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TRITERPENOID SAPONINS FROM *ZYGOPHYLLUM DECUMBENS*‡

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Key Word Index—*Zygophyllum decumbens*; Zygophyllaceae; triterpenoid saponins; zygophylosides I, J and K.

Abstract—Three new triterpenoid saponins, 3-*O*-[β-D-glucuronic acid pyranosyl]-arjunolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygophyloside I), 3-*O*-[β-D-glucuronic acid pyranosyl]-30-norajunolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygophyloside J) and 3-*O*-[β-D-glucuronic acid pyranosyl]-29-hydroxyoleanolic acid-28-*O*-[β-D-glucopyranosyl] ester (zygophyloside K) have been isolated from *Z. decumbens*. The structures were established primarily on the basis of NMR spectroscopy. The assignments of all NMR signals were performed by means of ^{1}H–^{1}H COSY, NOESY, ROESY, TOCSY, HMQC and HMBC experiments.

INTRODUCTION

As part of our investigations on the triterpenoid saponins from pharmacologically interesting *Zygophyllum* species [1] we now report the isolation and structure elucidation of three new triterpenoid saponins zygophyloside I (**1**), J (**2**) and K (**3**) from *Z. decumbens* Del. This plant, known in Arabic as "Bossel Kelab", grows wild in the arid zones of Egypt, Syria, Palestine and Sudan. The aqueous extract of this plant shows hypotensive, antipyretic, spasmolytic, diuretic and local anesthetic effects in animal tests [2].

RESULTS AND DISCUSSION

The crude saponin extracts of the whole plants of *Z. decumbens* were obtained as described in the Experimental. After purification by MPLC on LiChroprep RP-8, column chromatography on Sephaded LH-20, followed by preparative and semi-preparative HPLC on RP-18 yielded three new saponins.

The LSI mass spectrum of **1** exhibited the $[M-1]^-$ ion at *m/z* 825. The fragment ions at *m/z* 663 $[M-1-162]^-$ and 487 $[M-1-162-176]^-$ showed the sequential loss of a hexose moiety and a hexose together with a uronic acid fragment. The $[M-1]^-$ ion, together with ^{1}H NMR and ^{13}C NMR data, allowed us to propose the molecular formula $C_{42}H_{66}O_{16}$.

The ^{1}H NMR spectrum of **1** showed two anomeric proton signals at δ 4.60 (H-1′, $^3J_{1',2'}$ = 7.8 Hz) and δ 5.63 (H-1″, $^3J_{1'',2''}$ = 8.1 Hz) indicating the presence of two monosaccharides, one bonded as a glycoside (δ 4.60) and the other as a glycosyl ester (δ 5.63). The sugar moieties were identified as β-glucopyranosyl ester and a β-glucuronic acid pyranoside by ^{1}H–^{1}H COSY-45 and TOCSY spectra. The HMBC cross signals for H_3-24/C-3 and H-1′/C-3 showed the glycosidation with glucuronic acid pyranose in position C-3. The glucosidation of the position C-28 was indicated by the HMBC cross peak of the ^3J coupling between H-1″ glucose/C-28. Starting from H_3-24 (δ 0.70) the assignment of the methyl groups H_3-25 (δ 0.89), H_3-26 (δ 0.80) and H_3-27 (δ 1.06) was possible by the determination of the common ^2J- and ^3J-coupling partners in the HMBC spectrum (Fig. 1). The further assignment of the other ^{1}H and ^{13}C NMR resonances was realised by HMBC, HMQC, ^{1}H–^{1}H COSY and TOCSY experiments. The values of the coupling constants between H-18 and both protons of position C-19 ($^3J_{18,19eq}$ = 4.2 Hz, $^3J_{18,19ax}$ = 13.2 Hz) indicated that H-18 is in the axial position in ring E. This fact and the NOESY cross signal for H-16$_{ax}$/H-21$_{ax}$ showed the *cis*-connection of the rings D and E. The existence of arjunolic acid(2α-OH) instead of bayogenin (2β-OH) was established by the value of the coupling constant of H-3 ($^3J_{2,3}$ = 9.5 Hz), the NOESY cross signals for

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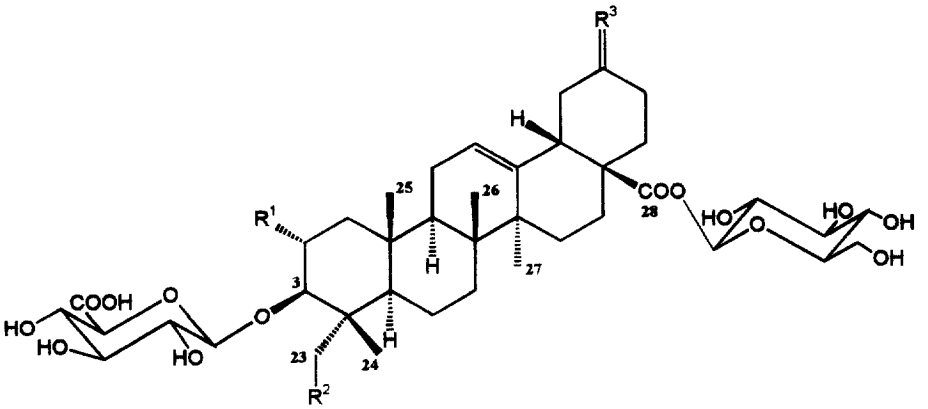
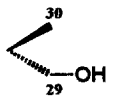


	1	2	3
R^1	OH	OH	H
R^2	OH	OH	H
R^3			

H-2/H_3-24 and H-2/H_3-25 and the good agreement of the ^{13}C NMR data with the published spectra [3].

The LSI mass spectrum of **2** showed the $[M-1]^-$ ion at *m/z* 809. This, compared to the mass spectrum of **1** is a decrease in mass of 16 units. The ^1H NMR and ^{13}C NMR data of **2** showed the presence of the same monosaccharides in positions C-3 and C-28 as in **1**. In the ^1H NMR spectrum only four methyl groups at δ 0.72 (*s*, H_3-24), 0.81 (*s*, H_3-26), 0.83 (*s*, H_3-25) and 0.99 (*s*, H_3-27) were observed for the aglycone. In comparison with **1** a further double bond was established in position C-20/C-29 due to the presence of two broad singlets in the ^1H NMR spectrum at δ 4.52 (H-29a) and 4.57 (H-29b) together with the ^{13}C NMR resonances at δ 107.7 (C-29) and 149.0 (C-20). The ^{13}C NMR resonances of C-1–C-17 and C-23–C-28 of **2** matched well with the appropriate data of **1**, which confirmed the identity of the rings A to D of **1** and **2**. The proton resonance at δ 2.80 was assigned to H-18 because of the cross signals with C-12, C-13, C-16 and C-28 in the HMBC spectrum. The NOESY cross signals H-18/H-22_{ax} and H-16_{ax}/H-21_{ax} enabled us to determine the spin system C-21/C-22 by use of the ^1H–^1H COSY spectrum. The cross signals of H-19_{eq} and H-19_{ax} with the ^{13}C NMR resonance at δ 149.0 (C-20) in the HMBC spectrum were only consistent with the $\Delta^{20(29)}$-double bond. The NOE correlations H-19_{eq}/H-29_a and H-21_{eq}/H-29_b and the good agreement of the ^{13}C NMR resonances of C-7–C-22, C-26, C-27 and C-29 with the published data of 30-norhederagenin [4] were further proof for the position of the $\Delta^{20(29)}$-double bond. The assignment of the ^{13}C NMR resonances of C-21 and C-22 in 30-norhederagenin should be reversed. The *cis*-connection between the rings D and E was shown by the NOESY cross signal H-19_{ax}/H_3-27.

The LSI mass spectrum of **3** showed additionally to the $[M-1]^-$ ion at *m/z* 809 two fragment ions at *m/z* 647 and 633 arising by the loss of a hexose and a uronic acid moiety, respectively. According to the ^1H NMR and ^{13}C NMR data the sugar moieties proved to be the same as in the saponins **1** and **2**. Starting with the ^1H NMR resonance of the anomeric proton of the glucuronic acid pyranoside (H-1′) full assignment of the ^1H NMR and ^{13}C NMR resonances of the rings A–D of the aglycone was possible with the aid of ^1H–^1H COSY, TOCSY, HMBC and HMQC spectra. The ROESY cross signal for H-18/H-22_{ax} allowed us to assign the ^1H and ^{13}C NMR signals of positions C-21 and C-22 by use of the ^1H–^1H COSY and the HMQC experiments. The determination of H_3-30 was possible by the ROESY cross signal for H-18/H_3-30, which enabled us to assign the methylene group at C-29 by the HMBC correlation of H_3-30/C-29. The hydroxy function in position C-29 of **3** explained the strong down field shifts of the proton and carbon

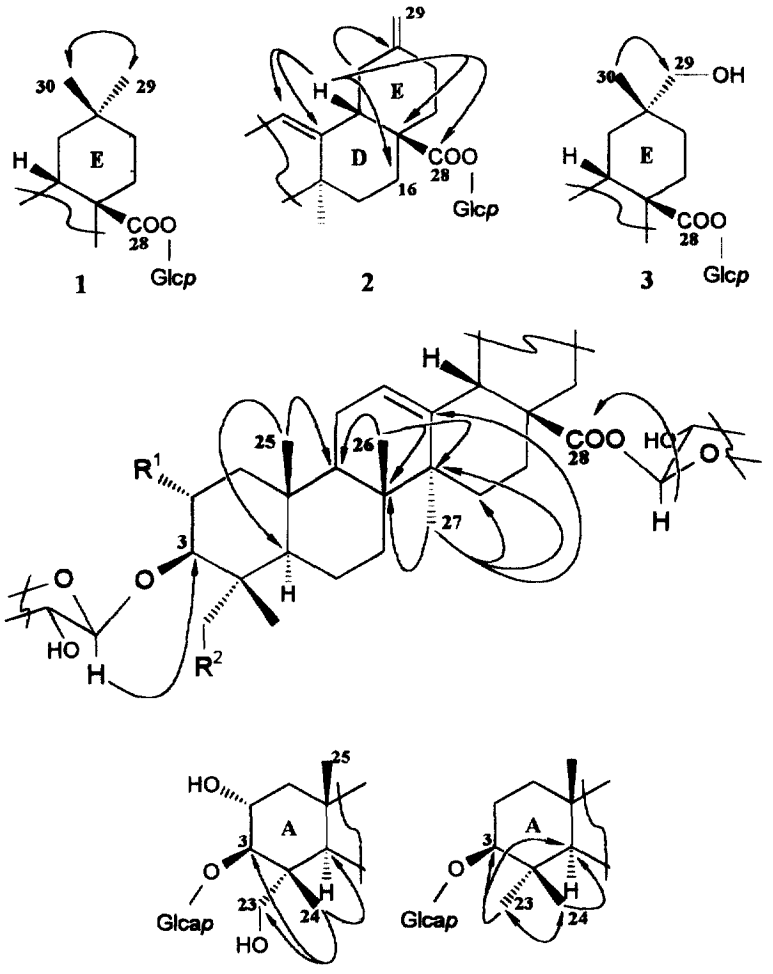


Fig. 1. ^2J and ^3J HMBC correlations of **1**, **2** and **3**.

resonances in comparison with **1** ($\Delta\delta = +2.55/+2.85$; $\Delta\delta = +37.6$).

The determination of the D- or L-form of the two monosaccharides were carried out as described in the Experimental and in Ref. [1]. (*R*)- and (*S*)-2-butanol and the D-forms of glucose (Glc) and glucuronic acid (Glca) were used. In the GLC the R_t values of the trimethylsilylated glycosides (*R*)-2-butyl-D-Glc, (*S*)-2-butyl-D-Glc, (*R*)-2-butyl-D-Glca and (*S*)-2-butyl-D-Glca showed a very good agreement (co-injection) with the trimethylsilylated reference compounds. Consequently it was shown that glucose and glucuronic acid belong to the D-series.

EXPERIMENTAL

General

Mps: uncorr.; negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C), reverse probehead, δ in ppm, solvent CD_3OD and mixture with pyridine-d_5, CD_3OD was used as int. standard (^{1}H: 3.30, ^{13}C: 49.0), temp. 290 and 313 K. NOESY: phase-sensitive using TPPI, mixing time 300 msec, ROESY: phase-sensitive using TPPI, spin lock cw pulse 310 msec. TOCSY: phase-sensitive using TPPI, mixing time 134.3 msec (80 MLEV-17 cycles plus 2 trim pulses of 2.5 msec each), HMQC: phase-sensitive using TPPI, BIRD sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 143 msec ($J_{C,H} = 7$ Hz).

TLC: silica gel (0.25 μm precoated plastic sheets SIL G/UV_{254} Macherey-Nagel). the spots were sprayed with 10% H_2SO_4 in MeOH, 'triterpene reagent' (1% soln of vanillin in 50% H_3PO_4) and phosphomolybdic acid reagent (Aldrich). R_f values were given for $CHCl_3$–MeOH–H_2O (14:11:3) as eluent. For isocratic MPLC on LiChroprep RP-8 (460 × 36 mm, 40–63 μm, Merck) 35% MeOH was used. For analytical, semi-prep. and prep. HPLC a Knauer system equipped with a variable wavelength monitor together with Spherisorb ODS II (250 × 4 mm, 250 × 8

Table 1. ^{1}H NMR spectral data for saponins **1**–**3** in CD_3OD/pyridine-d_5 = 3:2 (**1**, **2**) and CD_3OD (**3**)

	1	**2**	**3**
1 ax/eq	0.93/1.97	0.98/1.98	1.00/1.61
2 ax/eq	3.86/—	3.92/—	1.70/1.99
3 ax	3.67 *d* 9.5 Hz	3.85 *d* 9.7 Hz	3.18
5 ax	1.38	1.43	0.78
6 ax/eq	1.23/1.44	1.14/1.43	1.41/1.53
7 ax/eq	1.53/1.17	1.43/1.14	1.47/1.32
9 ax	1.62	1.58	1.58
11 ax/eq	1.81/1.81	1.75/1.75	1.90/1.90
12	5.20	5.21	5.28
15 ax/eq	1.88/0.98	1.98/0.98	1.80/1.08
16 ax/eq	1.92/1.69	1.98/1.80	2.09/1.77
18 ax	2.87 *dd*	2.80 *dd*	2.75 *dd*
	4.2/13.2 Hz	5.2/13.7 Hz	4.6/14.0 Hz
19 ax/eq	1.62/1.10	2.38/1.98	1.99/1.44
21 ax/eq	1.27/1.04	2.05/1.99	1.64/1.44
22 ax/eq	1.65/1.58	1.40/1.80	1.64/1.75
23			1.04 *s*
23_a	3.90 *d* 11.3 Hz	3.34 *d* 11.0 Hz	
23_b	3.26 *d* 11.3 Hz	4.6 *d* 11.0 Hz	
24	0.70 *s*	0.72 *s*	0.84 *s*
25	0.89 *s*	0.83 *s*	0.94 *s*
26	0.80 *s*	0.81 *s*	0.79 *s*
27	1.06 *s*	0.99	1.17 *s*
29	0.82 *s*	$29_a(\rightarrow 19_{aq})$: 4.52 *s*	29_a: 3.37 *d*
		$29_b(\rightarrow 21_{aq})$: 4.57 *s*	29_b: 3.67 *d*
			12.0 Hz
30	0.80 *s*	—	1.21 *s*
1′	4.60 *d*	4.77 *d*	4.32 *d*
	7.8 Hz	7.5 Hz	7.8 Hz
2′	3.51	3.67	3.23
3′	3.57	3.74	3.34
4′	3.56	3.74	3.43
5′	3.71	3.78	3.53
1″	5.63 *d*	5.79 *d*	5.37 *d*
	8.1 Hz	8.2 Hz	8.2 Hz
2″	3.56	3.74	3.31
3″	3.37	3.83	3.39
4″	3.61	3.82	3.34
5″	3.50	3.62	3.33
$6''_a$	3.83	3.96	3.67
	4.8/12.0 Hz	4.7/12.0 Hz	5.0/11.2 Hz
$6''_b$	3.95	4.06	3.80
	2.1/12.0 Hz	2.5/12.0 Hz	1.7/11.2 Hz

mm, 5 μm, Bischoff) and LiChroprep RP-18 (250 × 16 mm, 5–20 μm, Knauer) prepacked columns were used. GLC (He at 50 kPa; 3 min 80°, 80° to 300° with 3° min^{-1}) was performed on Fisons GC 8000 instrument using a fused silica capillary column coated with DB 1 phase (30 m × 0.32 mm, J&W).

Isolation of compounds

Z. decumbens was collected in November 1993 near Suez and identified by Dr L. Boulos from the National Research Centre (NRC) Cairo. A voucher specimen of the plant is deposited at the Herbarium of the NRC, Department of Chemotaxonomy.

The whole fresh plants of *Z. decumbens* (4 kg) were minced and extracted with 80% MeOH. The solvent was evaporated under red. pres. and the aq. phase extracted with petrol. After evaporation of the aq. phase the residue was successively partitioned between H_2O and EtOAc and H_2O and *n*-BuOH. The butanolic fr. was sepd and evaporated under red. pres. at 50° to afford the crude saponin mixture (10 g). Successive CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (18:6:1)

Table 2. ^{13}C NMR spectral data for saponins **1–3** and 30-norhederagenin in CD_3OD-/pyridine-d_5 = 3:2 (**1**, **2**) CD_3OD (**3**) and pyridine-d_5 (30-norhederagenin)

	1	**2**	**3**	30-norhederagenin [4]
1	47.9	48.0	39.8	38.8
2	68.1	67.8	26.9	27.7
3	87.2	86.9	90.6	73.5
4	45.3	45.3	40.1	42.9
5	47.8	47.5	57.0	48.7
6	18.8	18.7	19.3	18.6
7	33.1	33.0	33.9	33.0
8	40.6	40.4	40.7	39.8
9	47.0	48.5	49.2	48.1
10	38.5	38.3	37.9	37.2
11	24.6	24.4	24.5	23.8
12	123.4	123.4	122.3	122.6
13	145.0	144.1	142.0	144.9
14	42.9	42.2	42.9	42.0
15	28.8	28.6	28.8	28.3
16	24.0	23.9	24.1	23.8
17	47.7	47.9	47.5	47.1
18	42.4	48.2	44.7	48.0
19	47.0	42.2	47.8	42.0
20	30.6	149.0	33.2	148.5
21	34.7	30.6	35.6	38.4
22	33.2	38.1	34.7	30.4
23	64.1	63.81	28.5	68.1
24	14.7	14.9	17.0	13.1
25	17.7	17.8	16.1	16.0
26	17.9	17.7	17.7	17.5
27	26.4	26.4	26.2	26.2
28	177.5	177.5	175.6	179.4
29	33.5	107.7	71.1	107.0
30	24.0	—	24.8	
1′	105.1	105.1	106.8	
2′	75.1	75.1	75.5	
3′	78.1	78.2	78.0	
4′	73.7	73.8	73.9	
5′	75.6	75.5	76.6	
6′		not detected		
1″	95.9	96.0	98.8	
2″	74.1	74.2	73.8	
3″	78.7	78.8	78.3	
4″	71.3	71.2	71.3	
5″	79.1	79.3	78.7	
6″	62.5	62.4	62.4	

$CHCl_3$–MeOH–H_2O (18:7:1) yielded frs I (386 mg) and II (80 mg). 250 mg of fr. I were further chromatographed by MPLC eluting with 35% MeOH followed by prep. and semi-prep. HPLC with different MeOH–H_2O gradients (prep.: 27–30% MeOH in 21 min, 30–80% in 6 min, 80% isocratic, 16 ml min^{-1}; semi-prep.: 28–45% MeOH in 17 min, 45% for 3 min, 2.7 ml min^{-1}) to give pure saponins **1** (4.5 mg, R_f 0.44) and **2** (3.2 mg, R_f 0.42). For further purification of fr. II CC on Sephadex LH-20 eluting with MeOH was used to give frs III (3.5 mg), IV (7.6 mg) and V (27.6 mg). By use of semi-prep. and analytical HPLC (semi-prep.: 33% MeOH, 2.8 ml min^{-1}; analytical: 12–20% ACN in 30 min, 1.0 ml min^{-1}) 2.4 mg of **3** (R_f 0.41) was isolated from frs III and IV. Because of high losses of saponins **1–3** by irreversible adsorption on silica, the use of TLC on silica gel was impossible.

2-Butylglycosides

R_f according to Ref. [5]. To produce the (*R*)- or (*S*)-2-butylglycosides, (*R*)-(−)- or (*S*)- (+)-2-BuOH was used. A sample (ca 250 μg) of the appropriate saponin was hydrolysed with 0.5 ml 6% HCl for at least 2 h at

80°. After evaporation of the acid under red. pres., 0.5 ml of the appropriate 2-BuOH was added, dried HCl gas was bubbled through the soln for 30 s and the reaction mixture was heated for 3 h at 80° under N_2 in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. Because of formation of the lactone in the case of glucuronic acid, we prepared the standards starting with heating in 6% HCl. Identification of the sugars were done by comparison of the R_f values and coinjection with the appropriate standard. (*R*)-2-butyl-D-Glc: R_f 32.78, 36.17, R_i 1903, 2005; (*S*)-2-butyl-D-Glc: R_f 32.39, 35.73, R_i 1891, 1992; (*R*)-2-butyl-D-Glca: R_f 32.45, R_i 1893; (*S*)-2-butyl-D-Glca: R_f 32.82, R_i 1905.

Zygophyloside I (**1**). ($C_{42}H_{66}O_{16}$, M_r 826.93); Mp 186–191° (decomp.). LSI-MS negative ion mode *m/z* (rel. int.): 825 $[M-H]^-$ (31), 663 $[M-H-Glc]^-$ (4), 487 $[M-H-Glc-Glca]^-$ (4). ^1H NMR: Table 1, ^{13}C NMR: Table 2.

Zygophyloside J (**2**). ($C_{41}H_{62}O_{16}$, M_r 810.89); Mp 184–188° (decomp.). LSI-MS negative ion mode *m/z* (rel. int.): 809 $[M-H]^-$ (22), 647 $[M-H-Glc]^-$ (4). ^1H NMR: Table 1, ^{13}C NMR: Table 2.

Zygophyloside K (**3**). ($C_{42}H_{66}O_{15}$, M_r 810.92); Mp 192–196°. LSI-MS negative ion mode *m/z* (rel. int.): 809 $[M-H]^-$ (20), 647 $[M-H-Glc]^-$ (5), 633 $[M-H-Glca]^-$ (8). ^1H NMR: Table 1. ^{13}C NMR: Table 2.

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