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Lupane-Triterpenes from the Leaves of *Brassaiopsis glomerulata*

Phan Van Kiem, Nguyen Tien Dat, Chau Van Minh, Jung Joon Lee[1], and Young Ho Kim[2]

Institute of Natural Products Chemistry, NCST, 18 Hoang Quoc Viet, Nghiado, Caugiay, Hanoi, Vietnam, [1]Korea Research Institute of Bioscience and Biotechnology, P.O.Box 115, Yusong, Daejeon 305-600, Korea and [2]College of Pharmacy, Chungnam National University, Taejon 305-764, Korea

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Three known lupane-triterpenes, 3α-hydroxy-lup-20(29)-en-23,28-dioic acid (**1**), 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (acankoreoside A, **2**) and 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (**3**) were isolated from the leaves of *Brassaiopsis glomerulata* (Blume) Regel, a species of Araliaceae family growing in Vietnam. Their structures were determined on the basis of spectroscopic data.

Key words: *Brassaiopsis glomerulata*, Araliaceae, Lupane-triterpene

INTRODUCTION

Brassaiopsis glomerulata (Blume) Regel (Araliaceae) is widely distributed deciduous tree, which is found in the mountainous areas of North Vietnam in the Phucyen and Vinhphuc provinces. The plant is used as a traditional medicine to treat rheumatism and back pain in old people (Chi 1997). Up to now, the chemical constituents and bio-activities of this plant have not been studied. In this report, we describe the isolation and structure identification of three lupane-triterpenes from *B. glomerulata*. Based on spectroscopic data, their structures were verified as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid (**1**), 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (**2**), and 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (**3**).

MATERIALS AND METHODS

General experimental procedures

Melting points were determined using a Yanagimoto micro hot-stage melting point apparatus. IR spectra were obtained on a JASCO DIP-370 Digital polarimeter. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker-NMR DRX 300 unit and chemical shifts are expressed as δ values using TMS as an internal standard. Column chromatography (CC) was performed on silica gel 60, RP-18, YMC resin and Dianion HP-20 resin.

Correspondence to: Young Ho Kim Ph.D., College of Pharmacy, Chungnam National University, Taejon 305-764, Korea
Tel: 82-42-821-5933, Fax: 82-42-823-6566
E-mail: yhk@cnu.ac.kr

Plant material

B. glomerulata was collected from Tamdao mountain, Vinhphuc province, Vietnam on December 2002 and identified by Prof. Vu Van Chuyen, Hanoi University of Pharmacy, Vietnam. Voucher specimens (No 2846) were deposited at the herbarium of the Institute of Natural Product Chemistry, NCST, Vietnam.

Extraction and isolation

Dried and powdered leaves (2.5 kg) were extracted three times with hot MeOH. The combined solutions were then evaporated under reduced pressure to yield a MeOH extract (170 g), which was suspended in water and extracted using dichloromethane. The dichloromethane fraction (46 g) was subjected to repeated chromatography on a silica gel column (ϕ 70 mm × L 800 mm), eluted with *n*-hexane-acetone (6:1) yielding five fractions (Fr. A-E). Fraction B (5.4 g) was chromatographed on a silica gel column (ϕ 25 mm × L 700 mm), using chloroform-methanol (100:1) as eluent, obtained six subfractions (subfr. A-F). Subfractions B (1.1 g) and D (1.8 g) were recrystallized from MeOH yielding **1** (150 mg) and **3** (420 mg) as white crystals, respectively.

The water fraction (124 g) was adsorbed on highly porous polymer resin (Dianion HP-20, Mitsubishi Chem.

Ind. Co. Ltd, Tokyo, Japan; column ϕ 70 mm × L 800 mm) and eluted with water containing increasing concentrations of MeOH (100 % H_2O, 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH and 100% MeOH). The 40% MeOH fraction (64 g) was chromatographed on a silica gel column using $CHCl_3$-MeOH-H_2O (70:30:4) as eluent, and then the major fraction was purified on a YMC RP-18 column using MeOH-H_2O (6:3) as eluent to yield **2** (12 g, white powder) as the main component.

3α-Hydroxy-lup-20(29)-en-23,28-dioic acid (1)
White crystals, m.p. 260-262°C; FAB-MS (positive) *m/z* 487.27 $[M+H]^+$. IR (KBr) ν_{max} (cm^{-1}): 3390 (OH), 1702 (C=O), 2985 (C-H). ^{1}H-NMR (pyridine-d_5) δ: 0.93 (3H, s, H-27), 0.94 (3H, s, H-25), 1.12 (3H, s, H-26), 1.46 (3H, s, H-24), 1.59-1.86 (2H, m, H-11), 1.77 (1H, d, *J* = 11.6 Hz, H-9), 1.78 (3H, s, H-30), 2.70 (1H, m, H-13), 3.54 (1H, m, H-19), 4.26 (1H, m, H-3), 4.74 (1H, br s, H-29_a), 4.90 (1H, br s, H-29_b). ^{13}C-NMR (pyridine-d_5) δ: 14.9 (q, C-27), 16.8 (q, C-26 , 16.9 (q, C-25), 18.1 (q, C-24), 19.6 (q, C-30), 21.2 (t, C-11), 21.9 (t, C-6), 26.2 (t, C-12), 26.3 (t, C-2), 30.4 (t, C-15 , 31.3 (t, C-21), 32.9 (t, C-16), 33.0 (t, C-1), 34.9 (t, C-7), 37.6 (s, C-10), 37.6 (t, C-22), 38.2 (d, C-13), 41.9 (s, C-8), 43.1 (s, C-14), 45.1 (d, C-5), 47.9 (d, C-19), 49.9 (s, C-18 , 51.2 (d, C-9), 52.1 (s, C-4), 56.7 (s, C-17), 73.1 (d, C-3), 110.0 (t, C-29), 151.4 (s, C-20), 178.6 (s, C-28), 179.6 (s, C-23).

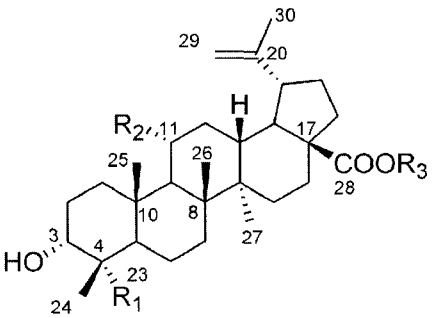
3α-Hydroxy-lup-20(29)-en-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (acankoreoside A, 2)
A white powder, m.p. 225-228 °C; FAB-MS m/z: 979.38 $[M+Na]^+$. IR (KBr) ν_{max} (cm^{-1}): 3417 (OH), 1724 (C=O), 2985 (C-H). ^{1}H-NMR (pyridine-d_5) δ: 0.93 (3H, s, H-27), 0.94 (3H, s, H-25), 1.12 (3H, s, H-26), 1.46 (3H, s, H-24), 1.59-1.86 (2H, m, H-11), 1.69 (3H, d, *J* = 6.1Hz, H-6 rha), 1.77 (1H, d, *J* = 11.6Hz, H-9), 1.78 (3H, s, H-30), 2.70 (1H, m, H-13), 3.54 (1H, m, H-19), 4.26 (1H, m, H-3), 4.74 (1H, br s, H-29_a), 4.90 (1H, br s, H-29_b), 4.95 (1H, d, *J* = 7.9Hz, H-1 glc), 5.84 (1H, br s, H-1 rha), 6.34 (1H, d, *J* = 7.9Hz, H-1 glc). ^{13}C-NMR (pyridine-d_5) δ: 14.9 (q, C-27), 16.8 (q, C-26), 16.9 (q, C-25), 18.1 (q, C-24), 18.6 (q, C-6 rha), 19.6 (q, C-30), 21.1 (t, C-11), 21.9 (t, C-6), 26.2 (t, C-12), 26.3 (t, C-2), 30.4 (t, C-15), 31.3 (t, C-21), 32.9 (t, C-16), 33.0 (t, C-1), 34.9 (t, C-7), 37.6 (s, C-10), 37.6 (t, C-22), 38.2 (d, C-13), 41.9 (s, C-8), 43.1 (s, C-14), 45.1 (d, C-5), 47.9 (d, C-19), 49.9 (s, C-18), 51.2 (d, C-9), 52.1 (s, C-4), 56.7 (s, C-17), 61.5 (t, C-6 glc'), 69.6 (t, C-6 glc), 70.4 (d, C-5 rha), 71.0 (d, C-4 glc), 72.7 (d, C-2 rha), 72.7 (d, C-3 rha), 73.1 (d, C-3), 74.1 (d, C-4 rha), 74.2 (d, C-2 glc), 75.4 (d, C-2 glc'), 76.6 (d, C-3 glc'), 77.3 (d, C-5 glc'), 78.1 (d, C-5 glc), 78.4 (d, C-4 glc'), 78.8 (d, C-3 glc), 95.4 (d, C-1 glc), 102.9 (d, C-1 rha), 105.3 (d, C-1 glc'), 110.0 (t, C-29), 151.4 (s, C-20), 175.1 (s, C-28), 179.6 (s, C-23).

3α,11α-Dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (3)
White crystals, m.p. 215-218°C; FAB-MS *m/z*: 509.25 $[M+Na]^+$. IR (KBr) ν_{max} (cm^{-1}): 3350 (OH), 1725 (C=O), 2985 (C-H). ^{1}H-NMR (pyridine-d_5) δ: 0.93 (3H, s, H-27), 0.94 (3H, s, H-25), 1.12 (3H, s, H-26), 1.46 (3H, s, H-24), 1.77 (1H, d, *J* = 11.6 Hz, H-9), 1.78 (3H, s, H-30), 2.70 (1H, m, H-13), 3.54 (1H, m, H-19), 3.92 (1H, m, H-11), 4.26 (1H, m, H-3), 4.63 (1H, br s, H-29_a), 4.85 (1H, br s, H-29_b), 10.00 (1H, s, H-23). ^{13}C-NMR (pyridine-d_5) δ: 14.9 (q, C-27), 15.1 (q, C-25), 16.8 (q, C-26), 17.9 (q, C-24), 19.7 (q, C-30), 21.5 (t, C-6), 27.3 (t, C-2), 30.2 (t, C-15), 31.4 (t, C-21), 33.0 (t, C-16), 35.5 (t, C-1), 35.7 (t, C-7), 37.7 (t. C-22), 37.8 (d, C-13), 38.4 (t, C-12), 39.2 (s, C-10), 43.1 (s, C-8), 43.4 (s, C-14), 44.4 (d, C-5), 47.9 (d, C-19), 49.5 (s, C-18), 53.1 (s, C-4), 56.1 (d, C-9), 56.7 (s, C-17), 69.9 (d, C-11), 73.2 (d, C-3), 110.2 (t, C-29), 151.4 (s, C-20), 178.9 (s, C-28). 210.1 (s, C-23).

RESULTS AND DISCUSSION

Compounds **1** and **3** were obtained as white crystals from the dichloromethane fraction of the MeOH extract of the dried leaves of *B. glomerulata*, and compound **2** was obtained as a white powder from the water fraction of the MeOH extract. The IR spectra of **1**, **2** and **3** showed absorptions assignable to hydroxyl, carbonyl and >C=CH_2 functionalities.

The positive FAB-MS spectra of **1**, **2** and **3** showed ion peaks at *m/z* 487.27 ($[M+H]^+$), 979.38 ($[M+Na]^+$) and 509.25 ($[M+Na]^+$), which were corresponded to the molecular formulas of $C_{30}H_{46}O_5$, $C_{48}H_{76}O_{19}$ and $C_{30}H_{46}O_5$, respectively. The ^{1}H-NMR spectrum (in pyridine-d_5) of **1** and **2** confirmed the presence of five tertiary methyl groups [δ 0.93 (3H, s), 0.94 (3H, s), 1.12 (3H, s), 1.46 (3H, s) and 1.78 (3H, s)], one secondary hydroxyl function [δ 4.26 (1H, m)] and two olefinic protons [δ 4.74 (1H, br s) and 4.90 (1H, br



1	COOH	H	H
2	COOH	H	glc-glc'-rha
3	CHO	OH	H

Fig. 1. Structures of compounds **1**, **2** and **3**

s)]. The ^{1}H-NMR spectra of **1** and **2** differed, in as much as **2** had more proton signals due to the presence of sugar protons, a sugar moiety with chemical shifts between δ 3.65 and 4.94, three anomeric protons at δ 4.95 (1H, d, J = 7.9 Hz), 5.84 (1H, br s) and 6.34 (1H, d, J = 7.9Hz), and one secondary methyl group at δ 1.69 (3H, d, J = 6.1 Hz). In the ^{13}C-NMR spectra (in pyridine-d_5) of **1** and **2**, 30 carbon signals, belonging to a lupane skeleton were similar. These included five methyl groups, a secondary hydroxyl group at δ 73.1, olefinic carbons (δ 110.0 and 151.4) and two carboxyl groups [δ 178.6 and 179.6 (**1**); 175.1 and 179.6 (**2**)]. The ^{13}C-NMR spectra of **1** and **2** also differed as **2** had more carbon signals due to the presence of two glucoses and one rhamnose. Based on these spectroscopic data and comparisons with reported data (Chang *et al.*, 1998), **1** and **2** were identified as 3α-hydroxy-lup-20(29)-en-23,28-dioic acid and 3α-hydroxy-lup-20(29)-en-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (acankoreoside A), respectively. The ^{1}H-NMR spectrum (in pyridine-d_5) of **3** also confirmed the presence of five tertiary methyl groups [δ 0.93 (3H, s), 0.94 (3H, s), 1.12 (3H, s), 1.46 (3H, s) and 1.78 (3H, s)], two secondary hydroxyl groups (δ 3.92 and 4.26), two olefinic protons [δ 4.63 (1H, br s) and 4.85 (1H, br s)], and one aldehydic proton [δ 10.00 (1H, s)]. In the ^{13}C-NMR spectrum (in pyridine-d_5) of **3**, 30 carbon signals were observed including five tertiary methyl groups (δ 14.9, 15.1, 16.8, 17.9 and 19.7), two secondary alcohol groups (δ 69.9 and 73.2), olefinic carbons (δ 110.2 and 151.4), a carboxyl group (δ 178.9) and an aldehydic carbon (δ 210.1). The signals of **3** were similar to those of 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid (Ty *et al.*, 1985). Based on the above spectroscopic data, **3** was identified as 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid. This is the first report of **1**, **2** and **3** in *Brassaiopsis* species.

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