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Oxidation, epoxidation and sulfoxidation reactions catalysed by haloperoxidases

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Contents

1. Introduction

Biological systems have evolved haloperoxidase (HaloPO) 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 chloroperoxidase (CPO) first discovered in the marine fungus *Caldariomyces fumago* in 1966,[14] myeroperoxidase, eosinophil peroxidase and lactoperoxidase from mammalian systems.[15] The third class of haloperoxidases is vanadium-containing peroxidases that require a vanadate ion (VO_4^{-3}). Vanadium peroxidase was fist discovered in the brown alga *Ascophyllum nodosum* in 1984[16] but since then it has also been found in lichen *Xanthoria parietina*[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 towards 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 *C. 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 a high enantiomeric excess.[26–31]

Current interest in catalytic oxidative transformations in industry is governed by two major issues: the first 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 and the second major issue is the need for high chemo-, regio- or enantioselectivies in order to improve chemical yields, to minimise 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.[32]

Keywords: haloperoxidase; chloroperoxidase; bromoperoxidase; oxidation; epoxidation; sulfoxidation; fungi; algae; microorganisms; enzyme reactions.

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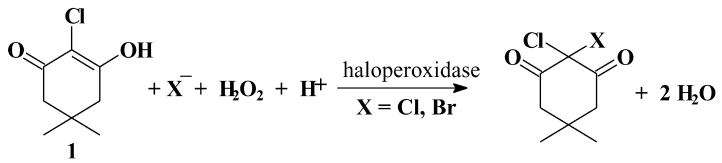


Scheme 1.

This is the first review which combines oxidation, epoxidation, and sulfoxidation catalysed by haloperoxidases isolated from different natural sources.

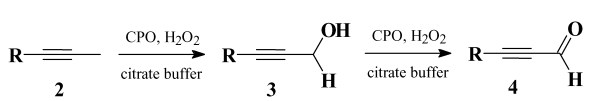
2. Oxidation reactions catalysed by chloro- and bromoperoxidases

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) and this has been used to investigate 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 catalysed the oxidation of 2-alkynes **2** to aldehydes **4** in the presence of hydrogen peroxide or *t*-butyl hydroperoxide as shown in 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%). It was additionally reported that CPO catalyzed highly enantioselective propargylic hydroxylations.[38]

CPO from the fungus *C. fumago* also catalysed the oxidation of primary alcohols selectively to the corresponding aldehydes **5**–**13** in a biphasic system of hexane or ethyl acetate and a buffer (pH=5.0) (Scheme 3).[39,40] A *cis* to *trans* isomerisation in the case of *cis*-2-hexenal was also observed.

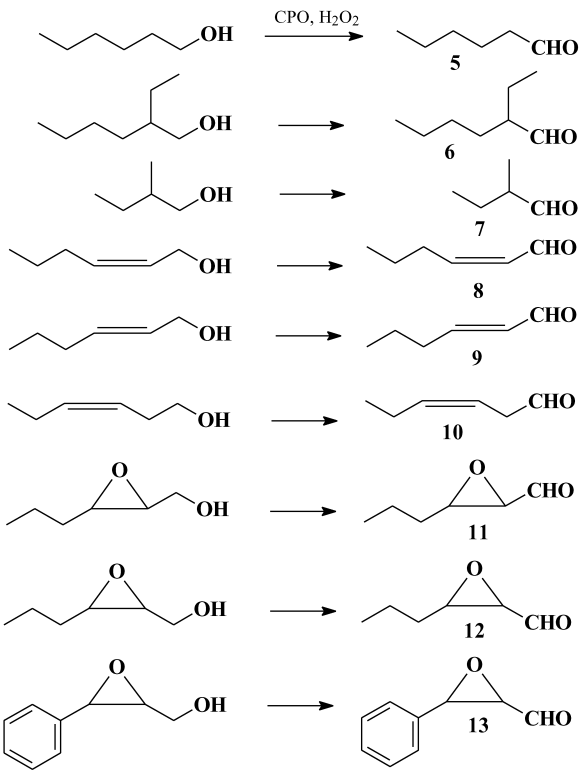
The asymmetric oxidation of prochiral 1,3-cyclohexadiene is catalysed by a CPO from *C. fumago*.[41] The process occurs enantioselectively and furnishes the non-racemic *trans*-diols 1,2- and 1,4-dihydroxycyclohexene, (−)-**16** and (+)-**17**, in good yield (Scheme 4). The oxidation of cyclohexadiene **14** was carried out in citrate buffer (0.1 M, pH=5.0). Although the cyclohexadiene epoxide **15** has not been detected in the reaction mixture, it is plausible that this is formed in the first step of the mechanism involving CPO



a) R = Me (ND)
b) R = Et (11% aldehyde)
c) R = Ph (6%)
d) R = CH_2OAc (45%)
e) R = CH_2Br (56%)
f) R = CH_2CH_2OAc (12%)
g) R = CH_2CH_2Br (5%)
h) R = COOEt (0%)

ND = not detected

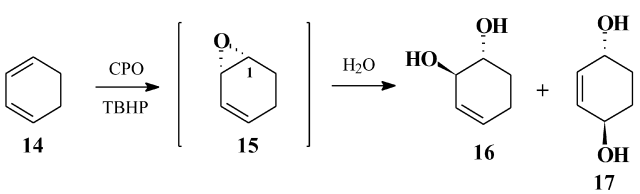
Scheme 2.



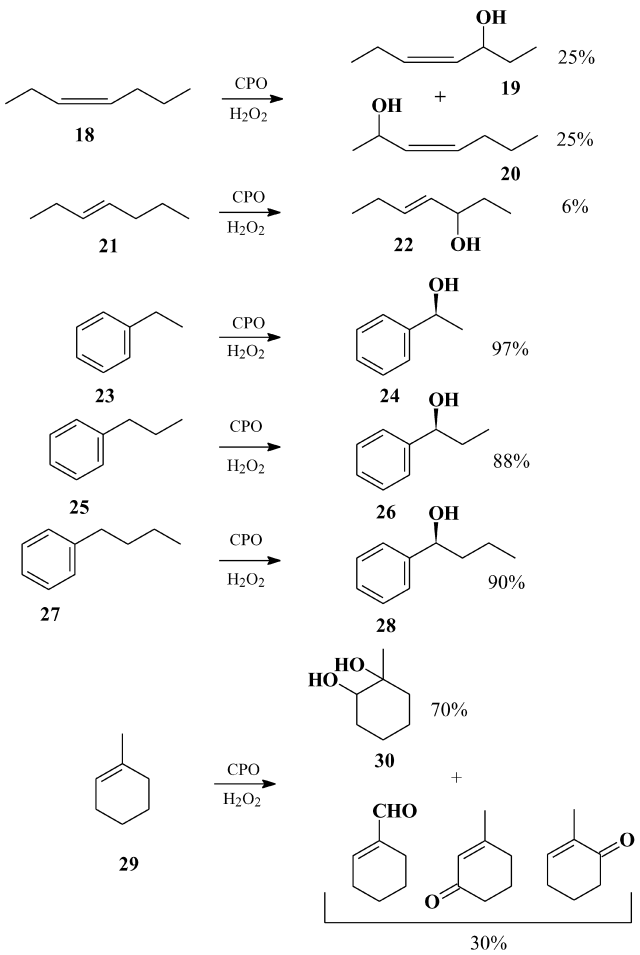
Scheme 3.

and undergoes fast nucleophilic attack by water, with partial rearrangement, giving the *trans*-diols **16** and **17**, respectively.

The substrate specificity of CPO from *C. fumago* in a number of halide-independent reactions has been investigated and the ability of this enzyme to perform benzylic hydroxylations with high enantioselectivity was revealed. The substrate range of CPO has been expanded and enantioselectivity data for synthetically useful oxidations was observed.[42] The enzyme oxidises straight-chain aliphatic and cyclic *cis*-olefins in a highly stereoselective manner, favouring small unsubstituted substrates in which the double bond is not more than two carbon atoms from the terminal **18** and **21** to **19** and **22**, respectively (Scheme 5). The oxidation of 1-methylcyclohexene **29** resulted in the



Scheme 4.



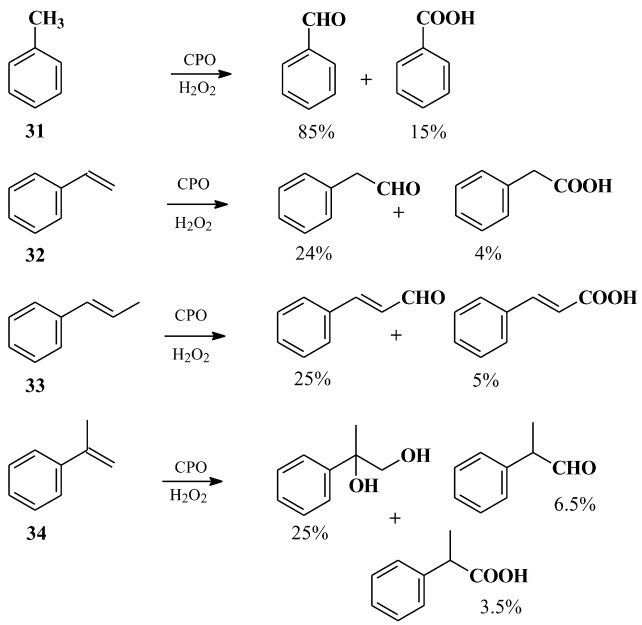
Scheme 5.

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 **24** in 97% ee. Surprisingly, the oxidation of propylbenzene **25** resulted in the alcohol product with the opposite stereochemistry **26**, (*S*)-1-phenyl-1-propanol, with an *ee* of 88%. Although the hydroxylation of butylbenzene **27** was rather inefficient, the product **28** had a good enantiomeric purity (*ee* 90%).

Oxidation of aromatic compounds **31**–**34** has also been 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 5-fold faster than that of the hydroxylation of toluene.

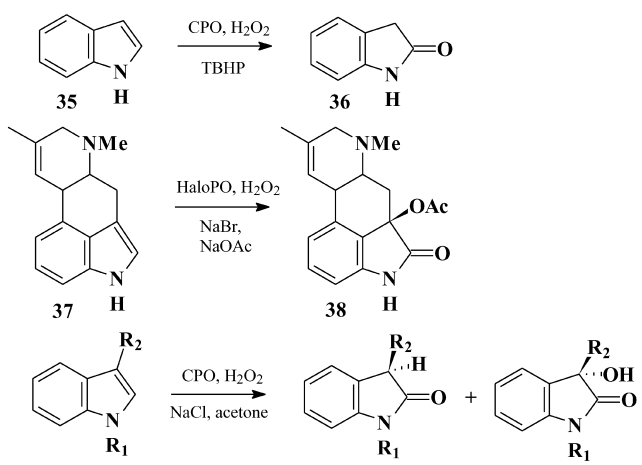
In the arylalkene series of substrates, styrene **32** was converted to a mixture that contained 24% phenylacetaldehyde (formed most likely via 1,2-rearrangement of the protoporphyrin-bound intermediate)[43] and 4% phenylacetic acid. The enantioselectivity of styrene epoxidation was significantly lower than in the epoxidation of *trans*-[^{2}H]-



Scheme 6.

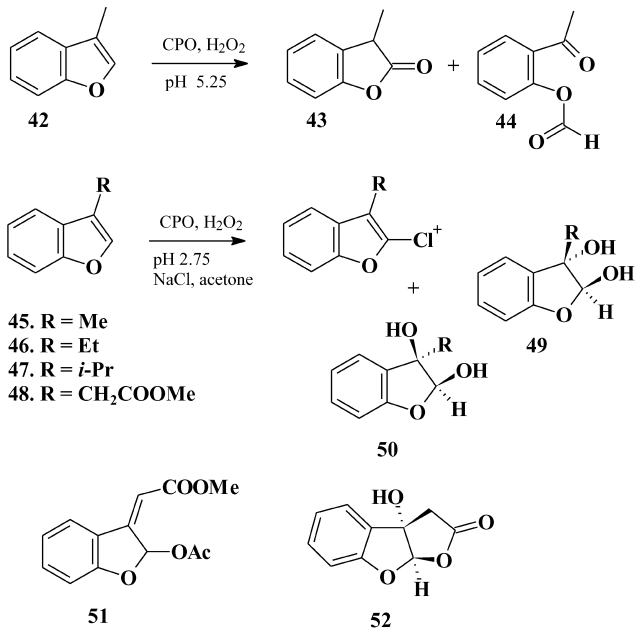
styrene that proceeded without detectable loss of stereochemistry.[43] α-Methylstyrene **34** was found to be a very reactive substrate. The olefin was efficiently converted to the corresponding epoxide which spontaneously hydrolysed to 2-phenyl-l,2-propanediol (Scheme 6). As in the case of styrene, this epoxidation was also accompanied by the formation of l-methyl-phenylacetaldehyde, which was further oxidised to l-methylphenylacetic acid.[42]

CPO from *C. fumago* was used in the oxidation of indole **35** to the lactone **36** (Scheme 7).[44] The same reaction was demonstrated by Kren et al.[45] as an unusual double oxidation catalysed by CPO during a study of the metabolism of the ergot alkaloid **37** to give **38**. The oxidation of the indole derivatives **39**–**41** by CPO from *C. fumago* has been investigated. Under conditions in which inactivitation of CPO was minimised by the presence of



39. $R_1 = R_2 = Me$
40. $R_1 = H$, $R_2 = Me$
41. $R_1 = H$, $R_2 = CH_2COOMe$

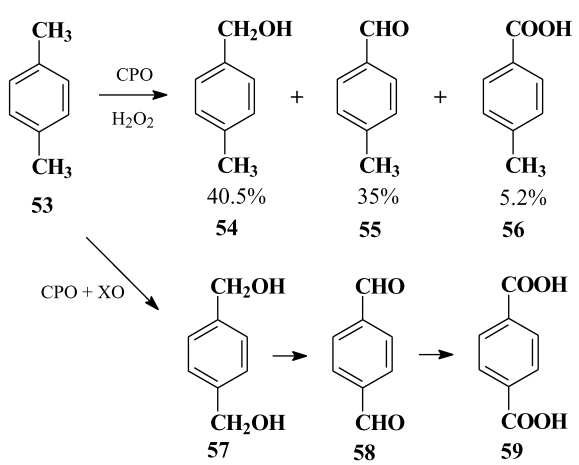
Scheme 7.



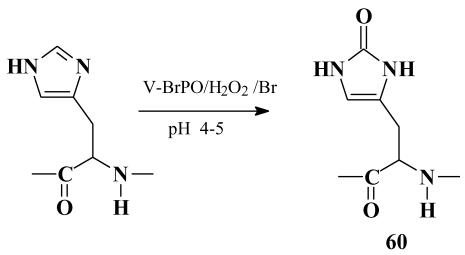
Scheme 8.

chloride and hydrogen peroxide,[46] the oxidation products of *N*-unsubstituted indoles tautomerised to give the corresponding lactam.

The 3-alkyl benzofurans **42**–**48** gave the 2,3-diols **49** and **50** as the 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 e.g. **42** were heterocyclic ring-cleaved compounds such as the ketoformate **44** (Scheme 8). At mildly acidic pH, however, in the presence of acetone and with careful control of both the enzyme and hydrogen peroxide concentration, it was possible to isolate significant quantities of the 1,2-diol oxidation products of benzofurans (derivatives **45**–**48**) by extraction of the reaction mixture with ethyl acetate. When dichloromethane was used for the 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 the diol



Scheme 9.



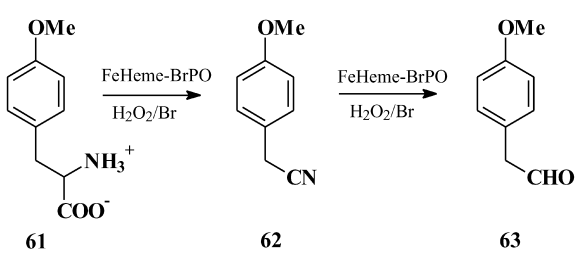
Scheme 10.

and the 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.

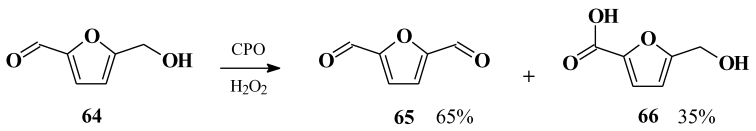
CPO from *C. fumago* was used to oxidise *p*-xylene **53**. Only one of the two aromatic methyl groups was oxidised, however, and 4-methylbenzyl alcohol **54**, *p*-tolualdehyde **55** and *p*-toluic acid **56** (Scheme 9) were formed. Investigation of numerous peroxidase and oxidase enzyme systems has shown that the route from 1,4-benzene-dimethanol **57** to terephthalic acid **59** *via* **58** is most efficient with a combination of two enzymes, CPO and xanthine oxidase (XO). Oxidation of **53** to a mixture of predominantly terephthaldicarboxaldehyde, 4-carboxy-benzaldehyde, and 4-hydroxymethylbenzaldehyde 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 a 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 (Rhodophyta) and green (Chlorophyta) algae, i.e. 76 and 71%, respectively. The bromoperoxidase (V-BrPO) isolated from the brown (Phaeophyta) marine alga *A. 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] Thus, **61** was converted to



Scheme 11.



Scheme 12.

p-methoxyphenyl-acetonitrile **62** and then to *p*-methoxyphenylacetoaldehyde **63** (Scheme 11).

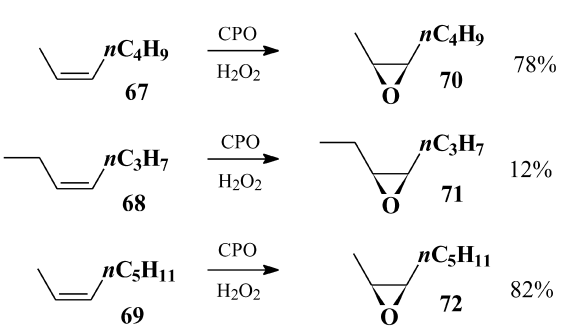
5-Hydroxymethyl-furfural **64** was oxidised by a CPO from *C. fumago* to the corresponding carboxylic acid **66**, but the major compound was found to be **65**.[54] Oxidation of the aldehyde to the acid proceeds via direct oxygen transfer, as indicated by the complete incorporation of $H_2^{18}O_2$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

3. 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 and 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 the rearranged aldehydes as byproducts. Similar results are obtained in the epoxidation of styrenes catalysed by cytochrome-c peroxidases.[58]

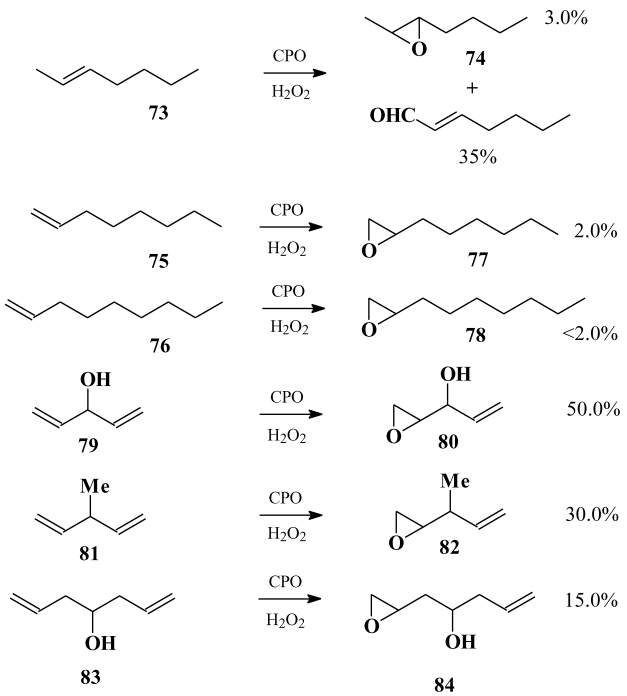
The CPO-catalysed epoxidation recently discovered by Colonna et al.[29] and Allain et al.[30] proceeds in high chemical and optical yields. Highly enantioselective epoxidation of the disubstituted alkenes **67**–**69** with hydrogen peroxide catalysed by CPO provided the R epoxides **70**–**72** preferentially. 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] The *trans*-olefins **73** (Scheme 14) 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 optimised by using branched 1-alkenes.[42]

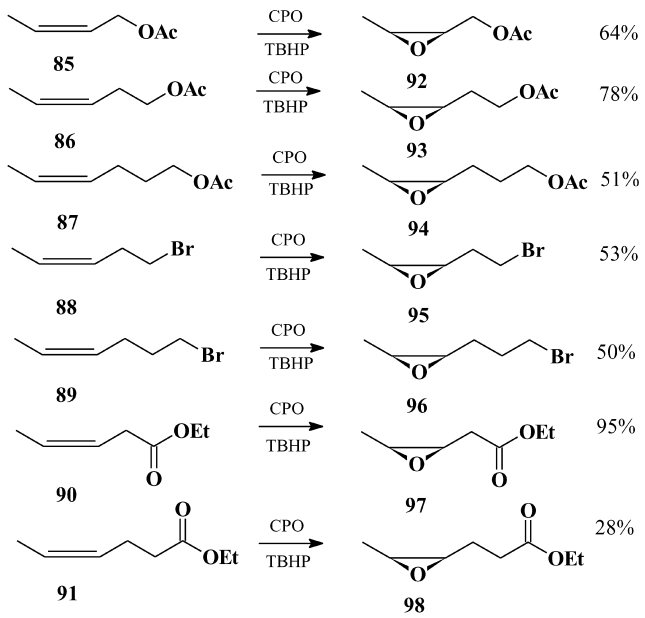


Scheme 13.

According to Allain et al.,[30] terminal alkenes such as 1-heptene and 1-octene **73**, **75** and **76** were epoxidised very poorly and non-selectively to **74**,**77** and **78** respectively (Scheme 14). Surprisingly, the oxidation of shorter terminal olefins such as the C-5 prochiral dienes **79** and **81** proceeded much more efficiently and resulted exclusively in the formation of the monoepoxides **80** and **82**, respectively. The oxidation of 3-hydroxy-1,4-pentadiene **79** proceeded with a high degree of diastereoselectivity (98%) and a modest enantioselectivity (65%). The predominant product (2*S*,3*R*)-1,2-epoxy-4-penten-3-ol **80** is an enantiomer of the epoxyalcohol produced *via* Sharpless epoxidation of the corresponding divinylcarbinol.[61] Substitution of the 3-hydroxyl moiety in the substrate with a methyl group as in **81** diminishes both the conversion and the 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 (**84**). It is apparent from the above results that the CPO catalyses 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.



Scheme 15.

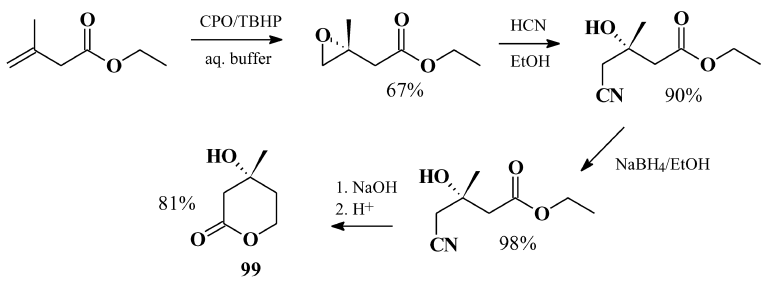
Asymmetric epoxidation of the functionalised *cis*-2-alkenes **85**–**91** catalysed by CPO used *tert*-butyl hydroperoxide (TBHP) as the terminal oxidant to form the corresponding epoxides **92**–**98** (Scheme 15).[26] The use of TBHP, especially in large-scale reactions appears to be more effective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

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 the prior methods required many steps to produce the lactone, in low overall yield, with moderate enantiomeric excess, required expensive starting materials, or various combinations of these. Meanwhile, a retrosynthetic analysis starting with an appropriately functionalised epoxide provided confidence that CPO could rescue the situation if used in the key stereogenic step.

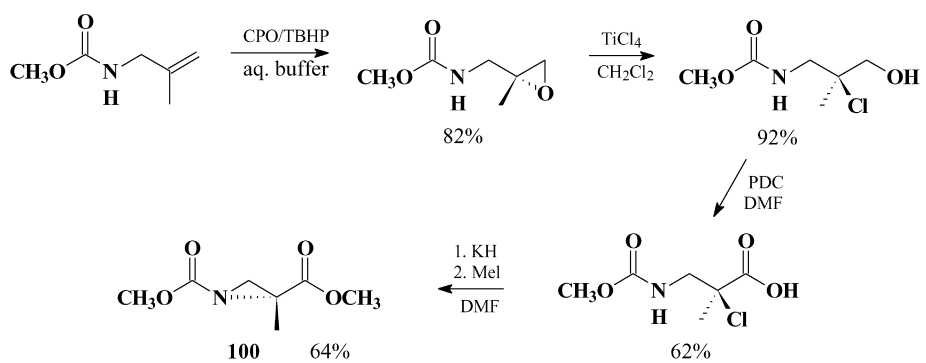
Another more recently completed synthesis is depicted in Scheme 17. Again, the epoxide is generated in high yield with conversion to (*R*)-dimethyl-2-methylaziridine-1,2-dicarboxylate **100** which may serve as a synthon for β-methylamino acids.[62]

Epoxidation of several monosubstituted olefins with CPO (Scheme 18, compounds **101**, **104**, **106**, **108** and **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, the ee was only moderate, in accordance with a previously published report.[29] 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%).

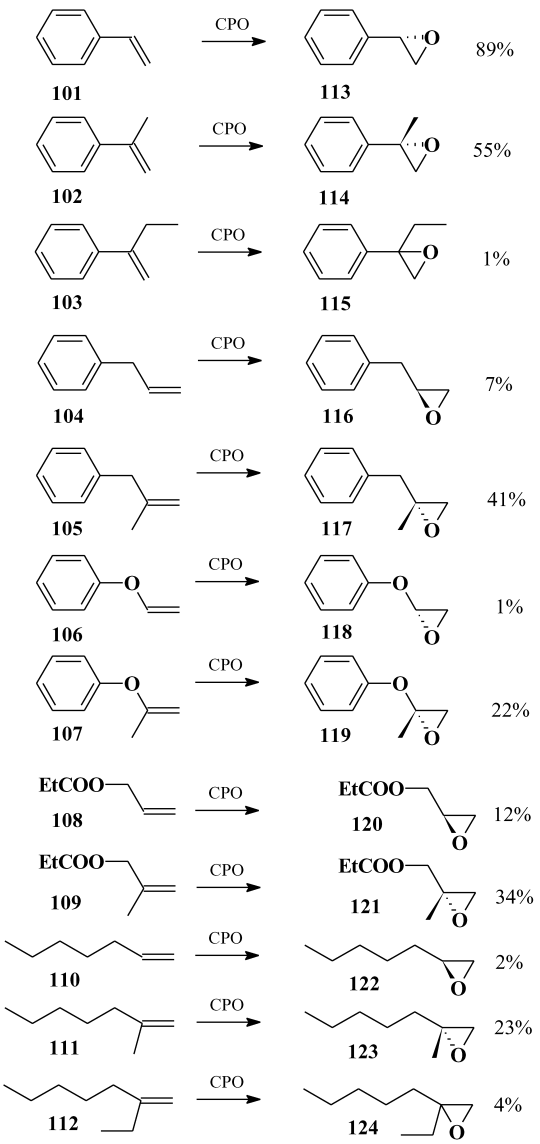
In contrast to the above results, epoxidation of matched 2-methylalkenes (Scheme 18, compounds **102**, **105**, **107**, **109** and **111**) showed a dramatic increase in both the turnover and the enantioselectivity. For the matched pair, allyl and methallyl propionate, an increase in the catalytic turnover of **103** by several orders of magnitude could be observed as a consequence of substitution of the double bond. In addition, while the epoxidation of allyl propionate with CPO leads rapidly to the formation of an inactive green



Scheme 16.



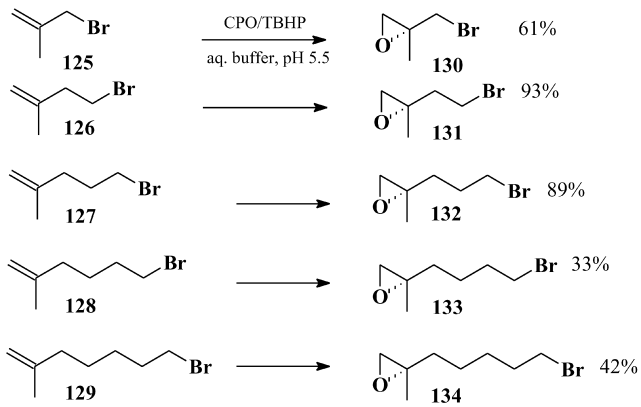
Scheme 17.



Scheme 18.

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 the turnover and the 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 the enantioselectivity.

A high epoxidation enantioselectivity was observed,[4] but the catalytic turnover declined for both compounds to a level similar to those observed for the monosubstituted alkenes **103** and **112** to **113** and **124**, respectively. For the ethyl-substituted terminal olefins, it appears that the greater steric size begins to limit the 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 a reduction of the



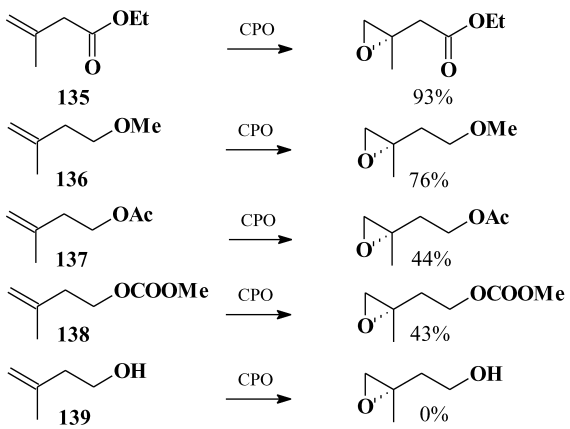
Scheme 19.

epoxidation turnovers. Conversely, the 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 the turnover number observed when the initial concentrations of facile substrates such as methallyl propionate are increased.

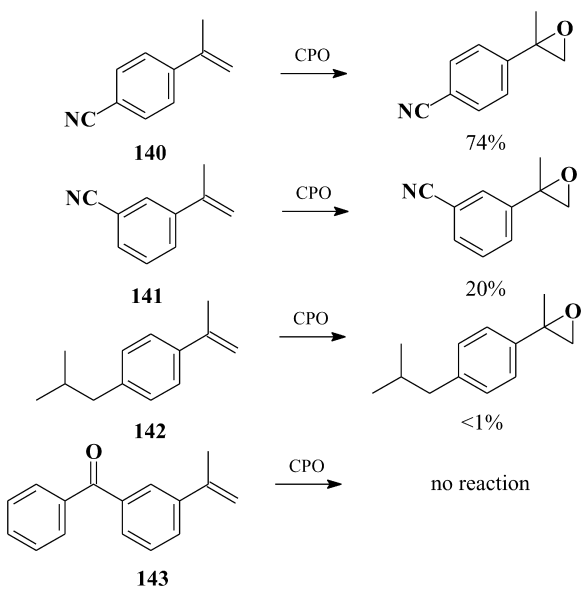
In the CPO-mediated epoxidation of the ω-bromo-2-methylalkenes **125**–**129** to the corresponding epoxides **130**–**134** the effect of the chain length on the enantioselectivity was investigated (Scheme 19).[28] The *ω*-bromo-2-methyl-1-alkenes were epoxidised under identical condition by using CPO from *C. fumago*. In all cases, the predominant enantiomer produced had the (*R*)-configuration, except 3-bromo-2-methylpropene oxide **126**, which was predominantly (*S*) only, because of a priority switch. The enantiomer of this latter compound was synthesised from the commercially available (*S*)-methylglycidol to confirm its stereochemistry. The substrate selectivity was approached by observing the effect of the chain length of the ω-bromo-2-methyl-alkenes on the substrate conversion. The compounds **125** and **126** in Scheme 19 were converted entirely to the products **130** and **131**, since no starting material could be observed in the reaction mixture extracts. The compounds **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 the increase conversions and it was found, for example, that doubling the initial quantity of CPO did not improve the conversion.

Lakner and Hager[59] have reported the epoxidation of the alkenes **135**–**139** to the corresponding epoxides. High yields were obtained for **135**, while the 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 oxygen is removed. The reaction mixture may be purged with nitrogen and sealed, under which conditions good results are obtained. Using a suitably substituted α-methylstyrene **102**, non-steroidal anti-inflammatory arylpropionic acids could be synthesised. Electron-donating substituents are to be avoided because of their tendency to promote solvolysis and rearrangement reactions. The cyano group is sterically and electronically



Scheme 20.



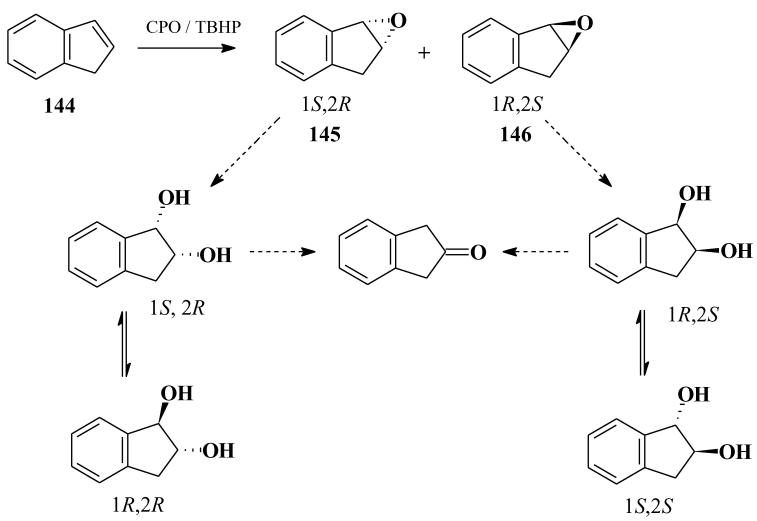
Scheme 21.

admissible, although it was discovered (Scheme 21) that the resulting epoxide possesses a moderate 74% *ee* for the *p*-cyano derivative **140** to low 20% ee for the *m*-cyano compound **141**. The compound **142** forms less than 1% epoxide and **143** has not been found to react with CPO.[27]

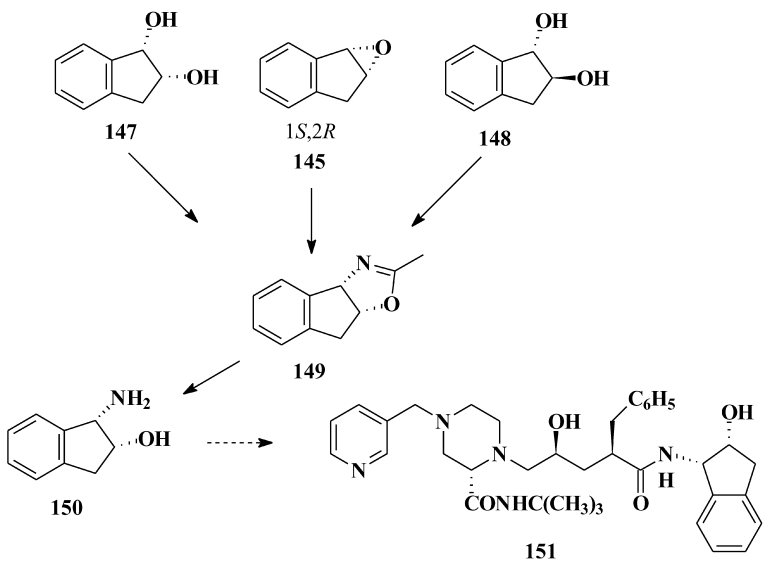
The stereochemistry of the CPO-catalysed epoxidation of indene **144** has been reported.[63] In aqueous, solution the intial epoxide is not stable and opens to form the *cis-trans*-diols. When the reaction was carried out in the absence of water, the epoxide enantiomers **145** and **146** were isolated, with the 1(*S*),2(*R*) enantiomer **145** being formed in 30% *ee* (Scheme 22).

1*S*,2*R*-Indene epoxide **145** is the precursor of *cis*-1*S*,2*R*-aminoindanol **150**, a key intermediate **149** 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 the 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 1*S*,2*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 a 10% yield of the chiral bromoindanol or epoxide from indene, and the yield was rapidly improved to 30% of *trans*-1*S*,2*S*-bromoindanol with an ee of 80%. Reaction mechanistic studies revealed that the stereo-selectivity observed was apparently due to a specific dehydrogenase activity present in MF5400, which was also found to resolve chemically synthesised racemic *trans*-2-bromoindanols.

The 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 epoxidised to produce the



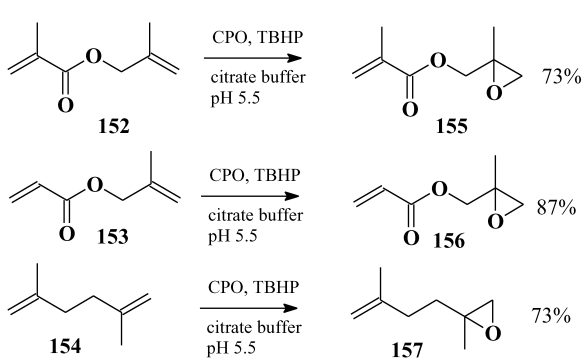
Scheme 22.



Scheme 23.

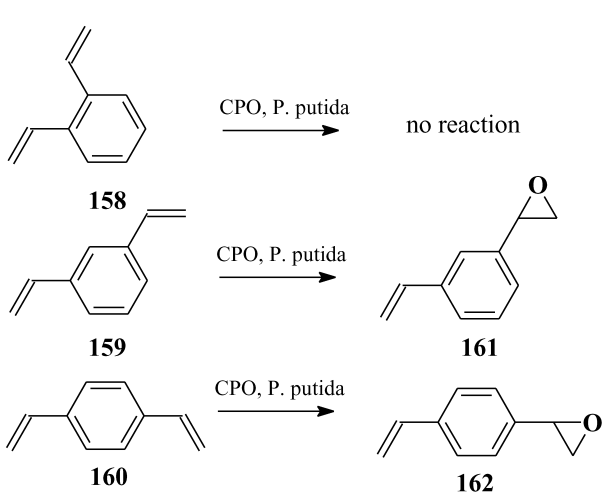
monoepoxide **155** in 73% yield and the conjugated α,β-unsaturated bond of the methacrylic acid moiety was untouched, as shown in Scheme 24, the enantioselectivity being in high yield. It was suggested that conjugated terminal olefins might have a low effect on the inhibition of CPO activity compared to other aliphatic terminal alkenes to give an inactive derivative in which the active heme site is *N*-alkylated.[60] Indeed, the 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 been proposed that CPO-catalysed epoxidation should produce only monoepoxides from symmetrical dienes and this was indeed the case. When the 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 *Pseudomonas* sp., such as xylene oxygenases, catalyse the epoxidation of styrene to styrene oxide with a high enantioselectivity.[71,72] CPO is a versatile and efficient biocatalyst that catalyses a variety of reactions, particularly asymmetric epoxidation and hydroxylation.[30,31,42]



Scheme 24.

The selective epoxidation of aryldienes catalysed by oxidases from *Pseudomonas putida* and the epoxidation of unsaturated acrylate derivatives catalysed by CPO[67] has been reported. Three isomers **158** (*ortho*-8%), **159** (*meta*-52%) and **160** (*para*-40%) which are presence in commercially available divinylbenzene were first used (Scheme 25) as model substrates for the investigation of selective epoxidation catalysed by oxidases from *P. putida*. For the *para*-isomer **160** and *meta*- **159**, the oxidases from *P. putida* demonstrated two types of selectivities. Firstly, the reaction stopped at the monoepoxide stage (**161** and **162**), with hardly any diepoxide and other products being detected or isolated from the reaction medium, and, secondly the epoxidation reactions showed excellent enantioselectivities (*ee* 95% for *para*- **160** and 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 the *para*- and *meta*-divinylbenzenes, the >*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]



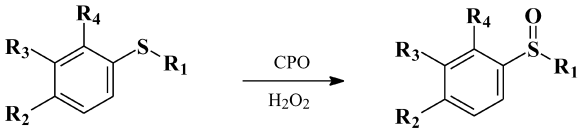
Scheme 25.

4. Sulfoxidation reactions

The heme-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 the 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 conditions.[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-fold lower (ca 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** showed little or no reaction.[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]

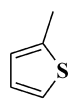
Lee et al.[78] studied the stereospecific sulfoxidation of the aryl alkyl sulfides **192** by purified toluene dioxygenase (TDO) from *P. patida* and showed that sulfoxidation yielded the (*S*)-sulfoxides **193** in 60–70%, yield, whereas sulfoxidation by CPO under the same conditions yielded > 98% (*R*)-sulfoxides **194** (Scheme 27).



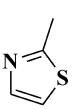
178-191

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, R_2 = 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

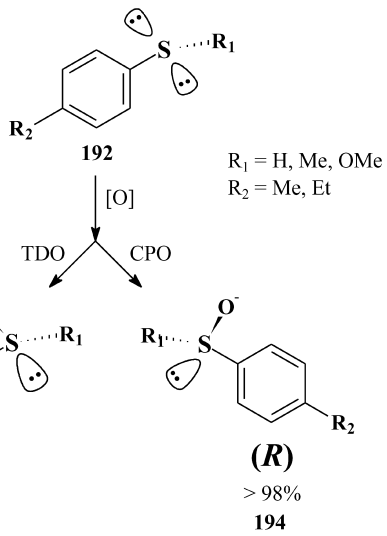
176 R_1 = Me, R_3 = R_4 = H, R_2 =



177 R_1 = Me, R_3 = R_4 = H, R_2 =

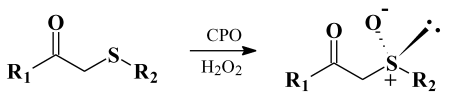


Scheme 26.



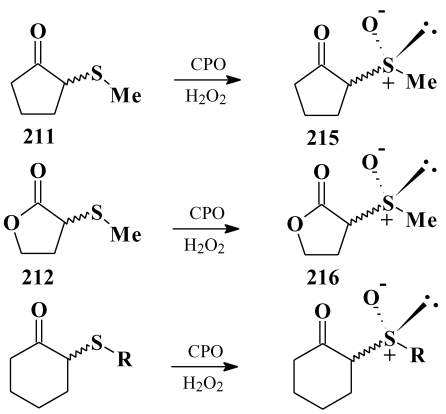
Scheme 27.

The CPO-catalysed oxidation of a series of β-carbonyl sulfides **195–202** and **211–214** to corresponding sulfoxides **203–210** and **215–218** has been studied at room temperature in aqueous citrate buffer (pH 5.0) at 25 °C.[79] For the dialkyl β-carbonyl sulfides, the products with methyl and ethyl substituents are obtained in ca. 100% yield (Scheme 28). When the alkyl group is *n*-propyl **197** or *iso*-propyl **198**, however, the yield drops dramatically (25%). An aryl sulfide derivative afforded the product in very low yield (4%), but when the phenyl group bears a carbonyl moiety and the sulfur substituents are methyl or ethyl, the oxidation occurs with high yields (91–95%).

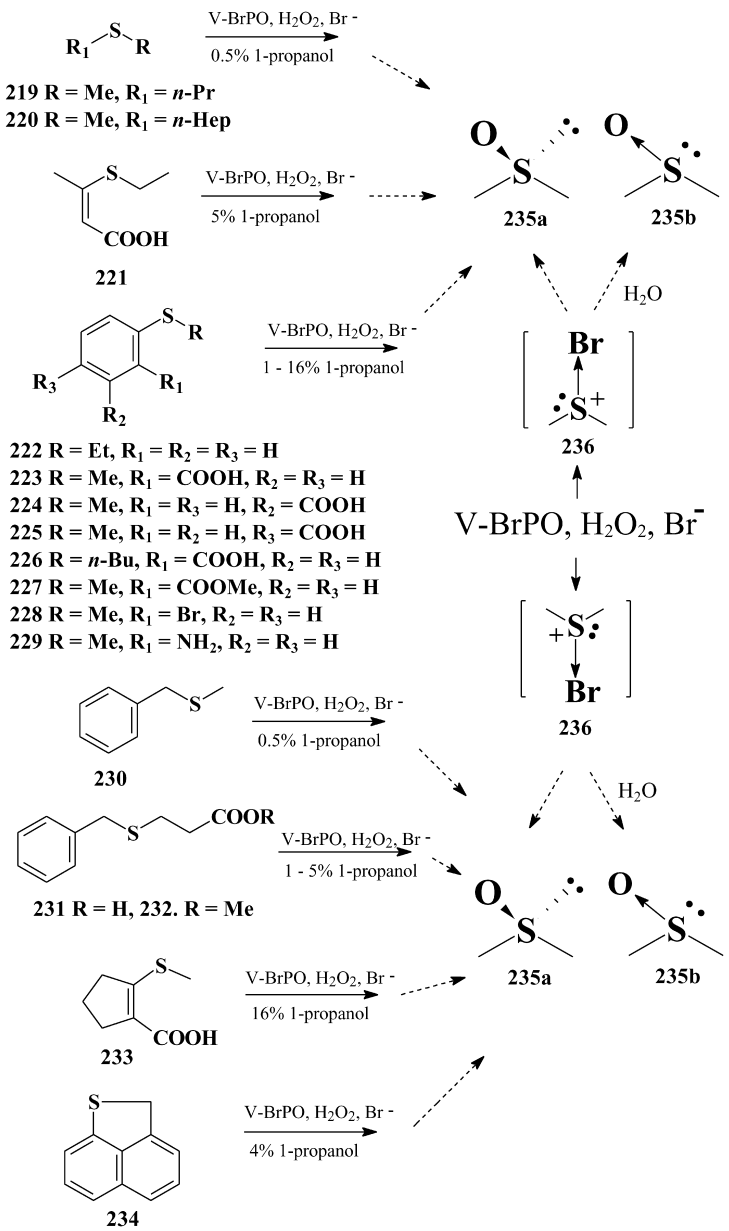


203-210

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

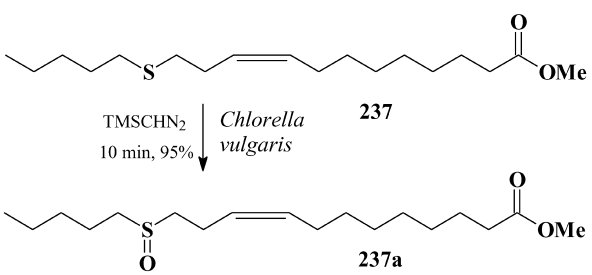


Scheme 28.



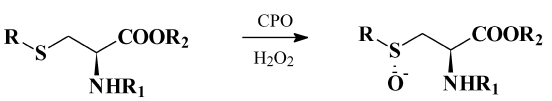
Scheme 29.

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



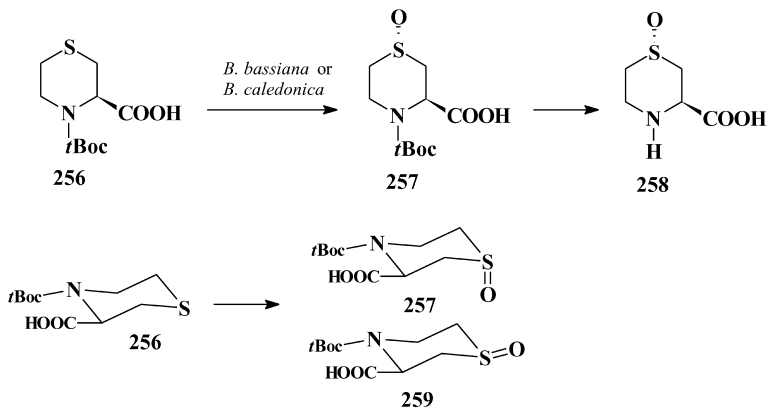
Scheme 30.

Experiments with a series of racemic cyclic carbonyl sulfides of differing size (compounds **211**, **212**, **213** and **214**) confirmed a positive influence on the product yields of a small size[80] to fit the CPO heme cleft. Indeed, the



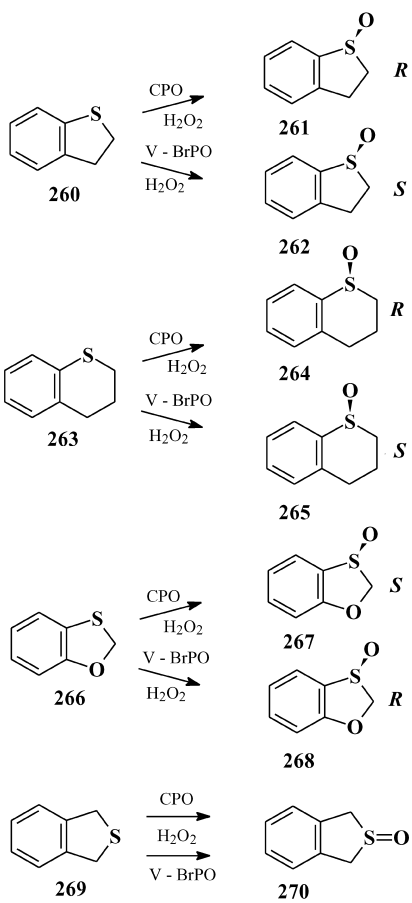
238 R = R2 = Me, R1 = MOC
239 R = Me, R1 = MOC, R2 = Et
240 R = Me, R1 = MOC, R2 = n-Pr
241 R = Me, R1 = MOC, R2 = n-Bu
242 R = Me, R1 = MOC, R2 = n-Pen
243 R = Me, R1 = ClAc, R2 = Me
244 R = Et, R1 = MOC, R2 = Me
245 R = n-Pr, R1 = MOC, R2 = Me
246 R = Allyl, R1 = MOC, R2 = Me

Scheme 31.



Scheme 32.

substrates **213** and **214**, being bulkier than the substrate **211**, gave a 2-fold 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 oxidised with CPO, yielding 99.5 and 7% of the sulfoxide product, respectively. Unexpectedly, the γ-butyrolactone sulfide **212** afforded the corresponding sulfoxide **216** in quantitative yields, indicating that an oxygen atom neighbour to the carbonyl group completely altered the enzyme selectivity. Oxidation of the racemic substrate **213** with 30% H_2O_2 in acetic acid gave 70% *de* of the sulfoxide **217**, albeit without optical activity. A similar result was reported elsewhere when the chiral sulfide **213** was oxidised with an oxaziridine derivative **217** and a 70% *de* product was also obtained.[82] The α-sulfinyl cyclic ketones **215**, **217** and **218** or the lactone **216**, containing an α-hydrogen, are known to exhibit a keto–enol tautomerism in organic solvents, and substrate enolisation may therefore be responsible for the observed de.[83] Thus, kinetic resolution of the cyclic carbonyl sulfides must be occurring to explain the ee, but product enolisation leads to de loss.



Scheme 33.

Using asymmetric sulfoxidation catalysed by a vanadium bromoperoxidase from the red alga *Carollina officinalis*, a series of prochiral sulfides **219**–**234** having a *cis*-positioned carboxyl group were oxidised rapidly, giving the sulfoxides **235** in >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. A rapid loss of stereoselectivity was found to occur when V-BrPO-catalysed 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 the oxidation of bromide and subsequent formation of a bromosulfonium ion intermediate **236**. The 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 a low bromine ion concentration will be due to either asymmetric sulfoxidation (type **235a**) or a slow halogen exchange in the bromosulfonium ion, which with H_2O give **235b**. The enantioselectivity was much less influenced by the presence of chloride ions, due to the low capability V-BrPO to oxidise 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 **237a** by crude extract of the alga *Chlorella vulgaris* (Scheme 30).[86]

The preparation of methionine sulfoxides and S-alkylcysteine sulfoxides with a defined stereochemistry at sulfur has been achieved in low yields by chemical resolution. All the stereoisomers of methionine and ethionine sulfoxides

Table 1. Some natural sources of haloperoxidases

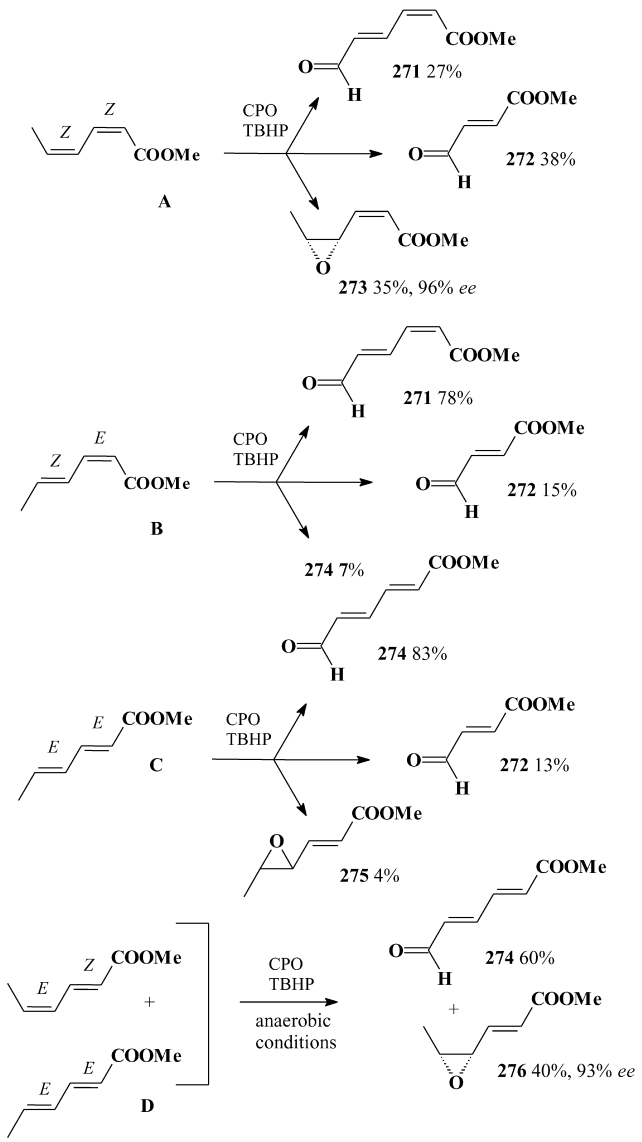
	Ref.
Bromoperoxidases	
Marine Green Algae (type Chlorophyta)	
Halimeda sp.	[111]
Penicillus capitatus	[105,106,108]
Penicillus lamourouxii	[105]
Rhipocephalus phoenix	[105]
Ulvella lens	[109]
Marine Red algae (type Rhodophyta)	
Ceramium rubrum	[110]
Corallina pilulifera	[96–98]
Corallina officinalis	[99,100,117]
Corallina vancouveriensis	[101]
Cystoclonium purpureum	[114]
Rhodomela larix	[107]
Ochtodes secundiramea	[138]
Marine Brown Algae (type Phaeophyta)	
Alaria esculenta	[112]
Ascophyllum nodosum	[95]
Chorda filum	[113]
Ecklonia stolonifera	[115]
Fucus distichus	[102]
Laminaria digitata	[103]
Laminaria hyperborea	[104]
Laminaria ochroleuca	[104]
Laminaria saccharina	[103,104]
Macrocystis pyrifera	[102]
Marine invertebrates	
Notomatus lobatus	[116]
Thelepus setosus	[107]
Ptychodera flava laysanica	[107]
Lichens	
Xanthoria parietina	[17]
Microorganisms	
Streptomyces aureofaciens	[120]
Streptomyces griseus	[118]
Streptomyces venezuelae	[119]
Streptomyces phaeochromogenes	[121]
Shigella flexneri	[123]
Salmonella enterica ser. typhimurium	[123]
Pseudomonas aureofaciens	[122]
Pseudomonas putida IF-3	[124]
Chloroperoxidases	
Fungus	
Aspergillus flavus	[125]
Caldariomyces fumago	[14]
Curvularia inaequalis	[126]
Embellisia didymospora	[127]
Fusarium oxysporum	[128]
Phanerochaete chrysosporium	[137]
Microorganisms	
Saccharomyces cerevisiae	[129]
Serratia marcescens	[134]
Streptomyces lividans	[130]
Streptomyces toyocaensis	[132]
Pseudomonas fluorescens	[94]
Pseudomonas pyrrocinia	[131]
Rhodococcus erythropolis	[136]
Marine invertebrates	
Notomastus lobatus	[116,133]
Haloperoxidases	
Freshwater algae	
Cladophora glomerata	[135]
Marine invertebrates	
Amphitrite ornata	[133]

have been prepared via biotrans-formation reactions involving the conversion of protected amino acid substrates to the corresponding sulfoxides by *Beauveria bassiana* or *B. 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*, *B. bassiana* and *B. caledonica* catalysed the oxidation of the *S*-alkyl-L-cysteine derivatives **238**–**246**, with the result that the maximum yields and diastereomeric excesses were obtained with the N-MOC C-carboxylate ester derivatives **247**–**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 suitable derivatives.

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 *B. bassiana* or *B. caledonica*. The sulfoxidation of the 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 *C. officinalis* oxidises several bicyclic sulfides to the corresponding sulfoxides with a high enantioselectivity (≤91%) in the absence of an added halide source. In addition, 2,3-dihydrobenzo[*b*]thiophene **260**, thiochroman **263**, 1,3-benzoxathiole **266** and 1,3-dihydrobenzo[*c*]thiophene **269** are all oxidised to the corresponding sulfoxide **261**, **262**, **264**, **265**, **267** and **268**, respectively, as shown in Scheme 33. With the exception of **270**, which is symmetrical, the oxidation of all the other substrates occurs stereo-specifically. The stereochemical orientation of all sulfoxide products of **262** and **265** are the same in Scheme 33, but, the *R* identity for the sulfoxide



Scheme 34.

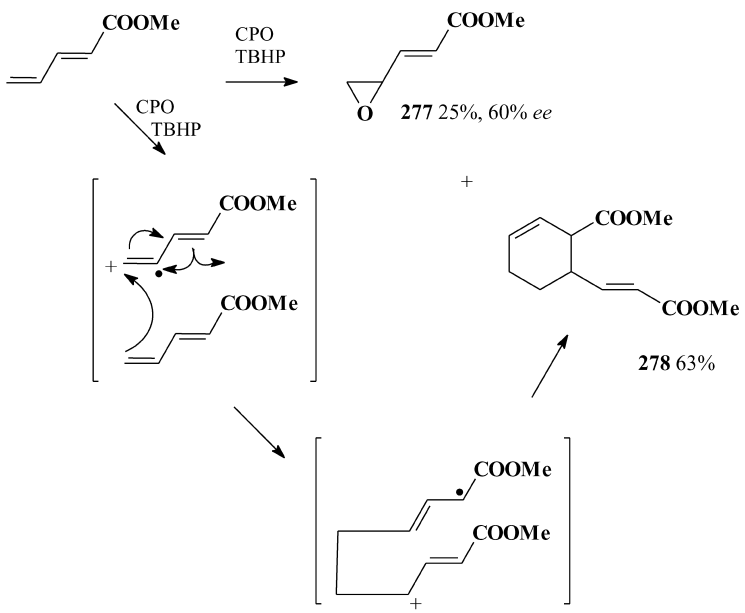
product of **268**, compared to the *S* identity of the sulfoxide product of **262** and **265**, is a result of the nomenclature rules.

Asymmetric sulfoxidation by means of a CPO from *C. 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*)-sulfoxide, replacement of a methylene group by either a more sterically demanding group or a heteroatom caused a substantial decrease in the reactivity or in the reactivity as well as the enantioselectivity. For the oxidation of thiochroman **263** and 1,3-benzoxathiole **266** by the CPO from *C. fumago* gave the (*R*)-**264** and (*S*)-sulfoxide **267** (Scheme 33).[93]

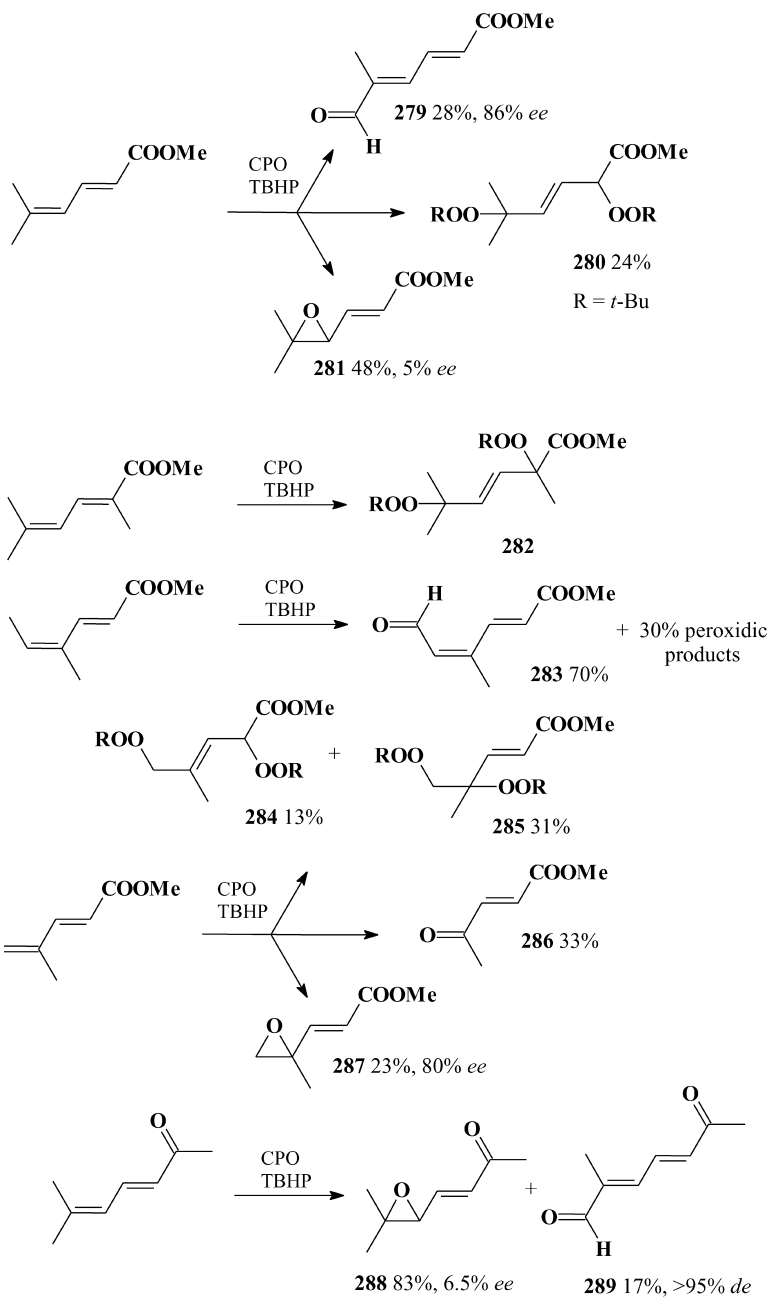
Chloroperoxidase was also found to be an effective catalyst in the oxidation of labile episulfides, yielding the corresponding antisulfoxides quantitatively and in the oxidation of sulfides (Scheme 33).

5. Distribution of haloperoxidases in nature

In the last decade, peroxidases, notably CPO from *C. fumago*, have been shown to catalyse 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] A major shortcoming of all heme-dependent peroxidases, such as CPO, however, 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 the vanadium-dependent haloperoxidases can



Scheme 35.



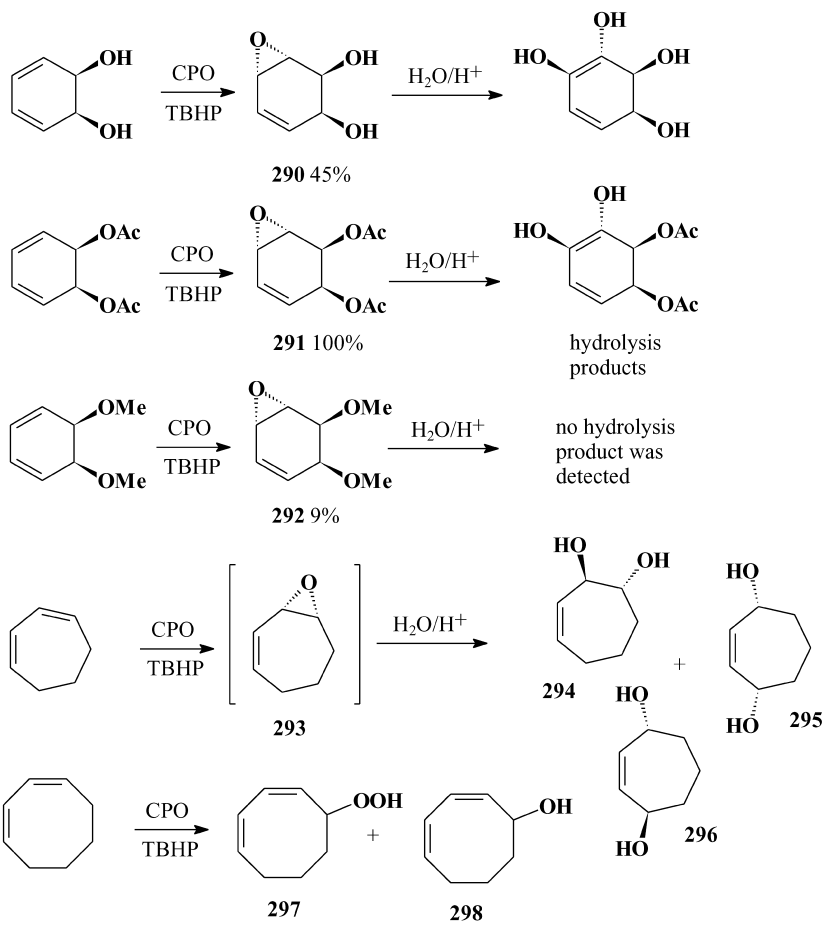
Scheme 36.

accommodate only very small substrates, such as halide ion, which severely curtails their utility. Nevertheless, enantioselective sulfoxidation was catalysed by vanadium-dependent bromoperoxidases from *C. officinalis*[84] and *A. 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 brief review has demonstrated that the haloperoxidases are successful reagents in organic synthesis.

6. Concluding remarks

Haloperoxidases are ubiquitous metalloenzymes that catalyse a variety of enantioselective oxygen-transfer reactions with hydrogen peroxide or alkyl peroxides. Advances have recently been made in using these enzymes 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.



Scheme 37.

7. Addition to distributions and reactions of haloperoxidases

Since this article was accepted for publication some new papers have been published. Chloroperoxidase having a similar enzyme type of *Caldariomyces fumago* was detected for the first time in bryophytes, namely in the liverwort *Bazzania trilobata*, using the monochlorodimedon assay.[139] Chloroperoxidase from bacterium *Serratia marcescens* W-250 was reported for the first time that bacterial non-heme haloperoxidases possess a phosphatase activity; a mechanism of phosphoesters hydrolysis by non-heme haloPO was proposed.[140] Presence of a vanadium-dependent haloperoxidase in *Botrytis cinerea* has also been reported.[141]

Some medicinal and catalytic potential of model complexes of vanadate-dependent haloperoxidases was observed.[142] Vanadium haloperoxidases catalyse the oxidation of halides leading to halogenation of substrates or, in the absence of suitable substrates, to oxidation of hydrogen peroxide into singlet oxygen and water; the most interesting model compounds that have been synthesised and studied as bromination catalysts, and catalysts for, i.e. epoxidation, hydroxylation, sulfoxidation and alcohol oxidation are discussed in recent review.[143] The chemistry of the formation of natural organohalogens catalysed by the group of haloperoxidases was discussed.[144] Structural and functional model compounds for these enzymes are introduced, and the use of haloperoxidase models in the enantioselective oxygenation of thioethers is discussed.[145]

The first examples of CPO-catalysed oxidations of dienes conjugated to an ester group, and possible mechanisms for these synthetically useful transformations were reported.[146] The reactions of the isomeric conjugated dienoic esters methyl 2*Z*,4*Z*-**A** (**271**–**273**), methyl 2*Z*,4*E*-**B** (**271**,**272** and **274**), methyl 2*E*,4*E*-**C** (**272** and **275**) and methyl 2*E*,4*Z*-**D**-hexadienoate (**274** and **276**) were investigated (Scheme 34). The stereoisomers **B**, **C** and **D** were prepared by photoisomerization[147] of **1** by irradiation at 254 nm.

The CPO-catalyzed oxidation of the methyl (2*E*)-2,4-pentadienoate gives the terminal double bond epoxide **277** (25%) and a cyclodimerization compound **278** (63%) as the major products showed in Scheme 35.[148]

Also the CPO-catalyzed oxidations of conjugated dienoic esters with a trisubstituted terminal double bond were studied by using TBHP as the terminal oxidant. Most of the substrates gave disubstituted mixed peroxides as the major products **279**–**289** (Scheme 36).[149]

Chloroperoxidase from *C. fumago* has been investigated as a catalyst for the oxidation of cyclic conjugated dienes.[150] As major products have been isolated epoxides **290**–**292**. The

nature of the substituents and the size of the carbocycle affect the enantioselectivity of the enzyme has also been observed. The study of the CPO-catalyzed oxidation was then extended to larger cyclic dienes, namely 1,3-cycloheptadiene and *cis*,*cis*-1,3-cyclooctadiene. For cycloheptadiene a 60% conversion of substrate was observed after 72 h. Cycloheptene epoxide **293** was not detected in the reaction mixture and only diols (−)-**294**, **295** and (+)-**296** in a ratio of 2:1:1 were observed (Scheme 37). The course of the CPO-catalysed oxidation of *cis*,*cis*-1,3-cyclooctadiene was quite surprising, since after 6 days a 53% conversion of the substrate was observed with formation of a product different from the expected epoxide or diols in 43% yield (Scheme 37), together with small amounts (5%) of the racemic alcohol **298**. The main product was identified as **297** by comparison with a synthetic standard.[150]

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