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Microbiological transformations. Part 46: Preparation of enantiopure (*S*)-2-pyridyloxirane via epoxide hydrolase-catalysed kinetic resolution

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Abstract

The hydrolytic kinetic resolution (HKR) of 2-pyridyloxirane is described, using the overexpressed epoxide hydrolase from the filamentous fungus *Aspergillus niger*. This allows the preparation of the (*S*)-enantiomer of this product in enantiopure form (ee >99%), which could not be obtained using conventional chemical methods. © 2000 Published by Elsevier Science Ltd.

1. Introduction

It is currently widely recognised that enantiopure epoxides are highly valuable chiral synthons, and several chemical methods have recently been developed in order to synthesise these important building blocks, or their corresponding vicinal diols, in enantiopure form.[†,1–3] We (and others) have recently developed a methodology that enables the preparation of such targets using a biocatalysed Hydrolytic Kinetic Resolution approach (HKR) performed by a 'new' type of enzyme, i.e. microbial epoxide hydrolases (EHs) (EC 3.3.2.3).[4–9] An application of this methodology to the synthesis of enantiomerically pure bioactive compounds has recently been illustrated.[10–12] Of course, this strategy may be even more attractive, if it allows the preparation of epoxides which cannot be obtained in enantiopure form by conventional chemical techniques. This is, for instance, the case for 2-pyridyloxirane **2**, a very valuable chiral synthon,[13,14] and we describe here the preparation of this target—in nearly enantiopure form—using a resolution process catalysed by the EH from the filamentous fungus *Aspergillus niger* (Scheme 1).

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† Several examples of enantioselective epoxidation of prochiral substrates have been described recently, most of them using heavy metal based catalysts. However, the obtained products rarely show excellent ees (>98%). Only a few applications of the new chemical HKR strategy have been described up to now. For a direct comparison of such strategies on one particular compound, see for instance Ref. 3.

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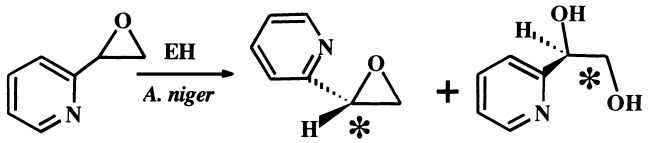

rac-2 (S)-2 (R)-3

Scheme 1. Biocatalysed HKR of *rac*-**2** using the *Aspegillus niger* epoxide hydrolase

2. Results and discussion

2.1. Chemical asymmetric oxidation

As stated above, some innovative metal-catalysed asymmetric oxidation procedures of prochiral olefins have been developed recently. However, these suffer from various intrinsic limitations and are therefore not applicable to all types of substrates. For instance, due to: (a) the fact that the olefinic precursor of **2** would be a monosubstituted (and therefore electron poor) olefin; and (b) the additional inductive electron-withdrawing power of the pyridine nitrogen atom, it can be predicted that 2-vinylpyridine **1**, the prochiral precursor of epoxide **2**, should be a bad substrate for such (electrophilic) catalysts. This was confirmed by the results we have obtained using these various chemical methodologies in order to prepare enantiomerically enriched epoxide **2** (or its corresponding vicinal diol **3**). Thus, in our hands: oxidation of **1** using the commercially available (salen)Mn-catalyst led to a very poor ee (9%) of the epoxide **2**,[15] the ADmix β catalysed dihydroxylation of **1** afforded diol **3** which showed an ee of only 79%[16] and the resolution of racemic **2** using the recently discovered HKR (salen)Co-catalyst was very slow and led, after 70 h, to epoxide **2** with an ee as low as 4%.[17]

2.2. Screening for appropriate epoxide hydrolase activity

Epoxide **2** was synthesised following the procedure described by Hanzlik et al.[18] In order to select the most suitable biocatalyst to carry out the resolution of **2**, we screened 14 enzyme extracts from our present collection of EH containing enzyme preparations, i.e. nine originating from fungal strains and five enzymes from various origins (overexpressed using a baculovirus system). Analytical scale experiments were pursued in order to determine their overall reaction profile, including their enantio- and regioselectivity. The results obtained throughout this study showed that the EH from *A. niger* (*An*EH) was the best choice to achieve the preparative scale resolution of **2**.[‡] We thus performed a more accurate determination of the parameters of this biocatalysed HKR process by using a crude enzyme extract obtained from an overexpressing fungal strain.[§,19–21]

[‡] These detailed results will be described in a separate paper.

[§] This recombinant strain has been constructed by Professor J. Visser (Wageningen Agricultural University, Section Molecular Genetics of Industrial Microorganisms, Wageningen, 6703H, The Netherlands) by cloning the epoxide hydrolase gene from the wild strain *Aspergillus niger* LCP 521 into an appropriate *Aspergillus* host (unpublished results, for the corresponding patent applications, see Ref. 19). The enzyme has been previously purified in our laboratory (see Ref. 20) and further on sequenced and cloned in an *Escherichia coli* host (see Ref. 22). The X-ray crystal structure of this enzyme has also been published recently (see Ref. 21).

2.3. Determination of the enantio- and regioselectivity

Interestingly, the *An*EH exhibited a rather high enantioselectivity towards **2**, hydrolysing preferentially the (*R*)-enantiomer and thus leading to the recovery of the slow reacting antipode (*S*)-**2**. The ee of this epoxide, as well as of the formed diol **3**, was determined using chiral GC analysis (using a chiral capillary column: Chirasil Dex CB; Chrompack). To allow this determination, **3** was first cyclised back into epoxide **2** (without change of its absolute configuration) following the procedure previously described by Golding et al.[23] The *E* value of this biohydrolysis was calculated, on the basis of both the substrate ee and the percentage of conversion, to be about 80 (at a 5 mM substrate concentration).[24] The absolute configuration of **2** and of the corresponding diol **3** were assigned on the basis of their specific rotation sign by comparison with previously described data.[25,26] Since determination of the regioselectivity is also an important factor in this type of reactions, we have also determined the $\alpha(S)/\beta(S)$ and $\alpha(R)/\beta(R)$ ratios by studying, separately, the stereochemical outcome of the enzymatic hydrolysis of enantiopure (*S*)-**2** and of racemic **2**, as described previously.[27] Both these values were about 3:97, indicating that the enzymatic attack occurred almost exclusively at the β-carbon atom for each enantiomer. As a result, biohydrolysis essentially led to *retention of configuration* for the formed diol **3**, an observation consistent with the fact that the ee of **3** was decreasing throughout the conversion of the racemic mixture down to 0% at total conversion.

A semi-preparative scale experiment was conducted using a 5 mM concentration of racemic **2** (0.61 g/L). Thus, 610 mg of **2** in 1 L pure water were treated with 990 mg of a crude enzyme extract of EH. After 97 min, the medium was extracted with chloroform. After normal work-up, this led to 260 mg (43% yield) of (*S*)-**2** whose ee was shown to be higher than 99%, and to 300 mg of (*R*)-**3** diol (43% yield) with 62% ee.

3. Conclusion

The biocatalytic hydrolytic kinetic resolution of 2-pyridyloxirane **2** was explored using 14 different epoxide hydrolases. We observed that the EH of the fungus *Aspergillus niger* GBCF 79 was a very efficient catalyst to achieve the resolution of this substrate, and (*S*)-**2** was obtained in enantiopure form (ee $>99\%$) and a 43% yield. It has to be emphasised that neither of the two enantiomers of this epoxide are accessible in good enantiomeric purity using the presently available conventional chemical procedures based on the use of heavy metal catalysts. Thus, this very simple 'green chemistry' procedure, which uses water as a reactant and solvent, currently appears to be the best and most direct way to prepare (*S*)-**2** in enantiopure form. Work is in progress in our laboratory in order to perform the scaling-up of this procedure, as well as to explore its applicability for other pyridine ring containing epoxides.

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