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 FADH₂, which can be produced from FAD and NADH by unspecific flavin reductases. The FADH₂-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-peroxidase (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-
nadium-containing peroxidases that require a vadan-
dium ion (VO$_4^{3-}$). Vanadium peroxidase was first dis-
covered in the brown alga Ascophyllum nodosum in
1984 [16] but since then it is also been found in li-
chen [17] and in fungi [18].

The preparation of chiral compounds in non-ra-
cemic form is a goal of great interest in organic syn-
thesis, due to the large application that these com-
ounds 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 stereo-
selective discrimination of alcohol functions so as to
achieve polyhydroxylated compounds in enantiopure
form [20-24]. The enantioselective direct introduc-
tion of oxygen onto olefins with biocatalysis by halo-
peroxidases, in oxygenase-type reactions, is very use-
ful and effective for this purpose [25]. In particular
the use of Caldariomyces fumago CPO is especially
advantageous, since this usually involves peroxides
(H$_2$O$_2$ or ROOH), without requiring expensive co-
factors. Moreover, due to its broad substrate accep-
tance, this CPO has great synthetic potential and has
allowed the stereoselective epoxidation and the hy-
droxylation of a wide range of olefins in satisfactory
yield and with high enantiomeric excess [26-31].

Current interest in catalytic oxidative transforma-
tions in industry is governed 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 oxy-
gen as the oxidant. A second major issue is the need
for high chemo-, regio- or enantioselectivities 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 oxi-
dation 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 re-
view 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 investi-
gate the enzyme kinetic mechanism of the vanadium
haloperoxidases [33-35].

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 hy-
drogen peroxide or t-butyl hydroperoxide as shown
below (Scheme 2). The CPO propargylic oxidation
of alkynes to aldehydes proceeds via an alcohol in-
termediate 3. When propargylic alcohols were incu-
bated with CPO in the presence of H$_2$O$_2$ [37], it was
observed that the alcohols were completely and rap-
idly 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 se-
lectivity 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-cyclo-
hexadiene is catalyzed by a CPO from Caldario-
myces fumago [41]. The process occurs enantio-
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 in the reaction mixture, it is plausible that in the first step in the mechanism involving CPO and undergoes fast nucleophilic attack by water, with partial rearrangement, giving the trans diols 16 and 17, respectively.

The substrate specificity of CPO from Caldoromyces 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 benzoic acid. Small amounts of benzyl alcohol detected during the early stages of the reaction disappeared due to oxidation to benzoic acid. 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-
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-[2H]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-1,2-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.

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

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 terephthaliccarboxaldehyde, 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].

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].

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].

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Thus, 61 was converted to \( p \)-methoxyphenylacetanilide 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 \( \mathrm{H}_2^{18} \)O2 (Scheme 12).

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**Epoxidation reactions**

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 \( \text{cis} \) 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]. \( \text{Trans} \) 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-1,4-pentadiene 79 proceeded with a high degree of diastereoselectivity (98%) and modest enantioselectivity (65%). The predominant product (2S,3R)-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.

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 H2O2 because CPO is relatively sensitive to H2O2, losing activity rapidly in the presence of excess reagent.

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

![Chemical structures and reactions]

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-methyl-alkenes (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 autooxidative 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-methyl-alkenes 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,
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 O₂ is removed. The reaction mixture may be purged with N₂ 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 initial epoxide is not stable and opens to form the cis-trans diols. When the reaction was
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carried out in the absence of water, the epoxide enantiomers 145 and 146 were isolated, with the 1R, 2S enantiomer 145 being formed in 30% ee (Scheme 22).

1S,2R-Indene oxide 145 is the precursor of cis-1S,2R-aminoundanol 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 2S,1S-bromoindanol 148 which could be chemically converted to the desired 1S,2R-epoxide through basification or used directly in the asymmetric synthesis of cis-1S,2R-amino-

indanol 150. The bioconversion rate and the enantio-meric 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-2S,1S-bromoindanol with an ee of 80%. Reaction mechanistic studies revealed that the stereoselectivity observed was apparently due to a specific dehydrogenase activity present in MF5400, which was also found to resolve chemically synthesized racemic trans-2-bromoindanols.

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 monooepoxide 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
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 \( \alpha,\beta \)-unsaturated double bond in enones using synzymes, viz polyleucine where the epoxidation takes place exclusively at the \( \alpha,\beta \)-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).

The oxidase systems from the bacteria \emph{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 \emph{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 (\emph{ortho} - 8%), 159 (\emph{meta} - 52%) and 160 (\emph{para} - 40%) were first used as a model substrates for the investigation of selective epoxidation catalyzed by oxidases from \emph{P.putida}. For \emph{para} 160 and \emph{meta} 159, the oxidases from \emph{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 (\textit{ee} 95% for \emph{para} 160, \textit{ee} 94% for \emph{meta} 159). Very surprisingly, the \emph{ortho}-isomer 158 is not a substrate for the oxidases from \emph{P.putida} (Scheme 25). In contrast to \emph{para}- and \emph{meta}-divinylbenzenes, \emph{para}- and \emph{meta}-allylstyrenes are not substrates for the oxidases from \emph{P.putida} or \emph{Poleovorans}, which suggested that these enzymes showed very high substrate specificities [27].
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 R₁ 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 stereospecific sulfoxidation of aryl alkyl sulfides 192 by purified toluene dioxygenase (TDO) from *Pseudomonas putida*, and shown that sulfoxidation yielded (S)-sulfoxides 193 in 60-70%, and sulfoxidation by CPO under the same conditions yielded more than 98% (R)-sulfoxides 194 (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 \( \beta \)-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%).

![Image](Image.png)

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 \( \gamma \)-butyrolactone sulfide 212.

Experiments with a series 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 \( \beta \)-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 \( \gamma \)-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% \( \text{H}_2\text{O}_2 \) 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% \( \text{d.e} \) product was obtained [82]. The \( \alpha \)-sulfinyl cyclic ketones 215, 217 and 218 or lactone 216, containing an \( \alpha \)-hydrogen, are known to exhibit a keto-enol tautomerism in organic solution, and therefore substrate enolization may be responsible for the observed \( \text{d.e} \) [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 oxidized 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 chloride 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].
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].

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 predominately *(R)-sulfoxides by sulfur oxidation of protected methionine substrates by CPO, and of the *(S)-sulfoxide from S-allylcysteine by the enzyme cyclohexane monoxygenase have been reported [87-91].

Treatment of N-methoxycarbonyl C-carboxylate ester derivatives of S-methyl-L-cysteine by CPO/H\(_2\)O\(_2\) resulted in oxidation at sulfur to produce the *(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.

\[
\begin{align*}
\text{R} & \quad \text{S} \quad \text{NHR}_1 \quad \text{COOR}_2 \quad \xrightarrow{\text{CPO/} \text{H}_2\text{O}_2} \quad \text{R} \quad \text{S} \quad \text{NHR}_1 \quad \text{COOR}_2 \\
238. & \quad \text{R} = \text{R}_2 = \text{Me}, \text{R}_1 = \text{MOC} \\
239. & \quad \text{R} = \text{Me}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{Et} \\
240. & \quad \text{R} = \text{Me}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{n-Pr} \\
241. & \quad \text{R} = \text{Me}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{n-Bu} \\
242. & \quad \text{R} = \text{Me}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{n-Pen} \\
243. & \quad \text{R} = \text{Me}, \text{R}_1 = \text{ClAc}, \text{R}_2 = \text{Me} \\
244. & \quad \text{R} = \text{Et}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{Me} \\
245. & \quad \text{R} = \text{n-Pr}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{Me} \\
246. & \quad \text{R} = \text{Allyl}, \text{R}_1 = \text{MOC}, \text{R}_2 = \text{Me}
\end{align*}
\]

Scheme 31

The *(S)_s natural product sulfoxide chondrine 258

was obtained via biotransformation of the N-tboc 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-Dihydrobenzo[\(\text{c}\)]thiophene 260, thiochroman 263, 1,3-benzoxathiolo 266 and 1,3-dihydrobenzo-\([\text{b}]\)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 stereochemical orientation of all sulfoxide products of 262, 265, and 268 are the same (Scheme 33), however, the *(R)_s identity for the sulfoxide product of 268, compared to the *(S)_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\(_2\)O\(_2\) 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[\text{b}]thiophene 260 is an excellent substrate giving 99.5% yield of the *(R)-sulfox-
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).

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 H₂O₂ [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 |
|------------------|-----------|----------------|----------|
| *Marine Green Algae (Phylum Chlorophyta)* | | | |
| Streptomyces aureofaciens | | Streptomyces griseus | [118] |
| Halimeda sp. | [111] | Streptomyces venezuelae | [119] |
| Penicillus capitatus | [105,106,108] | Streptomyces phaeochromogenes | [121] |
Table 1
Continued

| Bromoperoxidases         | Reference | Microorganisms       | Reference |
|--------------------------|-----------|----------------------|-----------|
| *Penicillium lamourouxii*| [105]     | Shigella flexneri    | [123]     |
| *Rhizocephalus phoenix*  | [105]     | *Salmonella enterica*| [123]     |
| *Ulvella lens*           | [109]     | *Pseudomonas aureofaciens* | [122] |
| *Pseudomonas putida IF-3*|           |                      |           |

**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] |
| Rhodomena larix          | [107]     | *Curvularia inaequalis* | [126] |
| Ochotodes secundiramea   | [138]     | *Embellisia didymospora* | [127] |
|                         |           | *Fusarium oxysporum* | [128]     |

**Marine Brown Algae (Phylum Phaeophyta)**

| Alaria esculenta         | [112]     |                      |           |
| Ascophyllum nodosum      | [95]      |                      |           |
| Chorda filum             | [113]     | *Saccharomyces cerevisiae* | [129] |
| Ecklonia stolonifera     | [115]     | *Serratia marcescens* | [134]     |
| Fucus distichus          | [102]     | *Streptomyces lividans* | [130] |
| Laminaria digitata       | [103]     | *Streptomyces toyoaensis* | [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**

| Notomastus lobatus       | [116,133] |                      |           |

**Haloperoxidases**

| Thelanus setosus         | [107]     | Freshwater algae     | [135]     |
| Prychoderia flava laysanica | [107] |                      |           |

**Lichens**

| Xanthoria parietina      | [17]      | Marine Invertebrates  | [133]     |
|                         |           | Amphitrite ornata     | [133]     |
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