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TRITERPENOID SAPONINS FROM *ZYGOPHYLLUM* SPECIES

M. HANI A. ELGAMAL, KAMEL H. SHAKER, KARL PÖLLMANN* and KARLHEINZ SEIFERT*†

National Research Centre, Laboratory of Natural Products, Dokki-Cairo, Egypt; *University of Bayreuth, Organic Chemistry I/2, NW II, D-95440 Bayreuth, Germany

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Key Word Index—*Zygophyllum* species; Zygophyllaceae; triterpenoid saponins; zygophyloside F.

Abstract—From the roots of *Zygophyllum coccineum* and the aerial parts of *Z. album* and *Z. dumosum*, the new triterpenoid saponin 3-*O*-[β-D-2-*O*-sulphonylquinovopyranosyl]-quinovic acid-27-*O*-[β-D-glucopyranosyl] ester (zygophyloside F) has been isolated. The known saponins, 3-*O*-[β-D-quinovopyranosyl]-quinovic acid 27-*O*-[β-D-glucopyranosyl] ester and 3-*O*-[β-D-quinovopyranosyl]-quinovic acid, were isolated from the aerial parts of *Z. dumosum*. The structures were determined primarily on the basis of NMR spectroscopy. The assignment of the NMR signals were performed by means of ^{1}H–^{1}H COSY–45, HMQC, HMBC and TOCSY experiments.

INTRODUCTION

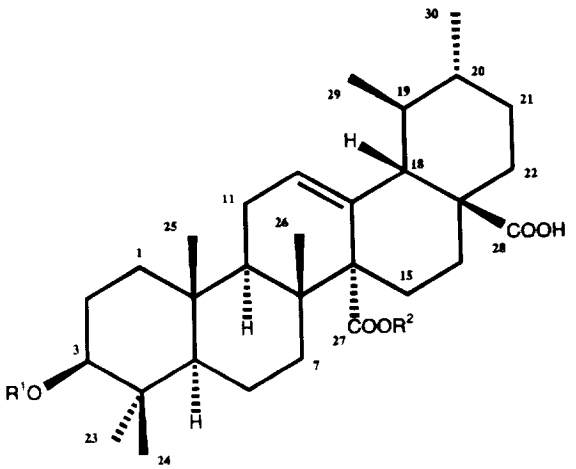
Zygophyllum coccineum L. grows wild in Egypt and in the neighbouring region of Sudan [1]. Leaves, stems and fruits of this plant are used in folk medicine as the drug 'Kammûn Quarâmâny'. This drug is active against rheumatism, gout, asthma and hypertension and is also used as a diuretic, anthelminthic and antidiabetic agent. In a previous investigation, quinovic acid, saponins and tannins of unknown structures have been found in the leaves, stems and roots of *Z. coccineum* [2, 3]. In this paper, we present the isolation and structural elucidation of the new triterpenoid saponin zygophyloside F from *Z. coccineum* and the other two *Zygophyllum* species *Z. album* and *Z. dumosum*.

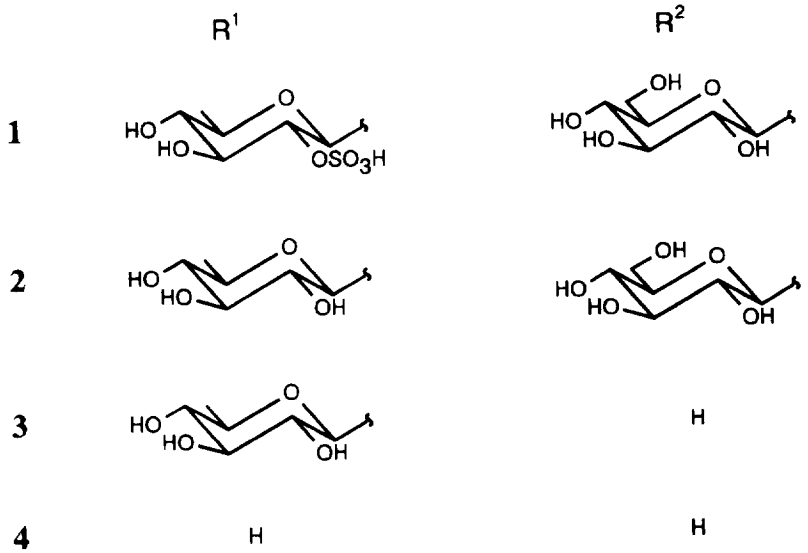
RESULTS AND DISCUSSION

The saponin fractions of *Z. coccineum*, *Z. album* and *Z. dumosum* were obtained as described in Experimental. These fractions were subjected to column chromatography on silica gel followed by HPLC or Sephadex LH-20 separation to afford zygophyloside F (**1**) from the roots of *Z. coccineum* and the aerial parts of *Z. album* and *Z. dumosum*. 3-*O*-[β-D-Quinovopyranosyl]-quinovic acid-27-*O*-[β-D-glucopyranosyl] ester (zygophyloside B, **2**) and 3-*O*-[β-D-quinovopyranosyl]-quinovic acid (**3**) have been isolated from the aerial parts of *Z. dumosum*. The saponins **2** and **3** were previously found in the leaves and stems of *Z. propinquum* [4] and **3** was obtained from cinchona bark [5]. The negative liquid secondary-ion mass spectrum of **1** exhibited the $[M-1]^-$ ion (*m/z* 873), which, together with ^{1}H and ^{13}C NMR data, allowed us to propose the molecular formula $C_{42}H_{66}O_{17}S$. The fragment ions at *m/z* 711 $[M-1-162]^-$, 667 $[M-1-162-144]^-$ and 587 $[M-1-162-44-80]$ showed the sequential loss of a hexose moiety, hexose moiety + CO_2 and hexose moiety + CO_2 + SO_3. The $-OSO_3H$ moiety is characterized by the fragment ions at *m/z* 97 $[SO_4H]^-$ and 80 $[SO_3]^-$. Acid hydrolysis with 10% H_2SO_4–dioxane (1:1) of **1** yielded quinovic acid (**4**), glucose and quinovose. The monosaccharides were detected by TLC.

The ^{1}H NMR spectrum (500 MHz, pyridine-d_5) of **1** showed the existence of four tertiary and two secondary methyl groups characterized by the singlets at δ0.87 (3H-25), 1.12 (3H-24), 1.19 (3H-26), 1.28 (3H-23) and the doublets at δ0.76 (J = 5.9 Hz, 3H-30), 1.16 (J = 6.3 Hz, 3H-29). The signal at δ5.99 was attributed to the olefinic proton H-12. The doublets of two anomeric proton signals at δ4.72 (J = 7.6 Hz), 6.35 (J = 8.1 Hz) and the doublet at δ1.57 (J = 5.9 Hz), due to a methyl group, indicated the presence of a β-D-quinovopyranose and a β-D-glucopyranose unit. Starting from the anomeric proton signals the other signals of the protons of the quionvopyranose and glucopyranose moiety could be assigned by ^{1}H–^{1}H COSY-45° and TOCSY experiments. The chemical shifts of the H-1 sugar signals at δ4.72 and 6.35 indicated the glycosylation of **4** with quinovose at C-3 (glycoside) and glucose at C-27 or C-28 (glucopyranosyl ester). In the ^{13}C NMR spectrum of **1** the signal of C-12 is downfield ($\Delta\delta$ + 0.6) and the signal of C-13 upfield shifted ($\Delta\delta$ − 0.9) in comparison with **4**. The chemical shifts of the C-12 signal (δ129.6) and the C-13 signal (δ133.2) were in agreement with the glycosylation at C-27. In the case of the glycosylation at C-28, the signals of C-12 and C-13 would have been expected at δ128.9 and 135.4, respectively [6]. The corresponding

†Author to whom correspondence should be addressed.





28-glucopyranosyl ester of **1** has been isolated from the aerial parts of *Z. propinquum* [7]. The downfield shifts of the H-2′ quinovose ($\Delta\delta$ + 1.00) and C-2′ quinovose ($\Delta\delta$ + 5.3) signals of **1** compared with those of **2** indicate that the sulphate group is in position C-2′ of the quinovose.

The protonated carbons of **1** were assigned by $^{1}H-^{13}C$ heteroscalar correlated 2D NMR spectra (HMQC). By a proton detected multiple bond $^{1}H-^{13}C$ correlation spectrum (HMBC) all quaternary carbon signals could be assigned. From these results the structure was elucidated to be 3-*O*-[β-D-2-*O*-sulphonylquinovopyranosyl]-quinovic acid 27-*O*-[β-D-glucopyranosyl] ester.

EXPERIMENTAL

General. NMR: 500 MHz (^{1}H), and 125 MHz (^{13}C), δ in ppm, solvent pyridine-d_5. Negative ion mass spectra: Finnigan MAT 8500. The matrix for the liquid SIMS was glycerol. CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 mm, $CHCl_3$–MeOH–H_2O, 14:7:1); the spots were sprayed with 10% methanolic H_2SO_4, 'triterpene reagent' (1% soln of vanillin in 50% H_3PO_4) and 'sugar reagent' (4% ethanolic aniline–4% ethanolic diphenylamine–H_3PO_4, 5:5:1). HPLC: LiChrosorb RP-18 prepacked column (250 × 8 mm, 5 μm, Knauer). The system was equipped with a Knauer differential refractometer.

Isolation. All *Zygophyllum* species were collected in 1991 in the North of Sinai and identified by Dr L. Boulos from the National Research Centre (NRC), Cairo. A voucher specimen of the plants is deposited at the Herbarium of the NRC, Department of Chemotaxonomy.

Z. coccineum. Dried powdered roots (1 kg) were extracted with petrol, EtOAc, MeOH and MeOH–H_2O (1:1). The methanolic residue was successively par-

Table 1. ^{13}C NMR spectral data for compounds **1**–**4** in pyridine-d_5

	1	2	3	4		1	2	3	4
1	39.6	39.4	39.5	39.2	22	37.5	37.5	37.6	37.6
2	26.7	26.8	26.9	28.2	23	28.1	28.0	28.1	28.6
3	89.2	88.5	88.6	77.9	24	17.1	17.0	17.1	16.6
4	40.2	40.2	40.1	40.0	25	16.5	16.6	16.6	16.6
5	55.8	55.8	55.9	55.7	26	19.2	19.2	19.0	18.9
6	18.5	18.5	18.7	18.9	27	176.5	176.5	180.2	180.1
7	36.4	36.4	37.2	37.1	28	178.0	178.0	178.1	178.0
8	40.2	40.2	40.1	40.0	29	18.1	18.1	18.3	18.2
9	47.2	47.2	47.3	47.3	30	21.2	21.2	21.4	21.3
10	37.0	37.0	37.6	37.3	1′	104.1	106.6	106.7	
11	23.4	23.2	23.4	23.3	2′	81.2	75.9	76.0	
12	129.6	129.5	129.3	129.0	3′	78.2	78.3	78.4	
13	133.2	133.2	134.2	134.1	4′	76.7	76.9	76.9	
14	56.7	56.7	56.9	56.8	5′	72.2	72.6	72.7	
15	26.1	26.1	26.4	26.4	6′	18.5	18.8	18.8	
16	25.5	25.5	25.6	25.5	1″	95.7	95.6		
17	48.9	48.9	48.9	48.7	2″	74.1	74.1		
18	54.7	54.7	55.0	54.9	3″	78.9	78.7		
19	37.5	37.5	37.8	37.7	4″	71.3	71.2		
20	39.1	39.0	39.4	39.3	5″	79.2	79.2		
21	30.3	30.6	30.7	30.6	6″	62.4	62.3		

titioned between H_2O and EtOAc, and H_2O and *n*-BuOH. The butanolic fr. was sepd and evpd under red. pres. at 50° to give a crude saponin mixt. (3.5 g). MPLC (330 mm) on LiChroprep RP-8, eluting with MeOH–H_2O (5 min 100% H_2O; 100%–90% H_2O in 5 min; 90%–60% in 30 min), yielded frs 64–80, which were subjected to HPLC on LiChrosorb RP-18, eluting with MeOH–H_2O (9:11) and detecting with a differential refractometer to afford pure saponin **1** (10.5 mg, R_f 0.31, R_t 17.5 min).

Z. album. Dried powdered aerial parts (300 g) were extracted as described for *Z. coccineum*. CC of the residue (1 g) on silica gel, eluting with $CHCl_3$, $CHCl_3$–MeOH (17:2) and $CHCl_3$–MeOH–H_2O (14:5:1 to 14:6:1), yielded 50 mg crude saponin **1**. CC on Sephadex LH-20, eluting with MeOH, afforded pure saponin **1** (12 mg, R_f 0.31).

Z. dumosum. The aq. extract of dried powdered stem parts (3 kg) was successively extracted with EtOAc and *n*-BuOH. Evapn of the butanolic fr. under red. pres. at 50° gave a crude saponin mixt. (13 g). CC (7 g) on silica gel, eluting with $CHCl_3$, $CHCl_3$–MeOH (9:1 to 17:1) and $CHCl_3$–MeOH–H_2O (14:4:1 to 12:5:1), yielded two frs, which were chromatographed on Sephadex LH-20 with MeOH as eluent to afford pure saponins **1** (23 mg, R_f 0.31), **2** (16 mg, R_f 0.71) and **3** (19 mg, R_f 0.82).

Acid hydrolysis. The appropriate saponin **1**–**3** (5 mg) was dissolved in 5 ml 10% H_2SO_4–dioxane (1:1) and refluxed for 3.5 hr at 100°. The reaction mixt. was diluted with H_2O and extracted with $CHCl_3$ to give **4**. The aq. layer was neutralized with $KHCO_3$ and sugars were identified by TLC ($CHCl_3$–MeOH–AcOH–H_2O, 8:3:5:2), spraying with 'sugar reagent'.

Zygophyloside F (**1**). ($C_{42}H_{66}O_{17}S$, M_r 874.40); $[\alpha]_D^{25}$ + 23° (MeOH; *c* 0.25). Liquid SIMS negative ion mode *m z* (rel. int.): 873 $[M - H]^-$ (75), 711 $[M - H - Glc]^-$ (6), 667 $[M - H - Glc - CO_2]^-$ (4), 587 $[M - H - Glc - CO_2 - SO_3]^-$ (2), 97 $[HSO_4]^-$ (78), 80 $[SO_3]^-$ (100). ^1H NMR: δ0.76 (*d*, *J* = 5.9 Hz, 3H-30), 0.87 (*s*, 3H-25), 0.90 (H-5, H-20), 1.08 (H-1), 1.12 (*s*, 3H-24), 1.16 (*d*, *J* = 6.3 Hz, 3H-29), 1.19 (*s*, 3H-26), 1.22 (H-6), 1.28 (*s*, 3H-23), 1.30 (H-21), 1.40 (H-19), 1.46 (H-6), 1.57 (*d*, *J* = 5.9 Hz, 3H-6′), 1.60 (H-1), 1.65 (H-22), 1.72 (H-7), 1.77 (H-22), 1.89 (H-2), 1.91 (H-7), 1.99–2.14 (H-11), 2.12 (H-2), 2.20 (H-15), 2.38 (H-16), 2.56 (H-15, H-16), 2.67 (H-18), 2.68 (H-9), 3.15 (*dd*, *J* = 4.2, 11.4 Hz, H-3), 3.61 (H-4′), 3.71 (H-5′), 4.04 (H-5″), 4.21 (H-2″), 4.27 (H-3′), 4.30 (H-3″), 4.32 (H-4″), 4.35 (*J* = 4.1, 11.9 Hz, H-6″$_a$), 4.42 (H-6″$_b$), 4.72 (*d*, *J* = 7.6 Hz, H-1′), 4.96 (H-2′), 5.99 (H-12), 6.35 (*d*, *J* = 8.1 Hz, H-1″); ^{13}C NMR: Table 1.

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REFERENCES

1. El-Hadidi, M. N. (1972) in *Flora des iranischen Hochgebirges und der umrahmenden Gebirge* (Rechinger, K. H., ed.), Vol. 98, p. 28. Akademische Druck- und Verlagsanstalt, Graz, Austria.
2. Saber, A. H. and El-Moghazy Shoaib, A. M. (1960) *J. Pharm. Sci. U. A. R.* **1**, 1.
3. Saber, A. H. and El-Moghazy Shoaib, A. M. (1966) *J. Pharm. Sci. U. A. R.* **7**, 117.

4. Ahmad, V. U., Ghazala, S. U. and Shaheen, B. (1990) *J. Nat. Prod.* **53**, 1193.
5. Tschesche, R., Duphorn, I. and Snatzke, G. (1963) *Liebigs Ann. Chem.* **667**, 151.
6. Hassanean, H. A., El-Hamouly, M. M. A., El-Moghazy, S. A. and Bishay, D. W. (1993) *Phytochemistry* **33**, 667.
7. Ahmad, V. U., Ghazala, S. U. and Ali, M. S. (1993) *Phytochemistry* **33**, 453.