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A New Cardenolide and Other Compounds from *Salsola tetragona*

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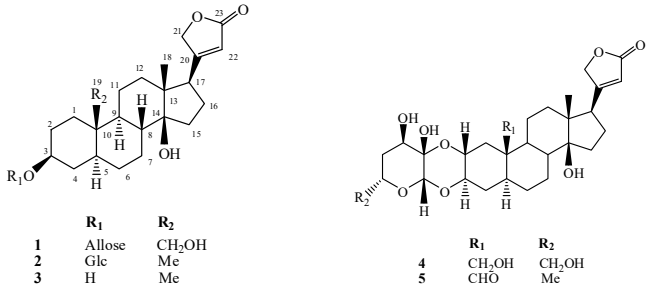
One new cardenolide, 3-*O*-β-D-allopyranosylcoroglaucigenin (salsotetragonin) (**1**), was isolated from the aerial parts of *Salsola tetragona* Delile with four known cardenolides (**2-5**), two known flavonoids (**6-7**), three known phenolic compounds (**8-10**) and two known fatty acids (**11-12**). Their structures were identified by spectroscopic analyses and by comparison of their spectral data with those reported in the literature. Compounds **1-5**, **7**, **10** and **12** were isolated from the genus *Salsola* for the first time. This is the first report on cardenolides identified in the Amaranthaceae family.

Keywords: *Salsola tetragona* Delile, Amaranthaceae, Cardenolides, Phenolic glycosides.

The genus *Salsola* (Amaranthaceae, ex. Chenopodiaceae) consists of over 174 species found in the arid regions of Asia, Europe and Africa [1]. *Salsola* species possess antihypertensive, anti-inflammatory, anticancer, antioxidant, anti-Alzheimer, antidepressant and antimicrobial activities [2-5]. Previous phytochemical investigations of this genus reported the isolation of flavonoids and other phenolic compounds [6], alkaloids [7], fatty acids [8] and triterpene glycosides [9]. In continuation of our works on plants from Algerian Septentrional Sahara [10-11], we report here the isolation and structural characterization of one new cardenolide, 3-*O*-β-D-allopyranosylcoroglaucigenin, named salsotetragonin (**1**), in addition to eleven known compounds from aerial parts of *S. tetragona* (Figure S1).

The hydromethanolic extract of the aerial parts of *S. tetragona* was partitioned successively with light petrolum, CH_2Cl_2, EtOAc and *n*-butanol to give four extracts. Purification of the *n*-butanol extract through repeated silica gel columns, flash chromatography and semi-prep HPLC led to the isolation and identification of a new cardenolide (**1**) and seven known compounds (**3**, **4**, **6-10**). The dichloromethane extract was fractionated by silica gel chromatography to give four known compounds (**2**, **5**, **11-12**). These compounds were identified by extensive spectroscopic methods including 1D-(^{1}H and ^{13}C) and 2D-NMR (COSY, HSQC, HMBC and NOESY) experiments, as well as HR-ESI-MS analysis and by comparison of their spectral data with the literature. The eleven known compounds were identified as four cardenolides: uzarigenin (**2**) [12], desglucouzarin (**3**) [13], 12-dehydroxyghalakinoside (**4**), and calactin (**5**) [14] (Figure 1), two flavonoids: kaempferol-3-*O*-β-D-glucopyranoside (**6**) [15] and quercetin-3-*O*-β-D-glucopyranosyl-(1→6)-glucopyranoside (**7**) [16], three phenolic compounds: vanillic acid (**8**) [17], canthoside C (**9**), and canthoside D (**10**) [18] and two fatty acids : oleic acid (**11**) [19], and 2,3-dihydroxypropylpalmitate (**12**) [20] (Figure S1).

Compound **1** was isolated as a white amorphous powder. Its positive HR-ESI-MS showed a pseudomolecular ion peak at *m/z* 575.2829 $[M+Na]^+$, corresponding to the molecular formula $C_{29}H_{44}O_{10}$. The



	R_1	R_2
1	Allose	CH_2OH
2	Glc	Me
3	H	Me

	R_1	R_2
4	CH_2OH	CH_2OH
5	CHO	Me

Figure 1: Structures of compounds **1-5**.

^{1}H NMR spectrum of compound **1** (Table 1) showed characteristic signals of a butenolactone ring at δ_H 4.95 (1H, dd, *J* =18.4, 1.6 Hz, H-21a), 5.07 (1H, dd, *J* = 18.4,1.4 Hz, H-21b) and 5.93 (1H, s, H-22), one oxymethylene at δ_H 3.74 (1H, d, *J* = 11.7 Hz, H-19a) and δ_H 3.88 (1H, d, *J* = 11.7 Hz, H-19b), one oxymethine at δ_H 3.78 (m, H-3), and one methyl signal at δ_H 0.95 (3H, s, H-18). Its ^{13}C NMR spectrum (Table 1) showed twenty-nine carbon signals including six of the sugar part (Figure 1).

The ^{1}H NMR and ^{13}C NMR data of **1** were very similar to those of 3-β-D-glucopyranosylcoroglaucigenin [21]. The COSY and HSQC spectra showed one anomeric proton of a sugar moiety at δ_H 4.78 (d, *J* = 7.9 Hz, H-1′) and δ_C 99.8 with five signals of an hexose unit [H-2′ [δ 3.28 (dd, *J* = 7.9, 3.0 Hz)], H-3′ [δ 4.06 (t, *J* = 3.0 Hz)], H-4′ [δ 3.48 (dd, *J* = 9.5, 3.0 Hz)], H-5′ [δ 3.69 (m)], and H_2-6′, [δ 3.66 (dd, *J* = 11.3, 5.7 Hz) and 3.85 (dd, *J* = 11.3, 1.8 Hz)]; the sugar was identified as an allopyranoside [22]. The large coupling constant (*J* = 7.9 Hz) of the anomeric proton at δ_H 4.78 indicated that the allose had a β-configuration. The linkage of the allose at C-3 of the aglycone was established from the HMBC correlation between allo-H-1′ (δ_H 4.78) and C-3 (δ_C 78.9) (Figure 2). The stereochemistry of **1** was confirmed by the NOE effect (Figure 2) between H-5/H-9 and H-3, which clearly established the α-orientation of H-5.The correlation of H_3-18 to H-21 and H-22 in the

Noesy spectrum indicated the β orientation of the γ lactone at C-17. The chemical shifts of the carbons of the C and D rings of **1** are in agreement with those of coroglaucigenin and madagascarrensilid (A) recorded in deuterated pyidine and in CD_3OD [23,24], indicating a 14-β hydroxy orientation. Thus compound **1** was identified as 3-*O*-β-D-allopyranosylcoroglaucigenin, which we named salsotetragonin.

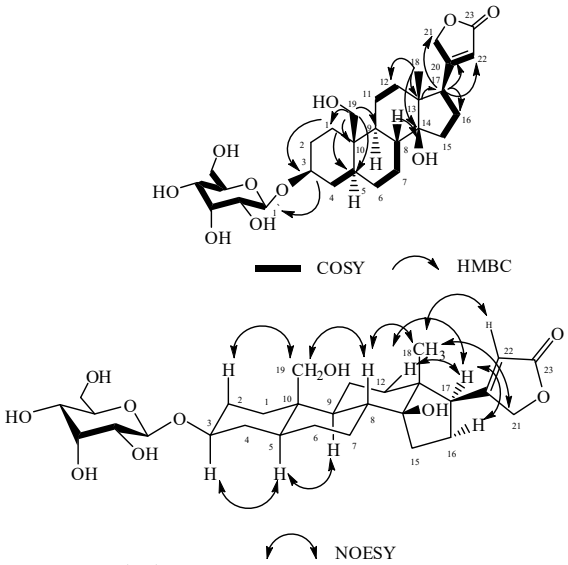


Figure 2: ^1H–^1H COSY, HMBC and NOESY correlations of **1**.

Table 1: ^1H and ^{13}C NMR data for compound **1** (in CD_3OD; ^{13}C: 150 MHz; ^1H: 600 MHz).

Position	**1**	
	δ_H (m, *J* in Hz)	δ_C
1	0.81 (td, 13.6, 3.5), 2.34 (dt, 13.3, 3.5)	32.8
2	1.57 (dm, 14.9), 1.91 (m)	30.9
3	3.78 (m)	78.9
4	1.44 (m), 1.82 (dd, 11.9, 3.4)	35.9
5	1.24 (m)	45.9
6	1.24 (m), 1.34 (m)	29.6
7	2.10 (dd, 12.3, 3.1), 1.15 (m)	28.8
8	1.82 (td, 11.9, 3.4)	43.1
9	1.03 (td, 13.5, 4.5)	51.5
10	-	40.6
11	1.65 (m)	24.1
12	1.41 (td, 13.6, 4.3), 1.53 (dt, 13.5, 3.0)	41.5
13	-	51.2
14	-	86.3
15	1.73 (dd, 11.6, 8.5), 2.14 (m)	33.5
16	2.16 (m), 1.90 (m)	28.1
17	2.85 (dd, 9.5, 5.8)	52.2
18	0.95 (s)	16.7
19	3.74 (d, 11.7), 3.88 (d, 11.7)	59.9
20	-	178.5
21	4.95 (dd, 18.4, 1.6), 5.07 (dd, 18.4, 1.4)	75.3
22	5.93 (s)	117.8
23	-	176.9
Allose (at C-3)		
1′	4.78 (d, 7.9)	99.8
2′	3.28 (dd, 7.9, 3.0)	72.4
3′	4.06 (t, 3.0)	73.1
4′	3.48 (dd, 9.5, 3.0)	69.1
5′	3.69 (m)	75.5
6′	3.66 (dd, 11.3, 5.7), 3.85 (dd, 11.3, 1.8)	63.3

Experimental

General: Optical rotations were measured in DMSO with a Perkin-Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX III 500 spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz) and on a Bruker Avance III-600 spectrometer equipped with a cryo platform (^1H at 600 MHz and ^{13}C at 150 MHz). 2D-NMR experiments were performed using standard Bruker-microprograms (TopSpin version 3.2 software). HR-ESI-MS and ESI-MS experiments were performed using a Micromass Q-TOF micro-instrument (Manchester, UK). TLC was carried out on pre-coated silica gel 60 F_{254} (Merck). Vacuum liquid chromatography (VLC) was carried out on LiChroprep RP-18 (40-63 µm, Merck). HPFC was performed on a Grace® Reveleris System using Grace® cartridges (Silica gel or RP-C_{18}). The semi-preparative HPLC was performed on a Dionex apparatus equipped with an ASI-100 automated sample injector, a STH 585 column oven, a LPG-3400A pump, a diode array detector UVD-340U and Chromeleon® software.

Plant material: *Salsola tetragona* Delile was collected in Ghardaïa (Algerian Septentrional Sahara) in May 2011 and was identified by Pr. Gérard De Belair (University of Annaba, Algeria). A voucher specimen (LOST.St05/09) was deposited at the herbarium of the Faculty of Sciences, University frères Mentouri- Constantine.

Extraction and isolation: Air-dried and powdered aerial parts (1980 g) of *S. tetragona* were extracted with 80% MeOH (10 L). After evaporating the MeOH under *vacuum*, the residue was dissolved in water (900 mL) and partitioned with PE, CH_2Cl_2, EtOAc, and *n*-BuOH, successively (3 x 300 mL). The *n*-BuOH extract (6 g) was subjected to VLC over RP-18 eluted with H_2O-MeOH (6:4, 5:5, 4:6, 3:7, 2:8 and 0:10). Fractions of 150 mL were collected and pooled according to their similarity in TLC profile to give 6 fractions (F1–F6 respectively). F1 (1.2 g) was submitted to RP-18 flash chromatography eluting with MeCN:H_2O (5:95 to 10:0) to afford 19 sub-fractions. Sub-fraction [20-23] (35 mg), was purified by semi-prep HPLC on RP-18 eluted with MeCN:H_2O (6:94 to 1:9), affording compounds **9** (3 mg) and **10** (1.5 mg). F2 (1.5 g) was chromatographed on a silica gel column eluted with EtOAc:MeOH:H_2O (20:2:1), giving 2 main sub-fractions. Sub-fraction [90] (180 mg) afforded **4** (22 mg) by silica gel CC ($CHCl_3$:MeOH, 9:1). Sub-fraction [115-119] (223 mg) was subjected to silica gel CC eluting with $CHCl_3$:MeOH (8:2) to afford **1** (27 mg) and sub-fraction [35-48] (100 mg), which was purified over silica gel using EtOAc:MeOH:H_2O (20:2:1) to yield compounds **6** (15 mg) and **7** (18 mg). F3 (750 mg) was fractionated by silica gel CC eluted with $CHCl_3$:MeOH (0 to 100% of MeOH). The main sub-fraction [59-63] (212 mg) was further separated by silica gel CC ($CHCl_3$:MeOH, 0 to 100% of MeOH) and **3** (25 mg) was isolated. F4 (302 mg) was subjected to silica gel CC using $CHCl_3$:MeOH (0 to 100% of MeOH) to give 3 sub-fractions. Sub-fraction [72] (111.2 mg) was selected for silica gel CC eluted with $CHCl_3$:MeOH (0 to 100% of MeOH) to yield compound **8** (3 mg).

The CH_2Cl_2 extract (2.5 g) was subjected to silica gel CC, eluted with toluene:$CHCl_3$ (0 to 100% of $CHCl_3$) and $CHCl_3$:EtOAc (0 to 100% of EtOAc). Sub-fraction [27-32] (123 mg) was purified over silica gel using diethyl ether:EtOAc (9:1) to yield compound **11** (1.4 mg). Sub-fraction [160-173] (212 mg) was passed through a silica gel column ($CHCl_3$:EtOAc, 9:1) yielding compounds **2** (15 mg) and **5** (5 mg). Sub-fraction [234-257] (162 mg) was chromatographed on silica gel, eluted with ($CHCl_3$:MeOH, 9:1) that afforded compound **12** (2 mg).

Acid hydrolysis: The pure compounds were treated with 2 M HCl at 100°C for 1 h. The hydrolysates were extracted with EtOAc and the sugars identified in the aqueous residue by comparison with authentic samples by TLC using silica gel impregnated with 0.2 M NaH_2PO_4 and a solvent system of Me_2CO–H_2O (9:1); the compounds were revealed with aniline malonate. The optical rotation of the purified sugar was measured and compared with an authentic sample of D-allose.

3-*O*-β-D-Allopyranosylcoroglaucigenin (salsotetragonin) (1)
White amorphous powder.
$[\alpha]_D^{20}$: -16.9 (*c* 0.18, DMSO).
^{1}H NMR (CD_3OD, 600 MHz): Table 1.
^{13}C NMR (CD_3OD, 150 MHz): Table 1.
HR-ESI-MS: $[M+Na]^+$ *m/z* 575.2829 (calcd for $C_{29}H_{44}O_{10}Na$, 575.2832).

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Supplementary data: Figure S1 and Supplementary Data are included in the Supporting information.

References

[1] Botschantzev V. (**1974**) A synopsis of *Salsola* (Chenopodiaceae) from South and South-West africa. *Kew Bulletin, **29***, 597-614.
[2] Ahmad Z, Mehmood S, Fatima I, Malik A, Ifzal R, Afza N, Iqbal L, Latif M, Nizami TA. (**2008**) Structural determination of salsolins A and B, new antioxidant polyoxygenated triterpenes from *Salsola baryosma* by 1D and 2D NMR spectroscopy. *Magnetic Resonance in Chemistry*, ***46***, 94-98.
[3] Woo WS, Lee EB, Chi HJ. (**1977**) Biological evaluation of some Saudi Arabian plants. *Journal* of *Korean Pharmaceutical Sciences*, ***21***, 141-145.
[4] Oueslati MH, Ben JH, Mighri Z, Chriaa J, Abreu PM. (**2006**) Phytochemical constituents from *Salsola tetrandra*. *Journal of Natural Products*, ***69***, 1366-1369.
[5] Tundis R, Loizzo MR, Statti GA, Menichini F. (**2007**) Inhibitory effects on the digestive enzyme alpha-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*. *Pharmazie*, ***62***, 473-475.
[6] Xiang Y, Li YB, Zhang J, Li P, Yao YZ. (**2007**) Studies on chemical constituents of *Salsola collina*. *Zhongguo Zhong Yao Za Zhi*, ***32***, 409-413.
[7] Xiang Y, Li YB, Zhang J, Li P, Yao YZ. (**2007**) A new alkaloid from *Salsola collina. Yao Xue Xue Bao*, ***42***, 618-620.
[8] Jin YS, Du JL, Yang Y, Jin L, Song Y, Zhang W, Chen HS. (**2011**) Chemical and biologically active constituents of *Salsola collina*. *Chemistry of Natural Compounds*, ***47***, 257-260.
[9] Hamed AI, Masullo M, Sheded MG, Mahalel UA, Tawfik MM, Perrone A, Piacente S. (**2011**) Triterpene saponins from *Salsola imbricata*. *Phytochemistry Letters*, ***4***, 353-356.
[10] Boudiar T, Lakhal H, Khalfallah A, Kabouche A, Kabouche Z, Brouard I, Bermejo J, Christian B. (**2010**) A new alkaloid and flavonoids from the aerial parts of *Euphorbia guyoniana*. *Natural Product Communications*, ***5***, 35-37.
[11] Lakhal H, Boudiar T, Kabouche A, Kabouche Z, Touzani R, Christian B. (**2010**) New sesquiterpene lactone and other constituents from *Centaurea sulphurea* (Asteraceae). *Natural Product Communications*, ***5***, 849-850.
[12] Shaker KH, Morsy N, Zinecker H, Imhoff JF, Schneider B. (**2010**) Secondary metabolites from *Calotropis procera* (Aiton). *Phytochemistry Letters*, ***3***, 212-216.
[13] Elgamal M, Hani A, Hanna AG, Morsy NAM, Duddeck H, Simon A, Gati T, Toth G. (**1999**) Complete ^{1}H and ^{13}C signal assignments of 5α cardenolides isolated from *Calotropis procera* R. B.R. *Journal of Molecular Structure*, ***477***, 201-208.
[14] Hamed AI, Plaza A, Balestrieri ML, Mahalel UA, Springuel IV, Oleszek W, Pizza C, Piacente S. (**2006**) Cardenolide glycosides from *Pergularia tomentosa* and their proapoptotic activity in Kaposi's sarcoma cells. *Journal of Natural Products*, ***69***, 1319-1322.
[15] Berrehal D, Khalfallah A, Kabouche A, Kabouche Z, Karioti A, Bilia AR. (**2010**) Flavonoid glycosides from *Randonia africana* Coss. (Resedaceae). *Biochemical Systematics and Ecology*, ***38***, 1007-1009.
[16] Shehab NG, Mahdy A, Khan SA, Noureddin SM. (**2011**) Chemical constituents and biological activities of *Fagonia indica* Burm F. *Research Journal* of *Medicinal Plant*, ***5***, 531-546.
[17] Wang XJ, Zhao YX, Jia XH, Ding XB. (**2011**) Studies on the chemical constituents of *Salsola collina*. *Zhong yao cai*, ***34***, 230-231.
[18] Kanchanapoom T, Kasai R, Yamasaki K. (**2002**) Iridoid and phenolic diglycosides from *Canthium berberidifolium*. *Phytochemistry*, ***61***, 461-464.
[19] Turki ZA. (**2000**) Chemotaxonomical studies of the genus *Salsola* (Chenopodiaceae) in Egypt. *Egyptian Journal of Botany*, ***38***, 47-61.
[20] Li Y, An K, Sun J, Wang J, Zhong Y, Sun L. (**2011**) Chemical constituents of vinegar-processed *Kansui radix*. *Food and Drug*, ***13***, 183-186.
[21] Jolad SD, Bastest RB, Cole JR, Hoffmann JJ, Siahaan TJ, Timmerann BN. (**1986**) Cardenolides and a lignan from *Asclepias subulata*. *Phytochemitry*, ***25***, 2581-2590.
[22] Murakami T, Emoto A, Matsuda H, Yoshikawa M. (**2001**) Medicinal foodstuffs. XXI. Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a, -b, -c, -d, -e, -f, -g, and -h, and new oleanane-type triterpene saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese *Momordica charantia* L. *Chemical and Pharmaceutical Bulletin*, ***49***, 54-63.
[23] Yamauchi T, Abe F, Nish M. (**1978**) ^{13}C NMR of 5α-cardenolides. *Chemical and Pharmaceutical Bulletin*, ***26***, 2894-2896.
[24] Pan E, Harinantenaina L, Brodie PJ, Callmander M, Rakotonandrasana S, Rakotobe E, Resamison VE, TenDyke K, Shen Y, Suh EM, Kingston DGI. (**2011**) Cardenolides of *Leptadenia madagascariensis* from the Madagascar dry forest. *Bioorganic and Medicinal Chemistry*, ***19***, 422-428.