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Enzyme-Triggered Opening of an Epoxide: Chemoenzymatic Synthesis of (2*R*,5*R*)- and (2*S*,5*R*)-Pityol

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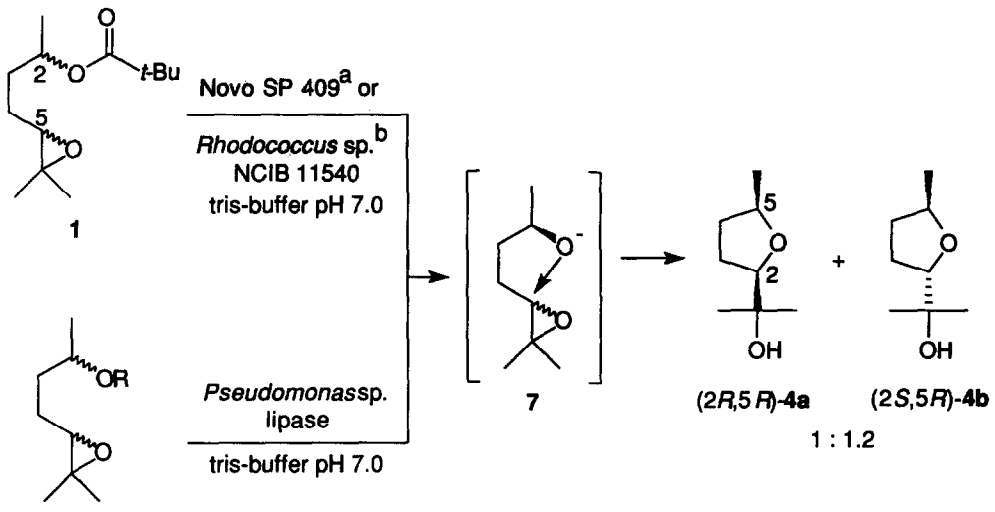
Abstract: Treatment of a diastereomeric mixture of (±)-epoxy-ester **1** with a crude immobilised enzyme preparation (Novo SP 409) or whole lyophilized cells of *Rhodococcus erythropolis* NCIB 11540 in aqueous buffer (pH 7.0) did not lead to the formation of the expected epoxy alcohol **2** or diol **5** but surprisingly furnished the rearranged products **4a** and **4b** in >98% enantiomeric excess. (2*R**,5*R**)-2-(1-Hydroxy-1-methylethyl)-5-methyltetrahydrofuran (pityol, **4a**) is a pheromone of the elm bark beetle, *Pteleobius vittatus*. The fact that pityol was not formed from epoxy-alcohol **2** in the absence of biocatalyst leads us to the conclusion that the rearrangement was triggered by an enzyme.

During the course of our study on the enzyme-catalysed asymmetric hydrolysis of epoxides making use of microbial epoxide hydrolases [1,2] we attempted to hydrolyse the epoxy moiety of a diastereomeric mixture of (±)-epoxy-ester **1**. Surprisingly, the expected diol **5** was only detected in trace amounts, and the two main products which were formed during the reaction were identified as the diastereomeric tetrahydrofuran derivatives **4a** [3] and **4b** [4] in a ratio of about 1:1.2. (2*R**,5*R**)-2-(1-Hydroxy-1-methylethyl)-5-methyltetrahydrofuran (**4a**) has been commonly denoted as pityol and is the aggregation pheromone of the elm bark beetle *Pteleobius vittatus* [5,6]. Both diastereomers have been separated by medium pressure liquid chromatography, and their structures were elucidated by comparison of their ^{1}H- and ^{13}C-NMR spectra with independently synthesized material. Their optical purity was shown to be >98% as analysed by chiral GLC [7]. The absolute configuration was determined on the basis of the reported retention times of all four possible pityol isomers [8] and was confirmed by co-injection with material obtained from (*R*)- and (*S*)-6-methylhept-5-en-2-ol (sulcatol) [9], respectively.

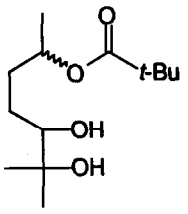


Scheme: Enzyme-triggered opening of an epoxide.

The unexpected outcome of this reaction prompted us to a more detailed investigation. It is known that epoxy-alcohol **2** undergoes ring closure in strongly alkaline medium forming a mixture of all possible stereoisomers of pityol together with the corresponding tetrahydropyran derivative **6** [10]. In contrast, the epoxy-alcohol **2** [11] proved to be remarkably stable at the conditions of the enzymatic reaction (tris-buffer, pH 7.0) in the absence of biocatalyst, and the starting material remained unchanged for more than one day. The addition of sodium hydroxide, however, led to rapid cyclisation.

From these observations the following sequence was assumed: Upon biocatalytic hydrolysis of the pivaloate ester of **1**, which proceeds with complete enantioselectivity with respect to the chiral center of the ester moiety on carbon 2, the intermediate oxy-anion species **7** performs a nucleophilic ring opening of the epoxide moiety. To verify this hypothesis, the corresponding acetate ester **3** was subjected to lipase-catalysed hydrolysis using *Pseudomonas* sp. lipase [12] under identical conditions, and the resulting rearranged products **4a** and **4b** were isolated in similar high enantiomeric purity. At no point of the reaction the alcohol **2** was present in significant amounts. This proves that an involvement of an epoxide-hydrolase can be ruled out. In addition, the rearrangement proceeds with low selectivity with respect to the chiral center located on the epoxy moiety (carbon-5), leading to the formation of diastereomeric products **4a** and **4b** in almost equal amounts.

Interestingly, no significant amounts of pyran derivative **6**, resulting from an attack of the C-2 oxygen on carbon 6, were detected. This is in contrast to a recent report on the microbial multistep transformation of 6-methyl-hept-5-en-2-one with fermenting cells of *Botrytis cinerea* [13].



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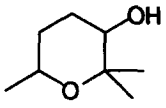

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Such reactions, during which an unstable intermediate prone to rearrangement or decomposition was generated by the aid of a hydrolytic enzyme are rare but not unprecedent. For instance, epoxy-diols formed during the enzymatic hydrolysis of di-epoxides catalysed by cytosolic epoxide hydrolase furnished tetrahydrofuran products [14]. Similarly, the pig liver esterase (PLE) catalysed asymmetric hydrolysis of a bicyclic *meso*-diester bearing an epoxy-moiety led to Meinwald rearrangement of the initially formed hemiester [15]. A closely related sequence involving a cyclisation cascade of a diepoxide was likewise triggered by PLE-catalysed ester-hydrolysis [16]. Finally, the enzyme-catalysed hydrolysis of a 1,2-dioxetane carboxylic ester by generating a chemiexcited intermediate which underwent decomposition with concomitant chemoluminescence was reported [17].

Summary

A synthesis of (2*R*,5*R*)-2-(1-hydroxy-1-methylethyl)-5-methyltetrahydrofuran (pityol, **4a**) in >98% enantiomeric excess was accomplished by using a crude immobilised enzyme preparation derived from *Rhodococcus* sp. (Novo SP 409) or whole lyophilised cells of *Rhodococcus erythropolis* NCIB 11540. The biocatalytic key step involved two unexpected features: (i) a biocatalytic resolution of a secondary pivaloate ester followed by (ii) an enzyme-triggered opening of an epoxide.

Experimental

(2*R*,5*R*)-2-(1-Hydroxy-1-methylethyl)-5-methyltetrahydrofuran (**4a**)
(2*S*,5*R*)-2-(1-Hydroxy-1-methylethyl)-5-methyltetrahydrofuran (**4b**)

Biocatalyst (Novo SP 409, batch no. PPK-2644, 10g, or lyophilized cells of *Rhodococcus erythropolis* NCIB 11540, 1g, resp.) was hydrated in tris-buffer (0.1N, pH 7.0, 1L) at r.t. for 1h with shaking. Then, substrate **1** (1g) was added and shaking was continued. When about half of the starting material was consumed after ~30h, the biocatalyst was filtered (cells were centrifuged and separately extracted with acetone) and products were extracted from the mixture with ether. Separation of the diastereomers **4a** and **4b** (crude yield ~70% based on 50% theoretical yield of a kinetic resolution of a racemate) was performed by sequential medium pressure liquid chromatography on silica gel Merck 60 (40-63μm) with pet.-ether/ethyl acetate (10/1) as eluent. Bp. 45-50°C bulb-to-bulb distilliation. ^{13}C-NMR

(75.47 MHz, $CDCl_3$) **4a** [3]: δ = 21.31, 24.51, 26.19, 27.42, 33.37, 71.09, 75.72, 86.23; **4b** [4]: δ = 21.42, 24.25, 27.30 (x2) 34.70, 71.90, 76.37, 85.55.

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7. J&W Cyclodex B (permethyl-β-cyclodextrin on OV 1701) 30m x 0.25mm, 0.25μ film, N_2, 80°C iso, pityol isomers: (2*R*,5*R*)-**4a** 15.5min, (2*S*,5*S*) 16.2min, (2*R*,5*S*) 18.3min, (2*S*,5*R*)-**4b** 20.2min.
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