Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 1839–1842

TETRAHEDRON: *ASYMMETRY*

Microbiological transformations. Part 42: A two-liquid-phase preparative scale process for an epoxide hydrolase catalysed resolution of *para*-bromo-α-methyl styrene oxide. Occurrence of a surprising enantioselectivity enhancement

M. Cleij, A. Archelas and R. Furstoss *

Groupe Biocatalyse et Chimie Fine, ERS 157 associée au CNRS, Faculté des Sciences de Luminy, Case 901, 163 avenue de Luminy, 13288 Marseille Cedex 9, France

Received 7 April 1998; accepted 5 May 1998

Abstract

A two-liquid-phase process allowing for the preparative scale enantioselective resolution of 80 g/L (i.e. 0.38 mol/L) *para*-bromo-α-methyl styrene oxide is described, using an enzymatic extract from the fungus *Aspergillus niger*. The lifetime of the enzyme, and therefore the efficiency of the biohydrolysis, was considerably improved by performing this biohydrolysis at 4°C. Surprisingly, the use of this procedure led to a dramatic enhancement of the reaction enantioselectivity.

1. Introduction

Enantiopure epoxides, as well as their corresponding vicinal diols, are highly valuable chiral synthons useful for the synthesis of various biologically active molecules. One of the presently emerging approaches, allowing for the preparation of these building blocks under environmentally gentle conditions, is the use of biocatalytic methods.[1] In this context, the enantioselective hydrolysis of racemic epoxides using enzymes — i.e. epoxide hydrolases (EHs) — appears to be highly promising, in particular due to the fact that these cofactor independent biocatalysts have been proven recently to be ubiquitous in nature, and can therefore be prepared in large scale from various microorganisms.[2] We have ourselves shown that EHs from fungal origin are often able to perform such resolution processes in a very efficient and enantioselective manner.[3] However, because of the generally very limited solubility of organic substrates in the buffer solution used, only small amounts (i.e. a low concentration) of substrate can be transformed per litre of solution. One way to overcome this solubility problem is to use water soluble

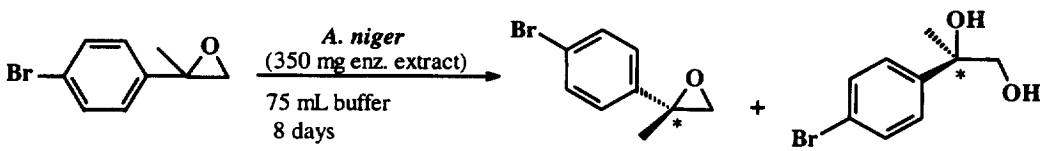
* Corresponding author. Tel: +33 04 91 82 91 55; fax: +33 04 91 82 91 45; e-mail: furstoss@lac.gulliver.fr

0957-4166/98/$19.00
PII: S0957-4166(98)00180-3

co-solvents, and we have shown that this approach can be used with a soluble EH extract from the fungus *Aspergillus niger* LCP 521 without too much loss of enzyme activity.[4] In this paper, we describe another strategy to circumvent the solubility problem — i.e. the use of a two-liquid-phase approach.[5] Very satisfactorily, this indeed allowed the 'high concentration' resolution of an aromatic epoxide substrate to be achieved efficiently. Furthermore, this experiment led us to observe a very unusual, and to the best of our knowledge unprecedented, dramatic increase of the reaction enantioselectivity due to the use of these specific experimental conditions.

2. Results and discussion

In the course of this work, we have investigated the possibility of achieving the enantioselective hydrolysis of *para*-bromo-α-methyl styrene oxide **1** using a soluble enzymatic extract of the fungus *Aspergillus niger* LCP 521 (Scheme 1). This substrate was chosen because, as a general feature, *para*-substituted α-aryl propionic acid derivatives are interesting building blocks for the synthesis of valuable drugs, such as for instance ibuprofen, one of the top-ten drugs sold (presently as a racemate) on a world wide scale.[6]



(±)-1 — 6 g

(*S*)-1 — 39 % Yld, 99.7 % ee

(*R*)-2 — 49 % Yld, 96 % ee

Scheme 1.

Preliminary analytical studies aimed at exploring the reactivity of **1** were performed at 27°C, using a substrate concentration of 8 mM (1.7 g/L) and a crude enzyme concentration of 30 mg/mL in a 0.2 M Tris/HCl buffer solution (pH 8.0). Chiral GC analysis indicated that one enantiomer of **1** was very preferentially hydrolysed. We later determined that the residual epoxide was of (*S*) absolute configuration, whereas the product formed was the (*R*)-**2** diol. Indeed, reduction of the obtained diol with sodium metal in EtOH[7] led to the corresponding α-methylphenylethanediol whose absolute configuration has been determined previously.[8] Recyclisation of (*R*)-**2** (TsCl/NaH in ether) led to (*R*)-**1**, which was used for chiral GC comparison with epoxide **1** obtained by biohydrolysis. Calculation of the apparent E value following Sih's equation[9] and using either the two ees or the ee of the substrate together with the conversion ratio, led to a similar value of about 20.[10] This indicates that the regioselectivity of the enzymatic attack is highly preferential (or even absolute) at the terminal carbon atom, which seems reasonable from the chemical point of view since, because of steric hindrance, the tertiary benzylic position should not be reactive towards a nucleophilic attack of the enzyme. Such a very high regioselectivity on similarly substituted substrates has been observed previously using ^{18}O labelled experiments.[11]

In order to set up a preparative scale process, we explored the possibility of achieving this reaction using a two-liquid-phase enzymatic reactor, where the substrate would also play the role of the organic phase. Thus, a reaction was conducted using the same experimental conditions as above, but increasing the substrate input up to 770 mM (164 mg for 1 mL of enzymatic solution). It should be emphasised that, this substrate being only scarcely soluble in water, the use of such an amount leads to the formation of a

separate organic layer. This thus allows spontaneous substrate hydrolysis to be minimised and, at the same time, to increase greatly the amount of substrate which can be resolved using a given enzymatic solution. Nevertheless, hydrolysis of **1** occurred quite satisfactorily. Very surprisingly, however, it appeared that the E value, calculated when the ee of remaining **1** reached about 95% (using both the ee of **1** and **2**), reached a value as high as 260, showing about a 13-fold increase. At the present time, we do not have any explanation for this very striking — and to the best of our knowledge unprecedented — result, which is obviously very interesting from a preparative point of view. In particular, this cannot be due to the protection provided by the biphasic system towards chemical hydrolysis, since this is quite negligible under these experimental conditions (0.6% per hour at 8 mM substrate concentration). Different hypotheses can of course be invoked, such as for instance enantioselective inhibition by the formed diol, which would be reminiscent of the results previously described by Sih et al. for lipase catalysed hydrolyses in the presence of dextromethorphan or levomethorphan[12] or by Carrea et al. for lipase catalysed syntheses in chlorinated solvents.[13]

We also decided to try to improve further this biohydrolysis by performing this reaction at low temperature instead of at room temperature. This modification was aimed at enhancing the half life of the epoxide hydrolase, which we observed to be significantly higher at 4°C as compared to room temperature. Thus, using these optimised conditions, a preparative scale experiment was conducted in a two-liquid-phase reactor on 6 g of **1**, in the presence of 350 mg enzyme extract dissolved in 75 mL buffer solution. This was stirred for 8 days at 4°C, and the residual epoxide was then extracted with pentane (4×25 mL) whereas the diol was further extracted with ether (4×30 mL). Both products were purified by flash chromatography, which led to the residual (*S*)-**1** epoxide (2.3 g; 10.8 mmol; 39% yield), and to the (*R*)-**2** diol (3.2 g; 13.8 mmol; 49% yield). Chiral GC analysis of both these products showed an ee as high as 99.7% ($[\alpha]_D^{20}=+16$ (c=1; $CHCl_3$)) and 96% ($[\alpha]_D^{20}=-11$ (c=1; $CHCl_3$)), respectively.

3. Conclusion

The results described in this work show that it is possible to achieve the very efficient resolution of *para*-bromo-α-methyl styrene oxide **1** by using a crude enzymatic extract of the fungus *A. niger*. Moreover, this biohydrolysis could be carried out using a two-liquid-phase reactor, which allowed the transformation of 6 g of substrate with 350 mg of crude enzymatic extract, obtained from the fungus *A. niger*, in 75 mL buffer solution. This opens the way to the easy and efficient use of epoxide hydrolases for straightforward preparative scale applications. Further work is in progress in our laboratory in order to explore the possibility of applying this two-liquid-phase process to other substrates, as well as to gain some further information on the intrinsic reasons governing the surprising enantioselectivity enhancement observed in the course of these experiments.

Acknowledgements

This work has been achieved in the context of the EC BIOC4-CT95-0005. Financial support to one of us (M.C.) in the frame of this contract is greatfully acknowledged.

References

1. Archelas, A.; Furstoss, R. *Ann. Rev. Microbiol.* **1997**, *51*, 491–525.
2. Archelas, A.; Furstoss, R. *Tibtech* **1998**, *16*, 108–116.
3. Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1996**, *61*, 7402–7407.
4. Pedragosa-Moreau, S.; Morisseau, C.; Baratti, J. C.; Zylber, J.; Archelas, A.; Furstoss, R. *Tetrahedron* **1997**, *53*, 9707–9714.
5. (a) Lilly, M. D. *J. Chem. Technol. Biotechnol.* **1982**, *32*, 162–169. (b) Carrea, G. *Tibtech* **1984**, *2*, 102–106.
6. (a) Wechter, W. J. *J. Clin. Pharmacol.* **1994**, *34*, 1036–1042. (b) Brune, K.; Geisslinger, G.; Menzel-Soglowek, S. *J. Clin. Pharmacol.* **1992**, *32*, 944–952.
7. Hudlicky, T.; Boros, E. E.; Boros, C. H. *Tetrahedron: Asymmetry* **1993**, *4*, 1365–1386.
8. (a) Nakajima, M.; Tomioka, K.; Iataka, Y.; Koga, K. *Tetrahedron* **1993**, *49*, 10793–10806. (b) Pedragosa-Moreau, S.; Archelas, A.; Furstoss, R. *Tetrahedron* **1996**, *52*, 4593–4606.
9. Chen, C. S.; Fujimoto, Y.; Girkaudas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
10. It must be emphasised that, as a general feature for EH catalysed resolutions, some loss of enantiomeric purity for the diol produced must be accounted to the spontaneous chemical hydrolysis of the substrate, thus leading to a measured E value lower than the 'real' (enzymatic) one. However, for this particular case, spontaneous hydrolysis was found to be rather slow, i.e. about 0.6% per hour (at 27°C). Thus, the 'real' E value must be almost identical to the one measured experimentally.
11. Mischitz, M.; Mirtl, C.; Saf, R.; Faber, K. *Tetrahedron: Asymmetry* **1996**, *7*, 2041–2046.
12. Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836–6841.
13. Secundo, F.; Ottolina, G.; Riva, S.; Carrea, G. *Tetrahedron: Asymmetry* **1997**, *13*, 2167–2173.