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# Enantioselective hydrolysis of unbranched aliphatic 1,2-epoxides by *Rhodotorula glutinis*

C. A. G. M. Weijers,<sup>a,\*</sup> A. L. Botes,<sup>b</sup> M. S. van Dyk<sup>b</sup> and J. A. M. de Bont<sup>a</sup>

<sup>a</sup>Division of Industrial Microbiology, Department of Food Technology and Nutrition Sciences, Wageningen Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands

<sup>b</sup>Department of Microbiology and Biochemistry, University of the Orange Free State, PO Box 339, Bloemfontein, South Africa

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## Abstract

Epoxide hydrolase catalysed resolution of aliphatic terminal epoxides has been demonstrated for the hydrolysis of a homologous range of unbranched 1,2-epoxyalkanes by the yeast *Rhodotorula glutinis*. Both enantioselectivity and reaction rate were strongly influenced by the chain length of the epoxide used. Enantioselectivity showed an optimum in the hydrolysis of 1,2-epoxyhexane (E=84). Resolution of ( $\pm$ )-1,2-epoxyhexane resulted in (*S*)-1,2-epoxyhexane (e.e.>98%, yield=48%) and (*R*)-1,2-hexanediol (e.e.=83%, yield=47%). © 1998 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

A great interest exists in the development of methods for the synthesis of enantiomerically pure epoxides, because they are important chiral building blocks in the preparation of more complex enantiopure bioactive compounds. Various chemical and biological production methods have been reviewed.<sup>1,2</sup> In biological production methods, special attention has been given to terminal aliphatic epoxides because of their relatively high chemical stability in water-containing reaction mixtures. Furthermore, the absence of reactive side groups other than the oxirane ring, in most cases excludes undesired side-reactions while using crude enzyme preparations or whole cell biocatalysts.

Most studies on the biological production of enantiopure aliphatic epoxides involved direct epoxidation of alkenes by mono-oxygenase containing bacterial cells.<sup>3–5</sup> In all cases, aliphatic 1,2-epoxides were produced either as a racemic mixture or with the (*R*)-configuration in excess. The method, however, was problematic due to product toxicity.<sup>6</sup> Resolution of several aliphatic 1,2-epoxides has been observed

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\* Corresponding author. E-mail: Carel.Weijers@algemeen.im.wau.nl

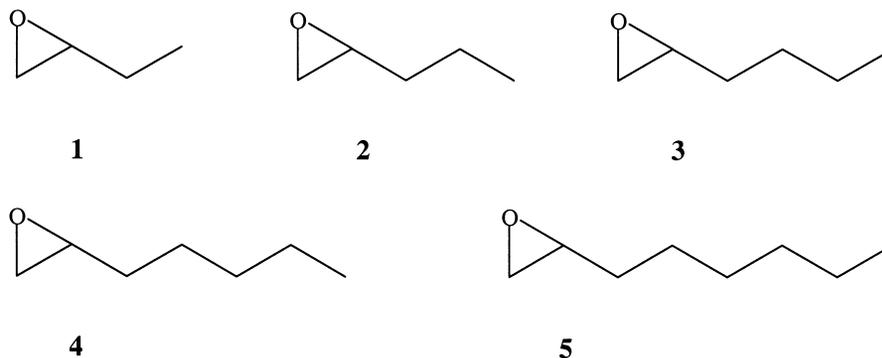
in a previous study for non-hydrolytic epoxide conversions by *Nocardia* H8.<sup>7</sup> However, because of insufficient enantioselectivities, the yields of the obtained enantiopure (*S*)-epoxides were low.

Epoxide hydrolase catalysed hydrolysis of aliphatic 1,2-epoxides has been studied with biocatalysts from different sources. Mammalian microsomal epoxide hydrolase (mEH) was used for the hydrolysis of 1,2-epoxyhexane and 1,2-epoxydecane.<sup>8</sup> However, very low enantioselectivities were observed and the formed diols were of low enantiomeric purity, even at very low conversions. Fungal epoxide hydrolase activities towards aliphatic 1,2-epoxides have been reported for the hydrolysis of C<sub>6</sub> to C<sub>14</sub> 1,2-epoxyalkanes by crude cell extracts from *Ulocladium atrum* and *Zopfiella karachiensis*.<sup>9</sup> Activities were at a maximum for C<sub>8</sub> and C<sub>10</sub> epoxides but, enantioselectivities were not given. Bacterial epoxide hydrolase activities were tested in a study for the resolution of 1,2-epoxyoctane with cell suspensions of the genera *Rhodococcus*, *Nocardia* and *Mycobacterium*.<sup>10</sup> From this screening it was concluded that the enantioselectivities of all biocatalysts tested were very low for the hydrolysis of this substrate (E<6).

Recently, we have investigated the presence of enantioselective epoxide hydrolases in yeasts. Enantioselective hydrolysis of various aryl, alicyclic and aliphatic epoxides by a strain of the yeast *Rhodotorula glutinis* has been demonstrated.<sup>11</sup> We have now extended our study by investigating substrate specificities and enantioselectivities for a homologous range of C<sub>4</sub> to C<sub>8</sub> aliphatic 1,2-epoxides.

## 2. Results and discussion

Substrate specificity and enantioselectivity of the yeast epoxide hydrolase towards aliphatic 1,2-epoxides were investigated by incubating epoxides (±)-**1** to (±)-**5** with resting cell suspensions of glucose-grown *Rhodotorula glutinis* (Scheme 1). The reactions were monitored by periodic sampling and followed by GLC analysis using chiral columns. The present study was initiated by optimizing the reaction conditions for epoxide **1**. In comparison with experiments performed in our previous study,<sup>11</sup> we have now raised the substrate concentration to a final concentration of 20 mM and lowered the biocatalyst concentration to a maximum of 0.5 grams dry weight per 10 ml of reaction mixture. Under these conditions the reaction rate as well as the enantioselectivity for epoxide **1** were improved (Table 1). The optimized reaction conditions were used in subsequent experiments for the hydrolysis of epoxides (±)-**2** to (±)-**5**.



Scheme 1. Aliphatic 1,2-epoxides used as substrates for hydrolysis by *Rhodotorula glutinis*

In the hydrolysis of racemic epoxides **1** to **5**, the reaction was terminated when the residual epoxide reached an e.e. of more than 98%. The reaction time, yield of the epoxide, e.e. and yield of the formed diol were then determined. Initial hydrolysis rates, absolute configurations, e.e. values and yields of the residual epoxides and of the formed diols are summarized in Table 1. Absolute configurations of the

Table 1  
Hydrolysis of linear-chain aliphatic 1,2-epoxides by *Rhodotorula glutinis*

Epoxide (residual substrate)						Diol product			
	reaction rate <sup>a</sup>	e.e.	abs. conf.	yield	reaction time (h)		e.e.	abs. conf.	yield
<b>1</b>	2.3	> 98 %	(S)	21 %	3.0	<b>1a</b>	29 %	(R)	78 %
<b>2</b>	7.2	> 98 %	(S)	40 %	0.7	<b>2a</b>	66 %	(R)	54 %
<b>3</b>	50.8	> 98 %	(S)	48 %	0.4	<b>3a</b>	83 %	(R)	47 %
<b>4</b>	106.1	> 98 %	(S)	44 %	0.3	<b>4a</b>	73 %	(R)	52 %
<b>5</b>	85.2	> 98 %	(S)	38 %	0.3	<b>5a</b>	55 %	(R)	60 %

<sup>a</sup>) Initial rate of epoxide hydrolysis in nmol/min, mg dw  
Hydrolysis of 20 mM epoxide in 10 ml reaction mixture with glucose-grown cells of *Rhodotorula glutinis* (dry weights ranging from 0.1 to 0.5 g).

epoxides and diols were determined by co-injection of commercially available reference compounds on chiral GLC and by comparison of the specific rotation values with data from the literature.

From the results summarized in Table 1 it is evident that the epoxide hydrolase from *Rhodotorula glutinis* has a preference for substrates with a chain length of six carbon atoms and more. The reaction rates for epoxides **3**, **4** and **5** are very much higher than those for epoxides **1** and **2**. The high enantioselectivity observed for hydrolysis of epoxide (±)-**4** and, in particular, for epoxide (±)-**3** is remarkable. Resolution of unbranched aliphatic terminal epoxides with moderate or high selectivities has not been reported for epoxide hydrolases from other sources.

All tested epoxides were hydrolysed to (*R*)-diols with *retention* of configuration at the more hindered carbon atom. The enantiomeric purities of the diols, obtained after complete resolution of the corresponding epoxides, were in all cases low. Enantiopure diols could only be obtained from the corresponding epoxides at low conversions.

For determination of the enantiomeric ratio *E*,  $\ln [R_0]/[R]$  versus  $\ln [S_0]/[S]$  was plotted. The slope of this curve represents the *E* value. The concentrations of the (*R*)-enantiomer at time 0 and *t* are represented by  $[R_0]$  and  $[R]$ , while  $[S_0]$  and  $[S]$  are the concentrations of the (*S*)-enantiomer at time 0 and *t*, respectively. Concentrations at time *t* were determined from samples taken at two minute intervals, during the course of the reaction. This method for determining *E* has previously been used in a study on the hydrolysis of *p*-nitrostyrene oxide by an epoxide hydrolase preparation from *Aspergillus niger*.<sup>12</sup> The influence of the substrate chain length on the enantiomeric ratio *E* in the resolution of epoxides (±)-**1** to (±)-**5** by *Rhodotorula glutinis*, is shown in Fig. 1. In other relevant studies on the hydrolysis of aliphatic terminal epoxides, it was found that enantioselectivities were only significantly higher when substrates with branched alkyl chains were used.<sup>8–10</sup>

In order to investigate the effect of increased substrate concentration on the biocatalyst, we raised the concentration of epoxide (±)-**3**. In this experiment 500 mg epoxide (±)-**3** was added to 1200 mg (dry weight) cells of *Rhodotorula glutinis* in a total volume of 10 ml. Such a substrate concentration, calculated to be theoretically 500 mM, exceeds the maximum solubility of epoxide-**3**. Under these

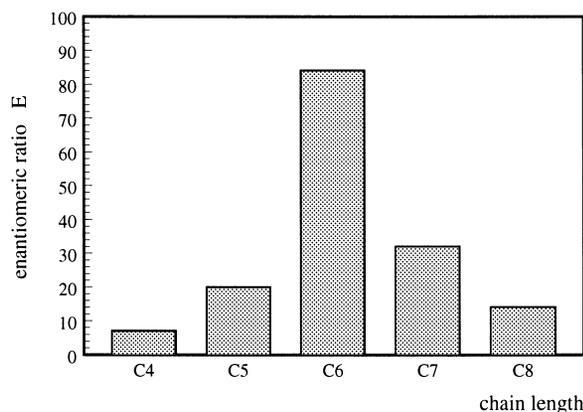


Fig. 1. Effect of the substrate chain length on the enantioselectivity in the hydrolysis of a range of unbranched aliphatic 1,2-epoxides by *Rhodotorula glutinis*

conditions, substantial amounts of epoxide will be separated from the aqueous phase during the reaction. Chemical hydrolysis of the epoxide will be minimized and larger quantities of substrate can thus be resolved. This high epoxide concentration had no significant adverse effect on the biocatalyst since there was only about a 10% decrease in reaction rate ( $r=45$  nmol/min, mg dw), compared with the rate for 20 mM epoxide ( $\pm$ )-**3**. The use of high substrate concentrations will greatly facilitate the application of this method on a preparative scale.

### 3. Conclusions

Epoxide hydrolysis with moderate to high enantioselectivities has, for the first time, been demonstrated for the resolution of a homologous range of unbranched aliphatic 1,2-epoxides by cells of the yeast *Rhodotorula glutinis*. (*R*)-Epoxides were preferentially hydrolysed to (*R*)-diols with retention of configuration at the more hindered carbon atom. Reaction rates as well as enantioselectivities were strongly influenced by the chain length of the epoxide used. High enantioselectivity ( $E=84$ ) in combination with relatively high activity was observed in the resolution of ( $\pm$ )-1,2-epoxyhexane.

### 4. Experimental section

#### 4.1. General

Gas chromatography (GLC) was performed on Chrompack CP9000 and Hewlett–Packard 6890 gas chromatographs equipped with FID detectors and using  $N_2$  as a 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  $\mu$ m film thickness). For epoxides **1** and **2** a  $\beta$ -DEX 225 column (Supelco Inc.) was used at oven temperatures of 50°C and 55°C, respectively. Enantiomer analysis of epoxides **3**, **4** and **5** was done on a  $\beta$ -DEX 120 column (Supelco Inc.) at oven temperatures of 45°C, 45°C and 55°C respectively. Chiral GLC analysis for diols **1a**, **2a**, **3a**, **4a** and **5a** was performed on a  $\beta$ -DEX 120 column at oven temperatures of 90°C, 100°C, 125°C, 125°C and 130°C. Concentrations of epoxides and diols were derived from calibration curves using heat-killed cells of *Rhodotorula glutinis*. Optical rotation

values were measured on a Perkin–Elmer 241 polarimeter at 589 nm.  $^1\text{H}$  NMR spectra were recorded on a Bruker 300 MHz spectrometer.

#### 4.2. Epoxides **1** to **5**

The commercially available racemic substrates ( $\pm$ )-1,2-epoxybutane **1**, ( $\pm$ )-1,2-epoxypentane **2**, ( $\pm$ )-1,2-epoxyhexane **3**, and ( $\pm$ )-1,2-epoxyoctane **5** were all obtained from Aldrich Chemie. Epoxide **5** was available from Fluka in enantiomeric pure (*R*) and (*S*) form as well.

( $\pm$ )-1,2-Epoxyheptane **4** was synthesized by direct epoxidation of the corresponding 1-heptene using *m*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane at 0°C. The structure of epoxide **4** was characterized by GC/MS analysis *m/z* 85 (12,  $\text{M}^+ - \text{CHO}$ ), 71 (100,  $\text{CH}_3(\text{CH}_2)_4^+$ ), 55 (43,  $\text{CH}_3(\text{CH}_2)_2^+$ ).  $^1\text{H}$  NMR of ( $\pm$ )-**4** in  $\text{CDCl}_3$ :  $\delta_{\text{H}}$  0.91 (3H, t,  $J=7$  Hz,  $\text{CH}_3$ ), 1.28–1.68 (8H, m,  $-(\text{CH}_2)_4-$ ), 2.48 (1H, dd,  $J=5.1$  and 2.8 Hz,  $\text{CH}_2(\text{O})\text{CH}-$ ), 2.76 (1H, dd,  $J=5.1$  and 4 Hz,  $\text{CH}_2(\text{O})\text{CH}$ ), 2.90–2.95 (1H, m,  $\text{CH}_2(\text{O})\text{CH}-$ ).

#### 4.3. Reference compounds **1a** to **5a**

For identification and determination of the yields of the formed diols from epoxides **1** to **5**, the corresponding racemic reference diol compounds **1a**, **2a**, **3a** and **5a** were purchased from Aldrich Chemie. Acid catalysed hydrolysis was used to prepare ( $\pm$ )-1,2-heptanediol **4a** from the corresponding epoxide ( $\pm$ )-**4**. GC/MS analysis *m/z* 101 (27,  $\text{M}^+ - \text{CH}_2\text{OH}$ ), 83 (77,  $\text{M}^+ - (\text{CH}_2\text{OH} + \text{H}_2\text{O})$ ), 55 (100,  $\text{CH}_3(\text{CH}_2)_2^+$ ).  $^1\text{H}$  NMR of ( $\pm$ )-**4a** in acetone- $d_6$ :  $\delta_{\text{H}}$  0.89 (3H, t,  $J=7$  Hz,  $\text{CH}_3$ ), 1.18–1.42 (6H, m,  $-(\text{CH}_2)_3-$ ), 1.42–1.60 (2H, m,  $-\text{CH}_2\text{CH}(\text{OH})-$ ), 2.04–2.08 (1H, m,  $-\text{CH}(\text{OH})-$ ), 3.30–3.53 (2H, m,  $-\text{CH}_2\text{OH}$ ).

#### 4.4. Growth conditions for *Rhodotorula glutinis*

The yeast *Rhodotorula glutinis* strain CIMW 147 was 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. *Rhodotorula 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  $\text{h}^{-1}$ . 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  $-20^\circ\text{C}$ .

#### 4.5. Epoxide hydrolysis by *Rhodotorula glutinis*

Hydrolysis of epoxides was routinely performed in 100 ml screw-capped bottles sealed with rubber septa. The bottles contained 1 to 5 ml concentrated washed cell suspension of *Rhodotorula glutinis* (0.1 to 0.5 g dry weight) and 50 mM potassium phosphate buffer pH 7.5 to a total volume of 10 ml. The bottles were placed in a shaking waterbath at 35°C and the reaction was started by addition of 0.20 mmol of epoxide. The course of the epoxide hydrolysis was followed by periodic headspace sampling followed by analysis by chiral GLC. 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%. Subsequently, diols were extracted with ethylacetate from NaCl saturated supernatants, obtained after centrifugation of the reaction mixture. Analysis of the diols was by chiral GLC.

#### 4.6. Absolute configuration of epoxides **1** to **5**

Absolute configurations were determined for the residual epoxides obtained after hydrolysis of 300 mg of the appropriate epoxide by 800 mg (dry weight) of *Rhodotorula glutinis* cells as described in this paper. The moment for terminating the reactions was determined by monitoring head space samples by chiral GLC. The reactions were stopped by removal of the yeast cells by centrifugation (20,000 g, 10 minutes, 4°C). Subsequently, the supernatants were extracted twice with an equal volume of cold pentane. The combined organic layers were dried over MgSO<sub>4</sub> and concentrated by evaporation at 40°C under atmospheric pressure. Because of the high volatility of most epoxides, concentration was not continued further and measurement of the specific optical rotation values was performed in the concentrated pentane fraction. Chiral GLC was used for determination of e.e.s and concentrations were derived from calibration curves. Data of chiral GLC analysis and specific optical rotation values of the residual epoxides are:

epoxide (*S*)-**1**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = -15.5 (c=0.20, pentane; e.e.=95%), [lit.<sup>13</sup>: (*R*)-**1**: [ $\alpha$ ]<sub>D</sub><sup>16</sup> = +12.4 (c=5.98, dioxane; e.e.>98%)];

epoxide (*S*)-**2**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = -16.8 (c=0.28, pentane; e.e.>98%);

epoxide (*S*)-**3**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = -18.7 (c=0.93, pentane; e.e.>98%), [lit.<sup>4</sup>: (*R*)-**3**: [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +12.1 (neat; e.e.=66%)];

epoxide (*S*)-**4**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = -12.3 (c=0.31, pentane; e.e.=78%) [lit.<sup>4</sup>: (*R*)-**4**: [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +15.1 (neat; e.e.=94%)].

Determination of the absolute configurations was by comparison of our results with the data reported in the literature.<sup>4,13</sup> Absolute configuration of the resolved residual epoxide (*S*)-**5** was determined by co-injection on chiral GLC with the enantiopure reference compounds (*R*)-**5** and (*S*)-**5** from Fluka.

#### 4.7. Absolute configuration of diols **1a** to **5a**

Absolute configurations were determined for the diols formed from duplicate hydrolysis of 300 mg of the appropriate epoxide by 800 mg (dry weight) of *Rhodotorula glutinis* cells as described. Experiments were carried out in duplicate as described in this paper for resolution of the epoxides. However, in the case of the diols, the reactions were already terminated at low conversions in order to obtain diols with higher enantiomeric purities. The residual epoxides are removed from the reaction mixture by extraction with pentane as described. The remaining aqueous reaction mixtures were subsequently saturated with NaCl and extracted twice with an equal amount of ethylacetate. The combined organic layers were dried over MgSO<sub>4</sub> and evaporated under reduced pressure to give an oily residue of diols **1a** to **4a**, and a white solid in the case of diol **5a**. For measurement of the specific rotation values and enantiomeric purities, the diols were redissolved in methanol. Chiral GLC analysis was performed for determination of the e.e. values. Data of chiral GLC analysis and specific optical rotation values of the formed diols are:

diol (*R*)-**1a**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = +3.6 (c=3.5, methanol; e.e.=36%), [lit.<sup>14</sup>: (*S*)-**1a**: [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -8.6 (c=1.0, methanol; e.e.>99%)];

diol (*R*)-**2a**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = +9.9 (c=5.4, methanol; e.e.=67%), [lit.<sup>14</sup>: (*S*)-**2a**: [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -17.3 (c=1.0, methanol; e.e.>99%)];

diol (*R*)-**3a**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = +13.5 (c=5.8, methanol; e.e.=91%), [lit.<sup>14</sup>: (*S*)-**3a**: [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -16.4 (c=1.0, methanol; e.e.>99%)];

diol (*R*)-**4a**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = +14.4 (c=2.4, methanol; e.e.=93%), [lit.<sup>14</sup>: (*S*)-**4a**: [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -15.4 (c=1.0, methanol; e.e.>99%)];

diol (*R*)-**5a**: [ $\alpha$ ]<sub>D</sub><sup>24</sup> = +12.8 (c=0.95, methanol; e.e.=80%), [lit.<sup>14</sup>: (*S*)-**5a**: [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -13.6 (c=1.0, methanol; e.e.>99%)].

Determination of the absolute configurations was by comparison of our results with the data reported in the literature.<sup>14</sup>

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