

Hydrolytic kinetic resolution of the enantiomers of the structural isomers *trans*-1-phenylpropene oxide and (2,3-epoxypropyl)benzene by yeast epoxide hydrolase

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Abstract

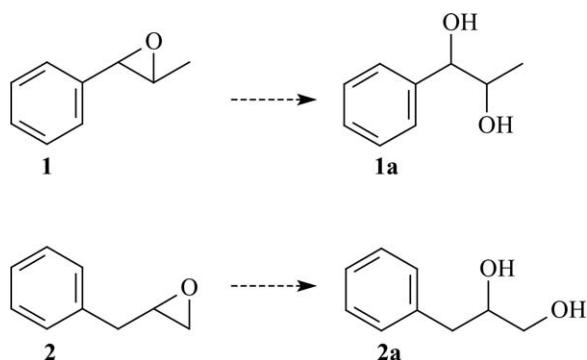
Kinetic resolution of the enantiomers of *trans*-1-phenylpropene oxide and (2,3-epoxypropyl)benzene was achieved by yeasts from the genus *Rhodotorula*. The resolution of *trans*-1-phenylpropene oxide by *Rhodotorula glutinis* UOFS Y-0123 yielded (1R,2R)-epoxide (ee >98%, yield 30%) and (1R,2S)-diol (ee 95%, yield 40%). The highest enantio- and regioselectivity toward (2,3-epoxypropyl)benzene resided in *Rhodotorula* sp. UOFS Y-0448 (E = 6.16), yielding (S)-epoxide (ee 64%, yield 33%) and (R)-diol (ee 67%, yield 28%). This confirms the superiority of yeasts from the *Basidiomycetes* genera in the enantioselective hydrolysis of epoxides from different structural classes.

Introduction

Epoxide hydrolases (EHs) are hydrolytic enzymes (EC 3.3.2.3), ubiquitous in nature, that catalyse the stereoselective hydrolysis of chiral epoxides, resulting in the formation of the corresponding vicinal diol(s) (Steinreiber & Faber 2001). Both enantiopure epoxides and vicinal diols are important chiral intermediates in the synthesis of more complex chiral compounds, including a significant number of known drugs (De Vries & Janssen 2003). The use of EHs from microbial sources as selective biocatalysts in synthetic regimes offers several advantages, of which the most important may be the fact that microbial cells can be cultivated on an almost unlimited scale by fermentation. Additionally, microbial EHs do not require co-factors or metal ions for catalytic activity (Archelas & Furstoss 2001) and enzyme induction is, in most cases, not necessary (Botes 1999).

Yeast EHs, especially those from red yeasts, exhibit excellent enantioselectivity toward a broad range of epoxide substrates (Steinreiber & Faber 2001, Weijers & De Bont 1999). Furthermore, they are extremely useful in hydrolytic kinetic resolution (HKR) of mono-substituted epoxides, although they are less selective for the hydrolysis of some mono-substituted epoxides with unsaturated hydrocarbon side chains (Botes *et al.* 1999).

For this purpose, a comparative study of the HKR of *trans*-1-phenylpropene oxide (structural class: styrene oxides), and a structural isomer (2,3-epoxypropyl)benzene [structural class: mono-substituted (with unsaturated hydrocarbon side-chain)] was conducted.



- 1** *trans*-1-Phenylpropene oxide
1a *trans*-1-Phenylpropane-1,2-diol
2 (2,3-Epoxypropyl)benzene
2a 3-Phenylpropane-1,2-diol

Fig. 1. Epoxide substrates and diol products.

Materials and methods

General

A total of 307 yeast strains from 31 genera were obtained from the Yeast Culture Collection of the University of the Free State, Bloemfontein, South Africa, and were screened for enantioselective EH activity toward *trans*-1-phenylpropene oxide **1** and (2,3-epoxypropyl)benzene **2** (Figure 1). Both substrates are commercially available from Aldrich (**1** as separate enantiomers and **2** as the racemate).

Yeast cultivation

Yeasts were cultivated in shake-flask cultures in medium consisting of 0.5% (w/v) yeast extract, 2% (w/v) malt extract, 1% (w/v) peptone and 1.5% (w/v) glucose. After incubation for 48 h at 25 °C, cells were harvested by centrifugation (6000 g, 10 min, 4 °C), washed with phosphate buffer (50 mM, pH 7.5) and resuspended to 20% (w/v) in the same buffer.

Initial activity screen

Substrates **1** and **2** were added (neat) to 500 μ l cell suspensions to give 20 mM. The reactions were incubated at 25 °C for 3 h. Reactions were terminated by extraction of unreacted substrate and formed product with 250 μ l ethyl acetate, after saturation with NaCl. After centrifugation (12 000 g, 10 min), organic layers were analysed for product formation by TLC.

Stereoselectivity assays

Strains yielding positive TLC results were assayed for EH stereoselectivity. Ten ml cell suspension was transferred to a 20 ml glass vial with a screw cap fitted with a PTFE/rubber septum. The reaction was started by addition of substrate at 20 mM, and the mixture was agitated at 25 °C for 3 h. Samples (500 μ l) were taken at intervals, extracted with 250 μ l ethyl acetate and centrifuged (12 000 g, 10 min, 4 °C). The organic layers were removed, dried over anhydrous Na₂SO₄ and analysed by chiral GC.

Analysis

TLC was performed using F₂₅₄ silica gel plates with chloroform ethyl acetate (1:1, v/v) as the mobile phase. A solution of vanillin in sulphuric acid (20 mg ml⁻¹) was used as a spray reagent.

Chiral GC was performed on a gas chromatograph equipped with a FID detector, using N₂ as the carrier gas at a column head pressure of 140 kPa. Determination of enantiomeric excesses (ee) of substrates and products was achieved using chiral fused silica cyclodextrin capillary columns (30 m \times 0.25 mm, 0.25 μ m film). **1** was analysed using an α -DEX 120 column and **2** using a β -DEX 225 column (both supplied by Supelco) at oven temperatures of 90 °C and 110 °C, respectively. The diol products **1a** and **2a** were analysed using a β -DEX 225 column at an oven temperature of 150 °C. Calibration curves for epoxide and diol concentrations were compiled from reactions employing heat-killed cells. Retention times and elution orders were established and confirmed by injection and co-injection of enantiomerically pure standards.

Results and discussion

trans-1-Phenylpropene oxide (**1**)

Good results for the enantioselective hydrolysis of **1** were achieved by a number of strains, the best of which delivered similar results and all belong to the *Basidiomycetes* genera of *Rhodotorula* and *Trichosporon*. To avoid repetition of results, only one representative strain, *Rhodotorula glutinis* UOFS Y-0123, will be discussed in detail. This strain converted enantioselectively (1S,2S)-**1** to (1R,2S)-**1a**, yielding (1R,2R)-**1** (ee > 98%, yield 30%) and (1R,2S)-**1a** (ee > 95%, yield > 40%). This reaction proceeds with

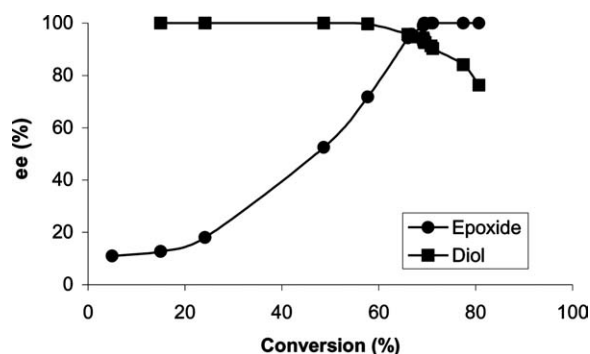


Fig. 2. Enantiomeric excess as a function of conversion during hydrolysis of *trans*-1-phenylpropene oxide by UOFS Y-0123.

the same stereochemistry as hydrolysis by *Rhodotorula glutinis* CIMW 147 (Weijers & De Bont 1999), as well as hydrolysis by the fungi *Aspergillus terreus* (Moussou *et al.* 1998a) and *Beauveria bassiana* (Pedragosa-Moreau *et al.* 1996a), but with different stereochemistry than hydrolysis by mammalian microsomal EH [(1*S*,2*S*)-**1** converted to (1*S*,2*R*)-**1a**] (Bellucci *et al.* 1993). The high ee's of both the residual epoxide **1** and diol-product **1a** are noteworthy, since **1** is not accessible in very high enantiomeric purity *via* chemical synthetic techniques such as Jacobsen/Katsuki asymmetric epoxidation, where epoxide enantiomeric excesses (ee) range from 81–83% (Chang *et al.* 1994).

A more detailed kinetic study of the resolution of **1** by UOFS Y-0123 was performed by plotting the ee's of the residual (1*R*,2*R*)-epoxide and the formed (1*R*,2*S*)-diol against proceeding conversion (Figure 2). This shows a near complete kinetic resolution of racemic **1** at around 70% conversion, where the ee of (1*R*,2*R*)-**1** approaches 100%. As the yield of **1** is obviously low (high conversion), the high ee and yield of the diol-product **1a** strongly indicate that UOFS Y-0123 is particularly useful for the selective production of (1*R*,2*S*)-**1a**.

However, the formation of (1*R*,2*S*)-**1a** at conversions exceeding 50% indicates that (1*R*,2*S*)-**1a** is not only formed by preferential hydrolysis of (1*S*,2*S*)-**1**, but also from (i) hydrolysis of (1*R*,2*R*)-**1**, and/or (ii) stereoinversion of (1*S*,2*R*)-**1a** to (1*R*,2*S*)-**1a** (Hasegawa *et al.* 1990). The decrease in diol-ee during reaction stages beyond 50% conversion indicates that the EH from UOFS Y-0123 is almost totally regioselective toward the faster reacting (1*S*,2*S*)-**1** (producing only one diol-enantiomer from the preferred substrate-enantiomer), while the slower reacting (1*R*,2*R*)-**1** may

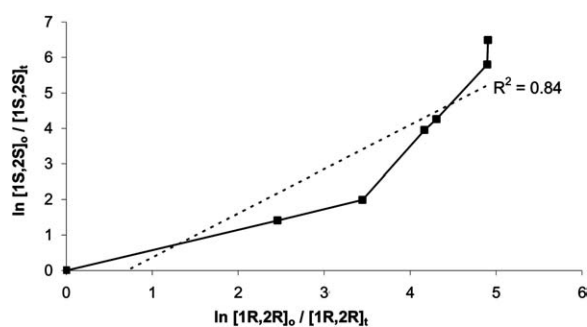


Fig. 3. Linear regression of the natural logarithms of the relative concentrations of (1*R*,2*R*)- and (1*S*,2*S*)-*trans*-1-phenylpropene oxide during hydrolysis by UOFS Y-0123.

be hydrolysed to both diol-enantiomers, resulting in a consequent decrease in diol-ee. This indicates low regioselectivity toward the slower reacting (1*R*,2*R*)-epoxide (Moussou *et al.* 1998b). Similar observations have been made for the hydrolysis of **1** by *Aspergillus terreus* (Moussou *et al.* 1998a) and *Beauveria bassiana* (Moussou *et al.* 2000).

Regioselectivity – or the lack thereof – is confirmed by a plot of $\ln[R]_0/[R]_t$ versus $\ln[S]_0/[S]_t$ (Equation (1): enantiomeric ratio as regioselectivity indicator) of which the slope describes the enantiomeric ratio, *E*, (Morisseau *et al.* 1997, Rakels *et al.* 1993). In the case of absolute regioselectivity, this logarithmic plot is linear with a high correlation coefficient (R^2) and therefore a constant slope (Moussou *et al.* 1998a). In cases of low regioselectivity, this plot is non-linear and the correlation coefficient low, indicating that the slope (*E*) is a variable, which cannot be calculated accurately and which should not be used as an indication of enantioselectivity (Moussou *et al.* 1998b, Orru & Faber 1999, Pedragosa-Moreau *et al.* 1996b).

$$E = \frac{\ln [R]_0 / [R]_t}{\ln [S]_0 / [S]_t}, \quad (1)$$

where $[R]_0$, $[R]_t$, $[S]_0$ and $[S]_t$ = concentrations of enantiomers (R) and (S) at times 0 and t, respectively, with (R) representing the faster reacting enantiomer. The lack of regioselectivity in UOFS Y-0123 was confirmed by a plot of $\ln[1*S*,2*S*]_0/[1*S*,2*S*]_t$ versus $\ln[1*R*,2*R*]_0/[1*R*,2*R*]_t$ (Figure 3). As expected in cases of low regioselectivity, the logarithmic plot for hydrolysis of **1** by UOFS Y-0123 is non-linear with a low correlation coefficient ($R^2 = 0.84$), confirming our existing suspicion of low regioselectivity, and an *E*-value for the hydrolysis of **1** is thus not reported.

(2,3-Epoxypropyl)benzene (2)

Yeasts displaying enantioselective EH-activity toward **2** displayed a definite preference toward conversion of (R)-**2**, with retention of configuration, to (R)-**2a**, which is in accordance with enantioselectivities published for the hydrolysis of other mono-substituted epoxides, by yeast EH (Botes *et al.* 1999) and fungal EH (Orri & Faber 1999). The best results were achieved by *Rhodotorula* sp. UOFS Y-0448, yielding (S)-**2** (ee 65%, yield 33%) and (R)-**2a** (ee 67%, yield 28%) ($E = 6.16$). Fairly similar results were achieved by *Rhodotorula* sp. UOFS Y-2043, which yielded (S)-**2** (ee 62%, yield 36%) and (R)-**2a** (ee 48%, yield 26%), although with low regioselectivity.

Conclusion

Excellent results for the enantioselective hydrolysis of *trans*-1-phenylpropene oxide **1** were obtained by the yeast *Rhodotorula glutinis* UOFS Y-0123. Although not regioselective, this yeast achieves good yields and enantioselectivity and may be particularly effective in the enantioselective production of (1R,2S)-*trans*-phenylpropane-1,2-diol. Reports of similar results for other strains from the same species exist in literature, and the degree of enantioselectivity in the hydrolysis of **1** seems to be well in favour of yeast EHs. Additionally, yeasts from the same genus appear to be less enantioselective (but more regioselective) in the HKR of (2,3-epoxypropyl)benzene **2**, a structural isomer of **1**. These selectivity differences may be due to differences in the catalytic mechanism of EHs toward substrates from different structural classes. As far as literature reports are concerned, no better success for the HKR of **2** has yet been reported. This further confirms the superiority of yeasts from *Basidiomycetes* genera in the enantioselective hydrolysis of epoxides from different structural classes.

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