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Multienzymatic preparation of (−)-[3-(oxiran-2-yl)phenyl]methanol and (−)-3-(oxiran-2-yl)benzoic acid

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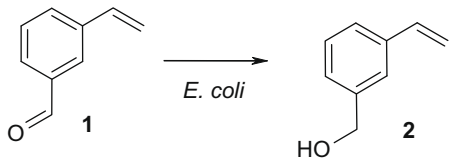
ABSTRACT

The cascade use of enzymatic activities allows for the preparation of enantiomerically pure epoxides. In particular, using whole-cell biocatalysts we can prepare both (−)-[3-(oxiran-2-yl)phenyl]methanol and (−)-3-(oxiran-2-yl)benzoic acid in one-pot, two or three steps procedure. The yield is quantitative and enantiomeric purity greater than 95%. The selected biocatalysts contain a styrene monoxygenase from *Pseudomonas fluorescens* ST and a naphthalene dihydrodiol dehydrogenase from *P. fluorescens* N3, cloned and expressed in *Escherichia coli*.

1. Introduction

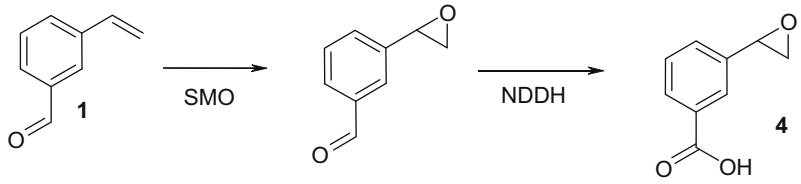
In the framework of our ongoing interest in biocatalysis we have studied the possibility of preparing enantiomerically pure epoxides by multienzymatic synthesis.[1–7] The enzyme activities were selected from our biocatalyst pool to perform the transformation of a styrene derivative into interesting new compounds. The substrate chosen was 3-vinyl benzaldehyde and is commercially available. Our intention was the preparation of 3-(oxiran-2-yl)benzoic acid **4**, in good yield and high enantiomeric purity. Moreover, we wanted to prepare this compound using only enzymatic activities. Both enzymes have been the subject of previous studies and had demonstrated high selectivity and good performance.[8,9] However, we knew that the styrene monoxygenase (SMO) cannot transform acid derivatives; therefore, the reaction sequence should be: epoxidation and oxidation (Scheme 1).

Another enzymatic activity is present in our biocatalysts that introduce a new factor of interest. As already described[10] *Escherichia coli*, our host organism, expresses a reductive activity that chemoselectively transforms aldehydes into the corresponding alcohols (Scheme 2).



Scheme 2. Reduction of 3-vinylbenzaldehyde into 3-(vinyl)phenylmethanol **2**.

This activity is highly efficient and it is impossible to prevent its action on any type of aldehyde that we tested. The consequence is that the designed transformation can proceed in three successive steps as reported in Scheme 3.



Scheme 1. Synthesis of (−)-3-(oxiran-2-yl)benzoic acid **4**.

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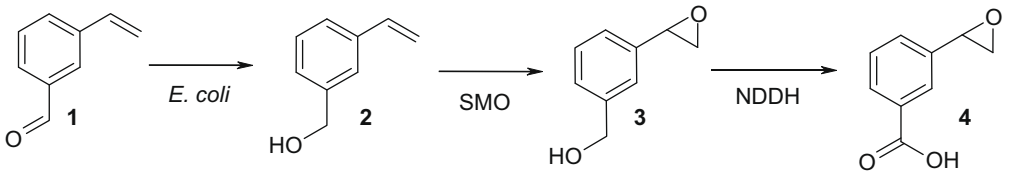

Scheme 3. Three-step preparation of (−)-3-(oxiran-2-yl)benzoic acid **4**.

The reaction sequence will work only if the last transformation occurs when the other two have finished.

2. Results and discussion

Initially, we separately tested all of the transformations, to assess their performance on the chosen substrate. The results were very good demonstrating that the enzymes correctly transform each substrate into the corresponding product. Then, we checked the reaction sequence using two different approaches whose final result was identical. The first approach uses three biocatalysts in a cascade reaction: *E. coli* JM109, *E. coli* JM109 (pTAB19), expressing monoxygenase activity, and *E. coli* JM109 (pVL2028), expressing alcohol dehydrogenase activity. The second uses the last two biocatalysts, only. The first approach was tested to demonstrate that it is possible to operate three successive transformations without a visible difference in comparison to the two-step sequence.

The procedure developed allows for the preparation of both (−)-[3-(oxiran-2-yl)phenyl]methanol **3** and (−)-3-(oxiran-2-yl)benzoic acid **4**, simply adding the third step if the acid is the desired product. Both products are the sole compounds present at the end of the respective reaction sequences; they are easily recovered from the cultures and they both are enantiomerically pure.

In Figure 1 the transformation of 2-vinylbenzaldehyde **1** to 3-(vinyl)phenylmethanol **2** by *E. coli* JM109 is shown. The reaction is very fast and goes to completion in less than 30 min. As can be observed from Figure 2, the same transformation occurs when *E. coli* JM109 (pTAB19) is used. However, using this biocatalyst the 3-(vinyl)phenylmethanol **2** is further transformed into the corresponding epoxide by the SMO activity. This reaction is slower and needs 3 h to reach completion. Using compound **2** directly with *E. coli* JM109 (pTAB19) we can observe the transformation to the epoxide at a similar rate (Fig. 3).

In Figure 4 the HPLC analysis of the three-step transformation of compound **1** into compound **4** is reported. In the figure it is possible to observe the initial fast transformation of **1** into **2** (30 min), followed by the transformation of **2** into **3** (3 h). At this time the second biocatalyst is added to the mixture and compound **3** begins its transformation into compound **4**. This last conversion is quite slow; however, the observed rate is in complete agreement with our previous experiments. NDDH is not a standard alcohol dehydrogenase and its activity towards primary and secondary alcohol highly depends on the substrate structure. Nevertheless, at the end of the transformation the final product is recovered. The complete sequence can and should be optimised to improve the rate and the time yield.

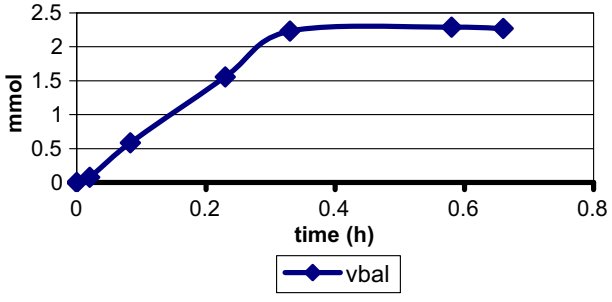

Figure 1. Synthesis of 3-(vinyl)phenylmethanol **2** (vbal, ♦).

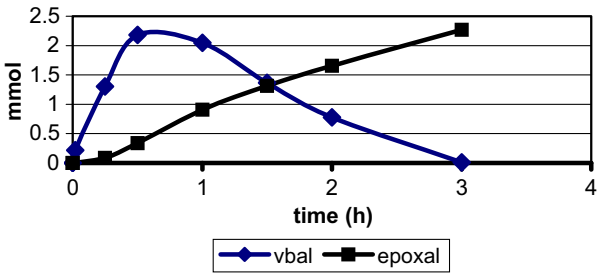

Figure 2. Synthesis of (−)-[3-(oxiran-2-yl)phenyl]methanol **3** (epoxal, ■) and 3-(vinyl)phenylmethanol **2** increase and decrease (vbal, ♦).

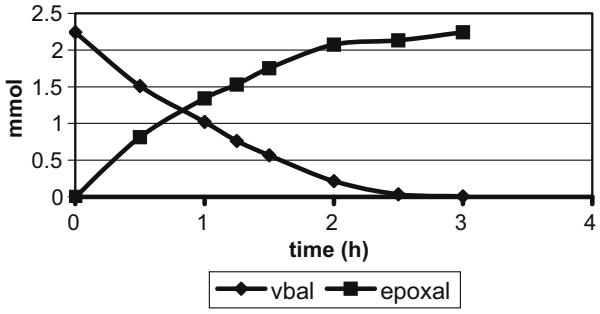

Figure 3. Synthesis of (−)-[3-(oxiran-2-yl)phenyl]methanol **3** (epoxal, ■) and 3-(vinyl)phenylmethanol **2** decrease (vbal, ♦).

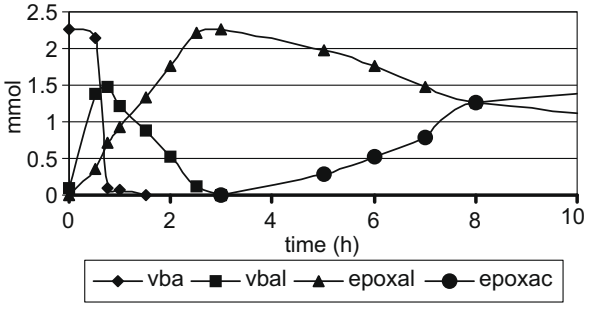

Figure 4. Synthesis of (−)-3-(oxiran-2-yl)benzoic acid **4**; (−)-[3-(oxiran-2-yl) phenyl]methanol **3** (epoxal, ▲) and 3-(vinyl)phenylmethanol **2** increase and decrease (vbal, ■), 3-vinylbenzaldehyde **1** (vba, ♦) decrease and (−)-3-(oxiran-2-yl)benzoic acid **4** (epoxac, ●) increase.

3. Conclusion

This preliminary study demonstrates the power of multienzymatic synthesis, indicating that it should be possible to manage many independent transformations without the need of intermediate isolation and purification. We are currently developing both new sequences and newly regulated biocatalysts that can express different activities at the desired time.

4. Experimental

4.1. Analytical methods

Substrates and products were monitored by analysing the water phase with HPLC, Hitachi-Merck, UV–visible detector at 220 nm, reverse phase column C18 (Hibar LICHROSORB 50334, 10 μm, 25 cm), H_2O/CH_3CN 1:1 eluent, 1 mL/min flow, Hitachi D2500 integrator.

The absolute (*S*)-configuration of biocatalytically prepared (*S*)-styrene oxide (proven via comparison with commercially available, enantiopure (*S*)-styrene oxide (Aldrich)) was used as the reference for all epoxides and the configuration accordingly presumed. Enantiomeric excesses were measured using a Chrompack ChiralDex-CB column.

^{1}H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ (Merck) using Bruker AC-200 instrument. All signals are expressed as ppm down field from tetramethylsilane.

HRMS spectra were obtained using Autospec 246M VG Fisons instrument.

Optical rotation was obtained in $CHCl_3$ or CH_3OH using JASCO P-1030 polarimeter.

4.2. Preparation procedures

4.2.1. Preparation of 3-(vinyl)phenylmethanol 2

At first, 2.27 mM 3-vinylbenzaldehyde **1**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (1 g/L CDW) in 50 mL of M9 medium, containing glucose (0.2% w/v), at 30 °C. After 30 min the cells were separated by centrifugation (10,000 rpm, 4 °C); the surnatant was extracted using AcOEt (three 30 mL portions), the organic phases were collected, dried over Na_2SO_4 and evaporated at reduced pressure. The crude product (12 mg, 79%) only contained 3-(vinyl)phenylmethanol **2**. Oil; δ_H (200 MHz, $CDCl_3$) 4.7 (2H, s), 5.3 (1H, d, *J* = 10.8 Hz), 5.8 (1H, d, *J* = 17.5 Hz), 6.7 (1H, dd, *J* = 10.8, 17.5 Hz), 7.3–7.45 (4H, m).

4.2.2. Preparation of [3-(oxiran-2-yl)phenyl]methanol 3

At first, 2.24 mM 3-(vinyl)phenylmethanol **2**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (pTAB19) (1 g/L CDW) in 50 mL of M9 medium, containing glucose (0.2% w/v), at 30 °C. After 3 h the cells were separated by centrifugation (10,000 rpm, 4 °C); the surnatant was extracted using AcOEt (three 30 mL portions), the organic phases were collected, dried over Na_2SO_4 and evaporated at reduced pressure. The crude product (14 mg, 83%) only contained [3-(oxiran-2-yl)phenyl]methanol **3**. Oil; δ_H (200 MHz, $CDCl_3$) 2.8 (1H, dd, *J* = 5.5, 2.7 Hz), 3.2 (1H, dd, *J* = 5.5, 4.1 Hz), 3.9 (1H, dd, *J* = 4.1, 2.7 Hz), 4.7 (2H, s), 7.2–7.4 (4H, m); δ_C (75.5 MHz, $CDCl_3$) 51.4 (t), 52.5 (d), 65.4 (t), 124 (d), 125.3 (d), 126.9 (d), 129 (d), 138.2 (s), 141.5 (s); *m*/*z* (EI) 150 (M^+, 12), 149 (12), 119 (100), 91 (83); HRMS (EI): M^+, found 150.06660. $C_9H_{10}O_2$ requires 150.06808; $[\alpha]_D = -22.25$ (*c* 4.8 mg, $CHCl_3$). Retention times in chiral GLC (t_0 = 110 °C for 5 min, t_f = 180 °C, 3 °C/min, P_{He} = 0.8 atm) of both enantiomers: 41.0 and 41.8 min.

4.2.3. Preparation of 3-(oxiran-2-yl)benzoic acid 4

At first, 2.27 mM 3-vinylbenzaldehyde **1**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (pTAB19) (1 g/L CDW) in 50 mL of M9 medium, containing glucose (0.2% w/v), at 30 °C. After 3 h an equal amount of *E. coli* JM109 (pVL2028) (1 g/L CDW) was added and the reaction continued, at 30 °C. The reaction was allowed to react for 24 h, then the cells were separated by centrifugation (10,000 rpm, 4 °C); the surnatant was acidified using HCl 1 M and extracted using AcOEt (three 30 mL portions). The organic phases were collected, washed with 2 × 50 mL portions of the $NaHCO_3$ water solution and with water to neutral pH, dried over Na_2SO_4 and evaporated at reduced pressure. The crude product (14.5 mg, 79%) only contained 3-(oxiran-2-yl)benzoic acid **4**. Oil; δ_H (200 MHz, $CDCl_3$) 2.85 (1H, dd, *J* = 5.4, 2.6 Hz), 3.2 (1H, dd, *J* = 5.2, 4.1 Hz), 3.9 (1H, dd, *J* = 4.1, 2.6 Hz), 7.5–7.6 (2H, m), 8.1–8.2 (2H, m); δ_C (75.5 MHz, $CDCl_3$) 51.4 (t), 52.0 (d), 127.6 (d), 128.6 (d), 130 (d), 130.8 (d), 134.7 (s), 138.5 (s), 171.3 (s); *m*/*z* (EI) 164 (M^+, 20), 163 (30), 148 (40), 119 (100), 105 (55), 91 (50); HRMS (EI): M^+, found 164.04321. $C_9H_{10}O_2$ requires 164.04734; $[\alpha]_D = -12.6$ (*c* 4.4 mg, CH_3OH). Retention times in chiral GLC (t_0 = 110 °C for 5 min, t_f = 180 °C, 3 °C/min, P_{He} = 0.8 atm) of both enantiomers: 72.6–73.8 min.

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