SHORT REPORT

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A New Dammarane-type Triterpene Saponin from the Root of *Aralia elata*

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Abstract: A new dammarane-type triterpene glycoside (**1**) and compounds Ginsenoside F_5 (**2**), Ginsenoside F_3 (**3**), Ginsenoside F_2 (**4**), Ginsenoside Rg_2 (**5**) were isolated from the root of *Aralia elata* Seem.. The new compound was established as 12-oxo-3β, 6α, 20 (*S*)-trihydroxydammar-24-ene-3-*O*-*β*-D-glucopranosyl (1→2) *β*-D-glucopyranoside. All the structures of the compounds were elucidated on the basis of extensive spectral and chemical evidence.

Keywords: *Aralia elata*; 12-oxo-dammarane type saponin; Araliaceae.

1. Introduction

Aralia elata Seem. (Araliaceae) is an ancient and well-known folk medicine in China. It is widely distributed in Asian areas and has been used as a traditional Chinese medicine for the treatment of rheumatism, diabetes, myocardial infarction, gastric ulcer, neurasthenia [1]. The total saponins of the *Aralia elata* have been proved to have improvement in cardiac function [2]. More than 100 triterpene saponins have been isolated from this plant, including seven dammarane-type triterpene saponins [3]. In a continuing study, we now report the compounds which are found in systematic research on chemical constituents from the root of *Aralia elata*.

2. Materials and Methods

2.1. Plant material

The root of *Aralia elata* were collected in Anshan from Liaoning Province, China and identified by researcher Bengang Zhang, Institute of Medicinal Plant Development. A voucher specimen (No. AE-201108) has been deposited at the Herbarium of the Institute of Medicinal Plant Development.

2.2 Extraction and isolation

Air-dried and powdered plant material (5kg) was extracted three times with 70% ethanol at room temperature. The 70% ethanol extract was evaporated to dryness under reduced pressure. Then

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extract was subjected to a macro-reticular absorption resin (AB-8) column using 20%, 80% MeOH-water and MeOH. The 80% extract was evaporated to dryness under reduced pressure. The total saponins were subjected to chromatography on a silica gel column and eluted in a stepwise manner with $CHCl_3$-MeOH-H_2O mixture (9:1:0.1-6:4:0.5) to afford nine fractions (Fraction A-I). Fraction B (15g) was chromatographed on Chromatorex ODS (eluted from 50 to 80% MeOH) to afford four fractions. The second fraction was further purified with a gradient of 30% MeOH-H_2O by preparative HPLC to get compound **2** (4.3 mg) and compound **3** (6.5 mg). The fourth fraction was purified with a gradient of 77% MeOH-H_2O by preparative HPLC to get compound **4** (8.7 mg). Fraction C (8g) was chromatographed on Chromatorex ODS (eluted from 50 to 80% MeOH) to afford a saponin fraction. This fraction was purified by HPLC with a gradient of 34% MeOH-H_2O to get compound **1** (6.7 mg) and compound **5** (7.0 mg).

Compound **1***:* A colorless gum; $[\alpha]_D^{25}$ -66° (c 0.1, MeOH); IR (KBr)ν_{max} 3326 (OH), 2913, 1642, 1592, 1414, 1123, 1075, 1040, 853, 802 cm^{-1}; ^{1}H-NMR (600MHz, CD_3OD): δ (ppm) = 0.80 (3H, s, H-30), 1.00 (3H, s, H-21), 1.04 (3H, s, H-19), 1.06 (3H, s, H-29), 1.32 (3H, s, H-18), 1.39 (3H, s, H-28), 1.62 (3H, s, H-27), 1.67 (3H, s, H-26), 4.12 (1H, td, *J*=10.2, 4.8 Hz, H-6), 3.20 (1H, dd, *J*=12.0, 4.8 Hz, H-3), 5.10 (1H, t, *J*=7.2 Hz, H-24), 4.44 (1H, d, *J*=7.8 Hz, H-1'), 4.68 (1H, d, *J*=7.8 Hz, H-1''); ^{13}C-NMR (150 MHz, CD_3OD): δ (ppm) = 40.2 (C-1), 27.1 (C-2), 91.2 (C-3), 41.4 (C-4), 62.3 (C-5), 68.9 (C-6), 46.9 (C-7), 42.7 (C-8), 55.2 (C-9), 39.8 (C-10), 40.8 (C-11), 215.1 (C-12), 57.3 (C-13), 56.8 (C-14), 32.8 (C-15), 25.2 (C-16), 44.8 (C-17), 17.8 (C-18), 17.7 (C-19), 75.1 (C-20), 26.0 (C-21), 42.2 (C-22), 24.1 (C-23), 126.1 (C-24), 132.2 (C-25), 25.9 (C-26), 17.9 (C-27), 31.3 (C-28), 16.9 (C-29), 17.6 (C-30), 105.8 (C-1'), 81.3 (C-2'), 78.5 (C-3'), 71.9 (C-4'), 77.9 (C-5'), 63.4 (C-6'), 104.7 (C-1''), 76.5 (C-2''), 78.7 (C-3''), 72.2 (C-4''), 78.1 (C-5''), 63.1 (C-6''); HR-ESI-MS: *m/z* 821.4724 $[M + Na]^+$ (calcd for $C_{42}H_{70}O_{14}Na$, 821.4663).

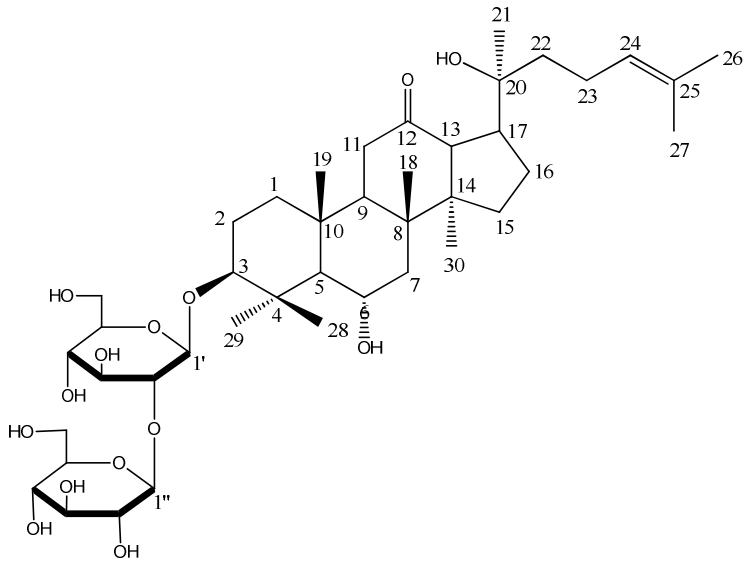

Figure 1. Chemical structure of compound **1**

3. Results and Discussion

Compound **1** was obtained as a colorless gum. The molecular formula was determined as $C_{42}H_{70}O_{14}$ based on its positive HR-ESI-MS spectral data at *m/z* 821.4663 $[M+Na]^+$ (calcd 821.4663). The IR spectrum of **1** indicated the presence of hydroxyl (3326 cm^{-1}) and olefinic (1642, 802cm^{-1}) functionalities. Its ^{1}H-NMR showed the presence of eight singlet methyls (δ 0.80 (3H, s, H-30), 1.00 (3H, s, H-21), 1.04 (3H, s, H-19), 1.06 (3H, s, H-29), 1.32 (3H, s, H-18), 1.39 (3H, s, H-28), 1.62 (3H, s, H-27), and 1.67 (3H, s, H-26) as well as two oxymethine protons at δ 4.12 (1H, td, *J*=10.2, 4.8 Hz) and 3.20 (1H, dd, *J*=12.0, 4.8 Hz). A trisubstituted double bond proton at δ 5.10 (1H, t, *J*=7.2 Hz, H-24) was also observed. Additionally, the resonances of two anomeric protons, indicative of the existence of two sugar moieties, were observed in the downfield region at δ 4.44 (1H, d, *J*=7.8 Hz, H-

1'), 4.68 (1H, d, *J*=7.8 Hz, H-1"). Acid hydrolysis of **1** followed by TLC analysis suggested the presence of two glucose units. The configuration of the two anomeric positions were determined to be *β* on basis of their coupling constant (*J*=7.8 Hz).The ^{13}C-NMR spectrum of **1** displayed 42 carbon signals. A set of signals (*δ* 105.8, 81.3, 78.5, 71.9, 77.9, 63.4, 104.7, 76.5, 78.7, 72.2, 78.1, 63.1) at the downfield region due to two *β*-D-glucopyranosyl units were observed in the ^{13}C-NMR spectrum of **1** [1-2]. The methyl signals at *δ* 16.9, 17.6, 17.7, 17.8, 17.9, 25.9, 26.0, 31.3 together with its ^{1}H-NMR spectral data indicated compound **1** was a triterpene saponin [4-5]. The observed olefinic carbon signals at *δ* 126.1 (C-24) and 132.2 (C-25) as well as in the point of biogenetic considerations, compound **1** was determined to be a dammarane-type structure [6-7]. Furthermore, the signal of C-5 at *δ* 62.3 was a characteristic of a protopanaxatriol-type aglycone [8-9]. The signal resonated at *δ* 215.1 was attributed to the carbon of C-12, which was confirmed by HMBC correlations (Figure 3) between *δ* 3.03 (H-13), 2.35 (H-17), and 2.18 (H-11) and *δ* 215.1 (C-12). The attachment of the sugar chain at the C-3 hydroxyl group of the aglycone was established by means of the diagnostic glycosidation shift of this carbon and was confirmed by the HMBC correlation between *δ* 4.44 (H-1') and *δ* 91.2 (C-3). Furthermore, a cross peak observed in the HMBC spectrum of **1** between *δ* 4.68 (H-1") and *δ* 81.3 (C-2') permitted the disaccharide chain to be defined as *β*-D-glucosyl (1-2)-*β*-D-glucopyranoside. Based on the information above, compound **1** was identified as a diglucoside whose sapogenol moiety was a propanaxtriol type triterpene with variations in the C-12 as well as the linkage between the two glucoside units. The stereochemistry of **1** was resolved by application of NOESY experiment (Figure 3). The observed correlations in the NOESY spectrum between H-3 and H_3-28, H-5 and H-9 and H-5, H_3-30 implied that these protons were cofacial and accepted *α* configuration. While observation of the NOESY couplings from H_3-29 to H_3-19 and from H_3-18 to H_3-19 and H-13 revealed that these protons occupied the *β*-face of the molecule. The orientation of 3*β*-OH based on the multiplicity of H-3 (*J*=12.0, 4.8 Hz) [10]. NOESY correlation between H-17 and H_3-21 facilitated assignment of the C-20 (*S*) configuration. On the basis of above evidence, the structure of **1** was established as 12-oxo-3*β*, 6*α*, 20 (*S*) -trihydroxydammar-24-ene-3-*O*-*β*-D-glucopyranosyl (1→2) *β*-D-glucopyranoside. To the best of our knowledge, only few 12-oxo-dammarane type saponins have previously been isolated [11]. Moreover, this is the first occurrence of 12-oxo-dammarane type saponin in Araliaceae.

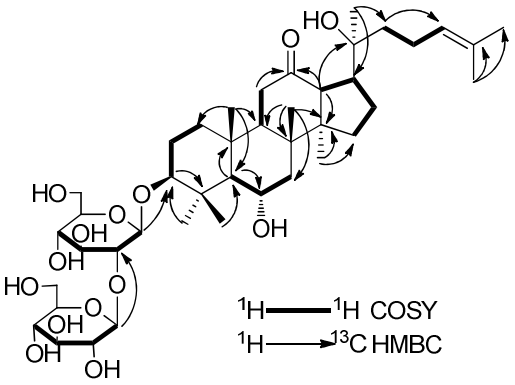

Figure 2. Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

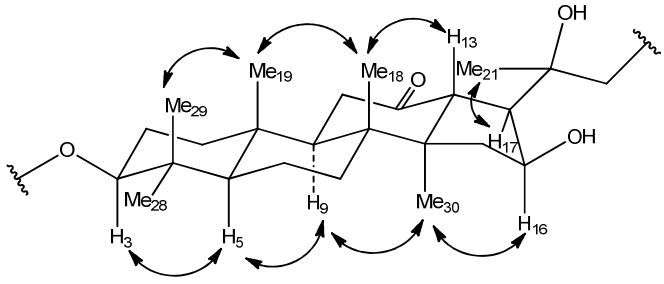

Figure 3. NOESY correlations of compound **1**

The known compounds ginsenoside F_5 **(2)** [12], ginsenoside F_3 **(3)** [13], ginsenoside F_2 **(4)** [14], ginsenoside Rg_2 **(5)** [15] were identified through their spectral data and by direct comparison with published data.

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