Highly selective asymmetric hydrolysis of 2,2-disubstituted epoxides using lyophilized cells of *Rhodococcus* sp. NCIMB 11216

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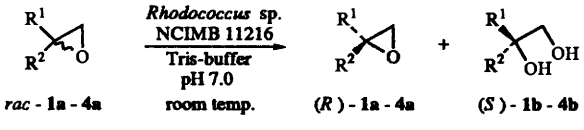
(±)-2-Methyl-2-alkyl epoxides have been resolved by biocatalytic hydrolysis employing lyophilized cells of *Rhodococcus* sp. NCIMB 11216. The reaction has been shown to proceed with retention of configuration leading to (*S*)-1,2-diols and (*R*)-epoxides with excellent selectivities (E > 100).

Chiral epoxides, extensively employed intermediates in organic synthesis because of their ability to react with a broad variety of nucleophiles have, in recent years, been the subject of much research effort directed towards the development of catalytic methods for their production.[1] Of the numerous protocols employing a variety of biocatalysts,[2,3] the use of epoxide hydrolases [EC 3.3.2.X] is particularly facile owing to the lack of co-factors. Until now, these enzymes have been predominantly isolated from mammalian systems and have been studied especially with respect to their role in the detoxification of xenobiotics.[4] Their application in biotransformations[5] on a preparative scale, however, has been impeded by lack of a sufficient supply of enzyme from (rat) liver microsomes. It was only recently, that whole microbial cells of *Aspergillus niger*[6] and *Beauveria bassiana* (formerly *B. sulfurescens*)[7] or a crude immobilized enzyme preparation derived from *Rhodococcus* sp.[8] were successfully used for the enantioselective hydrolysis of epoxides on a preparative scale.

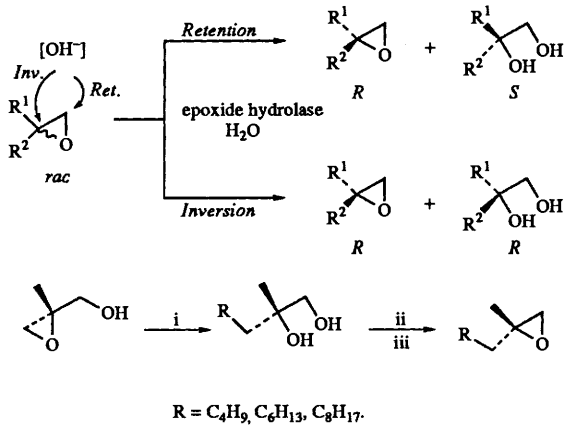
As part of our on-going search for epoxide hydrolases from microbial sources,† we discovered, during screening, that whole lyophilized cells of *Rhodococcus* sp. NCIMB 11216 can be used as a convenient and highly selective biocatalyst for the resolution of 2,2-disubstituted epoxides. In contrast to mammalian systems where, in general, epoxide hydrolases have to be induced, the epoxide hydrolase activity of *Rhodococcus* sp. NCIMB 11216 was present even when the cells were grown on a non-optimized standard medium (glucose, peptone, yeast extract). Thus, the epoxide hydrolase seems to be a constitutive enzyme.‡



	1a, b	2a, b	3a, b	4a, b
R^1	C_5H_{11}	C_5H_{11}	C_7H_{15}	C_9H_{19}
R^1	Me	Et	Me	Me

Scheme 1 Biocatalytic resolution of the epoxides **1a–4a**

The absolute configurations of the diols **1b–4b** and the residual epoxides **1a–4a** in the reaction mixtures were determined with great care for the following reason (Scheme 2). In contrast to reactions catalysed by carboxyl ester hydrolases, where the absolute configuration of the chiral centre always remains the same throughout the reaction, microbial hydrolysis of the



Scheme 2 Determination of absolute configuration. *Reagents and conditions:* i, RMgBr, THF, Li_2CuCl_4 cat., −80 °C; ii, TosCl, Py, CH_2Cl_2, room temp.; iii, K_2CO_3, acetone, H_2O.

epoxides may take place by one of two paths: that is (i) attack of the (formal) hydroxyl ion either on the less hindered oxirane carbon atom affecting *retention of configuration* or (ii) attack on the chiral centre, which leads to *inversion*.[7,9] Reference material for **1a**[8], **2a**, **3a** and **1b**, **2b**, **3b** was synthesized *via* Li_2CuCl_4-catalysed addition of the corresponding Grignard reagent onto (*R*)- or (*S*)-2-methylglycidol[10] and subsequent ring closure *via* the mono-tosylate. Absolute configurations were verified by co-injection on chiral GLC. Substrates **1a**, **3a** and **4a** were shown to be hydrolysed with retention, *i.e.* (*S*)-diols were formed while the (*R*)-epoxides remained. Substrate **2a** was not investigated because of the low selectivity of the reaction.

The determination of the selectivity of the reaction deserves a special comment. In general, the selectivity of a biocatalytic kinetic resolution of enantiomers is most conveniently expressed as the Enantiomeric Ratio (E).[11] This parameter has been primarily associated with ester hydrolyses and syntheses (catalysed by carboxyl ester hydrolases) and it is based on several requirements, such as the irreversibility of the reaction and non-transformation of the chiral centre. Although the first condition is clearly met with epoxide hydrolases being active in a bulk aqueous medium, only assumptions can be made about the involvement of the chiral centre in the reaction as long as the exact reaction mechanism remains unclear.§[12] However, using comparable sets of data (*i.e.* conversion, ee of product and substrate) obtained from experiments which were stopped at varying degrees of conversion, identical E values were

† To be published in a forthcoming full paper.
‡ Preliminary studies on the isolation of the enzyme indicate that no co-factor is involved.

§ A detailed mechanistic study on the reaction mechanism using $^{18}OH_2$ and purified enzyme is currently being carried out.

Table 1 Optical purities of products

Substrate	Conversion (%)	Epoxide		Diol		Selectivity (E)
		Configuration	ee (%)	Configuration	ee (%)	
(±)-**1a**	43	(*R*)	71	(*S*)	96	104
(±)-**2a**	26	N.d.	25	N.d.	70	7.2
(±)-**3a**	20	(*R*)	25	(*S*)	98	126
(±)-**4a**	36	(*R*)	55	(*S*)	>99	>200

N.d. = not determined.

obtained within experimental error. Furthermore, an inversion of configuration would require an S_N2 attack on a quaternary carbon atom which can be considered as highly disfavoured. These arguments suggest that the calculation of E values is valid in the present case.

Comparison of the results displayed in Table 1 show that chiral recognition of the enzyme depends mainly on the 2-alkyl substituents being of different size: thus, 2-methyl-2-pentyloxirane **1a** was resolved with high selectivity (E = 104) whilst with 2-ethyl-2-pentyloxirane, **2a**, the selectivity dropped considerably (E ~ 7). The reverse phenomenon was observed when the alkyl chain was extended in a stepwise manner leading to 2-methyl-2-nonyloxirane (substrate **4a**), which was resolved with complete specificity (E > 200).

These results demonstrate, that bacterial epoxide hydrolases are highly selective biocatalysts for the resolution of 2,2-disubstituted oxiranes and that they open a new way for the preparation of enantiomerically pure epoxides.

Experimental

Preparation of biocatalyst

Rhodococcus sp. NCIMB 11216 was grown in a 10 dm^3 fermenter at 30 °C with adequate aeration in a glucose medium [K_2HPO_4 (6 g dm^{-3}), KH_2PO_4 (3 g dm^{-3}), NaCl (0.5 g dm^{-3}), $MgSO_4$ (0.5 g dm^{-3}), $(NH_4)_2SO_4$ (1 g dm^{-3}), yeast extract (2.5 g dm^{-3}), peptone (2.5 g dm^{-3}), glucose (10 g dm^{-3}), and $CaCl_2$ (50 mg dm^{-3}); pH 7.5]. At the late exponential growth phase (~25–35 h) the pink cells were harvested by centrifugation (3000 g), resuspended in Tris buffer (0.1 mol dm^{-3}, pH 7.0), centrifuged again and lyophilized. Typical yields of dry cells were in the range 4–5 g dm^{-3}. The cells could be stored over several months at +5 °C without significant loss of activity.

General procedure for the asymmetric hydrolysis of the epoxides (±)-1a–4a

Lyophilized cells of *Rhodococcus* sp. NCIMB 11216 (2 g) were suspended in Tris buffer (pH 7.0, 0.1 mol dm^{-3}; 400 cm^3) for 30 min. The substrates (±)-**1a**–**4a** (1 g) were added to the mixture which was then agitated at room temperature while the reaction was monitored by TLC. After 8–12 h the cells were centrifuged, and the product diols **1b**–**4b** together with the residual epoxides **1a**–**4a** were extracted with ethyl acetate from the buffer medium and the cells in ~90% overall yield. The optical purity of products was analysed by chiral GLC on a β-cyclodextrin phase without derivatization (CP-Chirasil-DEX CB, 25 m, 0.32 mm, 0.25 μm film, H_2).

(*R*)-**1a**: $[\alpha]_D^{20}$ −6.35 (*c* 3.43, $CHCl_3$, 71% ee); (*S*)-**1b**: $[\alpha]_D^{20}$ −3.25 (*c* 3.6, $CHCl_3$, 96% ee), ref. 13: $[\alpha]_D^{23}$ −1.8 (*c* 0.98, $CHCl_3$, 76% ee); (*S*)-**3b**: $[\alpha]_D^{20}$ −5.0 (*c* 4.0, $CHCl_3$, 98% ee); (*R*)-**4a**: $[\alpha]_D^{20}$ −3.65 (*c* 3.6, $CHCl_3$, 55% ee), $[\alpha]_D^{20}$ −2.82 (*c* 2.3, EtOH, 55% ee), ref. 14: $[\alpha]_D$ −12.2 (*c* 0.3, EtOH, 90% ee) for (*S*)-**4a** is erroneous (after comparison of the ^1H NMR-spectra which were kindly provided by the authors); (*S*)-**4b**: $[\alpha]_D^{20}$ −4.6 (*c* 3.6, $CHCl_3$, >99% ee).

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