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Asymmetric Opening of an Epoxide by Azide Catalyzed by an Immobilized Enzyme Preparation from *Rhodococcus* sp.

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Abstract: The asymmetric nucleophilic opening of (±)-2-methyl-2-pentyloxirane (**1**) by azide yielding the azido-alcohol (*R*)-**3** (e.e. >60%) was achieved by using a crude immobilized enzyme preparation derived from *Rhodococcus* sp. (NOVO SP 409). Evidence for the reaction being catalysed by an enzyme is presented.

Recently we have shown that a crude immobilized enzyme preparation derived from *Rhodococcus* sp. (NOVO SP 409) which contains a number of enzymes such as nitrilase, nitrile hydratase, esterase and amidase, also exhibits epoxide-hydrolase activity[1]. The latter potential may be used for the asymmetric hydrolysis of *meso*- or the kinetic resolution of racemic epoxides[2]. Thus, when (±)-2-methyl-2-pentyloxirane (**1**) was subjected to the action of SP 409 in aqueous buffer, the diol (*S*)-**2** and remaining substrate (*R*)-**1** were obtained in 40% and 72% e.e., resp. The selectivity of this resolution (calculated as the enantiomeric ratio E[3]) was E = 4.7. Inspired by recent reports on the asymmetric nucleophilic opening of epoxides by amines catalyzed by crude enzyme preparations such as liver microsomes[4] and porcine pancreatic lipase[5], we investigated whether also azide could be employed as nucleophile. Thus, when the enzymatic hydrolysis of (±)-**1** was carried out in the presence of the non-natural nucleophile azide, the azido-alcohol (*R*)-**3** was formed in addition to the diol (*S*)-**2**[6] (Scheme 1). Bearing in mind that azido-alcohols are precursors of synthetically useful amino-alcohols and in search for evidence that the reaction is catalysed by an enzyme rather than of spontaneous nature, the details of this reaction were investigated by using chiral GLC[7].

Scheme 1. Simultaneous asymmetric hydrolysis and azidolysis of (±)-2-methyl-2-pentyloxirane.

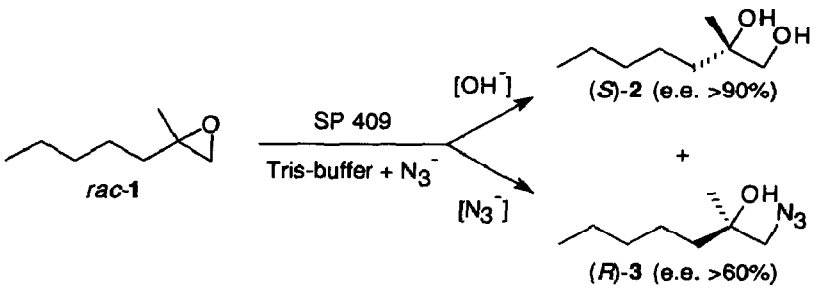
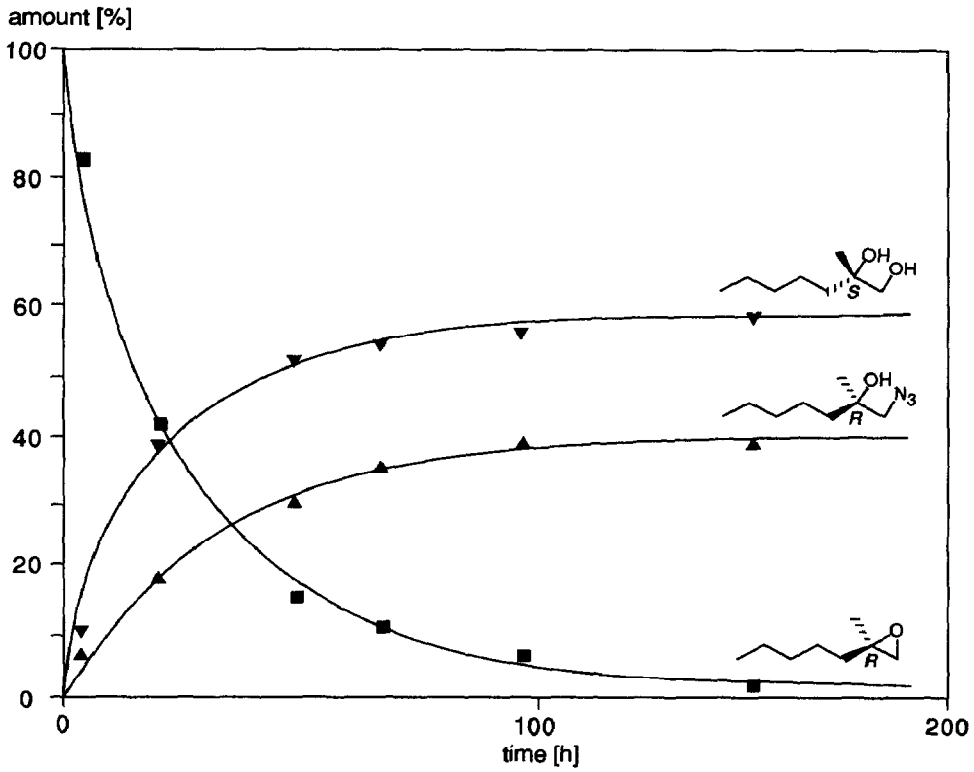


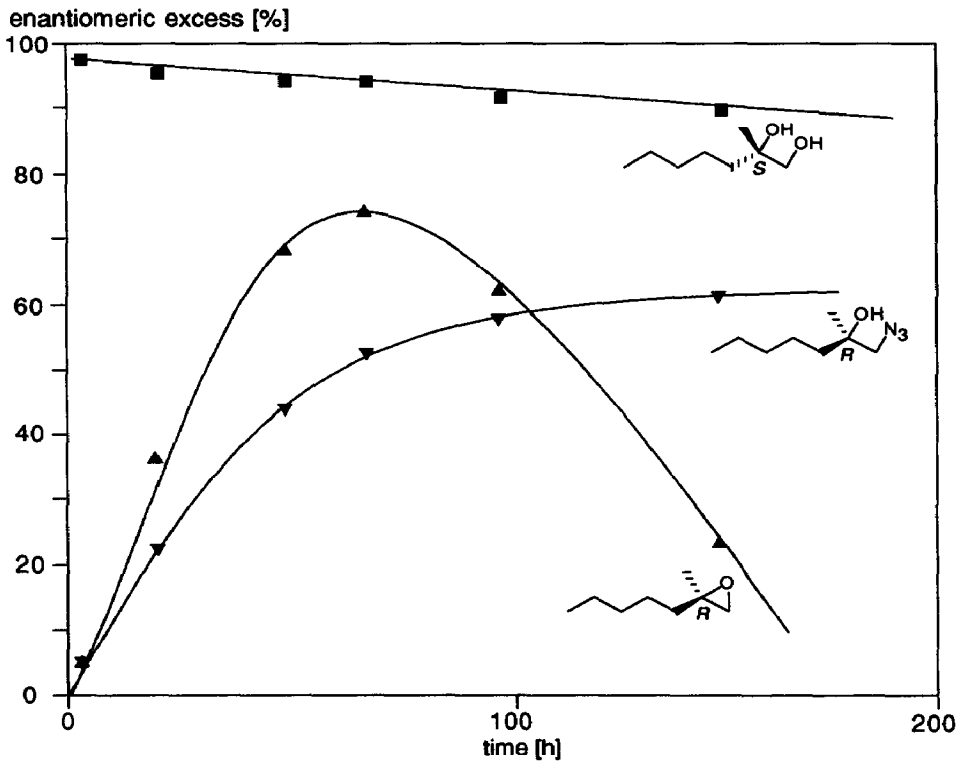
Figure 1 shows the gradual disappearance of epoxide (±)-**1** going in hand with the formation of diol (*S*)-**2** which was expected from our previous findings[1]. On the other hand, at the same time the azido-alcohol (**3**) of *opposite* (*R*)-configuration was formed as second product albeit at a slower rate. No side reactions were detected.

Figure 1. Products from the hydrolysis-azidolysis reaction.



When the optical purity of all components was monitored during the course of the reaction a complex pattern was observed (Figure 2): Diol (*S*)-(2) was formed in very high e.e. (>95%) at the beginning of the reaction. This value declined only slightly during the later stage (e.e. ~90%). The 'non-natural' product azido-alcohol (*R*)-(3) showed the opposite pattern: It was almost racemic at the beginning but reached an optical purity of >60% at a late stage. Finally, the racemic epoxide-substrate (±)-1 reached a maximum in optical purity (e.e. ~75%) at around 80% conversion but this value decreased significantly towards the end of the reaction.

Figure 2. Optical purity of compounds plotted versus time.



The observations described above lead us to the conclusion that the formation of azido-alcohol 3 is not due to a spontaneous reaction of azide with epoxide 1, but rather is *catalyzed by an enzyme*. This hypothesis is supported by the following arguments:

1) The formation of diol 2 and azido-alcohol 3 is negligible in the presence of heat-denaturated enzyme (<10% of the reaction rate).

2) The kinetics of the reaction do not show the typical pattern of a single-step kinetic resolution of an irreversible reaction[3] but rather follows two enantiodivergent independent reaction pathways[8,9]. The enantiomeric excess of the product diol 2 stays constantly high (>90%) throughout the reaction (it should decline towards 0% when the reaction is nearing completion). Furthermore, the e.e. of the substrate

epoxide **1** reaches a maximum at around 80% conversion and drops significantly during the late stage of the reaction (it should reach a maximum there).

3) Assuming that the formation of the azido-alcohol **3** is due to a spontaneous reaction (without asymmetric catalysis) the e.e. of the azido-alcohol formed is only dependent on the e.e. of the epoxide. In other words, both the curves for the dependence of enantiomeric excess *versus* conversion of epoxide **1** and azido-alcohol **3** in Figure 2 must be parallel. This is not the case. Thus, the (*S*)-epoxide - the 'right' enantiomer - is preferred from the racemate and hydrolysed to yield the (*S*)-diol. The (*R*)-epoxide - the 'wrong' antipode - is converted to the (*R*)-azido-alcohol.

The mechanism of this reaction, *i.e.* the question on whether an epoxide hydrolase is capable of accepting azide as 'non-natural nucleophile' (instead of water) or if another enzyme is involved is currently being studied.

Summary: When the biocatalytic asymmetric hydrolysis of (±)-2-methyl-2-pentyloxirane (**1**) using a crude immobilized enzyme preparation derived from *Rhodococcus* sp. was performed in a buffer system containing azide, the simultaneous formation of 2-methylheptane-1,2-diol (*S*)-(**2**) (e.e. >90%) and 1-azido-2-methylheptan-2-ol (*R*)-**3** (e.e. >60%) was observed.

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6. NOVO SP 409 (10g) was suspended in Tris-buffer (400mL, 0.1N, pH 7.0) containing sodium azide (0.25N) for 1h. Then (±)-**1** (1.3g) was added and the mixture was shaken at r.t. (180rpm). The reaction was monitored by chiral GLC (ref. 7) and the peaks of the corresponding enantiomeric products were identified by co-injection with independently synthesized material. Reference material for diol (*S*)-**2** was obtained as previously reported (ref. 1) and azido-alcohol (*R*)-**3** was prepared by reaction of (*R*)-**1** with azide (2 equ. NaN_3 in 85% aqu. EtOH containing 2 equ. NH_4Cl, reflux 12 h). 300 MHz-^{1}H-NMR ($CDCl_3$): δ = 0.8-0.92 (m, 3H, H on C-7), 1.18 (s, 3H, methyl on C-2), 1.22-1.38 (m, 6H, H on C-4, C-5, C-6), 1.44-1.50 (m, 2H, H on C-3), 1.98 (br s, 1H, OH), 3.18-3.30 (dd, 2H, diastereotopic H on C-1, ^{2}J=12 Hz). 75.47 MHz-^{13}C-NMR ($CDCl_3$): δ = 14.1 (C-7), 22.73 (C-6), 23.56 (C-5), 24.64 (C-4), 32.47 (methyl on C-2), 39.87 (C-3), 61.10 (C-1), 73.07 (C-2). IR (film): 2114 cm^{-1} (N_3).
7. Permethyl-β-cyclodextrin J&W Cyclodex B (30mx0.25mm, 0.25μm film, N_2): (±)-**2** 110°C iso, (*S*)-**2** 34.2 min, (*R*)-**2** 35.8 min. [Octakis-(2,6-diphenyl-3-trifluoroacetyl)]-γ-cyclodextrin Astec Chiraldex G-TA (30mx0.25mm, 0.25μm film, H_2): (±)-**1** 50°C iso, (*R*)-**1** 6.4 min, (*S*)-**1** 7.3 min; (±)-**3** 105°C iso, (*R*)-**3** 7.7 min, (*S*)-**3** 8.2 min.
8. For related examples see: Brooks, D.W.; Wilson, M.; Webb, M. *J. Org. Chem.* **1987**, *52*, 2244-2248; Okano, K.; Mizuhara, Y; Suemune, H.; Akita, H.; Sakai, K. *Chem. Pharm. Bull.* **1988**, *36*, 1358-1365. Königsberger, K.; Alphand, V.; Furstoss, R.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 499-500.
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