A new triterpenoid glucoside from *Leucas zeylanica*

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\section*{ABSTRACT}
A new triterpenoid glucoside, leuctriterpencoside (1), along with two known compounds (2–3) were isolated from *Leucas zeylanica*. The structure of the new compound was elucidated using comprehensive spectroscopic methods. Compound 1 showed significant inhibitory activity against \(\alpha\)-glucosidase (IC\textsubscript{50} value of 0.85 ± 0.12 \(\mu\)M).

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Leucas zeylanica; triterpenoid; \(\alpha\)-glucosidase activity

\section*{1. Introduction}
*Leucas zeylanica* belongs to *Leucas* genus of Labiatae, which grow in fields, open tidal wetlands and sandy grasslands. Local people use it as a non-toxic side effect herbal medicine. The crushed leaves of the plant can be applied to wounds, sores, especially those of the eyes and nose, chronic skin diseases, such as psoriasis and scabies. The plant is also used to treat mild fevers, colds, rheumatism and snake bites, and as a decoction against roundworm, mainly for children (Babu et al. 2016; Hossain et al. 2013; Qureshi et al. 2010). In our research for new bioactive natural compounds from the traditional medicinal plants, a new triterpenoid glucoside, leuctriterpencoside (1), along with two known compounds, (–)-epiloliolide (2) (Tianyun Jin et al. 2016), (E)-4-((1S,3R,4R)-1-hydroxy-4,5,5-trimethyl-7-oxabicyclo [4.1.0]heptan-1-yl)but-1-en-3-one (3)
(Yi Tao et al. 2012) (Figure 1) were isolated from the stems of L. zeylanica. The inhibitory activities of all compounds against seven pathogenic bacteria were evaluated. The anti-α-glucosidase activity of compound 1 was also tested.

2. Results and discussion

Compound 1 was obtained as a white amorphous powder with a molecular formula of C_{36}H_{54}O_{8} (ten degrees of unsaturation), deduced from HRESIMS (m/z 613.3733 [M - H]) and NMR data. In the 1H-NMR one aldose proton at δH 9.47 (1H, s, H-29), an anomeric proton δH 5.90 (1H, d, J = 2.0 Hz, H-1'), an olefinic proton δH 5.43 (1H, m, H-12), four oxygenated methine signals δH 3.72 (1H, dd, J = 3.2, 1.6 Hz, H-5'), 3.64 (1H, dd, J = 6.4, 1.2 Hz, H-4'), 3.62 (1H, dd, J = 3.2, 1.2 Hz, H-3'), 3.45 (1H, dd, J = 10.0, 9.6 Hz, H-2'), a sp³ methine signal δH 2.73 (1H, dd, J = 13.2, 6.0 Hz, H-18), two non-equivalent methylene protons at δH 2.58 (1H, m, H-2a) and 2.40 (1H, ddd, J = 14.0, 8.8, 4.0 Hz, H-2b), seven methyl singlets δH 1.24 (3H, d, J = 2.4 Hz, H-6'), 1.24 (3H, s, H-27), 1.08 (3H, s, H-24), 1.09 (3H, s, H-30), 1.06 (3H, s, H-23), 0.99 (3H, s, H-26), 0.85 (3H, s, H-25). The 13C-NMR and DEPT spectra exhibited 36 carbon resonances including seven methyls, ten methylenes, eight methines (including five oxygenated methines), seven quaternary carbons, two olefinic carbons and two carbonyl group carbons. These spectroscopic features suggested that 1 was comprised of one triterpenoid subunit and one rhamnopyranose subunit. The 1H- and 13C-NMR spectra of the triterpenoid subunit closely resembled those of 29-hydroxy-3-oxo-olean-12-en-28-oic acid (Begum et al. 2015; Duan et al. 2000), except for the disappearance of a hydroxymethyl group and the presence of an aldehyde group [δH 9.47(s), δC 207.6] for C-29 in 1. So the hydroxymethyl group was replaced by aldehyde group in 1. The location of this aldehyde group was confirmed to be C-29 by the HMBC correlations of H-29/C-19,20,21,30. On the other hand, the proton signal at δH 2.73 (H-18β) showed NOESY correlations with the signal at δH 9.47 (H-29). Based on the above observations and the conformation of the oleanane skeleton, a C-29 assignment of aldehyde group is proposed. The acidic hydrolysis of 1 afforded a rhamnopyranose and a triterpenoid. Based on the small coupling constant of anomeric proton at δH 5.90 (J = 2.0 Hz) and [α]^{25}_{D} +2.0 (c 0.1, MeOH), we deduced the rhamnopyranose was a α-L-rhamnopyranose. This was further confirmed by the same Rf value of an authentic sugar sample on co-TLC (Haskins et al. 1946). The location of the rhamnopyranose at C-28 was
confirmed by the HMBC correlation from H-1’ to C-28. Thus, compound 1 was identified as 29-hydroxy-3-oxo-olean-12-en-28-O-α-L-rhamnopyranosyl ester. And we named compound 1 as leuctriterpencoside.

The structures of known compounds 2-3 were identified by comparing the information of NMR and optical rotations with those from the reported studies.

All compounds were evaluated against the phytopathogenic bacterial Micrococcus tetragenus, Escherichia coli, Staphylococcus albus, Bacillus cereus, Saphylococcus aureus, Micrococcus luteus, Bacillus subtilis. However, these compounds had no inhibitory effect against all the tested bacteria.

The inhibitory activity against α-glucosidase of the compound 1 was determined. Compound 1 showed significant activity with the IC₅₀ value of 0.85 ± 0.12 μM (The IC₅₀ value of positive control acarbose was 2.35 ± 0.20 μM).

3. Experimental

3.1. General experimental procedures

IR spectra were recorded on a Nicolet 6700 spectrophotometer. UV spectra were recorded on a Beckman DU 640 spectrophotometer. Optical rotations were measured on a JASCO P-1020 digital polarimeter. NMR spectra were recorded on a Bruker AV spectrometer (400 MHz for ¹H and 100 MHz for ¹³C). TMS was used as an internal standard. HRESIMS spectra were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), octadecylsilyl silica gel (YMC; 12 nm–50 μm) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography (TLC). Semi-Preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5 μm).

3.2. Plant material

The stems of Leucas zeylanica were collected from Haikou, Hainan Province, China, in July 2016, and identified by Prof. Qiong-Xin Zhong, College of Life Science, Hainan Normal University. A voucher specimen (No. FOC20170115) has been deposited at the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

3.3. Extraction and isolation

The air-dried and powdered (4.0 kg) L. zeylanica were extracted three times with 75% EtOH (30 L) at room temperature (3 × 3 d) and filtered. The filtrate was evaporated under reduced pressure using a rotavapor to obtain the EtOH extract (351 g), which was suspended in distilled H₂O (2 L) and successively solvent partitioned with petroleum ether (PE) and EtOAc, yielding 120 g, 90 g, and aqueous layer (150 g). The PE extract (120g) was subjected to silica gel column chromatography (CC) eluting first with PE. The polarity was gradually increased with EtOAc. Each fraction (200 mL) was
analysed by TLC. Fractions with similar TLC patterns were combined to give five fractions (Frs. 1–5). Frs. 3 (12 g) was applied to silica gel CC eluted with PE-EtOAc (from 20:1 to 1:1) to afford three subfractions (3a–3c). Subfraction 3c was further purified by using Semi-Preparative HPLC (80% MeOH/H₂O) to obtain 1 as a white amorphous powder (31 mg) and 2 as a white amorphous solid (9 mg). Subfractions 3b was further separated by Semi-Preparative HPLC (CH₃CN/H₂O, 60:40 v/v) to obtain 3 as yellow oil (8 mg).

3.4. Physical properties of compound 1

White amorphous powder; [α]D²⁵ +38.0 (c 0.1, MeOH); UV (MeOH) λmax (log ε) 207 (4.2) nm; IR (KBr) νmax⁻¹ 3419, 2928, 1707, 1692, 1646, 1563 and 1547 cm⁻¹; HR-ESI-MS m/z 613.3733 [M - H]⁻ (C₃₆H₅₃O₈; calcd 613.3740); ¹H-NMR (MeOD-d₄, 400 MHz) δ: 9.47 (1 H, s, H-29), 5.43 (1 H, m, H-12), 5.90 (1 H, d, J = 2.0 Hz, H-1'), 3.72 (1 H, dd, J = 3.2, 1.6 Hz, H-5'), 3.64 (1 H, dd, J = 6.4, 1.2 Hz, H-4'), 3.62 (1 H, dd, J = 3.2, 1.2 Hz, H-3'), 3.45 (1 H, dd, J = 10.0, 9.6 Hz, H-2'), 2.73 (1 H, dd, J = 13.2, 6.0 Hz, H-18), 2.58 (1 H, m, H-2), 2.40 (1 H, ddd, J = 14.0, 8.8, 1.4 Hz, H-2), 1.24 (3 H, d, J = 2.4 Hz, H-6'), 1.24 (3 H, s, H-27), 1.08 (3 H, s, H-24), 1.09 (3 H, s, H-30), 1.06 (3 H, s, H-23), 0.99 (3 H, s, H-26), 0.85 (3 H, s, H-25); ¹³C NMR (100 MHz, MeOD-d₄) δ: 220.3 (C-3), 207.6 (C-29), 176.5 (C-28), 144.3 (C-13), 124.9 (C-12), 95.8 (C-1'), 73.4 (C-2'), 72.5 (C-3'), 72.3 (C-4'), 71.3 (C-5'), 56.5 (C-5), 48.5 (C-17), 48.1 (C-9), 48.0 (C-4), 47.7 (C-14), 43.9 (C-18), 43.0 (C-8), 40.7 (C-10), 40.4 (C-1), 40.2 (C-19), 37.9 (C-20), 35.1 (C-2), 34.3 (C-22), 33.5 (C-7), 28.6 (C-21), 28.3 (C-15), 27.0 (C-30), 26.4 (C-27), 24.6 (C-16), 24.2 (C-26), 23.8 (C-11), 22.0 (C-23), 20.7 (C-6), 18.2 (C-6'), 17.9 (C-25), 15.5 (C-24).

3.5. Hydrolysis of compound 1

A solution of 1 (10 mg) in 6 mol/L HCl (1 mL) was reacted 3 h at 100 °C. The reaction mixture was extracted with EtOAc repeatedly to remove the aglycone fraction. The H₂O layer was then concentrated to furnish the sugar residue (2.0 mg).

3.6. Biological assays

Antibacterial activity was determined by the conventional broth dilution assay (Pierce et al. 2008). Seven terrestrial pathogenic bacteria, *M. tetragenus*, *E. coli*, *S. albus*, *B. cereus*, *S. aureus*, *M. luteus*, and *B. subtilis* were used, and ciprofloxacin was used as a positive control.

The activity of α-glucosidase assay was performed employing the colorimetric method described by Ma (Ma K et al. 2014). The reference substances were acarbose. The activities of α-glucosidase were determined in 96-well plates, and the absorbance was determined at 405 nm measured with ELISA Microplate Reader (Bio Tek ELX800) (Ma K et al. 2014). Acarbose was utilized as the positive control with an IC₅₀ of 2.35 ± 0.20 μmol/mL. Reaction of α-glucosidase Inhibitory Activity consisted of 20 μL of compound 1 and 2 in DMSO (final concentrations of 0.2, 0.05 mg/mL), 10 μL of α-glucosidase (1 U/mL), 50 μL of phosphate buffer (pH 6.8), 20 μL of 2.5 mmol/L substrate...
(p-nitrophenyl-α-D-glucopyranoside) solution, the final volume of the reaction was 100 µL. The plates were incubated at 37 °C for 15 min, then added 150 µL. The blank was prepared by adding phosphate buffer instead of the α-glucosidase using the present method. The negative control was prepared by adding phosphate buffer instead of the sample in the same way as test. The inhibition rates (\%) = \frac{[(\text{OD}_{\text{control}} - \text{OD}_{\text{control blank}}) - (\text{OD}_{\text{test}} - \text{OD}_{\text{test blank}})]}{(\text{OD}_{\text{control blank}})} \times 100\%. IC_{50} values of the sample were calculated using the IC_{50} calculative software.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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