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TRITERPENOID SAPONINS FROM THE ROOTS OF *ZYGOPHYLLUM* SPECIES

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

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

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Key Word Index—*Zygophyllum* species; Zygophyllaceae; triterpenoid saponins; zygophylosides G and H.

Abstract—Two new triterpenoid saponins, 3-*O*-[β-D-2-*O*-sulphonylglucopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester (zygophyloside G), 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-quinovopyranosyl]-quinovic acid-28-O-[β-D-glucopyranosyl] ester (zygophyloside H), and seven known saponins have been isolated. The structures were established primarily on the basis of NMR spectroscopy. The assignment of all NMR signals was performed by means of ^{1}H–^{1}H COSY-45, NOESY, TOCSY, ^{13}C APT, HMQC and HMBC experiments. The determination of the glycosylation sites was possible by the HMBC experiments. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Zygophyllum coccineum L. is used in folk medicine as part of a drug against rheumatism, gout and hypertension [1]. This was the motivation to investigate the natural products of different *Zygophyllum* species, grown in Egypt. In this report we present the isolation of the two new triterpenoid saponins zygophylosides G (**1**) and H (**4**) and seven known saponins.

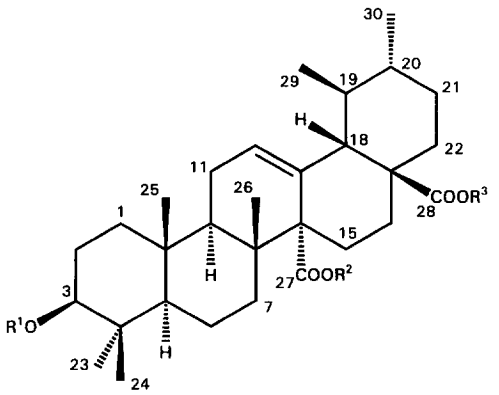
RESULTS AND DISCUSSION

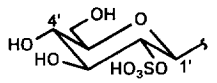
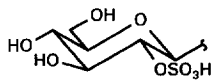
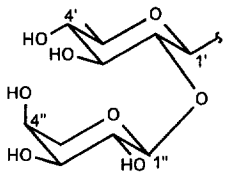
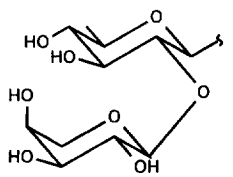
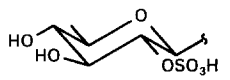
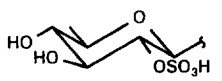
The crude saponin extracts of the roots of *Z. coccineum* and *Z. dumosum* Boiss. were obtained as described in the Experimental. Further purification was achieved by column chromatography on Sephadex G-25 or MPLC on LiChroprep RP-8 followed by TLC on silica gel and HPLC on RP-18 material. Zygophyloside G (**1**), 3-*O*-[β-D-glucopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester (**2**) [2], zygophyloside H (**4**), 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-quinovopyranosyl]-quinovic acid (**5**) [3], 3-*O*-[β-D-2-*O*-sulphonylquinovopyranosyl]-quinovic acid (**7**) [4], 3-*O*-[β-D-glucopyranosyl]-quinovic acid (**8**) [5], 3-*O*-[β-D-quinovopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester (**9**) [6] and 3-*O*-[β-D-quinovopyranosyl]-quinovic acid (**10**) [5] have been isolated from the roots of *Z. coccineum*. In the roots of *Z. dumosum*, compounds **1**, **2**, **4** and 3-*O*-[β-D-2-*O*-sulphonylquinovopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester (**6**) [4] were found.

The LSI mass spectrum of **1** exhibited the $[M-1]^-$ ion at *m/z* 889. This together with ^{1}H and ^{13}C NMR data allowed us to propose the molecular formula $C_{42}H_{66}O_{18}S$. The fragment ions at *m/z* 845 $[M-1-44]^-$, 727 $[M-1-162]^-$, 683 $[M-1-162-44]^-$ and 603 $[M-1-162-44-80]^-$ showed the sequential loss of CO_2, a hexose moiety, a hexose moiety plus CO_2 and a hexose moiety plus CO_2 and SO_3. The $-OSO_3H$ group is characterized by the fragment ions at *m/z* 97 $[SO_4H]^-$ and 80 $[SO_3]^-$.

The ^{1}H and ^{13}C NMR spectra of **1** showed the presence of quinovic acid as aglycone. Two anomeric proton signals at δ 4.82 and 6.34 indicated the presence of two monosaccharides, one bonded as a glycoside (δ 4.82) and the other as a glycosyl ester (δ 6.34). By use of ^{1}H–^{1}H COSY-45 spectra both monosaccharides were identified as glucose. The cross peaks of the ^3J long range couplings between H-18/C-28, H-1″ glucose/C-28 and H-1′ 2-*O*-sulphonylglucose/C-3 in the HMBC spectra enabled us to determine the resonance of C-28 and the glucosidations in positions C-28 and C-3. The downfield shifts of the H-2′ (Δδ + 1.03) and C-2′ (Δδ + 5.3) signals of **1** compared with those of **2** indicated the sulphate group was in position C-2′ of the glucose. The signals of the axial and equatorial oriented protons of the quinovic acid were assigned by NOESY experiments. The assignments of H_3-23, H_3-24 and the corresponding ^{13}C signals were obtained by NOESY and HMQC. The value of the coupling constant between H-18 and H-19 ($^3J_{18,19}$ = 11.4 Hz) indicated that both protons were in an axial position. This,

†Author to whom correspondence should be addressed.



	R^1	R^2	R^3
1		H	β-D-Glucopyranosyl
2	β-D-Glucopyranosyl	H	β-D-Glucopyranosyl
3		Me	β-D-Glucopyranosyl
4		H	β-D-Glucopyranosyl
5		H	H
6		H	β-D-Glucopyranosyl
7		H	H
8	β-D-Glucopyranosyl	H	H
9	β-D-Quinovopyranosyl	H	β-D-Glucopyranosyl
10	β-D-Quinovopyranosyl	H	H

and the NOESY cross peak between H-19 and H-16_{ax} showed the *cis*-connection of the rings D and E. The reaction of **1** with diazomethane yielded its methyl ester **3**. The ^{13}C NMR spectrum of **3**, in comparison with that of **1**, caused a highfield shift of the resonances of C-27 ($\Delta\delta - 2.3$), C-29 ($\Delta\delta - 0.6$) and C-13 ($\Delta\delta - 1.0$) and a downfield shift of the C-12 signal ($\Delta\delta + 0.5$). The other values of the ^{13}C resonances showed no significant changes. Additionally to **1** the heteronuclear ^{3}J couplings H-15/C-27 and -OC$\underline{H}_3$/C-27 occurred in the HMBC spectra of **3**, which ensures the glucosidation of **1** at position C-28.

The ^{13}C NMR resonances of the aglycone of **4** matched well with the signals of **1** indicating the same aglycone for both compounds. The occurrence of three anomeric signals at δ 4.65, 5.13 and 6.36 in the ^{1}H NMR spectrum of **4** showed the presence of three sugar units. Together with the LSI mass spectrum of **4** exhibiting the $[M-1]^-$ ion at *m/z* 925, we were able to propose $C_{47}H_{74}O_{18}$ as the molecular formula. This was consistent with the fragment ions at *m/z* 763 $[M-1-162]^-$, 719 $[M-1-162-44]^-$ and 587 $[M-1-162-44-132]^-$, corresponding to the elimination of a hexose, hexose plus CO_2 and hexose plus CO_2 plus pentose from the deprotonated molecular ion. The existence of a glucose ester at position C-28 was established by comparison of ^{1}H and ^{13}C signals of **4** and **1**. The other two sugars were determined as quinovose and arabinose by use of ^{1}H–^{1}H COSY-45 and TOCSY experiments. The cross peaks between H-1‴ glucose/C-28, H-1″ arabinose/C-2′ quinovose and H-1′ quinovose/C-3 occurring in the HMBC spectra gave evidence for the position of the glucose ester at position C-28 and the interglycosidic linkage of arabinose at position C-2′ of the quinovose, which is connected at position C-3 with quinovic acid. The determination of the D- or L-form of the sugars of **1**, **4** and **5** was carried out as follows: 250 μg of the corresponding saponin were hydrolysed under acidic conditions and the mixture was allowed to react with (*R*)-(−)-2-butanol to give the (*R*)-2-butylglycosides. The trimethylsilylated derivatives were investigated by GLC. The R_t values of the trimethylsilylated butylglycosides were determined and showed a very good agreement (co-injection) with the trimethylsilylated reference compounds (*R*)-2-butyl-D-glucopyranoside, (*R*)-2-butyl-D-quinovopyranoside and (*R*)-2-butyl-L-arabinopyranoside. From all the above given data the structures of **1** and **4** were elucidated to be 3-*O*-[β-D-2-*O*-sulphonylglucopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester and 3-*O*-[α-L-arabinopyranosyl-(1→2)-β-D-quinovopyranosyl]-quinovic acid-28-O-[β-D-glucopyranosyl] ester.

The ^{13}C NMR resonances of **6** matched well with the ^{13}C signals of the aglycone and the glucose ester of **1**. The HMBC cross peak H-1″ glucose/C-28 indicated undoubtedly the glucosylation of position C-28 of **6**. All physical and spectroscopic data of the saponin **6** are identical with zygophyloside F. Therefore, we have to revise our published structure of zygophyloside F. The correct glucosidation site of zygophyloside F and 3-*O*-[β-D-quinovopyranosyl]-quinovic acid-27-*O*-[β-D-glucopyranosyl] ester in ref. [7] is position C-28 instead of C-27. The structures of **6** and 3-*O*-[β-D-quinovopyranosyl]-quinovic acid-28-*O*-[β-D-glucopyranosyl] ester (**9**) were first published by Ahmad *et al.* [4] and Aquino *et al.* [6], respectively.

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 pyridine-d_5, temp. 303 K, pyridine-d_5 signals were used as int. standard (^{1}H: 8.71, ^{13}C: 149.9), NOESY: phase-sensitive using TPPI, mixing time 300 and 600 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: 71 msec ($J_{C,H}$ = 14 Hz).

CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 and 1 mm precoated plates 60 F_{254}, Merck, 0.25 mm 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), 'sugar reagent' (4% ethanolic aniline–4% ethanolic diphenylamine–H_3PO_4, 5:5:1) and phosphomolybdic acid reagent (Aldrich), R_f values are given for $CHCl_3$–MeOH and $CHCl_3$–MeOH–H_2O mixts as eluents. For centrifugal TLC a Chromatotron, model 894 (Harrison Research, U.S.A.) with self-coated rotors, was used (1 and 2 mm, silica gel 60 PF_{254} Merck, containing gypsum; flow rate: 3 ml min^{-1} (1 mm), 7 ml min^{-1} (2 mm), fr. size: 7 ml). For MPLC on LiChroprep RP-8 (460 × 36 mm, 40–63 μm, Merck) MeOH–H_2O gradients were used. For the analyt. and semi-prep. HPLC a Knauer HPLC system equipped with a variable wavelength monitor together with LiChrosorb RP-18 (250 × 8 mm, 5 μm, Knauer) and Spherisorb ODS II (250 × 4, 250 × 8 mm, 5 μm, Bischoff) prepacked columns were used. GLC (He at 50 kPa; 3 min 80°, 80–120° with 3° min^{-1}, 120–170° with 0.5° min^{-1}, 170–280° with 5° min^{-1}) was performed on a Fisons GC 8000 instrument using a fused silica capillary column coated with DB 1 phase (30 m × 0.32 mm, J&W).

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.

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

(1.5 g) on Sephadex G-25 eluting with H_2O yielded 3 saponin frs., which were further chromatographed by Chromatotron eluting with $CHCl_3$–MeOH gradients: fr. I (148.9 mg; 12:1, 10:1, 8:1, 6:1, each 120 ml, 1 mm) afforded **8** [frs 10–17, 5.2 mg, R_f 0.58 (4:1)] and **5** [frs 18–25, 5.0 mg, R_f 0.47 (4:1)]; fr. II (148.7 mg; 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, each 120 ml, 1 mm) yielded **10** [6.0 mg, R_f 0.73 (4:1)] and 39.1 mg crude saponin, which was purified by TLC [$CHCl_3$–MeOH–H_2O (14:7:1), containing 0.1% HCO_2H] to give **7** [10.0 mg, R_f 0.67 (14:7:1)]; fr. III (1166.7 mg; 12:1, 9:1, 7:1, 5:1, each 240 ml, 2 mm) yielded frs 1–11, which were purified by HPLC using a MeCN–H_2O gradient (20–32% MeCN in 22 min) on Spherisorb RP-18 to give pure **2** [9.5 mg, R_f 0.3 (4:1), R_t 11.2 min]. MPLC of 600 mg of the crude saponin extract on LiChroprep afforded 7 frs. Nearly pure **1** was found in frs 9–63 (25 mg). HPLC of frs 81–90 on LiChrosorb RP-18 eluting with MeCN–H_2O (20–40% MeCN in 20 min, 212 nm) yielded 8.2 mg **4** [R_f 0.68 (14:7:1), R_t 25.6 min] and 6.9 mg **9** [R_f 0.77 (14:7:1), R_t 18.2 min].

Dried powdered roots (2 kg) of *Z. dumosum* were

Table 1. ^{13}C NMR spectral data for compounds **1–8** in pyridine-d_5

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	39.6	39.0	39.1	39.2	39.2	39.1	39.0	39.0
2	26.6	26.8	26.6	26.9	28.4	26.8	26.8	26.8
3	89.5	88.8	89.5	88.5	88.5	89.3	89.4	88.7
4	40.2	39.5	39.6	39.5	39.5	39.4	39.7	39.4
5	55.8	55.7	56.1	55.9	55.9	55.8	55.8	55.7
6	18.5	18.6	18.4	18.5	18.5	18.5	18.4	18.6
7	37.5	37.6	37.6	37.6	37.6	37.6	37.8	37.8
8	40.2	40.1	40.4	40.2	40.1	40.2	40.0	40.0
9	47.2	47.1	47.5	47.3	47.2	47.3	47.1	47.2
10	37.0	37.0	36.9	37.0	37.1	37.0	37.3	37.1
11	23.4	23.5	23.2	23.4	23.4	23.5	23.4	23.3
12	129.6	129.1	130.1	129.6	129.0	129.6	128.6	128.9
13	133.3	133.8	132.3	133.2	134.2	133.3	134.7	134.1
14	56.8	57.1	56.6	56.8	56.8	56.8	57.1	56.8
15	25.5	25.7	25.3	25.5	25.4	25.5	25.7	25.5
16	26.2	26.4	25.8	26.2	25.5	26.2	26.6	26.4
17	49.0	49.1	48.8	48.9	48.7	49.0	48.9	48.7
18	54.7	54.9	54.2	54.7	55.0	54.7	55.2	54.9
19	37.5	37.5	37.5	37.5	37.8	37.5	37.7	37.5
20	39.1	39.2	39.0	39.1	39.4	39.1	39.5	39.4
21	30.3	30.4	30.1	30.3	30.0	30.3	30.0	30.6
22	36.5	36.6	36.2	36.4	37.0	36.5	37.1	37.0
23	28.1	28.1	28.2	27.7	27.7	28.1	28.2	28.0
24	17.1	17.1	17.1	16.5	16.6	17.1	17.1	17.1
25	16.6	16.7	16.5	16.7	16.5	16.6	16.6	16.5
26	19.2	19.3	19.0	19.2	18.9	19.2	19.0	18.9
27	178.0	178.6	175.7	178.0	178.1	178.1	178.5	178.1
28	176.6	176.7	176.3	176.5	180.2	176.7	180.7	180.1
29	18.2	18.3	17.6	18.1	18.2	18.2	18.6	18.3
30	21.2	21.3	21.3	21.2	21.4	21.2	21.5	21.4
-OMe			51.3					
1′	104.3	107.0	104.4	104.9	104.9	104.1	104.2	106.9
2′	81.0	75.7	83.9	84.2	84.2	81.2	81.1	75.8
3′	78.3	78.7	78.2	77.8	77.8	77.7	78.1	78.8
4′	71.8	71.8	71.7	76.6	76.6	76.7	76.8	71.8
5′	77.5	78.3	77.7	72.4	72.4	72.2	72.3	78.2
6′	62.8	63.1	62.7	18.5	18.5	18.5	18.6	63.1
1″	95.7	95.7	95.7	106.8	106.8	95.8		
2″	74.1	74.2	74.1	73.8	73.8	74.2		
3″	78.9	78.9	78.8	74.3	74.3	78.9		
4″	71.2	71.2	71.1	69.1	89.1	71.3		
5″	79.3	79.3	79.3	67.0	67.0	79.3		
6″	62.4	62.4	62.3			62.4		
1‴				95.7				
2‴				74.2				
3‴				78.9				
4‴				71.2				
5‴				79.3				
6‴				62.3				

extracted with petrol and MeOH–H_2O (4:1). The methanolic residue was partitioned as described for *Z. coccineum*. The butanolic fr. gave a crude saponin mixt. (5.0 g). Sepn of 1010 mg crude saponin extract by MPLC on LiChroprep RP-8 eluting with MeOH–H_2O (25 ml min^{-1}, 0–50% MeOH in 60 min; 50% for 10 min; 50–100% in 50 min) afforded 9 frs. Fr. 6 (186.8 mg) was purified by Chromatotron (C_6H_{12}–EtOAc–MeOH, 12:4:1, 6:2:1, 3:1:1, 3:1:2 and 3:1:3, 100 ml each) followed by HPLC to yield pure **6** [14.0 mg, R_f 0.50 (12:8:1), R_t 14.5 min] and **1** [11.4 mg, R_f 0.44 (12:8:1), R_t 10.5 min]. Prep. TLC of fr. 7 (114.3 mg) on silica gel ($CHCl_3$-MeOH-H_2O, 14:8:1 and HPLC afforded 5.8 mg **2**. For both HPLC sepns Spherisorb ODS II was used. Elution with a MeCN–H_2O gradient (3.0 ml min^{-1}, 13–16% MeCN in 1 min, 16–23% in 12 min, 23–28% in 3 min) yielded after TLC of fr. 8 (82.5 mg) on silica gel ($CHCl_3$–MeOH–H_2O, 13:8:1) 17.9 mg **4**.

Methylation. Compound **1** (20 mg) was dissolved in 5 ml MeOH, cooled in an ice bath and 3 ml ethereal CH_2N_2 were added. The soln was allowed to warm to room temp. in 10 min and the solvent was removed by bubbling of N_2 through the soln. Purification by HPLC on Spherisorb ODS II eluting with MeCN–H_2O [2.5 ml min^{-1}, 12–40% MeCN in 43 min; R_f 0.35 (12:7:1), R_t 22.6 min] yielded 4.8 mg pure **3**. LSI-MS negative ion mode *m/z* (rel. int.): 903 $[M-H]^-$ (100), 741 $[M-H-Glc]^-$ (15), 697 $[M-H-Glc-CO_2]^-$ (10), 97 $[SO_3H]^-$ (20), 80 $[SO_3]^-$ (20).

(R)-2-Butylglycosides. (R_i according to ref. [8].) A sample (250 μg) of the appropriate saponin was hydrolysed with 0.5 ml 5% HCl for at least 3 hr at 80°. After evapn of the acid under red. pres., 0.5 ml (*R*)-(−)-2-BuOH was added, HCl gas was bubbled through the soln for 30 sec and the reaction mixt. was heated for 3 hr at 80° under N_2 in a sealed vessel. Trimethylsilylation was performed with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide overnight. L-Ara: R_t 39.41, R_i 1775; D-Ara: R_t 38.39, R_i 1764; D-Qui: R_t 53.18, R_i 1888; L-Glc: R_t 81.78, R_i 2083; D-Glc: R_t 82.21, R_i 2086.

Zygophyloside G (**1**). ($C_{42}H_{66}O_{18}S$, M_r 891.03); mp 240–244°; $[\alpha]_D^{25}$ +20° (MeOH; *c* 0.16). LSI-MS negative ion mode *m/z* (rel. int.): 889 $[M-H]^-$ (100), 845 $[M-H-CO_2]^-$ (10), 727 $[M-H-Glc]^-$ (25), 683 $[M-H-Glc-CO_2]^-$ (20) and 603 $[M-H-Glc-CO_2-SO_3]^-$ (10), 97 $[SO_4H]^-$ (35), 80 $[SO_3]^-$ (30). ^1H NMR: δ 0.73 (*d*, $J_{20,30}$ = 6.3 Hz, H_3-30), 0.80 (*s*, H_3-25), 0.85 (H-5, H-20), 0.95 (H-1_{ax}), 1.11 (*s*, H_3-24), 1.14 (*d*, $J_{19,29}$ = 6.2 Hz, H_3-29), 1.16 (*s*, H_3-26), 1.18 (H-6_{ax}), 1.19-1.31 (H-$21_{ax/eq}$), 1.28 (*s*, H_3-23), 1.35 (H-19), 1.42 (H-1_{eq}, H-6_{eq}), 1.59 (H-7_{ax}), 1.70 (H-22_{ax}), 1.75 (H-7_{eq}), 1.77 (H-2_{ax}), 1.83 (H-22_{eq}), 1.92–2.10 (H-$11_{ax/eq}$), 2.10 (H-2_{eq}), 2.18 (H-16_{eq}), 2.37 (H-15_{ax}), 2.50 (H-15_{eq}, H-16_{ax}), 2.59 (*dd*, $J_{9,11eq}$ = 5.6 Hz, $J_{9,11ax}$ = 10.4 Hz, H-9), 2.65 (*d*, $J_{18,19}$ = 11.4 Hz, H-18), 3.12 (*dd*, $J_{2eq,3}$ = 3.7 Hz, $J_{2ax,3}$ = 11.5 Hz, H-3), 3.90 (H-5′), 4.03 (H-5″), 4.13 (H-4′), 4.21 (H-2″), 4.28 (H-$6'_a$), 4.30 (H-3″), 4.36 (H-4″), 4.38 (H-3′), 4.40 (H-$6''_a$), 4.46 ($J_{5'',6b''}$ = 2.5 Hz, $J_{6a'',6b''}$ = 12.0 Hz, H-$6''_b$), 4.48 ($J_{5',6b'}$ = 2.4 Hz, $J_{6a',6b'}$ = 11.9 Hz, H-$6'_b$), 4.82 (*d*, $J_{1',2'}$ = 7.4 Hz, H-1′), 5.01 (H-2′), 5.95 (H-12), 6.34 (*d*, $J_{1'',2''}$ = 8.2 Hz, H-1″); ^{13}C NMR: Table 1.

Zygophyloside H (**4**). ($C_{47}H_{74}O_{18}$, M_r 927.12); mp 215–219°; $[\alpha]_D^{25}$ +36° (MeOH; *c* 0.17). LSI-MS negative ion mode *m/z* (rel. int.): 925 $[M-H]^-$ (47), 763 $[M-H-Glc]^-$ (7), 719 $[M-H-Glc]^-$ (15), 587 $[M-H-Glc-CO_2-Ara]^-$ (10). ^1H NMR: δ 0.73 (*d*, $J_{20,30}$ = 6.3 Hz, H_3-30), 0.87 (H-20), 0.90 (H-5), 0.91 (*s*, H_3-25), 1.04 (*s*, H_3-24), 1.09 (H-1_{ax}), 1.12 (*s*, H_3-23), 1.16 (*d*, $J_{19,29}$ = 6.1 Hz, H_3-29), 1.21 (*s*, H_3-26), 1.23–1.30 (H-$21_{ax/eq}$), 1.27 (H-6_{ax}), 1.35 (H-19), 1.48 (H-6_{eq}), 1.58 (H-1_{eq}), 1.59 (*d*, $J_{5',6'}$ = 5.5 Hz, H_3-6′), 1.66 (H-7_{ax}), 1.73 (H-22_{ax}), 1.82 (H-7_{eq}), 1.86 (H-2_{ax}, H-22_{eq}), 2.00–2.10 (H-$11_{ax/eq}$), 2.10 (H-2_{eq}), 2.20 (H-16_{eq}), 2.42 (H-15_{ax}), 2.53 (H-15_{eq}, H-16_{ax}), 2.68 (d, $J_{18,19}$ = 11.3 Hz, H-18), 2.68 (H-9), 3.10 (*dd*, $J_{2eq,3}$ = 4.3 Hz, $J_{2ax,3}$ = 11.6 Hz, H-3), 3.64 (H-4′), 3.66 (H-5′), 3.75 (H-$5''_{ax}$), 4.03 (H-2′, H-5‴), 4.14 (H-3′), 4.19 (H-3″), 4.23 (H-2‴), 4.29 (H-4″), 4.30 (H-3‴), 4.32 (H-$5''_{eq}$), 4.36 (H-4‴), 4.40 (H-$6'''_a$), 4.43 (H-$6'''_b$), 4.53 (H-2″), 4.65 (*d*, $J_{1',2'}$ = 7.6 Hz, H-1′), 5.13 (*d*, $J_{1'',2''}$ = 6.6 Hz, H-1″), 5.99 (H-12), 6.36 (*d*, $J_{1''',2'''}$ = 8.1 Hz, H-1‴); ^{13}C NMR: Table 1.

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REFERENCES

1. Saber, A. H. and El-Moghazy Shoaib, A. M., *Journal of Pharmaceutical Science of the U.A.R.*, 1961, **1**, 135.
2. Matos, M. E. O., Sousa, M. P., Machado, M. I. L. and Filho, R. B., *Phytochemistry*, 1986, **25**, 1419.
3. Ahmad, V. U., Uddin, G. S. and Bano, S., *Journal of Natural Products*, 1990, **53**, 1193.
4. Ahmad, V. U., Uddin, G. S. and Ali, M. S., *Phytochemistry*, 1993, **33**, 453.
5. Tschesche, R., Duphorn, I. and Snatzke, G., *Liebigs Annals of Chemistry*, 1963, **667**, 151.
6. Aquino, R., De Simone, F., Pizza, C., Conti, C. and Stein, M. L., *Journal of Natural Products*, 1989, **52**, 679.
7. Elgamal, M. H. A., Shaker, K. H., Pöllmann, K. and Seifert, K., *Phytochemistry*, 1995, **40**, 1233.
8. van den Dool H. and Kratz, P. D., *Journal of Chromatography*, 1963, **11**, 463.