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Lactones 34 [1]. Application of alcohol dehydrogenase from horse liver (HLADH) in enantioselective synthesis of δ- and ε-lactones

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ABSTRACT

The ability of horse liver alcohol dehydrogenase (HLADH) to the enantioselective oxidation of primary–primary, primary–secondary and primary-tertiary aliphatic 1,5- and 1,6-diols **1a–i** was studied. No enantioselectivity of the transformations of primary–primary 1,6-diols **1a–d** to ε-lactones **4a–d** was observed. Regioselective oxidation of primary–secondary 1,6-diols **1e**,**f** and 1,5-diols **1h**,**i** afforded enantiomerically enriched ε-lactones **4e**,**f** and δ-lactones **4h**,**i**. ε-Lactones **4e**,**f** were formed with higher enantiomeric excesses (e.e. = 85–99%). Enzymatic oxidation of primary–tertiary 1,6-diol **1g** did not give lactone product.

1. Introduction

The synthesis of optically pure lactones has been the aim of many our research projects. Among different synthetic strategies we have applied so far, the synthesis from optically active starting materials was the most common one [2,3]. We have tried also to obtain enantiomerically pure lactones by enzymatic resolution of their racemic forms [4] or by application of biocatalysts in the lactonisation of racemic γ, δ-epoxyesters [5,6].

Here, we present another possibility, the synthesis of optically pure or enantiomerically enriched lactones via the oxidation of nonsymmetric acyclic racemic 1,5 and 1,6-diols by Horse Liver Alcohol Dehydrogenase (HLADH). We would like to mention that there is no literature data concerning the oxidation of aforementioned substrates to δ- and ε-lactones by HLADH.

The choice of this enzyme as the biocatalyst was not made by chance. Commercially available NAD^+-dependent Horse Liver Alcohol Dehydrogenase (HLADH) catalyzes oxidoreduction reactions of a broad spectrum of substrates i.e.: alcohols, aldehydes, ketones [7–12]. HLADH has been known to be effective catalyst in oxidation of only one selected hydroxy group in a polihydroxylated molecule [13,14]. The ability of HLADH to the stereospecific oxidation of only one of the enantiotopic hydroxy group of *meso* diols possessing acyclic, monocyclic and bicyclic structures, is well documented in the literature [15–17]. For that reason, HLADH-catalyzed oxidation of *meso* diols is direct and convenient one-step method of synthesis of a broad range of chiral γ- and δ-lactones.

Complete regiospecifity for primary hydroxy groups oxidation was observed with cyclopentyldiol and cyclohexyldiol substrates [13,14]. The reaction yields were satisfactory, but enantiomeric excesses were very low.

2. Materials and methods

2.1. Analysis

The purity of isolated products was checked by thin layer chromatography (TLC), using aluminum 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. The progress of reaction and enantiomeric excesses of the lactones were determined by gas chromatography (GC). 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). Chiral columns: CP7502 Chirasil-dex CB (25 m × 0.25 mm × 0.25 μm) and Astec Chiraldex B-PM (30 m × 0.25 mm × 0.12 μm) were used to the enantiomeric excesses determination.

The structures of the compounds were determined on the basis of ^1H NMR 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

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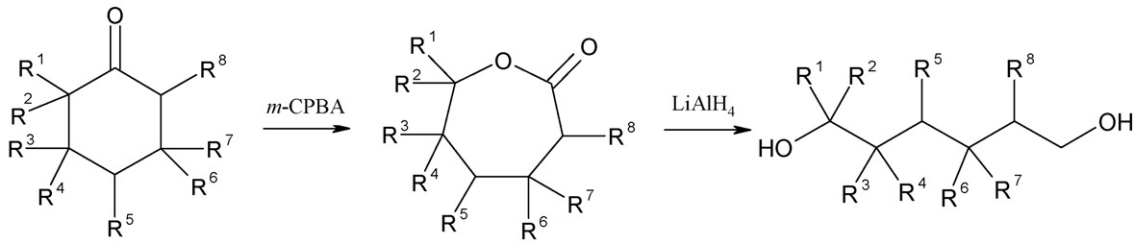


6 a-g **4 a-g** **1 a-g**

1, 4, 6a: $R^3 = CH_3$, $R^1, R^2, R^4, R^5, R^6, R^7, R^8 = H$ and **1b, 4j***: $R^6 = CH_3$, $R^1, R^2, R^3, R^4, R^5, R^7, R^8 = H$

1, 4, 6b: $R^5 = CH_3$, $R^1, R^2, R^3, R^4, R^6, R^7, R^8 = H$

1, 4, 6c: $R^3, R^4, R^6 = CH_3$, $R^1, R^2, R^5, R^7, R^8 = H$ and **1d, 4d**: $R^3, R^6, R^7 = CH_3$, $R^1, R^2, R^4, R^5, R^8 = H$

1, 4, 6e: $R^1 = CH_3$, $R^2, R^3, R^4, R^5, R^6, R^7, R^8 = H$

1, 4, 6f: $R^1 = (CH_3)_2$-CH, $R^6 = CH_3$, $R^2, R^3, R^4, R^5, R^7, R^8 = H$

1, 4, 6g: $R^1, R^2, R^8 = CH_3$, $R^3, R^4, R^5, R^6, R^7 = H$

* - not isolated

Scheme 1.

IV automatic polarimeter (Rudolph). The pH measurements were conducted on a HI 9321 Microprocesor pH Meter equipped with a glass electrode.

2.2. Chemicals and biocatalysts

4-Methylcyclohexanone (**6b**), 3,3,5-trimethylcyclohexanone (**6c**), 2-methylcyclo-hexanone (**6e**), menthone (**6f**), 1,5-hexanediol (**1h**), 2,4,4-trimethylcyclopentanone (**6i**), *m*-chloroperbenzoic acid (77%) and $LiAlH_4$ were purchased from Sigma–Aldrich Chemical Co., while 3-methylcyclohexanone (**6a**) and 2,2,6-trimethylcyclohexanone (**6g**) were purchased from Fluka Bio-Chemika. Horse Liver Alcohol Dehydrogenase (HLADH) and coenzymes: Nicotinamide Adenine Dinucleotide (NAD^+) and Flavin Mononucleotide (FMN) were purchased from Sigma Chemical Co.

2.3. Synthesis of racemic diols *1a–i*

Racemic nonsymmetric aliphatic 1,6-diols with primary–primary, primary–secondary and primary–tertiary hydroxy groups **1a–g** were obtained in two-step synthesis (Scheme 1). The saturated ketones **6e**,**f**,**g** were oxidized in Baeyer–Villiger reaction with 1.1 equiv. of *m*-chloroperbenzoic acid (*m*-CPBA) to the corresponding racemic ε-lactones **4e**,**f**,**g** in good yields (65–90%). The oxidation of other ketones **6a** and **6c** afforded the mixture of lactones **4a**,**j*** and **4c**,**d** respectively, which were separated by column chromatography. Pure racemic lactones **4a–g** were reduced with $LiAlH_4$ to the corresponding diols **1a–g** in good yields (62–90%).

3,3-Dimethylhexane-1,5-diol (**1i**) was obtained in the same sequence of two reactions as described above, starting from 2,4,4-trimethylcyclopentanone (**6i**) (Scheme 2). The 1,5-hexanediol (**1h**) was purchased from Sigma Chemical Co.

2.4. Oxidation of ketones *6a–i*

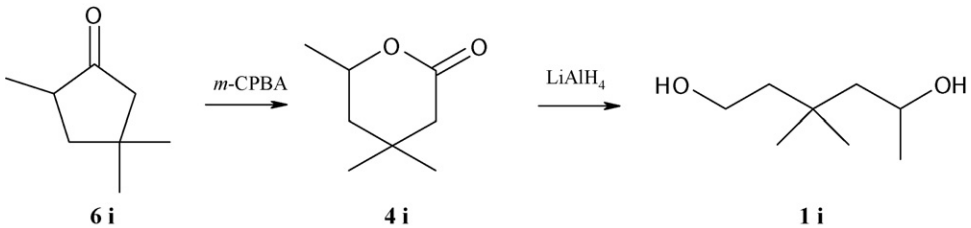
A solution of *m*-chloroperbenzoic acid (10 mmol) in methylene chloride (25 cm^3) was added dropwise to a solution of ketone **6a–i** (9 mmol) in methylene chloride (10 cm^3). When the reaction was completed (GC, TLC – 1–3 days) the excess of *m*-chloroperbenzoic acid was reduced with saturated Na_2SO_3 aqueous solution. The organic layer was separated and washed with saturated $NaHCO_3$, brine and dried over anhydrous $MgSO_4$. The crude product was purified by column chromatography with hexane/acetone (5:1) as the eluent. In case of lactones **4a**,**j*** and **4c**,**d** the mixture of hexane and acetone with changing polarity (from 19:1 to 9:1) was used. The spectral data for lactones **4a–g** were published earlier by our research group [1,18]. The yields of reactions and physical data of δ-lactone **4i** and ε-lactones **4a–g** obtained are given below.

2.4.1. (±)-6-Methyl-1-oxepan-2-one (*4a*)

Yield 97% (the mixture of **4a** and **4j***), $n_D^{20} = 1.4310$, Lit. [19]: $n_D^{30} = 1.4568$ (the mixture of **4a** and **4j***).

2.4.2. (±)-5-Methyl-1-oxepan-2-one (*4b*)

Yield 90%, $n_D^{20} = 1.4556$, Lit. [19]: $n_D^{30} = 1.4558$.



6 i **4 i** **1 i**

Scheme 2.

*2.4.3. (±)-4,6,6-Trimethyl-1-oxepan-2-one (**4c**)*
Yield 90% (the mixture of **4c** and **4d**), $n_D^{20} = 1.4247$, Lit. [19]: $n_D^{20} = 1.4565$ (the mixture of **4c** and **4d**).

*2.4.4. (±)-4,4,6-Trimethyl-1-oxepan-2-one (**4d**)*
mp = 30–31 °C.

*2.4.5. (±)-7-Methyl-1-oxepan-2-one (**4e**)*
Yield 70%, $n_D^{20} = 1.4561$, Lit. [20]: $n_D^{20} = 1.4564$.

*2.4.6. (±)-7-Isopropyl-4-methyl-1-oxepan-2-one (**4f**)*
Yield 72%, $n_D^{20} = 1.4521$, Lit. [21]: $n_D^{22} = 1.4596$.

*2.4.7. (±)-3,7,7-Trimethyl-1-oxepan-2-one (**4g**)*
Yield 81%, mp = 45–47 °C.

*2.4.8. (±)-4,4,6-Trimethyl-tetrahydropyran-2-one (**4i**)*
Yield 82%, $n_D^{20} = 1.4411$, Lit. [22]: $n_D^{20} = 1.4474$.

*2.5. Reduction of lactones **4a–i***

A solution of lactone **4a–i** (20 mmol) in diethyl ether (25 cm^3) was added dropwise to $LiAlH_4$ (25 mmol) in diethyl ether (25 cm^3). The mixture was stirred for 1–2 days at room temperature. When the reaction was completed (GC, TLC), water was added to decompose the excess of $LiAlH_4$. The mixture was then acidified with 0.01 M HCl and the product was extracted with diethyl ether. Then the extract was washed with saturated NaCl and dried ($MgSO_4$). The crude product was purified by column chromatography (silica gel, hexane/acetone, 5:1). The yields and physical data of 1,5-diol **1i** and 1,6-diols **1a–g** obtained are given below. The spectral data for compounds **1g**,**i**, not described in literature, are also presented.

*2.5.1. (±)-2-Methylhexane-1,6-diol (**1a**)*
Yield 62%, $n_D^{20} = 1.4512$, Lit. [23]: $n_D^{20} = 1.4556$.

*2.5.2. (±)-3-Methylhexane-1,6-diol (**1b**)*
Yield 40%, $n_D^{20} = 1.4547$, Lit. [23]: $n_D^{20} = 1.4554$.

*2.5.3. (±)-2,2,4-Trimethylhexane-1,6-diol (**1c**)*
Yield 85%, $n_D^{20} = 1.4611$, Lit. [24]: $n_D^{20} = 1.4626$.

*2.5.4. (±)-2,4,4-Trimethylhexane-1,6-diol (**1d**)*
Yield 88%, $n_D^{20} = 1.4620$, Lit. [24]: $n_D^{20} = 1.4628$.

*2.5.5. (±)-Heptane-1,6-diol (**1e**)*
Yield 74%, $n_D^{20} = 1.4522$, Lit. [25]: $n_D^{20} = 1.4530$.

*2.5.6. (±)-3,7-Dimethyloctane-1,6-diol (**1f**)*
Yield 95%, $n_D^{20} = 1.4594$, Lit. [26]: $n_D^{20} = 1.4615$.

*2.5.7. (±)-2,6-Dimethylheptane-1,6-diol (**1g**)*
Yield 90%, $n_D^{20} = 1.4541$; ^{1}H NMR (δ): 0.89 (d, *J* = 6.9 Hz, 3H, C**H$_3$**-CH<), 1.20 (s, 6H, (C**H$_3$**)$_2$C<), 1.25–1.70 (m, 7H, C**H$_2$**-3, C**H$_2$**-4, C**H$_2$**-5, **H**-2), 1.78 (s, 2H, two O**H**), 3.40 (dd, *J* = 10.5, 6.6 Hz, 1H, one of C**H$_2$**-1), 3.47 (dd, *J* = 10.5, 6.0 Hz, 1H, one of C**H$_2$**-1); IR (film, cm^{-1}): 3350 (s), 2967 (s), 1378 (s), 1040 (s).

*2.5.8. (±)-3,3-Dimethylhexane-1,5-diol (**1i**)*
Yield 76%, $n_D^{20} = 1.4520$; ^{1}H NMR (δ): 0.94 and 0.97 (two s, 6H, (C**H$_3$**)$_2$C<), 1.19 (d, *J* = 6.2 Hz, 3H, C**H$_3$**-CH<), 1.27 (dd, *J* = 14.8, 1.7 Hz, 1H, one of C**H$_2$**-4), 1.44 (dt, *J* = 14.3, 5.8 Hz, 1H, one of C**H$_2$**-2), 1.66 (dd, *J* = 14.8, 8.7 Hz, 1H, one of C**H$_2$**-4), 1.83 (ddd, *J* = 14.3, 7.9, 6.5 Hz, 1H, one of C**H$_2$**-2), 3.32 (s, 2H, two O**H**), 3.67–3.76 (two m, 2H, C**H$_2$**-1), 3.99 (m, 1H, **H**-5); IR (film, cm^{-1}): 3338 (s), 2960 (s), 1470 (s), 1367 (s), 1050 (s), 1023 (s).

*2.6. Enzymatic oxidation of racemic diols **1a–i** catalyzed by HLADH*

The reactions were carried out in a buffer at pH 9.0 using FMN as the effective agent in recycling process of the catalytic amounts of NAD^+ coenzyme used. In the screening experiments diols **1a–i** (0.02 g) and coenzymes: NAD^+ (0.007 g) and FMN (0.2 g) were dissolved in 0.1 M glycine-NaOH buffer (20 cm^3) at 20–25 °C. The pH of the mixture was readjusted to 9 with 2 M NaOH. HLADH (0.002 g) was added, and the mixture was stirred for appropriate time at room temperature, with periodic adjustment of the pH to 9. The mixture turned from its initial clear orange to almost black color as the reaction proceeded. Samples of the reaction mixtures were taken after several time intervals (2, 24, 48 and 120 h) and analyzed by GC.

The preparative-scale transformations were performed in the same conditions in five 20 cm^3 vials using 0.1 g of diols **1a–i** (5 × 0.02 g), 0.035 g of NAD^+ (5 × 0.007 g), 1 g FMN (5 × 0.2 g) and HLADH (5 × 0.002 g). When the reaction was completed the aqueous mixtures were pooled, acidified, washed with NaCl and extracted with $CHCl_3$. The extracts were dried with anhydrous $MgSO_4$ and evaporated *in vacuo*. The crude products were purified by column chromatography (silica gel, eluent: hexane/acetone, 8:1). The yields and optical rotations of products obtained are given below. The spectral data for new compounds are also presented.

*2.6.1. Preparative oxidation of (±)-**1a***
Oxidation of (±)-**1a** (0.1 g), after 48 h, gave a mixture of: unreacted (±)-diol **1a** (48%) and (±)-lactone **4a** (27%). The column chromatography of this mixture afforded 0.046 g (46% yield) of (±)-diol **1a** and 0.023 g (23% yield) of (±)-lactone **4a**.

*2.6.2. Preparative oxidation of (±)-**1b***
Oxidation of (±)-**1b** (0.1 g) gave complex mixture of products, which was not separable.

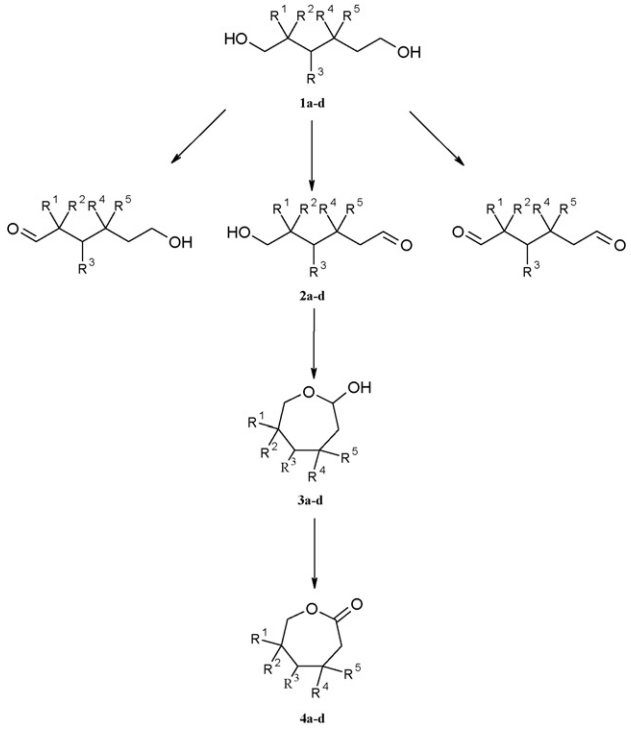
*2.6.3. Preparative oxidation of (±)-**1c***
Oxidation of (±)-**1c** (0.1 g), after 48 h, gave a mixture of: unreacted (±)-diol **1c** (53%), (±)-lactone **4c** (23%) and hemiacetal **3c** (24%). The column chromatography of this mixture afforded 0.044 g (44% yield) of (±)-diol **1c** and 0.02 g (20% yield) of (±)-lactone **4c**. The oxidation of (±)-**1c** for longer time (120 h) let to obtain (±)-lactone **4c** as the only product in high (92%) yield.

*2.6.4. Preparative oxidation of (±)-**1d***
Oxidation of (±)-**1d** (0.1 g), after 120 h, gave a mixture of: (±)-lactone **4d** (54%) and hemiacetal **3d** (21%). The column chromatography of this mixture afforded 0.043 g (43% yield) of (±)-lactone **4d**.

*2.6.5. Preparative oxidation of (±)-**1e***
Oxidation of (±)-**1e** (0.1 g), after 48 h, gave mixture of the following products: unreacted diol **1e** (9%), (+)-lactone **4e** (32%), hydroxyaldehyde **2e** (42%) and hydroxyketone **5e** (17%). The column chromatography of this mixture afforded 0.005 g (5% yield) of diol **1e**, 0.022 g (22% yield) of (+)-(*R*)-lactone **4e**, e.e. > 99%, $[\alpha]_D^{25} = +14.1°$ (*c* 1.5, $CHCl_3$), Lit. [27]: $[\alpha]_D^{20} = +16.2°$ (*c* 1.5, $CHCl_3$), 0.036 g (36% yield) of hydroxyaldehyde **2e** and 0.015 g (15% yield) of hydroxyketone **5e**.

6-Hydroxyheptan-1-al (**2e**): ^{1}H NMR (δ): 1.19 (d, *J* = 6.1 Hz, 3H, –C**H$_3$**), 1.25–1.70 (m, 6H, C**H$_2$**-3, C**H$_2$**-4, C**H$_2$**-5), 2.46 (t, *J* = 7.3 Hz, 2H, C**H$_2$**-2), 3.84 (m, 1H, **H**-6), 9.77 (m, 1H, –C**H**O); IR (film, cm^{-1}): 3420 (s), 2927 (s), 1373 (s), 1112 (s).

1-Hydroxyheptan-6-one (**5e**): ^{1}H NMR (δ): 1.30–1.60 (m, 6H, C**H$_2$**-2, C**H$_2$**-3, C**H$_2$**-4), 2.10 (s, 3H, C**H$_3$**-), 2.41 (t, *J* = 7.2 Hz, 2H, C**H$_2$**-



1-4a: $R^1 = CH_3$; $R^2, R^3, R^4, R^5 = H$ **1-4c**: $R^1, R^2, R^4 = CH_3$; $R^3, R^5 = H$

1-4b: $R^3 = CH_3$; $R^1, R^2, R^4, R^5 = H$ **1-4d**: $R^1, R^4, R^5 = CH_3$; $R^2, R^3 = H$

Scheme 3.

5), 3.61 (t, *J* = 6.6 Hz, 2H, C**H$_2$**-1); IR (film, cm^{-1}): 3407 (s), 2963 (s), 1711 (s), 1368 (s), 1054 (s).

*2.6.6. Preparative oxidation of (±)-**1f***

Oxidation of (±)-**1f** (0.1 g), after 48 h, afforded a mixture of diol **1f** (8%), (+)-lactone **4f** (20%) and hydroxyaldehyde **2f** (62%). The column chromatography of this mixture gave 0.005 g (5% yield) of diol **1f**, 0.018 g (18% yield) of (+)-(4*S*,7*R*)-lactone **4f**, e.e. = 85%, $[\alpha]_D^{25}$ = +19.5° (*c* 1.5, $CHCl_3$), Lit. [28]: $[\alpha]_D^{25}$ = +22.0° (*c* 2.0, $CHCl_3$) and 0.041 g (41% yield) of hydroxyaldehyde **2f**.

6-Hydroxy-3,7-dimethyloctan-1-al (**2f**): ^{1}H NMR (δ): 0.88 and 0.89 (two d, *J* = 7.7 Hz, 6H, (C**H$_3$**)$_2$CH-), 0.97 (d, *J* = 6.8 Hz, 3H, C**H$_3$**-3), 1.0–2.2 (m, 6H, **H**-3, C**H$_2$**-4, C**H$_2$**-5, **H**-7), 2.24 (ddd, *J* = 16.2, 7.9, 2.1 Hz, 1H, one of C**H$_2$**-2), 2.41 (ddd, *J* = 16.2, 5.5, 1.1 Hz, 1H, one of C**H$_2$**-2), 3.63 (m, 1H, **H**-6), 9.75 (m, 1H, –C**H**O); IR (film, cm^{-1}): 3423 (s), 2956 (s), 2718 (s), 2629 (w), 1711 (s), 1388 (s), 1011 (s).

*2.6.7. Preparative oxidation of (±)-**1h***

Oxidation of (±)-**1h** (0.1 g), after 48 h, afforded a mixture of diol **1h** (4%), (+)-lactone **4h** (43%) and hemiacetal **3h** (53%). The column chromatography of this mixture gave 0.04 g (40% yield) of (+)-(*R*)-lactone **4h**, e.e. = 62%, $[\alpha]_D^{25}$ = +26.1° (*c* 1.5, $CHCl_3$), Lit. [29]: $[\alpha]_D^{25}$ = +42° (*c* 2.0, EtOH) and 0.048 g (48% yield) of hemiacetal **3h**. After 120 h the only product of the reaction was racemic lactone **4h**.

*2.6.8. Preparative oxidation of (±)-**1i***

Oxidation of (±)-**1i** (0.1 g), after 48 h, afforded a mixture of (+)-lactone **4i** (28%) and hemiacetal **3i** (72%). The column chromatography of this mixture gave 0.023 g (23% yield) of (+)-lactone **4i**, e.e. = 64%, $[\alpha]_D^{25}$ = +21.2° (*c* 0.85, $CHCl_3$) and 0.061 g (61% yield) of hemiacetal **3i**.

cis-4,6,6-Trimethyltetrahydropyran-2-ol (**3i**): ^{1}H NMR (δ): 0.98 and 1.02 (two s, 6H, (C**H$_3$**)$_2$C<), 1.10–1.21 (m, 2H, one of C**H$_2$**-5 and one of C**H$_2$**-3), 1.22 (d, *J* = 6.2 Hz, 3H, C**H$_3$**-CH<), 1.28 (dt, *J* = 13.3, 2.1 Hz, 1H, one of C**H$_2$**-5), 1.63 (dt, *J* = 12.8, 2.1 Hz, 1H, one of C**H$_2$**-3), 3.77 (m, 1H, **H**-6), 3.95 (d, *J* = 5.7 Hz, 1H, O**H**), 4.92 (m, 1H, **H**-2).

trans-4,6,6-Trimethyltetrahydropyran-2-ol (**3i**): ^{1}H NMR (δ): 0.92 and 1.02 (two s, 6H, (C**H$_3$**)$_2$C<), 1.10–1.21 (m, 2H, one of C**H$_2$**-5 and one of C**H$_2$**-3), 1.17 (d, *J* = 6.2 Hz, 3H, C**H$_3$**–CH<), 1.38 (dt, *J* = 13.2, 2.0 Hz, 1H, one of C**H$_2$**-5), 1.57 (dt, *J* = 14.0, 1.5 Hz, 1H, one of C**H$_2$**-3), 4.05 (d, *J* = 5.7 Hz, 1H, O**H**), 4.21 (m, 1H, **H**-6), 5.36 (m, 1H, **H**-2).

3. Results and discussion

The substrates for enzymatic oxidation, diols **1a**–**i**, were synthesized in good yields by reduction of corresponding δ- or ε-lactones **4a**–**i**, which were products of Baeyer–Villiger oxidation of ketones **6a**–**i** (Schemes 1 and 2). The diols **1a**–**i** could be divided in four groups: primary–primary 1,6-diols **1a**–**d**, primary–secondary 1,6-diols **1e**,**f**, primary–tertiary 1,6-diol **1g** and primary–secondary 1,5-diols **1h**,**i**.

*3.1. Enzymatic oxidation of primary–primary 1,6-diols **1a**–**d***

In the first step of enzymatic oxidation of primary–primary 1,6-diols **1a**–**d** we expected the formation of three products shown in Scheme 3. The GC analysis of the reaction mixture showed the presence of many products. Unfortunately we did not isolate neither hydroxyaldehydes nor dialdehydes from the reaction mixture. Formation of hydroxyaldehydes **2a**–**d** is undirectly confirmed by the presence of final products of oxidation, lactones **4a**–**d**. Analyzing these results we can suppose that hydroxyaldehydes **2a**–**d** were formed in the first step of oxidation and in the second step they cyclized into hemiacetals **3a**–**d**. Two of them (**3c**,**d**)

Table 1
The composition (in % according to GC) of the products mixture in the course of the enzymatic oxidation of primary–primary 1,6-diols **1a–d** by HLADH.

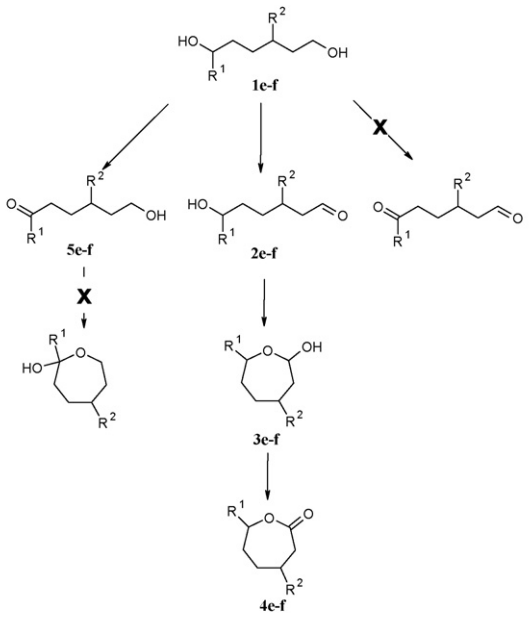
Substrate	Time (h)	**1a–d** (%)	**3a–d** (%)	**4a–d** (%)	Other products (%)
1a	2	85	–	15	–
	24	70	–	22	8
	48	48	–	27	25
1b	2	50	–	4	46
	24	15	–	8	77
	48	11	–	5	84
1c	2	95	5	–	–
	24	72	28	–	–
	48	53	24	23	–
	120	–	–	100	–
1d	2	87	11	2	11
	24	–	51	29	20
	48	–	42	33	25
	120	–	21	54	25

were detected by GC in the reaction mixture. Only **3c** was isolated together with lactone **4c** from the products mixture obtained after 48 h biotransformation. The multiplets of methine protons at hemiacetal carbon atoms of two diastereoisomeric hemiacetals **3c** are present in the range δ = 4.8–5.2 in the ^{1}H NMR spectrum of this mixture. Finally, the hemiacetals **3a–d** were oxidized to lactones **4a–d**.

The results presented in Table 1 indicate that beside lactones some other products were also formed. They usually composed the complex mixtures which were difficult to separate. We suspected that some of the components of these mixtures could be formed via further transformation of lactones. In separate experiments the stability of lactones **4a–c** in the reaction medium (glycine buffer, NAD^+, FMN, pH = 9) without HLADH was checked. As we suspected the lactones **4a**,**b** were unstable in such conditions and only lactone **4c** was stable. It explains the fact that only product **4c** of oxidation of diol **1c** was obtained in good (above 90%) yield. The GC analysis carried out with the application of chiral columns indicated that all lactones **4a–d** isolated as products of oxidation of respective diols **1a–d** were obtained as racemic mixtures.

3.2. Enzymatic oxidation of primary–secondary 1,6-diols *1e,f*

In the case of biotransformation of diol **1e** it was possible to detect by GC, products of the first step of oxidation: hydroxyaldehyde **2e** as the major and hydroxyketone **5e** as the minor one (Scheme 4; Table 2). The biotransformation of diol **1f** proceeded only via hydroxyaldehyde **2f**. These results confirmed the preference of oxidation of primary hydroxy group. Further immediate oxidation of hydroxyaldehydes **2e**,**f** proceeding via hemiacetals, led directly to the corresponding lactones **4e**,**f**. After 5 days of biotransformation lactone **4e** was obtained in 30% yield and lactone



Scheme 4.

4f in 45% yield. In these biotransformations we did not detect the presence of hemiacetals **3e**,**f** in the reaction mixture.

It is worth to point out that the (+)-(*R*)-enantiomer of lactone **4e** (e.e. > 99%) and (+)-(*4S*,*7R*)-mentholactone (**4f**) (e.e. = 85%) were isolated as the main products from reaction mixture. The configurations of chiral centers in lactones **4e** and **4f** were determined by comparison of their specific rotation values ($[\alpha]_D^{25}$ = +14.1° (*c* 1.5, $CHCl_3$) for **4e** and $[\alpha]_D^{25}$ = +19.5° (*c* 1.5, $CHCl_3$) for **4f**) with literature data ($[\alpha]_D^{20}$ = +16.2° (*c* 1.5, $CHCl_3$) for **4e** [27] and $[\alpha]_D^{25}$ = +22.0° (*c* 2.0, $CHCl_3$) for **4f** [28]).

3.3. Enzymatic oxidation of primary–tertiary 1,6-diol *1g*

There is only one possible pathway in biotransformations of primary–tertiary diol **1g** by HLADH. The oxidation of the primary hydroxy group leads to the hydroxyaldehyde **2g**. Its further oxidation, via hemiacetal form, should lead to the expected lactone **4g**. However, the GC analysis of the composition of the products mixture did not show the presence of lactone. After 5 days of biotransformation of diol **1g** the composition of reaction mixture contained the substrate **1g** (55%) and two products: the major one (28%) and the minor one (17%). We suspect that these products were hydroxyaldehyde **2g** and hemiacetal **3g**, but our attempts to isolate them did not give positive results.

Table 2
The composition (in % according to GC) of the products mixture in the course of the enzymatic oxidation of primary–secondary 1,6-diols **1e**,**f** by HLADH.

Substrate	Time (h)	**1e**,**f** (%)	**2e**,**f** (%)	**5e**,**f** (%)	**4e**,**f**		Other products (%)
					(%)	e.e.[a](%)	
1e	2	70	21	9	–	–	–
	24	45	34	16	5	>99 (*R*)	–
	48	9	42	17	32	>99 (*R*)	–
	120	2	37	18	43	>99 (*R*)	–
1f	2	70	30	–	–	–	–
	24	20	68	–	12	91 (4*S*,7*R*)	–
	48	8	62	–	20	85 (4*S*,7*R*)	10
	120	-	12	–	55	85 (4*S*,7*R*)	33

[a] e.e.: enantiomeric excess

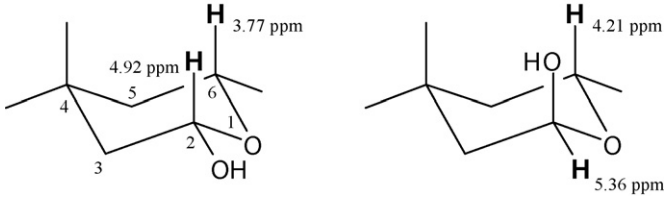
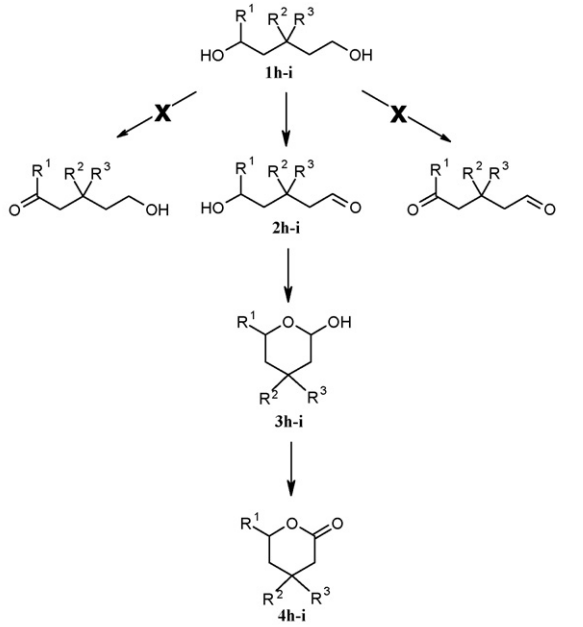


Fig. 1. The structure and chemical shifts of H-2 and H-6 multiplets of *cis* and *trans* isomer of **3i**.

3.4. Enzymatic oxidation of primary–secondary 1,5-diols *1h,i*

The HLADH showed complete chemoselectivity towards the oxidation of primary hydroxy group in the primary–secondary 1,5-diols **1h,i**. As detected by GC, the products of the first step of oxidation process, instead of the hydroxyaldehydes **2h,i** were their corresponding hemiacetals **3h,i**. Hemiacetal **3i**, as a diastereoisomeric *cis*/*trans* mixture, was isolated by column chromatography from products mixture after 48 h of biotransformation. The ratio of *cis*/*trans* isomers was determined from the integration of multiplets of protons H-6 and H-2. The signals of these protons were assigned to the corresponding isomers on the basis of their chemical shifts. Assuming the chair conformation and equatorial position of methyl group at C-6 (Fig. 1) in the *cis* isomer hydroxy group was also located in equatorial position, whereas in *trans* isomer it occupied axial position. In *trans* isomer the multiplet of H-6 is shifted downfield (δ = 4.21) by the axial hydroxy group, in comparison with its location in the ^{1}H NMR spectrum of *cis* isomer (δ = 3.77). On the other hand, the signal of equatorial proton H-2 in *trans* isomer is shifted downfield (δ = 5.36) by deshielding effect of neighboring oxygen atom, in comparison with the chemical shift (δ = 4.92) of axial proton H-2 found in the spectrum of *cis* isomer.

Further oxidation of lactols **3h,i** led directly to the corresponding lactones **4h,i**. (Table 3). Taking into consideration all these results, the following pathway of oxidation primary–secondary 1,5-diols **1h,i** can be proposed (Scheme 5).



1-4h: $R^1 = CH_3$; $R^2, R^3 = H$ **1-4i**: $R^1, R^2, R^3 = CH_3$

Scheme 5.

Table 3
The composition (in % according to GC) of the products mixture in the course of the enzymatic oxidation of primary–secondary 1,5-diols **1h,i** by HLADH.

Substrate	Time (h)	**1h,i** (%)	**3h,i** (%)	**4h,i**	
				(%)	e.e.[a](%)
1h	2	54	24	22	*nd*[b]
	24	26	35	39	*nd*[b]
	48	4	53	43	*nd*[b]
	120	–	3	97	62 (*R*)
1i	2	27	73	–	–
	24	–	95	5	72 (+)
	48	–	72	28	64 (+)
	120	–	70	30	64 (+)

[a] e.e.: enantiomeric excess.
[b] *nd*: not determined.

The high isolated yield of lactone **4h**, which reached almost 95% is attention worthy. Unfortunately, it was impossible to determine the enantiomeric excess of lactone **4h** by chromatographic methods with application of chiral columns, being at our disposal. Therefore, the optical purity of the lactone **4h** was estimated on the basis of its specific rotation data. The specific rotation value of enantiomerically pure (+)-(*R*)-lactone **4h** is known [29] ($[\alpha]_D^{25} = +42^\circ$, *c* 2.0, EtOH) and the specific rotation of our sample was as follows: $[\alpha]_D^{25} = +26.1^\circ$, *c* 1.5, $CHCl_3$. According to the formula given by Carey and Sundberg [30] the calculated enantiomeric excess of our lactone **4h** has value 62%. The enantiomeric excess of (+)-lactone **4i** ($[\alpha]_D^{25} = +21.2^\circ$, *c* 0.85, $CHCl_3$) reached 64%. We could not find in literature any information about the configuration of chiral centre of this compound.

4. Conclusions

The results presented above indicate that 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. Regioselective oxidation of diols studied afforded lactones **4e**,**f**,**h**,**i** with high enantiomeric excesses (62–99%). On the basis of the literature reports it was found that (*R*)-isomers of lactones **4e**,**h** and (*4S,7R*)-isomer of mentholactone (**4f**) were formed.

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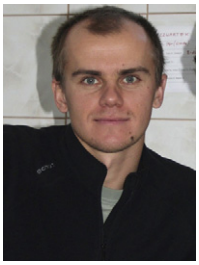

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