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Microbiological Transformations. 31: Synthesis of Enantiopure Epoxides and Vicinal Diols Using Fungal Epoxide Hydrolase Mediated Hydrolysis.

S. Pedragosa-Moreau, A. Archelas, R. Furstoss*

Groupe "Biocatalyse et Chimie Fine", Faculté des Sciences de Luminy,
Case 901, 163 Avenue de Luminy, F-13288 Marseille Cedex 9, France

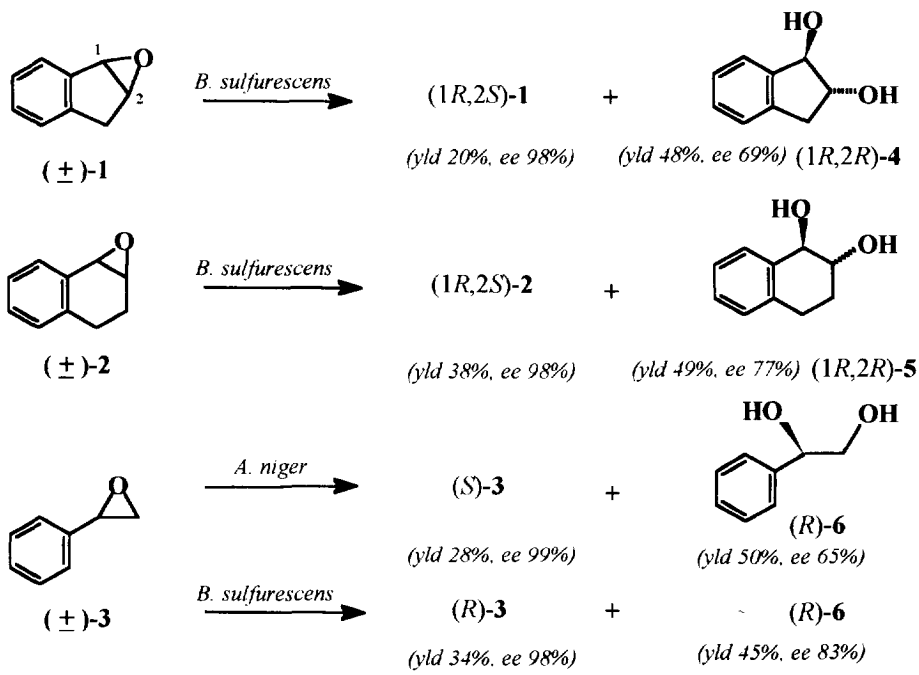
Abstract. The enantioselective hydrolysis of epoxyindene and dihydronaphtalene epoxides by the fungus *Beauveria sulfurescens* (ATCC 7159) is described. This allowed the preparation of both these epoxides, as well as of the corresponding diols, in good to excellent enantiomeric purity.

The asymmetric synthesis of enantiopure epoxides and/or of their corresponding vicinal diols is an important area in asymmetric synthesis. Very elegant and efficient work has been developed recently by different authors using transition metal catalysis, leading to important new processes allowing for the synthesis of such chirons.[1] However, these methods can be severely limited depending on the substrate structure. In this context, we have studied recently the possible use of biocatalytic methods and have focused our attention on the biocatalyzed hydrolysis of racemic epoxides, which can be conducted - by monitoring the conversion ratio - to obtain an extremely high ee of the remaining epoxide. This can be interesting from different points of view. First, this may allow the preparation of epoxides and vicinal diols in good to excellent enantiomeric purity. Moreover, such an approach can prove to be complementary to the chemical approaches in the case where the product obtained shows a limited ee. A method allowing to enhance the enantiomeric purity of such an already optically enriched product to a very high value (ee > 99.5 %) - essential to manufacture enantiopure biologically active compounds - would therefore be very helpful. In this context, a one-pot tandem asymmetric epoxidation/kinetic resolution process of 1,2-dihydronaphtalene has been recently described. However, the observed enantioselectivity observed was moderate, leading to a noticeable drop of the preparative yield.[2]

We here describe the results we have obtained performing the hydrolysis of racemic epoxyindene **1** and epoxydihydronaphtalene **2** using the fungus *Beauveria sulfurescens*. Both these compounds, which cannot be obtained in optically pure form *via* chemical epoxidation, are of interest for organic synthesis. For instance, epoxyindene has been described as being a key-intermediate for the synthesis of a highly efficient inhibitor of an enzyme involved in human immunodeficiency (HIV). This orally active HIV protease inhibitor Indinavir (L -

735,524) is one of a group of compounds that are in advanced chemical trials for the treatment of AIDS[3,4]. Also, both *cis*-1-amino-2-indanols obtainable from the enantiomers of **1** have been shown to be very useful chiral ligands in the catalytic asymmetric reduction of ketones with BH_3-Sme_2.[5]

We have previously described that the fungus *Aspergillus niger* is able to enantioselectively hydrolyse styrene oxide **3**, leading to the remaining (*S*)-epoxide and to the formed (*R*)-diol **6**[6]. Furthermore, we have noticed that the fungus *Beauveria sulfurescens* was enantiocomplementary and led to the (*R*)-epoxide and to the corresponding (*R*)-diol. Surprisingly enough, exploratory studies indicated that neither **1** nor **2**, which can be regarded as being rigid *cis*-substituted styrene oxide derivatives, were good substrates for the fungus *Aspergillus niger*, i.e. that no noticeable reaction occurred at all. However, we were very pleased to notice that both **1** and **2** were rapidly hydrolyzed by the fungus *Beauveria sulfurescens*. Thus, when **1** (at a concentration of 1 g/L) was treated for 1 hour with a resting-cells buffered suspension of this fungus prepared from a 1 L culture as described previously[6], both the remaining (1*R*,2*S*)-**1** enantiomer, and the (1*R*,2*R*)-**4** vicinal diol were obtained. The isolated yields were respectively of 20 and 48 %, and the corresponding ees were higher than 98 % for **1** and 69 % for **4**. Similarly, epoxydihydronaphtalene **2** (1 g/L) was hydrolyzed within 0.5 hour and led to the enantiopure remaining epoxide (1*R*,2*S*)-**2** (38 % yield, ee > 98 %) and to the corresponding vicinal (1*R*,2*R*)-**5** diol (49 % yield, 77 % ee).



The absolute configurations of the remaining epoxides and of the diols formed were established on the basis of their optical rotation previously described[7,8]. Their ees were determined using chiral GC [Lipodex E for compounds **1**, **3**, **4** and **5** and heptakis (6-O-methyl-2,3-di-O-pentyl)-β-cyclodextrin for epoxide **2**]. The ees of diols were measured after derivatisation into their dimethoxylated compounds.

It is interesting to compare these results with thoses we have obtained previously with styrene oxide **3**. We have described that biohydrolysis of this substrate necessitated, under identical experimental conditions, a 2 hours period to afford a 34 % yield of remaining (*R*)-epoxide (ee > 98 %) and a 45 % yield of the corresponding (*R*)-diol **6** (ee 83 %). Thus, both compounds **1** and **2** appear to be hydrolyzed faster than **3**.

As far as the E values of these reactions are concerned, we have previously shown that conducting the biohydrolysis of **3** in ^{18}O labelled water resulted in a 99 % incorporation of the labeled oxygen at the benzylic carbon atom for the fast reacting enantiomer, but to a 50/50 repartition of this label on both carbon atoms for the slow reacting antipode.[9] Thus by extrapolating these results for **1** and **2** it appears that, because of this eventual lack of regioselectivity, it is not possible to calculate a reasonably accurate E value for these reactions using the ee of the residual epoxide (ees) and of the formed diol (eep).[10] (For instance, calculation of the E value after 15 min bioconversion led to a value of 11, whereas a value of 20 was obtained after half an hour). However it must be emphasized that, in spite of this fact, the ees of both **1** and **2** can be conducted to values approaching 100 % by monitoring the reaction properly. Interestingly, it has been previously described that the chemical asymmetric epoxidation or dihydroxylation of indene and 1,2-dihydronaphtalene lead to epoxides **1** and **2** showing ees in a 92-96 % range.[11-13] Furthermore, this necessitated in some cases experimental conditions (-78°C) difficult to carry out on large scale preparations and may lead to delicate work-up procedures. Thus, our biocatalytic approach appears to be particularly attractive in this context. This is also the case as far as synthesis of the optically active diols **4** and **5** is concerned. Indeed, in both cases, the ees we obtained - and which could be further enhanced by stopping the reaction at a lower conversion ratio - are much higher than those obtained using the Sharpless dihydroxylation process.[13]

The obvious drawback of our approach is the intrinsic yield limitation (to 50%) of such resolution processes. Therefore it appears that the combined use of the Jacobsen/Katsuki asymmetric epoxidation and of our biocatalytic approach would constitute an excellent compromise to obtain epoxides 1 and 2 of (1*R*,2*S*) absolute configuration in high yields and with ees approaching 100 %. Interestingly, it has been shown recently that other fungal strain (for instance *D. gossipina* ATCC 16391 and *L. theobromae* MF 5215) similarly allowed to obtain the (1*S*,2*R*) indene oxide antipode in high enantiomeric purity[14] and that both these epoxides can be transformed further on into the corresponding cis-1,2 amino alcohol without loss of stereochemical integrity at C(2).[15]

Work is currently in progress in our laboratory in order to explore the scope and limitations of this biocatalyzed epoxide hydrolysis using other variously substituted styrene oxide derivatives.

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