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Journal of Molecular Catalysis B: Enzymatic 5 (1998) 79–85

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Fungal epoxide hydrolases: new tools for the synthesis of enantiopure epoxides and diols

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Received 17 October 1997; accepted 3 December 1997

Abstract

This presentation describes efficient means of preparing optically pure epoxides and diols using fungal epoxide hydrolases as biocatalysts. The biohydrolyses can be carried out in a preparative scale for different types of epoxides as terpenic-, aliphatic-, aromatic- and glycidyl acetal-derivatives bearing an epoxide moiety. In addition, in order to obtain these compounds in good yield, an efficient enzymatic reactor and different enantioconvergent processes were devised.

Keywords: Fungal epoxide hydrolase; Enantioselectivity; Optical resolution; Epoxide; 1,2-Diol

1. Introduction

Epoxides and diols are very interesting chiral building blocks which can be used in the asymmetric synthesis of biologically active drugs. Therefore, the asymmetric synthesis of these compounds is an important area in organic chemistry, and very elegant and efficient work have been developed using transition metal catalysis leading to such chirons [1–3]. However, these methods can show some limitations depending on the substrate structure. In this context, we have studied the possible use of biocatalytic methods to prepare these chiral compounds, and we have focused our attention on the enantioselective biohydrolysis of racemic epoxides using fungal epoxide hydrolases (EHs). These cofactor-independent enzymes are very interesting tools for the organic chemist, since they can specifically hydrolyze one enantiomer of a racemic mixture, leading to the formation of an optically active diol, leaving the unreacted enantiomer unchanged in the medium. It has been shown that EHs are almost ubiquitous in nature [4]. In particular, they have been detected in mammals [5], where they are located in liver for instance, in plants [6] like potatoes, soybean, maize or in microorganisms like bacteria [7], yeasts [8] and fungi [9]. Because of their involvement in the detoxication of various xenobiotics, mammalian enzymes have been extensively studied. On the contrary, very scarce results are available for such enzymes from plant or microbial origin.

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1381-1177/98/$19.00
PII: S1381-1177(98)00011-3

As our objective was to prepare chiral epoxides and diols on a several-gram scale, the biocatalysts used to carry out the resolution of racemic epoxides were those from fungi because they are easily available from large-scale cultures. In order to explore the scope and limitation of these EHs, four families of substrates were studied: (a) terpenic epoxides, (b) glycidyl acetal derivatives, (c) aliphatic epoxides and (d) aromatic epoxides. In addition, in order to obtain these compounds in a preparative scale and a good yield, we have devised an efficient enzymatic reactor, as well as different enantioconvergent processes.

2. Results and discussion

2.1. Enantioselective biohydrolysis of various epoxides

Very interesting results were obtained in the case of the biohydrolysis of terpenic epoxides using the fungus *Aspergillus niger* as biocatalyst. An application was the synthesis of both enantiomers of the Bower's compound **1**, a compound known to be a potent analogue of insect juvenile hormone [10] (Fig. 1). These hormones are necessary in insect development, and we wanted to prepare specifically both enantiomers of this compound in order to test separately their biological activity. The enantioselective biohydrolysis of the racemic Bower's compound **1** led to the (6*S*)-diol **2** in 48% isolated yield and 70% ee. The remaining epoxide of (6*S*) absolute configuration was isolated in a 36% yield and exhibited a high ee (96%). Its antipode was easily prepared by chemical way in two steps from this enantiomer. Indeed, acid hydrolysis led to diol (6S)-**2**, which, upon reaction with tosylchloride in the presence of sodium hydride, afforded epoxide (6*R*)-**1** after cyclisation involving total inversion at this stereogenic carbon atom. Each enantiomer of Bower's compound was tested for its juvenile hormone activity, and it was found that the (6*R*)-enantiomer was 10 times more active than its antipode against the yellow meal worm *Tenebrio molitor*.

Another application was the highly diastereoselective hydrolysis of the various stereoisomers of the exocyclic limonene epoxides **3** using this same fungus, thus opening the way to the synthesis of either enantiopure bisabolol

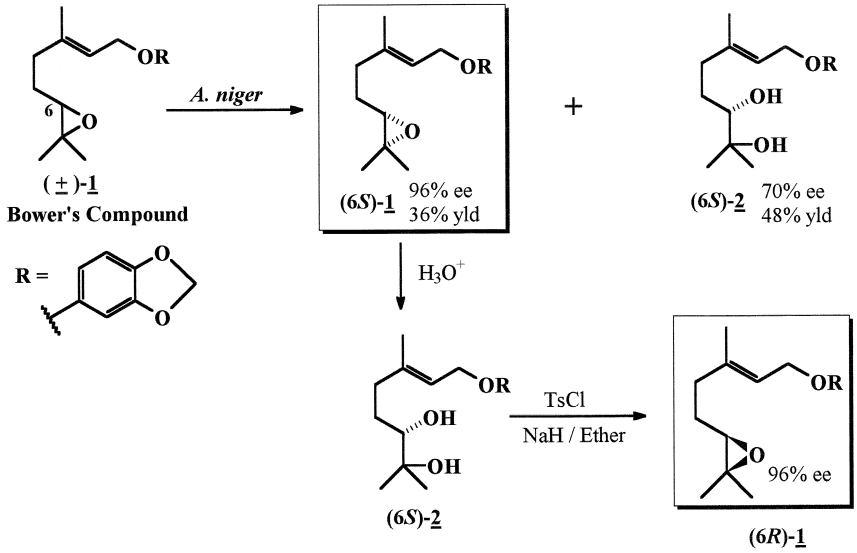

Fig. 1. Synthesis of both enantiomers of Bower's compound using the fungus *A. niger* as biocatalyst.

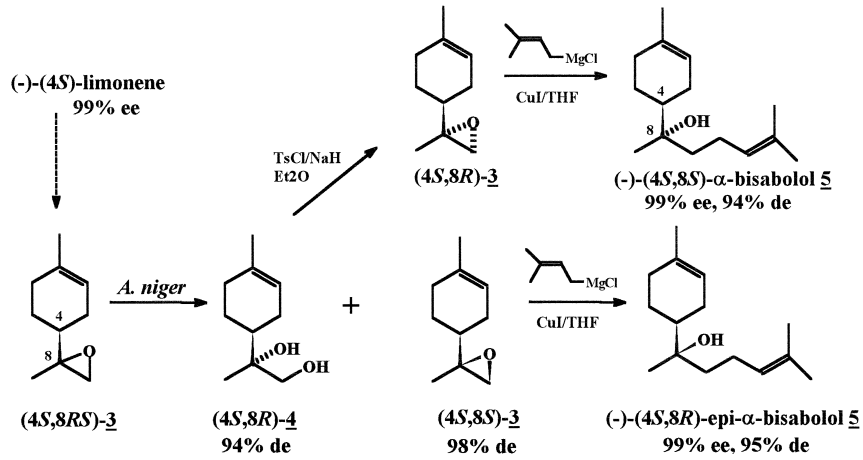

Fig. 2. Diastereoselective biohydrolysis of exocyclic limonene epoxides using whole cells of *A. niger*. Synthesis of α-Bisabolol.

stereoisomer **5** [11] (Fig. 2). One of these enantiomers, i.e. (4*S*,8*S*)-α-bisabolol **5**, is used on an industrial scale for the preparation of various skin-care creams, lotions and ointments.

Glycidyl acetal derivatives are very valuable chiral building blocks because these molecules are bearing two chemically different reactive active sites, i.e., one oxirane ring and one protected aldehyde. They have been used for instance by Effenberger [12] as C3 chirons, by Wong [13] to carry out a synthesis of azasugars, and by Bolte and Demuynck [14] to prepare glycosidase inhibitors. However, optically pure glycidyl acetal derivatives are very difficult to synthetise by chemical ways, and the best obtained yield was lower than 50% [15]. During the study of the enantioselective biohydrolysis of this type of epoxide, the fungus *A. niger* was found to be a good candidate to achieve their resolution [16]. It was observed that the enantioselectivity was higher when the acetal function was bulkier. The best result was obtained when the protecting group was a cyclic acetal (Fig. 3). From 5 g of racemic epoxide **6**, 2 g of optically pure (*R*)-**6** and 2.3 g of (*S*)-diol **7** exhibiting a high ee (92%) were isolated. The epoxide of (*S*) absolute configuration could be easily prepared from diol **7** by a chemical cyclisation. Indeed, reaction of tosylchloride in the presence of sodium hydride afforded (*S*)-**6** without loss of enantiomeric purity with an excellent yield of 90%.

As far as the biohydrolyses of aliphatic epoxides are concerned, 10 different epoxides were studied with seven fungi selected from a screening of about 40 strains [17,18]. In Table 1, the results obtained for four types of substrates corresponding to a monosubstituted-, a *gem*-, a *cis*- or a *trans*-epoxide are reported. We succeeded in finding in each case a fungus which was able to afford an optically pure epoxide. The corresponding diols were obtained with

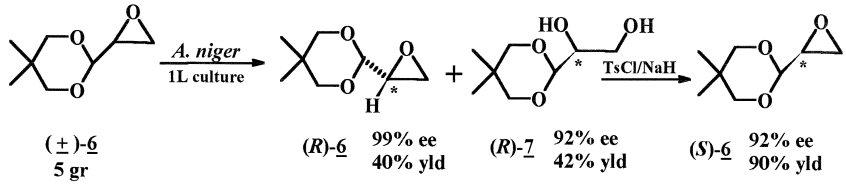

Fig. 3. Preparative biohydrolysis of glycidyl acetal derivatives using whole cells of the fungus *A. niger*.

Table 1
Preparative biohydrolysis of aliphatic epoxides using different fungal strains as biocatalyst

Substrates[a]	Strains	Epoxide		Diol	
		Yield (%)	ee (%)	Yield (%)	ee (%)
Hexyloxirane	*Mortierella isabellina*	18	> 97	54	35
1-Methyl-1-pentyloxirane	*A. niger*	22	> 99	62	32
trans-2-Methyl-1-phenyloxirane	*S. racemosum*	5	98	68	44
trans-2-Methyl-1-phenyloxirane	*C. globosum*	12	> 97	60	78
cis-2-Methyl-1-phenyloxirane	*C. globosum*	8	> 97	59	58

[a] Biohydrolysis carried out on 2 g of racemic epoxide using a whole cell culture.

only moderate ee (between 32 and 78%). However, an interesting result was obtained in the case of *trans*-2-methyl-1-pentyloxirane for which each corresponding enantiomer could be prepared by choosing the adequate strain. Indeed, the residual epoxide was (1*R*,2*R*) when *Syncephalastrum racemosum* was used as biocatalyst, while its antipode was isolated using *Chaetomium globosum*. Therefore, for this epoxide, these two strains are enantiocomplementary. It should be noted that these optically pure epoxides were obtained in low preparative yields (8 to 22%). Two reasons can explain such low yields: (a) the enantioselectivity of the biohydrolysis is not very high, (b) these epoxides are highly volatile, which led to important loss upon extraction/purification process. Nevertheless, these experiments show that this selection of strains allows to produce at least one optically pure enantiomer of various substituted alkyl epoxides via kinetic resolution of the racemates.

Comparative biohydrolyses of various substituted styrene oxide derivatives were carried out using the fungi *A. niger* and *Beauveria sulfurescens* (presently *B. bassiana*) [19]. These microorganisms proved to be equipped with EHs, which can achieve these hydrolyses with high enantioselectivity. In addition, these two strains were revealed to be enantiocomplementary, since they achieved the highly enantioselective hydrolysis of the (*R*)- or the (*S*)-enanti-

Table 2
Comparative biohydrolysis of *para*-substituted aromatic epoxides using the fungi *A. niger* and *B. sulfurescens*

Substrates[b]	*A. niger*[a]			*B. sulfurescens*[a]		
	Epoxide		Diol	Epoxide		Diol
	Yield (rt)[c]	ee (Abs. Conf.)	ee (Abs. Conf.)	Yield (rt)[c]	ee (Abs. Conf.)	ee (Abs. Conf.)
Styrene oxide	28% (2 h)	98% (*S*)	51% (*R*)	34% (2 h)	98% (*R*)	83% (*R*)
para-Methylstyrene oxide	34% (1 h)	95% (*S*)	66% (*R*)	30% (0.5 h)	> 98% (*R*)	76% (*R*)
para-Fluorostyrene oxide	35% (1 h)	98% (*S*)	81% (*R*)	25% (2 h)	96% (*R*)	78% (*R*)
para-Bromostyrene oxide	34% (1 h)	> 98% (*S*)	80% (*R*)	33% (3 h)	96% (*R*)	79% (*R*)
para-Cyanostyrene oxide	38% (1 h)	98% (*S*)	76% (*R*)	59% (24 h)	15% (*R*)	50% (*R*)
para-Nitrostyrene oxide	37% (1 h)	98% (*S*)	70% (*R*)	50% (24 h)	20% (*S*)	49% (*R*)

[a] Experimental conditions: All the biohydrolyses were carried out in a 1L fermentor using whole cell cultures of *A. niger* or *B. sulfurescens*.
[b] Substrate concentration: 1 g/l (pH 7, $T = 27$°C).
[c] (rt): reaction time.

omer of styrene oxide [20], respectively, as well as of several other *para*-substituted styrene oxides [21]. The results reported in Table 2 show that the enantioselectivity of the biohydrolysis was almost independent of the nature of the *para*-substituent in the case of *A. niger*, thus leading to the optically pure (*S*) epoxides. On the contrary, in the case of *B. sulfurescens*, the reaction rate and the enantioselectivity decreased when the electro-withdrawing effect of the *para*-substituent increased. These results and some other experiments conducted using labeled water and crude lyophilized enzymatic extract allowed to get more insight into the mechanism of the oxirane ring opening [22].

2.2. Process improvement

The previous results show that fungal epoxide hydrolases are very interesting tools to obtain optically pure epoxides of different structures. In order to set up an efficient and easy-to-use biotechnological process, a soluble crude lyophilised extract of *A. niger* was prepared that could be used in a batch reactor. Thus, 4 g (53 g/l) of *p*-nitrostyrene oxide **8** (dissolved in a 80/20 water/DMF solution) could be resolved within 32 h leading to a 45% yield and a 95% ee of the remaining epoxide (*S*)-**8**, whereas the ee of the (*R*)-formed diol **9** was about 86% (52% yield) [23] (Fig. 4).

For such resolution processes, the yield is intrinsically limited to 50% because the enantioselective biohydrolysis is a resolution process. In order to overcome this important limitation, three different strategies have been devised and are described hereunder.

2.2.1. Chemo-enzymatic enantioconvergent process

This method is based on the consecutive use of an enantioselective biohydrolysis of the epoxide moiety, followed by an acid-catalysed hydrolysis of the remaining epoxide, in order to obtain only one enantiomer of the diol. Improvement of this approach was devised by combining these two steps in a one-pot procedure and by optimising the yield and ee of the diol produced by means of a mathematical approach. An application was the synthesis of (*R*)-Nifenalol **10** (known as having β-blocker activity), from the *A. niger* catalysed biohydrolysis of *p*-nitrostyrene oxide **8** (Fig. 4). The controlled acid hydrolysis of the reaction mixture, which contained the unreacted (*S*)-epoxide **8** and the (*R*)-diol **9** resulting from the enzymatic hydrolysis, led to an overall yield of 94% of (*R*)-diol **9** (ee 80%), due to steric inversion upon acid hydrolysis of the (*S*)-epoxide **8**. After recrystallisation, this (*R*)-diol **9** (ee 99%) could be transformed into the biologically active enan-

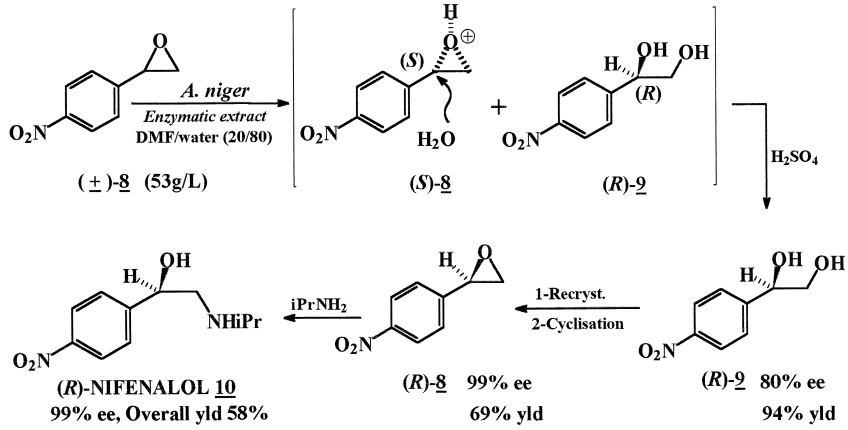

Fig. 4. Enantioconvergent synthesis of the β-blocker (*R*)-Niténalol® using a combined chemoenzymatic approach.

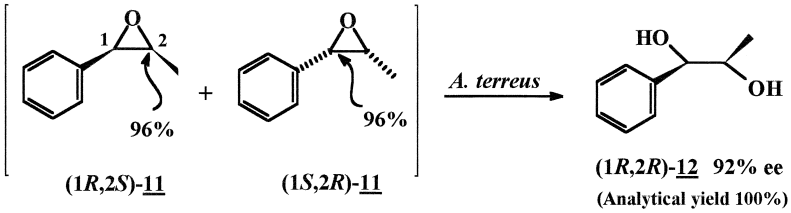

Fig. 5. Enantioconvergent biohydrolysis of *cis*-methylstyrene oxide using an enzymatic extract of the fungus *A. terreus*.

tiomer of (*R*)-Niténalol® **10** (overall yield 58%) [24].

2.2.2. Pure enzymatic enantioconvergent process

As mentioned previously, the two fungi, *A. niger* and *B. sulfurescens*, are able to perform highly enantioselective hydrolysis of racemic styrene oxide. Indeed, styrene oxide was very efficiently hydrolysed by *A. niger* affording the (*S*)-enantiomer in 96% ee within a few hours. Moreover, *B. sulfurescens* showed an opposite enantioselectivity, leading to the (*R*)-enantiomer in 98% ee. Both hydrolyses leading to the corresponding (*R*)-diol, an enantioconvergent process was obtained using a mixture of the two fungi. This led to a 92% overall yield of (*R*)-phenylethanediol in 89% ee [20].

In the two previous enantioconvergent processes, a combination of a chemical and an enzymatic hydrolysis or a combination of two biocatalysts have been used. However, a careful monitoring of the different reactions must be performed. This monitoring may be avoided using a biocatalyst, which can perform the enantioconvergent process by itself. This interesting case could be observed during biohydrolysis of the *cis*-methylstyrene oxide **11** using an enzymatic extract of *Aspergillus terreus* [25] (Fig. 5). Indeed, after 3 h, epoxide **11** was completely transformed into the corresponding *threo* diol **12**, which exhibited a 92% ee. During this process, the (1*R*,2*S*)-**11** enantiomer was opened with a regioselectivity of 96% at the carbon atom bearing the methyl group, and by inversion of configuration of this atom, the (1*R*,2*R*)-diol **12** was preferentially formed. At the same time, the oxirane ring of its antipode was opened with a regioselectivity of 96% on the benzylic carbon atom which led to the same (1*R*,2*R*)-diol **12** via inversion of configuration. Monitoring of this reaction showed that the ee of the formed diol stayed at 92% all over the reaction. A similar case was also observed during biohydrolysis of **11** using the whole fungus *B. sulfurescens* [19]. This reaction was carried out on 1 g of racemic epoxide, and the corresponding *threo*-(1*R*,2*R*)-diol **12** was isolated in a good preparative yield (85%) and an excellent ee (98%). This diol can be easily transformed into *trans*-methylstyrene oxide without loss of enantiomeric purity.

3. Conclusion

The use of fungal epoxide hydrolases seems to be a very promising method to prepare optically pure epoxides and diols. Indeed, these enzymes, which act directly on the epoxide ring, independently of any other functionality, offer several advantages, i.e., (a) they have been shown recently to be ubiquitous in nature, (b) they are cofactor independent enzymes, (c) they can be produced easily from various microorganisms, (d) they can be partially purified and used as an enzymatic powder, (e) they can act in the presence of organic solvents, thus allowing to handle water insoluble substrates, (f) they very often lead to excellent ee's of the remaining epoxide and, in certain cases, of the formed diol that can either be cyclized back to the enantiopure epoxide or derivatised into reactive 'epoxide-like' chiral synthons (cyclic sulfites or

sulfates). In some cases, because of the different regioselectivity of the oxirane ring opening of each enantiomer of a racemic mixture, the diol can be isolated with a high enantiomeric purity and a theoretical yield of 100% (enantioconvergent process

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