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Enantioselective hydrolysis of aryl, alicyclic and aliphatic epoxides by *Rhodotorula glutinis*

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Abstract: Enantioselective epoxide hydrolysis by yeasts has been demonstrated for the hydrolysis of several aryl, alicyclic and aliphatic epoxides by a strain of *Rhodotorula glutinis*. High enantioselectivity was obtained in the hydrolysis of methyl substituted aryl and aliphatic epoxides whereas selectivity towards terminal epoxides in all cases was lower. Homochiral vicinal diols were formed from several methyl substituted epoxides and also from *meso* epoxides. Kinetic resolution of *trans*-1-phenyl-1,2-epoxypropane was studied in more detail. © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

Enantiomerically pure epoxides are important chiral building blocks in organic synthesis and can be used as key intermediates in the synthesis of more complex enantiopure bioactive compounds. Therefore, a great interest exists in the development of methods for the synthesis of enantiopure epoxides. Various chemical and biological production methods have been reviewed[1,2]. Among biological production routes, enantioselective bioconversion might be a very promising method because epoxides with very high enantiomeric purities can be obtained and the enzymes involved seem to react with a homologous range of epoxides[3–5]. However, only limited information is available on the use of microbial enzymes in enantioselective bioconversion. Nevertheless, different types of reactions involved in microbial epoxide conversion are known[6]. Enantioselectivity in microbial epoxide conversion has been detected in the case of conversion of 1,2-epoxyalkanes by a *Nocardia* sp[7]. This organism and also a *Xanthobacter* sp contains an NAD-dependent enzyme which is able to resolve enantiomerically pure 2*R*-epoxyalkanes from racemic 2,3-epoxyalkanes[8].

Hydrolytic enzymes[9] may be more promising as enantioselective biocatalysts, because they are cofactor independent and because the epoxide enantiomer degraded may be recovered as enantiomerically pure diol. Enantioselectivity of epoxide hydrolases of mammalian origin (mEH and sEH) has been studied extensively with special attention to the microsomal enzyme[5,10]. However, biocatalysts from microbial sources might be more applicable on an industrial scale and therefore studies on microbial enantioselective epoxide hydrolysis have been set up recently. An enantioselective epoxide hydrolase from a bacterial source has been observed in a *Rhodococcus* sp which showed selectivity in the hydrolysis of 2,2-disubstituted epoxides[11]. Deracemization of epoxides using bacterial epoxide hydrolases has subsequently been reported for the hydrolysis of *cis*-2,3-epoxyheptane by a *Nocardia* sp[12] and of 1-methyl-1,2-epoxycyclohexane by a *Corynebacterium* sp[13]. Fungal epoxide hydrolysis has been described for *Aspergillus niger* and *Beauvaria sulfurescens*. These two strains showed different enantio- and regioselectivities in the hydrolysis of styrene oxide[14] and several other substituted styrene oxide derivatives[15,16]. Based on hypothetical active site models it was proposed that the two fungal enzymes operate with different mechanisms for oxirane ring opening. Other examples of fungal epoxide hydrolase activities have been reported for the enantioselective hydrolysis

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of indene oxide by *Diplodia gossipina*[17] and of aliphatic epoxides by *Ulocladium atrum* and *Zopfiella karachiensis*[18].

Recently, we have investigated the presence of enantioselective epoxide hydrolases in yeasts. These organisms are most promising because they are easy to cultivate which would make the production of epoxide hydrolases on a large scale more feasible. Enantioselective hydrolysis of various aryl, alicyclic and aliphatic epoxides by a strain of the yeast *Rhodotorula glutinis* has been observed.

Results and discussion

Scope of epoxide hydrolysis

Screening for epoxide hydrolase activity in yeasts was performed with styrene oxide and *trans*-1-phenyl-1,2-epoxypropane as potential substrates. These two aryl epoxides are known to be typical substrates for the microsomal (mEH), and soluble (sEH) mammalian epoxide hydrolase respectively[19,20]. We tested both substrates not knowing which type of enzyme was to be expected in yeasts. A number of yeast strains was tested for epoxide hydrolase activity and *Rhodotorula glutinis* strain CIMW 147 was eventually selected for further studies. *Rhodotorula glutinis* is able to hydrolyse both aryl epoxides tested, with relatively high activity and, in addition, the organism is very easy to cultivate. When grown on various simple carbon sources, the yeast possessed in all cases epoxide hydrolase activity which facilitates the production of large amounts of this biocatalyst. In the present study, *Rhodotorula glutinis* was routinely grown in a chemostat culture on a mineral medium supplemented with glucose as carbon source.

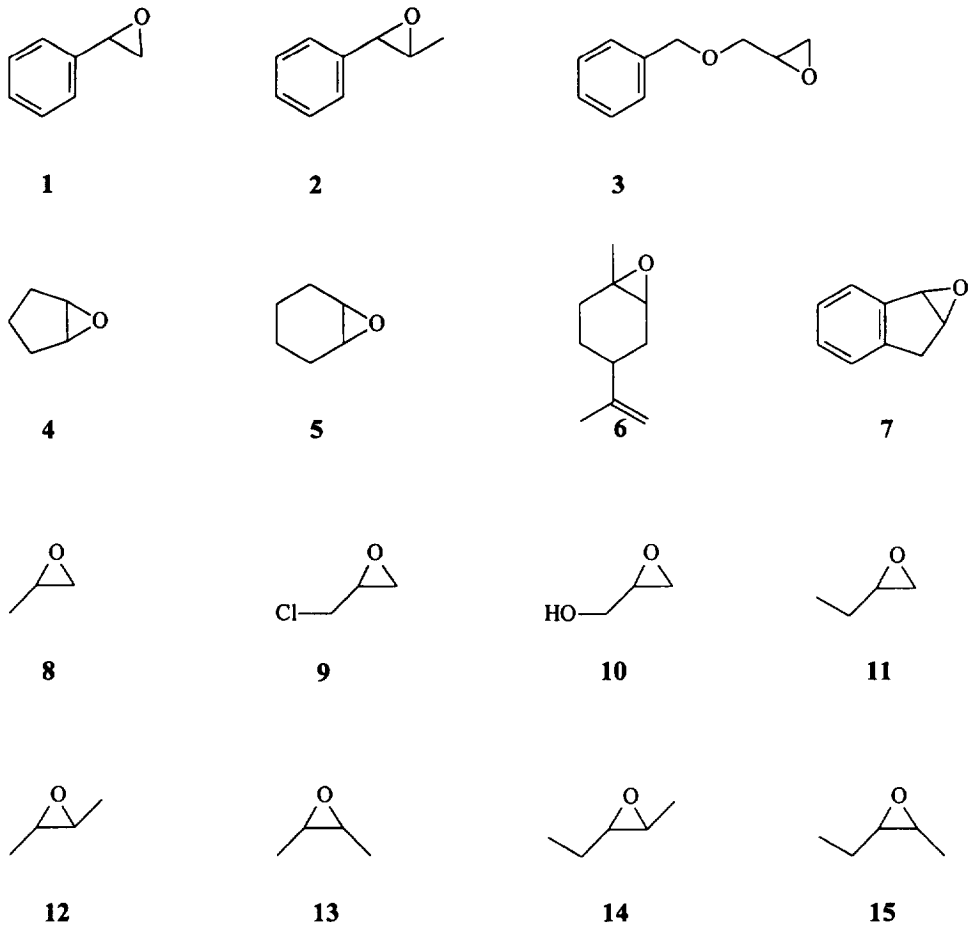
Substrate specificity and enantioselectivity of the yeast epoxide hydrolase was investigated by incubating epoxide (±)-**1** to (±)-**15** with resting cell suspensions of *Rhodotorula glutinis* (Scheme 1). The reactions were monitored by periodically taking samples and subsequent analysis by GLC using chiral columns.

In the hydrolysis of racemic epoxide **1** to **15**, the reaction was terminated when the residual epoxide reached an e.e. of more than 98%. Then the reaction time, yield and configuration of the epoxide and e.e. and configuration of the formed diol were determined. Reactions with *meso* epoxides were terminated when more than 98% conversion was reached. Initial hydrolysis rates, absolute configurations and yields of the residual epoxides **1** to **15** and e.e. values and absolute configurations of the formed diols **1a** to **15a** are summarized in Table 1. Absolute configurations of the diols were determined *via* prepared, or commercially available, compounds by co-injection on chiral GLC and, in specific cases, by comparison of the specific rotation values.

From the results of Table 1 it is concluded that the epoxide hydrolase from *Rhodotorula glutinis* has a broad substrate specificity because all tested epoxides, with the exception of epoxide **10**, are accepted by this enzyme. Nevertheless, among the accepted substrates there are significant differences in reaction rate and enantioselectivity, resulting in optically pure epoxides and diols with different yield and e.e. values respectively. Terminal epoxides were, as expected, hydrolysed with relatively low regioselectivity to (*R*) diols with *retention* of configuration at the more hindered carbon atom. In the case of methyl substituted epoxides the reaction proceeded with *inversion* of configuration at the more hindered carbon atom. Enantioselectivity in the second case was to such an extent that very high values of enantiomeric purity of the formed diol and of the yield of the residual epoxide were obtained.

Experiments for epoxide hydrolysis were routinely performed under aerobic conditions resulting in the data presented in Table 1. Under these conditions, however, it was observed that in the hydrolysis of some of the short-chain aliphatic epoxides **8** to **15** small amounts of the formed diols were subsequently oxidized to the corresponding α-hydroxy keto compounds. Fortunately, it was found that partial oxidation of vicinal diols by one or more oxidoreductases from *Rhodotorula glutinis* could be minimized under anaerobic conditions. No oxidation of the more complex diols **1a** to **7a** was detected in either reaction condition.

The aryl epoxides **1**, **2**, and **3** appeared to be very good substrates for the epoxide hydrolase of



Scheme 1. Epoxides used as substrates for epoxide hydrolysis by *Rhodotorula glutinis*.

Rhodotorula glutinis with maximum activity for benzyl glycidyl ether **3**. From the reaction rates determined for the various epoxides it can be concluded that the epoxide hydrolase has a preference for phenyl group containing substrates. Relatively high activity combined with high enantioselectivity was found for the hydrolysis of the *trans*-methyl substituted styrene oxide derivative **2**. Furthermore, biocatalytic preparation of this compound will be of interest because chemical synthesis using recently developed selective catalysts[21,22] is still less satisfactory. For these reasons kinetic resolution of epoxide **2** was eventually selected to be studied in more detail.

Alicyclic epoxides **4** to **7** were all hydrolysed to (1*R*,2*R*) diols with relatively high reaction rates. The hydrolyses of the *meso* epoxides **4** and **5** are of special interest because in these cases enantiopure *trans* diols can be obtained possibly in 100% theoretical yield. In this context, our results obtained for diol (1*R*,2*R*)-**4a** (>98% e.e. and 98% yield) and diol (1*R*,2*R*)-**5a** (90% e.e. and 93% yield) are rather encouraging. For the hydrolysis of substituted alicyclic epoxides, the (4*S*)-(−) and (4*R*)-(+) isomers of limonene oxide **6** were selected. It was observed that the position of the sidechain at the stereogenic C(4) carbon atom is of great influence on the activity and enantioselectivity. Very high selectivity was found when the alkyl substituent was in the (4*S*) configuration, leading to the single *trans*-(1*R*,2*R*,4*S*)-**6a** diol and 48% yield of epoxide (1*S*,2*R*,4*S*)-**6**. With the sidechain of epoxide **6** in the (4*R*) configuration a much lower activity and selectivity were obtained, resulting in a low yield for the residual epoxide. The diol formed was in this case a mixture of 65% (1*R*,2*R*,4*R*)-**6a** and 35%

Table 1. Hydrolysis of various epoxides by *Rhodotorula glutinis*

Epoxide (residual substrate)						Diol product		
	reaction rate [a]	e.e.	abs conf.	yield	reaction time (h)		e.e.	abs. conf.
1	6.5	> 98 %	(*S*)	18 %	0.8	**1a**	48 %	(*R*)
2	0.91	> 98 %	(1*R*,2*R*)	45 %	1.3	**2a**	> 98 %	(1*R*,2*S*)
3	12.7	> 98 %	(*R*)	14 %	0.2	**3a**	33 %	(*S*)
4	0.29	—	*meso*	< 2 %	11.0	**4a**	> 98 %	(1*R*,2*R*)
5	2.1	—	*meso*	< 2 %	1.4	**5a**	90 %	(1*R*,2*R*)
(-)-6	1.2	> 98 %	(1*S*,2*R*,4*S*)	48 %	1.2	**(-)-6a**	> 98 %	(1*R*,2*R*,4*S*)
(+)-6	0.64	> 98 %	(1*S*,2*R*,4*R*)	28 %	3.2	**(+)-6a**	30 %	(1*R*,2*R*,4*R*)
7	11.4[b]	> 98 %	(1*R*,2*S*)	22 %	0.2	**7a**	54 %	n.d.
8	0.50	> 98 %	(*S*)	15 %	3.0	**8a**	30 %	(*R*)
9	2.0	> 98 %	(*R*)	10 %	1.0	**9a**	22 %	(*S*)
10	—	no epoxide hydrolysis		—	—	**10a**	—	—
11	1.4	> 98 %	(*S*)	16 %	0.8	**11a**	25 %	(*R*)
12	0.07	> 98 %	(2*R*,3*R*)	47 %	18.3	**12a**	—[c]	*meso*
13	0.52	—	*meso*	< 2 %	13.0	**13a**	90 %	(2*R*,3*R*)
14	0.21	> 98 %	(2*R*,3*R*)	48 %	3.4	**14a**	54 %	(2*S*,3*R*)
15	1.2	> 98 %	(2*R*,3*S*)	48 %	0.6	**15a**	> 98 %	(2*R*,3*R*)

[a] Initial rate of epoxide hydrolysis in nmol/min, mg dw
[b] Under the present reaction conditions there was also significant chemical hydrolysis of epoxide 7
[c] > 98 % *cis* diol
n.d. not determined
Hydrolysis of 10 mM epoxide in 20 ml reaction mixture with glucose-grown cells of *Rhodotorula glutinis* (dry weights ranging from 1.0 to 1.5 g).

(1*S*,2*S*,4*R*)-**6a**. A similar effect of the orientation of the sidechain has been observed in the hydrolysis of limonene oxide by rabbit mEH[23]. The mammalian enzyme, however, showed a preference for the two other enantiomers of (−)- and (+)-limonene oxide. Indene oxide **7** was chosen as another example of a substituted alicyclic epoxide. Enantiomerically pure indene oxide is of commercial interest because it is a precursor to a side chain of a specific HIV protease inhibitor. In our experiments for biocatalytic resolution of epoxide **7** we observed, however, that the reaction was strongly hampered by the chemical

instability of this compound. As a result the yield of (1*R*,2*S*)-**7** as well as the e.e. of diol-**7a** were unsatisfactory. At the moment, experiments for enhancement the stability of indene oxide by use of organic solvents are in progress.

The terminal aliphatic epoxides **8**, **9**, and **11** were found to be poor substrates for the yeast epoxide hydrolase. Activity and enantioselectivity for these compounds was relatively low and was slightly increased by increasing length of the side chain. Glycidol **10** was not hydrolysed at all by the yeast epoxide hydrolase, nor at lower concentrations of 5 and 1 mM.

Activity for methyl substituted epoxides, represented by substrates **12** to **15**, was low in the case of *trans* substituted side chains and higher when the side chains were *cis* situated. Enantioselectivity was very high for the epoxides **12**, **14** and **15** resulting in residual (2*R*) epoxides with nearly the maximal feasible yields of 50%. Hydrolysis of the *meso* epoxide **13** was somewhat unsatisfactory concerning the relatively low yield of 78% of diol product **13a**. This was most probably caused by some subsequent oxidation of the diol since significant amounts of 3-hydroxy-2-butanone were detected in the final reaction mixture.

Kinetic resolution of epoxide-2

A detailed study of the resolution of epoxide (1*R*,2*R*)-**2** was performed by monitoring the course of the e.e. values of (1*R*,2*R*)-**2** in relation to the proceeding conversion in the hydrolysis of (±)-**2**. In Figure 1 it is shown that a complete kinetic resolution of (1*R*,2*R*)-**2** (e.e. >98%) is achieved at a conversion of 55% which is a rather encouraging result for a resolution process. During the initial phase of the reaction enantiomerically pure (1*R*,2*S*)-**2a** was found to be the product of the hydrolysis of (±)-**2** and was even detected slightly above 50% conversion, indicating that this diol was also formed in some very small amounts from (1*R*,2*R*)-**2**. From Figure 1 it is also obvious that at conversion values of 60% or more the decrease of e.e.'s seems to be dramatic. However, it must be considered that resolution of the initial reaction mixture takes place in about one hour whereas the final part of the reaction is still not completed after more than 40 hours. In this period, chemical hydrolysis of epoxides may be significant. Formation of other diol enantiomers was investigated by monitoring the chemical hydrolysis in the final part of the reaction. For this, the yeast cells were separated from the reaction mixture after complete hydrolysis of (1*S*,2*S*)-**2** after which the incubation of the reaction mixture was continued without cells. In Figure 1 it is shown that the loss of enantiomeric purity of (1*R*,2*S*)-**2a** in the reaction mixture is mainly caused by formation of the other diol enantiomers by chemical hydrolysis of the residual (1*R*,2*R*)-**2**.

Enantioselective hydrolysis of *trans*-1-phenyl-1,2-epoxypropane has been studied before with epoxide hydrolases from other sources. In a study on the substrate enantioselectivity of the rabbit microsomal epoxide hydrolase (mEH) only a low selectivity in favour of epoxide (1*S*,2*S*)-**2** has been observed, resulting in epoxide (1*R*,2*R*)-**2** and diol (1*S*,2*R*)-**2a** both with low enantiomeric purity and low yield[20]. Better results were obtained with an epoxide hydrolase from the fungus *Beauvaria sulfurescens*. It has been reported that with this organism resolution of (±)-**2** is possible resulting in epoxide (1*R*,2*R*)-**2** (e.e. 98%, yield 30%) and diol (1*R*,2*S*)-**2a** (e.e. 90%, yield 38%)[15]. The results from the present study indicate the yeast *Rhodotorula glutinis* performs even better than this fungus.

Epoxide hydrolases, in some cases showing high enantioselectivity, have been reported from mammalian sources[4,5,9], plants[24] and from fungal[15,17,18] and bacterial[11–13] microorganisms. The pool of sources of these useful biocatalytic enzymes has now been extended with the yeast *Rhodotorula glutinis*. By further research on the yeast enzyme and other epoxide hydrolases a better selection for a specific enzyme, based on characteristics of both enzyme as well as of source organism, will be made possible.

Conclusions

Enantioselective epoxide hydrolysis by yeasts has been first demonstrated for the hydrolysis of several aryl, alicyclic and aliphatic epoxides by cells of *Rhodotorula glutinis*. By use of this

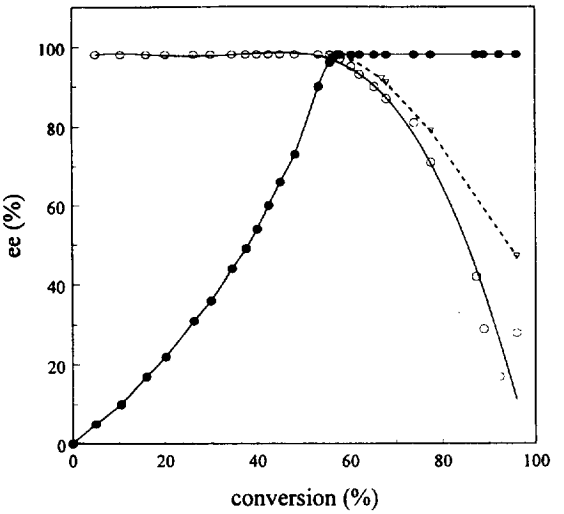


Figure 1. Resolution of 10 mM *trans*-1-phenyl-1,2-epoxypropane by *Rhodotorula glutinis* (2.3 g weight of dry cells in 50 ml). (●) Epoxide (1*R*,2*R*)-**2**; (○) diol (1*R*,2*S*)-**2a**; (▽) diol (1*R*,2*S*)-**2a** during chemical hydrolysis of residual (1*R*,2*R*)-**2** (dotted line).

biocatalyst enantiomerically pure forms of methyl substituted aryl and aliphatic epoxides as well as the corresponding diols can be prepared. Diol formation was also promising in the hydrolysis of (−)-limonene oxide and of alicyclic *meso* epoxides. At the moment, experiments for enhancement of the hydrolase activity and isolation and characterisation of the epoxide hydrolase from *Rhodotorula glutinis* are in progress.

Experimental section

General

Gas chromatography (GLC) was performed on Chrompack CP9000 and Hewlett–Packard 6890 gaschromatographs equipped with FID detectors and using N_2 as carrier gas. Determination of the enantiomeric excesses was performed by GLC using fused silica cyclodextrin capillary columns (30 m length, 0.25 mm ID and 0.25 μm film thickness). For epoxide **1**, **2**, **6** and **7** an α-DEX 120 column (Supelco Inc.) was used at an oven temperature of 85°C, 90°C, 80°C and 110°C respectively. Enantiomer analysis of epoxide **3** and **10** was done at a β-DEX 120 column (Supelco Inc.) at oven temperatures of 110°C and 60°C. Complexation gas chromatography[25,3] with a Chirametal-27-*R*-3-1-13 column was used at an oven temperature of 55°C to determine the enantiomeric composition of epoxide **8**, **9** and **11** to **15**. Chiral GLC analysis for diol **3a** and **7a** was performed on a β-DEX 120 column at an oven temperature of 145°C after derivatisation into their acetonides. Enantiomer analysis of the other diol compounds was carried out by direct analysis on a β-DEX 120 column at oven temperatures ranging from 70°C to 145°C. Quantification of epoxides and diols was by GLC analysis using the respective capillary columns and conditions as for determination of e.e.'s. The concentrations of epoxides and diols were derived from calibration curves. Mass spectra of diol compounds were recorded on a Hewlett–Packard 5970 mass selective detector coupled to an HP 5890 gas chromatograph and were in accordance with the proposed structures. Optical rotation values were measured on a Perkin–Elmer 241 polarimeter at 589 nm.

Epoxides 1 to 15

The commercially available racemic and *meso* substrates cyclopentene oxide **4**, cyclohexene oxide **5**, *cis*/*trans*-(4*S*)-limonene oxide **6**, (±)-1,2-epoxybutane **11**, (±)-*trans*-2,3-epoxybutane **12** and *cis*-2,3-epoxybutane **13** were all obtained from Aldrich Chemie. Several of the other epoxides were

available in enantiomeric pure form as well: (1*R*,2*R*)- and (1*S*,2*S*)-*trans*-1-phenyl-1,2-epoxypropane **2** (Aldrich), (*S*)- and (*R*)-benzyl glycidyl ether **3** (Fluka), (1*R*,2*S*,4*R*)- and (1*S*,2*R*,4*R*)-limonene oxide **6** (Fluka), (*R*)- and (*S*)-1,2-epoxypropane **8** (Fluka), (*S*)- and (*R*)-epichlorohydrin **9** (Fluka) and (*R*)- and (*S*)-glycidol **10** (Aldrich).

(±)-Indene oxide **7** was synthesized by cyclisation of the indene bromohydrin[26]. To 75 ml 13 N KOH solution 12.5 g *trans*-2-bromo-1-indanol (Aldrich) was added. After stirring for 1 hour at room temperature, the reaction mixture was extracted three times with ethylacetate. The ethylacetate extracts were combined, washed with water and dried over $MgSO_4$. Evaporation under reduced pressure yielded 7.2 g of a white solid of epoxide **7**. The structure of epoxide **7** was characterized by GC/MS analysis.

(±)-*trans*-2,3-Epoxypentane **14** and (±)-*cis*-2,3-epoxypentane **15** were prepared by direct epoxidation of the corresponding *trans*- and *cis*-2-pentene using *m*-chloroperoxybenzoic acid (MCPBA) as described in our previous studies[3,8].

Reference compounds 1a to 15a

For identification and determination of the absolute configurations and yields of the formed diols from epoxides **1** to **15**, the corresponding reference diol compounds were purchased or prepared. Commercially available were the enantiomerically pure forms of diol **1a** (Fluka), **3a** (Fluka), **4a** (Fluka), **5a** (Fluka), **8a** (Fluka), (*R*)-**9a** (Fluka), **10a** (Aldrich), **11a** (Aldrich), **12a** (Aldrich) and **13a** (Fluka).

A reference compound of optically pure (1*R*,2*S*)-**2a** was prepared according to the reported method of microbial asymmetric reduction of 1-phenyl-1,2-propanedione with *Saccharomyces cerevisiae*[27]. By using this method with *Saccharomyces cerevisiae* CBS 1394 (from Centraalbureau voor Schimmelcultures, Yeast Division, The Netherlands), 38 mg (1*R*,2*S*)-**2a** was obtained from 70 mg 1-phenyl-1,2-propanedione (Fluka Chemie). The structure of the reference compound (1*R*,2*S*)-**2a** was confirmed by GC/MS analysis and the enantiomeric excess and specific optical rotation were determined as 90% e.e. and $[\alpha]^{24}_D = -36.3$ (c=1.9, $CHCl_3$) respectively. Chiral GLC analysis with a β-DEX 120 column at 145°C resulted in a major peak at R_t=33.8 min (95%) and a minor peak at R_t=30.8 min (5%).

trans-8-*p*-Menthene-1,2-diols **6a** were prepared by hydrolysis of the corresponding epoxides[28]. For this, a solution of 150 mg epoxide in 10 ml of 0.1 M $HClO_4$ in 1:9 (v/v) dioxane–water was stirred for 5 minutes at room temperature. After addition of 2 ml water, the diols were extracted twice with ethylacetate. The ethylacetate extracts were combined, washed with 2 ml of water, and dried over $MgSO_4$. Removal of the solvent yielded about 90% diol compound. In this way, (1*R*,2*R*,4*R*)-**6a** was prepared from (1*R*,2*S*,4*R*)-**6** and (1*S*,2*S*,4*R*)-**6a** was the minor hydration product of (1*S*,2*R*,4*R*)-**6** respectively. Diol (1*R*,2*R*,4*S*)-**6a** was prepared from (1*R*,2*S*,4*S*)-**6**.

(±)-*trans*-Indan-1,2-diol **7a** was prepared by a reported method[29] from indene oxide **7**. A solution of 1.0 g epoxide **7** in 100 ml 2 N KOH was heated to 102°C for 4 hours. Subsequently, the reaction mixture was saturated with NaCl, extracted with ethylacetate, dried over $MgSO_4$ and evaporated under reduced pressure yielding 0.43 g of compound **7a**.

Acid catalysed epoxide hydrolysis was used in the synthesis of *erythro* (±)-2,3-pentanediol **14a** from *trans* **14** and *threo* (±)-2,3-pentanediol **15a** from *cis* **15** respectively. After derivatisation of the diols into their acetonides, determination of the absolute configurations was performed by complexation gas chromatography[30] using a Ni(II)-*bis*-(3-heptafluorobutyryl-(1*R*,2*S*)-pinan-4-onate column at 75°C. The obtained peak elution orders were used for correlation to those obtained after direct analysis on a β-DEX 120 column at 75°C.

Derivatisation of diols into their acetonides

Samples of 1.0 ml of diol-containing reaction mixture or 10 mM reference solution, were saturated with NaCl and subsequently extracted with an equal amount of 2,2-dimethoxypropane. To 0.5 ml of the organic phase, 200 mg Amberlite IR-120 H^+ (BDH, UK) was added and the mixture was stirred for an hour at room temperature. After neutralization with approximately 200 mg $NaHCO_3$, the mixture

was extracted with 1.0 ml ethylacetate and dried over $MgSO_4$. Analysis of the prepared acetonides was by chiral GLC.

Absolute configuration of diol **2a**

Absolute configurations were determined of the diols formed from hydrolysis of 0.50 mmol of epoxide (±)-**2**, (1*R*,2*R*)-**2** and (1*S*,2*S*)-**2** by *Rhodotorula glutinis* cells. Experiments for epoxide hydrolysis were carried out as described in this paper. By monitoring the duplicate reaction bottle, the moment for terminating the reaction of each epoxide was determined. The reactions were stopped by removal the yeast cells by centrifugation (20,000 *g*, 10 minutes). The supernatants were saturated with NaCl and subsequently extracted twice with an equal volume of ethylacetate containing decane as an internal standard. GLC analysis was performed for determination the yields and e.e. values of the formed diols.

The combined organic layers from the respective reaction mixtures were dried over $MgSO_4$ and evaporated under reduced pressure to give each an oily residue. For measurement of the specific optical rotation values the diols were redissolved in $CHCl_3$. Data of chiral GLC analysis (R_t's on β-DEX 120 column at 145°C) and specific optical rotation values are:

diol **2a** from (±)-**2**: R_t=33.8 min; $[\alpha]^{24}_D$=−31.6 (c=1.2, $CHCl_3$)

diol **2a** from (1*R*,2*R*)-**2**: R_t=30.8 and 32.7 min; $[\alpha]^{24}_D$=+8.8 (c=0.16, $CHCl_3$)

diol **2a** from (1*S*,2*S*)-**2**: R_t=33.8 min; $[\alpha]^{24}_D$=−32.8 (c=0.6, $CHCl_3$).

Determination of the absolute configurations was by comparison of the results with data from literature[27,31] and from the prepared reference compound of (1*R*,2*S*)-**2a**.

Growth conditions for Rhodotorula glutinis

The yeast *Rhodotorula glutinis* strain CIMW 147 was obtained from our own laboratory culture collection. A mineral medium supplemented with 0.2% ($^w/_v$) yeast extract and 1% ($^w/_v$) glucose was used for cultivation. In the present study, *R. glutinis* was routinely grown in a chemostat culture under aerobic conditions in a 2 l fermentor (with 1 l working volume) at 30°C, with a dilution rate of 0.15 h^{-1}. The pH of the culture was maintained at 6.0. The cells were harvested by centrifugation at 16,000 *g*, washed twice with 50 mM potassium phosphate buffer pH 7.5, concentrated, and stored at −20°C.

Epoxide hydrolysis by Rhodotorula glutinis

Hydrolysis of epoxides was performed in 100 ml screw-capped bottles sealed with rubber septa. The bottles contained 10 ml concentrated washed cell suspension of *Rhodotorula glutinis* (1.0 to 1.5 g dry weight) and 50 mM potassium phosphate buffer pH 7.5 to a total volume of 20 ml. The bottles were placed into a shaking waterbath at 35°C and the reaction was started by addition of 0.20 mmol epoxide. The course of the epoxide hydrolysis was followed by periodically taking samples from the reaction mixture, centrifuging for 4 minutes at 15.000 *g*, extracting 0.5 ml supernatants with 1.0 ml ethylacetate and analysis by chiral GLC. Diols were analysed similarly, however, extraction with ethylacetate was after saturation of the supernatants with NaCl. Chiral GLC of epoxide **8**, **9**, **11** and **12** to **15** was performed by analysis of headspace samples taken from the reaction mixture[3]. Initial reaction rates were determined from the epoxide disappearance and correlated to the dry weight of the used yeast suspension. In general, reactions were terminated when the residual epoxides reached e.e.'s of more than 98%.

The detailed kinetic resolution of (±)-**2** was performed in 250 ml screw-capped bottles with 25 ml concentrated washed cells, 0.50 mmol epoxide and phosphate buffer to a total volume of 50 ml. Chemical hydrolysis of residual epoxide from (±)-**2** was tested by removal of the yeast cells by centrifugation (20,000 *g*, 10 minutes) from the reaction mixture after complete hydrolysis of (1*S*,2*S*)-**2**. Subsequently, the obtained clear supernatant was further incubated at 35°C. Chemical hydrolysis was determined by monitoring the diol formation and epoxide conversion in the supernatant without cells. Eventually, four enantiomers of diol **2a** were detected in the final part of the reaction. There was no significant difference in the relative ratio of the four enantiomers in the final reaction mixtures with

and without cells (4:9:21:66, ratio of peaks with ascending elution order by chiral GLC). A detailed course of the kinetic resolution of (±)-**2** is shown in Figure 1.

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