Biocatalysis and Biotransformation, September–December 2010; 28(5–6): 408–414

SHORT COMMUNICATION

Biotransformations of bicyclic trimethylcyclohexane chloro-, bromo- and iodolactones using fungal strains

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Abstract
Several fungal strains (*Fusarium*, *Botrytis*, *Beauveria*) were screened for their ability to transform three bicyclic halo-γ-lactones with a trimethylcyclohexane ring. Most of the micro-organisms carried out hydrolytic dehalogenation and transformed these lactones into two hydroxy-γ-lactones: cis (-)-2-hydroxy-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one and trans (+)-2-hydroxy-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one. The structures of all substrates and products were established on the basis of their spectral data and X-ray analysis. The method presented offers an alternative route to obtaining hydroxy-lactones with high enantiomeric excess.

Keywords: *Lactones, biotransformations, hydrolytic dehalogenation, fusarium species*

Introduction

Halogenated organic compounds are often met in nature. They are used as herbicides, insecticides, pesticides, fungicides, solvents and intermediates for chemical syntheses. These compounds can be toxic for the environment, so biodegradation is an important topic to study (Janssen et al. 1994; De Wit 2002; Erable et al. 2005; Segev et al. 2007; Kim et al 2009).

Natural terpenoids including a lactone ring and hydroxy group in their structure are often isolated from plants and animals. Natural hydroxylactones have many specific biological activities like anti-malarial (Ortet et al. 2008), anti-fungal (Vajs et al. 1999; Pujar et al. 2000), cytotoxic (Zhang & Feng 1997; El Hassany et al. 2004; Liu et al. 2008), antibacterial (El Hassany et al. 2004), anti-cancer (El-Gamal 2001) and phytotoxicity (Fukushima et al. 1998).

There are many chemical routes to introduce a hydroxy group into a lactone molecule, but these often involve multiple steps (Kiegiel et al. 2000; Popsavin et al. 2003; Rodriguez et al. 2006; Antoniotti & Duñach 2009). A better method is direct biohydroxylation of chemically produced lactones with different functional groups. In our laboratory we have applied micro-organisms (fungal strains) for hydroxylation of iodolactones (Grotowska & Wawrzeńczyk 2002), saturated (Gładkowski et al. 2004; 2006) and unsaturated lactones (Gładkowski et al. 2006; 2007).

Here we present further examples of biohydroxylation of chloro-, bromo- and iodolactones containing a trimethylcyclohexane ring. These compounds were chosen for two reasons. Some terpenoid halolactones exhibit anti-feeding activity against insect pests, so they could be useful for insect pest control (Paruch et al. 2000; 2001). Secondly, these lactones may be good model substrates for finding micro-organisms which are able to replace the halogen with a hydroxy group in good yield. This would demonstrate the potential for detoxification using model toxic compounds (halolactone) by conversion to the environmentally safer hydroxylactone.

Materials and methods

Analysis

The progress of chemical reactions and biotransformations as well as the purity of isolated products

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(Received 21 June 2010; accepted 5 November 2010)

ISSN 1024-2422 print/ISSN 1029-2446 online © 2010 Informa UK, Ltd.
DOI: 10.3109/10242422.2010.538688

were monitored by TLC on silica gel-coated aluminium plates (DC–Alufolien Kieselgel 60 F254, Merck, Germany) and by GC analysis which was carried out on a Varian CP-3380 instrument using an HP-5 column (cross-linked methyl silicone gum, 30 m × 0.32 mm × 0.25 μm). The temperatures during GC analysis were: injector 150°C, detector (FID) 300°C, column temperature: 100°C held for 1 min, 100–200°C at 10°C min^{-1}, 200–300 at 50°C min^{-1}, 300°C held for 1 min. The enantiomeric excesses were determined by GC analysis using the chiral column CP-cyclodextrin-B-325, 30 m × 0.25 mm × 0.25 μm under the following conditions: injector 200°C, detector (FID) 250°C, column temperature: 140°C held for 45 min, 140–200°C at 20°C min^{-1}, 200°C held for 1 min. The products were purified by preparative column chromatography on silica gel (Kieselgel 60, 230–400 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$ solution on a Bruker Avance DRX 300 spectrometer. IR spectra were determined using a FTIR Thermo-Mattson IR 300 Spectrometer. Optical rotations were measured on an Autopol IV automatic polarimeter (Rudolf Research Analytical, NY, USA). All the melting points were measured on a Boetius apparatus.

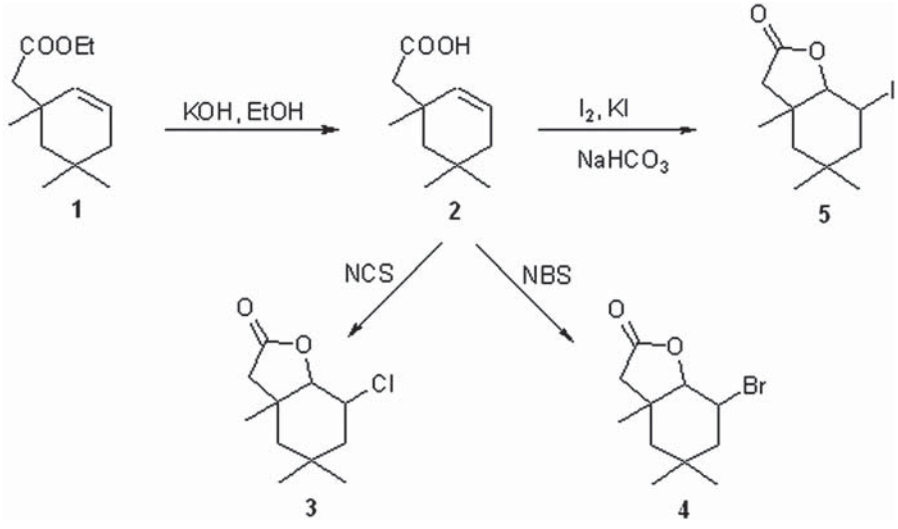
X-ray crystallographic data

X-ray data for 3 and 5 were collected on a Kuma KM4 CCD diffractometer (MoKα radiation; λ = 0.71073 Å). The data were collected at 100 K using an Oxford Cryosystem device. Data reduction and analysis were carried out with the CrysAlice 'RED' program (Oxford Diffraction 2001). The space groups were determined using the XPREP program. The structures were solved by direct methods using the XS program and refined using all F^2 data, as implemented by the XL program (Bruker AXS 1999). Non-hydrogen atoms were refined with anisotropic displacement parameters. All H atoms were placed at calculated positions. Before the last cycle of refinement all H atoms were fixed and were allowed to ride on their parent atoms. The Friedel pairs were merged before the final refinement.

Materials

The substrates for biotransformations, racemic halolactones 3–5 were synthesized from known γ,δ-unsaturated ester 1 (Grabarczyk et al. 2005) (Scheme 1). Lactone 3 was obtained from ester 1 in two steps: basic hydrolysis and iodolactonization according to a procedure described earlier (Grabarczyk et al. 2005). The two known lactones 4 and 5 (Pisarski & Wawrzeńczyk 2006) were also obtained in two steps: basic hydrolysis of ester 1 and bromo- or chlorolactonization. The spectral data given below are useful for studying biotransformation of the parent substrates.

2-Chloro-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one (3). A mixture of acid 2 (2.4 g, 0.013 mol) in 30 ml of THF and NCS (3.36 g, 0.025 mol) was stirred for 24 h at room temperature. Then water was added to the mixture and the product diluted with diethyl ether. The organic layer was washed with saturated $NaHCO_3$ solution, brine and dried with anhydrous magnesium sulphate. The crude product was chromatographed on silica gel (hexane:acetone, 3:1) and 1.98 g (69%) of chlorolactone 3 was obtained. The product is characterized by the data given: mp 97–98°C, ^{1}H NMR (300 MHz, $CDCl_3$): 1.05 and 1.09 (two s, 6H, $(CH_3)_2$ C-4), 1.23 (s, 3H, CH_3C-6), 1.40 (d, J = 15.0 Hz,



Scheme 1. Synthesis of halolactones 3-5.

1H, one of CH_2-5), 1.56–1.70 (m, 2H, one of CH_2-5 and H-3_{ax}), 1.92 (ddd, J = 13.7, 4.0 and 2.2 Hz, 1H, H-3_{eq}), 2.17 and 2.74 (two d, J = 17.5 Hz, 2H, CH_2-7), 3.98 (ddd, J = 12.5, 8.8 and 4.0 Hz, 1H, H-2), 4.11 (d, J = 8.7 Hz, 1H, H-1); IR (KBr, cm^{-1}): 1786 (s), 1455 (m), 1157 (s), 991 (m), 742 (m).

Crystal data for (3): $C_{11}H_{17}ClO_2$, Mr = 216.70, colourless plate, crystal dimensions 0.31 × 0.14 × 0.03 mm, orthorhombic, space group $P2_1/c$, a = 8.394(3), β = 7.700(2), c = 17.215(4) Å (anstrem), Å = 95.21(3) °, V = 1108.1(6) $Å^3$, Z = 4, D_c = 1.299 Mg m^{-3}, T = 100(2) K, R = 0.045, $wR2$ = 0.085 (2354 reflections with $I > 2\sigma(I)$) for 127 variables. CCDC No: 771511.

2-Bromo-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one (4). Acid 2 (2.1 g, 0.011 mol) was dissolved in 30 ml of THF and NBS (3.1 g, 0.092 mol) was added. The mixture was stirred for 24 h at room temperature and then water was added. The product was extracted with diethyl ether. The ethereal fractions were combined, washed with saturated $NaHCO_3$ solution, brine and dried with anhydrous magnesium sulphate. The crude product was chromatographed on silica gel (hexane:acetone, 3:1) giving 2.43 g (81%) of bromolactone 4 with the following physical and spectral data: mp 110–113°C; ^{1}H NMR (300 MHz, $CDCl_3$): 1.03 and 1.09 (two s, 2H, $(CH_3)_2$C-4), 1.22 (s, 3H, CH_3C-6), 1.38 (d, J = 15.0 Hz, 1H, one of CH_2-5), 1.70 (dd, J = 15.0 and 2.3 Hz, 1H, one of CH_2-5), 1.72 (d, J = 13.4 Hz, 1H, H-3_{ax}), 2.04 (ddd, J = 13.4, 4.1 and 2.3 Hz, H-3_{eq}), 2.15 and 2.77 (two d, J = 17.5 Hz, 2H, CH_2-7), 4.05 (ddd, J = 13.2, 9.2 and 4.1 Hz, 1H, H-2), 4.22 (d, J = 9.2 Hz, 1H, H-1); IR (KBr, cm^{-1}): 1784 (s), 1454 (m), 1143 (s), 1024 (s), 989 (s), 696 (m).

2-Iodo-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one (5). Melting point 99–100°C ; 1H NMR (300 MHz, $CDCl_3$), δ: 0.98 and 1.06 (two s, 6H, $(CH_3)_2$C-4), 1.17 (s, 3H, CH_3C-6), 1.37 (d, J = 15.0 Hz, 1H, one of CH_2-5), 1.73 (dd, J = 15.0, 2.4 Hz, 1H, one of CH_2-5), 1.95 (dd, J = 13.7, 13.5 Hz, 1H, H-3_{axial}), 2.09 and 2.85 (two d, J = 17.6 Hz, 2H, CH_2-7), 2.13 (ddd, J = 13.7, 4.0, 2.4 Hz, H-$3_{equatorial}$), 4.10 (ddd, J = 13.5, 9.9, 4.0 Hz, 1H, H-2), 4.32 (d, J = 9.9 Hz, 1H, H-1), IR (film, cm^{-1}): 2976 (s), 1788 (s), 1468 (m), 1160 (s), 1028 (s).

Micro-organisms

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

The fungal strains used came from the collection at the Institute of Biology and Botany, Medical University, Wrocław (*Fusarium culmorum* (AM 10), *Fusarium avenaceum* (AM 11), *Fusarium oxysporum* (AM 145), *Fusarium tricinctum* (AM 395), *Fusarium semitectum* (AM 20), *Fusarium solani* (AM 203), *Botrytis cinerea* (AM 235), *Beauveria bassiana* (AM 278)). 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.

Biotransformations

Screening procedure

The strains were cultivated at 25°C in Erlenmeyer flasks which contained 100 ml of medium consisting of 3 g glucose and 1 g peptobac in water. After 4 days, 10 mg of biotransformation substrate in 1 ml of acetone was added to the cultures. Incubation of the shaken cultures with substrate was continued for 14 days. After 5, 9 and 14 days of incubation the products of biotransformation were extracted with dichloromethane and analyzed by TLC (silica gel, hexane:acetone 3:1) and GC (HP-5 column). The results of GC analyses for lactone 3–5 are presented in Table I. During the screening procedure the priority was to identify those organisms which replaced the halogen with a hydroxy group with the highest yield. At this stage we were interested only in the degree of transformation of substrates into products.

Preparative biotransformation

Halolactones 3–5 (100 mg dissolved in 10 ml acetone) were added to 4-day old cultures prepared as described in the screening procedure. The cultures were shaken in 10 flasks with 100 ml of medium in each flask. After 14 days of shaking the products were extracted with dichloromethane. The organic solutions were dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The products were separated by column chromatography (silica gel, hexane:acetone 3:1). The preparative biotransformations of lactones 3, 4 and 5 gave a mixture of hydroxylactone 6 (in one case hydroxylactone 7) and untransformed substrate (Scheme 2). Results of the preparative biotransformations are presented in Tables II–IV. The physical and spectral data of hydroxylactone 6 and hydroxylactone 7 (Wawrzeńczyk et al. 2003) are given below.

Cis (-)-2-hydroxy-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one (6). mp 109–110°C, ^{1}H NMR (300 MHz, $CDCl_3$): 0.96 and 1.02 (two ss, 2H, $(CH_3)_2$C-4), 1.20 (d, J = 14.5 Hz, 1H, one of CH_2-5), 1.29 (s, 3H,

Table I. Composition (in percentage according to GC) of the product mixtures of screening biotransformations of lactones 3, 4, 5.

Entry	Strain	Time of incubation (days)	Products of transformations (%) 3		4		5	
			3	6	4	6	5	6
1	*F. culmorum*	5	94	6	84	16	86	14
		9	81	19	53	47	77	23
		14	63	37	35	65	37	63
2	*F. avenaceum*	5	85	15	57	43	28	72
		9	49	51	28	72	7	93
		14	43	57	25	75	3	97
3	*F. oxysporum*	5	95	5.0	62	38	60	40
		9	36	64	33	67	32	68
		14	35	65	24	76	31	69
4	*F. tricinctum*	5	78	22	59	41	36	64
		9	52	48	32	68	27	73
		14	46	54	27	73	13	87
5	*F. semitectum*	5	86	14	45	55	26	74
		9	85	15	32	68	17	83
		14	78	22	28	72	12	88
6	*F. solani*	5	63	37	50	50	80	20
		9	47	53	27	73	28	72
		14	40	60	24	76	25	75
7	*B. cinerea*	5	97	3	94	6	100	0
		9	35	65	70	30	82	18
		14	30	70	62	28	82	18
8	*B. bassiana*	5	98	2	99	1	96	4
		9	90	10	94	6	91	9
		14	84	16	88	12	88	12

CH_3C-6), 1.32 (dd, J = 14.5, 1.8 Hz, 1H, one of CH_2-5), 1.43 (dd, J = 12.7, 12.4 Hz, 1H, one of CH_2-3), 1.61 (ddd, J = 12.7, 4.5 and 1.8 Hz, one of CH_2-3), 2.07 (s, 1H, OH), 2.37 (s, 2H, CH_2-7), 3.99 (ddd, J = 12.4, 4.5 and 3.0 Hz, 1H, H-2), 4.31 (d, J = 3.0 Hz, 1H, H-1), ^{13}C NMR ($CDCl_3$): 23.84 (C-11), 26.53 (C-10), 31.99, 40.24 (C-4 and C-6), 33.81 (C-9), 40.31 (C-3), 45.04 (C-5), 48.02 (C-7), 65.72 (C-2), 85.96 (C-1), 175.64 (C-8), IR (film, cm^{-1}): 3480 (s), 2966 (s), 1773 (s), 1177 (s), 1058 (s).

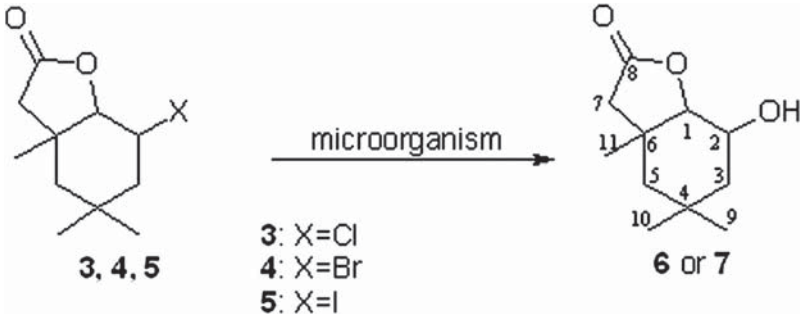
Crystal data for (6): $C_{11}H_{18}O_3$, Mr = 198.25, colourless cut-needle, crystal dimensions 0.23 × 0.18 × 0.14 mm, orthorhombic, space group $P2_12_12_1$, a = 8.657(2), b = 10.632(2), c = 22.952(5) Å, V = 2112.5(8) Å^3, Z = 8, D_c = 1.247 Mg m^{-3}, T = 100(2) K, R = 0.087, $wR2$ = 0.124 (2839 reflections with $I > 2\sigma(I)$) for 253 variables. CCDC No: 771512.

Trans (+)-2-hydroxy-4,4,6-trimethyl-9-oxabicyclo[4.3.0] nonan-8-one (7). mp 109–110°C, ^{1}H NMR (300 MHz, $CDCl_3$): 1.05 and 1.09 (two s, 2H, $(CH_3)_2$C-4), 1.23 (s, 3H, CH_3C-6), 1.37 (d, J = 15.0 Hz, 1H, one of CH_2-5), 1.61–1.70 (m 2H, one of CH_2-3 and one of CH_2-5), 1.92 (ddd, J = 13.7, 4.1, 2.2 Hz, 1H, one of CH_2-3), 2.18 and 2.74 (two d, J = 17.5 Hz, 2H, CH_2-7), 4.00 (ddd, J = 12.6, 8.7, 4.1 Hz, 1H, H-2), 4.11 (d, J = 8.7 Hz, 1H, H-1), IR (film, cm^{-1}): 3460 (s), 2968 (s), 1784 (s), 1164 (s), 1056 (s).

Results and discussion

The substrates for transformation were three racemic halo-γ-lactones (3, 4, 5) containing a trimethylcyclohexane ring. Iodo-γ-lactone 5 was obtained from known γ,δ-unsaturated ester 1 as described earlier (Grabarczyk et al. 2005). Two known halolactones (Pisarski & Wawrzeńczyk 2006), chloro-γ-lactone 3 and bromo-γ-lactone 4, were also obtained from ester 1, which was hydrolyzed in a 2.5% ethanolic solution of KOH to give acid 2. γ,δ-Unsaturated acid 2 was a substrate for chlorolactonization and bromolactonization using N-chlorosuccinimide or N-bromosuccinimide in THF (Scheme 1).

The structures of chloro-γ-lactone 3 and bromo-γ-lactone 4 were determined on the basis of their spectral (^{1}H NMR and IR) data and X-ray analysis (for 3). The absorption bands at 1786 cm^{-1} for 3 and 1784 cm^{-1} for 4 in their IR spectra confirmed the presence of the γ-lactone ring in the molecule of these compounds. X-ray analysis of 3 indicated that the cyclohexane ring exists in a slightly deformed chair conformation and the C-O bond of γ-lactone ring and C-Cl bond are situated in *trans* diequatorial positions. One of the methyl groups at C-4 is *cis* oriented in relation to the C-O bond (Structure 1). The



Scheme 2. Biotransformations of halolactones 3-5.

Table II. Composition (in percentage according to GC) of the product mixtures of preparative biotransformations of lactone 3.

Entry	Strain	Time of incubation (days)	3 (%)	6 (%)	7 (%)	Isolated yield (%)	ee (%)	a_D^{20}
1	*F. avenaceum*	14	51	—	49	44.2	92	+16.6° (c = 1.10%, $CHCl_3$)
2	*F. oxysporum*	10	22	78	—	40.4	24	−11.4° (c = 1.11%, $CHCl_3$)
3	*F. tricinctum*	14	40	60	—	48.0	51.7	−31.6° (c = 1.02%, $CHCl_3$)
4	*F. solani*	14	48	52	—	39.0	55.9	−38.2° (c = 1.20%, $CHCl_3$)
5	*B. cinerea*	14	38	62	—	47.0	27.5	−19.9° (c = 1.15%, $CHCl_3$)

similarity between spectral data (^{1}H NMR) of chlorolactone 3 and bromolactone 4 indicates that their structures are identical. The same results were obtained earlier for iodo-γ-lactone 5 (Grabarczyk et al. 2005).

The first step of the biotransformation procedure was screening using eight fungal strains of local origin: *F. culmorum*, *F. avenaceum*, *F. oxysporum*, *F. tricinctum*, *F. semitectum*, *F. solani*, *B. cinerea* and *B. bassiana*. The progress of the biotransformation was monitored by TLC and GC (Table I). These results indicated that almost all strains transformed the three substrates with a high or satisfactory conversion. Only *B. bassiana* gave a low conversion (12–16%) after 14 days. Chlorolactone 3 and bromolactone 4 were transformed by five and six micro-organisms, respectively, with good yields (49–78% and 57–75%). The best results were obtained with *F. oxysporum* for 3 and *F. culmorum*, *F. avenaceum* and *F. tricinctum* for 4. Iodolactone 5 was transformed by six strains, giving the highest yields (63–97%) of product after 14 days. The best results were observed when *F. avenaceum* and *F. semitectum* were used as biocatalysts. For all transformations only one product was observed.

Preparative scale transformations of 3, 4, 5 were performed using different *Fusarium* species, while *B. cinerea* was used for 3 only (Tables II–IV). The composition of product mixtures showed that, in almost all cases, hydroxylactone 6 was obtained as the sole product (Scheme 2). In a single case, when lactone 3 was transformed with *F. avenaceum* the alternative hydroxylactone 7 was observed (Scheme 2).

The structures of products 6 and 7 were established on the basis of their spectral and X-ray analysis data. The IR spectra showed that the γ-lactone ring had been retained during the biotransformation (absorption bands at 1773 cm^{-1} and 1784 cm^{-1}, respectively). Strong and broad bands found at 3480 cm^{-1} for 6 and 3460 cm^{-1} for 7 suggested the presence of a hydroxyl group in both molecules.

^{1}H NMR spectra of hydroxy-γ-lactone 6 gave very interesting results. A small coupling constant (J = 3.0 Hz) of H-1 indicated that this proton was located in an equatorial position. The signal from the H-2 proton at 3.99 ppm as a doublet of doublets (J = 12.2, 4.5 and 3.0 Hz) suggested that this proton was in an axial position. The location of signals of the H-1 proton and CH_3 C-6 group were shifted towards higher fields, however signals of the CH_2-3 and CH_2-5 groups moved in the opposite direction. The shape of the signal from the CH_2-7 group changed into singlet. All of this suggested a change of conformation of the molecule during the biotransformation.

X-ray analysis of hydroxy-γ-lactone 6 shows that the cyclohexane ring adopted a chair conformation. In this case, the OH group was in an equatorial position and *cis* oriented in relation to the C-O bond of the γ-lactone ring, which was in an axial position. One of the methyl group at C-4 was *trans* oriented in relation to the C-O bond and *cis* oriented to the methyl group at C-6 (Structure 2).

^{1}H NMR spectra of hydroxy-γ-lactone 7 and chlorolactone 3 indicated a similarity between them. In the case of lactone 7 a coupling constant (J = 8.7 Hz) of H-1 indicated that this proton was located in an axial position. The signal at 4.00 ppm as a doublet of doublets (J = 12.6, 8.7 and 4.1 Hz) from the H-2 proton suggested that this proton was in an axial position. The signal from the CH_2-7 group was present as two doublets with coupling constant J = 17.5 Hz. In the case of lactone 3 these signals and their multiplicity

Table III. Composition (in percentage according to GC) of the product mixtures of preparative biotransformations of lactone 4.

Entry	Strain	Time of incubation (days)	4 (%)	6 (%)	Isolated yield (%)	ee (%)	a_D^{20}
1	*F. culmorum*	14	19	75	25.1	0.4	−0.6° (c = 0.94%, $CHCl_3$)
2	*F. avenaceum*	10	25	75	59.5	81.0	−48.9° (c = 0.85%, $CHCl_3$)
3	*F. oxysporum*	14	33	67	52.7	74.4	−45.3° (c = 0.85%, $CHCl_3$)
4	*F. tricinctum*	10	26	74	69.0	70.0	−42.3° (c = 1.22%, $CHCl_3$)
5	*F. semitectum*	10	37	63	29.0	0	0°
6	*F. solani*	10	20	57	41.4	45.4	−29.4° (c = 1.18%, $CHCl_3$)

Table IV. Composition (in percentage according to GC) of the product mixtures of preparative biotransformations of lactone 5.

Entry	Strain	Time of incubation (days)	5 (%)	6 (%)	Isolated yield (%)	ee (%)	a_D^{20}
1	*F. avenaceum*	10	10	90	85.6	4.0	+3.6° (c = 0.96%, $CHCl_3$)
2	*F. oxysporum*	10	26	74	47.1	26.6	−25.7° (c = 0.85%, $CHCl_3$)
3	*F. tricinctum*	14	25	75	60.7	22.2	−21.6° (c = 1.09%, $CHCl_3$)
4	*F. semitectum*	14	11	89	79.0	14.7	−11.0° (c = 1.03%, $CHCl_3$)
5	*F. solani*	14	15	85	52.9	13.4	−10.5° (c = 1.07%, $CHCl_3$)

were identical. The signals from the other groups in both lactones were also identical. This indicates that the C-O bond of the γ-lactone ring is *cis* oriented in relation to axial methyl group at C-6. The OH group, however, is in an equatorial position and *trans* oriented in relation to both the axial methyl group at C-6 and C-O bond of the γ-lactone ring.

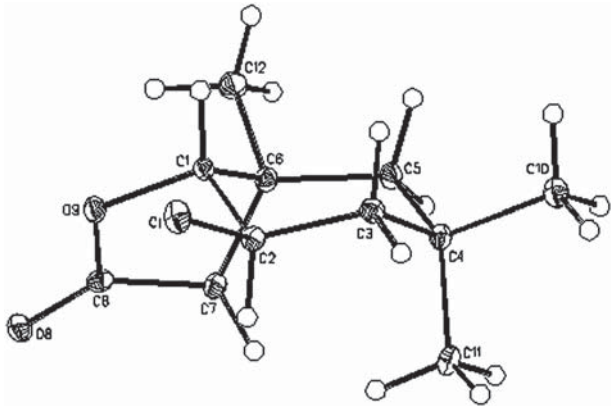
Products of biotransformation (6 and 7) were optically active with the exception of lactone 6 derived from bromolactone 5 using *F. semitectum*. Enantiomeric excess was determined by chiral GC (Beta Dex, 30 m × 0.25 mm × 0.25 μm). In almost every case formation of the (-) isomer of lactone 6 was preferred (Tables II–IV). Only iodolactone 5 transformed by *F. avenaceum* gave the (+) isomer of 6, but with a very small enantiomeric excess (4%). Lactone 7 obtained from chlorolactone 3 by *F. avenaceum* was the (+) isomer with a good enantiomeric excess (92%).

Only one micro-organism, *F. avenaceum*, gave two different products during transformations of chlorolactone 3 and bromolactone 4, namely hydroxylactone 6 and 7, respectively. The lactone 6 had an equatorial OH group *cis* oriented in relation to the axial C-O bond of the γ-lactone ring and *trans* oriented in relation to the axial methyl group at C-4 and the methyl group at C-6. In the lactone 7 the OH group was also in an equatorial position, but *trans* oriented in relation to both the axial methyl group at C-6 and C-O bond of the γ-lactone ring. For that reason both products (6, 7) show different stereo-selectivity for substrates 3 and 4 (Table II and III).

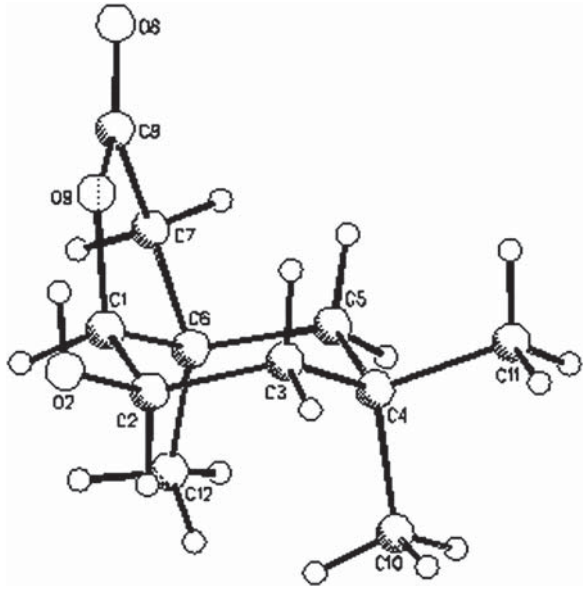
Conclusions

Most of the micro-organisms used for biotransformation transformed all three substrates into products with high yield. These micro-organisms showed a very high region-selectivity, the OH group was always introduced in an equatorial position at C-2. In almost every case (for hydoxylactone 6) a change in conformation of the cyclohexane ring was observed. Only hydroxylactone 7, produced by transformation of chlorolactone 3, retained its conformation. The best substrate, giving product with the highest yield and enantiomeric excess, was bromolactone 4. The best micro-organism for transformation of halolactones 3-5 was *F. avenaceum*.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.



Structure 1. 2-Chloro-4,4,6-trimethyl-9-oxabicyclo[4.3.0]nonan-8-one (3).



Structure 2. Cis (-)-2-hydroxy-4,4,6-trimethy-9-oxabicyclo[4.3.0]nonan-8-one (6).

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