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Fungistatic Activity of Bicyclo[4.3.0]-γ-lactones

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ABSTRACT: Five optically active and sixteen racemic lactones (nine of them new) of bicyclo[4.3.0]nonane structure were synthesized. IC_{50} values for the following phytopathogens were determined: *Aspergillus ochraceus* AM 456, *Fusarium culmorum* AM 282, *Fusarium oxysporum* AM 13, *Fusarium tricinctum* AM 16. Effect of compound structures, especially stereogenic centers, on fungistatic activity has been discussed. The highest fungistatic activity was observed for *trans*-7,8-dibromo-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3c**), IC_{50} = 30.1 μg/mL (0.10 μM/mL), and *cis*-7,8-epoxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3b**), IC_{50} = 72.2 μg/mL (0.47 μM/mL), toward *F. oxysporum* AM 13.

KEYWORDS: lactones, IC_{50}, HLADH, *Fusarium oxysporum*, *Fusarium culmorum*

INTRODUCTION

Species of *Aspergillus*, *Fusarium,* and *Penicilium* are commonly found in soil and air. Not only are they saprophytes and plant pathogens but they also may attack people with low immunoresistance.[1−4] Under unfavorable conditions numerous strains may produce mycotoxins.[5,6] Especially troublesome and difficult to combat are phytopathogens of the genus *Fusarium*, for example *F. culmorum* and *F. tricinctum* species are responsible for head blight infection,[7−10] and *F. oxysporum* is the main cause of wilt.[11,12] The microorganisms of the genus *Fusarium* cause huge loss in agriculture and product storage, and also they produce a wide range of toxic compounds, which are dangerous for humans, like trichocetin toxins (e.g., T-2 toxin, deoxynivalenone), zearalenone and fumonisins.[8,10,13,14] These compounds may attack liver, kidney, circulatory, endocrine or digestive systems, skin and blood.[8]

Lactones are a very interesting group of compounds, because of different properties and biological activities. They have been identified as natural ingredients of plant oils, vegetables, fruits and food products.[15−19] Terpenoid lactones are characterized with attractive, mainly fruity fragrances and usually demonstrate sweet, coconut or herbal taste.[20,21]

Compounds with lactone ring show a wide range of biological activities, among which the most widely known are fungistatic,[22−24] bacteriostatic[25,26] and cytostatic[27−30] ones. Moreover numerous insect pheromones consist of a lactone ring in their structure.[31] The lactones synthesized by our group proved also to have deterrent activity toward insects.[32−34] Therefore, it was purposeful to check whether they also indicate fungistatic activity toward fungi strains present in soil or in stored grain, vegetables and fruits.

We have synthesized 9-carbon lactones with structures similar to natural phthalides,[35−39] and their structural analogues. Fungistatic activity of these compounds was determined for the following strains: *Aspergillus ochraceus* AM 456, *Fusarium culmorum* AM 282, *F. oxysporum* AM 13, *F. tricinctum* AM 16 and *Penicillium citrinum* AM 354. Our study concerned structural aspects of fungistatic activity, so that we synthesized both racemic and optically active bicyclic lactones and we were searching for functional groups or other parts of molecules that are important with respect to their biological activity.

MATERIALS AND METHODS

Analytical Methods. Composition and purity of products were established by thin layer chromatography (TLC) and gas chromatography (GC). TLC was carried out on silica gel Kieselgel 60 F_{254} (Merck) with hexane:acetone:isopropanol:ethyl acetate (60:1:3:1) as a developing system. Compounds were visualized by spraying the plates with 1% $Ce(SO_4)_2$, 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4, followed by heating. Gas chromatography analyses were performed on an Agilent instrument, fitted with a flame ionization detector (FID), using a Carbowax column (30 m, 0.53 mm, 0.88 μm film thickness) or a chiral column: CP-cyclodextrin (25 m × 0.25 mm, 0.25 μm film thickness); H_2 at a flow rate of 2 mL/min was used as a carrier gas.

Molecular mass was confirmed on a Varian Chrompack GC MS CP-3800 Saturn 2000 GC/MS/MS with ionization energy of 70 eV, using HP-1 column (cross-linked methyl silicone gum, 25 m × 0.32 mm ×0.25 μm film thickness), and HRMS analysis was conducted on a micrOTOF-Q Bruker.

Optical rotations were measured on an Autopol IV automatic polarimeter (Rudolph) with thermostatic system, in chloroform solutions, at concentrations given in g/100 mL.

X-ray data for compound **3c** were collected on a Kuma KM4CCD diffractometer (Mo Kα radiation; λ = 0.71073 Å) at 100 K using an Oxford Cryosystem device. Data reduction and analysis were carried out with the CrysAlice "RED" program. The analytical correction for absorption was applied. The space groups were determined using the XPREP program. The structure was solved by direct methods using the XS program and refined using all F^2 data, as implemented by the XL program.[40] 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.

Structures of the substrates and the products were determined based on ^{1}H NMR, ^{13}C NMR, COSY, HMQC and IR spectra. NMR spectra were recorded at 500 MHz using $CDCl_3$ solution, on a Bruker Avance

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DRX-500 spectrometer. IR spectra were measured on a Mattson FTIR-300 Thermo Nicolet spectrometer.

Chemicals. The chemicals used for the synthesis were purchased from Aldrich, Sigma, and Fluka. PDA for fungistatic tests was purchased from BTL in Poland. DDAC, didecyldimethylammonium chloride (50% solution in propan-2-ol/water 2:3), was purchased from Merck.

Enzymes and Coenzymes. The following alcohol dehydrogenases were used: HLADH recombinant from *Escherichia coli* (EVO), PADH 3000, screening kit (Codexis), LKADH from *Lactobacillus kefir* (Fluka), YADH from Baker yeast (Sigma-Aldrich).

Coenzymes. Nicotinamide adenine dinucleotide (NAD^+) and flavin mononucleotide (FMN) were purchased from Sigma Chemical Co.

Microorganisms. The phytopathogens *Aspergillus ochraceus* AM 456, *Fusarium culmorum* AM 282, *F. oxysporum* AM 13, *F. tricinctum* AM 16, and *Penicillium citrinum* AM 354 were obtained from the collection of Institute of Botany of Medical University of Wrocław.

Synthesis of Lactones. *(±)-cis-3-Oxabicyclo[4.3.0]non-7-en-2-one (**3a**).* $LiAlH_4$ (0.20 g, 5.3×10^{-3} mol) in anhydrous diethyl ether (15 mL) was placed in a 2-neck flask equipped with a dropping funnel, a condenser and a magnetic stirrer. To the stirred mixture *cis*-1,2,3,6-tetrahydrophthalic anhydride **1** (820 mg, 5.4×10^{-3} mol) in 15 mL of diethyl ether was added dropwise (the substrate was dissolved in diethyl ether with heat and then cooled). After 20 min the reaction mixture was poured into 25 g of ice mixed with 50 mL of 6% hydrochloric acid and extracted three times with 50 mL of diethyl ether. The combined ethereal extracts were washed with brine and dried over anhydrous $MgSO_4$. After evaporation of the solvent the pure product **3a** (0.5434 g) was isolated by column chromatography (eluent hexane: acetone 4:1 v/v) with yield 73%.

*(−)-(1S,5R)-cis-3-Oxabicyclo[4.3.0]non-7-en-2-one ((−)-**3a**).* *cis*-4,5-Bis(hydroxymethyl)cyclohexene **16** (0.223 g, 1.4 mmol), NAD^+ (73 mg, 0.11 mmol) and FMN (0.8 g, 2.03 mmol) were dissolved in 40 mL of 0.1 M glycine−NaOH buffer in an Erlenmeyer flask (100 mL). pH of the mixture was readjusted to 9 with 20% NaOH. Then the HLADH (8 mg) was added and the mixture was kept at room temperature with periodic adjustment of pH to 9. The progress of oxidation was monitored by GC. When the reaction was completed (7 days), the pH was raised to 12 and the mixture was three times extracted with $CHCl_3$ (50 mL) to remove the unreacted diol. The aqueous phase was acidified to pH 3, then extracted with $CHCl_3$ (5 × 50 mL), and the extract was dried ($MgSO_4$). After solvent evaporation, the crude product was purified by column chromatography (eluent hexane:acetone 3:1 v/v) to give 130 mg (yield 58%) of (−)-**3a**: ee = 90% (GC, chiral column); $[\alpha]_{589}^{20} = -58.3°$ (*c* = 2.0, $CHCl_3$), lit.[41] $[\alpha]_{589}^{20} = -67.1°$. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.77−2.05 (m, 1, one of CH_2-6), 1.90−2.90 (m, 3, one of CH_2-6, H-5, one of CH_2-9), 2.74−2.80 (m, 2, one of CH_2-9, H-1), 4.00 (dd, 1, *J* = 2.0, 8.8 Hz, one of CH_2-4), 4.30 (dd, 1, *J* = 5.1, 8.8 Hz, 1H, one of CH_2-4), 5.60−5.70 (m, 2, H-8, H-7); IR (film, cm^{-1}) 1771 (s), 1134 (m); GC−EIMS 139 [M + 1].

*(±)-cis-3-Oxabicyclo[4.3.0]nonan-2-one (**3e**).* Lactone **3e** was obtained (0.5313 g) with yield 69%, by the same procedure as lactone **3a**, using $LiAlH_4$ (0.23 g, 6.0×10^{-3} mol) and *cis*-1,2-cyclohexanedicarboxylic anhydride **2** (850 mg, 5.5×10^{-3} mol).

*(+)-(1S,5R)-cis-3-Oxabicyclo[4.3.0]nonan-2-one ((+)-**3e**).* **3e** was obtained by the same procedure as lactone **3a**, using *cis*-1,2-bis-(hydroxymethyl)cyclohexane **17** (0.335 g, 2.1 mmol), NAD^+ (110 mg, 0.165 mmol), FMN (1.2 g, 3.05 mmol) and 0.1 M glycine−NaOH buffer (60 mL, pH = 9) at room temperature. A seven-day reaction gave 212 mg of (+)-**3e** (yield 65%): ee = 91% (GC, chiral column;) $[\alpha]_{589}^{20} = +42.5°$ (*c* = 2.2, $CHCl_3$), lit.[41] $[\alpha]_{589}^{20} = +48.8°$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.80−0.98 (m, 1, one of CH_2-6), 1.05−1.30 (m, 5, CH_2-8, CH_2-7, one of CH_2-6), 1.34 (d, 1, *J* = 10.2 Hz, one of CH_2-9), 1.45−1.95 (m, 1, one of CH_2-9), 2.10 (dd, 1, *J* = 11.0, 23.3 Hz, H-1), 2.35−2.70 (m, 1, H-5), 3.92 (d, 1, *J* = 8.8 Hz, one of CH_2-4), 4.16 (dd, 1, *J* = 5.0, 8.8 Hz, one of CH_2-4); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 22.4 (CH_2-6), 22.8 (CH_2-8), 23.3 (CH_2-7), 27.1 (CH_2-9), 35.3 (CH-5), 39.4 (CH-1), 71.7 (CH_2-4), 178.6 (C-2); IR (film, cm^{-1}) 2942 (s), 2254 (s), 1766 (s), 1210 (m), 989 (m); GC−EIMS 141 [M + 1].

The *cis*-diols: 4,5-bis(hydroxymethyl)cyclohexene **16** and 1,2-bis-(hydroxymethyl)cyclohexane **17** were prepared in good yields by the literature[41] procedures.

*(±)-4,4-Dimethyl-cis-3-oxabicyclo[4.3.0]non-7-en-2-one (**4a**).* To magnesium (215 mg, 8.96×10^{-3} mol) activated with iodine was added anhydrous diethyl ether (10 mL), followed by slow addition of methyl iodide (1.272 g, 8.96×10^{-3} mol). The reaction was continued until all the magnesium had reacted, and then *cis*-1,2,3,6-tetrahydrophthalic anhydride **1** (0.6809 g, 4.48×10^{-3} mol) was added dropwise at room temperature. After 3 h the reaction mixture was poured into 6% HCl mixed with ice and extracted with diethyl ether (5 × 50 mL), and the ethereal phase was washed with 6% HCl (2 × 30 mL) and brine until neutral. The extract was dried over $MgSO_4$, the solvent was evaporated off and the reaction mixture was purified by column chromatography using hexane:acetone 4:1 as eluent to give 0.5212 g of lactone **4a** (yield 70%): ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.38 (s, 6, C-4(CH_3)$_2$), 1.75−1.95 (m, 1, one of CH_2-6), 2.00−2.35 (m, 3, one of CH_2-9, one of CH_2-6, H-5), 2.36−2.56 (m, 1, H-1), 2.95−3.18 (m, 1, one of CH_2-9), 5.63−5.89 (m, 2, H-7, H-8); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 21.9 (CH_2-6), 22.3 (CH_2-9), 23.1 (CH_3−C-4), 27.1 (CH_3−C-4), 37.7 (CH-1), 40.8, (CH-5), 85.0 (C-4), 125.0 (CH-8), 125.3 (CH-7), 178.5 (C-2); IR (film, cm^{-1}) 3019 (s), 1782 (m), 1736 (w), 1216 (s); GC−EIMS 167 [M + 1].

*(±)-4,4-Dimethyl-cis-3-oxabicyclo[4.3.0]nonan-2-one (**4e**).* Lactone **4e** was obtained (0.4990 g) with yield 73%, by the same procedure as lactone **4a**, using magnesium (195 mg, 8.12×10^{-3} mol), methyl iodide (1.1540 g, 8.1×10^{-3} mol) and *cis*-1,2-cyclohexanedicarboxylic anhydride **2** (0.6257 g, 4.06×10^{-3} mol).

*(−)-(1R,5S)-4,4-Dimethyl-3-cis-oxabicyclo[4.3.0]nonan-2-one ((−)-**4e**).* To 30 mg (13.58×10^{-4} mol) of magnesium activated with iodine was added anhydrous diethyl ether (10 mL), followed by slow addition of methyl iodide (0.1725 g, 13.58×10^{-4} mol). The reaction was continued until all the magnesium had reacted, and then (+)-(1*S*,5*R*)-*cis*-3-oxabicyclo[4.3.0]nonan-2-one ((+)-**3e**) (80 mg, 5.70×10^{-4} mol) was added dropwise at room temperature. After 3 h the reaction mixture was poured into 2% HCl mixed with ice and extracted with diethyl ether (5 × 30 mL), and the ethereal phase was washed with 6% HCl (2 × 30 mL) and brine until neutral. The extract was dried over $MgSO_4$, the solvent was evaporated off and the crude product without purification was oxidized with dichromate pyridinium (0.215 g, 0.57 mmol) in CH_2Cl_2 (15 mL). When the substrate was consumed (TLC, GC, 36 h), the reaction mixture was filtered through Florisil, the solvent was evaporated off and the crude product was purified by column chromatography using hexane:acetone 4:1 as eluent to give 48 mg of lactone (−)-**4e** (yield 50%): ee = 91% (GC, chiral column); $[\alpha]_{589}^{20} = -35.2°$ (*c* = 1.2, $CHCl_3$), lit.[41] $[\alpha]_{589}^{20} = -40.1°$; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.00−1.17 (m, 1, one of CH_2-6), 1.33−1.35 (two s, 6, C-4-(CH_3)$_2$), 1.45−1.65 (m, 5, one of CH_2-6, CH_2-7, CH_2-8), 1.65−1.79 (m, 2, CH_2-9), 1.98−2.24 (m, 1, H-1), 2.96 (t, 1, *J* = 6.3 Hz, H-5); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 22.5 (CH_2-8), 22.8 (CH_2-6), 22.9 (CH_2-7), 23.6 (CH_3−C-4), 25.1 (CH_3−C-4), 26.1 (CH_2-9), 39.9 (CH-1), 43.5 (CH-5), 84.0 (C-4), 177.6 (C-2); IR (film, cm^{-1}) 2937 (m), 2254 (m), 1758 (s), 911 (s), 731 (s); GC−EIMS 169 [M + 1].

*(±)-cis-7,8-Epoxy-cis-3-oxabicyclo[4.3.0]nonan-2-one (**3b**).* Lactone **3a** (0.2503 g, 1.8×10^{-3} mol) dissolved in dichloromethane (15 mL) was placed in a round-bottom flask fitted with a magnetic stirrer, and 70% *m*-chloroperbenzoic acid (MCPBA) (0.70 g, 2.8×10^{-3} mol) was added to it portionwise. After 8 h of the reaction 20% Na_2SO_3 solution (15 mL) was added and the mixture was extracted with diethyl ether (3 × 50 mL). The organic layer was washed with 10% $NaHCO_3$ solution (5 mL) and brine

until neutral and dried over $MgSO_4$. After evaporation of solvent the product mixture was purified by column chromatography using hexane:aceton 2:1 as eluent to give 0.2482 g of **3b** (yield 89%).

*(−)-(1S,5R,7S,8R)-cis-7,8-Epoxy-cis-3-oxabicyclo[4.3.0]nonan-2-one ((−)-**3b**).* Epoxide (−)-**3b** was obtained (48.3 mg) with yield 88%, by the same procedure as lactone **3b**, using lactone (−)-**3a** (52 mg, 3.77×10^{-4} mol) and 70% MCPBA (0.14 g, 0.56×10^{-3} mol): ee = 91% (GC, chiral column); $[\alpha]_{589}^{20} = -11.1°$ (*c* = 2.0, $CHCl_3$) lit.[42] $[\alpha]_{589}^{20} = -12.9°$; ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.66 (dd, 1, *J* = 11.4, 15.0 Hz, one of CH_2-6), 2.17−2.26 (m, 1, one of CH_2-6), 2.31−2.37 (m, 1, one of CH_2-9), 2.51 (dd, 1, *J* = 7.2, 13.9 Hz, one of CH_2-9), 2.62−2.70 (m, 1, H-1), 3.08−3.11 (m, 1, H-5), 3.18 (t, 2, *J* = 7.0 Hz, H-7, H-8), 3.94 (dd, 1, *J* = 1.1, 9.0 Hz, one of CH_2-4), 4.17 (dd, 1, *J* = 5.1, 9.0 Hz, one of CH_2-4); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 20.5 (CH_2-6), 24.6 (CH_2-9), 30.5 (CH-1), 34.7 (CH-5), 49.6 (CH-7), 51.6 (CH-8), 71.2 (CH_2-4), 178.5 (C-2); IR (film, cm^{-1}) 1771 (s), 1158 (m); GC−EIMS 155 $[M + 1]$.

*(±)-4,4-Dimethyl-cis-7,8-epoxy-cis-3-oxabicyclo[4.3.0]nonan-2-one (**4b**).* Lactone **4b** was obtained (0.6024 g) with yield 76%, by the same procedure as lactone **3b**, using lactone **4a** (0.7234 g, 4.35×10^{-3} mol) and 70% MCPBA (1.18 g, 4.8×10^{-3} mol): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.22 (s, 1, one of CH_2-6), 1.31 and 1.40 (two s, 6, C-4(CH_3)$_2$), 1.56−1.74 (m, 1, one of CH_2-6), 2.10−2.25 (m, 1, one of CH_2-9), 2.31−2.47 (m, 1, one of CH_2-9), 2.75−2.97 (m, 1, H-1), 3.00−3.21 (m, 1, H-5), 3.25 (t, 2, *J* = 4.0 Hz, H-7, H-8); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 20.9 (CH_2-6), 22.5 (CH_3−C-4), 23.5 (CH_3−C-4), 26.7 (CH_2-9), 35.4 (CH-1), 38.8 (CH-5), 49.7 (CH-7), 51.6 (CH-8), 84.0 (C-4), 178.1 (C-2); IR (film, cm^{-1}) 3019 (w), 2399 (w), 1773 (s), 1215 (m), 756 (s); GC−EIMS 183 $[M + 1]^+$; HRMS 183.1 $[M + 1]^+$.

*(±)-trans-7,8-Dibromo-cis-3-oxabicyclo[4.3.0]nonan-2-one (**3c**).* The lactone **3a** (0.3020 g, 2.12×10^{-3} mol) was dissolved in tetrachloromethane (15 mL) and was placed in a sealed round-bottom flask fitted with septum and a magnetic stirrer. Then 0.7 mL (13.6×10^{-3} mol) of bromine was added dropwise using a syringe. The reaction mixture was stirred at room temperature for 2 h, and then 20 mL of 10% sodium thiosulfate solution was added. The product was extracted with diethyl ether (3 × 50 mL) and washed with brine and water until neutral. The organic layer was dried over $MgSO_4$, the solvent was evaporated off and the crude product was purified by column chromatography using dichloromethane:methanol (399:1) as eluent. 0.5079 g of lactone **3c** was obtained (yield 78%).

*(+)-(1S,5R,7R,8R)-trans-7,8-Dibromo-cis-3-oxabicyclo[4.3.0]nonan-2-one ((+)-**3c**).* Product (+)-**3c** was obtained (121 mg) with yield 70%, by the same procedure as lactone **3c**, using lactone (−)-**3a** (80 mg, 5.80×10^{-4} mol) and bromine (0.1 mL, 19.5×10^{-4} mol): $[\alpha]_{589}^{20} = +27.8°$ (*c* = 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.23 (s, 1, one of CH_2-6), 1.54 (s, 1, one of CH_2-6), 1.73−2.36 (m, 2, one of CH_2-9, H-1), 3.95−4.16 (m, 1, H-5), 4.17−4.40 (m, 2, H-7, H-8), 4.53 (d, 1, *J* = 1.6 Hz, one of CH_2-4), 4.62 (d, 1, *J* = 3.0 Hz, one of CH_2-4); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 25.4 (CH_2-6), 29.6 (CH_2-9), 32.8 (CH-5), 36.9 (CH-1), 47.1 (CH-7), 52.2 (CH-8), 70.5 (CH_2-4), 176.9 (C-2); IR (film, cm^{-1}) 2360 (w), 1767 (m), 1167 (w); GC−EIMS 299, 300 $[M + 1]^+$; HRMS 298.91, 300.90 $[M + 1]^+$.

*Crystal data for **3c**:* $C_8H_{10}Br_2O_2$, *M* = 297.98, colorless block, crystal dimensions 0.32 × 0.21 × 0.13 mm, monoclinic, space group $P2_1/c$, *a* = 6.646(2), *b* = 13.691(4), *c* = 10.087(3) Å, β = 99.12(3)°, *V* = 906.2(5) Å^3, *Z* = 4, D_c = 2.184 Mg m^{-3}, μ(Mo Kα) = 8.90 mm^{-1}, *T* = 100(2) K, *R* = 0.025, *wR* = 0.062 (1699 reflections with $I > 2\sigma(I)$) for 109 variables. CCDC reference number: 794604.

*(±)-trans-7,8-Dibromo-cis-4,4-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one (**4c**).* Lactone **4c** was obtained (1.1320 g) with yield 80%, by the same procedure as lactone **3c**, using 0.7216 g (4.30×10^{-3} mol) of **4a** and 1 mL (19.5×10^{-3} mol) of bromine: ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.32−1.50 (two s, 6, C-4(CH_3)$_2$), 1.72−1.90 (m, 1, one of CH_2-6), 2.10−2.41 (m, 2, one of CH_2-6, one of CH_2-9), 2.44−2.60 (m, 1, H-1), 2.89−3.04 (m, 1, one of CH_2-9), 3.05−3.25 (m, 1, H-5), 3.85−4.06 (m, 2, H-7, H-8); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 22.9 (CH_3−C-4), 25.5 (CH_3−C-4), 25.9 (CH_2-6), 26.7 (CH_2-9), 36.4 (CH-1), 38.8 (CH-5), 47.1 (CH-7), 50.8 (CH-8), 83.6 (C-4) and 176.9 (C-2); IR (film, cm^{-1}) 2977 (s), 1766 (s), 1274 (m), 1112 (m); GC−EIMS 326, 327 $[M + 1]^+$; HRMS 248.92, 350.92 $[M + Na]^+$.

*(±)-trans-7-Bromo-8-hydroxy-cis-3-oxabicyclo[4.3.0]nonan-2-one (**3d**).* To lactone **3a** (0.5941 g, 4.3×10^{-3} mol) dissolved in 15 mL of THF:H_2O solution (7:3) NBS (*N*-bromosuccinimide) (0.85 g, 4.7×10^{-3} mol) was added portionwise and the mixture was stirred until the substrate was consumed (TLC). Next 30 mL of water and 150 mL of diethyl ether were added. The organic layer was washed with brine (3 × 20 mL) until neutral and dried over anhydrous $MgSO_4$. Then the solvent was evaporated off and the mixture was purified by column chromatography, using a mixture of hexane:acetone 2:1 v/v as eluent. The bromohydrine **3d** (0.7783 g) was isolated with yield 77%: ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.94−2.10 (m, 1, one of CH_2-6), 2.11−2.25 (m, 1, one of CH_2-6), 2.26−2.56 (m, 3, CH_2-9, H-1), 2.57−2.73 (m, 1, H-5), 2.76−2.94 (m, 1, H-8), 3.95−4.17 (m, 1, H-7), 4.22 (dd, 1, *J* = 3.8, 7.6 Hz, one of CH_2-4), 4.28 (dd, 1, *J* = 5.3, 9.1 Hz, one of CH_2-4); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 25.0 (CH_2-6), 28.3 (CH_2-9), 31.6 (CH-5), 36.3 (CH-1), 50.2 (CH-7), 68.0 (CH-8), 71.1 (CH_2-4), 179.0 (C-2); IR (film, cm^{-1}) 3446 (w), 1767 (m); GC−EIMS 236, 237 $[M + 1]^+$; HRMS 234.99, 236.99 $[M + 1]^+$.

*(±)-trans-7-Bromo-8-hydroxy-cis-4,4-dimethyl-3-oxabicyclo[4.3.0]nonan-2-one (**4d**).* Bromohydrine **4d** (0.5795 g) was isolated with yield 75%, by the same procedure as lactone **3d**, using 0.3950 g (2.8×10^{-3} mol) of **4a** and 0.53 g (2.99×10^{-3} mol) of NBS: ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.38 and 1.42 (two s, 6, C-4(CH_3)$_2$), 1.87 (ddd, 1, *J* = 4.3, 4.4, 14.5 Hz, one of CH_2-6), 2.20−2.32 (m, 3, one of CH_2-6, one of CH_2-9, −OH), 2.40−2.70 (m, 2, H-1, one of CH_2-9), 2.96 (dd, 1, *J* = 7.0, 7.1 Hz, H-5), 4.06−4.12 (m, 1, H-8), 4.25−4.32 (m, 1, H-7); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 18.7 (CH_3−C-4), 20.5 (CH_3−C-4), 20.9 (CH_2-6), 22.0 (CH_2-9), 32.6 (CH-1), 34.8 (CH-5), 45.7 (CH-7), 63.3 (CH-8), 80.2 (C-4), 174.1 (C-2); IR (film, cm^{-1}) 3444 (w), 2253 (m), 1760 (m), 906 (s), and 730 (s); GC−EIMS 263, 264 $[M + 1]^+$; HRMS 285.00, 287.00 $[M + Na]^+$.

*(±)-8-Hydroxy-cis-3-oxabicyclo[4.3.0]nonan-2-one (**6**).* A round-bottom flask equipped with a magnetic stirrer was charged with 1.050 g (6.3×10^{-3} mol) of *cis*-1,2,3,6-tetrahydrophthalic anhydride **1** dissolved in 25 mL of dichloromethane, and 1.875 g (7.5×10^{-3} mol) of 70% MCPBA was added to it portionwise. The mixture was stirred for 12 h, then 25 mL of 20% Na_2SO_3 solution was added and the mixture was extracted with methylene chloride (4 × 50 mL). The organic layer was washed with 10% Na_2SO_3 (10 mL) solution and brine until neutral and dried over $MgSO_4$. The solvent was evaporated off, and the mixture was purified by column chromatography, using hexane:acetone 5:3 as eluent. 0.7184 g of the *cis*-4,5-epoxy-*cis*-1,2-cyclohexanedicarboxylic anhydride (**5**) was isolated (yield 65%). Next $LiAlH_4$ (0.60 g, 15.9×10^{-3} mol) was placed in a 2-neck flask equipped with a condenser, a dropping funnel and a magnetic stirrer, and then 20 mL of diethyl ether was added, followed by slow addition of *cis*-4,5-epoxy-*cis*-1,2-cyclohexanedicarboxylic anhydride **5** (530 mg, 3.15×10^{-4} mol), previously dissolved in 15 mL of diethyl ether. The mixture was refluxed until all the substrate was consumed (3 h, TLC, GC) and poured into 50 mg of ice mixed with 50 mL of 6% HCl solution. The product was extracted with diethyl ether (3 × 50 mL), and the organic layer was washed with brine and dried over $MgSO_4$. After evaporation of solvent the pure product was isolated by column chromatography (hexane:acetone 2:1) to give 0.199 g of lactone **6** (yield 45%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.09−1.12 (dt, 1, *J* = 3.8, 8.6 Hz, one of CH_2-6), 1.24−1.27 (m, 1, one of CH_2-7), 1.54−1.60 (m, 3, CH_2-9, one of H-6), 1.82−1.98 (m, 1, one of CH_2-7), 2.32−2.46 (m, 1, CH_2-1), 2.60−2.67 (m, 1, H-5), 3.65−3.84 (m, 1, H-8), 4.22 (dd, 1, *J* = 3.8, 7.6 Hz, one of CH_2-4), 4.32 (dd, 1,

J = 7.6, 9.2 Hz, one of CH_2-4); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 22.4 (CH_2-6), 25.3 (CH_2-7), 28.7 (CH_2-9), 32.1 (CH-5), 36.7 (CH-1), 69.5 (CH-8), 72.3 (CH_2-4), 176.2 (C-2); IR (film, cm^{-1}); 3443 (s), 2938 (s), 2248 (m), 1768 (s), 1172 (m), 1010 (m); GC−EIMS 157 $[M + 1]^+$; HRMS 157.98 $[M + 1]^+$.

*(±)-Cyclohex-2-en-1-ol (**8**).* A solution of cyclohex-2-en-1-one (**7**) (7.0 g, 72.8 mmol) in 15 mL of dry diethyl ether was added dropwise to a stirred $LiALH_4$ (1.4 g, 36.4 mmol) in 35 mL of diethyl ether, and the stirring was continued at room temperature. When reaction was completed (3 h, TLC, GC), the mixture was diluted with diethyl ether (150 mL) and washed successively with an aqueous solution of 10% HCl (15 mL) and water (until neutral). After solvent evaporation, the product was purified by column chromatography (hexane:acetone 3:1 v/v) to give 6.6 g of **8** (yield 93%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.55−1.62 (two m, 3, CH_2-5, one of CH_2-4), 1.84−1.91 (m, 1, one of CH_2-6), 2.27−2.32 (m, 2, one of CH_2-4, one of CH_2-6), 4.13−4.17 (m, 1, H-1), 5.69−5.71 (m, 1, H-3), 5.80 (dt, 1, J = 3.2, 10.1 Hz, H-2); IR (film, cm^{-1}) 3336 (s), 2934 (s), 1055 (s); GC−EIMS 99 $[M + 1]$.

*(±)-Ethyl(cyclohex-2-ene-1-yl)acetate (**9**).* A mixture of cyclohex-2-en-1-ol (**8**) (5.3 g, 54.1 mmol), ethyl orthoacetate (66.4 mL, 0.41 mol) and propionic acid (0.03 mL) was heated at 138 °C in a flask equipped with a distillation head. The reaction was continued until all the substrate was consumed. The excess of ethyl orthoacetate was distilled off, and the residue was purified by column chromatography (hexane:acetone 19:1) to give 7.2 g of ester **9** (yield 80%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.14−1.29 (m, 1, one of CH_2-6), 1.23 (t, 3, J = 7.0 Hz, $-CH_3$), 1.52−1.81 (two m, 3, CH_2-5, one of CH_2-6), 1.92−1.95 (m, 2, CH_2-4), 2.27 (dd, 1, J = 8.1, 15.2 Hz, one of $-CH_2-C(O)-$), 2.36 (dd, 1, J = 6.8, 15.2 Hz, one of $-CH_2-C(O)-$), 2.52−2.56 (m, 1, H-1), 4.09 (q, 2, J = 7.1 Hz, $>C(O)-O-CH_2-$), 5.50 (dddd, 1, J = 0.9, 2.0, 4.0, 10.1 Hz, CH-2); 5.50 (ddd, 1, J = 3.4, 5.8, 10.1 Hz, CH-3); IR (film, cm^{-1}) 2998 (s), 1748 (s), 1164 (s) 1036 (s); GC−EIMS 169 $[M + 1]$.

*(±)-cis-Ethyl(7-oxabicyclo[4.1.0]hept-2-yl)acetate (**10**).* To ester **9** (1.5235 g, 9.06 × 10^{-3} mol) dissolved in CH_2Cl_2 (20 mL), *m*-chloroperbenzoic acid (2.290 g, 9.29 × 10^{-3} mol) was added portionwise. The reaction mixture was stirred for 8 h, then the solvent was evaporated off and the residue was dissolved in 1.5 mL of DMF and purified by column chromatography (eluent hexane:acetone 25:1) to give 1.3848 g of pure **10** (yield 83%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.10−1.15 (m, 1, one of CH_2-4), 1.24 (t, 3, J = 7.2 Hz, $-CH_3$), 1.52−1.81 (two m, 3, CH_2-5, one of CH_2-4), 1.72−1.91 (m, 2, CH_2-6), 2.38−2.40 (m, 2, $-CH_2-C(O)-$), 2.55−2.58 (m, 1, H-3), 3.12−3.15 (m, 1, H-7); 3.20 (t, 1, J = 4.1 Hz, H-8), 4.18 (q, 2, J = 7.2 Hz, $>C(O)-O-CH_2-$); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 14.3 ($-CH_3$), 19.6 (CH_2-5), 23.6 (CH_2-6), 25.0 (CH_2-4), 31.9 ($-CH_2-C(O)-$), 37.9 (CH-3), 53.2 (CH-7), 55.1 (CH-8), 60.4 ($-O-CH_2-$), 172.7 ($-C(O)-O-$); IR (film, cm^{-1}) 1748 (s), 1182 (s), 1152 9 (s), 1032 (s); GC−EIMS 185 $[M + 1]$.

*(±)-trans-9-Hydroxy-cis-2-oxabicyclo[4.3.0]nonan-3-one (**11**).* Epoxy ester **10** (1.2430 g, 6.75 × 10^{-3} mol) was dissolved in 20 mL of a mixture of THF:H_2O:$HClO_4$ (10:5:0.5 v/v) and stirred at room temperature. The reaction was stopped after 4 h, when the intermediate diol ester was no longer observed by TLC and GC. The reaction mixture was neutralized with 20% solution of $NaHCO_3$, extracted with diethyl ether (4 × 50 mL) and purified by column chromatography to give 0.8231 g of **11** (yield 78%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.34−1.52 (m, 2, one of CH_2-6, −OH), 1.54−1.74 (m, 4, one of CH_2-8, CH_2-7, one of CH_2-6), 1.86−1.92 (m, 1, one of CH_2-8), 2.31 (dd, 1, J = 9.3, 17.1 Hz, one of CH_2-4), 2.48 (dd, 1, J = 7.8, 17.1 Hz, one of CH_2-4), 2.70−2.85 (m, 1, H-5), 3.82 (ddd, 1, J = 4.3, 6.5, 8.9 Hz, H-9), 4.28 (t, 1, J = 6.5 Hz, H-1); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 17.9 (CH_2-7), 25.4 (CH_2-6), 29.5 (CH_2-8), 33.8 (CH_2-4), 34.4 (CH-5), 69.3 (CH-9), 84.1 (CH-1), 177.1 (C-3); IR (film, cm^{-1}) 3441 (s), 2935 (s), 2252 (m), 1772 (s), 1179 (m), 1010 (m); GC−EIMS 157 $[M + 1]$.

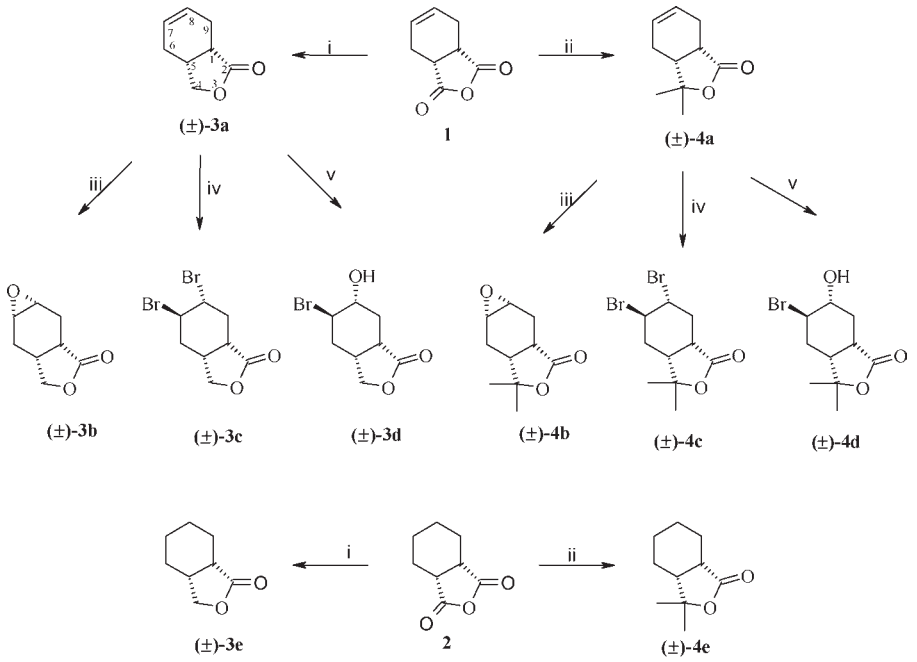
*(±)-trans-9-Bromo-cis-2-oxabicyclo[4.3.0]nonan-3-one (**12**).* To ester **9** (0.723 g, 4.3 × 10^{-3} mol) dissolved in 15 mL of a mixture of THF:H_2O (7:3) was added 0.8 g (5.3 × 10^{-3} mol) of NBS portionwise. The mixture was stirred until all the substrate was fully consumed (TLC) and then acidified with 10% HCl and stirred for a further 3 h. Thirty milliliters of water and 150 mL of diethyl ether were added to the reaction mixture, and the organic layer was separated, washed with brine until neutral (3 × 20 mL) and dried over $MgSO_4$. The solvent was evaporated off, and the crude product was purified by column chromatography with hexane:acetone 9:1 as eluent to give 0.6602 g of bromolactone **12** (yield 70%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.28−1.39 (m, 1, one of CH_2-6), 1.48−1.60 (m, 1, one of CH_2-7), 1.71−1.88 (m, 2, one of CH_2-7, one of CH_2-6), 1.90−2.11 (m, 2, CH_2-8), 2.28 (dd, 1, J = 3.3, 16.9 Hz, one of CH_2-4), 2.61 (dd, 1, J = 6.8, 16.9 Hz, one of CH_2-4), 2.70−2.83 (m, 1, H-5), 4.42−4.50 (m, 1, H-9), 4.60 (dd, 1, J = 4.3, 4.4 Hz, H-1); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 18.5 (CH_2-7), 26.1 (CH_2-6), 29.3 (CH_2-8), 32.5 (CH_2-4), 36.5 (CH-5), 48.2 (CH-9), 81.3 (CH-1), 175.8 (C-3); IR (film, cm^{-1}) 1785 (s), 1173 (s), 1019 (s); GC−EIMS 220, 221 $[M + 1]^+$; HRMS 240.98, 242.98 $[M + Na]^+$.

*(±)-trans-9-Acetyl-cis-2-oxabicyclo[4.3.0]nonan-3-one (**13**).* Hydroxy lactone **11** (0.4250 g, 2.72 × 10^{-3} mol) was dissolved in 2.5 mL of anhydrous pyridine. To the stirred mixture was slowly added 0.7 mL of acetyl chloride (0.77 g, 9.81 × 10^{-3} mol). Progress of the reaction was checked by TLC. After 4 h the reaction mixture was diluted with 150 mL of diethyl ether and the pyridine was washed off with 6% HCl solution (3 × 50 mL) and brine (3 × 20 mL). The organic layer of intensively yellow color was dried over $MgSO_4$, the solvent was evaporated off and the residue was purified three times by column chromatography using the following eluents: (1) hexane:acetone 4:1; (2) dichloromethane:methanol 399:1; (3) hexane:acetone 2:1. 0.3945 g of pure product **13** was obtained (yield 73%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.34−1.59 (m, 1, one CH_2-6), 1.60−1.76 (m, 4, one of CH_2-8, CH_2-7, one of CH_2-6), 1.79−1.93 (m, 1, one of CH_2-8), 2.07 (s, 3, $C(O)CH_3$), 2.33 (dd, 1, J = 7.2, 17.1 Hz, one of CH_2-4), 2.53 (dd, 1, J = 7.5, 17.0 Hz, one of CH_2-4), 2.65−2.82 (m, 1, H-5), 4.36 (t, 1, J = 5.9 Hz, H-1) 4.96 (m, 1, H-9); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 17.7 ($CH_3C(O)-$), 20.9 (CH_2-7), 25.5 (CH_2-8), 26.5 (CH_2-6), 34.0 (CH_2-4), 34.5 (CH_2-5), 70.4 (CH-9), 79.4 (CH-1), 169.8 (>C═O), 175.9 (C-3); IR (film, cm^{-1}) 1783 (s), 1730 (s), 1240 (m), 1021 (m); GC−EIMS 199 $[M + 1]$.

*(±)-trans-9-Butyryl-cis-2-oxabicyclo[4.3.0]nonan-3-one (**14**).* **14** was obtained by the same procedure as lactone **13**, using hydroxy lactone **11** (0.3133 g, 2.0 × 10^{-3} mol) and butyryl chloride (0.6 mL, 0.504 g, 4.75 × 10^{-3} mol) as reagents. The product was purified by column chromatography using hexane:acetone in 4:1 ratio to give 0.2450 g of pure **14** (65% yield): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 0.94 (t, 3, J = 7.4 Hz, CH_3-4′), 1.40−1.70 (m, 5, CH_2-6, CH_2-7, one of CH_2-8), 1.65 (sext, 2, J = 7.4 Hz, CH_2-3′), 2.30 (t, 2, J = 7.4 Hz, CH_2-2′), 2.31 (dd, 1, J = 6.9, 17.0 Hz, one of CH_2-4), 2.53 (dd, 1, J = 7.4, 17.0 Hz, 1H, one of CH_2-4), 2.64−2.76 (m, 1, H-5), 4.35 (t, 1, J = 5.8 Hz, H-1), 4.95−5.06 (m, 1, H-9); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 13.4 (CH_3-4′), 17.7 (CH_2-3′), 18.3 (CH_2-7), 25.6 (CH_2-8), 26.4 (CH_2-6), 34.0 (CH_2-2′), 34.7 (CH_2-4), 36.1 (CH-5), 69.9 (CH-9), 79.4 (CH-1), 172.5 (C-3), 176.0 (>C═O); IR (film, cm^{-1}) 1785 (m), 1735 (m) and 1153 (m); GC−EIMS 227 $[M + 1]^+$; HRMS 226.98 $[M + 1]^+$.

*(±)-trans-9-(p-Methoxyphenyl)acetoxy-cis-2-oxabicyclo[4.3.0]nonan-3-one (**15**).* **15** was obtained by the same procedure as lactone **13**, using hydroxy lactone **11** (0.4923 g, 3.15 × 10^{-3} mol) and 4-methoxyphenylacetyl chloride (0.9 mL, 0.672 g, 3.65 × 10^{-3} mol). After purification by column chromatography using hexane:acetone in 4:1 ratio 0.6780 g of pure **15** was obtained (yield 63%): ^1H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.30−1.85 (three m, 6, CH_2-6, CH_2-7, CH_2-8), 2.27 (dd, 1, J = 6.3, 16.8 Hz, one of CH_2-4), 2.27 (dd, 1, J = 7.3, 16.8 Hz, 1H, one of CH_2-4), 2.55−2.68 (m, 1, H-5), 3.56 (s, 2, $-CH_2-Ph-$),

Scheme 1[a]



[a] (i) $LiAlH_4$, Et_2O; (ii) 2 mol of MeMgI, Et_2O; (iii) MCPBA, CH_2Cl_2; (iv) Br_2, CCl_4; (v) NBS, $THF:H_2O$.

3.77 (s, 3, −O−CH_3), 4.31 (t, 1, J = 5.8 Hz, H-1), 4.98−5.05 (m, 1, CH-9), 6.80−7.20 (four m, 4, -C_6H_4-); ^{13}C NMR (151 Mz, $CDCl_3$) δ (ppm) 17.7 (CH_2-7), 25.6 (CH_2-8), 26.2 (CH_2-6), 33.9 (CH_2-4), 34.8 (CH-5), 40.4 (−CH_2−Ph), 55.1 (−O−CH_3), 70.4 (CH-9), 79.1 (CH-1), 113.8 (CH═3′, CH═5′), 125.7 (CH═2′, CH═6′), 130.1 (CH═1′), 158.6 (CH═4′), 170.7 (>C═O), 176.0 (C-3); IR (film, cm^{-1}) 3019 (s), 1782 (m), 1736 (w), 1216 (s); GC−EIMS 305 $[M + 1]^+$; HRMS 305.98 $[M + 1]^+$.

Antifungal Assays. The proper amounts of tested lactones were dissolved in DMSO and added to sterilized potato dextrose agar (PDA) (0.01 mL of DMSO for 1 mL of agar) in 9 cm Petri dishes to give lactone concentrations of 50, 100, 150, 200, 250, or 300 μg/mL. After transferring a mycelium of a fungus to the nutrient prepared in such a way, the testing dishes were incubated at 27 °C and 60−80% of relative humidity. When the mycelium of the fungi in the control dishes (nutrient with DMSO, without lactones added) reached 5.5−6.0 cm of diameter, a diameter of growth zone in control samples and in experimental dishes were measured. All the lactones were tested at concentration of 150 μL/mL and at three more concentrations, which were chosen individually. Each test was repeated three times. Didecyldimethylammonium chloride (DDAC) was used as reference compound. IC_{50} (the half maximal inhibitory concentration) values for each tested compound were determined based on four measured values, as a percent of inhibition of growth and calculated according to the formula $(1 - D_a/D_b) \times 100\%$, where D_a is the diameter of growth zone in experimental dish (cm) and D_b is diameter of growth zone in the control dish (cm).

Statistical analysis was done using ANOVA (p = 0.05), and the means were compared by calculating the least significant difference (LSD, Tukey).

RESULTS AND DISCUSSION

In the first stage of our work we synthesized racemic lactone derivatives of *cis*-3-oxabicyclo[4.3.0]nonan-2-one and *cis*-2-oxabicyclo[4.3.0]nonan-3-one structures.

Synthesis of *cis*-3-oxabicyclo[4.3.0]nonan-2-one derivatives (3a−e) and their *gem*-dimethyl derivatives (4a−e). Starting from *cis*-1,2,3,4-tetrahydrophthalic anhydride (**1**) in the reduction with lithium aluminum hydride racemic *cis*-3-oxabicyclo-[4.3.0]non-7-en-2-one (**3a**) was obtained (Scheme 1). The structure of the product was established by means of IR, ^{1}H NMR, and ^{13}C NMR. Formation of a lactone ring in compound **3a** was confirmed by the presence of a single band at 1771 cm^{-1} in the IR spectrum, the signal of a carbonyl carbon atom of δ = 175 ppm in the ^{13}C NMR, and two signals at 4.0 and 4.3 ppm (doublet of doublets) in the ^{1}H NMR, which indicate that there are two protons at C-4. The presence of a double bond was proved by the multiplet at 5.60−5.70 ppm in the ^{1}H NMR spectrum and two signals at 125.0 and 125.3 ppm in the ^{13}CNMR spectrum.

4,4-Dimethyl-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**4a**) the structural analogue of lactone **3a** with *gem*-dimethyl group at C-4 was obtained in a Grignard reaction of two equivalents of methylmagnesium iodide with one equivalent of anhydride **1**. The signals of the methyl groups were found in the ^{1}H NMR spectrum as a singlet of six protons at 1.38 ppm, whereas the double bond protons were visible as a multiplet at 5.63−5.89 ppm.

In the next stage of the synthesis, double bonds of lactones **3a** and **4a** were oxidized with *m*-chloroperbenzoic acid to give *cis*-epoxides **3b**[42,43] and **4b** (Scheme 1). In the ^{1}H NMR spectra the signals of protons at C-7 and C-8 are represented by separate triplets of a coupling constant J = 7.0 Hz for *cis*-7,8-epoxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3b**) and J = 4.0 Hz for 4,4-dimethyl-*cis*-7,8-epoxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**4b**). Bromination of unsaturated lactones **3a** and **4a** proceeded highly stereoselectively and gave *trans*-dibromo derivatives: *trans*-7,8-dibromo-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3c**) and *trans*-7,8-dibromo-4,4-dimethyl-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**4c**) (Scheme 1). In these compounds, similarly to epoxy lactones, the signals of protons at C-7 and C-8 in the

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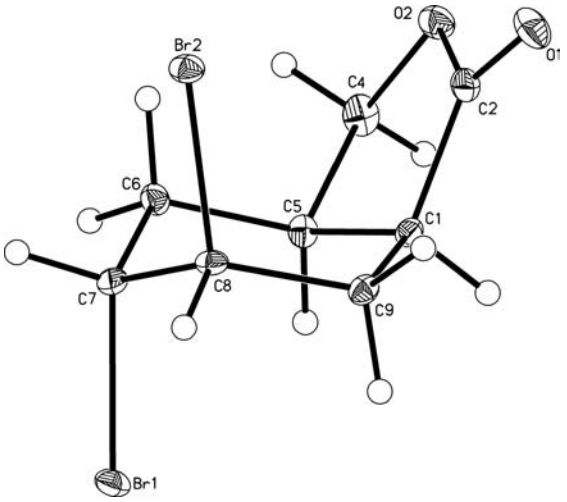


Figure 1. Crystal structure of *trans*-7,8-dibromo-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3c**). Crystal data: $C_8H_{10}Br_2O_2$; M = 297.98; colorless block; crystal dimensions 0.32 × 0.21 × 0.13 mm; monoclinic; space group $P2_1/c$; a = 6.646(2), b = 13.691(4), c = 10.087(3) Å; β = 99.12(3)°; V = 906.2(5) Å^3; Z = 4; D_c = 2.184 Mg m^{-3}; μ = 8.900 mm^{-1}; T = 100(2) K; R = 0.025, wR = 0.062 (1699 reflections with $I > 2\sigma(I)$) for 109 variables; CCDC reference number 794604.

^{1}H NMR overlap and give nondiagnostic multiplets. For dibromo lactone **3c** X-ray analysis has been done (Figure 1).

The crystal structure confirms the mechanism of bromine addition to *cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**3a**).

We also obtained bromohydrine derivatives of lactones **3a** and **4a** in the addition reactions. The mechanism of bromohydrine addition to unsaturated bond leads to *trans* product.

In both syntheses we isolated single diastereoisomer, which was confirmed by IR and ^{13}C NMR spectra. Because the significant diagnostic signals in ^{1}H NMR spectra were multiplets, the relative configuration of **3d** and **4d** was established on the basis of additional spectroscopic methods (COSY and HMQC for **4d**). The COSY spectra of **3d** and **4d** indicated the correlations between multiplets from H-7 (−CH−Br) and two signals from CH_2-6. The other correlation was observed for signals of H-8 (−CH−OH) and two multiplets from CH_2-9. We observed correlation between H-7 and H-8 too. In the HMQC spectrum of **4d** the correlation between signals from proton H-7 (4.28 ppm) and carbon atom C-7 (45.7 ppm), as well as signals from proton H-8 (4.09 ppm) and carbon atom C-8 (63.3 ppm), confirmed these assignations.

The saturated lactones **3e** and **4e** were obtained in an analogous way to their unsaturated analogues **3a** and **4a**.

Synthesis of Hydroxy Lactones 6 and 11. Two isomeric hydroxy lactones of different position of oxygen atom in the five-membered ring were synthesized. The first one, 8-hydroxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**6**), was obtained in a two-step synthesis (Scheme 2). In the first step *cis*-1,2,3,4-tetrahydrophthalic anhydride **1** was oxidized with *m*-chloroperbenzoic acid to *cis*-epoxy anhydride **5**.

Compound **5** was subjected to the reduction with lithium aluminum hydride which gave a mixture of two hydroxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-ones with a hydroxyl group at either C-7 or C-8 in a 15:85 ratio. Isomerism of these hydroxy lactones was confirmed by GC−MS [M$^+$ 156]. Only the major product **6** was isolated.

The presence of the hydroxyl group of hydroxy lactone **6** was confirmed by IR (3443 cm^{-1}). The location of the hydroxyl group at C-8 was established by means of spectral data: ^{1}H NMR, ^{13}C NMR and HMCQ. The signal from H-8 in the ^{1}H NMR spectrum was observed as not a diagnostic signal, so its position was appointed by HMCQ, where we observed coupling between multiplet from H-8 (3.65−3.84 ppm) and C-8 (69.5 ppm).

The second hydroxy lactone, *trans*-9-hydroxy-*cis*-2-oxabicyclo-[4.3.0]nonan-3-one (**11**), was obtained in a 4-step synthesis (Scheme 3), in which allyl alcohol **8**, obtained in the reduction of ketone **7**, was subjected to Claisen rearrangement leading to γ,δ-unsaturated ethyl ester **9**. This ester was oxidized with *m*-chloroperbenzoic acid to epoxide **10**, obtained as a mixture of diastereoisomers *cis*:*trans* in a 9:1 ratio. The structure of *cis* epoxy ester **10** is confirmed by the coupling constant (J = 4.1 Hz) of protons at C-7 and C-8 in the ^{1}H NMR. Under acidic conditions both epoxy esters underwent cyclization (via dihydroxy esters) to a single, diastereoisomerically pure hydroxy lactone **11**. Relative configuration of lactone **11** was established by ^{1}H NMR spectrum. Contrary to the small coupling constant (0−5 Hz) of diequatorial or axial−equatorial orientation of protons in a six-membered cyclohexane ring, the coupling constant of neighboring axial protons is in the range 6−14 Hz. The coupling constant (J = 6.5 Hz) between H-1 (δ = 4.28) and H-9 (δ = 3.82) in hydroxy lactone **11** convinced us that the OH group is located in the equatorial position.

Additionally, the presence of a doublet of doublets of doublets (δ = 3.82) with the large coupling constants at H-9 with neighboring axial proton H-8 (J = 8.9 Hz) and equatorial proton H-8 (J = 4.3 Hz) proved the equatorial orientation of the hydroxy group. It indicates that the OH group is situated *trans* to the γ-lactone ring.

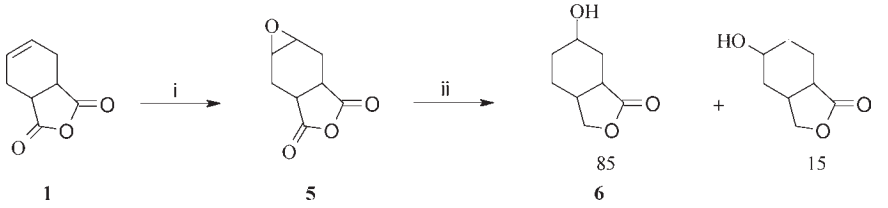
Synthesis of *cis*-2-Oxabicyclo[4.3.0]nonan-3-one Derivatives: 12−15. In order to check how the protection of the hydroxyl group in lactone **11** would influence the fungistatic activity, this lactone was transformed into three esters: **13**, **14** and **15**, in which the hydroxyl group was esterified with acetic acid chloride, butyric acid chloride and *p*-methoxyphenylacetic acid chloride, respectively (Scheme 3). Additionally, *trans*-9-bromo-*cis*-2-oxabicyclo[4.3.0]nonan-3-one (**12**) was synthesized, in which the hydroxyl group was replaced with the more bulky bromine atom. This lactone was synthesized in the reaction of γ,δ-unsaturated ester **9** with *N*-bromosuccinimide (Scheme 3).

Fungistatic Activity of Racemic Lactones 3a−e, 4a−d, 6 and 11−15. The racemic lactones were subjected to the tests of activity toward three phytopathogenic species: *Aspergillus ochraceus* AM 456, *Fusarium culmorum* AM 282 and *Penicillium citrinum* AM 354. The tested compounds were the most active toward *F. culmorum*, so the next two microorganisms of the genus *Fusarium* were subjected to the tests: *F. oxysporum* AM 13 and *F. tricinctum* AM 16. The half maximal inhibitory concentration (IC_{50}) was calculated based on degree of growth inhibition on the cultivation medium with tested compound added in comparison with a control sample (Table 1a,b).

Lactones of *cis*-3-oxabicyclo[4.3.0]nonan-2-one structure (**3a**−**e**) and their *gem*-dimethyl derivatives (**4a**−**e**) inhibit mycelium growth of *A. ochraceus* AM 456, *F. culmorum* AM 282 and *F. oxysporum* AM 13 stronger than of *F. tricinctum* AM 16 and *P. citrinum* AM 354.

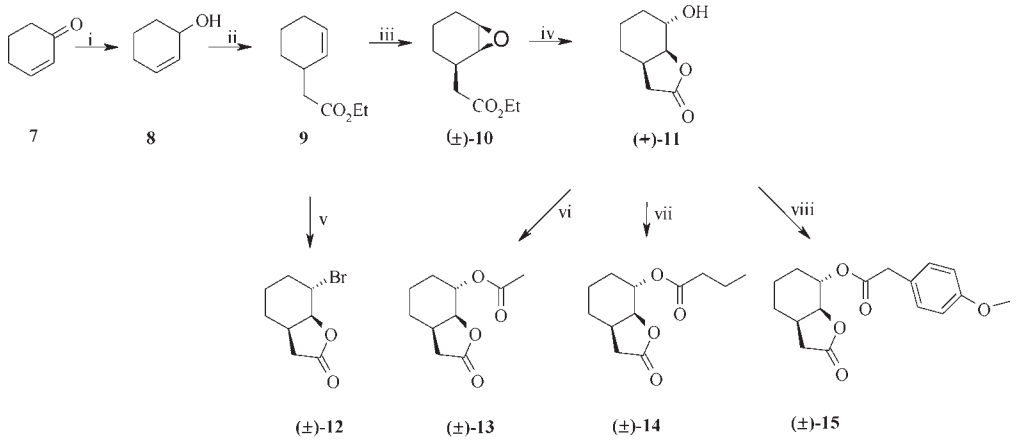
Lactones *cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**3a**) (entry 3), *cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3e**) (entry 7), 4,4-dimethyl-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**4a**) (entry 8) and

Scheme 2[a]



[a] (i) MCPBA, CH_2Cl_2; (ii) $LiAlH_4$, Et_2O.

Scheme 3[a]



[a] (i) $LiAlH_4$, Et_2O; (ii) $CH_3C(OC_2H_5)_3$, C_2H_5COOH; (iii) MCPBA, CH_2Cl_2; (iv) $THF:HClO_4:H_2O$; (v) NBS, $THF:H_2O$; (vii) $CH_3C(O)Cl$, Py; (vii) $C_3H_7C(O)Cl$, Py; (viii) $CH_3OPhCH_2C(O)Cl$, Py.

4,4-dimethyl-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**4e**) (entry 12) inhibit growth of mycelium of *A. ochraceus* AM 456, *F. culmorum* AM 282 and *F. oxysporum* AM 13 at IC_{50} = 134.5−314.3 μg/mL (0.80−2.25 μM/mL). An increase of fungistatic activity is attributed to the presence of the *gem*-dimethyl group (**4a**, **4e**). We analyzed the influence of double bond on two pairs of lactones: **3a**, **3e** and **4a**, **4e**. Fungistatic activity of those lactones is similar, with the exception of activity of saturated lactones **3e**, **4e** toward *F. culmorum* AM 282.

The highest fungistatic activity was observed for *trans*-7,8-dibromo-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3c**) (entry 5). This lactone inhibits growth of *F. oxysporum* AM 13 by 50% at a concentration of 30.1 μg/mL (0.10 μM/mL), whereas for the other *Fusarium* species this degree of inhibition is achieved for concentrations of 81.5−110.5 μg/mL (0.28−0.37 μM/mL). The structural analogue of **3c** with a *gem*-dimethyl group at C-4 (**4c**) (entry 11) exhibits two or three times lower activity. Activity of IC_{50} below 100 μg/mL toward *F. oxysporum* AM 13, *F. culmorum* AM 282 was also observed for *cis*-7,8-epoxy-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3b**) (entry 4), whereas its *gem*-dimethyl derivative (**4b**) (entry 10) was less effective. Replacement of one of the bromine atoms at C-8 with a hydroxyl group in *trans*-7-bromo-8-hydroxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3d**) and in *trans*-7-bromo-8-hydroxy-4,4-dimethyl-*cis*-3-oxabicyclo[4.3.0] nonan-2-one (**4d**) negatively affects the fungistatic activity.

We expected lower activity of 8-hydroxy-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**6**) (entry 13) compared to lactone **3d**. Such a correlation was observed for *A. ochraceus* AM 456 and *P. citrinum* AM 354; however, the IC_{50} values for the tested *Fusarium* strains were lower. An attempt to synthesize 7-bromo-*cis*-3-oxabicyclo[4.3.0]nonan-2-one was unsuccessful, therefore we included derivatives of *cis*-2-oxabicyclo[4.3.0]nonan-3-one in the tests. Additionally, this allowed us to check whether the location of oxygen atom in the lactone ring has an influence on the IC_{50} value. A negative effect of hydroxyl group on fungistatic activity was confirmed in tests with hydroxy lactone **11** (entry 14), which to a small degree inhibited growth of the tested phytopathogens.

When the hydroxyl group is protected with an ester moiety (**13**−**15**) or replaced with bromine (**12**), the fungistatic activity toward *F. culmorum* AM 282 and *F. tricinctum* AM 16 is increased, however, this considerably decreases the fungistatic activity toward *F. oxysporum* AM 13.

Influence of Stereogenic Centers on Fungistatic Activity. The derivatives of the *cis*-3-oxabicyclo[4.3.0]nonan-2-one structure, especially lactones **3b** and **3c**, showed higher activity toward *F. oxysporum* AM 13 and *F. culmorum* AM 282 than those of *cis*-2-oxabicyclo[4.3.0]nonan-3-one.

The methods of obtaining of two enantiomers of *cis*-3-oxabicyclo[4.3.0]non-7-en-2-one (**3a**) and *cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3e**) described in the literature include, among others, hydrolysis of anhydrides by means of lipases and oxidation of *meso* diols using HLADH.[41−43] HLADH isolated from horse liver is not commercially available, therefore we used the enzyme recombinated in *Escherichia coli*.

In the screening tests we have checked whether *cis*-1,2-bis(hydroxymethyl)cyclohexane **17** can be oxidized to both enantiomers of *cis*-lactone **3e** using commercial alcohol dehydrogenases (Scheme 4). The results are presented in Table 2.

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Table 1. The Half Maximal Inhibitory Concentration (IC_{50}) for Racemic Lactones (a) in μg/mL and (b) in μM/mL against *A. ochraceus* AM 456, *F. culmorum* AM 282, *F. oxysporum* AM 13, *F. tricinctum* AM 16, and *P. citrinum* AM 354[a]

		(a) IC_{50} [μg/mL]				
entry	compd	*A. ochraceus* AM 456	*F. culmorum* AM 282	*F. oxysporum* AM 13	*F. tricinctum* AM 16	*P. citrinum* AM 354
1	**1**	286.7	310.2	220.3	289.4	>350
2	**2**	342.3	291.5	240.5	302.9	>350
3	**3a**	170.9	184.0	282.0	220.7	>350
4	**3b**	141.0	90.4	72.2	156.5	312.5
5	**3c**	219.0	81.5	30.1	110.5	305.9
6	**3d**	140.9	172.4	141.0	211.2	292.0
7	**3e**	186.8	147.3	314.3	221.9	>350
8	**4a**	159.3	182.6	188.2	204.7	274.5
9	**4b**	142.1	141.5	95.3	180.5	>350
10	**4c**	120.9	154.7	80.2	191.0	254.8
11	**4d**	220.4	166.3	170.4	162.4	212.1
12	**4e**	159.7	134.5	179.1	212.6	291.8
13	**6**	212.5	120.7	126.6	161.1	>350
14	**11**	>350	>350	195.3	219.5	212.5
15	**12**	244.3	133.1	184.1	173.4	281.7
16	**13**	226.7	144.9	299.7	146.0	282.4
17	**14**	282.5	172.8	218.6	111.0	>350
18	**15**	172.8	159.2	262.5	181.6	263.6
	DDAC	51.3	24.5	24.2	27.9	41.6
		(b) IC_{50} [μM/mL]				
entry	compd	*A. ochraceus* AM 456	*F. culmorum* AM 282	*F. oxysporum* AM 13	*F. tricinctum* AM 16	*P. citrinum* AM 354
1	**1**	1.89	2.04	1.45	1.90	2.30
2	**2**	2.23	18.88	1.56	1.96	2.27
3	**3a**	1.25	1.33	2.04	1.60	2.54
4	**3b**	0.91	0.59	0.47	1.01	2.03
5	**3c**	0.73	0.28	0.10	0.37	1.03
6	**3d**	0.60	0.73	0.60	0.90	1.24
7	**3e**	1.33	1.05	2.25	1.58	2.50
8	**4a**	0.96	1.10	1.13	1.23	1.65
9	**4b**	0.78	0.77	0.52	0.99	1.92
10	**4c**	0.38	0.47	0.25	0.58	0.78
11	**4d**	0.85	0.63	0.65	0.62	0.81
12	**4e**	0.95	0.80	1.06	1.26	1.73
13	**6**	1.36	0.77	0.81	1.03	2.24
14	**11**	2.24	2.24	1.25	1.41	1.36
15	**12**	1.12	0.61	0.84	0.79	1.28
16	**13**	1.25	0.80	1.64	0.80	1.55
17	**14**	1.25	0.76	0.96	0.49	1.54
18	**15**	0.57	0.52	0.86	0.60	0.87
	DDAC	0.14	0.07	0.07	0.08	0.11

[a] Statistical analysis was done using ANOVA ($p = 0.05$), and the means were compared by calculating the least significant difference (LSD, Tukey). IC_{50} was calculated on the basis of standard curve with standard deviation $\pm$1.825−2.134.

The most effective enzyme, which was used in the next part of our work, was HLADH, which oxidized the *meso* diol **17** to (+)-isomer of (1*S*,5*R*)-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3e**). Alcohol dehydrogenase from *Lactobacillus kefir* (LKADH) oxidized the diol to the (−)-enantiomer, however, with an ee of 82% only.

Five enantiomerically enriched lactones were obtained *via* enzymatic oxidation of the respective diols (Scheme 5). For enantiomerically enriched lactones (−)-(1*S*,5*R*)-*cis*-3-oxabicyclo-[4.3.0]non-7-en-2-one ((−)-**3a**) and (+)-(1*S*,5*R*)-*cis*-3-oxabicyclo[4.3.0]nonan-2-one ((+)-**3e**) absolute configuration (1*S*,5*R*) were assigned by literature date.[41] (−)-(1*S*,5*R*,7*S*,8*R*)-*cis*-7,8-Epoxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one ((−)-**3b**) was obtained by oxidation of enantiomerically enriched lactone (−)-(1*S*,5*R*)-*cis*-3-oxabicyclo[4.3.0]non-7-en-2-one ((−)-**3a**)

with *m*-chloroperbenzoic acid, and its configuration was established on sign of optical rotation and literature date.[42]

The *gem*-dimethyl group was introduced in a two-step synthesis, including a Grignard reaction leading to diol, which was next oxidized to (−)-lactone **4e**. Absolute configuration (1*S*,5*R*) was assigned by known literature mechanism[44] and a sign of optical rotation.[41]

Configuration of stereogenic centers of new lactone (+)-(1*S*,5*R*,7*R*,8*R*)-*trans*-7,8-dibromo-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3c**) was established on the basis of absolute configuration of substrate, spectroscopic methods and X-ray diffraction study.

The fungistatic activity of the obtained 5 isomers of ee = 89−91% was determined toward *F. culmorum* 282 and *F. oxysporum* 13. The IC_{50} values and differences in activities of optical isomers compared to the racemic substrates are presented in Table 3.

Enantiomerically enriched **3a**, **3b**, **4e** showed essential increase in fungistatic activity compared to racemic compounds. Optically active (+)-(1*S*,5*R*,7*R*,8*R*)-*trans*-7,8-dibromo-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3c**) was less active than the racemic mixture against the strains of *F. oxysporum* AM 13 and *F. culmorum* AM 282.

In summary, we have synthesized and determined antifungical activity of three groups of lactones: two of them with *cis*-3-oxabicyclo[4.3.0]nonan-2-one skeleton and the third group

Scheme 4

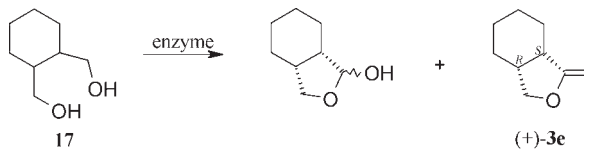


Table 2. Oxidation of 1,2-Bis(hydroxymethyl)cyclohexane (17) Using Commercially Available Alcohol Dehydrogenases

entry	biocatalyst	time [days]	conversion of **17** [%]	lactol [%]	**3e** [%]	ee of **3e** [%]
1	HLADH	1	95	67	28	(+) 92
		7	100	35	65	(+) 92
		14	100	5	95	(+) 92
2	PADH I	1	2	2	0	0
		7	11	11	0	0
		14	11	11	0	0
3	PADH II	1	36	0	36	(−) 26
		7	42	0	42	(−) 26
		14	43	0	43	(−) 25
4	PADH III	1	6	4	2	(+) 95
		7	26	0	26	(+) 54
		14	100	10	90	(+) 16
5	YADH	1	0	0	0	0
		7	14	5	9	(+) 58
		14	46	2	44	(+) 34
6	LKADH	1	0	0	0	0
		7	0	0	0	0
		14	83[a]	19	24	(−) 82

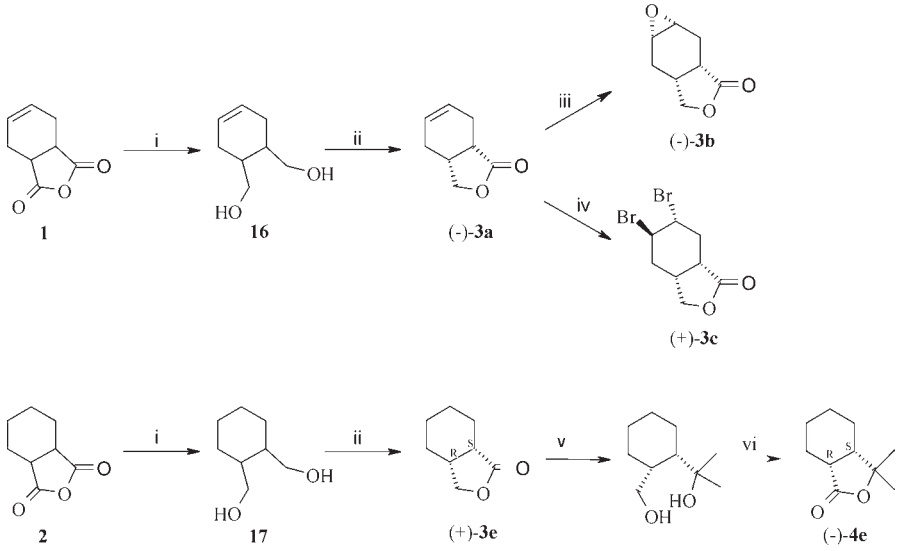
[a] : additional products.

Table 3. The Half Maximal Inhibitory Concentration (IC_{50}) Measured in μg/mL for Chiral Lactones against *F. culmorum* AM 282 and *F. oxysporum* AM 13[a]

		F. culmorum AM 282		*F. oxysporum* AM 13	
entry	compd	IC_{50}[μg/mL]	ΔIC_{50}[b]	IC_{50}[μg/mL]	ΔIC_{50}[b]
1	(−)-**3a**	171.0	+13	222.0	+60
2	(−)-**3b**	59.7	+31	51.9	+20
3	(+)-**3c**	90.5	−9	47.5	−17
4	(+)-**3e**	169.4	−22	261.0	+53
5	(−)-**4e**	120.6	+14	162.3	+17

[a] Statistical analysis was done using ANOVA ($p = 0.05$), and the means were compared by calculating the least significant difference (LSD, Tukey). IC_{50} was calculated on the basis of standard curve with standard deviation ±1.825−2.134. [b] $\Delta IC_{50} = IC_{50racemate} - IC_{50enantiomer}$.

Scheme 5[a]



[a] (i) $LiAlH_4$, Et_2O; (ii) HLADH; (iii) MCPBA, CH_2Cl_2; (iv) Br_2, CCl_4; (v) MeMgI, Et_2O; (vi) dichromate pyridine, CH_2Cl_2.

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with *cis*-2-oxabicyclo[4.3.0]nonan-3-one. These compounds inhibited growth of the fungi by 50% at concentrations from 30 μg/mL (0.10 μM/mL) to above 350 μg/mL (1.92 μM/mL) and were active toward *A. ochraceus* AM 456, *F. culmorum* AM 282, *F. oxysporum* AM 13, and *F. tricinctum* AM 16. The strain of *P. citrinum* AM 354 was found to be very resistant to these lactones. The tested lactones, due to their structural differences, allowed us to assess how the structure affects the biological activity. An impact of the following factors was determined: configuration of stereogenic centers; location of lactone function in the five-member γ-lactone ring of [4.3.0] structure; the presence and location of *gem*-dimethyl moiety; the presence of epoxide ring, bromine atoms, hydroxyl group and ester group.

As a result of the biological tests it was found that the compounds *cis*-7,8-epoxy-*cis*-3-oxabicyclo[4.3.0]nonan-2-one (**3b**), its *gem*-dimethyl derivative **4b** and *trans*-7,8-dibromo-*cis*-3-oxabicyclo-[4.3.0]nonan-2-one (**3c**) were active toward *F. culmorum* AM 282, *F. oxysporum* AM 13 and *F. tricinctum* AM 16.

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