Four new β-lactones from the endophytic Streptomyces sp. T1B1

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ABSTRACT

The detailed investigation of endophytic Streptomyces sp. T1B1 was performed during a search for new structural and active compounds. The strain T1B1 was isolated from the old bast tissue of Taxus yunnanensis and determined to be a member of Streptomyces, according to the 16S rRNA analysis. The extracts from the PDA solid fermentation media of Streptomyces sp. T1B1 were purified and four β-lactones were isolated. They were identified as 4α-(3,5-dihydroxy-hexyl)-3α-methyl-2-oxetanone (1), 4α-(3-methyl-4-formyloxy-hexyl)-3α-methyl-2-oxetanone (2), 4α-(3,5-dihydroxy-heptyl)-3α-methyl-2-oxetanone (3) and 4α-(3-methyl-4-formyloxy-heptyl)-3α-methyl-2-oxetanone (4) on the basis of spectral data.

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1. Introduction

Endophytes occurring in higher plants were relatively unstudied as potential sources of novel natural products for exploitation in medicine (Strobel, 2003), e.g. a novel taxol-producing endophytic fungus was discovered in Taxus brevifolia (Stierle et al., 1993). The products of endophytic microbes and their foreground for use in medicine, agriculture and industry have been discussed (Gutiérrez et al., 2012). The increasing number of new compounds recently discovered in endophytes demonstrated that their potential for producing many more unknown natural products, most of which are still unexploited for their potential applications.

Taxus yunnanensis Loes. (Taxaceae), which mostly distributed in southwest of China, is well recognized for producing anticancer compound taxoids (Zhang et al., 1995). In our experiments for searching new structural and active compounds from the endophytic microorganisms, a series of novel compounds were obtained (Zhao et al., 2005, 2007; Yang et al., 2012). During an ongoing search for new bioactive metabolites from plant endophytic microorganisms, an isolate of Streptomyces sp. T1B1, obtained from the old bast tissue of T. yunnanensis was investigated. Herein, we describe the isolation and structural elucidation of four new β-lactones from T1B1 (Fig. 1).

2. Results and discussion

Compound 1 was obtained as colorless oil. The HR-ESI-MS data indicated a molecular formula of C16H16O4 based on the [M]+ ion signal at m/z 202.1218 ([M]+, calc. 202.1205). The NMR data (Table 1) revealed one quaternary carbon at δC 178.1, four methines at δC 81.1 (δH 4.00), 77.1 (δH 4.19), 65.1 (δH 4.06) and 45.4 (δH 2.51), and four methylenes and two methyls, which suggested compound 1 was β-lactone (Zhao and Romo, 1997; Romo et al., 1998). According to the NMR and MS spectra, compound 1 had two more hydroxyls than 4-hexyl-3-methyloxetan-2-one (Romo et al., 1998). The HMBC experiment (Table 1) showed correlations between H-10 (δH 1.14) and the carbons at δC 178.1 (C-1), 81.1 (C-3), and 45.4 (C-2), and between H-2 (δH 2.51) and the carbons at δC 178.1 (C-1), 81.1 (C-3), 29.0 (C-4) and 13.7 (C-10), together with correlations between H-3 (δH 4.00) and the carbons at δC 178.1 (C-1), 13.7 (C-10) to establish the β-lactone unit. Two hydroxyls were located at C-6 and C-8 base on the correlations between H-7 (δH 4.19) and the carbons at δC 30.5 (C-5), 65.1 (C-8), between H-8 (δH 4.06) and the carbons at δC 77.1 (C-6), 22.9 (C-9), and other correlations (Table 1). ROESY experiment showed NOE interactions between H-3 and H-10 supporting the relative configurations of C-3 and C-2 (Fig. 2). Based on above data, compound 1 was elucidated to be 4α-(3,5-dihydroxy-hexyl)-3α-methyl-2-oxetanone.

Compound 2 was obtained as colorless oil. The HR-ESI-MS data indicated a molecular formula of C16H16O4 based on the [M]+ ion signal at m/z 230.1153 ([M]+, calc. 230.1154). The MS and NMR spectroscopic data of compound 2 were very similar to those of compound 1. After careful analysis of HMBC data (Table 1), the
Compound 4 was obtained as colorless oil. The HR-ESI-MS data indicated a molecular formula of C_{11}H_{16}O_{3} based on the [M]+ ion signal at m/z 244.1300 ([M]+, calc. 244.1311). The MS and NMR spectroscopic data of compound 4 were very similar to those of compound 3. After careful analysis of HMBC data (Table 2), the results showed that there was one formyloxy group (δ_C 161.2 (δ_H 8.09)) in compound 4 instead of one hydroxyl in compound 3 at C-8: the H-12 (δ_H 8.09) was correlated with the carbon at δ_C 73.7 (C-8). ROESY experiment showed NOE interactions between H-3 and H-10 supporting the relative configurations of C-3 and C-2 (Fig. 2). Based on above data, compound 4 was elucidated to be 4α-(3-methyl-4-formyloxy-heptyl)-3α-methyl-2-oxetanone.

Compounds 1–3 did not show any inhibitory activities to all the tested cell lines at 40 μM and compounds 1–4 did not show obvious activity against the tested nematodes.

3. Experimental

3.1. General

UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer, λ_max (log ε) in nm. NMR experiments were carried out on Bruker AM-400 and Bruker DRX-500 NMR spectrometers with TMS as internal standard. ESI-MS and HR-ESI-MS were recorded on a Finnigan LCQ-Advantage mass spectrometer and a VG Auto-Spec-3000 mass spectrometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Column chromatography was carried out on silica
gel (G, 200–300 mesh and H, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia). Thin-layer chromatography (TLC) was performed on silica gel (Sigel G, Qingdao Marine Chemical Factory, Qingdao, China). Solvents were of the industrial purity and distilled prior to use.

3.2. Fungal material

The old bast of *T. yunnanensis* was collected at Kunming Botanic Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan, China, in August 2008. The plant materials were washed under running tap water and were sterilized successively with 75% ethanol for 1 min and 0.1% mercury perchloride for 5 min, then rinsed five times in sterile water and cut into small pieces which were incubated at 25 °C on YMG media (yeast extract 4.0 g, malt extract 10.0 g, glucose 4.0 g, agar 15.0 g, distilled water 1000 mL) and cultured until colony or mycelium appeared surrounding the segments. After culturing about one month, a strain appeared and named T1B1, which was isolated from the sterilized bast. The identification of the T1B1 by amplification of 16S rRNA was followed before method (Zhao et al., 2007). It was identified as *Streptomyces* sp. according to the 16S rRNA analysis and the 16S partial sequence of the endophyte T1B1 was registered in the GenBank database with the accession number KC295572. It was deposited at the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

3.3. Extraction and isolation

The strain was cultured in 10 L PDA medium [consisting of potato (200 g/L), dextrose (20 g/L), and agar (15 g/L)]. After cultivation for two weeks at 28 °C, the cultures were exhaustively extracted three times with AcOEt/MeOH/AcOH (80:15:5, v/v/v) to obtain 14.7 g extract. The extract was chromatographed on silica gel (G, silica gel 200–300 mesh, 150 g) and eluted with petroleum ether/acetone (10:1–7:3) to afford four fractions (PA1–PA4), and eluted with CHCl3/MeOH (20:1–8:2) to five fractions (CH1–CH5). Fraction PA4 (2.9 g) was purified on silica gel column (G, 200–300 mesh, 120 g) eluting with petroleum ether/acetone (8.2:5:5 contains 1% formic acid) to afford three fractions (PA4-1–PA4-3). PA4-3 (1.4 g) was purified on silica gel column (G, 200–300 mesh, 50 g) eluting with petroleum ether/acetone (7:3 contains 1% formic acid) to afford four fractions (PA4-3-1–PA4-3-4). PA4-3-1 (25 mg) was purified on silica gel (G, 10 g) eluting with petroleum ether/acetone (10:1 contains 1% formic acid) to obtain compound 4 (13 mg). PA4-3-2 (119 mg) was purified on silica gel 200–300 mesh (25 g) eluting with petroleum ether/acetone (9:1 contains 1% formic acid) to afford fractions (PA4-3-2–1–PA4-3-2–4). PA4-3-2-1 (10 mg) was purified on silica gel H (4 g) eluting with petroleum ether/acetone (10:1 contains 1% formic acid) to obtain compound 2 (3.6 mg). PA4-3-3 (650 mg) was purified on silica gel 200–300 mesh (50 g) eluting with petroleum ether/acetone (9:1 contains 1% formic acid) to afford five fractions (PA4-3-3–1–PA4-3-3–5). PA4-3-3-4 (120 mg) was purified on silica gel G (10 g) eluting with petroleum ether/acetone (8:2 contains 1% formic acid) to obtain compound 3 (7 mg). PA4-3-3-5 (60 mg) was purified on silica gel G (15 g) eluting with CHCl3/acetone (8:2 contains 1% formic acid) to obtain compound 1 (17 mg).

3.3.1. 4α-(3,5-Dihydroxy-hexyl)-3α-methyl-2-oxetanone (1)

Colorless oil; [α]D 25 = −7.00 (c = 0.14, MeOH); UV (MeOH) λmax (log ε): 201.3 (2.78); NMR data see Table 1; ESI-MS: 203 [M+H]+; HR-ESI-MS: 202.1218 ([M]+, calc. 202.1205).

3.3.2. 4α-(3-Methyl-4-formyloxy-hexyl)-3α-methyl-2-oxetanone (2)

Colorless oil; [α]D 25 = −21.54 (c = 0.13, MeOH); UV (MeOH) λmax (log ε): 201.8 (3.29); NMR data see Table 1; ESI-MS: 231 [M+H]+; HR-ESI-MS: 230.1153 ([M]+, calc. 230.1154).

3.3.3. 4α-(3,5-Dihydroxy-heptyl)-3α-methyl-2-oxetanone (3)

Colorless oil; [α]D 25 = −21.79 (c = 0.13, MeOH); UV (MeOH) λmax (log ε): 214.1 (2.86); NMR data see Table 2; ESI-MS: 217 [M+H]+; HR-ESI-MS: 216.1369 ([M]+, calc. 216.1362).

3.3.4. 4α-(3-Methyl-4-formyloxy-heptyl)-3α-methyl-2-oxetanone (4)

Colorless oil; [α]D 25 = −15.87 (c = 0.15, MeOH); UV (MeOH) λmax (log ε): 201.2 (2.87); NMR data see Table 2; ESI-MS: 245 [M+H]+; HR-ESI-MS: 244.1300 ([M]+, calc. 244.1311).

3.4. Assay activities

Antitumor activity was measured by the microculture tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, MTT, Sigma] assay. Five cell lines were selected for testing (leukemia cell line HL-60, hepatocarcinoma cell line SMMC-7721, lung adenocarcinoma cell line A-549, breast cancer cell line MCF-7 and colon cancer cell line SW480), and MW300 used as control. The antitumor activities of compounds (1–3) against these cell lines were assayed in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. The nematodes *Panagrellus redivivus* and *Caoenorrhadinis elegans* were cultured on oatmeal medium (20 g oatmeal in 80 mL H2O) at 25 °C for 7 d. The cultured nematodes were separated from the culture medium using the Baerman funnel technique. Test samples (compounds 1–4) were dissolved in acetone or methanol respectively, and then diluted to different concentrations with sterilized water for the assay. The method of nematicidal activity was based on before method (Li et al., 2005).

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