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ORIGINAL ARTICLE

Antimicrobial activity of new bicyclic lactones with three or four methyl groups obtained both synthetically and biosynthetically

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Abstract Ten new derivatives of isophorone were obtained through a five-step synthesis. Among the products were several unsaturated, bicyclic lactones with three or four methyl groups. These lactones were used as the substrates for biotransformation mediated by selected fungal strains (*Fusarium* species, *Syncephalastrum racemosum*, *Cunninghamella japonica*, *Penicillium* species, *Absidia* species, *and Pleurotus ostreatus*). Four new hydroxylactones were obtained as a result of biotransformation. Because the unsaturated lactone with four methyl groups was a diastereoisomeric mixture, a structural analysis was conducted. The hydroxylactones were also included in this analysis. Both the unsaturated lactones and hydroxylactones were examined for their antimicrobial activity. It was found that some of these compounds exhibited growth inhibition against pathogenic strains of bacteria (*Staphylococcus aureus*, *Pseudomonas fluorescens*), yeasts (*Candida albicans*) and filamentous fungi (*Alternaria* sp., *Penicillium* sp.). All obtained compounds were also subjected to scent analysis.

1. Introduction

Modern medicine continues to search for new drugs and preparations to combat diseases caused by ubiquitous bacteria, viruses and fungi. Infections caused by these pathogens decrease the quality of life of the average inhabitant of the earth. Environmental pollution, poor nutrition and abuse of all kinds of pharmaceuticals leave us particularly susceptible to infections of all kinds of pathogens. Among the bacteria,

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the main group of unicellular prokaryotic organisms, *Staphylococcus aureus* is extremely dangerous. This Gram-positive bacterium can cause necrotizing pneumonia, endocarditis or toxic shock syndrome, among others. Strains of this microorganism that are resistant to methicillin cause the majority of hospital-acquired infections in the world [1]. Another commonly occurring drug-resistant bacterium, *Pseudomonas fluorescens*, may cause infections of the urinary tract, meninges, bones, joints, and others [2].

In addition, long-term use of antimicrobial therapy or chemotherapeutic agents can cause an imbalance of bacteria normally present in the colon. This results in weakened immunity, leaving the patient particularly susceptible to *Candida* fungal infections [3]. Populations particularly vulnerable to candidiasis include patients struggling with chronic illnesses such as diabetes, eating disorders and absorption, cancer and blood disorders [4]. Filamentous fungi, such as those of the genus *Alternaria* and *Penicillium* are equally dangerous, and their spores can cause inhaled allergies [5].

More and more mutating pathogens have become resistant to currently available drugs. For this reason, it is very important to search for new chemical compounds able to replace those compounds that are no longer active. Especially noteworthy to this search are plant extracts used in traditional folk medicine. Very often, the compounds of these isolates with specific biological properties are molecules containing a lactone ring. Particular attention should be paid to hydroxylactones, many of which exert anticancer [6–9], anti-inflammatory [10–12], antiviral [13,14], antifungal [15–17], or antibacterial [18,19] activities. Such compounds are not only derived solely from plant material; they may also be produced by chemical synthesis [20–22] or biotransformation [23–27].

For more than ten years our team has been obtaining new hydroxylactones from the chemically derived halolactones [23–27]. For this purpose we use filamentous fungi capable of performing hydrolytic dehalogenation. This method allows us to obtain compounds whose acquisition by standard chemical synthesis would be either impossible, or too complex to be viable. In the presented work, we used unsaturated lactones as substrates, hoping to obtain new derivatives with interesting biological properties.

2. Experimental

2.1. Chemistry

Analytical TLC was performed on silica gel-coated aluminium plates (DC-Alufolien Kieselgel 60 F254, Merck, Darmstadt, Germany) with a mixture of hexane, acetone and diethyl ether in various ratios. Compounds were detected by spraying the plates with 1% $Ce(SO_4)_2$, 2% $H_3[P(Mo_3O_{10})_4]$ in 10% H_2SO_4 or 20% ethanolic H_2SO_4, containing 0.1% of anisaldehyde, followed by heating to 120 °C. Preparative column chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh ASTM, Merck, Dramstadt, Germany) with a mixture of hexane, acetone and diethyl ether (in various ratios) as eluents. GC analysis was carried out on an Agilent Technologies 6890N (Varian, Agilent Technologies, Santa Clara, CA, USA) instrument using a DB-17 column (cross-linked methyl silicone gum, 30 m × 0.32 mm × 0.25 μm) or on a Varian CP3380 (Varian, Agilent Technologies, Santa Clara, CA, USA) instrument using the Thermo TR-5 (30 m × 0.32 mm × 1.0 μm) capillary columns:. The molar masses of the obtained compounds were confirmed by a high resolution mass spectrometry analysis using a Waters GCT Premier instrument (*ESI* ionization) (Waters Division, Milford, Massachusetts). An enantiomeric excess of the products obtained during biotransformation was 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) 220 °C, column temperature: 140 °C (hold 45 min), 140–200 °C (rate 20 °C/min), 200 °C (hold 1 min). Optical rotations were determined on a P-2000 polarimeter (Jasco Easton, PA, USA) in chloroform solutions, whose concentrations are denoted in g/100 mL. The melting points were determined on a Boetius apparatus. The refractive index was measured on a Carl Zeiss Abbe and Pulfrich refractometer (Jena, Germany).

NMR spectra were recorded in $CDCl_3$ solution on a Bruker Avance DRX 300 MHz spectrometer (Bruker, Billerica, MA, USA) or on a Bruker Avance 600 MHz spectrometer (Bruker, Billerica, MA, USA). IR spectra were recorded on a Thermo-Nicolet IR 300 FT-IR spectrometer (Waltham, MA, USA).

2.1.1. Synthesis

The commercially available ketone, isophorone (**1**), was purchased from Fluka. The known allyl alcohol **2** was obtained according to the method described by Magnusson and Thoren [28]. All of the subsequent products were obtained according to procedures that are described below:

*2.1.1.1. General procedure for the synthesis of ethyl esters (**3a-b**).* Ester **3a** (1.14 g, 5.42 mmol, yield 95%) was obtained as a product of the Claisen rearrangement with orthoacetate modification [29] and ester **3b** (two diastereoisomers) (1.44 g, 6.44 mmol, yield 80%) was obtained in the same manner as the first one, but with orthopropionate modification characterized by the data given below.

*2.1.1.1.1. Ethyl (3,5,5-trimethylcyclohex-2-en-1-yl)acetate (**3a**).* **3a**: n_D = 1.4618; IR (KBr): 2954, 1736, 1456, 1368, 1269, 1180, 1033 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.87 (s, 3H, CH_3-10), 0.95 (s, 3H, CH_3-11), 1.26 (t, J = 7.1 Hz, 3H, CH_3-13), 1.52 (m, 2H, CH_2-6), 1.58 (d, J = 17.3 Hz, 1H, one of CH_2-4), 1.63 (s, 3H, CH_3-9), 1.80 (dm, J = 17.3 Hz, 1H, one of CH_2-4), 2.23 (dd, J = 9.6 and 9.6 Hz, 2H, CH_2-7), 2.60 (m, 1H, H-1), 4.14 (q, J = 7.1 Hz, 2H, CH_2-12), 5.20 (s, 1H, H-2) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): 14.33 (C-13), 23.90 (C-9), 25.36 (C-10), 29.98 (C-5), 31.12 (C-3), 31.84 (C-11), 41.28 (C-7), 42.31 (C-4), 44.08 (C-6), 60.22 (C-12), 122.77 (C-2), 133.95 (C-1), 173.10 (C-8) ppm; *ESIHRMS*: calcd for $C_{13}H_{22}O_2$, m/z 211.1692 $(M+H)^+$, found 211.1698.

*2.1.1.2. Ethyl 2-(3,5,5-trimethylcyclohex-2-en-1-yl)propanoate (**3b**).* **3b**: n_D = 1.4542; IR (KBr): 2956, 1735, 1458, 1365, 1250, 1187, 1075 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3-10), 0.95 (s, 3H, CH_3-11), 1.00 (m, 1H, one of CH_2-6), 1.08 (d, J = 6.9 Hz, 3H, CH_3-12), 1.26 (t, J = 7.1 Hz, 3H, CH_3-14), 1.28 (m, 1H, one of CH_2-6), 1.49 (m, 1H, one of CH_2-4), 1.63 (s, 3H, CH_3-9), 1.81 (m, 1H, one of CH_2-4), 2.32 (m, 1H, CH_2-7), 2.42 (m, 1H, H-1), 4.15 (q, J = 7.1 Hz, 2H, CH_2-13), 5.12 (s, 1H, H-2) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): 13.50 (C-12), 14.35 (C-14), 24.04 (C-9), 25.26 (C-10), 29.90 (C-5), 31.98 (C-11), 36.77 (C-4),

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