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Journal of Molecular Catalysis B: Enzymatic 11 (2001) 243–247

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Lactones 7
1. Enantioselective lactonization of racemic ethyl (5,5-dimethyl-2,3-epoxycyclohex-1-yl) acetate

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Abstract

Seven fungi strains were checked as biocatalysts of lactonization of ethyl (5,5-dimethyl-2,3-epoxycyclohex-1-yl)acetate. Two of them transformed the racemic substrate with high efficiency and enantioselectivity. *Rhodotorula rubra* transformed preferentially the (−) enantiomer of substrate, whereas *Fusarium semitectum*, the (+) one. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Biotransformation; Enantioselective lactonization; Hydroxy lactones; *Rhodotorula rubra*; *Fusarium semitectum*

1. Introduction

Microorganisms are one of the most efficient biocatalysts. They have already found successful application in asymmetric synthesis [2,3] and in the resolution of racemic substrates [4,5]. We are interested in the application of microorganisms to the lactonization of epoxy esters. Recently, we have presented results of lactonization of acyclic γ,δ-epoxy esters with the presence of several fungi cultures as biocatalysts [1]. Three of them, *Rhodotorula rubra*, *Botrytis cinerea* and *Fusarium semitectum*, were the most effective. *R. rubra* transformed epoxy esters preferentially to *trans* δ-hydroxy-γ-lactones (ee above 70%) in 70% yield. Application of *B. cinerea* and *F. semitectum* to the lactonization of these substrates afforded γ-hydroxy-δ-lactone in 20–30% yield and 70–100% enantiomeric excess of isomer (−).

Here, we would like to present the results of biolactonization of (±)ethyl (5,5-dimethyl-2,3-epoxycyclohex-1-yl) acetate (**2**).

2. Materials and methods

The purity of intermediates and isolated products was checked by TLC: silica gel DC-Alufolien Kieselgel 6O F_{254} (Merck) with the application of hexane–acetone 9:1 (for epoxy ester) and hexane–acetone 2:1 (for hydroxy lactone) as the developing systems. The same eluents were applied in the course of preparative column chromatography (silica gel: Kieselgel 60, 230–400 mesh) for the separation of product mixtures. GC analysis were performed on Hewlett-Packard 5890 instrument using the follow-

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PII: S1381-1177(00)00081-3

ing capillary columns: HP-1 (racemic compounds), CP-Cyclodextrin (epoxy ester and lactone) and Chirasil-Val-L (diol ester).

All reagents were purchased from Fluka. The starting material — ethyl (5,5-dimetyl-2-cyclohexen-1-yl) acetate (**1**) — was obtained from dimedone as described earlier [6]. Further chemical reactions were carried out as follows:

2.1. Ethyl-(5,5-dimethyl-2,3-epoxycyclohex-1-yl) acetate (2)

m-Chloroperbenzoic acid (1.8 g, 70%, 4 mmol) was added to a solution of ester **1** (1 g, 5 mmol) in methylene chloride (70 cm^3). The reaction mixture was kept at room temperature. When the reaction was completed (TLC, 24 h) the mixture was diluted with ethyl ether (30 cm^3) and washed successively with aqueous solution of Na_2CO_3 and Na_2SO_3, and finally, with water (until neutral). The crude epoxy ester **2** was purified by column chromatography (silica gel, hexane–acetone 9:1) to give 0.87 g (81%) of desired product: $n_D = 1.4535$; ^{1}H NMR ($CDCl_3$) δ: 0.86 and 0.90 (two s, 6H, $-(CH_3)_2C-$, 1.00 (d, $J = 13.0$ Hz, 1H, one of CH_2-6 proton), 1.04 (ddd, $J = 13.0$, 5.7 and 1.9 Hz, 1H, one of CH_2-6 proton), 1.27 (t, $J = 7.0$ Hz, 3H, $-CH_3$), 1.54 (d, $J = 15.3$ Hz, 1H, one of CH_2-4), 1.60 (ddd, $J =$ 15.3, 5.0 and 1.8 Hz, one of CH_2-4), 2.29 (dd, $J = 14.9$ and 5.7 Hz, 1H, one of CH_2-7), 2.71 (m, 1H, H-1), 2.56 (dd, $J = 14.9$ and 7.5 Hz, 1H, one of CH_2-7), 3.10–3.16 (two m, 2H, CH of oxirane ring), 4.17 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3); IR (film, cm^{-1}): 1748 (s), 1188 (m), 1152 (m), 1032 (m).

Acidic lactonization was carried out with perchloric acid, tartaric acid and potassium tartrate. Procedures for these reactions were described earlier [7]. Here, we present the procedure of lactonization, which led to the mixture of diol ester **3** and hydroxy lactone **4**.

Epoxy ester **2** (1 g, 4.5 mmol) was stirred with 100 cm^3 of $HClO_4$ solution ($THF:H_2O:HClO_4$ 10:5:0.1) for 4 h. The products were extracted with ethyl ether. The ethereal solution was washed with saturated $NaHCO_3$ solution, water and brine, and dried ($MgSO_4$). After the separation of this mixture by column preparative chromatography (silica gel, hexane–acetone 2:1), 0.2 g (18%) of diol ester **3** and 0.5 g (63%) of δ-hydroxy-γ-lactone **4** were obtained. Their physical and spectral data are as follows.

2.2. Ethyl (2,3-dihydroxy-5,5-dimethylcyclohex-1-yl) acetate (3)

^{1}H NMR ($CDCl_3$) δ: 0.95 and 1.06 3H, (two s, 6H, $-C(CH_3)_2-$), 1.27 (t, $J = 7.1$ Hz, $-OCH_2CH_3$), 2.32 (m, 1H, one of $-CH_2$-7), 2.51–2.60 (three m, one of $-CH_2$-7, O*H* and H-1), 3.66 (dd, $J = 5.1$ and 3.1 Hz, 1H, H-2), 3.88 (td, $J = 5.1$ and 4.0 Hz, 1H, H-2), 4.15 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), IR (film, cm^{-1}): 3300 (s,b), 1712 (s), 1224 (s), 1296 (s).

2.3. 2-Hydroxy-4.4-dimethyl-9-oxabicyclo-[4.3.0]nonan-8-one (4)

mp. 53–54°C; ^{1}H NMR ($CDCl_3$) δ: 1.01 and 1.04 (two s, 6H, $-C(CH_3)_2-$), 1.24–1.42 (m, 3H, CH_2-5 and one of CH_2-3), 1.59 (dd, $J = 14.0$ and 4.2 Hz, 1H, one of CH_2-3), 2.26 (d, $J = 13.8$ Hz, 1H, one of CH_2-7), 2.35 (s, broad, 1H, $-OH$), 2.76 (dd, $J = 13.8$ and 8.3 Hz, 1H, one of CH_2-7), 4.06 (dddd, $J =$ 5.9, 5.5, 5.2 and 4.2 Hz, 1H, H-3), 4.40 (t, $J = 5.9$ Hz, 1H, H-1); IR (KBr, cm^{-1}): 3464 (s,b), 1784 (s), 1183 (s), 1028 (m).

Biotransformations were carried out as described below.

Cultivation of microorganisms was carried out in the medium containing peptone (10 g), glucose (40 g) and agar (80 g) in water solution (1 dm^3) at 28°C and stored in refrigerator at 4°C.

2.4. Screening procedure

The microorganisms were cultivated at 25°C in 300 cm^3 Erlenmayer flasks containing 75 cm^3 of the following nutrient: 1% solution of peptone and glucose (3%) in deionized water or brew from potatoes (750 g/1 dm^3) enriched with glucose (3%). After 3–5 days of growth, 10 mg of substrate in 0.5 cm^3 of ethanol was added to the shaken cultures. The transformation was continued for 4 days. The products were extracted with ethyl ether and analysed by

Table 1
Composition (in % according to GC) of the product mixtures of acidic lactonization of **2**

Entry	pH	**2**	**4**	**3**
1	7.00	100	–	–
2	6.38	100	–	–
3	5.55	100	–	–
4	4.55	100	–	–
5	3.83	94.7	5.3	–
6	2.48	18.4	68.5	–
7	1.50	5.3	51.5	43.3
8	1.15	0	17.0	83.0

TLC (eluent: hexane–isopropyl alcohol–acetone–ethyl acetate 60:3:1:1) and GC (Ultra-1, HP-5, CP-Cyclodextrin, Chirasil-Val-L). The results of GC analysis are presented in Table 1.

2.5. Preparative biotrasformation

Epoxy ester **2** (5×30 mg) in 0.5 cm^3 of ethanol was added to the culture of *R. rubra* (five flasks with 75 cm^3 of the nutrient brew from potatoes enriched with glucose). After 4 days of shaking at 26°C, the products were extracted with ethyl ether. The ethereal solution was washed with brine and dried ($MgSO_4$). The solvent was evaporated and the crude product (130 mg mixture of epoxy ester **2**, lactone **4** and metabolites of microorganism) was separated by column chromatography (silica gel, hexane–isopropyl alcohol–acetone–ethyl acetate 60:3:1:1). The pure (+)-epoxy ester **2** (24 mg) and δ-hydroxy-γ-lactone **4** (32 mg) were isolated.

Enatiomer (−) of epoxy ester **2** (35 mg) and δ-hydroxy-γ-lactone **4** (63 mg) were obtained from preparative biotransformation (9 days) of epoxy ester **2** (360 mg) with *F. semitectum.*

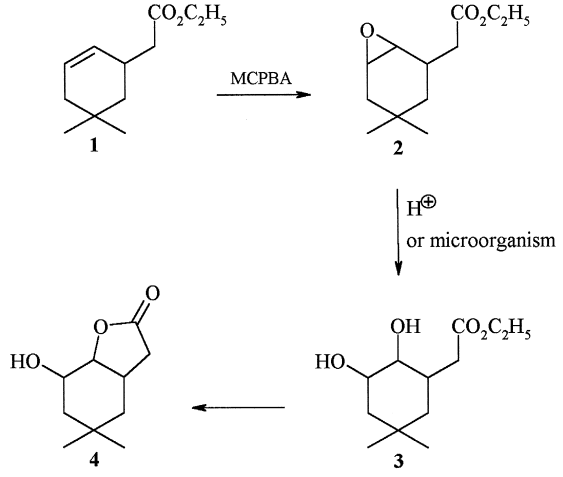
3. Results and discussion

The substrate for biotransformations — epoxy ester **2** — was obtained by of oxidation of known [6] ester **1** with m-chloroperbenzoic acid (Scheme 1). The formation of only one epoxide was indicated by both — thin layer and gas chromatography. It was also proved by ^{1}H NMR spectrum. The lack of coupling of H-1 with one of CH_2-6 group proton in ^{1}H NMR spectrum of epoxy ester **2** confirmed the *trans* orientation of oxirane ring to pseudoaxial methyl group at C-5, as well as the pseudoequatorial orientation of methylcarbethoxy group. Additionally, the small difference of chemical shifts of methyl groups ($\Delta\delta = 0.04$) confirmed the *trans* orientation of oxirane ring to the pseudoaxial methyl group at C-5, as well as a half-chair conformation of cyclohexane ring in which the C-1, C-2, C-3 and C-4 atoms are situated in the same plane.

Acidic ($HClO_4$, tartaric acid) lactonization of racemic epoxy ester **2** afforded mixture of hydroxy lactone **4** and diol ester **3**. The composition of the product mixture depended on the pH of the medium (Table 1).

The lactonization carried out at room temperature and pH = 1.15 afforded the mixture of hydroxy lactone **4** (83%) and diol ester **3** (17%). When the pH of the medium was 4.55 or above, the lactonization of epoxy ester **2** did not occur. Both the diol ester **3** and hydroxy lactone **4** were isolated in racemic forms. The monitoring of the progress of the lactonization by GC led to the conclusion that the transformation of epoxy ester **2** into hydroxy lactone **4** proceeds through the diol ester **3**, which is formed as a result of electrophilic opening of oxirane ring.

The *trans* diaxial orientation of hydroxy groups in **3** was confirmed by coupling constants between H-1,



Scheme 1.

H-2 and H-3 found in the ^{1}H NMR spectrum. Doublet ($J_{\text{H-2, H-3}} = 5.1$ Hz) of doublets ($J_{\text{H-2, H-1}} = 3.1$ Hz) was observed for proton H-2. The second coupling constant confirmed the axial orientation of H-1, as well as the equatorial position of carbethoxymethyl group at C-1. The coupling constants of H-3 with H-4a (4.0 Hz) and with H-4e (5.1 Hz) confirmed its equatorial orientation and axial orientation of hydroxyl group at C-3. Such orientation of this group and its nearness to axial methyl group at C-5 caused relatively considerable differentiation of methyl groups in respect to their chemical shift ($\Delta\delta = 0.11$). The axial orientation of hydroxyl group at C-2 in hydroxy lactone **4** was also confirmed by ^{1}H NMR spectral data. The coupling constants of H-2 with neighbouring protons (5.9, 5.5 and 4.2 Hz) indicated its equatorial orientation. The coupling ($J = 5.2$ Hz) of this proton with hydroxyl proton was also observed. This last coupling is not present in the spectrum of acetate of hydroxy lactone **4**. The axial orientation of C—O bond of the lactone ring was also confirmed by ^{1}H NMR data. The triplet of H-1 at $\delta = 4.40$ with coupling constant $J = 5.9$ Hz indicates its equatorial orientation.

In order to obtain hydroxy lactone **4** in enantiomerically pure forms, we carried out several lactonization with microorganisms as biocatalysts. Seven fungi cultures were checked as biocatalysts of lactonization of epoxy ester **2** (Table 2). The biotransformations were carried out in the medium with phosphorane buffer (pH = 6.98).

The results presented in the Table 2 indicate that most of the microorganisms transformed the substrate into δ-hydroxy-γ-lactone **4**. Diol ester **3** was observed in the product mixtures obtained from biotransformations with *B. cinerea* (entries 3,4) and *Pholiota aurivella* (entry 9). Two species: *Alternaria alternata* and *F. semitectum* transformed epoxy ester **2** in small extent. After 4 days, above 70% of substrates was still present in the product mixture. The most effective biotransformation was observed when *B. cinerea* (entries 3,4) and *Yarrowia lipolytica* (entry 12) were applied. Two microorganisms, *F. semitectum* (entries 7,8) and *R. rubra* (entries 10,11) transformed epoxy ester **2** mostly into δ-hydroxy-γ-lactone (**4**) with relatively high enantioselectivity (Fig. 1). *R. rubra* (entries 10,11) transformed preferentially enantiomer (−) of epoxy ester **2**, whereas *F. semitectum*, the enantiomer (+). In the lactonization with the first microorganism, the (+) enantiomer of hydroxy lactone **4** was formed with 78% ee, and during the transformation with the second

Table 2
Compositions (in % according to GC) of the product mixtures of microbial lactonization of (±) — **2**

Entry	Strains	Time (days)	**2**		**4**		**3**	
			(%)	ee (%)	(%)	ee (%)	(%)	ee (%)
1	*A. alternaria*	4	72	+(34)	8	+(42)	–	–[a]
2		6	56	+(54)	28	+(53)	–	–[a]
3	*B. cinerea*	4	3	−(20)	43	−(59)	46	(4)[a]
4		6	2	−(21)	32	−(22)	28	(2)[a]
5	*F. avenaceum*	3	69	+(56)	4	+(65)	–	–[a]
6		4	52	+(46)	3	+(51)	–	–[a]
7	*Fusarium semitectum*	4	82	−(20)	18	−(36)	–	–
8		11	19	−(100)	81	−(22)	–	–
9	*Pholiota aurivella*	4	19	−(56)	25	−(42)	23	(63)[a]
10	*R. Rubra*	3	45	+(100)	48	+(78)	–	–[a]
11		4	38	+(100)	54	+(62)	–	–[a]
12	*Yarrowia lipolytica*	4	2	+(100)	38	−(5.2)	–	–[a]

[a] Unidentified compounds were also present in the product mixture.

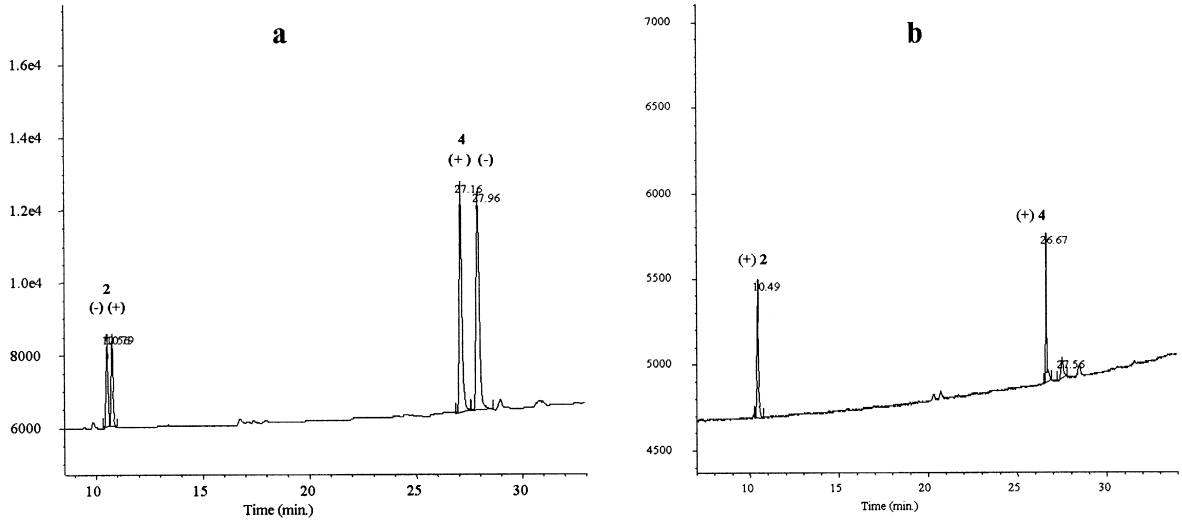


Fig. 1. Chromatogram (a) racemic micture of epoxy ester **2** and racemic hydroxy lactone **4**, (b) crude product mixture of preparative lactonization of **2** with *R. rubra*.

one, the formation of (−) enantiomer of **4** with 36% ee was observed.

The results of biotransformation with these microorganisms show, that there is a possibility to obtain the slowly transformed pure enantiomers of epoxy ester **2**, as well as after their chemical lactonization pure enantiomers of hydroxy lactones **4**. Preparative (150 mg) lactonization of **2** with *R. rubra* afforded slowly reacted enantiomer (+) of epoxy ester **2** (ee = 95%, $[\alpha]_D^{20} = +34.9$ ($c = 0.84$, $CHCl_3$) (Fig. 1), which when subjected to acidic ($HClO_4$) lactonization gave the (−)-δ-hydroxy-γ-lactone **4** (ee = 95%, $[\alpha]_D^{25} = -15.4$ ($c = 0.7$, $CHCl_3$).

The (−) enantiomer of epoxy ester **2** was also obtained, but with some difficulties. Preparative (360 mg) lactonization with *F. semitectum* after 7 days afforded the mixture of unreacted epoxy ester **2** (53%), hydroxy lactone **4** (29%), and diol ester **3** (17%). After 9 days, only unreacted (−) epoxy ester (43%) and hydroxy lactone **4** (57%) were present in the product mixture. However, slowly transformed (−) enantiomer of **2** was isolated in low yield (9.6%). The enantiomeric excess determined by GC was 97%, and optical rotation $[\alpha]_{589}^{27.9} = -28.8$ ($c =$ 1.07, $CHCl_3$). This epoxy ester was also lactonized under acidic conditions ($HClO_4$, pH = 1.15) to afford (+) enantiomer of δ-hydroxy-γ-lactone **4** in 47% yield. The optical rotation of the obtained sample (ee 97%) was $[\alpha]_{589}^{27.9} = +15.5$ ($c = 0.8$, $CHCl_3$).

Acknowledgements

We thank the Agricultural University of Wrocław for financial support (Grant no. 512).

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