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Triterpene saponins from *Salsola imbricata*

Arafa I. Hamed [a,*,1], Milena Masullo [b,1], Mohamed G. Sheded [a], Usama A. Mahalel [a], Moatz M. Tawfik [a], Angela Perrone [b], Sonia Piacente [b,**]

[a] Faculty of Science, South Valley University, Aswan 81528, Egypt
[b] Dipartimento di Scienze Farmaceutiche e Biomediche, Università degli Studi di Salerno, Via Ponte Don Melillo, I-84084 Fisciano, Salerno, Italy

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ABSTRACT

Continuing our investigations on medicinal plants of the Egyptian desert, two new triterpene glycoside derivatives, along with three known compounds have been isolated from the roots of *Salsola imbricata*, a shrub widely growing in Egypt. Their structures have been established as 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucuronopyranosyl-akebonic acid 28-*O*-β-D-glucopyranoside and 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucuronopyranosyl-29-hydroxyoleanolic acid 28-*O*-β-D-glucopyranoside on the basis of spectroscopic methods including 1D- (^{1}H, ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC) experiments as well as mass spectrometry analysis.

1. Introduction

Plants belonging to the genus *Salsola* (Family: Chenopodiaceae) are common in the arid and semiarid regions of our planet and about 15 different *Salsola* species are found in Egypt (Boulos, 1999). Typically, these plants grow on flat, often dry and/or somewhat saline soils, with some species in salt marshes. *Salsola imbricata* Forssk. subsp. *imbricata* (syns = *Chenopodium baryosmum* = *Salsola foetida* = *Caroxylon imbricata* = *Salsola baryosma*) is a shrub widely growing in Egypt, known with the Arabic name "Harm", and used as a good camel food (Täckholm, 1974; Batanouny, 1999). The aqueous extracts of *Salsola* species are used in traditional medicine as an oral contraceptive by Bushmen women in Namibia and in the Republic of South Africa (Swart et al., 2003). In the middle East, *Salsola baryosma* is used against inflammations and as a diuretic agent (Al-Saleh et al., 1993). It has also been reported to possess antioxidant effects (Ahmad et al., 2008) and CNS depressant activity (Woo et al., 1977).

At present, no pharmacological and phytochemical studies have been reported for *Salsola* spp. growing in Egypt. Only few species from the genus *Salsola* growing in other geographical areas have been analyzed from a chemical point of view. In particular, alkaloids from the aerial parts of *S. oppositofolia*, *S. soda*, *S. tragus* and *S. collina* (Tundis et al., 2009; Zhao and Ding, 2004), flavonoids and other phenolic compounds from *S. kali*, *S. soda*, *S. oppositifolia* and *S. collina* (Tundis et al., 2007; Xiang et al., 2007a, 2007b), and fatty acid derivatives from *S. tetrandra* (Oueslati et al., 2006) have been reported. Moreover, triterpenes with significant antioxidant activity were isolated from *S. baryosma* (Ahmad et al., 2008). As a part of our on going research on medicinal plants of the Egyptian desert (Hamed et al., 2006; Perrone et al., 2007; Piacente et al., 2009; Plaza et al., 2004, 2005), we carried out the phytochemical investigation of *S. imbricata*.

In the present paper we describe the isolation of two new triterpene glycosides (**1** and **2**) along with two known triterpene glycosides pseudoginsenoside RT1 and silphioside G (Tanaka et al., 1985), and one known nortriterpene glycoside boussingoside A2 (Espada et al., 1990). Their structures were elucidated by extensive spectroscopic methods including 1D- (^{1}H and ^{13}C) and 2D-NMR (DQF-COSY, HSQC, HMBC, and TOCSY) experiments as well as ESIMS analysis.

2. Results and discussion

The roots of *S. imbricata* were extracted with 80% MeOH and the crude extract was fractionated on RP-18 columns to yield two new compounds (**1** and **2**) (Fig. 1).

The HRMALDITOF mass spectrum of **1** showed a major ion peak at *m/z* 933.4065 $[M+Na]^+$ ascribable to a molecular formula $C_{46}H_{70}O_{18}$ (calcd. for $C_{46}H_{70}NaO_{18}$, 933.4060). The ESI-MS spectrum showed the major ion peak at *m/z* 933 $[M+Na]^+$ and significant fragments in MS/MS analysis at *m/z* 625 [M+Na-132-176]$^+$ corresponding to the loss of one pentose and one uronic acid

* Corresponding author. Tel.: +20 972319811; fax: +20 3480450.
** Corresponding author. Tel.: +39 089 969763; fax: +39 089 969602.
E-mail addresses: arafahamed75@yahoo.com (A.I. Hamed), piacente@unisa.it (S. Piacente).
[1] Contributed equally to this work.

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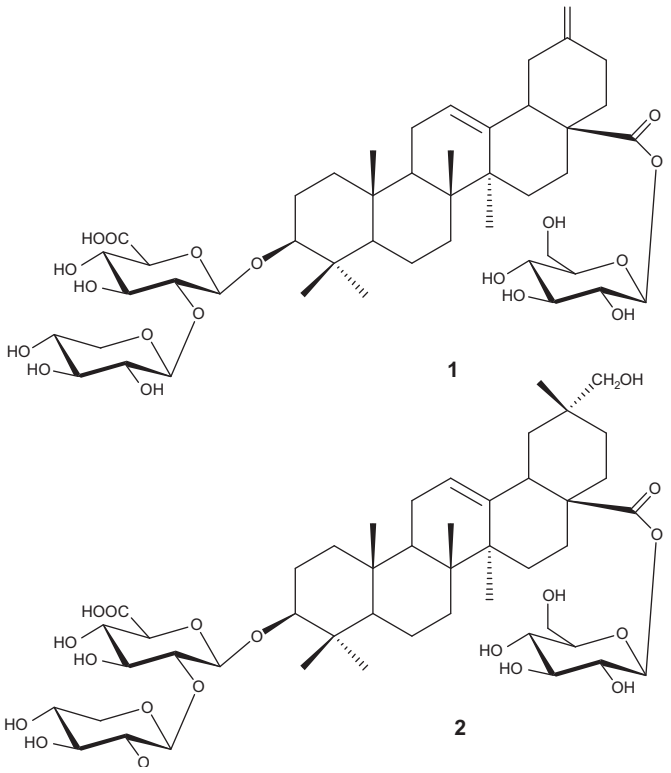

Fig. 1. Compounds **1** and **2** isolated from *Salsola imbricata.*

at *m*/*z* 463 [M+Na-132-176-162]$^+$, attributable to the subsequent loss of one hexose unit.

The ^{1}H NMR spectrum of the aglycone portion of **1** displayed signals for five tertiary methyl groups at δ 0.83, 0.87, 0.98, 1.08, and 1.22, two exo-methylene protons at δ 4.64, an olefinic proton at δ 5.35 (1H, t, J = 3.5 Hz), and for a proton at δ 3.22 (1H, dd, J = 11.5, 4.2) assigned to H-3 (Table 1). The ^{13}C NMR spectrum of **1** showed for the aglycone 29 carbon signals which suggested a noroleanane skeleton (Table 1). Two olefinic carbons at δ 124.0 (C-12) and δ 143.7 (C-13) indicated a typical Δ^{12} pentacyclic triterpene derivative (De Tommasi et al., 1998). In the HMBC spectrum of **1**, the 20(29)-exo-methylene proton signal at δ 4.64 correlated with two methylene carbons at δ 42.2 (C-19) and 30.8 (C-21) which confirmed a noroleanene-type skeleton. A detailed analysis of NMR data allowed us to determine the aglycone as the akebonic acid (Ikuta and Itokawa, 1989).

The ^{1}H NMR spectrum displayed in the sugar region signals corresponding to three anomeric protons at δ 5.40 (d, J = 7.8 Hz), 4.55 (d, J = 7.5 Hz), and 4.46 (d, J = 7.5 Hz), whose carbon resonances were assigned unambiguously by HSQC experiments to be at δ 95.4, 106.2, and 105.1, respectively (Table 1).

Complete assignments of the resonances of each sugar unit were achieved by extensive 1D-(^{1}H, ^{13}C, TOCSY) and 2D-(HSQC, HMBC) NMR analyzes. All these data showed the presence of one β-glucopyranosyl unit (δ 5.40), one β-xylopyranosyl unit (δ 4.55), and one β-glucuronic acid unit (δ 4.46). The D configuration of xylose, glucose and glucuronic acid units were established after hydrolysis of **1** with 1 N HCl, trimethylsilation and determination of the retention times by GC (De Marino et al., 2003).

Glycosidation shifts were observed for C-2$_{glcA}$ (δ 83.1), C-3 (δ 90.6) and C-28 (δ 177.8). All connectivities within **1** were confirmed by the HMBC spectrum, which showed a long-range correlation between H-1$_{xyl}$ (δ 4.55) and C-2$_{glcA}$ (δ 83.1), H-1$_{glcA}$ (δ 4.46) and C-3 (δ 90.6), and between H-1$_{glc}$ (δ 5.40) and C-28 (δ 177.8), confirming the bidesmosidic character of **1**.

Table 1
^{1}H and ^{13}C NMR data (J in Hz) of compounds **1** and **2** (600 MHz, δ ppm, in CD_3OD).

Position	**1**		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	39.5	1.63, 1.03, m	39.6	1.62,1.02, m
2	26.8	2.03, 1.73, m	26.8	2.03, 1.70, m
3	90.6	3.22, dd (11.5, 4.2)	90.4	3.20, dd (11.4, 4.2)
4	39.8	–	40.1	–
5	57.0	0.81, m	56.8	0.79, m
6	19.2	1.57, 1.43, m	19.1	1.56, 1.42, m
7	33.7	1.52, 1.35, m	33.7	1.51, 1.33, m
8	40.7	–	40.5	–
9	48.7	1.62, m	48.8	1.60, m
10	37.8	–	37.6	–
11	24.5	1.95 (2H), m	24.3	1.92 (2H), t (3.5)
12	124.0	5.35, t (3.5)	123.8	5.29, t (3.5)
13	143.7	–	144.6	–
14	42.7	–	42.5	–
15	29.0	1.88 1.17, m	28.7	1.83, 1.11, m
16	23.9	2.20, 1.88, m	23.7	2.09, td (13.7, 3.0) 1.75, m
17	47.8	–	47.8	–
18	48.4	2.77, dd (13.3, 4.7)	41.6	2.91, dd (13.7, 3.3)
19	42.2	2.59 t (13.3), 2.11, dd (13.3, 4.7)	41.0	1.84, 1.13, m
20	149.3	–	36.7	–
21	30.8	2.26, 2.16, m	29.1	1.53, 1.19, m
22	38.4	1.94, 1.58, m	32.3	1.77, 1.68, m
23	28.1	1.08, s	28.0	1.07, s
24	16.4	0.87, s	16.3	0.86, s
25	15.6	0.98, s	15.8	0.98, s
26	17.6	0.83, s	17.4	0.83, s
27	26.4	1.22, s	26.2	1.19, s
28	177.8	–	177.5	–
29	107.2	4.64 (2H), s	74.1	3.21 (2H), s
30	–	–	19.3	0.95, s
	β-D-GlcA (at C-3)		β-D-GlcA (at C-3)	
1	105.1	4.46, d (7.5)	105.0	4.45, d (7.5)
2	83.2	3.51, dd (7.5, 9.0)	83.1	3.51, dd (7.5, 9.0)
3	77.7	3.62, dd (9.0, 9.0)	77.6	3.62, dd (9.0, 9.0)
4	73.4	3.49, dd (9.0, 9.0)	73.3	3.49, dd (9.0, 9.0)
5	76.2	3.57, m	76.4	3.57, m
6	176.8	–	176.5	–
	β-D-Xyl (at C-2$_{GlcA}$)		β-D-Xyl (at C-2$_{GlcA}$)	
1	106.2	4.55, d (7.5)	106.1	4.55, d (7.5)
2	76.2	3.25, dd (9.2, 7.5)	75.8	3.25, dd (9.2, 7.5)
3	77.4	3.34, t (9.2)	77.8	3.34, t (9.2)
4	70.9	3.47, m	70.9	3.49, m
5	66.8	3.82, dd (11.7, 5.2) 3.16, t (11.7)	67.2	3.84, dd (11.7, 5.2) 3.17, t (11.7)
	β-D-Glc (at C-28)		β-D-Glc (at C-28)	
1	95.4	5.40, d (7.8)	95.4	5.42, d (7.8)
2	73.7	3.35, dd (7.8, 9.0)	73.6	3.36, dd (7.8, 9.0)
3	78.3	3.38, dd (9.0, 9.0)	78.1	3.38, dd (9.0, 9.0)
4	70.7	3.38, t (9.0)	70.9	3.39, t (9.0)
5	77.9	3.44, m	78.1	3.45, m
6	62.1	3.84, dd (2.5, 12.0) 3.71, dd (4.5, 12.0)	62.3	3.84, dd (2.5, 12.0) 3.70, dd (4.5, 12.0)

Therefore, compound **1** was identified as the new 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucuronopyranosyl akebonic acid 28-*O*-β-D-glucopyranoside.

To the best of our knowledge, this is the first report of compounds characterized by a C-30 noroleanane skeleton with a C20–C29 double bond in the *Salsola* genus.

The HRMALDITOF mass spectrum of **2** showed a major ion peak at *m*/*z* 965.4728 [M+Na]$^+$ ascribable to the molecular formula $C_{47}H_{74}O_{19}$ (calcd. for $C_{47}H_{74}NaO_{19}$, 965.4722). The ESI-MS spectrum showed the major ion peak at *m*/*z* 965 [M+Na]$^+$ and significant fragments in MS/MS analysis at *m*/*z* 657 [M+Na-132-176]$^+$ ascribable to the loss of one pentose and one uronic acid, and at *m*/*z* 495 [M+Na-132-176-162]$^+$ due to the subsequent loss of one hexose unit. The ^{1}H NMR spectrum of the aglycone portion of **2**

displayed signals attributable to six tertiary methyl groups at δ 0.83, 0.86, 0.95, 0.98, 1.07, and 1.19, a primary alcoholic function at δ 3.21 (2H, s), and an olefinic proton at δ 5.29 (1H, t, J = 3.5 Hz). A signal at δ 3.20 (1H, dd, J = 11.4, 4.2) was assigned to H-3 (Table 1).

The ^{13}C NMR spectrum of **2** showed for the aglycone portion 30 carbon signals. Two olefinic carbons at δ 123.8 (C-12) and δ 144.6 (C-13) indicated a typical Δ^{12} pentacyclic triterpene derivative (Table 1). In the HMBC spectrum of **2**, the correlations of the primary alcoholic proton signal at δ 3.21 with the methyl carbon at δ 19.3 (C-30) and with the two methylene carbons at δ 41.0 (C-19) and 29.1 (C-21) confirmed the location of the secondary alcoholic group at C-29. Thus, the aglycone of **2** was identified as 29-hydroxyoleanolic acid (mesembryanthemoidigenic acid), which was confirmed by comparison with literature data (Wenjuan et al., 1986).

The ^{1}H NMR spectrum displayed in the sugar region signals corresponding to three anomeric protons at δ 5.42 (d, J = 7.8 Hz), 4.55 (d, J = 7.5 Hz), and 4.45 (d, J = 7.5 Hz) (Table 1), corresponding to one β-glucopyranosyl unit, one β-xylopyranosyl unit, and one β-glucuronic acid unit, respectively. The ^{1}H NMR and ^{13}C chemical shifts of the sugar moiety were superimposable on those of **1**. In the HMBC spectrum long-range correlations between H-1$_{xyl}$ (δ 4.55) and C-2$_{glcA}$ (δ 83.1), H-1$_{glcA}$ (δ 4.45) and C-3 (δ 90.4), and H-1$_{glc}$ (δ 5.42) and C-28 (δ 177.5) were observed. Therefore, compound **2** was identified as the new 3-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucuronopyranosyl-29-hydroxyoleanolic acid 28-*O*-β-D-glucopyranoside. 29-Hydroxyoleanene glycosides, salsolins A and B, have been also isolated from another *Salsola* species, *S. baryosma* and have been reported to possess a significant antioxidant activity (Ahmad et al., 2008).

Pseudoginsenoside RT, silphioside G and boussingoside A2 were identified by comparison of their ^{1}H and ^{13}C NMR data with those reported in the literature (Tanaka et al., 1985; Espada et al., 1990). Boussingoside A2 is a C-30 noroleanane derivative with a C20–C29 double bond and, therefore, together with compound **1** represent the first report of this kind of compounds in a *Salsola* species, thus being potentially useful as chemotaxonomic markers to differentiate *S. imbricata* in the frame of *Salsola* genus.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP 1000 polarimeter. NMR experiments were performed on a Bruker DRX-600 spectrometer equipped with a Bruker 5 mm TCI CryoProbe at 300 K. All 2D-NMR spectra were acquired in CD_3OD (99.95%, Sigma Aldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, HMBC and ROESY spectra. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-off light (MALDITOF) mass spectrometry. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18–39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standards. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max ion-trap mass spectrometer equipped with Xcalibur software. Column chromatography was performed over RP-18 (LiChroprep_ RP-18, 40–63 lm, Merck, 2 cm × 50 cm). GC apparatus using a l-Chirasil-Val column (0.32 mm × 25 m). TLC was performed on both silica gel F254 (Merck) and RP-18 F_{254s} plates, and visualized with Liebermann–Burchard reagent.

3.2. Plant material

The roots of *S. imbricata* Forssk. subsp. *imbricata* were collected at Edfu-Marsa Alam road, South-Eastern Desert (Aswan, Egypt) in February 2009 and identified by Prof. M.G. Sheded according to Täckholm (1974) and Boulos (1999). The voucher specimen (No. 11373) was deposited at the Botany Department Herbarium, Faculty of Science of Aswan, Egypt.

3.3. Extraction and isolation

The roots of *S. imbricata* Forssk. subsp. *imbricata* (1.4 kg) were powdered and exhaustively extracted with 80% MeOH (3 L× 1.5 L) for 20 days by maceration at room temperature. The crude extract was concentrated under reduced pressure to a syrupy consistency (200 g). Part of the extract (2.5 g) was fractionated on an RP-18 column (LiChroprep_ RP-18, 25-40 lm, Merck, 10 cm × 25 cm) using H_2O: MeOH as the mobile phase. Six fractions (1000 mL) were collected 100% H_2O (550 mg), 20% MeOH (624 mg), 40% MeOH (226 mg), 60% MeOH (349 mg), 80% MeOH (333 mg), and 100% MeOH (1.411 g), respectively. Fraction obtained with 80% MeOH contained saponins. 100 mg of this fraction from 80% was fractioned on RP-18 column (50 × 2) eluted with 60–70% MeOH as mobile phase to yield compounds **1** (5 mg), **2** (4.8 mg), boussingoside A2 (8.2 mg), pseudoginsenoside RT1 (9.2 mg), and silphioside G (10.2 mg), respectively.

3.4. 3-*O*-β-*D*-xylopyranosyl-(1 → 2)-*O*-β-*D*-glucuronopyranosyl-akebonic acid 28-*O*-β-*D*-glucopyranoside (1)

Amorphous powder; $[\alpha]_D^{25} + 45.2$ (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; ESIMS *m/z* 933 $[M+Na]^+$; ESIMS/MS *m/z* 625 $[M+Na\text{-}132\text{-}176]^+$, 463 $[M+Na\text{-}132\text{-}176\text{-}162]^+$; HRMALDITOFMS *m/z* 933.4065 $[M+Na]^+$ (calcd. for $C_{46}H_{70}NaO_{18}$, 933.4060).

3.5. 3-*O*-β-*D*-xylopyranosyl-(1→2)-*O*-β-*D*-glucuronopyranosyl-29-hydroxyoleanolic acid-28-*O*-β-*D*-glucopyranoside (2)

Amorphous powder; $[\alpha]_D^{25} + 31$ (*c* 0.3, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; ESIMS *m/z* 965 $[M+Na]^+$; ESIMS/MS *m/z* 657 $[M+Na\text{-}132\text{-}176]^+$, 495 $[M+Na\text{-}132\text{-}176\text{-}162]^+$; HRMALDITOFMS *m/z* 965.4728 $[M+Na]^+$ (calcd. for $C_{47}H_{74}NaO_{19}$, 965.4722).

3.6. Acid hydrolysis

The configuration of sugar units were established after hydrolysis of **1** and **2** with 1 N HCl, trimethylsilation and determination of the retention times by GC operating in the experimental conditions previously reported by De Marino et al. (2003).

The peaks of the hydrolysate of **1** and **2** were detected at 10.99 and 12.02 (D-xylose), at 14.73 min (D-glucose) and at 15.83 min (D-glucuronic acid). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 10.98 and 12.00 min (D-xylose), and 14.71 min (D-glucose), 15.81 min (D-glucuronic acid).

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