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New clerodane diterpenes from fungal biotransformation of the 3,12-dioxo-15,16-epoxy-4-hydroxycleroda-13(16),14-diene

Abstract

The biotransformation of clerodane diterpene 3,12-dioxo-15,16-epoxy-4-hydroxycleroda-13(16),14-diene (1) by the endophytic fungi Lasiodiplodia gonubiensis, Neofusicoccum ribis and Pseudofusicoccum stromaticum provided four different hydroxylated diterpenes. The biotransformation performed by L. gonubiensis yielded two compounds formed through reduction of the carbonyl group at C-3 and hydroxylation at C-7, respectively. N. ribis produced the same compounds of L. gonubiensis as well as one with both the carbonyl reduction at C-3 and hydroxylation at C-7. P. stromaticum also produced the same compounds of L. gonubiensis in addition to the C-6 hydroxylated derivative. Among these compounds, three diterpenes are being described for the first time in literature. Additionally, two new chemical derivatives were prepared by esterification and benzylation reactions from one of the new biotransformed diterpene.

Keywords: biotransformation, endophytic fungi, clerodane diterpene, lasiodiplodia gonubiensis, neofusicoccum ribis, pseudofusicoccum stromaticum

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Abbreviations: PDA, potato-dextrose agar; DMSO, dimethyl sulfoxide; HPLC-DAD, high performance liquid chromatography--diode array detector; ODS, octadecylsilane; FTIR, fourier transform infrared spectroscopy; HRMS, high-resolution mass spectrometer; ^{1}HNMR, proton nuclear magnetic resonance; ^{13}CNMR, carbon nuclear magnetic resonance; NOESY, nuclear overhauser effect spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond correlation

Introduction

Terpenes, with approximately 30,000 compounds, are considered one of the most important classes of natural products isolated from plants. They have high economic value and have applications in several areas such as pharmaceutical and cosmetic industries. Among the terpenes, diterpenes are notable by exhibiting anti-microbial, insecticidal, anti-carcinogenic, anti-diabetic and neurobiological activities.[1–3] Microbial transformations of diterpenes have been reported as an alternative tool to furnish new derivatives.[4] Advantages of using this kind of enzyme transformation include high level of regio-and stereo-selectivity, require mild reaction conditions and are important steps to introduce functional groups into inaccessible sites of the molecules, producing rare structures.[3–6]

The clerodane diterpene (3R, 4S, 5S, 8S, 9R, 10S)-3,12-dioxo-15,16-epoxy-4-hydroxycleroda-13(16),14-diene (1) was isolated for the first time from Croton argyrophylloides (Euphorbiaceae). This compound was biotransformed by Cunninghamella echinulata and Rhizopus stolonifer fungi and produced a new diterpene, as previously described by Monte et al.[7] and Mafezoli et al.[8]

In this work, we report the isolation of one known (B1) and three new diterpenes (B2, B3 and B4) obtained from biotransformation of diterpene 1 by a fungal strain of Lasiodiplodia gonubiensis, Neofusicoccumum ribis and Pseudofusicoccum stromaticum. Additionally, two new chemical derivatives (Q1 and Q2) obtained by esterification and benzylation of B2. The structures of the diterpenes were established mainly based on their 1D and 2D NMR spectroscopic data and HRMS.

Materials and methods

General procedure

Melting points were determined on a Micro-Quimica MQAPF-302 and Mettler Toledo FP62 apparatus, and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Optical rotations were determined on Jasco P-2000 equipment. NMR spectra were recorded on Bruker Avance DPX 300 (300MHz) and Avance DPX 500 (500MHz) spectrometers. High-resolution MS were obtained on a Shimadzu LC-MS IT-TOF spectrometer equipped with an ESI source in positive and negative modes. Analytical thin-layer chromatography (TLC) was performed on pre-coated 0.25mm thick plates of silica gel 60 F254, and the spots were visualized under a UV lamp (254nm) and by spraying with a solution of perchloric acid-vanillin in EtOH, followed by heating. HPLC analyses were done on a Shimadzu instrument equipped with a LC-20AT high-pressure pump, a SPD-M20A photodiode array detector. Potato-dextrose-broth was purchased from HIMEDIA™ and all other chemical compounds were from Vetec™ and Synth™.

Substrate

The compound (3R,4S,5S,8S,9R,10S)-3,12-dioxo-15,16-epoxy-4-hydroxycleroda-13(16),14-diene (1) used as substrate in the biotransformation was isolated, as previously reported, from the n-hexane extract of the roots of Croton micans var. argyroglossum in about 4,0% yield.[8]

Fungal strains

Three strains of endophytic fungi were isolated from plants collected in Caatinga biome (Ceará, Brazil), and are deposited in the Laboratory of Phytopathology at Embrapa Tropical Agro-business (CNPAT, Fortaleza, Ceará, Brazil). The strains were identified by molecular analysis (DNA sequencing of the regions ITS1/ITS4) as: Lasiodiplodia gonubiensis (strain 474), Neofusicoccum ribis (strain 683), and Pseudofusicoccum stromaticum (strain 477).

Fungus cultivation

All strains were separately inoculated in Petri dishes containing PDA medium, and incubated for 7 days at 28°C in order to ensure that all of them were of the same age. Then, one pellet (diameter 6mm) of the strain was transferred to 250mL Erlenmeyer flasks each containing 100mL of potato-dextrose (24g/L) broth previously autoclaved at 120°C for 15min. After 7days of cultivation (150rpm and 28°C), the substrate (10mg) dissolved in DMSO (200µL) was added to the flasks and maintained in culture for 7 more days. One flask was used as control (no substrate was added).

Biotransformation products

After 7 days of cultivation, the mycelium was separated by filtration. The filtrate was extracted with EtOAc (3x50mL), and the organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated. The extract was dissolved in acetonitrile/methanol mixture (1:1) and partitioned with hexane (3x50mL) to remove the fats. The defatting extract was analyzed by semi-preparative HPLC-DAD as follows: 200µL as injection volume, Phenomenex™ ODS column (250mmx10mm, 100Å), eluent CH_3CN/H_2O (4:6) and flow rate 3.5mL/min, resulting in the isolation of compounds B1(20.2min), B2(7.5min), B3(4.5min), and B4(18.2 min).

(3R,4S,5S,8S,9R,10S)-3,4-dihydroxy-15,16-epoxy-12-oxocleroda-13(16),14-diene (B1)

The product B1 was the same compound obtained as biotransformation product by the fungi C. echinulate and R. stolonifer in previous work.[8]

(4S,5S,7R,8R,9S,10S)-4,7- dihydroxy-15,16-epoxy-3,-12-dioxocleroda-13(16),14-diene (B2)

White powder; $[\alpha]_D^{27}$ =-7.86 (c=0.1, $CDCl_3/CH_3OH$ 1:1); IR: 3648, 3132, 2934, 2698, 1708, 1601, 1158cm^{-1}; ^{1}HNMR (500MHz, $CDCl_3$) δ: 8.04 (1H, s, H-16), 7.46 (1H, s, H-15), 6.77 (1H, s, H-14), 3.54 (1H, ddd, J=10.9, 10.9 and 3.6Hz, H-7), 2.89 (1H, d, J=16.1Hz; H-11a), 2.84 (1H, dd, J=15.6 and 3.0Hz; H-10), 2.69 (1H, d, J=16.1Hz; H-11b), 2.56 (1H, m, H-2a), 2.45 (1H, dd, J=17.6 and 3.3Hz, H-2b), 2.03 (1H, m, H-1a), 1.88 (1H, dd, J=16.0 and 3.7Hz, H-6a), 1.79 (1H, m, H-8), 1.73 (1H, dd, J=18.1 and 5.1Hz, H-1b), 1.59 (1H, m, H-6b), 1.41 (3H, s, H-18), 1.09 (3H, d, J=6.6Hz, H-17), 0.89 (6H, s, H-19 and 20); ^{13}CNMR (125MHz, $CDCl_3$) δ: 215.7 (C-3), 197.4 (C-12), 149.9 (C-16), 146.1 (C-15), 131.0 (C-13), 109.4 (C-14), 83.0 (C-4), 70.1 (C-7), 48.3 (C-11), 46.7 (C-5), 45.9 (C-8), 44.0 (C-9), 42.7 (C-10), 41.7 (C-6), 37.6 (C-2), 24.6 (C-1), 22.2 (C-18), 19.2 (C-20), 16.7 (C-19), 12.2 (C-17); HRMS: m/z $[M+Na]^+$ calcd for $C_{20}H_{28}NaO_5$: m/z 371.1834; found: 371.1829.

(3R,4S,5S,7R,8S,9R,10S)-3,4,7-trihydroxy-15,16-epoxy-12-oxocleroda-13(16),14-diene (B3)

White powder; $[\alpha]_D^{27}$ =+12.80 (c=0.1, $CDCl_3/CH_3OH$ 1:1); IR: 3430, 2980, 1570cm^{-1}; ^{1}HNMR (300MHz, $CDCl_3$) δ: 8.00 (1H, s, H-16), 7.40 (1H, s, H-15), 6.73 (1H, s, H-14), 3.77 (1H, dd, J=12.0 and 4.8Hz, H-3), 3.54 (1H, ddd, J=10.9, 10.6 and 3.8Hz; H-7), 2.98 (1H, s, H-11a), 2.73 (1H, s, H-11b), 2.18 (1H, dd, J=12.3 and 3.0Hz, H-10), 1.88 (1H, m, H-2a), 1.85 (1H, dd, J=12.0 and 3.7Hz, H-6a), 1.79 (1H, m, H-8), 1.45 (2H, m, H-1), 1.44 (1H, m, H-2b), 1.38 (1H, dl, J=13.0Hz, H-6b), 1.14 (3H, s, H-18), 1.06 (3H, d, J=6.8Hz, H-17), 1.03 (3H, s, H-19), 0.85 (3H, s, H-20); ^{13}CNMR (75MHz, $CDCl_3$) δ: 194.8 (C-12), 147.0 (C-16), 144.5 (C-15), 129.6 (C-13), 108.8 (C-14), 79.0 (C-4), 72.2 (C-3), 70.0 (C-7), 47.4 (C-11), 44.9 (C-8), 43.1 (C-5), 42.8 (C-9), 42.0 (C-10), 41.4 (C-6), 30.2 (C-2), 21.4 (C-1), 18.8 (C-20), 16.1 (C-19), 16.0 (C-18), 11.8 (C-17); HRMS: m/z $[M+Na]^+$ calcd for $C_{20}H_{30}NaO_5$: m/z 373.1985; found: 373.1984.

(4S,5R,6R,8S,9R,10S)-4,6-dihydroxy-15,16-epoxy-3,-12-dioxocleroda-13(16),14-diene (B4)

White powder; $[\alpha]_D^{27}$ = -57.13 (c=0.1, $CDCl_3/CH_3OH$ 1:1); IR: 3430, 2980, 1570cm^{-1}; ^{1}HNMR (300MHz, $CDCl_3$) δ: 8.03 (1H, s, H-16), 7.46 (1H, s, H-15), 6.77 (1H, s, H-14), 4.08 (1H, dd, J=11.3 and 4.1Hz, H-6), 2.85 (1H, d, J=16.3Hz; H-11a), 2.80 (1H, dd, J=13.2 and 3.0Hz, H-10), 2.68 (1H, d, J=16.3Hz; H-11b), 2.55 (1H, ddl, J=13.8 and 7.4Hz, H-2a), 2.46 (1H, ddl, J=14.3 and 3.6Hz, H-2b), 2.03 (2H, m, H-8 and H-1b), 1.85 (1H, dd, J=12.9 and 4.4Hz, H-1a), 1.60 (1H, dl, J=13.4, H-7a), 1.52 (3H, s, H-18), 1.48 (1H, m, H-7b), 0.93 (3H, d, J=6.8Hz, H-17), 0.86 (3H, s, H-19), 0.85 (3H, s, H-20); ^{13}CNMR (75MHz, $CDCl_3$) δ: 213.5 (C-3), 196.8 (C-12), 147.1 (C-16), 144.7 (C-15), 129.6 (C-13), 108.8 (C-14), 83.0 (C-4), 71.9 (C-6), 48.9 (C-5), 46.8 (C-11), 41.2 (C-10), 42.0 (C-9), 35.8 (C-2), 35.3 (C-8), 34.4 (C-7), 23.5 (C-1), 22.0 (C-18), 17.8 (C-20), 16.3 (C-17), 9.4 (C-19); HRMS: m/z $[M+Na]^+$ calcd for $C_{20}H_{28}NaO_5$: m/z 371.1829; found: 371.1834.

Chemical derivatives

To a magnetically stirred solution of B2 (0.0143mmol, 5mg) in CH_2Cl_2 (143µL) triethylamine (0.0715mmol, 6µL) was added. After 10min., 11µL (0.143mmol) of the corresponding propionyl chloride was added. The mixture was stirred at room temperature for 3h, at which point no remaining starting material could be observed by TLC. Then, the solvent was evaporated under reduced pressure and the product was purified by flash chromatography on silica gel, using hexane/ethyl acetate (9:10) mixture as eluent. The product Q1 was obtained in 53.8% yield.

To a magnetically stirred solution of B2 (0.0143mmol, 5mg) and K_2CO_3 (0.0143mmol, 1.97mg) in acetone (44.7µL) benzyl chloride (0.0143mmol, 1.6µL) was added and benzyltriethylammonium chloride (0.00143mmol, 0.15mg). The reaction was refluxed for 36 hours. Thereafter, 20mL of saturated NaCl solution was added and the mixture was extracted with EtOAc (3x50mL), and the organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated. The product Q2 was purified by flash chromatography on silica gel, using hexane/ethyl acetate (8:2) as eluent. The product was obtained in 57.3% yield.

(4S,5S,7R,8R,9S,10S)-7-propionyloxy-4-hydroxy-15,-16-epoxy-3,12-dioxocleroda-13(16),14-diene (Q1)

White powder; $[\alpha]_D^{27}$ =+96.2 (c=0.1, $CDCl_3/CH_3OH$ 1:1); IR: 3476, 2974, 1713, 1667, 1186, 1156cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.04 (1H, s, H-16), 7.46 (1H, s, H-15), 6.78 (1H, s, H-14), 4.89 (1H, ddd, J=11.2, 11.2 and 4.0Hz, H-7), 2.89 (1H, dd, J=12.6 and 3.0Hz,

H-10), 2.88 (1H, d, J=16.4Hz; H-11a), 2.74 (1H, d, J=16.4Hz, H-11b), 2.52 (1H, m, H-2b), 2.46 (1H, dd, J=5.8 and 2.4Hz, H-2a), 2.30 (2H, q, J=7.6Hz, H-2'), 2.14 (1H, dq, J=11.0 and 6.6Hz, H-8), 2.0 (1H, m, H-1a), 1.85 (1H, dd, J=12.1 and 4.0Hz, H-6a), 1.75 (1H, dd, J=12.6 and 5.3Hz, H-1b), 1.66 (1H, m, H-6b), 1.40 (3H, s, H-18), 1.13 (3H, t, J=7.6Hz, H-3'), 0.93 (6H, s, H-19 and H-20), 0.91 (3H, d, J=5.5Hz, H-17); ^{13}CNMR (75MHz, $CDCl_3$) δ: 214.4 (C-3), 194.5 (C-12), 174.1 (C-1'), 147.0 (C-16), 144.6 (C-15), 129.5 (C-13), 108.8 (C-14), 81.4 (C-4), 72.2 (C-7), 47.1 (C-11), 45.6 (C-5), 42.7 (C-9), 41.8 (C-8), 41.0 (C-10), 37.3 (C-6), 36.1 (C-2), 28.1 (C-2'), 23.5 (C-1), 22.0 (C-18), 19.0 (C-20), 15.9 (C-19), 11.6 (C-17), 9.41 (C-3'); HRMS: m/z $[M+Na]^+$ calcd for $C_{23}H_{32}O_6Na$: m/z 427.2099; found: 427.2095.

(4S,5S,7R,8R,9S,10S)-7-benzyloxy-4-hydroxy-15,16-epoxy-3,12-dioxocleroda-13(16),14-diene (Q2)

White powder; $[\alpha]_D^{27}$ =-18.7 (c=0.1, $CDCl_3$/CH_3OH 1:1); IR: 3472, 2973, 1737, 1713, 1265cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ: 8.03 (1H, s, H-16), 7.46 (1H, s, H-15), 7.37 (5H, s, H-3', 4', 5', 6' and 7'), 6.76 (1H, s, H-14), 5.14 (2H, s, H-1'), 4.68 (1H, ddd, J=11.3, 11.3 and 3.9Hz, H-7), 2.88 (1H, d, J=16.1Hz, H-11a), 2.86 (1H, dd, J=12.1 and 3.6Hz, H-10), 2.70 (1H, d, J=16.1Hz, H-11b), 2.55 (1H, m, H-2a), 2.45 (1H, m, H-2b), 2.09 (1H, m, H-8), 2.01 (1H, m, H-1a), 1.98 (1H, dd, J=12.3 and 3.9Hz, H-6a), 1.77 (1H, m, H-1b), 1.71 (1H, m, H-6b), 1.40 (3H, s, H-18), 0.98 (3H, d, J=6.6Hz, H-17), 0.93 (3H, s, H-20), 0.92 (3H, s, H-19); ^{13}CNMR (75 MHz, $CDCl_3$) δ: 214.3 (C-3), 194.3 (C-12), 147.1 (C-16), 144.7 (C-15), 135.6 (C-2'), 129.5 (C-13), 128.7 (C-4' and C-6'), 128.6 (C-5'), 128.3 (C-3' and C-7'), 108.8 (C-14), 81.3 (C-4), 77.4 (C-7), 69.7 (C-1'), 47.0 (C-11), 45.6 (C-5), 42.7 (C-9), 41.8 (C-8), 40.9 (C-10), 37.1 (C-6), 36.1 (C-2), 23.5 (C-1), 21.9 (C-18), 19.0 (C-19), 15.8 (C-20), 11.6 (C-17); HRMS: m/z $[M+Na]^+$ calcd for $C_{20}H_{28}O_5Na$ – $[C_7H_7]$: m/z 371.1829; found: 371.1834.

Results and discussion

The natural product (3R,4S,5S,8S,9R,10S)-3,12-dioxo-15,16-epoxy-4-hydroxycleroda-13(16),14-diene (1) was previously biotransformed by C. echinulata var. elegans and R. stolonifer and the absolute configuration of the biotransformation product B1 was defined as depicted in Figure 1.[8] In this work, we used the whole cells of three fungi, L. gonubiensis, N. ribis and P. stromaticum, to biotransform 1. By L. gonubiensis it was possible to obtain the diterpenes B1 (1.0%) and B2 (1.6%); by N. ribis the diterpenes B1 (12.9%), B2 (4.9%) and B3 (8.1%) were obtained, and using P. stromaticum it was possible to obtain the diterpenes B1 (2.4%), B2 (6.1%) and B4 (1.9%). Chemical derivatization of B2 yielded Q1 and Q2. Among the five derivatives obtained (Figure 1), compounds B2-B4 and Q1-Q2 are being reported for the first time.

Biotransformation products B1 and B2 were obtained from all fungal strains. The ^{13}CNMR spectrum of B2 was very similar to that of the starting material 1. Unlike B1, in B2 the carbonyl group at C-3 was not reduced (δ215.7). Comparison of ^{13}CNMR spectra of substrate 1 and B2 evidenced the C-7 hydroxylation by the appearance of the signal at δ70.1 (CH) and the lack of the signal at δ26.8 (CH_2) in B2. This hydroxylation was corroborated by the appearance of the signal at δ3.54 (1H, ddd, J=10.9, 10.9 and 3.6Hz) on the ^{1}HNMR spectrum of B2. The hydroxylation at the C-7 position was confirmed based on the long-range correlations observed in the HMBC spectrum where the carbon at δ70.1 showed correlations with hydrogens at δ1.88 (1H, H-6a), 1.79 (1H, H-8), 1.59 (1H, H-6b) and 1.09 (3H, H-17).

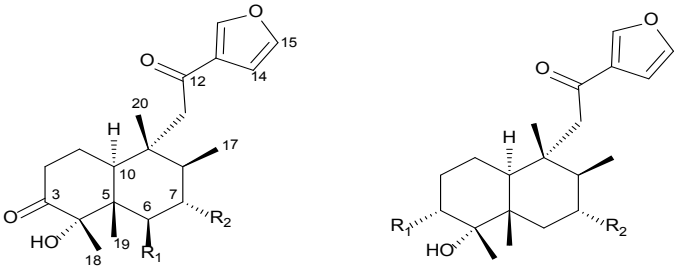
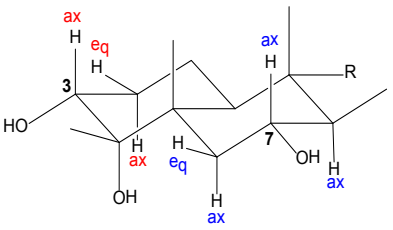


Figure 1 Chemical structures of the fungal and chemical derivatives of the clerodane diterpene 1.

The α-orientation of hydroxyl group was established by the NOESY experiment through nOe cross-peaks observed between the carbinol methine at δ3.54 (H-7) and the methyl groups at δ0.89 (C-19 and C-20). The data above established the structure of compound B2 as the new (4S,5S,7R,8R,9S,10S)-4,7-dihydroxy-15,16-epoxy-3,12-dioxocleroda-13(16),14-diene, and its molecular formula $C_{20}H_{28}O_5$ was confirmed by HRMS.

Compound B3 was isolated from *N. ribis* culture. The ^{13}CNMR spectrum of B3 showed the lack of the peak at δ215.7 and the presence of one at δ72.2 in the spectrum confirmed that compound 1 was regioselectively bioreduced at C-3. In addition, the appearance of the signal at δ70.0 suggested the C-7 hydroxylation like B2. The hydroxylation position at C-7 was confirmed through the correlation signal of methyl group at δ1.06 (3H, d, J=6.8Hz, H-17) with the carbon at δ70.0 on the HMBC spectrum. In the 1H NMR spectrum, the new carbinol methine groups were confirmed by signals at δ3.77 (1H, dd, J=12.0 and 4.8Hz, H-3) and 3.54 (1H, ddd, J=10.9, 10.6 and 3.8Hz; H-7) as well as by their correlations at HSQC spectrum. The β orientation of the hydrogens at C-3 and C-7 was defined by analysis of their coupling constant (Figure 2). The new compound B3 was named (3R,4S,5S,7R,8S,9R,10S)-3,4,7-trihydroxy-15,16-epoxy-12-oxocleroda-13(16),14-diene, which is in agreement with the molecular formula C_20H_30O5 determined by HRMS analysis.



H-3, dd, *J*=12.0 and 4.8 Hz
H-7, ddd, *J*=10.9, 10.6 and 3.8 Hz

Figure 2 Analysis of the coupling constant of H-3 and H-7 in B3.

The biotransformation product B4 was obtained only in the P. stromaticum culture. The ^{13}CNMR spectrum of B4 showed no reduction of carbonyl group at δ213.5 (C-3) and the appearance of carbinol methine group at δ71.8 (C-6). The position of hydroxylation in B4 was determined through the correlation of the signal of methyl group at δ0.86 (H-19) with carbons at δ83.0 (C-4), 71.9 (C-6) and 48.9 (C-5) observed on HMBC spectrum. The β orientation of hydroxyl group was based on the coupling constant values of hydrogen at δ4.08 (dd, J=11.3 and 4.1Hz, H-6), which are justified by axial-axial and axial-equatorial couplings. B4 is new compound

named (4S,5R,6R,8S,9R,10S)-4,6-dihydroxy-15,16-epoxy-3,12-dioxocleroda-13(16),14-diene, which is in agreement with the molecular formula $C_{20}H_{28}O_5$ determined by HRMS analysis.

The chemical derivative Q1 was obtained from B2 in 53.8% yield. The esterification of the hydroxyl group to incorporate the propanoyl group was confirmed by its ^{1}HNMR spectrum through the signals at δ 2.30 (2H, q, J=7.6Hz, H-2') and δ1.13 (3H, t, J=7.6Hz, H-3'), as well as the signals at δ174.1 (C-1'), 28.1 (C-2') and 9.41 (C-3') on the 13C NMR spectrum, and confirmed by HRMS ($C_{23}H_{32}O_6$). The new compound Q1 was named (4S,5S,7R,8R,9S,10S)-7-propionyloxy-4-hydroxy-15,16-epoxy-3,12-dioxocleroda-13(16),14-diene.

The chemical derivative Q2 was obtained from B2 in 57.3% yield. The HRMS of Q2 was in agreement with the molecular formula $C_{27}H_{34}O_5$, indicating the incorporation of benzyl group in the structure. The presence of benzyl group was stablished by its ^{1}HNMR spectrum through the signals at δ7.37 (5H, s, H-3', 4', 5', 6' and 7') and 5.14 (2H, s, H-1'), as well as the signals at δ135.6 (C-2'), 128.7 (C-4' and C-6'), 128.6 (C-5'), 128.3 (C-3' and C-7') and 69.7 (C-1') on the ^{13}CNMR spectrum, and the correlation observed between these signals at HSQC spectrum. The new derivative Q2 was named (4S,5S,7R,8R,9S,10S)-7-benzyloxy-4-hydroxy-15,16-epoxy-3,12-dioxocleroda-13(16),14-diene.

Conclusion

In summary, the clerodane diterpene 3,12-dioxo-15,16-epoxy-4-hydroxy-cleroda-13(16),14-diene (1) was stereo and regioselectively bioreduced and hydroxylated by whole-cells of L. gonubiensis, N. ribis and P. stromaticum. Four biotransformed products (B1-B4) and two chemical derivatives (Q1-Q2) were produced. Among all the derivatives obtained, only compound B1 has been previously reported in the literature. These results suggest the potential application of L. gonubiensis, N. ribis and P. stromaticum for the structural functionalization of clerodane diterpenes.

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Conflict of interest

The author declares no conflict of interest.

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