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Oleanane-type triterpenoid saponins from *Silene armeria*

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ABSTRACT

Twelve triterpenoid saponins, including seven compounds (i.e., armerosides A–G) hitherto unknown, were isolated from whole plants of *Silene armeria*. Their structures were established based on extensive spectroscopic analyses and chemical methods. From a biosynthetic perspective, C-23 oxidation of the sapogenin appears to be a key factor in the glycosylation pathway.

1. Introduction

The genus *Silene* belongs to the family Caryophyllaceae, which contains more than 700 species. These species include many herbaceous plants and are primarily distributed in temperate regions of the Northern Hemisphere (Mamadalieva et al., 2014). *Silene* plants contain triterpenoid saponins, and their surfactant properties have been exploited in some *Silene* plants as detergents since ancient times. However, phytochemical studies of triterpenoid saponins in plants of the *Silene* genus remain limited. Chemical investigations have been previously conducted on only six *Silene* species, including *Silene fortunei* (Lacaille-Dubois et al., 1999; Gaidi et al., 2002), *Silene jenisseensis* (Lacaille-Dubois et al., 1995, 1997), *Silene viscidula* (Xu et al., 2010, 2012), *Silene vulgaris* (Glensk et al., 1999; Bouguet-Bonnet et al., 2002; Larhsini et al., 2003), *Silene rubicunda* (Fu et al., 2005; Wu et al., 2015), and *Silene cucubalus* (Larhsini et al., 2003). These studies resulted in isolation and structural elucidation of approximately thirty types of triterpenoid saponins. As part of an ongoing phytochemical investigation of triterpenoid saponins from plants in the family Caryophyllaceae, isolation and structural elucidation of eight triterpenoid saponins from the roots of *S. rubicunda* was previously reported (Fu et al., 2005).

Silene armeria L., which is commonly known as Sweet William Catchfly, is an annual herbaceous plant that is native to Central and Northern Europe and widely distributed in temperate regions of Western Asia and America (Bajpai et al., 2008). This plant was introduced into Japan as an ornamental in the Edo period (1603-1868 CE). The chemical constituents of *S. armeria* are unknown, except for some essential oil compositions (Bajpai et al., 2008). Herein, the first phytochemical investigation of triterpenoid saponins from *S. armeria* is reported. Twelve triterpenoid saponins were isolated, including seven new compounds that were named armerosides A (**3**), B (**5**), C-F (**7**–**10**), and G (**12**).

2. Results and discussion

A methanol extract of whole plants of *S. armeria* was suspended in H_2O and then partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction was fractionated on a Diaion HP-20 column and eluted successively with water, aqueous MeOH (1:1) and MeOH. The MeOH eluate was concentrated to afford a crude saponin fraction. Further separation of this fraction by reversed-phase (RP) and normal-phase (NP) column chromatography, followed by repeated preparative HPLC purification, resulted in isolation of twelve triterpenoid saponins (**1**–**12**).

The known compounds were identified as 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-quillaic acid (**1**) (Lacaille-Dubois et al., 1995), 3-*O*-*β*-D-galactopyranosyl-(1 → 2)-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucuronopyranosyl-quillaic acid (**2**) (Guo et al., 1998), dianchinenoside D [3*β*,16*α*-dihydroxy-olean-12-en-23,28-dioic acid 28-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-

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glucopyranosyl ester] (**4**) (Li et al., 1994), sinocrassuloside I {3β,16α-dihydroxy-olean-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester} (**6**) (Zhao et al., 2004), and saponarioside K {3,16α-dihydroxy-3,4-seco-olean-4(24),12-dien-23,28-dioic acid 28-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester} (**11**) (Koike et al., 1999) based on detailed NMR spectroscopic analyses and comparison with literature data (Fig. 1). Most of the known compounds (**1**, **2**, **4**, **6**) were reported in other *Silene* species, i.e., **1** from *S. jenisseensis* (Lacaille-Dubois et al., 1995), **2** from *S. cucubalus* (Larhsini et al., 2003), and **4** and **6** from *S. viscidula* (Xu et al., 2012; Zhao et al., 2004). Saponin **11**, which was only isolated from *Saponaria officinalis* (Caryophyllaceae), was reported for the first time from a *Silene* species (Koike et al., 1999). The seven previously unknown compounds (**3**, **5**, **7**–**10**, and **12**) were given trivial names (i.e., armerosides A-G), and their chemical structures were elucidated by detailed spectroscopic analyses and chemical methods.

Armeroside A (**3**) was obtained as an amorphous powder. Its molecular formula was established as $C_{62}H_{96}O_{30}$, based on the positive-ion high-resolution (HR)-FAB-MS data that contained a $[M+Na]^+$ pseudo-molecular peak at *m*/*z* 1343.5846. In the ^{1}H- and ^{13}C-NMR spectra, characteristic resonances for the olean-12-ene skeleton were observed, including six tertiary methyl groups at δ_H 0.87, 0.96, 1.00, 1.10, 1.38, and 1.78 (each s) and δ_C 32.9, 26.8, 24.3, 17.2, 15.7, and 10.7 as well as an olefin moiety at δ_H 5.56 (br s) and δ_C 121.9 (C-12) and 144.5 (C-14) (Table 1 and Table 3). Other important NMR resonances, which were assigned to the sapogenin moiety, included: an aldehyde at δ_H 9.78 (s) and δ_C 209.7; an esterified carboxyl moiety at δ_C 175.8; and two oxygenated methines at δ_H 4.07 and δ_C 83.3, as well as δ_H 5.23 (br s) and δ_C 73.4, respectively. These data are in good agreement with those of 3,28-bisdesmosidic quillaic acid (3β,16α-dihydroxy-23-oxo-olean-12-en-28-oic acid; **17**) (Jia et al., 2002).

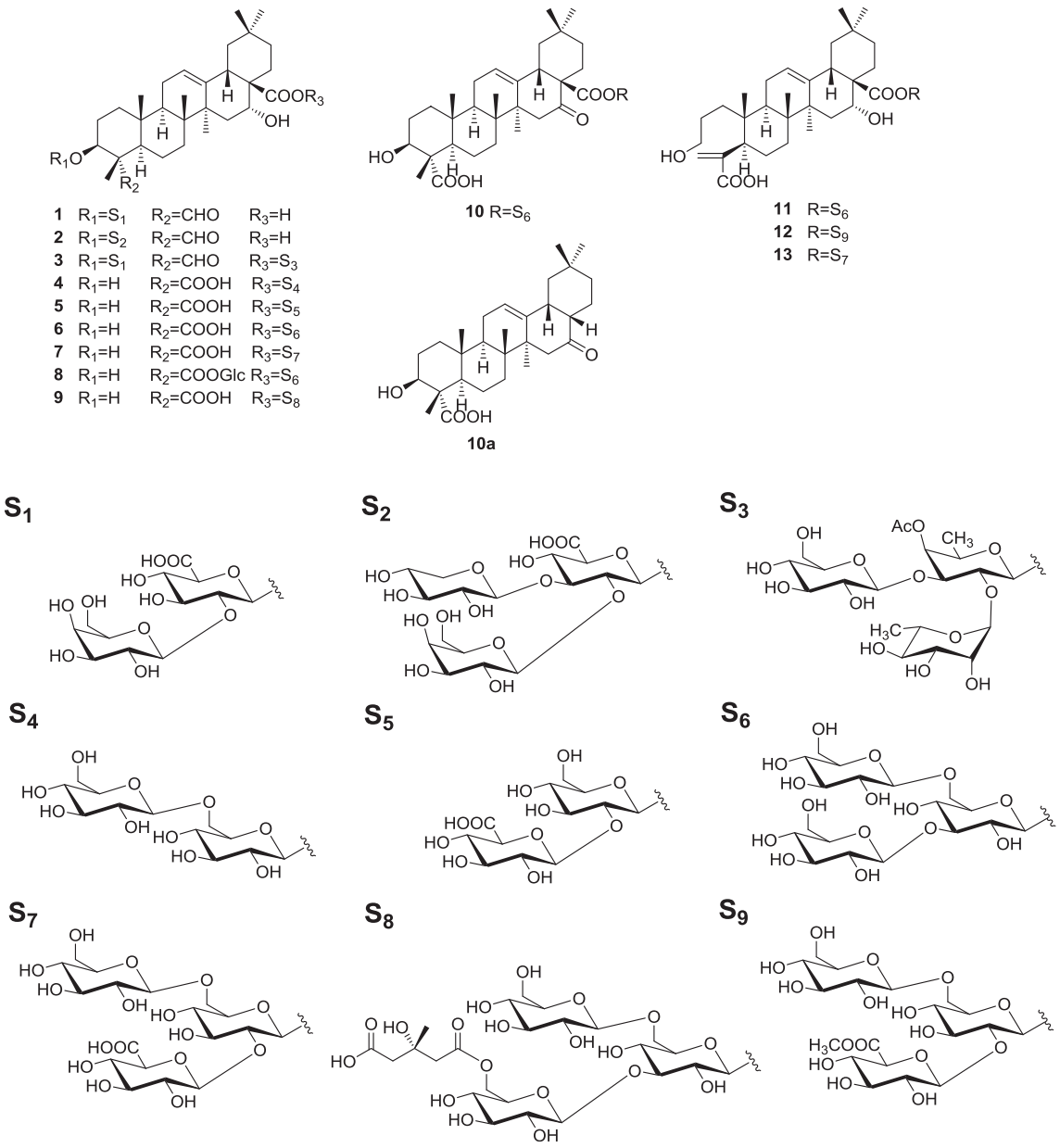

Fig. 1. Structures of the triterpenoid saponins isolated from *S. armeria*.

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Table 1
^{13}C NMR spectroscopic data (δ) for the aglycone moieties of **3**, **5**, **7**–**10**, **12**, and **10a** (125 MHz in pyridine-d_5).

Position	3	5	7	8	9	10	12	10a
1	38.1	37.8	39.2	39.0	39.3	39.0	37.3	38.6
2	24.8	26.4	27.8	27.5	27.9	27.6	27.6	27.6
3	83.3	74.1	75.6	75.2	75.6	75.6	63.4	75.2
4	54.9	53.1	54.7	55.2	54.5	54.3	146.3	54.2
5	48.3	50.6	52.0	52.3	52.1	51.7	43.7	51.9
6	20.3	20.4	21.8	21.3	21.8	21.6	26.1	21.5
7	31.6	32.0	33.4	33.1	33.3	32.8	32.6	33.0
8	40.2	39.0	40.5	40.4	40.5	40.7	39.8	39.1
9	46.8	46.2	47.6	47.5	47.6	47.2	37.6	47.7
10	36.1	35.5	36.9	37.0	37.0	36.8	39.9	36.9
11	23.6	22.5	23.9	23.9	23.9	23.9	24.5	23.4
12	121.9	120.6	122.0	122.7	122.7	124.8	122.5	117.6
13	144.5	143.5	144.7	144.5	144.4	140.9	144.8	142.7
14	42.1	40.6	42.0	42.0	42.1	48.6	42.6	42.8
15	36.1	34.8	36.2	36.1	36.2	46.3	36.3	44.0
16	73.4	72.7	74.0	74.2	74.2	208.4	75.6	213.4
17	49.4	47.6	49.0	49.1	49.2	59.0	49.2	49.6
18	41.4	40.0	41.3	41.2	41.3	46.8	41.4	40.0
19	47.4	46.0	47.4	47.2	47.2	46.7	47.5	42.8
20	30.5	29.3	30.7	30.8	30.8	30.6	30.8	30.5
21	35.8	34.7	36.0	35.9	35.9	34.9	31.9	38.5
22	32.6	30.2	31.6	32.1	32.1	27.1	36.0	23.7
23	209.7	179.2	180.6	177.6	180.6	181.2	171.5	180.2
24	10.7	10.8	12.2	12.0	12.2	12.4	124.2	11.9
25	15.7	14.8	16.3	16.3	16.3	16.1	19.1	15.8
26	17.2	16.1	17.5	17.5	17.6	17.5	17.8	16.7
27	26.8	25.6	27.0	27.1	27.2	27.4	27.2	25.4
28	175.8	174.4	175.9	175.8	175.8	171.7	176.0	
29	32.9	31.7	33.1	33.2	33.1	32.8	33.2	33.2
30	24.3	23.1	24.5	24.7	24.8	23.3	24.7	24.6

Table 2
^{13}C NMR spectroscopic data (δ) for the sugar moieties of **3**, **5**, **7**–**10**, and **12** (125 MHz in pyridine-d_5).

Position	3	5	7	8	9	10	12
3-*O*-sugar							
GlcA-1	103.1						
2	82.3						
3	77.5						
4	72.7						
5	77.3						
6	172.1						
Gal-1	106.2						
2	74.4						
3	77.0						
4	70.0						
5	74.7						
6	62.0						
28-*O*-sugar							
	Fuc	Glc	Glc 1	Glc 1	Glc 1	Glc 1	Glc 1
1	94.3	92.5	93.8	95.2	95.1	95.6	93.5
2	72.2	78.4	79.3	72.7	72.7	72.5	79.4
3	82.7	77.1	78.2	88.5	88.5	88.2	78.6
4	74.1	69.5	70.5	69.0	69.0	68.7	70.5
5	70.4	77.8	77.8	77.7	77.6	77.7	77.8
6	16.3	60.7	69.0	69.1	69.1	68.8	69.2
	Rha	GlcA	GlcA	Glc 2	Glc 2	Glc 2	GlcA
1	101.4	103.9	105.2	105.7	105.2	105.6	105.2
2	72.1	74.4	75.7	75.1	75.0	75.4	75.6
3	72.2	76.3	77.7	78.3	78.1	78.2	77.6
4	73.7	71.8	73.2	71.6	71.6	71.7	73.0
5	69.9	76.4	77.8	78.6	78.5	78.5	77.3
6	18.6	173.2	173.6	62.5	62.5	62.5	171.0
OCH_3							52.3
	Glc		Glc 2	Glc 3	Glc 3	Glc 3	Glc 2
1	105.4		105.3	105.4	105.6	105.4	105.4
2	75.0		75.7	75.4	75.2	75.1	75.2
3	78.2		78.3	78.3	78.2	78.3	78.4
4	71.0		71.6	71.7	71.7	71.7	71.7
5	78.2		78.3	78.4	75.4	78.3	78.4
6	62.5		62.6	62.8	64.7	62.8	62.8
23-*O*-sugar							
Glc-4-1				96.6			
2				74.4			
3				78.7			
4				71.3			
5				79.3			
6				62.4			
HMG-1					171.7		
2					46.3		
3					70.1		
4					46.6		
5					174.7		
6					28.1		
$\underline{C}OCH_3$	171.1						
$CO\underline{C}H_3$	20.6						

For the sugar moiety, the ^{1}H- and ^{13}C-NMR spectra of **3** contained resonances that were assigned to β-fucopyranosyl (Fuc), α-rhamnopyranosyl (Rha), β-glucopyranosyl (Glc), β-galactopyranosyl (Gal), and β-glucuronopyranosyl (GlcA) moieties with anomeric proton resonances at δ_H 6.03 (d, J = 7.8 Hz, Fuc-H-1), 6.47 (br s, Rha-H-1), 5.07 (d, J = 7.7 Hz, Glc-H-1), 5.23 (d, J = 7.5 Hz, Gal-H-1) and 4.94 (d, J = 7.5 Hz, GlcA-H-1), respectively. In addition, corresponding anomeric carbon resonances were observed at δ_C 94.3, 101.4, 105.4, 106.2 and 103.1 (Table 2 and Table 4). All of the NMR resonances that corresponded to sugars were assigned based on careful analyses of the ^{1}H-^{1}H COSY, TOCSY, HSQC, HSQC-TOCSY, HMBC and NOESY data. The sugar chain at C-3 of the sapogenin was established by HMBC correlations from δ_H 5.23 (Gal-H-1) to δ_C 82.3 (GlcA-C-2) and δ_H 4.94 (GlcA-H-1) to δ_C 83.3 (C-3). Similarly, the sugar chain at C-28 was established by HMBC correlations from δ_H 6.47 (Rha-H-1) to δ_C 72.2 (Fuc-C-2), δ_H 5.07 (Glc-H-1) to δ_C 82.7 (Fuc-C-3), and δ_H 6.03 (Fuc-H-1) to δ_C 175.8 (C-28). The sugar sequence was also confirmed by observation of NOESY correlations between the anomeric proton and the proton attached to the carbon where the sugar was linked. In addition to the resonances due to sapogenin and component sugars, the ^{1}H- and ^{13}C-NMR spectra also indicated presence of an acetyl moiety at δ_H 1.91 (s) and δ_C 171.1 and 20.6. Its position was deduced as Fuc-C-4 based on an HMBC correlation from the downfield shifted Fuc-H-4 at δ_H 5.85 to δ_C 171.1.

Acid hydrolysis of **3** with 1 M HCl in dioxane–H_2O (1:1) afforded a partially hydrolyzed product, 3-*O*-β-D-glucuronopyranosyl-quillaic acid (**21**) (Bouguet-Bonnet et al., 2002), and with 2 M aqueous trifluoroacetic acid (TFA) afforded the sapogenin, quillaic acid (**17**) (Iwamoto et al., 1985). Acid hydrolysis of structurally related saponins **1** and **2** yielded identical results. The component sugars were identified as D-galactose, L-rhamnose, D-glucose, D-fucose and D-glucuronic acid based on HPLC analyses of their 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives after acid hydrolysis and comparison to standard sugars (Li et al., 2005). Therefore, the chemical structure of armeroside A (**3**) was determined to be 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl-quillaic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-4-*O*-acetyl-β-D-fucopyranosyl ester.

Armerosides B (**5**), C (**7**), D (**8**), and E (**9**) were isolated as amorphous powders. Upon acid hydrolysis, these compounds afforded the same sapogenin (i.e., 3β,16α-dihydroxy-olean-12-en-23,28-dioic acid (**18**)) (Li et al., 1993) but different component sugars.

The molecular formula of armeroside B (**5**) was determined to be $C_{42}H_{64}O_{17}$ based on the positive-ion HR-FAB-MS data. In the ^{1}H- and ^{13}C-NMR spectra, in addition to the resonances assigned to the sapogenin, resonances for β-glucopyranosyl and β-glucuronopyranosyl moieties were observed with anomeric protons at δ_H 6.06 (d,

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Table 3
^{1}H NMR spectroscopic data (δ) for the aglycone moieties of **3**, **5**, **7**–**10**, **12**, and **1a** (500 MHz in pyridine-d_5).

Position	3	5	7	8	9	10	12	10a
1	0.90[a]	1.23 (m)	1.22 (m)	1.14 m	1.25[a]	1.18[a]	1.19[a]	1.17[a]
	1.49[a]	1.67[a]	1.68[a]	1.64[a]	1.69[a]	1.65 (br d, 13.4)	1.60 (m)	1.61[a]
2	1.18a	1.92[a] (2H)	1.92[a] (2H)	1.87 m	1.95[a] (2H)	1.93 (2H, m)	1.81 (m)	1.91[a](2H)
	2.17[a]						2.39[a]	
3	4.07[a]	4.64 (dd, 8.2, 6.8)	4.63 (dd, 9.4, 7.4)	4.63 (dd, 11.3, 5.3)	4.66 (dd, 10.2, 6.2)	4.59 (br d, 8.1)	3.78 (m)	4.62[a]
							3.90 (m)	
5	1.36[a]	1.98[a]	1.98 (br d, 11.2)	1.92[a]	2.04 (br d, 11.4)	1.94 (br d, 11.4)	3.24 (dd, 12.9, 2.1)	1.97 (dd, 11.4, 1.7)
6	0.90[a]	1.59[a]	1.62[a]	1.68[a]	1.72[a]	1.50 (m)	1.84[a]	1.57 (m)
	1.36[a]	1.51 (br d, 12.0, 3.4)	1.51 (br d, 10.3)	1.56[a]	1.53 (br d, 11.8)	1.61[a]	1.44 (dd, 13.0, 2.2)	1.68 (ddd, 16.1, 12.6, 3.4)
7	2.17[a]	1.51 (br d, 12.0, 3.4)	1.51 (br d, 10.3)	1.28[a]	1.34[a]	1.09 (br d, 11.1)	1.64 (m)	1.15[a]
	2.40[a]	1.69 (m)	1.68[a]	1.71[a]	1.72[a]	1.52[a]	1.71 (m)	1.41 (m)
9	1.82	1.92[a]	1.92[a]	1.90[a]	1.96[a]	1.71 (dd, 9.7, 7.7)	2.41[a]	1.80 (dd, 10.6, 6.8)
11	1.96[a] (2H)	2.04 (m)	2.03 (m)	2.01 m	2.06[a] (2H, m)	2.06 (m)	1.19[a]	2.10 (m)
		1.93[a]	1.91[a]			2.01 (m)	2.11[a]	1.90[a]
12	5.56 (br s)	5.61 (br s)	5.60 (br s)	5.56 (t, 3.3)	5.59 (br s)	5.62 (br s)	5.61 (br s)	5.43 (t, 2.6)
15	1.96[a]	1.82 (br d, 15.1)	1.82 (br d, 15.2)	1.62[a]	1.65[a]	2.05 (d, 14.6)	1.89 (dd, 15.2, 2.3)	2.08 (d, 15.4)
	2.19[a]	2.22 (dd, 16.3, 4.0)	2.20 (br d, 12.6)	2.33[a]	2.39 (dd, 14.8, 3.1)	3.39 (d, 14.6)	2.31 (dd, 14.9, 3.2)	2.58 (d, 16.0)
16	5.23 (br s)	5.49 (br s)	5.48 (br s)	5.15 (br s)	5.17 brs		5.31 (br s)	
17								1.88[a]
18	3.37 (dd, 3.7, 140)	3.34 (dd, 4.0, 14.0)	3.35 (dd, 14.2, 3.9)	3.45 (dd, 14.4, 4.0)	3.47 (dd, 14.3, 3.7)	3.64 (dd, 14.3, 3.6)	3.44 (dd, 14.1, 4.6)	2.34 (m)
19	2.74 (t, 13.6)	2.64 (t, 13.5)	2.62 (t, 13.5)	2.72 (t, 13.5)	2.76 (t, 13.5)	1.56[a]	1.36 (m)	0.92[a]
	1.34[a]	1.33[a]	1.32 (dd, 12.0, 3.2)	1.32[a]	1.34[a]	1.3[a]	2.72 (t, 13.5)	1.88[a]
21	1.28 (m)	2.26 (td, 13.1, 4.4)	2.23 (td, 12.8, 4.7)	2.33[a]	2.38 (td, 14.8, 3.1)	1.75 (td, 12.6, 3.8)	1.28 (m)	1.09 (dd, 12.9, 3.2)
	2.40	1.17 (m)	1.16 (br d, 13.4)	1.26[a]	1.28[a]	1.18[a]	2.36[a]	1.35 (m)
22	1.49[a]	2.37 (br d, 12.6)	2.37 (br d, 13.5)	2.33[a]	2.13 m	2.59 (dt, 13.2, 2.6)	2.11[a]	2.27 (2H, m)
	1.59 (br d, 12.0)	1.98[a]	2.02[a]	2.11[a]	2.33 (br d, 10.0)	1.66[a]	2.36[a]	
23	9.78 (s)							
24	1.38 (s)	1.59 (s)	1.59 (s)	1.58 s	1.63 s		5.44[a]	1.61 (s)
						1.59 (s)	6.49 (s)	
25	0.87 (s)	0.99 (s)	1.00 (s)	1.00 s	1.08 s	1.02 (s)	0.95 (s)	0.97 (s)
26	1.10 (s)	1.02 (s)	1.02 (s)	1.06 s	1.12 s	1.19 (s)	1.15 (s)	0.79 (s)
27	1.78 (s)	1.66 (s)	1.65 (s)	1.66 s	1.74 s	1.23 (s)	1.76 (s)	0.98 (s)
29	0.96 (s)	0.92 (s)	0.90 (s)	0.94 s	0.95 s	0.82 (s)	1.01 (s)	0.91 (s)
30	1.00 (s)	0.97 (s)	0.97 (s)	1.02 s	1.05 s	0.89 (s)	1.05 (s)	0.83 (s)

[a] Overlapping signals.

J = 7.7 Hz, Glc-H-1) and 5.65 (d, J = 7.7 Hz, GlcA-H-1). In addition, the corresponding anomeric carbons were observed at δ_C 92.5 and 103.9, respectively. The component sugars were identified as being in the D-form based on the same method used for compound **3**. The linkage of the sugar chain was deduced by the HMBC correlations from GlcA-H-1 (δ_H 5.65) to Glc-C-2 (δ_C 78.4) and Glc-H-1 (δ_H 6.06) to C-28 (δ_C 174.4), as well as from the NOESY correlation between GlcA-H-1 and Glc-H-2. Therefore, the structure of armeroside B (**5**) was elucidated to be 3β,16α-dihydroxy-olean-12-en-23,28-dioic acid 28-*O*-β-D-glucuronopyranosyl-(1 → 2)-β-D-glucopyranosyl ester.

The molecular formula of armeroside C (**7**) was determined to be $C_{48}H_{74}O_{22}$ based on the positive-ion HR-FAB-MS data. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of two β-glucopyranosyl and one β-glucuronopyranosyl moieties, and their absolute configurations were determined to be D by acid hydrolysis. The linkage of the sugar chain was deduced by HMBC correlations from GlcA-H-1 (δ_H 5.57) to Glc1-C-2 (δ_C 79.4), Glc2-H-1 (δ_H 4.93) to Glc1-C-6 (δ_C 69.0), and Glc1-H-1 (δ_H 5.97) to C-28 (δ_C 175.9), as well as NOESY correlations between GlcA-H-1 and Glc1-H-2, and Glc2-H-1 and Glc1-H$_{a,b}$-6. This conclusion was also confirmed by the glycosylation shifts for Glc1-C-6 (+8.3 ppm) compared to that for **5** and for Glc1-C-2 (+3.9 ppm) compared to that for **4**. Therefore, the structure of armeroside C (**7**) was elucidated to be 3β,16α-dihydroxy-olean-12-en-23,28-dioic acid 28-*O*-β-D-glucuronopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester.

The molecular formula of armeroside D (**8**) was determined to be $C_{54}H_{86}O_{26}$ based on the positive-ion HR-FAB-MS data. Upon acid hydrolysis, **8** afforded only D-glucose as the component sugar. The ^{1}H- and ^{13}C-NMR spectra indicated resonances that were assigned to four sets of β-glucopyranosyl moieties, and two of these moieties were attached to the sapogenin through ester linkages based on the upfield-shifted anomeric carbon resonances at δ_C 95.2 and 96.6 and downfield-shifted anomeric proton resonances at δ_H 6.13 and 6.36. A comparison of the ^{13}C-NMR data for **8** and **6** indicated that compound **8** has one more glucose unit than **6**, and a downfield shift was observed at C-23 (+2.9 ppm), suggesting that **8** was a 23-glucosyl ester of **6**. This assignment was further confirmed by HMBC correlations from Glc4-H-1 (δ_H 6.36) to C-23 (δ_C 177.6) and Glc1-H-1 (δ_H 6.13) to C-28 (δ_C 175.8). The remaining two glucopyranosyl moieties were attached to the C-3 and C-6 of the C-28-glucopyranosyl moiety based on the HMBC correlations from Glc2-H-1 (δ_H 5.19) to Glc1-C-3 (δ_C 88.5) and Glc3-H-1 (δ_H 4.95) to Glc1-C-6 (δ_C 69.1), as well as NOESY correlations between Glc2-H-1 and Glc1-H-3, and Glc3-H-1 and Glc1-H$_{a,b}$-6. Thus, the structure of armeroside D (**8**) was determined to be 23-*O*-β-D-glucopyranosyl-28-*O*-β-D-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl 3β,16α-dihydroxy-olean-12-en-23,28-dioic acid.

The molecular formula of armeroside E (**9**) was determined to be $C_{54}H_{84}O_{25}$ based on positive-ion HR-FAB-MS data. Acid hydrolysis of **9** resulted in only D-glucose as the component sugar. In the ^{1}H- and ^{13}C-NMR spectra, the resonances of the sugar moiety overlapped substantially, except for the anomeric resonances for Glc1 (δ_H 6.14, δ_C 95.1), Glc2 (δ_H 5.18, δ_C 105.6), and Glc3 (δ_H 4.93, δ_C 105.2). The TOCSY and HMQC-TOCSY spectra enabled the assignment of resonances for each glucose unit. The linkage of the sugar chain was deduced based on the HMBC correlations from Glc3-H-1 (δ_H 4.93) to Glc1-C-6 (δ_C 69.1), Glc2-H-1 (δ_H 5.18) to Glc1-C-3 (δ_C 88.5), and Glc1-H-1 (δ_H 6.14) to C-28 (δ_C 175.8), as well as NOESY correlations

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Table 4
^{1}H NMR spectroscopic data (δ) for the sugar moieties of **3**, **5**, **7**–**10**, and **12** (500 MHz in pyridine-d_5).

Position	**3**	**5**	**7**	**8**	**9**	**10**	**12**
3-*O*-sugar							
GlcA-1	4.94 (d, 7.5)						
2	4.22 (t, 8,5)						
3	4.31 (t, 8.8)						
4	4.54[a]						
5	4.54[a]						
Gal-1	5.23 (d, 7.5)						
2	4.59[a]						
3	4.15[a]						
4	4.59[a]						
5	4.16[a]						
6	4.59[a] (2H)						
28-*O*-sugar							
	Fuc	Glc	Glc 1	Glc 1	Glc 1	Glc 1	Glc 1
1	6.03 (d, 8.3)	6.06 (d, 7.7)	5.97 (d, 7.4)	6.13 (d, 8.3)	6.14 (d, 8.3)	6.14 (d, 8.3)	6.05 (d, 7.5)
2	4.67 (t, 8.8)	4.32[a]	4.20[a]	4.04 (t, 8.5)	4.02 (t, 8.7)	3.93 (t, 8.3)	4.19 (t, 8.6)
3	4.43 (dd, 9.7, 3.5)	4.28 (t, 8.9)	3.98[a]	4.16[a]	4.14 (t, 9.6)	4.17 (t, 7.9)	4.20[a]
4	5.85 (d, 3.2)	4.21 (t, 9.2)	4.23 (t, 9.2)	4.21[a]	4.18 (t, 9.1)	4.18 (t, 9.2)	4.20[a]
5	4.03[a]	3.91 (ddd, 9.4, 4.3, 2.6)	3.95[a]	4.01 (m)	3.99[a]	4.04 (m)	3.96 (m)
6	1.16 (d, 6.3)	4.30[a]	4.27 (dd, 11.8, 4.6)	4.17[a]	4.26 (dd, 11.9, 4.8)	4.24 (dd, 12.0, 4.5)	4.26 (dd, 11.5, 4.6)
		4.38 (dd, 11.8, 2.3)	4.61 (br d, 10.3)	4.56 (br d 10.3)	4.61 (br d, 10.1)	4.55 (br d, 12.0)	4.60 (dd, 11.4, 2.0)
	Rha	GlcA	GlcA	Glc 2	Glc 2	Glc 2	GlcA
1	6.47 (br s)	5.65 (d, 7.7)	5.57 (d, 8.1)	5.19 (d, 8.0)	4.93 (d, 8.0)	5.18 (d, 7.7)	5.60 (d, 7.8)
2	4.77 (br s)	4.12 (t, 8.5)	4.09 (t, 8.5)	3.97 (t, 8.2)	3.94[a]	3.93 (t, 8.3)	4.08 (t, 8.5)
3	4.47 (dd, 8.6, 3.2)	4.25 (t, 9.2)	4.23 (t, 9.2)	4.11 (t, 7.6)	4.12 (t, 9.6)	4.15[a]	4.20[a]
4	4.25[a]	4.51 (t, 9.6)	4.51[a]	4.14[a]	3.96 (t, 8.5)	4.06 (t, 9.0)	4.42[a]
5	4.50[a]	4.54 (t, 9.3)	4.51[a]	3.94 (m)	3.92[a]	3.92 (m)	4.43[a]
6	1.67 (d, 6.0)			4.27[a]	4.22 (dd, 11.4, 5.7)	4.20 (dd, 11.3, 5.0)	
				4.47 (dd, 11.9, 2.5)	4.46 (dd, 11.4, 2.6)	4.46 (dd, 11.8, 2.3)	3.84 (s)
	Glc		Glc 2	Glc 3	Glc 3	Glc 3	Glc 2
1	5.07 (d, 7.7)		4.93 (d, 8.8)	4.95 (d, 7.7)	5.18 (d, 7.7)	4.95 (d, 8.0)	4.95 (d, 7.7)
2	3.95 (t, 8.3)		3.95 (t, 7.9)	3.94 (d, 7.6)	3.94[a]	3.95 (t, 8.0)	3.97 (t, 8.3)
3	4.13 (t, 9.5)		4.13[a]	4.14[a]	4.12 (t, 9.6)	4.15[a]	4.17[a]
4	4.05 (t, 9.2)		4.13[a]	4.15[a]	4.12 (t, 9.0)	4.15[a]	4.16[a]
5	3.89 m		3.82 (m)	3.84 (m)	3.95[a]	3.83 (m)	3.84 (m)
6	4.26[a]		4.29 (dd, 11.9, 5.1)	4.30[a]	4.70 (dd, 11.4, 5.7)	4.28 (dd, 12.0, 5.4)	4.31 (dd, 12.3, 5.7)
	4.45[a]		4.42 (dd, 11.9, 2.4)	4.43 (dd, 11.7, 2.3)	4.96 (dd, 11.5, 1.4)	4.42 (dd, 12.0, 2.6)	4.44 (dd, 11.8, 2.0)
23-*O*-sugar							
Glc 4-1				6.36 (d, 8.1)			
2				4.13 (t, 8.4)			
3				4.23[a]			
4				4.25[a]			
5				3.97[a]			
6				4.27[a]			
				4.33 (dd, 12.0, 2.8)			
HMG-2					3.10 (d, 14.2)		
					3.15 (d, 14.2)		
4					3.12 (d, 14.9)		
					3.17 (d, 14.9)		
6					1.73 (s)		
$CO\underline{CH_3}$	1.91 (s)						

[a] Overlapping signals.

between Glc3-H-1 and Glc1-$H_{a,b}$-6, and Glc2-H-1 and Glc1-H-3.

The ^{1}H- and ^{13}C-NMR data also indicated presence of a 3-hydroxy-3-methylglutaryl (HMG) moiety, based on the characteristic resonances of the tertiary methyl group [δ_H 1.73 (s), and δ_C 28.1], two methylenes [δ_H 3.10 and 3.15 (d, J = 14.2 Hz), and δ_C 46.3; δ_H 3.12 and 3.17 (d, J = 14.9 Hz), and δ_C 46.6], two carbonyl groups (δ_C 171.7, and 174.7), and a quaternary carbon (δ_C 70.1). The location of the HMG moiety was deduced based on the HMBC correlations from Glc2-$H_{a,b}$-6 to HMG-C-1 and the acylation shifts at $H_{a,b}$-6 (+0.5 ppm) and C-6 (+2.2 ppm) of Glc2 compared to those for **6**. To determine the absolute configuration of the HMG moiety, the esterified carboxyl moiety was selectively reduced by $LiEt_3BH$ to afford mevalonolactone (Fujimoto et al., 2001), which was identical to 3*R*-mevalonolactone based on chiral GC-MS analysis. Therefore, the structure of armeroside E (**9**) was elucidated to be 3β,16α-dihydroxy-olean-12-en-23,28-dioic acid 28-*O*-[β-D-6-*O*-((3*S*)-3-hydroxy-3-methylglutaryl)-glucopyranosyl-(1 → 3)]-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester.

Ameroside F (**10**) was isolated as an amorphous powder, and its molecular formula was determined to be $C_{48}H_{74}O_{21}$ based on the positive-ion HR-FAB-MS data. A comparison of the ^{1}H- and ^{13}C-NMR spectra of **10** and **6** indicated identical resonances for the sugar moieties but differences in the sapogenin resonances. Further detailed analyses of the NMR data indicated that the sugar chains of **10** and **6** were identical.

The sapogenin resonances of compounds **10** and **6** differed because a resonance was observed for the ketone group (δ_C 208.4) in **10**, but hydroxylated methine resonances were also observed at C-16 in **6**. The ketone group was located at C-16 based on the HMBC correlations from $H_{a,b}$-22, H-18, and $H_{a,b}$-15 to C-16. Rather than the genuine sapogenin, acid hydrolysis of **10** afforded compound **10a**, and the structure of this compound was determined to be 3β-

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hydroxy-16-oxo-28-nor-olean-12-en-23-oic acid based on detailed 2D-NMR spectroscopic analysis (Fig. 2). The *cis* geometry of the D/E ring fusion for **10a** was deduced based on key NOESY correlations between H-17 and H-15β, H-18 and H-12, and H-22β and H_3-30. The generation of **10a** by decarboxylation upon acid hydrolysis is consistent with the β-keto carboxyl ester structure in **10**. Therefore, the structure of armeroside F (**10**) was determined to be 3β-hydroxy-16-oxo-olean-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester.

Armeroside G (**12**) was isolated as an amorphous powder, and its molecular formula was determined to be $C_{54}H_{84}O_{25}$ based on the positive-ion HR-FAB-MS data. In the ^{1}H- and ^{13}C-NMR spectra of **12**, the resonances that were assigned to the sapogenin moiety were identical to those in **11**. Detailed analyses of the 2D-NMR spectroscopic data led to elucidation of the sapogenin as 3,16α-dihydroxy-3,4-seco-olean-4(24),12-dien-23,28-dioic acid (Koike et al., 1999). This conclusion was further supported by acid hydrolysis of **12** and **11**, which afforded the same product. Detailed analyses of the ^{1}H-, ^{13}C- and 2D-NMR data also indicated the presence of two β-glucopyranosyl and one methylated β-glucuronopyranosyl moieties in **12** with anomeric resonances at δ_H 6.05 and δ_C 93.5 for Glc1, δ_H 5.60 and δ_C 105.2 for GlcA, and δ_H 4.95 and δ_C 105.4 for Glc2. All of the component sugars were determined to be in the D-form based on acid hydrolysis results. The sugar linkage was deduced based on the HMBC correlations from: Glc2-H-1 (δ_H 4.95) to Glc1-C-6 (δ_C 69.2); GlcA-H-1 (δ_H 5.60) to Glc1-C-2 (δ_C 79.4); Glc1-H-1 (δ_H 6.05) to C-28 (δ_C 176.0), as well as NOESY correlations between Glc2-H-1 and Glc1-$H_{a,b}$-6, and GlcA-H-1 and Glc1-H-2. Therefore, the structure of armeroside G (**12**) was elucidated to be 3,16α-dihydroxy-3,4-seco-olean-4(24),12-dien-23,28-dioic acid 28-*O*-[β-D-6-methyl-glucuronopyranosyl-(1 → 2)]-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranosyl ester. Because methanol was used for extraction, compound **12** may be a methylated artifact of corresponding saponin **13**.

A possible biogenesis pathway is shown in Figs. 3 and 4. Oxidation at C-16, C-23, and C-28 of β-amyrin (**16**) affords quillaic acid (**17**; sapogenin of **1**–**3**). Further oxidation at C-23 generates 3β,16α-dihydroxyolean-12-en-23,28-dioic acid (**18**; sapogenin of **4**–**9**) followed by oxidation of the 16α-OH group to generate 3β-hydroxy-16-oxo-olean-12-en-23,28-dioic acid (**19**; sapogenin of **10**) or oxidative cleavage at C-3 and C-4 to generate 3,16α-dihydroxy-3,4-seco-olean-4(24),12-dien-23,28-dioic acid (**20**; sapogenin of **11**, **12**). In accordance with C-23 substitution by an aldehyde or carboxyl group, glycosylation diverges based on the component sugar types and sugar linkage. C-23 oxidation may be a key step in the biosynthesis of *Silene* saponins, which is supported by the triterpenoid saponins reported from other *Silene* species that possess similar metabolic profiles (Lacaille-Dubois et al., 1995, 1997; Glensk et al., 1999; Larhsini et al., 2003; Fu et al., 2005).

Silene saponins have been reported to have inhibitory activity against lymphocyte proliferation (Gaidi et al., 2002). In this study, all of the compounds were screened for their cytotoxicity against human hepatocellular carcinoma HepG2 cells. None of the compounds exhibited cytotoxicity at a final concentration of 10 μM.

3. Conclusions

This first phytochemical investigation of triterpenoid saponins from *S. armeria* resulted in the isolation and structural elucidation of twelve oleanane-type triterpenoid saponins, including seven new compounds (armerosides A–G). Saponins **11** and **12** possess cleavage structures at C-3 and C-4 of the oleanane-type sapogenin, which extends the structural diversity of *Silene* saponins. From a biosynthetic perspective, the substitution at C-23 of the sapogenin appears to be a key factor in the glycosylation pathway. Further investigation is required to identify the biosynthetic enzymes involved in the biosynthesis of *Silene* saponins.

4. Experimental

4.1. General

IR spectra were recorded on a JASCO FT/IR-4100 spectrometer using the KBr disk method, and the optical rotations were measured with a JASCO P-2200 digital polarimeter in a 0.5 dm cell. The NMR spectra were measured with a JEOL ECA-500 NMR spectrometer using TMS as the internal reference, and the chemical shifts are expressed in δ (ppm). HR-FAB-MS was conducted using a JEOL JMS-700 MStation mass spectrometer. Diaion HP-20 resin (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (silica gel 60, Merck), and ODS (Chromatorex, 100–200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan) were used for column chromatography (CC). Preparative HPLC was performed on a Waters 515 HPLC Pump system equipped with a Shodex RI-101 differential refractometer detector. A Shiseido CAPCELL PAC RP-C_{18} column (150 × 20 mm i.d.) was used for RP HPLC, and an YMC-Pack SIL column (250 × 20 mm i.d.) was used for NP HPLC. TLC was conducted on Kieselgel 60 F254 plates (E. Merck).

4.2. Plant material

S. armeria plants were cultivated at the Botanical Garden of the Faculty of Pharmaceutical Sciences, Toho University, and collected in June 2005. The plants were identified by one of the authors (K. K.) A voucher sample of the plant (TH-SA-1) is deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Toho University.

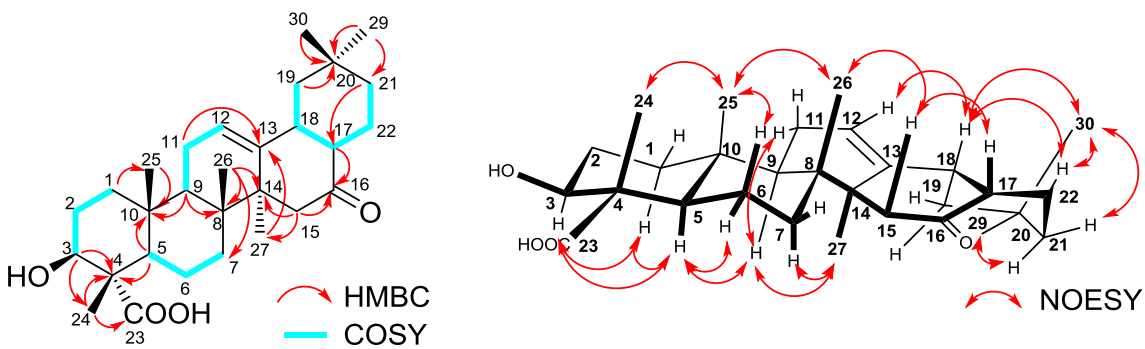

Fig. 2. Key COSY, HMBC, and NOESY correlations of **10a**.

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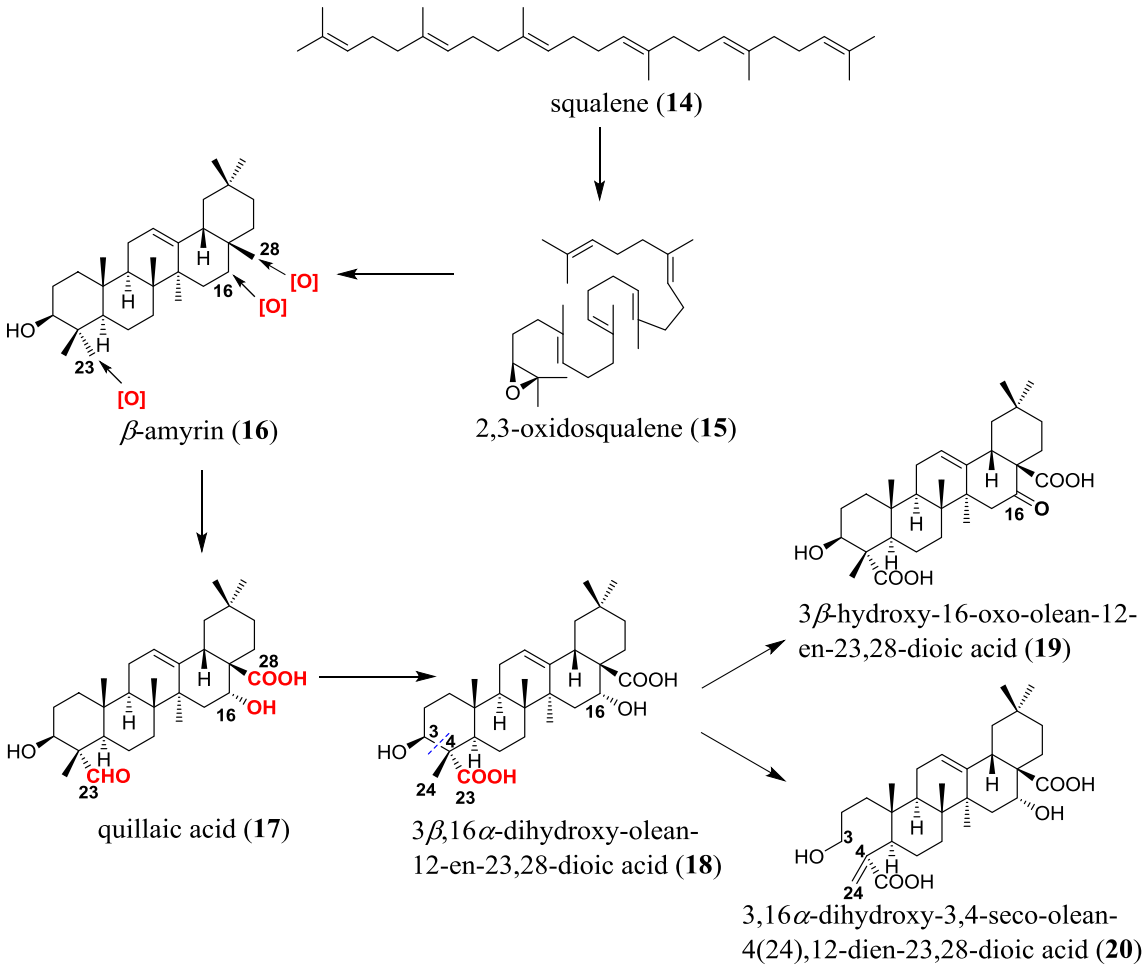

Fig. 3. Possible biosynthetic pathway for different aglycones of triterpenoid saponins isolated from *S. armeria*.

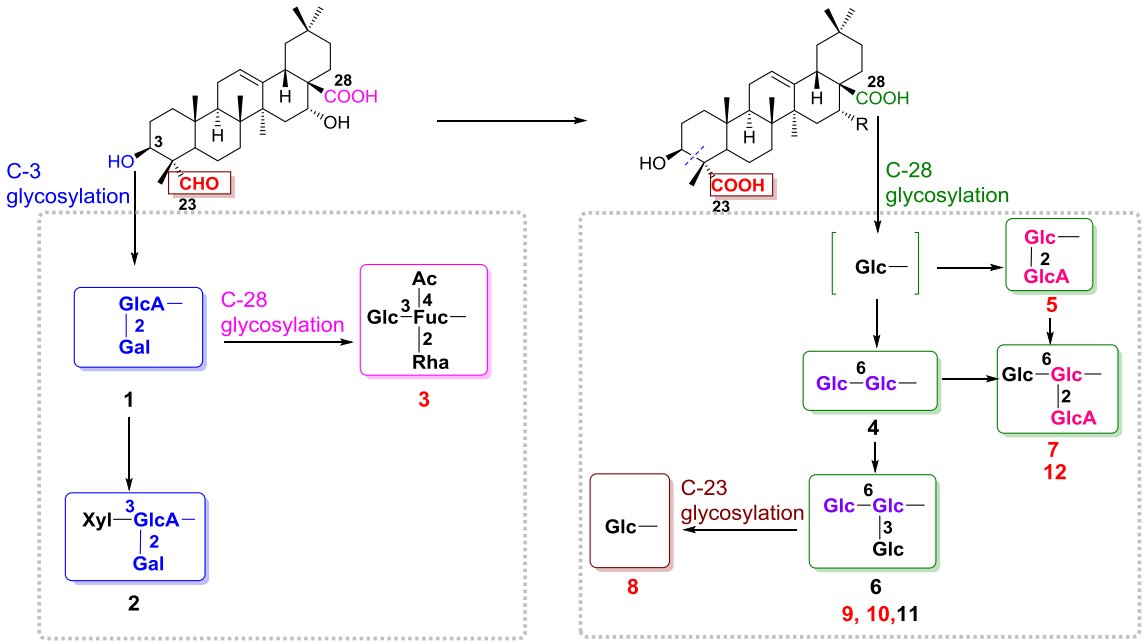

Fig. 4. Possible biosynthetic pathway for compounds **1**–**12** isolated from *S. armeria*.

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4.3. Extraction and isolation

Fresh whole plants of *S. armeria* (10 kg) were extracted with MeOH (80 L) at room temperature for one day. The MeOH extract was concentrated to afford an extract (580 g), which was then suspended in H_2O (1 L) and successively partitioned between EtOAc (1 L each, × 5) and *n*-BuOH (1 L each, × 5), respectively. The *n*-BuOH-soluble fraction (137 g) was applied to a Diaion HP-20 column (95 × 8.5 cm i.d.), eluted with H_2O, MeOH-H_2O (1:1), and MeOH (each 10 L). The MeOH-eluted fraction (crude saponin fraction, 34.6 g) was applied to an ODS column (50 × 5.5 cm i.d.) and eluted with a MeOH-H_2O gradient (4:6, 5:5, 6:4, 7:3, 8:2, 2 L each) followed by MeOH (2 L) to afford ten fractions (F1-F10). Further separation of fraction F4 (3.0 g) by preparative RP-HPLC using MeCN-H_2O containing 0.06% TFA (28:72, v/v) led to isolation of compounds **11** (8 mg) and **12** (6 mg). The separation of fraction F5 (1.3 g) by preparative RP-HPLC using MeCN-H_2O containing 0.06% TFA (30:70, v/v) led to isolation of compounds **7** (76 mg) and **8** (8 mg). Fraction F6 (7.5 g) was subjected to silica gel CC and eluted with $CHCl_3$-MeOH-H_2O (60:29:6, v/v/v) to afford seven sub-fractions (S1−S7) and $CHCl_3$-MeOH-H_2O (6:4:1, v/v/v) to afford three sub-fractions (S8−S10). Separation of sub-fraction S4 by preparative NP-HPLC with $CHCl_3$-MeOH-H_2O (60:33:7, v/v/v) led to isolation of compounds **4** (63 mg) and **10** (18 mg). Separation of sub-fraction S6 by preparative RP-HPLC using MeCN-H_2O containing 0.06% TFA (32:68, v/v) led to isolation of compound **6** (1634 mg). Separation of sub-fraction S8 by preparative RP-HPLC with MeOH-H_2O (65:35, v/v) led to isolation of compound **5** (76 mg). Separation of sub-fraction S9 by preparative RP-HPLC using MeCN-H_2O containing 0.06% TFA (35:65, v/v) led to isolation of compounds **1** (48 mg), **2** (71 mg), **3** (11 mg), and **9** (21 mg).

4.4. Armeroside A (*3*)

Amorphous white powder. $[\alpha]_D^{19}$ + 2.9 (*c* 0.22, EtOH-H_2O = 1:1). IR (KBr) υ_{max} cm^{-1}: 3410, 2924, 2855, 1734, 1636, 1420, 1376, 1160, 1060. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 1343.5846 $[M+Na]^+$ (Calcd for $C_{62}H_{96}O_{30}Na$, 1343.5884).

4.5. Armeroside B (*5*)

Amorphous white powder. $[\alpha]_D^{21}$ -1.0 (*c* 0.50, EtOH). IR (KBr) υ_{max} cm^{-1}: 3415, 2924, 2855, 1703, 1681, 1456, 1387, 1260, 1202, 1077. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 863.4049 $[M+Na]^+$ (Calcd for $C_{42}H_{64}O_{17}Na$, 863.4041).

4.6. Armeroside C (*7*)

Amorphous white powder. $[\alpha]_D^{22}$ -15.3 (*c* 0.50, EtOH). IR (KBr) υ_{max} cm^{-1}: 3412, 2925, 2856, 1677, 1636, 1457, 1377, 1204, 1077. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 1025.4592 $[M+Na]^+$ (Calcd for $C_{48}H_{74}O_{22}Na$, 1025.4569).

4.7. Armeroside D (*8*)

Amorphous white powder. $[\alpha]_D^{22}$ -1.7 (*c* 0.50, EtOH). IR (KBr) υ_{max} cm^{-1}: 3417, 2925, 2855, 1732, 1678, 1636, 1455, 1378, 1205, 1075. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 1173.5315 $[M+Na]^+$ (Calcd for $C_{54}H_{86}O_{26}Na$, 1173.5305).

4.8. Armeroside E (*9*)

Amorphous white powder. $[\alpha]_D^{22}$ +0.71 (*c* 0.50, EtOH). IR (KBr) υ_{max} cm^{-1}: 3406, 2925, 2856, 1710, 1678, 1458, 1384, 1204, 1074. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 1155.5224 $[M+Na]^+$ (Calcd for $C_{54}H_{84}O_{25}Na$, 1155.5199).

4.9. Armeroside F (*10*)

Amorphous white powder. $[\alpha]_D^{17}$ -19.3 (*c* 0.50, EtOH-H_2O = 1:1). IR (KBr) υ_{max} cm^{-1}: 3431, 2924, 2854, 1699, 1636, 1559, 1458, 1376, 1159, 1063. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 1009.4613 $[M+Na]^+$ (Calcd for $C_{48}H_{74}O_{21}Na$, 1009.4620).

4.10. Armeroside G (*12*)

Amorphous white powder. $[\alpha]_D^{22}$ -7.5 (*c* 0.50, EtOH). IR (KBr) υ_{max} cm^{-1}: 3416, 2925, 2858, 1735, 1683, 1431, 1381, 1204, 1136, 1078. For ^{1}H-NMR (500 MHz, pyridine-d_5) and ^{13}C-NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. HR-FAB-MS (positive) *m/z* 1039.4701 $[M+Na]^+$ (Calcd for $C_{49}H_{76}O_{22}Na$, 1039.4726).

4.11. Acid hydrolysis

Individual solutions of **1**–**10** (each 5 mg) and **11**–**12** (each 3 mg) in 1 M HCl (dioxane-H_2O, 1:1, 10 mL) were separately heated until reflux occurred, this being maintained for 1 h. After removal of dioxane by evaporation, the solution was extracted with EtOAc (10 mL × 3). The EtOAc fractions from compounds **1**, **2**, and **3** were then separated by preparative NP-HPLC with $CHCl_3$-MeOH-H_2O (60:29:6, v/v/v) as eluent to afford 3-*O*-*β*-D-glucuronopyranosylquillaic acid (**21**). EtOAc fractions from compounds **4**–**9** were separated by preparative NP-HPLC with $CHCl_3$-MeOH (95:5, v/v) as eluent to afford 3*β*,16*α*-dihydroxy-olean-12-en-23,28-dioic acid (**18**). The EtOAc fraction from compound **10** was separated by preparative NP-HPLC with $CHCl_3$-MeOH (96:4, v/v) as the eluent to afford 3*β*-hydroxy-16-oxo-28-nor-olean-12-en-23-oic acid (**10a**). EtOAc fractions from compounds **11**–**12** were separated by preparative NP-HPLC with $CHCl_3$-MeOH (95:5, v/v) as eluent to afford 3,16*α*-dihydroxy-3,4-seco-olean-4(24),12-dien-23,28-dioic acid (**20**). Acid hydrolysis of compounds **1**–**3** (each 5 mg) in 2 M aqueous TFA using the same procedure afforded quillaic acid. Spectroscopic data including NMR and MS for all of the compounds except **10a** were identical to those for authentic samples.

Compound **10a** (3*β*-hydroxy-16-oxo-28-nor-olean-12-en-23-oic acid): Amorphous white powder. $[\alpha]_D^{21}$ -10.8 (*c* 0.33, EtOH). For ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1 and 3. HR-FAB-MS (positive) *m/z* 479.3140 $[M+Na]^+$ (Calcd for $C_{29}H_{44}O_4Na$, 479.3137).

4.12. Determination of absolute configurations of sugars in compounds *1*–*12*

Aqueous layers obtained by each acid hydrolysis were neutralized by passage over an ion-exchange resin (DIAION W20) column and concentrated under reduced pressure to dryness to obtain corresponding sugar fractions. These were individually dissolved in H_2O (1 mL), and (*S*)-(−)-1-phenylethylamine (5 mg) and $NaBH_3CN$ (3 mg) in EtOH (1 mL) were added. After incubation at 40 °C for 4 h, glacial AcOH (0.2 mL) was added, with each reaction mixture evaporated to dryness. Each resulting solid was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) for 24 h at room temperature,

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with corresponding reaction mixture evaporated 5 times by adding H_2O to remove pyridine and passed through a Sep-Pak C18 cartridge (Waters) using CH_3CN-H_2O (20:80 and 50:50, v/v, each 10 mL) as solvents. Then, each CH_3CN-H_2O (1:1, v/v) eluate, which consisted of a mixture of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives of monosaccharides, was analyzed by HPLC under the following conditions: column, YMC Triart C18 (150 × 4.6 mm i.d.); solvent: CH_3CN-H_2O (40:60, v/v); flow rate, 0.8 mL/min; column temperature, 40 °C; detection, UV 230 nm. Derivatives of D-xylose, D-glucuronic acid, D-galactose, D-fucose, D-glucose and L-rhamnose were detected as follows: t_R (min) 17.3 (derivative of D-xylose), 19.0 (derivative of D-glucuronic acid), 20.4 (derivative of D-galactose), 20.7 (derivative of D-fucose), 24.4 (derivative of D-glucose), and 26.2 (derivative of L-rhamnose).

4.13. Determination of the absolute configuration of the 3-hydroxy-3-methylglutaryl (HMG) moiety in **9**

A solution of $LiEt_3$-BH (1.0 M) in dry THF (100 μL) (Aldrich) was added to a solution of **9** (3.0 mg) in dry THF (400 μL) in an ice bath. The reaction mixture was stirred under N_2 using ice-cooling for 30 min. After H_2O (3.0 mL) was added to the reaction mixture, 0.1 M HCl was added dropwise to adjust the pH to 3. The reaction mixture was stirred under N_2 for 48 h. Then, the reaction mixture was partitioned with *n*-hexane (3 mL × 3). The *n*-hexane layer containing mevalonolactone and standard compounds of (3*S*,3*R*)-mevalonolactone and (3*S*)-mevalonolactone were analyzed by chiral GC-FID. GC analysis was performed on a GC-2010 Plus (Shimadzu, Japan) equipped with a flame ionization detector (FID) and an Agilent J&W chiral capillary column CYCLOSIL (30 m × 0.25 mm i.d.). The column temperature was maintained at an initial temperature of 40 °C for 5 min and increased at 5 °C/min to 230 °C, which was maintained for 20 min. He was used as carrier gas at a constant flow rate of 2.11 mL/min. Authentic (3*S*)- and (3*R*)-mevalonolactone possessed retention times of 33.65 min and 33.54 min, respectively. The retention time of the sample was identical to that of (3*R*)-mevalonolactone, establishing that the absolute configuration of the HMG moiety in **9** was 3*S*.

4.14. Cytotoxic assays

Cytotoxic assays using the human hepatocellular carcinoma cell line HepG2 (RIKEN Cell Bank, Tsukuba, Japan) were performed using an MTT assay as previously reported (Chang et al., 2007).

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