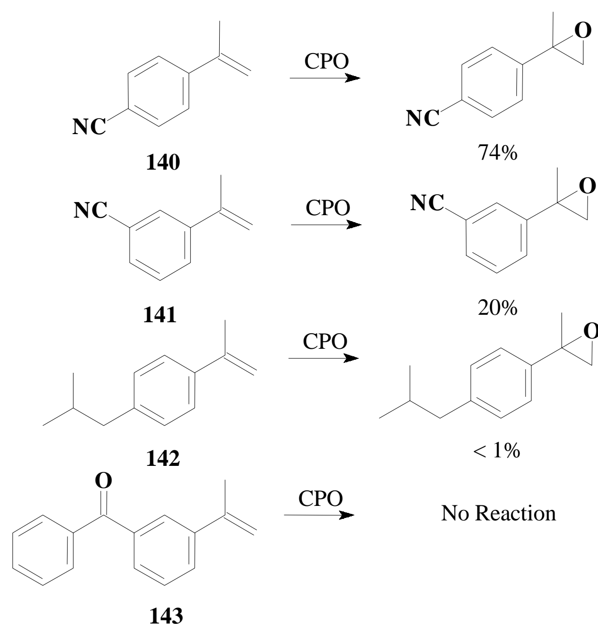
Scheme 20

since no starting material could be observed in reaction mixture extracts. Entries **127** - **129** illustrate a rapid decline in conversion with each additional carbon. For these latter three substrates that failed to convert completely, some attempts were made to increase conversions. Doubling the initial quantity of CPO did not improve conversion.

Lakner and Hager [59] have reported the epoxidation of **135** – **139** alkenes to corresponding epoxides. High yields were obtained for **135**, while epoxide **139** was not converted (Scheme 20).

α-Methylstyrene **102** is a somewhat slower CPO substrate than styrene but with a respectable yield (55-89%) of the corresponding epoxide. Overoxidation to acetophenone predominates unless O_2 is removed. The reaction mixture may be purged with N_2 and sealed under which conditions good results are obtained. Using a suitably substituted α-methylstyrene **102** non-steroidal anti-inflammatory aryl-propionic acids could be synthesized. Electron-donating substituents are to be avoided because of their tendency to promote solvolysis and rearrangement reactions. The cyano group is sterically and electronically admissible, though it was discovered (Scheme 21) that the resulting epoxide possesses moderate 74% ee for *p*-cyano **140** to low 20% *ee* for *m*-cyano **141**. Compound **142** forms less than 1% epoxide, and **143** has not reacted with CPO [27].

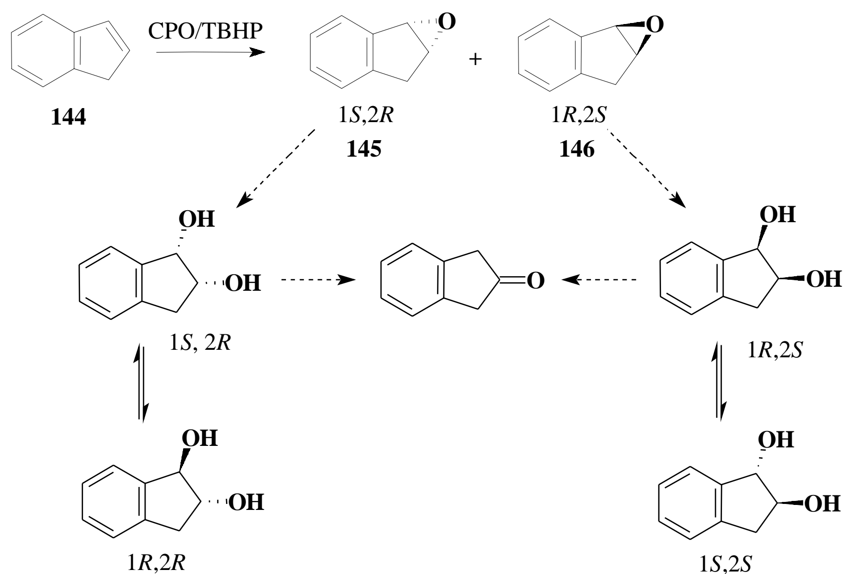
The stereochemistry of the CPO catalysed epoxidation of indene **144** has been reported [63]. In aqueous solution the intial epoxide is not stable and opens to form the *cis-trans* diols. When the reaction was



Scheme 21

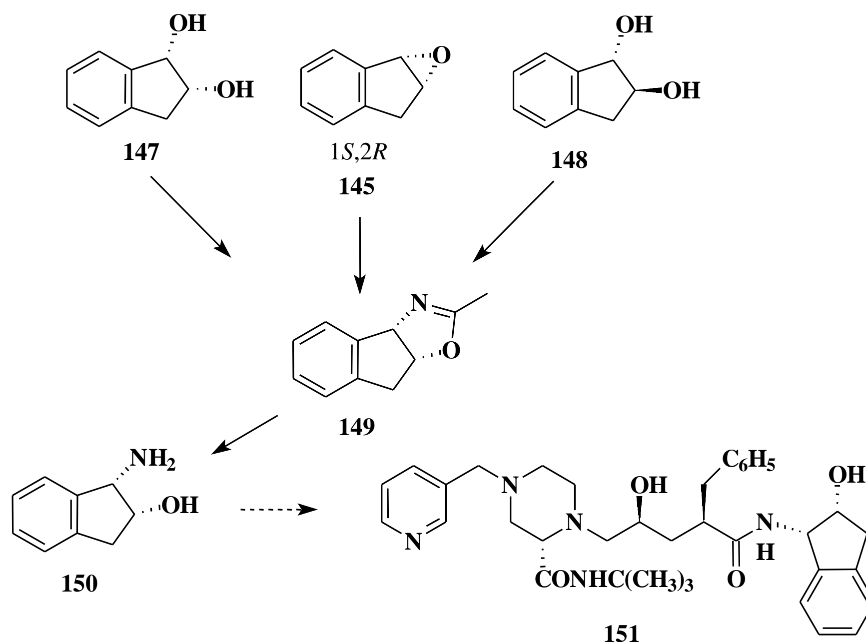
carried out in the absent of water, the epoxide enantiomers **145** and **146** were isolated, with the 1*R*, 2*S* enantiomer **145** being formed in 30% *ee* (Scheme 22).

1*S*,2*R*-Indene oxide **145** is the precursor of *cis*-1*S*,2*R*-aminoindanol **150**, a key intermediate of the Merck HIV–1 protease inhibitor, Crixivant **151** [64, 65]. As an alternative to the challenging chemical synthesis of this chiral epoxide from indene, the biotransformation route using an enzyme catalyst has been reported [66]. The products were generally racemic *trans*-bromoindanols **147** and **148,** which upon basification yielded racemic epoxides (Scheme 23). It was found that a crude enzyme preparation from the fungal culture *Curvularia protuberata* MF5400 converted indene to the chiral 2*S*,1*S*-bromoindanol **148** which could be chemically converted to the desired 1*S*,2*R*-epoxide through basification or used directly in the asymmetric synthesis of *cis*-1*S*,2*R*-aminoindanol **150**. The bioconversion rate and the enantiomeric excess (*ee*) achieved with this cell-free system were heavily pH dependent. An initial reaction at pH 7.0 gave - 10% yield of the chiral bromoindanol or epoxide from indene, and the yield was rapidly improved to 30% of *trans*-2*S*,1*S*-bromoindanol with an *ee* of 80%. Reaction mechanistic studies revealed that the stereoselectivity observed was apparently due to a specific dehydrogenase activity present in MF-5400, which was also found to resolve chemically synthesized racemic *trans*-2-bromoindanols.



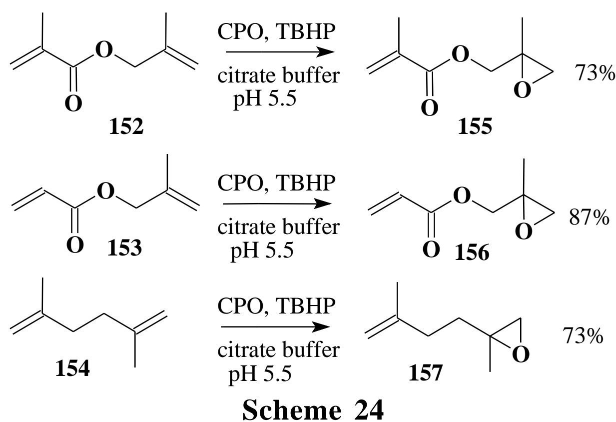
Scheme 22

Selective epoxidation of dienes by CPO from *C. fumago* has been reported [67]. The methacrylate **152** was a good substrate, which showed two types of selectivity: only the isolated double bond was epoxidized to produce monoepoxide **155** in 73% yield, and the conjugated α,β-unsaturated bond of the methacrylic acid moiety was untouched as shown in Scheme 24; the enantioselectivity was high yield. It suggested that conjugated terminal olefins might have



Scheme 23

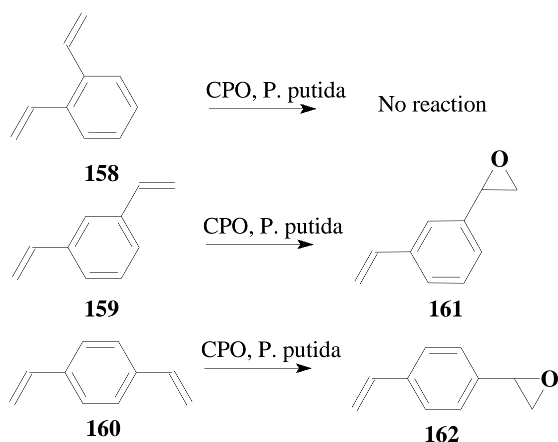
a low effect on the inhibition of CPO activity compared to other aliphatic terminal alkenes to give an inactive derivative in which the active heme site is *N*-alkylated [60]. Indeed, acrylate **153** was an excellent substrate for CPO epoxidation and selectively afforded the monoepoxide **156** (Scheme 24) in high yield and excellent enantioselectivity (87% yield). This is complementary to the epoxidation of the α,β-unsaturated double bond in enones using synzymes, viz polyleucine where the epoxidation takes place exclusively at the α,β-unsaturated double bond [68-70]. It has further proposed that CPO-catalyzed epoxidation should produce only monoepoxides from symmetrical dienes. This indeed was the case. When dimethylhexadiene **154** was used as a model substrate, biocatalytic epoxidation afforded exclusively the monoepoxide **157** as a unique product (Scheme 24).



Scheme 24

The oxidase systems from the bacteria *Pseudomonas* sp., such as xylene oxygenases catalyze the epoxidation of styrene to styrene oxide with high enantioselectivity [71,72]. CPO is a versatile and efficient biocatalyst that catalyzes a variety of reactions, particularly asymmetric epoxidation and hydroxylation [30,31,42].

The selective epoxidation of aryl dienes catalyzed by oxidases from *Pseudomonas putida* and the epoxidation of unsaturated acrylate derivatives catalyzed by CPO [67] has been reported. Commercially available divinylbenzene which are a mixture of three isomers **158** (*ortho* - 8%), **159** (*meta* - 52%) and **160** (*para* - 40%) were first used as a model substrates for the investigation of selective epoxidation catalyzed by oxidases from *P.putida*. For *para*- **160** and *meta*- **159**, the oxidases from *P.putida* demonstrated two types of selectivities. First, the reaction stopped at the monoepoxide stage, with hardly any diepoxide and other products were detected or isolated from the reaction medium. Second, the epoxidation reactions showed excellent enantioselectivities (*ee* 95% for *para*- **160**, *ee* 94% for *meta*- **159**). Very surprisingly, the *ortho*-isomer **158** is not a substrate for the oxidases from *P.putida* (**Scheme** 25). In contrast to *para*- and *meta*-divinylbenzenes, *para*- and *meta*-allylstyrenes are not substrates for the oxidases from *P.putida* or *P.oleovorans*, which suggested that these enzymes showed very high substrate specificities [27].



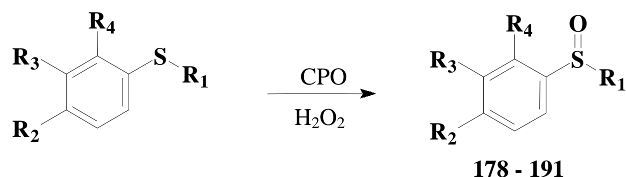
Scheme 25

Reactions of Sulfoxidation

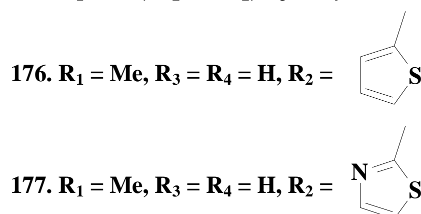
The haem-containing CPO from *C. fumago* has been shown to catalyse enantioselective sulfoxidation [73-76]. A number of peroxidases, in particular CPO, mediate the clean oxidation of dialkyl sulfides **163** – **177** to the corresponding sulfoxides **178** - **191**, without any further reaction to the sulfone (Scheme 26). The reaction is often performed to demonstrate the oxygen-transfer capabilities of peroxidases [4,5,27] and has also served, as a test-bed for reaction procedures. Hydrogen peroxide has almost universally been used as the oxidant. The slow, uncatalysed, oxidation that takes place in the background can be reduced to a minimum by keeping the hydrogen peroxide concentration as low as possible.

CPO mediates the oxidation of phenyl methyl sulfide, thioanisole, **163**, at a turnover frequency of 200 s^{-1} under saturating condition [77]. In a practical procedure, in which the catalyst was deliberately starved of hydrogen peroxide to extend its lifetime, the average turnover frequency was 10-times lower, near 15-20 s^{-1} [77]. Substituting the phenyl ring in **163** in the *meta*- or *para*-position with small electron donating or withdrawing substituents reduced the reaction rate but the *ortho*-methoxy derivative of **163** hardly reacted at all [77]. CPO is very sensitive to the size of the R_1 group in **163** - **174**; increasing its size from methyl to ethyl **164** had only a slight effect on the rate but the propyl sulfide **165** reacted quite sluggishly [77].

Lee et al. [78] studied stereopecific sulfoxidation of aryl alkyl sulfides **192** by purified toluene dioxygenase (TDO) from *Pseudomonas patida*, and shown that sulfoxidation yielded (*S*)-sulfoxides **193** in 60-70%, and sulfoxidation by CPO under the same

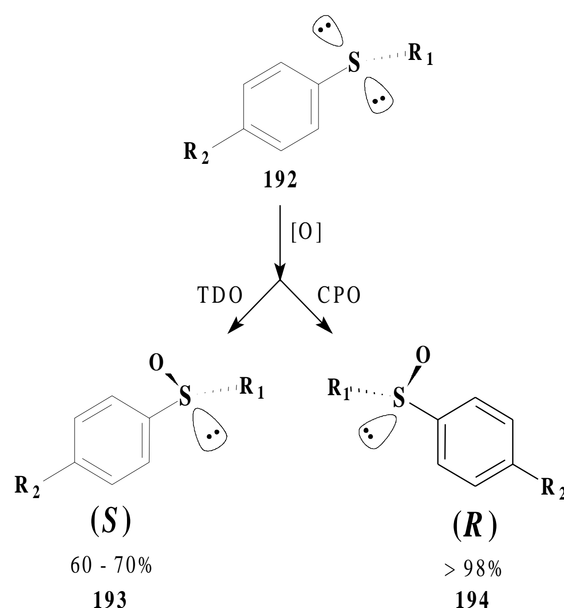


163. R_1 = Me, R_2 = R_3 = R_4 = H
164. R_1 = Et, R_2 = R_3 = R_4 = H
165. R_1 = *n*-Pr, R_2 = R_3 = R_4 = H
166. R_1 = Me, R_2 = Me, R_3 = R_4 = H
167. R_1 = Me, R_2 = OMe, R_3 = R_4 = H
168. R_1 = Me, R_2 = R_4 = H, R_3 = OMe
169. R_1 = Me, R2 = R_3 = H, R_4 = OMe
170. R_1 = Me, R_2 = Cl, R_3 = R_4 = H
171. R_1 = Me, R_3 = Cl, R_2 = R_4 = H
172. R_1 = Et, R_2 = Cl, R_3 = R_4 = H
173. R_1 = Me, R_2 = Br, R_3 = R_4 = H
174. R_1 = Me, R_3 = Br, R_2 = R_4 = H
175. R_1 = Me, R_2 = NO_2, R_3 = R_4 = H



Scheme 26

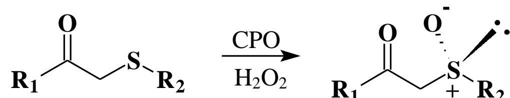
conditions yielded more than 98% (*R*)-sulfoxides **194** (Scheme 27).



Scheme 27

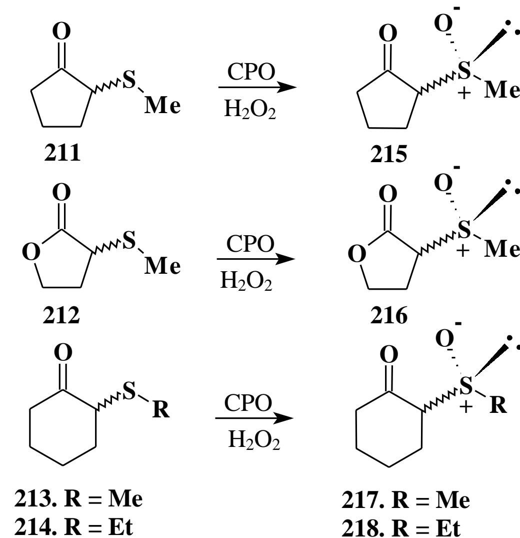
CPO catalyzed oxidation of a series of β-carbonyl sulfides **195** – **202** and **211** – **214** to corresponding sulfoxides **203** – **210** and **215** – **218** has been stud-

ied at room temperature in aqueous citrate buffer pH 5.0 at 25°C [79]. For dialkyl β-carbonyl sulfides, the products with methyl and ethyl substituents are obtained in ca. 100% yield (Scheme 28). However when the alkyl group is *n*-propyl **197** or *iso*-propyl **198** the yield drops dramatically (25%). An aryl sulfide derivative afforded product in very low yield (4%), but when the phenyl group bears a carbonyl, and the sulfur substituents are methyl or ethyl, the oxidation occurs with high yields (91–95%).



203 - 210

195. R_1 = Me, R_2 = Me
196. R_1 = Me, R_2 = Et
197. R_1 = Me, R_2 = *n*-Pr
198. R_1 = Me, R_2 = *i*-Pr
199. R_1 = Me, R_2 = Ph
200. R_1 = Ph, R_2 = Me
201. R_1 = *p*-Me-C_6H_4, R_2 = Et
202. R_1 = OC_2H_5, R_2 = Me

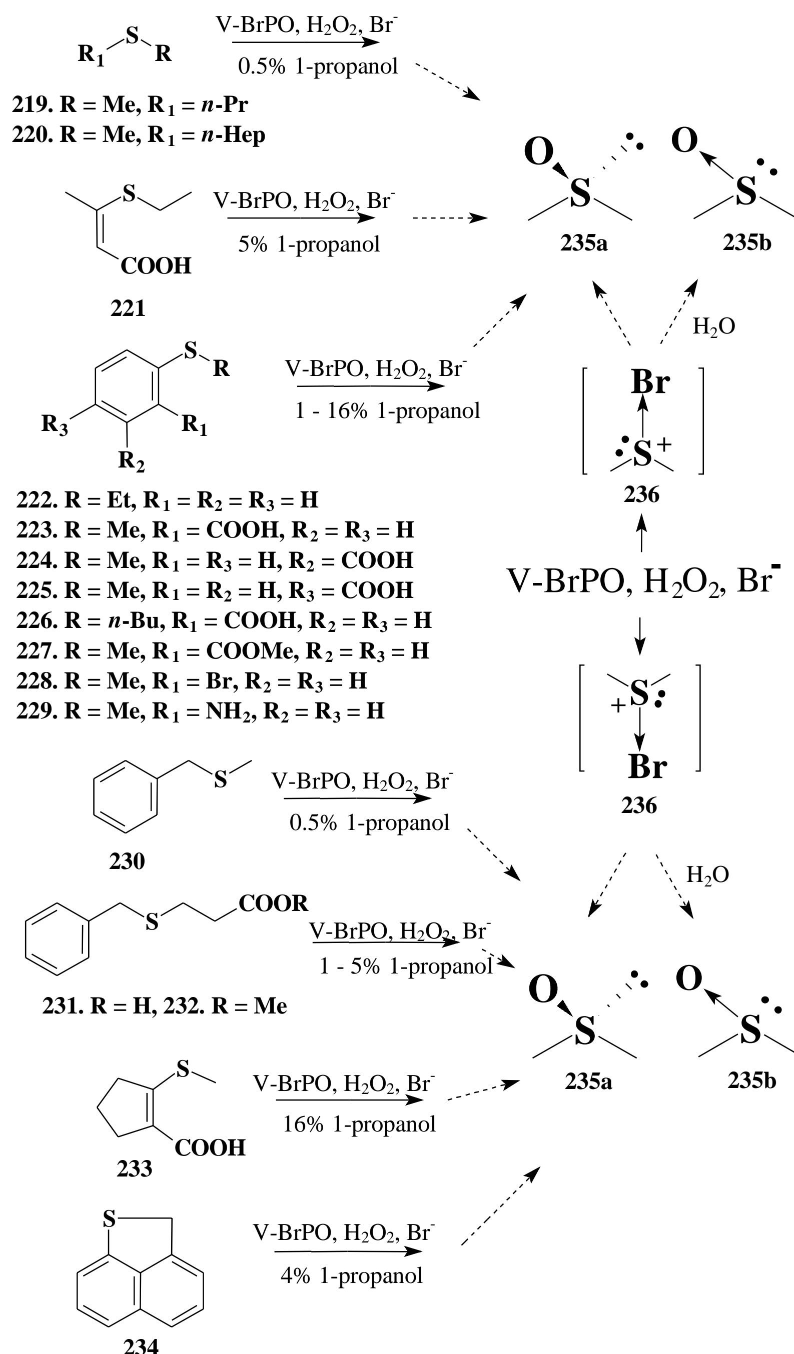


Scheme 28

Steric control of the sulfoxidation reaction is also confirmed with cyclohexanone derivatives, although a low product yield is observed even at high enzyme concentrations. Noteworthy are the yields obtained with cyclopentanone sulfide **211** (65%) and an unexpected quantitative yield obtained with the γ-butyrolactone sulfide **212**.

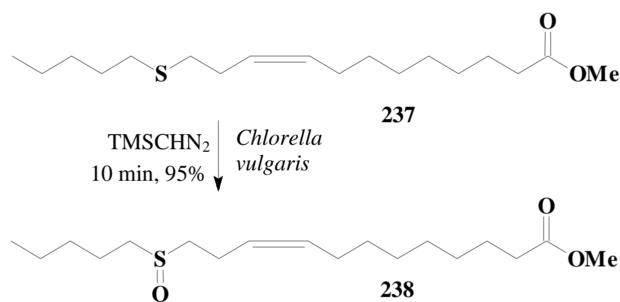
Experiments with taseries of racemic cyclic carbonyl sulfides of differing size (compounds **211**, **212**, **213**, **214**) confirmed a positive influence on the product yields of a small size [80] to fit the CPO heme cleft. Indeed, substrates **213** and **214** being bulkier than substrate **211** gave a twofold lower yield than the smaller substrate. An effect of a carbonyl group in the β-position was observed by Allenmark and Andersson [81] when 2,3-dihydrobenzo[*b*]thiophene and benzo[*b*]thiophen-3-one were oxidized with CPO yielding 99.5% and 7% sulfoxide product, respectively. Unexpectedly the γ-butyrolactone sulfide **212** afforded the corresponding sulfoxide **216** in quantitative yields, indicating that an oxygen atom neighbor to the carbonyl completely altered the enzyme selectivity. Oxidation of racemic substrate **213** with 30% H_2O_2 in acetic acid gave 70% d.e. sulfoxide **217**, albeit without optical activity. A similar result was reported elsewhere when the chiral sulfide **213** was oxidized with an oxaziridine derivative **217**: 70% *de* product was obtained [82]. The α-sulfinyl cyclic ketones **215**, **217** and **218** or lactone **216**,containing an α-hydrogen, are known to exhibit a keto-enol tautomerism in organic solution, and therefore substrate enolization may be responsible for the observed *de*. [83]. Thus, kinetic resolution of the cyclic carbonyl sulfides must be occurring to explain the *ee*, but product enolization leads to *de* loss.

Asymmetric sulfoxidation catalyzed by a vanadium bromoperoxidase from the red alga *Carollina officinalis* of a series of prochiral sulfides **219** – **234** having a *cis*-positioned carboxyl group **221**, **223**-**227**, **231**-**233** were oxidize rapidly, giving the sulfoxide **235** more than 95% *ee* (Scheme 29) [84]. The pH-rate profile shows a typical sharp sigmoidal curve, indicative of a deprotonation event at around pH 6.4. The corresponding, non-protolytic, methyl esters were not catalysed by the enzyme. Rapid loss of stereoselectivity was found to occur when V-BrPO-catalyzed oxidation was carried out in the presence of bromide ions. This has been interpreted as being due to the intervention of a competing reaction involving oxidation of bromide and the subsequent formation of a bromosulfonium ion intermediate **236**. Favoured oxidation of bromide is a subsequent step of sulfide bromination, leading to racemic sulfoxide via rapid halogen exchange in a bromosulfonium ion **236**. The formation of optically active sulfoxide at low bromine ion concentration will be due to either asymmetric sulfoxidation (type **235a**) or a slow halogen exchange in the bromosulfonium ion. The enantioselectivity was much less influenced by the presence of chloride ions, due to the low capability V-BrPO to oxidize halides more electronegative than bromide [85].



Scheme 29

The synthetic analogue of oleic acid, 13-thiaoleic acid, methyl ester **237**, was readily oxidised to the corresponding S-oxide **238** by the crude extract of alga *Chlorella vulgaris* (Scheme 30) [86].

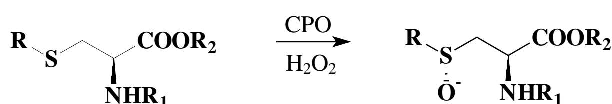


Scheme 30

The preparation of methionine sulfoxides and S-alkylcysteine sulfoxides with defined stereochemistry at sulfur has been achieved in low yields by chemical resolution. All the stereoisomers of methionine and ethionine sulfoxides have been prepared via biotransformation reactions involving the conversion of

protected amino acid substrates to the corresponding sulfoxides by *Beauveria bassiana* or *Beauveria caledonica*, while the selective formation of predominantly (*R*)-sulfoxides by sulfur oxidation of protected methionine substrates by CPO, and of the (*S*)-sulfoxide from S-allylcysteine by the enzyme cyclohexanone monooxygenase have been reported [87-91].

Treatment of N-methoxycarbonyl C-carboxylate ester derivatives of S-methyl-l-cysteine by CPO/H_2O_2 resulted in oxidation at sulfur to produce the (R_S)-sulfoxide in moderate to high diastereomeric excess. CPO's from *C. fumago, Beauveria bassiana* and *Beauveria caledonica* catalysed oxidation of S-alkyl-L-cysteine derivatives **238** - **246**, with the result that maximum yield and diastereomeric excesses were obtained with the N-MOC C-carboxylate ester derivatives **247 – 251, 252 - 255** (**Scheme** 31) [92]. The S-ethyl substrate **244** was also acceptable for CPO-catalysed oxidation, but the larger S-alkyl or S-alkenyl substrates **245** and **246** were not derivatives.



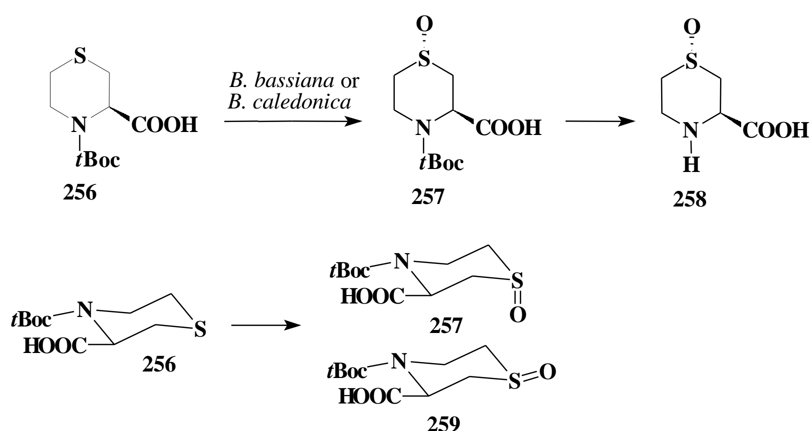
238. R = R$_2$ = Me, R$_1$ = MOC **247 - 255**
239. R = Me, R$_1$ = MOC, R$_2$ = Et
240. R = Me, R$_1$ = MOC, R$_2$ = *n*-Pr
241. R = Me, R$_1$ = MOC, R$_2$ = *n*-Bu
242. R = Me, R$_1$ = MOC, R$_2$ = *n*-Pen
243. R = Me, R$_1$ = ClAc, R$_2$ = Me
244. R = Et, R$_1$ = MOC, R$_2$ = Me
245. R = *n*-Pr, R$_1$ = MOC, R$_2$ = Me
246. R = Allyl, R$_1$ = MOC, R$_2$ = Me

Scheme 31

The (S_S) natural product sulfoxide chondrine **258** was obtained via biotransformation of the N-*t*boc derivative of L-4-(*S*)-morpholine-2-carboxylic acid **256** using *Beauveria bassiana* or *Beauveria caledonica*. The sulfoxidation of substrate **256** was performed by a *Beauveria* species (Scheme 32) in order to produce **257**, an intermediate in the synthesis of the natural product chondrine, **258** [92]. The configuration of the resulting sulfoxide was assigned as the axial (S_S) isomer **257** in preference to the equatorial sulfoxide **259**. The absolute configuration of the biocatalysis product **257** was reconfirmed by removal of the *t*-boc protecting group to give the natural product chondrine **258** possessing the (S_S) sulfoxide (**Scheme** 32).

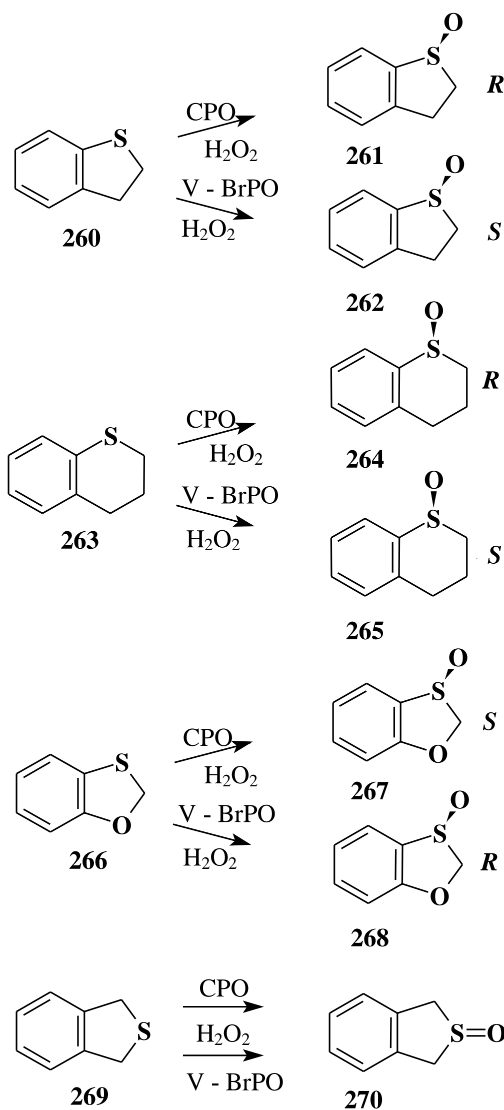
V–BrPO from the coralline red alga *Corallina officinalis* oxidizes several bicyclic sulfides to the corresponding sulfoxide with a high enantioselectivity, up to 91% in the absence of an added halide source. Also 2,3-Dihydrobenzothiophene **260**, thiochroman **263**, 1,3-benzoxathiole **266** and 1,3,dihydrobenzo-[*c*]thiophene **269** are all oxidized to the corresponding sulfoxide as shown in Scheme 33. With the exception of **269** which is symmetrical, the oxidation of all the other substrates occurs stereospecifically. The sterochemical orientation of all sulfoxide products of **262, 265**, and **268** are the same (Scheme 33), however, the *R* identity for the sulfoxide product of **268**, compared to the *S* identity of the sulfoxide product of **262** and **265** is a result of the nomenclature rules.

Asymmetric sulfoxidation by means of a CPO from *Caldariomyces fumago* and H_2O_2 as the oxygen source was studied for a series of sterically well-defined substrates. The stereochemistry of the sulfoxidation was the same for all substrates studied. While 2,3-dihydrobenzo[*b*]thiophene **260** is an excellent substrate giving 99.5% yield of the (*R*)-sulfox-



Scheme 32

ide, replacement of a methylene group by either a more sterically demanding group or a heteroatom caused a substantial decrease in reactivity or in reactivity as well as enantioselectivity. For the oxidation of thiochroman **263** and 1,3-benzoxathiole **266** by the CPO from *Caldariomyces fumago* gave (*R*)-**264** and (*S*)-sulfoxide **267** (Scheme 33) [93]. Chloroperoxidase was also found to be an effective catalyst in the oxidation of labile episulfides yielding the corresponding anti-sulfoxides quantitatively and in the oxidation sulfides (Scheme 33).



Scheme 33

Distribution of haloperoxidases in nature

In the last decade peroxidases, notably CPO from *Caldariomyces fumago,* have been shown to catalyze a wide variety of synthetically useful (enantioselective) oxygen transfer reactions with H_2O_2 [27,54, 77,94], *e.g*., asymmetric epoxidation of olefins [30,42,59], benzylic, propargylic, and allylic hydroxylation [26,31], asymmetric sulfoxidation [4,61,74,77], and oxidation of indoles to the corresponding 2-oxindoles [44-46]. However, a major shortcoming of all heme-dependent peroxidases, such as CPO, is their low operational stability [77], resulting from facile oxidative degradation of the porphyrin ring. In contrast, vanadium haloperoxidases, such as vanadium CPO from *Curvularia inaequalis* [18,35] are non-heme enzymes and, hence, are much more stable. Unfortunately, the active site of vanadium-dependent haloperoxidases can accommodate only very small substrates, such as halide ion, which severely curtails their utility. Nevertheless, enantioselective sulfoxidation was catalyzed by vanadium-dependent bromoperoxidases from *Corallina officinalis* [84] and *Ascophyllum nodosum* [8-10].

Haloperoxidases have been isolated from many natural sources (see Table 1). In addition haloperoxidase activity has been detected in many algal species [51] in other marine invertebrates, and microorganisms. One of the most interesting, yet unsolved problems in the area of terrestrial and marine halogenation, is the biogenesis of the chiral halogenated natural products [1]. This is brief review demonstrated that the haloperoxidases are successful reagents in organic synthesis.

Table 1
Some natural sources of haloperoxidases

Bromoperoxidases	Reference	*Microorganisms*	Reference
		Streptomyces aureofaciens	[120]
Marine Green Algae (Phylum Chlorophyta)		*Streptomyces griseus*	[118]
Halimeda sp.	[111]	*Streptomyces venezuelae*	[119]
Penicillus capitatus	[105,106,108]	*Streptomyces phaeochromogenes*	[121]

Table 1
Continued

Bromoperoxidases	**Reference**	***Microorganisms***	**Reference**
Penicillus lamourouxii	[105]	*Shigella flexneri*	[123]
Rhipocephalus phoenix	[105]	*Salmonella enterica ser. typhimurium*	[123]
Ulvella lens	[109]	*Pseudomonas aureofaciens*	[122]
		Pseudomonas putida IF-3	[124]
Marine Red algae (Phylum Rhodophyta)			
Ceramium rubrum	[110]	**Chloroperoxidases**	
Corallina pilulifera	[96-98]		
Corallina officinalis	[99,100]	***Fungus***	
Corallina vancouveriensis	[101]	*Aspergillus flavus*	[125]
Cystoclonium purpureum	[114]	*Caldariomyces fumago*	[14]
Rhodomela larix	[107]	*Curvularia inaequalis*	[126]
Ochtodes secundiramea	[138]	*Embellisia didymospora*	[127]
		Fusarium oxysporum	[128]
Marine Brown Algae (Phylum Phaeophyta)		*Phanerochaete chrysosporium*	[137]
Alaria esculenta	[112]		
Ascophyllum nodosum	[95]	***Microorganisms***	
Chorda filum	[113]	*Saccharomyces cerevisiae*	[129]
Ecklonia stolonifera	[115]	*Serratia marcescens*	[134]
Fucus distichus	[102]	*Streptomyces lividans*	[130]
Laminaria digitata	[103]	*Streptomyces toyocaensis*	[132]
Laminaria hyperborea	[104]	*Pseudomonas fluorescens*	[94]
Laminaria ochroleuca	[104]	*Pseudomonas pyrrocinia*	[131]
Laminaria saccharina	[103,104]	*Rhodococcus erythropolis*	[136]
Macrocystis pyrifera	[102]		
		Marine Invertebrates	
Marine Invertebrates		*Notomastus lobatus*	[116,133]
Notomatus lobatus	[116]		
Thelepus setosus	[107]	**Haloperoxidases**	
Ptychodera flava laysanica	[107]	***Freshwater algae***	
		Cladophora glomerata	[135]
Lichens			
Xanthoria parietina	[17]	***Marine Invertebrates***	
		Amphitrite ornata	[133]

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