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Triterpenoid glycosides from the rhizomes of *Allium ascalonicum* and their anoctamin-1 inhibitory activity

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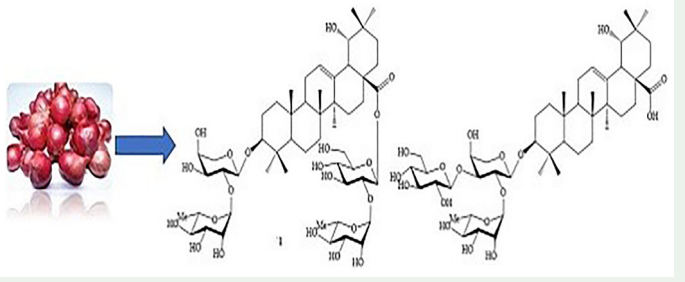
ABSTRACT

Ten triterpenoid glycosides including two undescribed compounds (**1** and **2**) were isolated from the methanol extract of *Allium ascalonicum* rhizomes. These compounds were structurally elucidated to be 3β-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-19α-hydroxyolean-12-ene-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester (**1**), 3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-3β,19α-dihydroxyoleanane-12-en-28-oic acid (**2**), lactifoloside C (**3**), lactifoloside H (**4**), randiasaponin IV (**5**), kudinoside G (**6**), ilexkudinoside W (**7**), lactifoloside G (**8**), kudinoside D (**9**), and ilexkudinoside T (**10**) by analyzing their HR-ESI-MS, NMR spectral data and by comparison with those reported in the literature. Compounds **1–10** were evaluated for anoctamin-1 (ANO1) inhibitory activity using yellow fluorescent protein reduction assays. At the concentration of 30 μM, compounds **2** and **9** displayed moderate ANO1 inhibitory percentages of 28.9 ± 0.85% and 26.2 ± 0.65%, respectively.



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1. Introduction

Allium is a large genus of the Alliaceae family, comprising approximately 500 species. Some of them such as *A. cepa* (onion), *A. sativum* (garlic), *A. porrum* (leek), *A. schoenoprasum* (chive), *A. fistulosum* (scallion), and *A. ascalonicum* (shallot) have been commonly used all over the world as vegetables or culinary supplements (Lanzotti et al. 2014; Singh et al. 2018). *Allium* vegetables as well as garlic were well known for thousands years in traditional medicines to treat inflammation and cardiovascular diseases. They have also been known as potent antitumor, antimicrobial, antiarthritic, hypolipidaemic, and hypoglycemic agents (Sengupta et al. 2004). *A. ascalonicum* is cultivated as a common vegetable in Viet Nam and many countries in Asia, Europe, America, and Africa (Loi 2012). In Vietnamese folk medicine, this plant is used to treat typhoid, fever, colds, headaches, bloating and poisoning, blurred vision, deaf ears, and breast swelling (Loi 2012). Previous phytochemical studies on *A. ascalonicum* indicated that it contains sulphur-containing compounds (Adeniyi and Anyiam 2004), flavonoids and furostanol saponins (Fattorusso et al. 2002). Herein, we report the isolation and structural elucidation of two new and eight known triterpenoid glycosides from the methanol extract of *A. ascalonicum* rhizomes. Additionally, the anoctamin-1 (ANO1) inhibitory activity of the isolated compounds was also investigated. ANO1, known as transmembrane protein 16A (TMEM16A), has been identified as a calcium-activated chloride channel expressed in various cell types (Yang et al. 2008; Seo et al. 2017). Since ANO1 plays pivotal roles in the regulation of various biological process, such as epithelia fluid secretion, smooth muscle contraction, cell proliferation and sensory signal transduction, the finding of ANO1 inhibitors would be considered as a new strategy for the treatment of several diseases such as pain, hypertension, asthma, diarrhea, and cancer (Bill and Gaither 2017). To date, only a few ANO1 inhibitors have been described, but several flavonoids and cycloartane triterpenoids have been reported to inhibit ANO1 (Seo et al. 2017; Thu et al. 2019). As mentioned above, inhibition and modulation of ANO1 function could be beneficial to therapeutics, so the chemical constituents of *A. ascalnoicum* were investigated for ANO1 inhibitory activity.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula of $C_{53}H_{86}O_{21}$ was deduced from the quasi molecular ion peaks $[M+^{35}Cl]^-$ at *m/z* 1093.5370 and $[M+^{37}Cl]^-$ at *m/z* 1095.5386 (calcd. for $C_{53}H_{86}O_{21}{}^{35}Cl$: 1093.5350 and calcd. for $C_{53}H_{86}O_{21}{}^{37}Cl$: 1095.5321) in the negative ion mode HR-ESI-MS. The ^1H NMR spectrum of **1** exhibited one olefin proton signal [δ_H 5.35 (br s)], seven methyl singlets [δ_H 0.78, 0.87, 0.95, 0.96, 0.98, 1.03, and 1.31 (each, 3H)], and two methyl doublets [δ_H 1.25 and 1.26 (each, 3H, d, $J = 6.0$ Hz)]. Four anomeric protons were identified at δ_H 4.58 (d, $J = 5.0$ Hz), 5.11 (d, $J = 1.5$ Hz), 5.39 (d, $J = 1.5$ Hz), and 5.44 (d, $J = 7.5$ Hz) suggesting the presence of four sugar units. The ^{13}C NMR and DEPT spectra revealed the signals of 53 carbons, including 8 non-protonated carbons, 25 methines, 11 methylenes, and 9 methyl groups. A double bond (C=C) was assigned by a pair of deshielded carbon signals at δ_C 124.8 and 144.3. The most deshielded carbon signal at δ_C 178.5 suggested the presence of one carboxyl group. Four carbon signals at δ_C

104.7, 102.1, 101.6, and 95.3 demonstrated the anomeric carbons of four sugar units. The aforementioned data suggested that compound **1** to be an oleanane-type triterpene glycoside having four sugar units. The HMBC correlations between H_3-23 (δ_H 1.03)/H_3-24 (δ_H 0.87) and C-3 (δ_C 90.8)/C-4 (δ_C 40.3)/C-5 (δ_C 57.2) suggested the position of the oxygenated group at C-3 and assignment of two methyl groups C-23/C-24 of the oleanane skeleton (Supplementary material, Figure S1). The HMBC correlations between H_3-25 (δ_H 0.95) and C-5 (δ_C 57.2)/C-9 (δ_C 49.1) and between H_3-26 (δ_H 0.78) and C-9 indicated assignment of two methyl groups C-25 and C-26. The HMBC correlations from H_3-26/H_3-27 (δ_H 1.31) to C-8 (δ_C 40.9)/C-9 (δ_C 49.1) and from H_3-27 to C-13 (δ_C 144.3) confirmed the presence of a methyl group at C-27 and a double bond at C-12/C-13, respectively. Moreover, COSY cross-peaks of H-9 (δ_H 1.75)/H_2-11 (δ_H 1.96 and 1.98)/H-12 (δ_H 5.35) further confirmed the position of double bond C-12/C-13. An additional hydroxy group at C-19 was deduced from the COSY cross peak of H-18 (δ_H 3.02)/H-19 (δ_H 3.28) and further reconfirmed by HMBC correlations between H_3-29 (δ_H 0.96)/H_3-30 (δ_H 0.98) and C-19 (δ_C 82.7). The carboxyl group was characterized by deshielded carbon signal at δ_C 178.5. Next, the orientation of two substituted groups (at C-3 and C-19) in the oleanane backbone was examined by *J* coupling constant of related carbinol protons as well as by NOESY analysis. The double doublet signals ($J = 11.5$, 4.5 Hz) of H-3 (δ_H 3.14) and the NOESY correlation of H-3/H-5 (δ_H 0.82) indicated *axial-alpha* orientation of H-3. NOESY correlations between H-18 (δ_H 3.02) and H_3-29 (δ_H 0.96)/H-19 (δ_H 3.28) and between H-19 and H_3-29 (δ_H 0.96) suggested *axial-alpha* orientation of OH-19 (Supplementary material, Figure S2). Therefore, the aglycone moiety of **1** was identified to be 3β,19α-dihydroxyolean-12-ene-28-oic acid. Except 30 carbon signals belonging aglycone moiety, remaining 23 carbons suggested the presence of three hexose and one pentose sugar units. Two broad singlets of anomeric protons (δ_H 5.11 and 5.39) implied α-linkages of two rhamnosyl sugars. The glucopyranosyl linkage must be in the β-form as judged from the large *J* coupling constant ($J = 7.5$ Hz) of the anomeric proton at δ_H 5.44. The pentose was assigned to be α-arabinopyranose indicated by $J_{H1\,-\,H2}$ coupling constant ($J = 5.0$ Hz) and a broad singlet of proton Ara H-4′ (δ_H 3.80). The presence of D-glucose, L-arabinose, and L-rhamnose in compound **1** was confirmed by acid hydrolysis. The sugars were converted into their TMS derivatives, and analyzed by GC in comparison with authentic sugar (Kiem et al. 2009). Continuously, the HMBC correlations between anomeric protons Ara H-1′ (δ_H 4.58) and C-3 (δ_C 90.8) and between Glc H-1‴ (δ_H 5.44) and C-28 (δ_C 178.5) suggested arabinopyranosyl group binding at C-3, and a glucopyranosyl group binding at C-28 of the aglycone. The COSY cross peak of Ara H-1′ (δ_H 4.58)/Ara H-2′ (δ_H 3.79) and HMBC correlation from RhaI H-1″ (δ_H 5.11) to Ara C-2′ (δ_C 76.9) indicated the first rhamnopyranosyl group link to C-2 of arabinopyranosyl group. Similarly, the COSY cross peak of Glc H-1‴ (δ_H 5.44)/Glc H-2‴ (δ_H 3.62) and the HMBC correlation from RhaII H-1⁗ (δ_H 5.39) to Glc C-2‴ (δ_C 77.0) indicated the second rhamnopyranosyl group link to C-2 of glucopyranosyl group. Consequently, the structure of compound **1** was determined to be 3β-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-19α-hydroxyolean-12-ene-28-oic acid 28-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester, a new compound named as alliumascaside A (Figure 1).

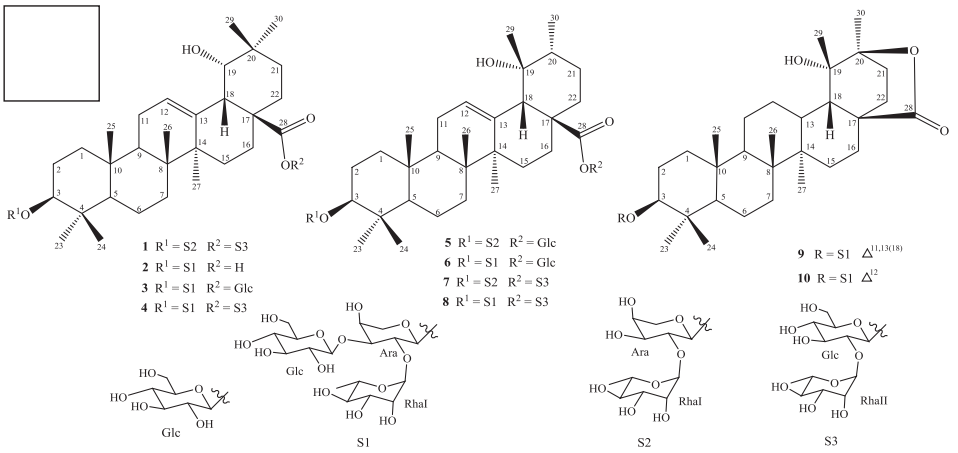

Figure 1. Chemical structures of compounds **1–10.**

The HR-ESI-MS analysis of **2** revealed quasi molecular ion peaks at *m/z* 947.4739 $[M+^{35}Cl]^-$ and *m/z* 949.4755 $[M+^{37}Cl]^-$ (calcd. for $C_{47}H_{76}O_{17}{}^{35}Cl$: 947.4771 and $C_{47}H_{76}O_{17}{}^{37}Cl$: 949.4742, respectively), indicating a molecular formula of $C_{47}H_{76}O_{17}$. The ^{1}H NMR spectrum of **2** exhibited one olefin proton signal [δ_H 5.33 (br s)], seven methyl singlets [δ_H 0.77, 0.88, 0.98, 1.05, 1.31 (each, 3H) and 0.96 (6H)], a methyl doublet [δ_H 1.25 (3H, d, $J = 6.0$ Hz)], and three anomeric protons [δ_H 4.51 (d, $J = 7.5$ Hz), 4.53 (d, $J = 5.0$ Hz), and 5.23 (br s)]. The ^{13}C NMR and DEPT spectra showed signals for 47 carbons, including 8 non-protonated carbons, 20 methines, 11 methylenes, and 8 methyl groups. A pair of deshielded carbon signals at δ_C 124.8 and 144.7 suggested the presence of a C=C double bond. Three anomeric carbons at δ_C 105.1, 102.0, and 104.3 further confirmed the presence of three sugar units. The ^{1}H and ^{13}C NMR spectral data of **2** were recognized to have close similarity with those of **1**, except signals corresponding to the sugar moieties (Supplementary material, Table S1). In particular, three pairs of anomeric protons/carbons containing a broad singlet of an anomeric proton (δ_H 5.23) and doublet methyl group (δ_H 1.25) indicated one rhamnose unit in compound **2**, which was different from the two rhamnose units in the structure of compound **1**. The presence of D-glucose, L-arabinose, and L-rhamnose sugars in compound **2** were also confirmed by GC analysis and comparison with authentic sugar standards after acid hydrolysis and TMS derivatization (Kiem et al. 2009). Furthermore, the multiplicity of ara H-1′ [4.58 (d, $J = 5.0$ Hz)], rha H-1″ [5.23 (br s)], and glc H-1‴ [4.51 (d, $J = 7.5$ Hz)] in the ^{1}H-NMR spectrum of compound **2** indicated α-form of the arabinopyranosyl, α-form of the rhamnopyranosyl, and β-form of the glucopyranosyl linkages, respectively. Additionally, the HMBC correlation from Rha H-1″ (δ_H 5.23) to Ara C-2′ (δ_C 75.3) and from Glc H-1‴ (δ_H 4.51) to Ara C-3′ (δ_C 81.9) indicated the structure of a trisaccharide in compound **2** as β-D-glucopyranosyl (1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl group. The HMBC correlation between Ara H-1′ (δ_H 4.58) and C-3 (δ_C 89.7) indicated the *O*-glycosidic linkage of that trisaccharide at C-3 of the aglycone moiety. Consequently, structure **2** was established to be 3-*O*-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-3β,19α-dihydroxyoleanane-12-en-28-oic acid, a new compound named as alliumascaside B.

Compounds **3–10** were identified as lactifoloside C (Ming An et al. 1997), lactifoloside H (Ouyang et al. 1998), randiasaponin IV (Sahpaz et al. 2000), kudinoside G (Ming An et al. 1997), ilexkudinoside W (Che et al. 2011), lactifoloside G (Ouyang et al. 1998), kudinoside D (Ouyang et al. 1996), and ilexkudinoside T (Tang et al. 2009), respectively, by analyses of their MS, 1D and 2D NMR spectroscopic data and by comparison with those reported in the literature (Figure 1). Compounds **1–10** were then evaluated for their ANO1 inhibitory effect using yellow fluorescent protein reduction assay (Seo et al. 2016). Experiments were performed in triplicate and the biological results were described in the percentage of inhibition (mean ± SD). Ani9 was purchased from ChemDiv (San Diego, CA) and the 3 μM Ani9 solution was used as positive control (97.5 ± 0.34% ANO1 inhibition). At the concentration of 30 μM, compounds **2** and **9** exhibited 28.9 ± 0.85% and 26.2 ± 0.65% ANO1 inhibitory activity. Other compounds (**1, 3–8**, **10**) were inactive. For the structure-activity relationship of ten isolated triterpene saponins, it is possible to suggest that compounds possessing free acid functional group at C-28 or having cyclic carboxylic ester between C-28 and C-20 showed moderate inhibitory activity against ANO1 while the attachment of sugar units at C-28 diminish the inhibitory activity against ANO1. Since modulation of ANO1 can play a critical role in the treatment of various diseases, screens of various compounds including natural compounds have been conducted to identify more potent and specific inhibitors of ANO1. However, many reported ANO1 inhibitors from natural products have been focused on small-sized phenolic compounds such as flavonoids and stilbenes (Chai et al. 2017; Seo et al. 2017). There have been few reports suggesting triterpene saponins as ANO1 inhibitors. In this study, ten triterpene saponins including two new compounds were identified from *A. ascalonicum* and two of them showed ANO1 inhibitory activity, suggesting the possibility of finding ANO1 inhibitors of various chemical structures from natural products.

3. Experimental

3.1. General

HR-ESI-MS were recorded on an Agilent 6530 accurate mass QTOF LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal Standard. Semi-preparative HPLC was performed on an Agilent 1100 HPLC system including degasser, quaternary pump, autosampler, DAD detector, J'sphere ODS M-80 column (150 × 20 mm), and flow rate of 3 mL/min. Silica gel, reverse phase C-18, and diaion HP-20 were used as adsorbent in flash column chromatography.

3.2. Plant material

The rhizomes of *A. ascalonicum* L. were collected in Hanoi City in December 2018, and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources. A voucher specimen (No: NCCT.2019.78) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powder of the rhizomes of *A. ascalonicum* L. (5.0 kg) was sonicated 3 times with methanol (10 L, each). After removal of solvent, the methanol extract (340 g) was suspended with water (4 L) and successively separated with dichloromethane to give dichloromethane residue (AA1, 150 g) and water layer (AA2). The water layer was chromatographed on a Diaion HP-20 column, eluting with water, and then methanol/water (1/3, 1/1, 3/1, and 1/0, v/v) to obtain four fractions W1-W4, respectively. The W2 fraction (38.0 g) was roughly separated on a silica gel column, eluting with CH_2Cl_2/MeOH (20/1, 10/1, 5/1, 2.5/1, 1/1, v/v) to give five sub-fractions W2A-W2E, respectively. Fraction W2C (16.0 g) was chromatographed on a RP-18 column, eluting with acetone/water (1/1.2, v/v) to give four fractions W2C1-W2C4. Fraction W2C2 (3.5 g) was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/4/0.2, v/v/v) to give two fractions W2C2A and W2C2B. Compounds **2** (3.2 mg) and **5** (3.6 mg) were obtained from W2C2A by HPLC running with ACN in H_2O (33%, v/v). Compounds **9** (3.6 mg) and **10** (9.1 mg) were obtained from fraction W2C2B by HPLC running with ACN in H_2O (40%, v/v). Fraction W2C3 was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/4/0.3, v/v/v) to give three fractions W2C3A-W2C3C. Fraction W2C3A was chromatographed on HPLC using ACN in H_2O (30%, v/v) to obtained compounds **1** (3.6 mg) and **7** (3.9 mg). Fraction W3 (48 g) was separated on a silica gel column eluting with CH_2Cl_2/MeOH (20/1, 5/1, 1/1, v/v) to give three sub-fractions W3A-W3C. Fraction W3A (14.0 g) was chromatographed on a RP-18 column eluting with acetone/water (1/1.2, v/v) to give four fractions W3A1-W3A4. Fraction W3A2 was first chromatographed on a RP-18 column chromatography eluting with acetone/water (4/1, v/v) and then purified on HPLC running with 30% acetonitrile to yield compounds **3** (4.1 mg) and **6** (6.4 mg). The W3B fraction (15.0 g) was chromatographed on a RP-18 column eluting with acetone/water (1/1.2, v/v) to give five fractions W3B2A-W3B2E. Fraction W3B2B (3.5 g) was first chromatographed on a RP-18 column chromatography eluting with acetone/water (4/1, v/v) and then purified on HPLC running with 35% acetonitrile to yield compounds **8** (16.6 mg) and **4** (5.0 mg).

3.3.1. Alliumascaside A (1)

Amorphous powder, $[\alpha]_D^{25}$: −95.0° (*c* 0.1, MeOH); HR-ESI-MS: *m/z* 1093.5370 $[M+^{35}Cl]^-$ (Calcd for $C_{53}H_{86}O_{21}{}^{35}Cl$, 1093.5350) and 1095.5386 $[M+^{37}Cl]^-$ (Calcd. for $C_{53}H_{86}O_{21}{}^{37}Cl$, 1095.5321); ^1H NMR (CD_3OD, 500 MHz) δ_H (ppm): 1.00 (m, H_a-1), 1.63 (m, H_b-1), 1.75 (m, H_a-2), 1.85 (m, H_b-2), 3.14 (dd, $J = 4.5$, 11.5 Hz, H-3), 0.82 (br d, 11.5 Hz, H-5), 1.45 (m, H_a-6), 1.58 (m, H_b-6), 1.43 (m, H_a-7), 1.51 (m, H_b-7), 1.75 (m, H-9), 1.96 (m, H_a-11), 1.98 (m, H_b-11), 5.35 (br s, H-12), 1.05 (m, H_a-15), 1.80 (m, H_b-15), 1.70 (m, H_a-16), 2.35 (m, H_b-16), 3.02 (br s, H-18), 3.28 (br d, $J = 3.5$ Hz, H-19), 1.16 (m, H_a-21), 1.47 (m, H_b-21), 1.66 (m, H_a-22), 1.84 (m, H_b-22), 1.03 (s, H-23), 0.87 (s, H-24), 0.95 (s, H-25), 0.78 (s, H-26), 1.31 (s, H-27), 0.96 (s, H-29), 0.98 (s, H-30), *Ara:* 4.58 (d, 5.0 Hz, H-1′), 3.79 (dd, $J = 5.0$, 8.5 Hz, H-2′), 3.76 (dd, $J = 3.0$, 8.5 Hz, H-3′), 3.80 (br s, H-4′), 3.87 (br d, $J = 11.5$ Hz, H_a-5′), 3.50 (dd, $J = 2.5$, 11.5 Hz, H_b-5′), *Rhal:* 5.11 (br s, H-1″), 3.96 (br d, $J = 3.0$ Hz, H-2″), 3.70 (dd, $J = 3.0$, 9.0) Hz, H-3″), 3.40 (t, $J = 9.0$ Hz, H-4″), 3.82 (m, H-5″), 1.25 (d, $J = 6.0$ Hz, H-6″), *Glc:* 5.44 (d, $J = 7.5$ Hz, H-1‴), 3.62 (dd, $J = 7.5$, 9.0 Hz, H-

2‴), 3.57 (t, $J = 9.0$ Hz, H-3‴), 3.43 (t, $J = 9.0$ Hz, H-4‴), 3.35 (m, H-5‴), 3.78 (dd, $J = 2.0$, 11.5 Hz, H_a-6‴), 3.70 (dd, $J = 5.5$, 11.5 Hz, H_b-6‴), *Rhall*: 5.39 (br s, H-1⁗), 3.91 (br d, $J = 3.0$ Hz, H-2⁗), 3.70 (dd, $J = 3.0$, 9.0 Hz, H-3⁗), 3.40 (t, $J = 9.0$ Hz, H-4⁗), 3.83 (m, H-5⁗), 1.26 (d, $J = 6.0$ Hz, H-6⁗). ^{13}C NMR (CD_3OD, 125 MHz) δ_C (ppm): 39.8 (C-1), 27.2 (C-2), 90.8 (C-3), 40.3 (C-4), 57.2 (C-5), 19.5 (C-6), 33.1 (C-7), 40.9 (C-8), 49.1 (C-9), 38.1 (C-10), 24.7 (C-11), 124.8 (C-12), 144.3 (C-13), 42.8 (C-14), 29.6 (C-15), 28.5 (C-16), 47.3 (C-17), 45.3 (C-18), 82.7 (C-19), 35.9 (C-20), 29.6 (C-21), 34.1 (C-22), 28.5 (C-23), 17.0 (C-24), 16.0 (C-25), 17.8 (C-26), 24.7 (C-27), 178.5 (C-28), 28.6 (C-29), 25.3 (C-30), *Ara:* 104.7 (C-1′), 76.9 (C-2′), 73.0 (C-3′), 68.3 (C-4′), 64.2 (C-5′), *Rhal:* 102.1 (C-1″), 71.9 (C-2″), 72.2 (C-3″), 73.8 (C-4″), 70.2 (C-5″), 18.0 (C-6″), *Glc:* 95.3 (C-1‴), 77.0 (C-2‴), 79.2 (C-3‴), 71.4 (C-4‴), 78.4 (C-5‴), 62.4 (C-6‴), *Rhall:* 101.6 (C-1⁗), 72.2 (C-2⁗), 72.2 (C-3⁗), 73.9 (C-4⁗), 70.3 (C-5⁗), 18.2 (C-6⁗).

3.3.2. Alliumascaside B (2)

Amorphous powder, $[\alpha]_D^{25}$: - 76.0° (*c* 0.1, MeOH); HR-ESI-MS: *m/z* 947.4739 $[M+^{35}Cl]^-$ (Calcd. for $C_{47}H_{76}O_{17}{}^{35}Cl$, 947.4771) and 949.4755 $[M+^{37}Cl]^-$ (Calcd. for $C_{47}H_{76}O_{17}{}^{37}Cl$, 949.4742); ^{1}H NMR ($CD_3OD$, 500 MHz) δ_H (ppm): 1.01 (m, H_a-1), 1.63 (m, H_b-1), 1.75 (m, H_a-2)/1.85 (m, H_b-2), 3.13 (dd, $J = 4.5$, 11.5 Hz, H-3), 0.82 (br d, $J = 11.5$ Hz, H-5), 1.45 (m, H_a-6), 1.58 (m, H_b-6), 1.32 (m, H_a-7), 1.54 (m, H_b-7), 1.75 (m, H-9), 1.92 (m, H_a-11), 1.97 (m, H_b-11), 5.33 (br s, H-12), 1.06 (m, H_a-15), 1.80 (m, H_b-15), 2.28 (m, H_a-16), 1.64 (m, H_b-16), 3.09 (br s, H-18), 3.28 (br d, $J = 3.0$ Hz, H-19), 1.66 (m, H_a-21), 1.78 (m, H_b-21), 1.31 (m, H_a-22), 1.63 (m, H_b-22), 1.05 (s, H-23), 0.88 (s, H-24), 0.96 (s, H-25), 0.77 (s, H-26), 1.31 (s, H-27), 0.96 (s, H-29), 0.98 (s, H-30), *Ara*: 4.58 (d, $J = 5.0$ Hz, H-1′), 3.90 (dd, $J = 5.0$, 8.5 Hz, H-2′), 3.88 (dd, $J = 3.0$, 8.5 Hz, H-3′), 4.04 (br s, H-4′), 3.91 (br d, $J = 11.5$ Hz, H_a-5′), 3.52 (dd, $J = 2.5$, 11.5 Hz, H_b-5′), *Rhal*: 5.23 (br s, H-1″), 3.93 (br d, $J = 3.0$ Hz, H-2″), 3.73 (dd, $J = 3.0$, 9.0 Hz, H-3″), 3.40 (t, $J = 9.0$ Hz, H-4″), 3.86 (m, H-5″), 1.25 (d, $J = 6.0$ Hz, H-6″), *Glc*: 4.51 (d, $J = 7.5$ Hz, H-1‴), 3.30 (dd, $J = 7.5$, 9.0 Hz, H-2‴), 3.32 (t, $J = 9.0$ Hz, H-3‴), 3.35 (t, $J = 9.0$ Hz, H-4‴), 3.38 (m, H-5‴), 3.83 (dd, $J = 2.5$, 11.5 Hz, H_a-6‴), 3.70 (dd, $J = 5.5$, 11.5 Hz, H_b-6‴). ^{13}C NMR (CD_3OD, 125 MHz) δ_C (ppm): 39.9 (C-1), 27.2 (C-2), 89.7 (C-3), 40.4 (C-4), 57.3 (C-5), 19.5 (C-6), 33.1 (C-7), 40.9 (C-8), 49.1 (C-9), 38.1 (C-10), 24.8 (C-11), 124.8 (C-12), 144.7 (C-13), 42.6 (C-14), 29.7 (C-15), 28.6 (C-16), 47.0 (C-17), 45.4 (C-18), 82.6 (C-19), 36.0 (C-20), 29.5 (C-21), 34.0 (C-22), 28.6 (C-23), 17.2 (C-24), 15.9 (C-25), 17.8 (C-26), 25.0 (C-27), (C-28, not detected), 28.7 (C-29), 25.2 (C-30), *Ara*: 105.1 (C-1′), 75.3 (C-2′), 81.9 (C-3′), 68.5 (C-4′), 64.6 (C-5′), *Rhal*: 102.0 (C-1″), 72.1 (C-2″), 72.1 (C-3″), 73.8 (C-4″), 70.3 (C-5″), 18.0 (C-6″), *Glc*: 104.3 (C-1‴), 75.1 (C-2‴), 78.0 (C-3‴), 71.2 (C-4‴), 78.0 (C-5‴), 62.4 (C-6‴).

4. Conclusions

Ten triterpene glycosides including alliumascaside A (**1**) and alliumascaside B (**2**), lactifoloside C (**3**), lactifoloside H (**4**), randiasaponin IV (**5**), kudinoside G (**6**), ilexkudinoside W (**7**), lactifoloside G (**8**), kudinoside D (**9**), and ilexkudinoside T (**10**) were isolated from the rhizomes of *A. ascalonicum*. Compounds **1** and **2** were elucidated to be new compounds. All of the isolated compounds were evaluated for ANO1 inhibitory activity

at a concentration of 30 μM. Compounds **2** and **9** moderately exhibited activity with inhibition rates of 28.9 ± 0.85% and 26.2 ± 0.65%, respectively.

Disclosure statement

The authors declare no conflict of interest.

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