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Short Communication

# Oxidation of 1,4-alkanediols into γ-lactones via γ-lactols using *Rhodococcus erythropolis* as biocatalyst

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This publication is dedicated to Prof. Dr. Hanswerner Dellweg on the occasion of his 85th birthday.

**Abstract**

Whole cells of *Rhodococcus erythropolis* DSM 44534 grown on ethanol, (*R*)- and (*S*)-1,2-propanediol were used for biotransformation of racemic 1,4-alkanediols into γ-lactones. The cells oxidized 1,4-decanediol (**1a**) and 1,4-nonanediol (**2a**) into the corresponding γ-lactones 5-hexyl-dihydro-2(3*H*)-furanone (γ-decalactone, **1c**) and 5-pentyl-dihydro-2(3*H*)-furanone (γ-nonalactone, **2c**), respectively, with an EE(*R*) of 40–75%. The transient formation of the γ-lactols 5-hexyl-tetrahydro-2-furanol (γ-decalactol, **1b**) and 5-pentyl-tetrahydro-2-furanol (γ-nonalactol, **2b**) as intermediates was observed by GC–MS. 1,4-Pentanediol (**3a**) was transformed into 5-methyl-dihydro-2(3*H*)-furanone (γ-valerolactone, **3c**) whereas (*R*)- and (*S*)-2-methyl-1,4-butanediol (**4a**) was converted to the methyl-substituted γ-butyrolactones 4-methyl-dihydro-2(3*H*)-furanone (**4c$_1$**) and 3-methyl-dihydro-2(3*H*)-furanone (**4c$_2$**) in a ratio of 80:20 with a yield of 55%. Also *cis*-2-buten-1,4-diol (**5a**) was transformed resulting in the formation of 2(5*H*)-furanone (γ-crotonolactone, **5c**). At the higher pH values of 8.8 the yield of lactone formed was improved; however, the enatiomeric excesses were slightly higher at the lower pH of 5.2.


*Keywords:* Biotransformation; Rhodococcus; 1,4-Diol; γ-Lactol; γ-Lactone; Oxidation

## 1. Introduction

The aroma compound γ-nonalactone (5-pentyl-dihydro-2(3*H*)furanone, **2c**) is present in many fruits and fermented products. The odorant attributes are described as strong sweet, soft coconut and fatty milky for the (*R*)-enantiomer whereas the (*S*)-enantiomer shows less intensive weak coconut and fatty-moldy aspects [1]. The oaky attribute of wines is associated among other odor active compounds also with **2c** [2] and Ito et al. [3] identified **2c** as potent sweet aroma component of Chinese jasmine green tea scented with flowers of *Jasminum sambac* while the cyclic hemiacetal γ-nonalactol (5-pentyl-tetrahydro-furan-2-ol, **2b**) was found in watermelon and is considered to contribute to the fresh note in its aroma [4]. However the precursors or the metabolic fate of **2b** remain unclear [5]. The formation of natural γ-decalactone (5-hexyl-dihydro-2(3*H*)furanone, **1c**) in the catabolism of ricinoleic acid by fungi was first observed by Okui et al. [6]. In general oxygenated fatty acids were characterized as the precursors of γ- and δ-lactones in yeasts. In beer **2c** is known as an aroma active compound and linoleic acid is identified as precursor [7]. However, the biosynthesis of substituted γ-lactones was also demonstrated by Baeyer–Villiger monooxygenase oxidation of substituted cyclobutanone structures [8] and enzymatic dehydrogenation of 1,4-diols [9].

In this study we investigated the biotransformation of racemic 1,4-diols by *Rhodococcus erythropolis* DSM 44534 into γ-lactols and γ-lactones. The use of *R. erythropolis* as a whole cell biocatalyst transforming 1,4-diols into biologically active γ-lactols and γ-lactones is of preparative interest as these compounds exhibit flavor properties and are chiral building blocks in synthetic organic chemistry.

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## 2. Experimental

### 2.1. Biotransformations

Growth of *R. erythropolis* DSM 44534 and biotransformation assays with washed cells were performed under the conditions described previously [10].

Biotransformations were carried out with ethanol grown cells if not noted otherwise.

### 2.2. Analytical methods

The concentration and purity of substrates were determined by GC-FID. Qualitative analysis was performed with GC/EI–MS. Enantioselective GC/EI-MS analysis was carried out with chiral GC-phase Lipodex® E. Details have been described previously [11].

### 2.3. Chemical syntheses

#### 2.3.1. Chemicals

Chemicals were from Sigma–Aldrich or Fluka and of highest purity available; all solvents were of analytical grade or distilled before use.

#### 2.3.2. Synthesis of rac 1,4-diol compounds

The synthesis of rac 1,4-diols from lactones was performed by a standard procedure. In brief, rac γ-lactones were reduced with lithium aluminum hydride in dry THF. Standard work up and silica gel chromatography revealed the 1,4-diols in 65–75% yield and 95% purity GC-FID. EI-MS *m/z* (intensity in %) of $C_{10}H_{22}O_2$ (**1a**) ($M_r = 174$), resulted in 115 (6), 97 (21), 89 (21) and 71 $[C_4H_9O_2–H_2O]^+$ (100). EI-MS *m/z* of $C_9H_{20}O_2$, (**2a**) ($M_r = 160$) resulted in 101 (10), 89 (46), 83 (53), 71 $[C_4H_9O_2–H_2O]^+$ (100), 55 (72), and 43 (49). EI-MS *m/z* of $C_5H_{12}O_2$ (**3a**) ($M_r = 104$) resulted in 89 (46), 71 $[C_4H_9O_2–H_2O]^+$ (100), 58 (14), 45 (90) and 42 (56).

#### 2.3.3. Synthesis of γ-nonalactol (*2b*)

Rac γ-nonalactone (rac-**2c**) (1.7 g, 10 mmol) was dissolved in toluene under $N_2$ and a solution of DIBAL-H (20 mmol) in dry THF (40 mL) was added dropwise during 5 min at −70 °C. After 20 min at −70 °C the solution was warmed to r.t. The excess DIBAL-H was hydrolyzed with 2 mL methanol and 25 mL $H_2O$ and acidified dropwise with 5 mL of a 2 M HCl solution and extracted with diethylether (2× 80 mL). The organic phase was washed with 5% $NaHCO_3$ and saturated NaCl solution and dried with $Na_2SO_4$. The organic solvent was removed by evaporation under reduced pressure to leave a colorless oil, which was purified on a LC (100 g aluminum oxide/silica gel 2:1) column. The column was eluted with 80 mL fractions of pentane/$CH_2Cl_2$ (9:1); pentane/$CH_2Cl_2$ (3:1); pentane/diethylether (9:1), and diethylether. γ-Nonalactol (**2b**) was eluted in the pentane/diethylether (9:1) fraction and after evaporation a colorless oil showing a fruity fresh odor was obtained (1.4 g 90% purity by GC-FID). EI-MS *m/z* (%) of γ-nonalactol (**2b**) $C_9H_{18}O_2$ ($M_r = 158$), 157 ($[M-1]^+$) (2), 140 ($[M-H_2O]^+$) (12), 128 (3), 87 (100), 69 (50), and 55 (45). MS-data are in accordance with data reported by [12].

## 3. Results and discussion

### 3.1. Biotransformation of 1,4-diols by R. erythropolis DSM 44534

Washed cell suspensions of *R. erythropolis* DSM 44534 pregrown on ethanol were used to study the oxidation of racemic 1,4-diols. During biotransformation of rac-1,4-decanediol (rac-**1a**) a sweet peach-like odor was observed, biotransformation of rac-1,4-nonanediol (rac-**2a**) resulted in a transient appearance of an fresh fruity-like odor, respectively, indicating the presence of flavor compounds. The flavor active γ-lactones 5-hexyl-dihydro-2(3H)-furanone (γ-decalactone, **1c**) and 5-pentyl-dihydro-2(3H)-furanone (γ-nonalactone, **2c**), respectively, were analyzed as products. GC–MS analysis also revealed the formation of γ-lactols 5-hexyl-tetrahydro-2-furanol (γ-decalactol, **1b**) and 5-pentyl-tetrahydro-2-furanol (γ-nonalactol, **2b**), respectively, as intermediates.

During biotransformation of diol **2a** the concentration of lactol **2b** was nearly constant and decreased finally when **2a** vanished. After 4 h a transient maximum of lactone **2c** was reached, however, after 16 h also **2c** was completely consumed (data not shown). We propose that lactols are formed from diols by oxidation of the primary hydroxyl group into 4-hydroxyalkanals and cyclic hemiacetal (lactol) formation of the aldehyde by

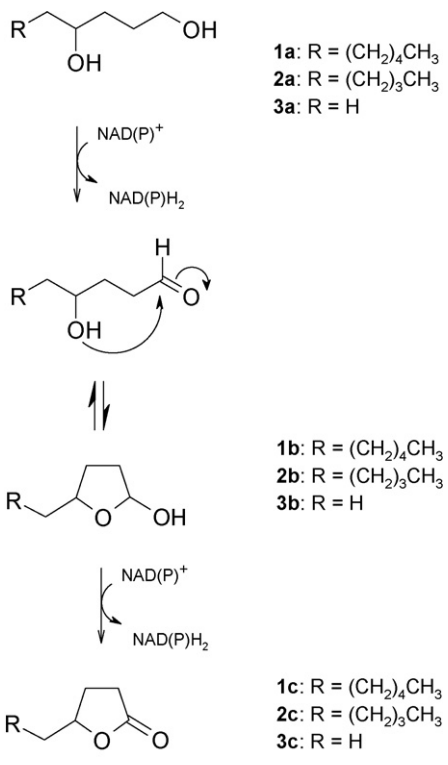


Fig. 1. Proposed formation pathway for γ-decalactone (**1c**), γ-nonalactone (**2c**), and γ-valerolactone (**3c**) by *R. erythropolis* DSM 44534 pregrown on ethanol. EI-MS: *m/z* (%) of γ-decalactol (**1b**) $C_{10}H_{20}O_2$ ($M_r = 172$), 154 ($[M-H_2O]^+$) (15), 136 (10) 87 (100) 69 (50) and 55 (40).

tautermerisation. Lactols are then further oxidized to lactones (Fig. 1).

An alternative to the formation of lactones via lactols is the oxidation of the aldehyde 4-hydroxyalkanal (Fig. 1) to 4-hydroxyalkanoic acid and subsequent lactonization. The lactonization of a 4-hydroxy acid to the lactone has been observed e.g. with *Saccharomyces cerevisiae*, which transforms 4-hydroxydecanoate to γ-decalactone **1c** [14]. However, with *R. erytropolis* DSM 44534 we did not observe the formation of γ-butyrolactone starting from 4-hydroxybutanoate, nevertheless, 1,4-butanediol was converted to γ-butyrolactone [10].

We assume, *R. erythropolis* DSM 44534 lacks an activity which enzymatically lactonizes 4-hydroxyalkanoic acids. Chemically, 4-hydroxyalkanoic acids do not lactonize spontaneously at neutral pH, however, 4-hydroxybutyraldehyde exhibited an equilibrium with the lactol 2-hydroxytetrahydrofuran as major ring-chain-tautomer (data not shown).

The results of the transformation of **1a** and **2a** by *R. erythropolis* DSM 44534 are summarized in Table 1. In all cases, the lactones exhibited (*R*)-configuration. High molar conversion yields of 80% were obtained with ethanol grown cells. With these cells also high $EE_R$ values were achieved in the case of **1a**. *R. erythropolis* DSM 44534 was grown on ethanol, (*R*)-1,2-propanediol or (*S*)-1,2-propanediol to see whether the growth substrate influences the stereochemical outcome and molar conversion yield (Table 1).

In addition, the complete conversion of 1 mM rac-1,4-pentanediol (rac-**3a**) after 100 min incubation (0.7 mg cells dry wt. $mL^{-1}$) resulted in the formation of 225 μM γ-valerolactone (**3c**) with an $EE_R$ of 6% (25% yield). γ-Valerolactol (**3b**) was not detected during incubation period. The experimental conditions used for this short carbon chain diol **3a** were in accord with substrates **4a** and **5a** (see Section 3.3).

In contrast to our findings Romano et al. [13] reported a one step oxidation of rac-**2a** into (*S*)-**2c** resulting in an $EE_S$ of max. 58% (molar yield 20%) by the acetic acid bacterium *Gluconobacter oxydans* MIM 1000/14.

The observed $EE_R$ enhances with increasing alkyl chain length of the 1,4-diol substrates. The $EE_R$ with *R. erythropolis* DSM 44534 may be due to a number of reasons: (I) the enzyme oxidizing rac-**2a** to lactol **2b** prefers the enantiomer (*R*)-**2a**; (II) the lactone (*S*)-**2c** is preferentially consumed by a lactone converting enzyme system; (III) a secondary alcohol dehydrogenase oxidizes C(4)-OH of (*S*)-**2a** into 4-oxo-1-nonanol, thereby

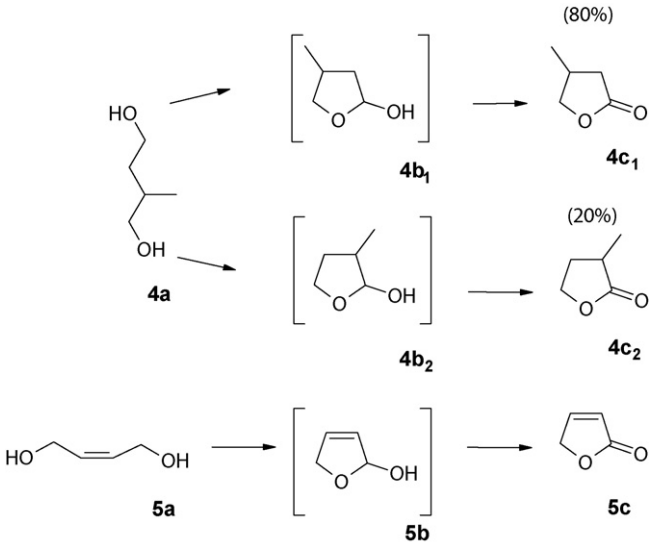


Fig. 2. Oxidation of primary 1,4 -diols into γ-lactones by ethanol pregrown cells of *R. erythropolis* DSM 44534 after 100 min; lactol intermediates **4b$_1$**, **4b$_2$**, and **5b** were not detected by the applied GC and GC–MS methods.

enriching the relative substrate concentration of (*R*)-**2a**; (IV) lactol (4*R*)-**2b** is preferentially oxidized into (4*R*)-**2c**.

The stereoselective oxidation of secondary (*S*)-configured alcohols from racemic substrates by a secondary alcohol dehydrogenase Q15 (ADH) from *Rhodococcus ruber* has been reported [15]. Therefore we assume the oxidation of the secondary alcohol group (C1) of the lactols with (*S*)-stereoselectivity. Tautomerization of remaining (4*R*)-lactols leads to a racemization at C1. The stereoisomers of the diols and lactols, respectively, did not separate on chiral GC. Consequently, we cannot determine the stereoselective step in (4*R*)-γ-lactone synthesis from 1,4-alkane diols by *R. erythropolis* DSM 44534.

### 3.2. pH-dependency

Biotransformation of rac-**2a** was studied also at different pH-values (Table 2). The pH-value did not significantly influence the stereochemical outcome of lactone formation with lactone **2c** showing $EE_R$ values ranging between 40 and 48%. However, we found higher lactone concentrations at pH 8.8 compared to biotransformation at pH of 5.2.

Table 1
Biotransformation of 2 mM rac-**1a** and rac-**2a** by *R. erythropolis* DSM 44534 (1.2 mg dry wt. $mL^{-1}$) pregrown on different substrates after 100 min incubation

| Growth substrate | **1a** | | | **2a** | | |
|---|---|---|---|---|---|---|
| | **1b**[b] | **1c**[b] (EE %) | Yield[a] (%) | **2b**[b] | **2c**[b] (EE %) | Yield[a] (%) |
| Ethanol | 230 | 170 (72 *R*) | 80 | 100 | 200 (43 *R*) | 83 |
| (*R*)-1,2-Propanediol | 80 | 200 (10 *R*) | 75 | 70 | 140 (24 *R*) | 76 |
| (*S*)-1,2-Propanediol | 110 | 240 (24 *R*) | 70 | <5 | 110 (45 *R*) | 57 |

[a] Yield was calculated as the ratio of the sum of products (**b** and **c**) and substrate (**a**) consumed.
[b] Concentration of compound (μM). Results are the average of duplicate experiments.

Table 2
Biotransformation of 1 mM **2a** by *R. erythropolis* DSM 44534 pregrown on ethanol (1.2 mg dry wt. $mL^{-1}$) at different pH-values

| | 90 min | | | 240 min | | |
|---|---|---|---|---|---|---|
| | **2b**[b] | **2c**[b] (EE %) | Yield[a] (%) | **2b**[b] | **2c**[b] (EE %) | Yield[a] (%) |
| pH 5.2 | 50 | 170 (43 *R*) | 65 | 30 | 150 (48 *R*) | 50 |
| pH 7.2 | 90 | 140 (40 *R*) | 85 | 60 | 440 (42 *R*) | 75 |
| pH 8.8 | 100 | 220 (41 *R*) | 75 | 80 | 550 (41 *R*) | 64 |

[a] Yield was calculated as the ratio of the sum of products (**b** and **c**) and substrate (**a**) consumed.
[b] Concentration of compound (μM). Results are the average of duplicate experiments.

### 3.3. Biotransformation of (S)- or (R)-*4a* and *5a*

The biotransformation of bis-primary 1,4-diols also resulted in the formation of γ-lactones. (*R*)- or (*S*)-2-Methyl-1,4-butanediol ((*R*)-**4a**, (*S*)-**4a**) and 2*Z*-butene-1,4-diol (**5a**) were incubated at 1 mM concentrations with *R. erythropolis* DSM 44534 (0.7 mg cells dry wt. $mL^{-1}$). All substrates were completely converted after 100 min; therefore a lower biomass load was used compared to the conversion of **1a/2a**. (*R*)- and (*S*)-**4a** were both transformed into 4-methyl-dihydro-2(3H)-furanone (**4c1**) and 3-methyl-dihydro-2(3H)-furanone (**4c2**), respectively. The configuration of the enantiomers of **4a** had no effect on the ratio of the products $\mathbf{4c_1}$ (80%) and $\mathbf{4c_2}$ (20%) (Fig. 2). Also the molar conversion yield was in both cases about 55%. The oxidation of the hydroxyl group which is further away from the methyl group seems to be oxidized preferentially thus resulting in 80% of the 4-methyl substituted lactone $\mathbf{4c_1}$. 2*Z*-Buten-1,4-diol (**5a**) was converted into γ-crotonolactone (2(5H)-furanone, **5c**) with a molar conversion yield of 20%.

## 4. Conclusions

The present study reveals *R. erythropolis* DSM 44534 grown on ethanol as versatile microorganism for stereoselective oxidation of racemic 1,4-diols into (*R*)-γ-lactones with enantiomeric excesses of up to $EE_R = 72\%$. In addition, biotransformation of 2 mM 1,4-diols **1a** and **2a** led to the formation of γ-lactols **1b** and **2b** up to 230 μM which were isolated and analyzed by GC–MS. The amount of lactone **2c** was highest at pH 8.8, whereas the $EE_R$ was slightly higher at pH 5.2.

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