Article

Antimicrobial Activity of Hydroxylactone obtained by Biotransformation of Bromo- and Iodolactone with Gem-Dimethylcyclohexane Ring

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Duas lactonas bicíclicas com anéis gem-dimetilciclohexano (δ-bromo-γ-lactona e δ-iodo-γ-lactona) foram utilizadas como substratos para biotransformação por células inteiras de várias estirpes fúngicas (cinco *Fusarium* strains, *Nigrospora oryzae*, *Syncephalastrum racemosum*, *Stemphylium botryosum* e *Cunninghamella japonica* e *Acremonium sp*). Alguns dos microorganismos selecionados (principalmente cepas *Fusarium*) transformam estas lactonas por desalogenação hidrolítica em *cis*-(−)-2-hidroxi-4,4-dimetil-9-oxabiciclo[4.3.0]nonano-8-ona. A conversão do substrato foi igual ou próxima de 100%, mostrando que o método permite a remoção completa do átomo de halogênio da molécula, substituindo-o por um grupo hidroxila. As estruturas de todos os substratos e produtos foram estabelecidas com base nos seus dados espectrais. A hidroxilactona obtida como resultado da biotransformação foi examinada por sua atividade biológica contra bactérias, leveduras e fungos. Este composto inibe o crescimento de alguns microorganismos testados.

Two bicyclic lactones with gem-dimethylcyclohexane rings (δ-bromo-γ-lactone and δ-iodo-γ-lactone) were used as substrates for biotransformation by whole cells of several fungal strains (five cepas *Fusarium*, *Nigrospora oryzae*, *Syncephalastrum racemosum*, *Stemphylium botryosum*, *Cunninghamella japonica* and *Acremonium sp*). Some of the selected microorganisms (mainly *Fusarium* strains) transformed these lactones by hydrolytic dehalogenation into *cis*-(−)-2-hydroxy-4,4-dimethyl-9-oxabicyclo[4.3.0]nonan-8-one. The conversion of the substrate was equal or close to 100%, showing that this method allows for the complete removal of the halogen atom from the molecule, replacing it by a hydroxy group. The structures of all substrates and products were established on the basis of their spectral data. Hydroxylactone obtained as a result of biotransformation was examined for its biological activity against bacteria, yeasts and fungi. This compound inhibits the growth of some tested microorganisms.

Keywords: lactones, biotransformations, hydrolytic dehalogenation, *Fusarium* species, antimicrobial activity

Introduction

Halogenated organic compounds are commonly used in agriculture as herbicides, insecticides or artificial fertilizers, in medicine as antibiotics and also in chemistry as solvents or intermediates.[1] Many of these compounds are used during a variety of industrial processes and may pollute our environment, including water and air, as they are difficult to degrade naturally. It is therefore important to find methods that can neutralize these compounds. For many years, different bacteria have been used to transform halocompounds into other compounds, and there are many reports on the transformation of haloalkanes,[2-4] halogenated ethenes[5] and halogenated aromatic compounds[1,6,7] by bacteria. Another enzyme widely used to utilize halocompounds is cytochrome P-450.[8,9] In our laboratory, we work with microorganisms that are able to degrade halogenated compounds including halolactones.[10-13]

Hydroxylactones can be obtained by means of chemical synthesis[14-18] or by biotransformation of saturated

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or unsaturated lactones.[19,20] Chemical synthesis of hydroxylactones usually yields one lactone or a mixture of lactones with specific structure. However, microorganisms may produce other compounds that are impossible to obtain synthetically when the hydroxy group is in an inactive place of the molecule[19,20] or in another site (for example, equatorial instead axial) in relation to the lactone ring.[12,13,21]

Here, we present further examples of biohydroxylation of bromolactone and iodolactone with a gem-dimethylcyclohexane ring. These lactones were chosen because in the previous studies we found that the location of gem-dimethyl groups in the cyclohexane ring significantly affected the structure of the biotransformation products. In the case of saturated lactones with gem-dimethyl groups at C-4, microorganisms were able to introduce a hydroxy group at C-3 in equatorial position.[19] In turn, the saturated lactone with the gem-dimethyl moiety at C-3 would yield two products with hydroxy group at C-2 or C-6, both in equatorial position.[20] It is worth noting that in this case, there was a change in cyclohexane ring conformation. Similar situation was observed during biotransformation of halolactones with gem-dimethyl moiety at C-5 or C-4 (with additional methyl group at C-6) when the hydroxy group was introduced in place of a halogen atom. However, the orientation of a hydroxy group dependes on the position of gem-dimethyl groups. In the first case, a hydroxy group was introduced in an axial position, and the conformation of cyclohexane ring was maintained. The presence of gem-dimethyl groups at C-4 resulted in an introduction of a hydroxy group in an equatorial position and also a change in cyclohexane ring conformation.[11,12]

Experimental

Analysis

The progress of all reactions and the purity of isolated products were checked by thin layer chromatography (TLC) on silica gel-coated aluminum plates (DC-Alufolien Kieselgel 60 F254, Merck), and by gas chromatography (GC) analysis which was carried out on a Hewlett Packard HP 5890 instrument using the HP-5 column (cross linked methyl silicone gum, 30 m × 0.32 mm × 0.25 μm) using the following conditions: injector 150 °C, detector (FID) 300 °C, column temperature: 100 °C (hold 1 min), 100-200 °C (rate 10 °C min^{-1}), 200-300 °C (rate 50 °C min^{-1}) and 300 °C (hold 1 min). The structures of the obtained compounds were also confirmed by gas chromatography-mass spectrometry (GC-MS) analysis using Varian Saturn 2000 instrument (*EI* ionization) with the HP-1 column (cross-linked methyl silicone gum, 25 m × 0.32 mm × 0.52 μm) under the

following conditions: injector 200 °C, detector 300 °C, column temperature: 120 °C (hold 2 min), 120-300 °C (rate 20 °C min^{-1}) and 300 °C (hold 3 min). The enantiomeric excess of the products obtained from biotransformation were determined by GC analysis using the chiral column CP-cyclodextrin-B-325 (30 m × 0.25 mm × 0.25 μm). The temperatures during these GC analyses were as follows: injector 200 °C, detector (FID) 250 °C, column temperature: 140 °C (hold 45 min), 140-200 °C (rate 20 °C min^{-1}) and 200 °C (hold 1 min). Preparative column chromatography on silica gel (Kieselgel 60, 230-400 mesh) was used for the purification of the products of the chemical synthesis and biotransformation.

[1]H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in a CDCl$_3$ solution on a Bruker Avance DRX 300 spectrometer. The assignments of ^{13}C NMR chemical shifts were made by means of C/H correlation heteronuclear multiple quantum coherence (HMQC). Infrared spectra were determined using an FTIR Thermo-Mattson IR 300 spectrometer. Optical rotations were measured on an Autopol IV automatic polarimeter (Rudolph).

Materials

The substrates for biotransformation were two racemic halolactones **2** and **3**, which were synthesized from a known γ,δ-unsaturated acid **1**.[15] The new bromolactone **2** was obtained by bromolactonisation of acid **1** using *N*-bromosuccinimide (NBS) in tetrahydrofuran (THF). The known iodolactone **3** was obtained by iodolactonisation of acid **1** according to the procedure described earlier.[15] Here, we describe the synthesis and spectral data of these compounds, and the use of these data for studying the changes in the molecules during biotransformation.

2-Bromo-4,4-dimethyl-9-oxabicyclo[4.3.0]nonan-8-one (**2**)

Acid **1** (2.3 g, 0.014 mol) was dissolved in 30 mL of THF, and then *N*-bromosuccinimide (NBS, 5.2 g, 0.029 mol) was added. This mixture was stirred for 24 h at room temperature and then water was added (30 mL). The product was extracted with diethyl ether (3 × 30 mL). The combined ethereal fractions were washed with saturated NaHCO$_3$ solution (20 mL), brine (20 mL) and dried with anhydrous magnesium sulphate. The crude product was purified on silica gel (hexane:acetone, 3:1) yielding 2.5 g (74%) of bromolactone **2** with the following physical and spectral properties: mp 59-60 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.00 and 1.06 (two s, 6H, (CH$_3$)$_2$C-4), 1.30 and 1.38 (two m, 2H, CH$_2$-5), 1.76 (dd, 1H, *J* 14.3, 10.7 Hz, H-3

axial), 1.96 (dd, *J* 14.3, 4.2 Hz, 1H, H-3 equatorial), 2.29 (dm, 1H, *J* 13.6 Hz, one of CH$_2$-7), 2.68-2.79 (two m, one of CH$_2$-7 and H-6), 4.18 (ddd, 1H, *J* 10.7, 6.5, 4.2 Hz, H-2), 4.72 (dd, 1H, *J* 6.5, 6.3 Hz, H-1); ^{13}C NMR (300 MHz, CDCl$_3$) δ 29.58 (C-10), 30.55 (C-9), 31.72 (C-4), 32.53 (C-6), 35.52 (C-7), 38.37 (C-5), 43.79 (C-3), 46.69 (C-2), 85.04 (C-1), 175.53 (C-8); IR (KBr) ν_{max}/cm^{-1} 2960, 1783, 1371, 1173, 999; EI-MS *m/z* (%) 248 [M + H], 167 [M-HBr], 167 (100), 149 (17), 123 (20), 95 (28), 81 (19), 55 (24), 39 (36).

2-Iodo-4,4-dimethyl-9-oxabicyclo[4.3.0]nonan-8-one (**3**)

^{1}H NMR (300 MHz, CDCl$_3$) δ 1.00 and 1.03 (two s, 6H, (CH$_3$)$_2$C-4), 1.36 (m, 2H, CH$_2$-5), 1.86 (dd, 1H, *J* 14.3, 12.2 Hz, H-3 axial), 2.05 (dd, 1H, *J* 14.3, 4.2 Hz, H-3 equatorial), 2.32 (m, 1H, H-6), 2.70 (m, 2H, CH$_2$-7), 4.19 (ddd, 1H, *J* 12.2, 7.8, 4.2 Hz, H-2), 4.83 (dd, 1H, *J* 7.8, 7.2 Hz, H-1); EI-MS *m/z* (%) 294 [M + H], 167 [M-HJ], 167 (100), 149 (21), 123 (50), 107 (54), 95 (28), 81 (31), 69 (45), 55 (24), 39 (57).

Microorganisms

The fungal strains used in all biotransformation reactions came from the collection of the Institute of Biology and Botany, Medical University, Wrocław (Poland): *Nigrospora oryzae* AM8, *Fusarium culmorum* AM10, *Fusarium avenaceum* AM11, *Fusarium tricinctum* AM16, *Fusarium semitectum* AM20, *Syncephalastrum racemosum* AM105, *Fusarium solani* AM203, *Stemphylium botryosum* AM279, *Cunninghamella japonica* AM472 and *Acremonium sp.* AM545. All these strains are available at the Department of Chemistry, Wrocław University of Environmental and Life Sciences. The fungi were cultivated on Sabouraud's medium containing 5 g of aminobac, 5 g of peptone, 40 g of glucose and 15 g of agar dissolved in 1 L of distilled water at 28 °C and stored in refrigerator at 4 °C.

Biotransformation

Screening procedure

The fungal strains used for biotransformation were cultivated at 25 °C in Erlenmeyer flasks containing 100 mL of a medium (3 g glucose and 1 g peptobac in 100 mL of water). After 4 days, 10 mg of the substrate dissolved in 1 mL of acetone were added to each culture flask. Incubation of the shaking cultures with the substrate was continued for 7 days. After 3, 5 and 7 days of incubation, the products of biotransformation were extracted with dichloromethane (15 mL), and analyzed by TLC (silica gel, hexane:acetone 3:1) and GC (HP-5 column).

Preparative biotransformations

Halolactone **2** or **3** (100 mg) were dissolved in 10 mL of acetone and dispensed into 10 Erlenmeyer flasks containing the four day cultures of fungal strains prepared as described in the screening procedure. The microorganisms selected during the screening transformations were incubated with the substrates for 7 days, then, the product mixtures were extracted with dichloromethane (3 × 40 mL). The organic solutions were dried (MgSO$_4$) and the solvent was evaporated *in vacuo*. Column chromatography (silica gel, hexane:acetone 3:1) was used to obtain pure product (hydroxylactone **4**), the unreacted substrate and the fungal metabolites. The physical and spectral data of hydroxylactone **4** (2-hydroxy-4,4-dimethyl-9-oxabicyclo[4.3.0]nonan-8-one) are presented below: mp 80-81 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 0.92 and 0.94 (two s, 6H, (CH$_3$)$_2$C-4), 0.98 (dd, 1H, *J* 13.8, 12.9 Hz, one of CH$_2$-5), 1.18 (dd, 1H, *J* 12.6, 12.3 Hz, one of CH$_2$-3), 1.42 (dd, 1H, *J* 13.8, 6.4 Hz, one of CH$_2$-5), 1.63 (dd, 1H, *J* 12.6, 5.0 Hz, one of CH$_2$-3), 2.23 (d, 1H, *J* 16.7 Hz, one of CH$_2$-7), 2.58 (ddd, 1H, *J* 13.0, 6.4, 6.3 Hz, H-6), 2.75 (dd, 1H, *J* 16.7, 6.3 Hz, one of CH$_2$-7), 3.92 (ddd, 1H, *J* 12.3, 5.0, 3.9 Hz, H-2), 4.58 (t, *J* 3.9 Hz, 1H, H-1); ^{13}C NMR (300 MHz, CDCl$_3$) δ 24.01 (C-10), 31.63 (C-4), 32.31 (C-9), 33.29 (C-6), 38.38 (C-7), 39.45 (C-5), 41.18 (C-3), 67.08 (C-2), 80.86 (C-1), 176.20 (C-8); IR (KBr) ν_{max}/cm^{-1} 3333, 2958, 1778, 1164, 1070; EI-MS *m/z* (%) 185 [M + H], 168 [M-H$_2$O], 168 (49), 150 (18), 125 (24), 109 (21), 105 (21), 98 (47), 85 (100), 81 (30), 69 (64), 55 (39), 41 (67), 39 (64).

Bioassay

The following strains of bacteria were used: *Micrococcus flavus* C1, *Bacillus cereus* C3, *Escherichia coli* C1, *Bacillus subtilis* B5, *Pseudomonas fluorescens* W1, yeast: *Debaryomyces hansenii* K12a, *Saccharomyces cerevisiae* SV30, *Yarrowia lipolytica* ATCC 20460, *Schizosaccharomyces pombe* C-1, *Rhodotorula rubra* C-9 and filamentous fungi: *Aspergillus niger* XP, *Fusarium linii* 3A, *Penicillium sp.*, *Alternaria sp.* These strains came from the own collection of the Department of Biotechnology and Food Microbiology, University of Environmental and Life Sciences. The bacterial cultures were carried out for 48 h in a liquid broth containing 15 g of dry bullion (Biocorp) and 10 g of glucose dissolved in 1 L of distilled water. Yeast and fungi were cultured in YPG medium (10 g of yeast extract, 10 g of bacteriological peptone and 10 g of glucose dissolved in 1 L of distilled water) for 48 and 96 h, respectively. The effects of hydroxylactone **4** on the growth of microorganisms

were tested in a microbiological apparatus Bioscreen C (Automated Growth Curve Analysis System, Lab Systems, Finland). The working volume in the wells of the Bioscreen plate was 300 μL, comprising 280 μL of culture medium, and 10 μL of cell or spore solution (final density 1×10^6 cells mL^{-1}). Hydroxylactone **4** was dissolved in 10 μL dimethyl sulfoxide and used at a final concentration of 0.1% (m/v). The temperature was controlled at 30 °C (bacteria, yeasts) and 25 °C (filamentous fungi), the optical density of the cell suspensions was measured automatically at 560 nm at regular intervals of 30 min, for 2-4 days. The cell cultures were placed on a continuous shaker. Each culture was performed in 3 replications.

The data were analyzed using a spreadsheet software (Excel 97) and the means for the triplicates of each culture medium type were calculated. The mean values were used to generate the growth curves for each investigated strain, constituting a function of the incubation time and the culture medium absorbance. The resulting microbial growth curves were compared to control cultures in the medium supplemented with dimethyl sulfoxide.

Results and Discussion

The substrates for transformation were two racemic halo-γ-lactones (**2**, **3**) that included a gem-dimethylcyclohexane ring. γ,δ-Unsaturated acid **1** was used to obtain two lactones: iodo-γ-lactone **3**[16] and the novel bromo-γ-lactone **2**. Lactone **2** was obtained using *N*-bromosuccinimide in tetrahydrofurane (Scheme 1).
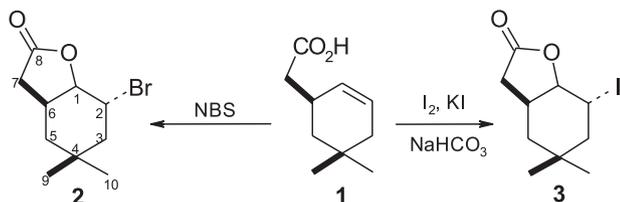
Scheme 1. Synthesis of halolactones **2** and **3**.

The structure of bromo-γ-lactone **2** was determined on the basis of its spectral (¹H NMR and IR) data. The absorption bands at 1783 cm^{-1} on IR spectra confirmed the presence of a γ-lactone ring in the molecule. The similarity between spectral data (¹H NMR) of bromolactone **2** and the previously obtained iodolactone **3**[15] suggests that their structures are similar, both lactones have the same skeleton. The analysis of ¹H NMR spectra of bromolactone **2** showed that the coupling constants of H-1 with H-2 (*J* 6.5 Hz), H-2 with H-3 axial (*J* 10.7 Hz) and H-2 with H-3 equatorial (*J* 4.2 Hz) are similar to coupling constants of iodolactone **3**: *J* 7.8 Hz (H-1 with H-2), *J* 12.1 Hz (H-2 with H-3 axial) and *J* 4.2 Hz

(H-2 with H-3 equatorial), respectively. This indicates that in both molecules, the cyclohexane ring exists in the chair conformation, H-1 and H-2 protons are in *trans* diaxial positions, and H-2 proton and lactone ring are *cis* oriented in relation to the axial methyl group at C-4 (Scheme 3).

In the first step of biotransformation, ten fungal strains of local origin were tested for their ability to transform halolactones: *Nigrospora oryzae* AM8, *Fusarium culmorum* AM10, *Fusarium avenaceum* AM11, *Fusarium tricinctum* AM16, *Fusarium semitectum* AM20, *Syncephalastrum racemosum* AM105, *Fusarium solani* AM203, *Stemphylium botryosum* AM279, *Cunninghamella japonica* AM472 and *Acremonium sp.* AM545. The progress of transformations was monitored by means of standard techniques (TLC and GC). The results of the biotransformation screening are shown in Table 1.

Table 1. Hydrolytic dehalogenation of bromolactone **2** and iodolactone **3** after 7 days of incubation

entry	Microorganism	Bromolactone **2**	Iodolactone **3**
1	*Nigrospora oryzae* AM8	(−)	(−)
2	*Fusarium culmorum* AM10	(+++)	(+++)
3	*Fusarium avenaceum* AM11	(++)	(++)
4	*Fusarium tricinctum* AM16	(−)	(++)
5	*Fusarium semitectum* AM20	(+++)	(++)
6	*Syncephalastrum racemosum* AM105	(+)	(+)
7	*Fusarium solani* AM203	(++)	(++)
8	*Stemphylium botryosum* AM279	(+)	(+)
9	*Cunninghamella japonica* AM472	(+)	(−)
10	*Acremonium* sp. AM545	(+++)	(+)

(−): the conversion 5-22%; (+): the conversion 32-51%; (++): the conversion 66-83%; (+++): the conversion 95-100%.

The best degree of conversion (100%) was observed when bromolactone **2** was incubated with *Fusarium semitectum* AM20 (entry 5) and *Acremonium sp*. AM545 (entry 10). Interestingly, the same microorganisms converted the second substrate, iodolactone **3** to a lesser extent (79 and 38%, respectively). The reverse situation was observed for *Fusarium tricinctum* AM16 (entry 4). This fungus preferred hydroxylation of iodolactone (79 %), bromolactone was transformed only in 15%. The other three fungal strains: *Fusarium culmorum* AM10 (entry 2), *Fusarium avenaceum* AM11 (entry 3) and *Fusarium solani* AM203 (entry 7) transformed both substrates with comparable efficiency (between 66 and 96%). The screening procedure assessed only the conversion degree. Both substrates

(bromolactone **2** and iodolactone **3**) were converted into the same product (Scheme 2).
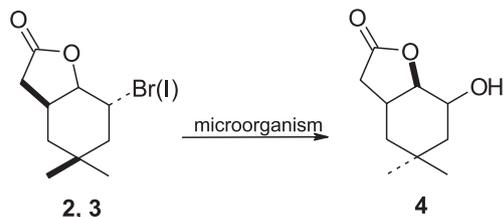

Scheme 2. Biotransformations of halolactones **2** and **3**.

In the next step, the fungal strains selected during the screening transformations were chosen for preparative biotransformation. These were mainly *Fusarium* species and *Acremonium sp.* that effectively converted the substrates into a product (over 66% efficacy). The results of this step are shown in Figures 1 and 2.
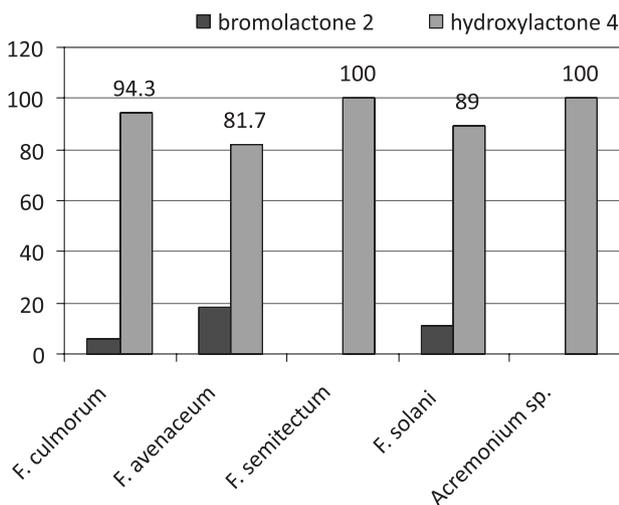
Figure 1. Composition (in %) of the product mixtures of preparative biotransformation of bromolactone **2**.
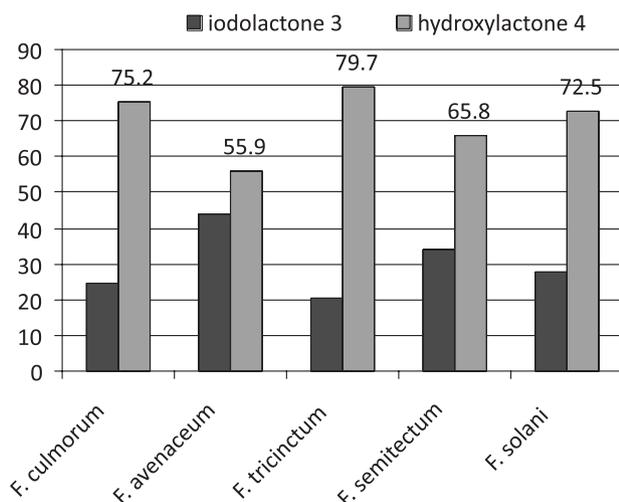
Figure 2. Composition (in %) of the product mixtures of preparative biotransformation of iodolactone **3**.

The above data indicate high substrate specificity. Fungal strains clearly preferred bromolactone **2**, this substrate was fully converted in two cases (*Fusarium semitectum* AM20 and *Acremonium sp.* AM545) and in 81.7 to 94.3% in three other cases, while iodolactone **3** was transformed with lower yield (55.9-79.7%).

The structure of the obtained product was established on the basis of its spectral data. The IR spectra showed that the γ-lactone ring was retained in the product during the biotransformation (absorption bands at 1778 cm⁻¹). A strong and broad band found at 3333 cm⁻¹ suggested the presence of a hydroxy group in the molecules.

Previous analyses of bromolactone **2** and iodolactone **3** structures showed that H-1 and H-2 protons were in *trans* diaxial positions, the bromine or iodine atom was in the equatorial position and *trans* oriented in relation to the C–O bond, and the axial methyl group was at C-4.

The analysis of NMR (¹H,¹³C COSY (correlation spectroscopy) and HMQC) spectra of the hydroxylactone **4** that was obtained microbiologically and bromolactone **2** allowed for the identification of differences between both molecules. The coupling constants of H-1 with H-2 (*J* 3.9 Hz), H-2 with H-3 axial (*J* 12.3 Hz) and H-2 with H-3 equatorial (*J* 5.0 Hz) indicated that the molecule of hydroxylactone **4** exists in a chair conformation. The coupling constants observed for the H-2 proton suggested its axial position, and for the H-1 proton indicated its equatorial position. Additionally, in the case of hydroxylactone **4,** it can be observed that the axial H-2 proton is *cis* oriented in relation to the equatorial H-1 proton and the axial methyl group at C-4. However, the C–O bond of the lactone ring is *trans* oriented to the axial methyl group at C-4. These data suggested that bromine or iodine atom was replaced with a hydroxy group in a mechanism resembling the S_N2 mechanism. In the obtained product **4**, the hydroxy group should be in an axial position. Spectral data showed, however, that the hydroxy group occupied an equatorial position and conformation of the cyclohexane ring was altered. This change can be explained by a tendency of the molecule to minimize energetically unfavorable 1,3-diaxial repulsions between the hydroxy group at C-2 and the axial methyl group at C-4 (Scheme 3).

Similar situations involving the change in hydroxylactone conformation as a result of biotransformation were observed in earlier experiments using similar substrates.[11-13]

During previous syntheses, the structural analogous of hydroxylactone **4** were obtained.[14,21] These methods allowed us to obtain a hydroxylactone in which the cyclohexane ring was in the boat conformation, and lactone ring was in *trans* position in relation to the axial methyl group at C-4. The hydroxy group at C-2 occupied the equatorial position and
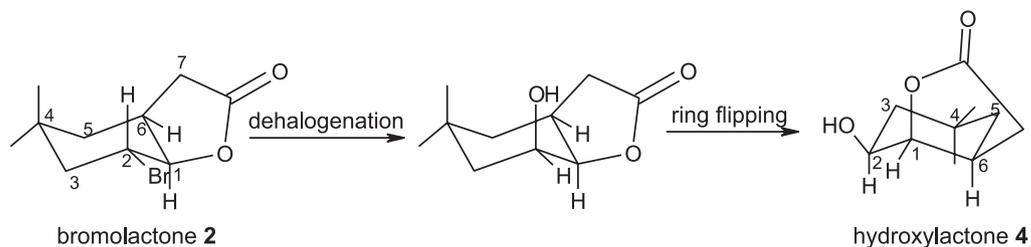
Scheme 3. Equatorial location of hydroxy group in hydroxylactone **4** as the stereochemical consequence of the microbial hydrolytic dehalogenation of bromolactone **2** or iodolactone **3** proceeding with the inversion at C-2.

Table 2. Results of preparative biotransformations of bromolactone **2**

entry	Strain	Isolated yield / g / %	ee / %	α_D^{20}
1	*F. culmorum* AM10	0.015 / 24.0	8.3	−11.28 (*c* = 0.69%, CHCl₃)
2	*F. avenaceum* AM11	0.0077 / 12.3	3.4	−5.54 (*c* = 0.52%, CHCl₃)
3	*F. semitectum* AM20	0.0235 / 37.5	14.4	−22.13 (*c* = 0.74%, CHCl₃)
4	*F. solani* AM203	0.014 / 22.4	12.4	−16.87 (*c* = 0.44%, CHCl₃)
5	*Acremonium sp.* AM545	0.0246 / 39.0	12.6	−17.22 (*c* = 0.54%, CHCl₃)

Table 3. Results of preparative biotransformations of iodolactone **3**

entry	Strain	Isolated yield / g / %	ee / %	α_D^{20}
1	*F. culmorum* AM10	0.009 / 14.4	9.0	−12.32 (*c* = 0.48%, CHCl₃)
2	*F. avenaceum* AM11	0.0195 / 31.2	18.6	−30.74 (*c* = 0.48%, CHCl₃)
3	*F. tricinctum* AM16	0.0133 / 21.2	16.9	−27.65 (*c* = 0.41%, CHCl₃)
4	*F. semitectum* AM20	0.0168 / 26.8	4.0	−6.01 (*c* = 0.43%, CHCl₃)
5	*F. solani* AM203	0.0186 / 29.7	19.4	−33.44 (*c* = 0.45%, CHCl₃)

was located in the *trans* position relative to the C−O bond of the lactone ring.

In the case of hydroxylactone **4**, the cyclohexane ring is in the chair conformation. The H-2 proton is in axial position, and the H-1 proton is in equatorial position. The equatorial hydroxy group at C-2 is *cis* oriented in relation to the C−O bond of the lactone ring and *trans* oriented relative to the axial methyl group at C-4.

For all hydroxylactones obtained from halolactones during the preparative biotransformations, their optical purity was determined in order to investigate the ability of microorganisms to transform the chosen substrates into the new products, and to test the product enantiospecificity. The results are shown in Tables 2 and 3.

In all cases, the formation of (−) isomer of hydroxylactone **4** was preferred, unfortunately the enantiomeric excess was very low (3.4-19.4%). Even in the case of low degree of conversion (55.9%) of iodolactone **3** by *F. avenaceum* AM11 (entry 2), the enantiomeric excess was low (18.6%). On the other hand, the best result was obtained for *F. solani* AM203 (entry 5), despite the fact that in this case, 72.5% of iodolactone **3** were converted into hydroxylactone (Figure 2).

Considering the fact that naturally occurring hydroxylactones have different biological properties including fungicidal and bactericidal,[22-25] we decided to test the obtained hydroxylactone **4** in this respect. Bioassays performed on selected strains of bacteria, yeasts and fungi are given in Tables 4-6 and in Figures S15-S21 in the Supplementary Information (SI) section.

The gathered data support the conclusion that the best results with hydroxylactone **4** on total growth inhibition was observed for the two strains of *M. flavus* C-1 (Table 4 entry 1) and *Alternaria sp.* (Table 6 entry 4). For five other strains of *B. cereus* and *P. fluorescens* W-1 (Table 4 entry 2 and 5), *D. hansenii* K12a and *S. cerevisiae* SV30 (Table 5 entry 1 and 2) and also *Penicillium sp.* (Table 6

Table 4. Effects of hydroxylactone **4** on the bacterial growth

entry	Microorganism	Effect
1	*Micrococcus flavus* C1	complete inhibition
2	*Bacillus cereus* C3	growth retardation (extension lag phase to 9 h), limiting growth, increase in OD = 0.87 (max. OD control = 1,037)
3	*Escherichia coli* C1	no influence on the growth
4	*Bacillus subtilis* B5	no influence on the growth
5	*Pseudomonas fluorescens* W1	inhibition of growth, OD = 0.27 (max. OD control = 0.75)

entry 3), delay of the growth of the microorganism was observed.

Table 5. Effects of hydroxylactone **4** on the yeat growth

entry	Microorganism	Effect
1	*Debaryomyces hansenii* K12a	delayed growth (around 20 h longer lag-phase), similar to the OD control
2	*Saccharomyces cerevisiae* SV30	growth inhibition, growth OD = 0.59 (max. OD control = 1,546)
3	*Yarrowia lipolytica* ATCC 20460	no influence on the growth
4	*Schizosaccharomyces pombe* C-1	no influence on the growth
5	*Rhodotorula rubra* C-9	no influence on the growth

Table 6. Effects of hydroxylactone **4** on the fungi growth

entry	Microorganism	Effect
1	*Aspergillus niger* XP	no influence on the growth
2	*Fusarium linii* 3A	no influence on the growth
3	*Penicillium sp.*	limits the growth, increase in OD = 1.264 (max. OD control = 1, 88)
4	*Alternaria sp.*	complete inhibition

Conclusions

All fungal strains used for the bioconversion of bromolactone **2** and iodolactone **3** transformed them into the same hydroxylactone **4** with a very high regioselectivity because the OH group was always introduced in the equatorial C-2 position. Halolactones were transformed by fungal strains with a good yield, mainly into the (–) isomer of hydroxylactone **4**. It should be noted that, in the case of bromolactone, two microorganisms (*F. semitectum* and *Acremonium sp*.) were able to complete the substrate dehalogenation. In addition, the performed biological tests proved that hydroxylactone **4** inhibited the growth of certain microorganism strains.

Supplementary Information

Supplementary Information (figures containing IR, ^{1}H, COSY, HMQC and ^{13}C NMR spectra, GC-MS chromatograms, figures illustrated the effect of hydroxylactone **4** on the growth of the microorganisms) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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