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A NEW TRITERPENE SAPONIN FROM *Ligularia veitchiana*

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*A new oleanane triterpene saponin **1** was isolated from* Ligularia veitchiana*. The structure of compound **1** was elucidated as olean-12-en-6β,16β,28-triol 3-*O*-β-D-glucuronopyranoside by spectroscopic data (^1H, ^{13}C NMR, HMBC, HSQC, COSY, and NOSEY) and chemical evidence. Additionally, compound **1** presented phytotoxic effect on the growth of lettuce at 200 ppm.*

Keywords: *Ligularia veitchiana*, oleanane saponin, phytotoxic.

Ligularia veitchiana (Hemsl.) Greenm., a well-known traditional herbal medicine, is grown in northwestern China [1]. It has been used in traditional Chinese medicine to treat influenza, cough, ulcer, and pulmonary tuberculosis [2]. Previous chemical studies of this plant have reported the isolation of several compounds including sesquiterpenes, triterpenes, neolignans, phenylpropanoids, benzofurans, and their derivatives [2–5]. Among them, some isolated compounds showed a variety of bioactivities, such as anti-inflammatory, anticancer, and nematocidal activities [4–6]. To find new active compounds, the chemical constituents of *L. veitchiana* were investigated. From the *n*-butanol-soluble fraction, we have isolated a new oleanane triterpene saponin (**1**). In this paper, we describe the isolation, structural elucidation, and phytotoxic effect of the new triterpene saponin.

Compound **1** was obtained as a yellow gum, was found to have the molecular formula $C_{36}H_{58}O_{10}$ by the quasi-molecular ion peak at *m/z* 651.4106 ($[M + H]^+$ calcd 651.4108) in the HR-ESI-MS, indicating eight degrees of unsaturation. Its IR spectrum showed absorption bands at 3348, 1708, and 1650 cm^{-1} owing to hydroxy, carboxy, and olefine functionalities. Acid hydrolysis of **1** revealed one unit of glucuronic acid (identified by co-TLC with the authentic sample and detailed studies of COSY, HSQC, and HMBC spectra) [7]. The ^1H NMR spectrum of **1** showed signals for seven tertiary methyl groups at δ 0.88, 0.92, 1.12, 1.13, 1.18, 1.23, and 1.28 (each 3H, s, H_3-29, 30, 25, 23, 27, 24, 26), three methines bearing oxygen function [δ 3.09 (1H, dd, J = 9.6, 5.5 Hz, H-3), 4.49 (1H, br.s, H-6), and 4.22 (1H, dd, J = 11.8, 4.5 Hz, H-16)], one methylene bearing oxygen function [δ 3.78 (1H, d, J = 9.5 Hz) and 3.25 (1H, m), H_2-28], and one olefinic proton at δ 5.25 (1H, br.s, H-12). The ^{13}C NMR spectrum showed signals corresponding to 36 carbons, including a carboxy carbon at δ 175.5 (C-6′), two olefinic carbons at δ 143.7 (C-13) and 124.2 (C-12), four oxygenated carbons at δ 91.1 (C-3), 68.6 (C-6), 67.9 (C-16), and 68.9 (C-28), and seven tertiary methyl carbons at δ 28.4 (C-23), 18.5 (C-24), 17.5 (C-25), 18.7 (C-26), 27.5 (C-27), 31.7 (C-29), and 24.3 (C-30). Furthermore, the spectrum also showed a signal at δ_C 105.6 (C-1′) assignable to an anomeric carbon in a sugar unit. These above proton and carbon data suggested that **1** might be assignable to an oleanane-type triterpene skeleton with a sugar moiety. Its aglycone was determined to be olean-12-en-3*β*,6*β*,16*β*,28-tetraol [8], owing to their similar NMR data, except for the presence of one set of resonances attributable to a D-glucuronopyranosyl moiety [H-1′ (δ_H 4.37, 1H, d, J = 8.0 Hz), δ_C 105.6, 75.4, 78.1, 74.1, 76.5, and 175.5] in **1** [9]. The *β*-anomeric configuration of the sugar unit was deduced from the coupling constant (8.0 Hz). In the HMBC experiment, the correlation between H-3 and C-1′ indicated the attachment of glucuronic acid at C-3 (Fig. 1). The three hydroxyl groups in **1** were assigned on C-6, C-16, and C-28, respectively, owing to the COSY correlations of H-5–H-6–H-7 and H-15–H-16 (Fig. 1), as well as HMBC correlations of H-6 to C-5/C-6/C-7, H-16 to C-15/C-16/C-17, and H_2-28 to C-16/C-17/C-22 (Fig. 1).

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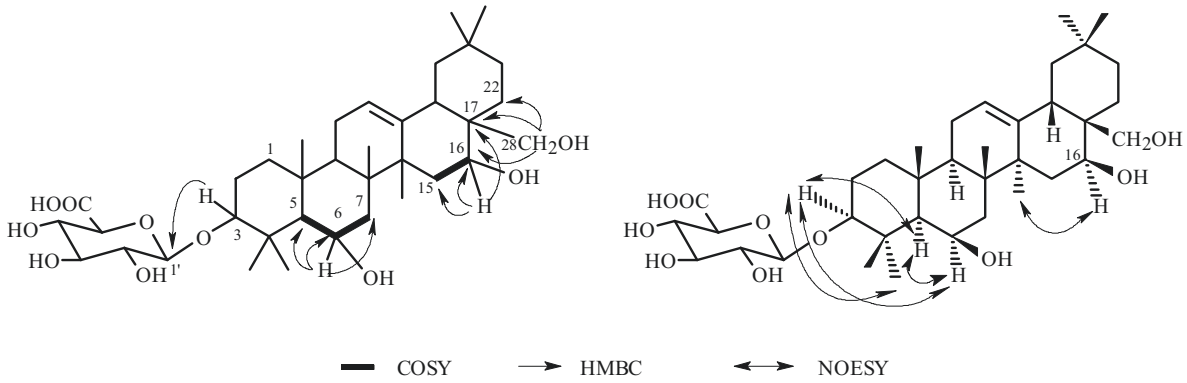

Fig. 1. Key ^1H–^1H COSY, HMBC, and NOESY correlations of compound **1**.

The relative configuration of **1** was confirmed by NOESY (Fig. 1). The α-orientation of H-3 and H-6 was deduced from the correlations of H-3 with H-5, H-6, and H_3-23 and the α-orientation of H-16 was deduced from the correlation of H-16 and H_3-27. Based on the above analysis, the structure of compound **1** (Fig. 1) was established as olean-12-en-6β,16β,28-triol 3-*O*-β-D-glucuronopyranoside.

The phytotoxic effect of compound **1** on lettuce growth was examined. Compound **1** inhibited the growth of root (66.4%) and hypocotyl (68.9%) of lettuce at 200 ppm.

EXPERIMENTAL

General Experimental Procedures. IR spectrum (neat or KBr) was recorded on a Perkin Elmer 2000 FT-IR spectrometer. HR-ESI-MS was recorded on a Bruker APEX II mass spectrometer. NMR spectra of the new compound were recorded on a Bruker Avance 600 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Silica gel (200–300 mesh; Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (40–70 μm; GE Healthcare, USA) were used for column chromatography (CC).

Plant Material. The plants of *L. veitchiana* were collected in Zhangjiajie, Hunan Province, People's Republic of China, in September, 2018, and identified by Hai-bo Wu. A voucher specimen (No. 20180906) was deposited at Minzu University of China.

Extraction and Isolation. The air-dried and powdered whole herbs of *L. veitchiana* (1.6 kg) were extracted at reflux three times with 95% ethanol, for 4 h each. The filtered and concentrated extract was dissolved in water and then sequentially partitioned with petroleum ether, chloroform, and *n*-butanol to yield four portions. The *n*-butanol fraction (80 g) was subjected to repeated CC using chloroform–methanol (50:1, 25:1, 10:1, 5:1, and 2:1) as a solvent system to produce five fractions (F1–F5). Fraction F3 was subjected to Sephadex LH-20 CC (chloroform–methanol, 1:1) and then was separated by silica gel CC using chloroform–methanol, 8:1 to afford **1** (2.3 mg).

Olean-12-en-6β,16β,28-triol 3-*O*-β-D-glucuronopyranoside (1), yellow gum, $[\alpha]_D^{20}$ –19.8° (*c* 0.1, MeOH). IR (ν_{max}, cm^{-1}): 3348, 1708, 1650. HR-ESI-MS *m/z* 651.4106 $[M + H]^+$ (calcd for $C_{36}H_{59}O_{10}$, 651.4108). ^1H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 5.25 (1H, br.s, H-12), 4.49 (1H, br.s, H-6), 4.37 (1H, d, J = 8.0, H-1′), 4.22 (1H, dd, J = 11.8, 4.5, H-16), 3.78 (1H, d, J = 9.5, H-28a), 3.61 (1H, d, J = 9.9, H-5′), 3.45 (1H, t, J = 9.1, H-4′), 3.38 (1H, t, J = 9.0, H-3′), 3.25 (1H, m, H-28b), 3.21 (1H, t, J = 7.9, H-2′), 3.09 (1H, dd, J = 9.6, 5.5, H-3), 2.16 (1H, dd, J = 13.1, 3.3, H-18), 2.15 (1H, m, H-22a), 1.99 (1H, m, H-11a), 1.88 (1H, m, H-11b), 1.79 (1H, m, H-2a), 1.73 (1H, m, H-7a), 1.73 (1H, m, H-19a), 1.73 (1H, m, H-2b), 1.57 (1H, dd, J = 10.4, 7.1, H-9), 1.55 (1H, m, H-1a), 1.55 (1H, m, H-7b), 1.41 (1H, m, H-21a), 1.41 (1H, m, H-22b), 1.38 (1H, d, J = 13.4, H-15a), 1.28 (3H, s, H-26), 1.23 (3H, s, H-24), 1.21 (1H, m, H-21b), 1.18 (3H, s, H-27), 1.18 (1H, d, J = 12.8, H-15b), 1.13 (3H, s, H-23), 1.12 (3H, s, H-25), 1.00 (H, d, J = 13.1, H-19b), 0.94 (1H, m, H-1b), 0.92 (3H, s, H-30),0.88 (3H, s, H-29), 0.77 (1H, br.d, J = 10.8, H-5). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 42.0 (C-1), 27.1 (C-2), 91.1 (C-3), 41.1 (C-4), 57.3 (C-5), 68.6 (C-6), 41.5 (C-7), 40.4 (C-8), 48.5 (C-9), 37.2 (C-10), 24.6 (C-11), 124.2 (C-12), 143.7 (C-13), 45.1 (C-14), 34.8 (C-15), 67.9 (C-16), 40.9 (C-17), 45.0 (C-18), 47.8 (C-19), 31.5 (C-20), 34.7 (C-21), 25.8 (C-22), 28.4 (C-23), 18.5 (C-24), 17.5 (C-25), 18.7 (C-26), 27.5 (C-27), 68.9 (C-28), 31.7 (C-29), 24.3 (C-30), 105.6 (C-1′), 75.4 (C-2′), 78.1 (C-3′), 74.1 (C-4′), 76.5 (C-5′), 175.5 (C-6′).

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