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Lactones 42. Stereoselective enzymatic/microbial synthesis of optically active isomers of whisky lactone ☆

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ABSTRACT

Two different methods, enzyme-mediated reactions and biotrasformations with microorganisms, were applied to obtain optically pure *cis*- and *trans*-isomers of whisky lactone **4a** and **4b**. In the first method, eight alcohol dehydrogenases were investigated as biocatalysts to enantioselective oxidation of racemic *erythro*- and *threo*-3-methyloctane-1,4-diols (**1a** and **1b**). Oxidation processes with three of them, alcohol dehydrogenases isolated from horse liver (HLADH) as well as recombinant from *Escherichia coli* and primary alcohol dehydrogenase (PADH I), were characterized by the highest degree of conversion with moderate enantioselectivity (*ee* = 27–82%) of the reaction. In all enzymatic reactions enantiomerically enriched not naturally occurring isomers of *trans*-(−)-(4*R*,5*S*)-**4b** or *cis*-(+)-(4*R*,5*R*)-**4a** were formed preferentially. In the second strategy, based on microbial lactonization of γ-oxoacids, naturally occurring opposite isomers of whisky lactones were obtained. *Trans*-(+)-(4*S*,5*R*)-isomer (*ee* = 99%) of whisky lactone **4b** was stereoselectively formed as the only product of biotransformations of 3-methyl-4-oxooctanoic acid (**5**) catalyzed by *Didimospheria igniaria* KCH6651, *Laetiporus sulphurens* AM525, *Chaetomium* sp.1 KCH6670 and *Saccharomyces cerevisiae* AM464. Biotransformation of γ-oxoacid **5**, in the culture of *Beauveria bassiana* AM278 and *Pycnidiella resinae* KCH50 afforded a mixtures of *trans*-(+)-(4*S*,5*R*)-**4b** with enantiomeric excess *ee* = 99% and *cis*-(−)-(4*S*,5*S*)-**4a** with enantiomeric excesses *ee* = 77% and *ee* = 45% respectively.

1. Introduction

First identified in 1970 by Suomalainen and Nykanen (1970) "oak lactone" was reported as a single compound in a range of alcoholic beverages that had been matured in oak barrels. It was later demonstrated that there were actually two oak lactone structures (Masuda & Nishimura, 1971). Two diastereoisomers of oak lactone were isolated and identified from three wood species; *Quercus mongolica*, *Quercus serrata* and a white oak (from North America, unreported species). The other term "whisky lactone" arise due to its initial isolation in aged whisky. It was identified as essential flavour component of aged alcoholic beverages such as whisky, cognac and brandy. Nowadays, it is also used as ingredient of flavours beverages, candy, biscuit, bread and variety of baked food, wine and tobacco.

Gunther and Mosandl separated four stereoisomers of the whisky lactone and determined their odoriferous properties (Brenna, Fuganti, Gatti, & Serra, 2011; Mosandl & Gunther, 1989). In general, the isomers odours were described as being reminiscent of coconut; the *cis*-isomers were woody and earthy, while the *trans*-isomers resembled celery. However, it has been established that oak wood contains only the (4*S*,5*S*)-*cis* and the (4*S*,5*R*)-*trans*-isomers of oak lactone (Abbott, Puech, Bayonove, & Baumes, 1995). Of the two, the *cis*-isomer is considered to be the more important in sensory terms, because of higher concentrations than the *trans*-isomer in all types of wines. It is worth to mention that commercially available oak lactone is a mixture of *cis* and *trans*-isomers, each as a racemate. There is the need to develop a concise synthetic pathway from which all four stereoisomers of oak lactone can be prepared.

During recent years biocatalysis is becoming key component in the toolbox available to the synthetic chemists for the synthesis of enantiomerically enriched compounds (Brenna et al., 2011). Under the term "biocatalysis" we distinguish transformations of a substrate catalyzed by (1) whole cell or resting cell systems (biotransformations) (Gładkowski et al., 2007; Olejniczak & Ciunik, 2004; Olejniczak, Gawroński, & Wawrzeńczyk, 2001; Olejniczak & Wawrzeńczyk, 2000; Ratuś, Gładkowski, & Wawrzeńczyk, 2009) or (2) by crude extracts or partially purified enzyme (enzyme catalysis) (Boratyński, Kiełbowicz, & Wawrzeńczyk, 2010; Chojnacka, Obara, & Wawrzeńczyk, 2007; Fajkowska, Obara, & Wawrzeńczyk, 2007).

Recently we have published the novel synthesis of δ- and ε-lactones with application of alcohol dehydrogenase from horse liver

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(Boratyński et al., 2010). The results obtained indicate that horse liver alcohol dehydrogenase (HLADH) can be successfully used as biocatalyst in enantioselective synthesis of δ- and ε-lactones from primary-secondary 1,5-diols and 1,6-diols, respectively. Therefore, it was proposed to check whether primary-secondary 1,4-diols **1a** and **1b** were a good substrates for a series of commercially available alcohol dehydrogenases to receive corresponding γ-lactones **4a** and **4b**.

Additionally, to explore effective and low-cost methods for the synthesis of enantiomerically pure lactones, our research task focused on evaluation the stereoselectivity of some microorganisms, mostly filamentous fungi, in the lactonisation of racemic γ-oxoacid **5**. It is widely accepted that baker's yeast plays the most traditional and useful role (Santaniello, Ferraboschi, Grisenti, & Manzocchi, 1992), although yeasts belonging to other genera have been found to be able to compete with the more common *Saccharomyces cerevisiae* (Hasegawa, Uosaki, Adachi, & Matsuno, 1996). The present study was aimed at the looking for other biocatalysts capable to synthesis of both enantiomers of the diastereomeric whisky lactones by the use of reductive enzymes in the enantiodifferentiating step.

In this paper, the results of the application of biocatalytic methods, either biotrasformations with whole cell systems of γ-oxoacid **5** or enzyme-mediated oxidation reactions of 1,4-diols **1a** and **1b**, to the preparation of enantiomerically enriched isomers of whisky lactones (**4a** and **4b)** are reported.

2. Materials and methods

2.1. Chemicals

Mixture of isomers of whisky lactones (**4a** and **4b**) were purchased from Sigma–Aldrich.

2.2. Biocatalysts

2.2.1. Enzymes and coenzymes

The following alcohol dehydrogenases were used: horse liver alcohol dehydrogenase (HLADH) from Sigma–Aldrich; yeast alcohol dehydrogenase (YADH) from Sigma–Aldrich; horse liver alcohol dehydrogenase recombinant from *Escherichia coli* (HLADH-rec) from Evocatal GMBH; *Lactobacillus kefir* alcohol dehydrogenase (LKADH) from Fluka; primary alcohol dehydrogenase screening kit (PADH I, II and III) from Codexis. *Bacillus stearothermophilus* alcohol dehydrogenase (BSADH) was a generous gift from Protein Biochemistry Institute, Naples, Italy.

Coenzymes: nicotinamide adenine dinucleotide (NAD^+), flavin mononucleotide (FMN) were purchased from Sigma–Aldrich, while nicotinamide adenine dinucleotide phosphate ($NADP^+$) were purchased from Codexis.

2.2.2. Microorganisms

Absidia cylindrospora AM336, *Aspergillus candidus* AM386, *Aspergillus glaucus* AM211, *Aspergillus nidulans* AM243, *Aspergillus ochraceus* AM370, *Aspergillus* sp. AM31, *Bacillus subtilis* 901, *Beauveria bassiana* AM278, *Candida utilis* AM469, *Candida viswanathi* AM120, *Cenangium ferruginosum* KCH56, *Chaetomium indicum* AM158, *Chaetomium* sp. AM432, *Chaetomium* sp.1 KCH6670, *Didimospheria igniaria* KCH6651, *Fusarium avenaceum* AM11, *Fusarium culmorum* AM7, *F. culmorum* AM9, *Fusarium equiseti* AM15, *Humicola grisea* AM560, *Laetiporus sulphurens* AM514, *L. sulphurens* AM524, *L. sulphurens* AM525, *Melogramma camphylosporum* AM563, *Mortierella isabellina* AM212, *Mucor circinelloides* AM148, *Mucor globosus* AM167, *Penicillium albidum* AM79, *Penicillium camembertii* AM83, *Penicillium citrinum* AM354, *Penicillium diversum* AM388, *Penicillium lilacinum* AM111, *Penicillium notatum* AM904, *Penicillium purpurogenum* AM80, *Penicillium spinulosum* AM114, *Penicillium thomi* AM91, *Pholiota aurivella* AM521, *Piptoporus betulinus* KCH39, *Pycnidiella resinae* KCH50, *Rhizopus nigricans* AM394, *Rhodotorula marina* AM77, *Rhodotorula rubra* AM4, *S. cerevisiae* AM464, *Sclerophoma pythiophila* KCH55 were obtained from Department of Chemistry at Wroclaw University of Environmental and Life Sciences (Poland) and Institute of Biology and Botany Medical University (Wroclaw, Poland). Microorganisms were cultivated on a Sabouraud agar consisting of: aminobac 5 g, peptone K 5 g, glucose 40 g and agar 15 g in distilled water 1 l at 28 °C and pH 5.5 and stored in refrigerator at 4 °C.

2.3. Analysis

The progress of the reaction and the purity of synthesized products was checked by thin layer chromatography (TLC) and gas chromatography (GC). The TLC analyses were carried out by using aluminium foil plates coated with silica gel. Compounds were detected by spraying the plates with 1% $Ce(SO_4)_2$ and 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4. The crude products were purified by preparative column chromatography using silica gel (Kieselgel 60, 230–400 mesh, Merck) with hexane–acetone mixture (various ratios) as an eluent.

Gas chromatography analyses were performed on Agilent Technologies 6890N and Varian Chrompack CP-3380 instruments, using HP-20M-Carbowax (cross linked phenyl methyl siloxane) capillary column (25 m × 0.32 mm × 0.3 μm); DB-17 (cross linked phenyl methyl siloxane) capillary column (30 m × 0.25 mm × 0.25 μm). Enantiomeric and diastereoisomeric excess were determined on a CP7502 Chirasil-DEX CB column (25 m × 0.25 mm × 0.25 μm) with the following temperature programme: 80–200 °C at 5 °C/min, hold at 200 °C for 3 min. The total run time was 27 min. The retention times for each whisky lactone isomers were identified as: t_R *trans*-(+)-(4*S*,5*R*)-**4b** = 10.75, t_R *trans*-(−)-(4*R*,5*S*)-**4b** = 11.01, t_R *cis*-(−)-(4*S*,5*S*)-**4a** = 11.90, t_R *cis*-(+)-(4*R*,5*R*)-**4a** = 12.06. The retention time values were determined for enantiomerically enriched samples and assigned to corresponding isomers taking into account assignations reported by Wilkinson (Wilkinson, Elsey, Prager, Tanaka, & Sefton, 2004).

The structures of the compounds were determined on the basis of ^1H NMR, ^{13}C NMR, Dept-135, HMQC, COSY and IR spectral data. ^1H NMR spectra were recorded for $CDCl_3$ solutions on Bruker Avance DRX 600 (600 MHz) spectrometer. IR spectra were determined using FTIR Thermo-Mattson IR 300 Spectrometer. Optical rotations were measured on an Autopol IV automatic polarimeter (Rudolph). The pH measurements were conducted on a HI 9321 Microprocesor pH Metre equipped with a glass electrode.

2.4. Synthesis of substrates for biotransformations

Erythro- and *threo*-3-methyloctane-1,4-diols (**1a** and **1b**), substrates for enzyme-mediated reactions, were prepared by the reduction of corresponding racemic *cis*- and *trans*-isomers of whisky lactones (**4a** and **4b**) with $LiAlH_4$, according to the method described by us earlier (Boratyński et al., 2010). Substrate for biotransformations, 3-methyl-4-oxooctanoic acid (**5**) was obtained as a result of five-step synthesis from 1-bromopropane and crotonaldehyde according to the procedure for synthesis of other γ-oxoacids homologues described previously (Obara et al., 2008; Smuga, 2008).

2.5. Biotransformations of racemic 3-methyl-4-oxooctanoic acid (**5**)

2.5.1. Screening procedure

The fungal and bacterial strains were transferred from the slants to 250-ml Erlenmeyer flask containing 75 ml of medium (3% glucose, 1% aminobac in water). After cultivation at 25 °C for

four days on a rotary shaker, 0.02 g of substrate **5**, dissolved in 1.0 ml of acetone was added to the grown cultures. In control experiments, the substrates were incubated in the medium without fungi. For the time-course analysis after 1, 2 and 4 days, 10 ml of the transformation mixture were taken out and extracted three times with dichloromethane. The extracts were dried over $MgSO_4$ and concentrated *in vacuo*. Residues were dissolved in 1 ml of acetone and analyzed by TLC and GC. Screening procedure was carried out for 44 strains on 3,7-dimethyl-4-oxooctanoic acid, a representative compound of series of oxoacids we have studied. The screening of microorganisms led to the selection of six strains (*B. bassiana* AM278, *Chaetomium* sp.1 KCH6670, *D. igniaria* KCH6651, *L. sulphurens* AM525, *P. resinae* KCH50, *S. cerevisiae* AM464) that had ability to biotransformation of 3,7-dimethyl-4-oxooctanoic acid. The selected strains have been applied in microbial lactonization of 3-methyl-4-oxooctanoic acid (**5**).

2.5.2. Preparative biotransformation

In order to isolate and identify the products, preparative-scale biotransformation were performed fivefold in the same conditions as screening procedure. After 2 days of incubation the mixtures were extracted three times with dichloromethane, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product mixtures were separated by column chromatography (silica gel, eluent hexane/diethyl ether 6:1). Pure biotransformation products were identified by means of spectral analyzes and optical rotation measurements.

*2.5.2.1. Biotransformation of (±)-**5** by B. bassiana AM278.* Biotransformation of (±)-oxoacid **5** (0.1 g), after 24 h, gave a mixture of: unreacted (−)-**5** (48%, *ee* = 10%), (−)-(4*S*,5*S*)-**4a** (16%, *ee* = 85%) and (+)-(4*S*,5*R*)-**4b** (36%, *ee* = 99%). The column chromatography of this mixture afforded 0.01 g (10% yield) of (−)-(4*S*,5*S*)-**4a**, *ee* = 85%, $[\alpha]_D^{20} = -65^\circ$ (*c* 0.33, $CHCl_3$), ((Wilkinson et al., 2004): (−)-(4*S*,5*S*)-**4a** *ee* = 99%, $[\alpha]_D^{20} = -74^\circ$, (*c* 0.42, CH_3OH)). The biotransformation of (±)-**5** for longer time (48 h) decrease the enantiomeric excess of (−)-(4*S*,5*S*)-**4a** to *ee* = 77%.

*2.5.2.2. Biotransformation of (±)-**5** by Chaetomium sp. 1 KCH6670.* Biotransformation of (±)-oxoacid **5** (0.1 g), after 24 h, gave (+)-(4*S*,5*R*)-**4b** (100%, *ee* = 99%) as the only product. The column chromatography afforded 0.04 g (40% yield) of (+)-(4*S*,5*R*)-**4b**, *ee* = 99%, $[\alpha]_D^{20} = +95^\circ$ (*c* 0.54, $CHCl_3$), ((Wilkinson et al., 2004): (+)-(4*S*,5*R*)-**4b** *ee* = 99%, $[\alpha]_D^{20} = +100^\circ$).

*2.6. Enzymatic oxidation of racemic erythro- and threo-3-methyloctane-1,4-diols (**1a** and **1b**)*

2.6.1. Screening procedure

In the screening experiments 3-methyloctane-1,4-diol (**1a**-**b**) (0.02 g) and coenzymes: $NAD(P)^+$ (0.007 g) and FMN (0.2 g) were dissolved in 0.1 M glycine–NaOH buffer (15 ml) at different value of pH (7.2, 8.5, 9.0) and temperature (25, 35, 45 °C). The pH of the mixture was readjusted with 2 M NaOH. Then, the enzyme solution (0.002 g) dissolved in buffer (2 ml) was added dropwise. To determine the stability of the substrate the control sample, containing a buffer with the substrate without the addition of the enzyme, was conducted. Changes in pH during the biotransformation was monitored using a pH-metre. Samples (1 ml) of the reaction mixtures were taken after several time intervals (2, 24, 48 and 120 h). The aqueous phase was acidified to pH = 3, washed with NaCl, then extracted with $CHCl_3$ (1 ml). The extract was dried ($MgSO_4$) and analyzed by GC.

2.6.2. Preparative enzymatic oxidation

The preparative-scale transformations were performed in the same conditions as screening experiments in five 20 ml vials using 0.1 g of 3-methyloctane-1,4-diol (**1a**–**b**) (5 × 0.02 g), 0.035 g of $NAD(P)^+$ (5 × 0.007 g), 1 g FMN (5 × 0.2 g) and 0.01 g enzyme (5 × 0.002 g). When the reaction was completed the reaction mixture was extracted by hydrodistillation method using a Deryng apparatus. The yields and optical rotations of products obtained are given below. The spectral data for compounds obtained are also presented.

*2.6.2.1. Preparative oxidation of (±)-**1a** by PADH II.* Oxidation of (±)-**1a** (0.1 g), after 48 h, gave a mixture of the following products: (+)-(4*R*,5*R*)-**4a** (45%, *ee* = 46%) and mixture of hemiacetals **2a** and **3a** (55%). The column chromatography of this mixture afforded 0.041 g (41% yield) of (+)-(4*R*,5*R*)-**4a**, *ee* = 46%, $[\alpha]_D^{20} = +16.2^\circ$ (*c* 4.0, $CHCl_3$) ((Wilkinson et al., 2004): (+)-(4*R*,5*R*)-**4a** *ee* > 99%, $[\alpha]_D^{20} = +79^\circ$ (*c* 0.5, CH_3OH)) and 0.04 g (40%) of hemiacetals **2a** and **3a**.

*2.6.2.2. Preparative oxidation of (±)-**1a** by HLADH recombinant from E. coli.* Oxidation of (±)-**1a** (0.1 g), after 24 h, gave a mixture of: unreacted (±)-diol **1a** (27%), (+)-(4*R*,5*R*)-**4a** (55%, *ee* = 64%) and mixture of hemiacetals **2a** and **3a** (18%). The column chromatography of this mixture afforded 0.048 g (48% yield) of (+)-(4*R*,5*R*)-**4a**, *ee* = 64%, $[\alpha]_D^{20} = +18.8^\circ$ (*c* 3.1, $CHCl_3$), 0.016 g (16%) of hemiacetals **2a** and **3a** and 0.021 g (21% yield) of unreacted (±)-diol **1a**. The oxidation of (±)-**1a** for longer time (120 h) let to obtain (±)-(4*R*,5*R*)-**4a** as the only product in high (94%) yield.

*2.6.2.3. Preparative oxidation of (±)-**1a** by HLADH.* Oxidation of (±)-**1a** (0.1 g), after 24 h, gave a mixture of the following products: (+)-(4*R*,5*R*)-**4a** (42%, *ee* = 56%) and mixture of hemiacetals **2a** and **3a** (58%). The column chromatography of this mixture afforded 0.038 g (38% yield) of (+)-(4*R*,5*R*)-**4a**, *ee* = 56%, $[\alpha]_D^{20} = +29.1^\circ$ (*c* 4.8, $CHCl_3$) and 0.055 g (55%) of hemiacetals **2a** and **3a**.

*2.6.2.3.1. Cis-5-butyl-4-methyltetrahydrofuran-2-ols (mixture of isomers; **2a** and **3a**) (Fig. 1).* Colorless liquid, ***trans*** (**3a**) and ***cis*** (**2a**) isomers of the composition 77 : 23%; **^{1}H NMR** (600 MHz, $CDCl_3$) δ: 0.89 (d, *J* = 7.1 Hz, 3H, CH_3-4 of **3a**); 0.90 (t, *J* = 7.1 Hz, 3H, CH_3-4′ of **3a**); 0.91 (t, *J* = 7.1 Hz, 3H, CH_3-4′ of **2a**); 1.05 (d, *J* = 7.0 Hz, 3H, CH_3-4 of **2a**); 1.25–1.48 (m, 12H, CH_2-1′, CH_2-2′, CH_2-3′ both isomers); 1.62 (m, 1H one of CH_2-3 z **2a**); 1.80 (dt, *J* = 13.3, 5.3 Hz, 1H, one of CH_2-3 of **3a**); 2.00 (ddd, *J* = 13.3, 7.4, 2.9 Hz, 1H, one of CH_2-3 of **3a**); 2.20–2.30 (m, 2H, one of CH_2-3 of 2, H-4 of **2a**); 2.38 (m, 1H, H-4 of **3a**) 2.87 (s, 1H, OH of **3a**); 2.99 (s, 1H,

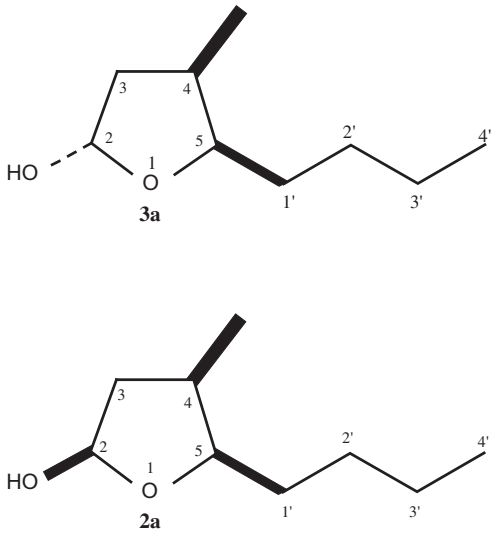


Fig. 1. *Cis*-5-butyl-4-methyltetrahydrofuran-2-ols (mixture of isomers; **2a** and **3a**).

OH of **2a**); 3.88 (ddd, J = 9.0, 5.5, 4.8 Hz, 1H, H-5 of **2a**); 4.12 (dt, J = 8.3, 5.3 Hz, 1H, H-5 of **3a**); 5.43 (dd, J = 5.6, 2.6 Hz, 1H, H-2 of **2a**); 5.52 (dd, J = 5.4, 2.9 Hz, 1H, H-2 of **3a**); **^{13}C NMR** (150 MHz, $CDCl_3$): δ: 14.06 (CH_3-4 of **3a**), 14.51 (C-4′ of **3a**), 14.52 (C-4′ of **2a**), 14.75 (CH_3-4 of **2a**), 22.70, 22.84, 28.72, 29.71, 30.04, 30.89 (C-1′, C-2′, C-3′ both isomers), 34.41 (C-4 of **3a**), 34.81 (C-4 of **2a**), 41.50 (C-3 of **2a**), 42.12 (C-3 of **3a**), 80.72 (C-5 of **3a**), 82.97 (C-5 of **2a**), 97.41 (C-2 of **3a**), 98.46 (C-2 of **2a**); **IR** (film, cm^{-1}): 3408 (m), 1456 (m), 1062 (m).

*2.6.2.4. Preparative oxidation of (±)-**1b** by PADH II.* Oxidation of (±)-**1b** (0.1 g), after 48 h, gave a mixture of: unreacted (±)-diol **1b** (41%), (−)-(4*R*,5*S*)-**4b** (56%, *ee* = 82%) and mixture of hemiacetals **2b** and **3b** (3%). The column chromatography of this mixture afforded 0.044 g (44% yield) of (−)-(4*R*,5*S*)-**4b**, *ee* = 82%, $[\alpha]_D^{20} = -84^\circ$ (*c* 0.2, $CHCl_3$), ((Wilkinson et al., 2004): (−)-(4*R*,5*S*)-**4b** *ee* > 99%, $[\alpha]_D^{20} = -97^\circ$ (*c* 0.34, CH_3OH)).

*2.6.2.5. Preparative oxidation of (±)-**1b** by HLADH.* Oxidation of (±)-**1b** (0.1 g), after 24 h, gave a mixture of: unreacted (±)-diol **1b** (55%), (−)-(4*R*,5*S*)-**4b** (31%, *ee* = 27%) and mixture of hemiacetals **2b** and **3b** (14%). The column chromatography of this mixture afforded 0.028 g (28% yield) of (−)-(4*R*,5*S*)-**4b**, *ee* = 27%, $[\alpha]_D^{20} = -8.4^\circ$ (*c* 5.2, $CHCl_3$) and 0.012 g (12%) of hemiacetals **2b** and **3b**. The oxidation of (±)-**1b** for longer time (120 h) let to obtain (±)-**4b** as the only product in high (87%) yield.

*2.6.2.6. Preparative oxidation of (±)-**1b** by HLADH recombinant from E. coli.* Oxidation of (±)-**1b** (0.1 g), after 24 h, gave a mixture of: unreacted (±)-diol **1b** (24%), (−)-(4*R*,5*S*)-**4b** (60%, *ee* = 34%) and mixture of hemiacetals **2b** and **3b** (16%). The column chromatography of this mixture afforded 0.051 g (51% yield) of (−)-(4*R*,5*S*)-**4b**, *ee* = 34%, $[\alpha]_D^{20} = -8.7^\circ$ (*c* 6.8, $CHCl_3$), 0.015 g (15%) of hemiacetals **2b** and **3b** and 0.02 g (20% yield) of unreacted (±)-diol **1b**. The oxidation of (±)-**1b** for longer time (120 h) let to obtain (±)-**4b** as the only product in high (92%) yield.

*2.6.2.6.1. Trans-5-butyl-4-methyltetrahydrofuran-2-ols (mixture of isomers; **2b** and **3b**) (Fig. 2).* Colorless liquid, ***trans*** (**3b**) and ***cis*** (**2b**) isomers of the composition 53 : 47%; **^{1}H NMR** (600 MHz, $CDCl_3$) δ: 0.90 (t, J = 6.9 Hz, 3H, CH_3-4′ of **2b**); 0.91 (t, J = 6.9 Hz, 3H, CH_3-4′ of **3b**); 1.03 (d, J = 6.5 Hz, 3H, CH_3-4 of **2b**); 1.06 (d, J = 6.7 Hz, 3H, CH_3-4 of **3b**); 1.23–1.68 (m, 14H, one of CH_2-3, CH_2-1′, CH_2-2′, CH_2-3′ both isomers); 1.80 (m, 1H, H-4 of **3b**); 2.06 (dd, J = 12.5, 6.7 Hz, 1H, one of CH_2-3 of **2b**); 2.13 (m, 1H, H-4 of **2b**); 2.38 (ddd, J = 14.0, 9.0, 5.6 Hz, 1H, one of CH_2-3 of **3b**); 2.59 (s, 1H, OH of **2b**); 2.75 (s, 1H, OH of **3b**); 3.48 (dt, J = 8.3, 4.0 Hz, 1H, H-5 of **2b**); 3.69 (dt, J = 8.0, 3.6 Hz, 1H, H-5 of **3b**); 5.42 (m, 1H, H-2 of **2b**); 5.50 (m, 1H, H-2 of **3b**); **IR** (film, cm^{-1}): 3408 (m), 1456 (m), 1062 (m).

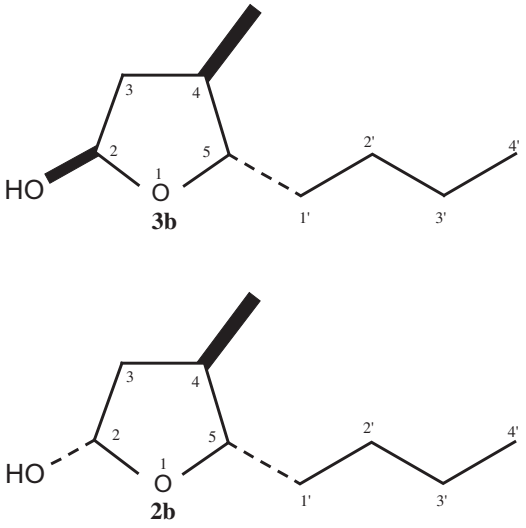


Fig. 2. *Trans*-5-butyl-4-methyltetrahydrofuran-2-ols (mixture of isomers; **2b** and **3b**).

2.7. Extraction procedure using a Deryng apparatus

After the biotransformation, the combined aqueous fractions were placed in a 500 ml round flask and 0.1 M NaOH was added portionwise to pH = 12. Sample flask was heated for 2 h, after the boiling point was reached. The vapours were condensed by means of a cold refrigerant. The main purpose of medium alkalization was conversion of obtained lactones **4a** and **4b**, by opening of lactone ring, into hydroxyacid salts, which are water-soluble and non-volatile compounds. After the extraction, the solvent, 1 ml of cyclohexane, containing the volatile compounds – hemiacetals **2a**–**3a** or **2b**–**3b**, was collected in a 2.5 ml vial. Then, the reaction mixture was acidified by 0.1 M HCl to pH = 3 and distilled again for 2 h in Deryng apparatus. During the hydrodistillation of volatile compounds, contained in the aqueous layer, whisky lactones **4a** and **4b** were extracted with 1 ml of cyclohexane. The unreacted diol **1a** and **1b**, remained in the aqueous medium, was used to next enzymatic oxidation. In this way, volatile products were separated from substrate **1a** without time-consuming and toxic solvent-free column chromatography.

*2.8. Oxidation of erythro-3-methylnonane-1,4-diol (**1a**) with TEMPO/BAIB*

To a stirred room temperature solution of diol **1a** (0.025 g, 0.04 mmol), dissolved in methylene chloride (3 ml) was added sequentially bis-acetoxyiodobenzene (BAIB, 0.014 g, 0.04 mmol) and 2,2,6,6-tetramethylpiperidinooxy (TEMPO, 0.001 g, 0.003 mmol). The reaction was conducted at room temperature. Progress of the reaction was monitored by TLC and GC. After 4 h of the reaction, a saturated solution of $Na_2S_2O_3$ (10 ml) was added and the mixture was extracted with diethyl ether (25 ml). The organic fraction was separated and washed with saturated $NaHCO_3$ (10 ml) solution and then, water (10 ml). The combined aqueous layers were extracted three times with diethyl ether (25 ml). Ether extract was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated by rotary evaporation. After GC analysis with application of chiral column racemic mixture of lactone **4a** was obtained.

*2.9. Oxidation of mixture of isomers cis-5-butyl-4-methyltetrahydrofuran-2-ols (**2a** and **3a**) with pyridine dichromate*

In a round bottom flask a mixture of diastereomeric hemiacetals **2a** and **3a** (0.05 g, 0.4 mmol), pyridine dichromate (0.3 g, 0.8 mmol) and anhydrous sodium acetate (0.022 g, 0.2 mmol) dissolved in anhydrous methylene chloride (20 ml) were placed. The reaction was carried out at room temperature with continuous stirring. Progress of the reaction was monitored by TLC and GC analysis. After 4 h of the reaction, solvent was evaporated, and the residue was extracted with hexane. The organic layer was filtered through Florisil, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to afforded 0.04 g (80% yield) of (−)-(4*S*,5*S*)-**4a**, *ee* = 38%, $[\alpha]_D^{20} = -23.1^\circ$ (*c* 5.2, $CHCl_3$).

3. Results and discussion

3.1. Enzymatic oxidation of racemic erythro- and threo-3-methyloctane-1,4-diols (**1a** and **1b**)

Substrates for enzyme-mediated reactions, racemic *erythro*- and *threo*-3-methyloctane-1,4-diols (**1a** and **1b**), were synthesized in good yields by reduction of corresponding *cis*- and *trans*-whisky lactones (**4a** and **4b**).

In our previous paper (Boratyński et al., 2010), we reported that HLADH showed complete chemoselectivity towards the oxidation of primary hydroxyl group in the primary-secondary 1,5-diols. In our current investigation, in the oxidation of primary-secondary 1,4-diols (Fig. 3) we isolated the same products as in the oxidation of primary-secondary 1,5-diols: the corresponding lactones and diastereoisomeric mixture of hemiacetals. They are formed in the same pathway as we proposed for the primary-secondary 1,5-diols oxidation (Boratyński et al., 2010). In the first step of oxidation, the primary hydroxyl group of diol is chemoselectively oxidized and hydroxyaldehyde is formed. Further immediate oxidation of hydroxyaldehyde proceeding via hemiacetals, led directly to the corresponding lactone.

It was of our interest to check whether **1a** and **1b** can be oxidized to both enantiomers of *cis*- and *trans*-lactone **4a** and **4b** using different enzymes. To achieve this purpose, eight commercially available alcohol dehydrogenases were investigated as biocatalysts. It was also interesting to determine the effect of stereochemical structure of substrates **1a** and **1b** on the enantioselectivity of the lactone products **4a** and **4b**. To increase the enantioselectivity and reaction efficiency, after previous analysis of spectrum of activity of each biocatalyst, the biotransformation conditions were optimized.

3.1.1. Enzymatic oxidation of racemic threo-3-methyloctane-1,4-diol (**1b**)

Screening transformations of **1b** showed that *trans*-isomer of **4b** can be obtained, although with different optical purity, using all tested enzymes. The total degree of conversion (100%) of substrate **1b** was observed in oxidation with both forms of enzyme HLADH isolated from horse liver as well as recombinant from *E. coli*. The highest optical activity (*ee* = 80%) showed enantiomerically enriched isomer of (−)-(4*R*,5*S*)-lactone **4b** obtained by the transformation catalyzed by PADH II. Some of the enzymes (BSADH, PADH I, PADH III, YADH) used, showed opposite enantioselectivity and the substrate **1b** was oxidized to the (+)-(4*S*,5*R*)-enantiomer of **4b**, however, with degree of conversion and enantiomeric excesses significantly lower. The results were presented in Table 1.

Taking into consideration the results of preliminary screening (Table 1), the preparative transformations of the diol **1b** were carried out with three selected alcohol dehydrogenases (Table 2).

After 1 day of oxidation of **1b**, catalyzed by HLADH isolated from the horse liver or recombinant from *E. coli*, enantiomerically enriched isomers of (−)-(4*R*,5*S*)-**4b** were obtained in 28% yield (*ee* = 27%) and 51% yield (*ee* = 34%) respectively (Table 2). Among mixture of products, hemiacetals **2b** and **3b** with a reaction yield of 12% and 15% respectively were isolated. The oxidation of (±)-**1b** for longer time (120 h) let to obtain racemic **4b**, as the only product in high (94%) yield.

The same stereoisomer of whisky lactone (−)-(4*R*,5*S*)-**4b**, but with a significantly higher enantioselectivity, was formed as a result of the preparative transformation of diol **1b** catalyzed by enzyme PADH II. After 2 days of biotransformation, carried out in glycine buffer at pH = 7.2, optically active (−)-(4*R*,5*S*)-**4b** was obtained with a yield of 44% and enantiomeric excess of 82% ($[\alpha]_D^{20} = -84^\circ$ (c 0.2, $CHCl_3$)).

All biocatalysts showed complete chemoselectivity towards the oxidation of primary hydroxyl group in the *threo*-3-methyloctane-1,4-diol (**1b**). Through spectroscopic analysis, the products of the first step of oxidation process were determined as diastereoisomeric *cis*/*trans* mixture of hemiacetals **2b** and **3b**. Identification of diastereomeric hemiacetals **2b** and **3b** were carried out on the basis of chemical shifts of the signals from protons H-2, H-4, H-5 and the protons of the methyl group at C-4 in the ^{1}H NMR spectrum (Fig. 4).

Considering the space structure of individual diastereoisomers **2b** and **3b**, the position of hydroxyl group and butyl group on the same side of the lactol ring will point to the *cis*-isomer of hemiacetal (**2b**). However, in the *trans*-isomer (**3b**) both substituents are located in opposite sides of the lactol ring. The ratio of *cis*/*trans*-isomers (47% of **2b** and 53% of **3b**) was evaluated from the integration of signals of protons H-2 and H-5.

Doublet of triplets from the proton H-5 in the ^{1}H NMR spectrum of the *trans*-isomer (**3b**) is shifted downfield (δ = 3.69), in comparison with its location in the ^{1}H NMR spectrum of *cis*-isomer (**2b**) (δ = 3.48). Such a difference in chemical shift due to deshielding effect of the oxygen atom of the hydroxyl group located on the same side of hemiacetal ring as the proton H-5 in the *trans*-isomer **3b**. The presence of one-proton multiplets from H-2 respectively at 5.42 and 5.50 ppm in the ^{1}H NMR spectrum indicates the presence of hemiacetal ring in the molecule. In *cis*-isomer **2b** the multiplet of H-4 is shifted downfield (δ = 2.13) by the deshielding effect of neighbouring hydroxyl group, in comparison with its location in the ^{1}H NMR spectrum of *trans*-isomer **3b** (δ = 1.80). The difference between the diastereoisomers can be also seen in the chemical shifts of doublets of protons from methyl groups at C-4 carbon atom (δ = 1.03 for **2b** and δ = 1.06 for **3b**).

3.1.2. Enzymatic oxidation of racemic erythro-3-methyloctane-1,4-diol (**1a**)

The screening studies have shown that all of the biocatalysts used, catalyze oxidation of *erythro*-3-methyloctane-1,4-diol (**1a**) to the corresponding *cis*-whisky lactone (**4a**) (Table 3) with various enantioselectivity.

For further preparative-scale transformations the same enzymes as for the preparative oxidation of *threo*-isomer **1b** were selected. After one day of preparative transformation of *erythro*-3-methyloctane-1,4-diol (**1a**) using HLADH recombinant in *E. coli* the following products: (+)-(4*R*,5*R*)-**4a** (*ee* = 64%, yield 48%), hemiacetals **2a** and **3a** (yield 16%) and unreacted substrate **1a** (yield 21%) were isolated (Table 4). The longer time (120 h) of oxidation of (±)-**1a** let to obtain racemic **4a**, as the only product in 100% yield according to GC.

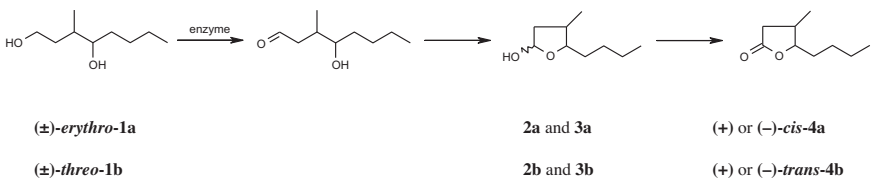


Fig. 3. Enzymatic oxidation of primary–secondary 1,5-diols **1a** and **1b**.

Table 1
The composition (in % according to GC) of products mixture after 5 days of enzymatic oxidation of racemic *threo*-3-methyloctane-1,4-diol (**1b**).

Enzyme	Oxidation conditions								Substrate	Products		
	Coenzyme		Temperature [°C]			pH			**1b**	**2b** and **3b**	**4b**	
	$NAD^+/NADP^+$	FMN	25	35	45	7.2	8.5	9.0	[%]	[%]	[%]	*ee* [%]
HLADH	√	√	√					√	0	0	100	0
HLADH*	√	√	√					√	0	0	100	0
	√	√		√			√		0	0	100	0
	√	√	√			√			0	29	71	32 (−)-(4*R*,5*S*)
	√		√					√	0	3	97	5 (−)-(4*R*,5*S*)
BSADH	√		√					√	82	12	6	90 (+)-(4*S*,5*R*)
	√	√			√			√	100	0	0	
LKADH	√	√	√			√			97	2	1	60 (−)-(4*R*,5*S*)
	√	√	√				√		88	5	7	92 (−)-(4*R*,5*S*)
PADH I	√	√	√			√			50	39	11	26 (+)-(4*S*,5*R*)
	√	√	√				√		97	0	3	0
PADH II	√	√	√			√			69	8	23	80 (−)-(4*R*,5*S*)
	√	√	√				√		88	2	10	46 (−)-(4*R*,5*S*)
PADH III	√	√	√			√			73	25	2	44 (+)-(4*S*,5*R*)
	√	√	√				√		56	4	40	6 (+)-(4*S*,5*R*)
YADH	√	√	√			√			73	11	16	5 (+)-(4*S*,5*R*)
	√	√	√				√		61	13	26	5 (+)-(4*S*,5*R*)

* Alcohol dehydrogenase recombinant from *E. coli* (HLADH).

Table 2
The composition (in % according to GC) of products mixture in the course of preparative oxidation of racemic *threo*-3-methyloctane-1,4-diol (**1b**) catalyzed by alcohol dehydrogenases.

Enzyme	Time	Substrate		Products			
		1b		**2b** and **3b**		**4b**	
	[h]	[%]	*ee* [%]	[%]	*de* [%]	[%]	*ee* [%]
HLADH	2	72		12		16	27 (−)-(4*R*,5*S*)
	24	55		14	6 (**3b**)	31	27 (−)-(4*R*,5*S*)
	120	0		0		100	0
HLADH*	2	58		17		25	62 (−)-(4*R*,5*S*)
	24	24	3	16		60	34 (−)-(4*R*,5*S*)
	120	0		0		100	0
PADH II	24	77		3		20	90 (−)-(4*R*,5*S*)
	48	41		3		56	82 (−)-(4*R*,5*S*)

* Alcohol dehydrogenase recombinant from *E. coli* (HLADH).

The same stereoisomer of whisky lactone (+)-(4*R*,5*R*)-**4a**, but with a slightly lower optical purity (*ee* = 56%) and yield of the reaction (38%), was one of the products of transformation of diol **1a** with HLADH isolated from the horse liver as biocatalyst. The intermediate products of first step of oxidation of diol **1a**, mixture of *trans* **3a** and *cis* **2a** hemiacetals were isolated with a yield of 55%.

The 2-days transformation of **1a**, involving PADH II as biocatalyst, gave a mixture of hemiacetals **2a** and **3a** (yield 40%) and (+)-(4*R*,5*R*)-**4a** with the lowest, compared to previous biotransformations, enantiomeric excess (*ee* = 46%).

The enantiomerically enriched mixture of hemiacetals **2a** and **3a**, obtained as a result of transformation catalyzed by HLADH, was oxidized with pyridine dichromate, in order to determine the enantiomeric excess of lactone product **4a** formed. As it turned out, the enantiomeric excess of (−)-(4*S*,5*S*)-**4a** was 38%. In next experiment, the unreacted diol **1a**, isolated after 1 day oxidation catalyzed by HLADH recombinant from *E. coli*, was oxidized with TEMPO/BAIB to racemic mixture of lactone **4a**. These studies confirm that the second stage of diol oxidation – oxidation of the corresponding hemiacetals to lactone – determines the enantioselectivity of the whole oxidation process.

The composition of the isolated mixture of hemiacetal isomers **2a** and **3a** was 23% of **2a** and 77% of **3a**. On the basis of previous description for hemiacetals **2b** and **3b** (Fig. 4) identification of single isomers was done (Fig. 5).

The signals of proton H-5, in both the *trans*-isomer (**3a**) (δ = 4.12) and *cis*-isomer (**2a**) (δ = 3.88), in the ^{1}H NMR spectrum of diastereomeric hemiacetals **2a** and **3a**, in comparison with the spectrum of the mixture of hemiacetals **2b** and **3b**, are shifted downfield. The presence of doublet of doublets from H-2 respectively at 5.43 ppm of **2a** and 5.52 ppm of **3a** in the ^{1}H NMR spectrum indicates the presence of hemiacetal ring in the molecule. The considerable difference (δ = 1.05 of **2a** and δ = 0.89 of **3a**) in chemical shifts of doublet of protons of methyl group at C-4 carbon atom was also observed.

3.2. Biotransformations of racemic 3-methyl-4-oxooctanoic acid (*5*)

A few syntheses of racemic and optically active whisky or cognac lactones is reported in the literature (Brenna, Dei Negri, Fuganti & Serra, 2001; Benedetti et al., 2001; Forzato et al., 2001; Ito, Yoshitake, & Katsuki, 1996; Nishikori, Ito, & Katsuki, 1998; Ozeki, Hashimoto, Nishide, Kajimoto, & Node, 2005; Santaniello et al., 1992; Schleth, Vogler, Harms, & Studer, 2004; Wilkinson et al., 2004). One of them, the enantioselective reduction of their corresponding γ-oxoacid or ester intermediates by means of enzymes, either isolated or belonging to a whole cell system, has found gen-

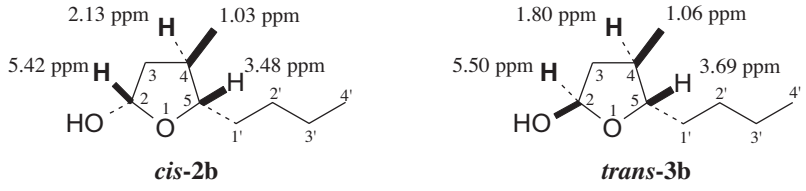


Fig. 4. Structures and chemical shifts of signals from protons H-2, H-4, H-5 and protons of the methyl group at carbon atom *C*-4 in hemiacetals **2b** and **3b**.

Table 3
The composition (in % according to GC) of products mixture after 5 days of enzymatic oxidation of racemic *erythro*-3-methyloctane-1,4-diol (**1a**).

Enzyme	Oxidation conditions								Substrate	Products		
	Coenzyme		Temperature [°C]			pH			**1a**	**2a** and **3a**	**4a**	
	$NAD^+/NADP^+$	FMN	25	35	45	7.2	8.5	9.0	[%]	[%]	[%]	*ee* [%]
HLADH	√	√	√					√	**0**	**0**	**100**	**0**
HLADH*	√	√	√					√	0	38	62	56 (+)-(4*R*,5*R*)
	√	√		√			√		0	35	65	54 (+)-(4*R*,5*R*)
	√	√	√			√			**32**	**13**	**55**	**46 (+)-(4*R*,5*R*)**
	√		√					√	0	0	100	0
BSADH	√		√					√	0	76	24	4 (−)-(4*S*,5*S*)
	√	√			√			√	100	0	0	
LKADH	√	√	√			√			38	14	48	38 (+)-(4*R*,5*R*)
	√	√	√				√		98	1	1	–
PADH II	√	√	√			√			**21**	**30**	**49**	**60 (+)-(4*R*,5*R*)**
	√	√	√				√		89	3	8	46 (+)-(4*R*,5*R*)
PADH III	√	√	√			√			84	0	16	52 (+)-(4*R*,5*R*)
	√	√	√				√		80	3	17	52 (+)-(4*R*,5*R*)
YADH	√	√	√			√			63	26	11	80 (+)-(4*R*,5*R*)
	√	√	√				√		10	15	75	22 (+)-(4*R*,5*R*)

* Alcohol dehydrogenase recombinant from *E. coli* (HLADH).

Table 4
The composition (in % according to GC) of products mixture in the course of preparative oxidation of racemic *erythro*-3-methyloctane-1,4-diol (**1a**) catalyzed by alcohol dehydrogenases.

Enzyme	Time	Substrate		Products			
		1a		**2a** and **3a**		**4a**	
	[h]	[%]	*ee* [%]	[%]	*de* [%]	[%]	*ee* [%]
HLADH	2	76		15		9	58 (+)-(4*R*,5*R*)
	24	0		58	54 (**3a**)	42	56 (+)-(4*R*,5*R*)
	120	0		0		100	0
HLADH*	2	54		14		32	64 (+)-(4*R*,5*R*)
	24	27	0	18		55	64 (+)-(4*R*,5*R*)
	120	0		0		100	0
PADH II	24	27		30		43	61 (+)-(4*R*,5*R*)
	48	0		55		45	46 (+)-(4*R*,5*R*)

* Alcohol dehydrogenase recombinant from *E. coli* (HLADH).

eral applicability (Santaniello et al., 1992). As a result of various biotransformation performed by Benedetti and co-workers, all four cognac lactones (whisky lactones homologues) have been obtained in high enantiomeric excess (Benedetti et al., 2001). Baker's yeast reductases showed a different enantiopreference for the oxoesters and oxoacids, while enzymatic hydrolyses were characterized by low conversion. In literature have been reported the reduction of γ-oxoacid (**5**) by *S. cerevisiae*, affording a single enantiopure diastereoisomer of *trans*-(+)-(4*S*,5*R*)-whisky lactone (Brenna, et al., 2001).

Due to the high cost of the enzyme and coenzymes required and the low, not satisfactory optical purity of obtained whisky lactones **4a** and **4b**, the application of diols **1a** and **1b**, as substrates for enzymatic oxidation is economically not attractive for large-scale process. In view of this, among the different biosynthetic strategies, we have applied microbial lactonization of γ-oxoacid to obtain enantiomerically pure whisky lactones (Fig. 6). There is the need to find other biocatalysts than *S. cerevisiae* capable to conduct microbial reduction of γ-oxoacids from which all four stereoisomers of whisky lactone can be obtained. Extending the pool of biocatalysts of the reduction – lactonization process we decided to examine some filamentous fungi strains for this purposes.

The substrate for biotransformations, 3-methyl-4-oxooctanoic acid (**5**) was obtained as a result of five-step synthesis according to the procedure for synthesis of other γ-oxoacids homologues described previously (Obara et al., 2008; Smuga, 2008). Six microorganisms, among 44 strains investigated as biocatalysts for the lactonization of racemic 3,7-dimethyl-4-oxooctanoic acid, showed good efficiency in the lactonization process (Smuga, 2008) (Table 5). Therefore, selected strains were applied in microbial lactonization of 3-methyl-4-oxooctanoic acid (**5**).

In contrast to enzymatic reactions, where mainly enantiomerically enriched not naturally occurring isomers of *trans*-(−)-(4*R*,5*S*)-**4b** or *cis*-(+)-(4*R*,5*R*)-**4a** were formed, in microbial lactonization the naturally occurring opposite isomers of *trans*-(+)-(4*S*,5*R*)-**4b** and *cis*-(−)-(4*S*,5*S*)-**4a** were obtained.

The highest degree of conversion (100% according to GC) in the shortest period of time (only 24 h) showed strains of *S. cerevisiae* AM464 and *Chaetomium* sp.1 KCH6670. Both strains completely converted **5** to *trans*-(+)-(4*S*,5*R*)-isomer of **4b**. Interesting results were also obtained during the biotransformation of **5** by *L. sulphurens* AM525 and *P. resinae* KCH50. These two microorganisms transformed substrate **5** with a yield of 92% into *trans*-(+)-(4*S*,5*R*)-isomer of **4b** and 78% into mixture of isomers: *cis*-(−)-(4*S*,5*S*)-**4a** and *trans*-(+)-(4*S*,5*R*)-**4b** respectively. However *D. igniaria* KCH6651 and *B. bassiana* AM278, after this period of time, transformed the racemic substrate **5** with a yield of just over 50%.

After 100% conversion of **5**, the *trans*-(+)-(4*S*,5*R*)-isomer of **4b** was stereoselectively formed as the only product of biotransformations catalyzed by *D. igniaria* KCH6651, *L. sulphurens* AM525, *Chaetomium* sp.1 KCH6670 and *S. cerevisiae* AM464. The formation of

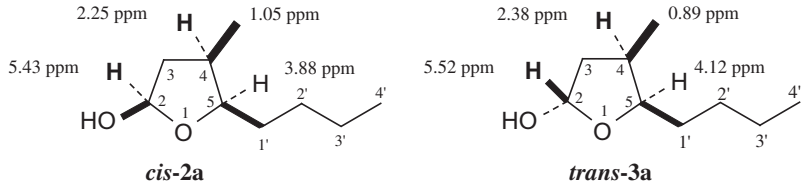
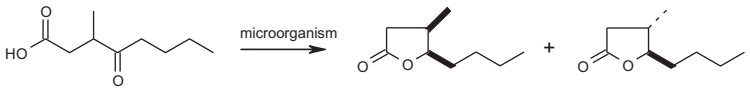


Fig. 5. Structures and chemical shifts of signals from protons H-2, H-4, H-5 and protons of the methyl group at carbon atom *C*-4 in hemiacetals ***2a*** and ***3a***.



(±)-5 (+) or (−)-*cis*-4a (+) or (−)-*trans*-4b

Fig. 6. Microbial lactonization of 4-oxoacid **5**.

Table 5
The composition (in % according to GC) of products mixture of biotransformation of racemic oxoacid **5**.

Microorganisms	Time	Substrate		Products			
		5		4a		4b	
	[h]	[%]	*ee* [%]	[%]	*ee* [%]	[%]	*ee* [%]
B. bassiana AM278	**24**	**48**	**10**	**16**	**85 (−)-(4*S*,5*S*)**	**36**	**99 (+)-(4*S*,5*R*)**
	48	0		45	77 (−)-(4*S*,5*S*)	55	99(+)-(4*S*,5*R*)
Chaetomium sp. 1 KCH6670	**24**	**0**		**0**		**100**	**99 (+)-(4*S*,5*R*)**
D. igniaria KCH6651	24	44	14 (−)	0		56	99 (+)-(4*S*,5*R*)
	48	0		0		100	99 (+)-(4*S*,5*R*)
L. sulphurens AM525	24	8	15 (−)	0		92	80(+)-(4*S*,5*R*)
	48	0		0		100	99(+)-(4*S*,5*R*)
P. resinae KCH50	24	22	27 (−)	34	54 (−)-(4*S*,5*S*)	44	99 (+)-(4*S*,5*R*)
	48	0		48	45 (−)-(4*S*,5*S*)	52	99 (+)-(4*S*,5*R*)
S. cerevisiae AM464	24	0		0		100	99 (+)-(4*S*,5*R*)

only one almost optically pure product from racemic 4-oxoacid (**5**) in these processes can be explained by preferential reduction of *R*-enantiomer of oxoacid and simultaneous racemization of *S*-enantiomer in the keto–enol tautomerization process. Such racemization has been observed in the microbial Baeyer-Villiger oxidation of cyclic ketones (Berezina, Alphand, & Furstoss, 2002). Lactonization in the culture of *B. bassiana* AM278 and *P. resinae* KCH50 afforded a mixture of *cis*- and *trans*-γ-lactones **4a** and **4b**.

The results presented in Table 5 indicate that in most of performed biotransformations, optically pure (+)-*trans*-γ-lactone **4b** (*ee* = 99%) was formed. Only the lactonization processes in the culture of *B. bassiana* AM278 and *P. resinae* KCH50 were afforded, besides (+)-*trans*-γ-lactone **4b** (*ee* = 99%), also (−)-*cis*-γ-lactone **4a** with an enantiomeric excess *ee* = 77% and *ee* = 45% respectively.

Two microorganisms were selected to catalyze the preparative biotransformation of racemic **5** (Table 5). It is worth to point out that the *trans*-(+)-(4*S*,5*R*)-enantiomer of lactone **4b** (*ee* = 99%) and enantiomerically enriched *cis*-(−)-(4*S*,5*S*)-isomer of lactone **4a** were isolated after 24 h from reaction mixture in the culture of *P. resinae* KCH50 and *B. bassiana* AM278 respectively. The configurations of the chiral centres in both isomers of lactones **4b** and **4a** were determined by comparison of their specific rotation values ($[\alpha]_D^{20} = +95^\circ$ (*c* 0.54, $CHCl_3$) for **4b** and $[\alpha]_D^{20} = -65^\circ$ (*c* 0.33, $CHCl_3$) for **4a** with literature data (Wilkinson et al., 2004) ($[\alpha]_D^{20} = +100^\circ$ (*c* 0.48, CH_3OH) for **4b** and $[\alpha]_D^{20} = -74^\circ$ (*c* 0.42, CH_3OH) for **4a**).

4. Conclusions

Two different approaches, the enzymatic oxidation of diols as well as the microbial lactonization of γ-oxoacid, in enantioselective synthesis of optically pure *cis*- and *trans*-isomers of whisky lactone **4a** and **4b** were studied. Interestingly, depending on the method, opposite enantiomerically enriched isomers of whisky lactone can be obtained.

As a result of enzymatic oxidation of diols, enantiomerically enriched not natural isomers of oak lactones were obtained. Among the different alcohol dehydrogenases, enzyme isolated from horse liver (HLADH) seems to be the best candidate to catalyze oxidation of primary-secondary 1,4-diols **1a** and **1b**.

Lactonization in the culture of whole cell of microorganisms seems to be a significant alternative method to enzyme-mediated oxidation processes. Biotransformations of γ-oxoacid **5** in the cultures of *S. cerevisiae* and various filamentous fungi strains exhibited a high degree of stereoselectivity with the overwhelming dominance of *trans*-(+)-(4*S*,5*R*)-isomer (*ee* = 99%) of whisky lactone **4b**. Moreover, we have selected two filamentous fungi, *B. bassiana* AM278 and *P. resinae* KCH50, capable to produce enantiomerically enriched *cis* and optically pure *trans*-isomer as well.

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