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Epoxide Hydrolases as Asymmetric Catalysts

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1. Introduction

About two thirds of biotransformations reported on non-natural compounds in the last twenty years used hydrolase enzymes.[1,2] There are a number of reasons for this:

(1) Hydrolases do not require cofactors (e.g. NAD(P)H/NAD(P)) other than water.

(2) They are widely available from a number of sources.[3]

(3) They remain catalytically active in non-aqueous media giving rise to, for example, ester formation rather than cleavage reactions.

(4) They frequently show remarkable chemo-, regio- and stereoselectivity whilst accepting a wide range of substrates.

While most of the biotransformations involving hydrolase enzymes described in the chemical literature have used esterases and lipases, in recent years various groups have turned their attention to a different type of hydrolase enzyme namely epoxide hydrolases [E.C. 3.3.2.X].

Epoxide hydrolases catalyse the hydrolysis of epoxides to the corresponding vicinal diols, **Scheme 1**.

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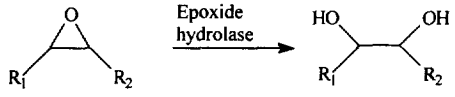


Scheme 1

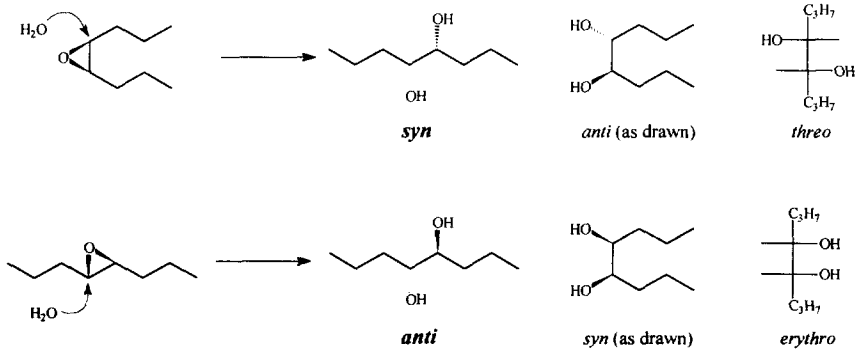
When the enzymes display product- or substrate-enantioselectivity, they may be used by the synthetic organic chemist to produce valuable enantiopure intermediates in the form of chiral epoxides and/or vicinal diols.

Epoxide hydrolases are sometimes called epoxide hydratase or epoxide hydrase in the literature. Hydratase or hydrase enzymes are a sub-class of the hydrolase category and catalyse the hydration of their substrates. For example, in the conversion of acrylonitrile to acrylamide, water is added across the nitrile bond but no sigma bonds are broken, rendering the term hydrolase unsuitable. For the reaction shown in **Scheme 1** water is added across the epoxide to produce a diol, however, since a C-O σ-bond is broken the terms hydrolysis and, hence, hydrolase are appropriate and will be used throughout this review.

Some authors in this field use the (dimensionless) E-value[4] in order to evaluate and compare stereoselectivities between enzymes and/or substrates. The E-value is defined as the ratio of the values of k_{cat}/K_M for the two enantiomers individually, i.e. the measure of the enzyme's catalytic efficiency towards transformation of one enantiomer over the other. However, the two enantiomers are not generally available for direct measurement of these parameters and when they are, the process of measurement of the values may be laborious. Other methods for the calculation of the E-value, based on the ee. of the product or residual substrate and the degree of conversion, have therefore been developed.[4] As a rule of thumb E-values below 50 indicate low selectivity, while those over 100 indicate high selectivity, values in between indicate moderate selectivity. Some interesting discussions on the utility of the E-value have been published recently.[5,6] For the purposes of this review, except where the value was used explicitly by the authors, E-values will not be used to assess enantioselectivity since the value can be deceptive to the synthetic chemist. Isolated yields and enantiomeric excesses will be reported instead.

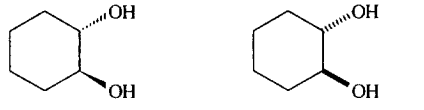
1.1 Nomenclature

The opening of epoxide rings occurs with attack of the nucleophile *anti* to the oxygen atom of the oxirane ring, with inversion of the carbon atom under attack.[7] Hydrolysis of *cis*-epoxides therefore proceeds to yield diols whose absolute configuration is *threo* when drawn in their Fischer projection, **Scheme 2**. The terms *syn* and *anti* will be used throughout this review and will refer to the compounds when drawn in zigzag conformation, **Scheme 2**. Thus, the hydrolysis of *cis*-epoxides yields *syn*-diols, while hydrolysis of *trans*-epoxides yields *anti*-diols. Epoxycycloalkanes are hydrolysed to diols whose two hydroxyl groups are on opposite faces of the carbocyclic ring, such diols will be described as *trans*.



Scheme 2

Absolute stereochemistry is denoted pictorially with dashes and wedges, while relative stereochemistry is denoted with straight bold and dashed bonds. In addition racemic compounds with a given relative chemistry are denoted in this manner, **Scheme 3**[8].



Absolute (1S,2S) Relative (and/or racemic)

Scheme 3

1.2 Chemical asymmetric synthesis of epoxides and diols

Enantiomerically pure epoxides and vicinal diols are valuable intermediates for the synthetic organic chemist and as a consequence there are many reported chemical and biochemical methods for their synthesis. The approaches taken by the various researchers in these fields have been reviewed in recent years and will be presented in outline only for the purposes of this review.

Asymmetric epoxidation of alkenes commenced with a report in 1965 that low levels of enantioselectivity could be achieved using percamphoric acid.[9,10] However, useful levels of asymmetric induction were not reported in this field until Katsuki and Sharpless reported the titanium-catalysed asymmetric epoxidation of a wide variety of allylic alcohols with optical yields usually greater than 90%.[11] The discovery that the addition of molecular sieves to the reaction media had an extremely beneficial effect on the course of the reactions,[12,13] such that nearly all allylic alcohols may be epoxidised catalytically, has led to the extensive use of this reaction in synthetic chemistry. An excellent review of the asymmetric catalytic epoxidation of allylic alcohols has been published by one of the discoverers of the reaction.[14] The reactions are compatible with an astonishing array of functionality; the one drawback of this exceptional reaction is the requirement of the alkenes to have hydroxyl functionality in the allylic position.

The search for reagents which perform the catalytic asymmetric epoxidation of unfunctionalised alkenes was continued independently by Katsuki and Jacobsen whose optically active (salen)manganese(III) complexes have been shown to be highly effective catalysts for the desired reaction. The optimisation and scope of the reagents involved have been reviewed recently by the principal researchers in the field.[15,16] While most references to these reactions describe the substrates as unfunctionalised, it is noticeable that, due to the steric and electronic nature of the catalysts, the best substrates are *cis*-alkenes conjugated with aryl, acetylenic and alkenyl groups. *cis*-Alkenes bearing only alkyl substituents are epoxidised with moderate enantioselectivity. Conjugated tri-substituted alkenes (*Z*-configuration) bearing a methyl group *cis* to the hydrogen are also good substrates, while epoxidation of *trans*-alkenes proceeds with only moderate enantioselectivity.[16] The limitation that the alkenes amenable to enantioselective epoxidation must be conjugated is, arguably, a greater drawback than the requirement that the previously described Sharpless-Katsuki titanium-catalysed epoxidation is exclusive for allylic alcohols.

The area of asymmetric synthesis of vicinal diols has been dominated by the Sharpless osmium-catalysed asymmetric dihydroxylation (AD) of alkenes in the presence of quinuclidine ligands. The process has been extensively reviewed in recent years by the principal discoverer of the reaction.[17,18] Included in one of the reviews is an interesting discussion of the uses of vicinal diols in asymmetric synthesis.[18] The reaction is highly effective for the AD of most of the possible alkene substitution patterns. Some mono-substituted (i.e. terminal) alkenes are dihydroxylated with good enantioselectivity depending on the choice of chiral quinuclidine ligand. *trans*-Disubstituted alkenes are particularly good substrates for the AD. A limited number of 1,1-disubstituted and tri-substituted alkenes have been shown to be good substrates while *cis*-disubstituted alkenes have proven to be the most difficult to dihydroxylate with high enantioselectivity.[17]

Biochemical approaches towards the asymmetric synthesis of epoxides have been reviewed in recent years by various workers.[19-22] Direct stereospecific epoxidation of alkenes by isolated monooxygenases (cytochromes P 450) and bacterial monooxygenases (from *pseudomonads*, *mycobacteria*, *xanthobacter*, *corynebacteria* and *nocardia*) has been reported. Typically yields are relatively low but enantiomeric excesses

may be high. However, almost all of the epoxides produced are of *R*-absolute configuration and the bacteria are usually highly substrate specific. Epoxides may be produced indirectly from alkenes by haloperoxidases, *via* initial halohydrin formation and subsequent ring closure. A final biocatalytic approach to chiral epoxides is that of enzymatic resolution; either by means of lipases, whose use necessitates the additional presence of ester, acid or alcohol functionality, or by the direct enantioselective degradation of epoxides by epoxide hydrolase enzymes.

1.3 The occurrence of epoxide hydrolases

Epoxides may be formed in vivo by the action of cytochrome P450 monooxygenases on alkene substrates.[23] Due to their electronic polarisation and strained structure, epoxides may react with nucleophilic groups in many tissue constituents, including macromolecules of crucial importance to normal cellular function such as DNA, RNA and proteins. Therefore epoxide hydrolases play an important role in the metabolism of xenobiotics, in particular of aromatic systems, the oxides of which are thought to be responsible for the carcinogenic nature of polycyclic aromatic systems.[24]

Epoxide hydrolases have been isolated from a wide range of organisms. They have been found in all mammalian species tested, the most widely studied being the mammalian liver microsomal epoxide hydrolase (mEH).[24] In addition, four other mammalian epoxide hydrolases have been identified: soluble epoxide hydrolase (sEH, sometimes referred to as cytosolic epoxide hydrolase, cEH, in the literature), cholesterol epoxide hydrolase, leukotriene A_4 epoxide hydrolase and hepoxilin epoxide hydrolase.[25]

Until recently it was thought that epoxide hydrolases from other sources were rather rare, in particular those from microbial sources which could be produced on a relatively large scale. However, a survey of the literature shows that a number of epoxide hydrolases have been purified from microbial,[26-30] and other sources, such as potatoes,[31] insects,[32] and soy beans.[33]

This review will concentrate on the potential synthetic applications of the various epoxide hydrolases with regard, in particular, to the substrate range and enantioselectivity shown.

2. Mammalian epoxide hydrolases

Of the five mammalian epoxide hydrolases mentioned above, by far the most widely studied, in terms of its biological role and its potential in synthetic chemistry, is that found in mammalian liver microsomes (mEH). The enzyme has been shown to be a single peptide chain with a molecular weight of 48.5-49.5 kDa. The pH optimum of the enzyme is between 8.9 and 9.4. MEH does not contain any prosthetic groups such as haem or flavin, and no significant content of iron, zinc, copper, manganese or molybdenum was found.[24]

The only other mammalian epoxide hydrolase whose chemistry has been studied is mammalian soluble epoxide hydrolase (sEH). Soluble epoxide hydrolase was until recently usually referred to as cytosolic epoxide hydrolase (cEH) to distinguish it from the membrane anchored mEH. However evidence is accumulating that the enzyme is located simultaneously in the cytosol and in the peroxismal matrix of liver cells, such that the term "soluble" seems to characterise the enzyme more precisely and is recommended.[34]

2.1 Mechanism of mammalian epoxide hydrolases

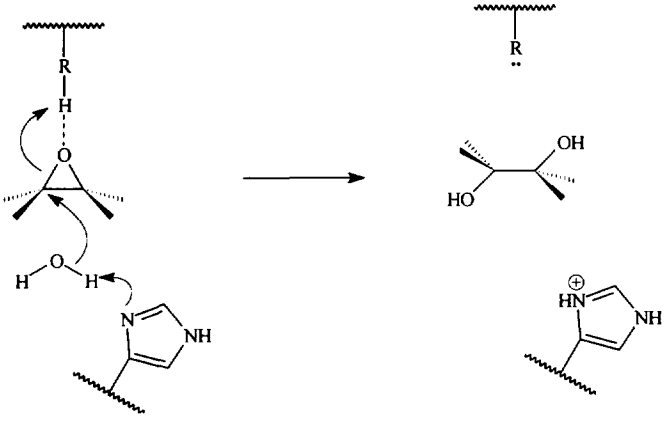
Before the chemistry of mEH is reviewed, a brief summary of its proposed mechanism will be presented.

Scheme 4 shows the two mechanisms which have been proposed for mEH over the last twenty years. Mechanism 1 is a general base-catalysed mechanism whereby a histidine residue abstracts a proton from a water molecule trapped in the active site of the enzyme. This generates a free hydroxyl anion which attacks one end of the epoxide, which in turn may have been activated by partial protonation from, for example, a lysine residue.

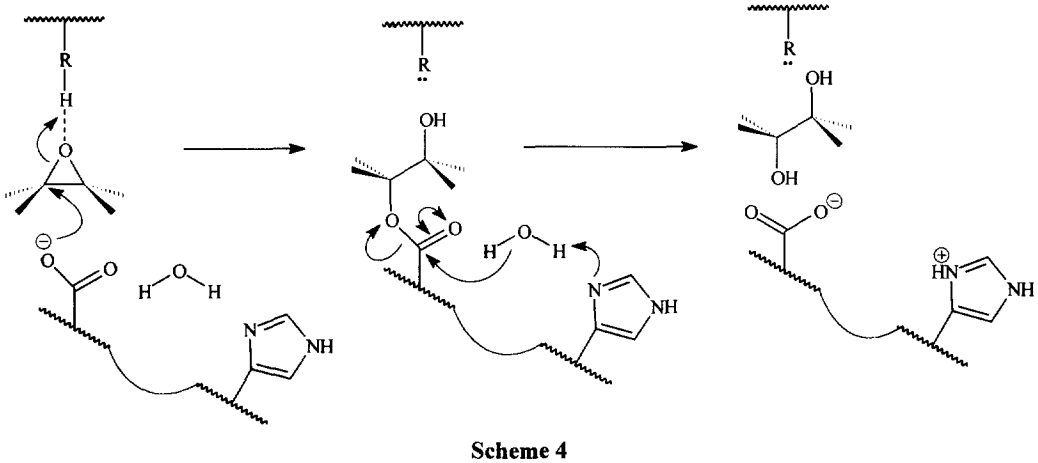
The second mechanism involves the attack of a nucleophilic carboxylate residue at one end of the epoxide which again has been activated by protonation. This gives rise to an α-hydroxyester intermediate

covalently bound at the active site of the enzyme. The intermediate is hydrolysed by a hydroxide anion, again generated by the deprotonation of a water molecule by a histidine residue, release of the diol product regenerates the nucleophilic carboxylate residue.

Mechanism 1



Mechanism 2



Scheme 4

Only recently has mechanism 2 been shown to be the more likely route for epoxide hydrolysis. Early work by Du Bois *et al.* [35] showed that mEH was inactivated irreversibly by specific alkylation of histidine residues, leading them to propose mechanism 1 as a possible pathway. Bell and Casper[36] used site directed mutagenesis to identify His 431 as essential for activity of the enzyme, again leading them to propose mechanism 1 as the route of epoxide hydrolysis. However, none of the observations excluded mechanism 2, which also requires a histidine residue to generate a hydroxide anion for hydrolysis of the ester intermediate.

Evidence for the likelihood of mechanism 2 came from an elegant set of experiments published by Armstrong.[37] The experiments involved single turn-over reactions of the enzyme in ^{18}O-labelled water and of ^{18}O-labelled enzyme in unlabelled water. The former reaction resulted in no incorporation of ^{18}O into the diol product whereas the latter reaction gave ^{18}O-labelled diol product. This was the first definitive evidence that mechanism 1 was probably not the likely course, and that the additional oxygen atom incorporated into the diol product had originated from the enzyme itself rather than from water bound at the active site.

Ironically, perhaps the best evidence for the existence of a covalently bound ester intermediate in the mechanism of mEH came from the elucidation of the mechanism of another enzyme, haloalkane dehalogenase (HAD). HAD catalyses the conversion of 1-haloalkanes to primary alcohols. Verschueren *et al.*[38] showed by X-ray crystallographic studies that the mechanism of HAD involves S_N2 displacement of one chlorine atom of 1,2-dichloroethane by an aspartate residue (Asp 124). This gives rise to an alkylated enzyme similar to that shown in mechanism 2. Hydrolysis of that intermediate occurs with the activation of a water molecule by a His-Asp pair of residues (His 289 and Asp 260) at the active site.

Arand *et al.*[34] observed that the C-termini of mEH and sEH have regions of high sequence homology with HAD (and a related enzyme haloacetate dehalogenase). In particular the regions around Asp 124, Asp 260 and His 289 (HAD); Asp 333, Asp 495 and His 523 (sEH) and Asp 226, Asp 352 and His 431 (mEH) showed significant homology. It was hypothesised that the three enzymes were related in evolutionary terms and shared the same type of mechanism (mechanism 2 for mEH and cEH). The same authors have since demonstrated for sEH that Asp 333, Asp 495 and His 523 are essential for catalytic activity and that variation in the amino acids flanking these residues causes significant changes in the kinetic parameters of the enzymatic reaction.[39]

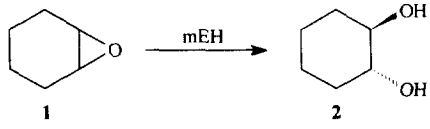
2.2 The chemistry of mammalian epoxide hydrolases

In addition to the studies on their biological role and mechanism, a great deal of attention has been focused on the use of mammalian epoxide hydrolases, in particular mEH, as a potential asymmetric catalyst in synthetic organic chemistry. This section will review the chemistry of mammalian epoxide hydrolases with a particular regard to the range of substrates accepted and the enantioselectivity of the enzymes. Generally mEH shows greater enantioselectivity than the related sEH, and as a result, has seen the most use as a potential asymmetric catalyst. Not surprisingly, the substitution pattern around the oxirane ring has a significant effect on the efficacy of enzymatic hydrolysis by mEH. Oesch *et al.* described structure-activity relationships for substrates of mEH.[40] Using partially purified mEH from guinea pig liver, they concluded that monosubstituted oxiranes with a large hydrophobic substituent were excellent substrates for mEH. *cis*-Disubstituted and 1,1-disubstituted epoxides are reasonable substrates while *trans*-disubstituted, trisubstituted and tetrasubstituted epoxides were extremely poor substrates for the enzyme. It was noted that alicyclic epoxides (i.e. oxides of cyclic alkenes) varied considerably in their ability to act as substrates for mEH. No details of product or substrate enantioselectivity were recorded. In addition, it should be noted that if both enantiomers of a racemic epoxide are good substrates for the enzyme and both are hydrolysed rapidly, it is unlikely that the enzyme will show a particularly high degree of enantioselectivity and will be of little use to the synthetic organic chemist.

The review of mammalian EH chemistry will be divided according to the type of substrate: alicyclic epoxides, epoxides of heterocycles, epoxybromocyclohexanes, aryl-substituted epoxides and aliphatic epoxides.

2.2.1 Alicyclic epoxides

Whilst not the first group to study the enzymatic hydrolysis of epoxides, Jerina *et al.*[41] were the first to study the enantioselectivity of mEH catalysis through the hydrolysis of cyclohexene oxide **1** and other substrates. On complete conversion of the epoxide the diol product was formed in ~70% ee, **Scheme 5**.



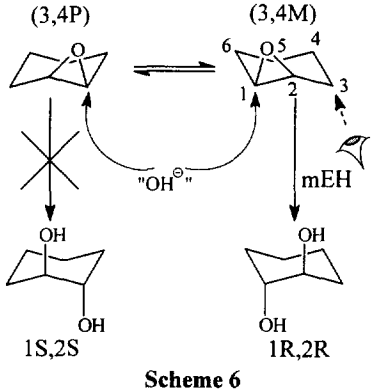
1 **2**

Scheme 5

The enantiomeric excess of the product diol **2** was calculated from the optical rotation by comparison with the rotation of the known optically pure compound.[42] Later Bellucci *et al.*[43] repeated the transformation

using rabbit liver mEH and the product optical purity was measured by HPLC analysis of diastereomeric derivatives. In this study *trans*-1*R*,2*R*-dihydroxycyclohexane **2** was formed in 90% enantiomeric excess. The reaction was stopped after seven hours as after this time enzyme inactivation had begun to occur and non-enzymatic hydrolysis of the epoxide began to be a competitive process. Allowing for a small amount of non-enzymatic hydrolysis, the enantioselectivity of the enzyme itself was 94%.

Cyclohexene oxide is a *meso* compound but exists as a rapidly equilibrating mixture of 3,4M and 3,4P conformers which are mirror images of each other,[44] where M (minus, anticlockwise) and P (plus, clockwise) refer to the helicity about the 3,4 C-C bond.



Scheme 6

The enantioselectivity of mEH with respect to the *meso* cyclohexene oxide, **Scheme 6**, was justified as the preferential binding of the 3,4M conformer of cyclohexene oxide followed by the expected *trans*-diaxial hydrolysis of the epoxide *via* inversion at the *S*-configured stereocentre, yielding the 1*R*,2*R* diol.

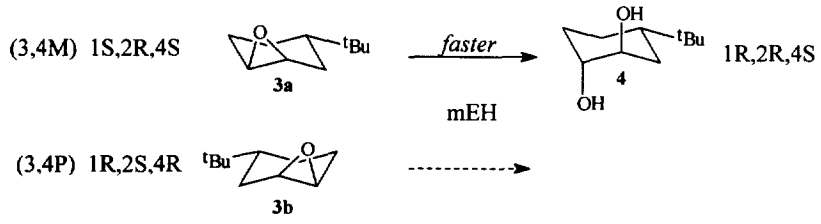
A related study by the same authors[45] analysed the product enantioselectivity of C_5 - C_8 epoxycycloalkanes with mEH and sEH. The results are summarised in **Table 1**.

Substrate	**mEH** % ee diol	Absolute configuration	**sEH** % ee diol	Absolute configuration
Cyclopentene oxide	90	1*R*,2*R*	60	1*R*,2*R*
Cyclohexene oxide	76	1*R*,2*R*	20	1*R*,2*R*
Cycloheptene oxide	40	1*R*,2*R*	30	1*R*,2*R*
Cyclooctene oxide	70	1*R*,2*R*	-	-

Table 1 Enantioselectivity in the hydrolysis of homologous carbocyclic epoxides by mEH and sEH

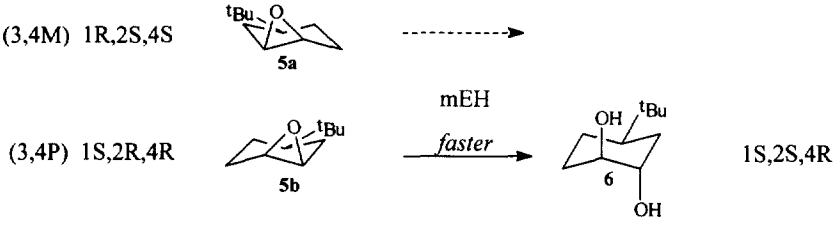
The absolute configuration of all diol products was 1*R*,2*R* indicating that all hydrolyses had proceeded with inversion at the *S*-configured stereocentre. The discrepancy between the ee of the diol formed from cyclohexene oxide obtained in this study and that described previously is not stated explicitly in either paper but is presumably due to competitive non-enzymatic hydrolysis in the latter example due to enzyme inactivation at the later stages of the reaction. Cyclooctene oxide was hydrolysed very slowly by mEH and was not a substrate for the soluble enzyme. The two enzymes show qualitatively similar enantioselectivity with regard to these substrates, indicating similar steric requirements of their active sites, although these are less strict in the case of sEH.

The preference for the 3,4M conformer of epoxycyclohexanes had been noted previously in the mEH-catalysed hydrolysis of *tert*-butylcyclohexene oxides. (±)-*trans*-4-*tert*-Butylcyclohexene oxide **3a/b** was hydrolysed with reasonable substrate enantioselectivity by rabbit liver mEH,[46] giving rise to (−)-1*R*,2*R*,4*S*-1,2-dihydroxy-4-*tert*-butylcyclohexane **4** in 69% ee at 51% conversion, **Scheme 7**.



Scheme 7

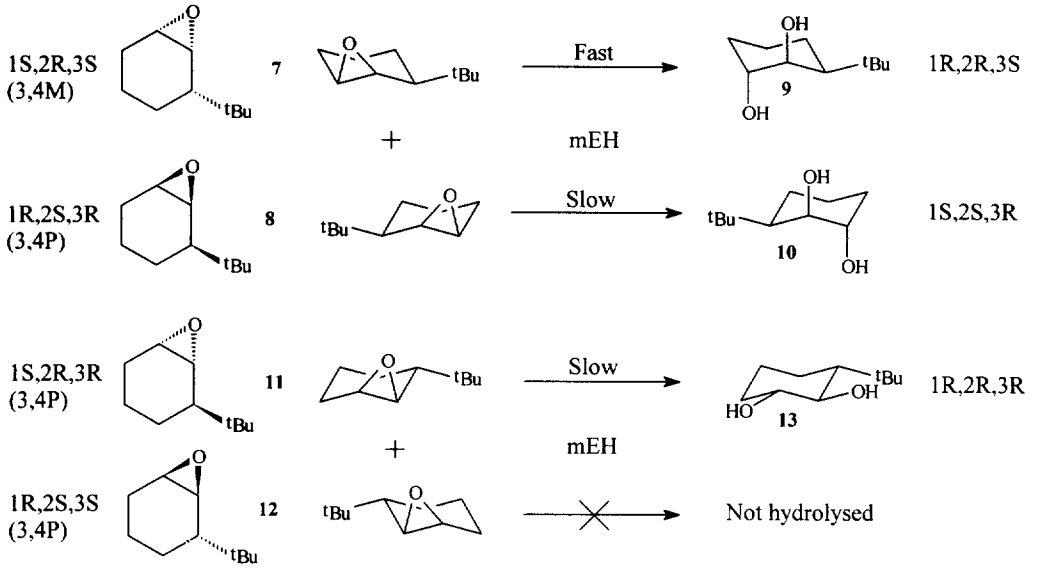
(±)-*cis*-4-*tert*-Butylcyclohexene oxide **5a/b** was hydrolysed with slightly higher enantioselectivity, **Scheme 8**. The 1*S*,2*R*,4*R*-enantiomer **5b** was hydrolysed more rapidly than its antipode and gave (+)-1*S*,2*S*,4*R*-1,2-dihydroxy-4-*tert*-butylcyclohexane **6** in 88% ee at 48% conversion.



Scheme 8

Unusually, the preferred enantiomer was hydrolysed in the 3,4P conformation *via* inversion at the *R*-configured oxirane carbon. It was concluded that while the conformation of the cyclic ring was of importance to the enantiomer-selectivity of the enzyme, a second factor namely a hydrophobic group being placed to the right of the epoxide functionality, when the compound is drawn as shown in **Schemes 7** and **8**, has a greater effect on the substrate binding. *trans*-1*S*,2*R*,4*S*-1,2-Epoxy-4-*tert*-butylcyclohexane **3a** fits both criteria for hydrolysis by mEH; existing in the 3,4M conformation and having the hydrophobic moiety to the right of the epoxide as drawn. *cis*-1*S*,2*R*,4*R*-1,2-Epoxy-4-*tert*-butylcyclohexane **5b** exists in the 3,4P conformer but is still preferentially hydrolysed by mEH due to the favourable interaction of the *tert*-butyl group to the right of the epoxide.

Studies on the hydrolysis of *cis*- and *trans*-1,2-epoxy-3-*tert*-butylcyclohexane seem to confirm the above hypothesis, **Scheme 9**.[47]



Scheme 9

Hydrolysis of (±)-*cis*-1,2-epoxy-3-*tert*-butylepoxycyclohexane **7/8** yielded 1*R*,2*R*,3*S*-1,2-dihydroxy-3-*tert*-butylcyclohexane **9** in >96% ee at 50% conversion from selective opening of the 1*S*,2*R*,3*S*-epoxide enantiomer **7**. Only when all of this enantiomer had been hydrolysed was any of the 1*S*,2*S*,3*R* diol **10** produced, indicating that both enantiomers had been hydrolysed with inversion at C-1. Despite being a poorer substrate in terms of the initial rate of reaction, mEH showed even higher enantiomer-specificity towards (±)-*trans*-1,2-epoxy-3-*tert*-butylepoxycyclohexane **11/12**. The reaction stopped completely at 50% conversion yielding 1*R*,2*R*,3*R*-1,2-dihydroxy-3-*tert*-butylcyclohexane **13** in >96% ee. None of the other diol enantiomer was produced even after prolonged incubation times. The diol was formed from di-equatorial opening of the 1*S*,2*R*,3*R*-epoxide enantiomer **11**. This unusual course of epoxide ring opening had been previously noted in the acid-catalysed hydrolysis of epoxy-*tert*-butylcyclohexanes,[48] and was rationalised by the steric shielding of *trans*-diaxial (anti-periplanar) opening by the adjacent bulky group.

The results of the enzyme-catalysed hydrolysis are partly consistent with the two requirements of the model for mEH-catalysed hydrolysis of epoxycyclohexanes; only the *cis*-1*S*,2*R*,3*S* epoxide **7** is in the 3,4M conformer and has a hydrophobic group situated to the right when the oxygen atom is drawn top-side and to the front. The *trans*-1*S*,2*R*,3*R*-epoxide enantiomer **11** is not in the preferred conformation but has the required positioning of the hydrophobic moiety. However, the model does not explain why the *cis*-1*R*,2*S*,3*R*-epoxide **8** is hydrolysed, albeit slowly - despite not having either requirement - while the *trans*-1*R*,2*S*,3*S*-epoxide **12** is not hydrolysed at all even though it exists in the required conformer. Hydrolysis of the latter epoxide would require *trans*-diaxial opening at the carbon adjacent to the bulky substituent, which is disfavoured, or the diequatorial opening shown by its antipode. Presumably the active site of the enzyme cannot accommodate either of these two modes of hydrolysis. Unfortunately the enantiomeric excesses of the residual epoxides were not reported for any of the *tert*-butylepoxycyclohexanes analysed.

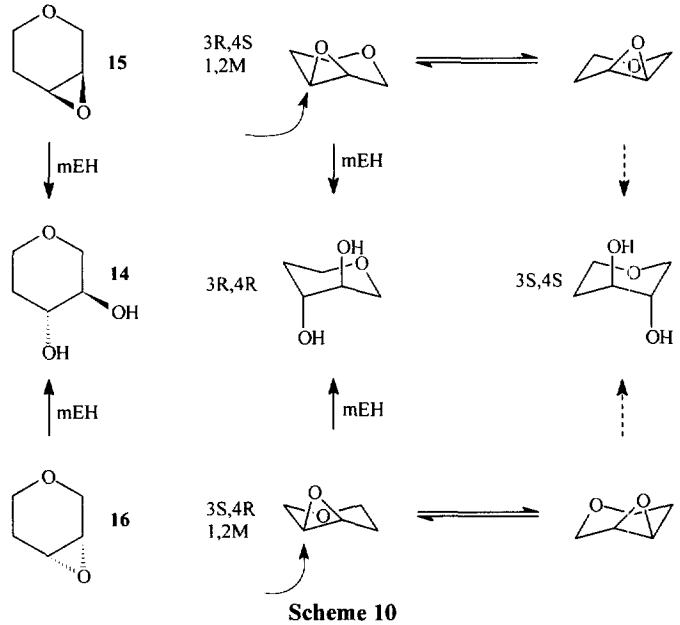
The hydrolysis of both enantiomers of *trans*-4,5-dimethyl-1,2-epoxycyclohexane separately showed once more the slight preference of mEH towards the 3,4M conformers of epoxycyclohexanes.[49] However, when supplied as a racemate the enantioselectivity of the enzyme was low, yielding 1*R*,2*R*,3*R*,4*R*-*trans*-1,2-dihydroxy-*trans*-3,4-dimethylcyclohexane in 40% ee at 20% substrate conversion, dropping to 22% at 55% conversion. An interesting feature of the reaction was that the rate of reaction increased after 50% conversion. One might expect the rate of a kinetic resolution process to drop once all of the favoured enantiomer has been

consumed and the hydrolysis of the residual unfavoured enantiomer to proceed more slowly. The observations during the hydrolysis of *trans*-4,5-dimethyl-1,2-epoxycyclohexane imply that the enantiomer which is hydrolysed first (lower K_M) is turned over in the active site at a slower rate (lower V_{max}) than the other enantiomer (higher K_M) which is turned over faster (higher V_{max}), the former acting as a competitive inhibitor of the latter.

2.2.2 Epoxides of heterocycles

Some interesting results have been obtained in the mEH-catalysed hydrolysis of various epoxytetrahydropyrans and epoxytetrahydrofurans.

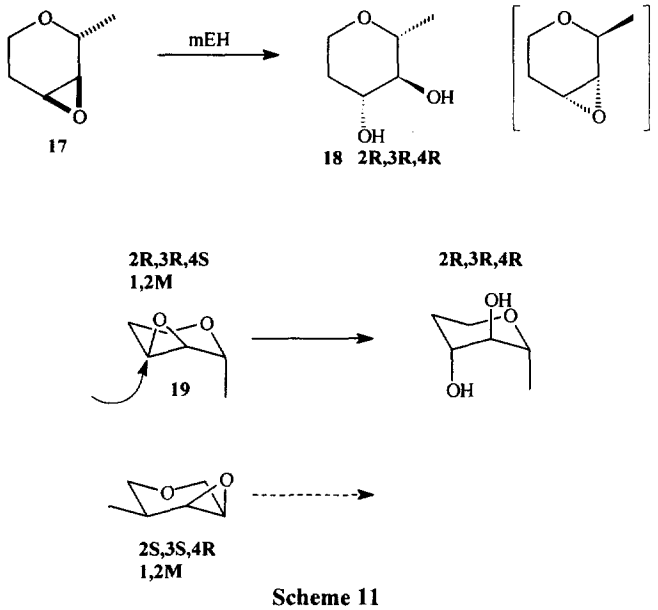
Bellucci *et al.* showed that the mEH-catalysed hydrolysis of (±)-3,4-epoxytetrahydrofuran **15/16** occurred with complete stereoconvergence such that at complete conversion the product of the reaction was (−)-3*R*,4*R*-3,4-dihydroxytetrahydropyran **14** in >96% ee, **Scheme 10**.[50]



Scheme 10

That a single enantiomeric product was obtained from a racemic starting material implies that the two enantiomers were hydrolysed with complete but opposite regioselectivity. Both enantiomers are hydrolysed in the 1,2M conformation (equivalent to 3,4M of cyclohexene oxide) but 3*R*,4*S*-epoxytetrahydropyran **15** is hydrolysed with inversion at C-4 while hydrolysis of the other enantiomer, 3*S*,4*R*-epoxytetrahydropyran **16**, occurs with inversion at C-3, such that the only product of the reaction is (−)-3*R*,4*R*-3,4-dihydroxytetrahydropyran **14**. Positions C-3 and C-4 exhibit different reactivity in non-enzymatic reactions, due to the inductive effect of the THP-ring oxygen atom disfavouring cleavage of the C3-O bond. For example both hydride reduction and reaction with hydrogen halides occurs with a 9:1 preference for attack at C-4, while dimethylamine shows a 2:1 preference for attack at the same carbon. The enzymatic hydrolysis of this epoxide is not sensitive to this inductive effect but is much more sensitive to ring helicity.

Related work by a group at the same university studied the mEH-catalysed hydrolysis of (±)-*trans*- and (±)-*cis*-2-methyl-3,4-epoxytetrahydropyran.[51] Both compounds were better substrates for mEH than the unsubstituted compound, as determined by the initial rates of reaction, confirming the preference of mEH for an additional lipophilic group for binding at the active site. The epoxides were attacked exclusively at C-4, attack at C-3 was not observed for either enantiomer possibly due to steric effects caused by the extra substituent at C-2. Hydrolysis of (±)-*trans*-2-methyl-3,4-epoxytetrahydropyran **17** is illustrated by **Scheme 11**.

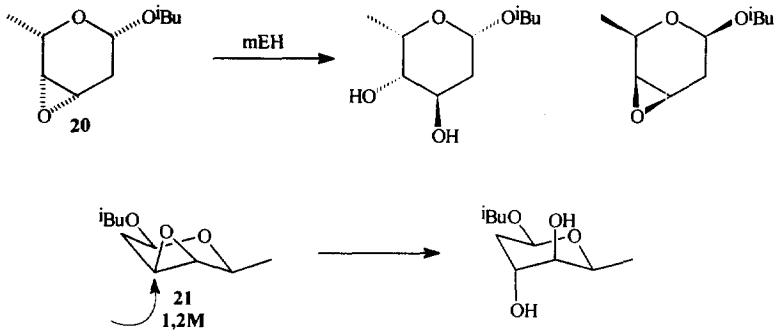


Scheme 11

At 50% conversion the product (−)-2*R*,3*R*,4*R*-2-methyl-3,4-dihydroxytetrahydropyran **18** was optically pure and was formed by *trans*-diaxial opening of the 2*R*,3*R*,4*S*-*trans*-2-methyl-3,4-epoxytetrahydropyran **19** in its 1,2M conformation. This conformation has the methyl substituent in the favoured position to the right of the epoxide when drawn with the oxirane oxygen atom at the front and topside, although this means the methyl group must sit in an unfavourable axial position. The antipode preferentially exists in the 1,2M conformer due the methyl group being equatorial. However, when drawn as described that methyl group is to the left of the epoxide ring and some kind of steric interaction with enzyme active site prevents hydrolysis almost entirely. Indeed the reaction stopped completely at 70% conversion.

The enzymatic hydrolysis of (±)-*cis*-2-methyl-3,4-epoxytetrahydropyran was not studied in such great detail. The initial rate of hydrolysis was greater than that of the *trans*-isomer. A sharp decrease in rate at 50% conversion with the reaction again stopping altogether at 70% conversion suggested that this reaction was also probably highly enantioselective, although no measurement of the enantiomeric excess was presented.

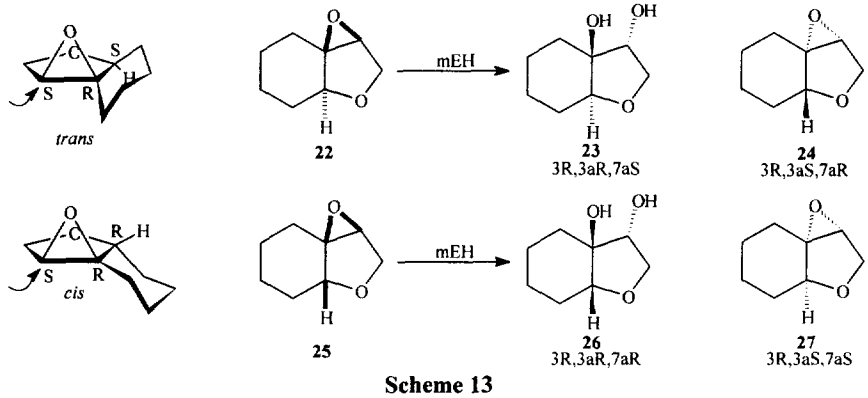
The application of the resolution of racemic 2-methyl-3,4-epoxytetrahydrofurans to the synthesis of optically pure deoxysugars was exploited by Barili *et al.*[52,53] Polysaccharides with non-conventional structures, such as deoxy and aminodeoxysugars, are often found as components of biologically active compounds. This group used mEH-catalysed kinetic resolution of isobutyl-3,4-anhydro-2,6-dideoxy-lyxo-β-DL-hexopyranoside **20** as the key resolution step in the synthesis of both enantiomers of isobutyl-β-L(or D)-boivinopyranoside, **Scheme 12**.



Scheme 12

At 50% conversion both residual epoxide and transformed diol were isolated in >96% ee. 3*S*,4*R*-Isobutyl-3,4-anhydro-2,6-dideoxy-lyxo-β-L-hexopyranoside **21** is hydrolysed preferentially in the required 1,2M conformation, with the methyl group in a position to fit into the proposed lipophilic pocket to the right of the epoxide ring as shown in **Scheme 12**. Beyond 50% conversion, the D-enantiomer was hydrolysed *via* the 1,2P conformer with inversion at the *R*-configured oxirane atom to give, ultimately, racemic diol.

MEH also shows good enantioselectivity towards epoxytetrahydrofurans. Barilli *et al.* showed that enzymatic hydrolysis of *meso*-3,4-epoxytetrahydrofuran proceeded rapidly to give 3*R*,4*R*-3,4-dihydroxytetrahydrofuran in 96.5% ee.[54] Hydrolysis of (±)-*trans*- **22** and (±)-*cis*-octahydro-3,3a-epoxybenzofuran **25** was also highly enantioselective. Only one enantiomer of each geometric isomer was a substrate for mEH, the reactions stopping altogether at 50% conversion. Both isomers were hydrolysed with inversion at C-3 of the 3*S*,3a*R*-enantiomer, leading to 3*R*,3a*R*-diols, **Scheme 13**. The reactions were performed on a preparative (200mg) scale although no yields were given.



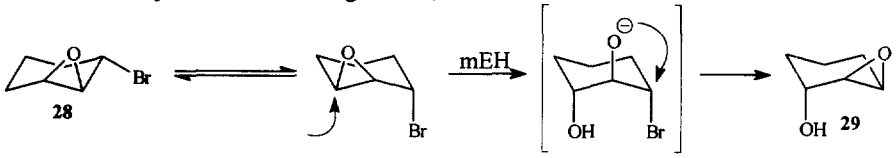
Scheme 13

The residual epoxides and diol products were isolated in high optical purity. Resolution of the *trans*-diastereomer **22** gave both 3*R*,3a*R*,7a*S*-diol **23** and 3*R*,3a*S*,7a*R*-epoxide **24** in >98% ee. Hydrolysis of the *cis*-diastereomers **25** gave rise to residual *cis*-3*R*,3a*S*,7a*S*-epoxide **27** in ~90% ee. The corresponding diol was found to be in >98% ee (3*R*,3a*R*,7a*R*, **26**). The results may be rationalised by the usual presence of a lipophilic group to the right of the oxirane ring.

2.2.3 Epoxybromocyclohexanes

Two studies have been published concerning the mEH-catalysed hydrolysis of (±)-*trans*-3-bromo-1,2-epoxycyclohexane[55] **28** and (±)-*cis*-3-bromo-1,2-epoxycyclohexane.[56] The enantioselectivity of hydrolysis of the *trans*-diastereomer was not measured but interestingly the only product of the enzymatic hydrolysis was

trans-2,3-epoxycyclohexanol **29**. The alcohol was formed from enzymatic hydrolysis of the epoxide and elimination of bromide by intramolecular ring closure, **Scheme 14**.



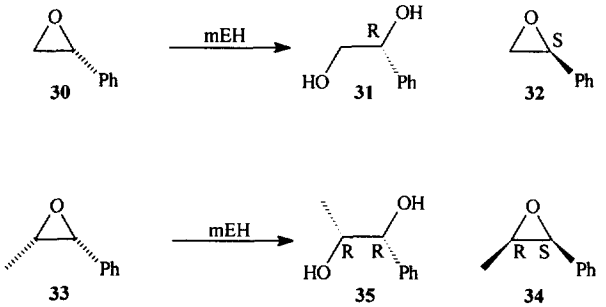
Scheme 14

Hydrolysis of (±)-*cis*-3-bromo-1,2-epoxycyclohexane by mEH was not very enantioselective, leading to 1*R*,2*S*,3*R*-diol in 66% ee during the early stages of the reaction (up to 30% conversion), dropping to just 33% ee at 58% conversion. The optical purity of the residual epoxide was not measured during the latter stages of the reaction, where one would expect it to be highest. The results are rationalised qualitatively using the usual model and indicate that the large bromine atom is probably not bound particularly well by the proposed lipophilic pocket, hence the low enantioselectivity of the reaction.

2.2.4 Aryl-substituted epoxides

A number of workers have studied the mEH-catalysed resolution of a range of aryl-substituted epoxides. Early work by Jerina *et al.*[41] indicated that hydrolysis of styrene oxide **30** by rabbit liver microsomes was not enantiomer-selective producing a slight (<1%) enantiomeric excess of (–)-1*R*-1-phenylethane-1,2-diol **31** at 18% conversion. The enantiomeric excess was calculated from the optical rotation of the product by comparison with the rotation of the known optically pure compound. The reaction is, however, highly regioselective. When the enzymatic hydrolysis is performed in $H_2{}^{18}O$, over 90% of the ^{18}O label was found to be incorporated into the primary hydroxyl group of the diol. Bellucci *et al.* repeated the work with more accurate chiral GLC analysis of the starting epoxide and diol.[57] Styrene oxide was hydrolysed with higher enantioselectivity than reported previously. At low conversion (12%) the diol product **31** was optically pure with *R*-absolute configuration. At high conversion (78%) the residual *S*-epoxide **32** was optically pure. At conversion closer to the halfway stage of the reaction (56%) the enantiomeric excesses of epoxide and diol were just 76% and 45% respectively, **Scheme 15**.

(±)-*cis*-β-Methyl styrene oxide **33** was hydrolysed by mEH with excellent enantioselectivity, giving rise to both 1*S*,2*R*-1-phenylpropene oxide **34** and 1*R*,2*R*-1-phenyl-1,2-dihydroxypropane **35** in optically pure form. Hydrolysis of both styrene oxide and (±)-*cis*-β-methyl styrene oxide occurs with attack at the less hindered end of the epoxide (C-2) during mEH mediated catalysis, **Scheme 15**, an observation which had been made previously by Hanzlik *et al.*[58]



Scheme 15

MEH was less enantioselective towards (±)-*trans*-β-methyl styrene oxide. The highest enantiomeric excess recorded for the residual 1*R*,2*R*-epoxide was 30% at 58% conversion, while the diol (1*S*,2*R*) was formed in just 23% ee even at low conversion (10%). The regiochemistry of hydrolysis was analysed by

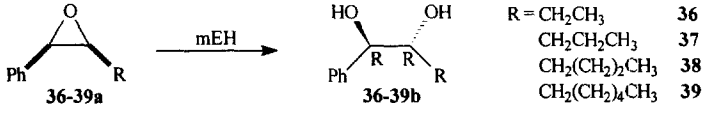
incubating the optically pure epoxides with mEH. Both *R*- and *S*-styrene oxide were hydrolysed with complete retention of stereochemistry, confirming attack at C-2 only. The latter epoxide was measured to have a K_M 2.6 times higher than the former, hence preferential hydrolysis of the *R*-enantiomer occurs during kinetic resolution. The *R*-enantiomer acts as a competitive inhibitor of the *S*-enantiomer despite the latter having a V_{max} 3.2 times higher than its antipode. A similar situation is observed in the hydrolysis of (±)-*cis*-β-methyl styrene oxide. The 1*R*,2*S*-enantiomer is hydrolysed with inversion at C-2 exclusively. The K_M of this enantiomer is lower, by a factor of 68, than that of its antipode. The result is that during kinetic resolution, the 1*R*,2*S*-enantiomer acts as a strong competitive inhibitor of its antipode, giving excellent enantioselectivity at 50% conversion. After this stage of the reaction the rate increases due to the higher V_{max} of the residual epoxide.

Calculation of K_M values for a range of optically pure substrates made it possible to hypothesise about the steric and electronic nature of the active site of the enzyme. They concluded, unsurprisingly, that all forms having a phenyl group to the right rearside of the oxirane ring, when the substrate is viewed with the oxygen topside and to the front, have similar low K_M values of around 10^{-5}M. This is obviously in agreement with the previous hypothesis describing the enantioselectivity of mEH towards the oxides of cyclic hydrocarbons. Furthermore, the presence of a *cis*-methyl or phenyl (see later) does not affect binding ability, suggesting the existence of a region to the left, rearside which is large enough to accommodate these groups but does little to enhance the binding of these substrates. However, the presence of a methyl group at the front leftside of the epoxide (*trans*-β-methylstyrene oxide) causes an unfavourable interaction with the corresponding region of the active site.

An investigation into the enantioselectivity of sEH towards similar substrates was reported by the same authors.[59] In both cases (styrene oxide and *trans*-β-methylstyrene oxide) sEH showed little enantioselectivity. Racemic styrene oxide and 1-phenyl-1,2-dihydroxyethane were recovered at all conversions. The rate of hydrolysis of the two enantiomers analysed individually, indicated that both enantiomers were hydrolysed at almost the same rate. Studies in ^{18}O-labelled water showed incorporation of the labelled oxygen atom on both C-1 (42%) and C-2 (58%).

Interestingly, when analysed individually 1*S*,2*S*-*trans*-β-methylstyrene oxide and 1*R*,2*R*-*trans*-β-methylstyrene oxide gave exclusively 1*R*,2*S*-1-phenyl-1,2-dihydroxypropane and 1*S*,2*R*-1-phenyl-1,2-dihydroxypropane respectively, indicating, for the first time with a mammalian epoxide hydrolase, inversion at the more hindered (benzylic) carbon of the epoxide. It was suggested from this observation that initial protonation of the epoxide, favouring attack at the benzylic position, plays a greater role for sEH than for mEH. While displaying unusual regioselectivity, sEH is not of any particular use to the synthetic chemist due to its pronounced lack of substrate or product enantioselectivity.

Recently, a study into the enantioselectivity of mEH towards homologous *cis*-β-alkylstyrene oxides was published by Bellucci's group.[60] As shown above, *cis*-β-methylstyrene oxide **33** was resolved completely at 50% conversion, similarly *cis*-β-ethylstyrene oxide **36a** was resolved to give both the 1*S*,2*R*-epoxide and 1*R*,2*R*-diol in optically pure form at 50% conversion. When the reaction of the latter epoxide was allowed to continue to complete conversion, the ee of the formed diol was >90% indicating that the reaction was stereoconvergent. As the length of the β-alkyl chain was increased beyond two carbons, substrate enantioselectivity by mEH diminished almost to zero. However, the same type of stereoconvergent reaction was observed for *cis*-β-propyl- **37a**, *cis*-β-*n*-butyl- **38a** and *cis*-β-*n*-hexylstyrene oxide **39a**, all of which yielded the corresponding 1*R*,2*R*-diols **37-39b**, in high optical purity, on complete hydrolysis by mEH, **Scheme 16**.

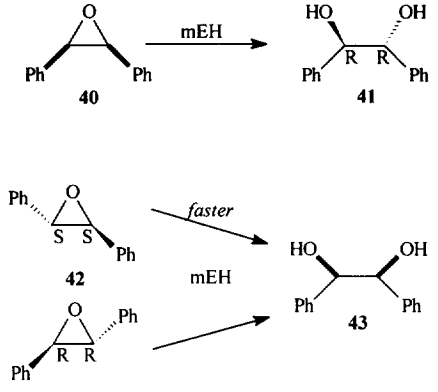


36-39a **36-39b**

R =	CH_2CH_3	**36**
	$CH_2CH_2CH_3$	**37**
	$CH_2(CH_2)_2CH_3$	**38**
	$CH_2(CH_2)_4CH_3$	**39**

Scheme 16

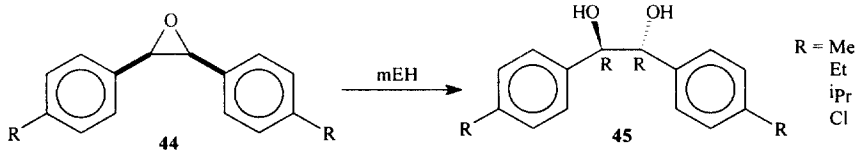
As early as 1972, Watabe *et al.* showed that the mEH-catalysed hydrolysis of *meso-cis*-stilbene oxide **40** was highly stereoselective, **Scheme 17**.[61]



Scheme 17

The sign and magnitude of the optical rotation of the 1,2-diphenyl-1,2-ethandiol **41** produced indicated that the product had 1*R*,2*R*-absolute configuration in >98% ee. *trans*-Stilbene oxide **42** was hydrolysed more slowly by a factor of almost 800, indicating the intolerance of mEH towards *trans*-substituted epoxides. Both 1*S*,2*S*-**42** and 1*R*,2*R*-**42** gave rise to *meso*-1,2-diphenyl-1,2-ethandiol (**43, Scheme 17**) the former at a higher rate than the latter and, although, the residual (+)-1*R*,2*R*-*trans*-stilbene oxide was optically active, no enantiomeric excess was calculated. Bellucci's group repeated the work using more accurate analytical techniques and described an enantiomeric excess of 88% for (+)-1*R*,2*R*-1,2-diphenyl-1,2-dihydroxyethane **41** formed from the mEH-catalysed hydrolysis of *cis*-stilbene oxide.[45] SEH-catalysed hydrolysis of the same epoxide proceeded more slowly, by a factor of 17, but qualitatively yielded the same diol in lower enantiomeric excess (70%).

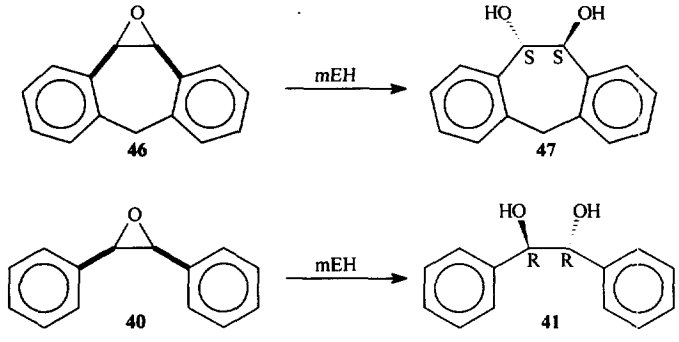
4,4'-Disubstituted stilbene oxides **44** were hydrolysed to the corresponding 1*R*,2*R*-1,2-dihydroxy-1,2-diarylethanes **45** in high optical purities[62] (>96%ee R=Me, Et, ^{i}Pr; 90% ee R=Cl), **Scheme 18**.



Scheme 18

The rates of hydrolysis of the substituted stilbene oxides were markedly reduced. Kinetic calculations showed that the additional substituents increased the K_M of the substrates by a factor of around 5, except in the case of 4,4'-diisopropyl stilbene oxide whose K_M was only slightly higher than that of the unsubstituted parent compound. The presence of a 4-methyl, ethyl or chloro group seems to inhibit sterically the accommodation of the aryl moiety in the right rearside pocket of the active site of mEH. The anomalous isopropyl substituted stilbene oxide reactivity (lower K_M) was rationalised by the increased binding of the second isopropyl group at the back, leftside of the active site countering the reduced binding at the usual right, rearside.

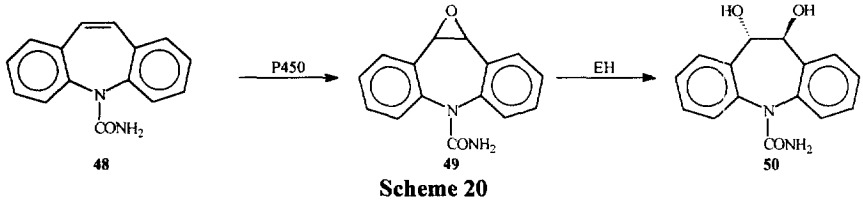
A related example, namely mEH-catalysed hydrolysis of 10,11-dihydro-10,11-epoxy-5H-dibenzo[*a*,*d*]cycloheptane **46**, showed unusual enantioselectivity.[63]



Scheme 19

The structural relationship to *cis*-stilbene oxide **40** is seems obvious but contrastingly mEH hydrolyses **46** to the corresponding *S,S*-diol **47** in 52% ee, **Scheme 19**. Formation of the *S,S* diol occurs *via* inversion at the *R*-configured carbon of the *meso*-epoxide. Hydrolysis was slow, requiring the addition of further portions of enzyme preparation every few hours to achieve 25% conversion in 24h. For comparison *cis*-stilbene oxide was hydrolysed under the same conditions in under four hours. Presumably, the conformational restraint in the benzofused system is responsible for the switch in enantioselectivity, since cycloheptene oxide is hydrolysed to 1*R*,2*R*-1,2-dihydroxycycloheptane. No explanation was offered by the authors.

Inversion at the *R*-configured carbon of *meso*-epoxides has been noted for two other substrates. Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide **48**), structurally related to 5H-dibenzo[a,d]-cycloheptane, is widely used in the treatment of epilepsy and trigeminal neuralgia. *In vivo* metabolism is proposed to proceed *via* cytochrome P450 dependant oxidation of the double bond to 10,11-dihydro-10,11-epoxycarbamazepine **49**, followed by epoxide hydrolase-catalysed hydrolysis to *trans*-10,11-dihydro-10,11-dihydroxycarbamazepine **50**, **Scheme 20**.



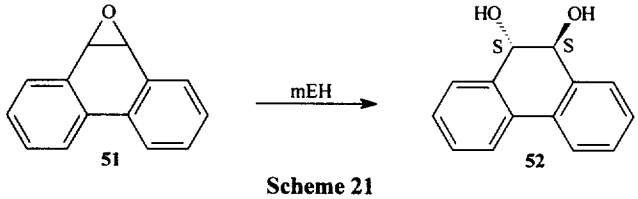
Scheme 20

Bellucci *et al.* proposed to study the stereochemistry of the latter reaction-catalysed by animal liver mEH.[64] Microsomes from rabbit, rat and guinea pig proved to be almost inactive towards epoxide **49**, in agreement with previous reports that human mEH is 100-fold more active. Due to the difficulties in securing sufficient quantities of the enzyme derived from human liver and the dangers of direct administration of the epoxide, it was decided to study the reaction by isolating the diol from the urine of patients under treatment with carbamazepine.

trans-10,11-Dihydro-10,11-dihydroxycarbamazepine **50** was isolated from human urine in 80% ee and shown to be of *S,S*-absolute configuration at the two asymmetric carbons, indicating that inversion had occurred at the *R*-configured oxirane carbon. Whether the enantioselectivity of the transformation of carbamazepine **48** to dihydroxy-carbamazepine **50** resides solely in mEH hydrolysis of an intermediate epoxide was not tested for the reasons described above. Such enantioselectivity could have arisen from, for example, non-selective epoxide hydrolysis followed by selective degradation of the 10*R*,11*R*-10,11-dihydro-10,11-dihydroxycarbamazepine enantiomer. In fungal species, transformation of a C-2 symmetric diol (*trans*-dihydroxycyclohexane) to its enantiomer by a stereoinversion/oxidation process has even been reported.[65] For most substrates only small differences in substrate range and enantioselectivity have been noted in the comparison of mEHs derived from different sources, indicating the possibility of a carbamazepine oxide-

specific hydrolase in humans. Qualitative similarities, however, in the absolute stereochemistry of both *trans*-10,11-dihydro-10,11-dihydroxy-5H-dibenzo[a]cycloheptane formed from mEH catalysis of the corresponding epoxide and the diol isolated from carbamazepine-treated patients may suggest that similar hydrolase enzymes are at work.

9,10-Dihydrophenanthrene-9,10-oxide **51** was hydrolysed by mEH to 9*S*,10*S*-9,10-dihydroxy-9,10-dihydrophenanthrene **52** in 25% ee,[66] **Scheme 21**.



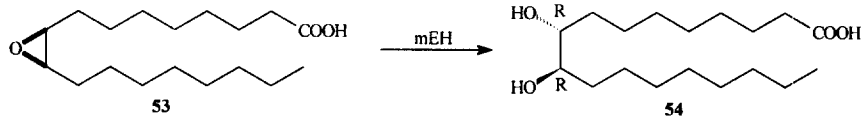
51 **52**

Scheme 21

Hydrolysis of these rather unusual tricyclic *meso*-epoxides seems to be the only case where inversion occurs preferentially at the *R*-configured asymmetric carbon of *meso*-epoxides in mEH-catalysed hydrolysis.

2.2.5 Aliphatic epoxides

In 1972 Watabe and Akamatsu reported the mEH-catalysed hydrolysis of (±)-*cis*-9,10-epoxystearic acid **53** (*cis*-9,10-epoxyoctadecanoic acid),[61] **Scheme 22**.



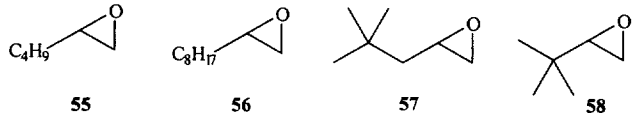
53 **54**

Scheme 22

The enantiomeric excess of the diol formed was estimated from its optical rotation following a recrystallisation step in the purification. Some of the data may, therefore, be unreliable. At 50% conversion, the ee of the product 9*R*,10*R*-9,10-dihydroxyoctadecanoic acid **54** was 24%. Slightly surprising was the observation that the product was optically active even after all of the (racemic) epoxide had been hydrolysed, indicating that the two enantiomers were hydrolysed at different rates with different regioselectivities but with overall preference for inversion at *S*-configured epoxide carbons.

Hanzlik *et al.*[58] studied the regiochemistry of rat liver mEH hydrolysis of *cis*-2,3-[^{18}O]-epoxyoctane. While no details of product or substrate enantioselectivity were given (described only as "low"), 85% of the radiolabel was found at C-3 of the diol product, indicating high regioselectivity in the inversion at the less hindered C-2.

A small range of mono-alkylsubstituted epoxides was assayed by Bellucci *et al.*,[67] **Scheme 23**.



55 **56** **57** **58**

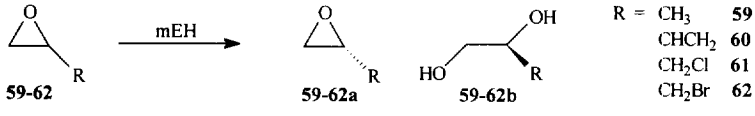
Scheme 23

Straight chain epoxides (1,2-epoxyhexane **55** and 1,2-epoxydecane **56**) were hydrolysed relatively quickly and with almost no enantioselectivity; the product diols had 24% and 16% ee respectively at just 16% and 14% conversion respectively. Branched chain 1,2-epoxyalkanes were found to induce greater enantioselectivity. (±)-4,4-Dimethyl-1,2-epoxypentane **57** was hydrolysed at a rate ~20-fold slower than the straight chain epoxides but the enantiomeric excess of the *R*-configured diol product was 40% at 10% conversion. The homologous epoxide (±)-3,3-dimethyl-1,2-epoxybutane **58** gave the corresponding *R*-diol in 50% ee at 14% conversion. An interesting and previously unrecorded phenomenon, for this enzyme, was that

for the latter two substrates increasing the ratio of epoxide to enzyme preparation, so that the epoxide concentration was above its saturation concentration, led to an impressive increase in the enantioselectivity of the transformation. Under these conditions (−)-2*R*-1,2-dihydroxy-3,3-dimethylbutane was formed from **58** in 92% ee up to 38% conversion. The explanation offered for this unusual result was that when present in a large excess, more substrate may accumulate in or around the microsomes and alter the environment of the enzyme, producing some modification in the enzyme itself which may increase its enantioselecting ability. While the authors admit they have no conclusive explanation of this result, it is clear that such a phenomenon is extremely useful for synthetic purposes.

A related study by the same group examined the enantioselectivity of sEH towards the same range of substrates, with remarkably similar results.[68] The straight chain epoxides showed little or no enantioselectivity, the longer chain giving rise to *R*-diol in low optical purity. At low substrate concentration (25mM), the branched chain epoxides showed were transformed to *R*-diols with low optical purity. When the concentration was raised (100mM) (−)-2*R*-1,2-dihydroxy-3,3-dimethylbutane (formed from hydrolysis of **58**) was present as a single enantiomer up to 45% conversion. A less dramatic though significant increase in enantioselectivity was observed in the case of 4,4-dimethyl-1,2-epoxypentane **57** at high concentrations.

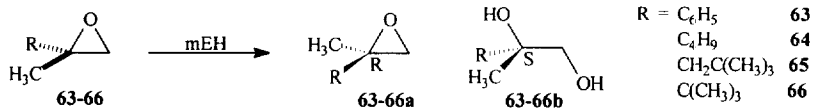
Wistuba and Shurig examined the enantioselectivity of mEH towards a different range of monosubstituted epoxides,[69] **Scheme 24**.



Scheme 24

While the enantiomeric excesses of the product diols were low from the hydrolysis of propene oxide **59** and 1,2-epoxybutene **60**, yielding a slight excess of the *S*-diol (4% ee at 25% conversion and 9% ee at 14% conversion for **59b** and **60b** respectively), mEH showed greater enantioselectivity in the case of epichlorohydrin **61** and epibromohydrin **62**. *R*-3-Chloro- and *R*-3-bromo-1,2-dihydroxypropane (**61b** and **62b** respectively) were formed in 46% ee (17% conversion) and 52% ee (25% conversion) respectively. A switch in the sequence rule due to the halogen atoms of **61** and **62** should be noted, the sense of hydrolysis with respect to the enzyme active site is identical for all four substrates. Unusually the enantiomer with the alkyl group on the front, rightside of the epoxide as drawn in the standard manner was hydrolysed preferentially, albeit with low enantioselectivity.

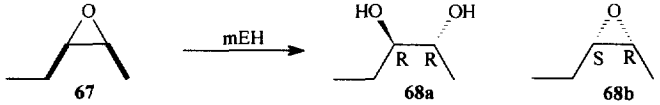
The enantioselectivity of mEH towards a range of 1,1-dialkylepoxides has been studied,[70] **Scheme 25**.



Scheme 25

Non-enzymatic hydrolysis of α-methylstyrene oxide **63** was found to be a competing reaction and the enzymatic hydrolysis was not studied in detail. The hydrolysis of 2-methyl-1,2-epoxyhexane **64** was moderately enantioselective, leading to residual *R*-epoxide **64a** in 40% ee and formed *S*-diol **64b** in 54% ee at 42% conversion. Interestingly, the absolute stereochemistries of both products were opposite to those seen in the hydrolysis of 1,2-epoxyhexane **55**. The presence of a geminal substituent produces a 5-fold increase in the rate of mEH-catalysed hydrolysis but also reverses which enantiomer was hydrolysed preferentially (*S*-2-methyl-1,2-epoxyhexane, *S*-**64**). The reverse in selectivity was attributed to the enhanced stability of the enzyme-substrate complex of the *S*-enantiomer over the *R*-enantiomer, since the position of attack, i.e. the terminal carbon, was unchanged. Enantioselectivity in the hydrolysis of 2-methyl-4,4-dimethyl-1,2-epoxypentane **65** and 3,3-dimethyl-1,2-epoxybutane **66** was very low.

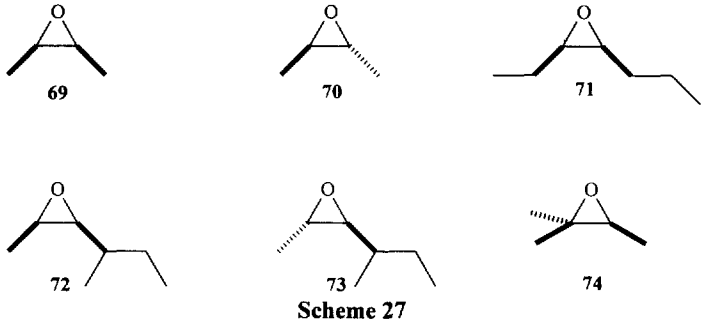
In a detailed study of the hydrolysis of the smallest aliphatic *cis*-epoxide containing differently configured carbon atoms, namely *cis*-2,3-epoxypentane **67**, mEH showed exceptionally good enantioselectivity.[71] 2*S*,3*R*-2,3-Epoxypentane was hydrolysed preferentially, with inversion exclusively at C-2, by mEH. The antipode is not a substrate for mEH leading to both 2*R*,3*S*-2,3-epoxypentane **68b** and 2*R*,3*R*-2,3-dihydroxypentane **68a** in >99% ee at 50% conversion, **Scheme 26**. The racemic *trans*-isomer was hydrolysed with lower enantioselectivity, although the 2*S*,3*S*-enantiomer was hydrolysed more rapidly, leading to the 2*R*,3*S*-diol (58% ee after two hours).



67 **68a** **68b**

Scheme 26

This work was followed by a more detailed analysis of a wider range of 1,2-disubstituted and trisubstituted epoxides,[72] **Scheme 27**.



69 **70** **71**

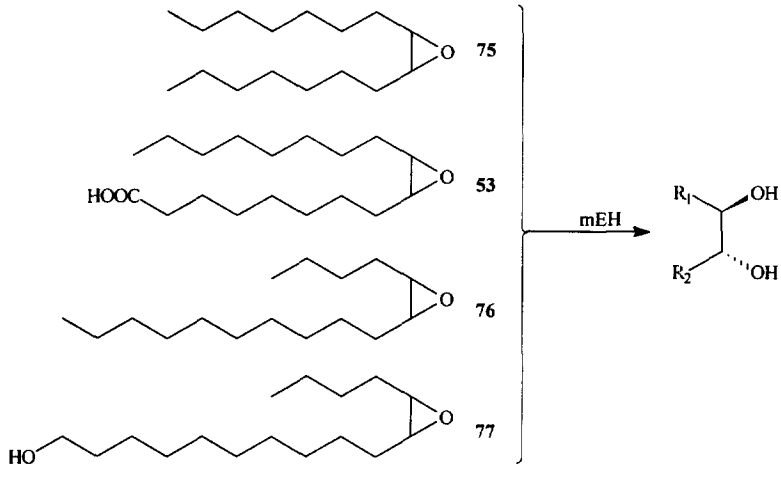
72 **73** **74**

Scheme 27

cis-2,3-Epoxybutane **69** was hydrolysed predominantly *via* inversion at the *S*-configured carbon giving rise to 2*R*,3*R*-2,3-dihydroxybutane in 86% ee. The racemic *trans*-isomer **70** was hydrolysed much more slowly and led to *meso*-2,3-dihydroxybutane as expected. The reaction showed high substrate specificity; 2*S*,3*S*-2,3-epoxybutane was hydrolysed slowly, whereas its antipode was not hydrolysed even after prolonged reaction times. The same sense of enantioselectivity was noted in the hydrolysis of *trans*-stilbene oxide **42**.[61] Both enantiomers of *cis*-3,4-epoxyheptane **71** were very poor substrates for mEH. The reaction proceeded very slowly and with very low enantioselectivity. 4-Methyl-2,3-epoxyhexane contains an additional stereocentre at C-4, the presence of which did not alter the expected enantioselectivity of enzymatic hydrolysis by comparison with *cis*- and *trans*-2,3-epoxypentane.[69] MEH-catalysed hydrolysis of the two (racemic) diastereomers of 4-methyl-*cis*-2,3-epoxyhexane **72** separately occurred *via* initial hydrolysis of the enantiomers with 2*S*,3*R*-absolute configuration. Inversion occurred at C-2 to yield 2*R*,3*R*,4*R*- and 2*R*,3*R*,4*S*-4-methyl-2,3-dihydroxyhexane from the two compounds respectively. Hydrolysis of the other enantiomers only occurred to a small extent (<5%) even after prolonged incubation times. MEH catalysed hydrolysis of 4-methyl-*trans*-2,3-epoxyhexane **73** was, not surprisingly, very slow and while the enantioselectivity was not studied in detail, the stereoisomers with 2*S*,3*S*-configuration were the favoured substrates, as expected. Oesch *et al.* had indicated that trisubstituted epoxides were poor substrates for mEH,[40] however, this study indicated that the smallest trisubstituted epoxide, 2-methyl-2,3-epoxybutane **74** was hydrolysed with high substrate enantioselectivity, yielding *R*-2-methyl-2,3-epoxybutane in 76% ee at 47% conversion, with inversion of the asymmetric mono-substituted oxirane carbon of the *S*-enantiomer predominantly, yielding the *R*-diol.

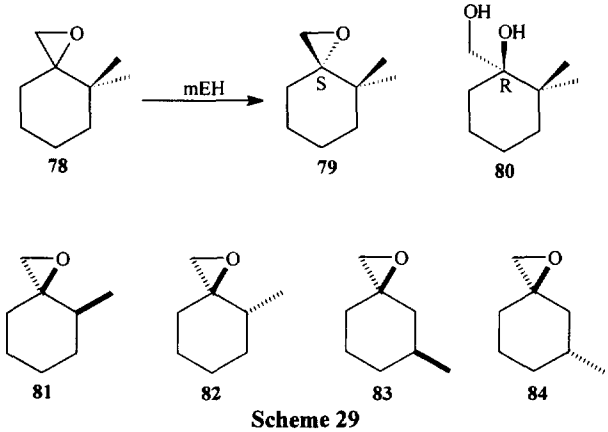
Enantioconvergent hydrolysis of racemic long chain dialkyl epoxides was reported recently by Bellucci's group, **Scheme 28**.[73] *cis*-9,10-Epoxyoctadecane **75** (*meso*), (±)-*cis*-9,10-epoxystearic acid **53**, (±)-

cis-5,6-epoxyhexadecane **76**, (±)-*cis*-11,12-epoxyhexadecan-1-ol **77** were all hydrolysed by mEH giving rise to the corresponding *R*,*R*-diol in >90% ee at 90% conversion **Scheme 28**.



Scheme 28

Recently Bellucci's group have studied the mEH-mediated resolution of racemic methyl substituted methylene cyclohexene oxides,[74] **Scheme 29**.



Scheme 29

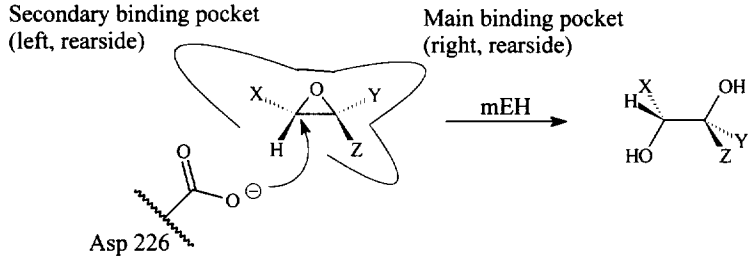
All five epoxides were good substrates for mEH, although significantly less good for sEH. The latter enzyme showed no enantioselectivity in the hydrolysis of any of the epoxides. The presence of a geminal dimethyl group resulted in complete enantioselectivity by mEH. 2,2-Dimethyl methylene cyclohexene oxide **78** was resolved to both (*S*)-2,2-dimethyl methylenecyclohexene oxide **79** and (*R*)-1-hydroxymethyl-2,2-dimethylcyclohexanol **80** in >95% ee at 50% conversion. When a single methyl group is present at C-2, **81** and **82**, enantioselectivity was lower for both diastereomers, while a single methyl group at C-3, **83** and **84** resulted in no enantioselectivity whatsoever for either diastereomer.

2.3 Summary and scope of mammalian epoxide hydrolases

The regio- and stereoselectivities of mEH-catalysed hydrolysis of epoxides obey certain guidelines, with few exceptions.

1) The epoxide ring is always opened by attack *anti* to the oxirane oxygen.
2) Attack is at the less hindered oxirane carbon. This is usually primary or secondary, never tertiary.
3) If the epoxide is viewed as drawn in **Scheme 30** hydrolysis occurs primarily at the left hand carbon.
4) In addition to the above rules, epoxides fused to six-membered rings are hydrolysed preferentially when the ring may adopt a 3,4M conformation.

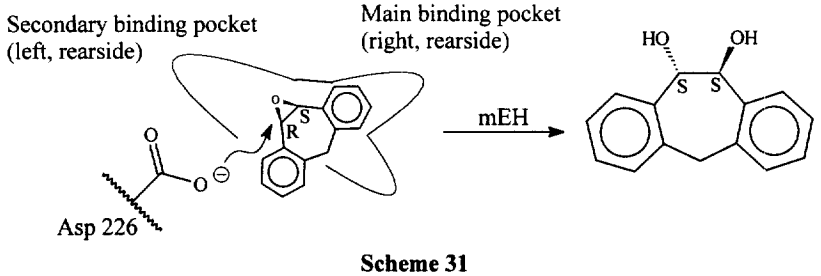
Taking the observations of various workers, and of the groups of Bellucci and Shurig in particular, a model for predicting the enantioselectivity of mEH may be proposed, **Scheme 30**. The rules are in part relevant to sEH, but almost without exception that enzyme shows much lower enantioselectivity.



Scheme 30

The results of Bellucci *et al.* and Shurig *et al.* are somewhat contradictory with respect to mono-substituted epoxides, the former stating that in general *R*-epoxides (Y=R, X=Z=H) are preferentially converted (to *R*-diols) over *S*-epoxides; the latter that *S*-epoxides (Z=R, Y=X=H) are converted to *S*-diols. It should be noted that enantioselectivity in all cases is low, except when R is very bulky (i.e. ^{t}Bu) when the *R*-epoxide is hydrolysed exclusively (yielding *R*-diol) until 50% conversion. In addition Bellucci's group tended to study larger more sterically demanding epoxides compared to the smaller epoxides such as propene oxide and epihalohydrins, studied by Shurig, indicating a possible switch in enantioselectivity as the size of the alkyl group of mono-substituted epoxides increases.

Meso-epoxides (X=Y, Z=H) bind as drawn such that the *S*-configured carbon is attacked by Asp 226 of mEH and undergoes inversion yielding *R,R*-diols of, usually, high optical purity. The only exceptions are the unusual tricyclic dibenzo[a]cycloheptene oxides which give *S,S*-diols, usually, of lower optical purity indicating different binding at the active site. This binding is possibly such that one of the aromatic rings occupies the main binding pocket to the right rearside, while the other protrudes away from the active site. This binding mode is depicted in **Scheme 31**. The oxygen atom towards the top ensures attack at the *R*-configured carbon.



Scheme 31

cis-Disubstituted epoxides, **Scheme 30**, (Y≠X, Y>X, Z=H) are hydrolysed in a similar manner to *meso*-epoxides such that the larger substituent (Y) occupies the main binding pocket leaving the smaller substituent (X) in the left, rearside space.

Arguably the most significant feature of the above, and other proposed models (after the requirement that attack occurs from the front, leftside of the pocket), is the lack of steric freedom on the front, leftside of the epoxide as drawn. It is this feature which prevents *trans*-epoxides from being hydrolysed readily. *S,S*-Configured *trans*-epoxides (Y=H, Z ≥ X) may fit into the active site with the larger of the two groups (Z) occupying the space at the right, frontside of the active site. This binding is not ideal, however, causing a decrease in the rate of hydrolysis, compared to *cis*-epoxides. Hydrolysis then occurs *via* attack at the less hindered end of the epoxide. The antipodal *trans*-epoxide cannot adopt a position with the oxygen at the top which does not cause a steric clash of one of its substituents with the proposed group(s) at the front, leftside of the active site. That interaction also provides the basis for the enantioselective hydrolysis of *S*-2-methyl-2,3-epoxybutane preferentially over its antipode. The *S*-enantiomer may bind as described above (X=Y=Z=Me) while its enantiomer cannot bind with the oxygen atom at the top without one methyl group occupying the unfavourable space at the front, leftside.

While it is evident that mEH, and to a lesser extent sEH, may show high enantioselectivity in the hydrolysis of a wide range of epoxides and that in many cases it should be possible to predict the outcome of these reactions, neither enzyme has ever been used for preparative transformations on a scale larger than a few hundred milligrams. Workers frequently refer to "yields" in the literature, when a close examination of the experimental details reveals a reaction only run on an analytical scale (micromolar range) and the yields were calculated by GLC. For the purpose of this review those "yields" have been reported as "conversions."

The reason for the lack of preparative scale reactions is the source of the enzyme(s), namely mammalian liver. Typically 2-3kg of rabbit liver is required for the preparation of rabbit mEH used in each study documented. In addition, while that preparation is stable indefinitely at -70°C, the activity of the preparation is less stable at working temperatures. Indeed several reports of repeated addition of fresh microsomal preparation have been noted during the hydrolysis of "poor" substrates, which are often those for which enantioselectivity is highest. Biocatalysts derived from microbial sources do not suffer from this lack of availability since they may be produced on an almost unlimited scale by conventional fermentation techniques. One solution to the problem of limited availability of mammalian epoxide hydrolases is therefore to express the protein in a microbial species (e.g. *E. coli*). Techniques are available that enable multiple copies of the same protein to be produced within a single cell such that the protein of interest can constitute a significant proportion of the total expressed protein. Some reports of expression of mEH in *E. coli*,[36] *pseudomonads*[75] and other "immortal" cell lines[76] have been made but no large scale biotechnology has been utilised for the production of synthetically useful amounts of mEH or a cell line overexpressing mEH.

3. Microbial epoxide hydrolases

The problems of availability of mammalian liver epoxide hydrolases do not exist for epoxide hydrolase enzymes derived from microbial sources. Conventional fermentation techniques enable the production of bacteria or fungi on an almost unlimited scale. Reports of epoxide hydrolases from fungi and bacteria were until recently relatively scarce in the chemical and biochemical literature, leading researchers to assume that such enzymes were equally scarce. In recent years, however, research from various groups, in particular those of K. Faber (Austria) and R. Furstoss (France), has shown that a large number of bacteria and fungi contain epoxide hydrolases, many of which show extremely good enantioselectivity.[77] In many cases, multigram-scale reactions have been performed giving rise to epoxides and diols of high optical purity.

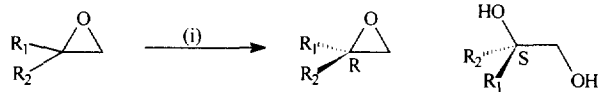
This section will be divided into two sections dealing with epoxide hydrolases from bacterial and fungal sources separately.

3.1 Bacterial epoxide hydrolases

As early as 1967 Schroepfer *et al.* showed that a soluble enzyme preparation from a *pseudomonad* (NRRL-2944), which catalysed the hydration of oleic acid to 10*R*-hydroxystearic acid, also catalysed the hydrolysis of *cis*- and *trans*-9,10-epoxystearic acid.[27,78] The reaction appeared to be stereo- and regiospecific

as hydrolysis stopped at 50% conversion and that the *cis*- and *trans*-epoxides gave *syn*- and *anti*-diols respectively. Both diol products were optically active, as were the residual *trans*- and *cis*-epoxides. Although no enantiomeric excesses were calculated, the values of the optical rotations of the diol products were in close agreement with those previously reported, indicating reasonable optical purity. Labelling studies in [^{18}O]-water indicated that hydrolysis had occurred *via* inversion at C-10.

Noting the scarcity of reports of bacterial epoxide hydrolases in the literature, Kurt Faber's group (Graz, Austria) began a screening programme of a large range of microbial organisms. The screen concentrated in particular on bacterial species, following the fortuitous discovery of epoxide hydrolase activity in a commercially available crude enzyme preparation from a *Rhodococcus* sp. (NOVO SP 409) which previously had been used for the hydrolysis of nitriles.[79] NOVO SP 409 was able to hydrolyse a range of mono- and 1,1-disubstituted epoxides with low to moderate enantioselectivity, **Scheme 32**.

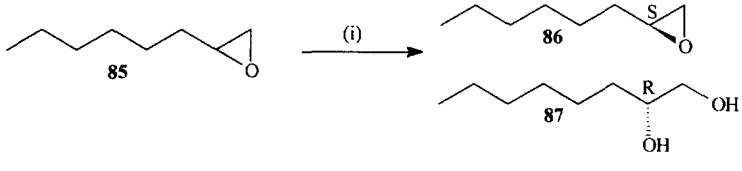


Scheme 32 Reagents: (i) *Rhodococcus* sp. NOVO SP 409

Reactions were generally slow (2-4 days) and were stopped at 50% conversion in order to assess the selectivity of the enzyme. Enantioselectivity was highest where R_1 was a methyl group and R_2 a longer alkyl chain, thus *R*-2-methylepoxyheptane was isolated in 72% ee, and the diol product, *S*-2-methyl-1,2-dihydroxyheptane in 40% ee. Hydrolysis of the *S*-epoxide occurred with retention of stereochemistry. Enantioselectivity was lower in the case of monosubstituted epoxides, for example, partial resolution of 1,2-epoxyoctane gave the *S*-epoxide in 38% ee and the *R*-diol in 22% ee. Interestingly, the enzyme displayed a switch in enantioselectivity on increasing the substitution around C-2, although levels of enantioselectivity were not high enough for synthetic purposes in either case. A range of *meso*-epoxides proved not to be substrates for this enzyme preparation.

Inspired by the discovery of a new bacterial epoxide hydrolase, the group then published the results of a large screen of available organisms.[80] Of 43 strains examined initially, seven showed interesting activity against a range of epoxides. Four bacterial strains, *Rhodococcus* spp. NCIMB 11216, NCIMB 11215 and NCIMB 11540 and *Corynebacterium* sp. UPT 9, were found to show activity along with three fungal species *Diploida gossypina* ATCC 10936, *Fusarium solani* DSM 62416 and *Glomerella cingulata* ATCC 10534. The activity of the fungal species will be discussed along with other fungal species later. Only species showing significant or interesting enantioselectivity will be discussed in this review.

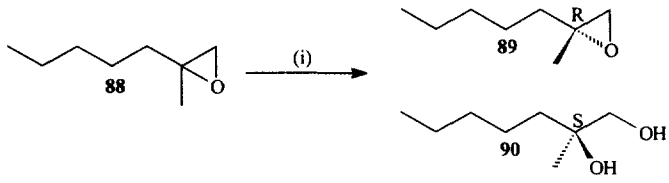
The enantioselectivity of *Rhodococcus* sp. NCIMB 11216 and *Corynebacterium* sp. UPT 9 was moderate towards 1,2-epoxyoctane **85**. Both species preferentially hydrolysed the *R*-epoxide giving *R*-1,2-dihydroxyoctane **87** in 39% ee and 41% ee respectively. The enantiomeric excesses of the residual *S*-epoxides **86** were low (21% ee and 10% ee respectively), **Scheme 33**.



Scheme 33 Reagents: (i) *Rhodococcus* sp. NCIMB 11216 or *Corynebacterium* sp. UPT9

Rather than quote the enantiomeric excesses of epoxide and diol along with the degree of conversion as an assessment of the selectivity of the resolutions, these authors favour the use of the E-value as an indication of selectivity. The E-values of the two reactions described above were 2.8 and 2.6 respectively.

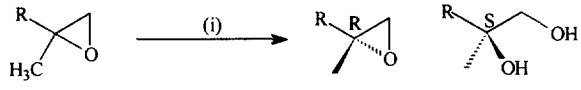
Selectivities were improved dramatically in the hydrolysis of the 1,1-disubstituted epoxide 2-methyl-1,2-epoxyheptane **88**.



Scheme 34 Reagents: (i) *Rhodococcus* spp. NCIMB 11216, NCIMB 11540, or *Corynebacterium* sp. UPT 9

The three bacteria depicted in **Scheme 34** showed the same sense of enantioselectivity giving rise to residual *R*-epoxide **89** and *S*-diol **90** with reasonable to excellent enantiomeric excesses. NCIMB 11216 and NCIMB 11540 gave rise to the diol in 96% ee and 89% ee respectively (E=104 and 29 respectively). The residual *R*-epoxide was found to be of lower optical purity (71% ee and 51% ee respectively). Again, the extra substituent at C-2 led to an interesting reverse in the enantioselectivity of enzymes. The enantioselectivity of the *Corynebacterium* sp. was lower (E=7).

The promising nature of the enantioselectivity exhibited by *Rhodococcus* sp. NCIMB 11216 led to a more detailed study of its substrate range,[81] **Scheme 35**.



Scheme 35 Reagents: (i) *Rhodococcus* sp. NCIMB 11216

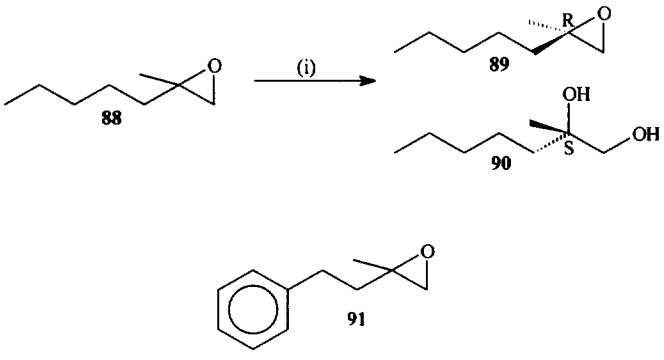
The results are summarised in **Table 2**.

Substrate R=	Conversion / %	Epoxide ee / %	Diol ee / %	Selectivity (E)
n-Pentyl	43	71 (*R*)	96 (*S*)	104
n-Heptyl	20	25 (*R*)	98 (*S*)	126
n-Nonyl	36	55 (*R*)	>99 (*S*)	>200

Table 2 Hydrolysis of homologous 2-methyl-2-alkyl-1,2-epoxides by *Rhodococcus* sp. NCIMB 11216

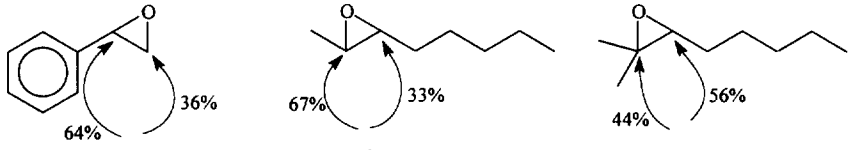
The ee values of the diol products were all in excess of 95%, increasing with increasing length of the alkyl chain. Since the reactions were stopped well before 50% conversion, the residual epoxides are of low optical purity. No details were given of the optical purity of the residual epoxide towards the latter stages of the reactions. While increasing the length of one alkyl chain had a beneficial effect on the selectivity, increasing the length of the shorter substituent dramatically decreased the level of selectivity. Thus, 2-ethyl-1,2-epoxyheptane was hydrolysed with low enantioselectivity, giving rise to the corresponding diol in 70% ee at 20% conversion.

Two other species, *Rhodoccocus* equi IFO 3730 and *Mycobacterium paraffinicum* NCIMB 10420 exhibit similar enantioselectivities towards the same range of 1,1-disubstituted epoxides (E>200).[77] The latter species also showed the same level of selectivity towards benzylglycidyl ether. Recently four further strains, *Nocardia* H8, *Nocardia* EH1, *Nocardia* TB1 and *Rhodococcus ruber* DSM 43338, have been reported to show excellent selectivity (E>200) in the hydrolysis of 2-methyl-1,2-epoxyheptane **88**, **Scheme 36**.[82] Previously, diols of high optical purity had only been observed at low substrate conversion, leading to low yields and more importantly residual epoxides of low optical purity. However, two of the new strains (*Nocardia*s EH1 and TB1) catalysed the complete resolution of this substrate such that at 50% conversion both residual *R*-epoxide **89** and transformed *S*-diol **90** were present in >99% ee. Introduction of an aryl moiety into the side chain (4-phenyl-2-methyl-1,2-epoxybutane **92**) all but destroyed the selectivity of the *Nocardia* enzymes (EH1 E=5.6, TB1 E=13).



Scheme 36 Reagents: (i) *Nocardia* EH1 or *Nocardia* TB1

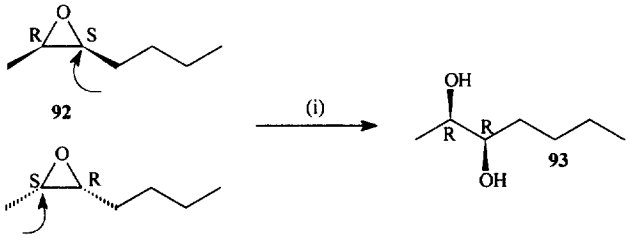
In all of the cases examined hydrolysis was found to proceed *via* inversion of the unsubstituted oxirane carbon leading to retention of configuration in the diol product. The same group have noted that *Rhodococcus* sp. NCIMB 11216 shows less regioselectivity in the hydrolysis of 2,3-epoxyalkanes and of styrene oxide, **Scheme 37**.[5]



Scheme 37 Regioselectivity of attack by *Rhodococcus* sp. NCIMB 11216

Incorporation of ^{18}O into the diol product of reactions run in ^{18}O-labelled water allows a direct measure of the regioselectivity of enzymatic hydrolysis, given by percentages in **Scheme 37**. No enantiomeric excesses or conversions were given so the data are not useful for predicting the synthetic utility of the enzyme. For example, if one enantiomer is hydrolysed with retention of configuration and the other with inversion of stereochemistry (i.e. the two enantiomers are hydrolysed with complete but opposite regiochemistry) the result is the deracemisation of the racemic epoxide to a single enantiomer diol.[50] However, the results, as presented, give no indication of this type of reaction since no data are given for the optical purity of the products.

Nocardia EH1 catalyses the enantioconvergent hydrolysis of *cis*-2,3-epoxyheptane **9** to 2*R*,3*R*-2,3-dihydroxyheptane **93** (85% yield, >90% ee),[82] **Scheme 38**.

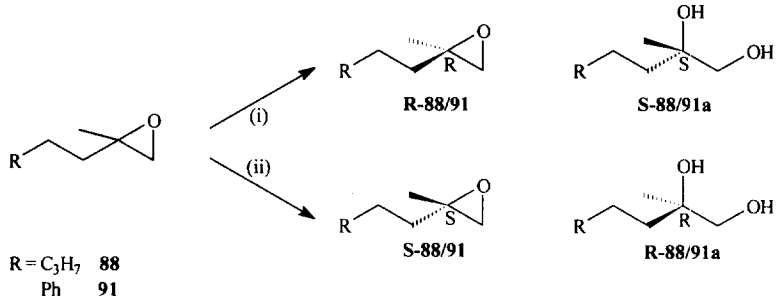


Scheme 38 Reagents (i) *Nocardia* EH1

The 2*S*,3*R*-enantiomer reacted 10-fold faster than the 2*R*,3*S*-enantiomer, however, hydrolysis of both enantiomers occurred *via* attack at the *S*-configured carbon atom of both enantiomers, leading exclusively to the 2*R*,3*R*-diol. Further evidence of this mechanism was obtained by ^{18}O-labelling studies.

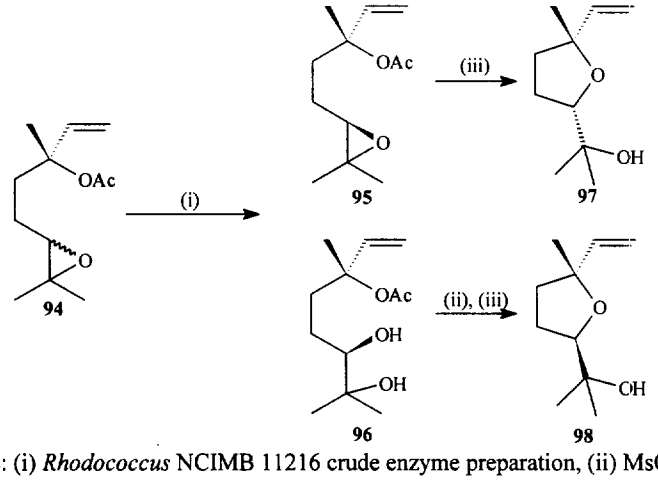
All of the bacterial enzymes described so far show the same sense of enantioselectivity (i.e. they all produce the *S*-diol from the *S*-enantiomer of 2-methyl-1,2-epoxyalkanes preferentially). Faber's group have

also isolated two strains which display complementary enantioselectivity, *Mycoplana rubra* and a second strain designated "Rot", whose taxonomy is still being assessed at the time of writing.[83] The enantioselectivity of the new strains was not quite as high with regard to 2-methyl-1,2-epoxyheptane **88** and 4-phenyl-2-methyl-1,2-epoxybutane **91** as some of the strains described previously (*Mycobacterium paraffinicum* NCIMB 10420 in particular[83,84]). However, these two epoxides were hydrolysed by the two strains, *Mycoplana rubra* and "Rot", giving R-2-methyl-1,2-dihydroxyheptane ***R*-88a** in 69% and 78% ee respectively, and *R*-4-phenyl-2-methyl-1,2-dihydroxybutane ***R*-91a** in 83% and 68% respectively, **Scheme 39**. The enantiomeric excesses of the residual epoxides ***S*-88/91** were very low for the two new strains (<10% ee) indicating that the reactions were probably stopped at very low conversion.



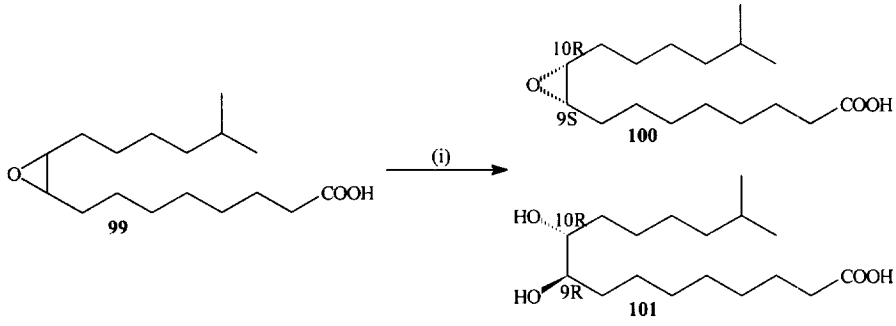
***Scheme* 39** Reagents: (i) *Mycobacterium paraffinicum* (ii) *Mycoplana rubra* or "Rot"

Faber's group is responsible for the only preparative scale synthesis of a natural product using a bacterial epoxide hydrolase as the key enantioselective step of the route.[85] A crude enzyme preparation from *Rhodococcus* NCIMB 11216 was used to distinguish epoxides **94** giving rise to both residual 3*R*,6*R*-epoxide **95** and transformed 3*R*,6*R*-diol **96** in 35% yield and 98% de, ***Scheme* 40**. Simple chemical transformations enabled the synthesis of 2*R*,5*S*- and 2*R*,5*R*-5-(1-hydroxy-1-methylethyl)-2-methyl-2-vinyltetrahydrofurans **97** and **98**, "linalool oxides", aroma compounds of several plants and fruits. Interestingly, the biotransformation was enantioconvergent: both diastereomers of the epoxide were transformed by the enzyme preparation to the 3*R*,6*R*-diol **96**. The 3*S*,6*R*-epoxide was hydrolysed with inversion of configuration *via* attack at C3, while the 3*R*,6*R*-epoxide was hydrolysed *via* attack at the more substituted oxirane carbon with overall retention of stereochemistry.



Scheme 40 Reagents: (i) *Rhodococcus* NCIMB 11216 crude enzyme preparation, (ii) MsCl, Et_3N, (iii) K_2CO_3, MeOH.

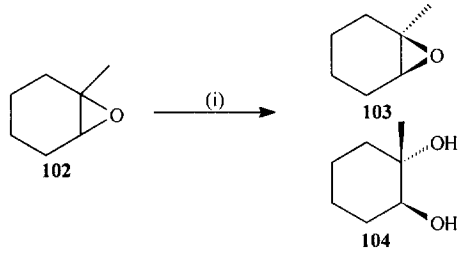
Otto *et al.* used an epoxide hydrolase derived from *Pseudomonas* NRRL B-2994 as the key step in the synthesis of (+)-disparlure, a sex pheromone of the Gypsy moth (*Lymantria dispar*),[86] **Scheme 41**.



Scheme 41 Reagents: (i) *Pseudomonas* NRRL B-2994

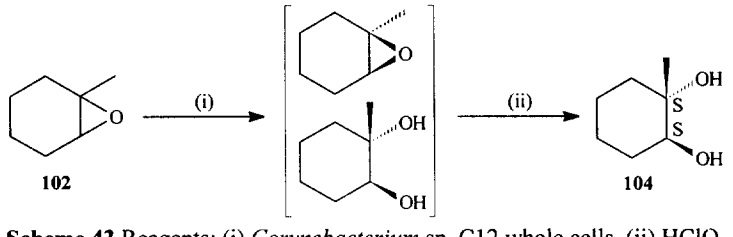
Resolution of (±)-9,10-epoxy-15-methylhexadecanoic acid **99** stopped automatically at 50% conversion yielding 9*S*,10*R*-9,10-epoxy-15-methylhexadecanoic acid **100** and the corresponding 9*R*,10*R*-diol **101**, indicating preferential hydrolysis of the 9*R*,10*S*-epoxide *via* inversion at C-10. The epoxide was converted in a single step to (+)-disparlure (7*R*,8*S*-7,8-epoxy-2-methyloctadecane), whose optical purity was greater than 90%.

Carter and Leak isolated a *Corynebacterium* sp. (designated C12) using cyclohexene oxide as sole carbon and energy source.[87] Archer *et al.* used this species to resolve 1-methyl-1,2-epoxycyclohexane **102** with exceptional enantioselectivity giving rise to 1*R*,2*S*-1-methyl-1,2-epoxycyclohexane **103** in >99% ee (30% yield) and 1*S*,2*S*-1-methyl-1,2-dihydroxycyclohexane **104** in 89% ee (42% yield),[88] **Scheme 42**.



Scheme 42 Reagents: (i) *Corynebacterium* sp. C12 whole cells

Acid-catalysed hydrolysis of the residual 1*R*,2*S*-epoxide gave rise to the same 1*S*,2*S*-diol, *via* inversion of at the more substituted oxirane carbon atom, as expected. The two reactions could be run in tandem, resulting in the novel chemo-enzymatic deracemisation of 1-methyl-1,2-epoxycyclohexane **102** and giving rise to 1*S*,2*S*-1-methyl-1,2-dihydroxycyclohexane **104** in high yield and optical purity (80% yield, >95% ee) **Scheme 43**.



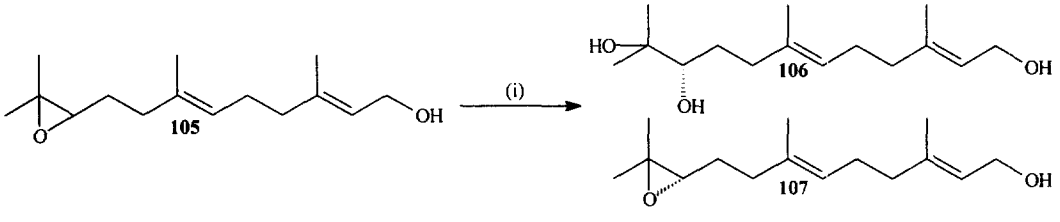
Scheme 43 Reagents: (i) *Corynebacterium* sp. C12 whole cells, (ii) $HClO_4$

3.1.1 Summary of bacterial epoxide hydrolases

It is apparent that epoxide hydrolases from bacterial sources are far more prevalent than was thought five years ago. Many of these organisms contain EHs that are highly enantioselective (*Rhodococcus* NCIMB 11216, *Nocardia* EH1 and *Nocardia* TB1 in particular). The substrate ranges of these enzymes in terms of their enantioselectivity have only just begun to be examined, with high enantioselectivity only occurring in a fairly limited number of cases where the substrate has a strict substitution pattern. For example hydrolysis of the related series of compounds 1,2-epoxyhexane, 2-methyl-1,2-epoxyheptane and 2-ethyl-1,2-epoxyheptane the selectivity is very poor, very good and poor respectively indicating that the methyl group at C-2 plays a significant role in the enantioselectivity when compared to the monosubstituted epoxide. However, increasing the length of the C-2 substituent by one carbon results in the loss of the enantioselectivity. These results seem to indicate a fairly tight active site capable of accommodating a reasonably small range of substrates. It is also significant that most of the enzymes show the same sense of enantioselectivity with regard to the substrates tested, even going as far as showing similar switches in enantioselectivity when comparing mono- and disubstituted epoxides. These similarities probably indicate that the enzymes are related in evolutionary terms.

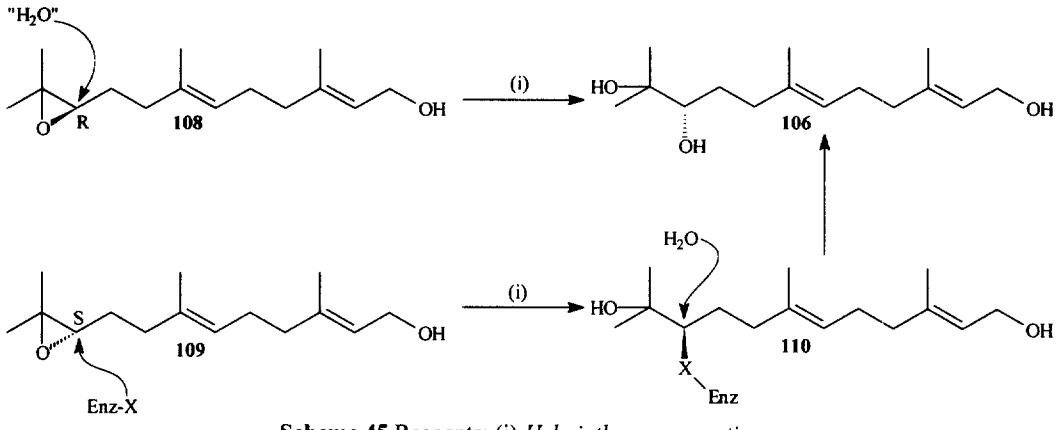
3.2 Fungal epoxide hydrolases

The earliest use of a fungal epoxide hydrolase for the synthesis of optically enriched compounds was reported by Suzuki *et al.*[89] The fungus *Helminthosporum sativum* was found to catalyse the asymmetric hydrolysis of racemic terpenoid 10,11-epoxyfarnesol **105**.



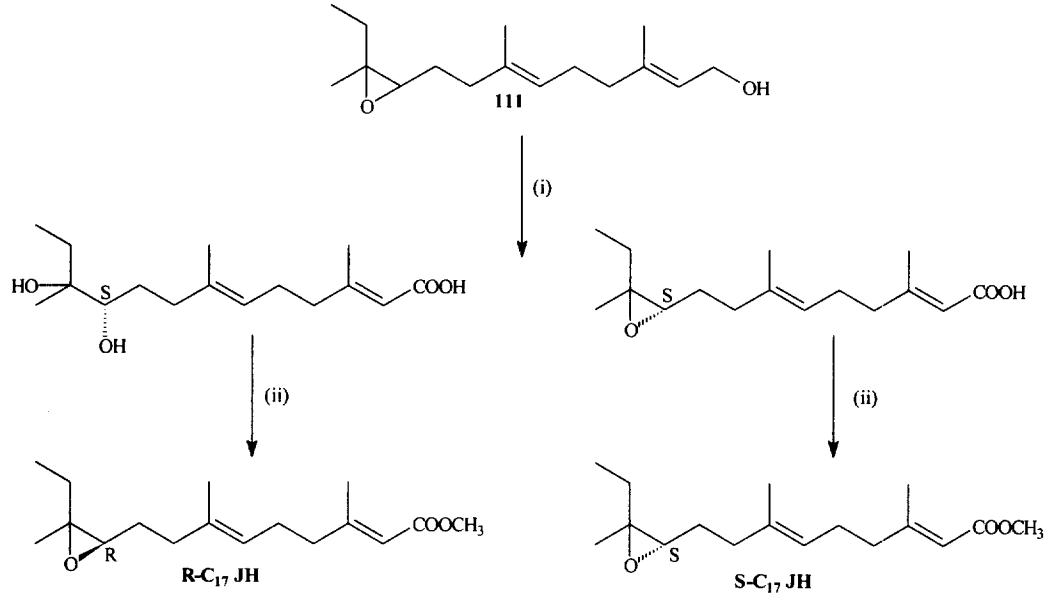
Scheme 44 Reagents: (i) *Helminthosporum sativum*

S-10,11-Dihydroxyfarnesol **106** and *S*-10,11-epoxyfarnesol **107** were isolated in optically active form, indicating transformation of the *R*-epoxide with inversion at the less substituted (secondary) oxirane carbon (C-10), **Scheme 44**. The same workers later showed that complete conversion of the epoxide by *H. sativum* occurred to yield *S*-10,11-dihydroxyfarnesol in 73% ee. Both *R*- and *S*-epoxides were hydrolysed to the *S*-diol **106**, the former more rapidly than the latter. Experiments using [^{18}O]-epoxyfarnesol showed that both enantiomers are hydrolysed with incorporation of the new oxygen atom at C-10. Examples of enantioconvergent epoxide hydrolyses described previously in this review have occurred where the enzyme has shown complete but opposite regioselectivity in the hydrolysis of the two enantiomeric epoxides individually.[50,82] Here the two enantiomers are hydrolysed with identical regiochemistry, i.e. attack at C-10, **Scheme 45**. *R*-10,11-Epoxyfarnesol **108** is converted to its corresponding diol through normal *trans*-hydrolysis with attack and inversion at C-10. Conversion of *S*-epoxyfarnesol **109** to the *S*-diol is more complicated. Studies using (±)-[10-^{2}H]-epoxyfarnesol proved that formation of a C-10 carbocation or C-10 ketone (redox process) was unlikely since all of the deuterium was retained in the product diol. When single enantiomer [^{18}O]-*S*-epoxyfarnesol was hydrolysed by *H. sativum* almost all of the label was found to be present at C-11 of the product diol, indicating 85-88% attack at C-10. The only explanation for these observations is that *S*-epoxyfarnesol is hydrolysed *via* a double inversion process, presumably with the involvement of a covalently bound enzyme substrate intermediate **110, Scheme 45**, ultimately leading to an unusual *cis*-hydrolysis of the epoxide .



Scheme 45 Reagents: (i) *Helminthosporum sativum*

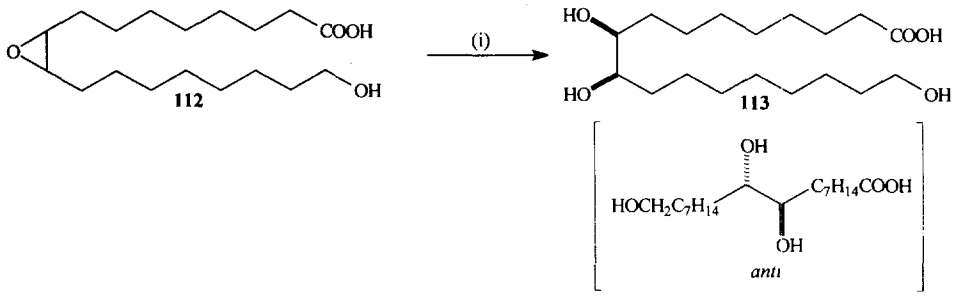
The same workers used the enantioselectivity of the fungus in the synthesis of both enantiomers of C_{17}-Juvenile Hormone (C_{17} JH) from racemic 10,11-epoxyhomofarnesol **111**,[90] **Scheme 46**.



Scheme 46 Reagents: (i) *Helminthosporum sativum,* (ii) Steps

The whole cells of the fungus also catalysed the oxidation of the primary allylic alcohol to the corresponding carboxylic acid.

The unusual *cis*-hydrolysis of epoxides was also noted by Kolattukudy *et al.*[91] A soluble epoxide hydrolase enzyme was isolated and characterised from *Fusarium solani pisi*. The enzyme was found to catalyse the formation of anti-9,10,18-trihydroxyoctadecanoic acid **113** from *cis*-9,10-epoxy-18-hydroxyoctadecanoic acid **112**, **Scheme 47**.



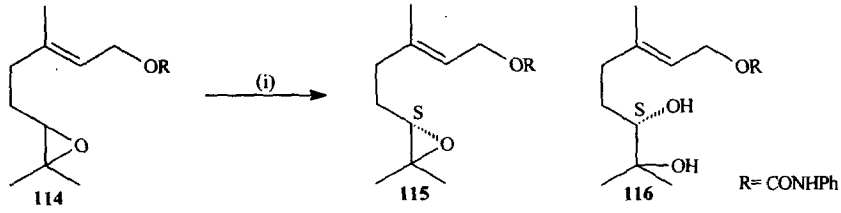
Scheme 47 Reagents: (i) *Fusarium solani pisi*

It was suggested that a transient carbocation generated by protonation of the oxygen atom of the epoxide is held on the back side by a nucleophilic functional group of the enzyme until hydrolysis of this adduct occurs by attack of a water molecule on the same side to which the oxirane oxygen was attached. They also note the strongly inhibitory effect of thiol specific reagents indicating that the nucleophile described may be a sulfhydryl moiety. The mechanism of the enzyme would therefore be *via* nucleophilic attack of the thiol to one end of the epoxide creating a β-hydroxythioether enzyme-substrate intermediate. Hydrolysis of the thioether linkage with a second inversion of the α-carbon yields the product of an overall *cis*-hydrolysis. Neither the optical purities nor the absolute configurations of any compounds were assessed.

Faber's group described the enantioselectivity of some fungal epoxide hydrolases during their screen for a range of microbial epoxide hydrolases.[80] *Diploida gossypina* ATCC 10936, *Fusarium solani* DSM 62416 and *Glomerella cingulata* ATCC 10534 showed activity against 1,2-epoxyoctane, benzyl glycidyl ether and 2-methyl-1,2-epoxyheptane. *Diploida gossypina* ATCC 10936 was also active against 3,3-dimethyl-1,2-epoxybutane. Only this latter fungus displayed reasonable enantioselectivity and then only towards the sterically demanding 3,3-dimethyl-1,2-epoxybutane, giving rise to 2*R*-1,2-dihydroxy-3,3-dimethylbutane in 88% ee albeit at low conversion (15%). Interestingly the diol formed in this reaction has the opposite absolute stereochemistry to the diol formed during reactions using bacterial species. Since the residual epoxide also had the opposite absolute stereochemistry, the fungal and bacterial enzymes showed opposite substrate enantioselectivity. The phenomenon was also noted in the fungal enzymatic hydrolysis of 2-methyl-1,2-epoxyheptane although enantioselectivities were much lower in the case of the fungal enzymes.

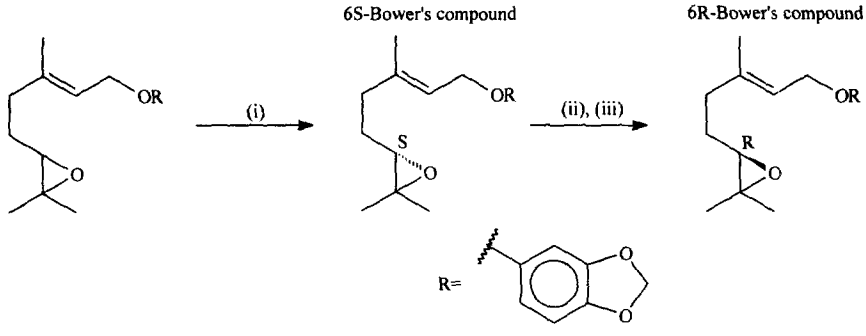
The first examples of preparative scale microbial enantioselective/diastereoselective epoxide hydrolyses utilised the fungus *Aspergillus niger* LCP 521 and were reported by Furstoss's group. The group had previously noted the 6,7-dihydroxylation of geraniol derivatives by this *A. niger* species, *via* an epoxidation/hydrolysis pathway.[92] This observation led the group to assess the enantioselectivity of the epoxide hydrolase enzyme(s) present towards the hydrolysis of a range of epoxides.

Hydrolysis of geraniol-*N*-phenylcarbamate **114**, mediated by *A. niger*, led to the *S*-epoxide in very high optical purity. It was found that reactions performed under an atmosphere of nitrogen gave better results in terms of yield and ee. This was possibly due to suppression of other oxygen-dependant catabolic pathways or decomposition of the fungus in the absence of air allowing only the hydrolysis of the faster reacting enantiomer. The reactions were performed on a multigram scale (5g starting racemate) and 6*S*-6,7-epoxy-3,7-dimethyl-2-octen-1-yl phenylcarbamate **115** was isolated in good yield (42%, 84% recovery) and 94% ee. The diol product was isolated in good yield but low enantiomeric excess (37%), **Scheme 48**.



Scheme 48 Reagents: (i) *Aspergillus niger* LCP 521

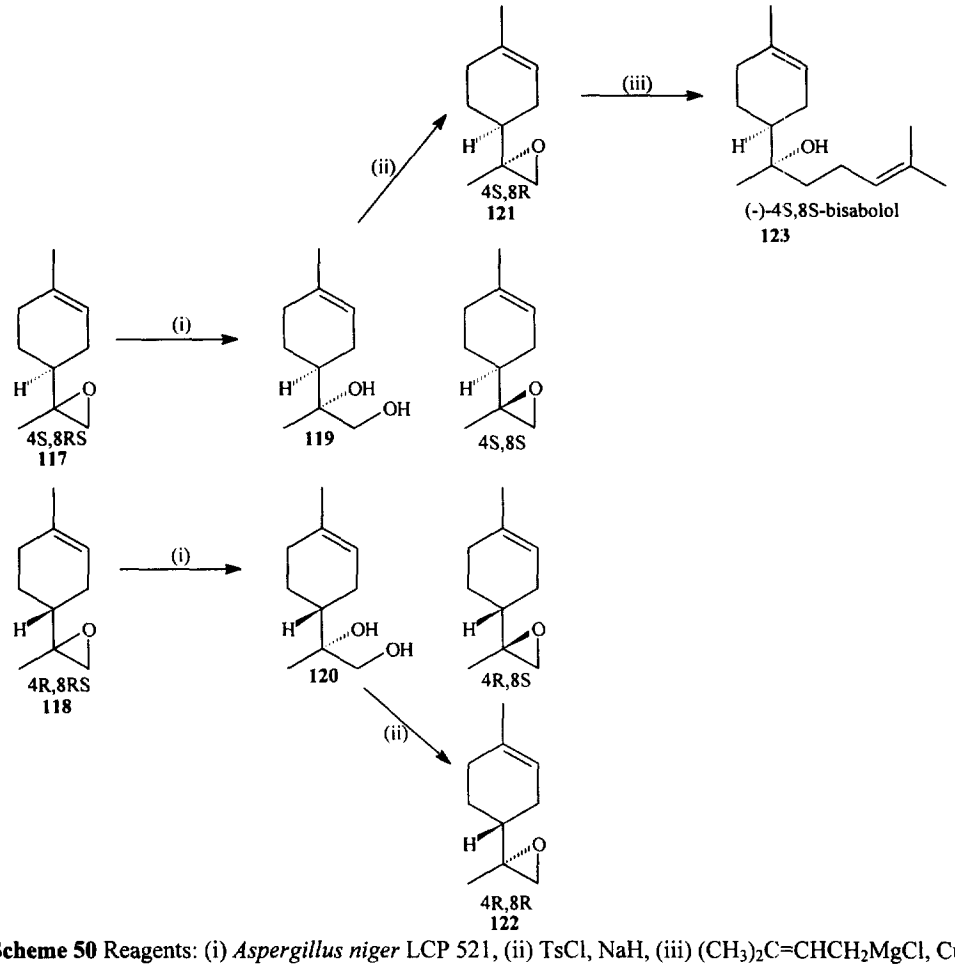
Simply changing the hydroxyl protecting group from phenyl carbamate to 3,4-methylenedioxyphenyl enabled the synthesis of Bower's compound, a potent analogue of insect juvenile hormone, using the same transformation, **Scheme 49**.[93]



Scheme 49 Reagents: (i) *Aspergillus niger* LCP 521, (ii) H^+, H_2O, (iii) TsCl, NaH

The *S*-enantiomer of Bower's compound was isolated directly from the resolution of its racemate. A simple acid-catalysed hydrolysis (retention of stereochemistry) followed by tosylation of the secondary hydroxyl group and ring closure (inversion of stereochemistry) gave *R*-Bower's compound. Both enantiomers were isolated in 96% enantiomeric excess.

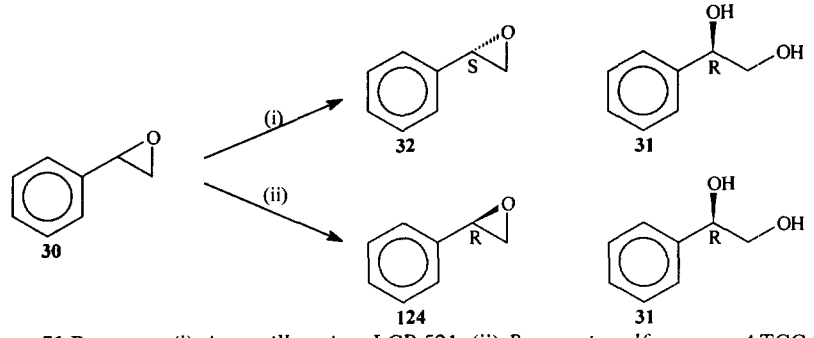
The same organism was also capable of the diastereoselective hydrolysis of the stereoisomers of 8,9-epoxylimonene, **Scheme 50**.[94]



Scheme 50 Reagents: (i) *Aspergillus niger* LCP 521, (ii) TsCl, NaH, (iii) $(CH_3)_2C{=}CHCH_2MgCl$, CuI

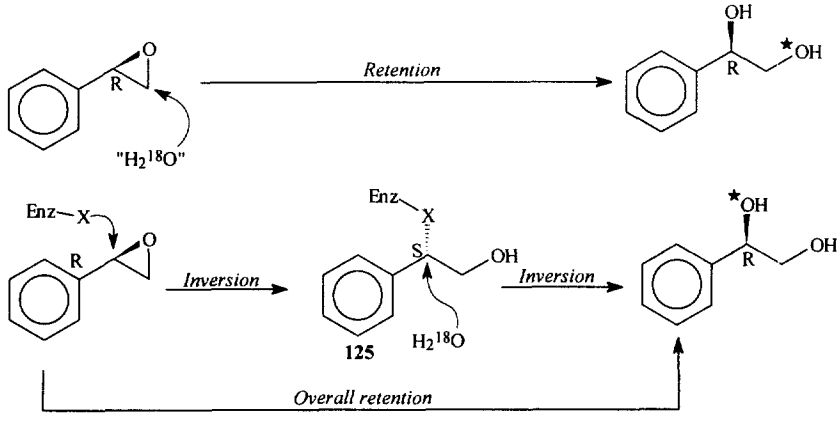
When 4*S*,8*RS*- **117** and 4*R*,8*RS*-epoxylimonenes **118** were incubated with *A. niger*, the epoxide and diol products of the diastereoselective hydrolyses were isolated in good yields (35% and 45% respectively) from both reactions. The epoxides were isolated in excellent diastereomeric excesses (both 98%) while the diol products were isolated in 89-94% de. The 4*S*,8*R*- and 4*R*,8*R*-diols (**119** and **120** respectively) formed biocatalytically were transformed in a single step, the reverse of the biocatalytic pathway, to 4*S*,8*R*- and 4*R*,8*R*-8,9-epoxylimonene (**121** and **122** respectively) by tosylation of the primary hydroxyl followed by ring closure to reform the epoxide. This allows access to all four 8,9-epoxylimonene diastereomers. The absolute stereochemistries of all products were as shown in **Scheme 50**. 4*S*,8*R*-8,9-Epoxylimonene was converted in a one step transformation with an organocuprate to (−)-4*S*,8*S*-α-bisabolol **123**, a natural product which is used on an industrial scale in skin care creams due to its anti-inflammatory, bactericidal and anti-mycotic properties. In fact, all four possible stereoisomers of bisabolol are natural products, all of which may be synthesised using this methodology.

The same *A. niger* strain was found to show good enantioselectivity in the hydrolysis of styrene oxide **30**, yielding *S*-styrene oxide **32** in 96% ee (23% yield). Additionally a second fungal strain *Beauveria sulfurescens* showed complementary and equally good selectivity giving rise to *R*-styrene oxide **124** (98% ee, 19% yield),[95] **Scheme 51**.



Scheme 51 Reagents: (i) *Aspergillus niger* LCP 521, (ii) *Beauveria sulfurescens* ATCC 7159

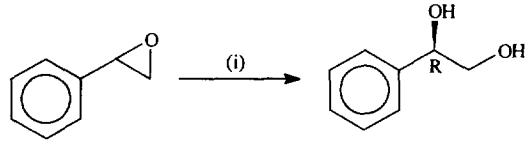
Interestingly, both species gave rise to the same *R*-diol enantiomer **31**. This result indicates that *A. niger* hydrolyses *R*-styrene oxide with retention of stereochemistry (i.e. *via* attack at C-2) and that *B. sulfurescens* hydrolyses *S*-styrene oxide with inversion of stereochemistry (i.e. *via* attack at C-1 benzylic position). A second explanation for the retention of stereochemistry observed for this hydrolysis with *A. niger*, i.e. that a double inversion process at the benzylic position had occurred, was discounted using ^{18}O-labelling experiments,[96] **Scheme 52**. Attack at the benzylic position by a nucleophilic group in the active site would give rise to a covalently bound enzyme-substrate intermediate **125**. A second inversion *via* hydrolysis of the adduct by attack of water at the same position would result in overall retention of stereochemistry. When run in [^{18}O]-water this mechanism would result in incorporation of the labelled oxygen atom at C-1 (benzylic position). Simple attack at C-2 would result in retention of stereochemistry but incorporation of the label at C-2.



Scheme 52

With *R*-styrene oxide as substrate, *R*-diol was formed as expected. The label was only found to be present in the primary hydroxyl group indicating a pathway with attack at C-2 only.

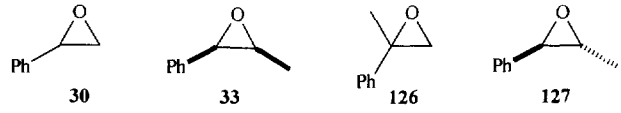
The fact that *A. niger* and *B. sulfurescens* hydrolyse different enantiomers of styrene oxide **30** but produce the same enantiomer of diol (*R*-1-phenyl-1,2-dihydroxyethane **31**) led to one of the most ingenious uses of epoxide hydrolases found in the literature. By using a mixture of both organisms in a single reactor vessel, racemic styrene oxide **30** was transformed to *R*-1-phenyl-1,2-dihydroxyethane **31** in 92% yield and in 89% enantiomeric excess, **Scheme 53**.



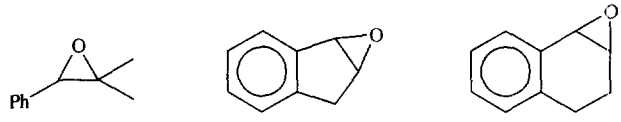
Scheme 53 Reagents: (i) *Aspergillus niger* LCP 521 and *Beauveria sulfurescens* ATCC 7159

p-Nitrostyrene oxide was also hydrolysed with high enantioselectivity using a crude enzyme preparation from *A. niger*. In a report describing the preliminary experiments with the view towards large scale synthesis of *p*-nitrostyrene oxide, a key chiron in the synthesis of the β-blocker Nifenalol®, Nelliah *et al.* report that the residual *S*-4-nitrostyrene oxide was optically pure (ee>99%) at just 58% conversion.[97]

A detailed study of the enantioselectivity of *A. niger* and *B. sulfurescens* towards substituted styrene oxide derivatives was published recently by the same group **Scheme 54**.[6]



30 **33** **126** **127**



128 **129** **130**

Scheme 54

The results obtained with *A. niger* are summarised in **Table 3**.

Aspergillus niger	Epoxide Yield/%	ee/%	Diol Yield/%	ee/%
Styrene oxide **30**	28	99 (*S*)	50	65 (*R*)
α-Methyl styrene oxide **126**	13	73 (*S*)	60	32 (*R*)
cis-β-Methylstyrene oxide **33**	No hydrolysis			
trans-β-Methylstyrene oxide **127**	No hydrolysis			
β,β-Dimethyl styrene oxide **128**	No hydrolysis			
Indene oxide **129**	No hydrolysis			
1,2-Epoxytetrahydronaphthalene **130**	No hydrolysis			

Table 3 Hydrolysis of related phenyl-substituted epoxides by *A. niger*

The additional methyl group at the benzylic position (α-methylstyrene oxide **126**) decreases the level of enantioselectivity by *A. niger*. The very low diol enantiomeric excess for this reaction was caused in part by significant non-enzymatic hydrolysis of the starting material. Substitution at the β-position (**33, 127-130**) had an even greater effect on the activity of the fungus in that none of the epoxides with this substitution pattern were substrates for the enzyme.

The results obtained with *Beauveria sulfurescens* were more promising and are described in **Table 4**.

Beauveria sulfurescens	Epoxide Yield/%	ee/%	Diol Yield/%	ee/%
Styrene oxide **30**	34	98 (*R*)	45	83 (*R*)
α-Methyl styrene oxide **126**	10	53 (*S*)	80	10 (*R*)
cis-β-Methylstyrene oxide **33**	42	20 (1*R*,2*S*)	42	99 (1*R*,2*R*)
trans-β-Methylstyrene oxide **127**	30	98 (1*R*,2*R*)	38	90 (1*R*,2*S*)
β,β-Dimethyl styrene oxide **128**	No hydrolysis			
Indene oxide **129**	20	98 (1*R*,2*S*)	48	69 (1*R*,2*R*)
1,2-Epoxytetrahydronaphthalene **130**	38	98 (1*R*,2*S*)	49	77 (1*R*,2*R*)

Table 4 Hydrolysis of related phenyl-substituted epoxides by *B. sulfurescens*

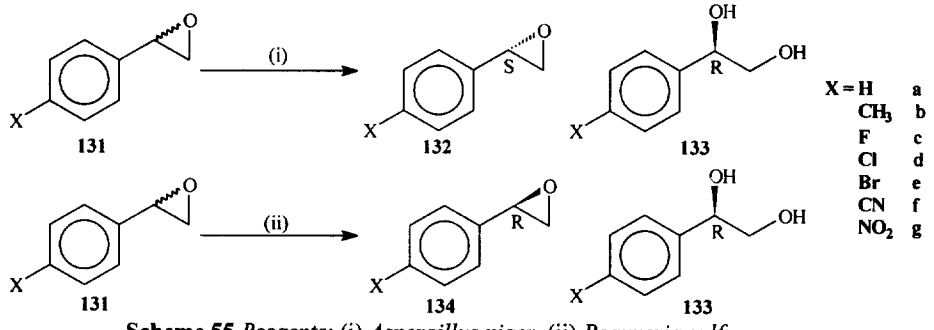
α-Methylstyrene oxide **126** was hydrolysed by *B. sulfurescens* yielding only a moderate enantiomeric excess (53%) of the residual epoxide at high conversion. The diol was formed in just 10% ee, but this low value again is partly explained by competitive non-enzymatic hydrolysis. The absolute configurations of epoxide (*S*) and diol (*R*) indicate that the diol was formed with retention of stereochemistry, in contrast to the hydrolysis of styrene oxide described above. Presumably steric crowding of the quaternary benzylic position disfavours attack at this position, leading to attack at C-2.

cis-β-Methylstyrene oxide **33** was hydrolysed relatively slowly, particularly when compared to the related cyclic examples, indene oxide **129** and 1,2-epoxytetrahydronaphthalene **130**. The stereochemistry of the former reaction also proved interesting. The diol product was found to be almost optically pure at all conversions, while the ee of the residual epoxide was very low at all conversions implying a stereoconvergent reaction. This was confirmed by the isolation of 1*R*,2*R*-1-phenyl-1,2-dihydroxypropane in 85% yield and 98% ee when the reaction had gone to complete conversion. The 1*S*,2*R*-epoxide enantiomer was hydrolysed with inversion at the benzylic position while its antipode was hydrolysed at C-2, both compounds being hydrolysed with inversion of the carbon with *S*-absolute configuration. β,β-Dimethylstyrene oxide **128** was not a substrate for *B. sulfurescens* .

Hydrolysis of racemic indene oxide **129** and 1,2-epoxytetrahydronaphthalene **130** was highly enantioselective leading to the 1*R*,2*R*-diols of excellent optical purity (>98% ee). The absolute configuration of the diol formed in each case indicated hydrolysis of the 1*S*,2*R*-epoxide enantiomers *via* inversion at the benzylic carbon (C-1).

Perhaps the most promising result in **Table 4** is the hydrolysis of *trans*-β-methylstyrene oxide **127** which proceeded to yield epoxide and diol in very high optical purity (98% ee and 90% ee respectively) in good yields (30% and 38% respectively). In addition to the fact that this is the only substrate which yields both product and starting material of high optical purity at conversions close to 50%, it should be noted that enantioselective hydrolyses of *trans*-epoxides are rare in the literature. *trans*-Epoxides are particularly poor substrates for mEH and *trans*-β-methylstyrene oxide is hydrolysed with very low enantioselectivity.

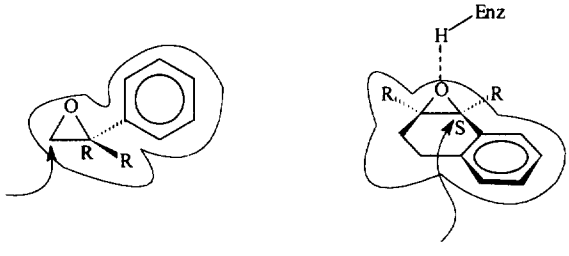
The group followed up this work with a mechanistic study into the hydrolysis of a range of *p*-substituted styrene oxides by *A. niger* and *B. sulfurescens*.[98]



Scheme 55 Reagents: (i) *Aspergillus niger*, (ii) *Beauveria sulfurescens*

A. niger gave residual *S*-epoxides in >96% ee in all cases (yields 28-38%) **132a-g**. The diols isolated from the reactions all had *R*-absolute configuration indicating attack solely at C-2 (non-benzylic) in all cases, **Scheme 55**. The *R*-epoxide enantiomer was shown to have a lower K_M and a higher V_{max} in all cases. *B. sulfurescens* again showed complementary selectivity with respect to the residual epoxide, giving rise to *R*-epoxides **134a-c** and **134e** in >96% ee. For all of the epoxides, except *p*-nitrostyrene oxide **131g**, the transformed diol also had *R*-configuration, indicating inversion of configuration by attack at C-1. Electron-donating groups (*p*-CH_3, **131b**) gave rise to a four-fold increase in reaction rate, while electron withdrawing groups were shown to decrease significantly both the enantioselectivity and rate of the reaction, both observations implying an acid-catalysed process in which some carbonium ion character would be created at C-1 during the transition state.

Some general features of these two fungal epoxide hydrolases may be noted. Firstly that the formed diol is always of *R*-absolute configuration, *A. niger* hydrolyses *R*-styrene oxide and *R*-α-methylstyrene oxide preferentially, while *B. sulfurescens* hydrolyses epoxides with *S*-configuration at C-1 (benzylic) with inversion of this carbon. Putative active site models for the two enzymes were also suggested, **Scheme 56**. The model for *A. niger* is similar to that of mEH, except no space is available to the left when the epoxide is drawn with the oxygen atom to the top at the front and the larger substituent sits in a pocket to the right rearside of the oxirane ring. This model allows *R*-epoxides to be consumed preferentially but does not allow substituents at the β-position of styrene oxide. However, the model does not explain the enantioselectivity of the trisubstituted 6,7-epoxygeraniol type compounds described previously by the same group.[93,94] The model for *B. sulfurescens* involves a large lipophilic pocket to the right frontside if the epoxide is drawn with the oxygen atom towards the top, allowing for selective hydrolysis of *S*-epoxides. The active site must be larger than that of *A. niger* and able to accommodate a range of substitution patterns.



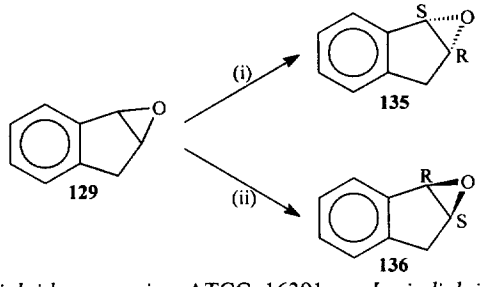
Aspergillus niger LCP 521 — *Beauveria sulfurescens* ATCC 7159

Scheme 56 Active site models of *A. niger* LCP 521 and *B. sulfurescens* ATCC 7159

The model for *B. sulfurescens* allows for the selective hydrolysis of *cis*-, *trans*- and bicyclic systems. 1,1-Disubstituted epoxides are not accommodated very well. A possible explanation for the low reactivity of *cis*-β-methylstyrene oxide and the lack of reactivity of β,β-dimethyl styrene oxide especially when compared to the two bicyclic compounds is that the latter exist in a planar conformation while the former are twisted such that the plane of the phenyl ring avoids interaction with the adjacent β-methyl group. Attack of the nucleophile, from underneath, is then hindered by one of the *ortho*-protons of the aromatic ring. The enantioconvergent hydrolysis of *cis*-β-methylstyrene oxide is rationalised by the unusual binding with the phenyl group of the 1*R*,2*S*-enatiomer in the front left pocket, rather than the front right, again possibly due to its twisted conformation.

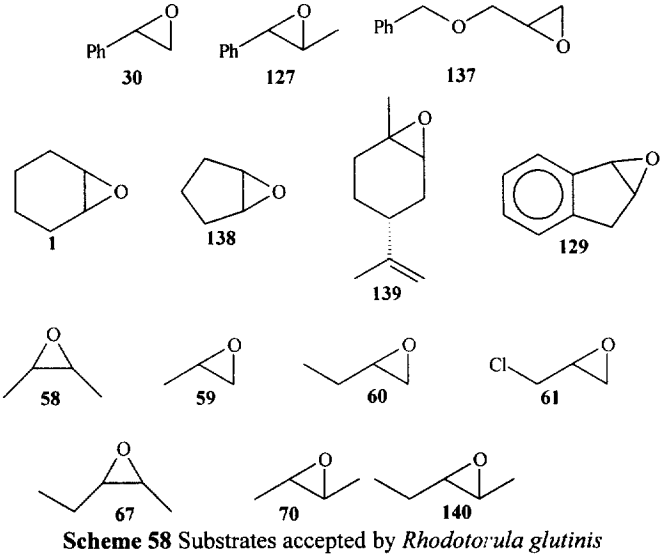
Workers at Merck Research evaluated the epoxide hydrolase activity of eighty fungal strains for enantioselective hydrolysis of indene oxide **129**.[99] 1*S*,2*R*-Indene oxide **135** is a precursor to the side chain of HIV protease inhibitor MK 639 and as such is a valuable chiral synthon. Of the strains examined, two were capable of giving rise to 1*S*,2*R*-indene oxide **135** in optically pure form (*Diploida gossipina* ATCC 16391 and *Lasiodiploida theobromae* MF 5215), two further strains were capable of performing the opposite bioresolution (*Gilmaniella humicola* MF 5363 and *Alentaria tenius* MF 4352), yielding 1*R*,2*S*-indene oxide

136 in very high optical purity (100% ee and 91% ee respectively), **Scheme 57**. Diploida gossipina ATCC 16391 was used in a preparative scale resolution of indene oxide. The residual 1*S*,2*R*-epoxide was isolated as a single enantiomer in a rather low 14% yield. No details of the diol products were given.



Scheme 57 Reagents: (i) *Diploida gossypina* ATCC 16391 or *Lasiodiploida theobromae* MF 521, (ii) *Gilmaniella humicola* MF 5363 or *Alternaria tenius* MF 4532

Very recently one of the most impressive examples of epoxide hydrolase activity, in terms of substrate range and enantioselectivity, was published by Weijers.[100] *Rhodotorula glutinis*, a yeast strain (which for the purpose of this review will be dealt with along with fungal strains), was found to hydrolyse a range of aryl-, alkyl- and alicyclic epoxides with exceptional enantioselectivity, **Scheme 58**.



Scheme 58 Substrates accepted by *Rhodotorula glutinis*

Aryl epoxides **30, 127** and **137** were hydrolysed with maximal activity by the whole cells of *Rhodotorula glutinis*, The ee of the residual epoxide was >98% in all cases, in yields as high as 48% (96% of the theoretical maximum). Terminal and internal, both cis and trans, epoxides were hydrolysed giving rise to diols with variable ee, often as high as 98%. Interestingly and unusually, for a microbial epoxide hydrolase, *meso*-epoxides cyclopentene oxide **138** and cyclohexene oxide **1** were hydrolysed and with excellent enantioselectivity. The corresponding 1*R*,2*R*-diols were isolated in 90% and >98% ee respectively. Inversion at the *S*-configured stereocentres implies the same mode of hydrolysis as was described for hydrolysis of cyclohexene oxide catalysed by microsomal epoxide hydrolase.[43] It should be stressed that hydrolysis of *meso*-epoxides by microbially-derived epoxide hydrolases is rare even before one takes into account the excellent enantioselectivity described here. Hydrolysis of (–)-limonene oxide **139** was highly

diastereoselective, giving rise to the residual 1*S*,2*R*,4*S*-epoxide and 1*R*,2*R*,4*S*-diol in >98% ee in good yield. When the *iso*-propenyl group of the epoxide had *S*-absolute configuration [(+)-limonene oxide, not drawn] both rate of hydrolysis and enantioselectivity decreased dramatically, possibly implying a preference for the 3,4M conformer of cyclohexyl rings.[43,44]

Carnell *et al.* have described the hydrolysis of cyclohexene oxide by a fungal species (*Corynosporium cassiicola*) which led to the formation of 1*S*,2*S*-dihydroxycyclohexane in high enantiomeric excess.[65] However, the high optical purity of the product diol was not a result of high enantioselectivity in the epoxide opening step but a double stereoinversion/oxidation process which converted the 1*R*,2*R*-diol to its enantiomer by the use of two dehydrogenase enzymes.

3.2.1 Summary of fungal epoxide hydrolases

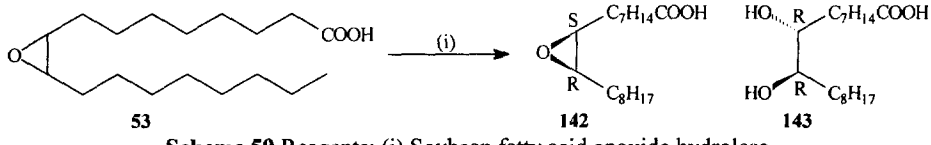
Like their bacterial counterparts, epoxide hydrolases from fungal sources are probably more widely distributed than was thought five years ago. Workers at Merck, who screened eighty fungal species for EH activity towards a single racemic compound,[99] rapidly identified two pairs of strains which showed excellent and complementary enantioselectivity. It may be possible to isolate or screen strains for the resolution of any particularly valuable chiral epoxide synthon in the same manner. The enantioselectivity of the various fungal epoxide hydrolases obviously varies with substrate structure, but also between strains such that enantio-complementary pairs are often available. The best example of which was described Furstoss's group, who used two separate species in a single bioreactor for the deracemisation of styrene oxide.[95]

4. Other epoxide hydrolases

The synthetic potential of several epoxide hydrolases from sources other than mammals and microbes have been evaluated.

4.1 Soybean fatty acid epoxide hydrolase (sbEH)

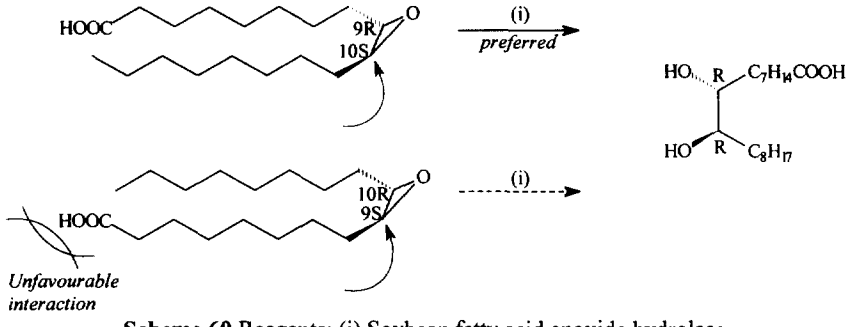
Two reports of a soluble epoxide hydrolase from soybeans have been published in which the stereochemistry of the reactions were described. Blee and Schuber studied the hydrolysis of *cis*-9,10-epoxystearic acid **53** (*cis*-9,10-epoxyoctadecanoic acid).[33] The absolute configuration of the diol product was 9*R*,10*R*-9,10-dihydroxyoctadecanoic acid **143**. The diol was formed in 80% ee at 50% conversion. Studies using ^{18}O-labelled epoxide indicated that both enantiomers were hydrolysed at the carbon with *S*-absolute configuration, leading to an enantioconvergent process. In a later paper[101] the same authors report that **143** was formed in >99% ee, **Scheme 59**.



53 **142** **143**

Scheme 59 Reagents: (i) Soybean fatty acid epoxide hydrolase

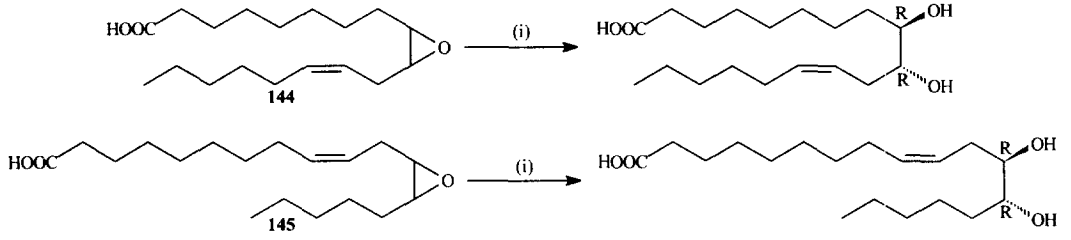
Since the steric and electronic nature of C-9 and C-10 of 9,10-epoxyoctadecanoic acid are almost identical neither of these factors is likely to be the cause of such enantioselectivity. It is more likely that the selectivity is a result entirely of the mode of interaction of the substrate within the active site and proper positioning of the oxirane ring with respect to the catalytic groups of the enzyme, **Scheme 60**.



Scheme 60 Reagents: (i) Soybean fatty acid epoxide hydrolase

While still resulting exclusively in the *R,R*-diol, sbEH did not show any substrate enantioselectivity towards the analogous ester, methyl *cis*-9,10-epoxystearate leading to the proposal that a repulsive interaction between the carboxylic acid moiety of the free acid and the enzyme active site was responsible for the lower reactivity of the 9*S*,10*R*-epoxide enantiomer. However, the epoxide may only be opened from below (drawn as in **Scheme 60**) and while the unfavourable interaction slows the reaction of this enantiomer, the regiochemistry (with respect to the substrate) is reversed, leading to attack at the 9*S*-carbon.

The enzyme was also capable of displaying the same degree of enantioconvergence in the hydrolysis of linoleic acid monoepoxides (9,10-epoxy-12-*Z*-octadecenoic acid **144** and 12,13-epoxy-9-*Z*-octadecenoic acid **145**),[101] **Scheme 61**.



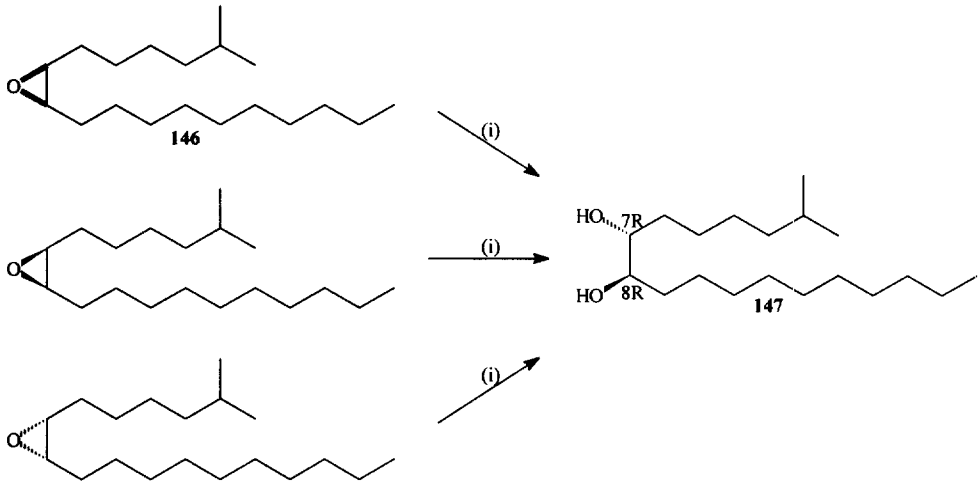
Scheme 61 Reagents: (i) Soybean fatty acid epoxide hydrolase

Irrespective of the position of the epoxide (9,10- **144** or 12,13- **145**) attack and inversion of the *S*-configured carbon atom of each enantiomer was observed, leading to the *R,R*-diols in high optical purity at complete conversion (>98% ee and >90% ee respectively). The enantiomer with the *S*-configured carbon further from the carboxylate moiety was the faster reacting enantiomer (9*R*,10*S*- and 12*R*,13*S*-epoxides) in both cases. This is analogous to the results described with 9,10-epoxystearic acid.

The enzyme is fairly specific towards epoxy-fatty acids so its use as an asymmetric catalyst in chemical synthesis would be limited. However, the enantioselectivity described towards the range of epoxides studied so far is exceptional. It is interesting to note that sbEH and the mammalian mEH show exactly the same dense of selectivity in the deracemisation of 9,10-epoxystearic acid leading to the corresponding *R,R*-diol in 90% ee.[73]

4.2 Gypsy moth antennae epoxide hydrolase

A crude enzyme preparation from the antennae of one-day old male gypsy moths was found to catalyse the enantioconvergent hydrolysis of (±)-disparlure (7,8-epoxy-2-methyloctadecane **146**) to the corresponding *R,R*-diol **147**, **Scheme 62**. The epoxide is a sex pheromone of the gypsy moth, so it is perhaps unsurprising that an epoxide hydrolase with activity towards pheromones with epoxide functionality should be found in the insect's sensory tissues.[102]



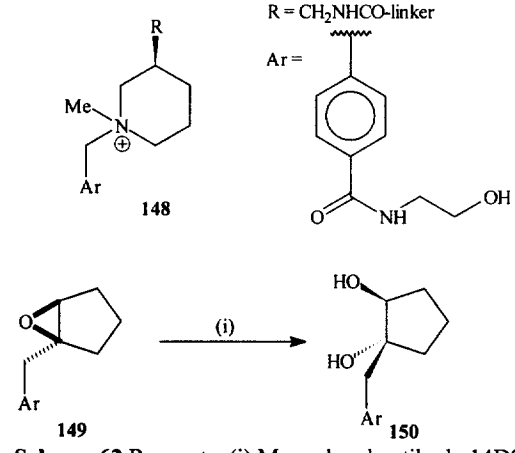
Scheme 62 Reagents: (i) Gypsy moth antennae preparation

Both enantiomers of disparlure, analysed individually, as well as racemic disparlure gave rise to optically pure 7*R*,8*R*-dihydroxy-2-methyloctadecane **147**, indicating attack at the *S*-configured carbon atoms in the case of both enantiomers.

For each hydrolysis the antennae of 50 gypsy moths were required to provide enough enzyme for the reactions. Even then long incubation times were necessary to provide enough of the diol product for GC analysis! Without extensive biotechnological study these enzymes, although displaying interesting enantioselectivity, will not be used on a preparative scale.

4.3 Antibody-catalysed epoxide hydrolysis

Sinha and Keinan raised monoclonal antibodies (Mabs) to the quaternary ammonium salt **148** to mimic the developing positive charge during hydrolsis of the epoxide **149, Scheme 63**.[103]



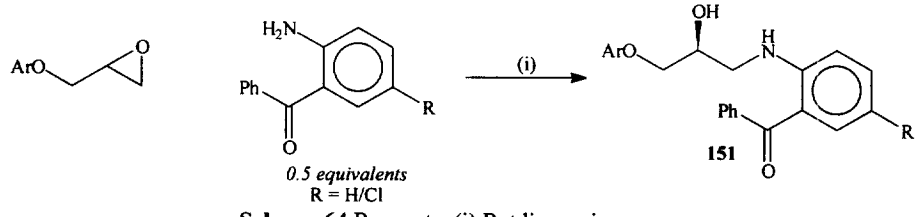
Scheme 63 Reagents: (i) Monoclonal antibody 14D9

Hydrolysis of the racemic epoxide **149** catalysed by Mabs 14D9 gave the diol **150** in 87% ee. No details of yield and/or conversion were recorded. Some structurally related epoxides were also hydrolysed by this antibody but without enantioselectivity.

5. Non-natural nucleophiles

Two studies have been published where the addition of a second nucleophilic reagent (other than water) has resulted in the enzyme-catalysed opening of the epoxide by the nucleophile.

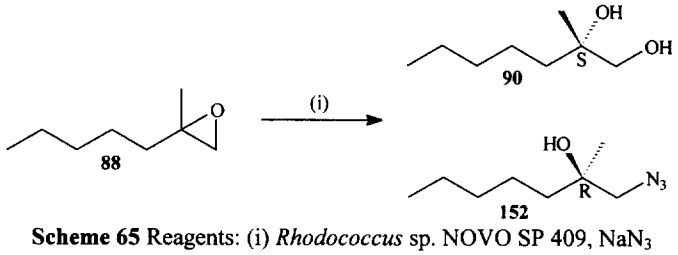
Kamal *et al.* showed that aryl-glycidyl ethers were selectively opened by amines in the presence of rat liver microsomes, **Scheme 64**.[104]



Scheme 64 Reagents: (i) Rat liver microsomes

The product 2-propanolamines **151** were always of *S*-absolute configuration. The enantiomeric excesses of the products were dependent on the structure of the starting materials. Good ees were noted where R was a chloro-substituent and the aryl moiety was phenyl, 2-$MeCOC_6H_4$ or α-naphthyl (86% ee, 88% ee and 85% ee respectively). When R was simply a proton the selectivities were generally less useful (ee ~ 50-65% for the range of nucleophiles tested) except where R was α-naphthyl and 4-$AcNHC_6H_4$ (75% ee and 77% ee respectively).

Mischitz and Faber demonstrated the asymmetric azidolysis of epoxides catalysed by *Rhodococcus* NOVO SP 409.[105] This species, described previously in this review, catalysed, amongst other reactions, the enantioselective hydrolysis of 2-methyl-1,2-epoxyheptane **88**. The enzyme was selective for the *S*-epoxide and produced the S-diol **90** in standard hydrolysis reactions. When the reaction was run under identical conditions, except with the addition of 25mM sodium azide, an additional second product was isolated, 1-azido-2-methyl-2-hydroxyheptane **152**. The absolute configurations of the product diol and azido-alcohol were found to be opposite (S-diol and *R*-azido-alcohol) indicating that each had originated from a different epoxide enantiomer, **Scheme 65**.



Scheme 65 Reagents: (i) *Rhodococcus* sp. NOVO SP 409, NaN_3

The diol product was formed in >90% ee, while the slower forming azido-alcohol was formed in 60% ee. At first glance the reaction seems to be simply non-enzymatic azidolysis of the residual epoxide, however, using heat denatured enzyme neither product was formed. In addition the kinetics of the reaction and enantiomeric excesses of the residual epoxide, diol and azido-alcohol indicated that both products were formed enzymatically.

While the enantiomeric excess of the azido alcohol was too low for synthetic purposes, the fact that the reaction occurs at all is interesting and may lead to entirely new uses for epoxide hydrolases in synthetic chemistry if this type of reaction is reasonably general.

6. Summary and conclusions

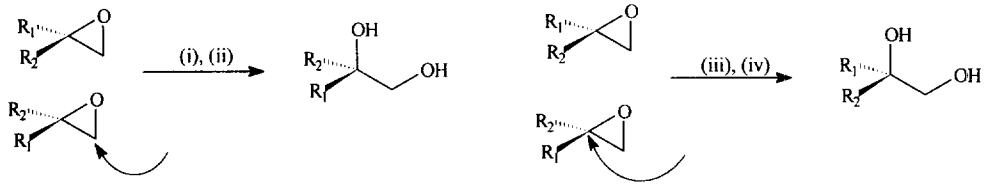
Epoxide hydrolase enzymes represent a relatively simple and efficient route towards the synthesis of optically enriched epoxides and diols with little or no other functionality. The relative merits and limitations of EHs from the various sources have been discussed.

Mammalian liver microsomal epoxide hydrolase (mEH) has been shown to display exceptionally high enantioselectivity towards a wide range of substrates. However, its limited availability has prevented chemists from viewing it as a potential asymmetric catalyst for the production of chiral epoxides and diols, in the same manner, for example, that lipases/esterases have been used in organic synthesis. Large-scale production of the enzyme ought to be possible *via* expression of the protein in a host bacteria (e.g. *E. coli*), followed by conventional fermentation of the genetically-engineered organism.

Epoxide hydrolases from microbial sources, which do not suffer from the drawback of limited availability, have been used to good effect for the production of a range of chiral epoxides and diols. In general, these enzymes do not exhibit such a broad substrate range as mEH, although, as studies continue, more EH enzymes will be discovered which will extend the range of substrates which may be hydrolysed with acceptable enantioselectivity. The recent paper by Weijers,[100] describing a yeast species (*Rhodotorula glutinis*) which displayed epoxide hydrolase activity with an exceptional substrate range in addition to superb enantioselectivity in most cases, represents an important achievement in this field.

The use of epoxide hydrolase enzymes for the production of enantiopure epoxides will always suffer from the drawback that such reactions are kinetic resolutions, and as such can result in a maximum 50% yield of the required epoxide. If, however, the target of the transformations is the transformed diol, a number of approaches have been adopted in order to optimise yields. The production of enantiopure reagents from *meso*-compounds or racemates (deracemisation) in high yield is a common goal of synthetic chemists. One area where mEH has exhibited much greater enantioselectivity than its microbial counterparts is in the asymmetric hydrolysis of *meso*-epoxides to chiral diols. Generally, *meso*-epoxides have proved to be relatively poor substrates for microbial EHs.

Some of the enzymes described catalyse the hydrolysis of both enantiomers of an epoxide to a single enantiomer of diol (deracemisation).[33,60,73,82,101] In most cases this activity has been discovered fortuitously, although Faber's group have screened a number of organisms for this type of ability.[82] However, Archer *et al.*[88] have described the synthesis of an enantiomerically pure diol in high yield and ee from the corresponding racemic epoxide, *via* sequential enzymic and acid catalysed reactions in a single pot.



Scheme 66 Reagents: (i) EH (inversion at less hindered oxirane carbon atom), (ii) low pH; (iii) EH (inversion at more hindered oxirane carbon), (iv) High pH. (Note: arrows indicate position of initial enzymatic hydrolysis.)

Provided the pH of the reaction may be altered such that the residual epoxide from the biotransformation is hydrolysed with complementary regioselectivity to the enzymatic reaction, then this protocol should provide a general route for the synthesis of enantiopure diols from racemic epoxides, **Scheme 66.**

Acknowledgements

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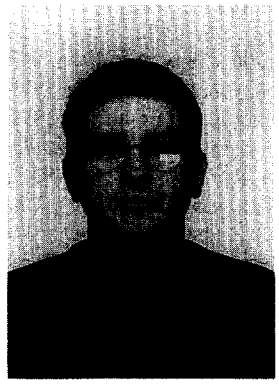
Biographical Sketch



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Ian Archer was born in Oxford, UK. He was educated at Emerson Park School, Essex, then at Imperial College, London, gaining his BSc. in 1993. Studies on the enantioselective production of oxygenated hydrocarbons by biotransformation, under the direction of Drs. D.A. Widdowson and D.J. Leak at Imperial College, led to a PhD. in 1996. His interests in Bioorganic Chemistry are continuing in his current post as a post-doctoral associate at the University of Edinburgh with Dr. N.J. Turner, investigating the biosynthesis of carbocyclic nucleosides.