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Asymmetric Hydrolysis of Epoxides using an Immobilized Enzyme Preparation from *Rhodococcus* sp.

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Abstract: An enzyme-catalyzed asymmetric hydrolysis of epoxides was achieved using an immobilized crude enzyme preparation derived from *Rhodococcus* sp. (NOVO SP 409). The mechanism of the reaction was shown to occur in a *trans*-specific hydrolytic opening *via* an S_N2-type of reaction. Depending on the substitutional pattern of the substrate, optically active epoxides and 1,2-diols with varying optical purities were obtained (in one case e.e. 72%).

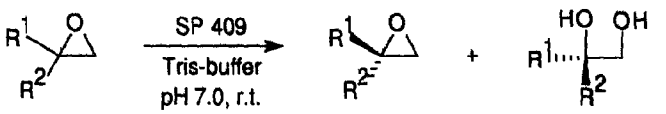
Chiral epoxides are extensively employed intermediates for the synthesis of enantiomerically pure compounds. Although several chemical methods are available for preparing them from optically active precursors, no efficient asymmetric syntheses involving asymmetrisation or resolution methods are known with exception of the Sharpless-epoxidation, which is limited to allylic alcohols[1].

For one of the existing alternatives, cofactor-independent enzymes catalysing the hydrolysis of epoxides (epoxide-hydrolases, occasionally also denoted as epoxide-hydrases [EC 3.3.2.X]) can be employed to achieve the kinetic resolution of racemic and the asymmetrisation of *meso*-epoxides[2]. The hitherto most intensively studied epoxide hydrolase system is that of mammalian liver microsomes[3]. Although high selectivities have been achieved in several cases using this type of enzymes, their applicability is impeded since they cannot be produced in sufficient amounts to make large-scale reactions feasible. Although microorganisms are known to possess epoxide hydrolases, the number of applications for preparative organic transformations is comparatively limited[2]. During a study on the enzyme-catalyzed hydrolysis of nitriles[4] using an immobilized enzyme preparation derived from *Rhodococcus* sp. (SP 409 from NOVO Industri, DK[5]) which was initially designed for the enzyme-catalyzed hydrolysis of nitriles under mild conditions, we discovered a previously unknown epoxide-hydrolase activity present in the crude

preparation. To verify that the reaction indeed proceeded under enzymatic catalysis the absence of reaction was carefully checked by control experiments (i) in the absence of enzyme and (ii) using heat-denaturated enzyme.

As depicted in scheme 1, several racemic epoxides (*rac*-**1a-7a**) were subjected to enzymatic hydrolysis[6]. Depending on the substitutional pattern of the substrate, various selectivities were observed. Low enantioselection was observed with terminal 1-substituted epoxides (*rac*-**1a-4a**), only 1-octenoxide (*S*-**3a**) was obtained with a moderate e.e. of 38%. Sterically more demanding 1-methyl-1-alkyl-epoxides were hydrolysed at a slower rate, but with better selectivities: 1-Methyl-1-pentyloxiran (*R*-**6a**) was obtained with 72% e.e. In addition to that, an interesting reversal in the stereochemical preference was observed. Whereas in case of the terminal epoxides *rac*-**3a** and *rac*-**4a** the (*R*)-epoxides were hydrolysed by leaving the (*S*)-enantiomers behind, the (*S*)-antipodes were preferably transformed in case of the branched methyl substituted derivatives *rac*-**6a** and *rac*-**7a**.

Scheme 1: Enzymatic resolution of racemic epoxides.



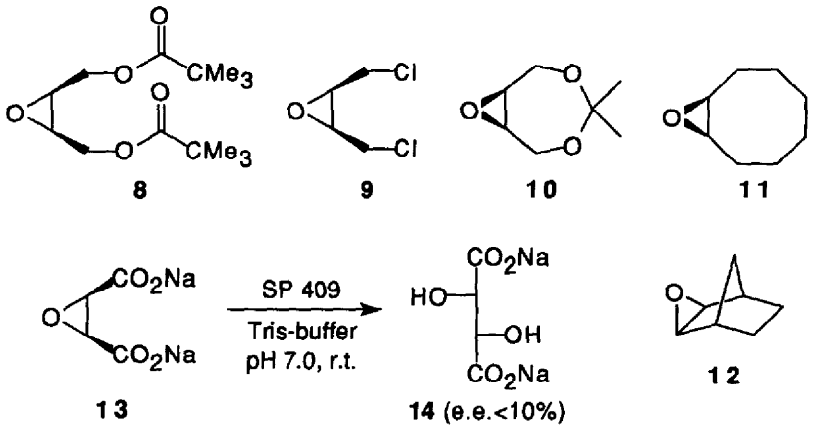
rac-**1a-7a** **1a-7a** **1b-7b**

Substrate	R^1	R^2	Epoxide (**1a-7a**)		Diol (**1b-7b**)	
			Config.	e.e. [%]	Config.	e.e. [%]
rac-**1a**	H	CH_2-O-allyl	n.d.	<5	n.d.	~5
rac-**2a**	H	$C(CH_3)_3$	n.d.	<5	n.d.	17
rac-**3a**	H	*n*-C_6H_{13}	*S*	38	*R*	22
rac-**4a**	H	*n*-$C_{14}H_{29}$	*S*	<10	*R*	<10
rac-**5a**	CH_3	CH_2-Ph	n.d.	<10	n.d.	<10
rac-**6a**	CH_3	*n*-C_5H_{11}	*R*	72	*S*	40[a]
rac-**7a**	CH_3	*n*-C_8H_{17}	*R*	23	*S*	10[a]

a) Opposite configuration as shown in scheme 1.

Quite unexpectedly, all of the *meso*-epoxides (**8-12**) tested proved to be non-substrates (Scheme 2). The only exception was disodium *cis*-2,3-epoxysuccinate **13**[7], which gave DL-tartaric acid (**14**) in low optical purity as the sole product (e.e. <10%, isolated and spectroscopically identified as its dimethyl ester). This observation (and the absence of *meso*-tartrate) proves that the reaction proceeds *via* an S_N2-type of reaction similar to the action of microsomal epoxide hydrolases[8,9].

Scheme 2: Enzymatic hydrolysis of *meso*-epoxides.



8 **9** **10** **11**

13 **14 (e.e.<10%)** **12**

The enantiomeric excess of products was determined as follows: GLC analysis using a γ-cyclodextrin column[10] was performed with compounds **2a**, **5a** and **7a**. Diols **5b** and **7b** were transformed into their corresponding acetonides (2,2-dimethoxypropane, H^+ cat., r.t.) prior to analysis. **1a**, **6a** and **6b** were analyzed on a β-cyclodextrin column[11], diols **1b** and **2b** were analyzed as the respective acetonides. Epoxides **3a** and **4a** were stereoselectively transformed into 1-methoxy-2-octanol and 1-methoxy-2-hexadecanol resp. (NaOMe cat., MeOH, Δ). The latter were analyzed by ^{1}H-NMR spectroscopy in the presence of Eu(hfc)$_3$ observing the methoxy signal. Diols **3b** and **4b** were protected as benzylidene acetals (PhCH=O, toluene, H^+ cat., Δ). ^{1}H-NMR spectroscopy in the presence of Eu(hfc)$_3$ revealed their optical purity and absolute configuration[12]. The latter results were confirmed by comparison of optical rotation values of (*R*)-**3b** ($[\alpha]_D^{20}$ +2.74 (c 2.9), EtOH, e.e. 22%) with literature data[13]. The configuration of **6a** was determined by comparison with authentic (*R*)-**6a** prepared from (*R*)-2-hydroxy-2-methylheptanoic acid (e.e. 85%) *via* the following sequence: $LiAlH_4$ reduction of the hydroxy acid gave (*R*)-**6b**, mono-tosylation involving the primary hydroxyl group furnished its corresponding 1-toluenesulfonate. The latter was finally subjected to ring closure (K_2CO_3, acetone, r.t.) to form epoxide (*R*)-**6a**. Comparison of optical rotation values of (*S*)-**6b** ($[\alpha]_D^{20}$ -1.38 (c 3.59), $CHCl_3$, 40% e.e.) with literature data[14] provided further evidence for the absolute configuration. Due to the low e.e. of **7a** an unambiguous determination of its absolute configuration was not possible, however, based on comparison of the elution order with both enantiomers of **6a** on chiral GC the configuration was assumed to be (*R*).

Summary: A crude immobilized enzyme preparation (NOVO SP 409) derived from *Rhodococcus* sp. (which was initially developed for the enzyme-catalyzed hydrolysis of nitriles) has been shown to possess also epoxide-hydrolase activity. Straight-chain terminal epoxides were well accepted as substrates but enantioselectivities were rather low. On the other hand, branched methyl-alkyl epoxides could be obtained with >70% e.e. A detailed study on the microbial asymmetric hydrolysis of epoxides is in progress in our laboratory.

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References and Notes

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5. SP 409 is a crude immobilized enzyme preparation designed for the enzymatic hydrolysis of nitriles. Besides its nitrile-hydratase and amidase it also contains ester-hydrolase(s) and epoxide-hydrolase(s).
6. General procedure: NOVO SP 409 (10g) was suspended in Tris-buffer (500mL, 0.1N, pH 7.0) for 1h. Then epoxide (1g) was added and the mixture was shaken at r.t. (180rpm). After the conversion reached ~50% (as monitored by TLC and/or GC, 2-4 days) the enzyme was filtered, washed with a small amount of buffer and the combined aqueous phase was extracted with ethyl acetate (3 x 50mL). Evaporation of the volatiles and column chromatography on silica gel gave optically enriched epoxides and diols.
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