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Oleanane-type triterpene saponins from *Aralia armata* leaves and their cytotoxic activity

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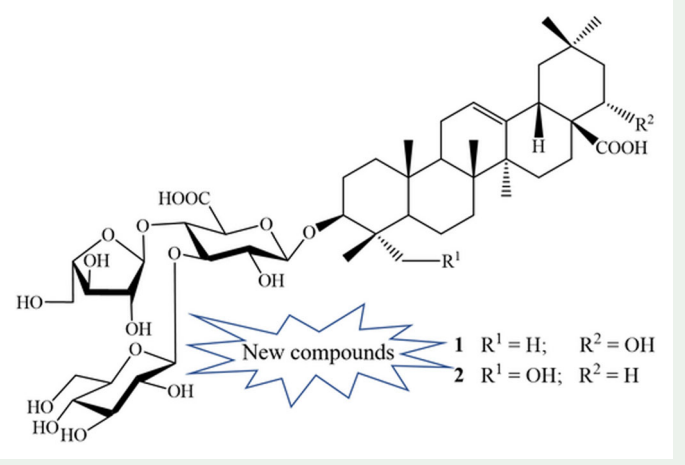
ABSTRACT

Two new, aramatosides A and B (**1** and **2**), together with seven known oleanane-type triterpene saponins (**3**–**9**) were isolated from the leaves of *Aralia armata*. Their structures were determined by combination of HR-ESI-MS, 1 D and 2 D NMR spectral data as well as comparison with the previous literature. Compounds **6**–**9** exhibited cytotoxic effects towards three human cancer cell lines (HT29, A2058, and A549) with IC_{50} values ranging from 2.01 ± 0.17 to 18.8 ± 1.17 μM. Especially, compound **7** (narcissiflorin) showed significant cytotoxic activity against HT29 and A549 cell lines with IC_{50} values of 2.02 ± 1.65 and 2.01 ± 0.17 μM, respectively, which are smaller than those of positive control irinotecan hydrochloride (IC_{50} values of 10.3 ± 1.32 and 9.89 ± 0.19 μM).



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1. Introduction

Genus *Aralia* comprises over 70 species that distributes mainly in the Asia, North and South America (Jason and Ella 2014). The dried leaves, roots and stem barks of several *Aralia* species have been used in the traditional medicines where they are indigenous, such as *A. armata* (Hu et al. 1995; Miao et al. 2016), *A. elata* (Song et al. 2001; Zhang et al. 2018), *A. chinensis* (Zhang et al. 2019), and *A. echinocaulis* (Li et al. 2017). Several *Aralia* plants have been chemically investigated and obtained substances belonging triterpenoid saponins, diterpenoids, phenolics, and acetylenic lipids. Especially, oleanane-type triterpene saponins were reviewed to be major chemical constituents of *Aralia* genus and expected responsibility for anti-inflammation, anti-diabetes, cardiovascular properties, and anti-proliferative effects (Jason and Ella 2014). The *A. armata* is common herbal in China and Viet Nam that has been used with tonic, anti-inflammation, and anti-bacterial properties. Up to date, a few chemical studies have been done and led to identification of triterpenes, triterpene saponins from the stem bark and root bark of *A. armata*, showing herbicidal and cytotoxic activities (Hu et al. 1995; Miao et al. 2016). Continuing research to find triterpene saponins from medicinal plant *A. armata* (Trang et al. 2015), herein, we report the identification of two new (**1** and **2**) and seven known oleanane-type triterpene saponins (**3**–**7**) from the leaves of *A. armata*. Cytotoxic activity of the isolated compounds on HT29 (human colorectal adenocarcinoma), A2058 (human caucasian metastatic melanoma), and A549 (human lung adenocarcinoma) cell lines were also evaluated.

2. Results and discussion

Compound **1** was isolated in form of white amorphous powder. The HR-ESI-MS (negative mode, Figure S3) of **1** showing quasi-molecular ion peaks at *m/z* 941.4767 $[M\text{-}H]^-$ and *m/z* 977.4533 $[M+Cl]^-$ indicated its molecular formula to be $C_{47}H_{74}O_{19}$ (calcd. for $C_{47}H_{73}O_{19}$, 941.4746; and calcd. for $C_{47}H_{74}O_{19}{}^{35}Cl$, 977.4513). The ^1H NMR spectrum of compound **1** (Figure S4) showed 7 singlet methyl signals (δ_H 1.19, 1.06, 1.01, 0.96, 0.96, 0.86, and 0.85), an olefinic proton [δ_H 5.26 (1H, br s)], and three anomeric protons [δ_H 5.20 (1H, br s), 4.88 (1H, d, $J = 8.0$ Hz), 4.37 (1H, d, $J = 8.0$ Hz)]. The ^{13}C NMR of compound **1** contained signals corresponding to 47 carbon atoms which were categorized by DEPT spectra into 9 non-protonated carbons, 20 methines, 11 methylenes, and 7 methyl groups (Figure S5 and S6, Table S1). In which, two deshielded signals (δ_C 181.0 and 176.5) corresponded to two carbonyl groups. Two olefinic carbon signals [δ_C 144.6 (C) and 123.7 (CH)] suggested the presence of a C = C double bond. Three anomeric carbon signals [δ_C 108.3, 106.3, and 104.3] indicated the presence of three monosaccharide units. Those characteristic NMR data suggested compound **1** belong to oleanane-type triterpene saponin that commonly reported in the *Aralia* genus (Jason and Ella 2014). Moreover, aglycon moiety of **1** were deduced to be 3β,21α-dihydroxyolean-12-ene-28-oic acid by analysis of HSQC, HMBC, and COSY spectra (Figure S1, S7-S9). Particularly, the chemical shift values of H-3 (δ_H 3.14)/C-3 (δ_C 90.9), HMBC correlations between H_3-23 (δ_H 1.06)/H_3-24 (δ_H 0.85) and C-3/C-4 (δ_C 40.2)/C-5 (δ_C 57.0) indicated an oxygenated group at C-3. HMBC correlations between H_3-25 (0.96)/H_3-26 (δ_H 0.86) and C-9 (δ_C 48.8), ^1H-^1H COSY cross peaks of H-9 (δ_H 1.58)/H_2-11 (δ_H 1.88 and

1.91)/H-12 (δ_H 5.26), and chemical shift value of C-12 (δ_C 123.7) demonstrated a C = C double bond at C-12/C-13. The location of this double bond was further supported by HMBC correlation between H_3-27 (δ_H 1.19) and C-13 (δ_C 144.6). COSY cross peaks of H-18 (δ_H 2.84)/H_2-19 (δ_H 1.11 and 1.74), HMBC correlations between H_3-29 (δ_H 0.96)/H_3-30 (δ_H 1.01) and C-19 (δ_C 46.9)/C-20 (δ_C 32.1)/C-21 (δ_C 42.9), COSY cross peaks of H_2-21 (δ_H 1.41 and 1.39)/H-22 (δ_H 3.86), and chemical shift value of C-22 (δ_C 72.5) indicated an additional oxygenated group at C-22. HMBC correlation between H-22 and carbonyl carbon C-28 (δ_C 181.0) revealed a carboxylic group C-28 and then established structure of 3,21-dihydroxyolean-12-ene-28-oic acid moiety. Based on the biosynthesis of oleanane skeleton, two methyl groups (C-25 and C-26) and a proton H-18 located at β-orientations meanwhile two protons (H-5 and H-9) and a methyl group (C-27) were at α-orientations. Therefore, NOESY correlation between H-18 (δ_H 2.84) and H-22 (δ_H 3.86) indicated α-orientation of hydroxy group at C-22. NOESY correlation between H-5 (δ_H 0.79) and H-3 (δ_H 3.14) confirmed β-orientation of oxygenated group at C-3 (Figure S1 and S10). Signals of 17 carbon atoms belonging sugar moiety indicated that it contained two hexose and one pentose units. Furthermore, observation of a carbonyl carbon (δ_C 176.5) suggested the presence of a glucuronic acid monosaccharide. Anomeric carbon and proton signals (δ_H 5.20/δ_C 108.3) were characterized for an arabinofuranosyl group (Rengifo et al. 2017). Another monosaccharide group was expected to be glucopyranosyl group by six carbinol signals (δ_C 104.3, 78.2, 77.9, 75.6, 71.1, 62.2). Additionally, HMBC correlation between Glu H-1′ (δ_H 4.37) and aglycon C-3 (δ_C 90.9) indicated that *O*-glucuronopyranosyl group link to the aglycon at C-3. The NMR spectral assignment of glucuronopyranosyl group was further clarified by COSY cross peaks of Glu H-1′ (δ_H 4.37)/Glu H-2′ (δ_H 3.56)/Glu H-3′ (δ_H 3.77)/Glu H-4′ (δ_H 3.86)/Glu H-5′ (δ_H 3.69). HMBC correlation between Glc H-1″ (δ_H 4.88) and Glu C-3′ (δ_C 82.1), Ara H-1‴ (δ_H 5.20) and Glu C-4′ (δ_C 75.4) indicated *O*-glucopyranosyl group at C-3′ and *O*-arabinofuranosyl group at C-4′ of glucuronopyranosyl moiety, respectively. Multiplicity of anomeric protons Glu H-1′ (d, $J = 8.0$ Hz), Glc H-1″ (d, $J = 8.0$ Hz), and Ara H-1‴ (br s) suggested β-linkage of glucuronopyranosyl, β-linkage of glucopyranosyl, and α-linkage of arabinofuranosyl groups, respectively. Monosaccharides in the sugar residue were further confirmed to be D-glucuronic acid, D-glucose, and L-arabinose by hydrolysis, convert to thiazolidine derivatives, HPLC analysis and comparison retention time with that of standard monosaccharide derivatives prepared in the same procedure (Tanaka et al. 2007). Consequently, compound **1** was determined to be 3β,21α-dihydroxyolean-12-ene-28-oic acid 3-*O*-[4′-*O*-α-L-arabinofuranosyl-(3′-*O*-β-D-glucopyranosyl)]-β-D-glucuronopyranoside, a new compound named as aramatoside A.

Compound **2** was isolated as a white amorphous powder. A pair of quasi-molecular ion peaks at *m/z* 941.4768 $[M\text{-}H]^-$ (calcd. for $C_{47}H_{73}O_{19}$, 941.4746) and *m/z* 977.4536 $[M + Cl]^-$ (calcd. for $C_{47}H_{74}O_{19}{}^{35}Cl$, 977.4513) in the HR-ESI-MS of **2** (Figure S11) indicated that compounds **2** and **1** shared the same molecular formula of $C_{47}H_{74}O_{19}$. The ^{1}H-NMR spectrum of **2** (Figure S12) observed 6 singlet methyl groups [δ_H 0.71, 0.88, 0.90, 0.97, 0.99, 1.17 (each 3H, s)], an olefinic proton [δ_H 5.23 (1H, br s)], and three anomeric protons [δ_H 4.47 (1H, d, $J = 8.0$ Hz), 4.84 (1H, d, $J = 8.0$ Hz), 5.20 (1H, br s)]. The ^{13}C-NMR and DEPT spectra of **2** showed signals corresponding to 47 carbon atoms including 9 non-protonated carbons, 19 methines, 13 methylenes, and 6 methyl groups

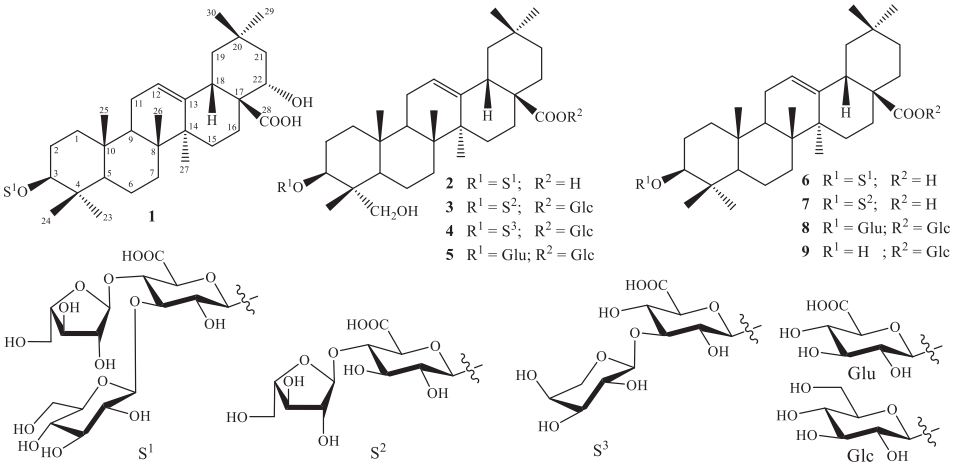

Figure 1. Chemical structures of oleanane-type triterpene glycosides isolated from *A. armata*.

(Figure S13-S17). Seventeen carbons in sugar moiety of **2** were in close similar chemical shift values with those of **1** which indicated compounds **1** and **2** containing the same trisaccharide moiety 4′-*O*-α-L-arabinofuranosyl-(3′-*O*-β-D-glucopyranosyl)]-β-D-glucuronopyranoside (Table S2). HMBC correlation between Glu H-1′ (δ_H 4.47) and C-3 (δ_C 83.1) confirmed *O*-glycosidic linkage at C-3 of the aglycone (Figure S2). Moreover, HMBC correlations between H_3-24 (δ_H 0.71) and C-3/C-4 (δ_C 43.9)/C-5 (δ_C 48.2)/C-23 (δ_C 65.0) indicated the presence of hydroxy group at C-23. The COSY cross peaks of H-9 (δ_H 1.74)/H-11 (δ_H 1.92)/H-12 (δ_H 5.23) and HMBC correlation of H_3-27 (δ_H 1.17)/C-13 (δ_C 146.6) supported for the position of C = C double bond at C-12/C-13. Therefore, the aglycone of **2** was established to be 3β,23-dihydroxyolean-12-ene-28-oic acid. Due to containing hydroxy group at C-23, the stereochemistry at C-4 of **2** was then demonstrated by NOESY analysis (Fig S2 and S18). NOESY correlations between H_3-24 (δ_H 0.71) and H_3-25 (δ_H 0.99), H-5 (δ_H 1.25) and H_2-23 (δ_H 3.62 and 3.28)/H-3 (δ_H 3.64) indicated that methyl groups C-24 and C-25 located at β-orientations meanwhile protons H-3, H-5 and hydroxymethylene group (C-23) located at α-orientations. Consequently, compound **2** was determined to be 3β,23-dihydroxyolean-12-ene-28-oic acid 3-*O*-[4′-*O*-α-L-arabinofuranosyl-(3′-*O*-β-D-glucopyranosyl)]-β-D-glucuronopyranoside, a new compound named as aramatoside B.

Other compounds were determined to be 3-*O*-α-L-arabinofuranosyl-(1→4)-β-D-glucuronopyranosylhederagenin 28-*O*-β-D-glucopyranosyl ester (**3**) (Rengifo et al. 2017), 3-*O*-[α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranosylhederagenin 28-*O*-β-D-glucopyranosyl ester (**4**) (Rastrelli et al. 1996), 3-*O*-β-D-glucuronopyranosylhederagenin 28-*O*-β-D-glucopyranosyl ester (**5**) (Dovgii et al. 2006), Stipuleanoside R1 (**6**) (Hu et al. 1995), Narcissiflorin (7) (Masood et al. 1981; Fujioka et al. 1989), Chikusetsusaponin IVa (8) (Liang et al. 2010), and oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**9**) (Young et al. 1997) by their good consistence with those reported in the literature (Figure 1).

The isolated compounds were then evaluated their cytotoxic activity. Compounds **1**-**9** were first screened their effects on the viability of HT29 cells. At a concentration of 30 μM, compounds **1**-**5** showed inactivity (cell viability over than 90%) meanwhile compounds **6**-**9** exhibited potential cytotoxic activity with cell viability ranging from

4.21 ± 0.06% to 7.56 ± 0.11% versus vehicle experiments (Table S3). Based on the screening results, compounds **6**-**9** were subjected to further study to find IC_{50} values and investigate their cytotoxicity against two additional human cancer cell lines including A2058 and A549 cell lines. As the results (Table S4), compounds **6**–**9** displayed cytotoxic activity toward HT29, A2058, and A549 cell lines with IC_{50} values from 2.01 ± 0.17 to 18.8 ± 1.17 μM. Compound **7** exhibited the best cytotoxic effect toward HT29, A2058, and A549 cell lines with the IC_{50} values of 2.02 ± 1.65, 4.26 ± 0.50, 2.01 ± 0.17 μM, respectively. Additionally, in the HT29 and A549 cell lines experiments, the IC_{50} values of compounds **7** (2.02 ± 1.65 and 2.01 ± 0.17) and **9** (6.51 ± 1.64 and 6.51 ± 1.6) were found smaller than those of known anti-cancer agent, irinotecan hydrochloride (10.3 ± 1.32 and 9.89 ± 0.19). For the structure and cytotoxicity relationship of compounds **1**-**9**, our results revealed that an additional of hydroxy group even at C-23 or C-22 of 3β-hydroxyolean-12-ene-28-oic moiety could be reduced their cytotoxic activity on HT29, A2058, and A549 cell lines.

3. Experimental

3.1. General

Optical rotation was measured on a Jasco P2000 polarimeter. NMR spectra were recorded on a Bruker 500 MHz spectrometer. HR-ESI-MS was carried out on an Agilent 6530 Accurate Mass Q-TOF LC/MS. The QTOF instrument was set at 2 GHz extended dynamic range resolution mode, negative ESI capillary voltage of 3500 V, fragmentor voltage of 175 V, MS scan ranging at *m/z* 100-1700, and MS acquisition rate of 1.0 spectra per second. Column chromatography was performed using silica gel, reverse phase C-18, and diaion HP-20 resins as stationary phase. Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} and RP-18 F_{254S} plates. The spots were visualized by spraying with solution of H_2SO_4 5% in ethanol followed by heating with a heat gun.

3.2. Plant material

The plant samples, *Aralia armata* leaves, were collected at Vinh Phuc province, Viet Nam in December 2017. Its scientific name was identified by botanist Nguyen The Cuong at the Institute of Ecology and Biological Resources, VAST. Voucher specimen (coded: NCCT-P71) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

Dried *Aralia armata* leaves (6 kg) were pulverized and then ultrasonically extracted with MeOH for three times (each 15 L of MeOH in 30 minutes). After filtration, the solvent was removed *in vacuo* to give 240 g of a dark methanolic residue. This crude extract was suspended in water and successively partitioned with dichloromethane and ethyl acetate to give organic soluble fractions and water layer. The water layer was poured on a diaion (HP-20) column and washed with water to remove salts and oligosaccharides. Saponin compounds were stepwise eluted by methanol/water (25%,

50%, 75%, and 100% volume of methanol) to give four fractions AAW1-AAW4. Fraction AAW2 (15.5 g) was separated by silica gel column chromatography, eluting with dichloromethane/methanol (1/0-0/1, v/v) to give five fractions AAW2A- AAW2E. Fraction AAW2B was further fractionated on a reverse phase C18 column, eluting with methanol/water (2/3, v/v) to give three fractions AAW2B1- AAW2B3. Fractions AAW2B1 and AAW2B3 were purified on silica gel column, eluting with dichloromethane/acetone/water (1/4/0.6, v/v/v) to yield compounds **3** (20 mg) and **4** (15 mg), respectively. Fraction AAW2B2 was purified on a reverse phase C18 column, eluting with acetone/water (2/5, v/v) to give compound **5** (11 mg). Fraction AAW4 (40 g) was also separated by silica gel column chromatography, eluting with dichloromethane/methanol (1/0-0/1, v/v) to give five fractions AAW4A- AAW4E. Fraction AAW4A was first chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/3/0.4, v/v/v) and then purified by reverse phase C18 column eluting with acetone/water (2/5, v/v) to yield compound **9** (9 mg). Fraction AAW4B was separated on a reverse phase C18 column, eluting with acetone/water (2/5, v/v) to obtain three fractions AAW4B1-AAW4B3. Fraction AAW4B1 and AAW4B2 were purified on a silica gel column, eluting with dichloromethane/acetone/water (1/3/0.4, v/v/v) to yield compounds **7** (8 mg) and **2** (8 mg), respectively. Fractions AAW4B3 was purified on a reverse phase column, eluting with methanol/water (1/1.2, v/v) to yield compound **6** (10 mg). Fraction AAW4C was purified on a reverse phase C18 column, eluting with methanol/water (1/1, v/v) to give compound **8** (12 mg). Compound **1** (20 mg) was isolated from fraction AAW4D by a reverse phase C18 column using acetone/water (2/5, v/v) as an eluent.

3.3.1. Aramatoside A (1)

White amorphous powder, $[\alpha]_D^{25}$: −73.6° (*c* 0.1, MeOH); HR-ESI-MS *m/z* 941.4767 $[M\text{-}H]^-$ (calcd. for $C_{47}H_{73}O_{19}$, 941.4746), *m/z* 977.4533 $[M+Cl]^-$ (calcd. for $C_{47}H_{74}O_{19}{}^{35}Cl$, 977.4513); ^1H NMR (CD_3OD, 500 MHz) δ_H ppm: 0.98 (m, H_a-1), 1.60 (m, H_b-1), 1.68 (m, H_a-2), 1.90 (m, H_b-2), 3.14 (dd, $J = 4.5$ and 12.0 Hz, H-3), 0.79 (m, H-5), 1.42 (m, H_a-6), 1.56 (m, H_b-6), 1.32 (m, H_a-7), 1.53 (m, H_b-7), 1.58 (m, H-9), 1.88 (m, H_a-11), 1.91 (m, H_b-11), 5.26 (br s, H-12), 1.11 (m, H_a-15), 1.90 (m, H_b-15), 1.69 (m, H_a-16), 1.96 (m, H_b-16), 2.84 (dd, 4.0, 14.0, H-18), 1.11 (m, H_a-19), 1.74 (m, H_b-19), 1.41 (m, H_a-21), 1.39 (m, H_b-21), 3.86 (m, H-22), 1.06 (s, H_3-23), 0.85 (s, H_3-24), 0.96 (s, H_3-25), 0.86 (s, H_3-26), 1.19 (s, H_3-27), 0.96 (s, H_3-29), 1.01 (s, H_3-30), **Glu**: 4.37 (d, $J = 8.0$ Hz, H-1′), 3.56 (dd, $J = 8.0$ and 9.0 Hz, H-2′), 3.77 (t, $J = 9.0$ Hz, H-3′), 3.86 (t, $J = 9.0$ Hz, H-4′), 3.69 (m, H-5′), **Glc**: 4.88 (d, $J = 8.0$ Hz, H-1″), 3.27 (dd, $J = 8.0$ and 9.0 Hz, H-2″), 3.38 (t, $J = 9.0$ Hz, H-3″), 3.36 (t, $J = 9.0$ Hz, H-4″), 3.28 (m,H-5″), 3.71 (overlapped, H_a-6″), 3.83 (overlapped, H_b-6″), **Ara**: 5.20 (br s, H-1‴), 4.02 (br s, H-2‴), 3.81 (br d, $J = 3.0$ Hz, H-3‴), 4.40 (m, H-4‴), 3.65 (dd, $J = 5.5$ and 11.5 Hz, H_a-5‴), 3.70 (dd, $J = 5.5$ and 11.5 Hz, H_b-5‴); ^{13}C NMR (CD_3OD, 125 MHz) δ_C ppm: 39.8 (C-1), 26.9 (C-2), 90.9 (C-3), 40.2 (C-4), 57.0 (C-5), 19.3 (C-6), 34.0 (C-7), 43.3 (C-8), 48.8 (C-9), 37.9 (C-10), 24.5 (C-11), 123.7 (C-12), 144.6 (C-13), 40.7 (C-14), 28.5 (C-15), 17.4 (C-16), 53.6 (C-17), 44.0 (C-18), 46.9 (C-19), 32.1 (C-20), 42.9 (C-21), 72.5 (C-22), 28.5 (C-23), 17.0 (C-24), 15.9 (C-25), 17.9 (C-26), 26.8 (C-27), 181.0 (C-28), 33.6 (C-29), 25.2 (C-30), **Glu**: 106.3 (C-1′), 76.5 (C-2′), 82.1 (C-3′), 75.4 (C-4′), 78.3 (C-5′), 176.5 (C-6′), **Glc**: 104.3 (C-1″), 75.6 (C-2″), 77.9 (C-3″), 71.1 (C-4″), 78.2 (C-5″), 62.2 (C-6″), **Ara**: 108.3 (C-1‴), 81.9 (C-2‴), 79.3 (C-3‴), 87.2 (C-4‴), 63.3 (C-5‴).

3.3.2. Aramatoside B (2)

White amorphous powder, $[\alpha]_D^{25}$: −38.2° (*c* 0.1, MeOH); HR-ESI-MS: *m/z* 941.4768 $[M-H]^-$ (calcd. for $C_{47}H_{73}O_{19}$, 941.4746), *m/z* 977.4536 $[M+Cl]^-$ (calcd. for $C_{47}H_{74}O_{19}{}^{35}Cl$, 977.4513); ^1H NMR (CD_3OD, 500 MHz) δ_H ppm: 0.96 (m, H_a-1), 1.60 (m, H_b-1), 1.73 (m, H_a-2), 1.91 (m, H_b-2), 3.64 (m, H-3), 1.25 (m, H-5), 1.39 (m, H_a-6), 1.49 (m, H_b-6), 1.29 (m, H_a-7), 1.61 (m, H_b-7), 1.64 (m, H-9), 1.92 (m, H_2-11), 5.23 (br s, H-12), 1.01 (m, H_a-15), 1.89 (m, H_b-15), 1.61 (m, H_a-16), 1.90 (m, H_b-16), 2.92 (dd, 3.5, 15.5, H-18), 1.11 (m, H_a-19), 1.69 (m, H_b-19), 1.17 (m, H_a-21), 1.37 (m, H_b-21), 1.51 (m, H_a-22), 1.76 (m, H_b-22), 3.28 (m, H_a-23), 3.62 (m, H_b-23), 0.71 (s, H_3-24), 0.99 (s, H_3-25), 0.88 (s, H_3-26), 1.17 (s, H_3-27), 0.90 (s, H_3-29), 0.97 (s, H_3-30), **Glu**: 4.47 (d, $J = 8.0$ Hz, H-1′), 3.55 (dd, $J = 8.0$ and 9.0 Hz, H-2′), 3.77 (t, $J = 9.0$ Hz, H-3′), 3.84 (t, $J = 9.0$ Hz, H-4′), 3.72 (m, H-5′), **Glc**: 4.84 (d, $J = 8.0$ Hz, H-1″), 3.28 (dd, $J = 8.0$ and 9.0 Hz, H-2″), 3.37 (t, $J = 9.0$ Hz, H-3″), 3.35 (t, $J = 9.0$ Hz, H-4″), 3.26 (m, H-5″), 3.71 (overlapped, H_a-6″), 3.84 (br d, $J = 12.0$ Hz, H_b-6″), **Ara**: 5.20 (br s, H-1‴), 4.02 (br s, H-2‴), 3.80 (br d, $J = 2.5$ Hz, H-3‴), 4.39 (m, H-4‴), 3.64 (dd, $J = 4.0$ and 12.0 Hz, H_a-5‴), 3.70 (dd, $J = 4.0$ and 12.0 Hz, H_b-5‴); ^{13}C NMR (CD_3OD, 125 MHz) δ_C ppm: 39.5 (C-1), 26.3 (C-2), 83.1 (C-3), 43.9 (C-4), 48.2 (C-5), 19.0 (C-6), 33.6 (C-7), 40.5 (C-8), 49.0 (C-9), 37.7 (C-10), 24.5 (C-11), 122.6 (C-12), 146.6 (C-13), 43.1 (C-14), 29.2 (C-15), 24.6 (C-16), 48.2 (C-17), 43.4 (C-18), 48.0 (C-19), 31.8 (C-20), 35.6 (C-21), 34.3 (C-22), 65.0 (C-23), 13.4 (C-24), 16.5 (C-25), 18.3 (C-26), 26.5 (C-27), 181.0 (C-28), 33.9 (C-29), 24.3 (C-30), **Glu**: 105.0 (C-1′), 76.1 (C-2′), 82.7 (C-3′), 75.2 (C-4′), 78.3 (C-5′), 176.6 (C-6′), **Glc**: 104.6 (C-1″), 75.6 (C-2″), 78.0 (C-3″), 71.1 (C-4″), 78.3 (C-5″), 62.2 (C-6″), **Ara**: 108.3 (C-1‴), 82.0 (C-2‴), 79.4 (C-3‴), 87.1 (C-4‴), 63.3 (Ara C-5‴).

4. Conclusions

Nine oleanane-type triterpene saponins (**1**-**9**) including two new compounds (**1** and **2**) were isolated from *Aralia armata* leaves. Their structures were elucidated by analysis of HR-ESI-MS, NMR spectral data, and comparison with those reported in the literature. Compounds **6**-**9** displayed great cytotoxic activity towards HT29, A2058, and A549 cell lines (IC_{50} from 2.01 ± 0.17 to 18.8 ± 1.17 μM) in compared to positive control (irinotecan hydrochloride, IC_{50} from 1.27 ± 0.56 to 10.3 ± 1.32 μM). Compounds **1**-**5** were considered as inactivity in our experiments. The results suggest that saponins containing 3β-hydroxyolean-12-ene-28-oic moiety could be potential cytotoxic agents against HT29, A2058, and A549 cell lines.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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