Pergamon

Tetrahedron, Vol. 52, No. 13, pp. 4593-4606, 1996

0040-4020/96 $15.00 + 0.00

S0040-4020(96)00135-4

Microbiological Transformations 32: Use of Epoxide Hydrolase Mediated Biohydrolysis as a Way to Enantiopure Epoxides and Vicinal Diols: Application to Substituted Styrene Oxide Derivatives.

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Abstract: The biohydrolyses of various substituted styrene oxide derivatives using the fungi *Aspergillus niger* or *Beauveria sulfurescens* are described. The results obtained show that this methodology allows the preparation of enantiomerically enriched epoxides and diols *via* enantioselective and regioselective hydration. The comparative study of the results obtained suggests that these hydrolyses operate following different mechanisms and a model of the corresponding active sites is proposed.

The preparation of enantiopure epoxides and of the corresponding vicinal diols is a very actively explored area, since these compounds are highly valuable chiral synthons. These can be prepared *via* various multistep ways. Two direct chemical procedures allowing for the synthesis of enantiopure epoxides have been particularly developed during the last decade: the Sharpless stereoselective epoxidation of olefins - which is restricted to allylic alcohols,[1] and the Jacobsen/Katsuki procedure which gives mainly good results with some *cis*-substituted olefins.[2] On the other hand, the Sharpless osmium catalyzed dihydroxylation approach which allows the direct preparation of enantiomerically enriched vicinal diols, has been proven to be essentially efficient for *trans*-disubstituted olefins.[3] However one drawback of these procedures is the fact that they are based on the use of heavy metal catalysts, which may be sources of industrial pollution.

The objective of our work was to develop a single-step biocatalytic approach for the simultaneous synthesis of enantiopure epoxides and vicinal diols by performing the enantioselective hydrolysis of epoxides using Epoxide Hydrolase enzymes (EH) from microbial origin. Indeed, whereas a large amount of work has been devoted to the study of mammalian EH[4] - which were shown to be either microsomal (mEH) or cytosolic (cEH) - the use of these biocatalysts is hampered by the highly limited supply of these enzymes. On the other hand, only scarce information is available concerning EH enzymes from microbial origin, although some very interesting results have been described recently.[5,6] In this context we were ourselves interested in using various fungi, and discovered that such cells are very often endowed with interesting EH activity.[7-9] Thus, we have previously described that two fungi, *A. niger* and *B. sulfurescens*, were able to perform the highly enantioselective hydrolysis of racemic styrene oxide.[8] Furthermore, these two strains were revealed to be enantiocomplementary, since they achieved the highly enantioselective hydrolysis of respectively the (*R*) or the (*S*)-enantiomer of styrene oxide **1**, and a mechanistic study showed that the EH of these two fungi operate *via* different regioselectivities.[9] Owing to the possibility to easily grow large quantities of biocatalyst, these two

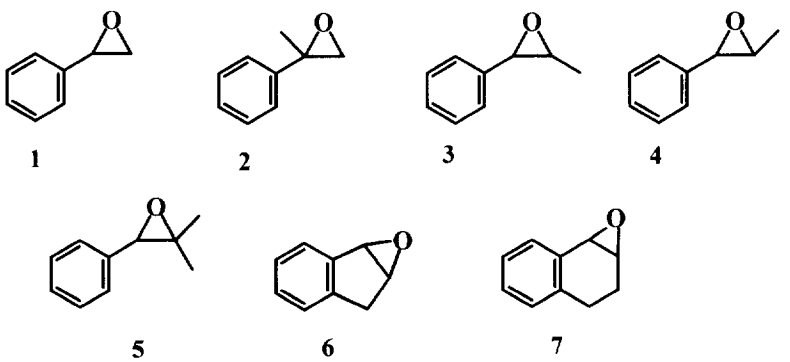
microbiological transformations could be carried out on large-scale quantities, thus allowing the multigram-scale production of either enantiomer.

Since these results clearly opened the way to the preparation of enantiopure epoxides using this new biocatalytic approach, it was of high interest to explore its scope and limitations. We therefore have carried on our work by studying different structurally related epoxides bearing substituents on the oxirane ring - i.e. by using substrates in which the oxirane moiety was either substituted by one or by several methyl groups, or involved in a "rigid" cyclic structure. This was aimed to explore the possible influence, on the enantiomer differentiation, of the steric and geometric factors implied in these fungal enzymes.

We here describe the results we have obtained, starting from racemic epoxides **1** to **7** which led respectively to the vicinal diols **8** to **14**. From these results, an attempt to develop a model taking into account the steric requirements involved at the catalytic binding site of the enzymes will be proposed.

RESULTS

The seven differently substituted styrene oxide derivatives **1** to **7** were used as substrates (Scheme 1). All the biohydrolyses were performed on a preparative scale by incubating 1 g of racemic epoxide in 1 L of buffered resting-cell suspension, using either *A. niger* or *B. sulfurescens* cells. Since the stereochemical outcome of these reactions are time dependent, the reactions were monitored by following the residual epoxide ee *versus* time, using chiral GLC analysis. The yields have been determined after isolation and purification of the residual epoxide and of the formed diol. The absolute configuration of the products have been assigned on the basis of their optical rotation by comparison with previously described data, and the ee of the diols have been measured, using chiral GLC, after derivatisation into their di-methylether, except in the case of diol **8** (obtained from **1**) where the ee has been determinated *via* the corresponding acetonide derivative.



Scheme 1

Biohydrolysis by *A. niger*

The results of the biohydrolysis of the styrene oxide derivatives **1** to 7 by the fungus *A. niger* are reported in Table 1. It clearly appears that the presence of an alkyl group located on the oxirane ring plays a critical role

on the outcome of the biohydrolysis. Thus, substitution of the oxirane ring by a methyl group at the C(1) carbon atom (compound **2**), seems to decrease the enantioselectivity of the reaction. Indeed, whereas in the case of **1** the residual epoxide exhibits an ee of 98% (for a 28% recovered yield), a lower ee of 73% (13% isolated yield) was obtained in the case of **2** after the same reaction period (2 h). The formed diol **9** also exhibited a lower enantiomeric purity (ee 32%). It should be noted that, as shown by blank experiments, noticeable chemical hydrolysis of **2** occurred (about 12% per hour) during the biohydrolysis, a fact which did not allow a good estimation of the influence of the methyl group on the real enantioselectivity of the enzymatic reaction. Things got even worse for all the β-substituted derivatives **3** to **7**, where no significant enzymatic hydrolysis was observed. Moreover, whereas in the case of the β-substituted mono- or dimethyl derivatives **3**, **4** and **5** chemical hydrolysis appeared to be relatively slow, both cyclic epoxides **6** and **7** showed significant chemical hydrolysis, leading to the corresponding racemic diol.

Table 1. Biohydrolysis of Styrene Oxide Derivatives 1 to 7 using *A. niger*.

Substrate	Yield epoxide (reaction time)	ee epoxide (Abs. Conf.)	Ref.	Product	Yield diol	ee diol (Abs. Conf.)	Ref.
1	28% (2 h)	99% (*R*)	8	**8**	50%	65% (*R*)	8
2	13% (2 h)	73% (*S*)	10[a]	**9**	60%	32% (*R*)	11
3-7	no biohydrolysis[b]	-		-	-	-	

(a) - The absolute configuration of **2** was not assigned on the basis of its optical rotation sign because some contradictory results are reported in literature.[10] Therefore, it was established by using a chemical correlation of **2** with the corresponding diol **9**. (b) - Enzymatic hydrolysis is too slow to be correctly quantified.

Biohydrolysis with *B. sulfurescens*

The results obtained after biohydrolysis of compounds **1** to **7** by the fungus *B. sulfurescens* are reported in Table 2. As in the case of *A. niger*, substitution of the oxirane ring appears to play a crucial role on the outcome of the biohydrolysis, although the consequences were completely different. Indeed, except for the *cis*-substituted epoxide **3** and the trisubstituted derivative **5**, a rapid and enantioselective enzymatic hydrolysis occurred in all cases.

The *trans*-methyl substituted derivative **4** - which incidentally is not accessible in high enantiomeric purity *via* the Jacobsen/Katsuki methodology[12,13] (maximum ee 81%) - reacted rapidly and afforded a 30% yield of remaining (1*R*,2*R*)-**4**, together with the (1*R*,2*S*)-**11** diol product, both compounds showing excellent ee's as high as 98 and 90% respectively. Also, for both epoxides **6** and **7**, a high enantioselectivity (similar to the one occurring for **1**) was observed and the rate of the biohydrolysis of these tricyclic compounds was increased by respectively two and four orders of magnitude relative to styrene oxide**1**. These biohydrolyses allowed to isolate optically pure residual epoxides (1*R*,2*S*)-**6** and (1*R*,2*S*)-**7**.

Table 2. Biohydrolysis of Styrene Oxide Derivatives using *B. sulfurescens*.

Substrate	Yield epoxide (reaction time)	ee epoxide (Abs. Conf.)	Ref.	Product	Yield diol	ee diol (Abs. Conf.)	Ref.
1	34% (2 h)	98% (*R*)	8	**8**	45%	83% (*R*)	8
2[a]	10% (5 h)	53% (*S*)	10[b]	**9**	80%	10% (*R*)	11
3	42% (4 h)	20% (1*R*,2*S*)	14	**10**	42%	99% (1*R*,2*R*)	15
4	30% (3 h)	98% (1*R*,2*R*)	15	**11**	38%	90% (1*R*,2*S*)	15
5	no hydrolysis[c]	-		**12**	-	-	
6	20% (1 h)[d]	>98% (1*R*,2*S*)	16	**13**	48%	69% (1*R*,2*R*)	16
7	38% (0.5 h)	>98% (1*R*,2*S*)	17	**14**	49%	77% (1*R*,2*R*)	17

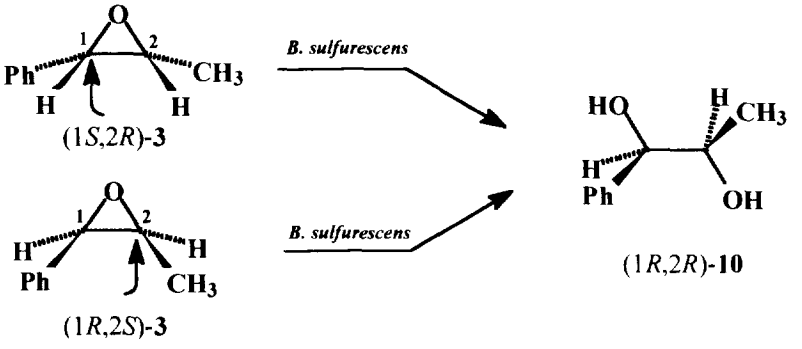
(a) - The biohydrolysis of **2** was carried out in shake flasks and only analytical yields were determined. (b) - The absolute configuration of **2** was not assigned on the basis of its optical rotation sign because some contradictory results are reported in the literature.[10] Therefore it was established by using a chemical correlation of **2** with the corresponding diol **9**. (c) - Enzymatic hydrolysis was too slow to be correctly quantified. (d) - Epoxide **6** was unstable under the reaction conditions and some 2-indanone was isolated with residual epoxide.

Interestingly, *cis*-epoxide **3** behave quite differently as compared to both cyclic epoxides **6** and **7** (which can be regarded as being structurally rigid *cis*-substituted compounds). Indeed, the reaction rate of **3** appeared to be relatively slow and, after 4 hours reaction, the residual epoxide exhibited an ee as low as 20% (44% isolated yield) whereas, very surprisingly, the formed diol **10** was shown to be almost optically pure (ee 99%, 42% isolated yield). One explanation to this puzzling result could be that the two antipodes were hydrolyzed with different regioselectivities, thus leading to an enantioconvergent process. In order to check this hypothesis, we carried out the biohydrolysis of **3** to completion by using 5 times the ratio of biocatalyst with respect to substrate **3**. This led to the formation of diol **10** in almost optically pure form (ee 98%) and a 85% preparative yield. This result is in perfect agreement with the previous hypothesis and it can thus be concluded that the diol (1*R*, 2*R*)-**10** resulted from a regiospecific water attack at the *more* hindered C(1) carbon atom on the (1*S*, 2*R*)-**3** enantiomer, whereas the same diol antipode was formed from (1*R*,2*S*)-**3** *via* an attack on the *less* hindered C(2) oxirane carbon atom (Scheme 2). It should be noted that a similar highly regioselective attack on the carbon atom of (*S*)-absolute configuration was observed in the case of the biohydrolysis of 3,4-epoxytetrahydropyran with mammalian EH[18] as well as for the biohydrolysis of both enantiomers of *cis*-9,10-epoxystearic acid by plant EH (soybean).[19]

The *gem*-substituted compound **2**, which bears a methyl group on the C(1) carbon atom of the oxirane ring, was rapidly hydrolysed (10% epoxide recovery after 5 hours). Unfortunately, the concomitant non-enzymatic hydrolysis did not allow to reach high ees for the reaction products even at high conversion ratios. Surprisingly enough however, this α-methyl substitution caused a dramatic switch in the substrate

enantioselectivity as compared to the result previously obtained with styrene oxide **1** itself. Thus, predominant hydrolysis of the (*R*)-enantiomer was observed in this case.

Finally, compound **5**, which bears two methyl groups at the β-position, proved to be a non-substrate for this fungus, since no noticeable reaction occurred under the standard experimental conditions used.



Scheme 2

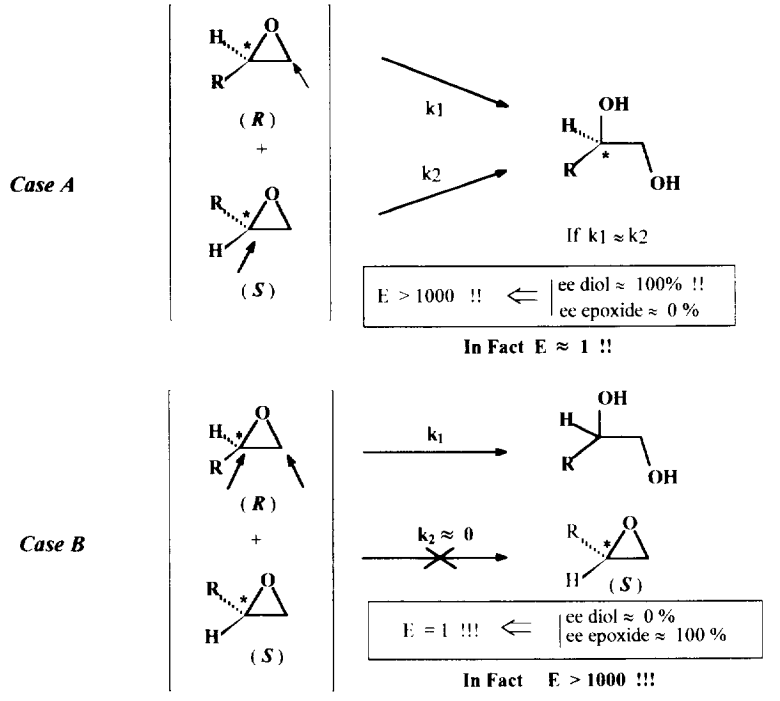
The determination of the E value related to these reactions deserves a special comment. According to Sih and coll.,[20] this E value is commonly used to characterize the enantioselectivity in enzyme catalyzed reactions. Several methods allowing for the determination of this E value have been developed and their applicability was recently studied in the case of either hydrolysis or synthesis of ester derivatives.[21] Usually, this E value is obtained through measuring the enantiomeric excess of the residual substrate (ees) - or of the corresponding product (eep) - at a certain conversion ratio (c) (Equations 1 and 2). However, in the case of biotransformations conducted using whole-cell cultures, it is very difficult to determine the degree of conversion with good accuracy[22] because of the heterogeneity of the medium. Therefore, the E values are very often determined using both ees and eep measurements (Equation 3).[22] However, this implies that the regioselectivity of the oxirane ring

$$E = \frac{\ln(1-c)(1-ees)}{\ln(1-c)(1+ees)} \qquad \textbf{Equation 1}$$

$$E = \frac{\ln[1-c(1+eep)]}{\ln[1-c(1-eep)]} \qquad \textbf{Equation 2}$$

$$E = \frac{\ln\left[\frac{eep(1-ees)}{(eep+ees)}\right]}{\ln\left[\frac{eep(1+ees)}{(eep+ees)}\right]} \qquad \textbf{Equation 3}$$

opening is identical for both enantiomers of the substrate. Indeed, as exemplified in scheme 3, a different regioselectivity in the attack of each enantiomer would lead to highly erroneous results. This can be illustrated by the two following cases.



Scheme 3

In case A, one should assume that the hydrolysis of a racemic epoxide is *not enantioselective*, but that each one of the two antipodes is attacked following a different regioselectivity - i.e. that one enantiomer is attacked at the *less* hindered carbon atom, whereas the other antipode is attacked at the *more* hindered carbon atom. This would lead to a diol showing theoretically a 100% ee, the remaining epoxide being always racemic (ees ≈ 0) whatever the extent of the reaction is. Using equation 3 would thus lead to a very high E value calculation (> 1000) which is incompatible with the starting hypothesis (non-enantioselective reaction). For example, calculation of the E factor in the case of **3** led to a high value (E > 200), which is quite surprising since the enantioselectivity of this reaction must be low - in regard of the 20% ee of the recovered epoxide at a 40% yield (i.e. 60% conversion).

The opposite case - case B - would be to consider a *totally enantioselective* reaction, where the two carbon atoms of the oxirane ring of one single enantiomer would be attacked similarly. This would lead to a remaining enantiopure epoxide but to a racemic diol. Using equation 3 would again lead to an erroneous - very low - E value (≈ 1) calculation which is obviously contradictory to the starting hypothesis.

Because of this ambiguity, and owing to the fact that we have no proof of the regioselectivities implied in these reactions, we did not, at the present stage, determine the E values of the biohydrolyses described in this study.

DISCUSSION

The results obtained in the course of this study deserve several comments. First, they confirm that the two fungi present marked differences as far as substrate specificity is concerned. Indeed, whereas the substrate selectivity seems to be very limited to non-substituted derivatives for *A. niger*, *B. sulfurescens* appears to much more easily accommodate the differently substituted styrene oxide derivatives. Another important observation relates to the striking fact that, without exception, the formed diol is of (*R*) absolute configuration. Using *B. sulfurescens* this obviously implies, since the fast reacting antipode is in most cases of (1*S*)-absolute configuration, that attack by the enzyme does occur at the most hindered C(1) carbon atom of (*S*) configuration, thus leading to inversion of configuration at this center. This observation is consistent with our previous results, where studies conducted using ^{18}O labelled water have shown that the enzymatic hydrolysis of the fast reacting (*S*)-enantiomer of styrene oxide **1** led to ^{18}O incorporation at the more substituted C(1) carbon atom.[9]

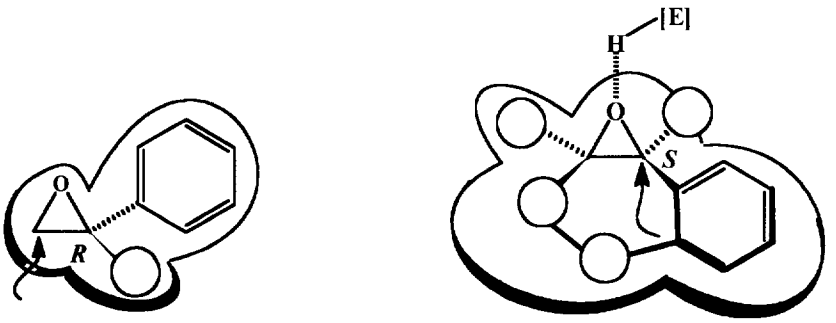
This regioselectivity relates interestingly to the possible mechanism implied in these hydrolyses. Indeed the mechanism of mammalian EH has been proposed by several authors to be a general base-catalyzed process, and it has been suggested that a histidine residue is involved for the deprotonation of the attacking water molecule.[23] Furthermore, Armstrong and coll.[24] have shown recently, from single turnover experiments conducted in isotopically enriched water, that the oxygen atom incorporated into the product, though ultimately derived from water, is proximally derived from the enzyme by way of an ester intermediate. On the other hand, it has been shown previously that acid-catalyzed opening of styrene oxide does essentially lead - *via* development of a positive charge at the benzylic carbon atom - to incorporation of water at this C(1) carbon atom.[25] Thus, our results suggest that the mechanism implied in the biohydrolyses mediated by *B. sulfurescens* should imply a similar acid-catalyzed process. Such a mechanism has been suggested previously for cEH.[26] On the other hand, because of the different regioselectivity observed for **1**,[9] a general base-catalyzed process should be operative for *A. niger* as in the case of mEH. However, since other factors (steric constraints for example) may also govern the regioselectivity of the oxirane opening, further studies are necessary in order to get a better insight into the enzymatic mechanism implied. In particularly, the use of substrates appropriately substituted on the phenyl ring may help to check these hypotheses.

Independently of the hypothesis that one or several EH's are operating in each of these fungi, it was of interest to build up models in order to shed some light on the space constraints involved in the EH's present in these two different microorganisms. This type of approach has been used previously to devise a model for the active site of the mammalian EH, and led to consider the existence of a lipophilic pocket located at the right backside of the active site space (taking as an hypothesis that the oxirane ring is oriented with the oxygen atom towards the top)[4,27] and that the nucleophilic entity (the carboxylate residue and/or the "activated" water molecule) is located near the less substituted carbon atom.

We had previously observed that our results obtained during the hydrolysis of **1** with *A. niger* could be well predicted by using this mammalian active site model. This observation shows to be not confirmed by the

present results obtained with compounds **2** to **7**. Indeed, these indicate that, in the case of *A. niger*, a β-substitution of the oxirane ring (with respect to the phenyl group) seems to prevent any good accommodation of both enantiomers into the active site. This lack of reactivity of these substrates could be due to an important steric hindrance between, on one hand, the alkyl group borne by the C(2) carbon atom and, on the other hand, an area of the active site. In addition, substitution at the α-position, as in **2**, led to a noticeable decrease of enantioselectivity, showing that the kinetic parameters (K_M and V_{max}) of either enantiomer were not sufficiently different to allow a high degree of enantiomeric differentiation. These observations led us to propose a model represented in scheme 4 for the EH active site of *A. niger*. This model is similar to the one related to the mEH except that no space happens to be available "around" the C(2) carbon atom allowing to fit alkyl groups.

In the case of *B. sulfurescens* it appears that, whatever the substitution in β-position (i.e. the β-substituted epoxides **3**, **4**, **6** and **7**), the fast reacting enantiomer is the one of (1*S*) absolute configuration, leading to accumulation of the (1*R*)-epoxide in the culture medium. Thus, no critical steric hindrance with the active site seems to take place when the C(2) carbon atom is monosubstituted by an alkyl group. Use of these results to build up a model led to conclude that a lipophilic pocket - which will accommodate the phenyl group of the (1*S*) enantiomer - must be located at the right frontside and that some space at the left frontside must also be available "around" the C(2) carbon atom in order to accommodate the β-alkyl group substituents (Scheme 4). In this case, we consider the nucleophilic entity to be located beneath the benzylic carbon atom.



(a) (b)

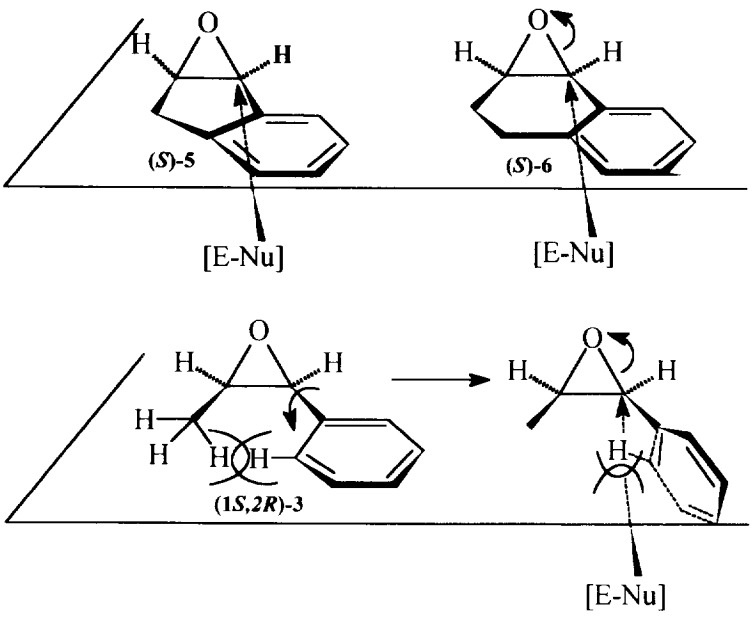
Scheme 4 : Proposed models for the active sites of *A. niger* (a) and *B. sulfurescens* (b) EH.

The peculiar result obtained in the case of the *gem*-epoxide **2** shows that this particular substrate does not fit into this model. One reasonable hypothesis would be to consider that, because of some steric hindrance, the (*S*) enantiomer has some difficulty to be accommodated into the proposed model. Therefore, a positioning where the phenyl group of the (*R*)-**2** antipode could fit into the left frontside pocket of the model seems to be preferred. This would explain that the enzymatic attack is reported at the less hindered C(2) carbon atom (which is thus well located to be attacked by the nucleophilic entity of the active site). The low degree of enantioselectivity

observed for epoxide **2** suggests that the two positionings must be somewhat equivalent. This hypothesis could also be taken into account in order to explain the observed stereoconvergency in the case of the *cis*-epoxide **3** (which gives exclusively the (1*R*,2*R*)-diol **10**). Indeed, a similar positioning of the less reactive (1*R*,2*S*) enantiomer of **3** (with the phenyl ring in the front left side of the model) would again localize the C(2) carbon atom in good place to be attacked by the nucleophilic entity of the active site. This is also consistent with our previous observation that the less reactive (1*R*)-styrene oxide **1** enantiomer is partly attacked at C(2) carbon atom.

The surprising differences observed between the reaction rates of the *cis*-epoxide **3** and those of the tricyclic epoxides **6** and **7** (which can both be considered as being also *cis*-substituted derivatives) may be explained on the basis of their different stereochemical arrangement. Indeed, examination of molecular models clearly indicates that the carbon framework of **6** and **7** are almost planar whereas - because of steric hindrance between the methyl and the phenyl groups - the aromatic cycle of **3** must be twisted of about 90° to adopt a perpendicular position (Scheme 5). Therefore, the low reactivity observed for **3** could be attributed to a disfavoured nucleophilic attack (by the enzymatic carboxylic or hydroxyl group) at the benzylic carbon atom because of the steric hindrance induced by this particular geometry. The same reason could account for the very slow biohydrolysis rate observed for the trisubstituted epoxide **5**. In the case of styrene oxide **1** or of *trans*-β-methyl styrene oxide **4** - for which high rates of hydrolysis were observed - it is interesting to notice that, owing to the lack of *cis*-β-substitution, a stereochemical arrangement of the phenyl group similar to the one involved in **6** and **7** could be easily adopted. These results are in agreement with the regioselectivity observed for the chemical nucleophilic opening of aromatic epoxides. Indeed, it has been shown[28] that *trans*-β-methyl(meta-trifluoromethyl)-styrene oxide, where the conjugation of the phenyl group with the oxirane ring is possible, is preferentially opened at the benzylic C(1) carbon atom whereas the nucleophilic attack is reported at the C(2) carbon atom in the case of its *cis*-isomer due to the steric hindrance of the phenyl group.

In conclusion, the results we have obtained in the course of this study, using differently substituted styrene oxide derivatives indicate clearly that, depending on the fungi, the substrate specificities, as well as the enantio- and regioselectivities of the oxirane ring opening, were completely different. For instance, the fungus *B. sulfurescens* presents a much broader substrate specificity than *A. niger*. Furthermore, using *B. sulfurescens*, it was shown that the enantioselectivity of the biohydrolysis was dependent of the oxirane ring substitution. Thus, the fast reactive enantiomer was always the (1*S*) one, except for the *gem*-epoxide **2**. Our experiments also show that this new methodology proves to be very interesting from a preparative point of view. Indeed, it allowed the preparation of either (1*R*)-**1**, -**4**, -**6** and -7 epoxides which could be isolated in optically pure form and with yields (20-40%) quite reasonable for a resolution process. Furthermore, this allowed the concomitant preparation of several diols - of (1*R*) absolute configuration- which exhibit high enantiomeric excess (70-90%). In particular, optically pure (1*R*,2*R*)-**10** could be obtained after complete biohydrolysis of *cis*-epoxide **3**.



Scheme 5

As far as the enzymatic mechanism is concerned, our results showed that the fast reactive (1*S*)-enantiomer was preferentially attacked at the benzylic C(1) carbon atom in the case of *B. sulfurescens*, a fact which strongly suggests that a general acido-basic catalyzed mechanism, similar to the one proposed for cytosolic EH, should be involved in this fungus. A comparison with the results obtained with mammalian EH also show that hydrolysis mediated by *A. niger* appears to be driven following a general base-catalyzed process as in mEH mediated hydrolyses. Further work, achieved to ascertain the nature (acid or basic) of the oxirane ring opening mechanism, will be described later on. From all these results, two active site models were proposed which allowed to describe some steric requirements implied in the catalytic active sites. Despite the fact that these models do not intend to represent the topology of the real active sites (several active EH's could be present in these fungi) they may be used as tools to predict the outcome of the hydrolysis of new aromatic epoxides. Work is in progress in our laboratory in order to confirm the validity of these models and to gain further information concerning the intimate mechanisms involved in these two fungi. Purification of the epoxide hydrolase of *A. niger* has been achieved also and will be described elsewhere.

EXPERIMENTAL SECTION

General. The strain of *A. niger* used in this work is registered at the "Museum d'Histoire Naturelle" (Paris) under n° LCP 521 (Lab. de Cryptogamie, 12 rue Buffon, 75005 Paris, France). Corn steep Liquor (CSL) is from Roquette SA. NMR spectra (^1H and ^{13}C) were recorded in $CDCl_3$ solution on a Bruker AC 250. Chemical shifts are reported in p.p.m. from TMS as internal standard. Optical rotation values were measured on a Perkin-Elmer 241C polarimeter at 589 nm. Vapor phase chromatography analyses were performed using either a 25 m

capillary column (OV 1701), or one of the three following "chiral" columns i.e.: Heptakis (6-O-methyl-2,3-di-O-pentyl)-β-cyclodextrin (col. I), Lipodex E [Octakis 3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin in OV 1701 (1/1)] (col. II) Octakis (6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin in OV 1701 (1/1) (col. III). Determination of the enantiomeric excesses of epoxides and diols were performed after derivatisation into their acetonide or dimethylethers. HPLC analyses were carried out using an UV detector (at 220 nm) and a column (12.5/0.4 cm) filled with 5 μm silica gel using hexane-ethanol as eluent. Separation and purification of the products were achieved by flash chromatography (silica gel 60 H from Merck and solvent mixtures consisting of pentane and ether in the range of 100% pentane to 100% ether) or by bulb-to-bulb distillation.

Synthesis of substrates 2 to 7

(+/-)-α-Methylstyrene oxide **2**. To a stirred solution of 4.4 g (20 mmoles) of trimethyloxosulfonium iodide in 20 mL of dry DMSO, placed under nitrogen, were added 0.87 g (20 mmoles) of sodium hydride (55% mineral oil dispersion).[29] After stirring for 30 min (formation of the dimethyloxosulfonium methylide), a solution of 2 g (16.7 mmoles) of acetophenone in 10 mL of DMSO was added and the reaction mixture stirred at room temperature for 5 h. After cooling and addition of water, the mixture was extracted with ether (3 times). The combined extracts were washed with brine, dried over magnesium sulfate and evaporated to yield 2.34 g of crude product. Purification by flash chromatography (pentane/ether) yielded 1.65 g (74% yield) of **2** as a colorless oil. ^{1}H-NMR δ: 1.72 (s, 3H); 2.80 (d, 1H, H_2, J_{gem} = 5.4 Hz); 2.96 (d, 1H, H_2, J_{gem} = 5.4 Hz); 7.2-7.4 (m, $5H_{arom}$). ^{13}C NMR δ: 21.9 (CH_3); 56.8 (C-1); 57.1 (C-2); 125.4; 127.4; 128.4; 141.2 (C-Ar).

(+/-)-cis-1-Phenylpropene oxide **3**. Racemic-**3** was prepared by cyclisation of the corresponding bromohydrin formed by reduction of α-bromopropiophenone. To a stirred solution of 3 g (14 mmoles) of α-bromopropiophenone in 30 mL of MeOH, cooled in an ice bath, was added 0.65 g of sodium borohydride. After removing the ice bath and stirring for 3 h, 2.3 g (17 mmoles) of potassium carbonate was added in the same flask. After 20 h stirring, 30 mL of water were added and the mixture was extracted with ether (3 times), washed twice with brine and dried over magnesium sulfate. Evaporation of the solvent yielded 1.75 g of crude product. Purification by bulb-to-bulb distillation allowed to isolate 1.6 g (85 % yield) of (±)-**3** as a colorless oil. ^{1}H-NMR δ: 1.07 (d, 3H, CH_3, J = 5.4 Hz); 3.33 (m, 1H, H_2); 4.05 (d, 1H, H_1, J_{1-2} = 4.2 Hz); 7.23-7.38 (m, $5H_{arom}$). ^{13}C NMR δ: 12.5 (C-3); 55.1 (C-2); 57.5 (C-1); 126.5; 126.6; 127.4; 128 (C-Ar). Using these experimental conditions a small proportion (5%) of *trans*-1-phenylpropene oxide was also formed.

(+/-)-trans-1-Phenylpropene oxide **4**. This epoxide was obtained by epoxidation of the corresponding olefin using *m*-chloroperoxybenzoic acid under biphasic conditions (CH_2Cl_2/phosphate buffer) for 5 h (96% yield) as described previously.[30] ^{1}H-NMR δ: 1.44 (d, 3H, CH_3, J = 5.1 Hz); 3.03 (q, 1H, H_2, J = 5.1 Hz; J_{1-2} = 2 Hz); 3.50 (d, 1H, H_1, J_{1-2} = 2 Hz); 7.22-7.36 (m, $5H_{arom}$). ^{13}C NMR δ: 17.9 (C-3); 58.9 (C-2); 59.5 (C-1); 125.5; 128.0; 128.4; 137.7 (C-Ar).

(+/-)-2,2-Dimethylstyrene oxide **5**. This epoxide was obtained using the same procedure described for **2** (97% yield). ^{1}H NMR δ: 1.07 (s, 3H); 1.48 (s, 3H); 3.86 (s, 1H); 7.26-7.36 (m, $5H_{arom}$). ^{13}C NMR δ: 17.9 (C-3); 24.7 (C-4); 61.1 (C-2); 64.6 (C-1); 126.4; 127.3; 128; 136.6 (C-Ar).

(+/-)-Indene oxide **6**. Racemic-**6** was obtained by cyclisation of the corresponding bromohydrin formed from indene. To a solution of indene (2 g, 17.2 mmoles) in acetone (60 mL) and water (10 mL), cooled in an ice bath, was added 3.67 g (20.6 mmoles) of N-bromosuccinimide. After stirring for 2 h at 0°C, the solution was stirred overnight at room temperature. Acetone was removed under vacuum, water was added and the mixture was extracted with ether (3 times). The combined organic phases were washed with brine, dried over magnesium sulfate and evaporated. The crude bromohydrin thus obtained was dissolved in methanol (50 mL) (without further purification) and treated with sodium bicarbonate (1.5 equiv.). After stirring overnight, the reaction mixture was filtered, methanol was removed in vacuo and water was added. The aqueous phase was extracted with ether (3 times) and the combined extracts were washed with brine and dried over magnesium sulfate. The solvent was removed in vacuo and the residue was purified by flash chromatography (pentane/ether) to give 1.7 g (75% overall yield) of racemic-**6** as a colorless oil. ^{1}H-NMR δ: 2.94 (dd, 1H, H_3, J_{3gem} = 18.0 Hz; J_{3-2} = 2.8 Hz); 3.20 (dd, 1H, H_3, J_{3gem} = 18.0 Hz); 4.13 (dd, 1H, H_2, J_{2-1} = 2.6 Hz; J_{2-3} = 2.9 Hz); 4.27 (d, 1H, H_1, J_{1-2} = 2.6 Hz); 7.24 (m, $3H_{arom}$); 7.46 (d, $1H_{arom}$, J = 7.1 Hz). ^{13}C NMR δ: 34.6 (C-3); 57.6; 59.1 (C-2; C-1); 125.1; 126.0; 126.2; 128.5; 140.8; 143.5 (C-Ar).

(+/-)-1,2-Dihydronaphtalene oxide **7**. This epoxide was obtained using the same procedure as described for **6** (74% overall yield). ^{1}H-NMR δ: 1.75 (m, 1H); 2.43 (m, 1H); 2.53 (m, 1H); 2.75 (m, 1H); 3.72 (m, 1H); 3.84 (d, 1H, J = 4.2 Hz); 7.09-7.40 (m, $4H_{arom}$). ^{13}C NMR δ: 21.8 and 24.4 (C-3; C-4); 52.7 (C-2); 55.1 (C-1); 126; 128.4; 128.4; 129.5; 132.5; 136.7 (C-Ar).

Synthesis of the racemic diols 9 to 14

These diols were prepared as follows by hydrolysis of the corresponding epoxides. To 1 g epoxide dissolved in a mixture of THF (50 mL) and water (10 mL) two drops of concentrated sulfuric acid were added. After stirring for 24 h, the hydrolysis was complete (checked by TLC) and the solution was neutralized by addition of saturated $NaHCO_3$ solution and extracted with ether (3 times). Evaporation of the washed (saturated salt solution) and dried ($MgSO_4$) extracts yielded a crude residue which was further purified by flash chromatography.

(+/-)-**9**: yield 80%, ^{1}H-NMR δ: 1.47 (s, 3H, CH_3); 2.74 (s large, 1H, OH); 3.19 (s large, 1H, OH); 3.58 (dd, 1H, H_2, J_{gem} = 10.9 Hz, $J_{H\text{-}OH}$ = 7.1 Hz); 3.75 (dd, 1H, H_2, J_{gem} = 11.1 Hz, $J_{H\text{-}OH}$ = 3.1 Hz); 7.23-7.45 (m, $5H_{arom}$). ^{13}C NMR δ: 26.0 (CH_3); 71.0 (C-2); 74.9 (C-1); 125.1; 127.2; 128.4; 144.9 (C-Ar).

(+/-)-**10**: yield 60%, ^{1}H-NMR δ: 1.05 (d, 3H, CH_3, J = 6.3 Hz); 2.66 (s large, 1H, OH); 2.95 (s large, 1H, OH); 3.84 (m, 1H, H_2); 4.34 (d, 1H, H_1, J = 7.4 Hz); 7.35-7.55 (m, $5H_{arom}$). ^{13}C NMR δ: 18.7 (C-3); 72.2 (C-2); 79.5 (C-1); 126.8; 128.4; 128.5; 141.1 (C-Ar). Using these experimental conditions, 12% of *erythro*-diol **11** were also formed.

(+/-)-**11**: yield 78%, mp 75-80°C, ^{1}H-NMR δ: 1.05 (d, 3H, CH_3, J = 6.4 Hz); 2.12 (s large, 1H, OH); 2.65 (s large, 1H, OH); 4.0 (m, 1H, H_2); 4.66 (d, 1H, H_1, J = 4.2 Hz); 7.33 (m, $5H_{arom}$). ^{13}C NMR δ: 17.2 (C-3); 71.3 (C-2); 77.5 (C-1); 126.6; 126.8; 127.8; 128.3; 128.5; 140.3 (C-Ar). Using these experimental conditions 20% of *threo*-diol **10** were also formed.

(+/-)-**12**: yield 86%, mp 63°C, ^{1}H NMR δ: 1.07 (s, 3H, CH_3); 1.20 (s, 3H, CH_3); 2.45 (s large, 1H, OH); 3.05 (d, 1H, OH, $J_{OH\text{-}H}$ = 3.1 Hz); 4.48 (d, 1H, H_1, $J_{H\text{-}OH}$ = 2.9 Hz); 7.25-7.36 (m, $5H_{arom}$). ^{13}C NMR δ: 23.7; 26.5 (C-3; C-4); 73.5 (C-2); 80.8 (C-1); 127.4; 127.8; 127.9; 140.6 (C-Ar).

(+/-)-**13**: yield 75%, mp 160°C, ^{1}H NMR (CD_3COCD_3) δ: 2.73 (dd, 1H, H_3, J_{gem} = 15.6 Hz , $J_{3\text{-}2}$ = 7.0 Hz); 3.16 (dd, 1H, H_3, J_{gem} = 15.6 Hz, $J_{3\text{-}2}$ = 7.0 Hz); 4.26 (m, 1H, H_2); 4.33 (d, 1H, OH, $J_{H\text{-}OH}$ = 4.8 Hz); 4.47 (d, 1H, OH, $J_{H\text{-}OH}$ = 6.1 Hz); 4.90 (m, 1H, H_1); 7.17-7.34 ($4H_{arom}$). ^{13}C NMR (CD_3COCD_3) δ: 38.7 (C-3); 81.5 (C-2); 82.1 (C-1); 125.1; 125.4; 127.4; 128.5. Using these experimental conditions 14% of *cis*-diol were also formed.

(+/-)-**14**: yield 77%, mp 103-105°C, ^{1}H-NMR (CD_3COCD_3) δ: 1.87 (m large, 3H (2OH + H_3); 2.16 (m, 1H, H_3); 2.92 (m, 2H, H_4); 3.83 (m, 1H, H_2); 4.58 (d, 1H, H_1, $J_{1\text{-}2}$ = 8.1 Hz); 7.17-7.60 ($4H_{arom}$). ^{13}C NMR (CD_3COCD_3) δ: 27.7 (C-3); 28.8 (C-4); 73.4 (C-2); 75.1 (C-1); 126.4; 126.9; 127.4; 128.2; 135.6; 137.1 (C-Ar). Using these experimental conditions a small proportion (17%) of *cis*-diol was also formed.

General procedure for derivatisation of the diols into the corresponding acetonides: 5 to 10 mg of the corresponding diol and 2,2-dimethoxypropane (100 µL) were stirred in the presence of a catalytic amount of TosOH for 30 min. After neutralization with a saturated $NaHCO_3$ solution, the reaction mixture was extracted with ether, dried over $MgSO_4$ and directly analyzed by chiral GLC.

General procedure for the derivatisation of the diols into the corresponding di-methylethers: to a stirred solution of the appropriate diol (20 mg) dissolved in dry DMSO, 50 mg of powdered KOH and 30 µL of methyl iodide were added. After stirring for 30 min, water was added and the reaction mixture was extracted with ether, dried over $MgSO_4$ and directly analyzed by chiral GLC.

Biohydrolysis of styrene oxide derivatives (1-7) with *A. niger* and *B. sulfurescens*.

General procedure. The fungal strains were cultured in a 2 L fermentor as previously described.[8] After incubation (40 h for *A. niger*, 48 h for *B. sulfurescens*) the mycelium was filtered off, washed with water, and

then placed back in the same fermenter filled with 1 L of a pH 8 phosphate buffer (0.1 M) solution. The medium was stirred at 700 rpm and maintained at 27°C. The appropriate racemic styrene oxide derivative (1 g) was added to the culture as a solution in ethanol (10 mL). The course of the bioconversion was followed by withdrawing two aliquots (2 mL) at time intervals. One of these samples was extracted with pentane (2 mL) and subjected to direct chiral GC analysis, which allows to determine the ee of the residual epoxide. After saturation with NaCl, the second sample was extracted with ether (2 mL) and after purification through silica gel and derivatisation to the acetonide or di-methylether, the ees of the diols were determined by chiral GC analysis. The bioconversion was stopped by addition of ether (500 mL). The medium was filtered off, and the fungal cake was separately extracted two times with ether (2 x 200 mL). After decantation, the aqueous phase was saturated with NaCl and then continuously extracted with dichloromethane (48 h). The combined organic layers were dried ($MgSO_4$) and purification of the products was achieved by flash chromatography or/and by bulb-to-bulb distillation. Preparative yields and ees of residual epoxides and formed diol are given in Table 1 and Table 2.
Optical rotations and chiral GLC data of the isolated products after biohydrolysis are as follows:

(*S*)-**2**: $[\alpha]_D^{20}$ +1.25 (c 0.2, EtOH); 73% ee [col(I) 60°C, t_R(*R*) 13.2 min and t_R(*S*) 14.6 min];

(1*R*,2*S*)-**3**: $[\alpha]_D^{20}$ -7.1 (c 2.09, $CHCl_3$) lit.[14] $[\alpha]_D^{20}$ +47.5 (c 1.17, $CHCl_3$) for (1*S*,2*R*)-**3**; 20% ee [col(II) 90°C, t_R(1*S*,2*R*) 6.1 min and t_R(1*R*,2*S*) 7.7 min];

(1*R*,2*R*)-**4**: $[\alpha]_D^{20}$ +45.7 (c 0.41, $CHCl_3$) lit.[15] $[\alpha]_D^{20}$ +48.5 (c 0.94, $CHCl_3$); 98% ee [col(II) 90°C t_R(1*S*,2*S*) 6.3 min and t_R(1*R*,2*R*) 6.9 min];

(1*R*,2*S*)-**6**: $[\alpha]_D^{20}$ -39 (c 0.62, $CHCl_3$) this value is not accurate because some 2-indanone, very difficult to separate from the epoxide, was present; lit.[16] $[\alpha]_D^{20}$ -55 (c 1, $CHCl_3$); 98% ee (col(II) 90°C t_R(1*S*,2*R*) 22.5 min and t_R(1*R*,2*S*) 24.2 min];

(1*R*,2*S*)-**7**: $[\alpha]_D^{20}$ +129 (c 0.81, $CHCl_3$) lit.[17] δ $[\alpha]_D^{20}$ +135 ($CHCl_3$); 98% ee [col(I) 100°C t_R(1*S*,2*R*) 13.1 min and t_R(1*R*,2*S*) 13.9 min];

(*R*)-**9**: $[\alpha]_D^{20}$ -1.36 (c 0.89, EtOH) lit.[11] $[\alpha]_D^{8}$ +1.7 (EtOH) for (*S*)-9; 32% ee [col(I) 60°C t_R(*S*) 48.4 min and t_R(*R*) 49.9 min];

(1*R*,2*R*)-**10**: $[\alpha]_D^{20}$ -30.9 (c 1.7, EtOH) lit.[15] $[\alpha]_D^{20}$ -32.6 (c 1.65, EtOH); 99% ee [col(III) 80°C t_R(1*S*,2*S*) 18.5 min and t_R(1*R*,2*R*) 18.8 min];

(1*R*,2*S*)-**11**: $[\alpha]_D^{20}$ -14.6 (c 0.68, EtOH) lit.[15] $[\alpha]_D^{20}$ -18.1 (c 0.44, EtOH); 90% ee [col(III) 80°C t_R(1*S*,2*R*) 14.9 min and t_R(1*R*,2*S*) 15.6 min];

(1*R*,2*R*)-**13**: $[\alpha]_D^{20}$ -20 (c 0.35, EtOH) lit.[16] $[\alpha]_D^{20}$ -29.4 (c 0.44, EtOH); 69% ee [col(II) 120°C t_R(1*S*,2*S*) 8.5 min and t_R(1*R*,2*R*) 12.5 min];

(1*R*,2*R*)-**14**: $[\alpha]_D^{20}$ +84.4 (c 1.04, $CHCl_3$) lit.[17] $[\alpha]_D^{20}$ +110 ($CHCl_3$); 77% ee [col(II) 130 °C t_R(1*S*,2*S*) 10.2 min and t_R(1*R*,2*R*) 16.2 min].

Total biohydrolysis of (±)-3 using *B. sulfurescens*

The fermentation was carried out in a 7 L fermentor jar containing 5 L of culture medium as previously described.[8] After 40 h of growth, the mycelium was filtered off, washed with water, and then replaced in the same fermentor containing 4 L of a pH 8 (0.1 M) phosphate buffer before addition of a solution of 1 g in 1.5 mL of ethanol. The medium was stirred at 500 rpm and maintained at 27°C without aeration. After incubation for 24 h, the medium was filtered off and the aqueous phase was saturated with NaCl and then continuously extracted with dichloromethane (36 h). The organic layer was dried ($MgSO_4$) and purification of diol **10** was achieved by flash chromatography followed by bulb-to-bulb distillation (200°C, 0.1 mm Hg) to afford 960 mg (85%) of (1*R*,2*R*)-**10** (ee 98%); $[\alpha]_D^{25}$ -30.1 (c 1.68, EtOH).

Acknowledgements. One of us (S. P.M.) is indebted to the Société Roussel-Uclaf for financial support.

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(*Received in Belgium* 10 *November* 1995; *accepted* 25 *January* 1996)