Pergamon

Tetrahedron Letters, Vol. 37, No. 46, pp. 8379-8382, 1996
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0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01935-1

Deracemization of (±)-*cis*-2,3-Epoxyheptane *via* Enantioconvergent Biocatalytic Hydrolysis using *Nocardia* EH1-Epoxide Hydrolase

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Abstract: Biocatalytic hydrolysis of (±)-*cis*-2,3-epoxyheptane using lyophilized bacterial cells of *Nocardia* EH1 gave (2*R*,3*R*)-heptane-2,3-diol as the sole product in 79% yield and 91% e.e. at 100% conversion. The pathway of this deracemization was elucidated by $^{18}OH_2$-labelling experiments using a partially purified epoxide hydrolase preparation and was shown to proceed in an enantioconvergent manner. Thus both substrate enantiomers were hydrolyzed *via* attack of [OH^-] at the respective (*S*)-configurated C-atom with concomitant inversion of configuration.
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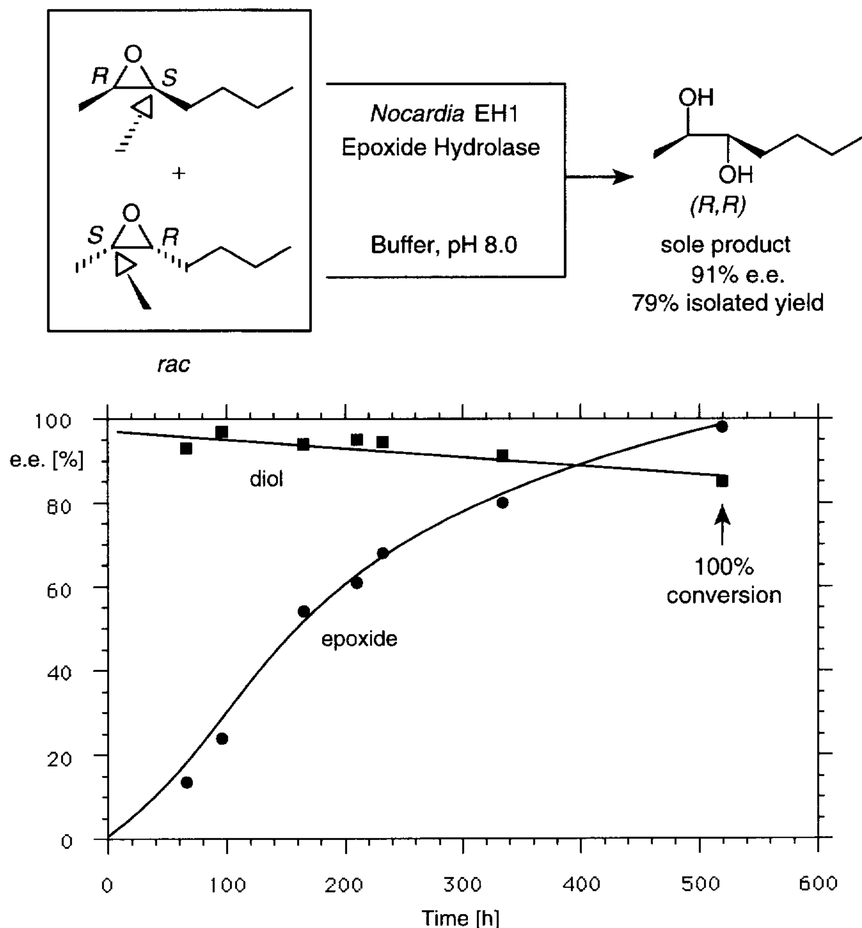
Chiral epoxides and vicinal diols (employed as their corresponding cyclic sulfite or sulfate esters as reactive intermediates) are extensively used high-value intermediates for the synthesis of chiral target compounds due to their ability to react with a broad variety of nucleophiles. Only recently, epoxide hydrolases from microbial sources such as bacteria[1] and fungi[2] were shown to be powerful chiral catalysts for the preparation of the title compounds on a preparative scale. As a rule, the enzymatic hydrolysis of racemic epoxides proceeds by following a classic kinetic resolution pattern, *i.e.* if the substrate enantiomers are hydrolyzed at different rates, optically enriched epoxide and enantiomeric 1,2-diol can be obtained in 50% theoretical yield at 50% conversion. Cases for a non-classic deracemization of epoxides, which lead to the highly desired formation of a *single product enantiomer* in 100% theoretical yield are rare. For instance, the hydrolysis of (±)-3,4-epoxytetrahydropyran[3] and several *cis*-β-alkyl substituted styrene oxides[4] by hepatic microsomal epoxide hydrolase proceeded in an enantioconvergent manner leading to the corresponding (*R*,*R*)-diols as the sole product. Similarly, soybean epoxide hydrolase converted (±)-*cis*-9,10-epoxy-12(*Z*)-octadecenoic and (±)-*cis*-12,13-epoxy-9(*Z*)-decenoic acid into the corresponding (*R*,*R*)-dihydroxy acids[5]. All of the above mentioned transformations, however, were aimed at the elucidation enzyme mechanisms and were performed only on an analytical scale. It was only recently, that preparative scale hydrolysis of (±)-*cis*-β-methylstyrene oxide by the fungus *Beauveria bassiana*[6] was reported to afford (1*R*,2*R*)-1-phenylpropane-1,2-diol in 85% yield and 98% e.e.

During our studies on the enzymatic resolution of 2,3-disubstituted epoxides using bacterial epoxide hydrolases we observed an unexpected phenomenon: Thus when (±)-*cis*-epoxyheptane was hydrolyzed using lyophilized whole cells of *Nocardia* EH1, the optical purity of the diol did not decline beyond a conversion of ~50% (which would be expected from a classic kinetic resolution) but rather remained at a constant level of ≥90% e.e. until the starting material was completely consumed[7,8] (Scheme 1).

Scheme 1. Enantioconvergent enzymatic hydrolysis of (±)-*cis*-2,3-epoxyheptene.



In order to eludicate the stereochemical pathway of the biotransformation, the reaction was performed in $^{18}OH_2$ by using a partially purified epoxide hydrolase preparation[9,10] (Scheme 2). GC/MS-Analysis[11] of the labelled diol products using a chiral stationary phase showed that (i) only one oxygen was incorporated into the substrate with (ii) inversion of configuration at the attacked carbon atom. The position of the ^{18}O-label was determined by analysis of the diol fragmentation pattern during glycol cleavage upon electron impact ionization. Thus analysis of the peaks with m/z of 87 (products C_2 and D_2) and 89 (C_3, D_3), resulting from O-incorporation at C_2 and C_3, resp., after background correction by comparison with unlabelled material, led to the ratios of C_2/D_3 and C_3/D_2. Additionally, the enantiomeric composition of epoxide (A/B) and diol (C_2+C_3/D_2+D_3) was analyzed by GC on a chiral phase (Table 1). From these data, the four first-order rate constants which determine the kinetics of the process were calculated at two independent points of the reaction[12]. As may be seen from Table 2, the values for the individual relative rate constants were within experimental error indicating the absence of enzyme deactivation and inhibition phenomena.

Scheme 2. Stereochemical course of the enantioconvergent enzymatic hydrolysis.

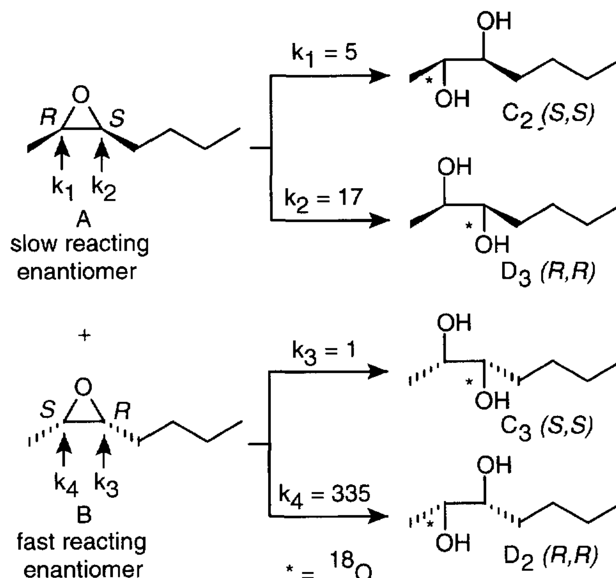


Table 1. Optical purities and label-distribution

Time [h]	e.e. [%]		Intensities (Counts)			
	epoxide	diol	C_2	C_3	D_2	D_3
22	3.3	95.9	62	10	2701	144
110	15.7	97.3	120	31	5309	308

Table 2. Relative first-order rate constants at two points of the reaction.

Time [h]	k_1	k_2	k_3	k_4
22	6	17	1	326
110	3.5	17	1	343

Analysis of the relative rate constants reveals that the (2*S*,3*R*)-epoxide (B) is the fast reacting enantiomer, which is hydrolyzed with excellent regioselectivity at C-2 (C-2/C-3 1:>300). On the contrary, the (2*R*,3*S*)-enantiomer is transformed about ten times slower and low regioselectivity in favor of the sterically more hindered C-3 atom is observed (C-3/C-2 3.4:1). From both epoxide enantiomers, the (2*R*,3*R*)-diol is predominantly formed.

The absolute configuration of products was determined as follows: Comparison of the diol by co-injection on chiral GLC with independently synthesized material[13] revealed its (2*R*,3*R*)-configuration. Reduction of optically enriched *cis*-2,3-epoxyheptane ($LiAlH_4$, Et_2O) gave a mixture of 2- and 3-heptanol. The configuration of 2-heptanol thus obtained was shown to be (*R*) via co-injection of the corresponding trifluoroacetate with a sample obtained from commercial (*R*)-2-heptanol.

Acknowledgements: The authors wish to express their cordial thanks to C. Syldatk (Stuttgart) for providing us with *Nocardia* EH1. This project was performed within the Spezialforschungsbereich Biokatalyse (SFB-A4). Financial support from the Fonds zur Förderung der wissenschaftlichen Forschung (F 104), the European Community (BIO4-CT95-0005) and the Austrian Federal Ministry of Sciences is gratefully acknowledged.

References and Notes

1. Faber, K.; Mischitz, M and Kroutil, W. *Acta Chem. Scand.* **1996**, *50*, 249.
2. Pedragosa-Moreau, S.; Archelas, A. and Furstoss, R. *Bull. Soc. Chim. Fr.* **1995**, *132*, 769.
3. Bellucci, G.; Berti, G.; Catelani, G. and Mastrorilli, E. *J. Org. Chem.* **1981**, *46*, 5148.
4. Bellucci, G.; Chiappe, C. and Cordoni, A. *Tetrahedron: Asymmetry* **1996**, *7*, 197.
5. Blée, E. and Schuber, F. *Eur. J. Biochem.* **1995**, *230*, 229.
6. Pedragosa-Moreau, S.; Archelas, A. and Furstoss, R. *Tetrahedron* **1996**, *52*, 4593.
7. The e.e.-time data of Scheme 1 were obtained from an analytical-scale experiment. No trace of spontaneous hydrolysis was observed in the absence of biocatalyst within 600 h.
8. Preparative scale reaction: *Nocardia* EH1 was grown in shake flasks at 30°C in a complex medium (yeast extract 10g/L, peptone 10g/L, glucose 10g/L, NaCl 2g/L, $MgSO_4 \cdot 7H_2O$ 0.147g/L, NaH_2PO_4 1.3g/L, K_2HPO_4 4.4g/L). After 36 h the biomass was centrifuged (5000U/min, 20 min) yielding 25-30g/L wet cells, which could be stored after lyophilization for several months without loss of activity. Lyophilized cells (4g) were rehydrated in Tris-buffer (50mL, 50mM, pH 8.0) for 1 h before addition of the epoxide (0.82g, 7.1mmol). The mixture was agitated on a rotary shaker at r.t. until the starting material was consumed (~64h). After addition of acetone (30mL), the mixture was centrifuged and the cells and the supernatant were separately extracted with ethyl acetate (3x). The combined organic layer was dried (Na_2SO_4), the volatiles evaporated and the residue was purified by silica gel chromatography to remove minor impurities which arose from the cells to furnish (*R*,*R*)-*threo*-2,3-dihydroxyheptane (yield 0.75g, 79%, e.e. 91%, $[\alpha]_D^{20}$ +20.45°, c=1, EtOH).
9. Obtained by hydrophobic interaction chromatography on Phenyl Sepharose CL-4B. Preliminary data indicate that the epoxide hydrolase from *Nocardia* EH1 is related to the enzyme from *Rhodococcus* sp. NCIMB 11216, *i. e.* a constitutive, soluble enzyme having a MW of ~35 kDa. See: Mischitz, M.; Faber, K. and Willetts, A. *Biotechnol. Lett.* **1995**, *17*, 893. Full details of the purification and characterization of the enzyme will be published in a separate paper.
10. Partially purified lyophilized enzyme (10mg) was rehydrated in $^{18}OH_2$ (97%, 120µL) at 30°C for 1 h. Then (±)-*cis*-2,3-epoxyheptane (4µL) was added and shaking was continued. Samples were extracted with ethyl acetate (120µL) analyzed by GC/MS on a Chirasil-DEX CB column (Chrompack, 25m x 0.25mm, 0.25µm film, 2.4mL/min He): 120°C iso, t_{ret}: (2*S*,3*S*)-diol 3.9min, (2*R*,3*R*)-diol 4.13min. Quadrupol-MS: EI 70eV, 130°C, auxiliary temp. 180°C.
11. For experimental details see: Mischitz, M.; Mirtl, C.; Saf, R. and Faber, K. *Tetrahedron: Asymmetry* **1996**, *7*, 2041.
12. A full treatment of the mathematical model describing enantioconvergent processes will be published in a full paper.
13. Treatment of (*S*)-ethyl lactate with *n*-butyl magnesium bromide in THF at r.t. gave (*S*)-2-hydroxy-3-heptanone (90%), which was reduced ($LiAlH_4$, Et_2O) to furnish (2*S*,3*S*)- and (2*S*,3*R*)-heptane-2,3-diol.

(*Received in Germany* 6 *September* 1996; *accepted* 26 *September* 1996)