SHORT REPORT

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Rec. Nat. Prod. 10:5 (2016) 645-648

records of natural products

Leucasinoside: A New Abietane Diterpenoid Glycoside from *Leucas zeylanica*

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(Received November 11, 2015; Revised December 12, 2015; Accepted December 12, 2015)

Abstract: A new abietane diterpenoid glycoside, leucasinoside, along with two known ones were obtained from the aerial parts of *Leucas zeylanica*. Its structure was characterized by comprehensive analyses of ^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, NOESY spectroscopic, and HREIMS mass spectrometric data. All the isolates were evaluated for their anti-inflammatory activities on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7, and the new compound showed moderate inhibitory activity.

Keywords: *Leucas zeylanica*; abietane diterpenoid; leucasinoside; anti-inflammatory.

1. Plant Source

During the process of finding secondary metabolites with interesting chemical structures and significant biological activities from Hainan Island of China, a new abietane diterpenoid glycoside along with two known ones were isolated from the aerial parts of *Leucas zeylanica* (Figure 1).

The aerial parts of *L. zeylanica* were collected in September 2014 from Changjiang, Hainan Province, China and identified by Prof. Niankai Zeng, School of Pharmaceutical Science, Hainan Medical University. A voucher specimen (NO. LZ201409) was deposited at the herbarium of School of Pharmaceutical Science, Hainan Medical University.

2. Previous Studies

Leucas zeylanica is a medicinal herb used in treating influenza, inflammation, and mainly distributed in southern regions of China. However, there is no report concerning its chemical constituents till now.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/01/2016 EISSN: 1307-6167

3. Present Study

The air-dried and powdered aerial parts of *Leucas zeylanica* (L.) R. Brown (2.0 kg), collected from Changjiang City in Hainan province of China, were extracted twice with methanol. Removal of the methanol under reduced pressure yielded a methanol extract (220 g). The residue was dissolved in water and partitioned with petroleum ether, ethyl acetate, and n-butanol, respectively. The n-butanol fraction (85 g) was subjected to silica gel column chromatography using a CH_2Cl_2-MeOH gradient (from 1:0 to 1:1) as eluent, to yield six fractions (Fr. A-F). Fr. D (12.5 g) was further separated by Sephadex LH-20 column eluting with MeOH to afford six subfractions (Sbuf. 1-6) Then, Subf. 4 was subjected to semi-preparative HPLC with MeOH-H_2O (35:65) as eluent to give compounds **2** (5.0 mg) and **3** (8.0 mg). Subf. 5 was further separated by semi-preparative HPLC with MeOH-H_2O (40: 60) as the eluent to give compound **1** (4.0 mg).

*leucasinoside **(1)***: White amorphous solid, $[\alpha]_D^{25}$ = +12.0 (*c* = 0.1, MeOH); UV ($CHCl_3$): λ_{max} (log ε): 206 (4.42), 280 (3.66); IR ν_{max} ($CHCl_3$): = 3430, 2930, 2865, 1725, 1621, 1280, 1076 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm)= 1.07 (1H, m, H-1α), 3.32 (1H, m, H-1β), 1.47 (1H, m, H-2α), 1.98 (1H, dd, *J* = 11.2, 7.2 Hz, H-2β), 1.06 (1H, m, H-3α), 2.23 (1H, d, *J* = 10.6 Hz, H-3β), 1.48 (1H, d, *J* = 10.6 Hz, H-5), 2.14 (1H, dd, *J* = 11.2, 3.6 Hz, H-6α), 1.88 (1H, dd, *J* = 11.2, 7.2 Hz, H-6β), 2.75 (2H, m, H-7), 6.37 (1H, s, H-14), 3.67 (1H, m, H-15), 3.60 (1H, dd, *J* = 12.0, 7.2 Hz, H-16a), 3.45 (1H, dd, *J* = 12.0, 2.4 Hz, H-16b), 1.13 (3H, d, *J* = 6.6 Hz, H-17), 1.28 (3H, s, H-18), 1.27 (3H, s, H-20), 4.38 (1H, d, *J* = 7.8 Hz, H-1'), 3.41 (1H, m, H-2'), 3.27 (1H, m, H-3'), 3.28 (1H, m, H-4'), 3.43 (1H, m, H-5'), 3.89 (1H, brd, *J* = 12.0 Hz, H-6'a), 3.70 (1H, dd, *J* = 12.0, 6.0 Hz, H-6'b); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 37.5 (CH_2, C-1), 21.2 (CH_2, C-2), 39.3 (CH_2, C-3), 45.3 (C, C-4), 57.1 (CH, C-5), 22.6 (CH_2, C-6), 35.0 (CH_2, C-7), 135.8 (C, C-8), 133.7 (C, C-9), 41.4 (C, C-10), 149.6 (C, C-11), 143.5 (C, C-12), 136.7 (C, C-13), 118.5 (CH, C-14), 35.1 (CH, C-15), 69.3 (CH_2, C-16), 18.6 (CH_3, C-17), 30.0 (CH_3, C-18), 182.3 (C, C-19), 17.5 (CH_3, C-20), 107.9 (CH, C-1'), 75.8 (CH, C-2'), 79.2 (CH, C-3'), 71.7 (CH, C-4'), 78.2 (CH, C-5'), 63.2 (CH, C-6'). HRESIMS: *m/z* 533.2369 ($[M+Na]^+$, calcd. $C_{26}H_{38}O_{10}Na^+$ for 533.2363).

Assay for inhibitory ability against LPS-induced NO production: RAW264.7macrophages were seeded in 24-well plates (105 cells/well). The cells were co-incubated with drugs and LPS (1 μg/mL) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW264.7 macrophage supernatants with Griess reagent. Aliquots of supernatants (100 μL) were incubated, in-sequence, with 50 μL of 1% sulfanilamide and 50 μL of 0.1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbance was recorded on a microplate reader at a wavelength of 570 nm [1].

The dried and powdered aerial parts of *L. zeylanica* were extracted with methanol. The crude extract obtained after evaporation of the solvent was subjected to conventional purification procedures and resulting in the isolation of a new abietane diterpenoid glycoside (**1**) along with two known ones (**2**, **3**) as shown in Figure 1.

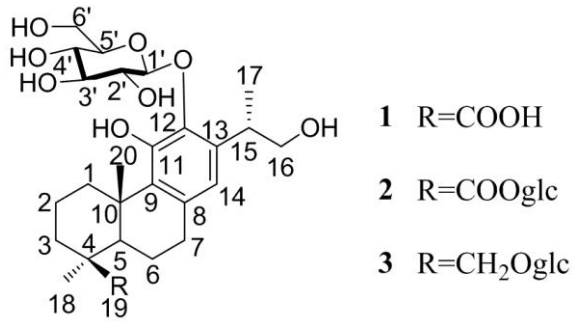

Figure 1. Structures of compounds **1-3** isolated from *L. zeylanica*.

Compound **1** was isolated as a white amorphous powder. Its molecular formula was established by the positive HRESIMS (*m*/*z* 533.2369 $[M + Na]^+$, calcd for $C_{26}H_{38}O_{10}Na$ 533.2363). Its ^1H NMR spectrum of **1** indicated the existence of three methyl group at δ = 1.13 (d, 6.6 Hz, H-17), 1.28 (s, H-18), 1.27 (s, H-20). Moreover, it showed signals owing to one methylene at δ = 3.45 (dd, 12.0, 2.4 Hz, H-16a), 3.60 (dd, 12.0, 7.2 Hz, H-16b), and one olefinic methine at δ 6.37 (s, H-14). The ^1H-NMR spectra data also exhibited signals due to a β-glucopyranosyl moiety with the anomeric proton at δ = 4.38 (d, 7.8 Hz). In addition to protonated carbon signals corresponding to the above protons, the ^{13}C NMR spectrum of **1** showed 20 carbon signals attributable to three methyls at δ = 18.6, 30.0, 17.5; six methylenes (one oxygenated) at δ = 37.5, 21.2, 39.3, 22.6, 35.0, 69.3; three methines (one olefinic) at δ_C 57.1, 118.5, 35.1; and eight quaternary carbons (five olefinic) at δ = 45.3, 135.8, 133.7, 41.4, 149.6, 143.5, 136.7, 182.3, along with six carbon signals for a sugar unit. These spectroscopic data are typical signals of a glycosidic diterpene with a tricyclic system including an aromatic ring [2, 3]. Detailed interpretation of ^1H-^1H COSY correlations from H-1 to H-2, H-2 to H-3, H-5 to H-6, H-6 to H-7, H-15 to H-16, and H-15 to H-17 indicated the existence of three isolated spin systems C-1-C-3, C-5-C-7, C-15-C-17 as depicted in Figure 2. In the HMBC spectrum, connections from H-18 (δ_H 1.28) to C-4 (δ_C 45.3), C-19 (δ_C 182.3), from H-20 (δ_H 1.27) to C-10 (δ_C 41.4), C-5 (57.1), C-9 (δ_C 133.7), from H-7 (δ_H 2.75) to C-8 (δ_C 135.8), and from H-15 (δ_H 3.67) to C-12 (δ_C 143.5), C-13 (δ_C 136.7), C-14 (δ_C 118.5), C-16 (δ_C 69.3), C-17 (δ_C 30.0) allowed the construction of 11, 16-dihydroxyabieta-8, 11, 13-triene framework in **1**. The HMBC correlations (Figure. 2) from H-1' (δ_H 4.38) to C-12 (δ_C 143.5) indicated that the β-D-glucopyranosyl unit is located at C-12. These data above were similar to those of 12-O-β-D-glucopyranosyl-3, 11, 16-trihydroxyabieta-8, 11, 13-triene, except for the methyl group was replaced by carboxyl group and the absence of C-3 hydroxyl group in **1** [4]. The relative configuration of **1** was determined on the basis of NOESY spectrum [5, 6]. The observed NOEs from H_3-18 to H-5 (δ_H 1.48), H-20 to H-2β (δ_H 3.32), H-6β (δ_H 1.88), and H-14 (δ_H 6.37) to H-7, H_3-18 allowed determination of the relative stereochemistry as depicted in Figure 1. The configuration of C-15 was tentatively determined to be S, since the NMR spectra of C-15, C-16, C-17 were identical with the referential compound. Collectively, **1** was characterized as 12-O-β-D-glucopyranosyl-11, 16-dihydroxyabieta-8, 11, 13-triene with a given name of leucasinoside. (Spectroscopic data provided in Supporting Information).

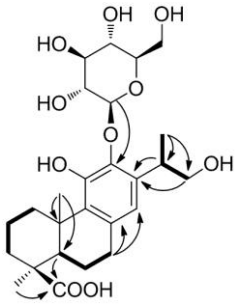

Figure 2. Important ^1H-^1H-COSY (—) and HMBC (⟶) correlations of leucasinoside (**1**).

The known compounds were identified as 19-O-β-D-carboxyglucopyranosyl-12-O-β-D-glucopyranosyl-11, 16-dihydroxyabieta-8, 11, 13-triene (**2**) [4], 12, 19-O-β-D-diglucopyranosyl-11, 16-dihydroxyabieta-8, 11, 13-triene (**3**) [7]. Both of them were obtained from this plant for the first time.

The three isolated compounds were tested on LPS-induced NO production in RAW 264.7 macrophages. Compounds **1-3** showed moderate inhibitory activities with IC_{50} values ranging from 12.6 to 18.8 μM as shown in Table 1. This result indicated that the new compound had the potent capability in curing inflammation.

Table 1. Inhibitory activity of compounds **1-3** on LPS-induced NO production.

Compounds	IC_{50} (μM)[a]
1	12.6 ± 1.2
2	18.8 ± 2.1
3	15.6 ± 1.8
Aminoguanidine[b]	1.8 ± 0.4

[a] Value present mean ± SD of triplicate experiments. [b] Positive control substance.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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