Aramatosides C and D, 2 Previously Undescribed Triterpene Glycosides Isolated From the Roots of *Aralia armata*

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Abstract

The 2 new oleanane-type triterpene glycosides, 23-hydroxyoleanolic acid-[28-Ọ-β-D-glucopyranosyl]-3-Ọ-{(β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside}, (1) and oleanolic acid-[28-Ọ-β-D-glucopyranosyl]-3-Ọ-{(β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside} (2) were isolated from the roots of *Aralia armata*. Their chemical structures were elucidated by using a combination of high resolution electrospray ionization mass spectrometry (HR-ESI-MS), 1 dimensional (1D), and 2 dimensional (2D) nuclear magnetic resonance spectral data, as well as by comparison with the previous literature. Compounds 1 and 2 displayed weak cytotoxic activity toward KB and HepG2 cell lines with IC₅₀ values of 25.1 ± 1.2 and 23.7 ± 0.9 µM (for 1) and 29.5 ± 1.3 and 23.9 ± 0.7 µM (for 2), respectively, compared to that of the positive control compound, ellipticine (IC₅₀: 1.3 ± 0.1 and 1.6 ± 0.1 µM, respectively) in an in vitro assay.

Keywords

Araliaceae, *Aralia armata*, aramatoside C, aramatoside D, triterpene glycoside, cytotoxic activity

Received: May 11th, 2021; Accepted: June 28th, 2021.

Introduction

*Aralia armata* (Wall.) Seem. (Araliaceae) is a common herbal in Vietnam that has been used in traditional medicine to cure hepatitis, arthritis, stomachache, malaria, and snake rolling.¹ Phytochemical study of *A armata* led to the isolation of oleanane-type triterpene glycosides as the main components of the leaves² and roots.³ From the leaves of this plant growing in Vietnam, 14 oleanane-type triterpene glycosides have been isolated and some of them exhibited cytotoxic activity against some cancer cell lines.⁴,⁶ However, no chemical and bioactive studies have been made on the roots of Vietnamese *A armata*. Continuing our search for bioactive compounds from *A armata*, herein, we report the isolation and structural elucidation of 2 previously undescribed oleanane-type triterpene saponins (named as aramatosides C and D) from the roots of this plant. The cytotoxic activity of these compounds on some cell lines was also evaluated.

Results and Discussion

Compound 1 was obtained as white amorphous powder from the methanol extract of the roots of *A armata*. The molecular formula of 1 was determined to be C₅₄H₸₈O₂₄ by its HR-ESI-MS results (m/z 1155.5341 [M + 35Cl]⁻ [calcd. for C₅₄H₸₈O₂₄Cl: 1155.5354, Δ = −1.1 ppm], m/z 1157.5289 [M + 37Cl]⁻ [calcd. for C₅₄H₸₆O₂₄Cl: 1157.5325, Δ = −3.1 ppm]) (Supplemental Figure S1), indicating 11° of unsaturation. Analyzing the ¹H nuclear magnetic resonance (NMR) and ¹³C NMR spectra, combined with the heteronuclear single quantum coherence spectroscopy (HSQC) spectrum of 1
showed 6 methyl groups \( \delta_{\text{H}} 0.76/\delta_{\text{C}} 13.2, \delta_{\text{H}} 1.00/\delta_{\text{C}} 16.4, \delta_{\text{H}} 0.83/\delta_{\text{C}} 18.7, \delta_{\text{H}} 1.19/\delta_{\text{C}} 26.4, \delta_{\text{H}} 0.95/\delta_{\text{C}} 24.0, \) and \( \delta_{\text{H}} 0.93/\delta_{\text{C}} 33.5 \), 1-CH\( \equiv \)C < double bond \( \delta_{\text{H}} 5.27 \) [\( J = 3.5 \text{ Hz} /\delta_{\text{C}} 123.8 \) and \( \delta_{\text{C}} 144.9 \), 1 carbonyl group \( \delta_{\text{C}} 178.1 \), 1 C-3 methine carbinol group \( \delta_{\text{H}} 3.67 \) [\( \text{dd}, J = 13.5, 5.4 \text{ Hz} /\delta_{\text{C}} 84.9 \), and 4 sugar moieties identified from the anomic signals at \( \delta_{\text{H}} 5.40 \) [\( \text{d}, J = 8.0 \text{ Hz} /\delta_{\text{C}} 95.7 \), \( \delta_{\text{H}} 4.53 \) [\( \text{d}, J = 7.5 \text{ Hz} /\delta_{\text{C}} 104.8 \), \( \delta_{\text{H}} 4.89 \) [\( \text{d}, J = 7.5 \text{ Hz} /\delta_{\text{C}} 103.5 \), and \( \delta_{\text{H}} 4.63 \) [\( \text{d}, J = 7.5 \text{ Hz} /\delta_{\text{C}} 105.3 \) (Supplemental Figures S2 to S11). The above data suggested that compound 1 was an oleane-type triterpene glycoside, a very typical subclass of \textit{Aralia} species.\(^{3,5,6}\) Comparing the NMR data of the aglycone of 1 with the corresponding data of the oleane-type saponis araliaasaponins XII to XVII found that the methyl at C-23 of these compound was replaced by an oxygenated methylene group (CH\(_2\)-O-H) in 1, which was confirmed by the additional signals at \( \delta_{\text{H}} 3.78 \) and \( 3.28 \) [\( \text{each}, 1\text{H}, \text{d}, J = 11.0 \text{ Hz} /\delta_{\text{C}} 64.8 \), as well as by the absence of CH\(_2\)-3 in the NMR spectra of 1. Furthermore, the heteronuclear multiple bond correlation (HMBC) correlations from H-24 \( (\delta_{\text{H}} 0.76) \) to carbons C-23 \( (\delta_{\text{C}} 64.8)/\text{C-3} (\delta_{\text{C}} 84.9)/\text{C-4} (\delta_{\text{C}} 44.2)/\text{C-5} (\delta_{\text{C}} 48.0) \), and from H-23 \( (\delta_{\text{H}} 3.28 \) and 3.78) to C-24 \( (\delta_{\text{C}} 13.2)/\text{C-4/\text{C-5} definitely indicated that the hydroxyl group was at C-23 and the methine carbinol carbon was at C-3. The double bond was determined at C-12/C-13, suggested from the similar biogenetic oleane-type saponis from \textit{Aralia} species\(^{3,5,6}\) and further confirmed by the observations of the HMBC correlations from H-26 \( (\delta_{\text{H}} 1.19) \) to C-13 \( (\delta_{\text{C}} 144.9) \) and from H-18 \( (\delta_{\text{H}} 2.87) \) to C-12 \( (\delta_{\text{C}} 123.8)/\text{C-13} \). In the NOESY spectrum, H-3 \( (\delta_{\text{H}} 3.67) \) had cross peaks to H-23 \( (\delta_{\text{H}} 3.28/3.78) \) and H-5 \( (\delta_{\text{H}} 1.23) \), and H-5 had a cross peak to H-23 (Supplemental Figure S15). This evidence, together with the larger coupling constant of H-2 and H-3 \( (J = 13.5 \text{ Hz}) \), indicated that H-3 \( (\alpha\text{-rhamnopyranosyl(1→2)β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]β-D-galactopyranoside}) \), a new compound named aramatocide C\(^{6}\) (Figure 1).

Compound 2 was also obtained as white amorphous powder and its molecular formula was determined to be C\(_{52}\)H\(_{88}\)O\(_{34}\) from the HR-ESI-MS results [\( m/z 1139.5402 \) [\( M+3\text{Cl}^+ \) ]-calcld. for C\(_{52}\)H\(_{88}\)O\(_{34}\)Cl: 1139.5405, \( \Delta = -0.2 \text{ ppm} \), \( m/z \)

| Table 1. NMR Spectroscopic Data for the Sugar Moieties of 1 and 2 in Deuterated Methanol. |
| --- |
| Pos. | \( ^{13}\text{C} \) | \( ^{1}\text{H} \) (mult., \( f \) in Hz) | \( ^{13}\text{C} \) | \( ^{1}\text{H} \) (mult., \( f \) in Hz) |
| 28-O-glc | 1' | 95.7 | 5.40 (d, 8.0) | 95.7 | 5.40 (d, 8.0) |
| | 2' | 74.0 | 3.33 (dd, 8.0, 9.0) | 74.0 | 3.33 (dd, 8.0, 9.0) |
| | 3' | 78.3 | 3.42 (q, 9.0, 9.0) | 78.4 | 3.43 (q, 9.0, 9.0) |
| | 4' | 71.2 | 3.35 (q, 9.0, 9.0) | 71.2 | 3.30 (q, 9.0, 9.0) |
| | 5' | 78.7 | 3.37 (m) | 78.7 | 3.38 (m) |
| | 6' | 62.5 | 3.70 (m)/3.84 (m) | 62.4 | 3.70 (m)/3.84 (m) |
| 3-O-gal | 104.8 | 4.53 (d, 7.5) | 105.9 | 4.42 (d, 7.5) |
| | 2' | 76.6 | 3.98 (t, 9.0, 7.5) | 77.5 | 3.89 (dd, 9.0, 7.5) |
| | 3' | 85.4 | 3.82 (q, 9.0, 3.0) | 85.4 | 3.78 (dd, 9.0, 3.0) |
| | 4' | 70.0 | 4.14 (br d, 3.0) | 70.0 | 4.13 (br d, 3.0) |
| | 5' | 76.0 | 3.56 (m) | 75.7 | 3.53 (m) |
| | 6' | 63.5 | 3.57 (m)/3.83 (m) | 62.5 | 3.72 (m)/3.84 (m) |
| 2'-O-glc | 103.5 | 4.89 (d, 7.5) | 104.7 | 4.74 (d, 7.5) |
| | 2' | 76.0 | 3.15 (d, 9.0, 7.5) | 76.1 | 3.14 (d, 9.0, 7.5) |
| | 3' | 78.2 | 3.31 (q, 9.0, 9.0) | 78.3 | 3.30 (q, 9.0, 9.0) |
| | 4' | 72.3 | 3.13 (q, 9.0, 9.0) | 71.5 | 3.42 (q, 9.0, 9.0) |
| | 5' | 78.2 | 3.35 (m) | 78.2 | 3.35 (m) |
| | 6' | 62.4 | 3.70 (m)/3.74 (m) | 62.3 | 3.70 (m)/3.79 (m) |
| 3'-O-gal | 105.3 | 4.63 (d, 7.5) | 105.3 | 4.62 (d, 7.5) |
| | 2' | 75.3 | 3.32 (d, 9.0, 7.5) | 75.3 | 3.31 (d, 9.0, 7.5) |
| | 3' | 78.3 | 3.30 (q, 9.0, 9.0) | 78.3 | 3.30 (q, 9.0, 9.0) |
| | 4' | 71.2 | 3.34 (q, 9.0, 9.0) | 71.2 | 3.34 (q, 9.0, 9.0) |
| | 5' | 78.0 | 3.36 (m) | 77.9 | 3.36 (m) |
| | 6' | 62.4 | 3.70 (m)/3.84 (m) | 62.4 | 3.70 (m)/3.84 (m) |

\(^{1}\)Measured at 100 MHz.

Abbreviation: NMR, nuclear magnetic resonance.
the methine carbinol group at C-3 (δ123.9/5.27 and carbon C-1) (Supplemental Figure S31). The lower α observed indicating that the H-3 orientation was acid hydrolysis of identified from the positive sign of optical rotation. Consequently, 23-CH2OH signals in corresponding 1s of 1, and further confirmed by HSQC, HMBC, and 1H-1H COSY spectra (Figure 2). The HMBC correlations from gal H-1′′ (δ_H 144.8), the carboxylate group (δC 178.1), the methine carbinol group at C-3 (δC 91.5, δ_H 3.14), and H-5 (δH 0.77) was observed indicating that the H-3 orientation was α/axial (Supplemental Figure S31). The lower field signal of the anomeric carbon C-1′ (δC 95.7), the higher field signal of the anomeric proton H-1′ (δ_H 5.40), as well as the HMBC correlation from H-1′ to C-28 (δC 178.1) confirmed that 1 sugar was attached to C-28 by an ester linkage. The remaining sugar moieties of 'dmeasured at 500 MHz.
3 Measured at 100 MHz.
4 Measured at 500 MHz.
5 Abbreviation: NMR, nuclear magnetic resonance.

Figure 2. The 1H-1H COSY and key heteronuclear multiple bond correlation (HMBC) correlations of compounds 1 and 2.
Table 3. The Cytotoxic Effects of Compounds 1 and 2 Towards KB and HepG2 Cell Lines.

| Compounds | IC_{50} (µM) KB | IC_{50} (µM) HepG2 |
|-----------|----------------|------------------|
| 1         | 25.1 ± 1.2     | 23.7 ± 0.9       |
| 2         | 29.5 ± 1.3     | 23.9 ± 0.7       |
| Ellipticine* | 1.3 ± 0.1     | 1.6 ± 0.1       |

*Positive control compound.

Compounds 1 and 2 were evaluated in vitro for their cytotoxic activity by using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide assay against 2 human cancer cell lines (KB and HepG2) acquired from the American Type Culture Collection. Compounds 1 and 2 displayed a weak cytotoxic activity toward KB and HepG2 cell lines with IC_{50} values of 25.1 ± 1.2 and 23.7 ± 0.9 µM (for 1) and 29.5 ± 1.3 and 23.9 ± 0.7 µM (for 2), respectively, compared to that of positive control compound, ellipticine (IC_{50}: 1.3 ± 0.1 and 1.2 ± 0.1, respectively) in vitro assay (Table 3).

Experimental

General

HR-ESI-MS was carried out on an Agilent 6530 Accurate Mass Q-TOF LC/MS. The QTOF instrument was set at 2 GHz in extended dynamic range resolution mode, a negative ESI capillary voltage of 3500 V, fragment or voltage of 175 V, MS scan range of m/z 100 to 1700, and MS acquisition rate of 1.0 spectra per second. The NMR spectra were recorded on a Bruker 500 MHz spectrometer and optical rotation on a Jasco P2000 polarimeter. Chromatography was performed using silica gel, reverse phase C-18, and diaion HP-20 resins as stationary phase. HPLC was carried out using an AGILENT 1100 HPLC system. For thin layer chromatography, precoated silica gel 60 F_{254} and RP-18 F_{254S} plates were used. The compounds were visualized by spraying with a solution of H_{2}SO_{4} 5% in ethanol, followed by heating with a heat gun.

Plant Material

*A armata* roots were collected in Danang province, Vietnam in January 2021. The plant was identified by Dr Nguyen The Cuong at the Institute of Ecology and Biological Resources, VAST. A voucher specimen (coded: NCCT-P71R) was deposited at the Institute of Marine Biochemistry, VAST.

Extraction and Isolation

The dried roots of *A armata* (5.0 kg) were cut into small species, powdered and then ultrasonically extracted with MeOH, 3 times (each 12 L of MeOH for 60 min). After filtration, the solvent was removed in vacuo to give 135 g of methanol extract. This was suspended in water and successively partitioned with dichloromethane and ethyl acetate (EtOAc) to give organic soluble fractions and a water layer. The water layer was chromatographed on a diatom (HP-20) column washing with water to remove salts and oligosaccharides. Saponins were stepwise eluted by methanol/water (25%, 50%, 75%, and 100%) volume of methanol) to give 4 fractions AA1-AA4. Fraction AA2 (12.0 g) was chromatographed on a silica gel column, eluting with dichloromethane/methanol (1/0-0/1, v/v) to give 4 fractions AA2A-AA2D. Fraction AA2C (3.0 g) was chromatographed on a reverse phase C18 column, eluting with methanol/water (3/5, v/v) to give 4 fractions AA2C1-AA2C4. Fraction AA2C2 was further chromatographed by HPLC (1’ sphere H-80 column, 250 mm length×20 mm ID), eluting with 16% acetonitrile in water, with a flow rate of 2 mL/min to give compounds 1 (9.7 mg) and 2 (11.3 mg).

23-Hydroxyoleanolic Acid-[28-O-β-D-Glucopyranosyl]-3-O-β-D-Glucopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→3)]-β-D-Galactopyranoside (Aramatossil C, 1). White amorphous powder, [α]_{D}^{25} + 51.0 (c 0.1, MeOH); HR-ESI-MS m/z 1155.5341 [M + 37Cl]^{+} (calcd. for C_{54}H_{88}O_{23}Cl: 1155.5354, Δ = -0.1 ppm), m/z 1157.5289 [M + 35Cl]^{+} (calcd. for C_{54}H_{86}O_{23}Cl: 1157.5325, Δ = -0.31 ppm); δ H NMR (CD_{3}OD, 500 MHz) and 13C NMR (CD_{3}OD, 125 MHz) data, see Tables 1 and 2.

Oleanolic Acid-[28-O-β-D-Glucopyranosyl]-3-O-β-D-Glucopyranosyl-(1→2)-[β-D-Glucopyranosyl-(1→3)]-β-D-Galactopyranoside (Aramatossil D, 2). White amorphous powder, [α]_{D}^{25} + 45.0 (c 0.1, MeOH); HR-ESI-MS m/z 1139.5402 [M + 35Cl]^{+} (calcd. for C_{54}H_{86}O_{23}Cl: 1139.5405, Δ = 0.2 ppm), m/z 1141.5414 [M + 37Cl]^{+} (calcd. for C_{54}H_{88}O_{23}Cl: 1141.5375, Δ = +3.4 ppm); 1H NMR (CD_{3}OD, 500 MHz) and 13C NMR (CD_{3}OD, 125 MHz) data, see Tables 1 and 2.

Acid Hydrolysis of Compounds 1 and 2. Compounds 1 and 2 (each, 8.0 mg) were dissolved in 0.5 mL of 6 N HCL and heated at 60 °C for 1.5 h. After cooling, the mixtures were extracted with EtOAc. The acid aqueous layer was neutralized with 1 N NaOH and freeze-dried. The 2 sugars were identified as glucose and galactose by comparison with authentic samples by TLC using MeCOEt–isoPrOH–Me_{2}CO–H_{2}O (20:10:7:6). After preparative TLC of the sugar mixture (for each 1 and 2, 3.5 mg) using this solvent, each isolated sugar was filtered to eliminate SiOH residue. The optical rotation of each purified sugar was measured to afford glucose and galactose as +19.1 (c 0.1, H_{2}O) and +46.2 (c 0.08, H_{2}O), respectively. By comparing the optical rotations with D-glucose [α]_{D}^{25} + 18.0 (c 0.1, H_{2}O) and D-galactose [α]_{D}^{25} + 45.0 (c 0.08, H_{2}O), the glucose and galactose in compounds 1 and 2 were determined to have β-D- and α-D- configurations.

Cytotoxic Assay

The cytotoxic assays are the same as described in our previous work (see Supplemental Material).
Conclusions
The 2 previously undescribed oleanane-type triterpene glycosides, oleanolic acid-[28-\(\beta\)-D-glucopyranosyl-3-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)2)-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)3)]-\(\beta\)-D-galactopyranoside (I) and 23-hydroxyoleanolic acid-[28-\(\beta\)-D-glucopyranosyl-3-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)2)-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)3)]-\(\beta\)-D-galactopyranoside (2) were isolated from the roots of *A. armata*. Their chemical structures were elucidated by using a combination of HR-ESI-MS, 1D NMR (1H, 13C NMR) and 2D NMR (HSQC, HMBC, 1H-1H COSY, NOESY) spectral data, as well as by comparison with the previous literature. Compounds 1 and 2 displayed moderate cytotoxic activity towards KB and HepG2 cell lines with IC\(_{50}\) values ranging from 23.7 ± 0.9 to 29.5 ± 1.3 µM, compared to that of the positive control compound, ellipticine (IC\(_{50}\): 1.3 ± 0.1 and 1.6 ± 0.1 µM, respectively) in in vitro assay.

Declaration of Conflicting Interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article. This work was supported by the Funds for Science and Technology of the University of Danang, University of Science and Education (grant number T2020-TĐ-01) and Vingroup Joint Stock Company and supported by the Domestic Master/ PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), Vingroup Big Data Institute (VINBIGDATA) for Nguyen Thi Hong Chuong (grant number: VINIF2020.TS.55).

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Supplemental Material
Supplemental material for this article is available online.

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