Chemistry of Natural Compounds, Vol. 48, No. 2, May, 2012 [Russian original No. 2, March–April, 2012]

A NEW TRITERPENOID SAPONIN AND OTHER SAPONINS FROM *Salicornia europaea*

Min Yin, Xiangyun Wang, Ming Wang, Yu Chen, Yunfa Dong, Youyi Zhao, and Xu Feng*

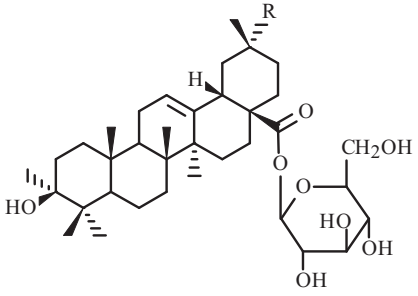
UDC 547.918

*A new triterpenoid saponin, 3β,29-dihydroxy-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**), together with four known triterpenoid saponins, i.e., oleanolic acid 28-*O*-β-D-glucoside (**2**), chikusetsusaponin IVa methyl ester (**3**), calenduloside E (**4**), and calenduloside E 6′-methyl ester (**5**), was isolated from* Salicornia europaea *Linn. Their structures were elucidated on the basis of spectral analysis.*

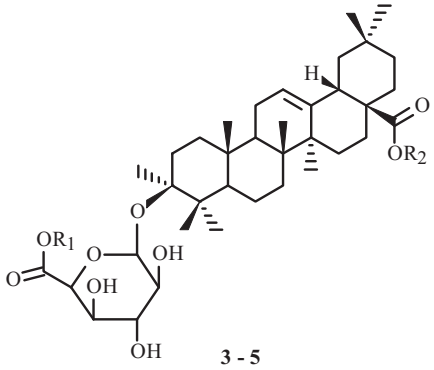
Keywords: *Salicornia europaea*, Chenopodiaceae, triterpenoid saponin.

Salicornia europaea Linn., marshfire glasswort, is a halophytic plant belonging to the family Chenopodiaceae growing on the sea coasts of temperate and subtropic zones, which has been used in Chinese folk medicine for the treatment of hypertension, cephalalgia, and scurvy [1]. Previous researches of chemical constituents of this plant resulted in the isolation of nine flavonoid compounds and four chromone compounds [2–5]. In the current research, a new triterpenoid saponin, namely 3*β*,29-dihydroxy-olean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester, was isolated from *Salicornia europaea* Linn., together with four known triterpenoid saponins, i.e., oleanolic acid 28-*O*-*β*-D-glucopyranoside (**2**), 3-*O*-(methyl-*β*-D-glucuronopyranosiduronoate)-28-*O*-*β*-D-glucopyranosyl oleanolate (chikusetsusaponin IVa methyl ester) (**3**), 3-*O*-*β*-D-glucuronopyranosyl oleanolic acid (calenduloside E) (**4**), and oleanolic acid 3-*O*-6′-*O*-methyl-β-D-glucuronopyranoside (calenduloside E 6′-methyl ester) (**5**). Their structures were established by spectral analysis.

Compound **1** was isolated as a white amorphous powder, $[\alpha]_D^{25}$ +31.3° (*c* 0.11, MeOH). Its molecular formula was identified as $C_{36}H_{58}O_9$ by HR-ESI-MS at *m/z* 657.3991 $[M + Na]^+$ (calcd 657.3979), indicating the presence of eight degrees of unsaturation. Its ^{1}H NMR spectrum showed the presence of six tertiary methyl groups (δ 0.91, 1.01, 1.08, 1.13, 1.20, and 1.25), a trisubstitued olefinic proton (5.48, t-like), and an anomeric proton signal (6.33, d, J = 8.1 Hz).



1, 2



3 - 5

1: R = CH_2OH
2: R = CH_3

3: $R_1 = CH_3$, R_2 = Glc
4: $R_1 = R_2 = H$
5: $R_1 = CH_3$, $R_2 = H$

Jiangsu Center for Research & Development of Medicinal Plants, Jiangsu Institute of Botany, Chinese Academy of Sciences, Nanjing Botanical Garden, Mem. Sun Yat-Sen, Nanjing 210014, P. R. China, fax: +86 25 84347084, e-mail: fengxu@mail.cnbg.net.
Published in *Khimiya Prirodnykh Soedinenii*, No. 2, March–April, 2012, pp. 232–234. Original article submitted January 12, 2011.

0009-3130/12/4802-0258 ©2012 Springer Science+Business Media, Inc.

TABLE 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and HMBC Data for Compound **1** (C_5D_5N, δ, ppm, J/Hz)

C atom	δ_C	δ_H	HMBC (H→C)
1	39.0	0.98 (m), 1.55 (m)	C-2, 10
2	28.1	1.84 (m)	C-3
3	78.1	3.40 (m)	C-23, 24
4	40.0		
5	55.9	0.83 (d, J = 11.3)	C-1, 3, 6, 9, 10, 23, 24, 25
6	18.9	1.37 (m), 1.52 (m)	C-5
7	33.2	1.37 (m), 1.48 (m)	C-26
8	39.4		
9	48.2	1.65 (m)	C-8, 10, 11, 25, 26
10	37.4		
11	23.9	1.92 (m)	C-12, 13
12	123.0	5.48 (br.s)	C-11, 13
13	144.4		
14	42.2		
15	28.4	2.37 (m), 1.16 (m)	C-14, 27
16	23.6	1.99 (m), 2.19 (m)	C-17
17	47.5		
18	41.3	3.32 (m)	C-12, 13, 14, 16, 17, 19, 28
19	41.0	2.13 (m)	C-18, 29, 30
20	36.4		
21	28.9	1.75 (m)	C-20, 22, 30
22	32.1	1.85 (br.d, J = 13.5), 1.92 (m)	C-21
23	28.8	1.20 (s)	C-3, 4, 5, 24
24	16.5	1.01 (s)	C-3, 4, 5, 23
25	15.7	0.91 (s)	C-1, 5, 9, 10
26	17.6	1.13 (s)	C-7, 8, 9, 14, 25, 27
27	26.1	1.25 (s)	C-13, 15, 26
28	176.5		
29	73.7	3.54 (s)	C-19, 20, 21, 30
30	19.7	1.08 (s)	C-19, 20, 21, 29
Glucose			
1′	95.8	6.33 (d, J = 8.1)	C-28, C-G-2, C-G-3
2′	74.2	4.19 (m)	C-G-1, 3
3′	79.0	4.27 (m)	C-G-2, 4
4′	71.2	4.32 (m)	C-G-3, 5
5′	79.3	4.02 (m)	C-G-4, 6
6′	62.3	4.40 (m), 4.43 (m)	C-G-5

The ^{13}C NMR spectrum (Table 1) displayed signals of one hydroxyl-bearing carbon at δ 78.1 (3-*β*OH) and one hydroxymethylene at δ 73.7, which gave a correlation in the HSQC spectrum with a 2H-singlet at δ_H 3.54. The ROESY spectrum of **1** showed a correlation between H-3 and H-23, confirming the *α*-equatorial orientation of the oxymethine proton at C-3 and consequently the *β*-axial orientation of the hydroxyl group. The above spectral data (^{1}H NMR and ^{13}C NMR), except for the ring E and the sugar moiety, were coincident with oleanolic acid [6].

In the HMBC experiment, the observation of cross-peaks between the 2H-singlet at δ_H 3.54 and four carbons, δ_C 19.7 (C-30), 28.9 (C-21), 36.4 (C-20), and 41.0 (C-19), suggested that C-29 or C-30 was oxygenated. Comparing with the NMR data [7], we found that C-29 (δ_C 73.7) was oxygenated. A cross-peak in the ROESY spectrum between δ_H 1.08 and 3.32 (H-18, *β*-axial) proved the *β*-axial orientation of C-30 and consequently the *α*-equatorial orientation of C-29. Therefore, the aglycone moiety was identified as 3*β*,29-dihydroxyolean-12-en-28-oic acid (mesembryanthemoidigenic acid) [8].

The ^{13}C NMR indicated a glucosyl group at δ 95.8 (Glc-1), 74.2 (Glc-2), 79.0 (Glc-3), 71.2 (Glc-4), 79.3 (Glc-5), and 62.3 (Glc-6). Additionally, acid hydrolysis of **1** afforded D-glucose as the sole sugar. Moreover, the HMBC correlation between H-1′ (δ_H 6.33) of glucose and C-28 (δ_C 176.5) of the aglycone indicated that the glucose was linked to C-28 of the aglycone. Based on the above studies, we conclude that compound **1** (Fig. 1) is 3*β*,29-dihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester.

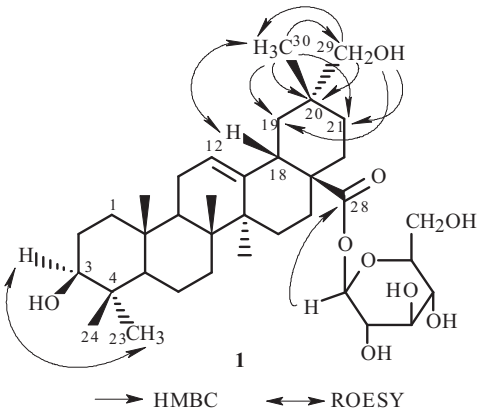


Fig. 1. The structure and key HMBC and ROESY correlations of compound **1**.

EXPERIMENTAL

General Procedures. NMR spectra were recorded on a Bruker AV-500 spectrometer with TMS as internal standard. HR-ESI-MS spectra were measured with an Agilent 1100 LC/MSD TOF mass spectrometer.

Plant Material. The whole plants of *S. europaea*, collected from Yancheng City, Jiangsu Province of China in May 2009, were taxonomically identified by Prof. Chang-Qi Yuan (Jiangsu Institute of Botany). A voucher specimen was deposited in the Herbarium of Nanjing Botanical Garden Mem. Sun Yat-Sen.

Extraction and Purification. The fresh whole plants (108 kg) were macerated with 95% ethanol three times, each time for 10 days, at room temperature. The combined three extracts were evaporated *in vacuo*, which afforded a fluid extract. Then the fluid extract was partitioned with ethyl acetate, and the remaining water solution passed through a D101 macroporous resin column with ethanol–water solutions to elute gradiently. The eluting fraction of 70% ethanol–water solution, after removal of methanol, afforded a residue (570 g), which was subjected to silica gel column chromatography to furnish fractions 1–15. Fraction 6 was subjected to silica gel column eluting with EtOAc–MeOH 10:1, and then on an ODS column with EtOH–H_2O (3:2, v/v) to ultimately afford a new compound **1** (11 mg). Fraction 5 was applied to the ODS column and eluted with EtOH–H_2O (1:1, v/v) to produce compound **2** (18 mg). Fraction 7 was isolated on the silica gel column eluting with a gradient of 10–20% MeOH in EtOAc to obtain compound **3** (10 mg). Fraction 8 was purified by column chromatography on silica gel eluting with EtOAc–MeOH 5:1 to afford compound **4** (10 mg). Fraction 9 was subjected to ODS column chromatography and eluted with EtOH–H_2O (1:4, v/v) to obtain compound **5** (11 mg).

Compound 1. White amorphous powder, $[\alpha]_D^{25}$+31.3° (*c* 0.11, MeOH). $C_{36}H_{58}O_9$ (MW: 634). HR-ESI-MS: 657.3991 ([M + Na]$^+$, calcd 657.3979). ^{1}H and ^{13}C NMR: see Table 1.

Compound 2. White amorphous powder. ESI-MS *m/z*: 617 [M – H]$^-$, 455 [M – 162 – H]$^-$. ^{1}H NMR (500 MHz, C_5D_5N, δ, ppm): 6.31 (d, Glc-1), 5.44 (s, H-12), 1.23 (3H, s), 1.21 (3H, s), 1.12 (3H, s), 1.01 (3H, s), 0.92 (3H, s), 0.89 (3H, s), 0.88 (3H, s). ^{13}C NMR (125 MHz, C_5D_5N, δ, ppm): 15.6 (C-25), 16.5 (C-24, 26), 18.9 (C-6), 23.5 (C-16), 23.7 (C-11), 23.9 (C-30), 26.1 (C-27), 28.1 (C-2), 28.3 (C-15), 28.8 (C-23), 30.8 (C-20), 32.6 (C-22), 33.1 (C-29), 33.2 (C-7), 34.0 (C-21), 37.4 (C-10), 39.0 (C-1), 39.4 (C-8), 40.0 (C-4), 41.8 (C-18), 42.2 (C-14), 46.4 (C-19), 47.0 (C-17), 48.2 (C-9), 55.9 (C-5), 62.3 (Glc-6), 71.2 (Glc-4), 74.2 (Glc-2), 78.1 (C-3), 78.9 (Glc-3), 79.3 (Glc-5), 95.8 (Glc-1), 122.6 (C-12), 144.2 (C-13) [9].

Compound 3. White needle crystals. ESI-MS *m/z*: 831 [M + Na]$^+$, 826 [M + NH_4]$^+$ (MW: 808). ^{1}H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 6.29 (d, J = 8.1), 5.40 (s, H-12), 4.96 (d, J = 6), 3.71 (3H, s), 1.27 (3H, s), 1.24 (3H, s), 1.07 (3H, s), 0.95 (3H, s), 0.89 (3H, s), 0.87 (3H, s), 0.82 (3H, s). ^{13}C NMR (125 MHz, C_5D_5N, δ, ppm): 15.5 (C-25), 16.9 (C-24), 17.5 (C-26), 18.5 (C-6), 23.4 (C-30), 23.6 (C-11, 16), 26.1 (C-15), 26.6 (C-27), 28.3 (C-23), 30.7 (C-20), 32.6 (C-22), 33.1 (C-7, 29), 34.0 (C-21), 37.0 (C-10), 39.5 (C-1), 39.9 (C-8), 41.7 (C-18), 42.2 (C-14), 46.2 (C-19), 47.0 (C-17), 48.1 (C-9), 52.0 (GlcUA-Me), 55.8 (C-5), 62.3 (Glc-6), 71.2 (Glc-4), 73.2 (GlcUA-4), 74.2 (GlcUA-2), 75.4 (GlcUA-3), 77.2 (GlcUA-2), 77.9 (GlcUA-5), 78.9 (Glc-3), 79.3 (Glc-3), 89.1 (C-3), 95.8 (GlcUA-1), 107.3 (GlcUA-1), 123.5 (C-12), 144.2 (C-13), 170.8 (GlcUA-6), 176.4 (C-28) [10].

Compound 4. White amorphous powder. ESI-MS *m/z*: 631 $[M - H]^-$, 655 $[M + Na]^+$ (MW: 632). ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 0.72 (br.d, H-5), 0.75 (s, Me-26), 0.87 (s, Me-24), 0.87 (s, Me-9), 0.97 (s, Me-30), 0.97 (s, Me-25), 1.08 (s, Me-23), 1.08 (s, Me-27), 1.23 (br.d, H-19), 1.49 (t, H-6), 1.82 (t, H-19), 2.50 (m, H-18), 3.12 (m, H-3), 3.33 (m, GlcUA-2, GlcUA-3, GlcUA-4, GlcUA-5), 4.13 (d, GlcUA-1), 5.15 (br.t, H-12). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 15.1 (C-25), 16.4 (C-24), 16.9 (C-26), 17.8 (C-6), 22.7 (C-30), 22.9 (C-16), 23.4 (C-11), 25.5 (C-2, 27), 27.2 (C-15), 27.6 (C-23), 30.4 (C-20), 32.2 (C-22), 32.4 (C-7), 32.8 (C-29), 33.4 (C-21), 36.3 (C-10), 38.2 (C-1), 38.7 (C-4), 38.9 (C-8), 40.8 (C-18), 41.3 (C-14), 45.4 (C-19), 45.8 (C-17), 47.1 (C-9), 55.0 (C-5), 72.2 (GlcUA-4), 73.9 (GlcUA -2), 76.62 (GlcUA-3, 5), 87.8 (C-3) 105.3 (GlcUA-1) 121.36 (C-12), 144.0 (C-13), 173.4 (GlcUA -6) [11].

Compound 5. White amorphous powder. ESI-MS *m/z*: 645 $[M - H]^-$, 669 $[M + Na]^+$ (MW: 646). ^1H NMR (300 MHz, C_5D_5N, δ, ppm): 0.79, 0.94, 0.95, 0.96, 0.99, 1.25, 1.29 (s, 7 × Me), 3.26 (dd, H-18), 3.35 (dd, H-3), 3.71 (s, CO_2Me), 4.07 (t, GlcUA H-2), 4.25 (t, GlcUA H-3), 4.46 (t, GlcUA H-4), 4.58 (d, GlcUA H-5), 4.98 (d, GlcUA H-1), 5.48 (t, H-12). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 15.5 (C-25), 16.9 (C-24), 17.4 (C-26), 18.4 (C-6), 23.8 (C-11, 16, 30), 26.2 (C-27), 26.6 (C-2), 28.2 (C-23), 28.3 (C-15), 31.0 (C-20), 33.2 (C-7), 33.3 (C-22, 29), 34.2 (C-21), 36.9 (C-10), 38.6 (C-1), 39.5 (C-4), 39.7 (C-8), 42.0 (C-14), 42.1 (C-18), 46.5 (C-19), 46.7 (C-17), 48.0 (C-9), 52.0 (GlcUA-Me), 55.8 (C-5), 73.2 (GlcUA-4), 75.4 (GlcUA-2), 77.2 (GlcUA-5), 77.9 (GlcUA-3), 89.1 (C-3), 107.3 (GlcUA-1), 122.5 (C-12), 144.8 (C-13), 170 (GlcUA-6), 180.2 (C-28) [12].

ACKNOWLEDGMENT

The research was supported by the Natural Science Foundation of Jiangsu Province, China (Grant No. BK2011424) and the Open Fund of Jiangsu Center for Research & Development of Medicinal Plants (No. 200901).

REFERENCES

1. State Administration of Traditional Chinese Medicine of PRC, *Dictionary of Chinese Herbal Medicine*, Shanghai Scientific and Technical Press, Shanghai (1999), 822 pp.
2. Y. Arakawa, Y. Asada, and H. Ishida, *J. Fac. Agric. Hokkaido Univ.*, **1**, 61 (1982).
3. M. Geslin and J. F. Verbist, *J. Nat. Prod.*, **48**, 111 (1985).
4. H. Chiji, T. Aiba, and M. Izawa, *Agric. Biol. Chem.*, **42**, 159 (1978).
5. Y. Arakawa, H. Chiji, and M. Izawa, *Agric. Biol. Chem*., **47**, 2029 (1983).
6. S. K. Srivastava and D. C. Jain, *Phytochemistry*, **28**, 644 (1989).
7. F. J. Guo, S. Lin, and Y. C. Li, *Chin. J. Med. Chem.*, **15**, 294 (2005) (in Chinese).
8. B. Tursch, J. Leclercq, and G. Chiurdoglu, *Tetrahedron Lett.*, **6**, 4161 (1965).
9. Y. P. Lin, M. H. Qiu, Z. R. Li, and X. H. Gao, *Acta Bot. Yunnan.*, **25**, 235 (2003) (in Chinese).
10. N. X. Qi, S. P. Jia, Z. F. Hao, and J. X. Li, *Chin. J. Med. Chem.*, **15**, 162 (2005) (in Chinese).
11. C. L. Ridout, K. R. Price, G. Parkin, M-G Dijoux, and C. Lavaud, *J. Agric. Food Chem.*, **42**, 279 (1994).
12. F. R. Melek, T. Miyase, O. D. El-Gindi, S. M. Abdel-Khalik, and M. Y. Haggag, *Phytochemistry*, **42**, 1405 (1996).