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Lactones 20 [1]: Biohydroxylation of saturated bicyclic γ-lactones with the substituted cyclohexane system

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Abstract

Three bicyclic saturated γ-lactones with various numbers of methyl groups at the cyclohexane ring were transformed into the corresponding hydroxylactones. The most effective biohydroxylations were performed by means of cultures *Fusarium culmorum* and *Absidia cylindrospora*. Biotransformations of lactones **4a** and **b** afforded the hydroxylactones with the secondary hydroxy groups (**5a** and **b**), whereas the hydroxy group introduced into the lactone **4c** turned out to be tertiary giving the product **5c**.

Keywords: Lactones; Biotransformations; Hydroxylations; *Fusarium culmorum*; *Absidia cylindrospora*

1. Introduction

Many lactones occurring in the nature exhibit specific biological activity, like antimicrobial [2–5], cytostatic [6–8] or anti-inflammatory [9]. They are also known as insect feeding deterrents [10–13] and plant growth regulators [14,15]. Studying the structures of natural biologically active lactones one can see that beside lactone moiety they usually possess additional functional group, mostly hydroxy or acetoxy. Being engaged in a research project concerning the synthesis of lactones as potential insect feeding deterrents we are interested in their transformation and functionalization. One of our synthetic paths leads from γ,δ-unsaturated acids to saturated lactones and hence there is a need for their further modification: hydroxylation and acetoxylation.

During the literature studies we have found that the chemical methods of introducing a hydroxy group into a molecule are not so efficient and mostly not stereospecific. So we directed our attention to biotransformation methods. We have found many examples of biohydroxylation of saturated hydrocarbons [16], acids [17,18], ketones [19] and also lactones [20]. In our laboratory we have applied microorganisms (fungi strains) for hydroxylation of campholenelactone [21] and some terpenoid iodolactones [22].

Here we present further examples of biohydroxylations of saturated lactones, this time the bicyclic γ-lactones with the substituted cyclohexane ring.

2. Materials and methods

2.1. Analysis

The progress of transformations as well as the purity of isolated products were checked by TLC technique on silica gel (DC-Alufolien Kieselgel 60 F_{254}, Merck). Chromatograms were developed by use of the solvent system: hexane:acetone 3:1. This eluent was also used for preparative column chromatography performed on silica gel (Kieselgel 60, 230–400 mesh).

Gas chromatography (GC) analysis was carried out on a Varian CP-3380 instrument with HP-1 column (crosslinked methyl silicone gum, 25 m × 0.32 mm × 0.25 μm) and HP-5 column (crosslinked methyl silicone gum, 30 m × 0.32 mm × 0.25 μm) and on a Hewlett-Packard 5890 instrument using a CP-cyclodextrin-B,3,6-M-19, 25 m × 0.25 × mm × 0.25 μm chiral column.

^{1}H NMR spectra were recorded in $CDCl_3$ solution on a Bruker Avance DRX 300 spectrometer. IR spectra were de-

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termined with a Specord M-80 infrared spectrophotometer (Carl Zeiss, Jena, Germany). Optical rotations were measured on a Autopol IV automatic polarimeter (Rudolph).

2.2. Materials

The substrates for biotransformations, racemic saturated lactones **4a**–**c** were synthesized from known γ,δ-unsaturated esters **1a**–**c**, respectively [23]. Here we present only their spectral data. They will be useful in studying the changes in the molecules of substrates caused by microorganisms.

2.2.1. 4,4,6-Trimethyl-9-oxabicyclo[4.3.0]nonan-8-one (4a)

Melting point 41 °C; ^{1}H NMR ($CDCl_3$), δ: 0.91 and 0.96 (2 s, 6H, $–(CH_3)_2C<$), 1.17–1.26 (m, 3H, one of $–CH_aH_e$-3 and CH_2-5, AB-system), 1.28 (s, 3H, $–(CH_3)C<$), 1.40 (td, J = 13.3 and 4.0 Hz, 1H, $–CH_aH_e$-3), 1.81 (tt, J = 13.3 and 4.0 Hz, 1H, $–CH_aH_e$-2), 1.96 (dq, J = 13.3 and 4.0 Hz, 1H, $–CH_aH_e$-2), 2.27 and 2.32 (2 d, J = 16.7 Hz, 2H, CH_2-7), 4.18 (t, J = 4.0 Hz, 1H, H-1); IR (cm^{-1}): 2952(s), 1800(s), 1468(s).

2.2.2. 4,4-Dimethyl-9-oxabicyclo[4.3.0]nonan-8-one (4b)

n_D = 1.4673, ^{1}H NMR ($CDCl_3$), δ: 0.90 and 0.93 (2 s, 6H, $(CH_3)_2C<$), 1.04 (dd, J = 13.0 and 12.9 Hz, 1H, one of CH_2-5), 1.26 (m, 1H, one of CH_2-3), 1.33–1.43 (m, 2H, one of CH_2-3 and one of CH_2-5), 1.78 (m, 1H, one of CH_2-2), 2.10 (m, 1H, one of CH_2-2), 2.16 (d, J = 16.7 Hz, 1H, one of CH_2-7), 2.43 (m, 1H, H-6), 2.69 (dd, J = 16.7 and 6.7 Hz, 1H, one of CH_2-7), 4.49 (m, 1H, H-1); IR (cm^{-1}): 2964(s), 1784(s), 1196(s), 1152(s).

2.2.3. 4-Methyl-9-oxabicyclo[4.3.0]nonan-8-one (4c)

n_D = 1.4895, ^{1}H NMR ($CDCl_3$), δ: 0.80 (m, 1H, one of CH_2-5), 0.88 (d, J = 6.5 Hz, 3H, $–(CH_3)CH–$), 1.11 (m, 1H, one of CH_2-5), 1.35 (m, 1H, H-4), 1.47–1.69 (m, 3H, CH_2-3 and one of CH_2-2), 2.16 (d, J = 16.7 Hz, 1H, one of CH_2-7), 2.23 (m, 1H, one of CH_2-2), 2.34 (m, 1H, H-6), 2.65 (dd, J = 16.7 and 6.6 Hz, 1H, one of CH_2-7), 4.45 (m, 1H, H-1); IR (cm^{-1}): 2927(s), 1782(s), 1209(s), 1165(s).

2.3. Microorganisms

The chemicals used for the preparation of the growing media were purchased from BTL in Poland, except glucose which was bought in POCh (Poland).

The strains we used in our research came from the collection of the Institute of Biology and Botany, Medical University, Wrocław (*Absidia cylindrospora* (AM 336), *Beauveria bassiana* (AM 446), *Fusarium culmorum* (AM 3/1), *Fusarium avenaceum* (AM 12), *Fusarium equiseti* (AM 22), *Nigrospora oryzae* (AM 8), *Rhodotorula rubra* (AM 82)) and from the collection of The Institute of Microbiology and Food Biotechnology, Agricultural University, Wrocław (*Yarrovia lypolityca* (A 101)).

The microorganisms were cultivated on Sabouraud agar containing aminobac (catalogue no. S-0002) 5 g, peptone K (S-0011) 5 g, glucose (459560117) 40 g and agar (S-0001) 15 g in distilled water (1 l) at 28 °C and stored in refrigerator at 4 °C.

3. Biotransformations

3.1. Screening procedure

The strains were cultivated at 25 °C in Erlenmayer flasks which contained 75 ml of medium consisting of glucose (459560117) 3 g and peptobac (S-0009) 1 g in water (100 ml). After 5 days, 10 mg of substrate in 1 ml of acetone were added to the grown cultures. The incubation of the shaken cultures with substrate was being continued for 12 days. After 4, 7 and 12 days of incubation the products of biotransformation were extracted with diethyl ether and analyzed by TLC (silica gel, hexane:acetone 3:1) and GC (HP-1 and HP-5 column). The results of GC analyses for lactone **4a**–**c** are presented in Table 1.

3.2. Preparative biotransformation

The saturated lactones **4a**–**c** (300 mg dissolved in 30 ml acetone) were added to the 5-day cultures of *F. culmorum* (in case of lactones **4a** and **b**) or *A. cylindrospora* (in case of lactone **4c**) prepared as described in the screening procedure. The cultures were shaken in three flasks with 500 ml of medium in each flask.

After 12 days of shaking the products were extracted with diethyl ether. The organic solutions were dried ($MgSO_4$) and the solvent was evaporated in vacuo. The products were separated by column chromatography (silica gel, hexane:acetone 3:1).

The preparative biotransformation of lactone **4a** gave the mixture of 70% of hydroxylactone **5a** (Scheme 2) and 30% of the starting material (according to GC).

The column chromatography afforded 106 mg hydroxylactone **5a** (35% isolated yield) and 50 mg of untransformed substrate (16% isolated yield).

The physical and spectral data of hydroxylactone **5a** are given below.

3.2.1. 3-Hydroxy-4,4,6-trimethyl-9-oxabicyclo[4.3.0] nonan-8-one (5a)

Melting point 110–120 °C; $[\alpha]_{546}^{26}$ = −13.5, (c = 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$), δ: 0.97–1.00 (2 s, 6H, $(CH_3)_2C<$), 1.25 (s, 3H, $–(CH_3)C<$), 1.38–1.44 (2 d, J = 14.8 Hz, 2H, CH_2-5, AB-system), 1.58 (s, 1H, –OH), 1.82 (ddd, J = 14.9, 11.5 and 3.3 Hz, 1H, $–CH_aH_e$-2), 2.18 (dt, J = 14.9 and 3.3 Hz, 1H, $–CH_aH_e$-2), 2.27 and 2.32 (2 d, J = 16.8 Hz, 2H, CH_2-7, AB-system), 3.62 (dd, J = 11.5 and 4.1 Hz, 1H, $–CH(OH)–$), 4.32 (t, J = 3.3 Hz, 1H, H-1); IR (cm^{-1}): 3490(s, b), 1756(s), 1236(s), 1068(m), 1040(m).

Table 1
Composition (in % according to GC) of the product mixtures of biotransformations of lactones **4a–c**

Entry	Strain	Time of incubation (days)	Products of transformation (%)					
			4a		**4b**		**4c**	
			4a	**5a**	**4b**	**5b**	**4c**	**5c**
1	*A. cylindrospora*	4	100	0	100	0	55	45
		7	100	0	100	0	13	87
		12	100	0	100	0	5	95
2	*F. culmorum*	4	57	43	80	20	92	8
		7	5	95	56	44	91	9
		12	5	95	11	89	83	17
3	*F. avenaceum*	4	100	0	100	0	100	0
		7	66	34	100	0	100	0
		12	28	72	100	0	100	0
4	*N. oryzae*	4	100	0	85	15	100	0
		7	100	0	70	30	100	0
		12	100	0	52	48	100	0

The preparative biotransformation of lactone **4b** gave the mixture of 61.5% of hydroxylactone **5b** (Scheme 2) and 38.5% of the starting material (according to GC).

The column chromatography afforded 80 mg hydroxylactone **5b** (27% isolated yield) and 40 mg of untransformed substrate (40% isolated yield).

The physical and spectral data of hydroxylactone **5b** are as follows.

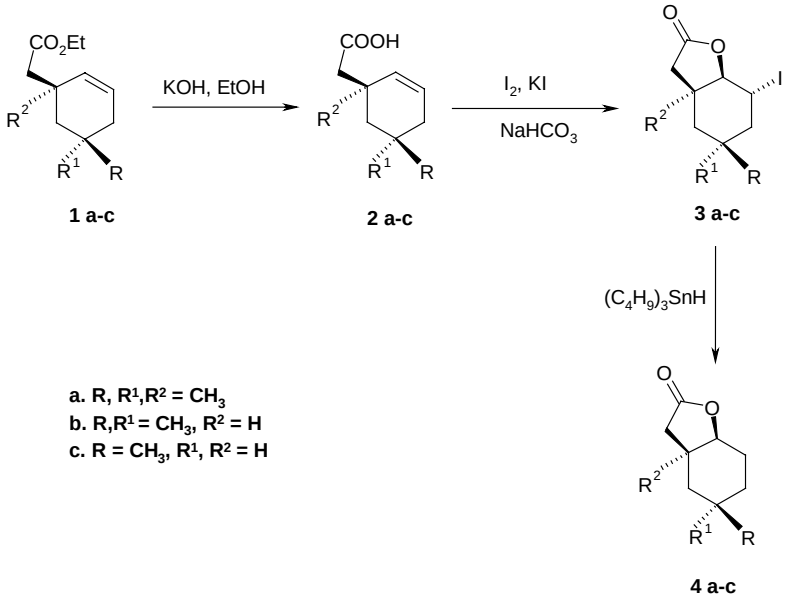
*3.2.2. 3-Hydroxy-4,4-dimethyl-9-oxabicyclo[4.3.0]nonan-8-one (**5b**)*

$[\alpha]_{546}^{26} = -75.4$ ($c = 0.8$, $CHCl_3$); ^{1}H NMR ($CDCl_3$), δ: 0.88 and 0.98 (2 s, 6H, $(CH_3)_2C<$), 1.14–1.23 (m, 1H, one of CH_2-5), 1.46–1.53 (m, 2H, one of CH_2-5 and OH), 1.70 (ddd, $J = 15.1$, 11.7 and 4.1 Hz, 1H, –CH_aH$_e$-2), 2.15 (d, $J = 16.8$ Hz, 1H, one of CH_2-7), 2.31 (ddd, $J = 15.1$, 4.9 and 2.1 Hz, 1H, –CH$_a$$H_e$-2), 2.45 (m, 1H, H-6), 2.66 (dd, $J = 16.8$ and 6.7 Hz, 1H, one of CH_2-7), 3.57 (dd, $J = 11.7$ and 4.9 Hz, 1H, –C*H*(OH)–), 4.60 (m, 1H, H-1); IR (cm^{-1}): 3302(s, b), 1769(s), 1193(m), 1059(m).

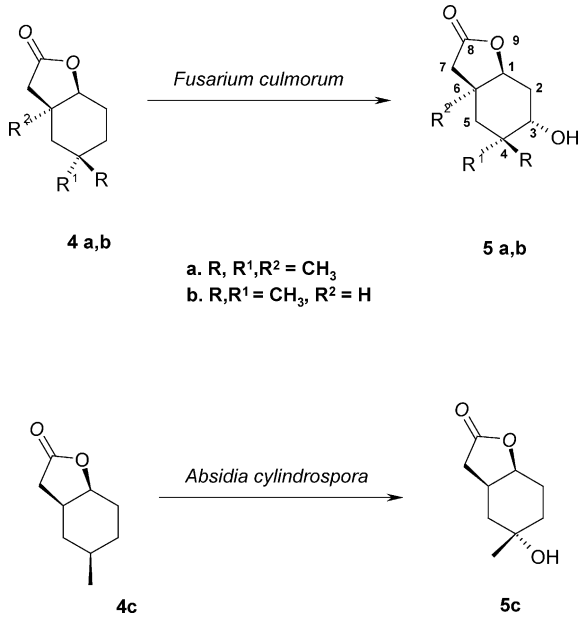
The preparative biotransformation of lactone **4c** gave the mixture of 41.7% of hydroxylactone **5c** (Scheme 2) and 58.3% of the starting material (according to GC).

After the column chromatography 30 mg of the hydroxylactone **5c** (15% isolated yield) and 80 mg of unreacted substrate (40% isolated yield) were obtained.

The physical and spectral data of the hydroxylactone **5c** are given below.



Scheme 1.



Scheme 2.

*3.2.3. 4-Hydroxy-4-methyl-9-oxabicyclo[4.3.0]nonan-8-on (**5c**)*

Oily liquid; $[\alpha]_{546}^{26} = -4.25$ ($c = 0.4$, $CHCl_3$); ^{1}H NMR ($CDCl_3$), δ: 1.17 (dd, $J = 14.1$ and 12.0 Hz, 1H, –CH_aH$_e$-5), 1.24 (s, 3H, –(CH_3)C(OH)–), 1.45–1.52 (m, 3H, CH_2-3 and OH), 1.68 (ddd, $J = 14.1$, 6.1 and 2.4 Hz, 1H, –CH$_a$$H_e$-5), 2.01–2.08 (m, 2H, CH_2-2), 2.17 (d, $J = 16.6$ Hz, 1H, one of CH_2-7), 2.62 (m, 1H, H-6), 2.70 (dd, $J = 16.6$ and 6.6 Hz, 1H, one of CH_2-7), 4.50 (m, 1H, H-1); IR (cm^{-1}): 3419(s, b), 1768(s), 1230(m), 1190(m).

4. Results and discussion

The substrates for biotransformations, racemic saturated lactones **4a–c**, were obtained in a three-step synthesis from known racemic γ,δ-unsaturated esters as described in our previous paper (Scheme 1) [23]. The relative orientations of the lactone ring and the substituents at C-6 and C-4 were established by X-ray analysis of corresponding 2-iodolactones (**3a–c**). These orientations did not change during the last step of this synthesis—reduction of iodolactones with tributyltin hydride [24]. Thanks to that we could ascribe the orientation of the hydroxy group introduced into molecule during the biohydroxylation (Scheme 2).

The first step of the biotransformation of saturated lactones **4a–c**, the screening procedure, was carried out using eight fungi species of local origin: *A. cylindrospora*, *B. bassiana*, *F. culmorum*, *F. avenaceum*, *F. equiseti*, *N. oryzae*, *R. rubra* and *Y. lypolytica*. The progress of the biotransformation was monitored by TLC and GC.

In the experiments with four microorganisms (*B. bassiana*, *F. equiseti*, *R. rubra* and *Y. lipolytica*) we did not observe any biotransformatic products. Even after twelve days of incubation only the substrates were present in the biotransformation mixture.

The results of the screening experiments for the next four species are given in Table 1. They indicate that only *F. culmorum* transformed all three lactones. The lactones **4a** and **b** were transformed to a high extent, whereas the lactone **4c** to rather low one. After 12 days only 5 and 11% of starting material remained unreacted. The third lactone (**4c**) was effectively transformed by *A. cylindrospora*. *F. avenaceum* transformed the lactone with three methyl groups at the cyclohexane ring (**4a**) whereas *N. oryzae* transformed the lactone **4b** (Table 1).

Preparative biotransformations of **4a** and **b** were performed by means of *F. culmorum*. The GC analysis confirmed that the products **5a** (35% isolated yield) and **5b** (27% yield) isolated from the preparative experiments were the same as those observed in screening experiments and no side products were detected. The structures of the products **5a** and **b** were established on the basis of IR and ^{1}H NMR data. They were identified as the products of biohydroxylation at C-3 (Scheme 2). The IR spectra showed the presence of a secondary hydroxy group (absorption bands: 3302, 1261, 1059 cm^{-1} for **5b** and 3490, 1236, 1068 cm^{-1} for **5a**) in the products. Bands at 1756 and 1769 cm^{-1} in the IR spectra of **5a** and **b**, respectively, indicate that the γ-lactone ring was untouched by microorganism. The place of biohydroxylation was established on the basis of comparative analysis of ^{1}H NMR spectra of substrates and products. In the case of lactones with gem-dimethyl groups at C-4 (**4a** and **b**), the hydroxy group was introduced in C-3 in equatorial position. This position is indicated by the lack of the signal of one of the CH_2-3 protons, the presence of multiplets at 3.57 (for **5b**) and 3.62 ppm (for **5a**) and similarities (chemical shift, multiplicity and coupling constants) of the signals of H-6 and H-1 observed in the spectra of both substrates (**4a** and **b**) and products (**5a** and **b**). Equatorial orientations of the hydroxy group in both hydroxylactones are indicated by the coupling constant values of H-3 with protons of the neighboring CH_2-2 group: 11.5 and 4.1 Hz for **5a**; 11.8 and 4.9 Hz for **5b**.

The preparative biotransformation of the lactone with only one methyl group at C-4 (**4c**), which was carried out with *A. cylindrospora* afforded one product (15% isolated yield), identical (according to GC) to the one observed during the screening experiments with *A. cylindrospora* and *F. culmorum*. Spectral data indicated that this time the biohydroxylation affected the C-4 position resulting in hydroxy lactone **5c** (Scheme 2). The presence of the hydroxy group as well as the γ-lactone ring in the molecule was confirmed by IR (absorption bands at 3419 and 1769 cm^{-1} in the spectrum of **5c**). The comparative analysis of the ^{1}H NMR spectra of **4c** and **5c** univocally show that the hydroxy group is located at the C-4 axial position. There

is no multiplet of H-4 in the spectrum of **5c**. The singlet of the methyl group at C-4 is shifted downfield by 0.3 ppm in the spectrum of **5c** in comparison with the doublet ($J = 6.6$ Hz) present at 0.89 ppm in the spectrum of substrate **4c**.

All three biotransformation products (**5a**–**c**) were optically active. They were found to be leavorotatory. The GC analyses performed on the chiral column (CP-cyclodextrin-B,3,6-M-19.25 m × 0.25 × mm × 0.25 μm) showed only two peaks for each product. Enantiomeric excess calculated on the basis of the data from these analyses are as follows: 28% for **5a**, 72% for **5b** and 30% for **5c**. These results suggest rather low enantioselectivity of biohydroxylation.

5. Conclusions

To sum up the results presented above, the following can be pointed out:

1. The microorganisms studied except *F. culmorum* showed substrate specificity.
2. They showed a very high regioselectivity of the biohydroxylation process.
3. The stereospecificity of these transformations was also very high. In the case of the biotransformation of **4a** and **b**, the hydroxy group was introduced in the equatorial position only.
4. Enantioselectivity of the transformations carried out was low.

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